SYNTHÈSE DE DÉRIVÉS STÉROÏDIENS ET NON STÉROÏDIENS COMME INHIBITEURS DES 17β-HYDROXYSTÉROÏDES DÉSHYDROGÉNASES TYPE 1 ET TYPE 3

Thèse

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Résumé

À cause de leur implication dans la biosynthèse des estrogènes et des androgènes, les enzymes de la famille des 17β-hydroxystéroïdes déshydrogénases (17β-HSDs) types 1, 3 et 5 sont des cibles thérapeutiques intéressantes pour le traitement des cancers estrogéno-dépendants et androgéno-dépendants. Malgré l'existence d'inhibiteurs de la 17β-HSD1, il n'y a pas encore de traitement du cancer du sein basé sur leur utilisation. Le CC-156 est un inhibiteur connu de la 17β-HSD1; cependant, à cause de son noyau stéroïdien, ce composé de type estrane stimule la prolifération des cellules cancéreuses sensibles aux estrogènes, limitant ainsi son utilisation thérapeutique. Afin de développer des inhibiteurs non estrogéniques de la 17β-HSD1, nous avons synthétisé trois mimiques non stéroïdiennes du CC-156 à partir du bromhydrate du tétrahydro-isoquinolinol. Bien que ces composés inhibent peu la 17β-HSD1, ils sont non estrogéniques. Nous avons aussi développé une voie de synthèse pour préparer deux chimiothèques possédant chacune 75 mimiques de l'estradiol. Ces derniers, plus flexibles que les dérivés précédents, ont été conçus et synthétisés comme potentiels inhibiteurs de la stéroïde sulfatase ou pour agir comme modulateurs sélectifs du récepteur des estrogènes.

L'inhibition de la 17β-HSD3 ou de la 17β-HSD5 permettrait de diminuer le taux des androgènes circulants et tumorals. En partant de l'androstérone (ADT), nous avons préparé une nouvelle famille d'inhibiteurs de la 17β-HSD3: des 3-spiromorpholinone-ADT et des 3-spirocarbamate-ADT ayant divers groupements hydrophobes sur leur cycle E supplémentaire. Afin de poser un premier jalon pour la synthèse d'inhibiteurs hybrides des 17β-HSD3 et 17β-HSD5, une spiromorpholinone ou un spirocarbamate a été ajouté en position C-3 d'une 17-spiro-δ-lactone. Brièvement, trente-deux 3-spiromorpholinone-ADT, cinq 3-spirocarbamate-ADT, trois 17-monospiro-δ-lactone-ADT et deux 3,17-dispiroandrostane-ADT ont été synthétisés. Quatre spiromorpholinones non stéroïdiennes ont aussi été synthétisées afin d'étudier le rôle du noyau androstane sur l'efficacité des inhibiteurs de la 17β-HSD3. Tous les produits finaux et les intermédiaires ont été caractérisés par spectrométries de RMN ^{1}H, RMN ^{13}C, IR et SM. Le potentiel inhibiteur de tous ces composés sur la 17β-HSD3 et leur androgénicité ont été mesurés. L'analyse des relations structure-activité a permis d'obtenir deux inhibiteurs efficaces et non androgéniques de la 17β-HSD3.

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Liste des abréviations et symboles

17α-OHPREG	17alpha-hydroxyprégnénolone
17α-OHPROG	17alpha-hydroxyprogestérone
5α-Red	5alpha-réductase
Å	Angström
ACTH	Adréno Cortico Trophic Hormone
ADN	acide désoxyribonucléique
ADNc	acide désoxyribonucléique cyclique
AKR	aldo-céto réductase
AKT	protéine kinase B
app.	apparent
AR	récepteur des androgènes
ARE	élément de réponse aux androgènes
ARN	acide ribonucléique
Asp	asparagine
17β-HSD	17bêta-hydroxystéroïde déshydrogénase
°C	degré celsius
^{13}C NMR	résonance magnétique nucléaire du carbone 13
CHOL	cholestérol
cm^{-1}	réciproque du centimètre
Δ^4-dione	4-androstène-3,17-dione
Δ^5-diol	5-androstène-3,17-diol
d	doublet
dd	doublet de doublet
DCM	dichlorométhane
DHEA	déhydroépiandrostérone
DHT	dihydrotestostérone
DIPEA	diisopropyléthylamine
DLA	domaine de liaison à l'ADN
DLL	domaine de liaison du ligand
DMF	*N*,*N*-diméthylformamide
E1S	estrone sulfate
E2	estradiol
EGFR	*epidermal growth factor receptor*
epi-ADT	épi-androstérone
equiv	équivalent
ERA	élément de réponse des androgènes
ERE	élément de réponse des estrogènes
FBS	*foetal bovine serum*
FDA	*Food and Drug Administration*
FESVG	fraction d'éjection systolique du ventricule gauche
Fmoc	9-fluorenylméthoxycarbonyle
Fmoc-OSu	N-(9-fluorenylméthoxycarbonyle)-succinimide
FSH	hormone folliculo-stimulante
FTN	facteur de transcription du domaine N-terminal
Glu	glutamate
GnRH	*gonadotropin-releasing hormone*
^{1}H NMR	résonance magnétique nucléaire du proton
h	heure

H12	hélice 12
HER2	*human epidermal growth factor receptor-2*
His	histidine
HPLC	*high performance liquid chromatography*
IC_{50}	concentration de produits nécessaire pour inhiber de 50 % la croissance cellulaire ou l'activité enzymatique
IR	infrarouge
J	constante de couplage
LDA	lithium diisopropylamine
LH	hormone lutéinisante
LHRH	*luteinizing hormone-releasing hormone*
LRMS	*low resolution mass spectrometry*
Lys	lysine
M	molaire
m	multiplet
m-CPBA	acide méta-chloroperbenzoïque
m/z	masse/charge
MAPK	*mitogen activated protein-kinase*
mg	milligramme
MHz	mégahertz
min	minute
mL	millilitre
MOM	méthoxy méthyl éther
MTS	3-(4,5-diméthylthiazol-2-yl)-5-(3-carboxyméthoxyphényl)2-(4-sulfophényl)- 2H-tétrazolium
NADH	nicotinamide adénine dinucléotide (forme réduite)
$NADP^+$	nicotinamide adénine dinucléotide (forme oxydée)
$NADP^+$	nicotinamide adénine dinucléotide phosphate (forme oxydée)
NADPH	nicotinamide adénine dinucléotide phosphate (forme réduite)
nM	nanomolaire
NMO	4-méthylmorpholine-*N*-oxide
NMR	résonance magnétique nucléaire
p-TSA	acide *para*-toluènesulfonique
Ph	groupe phényle
ppm	partie par million
PREG	prégnénolone
PROG	progestérone
PSA	antigène spécifique de la prostate
RA	recepteur des androgènes
RE	récepteur des estrogènes
RFA	radiofréquence
RP	recepteur de la progestérone
rt	*room temperature*
s	singulet
SAR	*structure-activity relationships*
SARM	*selective androgen receptor modulator*
SDR	*short chain dehydrogenase reductase*
Ser	sérine
SERM	*selective estrogen receptor modulator*
SG	survie globale

SSP	survie sans progression
STS	stéroïde sulfatase
T	testostérone
t	triplet
TBDMS	tert-butyldiméthylsilyle
TEA	triéthylamine
TFA	acide trifluoroacétique
THF	tétrahydrofuranne
TLC	*thin layer chromatography*
TMSC1	chlorotriméthylsilane
TPAP	tetrapropylammonium perruthénate
Tyr	tyrosine
VEGF	*vascular endothelial growth factor*
δ	déplacement chimique en ppm
µM	micromolaire
v	fréquence en Hz

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Avant-Propos

Depuis mon plus jeune âge, dans mon pays natal, j'ai été passionné par les sciences de la nature et de la vie. Je ne manquais pas d'utiliser des objets recyclés pour faire des expériences de chimie (cuves électrolytiques à l'aide de boites en plastique, oxydation des métaux avec dégagement gazeux dans des bouteilles transparentes…). Et durant mon adolescence, j'accompagnais mon père, phytothérapeute, dans la forêt pour faire la récolte des plantes médicinales.

Mon admission au programme de doctorat en physiologie-endocrinologie à partir de la session d'été 2009 sur la direction du Professeur Donald Poirier a été une merveilleuse nouvelle pour moi, mais aussi pour mon père, car il savait à quel point je tenais à poursuivre mes études dans un domaine qui a un impact plus ou moins direct sur les populations. Je suis entré pour la première fois dans le laboratoire de chimie médicinale du centre de recherche du CHU de Québec (alors appelé CRCHUQ) le 2 juillet 2009, date à laquelle j'ai commencé les travaux présentés dans cette thèse. Il y a de cela quatre ans et quatre mois.

En entente avec mon directeur, je présente les résultats de mes recherches sous forme d'articles. Étant donné que l'anglais est la langue permise par les journaux dans lesquels nous avons publié nos articles, et ceux dans lesquels nous soumettrons nos prochaines publications, tous nos articles ont été rédigés en anglais. Au moment de l'écriture de cette thèse, celle-ci contenait six articles au total, dont quatre articles publiés et deux manuscrits à soumettre. Une section «avant-propos» a été incluse en début de chaque chapitre pour présenter l'état de celui-ci ainsi que ma contribution aux résultats rapportés.

Les six chapitres qui constituent cette thèse sont séparés en deux parties. Dans la partie A, différentes approches sont décrites afin d'obtenir des mimiques non stéroïdiennes de E2, ou du CC-156, un puissant inhibiteur connu de la 17β – hydroxystéroïde déshydrogénase de type 1 (17β-HSD1), mais qui possède une activité estrogénique résiduelle non souhaitable. Bien que les mimiques non stéroïdiennes du CC-156 que nous avons synthétisé inhibent peu la 17β-HSD1, les évaluations biologiques présentées dans cette thèse montrent qu'ils ne possèdent pas d'activité estrogénique résiduelle, suggérant la potentielle utilisation de tels noyaux pour le développement d'autres médicaments pour le traitement des maladies sensibles aux estrogènes. La synthèse chimique, la caractérisation et l'évaluation biologique de toutes

ces mimiques non stéroïdiennes du CC-156 sont décrites. Le deuxième chapitre présente le développement d'une synthèse en parallèle simple en phase liquide de mimiques non stéroïdiennes de l'estradiol (E2). La voie de synthèse décrite dans ce chapitre, permet de générer un très grand nombre de mimiques non stéroïdiennes de l'estradiol. Dans le chapitre 2 nous présentons la synthèse chimique et la caractérisation de 150 mimiques non stéroïdiennes de l'estradiol, conçues comme potentiels inhibiteurs de la stéroïde sulfatase, une enzyme de la stéroïdogénèse ciblée pour différents cancers sensibles aux estrogènes, ou comme modulateurs sélectifs du récepteur des estrogènes. L'activité biologique de ces composés reste à confirmer.

La partie B de cette thèse présente le développement d'une nouvelle famille d'inhibiteurs efficaces et non androgéniques de la 17β-HSD3, à partir d'un noyau androstane. Une voie de synthèse des dérivés d'androstérone (ADT), portant en position C-3 un spirocycle (morpholinone ou carbamate) est décrite. Les résultats de la synthèse chimique, la caractérisation, l'évaluation biologique comme inhibiteur de la 17β-HSD3 et l'évaluation de l'activité proliférative (androgénicité) et antiproliférative (anti-androgénicité) sont présentés dans les quatre chapitres qui constituent cette deuxièma partie de ma thèse.

Afin de mieux situer les lecteurs de cette thèse dans le contexte de sa réalisation, et dans la compréhension des résultats obtenus, une introduction en français est abordée dès le début de la thèse, avec différentes sections traitant des généralités sur différents types de cancer. La première moitié de cette partie introductive décrit quelques options thérapeutiques couramment proposées aux patients qui souffrent de ces cancers et décrit quelques éléments de base pour la compréhension de leur mode d'action. Étant donné que le sujet principal de cette thèse concerne le développement d'inhibiteurs des 17β-HSD1 et 17β-HSD3, nous abordons dans la deuxième moitié de l'introduction, la stéroïdogénèse. Ces inhibiteurs pourront être utilisés en hormonothérapie pour bloquer la formation des estrogènes et des androgènes, et moduleront ainsi leur action sur leurs récepteurs respectifs.

Introduction générale

Introduction

Certains tissus montrent une sensibilité particulière aux effets des hormones sur la croissance, la différenciation ou la survie cellulaire. C'est le cas de la prostate, la glande mammaire, l'utérus et l'endomètre. Les hormones androgéniques et estrogéniques entretiennent la croissance des cellules cancéreuses de ces tissus dits hormonosensibles. La participation des estrogènes dans le maintien et l'évolution du cancer du sein et d'autres maladies estrogéno-dépendantes, par exemple, a grandement stimulé l'intérêt des chercheurs au cours des dernières décennies [1-3]. De la même manière, il a été établi que les androgènes stimulent la prolifération des cellules cancéreuses de la prostate [4, 5]. Afin d'apporter un traitement efficace aux cancers androgéno- et estrogéno-sensibles, on doit contrôler la formation et l'action des hormones androgéniques et estrogéniques les plus puissantes. Puisque ces hormones agissent très souvent en activant les récepteurs nucléaires, qui sont des facteurs promitotiques, le blocage de ces récepteurs est généralement utilisé comme approche thérapeutique. Parmi les facteurs qui contrôlent la formation des androgènes et des estrogènes (stéroïdogénèse), les 17β-hydroxystéroïde déshydrogénases (17β-HSDs) jouent un rôle très important en raison de leur position dans la voie de biosynthèse des androgènes et des estrogènes. La dernière étape de la biosynthèse de l'estradiol (E2), le plus puissant des estrogènes, est contrôlée par la 17β-HSD type 1 (17β-HSD1); cette enzyme catalyse la transformation de l'estrone (E1) en E2 en présence du cofacteur NADPH [6-8]. D'une manière similaire, la 17β-HSD type 3 (17β-HSD3) catalyse la dernière étape de la biosynthèse de la testostérone (T) à partir de la 4-androstène-3,17-dione (Δ^4-dione) en présence du NADPH [6, 9, 10].

La 17β-HSD3 existe presqu'exclusivement dans la fraction microsomale des cellules de Leydig testiculaire [11]. Ainsi, la 17β-HSD3 contribue approximativement à la production de 60 % des androgènes actifs chez l'homme [12]. L'inhibition de cette enzyme pourrait non seulement être une stratégie privilégiée dans le traitement des maladies androgéno-dépendantes, tel que le cancer de la prostate et l'hyperplasie bénigne prostatique, mais pourrait aussi contribuer d'une façon intéressante à développer des agents contraceptifs. Le blocage complet de la formation et de l'action des androgènes est crucial pour contrer la prolifération des cellules cancéreuses androgéno-dépendantes dont celles de la prostate [13]. Plusieurs traitements endocriniens existent déjà, ils consistent à bloquer la production testiculaire des

androgènes, à bloquer l'effet de la T ou de la dihydrotestostérone (DHT) sur le récepteur des androgènes (RA), ou à combiner les deux méthodes [14]. L'une des stratégies de traitement actuellement utilisée combine l'administration d'un agoniste de la LHRH pour produire une castration médicale et de l'anti-androgène pure flutamide pour bloquer le RA [13]. Par ailleurs, nous estimons que le ciblage spécifique de la 17β-HSD3, permettrait d'éviter les effets indésirables et les complications dues à la castration tant chirurgicale que chimique. À cause de la similitude des inhibiteurs avec les stimulateurs naturels du RA, ils sont souvent en mesure de ce lier à celui-ci et causer la prolifération des cellules cancéreuses de la prostate (effet androgénique).

1. Le cancer de l'ovaire

1.1 Généralités

Les ovaires représentent la partie essentielle de l'appareil reproducteur féminin. Ce sont deux petits organes ovoïdes situés au fond du bassin de la femme, près de l'extrémité des trompes de Fallope. L'ovaire exprime plusieurs enzymes de la stéroïdogénèse [15]. Ainsi, on y trouve la CYP17 responsable de la transformation du 17α-hydroxyprégnénolone (17α-OHPREG) et 17α-hydroxyprogesterone (17α-OHPROG) en déhydroépiandrostérone (DHEA) et Δ^4-dione respectivement, la CYP19 responsable de la transformation de Δ^4-dione et de T en E1 et E2 respectivement, la HSD3B1 (le gène de la 3β-hydroxystéroïde déshydrogénase) et la HSD17B1 (le gène de la 17β-HSD1). Des études ont montré qu'en plus de l'histoire familiale, les perturbateurs endocriniens peuvent constituer un facteur de risque important pour le cancer de l'ovaire : une utilisation du citrate de clomifène sur une durée de un an et plus par exemple pour favoriser l'ovulation chez les femmes augmente le risque de développer une tumeur ovarienne [16]. Le Danazol, employé pour le traitement de l'endométriose, est également un facteur de risque pour le cancer de l'ovaire [17]. Par ailleurs, les femmes qui utilisent des estrogènes après la ménopause sont exposées au risque de developper un cancer de l'ovaire [18]. Le cancer de l'ovaire est le cancer gynécologique le plus mortel et en raison de son difficile pronostique, ce cancer est considéré comme un « Silent killer » [19]. Cependant, bien que seulement 20 % des cancers de l'ovaire soient diagnostiqués au stage local (dans l'ovaire), 90 % des patientes diagnostiquées à ce stage peuvent être soignées par la chirurgie associée à la chimiothérapie commerciale [20].

1.2 Traitement du cancer de l'ovaire

Plusieurs options thérapeutiques sont proposées et discutées avec la patiente de façon à répondre aux besoins uniques de cette dernière. Les décisions relatives au traitement du cancer de l'ovaire se basent sur quatre éléments: le stade de la tumeur, le grade de la tumeur, le type de cancer de l'ovaire et le projet de grossesse de la femme atteinte après le traitement. Ainsi la chirurgie, la chimiothérapie, la thérapie ciblée, l'hormonothérapie et la radiothérapie sont souvent proposées.

1.2.1 La chirurgie

C'est le traitement principal proposé pour la plupart des cancers de l'ovaire. L'étendu de la chirurgie dépendra du stade d'avancement, mais aussi de l'état général de la patiente. La chirurgie conservatrice est généralement proposée à la femme désirant avoir une grossesse, se trouvant dans le stade IA, grade 1 [21]. Pour les patientes dans les stade IIA à IV, les meilleures chances de survie prolongée sont observées si l'exérèse est initiale et complète [22]. Cependant, la chirurgie doit être très souvent combinée à d'autres options thérapeutiques telles que la chimiothérapie, surtout dans les stades avancés de la maladie.

1.2.2 La chimiothérapie

La chimiothérapie utilisée pour soigner le cancer de l'ovaire est généralement une combinaison de deux ou plusieurs composés: les complexes à base de platine (Figure 1) tel que le cisplatine et le carboplatine, par exemple, et une taxane telle que le paclitaxel ou le docetaxel [23]. En dehors de ces quatre agents chimiothérapeutiques couramment utilisés, l'altretamine, le capecitabine, la cyclophosphamide composé dérivant de l'ifosfamide, etc. sont aussi utilisés (Figure 1). En plus des cellules cancéreuses, ces agents thérapeutiques tuent souvent une partie des cellules normales du corps et les effets secondaires liés à leur utilisation sont nombreux: la nausée et le vomissement, la perte d'appétit, la perte des cheveux, les ulcères buccaux etc. La chimiothérapie peut aussi endommager les cellules du sang produit dans la moelle osseuse. Cependant, la plupart des effets secondaires disparaissent à l'arrêt du traitement.

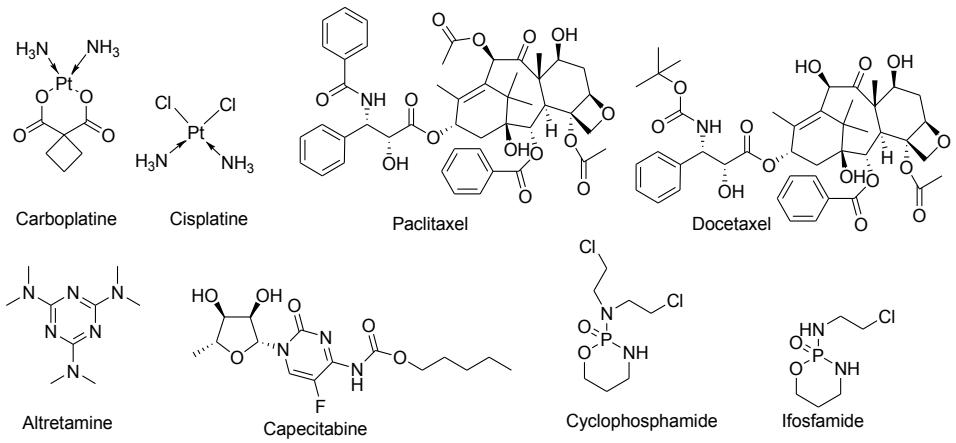


Figure 1: Quelques agents chimiothérapeutiques utilisés contre le cancer de l'ovaire

1.2.3 La thérapie ciblée

La thérapie ciblée est une variante de chimiothérapie. Elle utilise un composé capable d'agir de façon ciblée sur les programmes génétiques des cellules cancéreuses qui les différencient des cellules normales. Pour le cancer épithélial de l'ovaire, la thérapie ciblée la plus avancée est le bevacizumab (Avastin) [24]. C'est un anticorps monoclonal dirigé contre les facteurs de croissance de l'endothélium vasculaire (VEGF). Cette protéine va inhiber la vascularisation des cellules cancéreuses, et donc conduire à la mort ciblée de ces dernières. Bien que ce traitement soit déjà utilisé pour certains cancers, il n'a pas encore été validé dans le cas du cancer de l'ovaire.

1.2.4 L'hormonothérapie

L'hormonothérapie systémique est rarement utilisée dans le cas du cancer de l'ovaire épithélial, mais est très souvent utilisée quand il s'agit des tumeurs stromales ovariennes. Les agonistes de la LHRH sont utilisés pour baisser le niveau des estrogènes chez les femmes en préménopause. Les exemples d'agonistes très souvent utilisés sont la goséréline (Zoladex) et le leuprolide (Lupron) (Figure 2), injectés mensuellement ou au trimestre. Ce traitement peut causer l'ostéoporose s'il est utilisé sur une longue période [25]. Dans le but de baisser le niveau des estrogènes circulants chez les patientes, les inhibiteurs de l'aromatase peuvent être utilisés et ainsi permettre de traiter certaines tumeurs stromales de l'ovaire. Parmi les inhibiteurs utilisés, on peut citer le létrozole (Femara), l'anastrozole (Arimidex) et l'exémestane (Aromasin) (Figure 2), lesquels sont administrés sous forme de pilules une fois par jour [26-27]. Les effets indésirables reliés à l'utilisation des inhibiteurs de l'aromatase incluent les bouffées de chaleur, les douleurs articulaires et musculaires, ainsi que l'affaiblissement de la densité osseuse, pouvant conduire à l'ostéoporose [28].

2. Le cancer de l'endomètre

2.1 Généralités

L'endomètre est une membrane qui tapisse l'intérieur de l'utérus, l'organe dans lequel le fœtus se développe pendant la grossesse. Dans son fonctionnement normal chez une femme ayant atteint l'âge de la puberté, l'endomètre s'épaissit suivant le cycle menstruel et est évacué à la menstruation, à moins que ne survienne une grossesse [29]. Le cancer de l'utérus qui prend naissance dans l'endomètre est appelé carcinome de l'endomètre. Ces derniers constituent la majorité des cancers de l'utérus. Cependant, il existe un autre type de cancer de l'utérus qui se développe dans les couches musculaires de ce dernier; il s'agit du sarcome de l'utérus. Bien que l'endomètre ait longtemps été considéré comme un tissu cible pour les hormones stéroïdiennes non impliquées dans la stéroïdogénèse, une analyse transcriptomique et minutieuse des gènes [30, 31] a montré que ce tissu exprime l'activité de plusieurs enzymes de la stéroïdogénèse.

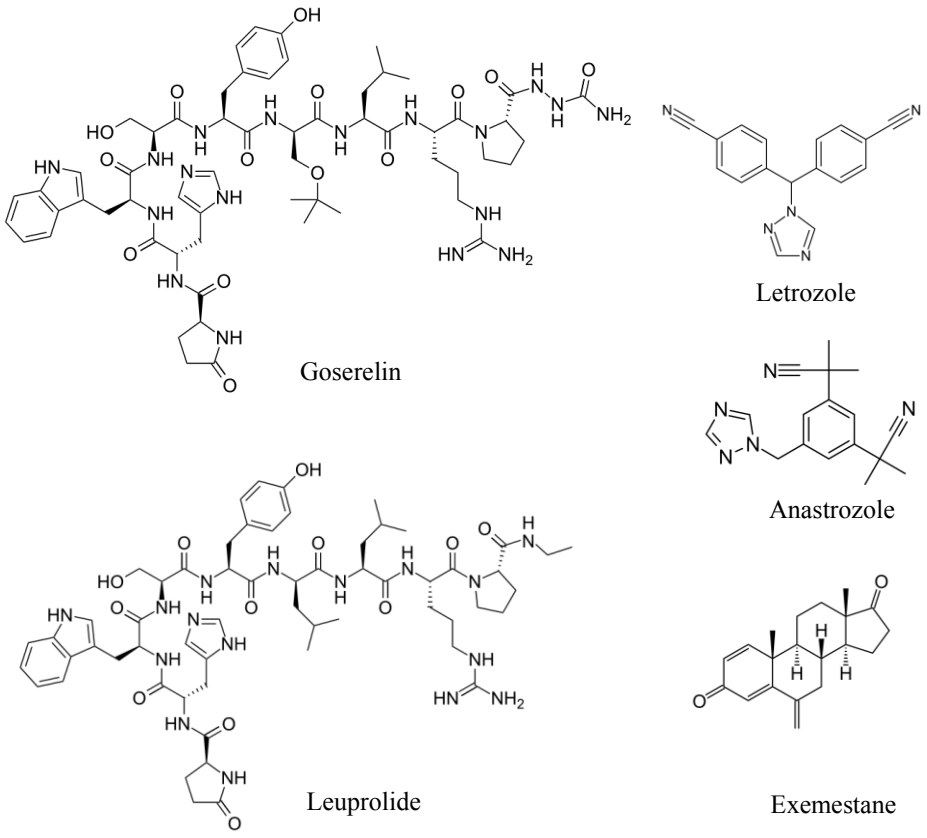


Figure 2: Quelques agents hormonothérapeutiques utilisés contre le cancer de l'ovaire

On y retrouve donc STAR, une protéine impliquée dans l'internalisation du cholestérol, CYP11A1, responsable du clivage de la chaine latérale du cholestérol et aussi HSD3B, CYP17A1, HSD17B1, HSD17B2 et CYP19A1 [32-40]. Le cancer de l'endomètre est divisé en deux types: le type I, estrogéno-dépendant est généralement moins sévère que le type II, non estrogeno-dépendant [41]. En plus de l'histoire familiale les principaux facteurs de risque sont l'obésité, le diabète, la ménopause tardive et certains traitements hormonaux dont le traitement par le tamoxifène [42-44].

2.2 Traitement du cancer de l'endomètre

Les options thérapeutiques pour les patientes atteintes du cancer de l'endomètre, prennent en compte leur âge, la présence de complications, l'étendue de la lésion et les caractéristiques du cancer. Dans tous les cas, la chirurgie est généralement le traitement de première ligne [45]. Ici l'objectif est d'éliminer complètement la tumeur ou d'en diminuer le volume, en vue de faciliter le pronostique, de déterminer le stade d'avancement et donc de donner une chance à d'autres options de traitement telles que la chimiothérapie, la radiothérapie, la curiethérapie et ou l'hormonothérapie. Nous discuterons très brièvement quelques unes de ces options thérapeutiques dans le paragraphe qui suit.

Lorsque la chimiothérapie est l'indication appropriée pour la patiente, plusieurs protocoles sont utilisés; nous nous limiterons ici à spécifier les molécules et associations courantes, sans toute fois indiquer le dosage [46-48]:

L'association cisplatine/doxorubicine (Figure 3) est la plus étudiée, mais en raison de sa toxicité hématologique, l'association carboplatine/paclitaxel est préférée surtout pour les patientes fragiles. En cas de carcinosarcomes, les associations très souvent utilisées sont ifosfamide/doxorubicine, cisplatine/ifosfamide, cisplatine/doxorubicine et cisplatine/ifosfamide/doxorubicine. L'hormonothérapie adjuvante n'est que très rarement recommandée pour le cancer de l'endomètre. Cependant, en situation de métastase, elle peut être indiquée, le plus souvent lorsque la chimiothérapie est contre-indiquée et la maladie a une évolution lente avec présence de récepteurs hormonaux. L'acétate de médroxyprogestérone (Figure 3) est administré par voie orale, et si celle-ci n'est pas indiquée, des antagonistes du RE peuvent être utilisés à sa place [49].

3. Le cancer du sein

3.1 Généralités

Parmi les différents types de cancers dont sont victimes les femmes, le cancer du sein est la deuxième cause de mortalité après le cancer du poumon [50]. La charge globale mondiale du cancer du sein est supérieure à celle de tous les autres types de cancer, et le taux d'incidence de cette pathologie est en augmentation [51]. L'âge avancé demeure l'un des plus grands facteurs de risque pour développer le cancer du sein; environ 50 % des femmes auront 65 ans et plus pendant qu'elles seront diagnostiquées. Le risque estimé chez des femmes âgées entre 60 et 79 ans est de 1 cas sur 14 femmes [52, 53].

Le carcinome canalaire infiltrant, encore appelé adénocarcinome canalaire envahissant, représente jusqu'à 80 % de tous les cancers du sein infiltrant diagnostiqués à la fois chez les patientes âgées et les plus jeunes [54]. Il est bien connu que les hormones estrogéniques sont d'importants stimulateurs de la plupart des cancers du sein [55]. Le profil histologique de cancer du sein étant le plus souvent dépendant de l'âge de la patiente, le pourcentage des tumeurs estrogéno-dépendantes (tumeurs RE+) augmente avec l'âge [56]. Chez les moins de 65 ans, on a 83 % de cas de tumeurs RE+, alors que chez les patientes de 65 ans et plus on détecte entre 87 % et 91 % de tumeurs RE+ et cette tendance est maintenue à la hausse chez les 85 ans et plus.

En plus de l'âge, les autres facteurs de risque recensés pour le cancer du sein sont: l'histoire familiale, l'indice de masse corporel, la prise excessive d'alcool, l'exposition aux radiations ionisantes, la prise de contraceptifs oraux et la thérapie hormonale de substitution [57, 58].

3.2 Traitement du cancer du sein

Une évaluation minutieuse du stage du cancer est établie selon le système TNM défini à cet effet. Dans ce système, (T) représente la taille de la tumeur, (N) représente l'état des nodules lymphatiques, et (M) signifie présence ou non de métastase; suite à cette évaluation, des options thérapeutiques sont proposées à la patiente [59].

3.2.1 La chirurgie

Malgré les grandes avancées de la chirurgie du cancer du sein durant les 20 dernières années qui donnent à beaucoup de femmes le choix d'une thérapie de conservation de la majeur partie du tissu mammaire [60], la plupart des médecins recommandent la mastectomie quand il existe un risque de récurrence plus grand que 10-15 %. La biopsie sentinelle des nodules lymphatiques permet la prédiction du statut du ganglion lymphatique axillaire sans dissection totale [61]. Le défit reste le traitement des tumeurs primaires sans faire de chirurgie ouverte, mais avec une procédure la moins invasive possible: l'ablation à la radiofréquence (RFA), l'ablation interstitielle au laser, l'ablation ciblée à l'ultrason ou la cryothérapie constituent des alternatives intéressantes à la chirurgie ouverte. Cependant, par souci de simplicité, nous nous limiterons pour la suite à la chimiothérapie, le traitement endocrinien et l'usage des anticorps monoclonaux adjuvants.

3.2.2 Les thérapies adjuvantes: Chimiothérapie, l'hormonothérapie et la thérapie ciblée au trastuzumab

La thérapie adjuvante systémique correspond à l'administration d'agents chimiothérapeutiques, le traitement hormonal et ou le trastuzumab (un anticorps monoclonal ciblant le récepteur des facteurs de croissance épidermique humain HER2) après la chirurgie primaire [62]. Le choix de ces thérapies adjuvantes repose sur la présence ou non du récepteur des estrogènes (RE) ou du récepteur de la progestérone (RP) avec un bénéfice visible chez les patientes qui présentent ces récepteurs [63].

Le rôle des taxanes (docétaxel et paclitaxel), des agents cytotoxiques, a été exploré dans plusieurs études [64, 65] (Figure 1). Une méta-analyse récente sur 15 500 patientes traitées par l'un ou l'autre de ces deux agents a montré un gain de survie de 3 % chez les patientes RE+ comparé à l'anthracycline.

Comme dans le cas du cancer de l'ovaire, les anthracyclines et les taxanes sont utilisés comme agents thérapeutiques cytotoxiques. Le docétaxel et le paclitaxel sont les taxanes qui ont démontré en clinique une activité cytotoxique importante avec des effets secondaires tolérables.

Le traitement endocrinien du cancer du sein est toujours d'actualité étant donné que dans la majorité des cas, les cellules de la tumeur sont RE+: l'usage des agonistes de la

GnRH ou de l'anti-estrogène tamoxifène est fait chez les femmes préménopausées tandis que chez les femmes ménopausées, un inhibiteur de la biosynthèse des estrogènes est généralement une alternative à l'utilisation du tamoxifène. L'utilisation des inhibiteurs n'a pas cependant connu le même succès chez les femmes préménopausées, car les agents utilisés ne sont pas assez puissants pour résoudre le problème des effets d'augmentation des pulsions gonadiques résultant de la rétroaction [67].

La source d'estrogènes est orientée par le statut de la patiente: la production ovarienne est prédominante chez les femmes en préménopause, alors que chez les femmes post-ménopausées, la biosynthèse par les tissus périphériques (muscles, tumeur elle-même) est plus importante [68]. L'utilisation des médicaments qui bloquent spécifiquement la biosynthèse des estrogènes dans son site de production est une stratégie intéressante. C'est à ce titre que les inhibiteurs d'aromatase sont mieux adaptés aux patientes ménopausées. On peut les classer en deux catégories: les dérivés stéroïdiens tels que le formestane et l'exémestane et les dérivés non stéroïdiens tels que le fadrozole, le vorozole (retiré du marché en raison de son efficacité relative), le létrozole et l'arimidex (anastrozole) (Figure 2 et 3) [69].

Le trastuzumab en monothérapie ou en association avec la chimiothérapie est la première thérapie ciblée à ce jour sur le marché. Cette dernière a confirmé son activité et son bénéfice dans le cancer du sein métastatique HER2-positif [70, 71]. En situation adjuvante, des essais randomisés ont évalué le bénéfice du trastuzumab en termes de survie sans progression (SSP) et de survie globale (SG). La toxicité la plus importante de cette thérapie ciblée est la toxicité cardiaque, secondaire à la baisse de la fraction d'éjection systolique du ventricule gauche (FESVG) [70]. Le trastuzumab a montré une action radio-sensibilisante en surmontant la résistance à l'apoptose induite par la radiothérapie sur des modèles expérimentaux utilisant les lignées cellulaires de cancer du sein surexprimant le récepteur HER2. Cet effet passe par le blocage de la phosphorylation des deux voies principales de signalisation, AKT (protéine kinase) et mitogen-activated protein kinase (MAPK) [72, 73].

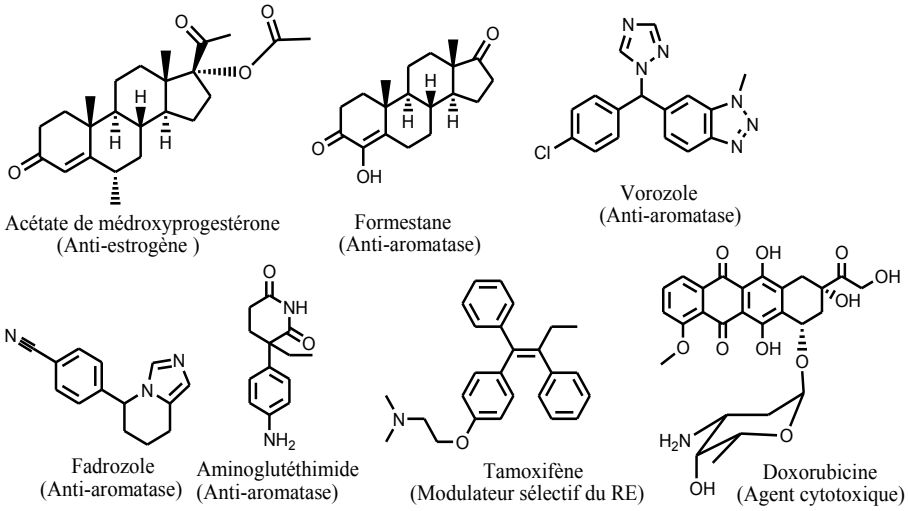


Figure 3: Structure de quelques inhibiteurs de l'aromatase, du modulateur sélectif du RE (SERM) tamoxifène et de l'agent cytotoxique doxorubicine

4. Le cancer de la prostate

4.1 Généralités

La prostate est une glande accessoire du système reproducteur masculin en forme de châtaigne, située profondément sous la vessie, en avant du rectum et entourant une partie du canal de l'urètre par lequel elle est traversée [74]. La taille de la prostate augmente de façon significative à la puberté, sous l'effet de la testostérone secrétée par les testicules. La prostate fabrique le liquide prostatique qui a un rôle important dans la maturation et la motilité des spermatozoïdes, d'où l'importance de la prostate pour la fertilité masculine. La prostate est formée de cellules glandulaires et stromales. On distingue trois types de cellules: les cellules sécrétoires, riches en antigène spécifique prostatique (PSA), sont situées à la surface et sont souvent le point de départ des cancers; les cellules basales (ou cellules souches) sont en profondeur et les cellules neuro-endocrines, hormono-résistantes, qui sont associées à une angiogénèse hormono-indépendante via les facteurs de croissance endothéliales vasculaires [75, 76].

Les androgènes jouent un rôle crucial dans le développement de la prostate chez l'homme, en agissant sur les cellules cibles par l'entremise du RA. Dans les cellules prostatiques, le métabolite le plus actif est la dihydrotestostérone (DHT) dont l'une des provenances est la réduction de la testostérone (T) par la 5α-réductase (5α-Red) [77, 78]. Les cellules prostatiques expriment toutes les enzymes nécessaires à la biosynthèse des androgènes actifs. Ceci relève de l'intracrinologie: en effet, bien que la castration chirurgicale chez l'homme cause la baisse de 90-95 % de la T sanguine, le niveau de DHT intra-prostatique ne baisse que de moitié, prouvant que dans la prostate la DHT ne

provient pas juste de la T testiculaire. Parmi les enzymes recensées, l'ADN complémentaire (ADNc) de la 3β-HSD a été isolé et caractérisé [79, 80]; la présence des deux types de 5α-Red (5α-Red1 et 5α-Red2) a été mise en évidence en clonant leurs ADNc respectives dans les chimiothèques d'ADNc de la prostate humaine [81, 82]. Par PCR quantitative, la présence de la 17β-HSD1, la 17β-HSD2, la 17β-HSD4 et la 17β-HSD5, reconnues pour métaboliser les stéroïdes, a été établie à la fois dans les tissus prostatiques sains et cancéreux [83]. De plus, il a été démontré que dans les tissus prostatiques cancéreux, la 17β-HSD3 est surexprimée à plus de 31 fois en comparaison à son niveau d'expression dans les tissus prostatiques normaux tandis que le niveau d'expression de la 17β-HSD2 est baissé de 7 fois. Puisque la 17β-HSD3 converti le Δ^4-dione en T actif sur le RA, et qu'à l'inverse la 17β-HSD2 converti la T en Δ^4-dione qui n'est pas directement actif sur RA [84], cette variation du niveau d'expression de ces enzymes contribue donc au maintien de la croissance des cellules cancéreuses de la prostate, même après le blocage de la source d'androgènes testiculaires [83]. Par ailleurs, la 17β-HSD15, une autre enzyme impliquée dans la biosynthèse de la DHT par une voie indépendante de la T a été récemment découverte dans la prostate et est présentement en étude pour en préciser le rôle physiologique [85].

4.2. Traitement du cancer de la prostate

Le protocole du traitement du cancer de la prostate commence par une évaluation du risque de progression, basée sur l'espérance de vie, la comorbidité, le grade de la biopsie (grade de Gleason), le stage clinique et le taux de PSA des patients. Le système de classement TNM du cancer de la prostate établie par l'*american joint committee for prostate cancer* a subi des améliorations au fil du temps [86]. En général le médecin proposera selon l'âge du patient, l'agressivité et le volume de la tumeur un traitement approprié (chirurgie ou radiothérapie, chimiothérapie, hormonothérapie, thérapie ciblée etc.), mais une surveillance médicale active peut aussi être proposée dans le cas des cancers localisés à évolution lente [87].

4.2.1 La chirurgie

Le diagnostic du cancer de la prostate a été révolutionné depuis la fin des années 70 à nos jours avec la découverte de l'antigène spécifique de la prostate (PSA), l'application de l'ultrason transrectale et la biopsie [88]. Cette avancée a été parallèlement suivie par des progrès dans la chirurgie thérapeutique de la prostate [89-91], faisant de la prostatectomie radicale un traitement initial approprié pour un nombre croissant de population jeune avec une morbidité minimale et un excellent contrôle de leur cancer. Cependant, les effets indésirables associés à la chirurgie restent nombreux: l'incontinence urinaire, le dysfonctionnement érectile et l'hémorragie sont les plus connus.

4.2.2 L'hormonothérapie

La place de la privation en androgène dans la gestion du cancer avancé de la prostate a été démontrée depuis 1941 par Huggins et Hodges [92, 93] et depuis ce temps, l'orchiectomie a été le traitement de référence contre le cancer de la prostate. La castration médicale réalisée par un agoniste de l'hormone de libération des gonadotrophines hypophysaires LHRH est équivalente à l'orchiectomie chirurgicale [94]. La leuprolide (Lupron) et l'acétate de goserelin (Zoladex) sont les agonistes les plus utilisés. Ils sont combinés aux anti-androgènes qui bloquent l'action intracrine de la DHT sur RA. Les anti-androgènes disponibles en clinique sont subdivisés en deux types: les stéroïdiens et les non stéroïdiens.

Les anti-androgènes stéroïdiens (Figure 4): L'acétate de cyprotérone (Androcur et Cyprostat), le premier anti-androgène utilisé cliniquement possède des propriétés antigonadotropiques [95]. Cette molécule est efficace sur la majorité des patients avec généralement moins d'effets indésirables que les estrogènes [97]. Cependant, elle cause la thrombolyse chez 5 % des patients, les œdèmes chez 4 %, la gynécomastie chez 13 %, la perte de libido chez la majorité des patients et bien d'autres effets indésirables ont été rapportés (cardio-vasculaires, gastro-intestinaux, toxicité hépatique sévère) [96, 97].

L'acétate de chlormadinone (Clordion, Gestafortin, Lormin, Normenon) (Figure 4), structuralement semblable au Cyprostat, agit de la même façon que ce dernier et possède les mêmes limitations. Ce composé n'est pas utilisé en Europe ni en Amérique, mais il est largement prescrit au Japon où il existe un brevet sur son utilisation [98].

L'acétate de mégestrol (Megace) (Figure 4), un dérivé synthétique de la progestérone, est un autre anti-androgène stéroïdien. En plus de son action antimitotique, ce composé supprime la sécrétion de l'hormone adrénocorticotrophique (ACTH), ce qui bloque ainsi la fonction surrénalienne et cause une diminution du cortisol et des androgènes [99].

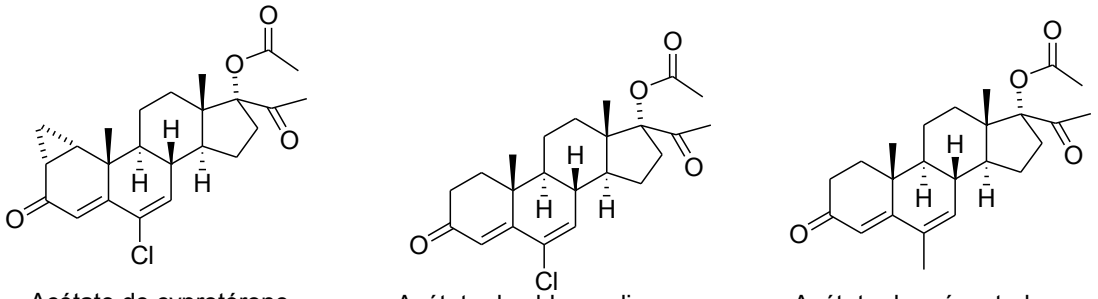


Figure 4: Structure de quelques anti-androgènes stéroïdiens synthétiques

Les anti-androgènes non stéroïdiens (Figure 5): Le flutamide fut le premier anti-androgène non stéroïdien disponible sur le marché. Il est efficace pour le traitement du cancer de la prostate et est mieux toléré que l'acétate de cyprotérone [100]. En effet, comme le flutamide est un anti-androgène pur, il ne cause pas les effets indésirables tels que la thrombose, l'embolisme et les œdèmes. Par ailleurs, comme ce n'est pas un progestatif, la fonction testiculaire sera maintenue et on observe que très rarement une perte de libido. Cependant, la gynécomastie et l'intolérance gastro-intestinale sont observées chez plusieurs patients [101, 102]. Il est important de mentionner que le flutamide est administré trois fois par jour. En effet, le métabolite actif l'hydroxyflutamide, aussi bien que la molécule mère (flutamide) possèdent une courte demi-vie ce qui explique le problème de biodisponibilité [103]. On associe ce traitement à la castration médicale ou chirurgicale, car l'utilisation du flutamide en monothérapie cause une hausse de l'hormone lutéinisante LH. Le nilutamide (Nilandron, Anandron) (Figure 5) est un anti-androgène non stéroïdien dont la structure est proche de celle du Flutamide. Cependant, puisque sa demi-vie (deux jours) est cinq fois plus grande que celle du flutamide [104], il peut être administré une seule fois par jour. Les effets indésirables observés avec le nilutamide incluent une diminution de l'adaptabilité à l'obscurité, la nausée, une intolérance à l'alcool et la perte de libido chez certains patients [105]. Le bicalutamide (Casodex) (Figure 5), l'un des derniers anti-androgènes à paraître sur le marché est très bien toléré par les patients et sa demi-vie, de 7 jours

[106], est presque 8 fois celle du flutamide. Cependant, une étude récente a montré que le bicalutamide a une affinité au RA inférieure à celle du ligand naturel DHT [107] et qu'il est capable de causer une surexpression du récepteur.

4.2.3 La chimiothérapie

Il arrive très souvent que des patients ayant subi un traitement hormonal (ablation des testicules ou traitement par un agoniste de la LHRH et administration d'un anti-androgène) présentent une récidive du cancer de la prostate [108]. Ceci passe par différents mécanismes complexes développés par les cellules cancéreuses pour produire une résistance au traitement hormonal. Ces mécanismes font intervenir non seulement les hormones sexuelles et leurs récepteurs [109], mais aussi plusieurs facteurs de croissance et leurs récepteurs suivant différentes voies de signalisation plus ou moins reliées [108]. Suite à cette résistance, la chimiothérapie est l'une des options proposées aux patients.

Les anthracyclines: Un agent thérapeutique fréquemment utilisé comme traitement de première ligne après l'insensibilité au traitement hormonal est la doxorubicine, administrée en petites doses hebdomadaires. En Europe, l'épirubicine, un analogue de la doxorubicine est très souvent utilisé. La mitoxantrone, un autre anthracycline a été combinée avec une dose de prednisone (Figure 5) et cette combinaison a montré une amélioration de la qualité de vie des patients [110].

Les analogues à base de platine: Le cisplatine et le carboplatine, deux complexes du platine (II), sont généralement utilisés. Ces agents ciblent l'ADN des cellules cancéreuses en alkylant l'azote N-7 de deux molécules adjacentes de guanine (liaison croisée inter-brin ou intra-brin) pour provoquer la morts de ces cellules [111]. Le grand problème lié à l'utilisation de ces agents thérapeutiques reste la faible sélectivité vis-à-vis des cellules saines. Il est donc nécessaire de développer d'autres agents thérapeutiques contre le cancer de la prostate.

4.2.4 La thérapie ciblée

L'angiogénèse tumorale représente un processus très important pendant la cascade métastatique. Des agents antiangiogéniques, opérant suivant différents mécanismes d'action, sont actuellement en développement [112-114]. Certains de ces agents sont même déjà utilisés dans des essais cliniques: le TNP-470, la suramine [113] et la vitamine D_3 sont des exemples connus (Figure 5). Très souvent, en plus d'empêcher

l'angiogénèse, la thérapie ciblée agit également sur la croissance et la survie cellulaire [115]. Parmi les molécules déjà avancées en essai clinique pour le cancer de la prostate, on peut citer: le géfitinib, un inhibiteur du récepteur du facteur de croissance épidermique (EGFR) de la tyrosine kinase [116]. Comme dans le cas du cancer du sein et de l'ovaire, le facteur de croissance endothélial vasculaire (VEGF) joue un rôle important dans l'angiogénèse prostatique et son niveau d'expression dans les tumeurs de prostate est élevé [117]. Le bévacizumab, un anti-VEGF a été utilisé dans plusieurs essais cliniques pour le traitement du cancer de la prostate, en monothérapie ou combiné avec un agent chimiothérapeutique classique [118-120]. Cependant, les idées convergent vers le fait que le cancer de la prostate est contrôlé seulement en partie par l'angiogénèse [121].

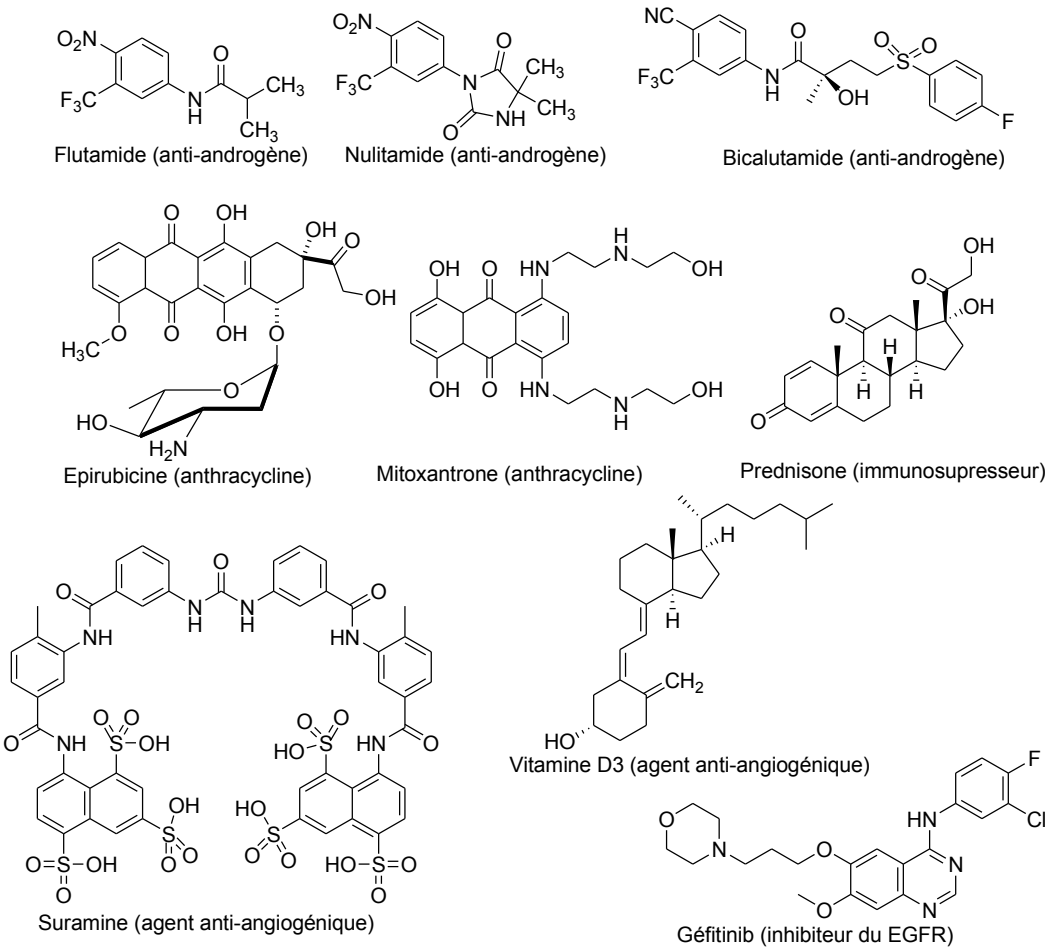


Figure 5: Structures de quelques anti-androgènes non stéroïdiens, d'anthracyclines, d'un immunosupresseur stéroïdien et d'agents anti-angiogéniques

On peut constater que malgré les grands succès qui ont été obtenus dans la recherche d'une thérapie contre les cancers en général, et le cancer de la prostate en particulier, ils restent incurables et il existe un besoin médical réel pour améliorer d'avantage la qualité de vie des malades. C'est dans ce sens que nous voulons développer des inhibiteurs de la biosynthèse des estrogènes et des androgènes, lesquels sont des facteurs importants dans le développement, la croissance et l'entretien des cellules cancéreuses.

5. Biosynthèse des estrogènes et des androgènes

Le point de départ de la biosynthèse de tous les stéroïdes, dont les stéroïdes sexuels, est l'internalisation du cholestérol (CHOL) de la membrane externe des mitochondries à leur membrane interne. Cette étape très rapide, contrôlée par une protéine appelée STAR (*steroidogenic acute regulatory protein*), est limitante pour la stéroïdogénèse [122]. Contrairement aux 3β-HSDs et aux 17β-HSDs, la plupart des enzymes de la stéroïdogénèse appartiennent à la famille des cytochromes P450. Une fois internalisé, le CHOL est converti en prégnénolone (PREG) par la P450scc, qui clive sa chaine latérale. Ce processus complexe implique trois séquences (deux réactions de monooxigénase et un clivage final) faisant intervenir des protéines de transfert d'électron (adrénodoxine réductase et adrénodoxine) localisées dans la mitochondrie; les électrons transférés proviennent au départ du NADPH [123-125]. Le schéma 1 est une illustration simplifiée de la biosynthèse de la PREG et de la progestérone (PROG) l'un de ses métabolites direct.

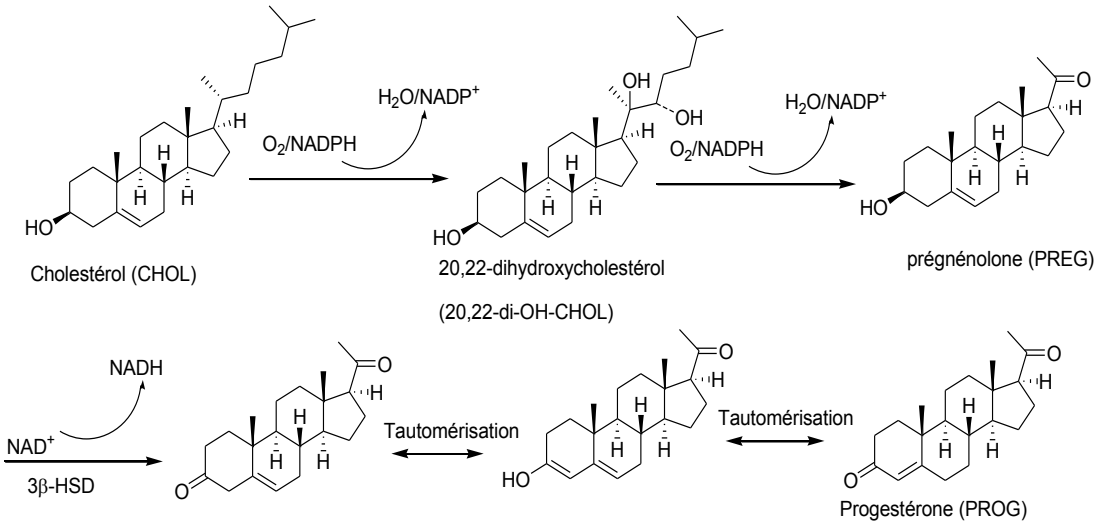


Schéma 1: Schéma simplifié de la biosynthèse de la PREG et de la PROG à partir du CHOL en présence de NADPH

La biosynthèse de la PROG implique deux étapes: l'oxydation du 3β-OH suivie d'une double tautomérisation céto/énol qui déplace la double liaison de la position C-5 à la position C-4. Ces deux étapes sont catalysées par la même enzyme appelée 3β-HSD/Δ^{4-5}-isomérase (Schéma 1) [79, 126]. La 3β-HSD assure également la conversion de la 17α-OHPREG en 17α-hydroxyprogestérone (17α-OHPROG). La biosynthèse de la 17α-OHPREG et de la 17α-OHPROG à partir de la PREG et de la PROG, respectivement, est catalysée par la P450c17 dont le gène encode à la fois l'activité hydroxylase et l'activité 17,20-lyase [127, 128]. Cette enzyme clive donc également la 17α-OHPREG et la 17α-OHPROG pour donner respectivement le DHEA et le Δ^4-dione (Figure 6). Le DHEA est ensuite réduit en position C-17 par la 17β-HSD1 pour donner le 5-androstène-3β,17β-diol (Δ^5-diol) qui est un stéroïde C19 possédant une faible activité estrogénique [129]. Parmi les voies de biosynthèse conduisant à la testostérone (T), on peut mentionner la transformation du Δ^5-diol par la 3β-HSD [130], mais aussi la réduction endocrine et intracrine du Δ^4-dione par la 17β-HSD3. En raison de notre intérêt pour cette enzyme, nous en discuterons davantage à la section 7. Une autre enzyme de la stéroïdogénèse qui présente beaucoup d'intérêt pour le traitement des cancers (estrogéno-dépendants) est l'aromatase (Cyp19). Elle catalyse la transformation du Δ^4-dione et de la T en E1 et en E2 respectivement (Figure 6).

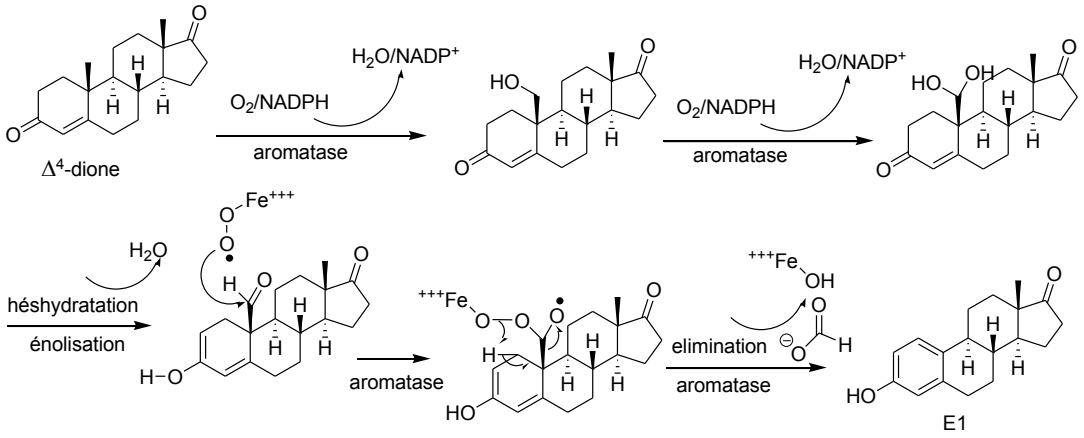


Schéma 2: Mécanisme de transformation du Δ^4-dione en E1 par l'aromatase en présence de NADPH

Plusieurs mécanismes d'aromatisation enzymatique du cycle A des stéroïdes C19 en stéroïdes C18 ont été proposés (Schéma 2), notamment par Wright et Akhtar [131-133]. Dans l'ensemble, trois oxydations séquentielles, impliquant chacune une mole d'oxygène et une mole de NADPH sont nécessaire pour cette transformation. Dans chacune des deux premières étapes, il y a une hydroxylation du méthyle C-19 qui conduit à un diol géminal. Ce dernier subit ensuite un réarrangement pour générer un aldéhyde en C-19; à ce niveau, une attaque radicalaire faisant intervenir un radical ferroxy (Fe-O$^{\bullet}$), ferro-péroxy (Fe-O-O$^{\bullet}$) ou un mélange des deux a été proposée. Le fer proviendrait de la porphyrine [134]. La dernière étape est un réarrangement (aromatisation) avec élimination de l'acide formique.

La dernière étape de la biosynthèse de E2 est la réduction du carbonyle en position C-17 de E1 par la 17β-HSD1. Le mécanisme proposé pour cette réduction fait intervenir des acides aminés du site catalytique qui favorisent la stabilisation de E1 [135]. Ainsi, l'histidine 221 (His221) fait une liaison hydrogène avec le phénol, la sérine 142 (Ser142) et la tyrosine 155 (Tyr155) font des liaisons hydrogènes avec le carbonyle de E1, mais c'est la Tyr155 qui donne le proton de l'alcool en C-17 et le cofacteur NADPH est le donneur de l'hydrure qui attaque le carbonyle en C-17 pour former E2 [136] (Schéma 3).

E2 et Δ^5-diol se lient au RE qui translocalise dans le noyau pour causer la prolifération des cellules sensibles aux estrogènes [137, 138]. Chez les femmes préménopausées, en raison de l'activité aromatase des cellules folliculaires, l'ovaire est la source principale de E2 [139]. L'aromatase est donc une cible privilégiée pour le traitement des cancers estrogéno-dépendants chez les femmes en préménopause. Cependant, à cause de la perte de la fonction ovarienne chez les femmes durant la ménopause, le taux de Δ^5-diol (environ 1.1 ng/mL) circulant est 100 fois supérieur à celui de E2 (5 à 10 pg/mL [129]. L'action intracrine de Δ^5-diol dans les cancers estrogéno-dépendants se trouve ainsi amplifiée.

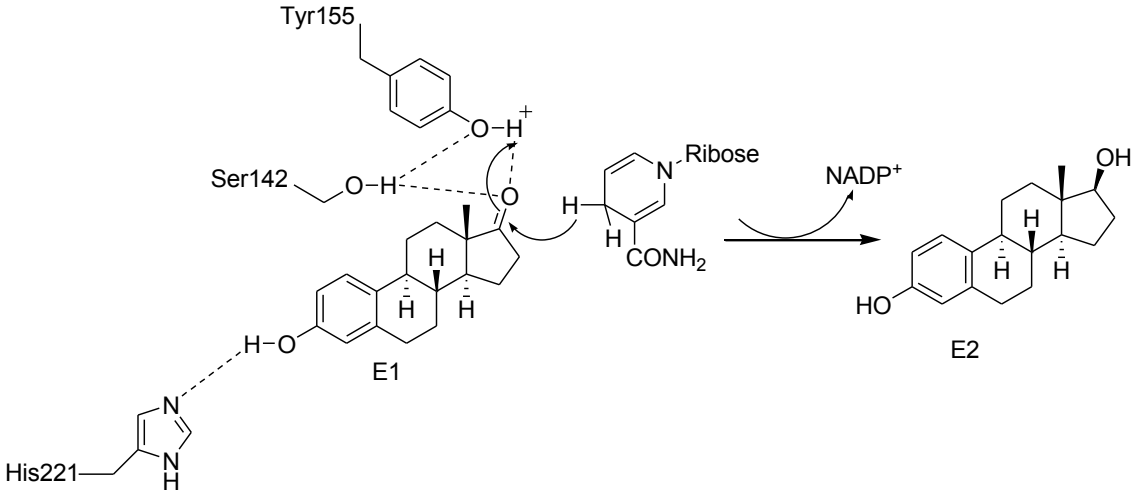


Schéma 3: Réduction de l'estrone (E1) en estradiol (E2) par la 17β-HSD1 en présence de NADPH

Bien que la T soit un stimulateur important du RA, l'androgène naturel le plus puissant est le DHT, qui est produit par la réduction de la T par la 5α-Red en présence du cofacteur NADPH qui agit comme donneur d'hydrure [141]. Le mécanisme de la réduction de T par la 5α-Red est identifié à celui de la réduction du Δ^4-dione par la 5β-Red. Ce dernier, décrit par Di Costanzo et collaborateurs [142], fait intervenir le glutamate 120 (Glu120), la lysine 87 (Lys87), la tyrosine 58 (Tyr58) et l'asparagine 50 (Asp50), des acides aminés du site catalytique qui assurent la stabilisation du stéroïde et le transfert d'un proton (17-OH du DHT).

En définitive, la biosynthèse des estrogènes et des androgènes est un processus complexe, faisant intervenir différentes enzymes et cofacteurs (Figure 6). Il est à noter que la famille des 17β-HSDs comporte plusieurs isozymes qui agissent comme oxydants ou comme réducteurs d'une façon tissus spécifique et selon le cofacteur disponible ($NADP^+$ ou NADPH). Plusieurs enzymes de la stéroïdogénèse, hormones hypothalamo-hypophysaires (FSH et LH) et récepteurs des stéroïdes sont des cibles thérapeutiques pour le traitement des maladies androgéno et estrogéno-dépendantes: on parle d'hormonothérapie. La section suivante présente quelques aspects de cette forme de thérapie.

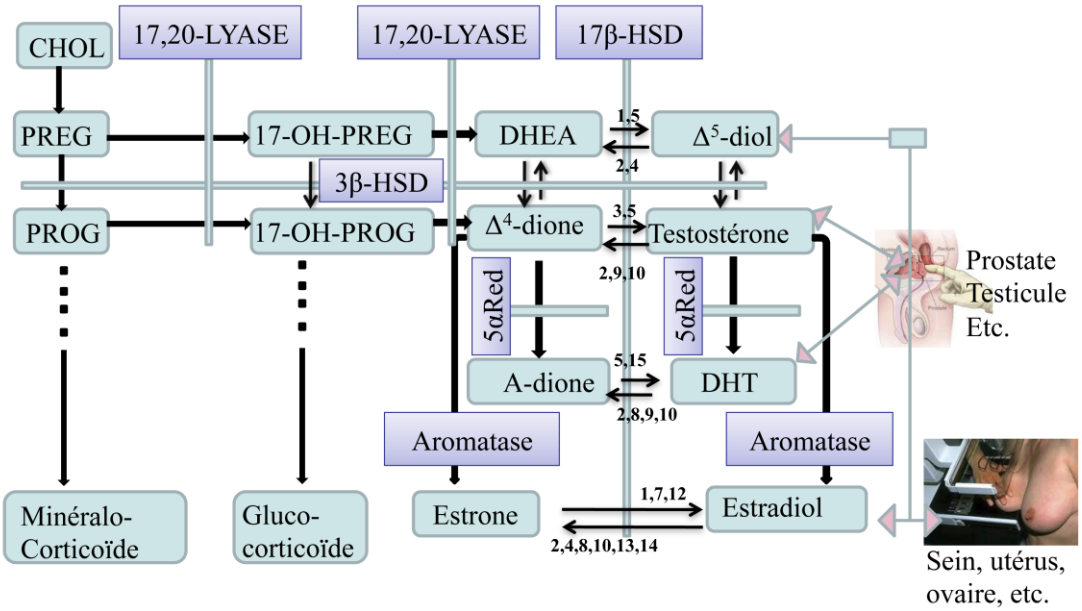


Figure 6: Représentation simplifiée de la biosynthèse des estrogènes et des androgènes

6. L'hormonothérapie

6.1 Généralités

Comme son nom l'indique, l'hormonothérapie est un traitement à base d'hormones naturelles, de leurs dérivés synthétiques ou d'autres perturbateurs de l'homéostasie des hormones. On peut donc ainsi limiter ou empêcher la formation d'une hormone ou bloquer l'action de celle-ci sur son récepteur [143]. L'extrait de la thyroïde de mouton a été utilisé durant le 19^{e} siècle pour soigner l'hypothyroïdie sévère. L'utilisation efficace de l'insuline pour le traitement du diabète et l'effet bénéfique des hormones de remplacement sur les symptômes de la ménopause sont des exemples connus de ce type de thérapie [144]. Un autre mode opératoire en hormonothérapie est de contrôler l'équilibre homéostatique en agissant sur le système hypothalamo-hypophysaire avec des agonistes des hormones gonadotropes (FSH et LH) et adrénotrope (ACTH) (rétroaction négative), contrôlant ainsi leur action sur des organes cibles [145]. Bien que plusieurs effets secondaires soient très souvent imputables à cette approche thérapeutique, son utilisation pour le traitement de plusieurs dysfonctionnements métaboliques et de différents cancers a connu de grands succès. Cependant, beaucoup reste à faire pour découvrir des cibles biologiques précises et des molécules hautement sélectives, dans le but d'éviter les effets indésirables éventuels qui peuvent être

observés. Afin de circonscrire notre champ de travail, nous nous limiterons dans ce paragraphe aux récepteurs des estrogènes (RE) et des androgènes (RA), leurs antagonistes, et nous terminerons par une brève description du mode d'action des modulateurs sélectifs des RE et RA (effet SERM et effet SARM).

6.2 Le récepteur des estrogènes et le récepteur des androgènes

Pour réaliser leurs fonctions physiologiques, les estrogènes et les androgènes se lient au RE et RA, respectivement; ces deux récepteurs appartiennent à la superfamille des récepteurs nucléaires [146, 147]. Dans l'ensemble, tous ces récepteurs possèdent trois principaux domaines: le domaine de liaison au ligand (DLL), le domaine de liaison à l'ADN (DLA) et le domaine N-terminal d'activation de la transcription, encore appelé facteur de transcription du domaine N-terminal (FTN).

Le récepteur des estrogènes (RA): Il existe deux types de RE, REα et REβ qui conservent 97 % d'homologie dans la séquence du domaine de liaison à l'ADN, mais diffèrent surtout dans le domaine d'activation de la transcription (16 % d'homologie) et le domaine de liaison du ligand (59 % d'homologie) [148]. Le site de l'ADN qui permet la liaison du récepteur s'appelle élément de réponse des estrogènes (ERE); il est constitué de 13 paires de bases inversées et répétées (5'-GGTCAnnnTGACC-3') et le récepteur se lie comme un homodimère pour exercer son activité transcriptionnelle [149, 150]. Une fois fixé sur l'ERE, le RE interagit avec la machinerie transcriptionnelle dont l'ARN-polymérase II à partir de son DLL et FTN pour favoriser la croissance cellulaire.

Le récepteur des androgènes (RE): Bien qu'il n'existe que très peu d'homologie dans la séquence du RA et du RE (< 20 %), les DLL de ces différents récepteurs possèdent un motif structural similaire constitué de 12 hélices α, ordonnées de façon antiparallèle [151]. La conformation de l'hélice 12 (H12) et son repliement par rapport à l'hélice 11 (H11) joue un rôle déterminant dans le recrutement des coactivateurs [152]. À l'origine, RA est lié à des protéines de choc thermique « heat shock protein (HSP)» (HSP 56, HSP 70 et HSP 90) ainsi qu'à des chaperonnes dans une conformation qui empêche leur interaction avec l'élément de réponse sur l'ADN [153]. La liaison du ligand androgénique déplace ces protéines et H12 adopte une nouvelle conformation favorable à sa translocalisation et son interaction avec l'ADN sur l'élément de réponse des

androgènes (ERA), qui comporte 15 paires de bases inversement répétées (5'-AGAACAnnnTGTTCT-3') [154].

6.3 Les antagonistes des récepteurs (RE et RA) et leur mode d'action

En général, les antagonistes du RE et du RA sont respectivement des mimiques stéroïdiennes ou non stéroïdiennes des estrogènes et des androgènes naturels. À cet effet, ils agissent comme inhibiteurs de la liaison de ces ligands naturels sur leur récepteur. Des études effectuées avec le tamoxifène et le composé ICI182,780 (Figure 7) ont révélé que ces derniers agissent comme anti-estrogène en induisant la sous-expression du RE [155]. Le tamoxifène module ainsi l'activité transcriptionnelle de RE en se fixant sur le DLL alors que le ICI182,780 se fixe plutôt dans un site allostérique du RE, diminue la vitesse de dimérisation et la répression de RE. D'une manière similaire, le composé EPI-001 (Figure 8), un anti-androgène non stéroïdien présentement en étude préclinique, exerce son antagonisme au niveau du domaine d'activation transcriptionnelle N-terminal [156]. Ceci est la preuve que les antagonistes de RE et de RA n'agissent pas toujours de façon classique en inhibant la liaison du ligand naturel. Alors que certains antagonistes de RE et de RA empêchent simplement la translocalisation de ces derniers du cytoplasme au noyau, d'autres empêchent simplement l'activation des domaines transcriptionnels situés hors du DLL [157]. Finalement, d'autres molécules comme le composé ICI164,384 (Figure 7), vont aussi empêcher la dimérisation du récepteur, une étape primordiale pour l'activité transcriptionnelle [158]. Pour leur part, le flutamide et le bicalutamide sont des anti-androgènes purs qui empêchent la liaison des androgènes au RA [159].

Dans un contexte thérapeutique comme le traitement du cancer, il est très important que la liaison de l'anti-estrogène et de l'anti-androgène ne soit pas un facteur favorable à la prolifération cellulaire. C'est dans l'optique de contourner cette difficulté que les antagonistes non stéroïdiens de RE et de RA ont été développés (Figure 7 et 8). L'évaluation de l'estrogénicité et de l'androgénicité d'un nouvel agent thérapeutique est donc primordiale. De ce fait, le niveau de croissance des cellules estrogéno- ou androgéno-sensibles est évalué en présence du composé d'intérêt. Parmi les lignées cellulaires sensibles aux estrogènes qui sont souvent utilisées on peut citer les cellules MCF-7, ZR-75-1, T47-D et OVCAR-3. Quant aux cellules sensibles aux androgènes, les cellules LNCaP, Shionogi et LAPC-4 sont les plus connues.

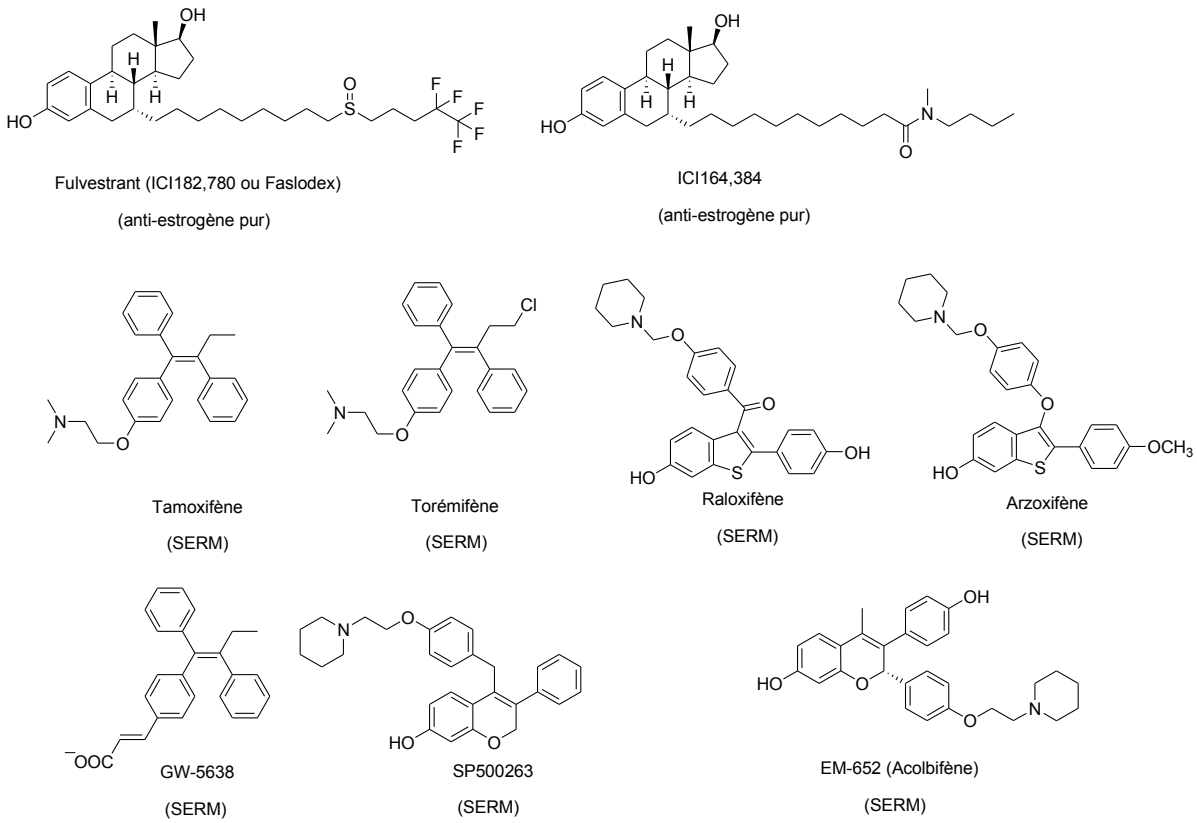


Figure 7: Structure moléculaire de deux anti-estrogènes purs stéroïdiens et de quelques SERMs non stéroïdiens

6.4 L'effet SERM et l'effet SARM

Les modulateurs non stéroïdiens de la formation et de l'action des hormones stéroïdiennes sont très adaptés pour leur spécificité à différents tissus et sont plus propices pour des modifications structurales. Avec l'avènement de ces types de composés, on a développé des modulateurs sélectifs du RE (SERMs) et du RA (SARMs) [160, 161]. Ces molécules ont des propriétés estrogéniques ou androgéniques d'une façon tissus spécifique. Par exemple, un SERM pourrait empêcher la croissance des cellules cancéreuses du sein et favoriser la croissance des cellules osseuses et ainsi permettre de lutter contre l'ostéoporose. C'est le cas du tamoxifène et du raloxifène qui agissent comme des anti-estrogènes dans le sein, mais agissent comme des estrogènes dans les os [162]. Dans le but de contrer les effets indésirables des anti-androgènes dans des tissus autres que ceux ciblés par un traitement, le développement des SARMs suscite de plus en plus d'intérêt [163]. Plusieurs analogues de la quinoline, des antagonistes de RA tels que les dérivés tricycliques de la pyridinodihydroquinoline ont

montré un effet anabolisant prometteur, sans pour autant agir de façon significative au niveau de la prostate [164].

Une compréhension simple du mode d'action des SERMs est de considérer une distribution tissulaire proportionnée de RE-α et de RE-β d'un tissu à l'autre [165, 166]. Les analogues du tamoxifène comme l'arzoxifène ou le torémifène qui, comme ce dernier, ont une affinité pour le RE-α supérieure à celle pour le RE-β, vont être anti-estrogèniques dans le tissu mammaire tout en étant estrogénique dans le tissu osseux. En effet, le sein et l'utérus sont plus riches en RE-α tandis que le tissu osseux et l'endothélium vasculaire sont plutôt riches en RE-β [166]. Le mécanisme d'action des SERMs et des SARMs peut cependant être plus complexe, en impliquant à la fois des facteurs de régulation (co-activateurs ou co-represseurs) dont la fonction est tissu-spécifique [167]. Ainsi, les SARMs, de la même façon que le DHT, recruteraient des co-activateurs dans les tissus anaboliques (os, muscles…) et majoritairement des co-represseurs dans les tissus androgéno-sensibles (prostate, testicules).

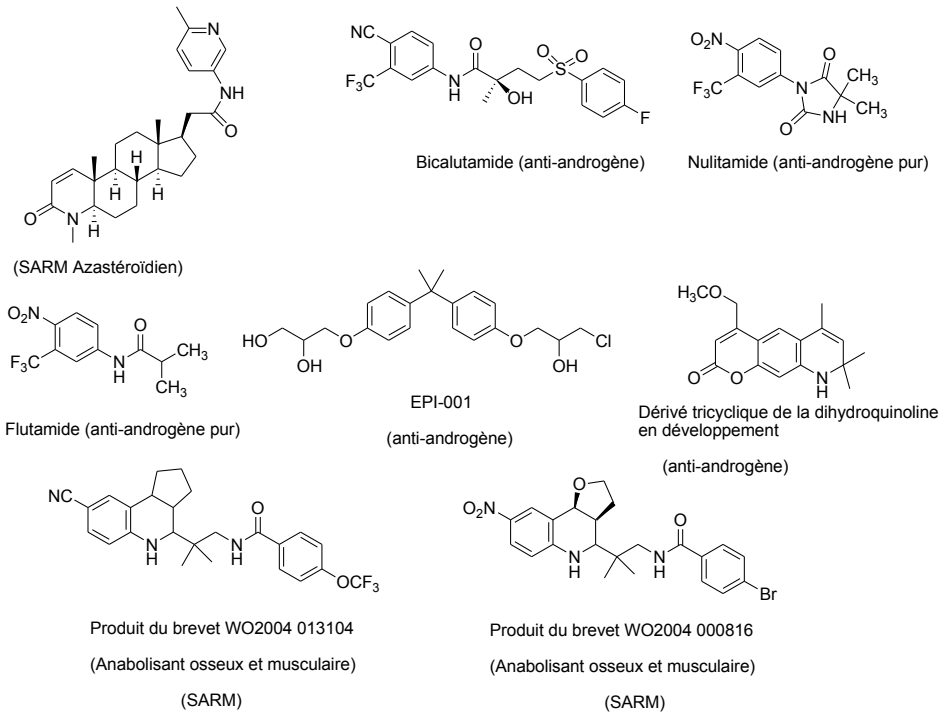


Figure 8: Structure de quelques anti-androgènes et SARMs azastéroïdiens et non stéroïdiens

7. Les enzymes de la stéroïdogénèse

7.1 Généralités

La stéroïdogénèse est un processus complexe qui débute par l'internalisation du CHOL. Les enzymes de la stéroïdogénèse sont d'une manière générale regroupées dans deux grandes familles: les cytochromes P450 et les oxido-réductases. Dans la voie métabolique conduisant aux corticostéroïdes (stéroïdes C-21), les cytochromes qui interviennent peuvent être cités dans l'ordre suivant: P450scc, P450c17, P450c21, P450c11 et P450c18. Il est cependant important de mentionner que la 3β-HSD qui n'est pas un cytochrome intervient juste après la P450scc, pour la transformation de la PREG et de 17α-OHPREG en PROG et en 17α-OHPROG respectivement. Pour la suite, nous nous intéresserons à l'aromatase, la stéroïde sulfatase (STS), la 17β-HSD1 et la 17β-HSD3, tous des enzymes qui sont d'un grand intérêt pour les cancers estrogéno- et androgéno-dépendants.

7.2 L'aromatase

L'aromatase est l'enzyme qui catalyse la transformation des stéroïdes C-19 en stéroïde C-18. Comme son nom l'indique, cette enzyme de 55 KDa est responsable de l'aromatisation du cycle A des stéroïdes C-19. Le gène CYP19 de l'aromatase est situé sur le chromosome 15q21 [169]. L'aromatase est ubiquitaire, c'est-à-dire qu'elle est présente dans plusieurs tissus sièges de la stéroïdogénèse (l'ovaire, le sein, le placenta et les testicules) mais aussi dans le tissus adipeux, la peau, l'os, le foie et le cerveau [127, 170-171]. Avec une meilleure compréhension de la structure et du fonctionnement de l'aromatase, et afin de bloquer la biosynthèse de E2 dans le cadre des pathologies estrogéno-dépendantes, trois générations d'inhibiteurs de l'aromatase ont été développées [172]. L'aminoglutéthimide et le formestane (Figure 3) ont été initialement utilisés dans plusieurs études pour valider la preuve de concept. Par la suite, le besoin d'inhibiteurs plus puissants et plus sélectifs a stimulé des études poussées conduisant à la synthèse du létrozole, de l'anastrozole et de l'exémestane (Figure 2) lesquels sont présentement utilisés en clinique.

7.3 La stéroïde sulfatase

La stéroïde sulfatase (STS) est l'enzyme responsable de l'hydrolyse des sulfates de stéroïdes en leur forme hydroxylée. Elle joue un rôle essentiel dans la régulation de la formation de stéroïdes biologiquement actifs [173] puisque les stéroïdes sulfatés ne sont pas actifs sur leur récepteur. L'enzyme est largement distribuée dans l'organisme et est impliquée dans différents processus physio-pathologiques. On la retrouve dans les testicules, les ovaires, la glande surrénale, l'endomètre, le placenta, la prostate, la peau, le cerveau, le foie et l'os [174-177]. Cependant, même si à ce jour la structure cristalline de l'enzyme a été définie, on a relativement peu d'information sur les facteurs qui régulent son expression ou son activité [178]. Cette enzyme, joue un rôle important dans la croissance de tumeurs estrogéno-dépendantes du sein et androgéno-dépendantes de la prostate. En effet, la STS est responsable de l'hydrolyse du sulfate de E1 et du sulfate de DHEA (le C-19 stéroïde le plus abondant en circulation) en E1 et DHEA respectivement. Ces deux stéroïdes peuvent être convertis par d'autres enzymes en estrogènes plus actifs (E2 et Δ5-diol), qui stimulent la croissance des tumeurs mammaires, ou en androgènes plus actifs (T et DHT), qui stimulent la croissance des tumeurs prostatiques. Il a été démontré que l'expression de la STS est augmentée dans les tumeurs du sein et que cette valeur a une signification pronostique [175]. Le rôle joué par la STS dans la croissance tumorale a incité le développement d'inhibiteurs. Le 667COUMATE (Figure 9), un inhibiteur non stéroïdien de la STS est présentement en phase clinique pour le traitement du cancer du sein hormono-dépendant [179]. Plusieurs études ont été menées en parallèles pour concevoir des inhibiteurs stéroïdiens et non stéroïdiens, sulfamoylés et non sulfamoylés, de la STS. Des travaux antérieurs dans notre laboratoire ont permis de démontrer l'importance d'un groupement hydrophobe en position C-17α d'un noyau estrane, et la conjugaison d'un inhibiteur non stéroïdien avec un groupement hydrophobe permet d'obtenir des inhibiteurs non-estrogéniques de la STS [180, 181]. La compagnie Nippon Organon a mis en application cette idée pour développer des inhibiteurs très actifs de la STS. La figure 9 illustre de la structure moléculaire de quelques inhibiteurs connus de la STS.

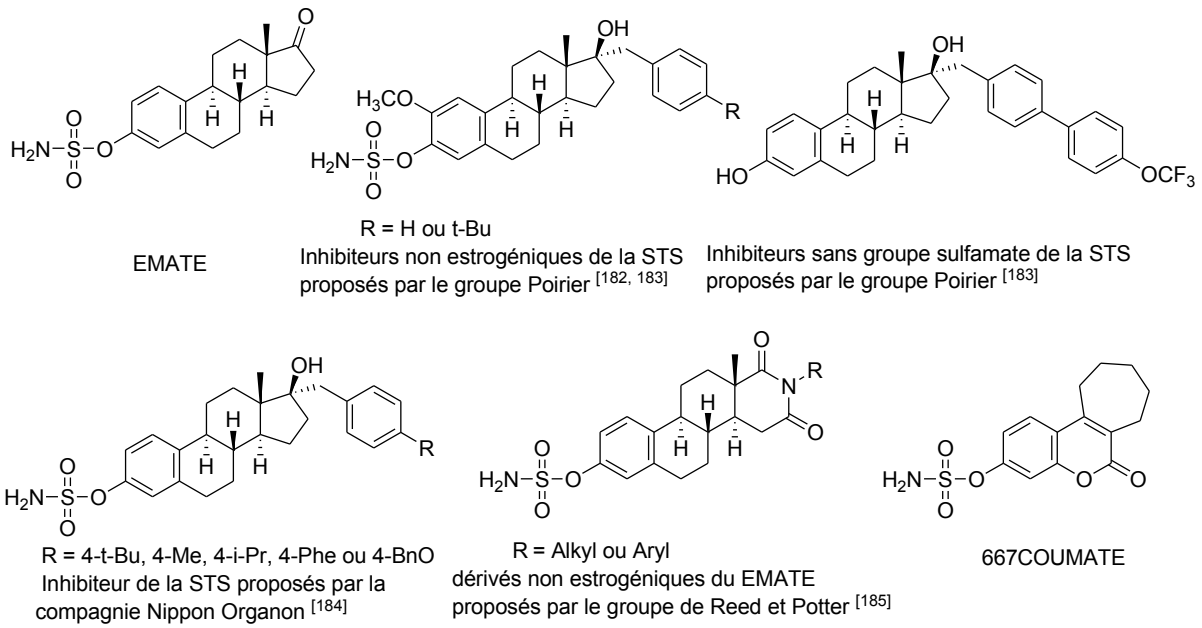


Figure 9: Quelques exemples d'inhibiteurs connus de la stéroïde sulfatase

7.4 Les 17β-hydroxystéroïde déshydrogénases (17β-HSDs)

Les 17β-HSDs sont considérés comme des pré-récepteurs (activateurs/inactivateurs) hormonaux *in vivo* [186]. Actuellement, 15 isozymes de cette famille d'enzymes ont été identifiés [187, 188]. On sait que la dernière étape de la biosynthèse de E2, l'estrogène le plus actif, est la réduction du carbonyle en position C-17 de E1 par les 17β-HSD1, 17β-HSD5, 17β-HSD7 et 17β-HSD12. Bien qu'il ait été suggéré que la 17β-HSD1 joue un rôle majeur chez la femme, des données récentes publiées par Haynes et collaborateurs [189] ont montré une corrélation positive significative entre les taux de E2 intra-tumorales et l'activité 17β-HSD7. Il a été démontré que des niveaux élevés d'ARNm de la 17β-HSD5 est lié à un risque significativement plus élevé de récidive tardive chez des patientes du cancer du sein RE+, jusqu'à cinq ans après le diagnostic [190]. Les inhibiteurs sélectifs des différents isozymes de la 17β-HSD ont été synthétisés dans le but d'étudier leur potentiel dans le traitement du cancer du sein [191]. À l'exception de la 17β-HSD5 qui est une aldo-céto réductase (AKR), les 17β-HSDs sont des déshydrogénases/réductases à chaine courte (SDR). Dans le tableau 1, nous avons résumé pour chaque isozyme, sa masse moléculaire, sa localisation cellulaire, sa localisation chromosomique, le cofacteur utilisé et sa fonction principale. Dans les prochains paragraphes, une description plus détaillée sera donnée sur le mode d'action de certains inhibiteurs de la 17β-HSD1 et de la 17β-HSD3.

Tableau 1: Quelques caractéristiques des isozymes de la 17β-HSD [6, 37, 191-196]

Nom de l'enzyme (Masse moléculaire)	**Localisation cellulaire**	**Localisation chromosomique**	**cofacteur**	**Fonction principale**
17β-HSD1 (34 kDa)	cytosol	17q11-q21	NADPH	E1→E2
17β-HSD2 (43 KDa)	réticulum endoplasmique	16q24.1-2	$NADP^+$	E2→E1*
17β-HSD3 (34,5 KDa)	réticulum endoplasmique	9q22	NADPH	Δ^4-dione→T**
17β-HSD4 (80 kDa)	peroxysome	5q2.3	NAD^+	multifonction
17β-HSD5 (37 kDa)	cytosol	10p14-15	NADPH	Δ^4-dione→T
17β-HSD6 (36 kDa)	membranes (chez le rat)	12q13.3	NAD^+	A-diol→DHT
17β-HSD7 (37 kDa)	membranes	10p11.2	NADPH	E1→E2
17β-HSD8	mitochondrie	6p21.3	NAD^+	E2→E1
17β-HSD9	----	----	NAD^+	multifonction
17β-HSD10 (108 kDa)	mitochondrie	Xp11.2	NAD^+	multifonction
17β-HSD11	réticulum endoplasmique	4q22.1	peu connue	peu connue
17β-HSD12	réticulum endoplasmique	11p11.2	NADPH	multifonction
17β-HSD13 (33,6 kDa)	cytosol	4q22.1	peu connue	peu connue
17β-HSD14 (28 kDa)	cytosol	19q13.33	NAD^+	E2→E1
17β-HSD15* **	peu connue	peu connue	NADPH	A-dione→DHT

* La 17β-HSD2 catalyse aussi majoritairement l'oxidation en C-17 de T et de DHT. ** La 17β-HSD3 catalyse aussi majoritairement la réduction en C-17 de l'androstanedione en DHT;

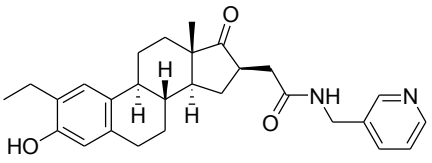
*** Cette enzyme n'a pas encore été complètement caractérisée.

7.5 La 17β-HSD type 1

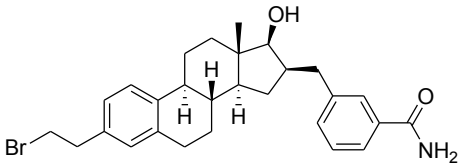
En présence du cofacteur NADPH, la 17β-HSD1 catalyse la dernière étape de la biosynthèse de E2, l'estrogène responsable de la stimulation de la croissance des cellules cancéreuses du sein et d'autres organes sensibles aux estrogènes par voie endocrine, autocrine, paracrine ou intracrine [12]. À cause du potentiel thérapeutique de cette enzyme et puisque sa structure cristalline était connue [197], plusieurs groupes de recherche ont mis l'accent sur le développement d'inhibiteurs spécifiques et non estrogéniques de la 17β-HSD1 [15, 142, 198-201]. Day et collaborateurs ont récemment développé le composé STX1040 (Figure 10), un inhibiteur sélectif de la 17β-HSD1, efficace *in vivo* dans un modèle de xénogreffe de cancer du sein chez des souris immunodéficientes, et ils ont montré que c'est la 17β-HSD1 et non pas la 17β-HSD12 qui est responsable de la transformation de E1 en E2 [187]. Dans notre laboratoire, différents inhibiteurs ont été développés et des résultats *in vitro* et *in vivo* intéressants ont été obtenus, notamment avec le composé PBRM, un inhibiteur non estrogénique de la 17β-HSD1 [202]. Un enjeu majeur dans la conception des inhibiteurs de la 17β-HSD1 était d'éliminer leur estrogénicité résiduelle. C'est avec cet objectif en tête que les dérivés (*N*,*N*-butylméthylheptanamide) de E2 (CC-1, CC-2 et CC-3 avaient été synthétisés en ajoutant une chaine alkylamide en position C-6 ou un groupement méthoxy en position C-2 [203]. Bien qu'ayant montré un très bon potentiel inhibiteur, CC-1 et CC-2 démeuraient des composés estrogéniques. Par contre, CC-3, qui porte un groupe méthoxy en position C-2, n'était pas estrogénique. Cependant, la méthoxylation en C-2 provoque une diminution importante de l'activité inhibitrice. Dans le but d'améliorer l'activité inhibitrice de ces premiers inhibiteurs, le composé CC-156 a été développé (IC_{50} de 44 nM) (Figure 10) [204]. Malgré le fort potentiel inhibiteur de ce composé, il possède une estrogénicité résiduelle non acceptable dans le contexte de traitement d'un cancer estrogéno-dépendant. C'est pour combler cette lacune que le composé non estrogénique PBRM a été developpé. Puisque l'estrogénicité des inhibiteurs stéroïdiens de la 17β-HSD1 était liée à leur similitude avec le squelette de E2, des inhibiteurs non stéroïdiens ont été préparés. De plus, plusieurs composés naturels non stéroïdiens de la famille des flavones, isoflavones, chalcones, coumestanes et lignanes inhibent la 17β-HSD1 [205]. La figure 10 présente quelques exemples

d'inhibiteurs stéroïdiens et non stéroïdiens développés à ce jour ainsi que des composés d'origine naturelle.

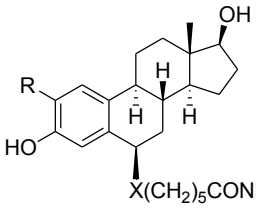
Inhibiteurs synthétiques stéroïdiens de la 17β-HSD1



STX1040 (IC_{50} = 27 nM *in vitro*) [187]



PBRM (IC_{50} = 68 nM *in vitro*) [202]

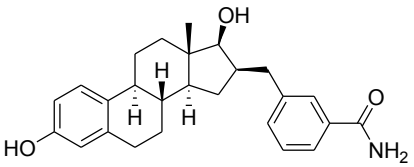


CC-1 (R = H, X = S) ; CC-2 (R = H, X = CH_2)

et CC-3 (R = OMe, X = CH_2)

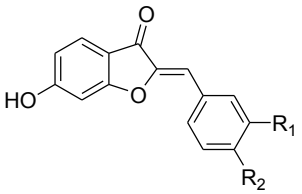
Potentiel inhibiteur identique (80 % à 1 µM)

Activité proliférative de CC-1 = 2,1 fois celle de CC-2 [203]

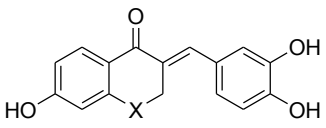


CC-156 (IC_{50} = 44 nM *in vitro*) [204]

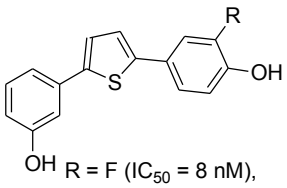
Inhibiteurs synthétiques non stéroïdiens de la 17β-HSD1



R_1 = Me, OMe; R_2 = H, OH [198]



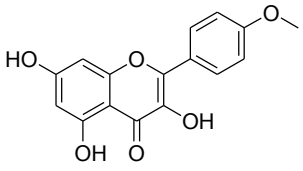
X = CH_2, O [198]



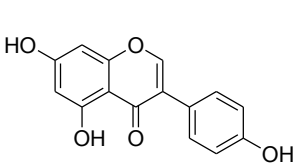
R = F (IC_{50} = 8 nM),

CH_3 (IC_{50} = 46 nM) [198]

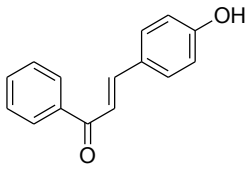
Inhibiteurs naturels non stéroïdiens de la 17β-HSD1



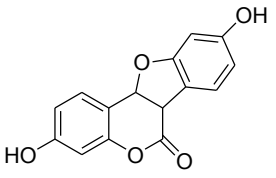
Kaempferide (flavone) [205]



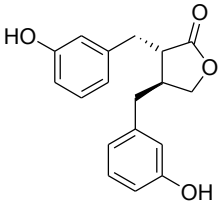
Genistein (isoflavone) [205]



4-hydroxychalcone (chalcone) [206]



Coumestrol (coumestane) [206]



Enterolactone (lignane) [207]

Figure 10: Quelques inhibiteurs synthétiques et naturels connus de la 17β-HSD1

7.6 La 17β-HSD type 3

La dernière étape de la biosynthèse de la T circulante est catalysée par la 17β-HSD3 en présence du cofacteur NADPH. Bien que cette enzyme membranaire existe presqu'exclusivement dans la fraction microsomale des cellules de Leydig testiculaires, où elle réduit la Δ^4-dione en T, certains chercheurs ont rapporté son expression dans d'autres tissus, tels que les plaquettes sanguines et les mégacaryocytes (cellules de la moelle hématopoïétique) [11, 208] et certaines tumeurs de la prostate. Ainsi, la 17β-HSD3 contribue approximativement à la production de 60 % des androgènes actifs chez l'homme [12]. L'importance de la 17β-HSD3 dans la production de la T a été démontrée par le pseudohermaphrodisme masculin. Dans ce cas, le défaut d'expression de cette enzyme [9] entraine une augmentation du taux relatif de Δ^4-dione par rapport au taux de la T [209]. L'inhibition de la 17β-HSD3 pourrait donc être une stratégie privilégiée dans le traitement des maladies androgéno-dépendantes telles que le cancer de la prostate et l'hyperplasie bénigne prostatique. Le blocage complet des androgènes est crucial pour contrer la prolifération des cellules cancéreuses androgéno-dépendantes dont celles de la prostate [13]. Plusieurs traitements endocriniens existent déjà, ils consistent à bloquer soit la production testiculaire des androgènes, à bloquer l'effet de T ou de la DHT sur le récepteur des androgènes (RA), ou à combiner les deux méthodes [14]. Le blocage actuellement utilisé en thérapie combine l'administration d'un agoniste de la LHRH, pour produire une castration médicale, et d'un anti-androgène (flutamide, casodex…) pour bloquer le RA [13]. Il a été démontré que dans l'ARNm obtenu des biopsies de prostates cancéreuses, la 17β-HSD3 est surexprimée plus de 30 fois par rapport au niveau normal, alors que le niveau d'expression de la 17β-HSD5, une autre enzyme capable de réduire la Δ^4-dione en T, ne changeait pas [83]. Par ailleurs, le niveau d'expression de la 17β-HSD3 est augmenté dans les cellules LNCaP après deux jours de traitement avec la dutastéride, un inhibiteur de la 5α-Red analogue au finastéride [210]. Des taux de T et de DHT relativement élevés ont été observés dans la prostate de certains patients récidivistes qui avaient pourtant subi les castrations androgéniques [211]. Il existe donc un réel besoin de développer d'autres stratégies de blocage intracrine de la production de T et de DHT. Day et collaborateurs [212] ont développé une famille d'inhibiteurs sélectifs et non stéroïdiens de la 17β-HSD3. Entre autres, le composé STX2171 (Figure 11) est capable de réduire le volume des tumeurs (xénogreffes de cellules LNCaP transfectées 17β-HSD3) dans un model murin. Des

coumarines d'origine naturelle tels que l'umbelliférone (IC_{50} = 1,4 μM) et le 4-méthylumbelliférone (IC_{50} = 0,91 μM) sont parmi les inhibiteurs non stéroïdiens connus de la 17β-HSD3 [213] (Figure 11). L'androstérone (ADT) et ses dérivés issus d'une substitution à différentes positions ont permis d'obtenir des inhibiteurs de la 17β-HSD3 [214-217]. Avec une valeur IC_{50} de 330 nM dans les microsomes de cellules HEK-293 transfectées 17β-HSD3, l'ADT est deux fois plus puissant que le substrat naturel Δ^4-dione. Les dérivés de l'ADT substitués en position C-16, bien qu'étant peu ou pas androgéniques, sont relativement moins actifs que les dérivés en position C-3 [217]. Par la suite, des dérivés 3α-éther et 3β-substitués de l'ADT ont été synthétisés [215]. Parmi ces composés, BTN1, BTN2 et BTN3 sont les meilleurs et ont tous un potentiel inhibiteur supérieur à celui du Δ^4-dione et de l'ADT (Figure 11). De ces travaux, il ressort que les longues chaines alkyles en position C-3 (>5 carbones) n'étaient pas favorables pour une bonne inhibition, mais que l'hydrophobicité du substituent à cette position est un facteur important. Les travaux publiés en 2002 dans notre laboratoire avaient permis de vérifier cette hypothèse [216] et de montrer que la rigidité du groupe hydrophobe est un facteur important. Ainsi, l'amine RM1 et le carbamate D-5-2 étaient les meilleurs inhibiteurs de cette étude. En raison de leur similitude avec les androgènes naturels, les dérivés stéroïdiens sont en mesure de se lier au RA et de causer la prolifération des cellules sensibles aux androgènes, constituant ainsi une limite à leur utilisation pour le traitement de certains cancers. Le composé RM-532-105 [4], est un inhibiteur stéroïdien et non androgénique qui a été développé plus récemment dans notre laboratoire. Nous l'avons utilisé comme inhibiteur de référence dans le cadre des travaux rapportés dans cette thèse.

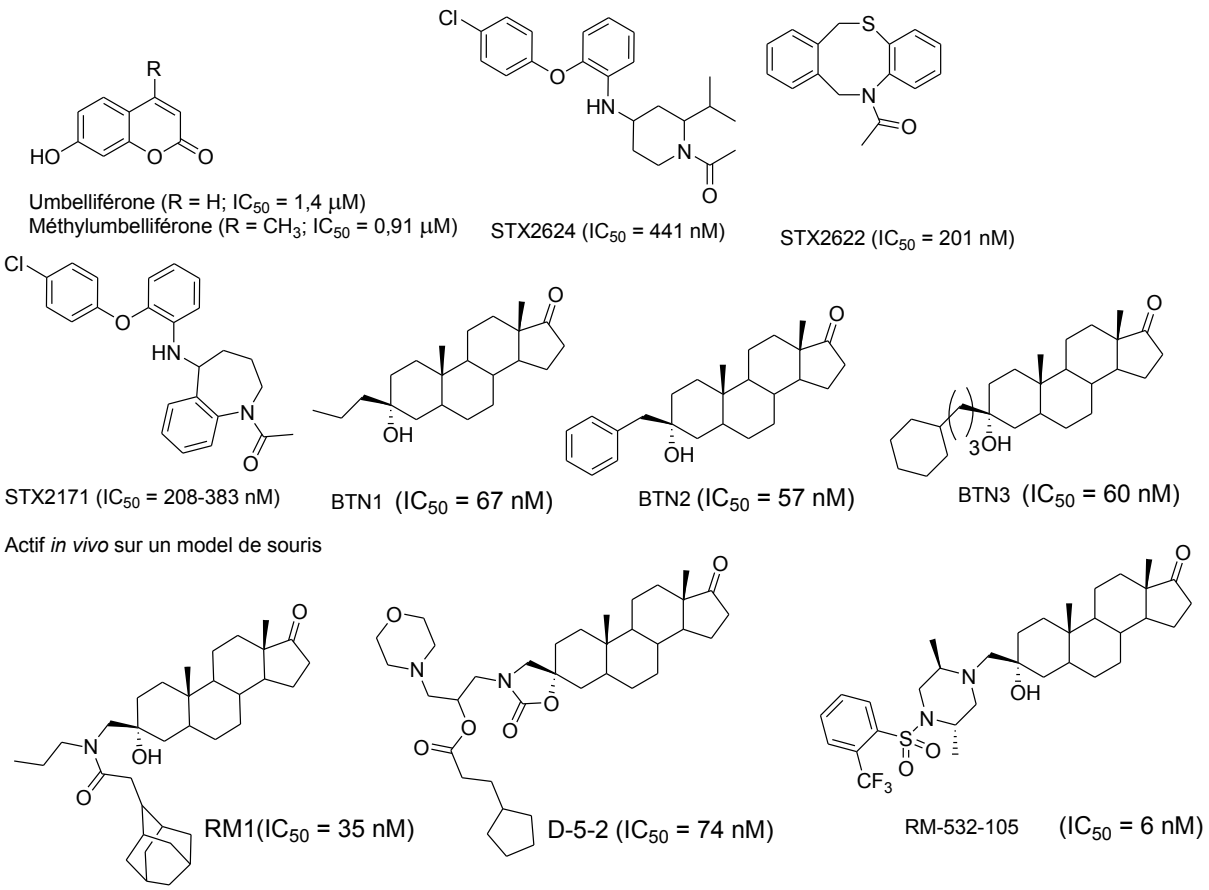


Figure 11: Quelques inhibiteurs connus de la 17β-HSD3

8. Aperçu du projet de recherche

Dans le premier volet (partie A) de cette thèse, différentes approches sont décrites afin d'obtenir des mimiques non stéroïdiennes de E2, ou du CC-156, un puissant inhibiteur de la 17β-HSD1 qui possède une activité estrogénique non souhaitable. Ces composés sont de potentiels inhibiteurs non estrogéniques de la 17β-HSD1, de la STS ou des noyaux de base pour le développement de nouveaux SERM. À cet effet, le chapitre 1 de cette thèse décrit la synthèse chimique et la caractérisation par RMN de mimiques non stéroïdiennes de l'inhibiteur stéroïdien CC-156. Le deuxième chapitre traite du développement d'une synthèse en parallèle et en phase liquide de mimiques non stéroïdiennes de E2.

Le deuxième volet de cette thèse vise principalement à développer une nouvelle famille d'inhibiteurs efficaces et non androgéniques de la 17β-HSD3, à partir d'un noyau androstérone (ADT). De ce fait, un spirocycle (cycle E) capable de contenir plusieurs niveaux de diversité moléculaire a été ajouté en position C-3 de l'ADT. L'étude des

relations structure-activité nous a permis de déterminer la meilleure stéréochimie ainsi que des modifications subtiles capables de rendre ces inhibiteurs plus efficaces et non androgéniques. Les quatre chapitres du deuxième volet (partie B) de cette thèse présentent les résultats de ces travaux. Dans le chapitre 3, nous utilisons les orientations spatiales observées dans la structure cristalline de deux composés pour expliquer le rôle joué par le groupe hydrophobe en C-3β sur l'activité inhibitrice observée sur la 17β-HSD3. Des travaux antérieurs dans notre laboratoire ayant démontré que les 17-spiro-δ-lactone-estrane sont de bons inhibiteurs de la 17β-HSD5, nous avons synthétisée des 3-spiromorpholinone-17-δ-spirolactone-ADT (chapitre 4), dans l'optique d'ouvrir la porte au développement d'inhibiteurs hybrides de la 17β-HSD3 et de la 17β-HSD5. Par ailleurs, nous avons démontré dans les chapitres 5 et 6 la nécessité de la présence du noyau androstane pour inhiber la 17β-HSD3. Nous avons aussi démontré (chapitre 6) que les composés synthétisés pouvaient être transformés par une réaction de couplage de Suzuki ou par «chimie click» donnant ainsi la possibilité de développer d'autres inhibiteurs.

Comme il a été mentionné dans cette introduction générale, l'implication des estrogènes et des androgènes dans différents cancers est une réalité indéniable. Il ressort de ces connaissances que des inhibiteurs de certaines enzymes (17β-HSD1, 17β-HSD3 et STS) impliqué dans la biosynthèse des estrogènes et des androgènes, ainsi que des modulateurs sélectifs de RE et RA, possèdent un potentiel thérapeutique très important. Les chapitres qui suivent traitent du développement de plusieurs inhibiteurs des 17β-HSD1, 17β-HSD3 et 17β-HSD5

Résultats

Partie A

Développement d'inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 1

Chapitre 1

Synthèse chimique et caractérisation par RMN de mimiques non stéroïdiennes d'un dérivé de l'estradiol utilisé comme inhibiteur de la 17β-hydroxystéroïde déshydrogénase type 1

1. Avant-propos

Le composé CC-156 est l'un des meilleurs inhibiteurs de la 17β-HSD1. Ce composé est malheureusement estrogénique et stimule la croissance des cellules cancéreuses sensibles aux estrogènes. La co-cristallisation du CC-156 avec l'enzyme et le $NADP^+$ a permis de comprendre sa forte affinité avec la 17β-HSD1 à partir des interactions qu'il fait avec les acides aminés présents dans le site catalytique.

Afin d'éliminer l'estrogénicité du CC-156, l'objectif de ce chapitre était de développer des mimiques non stéroïdiennes de ce dernier, un composé non stéroïdien n'étant généralement pas estrogénique.

Mon rôle dans ce projet a été de synthétiser des mimiques non stéroïdiennes du CC-156, de les purifier et de les caractériser par RMN ^1H, RMN ^{13}C, IR et SM, en vue de confirmer leur structure chimique. J'ai également fait en début du projet des modélisations qui nous ont aidées dans le choix des synthons.

Les essais enzymatiques pour ce projet ont été réalisés par Charles Ouellet et Alexandre Trottier, deux étudiants à la maitrise de notre laboratoire.

2. Résumé

Inhiber la biosynthèse de l'estradiol (E2) par le biais d'un inhibiteur de la 17β-hydroxystéroïde déshydrogénase de type 1 (17β-HSD1) est une stratégie prometteuse pour le traitement du cancer du sein. Nous avons conçu un noyau non stéroïdien pour mimer le CC-156, un puissant inhibiteur stéroïdien de la 17β-HSD1. A partir du bromhydrate du tétrahydro-isoquinolinol, deux composés représentatifs ont été synthétisés à l'aide d'une séquence de six réactions chimiques: la protection du groupe amine, la protection du phénol, l'hydrolyse du groupe protecteur *N*-Fmoc, la substitution nucléophile pour introduire un éthyloxirane, la phénolyse avec le *méta*- ou *para*-hydroxybenzamide et l'hydrolyse du groupe protecteur MOM. Bien que ces composés aient montré une stabilité *in silico* dans le site catalytique de l'enzyme, en conservant des interactions clés avec les acides aminés His221 et Ser142, déjà observées à partir de la structure cristalline de l'inhibiteur CC-156 avec la 17β-HSD1, ils inhibent faiblement cette dernière. Toutefois, ils n'ont pas montré d'activité estrogénique *in vitro*, suggérant que ce noyau non stéroïdien présente un potentiel pour la conception de médicaments. En outre, l'approche synthétique rapportée ici ouvre la voie à la préparation d'un grand nombre de mimiques non stéroïdiennes de dérivés de E2, lesquelles pourraient être testées sur la 17β-HSD1 et sur d'autres cibles biologiques.

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Chemical synthesis and NMR characterization of non steroidal mimics of an estradiol derivative used as inhibitor of 17β-hydroxysteroid dehydrogenase type 1

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Keywords: phenol derivative, tetrahydro-isoquinolinol, chemical synthesis, inhibitor, 17β-hydroxysteroid dehydrogenase

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Abstract

Inhibiting estradiol (E2) biosynthesis through 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) inhibitors is a promising strategy for breast cancer therapy. We have designed a non-steroidal template to mimic CC-156, a potent steroidal inhibitor of 17β-HSD1. Starting from tetrahydro-isoquinolinol hydrobromide, two representative compounds were synthesized in six chemical steps: protection of the amino group, protection of the phenol, hydrolysis of the N-protecting group Fmoc, nucleophilic substitution to introduce an ethyloxirane, phenolysis with *meta* or *para* hydroxybenzamide and the hydrolysis of the MOM protecting group. Although the compounds showed a good fit when docked in the catalytic site of the enzyme, conserving the key interactions with amino acids His221 and Ser142, observed from the crystalline structure of inhibitor CC-156 with 17β-HSD1, they weakly inhibited 17β-HSD1. However, they did not show estrogenic activity when tested *in vitro*, suggesting the potential of this non-steroidal template for drug design. Furthermore, the synthetic approach reported here opens a door to the preparation of additional non-steroidal mimics of E2 derivatives, which could be tested on 17β-HSD1 and others biological targets.

1. Introduction

Breast cancer is the leading cause of death worldwide among all the types of cancers affecting women (Jemal et al., 2011). Estrogenic hormones are important stimulators in most of these breast cancers (Dizerega et al., 1980; Travis & Key, 2003) and estradiol (E2), the most potent natural estrogen, stimulates cancer cell growth through endocrine, paracrine, and intracrine pathways (Labrie, 1991). The last step in E2 biosynthesis requires the enzyme 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). This enzyme also catalyses the reduction of dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (5-diol) (Figure 1), a weaker estrogen that becomes more important after menopause (Simard et al., 1988).

Blocking 17β-HSD1 thus appears to be an attractive strategy in breast cancer therapy (Poirier, 2008). To the best of our knowledge, there is still no 17β-HSD1 inhibitor under clinical trials. This is partly explained by the fact that many potent inhibitors of this enzyme have an estrane nucleus that can activate the estrogen receptors (ERs). Of course it is not desirable to use these estrogenic inhibitors in the treatment of hormone-dependent breast cancer, and synthesizing 17β-HSD1 inhibitors without estrogenic activity thus represents a great challenge (Poirier, 2010; Poirier, 2011; Day et al., 2008b). Compound CC-156 (Figure 2A) is a very potent inhibitor of 17β-HSD1 that was synthesized and reported from previous work in our laboratory (Laplante et al., 2008). The crystal structure of the ternary complex of CC-156 with 17β-HSD1 and cofactor NADP has already been described (Figure 2B) (Mazundar et al., 2009) and shown key interactions with amino acids in the enzyme catalytic site. However, this compound was found to stimulate the proliferation of MCF-7 and T-47D estrogen-sensitive (ER^+) breast cancer cell lines, thus greatly reducing its therapeutic potential. Three strategies were tried to reduce the proliferative (estrogenic) effect of this steroidal inhibitor: Replacing the hydroxy group at position 3 (C3) by a hydrogen atom; adding a methoxy at C2; or adding an alkylamide chain at C7, but all these methods did not give satisfactory results (Laplante et al., 2008).

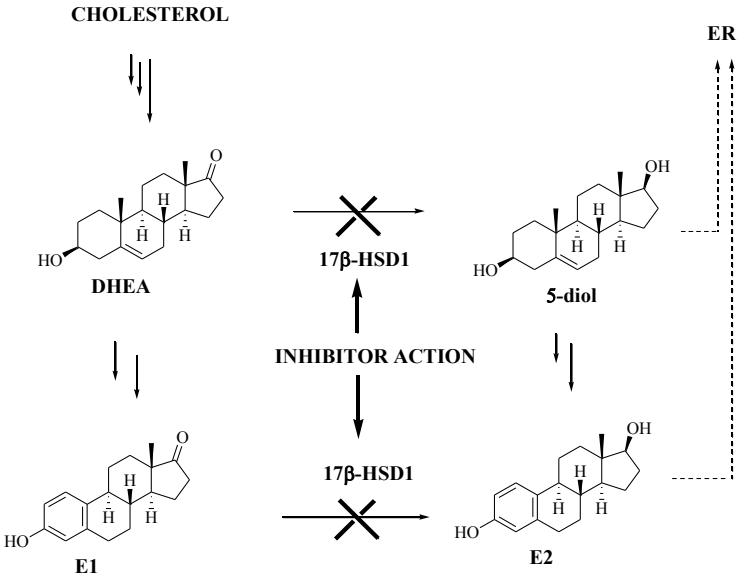

Figure 1. Last steps in the biosynthesis of active estrogens estradiol (E2) and 5-androstene-3β,17β-diol (5-diol), which stimulate the cell proliferation by their action on the estrogen receptor (ER)

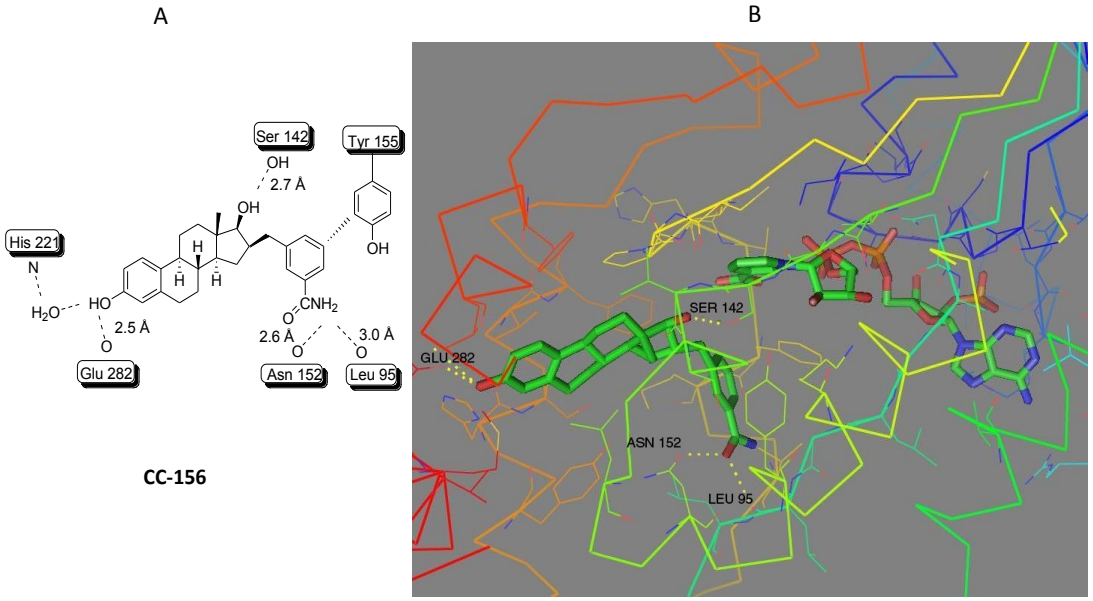

Figure 2. Analysis of the crystalline structure of the ternary complex 17β-HSD1/CC-156/NADP. 2D (A) and 3D (B) representations showing interactions (H-bond and π-π interactions) between the enzyme (17β-HSD1) and the inhibitor (CC-156) (Mazumdar et al., 2009)

Using AutoDock software (http://autodock.scripps.edu/), we found that compound 10 (Figure 3A) fits very well in the enzyme catalytic site of 17β-HSD1 (Figure 3B). We also observed that compound 10 keeps interactions with His221 and Ser142; its phenyl

ether moiety interacts with Tyr155 differently (with H-bond) regarding to CC-156 in which it binds via ππ interaction (Mazumdar et al., 2009); and new hydrogen bonds were observed with Val188, Thr190 and Val143. Such non-steroidal compound is interesting because it is expected to be an E2 mimic without residual estrogenic activity. Herein, we report the successful synthesis of 8 and 10, two mimics of CC-156, starting from tetrahydro-6-isoquinolinol hydrobromide (1) (Figure 4). We also report their characterization by IR, ^{1}H NMR, ^{13}C NMR and MS analyses, their assessment as inhibitor of 17β-HSD1, and their assessment as estrogenic compounds.

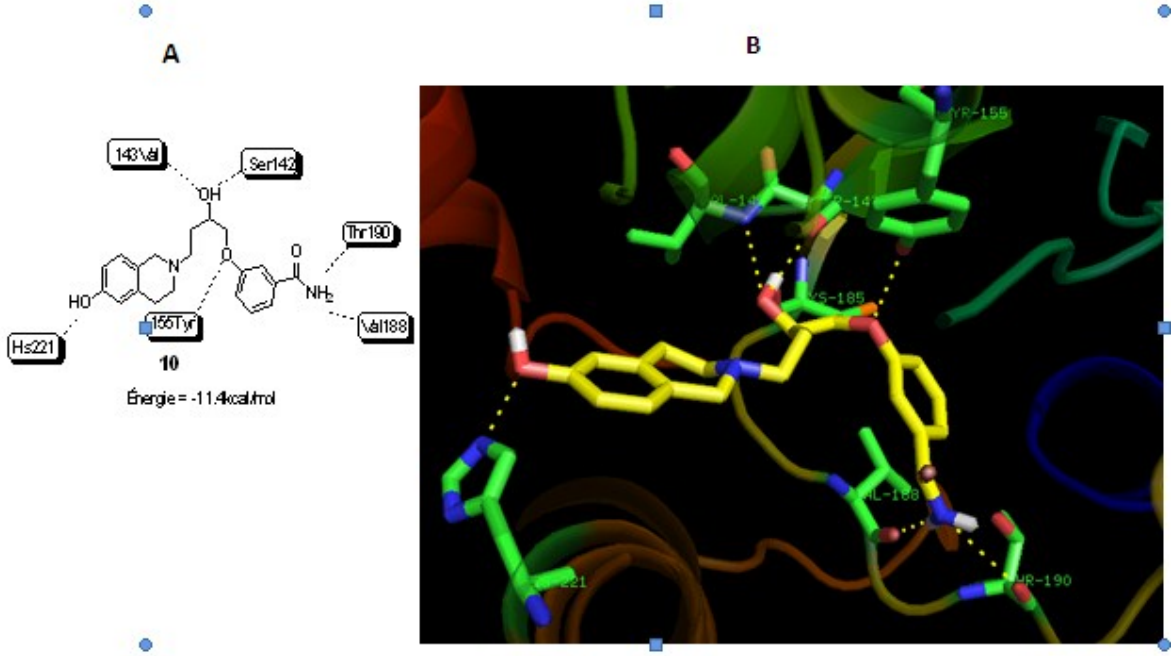

Figure 3. 2D (A) and 3D (B) representations of different interactions observed between the non-steroidal mimic of CC-156 (compound 10) and 17β-HSD1 after a docking experiment (AutoDock v.3). Calculated energy = -11.4 kcal/mol

2. Experimental

2.1 General

1,2,3,4-Tetrahydroisoquinolin-6-ol hydrobromide was purchased from Ark Pharm (Libertyville, IL, USA). 4-Hydroxybenzamide and chemical reagents of highest purity were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada), N-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu) was purchased from Novabiochem (Darmstadt, Germany). Solvents were obtained from Fisher Scientific (Montréal, QC, Canada). Reactions were run under an inert (argon) atmosphere in oven-

dried glassware. Analytical thin-layer chromatography (TLC) was performed on 0.20-mm silica gel 60 F254 plates (E. Merck, Darmstadt, Germany), and compounds were visualized by using ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with Silicycle R10030B 230–400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were obtained from a thin film of compound usually solubilised in CH_2Cl_2 (DCM) and deposited upon a NaCl pellet. They were recorded with a Horizon MB 3000 ABB FTIR spectrometer (ABB, Québec, QC, Canada) and bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and data reported in ppm. The $CDCl_3$ ^1H and ^{13}C NMR signals (7.26 and 77.0 ppm, respectively) and CD_3OD ^1H and ^{13}C NMR signals (3.31 and 49.0 ppm, respectively) were used as internal references. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ion-spray source.

2.2 Synthesis of Fmoc-Protected Compound 2

1,2,3,4-Tetrahydroisoquinolin-6-ol hydrobromide (1) (9.0 g, 39.1 mmol) was dissolved in a mixture of THF/water (3/1, v/v) and an aqueous 1N sodium bicarbonate solution (117 mL) was added. After the reaction mixture was stirred 5 min, Fmoc-OSu (13.9 g, 41.1 mmol) was added and the mixture stirred for 2 h at room temperature. Water (450 mL) was added and the crude product was extracted with EtOAc. The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. Compound 2 (14.0 g, 96% yield) was obtained as a white powder after purification by chromatography (hexanes/EtOAc, 1:1).

9*H*-Fluoren-9-ylmethyl 6-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (2):

Rf = 0.25 (hexanes/EtOAc, 8:2). IR (film) ν 3317 (OH), 2947, 2901, 1674 (NCOO), 1612, 1443, 1221. ^1H NMR ($CDCl_3$) δ 2.77 (m, Ar-$\underline{CH_2}$-CH_2), 3.67 (m, CH_2-$\underline{CH_2}$-N), 4.28 (t, J = 6.8 Hz, CH of Fmoc), 4.47 (d, J = 6.8 Hz, CH_2 of Fmoc), 4.55 (m, Ar-$\underline{CH_2}$-N), 6.63 (d, J = 2.5 Hz, CH of Ar), 6.64 and 6.83 (2m, 2 x CH of Ar), 7.31, 7.40, 7.60 and 7.77 (4m, 8 x CH of Fmoc). ^{13}C NMR ($CDCl_3$) δ 28.7 , 41.4 , 45.3 , 47.3 , 67.5 , 114.9, 115.1, 120.0 (2x), 125.0 (2x), 127.0 (2x), 127.4, 127.7 (2x), 133.1, 135.9, 141.3

(2x), 144.0 (2x), 154.2, 155.5. LRMS calculated for $C_{24}H_{22}NO_3$ (M+H) 372.15, found 372.30.

2.3 Synthesis of MOM and Fmoc-Protected Compound 3

To a solution of compound 2 (14.0 g, 37.6 mmol) in anhydrous DCM (1000 mL) under an argon atmosphere was added N,N-diisopropylethylamine (15.7 mL) and the mixture was stirred for 10 min at 0°C (in an ice/water bath). Chloromethyl methyl ether (5.43 g, 67.4 mmol) was then added slowly and the reaction mixture was brought to room temperature after 20 min and stirred for 24 h. Water was added to quench the reaction and the mixture extracted with DCM. The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude mixture was purified by flash chromatography (hexanes/EtOAc, 3:1) to provide compound 3 (11.0 g, 70% yield).

9*H*-Fluoren-9-ylmethyl 6-(methoxymethoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (3):
Rf = 0.38 (hexanes/EtOAc, 8:2). IR (film) ν 2947, 2901, 1697 (NCOO), 1612, 1504, 1427, 1227, 1149, 1095, 1011. ^{1}H NMR ($CDCl_3$) δ 2.81 (m, Ar-$\underline{CH_2}$-CH_2), 3.49 (s, CH_3O), 3.68 (m, CH_2-$\underline{CH_2}$-N), 4.28 (t, J = 6.8 Hz, CH of Fmoc), 4.47 (d, J = 6.8 Hz, CH_2 of Fmoc), 4.57 (m, Ar-CH_2-N), 5.17 (s, O-CH_2-O), 6.84 (d, J = 2.2 Hz, CH of Ar), 6.90 (m, CH of Ar), 7.03 (m, CH of Ar), 7.31, 7.39, 7.59 and 7.75 (4m, 8 x CH of Fmoc). ^{13}C NMR ($CDCl_3$) δ 28.7, 41.2, 45.1, 47.2, 55.8, 67.2, 94.3, 114.6, 115.8, 119.8 (2x), 124.8 (2x), 126.3, 126.9 (2x), 127.1, 127.5 (2x), 135.5, 141.2 (2x), 143.9 (2x), 155.2, 155.6. LRMS calculated for $C_{26}H_{26}NO_4$ (M+H) 416.18, found 416.30.

2.4 Fmoc Hydrolysis: Synthesis of Compound 4

Compound 3 (11.0 g, 26.4 mmol) was dissolved in anhydrous DCM (220 mL) and piperidine (55 mL). The reaction mixture was stirred for 1 h at room temperature and then quenched with water. The organic phase was washed with water and dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The yellowish powder was purified by flash chromatography (DCM/MeOH, 97:3) to provide compound 4 (2.9 g, 55% yield).

6-(Methoxymethoxy)-1,2,3,4-tetrahydroisoquinoline (4): Rf = 0.24 (hexanes/EtOAc, 3:1). IR (film) ν 3317 (NH), 2924, 1612, 1504, 1234, 1149, 1072, 1003. ^{1}H NMR (CD_3OD) δ 2.84 (t, J = 6.0 Hz, Ar-$\underline{CH_2}$-CH_2), 3.11 (t, J = 6.1 Hz, CH_2-$\underline{CH_2}$-N), 3.45 (s, CH_3O), 3.95 (s, Ar-$\underline{CH_2}$-N), 5.15 (s, O-CH_2-O), 6.80 (s, CH of Ar), 6.83 (d, J = 8.3 Hz, 1H, CH of pseudo A ring), 6.97 (d, J = 8.3 Hz, CH of Ar). ^{13}C NMR ($CDCl_3$) δ 29.3, 43.6, 47.6, 55.9, 94.5, 114.3, 116.5, 127.5, 129.3, 135.5, 155.4. LRMS calculated for $C_{11}H_{16}NO_2$ (M+H) 194.11, found 194.15.

2.5 Synthesis of Epoxide 5

To a solution of amine 4 (245 mg, 1.3 mmol) and potassium carbonate (261 mg, 1.9 mmol) in acetone (12 mL) was added 4-bromo-1,2-epoxybutane (476 mg, 3.2 mmol) and the mixture was stirred for 16 h at 55°C. The solution was filtered over a büchner and rinsed with DCM, then the collected filtrate was evaporated under reduced pressure and purified by flash chromatography (hexanes/EtOAc/triethylamine, 90:9:1) to yield epoxide 5 (216 mg, 65% yield).

6-(Methoxymethoxy)-2-[2-(oxiran-2-yl)ethyl]-1,2,3,4-tetrahydroisoquinoline (5):

Rf = 0.28 (DCM/MeOH, 9:1). IR (film) ν 2924, 1612, 1504, 1242, 1149, 1076, 1011. ^{1}H NMR ($CDCl_3$) δ 1.78 and 1.87 (2m, $\underline{CH_2}$-CH-O), 2.54 and 2.78 (2m, CH_2-O), 2.68 (t, J = 7.4 Hz, $CH_2\underline{CH_2}$-N), 2.72 (m, Ar-$CH_2\underline{CH_2}$-N), 2.88 (m, Ar- $\underline{CH_2}CH_2$-N), 3.03 (m, CH-O), 3.47 (s, CH_3O), 3.59 (s, Ar-$\underline{CH_2}$-N), 5.14 (s, O-CH_2-O), 6.79 (d, J = 3.0 Hz, CH of Ar), 6.81 (d, J = 8.3 Hz, CH of Ar), 6.94 (d, J = 8.2 Hz, CH of Ar). ^{13}C NMR ($CDCl_3$) δ 29.3, 30.5, 47.1, 50.8, 50.9, 54.7, 55.6, 55.9, 94.5, 114.2, 115.8, 127.5, 128.2, 135.5, 155.5. LRMS calculated for $C_{15}H_{22}NO_3$ (M+H) 264.15, found 264.15.

2.6 General Method for the Synthesis of Compounds 6 and 7

To a solution of 4-hydroxybenzamide or 3-hydroxybenzamide (104 mg, 0.76 mmol) in DMF (10 mL) was added potassium carbonate (158 mg, 1.14 mmol) and the mixture was stirred for 30 min under argon at 105°C. The epoxide 5 (100 mg, 0.38 mmol) was dissolved in DMF (3 mL) and added to the reaction mixture. After 22 h at 105°C, water (14 mL) and a saturated aqueous sodium bicarbonate solution (10 mL) were added and the mixture extracted with EtOAc. The organic phase was dried with Na_2SO_4 filtered, evaporated under reduced pressure and purified by flash chromatography (DCM/MeOH, 98:2 to 95:5) to yield the compound 6 (69 mg, 45% yield) or 7 (33 mg, 21% yield).

4-{2-Hydroxy-4-[6-(methoxymethoxy)-3,4-dihydroisoquinolin-2(1*H*)-yl] butoxy}benzamide (6):

Rf = 0.40 (DCM/MeOH, 9:1). IR (film) ν 3391 and 3190 (OH and NH_2), 2928, 2827, 2762, 1647 (CON, amide), 1612, 1574, 1508, 1423, 1400, 1311, 1261, 1149, 1076, 1022. ^{1}H NMR ($CDCl_3$) δ 1.90 (m, $\underline{CH_2}$-CH-O), 2.68 and 2.94 (2m, Ar-$CH_2\underline{CH_2}$-N), 2.87 (m, $CH_2\underline{CH_2}$-N and Ar-$\underline{CH_2}CH_2$-N), 3.47 (s, CH_3O), 3.62 and 3.74 (2d of AB system, J = 14.5 Hz, Ar-$\underline{CH_2}$-N), 3.94 (dd, J_1 = 5.9 Hz and J_2 = 9.3 Hz, 1H of OCH_2), 4.04 (dd, J_1 = 5.4 Hz and J_2 = 9.3 Hz, 1H of OCH_2), 4.23 (m, CHO), 5.14 (s, O-CH_2-O), 6.79 (d, J = 2.3 Hz, CH of Ar), 6.83 (dd, J_1 = 2.5 Hz and J_2 = 8.3 Hz, CH of Ar), 6.95 (d, J = 8.7 Hz, CH of Ar), 6.96 (d, J = 8.9 Hz, 2 x CH of benzamide), 7.76 (d, J = 8.8 Hz, 2 x CH of benzamide). ^{13}C NMR ($CDCl_3$) δ 28.5, 29.1, 50.5, 55.7, 55.9, 56.8, 71.5, 71.8, 94.5, 114.3 (2x), 114.4, 115.8, 125.7, 127.4, 127.5, 129.3 (2x), 135.1, 155.7, 161.9, 168.9. LRMS calculated for $C_{22}H_{28}N_2O_5$ (M+H) 401.20, found 401.20.

3-{2-Hydroxy-4-[6-(methoxymethoxy)-3,4-dihydroisoquinolin-2(1*H*)-yl]butoxy}benzamide (7):

Rf = 0.30 (DCM/MeOH, 9:1). IR (film) ν 3348 and 3198 (OH and NH_2), 2932, 2827, 1666 (CON, amide), 1612, 1582, 1504, 1447, 1381, 1246, 1153, 1076, 1011. ^{1}H NMR ($CDCl_3$) δ 1.88 (m, $\underline{CH_2}$-CH-O), 2.67 and 3.05 (2m, Ar-$CH_2\underline{CH_2}$-N), 2.87 (m, $CH_2\underline{CH_2}$-N and Ar-$\underline{CH_2}CH_2$-N), 3.47 (s, CH_3O), 3.63 and 3.74 (2d of AB system, J = 14.5 Hz, Ar-$\underline{CH_2}$-N), 3.93 (dd, J_1 = 5.6 Hz and J_2 = 9.4 Hz, 1H of OCH_2), 4.05 (dd, J_1 = 5.5 Hz and J_2 = 9.4 Hz, 1H of OCH_2), 4.23 (m, CH-O), 5.14 (s, O-CH_2-O), 6.78 (d, J = 2.3 Hz, CH of Ar), 6.82 (dd, J_1 = 2.5 Hz and J_2 = 8.4 Hz, CH of Ar), 6.95 (d, J = 8.4 Hz, CH of Ar), 7.10 (m, CH of benzamide), 7.33 (m, 2 x CH of benzamide) 7.41 (s, CH of

benzamide). ^{13}C NMR ($CDCl_3$) δ 28.5, 29.0, 50.5, 55.6, 55.9, 56.6, 71.4, 71.9, 94.5, 113.4, 114.5, 115.7, 118.6, 119.5, 127.2, 127.5, 129.6, 134.8, 135.1, 155.7, 159.0, 169.2. LRMS calculated for $C_{22}H_{28}N_2O_5$ (M+H) 401.20, found 401.20.

2.7 Hydrolysis of the MOM Group: Synthesis of 8 and 9

Compound 6 (68 mg, 0.17 mmol) was dissolved in methanol (2 mL), a solution of hydrochloric acid (20% in methanol) (6.2 mL) was added and the resulting mixture was heated 4.5 h at 65°C. Water was then added, the methanol evaporated under reduced pressure, the solution neutralised with a saturated aqueous solution of sodium bicarbonate, and the aqueous phase extracted with DCM. The organic phase was evaporated and the yellowish powder was purified by flash chromatography (DCM/MeOH, 98:2) to yield the expected compound 8 (18 mg, 29% yield) and the ester 9 (14.5 mg, 23% yield).

4-[2-Hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzamide (8):

Rf = 0.10 (DCM/MeOH, 9:1). IR (film) ν 3344 and 3178 (OH and NH_2), 2924, 2851, 1655 (CON, amide), 1605, 1570, 1512, 1458, 1381, 1254, 1180, 1126. ^1H NMR (CD_3OD) δ 2.00 (m, $\underline{CH_2}$-CH-O), 2.96 and 3.08 (2m, Ar-$\underline{CH_2}\underline{CH_2}$-N-$\underline{CH_2}$), 3.90 (s, Ar-$\underline{CH_2}$-N), 4.05 (m, Ph-$\underline{CH_2}\underline{CH}$O), 6.60 (d, J = 2.3 Hz, CH of Ar), 6.64 (dd, J_1 = 2.5 Hz and J_2 = 8.3 Hz, CH of Ar), 6.95 (d, J = 8.3 Hz, CH of Ar), 7.04 (d, J = 8.9 Hz, 2 x CH of benzamide), 7.87 (d, J = 8.9 Hz, 2 x CH of benzamide). ^{13}C NMR (CD_3OD) δ 28.6, 30.3, 51.7, 55.8, 55.9, 69.7, 73.2, 115.0, 115.3 (2x), 115.7, 123.8, 127.3, 128.8, 130.7 (2x), 135.1, 157.6, 163.3, 172.0. LRMS calculated for $C_{20}H_{25}N_2O_4$ (M+H) 357.17, found 357.15.

Methyl 3-[2-hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzoate (9):

Rf = 0.40 (DCM/MeOH, 9:1). IR (film) ν 3209 and 3055 (OH phenol and alcohol), 2947, 2831, 1713 (C=O, ester), 1605, 1512, 1435, 1281, 1257, 1173, 1111, 1034. ^1H NMR ($CDCl_3$) δ 1.87 (m, $\underline{CH_2}$-CH-O), 2.65 and 2.85 (2m, Ar-$\underline{CH_2}\underline{CH_2}$-N-$\underline{CH_2}$), 3.57 and 3.70 (2d of AB system, J = 14.1 Hz, Ar-$\underline{CH_2}$-N), 3.88 (s, CH_3O), 3.96 (dd, J_1 = 5.9 Hz and J_2 = 9.4 Hz, 1H of OCH_2), 4.06 (dd, J_1 = 5.3 Hz and J_2 = 9.4 Hz, 1H of Ph-OCH_2), 4.28 (m, CH-O), 6.43 (d, J = 2.4 Hz, CH of Ar), 6.56 (dd, J_1 = 2.5 Hz and J_2 = 10.8 Hz, CH of Ar), 6.79 (d, J = 8.3 Hz, CH of Ar), 6.94 (d, J = 8.9 Hz, 2 x CH of

methylbenzoate), 7.98 (d, J = 8.9 Hz, 2 x CH of methylbenzoate). ^{13}C NMR ($CDCl_3$) δ 28.1, 28.6, 50.6, 51.9, 55.5, 56.7, 71.5, 71.6, 113.7, 114.2 (2x), 114.9, 122.7, 124.5, 127.5, 131.6 (2x), 134.6, 155.2 (2x), 162.5, 166.9. LRMS calculated for $C_{21}H_{26}NO_5$ (M+H) 372.18, found 372.15.

2.8 Hydrolysis of the MOM Group: Synthesis of Compound 10

Compound 7 (27 mg, 0.07 mmol) was dissolved in acetone (3 mL), a solution of 10% hydrochloric acid in acetone (1/1, v/v) (8.4 mL) was added and the resulting mixture was heated for 4 h at 57°C. Water was then added, the acetone evaporated under reduced pressure, the solution neutralised with a saturated aqueous solution of sodium bicarbonate and the aqueous phase extracted with DCM. The organic phase was evaporated and purified by successive trituration in diethyl ether and hexanes to yield compound 10 (20 mg, 83% yield) as a white powder.

Methyl 4-[2-hydroxy-4-(6-hydroxy-3,4-dihydroisoquinolin-2(1*H*)-yl)butoxy]benzoate (10): Rf = 0.10 (DCM/MeOH, 9:1). IR (film) ν 3360 (OH and NH_2), 2924, 1666 (CON, amide), 1624, 1582, 1420, 1331, 1288, 1246, 1119. ^{1}H NMR (CD_3OD) δ 1.90 (m, $\underline{CH_2}$-CH-O), 2.68-2.90 (broad m, Ar-$\underline{CH_2}\underline{CH_2}$-N-$\underline{CH_2}$), 3.58 (s, Ar-$\underline{CH_2}$-N), 4.01 (m, O$\underline{CH_2}$-CH-O), 4.05 (m, OCH_2-$\underline{CH}$-O), 6.51 (d, J = 2.3 Hz, CH of Ar), 6.55 (dd, J_1 = 2.5 Hz and J_2 = 8.2 Hz, CH of Ar), 6.83 (d, J = 8.3 Hz, CH of Ar), 7.15 (d, J = 8.3 Hz, CH of benzamide), 7.37 (t, J = 8.2 Hz, CH of benzamide) 7.44 (m, 2 x CH of benzamide). ^{13}C NMR (CD_3OD) δ 29.8, 31.1, 52.1, 56.3, 56.8, 70.3, 73.3, 114.6, 115.1, 116.1, 119.5, 121.0, 125.2, 128.4, 130.6, 135.9, 136.3, 158.5, 160.5, 172.2. LRMS calculated for $C_{20}H_{25}N_2O_4$ (M+H) 357.17, found 357.15.

2.9 17β-HSD1 Inhibition of Compounds 8-10

The enzymatic assay was performed using the T-47D breast cancer cell line as source of 17β-HSD1 activity following a procedure previously published (Ayan et al., 2012).

2.10 Estrogenic Activity of Compounds 8 and 10

Using a procedure we previously published (Ayan et al., 2012), compounds 8 and 10 were tested to determine their potential proliferative (estrogenic) effect on estrogen-sensitive (ER^+) breast cancer T-47D cells.

2.11 ER-α Binding Affinity of Compounds 8 and 10

A comparative binding assay using a purified full-length recombinant human estrogen receptor alpha (ER-α) was done as previously described (Ayan et al., 2012).

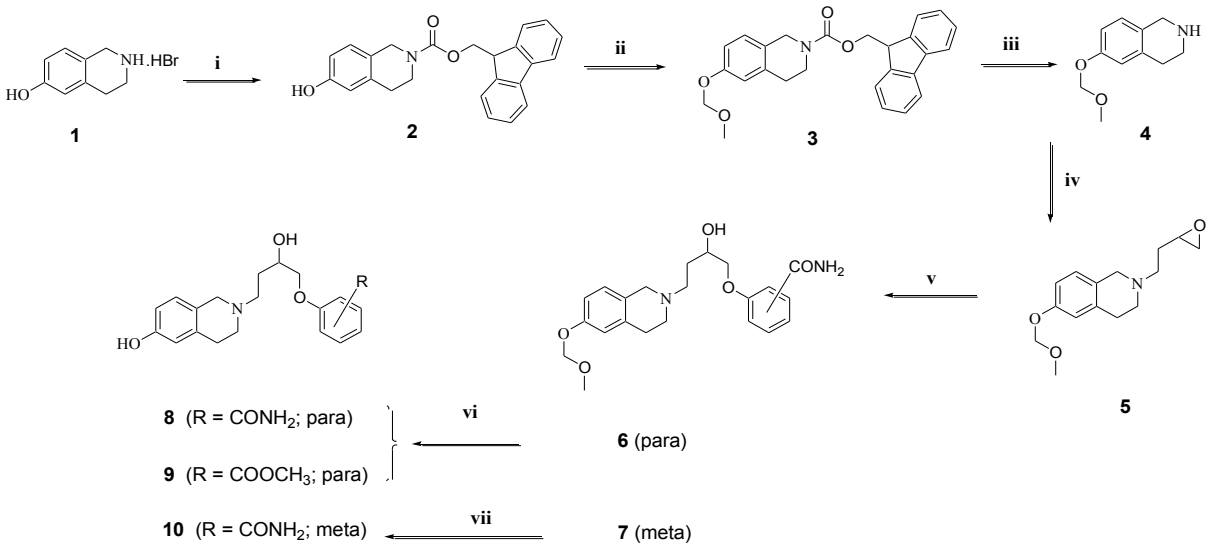

Figure 4. Synthesis of targeted compounds 8 and 10. Reagents and conditions: i) THF/H_2O, $NaHCO_3$, Fmoc-OSu, rt; ii) CH_2Cl_2, $ClCH_2OCH_3$, DIPEA, 0°C - rt; iii) piperidine; CH_2Cl_2, rt; iv) 4-bromo-1,2-epoxybutane, K_2CO_3, acetone, 55°C; v) K_2CO_3, *para*- or *meta*-hydroxybenzamide, DMF, 105°C; vi) MeOH/HCl, 65°C; vii) acetone/HCl, 57°C

3. Results and Discussion

3.1 Synthesis of Targeted Compounds 8 and 10 (Figure 4)

The free amine function of tetrahydro-6-isoquinolinol hydrobromide (1) was protected with Fmoc-OSu in presence of aqueous $NaHCO_3$. The hydroxyl group of 2 was next protected as a methoxymethyl (MOM) ether group to give compound 3. This compound was found to be unstable at room temperature and has to be used readily to the next step. The Fmoc protecting group of 3 was then hydrolysed by a treatment with a mixture of piperidine in methylene chloride to give compound 4. An undesirable adduct of compound 4 with dibenzofulvene was however observed, thus reducing the yield of the reaction. A nucleophilic substitution of 4-bromo-1,2-epoxybutane by the free amine function of 4 yielded the epoxide 5. *Para* and *meta* hydroxybenzamide were used to open the epoxide and generate the corresponding alcohols 6 and 7. The final compounds 8 and 10 were obtained from acidic hydrolysis of 6 and 7, respectively, but the use of solvent, methanol or acetone, greatly influenced the yield of each compound. In fact, the side product 9 (methyl ester) was isolated from the hydrolysis of 6 when methanol was used instead of acetone, thus reducing the yield of compound 8. A very good yield of

compound 10 (83%) was however obtained when we used acetone instead of methanol for the hydrolysis of 7.

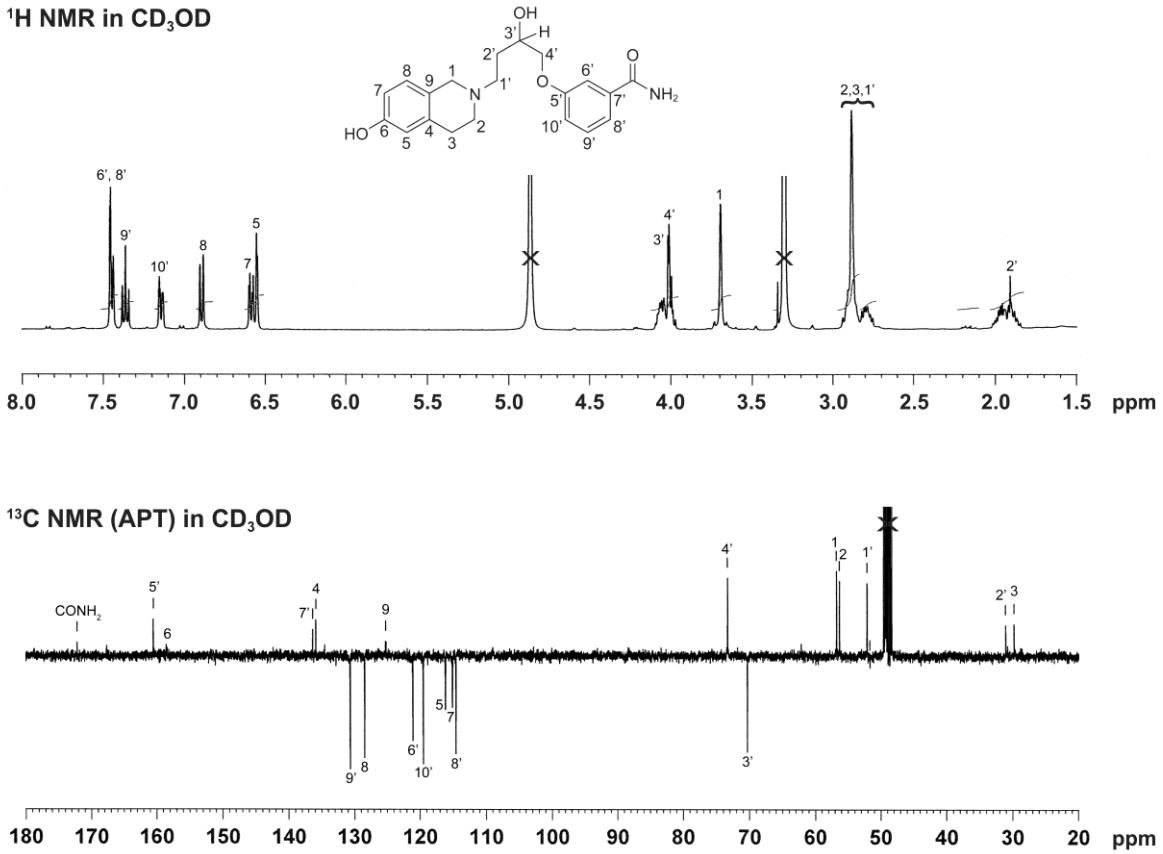

Figure 5. ^{1}H NMR and ^{13}C NMR (APT) spectra of final compound 10 dissolved in methanol-d_4. All protons and carbons were assigned to the appropriate signals

3.2 ^{13}C NMR Characterization of the Intermediate and Final Compounds

The assignment of ^{13}C NMR signals (Table 1) for all compounds was performed using a combination of 2D NMR (APT, HSQC, HMBC, COSY and NOESY) experiments (Claridge, 1999). To illustrate our strategy, we selected the final compound 10 (Figure 5). We started our analysis with the only CH non aromatic signal at 70.3 and 4.05 ppm, which were easily identified as C3' and H3', respectively, from APT and HSQC spectra. From these signals, protons and carbons 1, 2, 3, 1', 2' and 4' were distinctively assigned according to correlations observed in COSY, HSQC or/and HMBC. In fact, from cross-correlation in COSY spectrum, the H3' signal (4.05 ppm) showed two expected correlations: the first attributed to H2' (1.90 ppm) and the other to H4' (4.01 ppm), no more correlation being observed for H4' in COSY spectrum as expected. Proton H2' showed one more cross-correlation with the signal at 2.78 ppm, so we thus assigned this chemical shift value to H1'. Proton H1 (3.58 ppm) is distinguishable from H2 and H3, as it did not show cross-correlation in COSY experiment.

However, a correlation appears between the two signals at 2.74 and 2.83 ppm. To differentiate H2 from H3, we next used the results of HMBC experiment. In fact, proton H3 (2.85 ppm) distinctively showed four correlations in the HMBC spectrum (as expected with C2, C4, C5 and C9), and protons H2 (2.74 ppm) did not show long range coupling with C9, only one resolved signal being observed (attributed to C4). From each H signal reported above the corresponding C was easily identified using the HSQC spectrum.

After we identified the CH_2 signals and the non-aromatic CH signal, two groups of aromatic CH and C signals remained to be assigned: those in the left part of the molecule (A-ring: C4-C9) and those in the right part of the molecule (C-ring: C5'-C10'). For the former ring system, aromatic protons H5, H7 and H8 are obviously assigned from their multiplicity and COSY experiment. Moreover, a strong cross-correlation was observed in NOESY spectrum between the proton H1 (3.58 ppm) and a proton at 6.83 ppm. This doublet signal (J = 8.3 Hz) was then associated to H8. Using the HSQC data, the C8 was next assigned to the peak at 128.4 ppm. By combining the COSY correlations between H8 and H7 and the HSQC data, we identified H7 (6.55 ppm) and C7 (115.1 ppm). From the NOESY spectrum, a cross-correlation was observed between the known H3 signal and the unknown CH signal at 6.51 ppm, this later was thus assigned to H5. C5 (116.1 ppm) was then identified from H5 using the HSQC spectrum. In HMBC spectrum, only C4 (135.9 ppm) can correlate with H1, H2 and H3, only C9 (125.2 ppm) can correlate with both H1, H3 and H7 signals, and only C6 (158.5 ppm) correlated with H5, H7 and H8.

Table 1. ^{13}C NMR assignation of intermediates and final compounds

Cpds[a]	2	3	4	5	6	7	8	9	10
R_1	H	MOM[b]	MOM	MOM	MOM	MOM	H	H	H
R_2	----	----	----	----	*p*-$CONH_2$	*m*-$CONH_2$	*p*-$CONH_2$	*p*-$COOCH_3$	*m*-$CONH_2$
Solvent	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	CD_3OD	$CDCl_3$	CD_3OD
C1	45.3	45.1	43.6	54.7	55.7	55.6	55.9	55.5	56.8
C2	41.4	41.2	47.6	55.6	56.8	56.6	55.8	56.7	56.3
C3	28.7	28.7	29.3	29.3	29.1	29.0	28.6	28.6	29.8
C4	135.9	135.5	135.5	135.5	135.1	135.1	135.1	134.6	135.9
C5	115.1	115.8	116.5	115.8	115.8	115.7	115.7	114.9	116.1
C6	154.2	155.6	155.4	155.5	155.7	155.7	157.6	155.2	158.5
C7	114.9	114.6	114.3	114.2	114.4	114.5	115.0	113.7	115.1
C8	127.4	127.1	127.5	127.5	127.5	127.5	128.8	127.5	128.4
C9	133.1	126.3	129.3	128.2	127.4	127.2	127.3	124.5	125.2
C1’	155.5	155.2	----	50.8	50.5	50.5	51.7	50.6	52.1
C2’	67.5	67.2	----	30.5	28.5	28.5	30.3	28.1	31.1
C3’	47.3	47.2	----	50.9	71.5	71.4	69.7	71.5	70.3
C4’	144.0	143.9	----	47.1	71.8	71.9	73.2	71.6	73.3
C5’	125.0	124.8	----	----	161.9	159.0	163.3	162.5	160.5
C6’	127.0	126.9	----	----	114.3	119.5	115.3	114.2	121.0
C7’	127.7	127.5	----	----	129.3	134.8	130.7	131.6	136.3
C8’	120.0	119.8	----	----	125.7	113.4	123.8	122.7	114.6
C9’	141.3	141.2	----	----	129.3	129.6	130.7	131.6	130.6
C10’	----	----	----	----	114.3	118.6	115.3	114.2	119.5
COX	----	----	----	----	168.9	169.2	172.0	166.9	172.1
OCH_2O	----	94.3	94.5	94.5	94.5	94.5	----	----	----
OCH_3	----	55.8	55.9	55.9	55.9	55.9	----	51.9	----

a–See Figure 6 for structural formulae and carbon numbering. b–MOM: OCH_2OCH_3

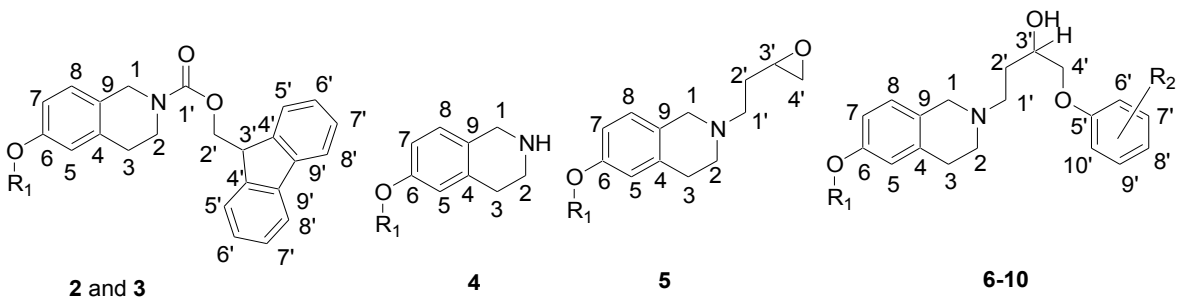

Figure 6. Structural formulae and carbon numbering of compounds 2 to 10

For the C-ring system, the C5' (160.5 ppm) was identified by a characteristic HBMC correlation with the previously assigned H4' signal. The carboxamide carbon (172.2 ppm) was also assigned by comparison with literature data for a similar benzamide group. We next identified H6' (7.47 ppm) because it is the only proton producing correlations with both C5' and $CONH_2$. From H6' we also identified C6' (121.0 ppm) with HSQC experiment. The apparent triplet at 7.37 ppm with a large coupling constant (8.2 Hz) was necessarily the H9', which correlated in HSQC experiment with the peak at 130.6 ppm (C9'). The H9' signal showed four cross-correlations in HMBC (as expected with C5', C7', C8' and C10'). Since C5' was previously assigned, the other quaternary carbon in this group is necessary C7' (136.3 ppm). To discriminate the two remaining H8' and H10' signals we used the NOESY experiment which showed a correlation with H10' (7.15 ppm) but not with H8' (7.45 ppm). Using the HSQC spectrum, we then assigned C10' at 119.5 ppm and C8' at 114.6 ppm.

3.3 Biological Evaluation of Compounds 8-10

The inhibitory potency of compounds 8, 9 and 10 on 17β-HSD1 was assessed at 0.1, 1 and 10 μM in intact T-47D cells, which are able to transform E1 to E2 and are known to be a good source of 17β-HSD1 (Day et al., 2008a; Laplante et al., 2009). Cells were co-incubated for 24 h with each compound to be tested and radiolabeled E1 (60 nM). Inhibitory effect of compounds was compared with unlabeled estrone and CC-156, each used as an inhibitor, after separation and quantification of radiolabeled steroids (E1 and E2). Although docking experiments gave us expectation of a good affinity of the target compound 10 with the enzyme (17β-HSD1), we were disappointed by the results of our enzymatic assay because compounds 8, 9 and 10 did not inhibit the transformation of E1 to E2 by 17β-HSD1 found in T-47D cells. This might be due to poor accessibility of these compounds to the cell cytoplasm where 17β-HSD1 is located, but this has to be verified by a biological test on homogenized cells. Moreover, the alkyl side chain between the phenyl rings render the molecules more flexible, with a higher degree of freedom compared to CC-156 where the rigidity is provided by the estrane scaffold. The electronic character of the pseudo-cycle B due to the presence of a nitrogen atom is another possible cause of the weaker affinity of compounds 8-10 with the enzyme.

One important objective of our project was to obtain a compound that is devoid of estrogenic activity. The proliferation of estrogen-sensitive (ER^+) T-47D cells was then carried out to determine the estrogenic activity of compounds 8 and 10. In this assay, an estrogenic compound will stimulate the proliferation of ER^+ cells, as observed when we used E2 as a reference compound. Thus, a treatment with 0.1 nM of E2 during seven days increased the cell proliferation from 100% (control) to 153%. Interestingly, compound 8 (*para*) did not show any proliferative (estrogenic) activity at the six concentrations tested (0.001, 0.01, 0.1, 1, 5 and 10 μM). Compound 10 (*meta*) showed no estrogenic activity at 0.001, 0.01, 0.1 and 1 μM but increased the cell proliferation to 125 and 129% at 5 and 10 μM, respectively. The binding affinity of compounds 8 and 10 was next carried out using the human ERα. In this competition binding assay, an estrogenic compound will displace the tritiated E2 ([^{3}H]-E2), and the remaining bound [^{3}H]-E2 will be measured and used to determine the affinity of this compound for ERα. Thus, when untritiated E2 was used as a reference compound in a range of concentration (10^{-12}-10^{-5} M), an effective concentration of 2.3 ± 0.4 nM was needed to displace 50% of 2.5 nM of [^{3}H]-E2. Interestingly, both compounds 8 and 10 did not show any affinity to ERα in concentrations ranging from 10^{-12} to 10^{-5} M.

4. Conclusion

We have synthesized non-steroidal mimics of CC-156, a potent steroidal inhibitor of 17β-HSD1, but they did not inhibit the transformation of E1 to E2 by 17β-HSD1 found in T-47D cells. The flexibility of compounds 8 and 10, compared to the rigid steroid backbone of CC-156, is potentially responsible for their lack of enzyme inhibitory activity. It is however interesting to mention that compounds 8 and 10 did not bind to ERα and did not induce the proliferation of estrogen-sensitive (ER^+) T-47D cells, except compound 10 at high concentrations of 5 and 10 μM, suggesting that they are not estrogenic compounds. We also fully characterized all intermediate and final compounds, and the synthetic approach we developed is now available to generate additional analogs.

Acknowledgements

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Chapitre 2

Développement d'une méthode de synthèse en parallèle et en solution, simple et efficace, de mimiques non stéroïdiennes flexibles de l'estradiol

1. Avant-propos

Encouragés par les résultats obtenus lors du développement des mimiques non stéroïdiennes dépourvues d'activité estrogénique (chapitre 1), nous avons développé une approche visant à synthétiser des mimiques non stéroïdiennes de l'estradiol (E2), qui pourront agir comme inhibiteurs de la 17β-HSD1, inhibiteurs de la stéroïde sulfatase (STS) ou comme modulateurs sélectifs du récepteur des estrogènes.

La conception des mimiques de E2 a été faite sur la base de superpositions avec le logiciel Chem 3D et des données de la littérature, en lien avec la co-cristallisation de la 17β-HSD1 avec E2. Les remarques pertinentes et les idées proposées par René Maltais (alors professionnel de recherche), ainsi que son assistance pour l'utilisation du synthétiseur robotisé ont été d'une grande utilité.

Dans le cadre de ce projet, j'ai réalisé la préparation de toutes les librairies, l'isolation des composés d'intérêt et leur caractérisation. L'activité biologique de ces composés reste à être évaluée. Ce manuscrit sera bientôt soumis pour publication dans la revue *Combinatorial Chemistry and High Throughput Screening*.

2. Résumé

Une méthode efficace de synthèse en parallèle a été développée pour la préparation de chimiothèques de mimiques de l'estradiol (E2) qui peuvent potentiellement interagir avec différentes cibles biologiques, telles que la 17β-hydroxystéroïde déshydrogénase de type 1, la stéroïde sulfatase et le récepteur des estrogènes. Deux chimiothèques de 75 membres chacune ont été préparées en ajoutant trois niveaux de diversité moléculaire sur un noyau non stéroïdien. Les hydroxybenzaldéhydes (*para* et *méta*) (premier niveau de diversité), protégés avec un groupe méthoxyméthyléther, ont d'abord été traités avec des amines primaires (deuxième niveau de diversité) dans des conditions d'amination réductrice. Les amines secondaires résultants ont ensuite réagi avec le 4-bromo-1,2-époxybutane pour fournir des époxydes en tant que précurseurs du troisième niveau de diversité. En utilisant différentes conditions expérimentales, des anilines, des amines, des phénols et des thiophénols ont été utilisés par la suite pour effectuer l'aminolyse de chaque époxyde. Une résine de type méthyl isocyanate a été utilisée pour piéger l'excès d'amine et le groupe protecteur a été éliminé par hydrolyse pour donner les composés phénoliques non stéroïdiens souhaités.

Combinatorial Chemistry and High Throughput Screening (Manuscript to be submitted)

Development of a simple and efficient solution-phase parallel synthesis of flexible non steroidal estradiol mimics

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Keywords: Estradiol mimic, phenol, reductive amination, epoxide opening, library.

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Abstract

An efficient parallel synthesis was designed and developed in order to provide libraries of estradiol mimics that can potentially interact with different biological targets associated with estradiol-related diseases, such as 17β-hydroxysteroid dehydrogenase type 1, steroid sulfatase and estrogen receptor. Two libraries of 75 members were synthesized around a non steroidal core by adding three levels of molecular diversity. Hydroxybenzaldehydes (1st level of diversity), protected as a methoxymethylether, first reacted with primary amines (2nd level of diversity) under reductive amination conditions. The resulting secondary amines next reacted with 4-bromo-1,2-epoxybutane to provide epoxide derivatives as precursors of the 3rd level of diversity. Using different experimental conditions, various anilines, amines, phenols and thiophenols were then used to open each epoxide. Methyl isocyanate scavenger was finally used to trap out the excess of amine and the protecting group was removed by hydrolysis to provide the phenolic non steroidal compounds.

INTRODUCTION

The role of estrogens in breast cancer and other estrogen-dependent diseases has been well established during the past decades [1-3]. Different key enzymes are involved in the formation of active estrogens including 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) and steroid sulfatase (STS) [4,5]. Estradiol (E2), the most potent estrogen resulting from these enzymatic transformations, binds the estrogen receptors (ERα and ERβ) in different tissues (breast, ovaries, bones, endometrial, brain) and can cause different responses [6-8]. For example, in women with estrogen-dependent breast cancer, E2 activates ER, causing proliferation of breast cancer cells (Figure 1). Therefore, estrogen deprivation therapy (EDT) represents one of the best known therapeutic options for women in advanced stage ER^+ breast cancer [9].

The development of molecules that interact with key targets of steroid hormone action (17β-HSD1 inhibitors, STS inhibitors and ER antagonists/agonists) represents an active field of research [10-14]. During the last years, many approaches were developed to target E2-related biological entities using steroidal derivatives. Most of these strategies failed because of the intrinsic estrogenic effect of those compounds. In fact, the estrane nucleus is often associated with carcinogenic effect of E2 derivatives [15-18]. As example, CC-156, a strong inhibitor of 17β-HSD1, was found to cause proliferation of ER^+ breast cancer cells [16]. In the same manner, first steroidal inhibitors of STS build around an estrane core were found to be estrogenic [18-20]. Indeed, a previous study in our laboratory showed that E2 derivatives bearing a hydrophobic substituent at C-17 position were good inhibitors of STS [19], but they were found to be estrogenic inhibitors. Integrating a hydrophobic substituent to a non steroidal E2 mimic was found to be an interesting way to limit the estrogenicity of the STS inhibitors [20]. Thus, the development of non steroidal E2 mimics that can specifically bind to 17β-HSD1, STS, ERα or ERβ has been reported with the advantage to be less estrogenic than their parent steroidal form [21-25].

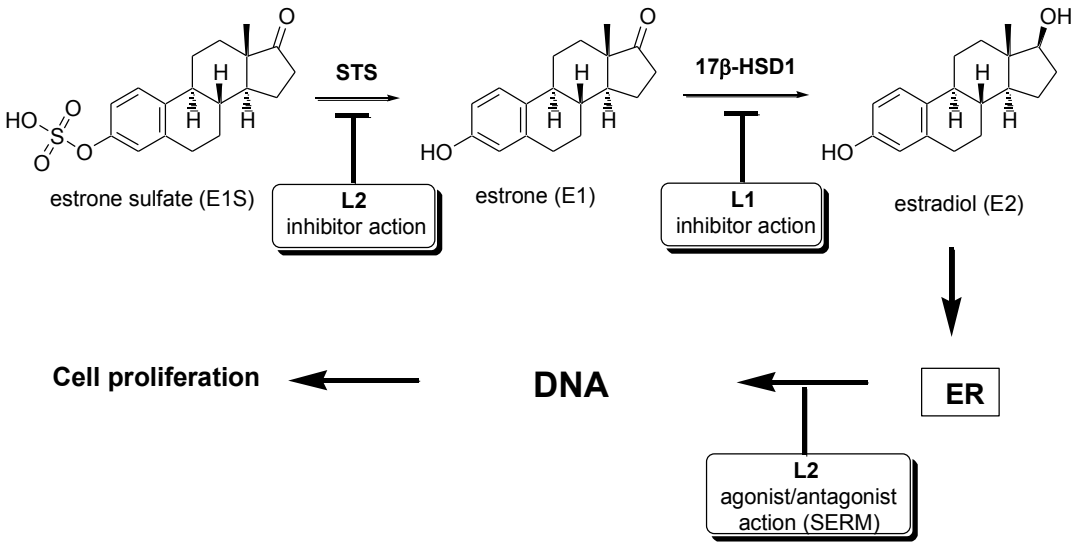


Figure 1: Sites of action (STS, 17β-HSD1 and ER) potentially targeted by non-steroidal E2 derivatives of libraries **L1** and **L2**. Cell proliferation is modulated by the formation of active steroid E2 and its action on estrogen receptor (ER).

Inspired by the success of non steroidal compounds that provide non estrogenic enzyme inhibitors as well as potent selective estrogen receptor modulators (SERM), we were interested to develop a simple synthetic approach to rapidly generate libraries of diversified non steroidal E2 mimics (Figure 2). Two diversified libraries (L1 and L2) of non steroidal derivatives were thus designed following parallel liquid-phase chemistry. The first library (L1) was build using element of diversity known to interact with 17β-HSD1 whereas the second library (L2) includes building blocks that could potentially promote STS inhibition or provide SERM activity.

RESULTS AND DISCUSSION

Library design

Ten epoxides (namely **6**, **7**, **8**, **9**, **10**, **6'**, **7'**, **8'**, **9'** and **10'** in Scheme 1) were first synthesized in three steps for introducing the level R_1 of molecular diversity, which will mimic the steroid cycles B and C (Figure 2). The pseudo cycle A, represented by the phenyl group of the initial precursor has been chosen to mimic the aromatic A-ring of E2. Once the series of ten epoxides was prepared, different nucleophilic building blocks were used for the opening of epoxides, thus introducing another level of molecular

diversity (R_2). The hydroxyl group was expected to mimic the 17β-OH of E2. The first library of 75 compounds (L1) was designed as potential inhibitors of 17β-HSD1 (Table 1). In this library, the amines **A**, **B**, **C**, **D**, **E**, **F**, **G**, **H**, **I**, **J** and phenols **K**, **L**, **M**, **N**, **O** were chosen in order to mimic the benzylamide moiety of inhibitor CC-156, an E2 derivative that interacts with certain amino acids inside 17β-HSD1 catalytic site [26]. In order to extend our methodology to the synthesis of more diversified compounds designed to bind STS, ERα or ERβ, the amines **P**, **Q**, **R**, **S**, **T** and thiophenols **U**, **V**, **W**, **X**, **Y** were selected to generate a second library of 75 compounds (L2) (Table 2).

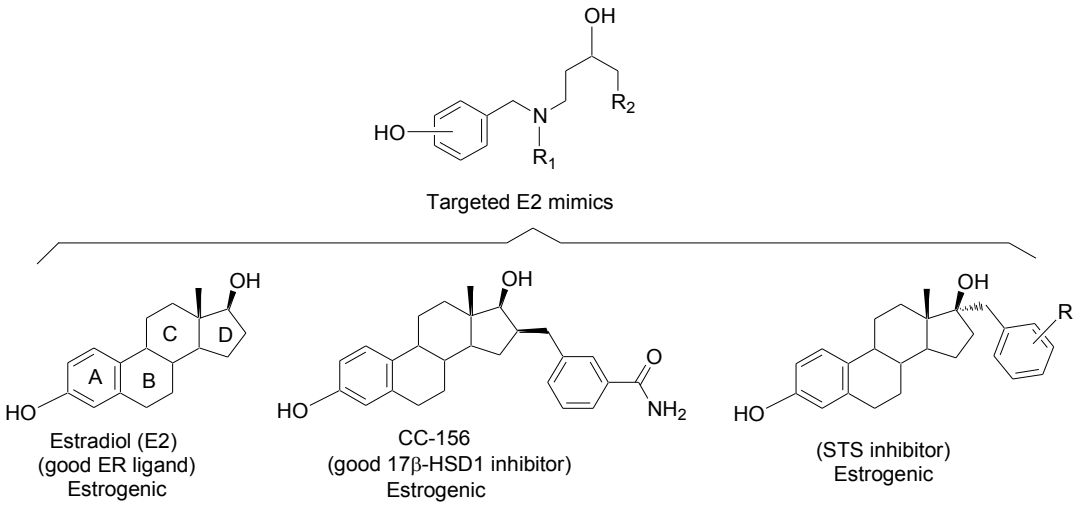
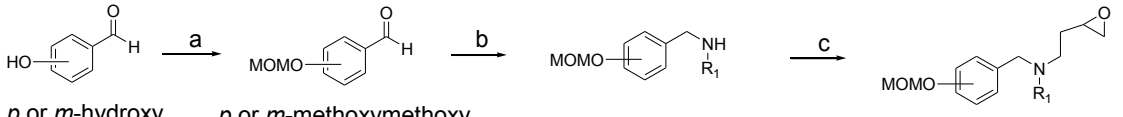


Figure 2: Design of flexible non steroidal estradiol (E2) mimics as potential 17β-HSD1 inhibitors, STS inhibitors or SERM. Steroid cycles are represented by A, B, C and D.

Chemistry

The synthesis of intermediate epoxides is reported in Scheme 1. The para- and meta-hydroxybenzaldehydes were first protected with a methoxymethyl (MOM) group. The products obtained were then submitted to a reductive amination with five primary amines to introduce the first level of diversity (R_1) by generating the secondary amines **1**, **2**, **3**, **4**, **5**, **1'**, **2'**, **3'**, **4'** and **5'** with good to excellent yields. These amines were further submitted to a nucleophilic substitution with 4-bromo-1,2-epoxybutane in refluxing acetone and potassium carbonate to give the tertiary amines **6**, **7**, **8**, **9**, **10**, **6'**, **7'**, **8'**, **9'** and **10'**, respectively. Such amino-epoxides represent key scaffolds for the synthesis of our two libraries of compounds (L1 and L2).



p or *m*-hydroxy benzaldehyde

p or *m*-methoxymethoxy benzaldehyde

R1:

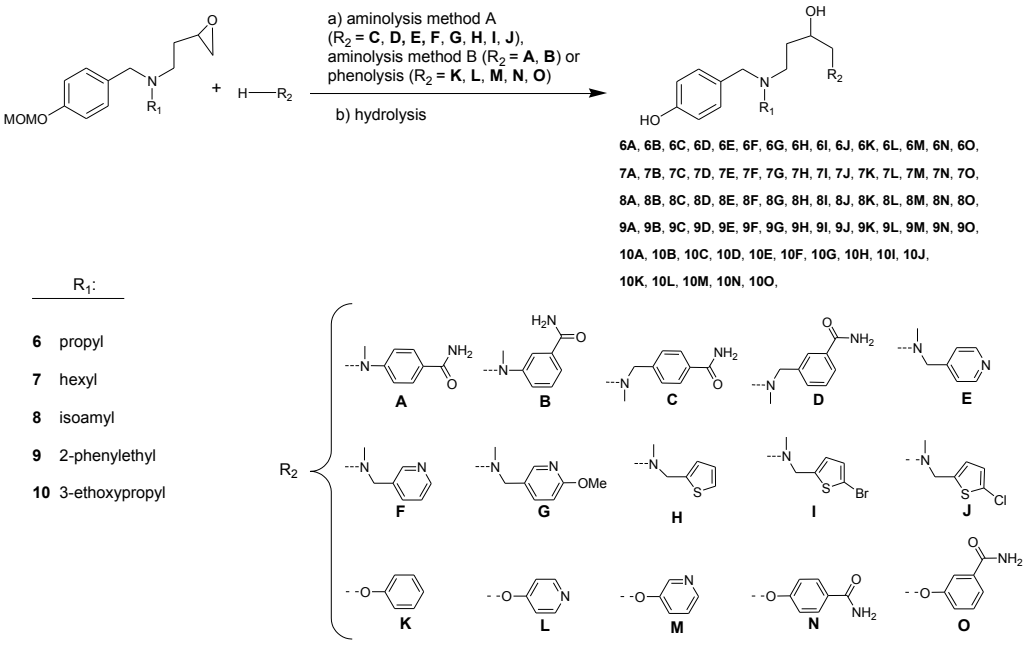
1 (*para*), 1' (*meta*)	propyl
2 (*para*), 2' (*meta*)	hexyl
3 (*para*), 3' (*meta*)	isoamyl
4 (*para*), 4' (*meta*)	2-phenylethyl
5 (*para*), 4' (*meta*)	3-ethoxypropyl

R1:

6 (*para*), 6' (*meta*)	propyl
7 (*para*), 7' (*meta*)	hexyl
8 (*para*), 8' (*meta*)	isoamyl
9 (*para*), 9' (*meta*)	2-phenylethyl
10 (*para*), 10' (*meta*)	3-ethoxypropyl

Scheme 1: Synthesis of epoxides **6**, **7**, **8**, **9**, **10**, **6'**, **7'**, **8'**, **9'** and **10'**. Reagents and conditions: (a) DIPEA, MOM-Cl, CH_2Cl_2, 0 °C to rt, overnight; (b) H_2N-R_1, molecular sieves, $NaBH_4$, EtOH, rt, overnight; (c) K_2CO_3, 4-bromo-1,2-epoxybutane, acetone, 50 °C, 48 h.

The aminolysis of epoxides **6**, **7**, **8**, **9** and **10** with the building blocks **C**, **D**, **E**, **F**, **G**, **H**, **I** and **J** (L1) or **P**, **Q**, **R**, **S** and **T** (L2) worked relatively well using the aminolysis method A (EtOH, 75 °C, 24 h) (Schemes 1 and 2). The amines **P**, **Q**, **R**, **S** and **T** were also used to open the epoxides **6'**, **7'**, **8'**, **9'** and **10'** with the conditions of method A (L2). However, it is well mentionning that the aminolysis with aniline derivatives **A** and **B** (L1) did not work under the conditions of method A, probably because of the deactivation of the free electrons of nitrogen doublet by the aromatic ring. Thus, a st***oe***chiometric quantity of magnesium perchlorate was added to the reaction in order to activate the epoxides (aminolysis method B). Epoxides **6**, **7**, **8**, **9** and **10** were also reacted with the phenolic building blocks **K**, **L**, **M**, **N** and **O** (L1). Each phenol was first mixed in a methanolic potassium carbonate solution, in order to form the phenolate, and this solution was added to the epoxide solution. The thiophenols **U**, **V**, **W**, **X** and **Y** reacted well with the epoxides **6**, **7**, **8**, **9** and **10** (L2) in methanol without base added to the reaction medium. Finally the methoxymethyl ether group of all compounds was hydrolyzed in acidic conditions to generate the library L1 members (Scheme 2, Table 1) and the library L2 members (Scheme 3, Table 2).

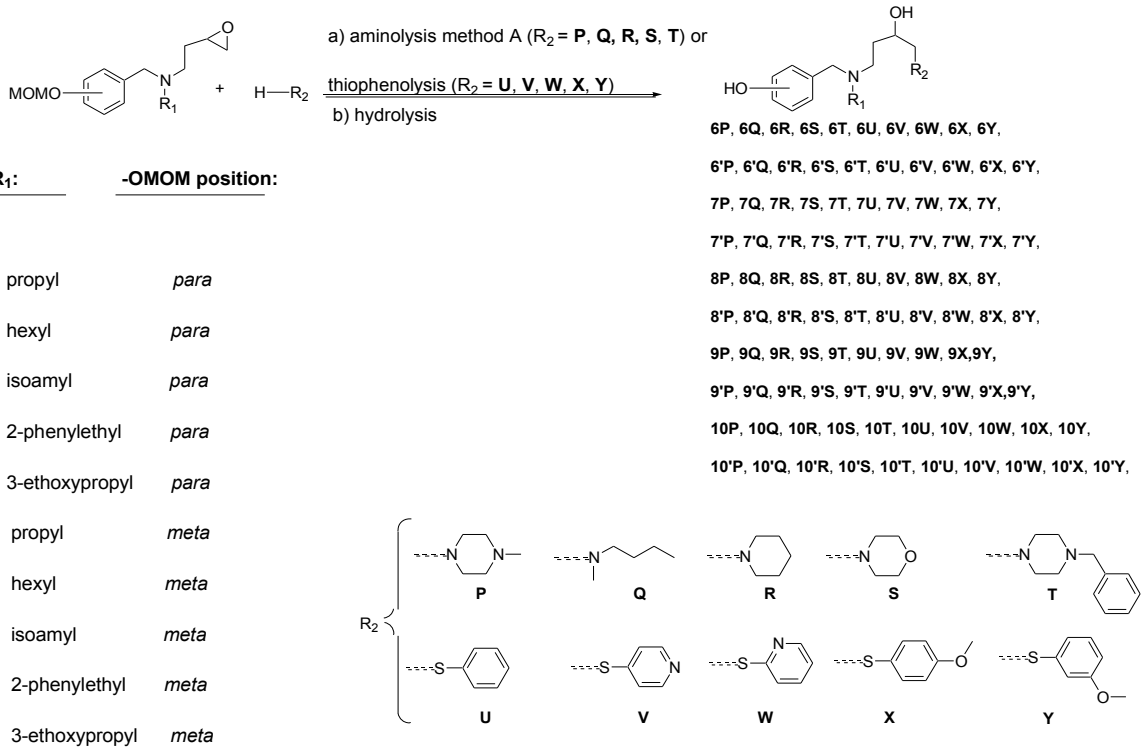


Scheme 2: Synthesis of the first library (L1) of E2-mimic compounds. Reagents and conditions: a) aminolysis method A: HR_2, EtOH, 75 °C, 24 h; aminolysis method B: $Mg(ClO_4)_2$, HR_2, CH_3CN, 55 °C, 24 h; phenolysis: K_2CO_3, HR_2, DMF, 106 °C, 26 h; b) hydrolysis: MeOH/HCl (8:2), 60 °C, 5 h.

Table 1: Library (L1) compounds designed to mimic CC-156, a strong inhibitor of 17β-HSD1.[a]

R_1/R_2	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
6	6A (71 %)	6B	6C	6D	6E	6F (66 %)	6G	6H	6I	6J	6K (60 %)	6L	6M	6N	6O
7	7A	7B (36 %)	7C	7D	7E	7F	7G (90 %)	7H	7I	7J	7K	7L (67 %)	7M	7N	7O
8	8A	8B	8C (71 %)	8D	8E	8F	8G	8H (80 %)	8I	8J	8K	8L	8M (79 %)	8N	8O
9	9A	9B	9C	9D (44 %)	9E	9F	9G	9H	9I (81 %)	9J	9K	9L	9M	9N (63 %)	9O
10	10A	10B	10C	10D	10E (77 %)	10F	10G	10H	10I	10J (93 %)	10K	10L	10M	10N	10O (50 %)

[a] Highlighted compounds have been chosen for determination of purity (%) by HPLC. See scheme 2 for the chemical structure of each library member.



R_1:		-OMOM position:
6	propyl	*para*
7	hexyl	*para*
8	isoamyl	*para*
9	2-phenylethyl	*para*
10	3-ethoxypropyl	*para*
6'	propyl	*meta*
7'	hexyl	*meta*
8'	isoamyl	*meta*
9'	2-phenylethyl	*meta*
10'	3-ethoxypropyl	*meta*

Scheme 3: Synthesis of the second library (L2) of E2-mimic compounds. Reagents and conditions: a) aminolysis method A: HR_2, EtOH, 75 °C, 24 h; thiophenolysis: HR_2, MeOH, 65 °C, 26 h; b) hydrolysis: MeOH/HCl (8:2), 60 °C, 5 h.

Table 2: Library (L2) compounds designed to target STS or ER.

R_1/R_2	P	Q	R	S	T	U	V	W	X	Y
6	**6P** (62 %)	**6Q**	**6R**	**6S**	**6T**	**6U**	**6V**	**6W**	**6X**	**6Y** (71 %)
7	**7P**	**7Q** (80 %)	**7R**	**7S**	**7T**	**7U**	**7V**	**7W**	**7X** (73 %)	**7Y**
8	**8P**	**8Q**	**8R** (88 %)	**8S**	**8T**	**8U**	**8V**	**8W** (70 %)	**8X**	**8Y**
9	**9P**	**9Q**	**9R**	**9S** (82 %)	**9T**	**9U**	**9V** (73 %)	**9W**	**9X**	**9Y**
10	**10P**	**10Q**	**10R**	**10S**	**10T** (74 %)	**10U** (77 %)	**10V**	**10W**	**10X**	**10Y**
6'	**6'P**	**6'Q**	**6'R**	**6'S**	**6'T** (88 %)	---	---	---	---	---
7'	**7'P**	**7'Q**	**7'R**	**7'S** (86 %)	**7'T**	---	---	---	---	---
8'	**8'P**	**8'Q**	**8'R** (88 %)	**8'S**	**8'T**	---	---	---	---	---
9'	**9'P**	**9'Q** (91 %)	**9'R**	**9'S**	**9'T**	---	---	---	---	---
10'	**10'P** (90 %)	**10'Q**	**10'R**	**10'S**	**10'T**	---	---	---	---	---

[a] Highlighted compounds have been chosen for determination of purity (%) by HPLC. See scheme 3 for the chemical structure of each library member.

CONCLUSION

Two 75-member libraries of E2 mimics were prepared following a simple and efficient solution phase parallel synthesis approach. Ten key epoxides were first synthesized in three steps and next reacted (epoxide opening) with various building blocks (anilines, amines, phenols and thiophenols). All compounds were characterized by ^{1}H NMR and LRMS to confirm the right structure. Representative compounds were also selected and analyzed by HPLC to provide purity index of the two libraries. Thus, the mean purity of library 1 members was 69 % (36 - 93 %) and the mean purity of library 2 was 80 % (62 – 91 %). These 150 compounds contain three levels of molecular diversity (*para*- or *meta*-hydroxybenzaldehyde, R_1 and R_2) and were designed to target steroidogenic

enzymes and steroid receptors. Most importantly, these first two libraries constitute a testing ground that validate the robustness and flexibility of this solution-phase parallel synthesis methodology toward the generation of more larger libraries dedicated to the exploration of E2-related targets in our research program. Their biological evaluation remain to be done and will be reported in due time.

EXPERIMENTAL

Chemistry

3-Hydroxybenzaldehyde, 4-hydroxybenzaldehyde, building blocks **F**, **H**, **I**, **K**, **L**, **M**, **N**, **O**, **P**, **Q**, **R**, **S**, **T**, **U**, **V**, **W**, **X**, **Y** and chemical reagents of highest purity were obtained from Sigma–Aldrich Canada Ltd. The building block **A** was available at ACC Corp. (San Diego, CA, USA) whereas the building blocks **B**, **C**, **D**, **E**, **G** and **J** were available at Aurora Fine Chemicals (San Diego, CA, USA). Solvents were obtained from Fisher Scientific (Montréal, Qc, Canada). The library 1 compounds (building blocks **C**-**O**) was prepared with the automated synthesizer 'The Solution' (aapptec; Louisville, KY, USA) using a solution-phase 96-reaction block. The library 2 compounds (building blocks **P**-**Y**) were made with an ACT LabTech manual synthesizer (Advanced ChemTech; Louisville, KY, USA) using either a 40 or 96 solution-phase reaction block.

Analytical thin-layer chromatography (TLC) was performed on 0.20-mm silica gel 60 F254 plates (E. Merck, Darmstadt, Germany) and compounds were visualized by using ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with Silicycle R10030B 230–400 mesh silica gel (Québec, Qc, Canada). Infrared spectra (IR) were obtained from a thin film of compound usually solubilised in CH_2Cl_2 and deposited upon a NaCl pellet. They were recorded with Horizon MB 3000 ABB FTIR spectrometer (ABB, Québec, Qc, Canada) and the stronger bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and data reported in ppm. The $CDCl_3$ ^1H and ^{13}C NMR signals (7.26 and 77.0 ppm, respectively), CD_3OD ^1H and ^{13}C NMR signals (3.31 and 49.0 ppm, respectively) or $(CD_3)_2CO$ ^1H and ^{13}C NMR signals (2.05 and 28.9 ppm respectively) were used as internal references. Low-resolution mass spectra (LRMS) were recorded on a Shimadzu prominence apparatus (Kyoto, Japan) equipped with a Shimadzu LCMS-2020. The HPLC purity of a sample

of library members was determined with a Shimadzu apparatus using a Shimadzu SPD-M20A Photodiode array detector, a Alltima HP C18 reversed-phase column (250 mm x 4.6 mm, 5 µm) and a solvent gradient of methanol (15 %) and water (85 %) in which 0.1 % HCl was added. The wave length of the UV detector was selected between 190-205 nm.

General procedure for the synthesis of secondary amines 1, 2, 3, 4, 5, 1', 2', 3', 4' and 5'

To a solution of methoxymethyloxybenzaldehyde [27] (*para* or *meta*) (1.50 mmol) and activated molecular sieves (4Å) (87 mg/mmol of aldehyde) in absolute ethanol (5 mL) was added a primary amine (propylamine, hexylamine, isoamylamine, 2-phenylethylamine or 3-ethoxylpropylamine) (2.26 mmol). The mixture was stirred at room temperature for 2 h and the molecular sieves filtered out with a Buchner, sodium borohydride powder (3.76 mmol) was then added to the reaction solution and the mixture was stirred overnight at room temperature. After filtration, ethanol was evaporated under reduced pressure and the products was purified by flash chromatography (hexanes/EtOAc, 1/1) to give the secondary amine **1**, **2**, **3**, **4**, **5**, **1'**, **2'**, **3'**, **4'** or **5'** with good yields.

***N*-[4-(methoxymethoxy)benzyl]propan-1-amine (1)**

Yellowish oil, yield 98 %. ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H, J = 7.4 Hz), 1.53 (m, 2H), 1.90 (br-s, 1H), 2.58 (t, 2H, J = 7.3 Hz), 3.48 (s, 3H), 3.72 (s, 2H), 5.16 (s, 2H), 6.99 (d, 2H, J = 8.6 Hz), 7.24 (d, 2H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$) δ 11.6, 22.8, 50.9, 53.1, 55.8, 94.4, 116.1 (2x), 129.3 (2x), 133.4, 156.1. LRMS calculated for $C_{12}H_{20}NO_2$ (M+H) 210.14, found 210.25.

***N*-[4-(methoxymethoxy)benzyl]hexan-1-amine (2)**

Yellowish oil, yield 93 %. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.6 Hz), 1.29 (m, 6H), 1.49 (m, 2H), 1.86 (br-s, 1H), 2.60 (t, 2H, J = 7.2 Hz), 3.48 (s, 3H), 3.73 (s, 2H), 5.16 (s, 2H), 6.99 (d, 2H, J = 8.4 Hz), 7.24 (d, 2H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 26.9, 29.8, 31.7, 49.2, 53.3, 55.8, 94.4, 116.1 (2x), 129.2 (2x), 133.7, 156.1. LRMS calculated for $C_{15}H_{26}NO_2$ (M+H) 252.19, found 252.25.

***N*-[4-(methoxymethoxy)benzyl]-3-methylbutan-1-amine (3)**

Yellowish oil, yield 98 %. ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 6H, J = 6.7 Hz), 1.40 (q, 2H, J = 7.1 Hz), 1.62 (m, 2H), 2.63 (t, 2H, J = 7.5 Hz), 3.48 (s, 3H), 3.72 (s, 2H), 5.16 (s, 2H), 6.99 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$) δ 22.4 (2x), 25.8, 38.8, 47.2, 53.2, 55.4, 94.1, 115.8 (2x), 128.9 (2x), 133.6, 155.9. LRMS calculated for $C_{14}H_{24}NO_2$ (M+H) 238.17, found 238.25.

***N*-[4-(methoxymethoxy)benzyl]-2-phenylethanamine (4)**

Brownish oil, yield 96 %. ^{1}H NMR ($CDCl_3$) δ 1.50 (br-s, 1H), 2.85 (m, 2H), 2.92 (m, 2H), 3.48 (s, 3H), 3.75 (s, 2H), 5.17 (s, 2H), 6.99 (d, 2H, J = 8.5 Hz), 7.22 (m, 5H), 7.29 (d, 2H, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$) δ 36.2, 50.3, 53.1, 55.7, 94.3, 116.0 (2x), 126.0, 128.3 (2x), 128.6 (2x), 129.1 (2x), 133.6, 139.9, 156.1. LRMS calculated for $C_{17}H_{22}NO_2$ (M+H) 272.16, found 272.25.

3-ethoxy-*N*-[4-(methoxymethoxy)benzyl]propan-1-amine (5)

Yellowish oil, yield 97 %. ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H, J = 7.0 Hz), 1.76 (m, 2H), 1.88 (br-s, 1H), 2.69 (t, 2H, J = 6.9 Hz), 3.44 (m, 7H), 3.70 (s, 2H), 5.13 (s, 2H), 6.97 (d, 2H, J = 8.6 Hz), 7.21 (d, 2H, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$) δ 14.8, 29.7, 46.4, 53.0, 55.4, 65.8, 68.8, 94.1, 115.8 (2x), 128.9 (2x), 133.5, 155.9. LRMS calculated for $C_{14}H_{24}NO_3$ (M+H) 254.17, found 254.20.

***N*-[3-(methoxymethoxy)benzyl]propan-1-amine (1')**

Yellowish oil, yield 98 %. ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.4 Hz), 1.53 (q, 2H, J = 7.3 Hz), 1.60 (br-s, 1H), 2.60 (t, 2H, J = 7.1 Hz), 3.48 (s, 3H), 3.76 (s, 2H), 5.18 (s, 2H), 6.93 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 6.96 (d, 1H, J = 7.5 Hz), 7.00 (s, 1H), 7.24 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 11.7, 23.21, 51.2, 53.7, 55.8, 94.3, 114.5, 115.8, 121.5, 129.3, 142.2, 157.3. LRMS calculated for $C_{12}H_{20}NO_2$ (M+H) 210.14, found 210.25.

***N*-[3-(methoxymethoxy)benzyl]hexan-1-amine (2')**

Yellowish oil, yield 69 %. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 1.32 (m, 6H), 1.50 (q, 2H, J = 7.5 Hz), 1.65 (br-s, 1H), 2.62 (t, 2H, J = 7.2 Hz), 3.48 (s, 3H), 3.76 (s, 2H), 5.18 (s, 2H), 6.92 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.2 Hz), 6.96 (d, 1H, J = 7.5 Hz), 7.00 (s, 1H), 7.24 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 13.9, 22.5, 26.9, 29.9, 31.7, 49.4,

53.8, 55.8, 94.3, 114.5, 115.8, 121.5, 129.2, 142.1, 157.3. LRMS calculated for $C_{15}H_{26}NO_2$ (M+H) 252.19, found 252.25.

***N*-[3-(methoxymethoxy)benzyl]-3-methylbutan-1-amine (3')**

Yellowish oil, yield 98 %. ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 6H, J = 6.6 Hz), 1.40 (q, 2H, J = 7.0 Hz), 1.53 (br-s, 1H), 1.63 (m, 1H), 2.64 (t, 2H, J = 7.6 Hz), 3.48 (s, 3H), 3.76 (s, 2H), 5.18 (s, 2H), 6.92 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.2 Hz), 6.96 (d, 1H, J = 7.6 Hz), 7.00 (s, 1H), 7.24 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 22.7 (2x), 26.1, 39.2, 47.6, 54.0, 56.0, 94.4, 114.6, 115.9, 121.6, 129.4, 142.29, 157.3. LRMS calculated for $C_{14}H_{24}NO_2$ (M+H) 238.17, found 238.20.

***N*-[3-(methoxymethoxy)benzyl]-2-phenylethanamine (4')**

Brownish oil, yield 97 %. ^{1}H NMR ($CDCl_3$) δ 1.60 (br-s, 1H), 2.86 (m, 2H), 2.92 (m, 2H), 3.48 (s, 3H), 3.79 (s, 2H), 5.17 (s, 2H), 6.93 (d, 2H, J = 7.87 Hz), 6.97 (s, 1H), 7.25 (m, 4H), 7.31 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 36.2, 50.3, 53.5, 55.8, 94.3, 114.6, 115.7, 121.4, 126.0, 128.3 (2x), 128.6 (2x), 129.2, 139.9, 141.9, 157.2. LRMS calculated for $C_{17}H_{22}NO_2$ (M+H) 272.16, found 272.20.

3-ethoxy-*N*-[3-(methoxymethoxy)benzyl]propan-1-amine (5')

Yellowish oil, yield 76 %. ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3H, J = 7.0 Hz), 1.79 (t, 2H, J = 6.6 Hz), 2.72 (t, 2H, J = 6.9 Hz), 3.47 (m, 8H), 3.76 (s, 2H), 5.17 (s, 2H), 6.92 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 6.96 (d, 1H, J = 7.6 Hz), 7.00 (d, 1H, J = 1.6 Hz), 7.23 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 15.0, 29.9, 46.6, 53.6, 55.6, 65.9, 68.9, 94.1, 114.3, 115.6, 121.3, 129.1, 142.0, 157.2. LRMS calculated for $C_{14}H_{24}NO_3$ (M+H) 254.17, found 254.20.

General procedure for the synthesis of epoxides 6, 7, 8, 9, 10, 6', 7', 8', 9' and 10'

To a solution of the secondary amine **1**, **2**, **3**, **4**, **5**, **1'**, **2'**, **3'**, **4'** or **5'** (20 mmol) and potassium carbonate (30 mmol) in acetone (50 mL) stirred at 40 °C was added 4-bromo-1,2-epoxybutane [28] (30 mmol) and the mixture was stirred for 48 h at 50 °C. The solution was filtered over a Büchner and rinsed with CH_2Cl_2, then the collected filtrate was evaporated under reduced pressure and purified by flash chromatography (hexanes/EtOAc/triethylamine; 90/9/1) to yield the epoxide **6**, **7**, **8**, **9**, **10**, **6'**, **7'**, **8'**, **9'** or **10'**.

***N*-[4-(methoxymethoxy)benzyl]-*N*-[2-(oxiran-2-yl)ethyl]propan-1-amine (6)**

Yellowish oil, yield 32 %. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7.4 Hz), 1.49 (q_{app}, 2H, J = 7.4 Hz), 1.68 (m, 2H), 2.38 (t_{app}, 2H, J = 7.1 Hz), 2.46 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.59 (t_{app}, 2H, J = 6.9 Hz), 2.73 (t, 1H, J = 4.5 Hz), 2.95 (m, 1H), 3.47 (s, 3H), 3.52 (d, 2H, J = 4.8 Hz), 5.16 (s, 2H), 6.97 (d, 2H, J = 8.6 Hz), 7.23 (d, 2H, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$) δ 11.8, 20.2, 30.5, 47.2, 50.3, 51.2, 55.7, 56.0, 57.9, 94.5, 116.0 (2x), 129.8 (2x), 133.2, 156.1.

***N*-[4-(methoxymethoxy)benzyl]-*N*-[2-(oxiran-2-yl)ethyl]hexan-1-amine (7)**

Yellowish oil, yield 51 %. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.28 (m, 6H), 1.47 (m, 2H), 1.68 (m, 2H), 2.41 (m, 2H), 2.46 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.59 (t_{app}, 2H, J = 6.9 Hz), 2.73 (t, 1H, J = 4.5 Hz), 2.95 (m, 1H), 3.49 (s, 3H), 3.51 (d, 2H, J = 4.8 Hz), 5.17 (s, 2H), 6.98 (d, 2H, J = 8.5 Hz), 7.23 (d, 2H, J = 8.3 Hz). ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 26.8, 27.0, 30.3, 31.7, 47.1, 50.2, 51.1, 53.6, 56.0, 57.8, 94.4, 115.8 (2x), 129.7 (2x), 133.1, 156.0.

***N*-[4-(methoxymethoxy)benzyl]-3-methyl-*N*-[2-(oxiran-2-yl)ethyl]butan-1-amine (8)**

Yellowish oil, yield 60 %. ^{1}H NMR ($CDCl_3$) δ 0.85 (dd, 6H, J_1 = 1.0 Hz, J_2 = 6.6 Hz), 1.36 (q, 2H, J = 7.2 Hz), 1.56 (m, 1H), 1.67 (m, 2H), 2.43 (m, 3H), 2.57 (t, 2H, J = 7.2 Hz), 2.73 (q_{app}, 1H, J = 4.2 Hz), 2.94 (dd, 1H, J_1 = 2.8 Hz, J_2 = 3.8 Hz), 3.48 (s, 3H), 3.50 (d, 2H, J = 3.2 Hz), 5.16 (s, 2H), 6.97 (d, 2H, J = 8.6 Hz), 7.22 (d, 2H, J = 8.6 Hz). ^{13}C NMR (CDCl3) δ 22.7 (2x), 25.7, 26.5, 30.3, 35.7, 47.0, 50.1, 51.6, 56.3, 57.8, 94.4, 115.8 (2x), 129.1, 133.0, 156.0.

***N*-[4-(methoxymethoxy)benzyl]-2-(oxiran-2-yl)-*N*-(2-phenylethyl)ethanamine (9)**

Yellowish oil, yield 57 %. ^{1}H NMR ($CDCl_3$) δ 1.68 (m, 2H), 2.43 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.72 (m, 5H), 2.85 (m, 2H), 2.91 (m, 1H), 3.50 (s, 3H), 3.61 (d, 2H, J = 8.2 Hz), 5.18 (s, 2H), 6.98 (d, 2H, J = 8.6 Hz), 7.15 (d, 2H, J = 8.2 Hz), 7.22 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 30.4, 33.4, 47.1, 50.2, 51.0, 55.4, 55.9, 57.7, 94.4, 115.8 (2x), 125.8, 128.2 (2x), 128.7 (2x), 129.7 (2x), 132.8, 140.5, 156.1.

3-ethoxy-*N*-[4-(methoxymethoxy)benzyl]-*N*-[2-(oxiran-2-yl)ethyl]propan-1-amine (10)

Yellowish oil, yield 51 %. ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, J = 7.0 Hz), 1.67 (m, 2H), 1.73 (m, 2H), 2.44 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.50 (m, 2H), 2.57 (t, 1H, J = 7.1

Hz), 2.71 (t_{app}, 2H, J = 4.5 Hz), 2.93 (m, 1H), 3.41 (m, 4H), 3.47 (s, 3H), 3.50 (d, 2H, J = 2.8 Hz), 5.15 (s, 2H), 6.96 (d, 2H, J = 8.6 Hz), 7.21 (d, 2H, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$) δ 15.1, 27.3, 30.4, 47.0, 50.3 (2x), 51.0, 55.8, 57.8, 66.0, 68.6, 94.4, 115.8 (2x), 129.7 (2x), 132.9, 156.1.

_N_-[3-(methoxymethoxy)benzyl]-_N_-[2-(oxiran-2-yl)ethyl]propan-1-amine (6')

Yellowish oil, yield 64 %. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 7.4 Hz), 1.51 (q_{app}, 2H, J = 6.9 Hz), 1.69 (m, 2H), 2.42 (m, 2H), 2.47 (m, 1H), 2.65 (m, 2H), 2.75 (t, 1H, J = 4.5 Hz), 2.97 (m, 1H), 3.48 (s, 3H), 3.56 (d, 2H, J = 8.0 Hz), 5.18 (s, 2H), 6.92 (d, 1H, J = 8.0 Hz), 6.98 (d, 1H, J = 7.4 Hz), 7.05 (s, 1H), 7.22 (t_{app}, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 11.8, 20.2, 30.5, 47.2, 50.5, 51.2, 55.9 (2x), 58.5, 94.4, 114.5, 116.4, 122.1, 129.1, 141.9, 157.3.

_N_-[3-(methoxymethoxy)benzyl]-_N_-[2-(oxiran-2-yl)ethyl]hexan-1-amine (7')

Yellowish oil, yield 71 %. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.28 (m, 6H), 1.50 (m, 2H), 1.69 (m, 2H), 2.43 (m, 3H), 2.68 (m, 2H), 2.75 (m, 1H), 2.97 (m, 1H), 3.48 (s, 3H), 3.55 (d, 2H, J = 3.6 Hz), 5.18 (s, 2H), 6.92 (d, 1H, J = 7.8 Hz), 6.98 (d, 1H, J = 7.0 Hz), 7.04 (s, 1H), 7.22 (t_{app}, 1H, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 27.0, 27.1, 30.5, 31.8, 47.2, 50.5, 51.2, 53.9, 56.0, 58.5, 94.4, 114.5, 116.4, 122.1, 129.1, 141.9, 157.3.

_N_-[3-(methoxymethoxy)benzyl]-3-methyl-_N_-[2-(oxiran-2-yl)ethyl]butan-1-amine (8')

Yellowish oil, yield 93 %. ^{1}H NMR ($CDCl_3$) δ 0.85 (dd, 6H, J_1 = 1.5 Hz, J_2 = 6.6 Hz), 1.37 (q, 2H, J = 7.3 Hz), 1.59 (m, 1H), 1.69 (m, 2H), 2.45 (m, 3H), 2.57 (t, 2H, J = 6.6 Hz), 2.74 (t_{app}, 1H, J = 4.5 Hz), 2.97 (m, 1H), 3.48 (s, 3H), 3.54 (d, 2H, J = 7.0 Hz), 5.17 (s, 2H), 6.91 (d, 1H, J = 1.9 Hz), 6.96 (s, 1H), 7.03 (s, 1H), 7.21 (t_{app}, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 22.8 (2x), 25.7, 26.6, 30.5, 36.0, 47.1, 50.5, 51.1, 56.3, 58.5, 94.4, 114.6, 116.5, 122.2, 129.1, 141.7, 157.3.

_N_-[3-(methoxymethoxy)benzyl]-2-(oxiran-2-yl)-_N_-(2-phenylethyl)ethanamine (9')

Yellowish oil, yield 61 %. ^{1}H NMR ($CDCl_3$) δ 1.69 (q, 2H, J = 6.5 Hz), 2.43 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.75 (m, 7H), 2.93 (dd, 1H, J_1 = 2.8 Hz, J_2 = 3.8 Hz), 3.49 (s, 3H), 3.66 (q_{app}, 2H, J = 12.1 Hz), 5.18 (s, 2H), 6.95 (m, 2H), 7.04 (s, 1H), 7.22 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 30.5, 33.5, 47.1, 50.4, 51.0, 55.6, 56.0, 58.4, 94.3, 114.6, 116.2, 122.0, 125.8, 128.2 (2x), 128.7 (2x), 129.1, 140.5, 141.4, 157.2.

3-ethoxy-*N*-[3-(methoxymethoxy)benzyl]-*N*-[2-(oxiran-2-yl)ethyl]propan-1-amine (10')

Yellowish oil, yield 73 %. ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H, J = 7.0 Hz), 1.69 (q, 2H, J = 6.7 Hz,), 1.75 (m, 2H), 2.45 (dd, 1H, J_1 = 2.7 Hz, J_2 = 5.0 Hz), 2.57 (m, 4H), 2.73 (m, 1H), 2.96 (m, 1H), 2.43 (m, 3H), 3.47 (s, 4H), 3.54 (d, 2H, J = 6.0 Hz), 5.17 (s, 2H), 6.93 (m, 2H), 7.02 (s, 1H), 7.21 (t, 1H, J = 7.8 Hz). ^{13}C NMR ($CDCl_3$) δ 15.2, 27.5, 30.5, 47.2, 50.6 (2x), 51.1, 56.0, 58.6, 66.1, 68.7, 94.4, 114.5, 116.4, 122.1, 129.1, 141.7, 157.3.

General procedures for the epoxide opening

a) Aniline derivatives: The epoxide **6**, **7**, **8**, **9** or **10** (0.2 mmol) and magnesium perchlorate (0.4 mmol) were dissolved in acetonitrile (1 mL). The mixture was stirred at 55 °C and, after the complete dissolution of magnesium perchlorate, the aniline derivative **A** or **B** (0.2 mmol) was added and continuously stirred at 55 °C for 20 h. Acetonitrile was then evaporated under vacuum, water added and the mixture extracted with CH_2Cl_2 over a 15 mL Telos phase separator (Kinesis Scientific Experts, Malta, NY, USA). After evaporation of the CH_2Cl_2, the protecting group of the oily product obtained was hydrolyzed following the general procedure described below.

b) Amines: The epoxide **6**, **7**, **8**, **9**, **10**, **6'**, **7'**, **8'**, **9'** or **10'** (0.2 mmol) in ethanol (1 mL) and the appropriate amine **C**, **D**, **E**, **F**, **G**, **H**, **I**, **J**, **P**, **Q**, **R**, **S** or **T** in ethanol (1 mL) were stirred at 75 °C. Ater 7 h, the ethanol was evaporated under vacuum. Methyl isocyanate polystyrene resin (0.6 mmol) and THF (2 mL) were added to the mixture and stirred for 5 h at 30 °C to scavenge the excess of amine. The resin was filtered under vacuum, the THF evaporated and replaced with ethanol and the protecting group of the oily product obtained was hydrolyzed following the general procedure described below.

c) Phenol derivatives: To a solution of the appropriate phenol **K**, **L**, **M**, **N** or **O** (0.4 mmol) in DMF (0.5 mL) was added a solution of the epoxide **6**, **7**, **8**, **9** or **10** (0.2 mmol) in DMF (1.5 mL) and potassium carbonate (0.6 mmol). The mixture was stirred at 106 °C for 26 h and then cooled to room temperature. A saturated solution of sodium bicarbonate (2 mL) was carefully added and the mixture extracted with CH_2Cl_2. After the solvent was evaporated under vacuum, the protecting group of the oily product obtained was hydrolyzed following the method described below.

d) Thiophenol derivatives: To a solution of the appropriate epoxide **6**, **7**, **8**, **9** or **10** (0.4 mmol) in methanol (1 mL) was added the appropriate thiophenol **U**, **V**, **W**, **X** or **Y** (0.4 mmol) in methanol (1 mL). The reaction mixture was stirred at 65 °C for 26 h and methanol was evaporated. The protecting group of the oily product obtained was hydrolyzed following the general procedure described below.

General procedure for the hydrolysis of the MOM protecting group

The compound obtained from the epoxide opening was dissolved in ethanol (2 mL), a methanolic solution of HCl (20 %) (1 mL) was added, and the mixture was stirred at 60 °C for 5 h. Ethanol and methanol were then evaporated under vacuum, water (2 mL) was added and the resulting solution treated with a saturated aqueous solution of sodium bicarbonate (6 mL). CH_2Cl_2 was used to extract the product over a 15 mL Telos phase separator (Kinesis Scientific Experts, Malta, NY, USA) and the final compound was recovered after evaporation of the solvent under vacuum.

^{1}H NMR and LRMS data for all members (150) of libraries L1 and L2 are reported in Supportive Material, but the full characterization (IR, ^{1}H NMR, ^{13}C NMR and LRMS) of a sampling of 10 representative compounds from each library (L1 and L2) are reported below.

Library L1 sampling (compounds 6A, 7G, 8M, 9I and 10O)

4-[{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butyl}(methyl) amino]benzamide (6A)

Yellowish powder. IR (film) ν 3340 and 3209 (OH and NH_2), 2955, 2831, 1651 (CON, amide), 1605, 1520, 1450, 1381, 1265, 1119, 833, 771, 733. ^{1}H NMR (CD_3OD) δ 0.88 (t, $\underline{CH_3}CH_2$, J = 7.4 Hz), 1.56 (m, $CH_3\underline{CH_2}$), 1.67 (m, $CH_2\underline{CH_2}CHOH$), 2.39, 2.45, 2.63 and 2.75 (4m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.07 (s, NCH_3), 3.43 (m, $HOCH\underline{CH_2}N$ and 1H of $Ar\underline{CH_2}$), 3.63 (d of AB system, 1H of $Ar\underline{CH_2}$, J = 12.6 Hz), 3.97 (m, $\underline{CH}OH$), 6.74 (m, 4xCH of phenol), 7.13 (d, 2xCH of benzamide, J = 8.3 Hz), 7.75 (d, 2xCH of benzamide, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$) δ 12.1 (CH_3), 20.7 (CH_2), 31.6 (CH_2), 40.0 (CH_3), 52.5 (CH_2), 56.7 (CH_2), 58.9 (CH_2), 59.3 (CH_2), 70.9 (CH), 111.9 (2xCH), 116.1 (2xCH), 120.8 (C), 129.9 (C), 130.4 (2xCH), 131.8 (2xCH), 153.6(C), 157.8 (C), 172.8 (C=O). LRMS calculated for $C_{22}H_{32}N_3O_3$ (M+H) 386.24, found 386.25.

4-{[hexyl(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl)amino}butyl) amino]methyl}phenol (7G)

Yellowish oil. IR (film) ν 3202 (OH), 2932, 1612, 1497, 1458, 1366, 1265, 1095, 1026, 825. ^{1}H NMR (CD_3OD) δ 0.90 (t, $\underline{CH_3}CH_2$, J = 6.9 Hz), 1.34 (m, $CH_3\underline{CH_2}\underline{CH_2}\underline{CH_2}$), 1.61 (m, $\underline{CH_2}CH_2NCH_2\underline{CH_2}CHOH$), 2.22 (s, NCH_3), 2.49 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2CHOH\underline{CH_2}$), 3.40 and 3.58 (2d of AB system, $Ar\underline{CH_2}$, J = 13.0 Hz), 3.49 (s, $CH_3N\underline{CH_2}$), 3.81 (m, $HO\underline{CH}$), 3.91 (s, OCH_3), 6.75 (d, 2xCH of phenol, J = 8.5 Hz), 6.79 (d, CH of meta pyridinyl, J = 8.6 Hz), 7.13 (d, 2xCH of phenol, J = 8.5 Hz), 7.68 (m, CH of meta pyridinyl), 8.03 (d, CH of meta pyridinyl, J = 2.1 Hz). ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 27.4 (CH_2), 28.2 (CH_2), 32.0 (CH_2), 32.8 (CH_2), 42.9 (CH_3), 52.3 (CH_2), 54.1 (CH_3), 54.5 (CH_2), 58.9 (CH_2), 60.1 (CH_2), 64.0 (CH_2), 70.3 (CH), 111.3 (CH), 116.1 (2xCH), 128.3 (C), 129.9 (C), 131.8 (2xCH), 141.8 (CH), 148.1 (CH), 157.8 (C), 165.1 (C). LRMS calculated for $C_{25}H_{40}N_3O_3$ (M+H) 430.30, found 430.30.

4-({[3-hydroxy-4-(pyridin-3-yloxy)butyl](3-methylbutyl)amino}methyl)phenol (8M)

Yellowish oil. IR (film) ν 3171 (OH), 2955, 2870, 1690, 1582, 1512, 1466, 1373, 1273, 1242, 1103, 1057, 833, 802. ^{1}H NMR (CD_3OD) δ 0.89 (dd, $(\underline{CH_3})_2CH$, J_1 = 5.0 Hz, J_2 = 6.5 Hz), 1.44 (q, $CH\underline{CH_2}CH_2N$, J = 7.2 Hz), 1.57 (m, $(CH_3)_2\underline{CH}$), 1.81 (q, $HOCH\underline{CH_2}CH_2N$, J = 6.2 Hz), 2.60 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.44 and 3.64 (2d of AB system, $Ar\underline{CH_2}$, J = 13.0 Hz), 3.96 (m, OCH_2), 4.07 (m, $HO\underline{CH}$), 6.74 (d, 2xCH of phenol, J = 8.5 Hz), 7.15 (d, 2xCH of phenol, J = 8.4 Hz), 7.39 (m, 2xCH of meta pyridinyl), 8.21 (m, 2xCH of meta pyridinyl). ^{13}C NMR (CD_3OD) δ 23.0 (CH_3), 23.1 (CH_3), 27.6 (CH), 30.0 (CH_2), 36.4 (CH_2), 52.0 (CH_2), 52.7 (CH_2), 58.9 (CH_2), 71.0 (CH), 73.2 (CH_2), 116.1 (2xCH), 116.3 (CH), 123.2 (CH), 129.6 (C), 131.9 (2xCH), 134.5 (C), 138.5 (CH), 142.2 (CH), 157.9 (C). LRMS calculated for $C_{21}H_{31}N_2O_3$ (M+H) 359.23, found 359.25.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(2-phenyl ethyl)amino] methyl}phenol (9I)

Yellowish oil. IR (film) ν 3202 (OH), 3024, 2939, 2816, 1666, 1605, 1512, 1450, 1366, 1250, 1103, 1026, 980, 833, 795, 702. ^{1}H NMR (CD_3OD) δ 1.61 and 1.76 (2m, $HOCH\underline{CH_2}CH_2N$), 2.27 (s, NCH_3), 2.44 (m, $Ph\underline{CH_2}$), 2.74 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2CHOH\underline{CH_2}$), 3.53 and 3.65 (2d of AB system, $Ar\underline{CH_2}$, J = 13.1 Hz), 3.70 (s, $CH_3N\underline{CH_2}$), 3.78 (m, $HO\underline{CH}$), 6.75 (m, 2xCH of phenol and 1xCH of thiophenyl), 6.94 (d, 1xCH of thiophenyl, J = 3.7 Hz), 7.16 (m, 2xCH of phenol and

3xCH of phenyl), 7.26 (m, 2xCH of phenyl). ^{13}C NMR (CD_3OD) δ 32.2 (CH_2), 33.8 (CH_2), 43.0 (CH), 52.0 (CH_2), 56.3 (CH_2), 58.0 (CH_2), 58.9 (CH_2), 63.5 (CH_2), 70.1 (CH), 112.2 (C), 116.1 (2xCH), 127.0 (CH), 127.8 (CH), 129.4 (2xCH), 129.7 (2xCH), 130.1 (C), 130.6 (CH), 131.8 (2xCH), 141.5 (C), 145.6 (C), 157.7 (C). LRMS calculated for $C_{25}H_{32}BrN_2O_2S$ (M+H) 503.13, found 503.15.

3-{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (10O)

Yellowish oil. IR (film) ν 3209 (OH and NH_2), 2939, 2870, 1666 (CON, amide), 1589, 1512, 1450, 1381, 1288, 1242, 1103, 1049, 833, 756. ^{1}H NMR (CD_3OD) δ 1.15 (t, $\underline{CH_3}CH_2$, J = 7.0 Hz), 1.80 (m, $\underline{CH_2}CH_2NCH_2\underline{CH_2}CHOH$), 2.64 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.45 (m, 1H of Ar$\underline{CH_2}$ and CH_2OCH_2), 3.64 (d of AB system, 1H of Ar$\underline{CH_2}$, J = 13.1 Hz), 3.91 (m, O$\underline{CH_2}$CHOH), 4.04 (m, HO$\underline{CH}$), 6.75 (m, 2xCH of phenol), 7.14 (m, 2xCH of phenol and 1xCH of benzamide), 7.46 (m, 3xCH of benzamide). ^{13}C NMR ($CDCl_3$) δ 15.4 (CH_3), 27.8 (CH_2), 30.4 (CH_2), 51.5 (CH_2), 52.1 (CH_2), 58.9 (CH_2), 67.2 (CH_2), 69.7 (CH_2), 70.9 (CH), 73.0 (CH_2), 114.6 (CH), 116.1 (2xCH), 119.4 (CH), 121.0 (CH), 130.7 (CH), 131.8 (2xCH), 129.9 (C), 136.3 (C), 157.8 (C), 160.4 (C), 172.2 (C=O). LRMS calculated for $C_{23}H_{33}N_2O_5$ (M+H) 417.23, found 417.25.

<u>Library L2 sampling (compounds 6T, 8U, 9X, 7'S and 10'P</u>)

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](propyl)amino}methyl)phenol (6T)

Yellowish oil. IR (film) ν 3194 and 3032 (OH and NH_2), 2939, 2816, 1612, 1512, 1458, 1358, 1265, 1157, 1095, 1011, 825, 741, 702. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, $\underline{CH_3}CH_2$, J = 7.4 Hz), 1.54 (m, $\underline{CH_2}CH_2NCH_2\underline{CH_2}CHOH$), 2.20-2.70 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$ and 4xCH_2 of piperazinyl), 3.35 and 3.55 (2d of AB system, Ar$\underline{CH_2}$, J = 13.3 Hz), 3.46 (s, N$\underline{CH_2}$CHOH), 3.75 (m, HO$\underline{CH}$), 6.78 (d, 2xCH of phenol, J = 8.5 Hz), 7.14 (d, 2xCH of phenol, J = 8.4 Hz), 7.22 (m, 1xCH of phenyl), 7.29 (m, 4xCH of phenyl). ^{13}C NMR (CD_3OD) δ 12.2 (CH_3), 20.7 (CH_2), 32.4 (CH_2), 52.2 (2xCH_2), 53.7 (2xCH_2), 54.3 (CH_2), 56.6 (CH_2), 58.9 (CH_2), 64.0 (CH_2), 65.6 (CH_2), 69.8 (CH), 116.1 (2xCH), 128.4 (CH), 129.3 (2xCH), 130.1 (C), 130.8 (2xCH), 131.8 (2xCH), 138.2 (C), 157.9 (C). LRMS calculated for $C_{25}H_{38}N_3O_2$ (M+H) 412.29, found 412.30.

4-({[3-hydroxy-4-(phenylsulfanyl)butyl](3-methylbutyl)amino}methyl)phenol (8U)

Yellowish oil. IR (film) ν 3202 (OH), 2955, 2862, 2831, 1612, 1512, 1466, 1373, 1242, 1173, 1088, 833, 694. ^{1}H NMR (CD_3OD) δ 0.86 (dd, $(\underline{CH_3})_2CH$, J_1 = 2.9 Hz, J_2 = 6.6 Hz), 1.39 (q, $CH\underline{CH_2}CH_2N$, J = 7.3 Hz), 1.53 (m, $(CH_3)_2\underline{CH}$), 1.69 (m, 1H of $HOCH\underline{CH_2}CH_2N$), 1.88 (m, 1H of $HOCH\underline{CH_2}CH_2N$), 2.34-2.71 (4m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 2.96 (m, $HOCH\underline{CH_2}S$), 3.38 and 3.57 (2d of AB system, $Ar\underline{CH_2}$, J = 12.9 Hz), 3.78 (m, $HO\underline{CH}$), 6.74 (d, 2xCH of phenol, J = 8.5 Hz), 7.11 (d, 2xCH of phenol, J = 8.5 Hz), 7.20 (m, CH of thiophenyl), 7.32 (m, 4H of thiophenyl). ^{13}C NMR (CD_3OD) δ 23.0 (CH_3), 23.1 (CH_3), 27.6 (CH), 32.3 (CH_2), 36.5 (CH_2), 41.3 (CH_2), 52.2 (CH_2), 52.6 (CH_2), 58.8 (CH_2), 71.8 (CH), 116.1 (2xCH), 127.0 (CH), 129.9 (C), 130.0 (2x CH), 130.3 (2xCH), 131.8 (2xCH), 137.9 (C), 157.8 (C). LRMS calculated for $C_{22}H_{32}NO_2S$ (M+H) 374.23, found 374.25.

4-{[{3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}(2-phenylethyl)amino]methyl} phenol (9X)

Yellowish oil. IR (film) ν 3202 (OH), 2939, 2831, 1597, 1497, 1458, 1366, 1242, 1173, 1103, 1034, 825, 741, 702. ^{1}H NMR (CD_3OD) δ 1.65 and 1.86 (2m, $HOCH\underline{CH_2}CH_2N$), 2.74 (m, $\underline{CH_2}N\underline{CH_2}CH_2CHOH\underline{CH_2}S$ and $Ph\underline{CH_2}$), 3.47 and 3.61 (2d of AB system, $Ar\underline{CH_2}$, J = 13.0 Hz), 3.72 (m, $HO\underline{CH}$), 3.77 (s, OCH_3), 6.75 (d, 2xCH of phenol, J = 8.5 Hz), 6.87 (d, 2xCH of thiophenol, J = 8.8 Hz), 7.13 (m, 2xCH of phenol and 3xCH of phenyl), 7.23 (d, 2xCH of phenyl, J = 7.5 Hz), 7.35 (d, 2xCH of thiophenol, J = 8.8 Hz).). ^{13}C NMR (CD_3OD) δ 32.6 (CH_2), 33.8 (CH_2), 43.5 (CH_2), 52.0 (CH_2), 55.8 (CH), 56.3 (CH_2), 58.8 (CH_2), 71.4 (CH), 115.7 (2xCH), 116.1 (2xCH), 127.0 (CH), 127.7 (C), 129.4 (2xCH), 129.7 (2xCH), 130.1 (C), 131.7 (2xCH), 134.3 (2xCH), 141.4 (C), 157.8 (C), 160.5 (C). LRMS calculated for $C_{26}H_{32}NO_3S$ (M+H) 438.20, found 438.20.

3-({hexyl[3-hydroxy-4-(morpholin-4-yl)butyl]amino}methyl)phenol (7'S)

Yellowish oil. IR (film) ν 3240 (OH), 2932, 2854, 2816, 1597, 1458, 1373, 1281, 1119, 864, 787. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, $\underline{CH_3}CH_2$, J = 6.9 Hz), 1.29 (m, $CH_3\underline{CH_2}\underline{CH_2}\underline{CH_2}$), 1.50 (m, $\underline{CH_2}CH_2NCH_2$), 1.60 (m, $CH_2NCH_2\underline{CH_2}CHOH$), 2.25 (m, $N\underline{CH_2}CHOH$), 2.30-2.70 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$ and $2xCH_2N$ of morpholinyl), 3.37 (d of AB system, 1H of $Ar\underline{CH_2}$, J = 13.5 Hz), 3.57 (m, 1H of $Ar\underline{CH_2}$ and $2xCH_2O$ of morpholinyl), 3.80 (m, $HO\underline{CH}$), 6.71 (dd, CH of phenol, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, CH of phenol, J = 7.5 Hz), 6.84 (d, CH of phenol, J = 1.6 Hz), 7.12 (t, CH of phenol,

J = 7.8 Hz). ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 27.7 (CH_2), 28.2 (CH_2), 32.5 (CH_2), 32.9 (CH_2), 52.6 (CH_2), 54.9 (CH_2), 55.3 (2xCH_2), 59.7 (CH_2), 66.1 (CH_2), 67.8 (2xCH_2), 69.4 (CH), 115.1 (CH), 117.2 (CH), 121.6 (CH), 130.3 (CH), 141.4 (C), 158.6 (C). LRMS calculated for $C_{21}H_{37}N_2O_3$ (M+H) 365.27, found 365.25.

3-({(3-ethoxypropyl)[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl]amino}methyl) phenol (10'P)

Yellowish oil. IR (film) ν 3194 (OH), 2939, 2816, 1597, 1458, 1373, 1288, 1157, 1119, 1011, 872, 787, 741, 694. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, $\underline{CH_3}CH_2$, J = 7.0 Hz), 1.57 (m, $CH_2\underline{CH_2}CH_2N$), 1.71 (m, $NCH_2\underline{CH_2}CHOH$), 2.17 (s, NCH_3), 2.24 (m, $N\underline{CH_2}CHOH$), 2.20-2.68 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$ and 4xNCH_2 of piperazinyl), 3.43 (m, 1H of Ar$\underline{CH_2}$ and CH_2OCH_2), 3.57 (d of AB system, 1H of Ar$\underline{CH_2}$, J = 13.1 Hz), 3.76 (m, HO$\underline{CH}$), 6.70 (dd, CH of phenol, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, CH of phenol, J = 7.5 Hz), 6.84 (s, CH of phenol), 7.11 (t, CH of phenol, J = 7.8 Hz). ^{13}C NMR (CD_3OD) δ 15.5 (CH_3), 28.1 (CH_2), 32.8 (CH_2), 46.0 (CH_3), 51.8 (CH_2), 52.5 (CH_2), 54.2 (CH_2), 55.7 (2xCH_2), 59.8 (CH_2), 65.5 (CH_2), 67.2 (CH_2), 69.3 (CH), 69.8 (2xCH_2), 115.1 (CH), 117.1 (CH), 121.5 (CH), 130.3 (CH), 141.6 (C), 158.6 (C). LRMS calculated for $C_{21}H_{38}N_3O_3$ (M+H) 380.28, found 380.25.

CONFLICT OF INTEREST

The authors confirm that this article content is not subjected to any conflict of interest.

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Supportive Material

Development of a simple and efficient solution-phase parallel synthesis of flexible non steroidal estradiol mimics

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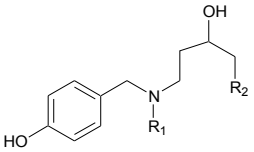
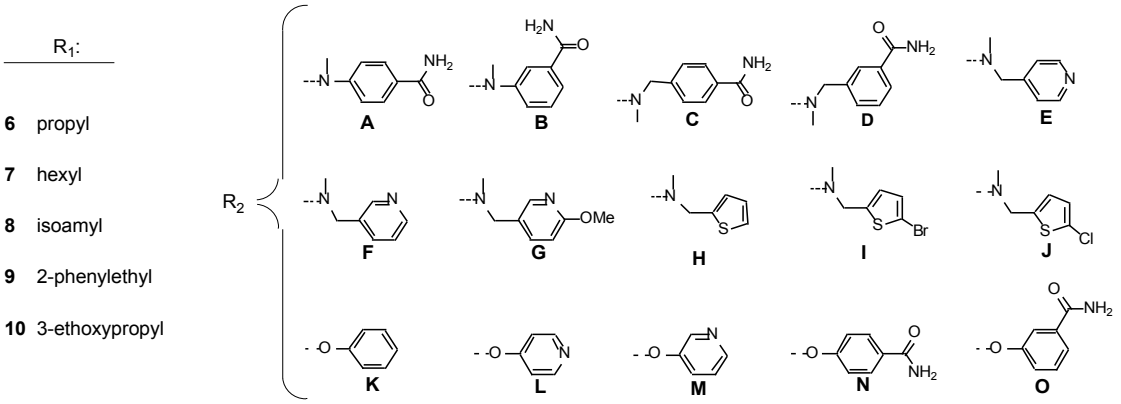
^{1}H NMR (400 MHz) and LRMS data for all members of libraries L1 and L2 (150 compounds)

Library L1 (75 members)



6A, **6B**, **6C**, **6D**, **6E**, **6F**, **6G**, **6H**, **6I**, **6J**, **6K**, **6L**, **6M**, **6N**, **6O**,
7A, **7B**, **7C**, **7D**, **7E**, **7F**, **7G**, **7H**, **7I**, **7J**, **7K**, **7L**, **7M**, **7N**, **7O**,
8A, **8B**, **8C**, **8D**, **8E**, **8F**, **8G**, **8H**, **8I**, **8J**, **8K**, **8L**, **8M**, **8N**, **8O**,
9A, **9B**, **9C**, **9D**, **9E**, **9F**, **9G**, **9H**, **9I**, **9J**, **9K**, **9L**, **9M**, **9N**, **9O**,
10A, **10B**, **10C**, **10D**, **10E**, **10F**, **10G**, **10H**, **10I**, **10J**,
10K, **10L**, **10M**, **10N**, **10O**,



4-[{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butyl}(methyl)amino] benzamide (6A)

Yellowish powder. IR (film) ν 3340 and 3209 (OH and NH_2), 2955, 2831, 1651 (CON, amide), 1605, 1520, 1450, 1381, 1265, 1119, 833, 771, 733. ^{1}H NMR (CD_3OD) δ 0.88 (t, $\underline{CH_3}CH_2$, J = 7.4 Hz), 1.56 (m, $CH_3\underline{CH_2}$), 1.67 (m, $CH_2\underline{CH_2}CHOH$), 2.39, 2.45, 2.63 and 2.75 (4m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.07 (s, NCH_3), 3.43 (m, $HOCH\underline{CH_2}N$ and 1H of $Ar\underline{CH_2}$), 3.63 (d of AB system, 1H of $Ar\underline{CH_2}$, J = 12.6 Hz), 3.97 (m, $\underline{CH}OH$), 6.74 (m, 4xCH of phenol), 7.13 (d, 2xCH of benzamide, J = 8.3 Hz), 7.75 (d, 2xCH of benzamide, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$) δ 12.1 (CH_3), 20.7 (CH_2), 31.6 (CH_2), 40.0 (CH_3), 52.5 (CH_2), 56.7 (CH_2), 58.9 (CH_2), 59.3 (CH_2), 70.9 (CH), 111.9 (2xCH), 116.1 (2xCH), 120.8 (C), 129.9 (C), 130.4 (2xCH), 131.8 (2xCH), 153.6(C), 157.8 (C), 172.8 (C=O). LRMS calculated for $C_{22}H_{32}N_3O_3$ (M+H) 386.24, found 386.25.

3-[{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butyl}(methyl)amino] benzamide (6B)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 7.3 Hz), 1.59 (m, 2H), 1.73 (m, 2H), 2.55, 2.67, 2.79, 2.91 (4m, 4H), 3.03 (s, 3H), 3.37 (m, 2H), 3.62 (m, 1H), 3.76 (d, 1H, J = 11.7 Hz), 3.95 (m, 1H), 6.76 (d, 2H, J = 8.5 Hz), 7.02 (m, 1H), 7.17 (d, 2H, J = 8.5 Hz), 7.30 (m, 2H), 7.38 (s, 1H). LRMS calculated for $C_{22}H_{32}N_3O_3$ (M+H) 386.24, found 386.25.

4-{[{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butyl}(methyl)amino] methyl}benzamide (6C)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7.4 Hz), 1.62 (m, 4H), 2.24 (s, 3H), 2.39 (m, 4H), 2.57 and 2.67 (2m, 2H), 3.42 (d, 1H, J = 13.0 Hz), 3.62 (m, 3H), 3.80 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 8.2 Hz), 7.85 (d, 2H, J = 8.3 Hz). LRMS calculated for $C_{23}H_{34}N_3O_3$ (M+H) 400.25, found 400.25.

3-{[{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butyl}(methyl)amino] methyl}benzamide (6D)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7.4 Hz), 1.62 (m, 4H), 2.23 (s, 3H), 2.41 (m, 4H), 2.57 and 2.67 (m, 2H), 3.41 (d, 1H, J = 13.0 Hz), 3.60 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.44 (m, 1H), 7.53 (d, 1H, J = 7.6 Hz), 7.79 (d, 1H, J = 7.7 Hz), 7.88 (s, 1H). LRMS calculated for $C_{23}H_{34}N_3O_3$ (M+H) 400.25, found 400.25.

4-{[{3-hydroxy-4-[methyl(pyridin-4-ylmethyl)amino]butyl}(propyl)amino] methyl}phenol (6E)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 7.4 Hz), 1.63 (m, 4H), 2.24 (s, 3H), 2.40 (m, 4H), 2.59 and 2.70 (2m, 2H), 3.42 (d, 1H, J = 13.0 Hz), 3.57 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 6.1 Hz), 8.47 (d, 2H, J = 6.1 Hz). LRMS calculated for $C_{21}H_{32}N_3O_2$ (M+H) 358.24, found 358.30.

4-{[{3-hydroxy-4-[methyl(pyridin-3-ylmethyl)amino]butyl}(propyl)amino] methyl}phenol (6F)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 7.4 Hz), 1.62 (m, 4H), 2.23 (s, 3H), 2.39 (m, 4H), 2.57 and 2.68 (m, 2H), 3.41 (d, 1H, J = 13.0 Hz), 3.61 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.42 (d, 1H, J = 6.1 Hz), 7.85 (m, 1H), 8.44 (m, 1H), 8.51 (d, 1H, J = 1.5 Hz). LRMS calculated for $C_{21}H_{32}N_3O_2$ (M+H) 358.24, found 358.25.

4-{[(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl)amino}butyl)(propyl) amino]methyl}phenol (6G)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7.4 Hz), 1.56 (m, 3H), 1.69 (m, 1H), 2.22 (s, 3H), 2.39 (m, 4H), 2.57 and 2.66 (2m, 2H), 3.41 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.48 (s, 2H), 3.81 (m, 1H), 3.91 (s, 3H), 6.75 (d, 2H, J = 8.5 Hz), 6.79 (d, 1H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.69 (m, 1H), 8.03 (d, 1H, J = 2.1 Hz). LRMS calculated for $C_{22}H_{34}N_3O_3$ (M+H) 388.25, found 388.20.

4-{[{3-hydroxy-4-[methyl(thiophen-2-ylmethyl)amino]butyl}(propyl)amino] methyl}phenol (6H)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 7.4 Hz), 1.60 (m, 3H), 1.74 (m, 1H), 2.27 (s, 3H), 2.40 (m, 4H), 2.58 and 2.68 (m, 2H), 3.42 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.78 (d, 2H, J = 4.7 Hz), 3.79 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.96 (d, 2H, J = 4.7 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.31 (dd, 1H, J_1 = 1.6 Hz, J_2 = 4.7 Hz). LRMS calculated for $C_{20}H_{31}N_2O_2S$ (M+H) 363.20, found 363.25.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(propyl) amino]methyl}phenol (6I)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 7.4 Hz), 1.61 (m, 3H), 1.75 (m, 1H), 2.27 (s, 3H), 2.40 (m, 4H), 2.59 and 2.69 (2m, 2H), 3.43 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.70 (d, 2H, J = 0.8 Hz), 3.78 (m, 1H), 6.76 (m, 3H), 6.94 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{20}H_{30}{}^{81}BrN_2O_2S$ (M+H) 443.11, found 443.15.

4-{[(4-{[(5-chlorothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(propyl) amino]methyl}phenol (6J)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 7.4 Hz), 1.57 (m, 3H), 1.72 (m, 1H), 2.27 (s, 3H), 2.40 (m, 4H), 2.59 and 2.69 (2m, 2H), 3.43 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.68 (d, 2H, J = 0.9 Hz), 3.78 (m, 1H), 6.76 (m, 3H), 6.81 (d, 1H, J = 3.7 Hz), 7.13 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{20}H_{30}ClN_2O_2S$ (M+H) 397.16, found 397.15.

4-{[(3-hydroxy-4-phenoxybutyl)(propyl)amino]methyl}phenol (6K)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 7.4 Hz), 1.61 (m, 3H), 1.81 (m, 1H), 2.44, 2.51, 2.68 and 2.78 (4m, 4H), 3.49 and 3.67 (2d of AB system, 2H, J = 13.0 Hz), 3.84 (dd, 2H, J_1 = 0.5 Hz, J_2 = 5.3 Hz), 4.01 (m, 1H), 6.75 (d, 4H, J = 8.6 Hz), 6.94 (m, 2H), 7.16 (d, 2H, J = 8.6 Hz), 7.27 (m, 1H). LRMS calculated for $C_{20}H_{28}NO_3$ (M+H) 330.20, found 330.25.

4-({[3-hydroxy-4-(pyridin-4-yloxy)butyl](propyl)amino}methyl)phenol (6L)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 7.2 Hz), 1.60 (m, 3H), 1.80 (m, 1H), 2.80 (m, 4H), 3.48 and 3.68 (2d of AB system, 2H, J = 13.1 Hz), 3.92 (m, 2H), 4.03 (m, 1H), 6.45 (d, 2H, J = 7.6 Hz), 6.76 (m, 2H), 7.15 (m, 2H), 7.72 (d, 2H, J = 7.6 Hz). LRMS calculated for $C_{19}H_{27}N_2O_3$ (M+H) 331.19, found 331.25.

4-({[3-hydroxy-4-(pyridin-3-yloxy)butyl](propyl)amino}methyl)phenol (6M)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 7.4 Hz), 1.58 (m, 3H), 1.80 (m, 1H), 2.41, 2.49, 2.64, and 2.74 (4m, 4H), 3.43 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.91 (m, 2H), 4.03 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.14 (d, 3H, J = 8.5 Hz), 7.38 (m, 2H), 8.14 (m, 1H), 8.24 (m, 1H). LRMS calculated for $C_{19}H_{27}N_2O_3$ (M+H) 331.19, found 331.20.

4-{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butoxy}benzamide (6N)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 7.4 Hz), 1.59 (m, 3H), 1.81 (q, 1H, J = 6.7 Hz), 2.40, 2.47, 2.66 and 2.74 (4m, 4H), 3.44 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.89 (d, 2H, J = 6.0 Hz), 4.03 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 6.97 (d, 2H, J = 8.9 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.85 (d, 2H, J = 8.9 Hz). LRMS calculated for $C_{21}H_{29}N_2O_4$ (M+H) 373.20, found 373.25.

3-{2-hydroxy-4-[(4-hydroxybenzyl)(propyl)amino]butoxy}benzamide (6O)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 7.3 Hz), 1.62 (m, 3H), 1.86 (q, 1H, J = 7.2 Hz), 2.55, 2.69, 2.86 and 2.93 (4m, 4H), 3.61 and 3.81 (2d of AB system, 2H, J = 13.2 Hz), 3.91 (d, 2H, J = 5.2 Hz), 4.02 (m, 1H), 6.76 (d, 2H, J = 8.5 Hz), 7.09 (dd, 1H, J_1 = 0.9 Hz, J_2 = 4.5 Hz), 7.18 (d, 2H, J = 8.4 Hz), 7.36 (t, 1H, J = 7.9 Hz), 7.43 (dd, 1H, J_1 = 1.2 Hz, J_2 = 4.2 Hz). LRMS calculated for $C_{21}H_{29}N_2O_4$ (M+H) 373.20, found 373.25.

4-[{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino]benzamide (7A)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.29 (m, 6H), 1.52 (m, 1H), 1.68 (m, 2H), 2.40-2.80 (4m, 4H), 3.07 (s, 3H), 3.44 (m, 3H), 3.63 (d, 1H, J = 13.0 Hz), 3.97 (m, 1H), 6.74 (d, 4H, J = 8.6 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.76 (d, 2H, J = 9.0 Hz). LRMS calculated for $C_{25}H_{38}N_3O_3$ (M+H) 428.28, found 428.30.

3-[{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino]benzamide (7B)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 6.9 Hz), 1.33 (m, 6H), 1.52 (m, 2H), 1.66 (m, 2H), 2.35-2.80 (4m, 4H), 3.03 (s, 3H), 3.43 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.96 (m, 1H), 6.74 (d, 2H, J = 8.4 Hz), 6.96 (m, 1H), 7.12 (d, 2H, J = 8.4 Hz), 7.24 (m, 2H), 7.39 (s, 1H). LRMS calculated for $C_{25}H_{38}N_3O_3$ (M+H) 428.28, found 428.30.

4-{[{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino] methyl}benzamide (7C)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.31 (m, 6H), 1.54 (m, 3H), 1.71 (m, 1H), 2.24 (s, 3H), 2.30-2.75 (m, 6H), 3.42 (d of AB system, 1H, J = 13.0 Hz), 3.58 (m, 3H), 3.82 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 8.3 Hz), 7.85 (d, 2H, J = 8.3 Hz). LRMS calculated for $C_{26}H_{40}N_3O_3$ (M+H) 442.30, found 442.25.

3-{[{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino]methyl} benzamide (7D)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 1.33 (m, 6H), 1.56 (m, 3H), 1.70 (m, 1H), 2.24 (s, 3H), 2.30-2.75 (m, 6H), 3.42 (d of AB system, 1H, J = 13.0 Hz), 3.58 (m, 3H), 3.83 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.44 (m, 1H), 7.53 (d, 1H, J = 7.7 Hz), 7.79 (d, 1H, J = 7.8 Hz), 7.88 (s, 1H). LRMS calculated for $C_{26}H_{40}N_3O_3$ (M+H) 442.30, found 442.30.

4-[(hexyl{3-hydroxy-4-[methyl(pyridin-4-ylmethyl)amino]butyl}amino) methyl]phenol (7E)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.31 (m, 6H), , 1.57 (m, 3H), 1.73 (m, 1H), 2.24 (s, 3H), 2.25-2.75 (m, 6H), 3.41 (d of AB system, 1H, J = 13.4 Hz), 3.60 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 6.0 Hz), 8.47 (d, 2H, J = 6.0 Hz). LRMS calculated for $C_{24}H_{38}N_3O_2$ (M+H) 400.29, found 400.30.

4-[(hexyl{3-hydroxy-4-[methyl(pyridin-3-ylmethyl)amino]butyl}amino) methyl]phenol (7F)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.29 (m, 6H), 1.57 (m, 3H), 1.70 (m, 1H), 2.23 (s, 3H), 2.25-2.70 (m, 6H), 3.41 (d of AB system, 1H, J = 12.9 Hz),

3.57 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.42 (m, 1H), 7.85 (m, 1H), 8.44 (m, 1H), 8.51 (s, 1H). LRMS calculated for $C_{24}H_{38}N_3O_2$ (M+H) 400.29, found 400.30.

4-{[hexyl(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl)amino}butyl) amino]methyl}phenol (7G)

Yellowish oil. IR (film) ν 3202 (OH), 2932, 1612, 1497, 1458, 1366, 1265, 1095, 1026, 825. ^{1}H NMR (CD_3OD) δ 0.90 (t, $\underline{CH_3}CH_2$, J = 6.9 Hz), 1.34 (m, $CH_3\underline{CH_2}\underline{CH_2}\underline{CH_2}$), 1.61 (m, $\underline{CH_2}CH_2NCH_2\underline{CH_2}CHOH$), 2.22 (s, NCH_3), 2.49 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2CHOH\underline{CH_2}$), 3.40 and 3.58 (2d of AB system, Ar$\underline{CH_2}$, J = 13.0 Hz), 3.49 (s, $CH_3N\underline{CH_2}$), 3.81 (m, HO$\underline{CH}$), 3.91 (s, OCH_3), 6.75 (d, 2xCH of phenol, J = 8.5 Hz), 6.79 (d, CH of meta pyridinyl, J = 8.6 Hz), 7.13 (d, 2xCH of phenol, J = 8.5 Hz), 7.68 (m, CH of meta pyridinyl), 8.03 (d, CH of meta pyridinyl, J = 2.1 Hz). ^{13}C NMR (CD_3OD) δ 14.4 (CH_3), 23.7 (CH_2), 27.4 (CH_2), 28.2 (CH_2), 32.0 (CH_2), 32.8 (CH_2), 42.9 (CH_3), 52.3 (CH_2), 54.1 (CH_3), 54.5 (CH_2), 58.9 (CH_2), 60.1 (CH_2), 64.0 (CH_2), 70.3 (CH), 111.3 (CH), 116.1 (2xCH), 128.3 (C), 129.9 (C), 131.8 (2xCH), 141.8 (CH), 148.1 (CH), 157.8 (C), 165.1 (C). LRMS calculated for $C_{25}H_{40}N_3O_3$ (M+H) 430.30, found 430.30.

4-[(hexyl{3-hydroxy-4-[methyl(thiophen-2-ylmethyl)amino]butyl}amino) methyl]phenol (7H)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 6.9 Hz), 1.29 (m, 6H), 1.60 (m, 3H), 1.74 (m, 1H), 2.28 (s, 3H), 2.33-2.70 (m, 6H), 3.42 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.77 (m, 1H), 3.78 (d, 2H, J = 4.4 Hz), 6.75 (d, 2H, J = 8.5 Hz), 6.97 (m, 2H), 7.13 (d, 2H, J = 8.4 Hz), 7.31 (dd, 1H, J_1 = 1.7 Hz, J_2 = 4.5 Hz). LRMS calculated for $C_{23}H_{37}N_2O_2S$ (M+H) 405.25, found 405.25.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(hexyl) amino]methyl}phenol (7I)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 6.9 Hz), 1.33 (m, 6H), 1.60 (m, 3H), 1.75 (m, 1H), 2.28 (s, 3H), 2.30-2.70 (m, 6H), 3.42 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.71 (s, 2H), 3.78 (m, 1H), 6.76 (m, 3H), 6.94 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{23}H_{36}{}^{79}BrN_2O_2S$ (M+H) 483.16, found 483.20.

4-{[(4-{[(5-chlorothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl) (hexyl)amino]methyl}phenol (7J)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 6.9 Hz), 1.36 (m, 6H), 1.58 (m, 3H), 1.75 (m, 1H), 2.28 (s, 3H), 2.30-2.72 (m, 6H), 3.43 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.69 (s, 2H), 3.79 (m, 1H), 6.76 (m, 3H), 6.81 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{23}H_{36}ClN_2O_2S$ (M+H) 439.21 found 439.25.

4-{[hexyl(3-hydroxy-4-phenoxybutyl)amino]methyl}phenol (7K)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.91 (t, 3H, J = 6.8 Hz), 1.34 (m, 6H), 1.61 (m, 2H), 1.92 (m, 2H), 2.75 (m, 2H), 3.02 (m, 2H), 3.78 (d, 1H, J = 12.9 Hz), 3.92 (m, 3H), 4.04 (m, 1H), 6.81 (d, 2H, J = 8.5 Hz), 6.94 (m, 3H), 7.25 (m, 4H). LRMS calculated for $C_{23}H_{34}NO_3$ (M+H) 372.25 found 372.25.

4-({hexyl[3-hydroxy-4-(pyridin-4-yloxy)butyl]amino}methyl)phenol (7L)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.31 (m, 6H), 1.53 (m, 2H), 1.63 (m, 2H), 2.35-2.79 (4m, 4H), 3.37 and 3.60 (2d of AB system, 2H, J = 13.0 Hz),

3.75 (m, 1H), 3.88 (m, 1H), 3.96 (m, 1H), 6.45 (d, 2H, J = 7.6 Hz), 6.76 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.73 (d, 2H, J = 7.6 Hz). LRMS calculated for $C_{22}H_{33}N_2O_3$ (M+H) 373.24 found 373.30.

4-({hexyl[3-hydroxy-4-(pyridin-3-yloxy)butyl]amino}methyl)phenol (7M)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 1.34 (m, 6H), 1.56 (m, 2H), 1.81 (q, 2H, J = 6.5 Hz), 2.36-2.80 (4m, 4H), 3.43 and 3.63 (2d of AB system, 2H, J = 13.1 Hz), 3.92 (m, 2H), 4.03 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.40 (m, 2H), 8.14 (d, 1H, J = 3.2 Hz), 8.25 (s, 1H). LRMS calculated for $C_{22}H_{33}N_2O_3$ (M+H) 373.24 found 373.25.

4-{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (7N)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.32 (m, 6H), 1.54 (m, 2H), 1.81 (q, 2H, J = 6.1 Hz), 2.40-2.79 (4m, 4H), 3.44 and 3.62 (2d of AB system, 2H, J = 12.9 Hz), 3.95 (m, 2H), 4.03 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.98 (d, 2H, J = 8.9 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.85 (d, 2H, J = 8.9 Hz). LRMS calculated for $C_{24}H_{35}N_2O_4$ (M+H) 415.25 found 415.25.

3-{4-[hexyl(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (7O)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 1.31 (m, 6H), 1.49 (m, 2H), 1.82 (q, 2H, J = 6.4 Hz), 2.38-2.81 (4m, 4H), 3.43 and 3.64 (2d of AB system, 2H, J = 12.8 Hz), 3.90 (m, 2H), 4.03 (m, 1H), 6.74 (m, 2H), 7.14 (m, 3H), 7.35-7.60 (m, 3H). LRMS calculated for $C_{24}H_{35}N_2O_4$ (M+H) 415.25 found 415.25.

4-[{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butyl}(methyl) amino]benzamide (8A)

Yellowish powder. ^{1}H NMR (CD_3OD) δ 0.87 (dd, 6H, J_1 = 3.2 Hz, J_2 = 6.5 Hz), 1.41 (q, 2H, J = 7.3 Hz), 1.53 (m, 1H), 1.67 (m, 2H), 2.37-2.78 (4m, 4H), 3.07 (s, 3H), 3.40 (m, 3H), 3.61 (d, 1H, J = 13.0 Hz), 3.97 (m, 1H), 6.74 (d, 4H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.4 Hz), 7.76 (d, 2H, J = 9.0 Hz). LRMS calculated for $C_{24}H_{36}N_3O_3$ (M+H) 414.27, found 414.25.

3-[{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butyl}(methyl) amino]benzamide (8B)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (dd, 6H, J_1 = 1.3 Hz, J_2 = 6.4 Hz), 1.44 (m, 2H), 1.54 (m, 1H), 1.68 (m, 2H), 2.36-2.78 (4m, 4H), 3.03 (s, 3H), 3.39 (m, 1H), 3.61 (m, 1H), 3.89 (s, 2H), 3.96 (m, 1H), 6.73 (d, 2H, J = 8.4 Hz), 6.94 (m, 1H), 7.12 (d, 2H, J = 8.3 Hz), 7.27 (m, 2H), 7.38 (d, 1H, J = 1.1 Hz). LRMS calculated for $C_{24}H_{36}N_3O_3$ (M+H) 414.27, found 414.30.

4-{[{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butyl}(methyl)amino] methyl}benzamide (8C)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.87 (dd, 6H, J_1 = 3.9 Hz, J_2 = 6.6 Hz), 1.41 (q, 2H, J = 7.3 Hz), 1.57 (m, 2H), 1.71 (m, 1H), 2.24 (s, 3H), 2.26-2.72 (m, 6H), 3.41 and 3.58 (2d, 2H, J = 13.0 Hz), 3.60 (s, 2H), 3.82 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 8.3 Hz), 7.85 (d, 2H, J = 8.3 Hz). LRMS calculated for $C_{25}H_{38}N_3O_3$ (M+H) 428.28, found 428.25.

3-{[{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butyl}(methyl)amino] methyl}benzamide (8D)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.87 (dd, 6H, J_1 = 4.0 Hz, J_2 = 6.5 Hz), 1.41 (q, 2H, J = 7.3 Hz), 1.58 (m, 2H), 1.73 (m, 1H), 2.24 (s, 3H), 2.30-2.73 (m, 6H), 3.41 (d, 1H, J

= 13.0 Hz), 3.58 (m, 3H), 3.83 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (m, 1H), 7.57 (m, 1H), 7.79 (d, 1H, J = 7.8 Hz), 7.88 (s, 1H). LRMS calculated for $C_{25}H_{38}N_3O_3$ (M+H) 428.28, found 428.30.

4-{[{3-hydroxy-4-[methyl(pyridin-4-ylmethyl)amino]butyl}(3-methylbutyl)amino] methyl}phenol (8E)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 4.3 Hz, J_2 = 6.6 Hz), 1.42 (q, 2H, J = 7.4 Hz), 1.58 (m, 2H), 1.73 (m, 1H), 2.25 (s, 3H), 2.27-2.74 (m, 6H), 3.41 (d, 1H, J = 13.0 Hz), 3.59 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 6.0 Hz), 8.47 (d, 2H, J = 5.9 Hz). LRMS calculated for $C_{23}H_{36}N_3O_2$ (M+H) 386.27, found 386.25.

4-{[{3-hydroxy-4-[methyl(pyridin-3-ylmethyl)amino]butyl}(3-methylbutyl) amino]methyl}phenol (8F)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 4.4 Hz, J_2 = 6.6 Hz), 1.41 (q, 2H, J = 7.3 Hz), 1.57 (m, 2H), 1.70 (m, 1H), 2.23 (s, 3H), 2.27-2.74 (m, 6H), 3.37 (d, 1H, J = 13.9 Hz), 3.58 (m, 3H), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.4 Hz), 7.42 (m, 1H), 7.85 (d, 1H, J = 7.8 Hz), 8.44 (d, 1H, J = 3.7 Hz), 8.51 (s, 1H). LRMS calculated for $C_{23}H_{36}N_3O_2$ (M+H) 386.27, found 386.25.

4-{[(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl)amino}butyl)(3-methylbutyl)amino]methyl}phenol (8G)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 4.0 Hz, J_2 = 6.6 Hz), 1.41 (q, 2H, J = 7.4 Hz), 1.59 (m, 2H), 1.70 (m, 1H), 2.23 (s, 3H), 2.28-2.72 (m, 6H), 3.41 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.48 (s, 2H), 3.81 (m, 1H), 3.91 (s, 3H), 6.75 (d, 2H, J = 8.5 Hz), 6.79 (d, 1H, J = 8.6 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.69 (m, 1H), 8.03 (d, 1H, J = 2.0 Hz). LRMS calculated for $C_{24}H_{38}N_3O_3$ (M+H) 416.28, found 416.30.

4-{[{3-hydroxy-4-[methyl(thiophen-2-ylmethyl)amino]butyl}(3-methylbutyl)amino]methyl}phenol (8H)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 3.7 Hz, J_2 = 6.6 Hz), 1.41 (q, 2H, J = 7.4 Hz), 1.60 (m, 2H), 1.74 (m, 1H), 2.28 (s, 3H), 2.35-2.72 (m, 6H), 3.42 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.78 (d, 2H, J = 4.9 Hz), 3.80 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.95 (d, 1H, J = 1.6 Hz), 6.96 (d, 1H, J = 4.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.31 (dd, 1H, J_1 = 1.8 Hz, J_2 = 4.5 Hz). LRMS calculated for $C_{22}H_{35}N_2O_2S$ (M+H) 391.23, found 391.25.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(3-methylbutyl)amino]methyl}phenol (8I)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 3.8 Hz, J_2 = 6.6 Hz), 1.42 (q, 2H, J = 7.4 Hz), 1.58 (m, 2H), 1.75 (m, 1H), 2.28 (s, 3H), 2.37 (dd, 2H, J_1 = 4.0 Hz, J_2 = 6.3 Hz), 2.40-2.73 (m, 4H), 3.42 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.71 (s, 2H), 3.78 (m, 1H), 6.75 (m, 3H), 6.94 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{22}H_{34}{}^{79}BrN_2O_2S$ (M+H) 469.14, found 469.15.

4-{[(4-{[(5-chlorothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(3-methylbutyl)amino]methyl}phenol (8J)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 3.8 Hz, J_2 = 6.6 Hz), 1.42 (q, 2H, J = 7.3 Hz), 1.58 (m, 2H), 1.76 (m, 1H), 2.28 (s, 3H), 2.37 (dd, 2H, J_1 = 4.1 Hz, J_2 = 6.3 Hz), 2.40-2.73 (4m, 4H), 3.43 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.69 (d,

2H, J = 0.9 Hz), 3.78 (m, 1H), 6.77 (m, 3H), 6.81 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{22}H_{34}ClN_2O_2S$ (M+H) 425.20, found 425.25.

4-{[(3-hydroxy-4-phenoxybutyl)(3-methylbutyl)amino]methyl}phenol (8K)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 4.9 Hz, J_2 = 6.5 Hz), 1.43 (q, 2H, J = 7.2 Hz), 1.57 (m, 1H), 1.81 (m, 2H), 2.39-2.79 (4m, 4H), 3.42 and 3.62 (2d of AB system, 2H, J = 12.9 Hz), 3.84 (m, 2H), 4.01 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.93 (m, 3H), 7.14 (d, 2H, J = 8.5 Hz), 7.27 (m, 2H). LRMS calculated for $C_{22}H_{32}NO_3$ (M+H) 358.23, found 358.25.

4-({[3-hydroxy-4-(pyridin-4-yloxy)butyl](3-methylbutyl)amino}methyl)phenol (8L)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 5.3 Hz, J_2 = 6.5 Hz), 1.44 (m, 2H), 1.58 (m, 3H), 2.38-2.80 (4m, 4H), 3.38 and 3.61 (2d of AB system, 2H, J = 12.9 Hz), 3.76 (dd, 1H, J_1 = 7.9 Hz, J_2 = 13.7 Hz), 3.90 (m, 1H), 3.98 (m, 1H), 6.45 (d, 2H, J = 7.6 Hz), 6.76 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.74 (d, 2H, J = 7.6 Hz). LRMS calculated for $C_{21}H_{31}N_2O_3$ (M+H) 359.23, found 359.25.

4-({[3-hydroxy-4-(pyridin-3-yloxy)butyl](3-methylbutyl)amino}methyl)phenol (8M)

Yellowish oil. IR (film) ν 3171 (OH), 2955, 2870, 1690, 1582, 1512, 1466, 1373, 1273, 1242, 1103, 1057, 833, 802. ^{1}H NMR (CD_3OD) δ 0.89 (dd, $(\underline{CH_3})_2CH$, J_1 = 5.0 Hz, J_2 = 6.5 Hz), 1.44 (q, $CH\underline{CH_2}CH_2N$, J = 7.2 Hz), 1.57 (m, $(CH_3)_2\underline{CH}$), 1.81 (q, $HOCH\underline{CH_2}CH_2N$, J = 6.2 Hz), 2.60 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.44 and 3.64 (2d of AB system, $Ar\underline{CH_2}$, J = 13.0 Hz), 3.96 (m, OCH_2), 4.07 (m, $HO\underline{CH}$), 6.74 (d, 2xCH of phenol, J = 8.5 Hz), 7.15 (d, 2xCH of phenol, J = 8.4 Hz), 7.39 (m, 2xCH of meta pyridinyl), 8.21 (m, 2xCH of meta pyridinyl). ^{13}C NMR (CD_3OD) δ 23.0 (CH_3), 23.1 (CH_3), 27.6 (CH), 30.0 (CH_2), 36.4 (CH_2), 52.0 (CH_2), 52.7 (CH_2), 58.9 (CH_2), 71.0 (CH), 73.2 (CH_2), 116.1 (2xCH), 116.3 (CH), 123.2 (CH), 129.6 (C), 131.9 (2xCH), 134.5 (C), 138.5 (CH), 142.2 (CH), 157.9 (C). LRMS calculated for $C_{21}H_{31}N_2O_3$ (M+H) 359.23, found 359.25.

4-{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butoxy}benzamide (8N)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 4.9 Hz, J_2 = 5.7 Hz), 1.44 (q, 2H, J = 7.3 Hz), 1.57 (m, 1H), 1.81 (q, 2H, J = 6.4 Hz), 2.41-2.80 (4m, 4H), 3.43 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.89 (m, 2H), 4.03 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.98 (d, 2H, J = 8.9 Hz), 7.15 (d, 2H, J = 8.5 Hz), 7.86 (d, 2H, J = 8.9 Hz). LRMS calculated for $C_{23}H_{33}N_2O_4$ (M+H) 401.24, found 401.25.

3-{2-hydroxy-4-[(4-hydroxybenzyl)(3-methylbutyl)amino]butoxy}benzamide (8O)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (dd, 6H, J_1 = 5.1 Hz, J_2 = 6.5 Hz), 1.44 (q, 2H, J = 7.3 Hz), 1.57 (m, 1H), 1.82 (q, 2H, J = 6.4 Hz), 2.40-2.82 (4m, 4H), 3.43 and 3.64 (2d of AB system, 2H, J = 13.0 Hz), 3.91 (m, 2H), 4.03 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.14 (m, 3H), 7.38 (t, 1H, J = 7.8 Hz), 7.45 (m, 2H). LRMS calculated for $C_{23}H_{33}N_2O_4$ (M+H) 401.24, found 401.25.

4-[{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butyl}(methyl) amino]benzamide (9A)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.70 (m, 2H), 2.76 (m, 6H), 3.07 (s, 3H), 3.43 (m, 2H), 3.52 and 3.66 (2d of AB system, 2H, J = 13.1 Hz), 3.97 (m, 1H), 6.74 (d, 4H, J = 8.4 Hz), 7.14 (m, 4H), 7.23 (m, 3H), 7.78 (m, 2H). LRMS calculated for $C_{27}H_{34}N_3O_3$ (M+H) 448.25, found 448.25.

3-[{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butyl}(methyl) amino]benzamide (9B)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.76 (m, 2H), 2.84 (m, 6H), 3.03 (s, 3H), 3.71 (m, 2H), 3.88 (s, 2H), 3.98 (m, 1H), 6.73 (d, 2H, J = 8.4 Hz), 6.93 (m, 1H), 7.06 (m, 1H), 7.14 (m, 2H), 7.30 (m, 6H), 7.42 (d, 1H, J = 1.5 Hz). LRMS calculated for $C_{27}H_{34}N_3O_3$ (M+H) 448.25, found 448.25.

4-{[{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butyl}(methyl) amino]methyl}benzamide (9C)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.66 (m, 2H), 1.74 (m, 1H), 2.23 (s, 3H), 2.34 (m, 2H), 2.75 (m, 6H), 3.51 and 3.65 (2d of AB system, 2H, J = 13.1 Hz), 3.59 (s, 2H), 3.82 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.15 (m, 4H), 7.25 (m, 3H), 7.45 (d, 2H, J = 8.2 Hz), 7.85 (d, 2H, J = 8.3 Hz). LRMS calculated for $C_{28}H_{36}N_3O_3$ (M+H) 462.27, found 462.25.

3-{[{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butyl}(methyl) amino]methyl}benzamide (9D)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.60 (m, 1H), 1.74 (m, 1H), 2.23 (s, 3H), 2.33 (m, 2H), 2.75 (m, 6H), 3.51 and 3.65 (2d of AB system, 2H, J = 13.0 Hz), 3.59 (d, 2H, J = 2.6 Hz), 3.83 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 7.14 (m, 4H), 7.24 (m, 3H), 7.42 (d, 1H, J = 7.6 Hz), 7.54 (d, 1H, J = 7.6 Hz), 7.78 (td, 1H, J_1 = 1.4 Hz, J_2 = 7.8 Hz), 7.88 (d, 1H, J = 1.4 Hz). LRMS calculated for $C_{28}H_{36}N_3O_3$ (M+H) 462.27, found 462.25.

4-{[{3-hydroxy-4-[methyl(pyridin-4-ylmethyl)amino]butyl}(2-phenylethyl) amino]methyl}phenol (9E)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.62 (m, 1H), 1.73 (m, 1H), 2.24 (s, 3H), 2.36 (m, 2H), 2.76 (m, 6H), 3.51 and 3.66 (2d of AB system, 2H, J = 13.1 Hz), 3.57 (d, 2H, J = 4.6 Hz), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.15 (m, 4H), 7.25 (m, 3H), 7.45 (d, 2H, J = 5.8 Hz), 8.46 (d, 2H, J = 5.9 Hz). LRMS calculated for $C_{26}H_{34}N_3O_2$ (M+H) 420.26, found 420.30.

4-{[{3-hydroxy-4-[methyl(pyridin-3-ylmethyl)amino]butyl}(2-phenylethyl) amino]methyl}phenol (9F)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.60 (m, 1H), 1.73 (m, 1H), 2.23 (s, 3H), 2.35 (m, 2H), 2.76 (m, 6H), 3.51 and 3.65 (2d of AB system, 2H, J = 13.0 Hz), 3.58 (d, 2H, J = 3.2 Hz), 3.82 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.15 (m, 4H), 7.26 (m, 3H), 7.41 (dd, 1H, J_1 = 4.9 Hz, J_2 = 7.6 Hz), 7.85 (m, 1H), 8.44 (td, 1H, J_1 = 1.8 Hz, J_2 = 7.9 Hz), 8.51 (d, 1H, J = 1.4 Hz). LRMS calculated for $C_{26}H_{34}N_3O_2$ (M+H) 420.26, found 420.30.

4-{[(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl)amino}butyl)(2-phenylethyl)amino]methyl}phenol (9G)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.59 (m, 1H), 1.71 (m, 1H), 2.22 (s, 3H), 2.33 (m, 2H), 2.71 (m, 6H), 3.48 (s, 2H), 3.51 and 3.64 (2d of AB system, 2H, J = 13.1 Hz), 3.81 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.78 (d, 1H, J = 8.6 Hz), 7.16 (m, 5H), 7.24 (d, 2H, J = 7.5 Hz), 7.68 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.5 Hz), 8.04 (d, 1H, J = 2.2 Hz). LRMS calculated for $C_{27}H_{36}N_3O_3$ (M+H) 450.27, found 450.25.

4-{[{3-hydroxy-4-[methyl(thiophen-2-ylmethyl)amino]butyl}(2-phenylethyl)amino] methyl}phenol (9H)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.61 (m, 1H), 1.75 (m, 1H), 2.28 (s, 3H), 2.37 (dd, 2H, J_1 = 4.3 Hz, J_2 = 6.2 Hz), 2.73 (m, 6H), 3.52 and 3.65 (2d of AB system, 2H, J =

13.0 Hz), 3.78 (d, 2H, J = 2.3 Hz), 3.79 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.97 (m, 2H), 7.16 (m, 5H), 7.24 (d, 2H, J = 7.3 Hz), 7.31 (dd, 1H, J_1 = 2.3 Hz, J_2 = 4.1 Hz). LRMS calculated for $C_{25}H_{33}N_2O_2S$ (M+H) 425.22, found 425.25.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(2-phenylethyl)amino] methyl}phenol (9I)

Yellowish oil. IR (film) ν 3202 (OH), 3024, 2939, 2816, 1666, 1605, 1512, 1450, 1366, 1250, 1103, 1026, 980, 833, 795, 702. ^{1}H NMR (CD_3OD) δ 1.61 and 1.76 (2m, HOCH$\underline{CH_2}CH_2N$), 2.27 (s, NCH_3), 2.44 (m, Ph$\underline{CH_2}$), 2.74 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2CHOH\underline{CH_2}$), 3.53 and 3.65 (2d of AB system, Ar$\underline{CH_2}$, J = 13.1 Hz), 3.70 (s, $CH_3N\underline{CH_2}$), 3.78 (m, HO$\underline{CH}$), 6.75 (m, 2xCH of phenol and 1xCH of thiophenyl), 6.94 (d, 1xCH of thiophenyl, J = 3.7 Hz), 7.16 (m, 2xCH of phenol and 3xCH of phenyl), 7.26 (m, 2xCH of phenyl). ^{13}C NMR (CD_3OD) δ 32.2 (CH_2), 33.8 (CH_2), 43.0 (CH), 52.0 (CH_2), 56.3 (CH_2), 58.0 (CH_2), 58.9 (CH_2), 63.5 (CH_2), 70.1 (CH), 112.2 (C), 116.1 (2xCH), 127.0 (CH), 127.8 (CH), 129.4 (2xCH), 129.7 (2xCH), 130.1 (C), 130.6 (CH), 131.8 (2xCH), 141.5 (C), 145.6 (C), 157.7 (C). LRMS calculated for $C_{25}H_{32}BrN_2O_2S$ (M+H) 503.13, found 503.15.

4-{[(4-{[(5-chlorothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(2-phenylethyl)amino]methyl}phenol (9J)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.62 (m, 1H), 1.76 (m, 1H), 2.27 (s, 3H), 2.36 (m, 2H), 2.73 (m, 6H), 3.53 and 3.65 (2d of AB system, 2H, J = 13.0 Hz), 3.68 (s, 2H), 3.79 (m, 1H), 6.75 (d, 2H, J = 8.5 Hz), 6.76 (d, 1H, J = 3.7 Hz), 6.81 (d, 1H, J = 3.7 Hz), 7.17 (m, 5H), 7.25 (m, 2H). LRMS calculated for $C_{25}H_{32}ClN_2O_2S$ (M+H) 459.18, found 459.20.

4-{[(3-hydroxy-4-phenoxybutyl)(2-phenylethyl)amino]methyl}phenol (9K)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.80 (m, 2H), 2.74 (m, 6H), 3.53 and 3.67 (2d of AB system, 2H, J = 13.0 Hz), 3.83 (d, 2H, J = 5.3 Hz), 4.00 (m, 1H), 6.77 (m, 3H), 6.94 (m, 2H), 7.15 (m, 5H), 7.25 (m, 4H). LRMS calculated for $C_{25}H_{30}NO_3$ (M+H) 392.21, found 392.20.

4-({[3-hydroxy-4-(pyridin-4-yloxy)butyl](2-phenylethyl)amino}methyl)phenol (9L)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.63 (m, 2H), 2.68 (m, 6H), 3.49 and 3.66 (2d of AB system, 2H, J = 13.0 Hz), 3.69 (m, 1H), 3.84 (m, 1H), 3.93 (m, 1H), 6.45 (d, 2H, J = 7.6 Hz), 6.76 (d, 2H, J = 8.5 Hz) , 7.15 (m, 5H), 7.26 (m, 2H), 7.67 (d, 2H, J = 7.6 Hz). LRMS calculated for $C_{24}H_{29}N_2O_3$ (M+H) 393.21, found 393.20.

4-({[3-hydroxy-4-(pyridin-3-yloxy)butyl](2-phenylethyl)amino}methyl)phenol (9M)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.69 (m, 1H), 1.81 (q, 1H, J = 6.6 Hz), 2.78 (m, 6H), 3.54 and 3.68 (2d of AB system, 2H, J = 12.9 Hz), 3.89 (m, 2H), 4.01 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.16 (m, 5H), 7.25 (m, 2H), 7.40 (m, 2H), 8.18 (m, 1H), 8.23 (s, 1H). LRMS calculated for $C_{24}H_{29}N_2O_3$ (M+H) 393.21, found 393.25.

4-{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butoxy}benzamide (9N)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.80 (m, 2H), 2.75 (m, 6H), 3.55 and 3.68 (2d of AB system, 2H, J = 12.8 Hz), 3.89 (m, 2H), 4.01 (m, 1H), 6.74 (d, 2H, J = 8.4 Hz), 6.97 (d, 2H, J = 8.9 Hz), 7.15 (m, 5H), 7.24 (d, 2H, J = 7.5 Hz), 7.85 (d, 2H, J = 8.8 Hz). LRMS calculated for $C_{26}H_{31}N_2O_4$ (M+H) 435.22, found 435.25.

3-{2-hydroxy-4-[(4-hydroxybenzyl)(2-phenylethyl)amino]butoxy}benzamide (9O)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.80 (m, 2H), 2.78 (m, 6H), 3.58 and 3.72 (2d of AB system, 2H, J = 13.0 Hz), 3.89 (m, 2H), 4.02 (m, 1H), 6.76 (m, 2H), 7.14 (m, 5H), 7.26 (m, 3H), 7.45 (m, 3H). LRMS calculated for $C_{26}H_{31}N_2O_4$ (M+H) 435.22, found 435.25.

4-[{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl) amino]benzamide (10A)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.70 (m, 4H), 2.43-2.74 (4m, 4H), 3.07 (s, 3H), 3.40 (m, 7H), 3.57 (d, 1H, J = 13.1 Hz), 3.99 (m, 1H), 6.75 (m, 4H), 7.12 (d, 2H, J = 8.4 Hz), 7.76 (d, 2H, J = 9.0 Hz). LRMS calculated for $C_{24}H_{36}N_3O_4$ (M+H) 430.26, found 430.25.

3-[{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl) amino]benzamide (10B)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.13 (t, 3H, J = 7.0 Hz), 1.69 (m, 4H), 2.43-2.80 (4m, 4H), 3.04 (s, 3H), 3.43 (m, 7H), 3.59 (d, 1H, J = 13.1 Hz), 3.96 (m, 1H), 6.73 (m, 2H), 6.92 (m, 1H), 7.13 (m, 3H), 7.31 (m, 2H). LRMS calculated for $C_{24}H_{36}N_3O_4$ (M+H) 430.26, found 430.25.

4-{[{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino] methyl}benzamide (10C)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.59 (m, 1H), 1.75 (m, 3H), 2.24 (s, 3H), 2.28 -2.70 (m, 6H), 3.44 (m, 5H), 3.58 (m, 3H), 3.83 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 8.2 Hz), 7.85 (d, 2H, J = 8.3 Hz). LRMS calculated for $C_{25}H_{38}N_3O_4$ (M+H) 444.28, found 444.25.

3-{[{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutyl}(methyl)amino] methyl}benzamide (10D)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.59 (m, 1H), 1.67 (m, 4H), 2.24 (s, 3H), 2.30-2.71 (m, 6H), 3.45 (m, 5H), 3.57 (d, 1H, J = 13.8 Hz), 3.59 (s, 2H), 3.83 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.44 (m, 1H), 7.54 (d, 1H, J = 7.7 Hz), 7.79 (m, 1H), 7.88 (d, 1H, J = 1.4 Hz). LRMS calculated for $C_{25}H_{38}N_3O_4$ (M+H) 444.28, found 444.25.

4-{[(3-ethoxypropyl){3-hydroxy-4-[methyl(pyridin-4-ylmethyl)amino]butyl} amino]methyl}phenol (10E)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.61 (m, 1H), 1.76 (m, 3H), 2.25 (s, 3H), 2.28-2.73 (m, 6H), 3.44 (m, 5H), 3.59 (d, 1H, J = 13.0 Hz), 3.60 (s, 2H), 3.83 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.45 (d, 2H, J = 6.0 Hz), 8.47 (d, 2H, J = 5.9 Hz). LRMS calculated for $C_{23}H_{36}N_3O_3$ (M+H) 402.27, found 402.30.

4-{[(3-ethoxypropyl){3-hydroxy-4-[methyl(pyridin-3-ylmethyl)amino]butyl} amino]methyl}phenol (10F)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.60 (m, 1H), 1.76 (m, 3H), 2.23 (s, 3H), 2.29-2.71 (m, 6H), 3.42 (m, 5H), 3.58 (d, 1H, J = 12.6 Hz), 3.60 (s, 2H), 3.83 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.42 (m, 1H), 8.85 (m, 1H), 8.44 (m, 1H), 8.51 (s, 1H). LRMS calculated for $C_{23}H_{36}N_3O_3$ (M+H) 402.27, found 402.25.

4-{[(3-ethoxypropyl)(3-hydroxy-4-{[(6-methoxypyridin-3-yl)methyl](methyl) amino}butyl)amino]methyl}phenol (10G)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.58 (m, 1H), 1.73 (m, 3H), 2.22 (s, 3H), 2.27-2.70 (m, 6H), 3.43 (m, 5H), 3.49 (s, 2H), 3.57 (d, 1H, J = 13.0 Hz), 3.82 (m, 1H), 3.91 (s, 3H), 6.74 (d, 2H, J = 8.5 Hz), 6.79 (d, 1H, J = 8.5 Hz), 7.13 (d, 2H, J = 8.5 Hz), 7.69 (m, 1H), 8.03 (d, 1H, J = 2.0 Hz). LRMS calculated for $C_{24}H_{38}N_3O_4$ (M+H) 432.28, found 432.25.

C4-{[(3-ethoxypropyl){3-hydroxy-4-[methyl(thiophen-2-ylmethyl)amino]butyl} amino]methyl}phenol (10H)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.59 (m, 1H), 1.75 (m, 3H), 2.28 (s, 3H), 2.37 (dd, 2H, J_1 = 3.9 Hz, J_2 = 6.3 Hz), 2.48 (m, 1H), 2.58 (m, 2H), 2.68 (m, 1H), 3.46 (m, 5H), 3.58 (d, 1H, J = 13.1 Hz), 3.79 (m, 3H), 6.74 (d, 2H, J = 8.5 Hz), 6.97 (m, 2H), 7.14 (d, 2H, J = 8.5 Hz), 7.32 (dd, 1H, J_1 = 1.8 Hz, J_2 = 4.5 Hz). LRMS calculated for $C_{22}H_{35}N_2O_3S$ (M+H) 407.23, found 407.20.

4-{[(4-{[(5-bromothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(3-ethoxypropyl)amino]methyl}phenol (10I)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.63 (m, 1H), 1.76 (m, 3H), 2.28 (s, 3H), 2.32-2.72 (4m, 6H), 3.45 (m, 5H), 3.58 (d, 1H, J = 13.0 Hz), 3.71 (s, 2H), 3.80 (m, 1H), 6.76 (m, 3H), 6.94 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{22}H_{34}{}^{79}BrN_2O_3S$ (M+H) 485.14, found 485.15.

4-{[(4-{[(5-chlorothiophen-2-yl)methyl](methyl)amino}-3-hydroxybutyl)(3-ethoxypropyl)amino]methyl}phenol (10J)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.61 (m, 1H), 1.77 (m, 3H), 2.28 (s, 3H), 2.35 (m, 2H), 2.57 (m, 4H), 3.46 (m, 5H), 3.58 (d, 1H, J = 13.0 Hz), 3.69 (s, 2H), 3.78 (m, 1H), 6.75 (m, 3H), 6.81 (d, 1H, J = 3.7 Hz), 7.14 (d, 2H, J = 7.8 Hz). LRMS calculated for $C_{22}H_{34}ClN_2O_3S$ (M+H) 441.19, found 441.20.

4-{[(3-ethoxypropyl)(3-hydroxy-4-phenoxybutyl)amino]methyl}phenol (10K)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.83 (m, 4H), 2.72 (m, 5H), 3.44 (m, 5H), 3.55 and 3.71 (2d of AB system, 2H, J = 13.0 Hz), 3.86 (d, 2H, J = 5.3 Hz), 4.02 (m, 1H), 6.76 (d, 2H, J = 8.5 Hz), 6.94 (m, 3H), 7.18 (d, 2H, J = 8.5 Hz), 7.27 (m, 2H). LRMS calculated for $C_{22}H_{32}NO_4$ (M+H) 374.23, found 374.25.

4-({(3-ethoxypropyl)[3-hydroxy-4-(pyridin-4-yloxy)butyl]amino}methyl)phenol (10L)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.64 (m, 2H), 1.78 (m, 2H), 2.62 (m, 4H), 3.45 (m, 5H), 3.60 (d, 1H, J = 13.1 Hz), 3.75 (dd, 1H, J_1 = 8.1 Hz, J_2 = 13.7 Hz), 3.91 (m, 1H), 4.00 (m, 1H), 6.45 (d, 2H, J = 7.6 Hz), 6.76 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 5.1 Hz), 7.73 (d, 2H, J = 7.6 Hz). LRMS calculated for $C_{21}H_{31}N_2O_4$ (M+H) 375.22, found 375.25.

4-({(3-ethoxypropyl)[3-hydroxy-4-(pyridin-3-yloxy)butyl]amino}methyl)phenol (10M)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.79 (m, 4H), 2.64 (m, 4H), 3.45 (m, 5H), 3.61 (d, 1H, J = 13.0 Hz), 3.95 (m, 2H), 4.05 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz), 7.39 (m, 2H), 8.14 (dd, 1H, J_1 = 1.4 Hz, J_2 = 4.3 Hz), 8.25 (d, 1H, J = 2.2 Hz). LRMS calculated for $C_{21}H_{31}N_2O_4$ (M+H) 375.22, found 375.25.

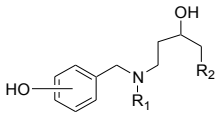
4-{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (10N)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.77 (m, 4H), 2.64 (m, 4H), 3.44 (m, 5H), 3.61 (d, 1H, J = 13.0 Hz), 3.89 (m, 2H), 4.04 (m, 1H), 6.74 (d, 2H, J = 8.4 Hz), 6.98 (d, 2H, J = 8.1 Hz), 7.15 (d, 2H, J = 8.3 Hz), 7.86 (d, 2H, J = 8.8 Hz). LRMS calculated for $C_{23}H_{33}N_2O_5$ (M+H) 417.23, found 417.25.

3-{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (10O)

Yellowish oil. IR (film) ν 3209 (OH and NH_2), 2939, 2870, 1666 (CON, amide), 1589, 1512, 1450, 1381, 1288, 1242, 1103, 1049, 833, 756. ^{1}H NMR (CD_3OD) δ 1.15 (t, $\underline{CH_3}CH_2$, J = 7.0 Hz), 1.80 (m, $\underline{CH_2}CH_2NCH_2\underline{CH_2}CHOH$), 2.64 (m, $CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 3.45 (m, 1H of Ar$\underline{CH_2}$ and CH_2OCH_2), 3.64 (d of AB system, 1H of Ar$\underline{CH_2}$, J = 13.1 Hz), 3.91 (m, O$\underline{CH_2}$CHOH), 4.04 (m, HO$\underline{CH}$), 6.75 (m, 2xCH of phenol), 7.14 (m, 2xCH of phenol and 1xCH of benzamide), 7.46 (m, 3xCH of benzamide). ^{13}C NMR ($CDCl_3$) δ 15.4 (CH_3), 27.8 (CH_2), 30.4 (CH_2), 51.5 (CH_2), 52.1 (CH_2), 58.9 (CH_2), 67.2 (CH_2), 69.7 (CH_2), 70.9 (CH), 73.0 (CH_2), 114.6 (CH), 116.1 (2xCH), 119.4 (CH), 121.0 (CH), 130.7 (CH), 131.8 (2xCH), 129.9 (C), 136.3 (C), 157.8 (C), 160.4 (C), 172.2 (C=O). LRMS calculated for $C_{23}H_{33}N_2O_5$ (M+H) 417.23, found 417.25.

Library L2 (75 members)



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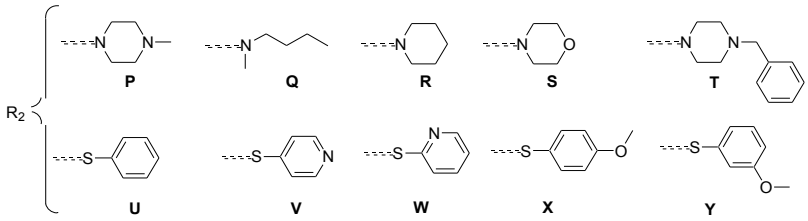
10'P, **10'Q**, **10'R**, **10'S**, **10'T**, **10'U**, **10'V**, **10'W**, **10'X**, **10'Y**,

R_1:		-OMOM position:
6	propyl	*para*
7	hexyl	*para*
8	isoamyl	*para*
9	2-phenylethyl	*para*
10	3-ethoxypropyl	*para*
6'	propyl	*meta*
7'	hexyl	*meta*
8'	isoamyl	*meta*
9'	2-phenylethyl	*meta*
10'	3-ethoxypropyl	*meta*



4-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](propyl)amino}methyl)phenol (6P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 1.54 (m, 4H), 2.16 (s, 3H), 2.23 (d, 2H, J = 7.3 Hz), 2.25-2.67 (m_{broad}, 12H), 3.35 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.74 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{19}H_{34}N_3O_2$ (M+H) 336.26, found 336.30.

3-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](propyl)amino}methyl)phenol (6'P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 1.57 (m, 4H), 2.17 (s, 3H), 2.20-2.70 (m_{broad}, 14H), 2.49 (m, 11H), 3.38 and 3.58 (2d of AB system, 2H, J = 13.6 Hz), 3.76 (m, 1H), 6.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.78 (d, 1H, J = 7.4 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{19}H_{34}N_3O_2$ (M+H) 336.26, found 336.30.

4-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(propyl)amino]methyl}phenol (6Q)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 0.90 (t, 3H, J = 7.3 Hz), 1.32 (m, 2H), 1.42 (m, 2H), 1.51 (m, 3H), 1.62 (m, 1H), 2.20 (s, 3H), 2.25 (m, 2H), 2.35 (m, 6H), 2.54 (m, 1H), 2.64 (m, 1H), 3.37 and 3.54 (2d of AB system, 2H, J = 13.3 Hz), 3.71 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{19}H_{35}N_2O_2$ (M+H) 323.26, found 323.35.

3-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(propyl)amino]methyl}phenol (6'Q)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 0.89 (t, 3H, J = 7.3 Hz), 1.31 (m, 2H), 1.41 (m, 2H), 1.51 (m, 3H), 1.64 (m, 1H), 2.19 (s, 3H), 2.25 (m, 2H), 2.40 (m, 4H), 2.56 (m, 1H), 2.65 (m, 1H), 3.40 and 3.57 (2d of AB system, 2H, J = 13.7 Hz), 3.71 (m, 1H), 6.71 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.86 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{19}H_{35}N_2O_2$ (M+H) 323.26, found 323.25.

4-({[3-hydroxy-4-(piperidin-1-yl)butyl](propyl)amino}methyl)phenol (6R)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 1.38 (m, 2H), 1.56 (m, 8H), 2.20 (d, 2H, J = 6.5 Hz), 2.38 (m, 6H), 2.54 (m, 1H), 2.63 (m, 1H), 3.36 and 3.54 (2d of AB system, 2H, J = 13.3 Hz), 3.74 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{19}H_{33}N_2O_2$ (M+H) 321.25, found 321.30.

3-({[3-hydroxy-4-(piperidin-1-yl)butyl](propyl)amino}methyl)phenol (6'R)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 1.38 (m, 2H), 1.55 (m, 8H), 2.20 (d, 2H, J = 6.6 Hz), 2.37 (m, 6H), 2.55 (m, 1H), 2.65 (m, 1H), 3.40 and 3.57 (2d of AB system, 2H, J = 13.8 Hz), 3.73 (m, 1H), 6.70 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.4 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{19}H_{33}N_2O_2$ (M+H) 321.25, found 321.25.

4-({[3-hydroxy-4-(morpholin-4-yl)butyl](propyl)amino}methyl)phenol (6S)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 1.55 (m, 4H), 2.24 (m, 2H), 2.31 (m, 1H), 2.40 (m, 5H), 2.55 (m, 1H), 2.64 (m, 1H), 3.35 and 3.57 (2d of AB system, 2H, J = 13.2 Hz), 3.59 (t, 4H, J = 4.6 Hz), 3.78 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{18}H_{31}N_2O_3$ (M+H) 323.23, found 323.30.

3-({[3-hydroxy-4-(morpholin-4-yl)butyl](propyl)amino}methyl)phenol (6'S)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 1.57 (m, 4H), 2.24 (m, 2H), 2.38 (m, 6H), 2.56 (m, 1H), 2.65 (m, 1H), 2.89 (s_{broad}, 1H), 3.38 (d, 1H, J = 13.6 Hz), 3.55 (m, 4H), 3.79 (m, 1H), 6.71 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.12 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{18}H_{31}N_2O_3$ (M+H) 323.23, found 323.25.

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](propyl)amino}methyl)phenol (6T)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 1.54 (m, 4H), 2.20-2.70 (m, 12H), 3.35 and 3.55 (2d of AB system, 2H, J = 13.3 Hz), 3.46 (s, 2H), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.4 Hz), 7.22 (m, 1H), 7.29 (m, 4H). LRMS calculated for $C_{25}H_{38}N_3O_2$ (M+H) 412.29, found 412.30.

3-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](propyl)amino}methyl)phenol (6'T)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 1.56 (m, 4H), 2.20-2.68 (m, 14H), 3.39 and 3.57 (2d of AB system, 2H, J = 13.6 Hz), 3.46 (s, 2H), 3.75 (m, 1H), 6.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.6 Hz), 6.85 (d, 1H, J = 1.5 Hz), 7.11 (t, 1H, J = 7.8 Hz), 7.25 (m, 1H), 7.34 (m, 4H). LRMS calculated for $C_{25}H_{38}N_3O_2$ (M+H) 412.29, found 412.30.

4-({[3-hydroxy-4-(phenylsulfanyl)butyl](propyl)amino}methyl)phenol (6U)
Yellowish oil. ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 7.3 Hz), 1.53 (m, 2H), 1.77 (m, 2H), 2.26 (m, 1H), 2.53 (m, 1H), 2.63 (m, 1H), 2.77 (m, 1H), 2.86 (dd, 1H, J_1 = 7.8 Hz, J_2 = 13.3 Hz), 3.12 (m, 1H), 3.20 and 3.73 (2d, 1H, J = 12.9 Hz), 3.93 (m, 1H), 6.75 (d, 2H,

J = 8.4 Hz), 7.12 (m, 3H), 7.23 (m, 2H), 7.30 (m, 4H). LRMS calculated for $C_{20}H_{27}NO_2S$ (M+H) 346.18, found 346.25.

4-({[3-hydroxy-4-(pyridin-4-ylsulfanyl)butyl](propyl)amino}methyl)phenol (6V)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7.4 Hz), 1.52 (m, 2H), 1.79 (m, 2H), 2.40 (m, 2H), 2.63 (m, 1H), 2.69 (m, 1H), 3.05 (d, 2H, J = 6.3 Hz), 3.40 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.92 (m, 1H), 6.73 (d, 2H, J = 8.4 Hz), 7.11 (d, 2H, J = 8.4 Hz), 7.29 (d, 2H, J = 5.5 Hz), 8.30 (d, 2H, J = 5.9 Hz). LRMS calculated for $C_{19}H_{27}N_2O_2S$ (M+H) 347.17, found 347.20.

4-({[3-hydroxy-4-(pyridin-2-ylsulfanyl)butyl](propyl)amino}methyl)phenol (6W)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.87 (t, 3H, J = 7.4 Hz), 1.54 (m, 2H), 1.79 (m, 2H), 2.41 (m, 2H), 2.62 (m, 1H), 2.69 (m, 1H), 3.23 (dd, 2H, J_1 = 1.6 Hz, J_2 = 5.9 Hz), 3.41 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.89 (m, 1H), 6.73 (d, 2H, J = 8.5 Hz), 7.11 (m, 3H), 7.29 (d, 1H, J = 8.1 Hz), 7.65 (m, 1H), 8.37 (m, 1H). LRMS calculated for $C_{19}H_{27}N_2O_2S$ (M+H) 347.17, found 347.20.

4-{[{3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}(propyl)amino]methyl}phenol (6X)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.87 (t, 3H, J = 7.4 Hz), 1.54 (m, 2H), 1.65 (m, 1H), 1.81 (m, 1H), 2.30-2.68 (m, 4H), 2.82 (m, 2H), 3.38 and 3.56 (2d of AB system, 2H, J = 13.0 Hz), 3.69 (m, 1H), 3.79 (s, 3H), 6.75 (d, 2H, J = 8.4 Hz), 6.88 (d, 2H, J = 8.7 Hz), 7.10 (d, 2H, J = 8.4 Hz), 7.35 (d, 2H, J = 8.7 Hz). LRMS calculated for $C_{21}H_{30}NO_3S$ (M+H) 376.19, found 376.20.

4-{[{3-hydroxy-4-[(3-methoxyphenyl)sulfanyl]butyl}(propyl)amino]methyl}phenol (6Y)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (t, 3H, J = 7.4 Hz), 1.55 (m, 2H), 1.68 (m, 1H), 1.84 (m, 1H), 2.30-2.70 (m, 4H), 2.96 (m, 2H), 3.39 and 3.57 (2d of AB system, 2H, J = 13.1 Hz), 3.77 (m, 1H), 3.78 (s, 3H), 6.77 (m, 3H), 6.92 (m, 2H), 7.10 (d, 2H, J = 8.4 Hz), 7.20 (t, 1H, J = 8.2 Hz). LRMS calculated for $C_{21}H_{30}NO_3S$ (M+H) 376.19, found 376.15.

4-({hexyl[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl]amino}methyl)phenol (7P)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, 3H, J = 6.9 Hz), 1.25 (m, 6H), 1.56 (m, 4H), 2.17 (s, 3H), 2.24 (m, 2H), 2.25-2.67 (m, 14H), 3.35 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{22}H_{40}N_3O_2$ (M+H) 378.30, found 378.35.

3-({hexyl[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl]amino}methyl)phenol (7'P)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, 3H, J = 6.9 Hz), 1.28 (m, 6H), 1.56 (m, 4H), 2.17 (s, 3H), 2.48 (m, 14H), 3.38 and 3.58 (2d of AB system, 2H, J = 13.5 Hz), 3.77 (m, 1H), 6.71 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.78 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{22}H_{40}N_3O_2$ (M+H) 378.30, found 378.30.

4-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(hexyl)amino]methyl}phenol (7Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.88 (m, 6H), 1.29 (m, 8H), 1.50 (m, 5H), 1.64 (m, 1H), 2.23 (s, 3H), 2.29 (d, 2H, J = 6.4 Hz), 2.39 (m, 4H), 2.55 (m, 1H), 2.65 (m, 1H), 3.38 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.74 (m, 1H), 6.79 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{22}H_{41}N_2O_2$ (M+H) 365.31, found 365.35.

3-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(hexyl)amino]methyl}phenol (7'Q)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.88 (m, 6H), 1.30 (m, 8H), 1.51 (m, 5H), 1.64 (m, 1H), 2.20 (s, 3H), 2.26 (m, 2H), 2.42 (m, 4H), 2.56 (m, 1H), 2.64 (m, 1H), 3.39 and 3.57 (2d of AB system, 2H, J = 13.6 Hz), 3.72 (m, 1H), 6.72 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.86 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{22}H_{41}N_2O_2$ (M+H) 365.31, found 365.30.

4-({hexyl[3-hydroxy-4-(piperidin-1-yl)butyl]amino}methyl)phenol (7R)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, 3H, J = 6.9 Hz), 1.28 (m, 6H), 1.53 (m, 10H), 2.23 (d, 2H, J = 6.5 Hz), 2.25-2.67 (m, 8H), 3.37 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.76 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{22}H_{39}N_2O_2$ (M+H) 363.29, found 363.35.

3-({hexyl[3-hydroxy-4-(piperidin-1-yl)butyl]amino}methyl)phenol (7'R)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, 3H, J = 6.9 Hz), 1.26 (m, 6H), 1.39 (m, 2H), 1.58 (m, 8H), 2.20 (d, 2H, J = 6.5 Hz), 2.30-2.68 (m, 8H), 3.39 and 3.57 (2d of AB system, 2H, J = 13.6 Hz), 3.75 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.78 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{22}H_{39}N_2O_2$ (M+H) 363.29, found 363.25.

4-({hexyl[3-hydroxy-4-(morpholin-4-yl)butyl]amino}methyl)phenol (7S)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.86 (t, 3H, J = 7.0 Hz), 1.30 (m, 6H), 1.48 (m, 2H), 1.60 (m, 2H), 2.25 (m, 2H), 2.28-2.70 (m, 8H), 3.34 (d, 1H, J = 13.2 Hz), 3.48 (m, 5H), 3.79 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{21}H_{37}N_2O_3$ (M+H) 365.27, found 365.30.

3-({hexyl[3-hydroxy-4-(morpholin-4-yl)butyl]amino}methyl)phenol (7'S)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.87 (t, 3H, J = 6.9 Hz), 1.29 (m, 6H), 1.50 (m, 2H), 1.60 (m, 2H), 2.25 (m, 2H), 2.30-2.70 (m, 8H), 3.37 (d of AB system, 1H, J = 13.5 Hz), 3.57 (m, 5H), 3.80 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.84 (d, 1H, J = 1.6 Hz), 7.12 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{37}N_2O_3$ (M+H) 365.27, found 365.25.

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](hexyl)amino}methyl)phenol (7T)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.86 (t, 3H, J = 6.9 Hz), 1.32 (m, 6H), 1.55 (m, 4H), 2.22-2.68 (m, 14H), 3.36 and 3.56 (2d of AB system, 2H, J = 13.1 Hz), 3.47 (s, 2H), 3.76 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz), 7.23 (m, 1H), 7.32 (m, 4H). LRMS calculated for $C_{28}H_{44}N_3O_2$ (M+H) 454.34, found 454.35.

3-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](hexyl)amino}methyl)phenol (7'T)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.86 (t, 3H, J = 6.9 Hz), 1.31 (m, 6H), 1.57 (m, 4H), 2.25 (m, 2H), 2.26-2.70 (m, 12H), 3.38 and 3.58 (2d of AB system, 2H, J = 13.5 Hz), 3.47 (s, 2H), 3.77 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.4 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz), 7.24 (m, 1H), 7.31 (m, 4H). LRMS calculated for $C_{28}H_{44}N_3O_2$ (M+H) 454.34, found 454.30.

4-({hexyl[3-hydroxy-4-(phenylsulfanyl)butyl]amino}methyl)phenol (7U)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.30 (m, 6H), 1.56 (m, 2H), 1.73 (m, 1H), 1.85 (m, 1H), 2.32-2.70 (4m, 4H), 2.96 (m, 2H), 3.38 and 3.57 (2d of AB system, 2H, J = 13.0 Hz), 3.78 (m, 1H), 6.74 (d, 2H, J = 8.4 Hz), 7.11 (d, 2H, J = 8.4 Hz), 7.17 (m, 1H), 7.33 (m, 4H). LRMS calculated for $C_{23}H_{34}NO_2S$ (M+H) 388.22 found 388.25.

4-({hexyl[3-hydroxy-4-(pyridin-4-ylsulfanyl)butyl]amino}methyl)phenol (7V)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 6.9 Hz), 1.31 (m, 6H), 1.49 (m, 2H), 1.82 (m, 2H), 2.34-2.74 (4m, 4H), 3.07 (m, 2H), 3.39 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.92 (m, 1H), 6.73 (d, 2H, J = 8.5 Hz), 7.11 (d, 2H, J = 8.4 Hz), 7.30 (d, 2H, J = 6.4 Hz), 8.30 (d, 2H, J = 6.3 Hz). LRMS calculated for $C_{22}H_{33}N_2O_2S$ (M+H) 389.22 found 389.25.

4-({hexyl[3-hydroxy-4-(pyridin-2-ylsulfanyl)butyl]amino}methyl)phenol (7W)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, J = 6.9 Hz), 1.29 (m, 6H), 1.56 (m, 2H), 1.80 (m, 2H), 2.32-2.74 (4m, 4H), 3.23 (d, 2H, J = 6.0 Hz), 3.40 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.90 (m, 1H), 6.73 (d, 2H, J = 8.5 Hz), 7.12 (m, 3H), 7.29 (d, 1H, J = 8.1 Hz), 7.67 (m, 1H), 8.37 (d, 1H, J = 4.1 Hz). LRMS calculated for $C_{22}H_{33}N_2O_2S$ (M+H) 389.22 found 389.20.

4-[(hexyl{3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}amino)methyl]phenol (7X)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 1.28 (m, 6H), 1.54 (m, 2H), 1.65 (m, 1H), 1.86 (m, 1H), 2.30-2.68 (4m, 4H), 2.83 (m, 2H), 3.36 and 3.56 (2d of AB system, 2H, J = 13.0 Hz), 3.71 (m, 1H), 3.78 (s, 3H), 6.75 (d, 2H, J = 8.4 Hz), 6.87 (d, 2H, J = 7.8 Hz), 7.10 (d, 2H, J = 8.3 Hz), 7.35 (d, 2H, J = 7.4 Hz). LRMS calculated for $C_{24}H_{36}NO_3S$ (M+H) 418.23 found 418.25.

4-[(hexyl{3-hydroxy-4-[(3-methoxyphenyl)sulfanyl]butyl}amino)methyl]phenol (7Y)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H, J = 6.9 Hz), 1.28 (m, 6H), 1.49 (m, 2H), 1.68 (m, 1H), 1.85 (m, 1H), 2.31-2.71 (4m, 4H), 2.96 (m, 2H), 3.38 and 3.57 (2d of AB system, 2H, J = 13.0 Hz), 3.78 (s, 3H), 3.80 (m, 1H), 6.77 (m, 3H), 6.92 (m, 2H), 7.10 (d, 2H, J = 8.5 Hz), 7.20 (t, 1H, J = 8.2 Hz). LRMS calculated for $C_{24}H_{36}NO_3S$ (M+H) 418.23 found 418.25.

4-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](3-methylbutyl)amino} methyl)phenol (8P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.83 (dd, 6H, J_1 = 3.3 Hz, J_2 = 6.6 Hz), 1.38 (q_{app}, 2H, J = 7.1 Hz), 1.60 (m, 3H), 2.17 (s, 3H), 2.24 (m, 2H), 2.20-2.68 (m, 10H), 3.35 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{21}H_{38}N_3O_2$ (M+H) 364.29, found 364.40.

3-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](3-methylbutyl)amino}methyl) phenol (8'P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (dd, 6H, J_1 = 2.7 Hz, J_2 = 6.6 Hz), 1.39 (q, 2H, J = 7.2 Hz), 1.59 (m, 3H), 2.17 (s, 3H), 2.24 (m, 2H), 2.20-2.75 (m, 10H), 3.38 and 3.58 (2d of AB system, 2H, J = 13.5 Hz), 3.76 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.78 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{38}N_3O_2$ (M+H) 364.29, found 364.30.

4-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(3-methylbutyl)amino]methyl}phenol (8Q)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.83 (dd, 6H, J_1 = 2.1 Hz, J_2 = 6.6 Hz), 0.90 (t, 3H, J = 7.3 Hz), 1.38 (m, 6H), 1.60 (m, 3H), 2.23 (s, 3H), 2.28 (d, 2H, J = 6.5 Hz), 2.30-2.68 (m, 6H), 3.37 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.73 (m, 1H), 6.79 (d,

2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{21}H_{39}N_2O_2$ (M+H) 351.29, found 351.35.

3-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(3-methylbutyl)amino]methyl}phenol (8'Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (dd, 6H, J_1 = 1.8 Hz, J_2 = 6.6 Hz), 0.90 (t, 3H, J = 7.3 Hz), 1.38 (m, 6H), 1.60 (m, 3H), 2.20 (s, 3H), 2.26 (m, 2H), 2.38 (m, 3H), 2.30-2.68 (m, 3H), 3.39 and 3.56 (2d of AB system, 2H, J = 13.6 Hz), 3.71 (m, 1H), 6.71 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{39}N_2O_2$ (M+H) 351.29, found 351.25.

4-({[3-hydroxy-4-(piperidin-1-yl)butyl](3-methylbutyl)amino}methyl)phenol (8R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.83 (dd, 6H, J_1 = 2.5 Hz, J_2 = 6.6 Hz), 1.40 (m, 4H), 1.55 (m, 7H), 2.24 (d, 2H, J = 6.5 Hz), 2.30-2.67 (m, 8H), 3.37 and 3.55 (2d of AB system, 2H, J = 13.2 Hz), 3.76 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{21}H_{37}N_2O_2$ (M+H) 349.28, found 349.35.

3-({[3-hydroxy-4-(piperidin-1-yl)butyl](3-methylbutyl)amino}methyl)phenol (8'R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (dd, 6H, J_1 = 2.2 Hz, J_2 = 6.6 Hz), 1.39 (m, 4H), 1.56 (m, 8H), 2.20 (d, 2H, J = 6.5 Hz), 2.25-2.68 (m, 8H), 3.39 and 3.57 (2d of AB system, 2H, J = 13.7 Hz), 3.74 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{37}N_2O_2$ (M+H) 349.28, found 349.25.

4-({[3-hydroxy-4-(morpholin-4-yl)butyl](3-methylbutyl)amino}methyl)phenol (8S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.83 (dd, 6H, J_1 = 4.0 Hz, J_2 = 6.6 Hz), 1.39 (m, 2H), 1.61 (m, 3H), 2.25 (d, 3H, J = 5.3 Hz), 2.29-2.70 (m, 8H), 3.35 (d, 1H, J = 13.2 Hz), 3.59 (m, 5H), 3.78 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{20}H_{35}N_2O_3$ (M+H) 351.26, found 351.25.

3-({[3-hydroxy-4-(morpholin-4-yl)butyl](3-methylbutyl)amino}methyl)phenol (8'S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (dd, 6H, J_1 = 3.2 Hz, J_2 = 6.6 Hz), 1.39 (q, 2H, J = 7.2 Hz), 1.60 (m, 3H), 2.25 (m, 2H), 2.33-2.60 (m, 8H), 3.38 (d, 1H, J = 13.5 Hz), 3.59 (m, 5H), 3.80 (m, 1H), 6.71 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.12 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{20}H_{35}N_2O_3$ (M+H) 351.26, found 351.25.

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](3-methylbutyl)amino} methyl)phenol (8T)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.83 (dd, 6H, J_1 = 3.2 Hz, J_2 = 5.8 Hz), 1.37 (m, 2H), 1.60 (m, 3H), 2.25 (m, 2H), 2.22-2.68 (m, 12H), 3.35 and 3.55 (2d of AB system, 2H, J = 13.4 Hz), 3.47 (s, 2H), 3.76 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz), 7.24 (m, 1H), 7.31 (m, 4H). LRMS calculated for $C_{27}H_{42}N_3O_2$ (M+H) 440.32, found 440.30.

3-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](3-methylbutyl)amino}methyl) phenol (8'T)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.84 (dd, 6H, J_1 = 2.5 Hz, J_2 = 6.6 Hz), 1.38 (q, 2H, J = 7.4 Hz), 1.59 (m, 3H), 2.26 (m, 2H), 2.30-2.68 (m, 12H), 3.39 and 3.58 (2d of AB system, 2H, J = 13.5 Hz), 3.47 (s, 2H), 3.77 (m, 1H), 6.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 2H, J = 7.5 Hz), 6.85 (d, 1H, J = 1.6 Hz), 7.11 (t, 1H, J = 7.7 Hz), 7.25 (m, 1H), 7.32 (m, 4H). LRMS calculated for $C_{27}H_{42}N_3O_2$ (M+H) 440.32, found 440.30.

4-({[3-hydroxy-4-(phenylsulfanyl)butyl](3-methylbutyl)amino}methyl)phenol (8U)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (dd, 6H, J_1 = 2.9 Hz, J_2 = 6.6 Hz), 1.39 (q, 2H, J = 7.3 Hz), 1.53 (m, 1H), 1.69 (m, 1H), 1.88 (m, 1H), 2.34-2.71 (4m, 4H), 2.96 (m, 2H), 3.38 and 3.57 (2d of AB system, 2H, J = 12.9 Hz), 3.78 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.11 (d, 2H, J = 8.5 Hz), 7.20 (m, 1H), 7.32 (m, 4H). LRMS calculated for $C_{22}H_{32}NO_2S$ (M+H) 374.23, found 374.25.

4-({[3-hydroxy-4-(pyridin-4-ylsulfanyl)butyl](3-methylbutyl)amino}methyl)phenol (8V)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.87 (dd, 6H, J_1 = 3.0 Hz, J_2 = 6.6 Hz), 1.41 (q, 2H, J = 7.2 Hz), 1.55 (m, 1H), 1.79 (m, 2H), 2.36-2.75 (4m, 4H), 3.07 (m, 2H), 3.39 and 3.59 (2d of AB system, 2H, J = 13.0 Hz), 3.93 (m, 1H), 6.73 (d, 2H, J = 8.5 Hz), 7.12 (d, 2H, J = 8.5 Hz), 8.30 (d, 2H, J = 6.4 Hz), 7.30 (d, 2H, J = 6.3 Hz). LRMS calculated for $C_{21}H_{31}N_2O_2S$ (M+H) 375.20, found 375.25.

4-({[3-hydroxy-4-(pyridin-2-ylsulfanyl)butyl](3-methylbutyl)amino}methyl)phenol (8W)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (dd, 6H, J_1 = 2.8 Hz, J_2 = 6.6 Hz), 1.40 (q, 2H, J = 7.3 Hz), 1.54 (m, 1H), 1.79 (m, 2H), 2.35-2.74 (4m, 4H), 3.24 (d, 2H, J = 5.9 Hz), 3.40 and 3.58 (2d of AB system, 2H, J = 13.0 Hz), 3.90 (m, 1H), 6.73 (d, 2H, J = 8.4 Hz), 7.14 (m, 3H), 7.29 (d, 1H, J = 8.1 Hz), 7.60 (m, 1H), 8.37 (d, 1H, J = 4.8 Hz). LRMS calculated for $C_{21}H_{31}N_2O_2S$ (M+H) 375.20, found 375.20.

4-{[{3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}(3-methylbutyl) amino]methyl}phenol (8X)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (dd, 6H, J_1 = 2.9 Hz, J_2 = 6.6 Hz), 1.38 (q, 2H, J = 7.2 Hz), 1.52 (m, 1H), 1.65 (m, 1H), 1.81 (m, 1H), 2.33-2.68 (4m, 4H), 2.83 (m, 2H), 3.36 and 3.56 (2d of AB system, 2H, J = 13.0 Hz), 3.70 (m, 1H), 3.78 (s, 3H), 6.75 (d, 2H, J = 8.5 Hz), 6.87 (d, 2H, J = 8.8 Hz), 7.10 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.9 Hz). LRMS calculated for $C_{23}H_{34}NO_3S$ (M+H) 404.22, found 404.20.

4-{[{3-hydroxy-4-[(3-methoxyphenyl)sulfanyl]butyl}(3-methylbutyl)amino] methyl}phenol (8Y)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 0.86 (dd, 6H, J_1 = 2.8 Hz, J_2 = 6.6 Hz), 1.40 (q, 2H, J = 7.1 Hz), 1.53 (m, 1H), 1.68 (m, 1H), 1.85 (m, 1H), 2.33-2.71 (4m, 4H), 2.97 (m, 2H), 3.38 and 3.57 (2d of AB system, 2H, J = 13.0 Hz), 3.78 (s, 3H), 3.79 (m, 1H), 6.77 (m, 3H), 6.92 (m, 2H), 7.10 (d, 2H, J = 8.5 Hz), 7.20 (t, 1H, J = 8.9 Hz). LRMS calculated for $C_{23}H_{34}NO_3S$ (M+H) 404.22, found 404.20.

4-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](2-phenylethyl)amino} methyl)phenol (9P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.59 (m, 2H), 2.17 (s, 3H), 2.23 (m, 2H), 2.42 (m, 7H), 2.71 (m, 7H), 3.50 and 3.62 (2d of AB system, 2H, J = 13.3 Hz), 3.74 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (m, 5H), 7.24 (m, 2H). LRMS calculated for $C_{24}H_{36}N_3O_2$ (M+H) 398.55, found 398.35.

3-({[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl](2-phenylethyl)amino}methyl) phenol (9'P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.60 (m, 2H), 2.17 (s, 3H), 2.24 (m, 2H), 2.48 (m, 7H), 2.75 (m, 7H), 3.53 and 3.66 (2d of AB system, 2H, J = 13.5 Hz), 3.76 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.86 (s, 1H), 7.11 (t,

1H, J = 7.8 Hz), 7.17 (m, 3H), 7.25 (m, 2H). LRMS calculated for $C_{24}H_{36}N_3O_2$ (M+H) 398.55, found 398.30.

4-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(2-phenylethyl)amino]methyl}phenol (9Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.90 (t, 3H, J = 7.3 Hz), 1.32 (m, 2H), 1.41 (m, 2H), 1.55 (m, 1H), 1.64 (m, 1H), 2.20 (s, 3H), 2.25 (m, 2H), 2.35 (m, 2H), 2.73 (m, 6H), 3.51 and 3.62 (2d of AB system, 2H, J = 13.3 Hz), 3.71 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.16 (m, 5H), 7.24 (m, 2H). LRMS calculated for $C_{24}H_{37}N_2O_2$ (M+H) 385.28, found 385.30.

3-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(2-phenylethyl)amino]methyl}phenol (9'Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.90 (t, 3H, J = 7.3 Hz), 1.31 (m, 2H), 1.41 (m, 2H), 1.55 (m, 1H), 1.66 (m, 1H), 2.20 (s, 3H), 2.24 (m, 2H), 2.34 (m, 2H), 2.73 (m, 6H), 3.54 and 3.65 (2d of AB system, 2H, J = 13.7 Hz), 3.71 (m, 1H), 6.72 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.80 (d, 1H, J = 7.5 Hz), 6.88 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz), 7.20 (m, 3H), 7.25 (m, 2H). LRMS calculated for $C_{24}H_{37}N_2O_2$ (M+H) 385.28, found 385.25.

4-({[3-hydroxy-4-(piperidin-1-yl)butyl](2-phenylethyl)amino}methyl)phenol (9R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.40 (m, 2H), 1.60 (m, 6H), 2.26 (d, 2H, J = 6.4 Hz), 2.40 (m, 2H), 2.50 (m, 2H), 2.72 (m, 6H), 3.51 and 3.61 (2d of AB system, 2H, J = 13.3 Hz), 3.78 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (m, 5H), 7.24 (m, 2H). LRMS calculated for $C_{24}H_{35}N_2O_2$ (M+H) 383.26, found 383.25.

3-({[3-hydroxy-4-(piperidin-1-yl)butyl](2-phenylethyl)amino}methyl)phenol (9'R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.39 (m, 2H), 1.55 (m, 6H), 2.20 (d, 2H, J = 6.6 Hz), 2.33 (m, 2H), 2.45 (m, 2H), 2.74 (m, 6H), 3.54 and 3.65 (2d of AB system, 2H, J = 13.7 Hz), 3.75 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.80 (d, 1H, J = 7.5 Hz), 6.87 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz), 7.17 (m, 3H), 7.24 (m, 2H). LRMS calculated for $C_{24}H_{35}N_2O_2$ (M+H) 383.26, found 383.20.

4-({[3-hydroxy-4-(morpholin-4-yl)butyl](2-phenylethyl)amino}methyl)phenol (9S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.61 (m, 2H), 2.24 (m, 2H), 2.38 (m, 2H), 2.44 (m, 2H), 2.75 (m, 6H), 3.49 and 3.64 (2d of AB system, 2H, J = 13.3 Hz), 3.57 (m, 4H), 3.78 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.16 (m, 5H), 7.25 (m, 2H). LRMS calculated for $C_{23}H_{33}N_2O_3$ (M+H) 385.24, found 385.25.

3-({[3-hydroxy-4-(morpholin-4-yl)butyl](2-phenylethyl)amino}methyl)phenol (9'S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.61 (m, 2H), 2.24 (m, 2H), 2.38 (m, 2H), 2.46 (m, 2H), 2.75 (m, 6H), 3.53 and 3.67 (2d of AB system, 2H, J = 13.6 Hz), 3.60 (m, 4H), 3.79 (m, 1H), 6.72 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.87 (d, 1H, J = 1.6 Hz), 7.11 (t, 1H, J = 7.8 Hz), 7.17 (m, 3H), 7.25 (m, 2H). LRMS calculated for $C_{23}H_{33}N_2O_3$ (M+H) 385.24, found 385.20.

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](2-phenylethyl)amino}methyl) phenol (9T)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.60 (m, 2H), 2.27 (m, 2H), 2.47 (m, 7H), 2.74 (m, 7H), 3.47 (s, 2H), 3.50 and 3.62 (2d of AB system, 2H, J = 13.4 Hz), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.16 (m, 5H), 7.24 (m, 2H), 7.32 (m, 4H). LRMS calculated for $C_{30}H_{40}N_3O_2$ (M+H) 474.30, found 474.30.

3-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](propyl)amino}methyl)phenol (9'T)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.60 (m, 2H), 2.26 (m, 2H), 2.50 (m, 7H), 2.75 (m, 7H), 3.47 (s, 2H), 3.54 and 3.66 (2d of AB system, 2H, J = 13.6 Hz), 3.77 (m, 1H), 6.71 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.86 (d, 1H, J = 1.4 Hz), 7.11 (t, 1H, J = 7.8 Hz), 7.20 (m, 6H), 7.32 (m, 4H). LRMS calculated for $C_{30}H_{40}N_3O_2$ (M+H) 474.30, found 474.30.

C4-({[3-hydroxy-4-(phenylsulfanyl)butyl](2-phenylethyl)amino}methyl)phenol (9U)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.68 (m, 1H), 1.87 (m, 1H), 2.74 (m, 6H), 2.97 (m, 2H), 3.49 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.79 (m, 1H), 6.74 (d, 2H, J = 8.5 Hz), 7.25 (m, 12H). LRMS calculated for $C_{25}H_{30}NO_2S$ (M+H) 408.19, found 408.20.

4-({[3-hydroxy-4-(pyridin-4-ylsulfanyl)butyl](2-phenylethyl)amino}methyl)phenol (9V)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.76 (m, 1H), 1.84 (m, 1H), 2.79 (m, 6H), 3.05 (m, 2H), 3.51 and 3.64 (2d of AB system, 2H, J = 13.0 Hz), 3.92 (m, 1H), 6.72 (d, 2H, J = 8.5 Hz), 7.13 (m, 5H), 7.24 (t, 2H, J = 7.3 Hz), 7.28 (d, 2H, J = 6.3 Hz), 8.29 (d, 2H, J = 6.3 Hz). LRMS calculated for $C_{24}H_{29}N_2O_2S$ (M+H) 409.19, found 409.20.

4-({[3-hydroxy-4-(pyridin-2-ylsulfanyl)butyl](2-phenylethyl)amino}methyl)phenol (9W)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.75 (m, 1H), 1.84 (m, 1H), 2.73 (m, 6H), 2.23 (dd, 2H, J_1 = 2.3 Hz, J_2 = 5.8 Hz), 3.51 and 3.64 (2d of AB system, 2H, J = 13.0 Hz), 3.90 (m, 1H), 6.73 (d, 2H, J = 8.4 Hz), 7.12 (m, 6H), 7.23 (m, 2H), 7.29 (d, 1H, J = 8.1 Hz), 7.55 (m, 1H), 8.36 (d, 1H, J = 4.9 Hz). LRMS calculated for $C_{24}H_{29}N_2O_2S$ (M+H) 409.19, found 409.15.

4-{[{3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}(2-phenylethyl)amino]methyl} phenol (9X)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.65 and 1.86 (2m, 2H), 2.74 (m, 8H), 3.47 and 3.61 (2d of AB system, 2H, J = 13.0 Hz), 3.72 (m, 1H), 3.77 (s, 3H), 6.75 (d, 2H, J = 8.5 Hz), 6.87 (d, 2H, J = 8.8 Hz), 7.13 (m, 5H), 7.23 (d, 2H, J = 7.5 Hz), 7.35 (d, 2H, J = 8.8 Hz).). LRMS calculated for $C_{26}H_{32}NO_3S$ (M+H) 438.20, found 438.20.

4-{[{3-hydroxy-4-[(3-methoxyphenyl)sulfanyl]butyl}(2-phenylethyl)amino]methyl} phenol (9Y)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.68 (m, 1H), 1.88 (m, 1H), 2.69 (m, 6H), 2.97 (m, 2H), 3.49 and 3.62 (2d of AB system, 2H, J = 13.0 Hz), 3.77 (s, 3H), 3.79 (m, 1H), 6.75 (m, 3H), 6.92 (m, 2H), 7.11 (m, 5H), 7.22 (m, 3H). LRMS calculated for $C_{26}H_{32}NO_3S$ (M+H) 438.20, found 438.20.

3-{4-[(3-ethoxypropyl)(4-hydroxybenzyl)amino]-2-hydroxybutoxy}benzamide (10O)
Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.15 (t, 3H, J = 7.0 Hz), 1.80 (m, 4H), 2.64 (m, 4H), 3.45 (m, 5H), 3.64 (d, 1H, J = 13.1 Hz), 3.91 (m, 2H), 4.04 (m, 1H), 6.75 (m, 2H), 7.14 (m, 3H), 7.46 (m, 3H). LRMS calculated for $C_{23}H_{33}N_2O_5$ (M+H) 417.23, found 417.25.

4-({(3-ethoxypropyl)[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl]amino}methyl) phenol (10P)
Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.08 (t, 3H, J = 7.0 Hz), 1.58 (m, 2H), 1.71 (m, 2H), 2.17 (s, 3H), 2.23 (m, 2H), 2.20-2.67 (m, 12H), 3.38 (m, 5H), 3.55 (d, 1H, J = 13.3

Hz), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{21}H_{38}N_3O_3$ (M+H) 380.28, found 380.35.

3-({(3-ethoxypropyl)[3-hydroxy-4-(4-methylpiperazin-1-yl)butyl]amino}methyl) phenol (10'P)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, 3H, J = 7.0 Hz), 1.57 (m, 2H), 1.71 (m, 2H), 2.17 (s, 3H), 2.24 (m,2H), 2.20-2.68 (m, 12H), 3.43 (m, 5H), 3.57 (d of AB system, 1H, J = 13.1 Hz), 3.76 (m, 1H), 6.70 (dd, 1H, J_1 = 1.7 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.84 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{38}N_3O_3$ (M+H) 380.28, found 380.25.

4-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(3-ethoxypropyl)amino]methyl} phenol (10Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.90 (t, 3H, J = 7.2 Hz), 1.08 (t, 3H, J = 7.0 Hz), 1.33 (m, 2H), 1.40 (m, 2H), 1.53 (m, 1H), 1.68 (m, 3H), 2.19 (s, 3H), 2.20-2.65 (m, 8H), 3.40 (m, 3H), 3.53 (d, 1H, J = 13.3 Hz), 3.70 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{21}H_{39}N_2O_3$ (M+H) 367.29, found 367.35.

3-{[{4-[butyl(methyl)amino]-3-hydroxybutyl}(3-ethoxypropyl)amino]methyl} phenol (10'Q)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 0.90 (t, 3H, J = 7.3 Hz), 1.09 (t, 3H, J = 7.1 Hz), 1.31 (m, 2H), 1.41 (m, 2H), 1.61 (m, 2H), 1.73 (m, 2H), 2.20 (s, 3H), 2.20-2.68 (m, 8H), 3.39 (m, 5H), 3.59 (d, 1H, J = 14.0 Hz), 3.72 (m, 1H), 6.71 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{39}N_2O_3$ (M+H) 367.29, found 367.30.

4-({(3-ethoxypropyl)[3-hydroxy-4-(piperidin-1-yl)butyl]amino}methyl)phenol (10R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, 3H, J = 7.0 Hz), 1.39 (m, 3H), 1.57 (m, 5H), 1.70 (m, 2H), 2.17 (m, 3H), 2.30-2.65 (m, 7H), 3.37 (m, 5H), 3.53 (d, 1H, J = 13.3 Hz), 3.74 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.4 Hz). LRMS calculated for $C_{21}H_{37}N_2O_3$ (M+H) 365.27, found 365.30.

3-({(3-ethoxypropyl)[3-hydroxy-4-(piperidin-1-yl)butyl]amino}methyl)phenol (10'R)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, 3H, J = 7.0 Hz), 1.41 (m, 2H), 1.59 (m, 6H), 1.72 (m, 2H), 2.20 (d, 2H, J = 6.6 Hz), 2.33 (m, 2H), 2.55 (m, 6H), 3.39 (m, 5H), 3.56 (d, 1H, J = 13.6 Hz), 3.75 (m, 1H), 6.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (d, 1H, J = 1.5 Hz), 7.11 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{37}N_2O_3$ (M+H) 365.27, found 365.25.

4-({(3-ethoxypropyl)[3-hydroxy-4-(morpholin-4-yl)butyl]amino}methyl)phenol (10S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, 3H, J = 7.0 Hz), 1.61 (m, 2H), 1.71 (m, 2H), 2.25 (d, 2H, J = 7.0 Hz), 2.42 (m, 5H), 2.55 (m, 2H), 2.64 (m, 1H), 3.38 (m, 5H), 3.59 (m, 4H), 3.78 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz). LRMS calculated for $C_{21}H_{35}N_2O_4$ (M+H) 367.25, found 367.25.

3-({(3-ethoxypropyl)[3-hydroxy-4-(morpholin-4-yl)butyl]amino}methyl)phenol (10'S)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.09 (t, 3H, J = 7.0 Hz), 1.60 (m, 2H), 1.71 (m, 2H), 2.24 (m, 2H), 2.30-2.68 (4m, 8H), 3.41 (m, 5H), 3.58 (m, 5H), 3.80 (m, 1H), 6.71 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (s, 1H), 7.12 (t, 1H, J = 7.8 Hz). LRMS calculated for $C_{21}H_{35}N_2O_4$ (M+H) 367.25, found 367.20.

4-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](3-ethoxypropyl)amino}methyl) phenol (10T)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.08 (t, 3H, J = 7.0 Hz), 1.60 (m, 2H), 1.70 (m, 2H), 2.26 (m, 2H), 2.25-2.70 (m, 14H), 3.38 (m, 4H), 3.47 (s, 2H), 3.54 (d, 1H, J = 13.3 Hz), 3.75 (m, 1H), 6.78 (d, 2H, J = 8.5 Hz), 7.14 (d, 2H, J = 8.4 Hz), 7.24 (m, 1H), 7.31 (m, 4H). LRMS calculated for $C_{27}H_{42}N_3O_3$ (M+H) 456.31, found 456.30.

3-({[4-(4-benzylpiperazin-1-yl)-3-hydroxybutyl](3-ethoxypropyl)amino}methyl) phenol (10'T)

Yellowish oil. ^{1}H NMR (Acetone-d_6) δ 1.08 (t, 3H, J = 7.0 Hz), 1.59 (m, 2H), 1.71 (m, 2H), 2.25 (m, 2H), 2.50 (m, 12H), 3.39 (m, 5H), 3.47 (s, 2H), 3.56 (d, 1H, J = 13.7 Hz), 3.77 (m, 1H), 6.70 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.79 (d, 1H, J = 7.5 Hz), 6.85 (d, 1H, J = 1.6 Hz), 7.11 (t, 1H, J = 7.8 Hz), 7.25 (m, 1H), 7.32 (m, 4H). LRMS calculated for $C_{27}H_{42}N_3O_3$ (M+H) 456.31, found 456.30.

4-({(3-ethoxypropyl)[3-hydroxy-4-(phenylsulfanyl)butyl]amino}methyl)phenol (10U)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.72 (m, 3H), 1.84 (m, 1H), 2.55 (m, 4H), 2.97 (s, 2H), 3.42 (m, 5H), 3.55 (d, 1H, J = 13.0 Hz), 3.80 (m, 1H), 6.73 (d, 2H, J = 8.5 Hz), 7.10 (d, 2H, J = 8.5 Hz), 7.22 (m, 1H), 7.33 (m, 4H). LRMS calculated for $C_{22}H_{32}NO_3S$ (M+H) 390.20, found 390.25.

4-({(3-ethoxypropyl)[3-hydroxy-4-(pyridin-4-ylsulfanyl)butyl]amino} methyl)phenol (10V)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.1 Hz), 1.80 (m, 4H), 2.59 (m, 4H), 3.07 (m, 2H), 3.42 (m, 5H), 3.56 (d, 1H, J = 13.0 Hz), 3.94 (m, 1H), 6.72 (d, 2H, J = 8.3 Hz), 7.11 (d, 2H, J = 8.3 Hz), 7.30 (m, 2H), 8.30 (d, 2H, J = 5.1 Hz). LRMS calculated for $C_{21}H_{31}N_2O_3S$ (M+H) 391.20, found 391.20.

4-({(3-ethoxypropyl)[3-hydroxy-4-(pyridin-2-ylsulfanyl)butyl]amino} methyl)phenol (10W)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.74 (m, 3H), 1.84 (m, 1H), 2.57 (m, 4H), 3.24 (dd, 2H, J_1 = 1.9 Hz, J_2 = 5.9 Hz), 3.43 (m, 5H), 3.56 (d, 1H, J = 13.0 Hz), 3.91 (m, 1H), 6.72 (d, 2H, J = 8.4 Hz), 7.11 (m, 3H), 7.30 (d, 1H, J = 8.1 Hz), 7.61 (m, 1H), 8.37 (d, 1H, J = 4.9 Hz). LRMS calculated for $C_{21}H_{31}N_2O_3S$ (M+H) 391.20, found 391.20.

4-{[(3-ethoxypropyl){3-hydroxy-4-[(4-methoxyphenyl)sulfanyl]butyl}amino] methyl}phenol (10X)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.74 (m, 4H), 2.54 (m, 4H), 2.85 (m, 2H), 3.44 (m, 5H), 3.53 (d, 1H, J = 13.1 Hz), 3.71 (m, 1H), 3.79 (s, 3H), 6.74 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.8 Hz), 7.10 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.8 Hz). LRMS calculated for $C_{23}H_{34}NO_4S$ (M+H) 420.21, found 420.20.

4-{[(3-ethoxypropyl){3-hydroxy-4-[(3-methoxyphenyl)sulfanyl]butyl}amino] methyl}phenol (10Y)

Yellowish oil. ^{1}H NMR (CD_3OD) δ 1.14 (t, 3H, J = 7.0 Hz), 1.70 (m, 3H), 1.86 (m, 1H), 2.55 (m, 4H), 2.97 (m, 2H), 3.44 (m, 5H), 3.54 (d, 1H, J = 13.0 Hz), 3.78 (s, 3H), 3.79 (m, 1H), 6.76 (m, 3H), 6.93 (m, 2H), 7.10 (d, 2H, J = 8.5 Hz), 7.21 (t, 1H, J = 8.2 Hz). LRMS calculated for $C_{23}H_{34}NO_4S$ (M+H) 420.21, found 420.20.

Partie B

Développement d'inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 3

Chapitre 3

Deux dérivés de l'androstérone comme inhibiteurs de la biosynthèse des androgènes

1. Avant-propos

Des criblages effectués sur différents noyaux stéroïdiens dans notre laboratoire avaient montré que le noyau androstérone possédait une affinité intéressante vis-à-vis de la 17β-HSD3 et que, particulièrement lorsqu'une substitution est réalisée en position C-3 par des groupements hydrophobes, ceci permettait d'obtenir des inhibiteurs efficaces de la 17β-HSD3. Dans ce chapitre, nous utilisons les orientations spatiales observées dans la structure cristalline de deux de nos inhibiteurs de la 17β-HSD3 (une spiromorpholinone et un spirocarbamate) pour expliquer le rôle joué par la disposition spatiale du groupement hydrophobe sur l'activité inhibitrice. Les évaluations biologiques réalisées par Lucie Carolle Kenmogne, ma collègue étudiante au doctorat dans le laboratoire, ainsi que l'analyse des données cristallographiques réalisées par Mr Michel Simard du laboratoire de diffraction des rayons X de l'Université de Montréal ont été très utiles pour ce projet.

Dans le cadre de ce projet, j'ai synthétisé, caractérisé et fait la cristallisation des deux composés décrits.

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2. Résumé

Les composés indiqués dans le titre: la (3R,5S,5'R,8R,9S,10S,13S,14S)-10,13-diméthyl-5'-(2-méthylpropyl)tétradécahydro-6'H-spiro[cyclopenta[a]phénanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione, $C_{26}H_{41}NO_3$ (I), et la méthyl (2R)-2-[(3R,5S,8R,9S,10S,13S,14S)-10,13-diméthyl-2',17-dioxohexadécahydro-3'H-spiro[cyclopenta[a]phénanthrene-3,5'-[1,3]oxazolidin-3'-yl]]-4-méthylpentanoate, $C_{28}H_{43}NO_5$ (II), possèdent la forme typique des stéroïdes (cycles A-D), mais ils diffèrent par leur cycle supplémentaire E. Le cycle E de l'azalactone (I) présente une conformation demi-chaise, tandis que le cycle E du carbamate (II) est plan. L'orientation du substituant sur le cycle E est clairement établie et permet une rationalisation des résultats biologiques obtenus avec ces dérivés de l'androstérone.

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Two androsterone derivatives as inhibitors of androgen biosynthesis

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Abstract

The title compounds, (3R,5S,5'R,8R,9S,10S,13S,14S)-10,13-dimethyl-5'-(2-methylpropyl)tetradecahydro-6'H-spiro[cyclopenta[*a*]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione, $C_{26}H_{41}NO_3$ (I), and methyl (2R)-2-[(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[*a*]phenanthrene-3,5'-[1,3]oxazolidin-3'-yl]]-4-methylpentanoate, $C_{28}H_{43}NO_5$ (II), possess the typical steroid shape (A–D rings), but they differ in their extra E ring. The azalactone E ring in (I) shows a half-chair conformation, while the carbamate E ring of (II) is planar. The orientation of the E-ring substituent is clearly established and allows a rationalization of the biological results obtained with such androsterone derivatives.

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Two androsterone derivatives as inhibitors of androgen biosynthesis

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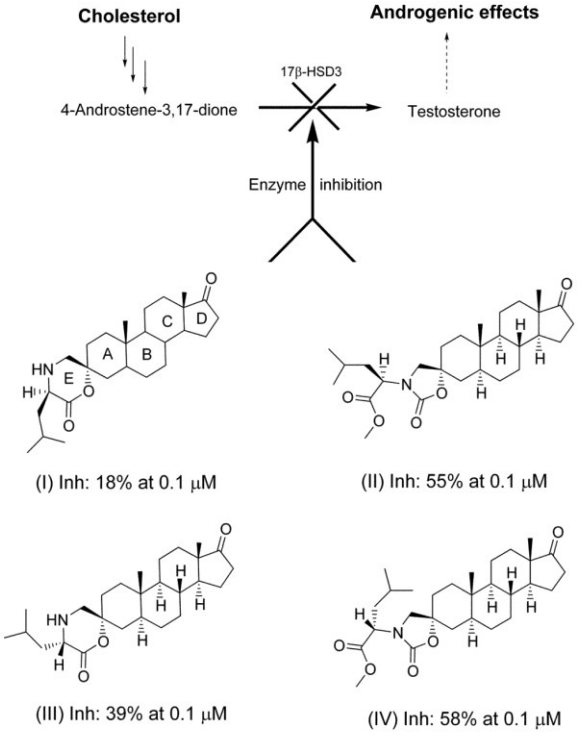
The title compounds, (3*R*,5*S*,5′*R*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-dimethyl-5′-(2-methylpropyl)tetradecahydro-6′*H*-spiro[cyclopenta[*a*]phenanthrene-3,2′-[1,4]oxazinane]-6′,17(2*H*)-dione, $C_{26}H_{41}NO_3$, (I), and methyl (2*R*)-2-[(3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-10,13-dimethyl-2′,17-dioxohexadecahydro-3′*H*-spiro[cyclopenta[*a*]phenanthrene-3,5′-[1,3]oxazolidin-3′-yl]]-4-methylpentanoate, $C_{28}H_{43}NO_5$, (II), possess the typical steroid shape (*A*–*D* rings), but they differ in their extra *E* ring. The azalactone *E* ring in (I) shows a half-chair conformation, while the carbamate *E* ring of (II) is planar. The orientation of the *E*-ring substituent is clearly established and allows a rationalization of the biological results obtained with such androsterone derivatives.

Comment

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) is an enzyme that catalyzes the reduction of 4-androstene-3,17-dione into the androgen testosterone (Poirier, 2010; Mohler *et al.*, 2007; Maltais *et al.*, 2011). This latter steroid hormone, as well as its natural metabolite dehydrotestosterone, is known to stimulate the proliferation of prostate cancer cells; the Scheme shows the biosynthesis of the androgenic hormone testosterone and a representation of azalactones (I) and (III) and carbamates (II) and (IV) tested as inhibitors of 17β-HSD3 (Inh = inhibition) (Poirier, 2008). Since 17β-HSD3 contributes to the production of androgens in men, inhibiting this enzyme is an interesting strategy to block androgen biosynthesis and to treat prostate cancer. Androsterone (ADT) derivatives substituted at position C3 were previously reported as inhibitors of 17β-HSD3 and it was established that a hydrophobic group is required to obtain good inhibition of 17β-HSD3 (Maltais *et al.*, 2002), a β-oriented group producing a better inhibition than an α-oriented group (Tchedam-Ngatcha *et al.*, 2005).

In our synthetic search for inhibitors of 17β-HSD3, we recently focused on the preparation of new ADT derivatives having an extra azalactone or carbamate *E* ring. For a better understanding of the structure–activity relationship (SAR) of



these new inhibitors, especially the side-chain orientation, we analyzed the structures of the representative compounds (I) and (II) (see Scheme and Fig. 1).

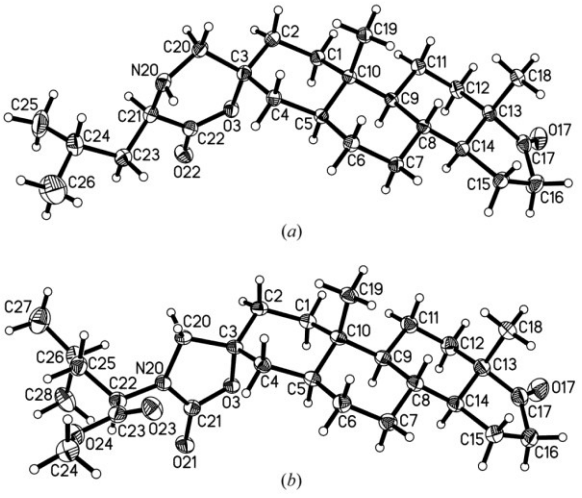


Figure 1
The molecular structures of (*a*) azalactone (I) and (*b*) carbamate (II), with non-H atoms represented by 50% probability displacement ellipsoids.

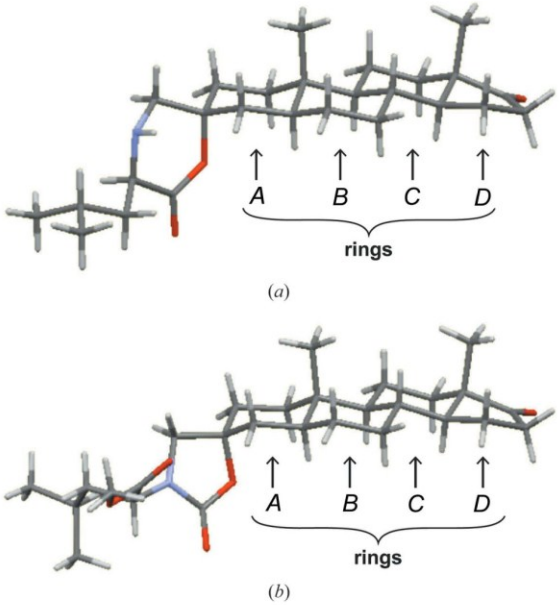


Figure 2
The structures of (*a*) (I) and (*b*) (II), showing the conformations of the steroid *A*, *B*, *C* and *D* rings.

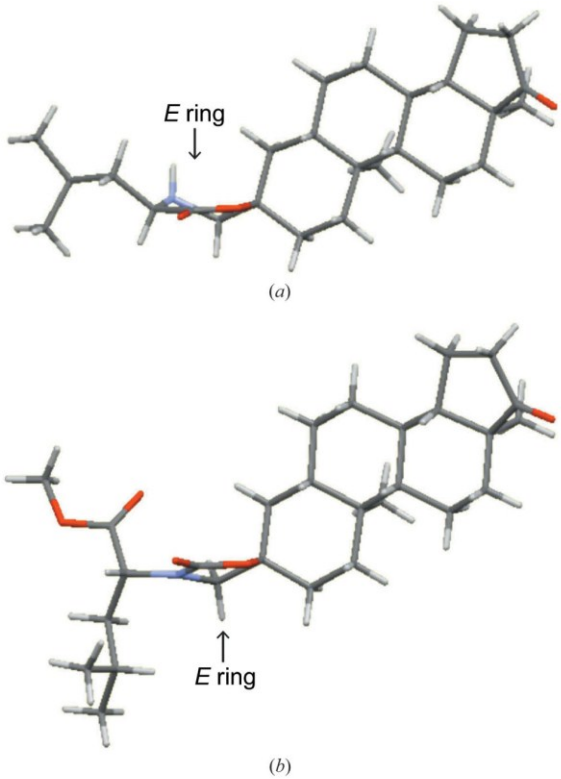


Figure 3
The *E*-ring conformation in (*a*) (I) and (*b*) (II).

The two compounds show fixed conformations of their extended side chains; no disorder is apparent in the structure analyses. Packing diagrams with van der Waals radii give the impression that these structures are not very tightly packed, in agreement with the low densities [1.162 Mg m^{-3} in (I) and 1.200 Mg m^{-3} in (II)]. The packing appears to be efficient enough, however, to be linked to the appearance of just one conformation of the side chain in each case.

The correct enantiomers, already known from the starting materials, were checked against the absolute structure parameters (Flack, 1983). For compound (I), the absolute structure parameter was found to be 0.12 (18) for 1747 Friedel pairs (83% Friedel coverage). For compound (II), it was 0.06 (18) for 2087 (100%) Friedel pairs. These two values are better than might have been expected considering that the highest anomalous contribution comes from O atoms (Flack & Bernardinelli, 2008). A Bayesian statistics analysis on Bijvoet differences was also performed using the program *PLATON* (Spek, 2009). The Hooft parameters obtained (Hooft *et al.*, 2008), *viz.* −0.04 (7) and −0.05 (6) for compounds (I) and (II), respectively, are also in very good agreement with the known molecular absolute configurations, and were perhaps influenced positively by the use of copper radiation. Probability levels of having a false attribution, *P*3(false), are essentially zero for both compounds.

The steroid six-membered *A*, *B* and *C* rings appear as the well known fused-ring system, all in chair conformations, while the five-membered *D* ring is in a slightly twisted envelope conformation in both azalactone (I) and carbamate (II) (Yan *et al.*, 2009) (Fig. 2). The heterocyclic ring of (I) is a half-chair perpendicular to the steroid backbone, while the heterocyclic ring of carbamate (II) is almost planar [maximum deviation = 0.147 (1) Å] and is perpendicular to the steroidal plane (Fig. 3). These extra heterocyclic *E* rings provide a restricted orientation to the isobutyl group in the azalactones (I) and (III) (see Scheme). In carbamates (II) and (IV), the methyl pentanoate group has a higher degree of liberty owing to the free rotation around the exocyclic bond to the N atom. In azalactone (I), the hydrophobic side chain has a distal orientation with respect to the longitudinal axis of the four-ring steroid system (to the right in Fig. 4*a*). Consequently, the isobutyl group of the other isomer, the azalactone (III), would be oriented to the left in a similar drawing, or in other words in a proximal disposition to the centerline of the four-ring system. Since the present compound (I) is a less potent inhibitor than its stereoisomer (III), it appears that the side chain must be proximal (on the left side in Fig. 4*a*) for better enzyme inhibition. The isobutyl group would thus point toward a hypothetical hydrophobic pocket of 17β-HSD3, which was previously deduced from SAR studies (Tchedam-Ngatcha *et al.*, 2005; Maltais *et al.*, 2011) but not confirmed because this membrane enzyme was never crystallized. In the case of carbamates (II) (Fig. 4*b*) and (IV), the inhibitory activity is the same (55 and 58% of inhibition at 0.1 μM) for both compounds due to the free rotation of the methyl pentanoate group, which promotes interaction with a hydrophobic pocket of the enzyme.

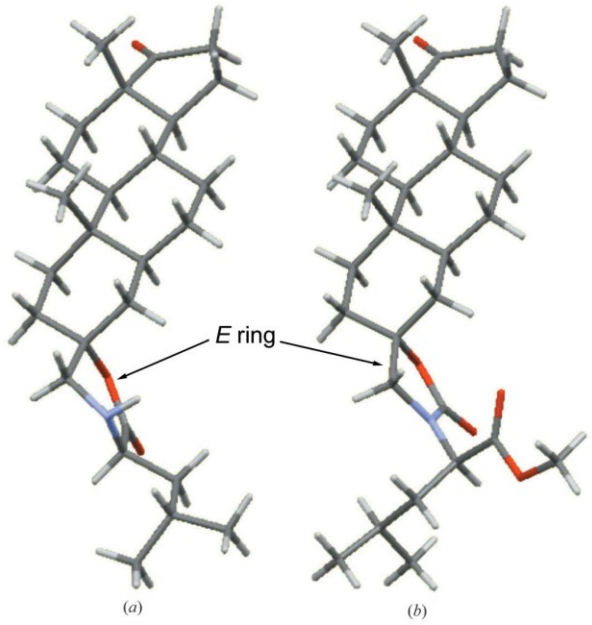


Figure 4
The *E*-ring substituent conformation in (*a*) azalactone (I) and (*b*) carbamate (II). The isobutyl group of (I) is oriented away from the longitudinal axis of the steroid ring system, while the methyl pentanoate group of (II) has free rotation.

Experimental

Compounds (I) and (III) were synthesized by modification of an approach developed in our laboratory (Rouillard *et al.*, 2008). Briefly, aminolysis of the oxirane at C3 of androsterone with L- and D-isoleucine methyl esters provided the corresponding amino alcohols, which were treated with sodium methoxide to afford azalactones (I) and (III). Compounds (II) and (IV) were prepared according to the method of Maltais *et al.* (2002) by treatment of each amino alcohol with triphosgene. X-ray quality crystals of (I) and (II) were obtained by slow diffusion of hexane into a methylene chloride solution of the compound (hexane–methylene chloride 1:1 *v*/*v*) followed by slow evaporation.

Azalactone (I)

Crystal data

$C_{26}H_{41}NO_3$
M_r = 415.60
Monoclinic, $P2_1$
a = 13.07929 (17) Å
b = 5.82873 (8) Å
c = 16.3140 (2) Å
β = 107.3095 (6)°
V = 1187.37 (3) Å^3
Z = 2
Cu $K\alpha$ radiation
μ = 0.58 mm^{-1}
T = 150 K
0.25 × 0.11 × 0.05 mm

Data collection

Bruker SMART 6000 diffractometer
Absorption correction: multi-scan (*SADABS*; Sheldrick, 2008)
T_{min} = 0.810, T_{max} = 0.971
36176 measured reflections
4334 independent reflections
4159 reflections with $I > 2\sigma(I)$
R_{int} = 0.038

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.040
$wR(F^2)$ = 0.107
S = 1.07
4334 reflections
279 parameters
1 restraint
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.18 e Å^{-3}
$\Delta\rho_{min}$ = −0.22 e Å^{-3}
Absolute structure: Flack (1983), 1733 Friedel pairs
Flack parameter: 0.12 (18)

Carbamate (II)

Crystal data

$C_{28}H_{43}NO_5$
M_r = 473.63
Orthorhombic, $P2_12_12_1$
a = 6.3941 (2) Å
b = 18.1440 (7) Å
c = 22.6047 (9) Å
V = 2622.47 (17) Å^3
Z = 4
Cu $K\alpha$ radiation
μ = 0.65 mm^{-1}
T = 150 K
0.12 × 0.05 × 0.05 mm

Data collection

Bruker Microstar diffractometer
Absorption correction: multi-scan (*SADABS*; Sheldrick, 2008)
T_{min} = 0.787, T_{max} = 0.968
43157 measured reflections
4933 independent reflections
4697 reflections with $I > 2\sigma(I)$
R_{int} = 0.052

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.037
$wR(F^2)$ = 0.101
S = 1.06
4933 reflections
312 parameters
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.23 e Å^{-3}
$\Delta\rho_{min}$ = −0.14 e Å^{-3}
Absolute structure: Flack (1983), 2087 Friedel pairs
Flack parameter: 0.06 (18)

H atoms were treated as riding, with C—H = 1.00 (methyl), 0.99 (methylene) and 0.98 Å (remaining H atoms), and with U_{iso}(H) = 1.5U_{eq}(C) for methyl H atoms and 1.2U_{eq}(C) otherwise. The N-bound H atom of (I) was located by difference Fourier synthesis; its coordinates were refined and its U_{iso}(H) value was constrained to 1.2U_{eq}(N).

Data collection: *APEX2* (Bruker, 2011); cell refinement: *SAINT* (Bruker, 2011); data reduction: *XPREP* (Bruker, 2008); program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *SHELXTL* (Sheldrick, 2008); software used to prepare material for publication: *UdMX* (Maris, 2004).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: FA3273). Services for accessing these data are described at the back of the journal.

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Chapitre 4

Synthèse des 5α-androstane-17-spiro-δ-lactones ayant en C-3 une cétone, un hydroxy, un spirocarbamate ou une spiromorpholinone comme inhibiteurs des 17β-hydroxystéroïdes déshydrogénases

1. Avant-propos

Il a été rapporté que les 17-monospiro-δ-lactone-estranes inhibent efficacement la 17β-HSD5. Dans ce chapitre, nous décrivons la synthèse d'une 3-spiromorpholinone-17-spiro-δ-lactone-ADT et d'une 3-spirocarbamate-17-spiro-δ-lactone-ADT. En effet en ajoutant une spiromorpholinone ou un spirocarbamate en position C-3 d'une 17-monospiro-δ-lactone-ADT, nous proposons une nouvelle stratégie qui ouvre la voie sur la synthèse d'inhibiteurs hybrides des 17β-HSD3 et 17β-HSD5, deux enzymes impliquées dans la formation des androgènes et par conséquent, des cibles thérapeutiques intéressantes pour le cancer de la prostate et autres maladies sensibles aux androgènes. Les évaluations biologiques réalisées par Jenny Roy ont contribuées au bon avancement de ce projet.

La synthèse des 17-spiro-δ-lactone-ADT et leur caractérisation ont été réalisées par Béatrice Tchédam Ngatcha pendant qu'elle poursuivait ses travaux de doctorat.

Dans le cadre de ce projet, j'ai synthétisé et caractérisé les 3-spiromorpholinone-ADT, les 3-spirocarbamate-ADT et les 3-spirocarbamate-17-spiro-δ-lactone-ADT. J'ai également participé au choix des pharmacophores en vue d'obtenir une meilleure activité inhibitrice.

2. Résumé

Nous avons synthétisé deux séries de dérivés androstane comme inhibiteurs des 17β-hydroxystéroïdes déshydrogénases (17β-HSD) de type 3 et de type 5. Dans la première série de composés, quatre dérivés monospiro en position C-17 ont été préparés à partir de l'androstérone (ADT) ou de l'épi-ADT. Après la protection de l'alcool en position C-3, la cétone en C-17 a été alkylée avec l'acétylure de lithium de la tétrahydro-2-(but-3-ynyl)-2-H-pyrane, la triple liaison a été hydrogénée, les groupes protecteurs hydrolysés et l'alcool oxydé pour donner le dérivé 3-céto-17-spiro-lactone correspondant. Les trois autres composés ont été générés à partir de cette céto-lactone par réduction de la cétone en position C-3, ou par l'introduction d'un ou deux groupes méthyles. Dans la deuxième série de composés, deux dérivés dispiro en C-3 et C-17 ont été préparés à partir de l'épi-ADT. Après l'introduction d'une spiro-δ-lactone en C-17 et un oxirane en C-3, une aminolyse de l'oxirane avec l'ester méthylique de la L-leucine a été réalisée pour obtenir un amino-alcool. Ce dernier a ensuite été traité avec du triphosgène ou le méthylate de sodium pour donner respectivement un dérivé carbamate-androstane ou morpholinone-androstane. Ces dérivés stéroïdiens inhibent la 17β-HSD3 (14-88 % à 1 μM; 46-94 % à 10 μM) et la 17β-HSD5 (54-73 % à 0,3 μM; 91-92 % à 3 μM). Ils ne possèdent pas d'activité androgénique et ne lient pas les récepteurs stéroïdiens (des androgènes, des estrogènes, de la progestérone et des glucocorticoïdes), ce qui en fait de bons candidats pour le traitement du cancer de la prostate.

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Synthesis of 5α-Androstane-17-spiro-δ-lactone with a 3-Keto, 3-Hydroxy, 3-Spiro-carbamate or 3-Spiro-morpholinone as Inhibitors of 17β-Hydroxysteroid Dehydrogenases

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Keywords: synthesis; steroids; spirolactone; enzyme inhibitor; 17β-hydroxysteroid dehydrogenase

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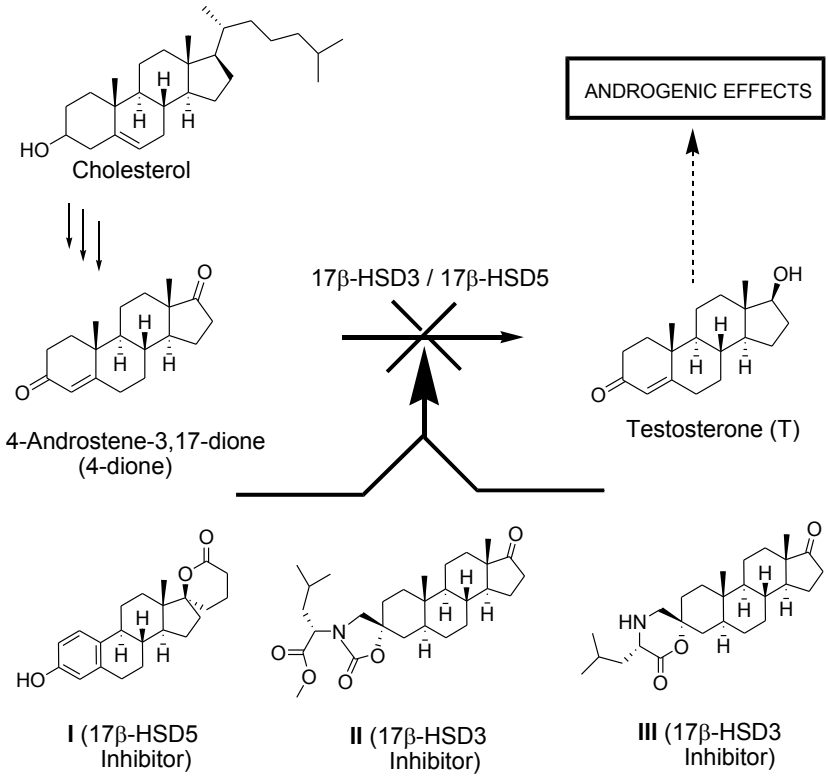
Abstract

We synthesized two series of androstane derivatives as inhibitors of type 3 and type 5 17β-hydroxysteroid dehydrogenases (17β-HSDs). In the first series, four monospiro derivatives at position C17 were prepared from androsterone (ADT) or *epi*-ADT. After the protection of the alcohol at C3, the C17-ketone was alkylated with the lithium acetylide of tetrahydro-2-(but-3-ynyl)-2-H-pyran, the triple bond was hydrogenated, the protecting groups hydrolysed and the alcohols oxidized to give the corresponding 3-keto-17-spiro-lactone derivative. The other three compounds were generated from this keto-lactone by reducing the ketone at C3, or by introducing one or two methyl groups. In the second series, two dispiro derivatives at C3 and C17 were prepared from *epi*-ADT. After introducing a spiro-δ-lactone at C17 and an oxirane at C3, an aminolysis of the oxirane with L-leucine methyl ester provided an amino alcohol, which was treated with triphosgene or sodium methylate to afford a carbamate- or a morpholinone-androstane derivative, respectively. These steroid derivatives inhibited 17β-HSD3 (14–88% at 1 μM; 46–94% at 10 μM) and 17β-HSD5 (54–73% at 0.3 μM; 91–92% at 3 μM). They did not produce any androgenic activity and did not bind steroid (androgen, estrogen, glucocorticoid and progestin) receptors, suggesting a good profile for prostate cancer therapy.

Introduction

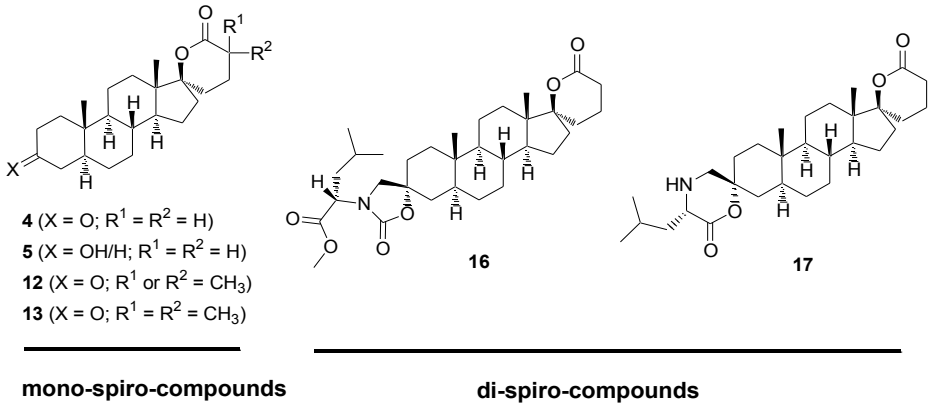
Prostate cancer is an androgen-dependent disease that is well known for its high sensitivity to androgen deprivation. In fact, for over 50 years, the exclusive treatment of advanced metastatic prostate cancer was androgen deprivation achieved through castration, as it was believed that 95% of androgens were of testicular origin [1,2]. However, it is now well known that peripheral tissues represent another important source of androgens [3,4]. In fact, the prostatic tissues efficiently convert the hormone precursor dehydroepiandrosterone (DHEA) into the active androgens testosterone (T) and dihydrotestosterone (DHT) [5–10]. Both type 3 and type 5 17β-hydroxysteroid dehydrogenases (17β-HSD3 and 17β-HSD5, respectively) catalyse the reduction of 4-androstene-3,17-dione (4-dione) to testosterone (T) (Figure 1). However, whereas type 3 is located mainly in the testis, type 5 is expressed in the peripheral tissues [10]. In order to control the peripheral formation of active androgens, which could enhance the efficacy of endocrine therapy (such as the use of a pure antiandrogen with an LHRH agonist), we focused on the development of inhibitors of 17β-HSD3 and 17β-HSD5.

Figure 1. Role of 17β-HSD3 and 17β-HSD5 in the synthesis of the androgenic hormone testosterone.



Our group has previously demonstrated that a spiro-δ-lactone at position 17 and an estrane backbone are two important requirements for the inhibition of 17β-HSD5 [11]. We also reported that introducing a hydrophobic group at position 3 of androsterone (ADT) provides potent inhibitors of 17β-HSD3 [12–16]. For example, we recently published the inhibitory potency of 3-spiro-carbamate and 3-spiro-morpholinone ADT derivatives and their respective stereoisomers on 17β-HSD3 [17]. In our pursuit of the optimization of new 17β-HSD inhibitors, we synthesized steroid derivatives **4**, **5**, **12**, **13**, **16** and **17** (Figure 2). The monospiro derivatives **4**, **5**, **12** and **13** were designed to inhibit 17β-HSD5 whereas dispiro derivatives **16** and **17** were designed to inhibit 17β-HSD3. In this article, we report the synthesis, NMR characterization and biological activity of these new spiro derivatives (compounds **4**, **5**, **12**, **13**, **16** and **17**) (Figure 2).

Figure 2. Chemical structures of potential inhibitors of 17β-HSD5 or 17β-HSD3 (monospiro-compounds: **4**, **5**, **12** and **13** and dispiro-compounds **16** and **17**).

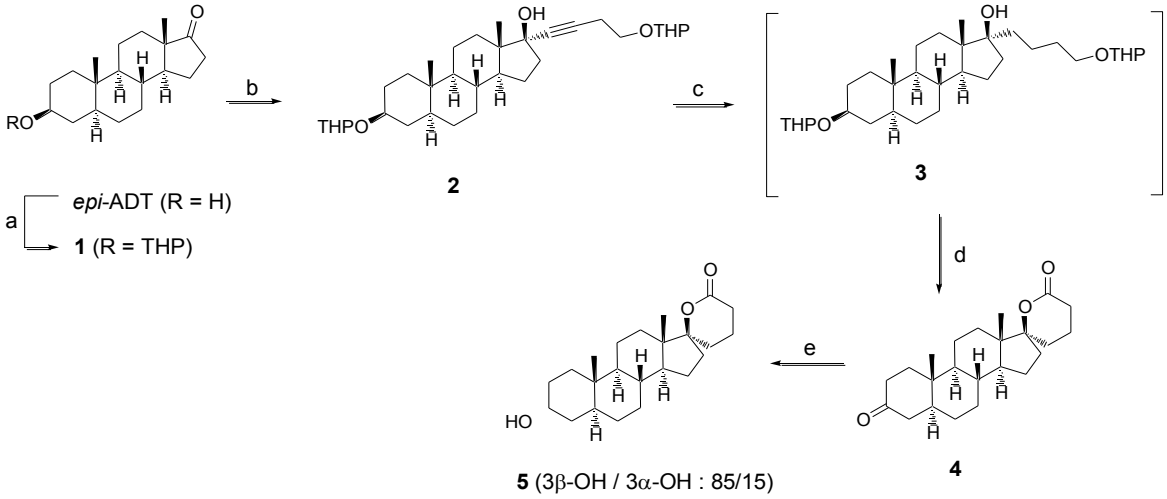


Results and Discussion

Synthesis of Spiro-δ-Lactones **4** *and* **5** *(Scheme 1)*

The natural C19-steroid *epi*-androsterone (epi-ADT) was the starting material used in the synthesis of spiro-δ-lactones **4** and **5**. After protecting the 3β-OH as a tetrahydropyranyl (THP) ether using dihydropyran in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA), the carbonyl group at position 17 was alkylated with the lithium acetylide generated from 2-(3-butynyloxy)-tetrahydro-2*H*-pyran and *n*-BuLi. As reported in the literature, the acetylide attacks the carbonyl group by the less hindered alpha face of the steroid, providing the alkyne derivative **2** [18–20]. The triple bond of the alkylated steroid **2** was hydrogenated using a mixture of Pd/C and

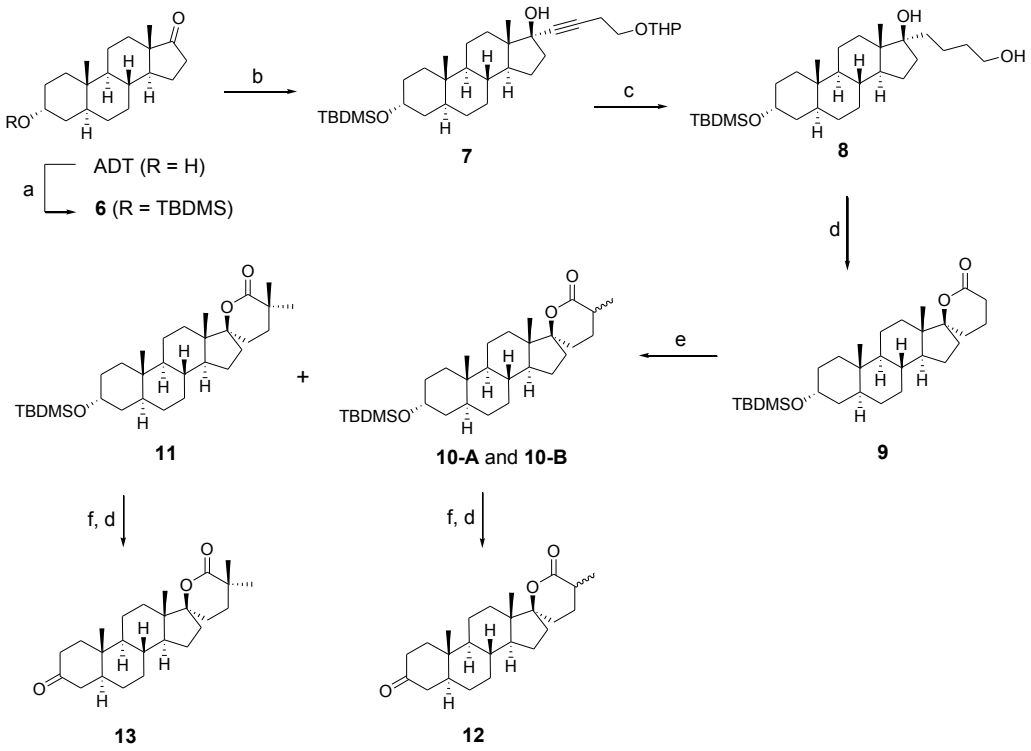
$Pd/CaCO_3$, under a hydrogen atmosphere to yield the corresponding alkane **3**. Without purification, the di-THP derivative **3** was treated with Jones' reagent leading to the spiro-δ-lactone **4**. Under these conditions, both deprotection and oxidation of secondary and primary alcohols occurred followed by lactonization between the 17β-OH and the carboxylic acid at the end of the side chain. Formation of the spiro-δ-lactone ring was confirmed by the presence of characteristic signals in ^{13}C-NMR (93.21 and 171.98 ppm for C-17 and COO, respectively), which are identical to those of the corresponding spiro-δ-lactone with an estrane nucleus [20]. The carbonyl group of ketone **4** was reduced with $NaBH_4$ in MeOH to yield **5** as an epimeric mixture of 3β- and 3α-alcohols (3β-OH/3α-OH: 85/15). The major 3β-OH product was identified by the 3α-CH signal at 3.55 ppm in ^{1}H NMR whereas the 3β-CH of the minor 3α-OH product appears at 4.01 ppm. These two signals are similar to those obtained from commercially available samples of *epi*-ADT (3α-CH: 3.60 ppm) and ADT (3β-CH: 4.07 ppm).



Scheme 1. Synthesis of spiro-δ-lactones **4** and **5**. *Reagents and conditions*: (a) dihydropyran, *p*-TSA, CH_2Cl_2, 0 °C; (b) $HC{\equiv}C(CH_2)_2OTHP$, *n*-BuLi, THF, −78 °C; (c) H_2, 10% Pd/C, 5% $Pd/CaCO_3$, EtOAc, rt; (d) Jones' reagent (2.7 M), acetone, 0 °C; (e) $NaBH_4$, MeOH, 0 °C.

Synthesis of Methylated Spiro-δ-Lactones **12** *and* **13** *(Scheme 2)*

The monomethylated and dimethylated spiro-δ-lactones **12** and **13** were obtained from androsterone (ADT) through the sequence of reactions depicted in Scheme 2. ADT was first protected as a silylated ether using *tert*-butyldimethylsilyl-chloride (TBDMS-Cl) and imidazole in DMF. The anion resulting from the reaction between *n*-BuLi and 2-(3-butynyloxy)tetrahydro-2*H*-pyran was used for the alkylation of the carbonyl of TBDMS-ADT (**6**). The alkyne **7** was submitted to hydrogenation conditions (H_2, Pd/C and Pd/$CaCO_3$) to yield the corresponding alkane, which was treated *in situ* with *p*-TSA in MeOH at room temperature to selectively hydrolyze the THP group. The diol **8** was then treated with Jones' reagent to yield the spiro-δ-lactone **9**. Alkylation in α-position of the lactone carbonyl was performed with lithium diisopropylamide (LDA) and methyl iodide. A mixture of three α-methylated lactones was obtained: the dimethylated lactone **11** and the two possible monomethylated spiro-δ-lactones **10A** and **10B**. Methylated spiro-lactones **10A**, **10B** and **11** were oxidized with Jones' reagent leading to compounds **12** and **13**. Unfortunately, epimerisation at position α of the lactone occurred when submitting each of the monomethylated compounds **10A** and **10B** to hydrolysis and oxidative conditions. The same mixture of the two possible monomethylated compounds was thus obtained, and this time, it was not possible to separate them by column chromatography.

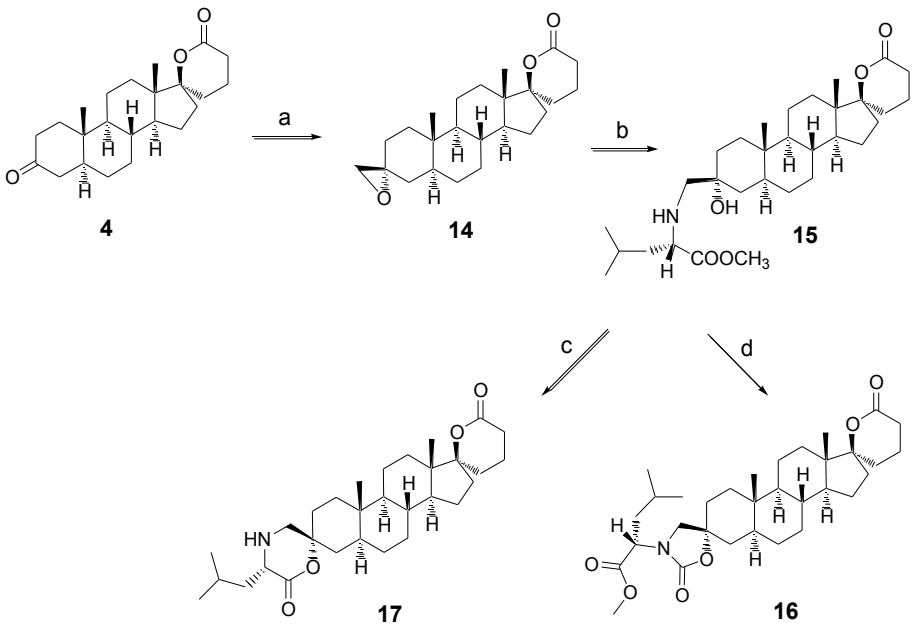


Scheme 2. Synthesis of methylated spiro-δ-lactones **12** and **13**. *Reagents and conditions:* (a) TBDMS-Cl, imidazole, DMF, rt; (b) $HC{\equiv}C(CH_2)_2OTHP$, *n*-BuLi, THF −78 °C; (c) *i.* H_2, 10% Pd/C, 5% $Pd/CaCO_3$, EtOAc, rt, *ii. p*-TSA, MeOH, rt; (d) Jones' reagent (2.7 M), acetone, 0 °C; (e) LDA, CH_3I, THF, 0 °C; (f) TBAF, THF, reflux.

Synthesis of Spiro-Carbamate **16** *and Spiro-Morpholinone* **17** *(Scheme 3)*

Following a slightly modified method reported previously in our laboratory [21], the 3-oxo-spiro-δ-lactone **4** was reacted with trimethylsulfoxonium iodide (four equivalents rather than two) to yield oxirane **14**. The 3β-CH_2 orientation of **14** was confirmed by a correlation with methyl 19 in NOESY spectrum. Compound **14** was then submitted to an aminolysis with L-leucine methyl ester to generate the amino alcohol **15**. The amino group of L-leucine methyl ester was previously generated from the commercially available chlorhydrate [21]. The spiro-carbamate **16** was obtained from the reaction of **15** with triphosgene. Because the reaction was very slow with only 0.5 equivalent of triphosgene, the quantity reported to produce a similar carbamate [14], we used one equivalent to complete the reaction generating **16**. The formation of a carbamate group was confirmed by a characteristic signal at 157.64 ppm in ^{13}C-NMR. The spiro-morpholinone **17** was obtained from the lactonization of the amino alcohol **15**. During

this step, unknown products are formed; this explains the poor yield of this reaction. This is probably due to the polymerization of the starting amino alcohol or the partial aminolysis of the spiro-δ-lactone. HPLC chromatogram showed four resolved peaks, integrating for 39% (expected compound **17**), 18% (the starting amino alcohol **15**) and two other peaks representing unknown products. The formation of the spiro-morpholinone moiety was confirmed by a characteristic signal at 171.99 ppm in ^{13}C-NMR.



Scheme 3. Synthesis of dispirolactone **16** (carbamate) and spiromorpholinone **17**. *Reagents and conditions:* (a) $(CH_3)_3SOI$, NaH, DMSO/THF, rt; (b) L-Leucine, MeOH, 90 °C; (c) CH_3ONa, THF, rt; (d) $(Cl_3CO)_2CO$, DIPEA, DCM, 0 °C to rt.

Biological Evaluation of Monospiro Derivatives **4**, **5**, **12** *and* **13**

Compounds **4**, **5**, **12** and **13** were evaluated for their ability to inhibit the 17β-HSD5 activity found in transfected HEK-293 cells by measuring the amount of labelled testosterone (T) formed from labelled natural substrate 4-dione (Table 1). All compounds inhibited the 17β-HSD5 (91–92% at 3 μM), but the C19-steroid (androstane) backbone seem to be less efficient than the C18-steroid (estrane) backbone. In fact, the androstane spiro-δ-lactone **4** is a less potent inhibitor than the corresponding estrane compound **I** (64 and 92% of inhibition at 0.3 μM, respectively). The monomethylation of lactone **4** (compound **12**) brought a slight increase in the

inhibitory activity (73% at 0.3 μM), whereas the dimethylation (compound **13**) brought a small decrease of inhibition (54% at 0.3 μM). The spiro-δ-lactone bearing a hydroxyl at position 3, compound **5**, gave a 56% inhibition of 17β-HSD5 at 0.3 μM, a value lower than that of the corresponding keto compound **4** (64% of inhibition). Compound **4**, **5**, **12** and **13** were also tested as inhibitor of 17β-HSD3 by measuring the transformation of labelled 4-dione to labelled T by a microsomal preparation of rat testis. No significant inhibition was observed at concentrations of 0.1 and 1 μM for all compounds, and only small inhibitory activities (46–58%) were obtained at the higher concentration of 10 μM. This is fully in accord with our first structure-activity relationship (SAR) results that identified the importance of a hydrophobic group at position 3 of ADT, instead of at position 16, to inhibit 17β-HSD3 [13,16].

After we determined the inhibitory potency of a spiro-δ-lactone at position 17 of an androstane backbone on 17β-HSD5 and 17β-HSD3, we evaluated their proliferative activity on Shionogi androgen-sensitive cell lines. In fact, for a potential use in prostate cancer, an enzyme inhibitor should be devoid of proliferative androgenic activity. The behaviour of compounds **4**, **5**, **12** and **13** on an androgen-sensitive Shionogi cell line was then evaluated and compared to that of hydroxyflutamide, a well known antiandrogen (Table 2) [22,23].

Except for the α-dimethylated lactone **13** which did not show any proliferative (androgenic) activity, the C19-steroid spiro-δ-lactones **4**, **5** and **12** exhibited a slight proliferative activity at 0.1 μM on Shionogi cells (12, 19 and 9%, respectively). However, at the higher concentration of 1 μM, no proliferative activity was observed for spiro-δ-lactones **4**, **5**, **12** and **13** suggesting that proliferative effects observed at 0.1 μM were not significant. The antiproliferative (antiandrogenic) activity was measured by the inhibition of DHT (0.3 nM)-induced proliferation on Shionogi cells. All the target compounds **4**, **5**, **12** and **13** showed an antiproliferative activity at 1 μM, the most important effect being observed with the spiro-δ-lactone **4** (100% antiproliferative activity). Its activity dropped down at lower concentration: 23% at 0.1 μM, compared to 69% for hydroxyflutamide at the same concentration.

Table 1. Inhibition of 17β-HSD5 and 17β-HSD3 by compounds **4**, **5**, **12**, **13**, **16** and **17**.

Compounds (characteristics) [a]	Inhibition of 17β-HSD5 at 0.3 µM (%) [b]	Inhibition of 17β-HSD5 at 3 µM (%) [b]	Inhibition of 17β-HSD3 at 0.1 µM (%) [c]	Inhibition of 17β-HSD3 at 1 µM (%) [c]	Inhibition of 17β-HSD3 at 10 µM (%) [c]
I (C18/17-lactone/ 3-OH	92	95	--	--	--
II (C19/17-oxo/ 3-carbamate	--	--	66.0 ± 1.7	88.3 ± 1.1	93.7 ± 0.8
III (C19/17-oxo/ 3-morpholinone	--	--	63.2 ± 2.6	81.0 ± 1.6	88.7 ± 4.0
4 (C19/17-lactone/ 3-oxo	64	92	4.9 ± 4.8	15.0 ± 0.9	56.7 ± 2.2
5 (C19/17-lactone/ 3-OH	56	91	0.6 ± 5.3	22.4 ± 2.8	45.7 ± 1.5
12 (C19/17-lactone; mono-	73	91	1.0 ± 3.3	22.2 ± 4.9	53.4 ± 5.0
13 (C19/17-lactone; bis-	54	91	1.0 ± 7.2	14.4 ± 3.3	58.0 ± 3
16 (C19/17-lactone/	--	--	32.0 ± 3.3	60.4 ± 4.4	60.9 ± 0.7
17 (C19/17-lactone/	--	--	11.2 ± 2.6	51.0 ± 1.5	87.2 ± 0.5

[a] C18: estrane nucleus (18 carbons) and C19: androstane nucleus (19 carbones); [b] For the transformation of [^{14}C]-4-dione to [^{14}C]-T by HEK-293 cells overexpressing human 17β-HSD5 (transfected cells in culture); [c] For the transformation of [^{14}C]-4-dione to [^{14}C]-T by rat testicular 17β-HSD3 (microsomal fraction).

Table 2. Proliferative an antiproliferative activities of monospiro-compounds **4**, **5**, **12** and **13** on Shionogi (AR^+) cells.

Compounds	Proliferative activity at 0.1 µM (%) [a]	Proliferative activity at 1 µM (%) [a]	Antiproliferative activity at 0.1 µM (%) [b]	Antiproliferative activity at 1 µM (%) [b]
4	12 ± 2	0 ± 5	23 ± 1	100 ± 1
5	19 ± 4	0 ± 5	10 ± 1	61 ± 3
12	9 ± 5	0 ± 3	0 ± 5	58 ± 2
13	0 ± 9	0 ± 5	1 ± 2	49 ± 7
OH-Flu [c]	0 ± 6	0 ± 2	69 ± 3	100 ± 3

[a] The proliferative activity expressed in percentage was calculated in comparison to the stimulation (100%) induced by 0.3 nM of potent androgen dihydrotestosterone (DHT); [b] The antiproliferative activity expressed in percentage is the ability of a compound to inhibit the 0.3 nM DHT-induced proliferation of cells; [c] The antiandrogen hydroxyflutamide was used as a reference compound [22,23].

To discriminate between two possible antiproliferative effects: an antiandrogenic activity mediated by the androgen receptor (AR) and a cytotoxic activity not mediated by AR, we measured the binding affinity on AR for each compound (Table 3). In fact, the C19-steroids **4**, **5**, **12** and **13** were expected to show some affinities with androgen receptor, as their chemical structures are similar to that of the natural substrate T and DHT. Compounds **4**, **5** and **12** showed a weak binding on AR suggesting an antiandrogenic effect instead of a cytotoxic effect. It is however possible that the antiproliferative effect we have observed on Shionogi cells was a mixture of both antiandrogenic and cytotoxic activities. Interestingly, the spiro-lactones **4**, **5**, **12** and **13** did not show affinities with other steroid (estrogen, glucocorticoid and progestin) receptors.

Table 3. Binding affinity (%) of compounds **4**, **5**, **12** and **13** on steroid receptors.

Cpds	Androgen receptor (%) [a]		Estrogen receptor-α (%) [a]		Glucocorticoid receptor (%) [a]		Progestin receptor (%) [a]	
	10 nM	1 μM	10 nM	1 μM	10 nM	1 μM	10 nM	1 μM
4	4 ± 1	9 ± 2	0 ± 1	1 ± 1	0 ± 3	0 ± 1	4 ± 2	0 ± 2
5	7 ± 1	5 ± 1	0 ± 1	0 ± 1	0 ± 1	1 ± 2	4 ± 1	1 ± 2
12	1 ± 2	4 ± 2	0 ± 2	1 ± 3	0 ± 3	0 ± 1	0 ± 2	0 ± 1
13	0 ± 2	0 ± 2	0 ± 2	0 ± 3	0 ± 2	1 ± 3	0 ± 3	1 ± 3
DHT [b]	70 ± 1	100 ± 1	2 ± 2	4 ± 1	2 ± 2	6 ±2	3 ± 2	40 ± 2
E2 [b]	0 ± 2	34 ± 1	75 ± 1	100 ± 1	5 ± 2	12 ± 2	6 ± 3	25 ± 2
DEX [b]	0 ± 1	2 ± 1	0 ± 3	0 ± 1	66 ± 2	99 ± 1	0 ± 3	1 ± 2
R5050 [b]	1 ± 4	28 ± 2	5 ± 2	4 ± 1	9 ± 2	85 ± 2	65 ± 2	99 ± 2

[a] Data are expressed in percentage (%) of the binding affinity obtained with a natural or synthetic ligand tested at a concentration of 1 μM (100% of binding); [b] Natural and synthetic ligands used for each receptor: DHT, dihydrotestosterone; E2, estradiol; DEX, dexamethasone; R5050, synthetic progestin.

Biological Evaluation of Dispiro Derivatives **16** *and* **17**

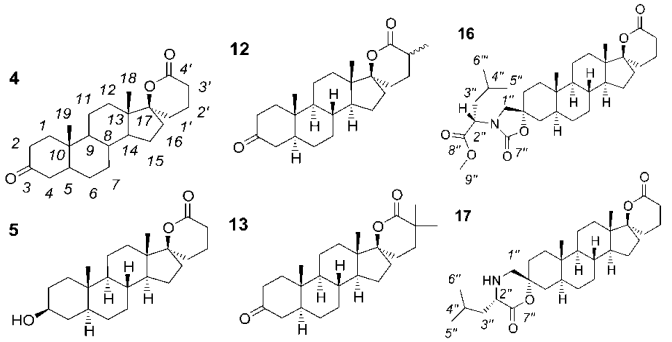
Compounds **16** and **17** were synthesized to determine the impact of two spiro-functionalities on the inhibition of 17β-HSD3 (Table 1). As mentioned above, a microsomal preparation of rat testis was used as source of enzyme activity transforming 4-dione to T. In the first series of monospiro derivatives (compounds **4**, **5**, **12** and **13**), the presence of a spiro-δ-lactone at position C-17 resulted in a very weak inhibition (1–5% at 0.1 μM). At the opposite, the presence of a carbamate or a morpholinone moiety at position C-3 generated a very good inhibitory activity (66 and 63% at 0.1 μM) for monospiro derivatives II and III, respectively. When we introduced a carbamate or a morpholinone at position C-3 of compound **4**, both dispiro derivatives **16** and **17** produced a moderate inhibitions of 17β-HSD3 (32 and 11% at 0.1 μM; 60 and 51% at 1 μM), which are less important than those of known inhibitors II and III (only C-3 derivatives) and more important than those of **4** and **5** (only C-17 derivatives).

Experimental

General

Chemical reagents as well as DMF and CH_2Cl_2, 99.8% anhydrous grade, were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Androsterone and *epi*-androsterone were obtained from Steraloids (Wilton, NH, USA). THF, used in anhydrous conditions, was distilled from sodium benzophenone ketyl. Solvents for chromatographies were purchased from BDH Chemicals (Montréal, QC, Canada) or Fisher Chemicals (Montréal, QC, Canada). Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) and 230–400 mesh ASTM silica gel 60 (E. Merck) was used for flash chromatography. Infrared spectra (IR) are reported in cm^{-1} and obtained on a Perkin-Elmer 1600 (FT-IR series) spectrophotometer. Nuclear magnetic resonance spectra (NMR) were recorded with a Bruker AC/F 300 spectrometer (Billerica, MA, USA) at 300 (^{1}H) and 75 (^{13}C) MHz or a Bruker AVANCE 400 spectrometer at 400 (^{1}H) and 100 (^{13}C) MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C). All ^{13}C-NMR signals of final compounds **4**, **5**, **12**, **13**, **16** and **17** (Table 4) were fully assigned using a series of NMR experiments (APT, HSQC, HMBC, COSY and NOESY) and data reported in literature [20,21,24–27]. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Laval University Chemistry Department (Québec, QC, Canada). The names of steroid derivatives were generated using ACD/Labs (Chemist'version) software (Toronto, ON, Canada).

Table 4. ^{13}C-NMR data of final compounds **4**, **5**, **12**, **13**, **16** and **17** dissolved in $CDCl_3$.



Cpds	4	5	12	13	16	17
C1	38.55	36.98	38.60	38.58	33.86	32.98
C2	38.07	31.38	38.12	38.11	32.75	31.48
C3	211.67	71.07	211.79	211.84	79.74	82.37
C4	44.59	38.03	44.63	44.62	39.48	38.11
C5	46.71	44.84	46.75	46.74	40.86	39.29
C6	28.72	28.47	28.77	28.75	28.01	28.00
C7	31.40	31.72	31.44	31.41	31.47	31.38
C8	35.75	35.82	35.81	35.81	35.84	35.85
C9	53.61	54.12	53.67	53.63	53.61	53.50
C10	35.75	35.49	35.81	35.81	35.31	36.02
C11	20.90	20.65	20.91 (20.98)	20.97	20.43	20.46
C12	31.86	31.92	31.93	31.92	31.93	31.96
C13	46.99	46.95	46.96	47.01	46.99	46.99
C14	49.64	49.77	49.45 (49.61)	49.49	49.77	49.83
C15	23.77	23.73	23.94 (23.72)	23.61	23.76	23.78
C16	33.93	33.90	33.99 (33.50)	34.77	33.95	33.92
C17	93.21	93.34	92.78 (93.65)	93.70	93.40	93.42
C18	14.40	14.37	14.52	14.56	14.43	14.45
C19	11.43	12.24	11.47	11.45	11.40	11.36
C1'	27.86	27.82	27.21 (28.58)	25.51	27.91	27.89
C2'	15.83	15.78	24.41 (24.21)	31.55	15.84	15.84
C3'	29.40	29.37	34.64 (36.16)	37.76	29.47	29.47
C4'	171.98	172.13	174.90 (175.89)	177.90	172.22	172.25
C5'	---	---	17.35 (17.26)	27.65	---	---
C6'	---	---	---	27.75	---	---
C1"	---	---	---	---	52.89	52.41
C2"	---	---	---	---	53.61	55.58
C3"	---	---	---	---	37.69	41.41
C4"	---	---	---	---	24.93	24.46
C5"/C6"	---	---	---	---	21.06/23.14	20.97/23.40
C7"	---	---	---	---	157.99	171.99
C8"	---	---	---	---	171.99	---
C9"	---	---	---	---	52.22	---

Synthesis of δ-Lactones **4** *and* **5** *(Scheme 1)*

(3β,5α)-3-(Tetrahydro-2H-pyran-2-yloxy)androstan-17-one (**1**).

To a solution of *epi*-androsterone (2.0 g, 6.89 mmol) in dry CH_2Cl_2 (150 mL) was added 3,4-dihydropyrane (1.9 mL, 3 eq) and *p*-toluene-sulfonic acid (0.130 g, 0.1 eq). The mixture was stirred for 2 h at 0 °C under an atmosphere of argon. The reaction was stopped by adding a saturated $NaHCO_3$ solution. Extraction was done with EtOAc, the organic layer was washed with water, then dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The yellow oil obtained was purified by flash chromatography, using a mixture of hexanes and EtOAc (8/2) as eluent, to give the compound **1** as a white amorphous solid in 82% yield. IR (film) υ 1740 (C=O, ketone); ^{1}H-NMR ($CDCl_3$) δ 0.83 and 0.85 (2s, CH_3-18 and CH_3-19), 0.60–2.10 (unresolved CH and CH_2), 2.43 (dd, J_1 = 19.0 Hz and J_2 = 8.8 Hz, CH-16β), 3.48 and 3.92 (2m, CH_2O of THP), 3.58 (m, CH-3α), 4.71 (t, J = 3.7 Hz, CH of THP); ^{13}C-NMR ($CDCl_3$) δ 12.28, 13.81, 20.04, 20.49, 21.78, 25.52, 27.79, 28.42, (28.56), 29.41, (29.70), 30.94, 31.30, 31.59, 34.29, 35.09, 35.85, (36.15), 36.96, (37.17), 44.80, (45.14), 47.80, 51.46, 54.52, 62.81, 75.09, (75.45), 96.60, (96.96), 221.32.

17β-Hydroxy-3β-(tetrahydro-2H-pyran-2-yloxy)-17α-[4'-(tetrahydro-2H-pyran-2-yloxy)butynyl]-5α-androstane (**2**).

To a solution of 2-(3-butynyloxy)-tetrahydro-2*H*-pyrane (3.1 mL, 19.86 mmol) in dry THF (100 mL) under an argon atmosphere was added a 1.6 M solution of *n*-BuLi in hexanes (12.4 mL, 19.65 mmol) and the reaction mixture was stirred at −78 °C for 20 min. A solution of ketone **1** (1.85 g, 4.95 mmol) in dry THF (50 mL) was then added dropwise over a period of 15 min and the mixture was allowed to react overnight, the temperature going from −78 °C to room temperature. A saturated $NaHCO_3$ solution was added and the extraction was done with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography using a mixture of hexanes and EtOAc (8/2) as eluent to give the alkylated compound **2** as an amorphous white solid in 68% yield. IR (film) υ 3429 (OH, alcohol); ^{1}H-NMR ($CDCl_3$) δ 0.76 and 0.77 (2s, CH_3-18 and CH_3-19), 0.55–2.20 (unresolved CH and CH_2), 2.49 (t, J = 6.9 Hz, C≡CCH_2), 3.50 and 3.84 (2m, 2 × CH_2O of THPs), 3.75 (m, CH-3α), 4.63 and 4.68 (2t, J = 3.1 Hz, 2 × CH of THPs); ^{13}C-NMR ($CDCl_3$) δ 12.18, 12.79, 19.13, 19.79, 19.85, 20.25, 20.79, 23.04, 25.36, 25.41, 27.70, 28.50, (28.65), 29.31, 30.44, 31.16, 31.50, 32.72, 34.22,

35.65, (36.06), 36.85, (37.07), 38.94, 44.66, (45.01), 46.78, 50.24, 53.90, 61.79, 62.49, (62.59), 65.71, 75.08, (75.41), 79.75, 82.76, 84.73, 96.37, (96.64), 98.43.

(5S,8R,9S,10S,13S,14S,17S)-10,13-Dimethylhexadecahydrospiro[cyclopenta [a]phenanthrene-17,2'-pyran]-3,6'(2H,3'H)-dione (**4**).

To a solution of alkyne **2** (1.5 g, 2.94 mmol) in EtOAc (100 mL) was added a 1:1 mixture of palladium on charcoal (10%) and palladium on calcium carbonate (5%) (150 mg). The reaction mixture was stirred overnight at room temperature under an atmosphere of hydrogen. The mixture was filtered through a pad of celite and the solvent evaporated to dryness under reduced pressure. Without purification, the white solid corresponding to hydrogenated compound **3** was directly used for the next step. To a stirred solution of **3** (1.4 g) in acetone (100 mL) stirred at 0 °C was added dropwise a 2.7 M solution of Jones' reagent (3.5 mL). The reaction was monitored by TLC and was completed after 30 min. Isopropyl alcohol was then added until a persistent green colour remained. Organic solvents were removed under reduced pressure and the resulting green concentrate dissolved in water. Extraction was done with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by column chromatography using a mixture of hexanes and EtOAc (9/1) gave the spiro-δ-lactone **4** as white solid in 98% yield (for the two steps). IR (film) υ 1714 (C=O, ketone and lactone); ^{1}H-NMR ($CDCl_3$) δ 0.98 (s, CH_3-19), 1.02 (s, CH_3-18), 0.60–2.58 (unresolved CH and CH_2); ^{13}C-NMR ($CDCl_3$) δ 11.43, 14.40, 15.83, 20.90, 23.77, 27.86, 28.72, 29.40, 31.40, 31.86, 33.93, 35.75 (2×), 38.07, 38.55, 44.59, 46.71, 46.99, 49.64, 53.61, 93.21, 171.98, 211.67; HRMS for $C_{23}H_{38}NO_3$ $[M+NH_4]^+$: calculated 376.2846, found 376.2850.

(3S,5S,8R,9S,10S,13S,14S,17S)-3-Hydroxy-10,13-dimethyloctadecahydrospiro[cyclopenta [a]phenanthrene-17,2'-pyran]-6'(3'H)-one (**5**).

Ketone **4** (200 mg, 0.549 mmol) was dissolved in MeOH (30 mL) and $NaBH_4$ (23 mg, 1.1 eq) was added. The solution was stirred at room temperature for 3 h. Water was then added, MeOH was evaporated under reduced pressure and the product was extracted with EtOAc. The organic layer was dried over $MgSO_4$, the solvent evaporated under reduced pressure and the crude product was purified by column chromatography using a mixture of hexanes and EtOAc (8/2) to give the alcohol **5** as an epimeric mixture at position 3 (3β-OH/3α-OH: 85/15, evaluated by ^{1}H-NMR). White solid (85% yield); IR (film) υ 3418 (OH, alcohol), 1716 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.77 and 0.79

(2s, CH_3-19 of both epimers), 0.93 (s, CH_3-18), 0.50–2.00 (unresolved CH and CH_2), 2.43 (m, CH_2COO), 3.55 and 4.01 (2m, CH-3β and CH-3α in proportions 85/15); ^{13}C-NMR ($CDCl_3$) *minor compound signals indicated between* [ ] δ [11.10], 12.24, 14.37, 15.78, 20.65, 23.73, 27.82, 28.47, 29.37, [29.61], 31.38, 31.72, 31.92, [32.15], 33.90, 35.49, 35.82, [36.08], 36.98, 38.03, [39.05], 44.84, 46.95, 49.77, 54.12, [66.31], 71.07, 93.34, 172.13; HRMS for $C_{23}H_{40}NO_3$ $[M+NH_4]^+$: calculated 378.3003, found 378.3008.

*3.3. Synthesis of α-Methylated δ-lactones **12** and **13** (Scheme 2)*

(*3α,5α)-3-{[tert-Butyl(dimethyl)silyl]oxy}androstan-17-one* (**6**).

The hydroxy group of androsterone (1.5 g, 5.17 mmol) was protected as a silylated ether in a mixture of dry DMF (100 mL), imidazole (1.76 g, 5 eq) and TBDMS-Cl (2.34 g, 3 eq). TBDMS-ADT (**6**) was thus obtained as a white solid in 94% yield and the IR, NMR and MS data are in accord with those reported in literature [28].

3α-(tert-Butyldimethylsilyloxy)-17β-hydroxy-17α-[4'-(tetrahydro-2H-pyran-2-yloxy)butynyl]-5α-androstane (**7**).

The carbonyl group of TBDMS-ADT (**6**) was alkylated with 2-(3-butynyloxy)tetrahydro-2*H*-pyran as described above for the synthesis of compound **2**. Compound **7** was obtained as colourless oil in 70% yield. IR (film) υ 3444 (OH, alcohol); ^{1}H-NMR ($CDCl_3$) δ 0.02 (s, $Si(CH_3)_2$), 0.76 (s, CH_3-19), 0.81 (s, CH_3-18), 0.89 (s, $SiC(CH_3)_3$), 0.70–2.25 (unresolved CH and CH_2), 2.55 (t, J = 6.8 Hz, $C{\equiv}CCH_2$), 3.55 and 3.84 (2m, 2 × CH_2O of THP and side chain), 3.95 (t, J = 2.1 Hz, CH-3β), 4.68 (t, J = 3.0 Hz, CH of THP); ^{13}C-NMR ($CDCl_3$) δ −4.85 (2×), 11.40, 12.90, 18.12, 19.26, 20.44, 23.15, 25.49, 25.63, 25.89 (3×), 28.59, 29.72, 30.57, 31.62, 32.35, 32.83, 36.09, 36.22, 36.78, 39.06, 46.24, 46.93, 50.37, 53.87, 61.92, 65.81, 66.86, 80.11, 83.16, 84.69, 98.62.

3α-(tert-Butyldimethylsilyloxy)-17β-hydroxy-17α-(4'-hydroxybutyl)-5α-androstane (**8**).

Compound **7** was submitted to hydrogenation conditions as described in the first part of the synthesis of **4**. The crude product was then used without purification for the next step, the hydrolysis of the THP group. The crude hydrogenated product (3.6 g, 6.406 mmol) was dissolved in MeOH and *p*-TSA (122 mg, 0.1 eq) was added. The reaction mixture was stirred at room temperature for 1 h. Water was added, the MeOH evaporated under reduced pressure and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography using a mixture of hexanes and EtOAc (5/5) as eluent to give the diol **8** as a white solid in 80% (for the two steps). IR (film) υ 3351 (OH, alcohol); ^{1}H-NMR ($CDCl_3$) δ 0.01 (s, $Si(CH_3)_2$), 0.77 (s, CH_3-19), 0.85 (s, CH_3-18), 0.89 (s, $SiC(CH_3)_3$), 0.62–2.05 (unresolved CH and CH_2), 3.69 (m, CH_2OH), 3.95 (t, J = 2.2 Hz, CH-3β); ^{13}C-NMR ($CDCl_3$) δ −4.86 (2×), 11.41, 14.41, 18.10, 19.80, 20.45, 23.65, 25.86 (3×), 28.56, 29.72, 31.61, 31.90, 32.43, 33.41, 34.51, 36.04, 36.16, 36.41, 36.74, 39.08, 46.45, 50.53, 54.36, 62.93, 66.83, 83.59.

(3S,5S,8R,9S,10S,13S,14S,17S)-3-{[tert-Butyl(dimethyl)silyl]oxy}-10,13-dimethyl octadecahydrospiro[cyclopenta[a]phenanthrene-17,2'-pyran]-6'(3'H)-one (**9**).

The diol **8** was oxidized with Jones' reagent as described in the second part of the synthesis of **4**. After purification by column chromatography using a mixture of hexanes and EtOAc (8/2) as eluent, lactone **9** was obtained in 95% yield as a white solid. IR (film) υ 1734 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.01 (s, $Si(CH_3)_2$), 0.77 (s, CH_3-19), 0.89 (s, $SiC(CH_3)_3$), 0.97 (s, CH_3-18), 0.62–2.00 (unresolved CH and CH_2), 2.45 (m, CH_2CO), 3.96 (t, J = 2.2 Hz, CH-3β); ^{13}C-NMR ($CDCl_3$) δ −4.85 (2×), 11.40, 14.45, 15.91, 18.12, 20.26, 23.78, 25.86 (3×), 27.91, 28.47, 29.50, 29.69, 31.87, 31.99, 32.44, 34.02, 35.92, 36.03, 36.69, 39.08, 47.04, 49.90, 54.22, 66.80, 93.55, 172.25.

Methylation of Lactone **9**

A solution of diisopropylamine (0.45 mL, 3.5 eq) in dry THF (2 mL) was stirred at 0 °C under an argon atmosphere and a 1.6 M solution of *n*-BuLi in hexanes (2.36 mL, 4.02 eq) was added dropwise. After 30 min, the resulting LDA solution was cooled at −78 °C and lactone **9** (0.445 g, 0.938 mmol) in dry THF (50 mL) was added. The mixture was allowed to stir 1 h at 0 °C and then cooled again at −78 °C before the addition of methyl iodide (4.02 mL, 6 eq) dropwise. The reaction mixture was stirred overnight from −78

°C to room temperature. Water was added to quench the reaction and the crude product was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over $MgSO_4$ and evaporated under reduced pressure. A column chromatography using a mixture of hexanes and EtOAc (9/1) as eluent allowed us to separate the three reaction products: the monomethylated lactone **10A**, the monomethylated lactone **10B** and the dimethylated lactone **11**, in proportions 2:2:1, respectively, in 70% yield.

(*3S,5S,8R,9S,10S,13S,14S,17R)-3-{[tert-Butyl(dimethyl)silyl]oxy}-5',10,13-trimethyloctadecahydrospiro[cyclopenta[a]phenanthrene-17,2'-pyran]-6'(3'H)-one* (**10**).
10A: White solid; IR (film) υ 1729 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.01 (s, $Si(CH_3)_2$), 0.76 (s, CH_3-19), 0.88 (s, $SiC(CH_3)_3$), 0.96 (s, CH_3-18), 1.26 (d, *J* = 7.1 Hz, $C\underline{H}_3$-CH), 0.62–2.00 (unresolved CH and CH_2), 2.38 (m, CHCO), 3.95 (t, *J* = 2.0 Hz, CH-3β); ^{13}C-NMR ($CDCl_3$) δ −4.86 (2×), 11.38, 14.52, 17.35, 18.08, 20.31, 23.65, 25.23, 25.85 (3×), 28.46, 28.56, 29.68, 31.85, 31.97, 32.43, 34.65, 35.93, 36.02, 36.12, 36.68, 39.07, 46.94, 49.80, 54.21, 66.78, 93.93, 175.06. **10B**: White solid; IR (film) υ 1729 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.004 (s, $Si(CH_3)_2$), 0.76 (s, CH_3-19), 0.88 (s, $SiC(CH_3)_3$), 0.95 (s, CH_3-18), 1.22 (d, *J* = 6.8 Hz, $C\underline{H}_3$-CH), 0.60–2.20 (unresolved CH and CH_2), 2.52 (m, CHCO), 3.95 (t, *J* = 2.0 Hz, CH-3β); ^{13}C-NMR ($CDCl_3$) δ −4.88 (2×), 11.37, 14.49, 17.25, 18.06, 20.21, 23.87, 24.43, 25.84 (3×), 27.23, 28.45, 29.66, 31.84, 31.99, 32.33, 33.50, 34.02, 35.88, 35.99, 36.66, 39.02, 46.99, 49.63, 54.18, 66.77, 93.02, 176.01.

(*3S,5S,8R,9S,10S,13S,14S,17R)-3-{[tert-Butyl(dimethyl)silyl]oxy}-5',5',10,13-tetramethyloctadecahydrospiro[cyclopenta[a]phenanthrene-17,2'-pyran]-6'(3'H)-one* (**11**).
White solid; IR (film) υ 1718 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.002 (s, $Si(CH_3)_2$), 0.76 (s, CH_3-19), 0.87 (s, $SiC(CH_3)_3$), 0.95 (s, CH_3-18), 1.23 and 1.25 (2s, 2 × CH_3), 0.62–2.15 (unresolved CH and CH_2), 3.94 (s, CH-3β); ^{13}C-NMR ($CDCl_3$) δ −4.88 (2×), 11.37, 14.54, 18.05, 20.29, 23.53, 25.52, 25.83 (3×), 27.61, 27.73, 28.44, 29.66, 31.58, 31.80, 31.99, 32.40, 34.82, 35.91, 36.00, 36.66, 37.71, 39.04, 47.01, 49.69, 54.17, 66.76, 93.91, 177.96.

Hydrolysis of the Silylated Ethers of **10A**, **10B** and **11**

Lactones **10A**, **10B** and **11** were respectively dissolved in dry THF. A 1 M solution of TBAF in THF (2 eq) was added and the resulting mixture was stirred overnight at refluxing temperature under an argon atmosphere. Water was then added and extraction was done with EtOAc. The organic phase was washed with a saturated NaCl solution and dried over $MgSO_4$. The crude products were respectively submitted to Jones' reagent as described above for the synthesis of **4**. A column chromatography using a mixture of hexanes and EtOAc (9/1) afforded the same mixture of monomethylated lactones **12**, in the case of **10A** and **10B**, and the dimethylated lactone **13** in the case of **11**.

(5S,8R,9S,10S,13S,14S,17R)-5',10,13-Trimethylhexadecahydrospiro[cyclopenta[a] phenanthrene-17,2'-pyran]-3,6'(2H,3'H)-dione (**12**).
White solid; 90% yield; IR (film) υ 1714 (C=O, ketone and lactone); ^{1}H-NMR ($CDCl_3$) δ 0.99 (s, CH_3-19), 1.03 (s, CH_3-18), 1.23 and 1.27 (2d, J = 7.0 Hz and J = 7.2 Hz, C<u>H</u>$_3$-CH in proportions 1/1), 0.62–2.60 (unresolved CH and CH_2); ^{13}C-NMR ($CDCl_3$) δ 11.47, 14.52, (17.26), 17.35, 20.91, (20.98), (23.72), 23.94, (24.21), 24.41, 27.21, (28.58), 28.77, 31.44, 31.93, (33.50), 33.99, 34.64, 35.81 (2 ×), (36.15), 38.12, 38.60, 44.63, 46.75, 46.96, 49.45, (49.61), 53.67, 92.78, (93.65), 174.90, (175.89), 211.79; HRMS for $C_{24}H_{37}O_3$ $[M+H]^+$: calculated 373.2737, found 373.2739.

(5S,8R,9S,10S,13S,14S,17R)-5',5',10,13-Tetramethylhexadecahydrospiro[cyclopenta[a]-phenanthrene-17,2'-pyran]-3,6'(2H,3'H)-dione (**13**).
White solid; 92% yield; IR (film) υ 1716 (C=O, ketone and lactone); ^{1}H-NMR ($CDCl_3$) δ 0.99 (s, CH_3-19), 1.03 (s, CH_3-18), 1.25 and 1.26 (2s, 2 × CH_3), 0.63–2.50 (unresolved CH and CH_2); ^{13}C-NMR ($CDCl_3$) δ 11.45, 14.56, 20.97, 23.61, 25.51, 27.65, 27.75, 28.75, 31.41, 31.55, 31.92, 34.77, 35.81, 37.76, 38.11, 38.58, 44.62, 46.74, 47.01, 49.49, 53.63, 93.70, 177.90, 211.84; HRMS for $C_{25}H_{39}O_3$ $[M+H]^+$: calculated 387.2894, 387.2900.

3.4. Synthesis of the Dispiro Compounds **16** *and* **17** *(Scheme 3)*

(2R,5'S,8'R,9'S,10'S,13'S,14'S,17'S)-10',13'-Dimethylhexadecahydro-2'H-dispiro[oxirane-2,3'-cyclopenta[a]phenanthrene-17',2''-pyran]-6''(3''H)-one (**14**).

Trimethyl sulfoxonium iodide (1.3 g, 5.9 mmol) and sodium hydride 60% in mineral oil (236 mg, 5.9 mmol) was dissolved in DMSO (15 mL) and the mixture was stirred for 1 h at room temperature under an argon atmosphere. Compound **4** (0.5 g, 1.4 mmol) dissolved in THF (10 mL) was then added and the mixture stirred for 3 h. The reaction was quenched with a saturated solution of NH_4Cl (35 mL) and the crude product was extracted with EtOAc and evaporated under reduced pressure. A column chromatography using a mixture of hexanes and EtOAc (8/2) afforded compound **14** (450 mg, 1.2 mmol) as a white solid in 72% yield. IR (film) υ 1720 (C=O, ketone and lactone); ^{1}H-NMR ($CDCl_3$) δ 0.86 (s, CH_3-19), 0.98 (s, CH_3-18), 0.70–2.15 (unresolved CH and CH_2), 2.45 (m, CH_2CO), 2.62 (s, CH_2O); ^{13}C-NMR ($CDCl_3$) δ 11.26, 14.43, 15.84, 20.48, 23.78, 27.89, 28.38, 29.12, 29.45, 31.53, 31.96, 33.95, 35.51, 35.82, 35.85, 35,90, 43.72, 46.98, 49.79, 53.54, 53.84, 58.49, 93.42, 172.19.

Methyl-N-{[(13S)-3-hydroxy-10,13-dimethyl-6'-oxoicosahydrospiro[cyclopenta[a] phenanthrene-17,2'-pyran]-3-yl]methyl}leucinate (**15**).

To a solution of the oxirane **14** (200 mg, 0.54 mmol) dissolved in MeOH (8 mL) was added L-leucine methyl ester (782 mg, 5.4 mmol) and the mixture was stirred in a Schlenck tube. After 22 h at 90 °C the mixture was dissolved in CH_2Cl_2 and concentrated under reduced pressure. The crude product was purified by a column chromatography using a mixture of hexanes and EtOAc (8/2) to give the amino alcohol **15** (276 mg) in 98% yield. IR (film) υ 3464 and 3333 (OH and NH); 1736 (C=O, ketone and lactone); ^{1}H-NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.90 and 0.92 (2d, J = 6.6 Hz, 2 × CH_3 from *i*-Pr), 0.96 (s, CH_3-18), 0.70–1.98 (unresolved CH and CH_2), 2.17 and 2.61 (2d of AB system, J = 11.9 Hz, CH_2N), 2.43 (m, CH_2CO), 3.22 (t, J = 7.3 Hz, CHC=O), 3.72 (s, CH_3O); ^{13}C-NMR ($CDCl_3$) δ 11.23, 14.44, 15.86, 20.42, 21.94, 22.87, 23.79, 24.83, 27.90, 28.46, 29.47, 31.67, 31.71, 32.01, 33.80, 33.96, 35.91, 35.99, 38.52, 40.64, 42.77, 44.10, 47.01, 49.88, 51.71, 53.99, 59.25, 60.87, 69.88, 93.48, 172.24, 176.33.

Methyl-(2S)-2-[(5R,5'S,8'R,9'S,10'S,13'S,14'S,17'S)-10',13'-dimethyl-2,6''-dioxoocta decahydro-2'H,3H-dispiro[1,3-oxazolidine-5,3'-cyclopenta[a]phenanthrene-17',2''-pyran]-3-yl]-4-methylpentanoate (**16**).

The amino alcohol **15** (69 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (4 mL) and DIPEA (47.5 μL) was added. The solution was stirred for 10 min at 0 °C and triphosgene (20 mg, 0.07 mmol) was added in two portions to the mixture, which was stirred for 5 min at 0 °C and for 2.5 h at room temperature. Another portion of triphosgene (20 mg, 0.07 mmol) was then added and the mixture was stirred for 2 h at room temperature. A saturated solution of $NaHCO_3$ was used to quench the reaction and the crude product was extracted with CH_2Cl_2. The organic phase was evaporated under reduced pressure and the crude product purified by column chromatography with hexanes and EtOAc (90/10 and 85/15) as eluent to give **16** (40 mg, 0.07 mmol) in 55% yield as a white solid. IR (film) υ 1740 (C=O, ketone, lactone and carbamate); ^{1}H-NMR ($CDCl_3$) δ 0.81 (s, CH_3-19), 0.96 (s, CH_3-18 and 2 × CH_3 from *i*-Pr), 0.70–2.00 (unresolved CH and CH_2), 2.44 (m, CH_2CO), 3.13 and 3.42 (2d of AB system, J = 8.1 Hz, CH_2N), 3.72 (s, CH_3O), 4.59 (m, CHC=O); ^{13}C-NMR ($CDCl_3$) δ 11.40, 14.43, 15.84, 20.43, 21.06, 23.14, 23.76, 24.93, 27.91, 28.01, 29.47, 31.47, 31.93, 32.75, 33.87, 33.95, 35.31, 35.84, 37.69, 39.48, 40.86, 46.99, 49.77, 52.22, 52.89, 53.6 2×), 79.74, 93.40, 157.64, 171.99, 172.22; HRMS for $C_{32}H_{50}NO_6$ $[M+H]^+$: calculated 544.3633, found 544.3643.

(2R,5S,5'S,8'R,9'S,10'S,13'S,14'S,17'S)-10',13'-Dimethyl-5-(2-methylpropyl)hexadeca hydro-2'H,6H-dispiro[1,4-oxazinane-2,3'-cyclopenta[a]phenanthrene-17',2''-pyran]-6,6''(3''H)-dione (**17**).

To a solution of sodium methoxide (33 mg, 0.61 mmol) in dry THF (17.5 mL) was added the amino alcohol **15** (100 mg, 0.19 mmol) and the reaction mixture was stirred for 2 h at room temperature. The reaction was stopped by adding a saturated solution of NH_4Cl and the crude product was extracted with EtOAc and purified by HPLC to generate the starting amino alcohol **15** (18%) and compound **17** (24 mg, 39%) as a white solid. IR (film) υ 1724 (C=O, lactone); ^{1}H-NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.92 and 0.95 (2d, J = 6.2 Hz, 2 × CH_3 from *i*-Pr), 0.96 (s, CH_3-18), 0.75–2.02 (unresolved CH and CH_2), 2.43 (m, CH_2CO), 2.80 and 2.88 (2d of AB system, J = 13.5 Hz, CH_2N), 3.50 (m, CHC=O); ^{13}C-NMR ($CDCl_3$) δ 11.36, 14.45, 15.84, 20.46, 23.40, 23.78, 24.46, 27.89, 28.00, 29.47, 31.38, 31.48, 31.96, 32.98, 33.92, 35.85, 36.02, 38.11, 39.29, 41.41, 46.99, 49.83, 52.41, 53.50, 55.58, 82.37, 93.42, 171.99, 172.25; HRMS $C_{30}H_{48}NO_4$ $[M+H]^+$: calculated 486.3578, found 486.3590.

3.5. Inhibition of 17β-HSD5

The enzymatic assay was performed using transfected (17β-HSD5) human embryonal kidney (HEK)-293 cells provided by Dr. Van Luu-The (CHUQ (CHUL)─Research Center) [29]. Briefly, 0.1 μM of the natural substrate [^{14}C]-4-androstene-3,17-dione (Dupont Inc., Mississauga, ON, Canada) and 10 μL of an ethanolic solution of inhibitor were added to freshly changed culture medium in a 6-well culture plate containing HEK-293 cells overexpressing human 17β-HSD5. After incubation for 18 h, the reaction was stopped by adding a solution of unlabelled 4-androstene-3,17-dione (4-dione) and testosterone (T) before extracting twice with 2 mL of diethyl ether. The organic phase was pooled and evaporated to dryness. The metabolites were solubilised in dichloromethane, applied to silica gel 60 thin layer chromatography (TLC) plate (Merck, Darmstadt, GE), and then separated by migration in the toluene/acetone (4/1) solvent system. Substrates and metabolites were identified by comparing them to reference steroids, revealed by autoradiography, and quantified using the Phosphoimager system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation (% *Transf*) and then the percentage of inhibition (% *Inh*) were calculated using the following equations: % *Transf* = 100 × [^{14}C]-T (cpm)/([^{14}C]-T (cpm) + [^{14}C]-4-dione (cpm)) and % *Inh* = 100 × [% *Transf* (without inhibitor) − % *Transf* (with inhibitor)]/% *Transf* (without inhibitor). To avoid the enzyme inhibition by the resulting product of reaction (T), the quantity of enzyme (intact cells) and the incubation time were both selected to give a percentage of transformation below 30%, which is in a linear range.

3.6. Inhibition of 17β-HSD3 (Microsomal Fraction of Rat Testes)

A microsomal preparation of rat testes was obtained using slightly modified previously described procedures [30–32]. In brief, rat testes were homogenized on ice with a Polytron in cold phosphate buffer (20 mM KH_2PO_4, 0.25 M sucrose, 1 mM EDTA, pH 7.5) containing protease inhibitors mini-complete (Roche Diagnostics, Laval, QC, Canada) and centrifugated at 12,500g for 15 min to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifugated at 100,000g for 45 min using an ultracentrifuge equipped with a 70.1 Ti rotor. The microsomal pellet was washed three times with phosphate buffer and centrifugated at 100,000g for 15 min. All these operations were conducted at 4 °C. The protein concentration of the

supernatant was determined by the Bradford method using bovine serum albumin as standard [33]. The enzymatic assay was performed at 37 °C for 2 h in 1 mL of a solution containing 860 µL of 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 100 µL of 5 mM NADPH in phosphate buffer, 10 µL of 5 µM [4-^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 µL of inhibitor dissolved in ethanol and 20 µL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in triplicate. Afterwards, radiolabelled steroids were extracted from the reaction mixture with diethyl ether. The organic phases evaporated to dryness with nitrogen stream. Residue was dissolved in 50 µL of dichloromethane and dropped on silica gel 60 F_{254} thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1) solvent system. Substrate ([^{14}C]-4-dione) and metabolite ([^{14}C]-T) were identified by comparison with reference steroids and quantified using the Storm 860 System (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and then the percentage of inhibition were calculated as reported above (Section 3.5).

3.7. Proliferative and Antiproliferative Shionogi (AR$^+$) Cell Assay

Assay for the proliferation of androgen-sensitive Shionogi mammary carcinoma cells as well as the inhibition of 0.3 nM DHT-induced proliferation was carried out according to the procedure described by Bydal and co-workers [11]. Calculations were performed according to the following equations and expressed as percentages: (a) Proliferative or androgenic activity = [(B − A)/(C − A)] × 100 and (b) Antiproliferative or antiandrogenic activity = [(C − D)/(C − A)] × 100, where A is the DNA content on cells incubated with control medium (µg), B is the DNA content of cells treated with the tested compound (µg), C is the DNA content of DHT-stimulated cells (µg) and D is the DNA content of DHT-stimulated cells treated with the tested compound (µg).

3.8. Steroids Receptor Binding Assays

The binding affinity assays on estrogen and progestin receptors from rat uterine tissue were carried out under the standard procedure established in our laboratory [34]. Assay for androgen receptor from rat ventral prostate was performed according to the procedure described by Luo and co-workers [35]. In the case of glucocorticoid receptors from rat liver tissue, the affinity binding assay was done using a slightly modified

procedure described by Asselin and co-workers [36]. A dextran-coated charcoal adsorption, instead of a protamine sulfate precipitation, was used to achieve the separation of bound and free steroids.

4. Conclusions

Monospiro and dispiro steroid derivatives were efficiently synthesized from ADT or *epi*-ADT and characterized by IR, ^{1}H-NMR, ^{13}C-NMR and MS spectroscopies. Careful analysis of NMR data, especially ^{13}C-NMR spectra, allowed the full assignment of all carbons for the series of monospiro and dispiro steroid derivatives. When tested as inhibitors of 17β-HSD5 and 17β-HSD3, the monospiro derivatives inhibited the enzyme according to the positioning and in accord with previously reported SAR studies. Thus, 17β-HSD5 was inhibited by the monospiro derivative at position C-17 whereas 17β-HSD3 was inhibited by a monospiro derivative at position C-3. For the first time, the presence of two spiro-functionalities was investigated as inhibitors of 17β-HSD3, but this strategy resulted in a lower inhibitory potency. Additional SAR results were generated for inhibiting 17β-HSD3 and 17β-HSD5, two key steroidogenic enzymes involved in biosynthesis of testosterone and in prostate cancer.

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Chapitre 5

Synthèse de dérivés 3-spiromorpholinone de l'androstérone comme inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 3

1. Avant-propos

Les résultats présentés dans ce chapitre découlent d'un criblage de différentes spiromorpholinones ayant une stéréochimie différente (*R* ou *S*) sur leur cycle E supplémentaire. Cependant, étant donné qu'il s'agit d'une communication courte (*Letters*), seul quelques exemples pertinents des résultats ont été rapportés, et l'ensemble des résultats ont été présentés dans le chapitre 6. Une étude des relations structure-activité (SAR) (synthèse chimique et évaluation biologique) nous a permis de confirmer que les spiromorpholinones de stéréochimie (*S*) sont de meilleurs inhibiteurs comparés à leurs analogues de stéréochimie inverse (*R*), et que les spiromorpholinones non stéroïdiennes sont très peu actives sur la 17β-HSD3. Les essais enzymatiques réalisés par Lucie Carolle Kenmogne ont été très utiles pour tirer les conclusions de ce projet.

J'ai réalisé la synthèse des spiromorpholinones stéroïdiennes et non stéroïdiennes ainsi que leur caractérisation et l'étude des relations structure-activité.

2. Résumé

Des spiromorpholinones ont été synthétisées à partir de l'androstérone ou de la cyclohexanone en 6 ou 3 étapes respectivement, et ces noyaux ont permis la mise en place d'un groupe hydrophobe par substitution nucléophile. Les spiromorpholinones non stéroïdiennes ne sont pas actives comme inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 3 (17β-HSD3), mais les morpholinones stéroïdiennes sont des inhibiteurs très puissants. En fait, les composés avec la stéréochimie (*S*) sont plus actifs que leurs homologues (*R*), tandis que les composés *N*-benzylés sont plus actifs que leurs précurseurs non substitués. Ces dérivés stéroïdiens ont montré une forte inhibition de la 17β-HSD3 dans un homogénat de testicules de rat (87-92 % d'inhibition à 1 μM).

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Synthesis of 3-Spiro-morpholinone androsterone derivatives as Inhibitors of 17β-Hydroxysteroid Dehydrogenase type 3

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Abstract

Spiromorpholinone derivatives were synthesized from androsterone or cyclohexanone in 6 or 3 steps, respectively, and these scaffolds were used for the introduction of a hydrophobic group via a nucleophilic substitution. Non-steroidal spiromorpholinones are not active as inhibitors of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), but steroidal morpholinones are very potent inhibitors. In fact, those with (*S*) stereochemistry are more active than their (*R*) homologues, whereas N-benzylated compounds are more active than their non substituted precursors. The target compounds exhibited strong inhibition of 17β-HSD3 in rat testis homogenate (87 – 92% inhibition at 1 μM).

The role of 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) in androgen-dependent prostate cancer is well established.[1–3] This enzyme converts 4-androstene-3,17-dione (Δ^4-dione) into the androgenic hormone testosterone (T) in the presence of cofactor NADPH.[4–6] Even though 17β-HSD3 is almost exclusively expressed in testes, it is up-regulated in prostate tumors.[7] In the classic pathway, T is further converted into the most active androgen dihydrotestosterone (DHT) by 5α-reductase (Figure 1). In fact, both T and DHT can activate the androgen receptor (AR) and, consequently, stimulate the proliferation of prostate cancer cells. To stop the androgen biosynthesis at the level of Δ4-dione, a steroid inactive on AR,[8] an inhibitor of 17β-HSD3 could be used. Since this membrane enzyme was not yet crystallized, structure-activity relationships (SAR) must be established for inhibitor development. From our previous laboratory work, it was established that the presence of a hydrophobic group at position C-3 of androsterone (ADT) is a good strategy for designing potent inhibitors of 17β-HSD3.[9–13]

In order to develop novel inhibitors of 17β-HSD3 (Scheme 1), we decided to build a new ring system (cycle E) at position 3 of the ADT nucleus. In fact, introducing a 3-spiroheterocyclic moiety is a good strategy for adding rigidity and introducing diversified hydrophobic groups with several orientations. It is thus expected that such groups can increase the affinity to 17β-HSD3 hydrophobic pocket.

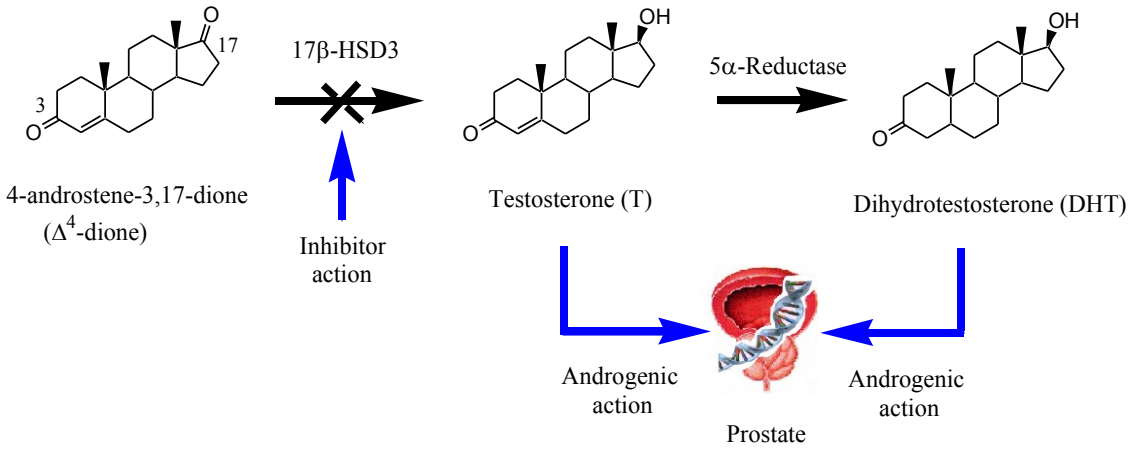


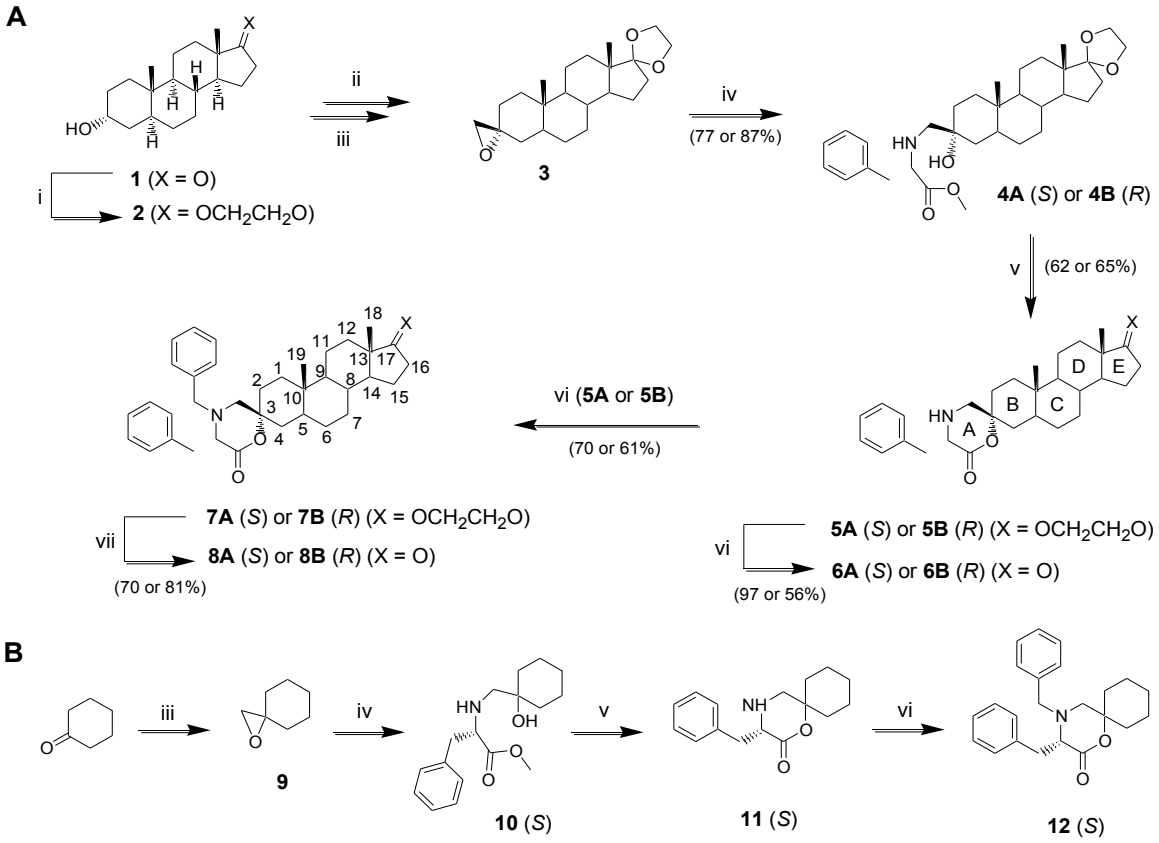
Figure 1. Blocking the biosynthesis of testosterone and dihydrotestosterone by using an inhibitor of 17β-HSD3.

The chemical steps involved in the synthesis of target compounds **6A**, **6B**, **8A** and **8B** are shown in Scheme 1A. Steroidal oxirane **3** was synthesized following three steps as previously reported.[11,12] Briefly, the C-17 ketone of ADT (**1**) was protected as a

dioxolane, the C-3 alcohol function of **2** was then oxidized in the presence of tetrapropylammonium perruthenate (TPAP) and N-methylmorpholine-N-oxide (NMO), and this ketone reacted regioselectively with trimethylsulfoxonium iodide to generate the oxirane **3**. This compound was subjected to aminolysis with (L) or (D) phenylalanine methyl ester to yield the amino-alcohol **4A** or **4B**. These two amino-alcohols were subjected to lactonization using sodium methoxide in THF at room temperature and the spiromorpholinones **5A** and **5B** were obtained. For this step, we adapted and optimized a method previously reported for position C-17 of the steroid.[14] In place of sodium hydride, low loading of sodium methoxide (0.6 equivalent) was used in a diluted reaction mixture to avoid racemization of the alpha-hydrogen of the amino acid ester as well as the formation of side products. The secondary amines **5A** and **5B** were benzylated following a nucleophilic substitution to give **7A** and **7B**. The hydrolysis of C-17-dioxolanes **5A**, **5B**, **7A** and **7B** in dioxane and aqueous 5% sulfuric acid generated the target products **6A**, **6B**, **8A** and **8B**.

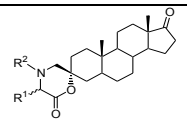
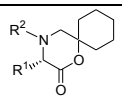
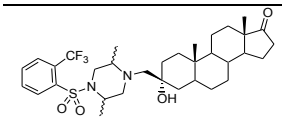
In order to verify the importance of the steroid scaffold for the inhibition of 17β-HSD3, non-steroidal spiromorpholinones **11** and **12** were synthesized starting from cyclohexanone and following the sequence of reactions reported in Scheme 1B. We selected only non-steroidal (S) isomers based on our preliminary results showing that the (*S*) spiromorpholinone isomer of steroidal derivatives produced a better 17β-HSD3 inhibitory activity than the (*R*) isomer. Compounds **11** and **12** were thus obtained with 76% and 61% global yields, in three and four steps, respectively.

All final steroidal and non-steroidal compounds as well as their intermediates were fully characterized (IR, ^{1}H NMR, ^{13}C NMR and LRMS) to confirm their chemical structure. To illustrate this, we reported the data from the steroidal and non-steroidal spiromorpholinones **6A**, **8A** and **12**.[15–17]



Scheme 1. Synthesis of spiromorpholinone derivatives. Reagents: i) $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux; ii) NMO, molecular sieves, TPAP, DCM, rt, 3 h; iii) $(CH_3)_3SOI$, NaH, DMSO/THF, rt; iv) (L) or (D)-phenylalanine methyl ester, MeOH, 90°C; v) CH_3ONa, THF, rt; vi) DIPEA, $C_6H_5CH_2Br$, DCM, 75°C, 22 h; vii) H_2O:H_2SO_4, dioxane, rt.

Table 1. Inhibitory activity toward 17β-HSD3 of the target compounds 6A, 6B, 8A, 8B, 11 and 12.

Structures	Names	*R* / *S*	R^1	R^2	Inhibition at 0.1 µM (%)[a]	Inhibition at 1 µM (%)[a]	Inhibition IC_{50} (nM)[b]
	**6A**	*S*	Bn	H	48.8 ± 2.0	92.0 ± 1.6	22
	6B	*R*	Bn	H	18.5 ± 26.9	63.2 ± 5.8[c]	---
	8A	*S*	Bn	Bn	58.2 ± 1.7	90.4 ± 0.7	58
	8B	*R*	Bn	Bn	25.6 ± 5.7	87.3 ± 2.8	---
	**11**	*S*	Bn	H	6.2 ± 6.3	19.5 ± 3.8	---
	12	*S*	Bn	Bn	24.2 ± 3.2	24.3 ± 4.2	---
	RM-532-105[d]	---	---	---	47.3 ± 12.4	92.1 ± 0.4	14

(a) Transformation of [^{14}C]-4-androstene-3,17-dione (50 nM) into testosterone by 17β-HSD3 in microsomal fraction of rat testes. (b) IC_{50} values were determined from the inhibition curves reported in Figure 2 using GraphPad-Prism 6 software (GraphPad Inc Solfware). (c) This inhibition value was obtained from another experiment performed under the same conditions. (d) Compound **15b** in reference 12).

The inhibitory activity of compounds **6A**, **6B**, **8A**, **8B**, **11** and **12** on 17β-HSD3 was evaluated in a microsomal fraction of rat testes using a known procedure.[18] These compounds were compared to RM-532-105, a known inhibitor of 17β-HSD3,[12] for their ability to inhibit the transformation of Δ^4-dione into T (Table 1). Non-steroidal spiromorpholinones **11** and **12** are not active, suggesting that the steroidal nucleus is essential for inhibitory activity, but steroidal morpholinones produced a very good inhibition of 17β-HSD3. Compounds with (*S*) conformation on the carbon bearing a benzyl (Bn) showed better inhibition than their (*R*) homologues. In fact, compound **6A** blocked 48.8% of the transformation of Δ^4-dione at 0.1 µM and consequently is a better inhibitor than compound **6B** (18.5%). Similarly, compound **8A** (58.2% inhibition at 0.1 µM) is more potent than its stereoisomer **8B** (25.6%). In this screening assay, compound **8A** seems to be a better inhibitor (58.2%) than the reference compound RM-532-105 (47.3% at 0.1 µM).

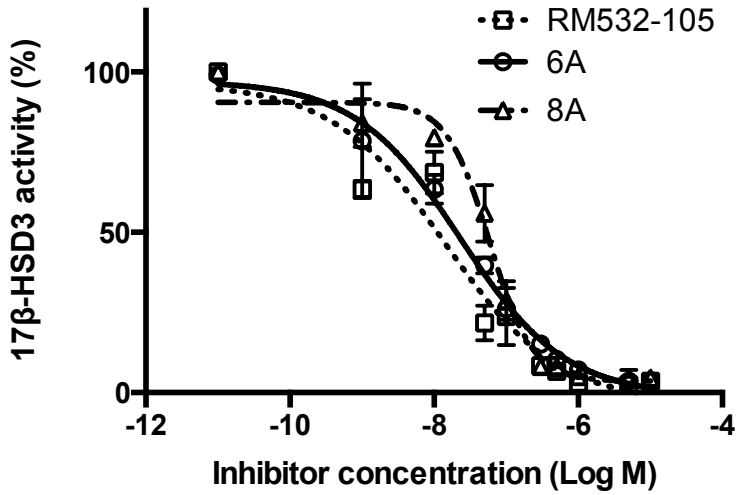


Figure 2. Effect of **6A**, **8A** and RM-532-105 on the transformation of [^{14}C] Δ^4-dione (50 nM) into [^{14}C]-T by 17β-HSD3. Results are expressed as mean ± SEM of triplicate.

For comparison purposes, we next tested the two steroidal (S)-spiromorpholinone derivatives **6A** and **8A** with the know 17β-HSD3 inhibitor RM-532-105 (Figure 2). Spiromorpholinones **6A** and **8A** (IC_{50} = 22 and 58 nM, respectively) are about 2-fold and 4-fold less potent than RM-532-105 (IC_{50} = 14 nM) (Table 1). These results confirm the potency of **6A** and **8A** as new lead compounds for inhibiting 17β-HSD3. Thus, we are confident that such 3-spiromorpholinone ADT derivatives can be improved by judicious diversification of the spirocycle as well as localization of the hydrophobic group. Further work reporting full details of the diversification of the 3-spiromorpholinone, including chemistry, SAR study and biological activity of the target compounds is underway and will be presented in a full paper in due course.

Acknowledgments

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15. Data for compound **6A**: White foam. IR (film, ν, cm^{-1}) 3333 (NH), 3032 (CH, Ph), 1736 (C=O, ketone and lactone). ^1H NMR-400 MHz (Acetone-d_6, δ, ppm) 0.81 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.37 (dd, J_1 = 8.7 Hz and J_2 = 18.2 Hz, CH-16β), 2.79 and 2.89 (2d of AB system, J = 13.3 Hz, CH_2N), 3.02 and 3.17 (2m, CHC$\underline{H}_2$Ph), 3.73 (dd, J_1 = 4.0 Hz and J_2 = 8.1 Hz,

NCHCO), 7.25 (m, Ph). ^{13}C NMR-100 MHz ($CDCl_3$, δ, ppm) 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.0, 31.5, 32.83, 35.0, 35.8, 36.0, 38.0, 39.2, 47.8, 51.4, 52.6, 53.7, 53.8, 58.7, 82.7, 127.1, 128.8 (2C), 129.5 (2C), 137.2, 170.8, 221.3. LRMS (m/z) calcd. for $C_{29}H_{40}NO_3$ $[M + H]^+$ 450.29, found 450.35.

16. Data for compound **8A**: White foam. IR (film, ν, cm^{-1}) 3063, 3032 (CH, Ph) and 1728 (C=O, ketone and lactone). ^{1}H NMR-400 MHz (Acetone-d_6, δ, ppm) 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.22 and 2.59 (2d of AB system, J = 12.5 Hz, CH_2N), 2.36 (dd, J_1 = 8.7 Hz and J_2 = 18.4 Hz, CH-16β), 3.27 and 4.40 (2d of AB system, J = 13.9 Hz, NC$\underline{H}_2$Ph), 3.37 (m, CHC$\underline{H}_2$Ph), 3.51 (dd, J_1 = 2.9 Hz and J_2 = 5.1 Hz, NCHCO), 7.31 (m, 2 x Ph). ^{13}C NMR-100 MHz (Acetone-d_6, δ, ppm) 10.7, 13.1, 20.0, 21.4, 27.7, 30.6, 30.6, 31.6, 33.0, 34.9, 35.1, 35.2, 35.8, 37.9, 39.6, 47.3, 51.2, 54.1, 57.4, 57.7, 65.9, 80.5, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5, 218.7. LRMS (m/z) calcd. for $C_{36}H_{46}NO_3$ $[M + H]^+$ 540.34, found 540.40.

17. Data for compound **12**: Yellowish oil. IR (film, ν, cm^{-1}) 3063, 3032 (CH, Ph) and 1720 (C=O, ketone and lactone). ^{1}H NMR-400 MHz ($CDCl_3$, δ, ppm) 0.87-1.74 (unassigned CH_2 of cyclohexyl), 2.08 and 2.65 (2d of AB system, J = 12.6 Hz, CH_2N), 3.18 and 4.31 (2d of AB system, J = 13.7 Hz, NC$\underline{H}_2$Ph), 3.26 and 3.54 (2m, CHC$\underline{H}_2$Ph); 3.52 (m, NCHCO), 7.28 (m, 2 x Ph). ^{13}C NMR-100 MHz ($CDCl_3$, δ, ppm) 21.2, 21.4, 25.3, 34.3, 35.7, 35.8, 56.0, 58.1, 66.3, 81.7, 126.7, 127.4, 128.0 (2C), 128.4 (2C), 128.5 (2C), 130.3 (2C), 137.5, 137.5, 171.0. LRMS (m/z) calcd. for $C_{23}H_{28}NO_2$ $[M + H]^+$ 350.20, found 350.20.

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Chapitre 6

Conception, synthèse chimique et évaluation biologique de dérives 3-spiromorpholinone/3-spirocarbamate de l'androstérone comme inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 3

1. Avant-propos

L'introduction d'une spriromorpholinone ou d'un sprirocarbamate portant un substituant hydrophobe en position C-3 de l'androstérone est la stratégie que nous avons adoptée pour le développement des inhibiteurs présentés dans ce chapitre. En effet, la restriction stérique offerte par le spirocycle et la présence d'une fonction amine sur celui-ci permettent à la fois d'ajouter différents groupements hydrophobes et de diriger l'orientation spaciale de ce dernier, selon la stéréochimie de la chaine lattérale de l'acide aminé de départ. Le présent chapitre montre en détail les résultats de la synthèse chimique de tous les composés ciblés, l'optimisation du processus de lactonisation, les criblages biologiques effectués et l'étude des relations structure-activité qui ont conduit au développement de nos meilleurs inhibiteurs. Les évaluations biologiques ont été effectuées par Lucie Carolle Kenmogne et Jenny Roy.

J'ai synthétisé et caractérisé tous les composés et intermédiaires nouveaux rapportés dans ce chapitre. J'ai également optimisé l'étape de la lactonisation et participé à l'analyse des relations structure-activité, en vue de choisir les meilleurs substituants hydrophobes présent sur le spirocycle.

2. Résumé

La 17β-hydroxysteroïde déshydrogénase de type 3 (17β-HSD3) est une enzyme clé dans la biosynthèse de la testostérone et de la dihydrotestostérone. Ces hormones stimulent les cancers de la prostate androgéno-dépendants. Afin de générer des inhibiteurs efficaces et non androgéniques de la biosynthèse des androgènes, nous avons synthétisé deux familles de 3-spiromorpholinones et de 3-spirocarbamates, tous dérivés de l'androstérone et portant des groupements hydrophobes diversifiés. Nous avons testé leur activité inhibitrice sur la 17β-HSD3 et mesuré leur effet androgénique sur les cellules androgéno-sensibles LAPC-4. De notre première étude des relations structure-activité (RSA), nous avons constaté que le composé **7E** a inhibé la 17β-HSD3 (77 % à 0,1 μM) de façon comparable à notre composé de référence RM-532-105 (76 % à 0,1 μM), mais présentait une activité androgénique résiduelle. Une librairie d'analogues du composé **7E** a été synthétisée afin de réduire l'effet androgénique observé. Dans cette nouvelle étude de RSA, les composés sulfonamide (**7E21**) et carboxamide (**7E22**) ont inhibé efficacement la 17β-HSD3 (IC_{50} = 28 et 88 nM, respectivement). Ces deux composés se sont révélés non-androgéniques et non-cytotoxiques, même à la plus forte concentration testée.

Journal of Medicinal Chemistry (Manuscript to be submitted)

Design, chemical synthesis and biological evaluation of 3-spiromorpholinone/3-spirocarbamate androsterone derivatives as inhibitors of 17β-hydroxysteroid dehydrogenase type 3

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Key words: Androgens, Spiromorpholinone, Synthesis, Prostate cancer, 17β-Hydroxysteroid dehydrogenase

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Abstract:

17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a key enzyme involved in the biosynthesis of testosterone and dihydrotestosterone. These hormones are known to stimulate the androgen-dependent prostate cancer. In order to generate effective inhibitors of androgen biosynthesis without androgenic effect, we synthesized a new family of 3-spiromorpholinone and 3-spirocarbamate androsterone derivatives bearing diversified hydrophobic groups. We also tested their inhibitory activity on 17β-HSD3 and their androgenic effect on androgeno-sensitive LAPC-4 cells. From our first structure-activity relationship (SAR) study, we noted that compound **7E** inhibited 17β-HSD3 (77 % at 0.1 µM) comparably to our reference compound RM-532-105 (76 % at 0.1 µM), but exhibited a residual androgenic effect. A library of **7E** analogues was next synthesized in order to generate compounds with reduced androgenic activity. In this new SAR study, the sulfonamide compound **7E21** and the carboxamide compound **7E22** inhibited 17β-HSD3 (IC_{50} = 28 and 88 nM, respectively). These two compounds were not androgenic and not cytotoxic even at the highest concentration tested. Thus, they are good candidates to be used further for *in vivo* assessment of their androgen deprivation activity.

Introduction

Prostate cancer is the second leading cause of death due to cancer in American men.[1] Androgens are known to play an important role in the development, growth, and the progression of prostate cancer.[2,3] The steroidogenic enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD3) converts 4-androstene-3,17-dione (Δ^4-dione) into the potent and major circulating androgen testosterone (T), in the presence of cofactor NADPH.[4] Both T and its metabolite dihydrotestosterone (DHT) bind the androgen receptor (AR), which dimerizes, translocalizes in the nucleus and activates the cell proliferation (Figure 1). Androgen deprivation has been recognized to be one of the most efficient treatments for advanced stage prostate cancer.[5] In fact, up to 80 % of patients with metastatic prostate cancer respond to androgen deprivation treatments.[6] Although many endocrine therapies are now available to block either the formation of testicular T and the action of T and DHT on AR,[7,8] there is still unmet medical needs for treatments using this approach. In fact, several side effects such as hot flush, erectile dysfunction, decrease muscle mass, hypertension and hypokalemia are usually observed.[9,10] Furthermore, abiraterone acetate, a recently approved CYP17 inhibitor used for patients with metastatic castration-resistant prostate cancer, may lead to adrenal insufficiency or adrenal crisis.[11] According to all these observations, and given that 17β-HSD3 acts in the last step of the biosynthesis of T, we hope that the development of a potent inhibitor of 17β-HSD3 without androgenic effect is a good therapeutic option for prostate cancer and other androgen-dependent diseases.

In previous works from our laboratory, androsterone (ADT) derivatives substituted at position C-3 were identified as new inhibitors of 17β-HSD3, and many important criteria for inhibition of 17β-HSD3 were established.[12-19] Although these inhibitors showed strong blockage of the enzyme activity, many of them produced an undesirable androgenic effect on Shionogi (AR^+) cells. In order to developed novel inhibitors of 17β-HSD3, we decided to build a new ring system (cycle E) at position 3 of the ADT nucleus. In fact, introducing a 3-spiroheterocyclic moiety is our strategy for adding rigidity and introducing diversified hydrophobic groups with different special orientations. This will enable the exploration of the hydrophobic pocket identified from our studies as important for 17β-HSD3 inhibition.

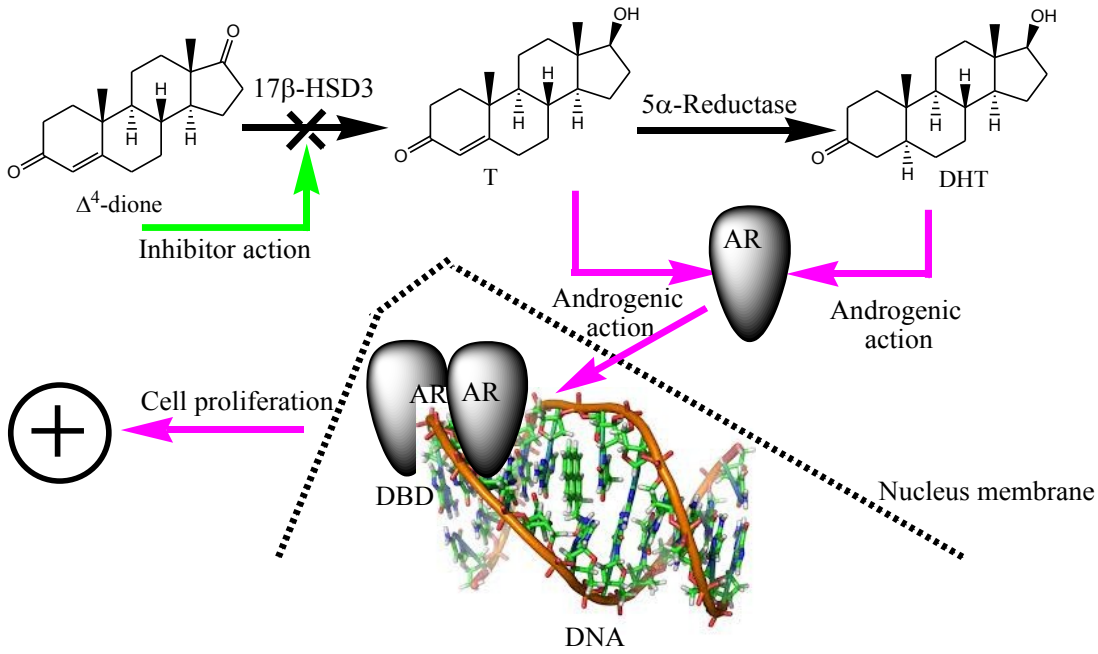
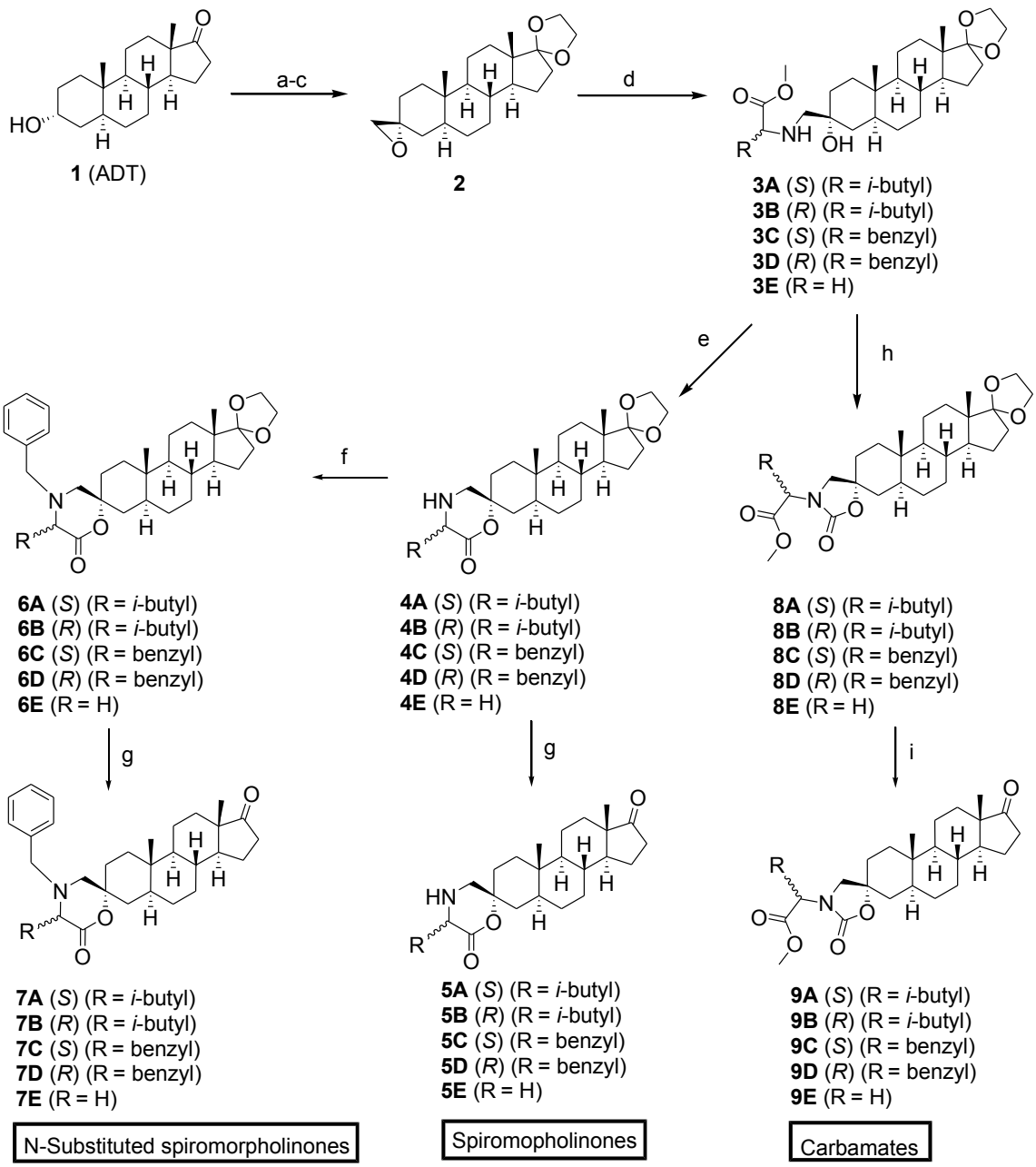


Figure 1: Biosynthesis of testosterone (T) and dihydrotestosterone (DHT) from 4-androstene-3,17-dione (Δ^4-dione) and their androgenic action. T and DHT bind to the androgen receptor (AR) that dimerizes and translocalizes in the cell nucleus, it then binds to the DNA binding domain (DBD) to co-activate cell proliferation.

In preliminary reports we have proved the impact of the stereochemistry on the carbon adjacent to the carbonyl of morpholinone.[20,21] We herein present full details of the chemical synthesis and characterization of a new ADT derivatives substituted at position C-3 by a spiromorpholinone or a spirocarbamate; the assessment of their ability to block the transformation of Δ^4-dione to T by 17β-HSD3 and the evaluation of their androgenic effect on androgen sensitive cells.



Scheme 1: Synthesis of target compounds. Reagents and conditions: (a) $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux, overnight; (b) NMO, molecular sieves, TPAP, DCM, rt, 3 h; (c) $(CH_3)_3SOI$, NaH, DMSO/THF, rt; (d) Methyl ester of L- or D-Leucine, L- or D-phenylalanine or glycine methyl esters, MeOH, 90°C, overnight; (e) CH_3ONa, THF, rt, 2 h; (f) DIPEA, $C_6H_5CH_2Br$, DCM, 75°C, 22 h; (g) H_2O:H_2SO_4, dioxane, rt, 2 h to 5 h; (h) $(Cl_3CO)_2CO$; DCM; 0°C to rt, 5 h; (i) HCl:MeOH (1:9), overnight.

Results and discussion

Chemical synthesis of steroidal compounds (scheme 1 and 2)

The oxirane **2** (3α-O) was obtained from a well known procedure already described in previous work (Scheme 1).[12,22] The inverse stereoisomer (3β-O) was the minor product isolated after recrystallization in acetone of the major product (stereoisomer 3α-O). Aminolysis of oxirane **2** in methanol yielded the aminoalcohols **3A-3E** with good to excellent yields (53-99 %). A similar aminolysis was previously done by Rouillard *et al.* at position C-17 of an estrane nucleus,[23] but in this work, since glycine methyl ester has high polarity, the authors failed in isolating the free aminoacid and to use it for the aminolysis of the C-17-oxirane. To overcome this problem, we generated glycine methyl ester *in situ* from its chlorhydrate salt, and the epoxide ring was opened successfully to form the glycine derivative **3E** in 53 % yield.

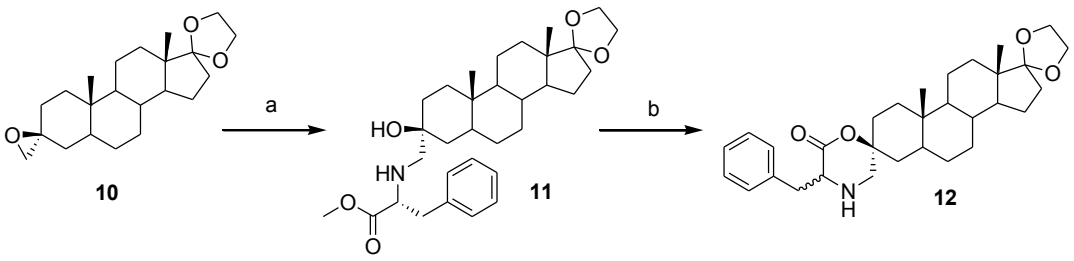
Table 1: Optimisation of the lactonisation process

Entry[a]	Base	Base Equiv.	THF (mL)	T (°C)	Crude mass isolated (mg)	Total mass of Pure *R/S*-isomers (mg)	Yield of *R/S*-isomers (%)	*R/S*-ratio[b]
1	NaH	0.9	1.5	22	129	17	17	[53:47]
2	NaH	1.1	5	22	50	17	24	[54:46]
3	*t*-BuOK	1.1	5	22	66	12	17	[49:51]
4	HMDSNa	1.1	5	22	55	25	36	[66:34]
5	LDA[c]	1.1	5	22	73	50	71	[52:48]
6	LDA[c]	2.2	5	22	----[e]	----[e]	----[e]	----[e]
7	LDA[c]	3.3	5	22	----[e]	----[e]	----[e]	----[e]
8	LDA[d]	<1.1	5	22	70	23	33	[86:14]
9	MeONa	1.1	5	22	83	63	90	[73:27]
10	MeONa	0.5	5	0	82	69	98	[77:23]
11	MeONa	0.6	12	22	85	69	98	[80:20]
12	MeONa	0.5	12	-10	84[f]	42	60	[76:24]

(a) We used 114 mg of starting 3α-O aminoalcohol **3D** for entry 1 and 75 mg of 3β-O aminoalcohol **11** for entries 2-12. (b) Two spots with variable importance on TLC plates. The ratio was calculated from ^{1}H NMR considering the integration of C-18 singlet. (c) LDA was generated separately in a large scale. (d) LDA was generated *in si*tu in a small scale (345 µL total volume of solution). (e) Products were formed but degraded readily; TLC showed total degradation after 4 h reaction time and no further work-up was done. (f) 25 % of starting aminoalcohol was observed.

The spiromorpholinones **4A-4E** were prepared by the intramolecular transesterification of the aminoalcohols **3A-3E**, respectively. In our first assay with 0.9 equivalent of sodium hydride (Table 1, entry 1), we observed a poor overall reaction yield (17 %), probably due to undesired intermolecular side reactions. TLC analysis showed two spots for two major products, while ^{1}H NMR characterization showed fingerprint for both products isolated, with a very slight difference on the chemical shift

of HC-α, CH_2N, $\underline{CH_2}Ph$ and 18-CH_3, suggesting that a racemisation occurred. In fact, since the HC-α of the lactone ring is slightly acid, especially when the geminal group is a benzyl, racemisation can occur in presence of NaH used as base. In order to optimize this crucial step and to reduce racemisation, several cyclisation conditions were performed (Table 1, entries 2-12) using the aminoalcohol **11**, obtained from the minor oxirane **10** (isomer 3β-O) (Scheme 2). The mixture of two major products formed were isolated, purified by chromatography to give the yield, and the *R/S*-ratio of both isomers was measured from the integration of the ^{1}H NMR spectra, which showed two distinctive singlets for 18-CH_3 (Figure 2A). Yield and *R/S*-ratio were not significantly improved using the same base (NaH) in a more diluted solution (entry 2). In order to investigate the effect of a more hindered base on the yield and *R/S*-ratio, we used potassium *tert*-butoxide (*t*-BuOK), sodium hexamethyldisilazide (HMDSNa) and lithium diisopropylamide (LDA).



Scheme 2: Chemical synthesis of **11** and **12** from epoxide **10**. Reagents and conditions: (a) D-Phenylalanine, MeOH, 90°C, overnight; (b) NaH, *t*-BuOK, HMDSNa, LDA or MeONa, THF, -10°C, 0°C or 22°C, 2 h (see Table 1).

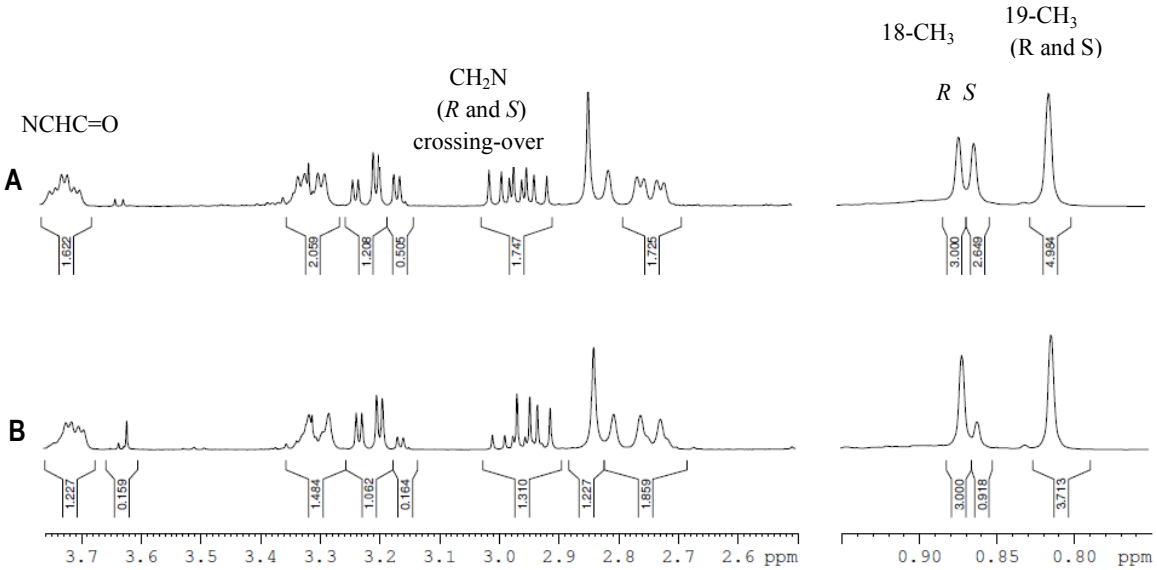


Figure 2: Fragments of ^{1}H NMR showing racemisation during the lactonisation of **11** to **12**. Distinctive 18-CH_3 peaks of the two isomers (*R* and *S*) were used to calculate the *R/S*-ratio of the isomeric mixture; (A) [52:48], (B) [77:23]

t-BuOK caused a total racemisation with only 17 % yield of both isomers (entry 3). Although the global yield was improved with HMDSNa ([66:34] *R/S*-ratio) and LDA ([52:48] *R/S*-ratio) when using these bases in the same conditions (36 % and 71 %, respectively) (entries 4 and 5), the *R/S*-ratio did not increase. In fact a drastic lost of global yield was observed with 2.2 and 3.3 equivalents of LDA (entries 6 and 7) whereas a moderate yield of 33 % and a very good *R/S*-ratio were obtained with less than 1 equivalent of LDA (entry 8). Sodium methoxide gave the best yield and few racemisation (entries 9-12, Figure 2B). Among all conditions tested, sodium methoxide (0.6 equivalent) used at 22°C in 12 mL of THF (entry 11) was selected for the lactonisation of **3A-3E** providing **4A-4E**. The high volume of THF and low quantity of base favor the intramolecular transesterification and avoid undesired intermolecular side reactions, thus providing the best yields of spiromorpholinones.

The *N*-benzylated spiromorpholinones **6A-6E** were obtained by a nucleophilic substitution of the secondary amines **4A-4E**. Finally, the C-17-dioxolane group of secondary amines **4A-4E** and tertiary amines **6A-6E** were hydrolyzed to provide the final compounds **5A-5E** and **7A-7E**. The spirocarbamates **8A-8E** were synthesized from the aminoalcohols **3A-3E**, respectively, following a treatment with triphosgene.[15] Intermediates **8A-8E** were not isolated from the reaction mixture and their C-17-dioxolane group was directly hydrolyzed to obtain **9A-9E** in 32 to 73 % yields.

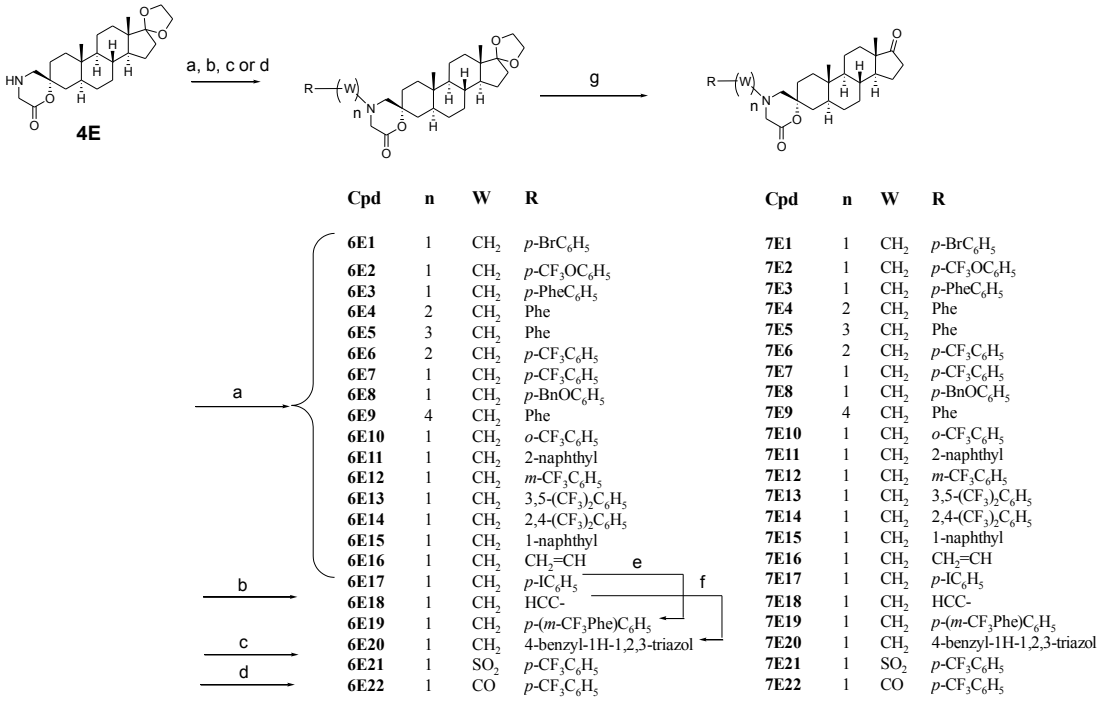
Chemical synthesis of steroidal compounds (scheme 3)

For our SAR study, different benzyl derivatives were also used to introduce molecular diversity on the spiromorpholinone ring. Starting from compound **4E**, a series of tertiary amines **6E1-6E18** were obtained by nucleophilic substitution (Scheme 3). We also investigated the Suzuki-Miyaura coupling[24] as an additional strategy for adding diversity. In fact, when compounds **6E17** was treated with 3-(trifluoromethyl)benzeneboronic acid, no reaction was observed whith $Pd(OAc)_2$ as catalyst, but using 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride ($Pd(dppf)Cl_2$) yielded the biaryl compound **6E19** in 23 % yield. This poor yield was probably due to the opening of the spirocycle E in aqueous Na_2CO_3 solution, but this reaction could be optimized. Starting from **6E18** we also experienced the "click chemistry" as a mean to diversify the *N*-substituent. Thus, the steroidal triazolic compound **6E20** was obtained by 1,3-dipolar cycloaddition of the terminal alkyne **6E18**

with benzyl azide.[25] It is well known that the 1,4-triazole regioisomer is exclusively obtained from catalysis with copper (I).[26-28] The sulfonamide **6E21** and the carboxamide **6E22** were also synthesized by nucleophilic substitution under conditions depicted in Scheme 3 and using benzyl sulfonyl chloride and benzoyl chloride, respectively. The C-17-dioxolane group of the tertiary amines **6E1**-**6E22** were finally hydrolyzed to generate the corresponding 17-keto products **7E1**-**7E22**.

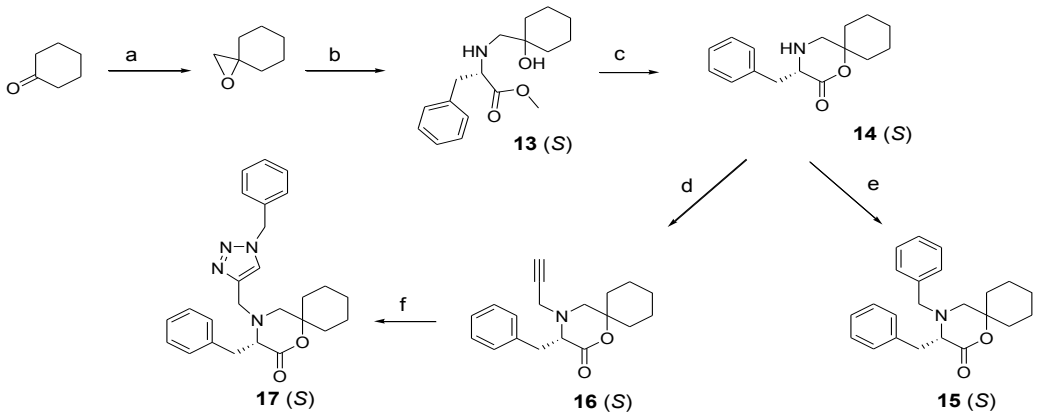
Chemical synthesis of non steroidal compounds (scheme 4)

In order to verify the importance of the steroid scaffold for the inhibition of 17β-HSD3, non-steroidal spiromorpholinones **14**, **15** and **17** were synthesized starting from cyclohexanone and following the sequence of reactions depicted in Scheme 4. Compound **14** was also used to prepare its propargylic derivative **16**, which was submitted to a 1,3-dipolar cycloaddition, under the same conditions used for the preparation of compound **6E20**, to generate compound **17**. The preparation of **17** using "click chemistry" represent an interesting additional strategy to the nucleophilic substitution for introducing molecular diversity on the spiromorpholinone ring.



Cpd	n	W	R	Cpd	n	W	R
6E1	1	CH_2	*p*-BrC_6H_5	**7E1**	1	CH_2	*p*-BrC_6H_5
6E2	1	CH_2	*p*-$CF_3OC_6H_5$	**7E2**	1	CH_2	*p*-$CF_3OC_6H_5$
6E3	1	CH_2	*p*-$PheC_6H_5$	**7E3**	1	CH_2	*p*-$PheC_6H_5$
6E4	2	CH_2	Phe	**7E4**	2	CH_2	Phe
6E5	3	CH_2	Phe	**7E5**	3	CH_2	Phe
6E6	2	CH_2	*p*-$CF_3C_6H_5$	**7E6**	2	CH_2	*p*-$CF_3C_6H_5$
6E7	1	CH_2	*p*-$CF_3C_6H_5$	**7E7**	1	CH_2	*p*-$CF_3C_6H_5$
6E8	1	CH_2	*p*-$BnOC_6H_5$	**7E8**	1	CH_2	*p*-$BnOC_6H_5$
6E9	4	CH_2	Phe	**7E9**	4	CH_2	Phe
6E10	1	CH_2	*o*-$CF_3C_6H_5$	**7E10**	1	CH_2	*o*-$CF_3C_6H_5$
6E11	1	CH_2	2-naphthyl	**7E11**	1	CH_2	2-naphthyl
6E12	1	CH_2	*m*-$CF_3C_6H_5$	**7E12**	1	CH_2	*m*-$CF_3C_6H_5$
6E13	1	CH_2	3,5-$(CF_3)_2C_6H_5$	**7E13**	1	CH_2	3,5-$(CF_3)_2C_6H_5$
6E14	1	CH_2	2,4-$(CF_3)_2C_6H_5$	**7E14**	1	CH_2	2,4-$(CF_3)_2C_6H_5$
6E15	1	CH_2	1-naphthyl	**7E15**	1	CH_2	1-naphthyl
6E16	1	CH_2	CH_2=CH	**7E16**	1	CH_2	CH_2=CH
6E17	1	CH_2	*p*-IC_6H_5	**7E17**	1	CH_2	*p*-IC_6H_5
6E18	1	CH_2	HCC-	**7E18**	1	CH_2	HCC-
6E19	1	CH_2	*p*-(*m*-CF_3Phe)C_6H_5	**7E19**	1	CH_2	*p*-(*m*-CF_3Phe)C_6H_5
6E20	1	CH_2	4-benzyl-1H-1,2,3-triazol	**7E20**	1	CH_2	4-benzyl-1H-1,2,3-triazol
6E21	1	SO_2	*p*-$CF_3C_6H_5$	**7E21**	1	SO_2	*p*-$CF_3C_6H_5$
6E22	1	CO	*p*-$CF_3C_6H_5$	**7E22**	1	CO	*p*-$CF_3C_6H_5$

Scheme 3: Reagents and conditions. (a) DIPEA, X-Ph-CH_2Br, DCM, 65-75°C, 22 h; (b) K_2CO_3, propargyl bromide, DMF, reflux, overnight; (c) 3-(Trifluoromethyl)phenylboronic acid, Pd(dppf)Cl_2, Na_2CO_3, H_2O:dioxane (3:1), 110°C, 5h; (d) Benzyl azide, aqueous $CuSO_4.5H_2O$, sodium ascorbate, BuOH:H_2O (1:1), rt, 3 h; (e) TEA, 4-(trifluoromethyl)benzenesulfonylchloride, DCM, 3 h, rt; (f) TEA, 4-(trifluoromethyl)benzoylchloride, DCM, rt, 3 h; (g) H_2O:H_2SO_4 (95:5), dioxane, rt, 2 to 5 h.



Scheme 4: Reagents and conditions. (a) $(CH_3)_3SOI$, NaH, DMSO/THF, rt, 24 h; (b) L-Phenylalanine methyl ester, MeOH, 90°C, overnight; (c) MeONa, THF, rt, 2 h; (d) K_2CO_3, propargyl bromide, DMF, reflux, overnight; (e) DIPEA, $C_6H_5CH_2Br$, DCM, 75°C, 22 h; (f) Benzyl azide, aqueous $CuSO_4.5H_2O$, sodium ascorbate, $BuOH:H_2O$ (1:1), rt, 3 h.

Biological activities

Inhibitory activity on 17β-HSD3 (screening study)

With the knowledge that the presence of a hydrophobic group on the C-3 position of ADT enhance the inhibition of 17β-HSD3, our choice of a rigid spirocycle at this position was our strategy to control the orientations of hydrophobic substituents. The amino acid used in the elaboration of the spirocycle at C-3 contains two diversifying factors, the first being the amino acid residue and the second being its stereochemistry. Finally the presence of a free amino group in the spiromorpholinone moiety is a third point of diversification. Thus, the new synthesized compounds can be grouped according to whether the stereochemistry on the C-α of the starting amino acid methyl ester was (*S*) or (*R*), whether they contain a secondary amino group on the spiromorpholinone (compounds **5A-5E**), whether the amino group is substituted (compounds **7A-7E**, **7E1-7E22**) and finally whether it is a non steroidal nucleus compounds **14-17**. The ability of all those compounds to inhibit the 17β-HSD3 contained in a microsomal fraction of homogenated rat testes was assessed by measuring the amount of [^{14}C]-T formed from natural substrate [^{14}C]-Δ^4–dione in the presence of NADPH as cofactor, and the result expressed as percent of inhibitory activity for each given compound (Table 2).

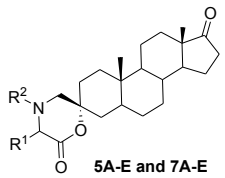
In the series **5A-5D** (secondary amines), the compounds with the (*S*) stereochemistry on the spiromorpholinone are better than their corresponding (*R*)

analogue. In fact the compound **5A** (39.4 % inhibition at 0.1 μM) with (*S*) stereochemistry is better than the analog **5B** (17.9 % inhibition at 0.1 μM) with (*R*) stereochemistry. Similarly, compound **5C** (48.8 % inhibition at 0.1 μM) is better than **5D** (18.5 % inhibition at 0.1 μM). This tendency is maintained in the *N*-benzylated spiromorpholinone series **7A-7D**. In fact, compounds **7A** and **7C** (62.9 % and 58.2 % inhibition at 0.1 μM) with (*S*) stereochemistry are better than the analogues **7B** and **7D** (45.9 % and 25.6 % inhibition at 0.1 μM) with the (*R*) stereochemistry. As we mentioned in our previous report supported by the crystalline structure of **9B**,[20] the stereochemistry on the C-α of the amino acid methyl ester moiety does not affect significantly the inhibitory potency of spirocarbamates **9A-9D**. This is probably due to the high degree of liberty of the methyl 4-methylpentanoate (**9A** and **9B**) and methyl 3-phenylpropanoate (**9C** and **9D**) that allows the free rotation around the exocyclic bond of the N atom. Thus compounds **9A** and **9C** (57.6 % and 48.3 % inhibition at 0.1 μM) with the (*S*) stereochemistry are not significantly different from their analogues **9B** and **9D** (55.4 % and 56.1 % inhibition at 0.1 μM) with the (*R*) stereochemistry.

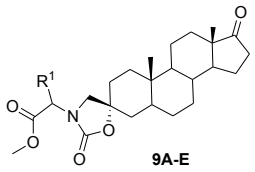
The logP value seems in some cases to correlate with the inhibitory potential, but the hydrophobicity is not the only parameter. As example, glycine-derived spiromorpholinone **5E** (logP = 3.29; 44.7 % inhibition at 1 μM) and spirocarbamate **9E** (logP = 3.72; 54.6 % inhibition at 1 μM) that have the lowest logP values are the less potent inhibitors in these series. In fact, ompound **5E** (logP = 3.29; 0 % inhibition at 0.1 μM) is very poor inhibitor as compared to its *N*-benzylated analogue **7E** (logP = 5.60; 56.0 % inhibition at 0.1 μM).

The *N*-benzylated compounds **7A-7E** showed better inhibition than their respective NH- analogues **5A-5E** suggesting the importance of a substituent at that position (tertiary amines instead of secondary amines). Thus, the secondary amines **5A** and **5B** (39.4 % and 17.9 % inhibition at 0.1 μM) are weaker inhibitors than the tertiary analogues **7A** and **7B** (62.9 % and 45.9 % inhibition at 0.1 μM). Many of new inhibitors have a comparable inhibitory potency to the reference compounds RM-532-105 and D-2-4 (47.3 % and 51.5 inhibition at 0.1 μM). The spiromorpholinones **7A, 7C** and **7E** (62.9 %, 58.2 % and 56.0 % inhibition at 0.1 μM, respectively) are even better than the reference compounds.

Table 2: Biological results for the first library of compounds (spiromorpholinones **5A-E**, **7A-E** and spirocarbamates **9A-E**



5A-E and 7A-E



9A-E

Cpds	*R/S*[a]	R^1	R^2	logP[b]	Protocol 1 17β-HSD3 inhibition (%)[c, d]		Protocol 2 17β-HSD3 inhibition (%)[c, e]		Androgenicity (%)[f]	
					0.1 µM	1 µM	1 µM	10 µM	0.1 µM	1 µM
5A	*S*	*i*Bu	H	5.21	39.4 ± 2.4	84.9 ± 1.1	69.3 ± 3.4	83.8 ± 2.6	14.5 ± 6.3	11.1 ± 10
5B	*R*	*i*Bu	H	5.21	17.9 ± 6.8	---	50.2 ± 2.4	77.7 ± 3.5	7.9 ± 2.2	23.6 ± 3.1
5C	*S*	Bn	H	5.66	48.8 ± 2.0	92.0 ± 1.6	75.0 ± 3.3	88.2 ± 1.6	3.9 ± 1.7	36.5 ± 1.6
5D	*R*	Bn	H	5.66	18.5 ± 26.9	---	63.2 ± 5.8	70.6 ± 3.1	0	15.3 ± 1.3
5E	-	H	H	3.29	0	---	44.7 ± 2.8	82.0 ± 3.5	9.7 ± 0.2	32 ± 10.5
7A	*S*	*i*Bu	Bn	7.32	62.9 ± 5.2	93.1 ± 0.6	77.3 ± 5.4	87.1 ± 3.1	8.4 ± 2.4	44.2 ± 8.4
7B	*R*	*i*Bu	Bn	7.32	45.9 ± 5.3	---	56.5 ± 1.3	82.3 ± 3.1	6.2 ± 7.3	47.7 ± 11.1
7C	*S*	Bn	Bn	7.77	58.2 ± 1.7	90.4 ± 0.7	81.0 ± 1.4	83.6 ± 3.1	6.1 ± 2.1	53.7 ± 4
7D	*R*	Bn	Bn	7.77	25.6 ± 5.7	87.3 ± 2.8	61.7 ± 4.4	84.7 ± 3.4	9.9 ± 5	50.1 ± 9
7E	-	H	Bn	5.60	56.0 ± 6.8	89.2 ± 1.8	90.9 ± 1.3	95.2 ± 2.2	8.1 ± 2.1	30.5 ± 11.1
9A	*S*	*i*Bu	---	5.45	57.6 ± 3.8	93.6 ± 1.2	82.3 ± 1.0	88.4 ± 3.5	0	8.4 ± 7.8
9B	*R*	*i*Bu	---	5.45	55.4 ± 3.2	73.6 ± 1.0	76.9 ± 1.8	67.8 ± 4.8	1.5 ± 1.4	21.9 ± 2.1
9C	*S*	Bn	---	5.89	48.3 ± 1.9	---	72.1 ±5.8	87.7 ± 2.0	0	0
9D	*R*	Bn	---	5.89	56.1 ± 2.6	---	74.3 ± 7.3	80.8 ± 3.5	0	29.2 ± 4.2
9E	-	H	---	3.72	9.0 ± 6.4	---	54.6 ± 1.4	80.8 ± 5.6	11 ± 3.5	33.8 ± 1
RM-532-105[g]	-	---	---	---	47.3 ± 12.4	92.1 ± 0.4	96.4 ± 0.6	97.1 ± 0.2	0	0
D-5-2[g]	-	---	---	---	51.5 ± 2.2	93.1 ± 1.1	93.9 ± 0.9	97.2 ± 0.4	4.8 ± 1.3	9.4 ± 15.5

(a) (*S*) or (*R*) Stereochemistry on the asymmetric carbone in the spiroheterocycle. (b) logP calculated with ChemDraw Ultra 9.0 (c) Transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by 17β-HSD3 of homogenated rat testes. (d) 18 % of initial enzyme transformation. (e) 4 % of initial enzyme transformation. (f) The androgenicity is the difference between the cell proliferation (in %) caused by a given compound and the basal proliferation fixed at 100 % (g) Known inhibitors of 17β-HSD3: D-5-2 (see reference 15) and RM-532-105 (see compound **15b** in reference 2).

Because of the impressive increase of 17β-HSD3 inhibition due to the *N*-benzylation of compound **5E** to **7E** (from 0 % to 56.0 % inhibition at 0.1 μM), we decided to diversify the spiromorpholinone **5E** with various alkyl and aryl groups (Table 3). The compounds in this series were studied regarding the nature of the substituent (R), its position (*ortho*, *meta* or *para*) on the phenyl group and the length of the methylenic chain (n). In order to obtain a less flexible spacer between the N-atom and the aryl group, the methylene group was replaced by a sulfonyl ($-SO_2$) in compound **7E21** and a carbonyl (C=O) in compound **7E22**. The results with the first series of compounds **7E1-7E9** showed that they have comparable 17β-HSD3 inhibitory potency than our reference compound RM-532-105. Although the 0.1 μM concentration tested did not allow a good comparison of compounds **7E1-7E9** of that first series as their inhibition potential was quite similar, the nature of the substituent (R) on the phenyl group and the length of the methylenic chain (n), slightly affect the inhibition potency (Table 3). In fact, compound **7E** (R = H, n = 1) with 80.8 % inhibition at 0.1 μM inhibited 17β-HSD3 better than **7E4** (R = H, n = 2) with 78.5 % and **7E5** (R = H, n = 3) with 74.9 % inhibition. The same tendency was observed with compounds **7E7** (R = *p*-CF_3, n = 1) with 83.4 % inhibition at 0.1 μM, compared to **7E6** (R = *p*-CF_3, n = 2) with 70.7 % inhibition at 0.1 μM. According to these observations, we decided to further the inhibitory activity optimization with a single methylene (n = 1) spacer.

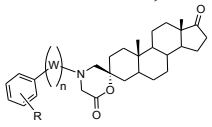
The second series of compounds (**7E10-7E22**) includes *N*-benzyl derivatives with different substituents (R) and orientations (*ortho*, *meta* or *para*) on the phenyl ring. Two compounds (**7E21** and **7E22**) in which the CH_2 (W) group is replaced by a sulfonyl group or a carbonyl group, respectively were also added. In order to refine our lead compound selection, the inhibitory potency was compared at a lower concentration (0.01 μM). At that concentration, the *ortho*-substituted compound **7E10** (24.4 % inhibition) is better than it *meta* analogue **7E12** (17.7 % inhibition). A disubstituted (CF_3) phenyl ring (**7E13** and **7E14**) and a fused naphtyl ring (**7E11** and **7E15**) did not improve the inhibitory potency at 0.01 μM. Adding a *para*-iodide on the benzyl group (compound **7E17**) reduced its inhibitory potency, but adding a *para* CF_3-phenyl on the benzyl (compound **7E19**) provided an inhibition similar to that of the lead compound **7E** (26.1 % and 27.2 % respectively). Interestingly at that concentration, the sulfonamide compound **7E21** (32.9 % inhibition) and carboxamide compound **7E22** (24.3 % inhibition) inhibited 17β-HSD3 comparably to the reference compound RM-532-105 (32.4 % inhibition).

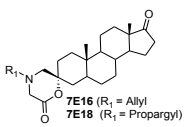
In order to verify the role of the androsterone scaffold, the inhibitory potency of non steroidal spiromorpholinones **14-17** were also assessed. Those compounds did not inhibit the conversion of Δ^4–dione into T by the 17β-HSD3 (6.2, 24.2, 0 and 9.4 % inhibition at 0.1 μM), proving the usefulness of the androgen core. This result suggests that the steroidal inhibitor competes better with the natural steroid substrate (Δ^4–dione) than the non steroidal derivative, which probably produces fewer interactions within the enzyme catalytic site. In fact, the non steroidal compounds did not possess a carbonyl group mimicking the C-17 ketone of the natural substrate, and this carbonyl is necessary for a good inhibition of 17β-HSD3.[15]

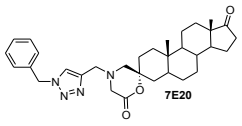
Proliferative (androgenic) activity on LAPC-4 cells (screening study)

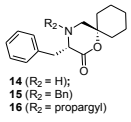
A good inhibitor of testosterone biosynthesis for prostate cancer therapy should be devoid of proliferative (androgenic) activity. Since most of our compounds inhibited similarly 17β-HSD3, we added another criterion, the androgenicity, to discriminate the new synthesized molecules. To achieve this, a prostate cancer cell line (LAPC-4 cells) expressing a non mutated AR was chosen and all new compounds were screened for their ability to stimulate or not the cell proliferation (Tables 2 and 3). The 17β-HSD3 inhibitor RM-532-105 and the natural androgen DHT were added as reference compounds and tested at two concentrations (0.1 and 1 μM). The androgenicity (in %) is the difference between the cell proliferation caused by a given inhibitor and the basal cell proliferation fixed at 100 %. Interestingly, only the sulfonamide derivative **7E21** and the carboxamide derivative **7E22**, which are the most rigid compounds in the *N*-substituted series, did not exhibit any androgenic effect at the two concentrations tested. These compounds were selected for further *in vitro* studies.

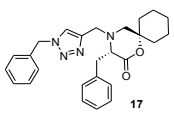
Table 3: Biological results for the second library of compounds (N-derivatives **7E1-7E22**, and **14-17)**











R	Cpds	n	w	17β-HSD3 inhibition (%)[a]				Androgenicity[b]	
				0.01 µM	0.1 µM	1 µM	10 µM	0.1 µM	1 µM
Protocol 3[c]									
H	**7E**	**1**	CH_2	----	80.8 ± 0.5	91.6 ± 1.3	94.3 ± 1.1	----	----
p-Br	**7E1**	**1**	CH_2	----	79.1 ± 2.7	92.9 ± 1.6	91.6 ± 1.6	21.8 ± 4.5	45.7 ± 4.5
p-OCF_3	**7E2**	**1**	CH_2	----	79.0 ± 2.4	93.8 ± 0.9	95.7 ± 2.2	45.9 ± 4.0	74.9 ± 5.2
p-Ph	**7E3**	**1**	CH_2	----	81.9 ± 3.6	93.1 ± 0.2	95.2 ± 0.7	47.7 ± 4.7	55.1 ± 5.7
H	**7E4**	**2**	CH_2	----	78.5 ± 1.5	92.2 ± 1.3	95.2 ± 1.1	48.9 ± 2.0	79.7 ± 2.2
H	**7E5**	**3**	CH_2	----	74.9 ± 2.3	90.5 ± 0.9	92.3 ± 0.6	64.9 ± 5.3	55.5 ± 5.2
p-CF_3	**7E6**	**2**	CH_2	----	70.7 ± 1.1	92.1 ± 0.8	93.3 ± 1.3	40.2 ± 1.4	76.1 ± 4.9
p-CF_3	**7E7**	**1**	CH_2	----	83.4 ± 2.2	94.0 ± 0.2	91.2 ± 2.1	12.3 ± 3.9	31.7 ± 5.2
p-OBn	**7E8**	**1**	CH_2	----	80.4 ± 1.3	90.8 ± 2.0	93.4 ± 0.7	0 ± 6.1	16.7 ± 6.0
H	**7E9**	**4**	CH_2	----	79.9 ± 2.4	91.1 ± 0.1	91.3 ± 1.4	5.3 ± 5.4	19.2 ± 5.2
----	**14**	**-**	----	----	6.2 ± 6.3	19.5 ± 3.8	20.6 ± 1.3	----	----
----	**15**	**-**	----	----	24.2 ± 3.2	24.3 ± 4.2	29.2 ± 2.7	----	----
----	**DHT**	**-**	----	----	----	----	----	28.8 ± 4.7	51.5 ± 8.0
RM-532-105[d]		**-**	----	----	89.4 ± 2.3	94.4 ± 1.6	94.0 ± 0.9	11.1 ± 4.0	0.4 ± 4.9
Protocol 4[e]									
H	**7E**	**1**	CH_2	27.2 ± 14.3	77.5 ± 2.2	95.0 ± 1.3	----	----	----
o-CF_3	**7E10**	**1**	CH_2	24.4 ± 8.3	77.9 ± 3.5	83.0 ± 0.6	----	0.5 ± 8.9	36.9 ± 2.3
2-naphthyl	**7E11**	**1**	CH_2	0	88.3 ± 7.1	93.2 ± 2.3	----	22.3 ± 5.8	16.5 ± 2.2
m-CF_3	**7E12**	**1**	CH_2	17.7 ± 6.8	54.1 ± 7.2	94.2 ± 1.1	----	0 ± 8.2	27.0 ± 9.7
3,5-bis(trifluoromethyl)	**7E13**	**1**	CH_2	15.1 ± 3.0	61.6 ± 13.5	94.4 ± 1.2	----	12.0 ± 4.6	5.9 ± 19.7
2,4-bis(trifluoromethyl)	**7E14**	**1**	CH_2	18.3 ± 41.9	64.1 ± 4.5	89.6 ± 3.5	----	0 ± 2.6	27.0 ± 3.5
1-naphthyl	**7E15**	**1**	CH_2	12.8 ± 2.8	79.1 ± 5.2	94.5 ± 0.8	----	3.8 ± 1.2	9.3 ± 0.3
----	**7E16**	**-**	----	8.4 ± 12	48.6 ± 7.2	89.0 ± 0.5	----	31.0 ± 4.5	11.3 ± 4.8
p-I	**7E17**	**1**	CH_2	12.2 ± 3.8	79.0 ± 2.6	95.3 ± 0.6	----	0.9 ± 3.7	15.9 ± 2.4
----	**7E18**	**1**	----	0.1 ± 4.1	34.1 ± 2.6	80.7 ± 1.4	----	20.1 ± 3.5	25.5 ± 7.8
p-(3-CF_3phenyl)	**7E19**	**1**	CH_2	26.1 ± 11.6	84.8 ± 3.5	84.9 ± 2.2	----	13.5 ± 4.2	14.3 ± 4.2
----	**7E20**	**-**	----	5.1 ± 3.1	42.2 ± 7.9	85.3 ± 3.0	----	10.0 ± 3.3	17.7 ± 6.6
p-CF_3	**7E21**	**1**	SO_2	32.9 ± 7.6	48.6 ± 10.9	85.1 ± 1.8	----	0 ± 3.1	0 ± 2.6
p-CF_3	**7E22**	**1**	CO	24.3 ± 15.8	45.4 ± 9.7	87.1 ± 2.9	----	0 ± 3.8	0 ± 3.3
----	**16**	**-**	----	0	0	0	----	11.1 ± 7.4	0 ± 3.5
----	**17**	**-**	----	0	9.4 ± 1.6	13.3 ± 31.4	----	2.5 ± 3.5	12.4 ± 4.0
----	**DHT**	**-**	----	----	----	----	----	28.8 ± 4.7	51.5 ± 8.0
RM-532-105[d]		**-**	----	32.4 ± 3.2	75.7 ± 6.3	95.2 ± 0.2	----	11.1 ± 4.0	0.4 ± 4.9

(a) Transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by 17β-HSD3 of homogenated rat testes. (b) The androgenicity is the difference between the cell proliferation (in %) cause by a given compound and the basal proliferation fixed at 100 %. (c) 33 % of initial enzyme transformation. (d) Known inhibitor of 17β-HSD3 (see compound **15b** in reference 2). (e) 18 % of initial enzyme transformation.

Biological evaluation of non-androgenic compounds 7E21 and 7E22

The IC_{50} values of **7E21** and **7E22** have been calculated for the inhibition of 17β-HSD3 and compared to the reference compound RM-532-105, Δ^4-dione and ADT (Table 4, see also Figure S1 and S2 in supporting information). Although, both compounds are less potent than RM-532-105 (IC_{50} = 13 nM), **7E21** (IC_{50} = 28 nM) is 6-fold better than the natural substrate Δ^4-dione (IC_{50} = 169 nM) and **7E22** (IC_{50} = 88 nM) is almost 2-fold better than Δ^4-dione.

Table 4: Inhibition of 17β-HSD3 by compounds **7E21**, **7E22**, RM-532-105, ADT and Δ^4-dione

Compounds	IC_{50} (nM)[a]			
	Protocol 1	**Protocol 2**	**Protocol 3**	**Average ± SEM**
7E21[b]	19	36	-	28 ± 9
7E22	69	106	-	88 ± 19
RM -532-105	16	~10	14	13 ± 2
ADT	69	111	-	90 ± 21
Δ4-dione	122	216	-	169 ± 47

(a) Transformation of [^{14}C]-Δ^4-dione to [^{14}C]-T by rat testicular 17β-HSD3 (microsomal fraction). (b) 30 % of compound **19** was present.

Antiproliferative (antiandrogenic) activity on LAPC-4 cells

The ability of compounds **7E21** and **7E22** to inhibit the proliferation of androgen-sensitive LAPC-4 cells induced by the most potent natural androgen DHT has been assessed (Figure 3). Thus, LAPC-4 cells incubated with DHT (0.1 μM) were treated with three concentrations (0.1, 1 and 5 μM) of **7E21** and **7E22**. The sulfonamide **7E21** at a concentration of 5 μM inhibited the DHT-induced proliferation below the basal cell proliferation level (control) like the reference compound RM-532-105 and better than hydroxyflutamide, the clinically used anti-androgen against metastatic prostate cancer. However the carboxamide **7E22** did not show a significant antiandrogenic effect at the three concentrations tested.

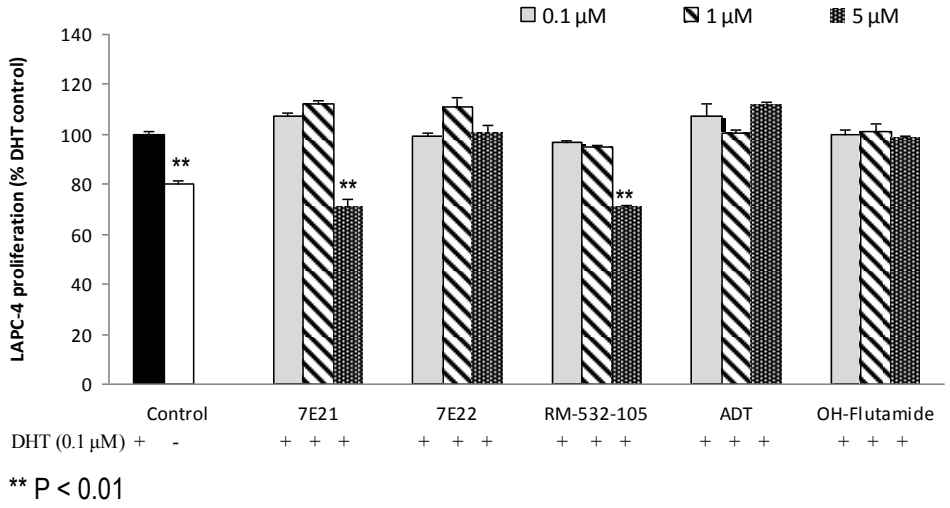


Figure 3: Antiproliferative (antiandrogenic) activity on androgen-sensitive LAPC-4 cells

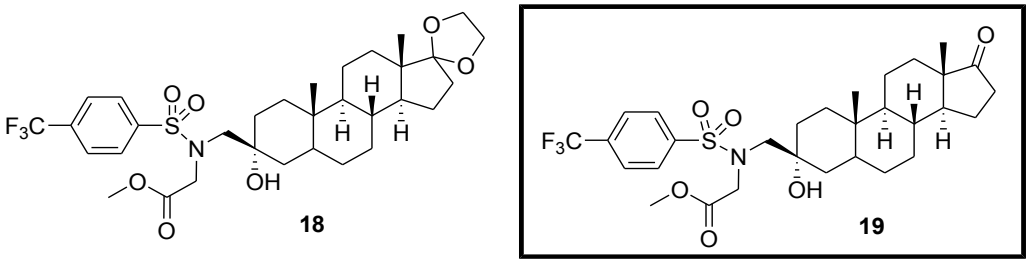


Figure 4: Chemical structure of the residual contaminant **19** and it intermediate **18**. (Also see scheme S2 of the supporting information for the synthesis of **18** and **19**.

Conclusions

Thirty-two 3-spiromorpholinone androsterone derivatives, five 3-spirocarbamate androsterone derivatives and four non steroidal spiromorpholinones have been synthesized. The chemical structure of all the final products and intermediates have been determined from ^{1}H NMR, ^{13}C NMR, IR and LR-MS. All target compounds were tested for their ability to inhibit the transformation of [^{14}C]-Δ^{4}-dione (50 nM) to [^{14}C]-T by 17β-HSD3 and their androgenic effect assessed on androgen sensitive LAPC-4 cells. SAR analysis showed the necessity of the steroidal (ADT) backbone in addition to an hydrophobic moiety for 17β-HSD3 inhibition. Stereochemistry, logP and steric indrance of the E-ring substituent affect the inhibition in a complex way. Although we did not succeed by generating a more potent inhibitor than RM-532-105, different synthetic strategy were developed for introducing a new molecular diversity on a rigid spiromorpholinone E ring, providing interesting possibility of optimization. From our study, we however identified one compound (the sulfonamide **7E21**) with a comparable

inhibitory potency than the reference compound RM-532-105 (IC_{50} = 28 and 14 nM respectively). This compound is non androgenic and inhibited the DHT-induced action proliferation of LAPC-4 cells below the basal proliferation, suggesting an antiandrogenic action. The sulfonamide morpholinone ADT derivative **7E21** is a good candidate for further *in vitro* and *in vivo* studies.

EXPERIMENTAL SECTION

General Remarks: Androsterone was purchased from Steraloids (Wilton, NH, USA). Benzyl bromide, benzyl bromide derivativatives, 4-(trifluoromethyl)benzenesulfonyl chloride, allyl bromide and propargyl bromide were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Solvents were obtained from Fisher Scientific (Montréal, QC, Canada). Reactions were run under an inert (argon) atmosphere in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on 0.20-mm silica gel 60 F254 plates (Fisher Scientific), and compounds were visualized using ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with Silicycle R10030B 230–400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were obtained from a thin film of compound usually solubilized in DCM and deposited upon a NaCl pellet. They were recorded with a Horizon MB 3000 ABB FTIR spectrometer (ABB, Canada) and only characteristic bands are reported. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and reported in ppm. The ^{1}H and ^{13}C NMR signals (7.26 and 77.00 ppm respectively) and acetone (2.05 and 28.9 ppm, respectively) were used as internal references. Low-resolution mass spectra (LR-MS) were recorded on a Shimadzu prominence apparatus (Kyoto, Japan) equipped with a Shimadzu LCMS-2020. The HPLC purity of compound **7E21** dissolved in DMSO was determined with a Shimadzu apparatus using a Shimadzu SPD-M20A Photodiode array detector, a Alltima HPC18 reversed-phase column (250 mm x 4.6 mm, 5 μm) and a solvent gradient of methanol (15 %) and water (85 %). The wave length of the UV detector was selected between 190-205 nm. The name of compounds was generated using ACD/Labs (chemist version) software (Toronto, ON, Canada).

General procedure for the synthesis of 3A-3D

In a schlenk tube, the oxirane **2** (1.7 mmol) was dissolved in dry MeOH (15 mL) and the freed amino acid methyl ester[23] (17.2 mmol) was added. The schlenk tube was screwed down hermetically, stirred and heated at 90 °C during 21 h. The MeOH was

then evaporated, the crude reaction mixture dissolved in DCM and pre-adsorbed on silica gel, and purified by flash column chromatography (hexanes / EtOAc / TEA (89:10:1).

methyl *N*-{[(3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-L-leucinate (3A)

99 % yield; Rf = 0.6 (hexanes/EtOAc, 1:1); IR (film) ν 3479 and 3333 (OH and NH), 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.74 (m, 1H), 0.78 (s, CH_3-19), 0.82 (s, 3H, CH_3-18), 0.91 and 0.92 (2d, J = 6.9 Hz, $(CH_3)_2$- from *i*Pr), 1.10-1.95 (unassigned CH and CH_2), 2.21 and 2.57 (2d of AB system, J = 11.6 Hz, CH_2N), 3.23 (dd, J_1 = 6.4 Hz, J_2 = 8.2 Hz, CHC=O), 3.67 (s, OCH_3), 3.83 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 21.9, 22.6, 22.9, 24.8, 28.5, 30.7, 31.2, 31.7, 33.8, 34.2, 35.8, 36.0, 38.6, 40.6, 42.8, 46.0, 50.3, 51.7, 53.9, 59.3, 60.9, 64.5, 65.1, 69.9, 119.5, 176.3 ppm.

methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-D-leucinate (3B)

99 % yield; Rf = 0.3 (hexanes/EtOAc, 1:1); IR (film) ν 3479 and 3333 (OH and NH), 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (m, 1H), 0.83 (s, CH_3-18), 0.90 and 0.93 (2d, J = 6.6 Hz, $(CH_3)_2$- from *i*Pr), 0.96-2.00 (unassigned CH and CH_2), 2.17 and 2.62 (2d of AB system, J = 11.9 Hz, CH_2N), 3.23 ($t_{app.}$, J = 6.7 Hz, CHC=O), 3.72 (s, OCH_3), 3.87 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 21.9, 22.6, 22.8, 24.8, 28.4, 30.7, 31.2, 31.5, 33.6, 34.1, 35.8, 35.9, 38.6, 40.6, 42.7, 45.9, 50.3, 51.6, 53.8, 59.3, 60.8, 64.5, 65.1, 69.9, 119.4, 176.3 ppm.

methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-L-phenylalaninate (3C)

77 % yield; Rf = 0.5 (hexanes/EtOAc, 1:1); IR (film) ν 3472 and 3340 (OH and NH), 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.72 (m, 1H), 0.75 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 (m, 1H), 1.12-1.97 (unassigned CH and CH_2), 2.24 and 2.56 (2d of AB system, J = 11.6 Hz, CH_2N), 2.92 (m, $\underline{CH_2}$-Ph), 3.45 (t, J = 6.6 Hz, CHC=O), 3.62 (s, OCH_3), 3.84 (m, OCH_2CH_2O), 7.25 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 22.7, 28.5, 30.7, 31.2, 31.5, 33.7, 34.2, 35.8, 35.9, 38.5, 39.8, 40.5, 46.0, 50.3, 51.7, 53.8, 59.3, 63.9, 64.5, 65.1, 70.0, 119.5, 126.7, 128.4 (2C), 129.1 (2C), 137.3, 175.0 ppm. LR-MS: calcd. for $C_{32}H_{48}NO_5$ $[M+H]^+$ 526.35, found 526.50.

methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-D-phenylalaninate (3D)

87 % yield; Rf = 0.43 (hexanes/EtOAc, 1:1); IR (film) ν 3472 and 3340 (OH and NH), 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.72 (m, 1H), 0.75 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 (m, 1H), 1.15-1.97 (unassigned CH and CH_2), 2.24 and 2.56 (2d of AB

system, J = 11.6 Hz, CH_2N), 2.92 (m, $\underline{CH_2}$-Ph), 3.43 (t, J = 6.6 Hz, CHC=O), 3.62 (s, OCH_3), 3.84 (m, OCH_2CH_2O), 7.25 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.4, 22.7, 28.4, 30.7, 31.2, 31.5, 33.6, 34.2, 35.8, 35.9, 38.4, 39.8, 40.5, 45.9, 50.3, 51.7, 53.8, 59.3, 64.0, 64.5, 65.1, 70.0, 119.4, 126.7, 128.4 (2C), 129.1 (2C), 137.3, 175.0 ppm. LR-MS: calcd. for $C_{32}H_{48}NO_5$ $[M+H]^+$ 526.35, found 526.50.

Synthesis of methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexa decahydrospiro[cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}glycinate (3E)

To a mixture of glycine methyl ester hydrochloride (1.1 g, 8.8 mmol) and DIPEA (2.2 g, 17.5 mmol) was added anhydrous MeOH (20 mL) and the solution was stirred during 30 min in a schlenk tube at room temperature. Oxirane **2** (0.3 g, 0.9 mmol) was then added and the solution heated to 95 °C during 22 h. The solution was cooled to room temperature, filtered on a büchner, the filtrate is evaporated, the crude mixture (1.8 g) dissolved in DCM, adsorbed on silica gel (7 g) (dry-pack) and then eluted with a mixture of hexanes / EtOAc / TEA (70:30:1). The yellowish product **3E** is obtained (0.2 g, 53 % yield) from the pure fractions. Rf = 0.17 (hexanes/EtOAc, 3:7); IR (film) ν 3464 and 3348 (OH and NH), 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.50 (s, CH_2N), 3.45 (s, NCH_2C=O), 3.73 (s, OCH_3), 3.83-3.95 (m, OCH_2CH_2O) ppm. ^{13}C NMR (acetone-d_6) δ 10.8, 13.9, 20.3, 22.4, 30.7, 31.4, 31.5, 33.3, 33.7, 33.9, 35.8, 35.9, 38.5, 40.5, 45.8, 50.3, 50.7, 51.2, 54.4, 61.1, 64.2, 64.8, 70.0, 118.9, 172.7 ppm.

Synthesis of the spiromorpholinones 4A-4E

To a solution of MeONa (0.37 mmol) in anhydrous THF (24 mL) at 0 °C was added a solution of the amino alcohol (**3A-3E)** (0.61 mmol) in dry THF (28 mL) under argon atmosphere. The solution was stirred 2 h at room temperature and the reaction mixture was quenched with a saturated ammonium chloride solution. The crude product was extracted 4 times with EtOAc and the organic layer was dried with anhydrous $MgSO_4$, filtered over vacuum and dried under reduced pressure. The residue was adsorbed on silica gel and purified by flash column chromatography with a mixture of hexanes/EtOAc/TEA (80:20:1).

(3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-5''-(2-methylpropyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4A)

89 % yield; Rf = 0.21 (hexanes/EtOAc 8:2); IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.79 (m, 1H), 0.83 (s, CH_3-19 and CH_3-18), 0.89 and 0.92 (2d, J = 6.6 Hz, $(CH_3)_2$- from *i*Pr), 0.95-1.98 (unassigned CH and CH_2), 2.83 and 2.93 (2d of AB system, J = 13.5 Hz, CH_2N), 3.43 (dd, J_1 = 4.0 Hz, J_2 = 9.6 Hz, NCHC=O), 3.85 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 21.0, 22.6, 23.4, 24.4, 28.1, 30.6, 31.5, 32.9, 34.1, 35.7, 36.0, 38.2, 39.2, 39.6, 41.4, 45.9, 50.1, 52.5, 53.3, 55.5, 64.5, 65.1, 82.3, 119.4, 171.9 ppm. LR-MS: calcd. for $C_{28}H_{46}NO_4$ $[M+H]^+$ 460.33, found 460.45.

(3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-5''-(2-methylpropyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4B)

84 % yield; Rf = 0.23 (hexanes/EtOAc 8:2); IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.83 (s, CH_3-18), 0.92 and 0.96 (2d, J = 6.3 Hz, $(CH_3)_2$- from *i*Pr), 0.80-1.99 (unassigned CH and CH_2), 2.80 and 2.88 (2d of AB system, J = 13.5 Hz, CH_2N), 3.49 (dd, J_1 = 3.5 Hz, J_2 = 10.0 Hz, NCHC=O), 3.89 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 20.9, 22.7, 23.4, 24.5, 28.2, 30.6, 31.5, 32.6, 34.2, 35.7, 36.0, 38.3, 39.3, 39.7, 41.5, 45.9, 50.1, 52.6, 53.4, 55.5, 64.5, 65.1, 82.3, 119.4, 171.9 ppm. LR-MS: calcd. for $C_{28}H_{46}NO_4$ $[M+H]^+$ 460.33, found 460.45.

(3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-5''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4C)

62 % yield; Rf = 0.15 (hexanes/EtOAc 8:2); IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.75 (m, 1H), 0.78 (s, CH_3-19), 0.82 (s, CH_3-18), 0.90-1.99 (unassigned CH and CH_2), 2.93 (m, CH_2N), 3.05 and 3.17 (2m, 2H, $\underline{CH_2}$Ph), 3.72 (dd, J_1 = 3.9 Hz, J_2 = 8.1 Hz, NCHC=O), 3.84 (m, OCH_2CH_2O), 7.22 (m, 1H of Ph), 7.29 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.6, 28.0, 30.6, 30.9, 31.1, 32.9, 34.2, 35.7, 35.9, 38.0 (2C), 39.2, 45.9, 50.1, 52.7, 53.3, 58.7, 64.5, 65.1, 82.8, 119.4, 127.0, 128.7 (2C), 129.5 (2C), 137.2, 170.8 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_4$ $[M+H]^+$ 494.32, found 494.45.

(3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-5''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4D)

65 % yield; Rf = 0.09 (hexanes/EtOAc 8:2); IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.77 (s, CH_3-19), 0.82 (s, CH_3-18), 0.85 (m, 1H), 0.88-1.98 (unassigned CH and CH_2), 2.85 (m, CH_2N), 3.05 and 3.15 (2m, $\underline{CH_2}$Ph), 3.74 (dd, J_1=

4.1 Hz, J_2 = 7.6 Hz, NCHC=O), 3.84 (m, OCH_2CH_2O), 7.24 (m, 1H of Ph), 7.27 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.7, 28.0, 30.6, 31.0, 31.3, 32.5, 34.2, 35.7, 35.9, 37.7, 37.9, 39.6, 45.9, 50.1, 52.7, 53.3, 58.7, 64.5, 65.1, 82.7, 119.4, 127.1, 128.8 (2C), 129.6 (2C), 137.2, 170.8 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_4$ $[M+H]^+$ 494.32, found 494.50.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4E)

52 % yield; Rf = 0.16 (hexanes/EtOAc 3:7); IR (film) ν 3340 (NH) and 1728 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.78 (s, CH_3-19), 0.83 (s, CH_3-18), 0.84-1.99 (unassigned CH and CH_2), 2.82 (s, CH_2N), 3.63 (s, NCH_2C=O), 3.90 (m, OCH_2-CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.4, 22.7, 28.1, 30.6, 31.0, 31.3, 32.7, 34.2, 35.7, 36.0, 38.0, 39.4, 45.9, 47.6, 50.1, 53.1, 53.4, 64.6, 65.2, 82.5, 119.4, 168.8 ppm. LR-MS: calcd. for $C_{24}H_{38}NO_4$ $[M+H]^+$ 404.27, found 404.35.

Synthesis of *N*-benzylated spiromorpholinones 6A-6E

To a solution **4A-4E** (0.1 mmol) in dry DCM (5mL) was added drop-wise DIPEA (0.17 mmol) in a schlenk tube. The tube was screwed down, stirred and heated at 75 °C during 10 min, then the reaction mixture was cooled to the room temperature and benzyl bromide (1.7 mmol) was added to the solution. The reaction mixture was stirred and heated at 75 °C during 48 h. After cooling the mixture, silica gel was added to the crude mixture, the solvent was evaporated under reduce pressure and the residue was purified by flash chromatography with a mixture of hexanes / EtOAc / TEA (95:5:1).

(3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyl-5''-(2-methylpropyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6A)

63 % yield; Rf = 0.83 (hexanes/EtOAc 1:1); ^1H NMR (acetone-d_6) δ 0.74 (s, CH_3-19), 0.81 (s, CH_3-18), 0.93 and 0.98 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 1.00-2.00 (unassigned CH and CH_2), 2.27 and 2.74 (2d of AB system, J = 12.5 Hz, CH_2N), 3.17 (dd, J_1 = 2.7 Hz, J_2 = 7.0 Hz, NCHC=O), 3.23 and 4.07 (2d of AB system, J = 13.7 Hz, N$\underline{CH_2}$-Ph), 3.83 (m, OCH_2CH_2O), 7.28 (m, 1H of Ph), 7.37 (m, 4H of Ph) ppm.

(3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyl-5''-(2-methylpropyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6B)

49 % yield; Rf = 0.83 (hexanes/EtOAc 1:1); ^1H NMR ($CDCl_3$) δ 0.68 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 and 0.99 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 0.80-2.11 (unassigned CH

and CH_2), 2.16 and 2.64 (2d of AB system, J = 12.4 Hz, CH_2N), 3.12 and 4.02 (2d of AB system, J = 13.5 Hz, N$\underline{CH_2}$-Ph), 3.18 (dd, J_1 = 2.8 Hz, J_2 = 7.0 Hz, NCHC=O), 3.89 (m, OCH_2CH_2O), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.1, 22.7, 23.9, 25.0, 28.3, 30.7, 31.1, 32.0, 32.7, 34.2, 35.7, 36.0, 38.3, 38.6, 39.8, 45.9, 50.1, 53.4, 57.8, 58.2, 63.0, 64.6, 65.1, 81.3, 119.4, 127.4, 128.5 (4C), 137.8, 171.4 ppm.

(3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-4'',5''-dibenzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6C)

70 % yield; Rf = 0.75 (hexanes/EtOAc 1:1); ^{1}H NMR (acetone-d_6) δ 0.62 (s, CH_3-19), 0.68 (m, 1H), 0.79 (s, CH_3-18), 0.88-1.95 (unassigned CH and CH_2), 2.21 and 2.58 (2d of AB system, J = 12.5 Hz, CH_2N), 3.28 and 4.39 (2d of AB system, J = 13.8 Hz, N$\underline{CH_2}$-Ph), 3.36 (m, $\underline{CH_2}$Ph), 3.51 (dd, J_1 = 3.0 Hz, J_2 = 5.1 Hz, NCHC=O), 3.85 (m, OCH_2CH_2O), 7.33 (m, Ph) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.9, 20.2, 22.4, 27.9, 29.5, 30.6, 31.2, 33.1, 33.9, 35.2, 35.7, 37.9, 39.5, 45.7, 50.2, 54.0, 57.5, 57.7, 61.0, 64.2, 64.8, 65.9, 80.5, 118.8, 126.4, 127.1, 127.8 (2C), 128.3 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5 ppm.

(3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-4'',5''-dibenzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6D)

61 % yield; Rf = 0.75 (hexanes/EtOAc 1:1); ^{1}H NMR (acetone-d_6) δ 0.62 (s, CH_3-19), 0.68 (m, 1H), 0.78 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.21 and 2.59 (2d of AB system, J = 12.4 Hz, CH_2N), 3.28 and 4.41 (2d of AB system, J = 13.8 Hz, N$\underline{CH_2}$-Ph), 3.38 (m, $\underline{CH_2}$Ph), 3.50 (dd, J_1 = 3.0 Hz, J_2 = 5.1 Hz, NCHC=O), 3.83 (m, OCH_2CH_2O), 7.30 (m, Ph) ppm. ^{13}CNMR (acetone-d_6) δ 10.8, 13.9, 20.2, 22.4, 27.6, 29.5, 30.6, 31.1, 31.2, 32.7, 33.9, 35.0, 35.7, 37.3, 39.9, 45.7, 50.2, 54.1, 57.5, 57.8, 64.2, 64.8, 65.8, 80.5, 118.8, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.6 (2C), 130.5 (2C), 137.9, 138.1, 169.4 ppm.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E)

70 % yield; Rf = 0.83 (hexanes/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 0.72 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.41 (s-broad, CH_2N), 3.26 (s, NCH_2C=O), 3.50 and 3.54 (2d of AB system, J = 13.3 Hz, N$\underline{CH_2}$Ph), 3.89 (m, OCH_2CH_2O), 7.31 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.7, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 61.4, 64.6, 65.1, 82.5, 119.4, 127.6, 128.5 (2C), 128.7 (2C), 136.7, 168.3 ppm.

General procedure to generate the spiromorpholinones 5A-5E and the N-benzylated spiromorpholinones 7A-7E

To a solution of compounds **4A**, **4B**, **4C**, **4D**, **4E**, **6A**, **6B**, **6C**, **6D** or **6E** (30 - 60 mg) in dioxane (2 mL) was added an aqueous solution of sulphuric acid (5 %) (2 mL) and the mixture was stirred at room temperature (2 h - 5 h). A saturated solution of Na_2CO_3 (12 mL) was added and the reaction mixture extracted with EtOAc. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The purification was performed by flash column chromatography. Elution with hexanes/EtOAc/TEA (90:10:1) (compounds **5A-5E**) or with hexanes/EtOAc/TEA (95:5:1) (compounds **7A-7E**).

(3R,5S,5'S,8R,9S,10S,13S,14S)-10,13-dimethyl-5'-(2-methylpropyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5A)

71 % yield; Rf = 0.5 (hexanes/EtOAc 1:1); IR (film) ν 3448 and 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.84 and 0.85 (2s, CH_3-18 and CH_3-19), 0.90 and 0.93 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 1.05 (m, 1H), 1.10-2.02 (unassigned CH and CH_2), 2.38 (m, CH-16β), 2.82 and 2.95 (2d of AB system, J = 13.4 Hz, CH_2N), 3.44 (dd, J_1 = 4.1 Hz, J_2 = 9.6 Hz, NCHC=O) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.2, 20.1, 20.6, 21.4, 22.9, 24.2, 27.9, 30.7, 31.2, 31.7, 33.2, 35.0, 35.1, 36.1, 37.9, 39.8, 41.3, 47.3, 51.3, 51.9, 54.3, 55.5, 81.8, 170.8, ~220 (too weak) ppm. LR-MS: calcd. for $C_{26}H_{42}NO_3$ $[M+H]^+$ 416.31, found 416.35.

(3R,5S,5'R,8R,9S,10S,13S,14S)-10,13-dimethyl-5'-(2-methylpropyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5B)

92 % yield; Rf = 0.44 (hexanes/EtOAc 1:1); IR (film) ν 3448 and 3340 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.93 and 0.97 (2d, J = 6.4 Hz, $(CH_3)_2$- from *i*Pr), 0.99-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.42 (m, CH-16β), 2.82 and 2.89 (2d of AB system, J = 13.5 Hz, CH_2N), 3.50 (dd, J_1 = 3.6 Hz and J_2 = 9.8 Hz, NCHC=O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.0, 21.8, 23.4, 24.5, 28.0, 30.5, 31.4, 31.5, 32.6, 35.0, 35.8, 36.1, 38.2, 39.7, 41.4, 47.8, 51.4, 52.5, 53.8, 55.6, 82.2, 171.8, 221.2 ppm. LR-MS: calcd. for $C_{26}H_{42}NO_3$ $[M+H]^+$ 416.31, found 416.35.

(3R,5S,5'S,8R,9S,10S,13S,14S)-5'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5C)

97 % yield; Rf = 0.7 (hexanes/EtOAc 3:7); IR (film) ν 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.81 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.38 (m, CH-16β), 2.79 and 2.90 (2d of AB system, J = 13.3 Hz, CH_2N), 3.02 and 3.16 (2m, $\underline{CH_2}Ph$), 3.73 (dd, J_1 = 4.0 Hz and J_2 = 8.1 Hz, NCHC=O), 7.24 (m, 1H of Ph), 7.29 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.0, 31.5, 32.8, 35.0, 35.8, 36.0, 38.0, 39.2, 47.8, 51.4, 52.6, 53.7, 53.8, 58.6, 58.7, 82.7, 127.1, 128.8 (2C), 129.5 (2C), 137.2, 170.8, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

(3R,5S,5'R,8R,9S,10S,13S,14S)-5'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5D)

56 % yield; Rf = 0.6 (hexanes/EtOAc 3:7); IR (film) ν 3448 and 3325 (NH), and 1728 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.80 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.37 (m, CH-16β), 2.79 and 2.88 (2d of AB system, J = 13.3 Hz, CH_2N), 3.10 (m, $\underline{CH_2}Ph$), 3.75 (dd, J_1 = 4.1 Hz and J_2 = 7.5 Hz, NCHC=O), 7.27 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.8, 27.8, 30.5, 31.3, 31.5, 32.5, 35.0, 35.8, 36.0, 37.7, 38.0, 39.6, 47.8, 51.4, 52.7, 53.8, 58.8, 82.7, 127.1, 128.8 (2C), 129.6 (2C), 137.2, 170.7, 221.2 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a] phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5E)

54 % yield; Rf = 0.29 (hexanes/EtOAc 1:1); IR (film) ν 3448 and 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.2 Hz, CH-16β), 2.83 (s, CH_2N), 3.63 (s, NCH_2C=O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.5, 31.2, 31.5, 32.7, 35.0, 35.8, 36.1, 37.9, 39.4, 47.6, 47.8, 51.4, 52.9, 53.8, 82.5, 168.7, 221.3 ppm. LR-MS: calcd. for $C_{22}H_{34}NO_3$ $[M+H]^+$ 360.25, found 360.25.

(3R,5S,5'S,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyl-5'-(2-methylpropyl)tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7A)

81 % yield; Rf = 0.77 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.93 and 0.98 (2d, J = 6.4 Hz, $(CH_3)_2$-from *i*Pr), 0.80-1.99 (unassigned CH and CH_2), 2.27 and 2.75 (2d of AB system, J = 12.5 Hz, CH_2N), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.3 Hz, CH-16β), 3.17 (dd, J_1 = 2.7 and J_2 = 6.9 Hz, 1H, NCHC=O), 3.21 and 4.07 (2d of AB system, J = 13.6 Hz, $\underline{CH_2}$-Ph), 7.34

(m, 1H of Ph), 7.37 (m, 4H of Ph) ppm. ^{13}C NMR (acetone-d_6) δ 10.8, 13.2, 20.1, 21.4, 21.6, 23.4, 24.9, 27.8, 30.6, 31.5, 31.7, 33.3, 34.9, 35.1, 36.0, 38.2, 38.3, 39.7, 47.3, 51.3, 54.3, 57.6, 57.7, 63.1, 80.5, 127.2, 128.4 (2C), 128.6 (2C), 138.2, 169.8, 218.7 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_3$ $[M+H]^+$ 506.36, found 506.40.

(3R,5S,5'R,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyl-5'-(2-methylpropyl)tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7B)

92 % yield; Rf = 0.85 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.84 (s, CH_3-18), 0.95 and 0.99 (2d, J = 6.6 Hz, $(CH_3)_2$-from *i*Pr), 0.80-2.15 (unassigned CH and CH_2), 2.18 and 2.65 (2d of AB system, J = 12.4 Hz, CH_2N), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, CH-16β), 3.12 and 4.03 (2d of AB system, J = 13.5 Hz, $\underline{CH_2}$-Ph), 3.18 (dd, J_1 = 2.9 Hz and J_2 = 6.8 Hz, NCHC=O), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 22.1, 24.0, 25.0, 28.1, 30.6, 31.5, 31.9, 32.7, 35.0, 35.8, 36.1, 38.2, 38.5, 39.8, 47.8, 51.4, 53.8, 57.7, 58.1, 63.1, 80.2, 127.4 , 128.5 (2C), 128.6 (2C), 137.7, 171.3, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_3$ $[M+H]^+$ 506.36, found 506.40.

(3R,5S,5'S,8R,9S,10S,13S,14S)-4',5'-dibenzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7C)

70 % yield; Rf = 0.61 (hexanes/EtOAc 8:2); IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.75-1.99 (unassigned CH and CH_2), 2.22 and 2.59 (2d of AB system, J = 12.5 Hz, CH_2N), 2.36 (dd, J_1 = 8.7 Hz, J_2 = 18.5 Hz, CH-16β), 3.27 and 4.40 (2d of AB system, J = 13.9 Hz, $N\underline{CH_2}$-Ph), 3.37 (m, $\underline{CH_2}$Ph); 3.51 (dd, J_1 = 2.9 Hz and J_2 = 5.1 Hz, NCHC=O), 7.31 (m, 2xPh) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.1, 20.0, 21.4, 27.7, 30.6, 30.6, 31.6, 33.0, 34.9, 35.1, 35.2, 35.8, 37.9, 39.6, 47.3, 51.2, 54.1, 57.4, 57.7, 65.9, 80.5, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5, 218.7 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_3$ $[M+H]^+$ 540.34, found 540.40.

(3R,5S,5'R,8R,9S,10S,13S,14S)-4',5'-dibenzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7D)

81 % yield; Rf = 0.59 (hexanes/EtOAc 8:2); IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.75-1.99 (unassigned CH and CH_2), 2.22 and 2.60 (2d of AB system, J = 12.4 Hz, CH_2N), 2.37 (dd, J_1 = 8.6 Hz, J_2 = 18.4 Hz, CH-16β), 3.29 and 4.42 (2d of AB system, J =13.8 Hz, $N\underline{CH_2}$-Ph), 3.41 (m, $\underline{CH_2}$Ph); 3.51 (dd, J_1 = 3 Hz and J_2 = 5.0 Hz, NCHC=O), 7.30 (m, 10H, 2xPh) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.2, 20.1, 21.4, 27.4, 30.6, 31.2, 31.7, 32.6, 34.9, 35.0, 35.1, 35.8, 37.2, 40.0, 47.3, 51.2, 54.2, 57.4, 57.8, 65.8, 80.5, 126.6, 127.1, 127.8 (2C), 128.4

(2C), 128.6 (2C), 130.5 (2C), 137.9, 138.1, 169.4, 218.7 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_3$ $[M+H]^+$ 540.34, found 540.40.

(3R,5S,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta [a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E)

71 % yield; Rf = 0.7 (DCM/MeOH 39:1); IR (film) ν 1725 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.99 (unassigned CH and CH_2), 2.11 (m, CH-16α), 2.43 (m, CH_2N and CH-16β), 3.27 (s, NCH_2C=O), 3.50 and 3.54 (2d of AB system, J = 4.4 Hz, $\underline{CH_2}$-Ph), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7, 61.4, 82.4, 127.6, 128.5 (2C), 128.7 (2C), 136.7, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(4-bromobenzyl)-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E1)

87 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1724 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.39 (s, CH_2N), 3.26 (s, NCH_2C=O), 3.46 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.19 (d, J = 8.3 Hz, 2H of Ph), 7.47 (d, J = 8.3 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.7, 60.6, 64.5, 65.1, 82.4, 119.4, 121.5, 130.3 (2C), 131.7 (2C), 135.8, 167.9 ppm. LR-MS: calcd. for $C_{31}H_{43}{}^{79}BrNO_4$ $[M+H]^+$ 572.23, found 572.35.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[4-(trifluoromethoxy)benzyl]

tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E2)

95 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.25 (s, NCH_2C=O), 3.51 (d, J = 2.4 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.19 (d, J = 8.0 Hz, 2H of Ph), 7.34 (d, J = 8.6 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.3, 59.8, 60.5, 64.5, 65.1, 82.4, 119.4, 120.4 (d, J_{C-F} = 257.1 Hz), 121.0 (2C), 129.9 (2C), 135.5, 148.6, 167.9 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_5$ $[M+H]^+$ 578.30, found 578.40.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(biphenyl-4-ylmethyl)-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E3)

91 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-1.99 (unassigned CH and CH_2), 2.46 (s, CH_2N), 3.30 (s, $NCH_2C{=}O$), 3.56 (d, J = 2.2 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.36 (m, 3H of Ar), 7.45 (m, 2H of Ar), 7.59 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 30.7, 31.0, 31.9, 32.9, 34.2, 35.8, 36.1, 38.5, 39.6, 46.0, 50.2, 53.4, 55.5, 59.8, 61.0, 64.6, 65.1, 82.5, 119.4, 127.0 (2C), 127.3 (2C), 127.4, 128.8 (2C), 129.1 (2C), 135.7, 140.5, 140.7, 168.2 ppm. LR-MS: calcd. for $C_{37}H_{48}NO_4$ $[M+H]^+$ 570.35, found 570.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(2-phenylethyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E4)

41 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.75-2.05 (unassigned CH and CH_2), 2.50 (s, CH_2N), 2.61 ($t_{app.}$, J = 7.5 Hz, $\underline{CH_2}$-Ph), 2.78 ($t_{app.}$, J = 7.6 Hz, $N\underline{CH_2}CH_2Ph$), 3.29 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.20 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 33.2, 34.2, 35.8, 36.0, 38.5, 39.6, 45.9, 50.2, 53.4, 55.3, 58.6, 60.4, 64.6, 65.1, 82.3, 119.4, 126.3, 128.4 (2C), 128.6 (2C), 139.5, 168.3 ppm. LR-MS: calcd. for $C_{32}H_{46}NO_4$ $[M+H]^+$ 508.33, found 508.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(3-phenylpropyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E5)

73 % yield; Rf = 0.9 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.78 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.34 (t, J = 7.0 Hz, $N\underline{CH_2}CH_2CH_2Ph$), 2.43 (s, CH_2N), 2.66 (t, J = 7.5 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.21 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.18 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 28.2, 30.7, 31.0, 31.9, 32.9, 33.0, 34.2, 35.8, 36.1, 38.6, 39.6, 46.0, 50.2, 53.4, 55.5, 56.0, 60.3, 64.6, 65.1, 82.3, 119.4, 125.9, 128.4 (4C), 141.7, 168.4 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_4$ $[M+H]^+$ 522.35, found 522.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{2-[4-(trifluoromethyl)phenyl] ethyl}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E6)

53 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.49 (s, CH_2N), 2.63 (t, J = 7.3 Hz, $\underline{CH_2}$-Ph), 2.83 (t, J = 7.3 Hz, $N\underline{CH_2}CH_2Ph$), 3.28 (d, J = 1.5 Hz, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.30 (d, J = 8.0 Hz, 2H of Ph), 7.54 (d, J = 8.1 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 30.6, 31.0, 31.8, 32.9, 33.0, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.2, 60.4, 64.5, 65.1, 82.3, 119.4, 124.2 (q, $J_{C\text{-}F}$ = 272.0 Hz), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz) (2C), 128.7 ($J_{C\text{-}C\text{-}F}$ = 32.5 Hz), 129.0 (2C), 143.6, 168.0 ppm. LR-MS: calcd. for $C_{33}H_{45}F_3NO_4$ $[M+H]^+$ 576.32, found 576.40.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[4-(trifluoromethyl)benzyl] tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E7)

85 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.42 (s, CH_2N), 3.28 (s, $NCH_2C{=}O$), 3.57 (d, J = 2.7 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.44 (d, J = 8.0 Hz, 2H of Ph), 7.61 (d, J = 8.1 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 60.8, 64.5, 65.1, 82.4, 119.4, 124.1 (q, $J_{C\text{-}F}$ = 272.0 Hz), 125.5 ($J_{C\text{-}C\text{-}C\text{-}F}$ = 3.8 Hz) (2C), 128.8 (2C), 130.0 ($J_{C\text{-}C\text{-}F}$ = 32.4 Hz), 140.9, 167.8 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.40.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[4-(benzyloxy)benzyl]-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E8)

70 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.40 (s, CH_2N), 3.24 (s, $NCH_2C{=}O$), 3.45 (d, J = 2.6 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 5.07 (s, CH_2O), 6.95 (d, J = 8.6 Hz, 2H of Ph), 7.21 (d, J = 8.6 Hz, 2H of Ph), 7.39 (m, 5H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.7, 31.0, 31.9, 32.9, 34.2, 35.8, 36.0, 38.4, 39.6, 46.0, 50.1, 53.4, 55.4, 59.7, 60.7, 64.6, 65.1, 70.0, 82.5, 114.8 (2C), 119.4, 127.5 (2C), 128.0, 128.6 (2C), 128.9, 129.9 (2C), 136.9, 158.3, 168.3 ppm. LR-MS: calcd. for $C_{38}H_{50}NO_5$ $[M+H]^+$ 600.36, found 600.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(4-phenylbutyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E9)

69 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.34 (t, J = 7.0 Hz, $N\underline{CH_2}CH_2$), 2.38 (s, CH_2N), 2.62 (t, J = 7.6 Hz, $CH_2\underline{CH_2}Ph$), 3.18 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.18 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 25.9, 28.1, 28.8, 30.7, 31.0, 31.9, 32.9, 34.2, 35.6, 35.8, 36.0, 38.5, 39.6, 46.0, 50.2, 53.4, 55.5, 56.7, 60.4, 64.6, 65.1, 82.3, 119.4, 125.8, 128.3 (2C), 128.4 (2C), 142.2, 168.5 ppm. LR-MS: calcd. for $C_{34}H_{50}NO_4$ $[M+H]^+$ 536.37, found 536.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[2-(trifluoromethyl)benzyl]tetra decahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E10)

57 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.48 (s, CH_2N), 3.28 (d, J = 2.5 Hz, $NCH_2C{=}O$), 3.67 (s, $\underline{CH_2}$-Ph), 3.88 (m, OCH_2CH_2O), 7.39 (t_{app}, J = 7.6 Hz, 1H of Ar), 7.55 (t, J = 7.5 Hz, 1H of Ar), 7.68 (m, 2H of Ar) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.3, 57.1, 60.1, 64.6, 65.1, 82.4, 119.4, 124.3 (q, $J_{C\text{-}F}$ = 273.9 Hz), 126.1 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 5.8 Hz), 127.6, 128.9 (q, $J_{C\text{-}C\text{-}F}$ = 32.8 Hz), 130.4, 132.0, 135.7, 167.9 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.40.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(naphthalen-2-ylmethyl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4] oxazinan]-6''-one (6E11)

74 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.69 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.44 (s, CH_2N), 3.33 (s, $NCH_2C{=}O$), 3.67 (d, J = 2.8 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.48 (m, 3H), 7.72 (s, 1H of Ar), 7.83 (m, 3H of Ar) ppm. LR-MS: calcd. for $C_{35}H_{46}NO_4$ $[M+H]^+$ 544.33, found 544.45.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[3-(trifluoromethyl)benzyl] tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E12)

92 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-2.00 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.29 (s, $NCH_2C{=}O$), 3.57 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.52 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 60.7, 64.5, 65.1, 82.4, 119.4, 124.0

(q, $J_{C\text{-}F}$ = 272.3 Hz), 124.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 129.0, 131.0 (q, $J_{C\text{-}C\text{-}F}$ = 32.3 Hz), 131.9, 137.9, 167.8 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.35.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[3,5-bis(trifluoromethyl)benzyl]-10',13'-dimethyl tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E13)

88 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-2.00 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.33 (s, $NCH_2C{=}O$), 3.65 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.82 (s, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.6, 28.0, 30.6, 31.0, 31.7, 32.8, 34.2, 35.7, 36.0, 38.5, 39.5, 45.9, 50.1, 53.4, 55.3, 59.7, 60.0, 64.5, 65.1, 82.4, 119.4, 121.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 123.2 (q, $J_{C\text{-}F}$ = 272.3 Hz) (2C), 128.5 (2C), 132.0 (q, $J_{C\text{-}C\text{-}F}$ = 33.6 Hz) (2C), 139.8, 167.3 ppm. LR-MS: calcd. for $C_{33}H_{42}F_6NO_4$ $[M+H]^+$ 630.29, found 630.30.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[2,4-bis(trifluoromethyl)benzyl]-10',13'-dimethyltetra decahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E14)

71 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.02 (unassigned CH and CH_2), 2.49 (s, CH_2N), 3.32 (d, J = 2.0 Hz, $NCH_2C{=}O$), 3.73 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.83 (d, J = 8.2 Hz, 1H of Ar), 7.92 (d, J = 7.5 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.9, 32.8, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.3, 56.6, 60.1, 64.5, 65.1, 82.3, 119.4, 121.9, 123.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = ~3 Hz), 123.4 (q, $J_{C\text{-}F}$ = 274.1 Hz) (2C), 128.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.2 Hz), 129.5 (q, $J_{C\text{-}C\text{-}F}$ = 31.1 Hz), 132.2 (q, $J_{C\text{-}C\text{-}F}$ = ~32.8 Hz), 140.2, 167.4 ppm. LR-MS: calcd. for $C_{33}H_{42}F_6NO_4$ $[M+H]^+$ 630.29, found 630.30.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(naphthalen-1-ylmethyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E15)

89 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.68 (s, CH_3-19), 0.81 (s, CH_3-18), 0.80-2.05 (unassigned CH and CH_2), 2.49 and 2.54 (2d of AB system, J = 12.1 Hz, CH_2N), 3.25 and 3.32 (2d of AB system, J = 17.4 Hz, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 3.93 (d, J = 3.5 Hz, $\underline{CH_2}$-Ph), 7.40 (m, 2H of Ar), 7.51 (m, 2H of Ar), 7.84 (m, 2H of Ar), 8.23 (m, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.4, 60.1, 60.1, 64.5, 65.1, 82.5, 119.4, 124.6, 125.1, 125.9 (2C),

127.6, 128.5, 128.7, 132.1, 132.2, 133.9, 168.2 ppm. LR-MS: calcd. for $C_{35}H_{46}NO_4$ $[M+H]^+$ 544.33, found 544.40.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(prop-2-en-1-yl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E16)

86 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.75-2.00 (unassigned CH and CH_2), 2.44 (s, CH_2N), 2.99 (d, J = 6.2 Hz, $N\underline{CH_2}CH=CH_2$), 3.22 (d, J = 2.6 Hz, $NCH_2C=O$), 3.89 (m, OCH_2CH_2O), 5.22 (m, $CH=\underline{CH_2}$), 5.78 (m, $\underline{CH}=CH_2$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.2, 59.9, 59.9, 64.5, 65.1, 82.4, 118.8, 119.4, 133.6, 168.3 ppm. LR-MS: calcd. for $C_{27}H_{42}NO_4$ $[M+H]^+$ 444.30, found 444.35.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(4-iodobenzyl)-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E17)

93 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.39 (s, CH_2N), 3.26 (s, $NCH_2C=O$), 3.45 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.06 (d, J = 8.2 Hz, 2H of Ar), 7.67 (d, J = 8.3 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.7, 60.7, 64.5, 65.1, 82.4, 93.0, 119.4, 130.6 (2C), 136.5, 137.7 (2C), 167.9 ppm. LR-MS: calcd. for $C_{31}H_{43}INO_4$ $[M+H]^+$ 620.22, found 620.25.

Synthesis of 6E18

To a solution of amine **4E** (0.15 mmol) in DMF (4 mL) was added K_2CO_3 (0.22 mmol) and a solution of propargyl bromide (0.45 mmol) in toluene (53 µL). The mixture was refluxed during 17 h and filtered. The DMF was then co-evaporated several times with EtOAc, and the oily crude product was purified by flash chromatography with a mixture of hexanes/EtOAc/TEA (93:6:1) to yield the yellowish product **6E18** (0.10 mmol).

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(prop-2-yn-1-yl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E18)

65 % yield; Rf = 0.4 (hexanes/EtOAc 7:3); IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-2.04 (unassigned CH and CH_2), 2.31 (t, J = 2.2 Hz, CH of alkyne), 2.55 (s, CH_2N), 3.35 (s, $NCH_2C=O$), 3.37 (d, J = 2.3 Hz, $N\underline{CH_2}CCH$), 3.88 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.2, 45.9, 50.1, 53.0, 53.4,

58.8, 64.5, 65.1, 74.7, 76.5, 82.0, 119.4, 168.1 ppm. LR-MS: calcd. for $C_{27}H_{40}NO_4$ $[M+H]^+$ 442.29, found 442.35.

Synthesis of 6E19

The tertiary amine **6E17** (0.07 mmol), 3-fluoromethylphenyl boronic acid (0.11 mmol), [1,1'-bis(diphenylphosphino)ferrocene]-dichloro palladium (II) (8 mol %) and Na_2CO_3 (0.11 mmol) were mixed in a 3:1 dioxane/water solution (4 mL) and the mixture refluxed under argon atmosphere during 5 h. The reaction mixture was then washed with water and extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4, filter and the crude mixture was purified by flash chromatography to yield the product **6E19** (0.02 mmol).

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[3'-(trifluoromethyl)biphenyl-4-yl]methyl}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a] phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E19)

23 % yield; Rf = 0.2 (hexanes/EtOAc 7:3); IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.05 (unassigned CH and CH_2), 2.46 (s, CH_2N), 3.30 (s, $NCH_2C{=}O$), 3.57 (d, J = 2.0 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.42 (d, J = 8.1 Hz, 2H of Ar), 7.58 (m, 4H of Ar), 7.78 (d, J = 7.5 Hz, 1H of Ar), 7.84 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.5, 59.8, 60.9, 64.6, 65.1, 82.5, 119.4, 123.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 124.0 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz), 127.2, 127.3 (2C), 129.3 (2C), 130.3, 131.2 (q, $J_{C\text{-}C\text{-}F}$ = 32.1 Hz), 136.7, 139.1, 141.5, 149.7, 168.1 ppm. LR-MS: calcd. for $C_{38}H_{47}F_3NO_3$ $[M+H]^+$ 638.34, found 638.40.

Synthesis of 6E20

To a solution of amine **6E18** (0.06 mmol) in a 1:1 mixture of *n*-butanol / water (3 mL) was added (azidomethyl)benzene (0.11 mmol), a molar solution of cupper (II) sulfate pentahydrate (63 μL) and a molar solution of sodium ascorbate (125 μL). The reaction mixture was stirred at room temperature for 3 h, washed with water and extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4 and filtered. The crude product was purified by flash chromatography to yield **6E20** (0.05 mmol).

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]-10',13'-di methyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E20)

82 % yield; Rf = 0.1 (hexanes/ EtOAc 1:1); IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.82 (s, CH_3-18), 0.70-2.04 (unassigned CH and CH_2), 2.47 (s, CH_2N); 3.26 (s, $NCH_2C{=}O$), 3.67 (s, $N\underline{CH_2}C{=}C\text{-}N$), 3.88 (m, OCH_2CH_2O), 5.53 (s, $N\underline{CH_2}Ph$), 7.27 (s, 1H of triazole), 7.37 (m, 5H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.8, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 51.6, 53.4, 54.2, 54.9, 59.6, 64.5, 65.1, 82.3, 119.4, 122.5, 128.0 (2C), 128.9, 129.2 (2C), 134.5, 167.9 ppm. LR-MS: calcd. for $C_{34}H_{47}N_4O_4$ $[M+H]^+$ 575.35, found 575.40.

Synthesis of N-sulfonamylated compound 6E21

To a solution of the amine **4E** (0.14 mmol) in DCM was added TEA (0.41 mmol and the mixture was stirred 5 min under argon atmosphere. 4-(Trifluoromethyl)benzenesulfonyl chloride (0.22 mmol) was then added and the solution stirred at room temperature. After 3 h, DCM is partially evaporated and silica gel added to make a dry-pack with the crude mixture. The residue was purified by flash chromatography with a mixture of hexanes/EtOAc/TEA (90:10:1) to yield **6E21** (0.11 mmol).

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[4-(trifluoromethyl)phenyl]sulfonyl} tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E21)

78 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 3.12 (d, J = 2.6 Hz, CH_2N), 3.89 (m, OCH_2CH_2O and $NCH_2C{=}O$), 7.85 (d, J = 8.4 Hz, 2H of Ar), 7.91 (d, J = 8.4 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.6, 27.9, 30.6, 30.9, 30.9, 32.6, 34.1, 35.7, 35.8, 37.4, 39.6, 45.9, 46.1, 50.1, 52.1, 53.4, 64.5, 65.2, 82.4, 119.3, 123.0 (q, $J_{C\text{-}F}$ = 273 Hz), 126.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.4 Hz) (2C), 128.1 (2C), 135.4 (q, $J_{C\text{-}C\text{-}F}$ = 33.3 Hz), 138.9, 163.9 ppm. LR-MS: calcd. for $C_{31}H_{41}F_3NO_6S$ $[M+H]^+$ 612.25, found 612.35.

(3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[4-(trifluoromethyl)phenyl]carbonyl} tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6E22)

86 % yield; Rf = 0.5 (hexanes/EtOAc 1:1); IR (film) ν 1740 (C=O, morpholinone), 1647 (C=O, amide) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19 and CH_3-18), 0.75-2.04 (unassigned CH and CH_2), 3.42 and 3.85 (2m, CH_2N), 3.89 (m, OCH_2CH_2O), 4.19 and 4.53 (2m, NCH_2C=O), 7.54 (d, J = 8.0 Hz, 2H of Ar), 7.74 (d, J = 8.0 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.6, 28.0, 30.5, 30.9, 32.6, 34.1, 35.7, 35.8, 37.1, 39.5, 45.9, 50.1, 53.4, 64.5, 65.1, 82.2, 119.3, 123.5 (q, J_{C-F} = 272.6 Hz), 126.0 (q, $J_{C-C-C-F}$ = 3.5 Hz) (2C), 127.6 (2C), 132.6 (q, J_{C-C-F} = 35.9 Hz), 137.7, 168.9 (2C) ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 576.29, found 576.35.

General procedure to generate compounds 7E1-7E22

To a solution of compounds **6E1-6E22** (30 - 60 mg) in dioxane (3 mL) was added an aqueous solution of sulphuric acid (5 %) (3 mL) and the mixture was stirred at room temperature (2 h - 5 h). A saturated solution of Na_2CO_3 (12 mL) was added and the reaction mixture extracted with EtOAc. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The purification was further done by flash column chromatography. Elution with hexanes/ EtOAc / TEA (95:5:1) to yield compounds **7A-7E**.

(3R,5S,8R,9S,10S,13S,14S)-4'-(4-bromobenzyl)-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E1)

78 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.84-1.99 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.40 (s, CH_2N), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.5 Hz, CH-16β), 3.26 (s, NCH_2C=O), 3.46 (d, J = 2.5 Hz, $\underline{CH_2}$-Ph), 7.19 (d, J = 8.3 Hz, 2H of Ar), 7.47 (d, J = 8.3 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.5, 60.6, 82.3, 121.5, 130.3 (2C), 131.7 (2C), 135.8, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{39}{}^{81}BrNO_3$ $[M+H]^+$ 530.20, found 530.15.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[4-(trifluoromethoxy)benzyl]tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E2)

70 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β); 2.44 (s, CH_2N), 3.25 (s, NCH_2C=O), 3.51 (d, J = 3.8 Hz, $\underline{CH_2}$-Ph), 7.19 (d, J = 8.1 Hz, 2H of Ar), 7.33 (d, J =

8.5 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 59.7, 60.5, 82.3, 120.4 (d, J_{C-F} = 257 Hz) 121.0 (2C), 129.9 (2C), 130.3, 135.4, 148.6, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_4$ $[M+H]^+$ 534.28, found 534.15.

(3R,5S,8R,9S,10S,13S,14S)-4'-(biphenyl-4-ylmethyl)-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E3)

94 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.47 (s, CH_2N), 3.30 (s, NCH_2C=O), 3.56 (d, J = 3.6 Hz, $\underline{CH_2}$-Ph), 7.36 (m, 3H of Ar), 7.45 (m, 2H of Ar), 7.60 (m, 4H of Ar) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.5, 59.7, 61.0, 82.4, 127.0 (2C), 127.3 (2C), 127.4, 128.8 (2C), 129.1 (2C), 135.7, 140.5, 140.6, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{35}H_{44}NO_3$ $[M+H]^+$ 526.32, found 526.30.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(2-phenylethyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E4)

60 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.85 (s, CH_3-18), 0.75-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 2.50 (s, CH_2N), 2.62 ($t_{app.}$, J = 7.5 Hz, $\underline{CH_2}$-Ph), 2.78 ($t_{app.}$, J = 7.6 Hz, $N\underline{CH_2}CH_2Ph$), 3.28 (d, J = 1.8 Hz, NCH_2C=O), 7.20 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.7, 32.9, 33.2, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.3, 58.6, 60.3, 82.2, 126.3, 128.4 (2C), 128.6 (2C), 139.5, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{42}NO_3$ $[M+H]^+$ 464.31, found 464.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(3-phenylpropyl)tetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E5)

51 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.96 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.34 (t, J = 7.0 Hz, $N\underline{CH_2}CH_2CH_2Ph$), 2.43 (m, CH-16β), 2.44 (s, CH_2N), 2.66 (t_{app}, J = 7.6 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.21 (s, NCH_2C=O), 7.18 (m, 3H of Ph), 7.29 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 28.2, 30.6, 31.5, 31.9, 32.9, 33.0, 35.0, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.4, 56.0, 60.1, 82.2, 126.0, 128.4 (4C), 141.6, 168.4, 221.3 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_3$ $[M+H]^+$ 478.32, found 478.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{2-[4-(trifluoromethyl)phenyl]ethyl}tetra decahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E6)

36 % yield; Rf = 0.5 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-2.02 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (dd, J_1 = 8.5 Hz, J_2 = 19.2 Hz, CH-16β), 2.50 (s, CH_2N), 2.64 ($t_{app.}$, J = 7.3 Hz, $\underline{CH_2}$-Ph), 2.84 ($t_{app.}$, J = 7.4 Hz, $N\underline{CH_2}CH_2Ph$), 3.29 (d, J = 4.1 Hz, $NCH_2C{=}O$), 7.31 (d, J = 8.0 Hz, 2H of Ar), 7.55 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 33.0, 35.0, 35.8, 36.1, 38.1, 39.6, 44.6, 47.8, 51.4, 53.8, 55.2, 57.9, 60.3, 82.2, 125.3 ($J_{c\text{-}c\text{-}c}$ = 3.6 Hz) (2C), 129.0 (2C), 133.1, 143.6, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{31}H_{41}F_3NO_3$ $[M+H]^+$ 532.30, found 532.20.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[4-(trifluoromethyl)benzyl]tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E7)

78 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-2.01 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.42 (m, CH-16β), 2.43 (s, CH_2N), 3.28 (s, $NCH_2C{=}O$), 3.57 (d, J = 2.7 Hz, $\underline{CH_2}$-Ph), 7.44 (d, J = 8.0 Hz, 2H of Ar), 7.61 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7, 60.8, 82.3, 124.0 (q, J = 270.3 Hz), 125.5 ($J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz) (2C), 128.8 (2C), 130.0 ($J_{C\text{-}C\text{-}F}$ = 32.3 Hz), 140.9, 167.8, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.25.

(3R,5S,8R,9S,10S,13S,14S)-4'-[4-(benzyloxy)benzyl]-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E8)

88 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.41 (s, CH_2N), 2.43 (dd, J_1 = 8.5 Hz, J_2 = 19.2 Hz, CH-16β), 3.24 (s, $NCH_2C{=}O$), 3.46 (d, J = 3.8 Hz, $\underline{CH_2}$-Ph), 5.07 (s, CH_2O), 6.95 (d, J = 8.6 Hz, 2H of Ph), 7.21 (d, J = 8.6 Hz, 2H of Ph), 7.39 (m, 5H, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.4, 59.6, 60.7, 70.1, 82.4, 114.8 (2C), 127.5 (2C), 128.0, 128.6 (2C), 128.9, 129.9 (2C), 136.9, 158.3, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_4$ $[M+H]^+$ 556.33, found 556.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(4-phenylbutyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E9)

77 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.34 (t, J = 7.0 Hz, N$\underline{CH_2}CH_2CH_2CH_2Ph$), 2.39 (s, CH_2N), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.4 Hz, CH-16β), 2.63 (t, J = 7.5 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.18 (d, J = 1.9 Hz, $NCH_2C{=}O$), 7.17 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 25.9, 27.9, 28.8, 30.6, 31.5, 31.8, 32.9, 35.0, 35.6, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.5, 56.7, 60.3, 82.2, 125.8, 128.3 (2C), 128.4 (2C), 142.2, 168.4, 221.3 ppm. LR-MS: calcd. for $C_{32}H_{46}NO_3$ $[M+H]^+$ 492.34, found 492.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[2-(trifluoromethyl)benzyl]tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E10)

67 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.86-2.03 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.49 (s, CH_2N), 3.29 (d, J = 3.9 Hz, $NCH_2C{=}O$), 3.68 (s, $\underline{CH_2}$-Ph), 7.40 (t, J = 7.6 Hz, 1H of Ar), 7.55 (t, J = 7.5 Hz, 1H of Ar), 7.67 (m, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 57.1, 60.0, 82.3, 124.3 (q, $J_{C\text{-}F}$ = 273.8 Hz), 126.1 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 5.7 Hz), 127.6, 128.9 (q, $J_{C\text{-}C\text{-}F}$ = 30.3 Hz), 130.5, 132.0, 135.7, 167.8, 221.2 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.50.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(naphthalen-2-ylmethyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E11)

86 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.44 (m, CH-16β), 2.45 (s, CH_2N), 3.34 (s, $NCH_2C{=}O$), 3.67 (d, J = 4.3 Hz, $\underline{CH_2}$-Ph), 7.50 (m, 3H of Ar), 7.71 (s, 1H of Ar), 7.83 (m, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 34.9, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.6, 59.6, 61.5, 82.4, 126.0, 126.3, 126.7, 127.6, 127.7 (2C), 128.4, 133.0, 133.2, 134.2, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{42}NO_3$ $[M+H]^+$ 500.31, found 500.20.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[3-(trifluoromethyl)benzyl]tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E12)

79 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.07

(m, CH-16α), 2.42 (m, CH-16β), 2.43 (s, CH_2N), 3.28 (s, $NCH_2C{=}O$), 3.57 (s, $\underline{CH_2}$-Ph), 7.52 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7, 60.7, 82.3, 124.0 (q, $J_{C\text{-}F}$ = 272.3 Hz), 124.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 129.1, 131.0 (q, $J_{C\text{-}C\text{-}F}$ = 32.4 Hz), 131.9, 137.9, 167.8, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.50.

(3R,5S,8R,9S,10S,13S,14S)-4'-[3,5-bis(trifluoromethyl)benzyl]-10,13-dimethyltetra decahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E13)

85 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.87-1.99 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.42 (m, CH-16β), 2.44 (s, CH_2N), 3.33 (d, J = 1.7 Hz, $NCH_2C{=}O$), 3.65 (s, $\underline{CH_2}$-Ph), 7.81 (s, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.6, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.3, 53.8, 55.3, 59.6, 60.0, 82.3, 121.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 123.2 (q, $J_{C\text{-}F}$ = 272.7 Hz) (2C), 128.5 (2C), 132.0 (q, $J_{C\text{-}C\text{-}F}$ = 33.4 Hz) (2C), 139.8, 167.3, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{38}F_6NO_3$ $[M+H]^+$ 586.27, found 586.15.

(3R,5S,8R,9S,10S,13S,14S)-4'-[2,4-bis(trifluoromethyl)benzyl]-10,13-dimethyltetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E14)

28 % yield; Rf = 0.8 (hexanes/EtOAc 1:1); IR (film) ν 1740 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 2.49 (s, CH_2N), 3.32 (d, J = 3.1 Hz, $NCH_2C{=}O$), 3.74 (s, $\underline{CH_2}$-Ph), 7.84 (d, J = 8.1 Hz, 1H of Ar), 7.91 (d, J = 8.7 Hz, 1H of Ar), 7.93 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.5, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 56.6, 60.0, 82.2, 123.3 (q, $J_{c\text{-}F}$ = 270.5 Hz), 123.5 (q, $J_{c\text{-}c\text{-}c\text{-}F}$ = 3.7 Hz), 124.4 (q, $J_{C\text{-}F}$ = 276.2 Hz), 124.8, 128.8, 129.6 (q, $J_{C\text{-}C\text{-}F}$ = 31.3 Hz), 130.2 ($J_{c\text{-}c\text{-}F}$ = 33.1 Hz), 131.0, 140.1, 167.3, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{38}F_6NO_3$ $[M+H]^+$ 586.27, found 586.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(naphthalen-1-ylmethyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E15)

86 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.69 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-1.93 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.41 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.52 (d, J = 5.5 Hz, CH_2N), 3.29 (d, J = 7.5 Hz, $NCH_2C{=}O$), 3.92 (d, J = 4.8 Hz, $\underline{CH_2}$-Ph), 7.40 (m, 2H of Ar), 7.51 (m, 2H of Ar), 7.85 (m, 2H of Ar), 8.22 (m, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4,

53.8, 55.4, 59.9, 60.1, 82.4, 124.6, 125.1, 125.9 (2C), 127.6, 128.5, 128.8, 132.1, 132.2, 133.9, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{42}NO_3$ $[M+H]^+$ 500.31, found 500.20.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(prop-2-en-1-yl)tetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E16)

55 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (m, CH-16β), 2.44 (s, CH_2N), 2.99 (d, J = 3.8 Hz, $N\underline{CH_2}CH{=}CH_2$), 3.21 (d, J = 6.3 Hz, $NCH_2C{=}O$), 5.21 (m, $CH{=}\underline{CH_2}$), 5.76 (m, $\underline{CH}{=}CH_2$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.2, 59.8, 59.9, 82.3, 118.9, 133.6, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{25}H_{38}NO_3$ $[M+H]^+$ 400.28, found 400.15.

(3R,5S,8R,9S,10S,13S,14S)-4'-(4-iodobenzyl)-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E17)

59 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.84-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.39 (s, CH_2N), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.4 Hz, CH-16β), 3.26 (s, $NCH_2C{=}O$), 3.45 (d, J = 2.5 Hz, $\underline{CH_2}$-Ph), 7.06 (d, J = 8.2 Hz, Ph), 7.67 (d, J = 8.2 Hz, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.5, 60.7, 82.3, 93.1, 130.6 (2C), 136.4, 137.7 (2C), 167.9, 221.2 ppm. LR-MS: calcd. for $C_{29}H_{39}INO_3$ $[M+H]^+$ 576.19, found 576.10.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(prop-2-yn-1-yl)tetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E18)

67 % yield; Rf = 0.3 (hexanes/EtOAc 7:3); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.75-2.04 (unassigned CH and CH_2), 2.09 (m, CH-16α), 2.31 (d, J = 2.3 Hz, CH of alkyne), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.57 (s, CH_2N), 3.36 (d, J = 1.0 Hz, $NCH_2C{=}O$), 3.38 (d, J = 2.4 Hz, $N\underline{CH_2}CCH$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 29.7, 30.6, 31.5, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 45.2, 47.8, 51.4, 53.1, 53.8, 58.7, 74.7, 76.5, 82.0, 168.1, 221.3 ppm. LR-MS: calcd. for $C_{25}H_{36}NO_3$ $[M+H]^+$ 398.26, found 398.15.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[3'-(trifluoromethyl)biphenyl-4-yl]methyl} tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E19)

59 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-2.04 (unassigned CH and CH_2), 2.09 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.47 (s-broad, CH_2N), 3.31

(s, $NCH_2C=O$), 3.58 (d, J = 3.4 Hz, $\underline{CH_2}$-Ph), 7.42 (d, J = 8.1 Hz, 2H of Ar), 7.59 (m, 4H of Ar), 7.78 (d, J = 7.5 Hz, 1H of Ar), 7.84 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.5, 59.7, 60.9, 82.4, 123.0 (q, $J_{c\text{-}F}$ = 270 Hz), 123.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.9 Hz), 124.0 (q, $J_{c\text{-}c\text{-}c\text{-}F}$ = 3.9 Hz), 127.3 (2C), 129.3 (2C), 130.3, 131.2 (q, $J_{c\text{-}c\text{-}F}$ = 34.0 Hz), 136.7, 139.1, 141.5, 149.7, 168.1, 221.3 ppm. LR-MS: calcd. for $C_{36}H_{43}F_3NO_3$ $[M+H]^+$ 594.31, found 594.20.

(3R,5S,8R,9S,10S,13S,14S)-4'-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E20)

90 % yield; Rf = 0.6 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O), 1454 (C-O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.50 (s, CH_2N), 3.27 (s, $NCH_2C=O$), 3.69 (s, $N\underline{CH_2}C=C\text{-}N$), 5.54 (s, $N\underline{CH_2}Ph$), 7.27 (m, 2H of Ph), 7.34 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 51.6, 53.8, 54.2, 54.9, 59.5, 82.2, 122.5, 128.0 (2C), 128.9, 129.2 (2C), 134.6, 143.6, 167.8, 221.2 ppm. LR-MS: calcd. for $C_{32}H_{43}N_4O_3$ $[M+H]^+$ 531.33, found 531.25.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[4-(trifluoromethyl)phenyl]sulfonyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E21)

42 % yield; Rf = 0.7 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-2.03 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.44 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 3.10 and 3.18 (2d of AB system, J = 12.5 Hz, CH_2N), 3.81 and 3.90 (2d of AB system, J = 17.5 Hz, $NCH_2C=O$), 7.87 and 7.91 (2d of AB system, J = 8.2 Hz, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.7, 30.5, 30.8, 31.5, 32.6, 35.0, 35.8, 36.0, 37.4, 39.6, 46.1, 47.7, 51.3, 52.0, 53.7, 82.3, 124.3 (q, $J_{C\text{-}F}$ = 273 Hz), 126.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.4 Hz) (2C), 128.1 (2C), 135.4 (q, $J_{C\text{-}C\text{-}F}$ = 33.4 Hz), 138.9, 163.8, 221.0 ppm. LR-MS: calcd. for $C_{29}H_{37}F_3NO_5S$ $[M+H]^+$ 568.23, found 568.10.

(3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[4-(trifluoromethyl)phenyl]carbonyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7E22)

83 % yield; Rf = 0.4 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O), 1647 (C=O, amide) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.85 (s-broad, CH_3-19 and CH_3-18), 0.80-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, CH-16β), 3.44 and

3.84 (2m, CH_2N), 4.20 and 4.53 (2m, $NCH_2C=O$), 7.54 (d, J = 8.0 Hz, 2H of Ar), 7.74 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.4, 32.5, 34.9, 35.8, 35.9, 36.9, 39.5, 47.7, 51.3, 53.7, 57.8, 64.5, 66.1, 123.5 (q, J_{C-F} = 273 Hz), 126.0 (q, $J_{C-C-C-F}$ = 3.5 Hz) (2C), 127.6 (2C), 132.8 (q, J_{C-C-F} = 35.9 Hz), 137.7, 168.9, 221.2 ppm. LR-MS: calcd. for $C_{30}H_{37}F_3NO_4$ $[M+H]^+$ 532.26, found 532.20.

Synthesis of carbamates 9A-9E

To a solution of the amino alcohol **3A**, **3B**, **3C**, **3D** or **3E** (0.12 mmol) in DCM (3 mL) was added diisopropylethylamine (0.24 mmol) at 0 °C, and the solution stirred under an atmosphere of argon for 10 min. Triphosgene (0.06 mmol) was added and the reaction mixture stirred during 5 h at room temperature. Then, an acidic solution (2 mL) of HCl:MeOH (10:90) was added to the reaction mixture which was stirred overnight at room temperature. The reaction was stopped by adding a saturated $NaHCO_3$ aqueous solution (10 mL) and the product extracted with DCM. The organic layer was dried using anhydrous Na_2SO_4 and evaporated under reduced pressure. The purification was done by flash chromatography, using hexanes/EtOAc/TEA (95:5:1) for the elution.

methyl (2S)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-4-methylpentanoate (9A)

73 % yield; Rf = 0.71 (hexanes/EtOAc 1:1); IR (film) ν 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.85 (s, CH_3-18), 0.96 and 0.97 (2d, J = 1.9 Hz, $(CH_3)_2$- from *i*Pr), 0.99-1.97 (unassigned CH and CH_2), 2.11 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.14 and 3.42 (2d of AB system, J = 8.1 Hz, CH_2N), 3.72 (s, OCH_3), 4.58 (dd, J_1 = 4.8 Hz and J_2 = 11.1 Hz, NCHCO) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.1, 21.8, 23.2, 24.9, 27.9, 30.6, 31.5, 32.7, 33.8, 35.0, 35.4, 35.8, 37.7, 39.5, 40.8, 47.7, 51.3, 52.2, 52.9, 53.6, 53.9, 79.6, 157.6, 172.0, 221.2 ppm. LR-MS: calcd. for $C_{28}H_{44}NO_5$ $[M+H]^+$ 474.31, found 474.35.

Methyl (2R)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-4-methylpentanoate (9B)

67 % yield; Rf = 0.57 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.83 (s, CH_3-19), 0.86 (s, CH_3-18), 0.97 (d, J = 6.5 Hz, $(CH_3)_2$- from *i*Pr), 0.80-1.97 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.44 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.14 and 3.44 (2d of AB system, J = 8.1 Hz, CH_2N), 3.72 (s, OCH_3), 4.60 (dd, J_1 = 4.8 Hz and J_2 = 11.1 Hz, NCHCO) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.1, 21.7, 23.1, 24.9, 27.8, 30.6, 31.5, 32.9, 33.9, 35.0, 35.4, 35.8, 37.7, 39.3, 40.8,

47.7, 51.3, 52.2, 52.8, 53.6, 53.9, 79.6, 157.5, 172.0, 221.1 ppm. LR-MS: calcd. for $C_{28}H_{44}NO_5$ $[M+H]^+$ 474.31, found 474.35.

Methyl (2S)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-3-phenylpropanoate (9C)

61 % yield; Rf = 0.40 (hexanes/EtOAc 1:1); IR (film) ν 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.84 (s, CH_3-18), 0.75-1.95 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.42 (dd, J_1 = 8.5 Hz, J_2 = 19.3 Hz, CH-16β), 2.95 and 3.35 (2m, $\underline{CH_2}$Ph), 3.09 and 3.33 (2d of AB system, J = 8.0 Hz, CH_2N), 3.76 (s, OCH_3), 4.88 (dd, J_1 = 5.5 Hz and J_2 = 11.3 Hz, NCHCO), 7.22 (m, 3H of Ph), 7.30 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.7, 30.5, 31.5, 32.4, 33.8, 35.0, 35.1, 35.3, 35.8, 38.8, 40.6, 47.7, 51.3, 52.4, 53.2, 53.8, 55.8, 79.7, 127.1, 128.6 (2C), 128.7 (2C), 136.0, 157.3, 171.0, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{42}NO_5$ $[M+H]^+$ 508.30, found 508.35.

Methyl (2R)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-3-phenylpropanoate (9D)

75 % yield; Rf = 0.50 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.84 (s, CH_3-18), 0.75-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.42 (dd, J_1 = 8.5 Hz, J_2 = 19.3 Hz, CH-16β), 2.96 and 3.35 (2m, $\underline{CH_2}$Ph), 3.10 and 3.33 (2d of AB system, J = 8.0 Hz, CH_2N), 3.76 (s, OCH_3), 4.87 (dd, J_1 = 5.4 Hz and J_2 = 11.2 Hz, 1H, NCHCO), 7.22 (m, 3H of Ph), 7.29 (m, 2H of Ph), ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.7, 30.5, 31.5, 32.4, 33.7, 35.0, 35.1, 35.3, 35.8, 39.1, 40.8, 47.7, 51.3, 52.4, 53.3, 53.8, 55.9, 79.7, 127.1, 128.5 (2C), 128.7 (2C), 136.0, 157.3, 171.0, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{42}NO_5$ $[M+H]^+$ 508.30, found 508.40.

Methyl [(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro [cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]acetate (9E)

32 % yield; Rf = 0.48 (hexanes/EtOAc 1:1); IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.10 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.33 (s, CH_2N), 3.75 (s, OCH_3), 3.99 and 4.04 (2d, J = 18.0 Hz, NCHCO) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.6, 31.5, 32.7, 33.8, 35.0, 35.4, 35.8, 39.3, 40.8, 45.0, 47.7, 51.3, 52.3, 53.9, 56.5, 79.6, 157.6, 169.0, 221.2 ppm. LR-MS: calcd. for $C_{24}H_{36}NO_5$ $[M+H]^+$ 418.25, found 418.30.

Inhibition of 17β-HSD3 (microsomal fraction of rat testes)

A microsomal preparation of rat testes was obtained using slightly modified previously described procedures [30]. In brief, rat testes were homogenized on ice with a Polytron in cold phosphate buffer (20 mM KH_2PO_4, 0.25 M sucrose, 1 mM EDTA, pH 7.5) containing protease inhibitors mini-complete (Roche Diagnostics, Laval, QC, Canada) and centrifugated at 12,500g for 15 min to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifugated at 100,000g for 45 min using an ultracentrifuge equipped with a 70.1 Ti rotor. The microsomal pellet was washed three times with phosphate buffer and centrifugated at 100,000g for 15 min. All these operations were conducted at 4 °C. The protein concentration of the supernatant was determined by the Bradford method using bovine serum albumin as standard. The enzymatic assay was performed at 37 °C for 2 h in 1 mL of a solution containing 860 µL of 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 100 µL of 5 mM NADPH in phosphate buffer, 10 µL of 5 µM [^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 µL of inhibitor dissolved in ethanol and 20 µL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in triplicate. Afterwards, radiolabelled steroids were extracted from the reaction mixture with diethyl ether. The organic phases evaporated to dryness with nitrogen stream. Residue was dissolved in 50 µL of dichloromethane and dropped on silica gel 60 F_{254} thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1) solvent system. Substrate ([^{14}C]-4-dione) and metabolite ([^{14}C]-T) were identified by comparison with reference steroids and quantified using the Storm 860 System (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and then the percentage of inhibition were calculated.

Proliferative and antiproliferative activities on LAPC-4 (AR^+) cells

Androgen-sensitive human prostate cancer cells LAPC-4, kindly provided by Dr. Chantal Guillemette (CHU de Québec- Research Center, Quebec, Canada) and maintained at 37 °C under 5 % CO_2 humidified atmosphere. Cells were grown in RPMI-1640 medium supplemented (v/v) with 10 % foetal bovine serum (FBS), 1 % L-glutamine, 1 % insuline and 1 % penicillin/streptomycin. To determine the effect of novel compounds on cell proliferation, LAPC-4 cells were resuspended with the medium supplemented with 5 % dextran-coated charcoal treated FBS rather than 10 % FBS to remove remaining hormones. Triplicate cultures of 10^4 cells in a total of 100 μL medium in 96-well microtiter plates (Becton–Dickinson Company, Lincoln Park, NJ, USA) were pre incubated at 37 °C, 5 % CO_2, for 24 h attachment period. Tested compounds were dissolved in DMSO to prepare the stock solution of 10^{-2} M. Those compounds were diluted at several concentrations with culture medium, added to corresponding wells and incubated for 3 days. Control wells were treated with vehicle DMSO. Quantification of cell growth was determined by MTS method, using CellTitter 96® AQueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) and following the manufacturer's instructions.

The proliferative (androgenic) activity was expressed as difference between the cell proliferation (in %) caused by a given compound and the basal cell proliferation fixed at 100 %. The antiproliferative (antiandrogenic) activity was determined as the ability of a compound to inhibit DHT (0.1 μM) -induced proliferation of LAPC-4 cells and was reported in comparison to the DHT stimulation fixed at 100 %.

ASSOCIATED CONTENT

S- Supporting information

Inhibitory curves of target compounds (IC_{50} values), and experimental details of chemical synthesis characterization of compound **11**, non steroidal compounds **13-17** the residual contaminant (compound **19**). This material is available, free of charge via Internet at: http://pub.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AR, androgen receptor; Ar, aryl group; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMSO, dimethylsulfoxide; 17β-HSD, 17-beta-hydroxysteroid dehydrogenase; NMO, *N*-methylmorpholine *N*-oxide; ($Pd(dppf)Cl_2$), 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride; Ph, phenyl group; *p*-TSA, para-toluene sulfonic acid; THF, tetrahydrofurane, TPAP, tetrapropylamonium perruthenate.

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Supporting Information

Design, chemical synthesis and biological evaluation of 3-spiromorpholinone/3-spirocarbamate androsterone derivatives as inhibitors of 17β-hydroxysteroid dehydrogenase type 3

G. B. Djigoué; L. C. Kenmogne; J. Roy; R. Maltais; D. Poirier

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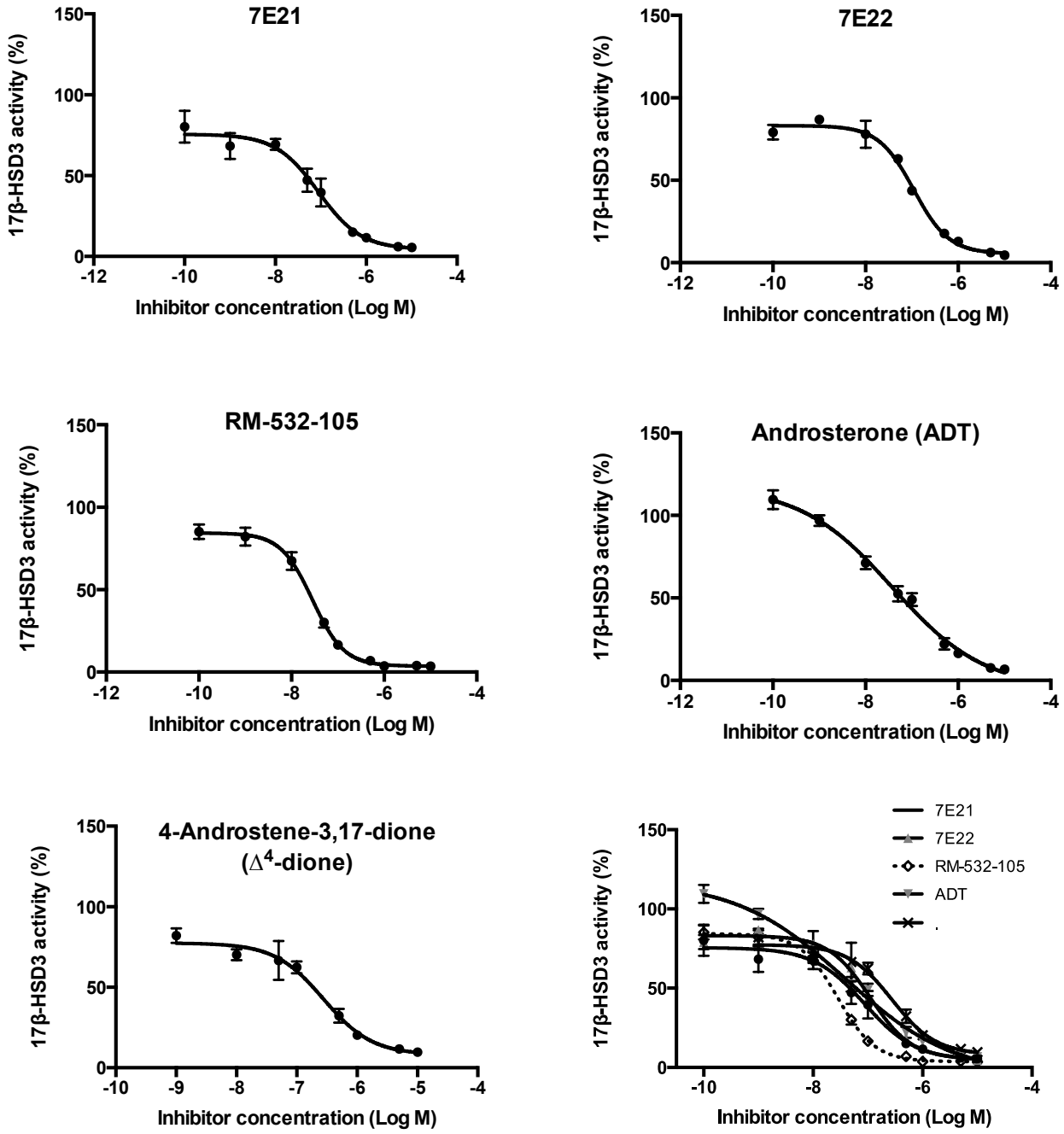


Figure S1: 17β-HSD3 inhibitory activity (Protocol 1). For the transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by rat testicular 17β -HSD3 (microsomal fraction). IC_{50} value for **7E21** (19 nM), **7E22** (69 nM), RM-532-105 (16 nM), ADT (69 nM) and Δ^4-dione (122 nM).

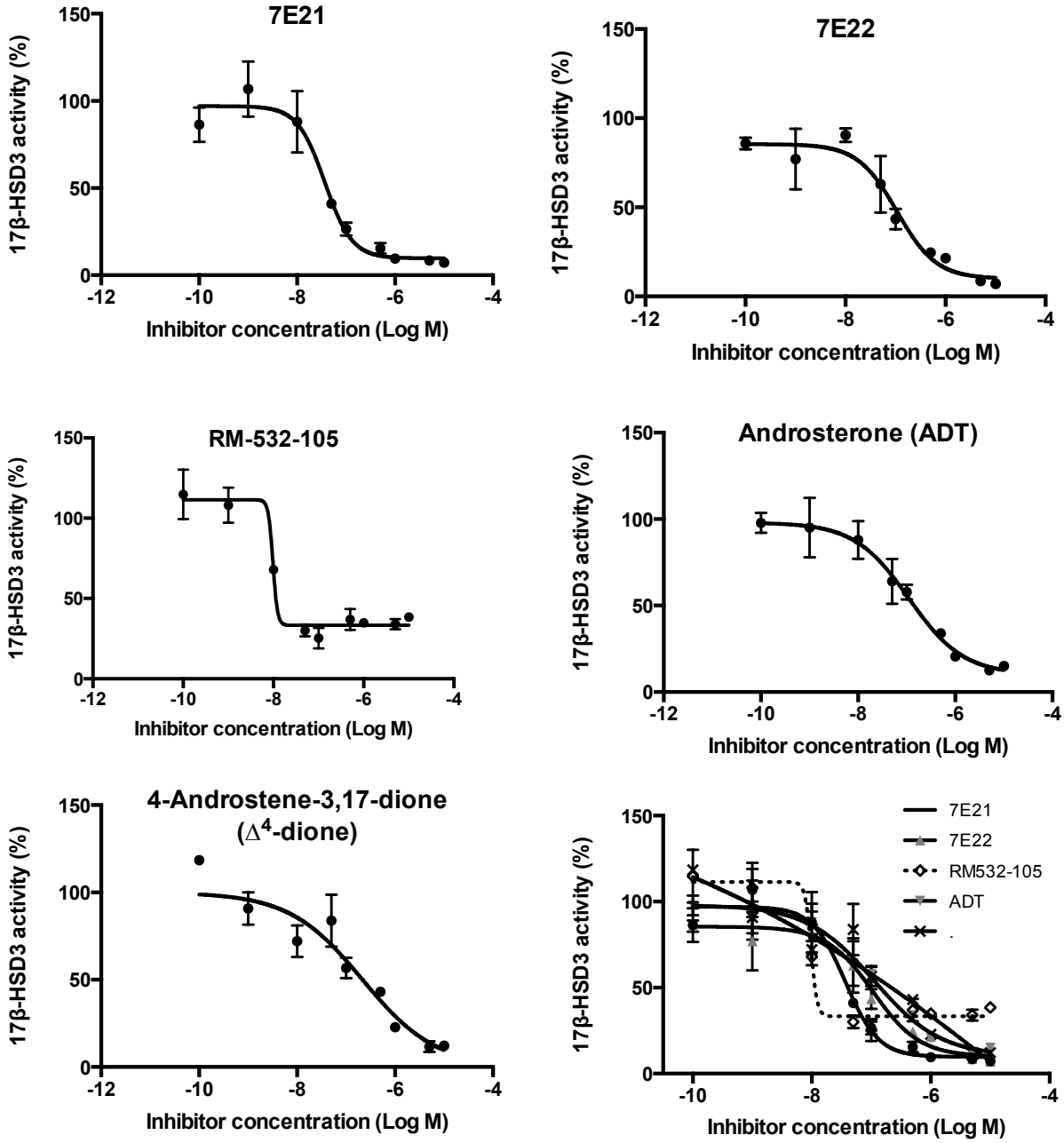


Figure S2: 17β-HSD3 inhibitory activity (Protocol 2). For the transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by rat testicular 17β-HSD3 (microsomal fraction). IC_{50} value for **7E21** (36 nM), **7E22** (106 nM), RM-532-105 (~10 nM), ADT (111 nM) and Δ^4-dione (216 nM).

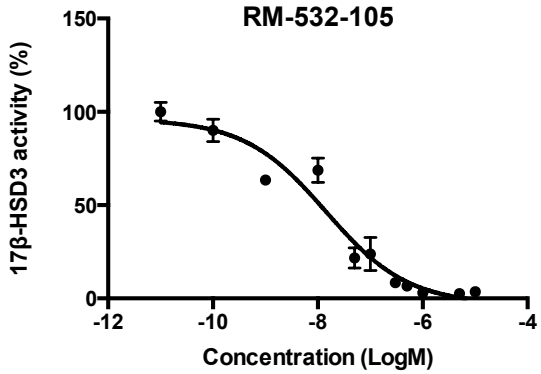
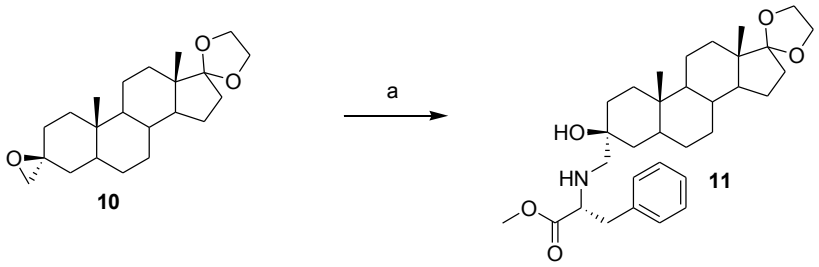


Figure S3: 17β-HSD3 inhibitory activity (Protocol 3). For the transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by rat testicular 17β-HSD3 (microsomal fraction). RM-532-105 (IC_{50} = 14 nM).



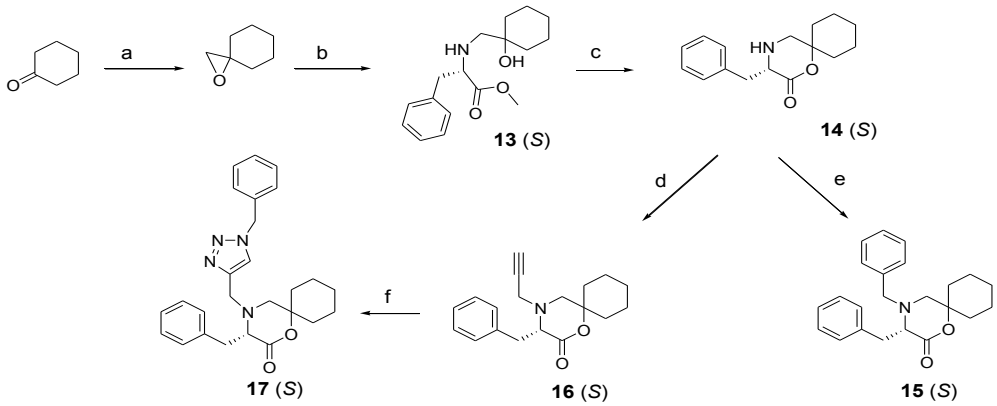
Scheme S1: Chemical synthesis of compound **11** from epoxide **10**. Reagents and conditions: (a) D-Phenylalanine, MeOH, 90°C, overnight.

Chemical synthesis of compound 11 (Scheme S1)

In a schlenk tube, the oxirane **10** (1.7 mmol) was dissolved in dry MeOH (15 mL) and the freed amino D-phenylalanine methyl ester (17.2 mmol) was added to the reaction mixture. The schlenk tube was then screwed down hermetically, stirred and heated at 90 °C during 21 h. The MeOH was evaporated, the crude reaction mixture dissolved in DCM pre-adsorbed on silica gel, and purified by flash column chromatography (hexanes/EtOAc/TEA (89:10:1) to yield compound **11** (1.16 mmol).

Methyl N-{[(3S,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-D-phenylalaninate (11)

68 % yield; Rf = 0.56 (hexanes/EtOAc, 7:3); ^{1}H NMR (acetone-d_6) δ 0.81 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-2.09 (unassigned CH and CH_2), 2.49 and 2.69 (2d of AB system, J = 11.8 Hz, CH_2N), 2.92 and 2.96 (2m, $\underline{CH_2}Ph$), 2.94 (t_{app}, J = 6.6 Hz, CHC=O), 3.31 (s, OH), 3.63 (s, OCH_3), 3.84 (m, OCH_2CH_2O), 7.24 (m, Ph) ppm.



Scheme S2: Reagents and conditions. (a) $(CH_3)_3SOI$, NaH, DMSO/THF, rt, 24 h; (b) L-Phenylalanine methyl ester, MeOH, 90°C, overnight; (c) MeONa, THF, rt, 2 h; (d) K_2CO_3, propargyl bromide, DMF, reflux, overnight; (e) DIPEA, $C_6H_5CH_2Br$, DCM, 75°C, 22 h; (f) Benzyl azide, aqueous $CuSO_4.5H_2O$, sodium ascorbate, $BuOH:H_2O$ (1:1), rt, 3 h.

Methyl N-[(1-hydroxycyclohexyl)methyl]-L-phenylalaninate (13)

73 % yield; Rf = 0.4 (hexanes/EtOAc 75:25); IR (film) ν 3464 and 3348 (OH and NH) and 1736 (C=O) cm^{-1}.^{1}H NMR ($CDCl_3$) δ 1.19-1.64 (unassigned CH_2 of cyclohexyl), 2.21 and 2.69 (2d of AB system, J = 12.2 Hz, CH_2N), 2.88 and 2.99 (2m, $CH\underline{CH_2}Ph$), 3.46 (dd, J_1 = 6.1 Hz, J_2 = 7.7 Hz, NCHCO), 3.68 (s, OCH_3), 7.23 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 22.0 (2C), 25.9, 35.7, 35.8, 39.9 (2C), 57.6, 64.0, 69.9, 126.8, 128.4 (2C), 129.1 (2C), 137.3, 175.1 ppm. LRMS (m/z) calcd. for $C_{17}H_{26}NO_3$ $[M + H]^+$ 292.18, found 292.20.

(3S)-3-benzyl-1-oxa-4-azaspiro[5.5]undecan-2-one (14)

76 % yield; Rf = 0.2 (hexanes/EtOAc 7:3); IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.24-1.81 (unassigned CH_2 of cyclohexyl), 2.73 and 2.93 (2d of AB system, J = 13.4 Hz, CH_2N), 3.10 and 3.31 (2m, $CH\underline{CH_2}Ph$), 3.73 (dd, J_1 = 3.9 Hz, J_2 = 8.4 Hz, NCHCO), 7.25 and 7.32 (2m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 21.3, 21.6, 25.4, 34.6, 35.8, 38.1, 50.8, 58.9, 83.1, 127.0, 128.7 (2C), 129.4 (2C), 137.4, 170.8 ppm. LRMS (m/z) calcd. for $C_{16}H_{22}NO_2$ $[M + H]^+$ 260.16, found 260.20.

(3S)-3,4-dibenzyl-1-oxa-4-azaspiro[5.5]undecan-2-one (15)

80 % yield; Rf = 0.7 (hexanes/EtOAc 7:3); IR (film) ν 1720 (C=O). ^{1}H NMR ($CDCl_3$) δ 0.87-1.74 (unassigned CH_2 of cyclohexyl), 2.08 and 2.65 (2d of AB system, J = 12.6 Hz, CH_2N), 3.18 and 4.31 (2d of AB system, J = 13.7 Hz, $N\underline{CH_2}Ph$), 3.26 and 3.54 (2m, $CH\underline{CH_2}Ph$); 3.52 (m, NCHCO), 7.28 (m, 2 x Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 21.2, 21.4, 25.3, 34.3, 35.7, 35.8, 56.0, 58.1, 66.3, 81.7, 126.7, 127.4, 128.0 (2C), 128.4 (2C), 128.5 (2C), 130.3 (2C), 137.5, 137.5, 171.0 ppm. LRMS (m/z) calcd. for $C_{23}H_{28}NO_2$ [M

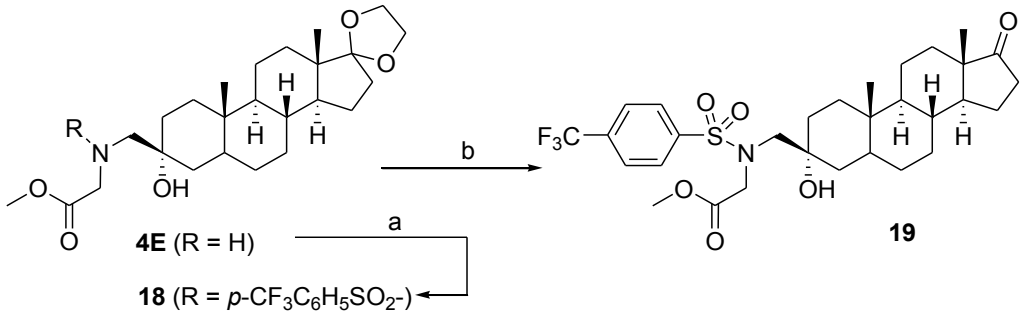
+ H]$^+$ 350.20, found 350.20.

(3S)-3-benzyl-4-(prop-2-yn-1-yl)-1-oxa-4-azaspiro[5.5]undecan-2-one (16)

44 % yield; Rf = 0.71 (hexanes/EtOAc 1:1); IR (film) ν 3279 (C-H of propargylic group) and 1720 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.88-1.75 (unassigned CH_2 of cyclohexyl), 2.30 (t, J = 2.3 Hz, CH of alkyne), 2.64 (d, J = 4.2 Hz, CH_2N), 3.05 and 3.41 (2m, $\underline{CH_2}Ph$), 3.46 (dd, J_1 = 2.4 Hz, J_2 = 17.6 Hz, NCHCO), 3.73 (m, HCC-$\underline{CH_2}$), 7.21 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 21.2, 21.6, 25.4, 34.4, 35.2 (2C), 35.8, 42.9 (2C), 56.8, 62.7, 81.1, 126.6, 128.0 (2C), 130.1 (2C), 137.1, 178.8 ppm. LRMS (m/z) calcd. for $C_{19}H_{24}NO_2$ [M + H]$^+$ 298.17, found 298.0.

(3S)-3-benzyl-4-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]-1-oxa-4-azaspiro[5.5] undecan-2-one (17)

69 % yield; Rf = 0.84 (hexanes/EtOAc 1:1); IR (film) ν 1720 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.81-1.65 (unassigned CH_2 of cyclohexyl), 2.29 and 2.72 (2d of AB system, J = 12.7 Hz, N$\underline{CH_2}$C-O), 3.25 and 3.50 (2m, NCH$\underline{CH_2}$Ph), 3.46 (dd, J_1 = 3.2 Hz, J_2 = 14.2 Hz, NCHCO), 3.71 and 4.05 (2d of AB system, J = 14.6 Hz, NCH_2C=C), 5.50 (s, Ph$\underline{CH_2}$N-N), 7.04 (s, H-C=C), 7.17 (m, Ph), 7.32 (2m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 21.3, 21.5, 25.2, 34.2, 35.7, 35.9, 48.4 (2C), 54.2, 56.0, 64.7, 81.6, 122.3, 126.6, 127.9 (2C), 128.1 (2C), 128.9, 129.2 (2C), 130.1 (2C), 134.5, 137.5, 170.8 ppm. LRMS (m/z) calcd. for $C_{26}H_{31}N_4O_2$ [M + H]$^+$ 431.24, found 431.10.



Scheme S3: Chemical synthesis of the residual contaminant **19**. (a) TEA, 4-(trifluoromethyl)benzene sulfonyl chloride, DCM, 3 h, rt; (b) H_2O:H_2SO_4 (95:5), dioxane, rt, 5 h.

Synthesis of *N*-sulfonamylated compounds 18 (Scheme S2)

To a solution of the amine **4E** (0.14 mmol) in DCM was added TEA (0.41 mmol and the mixture was stirred 5 min under argon atmosphere. 4-(trifluoromethyl)benzenesulfonyl chloride (0.22 mmol) was then added and the solution stirred at room temperature. After 3 h, DCM is partially evaporated and silica gel added to make a dry-pack with the crude mixture. The residue was purified over flash chromatography with a mixture of hexanes/EtOAc/TEA (90:10:1) to yield **18** (0.05 mmol).

methyl *N*-{[(3*R*,8*R*,9*S*,10*S*,13*S*,14*S*)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-*N*-{[4-(trifluoromethyl) phenyl]sulfonyl}glycinate (18)

36 % yield; Rf = 0.5 (hexanes/EtOAc 7:3); IR (film) ν 3526 (OH), 1751 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.84 (s, CH_3-18), 0.75-2.00 (unassigned CH and CH_2), 2.53 (s, OH), 3.22 (s, CH_2N), 3.65 (s, OCH_3), 3.85 (m, OCH_2CH_2O), 4.16 (s, $NCH_2C{=}O$), 7.79 (d, J = 8.3 Hz, 2H of Ar), 7.95 (d, J = 8.2 Hz, 2H of Ar) ppm. ^{13}C NMR (C DCl_3) δ 11.3, 14.4, 20.4, 22.6, 28.3, 30.7, 31.1, 31.2, 33.3, 34.2, 35.7, 36.0, 37.9, 40.5, 45.9, 50.3, 51.4, 52.4, 53.9, 60.7, 64.5, 65.2, 72.4, 119.4, 123.2 (q, $J_{C\text{-}F}$ = 273 Hz), 126.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.5 Hz) (2C), 128.0 (2C), 134.5 (q, $J_{C\text{-}C\text{-}F}$ = 33.1 Hz), 142.4, 170.4 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_6S$ $[M\text{-}HO]^+$ 626.28, found 626.40.

Hydrolysis of 18 to 19

To a solution of compound **18** (26 mg) in dioxane (3 mL) was added an aqueous solution of sulphuric acid (5 %) (3 mL) and the mixture was stirred at room temperature for 5 h. A saturated solution of Na_2CO_3 (12 mL) was added and the reaction mixture extracted with EtOAc. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The purification was further done by flash column chromatography. Elution with hexanes/EtOAc/TEA (90:10:1) hexanes/EtOAc/TEA (95:5:1) to yield compound **19** (21 mg).

methyl *N*-{[(3α)-3-hydroxy-17-oxoandrostan-3-yl]methyl}-*N*-{[4-(trifluoromethyl)phenyl] sulfonyl}glycinate (19)

87 % yield; Rf = 0.3 (hexanes/EtOAc 7:3); IR (film) ν 3518 (OH) and 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.86 (s, CH_3-18), 0.88-1.97 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.44 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 2.63 (s, OH), 3.22 (s, CH_2N), 3.66 (s, OCH_3), 4.14 (s, $NCH_2C{=}O$), 7.79 (d, J = 8.4 Hz, 2H of Ar), 7.95 (d, J = 8.3 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.3, 21.7, 28.1, 30.8, 31.1, 31.5, 33.2, 35.0, 35.8, 36.1, 37.8, 40.4, 47.8, 51.4, 51.5, 52.5, 54.3, 60.9, 72.3, 123.1 (q, $J_{C\text{-}F}$ = 273 Hz), 126.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.5 Hz) (2C), 128.0 (2C), 134.6 (q, $J_{C\text{-}C\text{-}F}$ = 33.1 Hz), 142.3, 170.5, 221.4 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_5S$ $[M\text{-}H_2O]^+$ 582.25, found 582.40.

Conclusion générale

La 17β-HSD1, la 17β-HSD3 et la 17β-HSD5 jouent un rôle clé dans la biosynthèse des hormones les plus actives sur les tumeurs cancéreuses estrogénosensibles et androgénosensibles. En catalysant la dernière étape de la biosynthèse des estrogènes et des androgènes, ces trois enzymes contrôlent les taux de certaines hormones circulantes et intratumorales. Les inhibiteurs présentés dans cette thèse, ont été conçus pour mieux interagir que leur substrat naturel avec les acides aminés du site catalytique de l'enzyme ciblée. Ces inhibiteurs pourront ainsi empêcher la transformation du substrat en hormone estrogénique ou androgénique.

En raison du rôle joué par la 17β-HSD1 dans les maladies sensibles aux estrogènes, le développement d'inhibiteurs sélectifs a connu un essor remarquable au cours de la dernière décennie. Cependant, il n'y a toujours pas d'inhibiteurs de la 17β-HSD1 utilisés en clinique. Ceci est en partie dû au fait que les inhibiteurs de la 17β-HSD1 possédaient un noyau estrane qui cause l'activation du récepteur des estrogènes.

Au début de mon premier projet de doctorat, le CC-156 était connu comme un inhibiteur très efficace de la 17β-HSD1 [204], mais celui-ci étant estrogénique, il ne pouvait de toute évidence pas être utilisé en clinique pour traiter le cancer du sein. Plusieurs stratégies avaient déjà été utilisées pour obtenir des analogues non estrogéniques du CC-156, notamment en ajoutant un groupement méthoxy en position C-2 ou en ajoutant une longue chaine d'akylamide en position C-7α. Cependant, ces modifications ont causé une baisse considérable de l'activité inhibitrice. Pour la première partie de mon projet de thèse, j'avais quatre défis à relever: développer une stratégie de synthèse de mimiques non stéroïdiennes du CC-156, les caractériser par RMN ^{1}H, RMN ^{13}C, IR et SM, optimiser leur affinité avec la 17β-HSD1 et, finalement, vérifier que ceux-ci n'activent pas le récepteur des estrogènes.

Sur la base des données cristallographiques de la 17β-HSD1 et d'un récent article sur la structure cristalline du complexe CC-156/17β-HSD1/NADP [135], et du «docking» moléculaire que nous avons effectué avec le logiciel Autodock v.3 (Figure 3; Chapitre 1), des mimiques non stéroïdiennes (composés **8** et **10**) de l'inhibiteur CC-156 ont été conçues. La synthèse de ces composés a été réalisée à l'aide d'une séquence de six réactions à partir du bromhydrate de tétrahydroisoquinolinol (**1**) (Figure 4; Chapitre 1). L'intermédiaire **3**, dont la fonction phénol et amine sont protégées respectivement par un groupement MOM et un groupement Fmoc s'est avéré être instable à la température ambiante. Nous recommandons d'utiliser cet intermédiaire rapidement pour la

préparation du composé **4**. De plus, le rendement de l'hydrolyse des composés **6** et **7** a été affecté par l'utilisation du méthanol, donnant le sous-produit **9** dont nous avons aussi évalué l'activité biologique. Nous recommandons l'utilisation de l'acétone pour cette hydrolyse, plutôt que le méthanol, afin d'éviter la transformation non désirée de la fonction carboxamide en ester de méthyle. La structure des composés **8**, **9** et **10** a été confirmée par RMN ^{1}H, RMN ^{13}C, IR et SM. Malgré les observations intéressantes obtenues à partir des expériences de «docking» avec le composé **10**, les composés **8**, **9** et **10** inhibent très faiblement la 17β-HSD1 présente dans les cellules T-47D. Cette faible inhibition pourrait être attribuée à différents facteurs. Notre première hypothèse est que les molécules synthétisées ne traversent pas la membrane plasmique des cellules T-47D, et donc n'atteignent pas la cible thérapeutique. Pour vérifier cette hypothèse, ces composés doivent être évalués sur un homogénat de cellules T-47D ou la 17β-HSD1 purifiée, et si elle est confirmée, on associera aux prochaines molécules synthétisées des pharmacophores capables d'améliorer leur biodisponibilité pour la 17β-HSD1. Notre deuxième hypothèse est que le caractère électronique du pseudo-cycle B, due à la présence d'un atome d'azot, diminue l'affinité de ces composés. Pour vérifier une telle hypothèse, nous suggérons de concevoir des inhibiteurs possédant un noyau dépourvu d'azote dans le pseudo-cycle B. Nous pensons également que la flexibilité relative de ces composés par rapport au squellette stéroïdien rigide du CC-156 les rend moins stables dans le site catalytique. Pour contrer ces problèmes, nous suggérons d'intégrer un bicycle non fusionné pour obtenir des inhibiteurs de la deuxième génération (Figure 12).

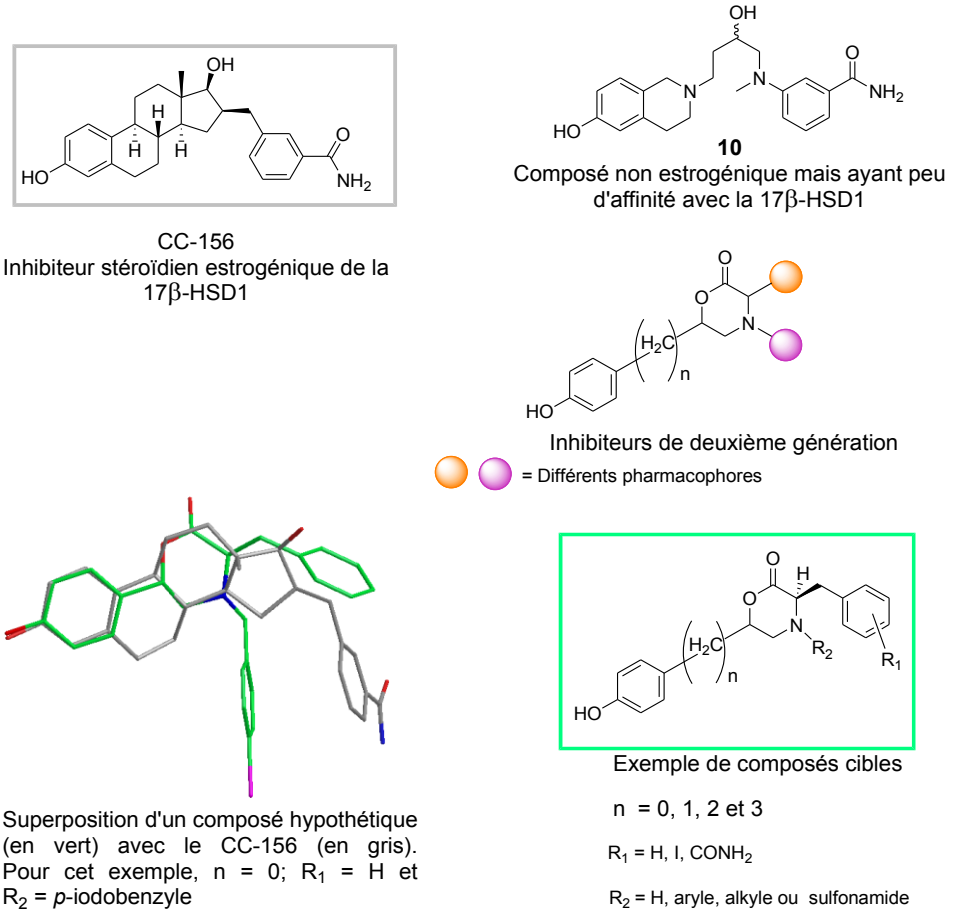


Figure 12: Conception d'un nouveau modèle de mimiques non stéroïdiennes de l'inhibiteur CC-156 qui pourraient être plus actives que le composé **10** sur la 17β-HSD1

Un des objectifs importants de mon projet était d'obtenir un composé dépourvu d'activité estrogénique. Cette activité a été évaluée sur les cellules (RE+) T-47D pour les produits **8** et **10**. Remarquablement, le composé **8** est dépourvu de toute activité estrogénique aux concentrations testées (0,001-10 µM) et le composé **10** n'est estrogénique qu'aux plus fortes concentrations de 5 et 10 µM. En définitive, malgré la faible affinité de ces composés avec l'enzyme 17β-HSD1, ils ne sont pas estrogéniques et, par conséquent, ils sont de bons candidats pour d'autres cibles biologiques. De plus la voie de synthèse que nous avons développée pourra être adaptée à un autre type de noyau.

Encouragés par nos premiers résultats, nous avons réalisé les travaux présentés au chapitre 2. Le but de notre second projet était de développer une méthode de synthèse en parallèle et en solution, simple et efficace, de mimiques non stéroïdiennes flexibles de l'estradiol (E2). En effet, de tels composés, dépourvus d'activité estrogénique seront

évalués sur des cibles thérapeutiques sensibles aux estrogènes. La méthode de synthèse que nous avons développée est simple, automatisable et pourrait permettre de générer dans un temps relativement court, un très grand nombre de molécules. Deux chimiothèques totalisant chacune 75 composés non stéroïdiens flexibles ont été conçues pour cibler la stéroïde sulfatase (STS) et les récepteurs des estrogènes (REα et REβ). Dix époxydes ont d'abord été préparés à partir du *para* et du *méta* hydroxybenzaldéhyde, avant d'être ensuite aminolysés, phénolysés ou thiophénolysés avec un grand nombre de «building blocks». En dehors des anilines (building blocks **A** et **B**) pour lesquels nous avons été obligé de trouver des conditions réactionnelles différentes pour l'ouverture des époxydes, les réactions en solution ont relativement bien fonctionnées. En effet, la faible réactivité des anilines pour l'ouverture des époxydes était due à la délocalisation du doublet d'électron libre de l'azote par l'effet du cycle aromatique. Nous avons donc ajouté une quantité stoechiométrique de perchlorate de manganèse, pour activer l'époxyde et cette stratégie a merveilleusement fonctionnée. En tout, 150 mimiques non stéroïdiennes de E2 ont été synthétisées et caractérisées par RMN ^{1}H et SM. Des molécules représentatives ont été sélectionnées dans chacune des chimiothèques et leur indice de pureté évalué, soit une moyenne de pureté de 69 % (36 - 93 %) et 80 % (62 – 91 %) repectivement pour les chimiothèques L1 et L2 (Tableaux 1 et 2, chapitre 2). Dix représentants ont ensuite été choisis pour en faire une caractérisation complète (RMN ^{1}H, RMN ^{13}C, IR et SM). L'évaluation biologique de ces composés sur la STS, REα et REβ sera éventuellement réalisée.

Pour le deuxième volet de ma thèse, portant sur la préparation d'une nouvelle famille d'inhibiteurs de la 17β-HSD3, nous sommes partis d'un ensemble d'idées découlant des travaux réalisés antérieurement dans notre laboratoire. En effet, différents stéroïdes avaient été criblés et parmi eux, l'androstérone avait montré une affinité intéressante vis-à-vis de la 17β-HSD3 [217], avec une valeur IC_{50} deux fois meilleure à celle du 4-androstène-3,17-dione (Δ^4-dione), le substrat naturel. Plusieurs modifications ont alors été faites sur différentes positions de ce noyau androstane et, finalement, il est ressorti que la substitution en position C-3 par des groupements hydrophobes permettait d'obtenir des inhibiteurs efficaces de la 17β-HSD3. Au point de départ de ce projet, nous est venue l'idée d'utiliser une spiromorpholinone comme moyen efficace pour introduire et positionner différents groupes (diversité moléculaire) à proximité de la position C-3, s'inspirant ainsi de ce qui avait été fait sur un noyau estrane en position C-

17 par Rouillard et collaborateurs (voir, référence 21 chapitre 4). En effet, avec la restriction stérique procurée par les spirocycles, nous avons pensé qu'une spiromorpholinone dérivant d'un ester d'acide aminé était une entité chimique offrant non seulement une possibilité de diversification moléculaire grace à la réactivité de l'amine, mais aussi une facilité de diversification des orientations si on considère les différents isomères (*L*) et (*D*) de chaque acide aminé utilisé. Les défis étaient de synthétiser, isoler et caractériser les produits ciblés, d'évaluer leur activité inhibitrice sur la 17β-HSD3 et déterminer s'ils étaient androgéniques ou non. Notre premier défi synthétique a été de réussir l'ouverture du C3-oxirane-ADT avec l'ester méthylique de la glycine, dont la forme moléculaire très polaire est difficilement isolable à partir du chlorhydrate correspondant. Cette réaction a été faite avec succès en générant la fonction amine de l'ester de glycine «*in situ*» en présence du DIPEA. Dans nos premières tentatives de lactonisation (trans-estérification intramoléculaire) nous avons constaté une racémisation, due à l'acidité du proton adjacent à la lactone. Nous avons évalué différentes conditions réactionnelles (bases, quantité de base, volume de solvant et température) (voir Tableau 1 chapitre 6, partie B), la meilleure condition découlant de ces essais était l'utilisation de 0,6 équivalent de CH_3ONa dans 12 mL de THF à la température ambiante pour 0,14 mmol d'amino-alcool de départ. Ces conditions ont permis d'obtenir 98 % de rendement et un ratio *R*/*S* de [80:20] partant de l'isomère *R*. À partir de l'ester méthylique de la glycine, la leucine (*L* et *D*) et la phénylalanine (*L* et *D*), nous avons synthétisé 10 spiromorpholinones, qui ont été caractérisées par RMN ^{1}H, RMN ^{13}C, IR et SM.

Le criblage biologique (inhibition de la 17β-HSD3) de ces composés nous a permis de remarquer que les composés ayant une stéréochimie *S* sur le spirocycle étaient de meilleurs inhibiteurs que leurs analogues de stéréochimie inverse (*R*). Les résultats présentés dans le chapitre 3 avec la cristallisation de deux candidats nous ont permis de mieux comprendre et de confirmer l'effet de l'orientation spaciale sur l'activité biologique observée.

Dans les résultats présentés au chapitre 4, une spiromorpholinone ou un spiro carbamate a été ajouté en position C-3 d'une 17-monospiro-δ-lactone-ADT, un inhibiteur de la 17β-HSD5. Suite à cette transformation, les composés obtenus ont été évalués comme inhibiteurs de la 17β-HSD3 avec des résultats concluants. Cette stratégie ouvre la voie pour la synthèse d'inhibiteurs hybrides des 17β-HSD3 et 17β-HSD5, deux enzymes qui sont impliquées dans la formation des androgènes et, par conséquent, des cibles

thérapeutiques intéressantes pour le cancer de la prostate et autres maladies androgénosensibles.

Les résultats présentés dans les chapitres 5 et 6 découlent d'une étude SAR (synthèse chimique et évaluation biologique). Nous avons démontré lors de nos synthèses chimiques qu'il est possible de réaliser une réaction de couplage (réaction de Suzuki) sur un halogénure d'aryle introduit dans la spiromorpholinone. Cependant, en utilisant le complexe ($Pd(dppf)Cl_2$) comme catalyseur et Na_2CO_3 comme base, nous avons seulement obtenu un rendement de 23 % pour cette réaction. Afin d'améliorer cette approche exploitable pour synthétiser d'autres inhibiteurs de la 17β-HSD3 et pour introduire des pharmacophores de plus en plus intéressants, nous suggérons d'investiguer les conditions de Suzuki en présence d'une base moins agressive. En effet, le faible rendement serait dû à l'ouverture de la morpholinone par la base. Toujours dans un souci de proposer d'autres possibilités de diversification moléculaire, le composé triazolique **6E20** a été synthétisé par «chimie click», une stratégie qui ouvre la porte à la préparation de plusieurs autres analogues. Enfin, dans le but d'étudier le rôle joué par le noyau androstane dans l'activité biologique, quatre dérivés non stéroïdiens ont été synthétisés et se sont avérés inactifs.

En résumé, l'ensemble des composés synthétisés ont été caractérisés pour confirmer leur structure et leur activité biologique (inhibition de la 17β-HSD3 et androgénicité) a été évaluée. Le sulfonamide **7E21** et le carboxamide **7E22** ont été identifiés comme étant des inhibiteurs de la 17β-HSD3 efficaces et non androgéniques. Ces deux produits issus de notre étude SAR peuvent maintenant être utilisés pour d'autres tests *in vitro* (pharmacocinétique et pharmacodynamique) et *in vivo* (modèles de xénogreffe de souris) plus élaborés. Ceci est un bon point de départ dans le processus de développement d'un médicament à base d'un inhibiteur de la 17β-HSD3 qui pourra être utilisé pour soigner le cancer de la prostate et d'autres maladies sensibles aux androgènes.

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