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SYNTHÈSE CHIMIQUE ET ACTIVITÉ BIOLOGIQUE D'AGENTS STÉROÏDIENS POUR LE TRAITEMENT DU CANCER DE LA PROSTATE ET DE LA LEUCÉMIE

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Résumé

Le cancer est l'une des maladies les plus répandues au Canada étant responsable de plus d'un quart de tous les décès. En effet chaque personne sera un jour ou l'autre touchée par cette maladie, soit personnellement ou par la souffrance d'un proche. Il y a de nombreux types de cancer, mais ils sont tous associés par la prolifération anarchique de cellules anormales dans le corps. Le cancer de la prostate et la leucémie sont deux types de cancer très fréquents dans la population.

Le cancer de la prostate est un cancer androgéno-sensible, c'est-à-dire son développement et sa croissance sont stimulés par la liaison des androgènes actifs au récepteur. L'utilisation d'un antiandrogène capable de bloquer cette interaction permet de diminuer la taille du cancer. Cependant, il n'existe présentement aucun antiandrogène capable de lier fortement le récepteur afin de traiter le cancer par sa seule utilisation. Pour cela, plusieurs agents thérapeutiques potentiels ont été synthétisés à l'aide de plusieurs réactions dont une métathèse utilisant le catalyseur de Grubbs. Parmi cet éventail de produits, le meilleur antiandrogène était un noyau 5α-androstane-3α,17β-diol avec, en positition 16α, une chaîne de 3 méthylènes avec un chlorure à son extrémité. Ce type de produit a de plus été modélisé afin de déterminer son interaction avec le récepteur.

La leucémie est le type de cancer le plus fréquent chez les enfants, mais peut aussi affecter les adultes. La majorité des traitements présentement utilisés induisent de nombreux effets secondaires pouvant parfois être fatals pour les patients. À l'aide de la chimie combinatoire ou de la chimie en solution, plusieurs librairies de dérivés 2β-amino-5α-androstane-3α,17β-diol ont été synthétisées. L'analyse de l'activité biologique de tout ces composés a permis d'en identifier six ayant une activité antiprolifération sur les cellules leucémiques HL-60 avec des IC_{50} variant de 0.58 à 6.4 μM. La plupart de ces produits affectent le cycle cellulaire en bloquant les cellules leucémiques dans la phase G_1 du cycle, tandis que d'autres induisent aussi la différenciation. De plus, quelques candidats ont démontré une sélectivité pour les cellules cancéreuses puisqu'ils n'ont aucun effet sur les cellules humaines normales.

Remerciements

La réalisation d'un doctorat exige beaucoup d'efforts et de travail. Mais, heureusement au cours de ces cinq années d'études, plusieurs personnes m'ont aidée et encouragée. Puisque leur soutien fût très important pour moi, j'aimerais les remercier.

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Avant-Propos

En tant que patient, parent ou ami, chacun d'entre nous connaît ou connaîtra un jour la douleur et la peine qui accompagne cette terrible maladie qu'est le cancer. C'est pourquoi, il est très important d'avoir des traitements très efficaces afin de contrer cette maladie. Par le passé, la découverte d'un nouvel agent thérapeutique découlait habituellement de l'évaluation biologique d'un large éventail de produits synthétisés par les industries chimiques et pharmaceutiques ou d'origine naturel. Tous ces produits étaient testés sans égard à leur structure. Maintenant, l'approche est un peu plus rationnelle. L'identification et la caractérisation de plusieurs cibles thérapeutiques ont permis l'élaboration et le développement de nouveaux agents présentement utilisés en cliniques. Des études structure-activités ont permis de cibler les groupements fonctionnels importants afin d'obtenir une meilleure activité thérapeutique.

Plusieurs chercheurs ont orientés leurs travaux vers l'élaboration de nouveaux traitements efficaces afin de diminuer le taux de mortalité causé par le cancer. Cette thèse de doctorat a donc été consacrée à la recherche de nouveaux agents thérapeutiques dans le cadre du traitement de deux cancers très importants, soit le cancer de la prostate et la leucémie. L'introduction de cette thèse passe en revue les généralités du cancer comme les facteurs de risques, son incidence ainsi que son développement. De plus, un résumé présentera le cancer de la prostate et la leucémie ainsi que les causes et les traitements actuels pour ces deux types de cancer. Par la suite, six articles scientifiques exposeront la majorité des résultats des recherches effectuées au cours de mon doctorat. Un avant-propos au début de chacun de ces chapitres mentionnera ma contribution à ces articles. Évidemment, ces manuscrits ont été rédigés en anglais puisque ceux-ci seront publiés dans des revues scientifiques de langue anglaise.

Le premier chapitre de cette thèse est consacré au cancer de la prostate. Il décrit la synthèse chimique en solution et l'évaluation biologique d'une série de dérivés du 5α-androstane-3α,17β-diol ayant une chaîne alkyle en position 16α, dans le but d'identifier un antiandrogène efficace. Dans le cadre de mon doctorat, un autre projet a aussi été abordé

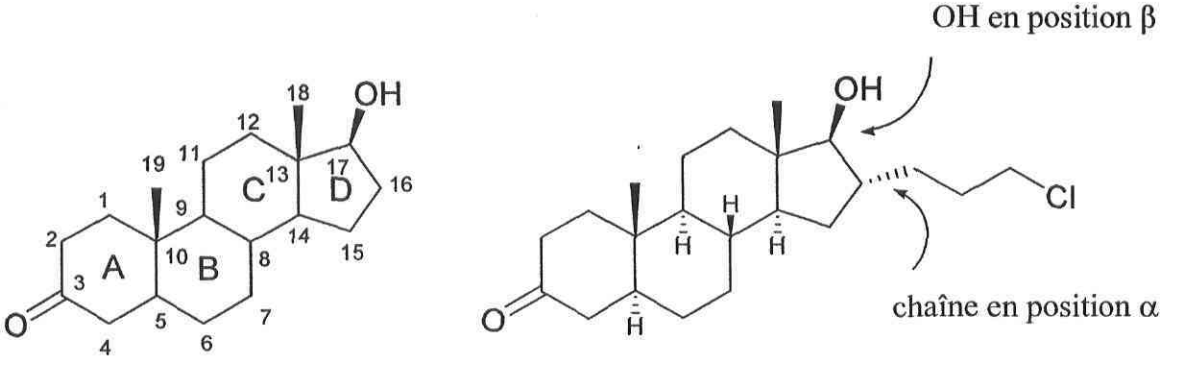
brièvement. Ce projet consistait à la synthèse sur support solide des dérivés de la dihydrotestostérone diversifiés en position 7α par la présence d'une chaîne de 11 méthylènes portant à son extrémité un acide aminé et un acide carboxylique. Les produits ont été préparés, mais puisque aucun ne présentaient d'activité antiandrogénique intéressante, les résultats n'ont pas été présentés dans cette thèse.

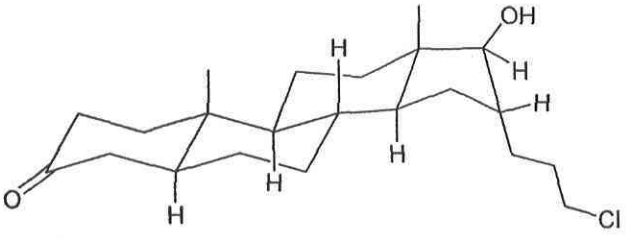
Les prochains chapitres traiteront du cancer le plus important chez les enfants, la leucémie. Tout d'abord, le deuxième chapitre rapportera la préparation d'une nouvelle famille d'aminostéroïdes dérivée du 2β-pipérazino-5α-androstane-3α,17β-diol à l'aide de la synthèse sur support solide ainsi que de leur activité antiproliférative sur les cellules leucémiques humaines (HL-60). Le troisième chapitre traitera de la synthèse, mais cette fois-ci en solution, de dérivés de la 2β-amino-5α-androstane-3α,17β-diol et de leur évaluation biologique. Le quatrième chapitre aura un volet totalement biologique puisque les meilleurs candidats issus des travaux des chapitres 2 et 3 seront utilisés afin d'étudier leur effet sur le cycle cellulaire, leur effet sur la différenciation cellulaire et leur sélectivité pour les cellules cancéreuses. À l'aide des informations obtenues dans les chapitres précédents, le cinquième chapitre présente la préparation de nouveaux dérivés du 2β-pipérazino-5α-androstane-3α,17β-diol ainsi que leur activité antiproliférative sur les cellules leucémiques HL-60. Finalement, le sixième chapitre traitera de la synthèse chimique et de l'action antiproliférative sur les cellules HL-60 de certains dérivés du 2-méthoxyestradiol, rapporté dans la littérature pour ces propriétés antileucémiques. Puisque ces recherches m'ont permis de développer une bonne expertise pour l'évaluation de l'activité antileucémique exprimée par différents agents, j'ai participé pendant mes études de doctorat à une collaboration internationale avec un chercheur de l'Université d'État de Moldavie (Aurelian Gulea). Ce dernier effectuait la synthèse de produits composés d'un noyau métallique et désirait savoir l'effet de ceux-ci sur les cellules HL-60. Les résultats de ces tests nous ont permis d'identifier quelques produits intéressants, mais ces résultats ne sont pas mentionnés dans cette thèse.

Tous les agents thérapeutiques présentés dans cette thèse possèdent une structure stéroïdienne. La structure générale d'un stéroïde est constituée de quatre cycles dont trois

de 6 carbones (A à C) et un de 5 carbones (D). La position de chaque carbone est numérotée par convention et lorsqu'un groupement est orienté au dessus du plan, celui-ci est en position β, et s'il est orienté dans le sens opposé, il est en position α. De plus, afin d'alléger la représentation des structures, la position des hydrogènes n'est pas représentée lorsque la jonction des deux cycles est trans.

Structure générale des C19-stéroïdes





Les références mentionnées dans l'introduction et la conclusion générale sont énumérées à la fin de cette thèse, alors que celles citées dans les manuscrits sont répertoriées à la fin de chacun de ceux-ci.

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Liste des abréviations et symboles

Å	Angström
ADN	acide désoxyribonucléique
APCI	«atmospheric pressure chemical ionisation»
APL	leucémie promyélocytique aiguë
AR	récepteur des androgènes
ARE	élément de réponse aux androgènes
Arg	arginine
ARN	acide ribonucléique
ATRA	acide rétinoïque totalement trans
^{13}C NMR	résonance magnétique nucléaire du carbone 13
°C	degré celsius
CD	«clusters» de différentiation
Cdk	protéines kinases dépendantes des cyclines
CFC	chlorofluorocarbone
cm^{-1}	réciproque du centimètre
m-CPBA	acide méta-chloroperbenzoique
CTL	contrôle
d	doublet
DBD	le domaine de liaison à l'ADN
DBMP	2,6-di-tert-butyl-4-méthylpyridine
dd	doublet de doublet
DHEA	déhydroépiandrostérone
DHEA-S	déhydroépiandrostérone sulfate
DHT	dihydrotestostérone
DIPEA	diisopropylethylamine
DMAP	diméthylaminopyridine
DMF	diméthylformamide
Δ^4-dione	4-androstène-3,17-dione
DOX	doxorubicine
epi-ADT	épi-androstérone
EPO	érythropoïétine
eq	équivalent
FAB	franco-américaine-britannique
FBS	«foetal bovine serum»
FDA	«Food and Drug Administration»
FITC	fluorescéine isothiocyanate
Fmoc	9-fluorenylméthoxycarbonyle
Fmoc-OSu	N-(9-fluorenylméthoxycarbonyle)-succinimide
FSH	hormone folliculo-stimulante
G-CSF	facteur stimulant le développement des granulocytes
Gly	glycine
h	heure
^{1}H NMR	résonance magnétique nucléaire du proton

H12	helice 12
HBTU	*O*-benzotriazol-1-yl-N,N,N',N'-tetraméthyluronium hexafluorophosphate
HOBt	*N*-hydroxybenzotriazole
HPLC	chromatographie liquide à haute performance
HY	2β-(4'-méthylpipérazino)-5α-androstane-3α,17β-diol
IC_{50}	concentration de produits nécessaire pour inhiber de 50 % la croissance cellulaire
IFN-α	interféron-α
IL-2	interleukine-2
IR	infrarouge
J	constante de couplage
LBD	domaine de liaison du ligand
LBP	«ligand-binding pocket»
LDA	lithium diisopropylamine
Leu	leucine
LH	hormone lutéinisante
LHRH	«luteinizing hormone-relasing hormone»
LLA	leucémie lymphoïde aiguë
LLC	leucémie lymphoïde chronique
LMA	leucémie myéloïde aiguë
LMC	leucémie myéloïde chronique
LRMS	spectre de masse basse résolution
NK	«natural killer»
nM	nanomolaire
NMO	4-méthylmorpholine-N-oxide
NMR	résonance magnétique nucléaire
M	molaire
m	multiplet
2-ME	2-méthoxyestradiol
MEM	«Eagle's minimal essential medium»
mg	milligramme
MHz	mégahertz
min	minute
ml	millilitre
MTS	3-(4,5-diméthylthiazol-2-yl)-5-(3-carboxyméthoxyphényl)2-(4-sulfophényl)-2H-tétrazolium
m/z	masse/charge
Phase G_1	intervalle de croissance
Phase S	intervalle de synthèse
Phase G_2	intervalle de croissance et de réorganisation
Phase M	intervalle de mitose
Phe	phénylalanine
Pro	proline
PSA	antigène spécifique de la prostate
PS-DES resin	polystyrene-diéthylsilyle
p-TSA	acide *para*-toluènesulfonique
PyBOP	benzotriazol-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate

PyBrOP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
RBA	«relative binding affinity»
RPE	R-phycoérythrine
rt	température de la pièce
s	singulet
SAR	«structure-activity relationships»
SARMs	«selective androgen receptor modulators»
S_N	substitution nucléophilique
SNC	système nerveux central
SPOS	«solid-phase organic synthesis»
t	triplet
TBDMS	*tert*-butyldiméthylsilyle
TFA	acide trifluoroacétique
THF	tétrahydrofuranne
TLC	chromatographie sur couche mince
TMSCl	chlorotriméthylsilane
TNF-α	facteur nécrosant des tumeurs-α
TPAP	tetrapropylammonium perruthénate
TPO	trombopoïétine
μM	micromolaire
ν	fréquence en Hz
v	volume
VD3	1,25-dihydroxyvitamine D3
VIH	virus de l'immunodéficience humaine
δ	déplacement chimique en ppm

Introduction

1 Le cancer

1.1 Introduction générale

Le cancer existe depuis toujours, aussi bien chez les animaux que chez les plantes. Un million d'année avant l'ère chrétienne, on a trouvé des tumeurs osseuses sur les squelettes d'animaux préhistoriques. Notre corps contient 50 000 à 100 000 milliards de cellules. Il est donc normal que parfois une cellule échappe aux mécanismes de régulation et devienne cancéreuse. Habituellement, notre organisme tue ces cellules devenues étrangères, par contre si notre système de défense est mis en échec, un cancer peut se développer pouvant mettre 4 à 10 ans pour arriver au stade clinique.

Un homme sur deux et une femme sur trois développeront un cancer au cours de leur vie. Cette maladie est la deuxième cause de mortalité après les maladies cardio-vasculaires. En 2006, on estimait qu'il y aurait 153 100 nouveaux cas de cancer au Canada et que 70 400 personnes allaient en mourir.[1] Environ 10 millions de personnes se voient diagnostiquer un cancer chaque année dans le monde, plus de 6 millions décèdent chaque année des suites de cette maladie et plus de 22 millions de personnes sont actuellement atteintes d'un cancer dans le monde. Malheureusement, on estime que le nombre de nouveaux cas va augmenter de 50 % dans les 20 prochaines années et atteindra 15 millions en 2020. Tous les pays sont touchés par le cancer, mais à des degrés différents. Les pays riches et industrialisés sont affectés davantage par cette maladie en raison de tumeurs associées au tabagisme et au mode de vie occidental qui peuvent causer des cancers du poumon, du côlon-rectum, du sein et de la prostate. Dans les pays en développement, plusieurs cancers découlent d'infections chroniques causant ainsi des tumeurs du foie, du col utérin et de l'estomac.[2] Les hommes semblent davantage touchés que les femmes par les cancers du poumon, de l'estomac, de l'œsophage et de la vessie. Ces écarts de distribution sont souvent causés par la différence d'exposition à certains agents déclencheurs plutôt qu'au sexe.

Une tumeur peut être soit de type bénin ou de type malin. Chez une tumeur bénigne, les cellules ressemblent à des cellules normales et peuvent fonctionner comme des cellules normales. Les tumeurs bénignes ne causent pas de problème médical important jusqu'à ce que leur taille interfère avec le fonctionnement normal d'un organe ou bien lorsqu'ils sécrètent des substances biologiques actives comme des hormones. Les tumeurs malignes se distinguent des tumeurs bénignes par leur capacité à envahir et à se disperser dans l'organisme. Ils se composent de cellules dont la croissance et les caractéristiques morphologiques sont nettement différentes des cellules normales. Les critères de malignité comprennent l'augmentation de la prolifération cellulaire, la disparition de la différenciation, l'infiltration lymphatique, une croissance infiltrante et la métastase à d'autres organes.

Le développement d'un cancer est un processus de plusieurs étapes, impliquant un grand nombre d'évènements génétiques (Figure 1). Tout d'abord, il y a l'initiation où une lésion à l'ADN de la cellule est induite par un agent cancérigène. Il est possible que l'organisme répare cette lésion et bloque ainsi le développement du cancer ou que cette cellule meurt par apoptose. Si la lésion n'est pas réparée, la cellule va se diviser et ainsi entraîner une modification irréversible de son potentiel de croissance. Finalement, la progression représente les nombreux cycles de réplication cellulaire entraînant le passage graduel d'une cellule modifiée vers une croissance autonome et cancéreuse. La dissémination finale des cellules malignes aboutissant à de multiples localisations tumorales est le processus métastatique.[3]

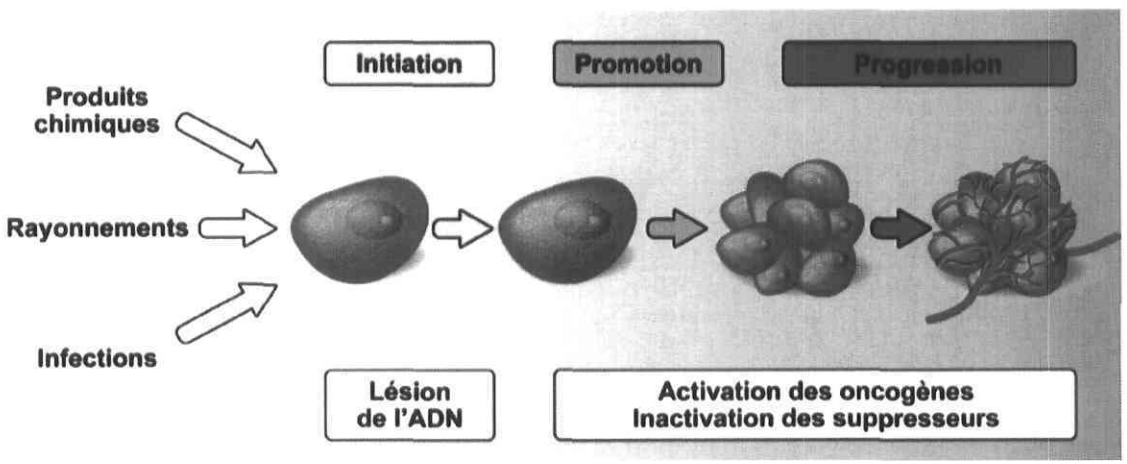


Figure 1. La cancérogenèse est un processus à multiples étapes.

1.2 Les facteurs de risque

Le cancer peut être causé par plusieurs facteurs qui ont été mis en évidence par l'étude de l'évolution de cette maladie dans les populations humaines, ainsi que de nombreuses expérimentations chez les animaux de laboratoire traités par plusieurs agents cancérigènes. Voici quelques exemples de substances et de facteurs pouvant initier le cancer.

Le tabac

Le tabagisme est la principale cause connue de décès lié au cancer dans le monde. La consommation du tabac cause, en plus du cancer du poumon, des tumeurs du larynx, du pancréas, du rein et de la vessie. La fumée du tabac contient un nombre élevé de cancérigènes chimiques. La fumée principale contient environ 4000 produits chimiques. Le goudron, son principal constituant, est formé majoritairement à partir de nicotine et d'hydrocarbures aromatiques polycycliques totalisant 3500 composés.[4] D'autres produits chimiques très cancérigènes comme le benzène, les amines aromatiques et les métaux lourds, sont aussi présents dans la fumée de tabac. Le cancer provoqué suite à l'usage du tabac ne peut être assigné à aucun des produits chimiques en particulier de la cigarette, mais est plutôt attribuable à l'ensemble de ses constituants.[5] La majorité des cancérigènes chimiques présents dans la fumée de cigarette requiert une activation métabolique afin d'entraîner leur effet. On retrouve les enzymes nécessaires à cette activation dans les poumons et dans d'autres organes cibles.[6] Le risque de chaque individu de développer un cancer peut varier avec l'activité et les niveaux de certaines enzymes telles que la glutathion-*S*-tranférase, le cytochrome P450 et les *N*-acétyl-tranférase. Suite à leur activation par ces enzymes, les formes réactives des hydrocarbures aromatiques polycycliques, nitrosamines et amines aromatiques sont générées et se lient par covalence sur l'ADN dans les différents tissus.[7] Les mutations mises en évidence chez les fumeurs sont provoquées, du moins en partie, à l'erreur de lecture causée par la liaison de certains hydrocarbures aromatiques polycycliques à l'ADN.[8]

L'alcool

Quoi qu'une faible consommation d'alcool semble exercer une protection contre les maladies cardiovasculaires, il a été clairement établi qu'une forte consommation peut entraîner le cancer de la cavité buccale, du pharynx, du larynx, de l'œsophage et du foie.[9] De plus cette consommation augmente le risque du cancer du sein et du cancer colorectal.[10-11] Comme l'éthanol en lui-même ne semble pas être cancérigène pour l'animal de laboratoire, d'autres hypothèses ont été envisagées. La plus importante est que l'acétaldéhyde, le principal métabolite de l'éthanol, serait cancérigène comme l'ont démontré certaines études *in vivo* et cliniques.[12] La consommation d'alcool combinée au tabagisme présente une augmentation du risque des cancers de la cavité buccale, du pharynx, du larynx et de l'œsophage. Il est possible que l'alcool joue un rôle de dissolvant favorisant ainsi l'absorption des cancérigènes contenus dans la fumée du tabac.[2] En conclusion, l'effet négatif de l'alcool sur la prédisposition au cancer dépend principalement, de son niveau de consommation.

Expositions professionnelles

Entre 1950 et 1975, de nombreuses études ont clairement établi un lien entre le risque élevé de cancer et certains environnements de travail.[13] Par contre, depuis un quart de siècle, peu de nouveaux cancérigènes professionnels ont été identifiés parce que les agents cancérigènes identifiés sont remplacés par d'autres agents, ou bien il y a eu l'introduction de nouveaux procédés ou matériaux industriels. Plusieurs professions et certains composés chimiques présents dans le milieu de travail sont reliés à une augmentation du risque de cancer. Comme par exemple les amines aromatiques, le benzène, l'amiante, les métaux, la poussière de bois et la peinture. On estime à environ 5 % la proportion de cancers attribuables aux expositions professionnelles les plus communément acceptées dans les pays développés.[14-15] Cependant, cette proportion peut atteindre 20 % chez les personnes réellement exposées aux agents cancérigènes comme les ouvriers travaillant dans les mines et certaines industries. De plus, certaines expositions peuvent avoir des répercutions à très long terme pouvant même aller jusqu'à 30 ans. Le cancer du poumon est le plus courant, mais ceux de la peau, des voies urinaires, de la cavité nasale et de la plèvre sont aussi très fréquents. Malheureusement, depuis quelques dizaines

d'années, la production industrielle s'est grandement déplacée vers des pays en développement tels que l'Amérique du Sud ou l'Asie puisque les normes et les exigences sanitaires et de sécurité y sont parfois moins sévères. Donc, l'industrialisation mondiale à comme conséquence d'augmenter les expositions professionnelles à des substances cancérigènes dans ces pays suite au transfert des industries à risque.[16]

La pollution environnementale

La pollution environnementale est causée par un ensemble spécifique de facteurs environnementaux cancérigènes, à savoir les polluants de l'air, de l'eau et du sol. Ces facteurs sont reliés par une caractéristique commune, c'est-à-dire l'absence de contrôle des individus sur leur niveau d'exposition. Parmi les polluants environnementaux, on retrouve les agents toxiques présents dans l'air urbain, les polluants de l'air intérieur des maisons, les sous-produits chlorés et tous les contaminants présents dans l'eau potable. Le lieu de résidence, rural ou urbain, ainsi que le contact avec des sources importantes d'émissions industrielles sont des facteurs influençant le risque de cancer. On évalue que la pollution de l'air, de l'eau et des sols serait responsable de 1 à 4 % de tous les cas de cancer.[15,17] La pollution de l'air ambiant peut causer certains cancers, donc celui du poumon. Depuis environ 20 ans, les niveaux d'émission de polluants atmosphériques industriels traditionnels, comme le dioxyde de soufre et les particules ont diminué. Malheureusement les gaz d'échappements des véhicules est un problème qui ne cesse d'augmenter. Les produits de l'essence sont constitués de composés organiques volatiles (benzène, toluène…), d'oxydes d'azote (NOx) et de fines particules (carbone, matériaux organiques absorbés, et des traces de composés métalliques) qui sont tous cancérigènes. Plusieurs études ont examiné la vie en zone urbaine, où l'air est considéré comme plus pollué par rapport à la vie en zone rurale, comme facteur de risque de cancer du poumon. Dans la majorité des cas, les taux de ce cancer étaient plus élevés dans les zones urbaines, et dans certains cas, ces taux étaient reliés aux niveaux de polluants spécifiques tels que les métaux et les particules.[18] De plus, la pollution de l'air par les chlorofluorocarbones (CFC) serait indirectement responsable de l'augmentation des cancers de la peau dans le monde. Les CFC sont transportés par les vents dans la stratosphère, où l'action d'un fort rayonnement solaire libère des atomes de chlore et de brome qui détruisent les molécules d'ozone en

réagissant avec elles. On estime donc que la dégradation de la couche d'ozone serait responsable de l'augmentation des rayonnements UVB.[19] La pollution de l'air intérieur peut avoir quant à elle des conséquences très importantes sur la santé. Comme par exemple, l'exposition chronique d'un non fumeur à la fumée de tabac dans l'air ambiant peut élever de 20 à 30 % le taux de mortalité causé par le cancer du poumon. Finalement, la présence de polluants dans l'eau potable ne représente pas un risque très important. Par contre, l'utilisation d'oxydants comme le chlore, l'hypochlorite, la chloramine et l'ozone pour la désinfecter peut entrainer la présence de niveaux élevés d'agents cancérigènes comme l'arsenic et des sous-produits chlorés qui ne sont toutefois pas négligeables.[20]

Les contaminants alimentaires

La nourriture peut être contaminée de différentes façons et ainsi causer des cancers des voies digestives ainsi que d'autres organes. Les contaminants peuvent être d'origine naturelle, comme par exemple des mycotoxines cancérigènes produites par certains champignons. Cette contamination se retrouve principalement en Afrique et en Asie, car en Europe et en Amérique du Nord elle est très suivie et réglementée.[21] De plus, la métabolisation d'un certain glucoside présent dans la fougère mangée par des vaches au Japon, au Costa Rica et au Royaume-Uni entraine des adduits d'alkylation cancérigènes. Ces vaches développent des tumeurs qui sont attribuables à ces agents et leur lait provoque le cancer chez des animaux de laboratoire.[22] La contamination alimentaire peut aussi être causée par les produits chimiques industriels. Certains de ces produits comme les pesticides ne sont pas biodégradables; ils demeurent dans l'environnement et s'accumulent tout le long de la chaîne alimentaire. Plusieurs de ces contaminants augmentent les risques de cancers du foie, du pancréas, du sein, de lymphome et de leucémie chez l'homme. Finalement, certains produits chimiques générés lors de la préparation alimentaire sont considérés comme cancérigènes. Les plus fréquents agiraient selon un mécanisme génotoxique.[23] Les amines hétérocycliques, pouvant se former lors de la cuisson de la viande et du poisson à haute température,[24] les hydrocarbures aromatiques polycycliques, générés dans la viande lorsque celle-ci est cuite sur une flamme, et les composés *N*-nitrosés, pouvant se former à l'intérieur des aliments contenant des nitrates et des nitrites ajoutés

(poisson et viande salés ou fumés directement au feu) ou ayant été en contact avec des engrais, sont tous différents produits cancérigènes formés lors de la préparation alimentaire.

La prise de médicaments

Il existe de nos jours une panoplie de médicaments essentiels pour le traitement de nombreuses pathologies plus ou moins gravent. Cependant, une faible quantité de ces médicaments expriment un effet secondaire cancérigène, principalement lorsque ceux-ci doivent être administrés à des doses élevées ou sur une longue période. Cet effet secondaire est toutefois compensé lorsque ces produits sont administrés à des patients atteints de maladies mortelles ou d'un cancer métastasé. Parmi ces médicaments cancérigènes, on compte certains antinéoplasiques, des hormones et des agonistes comme le tamoxifène, ainsi que des immunosuppresseurs. Les médecins et oncologues investissent beaucoup de temps à établir la meilleure posologie de ces médicaments afin de diminuer leurs effets secondaires tout en optimisant leur efficacité. Les dernières découvertes dans le domaine pharmaceutique n'ont toutefois pas été marquées par l'implication de médicaments dans le développement de cancers. Cela est probablement causé en partie par les nombreuses exigences envers les tests précliniques et cliniques des médicaments, puisqu'un médicament ayant une activité cancérigène lors de ces tests à peu de chance de se rendre en phase finale de développement et d'être commercialisé.

Les rayonnements

Des ondes électromagnétiques provenant de sources naturelles et artificielles sont générées quotidiennement. Ces ondes sont caractérisées par leur longueur d'ondes, leur fréquence ou leur énergie. Leur action sur le corps humain se produit au niveau cellulaire et les conséquences sont déterminées par l'intensité, l'énergie de ces rayonnements ainsi que par la quantité d'énergie absorbée par le corps. L'exposition aux rayonnements ionisants est très fréquente et inévitable. Il peut provenir soit de la radioactivité présente dans le sol, des rayons X médicaux (radiographie), des produits radiopharmaceutiques (radiothérapie) ou des rayons cosmiques. L'exposition à ces rayonnements peut entraîner une grande variété de néoplasme comme la leucémie, le cancer du sein et le cancer de la thyroïde.[25] Le rayonnement solaire constitue la principale source d'exposition aux ultraviolets et provoque

de nombreux types de cancers de la peau. L'exposition cutanée au rayonnement solaire altère l'ADN provoquant des lésions cellulaires qui peuvent aboutir à un cancer. L'incidence du cancer cutané augmente rapidement dans les populations à peau claire.[26] L'incidence de cette maladie a par exemple doublé au Canada au cours de ces 25 dernières années. Le centre international de Recherche sur le Cancer a estimé qu'au moins 85 % des mélanomes étaient causés par l'exposition au rayonnement solaire. Finalement, les champs électromagnétiques générés par l'ensemble des équipements électriques comme la télévision, la radio, les ordinateurs et les téléphones cellulaires sont possiblement associés à une augmentation du risque de leucémie chez l'enfant.[27] Cependant, les résultats des études chez l'adulte sont beaucoup moins clairs. De plus, l'effet des radiofréquences est encore moins clair. Les résultats expérimentaux sont limités, mais laissent croire que les radiofréquences ne peuvent provoquer de mutations de l'ADN.[28-29]

Infections chroniques

Depuis plus d'un siècle, il est connu que le cancer peut être provoqué par des agents infectieux.[30-31] En effet, ils sont responsables de 18 % des cas de cancer dans le monde, principalement dans les pays en développement. Les stratégies utilisées pour la prévention sont la vaccination et le dépistage.

L'alimentation

L'alimentation est un facteur important dans l'incidence du cancer. On estime qu'environ 30 % des cancers y sont probablement liés. Les aliments salés, fumés ou conservés dans la saumure sont associés à une augmentation du risque de cancer gastrique. De plus, la consommation de 80 grammes par jour de viande rouge (bœuf, agneau et porc) et la viande transformée (jambon, salami, bacon et autre charcuterie) peut augmenter de 25 % et 67 % respectivement, les risques de cancer colorectal.[32] Quelques hypothèses ont été soulevées pour expliquer comment la consommation de viande peut augmenter le risque de cancer. Tout d'abord, elle influencerait la production et le mécanisme d'action des sels et des acides biliaires par la flore intestinale.[33] De plus, certains composants peuvent se former dans la viande lors de la cuisson (amines hétérocycliques ou hydrocarbures aromatiques polycycliques) suite à la transformation de viande conservée (nitrates et

nitrites) ou du métabolisme endointestinal. Une alimentation riche en graisses saturées (viande grasse, beurre, fromage) augmente le risque des cancers du sein, du côlon et du pancréas. Ces graisses augmentent la concentration d'acides sécrétés par la bile (qui aide à digérer les graisses) et modifient la flore bactérienne du côlon. Lorsque ces acides sont présents en grande quantité, ils ont un effet toxique pour les cellules de l'intestin et se transforment en substances cancérigènes. Bien que les additifs alimentaires soient des produits chimiques, la proportion des cancers qui leur serait attribuable est très faible.[15] La plupart des données toxicologiques disponibles pour ces produits sont toutefois incomplètes. L'activité physique, la surveillance du poids pour éviter l'obésité et un apport quotidien fréquent de fruits et légumes frais réduisent le risque de certains cancers dont celui de la cavité buccale, du poumon et du col utérin.

Immunodépression

L'immunodépression est la diminution de la capacité du système immunitaire à répondre efficacement aux antigènes étrangers. Elle peut être induite par des médicaments, certains produits chimiques, des rayonnements ionisants et par différentes infections virales et parasitaires. Lorsque cette immunodépression est persistante, elle présente un risque de cancer. De plus, les médicaments utilisés pour diminuer le risque de rejet lors de greffes d'organes, augmentent l'incidence de lymphomes malins. Plusieurs agents cytotoxiques utilisés comme agents chimiothérapeutiques peuvent déclencher l'immunodépression et ainsi contribuer au développement d'un deuxième cancer, plus particulièrement chez les enfants. Finalement, les agents infectieux causant une immunodépression grave (VIH) augmente l'incidence de plusieurs tumeurs dont le lymphome non hodgkinien.[34]

Prédisposition génétique

Environ 5 % des cancers sont attribuables à des altérations génétiques héréditaires. Cette altération peut être présente chez un des deux parents ou a pu survenir dans une cellule germinale (ovule ou spermatozoïde) avant la fécondation et ainsi être transmise à la prochaine génération. Ces altérations peuvent éventuellement causer un cancer, principalement lorsque les gènes suppresseurs de tumeurs ou réparateurs de l'ADN sont impliqués. Comme par exemple, une femme porteuse d'un gène *BRCA1* muté possède un

risque d'environ 70 % de développer un cancer du sein ou de l'ovaire au cours de sa vie.[35] Heureusement, les mutations des gènes de prédisposition au cancer sont peu courantes. L'identification d'une de ces mutations permet d'utiliser certaines mesures préventives et un suivi médical.

Les hormones

Les hormones sexuelles, androgènes, estrogènes et progestérones, ont un rôle important dans le développement des cancers, particulièrement les cancers de l'endomètre, de l'ovaire, du sein et de la prostate. L'apparition de la ménarche à un âge précoce et une ménopause tardive augmente les risques du cancer du sein. Tandis qu'une grossesse à un jeune âge diminue les risques des cancers de l'endomètre, de l'ovaire et du sein.[36] L'utilisation de contraceptifs oraux est responsable d'une légère augmentation du risque de cancer du sein, mais exercerait un effet protecteur contre les cancers de l'ovaire et de l'endomètre, tandis que l'hormonothérapie augmenterait les risques de ces trois cancers.[37] Finalement, le risque de cancer de la prostate peut augmenter chez les hommes avec de fortes concentrations intra-prostatiques de dihydrotestostérone (DHT). La DHT est formée à partir de la testostérone dans la prostate. Elle se lie au récepteur des androgènes et l'active avec une affinité quatre fois supérieure à celle de la testostérone.[38-39] Un déterminant de la formation intra-prostatique de la dihydrotestostérone peut être la variation dans l'activité de la 5α-réductase intra-prostatique de type II qui catalyse la conversion de la testostérone en DHT.

1.3 L'action des stéroïdes sexuels

Certain cancers sont hormono-dépendants, c.-à-d. que ces cancers ont besoin de certains stéroïdes sexuels (estrogène ou androgène) pour leur développement, leur croissance et leur fonctionnement. Les cancers de l'utérus, de l'ovaire et des testicules sont des cancers hormono-dépendants, mais les plus communs sont les cancers du sein et de la prostate. 70 % des cancers du sein sont estrogéno-dépendants et plus de 80 % des cancers de la prostate sont androgéno-dépendants. L'action de ces différentes hormones s'effectue

via leur récepteur respectif. La deuxième section de cette introduction traitera d'un type de cancer androgéno-dépendant, le cancer de la prostate, et la troisième section traitera d'un type de cancer non-hormono-dépendant, la leucémie.

2 Le cancer de la prostate

2.1 Introduction et épidémiologie

De la grosseur d'une noix, la prostate est une glande du système reproducteur masculin située sous la vessie, en avant du rectum. Comme elle entoure l'urètre (le canal qui part de la vessie), son inflammation nuit à la miction. Pour fonctionner, la prostate a besoin d'hormones sexuelles, les androgènes, qui sont produites dans les testicules, principalement, et par les glandes surrénales. Le cancer de la prostate est le résultat d'une croissance non maîtrisée de cellules devenues malignes. Par ailleurs, parmi tous les cancers hormono-dépendants, le cancer de la prostate est celui qui est le plus sensible aux hormones. Au début, il ne provoque aucun symptôme. Puis, le patient peut avoir certains problèmes qui peuvent aussi être causés par une autre maladie de la prostate comme l'hypertrophie bénigne. Ces symptômes sont un fréquent besoin d'uriner (principalement la nuit), l'incapacité ou de la difficulté à commencer à uriner, de la difficulté à obtenir une érection, de la douleur pendant l'éjaculation ainsi que des douleurs dans le bas du dos et les hanches. Il est le cancer le plus fréquemment diagnostiqué chez l'homme et est la seconde cause de décès par cancer après celui du poumon. En effet, un homme sur huit sera diagnostiqué avec un cancer de la prostate au cours de sa vie et on estime que 4200[1] et 27 350[40] hommes au Canada et aux États-Unis, respectivement vont en mourir en 2006. Depuis 1994, le taux de mortalité associé au cancer de la prostate a diminué. Cette diminution coïncide avec l'utilisation du dosage de l'antigène spécifique de la prostate (PSA) lors du dépistage.[41]

2.2 Les facteurs de risque

Plusieurs facteurs peuvent augmenter les possibilités de développer un cancer de la prostate.[42] L'âge est le facteur de risque le plus fortement associé au cancer de la prostate puisque plus de 80 % des cancers de la prostate sont diagnostiqués chez des hommes de plus de 65 ans. Les antécédents familiaux ont eux aussi beaucoup d'importance puisque le

fait d'avoir un père ou un frère atteint de ce cancer double le risque. On estime que 10 % des cas de cancer de la prostate peuvent être héréditaires. Évidemment, un déséquilibre hormonal, en particulier des androgènes, aura aussi pour effet d'augmenter ce risque. De plus, un apport calorique élevé et un faible niveau d'activité physique augmentent les probabilités de développer un cancer de la prostate. La situation géographique peut aussi influencer ce risque puisque ce type de cancer est rare en Chine, en Inde et au Japon. Finalement, les hommes de race noire sont plus susceptibles d'être atteints du cancer de la prostate.[2]

2.3 La détection

Il existe plusieurs méthodes de détection du cancer de la prostate. Le toucher rectal est la manière la plus simple de détecter des anomalies anatomiques de la prostate car une asymétrie avec induction indique un cancer de la prostate. Depuis les années 80, le dosage du PSA est le second outil de dépistage du cancer de la prostate. Cette substance est fabriquée par la glande prostatique et est retrouvée à l'état normal en faible quantité dans le sang. Un des rôles reconnus de cet antigène est de maintenir le sperme à l'état liquide. Dans le cancer de la prostate, la désorganisation de l'architecture du tissu prostatique est à l'origine d'un passage plus important de PSA dans la circulation générale. Son taux sanguin est donc plus élevé. Par contre, l'inflammation, l'adénome de la prostate qui est une tumeur totalement bénigne, l'éjaculation ou une intervention sur la prostate peuvent également augmenter le PSA, mais habituellement de façon temporaire. Lorsque la concentration de PSA est élevée, le risque d'un cancer de la prostate augmente aussi. Un PSA élevé ne confirme pas nécessairement la présence d'un cancer de la prostate, mais il indique une anomalie de la prostate, qui peut être bénigne ou maligne. Le dosage du PSA, associé à un toucher rectal, contribue à établir le diagnostic de cancer de la prostate. Le PSA est actuellement le test le plus efficace pour la détection précoce où il n'y a pas encore de signes cliniques. Le dosage de cet antigène sert également à surveiller l'efficacité du traitement.

Chez une personne saine, sans cancer de la prostate, la limite normale de PSA est de 4 ng/ml.[43] Puisque le PSA peut fluctuer au cours du temps, il est donc important de faire un second dosage afin de valider une élévation de PSA. Lorsque ce dosage confirme l'élévation, il est possible d'effectuer le dosage du PSA libre pour améliorer la performance du diagnostic du PSA. Le PSA libre est une fraction du PSA (PSA= PSA libre + PSA lié) et plus le taux de PSA libre est faible, plus le risque de cancer est élevé. Cependant, ce dosage seul ne permet pas d'affirmer le diagnostic de cancer de la prostate, en particulier lorsque la concentration est inférieure à 10 ng/ml. À ce moment on peut effectuer une échographie. Cette technique utilise des ultrasons pour produire une image de la prostate, ces ultrasons sont émis par une sonde introduite dans l'anus. On peut ainsi guider très précisément une aiguille pour faire des prélèvements à un endroit déterminé de la prostate. Puisque les cellules cancéreuses sont visibles au microscope, l'analyse d'une biopsie prostatique permettra donc d'établir le diagnostic exact et ainsi d'élaborer une stratégie de traitement adéquate. Habituellement, cette biopsie est effectuée lorsque le toucher rectal et/ou le dosage du PSA ont permis de détecter une anomalie de la prostate.

Par la suite, d'autres analyses seront nécessaires afin de vérifier l'étendu du cancer à d'autres organes. Ces analyses sont le scanner, la résonnance magnétique et la scintigraphie osseuse.

2.4 Les différents stades

Le stade du cancer de la prostate marque le degré d'extension du cancer, aux avoisinants et aux autres organes. Reflétant le pronostic du cancer, il permet d'aider les urologues dans leur pratique afin de déterminer le traitement approprié. Les stades du cancer de la prostate sont habituellement définis par le système «TNM» qui décrit l'extension de la tumeur primitive (T), l'absence ou la présence d'invasion lymphatique (N), et l'absence ou la présence de métastase à distance (M).

Stade T

- T1 correspond à une tumeur non perçue au toucher rectal, mais confirmée suite à une valeur de PSA élevée et une biopsie. T1a et T1b correspondent à des cancers découverts incidemment lors de l'examen du tissu prostatique obtenu au cours d'une intervention pour un adénome bénin de la prostate. T1c correspond à des cancers découverts par biopsie faite à la suite d'une élévation suspecte du taux de PSA.
- T2 correspond à un cancer palpable au toucher rectal et qui semble localisé à la glande. T2a touche un lobe, tandis que T2b touche les deux lobes.
- T3 correspond à un cancer qui s'étend en dehors de la prostate et/ou aux vésicules séminales.
- T4 correspond à un cancer qui envahit les organes adjacents à la prostate (vessie, rectum, muscle releveur).

Stade N

- N0 correspond à l'absence d'envahissement lymphatique, et N1 à l'envahissement d'un ou de plusieurs ganglions régionaux.

Stade M

- M0 correspond à l'absence de métastases à distance.
- M1 correspond à la présence de métastases à distance. M1a implique des ganglions non régionaux. M1b correspond à la présence de métastases osseuses et M1c implique d'autres métastases.

Le pronostic est très favorable lorsque le cancer est détecté à un stade précoce et lorsqu'il est limité aux tissus prostatiques.

2.5 Les stéroïdes sexuels et leurs précurseurs

L'endocrinologie est l'étude de l'action des hormones tant au niveau moléculaire que cellulaire. La nature chimique des différentes hormones est très variée. Il y a cinq

classes d'hormones stéroïdiennes. Les minéralocorticoïdes et les glucocorticoïdes sont les deux premières classes dont les représentants actifs sont l'aldostérone et le cortisol. La progestérone constitue une classe en elle-même. La transformation de la prégnénolone et de la progestérone à l'aide des différentes enzymes, mène aux androgènes ainsi qu'aux estrogènes, qui sont les deux dernières classes d'hormones stéroïdiennes (Figure 2). Ces trois dernières classes sont appelées les stéroïdes sexuels, car à l'origine on croyait que les gonades (ovaires et testicules) étaient les seules sources de ces stéroïdes; les stéroïdes produits par la glande surrénale n'étaient pas encore étudiés à ce moment.

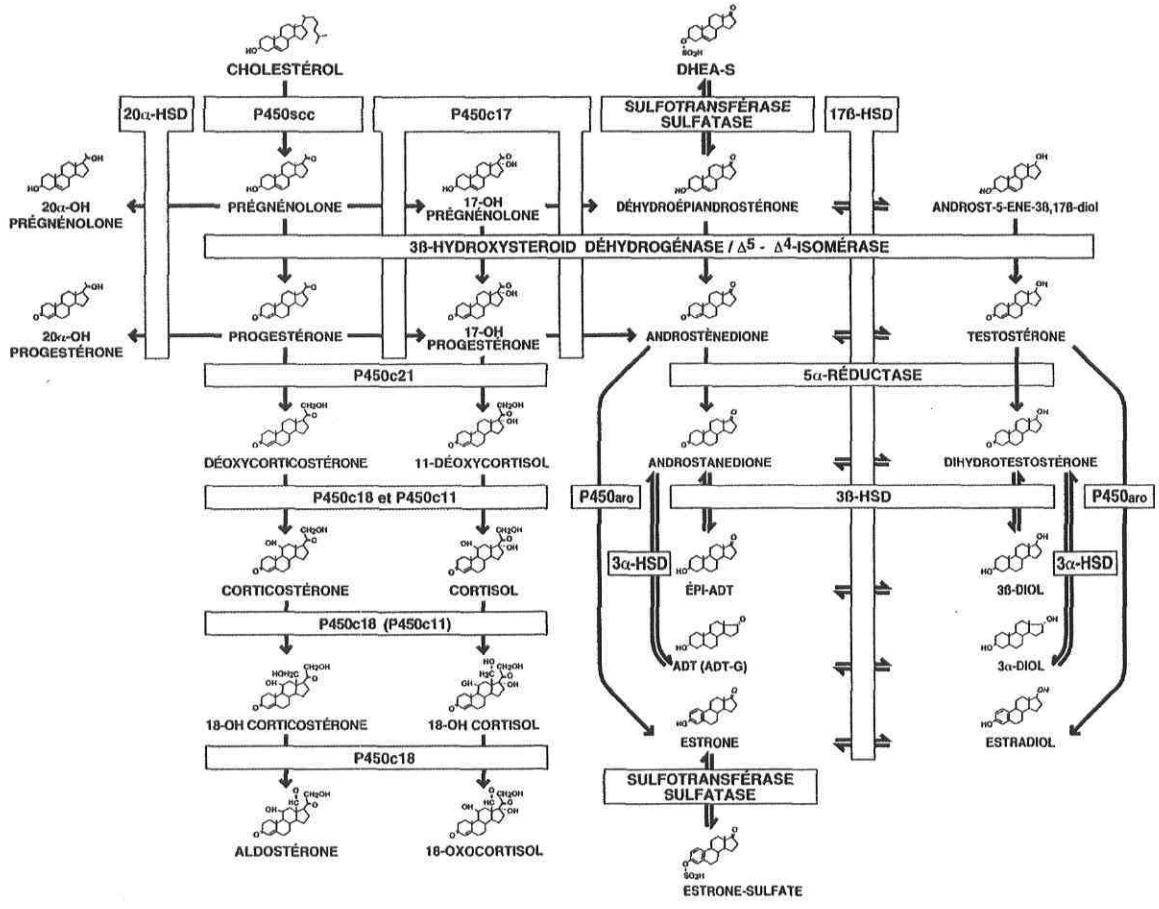


Figure 2. Formation des stéroïdes sexuels à partir de leurs précurseurs chez l'humain (stéroïdogenèse).

Chez l'homme, les androgènes les plus actifs sont la dihydrotestostérone (DHT) et la testostérone. En 1960, une équipe a cependant observé que les niveaux de sulfate de

déhydroépiandrostérone (DHEA-S) en circulation chez l'homme et la femme adultes étaient plus élevés que tout autre stéroïde à l'exception du cholestérol.[44] Quelques années plus tard, en 1968, l'équipe de Baird introduisait le concept de préhormones ou de précurseurs d'hormones actives.[45] Les stéroïdes surrénaliens précurseurs d'androgènes actifs sont la déhydroépiandrostérone (DHEA), le sulfate de déhydroépiandrostérone (DHEA-S) et l'androstènedione (Δ^4-dione). Ces stéroïdes surrénaliens possèdent un peu ou pas d'activité biologique intrinsèque et doivent être transformés pour exercer une action biologique.[46] L'importance de ces précurseurs surrénaliens vient du fait que chez l'homme, on estime que 30 à 50 % des androgènes résultent de la conversion périphérique de ces précurseurs. Des études ont démontré la présence de toutes les enzymes nécessaires à la stéroïdogenèse dans certains tissus périphériques, dont la prostate, ce qui permet de convertir les précurseurs stéroïdiens en androgènes actifs.[47] L'expression des enzymes n'est pas uniforme dans tous les tissus ce qui permet, du moins en partie, de moduler la formation de stéroïdes en fonction des besoins spécifiques de chaque tissu par intracrinologie. En 1991, le Dr Fernand Labrie a décrit l'intracrinologie comme un processus où l'hormone stéroïdienne active exerce son action à l'intérieur même de la cellule qui a participé à sa synthèse, à partir des précurseurs, sans être obligatoirement relâchée dans l'espace extracellulaire.[48] Ce processus est donc un système économique ne requérant que de petites quantités d'hormones pour exercer un effet maximal en très peu de temps puisque, contrairement au système endocrinien classique, l'hormone n'est pas diluée dans 10 à 40 litres de liquide corporel avant d'atteindre l'organe cible.

2.6 Les androgènes dans le cancer de la prostate

La première observation du rôle des hormones mâles dans le cancer de la prostate a été faite par Huggins et ses collaborateurs en 1941.[49-50] Puisque la majorité des cancers de la prostate sont hormono-sensibles, ils ont besoin des androgènes pour leur développement et leur croissance tout comme une prostate normale. La testostérone et la DHT exercent donc leur effet via le récepteur des androgènes (AR). De plus, la DHT est l'androgène le plus puissant et sa liaison avec le AR est plus forte que celle de la testostérone.[51] Le AR est

un récepteur nucléaire constitué de 3 principaux domaines: 1) la région N-terminale, qui comporte les fonctions nécessaires à la transcription, 2) le domaine de liaison du ligand (LBD) qui se trouve dans la région C-terminale et 3) le domaine de liaison à l'ADN (DBD) (Figure 3).[52] La séquence primaire des deux premiers domaines est hautement conservée parmi la famille des récepteurs hormonaux nucléaires. Le AR est activé par la liaison de son ligand[53] et agit alors comme facteur de transcription. Dans sa forme inactive, il est associé avec deux protéines nommées «Heat Shock Protein» et une fois le stéroïde lié au AR, le complexe se dissocie pour former un homodimère. Par la suite, cet homodimère est rapidement transloqué dans le noyau où il interagit avec une région spécifique de l'ADN, l'élément de réponse aux androgènes (ARE). Ce complexe peut alors recruter certains cofacteurs (coactivateurs ou corépresseurs) et ainsi contrôler la transcription de ce gène.[54]

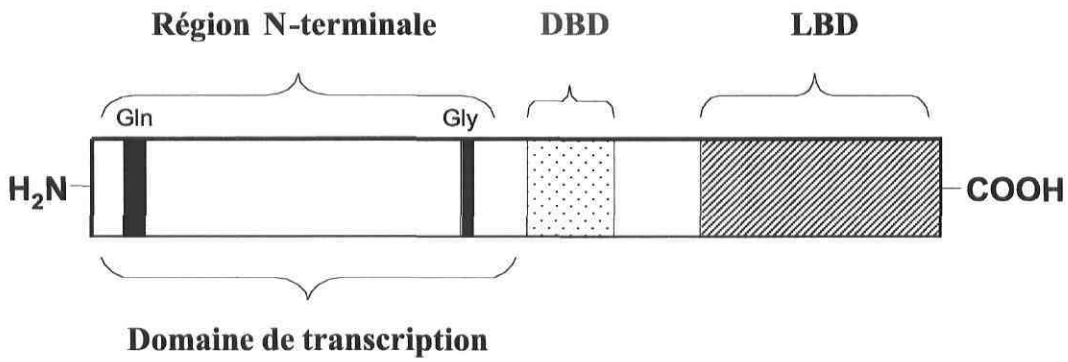


Figure 3. Le récepteur des androgènes.

2.7 Les traitements

2.7.1 Castration chirurgicale et oestrogénothérapie

Pendant longtemps et jusqu'à tout récemment, le blocage de l'action des androgènes testiculaires par la castration chirurgicale ou par l'administration de fortes doses d'estrogènes furent les traitements utilisés pour le cancer de la prostate.[49] L'inhibition de l'activité androgénique nuisible était partielle, puisque l'action de ces traitements était limitée aux androgènes d'origine testiculaire. Malgré cette inhibition partielle, plusieurs

groupes observaient des réponses positives dans 60 à 70 % des patients[55-56] puisque le cancer de la prostate est très sensible aux hormones, cependant ces réponses étaient de courte durée (3-7 mois). De plus, ces deux traitements n'avaient aucun effet sur la survie des patients et comportaient certains effets secondaires. En effet, la castration chirurgicale rencontrait des limitations d'ordre psychologique[57-58] et physique chez la plupart des patients puisque 2-10 % souffraient d'incontinence et environ 70 % d'impuissance. Quant à l'oestrogénothérapie, elle entraînait des complications cardio- et cérébrovasculaires importantes pouvant être fatales.[59-60]

2.7.2 Castration chimique

Il y a plus de 30 ans, découvrant l'importance de l'action de la LHRH pour la synthèse des différents stéroïdes sexuels dans les gonades,[61] plusieurs groupes de recherche ont synthétisés des supers agonistes de la LHRH dans le but de traiter l'infertilité.[62-63] Par contre, l'effet inverse a été observé dans différents tissus androgéno-sensibles du rat puisque des doses élevées de ces agonistes entraînaient une inhibition des fonctions pituitaires et gonadiques (diminution des stéroïdes sexuels).[64] Finalement, il a été rapidement prouvé que ce traitement, appelé castration chimique, pouvait remplacer la castration chirurgicale ainsi que l'oestrogénothérapie.[65] Au début du traitement, les cellules hypophysaires répondent à la stimulation de ces agonistes par une augmentation de la sécrétion de LHRH induisant une production de testostérone par les testicules. Cette réponse peut entraîner dans certains cas une aggravation du cancer, d'où l'importance de débuter le traitement par un antiandrogène afin de bloquer l'action androgénique de la testostérone. Puis, l'administration continue à fortes doses de ces agonistes entraîne un blocage de la sécrétion hypophysaire de LH et de FSH, par désensibilisation des récepteurs hypophysaires. Par la suite, ils conduisent à un arrêt de la sécrétion testiculaire, causant ainsi l'atrophie secondaire de la prostate, réversible à la fin du traitement. Même si l'utilisation d'un agoniste de la LHRH a pu remplacer la castration chirurgicale et l'administration d'estrogènes, elle ne permet toujours pas d'améliorer la survie des patients puisque ce traitement inhibe seulement la formation des androgènes testiculaires, sans

affecter ceux d'origine surrénalienne. Les principaux agonistes de la LHRH utilisés sont le busséréline (Bigonist™), le goséréline (Zoladex™) et le leuproline (Enantone™).

Bien que cette castration médicale entraîne une réduction de l'ordre de 90 à 95 % de la concentration de la testostérone dans la circulation,[65-66] cette diminution est beaucoup moins importante dans la prostate, où la concentration de DHT ne diminue que de 50 à 70 %. La différence entre ces deux concentrations est évidemment causée par l'action des précurseurs d'origines surrénaliennes à l'intérieure de la prostate dont l'action pourrait être bloquée par l'utilisation d'un antiandrogène.

2.7.3 Les antiandrogènes

Puisque l'étape importante dans l'action des androgènes est leur liaison aux ARs, il est évident que l'utilisation d'un antiandrogène, qui bloquera le LBD de ce récepteur, préviendra l'action de la testostérone et de la DHT. Donc, les antiandrogènes sont en compétition avec ces stéroïdes pour lier le site actif du AR (Figure 4).

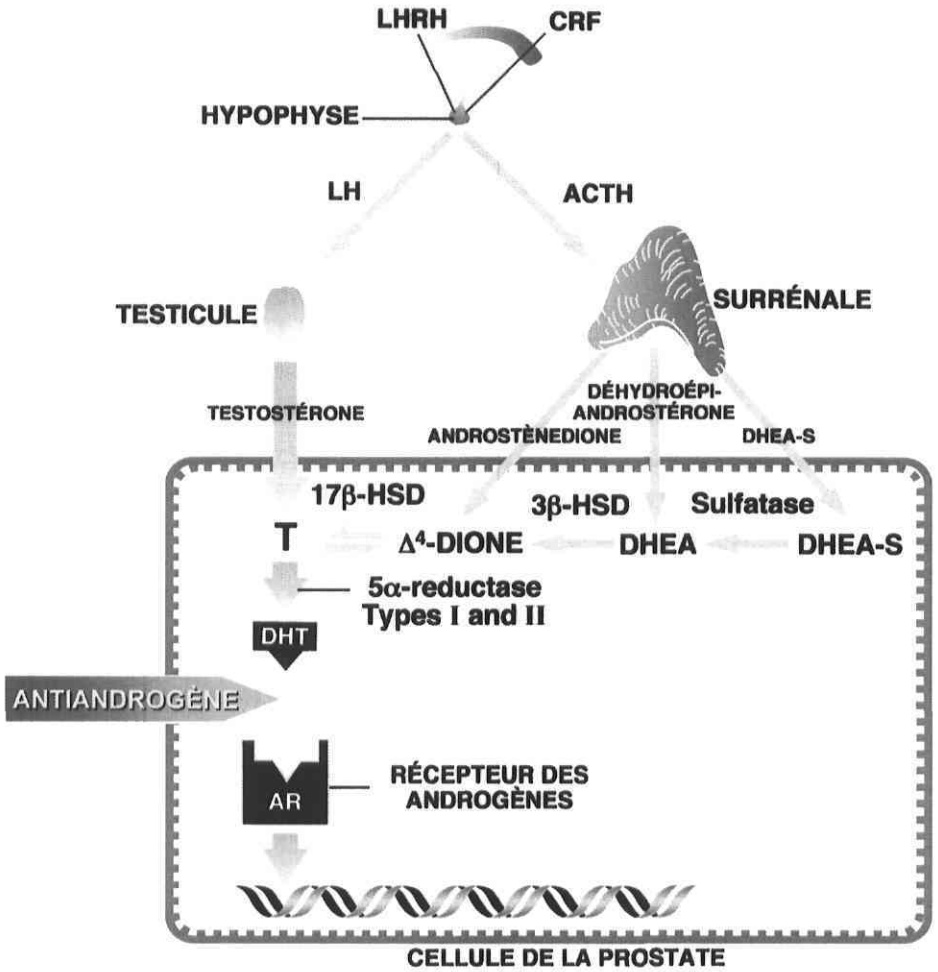


Figure 4. Activité intracrine dans la prostate et action d'un antiandrogène.

Il existe deux classes d'antiandrogènes : les stéroïdiens (les progestatifs) et les non-stéroïdiens (Figure 5). Généralement, les antiandrogènes stéroïdiens ont une bonne affinité pour le récepteur, mais sont parfois agonistes ou peuvent se lier à d'autres récepteurs et ainsi causer de graves problèmes. Habituellement, l'affinité d'un produit pour le AR est comparée à l'affinité d'un ligand synthétique stéroïdien, le R1881 qui possède un RBA (relative binding affinity) fixé à 100 %. Donc, plus un produit possède un RBA élevé (près de 100 %), plus il a une forte affinité avec le AR. L'un des antiandrogènes stéroïdiens le plus connu est le cyprotérone acetate découvert depuis 1963. Ce composé diminue la synthèse des stéroïdes sexuels par un rétrocontrôle sur la sécrétion d'androgènes par les testicules tout en bloquant le LBD. Malgré le fait qu'il induit des problèmes

cardiovasculaires, une perte de libido[67] et que son RBA est assez faible[68] cet antiandrogène est encore utilisé aujourd'hui.

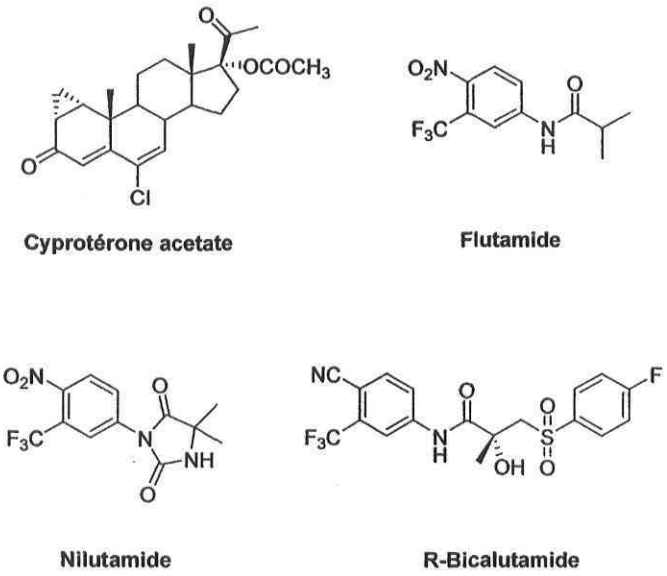


Figure 5. Exemples d'antiandrogènes.

Les antiandrogènes non-stéroïdiens n'ont habituellement aucun effet agoniste (antiandrogènes purs), mais possèdent une très faible affinité avec le récepteur. L'effet toxique le plus important induit par cette famille est une gynécomastie (hypertrophie des seins), parfois douloureuse. Les antiandrogènes non-stéroïdiens les plus utilisés sont le flutamide (Eulexine™), le nilutamide (Anandron™) et le bicalutamide (Casodex™). Le flutamide fût découvert dans les années 70 et au départ était destiné à être utilisé comme bactéricide contre le Staphylococcus aureus. Cependant ces propriétés intéressantes pour inhiber l'effet de la testostérone et du DHT ont fait de lui le premier antiandrogène pur.[69] Le flutamide est une prodrogue et son métabolite actif est l'hydroxyflutamide. Puisque ce métabolite possède une demi-vie très courte (5-6 heures), il doit être administré trois fois par jour (3 x 125 mg) afin de maintenir son niveau thérapeutique dans le sérum.[70] Son affinité pour le récepteur est très faible et il agit sur l'axe hypothalamo-hypophysaire en augmentant les concentrations sériques de LH et évidemment de testostérone. Un produit similaire, le nilutamide, est un herbicide possédant les mêmes propriétés que le flutamide.

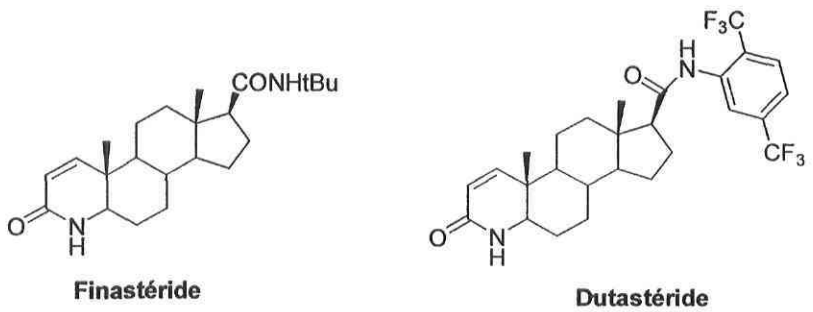
Une dose quotidienne de nilutamide est 300 mg/jour (6 x 50 mg) et ces effets secondaires les plus importants sont une intolérance à l'alcool (19 %) et des problèmes visuels (31 %).[71-72] Finalement, l'antiandrogène non-stéroïdien le plus efficace et le plus utilisé est le bicalutamide dont le RBA est supérieur à l'hydroxyflutamide. L'action de ce composé sur l'axe hypothalamo-hypophysaire est beaucoup moins importante et sa demie-vie est longue (7 jours), ce qui permet d'utiliser seulement une dose journalière de 50 mg. Les effets secondaires du bicalutamide sont moins sérieux que ceux du cyprotérone acetate et des antiandrogènes non-stéroïdiens et, de plus, il maintient la libido ainsi que les capacités physiques.

Habituellement, les antiandrogènes sont utilisés en combinaison avec un agoniste de la LHRH car les rémissions obtenues suite à l'utilisation d'un antiandrogène seul sont de plus courte durée que celles obtenues par la castration physique ou chimique. Cette faible période de rémission est certainement causée par la faible affinité pour le AR des antiandrogènes présentement utilisés ainsi que l'augmentation des taux sériques de gonadotropines et de testostérone. Plusieurs études ont confirmé que l'utilisation du blocage combiné utilisant un antiandrogène pur avec la castration chirurgicale ou chimique prolongeait la vie des patients ayant un cancer avancé de la prostate.[73-74] Au stade métastatique, le seul traitement efficace du cancer de la prostate est le traitement hormonal. De plus, lorsque le cancer est cliniquement localisé, le traitement hormonal a montré un succès surprenant, sous forme d'un contrôle à long terme ou même d'une guérison probable dans 90 % des cas.[75] Il y a donc de bonnes raisons de croire que le traitement hormonal va rapidement prendre une place de plus en plus importante dans le traitement du cancer de la prostate et ce, à tous les stades de la maladie.[76]

2.7.4 Les inhibiteurs de la 5α-réductase

Dans la prostate, la testostérone, à l'aide de l'enzyme 5α-réductase, est convertie en DHT qui est l'androgène le plus actif. De plus, les hommes avec une déficience en 5α-réductase type 2 ne sont pas affectés par l'hyperplasie bénigne de la prostate ou par le

cancer de la prostate. Cette observation a donc conduit à l'élaboration d'inhibiteurs de cette enzyme dont le finastéride et le dustastéride (Figure 6). Le finastéride, un dérivé de la testostérone, est un inhibiteur de type compétitif et irréversible. Étant présentement utilisé pour le traitement de l'hyperplasie bénigne de la prostate, les rares effets secondaires observés sont l'impuissance et une diminution de la libido. Une importante étude visant à établir le potentiel du finastéride comme agent préventif du cancer de la prostate a démontré qu'après sept ans d'administration (5 mg/jour) il avait diminué les risques d'être atteint d'un cancer de la prostate.[77] Le second composé, le dutastéride, inhibe les 5α-réductases type 1 et type 2 et induit la mort des cellules cancéreuses de la prostate.[78] Cet inhibiteur diminue le risque de cancer de la prostate et un traitement de 6 à 10 semaines (5 mg/jour) réduit de 40 % la concentration de l'androgène DHT à l'intérieur de la prostate.[79] Donc ces observations nous portent à croire que cet inhibiteur pourrait être utilisé éventuellement pour la prévention et le traitement du cancer de la prostate.



Finastéride **Dutastéride**

Figure 6. Inhibiteurs de la 5α-réductase.

2.7.5 La radiothérapie externe

La radiothérapie externe utilise un rayonnement à haute énergie pour détruire les cellules cancéreuses. Ce traitement affecte également les cellules saines mais elles sont plus résistantes que les cellules cancéreuses. La radiothérapie est utilisée pour traiter les cancers de la prostate localisés, ou qui ont atteint les tissus voisins. Elle peut être utilisée pour diminuer le volume de la tumeur ou pour éviter des complications locales. La radiothérapie

est efficace et peut être recommandée aux patients inopérables. Par contre, il est impossible d'effectuer une chirurgie en cas d'échec de la radiothérapie dû à la mauvaise cicatrisation des tissus traités par rayons. Un cancer localement avancé est habituellement traité par l'association d'un traitement endocrinien et d'une radiothérapie. Le traitement endocrinien peut comprendre des agonistes de la LHRH, des antiandrogènes ou parfois une castration physique. La dose totale des rayons appliquée sur la prostate doit être fractionnée dans le temps, ce qui explique la nécessité d'avoir des traitements à chaque jour (de 6 à 8 semaines consécutives). De plus, cette technique a bénéficié des progrès récents de l'informatique et la cible des rayons est maintenant plus précise.

Les effets secondaires de ce traitement sont habituellement mineurs et transitoires. L'irritation cutanée, la fatigue, la diarrhée, le sang dans les selles, l'irritation de la vessie, la proctite (inflammation du rectum)[80] et les troubles digestifs en sont quelques-uns. Évidemment, le risque de séquelles permanentes ne peut être exclu puisque des problèmes érectiles peuvent apparaître suite à ce traitement (risque de 40 %).

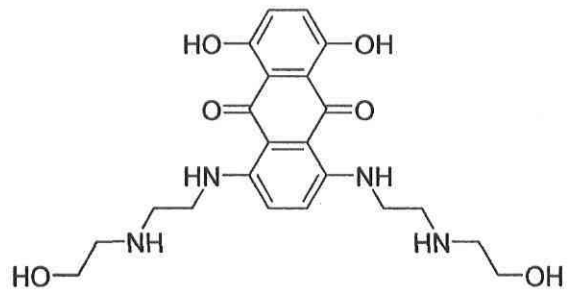
2.7.6 La curiethérapie

La curiethérapie est une technique d'irradiation grâce à des grains radioactifs insérés directement dans la prostate sous contrôle échographique. Cette intervention, qui est peu agressive, est d'une durée de 1 à 2 heures sous anesthésie générale et nécessite une hospitalisation d'environ 2 jours. Les particules radioactives sont placées dans la prostate de façon définitive et délivrent des radiations continues dont l'intensité diminue avec le temps. Après 6 mois, ces radiations sont négligeables. Ce traitement est présentement utilisé pour les cancers peu évolués (localisé et peu agressif), mais une combinaison avec l'hormonothérapie ou la radiothérapie externe est possible pour des cancers plus avancés. Les résultats obtenus sont aussi bons que ceux observés avec la radiothérapie externe qui est beaucoup plus coûteuse et les effets secondaires provoqués par ces deux techniques sont les mêmes.

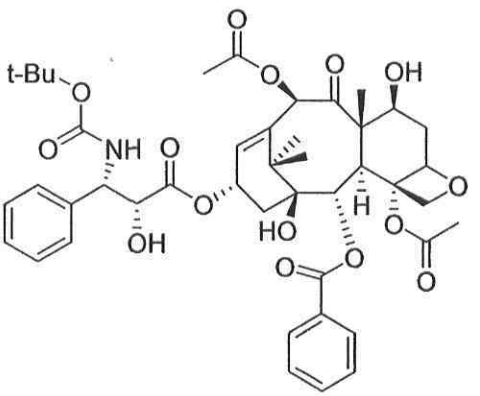
2.7.7 La chimiothérapie

En fonction du stade de leur cancer et de leur état, certains patients reçoivent un traitement hormonal, généralement accompagné de la castration et/ou de radiothérapie. Mais parfois et habituellement après deux ou trois ans, une résistance à ce traitement peut apparaître. Cette résistance est principalement rencontrée lors des cancers métastatiques. À ce moment, différents protocoles de chimiothérapie peuvent être utilisés. La chimiothérapie peut diminuer la croissance du cancer et alléger les douleurs du patient.

L'un des agents chimiothérapeutiques utilisés est le mitoxantrone (Figure 7).[81] De structure similaire à la doxorubicine, ce produit s'intercale dans l'ADN et empêche la formation d'ARN et la réplication de l'ADN. Ces principaux effets secondaires sont la nausée, la fatigue et la perte des cheveux. Cette anthracènedione produit moins de radicaux libres que les anthracyclines (comme la doxorubicine) ce qui réduit la toxicité cardiaque. Finalement, un nouvel agent très prometteur est présentement à l'étude. Ce produit est le taxotère, et est présentement utilisé pour le traitement des cancers du sein et du poumon (Figure 7). Il semble que ce produit soit plus efficace que le mitoxandrone chez les patients atteint d'un cancer métastatique hormono-résistant puisque les patients traités avec le taxotère ont bénéficié d'une amélioration de la qualité de vie et d'une diminution importante des douleurs. En outre, la durée de vie est allongée d'environ trois mois.



Mitoxantrone



Taxotère

Figure 7. Exemples d'agents chimiothérapeutiques.

2.8 Aperçu du projet de recherche

Le premier volet de cette thèse de doctorat a pour objectif d'effectuer la synthèse de nouveaux antiandrogènes dérivés du noyau 5α-androstane-3α,17β-diol. Comme il existe peu d'antagonistes ayant une forte affinité pour le récepteur des androgènes, nous croyons que cette nouvelle famille d'antiandrogènes pourrait avoir des RBA intéressants. Notre intérêt pour développer ces nouveaux produits est venue à la suite de résultats obtenus par notre groupe de recherche lors d'une étude portant sur le développement d'inhibiteurs de la 17β-hydroxystéroïde déshydrogénase type 3 pour le traitement du cancer de la prostate.[82] L'un des dérivés, le 16α-(3'-bromopropyl)-5α-androstane-3α.17β-diol (**1**) (Figure 8), présentait une affinité avec le récepteur des androgènes sans toutefois avoir d'affinité pour d'autres récepteurs stéroïdiens. De plus, il inhibait 86 % de la prolifération cellulaire des cellules Shionogi (AR^+) à 1 μM sans exercer d'effet agoniste. C'est pourquoi nous avons envisagé la préparation d'analogues du produit **1** (structures générales **2** et **3**) afin d'en améliorer l'efficacité. Notre hypothèse est que le noyau stéroïdien nous permettra d'avoir une bonne affinité avec le récepteur, mais que la chaîne en position 16 empêchera son activation (Chapitre 1).

Nous avons tout d'abord voulu vérifier l'importance de l'atome de brome en bout de chaîne. Pour ce faire, nous avons introduit, à l'aide de la chimie classique, une diversité moléculaire à cette position. L'effet du groupement hydroxy en position 3α aussi été étudié en le remplaçant par un carbonyle.

Par la suite, nous avons déterminé la longueur optimale de la chaîne en position 16α afin d'obtenir la meilleure activité antiandrogénique.

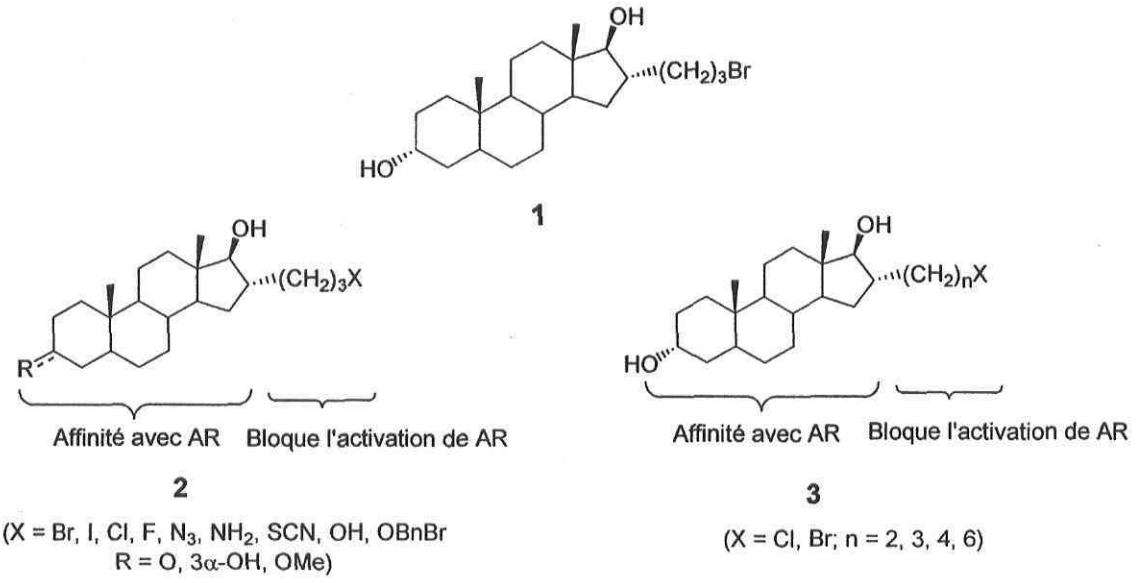


Figure 8. Nouveaux antiandrogènes stéroïdiens.

3 La leucémie

3.1 Composition du sang

Le sang est composé de plasma et de cellules sanguines. Le plasma est un fluide clair qui transporte les cellules sanguines. Il y a trois types différents de cellules sanguines: les globules rouges (érythrocytes), les globules blancs (leucocytes) et les plaquettes (thrombocytes). Les globules blancs sont les seules cellules possédant un noyau. La figure 9 démontre la taille relative et la quantité de ces trois types de cellules.

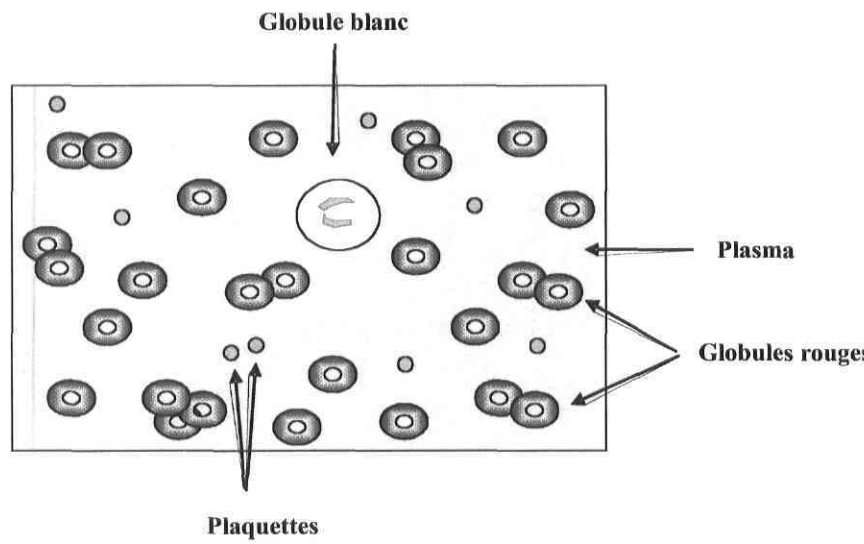


Figure 9. Cellules sanguines normales.

Les cellules sanguines sont formées dans la moelle osseuse (le tissu spongieux à l'intérieur des os) à partir de cellules souches. Les cellules souches peuvent se différencier et donner naissance à plusieurs types de cellules sanguines: les globules rouges, tous les types de globules blancs et les plaquettes. Chaque cellule souche peut se reproduire elle-même et donner naissance à plusieurs cellules immatures (blastes) et, par la suite, ces cellules se différencieront en un type particulier de cellules sanguines (précurseurs). Au départ, une cellule souche a le potentiel de devenir n'importe quel type de cellule. Sous

l'action de certaines substances, comme des hormones, les cellules souches peuvent devenir des cellules lymphoïdes ou des cellules myéloïdes (Figure 10).

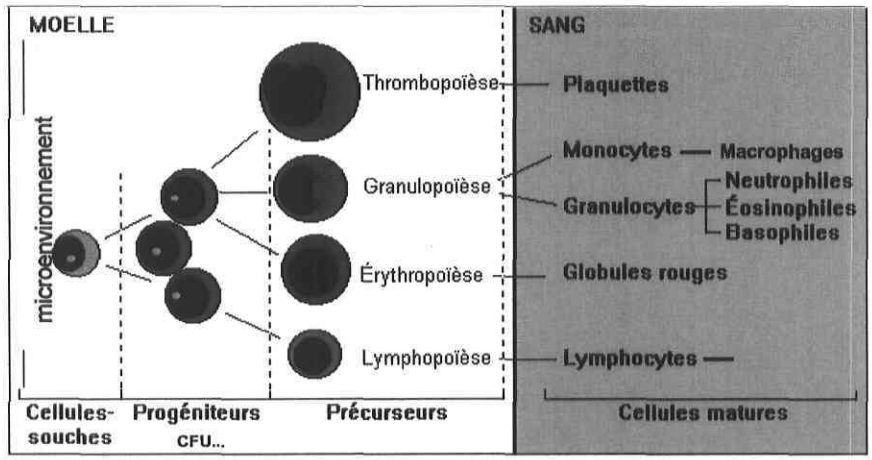


Figure 10. Progression du développement des cellules souches en une lignée cellulaire définitive. (Figure tirée du site internet http://www.fmed.ulaval.ca/med-17112)

Une moelle osseuse normale est composée de plusieurs types de cellules pouvant se reproduire elles-mêmes et ainsi former de nouvelles cellules qui pourront à leur tour se développer en un type cellulaire bien spécifique. Une moelle normale balance la production de ces différents types de cellules qui sont présentes dans le sang en quantité convenable. Chez un patient atteint de leucémie, cette production est débalancée.

3.2 Qu'est-ce que la leucémie?

La leucémie est un cancer du sang et à été découverte en 1847 par le pathologiste Rudolf Virchow. Elle implique des globules blancs immatures qui se reproduisent trop rapidement. Chaque type de leucémie concerne un type particulier de globule blanc et reflète son niveau de différentiation. Les cellules leucémiques sont différentes des cellules normales et sont incapables d'accomplir leurs tâches respectives. D'autres cellules

leucémiques ressemblent davantage aux cellules normales, mais elles ne meurent pas et s'accumulent. Dans la majorité des leucémies, sauf pour la leucémie lymphoïde chronique, les cellules souches arrêtent de se différencier ou le font de façon anormale. Ces cellules immatures prolifèrent et s'accumulent premièrement au niveau de la moelle osseuse et dans la circulation sanguine, laissant ainsi insuffisamment de place pour les «bonnes» cellules, c'est-à-dire les globules rouges, les globules blancs normaux et les plaquettes. Toutes ces cellules sont vitales pour le bon fonctionnement du corps humain. Les cellules leucémiques peuvent atteindre, après un certain temps, des organes vitaux.

Les organes les plus couramment affectés sont les ganglions lymphatiques, la rate, les poumons, le foie et la peau. Parfois les reins, le cerveau ou d'autres parties du système nerveux peuvent être touchés. Finalement, tous les tissus et organes sont susceptibles d'être infiltrés par la leucémie. L'accumulation de ces cellules immatures inhibe l'hématopoïèse normale (Figure 10) pouvant causer une leucopénie (diminution du nombre de globules blancs dans le sang), l'anémie (diminution du nombre de globules rouges dans le sang) ou une thrombocytopénie (diminution du nombre de plaquettes dans le sang) ayant pour conséquence des infections, de la fatigue et des saignements.

Habituellement, un diagnostic de leucémie est posé suite à une évaluation sanguine. Comme les premiers symptômes des différents types de leucémie sont similaires, des analyses en laboratoire sont nécessaires afin de déterminer le type de leucémie en particulier. Souvent ces tests sont complexes et permettent de découvrir quel chromosome est affecté ou le problème génétique présent. D'autres tests, comme la biopsie, peuvent être effectués. Une ponction lombaire peut être utilisée afin de confirmer les résultats des tests sanguins. Le diagnostic sera basé sur les résultats sanguins, la description des symptômes du patient, le rapport pathologique et l'examen physique. L'historique familial ainsi que l'environnement de vie et de travail seront considérés. Le stade et le niveau de risque seront déterminés à cette étape et serviront au choix du traitement.

3.3 Les risques et les causes

La leucémie représente 3 % de l'ensemble de tous les cancers. On estime qu'en 2006 il y aura 4000 nouveaux cas au Canada[1] et 32 810 au États-Unis[40] et que plus de la moitié vont en décéder. Cette maladie représente environ 195 000 décès dans le monde chaque année. L'incidence de ce cancer est plus élevée aux États-Unis, au Canada, en Europe occidentale, en Australie et en Nouvelle-Zélande, alors que ces taux sont généralement plus de deux fois inférieurs dans la plupart des pays d'Afrique et d'Asie.[34] De plus, les hommes seraient plus fréquemment touchés que les femmes.[83] Ce cancer est toutefois plus important chez les enfants puisqu'il représente 26 % des cas de cancer et est la deuxième cause de mortalité après les accidents. Cependant, une réduction importante de ce taux de mortalité, en particulier chez les enfants, est observée depuis les années 1960 grâce aux nombreux progrès pharmaceutiques.

De nos jours, on observe un pic de l'incidence au cours des quatre premières années de la vie d'un enfant, principalement causé par la leucémie lymphoïde aiguë, l'affection la plus fréquente en pédiatrie. Après l'enfance, l'incidence diminue brutalement jusqu'à 25 ans, après quoi on observe une augmentation importante jusqu'à l'âge de 85 ans. Environ 63 % des patients vont survivre une année après avoir été diagnostiqués d'une leucémie. Cependant, le nombre de ces patients toujours vivants cinq ans après avoir été diagnostiqués diminue jusqu'à environ 43 %, principalement à cause du faible taux de survie attribuable à certains types de leucémie, dont la leucémie myéloïde aiguë. Bien qu'il y ait eu de nombreux progrès dans le diagnostic et le traitement de la leucémie, la cause de cette maladie est encore inconnue. Plusieurs facteurs de risque sont toutefois connus.

Tout d'abord, certains virus peuvent être impliqués dans le développement de la leucémie. Le virus D'Epstein-Barr, qui attaque électivement les lymphocytes B, en est un. Il infecte principalement les enfants des pays à niveau de vie peu élevé. Le virus HTLV-1 en est un autre. Celui-ci est responsable dans moins de 4 % des cas de la leucémie lymphoïde à cellules T chez l'adulte, principalement au Japon, en Afrique et dans les Caraïbes. De plus, le virus HIV serait peut-être impliqué dans le développement de

certaines leucémies.[83] De même, des rayonnements ionisants seraient impliqués dans la leucémie. Ce phénomène est très bien connu et étudié.[84-85] Comme par exemple, les gens exposés à ces rayonnements pendant la guerre (Hiroshima), suite à une catastrophe (Tchernobyl) ou suivant un traitement médical (radiothérapie) sont plus fréquemment atteints d'une leucémie. Un autre facteur susceptible d'induire le développement d'une leucémie serait l'exposition professionnelle à certains produits chimiques, comme le benzène qui augmenterait ce risque de 2 à 10 fois.[83] De plus, l'utilisation d'agents antinéoplasiques en chimiothérapie (chlorambucil, cyclophosphamide) favoriserait son développement, tandis que certaines pathologies génétiques, comme la trisomie 21, pourraient augmenter le risque d'avoir une leucémie.

3.4 Les différents types de leucémie

Il y a plusieurs similarités entre les différents types de leucémie. Toutes les leucémies sont caractérisées par une production excessive de globules blancs immatures. Elles produisent des symptômes similaires et présentes toutes des anomalies génétiques et chromosomiques. Toutes ces analogies rendent donc le diagnostic encore plus difficile. Lorsque les analyses génétiques sont complétées, il est possible d'identifier clairement le type de leucémie puisque la carte chromosomique de chacun est différente. La composition chimique ou la position unique des constituants à la surface des cellules peuvent aussi aider à identifier le type de leucémie.

Il y a deux classifications principales de leucémie: la leucémie aiguë, qui progresse rapidement; et la leucémie chronique, qui progresse lentement. La détermination de cette classe est basée sur le niveau de différenciation des cellules impliquées. Par la suite, chacune de ces classes est divisée selon le type de cellules hématopoïétiques qui est touché, c'est-à-dire les leucémies lymphoïdes et les leucémies myéloïdes (Figure 11). Un diagnostic précis permet la sélection du traitement le plus efficace pour un type particulier de leucémie.

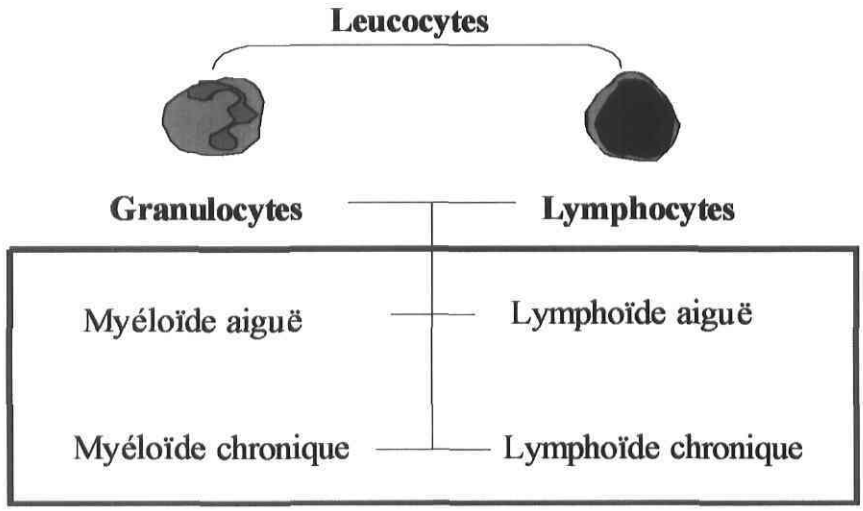


Figure 11. Types de leucémie. (Figure tirée du site internet http://www.fmed.ulaval.ca/med-17112)

La leucémie aiguë se développe soudainement. En l'absence de traitement, elle est fatale en moins de quelques semaines ou de quelques mois. Dans ce type de leucémie, la majorité des cellules sont peu différenciées, ce sont des cellules blastiques. La quantité de ces cellules augmente rapidement, prenant la place des cellules saines et aggravant ainsi la maladie. Qu'elles soient de type myéloïde ou de type lymphoïde, les symptômes sont identiques. La leucémie lymphoïde aiguë (LLA) touche principalement les enfants (75 à 80 % des leucémies infantiles). Seulement 20 % des leucémies chez l'adulte sont de ce type. Ce nombre augmente chez les patients de plus de 50 ans. Ce type est caractérisé par l'extrême prolifération des lymphocytes B et T dans la moelle osseuse et dans le système lymphatique. Quant à la leucémie myéloïde aiguë (LMA), on la retrouve chez les adultes ainsi que chez les enfants, mais dans une plus faible proportion puisque l'incidence de la LMA augmente avec l'âge. Les cellules impliquées dans ce type de leucémie sont les myéloblastes et la prolifération de ces derniers est incontrôlable empêchant donc les autres cellules d'effectuer leur tâche respective.

La leucémie chronique évolue sur plusieurs mois à quelques années. Dans ce type de leucémie, les cellules sont davantage différenciées et peuvent ainsi effectuées certaines

de leurs tâches sur une assez longue période. Le nombre de ces cellules augmente graduellement et c'est pourquoi elle est qualifiée de chronique. La leucémie myéloïde chronique (LMC) représente environ 15 % des leucémies chez l'adulte et peut éventuellement se transformer en leucémie aigüe. Son unique anomalie chromosomique est connue sous le nom de chromosome Philadelphia présent dans 90 % des LMC. Cette leucémie affecte les précurseurs des granulocytes et des plaquettes. Dans les débuts de la maladie, ces cellules fonctionnent normalement et le patient n'a aucun symptôme. Cette situation peut durer de 3 à 4 ans. Éventuellement, la nature de cette leucémie change et la LMC se transforme en leucémie aigüe. Finalement, la leucémie lymphoïde chronique (LLC) est le type le plus fréquent chez l'adulte représentant entre 25 et 30 % de tous les cas de leucémies chez l'adulte et 17 % de toutes les leucémies. Elle affecte deux fois plus les hommes âgés que les femmes. De plus, une augmentation du nombre de cas chez les enfants a été remarquée, ce qui est probablement dû à l'augmentation de la fréquence des examens sanguins de routine qui permettent une détection plus précoce. La LLC affecte les lymphocytes partiellement différenciés qui se divisent lentement, ce qui leur permet de vivre plus longtemps mais sans toutefois être capable d'exercer leur fonction spécifique. La grande majorité des LLC (95 %) concerne les lymphocytes B et les autres, les lymphocytes T. Il existe toutefois des formes plus rares de leucémie lymphoïde chronique comme la leucémie à cellules chevelues (2 %) et la leucémie prolymphocytaire.

3.5 Classification des différents types de leucémie

Afin de déterminer le traitement idéal pour chaque leucémie, il est important de déterminer le type (aigüe ou chronique), le sous-type (myéloïde ou lymphoïde) ainsi que le stade de la maladie. La sévérité de la leucémie et son pronostic vont avoir une très grande importance dans la décision de la thérapie. Le choix d'un traitement plus ou moins agressif sera donc basé sur certains facteurs pronostiques.

Leucémie lymphoïde aiguë

La leucémie lymphoïde aiguë est classée selon la morphologie des cellules malignes. La classification franco-américaine-britannique (FAB) reconnaît trois catégories qui, cependant, ne sont pas encore tout à fait claires.

-L1, plus fréquente chez l'enfant et ayant un meilleur pronostic. Une rémission complète est plus couramment observée pour ce type.

-L2, 80 % à cellules pré-B et 20 % à cellules pré-T.

-L3 qui est plus rare.

Habituellement, on évalue l'immunophénotype pour déterminer le type de traitement à privilégier. C'est-à-dire que l'on détermine les cellules présentes dans cette leucémie en évaluant le type d'antigènes à la surface des globules blancs. Ces marqueurs cellulaires sont appelés CD qui signifie «clusters» de différenciation et chacun d'eux sont associés avec un type cellulaire en particulier.

-Les principaux marqueurs pour les cellules B sont:
CD10, CD19, CD20, CD21, CD22 et CD24.

-Les principaux marqueurs pour les cellules T sont:
CD1, CD2, CD3, CD5, CD7 et CD4/CD8.

L'intensité du signal indique la puissance des CD présents à la surface des cellules. Donc, la réponse à un traitement dépend plus de l'intensité du signal (de l'expression) que du pourcentage de cellules qui exprimant cet antigène.

Leucémie myéloïde aiguë

Dans la plupart des leucémies myéloïdes aiguës, on détecte des anomalies chromosomiques. Ces anomalies, qui touchent les bras longs et courts des chromosomes, sont des translocations (une partie d'un chromosome se déplace sur un autre chromosome), des inversions et des insertions du matériel génétique. Ces anomalies déterminent le diagnostic et pronostic et conditionnent le choix du traitement. Les marqueurs cellulaires sont toutefois moins importants dans ce type de leucémie et souvent son diagnostic est

associé avec l'absence d'antigènes lymphocytaires. La classification de ce type de leucémie est plus complexe, mais la FAB en a fait 8 catégories. M0 à M7 (Tableau 1). Bien que la leucémie promyélocytique aiguë (APL) soit une maladie plus rare, elle est un modèle très utilisé dans la recherche de traitement en oncologie.

Tableau 1. Classification FAB de la leucémie myéloïde aiguë en 8 catégories.[86]

Sous-type FAB	% de cas	Morphologie	Cytométrie en flux	Cytogénétique
M0: leucémie indifférenciée	2-3	Aspect immature	CD13, CD14, or CD33 et CD34	--
M1: leucémie myéloblastique sans différenciation	20	Quelques blastes avec des granulations	CD13, CD33 et CD34	--
M2: Leucémie myéloblastique avec différenciation	25-30	Granulations, les corps d'Auer	CD13, CD15, CD33 et CD34	t(8;21) (q22;q22)
M3: Leucémie promyélocitaire hypergranuleuse	8-15	Promyélocitaire hypergranuleuse avec de multiples corps d'Auer	CD13, CD15 et CD33	t(15;17) (q22;q11-12)
M4: Leucémie myélomonocytaire	20-25	Blastes granuleux et monocytaires	CD11b, CD13, CD14 et CD15	M4Eo: inv(16) (p13q22)
M5: Leucémie monocytaire	20-25	M5A: indifférenciée M5B: différenciée	CD11b, CD13, CD14, CD15 et CD33	Translocation 11q23
M6: Érythroleucémie	5	Érythroblastes > 50 % de cellules nuclées, myéloblastes > 30 % de cellules non érythroblastiques	CD33	--
M7 : Leucémie à mégacaryotes	1-2	Mégacaryoblastes > 30 % de l'ensemble des cellules nucléées	CD33 et CD41	--

Leucémie lymphoïde chronique

La leucémie chronique peut causer différents problèmes, ce qui rend le diagnostic plus difficile. Pour qu'un patient soit atteint d'une leucémie lymphoïde chronique, son décompte lymphocytaire doit être supérieur à 5000 cellules/µl de sang et toutes ces cellules malignes doivent être monoclonales, c.-à-d. qu'elles proviennent de la même cellule souche. Ce dernier critère peut être vérifié grâce aux anticorps et aux marqueurs de surface CD. Certaines anomalies chromosomiques (délétions) caractéristiques de la LLC peuvent aussi être présentes et la trisomie 12 est présente dans 30 à 50 % des LLC. Une délétion ou une mutation du gène suppresseur de tumeur P53 peut aussi être observée. Comme ce gène intervient dans les mécanismes de détection des dommages à l'ADN et à la mise en branle de sa réparation, son absence permet donc aux cellules dont l'ADN est défectueux de se reproduire indéfiniment et d'accumuler de nombreuses mutations. De plus, le gène bcl-2 est surexprimé dans plus de 70 % des LLC. La protéine bcl-2, fabriquée par ce gène, est habituellement bénéfique pour les cellules en prévenant l'apoptose suite à de légères infections. Cependant, lorsqu'un cancer exprime en trop grande quantité cette protéine, les cellules sont protégées contre l'apoptose permettant donc une prolifération incontrôlée de ces cellules. Dans plusieurs cas, la présence de cette protéine en grande quantité peut induire une résistance à la chimiothérapie.

Il existe deux systèmes de classification pour les stades de cette leucémie, soit le système de Rai et celui de Binet. Le premier système utilise l'examen sanguin, de la moelle et l'examen physique. Le second, quant à lui, utilise les niveaux d'hémoglobine et de plaquettes ainsi que le nombre d'endroits envahis par les lymphocytes, soit la tête, le cou, les aisselles, les aines, la rate (palpation) et le foie (palpation). La classification suivante présente la classification selon le système de Rai:

-Stade 0. Lymphocytose absolue. Plus de 15 000 lymphocytes/µL.
-Stade 1. Lymphocytose absolue et lymphadénopathie (grossissement des ganglions lymphatiques.
-Stade 2. Lymphocytose absolue et lymphadénopathie avec soit une splénomégalie (rate volumineuse) ou une hépatomégalie (foie volumineux).

-Stade 3. Lymphocytose absolue et anémie (hémoglobine inférieure à 110 g/L).
-Stade 4. Lymphocytose absolue et thrombocytopénie (plaquettes inférieures à 100 000/µl) avec ou sans symptome d'anémie, de lymphadénopathie, de splénomégalie ou d'hépatomégalie.

Finalement, le pronostic est considéré comme excellent lorsque le temps nécessaire pour doubler la concentration sanguine de lymphocytes est supérieur à une année, que la concentration sanguine de β2-microglobuline est faible, qu'il y a absence de translocation entre les chromosomes 11 et 7 et que la présence de l'antigène CD23 est faible. Par conséquent, la thérapie peut être moins agressive.

Leucémie myéloïde chronique

L'analyse chromosomique de cette maladie montre la présence dans 90 % des cas du chromosome anormal Philadelphie, ce qui en fait un très bon indicateur pour le diagnostic de la LMC. Ce chromosome anormal est le résultat d'une translocation de la partie inférieure (bras long; q) du chromosome 22 sur la partie inférieure du chromosome 9 créant ainsi un gène hybride nommé bcr/abl. Ce gène qui mène à la formation d'ARN messager, nommé BCR-ABL, conduit à la production d'une protéine de fusion nommée p210. Cette protéine, qui est une tyrosine kinase, est la cible de certain traitement visant à la détruire.

3.6 Les traitements

Dans cette section, nous allons discuter des différentes options envisageables pour le traitement de la leucémie. Au cours du vingtième siècle, de nombreux progrès ont été faits dans la compréhension et le traitement de la leucémie.[87] Si la leucémie est aiguë, le traitement doit débuter immédiatement, par contre si elle est chronique, le traitement peut être repoussé de plusieurs mois allant jusqu'à plusieurs années. Le type de la leucémie, son sous-type, le profil sanguin établi suite à son analyse ainsi que les symptômes du patient sont tous des critères importants dans le choix du traitement. De plus, le traitement doit

prendre en considération l'âge ainsi que l'état de santé général du patient. Les traitements standards sont la chimiothérapie, les thérapies biologiques, la radiothérapie et la greffe. Évidemment, ces différentes options peuvent être combinées afin d'augmenter les chances de guérison.

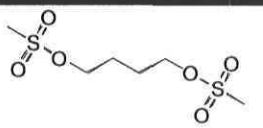
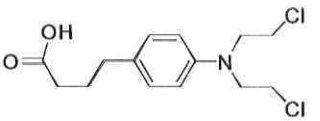
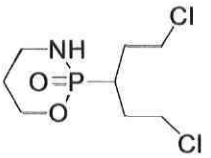
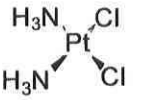
3.6.1 La chimiothérapie

La chimiothérapie est sans contredit le traitement de choix pour la majorité des leucémies. Ces agents agissent contre cette maladie en empêchant les cellules leucémiques de survive et de se reproduire. Il y a plusieurs familles d'agents chimiothérapeutiques, dont: les agents alkylants, les intercalants, les antimétabolites, les immunosuppresseurs, les inhibiteurs de la topoisomérase et les agents interagissant avec la tubuline. Les agents anticancéreux utilisés en hématologie sont régulièrement combinés et cette combinaison est communément nommée protocole de chimiothérapie.

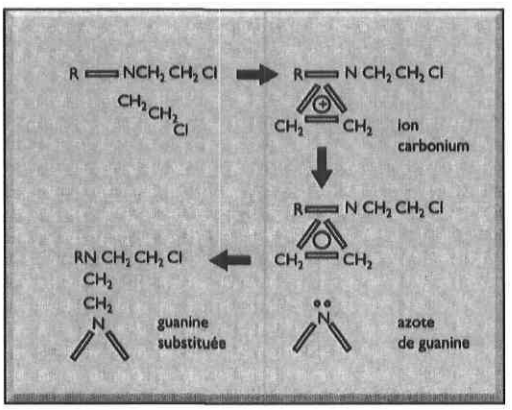
1) Les agents alkylants

Ces molécules (Tableau 2) ont la capacité d'attaquer les régions riches en électron des macromolécules, aboutissant à la formation de liaisons covalentes irréversibles. Ceci se traduit par la formation de ponts interbrins ou intrabrins avec un ou plusieurs acides nucléiques, entraînant des modifications structurales de l'ADN qui altèrent les fonctions de réplication et de transcription (Figure 12). La cellule est alors incapable de dupliquer son ADN bloquant ainsi la division cellulaire.[88]

Tableau 2. Exemples d'agents alkylants utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Busulfan	Myleran®	
Chlorambucile	Leukeran®	
Cyclophosphamide	Cytoxan®	
Cisplatine	Cisplatine®	

A)



B)

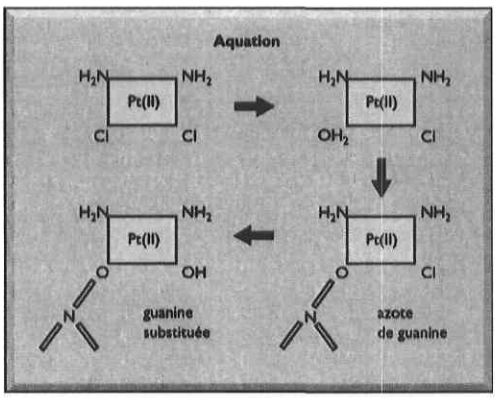
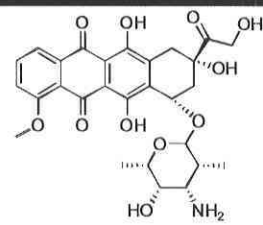
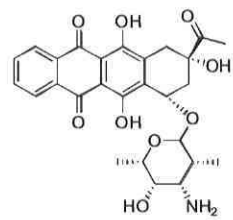
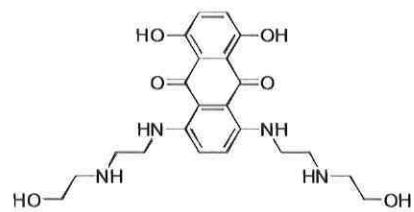


Figure 12. Mécanismes d'action de la moutarde azotée A) et du cisplatin B). (Figure tirée de Pharmacologie intégrée, Page et al.[89])

2) Les intercalants

Les agents intercalants (Tableau 3) sont des molécules polycycliques planes capables de s'intercaler au niveau de l'ADN entre deux bases adjacentes. Ceci conduit à un éloignement de ces bases, une déspiralisation de l'ADN et une inhibition de la réplication et de la transcription de l'ADN. La liaison des intercalants à l'ADN est réversible contrairement à celle des agents alkylants.

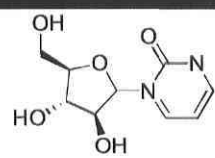
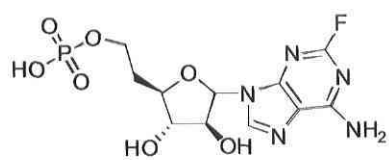
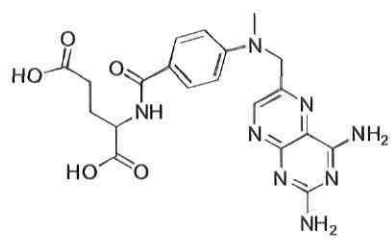
Tableau 3. Exemples d'agents intercalants utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Doxorubicine	Adriamycine®	
Idarubicine	Zavedos®	
Mitoxantrone	Novantrone®	

3) Les antimétabolites

Les antimétabolites interfèrent avec des étapes enzymatiques essentielles à la synthèse et/ou la transcription de l'ADN. Ce sont le plus souvent des analogues des substrats physiologiques des enzymes. Les mécanismes des antimétabolites sont variés: inhibition compétitive des réactions enzymatiques, inhibition d'une enzyme, incorporation dans les acides nucléiques.[88] Ils peuvent affecter l'ADN, l'ARN, la synthèse des protéines et la réplication cellulaire. Par exemple, la cytarabine (Tableau 4) est un antimétabolite qui, lorsque activé par le métabolisme cellulaire, ressemble à la cytosine triphosphate utilisée normalement par la cellule. Il peut alors être ajouté à l'ADN en formation à la place d'une cytosine triphosphate normale et ainsi empêcher sa reproduction. La fludarabine, quant à elle, est un analogue de l'adénosine. Elle interfère avec certaines enzymes et conduit les cellules vers l'apoptose. Le méthotrexate, un analogue de l'acide folique qui inhibe l'enzyme dyhydrofolate réductase nécessaire pour le métabolisme des acides foliques. Puisque ces acides sont nécessaires à la fabrication de l'ADN, sa synthèse est bloquée.

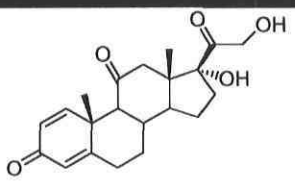
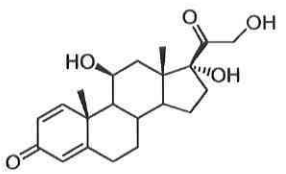
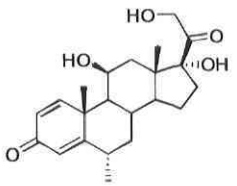
Tableau 4. Exemples d'antimétabolites utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Cytarabine	Cytarabine®	
Fludarabine	Fludara®	
Méthotrexate	Méthotrexate®	

4) Les immunosuppresseurs

Les immunosuppresseurs prednisone et méthylprednisolone sont des corticostéroïdes capables d'induire l'apoptose des lymphocytes en modulant l'expression de certaines protéines régulatrices comme Bcl-2, Bcl-xl et Bax (Tableau 5). De plus, la production de cytokines stimulant la division cellulaire des lymphocytes T par eux-mêmes est diminuée grâce aux corticostéroïdes. Ces composés stimulent les globules rouges de la moelle osseuse et augmentent leur temps de survie ainsi que celui des plaquettes.

Tableau 5. Exemples d'immunosuppresseurs utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Prednisone	Deltasone®	
Prednisolone	Solupred®	
6-Méthylprednisolone	Depot-Medrol®	

5) Les inhibiteurs de la topoisomérase

Les topoisomérases sont des enzymes capables d'effectuer la réparation conformationnelle de l'ADN. Ils ont un rôle important dans l'initiation de la réplication de l'ADN en assurant sa spiralisation/déspiralisation après avoir créé des cassures soit sur un seul brin d'ADN (topoisomérase du type I), soit des deux brins (topoisomérase du type II).

L'inhibiteur se lie au complexe ADN/topoisomérase. À ce moment, L'ADN double brin se brise et l'enzyme topoisomérase demeure liée à l'ADN brisé. Les dommages à L'ADN s'accumulent et causent le décès de la cellule (Figure 13). Les cellules en phases S et M du cycle cellulaire sont davantage sensibles à ces inhibiteurs, puisque l'activité des topoisomérases est importante dans ces périodes. Il y a des inhibiteurs spécifiques pour chaque type de topoisomérase. Le topotécan[90] et l'irinotécan[91] (Tableau 6) sont des inhibiteurs du type I, tandis que l'étoposide et le téniposide sont des inhibiteurs du type II.[92] La doxorubicine, en plus d'être un agent intercalant, peut également inhiber la topoisomérase II.

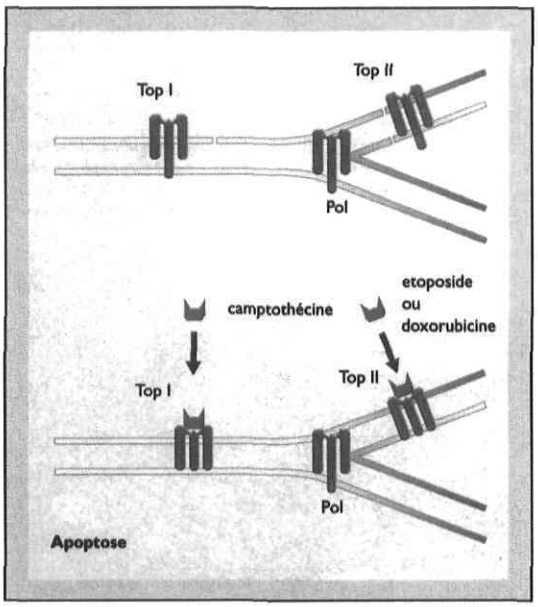
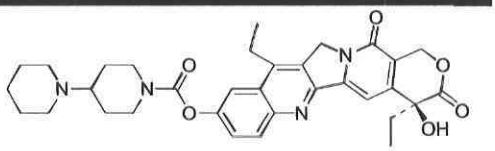
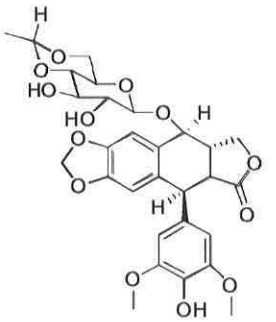


Figure 13. Mécanisme d'action d'inhibiteurs de la topoisomérase (TOP). (Figure tirée de Pharmacologie intégrée, Page et al.[89])

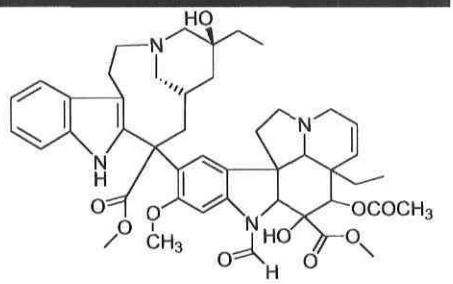
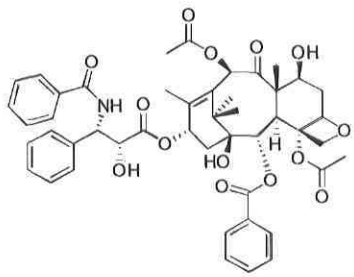
Tableau 6. Exemples d'inhibiteurs de la topoisomérase II utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Irinotécan	Camptosar®	
Étoposide	Vepeside®	

6) Les agents interagissant avec la tubuline

La tubuline est une protéine formant des fibres dans les cellules. Les agents chimiques vont bloquer les cellules en mitose en se liant à la tubuline et en empêchant sa polymérisation (Tableau 7). Cela a pour effet d'empêcher la séparation des chromosomes aux deux cellules filles puisque ceux-ci sont normalement tirés vers les deux pôles de la cellule en mitose grâce aux fuseaux de division formés par les polymères de tubuline. Le fonctionnement cellulaire est aussi touché puisque les microtubules forment la charpente de la cellule. Comme ces microtubules sont nécessaires à la mitose (phase M du cycle cellulaire), ces agents bloquent les cellules soit dans la phase S du cycle, où la formation des fuseaux est initiée (vincristine), soit dans la phase G2, où la tubuline est stabilisée (paclitaxel).

Tableau 7. Exemples d'agents interagissant avec la tubuline utilisés dans le traitement de la leucémie.

Noms génériques	Noms commerciaux	Structures
Vincristine	Vincristine®	
Paclitaxel	Taxole®	

3.6.2 Les thérapies biologiques

Les thérapies biologiques utilisent des protéines humaines, mais générées *in vitro*. Ces protéines sont normalement importantes dans les réponses immunitaires. Ces agents thérapeutiques agissent de plusieurs façons, mais généralement la protéine synthétique a pour effet d'augmenter simplement l'activité de la protéine naturelle. Par contre, d'autres thérapies ont pour effet de modifier la réponse biologique afin de changer la réponse cellulaire. Les anticorps monoclonaux et les cytokines sont les principaux traitements utilisés pour la leucémie.

1) Les anticorps monoclonaux

Ces agents sont des copies d'anticorps sécrétés par les globules blancs. Comme chaque antigène attire un anticorps en particulier, cette thérapie peut être plus sélective en

touchant spécifiquement un type de cellule (monoclonal), les cancéreuses. Elle augmente donc la réponse immunitaire naturelle contre le cancer. Ces agents peuvent aussi bloquer les récepteurs de certains facteurs de croissance importants ou bien générer un signal intracellulaire et ainsi induire l'apoptose des cellules cancéreuses. Ces anticorps peuvent être utilisés seuls ou conjugués avec une substance toxique, comme l'iode-131. Lorsque l'anticorps sera couplé à l'antigène, la proximité de la substance toxique causera des dommages à la cellule cancéreuse. Les anticorps monoclonaux sont aussi utilisés pour éliminer toutes les cellules cancéreuses de la moelle osseuse lors d'une autogreffe.

Les anticorps monoclonaux couramment utilisés pour le traitement de la leucémie sont le campath-1H, la B43-genistein et le bexxar. Ces trois produits sont présentement en études cliniques. De plus, le mylotarg est un anticorps monoclonal très prometteur pour le traitement des LMA qui a été approuvé par la «Food and Drug Administration» (FDA).

2) Les cytokines

Les cytokines régulent certains aspects du système immunitaire. Plusieurs de ces cytokines sont reconnues pour exercer une activité antitumorale et en voici quelques exemples.

-Interféron-α (IFN-α): il inhibe la prolifération cellulaire et augmente la cytotoxicité des cellules NK (natural killer). De plus, il prolonge la période de rémission obtenue avec la chiomiothérapie chez les patients atteints de LMC.
-Interleukine-2 (IL-2): il stimule le développement et la différenciation des lymphocytes. Il active aussi les cellules NK.
-Facteur nécrosant des tumeurs-α (TNF-α): Il entraîne la nécrose cellulaire.

Ces cytokines peuvent avoir des effets très bénéfiques pour les patients, comme diminuer le taux de récidive et prolonger la durée de leur survie, mais leurs effets secondaires semblent importants.

3) Facteurs de croissance

Les facteurs de croissance sont normalement des substances naturelles qui stimulent la croissance de nouvelles cellules. Dans le traitement de la leucémie, ces agents synthétiques, administrés par injections sous-cutanées, peuvent être utilisés afin d'aider la croissance de nouveaux globules blancs ou de plaquettes.[83]

-Facteur stimulant le développement des granulocytes (G-CSF): Cette protéine stimule la production de nouveaux globules blancs par la moelle osseuse.
-Érythropoïétine (EPO): Ce facteur est une protéine normalement synthétisée par les reins et en plus faible quantité par le foie. L'EPO stimule la moelle osseuse à produire des nouveaux globules rouges.
-Trombopoïétine (TPO): Cette protéine, synthétisée normalement par le foie et les reins, a une structure similaire à l'EPO. Son activité est toutefois différente puisqu'elle stimule la moelle osseuse à produire des nouvelles plaquettes.

Ces facteurs de croissance peuvent être administrés en combinaison ou à la suite du traitement de chimiothérapie afin de diminuer ses effets secondaires.

3.6.3 La radiothérapie

La radiothérapie peut ralentir la progression de la leucémie et aider à la traiter. Elle est habituellement utilisée en combinaison avec la chimiothérapie. Comme la plupart des drogues ont de la difficulté à atteindre le système nerveux central (SNC), la radiothérapie est souvent utilisée pour irradier l'encéphale en combinaison avec le méthotrexate, injecté dans le corps entier et dans le canal rachidien afin de réduire les risques de rechute au SNC. La radiothérapie est souvent utilisée lors de greffe de moelle osseuse. À ce moment, tout le corps est irradié. Cependant, l'utilisation de la radiothérapie est limitée en raison des effets secondaires possibles à long terme, en particulier à cause des effets sur la croissance des jeunes enfants et du risque d'affections malignes secondaires

3.6.4 La greffe

La greffe de moelle osseuse ou de cellules souches est souvent envisagée lorsque qu'il y a rechute suite à l'administration de chimiothérapie. Il y a deux types de greffe, soit la greffe allogénique et la greffe autologue. Ces deux types sont utilisés en combinaison avec de fortes doses de chimiothérapie et de radiothérapie. Lorsque le greffon est prélevé à partir d'un donneur compatible, il s'agit d'une greffe allogénique. Lorsque le greffon provient du patient lui-même, on parle alors de greffe autologue. L'utilisation de cellules souches provenant du sang périphérique est couramment utilisée en greffe autologue puisqu'elle diminue le temps d'hospitalisation par comparaison à la greffe de moelle.

-La greffe allogénique: ce type de greffe pour une première rémission complète de LMA donne une guérison dans 40 à 60 % des cas et est un traitement de référence pour les patients de moins de 40 ans. Cependant, le taux de rechute est de 25 % après 5 ans. La LLA de l'enfant est guérie dans 50 à 80 % des cas et celle de l'adulte présente un bon pronostic suite à la chimiothérapie classique. La greffe allogénique est donc moins utilisée. Par contre, pour la LMC, cette greffe est le seul traitement curatif.

-La greffe autologue: La chimiothérapie intensive suivie de réinjections de cellules souches hématopoïétiques autologues ne possède pas la toxicité de la greffe allogénique puisqu'il n'existe pas de conflit immunitaire entre le donneur et le receveur. En général, ce type de traitement présente un taux de mortalité plus faible puisqu'il n'y a aucun risque de rejet. Par contre, la greffe allogénique est plus efficace pour le traitement de la leucémie et diminue davantage la possibilité de rechute tout en impliquant plus de risque pour le patient.

3.6.5 Les nouveaux traitements

Le but de la chimiothérapie conventionnelle est de prévenir la croissance cellulaire en inhibant la synthèse des précurseurs d'ADN, en causant des dommages à la structure de l'ADN ou en perturbant la séparation des chromosomes. Ces modes d'actions non-spécifiques ont inévitablement conduit à des traitements ayant une faible sélectivité et un faible indice thérapeutique. Plusieurs réactions biochimiques et moléculaires se déroulant à l'intérieur de certains cancers sont maintenant connues et mieux définies. Donc, avec ces découvertes il apparaît des agents thérapeutiques possédant une meilleure sélectivité. Depuis plus de dix ans, les différentes recherches sur le cancer étudient le génome humain afin de comprendre les changements génétiques conduisant à une leucémie. Ils essaient de mettre au point différents traitements spécifiques aux cellules leucémiques avec un indice thérapeutique plus élevé, c.-à-d. avec moins d'effets secondaires à des doses thérapeutiques pour les patients. Il existe plusieurs cibles potentielles pour le développement de ces nouveaux agents et en voici quelques-unes.

Le traitement de la leucémie à l'aide des anticorps monoclonaux est une thérapie biologique encourageante puisque plusieurs anticorps sont présentement en étude clinique. L'angiogenèse est une autre cible des nouveaux traitements. La plupart des tumeurs ont besoin de plusieurs vaisseaux sanguins afin de pouvoir approvisionner leur métabolisme. Le développement de ces nouveaux vaisseaux est appelé angiogenèse. Ce procédé est aussi nécessaire à la leucémie, puisque ce cancer nécessite la formation de nouveaux vaisseaux sanguins à l'intérieur de la moelle osseuse. Des agents antiangiogéniques vont alors bloquer la synthèse de ces vaisseaux et ainsi pourraient faire diminuer le cancer. L'angiostatine et l'endostatine sont deux exemples d'agents antiangiogéniques. Ces deux produits peuvent diminuer ou inhiber la croissance des tumeurs chez la souris.[93] La thalidomide, qui possède des propriétés antiangiogéniques, est présentement en étude clinique. Cependant, ce produit ne peut être utilisé par une patiente désirant avoir des enfants puisqu'il est tératogène. Généralement, dans un cancer, le degré de différenciation cellulaire est inversement proportionnel au taux de prolifération des cellules cancéreuses. Malgré que ce processus de différenciation ne soit pas totalement élucidé, il existe plusieurs agents, dont l'acide

rétinoïque, certaines cytokines, et des analogues de la vitamine D, qui stimulent la différenciation des cellules cancéreuses, donc inhibant leur prolifération.[94] L'acide rétinoïque totalement trans (ATRA) permet d'obtenir un taux élevé de rémission complète lors du traitement de la leucémie promyélocytaire. Chez ce type de leucémie, qui représente environ 10 % des LMA, deux gènes du récepteur-α de l'acide rétinoïque sont fusionnés et c'est probablement la liaison de ce complexe avec ATRA qui induit la différenciation.[95] Cet acide est malheureusement efficace seulement lors du traitement de ce type de leucémie, de quelques cancers de la peau, et du cancer de la vessie. De plus, ce produit doit être administré en combinaison avec une forte dose de corticostéroïde afin de diminuer le syndrome de l'acide rétinoïque (syndrome d'activation leucocytaire) caractérisé par une fièvre et une détresse respiratoire qui peuvent parfois être fatales pour le patient. Les inhibiteurs de l'angiogenèse et les agents induisant la différenciation cellulaire ne causent probablement pas la mort des cellules cancéreuses. L'effet de ces deux thérapies est plutôt d'inhiber la prolifération de ces cellules pendant que le processus de mort cellulaire se poursuit conduisant ainsi à la diminution du cancer.[3]

Une autre cible importante des nouvelles thérapies est le cycle cellulaire (Figure 14). Il se divise en 4 phases successives soit la phase G_1 (intervalle de croissance), la phase S (intervalle de synthèse), la phase G_2 (intervalle de croissance et de réorganisation) et la phase M (intervalle de mitose). La progression à travers le cycle cellulaire est régulée par une série d'évènements très bien coordonnés. Les évènements importants de cette progression sont la synthèse, l'assemblage et l'activation de complexes formés de cyclines et de protéines kinases dépendantes des cyclines (Cdk), suivie par leur inactivation, dissociation et dégradation. Plusieurs mécanismes régulent la précision de ces étapes. Dans les cellules normales, les dommages de l'ADN sont repérés et le cycle est arrêté en G_1 par l'inhibition d'une Cdk. L'inhibition de cette Cdk est causée par la protéine p21, une protéine régulée par p53 qui est un gène suppresseur de tumeur. Il sert à détecter des dommages à l'ADN et à la mise en branle de sa réparation. Dans le cas où les dommages sont trop importants, p53 aura un rôle à jouer pour mener la cellule en apoptose. Il ne permet pas la division cellulaire tant que les réparations n'auront pas été faites. Ce gène est

muté dans plus de 50 % de tous les cancers humains, ce qui permet à l'ADN défectueux de se reproduire indéfiniment et ainsi accumuler plusieurs mutations.[96]

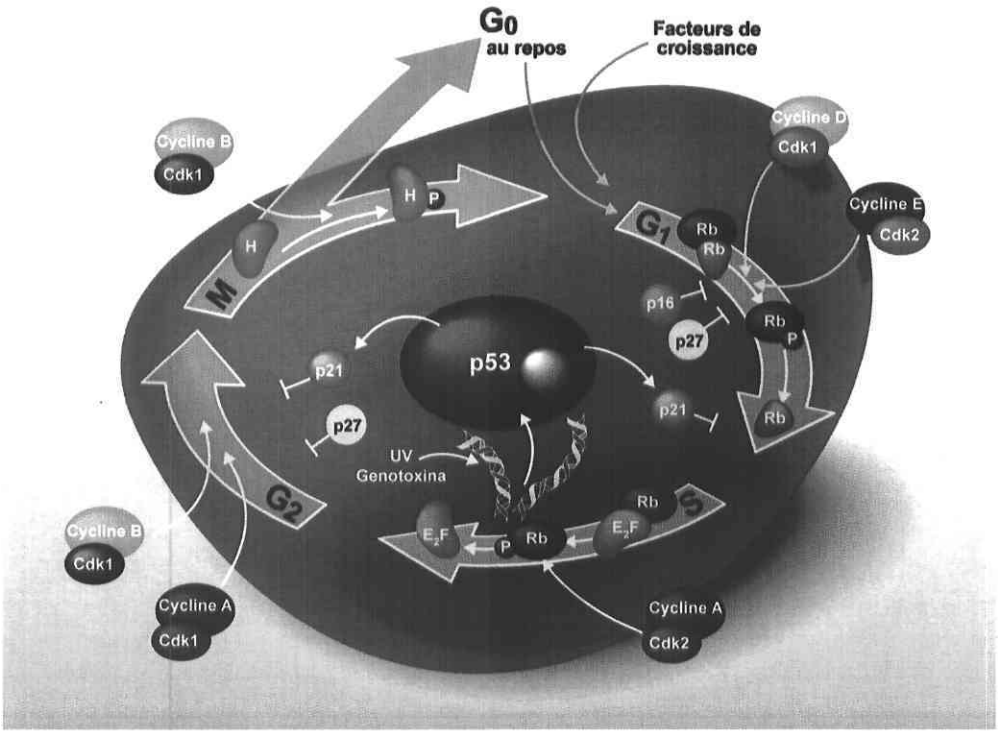


Figure 14. Le cycle cellulaire et les différents complexes Cdk/cycline ainsi que les quelques protéines inhibant les Cdks (p16, p27, p21).

Les cyclines sont évidemment une cible potentielle pour le traitement de la leucémie puisqu'elles ont la responsabilité de contrôler le cycle cellulaire. Donc, un inhibiteur de l'expression des cyclines D ou de leur interaction avec d'autres molécules, comme les Cdks, pourrait être un traitement efficace. Le UCN-01[97] et le flavopirodole[98] sont deux exemples d'inhibiteurs des Cdks. Ces produits sont présentement en études cliniques pour le traitement de la leucémie.

Une autre façon de perturber le cycle cellulaire serait d'inhiber une tyrosine kinase. Les tyrosines kinases ont un rôle important dans le développement des cancers en induisant la croissance des cellules cancéreuses par la phosphorylation des protéines de signalisation.

Le Gleevec est le premier inhibiteur tyrosine kinase à être utilisé en clinique. Ce produit, est un inhibiteur sélectif de la tyrosine kinase Bcr-Abl et un inhibiteur compétitif pour le site de liaison de l'ATP sur cette tyrosine kinase.[99] Il bloque le cycle cellulaire en G_0/G_1[100] et ne cause aucune inhibition lorsque la leucémie n'exprime pas la tyrosine Bcr-Abl.[101] Le Gleevec augmente le taux de rémission chez les patients atteints de LMC, comparé à la chimiothérapie conventionnelle.[102] La dose journalière recommandée lors d'un traitement avec le Gleevec est de 400 à 600 mg. Malgré son efficacité, plus de 10 % des patients ont ressenti plusieurs effets secondaires dont des nausées, des douleurs abdominales, des maux de tête et des vomissements. De plus, le développement d'une résistance au Gleevec peut entraîner une récidive en quelques mois.[103] Finalement, le coût annuel pour traiter une personne est d'environ 36 000$.

3.6.6 Les agents stéroïdiens

La famille des immunosuppresseurs est la seule à utiliser des agents composés d'un noyau stéroïdien pour le traitement de la leucémie. Ces stéroïdes, la prednisone, la prednisolone et le 6-méthylprednisolone induisent cependant de nombreux effets secondaires.[104] Mentionné précédemment, la 1,25-dihydroxyvitamine D_3, dérivée de la vitamine D, est utilisée comme agent de différenciation ou comme agent de chimioprévention puisque son action est très lente. Finalement, depuis plusieurs années, le 2-méthoxyestradiol est reconnu pour son potentiel dans le traitement du cancer du sein et de la prostate. Dernièrement, on a démontré qu'il induisait sélectivement l'apoptose des cellules leucémiques en causant des dommages oxydatifs et en perturbant la signalisation cellulaire.[105] Ce produit est toujours à l'étude.

3.7 Effets secondaires des traitements

La radiothérapie et la chimiothérapie cibles des cellules qui se divisent rapidement, comme la plupart des cellules cancéreuses. Cependant, cela implique que plusieurs cellules

saines à division rapide seront touchées. Ces cellules sont celles de la bouche, du transit intestinal, des cheveux, des ongles. Après un traitement, ces cellules meurent rapidement et se renouvellent par la suite. C'est ce renouvellement rapide qui entraîne les effets secondaires les plus courants comme la perte des cheveux et des lésions buccales.

Certains produits peuvent causer des douleurs abdominales et des douleurs osseuses (prednisolone). Parfois, certains traitements comme la doxorubicine, les anticorps monoclonaux et la radiothérapie peuvent provoquer des difficultés respiratoires, causées par des problèmes cardiaques. D'autres produits (vincristine, vinblastine,...) vont ralentir ou paralyser les intestins provoquant de la constipation. La diarrhée est fréquemment causée par la radiothérapie de l'abdomen ainsi que par des agents de chimiothérapie qui provoquent un débalancement des électrolytes, tels que le potassium et le sodium. Certains problèmes oculaires, comme des cataractes, peuvent être causés par la prednisone.

Il existe encore beaucoup d'autres effets secondaires, mais heureusement pour les patients, certains de ces effets peuvent être diminués ou traités par la prise de certains médicaments. Par exemple, le leucovorine permet de diminuer les effets secondaires du méthotrexate et le mesna protège la vessie des effets négatifs causés par des métabolites de la cyclophosphamide qui sont excrétés dans l'urine.

3.8 Aperçu du projet de recherche

L'un des deux projets de cette thèse de doctorat consistait à développer de nouveaux agents ayant un potentiel pour le traitement de la leucémie. La particularité de ces agents était leur structure générale puisqu'ils étaient tous composés d'un noyau stéroïdien. Comme mentionné précédemment, très peu d'agents thérapeutiques utilisés dans le traitement de la leucémie sont constitués d'un tel noyau. Donc, nous croyons que l'utilisation de ce noyau pourrait engendrer une nouvelle classe de produits qui, en plus d'agir selon un nouveau mécanisme, possèderait une sélectivité pour les cellules cancéreuses afin de diminuer leurs effets secondaires.

L'origine de ce projet est la publication en 1999, par le groupe de He et Jiang,[106] de résultats rapportant l'activité antiproliférative d'un aminostéroïde, soit le 2β-(4'-methylpipérazino)-5α-androstane-3α,17β-diol (HY) (Figure 15). Selon ces chercheurs, le HY inhibait la prolifération des cellules leucémiques humaines, HL-60, et induisait la différenciation chez ces mêmes cellules. Ils observaient une inhibition de 56 et 69 % à 1 et 10 μM respectivement, mais sans toutefois connaître son mécanisme d'action. Deux ans plus tard, la même équipe de recherche semblait abandonner le HY et publiait les résultats biologiques obtenus avec un deuxième aminostéroïde, le KH (Figure 15). Ce produit possédait la même structure que le HY, mais avec un carbonyle à la place d'un hydroxy en position 17. L'évaluation biologique du KH a été réalisée sur des cellules leucémiques murines, les WEHI-3B. Ces résultats démontraient que ce produit induisait lui aussi la différenciation cellulaire, mais que son pouvoir antiprolifératif semblait beaucoup moins efficace que celui du HY. Cependant, puisque ces deux composés ont été testés sur des lignées cellulaires différentes, il est difficile de comparer leur potentiel thérapeutique. Finalement, en 2005 cette équipe publia une nouvelle étude rapportant l'activité biologique du sel du KH.[107] L'activité antiproliférative de ce composé sur les HL-60 était comparable aux résultats obtenus au départ avec le HY.

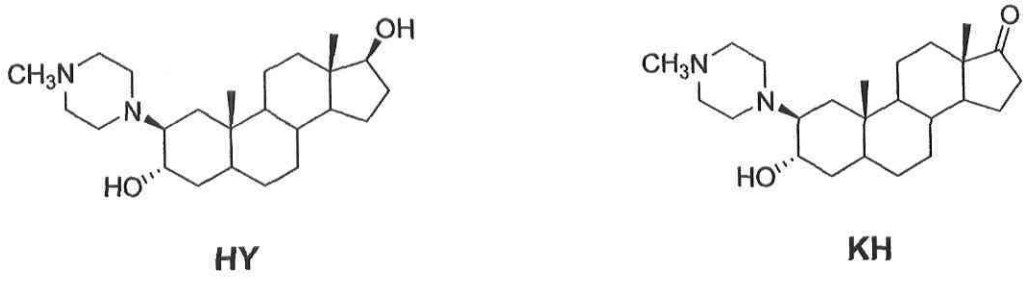


Figure 15. Structures des molécules du groupe de He Q.

Puisque notre laboratoire possédait une très bonne expertise dans le domaine de la chimie des stéroïdes[108-116] et que notre groupe avait développé une expertise pour la synthèse accélérée de dérivés stéroïdiens sur support solide,[117-123] dont certains avec un noyau pipérazine,[124] nous avons donc décidé d'élaborer des analogues du HY afin d'en

améliorer l'activité biologique. Dans un premier temps, nous avons effectué la synthèse du HY et du KH et évalué leur potentiel pour inhiber la croissance des cellules HL-60. Après plusieurs tests et à notre grande surprise, ces deux agents n'exerçaient pratiquement aucune inhibition. Par la suite, nous avons élaboré trois premières banques de produits dérivés du HY à l'aide de la chimie combinatoire sur support solide. La chimie combinatoire est une technique permettant de synthétiser rapidement de nouveaux composés, accélérant ainsi la découverte d'agents thérapeutiques. Par conséquent, à l'aide de cette technique, nous avons introduit un (composés **1**), deux (composés **2**) ou trois (composés **3**) niveaux de diversités sur le noyau pipérazine du HY (Figure 16). Ses niveaux étaient composés d'un ou deux acides aminés et/ou d'un acide carboxylique (Chapitre 2).

Par la suite, afin d'évaluer l'importance du noyau pipérazine ainsi que l'utilisation de différentes amines (cycliques, aliphatiques ou non), une nouvelle série de composés fut synthétisée mais cette fois-ci en utilisant la synthèse classique en solution ainsi qu'une nouvelle méthode d'aminolyse développée par notre équipe.[125] Cette étude nous a permis de générer environ 80 nouveaux aminostéroïdes (composés **4** et **5**, Chapitre 3) (Figure 16).

Évidemment, l'activité biologique des meilleurs représentants de ces deux familles a été approfondie. Lors de cette étude, l'activité cytotoxique, la sélectivité, leur effet sur le cycle cellulaire ainsi que le pouvoir d'induire la différenciation cellulaire ont été étudiés pour chacun de ces composés (Chapitre 4). Finalement, l'analyse de tous les résultats obtenus nous a conduit vers l'élaboration de trois nouvelles banques de composés à l'aide de la chimie combinatoire sur support solide (composés **6**) (Figure 16). L'activité antiproliférative de ces nouveaux dérivés du HY fut analysée (Chapitre 5).

Les travaux rapportés au cours de ces quatre chapitres nous ont permis d'obtenir quelques produits manifestant des activités antiprolifératives intéressantes avec des IC_{50} allant de 0.58 à 6.4 μM. L'étude exhaustive de l'activité biologique des meilleurs produits nous a permis de vérifier leur sélectivité et d'émettre quelques hypothèses pour leur mode d'action.

Finalement, depuis quelques années, le 2-méthoxyestradiol est connu pour avoir des propriétés antiangiogénique et antiproliférative sur plusieurs lignées cancéreuses. De plus, il induit l'apoptose chez différents types cellulaires et récemment, il a été rapporté pour induire l'apoptose chez les cellules leucémiques. Cependant sa faible biodisponibilité et son inactivation rapide nécessitent l'administration de grande quantité de produit pour inhiber la croissance des tumeurs. Nous savions que l'addition d'un sulfamate en position 3 du 2-méthoxyestradiol augmentait son activité biologique sur plusieurs lignées cancéreuses[126] mais l'effet de cette substitution sur les cellules leucémiques était encore inconnu. Nous avons donc décidé de vérifier cette activité sur les cellules leucémiques HL-60 ainsi que l'activité de dérivés du 2-méthoxyestradiol (composés **7**) (Figure 16). Certains de ces dérivés avaient déjà été synthétisés précédemment dans le cadre de d'autres projets, tandis que les nouveaux ont été réalisés à l'aide de la chimie classique en solution. Nous avons donc vérifié l'effet sur les cellules leucémiques de différentes substitutions sur les cycles A et D du 2-méthoxyestradiol (Chapitre 6).

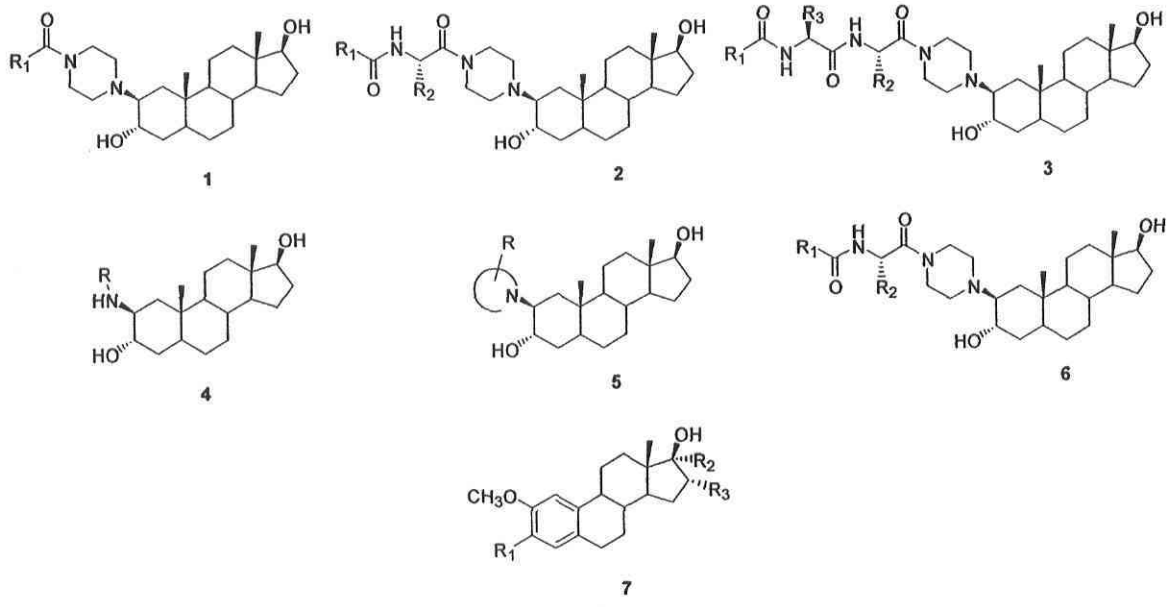


Figure 16. Structures générales des différents dérivés stéroïdiens (composés **1-7**) envisagés pour le traitement de la leucémie.

Résultats

Chapitre 1

Synthèse chimique et activité biologique de dérivés en position 16α du 5α-androstane-3α,17β-diol comme antiandrogènes

1.1 Avant-propos

Ma contribution à ce premier projet a été de réaliser la synthèse de la majorité des produits mentionnés dans ce manuscrit et Hervé Do, un stagiaire, a effectué la synthèse de quatre composés. J'ai de plus caractérisé par IR, RMN ^{1}H, RMN ^{13}C et LRMS tous les intermédiaires de synthèse et les produits finaux afin de confirmer leur structure. Rock Breton a effectué la modélisation moléculaire, Céline Martel a supervisé l'évaluation biologique et Fernand Labrie a accordé son soutien financier pour cette évaluation biologique. J'ai finalement rédigé ce manuscrit en collaboration avec mon directeur de recherche Donald Poirier et il sera bientôt soumis pour publication à la revue *Bioorganic & Medicinal Chemistry.*

1.2 Résumé

Dans le but de développer des nouveaux antiandrogènes plus efficaces, nous avons envisagé que certaines modifications sur les cycles A et D de la dihydrotestostérone (DHT) permettrait d'obtenir des produits ayant une forte affinité avec le récepteur des androgènes de même que des propriétés antagonistes intéressantes. Nous avons donc synthétisé une série de dérivés du 5α-androstane-3α,17β-diol avec une chaîne alkyle de 3 méthylènes en position 16α, mais portant différents groupements fonctionnels à son extrémité. Parmi tous ces dérivés, le chlorure induisait l'activité antiproliférative la plus importante sur les cellules androgéno-sensibles Shionogi. La substitution du OH en position 3 par un groupement méthoxy a montré l'importance de ce OH. D'ailleurs, sa transformation en cétone a augmenté la force de la liaison avec le récepteur des androgènes, mais a diminué l'activité antiproliférative en induisant un effet agoniste. Ces résultats ont confirmé l'importance d'utiliser un noyau 5α-androstane-3α,17β-diol à la place d'un noyau DHT. Des chaînes de longueurs variables, 2, 3, 4 et 6 méthylènes en position C-16α, ont été évaluées et la longueur optimale a été celle de 3 méthylènes. Bien que possédant une faible affinité avec le récepteur des androgènes, le 16α-(3'-chloropropyl)-5α-androstane-3α,17β-diol (**15**) exerce une activité antiproliférative sur les cellules androgéno-sensibles Shionogi similaire à celle obtenue avec le pur antiandrogène non-stéroïdien hydroxy-flutamide (77 et 67 %, respectivement à 0.1 μM). Le nouveau composé stéroïdien **15** est un bon point de départ pour le développement de futurs antiandrogènes ayant un potentiel thérapeutique contre le cancer de la prostate.

Bioorg. Med. Chem. (submitted)

Chemical synthesis and biological activities of 16α-derivatives of 5α-androstane-3α,17β-diol as antiandrogens

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Abstract

In our efforts to develop compounds with therapeutic potential as antiandrogens we examined the possibility that suitable modifications on the A and D-ring of dihydrotestosterone (DHT) nucleus could provide high affinity to the androgen receptor (AR) as well as antagonist properties. We thus synthesized a series of 5α-androstane-3α,17β-diol derivatives with a fixed side chain length of 3 methylenes at C-16α, but bearing a diversity of functional groups at the end. Among these, the chloride induced the best antiproliferative activity on androgen-sensitive Shionogi cells. Substituting the OH at C-3 by a methoxy group showed the importance of the OH. Moreover, its transformation into a ketone increased AR binding but decreased the antiproliferative activity and induced an agonist effect on AR. These results confirm the importance of keeping a 3α,17β-diol 5α-androstane nucleus instead of a DHT nucleus. Variable side-chain lengths of 2, 3, 4 and 6 methylenes at C-16α were investigated and the optimal length was found to be 3 methylenes. Although exhibiting a weak AR binding affinity, 16α-(3'-chloropropyl)-5α-androstane-3α,17β-diol (**15**) provided an antiproliferative activity on androgen-sensitive Shionogi cells similar to that of pure non steroidal antiandrogen hydroxyl-flutamide (77 and 67 %, respectively at 0.1 μM). The new steroidal compound **15**, thus constitutes a good starting point for development of future antiandrogens with a therapeutic potential against prostate cancer.

Introduction

Prostate cancer is the most frequently diagnosed cancer in men in the United States, accounting for 33 % of all cancers. It is estimated that 232,090 new cases of prostate cancer will be diagnosed and 30,350 men will die from this disease in 2005.[1] Androgens testosterone (T) and dihydrotestosterone (DHT) play an important role in the development, growth, and progression of prostate cancer.[2-5] Androgen receptor (AR) binds the male sex steroids, DHT and T, and regulates genes for male differentiation and development.[6] Therefore, mutations in the AR gene may lead to several diseases or conditions like prostate cancer or the androgen insensitivity syndrome.[7] Since an essential step in the action of androgens in target cells is binding to the receptor, a logical approach for neutralizing the action of androgens is the use of antiandrogens or compounds which prevent the interaction of T and DHT with the AR. Androgen ablation therapy has been shown to produce the most beneficial responses in multiple settings in prostate cancer patients.[8] Several studies have reported that a combination therapy of orchidectomy with antiandrogens, to inhibit the action of adrenal androgens, significantly prolongs the survival.[7-13] Since prostate cancer is so highly sensitive to androgens, the antiandrogen used must be a compound having high specificity and affinity for the AR while not possessing any androgenic, estrogenic, progestational, glucocorticoid or any other hormonal and antihormonal activities.[14,15]

Since even the best treatment of advanced or metastatic prostate cancer can only prolong life with minimal or no possibility of cure,[9,16-20] it is important to increase the efficiency of known treatments. One of the strategies investigated is to develop a much more potent antiandrogen than flutamide, a compound known to have a very weak AR binding affinity.[14] An interesting improvement was the development of selective androgen receptor modulators (SARMs), which required the synthesis of numerous non-steroidal compounds.[21,22] Several steroid scaffolds, such as cyproterone acetate,[23] spironolactone[24] and 4-aza-heterocycle steroid,[25] have also been reported as antiandrogens in the past, but their development was less impressive than non steroidal antiandrogens.

From a study about inhibition of the steroidogenic enzyme type 3 17β-hydroxysteroid dehydrogenase,[26] we recently reported an interesting antiandrogenic profile for the synthetic 16α-androstane derivative **1** (16α-(3'-bromopropyl)-5α-androstane-3α,17β-diol) (Figure 1). Furthermore, its antiproliferative activity on androgen-sensitive Shionogi cells is mediated through direct interaction with AR as supported by the relative AR binding affinity of compound **1**. With the aim of extending this exploratory work, a series of 16α-peptidosteroids represented by structure **2** was synthesized using solid-phase synthesis in parallel fashion.[27] The screening of the generated model libraries revealed interesting preliminary structure-activity relationships (SAR) related to their antiproliferative activities on Shionogi cells, but these activities were weak and not mediated by AR. We then decided to extend our SAR study by focusing more closely on the lead compound **1**. In this article, we report the synthesis of androstane derivatives **3**, which are variously substituted at the end of the C-16α-side chain of 3 methylenes or differently modified at C-3 to modulate the biological activities. Focusing on two optimal substitutions, another series of 5α-androstane-3α,17β-OH derivatives bearing a bromoalkyl or chloroalkyl chain of variable length at C-16α (compounds **4**) was synthesized. In addition to the chemical synthesis, proliferative and antiproliferative activities on Shionogi cells as well as the binding affinities for AR were determined. A modeling study was also performed in an attempt to analyze interactions between a representative compound and AR.

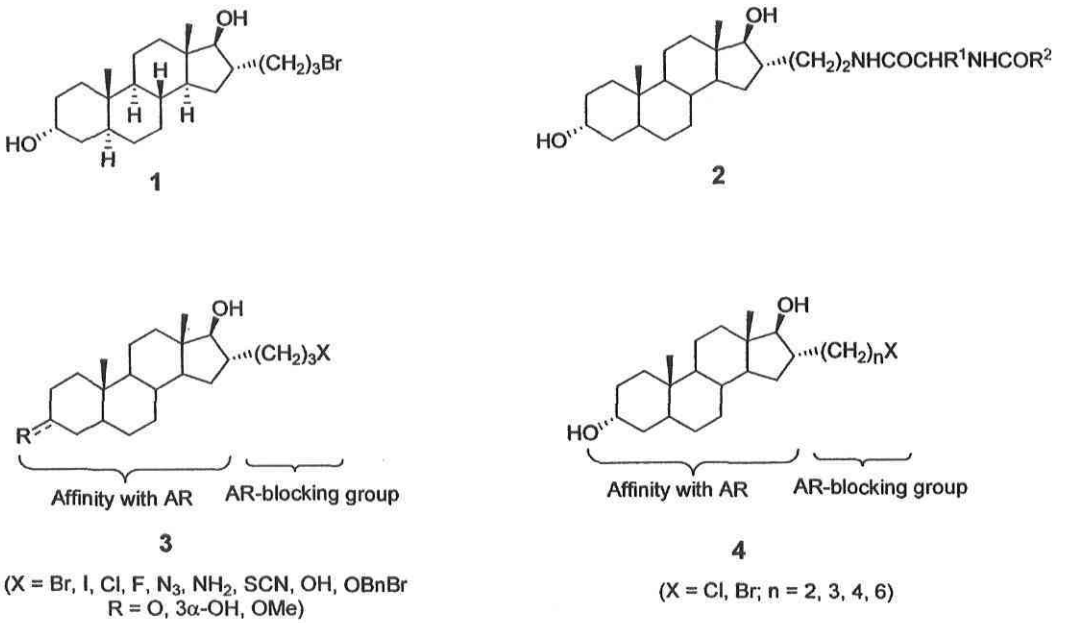


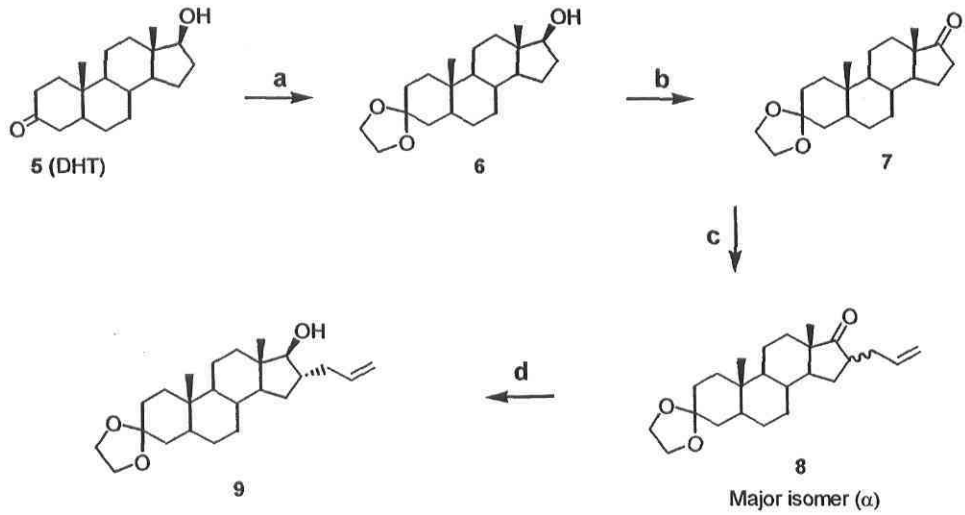
Figure 1. Chemical structures of 5α-androstane-3α,17β-diol derivatives **1** and **2** previously prepared as potential antiandrogens and general structures of analogues **3** and **4** that will be synthesized to extend our SAR study. The stereogenic centers are illustrated only for steroid **1**, but they are the same for all other steroid derivatives reported in this paper.

Results and discussion

Chemical Synthesis

Dihydrotestosterone (**5**) was the starting material for the synthesis of all compounds. First, we synthesized steroid precursor **9** through a sequence of reactions described in Scheme 1. The carbonyl group of **5** was protected as ketal and the 17β-hydroxy group of **6** was oxidized with TPAP and NMO to obtain ketone **7**. An alkylation in alpha position of the carbonyl was then performed to give **8**, a mixture of 16α-allyl and 16β-allyl stereoisomers (90/10, evaluated by the ^{1}H NMR signal of CH_3-18). After purification by chromatography, a mixture of the major isomer α and minor isomer β (2 %) was stereoselectively reduced using $LiAlH_4$ at low temperature to afford the secondary alcohol

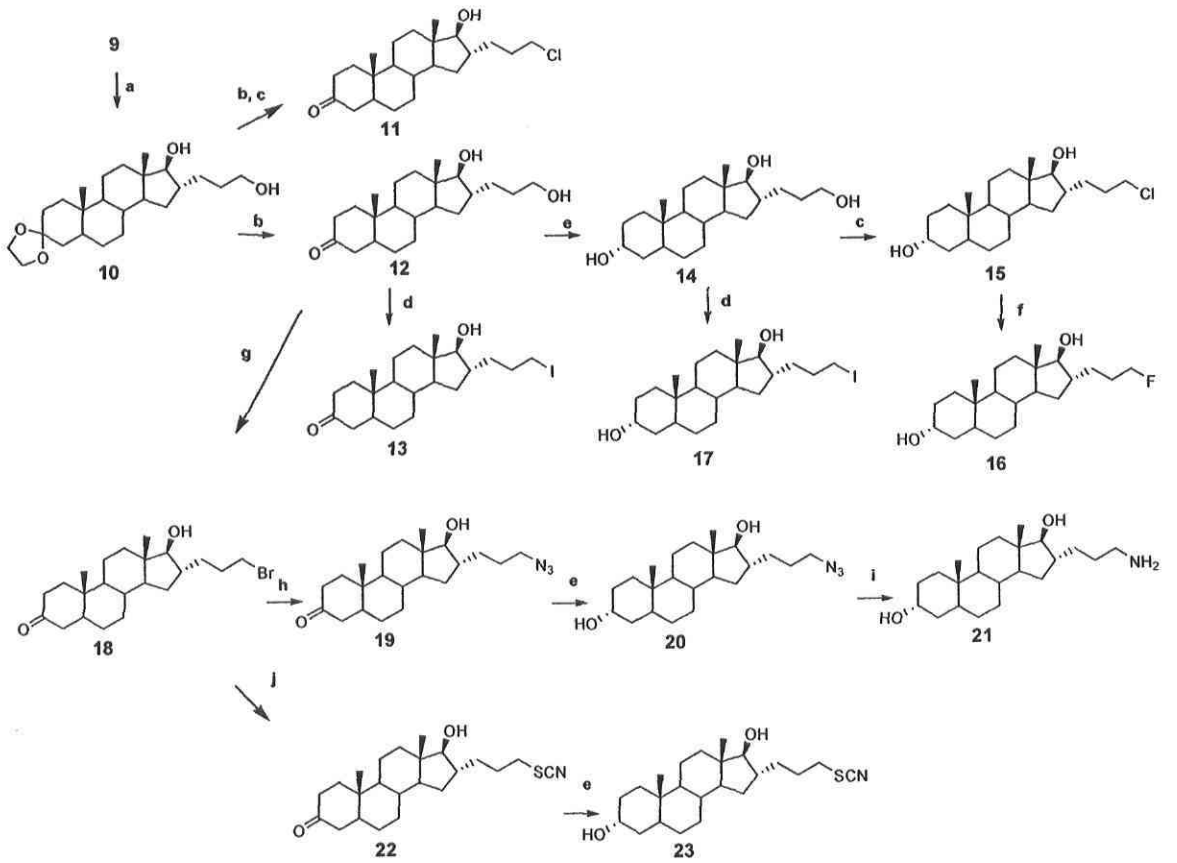
9. As previously observed for related compounds,[26, 28, 29] ^{1}H and ^{13}C NMR signals at C-17 confirmed the C-16α and 17β orientation of the allyl and OH groups, respectively.



Scheme 1. Synthesis of **9**. Reagents and conditions: **(a)** $(CH_2OH)_2$, p-TSA, benzene, Dean-Stark, reflux, 24 h; **(b)** TPAP, NMO, molecular sieves, CH_2Cl_2, rt, 1.5 h; **(c)** LDA (DIPA + BuLi), CH_2=$CHCH_2Br$, THF, -78 °C, 16 h; **(d)** $LiAlH_4$, THF, -78 °C, 6 h.

For the first part of our study, compound **9** was submitted to an oxidative hydroboration yielding diol **10**. Subsequently, several derivatives having a different functionality at the end of a propyl side-chain were generated using strategies reported in Scheme 2. Chloride **11** was obtained from **10** after ketal hydrolysis and substitution of the hydroxy group using CCl_4 and PPh_3. Under these conditions, the hindered secondary alcohol at the C-17β position was not reactive. Diol **12** was easily obtained after deprotection of **10**. The primary alcohol of **12** was either substituted by an iodide giving **13** or reduced to give triol **14**. We selected potassium tri-*sec*-butyl-borohydride (K-Selectride) as the reducing agent, because this reagent[30] gave mainly the 3α-OH isomer, which was confirmed using ^{1}H and ^{13}C NMR data reported in the literature.[31,32] Alcohol **14** was substituted by a chloride to afford **15**, which was substituted by a fluoride to obtain **16**. Iodide **17** was generated directly from alcohol **14**, because it was not possible to reduce **13** into **17**. Indeed, this kind of iodoalkyl is sensitive to K-Selectride reducing conditions. The intermediate compound **12** was also substituted using CBr_4 and PPh_3 to produce bromide

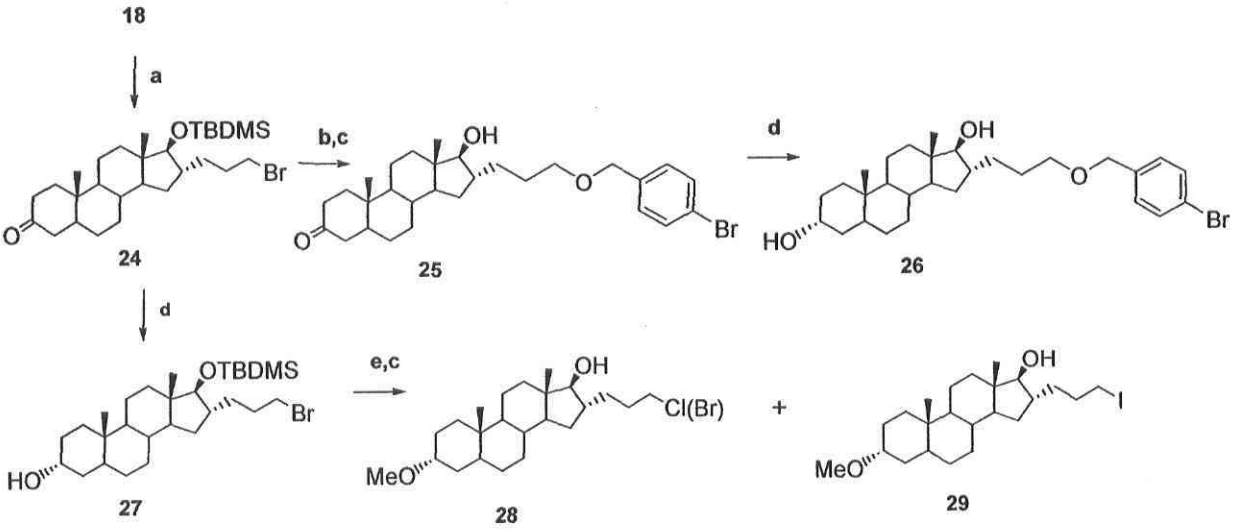
18. This compound was substituted by an azide to give **19**, which was reduced at the C-3 position giving **20**. Catalytic hydrogenation of azide group provided the free amine **21**. The bromide of **18** was also substituted by KSCN in ethanol to give **22** and this ketone reduced to generate alcohol **23**.



Scheme 2. Synthesis of **10-23**. Reagents and conditions: **(a)** *i*) BH_3·THF, 0 °C, 3 h, *ii*) H_2O_2, NaOAc, H_2O, 0 °C to rt, 3 h; **(b)** HCl 10 %, acetone, rt, 5 h; **(c)** PPh_3, CCl_4, CH_2Cl_2, 0 °C to rt, 1 h; **(d)** PPh_3, I_2, CH_2Cl_2, imidazole, 0 °C to rt, argon, 2 h; **(e)** K-Selectride/THF (1 M), -80 °C, 2 h; **(f)** TBAF, THF, reflux, argon, 5 h; **(g)** PPh_3, CBr_4, CH_2Cl_2, 0 °C to rt, 2 h; **(h)** NaN_3, CH_3CN, argon, 80 °C 48 h; **(i)** Pd/C, MeOH, H_2, 3 h; **(j)** KSCN, EtOH, reflux.

We next tried introducing an aromatic group at the end of the 16α side-chain by preparing compounds **25** and **26** (Scheme 3). The secondary alcohol of **18** was first protected as a tert-butyldimethylsilyl ether derivative **24** using TBDMS-OTf and 2,6-lutidine. The bromide of **24** was substituted by a 4-bromobenzyloxy group and the TBDMS

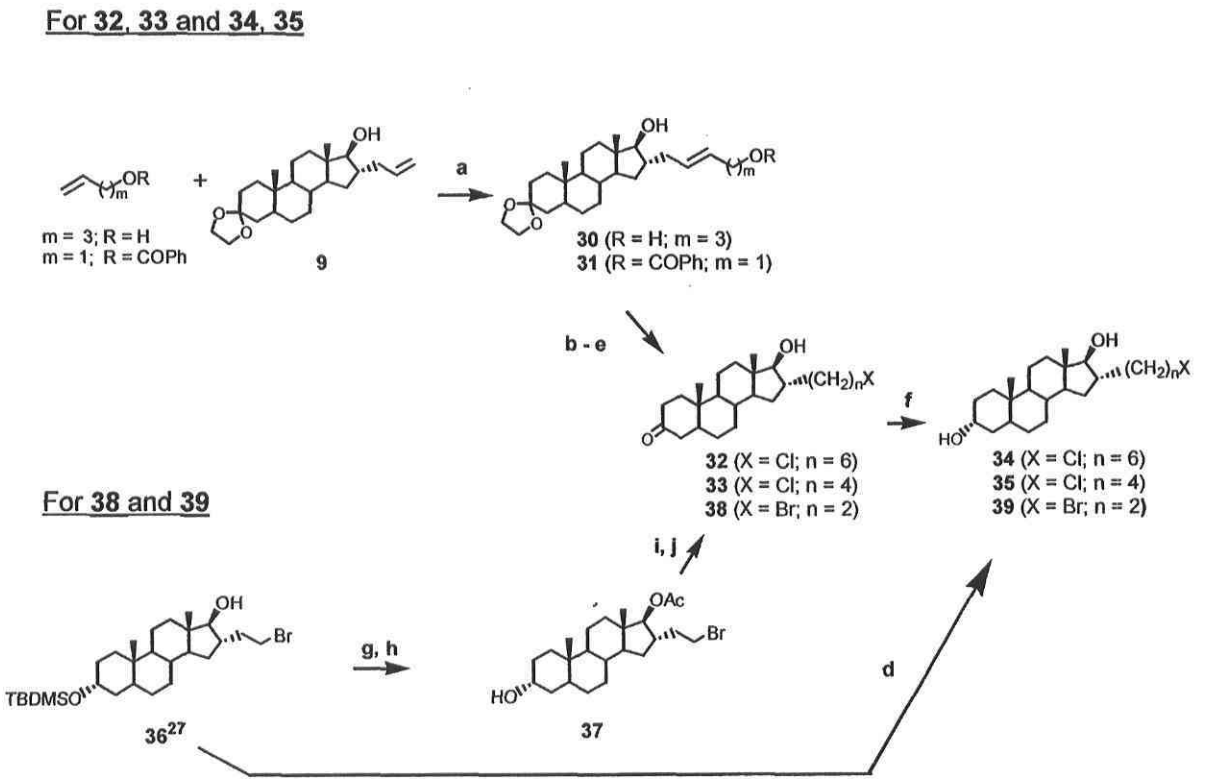
intermediate was hydrolysed to afford **25**, which, after carbonyl reduction with K-Selectride, afforded **26**. The methylation of the OH at position 3 of the steroid was also investigated. To obtain **28**, ketone **24** was reduced and intermediate **27** was methylated using NaH and MeI. This S_N2 reaction also gives the iodo analogue. The mixture of bromide/chloride and iodo derivatives was then hydrolysed to remove the TMDBS group, giving two new target compounds **28** and **29**.



Scheme 3. Synthesis of **24-29**. Reagents and conditions: **(a)** *i*) 2,6-lutidine, TBDMS-OTf, CH_2Cl_2, -78 °C, argon, 6 h, *ii*) aqueous HCl ; **(b)** AgOTf, 4-bromobenzyl alcohol, 2,6-di-tert-butyl-4-methyl-pyridine, CH_2Cl_2, 0 °C to rt, argon; **(c)** HCl, MeOH, CH_2Cl_2, 2 h; **(d)** K-Selectride, THF (1 M), -80 °C, 2 h; **(e)** NaH, MeI, 15-crown-5, THF, 0 °C to rt, argon.

For the second part of our study, we needed to prepare compounds with various side-chain lengths at C-16α. The synthesis of the compounds with the two longer side-chains required a Grubbs's metathesis between the key intermediate **9** and alcohols protected or not as a benzyl ester (Scheme 4). Using a second-generation ruthenium catalyst[33-35] allowed us to generate in reasonable yields compounds **30** and **31** with 6- and 4-carbon chains respectively at position 16α. Chloride **32** and **33** were generated by the following sequence of reactions: a double bond hydrogenation, a subsequent ester hydrolysis (for **31** only), a ketal hydrolysis and the substitution of the primary alcohol using CCl_4 and PPh_3. At this point, part of ketones **32** and **33** was reduced at C-3 to give alcohols

34 and **35**. Compounds **38** and **39** bearing a bromoethyl chain were synthesized from **36**, which was available in our laboratory.[27] This alcohol was first acetylated and the TBDMS protecting group was thereafter removed in a THF solution of HF/pyridine leading to hydroxyl derivative **37**. This later was oxidized using TPAP and NMO and the acetyl group was removed with potassium carbonate to give **38**. To obtain **39**, the TBDMS of **36** was hydrolysed under acid conditions.



Scheme 4. Synthesis of **30-39**. Reagents and conditions: **(a)** Grubbs's catalyst, CH_2Cl_2, reflux, 16 h; **(b)** H_2, Pd/C, EtOAc, rt, 16 h; **(c)** NaOH (10 % aq.), MeOH, rt, 1.5 h; **(d)** HCl 10 %, acetone, rt, 5 h; **(e)** PPh_3, CCl_4, CH_2Cl_2, 0 °C to rt, 1 h; **(f)** K-Selectride, THF (1 M), -78 °C, 1.5 h; **(g)** Ac_2O, pyridine, DMAP, rt, 2 h; **(h)** HF/pyridine, THF, rt, 60 h; **(i)** TPAP, NMO, molecular sieves, CH_2Cl_2; **(j)** K_2CO_3, MeOH.

Biological activity

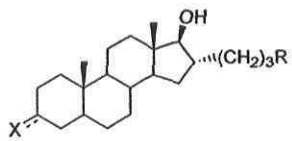
The antiproliferative activity of synthesized compounds on androgen-sensitive mammary carcinoma Shionogi cells is reported as the percentage (%) of inhibition. It corresponds to the ability of a compound to inhibit the cell proliferation induced by 0.3 nM of the natural potent androgen DHT and is compared to that of hydroxyflutamide, which is a potent *in vitro* antiandrogen.[36,37] The proliferative activity is reported as the percentage (%) of cell stimulation induced by a tested compound relative to stimulation (100 %) induced by 0.3 nM of DHT. Moreover, it is important to determine whether or not these compounds bind to androgen receptor (AR), in order to find out whether the antiproliferative activity is mediated by this receptor. The results are presented in Table 1.

First of all, substituting the bromide on the side-chain end with a diversity of functional groups leads to interesting SAR information. Clearly the substitution of the bromide atom of **1** by a more polar group such as OH, NH_2 and SCN (compounds **14**, **21** and **23**) is detrimental to the antiproliferative activity. The same result is also observed with a very bulky group such as the bromobenzyloxy of **26**. Among the series of halogeno derivatives **15**, **16** and **17**, chloride **15** induced a higher antiproliferative activity – 77 % – than all other compounds at 0.1 μM. Although halogens are good leaving groups, the results obtained with **1**, **15**, **16** and **17** suggest that these compounds do not work by formation of a covalent bond with AR or another target. Like chloride **15**, azide **20** also showed a good antiproliferative activity of 60 % at 0.1 μM. Among all these new compounds, none produced proliferative activity at 0.1 μM, except compound **26**. Binding affinity of all these 3α-OH derivatives for AR was also evaluated but none showed an important affinity.

In the light of these results, we decided to evaluate the importance of the 3α-OH group of these new derivatives by performing two modifications. Firstly, substituting the 3α-alcohol by a methoxy group (**28** and **29**) induced a drop of antiproliferative activity and binding affinity for AR. Secondly, with the aim of increasing the antagonistic effect and AR binding affinity, we prepared and tested a series of ketones. As expected, the presence

of a carbonyl at C3 greatly improved the AR binding. These better affinities to AR were however associated with a proliferative activity ranging from 9 to 153 % (100 % for DHT). The increase of binding and proliferative activity was the least important for alcohol **12** but remarkable for **25**. In fact, the proliferative activity of **25** (153 %) is stronger than that of DHT (100 %) and similar to that of **26** (163 %), the 3α-OH analogue. Thus the effects of **25** and **26** on Shionogi cell proliferation are not mediated by AR.

Table 1. Antiproliferative activity, proliferative activity and AR binding affinity[a].



Compounds	X	R	Antiproliferative activity (%)[b]		Proliferative activity (%)[c]	AR binding[d]	
			0.1 µM	1 µM	0.1 µM	0.1 µM	1 µM
1	3α-OH	Br	42	88	0	25	68
14	3α-OH	OH	21	45	0	3	4
15	3α-OH	Cl	77	97	0	5	36
16	3α-OH	F	43	76	0	1	12
17	3α-OH	I	39	96	0	17	53
20	3α-OH	N_3	60	93	0	8	33
21	3α-OH	NH_2	0	0	1	2	4
23	3α-OH	SCN	2	50	5	11	38
26	3α-OH	OCH_2PhBr	0	7	163	10	34
28	3α-OMe	Cl (Br)	18	99	0	5	11
29	3α-OMe	I	0	46	0	3	9
11	O	Cl	44	42	36	93	99
12	O	OH	54	46	9	17	66
13	O	I	41	76	22	95	99
19	O	N_3	73	58	33	92	99
22	O	SCN	0	45	21	90	98
25	O	OCH_2PhBr	0	0	153	95	99
OH-Flu[e]			67	100	0	5	33
RU1881[f]			-	-	-	97	100
T[g]			-	-	-	72	95
DHT[g]			-	-	100	94	95

[a]Error was ± 5 %

[b]Percentage of antiproliferative activity on Shionogi cells at 0.1 and 1 µM of tested compounds. The cells were previously stimulated by 0.3 nM of androgen DHT.

[c]Percentage of proliferative activity on Shionogi cells at 0.1 µM of tested compounds. The stimulation induced by 0.3 nM of androgen DHT was set as 100 %.

[d]Percentage of binding affinity on AR at 0.1 and 1 µM of tested compounds. The binding of RU1881 set as 100 %.

[e]OH-Flu: hydroxy-flutamide (pure antiandrogen).[36-38]

[f]Synthetic potent androgen.

[g]Natural androgen.

Compared to **25** or DHT, the proliferative effects of ketones **11**, **13**, **19** and **22** were weaker (21-36 % at 0.1 μM), but are representative of compounds with both agonist and antagonist activities on AR. Indeed, the presence of a carbonyl group at position 3 of the androstane derivatives leads to mixed antiandrogens (Figure 2), confirming the importance of keeping the 3α-OH.

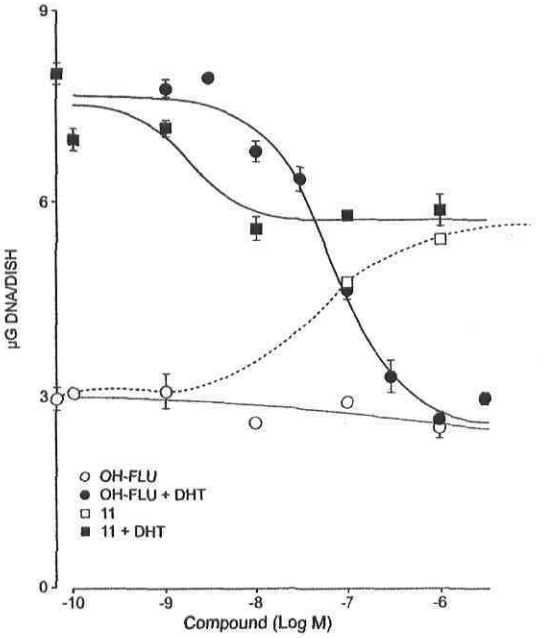


Figure 2. Proliferative and antiproliferative effects induced by ketone **11** on Shionogi cells. The concentration of DHT is 0.3 nM. The pure antiandrogen OH-Flu was used as control.

After we determined that a 3α-OH and a Cl atom at the end of the 16α-side chain formed the best combination, we wanted to find the optimal side-chain length (2, 3, 4 or 6 methylenes), the one that induces the best antiproliferative activity (Figure 3). Compound **15** with a side-chain length of 3 methylenes displayed the greater potency. In fact, at concentrations of 0.1 μM and 1 μM, this chloride derivative inhibited 77 and 97 % of DHT stimulation, respectively. The antiproliferative activity was however reduced with both a shorter and a longer side chain. As reported in Figure 4, the curve of **15** is comparable to those obtained with the non-steroidal pure antiandrogen hydroxy-flutamide. Furthermore, these two compounds have a weak binding affinity on AR (2.1 and 0.16 for OH-Flu and **15**, respectively).

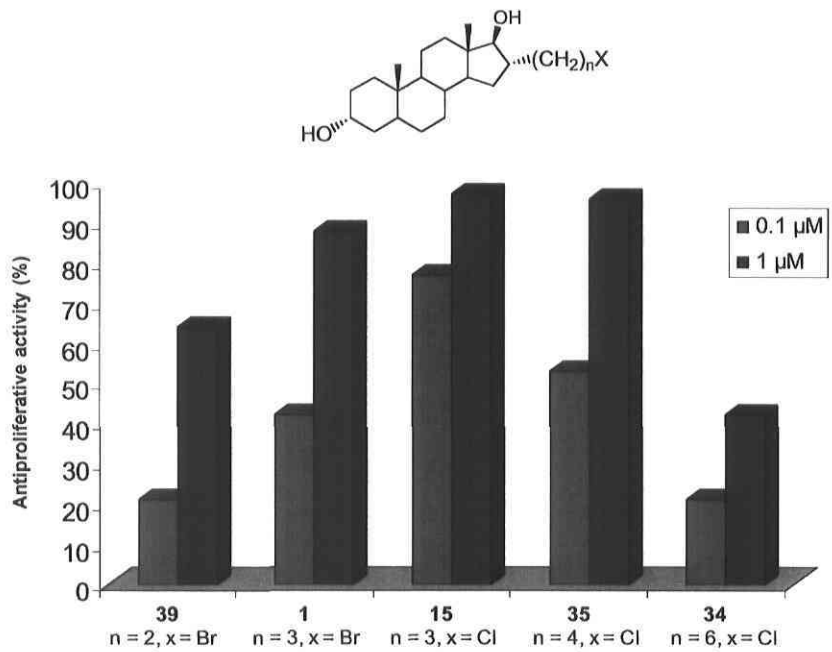


Figure 3. Antiproliferative activity on Shionogi cells as a function of the side-chain length at C16α. The cells were previously stimulated by 0.3 nM of DHT. Error was ± 4 %.

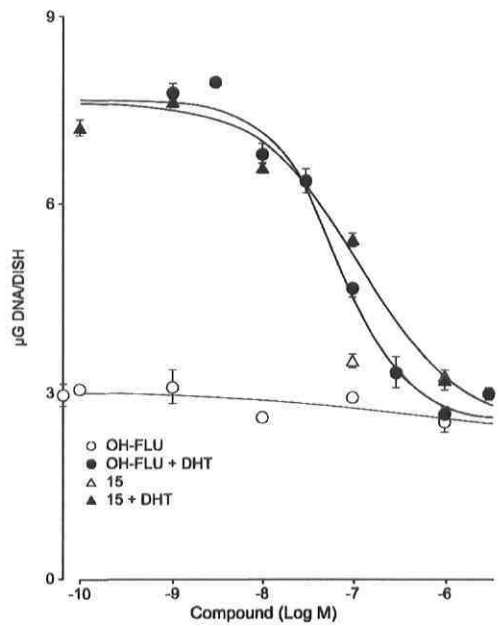


Figure 4. Proliferative and antiproliferative effects induced by a ketone in position 3 (compound **15**) on Shionogi cells. The concentration of DHT is 0.3 nM. The pure antiandrogen OH-Flu was used as control.

Molecular modelling

In order to understand the antiproliferative activity demonstrated by 16α-(3'-chloropropyl)-5α-androstane-3α,17β-diol (**15**), we tried to identify the preferred binding position of lead compound **1** within the AR ligand-binding domain (LBD) by modeling and energy minimization (Figure 5). The AR-LBD is mainly composed of α-helices arranged as a three-layered antiparallel α-helical sandwich, a fold common to all the steroid receptors.[39] The ligand-binding pocket (LBP) is mainly composed of hydrophobic residues, the side chains of which can easily adopt variable positions in order to better fit the hydrophobic core of the steroid and stabilize it. When binding an agonist, the top of the LBP is closed by an α-helix structure at the carboxyl-terminal end of the receptor, helix 12 (H12).[7, 40-42] A precise positioning of this helix is required for activation of AR[40] and, as shown by crystallographic studies made on the human estrogen receptor, a different positioning of H12 could account for the functional differences between agonists and antagonists.[43] This position shift would prevent coactivators from binding to the receptor. Structural modeling of **1** bound inside the LBP of AR and comparison with crystal structures of AR-LBD in complex with agonist ligands, DHT and testosterone,[42] suggest that **1** is well stabilized through numerous van der Waals contacts with hydrophobic residues delineating the LBP and by hydrogen bonds established at both extremities of its steroidal nucleus. Its 16α-(3'-bromopropyl) side chain is oriented toward the main chain of helix 11 and clashes with the side chain of one of its residues, Phe876. However, Söderholm et al[44] reported that the presence of a small favored volume for acceptor interactions close to the solvent accessible surface, located near the amide group of residue Leu880 and the carbonyl oxygen of Phe876. The possibility thus exists that this interference with helix 11 has an impact on the positioning of the C-terminal end of the receptor and causes a misplacement of H12 leading to the generation of the antagonist-like conformation of AR. These interactions could explain the antagonistic effect of compounds **1** and **15**. Moreover, the agonistic effect of the latter's analogue, compound **11**, could be explained by a higher AR binding affinity due to the presence of a ketone function at C-3, like in DHT. Since the oxygen atom of this ketone group has a lone pair of electrons, it could act as a hydrogen bond acceptor able to establish a strong interaction with a charged amino acid, Arg752 like DHT does.[42] Thus, the specific

interaction involving the C-3 ketone may orient the steroid core similarly to DHT and thus reduce the efficiency of the alkyl chain in repositioning Helix 12.

A)

B)

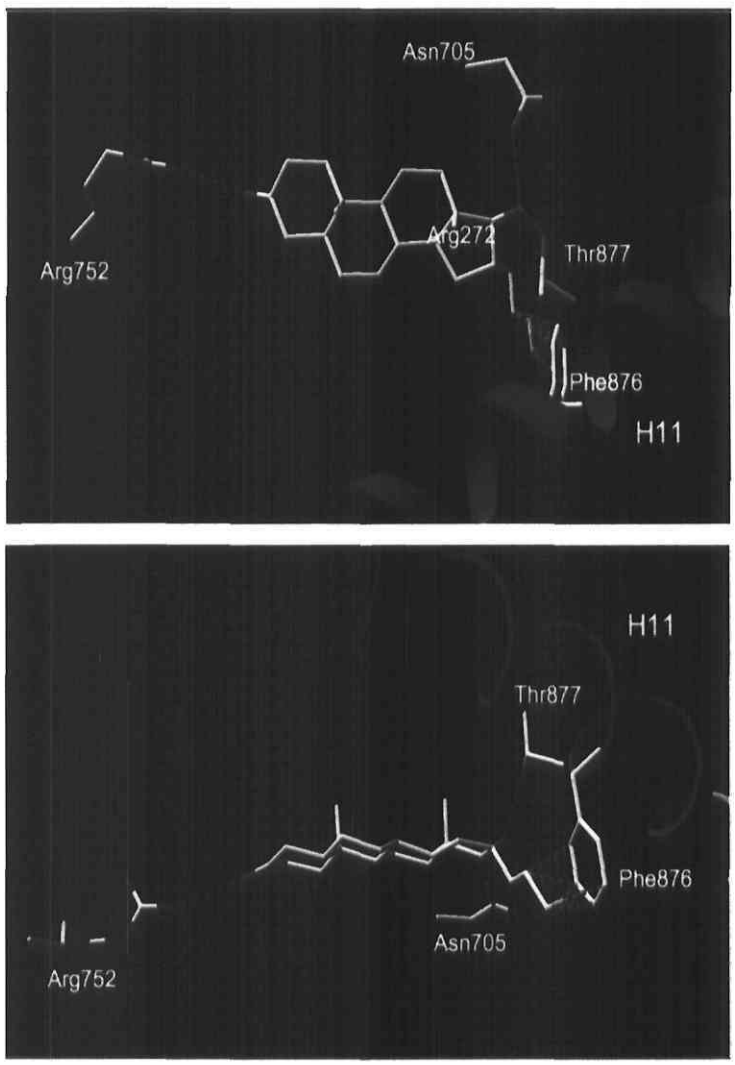


Figure 5. Hypothetical position of compound **1** in the hAR ligand binding site.
Schematic views (β-face of the steroid nucleus (A) and about 90° rotated view (B)) of **1** in the optimal binding position. Putative hydrogen bonds established by the ligand with the receptor (residues Arg752, Asn705, and Thr877) are indicated by broken green lines. Possible contacts between the C16α-side chain of the ligand and the Phe876 residue of helix 11 (see text) are indicated by broken pink lines. Carbon atoms are depicted in white, oxygen atoms in red, nitrogen atoms in blue, and bromine atom in orange. The Figures were generated with Swiss-PdbViewer.[45]

Conclusion

We have synthesized a novel series of antiandrogens by adding a short halogen alkyl side chain at position 16α of a 5α-androstane-3α,17β-diol nucleus. Among the series of functional groups attached at the end of the side chain, we found that an azide or halogen best improves the antiproliferative activity on Shionogi cells, especially when this element of diversity is a chloride. Moreover, the optimal side chain length was found to be 3 methylenes. However, all of these compounds displayed a weak binding affinity for the AR. We also showed the importance of a 3α-hydroxy group since its replacement with a methyl decreased biological activity. Moreover, the presence of a ketone at position 3 brought a better binding to AR, but unfortunately induced proliferation of androgen-sensitive Shionogi cells. These opposite effects certainly explain the poor antiandrogenic activity of these keto compounds at higher concentration. Among the compounds tested, the 3α-hydroxy compound **15** with a chloropropyl 16α-side chain displayed the highest antiandrogenic activity on Shionogi cells. Its inhibition of DHT cells stimulation is comparable to that obtained with the non-steroidal antiandrogen hydroxy-flutamide. Furthermore, compound **15** showed no proliferative agonistic activity on Shionogi cells.

Based on modeling studies we tried to explain how compound **1** antagonizes the AR. It was proposed that the halogen atom of the 16α chain of the steroid likely makes contacts with residues of helix 11, leading to a different positioning of helix 12 and thus preventing coactivators to bind to the receptor. Additional studies will however be necessary to better understand the mechanism of action of compounds **1** and **15**. Moreover, a better understanding of the key interactions between **15** and AR will contribute to the design of a second generation of this new family of antiandrogens with improved biological activity.

Experimental

General methods for chemical synthesis

Dihydrotestosterone (DHT) was obtained from Steraloids (Newport, RI, USA) and chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Nepean, ON, Canada) and VWR International (Montréal, Qc, Canada) and were used as received. Anhydrous solvents were purchased from Aldrich and VWR in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. Flash chromatography was performed on Silicycle 60 (Québec, Qc, Canada) 230-400 mesh silica gel. Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel 60 F_{254} plates (Whatman, Madison, UK) and compounds were visualized by exposure to UV light (254 nm) or a heated solution of ammonium/sulphuric acid/water. Infrared (IR) spectra were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only the significant bands reported in cm^{-1}. ^{1}H and ^{13}C spectra were recorded with a Brucker AC/F300 spectrometer (Billerica, MA, USA) at 300 and 75.5 MHz, respectively, and a Bruker AVANCE 400 spectrometer at 400 (^{1}H) and 100.6 (^{13}C) MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.16 ppm), acetone (2.05 and 206.26 ppm) or methanol (3.31 and 49.0 ppm) for ^{1}H and ^{13}C, respectively. Low-resolution mass spectra (LRMS) were recorded with an LCQ Finnigan apparatus (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionisation (APCI) source on positive or negative mode.

Synthesis of 3-dioxolane-5α-androstan-17β-ol (**6**)[46]

To a solution of DHT (**5**) (5.1 g, 17.7 mmol) in dry benzene (80 ml), were added under an argon atmosphere ethylene glycol (29.6 ml, 529.8 mmol) and *p*-TSA (33.6 mg, 0.177 mmol). The mixture was refluxed overnight using the Dean-Stark/water separator. The solution was quenched by the addition of saturated aqueous $NaHCO_3$ and concentrated under vacuum. The product was extracted with EtOAc, washed with brine, dried over

$MgSO_4$ and evaporated to dryness. Flash chromatography (hexanes/EtOAc, 95:5) afforded **6** as a white solid (5.2 g, 88 %). IR (film) $\nu = 3384$ (OH, alcohol); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.73 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.85-2.15 (m, 23 H), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.94 (s, 4 H, OCH_2CH_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.3 (C19), 11.6 (C18), 20.9 (C11), 22,8 (C6), 23.5 (C15), 28.6 (C16), 30.7 (C2), 31.3 (C7), 31.6 (C10), 35.7 (C8), 36.2 (C1), 36.9 (C12), 38.1 (C4), 43.1 (C13), 43.9 (C5), 51.1 (C14), 54.3 (C9), 64.1 (OCH_2CH_2O), 82.0 (C17), 109.3 (C3); LRMS for $C_{21}H_{35}O_3$ [MH^+]: 335.2 *m/z*.

Synthesis of 3-dioxolane-5α-androstan-17-one (**7**)

To a solution of alcohol **6** (5.2 g, 15.6 mmol) in dry CH_2Cl_2 (70 ml) under argon were added molecular sieves (4 Å) (7.8 g) and 4-methylmorpholine-N-oxide (2.7 g, 23.3 mmol) and the mixture was stirred for 15 min at rt. Tetrapropylammonium perruthenate (273 mg, 0.8 mmol) was added and the solution was stirred for 1 h. The resulting mixture was filtered on silica gel column, using hexanes/acetone (80:20) as eluent to give 5.1 g (98 %) of ketone **7**. IR (film) ν = 1740 (C=O, ketone); ^1H NMR (400 MHz, $CDCl_3$) δ = 0.83 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70-2.00 (m, 20 H), 2.07 (m, 16α-CH), 2.43 (dd, J_1 = 8.8 Hz, J_2 = 19.2, 16β-CH), 3.93 (s, 4 H, OCH_2CH_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.5 (C19), 14.0 (C18), 20.6 (C11), 21.9, 28.4, 30.9 (C2), 31.2 (C7), 31.7 (C10), 35.2, 35.8 (C8), 36.0, 36.1, 38.1 (C4), 43.8 (C5), 47.9, 51.6 (C14), 54.3 (C9), 64.3 (OCH_2CH_2O), 109.4 (C3), 221.5 (C17); LRMS for $C_{21}H_{33}O_3$ [MH^+]: 333.1 *m/z*.

Synthesis of 3-dioxolane-16α-allyl-5α-androstan-17-one (**8**)

A solution of diisopropylamine (2.35 ml, 16.8 mmol) in dry THF was stirred under argon at 0 °C and a 2.4 M solution of butyllithium in hexanes (7.0 ml, 16.8 mmol) was added dropwise. After 30 min, ketone **7** (5.1 g, 15.3 mmol) dissolved in dry THF was added dropwise in the resulting lithium diisopropylamine (LDA) solution. This mixture was allowed to stir for 30 min at 0 °C, then cooled at -78 °C and allyl bromide (1.45 ml, 16.8 mmol) was added dropwise. The reaction mixture was stirred overnight from -78 °C to 0 °C. Water was added to quench the reaction and the crude product was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 92:8) afforded the

major 16α-isomer **8** containing only 1.5 % of minor 16β-isomer (2.2 g, 38 %) and starting material (60 %). IR (film) ν = 1740 (C=O, ketone); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.83 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70-1.85 (m, 20 H), 2.05 (m, 1 H of CH_2-1'), 2.50 (m, 16β-OH and 1 H of CH_2-1'), 3.93 (s, 4 H, OCH_2CH_2O) 5.03 (m, CH_2-3'), 5.77 (m, CH-2'); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.5 (C19), 14.7 (C18), 20.5 (C11), 26.9 (C6), 28.4 (C15), 30.8, 31.2, 31.7, 35.1, 35.2, 35.8, 36.1, 38.0, 43.7 (C16), 44.2 (C5), 48.6 (C13), 49.1 (C14), 54.3 (C9), 64.3 (OCH_2CH_2O), 109.4 (C3), 116.5 (C-3'), 136.6 (C-2'), 222.0 (C17); LRMS for $C_{24}H_{37}O_3$ [MH^+]: 373.1 *m/z*.

Synthesis of 3-dioxolane-16α-allyl-5α-androstan-17β-ol (**9**)

A solution of ketone **8** (2.2 g, 5.8 mmol) in dry THF (50 ml) and $LiAlH_4$ (329.4 mg, 8.7 mmol) was stirred under argon at -78 °C. After 10 h, the reaction was quenched by the addition of water and 15 % aqueous NaOH solution. Rochelle salt (100 ml, 1M solution in water) was added and the mixture extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 85:15) yielded 2.0 g (92 %) of alcohol **9**. IR (film) ν = 3358 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.77 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.60-1.90 (m, 20 H), 1.99 and 2.36 (2m, CH_2-1'), 3.18 (dd, J_1 = 5.6 Hz, J_2 = 7.6 Hz, CH-17α), 3.60 (d, J = 5.4 Hz, 17β-OH), 3.87 (s, 4 H, OCH_2CH_2O), 4.98 (m, CH_2-3'), 5.85 (m, CH-2'); ^{13}C NMR (75.5 MHz, $(CDCl_3)$ δ = 11.6 (C19), 12.1 (C18), 20.8 (C11), 28.6 (C6), 30.1 (C15), 31.3 (C2), 31.6 (C7), 35.4 (C8), 35.7 (C10), 36.1 (C1), 36.9 (C12), 38.1 (C4), 39.8 (C1'), 43.4 (C16), 43.9 (C5), 44.0 (C13), 49.4 (C14), 54.3 (C9), 64.3 (OCH_2CH_2O), 87.6 (C17), 109.5 (C3), 115.8 (C-3'), 138.1 (C-2'); LRMS for $C_{24}H_{39}O_3$ [MH^+]: 375.1 *m/z*.

Synthesis of 3-dioxolane-16α-(3'-hydroxypropyl)-5α-androstan-17β-ol (**10**)

To a stirred solution of alkene **9** (113 mg, 0.30 mmol) in dry THF (15 ml) at 0 °C was added dropwise a 1 M borane solution in THF (1.81 ml, 1.81 mmol). The mixture was allowed to react under argon for 5 h, then a 1 N aqueous NaOAc solution (1.81 ml) and H_2O_2 (33 % w/v, 0.05 ml) were added and the resulting mixture was stirred at rt for 1 h.

The reaction was quenched by addition of water, extracted with EtOAc and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (70:30) as eluent to give diol **10** (86 mg, 73 %). IR (film) ν = 3256 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.67-1.79 (m, 25 H), 3.12 (dd, J_1 = 5.4 Hz, J_2 = 7.5 Hz, CH-17α), 3.41 (t, J = 5.2 Hz, CH_2-OH), 3.53 (dd, J_1 = 1.3 Hz, J_2 = 4.9 Hz, CH_2-OH), 3.58 (d, J = 5.32 Hz, 17β-OH), 3.86 (m, 4 H, ketal-H); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ = 11.9 (C19), 12.7 (C18), 21.6 (C11), (C6) and (C1′) under solvent, 31.4 (C15), 32.1 (C2), 32.5 (C7), 32.8, 33.2, 36.3, 37.0, 38.0, 38.9, 44.0 (C16), 44.6 (C5), 44.9 (C13), 50.4 (C14), 55.6 (C9), 63.0 (CH_2OH), 64.8 (OCH_2CH_2O), 88.4 (C17), 109.6 (C3); LRMS for $C_{24}H_{41}O_4$ [MH^+]: 393.0 *m/z*.

Synthesis of 16α-(3′-chloropropyl)-17β-hydroxy-5α-androstan-3-one (**11**)

Diol **10** (79 mg, 0.20 mmol) was dissolved in acetone (10 ml) and concentrated HCl (7 ml) was slowly added with a syringe, and the reaction mixture was stirred for 1 h at rt. An aqueous saturated $NaHCO_3$ solution was added and the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and solvent evaporated under reduced pressure. This crude keto diol, PPh_3 (106 mg, 0.40 mmol) and CCl_4 (133 mg, 0.40 mmol) in dry CH_2Cl_2 (15 ml) were added at 0 °C and stirred at rt under argon for 1 h. The reaction mixture was quenched by addition of water, extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/acetone (80:20) as eluent to yield chloride **11** (36 mg, 49 % for 2 steps). IR (film) ν = 3436 (OH), 1708 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.70-2.60 (m, 25 H), 3.16 (dd, J_1 = 5.8 Hz, J_2 = 7.3 Hz, CH-17α), 3.61 (t, J = 6.7 Hz, CH_2-Cl), 3.67 (d, J = 5.48 Hz, OH); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.7 (C19), 12.6 (C18), 21.7 (C11), 30.9 (C15), 31.3 (C7), 32.2 (C1′), 32.5 (C6), 33.9 (C2′), 36.2, 36.6, 37.8, 38.6, 39.4, 43.4 (C16), 44.9 (C13), 45.2, 46.2 (C3′), 47.7, 50.2 (C14), 55.0 (C9), 88.2 (C17), 210.2 (C3); LRMS for $C_{22}H_{35}O_2$ [M-Cl]$^+$: 331.3 *m/z*.

Synthesis of 16α-(3'-hydroxypropyl)-17β-hydroxy-5α-androstan-3-one (**12**)

Diol **10** (994 mg, 2.5 mmol) was dissolved in acetone (100 ml). Concentrated HCl (10 ml) was slowly added with a syringe and the reaction mixture was stirred for 2 h at rt. An aqueous saturated $NaHCO_3$ solution was then added and the reaction mixture was extracted with EtOAc. The organic phase was dried over $MgSO_4$ and solvent evaporated under reduced pressure. The solution was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography with hexanes/EtOAc (80:20) as eluent to yield diol **12** (688 mg, 70 %). IR (film) ν = 3396 and 3223 (OH), 1707 (C=O); ^{1}H NMR (400 MHz, CD_3OD) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.75-2.60 (m, 25 H), 3.13 (d, J = 7.7 Hz, CH-17α), 3.55 (dd, J_1 = 5.6 Hz, J_2 = 6.6 Hz, $C\underline{H_2}$-OH); ^{13}C NMR (75.5 MHz, (CD_3OD) δ = 11.7 (C19), 12.6 (C18), 22.0 (C11), 30.0 (15), 31.4 (C7), 32.5 (C1'), 33.1 (C6), 36.6, 36.9, 38.1, 38.9, 39.8, 43.8 (C16), 45.1 (C13), 45.5, ~49 (3C under solvent peaks), 50.4 (C14), 55.4 (C9), 63.2 (C3'), 88.8 (C17), 214.8 (C3); LRMS for $C_{22}H_{37}O_3$ [MH^+]: 349.1 *m/z*.

Synthesis of 16α-(3'-iodopropyl)-17β-hydroxy-5α-androstan-3-one (**13**)

Diol **12** *(85 mg, 0.24 mmol) was dissolved in dry* CH_2Cl_2 *(10 ml) and* PPh_3 *(95 mg, 0.36* mmol); I_2 (92 mg, 0.36 mmol) and imidazole (43 mg, 0.63 mmol) were added and stirred at rt under argon for 2 h. The mixture was quenched by addition of water, extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. After evaporation of solvent under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield iodide **13** (61 mg, 55 %). IR (KBr) ν = 3350 (OH), 1709 (C=O); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.78 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.75-2.50 (m, 26 H), 3.20 (m, CH_2-I and CH-17α); ^{13}C NMR, the compound was too unstable; LRMS for $C_{22}H_{36}O_2I$ [MH^+]: 459.1 *m/z*.

Synthesis of 16α-(3'-hydroxypropyl)-5α-androstan-3α,17β-diol (**14**)

Ketone **12** (957 mg, 2.75 mmol) was dissolved in dry THF (92 ml) and cooled at -78 °C. K-Selectride (3.29 ml, 3.29 mmol) was added dropwise under argon. After 1 h, the reaction was quenched by addition of a saturated aqueous NH_4Cl solution (20 ml) and allowed to

warm up to rt. The solution was concentrated under reduced pressure and the mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography (hexanes/acetone, 80:20) to afford **14**. (481 mg, 50 %). IR (KBr) ν = 3364 (OH); ^{1}H NMR (400 MHz, (CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70-1.85 (m, 25 H), 3.12 (d, *J* = 7.8 Hz, CH-17α), 3.55 (t, *J* = 6.0 Hz, $C\underline{H_2}$-OH), 3.95 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, (CD_3OD) δ = 11.7 (C19), 12.6 (C18), 21.4 (C11), 29.6 (C15), 29.7 (C6), 31.4 (C2), 32.5, 32.9 (C1), 33.1 (C2), 33.5 (C8), 36.7 (C4), 37.3 (C10), 38.2 (C12), 40.4 (C5), 43.8 (C16), 45.1 (C13), ~49 (1C under solvent peaks), 50.7 (C14), 56.1 (C9), 63.2 (C3′), 67.2 (C3), 88.9 (C17); LRMS for $C_{22}H_{39}O_2$ [MH^+]: 351.0 *m/z*.

Synthesis of 16α-(3′-chloropropyl)-5α-androstan-3α,17β-diol (**15**)

To a solution of diol **14** (117 mg, 0.33 mmol) in dry CH_2Cl_2 (18 ml) were added PPh_3 (350 mg, 1.34 mmol) and CCl_4 (205 mg, 1.34 mmol). After 2 h of stirring at rt under argon, the reaction mixture was quenched by addition of water, extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield **15** (92 mg, 75 %). IR (film) ν = 3356 (OH); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.75 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.70-2.00 (m, 25 H), 3.19 (d, *J* = 7.1 Hz, CH-17α), 3.55 (d, *J* = 6.62 Hz, CH_2-Cl), 4.04 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, ($CDCl_3$) δ = 11.4 (C19), 12.1 (C18), 20.4 (C11), 28.5 (C15), 29.1 (C1′), 30.5 (C6), 31.7 (C2′ and C7), 32.3 (C1), 33.1 (C2), 35.4 (C8), 36.0 (C4), 36.3 (C10), 36.9 (C12), 39.3 (C5), 43.2 (C16), 44.1 (C13), 45.5 (C3′), 49.5 (C14), 54.6 (C9), 66.7 (C3), 88.3 (C17); LRMS for $C_{22}H_{38}O_2Cl$-H_2O [MH-H_2O]$^+$: 351.1 *m/z*.

Synthesis of 16α-(3′-fluoropropyl)-5α-androstan-3α,17β-diol (**16**)

Chloride **15** (23 mg, 0.06 mmol) was dissolved in dry THF (18 ml) and the solution was cooled at 0 °C and treated with tetrabutylammonium fluoride (1.0 M in THF, 150 µl). This mixture was refluxed overnight and then quenched by addition of water. The product was extracted with EtOAc and dried over $MgSO_4$. After evaporation under reduced pressure, the

crude product was purified by flash chromatography with hexanes/acetone 95:5 to give **16** (16 mg, 73 %). IR (film) ν = 3358 (OH); ^{1}H NMR (400 MHz, (CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70-1.90 (m, 25 H), 3.12 (d, *J* = 7.6 Hz, CH-17α), 3.96 (narrow m, CH-3β), 4.36 (t, *J* = 5.9 Hz, 1 H of CH_2-F), 4.48 (t, *J* = 6.0 Hz, 1 H of CH_2-F); ^{13}C NMR (75.5 MHz, (CD_3OD) δ = 11.7 (C19), 12.6 (C18), 21.4 (C11), 29.6 (C15), 29.7 (C6), 30.4, 31.3, 32.3, 32.4, 32.9, 33.5 (C8), 36.7 (C4), 37.2 (C10), 38.2 (C12), 40.4 (C15), 43.7 (C16), 45.1 (C13), 50.7 (C14), 56.1 (C9), 67.2 (C3), 83.9 (C3′), 88.9 (C17); LRMS for $C_{22}H_{38}O_2F$ - H_2O [MH-H_2O]: 335.2 *m/z*.

Synthesis of 16α-(3′-iodopropyl)-5α-androstan-3α,17β-diol (**17**)

Using the same protocol as for the synthesis of **13**, the primary alcohol of **14** was substituted and the compound purified with hexanes/EtOAc (92:8) as eluent to yield **17** (61 mg, 30 %). IR (film) ν = 3354 (OH); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.75 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.75-2.00 (m, 25 H), 3.20 (m, CH_2-I and CH-17α), 4.15 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, ($CDCl_3$) δ = 7.5 (C3′), 11.2 (C19), 12.0 (C18), 20.2 (C11), 28.4 (C15), 29.0 (C7), 30.4 (C6), 31.5 (C2′), 32.1 (C1), 32.4 (C2), 35.2 (C8), 35.8 (C4), 36.2 (C10), 36.6, 36.7 (C12), 39.2 (C5), 42.8 (C16), 43.9 (C13), 49.3 (C14), 54.4 (C9), 66.5 (C3), 88.1 (C17); LRMS for $C_{22}H_{37}O_2I$ + NH_4 $[M + NH_4]^+$: 477.9 *m/z*.

Synthesis of 16α-(3′-bromopropyl)-17β-hydroxy-5α-androstan-3-one (**18**)

To a solution of diol **12** (947 mg, 2.72 mmol) in dry CH_2Cl_2 (18 ml) were added PPh_3 (1.42 g, 5.43 mmol) and CBr_4 (1.80 g, 5.43 mmol) at 0 °C. After 2 h of stirring at rt under argon, the reaction mixture was quenched by addition of water, extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/acetone (80:20) as eluent to yield **18** (862 mg, 77 %). IR (film) ν = 3426 (OH), 1705 (C=O); ^{1}H NMR (400 MHz, ($CD_3)_2CO$) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.70-2.50 (m, 31 H), 3.17 (dd, J_1 = 5.9 Hz, J_2 = 7.3 Hz, CH-17α), 3.51 (t, *J* = 6.8 Hz, CH_2-Br), 3.68 (d, *J* = 5.5 Hz, OH); ^{13}C NMR (75.5 MHz, ($CD_3)_2CO$) δ = 11.8 (C19), 12.6 (C18), 21.8 (C11), ~29 (2C under solvent

peaks), 30.9 (C15), 31.3 (C7), 32.2 (C1'), 32.8 (C6), 35.3 (C2'), 36.2, 37.9, 38.7, 39.4, 39.4, 43.3 (C16), 45.3, 47.7, 50.2 (C14), 55.1 (C9), 88.3 (C17), 210.2 (C3).

Synthesis of 16α-(3'-azidopropyl)-17β-hydroxy-5α-androstan-3-one (**19**)

To a solution of bromide **18** (243 mg, 0.59 mmol) in dry CH_3CN (20 ml) was added NaN_3 (154 mg, 2.37 mmol) under an argon atmosphere. The mixture was stirred at 80 °C for 24 h, and the reaction quenched by addition of water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 80:20) yielded 212 mg (95 %) of azide **19**. IR (film) ν = 3436 (OH), 2094 (N_3), 1711 (C=O); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.79 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70-2.50 (m, 26 H), 3.20 (d, J = 7.5 Hz, CH-17α), 3.29 (t, J = 6.6 Hz, CH_2-N_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.6 (C19), 12.1 (C18), 21.0 (C11), 27.9 (C15), 28.9, 30.5 (C15), 31.3 (C7), 32.9, 35.3, 35.9, 36.8, 38.3, 38.7, 43.4 (C16), 44.1 (C13), 44.8, 46.9, 49.3 (C14), 51.8 (C3'), 54.1 (C9), 88.2 (C17), 212.2 (C3); LRMS for $C_{22}H_{36}O_2N_3$ [MH^+]: 374.2 *m/z*.

Synthesis of 16α-(3'-azidopropyl)-5α-androstan-3α,17β-diol (**20**)

Using the same protocol as for the synthesis of **14**, ketone **19** was reduced into alcohol **20** and purified by flash chromatography with hexanes/EtOAc/CH_2Cl_2 (80:19:1) to give 63 mg (44 %) of **20**. IR (KBr) ν = 3450 (OH), 2084 (N_3); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.75-1.80 (m, 25 H), 3.19 (d, J = 7.4 Hz, CH-17α), 3.29 (t, J = 6.6 Hz, CH_2-N_3), 4.04 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 11.4 (C19), 12.1 (C18), 20.3 (C11), 27.9 (C15), 28.5 (C1'), 29.1 (C7), 30.5 (C6), 31.7 (C2'), 32.3 (C1), 32.9 (C2), 35.4 (C8), 36.0 (C4), 36.3 (C10), 36.9 (C12), 39.3 (C5), 43.4 (C16), 44.1 (C13), 49.5 (C14), 51.8 (C3'), 54.6 (C9), 66.7 (C3), 88.3 (C17); LRMS for $C_{22}H_{38}O_2N_3$ [MH^+]: 376.0 *m/z*.

Synthesis of 16α-(3'-aminopropyl)-5α-androstan-3α,17β-diol (**21**)

To a solution of azide **20** (104 mg, 0.28 mmol) in MeOH (16 ml) was added 10 % Pd-C (20 mg) and the mixture was stirred for 3 h under one atmosphere of H_2. After filtration

through Celite, the solvent was removed under reduced pressure. Purification by flash chromatography (acetone/MeOH, 90:10) afforded 87 mg (90 %) of amine **21**. IR (KBr) ν = 3348 (OH and NH_2); ^{1}H NMR (400 MHz, CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70-1.90 (m, 27 H), 2.66 (t, *J* = 6.0 Hz, $C\underline{H_2}$-NH_2), 3.12 (d, *J* = 7.6 Hz, CH-17α), 3.96 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, (CD_3OD) δ = 11.7 (C19), 12.6 (C18), 21.4 (C11), 29.6, 29.7 (C7), 30.9 (C6), 31.3 (C2′), 32.0 (C1), 32.9 (C2), 33.5, 33.9, 36.7, 37.2, 38.2, 40.4, 42.5, 43.8 (C16), 45.0 (C13), 50.7 (C14), 56.1 (C9), 67.2 (C3), 88.8 (C17); LRMS for $C_{22}H_{40}O_2N$ [MH^+]: 350.3 *m/z*.

Synthesis of 16α-(3′-thiocyanatopropyl)-17β-hydroxy-5α-androstan-3-one (**22**)

A mixture of bromide **18** (72 mg, 0.18 mmol) and KSCN (34 mg, 0.36 mmol) in EtOH (15 ml) was refluxed overnight. The reaction mixture was quenched by addition of water and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 70:30) yielded 56 mg (82 %) of **22**. IR (KBr) ν = 3519 (OH), 2145 (SC≡N), 1705 (C=O); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.79 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70-2.50 (m, 25 H), 2.97 (t, *J* = 7.2 Hz, CH_2-SCN), 3.21 (d, *J* = 7.4 Hz, CH-17α); ^{13}C NMR (75.5 MHz, ($CDCl_3$) δ = 11.6 (C19), 12.1 (C18), 21.0 (C11), 28.9 (2X), 30.5 (C15), 31.3 (C7), 34.1, 34.2 (C3′), 35.3, 35.9, 36.7, 38.2, 38.7, 43.0 (C16), 44.1 (C13), 44.8, 46.9, 49.3 (C14), 54.1(C9), 88.1 (C17), ~112 (C4', too weak) 212.2 (C3); LRMS for $C_{23}H_{36}O_2NS$ [MH^+]: 390.0 *m/z*.

Synthesis of 16α-(3′-thiocyanatopropyl)-5α-androstan-3α,17β-diol (**23**)

Using the same protocol as for the synthesis of **14**, the ketone of **22** (26 mg, 0.066 mmol) was reduced and purified using hexanes/EtOAc (70:30) as eluent to yield **23** (16.2 mg, 62 %). IR (KBr) ν = 3475 (OH), 2160(SC≡N); ^{1}H NMR (400 MHz, CD_3OD) δ = 0.76 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.70-2.00 (m, 25 H), 3.04 (t, *J* = 7.2 Hz, CH_2-SCN), 3.14 (d, *J* = 7.4 Hz, CH-17α), 3.96 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, (CD_3OD) δ = 11.7 (C19), 12.6 (C18), 21.4 (C11), 29.6, 29.7 (C7), 30.1 (C6), 31.4 (C2′), 32.9 (C2), 33.5, 35.1, 36.7, 37.2, 38.1, 40.4 (C16), 43.4, 45.2, ~49 (2C under solvent peaks), 50.7 (C14), 56.1

(C9), 67.2 (C3), 88.8 (C17), 113.7 (C4'); LRMS for $C_{23}H_{37}O_2NS + NH_4$ $[M + NH_4]^+$: 409.1 *m/z*.

Synthesis of 16α-(3'-bromopropyl)-17β -(t-butyldimethylsilyloxy)-5α-androstan-3-one (**24**) To a solution of **18** (118 mg, 0.29 mmol) in dry CH_2Cl_2 (30 ml), cooled at -78 °C, were added 2,6-lutidine (0.07 ml, 0.63 mmol) and TBDMS-OTf (0.06 ml, 0.26 mmol). The reaction mixture was stirred for 3 h under argon, then quenched by addition of water and 10 % HCl. The acidification step was performed to recover the ketone in position 3. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude compound by flash chromatography (hexanes/acetone, 95:5) afforded **24** (128 mg, 85 %). IR (film) ν = 1715 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.08 and 0.10 (2s $Si(CH_3)_2$), 0.80 (s, 18-CH_3), 0.91 (s, $SiC(CH_3)_3$), 1.07 (s, 19-CH_3), 0.70-2.50 (m, 40 H), 3.26 (d, J = 7.3 Hz, CH-17α), 3.51 (t, J = 6.4 Hz, CH_2-Br); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = -3.7 and -3.6 ($Si(CH_3)_2$), 11.7 (C19), 12.8 (C18), 18.8 ($SiC(CH_3)_3$), 21.8 (C11), 26.5 ($SiC(\underline{CH_3})_3$), ~30 (3C under solvent peaks), 32.2, 32.8, 34.1, 35.3, 36.2, 36.6, 38.3, 38.6, 39.4, 44.2 (C16), 45.2, 47.7, 49.9, 55.0 (C9), 88.8 (C17), 210.2 (C3); LRMS for $C_{28}H_{49}BrO_2Si$ $[M-Br]^+$: 445.2 *m/z*.

Synthesis of 16α-(3'-(4''-bromobenzyloxy)propyl)-17β-hydroxy-5α-androstan-3-one (**25**) AgOTf (57 mg, 0.22 mmol), 4-bromobenzyl alcohol (56 mg, 0.30 mmol) and 2,6-di-tert-butyl-4-methyl pyridine (91 mg, 0.44 mmol) were dissolved in dry CH_2Cl_2 (30 ml) and cooled at 0 °C. To this solution was added bromide **24** (78 mg, 0.15 mmol) and the mixture was stirred for 24 h. After filtration and evaporation under reduced pressure, the crude product was purified by flash chromatography with hexanes/ EtOAc 98:2 to afford the arylether derivative (71 mg, 76 %). The TBDMS group of this compound was hydrolysed with HCl (3 % v/v). After 2 h at rt, water was added; the methanol was evaporated under reduced pressure, the aqueous phase extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. Flash chromatography using hexanes/acetone 90:10 yielded **25** (48 mg, 83 %). IR (KBr) ν = 3423 (OH), 1700 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.79 (s, 18-CH_3), 1.07 (s, 19-CH_3), 0.75-2.55 (m, 25 H), 3.15 (dd, J_1 = 5.6 Hz, J_2 = 7.5 Hz, CH-17α), 3.48 (t, J = 6.1 Hz CH_2O), 3.62 (d, J = 5.4 Hz, OH), 4.46 (s, $O\underline{CH_2}Ph$), 7.26 (d, J = 8.5 Hz,

2H of Ph), 7.47 (d, J = 8.4 Hz, 2H of Ph); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.7 (C19), 12.6 (C18), 21.7 (C11), ~30 (2C under solvent peaks), 31.2 (C7), 32.2 (C1′), 33.2, 36.2, 36.6, 37.9, 38.6, 39.4, 43.9 (C16), 44.8 (C13), 45.2, 47.7, 50.2 (C14), 55.1 (C9), 71.5 (C4′), 72.4 (C3′), 88.3 (C17), 121.4 (1C of Ph), 130.3 (2C of Ph), 132.1 (2C of Ph), 139.7 (1C of Ph), 210.2 (C3); LRMS for $C_{29}H_{42}O_3Br$ [MH^+]: 517.0 and 518.9 *m/z*.

Synthesis of 16α-(3′-(4′′-bromobenzyloxy)propyl)-5α-androstan-3α,17β-diol (**26**)

Using the same protocol as for the synthesis of **14**, ketone **25** (45 mg, 0.087 mmol) was reduced into alcohol **26**. The alcohol was recovered in the organic layer and purified by flash chromatography (hexanes/acetone, 95:5) to afford **26** (27 mg, 60 %). IR (KBr) ν = 3384 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70-1.85 (m, 25 H), 3.14 (dd, J_1 = 5.7 Hz, J_2 = 7.3 Hz, CH-17α), 3.29 (d, J = 3.1 Hz, OH), 3.48 (t, J = 6.1 Hz, CH_2O), 3.59 (d, J = 5.4 Hz, OH), 3.93 (m, CH-3β), 4.47 (s, O$\underline{CH_2}$Ph), 7.31 (d, J = 8.4 Hz, 2H of Ph), 7.52 (d, J = 8.4 Hz, 2H of Ph); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8 (C19), 12.7 (C18), 21.2 (C11), ~30 (4C under solvent peaks), 30.8, 31.3 (C6), 32.8, 33.3, 36.4, 37.1, 38.0, 40.0, 44.0, 44.9, 50.5 (C14), 55.9 (C9), 66.1 (C3), 71.6 (C4′), 72.4 (C3′), 88.4 (C17), 121.5 (1C of Ph), 130.4 (2C of Ph), 132.2 (2C of Ph), 139.8 (1C of Ph); LRMS for $C_{29}H_{44}O_3Br$ [MH^+]: 518.9 and 521.0 *m/z*.

Synthesis of 16α-(3′-bromopropyl)-17β-(t-butyldimethylsilyloxy)-5α-androstan-3α-ol (**27**)

Using the same protocol as for the synthesis of **14**, ketone **24** (69 mg) was reduced into alcohol **27**. The alcohol was recovered in the organic layer and purified by flash chromatography (hexanes/acetone, 95:5) to afford **27** (40 mg, 58 %). IR (film) ν = 3352 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.07 and 0.10 (2s, $Si(CH_3)_2$), 0.77 (s, 18-CH_3), 0.80 (s, 19-CH_3), 0.91 (s, $SiC(CH_3)_3$), 0.70-2.00 (m, 40 H), 3.23 (m, CH-17α), 3.52 (t, J = 6.6 Hz, CH_2-Br), 3.80 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = -3.7 and -3.6 ($Si(CH_3)_2$), 8.4 (C19), 11.8 (C18), 18.8 ($SiC(CH_3)_3$), 21.2 (C11), 26.5 ($SiC(\underline{CH_3})_3$), ~30 (2C under solvent peaks), 32.1, 32.6, 32.8, 33.2, 33.5, 34.1, 35.3, 36.4, 37.0, 38.4, 38.6, 40.0, 44.1 (C16), 44.9, 46.2, 50.2 (C14), 55.7 (C9), 66.1 (C3), 88.9 (C17); LRMS for $C_{28}H_{51}O_2Br$ [MH^+]: 526.1 and 528.2 *m/z*.

Synthesis of 16α-(3'-bromo/chloropropyl)-3α-methoxy-5α-androstan-17β-ol (**28**) and *16α-(3'-iodopropyl)-3α-methoxy-5α-androstan-17β-ol* (**29**)

Bromide **27** (40 mg, 0.08 mmol) was dissolved in dry THF (18 ml) and the solution was cooled at 0 °C. NaH (18 mg, 0.45 mmol) was added and the mixture was stirred for 30 min before the addition of MeI (43 µl, 0.68 mmol) and 15-crown-5 ether. The reaction mixture was stirred at rt for 24 h and then quenched by addition of water. The product was extracted with CH_2Cl_2 and dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography with hexanes/EtOAc 98:2 giving a mixture of two methoxy derivatives (38 mg, 90 %). The TBDMS group of these compounds was removed with a solution of HCl 2 % in MeOH and, after 2 h, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The crude compound was purified by flash chromatography using hexanes/ EtOAc 95:5 to give **28** (21 mg, 70 %) and **29**. **28:** IR (film) ν = 3436 (OH); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.75 (s, 18-CH_3), 0.79 (s, 19-CH_3), 0.70-2.00 (m, 25 H), 3.18 (d, *J* = 7.2 Hz, CH-17α), 3.29 (s, OCH_3), 3.48 (m, 1 H of CH_2-Cl and CH-3β), 3.55 (t, *J* = 6.6 Hz, 1 H of CH_2Cl); ^{13}C NMR (75 MHz, ($CDCl_3$) δ = 11.6 (C19), 12.1 (C18), 20.3 (C11), 25.2, 28.6 (C15), 30.5 (C7), 31.6 (C1'), 31.9, 32.7, 32.9, 34.4, 35.4, 36.1, 36.9, 39.7 (C5), 43.1 (C16), 43.2, 44.1, 49.5 (C14), 54.5 (C9), 55.8 (OCH_3), 75.7 (C3), 88.3 (C17); LRMS for $C_{23}H_{39}O_2Cl$–CH_3O [M-OCH_3]$^+$: 351.2 *m/z*. **29:** IR (NaCl) ν = 3474 (OH); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.74 (s, 18-CH_3), 0.79 (s, 19-CH_3), 0.70-2.00 (m, 25 H), 3.20 (m, CH-17α and CH_2-I), 3.29 (s, OCH_3), 3.42 (m, CH-3β); ^{13}C NMR (75.5 MHz, ($CDCl_3$) δ = 7.6 (C3'), 11.6 (C19), 12.1 (C18), 20.3 (C11), 25.2, 28.6 (C15), 30.5 (C7), 31.6 (C1'), 32.6, 32.7, 32.9, 35.4, 36.1, 36.8, 36.9, 39.7 (C5), 43.0 (C16), 44.1, 49.5 (C14), 54.5 (C9), 55.8 (OCH_3), 75.7(C3), 88.2 (C17); LRMS for $C_{23}H_{39}O_2I$–CH_3O [M-OCH_3]$^+$: 443.0 *m/z*.

6'-(3''-dioxolane-17''β-hydroxy-5''α-androstan-16''α-yl)-hex-2'-enol (**30**)

This compound was prepared as reported below for the synthesis of **31**. 630 mg (36 %). ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.75 (s, 18''-CH_3), 0.81 (s, 19''-CH_3), 0.60-2.50 (m, 33 H), 3.20 (d, *J* = 7.5 Hz, CH-17''α), 3.65 (m, CH_2-OH and OH), 3.93 (s, 4 H, OCH_2CH_2O), 5.48 (m, CH-2' and CH-3'); LRMS for $C_{27}H_{44}O_4$ [MH^+]: 433.2 *m/z*.

Synthesis of 4'-(3''-dioxolane-17''β-hydroxy-5''α-androstan-16''α-yl)-but-2'-enyl-benzoate (**31**)

To a solution of alkene **9** (300 mg, 0.80 mmol) in dry CH_2Cl_2 (12 ml) were added benzoic acid allyl ester (390 mg, 2.4 mmol) and 2nd-generation Grubbs catalyst (tricyclohexylphosphine[1,3-bis(2,4,6-tri-methylphenyl)-4,5-dihydroimidazol-2-ylidene][benzylidine]rethenium(IV)dichloride (102 mg, 0.12 mmol). This mixture was refluxed overnight and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (hexanes/EtOAc, 95:5) to yield 190 mg (47 %) of **31**. IR (film) ν = 3423 (OH), 1713 (C=O, ester); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18''-CH_3), 0.82 (s, 19''-CH_3), 0.60-2.50 (m, 23 H), 3.21 (d, *J* = 7.7 Hz, CH-17''α), 3.88 (s, 4 H, OCH_2CH_2O), 4.77 (d, *J* = 6.2 Hz, CH_2-4'), 5.76 and 5.95 (2m, CH-2' and CH-3'), 7.52 (m, 2H of Ph), 7.64 (m, 1H of Ph), 8.04 (m, 2H of Ph); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8 (C19''), 12.5 (C18''), 21.4 (C11''), ~29.5 (C6 under solvent peaks), 31.0 (C15''), 31.8 (C2''), 32.2 (C7''), 36.0, 36.1, 36.9, 37.7, 37.9, 38.7, 43.5 (C16''), 44.0 (C5''), 44.3 (C13''), 50.2 (C14''), 55.2 (C9''), 64.5 (OCH_2CH_2O), 65.9 (C-4'), 86.8 (C17''), 109.4 (C3''), 125.8 (C-3'), (129.2, 129.3) (twin) (2x Ph), (130.1,130.3) (twin) (2x Ph), (131.3,131.4) (twin) (1x Ph), (133.6, 133.8) (twin) (1x Ph), 135.8 (C-2'), 166.4 (COO); LRMS for $C_{32}H_{45}O_5$ [MH^+]: 508.9 *m/z*.

16α-(6'-chlorohexyl)-17β-hydroxy-5α-androstan-3-one (**32**)

This compound was prepared as reported below for the synthesis of **33** except that there was no benzoic acid ester to be hydrolyzed. 16 mg (18 % for 3 steps). IR (film) ν = 3444 (OH), 1712 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO/D_2O$) δ = 0.78 (s, 18-CH_3), 1.06 (s, 19-CH_3), 0.70-2.60 (m, 31 H), 3.12 (d, *J* = 7.6 Hz, CH-17α), 3.59 (t, *J* = 6.7 Hz CH_2-Cl); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.7 (C19), 12.6 (C18), 21.8 (C11), 27.7 (C15), ~ 30 (4 C under solvent peaks), 31.2 (C1'), 32.2 (C6), 33.5 (C2'), 36.2, 36.6, 37.9, 38.6, 39.4, 44.1 (C16), 44.8 (C13), 45.2, 45.9 (C3'), 47.7, 50.2 (C14), 55.1 (C9), 88.3 (C17), 210.2 (C3); LRMS for $C_{25}H_{41}O_2$ [M-Cl]$^+$: 373.3 *m/z*.

Synthesis of 16α-(4'-chlorobutyl)-17β-hydroxy-5α-androstan-3-one (**33**)

A suspension of alkene **31** (190 mg, 0.4 mmol) and 10 % Pd-C (28 mg) in EtOAc was hydrogenated at 1 atmosphere for 24 h. After filtration through Celite, the solvent was removed under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 90:10) afforded 157 mg (82 %) of alkane derivative. An aqueous 10 % NaOH solution (8 ml) was added to this intermediate (157 mg, 0.31 mmol) dissolved in methanol (15 ml) and the reaction mixture was stirred for 1.5 h at rt. The product was extracted with CH_2Cl_2, the organic phase dried over $MgSO_4$ and the solvent was removed under reduced pressure to give the crude diol (124 mg). This diol was dissolved in acetone (15 ml) and concentrated HCl (8 ml) was slowly added with a syringe. The reaction mixture was stirred for 1 h at rt. An aqueous saturated $NaHCO_3$ solution was then added and the reaction mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. This alcohol (70 mg, 0.2 mmol), PPh_3 (101 mg, 0.4 mmol) and CCl_4 (101 mg, 0.4 mmol) in dry CH_2Cl_2 (10 ml) were then stirred at 0°C under argon for 1 h. The reaction was quenched by addition of water, extracted with CH_2Cl_2 and the organic phase dried over $MgSO_4$. After evaporation under reduced pressure, the crude product was purified by flash chromatography using hexanes/EtOAc (80:20) as eluent to yield chloride **33** (34 mg, 24 % for 4 steps). IR (film) ν = 3420 (OH), 1710 (C=O); ^{1}H NMR (400 MHz, $(CD_3)_2CO/D_2O$) δ = 0.78 (s, 18-CH_3), 1.06 (s, 19-CH_3), 0.70-2.60 (m, 27 H), 3.13 (d, J = 7.61 Hz, CH-17α), 3.59 (t, J = 6.7 Hz, CH_2-Cl); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.7 (C19), 12.6 (C18), 21.7 (C11), 26.4 (C15), 30.6 (C7), 31.1 (C1'), 32.2 (C6), 33.7 (C2'), 35.8, 36.1, 36.6, 37.8, 38.5, 39.3, 43.9 (C16), 44.7 (C13), 45.2, 45.8 (C3'), 47.6, 50.2 (C14), 55.0 (C9), 88.2 (C17), 210.2 (C3); LRMS for $C_{23}H_{39}O_2Cl$ $[M]^+$: 380.5 *m/z*.

16α-(6'-chlorohexyl)-5α-androstan-3α,17β-diol (**34**)

This compound was prepared as reported below for **35**. 15 mg (75 %). IR (film) ν = 3358 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70-1.90 (m, 31 H), 3.12 (dd, J_1 = 5.6 Hz, J_2 = 7.5 Hz, CH-17α), 3.28 (d, J = 3.1 Hz, OH), 3.55 (m, CH_2-Cl and OH), 3.94 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8 (C19), 12.7 (C18), 21.1 (C11), 27.7 (C15), 28.9 (C7), ~30 (4C under solvent peaks), 31.2,

32.7, 33.2, 33.5, 36.4 (C4), 36.7 (C10), 37.0 (C12), 38.0, 39.9 (C5), 44.1 (C16), 44.8 (C13), 45.9 (C3′), 50.5 (C14), 55.8 (C9), 66.0 (C3), 88.4 (C17); LRMS for $C_{25}H_{43}O_2$ $[M\text{-}Cl]^+$: 375.3 *m/z*.

Synthesis of 16α-(4′-chlorobutyl)-5α-androstan-3α,17β-diol (**35**)

Using the same protocol as for the synthesis of **14,** chloride **33** (26 mg, 0.06 mmol) was dissolved in dry THF (2 ml) and the solution cooled at -78 °C. K-Selectride (60 µl, 0.06 mmol) was added dropwise under argon. After 1 h, the reaction was quenched by the addition of a saturated aqueous NH_4Cl solution (1 ml). The mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the crude product was purified by flash chromatography with hexanes/EtOAc/CH_2Cl_2 (80:19:1) as eluent to afford **35** (17 mg, 72 %). IR (film) ν = 3355 (OH); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.70-1.90 (m, 27 H), 3.28 (dd, J_1 = 5.6 Hz, J_2 = 7.5 Hz, CH-17α), 3.28 (d, J = 3.1 Hz, OH), 3.60 (m, CH_2-Cl and OH), 3.93 (narrow m, CH-3β); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$) δ = 11.8 (C19), 12.6 (C18), 21.1 (C11), 26.5 (C15), ~30 (3 C under solvent peaks), 31.1 (C6), 32.7 (C2′), 33.2, 33.8, 35.8, 36.3 (C4), 37.0 (C10), 38.0 (C12), 39.9 (C5), 44.0 (C16), 44.8 (C13), 45.9 (C3′), 50.5 (C14), 55.8 (C9), 66.0 (C3), 88.3 (C17); LRMS for $C_{23}H_{39}O_2$ $[M\text{-}Cl]^+$: 347.3 *m/z*.

Synthesis of 16α-(2′-bromoethyl)-17β-acetoxy-5α-androstan-3α-ol (**37**)

To a solution of bromide **36** (180 mg, 0.35 mmol) in pyridine (5 ml) were added Ac_2O (357 mg, 0.35 mmol) and DMAP (1.2 mg, 0.01 mmol) under an argon atmosphere. After 2 h, the reaction was quenched by addition of a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc and the extracts washed with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure, yielding 190 mg of crude intermediate. The TBDMS group of this intermediate (180 mg) was removed with HF/pyridine (276 µl, 6.48 mmol) in THF at rt for 60 h and quenched by addition of a saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted with EtOAc and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 85:15) yielded 71 mg (49 % for two steps) of alcohol **37**.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 3.33 (s, $COCH_3$), 0.70-2.30 (m, 32 H), 3.35 (m, CH_2-Br), 4.03 (narrow m, CH-3β), 4.53 (d, *J* = 7.6 Hz, CH-17α).

Synthesis of 16α-(2'-bromoethyl)-17β-hydroxy-5α-androstan-3-one (**38**)

To a solution of alcohol **37** (30 mg, 0.07 mmol) in dry CH_2Cl_2 (2 ml) under argon were added molecular sieves (4 Å) (50 mg) and 4-methylmorpholine-N-oxide (12 mg, 0.10 mmol) and the mixture was stirred for 15 min at rt. Tetrapropylammonium perruthenate (2.4 mg, 0.07 mmol) was added and the solution was stirred for 2 h. The resulting mixture was filtered on a silica gel column, using hexanes/acetone (85:15) as eluent to give 29 mg (96 %) of the corresponding ketone. Subsequently, a solution of K_2CO_3 (36 mg, 0.26 mmol) in MeOH (1 ml) was added to a solution of ketone (29 mg, 0.06 mmol) in MeOH (1 ml) and the mixture was refluxed for 2 h. The MeOH was evaporated, the compound was dissolved in EtOAc and washed with a saturated aqueous NH_4Cl solution. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude product by flash chromatography (hexanes/EtOAc, 85:15) yielded 9 mg (35 %) of ketone **38**. IR (film) ν = 3422 (OH), 1707 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.80 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70-2.50 (m, 23 H), 3.22 (d, *J* = 7.1 Hz, CH-17α), 3.48 (m, CH_2-Br); LRMS for $C_{21}H_{34}O_2Br$ [MH^+]: 397.0 and 399.0 *m/z*.

16α-(2'-bromoethyl)-5α-androstan-3α,17β-diol (**39**)

The TBDMS group of ketone **36** (30 mg, 0.06 mmol) was hydrolysed with HCl (3 % v/v) in MeOH. After 2 h at rt, water was added, the methanol was evaporated under reduced pressure, the aqueous phase extracted with EtOAc and the organic phase dried over $MgSO_4$. Flash chromatography using hexanes/EtOAc 85:15 yielded **39** (19 mg, 82 %). IR (film) ν = 3374 (OH); ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 0.76 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.70-2.20 (m, 23 H), 3.22 (d, *J* = 7.1 Hz, CH-17α), 3.55 (m, CH_2-Br), 4.05 (narrow m, CH-3β); LRMS for $C_{21}H_{35}O_2Br$ -2H2O [MH-$2H_2O$]$^+$: 363.1 and 365.1 *m/z*.

Assessment of proliferative and antiproliferative activities on Shionogi (AR^+) cells

These cell culture assays were performed as previously reported in the literature.[27] The antiproliferative assay on androgen-sensitive (AR^+) Shionogi mammary carcinoma cells was carried out at two concentrations of the synthesized compounds, 0.1 and 0.1 μM, and the results are reported as the percentage (%) of inhibition relative to the proliferation induced by 0.3 nM of DHT. The proliferation assay was carried out at 0.1 μM, and results are reported as the percentage (%) of stimulation induced by 0.3 nM of androgen DHT (100 %).

Steroid receptor affinity assay

The binding affinity assay on androgen receptor from rat ventral prostate was performed according to the procedure described by Luo et al,[47] except that the results were expressed as the binding affinity at 0.1 and 1.0 μM of tested compound (the binding affinity of RU1881 was set as 100 %) .

Molecular modeling

To predict the optimal position of ligands in the steroid binding site of hAR, we have used a computational strategy similar to that proposed by Zhorov and Lin.[48] The receptor was represented by a double-shell model based on our hAR crystallographic structures.[42] The inner flexible shell was composed of hARLDB amino acids having at least one atom within 8 Å of the ligand. Residue internal coordinates of the flexible shell were allowed to move during minimization steps. The other amino acids of the model were included in the outer rigid shell, in which they were not allowed to vary during energy minimization.

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Chapitre 2

Dérivés du 2β-pipérazino-5α-androstane-3α,17β-diol : synthèse en parallèle sur support solide et activité antiproliférative contre les cellules leucémiques HL-60

2.1 Avant-propos

Ma contribution à ce projet a été tout d'abord de caractériser par IR, RMN ^{1}H, RMN ^{13}C et LRMS les intermédiaires de synthèse et l'échantillonnage des produits finaux afin de confirmer leur structure. Tous ces produits ont été synthétisés par Patrick DeRoy. J'ai effectué l'évaluation biologique de tous les produits finaux afin de déterminer leur pouvoir antiprolifératif sur les cellules leucémiques HL-60. Par la suite, j'ai synthétisé en plus grande quantité les trois meilleurs produits afin d'en faire la caractérisation complète et de confirmer leur activité biologique. J'ai aussi rédigé ce manuscrit en collaboration avec mon directeur de recherche Donald Poirier et il sera bientôt soumis pour publication à la revue *Journal of Combinatorial Chemistry*.

2.2 Résumé

La leucémie est le cancer le plus commun chez les enfants. Une étude préliminaire a identifiée qu'un stéroïde ayant un noyau méthylpipérazine était capable d'inhiber la prolifération des cellules leucémiques HL-60. Pour accélérer le développement de ce nouveau composé, nous avons généré des librairies d'analogues à l'aide de la synthèse organique en parallèle sur support solide (SPOS). Débutant avec la dihydrotestostérone, une séquence de 6 étapes à permis d'obtenir un stéroïde 2α,3α-époxydé, lequel a été ouvert sélectivement, pour donner après une protection avec le Fmoc, un diol avec la stéréochimie désirée. La différence de réactivité entre les alcools 3α et 17β a ensuite été utilisée pour permettre le couplage régiosélectif du 17β-OH à la résine PS-DES. Par la suite, nous avons généré trois librairies de dérivés du 2β-piperazino-5α-androstane-3α,17β-diol avec 1, 2 ou 3 niveaux de diversité moléculaire dans des rendements et des puretés acceptables pour en faire l'évaluation biologique. Plusieurs membres de ces librairies ont été plus efficaces que le composé de départ, principalement trois dérivés possédant une proline comme premier niveau de diversité. Ils ont efficacement inhibé la prolifération des cellules HL-60 avec des IC_{50} de 0.58, 0.66 et 1.78 μM selon le dernier niveau de diversité introduit. Ce travail a démontré le potentiel de notre approche SPOS dans l'optimisation d'une nouvelle classe d'agents cytotoxiques.

J. Comb. Chem. (submitted)

2β-(*N*-substituted piperazino)-androstane-3α,17β-diols: parallel solid-phase synthesis and antiproliferative activity on human leukemia HL-60 cells

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Keywords: Steroid, amine, solid-phase synthesis, leukemia, HL-60 cells.

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Abstract

Leukemia is the most common cancer affecting children. A steroid possessing a methylpiperazine nucleus was recently reported to inhibit the proliferation of HL-60 leukemia cells. To speed up the development of this promising potential new drug, we generated libraries of analogues using parallel solid-phase organic synthesis (SPOS). Starting from dihydrotestosterone, a 6-step sequence of reactions afforded a steroidal 2α,3α-epoxide, which was selectively opened to give, after *N*-Fmoc protection, a diol with the suitable stereochemistry. The difference of reactivity between 3α-OHs and 17β-OHs was then used to allow the regioselective 17β-OH coupling to PS-DES resin. We next generated three libraries of 2β-piperazino-5α-androstane-3α,17β-diol having 1, 2 or 3 levels of molecular diversity in acceptable yields and purity for our biological assay. Several members of these libraries were more potent than the lead compound, especially three members having a proline as the first level of diversity and a cyclohexylcarbonyl, a methylbutyryl or a cyclohexylacetyl as the second level of diversity. They efficiently inhibited the HL-60 cell proliferation with IC_{50} values of 0.58, 0.66 and 1.78 μM. The present work demonstrates the potential of our SPOS approach for optimizing a new class of cytotoxic agents.

Introduction

Leukemia affects both sexes and all ages. Although often thought of as primarily a childhood disease, it is diagnosed 10 times more often in adults than in children. Despite its rarity, leukemia is the chief cause of death in children between ages 1 and 14 years old.[1]

Killing cancerous cells can be done by irradiation, but this method is not selective and damages all cells in the body. Antibiotics and transfusions of blood components are used as supportive treatments. Under appropriate conditions, bone marrow transplantation may be useful in treating certain leukemias. However, chemotherapy is the most effective method.[2] Various anticancer drugs are used, either in combinations or as single agents. The anthracycline glycosides, especially doxorubicin (adriamycin) and daunorubicin constitute potent chemotherapeutic agents, with clinical utility against a wide range of human malignancies.[3] However, their long-term effectiveness is often limited by a dose-related cumulative cardiotoxicity and the development of acquired drug resistance, mediated by overexpression of the ATP-dependent efflux proteins P-glycoprotein and multidrug-resistance protein in chemosensitive tumors of the multidrug-resistance phenotype.[4,5] For acute lymphoblastic leukemia, prednisone, a steroid hormone, combined with chemotherapeutic agents can bring remission in at least a third of children and half the adults struck by this disease.[6,7] Such an effect is rare in other forms of acute leukemia.

Moreover, a prominent phenotypic abnormality of human acute leukemia cells is the inability of the cells to differentiate to functional mature cells; instead, the cells are blocked at an early stage of development and remain in the proliferative pool and rapidly accumulate.[8] Extensive studies on differentiation of myeloid cells to monocytes/macrophages or neutrophils in response to retinoic acid,[9,10] dimethyl sulfoxide, phorbol-12-myristate-13-acetate,[10] vitamin D_3,[9] and dimethylformamide[11] have been described. Unfortunately, these compounds are rarely potent enough to induce differentiation in vivo when used at doses that do not cause serious clinical side effects. Thus, treatment options are still limited and it is important to find more effective treatments

that act differently and with fewer side effects. We focused our attention on the aminosteroid HY – 2β-(4'-methyl-1'-piperazino)-5α-androstane-3α,17β-diol (**1**) – that He's research group[12,13] previously reported to exert an inhibitory activity on leukemic cells (Figure 1). More specifically, this molecule inhibits the proliferation of HL-60 cells, a human promyelotic leukemia cell line, and promotes cell differentiation. This 5α-androstane derivative possesses also some structural similarity with androsterone, a steroid that was reported to stimulate hematopoiesis both in mice and in humans.[14]

Solid-phase combinatorial chemistry is a valuable tool in the development and optimization of compounds with relevant biological applications in different fields of medicine.[15-22] Given the high interest of our group for the solid-phase chemistry of steroid derivatives,[23-27] and our previous work with a piperazine nucleus,[28] we decided to generate a series of HY derivatives using parallel solid-phase organic synthesis in order to optimize the biological activity of this lead compound. In this report, we describe the chemical synthesis of three libraries of aminosteroids **2** (Figure 1) and present data on their antiproliferative activity on HL-60 cells. We also give data on structure-activity relationships that will be useful to optimize the biological activity of this new family of 2β-substituted aminosteroids with potential for treatment of leukemia.

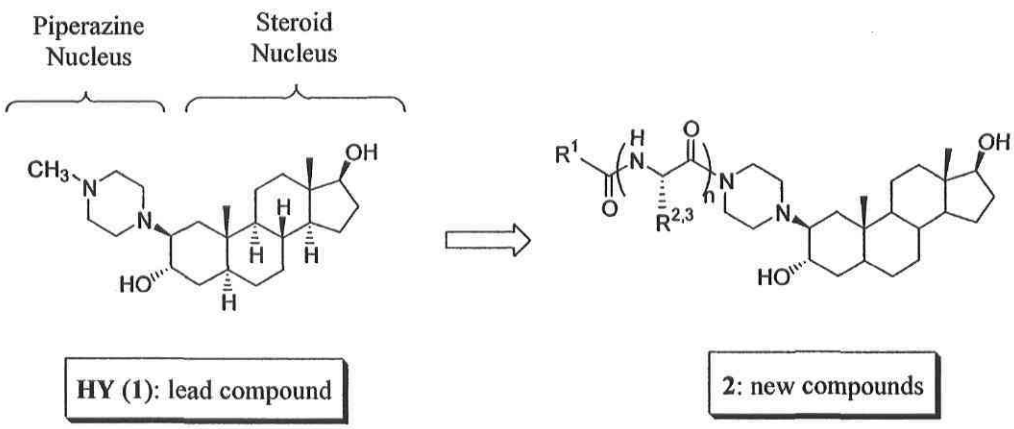
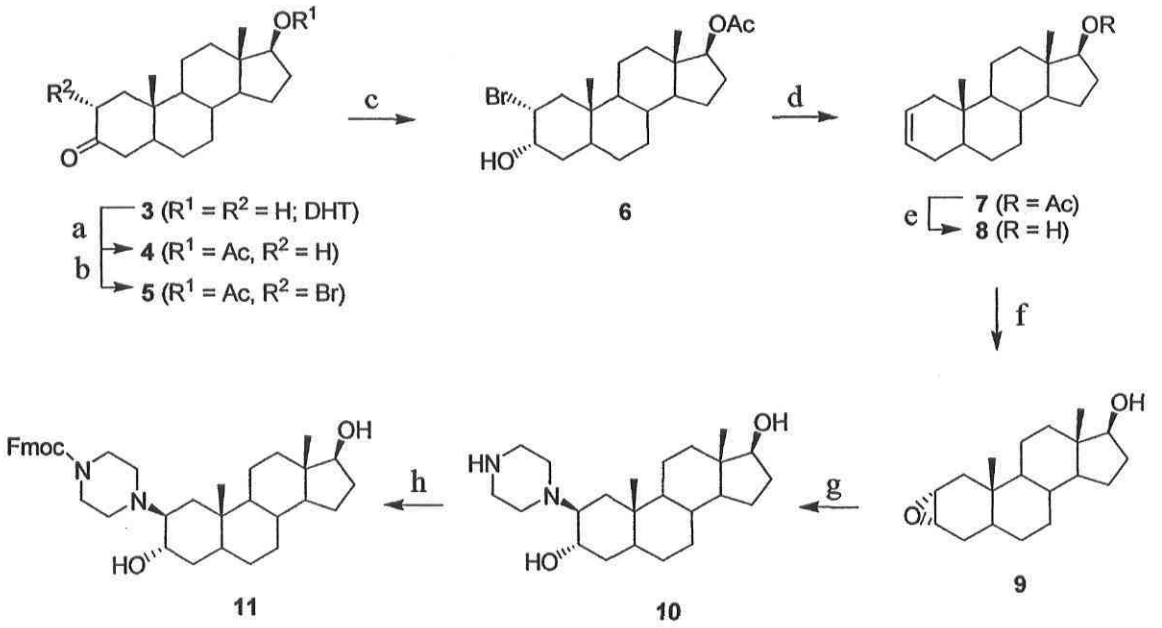


Figure 1. Chemical structure of 2β-(4'-methyl-1'-piperazinyl)-5α-androstane-3α,17β-diol, identified as HY (**1**), and of analogue compounds represented by the general structure **2** (n = 0, 1 or 2). The stereogenic centers are illustrated only for steroid **1**, but they are the same for all other steroid derivatives reported in this paper.

Results and Discussion

1. Synthesis of compound 11

The libraries of aminosteroids **2** were obtained from a common precursor, the piperazino derivative **11**, which was loaded on a polymeric support for the purpose of solid-phase synthesis. The key step in the preparation of **11** is a diastereoselective opening of epoxide **9** generated from the corresponding alkene **7** (Scheme 1). Synthetic approaches for generating **7** from dihydrotestosterone (DHT) can be found in the literature,[29] but they lead to the formation of a mixture of regioisomers (2,3 and 3,4 double bonds). It was however possible to obtain the 2,3-alkene by rigorously controlling the bromation and reduction steps of the reaction sequence. Thus the acetylation of DHT (**3**), followed by a selective bromination of DHT acetate (**4**) at C-2 with Br_2 in acetic acid, afforded bromoketone **5**. Using a stoichiometric equivalent of Br_2 is important in order to avoid a second bromination at C-4. K-selectride in THF, rather than the classical $NaBH_4$, was next used to obtain the bromohydrine **6**. Indeed, a better stereoselectivity for the reduction of bromoketone **5** was obtained with K-selectride, giving mainly the 3α-OH. This result was confirmed using ^1H NMR data of the well-known 3α-OH and 3β-OH 5α-androstanes reported in the literature.[30] The 3α-OH-androstane shows a fine signal at about 4.1 ppm, whereas the 3β-OH analogue gives a broad signal at 3.4 ppm. A flash chromatography is required at this step and a high purity of bromohydrine **6** is crucial for generating the alkene **7** in excellent C-2,3 isomeric purity. In fact, the residual non-brominated compound **4** will be reduced to a 3α-OH compound (3α-hydroxy-17β-acetoxy-5α-androstane). However, the latter product must be totally absent during the next step, because the elimination of this alcohol will give a mixture of C-2,3 and C-3,4 alkenes. Unfortunately, our attempt to purify this mixture by crystallization or chromatography was unsuccessful. However, the elimination of pure bromohydrine **6** in refluxing acetic acid with zinc powder gave the C-2,3 alkene **7**. Hydrolysis of the acetate and epoxidation with *m*-CPBA provided the 2,3α-epoxide **9**. This was later regio- and stereo-selectively opened to give,[31] after *N*-Fmoc protection of the piperazino nucleus of **10**, the solid-phase precursor **11**.



Scheme 1. Synthesis of solid-phase precursor **9**. Reagents and conditions are: (a) Ac_2O, pyridine, DMAP, rt (99%); (b) Br_2, AcOH, rt (95%); (c) K-selectride, -78°C, THF (71%); (d) Zn dust, AcOH, reflux (83%); (e) K_2CO_3/H_2O, MeOH, reflux (98%); (f) *m*-CPBA, CH_2Cl_2, 0°C (85%); (g) piperazine, H_2O, reflux (69%); (h) Fmoc-OSu, $NaHCO_3$ 1M, H_2O/THF (5:1), rt (70%).

The C2β- and C3α-stereochemistry of **11** was determined by NMR analysis. After having identified the key signals at C2 and C3, they were compared with ^1H and ^{13}C NMR data available in the literature for a steroid acting as a neuromuscular drug and possessing a 2β-morpholine and a 3α-OH group.[32] Our data for piperazino derivative **11** (2α-CH: 2.75 and 64.9 ppm; 3β-CH: 3.85 and 63.7 ppm) agree very well with reported data for the morpholino derivative (2α-CH: 2.54 and 65.2 ppm; 3β-CH: 3.89 and 63.9 ppm). X-ray analysis of aminosteroid **10** (Figure 2), which is the direct precursor of **11**, unambiguously confirmed that the stereochemistry of all centers was the expected one.

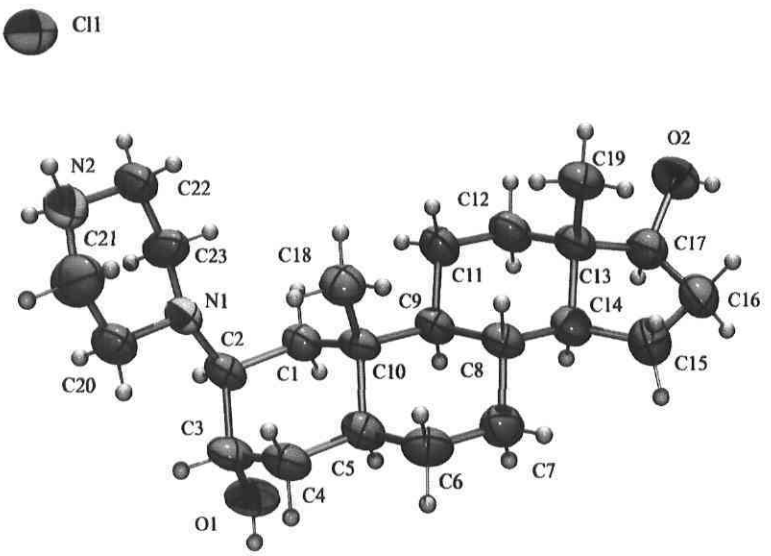
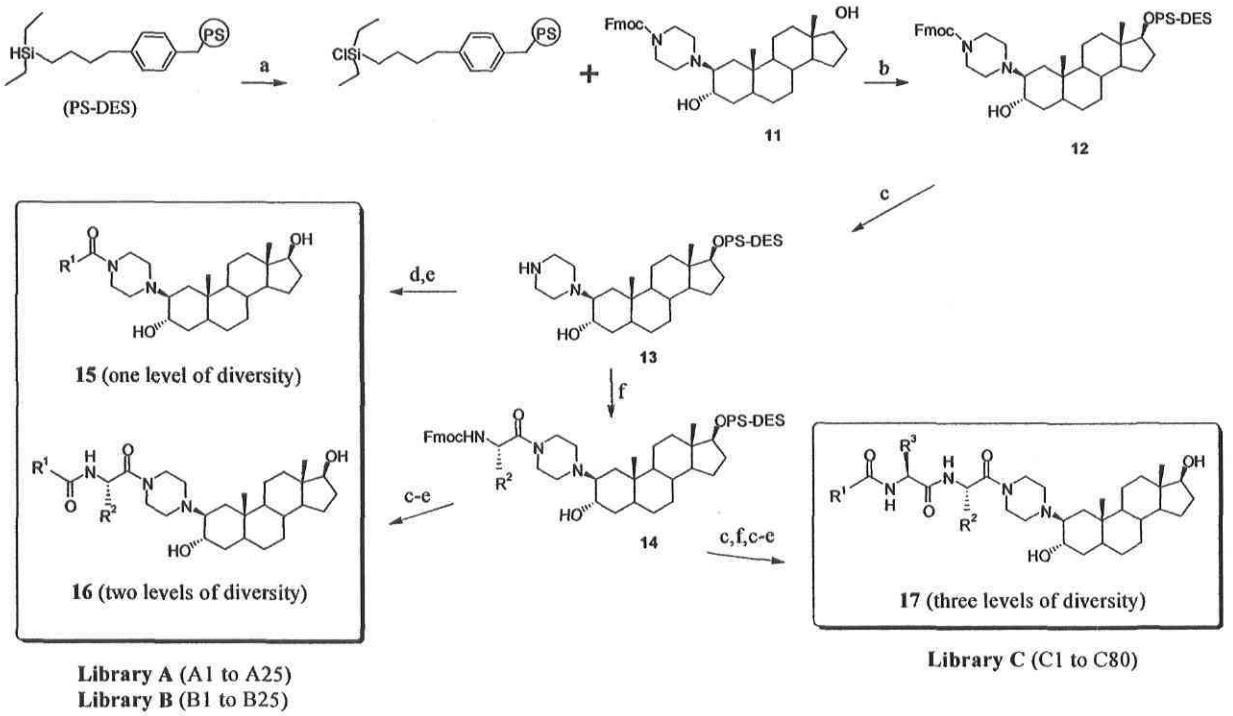


Figure 2. 3D-crystal structure of **10** showing the right stereochemistry at the two newly generated stereocenters (2β-piperazine and 3α-OH).

2. Solid-phase synthesis of libraries A, B and C

N-Fmoc-2β-piperazino-5α-androstane-3α,17β-diol (**11**) was coupled onto chlorosilyl resin, previously generated in situ from diethylsilyloxy linker (PS-DES resin). In the coupling reaction,[24] the resin was swelled in dry dichloromethane and treated with imidazole and the steroid (Scheme 2). The coupling yields of **11** giving **12**, calculated either by the increase of the resin weight or by the difference between the initial amount of steroid **11** and the amount of uncoupled **11**, were in the range of 50-80%, depending on the batch of resin **12**. The IR spectra of resin **12** showed the presence of the characteristic carbamate band. The Fmoc protecting group was easily cleaved with a solution of 20% piperidine in dichloromethane to afford resin **13**, which showed the characteristic IR and ^{13}C NMR signals of steroid **10** linked on PS-DES resin. Resin **13** was split and placed in the vessels of the reaction block of an ACT-Labtech semi-automated synthesizer for parallel synthesis. Both libraries A and B contain two series of compounds represented by **15** and **16**. Compounds with only one level of diversity were obtained by an acylation (PyBOP/HOBt) of each resin **13** sample with one of the selected carboxylic acids (R^1; see

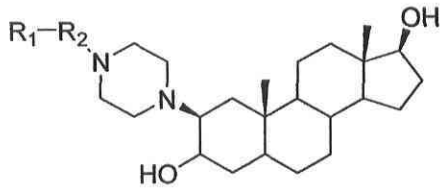
tables 1 and 2) giving **15** after cleavage of the silyl bond by vortexing the resin with a solution of HF-pyridine in dichloromethane. On the other hand, compounds with two levels of diversity were generated by an acylation of each resin **13** sample with one of the selected Fmoc-protected aminoacids (R^2; see Table 1), followed by the cleavage of Fmoc, an acylation with selected carboxylic acids (R^1) and final cleavage of the steroid from the resin with a solution of HF-pyridine in dichloromethane to afford **16**. Library C contains eighty compounds **17** with three levels of diversity. They were obtained from the intermediate resin **14** following the sequence of reactions reported above for the synthesis of **16**. Briefly, cleavage of the Fmoc protecting group of **14**, introduction of a second level of diversity by coupling a Fmoc-protected aminoacid (R^3) activated with PyBOP and HOBt, cleavage of the Fmoc protecting group, introduction of the last level of diversity with a carboxylic acid (R^1) and release of the steroid from the solid support afforded **17**.



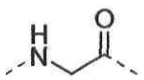
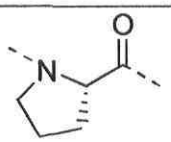
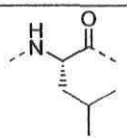
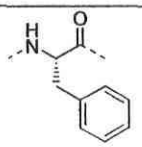
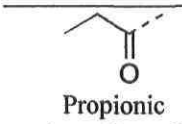
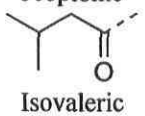
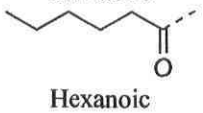
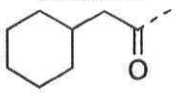
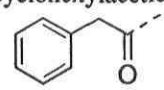
Scheme 2. Strategy for the synthesis of libraries of aminosteroids. Reagents and conditions are: (a) 1,3-dichloro-5,5-dimethylhydantoin, CH_2Cl_2, rt; (b) imidazole, CH_2Cl_2, rt (50-80%); (c) 20% piperidine in CH_2Cl_2, (v:v), rt; (d) carboxylic acid (R^1COOH), PyBOP, HOBt, DIPEA, DMF, rt; (e) *1*) HF/pyridine, CH_2Cl_2, *2*) $NaHCO_3$, *3*) $MgSO_4$ anh.; (f) *N*-Fmoc-*L*-amino acid (FmocNHCH(R^2 or R^3)COOH), PyBOP, HOBt, DIPEA, DMF, rt.

The three libraries were submitted to a random sampling and the selected members characterized by TLC, IR, ^1H NMR and MS analysis. These results confirmed the reactivity of all building blocks used in the elaboration of each library and allowed us to validate the rest of the library members. The overall mean crude yields for the solid-phase sequence of reactions were 75% for library A (6 steps), 94% for library B (6 steps) and 93% for library C (8 steps). The purity of aminosteroids released from the resin was estimated by TLC and NMR and found to be 70-90% for library A, 80-90% for library B and 50-80% for library C. In order to identity some hits, all members of libraries A-C were then submitted to a preliminary antiproliferative assay in HL-60 cells.

Table 1. Compound number (in bold) and inhibition (%) of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library A members.[a]

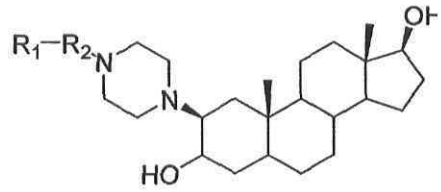


A1 - A25

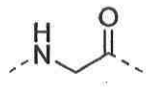
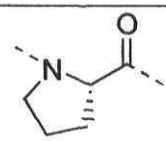
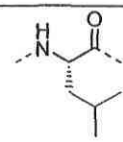
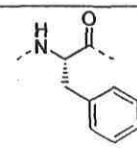
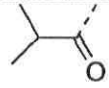
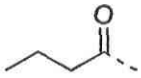
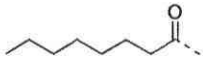
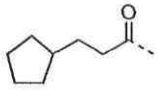
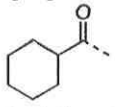
Amino acids (R_2) / **Carboxylic acids (R_1)**	 Gly	 Pro	 Leu	 Phe	NO Amino acid
 Propionic	**A1** 0 / 9	**A6** 0 / 72	**A11** 14 / 36	**A16** 0 / 76	**A21** 9 / 13
 Isovaleric	**A2** 9 / 13	**A7** 44 / 93	**A12** ND	**A17** 7 / 87	**A22** 18 / 24
 Hexanoic	**A3** 0 / 44	**A8** 49 / 91	**A13** 17 / 86	**A18** 8 / 98	**A23** 37 / 69
 Cyclohexylacetic	**A4** 6 / 61	**A9** 40 / 96	**A14** 14 / 93	**A19** 12 / 87	**A24** 39 / 95
 Phenylacetic	**A5** 0 / 3	**A10** 34 / 79	**A15** 12 / 70	**A20** 16 / 96	**A25** 18 / 75

[a] HY inhibited 20% of cell proliferation at 10 μM. Potent cytotoxic agent doxorubicin inhibited 96 and 99% of cell proliferation at 1 and 10 μM, respectively.

Table 2. Compound number (in bold) and inhibition (%) of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library B members.



B1 - B25

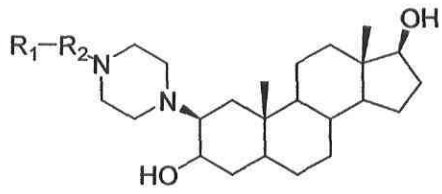
Amino acids (R_2) / **Carboxylic acids (R^1)**	 Gly	 Pro	 Leu	 Phe	NO Amino acid
 Isobutyric	**B1** 0 / 0	**B6** 16 / 87	**B11** 22 / 65	**B16** 0 / 84	**B21** 0 / 0
 Butyric	**B2** 0 / 9	**B7** 24 / 88	**B12** 18 / 43	**B17** 0 / 68	**B22** 0 / 8
 Octanoic	**B3** 0 / 45	**B8** 15 / 91	**B13** 0 / 51	**B18** 1 / 11	**B23** 36 / 95
 Cyclopentyl propionic	**B4** 5 / 55	**B9** 49 / 95	**B14** 4 / 73	**B19** 2 / 54	**B24** 36 / 84
 Cyclohexyl carboxylic	**B5** 0 / 16	**B10** 58 / 93	**B15** 15 / 70	**B20** 0 / 82	**B25** 0 / 0

3. Antiproliferative effect of aminosteroids on HL-60 cells

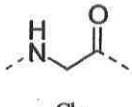
New synthesized aminosteroids from libraries A-C were tested to evaluate their effect on human myeloid leukemia HL-60 cell growth. The assay was performed at concentrations of 1 and 10 μM for each compound and the results are expressed as the percentage of cell growth inhibition (Tables 1-3). Interesting SAR results were obtained from the 130 aminosteroids. Thus, for compounds **A21-A25** and **B21-B25**, with only one level of diversity (R^1), the cyclohexylacetic and octanoic groups gave the best inhibition, compounds **A24** and **B23** inhibiting > 90% of the cell proliferation at 10 μM. For compounds **A1-A20** and **B1-B20**, with two levels of diversity (R^1 and R^2), the results also demonstrated the efficiency of a cyclohexylacetyl group, although similar groups, such as cyclopentylpropionic and octanoic groups also gave good results. However, the antiproliferative effect is strongly modulated by the presence and nature of an aminoacid. Indeed, compounds bearing a proline at R^2 showed the highest antiproliferative activity, better than the effect obtained with a leucine or a phenylalanine. Only a weak activity was achieved by compounds bearing a glycine at the same position. Compounds **A7-A9**, **A14**, **A18**, **A20** and **B8-B10** showed > 90% of cell growth inhibition at 10 μM, but at the lower concentration of 1 μM, only compounds with a proline as amino acid gave interesting results.

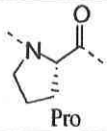
The aminosteroids from library C contain three levels of diversity, the first two levels (R^2 and R^3) consisting of Gly, Pro, Leu or Phe amino acids and the last level (R^1), of one of the five carboxylic acids previously used for the elaboration of library A. Most of them were clearly less active than analogue compounds of library A bearing the same element of diversity but only one or two levels of diversity. The Gly-Gly arrangement gave practically inactive compounds (0-30 % of inhibition). Only seven compounds gave over 90% of inhibition at 10 μM, the best combination of amino acids being Phe (as level R^2) and Pro (as level R^3) with 66-96% of growth inhibition and Phe-Gly with 37-94%. Five hits were obtained with these combinations of amino acids (**C63**, **C64**, **C65**, **C67**, **C-69** and **C70**). The combination Leu-Pro, with a cyclohexylacetic capping group (**C49**), and the combination Pro-Phe, with a phenylacetic capping group (**C40**), gave two more hits. At the lower concentration of 1 μM, however, their cell growth inhibition was negligible (0-10%).

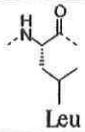
Table 3. Compound number (in bold) and inhibition (%) of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library C members.

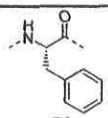


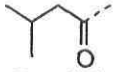
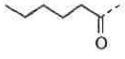
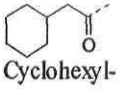
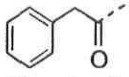
C1 - C80


Gly


Pro


Leu


Phe

	Amino acids (R_3) second level of diversity															
Carboxylic acids (R_1)	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe	Gly	Pro	Leu	Phe
 Propionic	**C1** 0/6	**C6** 8/8	**C11** 5/15	**C16** 0/0	**C21** 0/17	**C26** 0/0	**C31** 13/22	**C36** 0/10	**C41** 0/12	**C46** 0/10	**C51** 8/43	**C56** 3/18	**C61** 5/76	**C66** 10/66	**C71** 4/72	**C76** 0/41
 Isovaleric	**C2** 6/8	**C7** 0/28	**C12** 9/13	**C17** 0/0	**C22** 1/7	**C27** 0/60	**C32** 0/29	**C37** 0/46	**C42** 8/4	**C47** 0/48	**C52** 19/58	**C57** 0/29	**C62** 0/37	**C67** 8/96	**C72** 10/82	**C77** 0/37
 Hexanoic	**C3** 0/8	**C8** 21/23	**C13** 0/0	**C18** 0/6	**C23** 0/17	**C28** 3/52	**C33** 1/31	**C38** 1/73	**C43** 0/44	**C48** 0/63	**C53** 20/55	**C58** 2/5	**C63** 0/92	**C68** 10/89	**C73** 7/56	**C78** 0/14
 Cyclohexyl-acetic	**C4** 2/12	**C9** 4/30	**C14** 0/2	**C19** 0/28	**C24** 3/13	**C29** 0/41	**C34** 4/46	**C39** 0/82	**C44** 0/71	**C49** 0/98	**C54** 8/33	**C59** 2/8	**C64** 0/92	**C69** 9/95	**C74** 9/47	**C79** 0/1
 Phenylacetic	**C5** 0/6	**C10** 12/16	**C15** 5/4	**C20** 0/17	**C25** 0/22	**C30** 6/4	**C35** 5/51	**C40** 10/90	**C45** 4/13	**C50** 2/27	**C55** 14/31	**C60** 8/30	**C65** 5/94	**C70** 11/86	**C75** 9/48	**C80** 2/34

We also tested aminosteroid HY (**1**); and used doxorubicin, a well known potent cytotoxic agent,[33] as a positive control. Contrary to data previously reported in the literature,[12] the results indicated that the lead compound HY only has a weak effect on cell growth. Indeed, it inhibited about 20% of cell growth after 3 days of treatment at 10 μM. The aminosteroids from libraries A and B produced a much better inhibition of cell growth as represented by compounds **A7**, **A9** and **B10**, which inhibited 40 & 58% and 93 & 96% of cell proliferation at 1 and 10 μM, respectively. At these two concentrations, their percentages of growth inhibition are just slightly lower than those of doxorubicin (96 and 99%).

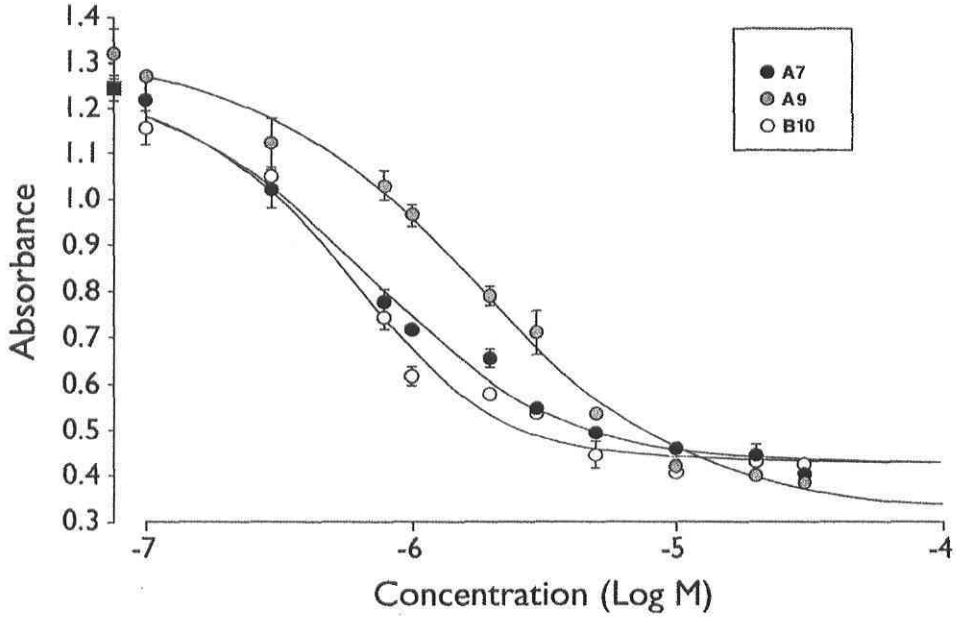


Figure 3. Effect of increasing concentrations of three selected aminosteroids on HL-60 cell growth represented by the absorbance (see Experimental section). The IC_{50} values are 0.66, 1.78 and 0.58 μM for **A7**, **A9** and **B10**, respectively.

After we identified the potential of aminosteroids **A7**, **A9** and **B10**, they were synthesized using the same approach discussed above, but in larger amounts for purposes of purification, characterization and validation of biological activity. The three compounds have a proline as amino acid element of diversity (R^2), suggesting its important contribution

to the cytotoxic activity. The yields (~65% for 5 steps after final purification) and purity obtained after this second synthesis were representative of the previously synthesized compounds. With pure compounds in our hands, we determined their IC_{50} values against HL-60 cells (Figure 3). Compounds **A7** and **B10** showed a better antiproliferative activity than **A9** since they displayed an IC_{50} of 0.66 ± 0.07 and 0.58 ± 0.07 μM, respectively, compared to 1.78 ± 0.21 μM for **A9**.

Conclusion

To speed up the development of aminosteroids with structural similarity to HY (Figure 1), we carried out a sequence of reactions taking advantage of our expertise in the solid-phase parallel synthesis of steroid derivatives. The key intermediate **11** (Scheme 1) was efficiently synthesized from DHT in 8 steps with an overall yield of 40%. Three libraries were then rapidly generated following a parallel approach (Scheme 2) giving individual compounds pure enough to be screened in our proliferative assay in HL-60 leukemia cells. Compounds **16** with two levels of diversity gave the best antiproliferative activity, especially for those bearing a proline at R^2 and a hydrophobic carboxylic acid with a six-member ring (aromatic or not) at R^1. In addition to generating SAR data, our results suggest that it is possible to modulate the antiproliferative activity by adding on a 3β-piperazino-5α-androstane-3β,17β-diol steroidal core an appropriate combination of an amino acid and of a carboxylic acid. We thus obtained several compounds with more potent cytotoxic activity than the lead compound HY. We also confirmed our results with pure aminosteroids **A7**, **A9** and **B10**, thus establishing the usefulness of our solid-phase strategy.

The next step for us is to extend our SAR study by synthesizing additional libraries of aminosteroids **16** taking advantage of the large diversity of amino acids (natural and non natural) and carboxylic acids commercially available. Although our preliminary results seem to indicate the efficiency of hydrophobic building blocks, our selection for the elaboration of libraries A-C was limited to only four aminoacids, mainly for the purpose of developing our chemical strategy. We must now extend this selection to a wider variety of groups. Thus the model libraries reported herein constitute a basic study for the preparation

of future more voluminous libraries of aminosteroids exhibiting antiproliferative effects against leukemia cells.

Experimental Section

General Methods

Dihydrotestosterone (DHT) was purchased from Steraloids (Wilton, NH, USA). The butyldiethylsilane polystyrene (PS-DES resin) with a loading of 1.58 mmol/g was supplied by Argonaut Technologies (San Carlos, CA, USA). Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and Calbiochem-Novabiochem Corp. (San Diego, CA, USA). The usual solvents were obtained from Fisher Scientific (Montreal, Qc, Canada) and were used as received. Anhydrous dichloromethane (CH_2Cl_2), dimethylformamide (DMF) and pyridine were obtained from Sigma-Aldrich. The loading of steroid **11** on resin was performed in peptide synthesis vessels (25 mL) with frit equipped for vacuum filtration (ChemGlass Inc.; Vineland, NJ, USA). The reaction vessels were shaken with a Burrell wrist-action shaker model 75 (Pittsburgh, PA, USA); the libraries of steroid derivatives were produced with an ACT LabTech manual synthesizer (Advanced ChemTech; Louisville, KY, USA) using either 40 or 96 solid-phase reaction blocks. The completion of solid-phase reactions were monitored after a micro-cleavage by thin-layer chromatography (TLC). TLC and flash-column chromatography were performed on 0.20-mm silica gel 60 F254 plates and with 230-400 mesh ASTM silica gel 60, respectively (E. Merck; Darmstadt, Germany). The purity of a sampling of final compounds released from solid support was determined by HPLC (Waters Associates Milford, MA, USA) using a Nova Pak C18 reversed-phase column (150 mm x 3.9 mm id) and an ultraviolet detector (205 nM or 225nM). Infrared spectra (IR) were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and the significant band reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C on a Bruker AC/F300 spectrometer (Billerica, MA, USA) or 400 MHz for ^{1}H and 100.6 MHz ^{13}C on a Bruker Avance 400 digital spectrometer and reported in ppm. 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. The X-ray analysis was performed by Marc Drouin at the *Laboratoire de diffraction des rayons-X de l'Université de Sherbrooke* (Sherbrooke Qc, Canada). Elemental analyses were provided by the Regional Laboratory for Instrumental Analysis (Université de Montréal, Montréal Qc, Canada).

17β-Acetoxy-5α-androstan-3-one (**4**)

Acetic anhydride (68 mL, 723 mmol) was added under argon at room temperature to a solution of dihydrotestosterone (**3**) (21.02 g, 72.4 mmol) and dimethylaminopyridine (DMAP) (177 mg, 1.45 mmol) in dry pyridine (50 mL). The resulting mixture was stirred for 3 h, concentrated under vacuum and the residue treated with HCl 20% (800 mL). The product was extracted with EtOAc and the organic phase washed with a saturated aqueous solution of $NaHCO_3$ (2 x 800 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 1:5) yielded 23.9 g (99%) of **4** as a white solid. IR (film): 1736 (C=O, ester), 1719 (C=O, ketone); ^{1}H NMR ($CDCl_3$): 0.80 (s, 18-CH_3), 1.01 (s, 19-CH_3), 0.75-2.40 (residual CH and CH_2), 2.04 (s, CH_3CO), 4.59 (dd, J = 9.0 Hz, J = 8.0 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.42, 12.08, 20.85, 21.16, 23.47, 27.48, 28.71, 31.16, 35.10, 35.66, 36.76, 38.08, 38.43, 42.57, 44.61, 46.55, 50.50, 53.64, 82.66, 171.16, 211.88; LRMS for $C_{21}H_{33}O_3$ $[MH]^+$: 333.4 m/z.

2α-Bromo-17β-acetoxy-5α-androstan-3-one (**5**)

To a solution of **4** (15.34 g, 46.2 mmol) in glacial acetic acid (460 mL) was added a 1 M solution of bromine (2.4 mL of Br_2 in 47 mL of AcOH) dropwise at room temperature. The resulting mixture was stirred for 1.5 h and concentrated under reduced pressure. The resulting red solution was dissolved in EtOAc (500 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 x 500 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 1:5) yielded 18.0 g (95%) of **5** as a white solid. IR (film): 1729 (C=O, ketone and ester), 732 (C-Br); ^{1}H NMR ($CDCl_3$): 0.80 (s, 18-CH_3), 1.09 (s, 19-CH_3), 0.80-2.45 (residual CH and CH_2), 2.04 (s, CH_3CO), 2.63 (dd, J = 13.4 Hz, J = 6.3 Hz, 1H), 4.58 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH),

4.74 (J = 13.4 Hz, J = 6.3 Hz, 2β-CH); ^{13}C NMR ($CDCl_3$): 12.10 (2x), 20.96, 21.18, 23.47, 27.46, 28.18, 30.95, 34.65, 36.58, 38.96, 42.53, 43.82, 47.37, 50.33, 51.56, 53.45, 54.39, 82.52, 171.18, 201.01; LRMS for $C_{21}H_{32}{}^{79}BrO_3$ $[MH]^+$: 411.3 m/z.

2α-Bromo-17β-acetoxy-5α-androstan-3α-ol (**6**)

A 1 M solution of K-selectride (21 mL, 21 mmol) was added dropwise under argon at –78 ^{O}C to a solution of **5** (4.3 g, 10.5 mmol) in dry THF (100 mL). The resulting mixture was stirred for 1 h and the temperature warmed to 0 ^{O}C; a saturated aqueous solution of NH_4Cl (400 mL) was then added. The resulting mixture was extracted with CH_2Cl_2 (3 x 200 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting crude bromohydrin was purified by flash chromatography (EtOAc:hexanes, 10:90) to afford 3.07g (71%) of **6** as a white solid. IR (film): 3447 (OH), 1717 (C=O), 735 (C-Br); ^{1}H NMR ($CDCl_3$): 0.77 (s, 19-CH_3), 0.83 (s, 18-CH_3), 2.03 (s, CH_3CO), 0.60-2.20 (residual CH and CH_2), 4.05 (s, 3β-CH), 4.46 (m, 2β-CH), 4.58 (t, J = 8.4 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.79, 12.03, 20.20, 21.11, 23.37, 27.29, 27.38, 31.06, 34.57, 34.76, 36.59, 37.40, 39.56, 42.44, 43.42, 50.47, 53.80, 58.33, 69.57, 82.60, 171.10; LRMS for $C_{21}H_{37}{}^{79}BrNO_3$ $[M + NH_4]^+$: 429.9 m/z.

17β-Acetoxy-5α-androst-2-ene (**7**)

To a solution of bromohydrin **6** (3.4 g, 8.2 mmol) in acetic acid (100 mL) was added zinc dust (3.77 g, 57.7 mmol) and the mixture was refluxed for 2 h. The solution was filtered and concentrated under vacuum, diluted in water, and the product extracted with EtOAc. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ until neutralisation, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 5:95) yielded 2.15 g (83%) of alkene **7** as a white solid. IR (film): 3018 (C=C), 1737 (C=O); ^{1}H NMR ($CDCl_3$): 0.75 (s, 19-CH_3), 0.78 (s, 18-CH_3), 0.75-2.20 (residual CH and CH_2), 2.04 (s, CH_3CO), 4.58 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH), 5.58 (m, 2H alkene); ^{13}C NMR ($CDCl_3$): 11.66, 12.04, 20.34, 21.21, 23.48, 27.49, 28.55, 30.23, 31.32, 34.66, 35.33, 36.89, 39.72, 41.39, 42.47, 50.67, 53.94, 82.90, 125.80 (2x), 171.31; LRMS for $C_{21}H_{33}O_2$ $[MH]^+$: 317.3 m/z.

5α-Androst-2-en-17β-ol (**8**)

A solution of K_2CO_3 (3.15 g, 22.8 mmol) in water (20 mL) was added to a solution of **7** (1.80 g, 5.7 mmol) in methanol (100 mL) and the mixture was refluxed for 1.5 h. The reaction mixture was diluted in water and extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 1:5) yielded 1.55 g (98%) of **8** as a white solid. IR (film): 3252 (OH), 3017 (C=C); ^{1}H NMR ($CDCl_3$): 0.74 (s, 19-CH_3), 0.76 (s, 18-CH_3), 0.70-2.10 (residual CH and CH_2), 3.63 (t, J = 8.5 Hz, 17α-CH), 5.58 (m, 2H alkene); ^{13}C NMR ($CDCl_3$): 11.05, 11.71, 20.48, 23.37, 28.60, 30.28, 30.50, 31.37, 34.70, 35.63, 36.75, 39.79, 41.51, 42.86, 51.00, 54.17, 82.01, 125.85 (2x); LRMS for $C_{19}H_{34}NO$ $[MH_4]^+$: 292.3 m/z.

2α,3α-Epoxy-5α-androstan-17β-ol (**9**)

To a solution of **8** (1.02 g, 3.7 mmol) in dry CH_2Cl_2 (35 mL) at 0 °C was added *m*-chloroperbenzoic acid (*m*-CPBA) 77% pure (1.23 g, 5.46 mmol) in six portions. The mixture was stirred for 1 h at 0 °C, then allowed to warm at room temperature and stirred overnight. The mixture was concentrated under reduced pressure, the residue was diluted in EtOAc and the solution washed successively with a saturated aqueous solution of $Na_2S_2O_3$ (2 x 100 mL) and a saturated aqueous solution of Na_2CO_3 (2 x 100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 1:5) yielded 909 mg (85%) of **9** as a white solid. IR (film): 3262 (OH); ^{1}H NMR ($CDCl_3$): 0.72 (s, 19-CH_3), 0.76 (s, 18-CH_3), 0.55-2.10 (residual CH and CH_2), 3.12 (m, 2H epoxide), 3.63 (t, J = 8.5 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.02, 12.95, 20.45, 23.34, 28.26, 29.00, 30.44, 31.20, 33.70, 35.67, 36.28, 36.60, 38.27, 42.78, 50.78, 51.04, 52.42, 53.79, 81.88; LRMS for $C_{19}H_{31}O_2$ $[MH^+]$: 291.3 m/z.

2β-Piperazino-5α-androstane-3α,17β-diol (**10**)

A solution of **9** (6.46 g, 22.3 mmol) in piperazine (50 g, 582 mmol) and water (6.3 mL) was refluxed (160 °C) for 24 h. The mixture was poured in water (500 mL) and the precipitate was filtered. The solid was dissolved in CH_2Cl_2 and the solution dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography

(MeOH:Et_3N:CH_2Cl_2, 14:1:85) yielded 5.76 g (69%) of **10** as a white solid. IR (NaCl film): 3370 (OH, alcohols and NH, amine). ^1H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.65-2.15 (residual CH and CH_2), 2.42, 2.58 and 2.90 (3m, 4 x CH_2N and 2α-CH), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH). ^{13}C NMR ($CDCl_3$): 11.2, 17.3, 20.9, 23.3, 28.2, 30.5, 31.1, 32.6, 34.7, 35.5, 35.7, 36.9, 38.4, 43.0, 46.7 (2x), 49.4 (2x), 50.9, 56.2, 63.3, 65.0, 81.6; LRMS for $C_{23}H_{41}N_2O_2$ $[MH]^+$: 377.3 m/z.

2β-[(N-(9-fluorenylmethoxycarbonyl)-piperazino]-5α-androstane-3α,17β-diol (**11**)

To a solution of **10** (5.76 g, 15.3 mmol) in a mixture of THF:water, 5:1 (275 mL) was added successively aqueous $NaHCO_3$ 1 M (37 mL) and *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu) in six portions. The mixture was stirred for 3 h then diluted in water and extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (EtOAc:hexanes, 1:1) and crystallization from a mixture of CH_2Cl_2 and hexanes yielded 6.1 g (70%) of **11** as a white solid. IR (NaCl film): 3423 (OH, alcohol), 1690 (C=O, carbamate), 1448 (aromatic ring), 1243 (C-O-C, carbamate); ^1H NMR ($CDCl_3$): 0.74 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.20 (residual CH and CH_2), 2.4-2.9 (broad, 2 x CH_2N and 2α-CH), 3.4-3.7 (broad, 2 x CH_2NCO), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.85 (m, 3β-CH), 4.24 (t, J = 6.6 Hz, C<u>H</u>CH_2 of Fmoc), 4.45 (d, J = 6.3 Hz, CH_2O of Fmoc), 7.34 (t, J = 7.4 Hz, 2H of Fmoc), 7.40 (t, J = 7.4 Hz, 2H of Fmoc), 7.57 (d, J = 7.4 Hz, 2H of Fmoc), 7.77 (d, J = 7.5 Hz, 2H of Fmoc); ^{13}C NMR ($CDCl_3$): 11.2, 17.3, 20.9, 23.3, 28.1, 30.5, 31.1, 32.9, 34.7, 35.5, 35.8, 36.8, 38.4, 43.1, 44.1, 47.3, 48.0, 50.8, 56.1, 63.7, 64.9, 67.2, 81.8, 119.9 (2x), 124.9 (2x), 127.0 (2x), 127.7 (2x), 141.3, 143.9, 155.0; LRMS for $C_{38}H_{51}N_2O_4$ $[MH]^+$: 599.3 m/z.

Synthesis of resin **12**. To PS-DES resin (2.00 g, 1.58 mmol/g theoretical loading) previously dried under vacuum during 2 days, added in a 50 mL peptide flask under argon and swollen in dry CH_2Cl_2 (10 mL), was added 1,3-dichloro-5,5-dimethylhydantoin (1.86 g, 9.47 mmol) in dry CH_2Cl_2 (10 mL). After 1 h, the resulting chlorosilyl resin was washed under argon with dry CH_2Cl_2 (3 x 20 mL). The disappearance of the SiH band at 2100 cm^{-1}

was confirmed in IR spectrum and the resin was next used for the loading step. Under argon, the resin was swollen in dry CH_2Cl_2 (10 mL) and a solution of imidazole (645 mg, 9.46 mmol) and hydroxysteroid **11** (5.66 g, 9.47 mmol) in CH_2Cl_2 (10 mL) was added. The solution was vortexed with a Burrell wrist-action shaker for 4 h at room temperature. The resin was washed with CH_2Cl_2 (5 x 20 mL), MeOH (3 x 20 mL), and dried overnight under vacuum to give 3.24 g of **12** with a loading of 0.64 mmol/g. IR (KBr): 3465 (OH, alcohol), 1702 (C=O, carbamate). The free steroid **11** (4.26 g) was easily recovered after a flash chromatography with EtOAc:hexanes (1:1).

Synthesis of resin **13**. A solution of piperidine in CH_2Cl_2 (1:5, v/v) (30 mL) was added to the resin **12** (3.24 g, 2.07 mmol) and the resulting solution was vortexed for 1 h at room temperature. The resin was then washed with CH_2Cl_2 (3 x 30 mL), MeOH (3 x 30 mL), and dried overnight under vacuum to give 2.75 g of **13**. IR (KBr): 3435 (OH, alcohol and NH, amine), no C=O band of carbamate at 1702 cm^{-1}; ^{13}C NMR ($CDCl_3$) (using the conditions reported in reference 24): 4.40, 5.33, 6.24, 6.76, 6.95, 11.46, 12.25, 13.09, 14.06, 17.25, 20.95, 22.41, 22.97, 23.45, 28.27, 31.19, 32.65, 34.64, 35.53, 35.73, 37.21, 38.44, 40.27, 43.41, 46.54, 49.38, 50.62, 56.29, 63.44, 65.08, 81.62, 127.90.

Synthesis of **library A** (Table 1). *First part, synthesis of 15:* To five samples of resin **13** (5 x 50 mg, 0.032 mmol) under argon was added a solution of benzotriazole-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (33.4 mg, 0.064 mmol), *N*-hydroxybenzotriazole (HOBt) (8.7 mg, 0.064 mmol), carboxylic acid (0.064 mmol) (propionic acid, isovaleric acid, hexanoic acid, cyclohexylacetic acid or phenylacetic acid) and diisopropylethylamine (DIPEA) (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL). The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 x 2 mL), MeOH (2 x 2 mL) and dried under vacuum. To release the diversified steroid **15**, each resin was treated with a mixture of CH_2Cl_2:THF (10:1) (0.5 mL) and a solution of HF/pyridine (20 μL) for 45 min. To the resulting solution were next added CH_2Cl_2 (0.1 mL), $NaHCO_3$ (100 mg); the mixture was vortexed for 30 min. CH_2Cl_2 (0.2 mL), anhydrous $MgSO_4$ (100 mg) were added and the resin vortexed for 2 min before being filtered. The

filtrate was washed with a saturated solution of $NaHCO_3$ (5 mL) and finally with water (5 mL). The organic layer was evaporated under reduced pressure to give **15** (see Table 1, column E). *Second part: synthesis of 16*. To twenty samples of resin **13** (20 x 50 mg, 0.032 mmol) under argon was added a solution of PyBOP (33.4 mg, 0.064 mmol), HOBt (8.7 mg, 0.064 mmol), *N*-Fmoc protected *L*-amino acid (0.064 mmol) (glycine, proline, leucine, or phenylalanine) and DIPEA (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL). The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 x 2 mL), MeOH (2 x 2 mL) and dried under vacuum to give **14**. To these resins was added a solution of piperidine (20%, v/v) in CH_2Cl_2 (0.5 mL). The mixture was vortexed for 1 h at room temperature, washed with CH_2Cl_2 (5 x 2 mL), MeOH (2 x 2 mL) and dried under vacuum overnight to give the resins with a free secondary amine. To each resin under argon was added a solution of PyBOP (33.4 mg, 0.064 mmol), HOBt (8.7 mg, 0.064 mmol), a carboxylic acid (0.064 mmol) (propionic acid, isovaleric acid, hexanoic acid, cyclohexylacetic acid, or phenylacetic acid) and DIPEA (22.4 μL, 0.128 mmol) in dry DMF (0.5 mL). The suspension was vortexed for 2 h at room temperature. The resin was washed with CH_2Cl_2 (5 x 2 mL), and MeOH (2 x 2 mL) and dried under vacuum to give resin. The procedure reported above for the synthesis of **15** was used to release the free steroids **16** (see Table 1, columns A-D). After a TLC analysis of each member of library A that confirmed the presence of a major compound, the twenty-five compounds with one and two levels of molecular diversity were submitted to a sampling that selected five compounds (**A5**, **A8**, **A14**, **A16** and **A22**), which were characterized by ^{1}H NMR and LRMS.

A5 (yield 43%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.70-2.20 (residual CH and CH_2), 2.3-2.8 (broad, 4 x CH_2N and 2α-CH), 3.2-3.8 (broad, 17α-CH and 2 x CH_2NCO), 3.63 (s, $\underline{CH_2}Ph$), 4.00 (m, 3β-CH and $NH\underline{CH_2}CO$), 6.55 (NH), 7.30 and 7.37 (2m, 5H, $CH_2\underline{Ph}$); LRMS for $C_{33}H_{50}N_3O_4$ $[MH]^+$: 552.3 m/z.

A8 (yield 56%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.89 (t, J = 6.8 Hz, CH_3CH_2), 0.70-2.30 (residual CH and CH_2), 2.25 (q, J = 7.6 Hz, CH_2CO), 2.4-3.0 (broad, 2 x CH_2N and 2α-CH), 3.54 and 3.65 (2m, 17α-CH, 2 x CH_2NCO and CH_2N of proline), 3.95 (m, 3β-CH), 4.82 (m, NCHCO); LRMS for $C_{34}H_{58}N_3O_4$ $[MH]^+$: 572.3 m/z.

A14 (yield 65%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.91 (d, J = 6.6 Hz, ($\underline{CH_3}$)$_2$CH), 0.70-2.10 (residual CH and CH_2), 2.06 (d, J = 6.8 Hz, NHCO$\underline{CH_2}$), 2.3-3.0 (broad, 2 x CH_2N and 2α-CH), 3.3-3.9 (broad, 2 x CH_2NCO), 3.63 (q, J = 6.1 Hz, 17α-CH), 3.95 (m, 3β-CH), 4.95 (m, NH$\underline{CH}$CO), 6.20 (NH); LRMS for $C_{37}H_{64}N_3O_4$ [MH^+] : 614.3 m/z.

A16 (yield 69%). ^{1}H NMR ($CDCl_3$): 0.75 (s, 18-CH_3), 0.83 (s, 19-CH_3), 1.14 (t, J = 7.6 Hz $CH_2\underline{CH_3}$), 0.70-2.20 (residual CH and CH_2), 2.22 (q, J = 7.6 Hz, NHCO$\underline{CH_2}$), 2.4-3.1 (broad, 2 x CH_2N, 2α-CH, and $\underline{CH_2}$-Ph), 3.2-3.6 (broad, 2 x CH_2NCO), 3.64 (q, J = 6.4 Hz, 17α-CH), 3.82 (m, 3β-CH), 5.17 (m, NH$\underline{CH}$CO), 6.38 (NH), 7.20 (m, 2H of Ph), 7.30 (m, 3H of Ph); LRMS for $C_{35}H_{54}N_3O_4$ [$MH]^+$: 580.3 m/z.

A22 (yield 64%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.97 (d, J = 6.5 Hz, ($\underline{CH_3}$)$_2$CH), 0.70-2.20 (residual CH and CH_2), 2.22 (d, J = 6.8 Hz, $COCH_2$), 2.4-2.9 (broad, 2 x CH_2N and 2α-CH), 3.4-3.8 (broad, 2 x CH_2NCO), 3.63 (q, J = 6.4 Hz, 17α-CH), 3.91 (m, 3β-CH); LRMS for $C_{28}H_{49}N_2O_3$ [$MH]^+$: 461.2 m/z.

Synthesis of **library B** (Table 2). For the preparation of this library, we used the same procedure as for library A (see above), but the carboxylic acids (R^1 level) were changed for isobutyric acid, butyric acid, octanoic acid, cyclopentylpropionic acid and cyclohexylcarboxylic acid. The loading of resin **12** was 0.55 mmol/g and we used 48 mg of resin for the synthesis of each library member. For the cleavage step giving **15** and **16**, we performed the reaction only in CH_2Cl_2 instead of CH_2Cl_2:THF (10:1). TLC analysis of each member confirmed the presence of a major compound and the sampling selected five compounds (**B5**, **B8**, **B14**, **B16** and **B22**), which were characterized by ^{1}H NMR and LRMS.

B5 (yield 95%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.70-2.30 (residual CH and CH_2), 2.6-3.3 (broad, 2 x CH_2N and 2α-CH), 3.5-4.2 (broad, 2 x CH_2NCO, NH$\underline{CH_2}$CO and 3β-CH), 3.63 (q, J = 6.1 Hz, 17α-CH), 6.50 (NH); LRMS for $C_{32}H_{54}N_3O_4$ [$MH]^+$: 544.3 m/z.

B8 (yield 92%). ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.87 (t, J = 7.0 Hz, CH_3CH_2), 0.88 (s, 19-CH_3), 0.70-2.20 (residual CH and CH_2), 2.30 (m, CH_2CON), 2.5-3.2 (broad, 2 x CH_2N

and 2α-CH), 3.55 and 3.66 (2m, (m, 17α-CH and CH_2N of proline), 3.3-4.0 (broad, 2 x CH_2NCO), 4.02 (m, 3β-CH), 4.80 (m, NCHCO); LRMS for $C_{36}H_{62}N_3O_4$ $[MH]^+$: 600.3 m/z.

B14 (yield 96%). ^1H NMR ($CDCl_3$): 0.74 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.92 and 0.96 (2d, J = 6.5 Hz, ($\underline{CH_3}$)$_2$CH), 0.70-2.15 (residual CH and CH_2), 2.21 (dd, J_1 = 7.0 Hz and J_2 = 8.6 Hz, $\underline{CH_2}$CONH), 2.6-3.1 (broad, 2 x CH_2N and 2α-CH), 3.63 (q, J = 6.3 Hz, 17α-CH), 3.4-4.2 (broad, 2 x CH_2NCO), 4.02 (m, 3β-CH), 4.94 (m, NH$\underline{CH}$CO), 6.10 (NH); LRMS for $C_{37}H_{64}N_3O_4$ $[MH]^+$: 614.2 m/z.

B16 (yield 95%). ^1H NMR ($CDCl_3$) : 0.75 (s, 18-CH_3), 0.88 (s, 19-CH_3), 1.13 and 1.14 (2d, J = 6.8 Hz, ($\underline{CH_3}$)$_2$CH), 0.70-2.2 (residual CH and CH_2), 2.37 (quintet, J = 6.9 Hz, (CH$_3$)$_2\underline{CH}$), 2.7-3.2 (broad, 2 x CH_2N and 2α-CH), 3.65 (m, 17α-CH), 3.3-4.2 (broad, 2 x CH_2NCO, $\underline{CH_2}$Ph and 3β-CH), 5.05 (m, NH$\underline{CH}$CO), 7.32 (m, CH$_2\underline{Ph}$), 6.25 (NH); LRMS for $C_{36}H_{56}N_3O_4$ $[MH]^+$: 594.2 m/z.

B22 (yield 95%). ^1H NMR ($CDCl_3$): 0.74 (s, 18-CH_3), 0.88 (s, 19-CH_3), 0.97 (t, J = 7.4 Hz, $\underline{CH_3}CH_2$), 0.70-2.20 (residual CH and CH_2), 2.29 (t, J = 7.5 Hz, $\underline{CH_2}$CO), 2.5-3.2 (broad, 2 x CH_2N and 2α-CH), 3.63 (q, J = 6.3 Hz, 17α-CH), 3.4-4.1 (broad, 2 x CH_2NCO), 4.00 (m, 3β-CH); LRMS for $C_{27}H_{47}N_2O_3$ $[MH]^+$: 447.4 m/z.

Synthesis of **library C** (Table 3). For the synthesis of library C, we used the same building blocks and procedure as for library A (see above), but only 27 mg of resin **13** (80 x 0.0134 mmol). Furthermore, two levels of amino acids (R^2 and R^3) were successively introduced before adding the final carboxylic acids (R^1). To release **17** we used pure CH_2Cl_2 (0.3 mL) instead of a mixture of CH_2Cl_2:THF (10:1). TLC analysis of each member of library C confirmed the presence of a major compound. From the eighty compounds with three levels of molecular diversity, a sampling selected ten compounds (**C9**, **C20**, **C21**, **C32**, **C42**, **C53**, **C56**, **C64**, **C70** and **C78**), which were characterized by ^1H NMR and LRMS.

C9 (yield 98%). ^1H NMR (CD_3OD): 0.72 (s, 18-CH_3), 1.01 (s, 19-CH_3), 0.70-2.2 (residual CH and CH_2), 2.26 (t, J = 6.8 Hz, CH_2CO), 2.4-2.8 (broad, 2 x CH_2N and 2α-CH), 3.3-3.8 (m, 2 x CH_2NCO, NH$\underline{CH_2}$CO, 17α-CH and CH_2N of proline), 4.08 (m sharp, 3β-CH), 4.50 (m, NCHCO); LRMS for $C_{38}H_{63}N_4O_5$ $[MH]^+$: 655.6 m/z.

C20 (yield 98%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70-2.1 (residual CH and CH_2), 2.4-2.7 (broad, 2 x CH_2N and 2α-CH), 3.21 (dd, J_1 = 5.0 Hz and J_2 = 14.0 Hz, CH$\underline{CH_2}$Ph), 3.47 (d, J = 6.6 Hz CO$\underline{CH_2}$Ph), 3.55 (m, 2 x CH_2NCO and 17α-CH), 4.05 (m sharp, 3β-CH and NH$\underline{CH_2}$CO), 4.70 (dd, J_1 = 5.0 Hz and J_2 = 9.7 Hz, NH$\underline{CH}$CO), 7.08 (d, J = 7.8 Hz, 2H of Ph), 7.20 (m, 8H of 2 x Ph); LRMS for $C_{42}H_{59}N_4O_5$ $[MH]^+$: 699.5 m/z.

C21 (yield 98%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 1.01 (s, 19-CH_3), 1.13 (t, J = 7.6 Hz, $\underline{CH_3}CH_2$), 0.7-2.1 (residual CH and CH_2), 2.28 (q, J = 7.6 Hz, $CH_3\underline{CH_2}$CO), 2.4-2.8 (broad, 2 x CH_2N and 2α-CH), 3.5-3.8 (m, 2 x CH_2NCO, 17α-CH, and $\underline{CH_2}$N of proline), 3.9-4.2 (m, 3β-CH and NH$\underline{CH_2}$CO), 4.95 (m, NCHCO); LRMS for $C_{33}H_{55}N_4O_5$ $[MH]^+$: 587.3 m/z.

C32 (yield 98%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.92-0.99 (8s, 2 x $(CH_3)_2$CH), 1.01 (s, 19-CH_3), 0.7-2.3 (residual CH and CH_2), 2.5-2.9 (broad, 2 x CH_2N and 2α-CH), 3.4-3.8 (broad, 2 x CH_2NCO and 17α-CH), 4.09 (m, 3β-CH), 4.70 (m, NH$\underline{CH}$CO), 4.90 (m, NCHCO); LRMS for $C_{39}H_{67}N_4O_5$ $[MH]^+$: 671.5 m/z.

C42 (yield 91%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.95 (m, 2 x $(\underline{CH_3})_2$CH), 1.01 (s, 19-CH_3), 0.7-2.1 (residual CH and CH_2), 2.13 (d, J = 6.3 Hz, CH$\underline{CH_2}$CO), 2.5-2.9 (broad, 2 x CH_2N and 2α-CH), 3.4-3.7 (broad, 2 x CH_2NCO), 3.55 (t, J = 8.7 Hz, 17α-CH), 3.80 (m, NHCHCO), 3.85 (d, J = 7.2 Hz, NH$\underline{CH_2}$CO), 4.10 (m, 3β-CH); LRMS for $C_{36}H_{63}N_4O_5$ $[MH]^+$: 631.6 m/z.

C53 (yield 95%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.90-0.97 (7s, 2 x $(\underline{CH_3})_2$)CH and $\underline{CH_3}CH_2$), 1.01 (s, 19-CH_3), 0.7-2.1 (residual CH and CH_2), 2.22 (t, J = 7.6 Hz, CH_2CO), 2.5-2.9 (broad, CH_2N and 2α-CH), 3.4-3.9 (broad, 2 x CH_2NCO), 3.55 (t, J = 8.6 Hz, 17α-CH), 4.09 (3β-CH), 4.39 (t, J = 7.6 Hz, NH$\underline{CH}$CO), 4.95 (m, NH$\underline{CH}$CO); LRMS for $C_{41}H_{73}N_4O_5$ $[MH]^+$: 701.4 m/z.

C56 (yield 97%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.94 (d, J = 6.4 Hz, $(\underline{CH_3})_2$CH), 1.01 (s, 19-CH_3), 1.01 (t, J = 7.6 Hz, $\underline{CH_3}CH_2$), 0.7-2.1 (residual CH and CH_2), 2.15 (q, J = 7.6 Hz, $\underline{CH_2}$CO), 2.5-2.9 (broad, 2 x CH_2N and 2α-CH), 2.85 and 3.15 (2 dd, $\underline{CH_2}$Ph), 3.55 (t, J = 8.6 Hz, 17α-CH), 3.4-3.9 (broad, 2 x CH_2NCO), 4.10 (3β-CH), 4.64 (m, NH$\underline{CH}$CO), 3.90 (m, NH$\underline{CH}$CO); LRMS for $C_{41}H_{65}N_4O_5$ $[MH]^+$: 693.4 m/z.

C64 (yield 96%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.95 (s, 19-CH_3), 0.7-2.1 (residual CH and CH_2), 2.12 (d, J = 6.9 Hz, $CHCH_2CO$), 2.3-2.7 (m, 2 x CH_2N and 2α-CH), 2.95 (m, CH_2Ph), 3.1-3.5 (broad, 2 x CH_2NCO), 3.56 (t, J = 8.6 Hz, 17α-CH), 3.82 (s, $NHCH_2CO$), 4.00 (3β-CH), 5.07 (t, J = 7.0 Hz, $NHCHCO$), 7.23 (d, J = 6.5 Hz, 2H of Ph), 7.29 (m, 3H of Ph); LRMS for $C_{42}H_{65}N_4O_5$ $[MH]^+$: 705.5 m/z.

C70 (yield 98%). ^{1}H NMR (CD_3OD): 0.72 (s, 18-CH_3), 0.94 (s, 19-CH_3), 0.7-2.2 (residual CH and CH_2), 2.25-2.75 (broad, 2 x CH_2N and 2α-CH), 3.00 (d, J = 7.6 Hz, CH_2Ph), 3.4-3.7 (broad, 2 x CH_2NCO and CH_2N of proline), 3.56 (t, J = 8.4 Hz, 17α-CH), 3.75 (s, CH_2Ph), 3.97 (m, 3β-CH), 4.42 (m, NCHCO), 5.04 (t, J = 7.7 Hz, $NHCHCO$), 7.25 (m, 2 x Ph); LRMS for $C_{45}H_{63}N_4O_5$ $[MH]^+$: 739.3 m/z.

C78 (yield 84%). ^{1}H NMR (CD_3OD): 0.74 (s, 18-CH_3), 0.86 (t, J = 7.4 Hz, CH_3CH_2), 0.95 (s, 19-CH_3), 0.70-2.10 (residual CH and CH_2), 2.13 (t, J = 7.5 Hz, CH_2CO), 2.5-3.7 (broad, 2 x CH_2N, 2 x CH_2NCO and 2α-CH), 3.58 (m, 17α–CH), 4.07 (3β-CH), 4.60 (m, $NHCHCO$), 5.02 (m, $NHCHCO$), 7.25 (m, 2 x Ph); LRMS for $C_{47}H_{69}N_4O_5$ $[MH]^+$: 769.6 m/z.

Synthesis and full characterization of **A7, A9** and **B10**. These three compounds were generated from resin **12** (256 mg, 0.52 mmol/g) following the sequence of reactions reported in scheme 2 for the synthesis of **16**. The final purification by flash chromatography with a mixture of CH_2Cl_2 and MeOH (10:1) yielded 44 mg of **A7**, 54 mg of **A9** and 66 mg of **B10**.

A7: 2β-{4-[1-(3-Methyl-butyryl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid (yield 59%). IR (KBr): 3420 (OH), 1636 (C=O, amides); ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.96 and 0.97 (2d, J = 6.1 Hz, $(CH_3)_2CH$), 0.70-2.30 (residual CH and CH_2), 2.4-3.0 (broad, 2 x CH_2N and 2α-CH), 3.54, 3.63, 3.68 and 3.85 (4m, 17α-CH, 2 x CH_2NCO and CH_2N of proline), 3.90 (m, 3β-CH), 4.84 (m, NCHCO); ^{13}C NMR ($CDCl_3$): 11.2, 17.9, 21.1, 22.7 (2x), 23.3, 25.3, 25.4, 27.9, 29.3, 30.5, 30.7, 35.3, 36.2, 36.6 (2x), 36.8, 39.0, 43.1, 43.3, 44.2 (2x), 47.5, 49.8 (2x), 50.6, 54.9, 56.2, 64.4, 66.4, 81.7, 171.4 (2x); LRMS for $C_{33}H_{56}N_3O_4$ $[MH]^+$:

558.4 m/z; Anal.($C_{33}H_{55}N_3O_4 \cdot 0.5\ H_2O$) CHN: calcd 69.93, 9.96, 7.41 and found 69.62, 10.15, 7.37.

A9: 2β-{4-[1-(2-Cyclohexyl-acetyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane-3α,17β-diol. Amorphous white solid (yield 67%). IR (KBr): 3442 (OH), 1631 (C=O, amides); ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70-2.30 (residual CH and CH_2), 2.4-3.0 (broad, 2 x CH_2N and 2α-CH), 3.54, 3.60, 3.68 and 3.80 (4m, 17α-CH, 2 x CH_2NCO and CH_2N of proline), 3.89 (m, 3β-CH), 4.85 (m, NCHCO); ^{13}C NMR ($CDCl_3$): 11.2, 17.3, 21.0, 23.3, 24.8, 26.1, 26.2, 26.3, 28.1, 29.2, 30.4, 31.0, 33.3, 33.4, 33.6, 34.6, 35.0, 35.5, 35.9, 36.7, 38.6, 42.1, 43.1, 45.5 (2x), 47.4, 48.8 (2x), 50.8, 55.6, 56.1, 63.9, 65.1, 81.8, 170.6, 171.3; LRMS for $C_{36}H_{60}N_3O_4$ $[MH]^+$: 598.4 m/z; Anal. ($C_{36}H_{59}N_3O_4 \cdot H_2O$) CHN: calcd 70.21, 9.98, 6.82 and found 69.19, 10.00, 6.72.

B10:2β-{4-[1-(Cyclohexyl-carbonyl)-pyrrolidine-3-carbonyl]-piperazin-1-yl}-5α-androstane -3α,17β-diol. Amorphous white solid (yield 88%). IR (KBr): 3427 (OH), 1632 (C=O, amides); ^{1}H NMR ($CDCl_3$): 0.73 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.30 (residual CH and CH_2), 2.37 (td, J_1 = 3.0 Hz, J_2 = 11.4 Hz, CHCON), 2.4-3.1 (broad, 2 x CH_2N and 2α-CH), 3.60, 3.72 and 3.95 (3m, 17α-CH, 3β-CH, 2 x CH_2NCO and CH_2N of proline), 4.80 (m, NCHCO); ^{13}C NMR ($CDCl_3$): 11.2, 17.8, 21.2, 23.3, 25.2, 25.7 (2x), 25.8, 27.9, 28.7, 28.9, 29.1, 30.5, 30.8, 35.5 (2x), 36.1, 36.7 (2x), 38.9, 42.5, 43.1, 44.2 (2x), 47.1, 49.3 (2x), 50.6, 55.1, 56.3, 64.4, 66.0, 81.7, 172.0, 174.9; LRMS for $C_{35}H_{58}N_3O_4$ $[MH]^+$: 584.5 m/z; Anal. ($C_{35}H_{57}N_3O_4 \cdot H_2O$) CHN: calcd 69.65, 9.99, 6.82 and found 69.85, 9.88, 6.98.

Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing *L*-glutamine (2 nM), antibiotics (100 IU penicillin/mL, 100 μg streptomycin/mL) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified

atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

Cell proliferation assay

The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 1 x 10^4 cells in a total of 100 μL medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, 5% CO_2. Compounds were dissolved in ethanol to prepare the stock solution of 1 x 10^{-2} M. These compounds and doxorubicin (Novapharm, Toronto, Canada) was diluted at multiple concentrations with culture media, added to each well and incubated for 3 days. Following each treatment, MTS (20 μl) was added to each well and incubated for 4 h. MTS is converted to water-soluble colored formazan by a dehydrogenase enzyme present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Supporting Information Available

^{13}C NMR assignment of key intermediate compound **10** and the representative final aminosteroid **A9** (Table 1). Crystallographic details (Tables 2-8) for the X-ray analysis of **10**.

Acknowledgments

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Chapitre 3

Synthèse d'une famille de 2β-aminoandrostane-3α,17β-diols et évaluation biologique de leur effet antiprolifératif sur les cellules de la leucémie myéloïde humaine HL-60

3.1 Avant-propos

Mon rôle dans ce projet a été d'effectuer l'évaluation biologique de tous les produits synthétisés par Dominic Thibeault afin de déterminer leur pouvoir antiprolifératif sur les cellules leucémiques HL-60. Patrick DeRoy a collaboré à l'élaboration de la synthèse chimique de certains produits. J'ai aussi participé à la rédaction de la partie expérimentale biologique de ce manuscrit qui sera soumis pour publication à la revue *Journal of Medicinal Chemistry*.

3.2 Résumé

Plusieurs agents thérapeutiques sont disponibles pour le traitement de la leucémie, mais ces composés sont fortement toxiques, provoquent beaucoup d'effets secondaires et ne permettent pas nécessairement la rémission de la maladie. Bien que peu de stéroïdes soient utilisés pour le traitement de la leucémie, le 2β-(4'-méthylpiperazino)-5α-androstane-3α,17β-diol (HY) a été rapporté récemment pour sa propriété à inhiber la prolifération des cellules HL-60 de leucémie humaine. Grâce à une procédure efficace que nous avons développée pour l'aminolyse des époxydes stéroïdiens encombrés, nous avons synthétisé une série de 2β-amino-5α-androstane-3α,17β-diols ayant une structure apparentée à celle du HY. Ainsi, l'ouverture du 2,3α-époxy-5α-androstan-17β-ol avec des amines primaires et secondaires a permis de générer plusieurs 2β-aminostéroïdes possédant différentes caractéristiques (allongement, ramification et fonctionnalisation de la chaîne latérale). Environ soixante-dix dérivés stéroïdiens ont par la suite été testés pour déterminer leur capacité à inhiber la prolifération des cellules HL-60 à 1 et 10 μM, et ainsi obtenir une première étude SAR (Structure Activity Relationship). Certains composés dotés de longues chaîne alkyles (6 à 16 carbones) ou de groupements volumineux (diphényle ou adamantatyle) ont démontré une activité antiprolifératives supérieure à celle du composé HY de départ.

Journal of Medicinal Chemistry (to be submitted)

Synthesis of a family of 2β-aminoandrostane-3α,17β-diols and biological evaluation of their antiproliferative effect on HL-60 acute myeloid leukemia cells

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Abstract

Many therapeutic agents are available for treatment of leukemia but these compounds are highly toxic, induce many secondary effects and they do not necessarily allow the remission of the disease. Even if few steroids are used for the treatment of leukemia, the 2β-(4'-methylpiperazino)-5α-androstane-3α,17β-diol (HY) was recently reported for his property to inhibit the proliferation of human leukemia HL-60 cells. With an efficient procedure that we have developped for the aminolysis of hindered steroidal epoxides, we synthesized a series of 2β-amino-5α-androstane-3α,17β-diols having a similar structure to HY. Hence, the opening of 2,3α-epoxy-5α-androstan-17β-ol with primary and secondary amines allowed generation of many 2β-aminosteroids with different lengthening, ramification and functionalization of the side chain. About seventy steroidal derivatives were next tested for determining their capacity to inhibit the proliferation of HL-60 cells at 1 and 10 μM and thus obtaining a first structure-activity relationship (SAR) study. Some compounds with long alkyl chains (6 to 16 carbons) or bulky groups (diphenyl or adamantyl) have shown antiproliferative properties superior to that of the starting HY compound.

Introduction

Leukemia is a major type of cancer affecting a significant segment of the population, and especially children. In fact, leukemia is the most frequent childhood cancer, with 26 % of all cases, and 32 % mortality.[1] The American Cancer Society (ACS) estimates that 30,600 new cases of leukemia will be diagnosed in the United States in 2003.[2] About 21,900 adults and children in the United States will die of leukemia during 2003. The ACS also predicts that about 2,200 children will be diagnosed with acute lymphocytic leukemia in the United States during the year 2003.[2] Fortunately, some success has been attained in cancer treatment, although the incidence rate for this disease remains relatively unchanged. Since the early 50s, mortality rates for childhood cancer have declined by more than 50 %, with most of the improvement occurring after 1970.[3] Forty years ago,[4] nearly no one could survive acute lymphoid leukemia or "childhood leukemia", whereas today, 80 % of children and teens affected are still alive 5 years after the diagnosis.[2] Five-year survival rates of children with acute myeloid leukemia have also increased over time to about 40 %.[2] But even if the success of clinical trials in identifying new agents and treatment modalities has been significant, actual treatments have many limitations. Combinations of chemotherapeutic compounds are used but these cytotoxic agents induce many physiological side effects and remission is not sure. Irradiation affords the same problem. Bone marrow transplant can also be performed but success is not guaranteed. Of course, combinations of treatments are employed for best results. Also, researches in genetic begins to appear for the treatment of leukemia.[5] It would be important to develop new therapeutic agents, which should be more active and produce less side effects or acting through a different mechanism than that of cytotoxic agents already used in leukemia treatments.

Up to now, new steroidal compounds have been investigated for potential antileukemic activity. Corticosteroids like prednisone or prednisolone are well documented for treatment of lymphoid leukemia.[6] Other steroidal derivatives were investigated for potential inhibitory effect on leukemic cells, such as 7-keto hybrid steroidal esters of nitrogen mustard.[7] Estrogen derivatives,[8] 1,25-dihydroxyvitamin D_3[9] and its derivatives[10] were also studied and good results were obtained but they are not always sufficient for the

purpose of cancer therapy. Recently, He and Jiang[11] reported the antiproliferative properties against human myeloid leukemia HL-60 cells of an aminosteroid, 2β-(4'-methylpiperazino)-5α-androstane-3α,17β-diol (**1**). This compound also induces differentiation of these cells. No structure-activity relationship (SAR) study was however conducted in order to optimize the antileukemic potential of this newly reported potential lead compound.

In search of a novel family of anticancer agents with fewer side effects and/or a different mechanism of action, we first verified the antileukemic activity of compound **1**, in our hands, and second synthesized analogous products with the aim of improving potency. Since the synthesis of aminosteroids has some limitations, we previously developed a new method for the efficient aminolysis of 2,3α-steroidal epoxides,[12] which allows us to obtain more easily a large series of 2β-aminosteroids and to extend our SAR study. In this paper, we report the chemical synthesis and characterization of more than seventy compounds of general structure **I** and **II** (Figure 1) and their level of antiproliferative activity against HL-60 cells.

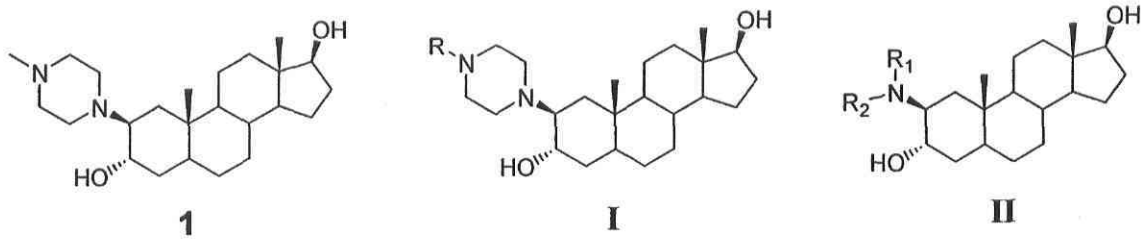


Figure 1. The lead compound **1** and general structures (**I** and **II**) of aminosteroids synthesized and tested.

Chemical results

Synthesis of epoxide 8 from DHT (scheme 1)

The first two steps of the synthesis were performed as reported in the literature.[13] Thus the acetylation of dihydrotestosterone (**2**), followed by a selective bromination of

DHT acetate (**3**) at C-2 with Br_2 in acetic acid, afforded the bromoketone **4**. A stoichiometric equivalent of Br_2 is important in order to avoid a second bromination at C-4. Even if the reaction is not complete, no purification is required at this step. K-selectride in THF, rather than classical $NaBH_4$, was next used to obtain the bromohydrine **5**. In fact, a better stereo-selectivity for the reduction of bromoketone **4** was obtained with K-selectride, giving mainly the 3α-OH. This result was confirmed using ^1H NMR data of the well-known 3α-OH and 3β-OH 5α-androstanes reported in the literature.[14] The 3α-OH-androstane shows a fine signal at about 4.1 ppm, whereas the 3β-OH analog gives a broad signal at 3.4 ppm. A flash chromatography is required at this step and a high purity of bromohydrine **5** is crucial for generating the alkene **6** in excellent C-2,3 isomeric purity. In fact, the residual non-brominated compound **3** will be reduced to a 3α-OH compound (3α-hydroxy-17β-acetoxy-5α-androstane). However, the latter product must be totally absent during the next step, because the elimination of this alcohol will give a mixture of C-2,3 and C-3,4 alkenes. Unfortunately, our attempt to purify this mixture by crystallization or chromatography was unsuccessful. However, the elimination of pure bromohydrine **5** in refluxing acetic acid with zinc powder gave the C-2,3 alkene **6**. Hydrolysis of the acetate and epoxidation with *m*-CPBA finally provided the 2,3α-epoxide **8**.

Alternative synthesis for alkene 6

Although the classical way reported above afforded the epoxide **8** in acceptable yield and purity when carefully carried out, we also explored various other possibilities for the synthesis of compound **8** with fewer synthetic steps and better purity. The Shapiro's reaction[15] was first studied as a straightforward approach for obtaining **6** from the corresponding tosylhydrazone derivative of DHT. Various bases and conditions were then used in order to improve the regioselectivity of the elimination step, but a mixture of two alkenes was always observed. These elimination reactions never afforded less than 6 % of C-3,4 alkene, which was found unsatisfactory for our purpose. A second strategy was the elimination of a good C-3 leaving group like a tosylate or a bromide, but at least 10 % of C-3,4 isomer was obtained in both cases. Since we were able to produce efficiently and rapidly a mixture of alkenes, we considered the possibility of isomerising the C-3,4 alkene

into C-2,3 alkene. Different catalysts, known to facilitate a double bond isomerization,[16] were thus tried with the substrate in refluxing toluene or DMF. Although an enrichment in C-2,3 isomer (compound **6**) was observed, no significant result was obtained because much degradation occurred. The low rate of isomerization and the loss of products convinced us to generate the alkene **6** by the classical method described above and using the bromination pathway.

Synthesis of aminosteroids 9-59 by opening of epoxide 8 (scheme 2)

To start our SAR study, we first diversified the cyclic amine on the C-2 carbon using the classical aminolysis procedure known in the literature.[17] An excess of amine (30 equivalents) used as solvent and water (18 equivalents) were refluxed with epoxide **8** to afford the desired products **9-22** with low to medium yields (0-64 %) after tedious purification. However, some amines did not react, thus limiting the possibility of diversification. Consequently, we applied the new procedure we developed for the aminolysis of a hindered steroidal epoxide,[12] using a larger diversity of amines to afford the targeted aminosteroids **23-59** in medium to high yields (48-97 %) with easier work-up procedure and purification. Only 3 equivalents of amine with 20 % mol. of $Gd(OTf)_3$ as catalyst were needed in a Schlenk tube to provide the desired product by heating (150 – 190 °C) the epoxide **8** for 2 hours (primary amines) or overnight (secondary and cyclic amines). In addition to cyclic amines, various aliphatic amines were also used to generate aminosteroids and to extend our SAR study, which is not possible with the classical aminolysis procedure.

Synthesis of aminosteroids 60-79 by amine derivatization (schemes 3 and 4)

The N-alkylpiperazine derivatives **61-77** were obtained from **60**. The latter was first synthesized from epoxide **8** in 70 % yield by a modification of the classical method of aminolysis, which utilizes a Schlenk tube. The amidation of **60** with a carboxylic acid, HBTU, as coupling agent, and DIPEA as base gave good yields (44-90 %) of amides **61-69**. The carbonyl reduction with BH_3-THF afforded the alkylamine chelated with boron.[18]

This complex was then broken in refluxing methanol overnight to give the desired products **70-77** in good yields after purification. In the formation of the last two compounds, **78** and **79**, from the secondary amine **35** and **37**, no complexation occurred during the reduction step, probably due to high steric hindrance. A simple work-up followed by flash chromatography gave the tertiary amines **78** and **79**.

Biological results

HL-60 cells were incubated at 37 °C with synthesized compounds in a humidified atmosphere of 95 % air / 5 % CO_2. Doxorubicin, a well known cytotoxic agent used for leukemia treatment, and of course HY (**1**), were used as references for a comparison of the antiproliferative properties of synthesized compounds. The antiproliferative activity of HY we then measured was lower than before. Thus, these results prompted us to begin an SAR study in order to generate 2β-aminosteroids with better antiproliferative properties. The first strategy was to diversify the amines on the C-2 carbon. In fact, we were not sure whether the piperazine moiety was the only possible building block for a good antileukemic compound. We then tried several cyclic amines showed in figure 2. At a concentration of 1 μM, no antiproliferative activity was observed for any compound tested, including HY (**1**). However, it seems that functional groups distant from the steroidal backbone afforded an inhibition at 10 μM. Although the large diversity of cyclic amines represented in figure 2 did not give expected results, three compounds (2β-(4'-benzylpiperidino)-5α-androstane-3α,17β-diol (**17**), 2β-(4'-(4"-nitrophenyl)-piperazino)-5α-androstane-3α,17β-diol (**20**) and 2β-(4'-piperidinopiperidino)-5α-androstane-3α,17β-diol (**59**)) were however identified to be potential new lead compounds. These results prompted us to extend our SAR study by elaborating a series of compounds with various alkyl chains on the piperazine moiety. This allowed us to determine whether distant functional groups are needed to exert an antiproliferative activity on HL-60 cells.

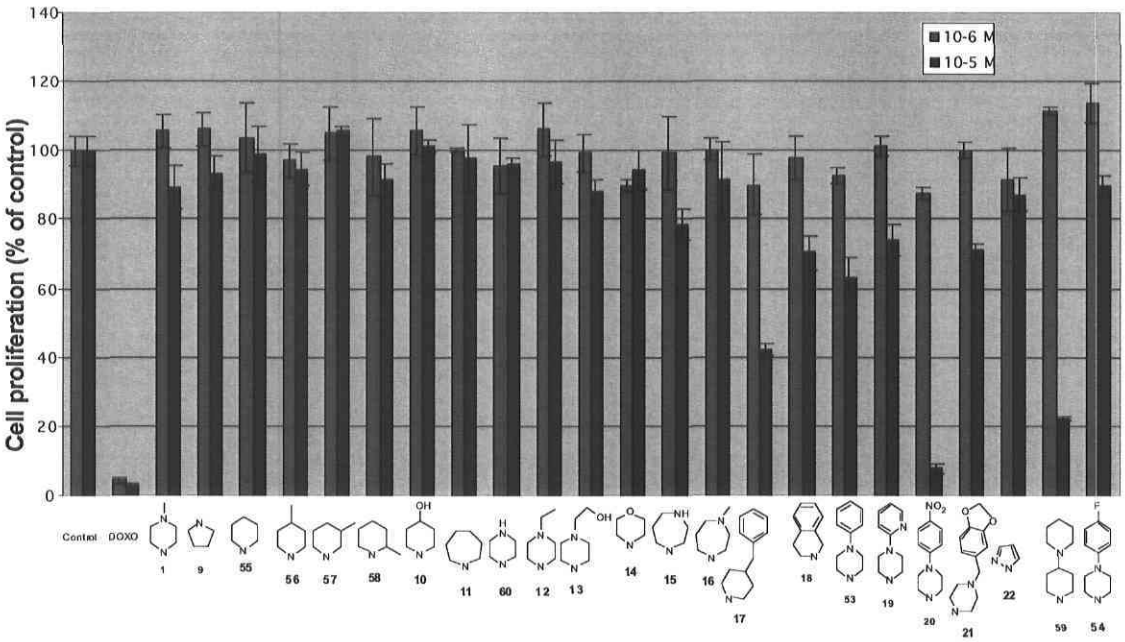


Figure 2. % of cell proliferation after a 3-days treatment at 2 concentrations of tested compounds.

To achieve our new objective, we started with compound **60** and added different carboxylic acids on the piperazine moiety to generate amide derivatives. The corresponding amine derivatives, without a carbonyl group, were also obtained and tested in the same conditions. Results showed in figure 3 are similar to our first series of compounds tested before (figure 2), except at 10 μM for the amine **75** with a long alkyl chain and his amide equivalent **66**. Since retinoic acid is used as an antitumor agent,[19] and to induce leukemia differentiation in the treatment of acute myeloid leukemia,[20] it was interesting to synthesize an aminosteroid with the retinoic acid moiety (compound **69**). We then added the retinoic acid on our precursor (compound **60**), and the amide **69** was the best compound at 1 μM and also the best compound synthesized in our SAR study. In fact, the activity of compound **69** was superior to retinoic acid itself.

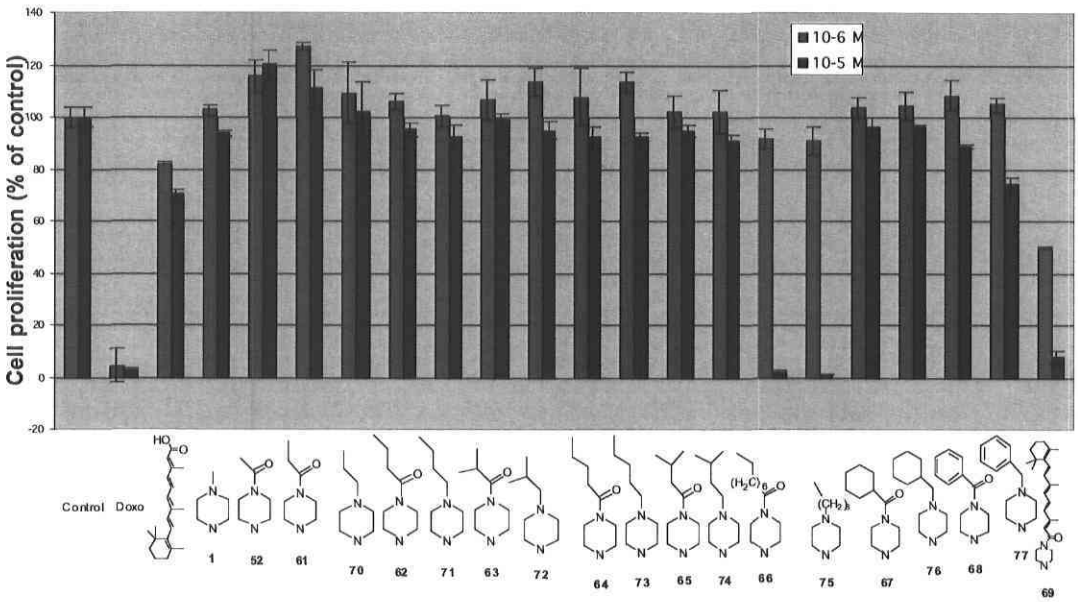


Figure 3. % of cell proliferation after a 3-days treatment at 2 concentrations of tested compounds.

We next elaborated and tested a third series of compounds synthesized by the opening of epoxide **8** using a series of long n-alkyl or small ramified primary amines (figure 4). Altough they were not active at 1 μM, best results were attained with long *n*-alkylamines at 10 μM (compounds **33** to **39**). However, too long chain did not increase inhibition since results obtained were nearly the same for a chain of 8 or 16 carbons.

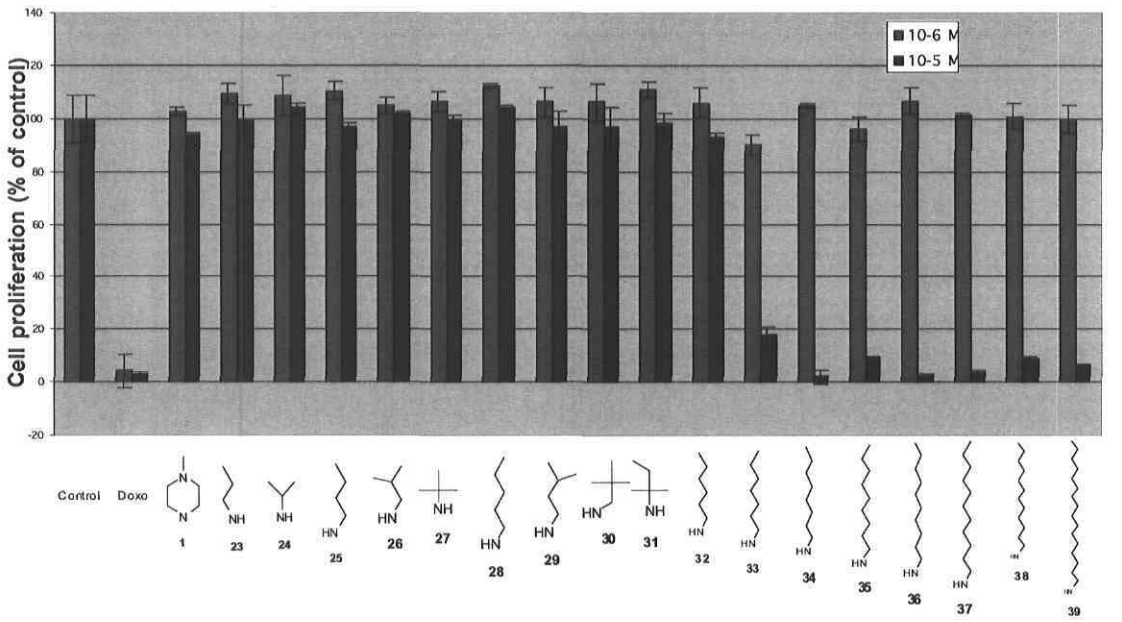


Figure 4. % of cell proliferation after a 3-days treatment at 2 concentrations of tested compounds.

In the last series of aminosteroids (figure 5), we tested additional secondary amines such as cycloalkylamines, alkyl amines with an oxygen atom (ether function), and amines containing an aromatic at the end of the chain or a bulky group like adamantane. We also tested dialkylamines and two other compounds to verify the importance of the steroid moiety. Compound **50** was published by the same group who reported HY (**1**) as an antileukemic compound but on a different cell system (WEHI-3B cells).[21] In our HL-60 cells, it was not better than HY. Dialkyl compounds were synthesized for their potential inhibition properties since corresponding monoalkyl chain (compounds **35** and **37**) afforded good results at 10 μM. But these dialkyl compounds (**78** and **79**) did not afford any good inhibition at both tested concentrations. With their two long *n*-alkyl chains, these tertiary aminosteroids are too hydrophobic and they probably remain in the phospholipid bilayer of the membrane cells.[22] The only compounds that gave a good inhibition in this fourth screening were compounds **47** and **48**, with hindered and bulky building blocks like 3,3-diphenylpropylamine and adamantyl methylamine respectively. Even with hydrophobic groups, these compounds are probably not retained in the phospholipid bilayer since these

bulky groups do not have a structural similitude to that of the bilayer with long alkyl chains like compounds **78** and **79**.[22]

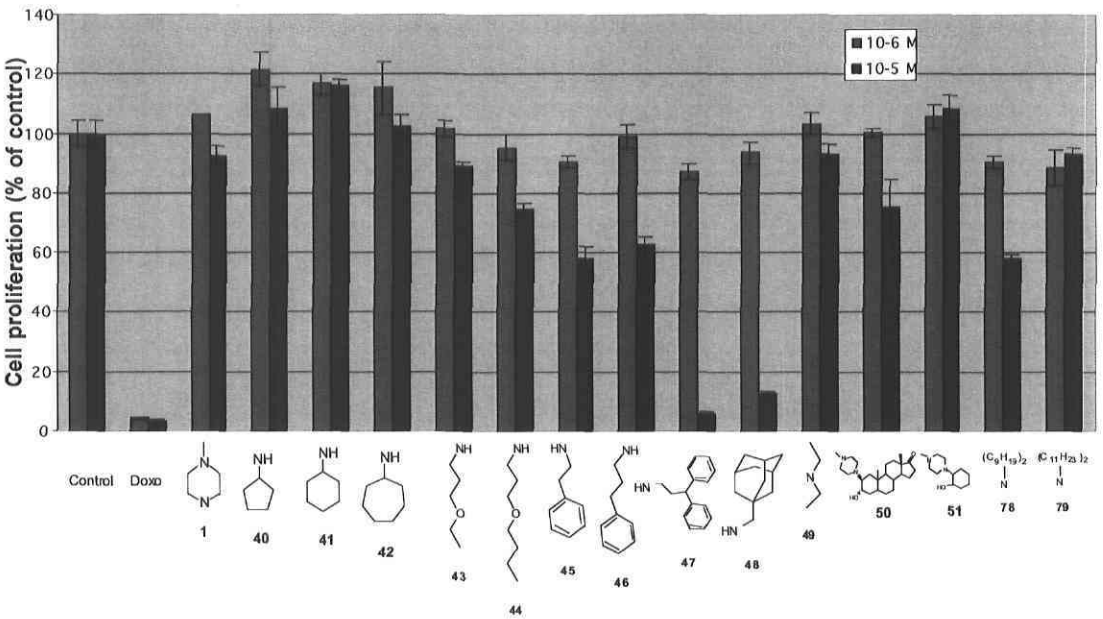
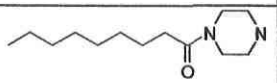
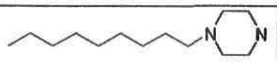
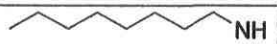
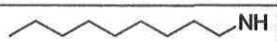
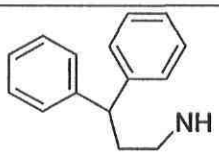
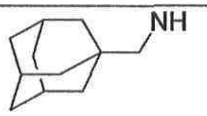
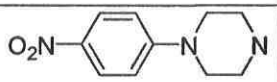
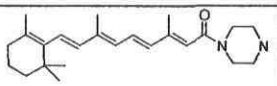


Figure 5. % of cell proliferation after a 3-days treatment at 2 concentrations of tested compounds.

The table 1 summarize the more active compounds synthesized in this SAR study. There are some similarities among different series of compounds. In figure 2, only one compound shows an interesting antiproliferative activity. Compound **20**, like other good compounds synthesized, has a distant and linear functional group. In figure 3, we observed a good inhibition at 10 μM for compounds **66** and **75** with long alkylamide and alkyl chain. Same result is obtained with the retinoyl compound **69** which is the best compound reported here with an antiproliferative activity of 33 % at 1 μM. The same inhibitory tendency is observed for compounds **34-39** in figure 4. In figure 5, only compounds **47** and **48** with bulky building blocks afforded inhibition. This SAR showed us that long, hydrophobic alkyl chains or bulky hydrophobic amines afford good antiproliferative properties but only at 10 μM. We succeeded to making compounds better than HY but good inhibition at 1 μM remain the next objective to achieve.

Table 1. Compilation of the best compounds from our study.

Compounds	Building blocks at C-2β	Cell proliferation (%) at 1 μM	Cell proliferation (%) at 10 μM
66		91.9 ± 3.7	2.0 ± 0.7
75		90.9 ± 5.0	1.0 ± 0.2
34		105.1 ± 0.8	2.0 ± 2.9
35		96.1 ± 4.5	9.8 ± 0.2
38		100.9 ± 4.9	9.2 ± 0.3
47		87.1 ± 2.5	6.1 ± 0.5
48		93.6 ± 3.7	12.5 ± 0.8
20		87.5 ± 1.6	7.8 ± 1.6
69		49.7 ± 0.4	7.9 ± 2.1

Conclusion

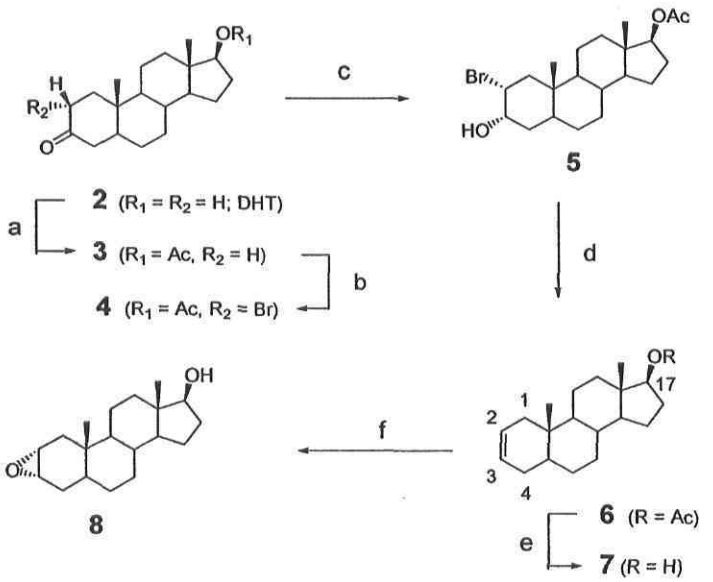
The chemical synthesis of a series of 2β-amino-5α-androstane-3α,17β-diols (**9-79**) was performed and a SAR study was made in order to improve the antiproliferative properties of this family of compounds on HL-60 leukemia cells. We first verified the potency of our lead compound and concluded that the HY (**1**) was not as good as it seemed in literature report. We next succeeded in optimizing the antiproliferative activities of this newly reported antileukemic agent. Good inhibitions of cellular proliferation were obtained for some compounds at 10 μM, but results are not so good at 1 μM. However, some of these synthesized products showed interesting antiproliferative properties compared to HY

and others compounds synthesized. Other works will be needed to improve the potency of these new therapeutic agents, but also to elucidate their mechanism of action. This last one could help us to design other active compounds more efficiently and more rapidly.

Experimental section

General methods for chemistry

Dihydrotestosterone (DHT) was purchased from Steraloids (Wilton, NH, USA) or Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada), while chemical reagents were from Sigma-Aldrich Canada Ltd. The usual solvents were obtained from Fisher Scientific (Montreal, QC, Canada) and were used as received. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. All anhydrous reactions were performed in oven-dried glassware under positive argon pressure. Thin-layer chromatographies were performed on 0.20 mm silica gel 60 F254 plates (Fisher, Montreal, Canada) and compounds were visualized using ammonium heptamolybdate tetrahydrate. Flash column chromatographies were performed on Silicycle R10030B 230-400 mesh silica gel (Quebec, QC, Canada). Infrared spectra (IR) were obtained from a thin film obtained with compound usually solubilized in $CDCl_3$ and layered over a NaCl pellet. They were recorded with a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz (^1H) and 75.5 MHz (^{13}C) on a Brucker AC/F300 spectrometer (Billerica, MA, USA) or a 400 MHz (^1H) and 100.6 MHz (^{13}C) on a Brucker Avance 400 digital spectrometer or a Inova Varian AS 400 (Palo Alto, CA, USA) and reported in ppm. The labile OH and NH NMR signals that were sometimes appearing were not listed. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ionspray source.



Scheme 1. Synthesis of epoxide **8**. **(a)** Ac_2O, pyridine, DMAP cat., rt (99 %); **(b)** Br_2, AcOH, rt; **(c)** K-selectride, -78 oC, THF (71 %, 2 steps); **(d)** Zn dust, AcOH, reflux (82 %); **(e)** K_2CO_3/H_2O, MeOH, reflux (98 %); **(f)** m-CPBA, CH_2Cl_2, 0 °C (85 %).

Chemical synthesis of epoxide 8 from DHT (2)

3-oxo-17β-acetoxy-5α-androstane (**3**)

Acetic anhydride (68 mL, 723 mmol) was added under argon at room temperature to a solution of dihydrotestosterone (**2**) (21.02 g, 72.3 mmol) and dimethylaminopyridine (DMAP) (176.7 mg, 1.45 mmol) in anhydrous pyridine (50 mL). The resulting mixture was stirred for 3 h, concentrated under vacuum and the residue treated with HCl 20 % (800 mL). The product is extracted with ethyl acetate and the extracts washed with a solution of saturated sodium bicarbonate (2 x 800 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude product by flash chromatography (EtOAc : hexanes, 1:5) yielded 23.98 g (99 %) of compound **3** as a white solid. IR (film): 1736 (C=O ester), 1716 (C=O, ketone) ; ^{1}H NMR ($CDCl_3$): 0.83 (s, 18-CH_3), 1.04 (s, 19-CH_3), 0.75-2.40 (22H), 2.06 (s, CH_3-acetyl), 4.61 (dd, J = 9.0 Hz, J = 8.0 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.42,

12.08, 20.85, 21.16, 23.47, 27.48, 28.71, 31.16, 35.10, 35.66, 36.76, 38.08, 38.43, 42.57, 44.61, 46.55, 50.50, 53.64, 82.66, 171.16, 211.88; LRMS for $C_{21}H_{33}O_3$ [MH^+]: 333.4 m/z.

2α-bromo-3-oxo-17β-acetoxy-5α-androstane (**4**)

To a solution of **3** (15.34 g, 46.2 mmol) in glacial acetic acid (460 mL) is added a 1 M solution of bromine (2.4 mL of Br_2 in 47 mL of AcOH) dropwise at room temperature. The resulting mixture was stirred for 1.5 h and concentrated under reduced pressure. The resulting red solution was dissolved in ethyl acetate (500 mL) and washed with a saturated solution of sodium bicarbonate (2 x 500 mL), dried over $MgSO_4$, filtered and evaporated to dryness. No purification was required at this step. IR (film): 1729 (C=O, ketone and ester), 732 (C-Br); ^{1}H NMR ($CDCl_3$): 0.83 (s, 18-CH_3), 1.11 (s, 19-CH_3), 0.80-2.45 (m, 19H), 2.06 (s, CH_3-acetyl), 2.65 (J = 13.4 Hz, J = 6.3 Hz, 1H), 4.61 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH), 4.76 (J = 13.4 Hz, J = 6.3 Hz, 2β-CH); ^{13}C NMR ($CDCl_3$): 12.10 (2x), 20.96, 21.18, 23.47, 27.46, 28.18, 30.95, 34.65, 36.58, 38.96, 42.53, 43.82, 47.37, 50.33, 51.56, 53.45, 54.39, 82.52, 171.18, 201.01; LRMS for $C_{21}H_{32}{}^{79}BrO_3$ [MH^+]: 411.3 m/z.

2α-bromo-3α-hydroxy-17β-acetoxy-5α-androstane (**5**)

A 1 M solution of K-selectride (21 mL, 21 mmol) was added dropwise under argon at –78 °C to a solution of compound **4** (4.3 g, 10.5 mmol) in anhydrous THF (100 mL). The resulting mixture was stirred for 1 h and the temperature is warm to 0 °C and neutralized with a solution of saturated NH_4Cl (400 mL). The resulting mixture was extracted with CH_2Cl_2 (3 x 200 mL). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated to dryness. The resulting crude bromohydrin was purified by flash chromatography (EtOAc:hexanes, 10:90) and yielded 3.07g (58 %, 2 steps) of compound **5** as a white solid. IR (film): 3470 (OH), 1717 (C=O), 735 (C-Br); ^{1}H NMR ($CDCl_3$): 0.73 (s, 19-CH_3), 0.80 (s, 18-CH_3), 2.00 (s, CH_3-acetyl), 0.60-2.20 (20H), 2.32 (s, 1H), 4.02 (s, 3α-CH), 4.43 (m, CH-Br), 4.54 (t, J = 8.4 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.79, 12.03, 20.20, 21.11, 23.37, 27.29, 27.38, 31.06, 34.57, 34.76, 36.59, 37.40, 39.56, 42.44, 43.42, 50.47, 53.80, 58.33, 69.57, 82.60, 171.10; LRMS for $C_{21}H_{34}{}^{79}BrO_3$ + NH_4 [MH^+]: 429.9 m/z.

17β-acetoxy-5α-androst-2-ene (**6**)

The bromohydrin **5** (3.4 g, 8.2 mmol), was dissolved in acetic acid (100 mL) and zinc dust (3.77 g, 57.7 mmol) was added and the mixture refluxed for 2 h. The solution was filtered and concentrated under vacuum, diluted in water, and the product extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate until neutralisation, dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the crude product by flash chromatography (EtOAc : hexanes, 5:95) yielded 2.15 g (82 %) of alkene **6** as a white solid. IR (film): 3017 (C=C), 1737 (C=O); ^{1}H NMR ($CDCl_3$): 0.78 (s, 19-CH_3), 0.81 (s, 18-CH_3), 0.75-2.20 (m, 20H), 2.06 (s, CH_3-acetyl), 4.60 (dd, J = 9.0 Hz, J = 7.9 Hz, 17α-CH), 5.60 (m, 2H alkene); ^{13}C NMR ($CDCl_3$): 11.66, 12.04, 20.34, 21.21, 23.48, 27.49, 28.55, 30.23, 31.32, 34.66, 35.33, 36.89, 39.72, 41.39, 42.47, 50.67, 53.94, 82.90, 125.80 (2x), 171.31; LRMS for $C_{21}H_{33}O_2$ [MH^+]: 317.3 m/z.

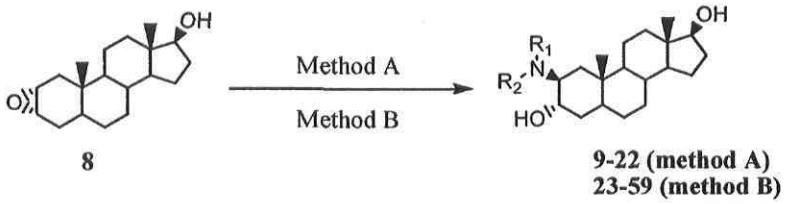
17β-hydroxy-5α-androst-2-ene (**7**)

A solution of potassium carbonate (3.15 g, 22.8 mmol) in water (20 mL) was added to a solution of compound **6** (1.80 g, 5.7 mmol) in methanol (100 mL) and the mixture refluxed for 1.5 h. The resulting mixture was diluted in water and extracted with ethyl acetate (3 x 75 mL). The organic layers are combined, dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude product by flash chromatography (EtOAc : hexanes, 1:5) yielded 1.55 g (98 %) of alcohol **7** as a white solid. IR (film): 3252 (OH), 3017 (C=C); ^{1}H NMR ($CDCl_3$): 0.76 (s, 19-CH_3), 0.78 (s, 18-CH_3), 0.70-2.10 (m, 21H), 3.65 (t, J = 8.5 Hz, 17α-CH), 5.60 (m, 2H alkene); ^{13}C NMR ($CDCl_3$): 11.05, 11.71, 20.48, 23.37, 28.60, 30.28, 30.50, 31.37, 34.70, 35.63, 36.75, 39.79, 41.51, 42.86, 51.00, 54.17, 82.01, 125.85 (2x); LRMS for $C_{19}H_{30}O$ + NH_4 [M^+]: 292.2 m/z.

17β-hydroxy-2α,3α-epoxy-5α-androstane (**8**)

To a solution of **7** (1.01 g, 3.64 mmol) in anhydrous CH_2Cl_2 (35 mL) at 0 °C was added *m*-CPBA 77 % pure (1.23 g, 5.46 mmol) in six portions. The mixture was stirred for 1 h at 0 °C, then allowed to warm at room temperature and stirred overnight. The mixture was concentrated under reduced pressure, the residue was diluted in ethyl acetate and the solution washed successively with a solution of saturated $Na_2S_2O_3$ (2 x 100 mL) and a

saturated solution of sodium carbonate (2 x 100 mL), dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the crude product by flash chromatography (EtOAc : hexanes, 1:5) yielded 909 mg (85 %) of epoxide **8** as a white solid. IR (film): 3252 (OH); ^{1}H NMR ($CDCl_3$): 0.74 (s, 19-CH_3), 0.78 (s, 18-CH_3), 0.55-2.10 (m, 21H), 3.14 (m, 2H epoxide), 3.65 (t, J = 8.5 Hz, 17α-CH); ^{13}C NMR ($CDCl_3$): 11.02, 12.95, 20.45, 23.34, 28.26, 29.00, 30.44, 31.20, 33.70, 35.67, 36.28, 36.60, 38.27, 42.78, 50.78, 51.04, 52.42, 53.79, 81.88; LRMS for $C_{19}H_{31}O_2$ [MH^+]: 291.3 m/z.



8 **9-22 (method A)** **23-59 (method B)**

Scheme 2. Synthesis of aminosteroids **9-59** by method A and B. **(A)** Amine (30 eq.), H_2O (18 eq.), reflux, 24 hours. **(B)** Amine (3 eq.), $Gd(OTf)_3$ (0.2 eq), toluene, Schlenk tube, 150-190 °C, 2 hours or overnight.

Classical method for aminolysis of epoxide 8 (synthesis of 9-22).

The epoxide **8** (1 mmol), the desired amine (30 mmol) and H_2O (18 eq.) were refluxed 24 h. The reaction mixture was then cooled, poured in water and the precipitate was filtered. The solid was dissolved in CH_2Cl_2 and the solution dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was then purified by flash chromatography using an appropriate mixture of MeOH : CH_2Cl_2.

2β-(pyrrolidino)-5α-androstane-3α,17β-diol (**9**)

Yield: 41 %; IR (film): 3321 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 1.06 (s, 19-CH_3), 0.70-2.10 (24H), 2.40 (m, 2α-CH), 2.54 (m, 4H, 2'-CH_2), 3.64 (m, 17α-CH), 4.06 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.15, 13.23, 20.60, 23.43 (3x), 27.86, 30.53,

31.53, 32.57, 34.89, 35.07, 36.41, 36.78, 39.35, 43.01, 51.07, 51.47 (2x), 55.40, 65.98, 67.91, 81.98; LRMS for $C_{23}H_{40}NO_2$ [MH^+]: 362.2 m/z.

2β-(4'-hydroxypiperidino)-5α-androstane-3α,17β-diol (**10**)

Yield: 26 %; IR (film): 3386 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.70-2.30 (25H), 2.58, 2.69 and 2.81 (3m, 2α-CH and 1'-CH_2), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.72 (m, 1H, 4'-CH), 3.83 (m, 3β-CH); ^{13}C NMR, 300 MHz (acetone-d_6): 11.70, 15.87, 21.56, 24.02, 28.90, 30.95, 32.26, 34.04, 35.18, 35.43, 35.68, 36.24, 36.74, 37.85, 39.65, 43.87, 47.00, 51.98, 56.80, 65.13, 66.14, 81.76; LRMS for $C_{24}H_{42}NO_3$ [MH^+]: 392.0 m/z.

2β-(homopiperidino)-5α-androstane-3α,17β-diol (**11**)

Yield: 18 %; IR (film): 3366 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.20 (28H), 2.40-3.20 (broad, 2α-CH and 2 x 2' –CH_2), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.82 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.20, 17.78, 20.98, 23.31, 26.56, 28.29, 29.25, 30.47, 30.56, 31.02, 31.74, 34.16, 35.42, 35.57, 35.81, 36.77, 38.65, 38.99, 43.09, 50.78, 53.73, 56.41, 64.56, 66.40, 81.88; LRMS for $C_{25}H_{44}NO_2$ [MH^+]: 390.0 m/z.

2β-(4'-ethylpiperazino)-5α-androstane-3α,17β-diol (**12**)

Yield: 58 %; IR (film): 3396 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.86 (s, 19-CH_3), 1.12 (t, J = 7.1 Hz, 2''-CH_3), 0.60-2.20 (20H), 2.45, 2.55 and 2.70 (3m, 2α-CH, 1''-CH_2 and 8H piperazine), 3.63 (m, 17α-CH), 3.85 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.19, 11.87, 17.28, 20.79, 23.37, 28.26, 30.65, 31.18, 32.55, 34.66, 35.47, 35.72, 36.90, 38.43, 43.00, 50.87, 52.29, 53.32 (4x), 56.15, 63.53, 64.34, 81.41; LRMS for $C_{25}H_{45}N_2O_2$ [MH^+]: 405.6 m/z.

2β-(4'-hydroxyethylpiperazino)-5α-androstane-3α,17β-diol (**13**)

Yield: 67 %; IR (film): 3328 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.20 (19H), 2.45-2.75 (broad, 8H piperazine and 1'' –CH_2), 3.07 (m, 2α-CH), 3.64 (m, 17α-CH and 2''-CH_2), 3.86 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$):

11.19, 17.26, 20.90, 23.31, 28.21, 30.48, 31.13, 32.75, 34.65, 35.51, 35.77, 36.79, 38.44, 43.08, 45.69, 50.86, 53.35 (2x), 56.15, 57.60 (2x), 59.21, 63.66, 64.45, 81.83; LRMS for $C_{25}H_{45}N_2O_3$ [MH^+]: 421.6 m/z.

2β-(morpholino)-5α-androstane-3α,17β-diol (**14**)

Yield: 42 %; IR (film): 3442 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.65-2.15 (20H), 2.47 (m, 2'-CH_2), 2.65 (m, 2α-CH and 2'-CH_2), 3.75 (m, 17α-CH, 2 x 3'-CH_2), 3.88 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 16.94, 20.89, 23.33, 28.16, 30.53, 31.18, 32.58, 34.42, 35.50, 35.84, 36.80, 38.53, 43.10, 48.88 (2x), 50.90, 56.07, 63.65, 65.02, 67.48 (2x), 81.91; LRMS for $C_{23}H_{40}NO_3$ [MH^+]: 378.3 m/z.

2β-(homopiperazino)-5α-androstane-3α,17β-diol (**15**)

Yield: 62 %; IR (film): 3372 (OH and NH); ^{1}H NMR, 300 MHz (CD_3OD): 0.72 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70-2.10 (22H), 2.60-3.40 (m, 8H homopiperazine and 2α-CH), 3.55 (t, J = 8.5 Hz, 17α-CH), 3.90 (m, 3β-CH); ^{13}C NMR, 300 MHz (CD_3OD): 11.76, 17.15, 22.03, 24.28, 27.82, 29.40, 30.65, 32.41, 36.68, 36.87, 37.01, 38.12, 40.13, 43.53, 44.21, 46.05, 47.92, 48.88, 51.61, 52.23, 57.46, 66.97, 67.07, 82.47; LRMS for $C_{24}H_{43}N_2O_2$ [MH^+]: 391.3 m/z.

2β-(4'-methylhomopiperazino)-5α-androstane-3α,17β-diol (**16**)

IR (film): 3387 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.74 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.65-2.15 (22H), 2.48 (s, 4'-CH_3), 2.60-3.00 (m, 8H homopiperazine and 2α-CH), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.78 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.56, 20.91, 23.28, 27.38, 28.30, 30.46, 31.06, 34.17, 35.04, 35.54, 35.65, 36.78, 38.55, 43.06, 46.43, 48.66, 49.62, 50.81, 55.88, 56.27, 59.52, 64.46, 65.57, 81.79; LRMS for $C_{25}H_{45}N_2O_2$ [MH^+]: 405.4 m/z.

2β-(4'-benzylpiperidino)-5α-androstane-3α,17β-diol (**17**)

IR (film): 3350 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.20 (25H), 2.59 (d, J = 4.8 Hz, 5'-CH_2), 2.30-3.40 (m broad, 2α-CH and 2 x 2'-CH_2), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.90 (m, 3β-CH), 7.14 (d, J = 7.1 Hz, 2H aromatic), 7.22 (t, J

= 7.3 Hz, 1H aromatic), 7.31 (d, J = 7.1 Hz, 2H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.59, 18.11, 21.40, 23.72, 28.60, 30.94, 31.43, 32.13, 32.30, 33.88, 35.82, 35.98, 36.31, 37.19, 38.15, 38.90, 43.16, 43.53, 45.37, 51.24, 52.75, 56.70, 64.16, 65.54, 82.21, 126.39, 128.67 (2x), 129.41 (2x), 140.50; LRMS for $C_{31}H_{48}NO_2$ [MH^+]: 466.5 m/z.

2β-(1,2,3,4-tetrahydroisoquinolino)-5α-androstane-3α,17β-diol (**18**)

Yield: 10 %; IR (film): 3390 (OH); ^{1}H NMR, 400 MHz (acetone-d_6): 0.76 (s, 18-CH_3), 0.92 (s, 19-CH_3), 0.70-2.15 (20H), 2.66 (m, 2α-CH), 2.95 (m, NCH_2-CH_2-Ar), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.76 and 3.92 (2d, J = 14.7 Hz, NCH_2-Ar), 3.98 (m, 3β-CH), 7.04 (m, 1H aromatic), 7.15 (m, 3H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.41, 20.93, 23.31, 28.22, 29.64, 30.50, 31.11, 32.74, 34.88, 35.54, 35.84, 36.78, 38.55, 43.07, 45.62, 50.84, 51.19, 56.23, 63.97, 64.35, 81.85, 125.69, 126.23, 126.52, 128.73, 134.30 (2x); LRMS for $C_{28}H_{42}NO_2$ [MH^+]: 424.0 m/z.

2β-(1-(2-pyridyl)piperazino)-5α-androstane-3α,17β-diol (**19**)

IR (film): 3401 (OH), 1596 (C=C); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.65-2.15 (20H), 2.58 (m, 2H piperazine), 2.75 (m, 3H, 2α-CH and 2H piperazine), 3.55 (m, 4H piperazine), 3.64 (t, J = 8.6 Hz, 17α-CH), 3.90 (m, 3β-CH), 6.64 (m, 2H aromatic), 7.49 (m, 1H aromatic), 8.21 (m, 1H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.20, 17.26, 20.88, 23.32, 28.20, 30.48, 31.14, 32.66, 34.62, 35.49, 36.00, 36.76, 38.44, 43.10, 45.78 (2x), 48.05 (2x), 50.85, 56.11, 63.73, 64.72, 81.87, 107.09, 113.34, 137.46, 147.93, 160.00; LRMS for $C_{28}H_{44}N_3O_2$ [MH^+]: 454.1 m/z.

2β-(4'-(4''-nitrophenyl)-piperazino)-5α-androstane-3α,17β-diol (**20**)

Yield: 12 %; IR (film): 3406 (OH), 1597, 1327 (nitro); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.74 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.70-2.10 (21H), 2.63 (m, 2H piperazine), 2.71 (m, 2α-CH), 2.81 (m, 2H piperazine), 3.44 (m, 4H piperazine), 3.63 (t, J = 8.5 Hz, 17α-CH), 3.93 (m, 3β-CH), 6.83 (d, J = 9.4 Hz, 2H aromatic), 8.15 (d, J = 9.3 Hz, 2H aromatic); ^{13}C NMR, 300 MHz (CD_3OD): 11.74, 14.28, 21.76, 24.30, 29.00, 30.62, 32.79, 33.90, 34.36, 36.53, 37.36, 38.11, 40.66, 44.15, 47.10, 48.21, 51.52 (2x), 52.42, 56.84, 66.57, 66.76, 82.50, 113.62 (2x), 126.73 (2x), 139.26, 156.59; LRMS for $C_{29}H_{44}N_3O_4$ [MH^+]: 498.5 m/z.

2β-(4'-piperonylpiperazino)-5α-androstane-3α,17β-diol (**21**)

Yield: 22 %; IR (film): 3378 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.15 (m, 20H), 2.40-3.00 (m broad, 2α-CH and 8H piperazine), 3.48 (s broad, NCH_2Ar), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.83 (m, 3β-CH), 5.95 (s, CH_2 acetal), 6.75 (m, 2H aromatic), 6.86 (s, 1H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.59, 17.65, 21.31, 23.75, 28.67, 30.96, 31.60, 33.28, 35.13, 35.97, 36.20, 37.27, 38.92, 43.53, 51.35, 53.86 (4x), 56.64, 63.12, 64.09, 64.88, 82.23, 101.26, 108.24, 109.91, 122.63, 132.36, 147.01, 148.03; LRMS for $C_{31}H_{47}N_2O_4$ [MH^+]: 511.3 m/z.

2β-(pyrazol)-5α-androstane-3α,17β-diol (**22**)

Yield : 76 %; IR (film): 3358 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.74 (s, 18-CH_3), 0.78 (s, 19-CH_3), 0.70-2.20 (20H), 3.65 (t, J = 8.5 Hz, 17α-CH), 4.55 (m, 2H, 2α-CH and 3β-CH); 6.38 (t, J = 2.2 Hz, 1H aromatic), 7.56 (d, J = 2.3 Hz, 1H aromatic), 7.65 (d, J = 1.8 Hz, 1H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.15, 14.82, 20.79, 23.28, 27.89, 30.38, 31.15, 34.17, 35.22, 35.76, 36.69, 39.27, 41.57, 43.01, 50.84, 55.41, 62.22, 67.38, 81.76, 105.23, 128.76, 138.83; LRMS for $C_{22}H_{35}N_2O_2$ [MH^+]: 359.2 m/z.

New method for aminolysis of epoxide 8 (synthesis of 23-59)

In a Schlenk tube purged with argon were added the epoxide **8** (1 mmol) dissolved in toluene (30 mL), the desired amine (3 mmol) and $Gd(OTf)_3$ (0.2 mmol). The reaction mixture was heated at 150-190 °C for 2 h (primary amines) or overnight (secondary and cyclic amines). The mixture was then cooled, poured on a silica gel column, and the chromatography performed with an appropriate mixture of MeOH:CH_2Cl_2.

2β-(n-propylamino)-5α-androstane-3α,17β-diol (**23**)

Yield: 84 %; IR (film): 3428 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.74 (s, 18-CH_3), 0.94 (t, J = 7.4 Hz, 3'-CH_3), 0.96 (s, 19-CH_3), 0.70-2.40 (24H), 2.60 and 2.70 (2m, 1'-CH_2), 2.79 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.61, 12.14, 15.81, 21.07, 23.36, 23.75, 28.45, 30.90, 31.74, 33.63, 35.60,

36.51, 37.18, 39.66, 39.91, 43.46, 50.22, 51.34, 56.06, 60.07, 69.39, 82.31; LRMS for $C_{22}H_{40}NO_2$ [MH^+]: 350.2 m/z.

2β-(isopropylamino)-5α-androstane-3α,17β-diol (**24**)

Yield: 46 %; IR (film): 3441 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.94 (s, 19-CH_3), 0.70-2.20 (20H), 1.36 (d, J = 6.4 Hz, 2'-CH_3), 1.47 (d, J = 6.4 Hz, 2'-CH_3), 3.33 (m, 1'-CH), 3.43 (m, 2α-CH), 3.65 (t, J = 8.5 Hz, 17α-CH), 4.12 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.58, 17.54, 18.21, 20.68, 21.50, 23.68, 28.31, 30.88, 31.07, 35.83, 36.35 (2x), 37.01, 39.33, 39.37, 43.48, 48.37, 51.05, 56.64, 57.20, 66.54, 82.20; LRMS for $C_{22}H_{40}NO_2$ [MH^+]: 350.4 m/z.

2β-(n-butylamino)-5α-androstane-3α,17β-diol (**25**)

Yield: 55 %; IR (film): 3358 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.74 (s, 18-CH_3), 0.93 (t, J = 7.3 Hz, 4'-CH_3), 0.97 (s, 19-CH_3), 0.70-2.20 (24H), 2.58 and 2.70 (2m, 1'-CH_2), 2.76 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.61, 14.43, 15.64, 20.88, 21.04, 23.75, 28.46, 30.91, 31.79, 32.63, 33.41, 35.58, 36.51, 37.19, 39.70, 39.93, 43.46, 48.34, 51.36, 56.00, 60.22, 69.57, 82.32; LRMS for $C_{23}H_{42}NO_2$ [MH^+]: 364.4 m/z.

2β-(isobutylamino)-5α-androstane-3α,17β-diol (**26**)

Yield: 47 %; IR (film): 3384 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.98 (m, 19-CH_3 and 2 x 3'-CH_3), 0.70-2.20 (21H), 2.50 and 2.65 (2m, 1'-CH_2), 2.91 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.94 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.61, 16.25, 20.79, 20.96, 21.18, 23.73, 28.08, 28.43, 30.89, 31.60, 34.25, 35.66, 36.53, 37.15, 39.54, 39.63, 43.47, 51.28, 55.46, 56.16, 60.43, 68.39, 82.30; LRMS for $C_{23}H_{42}NO_2$ [MH^+]: 364.3 m/z.

2β-(tert-butylamino)-5α-androstane-3α,17β-diol (**27**)

Yield: 89 %; IR (film): 3427 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.95 (s, 19-CH_3), 1.47 (s, 3 x 2'-CH_3), 0.60-2.20 (21H), 3.26 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 4.12 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.58, 17.29, 21.28, 23.71,

27.29 (3x), 28.42, 30.82, 31.25, 35.83, 35.92, 36.86, 37.07, 39.39, 42.02, 43.50, 51.13, 56.68, 57.25, 59.61, 65.65, 82.24; LRMS for $C_{23}H_{42}NO_2$ [MH^+]: 364.5 m/z.

2β-(n-pentylamino)-5α-androstane-3α,17β-diol (**28**)

Yield: 48 %; IR (film): 3337 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.92 (t, J = 6.9 Hz, 5'-CH_3), 0.97 (s, 19-CH_3), 0.70-2.20 (29H), 2.62 and 2.76 (2m, 1'-CH_2), 2.85 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.89 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.60, 14.44, 15.96, 21.12, 22.92, 23.75, 28.44, 29.51, 29.77, 30.92, 31.69, 33.86, 35.63, 36.50, 37.17, 39.66, 39.71, 43.47, 48.21, 51.32, 56.09, 60.23, 69.03, 82.32; LRMS for $C_{24}H_{44}NO_2$ [MH^+]: 378.4 m/z.

2β-(isopentylamino)-5α-androstane-3α,17β-diol (**29**)

Yield: qt; IR (film): 3416 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.96 (m, 19-CH_3 and 2 x 4'-CH_3), 0.70-2.20 (23H), 2.90 and 3.10 (2m, 1'-CH_2), 3.27 (m, 2α-CH), 3.65 (t, J = 8.5 Hz, 17α-CH), 4.20 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.59, 17.49, 21.47, 22.42, 22.80, 23.70, 26.38, 28.36, 30.89, 31.18, 34.91, 35.84, 36.06, 36.40, 37.04, 38.62, 39.43, 43.50, 44.67, 51.10, 56.43, 60.27, 66.32, 82.21; LRMS for $C_{24}H_{44}NO_2$ [M^+]: 378.4 m/z.

2β-(neopentylamino)-5α-androstane-3α,17β-diol (**30**)

Yield: 85 %; IR (film): 3363 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$ + CD_3OD): 0.71 (s, 18-CH_3), 0.89 (s, 3 x CH_3), 0.95 (s, 19-CH_3), 0.65-2.10 (20H), 2.19 and 2.50 (2d, J = 11.4 Hz, 1'-CH_2), 2.66 (m, 2α-CH), 3.59 (t, J = 8.5 Hz, 17α-CH), 3.77 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$ + CD_3OD): 11.51, 15.56, 20.98, 23.65, 28.01 (3x), 28.44, 30.41, 31.72, 31.77, 33.14, 35.52, 36.40, 37.13, 39.63, 40.12, 43.36, 51.31, 55.96, 60.31, 60.74, 69.09, 81.97; LRMS for $C_{24}H_{44}NO_2$ [MH^+]: 378.3 m/z.

2β-(tert-pentylamino)-5α-androstane-3α,17β-diol (**31**)

Yield: 35 %; IR (film): 3426 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.96 (s, 19-CH_3), 1.03 (t, J = 7.5 Hz, 4'-CH_3), 1.41 (s, 2'-CH_3), 1.42 (s, 2'-CH_3), 0.70-2.20 (22H), 3.28 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 4.19 (m, 3β-CH); ^{13}C NMR, 300

MHz ($CDCl_3$): 7.92, 11.15, 16.91, 20.86, 23.25, 23.73, 24.06, 28.00, 30.31, 30.78, 31.84, 35.35, 35.45, 36.46, 36.60, 38.92, 41.72, 43.03, 50.63, 56.30 (2x), 62.17, 65.02, 81.76; LRMS for $C_{24}H_{44}NO_2$ [MH^+] : 378.2 m/z.

2β-(n-hexylamino)-5α-androstane-3α,17β-diol (**32**)

Yield: 74 %; IR (film): 3382 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.90 (t, J = 6.8 Hz, 6'-CH_3), 0.95 (s, 19-CH_3), 0.70-2.20 (28H), 2.74 and 2.87 (2m, 1'-CH_2), 3.02 (m, 2α-CH) 3.64 (t, J = 8.5 Hz, 17α-CH), 3.98 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.60, 14.41, 16.64, 21.27, 22.89, 23.72, 26.97, 28.24, 28.39, 30.88, 31.44, 31.83, 34.87, 35.71, 36.44, 37.11, 39.24, 39.52, 43.48, 47.27, 51.21, 56.25, 60.14, 67.84, 82.25; LRMS for $C_{25}H_{46}NO_2$ [MH^+] : 392.4 m/z.

2β-(n-heptylamino)-5α-androstane-3α,17β-diol (**33**)

Yield: 77 %; IR (film): 3376 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.90 (t, J = 6.8 Hz, 7'-CH_3), 0.96 (s, 19-CH_3), 0.70-2.20 (30H), 2.70 and 2.83 (2m, 1'-CH_2), 2.96 (m, 2α-CH), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.97 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.60, 14.50, 16.47, 21.23, 23.00, 23.73, 27.36, 28.40, 28.72, 29.40, 30.90, 31.52, 32.09, 34.61, 35.69, 36.46, 37.13, 39.40, 39.57, 43.48, 47.54, 51.25, 56.21, 60.19, 68.17, 82.28; LRMS for $C_{26}H_{48}NO_2$ [MH^+] : 406.4 m/z.

2β-(n-octylamino)-5α-androstane-3α,17β-diol (**34**)

Yield: 93 %; IR (film): 3430 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.70 (s, 18-CH_3), 0.85 (t, J = 6.8 Hz, 8'-CH_3), 0.88 (s, 19-CH_3), 0.60-2.10 (32H), 2.83 and 2.99 (2m, 1'-CH_2), 3.22 (m, 2α-CH), 3.59 (t, J = 8.1 Hz, 17α-CH), 4.05 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.38, 14.27, 17.11, 21.35, 22.80, 23.61, 26.60, 26.71, 28.29, 29.16, 29.20, 30.66, 30.96, 31.88, 35.60, 35.75, 36.17, 36.83, 38.64, 39.17, 43.27, 46.12, 50.90, 56.22, 59.91, 66.51, 81.99; LRMS for $C_{27}H_{50}NO_2$ [MH^+] : 420.4 m/z

2β-(n-nonylamino)-5α-androstane-3α,17β-diol (**35**)

Yield: 85 %; IR (film): 3383 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.84 (t, J = 6.4 Hz, 9'-CH_3), 0.90 (s, 19-CH_3), 0.60-2.10 (34H), 2.63 and 2.76 (2m, 1'-

CH_2), 2.90 (m, 2α-CH), 3.58 (t, J = 8.4 Hz, 17α-CH), 3.88 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.40, 14.32, 16.08, 20.98, 22.86, 23.52, 27.22, 28.20, 28.78, 29.44, 29.55, 29.65, 30.66, 31.34, 32.06, 34.20, 35.46, 36.25, 36.95, 39.26, 39.36, 43.26, 47.54, 51.06, 55.96, 59.94, 68.22, 82.05; LRMS for $C_{28}H_{52}NO_2$ [MH^+] : 434.4 m/z.

2β-(n-decylamino)-5α-androstane-3α,17β-diol (**36**)

Yield: 88 %; IR (film): 3423 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.70 (s, 18-CH_3), 0.85 (t, J = 6.8 Hz, 10'-CH_3), 0.89 (s, 19-CH_3), 0.60-2.10 (36H), 2.78 and 2.93 (2m, 1'-CH_2), 3.14 (m, 2α-CH), 3.59 (t, J = 8.4 Hz, 17α-CH), 4.01 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.38, 14.31, 16.92, 21.17, 22.87, 23.49, 26.85, 26.92, 28.15, 29.33, 29.49, 29.58, 29.70, 30.65, 31.04, 32.07, 35.44, 35.57, 36.19, 36.85, 38.71, 39.21, 43.27, 46.37, 50.93, 56.16, 59.89, 66.80, 81.99; $C_{29}H_{54}NO_2$ [MH^+] : 448.5 m/z.

2β-(n-undecylamino)-5α-androstane-3α,17β-diol (**37**)

Yield: 83 %; IR (film): 3420 (OH and NH); ^1H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.87 (t, J = 6.4 Hz, 11'-CH_3), 0.92 (s, 19-CH_3), 0.70-2.20 (38H), 2.77 and 2.88 (2m, 1'-CH_2), 3.08 (m, 2α-CH), 3.62 (t, J = 8.3 Hz, 17α-CH), 4.00 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.15, 14.11, 16.43, 20.86, 22.66, 23.25, 26.73, 27.25, 27.92, 29.19, 29.31, 29.40, 29.55 (2x), 30.38, 30.88, 31.87, 34.82, 35.29, 35.94, 36.62, 38.60, 38.99, 43.03, 46.46, 50.70, 55.84, 59.62, 66.96, 81.76; LRMS for $C_{30}H_{56}NO_2$ [MH^+] : 462.4 m/z.

2β-(n-dodecylamino)-5α-androstane-3α,17β-diol (**38**)

Yield: 78 %; IR (film): 3421 (OH and NH); ^1H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.84 (t, J = 6.6 Hz, 12'-CH_3), 0.89 (s, 19-CH_3), 0.60-2.10 (40H), 2.68 and 2.81 (2m, 1'-CH_2), 2.97 (m, 2α-CH), 3.58 (t, J = 8.1 Hz, 17α-CH), 3.91 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.40, 14.33, 16.35, 21.04, 22.89, 23.51, 27.10, 28.16, 28.19, 29.49, 29.56, 29.67, 29.78, 29.85, 29.86, 30.63, 31.24, 32.12, 34.61, 35.50, 36.22, 36.92, 39.04, 39.29, 43.26, 47.14, 51.01, 56.02, 59.89, 67.72, 82.01; LRMS for $C_{31}H_{58}NO_2$ [MH^+] : 476.4 m/z.

2β-(n-hexadecylamino)-5α-androstane-3α,17β-diol (**39**)

Yield: 82 % ; IR (film): 3426 (OH and NH) ; ^{1}H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.84 (t broad, 16'-CH_3), 0.89 (s, 19-CH_3), 0.60-2.10 (48H), 2.73 and 2.87 (2m, 1'-CH_2), 3.05 (m, 2α-CH), 3.59 (t, J = 8.3 Hz, 17α-CH), 3.96 (m, 3β-CH) ; ^{13}C NMR, 300 MHz ($CDCl_3$): 11.40, 14.39, 16.63, 21.10, 22.90, 23.50, 26.98, 27.57, 28.17, 29.43, 29.57, 29.64, 29.79, 29.87, 29.90, 29.92 (4x), 30.66, 31.16, 32.18, 35.01, 35.57, 36.23, 36.89, 38.91, 39.26, 43.26, 46.80, 51.00, 56.11, 59.91, 67.35, 82.04 ; LRMS for $C_{35}H_{66}NO_2$ [MH^+] : 532.5 m/z.

2β-(cyclopentylamino)-5α-androstane-3α,17β-diol (**40**)

Yield: qt ; IR (film): 3383 (OH and NH) ; ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.95 (s, 19-CH_3), 0.70-2.20 (28H), 3.00 (m, 2α-CH), 3.38 (m, 1'-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.96 (m, 3β-CH) ; ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 16.19, 20.81, 23.31, 23.79 (2x), 28.01, 30.50, 31.12, 31.78, 32.68, 34.00, 35.28, 36.10, 36.72, 39.11, 39.98, 43.07, 50.84, 55.85, 57.37, 58.10, 68.47, 81.88 ; LRMS for $C_{24}H_{42}NO_2$ [MH^+] : 376.4 m/z.

2β-(cyclohexylamino)-5α-androstane-3α,17β-diol (**41**)

Yield: 91 %; IR (film): 3386 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.95 (s, 19-CH_3), 0.70-2.20 (30H), 2.83 (m, 1'-CH), 3.13 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.98 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.61, 16.93, 21.32, 23.72, 25.07, 25.29, 25.77, 28.43, 30.90, 31.41, 32.43 (2x), 35.02, 35.75, 36.50, 37.10, 39.50, 40.08, 43.49, 51.19, 55.22, 56.35, 56.60, 67.95, 82.27; LRMS for $C_{25}H_{44}NO_2$ [MH^+] : 390.3 m/z.

2β-(cycloheptylamino)-5α-androstane-3α,17β-diol (**42**)

Yield: 56 %; IR (film): 3396 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.91 (s, 19-CH_3), 0.70-2.20 (32H) 2.98 (m, 2α-CH and 1'-CH), 3.60 (t, J = 8.5 Hz, 17α-CH), 3.93 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$ + CD_3OD): 11.55, 16.67, 21.24, 23.66, 24.18, 24.50, 28.07, 28.11, 28.41, 30.59, 31.42, 32.18, 34.12, 34.70, 35.69, 36.41, 37.07, 39.31, 39.40, 43.42, 51.17, 56.25, 56.87, 57.44, 67.44, 82.02; LRMS for $C_{26}H_{46}NO_2$ [MH^+] : 404.4 m/z.

2β-(3'-ethoxypropylamino)-5α-androstane-3α,17β-diol (**43**)

Yield: 39 %; IR (film): 3426 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.70 (s, 18-CH_3), 0.90 (s, 19-CH_3), 1.18 (t, J = 8.0 Hz, 6'-CH_3), 0.60-2.10 (22H), 3.03 (m, 1'-CH_2), 3.18 (m, 2α-CH), 3.32 (m, 1'-CH_2), 3.51 (m, CH_2-O), 3.60 (m, 17α-CH and CH_2-O), 4.07 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.41, 15.20, 16.66, 21.08, 23.48, 25.63, 28.13, 30.61, 31.09, 34.83, 35.56, 36.02, 36.84, 37.92, 39.07, 43.28, 46.66, 50.95, 56.04, 59.99, 65.57, 67.53, 70.09, 81.98; LRMS for $C_{24}H_{44}NO_3$ [MH^+] : 394.4 m/z.

2β-(3'-butoxypropylamino)-5α-androstane-3α,17β-diol (**44**)

Yield: 82 %; IR (film): 3420 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.87 (s and t, 19-CH_3 and 8'-CH_3), 0.60-2.10 (26H), 3.05 (m, 1'-CH_2), 3.18 (m, 2α-CH), 3.30 (m, 1'-CH_2), 3.42 (m, 2H, CH_2-O), 3.59 (m, 17α-CH and CH_2-O), 4.05 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.42, 14.07, 16.58, 19.42, 21.04, 23.51, 25.69, 28.06, 30.58, 31.09, 31.60, 34.71, 35.50, 35.97, 36.85, 37.97, 38.99, 43.27, 46.60, 50.93, 55.95, 59.99, 65.57, 70.13, 71.99, 81.94; LRMS for $C_{26}H_{48}NO_3$ [MH^+]: 422.3 m/z.

2β-(2'-phenylethylamino)-5α-androstane-3α,17β-diol (**45**)

Yield: 81 %; IR (film): 3370 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.65 (s, 18-CH_3), 0.82 (s, 19-CH_3), 0.60-2.10 (20H), 2.80 (m, 1'-CH_2 and 2'-CH_2), 2.95 (m, 2α-CH), 3.55 (t, J = 8.7 Hz, 17α-CH), 3.78 (m, 3β-CH), 7.10-7.35 (m, 5H aromatic); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.39, 15.67, 20.89, 23.55, 28.19, 30.75, 31.43, 33.64, 35.40, 35.77, 36.25, 36.83, 39.35, 39.41, 43.26, 49.19, 51.14, 55.87, 59.94, 68.78, 82.10, 126.73, 128.83 (2x), 128.96 (2x), 138.77; LRMS for $C_{27}H_{42}NO_2$ [MH^+]: 412.3 m/z.

2β-(3'-phenylpropylamino)-5α-androstane-3α,17β-diol (**46**)

Yield: 83 %; IR (film): 3392 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.60-2.10 (22H), 2.65 (m, 1H of 1'-CH_2 and 3' –CH_2), 2.82 (m, 1H of 1'-CH_2), 2.90 (m, 2α-CH), 3.59 (t, J = 8.5 Hz, 17α-CH), 3.87 (m, 3β-CH), 7.12-7.20 (m, 3H aromatic), 7.22-7.30 (m, 2H aromatic); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.41, 16.15, 20.99, 23.52, 28.15, 29.92, 30.68, 31.32, 33.26, 34.34, 35.47, 36.22, 36.92, 39.23, 39.34, 43.27,

46.72, 51.08, 55.98, 59.96, 67.97, 82.07, 126.36, 128.56 (2x), 128.74 (2x), 141.16. LRMS for $C_{28}H_{44}NO_2$ [MH^+]: 426.3 m/z.

2β-(3'-diphenylpropylamino)-5α-androstane-3α,17β-diol (**47**)

Yield: 82 %; IR (film): 3360 (OH and NH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.89 (s, 19-CH_3), 0.60-2.10 (20H), 2.24 (m, 2'-CH_2), 2.52 (m, 1H of 1'-CH_2), 2.67 (m, 2α-CH and 1H of 1'-CH_2), 3.62 (t, J = 8.5 Hz, 17α-CH), 3.71 (m, 3β-CH), 4.04 (t, J = 7.8 Hz, 3'-CH), 7.15-7.30 (m, 10H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 15.19, 20.54, 23.31, 27.98, 30.46, 31.31, 32.89, 35.10, 35.64, 36.00, 36.72, 39.20, 39.59, 43.00, 46.46, 48.94, 50.89, 55.47, 59.69, 68.76, 81.86, 126.24 (2x), 127.76 (4x), 128.48 (4x), 144.53 (2x); LRMS for $C_{34}H_{48}NO_2$ [MH^+]: 502.3 m/z.

2β-(adamantanemethylamino)-5α-androstane-3α,17β-diol (**48**)

Yield: 72 %; IR (film): 3356 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.93 (s, 19-CH_3), 0.60-2.00 (35H), 2.06 (d, J = 11.6 Hz, 1H of 1'-CH_2), 2.36 (d, J = 11.2 Hz, 1H of 1'-CH_2), 2.60 (m, 2α-CH), 3.59 (t, J = 8.7 Hz, 17α-CH), 3.71 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.40, 15.45, 20.84, 23.56, 28.29, 28.66 (3x), 30.73, 31.64, 33.04, 33.58, 35.39, 36.40, 37.02, 37.46 (3x), 39.59, 40.17, 41.06 (3x), 43.27, 51.24, 55.81, 60.53, 61.31, 69.49, 82.18; LRMS for $C_{30}H_{50}NO_2$ [MH^+]: 456.4 m/z.

2β-(diethylamino)-5α-androstane-3α,17β-diol (**49**)

Yield: N.A.; IR (film): 3444 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.95 (s, 19-CH_3), 1.49 (t, J = 7.1 Hz, 2 x 2'-CH_3), 0.70-2.20 (20H), 2.85 (m, 2α-CH), 3.07 and 3.41 (2m, 2 x 1'-CH_2), 3.66 (t, J = 8.5 Hz, 17α-CH), 4.12 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 10.67, 11.62, 11.80, 18.34, 21.52, 23.68, 28.33, 30.87, 31.01, 35.36, 35.88, 36.61, 36.75, 36.95, 39.21, 43.43, 43.47, 44.71, 48.51, 50.95, 56.72, 64.14, 64.19, 82.13; LRMS for $C_{23}H_{42}NO_2$ [MH^+]: 364.3 m/z.

2β-(4'-methylpiperazino)-3α-hydroxy-5α-androstan -17-one (**50**)[21]

Yield: 81 %; IR (film): 3418 (OH), 1738 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.83 (s, CH_3), 0.85 (s, CH_3), 0.65-2.15 (19H), 2.27 (s, 4'-CH_3), 2.40 and 2.65 (2m broad, 2α-CH,

1H of 16-CH_2 and 8H piperazine), 3.84 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 13.76, 16.89, 20.43, 21.64, 27.95, 30.38, 31.53, 32.55, 34.40, 34.89, 35.77 (2x), 38.37, 45.86, 47.82, 51.21, 55.48 (4x), 55.91, 63.60, 64.34, 221.18; LRMS for $C_{24}H_{41}N_2O_2$ [MH^+]: 389.3 m/z.

Trans-2-(4'-methylpiperazino)-cyclohexanol (**51**)
Yield: quantitative; IR (film): 3448 (OH); ^1H NMR, 300 MHz ($CDCl_3$): 1.18 (m, 4H), 1.77 (m, 3H), 2.18 (m, 2H), 2.27 (s, 1''-CH_3), 2.43 (m, 5H), 2.74 (m, 2H), 3.33 (m, 2α-CH), 3.94 (m, CH-OH); ^{13}C NMR, 300 MHz ($CDCl_3$): 22.01, 23.86, 25.31, 32.99, 45.86, 55.48 (4x), 68.32, 69.82; LRMS for $C_{11}H_{23}N_2O$ [MH^+]: 199.1 m/z.

2β-(4'-methylpiperazino)-5α-androstane-3α,17β-diol (**1**)[11]
Yield: 88 %; IR (film): 3369 (OH); ^1H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.70-2.10 (20H), 2.34 (s, 4'-CH_3), 2.54 (m, 4H piperazine), 2.73 (m, 2α-CH and 4H piperazine), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.86 (m, 3β-CH); ^{13}C NMR 300 MHz ($CDCl_3$): 11.18, 17.15, 20.78, 23.31, 28.16, 30.56, 31.09, 32.70, 34.64, 35.41, 35.72, 36.88, 38.43, 42.94, 45.58, 50.84, 55.22 (4x), 56.10, 63.60, 64.37, 81.26; LRMS for $C_{24}H_{43}N_2O_2$ [MH^+]: 391.3 m/z.

2β-(4'-acetylpiperazino)-5α-androstane-3α,17β-diol (**52**)
Yield: 83 %; IR (film): 3384 (OH), 1630 (C=O); ^1H NMR, 300 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 2.10 (s, 2''-CH_3), 0.65-2.15 (20H), 2.50 (m, 2H piperazine), 2.70 (m, 2α-CH and 2H piperazine), 3.56 (m, 17α-CH and 4H piperazine), 3.87 (m, 3β-CH); ^{13}C NMR, 400MHz ($CDCl_3$): 11.62, 17.79, 21.38, 21.71, 23.73, 28.51, 30.91, 31.46, 33.72, 35.38, 35.92, 36.31, 37.15, 38.95, 41.75, 43.51, 46.62, 48.34, 48.96, 51.24, 56.53, 64.32, 65.43, 82.24, 169.30; LRMS for $C_{25}H_{43}N_2O_3$ [MH^+]: 419.3 m/z.

2β-(4'-phenylpiperazino)-5α-androstane-3α,17β-diol (**53**)
Yield: 80 %; IR (film): 3364 (OH), 1600 (CH aromatic); ^1H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 19-CH_3), 0.91 (s, 19-CH_3), 0.70-2.20 (20H), 2.77 (m, 2H piperazine), 2.95 (m, 2α-CH and 2H piperazine), 3.30 (m, 4H piperazine), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.96 (m, 3β-

CH), 6.93 (m, 3H aromatic), 7.30 (m, 2H aromatic); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.18, 17.43, 20.95, 23.29, 28.09, 30.48, 31.03, 33.32, 35.12, 35.48, 35.91, 36.72, 38.55, 43.07, 48.04 (2x), 49.23 (2x), 50.78, 56.16, 63.97, 64.68, 81.82, 116.44 (2x), 120.29, 129.16 (2x), 152.17; LRMS for $C_{29}H_{45}N_2O_2$ [MH^+]: 453.3 m/z.

2β-(4'-(4''fluorophenyl)-piperazino)-5α-androstane-3α,17β-diol (**54**)

Yield: 97 %; IR (film): 3368 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.76 (s, 18-CH_3), 0.90 (s, 19-CH_3), 0.70-2.20 (20H), 2.68 (m, 2H piperazine), 2.86 (m, 2α-CH and 2H piperazine), 3.15 (m, 4H piperazine), 3.64 (m, 17α-CH), 3.93 (m, 3β-CH), 6.89 (m, 2H aromatic), 6.98 (m, 2H aromatic); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.65, 17.70, 21.35, 23.75, 28.60, 30.94, 31.56, 33.36, 35.18, 35.94, 36.29, 37.21, 38.96, 43.52, 48.62 (2x), 51.06 (2x), 51.28, 56.57, 64.29, 65.04, 82.30, 115.86, 116.08, 118.44, 118.51, 148.24, (156.52 and 158.90) (C-F coupling); LRMS for $C_{29}H_{44}FN_2O_2$ [MH^+]: 471.3 m/z.

2β-(piperidino)-5α-androstane-3α,17β-diol (**55**)

Yield: 86 %; IR (film): 3424 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.88 (s, 19-CH_3), 3.04 (m broad, 2 x 2'-CH_2), 3.49 (m, 2α-CH), 3.62 (t, J = 7.8 Hz, 17α-CH), 3.98 (m, 3β-CH); ^{13}C NMR 300 MHz ($CDCl_3$): 11.21, 17.98, 21.05, 22.32, 23.22, 23.56 (2x), 27.86, 30.36, 30.59, 34.94, 35.41, 36.16, 36.26, 36.53, 38.59, 43.03, 50.53, 56.13, 63.78, 66.12, 81.63; LRMS for $C_{24}H_{42}NO_2$ [MH^+]: 376.4 m/z.

Note : C-2' did not appear or is located under another peak.

2β-(4'-methylpiperidino)-5α-androstane-3α,17β-diol (**56**)

Yield: 87 %; IR (film): 3416 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.73 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.98 (d, J = 5.7 Hz, 4'-CH_3), 0.65-2.20 (25H), 2.58 and 2.79 (2m, 2'-CH_2), 3.11 (m, 1H of 2'-CH_2), 3.26 (m, 2α-CH and 1H of 2'-CH_2), 3.62 (m, 17α-CH), 3.91 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.60, 18.32, 21.48, 21.55, 23.70, 28.46, 30.34, 30.91, 31.21, 33.05, 34.65, 35.95, 36.29, 36.50 (2x), 37.10, 38.98, 43.51, 45.81 (2x), 51.12, 56.68, 64.27, 65.91, 82.17; LRMS for $C_{25}H_{44}NO_2$ [MH^+]: 390.4 m/z.

2β-(3'-methylpiperidino)-5α-androstane-3α,17β-diol (**57**)

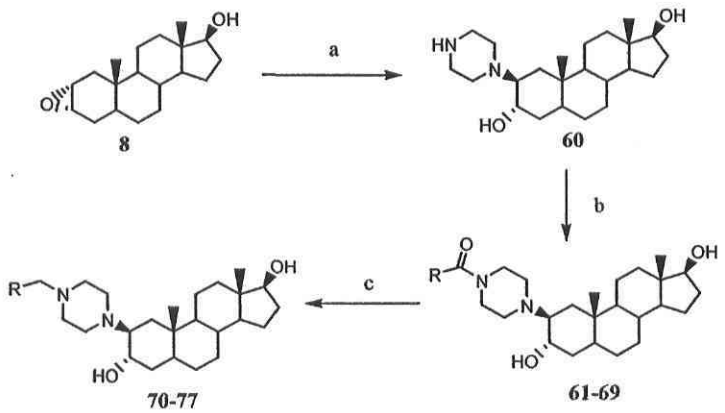
Yield: 78 %; IR (film): 3379 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.82 (s, 19-CH_3), 1.18 (d, 3'-CH_3), 0.60-2.10 (25H), 2.15 and 2.45 (2m, 2'-CH_2), 2.60 (m, 2α-CH and 2'-CH_2), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.79 (m, 3β-CH); ^{13}C NMR 400 MHz ($CDCl_3$): 11.18, 17.71, (19.45, 19.68) (twin), 20.92, 23.29, (25.83, 26.17) (twin), 28.28, 30.50, 31.09 (31.66, 31.87) (twin), 32.59, (33.24, 33.32) (twin), 35.04, 35.54, 35.66, 36.80, 38.30, 43.09, 44.91, 50.84, (52.64, 52.73) (twin), 56.31, (63.38, 63.47) (twin), (64.73, 64.96) (twin), 81.85; LRMS for $C_{25}H_{44}NO_2$ [MH^+]: 390.3 m/z.

2β-(2'-methylpiperidino)-5α-androstane-3α,17β-diol (**58**)

Yield: 48 %; IR (film): 3412 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, splitted in two, 18-CH_3), 0.88 (s, splitted in two, 19-CH_3), 0.84 (s, 2'-CH_3), 2.80 (m, 2α-CH), 3.20 and 3.50 (m, 6'-CH_2), 3.64 (m, 17α-CH), 3.95 (m, 3β-CH); ^{13}C NMR 400 MHz ($CDCl_3$): 11.61, 14.07, (18.06, 18.35) (twin), (21.34, 21.50) (twin), 23.71, 28.55, 28.65, 30.84, 30.97, 31.30, 31.40, 33.78, 35.94, 36.01, 36.38, 36.58, 37.15, 39.08, 43.53, 45.61, (51.14, 51.19) (twin), 57.10, 63.71, (64.87, 65.04) (twin), 82.21; LRMS for $C_{25}H_{44}NO_2$ [MH^+]: 390.3 m/z.

2β-(4'-piperidinopiperidino)-5α-androstane-3α,17β-diol (**59**)

Yield: 73 %; IR (film): 3423 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.70-2.10 (30H), 2.22 (m, 1H), 2.61 (m, 1H), 2.75 (m, 2α-CH), 2.92 (m, 7H), 3.64 (t, J = 8.4 Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR 400 MHz ($CDCl_3$): 11.59, 17.77, 21.35, 23.56, 23.73, 24.61 (2x), 27.47, 27.88, 28.62, 30.92, 31.52, 33.49, 35.30, 35.97, 36.20, 37.21, 38.88, 43.53, 50.64 (4x), 51.31, 56.63, 64.34 (2x), 64.99, 82.24 ; LRMS for $C_{29}H_{51}N_2O_2$ [M^+]: 459.4 m/z.



Scheme 3. Synthesis of aminosteroids **60** to **77**. **(a)** Piperazine, H_2O, Schlenk tube (150-190 °C); **(b)** HBTU, carboxylic acid, DIPEA, DMF, 0°C to rt; **(c)** BH_3*THF, THF, 0°C to rt then reflux in methanol overnight.

N-derivatization of 2β-(piperazino)-5α-androstane-3α,17β-diol (synthesis of 61-79)

2β-piperazino-5α-androstane-3α,17β-diol **(60).** A solution of epoxide **8** (6.46 g, 19.4 mmol) in piperazine (33.3 g, 388 mmol) and H_2O (5 mL) was heated in a Schlenk tube at 150-190 °C overnight, then poured in water (500 mL) and the precipitate was filtered. The solid is dissolved in CH_2Cl_2 and the solution dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the crude product by flash chromatography (MeOH : Et_3N : CH_2Cl_2, 14:1:85) yielded 5.76 g (70 %) of compound **60** as a white solid. IR (film): 3370 (OH and NH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.65-2.20 (22H), 2.42, 2.60 and 2.90 (3m, 2α-CH and 8H piperazine), 3.65 (t, J = 8.7 Hz, 17α-CH), 3.85 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.31, 20.86, 23.31, 28.23, 30.52, 31.15, 32.58, 34.67, 35.50, 35.72, 36.87, 38.40, 43.04, 46.14, 46.67 (2x), 49.38, 50.87, 56.17, 63.35, 65.03, 81.60; LRMS for $C_{23}H_{41}N_2O_2$ [MH^+]: 377.3 m/z.

Synthesis of *N*-amide derivatives of **60** (general procedure for synthesis of **61-69**).

HBTU (*O*-Benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate) (1 eq.) and carboxylic acid (1.1 eq.) was dissolved in DMF at 0 °C. Then, diisopropylethylamine (DIPEA) was added to the mixture and allowed to react for 5 min. Thereafter, compound **60** (1 eq.) dissolved in DMF was added to the solution and the temperature was raised to room temperature for 2–3 h. The resulting mixture was diluted in ethyl acetate and washed with water (4x). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to dryness. Purification of the crude product by flash chromatography (1-4 % MeOH in CH_2Cl_2) yielded the desired amide as a white solid.

2β-(4'-propanoylpiperazino)-5α-androstane-3α,17β-diol (**61**)

Yield: 85 %; IR (film): 3419 (OH), 1629 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 1.13 (t, J = 7.4 Hz, 3''-CH_3), 0.60-2.20 (20H), 2.32 (q, J = 7.5 Hz, 2''-CH_2), 2.48 (m, 2H piperazine), 2.69 (m, 2α-CH and 2H piperazine), 3.49 (m, 2H piperazine), 3.63 (m, 17α-CH and 2H piperazine), 3.88 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 9.45, 11.17, 17.22, 20.89, 23.28, 26.39, 28.10, 30.44, 31.05, 32.97, 34.72, 35.45, 35.80, 36.72, 38.44, 41.70, 43.06, 45.58, 48.04, 48.57, 50.81, 56.04, 63.75, 64.90, 81.79, 172.19; LRMS for $C_{26}H_{45}N_2O_3$ [MH^+]: 433.3 m/z.

2β-(4'-butanoylpiperazino)-5α-androstane-3α,17β-diol (**62**)

Yield: 82 %; IR (film): 3390 (OH), 1634 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.96 (t, J = 7.4 Hz, 4''-CH_3), 0.60-2.20 (22H), 2.29 (t, J = 7.5 Hz, 2''-CH_2), 2.55 (m broad, 2H piperazine), 2.75 (m broad, 2α-CH and 2H piperazine), 3.65 (m broad, 17α-CH and 4H piperazine), 3.90 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 13.98, 17.41, 18.66, 20.95, 23.28, 28.04, 30.46, 30.96, 33.43, 35.08 (2x), 35.46, 35.91, 36.68, 38.50, 41.03, 43.06, 45.16, 47.93, 48.58, 50.75, 56.06, 63.88, 65.13, 81.79, 171.43; LRMS for $C_{27}H_{47}N_2O_3$ [MH^+]: 447.3 m/z.

2β-(4'-isobutanoylpiperazino)-5α-androstane-3α,17β-diol (**63**)

Yield: 75 %; IR (film): 3402 (OH), 1624 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 1.11 (d, J = 6.6 Hz, 2 x 3''-CH_3), 0.60-2.20 (20H), 2.50 (m, 2H

piperazine), 2.75 (m, 2α-CH, 2''-CH and 2H piperazine), 3.60 (m broad, 17α-CH and 4H piperazine), 3.88 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.18, 19.38 (2x), 20.89, 23.28, 28.11, 29.94, 30.45, 31.07, 32.87, 34.62, 35.46, 35.79, 36.72, 38.43, 41.90, 43.06, 45.73, 48.15, 48.81, 50.81, 56.04, 63.75, 64.81, 81.79, 175.30; LRMS for $C_{27}H_{47}N_2O_3$ [MH^+]: 447.3 m/z.

2β-(4'-pentanoylpiperazino)-5α-androstane-3α,17β-diol (**64**)

Yield: 80 %; IR (film): 3398 (OH), 1624 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.92 (t, J = 7.3 Hz, 5''-CH_3), 0.60-2.20 (24H), 2.31 (t, J = 7.7 Hz, 2''-CH_2), 2.60 and 2.80 (2m broad, 2α-CH and 4H piperazine), 3.60 (m broad, 17α-CH and 4H piperazine), 3.91 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 13.86, 17.40, 20.94, 22.53, 23.28, 27.33, 28.04, 30.44, 30.96, 32.90, 33.46, 35.11, 35.45, 35.91, 36.68, 38.49, 40.99, 43.06, 45.14, 47.97, 48.57, 50.75, 56.04, 63.87, 65.14, 81.76, 171.62, LRMS for $C_{28}H_{49}N_2O_3$ [MH^+]: 461.3 m/z.

2β-(4'-isopentanoylpiperazino)-5α-androstane-3α,17β-diol (**65**)

Yield: 79 %; IR (film): 3402 (OH), 1626 (C=O); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.69 (s, 18-CH_3), 0.81 (s, 19-CH_3), 0.93 (d, J = 6.6 Hz, 2 x 3''-CH_3), 0.60-2.10 (21H), 2.16 (d, J = 8.2 Hz, 2'-CH_2), 2.40 (m, 2H piperazine), 2.60 (m, 2H piperazine and 2α-CH), 3.43 (m, 2H piperazine), 3.58 (m, 2H piperazine and 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR, 400 MHz ($CDCl_3$): 11.20, 17.21, 20.91, 22.77 (2x), 23.32, 25.80, 28.17, 30.52, 31.12, 32.80, 34.59, 35.51, 35.81, 36.77, 38.46, 41.95, 42.03, 43.09, 46.31, 48.20, 48.73, 50.87, 56.10, 63.75, 64.81, 81.88, 170.97; LRMS for $C_{28}H_{49}N_2O_3$ [MH^+]: 461.3 m/z.

2β-(4'-nonanoylpiperazino)-5α-androstane-3α,17β-diol (**66**)

Yield: 70 %; IR (film): 3394 (OH), 1628 (C=O); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.87 (s, 19-CH_3), 0.89 (t, J = 7.0 Hz, 9''-CH_3), 0.65-2.15 (32H), 2.32 (t, J = 7.7 Hz, 2''-CH_2), 2.43 (m, 2H piperazine), 2.66 (m, 2α-CH and 2H piperazine), 3.48 (m, 2H piperazine), 3.64 (m, 17α-CH and 2H piperazine), 3.90 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.17, 14.08, 17.18, 20.88, 22.63, 23.28, 25.34, 28.13, 29.15, 29.35, 29.47, 30.46,

31.09, 31.79, 32.80, 33.30, 34.57, 35.47, 35.76, 36.73, 38.43, 41.91, 43.06, 46.07, 48.09, 48.63, 50.83, 56.07, 63.73, 64.76, 81.82, 171.68; LRMS for $C_{32}H_{57}N_2O_3$ [MH^+]: 517.5 m/z.

2β-(4'-cyclohexylcarbonylpiperazino)-5α-androstane-3α,17β-diol (**67**)

Yield: 84 %; IR (film): 3382 (OH), 1628 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.73 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.60-2.10 (30H), 2.43 (m, 2''-CH), 2.40-3.20 (m broad, 2α-CH and 4H piperazine), 3.65 (m broad, 17α-CH and 4H piperazine), 3.93 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.56, 18.59, 21.01, 23.26, 25.71 (2x), 27.98, 29.33 (2x), 30.45, 30.85, 33.92, 34.69, 35.45, 36.03, 36.63, 38.52, 40.23 (2x), 42.64, 43.06, 44.59, 48.85 (2x), 50.68, 54.51, 56.02, 63.96, 65.45, 81.75, 174.52; LRMS for $C_{30}H_{51}N_2O_3$ [MH^+]: 487.5 m/z.

2β-(4'-benzoylpiperazino)-5α-androstane-3α,17β-diol (**68**)

Yield: 90 %; IR (film): 3392 (OH), 1622 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.73 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.60-2.20 (20H), 2.40-2.90 (m broad, 2α-CH and 4H piperazine), 3.55 (m broad, 17α-CH and 4H piperazine), 3.87 (m, 3β-CH), 7.40 (s, 5H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 17.28, 20.92, 23.28, 28.08, 30.45, 31.02, 33.15, 34.81, 35.47, 35.84, 36.72, 38.45, 42.18 (2x), 43.06, 48.07, 48.66, 50.79, 56.06, 63.78, 65.01, 81.79, 127.08 (2x), 128.51 (2x), 129.85, 135.37, 170.29; LRMS for $C_{30}H_{45}N_2O_2$ [MH^+]: 481.5 m/z.

2β-(4'-retinoylpiperazino)-5α-androstane-3α,17β-diol (**69**)

Yield: 44 %; IR (film): 3395 (OH), 1618 (C=O); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.71 (s, 18-CH_3), 0.84 (s, 19-CH_3), 1.01 (s, 2 x CH_3), 1.96, 2.00 and 2.07 (3s, 3 x CH_3), 0.60-2.20 (35H), 2.56-2.95 (m, 2α-CH and 4H piperazine), 3.50-4.00 (3m, 17α-CH, 4H piperazine and 3β-CH), 5.60-7.00 (m, 6H, alkene); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.18, 12.82, 14.48, 17.33, 19.17, 20.29, 20.92, 21.73, 23.28, 28.05, 28.91, 30.41, 31.00, 33.04, 33.39, 34.20, 34.98, 35.44, 35.85, 36.69, 38.49, 39.51, 41.07, 43.05, 46.04, 48.03, 48.63, 50.77, 56.06, 63.87, 64.97, 81.76, 120.55, 128.01, 128.88, 129.42, 129.72, 129.82, 129.94, 134.68, 137.30, 137.67, 166.86; LRMS for $C_{43}H_{67}N_2O_3$ [MH^+]: 659.3 m/z.

Synthesis of N-alkylpiperazino derivatives **70-77** (general procedure).

To an amidopiperazino derivative selected from 61-69 and dissolved in THF at 0 °C, BH_3-THF (10 eq.) was added and temperature raised to room temperature for 90 min. Water was then added and the mixture was extracted with CH_2Cl_2, the organic layer dried over $MgSO_4$, filtered and evaporated to dryness. An overnight reflux in methanol was then needed to break the boron-nitrogen complex. The resulting amine was purified by flash chromatography (2-7 % MeOH in CH_2Cl_2) to yield the desired amine as a white solid.

2β-(4'-propylpiperazino)-5α-androstane-3α,17β-diol (**70**)

Yield: 66 %; IR (film): 3380 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (t, J = 7.2 Hz, 3''-CH_3), 0.60-2.10 (22H), 2.30, 2.50 and 2.70 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.58 (m, 17α-CH), 3.82 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.21, 11.90, 17.25, 19.71, 20.80, 23.36, 28.24, 30.63, 31.15, 32.64, 34.67, 35.47, 35.73, 36.90, 38.43, 43.00, 50.86, 53.60 (4x), 56.13, 60.57, 63.56, 64.34, 81.36; LRMS for $C_{26}H_{47}N_2O_2$ [MH^+]: 419.3 m/z.

2β-(4'-butylpiperazino)-5α-androstane-3α,17β-diol (**71**)

Yield: 47 %; IR (film): 3380 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.93 (t, J = 7.3 Hz, 4''-CH_3), 0.60-2.20 (24H), 2.55, 2.70 and 2.85 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.61 (m, 17α-CH), 3.85 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.21, 13.98, 17.25, 20.70, 20.79, 23.36, 28.23, 28.60, 30.62, 31.15, 32.65, 34.67, 35.47, 35.74, 36.89, 38.43, 43.00, 50.85, 53.60 (4x), 56.13, 58.38, 63.57, 64.34, 81.37; LRMS for $C_{27}H_{49}N_2O_2$ [MH^+]: 433.4 m/z.

2β-(4'-isobutylpiperazino)-5α-androstane-3α,17β-diol (**72**)

Yield: 35 %; IR (film): 3370 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.84 (s, 19-CH_3), 0.90 (d, J = 6.5 Hz, 2 x 3''-CH_3), 0.60-2.15 (21H), 2.20, 2.60 and 2.80 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.62 (m, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.19, 17.41, 20.92 (3x), 23.28, 25.20, 28.13, 30.46, 31.05, 33.20, 35.03, 35.48, 35.82, 36.74, 38.49, 43.07, 50.80, 53.33 (4x), 56.18, 63.81, 64.41, 66.44, 81.79; LRMS for $C_{27}H_{49}N_2O_2$ [MH^+]: 433.4 m/z.

2β-(4'-pentylpiperazino)-5α-androstane-3α,17β-diol (**73**)

Yield: 62 %; IR (film): 3385 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (t, J = 6.9 Hz, 5''-CH_3), 0.60-2.15 (26H), 2.40, 2.60 and 2.75 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.60, (t, J = 8.5 Hz, 17α-CH), 3.84 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.19, 13.98, 17.21, 20.83, 22.47, 23.32, 25.84, 28.19, 29.56, 30.53, 31.12, 32.84, 34.70, 35.47, 35.77, 36.81, 38.46, 43.03, 50.84, 53.34 (4x), 56.10, 58.46, 63.71, 64.42, 81.59; LRMS for $C_{28}H_{51}N_2O_2$ [MH^+]: 447.4 m/z.

2β-(4'-isopentylpiperazino)-5α-androstane-3α,17β-diol (**74**)

Yield: 70 %; IR (film): 3382 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.88 (d, J = 6.4 Hz, 2 x 4''-CH_3), 0.65-2.15 (23H), 2.40, 2.55 and 2.70 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.58 (t, J = 8,5 Hz, 17α-CH), 3.83 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.21, 17.22, 20.82, 22.63 (2x), 23.35, 26.64, 28.22, 30.61, 31.14, 32.74, 34.69, 35.15, 35.47, 35.76, 36.87, 38.46, 43.00, 50.85, 53.51 (4x), 56.12, 56.84, 63.64, 64.37, 81.42. LRMS for $C_{28}H_{51}N_2O_2$ [MH^+]: 447.5 m/z.

2β-(4'-nonylpiperazino)-5α-androstane-3α,17β-diol (**75**)

Yield: 44 %; IR (film): 3386 (OH); ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.90 (t, J = 6.9 Hz, 9''-CH_3), 0.65-2.10 (26H), 2.35, 2.50 and 2.70 (3m broad, 2α-CH, 1''-CH_2 and 8H piperazine), 3.63, (t, J = 8.5 Hz, 17α-CH), 3.85 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.20, 14.13, 17.31, 20.84, 22.68, 23.35, 26.81, 27.59, 28.27, 29.28, 29.55 (2x), 30.56, 31.18, 31.87, 32.65, 34.69, 35.51, 35.76, 36.84, 38.46, 43.07, 50.87, 53.79 (4x), 56.16, 58.81, 63.59, 64.39, 81.75; LRMS for $C_{32}H_{59}N_2O_2$ [MH^+]: 503.5 m/z.

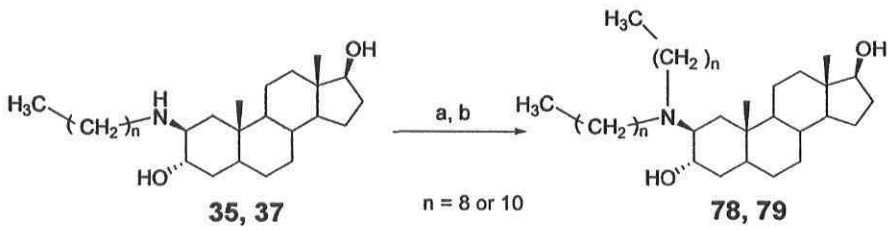
2β-(4'-cyclohexylmethylpiperazino)-5α-androstane-3α,17β-diol (**76**)

Yield: 78 %; IR (film): 3380 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.60-2.10 (29H), 2.16 (d, J = 6.6 Hz, 1''-CH_2), 2.50 and 2.75 (2m broad, 2α-CH and 8H piperazine), 3.61 (m, 17α-CH), 3.82 (m, 3β-CH); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.19, 17.36, 20.90, 23.30, 26.04 (2x), 26.64, 28.18, 30.48, 31.10, 31.87 (3x), 32.99, 34.81,

35.49 (2x), 35.78, 36.76, 38.45, 43.08, 50.82, 53.77 (4x), 56.17, 63.70, 64.38, 65.38, 81.80; LRMS for $C_{30}H_{53}N_2O_2$ [MH^+]: 473.5 m/z.

2β-(4'-benzylpiperazino)-5α-androstane-3α,17β-diol (**77**)

Yield: 46 %; IR (film): 3372 (OH); ^{1}H NMR, 300 MHz ($CDCl_3$): 0.72 (s, 18-CH_3), 0.83 (s, 19-CH_3), 0.65-2.20 (20H), 2.55 and 2.75 (2m broad, 2α-CH and 8H piperazine), 3.53 (s, benzylic CH_2), 3.61 (t, J = 8.5 Hz, 17α-CH), 3.82 (m, 3β-CH), 7.30 (s, 5H aromatic); ^{13}C NMR, 300 MHz ($CDCl_3$): 11.43, 17.65, 21.16, 23.54, 28.39, 30.72, 31.31, 33.37, 35.22, 35.73, 36.06, 37.00, 38.71, 43.31, 51.05, 53.23 (4x), 56.41, 63.03, 64.00, 64.66, 82.01, 127.54, 128.54 (2x), 129.54 (2x), 137.59; LRMS for $C_{30}H_{47}N_2O_2$ [MH^+]: 467.5 m/z.



Scheme 4. Synthesis of *n*-dialkyl aminosteroids **78** and **79**. **(a)** HBTU, carboxylic acid, DIPEA, DMF, 0 °C to rt; **(b)** BH_3*THF, THF, 0 °C to rt.

Synthesis of *N,N*-dialkylamino derivatives **78** and **79**.

HBTU (*O*-Benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) (1 eq.) and carboxylic acid (1.1 eq.) was dissolved in DMF at 0 °C. Then, diisopropylethylamine (DIPEA) was added in the mixture and allowed to react 5 min. Thereafter, compound **35** or **37** (1 eq.) dissolved in DMF was added to the solution and the temperature was raised to room temperature for 2–3 h. The resulting mixture was diluted in ethyl acetate and washed with water (4x). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to dryness. Purification of the crude product by flash chromatography (1-2 % MeOH in CH_2Cl_2) yielded the amide as a pale yellow oil. This, compound was dissolved

in THF at 0 °C, BH_3 - THF (10 eq.) was added to the solution and temperature raised to room temperature for 90 min. Water was then added and mixture was extracted with CH_2Cl_2, the organic layer dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography (8 % acetone in hexanes) gave the desired amine as a pale yellow oil.

2β-(dinonylamino)-5α-androstane-3α,17β-diol (**78**)

Yield: 47 % ; IR: 3397 (OH) ; ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.86 (s, 19-CH_3), 0.90 (t, J = 6.4 Hz, 2 x 9' CH_3), 0.70-2.10 (48H), 2.31 and 2.44 (2m, 2 x 1'-CH_2), 2.77 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.75 (m, 3β-CH) ; ^{13}C NMR, 400 MHz ($CDCl_3$): 11.57, 14.49 (2x), 18.01, 21.34, 23.06 (2x), 23.76, 27.84 (2x), 28.88, 29.70 (2x), 29.77 (2x), 30.01 (4x), 31.00, 31.58, 32.29 (2x), 33.72, 35.33, 36.05, 36.13, 37.25, 38.90, 43.53, 50.63 (2x), 51.32, 56.84, 61.30, 64.37, 82.34 ; LRMS for $C_{37}H_{70}NO_2$ [MH^+] : 560.5 m/z.

2β-(diundecylamino)-5α-androstane-3α,17β-diol (**79**)

Yield: 59 % ; IR: 3407 (OH) ; ^{1}H NMR, 400 MHz ($CDCl_3$): 0.75 (s, 18-CH_3), 0.85 (s, 19-CH_3), 0.89 (t, J = 6.8 Hz, 2 x 11' CH_3), 0.70-2.10 (56H), 2.31 and 2.44 (2m, 2 x 1'-CH_2), 2.80 (m, 2α-CH), 3.64 (t, J = 8.5 Hz, 17α-CH), 3.75 (m, 3β-CH) ; ^{13}C NMR, 400 MHz ($CDCl_3$): 11.58, 14.50 (2x), 18.02, 21.35, 23.08 (2x), 23.75, 27.83 (2x), 28.87, 29.68 (2x), 29.74 (2x), 29.84 (2x), 30.00 (2x), 30.05 (4x), 30.98, 31.57, 32.32 (2x), 33.74, 35.36, 36.05, 36.13, 37.28, 38.90, 43.52, 50.62 (2x), 51.31, 56.84, 61.29, 64.37, 82.32 ; LRMS for $C_{41}H_{78}NO_2$ [MH^+] : 616.6 m/z.

Biological evaluation of synthesized compounds

Cells culture

Human promyelocytic leukemia cells HL-60 (ATCC CCL-240) were maintained as logarithmically growing cultures in 90 % RPMI 1640 (Sigma, Saint Louis, USA). Cells were supplemented with 2 mM L-glutamine, 100 IU penicillin/mL, 100 μg streptomycin/mL and 10 % (v/v) of fetal bovine serum (FBS) and incubated in humidified atmosphere of 95 % air/ 5 % CO_2 at 37 °C.

Cytotoxicity assay

The dose-dependent effect of a compound on the viability of HL-60 cells was tested using the Cell Titer 96AQ kit (Promega, Madison, WI). In brief, the cell suspension containing 1 x 10^4 cells in 100 μL of medium was plated into a 96-well plate (Becton Dickinson and Company, Lincoln Park, NJ) and was incubated with an ethanolic solution of the compound at 1 μM or 10 μM. After 3 days, 20 μL of a solution containing 1.9 mg/mL 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) reagent and 300 μM phenazine ethosulfate was added to the cells. MTS is converted to water-soluble colored formazan by a dehydrogenase enzyme present in metabolically active cells. Following color development (~ 4 h), absorbance at 490 nm was taken with a microplate reader (Molecular Devices, Sunnyvale, CA). Data were reported as the % of cell proliferation compared to the control (100 % of proliferation).

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Supporting Information Available :

Table 1. ^{13}C NMR assignment for synthesized compounds*

	8 (epoxide)	**9**	**10[1]**	**11**	**12 (2D)**	**13**	**14**	**15[2]**	**16**
# of C									
1	38.27	32.57	34.04		32.55	32.75	32.58		
2	(50.78)[a]	67.91	66.14	66.40	64.34	64.45	65.02	67.07	65.57
3	(51.04)[a]	65.98	65.13	64.56	63.53	63.66	63.65	66.97	64.46
4	29.00	34.88			34.67	34.65	34.42		
5	(36.28)[b]	39.36	39.65	38.65	38.43	38.44	38.53	40.13	38.55
6	38.26	27.86	28.90	28.29	28.26	28.21	28.16	27.82	28.30
7	31.20	31.52	32.26	31.02	31.48	31.13	31.18	32.41	31.06
8	(35.67)[b]	35.07		35.57	35.36	(35.51)[a]	35.50	37.01	
9	(53.79)[a]	55.39	56.80	56.41	56.15	56.15	56.07	57.46	56.28
10	33.70	36.41		35.81	35.47	(35.77)[a]	35.84		
11	20.45	20.60	21.56	20.98	20.79	20.90	20.88	22.02	20.91
12	36.60	36.78		36.77	35.72	36.79	36.80		
13	42.78	43.01	43.87	43.09	43.00	43.08	43.10	43.53	43.06
14	(52.42)[b]	51.07	51.98	50.78	50.87	50.86	50.90	51.51	50.81
15	23.34	23.43	24.02	23.31	23.37	23.31	23.33	24.28	23.28
16	30.44	30.53	30.95	30.56	30.53	30.48	30.53	30.65	30.46
17	81.88	81.98	81.76	81.88	81.41	81.83	81.91	82.47	81.79
18	11.02	11.15	11.70	11.20	11.19	11.19	11.18	11.76	11.18
19	12.95	13.28	15.87	17.78	17.28	17.26	16.93	17.15	17.56
2'	---	51.47 (2x)	**47.00**	**53.73**	53.32 (2x)	**59.21**	48.88 (2x)	**52.23**	**59.52**
3'	---	23.43 (2x)	**37.85**	**38.99**	53.32 (2x)	**57.60**	67.48 (2x)	**48.88**	**55.88**
			36.74	**35.42**				**47.92**	**49.62**
1''	---	---	**36.24**	**30.47**	52.29	**53.35**	---	**46.05**	**48.66**
2''	---	---	**35.68**	**29.25**	11.87	**45.69**	---	**44.21**	**46.43**
			35.43	**26.56**				**38.12**	**36.78**
			35.18	**31.74**				**36.87**	**35.65**
				34.16				**36.58**	**35.54**
								29.40	**35.04**
									34.18
									27.38

* : $CDCl_3$ as solvent except other indication (1 : acetone-D_6 ; 2 : CD_3OD.) Bold values are non-assigned carbons; Values between brackets can be changed according to the subscript; (2D) : 2D spectral data available.

Table 2. ^{13}C NMR assignment for synthesized compounds*

	17	**18**	**19**	**20**[1]	**21**	**22**
# of C						
1	33.88	32.74	32.66	36.40	33.28	(34.17)[a]
2	65.54	64.35	64.72	66.76	64.88	(67.38)[b]
3	64.16	63.97	63.73	66.57	64.09	(62.22)[b]
4	35.82	34.88	34.62	38.11	35.13	(35.22)[a]
5	38.90	38.55	38.44	37.36	38.92	(41.57)[a]
6	28.60	28.22	28.20	29.00	28.67	27.89
7	31.40	31.11	31.14	32.79	31.60	31.15
8	35.98	35.54	35.49	36.53	35.97	(35.76)[a]
9	56.70	56.23	56.11	56.84	56.64	55.41
10	36.31	35.84	36.00	34.36	36.20	(36.69)[a]
11	21.40	20.93	20.88	21.76	21.31	20.79
12	37.19	36.78	36.76	33.90	37.28	(39.27)[a]
13	43.53	43.07	43.10	44.15	43.53	43.01
14	51.24	50.84	50.85	51.52	51.35	50.84
15	23.72	23.31	23.32	24.30	23.75	23.28
16	30.94	30.50	30.48	30.62	30.96	30.38
17	82.21	81.85	81.87	82.50	82.23	81.76
18	11.59	11.18	11.20	11.74	11.59	11.50
19	18.11	17.41	17.26	14.28	17.65	14.83
2'	52.75 45.37	**51.19**	(45.78)[a] (2x)	48.21 47.10	53.84 (2x)	---
3'	32.30 32.13	**45.62**	(48.05)[a] (2x)	52.42 51.52	53.86 (2x)	**138.83**
4'	38.15	**29.64**	---	---		**128.76**
						105.23
1''	43.16 = 5'	**125.69**	---	(139.26)[a]	63.12	---
2''	140.50 = 6'	**126.23**	160.00	(113.62)[a] (2x)	132.36	---
3''	(129.41)[a] (2x) = 7'	**126.52**	107.09	(126.73)[a] (2x)	109.91	---
4''	(128.67)[a] (2x) = 8'	**128.73**	137.46	(156.59)[a]	148.03	---
5''	126.39 = 9'	**134.30 (2x)**	113.34	---	147.01	---
6''	---	---	147.93	---	108.24	---
7''	---	---	---	---	122.63	---
OCH_2O	---	---	---	---	101.26	---

* : $CDCl_3$ as solvent except other indication (1 : CD_3OD.); Bold values are non-assigned carbons; Values between brackets can be changed according to the subscript.

Table 3. ^{13}C NMR assignment for synthesized compounds*

	23	**24**	**25**	**26**	**27**	**28**	**29 (2D)**	**30**
# of C								
1	39.91	39.33	39.93	39.54	42.01	39.71	38.62	40.12
2	60.07	56.64	60.22	60.43	57.25	60.23	60.27	60.31
3	69.39	66.54	69.57	68.39	65.65	69.03	66.32	69.09
4	33.63	36.35	33.41	34.25	35.91	33.86	34.91	33.14
5	39.66	39.73	39.69	39.63	39.39	39.66	39.43	39.63
6	28.45	28.31	28.46	28.43	28.42	28.44	28.36	28.43
7	31.74	31.07	31.79	31.60	31.25	31.69	31.18	(31.72)[a]
8	35.60	35.83	35.58	35.66	35.83	35.63	35.84	35.52
9	56.06	51.05	56.00	56.16	56.68	56.09	56.43	55.96
10	36.51	36.35	36.51	36.53	36.86	36.50	36.40	36.40
11	21.07	21.50	(21.04)[a]	21.18	21.28	21.16	21.46	20.91
12	37.18	37.01	37.19	37.15	37.07	37.17	37.04	37.13
13	43.46	43.48	43.46	43.47	43.50	43.47	43.50	43.35
14	51.34	48.37	51.36	51.28	51.13	51.32	51.10	51.31
15	23.75	23.68	23.75	23.73	23.71	22.92	23.70	23.65
16	30.90	30.88	30.91	30.90	30.82	29.77	30.89	30.41
17	82.31	82.20	82.32	82.30	82.24	82.39	82.21	81.97
18	11.61	11.58	11.61	11.60	11.58	11.60	11.59	11.51
19	15.81	17.54	15.64	16.25	17.29	15.96	17.49	15.56
1'	50.22	57.20	48.34	55.46	59.61	48.21	44.67	60.74
2'	23.36	18.21 20.68	32.63	28.07	27.29 (3x)	(30.92)[a]	36.06	(31.78)[a]
3'	12.14	---	(20.88)[a]	20.78 20.95	---	(29.51)[a]	26.38	28.01 (3x)
4'	---	---	14.43	---	---	23.75	22.42 22.80	
5'	---	---	---	---	---	14.44	---	

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; (2D) : 2D spectral data available.

Table 4. ^{13}C NMR assignment for synthesized compounds*

	31	32	33	34	35	36	37	38	39
# of C									
1	41.72	39.24	39.40	(38.64)[a]	(39.26)[a]	39.21	35.99	39.29	39.26
2	56.30	60.14	60.19	59.91	59.94	59.89	59.62	59.89	59.91
3	65.02	67.84	68.17	66.51	68.22	66.80	66.96	67.72	67.35
4	(35.35)[a]	34.87	34.61	(35.75)[b]	34.20	(32.07)[a]	34.82	34.61	(32.18)[a]
5	38.92	39.52	39.57	(39.17)[a]	(39.36)[a]	38.71	38.60	39.04	38.91
6	28.00	(28.39)[a]	28.40	28.29	(28.20)[b]	28.15	(27.92)[a]	28.19	(28.17)[b]
7	31.84	31.83	31.52	31.88	(31.34)[b]	(31.04)[a]	(31.87)[b]	31.24	(31.16)[a]
8	(35.45)[a]	35.71	35.69	35.60	35.46	(35.58)[b]	(35.29)[c]	35.50	35.01
9	56.30	56.25	56.21	56.22	55.96	56.16	55.83	56.02	56.11
10	(36.46)[b]	36.44	36.46	(36.17)[b]	36.25	36.19	(35.94)[c]	36.22	36.33
11	20.86	21.27	21.23	21.35	20.98	21.17	20.86	21.04	21.10
12	(36.60)[b]	37.10	37.13	(36.83)[b]	36.95	36.85	(36.62)[c]	36.92	36.89
13	43.03	43.48	43.47	43.27	43.26	43.27	43.03	43.26	43.26
14	50.63	51.21	51.25	50.90	51.06	50.93	50.70	51.01	51.00
15	23.25	23.72	23.73	23.61	23.52	23.49	23.25	23.51	23.50
16	30.31	30.88	30.90	(30.96)[b]	(30.66)[b]	30.65	(30.88)[b]	30.63	(30.66)[a]
17	81.76	82.25	82.28	81.99	82.05	81.99	81.76	82.01	82.04
18	11.14	11.60	11.60	11.38	11.40	11.38	11.15	11.40	11.40
19	16.91	16.64	16.47	17.11	16.08	16.92	16.43	16.35	16.63
1'	30.78	47.27	47.54	46.12	47.54	46.37	46.46	47.14	46.80
2'	23.28 24.06	31.44	32.09	(30.66)[b]	32.06	(35.44)[b]	36.62	32.12	(35.37)[a]
3'	62.17	(26.97)[a]	(29.40)[a]	(29.20)[b]	(29.64)[b]	(29.70)[c]	(30.38)[b]	29.86	(29.92)[c]
4'	7.92	(28.24)[a]	(28.72)[a]	(29.16)[b]	(29.55)[b]	(29.58)[c]	(29.55)[a]	29.85	(29.90)[c]
5'	---	(22.89)[a]	(27.36)[a]	(26.71)[b]	(29.44)[b]	(29.49)[c]	(29.40)[a]	29.78	(29.87)[c]
6'	---	14.41	23.00	(26.60)[b]	(28.78)[b]	(29.33)[c]	(29.31)[a]	29.67	(29.79)[c]
7'	---	---	14.50	22.80	(27.22)[b]	(26.92)[c]	(29.19)[a]	29.56	(29.64)[c]
8'	---	---	---	14.27	22.86	(26.85)[c]	(27.25)[a]	29.49	(29.57)[c]
9'	---	---	---	---	14.32	22.87	(26.73)[a]	28.16	(29.43)[c]
10'	---	---	---	---	---	14.31	22.66	27.10	(27.57)[b]
11'	---	---	---	---	---	---	14.11	22.89	(26.98)[b]
12'	---	---	---	---	---	---	---	14.33	22.90
16'	---	---	---	---	---	---	---	---	14.34

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript.

Table 5. ^{13}C NMR assignment for synthesized compounds*

	40	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
# of C									
1	(39.98)[a]	40.08	39.31	37.92	37.97	36.83	36.92	36.72	40.17
2	58.10	56.60	(57.44)[a]	59.99	59.99	59.94	59.96	59.69	(61.31)[a]
3	68.47	67.95	67.44	70.09	71.99	68.73	67.97	68.76	69.49
4	34.00	35.02	34.70	34.83	34.71	33.64	33.26	32.89	(33.04)[b]
5	(39.11)[a]	39.50	39.40	39.07	38.99	(39.41)[a]	(39.34)[a]	(39.59)[a]	39.59
6	28.01	28.43	(28.06)[b]	28.13	28.06	28.19	28.15	27.98	28.29
7	31.78	31.41	(31.42)[c]	31.09	31.60	31.43	31.32	31.31	31.64
8	35.28	35.75	35.69	35.56	35.50	35.40	34.34	35.10	35.39
9	55.85	55.22	56.25	56.04	55.95	55.87	55.98	55.47	55.81
10	36.10	36.50	36.41	36.02	35.97	35.77	35.47	35.64	36.40
11	20.81	21.31	21.24	21.08	21.04	20.98	20.99	20.54	28.04
12	36.72	37.10	37.07	36.84	36.85	36.25	36.22	36.00	37.02
13	43.07	43.49	43.42	43.28	43.27	43.26	43.27	43.01	43.27
14	50.84	51.19	51.17	50.95	50.93	51.14	51.08	50.89	51.24
15	23.31	23.72	23.66	23.48	23.51	23.55	23.52	23.31	23.56
16	30.50	30.90	30.59	30.61	30.98	30.75	30.68	30.46	30.73
17	81.88	82.27	82.02	81.98	81.94	82.10	82.07	81.86	82.18
18	11.18	11.61	11.55	11.41	11.42	11.39	11.41	11.18	11.40
19	16.19	16.93	16.67	(16.66)[a]	(16.58)[a]	15.67	16.15	15.19	15.45
1'	57.37	56.35	(56.87)[a]	46.66	46.60	49.19	46.72	(46.46)[b]	(60.53)[a]
2'	(31.12)[b] (32.68)[b]	32.43 (2x)	(34.15)[c] (30.59)[c]	25.63	25.69	(39.35)[a]	(29.92)[a]	(39.20)[a]	(33.58)[b] (2x)
3'	(23.79)[b] (2x)	(25.77)[a] (25.29)[a]	(28.11)[b] 32.18	(67.53)[b]	(70.13)[b]	(138.77)[b]	(39.23)[a]	(48.94)[b]	(41.06)[c] (2x)
4'	---	(25.07)[a]	24.18 24.50	---	---	(128.96)[b]	(141.16)[b]	(126.24)[c]	(37.46)[c] (2x)
5'	---	---	---	(65.57)[b]	(65.57)[b]	(128.83)[b]	(128.74)[b]	(127.76)[c]	(28.66)[c]
6'	---	---	---	(15.20)[a]	(31.09)[c]	(126.73)[b]	(128.56)[b]	(128.48)[c]	---
7'	---	---	---	---	(19.42)[c]	---	(126.36)[b]	(144.53)[c]	---
8'	---	---	---	---	(14.07)[a]	---	---	---	---

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript.

Table 6. ^{13}C NMR assignment for synthesized compounds*

	49	**50**	**1**	**52**	**53**	**54**
# of C						
1	(35.36)[a]	32.55	32.70	33.72	33.32	33.36
2	(64.19)[b]	64.34	64.37	65.43	64.68	65.04
3	(64.14)[b]	63.60	63.60	64.32	63.97	64.29
4	(36.61)[a]	34.40	34.64	35.38	35.12	35.18
5	39.21	38.37	38.43	38.95	38.55	38.96
6	28.33	27.95	28.16	28.51	28.09	28.60
7	31.01	31.53	31.09	31.46	31.03	31.56
8	35.88	34.89	35.41	35.92	35.48	35.94
9	56.79	55.91	56.10	56.53	56.16	56.57
10	(36.75)[a]	35.77	35.72	36.31	35.91	36.29
11	21.52	20.43	20.78	21.38	20.95	21.35
12	(36.95)[a]	30.38	36.88	37.15	36.79	37.21
13	43.47	47.82	42.94	43.51	43.07	43.52
14	50.95	51.20	50.84	51.24	50.78	51.28
15	23.68	21.64	23.31	23.73	23.29	23.75
16	30.87	35.77	30.56	30.91	30.48	30.94
17	82.13	221.18	81.26	82.23	81.82	82.30
18	10.67	13.76	11.18	11.62	11.18	11.63
19	18.34	16.89	17.15	17.79	17.43	17.70
2'	44.71 48.51	55.48 (2x)	55.22 (2x)	48.96 48.34	(49.23)[a] (2x)	48.62 (2x)
3'	11.62 11.80	55.48 (2x)	55.22 (2x)	46.62 41.75	(48.04)[a] (2x)	51.06 (2x)
1''	---	45.86	45.58	169.30	151.35	148.24
2''	---	---	---	21.71	116.44 (2x)	116.08 115.86
3''	---	---	---	---	129.16 (2x)	118.51 118.44
4''	---	---	---	---	120.29	*158.90* *156.52*

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; Italic values show a carbon-fluor coupling.

Table 7. ^{13}C NMR assignment for synthesized compounds*

	55 (2D)	**56 (2D)**	**57**	**58**	**59 (2D)**
# of C					
1	(34.94)[a]	33.05	32.59	33.78	33.49
2	66.12	65.91	[64.73] [64.96]	64.87 65.04	64.99
3	63.78	64.27	[63.47] [63.38]	63.71	64.34
4	(36.16)[a]	34.65	35.04	36.38	35.30
5	38.59	38.98	38.30	39.08	38.88
6	27.86	28.46	28.28	(28.55)[a]	28.62
7	30.59	31.21	31.09	(31.30)[b]	31.52
8	35.41	35.95	35.54	(36.00)[c]	35.97
9	56.13	56.68	56.31	57.10	56.63
10	36.26	36.29	35.86	36.58	36.20
11	21.05	21.48	20.92	21.34 21.50	21.35
12	36.53	37.10	36.80	37.15	37.21
13	43.03	43.51	43.09	43.53	43.53
14	50.53	51.12	50.84	51.14 51.19	51.31
15	23.22	23.70	23.29	23.71	23.73
16	30.36	30.92	30.50	(30.97)[d]	30.92
17	81.63	82.16	81.85	82.21	82.24
18	11.21	11.60	11.18	11.61	11.59
19	17.98	18.32	17.71	18.06 18.35	17.77
2'	? ?	45.81 (2x)	[52.73] [52.64]	(35.94)[c]	50.64 (2x)
3'	(23.56)[a] (2x)	36.50 (2x)	44.91	**(28.65)[a]**	27.47 27.88
4'	22.32	30.34	**[33.32] [33.24]**	**(30.85)[d]**	64.34
			[31.87] [31.66]	**(31.40)[b]**	
1''	---	---	**[26.17] [25.83]**	45.61 = 6'	---
2''	---	---	---	14.07	50.64 (2x) = 6'
3''	---	---	[19.68] [19.45]	---	24.61 (2x) = 7'
4''	---	21.55	---	---	23.56 = 8'

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; Values between square brackets show signals splitted into two; Bold values are non-assigned carbons; (2D) : 2D spectral data available.

Table 8. ^{13}C NMR assignment for synthesized compounds*

	60	**61 (2D)**	**70**	**62**	**71**	**63**	**72**
# of C							
1	32.58	33.26	32.64	33.43	32.65	32.87	33.20
2	(63.03)[a]	65.27	64.34	65.13	64.34	64.81	64.41
3	(63.35)[a]	64.20	63.56	63.88	63.57	63.75	63.81
4	34.67	35.00	34.67	35.08	34.67	34.62	35.03
5	38.40	38.90	38.43	38.50	38.43	38.43	38.49
6	28.23	28.58	28.24	28.04	28.23	28.11	28.13
7	31.15	31.55	31.15	30.96	31.15	31.08	31.05
8	35.50	35.95	35.47	35.46	35.47	35.46	35.48
9	56.17	56.52	56.13	56.06	56.13	56.04	56.18
10	35.72	36.22	35.73	35.91	35.74	35.79	35.82
11	20.86	21.32	20.80	20.95	20.79	20.89	20.92
12	36.87	37.20	36.90	36.68	36.89	36.72	36.74
13	43.04	43.51	43.00	43.06	43.00	43.06	43.07
14	50.87	51.30	50.86	50.75	50.84	50.81	50.80
15	23.31	23.73	23.36	23.28	23.36	23.28	23.28
16	30.52	30.92	30.63	30.46	30.62	30.45	30.46
17	81.60	82.26	81.36	81.79	81.37	81.79	81.79
18	11.18	11.60	11.21	11.18	11.21	11.18	11.18
19	17.31	17.58	17.25	17.41	17.25	17.18	17.41
2'	(49.38)[b] (46.14)[b]	49.07 48.56	53.60 (x)	48.58 47.93	53.60 (2x)	48.81 48.15	53.33 (2x)
3'	(46.67)[b] (2x)	46.33 42.44	53.60 (2x)	45.16 41.03	53.60 (2x)	45.73 41.91	53.33 (2x)
1''	---	172.64	60.57	171.43	58.38	175.30	66.44
2''	---	26.85	19.71	35.08	28.60	29.94	25.20
3''	---	9.91	11.90	18.66	20.70	19.38 (2x)	20.92 (2x)
4''	---	---	---	13.98	13.98	---	---
5''	---	---	---	---	---	---	---

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; (2D) : 2D spectral data available.

Table 9. ^{13}C NMR assignment for synthesized compounds*

	64	**73**	**65**	**74**	**66**	**75**	**67**
# of C							
1	33.46	32.84	32.80	32.74	(33.30)[a]	32.65	33.92
2	65.14	64.42	64.81	64.37	64.76	64.39	65.63
3	63.87	63.71	63.75	63.64	63.73	63.59	63.96
4	35.11	34.70	34.59	34.69	34.57	34.69	34.69
5	38.50	38.46	38.46	38.46	38.43	38.46	38.52
6	28.04	28.18	28.17	28.22	28.13	28.27	27.98
7	30.96	31.19	31.12	31.14	31.09	31.18	30.85
8	35.45	35.47	35.51	35.47	35.47	35.51	35.43
9	56.04	56.10	56.10	56.12	56.07	56.16	56.02
10	35.91	35.77	35.81	35.76	35.76	35.76	36.03
11	20.94	20.83	20.91	20.82	20.88	20.84	21.00
12	36.68	36.81	36.77	36.84	36.73	36.84	36.63
13	43.06	43.03	43.10	43.00	43.06	43.07	43.06
14	50.75	50.84	50.87	50.85	50.83	50.87	50.68
15	23.28	23.32	23.32	23.35	23.28	23.35	23.26
16	30.44	30.52	30.52	30.61	30.46	30.56	30.45
17	81.76	81.59	81.88	81.42	81.82	81.75	81.75
18	11.18	11.19	11.20	11.21	11.17	11.20	11.18
19	17.40	17.21	17.21	17.22	17.18	17.31	17.56
2'	48.75 47.93	53.34 (2x)	48.73 48.20	53.51 (2x)	48.63 48.09	53.79 (2x)	48.85 (2x)
3'	45.14 40.99	53.34 (2x)	46.31 42.03	53.51 (2x)	46.07 41.91	53.79 (2x)	44.59 42.64
1''	171.62	58.46	170.97	56.84	171.68	58.81	174.52
2''	32.90	29.56	41.95	(35.15)[a]	**(32.80)[a]**	**31.87**	54.51
3''	(27.33)[a]	(25.84)[a]	25.80	(26.64)[a]	**(31.79)[a]**	**29.55 (2x)**	40.23 (2x)
4''	(22.54)[a]	(22.47)[a]	22.77 (2x)	22.63 (2x)	**(29.47)[a]**	**29.28**	29.33 (2x)
5''	13.86	13.98	---		**(29.35)[a]**	**27.59**	18.59
6''	---	---	---	---	**(29.15)[a]**	**26.81**	---
7''	---	---	---	---	**(25.34)[a]**	**22.68**	---
8''	---	---	---	---	**(22.63)[a]**	**14.13**	---
9''	---	---	---	---	**(14.08)[a]**	---	---

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; Bold values are non-assigned carbons.

Table 10. ^{13}C NMR assignment for synthesized compounds*

	76	**68**	**77**	**69**	**69 (suite)**	**78**	**79**
# of C					C-SP_2		
1	32.99	33.15	33.37		120.55	33.72	33.74
2	64.38	65.01	64.00	64.97	128.01	64.37	64.37
3	63.70	63.78	63.03	63.87	128.88	61.30	61.29
4	34.81	34.81	35.21		129.42	35.33	35.36
5	38.45	38.45	38.71	38.49	129.72	38.90	38.90
6	28.18	28.08	28.39	28.05	129.82	28.88	28.87
7	31.10	31.02	31.31	31.00	129.94	31.58	31.57
8	35.49	35.47	35.73	35.45	134.68	36.05	36.05
9	56.17	56.06	56.41	56.06	137.30	56.84	56.84
10	35.78	35.84	36.06	35.85	137.62	36.13	36.13
11	20.90	20.92	21.16	20.92		21.34	21.35
12	36.76	36.72	37.00	36.69	C-SP_3 + C 1 et 4	37.28	37.28
13	43.08	43.06	43.31	43.05	12.82	43.53	43.52
14	50.82	50.79	51.05	50.77	14.48	51.32	51.31
15	23.30	23.28	23.54	23.28	19.17	23.76	23.75
16	30.48	30.45	30.72	30.41	20.29	31.00	30.98
17	81.80	81.79	82.01	81.76	21.73	82.34	82.32
18	11.19	11.18	11.43	11.18	28.91	11.57	11.58
19	17.36	17.28	17.65	17.31	33.04	18.01	18.02
					33.39		
2'	53.77 (2x)	48.66 48.07	53.23 (2x)	48.63 48.03	34.20	---	---
3'	53.77 (2x)	42.18 (2x)	53.23 (2x)	46.04 41.07	34.98	---	---
					39.51		
1''	65.38	170.79	64.66	166.87	---	**50.63 (2x)**	**50.62 (2x)**
2''	**31.88 (3x)**	**135.37**	**137.59**	---	---	**32.29 (2x)**	**32.32 (2x)**
3''	**26.64**	**129.85**	**129.54 (2x)**	---	---	**30.01 (4x)**	**30.05 (2x)**
4''	**26.04 (2x)**	**128.51 (2x)**	**128.54 (2x)**	---	---	**29.77 (2x)**	**30.00 (4x)**
5''	---	**127.08 (2x)**	**127.54**	---	---	**29.70 (2x)**	**29.83 (2x)**
6''	---	---	---	---	---	**27.83 (2x)**	**29.74 (2x)**
7''	---	---	---	---	---	**23.06 (2x)**	**29.68 (2x)**
8''	---	---	---	---	---	**14.48 (2x)**	**27.83 (2x)**
9''	---	---	---	---	---	---	**23.08 (2x)**
							14.50 (2x)

* : $CDCl_3$ as solvent; Values between brackets can be changed according to the subscript; Bold values are non-assigned carbons.

Chapitre 4

L'utilisation de dérivés du 2β-amino-5α-androstane-3α,17β-diol comme agents antinéoplasiques: Évaluation de la sélectivité, de l'effet sur le cycle cellulaire et de l'induction de la différenciation des cellules leucémiques HL-60

4.1 Avant-propos

Ma contribution à ce projet a été tout d'abord de mettre au point les tests biologiques nécessaires, pour l'évaluation de la sélectivité des différents composés, l'étude de leur effet sur le cycle cellulaire et l'étude de différenciation cellulaire. Par la suite, j'ai effectué l'évaluation biologique complète des six produits sélectionnés. J'ai finalement rédigé ce manuscrit en collaboration avec mon directeur de recherche Donald Poirier et il sera bientôt soumis pour publication dans une revue scientifique.

4.2 Résumé

La leucémie est le cancer le plus fréquent chez les enfants. Plusieurs agents thérapeutiques sont disponibles pour traiter cette maladie, mais ceux-ci sont très toxiques pour les patients causant ainsi de nombreux effets secondaires. Voilà donc l'importance de découvrir de nouveaux agents anticancéreux efficaces ayant une bonne sélectivité pour les cellules cancéreuses et agissant selon un mécanisme d'action différent des produits actuellement utilisés. Lors de travaux antérieurs, nous avons identifié quelques aminostéroïdes, des dérivés du 2β-amino-5α-androstane-3α,17β-diol, possédant une activité antiprolifération contre les cellules leucémiques humaines HL-60. Dans la présente étude, nous avons évalué l'effet des 6 meilleurs représentants de cette famille sur la croissance des cellules HL-60. Nos résultats démontrent que ces produits inhibent la prolifération cellulaire avec des IC_{50} variant de 0.58 à 6.4 μM. De plus, 4 de ces 6 composés sont sélectifs puisqu'ils n'exercent aucune activité toxique contre les cellules normales WI-38. Cette étude nous a également permis de constater que l'activité cytotoxique de ces aminostéroïdes est reliée à l'arrêt du cycle cellulaire causant ainsi l'accumulation des cellules HL-60 dans la phase G_0/G_1 du cycle et, de ce fait, l'augmentation du niveau d'apoptose de ces cellules. Nous avons aussi évalué l'effet de ces produits sur la différenciation cellulaire en mesurant l'expression des antigènes de surface CD11b et CD14. Trois de ces aminostéroïdes induisaient la différenciation des cellules HL-60.

Journal (to be submitted)

2β-Amino derivatives of 5α-androstane-3α,17β-diol as antineoplasic agents: Evaluation of selectivity, effect on the cell cycle and induction of differentiation in human leukemia HL-60 cells

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Abstract

Leukemia is the most commonly occurring cancer in children. Many therapeutic agents are available but they are highly toxic and induce several side effects. Thus there is a strong interest in the discovery of new anticancer agents which not only exhibit potent cytotoxic activity with high selectivity for cancer cells but also function by biochemical mechanisms which are distinct from those of the known anticancer drugs. In previous works, we have identified compounds with antiproliferative activity on human myeloid leukemia HL-60 cells and all of them were 2β-amino derivatives of 5α-androstane-3α,17β-diol. In the present study, we examined the effects of treatment with the 6 best aminosteroids on the cell growth of the HL-60 cell line. Proliferation of leukemia cells was inhibited with IC_{50} values of 0.58 to 6.4 μM. Moreover, 4 of these 6 compounds were selective for cancer cells since they are non-toxic toward normal human cell line WI-38. Interestingly, we showed that growth inhibition of all these compounds was associated with a cell cycle arrest that caused HL-60 cells to accumulate in G_0/G_1 phase and increased the amount of apoptotic cells. We also studied their effect on cellular differentiation by measuring expression of CD11b and CD14 surface antigens. Three compounds were found to induce the maturation of HL-60 cells.

Introduction

Acute myeloid leukemia (AML) is the most common leukemia in adults with 11,960 new cases in the United States in 2005.[1] A diagnosis of AML in adults is ominous because the median survival time of untreated patients is 3 months and survival at 1 year is less than 5 %.[2] It is also the type of leukemia with the lowest 5-year relative survival rate in children.[1] Chemotherapy induces a complete remission in 70 to 80 percent of younger patients with AML, but many of them have a relapse and die of their disease.[3] In general, 60-80 % of treated AML patients achieve remission; however, the 5-year survival rate is only 15 %.[1] Like several diseases, acute leukemia is treated with multiple aggressive therapies. Although these therapeutic agents have proven to be powerful, they often cause side effects. Thus, it is essential that the cytotoxic effect of a drug be selective for cancerous cells. Unfortunately, it is rare to find compounds selective enough to merit serious consideration as chemotherapeutic agents.[4] Hence the importance of having at our disposal several agents with different and complementary modes of action without or with fewer side effects.

At this time, most of the anticancer agents act through inhibition of cell proliferation by inducing a block in one phase of the cell cycle, and then activation of cell elimination by inducing active cell death.[5] The cell cycle is thus a critical regulator of the processes of cell proliferation and growth as well as of cell division following DNA damage. This has led to the development of new anticancer therapeutics, namely drugs that target the motor of the cell cycle, as example, the cyclin-dependent kinases (CDKs). These inhibitors were first applied to the treatment of malignancy as single agents, which are now commonly used in combination with traditional cytotoxic drugs.[6] Another strategy to treat non-lymphocytic leukemia is to use differentiation agents,[7] such as all-*trans*-retinoic acid (ATRA)[8] and 1,25-dihydroxyvitamin D3 (VD3).[9] Indeed, AML, is characterized by the arrest of cell differentiation, which leads to accumulation of immature cells. Although some interesting results were obtained with these compounds, the use of ATRA is hampered by its toxicity,[10,11] and that of VD3, by its hypercalcemic effects. Moreover, in the series of clinical studies in which ATRA was used alone, rarely did any patient maintain a remission

for more than 1 year.[12,13] Even though induction of differentiation has been shown to be possible with various inducers in vitro, it is generally considered that no effective agent has yet been provided for clinical use.[14-16]

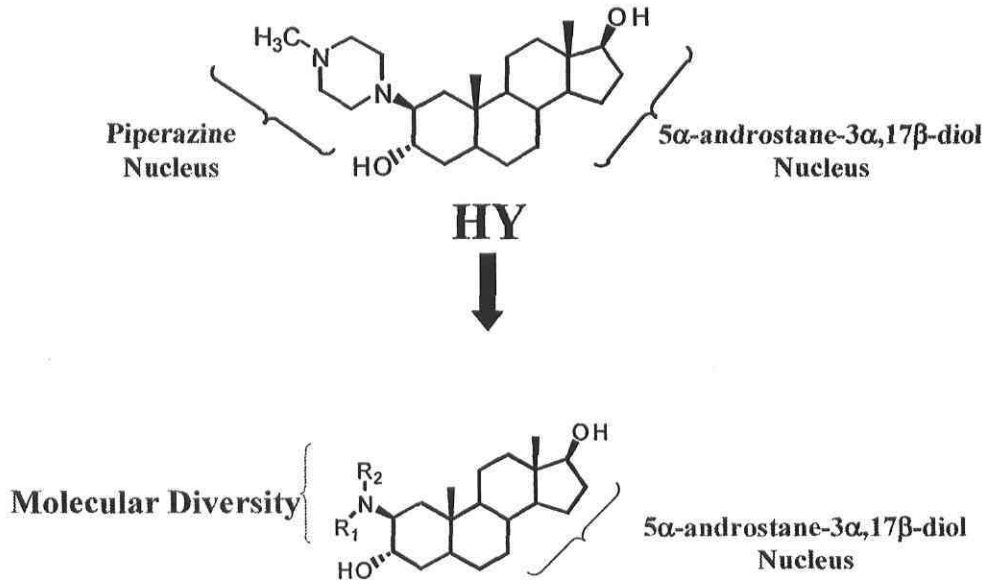


Figure 1. The lead compound HY and general structure of aminosteroid analogues.

The aminosteroid HY (Figure 1) is a compound recently reported to inhibit the cell proliferation as well as to induce the cell differentiation of human promyelocytic leukemia cell line, HL-60.[17] The dual action of HY and the fact that it is a steroid compound attracted our interest for this new family of potential anticancer agents. In previous studies, we identified novel 2-aminosteroids that better inhibited the proliferation of HL-60 cells than lead compound HY,[18,19] but we did not tested their effect on cell differentiation. From these results, we however selected six potent candidates (Figure 2), which can be roughly divided in two categories: Compounds **1** - **3**, having the amino acid proline as structural element, were prepared by solid-phase chemistry whereas compounds **4** - **6**, bearing a hydrophobic side chain added on a secondary or a tertiary amine, were prepared by classical chemistry.

In this paper, we established antileukemic activities (IC_{50} values) of aminosteroids **1 - 6** on HL-60 cells. Furthermore, with the aim of selecting compounds toxic only to cancerous cells, we evaluated the effects of each one on normal human lung WI-38 cells.[20] We also investigated their potential in the modulation of the cell cycle progression and induction of apoptosis. Finally, we assessed whether the compounds could induce differentiation of leukemic cells. The results allowed a better characterization of a new class of aminosteroid derivatives for potential use in leukemia therapy.

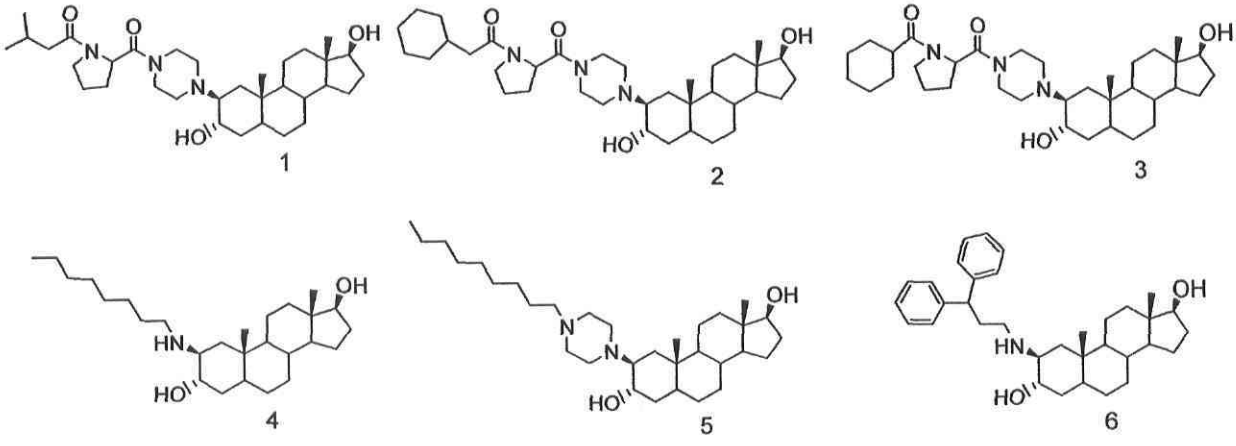


Figure 2. The chemical structures of selected aminosteroids **1 - 6** that efficiently inhibit HL-60 cells growth.

Experimental

Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90 % RPMI-1640 (Sigma, Saint Louis, USA) containing L-glutamine (2 nM), and antibiotics (100 IU penicillin/ml, 100 µg streptomycin/ml) and supplemented with 10 % (v/v) foetal bovine serum (FBS), in a 5 % CO_2 humidified atmosphere at 37°C. Cells were maintained twice a week by diluting them in RPMI-1640 medium containing 10 % FBS. WI-38 diploid fibroblasts derived from embryonic human lung (ATCC, Rockville, MD, USA) were cultured in Eagle's minimal essential medium (MEM) supplemented with 10 % (v/v) FBS, 100 IU penicillin/ml, 100 µg streptomycin/ml, 1 mM sodium pyruvate and 0.1 mM nonessential amino acids. Culture media were changed every 3-4 days and cells were split once a week.

Cell proliferation assay

The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA). MTS is converted to water-soluble coloured formazan by a dehydrogenase enzyme present in metabolically active cells, which allows us to measure the number of viable cells. In brief, triplicate cultures of 1×10^4 cells in a total of 100 µl medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, 5 % CO_2. Aminosteroids synthesized in our laboratory[18,19] were dissolved in ethanol to prepare the 1×10^{-2} M stock solution. These compounds and doxorubicin (DOX) (Novapharm, Toronto, Canada) were diluted at multiple concentrations with culture media, added to each well, as indicated, and incubated for 3 days. Following each treatment, 20 µl MTS were added to each well and the mix was incubated for 4 h. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Cell cycle analysis

Cell cycle analysis was performed on HL-60 cells treated with one of the aminosteroids at 5 or 10 μM. The cells were harvested at the indicated time, suspended in 300 μl of cold PBS (phosphate buffer saline) and fixed in 95 % ethanol at -20°C for at least 30 minutes. Cells were collected by centrifugation and the pellets were washed and resuspended in PBS with RNase A (Roche, Montréal, Canada) (1 mg/ml) for 30 additional minutes at room temperature. After centrifugation, cellular DNA was stained with propidium iodide (PI) (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and incubated for 20 minutes on ice. Analysis of DNA content was done using a Coulter EPICS XL (Beckman-Coulter, Miami, FL, USA). The effect on the cell cycle was determined by the percentages of cells in each phase of the cycle, as displayed by histograms generated by the MULTICYCLEAV software (Phoenix Flow System, San Diego, CA, USA). The effect on apoptosis was determined by the degree of increase in the proportion of sub G_1 hypo-diploid cells.

CD11b and CD14 cell surface antigens

For analysis of cellular differentiation, expression of cell surface antigens was measured using immunofluorescence staining. Cells were seeded in RPMI-1640 medium in 25-cm^2 tissue culture flasks (VWR, Montréal, Canada), containing 10 μM of one of our aminosteroids, all-trans retinoic acid (ATRA) or 1alpha,25-dihydroxyvitamin D3 (VD3) (both from Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) and incubated for different time periods. After treatment, cells were harvested, counted and washed with cold PBS. They were stained using simultaneously mouse anti-human CD11b antibody conjugated with R-phycoerythrin (RPE) and mouse anti-human CD14 antibody conjugated with fluorescein isothiocyanate (FITC) (both from Becton Dickinson Biosciences, San Jose, CA, USA). Controls were stained with mouse RPE-conjugated IgG_1 and FITC-conjugated IgG_2. Following incubation for 45 minutes, cells were washed twice with cold PBS, resuspended in 500 μl of PBS and analyzed on a Coulter EPICS XL (Beckman-Coulter, Miami, FL,

USA) giving the percentage of cells marked with CD11b only, CD14 only, and CD11b/CD14 (double-marked).

Statistical analysis

Data are presented as means ± S.E.M. Statistical significance was determined according to the multiple-range test of Duncan-Kramer.[21] *P* values which were less than 0.05 were considered as statistically significant.

Results

Cytotoxic activity and selectivity of aminosteroids 1 – 6

We examined the effect of six recently synthesized aminosteroids on the growth of human leukemia and normal lung fibroblast cells. To do so, we used human promyelocytic leukemia cells HL-60, a cell line often utilized as leukemic cells model for research. The concentrations that inhibited 50 % of HL-60 cell growth (IC_{50}) are summarized in Figure 3. All these compounds markedly inhibit leukemia cell proliferation after a 3-day treatment. However, compounds **1** - **3** (IC_{50} = 0.58 – 1.78 μM) are more efficient than **4** - **6** (IC_{50} = 3.2 – 6.4 μM). Compound **3** is thus approximately 11.0, 7.2 and 5.3-fold more potent than **4**, **5** and **6** respectively, in cell growth inhibition.

The cytotoxicity of compounds **1** - **6** was next examined in the WI-38 cell line, a normal cell line often used to determine the selectivity of action of cytotoxic agents.[20,22-24] As a positive control we used doxorubicin, a well-known and potent chemotherapeutic agent (Figure 4). After a 3-day treatment, aminosteroids **1** - **4** showed no toxicity toward the normal cells at the three concentrations tested (0.1, 1 and 10 μM). Although **5** and **6** did not induce significant changes in cell proliferation at 0.1 and 1 μM, they killed all the normal cells at 10 μM ($P<0.01$). By comparison, at concentrations of 1 and 10 μM, doxorubicin induced the death of 30 and 85 % of WI-38 cells, respectively ($P<0.01$). The

result reported above clearly showed that aminosteroids **1** - **4** have a selective cytotoxic action against the cancerous cancer cell line contrary to compounds **5**, **6** and doxorubicin, which inhibited the proliferation of both cancerous and normal cell lines.

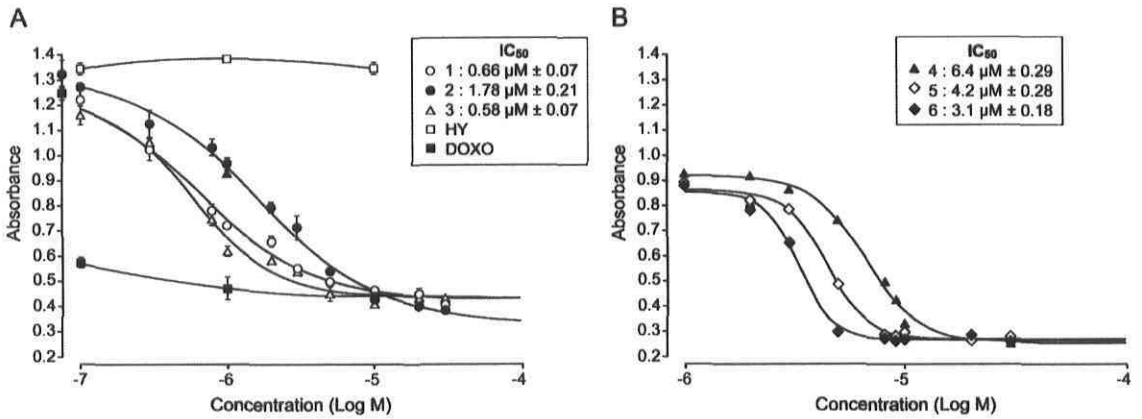


Figure 3. Inhibitory potency of aminosteroids **1** - **3** (A) and **4** - **6** (B) on the human leukemia (HL-60) cell growth. Cells (1 x 10^4 cells) were incubated with various concentrations of **1** - **6** for 3 days. IC_{50} represents the concentrations that inhibited cell growth by 50 %. Results are the means (± SEM) of triplicates.

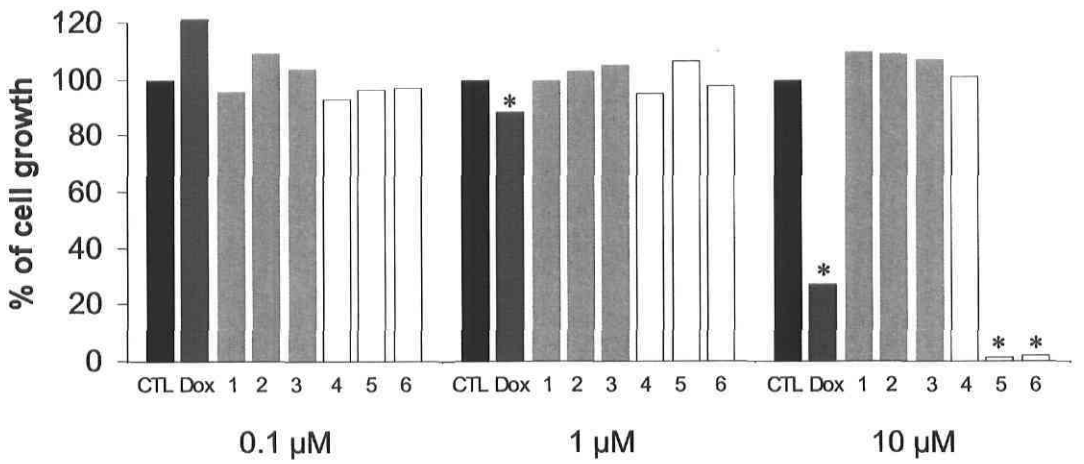
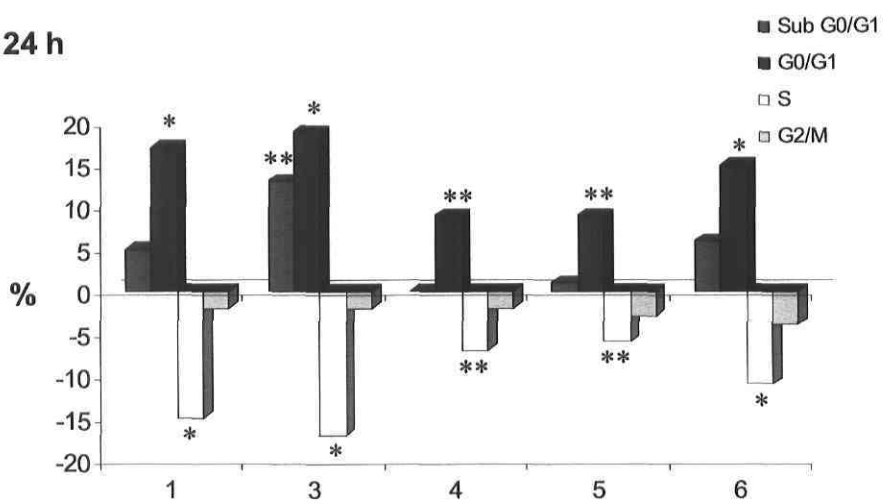


Figure 4. Cytotoxicity of aminosteroids **1** - **6** and doxorubicin (Dox) in normal cells, WI-38. Results are the means of triplicates (SEM < 0.1). Cell growth was assessed by MTS-based viability assay. * Indicates a result significantly different (P< 0.01) from the control (CTL).

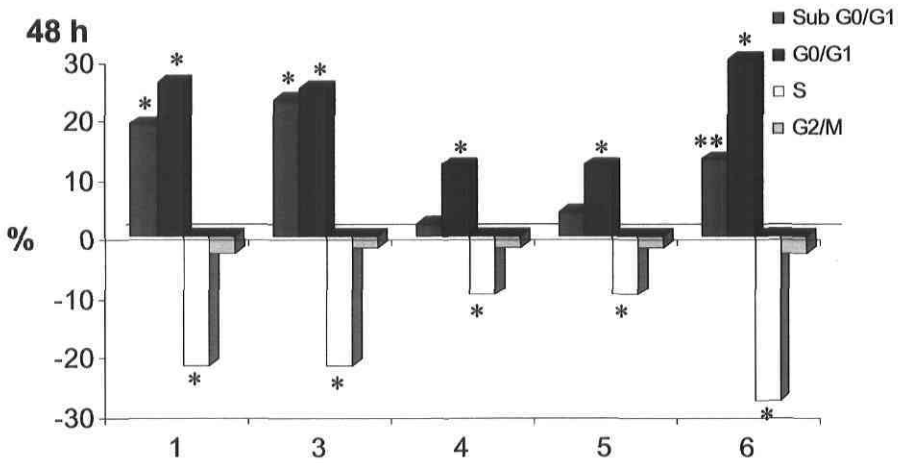
Cell cycle analysis

Having determined the efficiency of our compounds, we next investigated their putative mechanisms of action. We thus assessed their effects on the cell cycle and apoptosis induction. The cell cycle analysis was first performed with compounds **1** and **3**, the most active aminosteroids of the first category at 10 μM and next with compounds **4** - **6** at 5 μM (Figure 5). Treatments with compounds **4** - **6** were performed at 5 μM because a higher concentration induced complete apoptosis of all HL-60 cells after 12 h of incubation (data not shown). All aminosteroids caused similar effects after 24 and 48 h. Thus, when comparing to control, we observed an accumulation in the G_0/G_1 phase, the percentage of cells in the S phase decreased, the amount of cells in G_2/M phase slightly decreased, and the number of those in sub-G_0/G_1 increased. The increase of the Sub G_0/G_1 population suggests a higher occurrence of apoptosis. After 72 h, the pattern of cell cycle distribution changes for all aminosteroids, except for **6**. In fact, compared to results at 48 h the percentage of cells in the G_0/G_1 and S phases was lower. Moreover, compounds **1** and **3** augmented further the proportion of cells in sub-G_0/G_1. However, compounds **4** and **5** seem to be ineffective after 72 h because the cell cycle distribution is at the same level as that of control cells. So, although compounds **4** and **5** seem to induce cell cycle arrest in G_0/G_1, their action is clearly less efficient than that of aminosteroids **1**, **3** and **6,** especially at 72 h. These results show that aminosteroids tested modulate the mechanisms of cell cycle regulation by inducing G_0/G_1 arrest, thus inhibiting cell proliferation, and they appear to enhance apoptosis as well.

A)



B)



C)

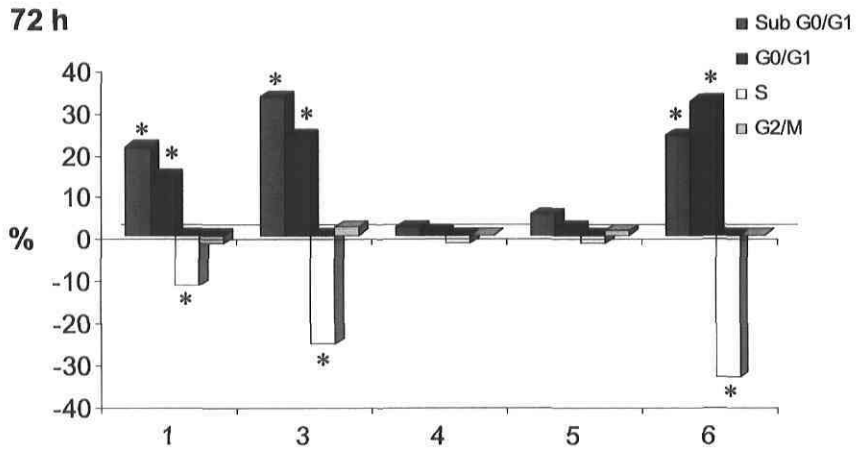


Figure 5. Effect of aminosteroids **1** and **3** (10 μM), and **4** - **6** (5 μM) on cell cycle distribution in HL-60 cells after 24 h (A), 48 h (B) or 72 h (C). Results are expressed as percentage of change relative to the control group. Compounds **1** and **3** were tested in a different experiment than compounds **4** – **6**. The percentage of cells in each cycle phase was determined by flow cytometry. Results are the means of triplicates (SEM < 2 %). * and ** Indicate a result significantly different from the control; *, $p< 0.01$; **, $p< 0.05$.

Analysis of cell differentiation induction by aminosteroids

Pursuing our investigation of modes of action, we next assessed the effect of the aminosteroids on cell differentiation. In order to do so, we evaluated expression of surface antigens CD11b (both granulocyte- and monocyte-like cell marker) and CD14 (monocyte-like cell marker) because in leukemia, the differentiation begins with an amplification of the expression of these two antigens. We again utilized the HL-60 cells because they differentiate along the granulocytic and/or monocytic pathway when treated with certain inducer agents.[25,26] In fact, this cell line has been widely used as a model for studying the molecular and cellular aspects of hematopoietic differentiation.[27]

The markers of differentiation stages were analyzed using flow cytometry after incubation for 24, 48 and 72 h with medium supplemented with a 10 μM concentration of aminosteroids. The two markers are found on approximately 3-10 % of untreated HL-60 cells (CTL). A 3-day exposure to compound **1**, **2** or **3** did not modify the levels of expression of CD11b, CD14 or CD11b/CD14 (double-marked) cell surface markers as indicated by flow cytometry analysis (Figure 6A). Thus, these 3 compounds do not act through induction of differentiation. On the other hand, we observed an increased differentiation of HL-60 cells following treatments with compounds **4**, **5** and **6**. As demonstrated in figure 6B, CD11b and double-marked CD11b/CD14 were significantly up-regulated during these treatments, especially after 48 and 72 h exposure, but the percentage of CD14 positive cells was not affected. After one day of incubation with **6**, we measured 30 % and 22 % of cells expressing CD11b and double-marked CD11b/CD14, respectively ($P<0.05$). This differentiation gradually increased to reach 58 % and 39 % after 3 days ($P<0.01$) and these results were slightly higher than those obtained after treatment with **5** ($P<0.01$) after the same time. HL-60 cells exposed for 24 and 48 h to **4** showed no statistically significant increase in expression surface markers, but after 72 h it induced expression of CD11b in 29 % of cells ($P<0.05$) and expression of CD14 in 10 % of cells ($P<0.05$). Compound **4** was thus less efficient than **5** and **6** in the induction of differentiation.

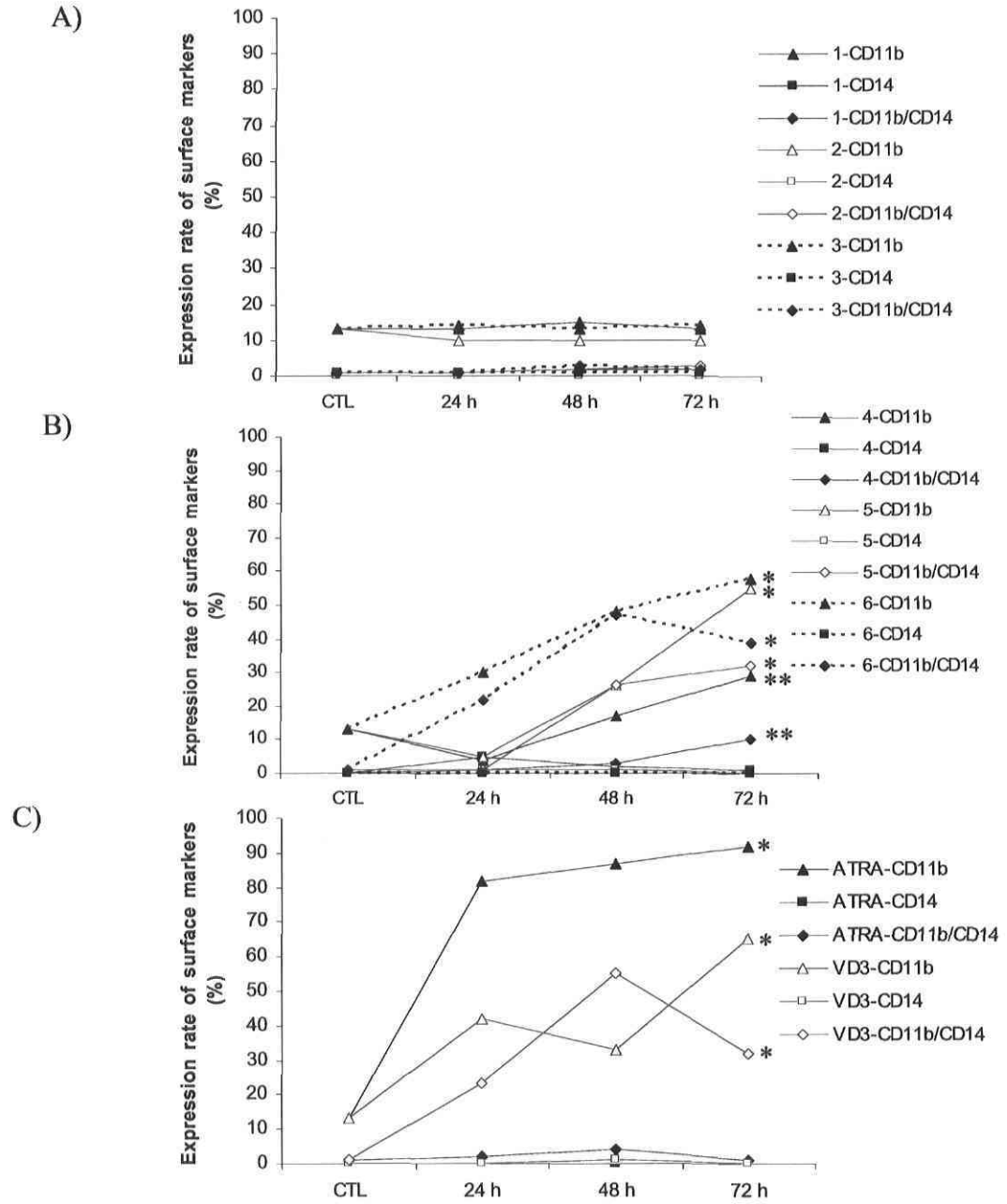


Figure 6. Time-dependent expression changes in CD11b, CD14 and CD11b/CD14 cell surface markers in HL-60 cells after a treatment with aminosteroids **1**, **2** or **3** (A), a treatment with aminosteroids **4**, **5** or **6** (B) and a treatment with retinoic acid (ATRA) or vitamin D3 (VD3) (C). Results are the means of triplicates (SEM < 3 %). * Indicates a result significantly different from the control (P< 0.01). ** Indicates a result significantly different (P< 0.05) from the control (CTL).

ATRA and VD3, two chemical agents well known to induce differentiation, served as positive controls (Figure 6C). Treatment with ATRA at 10 μM rapidly induced HL-60 cell differentiation, but only the expression of CD11b was increased. In fact, after one day the ATRA treatment increased CD11b from 13 to 83 % (P<0.01), reaching 92 % after 3 days of treatment. This increase was higher than those obtained with aminosteroids **5** and **6**. Moreover, incubation of HL-60 with VD3 for 3 days increased CD11b and double-marked CD11b/CD14 expressing cells to 65 % and 32 % (P<0.01), respectively. This level of differentiation is roughly the same as the effects obtained with compounds **5** and **6**. Clearly, the cell antigen expression analysis showed that the first category of aminosteroids, compounds **1** - **3**, does not induce HL-60 cell differentiation, contrary to the second category, compounds **4** - **6**, which has a similar potency as that of VD3.

Discussion

We recently reported that certain compounds with a steroidal nucleus inhibited the growth of HL-60 leukemia cells.[18,19] After selecting a series of 6 representative aminosteroids, we analyzed their biological effects and their mechanism of action. The aim of this preliminary study was to evaluate cytotoxic effects of this new class of compounds and their selectivity for cancerous cells vs. normal ones. Moreover, we examined their effects on the cell cycle, apoptosis induction and differentiation in HL-60 cells. Our results show that aminosteroids **1** - **6** inhibited HL-60 cell growth with IC_{50} values of 0.58 to 6.40 μM. Compound **3** was the most potent against myeloid leukemia cells. Interestingly, it was also found to inhibit the cell growth of prostate cancer Shionogi cells (data not shown). Compound **3** consists of a C19-steroidal nucleus (5α-androstane-3α,17β-diol) bearing a piperazine moiety on C-2β successively acylated with proline and cyclohexyl carboxylic acid. Contrary to **5** and **6**, aminosteroids **1** - **4** did not affect the growth of normal cells WI-38. This selectivity is important since standard chemotherapeutic agents, such as doxorubicin, are toxic on normal cells and cause severe side effects.

After 24 h of exposure of HL-60 cells to aminosteroids, all these tested compounds induced accumulation of cell cycle at G_0/G_1 phase and a concomitant reduction of S phase

cells. Furthermore, apoptosis started to increase after treatment with **1**, **3** or **6** as evidenced by the increase of Sub G_0/G_1 cells. Compounds **4** and **5** were less efficient than the others for the arrest of cell cycle in G_0/G_1 similarly as observed for their cytotoxic effect on HL-60 cells. Following a treatment of 72 h, **6** produced higher accumulation of cells in G_0/G_1 than **1** or **3**.

The differentiation of myeloid leukemia cells has often been associated with cell-cycle block in G_0/G_1 phase; however these two effects might be sometimes independent. Our results support this theory since compounds **1** - **3** did not demonstrate any morphological feature of differentiation in HL-60 cells, although inducing G_0/G_1 arrest. On the other hand, **4**, **5** and **6** generated a G_0/G_1 arrest and induced cell differentiation at a level similar to that of VD3. The analysis of myelomonocytic differentiation markers CD11b and CD11b/CD14 HL-60 leukemia cells indicated an augmentation of their expression for **4**, **5** and **6**. These results suggest that aminosteroids **1** - **6** possess two different mechanisms of action: one for compounds **1** - **3**, and an additional one for compounds **4** - **6**.

The exact mechanism by which these new aminosteroids induce their cytotoxic or/and differentiation effects is not yet understood. Indeed, there are a number of target enzymes and regulatory proteins that could be proposed to play a critical role in these processes.[28-30] Perturbation of cell cycle progression and inappropriate activity of the cyclin-dependent kinases are signals that can trigger apoptotic cell death.[31,32] It is possible that our aminosteroids act through regulation of a cyclin (cyclins D1-D3) or of cyclin-dependent kinases (CDKs) (CDK2, -4 or 6) driving progression through G_0/G_1 phase. We cannot at the present time formulate any hypothesis about the mechanism of action of the aminosteroids tested on leukemia cells. We however know that they act differently from Gleevec (imatinib mesylate), even if the latter also contains a piperazine moiety in its chemical structure. Indeed, this agent is a specific inhibitor of the Abl tyrosine kinase, which potently blocks the proliferation and cell cycle progression by inducing apoptosis of Bcr-Abl-positive K562 cells but not that of Bcr-Abl-negative HL-60 cells.[33]

The process by which cell cycle progression is controlled by aminosteroids **1** - **6** remains to be clarified, but the preliminary results discussed above indicated that a 5α-androstane-3α,17β-diol steroid nucleus with an appropriate group at position C-2β has antileukemic activity, possibly with a new mechanism of action. Using a long alkyl chain as in compound **5** or a bulky hydrophobic group as in compound **6** resulted in decreased cytotoxic selectivity (cancerous vs. normal cells) but increased the differentiation of the leukemic cell line. Moreover, by adding an amino acid and a carboxylic acid on the piperazine moiety, we generated compounds with stronger activity and selectivity. In summary, we have identified a category of aminosteroids that induces a cytotoxic effect in cancerous HL-60 cells but are non-toxic to normal WI-38 cells. These compounds produce G_0/G_1 arrest associated with elevated levels of apoptosis. Moreover, a number of them induced myeloid differentiation in HL-60 cells. We believe that this class of steroidal compounds may provide new therapeutic agents for myeloid leukemia or other types of cancers, but additional studies will be necessary to determine their mechanism of action.

Acknowledgments

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Chapitre 5

Synthèse de dérivés du 2β-pipérazino-5α-androstane-3α,17β-diol à l'aide de la chimie en parallèle sur support solide et leur effet sur les cellules leucémiques HL-60

5.1 Avant-propos

Ma contribution à ce projet a été premièrement de faire le design des librairies d'aminostéroïdes et d'en effectuer la synthèse. Par la suite, j'ai caractérisé par IR, RMN ^{1}H, RMN ^{13}C et LRMS tous les intermédiaires de synthèse et un échantillonnage des produits finaux afin de confirmer leur structure. J'ai également déterminé l'activité antiproliférative de tous ces dérivés sur les cellules leucémiques HL-60. Finalement, j'ai rédigé ce manuscrit en collaboration avec mon directeur de recherche Donald Poirier.

5.2 Résumé

La leucémie est le cancer le plus important chez les enfants. Nous avons déjà rapporté que l'utilisation de certains aminostéroïdes inhibait la croissance des cellules leucémiques HL-60 en induisant une toxicité sélective et un arrêt du cycle cellulaire dans la phase G_0/G_1. Dans la présente étude, nous avons utilisé la synthèse chimique en parallèle sur support solide pour synthétiser trois librairies d'aminostéroïdes. Ces librairies avec deux niveaux de diversités ont été élaborées à l'aide des résultats obtenus lors de nos travaux précédents. Le stéroïde précurseur pour la synthèse sur support solide, un 2β-pipérazino-5α-androstane-3α,17β-diol portant un groupement protecteur Fmoc, a été synthétisé en solution, à partir de l'épi-androstérone (épi-ADT) et par une séquence de trois étapes donnant l'alcène-2,3. Après le traitement de cet alcène avec *m*-CPBA, l'époxyde résultant a été sélectivement ouvert pour donner, après une protection N-Fmoc, un diol avec la stéréochimie souhaitée. La différence de réactivité entre le 3α et le 17β OHs a été utilisée pour permettre le couplage régiosélectif du 17β-OH sur la résine PS-DES. Après l'élimination du groupement protecteur Fmoc, des acylations séquentielles avec une diversité moléculaire appropriée ont été effectuées. Des clivages acides ont permis de générer trois librairies de 2β-pipérazino-5α-androstane-3α,17β-diols dans des rendements et des puretés acceptables pour l'évaluation biologique. Les librairies A-C étaient élaborées à partir de différents acides aminés (45 composés pour A), de dérivés de la phénylalanine (45 composés pour B) ou de dérivés de la proline (20 composés pour C). L'évaluation biologique de ces librairies a révélée des éléments structure-activité intéressants concernant leur effet cytotoxique sur les cellules HL-60. Les composés portant un résidu tétrahydroisoquinolone comme premier élément de diversité montraient une cytotoxicité importante, principalement lorsque les acides isovalérique ou cyclohexyl acétique étaient utilisés comme deuxième élément de diversité puisque ces dérivés inhibaient presque 40 % de la croissance cellulaire à 1 μM. D'ailleurs, l'utilisation de la forme naturelle *L* des acides aminés semble être plus efficace puisque les analogues ayant la configuration *D* induisent un activité cytotoxique plus faible. De plus, les dérivés de la phénylalanine portant un groupement cyano induisaient une plus grande inhibition que les autres dérivés. Finalement, la dernière librairie indiquait que l'effet cytotoxique induit par la proline pouvait être modulé par l'ajout d'un substituant.

Synthesis of 2β-piperazino derivatives of 5α-androstane-3α,17β-diol by solid-phase parallel chemistry and their effect in human HL-60 leukemia cells

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Abstract

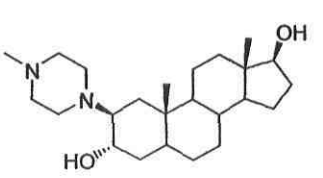
Leukemia remains the most commonly occurring cancers in children. We previously reported that the use of particular aminosteroids inhibited HL-60 leukemia cells growth by selective toxicity and produced cell cycle arrest in the G_0/G_1 phase. In the present study, we used solid-phase parallel chemistry to synthesize three additional libraries of aminosteroids. These libraries with two levels of diversity were elaborated based on the results obtained in our previous works. The solid-phase precursor, a 2β-piperazino-5α-androstane-3α,17β-diol bearing Fmoc protecting group, was synthesized in solution, starting from epi-androsterone (epi-ADT) by a sequence of three steps that afforded a 2,3-alkene. After treatment of this alkene with *m*-CPBA, the resulting epoxide was selectively opened to give, after N-Fmoc protection, a diol with suitable stereochemistry. The difference of reactivity between 3α and 17β OHs was then used to allow the regioselective 17β-OH coupling to PS-DES resin. After cleavage of Fmoc protecting group, sequential acylation reactions with appropriate molecular diversity were performed. Acidic cleavages were used to generate three libraries of 2β-piperazino-5α-androstane-3α,17β-diol derivatives in acceptable yields and purity for biological assays. Libraries A-C were composed either various amino acids (45 compounds for A), derivatives of phenylalanine (45 compounds for B) or derivatives of proline (20 compounds for C). The screening of these libraries revealed interesting structure-activity relationships related to their cytotoxic effects on HL-60 leukemia cells. Compounds bearing tetrahydroisoquinolone residue as the first element of diversity showed potent cytotoxicity, principally when isovaleric or cyclohexyl acetic acid were used as capping group since these derivatives inhibited almost 40 % of cell growth at 1 μM. Moreover, the use of natural *L* form of amino acids seems to be more effective while their analogues having *D* configuration induced a lower cytotoxic activity. Furthermore the phenylalanine derivatives bearing a cyano group induced a higher growth inhibition than other derivatives. Finally, the last library indicated that the increase of hydrophobicity of proline by different substituents seems to preserve cytotoxic effect achieved by the lead compound.

Introduction

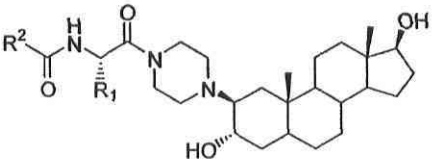
Cancer is the second leading cause of death among children and leukemia is the most common of this one. In 2005, acute myeloid leukemia occurs with an incidence of approximately 4.2 per 100,000 population overall in the Unites States with a slight predominance in men.[1] Like several diseases, acute leukemia is treated with multiple aggressive therapies. Many chemotherapeutic agents are available but high-doses are frequently essential for the cure of leukemia. Standard induction chemotherapy, depending on patient tolerability, is at present the treatment of choice in patients with myeloid leukemia but is limited by toxicity to normal cells. However, dosage reduction from the usual levels in the aim to decrease side effects led to reduction in the complete remission proportion. Consequently, it is imperative to find new anticancer agents with different and complementary modes of action and displaying effective selective toxicity toward cancer cells versus normal cells.

Recent studies have identified a C-19 steroid with potential as antileukemic agent (**1**, HY) (Figure 1).[2] During previous works, we have synthesized seventy aminosteroids using efficient procedure that we have developed for the aminolysis of hindered steroidal epoxides with primary and secondary amines.[3,4] Some of these compounds exerted a higher inhibition than the lead compound **1** on human acute myelocytic leukemia (HL-60) cell growth at 10 μM. These results demonstrated that steroidal compounds had potential for the treatment of acute myeloid leukemia. We also used our expertise in solid-phase synthesis of steroid derivatives[5-10] to elaborate and synthesize 150 compounds with several elements of diversity previously identified as potentially interesting (see general structure of library A members, Figure 1).[11] Biological evaluation of these steroids allowed us to discover three potential antileukemic agents with IC_{50} of 0.58, 0.66 and 1.78 μM on HL-60 cells. Moreover, these activities bring several significant elements of structure-activity relationships for the improvement of this class of compounds. We observed that steroidal nucleus bearing an amino acid, proline or phenylalanine, at R^1 provided the best cytotoxic activity against HL-60. The presence of an appropriate capping group at R^2, principally a six-membered ring, seems to be important for antiproliferative activity although extension

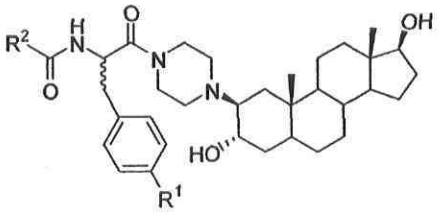
of molecular diversity with a dipeptide does not induce any cytotoxic effect on HL-60 cell growth at 1 μM. A preliminary analysis of the mechanism of action of these new class of aminosteroids proved that they induced cell cycle arrest in the G_0/G_1 phase associated with elevated levels of apoptosis.[12] Some of these compounds produced myeloid differentiation in HL-60 cells, whereas others were found to be selective for cancer cells when compared to normal cells WI-38.[12] This new amino steroid derivatives may offer a potential therapy for treatment of myeloid leukemia or other type of cancers through a mechanism of action that remain to be fully defined. Based on these previous results, we elaborated three new more focused libraries (A, B and C) and determined the antiproliferative activity of each library member on HL-60 cells in order to obtain additional structure-activity relationships for optimizing the efficiency of this new family of aminosteroids (Figure 1).



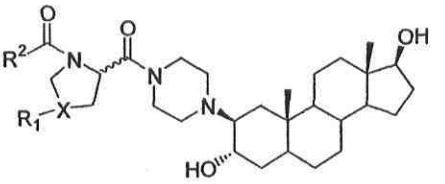
1



Library A



Library B



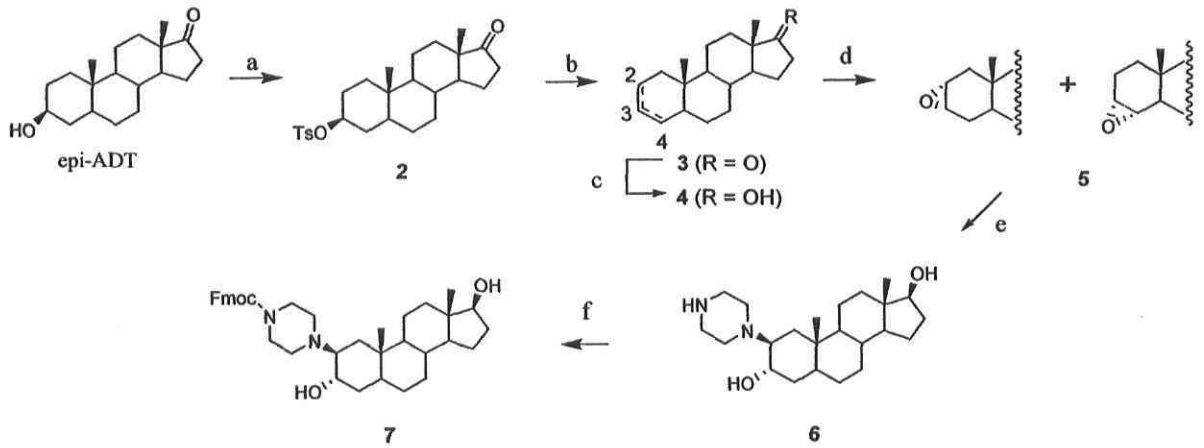
Library C

Figure 1. The lead compound **1** and general structure of members of libraries A, B and C, which will be prepared and tested in this study.

Results and discussion

Chemical Synthesis

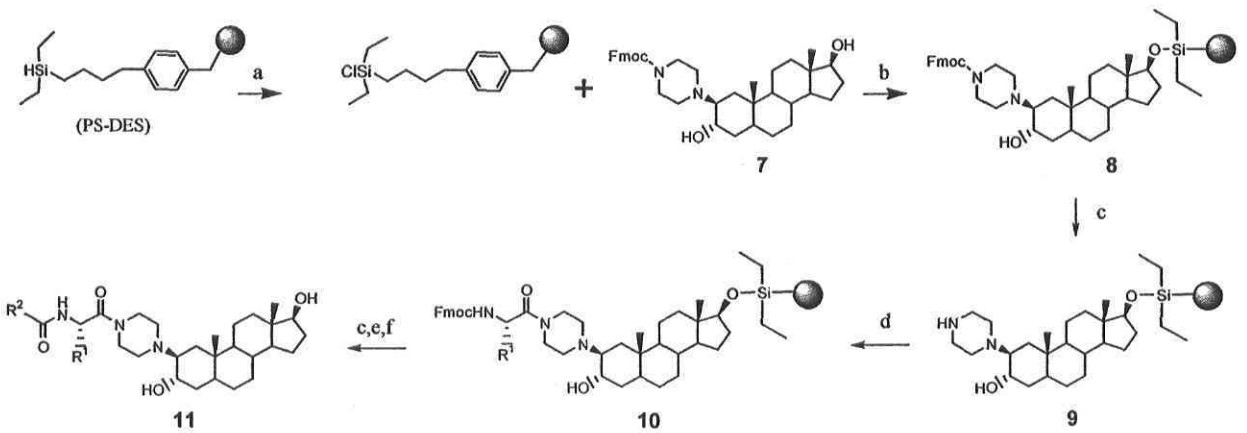
The synthesis of different members of libraries A, B and C was performed in two parts. The steroid precursor **7** was initially prepared in solution and then used for the synthesis of libraries in solid-phase. The synthesis of **7** was performed as described in figure 2 but differs from the way that we previously reported.[4,11] Briefly, treatment of epi-androsterone (epi-ADT) with tosyl chloride in pyridine afforded **2**, which under conditions of elimination (2,4,6-collidine at reflux) gave a 7:1 ratio of 2,3- and 3,4-alkenes, respectively. Subsequently, the ketone **3** was reduced into **4**, and this later was epoxidized giving a mixture of 2α,3α- and 3α,4α-epoxides **5**. To promote isolation of the wanted amino steroid **6**, we used an aminolysis procedure reported in literature.[13] In our case, the reaction was performed at 130 °C in ethylene glycol with only 2 equivalents of piperazine instead of morpholine. This way allowed selective ring opening of the steroidal 2α,3α-epoxide to give **6** whereas the corresponding 3α,4α-epoxide did not reacted. However, with piperazine the opening of 2α,3α-epoxide was not completed and increasing the temperature was not possible because it allowed 3α,4α-epoxide to react. Finally, the secondary amine of **6** was protected as Fmoc derivative providing the steroidal precursor **7** in an acceptable overall yield of 14 % for the last 5 steps. Thus, we used a shorter sequence of reactions that the other one already reported by our group[4,11] (6 vs 8 steps) for the synthesis of **7**. In this new sequence of reactions and due to application of selective aminolysis, it was not important to obtain exclusively the 2,3-alkene or 2,3-epoxide, which needed critical steps and meticulous purifications.



Scheme 1. Synthesis of **7**. Reagents and conditions: **(a)** Pyridine, DMAP cat.,Ts-Cl, rt; **(b)** 2,4,6-collidine, reflux; **(c)** $NaBH_4$, $MeOH/CH_2Cl_2$, 0°C; **(d)** *m*-CPBA, CH_2Cl_2, 0°C; **(e)** ethylene glycol, piperazine, 130°C; **(f)** $NaHCO_3$, Fmoc-OSu, $THF:H_2O$ (5:1), rt.

The linker used to achieve the planned solid-phase synthesis of libraries A-C was diethylsilyloxy linker, which was previously used in our group for the synthesis of libraries of 2β-aminosteroids[11] and other peptidosteroids.[6,8] The activated resin was generated in situ by a treatment with 1,3-dichloro-5,5-dimethylhydantoin. After a simple filtration and washing with dry dichloromethane, this wet resin was anchored to the less hindered secondary alcohol of **7**. The coupling yield of **8**, calculated either by the increase of the resin weight or by the difference between the initial amount of steroid and the amount of uncoupled steroid, was in the range of 70 %. After removal of the Fmoc protecting group with a solution of 20 % piperidine in dichloromethane, the first level of diversity (R^1) was introduced by an acylation of resin **9** with selected Fmoc-protected amino acid to give the resin **10**. Moreover, a mini-clivage test easily provided step-by-step information about the course of the solid-phase reactions performed. The Fmoc protecting group was then removed and the second level of molecular diversity (R^2) was introduced from resin **10** by an amidation step using chosen carboxylic acids. At the end of this second acylation step, the cleavage of the steroid derivatives was performed using a solution of HF/pyridine in dichloromethane:THF (10:1). To this resulting solution were added sodium bicarbonate and subsequently magnesium sulfate. The mixture was filtered and the filtrate was washed with a saturated solution of sodium bicarbonate and finally with water to remove the excess of

pyridine. After evaporation of the organic layer, the compound **11** was obtained in 17 to 95 % yields (5 steps). The average purity of the final compounds estimated by TLC (each library members) and NMR (sampling) was more than 80 %. Libraries A-C (Tables 1-3) were generated using the conditions reported above. The three libraries differ by the R^1 level of diversity (different type of amino acids) but we used the same building blocks at R^2.



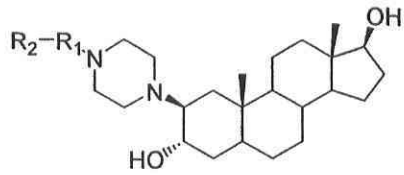
Scheme 2. Synthesis of libraries A, B and C. Reagents and conditions: (**a**) 1,3-dichloro-5,5-dimethylhydantoin, CH_2Cl_2, rt; (**b**) imidazole, CH_2Cl_2, rt; (**c**) 20 % piperidine in CH_2Cl_2, (v:v), rt; (**d**) Fmoc-amino acid, PyBrOP, HOBt, DIPEA, DMF, rt; (**e**) carboxylic acid, PyBOP, HOBt, DIPEA, DMF, rt; (**f**) *1*) HF/pyridine, CH_2Cl_2, *2*) $NaHCO_3$, *3*) $MgSO_4$ anh.

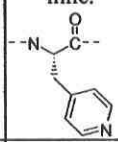
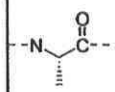
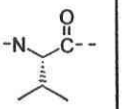
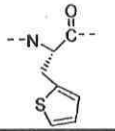
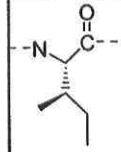
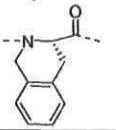
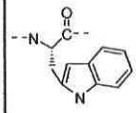
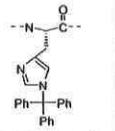
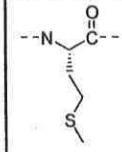
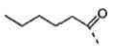
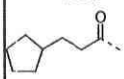
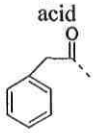
Antiproliferative activity on HL-60 cells

Compounds from libraries A, B and C, were evaluated for their activities against cell growth of leukemia HL-60 cells. The antiproliferative activity is reported in percentage of inhibition at 1 and 10 μM. Since in the previous study we have used only four amino acid as building blocks,[11] then the library A was devoted to extend this diversity with nine new amino acids. The results reported in Table 1 show that the presence of a tetrahydroisoquinolone amino acid produced the most potent antiproliferative activity. The combination of this amino acid with one of two building blocks reported important as capping group in our first study,[11] isovaleric acid (**A26**) and cyclohexyl carboxylic acid

(**A29**), displayed the best antiproliferative activities. After three days of treatment at 1 and 10 μM, these compounds inhibited an average 35 % and 96 % of cell growth, respectively. They were more potent than phenylalanine analogues **12** and **13** but less potent than the proline analogues **14** and **15** obtained in our first study (Table 2).[11]

Table 1. Yields (Y)[a] and inhibitions (%)[b,c] of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library A members.



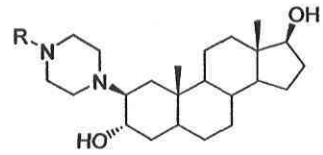
Amino acids (R^1) / **Carboxylic acids (R^2)**	Pyridylalanine. 	Alanine 	Valine 	Thienylalanine 	Isoleucine 	Tetrahydroisoquinolone 	Tryptophan 	N-trityl-Histidine 	Methionine 
Isovaleric acid 	A1 Y: 50 % 0/32	A6 Y: 63 % 0/21	A11 Y: 70 % 0/2	A16 Y: 81 % 1/17	A21 Y: 70 % 0/0	A26 Y: 73 % 33/98	A31 Y: 48 % 0/0	A36 Y: 67 % 0/38	A41 Y: 70 % 0/48
Hexanoic acid 	A2 Y: 50 % 0/38	A7 Y: 63 % 0/7	A12 Y: 70 % 17/18	A17 Y: 77 % 0/0	A22 Y: 76 % 0/13	A27 Y: 68 % 13/96	A32 Y: 43 % 0/29	A37 Y: 67 % 0/7	A42 Y: 72 % 0/44
Cyclopentyl propionic acid 	A3 Y: 70 % 0/51	A8 Y: 70 % 0/28	A13 Y: 76 % 2/50	A18 Y: 78 % 0/0	A23 Y: 81 % 0/54	A28 Y: 65 % 9/99	A33 Y: 21 % 12/31	A38 Y: 62 % 0/15	A43 Y: 65 % 0/37
Cyclohexyl carboxylic acid 	A4 Y: 73 % 0/51	A9 Y: 70 % 0/23	A14 Y: 71 % 8/47	A19 Y: 82 % 0/28	A24 Y: 76 % 6/32	A29 Y: 61 % 37/95	A34 Y: 17 % 0/38	A39 Y: 68 % 0/41	A44 Y: 70 % 0/38
Phenylacetic acid 	A5 Y: 74 % 0/21	A10 Y: 55 % 0/0	A15 Y: 76 % 0/15	A20 Y: 65 % 0/0	A25 Y: 71 % 0/0	A30 Y: 35 % 0/64	A35 Y: 25 % 27/17	A40 Y: 60% 18/33	A45 Y: 66 % 0/23

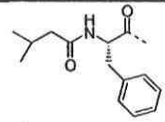
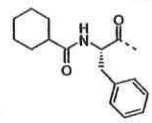
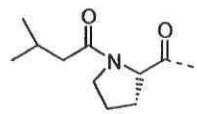
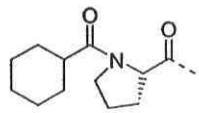
[a] Yields calculated for the solid-phase sequence of reactions.

[b] Experimental error ± 5 %.

[c] Antiproliferative activity (%) of reference compounds **1** (0/0) and doxorubicine (92/93) at two concentrations (1 μM/10 μM) in this assay.

Table 2. HL-60 cell growth inhibition of reference compounds.[a]



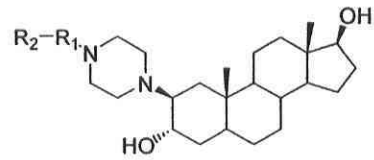
#	R	Cell growth inhibition (%)	
		1 µM	10 µM
12		7	87
13		0	82
14		44	93
15		58	93
1 (HY)	CH_3	0	20
DOXO	-------	96	99

[a] Data from reference 11.

The library B (Table 3) was prepared because *L*-phenylalanine (*L*-Phe) had some interesting effects on antiproliferative activity.[11] A series of *L*-Phe having different substituents in para position were then selected to extend our SAR study. We also added *D*-phenylalanine (*D*-Phe) as building block. Carboxylic acids were the same as for library A. First of all, these results showed that the use of *D*-Phe (**B1-B5**) instead of the natural *L* form did not increase the cytotoxic effect of these compounds. Indeed, compounds **B1-B5** (*D*-Phe) inhibited 57-97 % of cell proliferation at 10 µM while *L*-Phe analogues inhibited 54-98 % at the same concentration (data from ref. 11). On the other hand, the *L*-Phe derivatives bearing a cyano group (**B36-B40**) induced a higher inhibition than other

derivatives at position 4 (para) of the phenyl residue. Indeed, addition of various halogens like bromide, chloride, fluoride or iodide to *L*-Phe moiety showed poor cytotoxic activity. Furthermore, among all carboxylic acids used as building blocks, the cyclohexyl carboxylic acid at R^2 induced the better inhibitions. Compound **B39** resulting from combination of a cyanophenylalanine and a cyclohexylcarboxylic acid higher inhibited the cell growth than the phenylalanine analogue **13**, but it is less potent than lead compounds **14** and **15** having a proline (Table 2).

Table 3. Yields (Y)[a] and inhibitions (%)[b,c] of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library B members.



Amino acids (R^1) / **Carboxylic acids (R^2)**	D-Phe	Biphenylalanine	L-Phe (4Br)	Phe (4Cl)	Phe (4F)	Phe (4I)	Phe (4Me)	Phe (4CN)	Tyr (Me)
Isovaleric acid	B1 Y: 93 % 0/92	B6 Y: 87 % 4/69	B11 Y: 72 % 0/59	B16 Y: 69 % 0/27	B21 Y: 60 % 0/24	B26 Y: 88 % 0/0	B31 Y: 80 % 0/69	B36 Y: 86 % 0/66	B41 Y: 68 % 0/8
Hexanoic acid	B 2 Y: 95 % 0/79	B7 Y: 87 % 1/37	B12 Y: 80 % 0/45	B17 Y: 67 % 0/53	B22 Y: 67% 0/71	B27 Y: 73 % 0/43	B32 Y: 90 % 0/71	B37 Y: 82 % 0/91	B42 Y: 73 % 0/7
Cyclopentyl propionic acid	B3 Y: 94 % 0/57	B8 Y: 84 % 21/19	B13 Y: 79 % 0/40	B18 Y: 71 % 0/42	B23 Y: 64 % 0/65	B28 Y: 77 % 0/34	B33 Y: 85 % 9/32	B38 Y: 71 % 0/80	B43 Y: 71 % 0/3
Cyclohexyl carboxylic acid	B4 Y: 84 % 15/97	B9 Y: 80 % 9/36	B14 Y: 86 % 9/79	B19 Y: 69 % 8/81	B24 Y: 49 % 0/78	B29 Y: 72 % 2/63	B34 Y: 84 % 0/75	B39 Y: 81 % 0/98	B44 Y: 69 % 0/39
Phenylacetic acid	B5 Y: 94 % 0/89	B10 Y: 50 % 8/45	B15 Y: 78 % 8/73	B20 Y: 55 % 0/51	B25 Y: 31 % 0/73	B30 Y: 37 % 3/40	B35 Y: 72 % 0/66	B40 Y: 48 % 0/77	B45 Y: 61 % 0/25

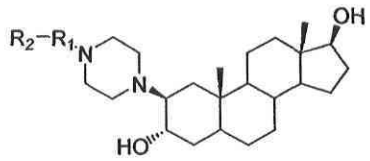
[a] Yields calculated for the solid-phase sequence of reactions.

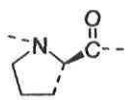
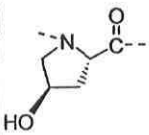
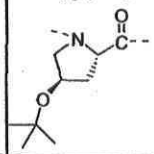
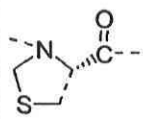
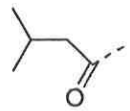
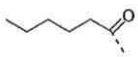
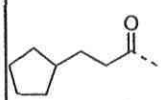
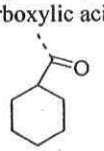
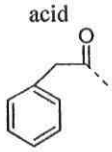
[b] Experimental error ± 5 %.

[c] Antiproliferative activity (%) of reference compounds **1** (0/18), doxorubicine (96/95), **14** (23/84) and **15** (47/89) at two concentrations (1 μM/10 μM) in this assay.

Until now, a proline as the first level of diversity was the most important element for antiproliferative activity of aminosteroids. We then synthesized a library with some derivatives of this amino acid. Due to the rarity of commercially available proline derivatives, only four amino acids were selected for library C (Table 3). For this third library (C), the activities of compounds **C1** and **C4** demonstrated that the use of *D*-proline induced a loss of cytotoxic activity against HL-60 cells when compared to reference compounds **14** and **15** having the same carboxylic acid as capping group. This important decrease of activity confirmed the importance of using *L*-proline in preparation of future aminosteroids. Among the three *L*-proline derivatives, the use of a substituent like a tert-butyloxy group or a sulfur atom, engendered a higher antiproliferative activity, especially for library members **C12**, **C13**, **C14** and **C18**. These aminosteroids were as potent as reference compounds **14** and **15** previously reported as the most potent antiproliferative agents on HL-60 cells.[11] These results suggest that introduction of different substituents on proline could be an interesting strategy for the optimization and development of further libraries. However, this substitution must not be to polar since addition of OH group on proline (**C6-C10**) generated the less potent compounds in library C.

Table 4. Yields (Y)[a] and inhibitions (%)[b,c] of HL-60 cell growth at two concentrations (1 μM / 10 μM) of library C members.



Amino acid (R^1) / **Carboxylic acids (R^2)**	D-Pro 	HydroxyPro 	Hyp(tBu) 	Thiazolidine 
Isovaleric acid 	C1 Y: 72 % 0/8	C6 Y: 64 % 0/56	C11 Y: 64 % 11/67	C16 Y: 64 % 20/78
Hexanoic acid 	C2 Y: 68 % 0/25	C7 Y: 67 % 6/66	C12 Y: 64 % 31/88	C17 Y: 66 % 20/79
Cyclopentyl propionic acid 	C3 Y: 69 % 0/56	C8 Y: 60 % 24/84	C13 Y: 68 % 50/95	C18 Y: 64 % 48/90
Cyclohexyl carboxylic acid 	C4 Y: 74 % 0/33	C9 Y: 69 % 9/74	C14 Y: 70 % 49/87	C19 Y: 58 % 2/61
Phenylacetic acid 	C5 Y: 70 % 0/20	C10 Y: 75 % 0/33	C15 Y: 69 % 1/52	C20 Y: 68 % 0/39

[a]Yields calculated for the solid-phase sequence of reactions.
[b]Experimental error ± 5 %.
[c]Antiproliferative activity (%) of reference compounds **1** (0/0), doxorubicin (82/95), **14** (33/76) and **15** (53/88) at two concentrations (1 μM/10 μM) in this assay.

Conclusion

The aim of the present work was to evaluate the antiproliferative effect on HL-60 cells of introducing either different amino acids, phenylalanine derivatives or proline derivatives on a 2β-piperazino-5α-androstane-3α,17β-diol nucleus. The intermediate steroid to be loaded onto solid support was produced from epi-ADT in a six steps sequence of reaction in solution (12 % yield). Following coupling to PS-DES resin, two levels of molecular diversity were introduced by successive acylation reactions using parallel solid-phase chemistry. The aminosteroids were finally released by an acidic treatment and, after washing, the purity of compounds from those libraries was very good and acceptable for cell growth assay.

The biological evaluation of all library members revealed interesting results regarding their cytotoxic activity against HL-60 leukemia cells. For the first library, the presence of tetrahydroisoquinolone at R^1 with selected capping group at R^2 seems to inhibit better cell growth, contrary to other amino acids of this library. In the second library, the presence of *D*-Phe does not seem to improve the cytotoxic effect when compared to natural version of this amino acid. Furthermore, addition of cyano group in para position of *L*-Phe resulted in better inhibition at 10 μM than all other derivatives of this second library. Finally, the third library clearly showed the importance to use natural configuration of Pro since the presence of *D*-Pro at R^1 induced a complete loss of cytotoxic activity. The results obtained with this last library suggest that the introduction of a substituent on *L*-Pro can modulated the biological activity. Our results and all observations suggest that two strategies could be investigated for the preparation of additional libraries of aminosteroids: 1) extend the diversity on *L*-Pro and 2) use new capping groups in combination with the most promising amino acid. Since the importance of using a steroidal nucleus was previously established by synthesizing compound **16**,[4] an analogue of HY without a steroid nucleus, and compounds **17** and **18**, both analogues without a steroid nucleus of the best aminosteroids **14** and **15** (data not shown), it is clear that the 5α-androstane-3α,17β-diol nucleus is a key element of biological activity (Figure 2). Additional strategy of development could also focus on moving the 2β-side chain at another steroidal position

and/or use of another steroidal nucleus. We believe that these modifications could improve the potency of this new class of aminosteroids to potentially provide an effective therapy for myeloid leukemia.

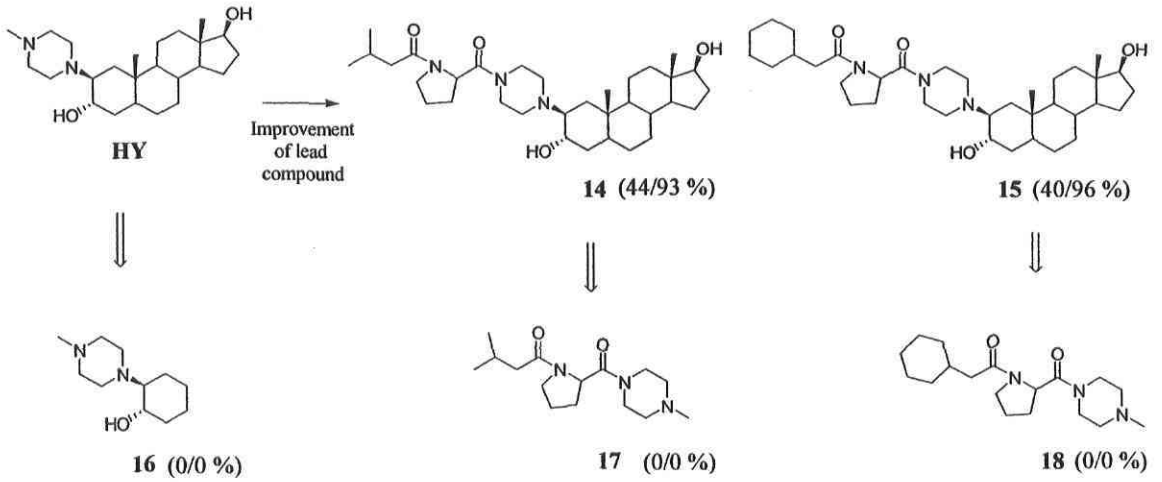


Figure 2. Antiproliferative activity of aminosteroids HY, **14** and **15** and their analogues **16-18** without a steroidal nucleus.

Experimental

General methods for chemical synthesis

Epi-ADT was obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The butyldiethylsilane polystyrene (PS-DES resin) with a loading of 1.37 mmol/g was supplied by Argonaut Technologie (San Carlos, CA). Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada), Calbiochem-Novabiochem Corp. (San Diego, CA) and Advanced Chemtech (Louisville, KY). The usual solvents were obtained from Fisher Scientific (Montréal, Qc, Canada) and were used as received. Anhydrous dichloromethane (CH_2Cl_2), dimethylformamide (DMF) and pyridine were obtained from Sigma-Aldrich. The loading of steroid **7** on resin was performed in peptide synthesis vessels (25 mL) with frit equipped for vacuum filtration (ChemGlass Inc.;

Vineland, NJ). The reaction vessels were shaken with a Burrell wrist-action shaker model 75 (Pittsburgh, PA) the libraries of steroid derivatives were realized with an ACT LabTech manual synthetizer (Advanced ChemTech; Louisville, KY) using either 40 or 96 solid-phase reaction block. The completion of solid-phase reactions were monitored after a micro cleavage by thin-layer chromatography (TLC). TLC and flash-column chromatography were performed on 0.20 mm silica gel 60 F254 plates and with 230-400 mesh ASTM silica gel 60, respectively (E. Merck; Darmstadt, Germany). Infrared spectra (IR) were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT) and the significant band reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz ^{13}C on a Bruker Avance 400 digital spectrometer and reported in ppm. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA) equipped with a turbo ion-spray source.

Synthesis of 3β-(4-methylphenylsulfonyloxy)-5α-androstan-17-one (**2**)[14]

4-Methylphenylsulfonylchloride (23.82 g, 125 mmol) was added to a solution of epi-ADT (20.16 g, 69.4 mmol) and 4-dimethylaminopyridine (500 mg) in pyridine (100 ml) under argon. This solution was stirred overnight at room temperature concentrated under vacuum and the residue treated with HCl 10 % (500 ml). The product was extracted with EtOAc and the organic phase dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the crude product by flash chromatography (acetone:hexanes, 5:95) yielded 30.36 g (98 %) of **2**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.80 (s, 19-CH_3), 0.83 (s, 18-CH_3), 0.60-2.10 (26 H), 2.05 (m, 1H of 16-CH_2), 2.43 (dd, J_1 = 8.8 Hz and J_2 = 19.2 Hz, 1 H of 16-CH_2), 2.44 (s, CH_3-Ph), 4.40 (m, 3α-CH), 7.33 (d, J = 8.1 Hz, 2 H of Ph), 7.79 (d, J = 8.2 Hz, 2 H of Ph).

Synthesis of 5α-androstan-2-en-17-one and its minor Δ^3 isomer (**3**)

A solution of **2** (30.29 g, 68.12 mmol) in 2,4,6-collidine (120 ml) under argon was heated under reflux for 3 h. The mixture was treated with HCl 10 %, extracted with EtOAc. The combined organic layer was dried over $MgSO_4$, filtered and evaporated to dryness to give a mixture of **3** (18.5 g) as a mixture of 2,3 and 3,4 isomer in proportions 7:1. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.78 and 0.80 (s, 19-CH_3 of Δ^2 and Δ^3-isomers), 0.87 (s, 18-CH_3), 0.75-

2.15 (m, 25 H), 2.44 (dd, J_1 = 8.8 Hz and J_2 = 20.0 Hz, 1 H of 16-CH_2), 5.3 (m, 0.24 H, 4-CH of Δ^3-isomer), 5.59 (m, 1.76 H, 2-and 3-CH of Δ^2 and Δ^3-isomers.

Synthesis of 17β-hydroxy-5α-androstan-2-ene and its minor Δ^3 isomer (**4**)

To a solution of crude ketone **3** (18.2 g) in a mixture of CH_2Cl_2:MeOH (1:10) (450 ml) at 0 °C was added sodium borohydride (3.03 g, 802 mmol) and the mixture was stirred under argon for 1 h. The reaction was quenched by the addition of a saturated aqueous solution of NH_4Cl (50 ml). The resulting solution was partially extracted with EtOAc and the precipitated was filtered. Evaporation of solvent afforded 18.9 g of crude alcohol **4** (Δ^2-isomer) and its minor Δ^3 isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.74 (s, 18-CH_3), 0.76 and 0.78 (2s, 19-CH_3 of Δ^2 and Δ^3 isomers), 0.60-2.10 (m, 26 H), 3.63 (t, J = 8. 6 Hz, 17α-CH), 5.3 (m, 0.24 H, 4-CH of Δ^3-isomer), 5.59 (m, 1.76 H, 2- and 3-CH of Δ^2 and Δ^3 -isomers). The characterization of pure Δ^2 isomer was previously reported.[4,9]

Synthesis of 17β-hydroxy-2α,3α-epoxy-5α-androstane and its minor Δ^3 isomer (**5**)

To a stirred solution of **4** (18.9 g) in dichloromethane (900 ml) was added 3-chloroperbenzoic acid (17.83 g, 103 mmol) under argon, while the temperature was maintained at 0°C. The solution was stirred for 5 h and concentrated under reduced pressure. The residue is diluted in EtOAc and washed successively with a solution of saturated aqueous $Na_2S_2O_3$ (1 x 500 ml) and a solution of aqueous $NaHCO_3$ (1 x 500 ml) and evaporated to dryness to give 21.8 g of crude epoxides **5**. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.72 (s, 18-CH_3), 0.76 and 0.77 (2s, 19-CH_3 of Δ^2 and Δ^3-isomers), 0.55-2.15 (m, 21 H), 2.70 (m, 0.26 H of 4-CH of Δ^3-isomer), 3.14 (m, 1.74 H of 2-CH and 3-CH of Δ^2 and Δ^3-isomers), 3.63 (t, J = 8.6 Hz, 17α-CH). Complete characterization of pure Δ^2 isomer was previously reported.[4,9]

Synthesis of 2β-piperazino-5α-androstane-3α,17β-diol (**6**)

The epoxides **5** (21.6 g) were dissolved in ethylene glycol (700 ml) and piperazine (6.36 g, 74.00 mmol) was added. The mixture was heated at 130 °C under an argon atmosphere for 7 h and then poured into cold water (4 L). The precipitated solid was filtered off and

washed with water. After the aqueous layer was extracted with dichloromethane, the organic phase was dried over $MgSO_4$, filtered and the filtrate combined with the precipitate initially obtained. The solvent was removed under reduced pressure to give 23.9 g of crude amine **6** and epoxide **5**. Only one isomer was observed by NMR analysis and data were identical as previously reported.[4,9]

Synthesis of 2β-[N-(9-fluoroenylmethoxycarbonyl)-piperazino]-5α-androstane-3α,17β-diol (**7**)

To a solution of crude **6** (23.9 g) in a mixture of THF:H_2O, 5:1 (500 ml) was added successively aqueous $NaHCO_3$ 1M (192 ml) and (Fmoc-OSu) in six portions. The mixture was stirred at rt overnight and poured into water. The crude product was extracted with dichloromethane and the combined organic layer was dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography with CH_2Cl_2:MeOH (98:2) yielded the epoxide **5** and 6.03 g of **7** (overall yield of 14 % for 5 steps) as a white solid. ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.73 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.70-2.15 (m, 23 H), 2.40 and 2.55 (2m, 2α-CH and 2X CH_2N), 3.43 (m, 2X CH_2NCO), 3.58 (m, 17α-CH), 4.04 (m, 3β-CH), 4.28 (t, J = 6.6 Hz, $\underline{CH}CH_2$ of Fmoc), 4.41 (d, J = 6.6 Hz, CH_2O of Fmoc), 7.34 (t_{app}, J = 7.4 Hz, 2H of Fmoc), 7.43 (t, J = 7.3 Hz, 2H Fmoc), 7.67 (d, J = 7.3 Hz, 2H of Fmoc), 7.87 (d, J = 7.5 Hz, 2H of Fmoc). Complete characterization of this compound (NMR in $CDCl_3$) was previously reported.[4,9]

Coupling of steroid 7 to PS-DES resin (synthesis of **8**)

The procedure differs slightly than previously reported.[11]

To dry PS-DES resin (2.48 g, 3.4 mmol) previously dried under vacuum for 2 days, a solution of 1,3-dichloro-5,5-dimethyldantoin (1.97 g, 10.0 mmol) in dry CH_2Cl_2 (15 ml) was added under argon atmosphere. The suspension was stirred with a Burrell wrist-action shaker for 1 h at rt. After, the resin was washed with dry CH_2Cl_2 (3 x 30 ml) under argon. The resin was directly used for the next step. To this resin, were added a solution of imidazole (681 mg, 10.0 mmol) in dry CH_2Cl_2 (3 ml) and a solution of **7** (6.03 g, 10 mmol) in dry CH_2Cl_2 (7 ml). The mixture was stirred overnight at rt under argon atmosphere. Then, the resin was washed with CH_2Cl_2 (4 x 30 ml), MeOH (3 x 30 ml), and dried

overnight under vacuum to give 3.61 g (67 % of loading by increasing weight) of resin **8**. We recovered 4.73 g of **7** after flash chromatography with EtOAc:hexanes (1:1) suggesting that 1.3 g of **7** was loaded for a loading of 63 %. IR (KBr): 3465 cm^{-1} (OH, alcohol), 1701 cm^{-1} (C=O, amide).

Synthesis of libraries A-C

Cleavage of Fmoc protective group. To the resin **8** (3.61 g, 1.886 mmol), was added a solution of piperidine (20 %, v/v) in CH_2Cl_2 (20 ml). The mixture was stirred for 1 h at rt. The resin was washed with CH_2Cl_2 (3 x 30 ml), MeOH (3 x 30 ml) and dried under vacuum to give the resin **9** (2.89 g). IR (KBr) no C=O (amide) bond of N-Fmoc at 1701 cm^{-1}. The libraries were done in parallel fashion using an ACT Labtech synthesizers. Coupling of an amino acid (R^1). To the resin **9** (74 mg, ~0.046 mmol) under an argon atmosphere, a solution of Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (64 mg; 0.138 mmol), 1-Hydroxybenzotriazole hydrate (HOBt) (19 mg, 0.138 mmol), and the Fmoc-protected amino acid (0.138 mmol) in dry DMF (1 ml) was added and the mixture was stirred for 3 min. *N,N*-Diisopropylethylamine (DIPEA) (47 µl, 0.276 mmol) was then added and the suspension was stirred for 5 h at rt. The resin was washed with CH_2Cl_2 (6 x 3 ml), MeOH (3 x 3 ml) and dried under vacuum to give the resin **10**. Coupling of a carboxylic acid (R^2). The resin **10** was treated as above to hydrolyse the N-Fmoc protecting group. To this resin **10,** a solution of Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (72 mg, 0.138 mmol), HOBt (19 mg, 0.138 mmol), and the carboxylic acid (0.138 mmol) in dry DMF (1 ml) were added and the mixture was stirred for 3 min under argon atmosphere. DIPEA (47 µl; 0.276 mmol) was added and the suspension was stirred for 5 h at rt. The resin was washed with CH_2Cl_2 (6 x 3 ml), MeOH (3 x 3 ml) and dried under vacuum. Decoupling of **11** from resin **10**. To the resin were added successively 1 ml of a mixture CH_2Cl_2:THF (10:1) and a solution of HF/pyridine (40 µL), and the mixture was stirred for 2 h. CH_2Cl_2 (0.3 ml) and $NaHCO_3$ solid (150 mg) were added and the mixture was stirred for 30 min. After we added CH_2Cl_2 (0.2 ml) and $MgSO_4$ anh. (100 mg) and stirred for 15 min. The resin was filtered and the filtrate was washed

with a saturated aqueous solution of $NaHCO_3$ (5 ml) and finally with water (5 ml). The organic layer was evaporated under reduced pressure to give compounds **11** (Tables 1-3).

Sampling of library A

A representative sampling of the first library members, namely **A4, A7, A 11, A 13, A 16, A 18, A 25, A 27, A 29, A 32, A 37** and **A 43** was taken and each compound characterized by ^{1}H NMR, IR and LRMS.

A4: Yield: 73 % (16 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 1.00 (s, 19-CH_3), 0.70-2.05 (37 H), 2.10-2.65 (broad, 2α-CH, 2X CH_2N and NHCOC<u>H</u>), 3.05 (dd, J_1 = 6.4 Hz, J_2 = 13.4 Hz, 1H of COCH<u>C</u>H_2), 3.40-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.04 (m, 3β-CH), 5.15 (m, NHC<u>H</u>CO), 7.17 (d, J = 8.7 Hz, NH), 7.22 (m, 2H of Pyr), 8.45 (m, 2H of Pyr). IR (film) ν = 3345 (OH and NH), 1633 (C=O, amides). LRMS for $C_{38}H_{59}N_4O_4$ [MH^+]: 635.5 *m/z*.

A7: Yield: 63 % (12 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.88 (t, J = 6.9 Hz, CH_2<u>CH_3</u>), 1.02 (s, 19-CH_3), 1.19 (d, J= 6.8 Hz, CH<u>CH_3</u>), 0.70-2.10 (36 H), 2.18 (t, J = 7.5 Hz, NHCO<u>CH_2</u>), 2.30-2.75 (broad, 2α-CH and 2X CH_2N), 3.40-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.07 (m, 3β-CH), 4.83 (m, NHC<u>H</u>CO), 7.11 (d, J = 7.4, NH). IR (film) ν = 3385 (OH and NH), 1632 (C=O, amides). LRMS for $C_{32}H_{56}N_3O_4$ [MH^+]: 546.4 *m/z*.

A11: Yield: 70 % (14 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.90 (6 s, 2 x (<u>CH_3</u>)$_2$CH), 1.02 (s, 19-CH_3), 0.70-2.25 (42 H), 2.30-2.75 (broad, 2α-CH, 2X CH_2N and COCH<u>C</u>H), 3.45-3.75 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.07 (m, 3β-CH), 4.74 (m, CO<u>C</u>HNH), 6.99 (d, J = 9.3, NH). IR (film) ν = 3347 (OH and NH), 1627 (C=O, amides). LRMS for $C_{33}H_{58}N_3O_4$ [MH^+]: 560.7 *m/z*.

A13: Yield: 76 % (16 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.86 and 0.88 (2d, J= 6.8 Hz, CH(<u>CH_3</u>)$_2$), 1.02 (s, 19-CH_3), 0.70-2.15 (43 H), 2.20-2.75 (broad, 2α-

CH, 2X CH_2N, COCHCH and NHCOCH_2), 3.40-3.80 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.07 (m, 3β-CH), 4.72 (dd, J_1 = 7.0 Hz, J_2 = 9.1 Hz, COCHNH), 7.02 (d, J = 9.0, NH). IR (film) ν = 3348 (OH and NH), 1626 (C=O, amides). LRMS for $C_{36}H_{62}N_3O_4$ [MH^+]: 600.5 *m/z*.

A16: Yield: 81 % (17 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.89 (d, J = 6.0 Hz, CH(CH_3)$_2$), 1.00 (s, 19-CH_3), 0.65-2.15 (35 H), 2.20-2.65 (broad, 2α-CH and 2X CH_2N), 3.08 (dd, J_1 = 6.3 Hz, J_2 = 14.3 Hz, 1H of CHCH_2), 3.26 (dd, J_1 = 7.10 Hz, J_2 = 14.4 Hz, 1H of CHCH_2), 3.35-3.70 (broad, 2X CH_2N, 17α-CH and 17β-OH), 4.04 (m, 3β-CH), 5.10 (dd, J_1 = 6.90 Hz, J_2 = 15.1 Hz, COCHNH), 6.90 (m, SCHCHCHC), 7.30 (m, SCHCHCHC and NH). IR (film) ν = 3410 and 3310 (OH and NH), 1633 (C=O, amides). LRMS for $C_{35}H_{56}N_3O_4S$ [MH^+]: 614.5 *m/z*.

A18: Yield: 78 % (18 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.00 (s, 19-CH_3), 0.60-2.15 (38 H), 2.22 (t, J = 7.6 Hz, $COCH_2$), 2.30-2.65 (broad, 2α-CH, 2X CH_2N), 3.07 (dd, J_1 = 6.3 Hz, J_2 = 14.5 Hz, 1H of CHCH_2), 3.26 (dd, J_1 = 7.1 Hz, J_2 = 14.6 Hz, 1H of CHCH_2), 3.35-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.03 (m, 3β-CH), 5.09 (dd, J_1 = 6.8 Hz, J_2 = 15.3 Hz, COCHNH), 6.90 (m, SCHCHCHC), 7.27 (m, SCHCHCHC and NH). IR (film) ν = 3410 and 3310 (OH and NH), 1626 (C=O, amides). LRMS for $C_{38}H_{60}N_3O_4S$ [MH^+]: 654.5 *m/z*.

A25: Yield: 71 % (15 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.81 (t, J = 7.6 Hz, CH_3CH_2), 0.84 (d, J= 6.8 Hz, CH_3CH), 1.02 (s, 19-CH_3), 0.70-2.15 (34 H), 2.20-2.70 (broad, 2α-CH, 2X CH_2N and COCHCH), 3.40-3.80 (broad, 2X CH_2NCO, 17α-CH, 17β-OH and CH_2Ph), 4.06 (m, 3β-CH), 4.74 (dd, J_1 = 7.4 Hz, J_2 = 9.0 Hz, COCHNH), 7.21 (m, 2H of Ph), 7.31 (m, 3H of Ph and NH). IR (film) ν = 3410 and 3292 (OH and NH), 1624 (C=O, amides). LRMS for $C_{37}H_{58}N_3O_4$ [MH^+]: 608.5 *m/z*.

A27: Yield: 68 % (15 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.88 (t, J = 7.0 Hz, CH_3CH_2), 1.03 (s, 19-CH_3), 0.60-2.25 (36 H), 2.30-2.80 (broad, 2α-CH, 2X

CH_2N and $CO\underline{CH_2}$), 3.05 (d, J= 5.2 Hz, $COCH\underline{CH_2}$), 3.25-3.75 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.08 (m, 3β-CH), 4.57 and 4.85 (2d, J = 16.0 Hz, $N\underline{CH_2}Ph$), 5.58 (t, J = 5.2 Hz, COCHN), 7.19 (m, 4H of Ph). IR (film) ν = 3423 (OH and NH), 1644 (C=O, amides). LRMS for $C_{39}H_{60}N_3O_4$ [MH^+]: 634.4 *m/z*.

A29: Yield: 61 % (14 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.03 (s, 19-CH_3), 0.60-2.25 (36 H), 2.30-2.80 (broad, 2α-CH, 2X CH_2N), 3.30-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.07 (m, 3β-CH), 4.53 and 4.94 (2d, J = 16. 0 Hz, $N\underline{CH_2}Ph$), 5.55 (t, J = 5.4 Hz, COCHN), 7.16 (m, 4H of Ph). IR (film) ν = 3422 (OH and NH), 1641 (C=O, amides). LRMS for $C_{40}H_{60}N_3O_4$ [MH^+]: 646.3 *m/z*.

A32: Yield: 43 % (10 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.87 (t, J = 7.0 Hz, $CH_2\underline{CH_3}$), 0.94 (s, 19-CH_3), 0.60-2.15 (36 H), 2.15-2.65 (broad, 2α-CH and $COCH_2$), 3.10 (m, 2X CH_2N), 3.30 (m, $CH\underline{CH_2}$), 3.55 (m, 2X CH_2NCO and 17α-CH), 3.94 (m, 3β-CH), 5.18 (m, $CO\underline{CH}NH$), 7.02, 7.09 and 7.22 (3m, 3H aromatic and NH), 7.37 and 7.63 (2d, J = 7.9 Hz, 2H aromatic), 10.1 (s, NH). IR (film) ν = 3395 (OH and NH), 1626 (C=O, amides). LRMS for $C_{40}H_{61}N_4O_4$ [MH^+]: 661.4 *m/z*.

A37: Yield: 67 % (26 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.85 (t, J = 6.9 Hz, $CH_2\underline{CH_3}$), 1.01 (s, 19-CH_3), 0.65-2.25 (37 H), 2.30-2.65 (broad, 2α-CH and 2X CH_2N), 2.70-2.95 (m, $CHCH_2$), 3.35-3.70 (broad, 2X CH_2N, 17α-CH and 17β-OH), 4.05 (m, 3β-CH), 5.14 (dd, J_1 = 6.5 Hz, J_2 = 15.0 Hz, $CO\underline{CH}NH$), 6.63 (d, J = 1.2Hz, CH of His), 7.15 (m, 6H of Ph_3), 7.25 (d, J = 1.4 Hz, CH of His), 7.41 (m, 9H of Ph_3). IR (film) ν = 3418 and 3304 (OH and NH), 1632 (C=O, amides). LRMS for $C_{54}H_{72}N_5O_4$ [MH^+]: 854.2 *m/z*.

A43: Yield: 71 % (22 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.65-2.10 (40 H), 2.20-2.70 (broad, 2α-CH, 2X CH_2N, $COCH\underline{CH_2}$ and $COCH_2$), 2.80 (s, CH_3S), 3.40-3.70 (broad, 2X CH_2NCO, 17α-CH, 17β-OH and $\underline{CH_2}S$), 4.07 (m, 3β-

CH), 5.03 (m, COCHNH), 7.17 (d, J = Hz, NH). IR (film) ν = 3408 and 3304 (OH and NH), 1632 (C=O, amides). LRMS for $C_{36}H_{62}N_3O_4S$ [MH^+]: 632.5 *m/z.*

Sampling of library B

A representative sampling of the second library members, namely **B4, B7, B11, B18, B25, B26, B34, B7,** and **B41** was taken and each compound characterized by ^{1}H NMR, IR and LRMS.

B4: Yield: 84 % (37 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.99 (s, 19-CH_3), 0.65-2.10 (36 H), 2.15-2.60 (broad, 2α-CH, 2X CH_2N and NHCOCH), 2.88 and 3.02 (2m, CH_2Ph), 3.30-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.02 (m, 3β-CH), 5.08 (dd, J_1 = 7.3 Hz, J_2 = 15.4 Hz, COCHNH), 7.02 (d, J= 8.4 Hz, NH), 7.24 (m, 5H of Ph). IR (film) ν = 3384 (OH and NH), 1626 (C=O, amides). LRMS for $C_{39}H_{60}N_3O_4$ [MH^+]: 634.5 *m/z.*

B7: Yield: 87 % (42 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.71 (s, 18-CH_3), 0.85 (t, J = 7.0 Hz, CH_3CH_2), 0.97 (s, 19-CH_3), 0.60-2.15 (35 H), 2.18 (t, J = 7.6 Hz, $COCH_2$), 2.25-2.70 (broad, 2α-CH and 2X CH_2N), 2.92 and 3.05 (2dd, J_1 = 7.1 Hz, J_2 = 13.3 Hz, $CHCH_2$Ph), 3.30-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.04 (m, 3β-CH), 5.15 (t_{app}, J 6.8 = Hz, COCHNH), 7.19 (d, J= 7.9 Hz, NH), 7.34 (m, 3H of Ph), 7.45 (t, J = 7.6 Hz, 2H of Ph), 7.56 (d, J = 8.2 Hz, 2H of Ph), 7.63 (d, J = 8.4 Hz, 2H of Ph). IR (film) ν = 3400 and 3304 (OH and NH), 1626 (C=O, amides). LRMS for $C_{44}H_{64}N_3O_4$ [MH^+]: 698.5 *m/z.*

B11: Yield: 72 % (34 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.85 (t_{app}, J = 6.7 Hz, CH_3CH), 0.92 (d, J = 6.6 Hz, 1x CH_3CH), 1.00 and 1.02 (2s, 19-CH_3), 0.70-2.20 (33 H), 2.30-2.70 (broad, 2α-CH and 2X CH_2N), 2.85 (m, 1 H of $CHCH_2$Ph), 2.99 (dd, J_1 = 7.1 Hz, J_2 = 13.4 Hz, 1H of $CHCH_2$Ph), 3.35-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.04 (m, 3β-CH), 5.12 (m, COCHNH), 7.21 (d, J = 8.4 Hz, 2H of PhBr), 7.44 (t, J = 8.4 Hz, 2H of PhBr). IR (film) ν = 3389 (OH and NH), 1625 (C=O, amides). LRMS for $C_{37}H_{57}BrN_3O_4$ [MH^+]: 686.3 and 688.3 *m/z.*

B18: Yield: 71 % (33 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.99 (s, 19-CH_3), 0.65-2.15 (37 H), 2.17 (t, J = 7.6 Hz, $COCH_2$), 2.30-2.60 (broad, 2α-CH and 2X CH_2N), 2.85 (m, 1 H of $CHCH_2Ph$), 3.01 (dd, J_1 = 7.0 Hz, J_2 = 13.4 Hz, 1H of $CHCH_2Ph$), 3.30-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.03 (m, 3β-CH), 5.11 (m, $COCHNH$), 7.20 (d, J = 8.5 Hz, NH), 7.27 (d, J = 5.6 Hz, 4H of PhCl). IR (film) ν = 3425 and 3307 (OH and NH), 1633 (C=O, amides). LRMS for $C_{40}H_{61}ClN_3O_4$ [MH^+]: 682.3 *m/z*.

B25: Yield: 31 % (14 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.98 (s, 19-CH_3), 0.70-2.15 (26 H), 2.20-2.70 (broad, 2α-CH and 2X CH_2N), 2.85 (under solvent, 1 H of $CHCH_2Ph$), 2.99 (dd, J_1 = 7.0 Hz, J_2 = 13.4 Hz, 1H of $CHCH_2Ph$), 3.25-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.05 (m, 3β-CH), 5.05 (m, $COCHNH$), 6.96 (t, J = 8.9 Hz, 2H of PhF), 7.15-7.35 (3H of PhF and 4H of Ph). IR (film) ν = 3400 and 3304 (OH and NH), 1626 (C=O, amides). LRMS for $C_{40}H_{55}FN_3O_4$ [MH^+]: 660.3 *m/z*.

B26: Yield: 88 % (44 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.85 (t_{app}, J = 6.9 Hz), 0.92 (d_{app}, J = 6.6 Hz, $(CH_3)_2CH$), 1.00 and 1.02 (2s, 19-CH_3), 0.60-2.15 (35 H), 2.20-2.70 (broad, 2α-CH and 2X CH_2N), 2.85 (under solvent, 1 H of $CHCH_2PH$), 2.97 (dd, J_1 = 7.1 Hz, J_2 = 13.4 Hz, 1H of $CHCH_2Ph$), 3.35-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.04 (m, 3β-CH), 5.11 (m, $COCHNH$), 7.07 (d, J = 8.2 Hz, 2H of PhI), 7.17 (d, J = 8.2 Hz, NH), 7.64 (d, J = 8.3 Hz, 2H of PhI). IR (film) ν = 3413 and 3319 (OH and NH), 1625 (C=O, amides). LRMS for $C_{37}H_{57}IN_3O_4$ [MH^+]: 734.3 *m/z*.

B34: Yield: 84 % (37 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.99 (s, 19-CH_3), 0.65-2.15 (36 H), 2.28 (s, $PhCH_3$), 2.15-2.65 (broad, 2α-CH and 2X CH_2N and $NHCOCH$), 2.85 (under solvent, 1 H of $CHCH_2Ph$), 2.94 (dd, J_1 = 7.50 Hz, J_2 = 13.3 Hz, 1H of $CHCH_2Ph$), 3.25-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.02 (m, 3β-CH), 5.03 (m, $COCHNH$), 6.99 (d, J = 8.6Hz, NH), 7.09 (d, J = 3.1 Hz, 4H of PhMe). IR (film) ν = 3411 (OH and NH), 1626 (C=O, amides). LRMS for $C_{40}H_{62}N_3O_4$ [MH^+]: 648.4 *m/z*.

B37: Yield: 82 % (37 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.86 (t, J = 7.2 Hz, $\underline{CH_3}CH_2$), 1.00 (s, 19-CH_3), 0.65-2.10 (36 H), 2.14 (t, J = 7.4 Hz, $COCH_2$), 2.25-2.70 (broad, 2α-CH and 2X CH_2N), 2.94 (dd, J_1 = 7.7 Hz, J_2 = 13.5 Hz 1H of $COCH\underline{CH_2}$), 3.13 (dd, J_1 = 6.3 Hz, J_2 = 13.4 Hz, 1H of $COCH\underline{CH_2}$), 3.35-3.70 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 4.05 (m, 3β-CH), 5.17 (m, $CO\underline{CH}NH$), 7.24 (d, J = 8.1 Hz, NH), 7.47 (d, J = 8.2 Hz, 2H of PhCN), 7.67 (d, J = 8.3 Hz, 2H of PhCN). IR (film) ν = 3389 (OH and NH), 1634 (C=O, amides). LRMS for $C_{39}H_{59}N_4O_4$ [MH^+]: 647.4 *m/z*.

B41: Yield: 68 % (20 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.87 (m, $(\underline{CH_3})_2CH$), 0.98 (s, 19-CH_3), 0.65-2.15 (35 H), 2.20-2.60 (broad, 2α-CH and 2X CH_2N), 2.92 (m, $\underline{CH_2}Ph$), 3.25-3.65 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 3.76 (s, CH_3O), 4.02 (m, 3β-CH), 5.10 (dd, J_1 = 7.5 Hz, J_2 = 15.2 Hz, $CO\underline{CH}NH$), 6.83 (d, J = 8.6 Hz, 2H of $\underline{Ph}OCH_3$), 7.15 (d, J = 8.7 Hz, 2H of $\underline{Ph}OCH_3$). IR (film) ν = 3353 (OH and NH), 1627 (C=O, amides). LRMS for $C_{38}H_{60}N_3O_5$ [MH^+]: 638.5 *m/z*.

Sampling of library C

A representative sampling of the third library members, namely **C4**, **C8**, **C11**, and **C20** was taken and each compound characterized by ^{1}H NMR, IR and LRMS.

C4: Yield: 74 % (20 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.01 and 1.02 (2s, 19-CH_3), 0.65-2.20 (39 H), 2.25-2.85 (broad, 2α-CH, 2X CH_2N and NCOCH), 3.20-3.70 (broad, 2X CH_2NCO, 17α-CH, 17β-OH and NCH_2 of Pro), 4.08 (m, 3β-CH), 4.83 and 5.01 (2dd, J_1 = 3.0 Hz, J_2 = 8.4 Hz, $CO\underline{CH}N$). IR (film) ν = 3401 (OH and NH), 1629 (C=O, amides). LRMS for $C_{35}H_{58}N_3O_4$ [MH^+]: 584.3 *m/z*.

C8: Yield: 60 % (17 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.01 and 1.03 (2s, 19-CH_3), 0.65-2.15 (40 H), 2.20-2.70 (broad, 2α-CH, 2X CH_2N and $COCH\underline{CH_2}$), 3.30-3.90 (broad, 2X CH_2NCO, 17α-CH, 17β-OH and $N\underline{CH_2}CHO$), 4.08 (m, 3β-CH), 4.4 and 4.55 (2m, CHO), 4.92 and 5.03 (2dd, J_1 = 6.4 Hz, J_2 = 8.1 Hz, COCHN). IR (film) ν = 3390 (OH and NH), 1634 (C=O, amides). LRMS for $C_{36}H_{60}N_3O_5$ [MH^+]: 614.5 *m/z*.

C11: Yield: 64 % (19 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 0.94 (m, ($\underline{CH_3}$)$_2$CH), 1.03 (s, 19-CH_3), 1.17 (s, $(CH_3)_3C$), 0.65-2.20 (44 H), 2.30-2.75 (broad, 2α-CH, 2X CH_2N and COCH$\underline{CH_2}$), 3.25-3.90 (broad, 2X CH_2N, 17α-CH, 17β-OH and N$\underline{CH_2}$CHO), 4.10 (m, 3β-CH), 4.5 (m, CHO), 4.94 and 5.01 (2m, COCHN). IR (film) ν = 3400 (OH and NH), 1634 (C=O, amides). LRMS for $C_{37}H_{64}N_3O_5$ [MH^+]: 630.5 *m/z*.

C20: Yield: 68 % (19 mg). ^{1}H NMR (400 MHz, acetone-d_6) δ = 0.72 (s, 18-CH_3), 1.02 (s, 19-CH_3), 0.65-2.25 (26 H), 2.30-2.75 (broad, 2α-CH and 2X CH_2N), 3.10 (m, CH$\underline{CH_2}$S), 3.35-3.75 (broad, 2X CH_2NCO, 17α-CH and 17β-OH), 3.82 (s, $\underline{CH_2}$Ph), 4.03 (m, 3β-CH), 4.56 and 4.91 (2d, J = 8.7 Hz, NCH_2S), 5.40 (m, COCHN), 7.26 (m, 5H of Ph). IR (film) ν = 3408 (OH and NH), 1646 (C=O, amides). LRMS for $C_{35}H_{52}N_3O_4S$ [MH^+]: 610.9 *m/z*.

Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD) were routinely grown in suspension in 90 % RPMI-1640 (Roswell Park Memorial Institute) (Sigma, Saint Louis, USA) containing L-glutamine (2 nM), antibiotics (100 IU penicillin/ml, 100 μg streptomycin/ml) and supplemented with 10 % (v/v) fetal bovine serum (FBS), in a 5 % CO_2 humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10 % FBS.

Cell proliferation assay

The cell proliferation assay was determined by MTS assay according to the procedure described previously[11] with some modification. In brief, triplicate cultures of 1 x 10^4 HL-60 cells in a total of 100 μl medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ) were incubated at 37°C, 5 % CO_2. Aminosteroids was dissolved in ethanol to prepare the stock solution of 1 x 10^{-2} M. These compounds was diluted at two concentrations with culture media, added to each well and incubated for 3

days. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Acknowledgments

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Chapitre 6

Dérivés du 2-méthoxyestradiol: Synthèse chimique et effet cytotoxique contre les cellules de la leucémie myéloïde humaine HL-60

6.1 Avant-propos

Ma contribution à ce projet a été d'effectuer la synthèse de certains dérivés du 2-méthoxyestradiol et d'en faire la caractérisation par IR, RMN ^{1}H, RMN ^{13}C et LRMS afin de confirmer leur structure. Par la suite, j'ai déterminé l'activité antiproliférative de tous les dérivés sur les cellules leucémiques HL-60 et rédigé ce manuscrit en collaboration avec mon directeur de recherche Donald Poirier.

6.2 Résumé

Il a été rapporté que le 2-méthoxyestradiol (2-ME), un stéroïde endogène chez l'humain, induisait, à fortes doses, des effets antiprolifératifs sur plusieurs cancers dont la leucémie. Il existe plusieurs agents pour traiter la leucémie, mais ces derniers ne sont pas toujours efficaces et sont souvent très toxiques. Dans cette étude, nous avons étudié le pouvoir antiprolifératif d'une série de dérivés du 2-ME sur les cellules leucémiques HL-60 à trois concentrations (0.1, 1 et 10 μM). Nos résultats ont montré que la présence du noyau stéroïdien est nécessaire pour l'inhibition de la prolifération des cellules HL-60, puisque l'évaluation biologique de composés constitués seulement des cycles A et B du 2-ME induisait aucun effet cytotoxique. De plus, une substitution en position 16 ou 17 par différents groupements diminue le potentiel cytotoxique du 2-ME. Par contre, l'introduction d'un groupement sulfamate en C-3 permet de contrebalancer l'effet négatif de la substitution du cycle D de manière à induire une activité antiproliférative similaire au 2-ME à 10 μM. De façon intéressante, l'addition d'un sulfamate en position 3 du 2-ME induisait une inhibition supérieure à celle du 2-ME contre la croissance des cellules HL-60, mais cet effet disparaît lorsque le groupe sulfamate est substitué. Ces résultats suggèrent que le dérivé 3-*O*-sulfamate du 2-ME n'agit pas seulement comme une prodrogue. De plus, l'introduction d'un cyclohexylméthyl ou d'un benzyl en position 3 du 2-ME par un lien éther inhibe la croissance des cellules HL-60 à 10 μM. Finalement, ces résultats suggèrent qu'une substitution appropriée du groupement hydroxyle en position 3 du 2-ME peut conduire à une nouvelle famille de stéroïdes potentiellement intéressants contre la leucémie myéloïde.

Journal (to be submitted)

2-Methoxyestradiol derivatives: chemical synthesis and cytotoxic effect against acute myeloid leukemia (HL-60) cells

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Keywords: Leukemia, 2-Methoxyestradiol, steroid, HL-60 cells.

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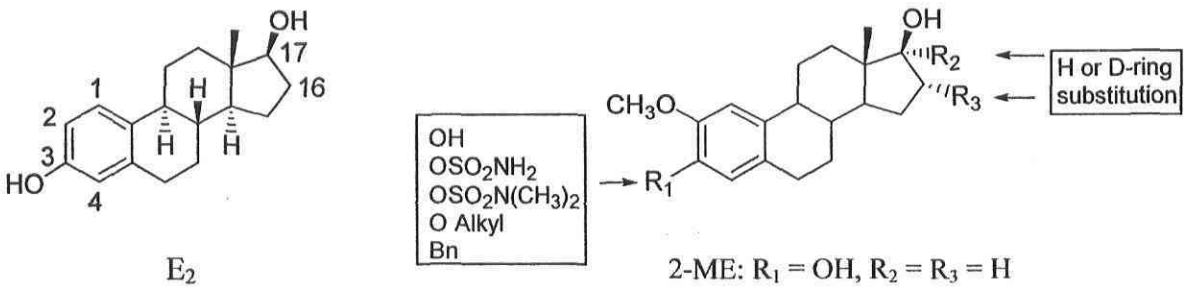
Abstract

It was reported that 2-methoxyestradiol (2-ME), a natural mammalian steroid, had some antiproliferative effects at high doses in several cancer including leukemia. Leukemia is an important cancer who affects a large population. There are several therapeutics agents available but these compounds are not always efficient and are highly toxics. In this study, we investigated the potential antiproliferative effect of a series of 2-ME derivatives on human leukemia cells. The effects of 0.1, 1 and 10 μM of all steroids were measured in HL-60 cells line using a cell proliferation assay (MTS). First of all, our results have shown that the presence of a steroidal nucleus is required to inhibit the proliferation of HL-60 cells, since the biological evaluation of a compound constituted only of A and B rings of 2-ME induced no cytotoxic effect at all. Furthermore, the substitution at position 16 or 17 by different groups decreased the cytotoxic potential of 2-ME. The introduction of a sulfamate group at C-3 allows however counterbalancing the negative effect of D-ring substitution, thus displaying a similar antiproliferative activity than 2-ME at 10 μM. Interestingly, addition of a sulfamate moiety in position 3 of 2-ME induced a greater inhibition than 2-ME against leukemia cell growth, but this effect is cancelled when this sulfamate was substituted. These results suggest that the 3-*O*-sulfamate derivative of 2-ME is unlikely to act simply as prodrug of the 2-ME. Furthermore, the introduction of a cyclohexylmethyl or benzyl group at position 3 of 2-ME by an ether link inhibited the HL-60 cell growth at 10 μM. Finally, these results suggest that an appropriate substitution of the hydroxyl group in position 3 of 2-ME can lead to a new family of agents against myeloid leukemia.

Introduction

The mammalian steroid 2-methoxyestradiol (2-ME) is a physiological nonpolar metabolite of the endogenous estrogen estradiol-17β (E_2) (Figure 1). E_2 is hydroxylated by NADPH-dependent cytochrome P450 enzymes mainly in the liver to 2-hydroxyestradiol, a major catechol E_2 metabolite formed in humans. This catechol estrogen is metabolically *O*-methylated to monomethyl ether such as 2-ME by catechol-*O*-methyltransferase.[1-4] This metabolite fails to bind the estrogen receptor and has a little or no uterotropic activity in ovariectomized female rats.[5-8] At the beginning, 2-ME was viewed as a natural compound without any potent biological activity. But, since a few years, it was reported to have anticarcinogenic activity. Indeed, in 1994, scientists recognized two properties of 2-ME: 1) it inhibits neovascularization of tumor xenografts and 2) it directly inhibits tumor cell proliferation.[9] Therefore, this E_2 derivative steroid has a strong antiangiogenic effect at pharmacological concentration.[10,11]



E_2 2-ME: $R_1 = OH$, $R_2 = R_3 = H$

Figure 1. General structure of E_2, 2-ME and 2-ME derivatives that will be tested in our study. The stereogenic centers are illustrated only for E_2, but they are the same for all often E_2 derivatives reported in this paper.

It has also been reported that 2-ME induces apoptosis in various cell types.[12-14] Thus, in a recent study, 2-ME potently induced apoptosis in human leukemia cells through a mechanism that involves the selective inhibition of superoxide dismutase and accumulation of reactive oxygen species.[15] Moreover, it was demonstrated that 2-ME inhibits tubulin polymerization by binding to the colchicine site of the tubulin protein, resulting in cell cycle arrest in the G2/M phase of the cell cycle and induction of

apoptosis.[16,17] Since 2-ME is relatively nontoxic to normal tissues,[15] it can offer an effective therapy against acute leukemia and others types of cancers. At this time, it is included in phase I clinical trials for breast cancer and phase II clinical trials for prostate cancer and multiple myeloma.[18,19] Even though 2-ME is active in vivo, important dose of the compound (75-150 mg/kg) have been used in animal studies to inhibit tumor growth.[10,11] In clinical trials, doses in the 6-8 g/day range have been administrated. Potential causes to use such high doses are its relative poor bioavailability and rapid inactivation by conjugation and oxidation of the 17β-hydroxyl group. Indeed, the 3-sulphated and 17-keto derivatives of 2-ME are reported to be inactive for inhibiting the in vitro proliferation of breast cancer cells.[18] However, it was known that the E_2-3-O-sulfamate, has a higher systemic estrogenic activity after oral administration than E_2 due to reduced hepatic metabolism of the drug.[20] Moreover, addition of this sulfamate group at position 3 of 2-ME induce a mean activity over several human cancer cell lines 80-fold greater than that of the established anticancer agent,[21] but activity of these derivatives against leukemia cells lines was not reported.

Acute myeloid leukemia (AML) is a disease which is characterized by abnormalities in the proliferation/differentiation cellular ratio. This type of leukemia is one of the most common types, with 11, 930 new cases in the United States in 2006[22] accounting for 80 % of all acute leukemia in adults.[23] Like several diseases, acute leukemia is treated with multiple aggressive therapies. Standard induction chemotherapy, depending on patient tolerability, is at present the treatment of choice in patients with myeloid leukemia but its efficacy is limited because of toxicity to normal cells. Although that current therapies result in 60 % to 80 % complete remissions in newly diagnosed patients, most of them will relapse with resistant disease.[24] Therefore, it is important to discover new efficient compounds with weak adverse side effects.

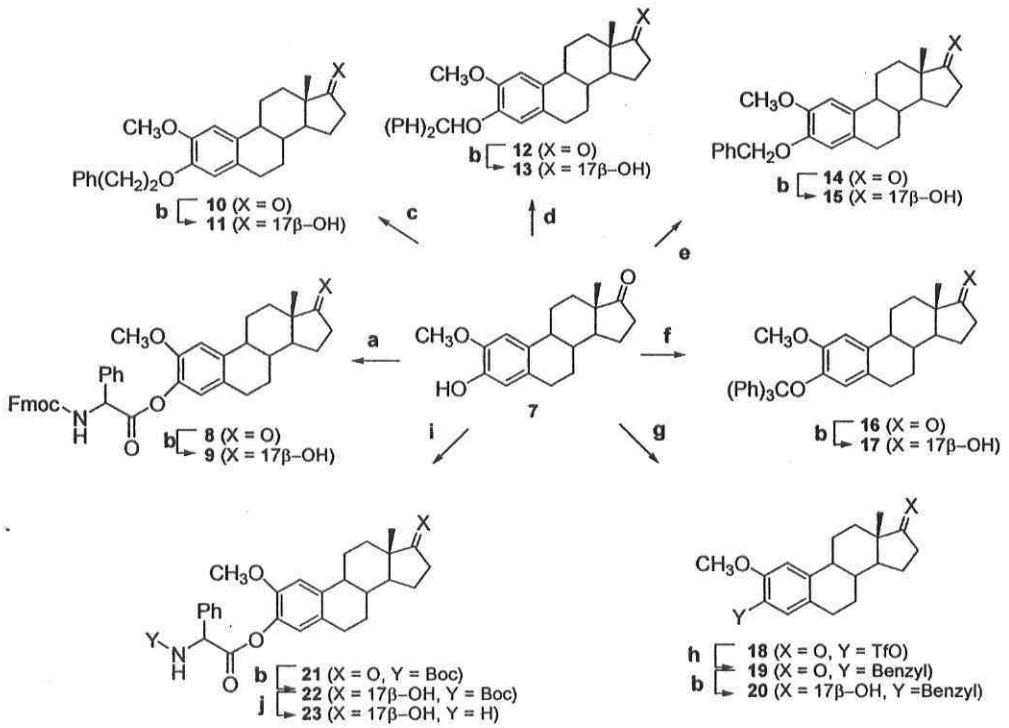
In this study, we selected a series 2-ME derivatives (Figure 1 and Table 1) and evaluated their antiproliferative potentials against human leukemia HL-60 cells.

Results and discussion

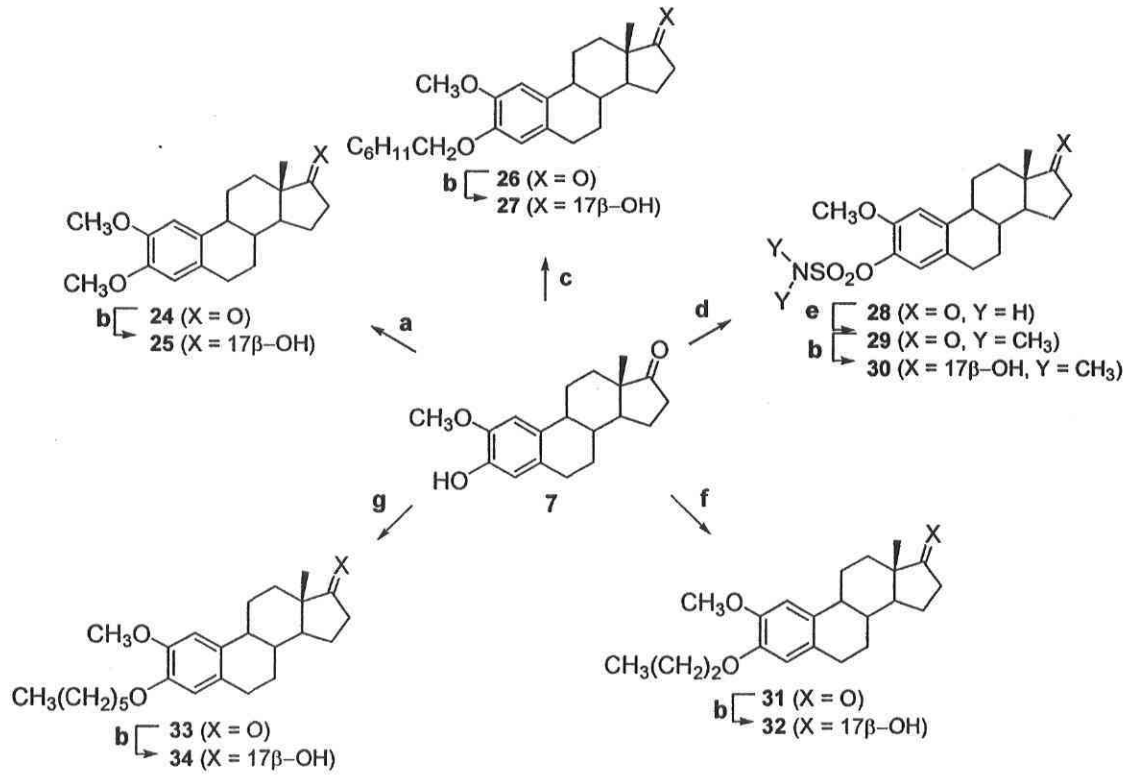
Chemical synthesis

The syntheses of compounds **1**, **3**, **4**, **5** and **6** (table 1) have been already reported in literature.[25-28] All other compounds were synthesized from 2-methoxyestrone (**7**), which was obtained similarly as reported in literature,[29] by using esterification, nucleophilic substitution, sulfamoylation reaction and classical reduction as reported in schemes 1 and 2. Briefly compounds **9** and **22** were obtained from **7** following an esterification and reduction with sodium borohydride. The free amino acid derivative of **23** was obtained from **22** after acid hydrolysis of the Boc group. For the synthesis of compounds **11**, **13**, **15**, **17**, **25**, **27**, **32** and **34** we used different nucleophilic substitution reactions followed by reduction with sodium borohydride. To obtain compound **20**, we generated the triflate derivative **18** from the corresponding phenol **7**, introduced the benzyl group with palladium/phosphino-catalyzed Negishi coupling,[30] and reduced the ketone in position 17. A sulfamoylation of **7** was necessary for the synthesis of **28**, which was di-N-methylated and reduced to give **30**. The compound **2,** constituted by only A and B rings of 2-ME, was synthesized in the same manner as for compound **7** starting from 5,6,7,8-tetrahydro-2-naphtol.

These 2-ME derivatives were obtained in good to excellent yields and were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS analysis.



Scheme 1. Synthesis of **8-23**. Reagents and conditions: **(a)** Fmoc-Phg-OH, PyBOP, HOBt, DIPEA, DMF, rt; **(b)** $NaBH_4$, $MeOH/CH_2Cl_2$, 0 °C; **(c)** $Ph(CH_2)_2Br$, Cs_2CO_3, DMF, 110 °C; **(d)** Ph_2CHBr, Cs_2CO_3, DIPEA, CH_2Cl_2, reflux; **(e)** $PhCH_2Br$, Cs_2CO_3, CH_3CN, rt; **(f)** Ph_3CCl, DIPEA, CH_2Cl_2, rt; **(g)** 4-nitrophenyl trifluoromethanesulfonate, K_2CO_3, DMF, rt; **(h)** *i)*$PhCH_2Br$, Zn^0, $Br(CH_2)_2Br$, TMSCl, DMF, 80 °C; *ii)*$PhCH_2ZnBr$, $Pd_2(dba)_3$, dppf, Bu_4NI, DMF,100 °C; **(i)** Boc-Phg-OH, PyBrOP, HOBt, DIPEA, DMF, rt; **(j)** TFA, CH_2Cl_2, rt.



Scheme 2. Synthesis of **24-34**. Reagents and conditions: **(a)** MeI, Cs_2CO_3, THF, reflux; **(b)** $NaBH_4$, MeOH/CH_2Cl_2, 0 °C; **(c)** $C_6H_{11}CH_2Br$, Cs_2CO_3, DMF, 110 °C; **(d)** NH_2SO_2Cl, DBMP, CH_2Cl_2, rt; **(e)** MeI, Cs_2CO_3, THF, reflux; **(f)** $CH_3(CH_2)_2Br$, Cs_2CO_3, DMF, 110 °C; **(g)** $CH_3(CH_2)_5Br$, Cs_2CO_3, DMF, 110 °C.

Biological results

These 2-ME derivatives were evaluated for their capacity to inhibit the proliferation of HL-60 cells growth, a human leukemia cell line and the results are presented in Table 1. Previous study had shown that 2-ME (**1**) induced apoptosis in several human leukemia cell lines (HL-60, U937 and Jurkat).[31] In accord with these results, 2-ME (**1**) caused 50 % growth inhibition at a concentration of 1 μM in our cell proliferation assay. To reach this

activity, the steroidal nucleus is required since biological evaluation of compound **2**, that consist only of A and B rings of 2-ME, induced no cytotoxic activity at all on HL-60 cell growth. Moreover, this antiproliferative activity was almost disappeared when the D-ring of 2-ME was substituted. In fact, introducing a bromopropyl or a benzyl group at position 16 or 17 of 2-ME, compounds **3** and **4** respectively, caused a lost of all cytotoxic activity against HL-60 cells. Thus, the antiproliferative effect of a 2-methoxy group is destroyed by the presence of a substitution at C16 or C17. Interestingly the addition of a sulfamate group at C-3 allowed to recover a part of the efficiency of 2-ME (58 % at 10 μM for **5**). Since a sulmafate moiety increased the cell growth inhibition of **4**, we decided to evaluate the sulfamoylation of 2-ME. Thus, compound **6** better inhibited (10, 62 and 61 %) than 2-ME (**6**, 50 and 55 %) the cell proliferation after treatment at concentrations of 0.1, 1 and 10 μM, respectively. However, this inhibitory potency can be abolished by alkylating the sulfamate nitrogen since compound **30** caused only 10 % cell growth inhibition at the higher concentration of 10 μM. This biological result correlates with those from an earlier study that clearly suggested that an unsubstitued sulfamate group is crucial for cell growth inhibition and that the compound **6** is unlikely to act just as a prodrug of 2-ME.[21]

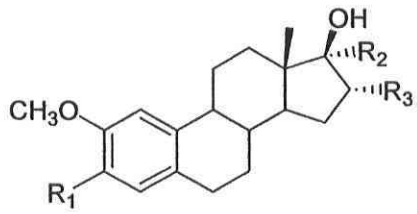
After these substitutions on D-ring, we decided to evaluate other modifications at position 3 of steroidal A-ring. First of all, it was obvious that a second methoxy group at position 3 (or a 3-*O*-methylation) induced a total loss of biological activity as demonstrated by the results achieved with compound **25**. Furthermore, addition of a short or a long alkyl chain at position 3, compounds **32** and **34** respectively, showed weak inhibitions of cell growth when compared to 2-ME. Interestingly, when the OH was substituted by a cyclohexylmethoxy or benzyloxy group, compounds **27** and **15** caused 50 and 65 % of cell growth inhibition respectively. These effects were similar to that of 2-ME at 10 μM, but lower at 1 μM. An additional methylene before the phenyl group of **15** or the absence of an ether link, compounds **11** and **20** respectively, displayed any inhibition of cell growth. Also, a bulky ether group although containing a phenyl group was less effective than **15**. Indeed addition of a second phenyl group, compound **13**, displayed no cytotoxic activity. The addition of a third phenyl group, compound **17**, induced a cytotoxic effect, but this

activity was the result of the cleavage of the trityl group in culture medium (recovery of 2-ME).

Finally, the introduction of an amino acid having a phenyl group by generating an ester link at C-3 of 2-ME was carried out. These results reveal that compounds **9**, **22** and **23** give interesting cytotoxic activities at 1 and 10 μM. However, the ester link of these three compounds was cleaved after 3 days of treatment. This degradation induces the formation of metabolite 2-ME in the culture medium, suggesting that the biological activity is displayed by this drug.

In summary, thirteen new derivatives of 2-ME were synthesized. Subsequently, all derivatives were evaluated and the presence of a cyclohexylmethyl or a benzyl groups at position 3 of 2-ME led to cytotoxic agents. However, the presence of a sulfamate group displayed higher antiproliferative activity against HL-60 cells. This biological result suggests that this 3-*O*-sulfamate derivative is unlikely to act simply as prodrug of the 2-ME as observed for the ester or trityl derivatives of 2-ME. Our observations prove that the 2-ME nucleus is essential and that the sulfamate moiety in position 3 is important to obtain a better inhibition of cell growth. However, we have no explanation for the cytotoxic effect obtained by the cyclohexylmethyl or benzyl group of compounds **27** and **15**, respectively. Further study will be necessary to better understand the role of a cyclohexylmethoxy, benzyloxy or sulfamate at position 3 of 2-ME on the proliferation of HL-60 cells. It will be also important to extend our SAR study in A-, B- or C-ring of 2-ME in order to provide better agents against myeloid leukemia.

Table 1. Chemical studies of 2-ME derivatives and their effect on HL-60 leukemia cell growth.



Compound	R_1	R_2	R_3	*Inhibition (%)**		
				10 μM	1 μM	0.1 μM
1 (2-ME)	OH	H	H	55	50	6
2**	OH	-	-	0	0	0
3	OH	H	$(CH_2)_3Br$	0	9	6
4	OH	CH_2Ph	H	4	9	3
5	OSO_2NH_2	CH_2Ph	H	58	7	8
6	OSO_2NH_2	H	H	61	62	10
30	$OSO_2N(CH_3)_2$	H	H	10	0	0
25	OCH_3	H	H	6	0	0
32	$O(CH_2)_2CH_3$	H	H	20	0	0
34	$O(CH_2)_5CH_3$	H	H	7	2	4
27	$OCH_2C_6H_{11}$	H	H	50	0	0
15	OCH_2Ph	H	H	65	0	0
11	OCH_2CH_2Ph	H	H	0	0	0
20	CH_2Ph	H	H	1	0	0
13	$OCHPh_2$	H	H	0	0	0
17	$OCPh_3$	H	H	46	14	2
9	OCOCH(Ph)NH-Fmoc	H	H	50	37	0
22	OCOCH(Ph)NH -Boc	H	H	50	33	0
23	$OCOCH(Ph)NH_2$	H	H	52	29	0

(*) Inhibition are the mean values obtained from experiments performed in triplicate, SEM = ± 7 %.

(**) 5,6,7,8-tetrahydro-3-methoxynaphthalen-2-ol (a 2-ME derivative without B and C ring

Experimental Section

Chemistry

Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montréal, Qc, Canada) and were used as received. Anhydrous CH_2Cl_2, CH_3CN and DMF were obtained from Sigma-Aldrich in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF), used in anhydrous conditions, was distilled from sodium/benzophenone ketyl under argon. Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel 60 F_{254} plates (Whatman, Madison, UK) and 230-400 mesh silica gel was used for flash chromatography (Silicycle, Québec, Qc, Canada). Infrared spectra (IR) were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only the significant bands reported in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded with a Brucker Avance 400 digital spectrometer (Billerica, MA, USA) at 400 and 100 MHz, respectively. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.16 ppm) or acetone (2.05 and 206.26 ppm) for ^1H and ^{13}C, respectively. Low-resolution mass spectra (LRMS) spectra were recorded on a PE Sciex API-150ex appartus (Foster City, CA, USA) equipped with a turbo ion spray source.

Synthesis of 2-methoxy-3-[2-(Fmoc-amino)-2S-phenyl-acetoxy]-estra-1,3,5(10)-trien-17-one (**8**)

N-Fmoc-L-phenyl glycine (217 mg, 0.58 mmol), PyBOP (302 mg, 0.58 mmol) and HOBt (78 mg, 0.58 mmol) were dissolved in DMF under argon and DIPEA (202 µl, 1.16 mmol) was subsequently added. This mixture was added to a solution of 2-methoxyestrone (**7**) (87 mg, 0.29 mmol) and stirred at rt overnight. The reaction was quenched with water and the crude product was extracted with EtOAc. The combine organic layer was washed with water, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography with hexanes:EtOAc (80:20) yielded 189 mg (99 %) of compound **8**. ^1H NMR ($(CD_3)_2CO$) δ = 0.90 and 0.91 (2 s, 18-CH_3), 1.30-2.50 (13H), 2.78 (m, 6-CH_2), 3.66 and 3.67 (2 s, OCH_3), 4.25 (m, $OCH_2\underline{CH}$), 4.36 (m, $O\underline{CH_2}CH$), 5.65 (d, J = 8.1 Hz, $\underline{CH}$Ph), 6.69 (d_{app}, J =

6.2 Hz, 1-CH), 6.98 (s, 4-CH), 7.29 (m, 2H of Fmoc), 7.43 (m, 5H), 7.63 (d, J = 7.4 Hz, 2H of Fmoc), 7.74 (d, J = 7.4 Hz, 2H of Fmoc), 7.86 (d, J = 7.5 Hz, 2H of Fmoc).

Synthesis of 2-methoxy-3-[2-(Fmoc-amino)-2S-phenyl-acetoxy]-1,3,5(10)-estratrien-17β-ol (**9**)

To a solution of **8** (179 mg, 0.27 mmol) in MeOH (10 ml) and CH_2Cl_2 (5 ml) was added sodium borohydride (21 mg, 0.55 mmol). The reaction mixture was stirred at rt for 2 h then quenched by the addition of saturated NH_4Cl (20 ml). The resulting solution was extracted with CH_2Cl_2 dried over $MgSO_4$ and evaporated to dryness. The desired product was purified by chromatography (hexanes:EtOAc (80:20)) to give 70 mg (40 %) of **9** and about 30 % of 2-ME. IR (film) ν = 3422 (OH and NH), 1760 (C=O ester), 1713 (C=O, carbamate); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.777 and 0.781 (2 s, 18-CH_3), 1.10-2.40 (13H), 2.72 (m 6-CH_2), 3.64 (m, OCH_3, 17α-CH, 17β-OH), 4.25 (m, OCH_2CH), 4.36 (m, OCH_2CH), 5.65 (d, J = 8.1 Hz, CHPh), 6.67 (d_{app}, J = 4.4 Hz, 1-CH), 6.96 (s, 4-CH), 7.30 (t, J = 7.2 Hz, 2H of Fmoc), 7.45 (m, 5H of Ph), 7.63 (d, J = 7.5 Hz, 2H of Fmoc), 7.74 (d, J = 7.5 Hz, 2H of Fmoc), 7.86 (d, J = 7.5 Hz, 2H of Fmoc); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.8 (C18), 24.0 (C15), 27.3, 28.1, ~30 (2C under solvent peaks), 31.2 (C12), 37.9 (C16), 39.7 (C8), 44.2 (C13), 45.6 (C9), 48.2 (C5'), 51.2 (C14), 56.4 (OCH_3), 59.5 (C2'), 67.7 (C4'), 81.9 (C17), 112.3 (C1), 121.0 (2x), 123.4 (C4), 126.3, 128.1, 128.7 (2x), 129.1, 129.4, 129.7 (2x), 130.0 (C5), 137.7 (2x), 138.7 (C3), 140.1 (C10), 142.3, 145.2 (2x), 150.1 (C2), 156.9 (C3'), 170.1 (C1'); LRMS for $C_{42}H_{44}NO_6$ [MH^+]: 658.6 *m/z*.

Synthesis of 2-methoxy-3-phenethyloxy-estra-1,3,5(10)-trien-17-one (**10**)

2-Methoxyestrone (**7**) (65 mg, 0.22 mmol) and Cs_2CO_3 (140 mg, 0.43 mmol) were dissolved in DMF (6 ml) under argon in a Schlenk tube. The mixture was stirred for 5 min and 2-bromoethylbenzene (44 µl, 0.32 mmol) was added. The solution was then heated at 110 °C overnight. The reaction was quenched with water and the crude product was extracted with EtOAc. The combine organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (93:7)) yielded 45 mg (52 %) of **10**. ^{1}H NMR ($(CD_3)_2CO$) δ = 0.89 (s, 18-CH_3), 1.20-2.55 (13H), 2.78 (m, 6-CH_2), 3.06 (t, J = 7.0 Hz,

$\underline{CH_2}Ph$), 3.76 (s, OCH_3), 4.14 (t, J = 7.0 Hz, OCH_2), 6.65 (s, 1-CH), 6.87 (s, 4-CH), 7.22 (t_{app}, 1H of Ph), 7.33 (m, 4H of Ph).

Synthesis of 2-methoxy-3-phenethyloxy-1,3,5(10)-estratrien-17β-ol (**11**)

To a solution of **10** (43 mg, 0.11 mmol) dissolved in MeOH (6 ml) and CH_2Cl_2 (1.5 ml) was added sodium borohydride (8.1 mg, 0.21 mmol) at 0 °C. After 2 h, the reaction was quenched by the addition of saturated NH_4Cl (10 ml). The resulting solution was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to dryness. Purification of the alcohol by flash chromatography (hexanes:EtOAc (80:20)) yielded 38 mg (88 %) of **11**. IR (film) ν = 3406 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.76 (s, 18-CH_3), 1.10-2.40 (13H), 2.73 (m, 6-CH_2), 3.06 (t, J = 7.0 Hz, $\underline{CH_2}Ph$), 3.64 (m, 17α-CH and 17β-OH), 3.76 (s, OCH_3), 4.14 (t, J = 7.0 Hz, OCH_2), 6.63 (s,1-CH), 6.86 (s, 4-CH), 7.28 (t_{app}, 1H of Ph), 7.32 (m, 4H of Ph); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.8 (C18), 24.0 (C15), 27.5, 28.4, ~30 (2C under solvent peaks), 31.3 (C12), 36.8 (C2'), 38.0 (C16), 40.1 (C8), 44.3 (C13), 45.5 (C9), 51.2 (C14), 56.8 (OCH_3), 70.8 (C1'), 82.0 (C17), 111.7 (C1), 115.7 (C4), 127.2, 129.3, 129.9 (C5), 130.1, 133.9 (C10), 140.0, 147.9 (C2), 149.0 (C3); LRMS for $C_{27}H_{35}O_3$ [MH^+]: 407.4 *m/z*.

Synthesis of 2-methoxy-3-benzhydryloxy-oxoestra-1,3,5(10)-trien-17-one (**12**)

A solution of **7** (58 mg, 0.19 mmol) and Cs_2CO_3 (94 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (5 ml) under argon. After 5 min, bromodiphenylmethane (71 mg, 0.29 mmol) was added and the solution was refluxed for 18 h. The resulting solution was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (94:6)) yielded 73 mg (81 %) of **12**. ^{1}H NMR ($CDCl_3$) δ = 0.89 (s, 18-CH_3), 0.90-2.60 (13H), 2.67 (m, 6-CH_2), 3.84 (s, OCH_3), 6.18 (s, $O\underline{CH}Ph_2$), 6.54 (s, 1-CH), 6.82 (s, 4-CH), 7.27 (m, 2H of Ph_2), 7.33 (m, 4H of Ph_2), 7.44 (m, 4H of Ph_2).

Synthesis of 2-methoxy-3-benzylhydroxy-1,3,5(10)-estratrien-17β-ol (**13**)

To a solution of **12** (73 mg, 0.16 mmol) dissolved in MeOH (7 ml) and CH_2Cl_2 (3 ml) was added sodium borohydride (12 mg, 0.31 mmol) at 0 °C. After 2 h, the reaction was

quenched by the addition of saturated NH_4Cl (10 ml). The resulting solution was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography (hexanes:EtOAc (80:20)) yielded 66 mg (90 %) of **13**. IR (film) ν = 3316 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.75 (s, 18-CH_3), 1.10-2.40 (13H), 2.60 (m, 6-CH_2), 3.65 (m, 17α-CH and 17β-OH), 3.84 (s, OCH_3), 6.41 (s, O<u>CH</u>Ph_2), 6.64 (s, 1-CH), 6.88 (s, 4-CH), 7.24 (t_{app}, 2H of Ph_2) 7.34 (m, 4H of Ph_2), 7.53 (m, 4H of Ph_2); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.8 (C18), 23.9 (C15), 27.4, 28.3, ~30 (C6 under solvent peaks), 31.2 (C12), 38.0 (C16), 39.9 (C8), 44.2 (C13), 45.4 (C9), 51.1 (C14), 56.8 (OCH_3), 81.8 (C17), 82.9 (C1'), 111.6 (C1), 118.2 (C4), 127.9 (2x), 128.1, 128.4, 128.7 (2x), 129.0, 129.3 (2x), 129.5 (C5), 134.4 (C10), 143.4 (2x), 146.4 (C2), 149.6 (C3); LRMS for $C_{32}H_{36}O_3$ + Na [MNa^+]: 491.4 *m/z*.

Synthesis of 2-methoxy-3-benzyloxy-estra-1,3,5(10)-trien-17-one (**14**)

A solution of **7** (75 mg, 0.25 mmol) and Cs_2CO_3 (122 mg, 0.38 mmol) was dissolved in CH_3CN (7 ml) under argon. After 5 min, 74 µl of benzylbromide (0.62 mmol) was added and the solution was stirred for 7 h under argon. The resulting solution was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to give 98 mg of **14** which was used without further purification for the next step. ^{1}H NMR ($CDCl_3$) δ = 0.91 (s, 18-CH_3), 1.20-2.60 (13H), 2.78 (m, 6-CH_2), 3.86 (s, OCH_3), 5.11 (s, <u>CH_2</u>Ph), 6.63 (s, 1-CH), 6.84 (s, 4-CH), 7.37 (m, 5H of Ph).

Synthesis of 2-methoxy-3-benzyloxy-1,3,5(10)-estratrien-17β-ol (**15**)

A solution of **14** (98 mg, 0.25 mmol) in MeOH (8 ml) and CH_2Cl_2 (3 ml) was treated with sodium borohydride (19 mg, 0.50 mmol) at 0 °C. After 2 h, the reaction was quenched by the addition of saturated NH_4Cl (10 ml). The resulting solution was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purification by flash chromatography (hexanes:EtOAc (85:15)) to give **15** (80 mg, 81%). ^{1}H NMR ($CDCl_3$) δ = 0.78 (s,18-CH_3), 1.10-2.40 (13H), 2.72 (m, 6-CH_2), 3.73 (t, J = 8.5 Hz, 17α-CH), 3.86 (s, OCH_3), 5.11 (s, O<u>CH_2</u>Ph), 6.62 (s, 1-CH), 6.85 (s, 4-CH), 7.37 (m, 5H of Ph); ^{13}C NMR ($CDCl_3$) δ = 11.2 (C18), 23.3 (C15), 26.7, 27.5, 29.3 (C6), 30.8 (C12), 36.9 (C16), 38.9 (C8), 43.4 (C13), 44.4 (C9), 50.2 (C14), 56.5 (OCH_3), 71.3 (C1'), 82.1 (C17),

110.1 (C1), 115.0 (C4), 127.5 (3' and 7'), 127.8 (5'), 128.6 (4' and 6'), 129.1 (C5), 133.2 (C10), 137.7 (3'), 146.6 (C2), 147.8 (C3); IR (film) ν = 3420 (OH); LRMS for $C_{26}H_{33}O_3$ [MH^+]: 393.0 *m/z*.

Synthesis of 2-methoxy-3-trytyloxy-estra-1,3,5(10)-trien-17-one (**16**)

A solution of **7** (40 mg, 0.13 mmol) in CH_2Cl_2 (9 ml) under argon was reacted with DIPEA (104 µl, 0.600 mmol) for 5 min. Trityl chloride (74 mg, 0.27 mmol) was added and the solution was stirred overnight at rt under argon. The solution was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The crude compound was purified by flash chromatography (hexanes:EtOAc (90:10)) to give **16** (59 mg, 82 %). ^{1}H NMR ($(CD_3)_2CO$) δ 0.86 (s, 18-CH_3), 1.00-2.60 (15H), 3.58 (s, OCH_3), 6.31 (s, 1-CH), 6.68 (s, 4-CH), 7.28 (m, 9H of Ph_3), 7.48 (m, 6H of Ph_3).

Synthesis of 2-methoxy-3-trytyloxy-1,3,5(10)-estratrien-17β-ol (**17**)

A solution of **16** (53 mg, 0.10 mmol) in MeOH (7 ml) and CH_2Cl_2 (3 ml) was added sodium borohydride (7 mg, 0.20 mmol) at 0 °C. After 2 h, the reaction was quenched with saturated NH_4Cl solution (10 ml). The mixture was then extracted with EtOAc, dried over $MgSO_4$, filtered and evaporated to dryness. The desired product was purified by flash chromatography (hexanes:EtOAc (80:20)) to give 46 mg of **17** (86 %). Be careful, this compound was found instable in $CDCl_3$ or in CH_2Cl_2. IR (film) ν = 3422 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.73 (s,18-CH_3), 1.10-2.50 (15H), 3.57 (s, OCH_3), 3.62 (m, 17α-CH and 17β-OH), 6.29 (s, 1-CH), 6.67 (s, 4-CH), 7.36 (m, 9H of Ph_3), 7.49 (d, J = 1.5 Hz, 6H of Ph_3); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.8 (C18), 23.9 (C15), 27.3, 28.2, ~30 (C6 under solvent peaks), 31.2 (C12), 37.9 (C16), 39.9 (C8), 44.2 (C13), 45.4 (C9), 51.1 (C14), 56.5 (OCH_3), 81.9 (C17), 91.4 (C1'), 110.8 (C1), 123.4 (C4), 128.1 (3C of Ph_3), 128.4 (6C of Ph_3), 130.2 (6C of Ph_3), 135.2 (C5), 143.3 (C10), 145.7 (3C of Ph_3), 151.3 (C2 and C3); LRMS for $C_{38}H_{40}O_3$ + Na [MNa^+]: 567.2 *m/z*.

Synthesis of 2-methoxy-3-(trifluoromethanesulfonyloxy)-estra-1,3,5(10)-trien-17-one (**18**)
A solution of **7** (103 mg, 0.34 mmol) in dry DMF (7 ml) was treated at rt with K_2CO_3 (141 mg, 1.02 mmol). 4-Nitrophenyl trifluoromethanesulfonate (213 mg, 0.78 mmol) was then added and the mixture was stirred for 4 h. The reaction was quenched by addition of water and the solution was extracted with Et_2O. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (90:10)) yielded **18** (132 mg, 90 %). ^{1}H NMR ($CDCl_3$) δ = 0.92 (s, 18-CH_3), 1.20-2.60 (13H), 2.85 (6-CH_2), 3.88 (s, OCH_3), 6.92 (s, 1-CH), 6.94 (s, 4-CH).

Synthesis of 2-methoxy-3-benzyl-estra-1,3,5(10)-trien-17-one (**19**)
Dibromoethane (7.2 µl, 0.08 mmol) was added to Zn (110 mg, 1.68 mmol) in DMF (4 ml) and the mixture was heated at 100 °C for 20 min. The reaction was allowed to return at rt and chlorotrimethylsilane (TMSCl) (2 µl, 0.02 mmol) and benzylbromide (33 µl, 0.28 mmol) were added. This mixture was reheated at 80 °C for 15 min, cooled to rt and used without purification for the next step. Steroid **18** (12 mg, 0.03 mmol) was dissolved in dry DMF (1 ml) degased with argon, and $Pd_2(dba)_3$ (2 mg, 0.002 mmol), 1,1'-bis(diphenylphosphino)ferrocene (1 mg, 0.002 mmol) and Bu_4NI (52 mg, 0.140 mmol) were added. Subsequently, a solution of the crude benzylzinc bromide in dry DMF was added and the reaction was heated at 100 °C under argon. After 5 h, the mixture was quenched with saturated NH_4Cl solution (10 ml). The solution was extracted with EtOAc, the organic layer was washed with water, dried over $MgSO_4$ and evaporated for dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (88:12)) yielded 10 mg of **19** (98 %). ^{1}H NMR ($(CD_3)_2CO$) δ = 0.90 (s, 18-CH_3), 1.25-2.50 (13H), 2.74 (m, 6-CH_2), 3.81 (s, OCH_3), 3.87 and 3.88 (2 s, $\underline{CH_2}$Ph), 6.82 (s, 1-CH), 6.89 (s, 4-CH), 7.15 (m, 1H of Ph), 7.23 (m sharp, 4H of Ph).

Synthesis of 2-methoxy-3-benzyl-1,3,5(10)-estratrien-17β-ol (**20**)
An ice cold solution of **19** (14 mg, 0.04 mmol) in MeOH (3 ml) and CH_2Cl_2 (1 ml) was treated with sodium borohydride (6 mg, 0.15 mmol) as described for the synthesis of **15**. The final product was purified by flash chromatography (hexanes:EtOAc (90:10)) to give

15 mg of **20** (98 %). IR (film) ν = 3358 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.78 (s,18-CH_3), 1.10-2.45 (13H), 2.70 (m, 6-CH_2), 3.64 (m, 17α-CH and 17β-OH), 3.80 (s, OCH_3), 3.87 (s, $\underline{CH_2}$-Ph), 6.79 (s, 1-CH), 6.87 (s, 4-CH), 7.15 (m, 1H of Ph), 7.23 (m sharp, 4H of Ph); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.8 (C18), 24.0 (C15), 27.4, 28.4, ~30 (C6 under solvent peaks), 31.3 (C12), 36.3 (C1'), 38.1 (C16), 40.0 (C8), 44.2 (C13), 45.9 (C9), 51.3 (C14), 55.9 (OCH_3), 82.0 (C17), 108.9 (C1), 126.6 (C3 and C4), 127.9 (C5), 129.1 (C10 and 1C of Ph), 129.8 (2C of Ph), 131.7 (2C of Ph), 140.4 (C2'), 142.7 (C3); LRMS for $C_{26}H_{33}O_2$ [MH^+]: 377.2 *m/z*.

Synthesis of 2-methoxy-3-[2-(Boc-amino)- 2S-phenyl-acetoxy]-estra-1,3,5(10)-trien-17-one (**21**)

To a solution containing N-Boc-L-Phenylglycine (75 mg, 0.30 mmol), PyBrOP (139 mg, 0.30 mmol) and HOBt (40 mg, 0.30 mmol) in dry DMF (2 ml) was added DIPEA (0.1 ml, 0.60 mmol). Shortly after the addition of DIPEA, the solution was added to a solution of **7** (45 mg, 0.15 mmol) in dry DMF (2 ml). The mixture was stirred overnight under argon and quenched with water. The crude product was extracted with EtOAc and the organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography with hexanes:EtOAc (80:20) yielded 64 mg (80 %) of **21**. ^{1}H NMR ($(CD_3)_2CO$) δ = 0.90 and 0.91 (2 s, 18-CH_3), 1.10-2.50 (24H), 1.43 (s, $C(CH_3)_3$), 2.79 (m, 6-CH_2), 3.66 and 3.67 (2 s, OCH_3), 5.55 (d, J = 7.9 Hz, $\underline{CH}$Ph), 6.69 ($d_{app.}$, J = 5.3 Hz, 1-CH), 6.97 (s, 4-CH), 7.41 (m, 3H of Ph), 7.59 (d, J = 7.4 Hz, 2 H of Ph).

Synthesis of 2-methoxy-3-[2-(Boc-amino)-2S-phenyl-acetoxy]-1,3,5(10)-estratrien-17β-ol (**22**)

A solution of **21** (64 mg, 0.12 mmol) in MeOH (10 ml) and CH_2Cl_2 (4 ml) at 0 °C was treated with sodium borohydride (13 mg; 0.34 mmol) as described for the synthesis of **15**. Purification of the crude product by flash chromatography (hexanes:EtOAc (80:20)) afforded **22** (60 mg, 93 %). IR (film) ν = 3447 (OH and NH), 1766 (C=O, ester), 1706 (C=O, carbamate); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.78 and 0.79 (2 s, 18-CH_3), 1.15-2.40 (13H), 1.43 (s, $C(\underline{CH_3})_3$), 2.73 (m, 6-CH_2), 3.64 (m, OCH_3, 17α-CH and 17β-OH), 5.55 (d, J = 8.2 Hz, $\underline{CH}$Ph), 6.66 ($d_{app.}$, J = 4.0 Hz, 1-CH), 6.84 (d, J = 7.6 Hz, NH), 6.97 (s, 4-CH), 7.41

(m, 3H of Ph), 7.59 (d, J = 7.3 Hz, 2H of Ph); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.7 (C18), 23.9 (C15), 27.2, 28.0, 28.6 (3x), ~30 (C6 under solvent peaks), 31.1 (C12), 37.9 (C16), 39.6 (C8), 44.1 (C13), 45.6 (C9), 51.0 (C14), 56.3 (OCH_3), 59.0 (C2'), 79.8, 81.9 (C17), 111.1 (C1), 123.3 (C4), 129.0 (2C of Ph), 129.2 (1C of Ph), 129.5 (2C of Ph), 129.9 (C5), 137.9 (C3), 138.7 (1C of Ph), 140.0 (C10), 150.0 (C2), 156.1, 170.2 (C1'); LRMS for $C_{32}H_{42}NO_6$ [MH^+]: 536.5 *m/z*.

Synthesis of 2-methoxy-3-(2-amino-2-phenyl-acetoxy)-1,3,5(10)-estratrien-17β-ol (**23**)

To a solution of **22** (13 mg, 0.02 mmol) in dry CH_2Cl_2 (3 ml) was added at 0 °C and under argon trifluoroacetic acid (TFA) (5.5 µl, 0.07 mmol). The reaction mixture was stirred for 12 h, evaporated under reduced pressure and the crude product was dissolved in CH_2Cl_2. This solution was treated with Et_3N (0.07 mmol) for 10 min, then extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by chromatography (hexanes:acetone (70:30)) to give **23** (8 mg, 77 %). IR (film) ν = 3340 (OH and NH_2), 1728 (C=O, ester); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.78 (s, 18-CH_3), 1.10-2.40 (13 H), 2.70 (m, 6-CH_2), 3.65 (m, OCH_3, 17α-CH and 17β-OH), 5.49 (s, CH-Ph), 6.57 (s, 1-CH), 6.95 (s, 4-CH), 7.31 (m, 1H of Ph), 7.38 (m, 2H of Ph), 7.63 (d, J = 8.0 Hz, 2H of Ph); LRMS for $C_{27}H_{34}NO_4$ [MH^+]: 436.1 *m/z*.

Synthesis of 2,3-dimethoxy-estra-1,3,5(10)-trien-17-one (**24**)

To a solution of **7** (51 mg, 0.17 mmol) in dry THF (3 ml) under argon were added successively cesium carbonate (165 mg, 0.51 mmol) and methyl iodide (26 µl, 0.42 mmol). This mixture was stirred overnight at reflux. After the reaction mixture was cooled at rt and quenched with water, the crude product was extracted with EtOAc, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography with hexanes:EtOAc (85:15) yielded 41 mg (80 %) of **24**. ^{1}H NMR ($CDCl_3$) δ = 0.92 (s,18-CH_3), 1.35-2.60 (13 H), 2.88 (m, 6-CH_2), 3.85 (s, OCH_3), 3.86 (s, OCH_3), 6.60 (s, 1-CH), 6.81 (s, 4-CH).

Synthesis of 2,3-dimethoxy-1,3,5(10)-estratrien-17β-ol (**25**)

A solution of **24** (40 mg, 0.13 mmol) in MeOH (8 ml) at 0 °C was treated with sodium borohydride (10 mg, 0.26 mmol) as described for the synthesis of **15**. Purification of the

crude product by flash chromatography (hexanes:EtOAc (70:30)) gave **25** (37 mg, 90 %). IR (film) ν = 3538 (OH); ^{1}H NMR ($CDCl_3$) δ = 0.79 (s, 18-CH_3), 1.20-2.40 (13 H), 2.80 (m, 6-CH_2), 3.74 (t, J = 8.5 Hz, 17α-CH), 3.84 (s, OCH_3), 3.86 (s, OCH_3), 6.59 (s, 1-CH), 6.82 (s, 4-CH); ^{13}C NMR ($CDCl_3$) δ = 11.3 (C18), 23.3 (C15), 26.7, 27.5, 29.4 (C6), 30.8 (C12), 36.9 (C16), 39.0 (C8), 43.4 (C13), 44.4 (C9), 50.2 (C14), 56.0 (OCH_3), 56.2 (OCH_3), 82.1 (C17), 109.1 (C1), 112.1 (C4), 128.9 (C5 and C10), 132.4 (C2 and C3); LRMS for $C_{20}H_{29}O_3$ [MH^+]: 317.1 *m/z*.

Synthesis of 2-methoxy-3-cyclohexylmethyloxy-estra-1,3,5(10)-trien-17-one (**26**)

To a solution of **7** (66 mg, 0.22 mmol) in dry DMF (20 ml) were added successively cesium carbonate (178 mg, 0.55 mmol) and cyclohexylmethyl bromide (46 µl; 0.33 mmol) in a Schlenk tube purged with argon. After 2 h at 110 °C, the reaction mixture was cooled to rt and quenched with water. The crude product was extracted with EtOAc and the combined organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography with hexanes:EtOAc (92:8) yielded 67 mg (77 %) of **26**. ^{1}H NMR ($CDCl_3$) δ = 0.91 (s,18-CH_3), 0.80-2.60 (24 H), 2.82 (m, 6-CH_2), 3.76 (d, J = 6.3 Hz, OCH_2), 3.83 (s, OCH_3), 6.60 (s, 1-CH), 6.82 (s, 4-CH).

Synthesis of 2-methoxy-3-cyclohexylmethyloxy-1,3,5(10)-estratrien-17β-ol (**27**)

A solution of **26** (58 mg, 0.15 mmol) in MeOH (6 ml) and CH_2Cl_2 (2.5 ml) at 0 °C was treated with sodium borohydride (11 mg, 0.30 mmol) as described for the synthesis of **15**. Purification of the crude product by flash chromatography (hexanes:EtOAc (80:20)) yielded 57 mg of **27** (98 %). IR (film) ν = 3422 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.77 (s, 18-CH_3), 1.00-2.40 (24 H), 2.73 (m, 6-CH_2), 3.65 (t, J = 5.8 Hz, 17α-CH), 3.72 (dd, J_1 = 1.5 Hz, J_2 = 6.5 Hz, $\underline{CH_2}$-Ph), 3.77 (s, OCH_3), 6.60 (s, 1-CH), 6.85 (s, 4-CH); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.5 (C18), 23.7 (C15), 26.4 (2C), 27.2, 28.1, ~30 (3C under solvent peaks), 30.5 (2C), 30.9 (C12), 37.7 (C16), 38.6 (C2'), 39.8 (C8), 43.9 (C13), 45.1 (C9), 50.8 (C14), 56.5 (OCH_3), 75.0 (C1'), 81.7 (C17), 111.3 (C1), 115.0 (C4), 129.5 (C5), 133.1 (C10), 148.0 (C2), 148.6 (C3); LRMS for $C_{26}H_{39}O_3$ [MH^+]: 399.3 *m/z*.

Synthesis of 2-methoxy-3-sulfamoyloxy-estra-1,3,5(10)-trien-17-one (**28**)

To a solution of **7** (92 mg, 0.31 mmol) in dry CH_2Cl_2 (10 ml) were added successively 2,6-di-tert-butyl-4-methylpyridine (DBMP) (188 mg, 0.92 mmol) and sulfamoyl chloride (106 mg, 0.92 mmol) under argon. The reaction mixture was stirred overnight and then quenched with water. The crude product was extracted with CH_2Cl_2, the organic phase dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography with hexanes:EtOAc (75:25) yielded 111 mg (95 %) of **28**. ^{1}H NMR ($CDCl_3$) δ = 0.92 (s,18-CH_3), 1.20-2.60 (13 H), 2.86 (m, 6-CH_2), 3.88 (s, OCH_3), 4.95 (OSO_2NH_2), 6.93 (s, 1-CH), 7.06 (s, 4-CH).

Synthesis of 2-methoxy-3-dimethylsulfamoyloxy-estra-1,3,5(10)-trien-17-one (**29**)

To a solution of **28** (63 mg, 0.17 mmol) in dry THF (3 ml) under argon was added successively cesium carbonate (162 mg, 0.50 mmol) and methyl iodide (26 µl, 0.42 mmol) in a Schlenk tube purged with argon. After 4 h at reflux, the reaction mixture was cooled at rt and quenched with water. The product was extracted with EtOAc, the organic phase dried over $MgSO_4$ and evaporated to dryness giving **29** (70 mg) which was used without purification. ^{1}H NMR ($CDCl_3$) δ = 0.92 (s, 18-CH_3), 1.20-2.60 (13 H), 2.84 (m, 6-CH_2), 2.98 (s, NCH_3), 3.86 (s, OCH_3), 6.88 (s, 1-CH), 7.07 (s, 4-CH).

Synthesis of 2-methoxy-3-dimethylsulfamoyloxy-1,3,5(10)-estratrien-17β-ol (**30**)

A solution of **29** (70 mg, 0.17 mmol) in MeOH (5 ml) and CH_2Cl_2 (3 ml) at 0 °C was treated with sodium borohydride (13 mg, 0.34 mmol) as described for the synthesis of **15**. Purification of the crude product by flash chromatography (hexanes:EtOAc (85:15)) yielded **30** (57 mg, 81 %). IR (film) ν = 3527 (OH); ^{1}H NMR ($CDCl_3$) δ = 0.79 (s, 18-CH_3), 1.15-2.40 (13 H), 2.79 (m, 6-CH_2), 2.97 (s, $N(CH_3)_2$), 3.74 (t, J = 8.5 Hz, 17α-CH), 3.85 (s, OCH_3), 6.88 (s, 1-CH), 7.04 (s, 4-CH); ^{13}C NMR ($CDCl_3$) δ = 11.2 (C18), 23.3 (C15), 26.5, 27.2, 28.8 (C6), 30.8 (C12), 36.9 (C16), 38.5 ($(CH_3)_2N$), 38.8 (C8), 43.3 (C13), 44.6 (C9), 50.3 (C14), 56.4 (OCH_3), 82.0 (C17), 110.5 (C1), 123.7 (C4), 129.7 (C5), 137.3 (C10), 139.7 (C3), 149.3 (C2); LRMS for $C_{21}H_{32}NO_5S$ [MH^+]: 409.9 *m/z*.

Synthesis of 2-methoxy-3-propyloxy-oxoestra-1,3,5(10)-trien-17-one (**31**)

A solution of **7** (80 mg, 0.27 mmol) in dry DMF (6 ml) under argon was treated successively with cesium carbonate (216 mg, 0.66 mmol) and 1-bromopropane (36 µl, 0.40 mmol) in a Schlenk tube purged with argon. After 2 h at 110 °C, the reaction mixture was cooled at rt and quenched with water. The crude product was extracted with EtOAc and the combined organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (90:10)) yielded **31** (87 mg; 97 %). ^{1}H NMR ($(CD_3)_2CO$) δ = 0.90 (s, 18-CH_3), 1.01 (t, *J* = 8.5 Hz, $CH_2\underline{CH_3}$), 1.30-2.50 (15 H), 2.80 (m, 6-CH_2), 3.78 (s, OCH_3), 3.89 (t, *J* = 6.5 Hz, OCH_2), 6.64 (s, 1-CH), 6.87 (s, 4-CH).

Synthesis of 2-methoxy-3- propyloxy-1,3,5(10)-estratrien-17β-ol (**32**)

A solution of **31** (77 mg, 0.23 mmol) in MeOH (6 ml) and CH_2Cl_2 (3 ml) at 0 °C was treated with sodium borohydride (17 mg, 0.45 mmol) as described for the synthesis of **15**. Purification of the crude product by flash chromatography (hexanes:EtOAc (80:20)) yielded 74 mg of **32** (95 %). IR (film) ν = 3520 (OH); ^{1}H NMR ($(CD_3)_2CO$) δ = 0.77 (s, 18-CH_3), 1.01 (t, *J* = 7.4 Hz, $CH_2\underline{CH_3}$), 1.10-2.40 (15 H), 2.73 (m, 6-CH_2), 3.65 (m, 17α-CH and17β-OH), 3.77 (s, OCH_3), 3.88 (t, *J* = 6.5 Hz, OCH_2), 6.61 (s, 1-CH), 6.85 (s, 4-CH); ^{13}C NMR ($(CD_3)_2CO$) δ = 11.0 (C3'), 11.8 (C18), 23.6 (C2'), 24.0 (C15), 27.5, 28.4, ~30 (C6 under solvent peaks), 31.3 (C12), 38.0 (C16), 40.1 (C8), 44.2 (C13), 45.4 (C9), 51.1 (C14), 56.7 (OCH_3), 71.3 (C1'), 82.0 (C17), 111.5 (C1), 115.4 (C4), 129.8 (C5), 133.5 (C10), 148.1 (C3), 148.9 (C2); LRMS for $C_{22}H_{33}O_3$ [MH^+]: 345.2 *m/z*.

Synthesis of 2-methoxy-3-hexyloxy-estra-1,3,5(10)-trien-17-one (**33**)

Compound **7** (62 mg, 0.21 mmol) was dissolved in dry DMF (6 ml) under argon and treated successively with cesium carbonate (134 mg, 0.41 mmol) and 1-bromohexane (43 µl, 0.31 mmol) in a Schlenk tube purged with argon. After 3 h at 110 °C, the reaction mixture was cooled at rt and quenched with water. The crude product was extracted with EtOAc and the combined organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude product by flash chromatography (hexanes:EtOAc (90:10)) yielded **33** (70 mg, 88 %). ^{1}H NMR ($(CD_3)_2CO$) δ = 0.88 (s, 18-CH_3), 0.90 (t, *J* =

7.0 Hz, $CH_2\underline{CH_3}$), 1.25-2.50 (21 H), 2.78 (m, 6-CH_2), 3.77 (s, OCH_3), 3.92 (t, J = 6.5 Hz, OCH_2), 6.63 (s, 1-CH), 6.85 (s, 4-CH).

Synthesis of 2-methoxy-3-hexyloxy-1,3,5(10)-estratrien-17β-ol (**34**)

A solution of **33** (62 mg, 0.16 mmol) in MeOH (6 ml) and CH_2Cl_2 (1.5 ml) at 0 °C was treated with sodium borohydride (12 mg, 0.32 mmol) as described for the synthesis of **15**. Purification of the crude product by flash chromatography (hexanes:EtOAc (80:20)) yielded **34** (57 mg,; 91 %). IR (film) ν = 3410 (OH); ^{1}H NMR ($CDCl_3$) δ = 0.78 (s, 18-CH_3), 0.89 (t, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.15-2.40 (21 H), 2.78 (m, 6-CH_2), 3.74 (t, J = 8.5 Hz, 17α-CH), 3.84 (s, OCH_3), 3.97 (t, J= 6.9 Hz, OCH_2), 6.59 (s, 1-CH), 6.82 (s, 4-CH); ^{13}C NMR ($CDCl_3$) δ = 11.2 (C18), 14.2 (C6'), 22.8 (C5'), 23.3 (C15), 25.8 (C3'), 26.7, 27.5, 29.4 (C6 and C2'), 30.8 (C12), 31.8 (C4'), 36.9 (C16), 39.0 (C8), 43.4 (C13), 44.4 (C9), 50.2 (C14), 56.5 (OCH_3), 69.3 (C1'), 82.1 (C17), 110.0 (C1), 114.0 (C4), 129.0 (C5), 132.4 (C10), 146.9 (C3), 147.6 (C2); LRMS for $C_{25}H_{39}O_3$ [MH^+]: 387.6 *m/z*.

Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD) were routinely grown in suspension in 90 % RPMI (Roswell Park Memorial Institute) 1640 (Sigma, Saint Louis, USA) containing L-glutamine (2 nM), antibiotics (100 IU penicillin/ml, 100 μg streptomycin/ml) and supplemented with 10 % (v/v) fetal bovine serum (FBS), in a 5 % CO_2 humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10 % FBS.

Cell proliferation assay

The cell proliferation assays were performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In

brief, triplicate cultures of 1 x 10^4 cells in a total of 100 µl medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ) at 37°C, 5 % CO_2. Steroidal-derivatives synthesized in our laboratory were dissolved in ethanol to prepare the stock solution of 1 x 10^{-2} mol/L. These compounds was diluted at three concentrations with culture media, added to each well and incubated for 3 days. Following each treatment, 20 µl MTS was added to all well and incubated for 4 h. MTS is converted to water-soluble colored formazan by a dehydrogenase enzyme present in metabolically active cells. Subsequently, the absorbance (abs) of each well was measured at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). Data were expressed as percentage inhibition of HL-60 cell proliferation.

$$\% \text{ inh. of compound z} = 100 - \left(\frac{\text{abs. of compound z X 100}}{\text{abs. of control}} \right)$$

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Conclusion générale

Le cancer est une maladie très importante et on estime que plus de 70 000 canadiens vont en mourir cette année. Quoi qu'il y ait eu de nombreux progrès ces dernières années dans ce domaine, les taux d'incidence et de mortalité sont encore très élevés justifiant la poursuite intensive des recherches. Le cancer de la prostate et la leucémie sont deux cancers très présents dans notre société touchant de nombreuses personnes. Malgré qu'il existe des agents pour traiter ces cancers, ceux-ci ont parfois une efficacité limitée ou bien induisent de nombreux effets secondaires. Cette thèse de doctorat a donc été consacrée à l'élaboration et la synthèse chimique de nouveaux agents thérapeutiques pour ces cancers, ainsi qu'à leur évaluation biologique.

Les androgènes occupent un rôle très important dans le développement et la croissance du cancer de la prostate. L'action de ces hormones étant médiée par le récepteur des androgènes, il est logique que l'utilisation d'un antiandrogène ayant une forte liaison avec le récepteur empêche la stimulation du cancer induite par la testostérone et la dihydrotestosterone (DHT). La première partie de mes travaux de doctorat fût donc axée sur la synthèse d'antiandrogènes dérivés du 5α-androstane-3α,17β-diol. Tout d'abord, la synthèse chimique m'a permis de générer, après plusieurs étapes, l'éventail de produits rapportés dans le chapitre 1. L'utilisation du catalyseur de Grubbs de seconde génération m'a été très utile pour la réaction de métathèse, une étape clé au cours de la synthèse des produits ciblés. En effet, à l'aide de ce nouveau catalyseur j'ai pu effectuer avec succès l'élongation de la chaîne en position 16α du noyau 5α-androstane-3α,17β-diol.

L'évaluation biologique de tous ces nouveaux dérivés stéroïdiens nous a permis d'identifier certains éléments structuraux nécessaires pour obtenir une activité antiprolifération importante sur les cellules AR^+ Shionogi ainsi qu'une bonne affinité avec le récepteur des androgènes. Nous avons confirmé que l'utilisation du noyau DHT pour ce type de dérivés n'était pas favorable puisque les liaisons avec le récepteur des androgènes (AR) étaient trop fortes, il y avait induction de la prolifération cellulaire (effet agoniste). Tous ces résultats nous ont permis de conclure que l'alcool en position 3 du noyau stéroïdien était important pour l'activité antiprolifération (effet antagoniste), mais d'autres travaux seront nécessaires pour confirmer son orientation (α ou β) optimale. De plus, la

modélisation moléculaire d'un des meilleurs candidats nous a permis d'énoncer une hypothèse pour le mécanisme d'action de ces produits ainsi que d'identifier les différentes interactions avec certains acides aminés du AR, lesquelles seraient potentiellement responsables de l'activité antiandrogénique obtenue. Ainsi le contact entre l'halogène de la chaîne latérale du stéroïde et l'hélice 11 du récepteur semble permettre un déplacement de l'hélice 12, causant ainsi l'activité antagoniste observée. Toutes les informations recueillies lors de nos expérimentations nous ont permis de synthétiser un composé légèrement plus efficace que le produit de référence et d'élaborer une hypothèse pour le mécanisme d'action de cette nouvelle famille.

La seconde partie de mes travaux de doctorat, et la plus importante, a été consacrée à la leucémie. Ce cancer, qui est le plus important chez les enfants, affecte aussi les adultes. Il existe actuellement sur le marché plusieurs agents thérapeutiques pour cette maladie, cependant ceux-ci s'avèrent habituellement très toxiques pour les patients provoquant ainsi de nombreux effets secondaires, pouvant parfois être fatales. L'objectif que je poursuivais était l'élaboration et la synthèse d'une toute nouvelle famille d'agents antileucémiques constitués d'un noyau stéroïdien. À l'aide de la chimie en parallèle sur support solide, plusieurs librairies de dérivés stéroïdiens, des aminostéroïdes, ont été synthétisées rapidement et avec succès puisque les rendements et les puretés étaient acceptables pour en permettre l'évaluation biologique sans purification. Pendant mon doctorat, j'ai mis au point la technique nécessaire pour l'évaluation biologique de tous ces dérivés, puisque cette méthode n'était pas clairement rapportée dans la littérature ni disponible dans notre laboratoire. Ces résultats nous ont permis d'identifier dans un premier temps quelques éléments nécessaires pour obtenir une activité antiproliférative intéressante sur les cellules leucémiques humaines HL-60. La proline semble être un acide aminé important pour l'obtention de cette activité, principalement lorsqu'il est lié à un acide carboxylique hydrophobe. L'utilisation de la phénylalanine induit aussi une activité intéressante, mais toutefois moindre que celle induite par la proline. Par la suite, et à l'aide d'une technique mise au point par notre groupe, l'aminolyse des époxydes stéroïdiens encombrés a permis la synthèse en solution de plusieurs produits dont trois autres candidats ayant un potentiel antiprolifératif intéressant sur les cellules HL-60.

Ainsi, l'évaluation biologique complète de ces meilleurs agents m'a permis d'accumuler quelques informations sur le mode de fonctionnement de cette nouvelle famille. J'ai tout d'abord identifié que quatre de ces produits, contrairement à la doxorubicine, étaient sélectifs pour les cellules cancéreuses puisque ceux-ci n'avaient aucun effet sur les cellules normales WI-38. Ce résultat est très intéressant puisqu'il est rarement observé avec les agents chimiothérapeutiques utilisés couramment en clinique.

Cette évaluation biologique nous a également permis de constater que certains de ces agents causaient l'arrêt du cycle cellulaire en G_1 et induisaient l'apoptose de ces cellules. Ce blocage du cycle dans une phase précise peut nous guider vers un mécanisme d'action possible utilisant les différentes cytokines et cyclines spécifiques à la phase G_1 du cycle. Des analyses en biologie moléculaire seront donc nécessaires afin de confirmer cette hypothèse. De plus, trois de ces candidats induisent la différenciation cellulaire de cellules leucémiques, dont deux au même niveau d'efficacité que la vitamine D3. Tous ces résultats biologiques ont donc permis de confirmer le pouvoir thérapeutique de cette nouvelle famille en indiquant quelques pistes pour l'élucidation de son mécanisme d'action.

La synthèse des dernières librairies d'aminostéroïdes m'a permis de découvrir que l'utilisation des acides aminés naturels *L* sont plus efficaces que les formes *D* pour inhiber la prolifération des cellules leucémiques et que l'ajout d'un substituant sur la proline pouvait moduler son activité antiproliférative. Ces nouveaux résultats ouvriront la porte à de nombreux projets tant au niveau chimique, par la synthèse de nouvelles familles de composés, qu'au niveau biologique, par l'évaluation de l'action de ces produits sur d'autres lignés cancéreuses (leucémiques ou non) et par l'étude de la régulation de certaines protéines.

Finalement, un dernier projet de recherche visait à déterminer l'action de plusieurs dérivés du 2-méthoxyestradiol (2-ME) sur les cellules HL-60. Ces résultats ont montré que le dérivé 3-*O*-sulfamate du 2-ME était plus efficace pour inhiber la croissance des cellules leucémiques et qu'il n'agissait pas seulement comme une prodrogue. Cela suggère donc

qu'un substituant approprié en position 3 du noyau 2-ME pourrait conduire à un agent potentiellement intéressant contre la leucémie myéloïde.

Tous les résultats discutés dans la deuxième partie de cette thèse nous ont permis d'en apprendre davantage sur une nouvelle famille d'aminostéroïdes ayant un potentiel thérapeutique pour le traitement de la leucémie. L'étude préliminaire du mécanisme d'action de ces produits nous a donné certains indices et permis d'émettre quelques hypothèses. Tout d'abord, il est possible que certains de nos produits inhibent une cycline ou une protéine kinase dépendant des cyclines. Cette régulation aurait donc comme conséquence de bloquer le cycle cellulaire dans la phase G_1. Cependant, afin de confirmer cette hypothèse, une étude plus approfondie du mécanisme d'action sera nécessaire. Dans le but d'obtenir des candidats plus actifs, la modification de la chaîne en position 2β de l'androstane-3α,17β-diols ou son déplacement sur le noyau stéroïdien pourra ainsi être guidé par la compréhension de ce mécanisme d'action.

Jusqu'à maintenant nos résultats ont toutefois amenés plusieurs éléments intéressants pouvant orienter la synthèse des prochaines banques de produits. L'utilisation de différents noyaux de la proline serait sans aucun doute l'une des modifications les plus intéressantes. Ces différents noyaux devront par contre être synthétisés puisqu'ils en existent très peu sur le marché. Donc en utilisant le groupement hydroxy sur la proline, il sera possible d'inséré plusieurs éléments et ainsi obtenir un large éventail de dérivés. De plus, la position (C-2 ou C-3) et l'orientation (α ou β) de la chaîne sur le noyau stéroïdien sont d'autres éléments potentiellement intéressants pouvant nous conduire à des agents possédants des meilleures activités biologiques. Par ailleurs, puisque nos résultats ont clairement démontré l'importance du noyau stéroïdien pour l'obtention de l'activité cytotoxique, l'utilisation d'un noyau stéroïdien différent pourrait peut-être augmenter leur efficacité.

Malgré le fait que nos travaux sur la leucémie n'en sont qu'à leurs débuts, je crois que les résultats obtenus jusqu'à maintenant sont très intéressants et prometteurs. Les années d'études que je lui ais consacré auront donc permis d'établir des bases solides pour

la continuation de ce projet. La fusion de la chimie médicinale et de la biologie cellulaire a permis d'accumuler de nombreux résultats menant à des hypothèses intéressantes. La conclusion globale de cette thèse supporte donc l'importance indéniable des dérivés stéroïdiens pour le traitement de plusieurs types de cancers allant du cancer de la prostate à la leucémie.

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