Chapitre 2

Hypothèses de travail et objectifs

2.1. Hypothèses de travail

Mon projet de maîtrise est divisé en deux sections toutes deux axées sur la conception, la synthèse et l'évaluation de l'activité biologique de dérivés stéroïdiens en vue de développer de nouveaux agents anticancéreux. Les cibles biologiques sont néanmoins très différentes pour chacune de ces deux sections : la première porte sur la synthèse sur support solide d'aminostéroïdes avec un noyau C18-stéroïdien pour le développement d'agents cytotoxiques tandis que la seconde est centrée, quant à elle, sur le développement d'inhibiteurs stéroïdiens de la CYP1B1, une enzyme présentant un potentiel comme cible thérapeutique.

Section A : Tel qu'énoncé précédemment, les aminostéroïdes constituent une classe de molécules aux propriétés biologiques uniques nécessitant une attention particulière. Le RM-581, un analogue du RM-133 (C19-stéroïde) avec un noyau C18-stéroïdien amino-substitué en C2 et possédant un groupe méthoxy en C3, a présenté une activité cytotoxique très intéressante sur plusieurs lignées cellulaires issues de différents cancers et son activité a été confirmée *in vivo* sur un modèle de xénogreffe. Il est par ailleurs deux fois plus stable métaboliquement que le RM-133 et aussi plus sélectif pour les cellules cancéreuses. Le mécanisme d'action du RM-581 n'a pas encore été identifié mais notre groupe a récemment découvert que le RM-133 agit comme un aggravateur du stress du réticulum endoplasmique.

Plusieurs dérivés du RM-133 avaient été développés par synthèse sur support solide afin d'introduire rapidement différents groupes aminés en C2 du noyau C19-stéroïdien. Ceci avait été effectué au moyen d'un ancrage diéthylsilylacétylénique en C17 du stéroïde sur une résine de polystyrène (PS-DES). Au vu du potentiel prometteur du RM-581, nous avons pensé qu'utiliser la même stratégie de synthèse pour développer rapidement des analogues du RM-581 pourrait aboutir à de bons résultats. Nous espérons, en effet, que faire varier les groupes chimiques constituant la chaîne latérale du RM-581 (**Figure 1**) pourrait aboutir à l'identification de candidats avec un profil plus intéressant pour le développement d'agents anticancéreux.

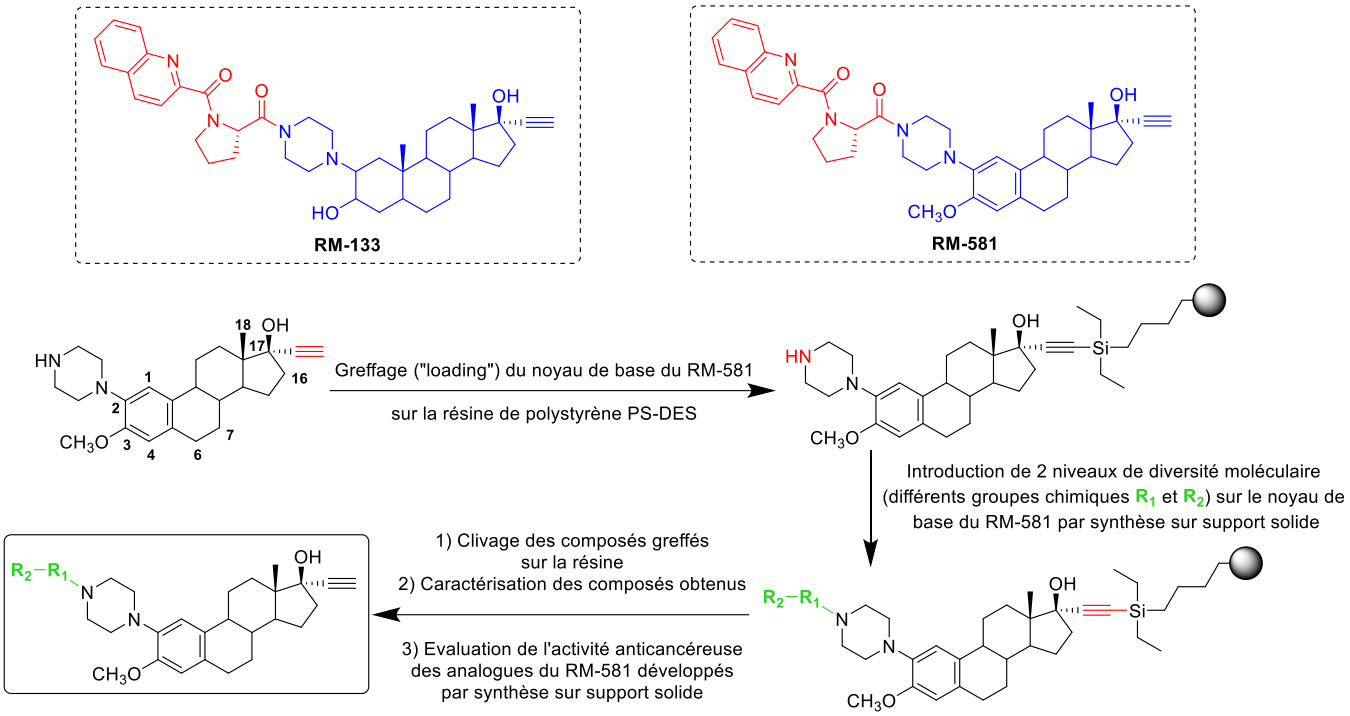

Figure 1. Représentation générale de la stratégie de synthèse sur support solide d'analogues du RM-581 qui avait été employée pour la synthèse de dérivés du RM-133. La chaîne latérale du RM-133 et du RM-581 est représentée en rouge et leur noyau de base en bleu. La résine de polystyrène PS-DES correspond aux sphères noires. Une numérotation partielle du noyau C18-stéroïdien du RM-581 est indiquée sur la structure de départ de la synthèse sur support solide. Les fonctions réactives de cette synthèse sont identifiées en rouge.

Section B : La seconde partie de mon projet de recherche s'articule autour du développement d'inhibiteurs de la CYP1B1, une enzyme présentant un potentiel thérapeutique par son aptitude à activer des composés procarcinogènes, son rôle dans le métabolisme de la 17β-estradiol (E2) et sa capacité à inactiver plusieurs médicaments, notamment des agents anticancéreux. Cette enzyme est par ailleurs surexprimée dans plusieurs cas de cancer, nous pensons donc qu'un inhibiteur en combinaison avec un agent anticancéreux pourrait être une stratégie très intéressante pour le traitement des cancers où cette enzyme est surexprimée.

Plusieurs études ont été menées sur le développement d'inhibiteurs de la CYP1B1, notamment des flavonoïdes et des stilbènes, mais peu d'inhibiteurs stéroïdiens ont été rapportés. Considérant que l'estrone (E1) et l'E2 sont tous deux des substrats de la CYP1B1, des dérivés stéroïdiens pourraient être des candidats intéressants pour l'inhibition de la CYP1B1. Notre groupe étant spécialisé dans le développement de stéroïdes, nous pensons qu'un criblage de l'activité inhibitrice de la CYP1B1 effectué sur une large variété de molécules sélectionnées

dans notre collection disponible au laboratoire pourrait aboutir à l'identification d'inhibiteurs potentiels de cette enzyme. L'identification de relations structure-activité permettra ensuite d'orienter la synthèse de nouveaux inhibiteurs stéroïdiens de la CYP1B1.

2.2. Objectifs

Section A : Basé sur l'hypothèse que la méthodologie de synthèse sur support solide précédemment employée par notre groupe pour la synthèse de dérivés du RM-133 est applicable à la synthèse d'analogues du RM-581; l'objectif de cette première section de mon projet va être de développer des librairies d'analogues du RM-581 par synthèse sur support solide (**Figure 1**) en faisant varier les groupes chimiques constituant la chaîne latérale (représentée en rouge ci-dessous) du noyau de base du RM-581 (en bleu). L'activité des composés ainsi synthétisés sera ensuite évaluée sur plusieurs lignées cellulaires issues de différents cancers en comparaison avec le RM-581, afin d'identifier des candidats avec un profil d'agent anticancéreux plus intéressant. À partir des résultats biologiques, nous espérons également pouvoir identifier des relations structure-activité pour cette nouvelle famille d'aminostéroïdes avec un noyau estrane.

Le chapitre 3 de mon mémoire porte sur la synthèse sur support solide de dérivés du RM-581, les objectifs spécifiques à atteindre dans cette section sont les suivants:

1) Synthétiser le noyau de base du RM-581 par chimie en solution puisl'introduire sur la résine de polystyrène PS-DES et optimiser certaines étapes de cette synthèse.

2) Introduire par synthèse sur support solide un premier niveau de diversité moléculaire par l'ajout de différents acides aminés sur le noyau de base du RM-581.

3) Introduire par synthèse sur support solide un second niveau de diversité moléculaire par l'ajout de différents acides carboxyliques sur l'acide aminé précédemment introduit.

4) Cliver les composés ainsi synthétisés de la résine afin de les caractériser.

5) Évaluer l'activité cytotoxique de ces différentes librairies d'analogues du RM-581 sur différentes lignées cellulaires cancéreuses.

6) Identifier des relations structure-activité à partir des niveaux de diversité moléculaire introduits et des résultats biologiques obtenus; identifier des candidats prometteurs pour le développement d'agents anticancéreux.

Section B : À partir de l'hypothèse selon laquelle des dérivés stéroïdiens pourraient constituer de puissants inhibiteurs de la CYP1B1, une enzyme jouant un rôle dans le développement de plusieurs cancers, l'objectif de cette deuxième section de mon projet va être d'effectuer un criblage biologique de l'activité enzymatique de la CYP1B1 à partir d'un large éventail de dérivés stéroïdiens disponibles au laboratoire. Les résultats obtenus permettront d'identifier des relations SAR qui orienteront la synthèse de nouveaux composés avec un profil prometteur pour le développement d'agents anticancéreux via leur capacité à inhiber l'activité de la CYP1B1. La structure cristalline de cette enzyme ayant été caractérisée, des études de "docking" seront effectuées en amont afin d'orienter le design de nouveaux inhibiteurs de la CYP1B1 (**Figure 2**).

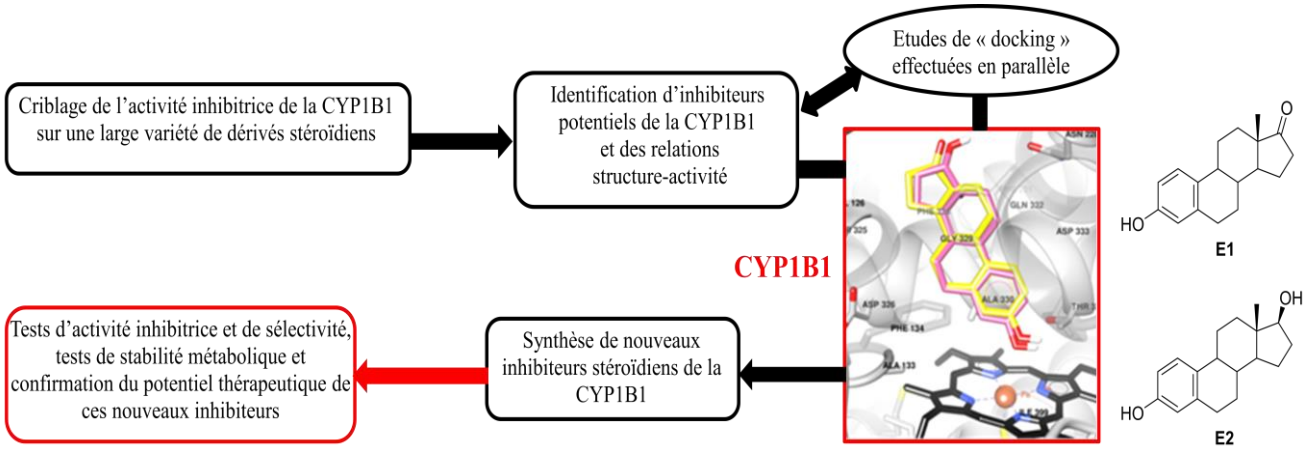

Figure 2. Approche méthodologique générale pour identifier des dérivés stéroïdiens présentant un bon potentiel d'inhibition de la CYP1B1. Le site catalytique de la CYP1B1 avec E1 (en jaune) et E2 (en rose) est également représenté. L'atome de fer (hème) est représenté par une sphère rouge et le noyau porphyrine est représenté en noir. Les structures chimiques d'E1 et E2 ont également été ajoutées en complément d'information.Cette seconde section de mon projet de recherche est composée de deux chapitres; le chapitre 4 est axé autour de la recherche bibliographique des inhibiteurs de la CYP1B1 identifiés jusqu'à aujourd'hui au sein d'une large variété de familles chimiques. L'objectif de ce projet est de rédiger une revue scientifique rapportant les inhibiteurs de la CYP1B1 actuellement connus et permettant ainsi de bien cerner le projet de recherche.

Le chapitre 5 est la mise en pratique des hypothèses énoncées ci-haut dont les objectifs sont les suivants :

1) Évaluer au moyen d'un test enzymatique standard l'activité inhibitrice de plusieurs dérivés stéroïdiens disponibles au laboratoire sur la CYP1B1.

2) Dégager des relations structure-activité qui vont orienter la synthèse de nouveaux composés prometteurs pour l'inhibition de la CYP1B1.

3) Effectuer des études de "docking" afin d'analyser et de rationnaliser les résultats biologiques obtenus ainsi que d'orienter le design d'une nouvelle série de dérivés stéroïdiens.

3) Évaluer l'activité inhibitrice de ces nouveaux dérivés stéroïdiens à l'encontre de la CYP1B1; tester la sélectivité de ces composés pour la CYP1B1 en comparaison avec la CYP1A1 et la CYP1A2; et évaluer la stabilité métabolique de ces composés.

4) Mettre en évidence le potentiel thérapeutique de ces composés dans le cadre de certains cancers au moyen d'un modèle biologique adapté, en mesurant notamment leur activité sur des cellules surexprimant la CYP1B1.

Chapitre 3

Synthèse sur support solide de dérivés aminostéroïdiens présentant des activités antiprolifératives significatives sur de multiples lignées cellulaires cancéreuses au moyen d'un ancrage diéthylsilylacétylénique

Avant-propos

Ce chapitre 3 est constitué d'un manuscrit qui a été soumis au journal *Anti-Cancer Agents in Medicinal Chemistry* le 12 octobre 2017 et fait actuellement l'objet d'une évaluation.

Plusieurs auteurs ont contribué au bon déroulement de ce projet, notamment René Maltais qui m'a apporté un grand soutien pour la mise en place des différentes étapes et paramètres nécessaires à la synthèse sur support solide des dérivés aminostéroïdiens. Il m'a également aidé dans le choix des groupes introduits sur le mestranol, le noyau de base du RM-581, et dans l'interprétation des résultats biologiques obtenus. Martin Perreault et Jenny Roy ont tous deux réalisé les essais biologiques présentés dans cet article et m'ont apporté différentes suggestions pour l'avancée de mon projet.

Mon directeur de recherche, Donald Poirier, a supervisé et participé à l'orientation des travaux en suggérant plusieurs idées pour le design des molécules, l'optimisation de leur synthèse ainsi que le choix des essais biologiques appropriés pour supporter l'intérêt thérapeutique de ces nouveaux dérivés aminostéroïdiens. Mon directeur a également participé à la rédaction du manuscrit en apportant plusieurs suggestions et corrections à ma première version.

Pour ma part, j'ai réalisé la synthèse de plusieurs grammes de résine comportant le noyau de base du RM-581. En utilisant cette résine, j'ai ensuite effectué la synthèse sur support solide et la caractérisation des 40 analogues du RM-581 décrits dans ce manuscrit. J'ai également resynthétiser les deux composés les plus actifs par chimie en solution. Basé sur les résultats obtenus, j'ai finalement participé à l'orientation des travaux et j'ai rédigé l'article.

Résumé

Le RM-133 appartient à une nouvelle famille de dérivés aminostéroïdiens démontrant des propriétés anticancéreuses très intéressantes confirmées *in vivo* dans quatre modèles de xénogreffes de cancers chez la souris. Cependant, la stabilité métabolique de RM-133 doit être améliorée. Après une étude, le remplacement de son noyau androstane par un noyau estrane plus stable a conduit au développement du RM-581, un analogue du RM-133 avec un noyau mestranol. En utilisant une stratégie de synthèse en phase solide impliquant cinq étapes, nous avons rapidement synthétisé une chimiothèque d'analogues du RM-581 en utilisant l'ancrage ("linker") diéthylsilylacétylénique récemment développé. Afin d'établir des relations structure-activité, nous avons ensuite mesuré leur activité antiproliférative sur un panel de lignées cellulaires provenant de divers cancers (sein, prostate, ovaire et pancréas). Certains dérivés du mestranol ont montré des activités anticancéreuses *in vitro* proches ou supérieures à celles observées pour le RM-581. Le composé **23**, un dérivé du mestranol présentant une chaîne latérale avec un groupe ((3,5-diméthylbenzoyl)-L-prolyl)pipérazine en position C2, s'est avéré très actif comme agent antiprolifératif sur les cellules cancéreuses LNCaP, PC-3, MCF-7, PANC-1 et OVCAR-3 (IC_{50} = 0.56, 0.89, 1.36, 2.47 et 3.17 μM, respectivement) et deux fois plus actif que le RM-581. Facilement synthétisé avec de bons rendements tant par synthèse organique sur support solide que par chimie classique en solution, ce candidat prometteur pourrait être utilisé comme agent antiprolifératif pour le traitement de divers cancers, notamment pancréatiques et ovariens, tous deux ayant de très mauvais pronostic.

Anti-Cancer Agents Med. Chem. (submitted)

Parallel Solid-Phase Synthesis Using a New Diethylsilylacetylenic Linker and Leading to Mestranol Derivatives with Potent Antiproliferative Activities on Multiple Cancer Cell Lines

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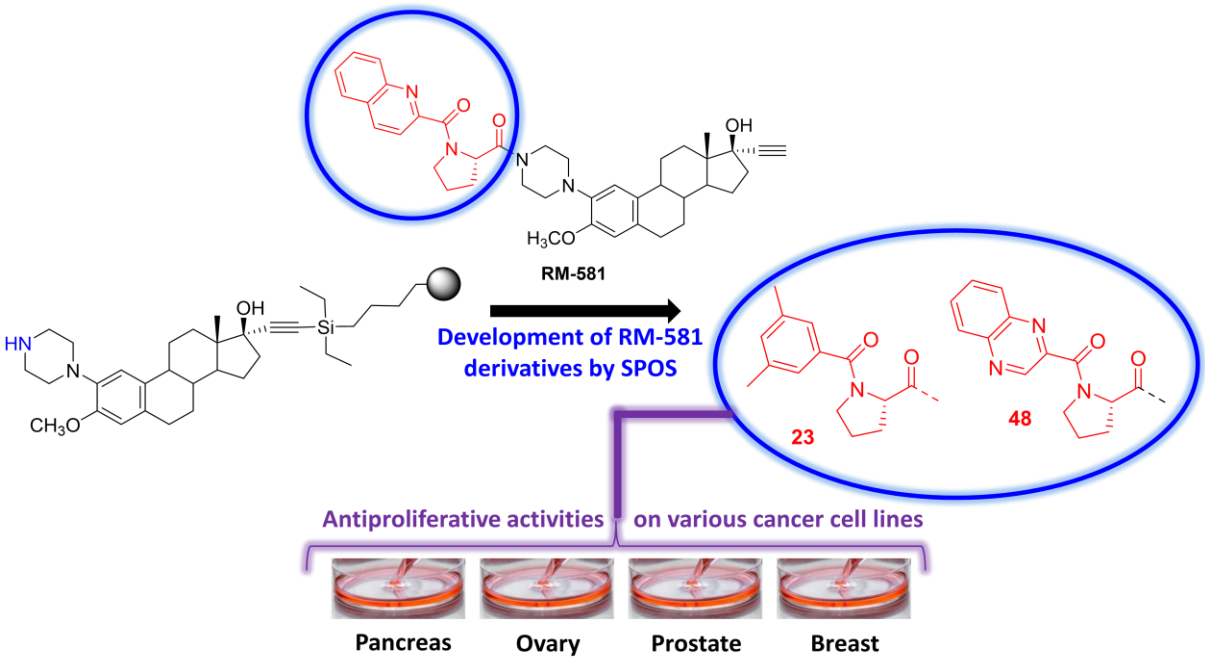
Abstract

RM-133 belongs to a new family of aminosteroid derivatives demonstrating very interesting anticancer properties, as confirmed *in vivo* in four mouse cancer xenograft models. However, the metabolic stability of RM-133 needs to be improved. After investigation, the replacement of its androstane scaffold by a more stable estrane scaffold led to the development of the mestranol derivative RM-581. Using solid-phase strategy involving five steps, we readily synthesized a series of RM-581 analogs using the recently-developed diethylsilyl acetylenic linker. To establish structure-activity relationships, we then investigated their antiproliferative potency on a panel of cancer cell lines from various cancers (breast, prostate, ovarian and pancreatic). Some of the mestranol derivatives have shown *in vitro* anticancer activities that are close to, or better than those observed for RM-581. Compound **23**, a mestranol derivative having a ((3,5-dimethylbenzoyl)-L-prolyl)piperazine side chain at position C2, was found to be very active as an antiproliferative agent on LNCaP, PC-3, MCF-7, PANC-1 and OVCAR-3 cancer cells (IC_{50} = 0.56, 0.89, 1.36, 2.47 and 3.17 µM, respectively) and to be twice as active as RM-581. Readily synthesized in good yields by both solid-phase organic synthesis and classic solution-phase chemistry, this promising candidate could be used as an antiproliferative agent on a variety of cancers, notably pancreatic and ovarian cancers, that both have very bad prognoses.



1. INTRODUCTION

Cancer is still one of the main causes of death, despite multiple efforts to fight this disease. Several anticancer drugs have been developed over the past years, but the efficiency and selectivity of these treatments need to be improved. Indeed, poor selectivity of such anticancer agents for cancer cells over normal cells can lead to severe side-effects, making the lives of patients even more difficult [1-3].

Our research group has generated aminosteroid derivatives showing cytotoxic properties on several cancer cells [4-6]. Based on structure-activity relationship studies, a promising candidate called RM-133 emerged (Fig. **1A**) [4]. In fact, this 5α-androstane-3α,17β-diol derivative displayed *in vitro* anticancer activity like Doxorubicin in several cancer cell lines (MCF-7, T-47D, OVCAR-3, HL-60, LNCaP, WEHI-3 and PANC-1) but showed weak toxicity over normal human cells [4, 7]. Moreover, it should be emphasized that the anticancer potency of RM-133 was validated *in vivo* on three cancer xenograft models (HL-60, OVCAR-3 and PANC-1 cell lines) [7, 8]. RM-133's mechanism of action was recently investigated, showing that RM-133 displays cytotoxic activities by acting as an endoplasmic reticulum (ER) stress aggravator through the disruption of cholesterol homeostasis (ERSA) [9-11].

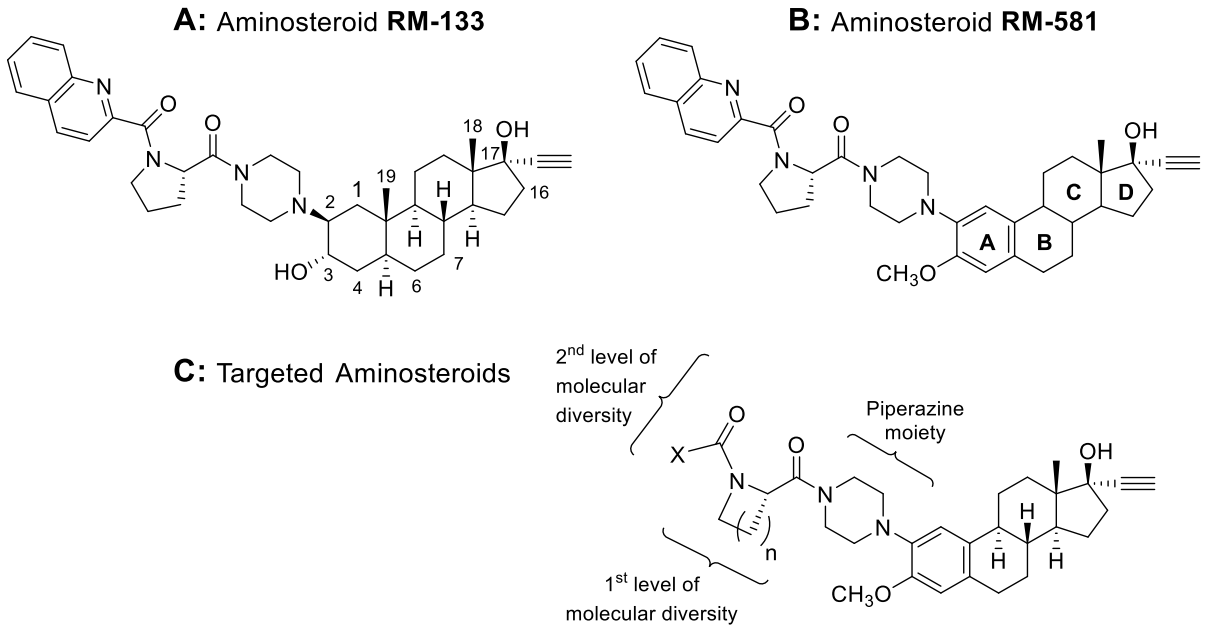


Fig. (1). Chemical structures of C19-steroid derivative RM-133 (**A**), C18-steroid derivative RM-581 (**B**) and targeted aminosteroids (**C**). RM-133 and RM-581 share the same quinoline-proline-piperazine side chain, but with two different steroid backbones. The new

aminosteroids are designed with a C18-steroid core and a new diversified side chain with two levels of molecular diversity. Partial numbering of carbons and identification of steroid A-D rings are reported in A and B, respectively.

Nevertheless, due to the weak tolerance of RM-133 for hepatic clearance, a high dose was required to obtain a significant cytotoxic effect in mouse tumor models [7]. After further structure-activity relationship (SAR) investigation, it was demonstrated that the protection of the alcohol at position 3α of the androstane steroid backbone could increase RM-133's metabolic stability [12]. Moreover, replacement of the 5α-androstane backbone (C19-steroid) of RM-133 by an estra-1,3,5(10)-trien backbone (C18-steroid) led to the development of RM-581, the C18-steroid analog of RM-133 (same side chain in C2) with a methoxy group in C3 instead of an alcohol (Fig. **1B**) [13]. In fact, RM-581 is a mestranol-based derivative that is known to be a more stable version of ethynyl-estradiol derivatives which do not have this methoxy group in C3 [14-16].

Biological results obtained with the new lead compound RM-581 were encouraging, because it was found to be two-fold more stable than RM-133 [13]. Furthermore, the anticancer potency of its predecessor RM-133 was conserved and, interestingly, RM-581 has also shown a signicant increase in selectivity for cancer cells, over normal cells. Finally, the *in vitro* properties of RM-581 were confirmed *in vivo* on a mouse xenograft (human MCF-7 cells) model [13]. It should be noted that, unlike C19-steroids, the A-ring of C18-steroids has a planar geometry due to its aromaticity, thus confering a distinct orientation to the side chain at position 2. However, the antiproliferative activity of RM-581 has only been tested on MCF-7 breast cancer cells and its distinct steroid nucleus (C18-steroid) bearing a lateral chain at position 2 should be validated and optimized for other cancers.

Thereby, the objective of this study is to synthesize new analogs of aminosteroid RM-581 by modifying the side chain at position 2 of the steroid backbone to identify promising candidates, which could be used for the treatment of several cancers, notably for poor prognostic cancers such as ovarian and pancreatic cancers [17, 18]. To accelerate the synthesis of these aminosteroid derivatives owning an ethynyl group at position 17α, our research group developed a diethylsilyl acetylenic linker for the solid-phase organic synthesis (SPOS) in parallel [19]. This methodology was previously used to generate libraries of RM-133 analogs and gave relevant results [19, 20]. We therefore planned to use the same approach for the preparation of RM-581 analogs by trying different combinations of amino

acids (1st level of molecular diversity) and carboxylic acids (2nd level of molecular diversity) to identify promising candidates. Herein, we report the chemical synthesis of a series of targeted mestranol derivatives (Fig. **1C**) and their cytotoxic activities on a pannel of nine cancer cell lines representative of four cancers among the most common (breast and prostate cancers) or with a very bad prognosis (ovarian and pancreatic cancers).

2. MATERIALS AND METHODS

2.1. Chemistry

2.1.1 General

The polystyrene butyldiethylsilane (PS-DES) resin with a loading of 1.47 mmol/g was supplied by Biotage (Charlotte, NC, USA). Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada), Matrix Innovation (Québec, QC, Canada), Alfa Aesar (Wood Hill, MA, USA), Chem-Impex Int'l. Inc. (Wood Dale, IL, USA) and AAPPTec (Louisville, KY, USA). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous dichloromethane (DCM), diethylether, dimethylformamide (DMF), dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) were obtained from Sigma-Aldrich. Ethyl acetate (EtOAc), hexanes and methanol (MeOH) were purchased from Fisher Scientific. The loading of steroid **1** on PS-DES-Cl (resin **4**) was performed in peptide synthesis vessels with frit equipped for vacuum filtration (ChemGlass Inc.; Vineland, NJ, USA). The steps giving final compound **9** were realized with an AAPPTec Solution automated organic synthesizer (Louisville, KY, USA) using a solid-phase reaction block (96 wells). Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20-mm silica gel 60 F_{254} plates (E. Merck; Darmstadt, Germany) and with 230-400 mesh ASTM silica gel 60 (Silicycle; Québec, QC, Canada), respectively. Infrared (IR) spectra were recorded on a MB 3000 ABB FTIR spectrometer (Québec, QC, Canada) and only the significant bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). Chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm) for ^{1}H and ^{13}C NMR, respectively. HPLC purities of final compounds released from solid support were determined with a Shimadzu apparatus (Kyoto, Japan) using a Shimadzu SPD-M20A Photodiode array detector, a Alltima

HP C18 reversed-phase column (250 mm x 4.6 mm, 5 μm) and a solvent gradient of MeOH-H_2O. The wavelength of the UV detector was selected between 190-205 nm. Low resolution mass spectra (LRMS) were recorded on a Shimadzu apparatus equipped with APCI (atmospheric pressure chemical ionization). High-resolution mass spectra (HRMS) were provided by Pierre Audet from the Department of Chemistry at Université Laval (Québec, QC, Canada).

2.1.2. Solid-phase synthesis of mestranol derivatives 10-49

*2.1.2.1. Coupling of steroid **1** to PS-DES resin **3** (synthesis of resin **5**)*

To a solution of compound **1** [13] (2.88 g, 7.3 mmol) in dry THF (35 mL) under an argon atmosphere at 0 °C was added dropwise a methyl lithium solution (1.6 M) in diethyl ether (13.7 mL, 21.9 mmol). DMSO (10 mL) was then added and the resulting mixture was stirred for 1.5 h at room temperature (rt) to generate compound **2**. To PS-DES resin **3** (2.48 g, 3.65 mmol) previously dried under vacuum and swollen in dry DCM (14 mL) was added a solution of 1,3-dichloro-5,5-dimethylhydantoin (2.09 g, 10.6 mmol) in dry DCM (26 mL) under an argon atmosphere. The resulting solution was stirred for 1 h at rt and the activated PS-DES-Cl resin **4** was washed twice with dry DCM (100 mL) and once with dry THF (100 mL). The organolithium **2,** obtained from compound **1**, was immediately added to the activated resin **4** and the mixture was stirred overnight at rt under an argon atmosphere. The resin was washed successively with DCM (100 mL), MeOH (100 mL), H_2O (100 mL), MeOH (100 mL), DCM (100 mL) and dried overnight under vaccum to give resin **5** (36% of loading by increasing weight).

*2.1.2.2. Addition of amino acids and carboxylic acids (synthesis of resins **8**)*

Portions of resin **5** (~ 100 mg) were placed in 4 mL-reactor wells of an automated synthesizer reaction block (96-well format; AAPPTec). A solution of the appropriate Fmoc-protected amino acid (Fmoc-L-azetidine, Fmoc-L-proline, Fmoc-L-homoproline) (0.3 M), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (0.5 M) and *N*,*N*-diisopropylethylamine (DIPEA) (1 M) was added to each well in DMF (1 mL). The suspensions were vortexed at 600 rpm for 4 h; the wells were then filtered using the vacuum system and resins **6** were washed with DMF (2 mL). The wells were filtered again and a

second cycle of coupling of the resins **6** with amino acids was achieved, as reported above. The deprotection of the Fmoc group of resins **6** was carried out by adding a solution of piperidine (20%, v/v) to each well in DMF (5 mL) (two deprotection cycles). The suspensions were vortexed at 600 rpm for 1 h; the wells were filtered and the resins **7** washed with DMF and ethanol (EtOH). To introduce a second level of molecular diversity, a solution of the appropriate carboxylic acid (0.5 M) (Supporting Information; **Table S1**), HBTU (0.5 M) and DIPEA (1 M) in DMF (1 mL) was added to each well. The suspensions were vortexed at 600 rpm for 3 h, the wells were filtered and the resins **8** washed with DCM and EtOH.

*2.1.2.3. Cleavage of the resin-bound aminosteroid derivatives **10-49** (synthesis of **9** from resins **8**)*

To each resin **8** was added a solution of HCl-MeOH-DCM (1:9:30) (2.5 mL) and the resulting suspensions were vortexed at 600 rpm over a total period of 24 h. The resins were then filtered, washed with DCM, and washed again with a solution of MeOH-DCM (1:1) (1.5 mL). The filtrates were neutralized with a saturated aqueous solution of $NaHCO_3$ (2 mL). The biphasic solutions were filtered with a phase separator syringe (Biotage, Uppsala, Sweden) and each organic solution was evaporated under reduced pressure to give aminosteroid derivatives **9**.

In summary, we performed the synthesis of three libraries (A-C) of 21 products. In the first one (library A), we used three different amino acids (L-azetidine, L-homoproline and L-proline) and seven carboxylic acids. For the two others (libraries B and C), only L-proline was selected as amino acid, whereas 21 different carboxylic acids were used in each case. The purity of the 63 compounds thus obtained was determined by HPLC and only aminosteroid derivatives with a purity higher than 70% were selected for a first round of biological assays. Forty (40) final compounds **10-49** with L-proline as amino acid were thus selected and characterized by ^{1}H NMR and LRMS (Fig. **2**).

*2.1.2.4. Characterisation of aminosteroid derivatives **10-49***

(2E)-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]but-2-en-1-one (**10**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.40 (m, residual CH and CH_2), 1.87 and 1.88 (2d, J = 6.9 Hz, C$\underline{H}_3$CH=CH),

2.606 and 2.611 (2s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.60-3.90 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.77 and 5.00 (2m, NHCO of Pro), 6.19 (d, J = 15.0 Hz, CH=C<u>H</u>CO), 6.58 (s, CH-1), 6.85 (s, CH-4), 6.94 (m, CH_3C<u>H</u>=CH); LRMS for $C_{34}H_{46}N_3O_4$ $[M + H]^+$: 560.7; HPLC purity: 87.5%.

1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-3-methylbut-2-en-1-one (**11**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 1.85 and 1.86 (2d, CH_3C), 2.08 and 2.09 (2d, J = 1.0 Hz, C<u>H</u>$_3$CCH), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.90-3.20 (m, 2 x CH_2N), 3.55-3.88 (m, 3 x CH_2NCO), 3.83 and 3.84 (2 s, OCH_3), 4.69 and 4.99 (2m, NHCO of Pro), 5.86 (s, $(CH_3)_2C$=C<u>H</u>), 6.58 (s, CH-1), 6.85 (s, CH-4); LRMS for $C_{35}H_{48}N_3O_4$ $[M + H]^+$: 574.8; HPLC purity: 83.7%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[(2S)-tetrahydrofuran-2-ylcarbonyl]pyrrolidin-2-yl}methanone (**12**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.60-3.90 (m, 3 x CH_2NCO), 3.83 and 3.84 (2s, OCH_3), 3.85 (t, J = 8.5 Hz, CH_2O), 4.25 and 4.61 (2m, CH_2C<u>H</u>O), 4.63 and 4.94 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.85 (s, CH-4); LRMS for $C_{35}H_{48}N_3O_5$ $[M + H]^+$: 590.8; HPLC purity: 82.0%.

1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-2-(phenylsulfanyl)ethanone (**13**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.40 (m, residual CH and CH_2), 2.60 and 2.61 (2 s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.18 (m, 2 x CH_2N), 3.60-3.90 (m, 3 x CH_2NCO), 3.72 and 3.76 (2s, CH_2S), 3.83 and 3.84 (2s, OCH_3), 4.84 and 4.94 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.84 (s, CH-4), 7.24 (m, 3 x CH of Ph), 7.46 (d, J = 8.3 Hz, 2 x CH of Ph); LRMS for $C_{38}H_{48}N_3O_4S$ $[M + H]^+$: 642.3; HPLC purity: 74.7%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[4-(methylamino)benzoyl]pyrrolidin-2-yl}methanone (**14**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.40 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.81 (m, CH_2-6), 2.86 (s, C<u>H</u>$_3$NH), 2.95-3.20 (m, 2 x CH_2N), 3.62-3.98 (m, 3 x CH_2NCO), 3.82 and 3.83 (2s,

OCH_3), 4.93 and 5.17 (2m, NHCO of proline), 6.55 (d, J = 8.7 Hz, 2 x CH of Ar), 6.57 (s, CH-1), 6.85 (s, CH-4), 7.53 (d, J = 6.9 Hz, 2 x CH of Ar); LRMS for $C_{38}H_{49}N_4O_4$ $[M + H]^+$: 625.8; HPLC purity: 81.3%.

cyclopropyl[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**15**). ^1H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 0.77 and 0.79 (2s, 2 x H of ($C\underline{H}_2$)$_2$CHCO), 0.85-2.40 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.60-3.95 (m, 3 x CH_2NCO), 3.83 and 3.84 (2s, OCH_3), 4.90 and 4.95 (2m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{34}H_{46}N_3O_4$ $[M + H]^+$: 560.3; HPLC purity: 82.2%.

1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-2-(4-hydroxyphenyl)ethanone (**16**). ^1H NMR ($CDCl_3$) δ: 0.87 (s, CH_3-18), 1.23-2.37 (m, residual CH and CH_2), 2.61 and 2.62 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.52-4.00 (m, 3 x CH_2NCO), 3.55 (s, PhC$\underline{H}_2$CON), 3.82 and 3.84 (2s, OCH_3), 4.62 and 4.96 (2m, NHCO of Pro), 6.56 (s, CH-1), 6.63 and 6.77 (2d, J = 8.4 Hz, 2 x CH Ar), 6.83 (s, CH-4), 7.02 and 7.12 (2d, J = 8.4 Hz, 2 x CH of Ar); LRMS for $C_{38}H_{48}N_3O_5$ $[M + H]^+$: 626.4; HPLC purity: 79.2%.

2-(4-aminophenyl)-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]ethanone (**17**). ^1H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.48-3.85 (m, 3 x CH_2NCO), 3.61 (s, PhC$\underline{H}_2$CON), 3.83 and 3.84 (2s, OCH_3), 4.56 and 4.94 (2m, NHCO of Pro), 6.57 (s, CH-1), 6.64 and 7.08 (2d, J = 8.3 Hz, 2 x CH of Ar), 6.84 (s, CH-4), 6.71 and 7.35 (2d, J = 8.3 Hz, 2 x CH of Ar); LRMS for $C_{38}H_{49}N_4O_4$ $[M + H]^+$: 625.4; HPLC purity: 73.5%.

2,2-dicyclohexyl-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]ethanone (**18**). ^1H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.90-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.90-3.20 (m, 2 x CH_2N), 3.58-3.90 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 5.01 (m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{44}H_{64}N_3O_4$ $[M + H]^+$: 699.6; HPLC purity: 77.2%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[(3-methylcyclohexyl)carbonyl]pyrrolidin-2-yl}methanone (**19**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.90 (d, J = 6.6 Hz, C<u>H</u>$_3$CH), 0.90-2.47 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.55-3.90 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.93 (m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{38}H_{54}N_3O_4$ $[M + H]^+$: 616.8; HPLC purity: 78.4%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[(4-methylcyclohexyl)carbonyl]pyrrolidin-2-yl}methanone (**20**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.97 (d, J = 7.1 Hz, C<u>H</u>$_3$CH), 1.25-2.50 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.55-3.85 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.94 (m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{38}H_{54}N_3O_4$ $[M + H]^+$: 616.8; HPLC purity: 77.9%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[(2-methylcyclohexyl)carbonyl]pyrrolidin-2-yl}methanone (**21**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.95 and 0.99 (2d, J = 7.0 Hz, C<u>H</u>$_3$CH), 1.25-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.60-3.85 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.96 (m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{38}H_{54}N_3O_4$ $[M + H]^+$: 616.8; HPLC purity: 81.4%.

(4-tert-butylcyclohexyl)[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**22**). ^{1}H NMR ($CDCl_3$) δ: 0.84 (s, $(CH_3)_3$C), 0.89 (s, CH_3-18), 0.90-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.55-3.88 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.95 (m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{41}H_{60}N_3O_4$ $[M + H]^+$: 658.5; HPLC purity: 80.6%.

[(2S)-1-(3,5-dimethylbenzoyl)pyrrolidin-2-yl]{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**23**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.32 (s, 2 x CH_3 of Ar), 2.60 and 2.62 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.50-4.00 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.57

and 5.14 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 7.04 (s, CH of Ar), 7.19 (s, 2 x CH of Ar); LRMS for $C_{39}H_{50}N_3O_4$ $[M + H]^+$: 624.4; HPLC purity: 79.4%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-{[(1R,2R)-2-phenylcyclopropyl]carbonyl}pyrrolidin-2-yl]methanone (**24**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.50 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m, 2 x CH_2N), 3.50-3.95 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.78 and 4.97 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.85 (s, CH-4), 7.10-7.30 (m, 5 x CH of Ar); LRMS for $C_{40}H_{50}N_3O_4$ $[M + H]^+$: 636.8; HPLC purity: 85.6%.

biphenyl-4-yl[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**25**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.35 (m, residual CH and CH_2), 2.60 (s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.22 (m, 2 x CH_2N), 3.60-4.00 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.65 and 5.18 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.87 (s, CH-4), 7.35-7.70 (m, 9 x CH of biphenyl); LRMS for $C_{43}H_{50}N_3O_4$ $[M + H]^+$: 672.8; HPLC purity: 83.2%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[2-(phenylamino)benzoyl]pyrrolidin-2-yl}methanone (**26**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.45-3.97 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.74 and 5.14 (2m, NHCO of Pro), 6.59 (s, CH-1), 6.83 (t, J = 7.4 Hz, 2 x CH of diphenylamine), 6.87 (s, CH-4), 6.93 (m, CH of diphenylamine), 7.25 (m, 4 x CH of diphenylamine), 7.33 and 7.40 (2d, J_A = 7.5 Hz and J_B = 8.3 Hz, 2 x CH of diphenylamine), 8.04 (s, NH); LRMS for $C_{43}H_{51}N_4O_4$ $[M + H]^+$: 687.8; HPLC purity: 81.8%.

{(2S)-1-[3,5-bis(trifluoromethyl)benzoyl]pyrrolidin-2-yl}{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**27**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.48-4.00 (m, 3 x CH_2NCO), 3.84 and 3.85 (2s, OCH_3), 4.46 and 5.12 (2m, NHCO of Pro), 6.59 (s, CH-1), 6.87 (s, CH-4), 7.94 (s, CH of Ar), 8.08 (s, 2 x CH of Ar); LRMS for $C_{39}H_{44}F_6N_3O_4$ $[M + H]^+$: 733.1; HPLC purity: 77.1%.

(2E)-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-3-phenylprop-2-en-1-one (**28**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.70-3.97 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.89 and 5.07 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.79 (d, J = 15.5 Hz, CH=C<u>H</u>CO), 6.87 (s, CH-4), 7.36 and 7.52 (2m, 5 x CH of Ph), 7.71 (d, J = 15.5 Hz, C<u>H</u>=CHCO); LRMS for $C_{39}H_{48}N_3O_4$ $[M + H]^+$: 622.8; HPLC purity: 85.1%.

[(2S)-1-(4-benzoylbenzoyl)pyrrolidin-2-yl]{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**29**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.50-4.00 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.58 and 5.15 (2m, NHCO of Pro), 6.59 (s, CH-1), 6.87 (s, CH-4), 7.47-7.85 (m, 9 x CH of benzophenone); LRMS for $C_{44}H_{50}N_3O_5$ $[M + H]^+$: 700.4; HPLC purity: 81.6%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[(4-methoxycyclohexyl)carbonyl]pyrrolidin-2-yl}methanone (**30**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.10-2.50 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.27 and 3.35 (2s, C<u>H</u>$_3$OCH), 3.45-3.90 (m, 3 x CH_2NCO and CH_3OC<u>H</u>), 3.83 (s, OCH_3), 4.73 and 4.94 (2m, NHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4); LRMS for $C_{38}H_{54}N_3O_5$ $[M + H]^+$: 632.9; HPLC purity: 80.1%.

2-[(1R)-bicyclo[2.2.1]hept-2-yl]-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]ethanone (**31**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.95-2.40 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.81 (m, CH_2-6), 2.90-3.15 (m, 2 x CH_2N), 3.48-3.90 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.66 and 4.96 (2m, NHCO of Pro), 6.57 (s, CH-1), 6.85 (s, CH-4); LRMS for $C_{39}H_{54}N_3O_4$ $[M + H]^+$: 629.1; HPLC purity: 79.5%.

{(2S)-1-[4-(diethylamino)benzoyl]pyrrolidin-2-yl}{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**32**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.16 (t, J = 6.8 Hz, 2 x C<u>H</u>$_3$$CH_2N$), 1.25-2.38 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m, 2 x CH_2N), 3.37 (q, J = 6.6 Hz, 2 x

$CH_3C\underline{H_2}N$), 3.60-4.02 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 5.19 (m, NHCO of Pro), 6.57 (s, CH-1), 6.60 (d, J = 8.5 Hz, 2 x CH of Ar), 6.85 (s, CH-4), 7.55 (d, J = 6.6 Hz, 2 x CH of Ar); LRMS for $C_{41}H_{55}N_4O_4$ $[M + H]^+$: 667.8; HPLC purity: 81.1%.

1-(3-{[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]carbonyl}phenyl)ethanone (**33**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.58 and 2.63 (2s, CH_3CO), 2.82 (m, CH_2-6), 2.95-3.21 (m, 2 x CH_2N), 3.52-4.00 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.56 and 5.14 (2m, NHCO of Pro), 6.58 (s, CH-1), 6.87 (s, CH-4), 7.52 (t, J = 7.7 Hz, CH of Ar), 7.81 (d, J = 7.7 Hz, CH of Ar), 8.03 (d, J = 7.9 Hz, CH of Ar), 8.17 (s, CH of Ar); LRMS for $C_{39}H_{48}N_3O_5$ $[M + H]^+$: 638.7; HPLC purity: 84.7%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-(quinolin-6-ylcarbonyl)pyrrolidin-2-yl]methanone (**34**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.36 (m, residual CH and CH_2), 2.60 (s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.22 (m broad, 2 x CH_2N), 3.54-4.02 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.60 and 5.20 (2m, NCHCO of Pro), 6.59 (s, CH-1), 6.87 (s, CH-4), 7.45, 7.93 and 8.15 and 8.97 (4m, 6 x CH of quinoline); LRMS for $C_{40}H_{47}N_4O_4$ $[M + H]^+$: 647.3; HPLC purity: 86.9%.

(4-fluoronaphthalen-1-yl)[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**35**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 (s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.23 (m broad, 2 x CH_2N), 3.74-4.03 (m, 3 x CH_2NCO), 3.86 (s, OCH_3), 4.92 and 5.23 (2m, NCHCO of Pro), 6.59 (s, CH-1), 6.88 (s, CH-4), 7.15 (m, CH of Ar), 7.48 (m, CH of Ar), 7.58 (t, J = 8.1 Hz, CH of Ar), 7.66 (t, J = 7.1 Hz, CH of Ar), 8.13 (d, J = 8.3 Hz, CH of Ar), 8.37 (d, J = 7.3 Hz, CH of Ar); LRMS for $C_{41}H_{47}FN_3O_4$ $[M + H]^+$: 664.7; HPLC purity: 86.5%.

1-benzothiophen-2-yl[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**36**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.36 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.72-4.12 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 5.00 and 5.19 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.87 (s, CH-4), 7.40 (m, 2 x CH of Ar), 7.80 (s,

CH=CS), 7.84 (m, 2 x CH of Ar); LRMS for $C_{39}H_{46}N_3O_4S$ $[M + H]^+$: 652.8; HPLC purity: 82.7%.

[(2S)-1-(2-bromobenzoyl)pyrrolidin-2-yl]{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**37**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 and 2.62 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.25-4.03 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.45 and 5.15 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 7.14-7.44 (m, 3 x CH of Ar), 7.53 and 7.57 (2d, J = 7.5 Hz, CH of Ar); LRMS for $C_{37}H_{45}Br^{81}N_3O_4$ $[M + H]^+$: 676.0; HPLC purity: 82.8%.

1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-2-phenoxyethanone (**38**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m broad, 2 x CH_2N), 3.64-3.95 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.65 and 4.75 (2d of AB system, J = 14.0 Hz, OCH_2), 4.92 and 4.98 (2m, NCHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4), 6.88-7.00 (m, 3 x CH of Ar), 7.29 (m, 2 x CH of Ar); LRMS for $C_{38}H_{48}N_3O_5$ $[M + H]^+$: 626.3; HPLC purity: 77.0%.

[(2S)-1-(3,5-difluorobenzoyl)pyrrolidin-2-yl]{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**39**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.50-4.00 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.50 and 5.09 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 6.75-6.90 (m, CH of Ar), 6.96 and 7.13 (2m, 2 x CH of Ar); LRMS for $C_{37}H_{44}F_2N_3O_4$ $[M + H]^+$: 632.5; HPLC purity: 85.0%.

[(2S)-1-(2,4-dimethoxybenzoyl)pyrrolidin-2-yl]{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}methanone (**40**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.37-4.00 (m, 3 x CH_2NCO), 3.82, 3.83 and 3.84 (3s, 3 x OCH_3), 4.54 and 5.12 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 6.41-6.51 (m, 2 x CH of Ar), 7.18 and 7.32 (2d, J = 8.3 Hz, CH of Ar); LRMS for $C_{39}H_{50}N_3O_6$ $[M + H]^+$: 656.4; HPLC purity: 88.7%.

furan-2-yl[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**41**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.68-4.15 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.93 and 5.12 (2m, NCHCO of Pro), 6.48 (m, CH of furan), 6.58 (s, CH-1), 6.86 (s, CH-4), 7.11 (d, J = 3.2 Hz, CH of furan), 7.51 (d, J = 0.8 Hz, CH of furan); LRMS for $C_{35}H_{44}N_3O_5$ $[M + H]^+$: 586.7; HPLC purity: 83.8%.

1,3-benzodioxol-5-yl[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]methanone (**42**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.36 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.60-4.00 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.61 and 5.12 (2m, NCHCO of Pro), 5.99 (s, OCH_2O), 6.58 (s, CH-1), 6.81 (d, J = 8.0 Hz, CH of Ar), 6.86 (s, CH-4), 7.11 (s, CH of Ar), 7.16 (d, J = 8.1 Hz, CH of Ar); LRMS for $C_{38}H_{46}N_3O_6$ $[M + H]^+$: 640.8; HPLC purity: 85.4%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-(pyridin-3-ylcarbonyl)pyrrolidin-2-yl]methanone (**43**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.38 (m, residual CH and CH_2), 2.60 (s, C≡CH), 2.81 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.60-3.98 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.58 and 5.14 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 7.35 (m, CH of pyr), 7.94 (m, CH of pyr), 8.67 (m, CH of pyr), 8.86 (s, CH of pyr); LRMS for $C_{36}H_{45}N_4O_4$ $[M + H]^+$: 597.3; HPLC purity: 77.0%.

2-cyclohexyl-1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]ethanone (**44**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 0.80-2.38 (m, residual CH and CH_2), 2.19 and 2.23 (2d, J = 7.2 Hz, CH_2Cy), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m broad, 2 x CH_2N), 3.51-3.90 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.69 and 4.95 (2m, NCHCO of Pro), 6.57 (s, CH-1), 6.85 (s, CH-4); LRMS for $C_{38}H_{54}N_3O_4$ $[M + H]^+$: 616.4; HPLC purity: 87.1%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-(pyrazin-2-ylcarbonyl)pyrrolidin-2-yl]methanone (**45**). ^{1}H NMR ($CDCl_3$) δ: 0.89 and 0.90 (2s, CH_3-18), 1.25-2.40 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.82 (m, CH_2-6), 2.88-3.23 (m broad, 2 x CH_2N), 3.55-4.12 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 5.14 and 5.61 (2m, NCHCO of Pro), 6.59 and 6.60 (2s, CH-1), 6.83 and 6.87 (2s, CH-4), 8.42, 8.57 and 8.64 (3m, 2 x CH of pyrazine), 9.19 and 9.31 (2s, CH of pyrazine); LRMS for $C_{35}H_{44}N_5O_4$ $[M + H]^+$: 598.7; HPLC purity: 85.4%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}{(2S)-1-[4-(methylsulfanyl)benzoyl]pyrrolidin-2-yl}methanone (**46**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.40 (m, residual CH and CH_2), 2.50 (s, SCH_3), 2.60 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.20 (m broad, 2 x CH_2N), 3.55-3.98 (m, 3 x CH_2NCO), 3.84 (s, OCH_3), 4.58 and 5.14 (2m, NCHCO of Pro), 6.58 (s, CH-1), 6.86 (s, CH-4), 7.24 (d, J = 8.3 Hz, 2 x CH of Ar), 7.54 (d, J = 8.3 Hz, 2 x CH of Ar); LRMS for $C_{38}H_{48}N_3O_4S$ $[M + H]^+$: 642.8; HPLC purity: 81.8%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-(1H-indol-2-ylcarbonyl)pyrrolidin-2-yl]methanone (**47**). ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.60 and 2.61 (2s, C≡CH), 2.82 (m, CH_2-6), 2.95-3.25 (m broad, 2 x CH_2N), 3.60-4.20 (m, 3 x CH_2NCO), 3.85 (s, OCH_3), 4.92 and 5.19 (2m, NCHCO of Pro), 6.59 (s, CH-1), 6.87 (s, CH-4), 6.98 (s, CH=CNH), 7.14 (t, J = 7.4 Hz, CH of indole), 7.28 (d, J = 7.3 Hz, CH of indole), 7.40 (d, J = 8.2 Hz, CH of indole), 7.68 (d, J = 7.9 Hz, CH of indole), 9.29 (s, NH); LRMS for $C_{39}H_{47}N_4O_4$ $[M + H]^+$: 635.5; HPLC purity: 84.2%.

{4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}[(2S)-1-(quinoxalin-2-ylcarbonyl)pyrrolidin-2-yl]methanone (**48**). ^{1}H NMR ($CDCl_3$) δ: 0.89 and 0.91 (2s, CH_3-18), 1.25-2.50 (m, residual CH and CH_2), 2.61 and 2.63 (2s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.37 (m broad, 2 x CH_2N), 3.60-4.30 (m, 3 x CH_2NCO), 3.81 and 3.85 (2s, OCH_3), 5.20 and 5.74 (2m, NCHCO of Pro), 6.57, 6.59, 6.65 and 6.88 (4s, CH-1 and CH-4), 7.75-8.18 (m broad, 4 x CH of quinoxaline), 9.43 and 9.46 (2s, CH of quinoxaline); LRMS for $C_{39}H_{46}N_5O_4$ $[M + H]^+$: 648.4; HPLC purity: 80.1%.

1-[(2S)-2-({4-[(17α)-17-hydroxy-3-methoxy-19-norpregna-1,3,5(10)-trien-20-yn-2-yl]piperazin-1-yl}carbonyl)pyrrolidin-1-yl]-2-(pyridin-4-yl)ethanone (**49**). ^{1}H NMR ($CDCl_3$) δ: 0.88 (s, CH_3-18), 1.25-2.37 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.80 (m, CH_2-6), 2.95-3.15 (m broad, 2 x CH_2N), 3.52-3.90 (m, 3 x CH_2NCO), 3.83 (s, OCH_3), 4.60 and 4.96 (2m, NCHCO of Pro), 6.57 (s, CH-1), 6.84 (s, CH-4), 7.27 (m, 2 x CH of pyr), 8.55 (d, J = 6.1 Hz, 2 x CH of pyr); LRMS for $C_{37}H_{47}N_4O_4$ $[M + H]^+$: 611.4; HPLC purity: 80.7%.

2.1.3. Re-synthesis of mestranol derivative 23

*2.1.3.1. Synthesis of ester **50***

To a solution of 3,5-dimethylbenzoic acid (270 mg, 1.8 mmol) in anhydrous DMF (10 mL) was added HBTU (683 mg, 1.8 mmol) under an argon atmosphere. The solution was stirred for 10 min and L-proline-OtBu (250 mg, 1.5 mmol) was added before the addition of DIPEA (1 mL, 6 mmol). The resulting solution was stirred at rt under an argon atmosphere overnight. The reaction mixture was poured into water, extracted with EtOAc, and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes-EtOAc (9:1 to 5:5) as eluent to give the desired ester **50** (440 mg, 97%). IR (KBr) *v*: 1736 (C=O, ester), 1636 (C=O, amide); ^{1}H NMR ($CDCl_3$) δ: 1.35 and 1.49 (2s, $C(CH_3)_3$), 1.98 (m, $NCH_2C\underline{H}_2$), 1.67, 1.83, 2.18 and 2.30 (4m, $C\underline{H}_2CHCO$), 2.29 and 2.32 (2s, 2 x CH_3 of Ar), 3.48, 3.61 and 3.77 (3m, NCH_2), 4.22 and 4.53 (2m, NCHCO), 7.00, 7.03 and 7.15 (3s, 3 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 21.2 (2C), 22.5 and 25.3, 27.8 and 28.0 (3C), 29.4 and 31.5, 46.4, 49.9, 59.8 and 62.1, 81.2 and 81.6, 124.3 and 124.8 (2C), 131.2 and 131.5, 136.5 and 137.0, 137.7 and 137.9 (2C), 169.9 and 170.7, 171.5; LRMS for $C_{18}H_{26}NO_3$ $[M + H]^+$: 304.15.

*2.1.3.2. Synthesis of acid **51***

To a solution of ester **50** (430 mg, 1.4 mmol) was added a solution of DCM-TFA (95:5) (5 mL). The solution was stirred at rt for 3 h and was then evaporated under reduced pressure. The resulting oil was co-evaporated with a mixture of THF/H_2O five times, and triturated with diethyl ether to give the acid **51** (340 mg, 97%) as a white powder. IR (KBr) *v*:

2400-3500 (OH, acid), 1744 (C=O, acid); ^{1}H NMR ($CDCl_3$) δ: 1.90, 2.01, 2.19, 2.30 and 2.46 (5m, $NCH_2C\underline{H}_2C\underline{H}_2CH$), 2.34 (s, 2 x CH_3 of Ar), 3.57 (m, NCH_2), 4.76 (m, $NC\underline{H}CO$), 7.10 (s, CH of Ar), 7.14 (s, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 21.2 (2C), 25.1, 28.5, 50.4, 53.7, 124.7 (2C), 132.0, 135.4, 138.0 (2C), 171.1, 171.5; LRMS for $C_{14}H_{18}NO_3$ $[M + H]^+$: 248.1.

*2.1.3.3. Synthesis of mestranol derivative **23***

To a solution of acid **51** (114 mg, 0.46 mmol) in anhydrous DMF (10 mL) was added HBTU (174 mg, 0.46 mmol) at rt. The solution was stirred for 10 min and compound **1** (150 mg, 0.38 mmol) was added before the addition of DIPEA (0.4 mL, 6 mmol). The solution was stirred overnight at rt under an argon atmosphere. The reaction mixture was poured into water, extracted with EtOAc and the organic phase was washed with water, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude compound was purified twice by flash chromatography with DCM-MeOH (99:1 to 97:3) and then with acetone/hexanes (5:5) as eluent to give the desired compound **23** as a light-yellow amorphous solid (74 mg, 31%). IR (KBr) *v*: 3410 (OH, alcohol), 1636 (C=O, amides); ^{1}H NMR ($CDCl_3$) δ: see section 4.1.2.4; ^{13}C NMR ($CDCl_3$) δ: 12.7, 21.2 (2C), 22.8, 25.4, 26.5, 27.3, 29.4, 32.7, 38.9, 39.4 (2C), 42.5, 43.7, 46.1, 47.1, 49.4, 50.2, 51.0, 51.2, 55.4, 56.0, 59.4, 74.0, 79.9, 87.5, 111.7, 115.8, 124.9 (2C), 131.5 131.6, 132.2, 136.5, 137.8 (2C), 138.4, 150.2, 169.8, 170.4; HRMS for $C_{39}H_{50}N_3O_4$ $[M + H]^+$: calc 624.37958, found 624.37701; HPLC purity: 100.0%.

2.1.4. Re-synthesis of mestranol derivative 48

*2.1.4.1. Synthesis of ester **52***

To a solution of 2-quinoxalinecarboxylic acid (313 mg, 1.8 mmol) in anhydrous DMF (10 mL) was added HBTU (683 mg, 1.8 mmol) under an argon atmosphere. The solution was stirred for 10 min and L-proline-OtBu (250 mg, 1.5 mmol) was added before the addition of DIPEA (1 mL, 6 mmol). The resulting solution was stirred at rt under an argon atmosphere overnight. The reaction mixture was poured into water, extracted with EtOAc and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes-EtOAc (8:2 to 6:4) + 1% of triethylamine (TEA) as eluent to give the desired ester **52** (340 mg, 71%).

IR (KBr) *v*: 1736 (C=O, ester), 1628 (C=O, amide); ^{1}H NMR ($CDCl_3$) δ: 1.22 and 1.51 (2s, $C(CH_3)_3$), 1.99 (m, $NCH_2C\underline{H}_2$), 2.05-2.40 (m, $C\underline{H}_2CHCO$), 3.90 and 4.14 (2m, NCH_2), 4.65 and 5.30 (2dd, J_1 = 3.6 Hz and J_2 = 8.5 Hz, $NC\underline{H}CO$), 7.83 (m, 2 x CH of quinoxaline), 8.12 (m, 2 x CH of quinoxaline), 9.41 and 9.52 (2s, $NC\underline{H}C$ of quinoxaline); ^{13}C NMR ($CDCl_3$) δ: 22.1 and 25.4, 27.7 and 28.0 (2C), 28.9 and 32.1, 48.4 and 49.8, 61.0 and 62.3, 81.4 and 81.5, 129.3, 129.4, 129.6, 129.9, 130.4, 131.2, 131.3, 139.7, 140.2, 142.4, 142.5, 145.6, 145.9, 146.9, 147.5, 164.0, 164.3, 171.0, 171.8; LRMS for $C_{18}H_{22}N_3O_3$ $[M + H]^+$: 328.2.

*2.1.4.2. Synthesis of acid **53***

To a solution of ester **52** (330 mg, 1 mmol) was added a solution of DCM-TFA (95:5) (5 mL). The solution was stirred at rt for 3 h. The resulting solution was evaporated under reduced pressure. The resulting brown oil was co-evaporated with a mixture of THF/H_2O five times, triturated two times with diethyl ether and one time with EtOAc to give the acid **53** (296 mg, 76%) as a brown solid. IR (KBr) *v*: 2300-3400 (OH, acid), 1728 (C=O, acid), 1628 (C=O, amide); ^{1}H NMR ($CDCl_3$) δ: 2.10 (m, $NCH_2C\underline{H}_2$), 2.27, 2.37 and 2.50 (3m, $C\underline{H}_2CHCO$), 3.91, 4.02, 4.19 and 4.36 (4m, NCH_2), 4.86 and 5.14 (2dd, J_1 = 3.0 Hz and J_2 = 7.9 Hz, NCHCO), 7.38 (t, J = 7.1 Hz), 7.39 (t, J = 7.2 Hz), 7.70 (d, J = 8.3 Hz), 7.90 (m) and 8.17 (m) (4 x CH of of quinoxaline), 9.44 and 9.52 (2s, NCHC of quinoxaline); ^{13}C NMR ($CDCl_3$) δ: 22.1 and 25.5, 28.5 and 31.8, 48.5 and 50.1, 53.1, 128.8, 129.3, 129.4, 129.8, 130.2, 130.6, 131.2, 131.5, 139.4, 140.1, 142.0, 145.4, 145.7, 146.7, 147.2, 164.1 164.9; LRMS for $C_{14}H_{14}N_3O_3$ $[M + H]^+$: 272.1.

*2.1.4.3. Synthesis of mestranol derivative **48***

To a solution of acid **53** (177 mg, 0.46 mmol) in anhydrous DMF (10 mL) was added HBTU (174 mg, 0.46 mmol) at rt. The solution was stirred for 10 min and compound **1** (150 mg, 0.38 mmol) was added before the addition of DIPEA (0.4 mL, 6 mmol). The solution was stirred overnight at rt under an argon atmosphere. The reaction mixture was poured into water, extracted with EtOAc, and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude compound was purified twice by flash chromatography with DCM-MeOH (99:1 to 97:3) + 1% of TEA and then with acetone/hexanes (5:5) to give the desired compound **48** as a light-yellow amorphous solid (100 mg, 41%). IR (KBr) *v*: 3418 (OH, alcohol), 1643 (C=O, amides); ^{1}H NMR ($CDCl_3$) δ:

see section 4.1.2.4; ^{13}C NMR ($CDCl_3$) δ: 12.7, 22.3, 22.8, 25.4, 26.5, 26.7, 27.3, 28.8, 29.4, 31.8, 32.7, 38.9, 39.4, 41.7, 42.1, 42.5, 43.7, 45.3, 46.0, 46.6, 47.1, 48.5, 49.4, 49.9, 50.7, 50.8, 50.9, 51.2, 55.4, 55.5, 58.0, 59.5, 74.1, 79.8, 87.5, 111.6, 111.7, 115.5, 115.8, 129.2, 129.3, 129.6, 129.9, 130.4, 130.5, 130.9, 131.2, 131.7, 131.9, 132.2, 132.3, 138.0, 138.4, 139.5, 140.2, 142.3, 142.5, 145.7, 146.3, 147.5, 147.9, 150.1, 150.2, 164.1, 164.7, 169.6, 169.9; HRMS for $C_{39}H_{46}N_5O_4$ $[M + H]^+$: calc 648.35443, found 648.35145; HPLC purity: 94.9%.

2.2. Biological assays

2.2.1. Cell culture

The following cell lines were all maintained under a 5% CO_2 humidified atmosphere at 37 °C. The culture media were changed every two to three days, and the cells were split once a week to maintain cell propagation. Cancer cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and each culture medium was supplemented with L-glutamine (2 nM), penicillin (100 IU/mL) and streptomycin sulphate (50 µg/mL). OVCAR-3 (ovarian cancer) cells were maintained in RPMI-1640 supplemented with 20% fetal bovine serum (FBS), bovine insulin (50 ng/mL) and 17β-estradiol (E2) (1 nM). Caov-3 (ovarian cancer) and PANC-1 (pancreatic cancer) cells were maintained in Dulbecco's modified Eagle's medium (DMEM), containing a mixture of F-12 Ham nutriment (DMEM-F12) (Sigma, Saint Louis, MO, USA) supplemented with 10% FBS. Hs 766T (pancreas cancer) cells were cultured in DMEM supplemented with 10% FBS. SK-OV-3 (ovarian cancer) cells were maintained in McCoy's 5a medium modified and supplemented with 10% FBS. BxPC-3 (pancreatic cancer), PC-3 (prostate cancer) and LNCaP (prostate cancer) cells were maintained in RPMI-1640 supplemented with 10% FBS. T-47D (breast cancer) cells were cultured in RPMI-1640 supplemented with bovine insulin (50 ng/mL) and 10% FBS. MCF-7 (breast cancer) cells were maintained in DMEM-F12 supplemented with 5% FBS, bovine insulin (50 ng/mL) and E2 (1 nM). LAPC4 (prostate cancer) cells were cultured in Iscove's modified Dulbecco's medium supplemented with 10% FBS and bovine insulin (50 ng/mL).