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Synthesis and identification of novel oxa-steroids as progesterone receptor antagonists

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Abstract—A novel series of oxa-steroids **6** derived from (8*S*, 13*S*, 14*R*)-7-oxa-estra-4,9-diene-3,17-dione **1** have been synthesized and identified as potent and selective progesterone receptor antagonists. These novel oxa-steroids showed similar potency to mifepristone. Preliminary SAR study resulted in the most potent 17-phenylethynyl oxa-steroid **6i** wih an IC_{50} of 1.4 nM. In contrast to the equipotent mifepristone toward the progesterone receptor (PR) and glucocorticoid receptor (GR), compound **6i** had over 200-fold selectivity for PR over GR.

The progesterone receptor (PR) is a member of the steroid receptor sub-family of the nuclear hormone receptor super-family which is a group of ligand-dependent nuclear transcription factors.[1] Progesterone is the endogenous ligand for PR, which regulates ovulation and prepares uterus to support pregnancy. PR modulators exist clinically as both agonists and antagonists. PR agonists such as medroxy-progesterone acetate (MPA)[2] are mainly used in contraception and hormone therapy, typically co-administered with an estrogen. One of the main issues with PR agonists is that they often bind and modulate the function of other members of the nuclear hormone receptor super-family, for example, the androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. In principle, a PR antagonist may have potential utility as a contraceptive.[3] However, current PR antagonists, such as mifepristone (RU-486),[4] are compromised as clinically useful contraceptive agents due to overt glucocorticoid receptor antagonism.[5]

With few clinically successful selective PR antagonists being available, their therapeutic potential has not yet been fully realized. A selective PR antagonist may be used not only in female contraception, but also potentially for the treatment of various gynecological and obstetric diseases including hormone-dependent cancers and non-malignant chronic conditions, such as fibroids[6] and endometriosis.[7] A variety of steroidal[8] and non-steroidal[9] PR modulators have been reported in recent years. As part of our interest in developing novel PR modulators,[10] herein we report the synthesis and

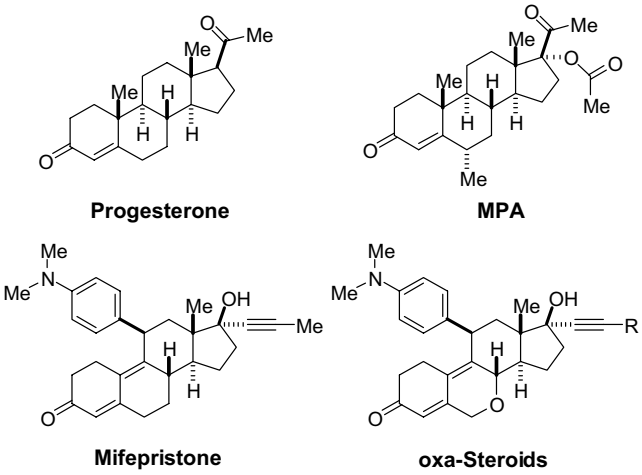

Figure 1. Structures of natural and unnatural steroids.

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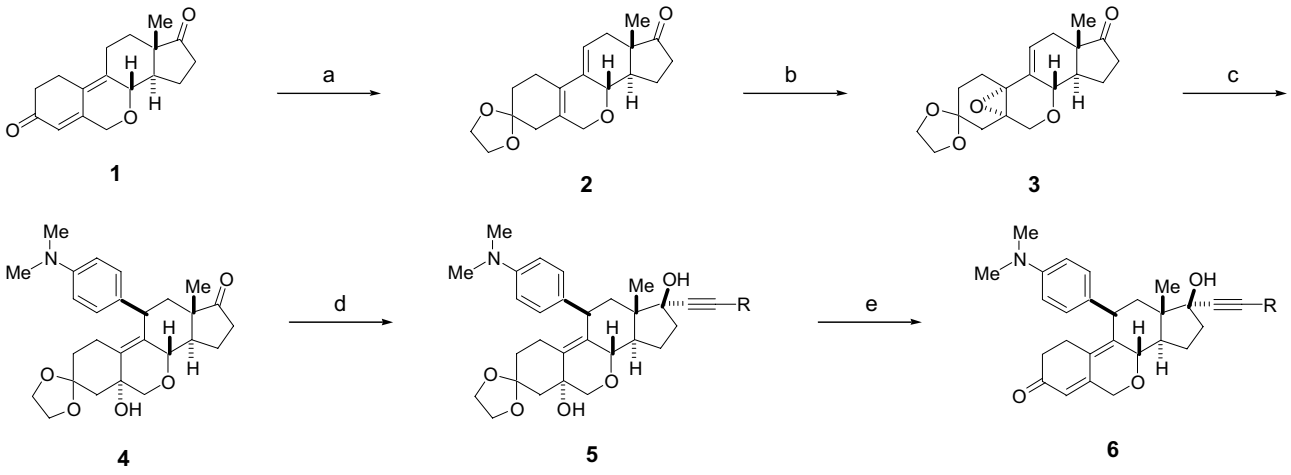
identification of a novel series of oxa-steroids (Fig. 1) as potent and selective PR antagonists.

Recently, we have achieved the first enantioselective synthesis of (8*S*, 13*S*, 14*R*)-7-oxa-estra-4,9-diene-3,17-dione **1** with the unambiguous *trans*-C/D ring junction[11] (Scheme 1). We were intrigued in applying this unnatural oxa-steroidal template to the discovery of novel PR modulators. Selective mono-ketallation of the 3-carbonyl group of compound **1** was achieved in the presence of pyridinium hydrochloride with azeotropic removal of water in refluxing benzene. Simultaneously, under the thermal and acidic reaction condition, the [5,6]-spiro-ring system induced the 4,9-diene to isomerize to the thermodynamically more stable 5,11-diene affording compound **2**. A highly regio-/stereo-selective epoxidation with *m*-CPBA at −30 °C generated predominantly α-epoxide **3** (α:β = 8:1). It is interesting to note that epoxidation of the oxa-steroid tended to result in better stereoselectivity than that of the natural steroid.[12] *N*,*N*-Dimethylaniline magnesium bromide was treated with the homogeneous CuCN–2LiCl complex[13] in THF at 0 °C to form the organocuprate which was immediately added to α-epoxide **3** at 0 °C, stereoselectively producing the 11-β-substituted oxa-steroid **4**. Stereoselective addition of 1-propynyl magnesium bromide to the carbonyl group of compound **4** at ambient temperature delivered compound **5a** (R = Me). Finally, deprotection and dehydration under the acidic condition furnished the oxa-steroid **6a** (R = Me). Thus, an efficient and stereoselective synthesis of novel oxa-steroids **6** was achieved in five steps from compound **1**.

Compound **6a** was evaluated for PR antagonist activity based on its ability to block progesterone induction of alkaline phosphatase activity in the human breast cancer cell line T47D.[14] It was also tested for GR antagonist activity based on its ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549.[15] The IC_{50} values of the compounds from the T47D and A549 assays are listed in Table 1. The ratio of their IC_{50} values was calculated as a measure of the separation of PR and GR antagonist activities. Mifepristone (RU-486) was tested as a control. It was determined that compound **6a** was a potent PR antagonist with an IC_{50} of 7.5 nM and it was about 10-fold more selective to PR over GR, which exhibited a slightly better selective profile to that of mifepristone.

Although compound **6a** was somewhat less potent than mifepristone, their similar PR activity was suggested by our computational study. Shown in Figure 2 is the comparison of the possible modes of mifepristone and compound **6a** bound in the ligand-binding domain of progesterone receptor. The model was built based on the X-ray crystal structures of hPR-norenthindrone[16] and hGR-mifepristone[17] complexes. In the construction of these models, helix 12 of the hPR-norenthindrone crystal structure was removed in order to open the ligand-binding site. Mifepristone and compound **6a** were then docked into the ligand-binding site using program Glide,[18] followed by re-packing of helix 12 back to the antagonism position in reference to the hGR-mifepristone crystal structure. The loop between helices 12 and 11 was re-built using program Prime.[18] The final complex structures were optimized by energy minimization using program MacroModel[18]. In these models, mifepristone and compound **6a** were predicted to bind in a similar way to PR, with a water molecule bridging the D-ring hydroxyl group and ASN719 through hydrogen bond. At the other end, another hydrogen bond may also exist between the A-ring carbonyl group and GLN725. Compared to the 7-methylene group in mifepristone, the polar and eletronegative 7-oxygen atom in oxa-steroid **6a** may play a slightly different role in the ligand–protein interactions with the nearby MET756 (not shown).

As a preliminary SAR study, a number of substituted alkyns were added to the carbonyl group of compound **4**, which led to compounds **6b–i** (Table 1). Replacement of the methyl group in compound **6a** with the smaller hydrogen atom in compound **6b** lowered the PR activity.



Scheme 1. Synthesis of oxa-steroids. Reagents and conditions: (a) $(CH_2OH)_2$, pyridine-HCl, benzene, refluxing, 82%; (b) *m*-CPBA, $NaHCO_3$, DCM, −30 °C, 70%; (c) $Me_2NC_6H_4MgBr$, CuCN–2LiCl, THF, 0 °C, 88%; (d) MeCCMgBr (**6a**, R = Me), THF, rt, 92%; (e) 3 N HCl aq, acetone, 95%.

Table 1. SAR study at the 17-substituted-ethynyl groups of compounds **6**

Compound	R	T47D (PR), IC_{50} (nM)	A549 (GR), IC_{50} (nM)	Ratio (GR/PR)
Mifepristone	—	1.4	1.6	1
6a	Me	7.5	86.5	12
6b	H	19.0	204.3	11
6c	Cyclopropyl	9.6	33.4	4
6d	CF_3	9.3	55.5	6
6e	CMe_3	185.0	166.9	1
6f	$CMe_2(OH)$	>1000	558.0	—
6g	CH_2OMe	600	315	0.5
6h	CH_2NMe_2	>1000	>3000	—
6i	Ph	1.4	304.0	217

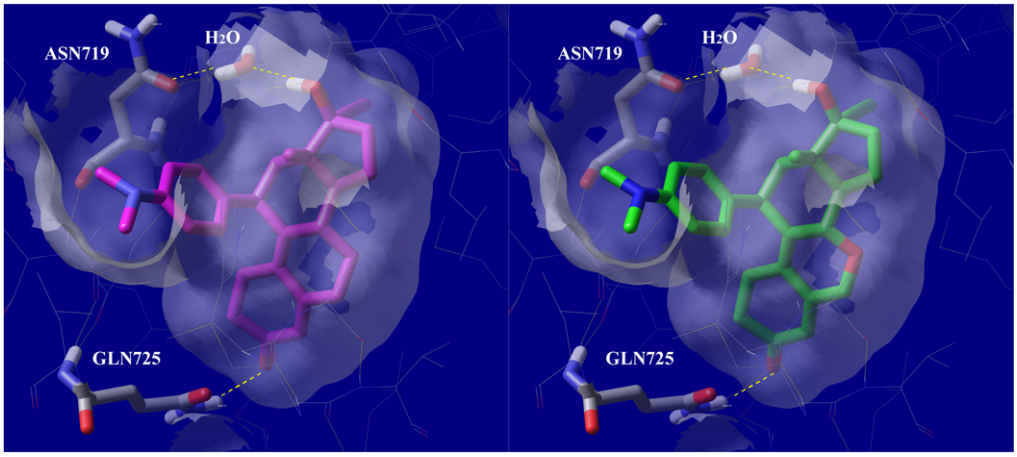


Figure 2.

The cyclopropyl group in compound **6c** and trifluoromethyl group in compound **6d** retained the PR activity. The bulky *tert*-butyl group in compound **6e** significantly decreased the PR activity. The polar functional groups such as 2-hydroxy-2-propyl group in compound **6f**, methoxymethyl group in compound **6g**, and *N*,*N*-dimethylaminomethyl group in compound **6h** resulted in much less active or literally inactive compounds. Surprisingly, it was found that change of the methyl group in compound **6a** to the phenyl group led to the most potent and selective PR antagonist, compound **6i**, which had an IC_{50} of 1.4 nM and was over 200-fold more selective for PR over GR.

It should be pointed out that oxa-steroids related to compound **1** were reported before in the literature,[19] but the structure and stereochemistry were unfortunately not clearly characterized and established in the report. In addition, the biological activities of those oxa-steroids were also evaluated and they were found to be inactive toward PR. Therefore, it was concluded that 'the insertion of an oxygen atom into the steroid backbone in place of the 7-methylene group has practically abolished the biological properties of this type of compounds.'

With our first enantioselective synthesis of compound **1** with the unambiguous stereochemistry of the *trans*-C/D ring junction, we have discovered for the first time that the oxa-steroids with an oxygen atom in place of the 7-methylene group have retained the biological properties of this type of compounds, as it has been demonstrated by the biological activities of oxa-steroids **6** toward PR and GR. More significantly, some of the oxa-steroids, such as compound **6i**, have shown outstanding better selectivity toward PR over GR than that of the natural steroids such as mifepristone.

In conclusion, a novel series of oxa-steroids **6** derived from (8*S*, 13*S*, 14*R*)-7-oxa-estra-4,9-diene-3,17-dione **1** have been synthesized. For the first time, the 7-oxa-steroids have been identified as potent and selective progesterone receptor antagonists. These novel oxa-steroids showed similar or comparable potency to that of mifepristone. Preliminary SAR study resulted in the discovery of the most potent 17-phenylethynyl oxa-steroid **6i** as a new potent selective PR antagonist. In contrast to non-selective mifepristone, compound **6i** had over 200-fold selectivity for PR over GR. More SAR study and their in vivo activity will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.062.

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13. The homogeneous CuCN–2LiCl complex in THF was found to be superior to CuCl[12] to ensure a reproducible and scalable reaction.
14. *T47D alkaline phosphatase assay*. T47D human breast cancer cells were plated in 96-well tissue culture plates at 10,000 cells per well in assay medium [RPMI medium without phenol red (Invitrogen) containing 5% (v/v) charcoal-treated FBS (Hyclone) and 1% (v/v) penicillin–streptomycin (Invitrogen)]. Two days later, the medium was decanted and test compound at various concentrations (0.01–1000 nM), 0.1 nM progesterone, or vehicle control (dimethyl sulfoxide) at a final concentration of 0.1% (v/v), was added in fresh assay medium. Twenty-four hours later, an alkaline phosphatase assay was performed using a SEAP kit (BD Biosciences Clontech, Palo Alto, CA). Briefly, the medium was decanted and the cells were fixed for 30 min at room temperature with 5% (v/v) formalin (Sigma). The cells were washed once at room temperature with Hanks' buffered saline solution (Invitrogen). Equal volumes (0.05 mL) of 1× dilution buffer, assay buffer, and 1:20 substrate/enhancer mixture were then added. After a 1-h incubation at room temperature in the dark, the lysate was transferred to a white 96-well plate (Dynex) and luminescence was read using a LuminoSkan Ascent (Thermo Electron, Woburn, MA).
15. *A549 reporter assay*. A549 human lung carcinoma cells were washed with OPTI-MEM I (Gibco). The medium was removed and lipid–DNA complex solution (1.5 μg/mL of GRE-luciferase reporter DNA in 8.5 mL OPTI-MEM I plus 6 μL/mL DMRIE-C reagent in 8.5 mL OPTI-MEM I, combined and mixed, then incubated at room temperature for 40 min) was overlayed onto the cells in a T160 flask. The cells were incubated for 16 h at 37 °C in a CO_2 incubator. The DNA-containing medium was removed and 30 mL of growth medium containing 5% (v/v) charcoal-treated fetal bovine serum was added. After 5–6 h, the cells were seeded in 96-well plates and incubated overnight at 37 °C. To each well were then added test compounds followed by dexamethasone as a corticoid challenge. The cells were incubated for 24 h. Luciferase assay buffer (Promega) was added to each well and the cell were incubated for 30 min at room temperature. Luciferase activity was measured in a DYNEX Microlite plate on a TopCount (Packard).
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