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Parallel synthesis and SAR study of novel oxa-steroids as potent and selective progesterone receptor antagonists

Fu-An Kang,* Jihua Guan, Nareshkumar Jain, George Allan, Olivia Linton, Pamela Tannenbaum, Xin Chen, Jun Xu, Peifang Zhu, Joseph Gunnet, Keith Demarest, Scott Lundeen and Zhihua Sui

Johnson and Johnson Pharmaceutical Research and Development, LLC, 665 Stockton Drive, Exton, PA 19341, USA

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Abstract—Efficient parallel synthesis of novel 7-oxa-steroids **4** has been achieved from the key intermediate **3** via a one-pot four-step sequence. oxa-Steroids **4** with various *ortho*-, *meta*-, and *para*-monosubstituents on the phenyl ring, as well as disubstituted phenyl and heterocycles, were evaluated for progesterone receptor (PR) and glucocorticoid receptor (GR) antagonist activities. SAR study demonstrated that the *para*-fluorinated substituents on the phenyl ring not only increased the potency for PR in a T47D cell functional assay, but also improved the selectivity over GR in an A549 cell functional assay. The *para*-fluorophenyl oxa-steroid **4l** and the *para*-trifluoromethylphenyl oxa-steroid **4p** were found to be remarkably more potent and more selective PR antagonists than mifepristone, with subnanomolar potency and about 140-fold selectivity over GR. Molecular modeling of the oxa-steroid bound to PR provided meaningful insight for the SAR study. oxa-Steroids **4a** and **4b** were found to be more efficacious than mifepristone in vivo in a rat uterine complement C3 assay via the oral route, although they were less than or equally potent to mifepristone in the T47D assay.

The progesterone receptor (PR), like other steroid receptors, plays a unique and crucial role in mammalian development and homeostasis. Progesterone is known to be required for mammary gland development, ovulation, and the maintenance of pregnancy. It has less clearly defined functions in bone, the cardiovascular system, and the central nervous system. In terms of opportunities for pharmacological intervention, it has arguably the greatest unexploited potential of the steroids. Currently, steroidal progestin agonists and antagonists are clinically approved for contraception, hormone replacement therapy, and therapeutic abortion. There is strong preclinical and clinical evidence for the value of progestin antagonists for treating endometriosis, uterine fibroids, dysfunctional uterine bleeding, and breast cancer.[1]

The discovery of the first PR antagonist, mifepristone (RU-486),[2] has stimulated an intensive search for more potent and selective antiprogestins. This has led to the identification of a variety of steroidal[3] and nonsteroidal[4] PR modulators in the past years. However, current PR antagonists, such as mifepristone, are compromised as clinically useful agents due to overt glucocorticoid receptor (GR) antagonism.[5] Therefore, new compounds with antiprogestational activity devoid of antiglucocorticoid activity are highly desirable for both clinical applications and basic endocrine research.[6] The recent discovery that treatment of mifepristone can potentially prevent *BRCA1*-mediated breast cancer[7] makes it more urgent to discover novel potent and selective PR antagonists to address unmet medical needs.

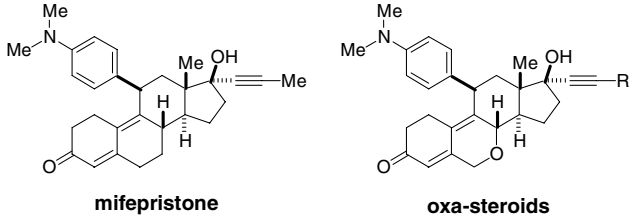

Figure 1. Structures of mifepristone and oxa-steroids.

Keywords: Progesterone; Receptor; Antagonist; oxa-Steroid; Glucocorticoid; Parallel synthesis; SAR study; In vitro; In vivo.
* Corresponding author. Tel.: +1 610 458 4147; e-mail: fkang@prdus.jnj.com

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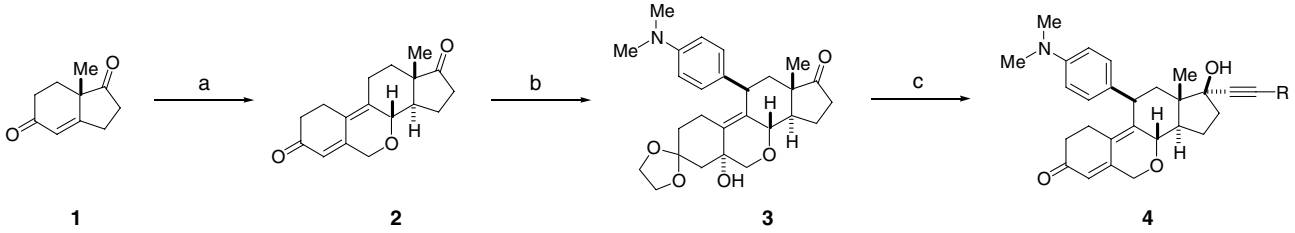
We have recently achieved the first synthesis and identification of novel 7-oxa-steroids as promising potent and selective PR antagonists (Fig. 1).[8] Herein we report the parallel synthesis and SAR study of a series of new oxa-steroids along this line for the development of potent and selective PR antagonists.

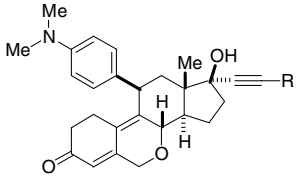
The oxa-steroids **4** were synthesized from (8*S*,13*S*,14*R*)-7-oxa-estra-4,9-diene-3,17-dione **2** (Scheme 1),[8] which was prepared from the Hajos–Parrish ketone **1**.[9] It is interesting to note that the synthesis of oxa-steroids **4** with five chiral centers in the congested heteropolycyclic structure was efficiently achieved in a highly stereoselective fashion, with the stereochemistry of the four new chiral centers being excellently controlled by the single chirality of the starting material, the Hajos–Parrish ketone **1**.

The mifepristone-like oxa-steroid **4a** has an IC_{50} of 7.5 nM for PR and over 10-fold selectivity over GR. Although it is less potent than mifepristone, it is slightly more selective than the equipotent mifepristone (Table 1). A preliminary SAR study at the C17-ethynyl position with various substituents has led to the discovery of the remarkably more potent and more selective PR antagonist **4b** with the phenyl group at the C17-ethynyl position. Compound **4b** has an IC_{50} of 1.4 nM for PR and over 200-fold selectivity over GR.[8] Apparently,



Scheme 1. (a) Ref. 9; (b) Ref. 8; (c) parallel synthesis of oxa-steroids **4** via the one-pot four-step sequence. Reagents and conditions: (1) RCCH, LiHMDS, THF, rt, 15 min; (2) compound **3**, THF, rt, 4 h; (3) 3 N HCl aq, acetone, rt, 4 h, 60–80%.

Table 1. SAR study of oxa-steroids **4**



Compound	R	T47D (PR), IC_{50} (nM)	A549 (GR), IC_{50} (nM)	Selectivity (GR/PR)
Mifepristone	—	1.4	1.6	1
4a	Methyl	7.5	86.5	12
4b	Phenyl	1.4	304.0	217
4c	2-F–phenyl	1.4	65.1	47
4d	2-Cl–phenyl	1.1	64.8	59
4e	2-Br–phenyl	8.6	62.7	7
4f	2-Me–phenyl	5.8	113.2	20
4g	2-F_3C–phenyl	3.2	51.0	16
4h	3-F–phenyl	1.0	56.6	57
4i	3-Cl–phenyl	12.5	48.9	4
4j	3-Me–phenyl	2.9	36.3	13
4k	3-F_3C–phenyl	2.7	45.4	17
4l	4-F–phenyl	0.27	37.6	139
4m	4-Cl–phenyl	0.61	43.5	71
4n	4-Br–phenyl	0.94	31.3	33
4o	4-Me–phenyl	1.6	22.1	14
4p	4-F_3C–phenyl	0.75	111.0	148
4q	4-MeO–phenyl	3.2	80.3	25
4r	4-NC–phenyl	3.7	41.3	11
4s	4-Me_3C–phenyl	19.5	65.8	3
4t	3,5-di-F–phenyl	0.91	34.4	38
4u	2-Pyridyl	34.0	175.3	5
4v	3-Pyridyl	21.0	341.6	16
4w	4-Pyridyl	39.0	246.5	6
4x	3-Thienyl	1.6	56.0	35

compound **4b** is a new PR antagonist that is as potent as, but much more selective than, mifepristone. Therefore, it became the new lead for further SAR study. For a focused SAR study, we were interested in fine-tuning the substitution pattern at the C17-ethynyl position of these oxa-steroids by installing various substituted phenyl groups and heterocycles, while keeping the rest of the molecule the same as that of mifepristone.

The oxa-steroids **4** were originally synthesized in two steps from compound **3**, including stereoselective Grignard addition and subsequent deprotection and dehydration.[8] For a more efficient parallel synthesis of oxa-steroids **4**, we developed a one-pot four-step sequence from compound **3**, which includes in situ generation of arylethynyl lithium, stereoselective addition to the C17-carbonyl group, deprotection of the C3-ketal group, and dehydration of the C5-hydroxy group. Thus, new oxa-steroids **4c**–**4x** were efficiently synthesized through this one-pot reaction in 60–80% yields after preparative TLC purification.[10]

Compounds **4c**–**4x** were evaluated for PR antagonist activity based on their ability to block progesterone induction of alkaline phosphatase activity in the human breast cancer cell line T47D. They were also tested for GR antagonist activity based on their ability to inhibit corticoid-induced transcription from a glucocorticoid response element (GRE)-linked luciferase reporter gene in the human lung carcinoma cell line A549. 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 was tested as a control.

The PR and GR activities of oxa-steroids **4c**–**4g** with *ortho*-substituted phenyl groups at the C17-ethynyl position are listed in Table 1. It seems that PR activity tolerates the *ortho*-substitution with F, Cl, Br, Me, and CF_3 well, which results in potent PR antagonists with IC_{50}s ranging from 1 to 10 nM. However, the *ortho*-substitution with these substituents also gives rise to relatively potent GR antagonists, with only a small separation between PR and GR. Compared to the phenyl group of compound **4b**, the small substituents on the phenyl group such as F and Cl retain PR potency (1–2 nM) and moderate selectivity over GR (about 50-fold selectivity).

The PR and GR activities of oxa-steroids **4h**–**4k** with *meta*-substituted phenyl groups at the C17-ethynyl position are listed in Table 1. Similar to the *ortho*-substitution, the *meta*-substitution with F, Cl, Me, and CF_3 leads to potent but less selective PR antagonists. Interestingly, the F substituent makes the oxa-steroid slightly more potent and selective than the Cl substituent, with an IC_{50} of 1.0 nM for PR and over 50-fold selectivity over GR.

The PR and GR activities of oxa-steroids **4l**–**4s** with *para*-substituted phenyl groups at the C17-ethynyl position are listed in Table 1. One half of these *para*-substituted phenyl groups make the oxa-steroids even more potent than mifepristone and compound **4b**. The F, Cl, Br, and CF_3 substituents result in PR antagonists with subnanomolar potency (0.27–0.94 nM). The F and CF_3 substituents also lead to about 140-fold selectivity for PR over GR. Therefore, compounds **4l** and **4p** are promising potent and selective PR antagonists identified in the oxa-steroid series after compound **4b**. In addition, the bulky *tert*-butyl group causes more than 10-fold reduction in PR potency suggesting a size limitation at this position.

The PR and GR activities of oxa-steroids **4t**–**4x** with disubstituted phenyl and heterocycles at the C17-ethynyl position are listed in Table 1. It appears that 2-, 3-, and 4-pyridyl groups result in up to 20-fold loss of PR potency, while 3,5-difluorophenyl and 3-thienyl groups exhibit similar potency but less selectivity for PR versus GR.

On the basis of the SAR of the minor modifications at the C17-ethynyl position, the trend is that small substituents such as F, Cl, Me, and CF_3 at the *ortho*-, *meta*-, and *para*-positions of the phenyl group retain or increase PR potency, compared to the unsubstituted phenyl group in compound **4b**. Interestingly, the small and electron-withdrawing substituents, such as F and CF_3, on the *para*-substituted phenyl groups not only enhance PR potency, but retain good selectivity over GR.

The possible binding modes of compound **4a** in the ligand-binding domain of PR suggested by molecular modeling are shown in Figure 2. The model was built based on the X-ray crystal structures of hPR-norenthindrone and hGR-mifepristone complexes.[8] The molecular modeling suggests that there is an open space available around the C17-ethynylmethyl group, which is consistent with our SAR study. Therefore, bigger groups, such as phenyls and heterocycles in compounds **4b**–**4x**, can be accommodated at this binding site. Compared to the methyl groups at the C17-ethynyl position of mifepristone and compound **4a** with up to about 10-fold selectivity toward

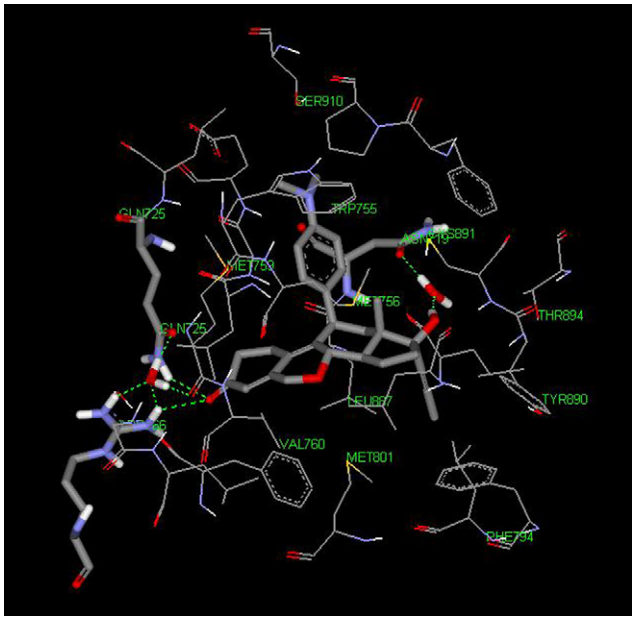


Figure 2. Molecular modeling of compound **4a** bound to PR.

PR over GR, the phenyl groups in compounds **4b**, **4l**, and **4p** with up to about 200-fold selectivity for PR over GR should play an important role in increasing PR potency and simultaneously decreasing GR potency. In contrast to the equipotent mifepristone, this potency and selectivity profile might result from the unique combination of ligand–protein interactions between the O7-oxygen atom and the nearby MET756, VAL760, LEU887, and MET801, as well as those between the C17-ethynylphenyl group and the phenyl groups of the nearby TYR890 and PHE794.

To investigate the in vivo profile of this novel series of oxa-steroids, compounds **4a** and **4b** were tested orally in ovariectomized Sprague–Dawley rats in a rat uterine complement C3 assay.[11] In this assay, ethynyl estradiol (EE) is used to stimulate C3 expression. Progestins inhibit EE-induced expression. In turn, antiprogestins counteract progestin-dependent inhibition. When compounds **4a** and **4b** were administered via the oral route along with EE and progesterone, they were found to be more efficacious than mifepristone, although they were less than or equally potent to mifepristone in vitro. This outcome demonstrated that these oxa-steroids, particularly the more potent ones with the relatively large phenyl groups at the C17-ethynylmethyl position, still have good oral exposure from the pharmacokinetic perspective.

In summary, efficient parallel synthesis via a one-pot four-step sequence led to a series of novel oxa-steroids **4**. SAR study on the C17-ethynyl position with various phenyl groups and heterocycles resulted in new PR antagonists **4l** and **4p** that are more potent and more selective than mifepristone. Molecular modeling of the oxa-steroid in PR provided meaningful insight for the SAR study. oxa-Steroids **4a** and **4b** were found to be more efficacious than mifepristone in vivo in a rat uterine complement C3 assay via the oral route, although they were less than or equally potent to mifepristone in vitro. Our study of 7-oxa-steroids as potent and selective PR antagonists demonstrated that the unnatural 7-oxa-steroids not only excellently mimic the natural steroids in terms of shape and activity, but also exhibit significant differentiation from similar steroid receptors and excellent in vivo efficacy. Further profiling of selected compounds in this series will be reported in due course.

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10. General procedure for the one-pot parallel synthesis of oxa-steroids **4**: 1 M LiHMDS in THF (48.6 μL, 0.048 mmol) was added to the stirred solution of the aryl alkyns (0.06 mmol) in THF (0.5 mL) at room temperature. After 15 min, compound **3** (5.5 mg, 0.012 mmol) in THF (0.5 mL) was added to the reaction mixture. After 4 h, acetone (1.0 mL) and 3 M HCl (0.15 mL) were added to the reaction mixture. After 4 h, sat. $NaHCO_3$ aq solution (5 mL) and EtOAc (5 mL) were added to the reaction mixture. The aqueous layer was extracted with EtOAc (2× 5 mL) and the combined organic layer was dried with brine and $MgSO_4$. Purification by preparative TLC eluted with 50–70% EtOAc in hexane gave compounds **4** in 60–80% yields.
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