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Synthesis and SAR study of novel pseudo-steroids as potent and selective progesterone receptor antagonists

Nareshkumar Jain *, George Allan, Olivia Linton, Pamela Tannenbaum, Xin Chen, Jun Xu, Peifang Zhu, Joseph Gunnet, Keith Demarest, Scott Lundeen, William Murray, Zhihua Sui

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

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ABSTRACT

Synthesis of novel 7-pseudo-steroids **1c** has been achieved from trenbolone **3** via an efficient 14 step sequence with overall yields of 10–15%. Various substitutions were incorporated at both the aromatic side chain as well as the D ring. The orientation of aromatic side chain at C10 plays a crucial role for progesterone receptor (PR) activity. Compound **2a** (T47D = 1 nM) with $-NMe_2$ para to the aromatic group along with spirofurane groups in the D ring was the optimal substitution. All compounds were also evaluated for glucocorticoid receptor (GR) antagonist activities in vivo in a rat and found efficacious in uterine complement C3 assay via the oral route of administrations.

There exist numerous proven and potential applications for progesterone antagonists (PA) in women's health care.

Mifepristone (RU-486),[1,2] one of the most widely prescribed PAs, is of limited clinical use due lack of selectivity[5,6] for the progesterone (PR) over the glucocorticoid (GR) receptor. The resulting undesirable side effect profile has provided the stimulus to search for more selective PAs. These efforts have led to the discovery of a variety of both steroidal[3] and nonsteroidal[4] entities in recent years.[7,8] Herein we report the synthesis and SAR of a novel series of pseudo-steroids with an improved selectivity of PR over GR (Fig. 1).

The pseudo-steroids **1c** were synthesized in 14 steps starting from commercially available trenbolone **3**. Several possible approaches were evaluated starting from **3** where the strategy was to oxidize C-ring double bond to yield the dialdehyde or equivalent functional group. Scheme 1 represents our optimal medicinal chemistry route which led to the common intermediate **10**. The synthesis began with Swern oxidation of the C17 hydroxyl group of **3** to yield diketone **4** in nearly quantitative yield. The crude product obtained from oxidation was converted selectively to ketal **5** by reacting with excess ethylene glycol in the presence of ethylformate and a catalytic amount of TsOH. At lower temperature, that is, 0–5 °C, almost no diketalization was observed even when an excess amount of ethylene glycol was used. The next step involved the dihydroxylation of **5**. Several catalytic dihydroxylation

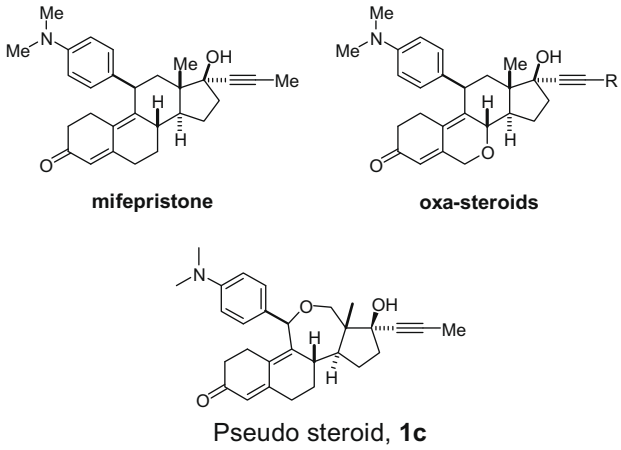

Figure 1. Structures of PRMs.

processes were evaluated, including number of asymmetric Sharpless oxidations. The dihydroxylation using catalytic amount of OsO_4 along with NMO as co-oxidant and a mixed solvent system (*t*-BuOH, THF, H_2O) was most efficient to yield compound **6** selectively in more than 55% yield. In this reaction 44% of starting material was recovered. The cleavage of the diol **6** to the dialdehyde **7** was efficiently carried out with lead tetracetate followed by reduction of dialdehyde using $NaBH_4$ to triol **8** in >95% yield for two steps. The most crucial step for the synthesis was achieving a

* Corresponding author. Tel.: +1 908 268 2336.
E-mail address: drnfjain@gmail.com (N. Jain).

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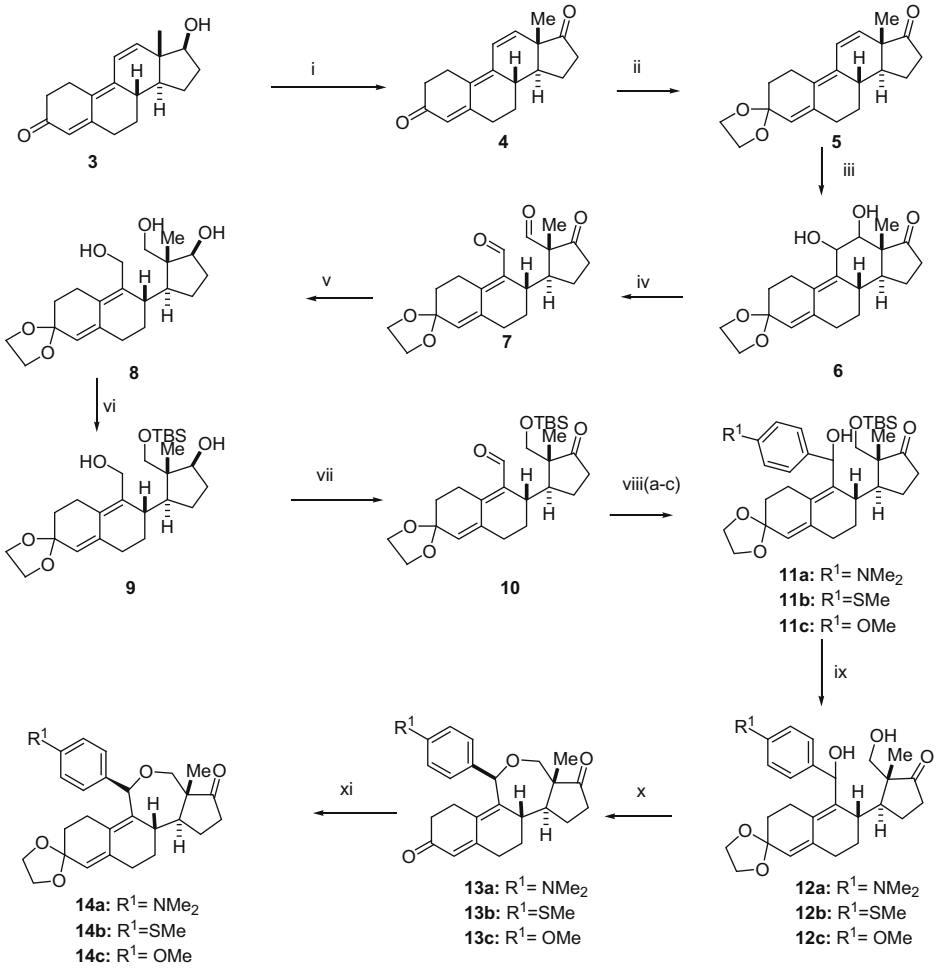


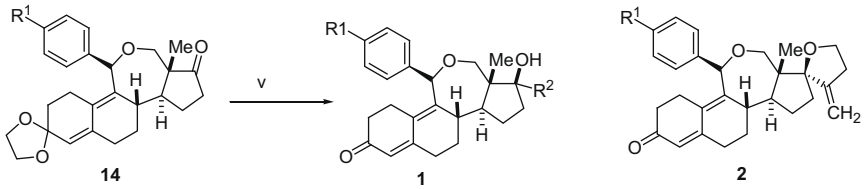
Scheme 1. Reagents and conditions: (i) DMSO, $(COCl)_2$ Et_3N; (ii) ethylorthoformate, $(CH_2OH)_2$, TsOH; (iii) OsO_4, NMO, *t*-BuOH:THF:H_2O (1:1:1); (iv) lead acetate, THF, 0 °C; (v) $NaBH_4$, THF:H_2O (10:1); (vi) TBSOTf, 2.6 lutidine, CH_2Cl_2, −78 °C, 44%; (vii) DMSO, $(COCl)_2$ Et_3N, CH_2Cl_2, −78 °C to rt, 94%; (viiia) *p*-$Me_2NPhMgBr$, −78 °C, 10 h, 87% (ds 3:1); (viiib) *p*-MeSPhMgBr, −78 °C, 10 h, 71% (ds 2:1); (viiic) *p*-MeOPhMgBr, −78 °C, 10 h, 91% (ds 3:1); (ix) TBAF, THF, −0 °C; (x) TsOH (2 mol %), CH_2Cl_2, 0 °C to rt, 36 h, 90–95% (ds >30:1); (xi) ethylorthoformate, $(CH_2OH)_2$, TsOH, 0 °C, 80–88%; (xii) MeCCMgBr, THF, 0 °C to rt, 12 h, 70–87%; (xiii) TsOH, acetone:H_2O (1:1), rt, 12 h.

selective protection of primary alcohol **8**. Among several reaction conditions surveyed, the 1.5 equiv of TBSOTf and 1.4 equiv of 2,6-lutidine in CH_2Cl_2 yielded desired mono TBS protected compounds **9** along with other mono or bis TBS protected alcohols (structure not shown). The mixture of these side products were recycled by converting back to triol **8** using *n*-Bu_4NF in THF. The TBS-diol **9** was converted to ketoaldehyde **10** efficiently using Swern oxidation conditions. The aromatic side chain was introduced during this step using the appropriate Grignard reagent. An excess freshly prepared Grignard reagent was added to aldehyde at −20 °C to yield addition product in 3–4:1diastereomeric ratios. The yield of these Grignard additions ranged from 80% to 97% depending on quality and type of reagent prepared. Using Grignards reagents, compound **11a** (R^1 = NMe_2), **11b** (R^1 = OMe) and **11c** (R^1 = SMe) were prepared. The deprotection of the diol **11a** yielded diol **12a** in >90% yield. At this point, the diastereoisomers were not separated. When the diol was treated with TsOH in acetone:water (4:1), it gave almost exclusively ketone **13a**. The stereochemistry of **13a** was determined using multiples 1D and 2D NOE experiments. The A ring of diketone **13a** was reprotected using ethylene glycol and methyl orthoformate in the presence of catalytic amount of TsOH to yield **14a**. Similarly compound **14b** (R^1 = OMe) and **14c** (R^1 = SMe) were also prepared starting from **11b** and **11c**, respectively, using similar sequential reactions. The intermediates **14**(**a–c**) were converted to compounds **1**(**b–k**) in two steps which included the addition of corresponding Grignard reagent followed by hydrolysis of ketal to the yield final ketone. Compound **1a** was prepared using LAH reduction of ketone **14a** followed by hydrolysis of the ketal using 1 N HCl. Details of these steps are in Table 1.

The spirofuranes **2a** and **2b** were prepared in three steps from **14a** and **14b** as shown in Scheme 2. The iodide **16** (*t*-butyl-(3-iodo-but-3-enyloxy)-dimethyl-silane was prepared according to the procedure described by Piers and Karunaratne.[9] The halogen metal exchange was carried out at −100 °C in THF using 1.1 equiv of *n*-BuLi. The ketones **14a** or **14b** were added to this reaction mixture to yield addition products **17a** (77%) and **17b** (80%), respectively. The TBS group was exchanged in situ first by treating compound **17a** or **17b** with TBAF (1 M, in THF) followed by reaction with MsCl and pyridine to yield the spirofuranes **18a** and **18b**. Compound **18a** and **18b** were converted to corresponding ketones by hydrolysis using catalytic amount of TsOH in acetone/water to yield spirofuranes **2a** and **2b**, respectively.

Compounds **1**(**a–k**) and **2**(**a–b**) 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

Table 1
SAR study of Pseudo-steroids



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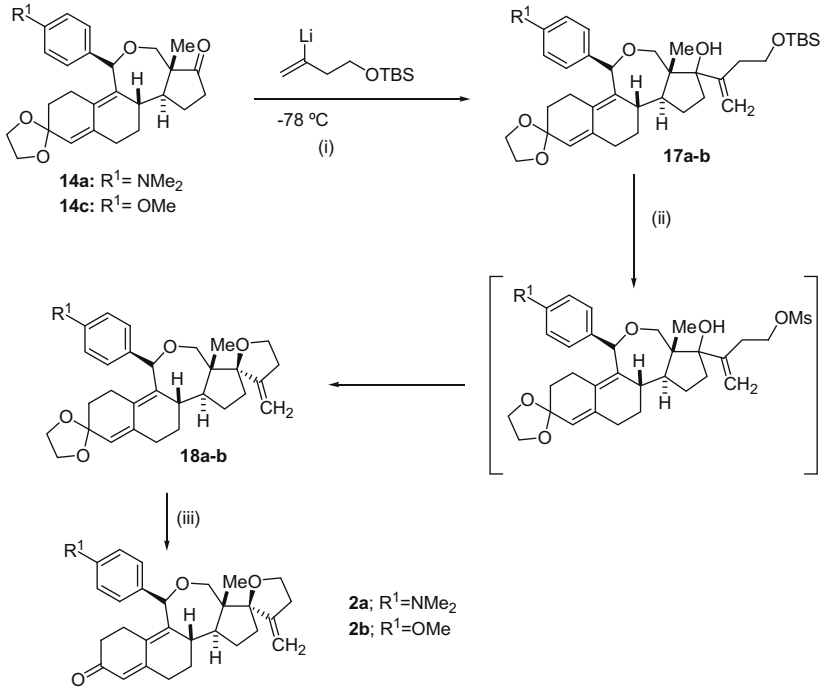
Compound	R^1	R^2	Reagent (v)	Yield (%)	T47D (PR) IC_{50} (nM)	A549 (GR) IC_{50} (nM)
1a	NMe_2	–H	$LiAlH_4$	77	930	1400
1b	NMe_2	–CCH	HCCMgBr	65	33	68
1c	NMe_2	–CCMe	MeCCMgBr	51	34	17
1d	NMe_2	–CCPh	PhCCMgBr	81	60	102
1e	NMe_2	$-CF_2CF_3$	$LiCF_2CF_3$	41	36	20
1f	NMe_2	$-C(Me)CH_2$	$BrMgC(Me)CH_2$	81	0.3	150
1g	SMe	$-CF_2CF_3$	$LiCF_2CF_3$	30	56	7
1h	SMe	$-C(Me)CH_2$	$BrMgC(Me)CH_2$	61	3.3	58
1i	SO_2Me	$-C(Me)CH_2$	NA	—	>1000	>3000
1j	OMe	CCH	HCCMgBr	55	>1000	110
1k	OMe	CCMe	MeCCMgBr	54	>1000	41
1l	OMe	CCPh	BrMgCCPh	61	280	21
1m	OMe	$CCPhpCF_3$	$BrMgCCPhpCF_3$	41	82	26
2a	NMe_2	—	—	—	1	62
2b	OMe	—	—	—	7	132
Mifepristone					1.4	1.6

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 pseudo-steroids **1**(**a**–**l**) with various substitutions at the C17-ethynyl position and various modifications at C10 are listed in Table 1. Compound **1a** → **1f** have a similar aromatic side chain as that of mifepristone. Compound **1a** (R^2 = H) showed poor PR antagonist activity in T47D cell-based functional assay. Similarly, poor activity was also observed in GR antagonist activity in A549 cell-based functional assay. Moderate gain in PR activity (IC_{50} 33–60 nM) was observed when H was replaced by various substituted alkynes (**1a** vs **1b**–**1c**). Similar modest gain in activity was observed for compound **1e** ($R^2 = CF_2CF_3$). These compounds also showed modest gain in GR antagonist activity (IC_{50} 20–102 nM) with very little signs of separation between PR and GR antagonist activity. When R^2 = H was replaced by an isopropylene group $R^2 = -C(Me)=CH_2$ (**1a** vs **1f**) significant increase in PR



Scheme 2. Reagents and conditions: (i) $CH_2=C(I)CH_2CH_2OTBS$, *n*-BuLi, −78 °C, 78–91%; (ii) *n*-Bu_4NF (5 equiv), THF, 3 h followed by pyridine (excess); MsCl, rt, 24 h, 61–77%; (iii) TsOH, acetone/H_2O; 93–95%.

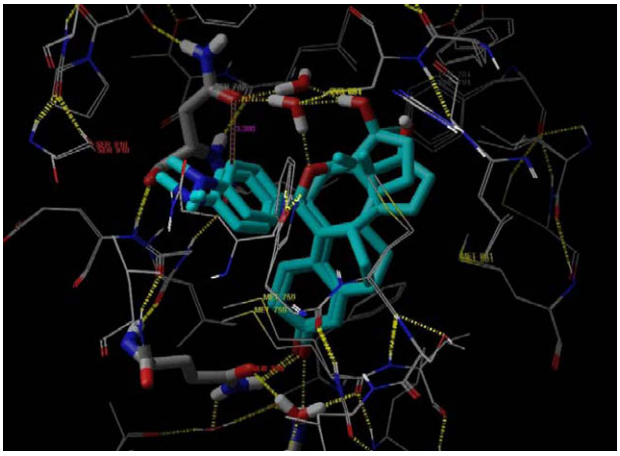


Figure 2. Molecular modeling of compound **1** and mifepristone bound to PR based on the X-ray crystal structures of hPR-norenthindrone and hGR-mifepristone complexes.

antagonist activity was observed (IC_{50} = 0.3 nM) with only modest gain in GR antagonist activity (IC_{50} = 150 nM).

In compounds **1g** and **1h** $-NMe_2$ was replaced by SMe in the aromatic side chain at C10 of pseudo-steroid scaffold. As observed previously for compound with an isopropene group **1h** (**1g** vs **1h**), when the R^1 = SMe attached to the aromatic group was replaced by $-SO_2Me$, complete loss of PR as well as GR activity was observed.

In compounds **14j**–**14m**, the $R^1 = NMe_2$ group was replaced by OMe in the aromatic side chain at C10 of pseudo-steroid scaffold. Interestingly complete loss of the PR antagonist activity was observed in small alkyne substituted compounds (**1j**: R^2 = CCH and **1k**: R^2 = CCMe) while GR antagonist activity was unchanged in these compounds. In bulky alkyne group, a moderate gain in PR antagonist activity was observed.

Compounds **2a** and **2b**, with spirofurane at C17 position and with two different substitution groups at C10 aromatic side chain were potent in the PR antagonist T47D cell-based functional assay (IC_{50} = 1–7 nM) while modest activity in GR antagonist A549 cell-based functional assay (IC_{50} = 62–132 nM) was observed as shown in Table 1.

Compound **1c** was tested orally in ovariectomized Sprague–Dawley rats in a rat uterine complement C3 assay.[10] In this assay, ethinyl estradiol (EE) was used to stimulate C3 expression. Progestins inhibited EE-induced expression. In turn, antiprogestins counteracted progestin-dependent inhibition. When compound **1c** was administered via the oral route along with EE and progesterone, it was found to be efficacious. An ID_{50} = 16 mg/kg was calculated for this compound **1c**. The ID_{50} for the mifepristone was 4.6 mg/kg.

The possible binding modes of compounds **1** and **2** 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.[7] The molecular modeling suggests that these novel pseudo-steroids have different mode of binding compared to normal progesterone derived compounds such as mifepristone. In this compound (**1a**), the oxygen in the C-ring available for the hydrogen bonding with water molecule bridges with amino acid ANS710. There is wider open space available around D ring and this finding is consistent with our SAR study. The presence of phenylalanine amino acid 797 could possibly induce the π–π interaction and hence provide better potency for compounds with isopropene and spirofurane in D ring system. The A ring system as well as side chain of the pseudo-steroids have a similar binding pocket as mifepristone.

In summary, several novel pseudo-steroid analogs were prepared in 14–16 steps. Modifications of the C10 side chain as well as D ring system lead to changes in both PR as well as GR antagonist activity in cell-based functional assays. Molecular modeling using known X-ray crystal structure revealed that these compounds have different mode of binding. The oxygen atom in C-ring played a similar role as the C17 hydroxy group in D ring of the mifeprestone. The SAR study executed on the C17 as well as C10 positions resulted in several potent and novel PR antagonists. Analog **1c** showed in vivo efficacy in the C3 model via oral route.

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