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Improving the developability profile of pyrrolidine progesterone receptor partial agonists

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ABSTRACT

The previously reported pyrrolidine class of progesterone receptor partial agonists demonstrated excellent potency but suffered from serious liabilities including hERG blockade and high volume of distribution in the rat. The basic pyrrolidine amine was intentionally converted to a sulfonamide, carbamate, or amide to address these liabilities. The evaluation of the degree of partial agonism for these non-basic pyrrolidine derivatives and demonstration of their efficacy in an in vivo model of endometriosis is disclosed herein.

The progesterone receptor (PR) is a ligand-activated transcription factor and a member of the nuclear receptor super-family.[1] PR and its endogenous ligand, progesterone (**1**, Chart 1), have essential roles in ovulation and implantation.[2] For this reason, synthetic PR agonists are used for oral contraception, hormone therapy, leiomyomas, and endometriosis.[3]

For the treatment of endometriosis, medroxyprogesterone acetate (MPA, **2**), **1**, and other agonists can act by opposing estrogen-stimulated endometrial tissue proliferation.[4] Side-effects associated with this therapy, including weight gain, break-through bleeding, and mood disturbances, have been attributed to PR full agonist activity and/or lack of nuclear receptor selectivity.[5] Despite recent advances in the identification of non-steroidal PR agonists,[6]

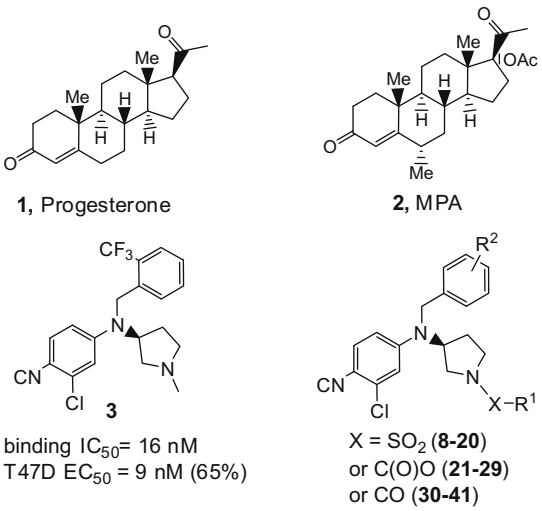

Chart 1. Steroidal and non-steroidal PR ligands.

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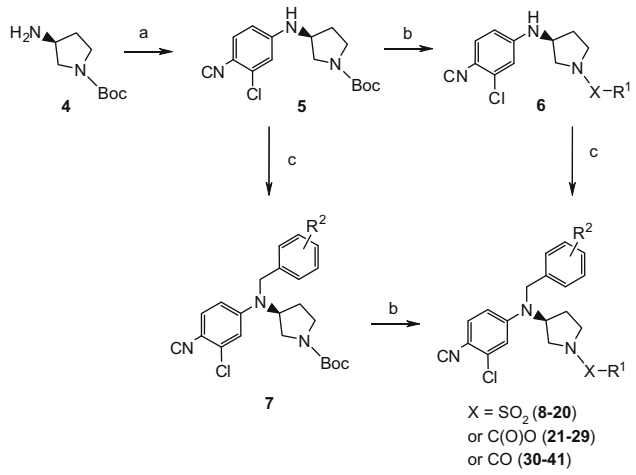
these compounds are likely to induce many of the same side-effects as full PR agonists. However, a selective partial agonist of PR that would be able to fully suppress the action of estrogen on endometrial tissue may reduce the side-effects associated with full agonists.

As part of this effort, we recently reported that ligands containing a (3*S*)-3-amino-1-pyrrolidine template (e.g., **3**) had excellent PR binding potency and partial agonist activity as measured in a human T47D-based alkaline phosphatase PR agonist assay, and >100-fold selectivity over several nuclear receptors, including the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and estrogen receptor (ER).[7] However, in the course of profiling these compounds, it was determined that compound **3** suffered from potent hERG channel blockade (0.78 μM), potent androgen receptor (AR) binding affinity (250 nM), and poor rat PK parameters including high clearance, a large volume of distribution and low oral exposure. In addition, compound **3** demonstrated modest inhibition of the human cytochrome P450 2D6 (1.6 μM) and 2C9 (1.1 μM) isoenzymes. Because compounds with basic amines are known to be associated with both hERG inhibition and high volumes of distribution, we explored analogs where the basicity of the pyrrolidine nitrogen was attenuated.[8] We have previously disclosed that conversion of the pyrrolidine ring to a pyrrolidinone, thereby reducing the basicity of the ring nitrogen, improved all of the aforementioned liabilities while maintaining excellent potency and partial agonism.[9] We now report that pyrrolidine sulfonamides, carbamates, and amides share the same properties while having the added benefit of ease of synthesis as well as the ability to deliver compounds with a broad range of agonist efficacy (37–77%).

The short synthetic route to prepare compounds **8**–**41** is shown in Scheme 1.[13] It is straightforward to diversify at two different positions, R^1 or R^2, by bringing in either group in the final step; steps b and c can be performed in either order. In either case, the first step involved the primary amine of (*S*)-3-amino-1-*tert*-butoxycarbonylpyrrolidine displacing the fluoride of 2-chloro-4-fluorobenzonitrile to give the secondary amine **5**. For diversification of the aniline nitrogen, step b was conducted next, beginning with removal of the BOC protecting group from the pyrrolidine **5** under acidic conditions to yield the amine, which was then reacted with a sulfonyl chloride, acid chloride or alkyl chloroformate to yield intermediate **6**. Lastly, benzylation of the aniline nitrogen under basic conditions afforded the target products. Alternatively, diversification of the pyrrolidine nitrogen in the last step began with incorporation of the substituted benzyl group onto the aniline nitrogen of **5** to form intermediate **7**. BOC removal under acidic conditions afforded the free pyrrolidine which was then transformed to the target analogs via reaction with a sulfonyl chloride, acid chloride or alkyl chloroformate.



Scheme 1. Reagents and conditions: (a) 2-chloro-4-fluorobenzonitrile, $NaHCO_3$, H_2O/DMSO (78%); (b) (i) TFA, CH_2Cl_2; (ii) Et_3N/DIEA/pyridine, CH_2Cl_2, R^1XCl [or for **38**–**41** (2*S*)-2-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}propanoic acid, NMM, *i*-butyl chloroformate, then TBAF]; (c) NaH, DMF, R^2BnBr.

The sulfonylpyrrolidines were tested initially in a binding assay for the progesterone receptor; those results are listed in Table 1. Since the limit of detection in that assay is around 20 nM, most compounds were also tested in a cellular assay format (T47D) which reports potency and efficacy relative to progesterone (100%). It is interesting to note that the potency at the PR receptor generally decreases by extending the R^1 alkyl chain from methyl to ethyl, propyl, and butyl. In the cellular assay, low nanomolar potency is achieved by both methanesulfonamide **8** and isobutanesulfonamide **12.** Two of the most important benefits achieved with the alkanesulfonamides were the improvement in AR selectivity and reduced P450 inhibition, specifically the 2D6 isoform. Consistent with the SAR observed with the *N*-alkyl substituted pyrrolidines, ortho substitution of the aniline benzyl group afforded optimal PR binding potency.[7] Though the chloro (**18**) and bromo (**19**) substituted compounds are exceptionally potent, they are less preferred due to their high levels of intrinsic agonism as measured by the T47D assay (87/88%). Both compounds **17** and **20** have a very promising overall in vitro profile.

Data for pyrrolidine carbamates is given in Table 2. Similar to the pyrrolidine sulfonamides, substitution of the benzyl 2-position led to compounds with improved PR binding affinity and AR selectivity (**22**–**25**). Disubstitution of the R^2 benzyl ring (**26**–**27**) reduces the cell potency and efficacy and increasing the size of the R^1 sub-

Table 1
In vitro data for **3** and sulfonamide compounds (X = SO_2) **8**–**20**[a]

Compd	R^1	R^2	PR IC_{50} (nM)	AR/PR	T47D EC_{50}, nM (% P4)	2D6 IC_{50} (μM)	2C9 IC_{50} (μM)
3	Chart 1		16[b]	16	9 (65%)	1.6	1.1
8	Me	2-CF_3	13[b]	480	11 (82)	15	0.33
9	Et	2-CF_3	40	62	3160 (26)	13	0.35
10	*i*-Pr	2-CF_3	40	62	>10,000	25	0.28
11	*n*-Pr	2-CF_3	80	>125	2630 (41)	9.0	0.11
12	*i*-Bu	2-CF_3	80	>125	10 (65)	13	0.16
13	*n*-Bu	2-CF_3	63	>160	NA	NA	NA
14	Me	H	63	160	843 (53)	14	2.4
15	Me	3-CF_3	100	16	NA	NA	NA
16	Me	2-CN	100	>100	1130 (53)	>33	1.5
17	Me	2-F	20[b]	315	36 (66)	18	1.7
18	Me	2-Cl	6[b]	>1000	1 (88)	31	0.87
19	Me	2-Br	5[b]	>600	3 (87)	31	0.69
20	Me	2-Me	16[b]	250	25 (77)	22	2.4

[a] Values are the mean of ⩾2 determinations.
[b] Tight-binding limit of the assay.

Table 2
In vitro data for carbamate compounds (X = C(O)O) **21**–**29**[a]

Compd	R^1	R^2	PR IC_{50} (nM)	AR/PR	T47D EC_{50}, nM (% P4)	2D6 IC_{50} (μM)	2C9 IC_{50} (μM)
21	Me	H	63	50	NA	7.5	1.3
22	Me	2-CF_3	12[b]	333	8 (66)	8.0	0.16
23	Me	2-F	12[b]	260	165 (51)	7.3	1.4
24	Me	2-Me	12[b]	260	4 (64)	22.0	0.68
25	Me	2-Cl	8[b]	160	0.1 (78)	11.0	1.1
26	Me	2-F, 3-F	20	125	137 (51)	6.7	1.2
27	Me	2-Me, 5-F	20	250	41 (62)	28.0	1.1
28	Et	2-Me, 5-F	30	167	364 (43)	22.0	1.5
29	*i*-Pr	2-Me, 5-F	50	32	233 (44)	15.0	1.1

[a] Values are the mean of ⩾2 determinations.
[b] Tight-binding limit of the assay.

Table 3
In vitro data for amide compounds (X = CO) **30**–**41**[a]

Compd	R^1	R^2	PR IC_{50} (nM)	AR/PR	T47D EC_{50}, nM (% P4)	2D6 IC_{50} (μM)	2C9 IC_{50} (μM)
30	Me	2-CF_3	30	333	35 (78)	17	1.8
31	Et	2-CF_3	25	320	24 (69)	10	1.2
32	*i*-Pr	2-CF_3	25	318	290 (35)	6.0	0.72
33	*t*-Bu	2-CF_3	50	120	400 (6)	16	0.44
34	Et	2-Br	8[b]	310	0.4 (95)	7.5	3.0
35	Et	2-Me	16[b]	490	2.1 (66)	20	4.0
36	Et	2-Me, 5-F	25	320	75 (69)	16	2.9
37	Et	2-Cl, 5-Cl	25	60	163 (81)	13	2.9
38	(*S*)-CH (OH)CH_3	2-CF_3	63	160	208 (47)	19	2.4
39	(*S*)-CH (OH)CH_3	2-Me	32	>320	3 (60)	>33	14
40	(*S*)-CH (OH)CH_3	2-Me, 5-F	79	>120	183 (35)	>33	11
41	(*S*)-CH (OH)CH_3	2-Cl, 5-Cl	50	160	341 (37)	20	7.9

[a] Values are the mean of ⩾2 determinations.
[b] Tight-binding limit of the assay.

stituent also led to reduced cellular potency and AR selectivity (**28**–**29**). In general, carbamate analogs showed a significant reduction in inhibition of the 2D6 isozyme but only modest improvement in 2C9 inhibition, similar to the sulfonamides. Compound **26** best exemplified the targeted potency, selectivity, and intrinsic agonism.

In the pyrrolidine amide class, a number of ligands with excellent PR binding affinity and AR selectivity were identified (Table 3). In general, 2D6 inhibition was less of an issue in this class. Again consistent with properties of other pyrrolidine derived analogs, increasing the size of R^1 led to reduced agonist potency and efficacy, and increased 2C9 inhibition (**30**–**33**). Significant decrease in 2C9 inhibition was achieved by replacement of the 2-CF_3 benzyl substituent which produced potent PR partial agonists (**34**–**37**). Since P450 inhibition can often be attributed to lipophilicity, an effort to introduce polarity into these compounds was undertaken. To this end, incorporation of a hydroxyl group onto the α-carbon of the amide moiety (**38**–**41**) led to PR partial agonists with further reduced P450 inhibition at 2C9. Compound **41** was selected for additional profiling because the intrinsic agonism (37%) was closest to the low end of the target range and was significantly different from the other compounds chosen for profiling.

An X-ray co-crystal structure of sulfonamide **20** bound to the PR ligand binding domain was solved to a resolution of 2.0 Å.[10] An overlay of progesterone (**1**) and compound **20** bound to PR (Fig. 1) shows that the benzonitrile group of **20** occupies the same space and interacts with the same residues (Gln725 and Arg766) as the A-ring carbonyl oxygen of **1**. In addition, the pyrrolidine ring of **20** lies perpendicular to the core steroidal backbone of **1** and extends out towards the AF2 region of the protein. In order to accommodate this group, the Met909 and Trp755 are slightly displaced relative to their position when **1** is bound. The movement of Met909 is notable as this residue is likely associated with the degree of agonism.[11]

Since the driving force for making these reduced-basicity analogs was to improve hERG and PK properties, it was gratifying to see improvement in both areas. Sulfonamide **20**, carbamate **26**, and amide **41** all showed reduced blockade of the hERG channel relative to **3** in a whole cell patch clamp assay (Table 4).[12] Additionally, rat exposure data indicates that the volume of distribution was reduced by more than threefold for all analogs. All three compounds also showed significant improvement in oral bioavailability, while **20** and **26** also showed reduced clearance.

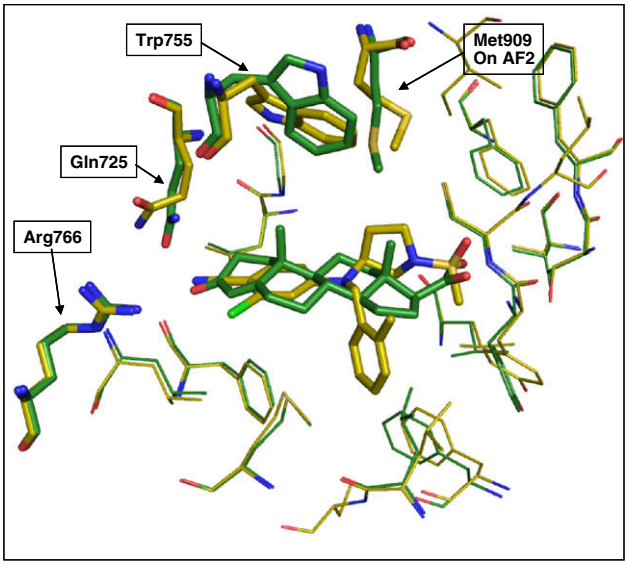


Figure 1. Superposition of **20** (yellow) & **1** (green) crystal structures. Enzyme backbone and key backbone residues are shown in the color of the ligand to which they correspond.

Table 4
Rat pharmacokinetic and hERG channel data[a]

Parameter	Compound			
	3[b]	**20**[b]	**26**[c]	**41**[b]
Dose (iv, po, mg/kg)	1.1/2.1	2.1/4.2	1.3/0.85	1.2/1.8
CLp, iv (mL/min/kg)	120	98	59.7	150
Vd_{ss} (L/kg)	39	12	11.7	10
C_{max}, iv (ng/mL)	39	221	290	134
$t_{1/2}$, iv (h)	3.6	1.6	5.0	1.1
Oral %F	23	~100	~100	~100
hERG IC_{50} (μM)	0.78	2.8	4.0	2.2

[a] Values are means of two experiments.
[b] Discrete study.
[c] Cassette study (conducted as a mixture).

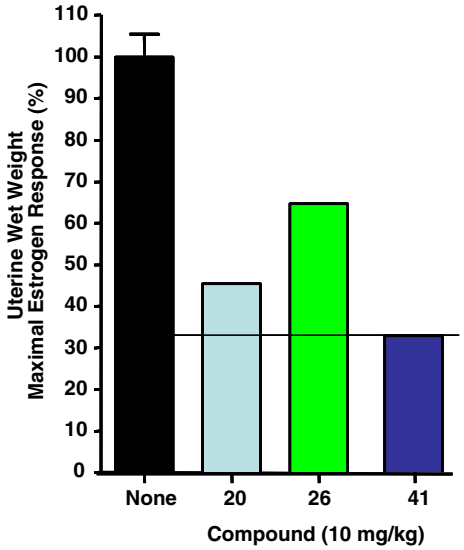


Chart 2. Rat Uterus Wet Weight. Line corresponds to reduction for medroxyprogesterone acetate (**2**, 10 mg/kg).

Previous work has demonstrated that PR partial agonists oppose the effects of estrogen-stimulated uterine growth in an ovariectomized rat uterotrophic model.[7,9] Three new compounds, sulfonamide **20**, carbamate **26** and amide **41** were tested at a 10 mg/kg dose and the reduction in uterine wet weight relative to estrogen alone was determined (Chart 2). All three compounds were efficacious; in fact, compound **41** demonstrated a decrease in uterine wet weight comparable to the full agonist MPA (**2**). This result suggests that compounds with a range of PR agonism levels could be developed and used to treat endometriosis. Whether such compounds will achieve an improved side-effect profile compared to full progestins will likely require clinical evaluation because there are no validated preclinical models for common PR-related side-effects such as break-through bleeding.

In summary, pyrrolidine derivatives designed to be less basic by incorporation of sulfonamide, carbamate or amide substituents demonstrate reduced hERG channel blockade and improved rat PK properties. These compounds were found to be potent PR partial agonists with good AR selectivity and reduced activity at two P450 isozymes. Three high quality compounds **20**, **26**, and **41** were progressed to an in vivo model to measure their ability to reduce estrogen-stimulated uterine growth and all were efficacious.

Acknowledgments

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13. Example preparation (compound **23**); step b (i): TFA (9.2 mL) was added to a solution of 1-dimethylethyl (3*S*)-3-[(3-chloro-4-cyanophenyl)amino]-1-pyrrolidinecarboxylate (6 g, 18.7 mmol) in CH_2Cl_2 (40 mL) and the mixture was stirred for 5 h. Toluene (20 mL) was added and the reaction mixture was concentrated. Saturated aqueous $NaHCO_3$ (50 mL) was added to the residue and the mixture was extracted with EtOAc (5 × 100 mL). The organic extracts were dried over $MgSO_4$ and concentrated to yield the product as a red–brown solid (6.8 g). MS(ES) *m*/*e* 220.0 $[M+H]^+$. (ii): Methyl chloroformate (0.78 g, 8.1 mmol) was added to a solution of 2-chloro-4-[(3*S*)-3-pyrrolidinylamino]benzonitrile (1.88 g, 8.48 mmol) and Et_3N (2 mL, 12.7 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After stirring at 0 °C for 3 h, saturated aqueous $NaHCO_3$ was added and the layers were separated. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified via flash chromatography to give the product as a white foam (2.12 g, 89%). MS(ES) *m*/*e* 280.2 $[M+H]^+$. Step c: NaH (60% in mineral oil, 0.043 g, 1.08 mmol) was added to a solution of methyl (3*S*)-3-[(3-chloro-4-cyanophenyl)amino]-1-pyrrolidinecarboxylate (0.1 g, 0.36 mmol) in DMF (3 mL) at 0 °C. After stirring for 5 min, 1-(bromomethyl)-2-fluorobenzene (0.072 g, 0.38 mmol) was added and the mixture was stirred for 2 h. Saturated aqueous $NaHCO_3$ was added and the reaction mixture was extracted with EtOAc (2×). The organic layers were dried over Na_2SO_4 and concentrated. The residue was purified via flash chromatography to give the product as a white foam (0.072 g, 52%).^{1}H NMR ($CDCl_3$): δ 7.44 (d, *J* = 9.2 Hz, 1H), 7.30 (m, 1H), 7.12 (m, 2H), 7.01 (m, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.60 (m, 3H), 3.83 (m, 1H), 3.72 (s, 3H), 3.60 (m, 1H), 3.45 (m, 1H), 3.31 (m, 1H), 2.23 (m, 1H), 2.05 (m, 1H). MS(ES) *m*/*e* 388.6 $[M+H]^+$.