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Rational design of orally-active, pyrrolidine-based progesterone receptor partial agonists

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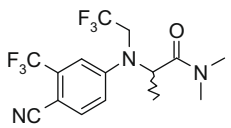
ABSTRACT

Using the X-ray crystal structure of an amide-based progesterone receptor (PR) partial agonist bound to the PR ligand binding domain, a novel PR partial agonist class containing a pyrrolidine ring was designed. Members of this class of *N*-alkylpyrrolidines demonstrate potent and highly selective partial agonism of the progesterone receptor, and one of these analogs was shown to be efficacious upon oral dosing in the OVX rat model of estrogen opposition.

The progesterone receptor (PR) is a ligand-activated transcription factor that, along with the estrogen receptor, plays an important role in modulating endometriosis. In the pathogenesis of this disorder, the ectopic endometrial tissue proliferates in response to estrogen,[1] and current therapies that functionally antagonize estrogen production or action are efficacious in alleviating symptoms. One such therapy that acts by antagonizing estrogen action is treatment with full progesterone receptor agonists (progestins). However, this method of therapy has associated with it undesirable side effects such as break-through bleeding and breast tenderness,[2] as well as additional adverse effects attributable to the poor steroid receptor selectivity of the marketed progestins.[3] Since the level of intrinsic PR agonism required to fully oppose estrogen action is unknown, a selective PR partial agonist has the potential to match the efficacy of progestin therapy, but have reduced side effects related to PR agonism and steroid receptor selectivity. The work described herein is the result of efforts directed at this strategy.

The goal of this effort was to identify small molecule PR partial agonists with high potency at PR and high selectivity over other steroid receptors: the androgen (AR), glucocorticoid (GR), mineralocorticoid (MR), and estrogen (ER) receptors. The chemistry effort initiated with the racemic dimethyl amide **1** (Fig. 1). This compound demonstrated a PR binding IC_{50} of 125 nM in the FP competition binding assay, partial agonist activity in a CV-1 reporter assay, and 10–100-fold selectivity over other steroid receptors.[4] However, it was found that the dimethyl amide functionality was



1
PR IC_{50} = 125 nM
PR CV-1 EC_{50} = 1.3 uM (68%)
AR IC_{50} = 1.6 uM
GR, MR, ER IC_{50}s >10 uM

Figure 1. Activity and selectivity data for racemic PR partial agonist ligand 1.

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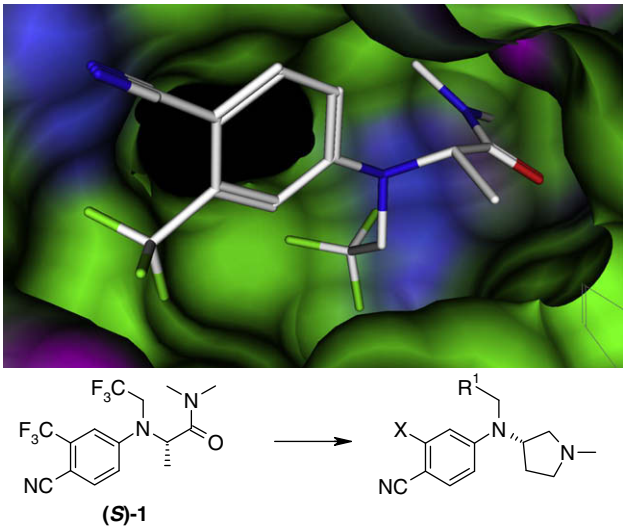
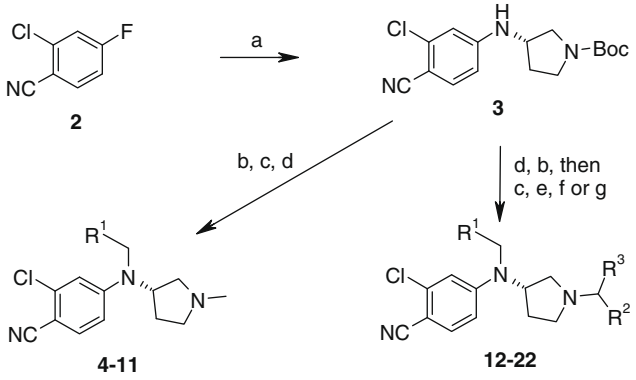


Figure 2. Bound conformation of (*S*)-**1** in the ligand binding domain of PR and proposed structure of a pyrrolidine-based compound.

metabolically unstable, making compounds such as **1** unsuitable for use in animal pharmacology models. Any attempts to modify the groups on the amide nitrogen resulted in substantial decreases in PR binding potency or selectivity. Therefore, efforts were focused on identification of an isosteric replacement for the amide functionality.

Compound **1** was co-crystallized with the PR ligand binding domain (PR LBD), and the *S*-enantiomer was found to be bound in the binding site.[5] Additionally, there were two key observations regarding the conformation of the bound ligand that led to the design of an isosteric replacement for the amide group (Fig. 2). First, the carbonyl oxygen of the amide in **1** did not appear to participate in any interactions with residues within the binding pocket, suggesting that it could be replaced with another atom or functionality. The second key observation was that the amide carbonyl bond and the Cα-methyl bond existed in an eclipsed conformation relative to one another, suggesting that the four atoms spanning the α-methyl carbon and the carbonyl oxygen could be tied together into a five-membered ring. This hypothesis led to the design of a series of *N*-alkylpyrrolidine-based compounds.

The *N*-alkylpyrrolidines were synthesized as shown in Scheme 1.[12] Reaction of 2-chloro-4-fluorobenzonitrile (**2**) and (*S*)-3-amino-1-*tert*-butoxycarbonylpyrrolidine provided pyrrolidine **3**. Removal of the Boc protecting group from **3** followed by reductive methylation on the pyrrolidine nitrogen and subsequent alkylation on the aniline nitrogen provided compounds **4–11**. Target compounds **12–22** were prepared by alkylation of **3** on the aniline nitrogen with an alkyl or benzyl halide, followed by removal of the Boc protecting group and alkylation of the resulting free pyrrolidine.



Scheme 1. Reagents and conditions: (a) (*S*)-3-amino-1-*tert*-butoxycarbonylpyrrolidine, $NaHCO_3$, H_2O/DMSO, 90 °C, 22 h; (b) trifluoroacetic acid, CH_2Cl_2, 25 °C, 1.5 h; (c) H_2CO, $NaBH_4$, MeOH/H_2O, 25 °C, 30 min; (d) R^1CH_2-halide, NaH, DMF, 25 °C, 1 h; (e) R^1CHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 25 °C, 2 h (R^3 = H); (f) R^2CH_2Br, K_2CO_3, CH_3CN, 75 °C, 15 min (R^3 = H); (g) (i) $R^2R^3C(CN)OH$, 4 Å sieves, EtOH, 60 °C, 2 h; (ii) $NaBH_4$, EtOH, 25 °C, 16 h.

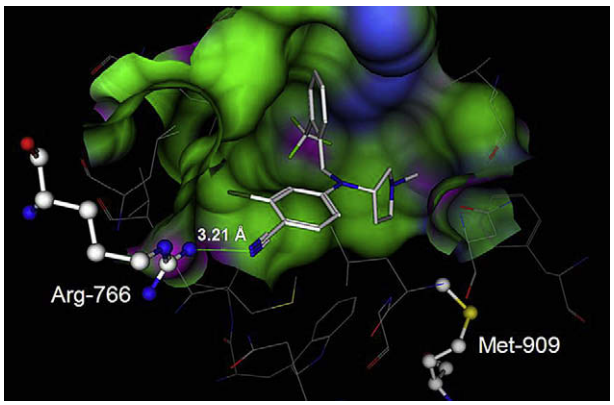
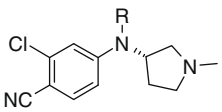


Figure 3. Bound conformation of compound **22** in the PR LBD.

Modification of the substituents on the pyrrolidine and aniline nitrogens revealed some interesting SAR trends, much of which can be explained in light of the X-ray crystal structure of compound **22** bound in the PR LBD (Fig. 3).[5] The preference for benzyl groups on the aniline nitrogen, particularly those with *ortho* substitutions (Table 1), can be understood by examining this structure. Smaller groups such as those found in compounds **4–6** clearly would not adequately fill the binding pocket for the substituent on the aniline nitrogen, but the shape of the pocket does appear well suited to accommodate a benzyl group. Within this pocket, there is a well-defined binding site for a relatively small *ortho* substituent such as chlorine, methyl or trifluoromethyl. Interestingly, the trifluoromethyl substitution led to a significant decrease in partial agonist efficacy. However, *para* substitution is not well accommodated in this pocket, and *meta* substitution is blocked by the back of the pocket and by the surface created by Leu-718 and Phe-794 (not shown). The sharp decrease in binding affinity observed when the phenyl portion of the benzyl group is replaced with a 2-pyridyl group (compound **8** vs compound **20**) is likely a

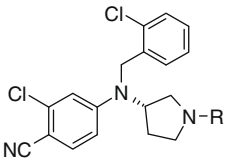
Table 1
In vitro data for substituted aniline analogs



Compds	R	PR binding IC_{50}, nM	PR CV-1 EC_{50}, nM (%P4)
4	Et	1300	>10,000
5	Pr	400	>10,000
6	Bu	250	>10,000
7	Cyclohexylmethyl	79	>10,000
20	Benzyl	40	1300 (58)
8	CH_2-2-Pyridyl	3000	>10,000
12	*o*-Cl-Benzyl	20[a]	1.6 (67)
9	*m*-Cl-Benzyl	160	>10,000
10	*p*-Cl-Benzyl	400	>10,000
11	*o*,*o*-Di-Cl-benzyl	40	>10,000
21	*o*-Me-Benzyl	50	200 (73)
22	*o*-CF_3-Benzyl	16[a]	3 (44)

[a] At or below the tight binding limit of the PR binding assay.

Table 2
In vitro data for substituted pyrrolidine analogs



Compds	R	PR binding IC_{50}, nM	PR CV-1 EC_{50}, nM (%P4)
12	Me	20[a]	1.6 (67)
13	*i*-Pr	20[a]	200 (47)
14	$(CH_2)_2CF_3$	13[a]	200 (52)
15	$(CH_2)_2OMe$	25	50 (40)
16	CH_2CN	8[a]	0.5 (64)
17	CH_2CO_2Et	8[a]	10 (45)
18	CH_2CONH_2	63	200 (47)
19	CH_2-2-pyridyl	20[a]	>10,000

[a] At or below the tight binding limit of the PR binding assay.

result of placement of a polar group in a hydrophobic binding environment.

The compounds for which the substituent on the pyrrolidine ring was varied were similar in binding affinity as measured in the PR FP binding assay, although for all but two of the compounds, the measured IC_{50} was at or below the tight binding limit of this assay. Therefore, for this compound set, the CV-1 functional assay may be a better measure of the true potency of the compounds. While a decrease in potency was generally observed with increasing size or polarity of the substituent on the aniline nitrogen, perhaps the more interesting trend was the decrease in partial agonist efficacy when the size was increased beyond cyanomethyl (Table 2). This may also be explained by examining the structure.

The binding pocket shown in Figure 3 that is occupied by the pyrrolidine *N*-methyl group in compound **22** does not appear to be large enough to accommodate a group larger than cyanomethylene. Although the ligand binding site of PR is known to have some degree of flexibility,[5] for larger substituents to be accommodated the ligand would need to undergo global movement within the binding site. Such shifts in the position of ligands in the PR LBD have been observed crystallographically.[5] A movement of this type might be expected to cause a perturbation of the position of Met-909 and induce a movement of helix 12 in the AF-2 region,[6] leading to a partial agonist response as has been observed with the partial agonist genistein bound to ERβ.[7] In fact, displacement of Met-909 from its position in the agonist conformation is observed in the structure of compound **22** bound in the PR LBD and is illustrated in the superposition of the PR LBD-bound conformations of progesterone[8] and compound **22** (Fig. 4). The pyrrolidine ring in compound **22** forces a significant movement in the Met-909 side chain from its position in the progesterone structure, which is expected to produce the partial agonist response that is observed for compound **22**.

Compound **22** was further evaluated in additional binding and functional assays to determine its selectivity for PR vs. the other steroid receptors (Table 3). Compound **22** binds to PR with ⩾500-fold higher affinity than for estrogen receptor β and >15-fold over AR. Since compound **22** is at or near the tight binding limit in the PR binding assay, a better measure of selectivity over AR and other NRs may be by the CV-1 assay. Accordingly, compound **22** is >400-fold selective for PR in AR, MR, and GR functional assays, with >2500-fold selectivity being observed versus GR in the antagonist assay. Compound **22** was inactive in the GR agonist assay (data not shown).

The PR partial agonist activity of compound **22** was also evaluated in T47D breast cancer cells expressing native PR.[9] In this assay, compound **22** is a potent partial agonist with an EC_{50} value of 9 nM and a maximal agonist response equivalent to 65% of the full progesterone response. In addition to measuring its partial agonist activity at PR, the ability of compound **22** to inhibit the estrogen-induced expression of SDF-1 (stromal cell-derived factor-1) in ELT-3 rat myometrial cells was evaluated.[10] Compound **22** was a potent inhibitor in this assay (max inh. = 91%), demonstrating an IC_{50} of 0.12 nM .

Having demonstrated potent partial agonist activity, high selectivity and potent inhibition of estrogen-stimulated SDF-1 expression in ELT-3 cells, compound **22** appeared to be an ideal candidate for evaluation in an animal model. The OVX rat model, a well-validated model for the measurement of estrogen opposition,[11] was chosen for this study, using uterine wet weight as the

Table 3
Steroid receptor selectivity data for compound **22**

FP binding assays			Functional assays			
PR	AR	ERβ	PR CV-1 agonist	AR CV-1 agonist	MR CV-1 agonist	GR A549 antagonist
0.016[a,c]	0.250[a]	>10[a]	0.003 (44%)[b]	1.3 (77%)[b]	5.0 (100%)[b]	7.9 (66%)

[a] IC_{50} (binding assays) and EC_{50} (functional assays) values are given in μM.
[b] For the functional assays, percent activation or inhibition relative to the control agonist is given in parentheses.
[c] At or below the tight binding limit of the assay.

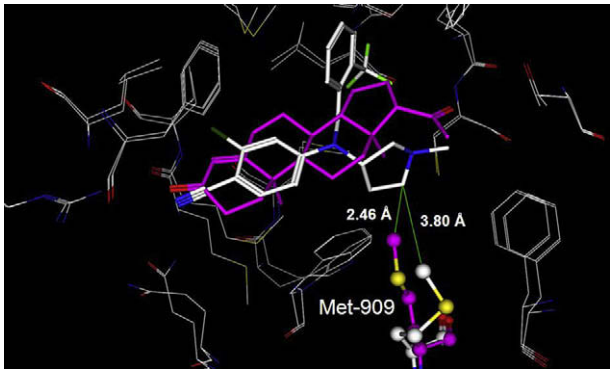


Figure 4. Superposition of the bound conformations of progesterone (carbon atoms colored magenta) and compound **22** in the PR LBD.

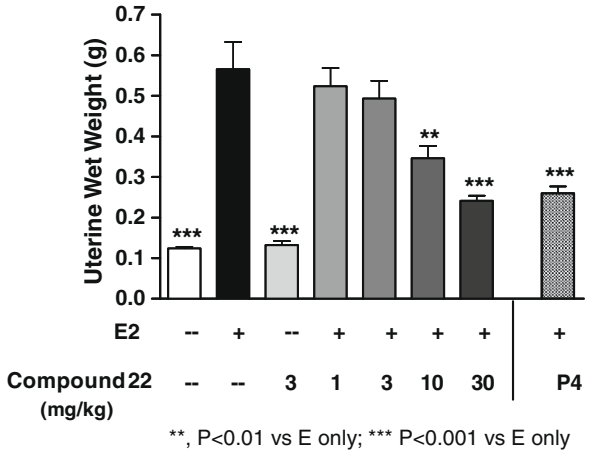


Figure 5. Inhibition of estrogen-stimulated uterine wet weight gain in ovariectomized rats by compound **22**.

phenotypic readout. As shown in Figure 5, oral administration (u.i.d.) of compound **22** resulted in a dose-dependent reduction in estrogen-stimulated uterine growth, with statistically-significant reductions at 10 and 30 mg/kg/day and an effect at 30 mg/kg/day comparable to that of progesterone (P4) administered at 10 mg/kg/day.

In summary, structure-based design has been successfully implemented in the design of a new class of highly potent and selective pyrrolidine-based progesterone receptor partial agonists, one of which has demonstrated dose-dependent efficacy in an animal model of estrogen opposition. This work has provided a solid foundation for further optimization of this compound class, the results of which will be reported elsewhere.

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12. *Example preparation*: Compound **22**. Step 1: A mixture of 2-chloro-4-fluorobenzonitrile (4.26 g, 27.5 mmol), 1,1-dimethylethyl (3*S*)-3-amino-1-pyrrolidinecarboxylate (5.11 g, 27.5 mmol) and $NaHCO_3$ (4.62 g, 55 mmol) in 45 mL of DMSO and 5 mL of H_2O was heated with stirring at 96 °C for 6 h and 86 °C for 16 h. The reaction was diluted with 200 mL of H_2O and extracted with Et_2O (3×). The extracts were washed with H_2O (2×), dried over Na_2SO_4, filtered, and concentrated. The residue was crystallized from Et_2O/hexane to yield 1,1-dimethylethyl (3*S*)-3-[(3-chloro-4-cyanophenyl)amino]-1-pyrrolidinecarboxylate (6.88 g, 78%). LC–MS (ES) *m/e* 322 $[M+H]^+$. Step 2: NaH (60% dispersion in mineral oil, 1.95 g, 48.7 mmol) was washed free of mineral oil with hexane, suspended in 100 mL of DMF stirred, and cooled in an ice bath. A solution of 1,1-dimethylethyl (3*S*)-3-[(3-chloro-4-cyanophenyl)amino]-1-pyrrolidinecarboxylate (10.43 g, 32.5 mmol) in 40 mL of DMF was added dropwise over 20 min. The reaction was stirred an additional 40 min, and a solution of 1-(bromomethyl)-2-(trifluoromethyl)benzene (11.65 g, 48.7 mmol) in 25 mL of DMF was rapidly added. The reaction mixture was stirred for 30 min at 0 °C and 30 min at rt. The mixture was poured into 200 mL cold aqueous NH_4Cl, and extracted with Et_2O (3×). The extracts were washed with H_2O (2×), dried over $NaSO_4$, filtered, and concentrated. The residue was purified by column chromatography (eluted with 25% EtOAc/hexane) to yield 1,1-dimethylethyl (3*S*)-3-((3-chloro-4-cyanophenyl){[2-(trifluoromethyl)phenyl]methyl}amino)-1-pyrrolidinecarboxylate (14.28 g, 92%). LC–MS (ES) *m/e* 480 $[M+H]^+$. Step 3: A solution of 1,1-dimethylethyl (3*S*)-3-((3-chloro-4-cyanophenyl){[2-(trifluoromethyl)phenyl]* methyl}amino)-1-pyrrolidinecarboxylate (14.0 g, 29 mmol) in 16 mL of CH_2Cl_2 was treated with TFA (15 mL) and stirred for 1.5 h. The reaction mixture was concentrated, and the residue was triturated with Et_2O and MeOH. After the solids were collected by filtration, washed with Et_2O, and dried, they were dissolved in 20 mL of MeOH and added to a stirred mixture of aqueous K_2CO_3 and Et_2O. The Et_2O was separated and the aqueous phase was extracted with Et_2O (2×). The combined Et_2O extracts were washed with H_2O and saturated NaCl, dried over Na_2SO_4, filtered, and concentrated to yield 2-chloro-4-((3*S*)-3-pyrrolidinyl{[2-(trifluoromethyl)phenyl] methyl}amino)benzonitrile (10.74 g, 98%). LC–MS (ES) *m/e* 380 $[M+H]^+$. Step 4: A solution of 2-chloro-4-((3*S*)-3-pyrrolidinyl{[2-(trifluoromethyl)phenyl] methyl}amino)benzonitrile (9.0 g, 24 mmol) in 125 mL of MeOH was treated with formaldehyde (10.9 mL of a 37% aqueous solution, 142 mmol). After 30 min, the solution was cooled in an ice bath. $NaBH_4$ (2.0 g, 52 mmol) was slowly added and the reaction mixture was stirred for 30 min. Cold H_2O (200 mL) and aqueous NH_4Cl (200 mL) were added to decompose excess $NaBH_4$. The mixture was extracted with Et_2O and the combined extracts were washed with H_2O and concentrated. The residue was purified on a short Al_2O_3 (neutral, Brockman 2.8) column eluting with Et_2O to yield 2-chloro-4-((1-methyl-3-pyrrolidinyl){[2-(trifluoromethyl)-phenyl]methyl}amino)benzonitrile (8.31 g, 88%). LC–MS (ES) *m/e* 394 $[M+H]^+$. ^1H NMR ($CDCl_3$)δ: 7.75 (d, J = 3.6 Hz, 1H), 7.55–7.35 (m, 3H), 7.2 (d, J = 3.6 Hz, 1H), 6.75 (d, J = 2.8 Hz, 1H), 6.52 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 5.07 (d, J = 18.8 Hz, 1H), 4.72 (d, J = 18.8 Hz, 1H), 4.64 (m, 1H), 2.90 (m, 1H), 2.7 (m, 2H), 2.6–2.4 (m, 5H), 1.87 (m, 1H).