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Discovery of orally active, pyrrolidinone-based progesterone receptor partial agonists

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ABSTRACT

We have designed and synthesized a novel series of pyrrolidinones as progesterone receptor partial agonists. Compounds from this series had improved AR selectivity, rat pharmacokinetic properties, and in vivo potency compared to the lead compound. In addition, these compounds had improved selectivity against hERG channel inhibition.

The progesterone receptor (PR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors.[1] In addition to its numerous other physiological actions, progesterone (P4, 1)-bound PR attenuates estrogen (E2)-stimulated proliferation of endometrial tissue (Chart 1).[2] Progesterone and other progestins such as medroxyprogesterone acetate (MPA, 2) are commonly used in hormone replacement therapy, secondary amenorrhea, and oral contraception; moreover, because of their ability to block the proliferation of endometrial tissue, they are also effective treatments for endometriosis.[3–5] Unfortunately, these therapies have multiple side effects associated with their full agonist activity and/or poor nuclear hormone receptor (NHR) selectivity such as weight gain, breakthrough bleeding, and mood disturbances.[3–5]

Recently, a number of selective non steroidal PR agonists designed to treat endometriosis have been reported.[6,7] While these ligands will likely reduce selectivity-related side effects, those associated with full agonism will persist. In an effort to diminish PR-related side effects, we initiated a chemistry effort to develop a selective PR partial agonist. It is hypothesized that a partial

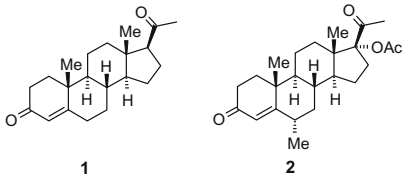

Chart 1. Steroidal PR ligands.

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agonist could reduce the level of PR-regulated gene products to such a level to oppose E2 but not to induce side effects associated with full agonism. Consequently, these ligands would fully suppress the action of E2 on endometrial tissue but minimize the side effects associated with both poor NHR selectivity and full PR agonism.

The pyrrolidine **3**, identified from a structure-based design strategy, was the starting point for our chemistry effort (Chart 2).[7] Compound **3** had excellent PR binding potency and partial agonist activity as measured by a T47D-based alkaline phosphatase PR agonist assay and >100-fold selectivity over several nuclear receptors, including, estrogen (ER), mineralocorticoid (MR), and glucocorticoid (GR) receptors.[8] Unfortunately, **3** and its *N*-alkyl analogs all suffered from potent androgen receptor (AR) binding affinity, potent hERG channel blockade, and poor rat and monkey PK parameters including high clearance, large volumes of distributions and low oral exposure. The latter two (hERG and PK), we attributed to the inherent lipophilic, basic nature of the pyrrolidine ring.[9]

Examination of the binding pocket surface of an X-ray crystal structure of **3** bound to the PR ligand binding domain (LBD) showed an unoccupied region of the protein corresponding to the carbon 5 of the pyrrolidine ring (Fig. 1).[7] It was believed that incorporation of a carbonyl group at this position would adequately fill this region of the LBD and possibly interact with Cys891. Furthermore, a carbonyl group at this position would have an additional benefit of reducing the basicity of the ring nitrogen. Toward this end, we investigated this novel pyrrolidinone core template.

The pyrrolidinone derivatives **10**–**22** were prepared according to Scheme 1. Aryl fluoride displacement of **4** with (2*S*)-2-amino-4-[(1,1-dimethylethyl)oxy]-4-oxobutanoic acid **5** followed by a reduction of the resultant acid afforded the primary alcohol **6**. Tosylation of **6** followed by nucleophilic displacement with sodium azide and then hydrogenation of the resultant azide provided the desired amine **7**. Incorporation of an alkyl group onto the amine **7** was accomplished via a 3-step process consisting of nosylation of the amine, alkylation, and then removal of the nosyl group with thiophenol. Alternatively, incorporation of more sterically hindered alkyl groups was accomplished either through reaction with a cyanohydrin and $NaBH_4$ or reductive amination to afford **8**. Transesterification of **8** to its methyl ester followed by cyclization under basic conditions yielded pyrrolidinone **9** which was then benzylated to provide the target analogs **10**–**23**.

The *N*-methyl pyrrolidinones synthesized were tested for PR and AR binding (AR selectivity is shown as a ratio of AR to PR binding) and for functional activity in the human T47D cell alkaline phosphatase assay (Table 1).[8] The unsubstituted benzyl analog **10** showed poor PR binding, AR selectivity, and weak activity in the T47D cellular assay. Incorporation of a halogen at the 2-position of the benzyl substituent produced partial agonists with improved PR binding affinity, AR selectivity, and cellular potency (**11** and **12**). Interestingly, the 2,3 di-fluoro substituted benzyl analog **14** demonstrated increased cellular potency relative to the 2,4 di-fluoro substituted benzyl analog **13**.

We next examined the effect of varying not only the benzyl group substitution but the nature of the alkyl group on the pyrrolidinone nitrogen (Table 2). Incorporation of either an ethyl or isopropyl group onto the pyrrolidinone nitrogen produced a number of partial agonists with excellent PR binding affinity (with several analogs reaching the tight binding limit of the assay), AR selectivity, and cellular potency. Compounds with larger alkyl groups (than ethyl or isopropyl) at this position demonstrated reduced

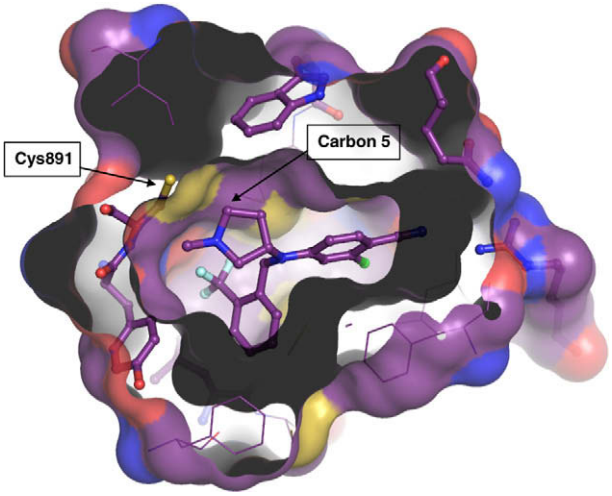
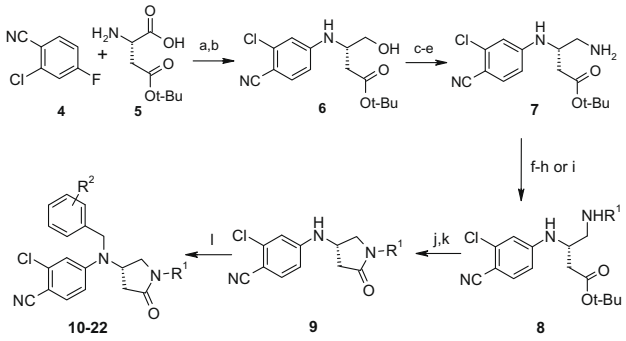
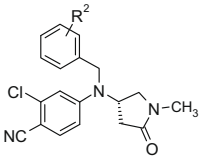


Figure 1. Binding pocket surface of compound **3**. Compound **3** shown in purple. Key backbone residues are highlighted as ball and stick.



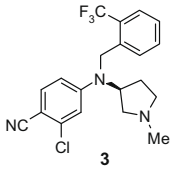
Scheme 1. Reaction conditions: (a) $NaHCO_3$, DMSO, H_2O, Δ; (b) CDI, $NaBH_4$, THF; (c) TsCl, pyr; (d) NaN_3, DMF; (e) H_2, PtO_2, EtOH; (f) *o*-$NO_2C_6H_4SO_2Cl$, Et_3N, CH_2Cl_2; (g) R^1X, K_2CO_3, DMF; (h) PhSH, K_2CO_3, DMF; (i) acetone cyanohydrin, 4 Å molecular sieves, $NaBH_4$; or $NaB(OAc)_3H$, DCE, 2-butanone or 3-pentanone; (j) 4 N HCl/dioxane, MeOH; (k) K_2CO_3, CH_3CN/CH_3OH, Δ or HMDS, CH_3CN; (l) NaH, DMF, R^2BnX.

Table 1
Selected in vitro data for compounds **10-15**[a]



Compd	R^2	PR binding IC_{50}, nM	AR/PR	PR T47D EC_{50}, nM (% P4)
10	—	200	50	1265 (39)
11	2-Cl	25	250	0.3 (62)
12	2-F	20	330	135 (50)
13	2-F,4-F	40	205	625 (46)
14	2-F,3-F	25	100	35 (53)
15	2-CF_3	25	80	70 (65)

[a] Values are the mean of ⩾2 determinations.

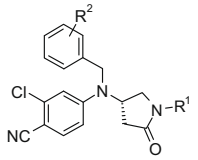


PR binding IC_{50}= 15 nM
PR T47D EC_{50} (%P4) = 30 nM (61%
AR binding IC_{50} = 250 nM
hERG IC_{50} = 0.78 μM

3

Chart 2. Profile of lead alkylpyrrolidine **3**.

Table 2
Selected in vitro data for compounds **15–22**[a]



Compd	R^2	R^1	PR binding IC_{50} (nM)	AR/PR	PR T47D EC_{50} (nM) (% P4)
16	2-CH_3	CH_2CH_3	15[b]	170	0.8 (57)
17	2-CH_3	$CH(CH_3)_2$	20	265	2 (71)
18	2-CH_3	Propyl	80	80	1415 (49)
19	2-CH_3	$CH(CH_3)CH_2CH_3$	125	80	750 (18)
20	2-Cl	$CH(CH_3)_2$	10[b]	830	0.3 (63)
21	2-Cl,5-Cl	CH_2CH_3	10[b]	210	0.8 (52)
22	2-Cl,5-F	CH_2CH_3	10[b]	525	0.3 (53)

[a] Values are the mean of ⩾2 determinations.
[b] Tight-binding limit of the assay. ND = no data.

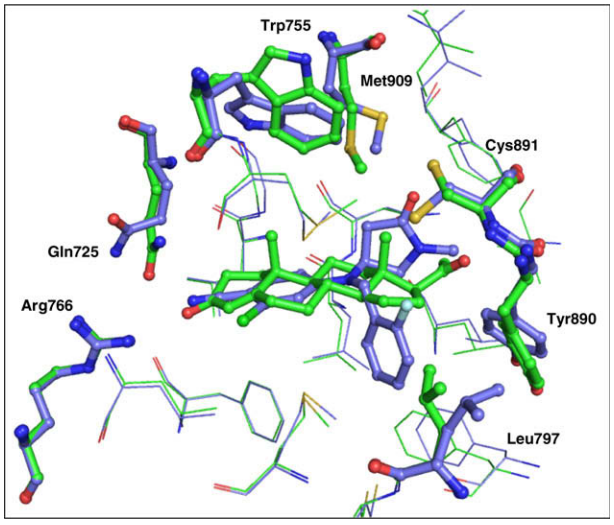


Figure 2. Superposition of **12** (cyan) & **1** (green) crystal structures. Enzyme backbone and key backbone residues are shown in the color of the ligand to which they correspond.

PR binding affinity, AR selectivity, and cellular potency (e.g., **18**–**19**).

Figure 2 shows overlay of the crystal structures of **1** and **12** bound to PR-LBD. These ligands share similar protein/ligand contacts, most notably the benzonitrile group of **12** interacting with the same residues (Gln725 and Arg766) as the A-ring carbonyl oxygen of 1.[7,10,11] However, compound **12** differs from **1** such that the pyrrolidinone ring resides essentially perpendicular to the backbone of **1** which appears to shift the positioning of the binding pocket residues Met909 and Trp755. Likewise, the carbonyl group of **12** alters the orientation of Cys891 compared to its position when compounds **1** and **3** (structure not shown) are bound to the PR LBD.[7] The movement of Met909 could be significant since this residue is part of AF2 region of the c-terminal helix 12 which has been associated with ligand functionality.[10] In addition, 2-F benzyl group of **12** extends towards the 17-α pocket which is unoccupied by **1** and subsequently displaces Tyr890 and Leu797 relative to their position when **1** is bound. Since the corresponding 17-α pocket of AR is smaller and less flexible, this interaction is a possible explanation for the improved AR selectivity of **12** over **1**.[11]

The pharmacokinetic properties (rat and monkey) of selected analogs are shown in Tables 3 and 4, respectively. In the rat, analogs **14**, **20**, and **22** demonstrated lower oral plasma clearances, reduced volumes of distributions, and improved oral bioavailability with respect to pyrrolidine **3**. In addition, the *N*-methyl and *N*-ethyl analogs **14** and **22** exhibited increased oral exposures, while the *N*-isopropyl analog **20** showed no significant improvement with respect to **3**. In the monkey, pyrrolidinone analogs **14** and **21** displayed reduced volumes of distribution and lower oral plasma clearances compared to **3**; however, only the *N*-methyl analog **14** showed significantly improved oral exposure.

To further profile these partial agonists, hERG channel blocking data via whole-cell patch clamp assay format were collected for selected analogs (Table 5).[12] Both of these compounds were markedly less potent than the lead **3**, with compound **12** having the most significant improvement.

With a set of PR ligands having both potent partial agonism and attractive pharmacokinetic properties in hand, we next measured their E2 opposition effect in an ovariectomized rat uterotrophic model.[11,13] It has been previously reported that in this model progestins inhibit the E2-induced increase of uterine wet weight. Along with the wet weight measurement, gene expression of complement C3 (an E2-regulated gene) was also

Table 3
Rat pharmacokinetic properties for **3**, **14**, **20**, **21** and **22**[a]

Parameter	Compound				
	3	**14**	**20**	**21**	**22**
Dose (iv; po; mg/kg)	1.1; 1.9	1.0; 2.2	1.1; 0.12	1.1;2.0	1.0; 1.3
Oral CLp (mL/min/kg)	510 (210)	16 (2)	40 (14)	230 (27)	75 (14)
Vd_{ss} (L/kg)	55.9 (22)	3.4 (0.6)	42 (10)	35 (30	15 (3)
Oral Cmax (ng/mL)	13 (6)	660 (160)	10 (4)	84 (33)	97 (20)
Oral AUC_{0-t}, (μg h/mL)	0.05 (0.02)	2.3 (0.2)	0.03 (0.01)	0.15 (0.02)	0.29 (0.06)
$t_{½}$, po (h)	5.8 (3.3)	6.2 (0.6)	5.8 (2.3)	1.8 (0.2)	2.0 (0.1)
Oral%F	~29	~ 100	~100	~100	~100

[a] Values are means of three experiments, standard deviation is given in parentheses.

Table 4
Monkey pharmacokinetic properties for **3**, **14**, and **21**[a]

Parameter	Compound		
	3	**14**	**21**
Dose (iv; po; mg/kg)	2.1; 4.0	1.0; 1.6	1.0; 1.3
Oral CLp (mL/min/kg)	1351 (100)	110 (38)	480 (110)
Vd_{ss} (L/kg)	9.3 (1.7)	1.7 (0.2)	2.8 (0.5)
Oral Cmax (ng/mL)	12 (2)	92 (20)	15 (1)
Oral AUC_{0-t}, (μg h/mL)	0.039 (0.006)	0.27 (0.08)	0.032 (0.002)
$t_{½}$, po (h)	1.9 (0.3)	1.4 (0.4)	1.5 (0.5)
Oral%F	2.8%	15%	4.9%

[a] Values are means of three experiments, standard deviation is given in parentheses.

Table 5
hERG IC50 data for **3**, **12**, **22**

Compound	hERG, IC_{50} (μM)
3	0.78
12	6.5
22	3.1

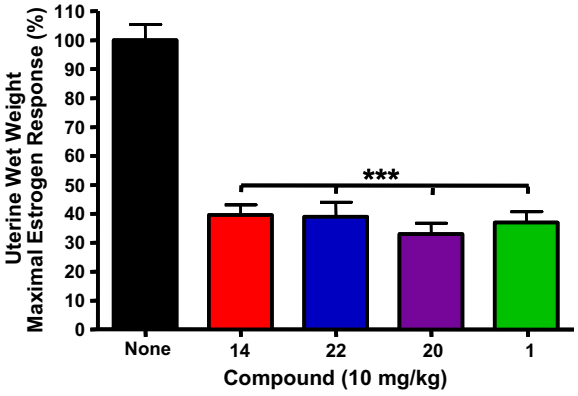


Figure 3. Estrogen-induced wet weight model. ***P <0.001 by One-way ANOVA with Tukey's post-test. Compounds were orally dosed at 10 mg/kg.

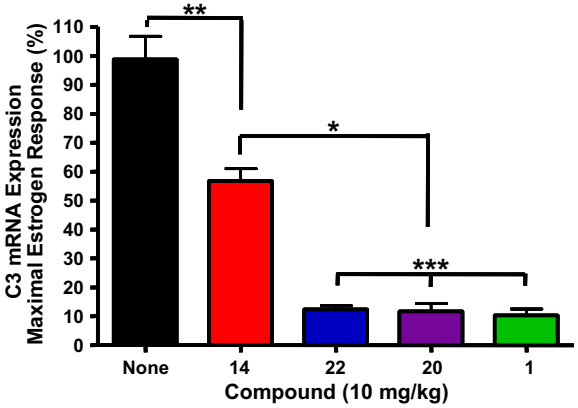


Figure 4. Gene expression in the estrogen-induced wet weight model. *P <0.05, *P <0.01, and ***P <0.001 by One-way ANOVA with Tukey's post-test. Compounds were orally dosed at 10 mg/kg.

measured.[11,13] Initial screening of the *N*-methyl pyrrolidinone **14** at a single oral dose (10 mg/kg) showed a reduction in E2-driven wet weight gain equivalent to 1 (~60% reduction) and a 43% reduction in complement C3 gene expression (Figs. 3 and 4). Under the same dosing conditions, the *N*-ethyl and *N*-isopropyl analogs **22** and **20** also demonstrated a decrease in uterine wet weight and reduction of complement C3 gene expression comparable to the full agonist **1**.

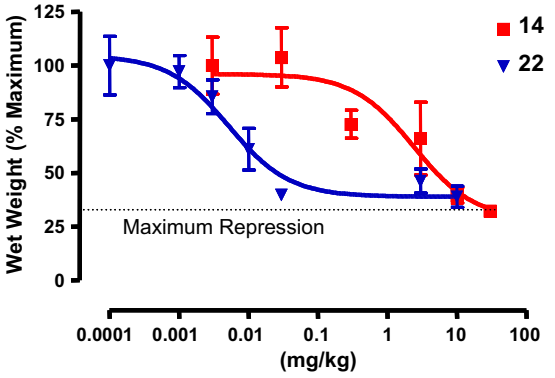


Figure 5. Full dose response study in ovariectomized rat uterotrophic model.

To obtain a better measure of the in vivo efficacy of this series of compounds, a full dose response study was conducted on **14** and **22** (Fig. 5). Both compounds showed a robust and dose-dependent decrease in uterine wet weight; however, the *N*-ethyl analog **22** (ED_{50} = 0.007 mg/kg) proved to be approximately 350-fold more potent than **14** (ED_{50} = 2.5 mg/kg).

In summary, we have described a series of selective and potent PR partial agonists. In general this series has improved AR selectivity, reduced hERG channel blocking, and improved PK parameters in the rat and monkey relative to lead pyrrolidine **3**. Optimization of several of these analogs demonstrated attractive rat pharmacokinetic properties and in vivo efficacy in an estrogen-induced uterotrophic model.

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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.2009.06.081.

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