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Optimisation of a pyrazole series of progesterone antagonists; Part 1

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ABSTRACT

The design and synthesis of a novel series of non-steroidal progesterone receptor antagonists is described. Ligand-lipophilicity efficiency (LLE) was used in the selection of a prototype agent for in vivo pharmacology studies.

The progesterone receptor (PR) is a member of the family of ligand-activated transcription factors that includes the estrogen (ER), androgen (AR), glucocorticoid (GR) and mineralocorticoid (MR) receptors.[1] The use of antagonists for the treatment of a variety of progesterone-related diseases and disorders is of considerable interest. Recent studies have shown PR antagonists to have application in the treatment of endometriosis and uterine fibroids.[2] At present, RU-486 (mifepristone, **1**) is the only PR antagonist approved for clinical use.[3] RU-486 is an 11β-substituted steroid and displays potent antagonist activity at other steroidal receptors, in particular the glucocorticoid receptor (GR).[4] This lack of selectivity limits its chronic use.

As part of a program to identify non-steroidal PR antagonists, we ran a high-throughput screen (HTS) using a PR binding assay. Triage of the HTS data was guided by knowledge of target class ligands. Specifically, compounds containing a cyanoaryl group were prioritised as this group is a known ketosteroid A-ring isostere in non-steroidal PR ligands such as the PR agonist Tanaproget (**2**)[5] (Fig. 1).

The HTS identified a series of phenoxypyrazoles, exemplified by **4** and **5** (Fig. 2). These were related to a series of proprietary non-nucleoside HIV reverse transcriptase inhibitors (NNRTi's)[6–8] that included the clinical candidate Lersivirine[8] (**3**). In a functional PR assay, using recombinant human PR expressed in CHO-MMTV-beta-lactamase, both **4** and **5** were shown to be moderately potent antagonists (IC_{50} of 224 nM and 131 nM, respectively).

Follow-up file screening around hits **4** and **5** demonstrated that 4-CN on the phenoxy moiety was superior to other mono-substituents assessed (data not shown). Replacing 4-CN of **5** with either 4-H, 4-Cl, 4-F or 4-MeO resulted in a >10-fold reduction in PR antagonist activity. The 3- and 2-CN analogues showed no PR antagonism at 10 μM. Lersivirine (**3**) was also devoid of PR activity. The selectivity profile of compound **5** was assessed and it was found to be >40-fold selective for PR over AR, ER, GR and MR (IC_{50}s all >5 μM).

We decided to retain an N-substituent bearing a polar functionality in our optimisation work. We hoped this would act not only as a selectivity handle but as a method of modulating potency, lipophilicity, and metabolic stability. Previous experience with this class of compound had shown that lipophilic analogues such as **5** often had an impaired metabolic stability profile.[8] Therefore, in order to deliver a candidate compound suitable for in vivo investigation, we felt it would be crucial to both improve PR potency and reduce compound lipophilicity. To assess success against these

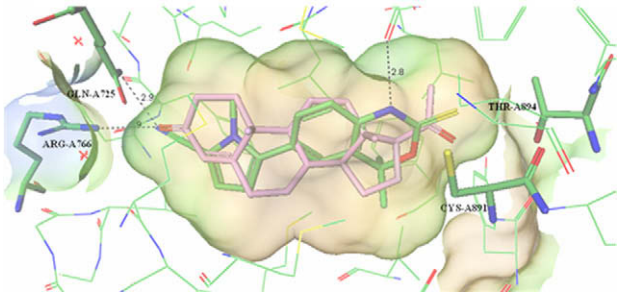

Figure 1. Superposition of Tanaproget and progesterone-bound PR ligand binding domain (LBD). The PR-LBD/Tanoproget (2) structure is shown in green (PDB code 1ZUC) and the PR-LBD/progesterone structure is shown in pink (PDB code 1A28). Hydrogens bonds are shown as black dotted lines.

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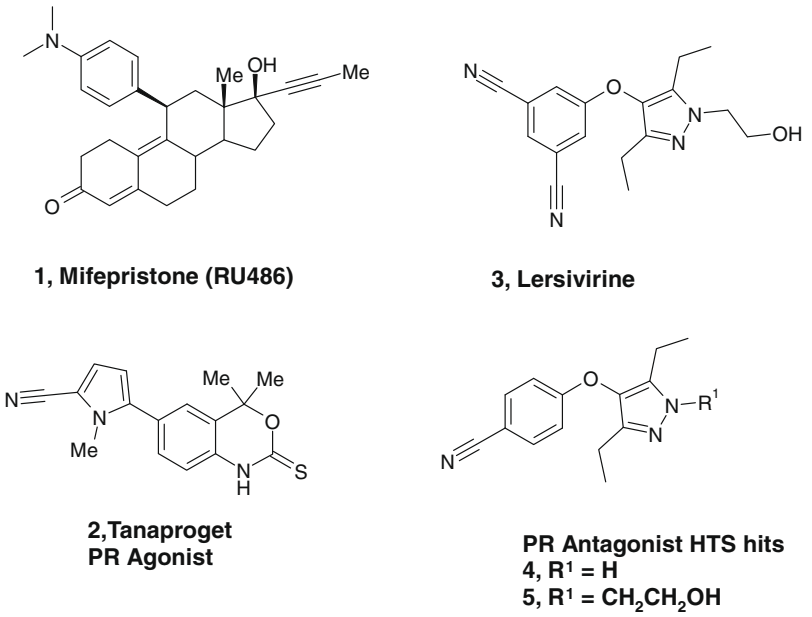
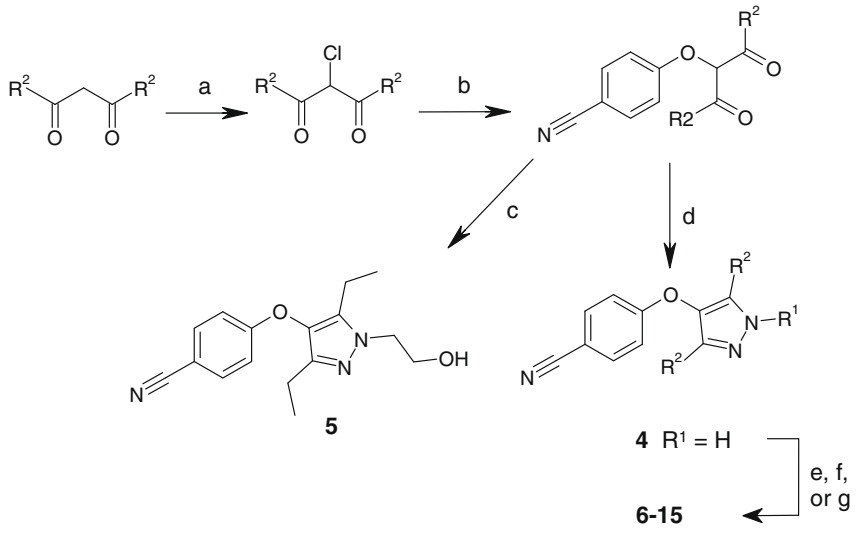


Figure 2. Reference structures.



Scheme 1. Synthesis of phenoxypyrazoles. Reagents and conditions: (a) NCS, TMSCl, DCM, 0–5 °C; (b) 4-cyanophenol, Cs_2CO_3, acetone, reflux, 30–80% for steps (a) and (b); (c) 2-hydroxyethylhydrazine, AcOH, 50–80%; (d) $H_2NNH_2{\cdot}H_2O$, AcOH, 25 °C, 85–95%; (e) R^1-halide, KO*t*Bu, 1,2-DME, 0→25 °C to give **6**, **12** and **13**; (f) ethylbromoacetate, KO*t*Bu, 1,2-DME, 0→25 °C, followed by either hydrolysis using NaOH in MeOH (to give **7**) or aminolysis with either saturated NH_3/MeOH (to give 8), $MeNH_2$/EtOH (to give **9** and **14**) or Me_2NH/MeOH (to give **10**); (g), $ClCH_2SCH_3$, KO*t*Bu, 1,2-DME, 0→25 °C, followed by Oxone, MeOH, H_2O, 25 °C to give **11** and **15** (45–55%).

criteria, we monitored compound ligand-lipophilicity efficiency (LLE = $-\log$ (PR IC_{50}) $-$ $\log D$),[9] in addition to PR potency.

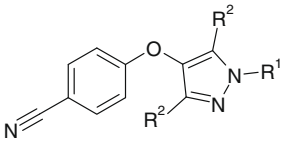
A range of pyrazole analogues with polar N-substituents were prepared according to Scheme 1.

Replacement of the hydroxyl group with a methyl ether (compound **6**, Table 1) gave an increase in PR potency, but not LLE. In addition, the increase in lipophilicity rendered the compound vulnerable to increased metabolism. Oxidation of alcohol **5** to acid **7** resulted in PR inactivity. Pleasingly however, simple amides **8–10** restored PR antagonism, with secondary mono-methyl amide **9** having the highest LLE (4.9). Sulfone **11** also demonstrated a good balance of properties, with an improved potency and LLE profile over compounds **4** and **5**. Aminoethyl **12** and homologated alcohol **13** were detrimental to PR potency.

Metabolite identification studies with **9** and **11** showed that oxidation of the ethyl R^2 groups were occurring during microsomal incubation. Replacement of the ethyl groups with cyclopropyl moieties generated mono-methyl amide **14** and methyl sulfone **15**. These compounds retained the improved PR potency and LLE profiles of compounds **9** and **11** but had improved metabolic stability.

Because of the potency and LLE advantage, coupled with acceptable metabolic stability, the amide **14** (PF-02367982) was selected for further study. It was evaluated in a number of nuclear hormone receptor binding and functional assays, and was found to be highly selective for PR (IC_{50} >10 μM against GR, AR, MR, ER). It was also selective (all IC_{50} values >10 μM) in wide-ligand profiling over a wide range of >70 targets (CEREP, Bioprint™, http://www.cerep.fr). The PR potency of **14** was confirmed (IC_{50} 40 nM) with an alkaline

Table 1
Pyrazole analogues **4**–**15**



Entry	R^1	R^2	PR IC_{50}[a] (nM)	Log *D*[b]	LLE[c]	HLM Cl_{int}[d]	RLM Cl_{int}[d]
4	H	Et	224	3.4	3.3	26	223
5	CH_2CH_2OH	Et	131	3.0	3.9	25	—
6	$CH_2CH_2OCH_3$	Et	13	>3.9	<4.0	>150	>500
7	CH_2CO_2H	Et	>10,000	(*3.0*)	<2	—	—
8	CH_2CONH_2	Et	173	(*2.2*)	4.6	<10	—
9	$CH_2CONHCH_3$	Et	35	2.6	4.9	22	51
10	$CH_2CON(CH_3)_2$	Et	886	(*2.9*)	3.2	35	144
11	$CH_2SO_2CH_3$	Et	56	2.7	4.6	60	—
12	$CH_2CH_2NH_2$	Et	1130	1.1	4.5	17	37
13	$CH_2CH_2CH_2OH$	Et	204	(*3.0*)	3.7	27	65
14	**$CH_2CONHCH_3$**	***c*Pr**	**47**	**2.5**	**4.8**	**<10**	**22**
15	**$CH_2SO_2CH_3$**	***c*Pr**	**71**	**2.6**	**4.5**	**<7**	**<10**

[a] Concentration to inhibit by 50% the fluorescence from the beta-lactamase produced by progesterone (10 nM) stimulation of recombinant human PR expressed in a CHO-MMTV-beta-lactamase cell line. Geometric mean of at least duplicate determinations.
[b] Log *D* measured in octanol:pH 7.4 buffer. If log *D* was not obtained, then (clog *P*) is shown.
[c] Ligand-lipophilicity efficiency (LLE) = −log (PR IC_{50}) − log *D*. *c*Log *P* was used if measured log *D* was not available.
[d] Cl_{int} is the intrinsic metabolic clearance in microsomes, in μl/min/mg of microsomal protein (HLM is human, RLM is rat).

Table 2
Pharmacokinetics of **14** dosed at 0.2 mg/kg iv and po

Species	Rat	Dog
Cl[a] (mL/min/kg)	27	3
Vd[b] (L/kg)	0.9	0.4
T (h)[c]	0.9	2.1
F[d] (%)	78	90

[a] In vivo clearance after iv dosing.
[b] Volume of distribution at steady state after iv dosing.
[c] Half-life after iv dosing.
[d] Bioavailability after oral dosing.

phosphatase assay using a human breast cancer cell line (T 47D) that endogenously expresses PR.

The pharmacokinetics of amide **14** was determined in rat and dog (Table 2). Amide **14** was progressed as a prototype non-steroidal PR antagonist into in vivo pharmacology studies, and was shown to block progesterone-induced arborisation of rabbit and cynomolgus macaque endometrium at 3 mg/kg po *qd* and at 2.5 mg/kg po *bid*.[10] Taken together, these data confirmed the in vivo pharmacological credentials of amide **14** as a specific PR antagonist and support the utility of this class of agents in the treatment of gynecological conditions such as endometriosis and uterine fibroids.[10]

In summary, the optimisation of a novel series of non-steroidal progesterone receptor antagonists using functional activity and LLE to guide compound selection is described. Starting with the HTS Hit **4** (R^1 = H), we introduced polar side chains at R^1 to improve potency and selectivity, lower lipophilicity and increase LLE. Changing the metabolically vulnerable ethyl groups at R^2 to cyclopropyls improved the overall metabolic stability. Compound **14** was progressed as a prototype non-steroidal PR antagonist into in vivo pharmacology studies, and was shown to block progesterone-induced arborisation of rabbit and cynomolgus macaque endometrium.[10] Sulfone **15** was subject to further modifications to optimise PR potency and physicochemical properties, and will be reported in due course.

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References and notes

1. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schuetz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M. *Cell (Cambridge, MA)* **1995**, *83*, 835.
2. (a) Spitz, I. M. *Curr. Opin. Obst. Gyn.* **2009**, *21*, 318; (b) Ioffe, O. B.; Zaino, R. L.; Mutter, G. L. *Mod. Path.* **2009**, *22*, 450; (c) Moller, C.; Hoffmann, J.; Kirkland, T. A.; Schwede, W. *Expert Opin. Investig. Drugs* **2008**, *17*, 469.
3. Brown, A.; Cheng, L.; Lin, S.; Baird, D. T. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 63.
4. Brogden, R. N.; Goa, K. L.; Faulds, D. *Drugs* **1993**, *45*, 384.
5. Fensome, A. et al *J. Med. Chem.* **2005**, *48*, 5092 (PDB code 1ZUC).
6. Burt, C.; Corbau, R.; Mowbray, C. E.; Perros, M.; Tran, I.; Stupple, P. A.; Webster, R.; Wood, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5599.
7. Corbau, R.; Hawes, M.; Jones, L. H.; Mills, J. E.; Mowbray, C. E.; Perros, M.; Selby, M. D.; Stupple, P. A.; Webster, R.; Wood, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5603.
8. Mowbray, C. E.; Burt, C.; Corbau, R.; Gayton, S.; Hawes, M.; Perros, M.; Tran, I.; Price, D. A.; Quinton, F.; Selby, M. D.; Stupple, P. A.; Webster, R.; Wood, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5857.
9. (a) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881; The same concept was independently proposed by researchers at Pfizer and termed LipE. (b) Ryckmans, T.; Edwards, M. P.; Horne, V. A.; Monica Correia, A.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. *Bioorg. Med. Chem. Lett.* **2009**, *15*, 4406.
10. de Giorgio-Miller, A.; Bungay, P.; Tutt, M.; Owen, J.; Goodwin, D.; Pullen, N. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 78.