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2-Amino-9-aryl-3-cyano-4-methyl-7-oxo-6,7,8,9-tetrahydropyrido[2′,3′:4,5]thieno[2,3-*b*]pyridine derivatives as selective progesterone receptor agonists

Yonghui Wang [a,*], Chaya Duraiswami [a], Kevin P. Madauss [b], Thuy B. Tran [b], Shawn P. Williams [b], Su-Jun Deng [b], Todd L. Graybill [a], Marlys Hammond [c], David G. Jones [b], Eugene T. Grygielko [c], Jeffrey D. Bray [c], Scott K. Thompson [c]

[a] *R&D, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[b] *R&D, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA*
[c] *R&D, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*

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ABSTRACT

High throughput screening of the corporate compound collection led to the identification of a novel series of 2-amino-9-aryl-3-cyano-4-methyl-7-oxo-6,7,8,9-tetrahydropyrido[2′,3′:4,5]thieno[2,3-*b*]pyridine derivatives as selective PR agonists. Initial SAR exploration leading to potent and selective agonists **9** and **11**, X-ray crystal structure of **9** bound to PR-LBD and preliminary developability data are described.

The progesterone receptor (PR) is a member of the nuclear receptor super-family and is a ligand dependent transcription factor.[1] PR and its endogenous ligand, progesterone (P4, **1** in Fig. 1), play an important role in uterine ovulation and implantation.[2] Synthetic PR ligands are commonly used in hormone replacement therapy, oral contraception, and treatment of endometriosis and uterine fibroids.[3] Endometriosis is a painful disease characterized by the growth of endometrial tissue outside the uterus. It is associated with dysmenorrhea, chronic pelvic pain, fatigue and a host of other symptoms. Progestins such as P4 and medroxyprogesterone acetate (MPA, **2**) act by opposing estrogen-stimulated endometrial tissue proliferation in the treatment of endometriosis. Some side-effects associated with this therapy such as weight gain, break through bleeding, and mood disturbances, are associated with the full agonist activity or poor steroid receptor selectivity of the progestins.[3–5] There has been great interest in development of selective non-steroidal PR agonists, and several small molecule non-steroidal PR agonists have been reported in the literature (e.g., **3**–**5**).[6,7] Herein we describe discovery of a novel series of 2-amino-9-aryl-3-cyano-4-methyl-7-oxo-6,7,8,9-tetrahydropyrido[2′,3′:4,5]thieno[2,3-*b*]pyridine derivatives as selective PR agonists.

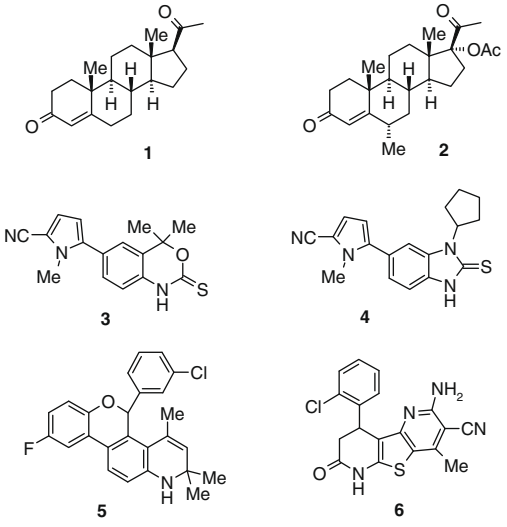

Figure 1. Steroidal (**1**, **2**) and non-steroidal (**3**–**6**) PR agonists.

* Corresponding author at present address: Medicinal Chemistry, R&D China, GlaxoSmithKline, 898 Halei Road, Shanghai 201203, China. Tel.: +1 86 21 61590761; fax: +1 86 21 61590730.
E-mail address: yonghui.2.wang@gsk.com (Y. Wang).

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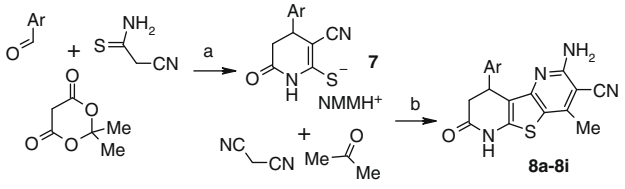
High throughput screening (HTS) of the corporate compound collection using a PR binding assay[8] led to the identification of the tricyclic pyridine **6** as a hit with a PR pIC_{50} of 6.7. The compound was subsequently found to be active in a PR CV-1 agonism assay[9] and in the more definitive T47D alkaline phosphatase cellular assay[10] with a pEC_{50} of 6.2 and 7.1, respectively. Furthermore, compound **6** showed great selectivity for PR over AR (AR pIC_{50} <5.0 in the binding assay, AR pEC_{50} <5.0 in the CV-1 agonism assay).

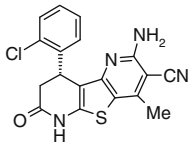
In order to assess the tractability of the HTS hit, several close analogs of **6** were synthesized via multi-component condensation of malononitrile, acetone and *N*-methylmorpholinium tetrahydropyridine-6-thiolates (**7**). The required tetrahydropyridine-6-thiolates **7** were generated in situ from arylaldehydes, cyanothioacetamide and Meldrum's acid in the presence of *N*-methylmorpholine (NMM) (Scheme 1).[11] These tricyclic pyridine analogs (**8a–8i**)[12] were tested in the PR binding and the CV-1 agonism assays to provide an initial SAR.

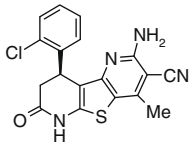
As shown in Table 1, the nature and position of substituents on the 9-aryl moiety of these tricyclic compounds had a significant effect on PR potency. The analogs with substituents on the ortho-position of phenyl (**6, 8b–8d**) increased binding affinities in the order of 2-OMe < 2-Me < 2-F < 2-Cl. Introduction of a second halogen on the 3-, 4-, 5-, or 6-position of 2-Cl-phenyl group attenuated its PR binding affinity (**8f–8i**).

The synthetic route described in Scheme 1 provided analogs, such as compound **6**, as a 1:1 mixture of enantiomers owing to the chiral center on 9-position of the tricyclic ring. Computational docking studies[13] were conducted to predict the more active isomer and postulate plausible binding modes for both isomers within PR. Docking studies predicted the *S*-isomer to be the more active enantiomer as the lactam NH group of the *S*-isomer could form a hydrogen bond with Asn-719 while the *R*-isomer could not due to steric constraints. The two enantiomers of the compound **6** were then separated by chiral HPLC and absolute stereochemistry of each isomer was subsequently determined by VCD analysis.[14] Both *S*- and *R*-isomers, **9** and **10**, were tested in the PR binding and the CV-1 agonism assays. Consistent with the computational docking predictions, the *S*-isomer (**9**) was found to be much more potent (PR binding pIC_{50} = 6.8 and CV-1 pEC_{50} = 6.7) than the *R*-isomer (**10**, PR binding pIC_{50} = 5.2, CV-1 pEC_{50} <5.0). Compound **9** also showed good activity in the T47D alkaline phosphatase cellular assay with a pEC_{50} of 7.4.



Scheme 1. Reaction conditions: (a) NMM, ethanol, rt then reflux; (b) ethanol, reflux.

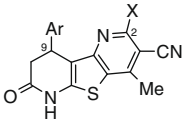




To investigate the binding mode of the tricyclic pyridines, the crystal structure of **9** bound to the PR-LBD (ligand binding domain) was solved to a resolution of 2.24 Å.[15] Figure 2 displays an overlay of progesterone **1** and compound **9** bound to PR-LBD and shows that compound **9** occupies the same binding pocket as PR's natural ligand, progesterone. The charged clamp region of Q725 and R766 overlays well with little notable movement. The 2-chlorophenyl moiety pushes the angle of the ligand towards M909, which is a residue associated with decreased agonism.[16] The ligand slightly moves M909, but the Cα carbon is not moved from the agonist position in progesterone. The distance from the amide nitrogen in compound **9** and the oxygen on N719 is 2.7 Å, which confirmed the docking predictions for the *S*-isomer.

Both the docked binding mode and crystal structure of **9** suggested that the binding affinity might be enhanced by replacement of the 2-amino group with a less polar group. To test this hypothesis, both 2-chloro-analog **11** and 2-hydroxyl-analog **12** were synthesized from **6** via Sandmeyer reaction (Scheme 2).[17] Compound **11** demonstrated greater potency than compound **6** in both the PR binding and the CV-1 assays (pIC_{50} = 7.3, pEC_{50} = 9.1, respectively) while compound **12** found to be inactive in both assays. In addition, compound **11** was found to be more potent than **6** in T47D alkaline phosphatase cellular assay with a pEC_{50} of 8.6. Fur-

Table 1
PR binding, CV-1 agonism and T47D alkaline phosphatase data for the tricyclic pyridine compounds



Compds	Ar	X	PR binding pIC_{50}[a]	PR CV-1 pEC_{50}[a]	AR binding pIC_{50}[a]	PR T47D pEC_{50}[a] (% P4)
8a	Ph	NH_2	5.7	<5.0	<5.0	–
8b	2-F–Ph	NH_2	6.2	5.4	–	6.4 (47)
6	2-Cl–Ph	NH_2	6.7	6.2	<5.0	7.1 (80)
8c	2-Me–Ph	NH_2	5.9	<5.0	<5.0	–
8d	2-OMe–Ph	NH_2	<5.0	<5.0	–	–
8e	3-OMe–Ph	NH_2	<5.0	< 5.0	–	–
8f	2,3-Di-Cl–Ph	NH_2	6.1	<5.0	<5.0	–
8g	2,4-Di-Cl–Ph	NH_2	6.1	<5.0	<5.0	–
8h	2,5-Di-Cl–Ph	NH_2	6.0	<5.0	<5.0	–
8i	2-Cl-6-F–Ph	NH_2	5.9	5.4	<5.0	6.1 (13)
9	(*S*)-2-Cl–Ph	NH_2	6.8	6.7	<5.0	7.4 (83)
10	(*R*)-2-Cl–Ph	NH_2	5.2	<5.0	<5.0	–
11	2-Cl–Ph	Cl	7.3	9.1	5.7	8.6 (116)
12	2-Cl–Ph	OH	<5.0	<5.0	<5.0	–

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

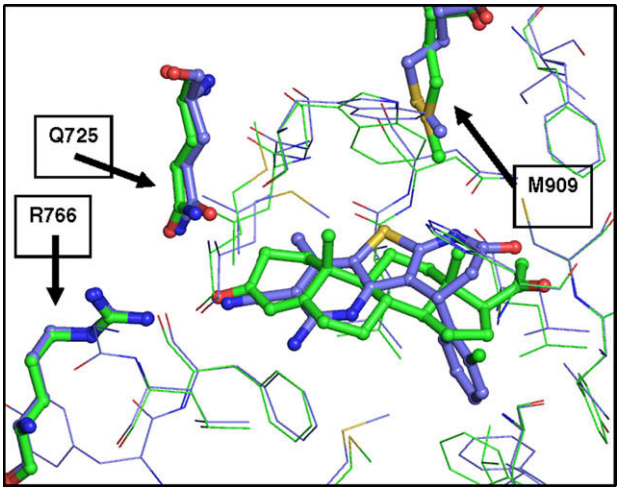
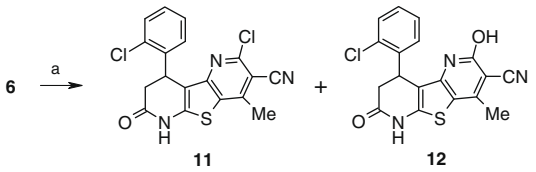


Figure 2. Superposition of 9 (blue) and 1 (green) crystal structures. Enzyme backbone and key backbone residues are shown in the color of the ligand to which they correspond.



Scheme 2. Reagents: (a) $NaNO_2$, HCl, H_2O.

ther, compound **11** demonstrated good selectivity for PR over AR (AR pIC_{50} = 5.7 in the binding assay, pEC_{50} <5.0 in the CV-1 agonism assay). Docking studies showed that the chloro group in **11** can form good hydrophobic interactions with Leu-763, Val-760 and Met-801 as well as an electrostatic interaction with the sulfur of Met-801. The amino group in **6** can also form a weak hydrogen bond with Met-801, however, this interaction is absent with the hydroxyl analog **12** or its tautomer.

Compound **6** was evaluated in general selectivity, CYP450, hERG, and in vitro and in vivo PK studies. Compound **6** demonstrated high selectivity in a CEREP screen of 50 receptors, transporters and ion-channels (<10% of inhibition at 1 μM against all 50 targets in the panel). In a CYP450 screen, compound **6** showed no or little inhibition for 4 major P450 isozymes (pIC_{50} <5.0 for 1A2, 2C19, 2D6, 3A4) and moderate inhibition for 2C9 (pIC_{50} = 5.9). Compound **6** did not show activity in a hERG binding assay. In vitro human and rat liver microsome stability studies revealed moderate intrinsic clearance for compound **6** (human: 0.019 mL/min/mg liver; rat 0.083 mL/min/mg liver). Compound **6** also demonstrated moderate clearance and oral bioavailability (Clb = 24 mL/min/kg, F = 36%, $T_{1/2}$ = 1.1 h, V_{dss} = 2.5 L/kg) in in vivo mouse PK studies (0.5 mg/kg iv, 1.0 mg/kg po). These PR potency, selectivity and initial developability data suggest that this series constitutes a reasonable starting point for further medicinal chemistry investigation.

In summary, we have identified a novel series of tricyclic pyridines as non-steroidal small molecular PR agonists. Initial SAR exploration led to potent and selective PR agonists such as **9** and **11**. The crystal structure of **9** bound to the PR-LBD was solved and provided direction for subsequent lead optimization.

Acknowledgements

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8. The PR binding assay was performed according to the manufacturers protocol (PR Competitor Assay Kit, Red—(Invitrogen—Product No. P2962)) with minor amendments. Briefly, 40 nM PR-Ligand Binding Domain, 2 nM Fluormone PL Red and 1 mM DTT were dissolved and mixed in Complete PR RED Buffer supplemented with 2 mM CHAPS. Ten microlitres of the mix were dispensed to each well of Greiner low volume plates, containing compounds at the required concentration. The plates were spun for 1 min at 200 g, covered to protect the reagents from light, and then incubated at room temperature for approximately 2 h. Plates were read on an Acquest using a 530–25 nm excitation and 580–10 nm emission interference filter and a 561 nm Dichroic mirror.
9. The CV-1 functional assay measures compound-mediated interaction of the PR-B isoform with the MMTV luciferase reporter to calculate compound potency and efficacy in BacMam transduced, progesterone-stimulated (4 nM) CV-1 cells. The antagonist response is expressed as a pIC50 (RU-486 pIC_{50} = 9.8, 100% efficacy).
10. The T47D alkaline phosphatase PR agonist assay was performed according to Ref.[16]. In short, 120 μL of a T47D cell suspension was seeded into a 96-well plate and cells were allowed to attach to the plate overnight. On the next day, the cells were treated with compound and incubated overnight. On the following day, 100 μL of pNPP-SPAP was added and there were allowed to stand in the dark for 2 h. Optical density was then measured at a wavelength of 405 nm on a plate reader.
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12. All tricyclic pyridines were prepared and tested as 1:1 mixtures of two enantiomers unless stated otherwise.
13. The co-crystal structure of progesterone in PR (PDB code 1A28) was used as the starting point for the initial docking calculations using the program, Flo+, version 0203. Residues within a 15 Å sphere of progesterone in the binding site were retained. The mcdock algorithm, which relies on a Monte Carlo perturbation/fast search/energy minimization algorithm was used. Only those residues that were known to be flexible from other crystal structures were made flexible during the energy minimization step. Two thousand steps of perturbation were performed and the 25 top-ranked poses were retained. Visual inspection of the interactions made by the ligand within the active site and the relative strain energies of the ligand calculated using Flo+ in each pose were used to determine the best docked pose. Also see: McMartin, C.; Bohacek, R. S. *J. Comput. Aided Mol. Des.* **1997**, *11*, 333.
14. (a) The two enantiomers of the compound **6** were separated by chiral HPLC via the following (SFC) conditions: 20 μm, 10 mm AD column, 10 mL/min total flow, 35% MeOH/$CHCl_3$ (4:1) and 65% CO_2, 140 bar, 40 °C, UV @ 280 nm; (b) Stereochemistry of **9** and **10** was assigned by Vibrational Circular Dichroism (VCD) analysis of both enantiomers.
15. The crystal of PR LBD bound to compound **9** had a data set collected at 17-ID of the APS, and were solved using the programs HKL2000, CCP4 (MOLREP), COOT, and REFMAC.
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17. Synthesis of **11** and **12**: A solution of 375 mg (5.43 mmol) of $NaNO_2$ in water (5 mL) was slowly added to a suspension of 200 mg (0.54 mmol) of **6** in concd HCl (10 mL) with stirring over a period of 1 h. The mixture was stirred at rt for another 12 h and then filtered. The filtered solid was washed with water and dried under vacuum. The HPLC purification of the crude products yielded 52 mg of **11** and 36 mg of **12**. Compound **11**: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.71 (s, 3H) 3.39 (dd, J = 16.67, 8.34 Hz, 2H) 4.94 (dd, J = 8.34, 1.77 Hz, 1H) 6.70 (dd, J = 7.71, 1.64 Hz, 1H) 7.11–7.24 (m, 1H) 7.29 (td, J = 7.64, 1.64 Hz, 1H) 7.55 (dd, J = 7.83, 1.26 Hz, 1H) 11.58 (s, 1H); MS m/e 388.1 (MH^+).