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Novel progesterone receptor modulators: 4-Aryl-phenylsulfonamides

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ABSTRACT

We have developed a new series of progesterone receptor modulators based upon the 4-aryl-phenylsulfonamide. Initial work in the series afforded potent compounds with good properties, however an advanced intermediate proved to be genotoxic in a non-GLP Ames assay following metabolic activation. We subsequently solved this problem and identified advanced leads which demonstrated oral efficacy in rhesus monkey pharmacodynamic and kinetics models.

The progesterone receptor (PR) is a member of the steroid receptor sub-family of the nuclear hormone receptor super-family, a group of ligand-dependent nuclear transcription factors and plays an important role in the female reproductive system.[1] Regulation of the receptor through it's ligand binding domain and endogenous hormone progesterone (P_4) has proven to be useful for effecting contraception and for providing hormonal therapy during the menopause.[2] Steroidal antagonists have been studied in the clinic as novel therapeutics for treating women with endometriosis[3–5] and uterine fibroids.[6–8] Of particular interest is the steroid asoprisnil **2** which reduced bleeding and uterine volume in a fibroid population (Chart 1).[9]

Previously we have published several series of non-steroidal progesterone receptor modulators: benzoxazinones, benzimidazolinones and oxindoles, typified by compounds **3**, **4** and **5**, Chart 2.[10–12]

The most advanced of these derivatives was the oxindole PRA-348 **6**,[13] which was the starting point for the current work, Scheme 1.

We wanted to explore the consequences of opening the heterocyclic ring to afford the open chain amides **7** and sulfonamides **8**, which are the focus of this report. We had previously established the 5-cyano-pyrrol-2yl functional group as a privileged steroid A-ring mimetic, and kept this functionality as a constant.

The amide **7** and sulfonamide target molecules **8** were prepared according to Scheme 2. 1-Methyl pyrrole **9** was reacted with chlorosulfonyl isocyanate followed by a DMF quench to afford the nitrile **10** in 69% yield, Scheme 2. Compound **10** was then lithiated (LDA in THF at −78 °C) and reacted with tri-isopropyl borate to afford, after work-up, the boronic acid **11**. Compound **11** was coupled under palladium catalysis ($Pd_2(dba)_3$/$P(tBu)_3$, KF, THF) with appropriately substituted anilines **12** to afford the biaryl derivatives **13**. Yields were typically good to excellent (57–98%), except in the case of 4-bromo-3-fluoroaniline (27%). Reaction of the intermediate anilines **13** with functionalized acid chlorides **14** afforded the amides **7**, while sulfonyl chlorides **15** then provided the target compounds **8**. Yields were generally in the moderate to high range, except for the *iso*-propyl sulfoanmide **23**, which was very low (2%).

Compounds were assessed for functional antagonism of progesterone in the human T47D breast cancer cell line, using alkaline phosphatase activity as the readout.[14] The T47D cell line endogenously expresses both A and B forms of the progesterone receptor. Selectivity between the isoforms was not determined.

We initially prepared compounds from both the amide **7** and sulfonamide **8** classes, Table 1. It was apparent that both functionalities support progesterone antagonism in the T47D cell line, however it was clear that the sulfonamides were considerably more potent. For example, the acetamide **16** was approximately 10-fold less active than the methane sulfonamide **17** (T47D IC_{50} = 75 nM and 8 nM, respectively). This was also true for the ethyl and

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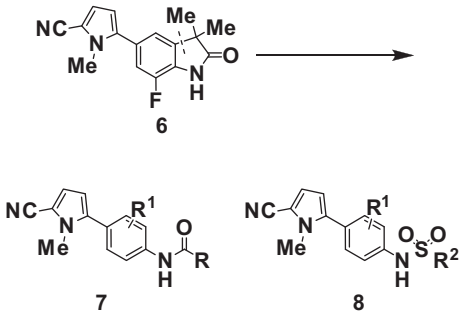
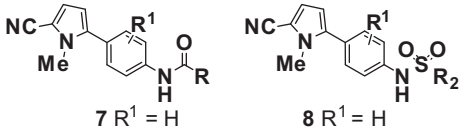


Scheme 1. PRA-348 **6** and ring opened derivatives **7** and **8**.

n-propyl derivatives, where the amides **18** and **20** were again approximately 8-fold less active than their sulfonamide counterparts **19** and **21** (IC_{50} = 9 and 10 nM, respectively). Interestingly in the amide series the *iso*-propyl derivative **22** lost further activity (IC_{50} = 167 nM) whilst the sulfonamide **23** was one of the most potent in the series (IC_{50} = 1.5 nM). Having observed this general trend, efforts were concentrated on the sulfonamide series, which were found to be poorly tolerant of substituted alkyl R_2 groups, e.g. the trifluoroethyl derivative **25** (IC_{50} = 58 nM). Considerable activity was lost with larger substituents, for example the aryl sulfonamides **25** and **26**, which showed only modest antagonism of progesterone induced alkaline phosphatase activity at the highest dose tested (3 μM).

An early concern that we had with compounds from this series was the presence of the embedded aniline, which could be a potential mutagen following hydrolysis of the sulfonamide bond. The sulfonamide **19** was chosen to assess this risk. The pharmacokinetic profile of **19**, and of its metabolites (Scheme 3) was studied in male rats, Table 2. Following a single oral administration of **19**, significant levels of the aniline **13** (C_{max} = 633 ng/mL or 3.2 μM) and the secondary metabolite **16** (C_{max} = 180 ng/mL or 0.7 μM) were found in circulation. Parent sulfonamide **19** was still the predominant species (C_{max} = 1926 ng/mL or 6.7 μM), however,

Table 1
Functional activity data in the T47D alkaline phosphatase assay for amides **7** and sulfonamides **8**, where R_1 = H



Compound	Template **7** or **8**	R^2	Alk. phos. IC_{50}[a] (nM)
2			0.3
16	7	Me	75
17	8	Me	8
18	7	Et	76
19	8	Et	9
20	7	*n*-Pr	78
21	8	*n*-Pr	10
22	7	*i*-Pr	167
23	8	*i*-Pr	1.5
24	8	$-CH_2CF_3$	58
25	8	4-tolyl	55% Inhib at 3 μM
26	8	4-C_6H_4-*i*-Pr	54% Inhib at 3 μM

[a] 50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations.

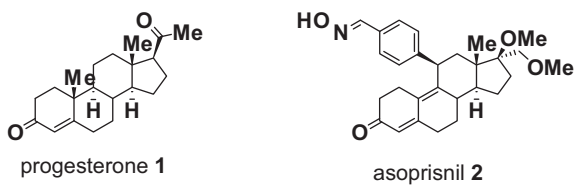


Chart 1. Structures of progesterone **1** and asoprisnil **2**.

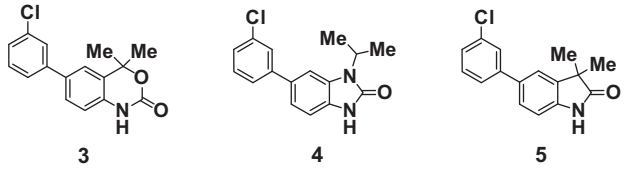
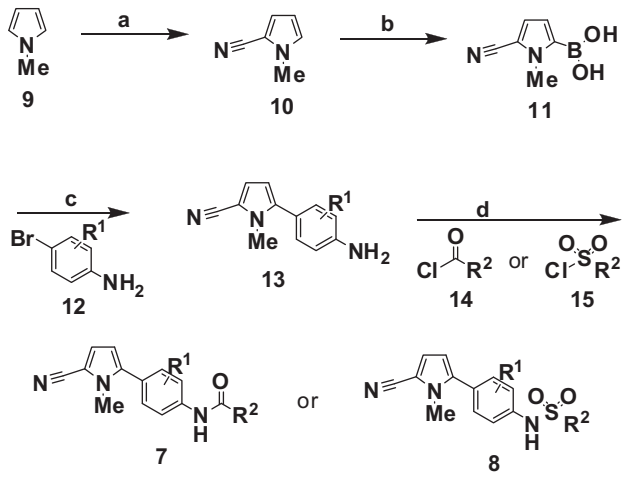


Chart 2. Non-steroidal heterocyclic PR modulators **3**–**5**.

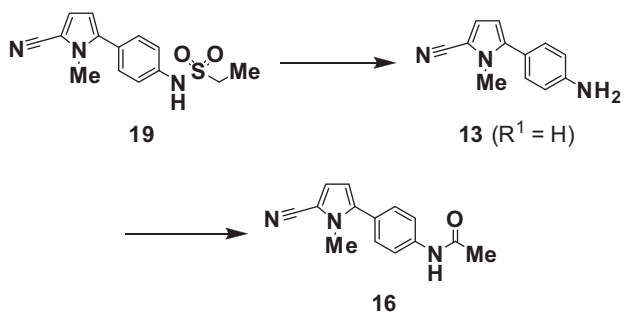


Scheme 2. Synthesis of amides **7** and sulfonamides **8**. Reagents and conditions: (a) Chlorosulfonyl isocyanate, MeCN −78 °C, then DMF; (b) LDA, THF −78 °C, then $B(OiPr)_3$; (c) KF, $Pd_2(dba)_3$, $P(tBu)_3$, THF; (d) pyridine.

the substantial amounts of the aniline and the acetamide warranted further investigation.

In the non-GLP Ames assay compound **19** was found to be non-reactive in the absence of metabolic activation, but gave a positive mutagenicity response in the presence of S-9 activation. A discrete study with the aniline **13** showed the same behavior.

Having established the aniline as the culprit species in the Ames test, we then sought to resolve the problem. As the mutagenic result was only positive upon metabolic activation, we decided to

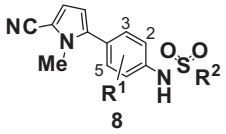


Scheme 3. In vivo metabolic fate of **19** in male rats.

Table 2
Selected pharmacokinetic parameters of compounds **19**, **13** and **16** in male rats following a single oral dose (20 mg/kg) of **19**

Compound	Cmax (ng/mL)	$t_{1/2}$ (h)	$AUC_{0\text{-}inf}$ (ng h/mL)
19	1926	2.5	12267
13	633	2.5	3084
16	180	5.0	1640

Table 3
Functional activity data in the T47D alkaline phosphatase assay for sulfonamides **8** where R_1 is an electron withdrawing group

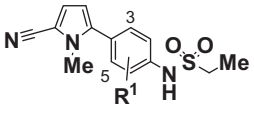


8

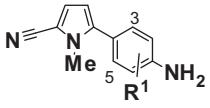
Compound	R_1	R_2	Alk. phos. IC_{50}[a] (nM)
27	2-F	Et	30
28	2-OCF3	Et	36
29	2-CN	Me	>3000
30	3-F	Et	6
31	3-F	Me	8
32	3-CF3	Et	12
33	3-CN	Et	128
34	3-F, 5-F	Et	12
35	2-F, 5-F	Et	57

[a] 50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations.

Table 4
Mutagenicity data determined in the non-GLP Ames test for compounds **31**, **32** and **34** and their parent anilines



31 R^1 = 3-F, 5-H
32 R^1 = 3-CF_3, 5-H
34 R^1 = 3-F, 5-F



13

Sulfonamide	**31**	**32**	**34**
Mutagenic[a]	NA	ND	NA
Parent aniline	**13**, R^1 = 3-F	**13**, R^1 = 3-CF3	**13**, R^1 = 3-F, 5-F
Mutagenic	NA	NA	NA

[a] NA denotes Not Active, ND denotes not determined.

evaluate the effect of de-activating the aniline nitrogen through adding electron withdrawing groups to the aryl ring, Table 3.

Substitution of the aryl ring was tolerated but within well defined boundaries, Table 3. For example the 2-fluoro **27** and 2-trifluoromethoxy **28** derivatives of compound **19** were reasonably potent antagonists in the T47D alkaline phosphatase assay (IC_{50} = 30 and 36 nM for **27** and **28**, respectively), however the nitrile **29** was very weak (IC_{50} >3 μM). The potency was regained at the 3-position using a fluoride substituent, with either the ethane

Table 5
Steroid receptor selectivity[a] in vitro for compound **34** and comparator asoprisinil **2**

Sulfonamide IC_{50} (nM)	**2**		**34**	
	Agonist	Antagonist	Agonist	Antagonist
Progesterone	IA	0.3	~20%@ 3 μM	11.7
Androgen	IA	9.0	IA	100
Glucocorticoid	IA	120	>10,000	70% @ 1 μM
Mineralocorticoid	IA	1600	IA	60% @ 1 μM
Estrogen	IA	2000	IA	IA

[a] Steroid receptor cross-reactivity data from cellular reporter assays.[13]

Table 6
Pharmacokinetic properties of compound **34**

34	Cyno.	Rat
Dose mg/kg (iv)	2	2
Cl (ml/min/kg)	2	2.6
V_{ss} (l/kg)	0.3	0.5
$t_{1/2}$ (h)	2.8	2.0
AUC_{inf} (ng h/ml)	18035	12977
Dose mg/kg, (p.o.)	2	2
C_{max} (ng/ml)	5102	2805
$T_{1/2}$ (h)	2.7	2.3
AUC_{inf} (ng h/ml)	13679	15164
F (%)	69	98.5

30 or methane sulfonamides **31** (IC_{50} = 6 and 8 nM, respectively). The trifluoromethyl derivative **32** was only slightly weaker (IC_{50} = 12 nM), however the 3-nitrile compound was much less active (IC_{50} = 128 nM). This latter observation would imply that the nitrile derivatives are too electron deficient with respect to the parent system or fluoride derivatives. Steric limitations are apparently not the limiting factor in either the 2- or 3-positions due to the activity of the 2-OCF_3 compound **28** and 3-CF_3 **32**. Additional substitution with fluorine was also tolerated well in the 3,5-position (**34**, IC_{50} = 12 nM), but not in the 2,5-difluoro system (**35**, IC_{50} = 57 nM) in concordance with the activity of compound **27**.

We then assessed the key mutagenicity question of these derivatives, Table 4. Addition of electron withdrawing groups to the parent anilines **13** in the 3 and or 5-positions was sufficient to mitigate the Ames response of the parent aniline **19,** presumably through inhibiting the metabolic transformation of the aniline to a reactive nitroso species. Consequently the sulfonamides **31**, **32** and **34** were also Ames negative as well.

Having addressed this liability, compound **34** was selected for further evaluation. The steroid receptor cross-reactivity of compound **34** was assessed in cellular reporter assays, Table 5. The closest antagonist cross-reactivity noted was against the androgen receptor (IC_{50} = 100 nM). Weak high concentration agonist activity was noted at the progesterone rector, otherwise the compound had a clean profile. The pharmacokinetic profile was assessed in both rats and cynomolgus monkeys, Table 6. Following iv administration of compound **34**, in both species, the compound showed low clearance, low volume of distribution and high exposure (*ca.* 18 and 13 μg h/mL in cyno. monkeys and rats, respectively). The half life was moderate, 2.8 and 2.0 h for cyno. monkey and rat respectively, following a 2 mg iv dose. Following oral dosing, the kinetics showed high exposure, a moderate half life ($t_{1/2}$ = 2.7 and 2.3 h in monkeys and rats, respectively) with good to excellent bioavailability (F = 69 and 98% in cyno. monkeys and rats, respectively). The kinetic profile is in accord with a molecule that displays high protein binding: at 10 μM, compound **34** has 0.3% free drug in human plasma protein and 1.9% free drug in rat plasma protein.

The efficacy of sulfonamide **34** was assessed in a rhesus macaque pharmacodynamic model of menses induction.[15] Ovariectomized

rhesus monkeys were cycled artificially with 28 days of exposure to estradiol (silastic implant). On day 14 they received a 14 day progesterone implant to simulate the follicular phase of the menstrual cycle. In this paradigm an active progesterone receptor antagonist (e.g. mifepristone) will block the effect of the progesterone implant and induce early menstruation, usually within 3 days of administration. Following oral administration (5 mg/kg/day) sulfonamide **34** induced early menstruation in all of the monkeys tested ($n = 3$). In a satellite PK study, ovariectomized female rhesus monkeys received the same dose of compound **34**. The circulating drug reached a C_{max} = 2888 ng/mL (8.8 μM) with high exposure ($AUC_{0\text{-last}}$ = 28485 ng h/mL). The estimated C_{av} ($AUC_{0\text{-last}}$/24 h) = 1187 ng/mL or 3.6 μM. The free drug available at this concentration would be approximately 11 nM (using the human protein binding value), consistent with the IC_{50} observed in T47D cells.

In summary we have developed a new series of progesterone receptor modulators, using our older chemical equity as the starting structural motif. Initial examples from the series were mutagenic in a non GLP Ames test, following metabolic activation. This finding was circumvented by modulating the electronics of the aniline moiety which presumably prevented the aniline metabolite from oxidizing to the reactive species. Compound **34** was further profiled in pharmacokinetic models in rats and non-human primates, with exposure sufficient to afford efficacy in a non-human primate model of the menstrual cycle.

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