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Novel 5-Aryl-1,3-dihydro-indole-2-thiones: Potent, Orally Active Progesterone Receptor Agonists

Andrew Fensome,[a,*] Marci Koko,[a] Jay Wrobel, Puwen Zhang,[a] Zhiming Zhang,[b] Jeffrey Cohen,[b] Scott Lundeen,[b] Kelly Rudnick,[b] Yuan Zhu[b] and Richard Winneker[b]

[a]*Chemical Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[b]*Women's Health Research Institute, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

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Abstract—During the course of our studies on 3,3-disubstituted-5-aryloxindoles derived progesterone receptor (PR) antagonists we discovered that changing the amide funtionality to a thio-amide resulted in compounds displaying potent PR agonist activity. In this communication, the synthesis, structure activity relationships (SAR) and in vivo activity of various 5-arylthio-oxindoles will be discussed.
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The progesterone receptor (PR) is a member of the steroid receptor sub-family of the nuclear hormone receptor super-family, a group of nuclear transcription factors (Chart 1).[1] Progesterone **1**, the endogenous ligand for the PR, is involved in the control of ovulation and preparation of the uterus for implantation and maintenance of pregnancy. Clinically, progestin agonists are mainly used in contraception and hormone replacement therapy, typically co-administered with an estrogen.

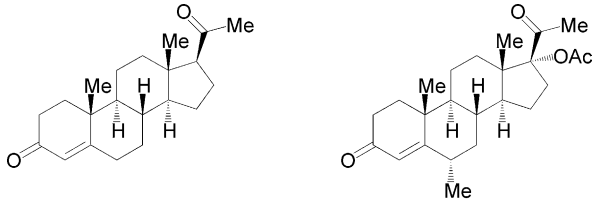

Chart 1. Steroid PR agonists.

Over the last few decades considerable synthetic effort has been aimed towards preparing new steroidal PR agonists which are more potent and bioavailable than progesterone. One of the most studied is medroxyprogesterone acetate (MPA) **2**.[2]

More recently, increasing interest has focussed on identifying and characterizing non-steroidal PR agonists,[3] (Chart 2).

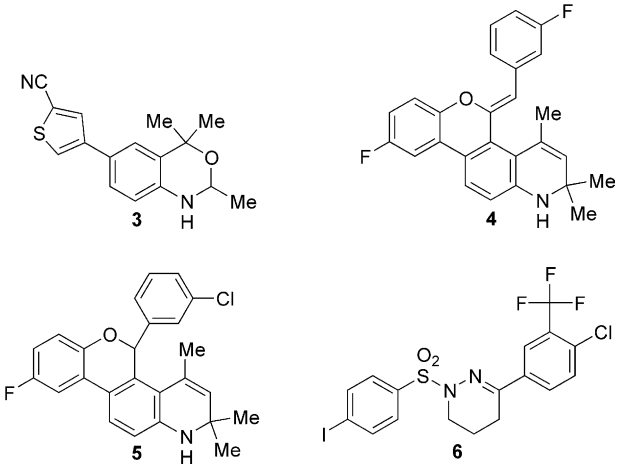

Chart 2. Non-steroidal PR agonists.

During the course of SAR studies with oxindole derived PR antagonists **I**,[4] we discovered that transposing the oxindole carbonyl group to a thio-carbonyl moiety **II**

*Corresponding author. Fax: +1-484-865-9398; e-mail: fensoma@wyeth.com

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turned the molecules into potent selective PR agonists (Scheme 1).

Chemistry

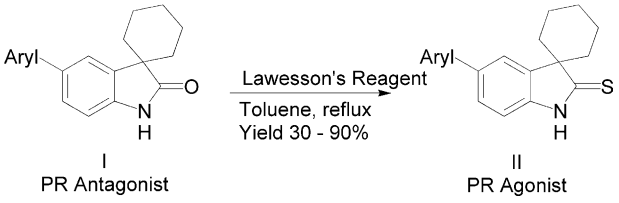
The target thio-oxindole derivatives **II**, were synthesized by treatment of the parent oxindole antagonists **I** with Lawesson's reagent in refluxing toluene (Scheme 1). Reactions were carried out in parallel in sealed tubes on a 100 mg scale. The products were then isolated by normal phase HPLC.

The compounds were evaluated for PR agonist activity based on their ability to induce alkaline phosphatase activity in the human breast cancer cell line T47D, Table 1.[5] As previously mentioned transposing the amide oxygen of oxindole **7** to a sulfur atom dramatically switched the activity of the molecule from antagonist ($IC_{50}=56$ nM, **7**) to agonist ($EC_{50}=1.6$ nM, **8**), with excellent potency. We then surveyed the basic substitution pattern of compound **8** to optimize the core thio-oxindole template.

Substituting the *N*-H of the thioamide moiety for an *N*-methyl group **9**, caused a dramatic loss of potency (T47D alkaline phosphatase $EC_{50} > 3000$ nM).

Moving the 3′-chlorine functionality to the 4′-position also caused a severe decline in potency ($EC_{50} > 2000$ nM, **11**), whereas the 2′-chloride, **10**, was only slightly weaker ($EC_{50}=5.3$ nM) than the parent thio-oxindole **8**.

The 3,3-dialkyl substitution pattern on the thio-oxindole core was examined next. Replacing the spirocyclohexyl group with a 3,3-dimethyl functionality **12** had little effect on potency, whereas the 3,3-diethyl derivative **13** was 100 fold weaker ($EC_{50}=5.3$ nM and 200 nM, respectively). There is an apparent trend in ring size in the spirocycle series, thus the spirocylobutane **14** was the least potent ($EC_{50}=28.7$ nM), the spirocyclopentyl **15** was intermediate ($EC_{50}=9.8$ nM) and the spirocyclohexyl derivative was the most potent ($EC_{50}=1.6$ nM, **8**). In the case of the mono-methyl derivative **16**, NMR evidence suggested that the isomeric 2-mercaptoindole moiety was the observed tautomer and this may account for its very weak activity (T47D alkaline phosphatase $EC_{50} > 3000$ nM). The 3-methyl-3-benzyl derivative was also poor ($EC_{50}=1200$ nM).



Scheme 1.

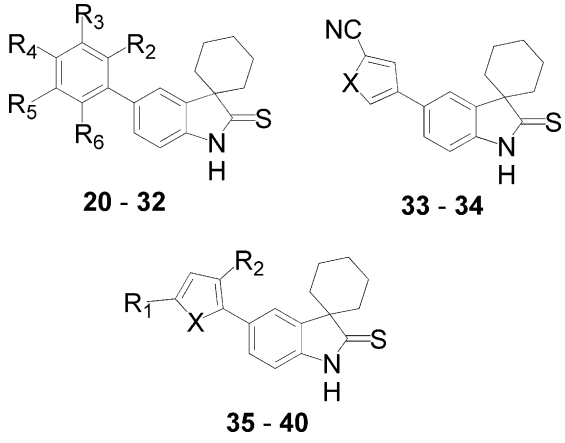
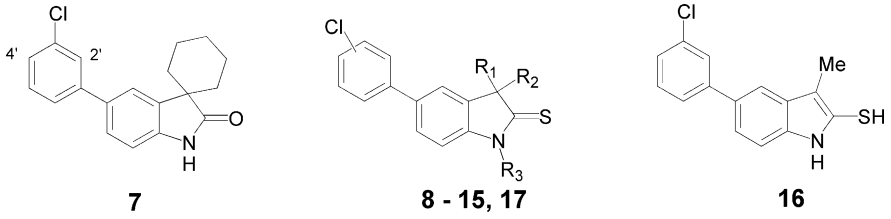


Table 1. T47D cell alkaline phosphatase assay data for compounds **2** and **7–17**



Compd	R_1	R_2	R_3	Chlorine substituent	PR Alkaline phosphatase[4] EC_{50} (nM)[a]
2, mpa					0.5
7, oxindole	$-CH_2CH_2CH_2$	CH_2CH_2-	H	3′	$IC_{50}=56$
8	$-CH_2CH_2CH_2$	CH_2CH_2-	H	3′	1.6
9	$-CH_2CH_2CH_2$	CH_2CH_2-	Me	3′	>3000
10	$-CH_2CH_2CH_2$	CH_2CH_2-	H	2′	5.3
11	$-CH_2CH_2CH_2$	CH_2CH_2-	H	4′	2000
12	Me	Me	H	3′	5
13	Et	Et	H	3′	200
14	$-CH_2CH_2CH_2-$		H	3′	28.7
15	$-CH_2CH_2CH_2CH_2-$		H	3′	9.8
16	Me	H	H	3′	>3000
17	Me	Bn	H	3′	1200

[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. The standard deviations for these assays were typically ±15% of mean or less.

With the spirocyclohexyl thio-oxindole as the template, we then turned our attention to the 5-aryl group, which was examined next, Table 2. Replacing the 3′-chlorine atom of thio-oxindole **8** with a fluorine atom to afford **20** had no effect on potency ($EC_{50}=1.6$ nM, **8** and **20**). Similarly 3-hydroxyl **21** and 3-cyano **22** analogues were also very potent ($EC_{50}=2.5$ nM and 2.0 nM respectively), however the 3-biphenyl derivative **23** was much weaker ($EC_{50}=71$ nM).

Based on analogue **11**, compounds having a single substituent at the 4′-position appeared to result in substantially reduced activity. However, adding a second substituent to the 4′-position of compound **8** (in the case of **24**, a fluorine atom) resulted in a compound with only slightly reduced activity over **8** (T47D alkaline phosphatase $EC_{50}=4.1$ nM). Thus substitution at the 4′ position is only deleterious if a suitable 3′-substituent is not present. Moving the fluorine to the 5′-carbon gave a small increase in potency ($EC_{50}=0.5$ nM, **25**). The same trend is also true for the 3′-fluoro **20** and 3′-cyano **22** derivatives. Thus adding a 4′-F to compound **20** or **22** caused only a minor reduction in potency (**26** $EC_{50}=5.4$ nM and **28** $EC_{50}=4.2$ nM). The 3′, 5′-difluorophenyl derivative **27** was a little more potent than the parent fluoride **20** ($EC_{50}=0.8$ nM) and the 3′-cyano-5′-fluorophenyl derivative **29**[7] was the most potent in the aryl series ($EC_{50}=0.36$ nM). Reversing the cyano and fluorine functionality in compound **28** to afford the 3′-fluoro-4′-cyanophenyl derivative **31** caused a drop in activity ($EC_{50}=14.8$ nM). Replacing the cyano group in compound **29** with a methoxy, to afford the congener **32**, resulted in nearly a 10 fold drop in potency ($EC_{50}=2.6$ nM).

Incorporating a thiophene ring in the 5-position of the thio-oxindole platform gave opportunities for different substitution patterns. The 2′-cyano-thiophen-4-yl derivative **33**[8] was as active as the most potent phenyl compound **29** ($EC_{50}=0.3$ nM), Table 3. Exchanging the sulfur in compound **33** with an oxygen atom gave the furan **34** which was about 5 fold weaker ($EC_{50}=1.9$ nM). In the isomeric thiophen-5-yl series, the effect of substitution at the 4′-position was explored. Thus the parent 2′-cyano-5-thiophene **35** had potent activity in the T47D assay ($EC_{50}=1.0$ nM). Adding a methyl group in the 4-position **36** slightly increased potency ($EC_{50}=0.5$ nM), but going to larger substituents reduced potency (n-propyl **37** $EC_{50}=7.7$ nM and n-butyl **38** $EC_{50}=23.5$ nM). Replacing the cyano group in compound **35** with a chloride **39** had little effect on activity ($EC_{50}=1.4$ nM). Also the furan **40** was nearly equipotent with thiophene **35** ($EC_{50}=1.9$ nM).

Some of the more potent molecules were tested in rat decidualization and uterine C3 models for progestational activity, Table 4.[6] Compounds were dosed orally, in a 2% Tween 80/0.5% methyl cellulose aqueous vehicle.

In the decidualization model, the parent thio-oxindole **8** was inactive at the highest dose tested, 3 mg/kg/day. However the 3′-cyano analogue **22** demonstrated potency equivalent to MPA ($EC_{50}=0.4$ mg/kg). The 3′-cyano-5′-fluorophenyl congener **29** was the most potent in the aryl series (decidual $EC_{50}=0.1$ mg/kg). In the heteroaryl series, the 2′-cyano-5′-thienyl thio-oxindole **35** was an order of magnitude more potent than it's 4′-methyl substituted analogue **36** ($EC_{50}=0.1$ mg/kg and 1 mg/kg, respectively). The furan **40** was also 10 fold weaker than the parent thiophene **35** (rat decidual $EC_{50}=1$ mg/kg).

The rat compliment component C3 assay measures the ability of a progestin agonist to block the estrogen stimulated expression of C3 mRNA.[6] The parent thio-oxindole **8** and cyano derivative **22** were an order of magnitude less potent than MPA (C3 $IC_{50}=0.37$, 0.3

Table 2. T47D cell alkaline phosphatase assay data for compounds **20–32**

Compd	R_3	R_4	R_5	R_6	PR Alkaline phosphatase[4] EC_{50} (nM)[a]
20	F	H	H	H	1.6
21	OH	H	H	H	2.5
22	CN	H	H	H	2.0
23	Ph	H	H	H	71.4
24	Cl	F	H	H	4.3
25	Cl	H	F	H	0.5
26	F	F	H	H	5.4
27	F	H	F	H	0.8
28	CN	F	H	H	4.2
29	CN	H	F	H	0.36
30	CN	H	H	F	1.4
31	F	CN	H	H	14.8
32	MeO	H	F	H	2.6

[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. The standard deviations for these assays were typically ±15% of mean or less.

Table 3. T47D cell alkaline phosphatase assay data for compounds **33–40**[a]

Compd	X	R1	R2	Alkaline phosphatase[4] EC_{50} (nM)[a]
33	S			0.3
34	O			1.9
35	S	CN	H	1.0
36	S	CN	Me	0.5
37	S	CN	*n*-Pr	7.7
38	S	CN	*n*-Bu	23.5
39	S	Cl	H	1.4
40	O	CN	H	1.9

[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. The standard deviations for these assays were typically ±15% of mean or less.

Table 4. Rat decidual and C3 data for MPA and compounds **8**, **22**, **29**, **35**, **36** and **40**

Compd	MPA	**8**	**22**	**29**	**35**	**36**	**40**
Decidualisation[a] ED_{50} (mg/kg)	0.4	>3	0.4	0.1	0.1	1	1
C3[b] IC_{50} (mg/kg)	0.026	0.37	0.3	0.025	0.03		

[a]50% effective dose of test compounds in the rat decidualization.
[b]C3 models.

Table 5. Cross-reactivity data for MPA and 29

Compd	AR EC_{50} (nM)	AR IC_{50} (nM)	GR EC_{50} (nM)	GR IC_{50} (nM)
MPA[a]	6.1		10	
29[b]	>10000	>10000	>10000	37

[a]Data from ref 9.
[b]Experimental values represent the mean of at least duplicate determinations. The standard deviation was typically +/−15% of mean.

and 0.026 mg/kg respectively). As with the decidualisation data, the 3′-cyano-5′-fluorophenyl derivative **29** and the 2′-cyano-5′-thienyl thio-oxindole **35** were the most potent in the series (IC_{50} = 0.025 and 0.03 mg/kg) and possessed efficacy and potency similar to MPA.

Compound **29** was evaluated in the PR competition binding assay using human T47D cell cytosol in the presence of 3 nM ^{3}H-R5020 as the radioligand.[5] In this assay thio-oxindole **29** had an IC_{50} = 15.9 nM. In comparison MPA **2** has an IC_{50} = 10.8 nM. Compound **29** was also evaluated for its androgen receptor (AR) (mouse fibroblast L929 cell line) and glucocorticoid receptor (GR) (human lung A549 cell line) activity.[6] It is clear from Table 5 that compound **29** has no AR agonist or antagonist activity. It does however have GR antagonist activity.

In conclusion, we have developed a new series of potent, orally active non-steroidal progesterone receptor agonists, based upon the 5-aryl thio-oxindole template.

Initial SAR studies settled on the spirocyclohexyl substitution pattern for the 3,3-dialkyl substituent on the thio-oxindole backbone. Although the template was relatively insensitive to the nature of the 3′-substituent on the 5-aryl group, an additional fluorine substituent enhanced both in vitro and in vivo potency. A variety of heteroaromatic groups were also explored and the 2′-cyanothien-5-yl moiety was found to be the most potent.

The 3′-cyano-5′-fluorophenyl **29** and 2′-cyano-thien-5′yl **35** spirocyclohexylthio-oxindoles were found to be equipotent to the steroidal progestin agonist MPA in the rat decidualisation and C3 models.

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7. 3-(1,2-Dihydro-2-thioxospiro[cyclohexane-1,3-[3H]indol]-5-yl)-5-fluorobenzonitrile **29**: mp. 236–250 °C; ^{1}H NMR ($CDCl_3$) δ 10.05 (s, 1H), 7.85 (d, 1H, J = 1 Hz), 7.65 (s, 1H), 7.46–7.52 (m, 2H), 7.33–7.36 (m, 1H), 7.16 (d, 1H, J = 8.1 Hz), 1.86–2,18 (m, 7H) and 1.54–1.66 (m, 3H); MS ((+)-APCI) m/z 337 [M + H]+.
8. (1′,2′-Dihydro-2′-thioxospiro[cyclohexane-1,3′-[3H]indol]-5′-yl)-2-thiophenecarbonitrile **35**: mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 12.82 (s, 1H), 8.0–7.98 (m, 2H), 7.73 (d, 1H, J = 4 Hz), 7.69 (dd, 1H, J = 8.2 and 1.5 Hz), 7.10 (d, 1H, J = 8.2 Hz), 1.98–1.77 (m, 7H), 1.43–1.33 (m, 3H); MS (EI) M+ @ m/z 324.
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