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New Progesterone Receptor Antagonists: 3,3-Disubstituted-5-aryloxindoles

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Abstract—A new series of 3,3-disubstituted-5-aryloxindoles has been synthesized and evaluated for progesterone receptor antagonist (PR) activity in a T47D cell alkaline phosphatase assay and for their ability to bind PR in competition binding studies. In this communication, the synthesis and structure–activity relationships (SARs) of various 3,3-substituents are discussed where it is clear that small alkyl and spiroalkyl groups are required to achieve better PR antagonist activity.

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.[1] Progesterone **1**, the endogenous ligand for the PR, is involved in the control of ovulation and preparation of the uterus to support pregnancy (Fig. 1). In principal a PR antagonist, may therefore have potential utility as a contraceptive.[2] In addition, PR antagonists have potential applications in the treatment of reproductive disorders such as uterine leiomyomas and endometriosis, as well as hormone dependent tumours.[3–5]

The steroidal PR antagonist Mifepristone (RU-486) **2**, is potentially compromised as a clinically useful contraceptive due to overt glucocorticoid receptor antagonism.[6] The goal of our study was to identify more receptor specific non-steroidal PR antagonists. Examples of non-steroidal PR antagonists have been described previously.[7] We decided to utilize the 5-aryldihydroquinoline **3** as a template,[7f] and during the course of our work have succeeded in replacing the dihydroquinoline ring with an oxindole **4**. This modification has allowed us to further explore the 3,3-alkyl region of the molecule.

Most of the compounds were prepared by either method A (Scheme 1, **5**–**13** and **15**–**20**) or method B (Scheme 2, **21**–**32**). The compounds exploring the 3,3′ alkyl substitution pattern were prepared according to Scheme 1. Oxindole **33** was alkylated according to Kende's procedure:[8] thus treatment with *n*-butyllithium in the presence of TMEDA, followed by quenching the anion with the appropriate alkyl iodide afforded the substituted oxindoles **34**. In the case of the spirocyclic compounds, the dianion was formed with excess *n*-butyllithium/TMEDA followed by addition of a diiodide. The bromides **35** were prepared by reaction of

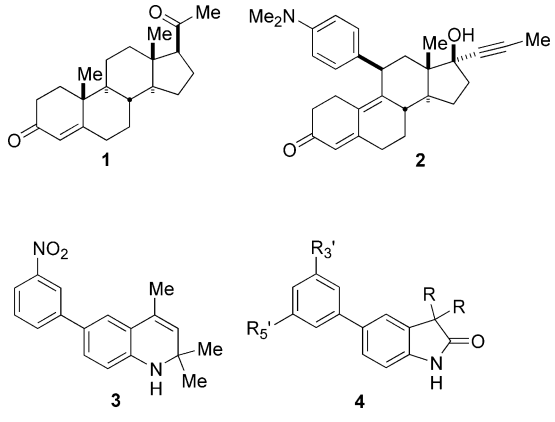

Figure 1. Ligands for the PR.

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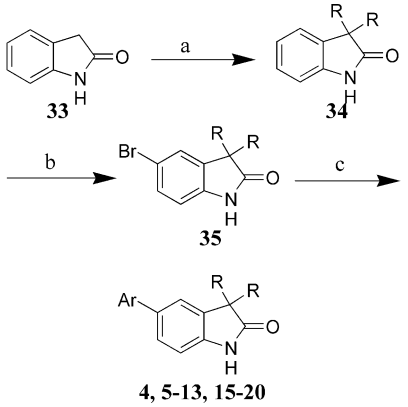
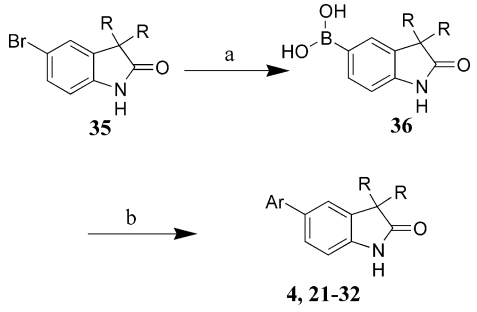


Scheme 1. Reagents and conditions: (a) *n*-BuLi, TMEDA, THF −78 °C, then R-I; (b) Br_2, NaOAc, AcOH, rt; (c) $Pd(Ph_3P)_4$, $ArB(OH)_2$, Na_2CO_3, DME–H_2O, 90 °C.



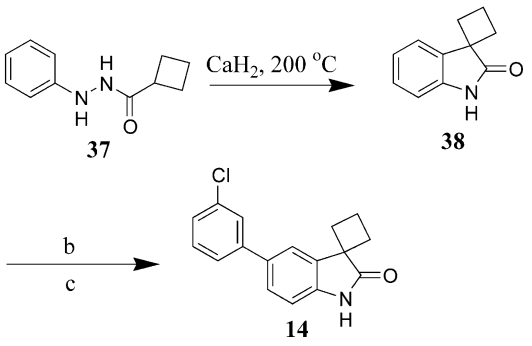
Scheme 2. Reagents and conditions: NaH, THF, *n*-BuLi, $(iPrO)_3B$; (b) $Pd(Ph_3P)_4$, ArBr, Na_2CO_3, DME–H_2O, 90 °C.

the precursor oxindole **34** with bromine and sodium acetate in acetic acid. Finally a Suzuki coupling with the appropriate bromide **35** and a phenylboronic acid in the presence of tetrakis(triphenylphosphine)palladium and sodium carbonate in either dimethoxyethane (DME)/water or toluene/ethanol/water gave the desired 5-aryloxindoles **4**.

Alternately, where the aryl boronic acid is not readily available, Scheme 2 was followed, method B. Reaction of the bromide **35** with sodium hydride in THF, then by *n*-butyl lithium at −78 °C was followed by quenching with tri-*iso*-propyl borate to afford the boronic acid **36**. Coupling of **36** with an aryl or heteroaryl bromide then gave the product **4**.

A different synthesis of the spirocyclobutane **14** was followed (Scheme 3).[9] The hydrazide **37** (prepared from phenylhydrazine and cyclobutanecarbonylchloride in DMF/pyridine) was mixed with CaH_2 and heated to 200 °C in the absence of solvent to afford the oxindole **38**. Compound **38** was then brominated and coupled with 3-chlorophenylboronic acid as described for Scheme 1 to afford compound **14**.

The compounds were evaluated for PR antagonist activity based on their ability to block progesterone induced alkaline phosphatase in the human breast cancer cell line T47D (Table 1).[10] PR competition binding studies were carried out using human T47D cell cytosol in the presence of 3 nM ^{3}H-R5020 as the radioligand.[10]



Scheme 3. Reagents and conditions: (a) Br_2, NaOAc, AcOH, rt; (b) $Pd(Ph_3P)_4$, 3-$ClC_6H_4B(OH)_2$, Na_2CO_3, DME–H_2O, 90 °C.

In the mono-substituted series the differences in hPR antagonist activity were small. The unsubstituted oxindole **6** had an $IC_{50} = 296$ nM in the T47D alkaline phosphatase assay and the mono-methyl compound **8** and mono-ethyl derivative **9** had $IC_{50} = 185$ and 130 nM, respectively. The mono-alkyl derivatives were all tested as racemates. Addition of a second 3-methyl group to compounds **7** and **8** enhanced the potency (T47D alkaline phosphatase $IC_{50} = 30.6$ and 66.4 nM for dimethyl analogues **10** and **11**, respectively). The 3,3-diethyl derivative **12** lost about 3-fold in potency compared to the dimethyl analogue **11** ($IC_{50} = 229$ nM).

We then looked at ring constrained spirocyclic oxindoles. The spirocyclopropane **13** and the spirocyclobutane **14** had comparable activity ($IC_{50} = 85$ and 70.7 nM, respectively). Increasing the ring size to a spirocyclopentane improved potency; thus the 3-nitrophenyl derivative **15** and its 3-chlorophenyl analogue **16** had similar potency ($IC_{50} = 38$ and 32 nM, respectively). The spirocyclohexanes **17** and **18** were similar in activity to the spirocyclopentanes [$IC_{50} = 36$ nM (**17**) and 56 nM (**18**)].

We prepared one unsymmetrical analogue, the 3-methyl-3′-benzyloxindole **19**, which was less potent than the 3,3′-dimethyl analogue **11** ($IC_{50} = 267$ nM).

Having optimized the 3,3-dialkyl oxindole core template, attention turned to functionalisation of the 5-aryl substituent (Table 1). The nature of the substituent on the 5-phenyl group was important for activity. For example replacing the nitro group with either a methoxyl (**20**) or acetyl (**21**) substituent reduced activity (T47D alkaline phosphatase $IC_{50} = 30.6$, 300 and 206 nM for compounds **10**, **20** and **21**, respectively). In contrast a 3′-cyano group retained activity (**22**, $IC_{50} = 27$ nM). A second small substituent could be added to the 5′-position of the phenyl group. Thus adding a fluorine to compound **11** to give the analogue **23** enhanced potency ($IC_{50} = 66.4$ and 29 nM, respectively). This was also true for the 3′-cyano-5′-fluoro (**24**) which had $IC_{50} = 13.2$ nM but not for the 3′-nitro-5′-fluoro (**25**) derivative which was equipotent with compound **10** ($IC_{50} = 27$ and 30 nM, respectively). Increasing the size of the second substituent from a fluorine to a chlorine reduced activity (**26** vs **10**, $IC_{50} = 100.1$ and 66.4 nM respectively).

Table 1. Functional activity data in the T47D alkaline phosphatase data for compounds **2**–**3** and **5**–**26**

Compd	R1[a]	R2[a]	R3′[a]	R5′[a]	Synthetic method	Alkaline phosphatase IC_{50} (nM)[b]	hPR competition IC_{50} (nM)
2						0.1	
3						41	
5	H	H	NO_2	H	A	299	
6	H	H	Cl	H	A	296	
7	Me	H	NO_2	H	A	102	
8	Me	H	Cl	H	A	185	
9	Et	H	Cl	H	A	130	
10	Me	Me	NO_2	H	A	30.6	509
11	Me	Me	Cl	H	A	66.4	
12	Et	Et	Cl	H	A	229	
13	$-CH_2CH_2-$		Cl	H	A	85	
14	$-CH_2CH_2CH_2-$		Cl	H	A	70.7	
15	$-CH_2CH_2CH_2CH_2-$		NO_2	H	A	38	193
16	$-CH_2CH_2CH_2CH_2-$		Cl	H	A	32	
17	$-CH_2CH_2CH_2\ CH_2CH_2-$		NO_2	H	A	36	96
18	$-CH_2CH_2CH_2\ CH_2CH_2-$		Cl	H	A	56	
19	Me	Bn	Cl	H	A	267	
20	Me	Me	MeO	H	A	300	
21	Me	Me	Ac	H	B	206	
22	Me	Me	CN	H	B	27	
23	Me	Me	Cl	F	B	29	
24	Me	Me	NO_2	F	B	27	
25	Me	Me	CN	F	B	13	
26	Me	Me	Cl	Cl	B	100.1	
27[c]	Me	Me	2-Cyanothien-5-yl		B	28	
28[c]	Me	Me	2-Nitrothien-5-yl		B	29	
29[c]	Me	Me	2-Cyano-4-methylthien-5-yl		B	29	
30	$-CH_2CH_2CH_2CH_2-$		CN	H	B	20.6	
31	$-CH_2CH_2CH_2\ CH_2CH_2-$		CN	H	B	14.7	
32	$-CH_2CH_2CH_2\ CH_2-$		CN	F	B	13.6	230
33	$-CH_2CH_2CH_2CH_2CH_2-$		CN	F	B	20	51

[a]Refer to structure (4) for numbering system.
[b]Values represent the average of at least duplicate determinations. The standard deviations for the assay was typically ±20% of mean or less.
[c]Structures shown in Figure 2.

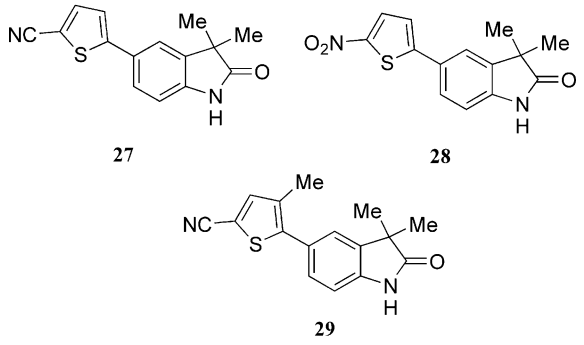


Figure 2.

We then looked at replacing the 5-phenyl group with a 2-substituted 5-thiophene, (Fig. 2). Again cyano and nitro derivatives were the most potent. The 5-cyanothiophene **27** had similar potency to the corresponding phenyl derivative **22** (IC_{50} = 28 and 27 nM, respectively). Similarly the nitro derivatives **28** and **10** were equipotent (IC_{50} = 29 and 30.6 nM, respectively). The addition of a 3′-methyl group to compound **27** had no effect on in-vitro potency (**29**, IC_{50} = 29 nM).

As was the case with the 3′-chloro and 3′-nitrophenyl derivatives, the activities of the 3′-cyano-5′-fluorophenyl compounds did not vary too much with the nature of the 3,3-dialkyl substituent. Thus, the dimethyl derivative **22** was active at 27 nM, the spirocyclopentyl and spirocyclohexyl derivatives **29** and **30** had IC_{50} = 20.6 and 14.7 nM, respectively.

Selected compounds were tested in the hPR competition binding assay. The 3-nitrophenyl substituted compounds were typically more potent than the 3-chlorophenyl derivatives. For example, the 3-nitrophenyl spirocylohexyl oxindole **17** had an IC_{50} = 96 nM, whereas its 3-chlorophenyl congener **18** had an IC_{50} = 477 nM. It was also found that potency in the competition assay increases across the series 3,3-dimethyl **10**, spirocyclopentyl **15** and spirocyclohexyl **17** (hPR competition IC_{50} = 509, 193, and 96 nM, respectively). The most potent compound in the competition assay was the spirocyclohexane **33** (IC_{50} = 51 nM). The spirocyclopentyl analogue **32** was less potent (IC_{50} = 230 nM).

Selected molecules were evaluated for activity in the rat decidualisation assay[11] (Table 2). The decidualisation assay measures the ability of a test compound to block the progesterone induced stimulation of the luminal cells of the uterus. All of the compounds in Table 2 showed statistically significant ($p < 0.05$) levels of inhibition when administered orally at a dose of 3 mg/kg. Generally it would appear that the spirocyclopentyl derivatives are the most potent. For example the

Table 2. Activity in the rat decidual assay for compounds **2**, **25** and **30–33**

Compd	**2**	**25**	**30**	**31**	**32**	**33**
% inhib.[a]	100	40	60	40	70	50

[a]Compounds were dosed orally at 3 mg/kg. ($p < 0.05$).

dimethyl substituted 3′cyano-5′fluoro compound **25** showed 40% inhibition at 3 mg/kg po, whereas the spiropentyl analogue **32** displayed 70% inhibition at the same dose. The spirocyclohexane **33** was similar to compound **32** (60% inhibition at 3 mg/k po). None of the compounds in the series were as potent as mifepristone **2** (100% inhibition at 3 mg/kg po, $IC_{50} = 0.5$ mg/kg po).

In summary, we have prepared a novel series of hPR antagonists based upon the 5-aryl oxindole scaffold. The most active of these derivatives had either a 3,3′-dimethyl or 3,3′-spirocyclopentyl or spirocyclohexyl substitution pattern. In-vivo activity has been achieved in the rat decidualisation assay where the most potent compound was the 3′-cyano-5′-fluorophenyl derivative **32**.

Acknowledgements

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