Novel 3-Aryl Indoles as Progesterone Receptor Antagonists for Uterine Fibroids


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ABSTRACT We report the synthesis and characterization of novel 3-aryl indoles as potent and efficacious progesterone receptor (PR) antagonists with potential for the treatment of uterine fibroids. These compounds demonstrated excellent selectivity over other steroid nuclear hormone receptors such as the mineralocorticoid receptor (MR). They were prepared from 2-bromo-6-nitro indole in four to six steps using a Suzuki cross-coupling as the key step. Compound 8f was orally active in the complement 3 model of progesterone antagonism in the rat uterus and demonstrated partial antagonism in the McPhail model of progesterone activity.

KEYWORDS Progesterone, progesterone receptor, NR3C3, progesterone receptor antagonist, uterine fibroids, Suzuki cross-coupling reaction

Uterine fibroids (leiomyomas) are benign tumors that develop from smooth muscle cells and fibrous connective tissues of the uterus.1–3 Although most are asymptomatic, in some women fibroids cause abnormal menstrual bleeding, pelvic pain, and reproductive dysfunction.4 The incidence of fibroids increases with age during the reproductive years and peaks between 35 and 40 years old.5 For those women whose quality of life is negatively impacted, hysterectomy is often necessary. As a result, fibroids are the primary indication for over 200,000 hysterectomies in the U.S. per year.6,7

An evaluation of hysterectomy cases revealed a similar incidence (77%) in both post- and premenopausal women.8 The fertility of premenopausal women can be decreased by the presence of submucosal myomas, which are fibroids partially in the cavity and partially in the wall of the uterus.9 Since hysterectomy is unacceptable for a woman who desires a future pregnancy, surgical procedures have been developed that preserve the uterus, such as myomectomy (fibroid removal with uterine retention), laser ablation, or embolization. Removal of fibroid growths can restore fertility.9 However, these treatments are invasive, expensive, and associated with a high rate of fibroid recurrence.10 Therefore, there is an unmet medical need for a noninvasive, pharmaceutical treatment of uterine fibroids in both post- and premenopausal women.

Currently the only pharmaceutical treatment for uterine fibroids involves the use of gonadotropin-releasing hormone (GnRH) agonists such as Lupron. These peptide hormones act on the pituitary gland, resulting in a down-regulation of the hypothalamic-pituitary-ovarian (HPO) axis, which decreases the release of gonadotropins (FSH and LH) and subsequently reduces the production of the ovarian hormones estrogen and progesterone. Withdrawal of ovarian hormone stimulation reduces uterine volume and fibroid size.11 Unfortunately, this benefit is accompanied by side effects, most notably bone loss, which limits treatment duration. Once therapy is discontinued, fibroids usually return. As a result, GnRH agonists are primarily used to reduce fibroid size prior to surgical removal.

Clinically, fibroids enlarge in women treated with norethindrone, a steroidal progesterone agonist.12 Progestins also block the decrease in uterine size associated with GnRH agonists.13 Furthermore, it has been demonstrated preclinically that progestins increase the mitotic index of myomas and myometrial cells both in vitro and in vivo.14 Conversely, clinical studies with the steroidal antiprogestin mifepristone have demonstrated a decrease in fibroid volume by 50% after 12 weeks of therapy.15 Another steroidal antiprogestin, Proellex (CDB-4124), has been in clinical trials for uterine fibroids, associated anemia, and endometriosis.16–19

The progesterone receptor (PR, NR3C3) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Two isoforms, PR-A and PR-B, have been described.20 The PRs can be modulated by a wide variety of ligands, ranging from full agonists such as progesterone (1) and promegestone (R-5020, 2) to full antagonists such as mifepristone (RU-486, 3) and Proellex (CDB-4124, 4). See

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Chart 1. In between these two extremes are selective progestosterone receptor modulators (SPRMs). Like their cousins, the selective estrogen receptor modulators (SERMs), these compounds elicit functional activities that depend on the cell context in which ligand induced receptor conformations recruit an ensemble of coactivators, resulting in promoter-specific interactions and subsequent selective gene activations. Results from a recent phase III clinical study with the steroidal SPRM asoprisnil (J-867, 5) suggest that PR modulation can affect dysfunctional bleeding and reduce fibroid size.

We have recently described the development of SERMs for the treatment of uterine fibroids in premenopausal women. Since clinical experience with GnRH agonists demonstrates that both estrogen and progesterone promote fibroid growth, we have also pursued the development of SPRMs for this indication. Here we describe for the first time a novel series of 3-aryl indoles as nonsteroidal, highly selective PR ligands.

Through screening efforts, we discovered that 3-[(4-methoxyphenyl)phenylmethyl]indole binds weakly across the steroidal nuclear hormone class of receptors (6, Figure 1). We recently described a selective Mineralocorticoid Receptor (MR, NR3C2) antagonist 7a based on this same nonsteroidal indole scaffold. We embarked on an extensive SAR study around compound 7a, with the primary goal being the removal of the tetrasubstituted, chiral carbon atom at C3 on the indole ring. During the course of this work, we unexpectedly discovered the highly selective PR ligands 8.

Figure 1 shows the previously described crystal structure of 7a overlaid with a representative of the new, achiral PR selective series (8c). The compounds are easily superimposable; however, in order to place the three common substituents (alkyl, aryl, and methylsulfonamide) in the same positions in chemical space, the indole rings must be rotated significantly with respect to each other. The resulting compounds showed reduced MR binding, increased PR binding, and even greater selectivity for PR over the androgen (AR, NR3C4) and glucocorticoid (GR, NR3C1) receptors.

The syntheses of compounds 8a-h are outlined in Scheme 1. In addition to being achiral, a significant advantage of this platform was the ease with which a three point SAR could be executed in rapid fashion. Therefore, several flexible routes were developed.

The initial route began with 3-bromo-6-nitroindole (9), which was deprotonated with LiHMDS followed by alkylation with alkyl halides to give 1-alkyl-3-bromo-6-nitroindoles 10. Alternatively, the indole nitrogen could be alkylated with alkyl alcohols using standard Mitsunobu conditions. Although many aryl coupling methods were effective with the bromoindoles 10, Suzuki conditions using the air-stable trialkylphosphonium tetrafluoroborate salt and tris(dibenzylideneacetone) dipalladium proved most versatile, allowing for a wide variety of aryl substitutions at C3 on the indole ring. Thus, 1-alkyl-3-bromo-6-nitroindoles 10 were coupled to aryl boronic acids to give 1-alkyl-3-aryl-6-nitroindoles 11. The nitro group was then reduced using standard conditions [hydrogenation or tin(II) chloride] followed by reaction with methanesulfonyl chloride to give final compounds 8 for biological assays.

Alternatively, the indole nitrogen could be protected as the phenyl sulfonamide 12 followed by Suzuki coupling and then deprotection of the phenyl sulfonamide with TBAF to give 3-aryl-indoles 14. The indole nitrogen of 14 could then be alkylated followed by reduction of the nitro group and sulfonylation of the subsequent amine to give final compounds 8.

In a third approach, 3-bromo-6-nitroindole was converted to the pinacolboryl derivative 15. This allowed direct coupling of more readily available aryl bromides without conversion to
the corresponding boronic acids. Coupling of aryl bromides to intermediate 15 worked well using standard, aqueous Suzuki conditions [tetrais(triphenylphosphine) palladium(0), bicarbonate, and lithium chloride]. This was followed by reduction and sulfonylation to give final compounds 8.

Biological data for compounds 8a–h are shown in Table 1. The binding affinity data were generated using appropriate tritium labeled standards and recombinant, full-length human receptors in competitive binding assays. The functional activity was measured using a transcription assay with full-length human PR cotransfected into a HEK293 cell line.

In general, the more rigid achiral series displayed much higher selectivities than the chiral series with its flexible linker between the aryl group and the indole ring. The screening hit, indole 6, possesses a binding affinity for PR of 478 nM but is 6- to 10-fold more potent at the other steroid receptors. It is nearly a full antagonist of PR but with a re-

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The alkyl substituent at N1 played a very important role in binding potency. Changing this substituent from methyl (8a) to ethyl (8b) and then from ethyl to isopropyl (8c) improved the binding affinity 7- and 5-fold, respectively. The functional activities follow this same trend. We also noted a preference for substitution of select small functional groups ortho to the cyano at the 4’ position on the aromatic ring at carbon 3 of the indole. For example, addition of a methyl group (8d) improved binding affinity by 3-fold. However, addition of a methoxy group at this position (8e) decreased binding affinity, while addition of fluorine (8f) improved affinity for PR. The same trend, although with somewhat muted differences, was also noted in the functional assays.

In our previous studies, we discovered a clear preference of MR for the S-enantiomers of compounds 7. Compare, for example, 7b, which has 5-fold better affinity than 7c. We wondered if PR would have a similar preference for one enantiomer over the other if chiral substituents were placed at the N1 position of indoles 8. In practice, substituting chiral groups at N1, such as the secondary butyl substituted indoles 8g and 8h, demonstrated a slight but consistent preference of PR for the S-enantiomers.

Compound 8f was advanced to further testing in vivo. The ovariectomized rat complement C3 assay was used to evaluate its ability to reverse the progesterin (R5020) dependent down-regulation of estrogen induced expression of complement C3 mRNA in the rat uterus (Figure 2). In this model, when compound 8f was dosed orally, it demonstrated potent antagonist activity, with an ED₅₀ of 0.33 mg/kg, which is comparable to that of asoprisnil (ED₅₀ = 0.31 mg/kg).

The activity of 8f was also assessed in the McPhail model. Progesterone treatment of immature estrogen-primed rabbits induces endometrial transformation, which is scored using the McPhail Index (Figure 3). The animals were dosed subcutaneously (sc) with or without progesterone to evaluate the agonist or antagonist activity of the compound. In the antagonist mode, mifepristone, asoprisnil, and 8f antagonize the effects of progesterone dosed at 1 mg/kg.

**Scheme 1**

Reagents and conditions: (a) LiHMDS, alkyl halide, DMF 0 °C to RT; (b) alkyl alcohol, DIAD, PPh₃, CH₂Cl₂, 0 °C to RT; (c) aryl boronic acid, Pd₂(dba)₃, [tBu]₃PBF₄, KF, THF, 40 °C; (d) H₂, Pd/C or PdO₂, THF or SnCl₂ -2H₂O, DMF, 60 °C; (e) MeCl, pyr, CH₂Cl₂; (f) PhSO₂Cl, DMAP, Et₃N, CH₂Cl₂; (g) TBAF, THF; (h) bis(pinacolato)diboron, PdCl₂(dppf), C₂H₂N₂, KOAc, DMSO, 90 °C; (i) aryl bromide, Pd(PPh₃)₃, Na₂CO₃, LiOH, H₂O, toluene, EtOH.

![Scheme 1](image-url)
Table 1. PR Binding and Functional Activities of 6, 7a–c, and 8a–h

<table>
<thead>
<tr>
<th>cmpd</th>
<th>R1</th>
<th>R2</th>
<th>isomer</th>
<th>PR</th>
<th>MR</th>
<th>AR</th>
<th>GR</th>
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<tr>
<td>6</td>
<td>8b</td>
<td>R</td>
<td>0.83 ± 0.33</td>
<td>1440 ± 223</td>
<td>2550 ± 666</td>
<td>780 ± 147</td>
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<tr>
<td>7a</td>
<td>F</td>
<td>S</td>
<td>2.0 ± 10.7</td>
<td>&gt;4170</td>
<td>&gt;4020</td>
<td>&gt;4290</td>
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<tr>
<td>7b</td>
<td>F</td>
<td>R</td>
<td>1.23 ± 0.260</td>
<td>1.51 ± 0.856</td>
<td>676 ± 306</td>
<td>54.4 ± 8.33</td>
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<tr>
<td>7c</td>
<td>Me</td>
<td>H</td>
<td>28.0 ± 10.7</td>
<td>&gt;4170</td>
<td>&gt;4020</td>
<td>&gt;4290</td>
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<tr>
<td>7f</td>
<td>H</td>
<td>H</td>
<td>0.42 ± 1.95</td>
<td>1770 ± 640</td>
<td>2590</td>
<td>927 ± 93.8</td>
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<tr>
<td>8a</td>
<td>H</td>
<td>H</td>
<td>0.079 ± 0.286</td>
<td>1490 ± 123</td>
<td>&gt;3900</td>
<td>1530 ± 156</td>
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<tr>
<td>8b</td>
<td>H</td>
<td>H</td>
<td>0.298 ± 0.150</td>
<td>126 ± 12.1</td>
<td>403 ± 212</td>
<td>157 ± 15.2</td>
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<tr>
<td>8c</td>
<td>H</td>
<td>H</td>
<td>1.45 ± 0.760</td>
<td>1390 ± 403</td>
<td>490 ± 172</td>
<td>977 ± 65.7</td>
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<tr>
<td>8d</td>
<td>H</td>
<td>Me</td>
<td>0.298 ± 0.150</td>
<td>126 ± 12.1</td>
<td>403 ± 212</td>
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<tr>
<td>8e</td>
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<td>126 ± 12.1</td>
<td>403 ± 212</td>
<td>157 ± 15.2</td>
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*Experimental values represent the average of at least duplicate determinations. Standard deviations are indicated by ± of the geometric mean.

Figure 2. Dose response (mg/kg, po) of asoprisnil and 8f in the ovariectomized rat complement C3 assay. E2 is estrogen. R5020 is promegestone. By comparison, the fold over asoprisnil (5 mg/kg) for vehicle control was 0.018 ± 0.011; while E2 (0.05 mg/kg) alone was 0.73 ± 0.05, and E2 (0.05 mg/kg) + R5020 (0.1 mg/kg) was 0.12 ± 0.03.

Mifepristone reached full antagonism at 1 mg/kg, whereas asoprisnil and 8f demonstrated only partial antagonism with a McPhail score of 3.1 for asoprisnil at 10 mg/kg and 2.0 for 8f at 5 mg/kg. At 30 mg/kg, 8f resulted in a McPhail score of 2.5 in the antagonist mode. In the agonist mode, mifepristone had no effect, but asoprisnil and 8f increased the McPhail Index to 3.1 and 1.5, respectively, at 30 mg/kg.

The oral bioavailability of 8f (dosed as a suspension) in rats was 31.4 ± 7.3% with a t_{max} of 4.0 h and an elimination half-life (t_1/2) of 19.8 ± 5.0 h. The volume of distribution (V_d) was 2.3 ± 0.7 L/kg, while its clearance was 2.5 ± 0.6 mL/min/kg.

Figure 3. Dose responses (mg/kg, sc) of asoprisnil, mifepristone, and 8f in the immature, estrogen-stimulated rabbit McPhail assay: (a) antagonist mode with 1 mg/kg progesterone (P4) + test compound at various doses; (b) agonist mode with progesterone (P4) alone at 1 mg/kg compared to test compound alone at 1, 10, and 30 mg/kg. The indole 8f is a potent, selective antagonist of PR in vitro. It is orally efficacious in an in vivo rat uterine model of PR.
antagonist activity. In the McPhail model, in the antagonist mode, it demonstrated activity less efficacious than the full antagonists mifepristone but more efficacious than the partial antagonist atraspiron.

**SUPPORTING INFORMATION AVAILABLE** Synthesis procedures and characterization data for compounds 8a–h, and a description of the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Author Contributions:** T.I.R. designed and interpreted experiments, synthesized compounds, wrote the manuscript, and contributed to the Supporting Information; C.A.C. designed experiments, synthesized compounds, wrote the Supporting Information, and contributed to the manuscript; Y.K.Y., T.J.B., J.E.L., S.A.J., N.E.H., B.S.M., and C.W.L. designed experiments, synthesized compounds, and contributed to the Supporting Information; K.L.Y. led the chemistry team, designed and interpreted experiments, synthesized compounds, and contributed to the Supporting Information; A.G.G., T.L.M. and P.K.S. designed, performed, and interpreted in vitro experiments; R.W.Z., J.J.O., and C.M.-R. designed, performed, and interpreted in vitro experiments and contributed to the Supporting Information; N.P. designed and interpreted animal exposure experiments, R.J.S.G. led the biology team, designed and interpreted experiments; and contributed to the manuscript and the Supporting Information; J.A.D. led the discovery team, designed and interpreted experiments, and contributed to the manuscript.

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