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Letters

6-Aryl-1,4-dihydro-benzo[*d*][1,3]oxazin-2-ones: A Novel Class of Potent, Selective, and Orally Active Nonsteroidal Progesterone Receptor Antagonists†

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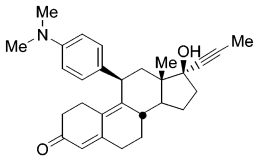
Abstract: Novel 6-aryl-1,4-dihydro-benzo[*d*][1,3]oxazin-2-ones were synthesized and tested as progesterone receptor (PR) antagonists. These compounds were potent and showed good selectivity for PR over other steroid receptors such as the glucocorticoid and androgen receptors (e.g., greater than 80-fold selectivity at PR for **4h**). Numerous 6-aryl benzoxazinones (e.g., **4h**−**j**) were active orally in the uterine decidualization and component C3 assays in the rats. In these in vivo models, **4h** had potencies comparable to mifepristone (**1**).

Introduction. The progesterone receptor (PR) is a member of the intracellular superfamily of ligand-dependent transcription factors.[1] PR agonists play an important role in female reproduction and have been used extensively in female contraception and hormone replacement therapy. PR antagonists, on the other hand, have only found limited utility, and their therapeutic potential has not yet been fully realized. A selective PR antagonist may be potentially useful in female contraception[2] and for the treatment of various gynecological and obstetric diseases including hormone-dependent breast and prostate cancers,[3−5] nonmalignant chronic conditions such as fibroids,[6,7] and endometriosis.[8,9]

Mifepristone (**1**), a clinically available steroidal PR antagonist, demonstrated potent activity at other steroid receptors such as the glucocorticoid receptor (GR), and this potentially limits its chronic use. Novel PR antagonists that are structurally distinct from the steroid class may have greater potential for selectivity against other steroid receptors (e.g., GR).[10−12] A number of nonsteroidal PR antagonists have been reported.[13−18] We have recently described novel PR antagonist 6-aryl benzimidazolones (**2**) and PR agonist 6-aryl benzoxazines (**3**).[19,20] The structure−activity relationship (SAR) studies from our reports indicated that the benzene-fused heterocyclic core played an important role in PR potency and functional activity. Further investigation on other benzene-fused ring systems uncovered a novel class of potent, selective, and orally active PR antagonists, 6-aryl-1,4-dihydrobenzoxazin-2-ones (**4**), which will be discussed herein.



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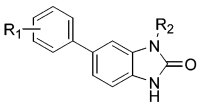

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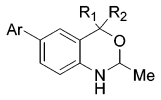

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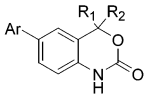

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Chemistry. A general synthesis of **4a**−**m** is described in Scheme 1. Brofoxine **5**[21] was reacted with a commercially available substituted aryl boronic acid, using a standard Suzuki coupling protocol, to afford the desired target compounds **4**. Alternatively, when the pendent aryl boronic acid was not available, brofoxine **5** was converted to its corresponding boronic acid **6** by a lithium−halogen exchange followed by quenching the reaction solution with triisopropyl borate and then

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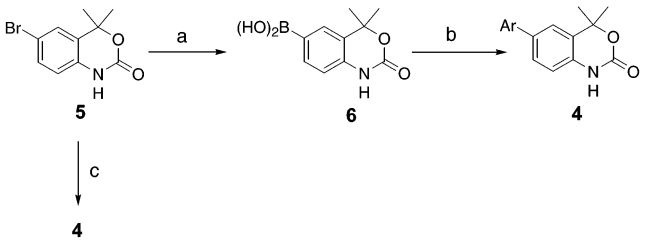
Scheme 1[a]



[a] Reagents: (a) *n*-BuLi, THF, −78 °C; B(O*i*-Pro)$_3$, −78 °C; 3 N HCl, room temperature, 65%. (b) ArX, Pd(Ph$_3$P)$_4$, Na$_2$CO$_3$, DME/H$_2$O, 85 °C, N$_2$, 40−80%. (c) ArB(OH)$_2$, Pd(Ph$_3$P)$_4$, Na$_2$CO$_3$, DME/H$_2$O, 85 °C, N$_2$, 40−80%.

acidic workup. Boronic acid **6** was then coupled with the appropriate aryl halide to afford **4**.

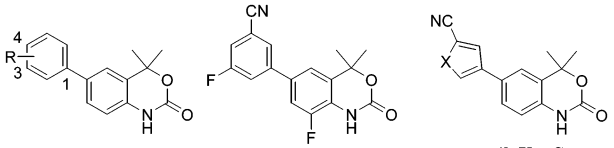
Results and Discussion. Recently, we disclosed 6-aryl benzimidazolones and 6-aryl benzoxazines as PR modulators.[19,20] The biological profile changed from PR antagonism to PR agonism when the benzoimidiazolone ring was replaced by a benzoxazine scaffold. This finding indicated that the benzene-fused heterocyclic ring played an important role in the direction of SAR for these series. To search for more potent and selective PR modulators, we continued to examine other benzene-fused scaffolds and discovered that 6-aryl benzoxazinones are potent and selective PR antagonists.

These 6-aryl benzoxazinones were tested in an assay using hPR/PRE-luciferase plasmid cotransfected CV-1 cells[22] and in an alkaline phosphatase assay in the T47D human breast carcinoma cell line.[23,24] The results are listed in Table 1. The nature of the substituent and substitution pattern on the pendent 6-aryl group played an important role in the PRE-luciferase activity of the compounds. For example, 3-chlorophenyl benzoxazinone **4c** (IC_{50} = 9.3 nM) was over 50-fold more potent than its 2- or 4-substituted congeners **4b**,**d** (IC_{50} values were greater than 500 nM) indicating that a substituent in the 3-position of the 6-aryl moiety was favored. Consequently, a number of compounds (**4a**,**e**,**f**) with different substituents at the 3-position of the 6-aryl group were prepared and showed low nonamolar activity in the PRE-luciferase assay (IC_{50} values were in the range of 3.8−38.3 nM).

Several 6-aryl disubstituted analogues (**4g**−**k**) were prepared. The SAR results in the PRE-luciferase assay showed that 3,5-disubstituted compounds **4g**,**h** were more potent than the corresponding 3,4-disubstituted analogues **4i**,**j**. The 8-fluoro-substituted analogue **4k** was less active than its parent congener **4h**. The good potency seen with compounds **4l**,**m** demonstrated that the thiophene and furan ring systems could successfully replace the substituted phenyl system.

Considerable differences[16,25] in potency and functional activity were observed with the compounds in the T47D alkaline phosphatase assay when compared to their PRE-luciferase results. Most notably, compounds **4g**,**h**,**l** were PR agonists with moderate potency in this assay in contrast to being potent PR antagonists in the CV-1 PRE-luciferase assay. The discrepancy in the functional preferences of the compounds from these two assays may have resulted from the different cell background or gene promoter context, though the mechanism is unclear.[26]

Table 1. PR Functional Activities of **1** and **4a**−**m** in CV-1 PRE-Luciferase and T47D Alkaline Phosphatase Assays



compd	R	CV-1 PRE-luciferase IC_{50} (nM)[a]	T47D alkaline phosphatase IC_{50} (nM)[a]
1		0.30	0.13
4a	3-F	3.8	8.2
4b	2-Cl	500.0	300.0
4c	3-Cl	9.3	30.0
4d	4-Cl	642.0	30.0
4e	3-CN	9.9	17.0
4f	3-OCH_3	38.3	19.5
4g	5-F, 3-Cl	2.6	(500.0)[b]
4h	5-F, 3-CN	15.9	(90.0)[b]
4i	4-F, 3-CN	55.0	15.1
4j	4-F, 3-Cl	37.0	23.0
4k[c]		98.8	26.2
4l		6.7	(61.1)[b]
4m		40.2	8.0

[a] Experimental values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ±20% of mean or less. The efficacy was ≥90% unless otherwise noted. The agonist efficacy for **4h** in the T47D alkaline phosphatase assay was 83%. The antagonist efficacy for **4l** in the PRE-luciferase assay was 60%. [b] The number in parentheses represented the EC_{50} values from the alkaline phosphatase assay. [c] Compound **4k** was prepared in a similar fashion as described in Scheme 1 starting from the corresponding fluorine-substituted analogue of **5**.

Several additional noteworthy results emerged from the T47D assay. The rearrangement of the fluorine atom from position 5 to position 4 on the 6-aryl groups (compare **4h** with **4i** and **4g** with **4j**) caused a change from PR agonism to PR antagonism. Similarly, a one atom change (oxygen to sulfur) caused a switch from antagonism to agonism in furan congener **4m** as compared to thiophene analogue **4l**.

Benzoxazinones **4a**,**c**,**e**,**g**−**m** were evaluated in the ovariectomized mature female rat decidualization model[27] and compared to mifepristone (**1**) (Figure 1 and Table 2). Analogues **4a**,**g**−**m**, containing a diverse array of 6-aryl moieties (i.e., substituted phenyl and heterocylic), showed robust oral activity. Compounds **4h**,**i**,**k** were similar to mifepristone (**1**) in potency while **4a**,**j**,**l**,**m** were marginally less potent than **1**. Interestingly, 6-(fluoro-cyanophenyl)-substituted analogue **4h**[28,29] was over 20-fold more potent than its des-fluorine congener **4e**. This result suggested that the extra fluorine atom on the 6-aryl group of **4h** significantly improved its pharmacokinetic properties over **4e** since both compounds had similar potency in the PRE-luciferase assay. As illustrated by the data in Table 3, compound **4e** was cleared at a substantially higher rate than the fluorine-substituted congener **4h** when both were given at a single 1 mg/kg intravenous dose to female rats. Furthermore, the bioavailability in rats of **4h** (72%) was significantly greater than **4e** (36%) suggesting that **4h** was also absorbed better than **4e**. The other 6-(fluoro-cyano)-substituted isomer **4i** also demonstrated a considerable boost in potency over des-fluoro parent **4e**.

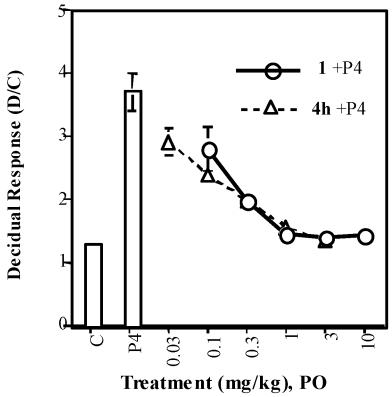


Figure 1. Representative dose−response curves for **4h** and **1** in the rat uterine decidualization model. Overiectomized mature rats were treated with vehicle (C), progesterone (P4, 5.6 mg/kg, SC), P4 + **1**, and P4 + **4h** for 7 days. Decidual response (D/C) was measured 24 h after the final treatment as described in ref 27. Each data point represents mean ± SE from seven animals.

Table 2. Oral Activities of **1** and **4a**,**c**,**e**,**g**−**m** in Rat Decidualization and C3 Models

compd	decidualization ED_{50} (mg/kg)[a]	component C3 ED_{50} (mg/kg)[a]
1	0.3	1.7
4a	1.5[b]	ND[c]
4c	>30	ND
4e	7.0	ND
4g	2.0[b]	ND
4h	0.3	1.0
4i	0.6	4.8
4j	1.0	2.5
4k	0.6	ND
4l	1.7	ND
4m	0.9	ND

[a] Experimental values represented the average of at least duplicate determinations. The standard deviation for the decidualization and C3 assays was typically ±15% of mean or less. [b] The estimated ED_{50} value. [c] Not determined.

Table 3. Pharmacokinetic Parameters of **4e**,**h** in Female Rats Following a 1 mg/kg IV Dose[a]

compd	$t_{1/2}$ (hr)	$AUC_{0\to\infty}$ (μg hr/mL)	Clp (L/hr kg)
4e	0.6 ± 0.1	0.46 ± 0.06	2.18 ± 0.29
4h	7.7 ± 2.2	3.69 ± 1.42	0.22 ± 0.06

[a] Experimental values represented the average of three animals.

Similarly, 6-(fluoro-chloro) isomers **4g**,**j** were over 15-fold more potent than their des-fluoro parent **4c**.

Selected compounds (**4h**−**j**) were tested in an additional in vivo PR antagonist model, the adult ovariectomized rat uterine component C3 assay.[30] PR antagonists are known to reverse the progestin down-regulation of estrogen-induced synthesis of complement component C3 in the epithelial cells of the rat uterus. As shown in Table 2, **4h**,**j** had comparable potency to mifepristone (**1**) while **4i** was marginally less potent.

Mifepristone (**1**) is also a potent GR antagonist (Table 4), and this is a cause of major concern with respect to its chronic use. In this regard, the benzoxazinones (**4g**,**h**,**j**−**m**) were tested for their cross-reactivities with estrogen (ER), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors[18,24] and compared to mifepristone (**1**). As illustrated in Table 4, the novel benzoxazinones demonstrated good selectivity for PR over other steroid receptors. For example, **4g**,**h** showed

Table 4. Antagonist Cross-Activities of **1** and **4g**,**h**,**j**−**m**

	IC_{50} (nM)				
compd	PR	ER	AR	GR	MR
1[a]	0.3 (99)	>1000 (40)	5 (7)	0.8 (98)	>1000 (77)
4g[b]	2.6	ND[c]	319	2126	839
4h[b]	15.9	>10 000	1292	1756	2369
4j[b]	37.0	>10 000	207	2581	2492
4l[b]	6.7	ND	280	1856	1386
4m[b]	40.2	ND	129	>10 000	>10 000

[a] See ref 18 for the experimental data; values in the parentheses represent percentage of efficacy. [b] Experimental values represent the average of at least duplicate determinations. The standard deviation for these assays was typically ±30% of mean or less. The efficacy was ≥80% unless otherwise noted. See refs 18 and 24 for experimental details. [c] Not determined.

greater than 80-fold selectivity for PR over other steroid receptors. More importantly, all benzoxazinones tested had greater than 100-fold selectivity for PR over GR in contrast to mifepristone, which was nearly equipotent on both receptors. In addition, none of these compounds showed any significant agonist activity at these steroid receptors. When tested orally in a rat thymic involution model,[31] **4h** did not show any GR antagonist activity at 30 mg/kg indicating at least a 100-fold selectivity for PR over GR in vivo.

These benzoxazinones demonstrated competitive binding when tested in a PR competition binding assay using cytosol from the human T47D breast carcinoma cell line.[24] For example, compounds **4g**,**h** had binding affinity (K_i) at PR of 5.5 and 25 nM, respectively.

Conclusion. 6-Aryl benzoxazinones were evaluated and found to be potent, selective, and orally active PR antagonists. As a novel class of nonsteroidal PR antagonists, 6-aryl benzoxazinones provided a substantial selectivity advantage over mifepristone and were found to be potentially useful as novel contraceptives and for the treatment of hormone-dependent cancers, nonmalignant chronic conditions such as uterine fibroids, and endometriosis.

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Supporting Information Available: Experimental details and data of **4a**−**m**. The procedures of rat uterine decidualization and component C3 models. This material is available free of charge via the Internet at http://pubs.acs.org.

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