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Novel 6-Aryl-1,4-dihydrobenzo[*d*][1,3]oxazine-2-thiones as Potent, Selective, and Orally Active Nonsteroidal Progesterone Receptor Agonists†

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Abstract—The functional activity of 6-aryl benzoxazinone-based progesterone (PR) antagonists changed to PR agonism when the 2-carbonyl group was replaced by a 2-thiocarbonyl moiety. Based on this finding novel 6-aryl benzoxazine-2-thiones were synthesized and evaluated as PR agonists in various in vitro and in vivo assays. Several analogues had sub-nanomolar in vitro potency and showed excellent oral activities in rats. Compounds **15** and **29** had similar potencies to medroxyprogesterone acetate (MPA) in the in vitro T47D alkaline phosphatase assay and in vivo rat decidualization model. In contrast to MPA, **29** was highly selective (>500-fold) for PR over glucocorticoid and androgen receptors.
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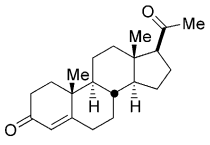
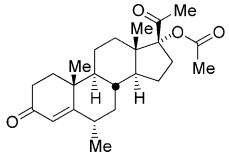
The progesterone receptor (PR) is one of the intracellular gene regulators known as 'ligand dependent transcription factors'.[1] Progesterone plays an important role in female reproduction and PR agonists have been used extensively in female contraception and hormone replacement therapy in combination with estrogens. However, many of the steroidal PR agonists are associated with well-recognized side effects such as mastalgia, nausea, headaches, and other CNS effects as well as concerns about cardiovascular complications.[2–5] A PR agonist that is selective for the uterus would greatly reduce these side effects. Also, many of the steroid-based agonists have demonstrated undesirable cross-reactivity with other steroid receptors such as glucocorticoid (GR), estrogen receptor (ER), and androgen receptors (AR). Novel PR agonists that are structurally distinct from the steroid class may have greater potential for tissue selectivity and selectivity against other steroid receptors (e.g., GR and AR).



Progesterone (P4)



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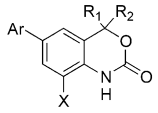
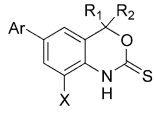


6-Aryl benzoxazin-2-ones PR antagonists (**1**)



6-Aryl benzoxazine-2-thiones PR agonists (**2-47**)

Recently, we disclosed 6-aryl benzimidazolones and 6-aryl benzoxazines as PR modulators.[6,7] The benzene-fused heterocyclic ring of these compounds played an important role in their PR functional activity and the direction of SAR. In our effort to examine other benzene-fused scaffolds for more potent and selective nonsteroidal PR modulators, we discovered that 6-aryl benzoxazinones (**1**) were potent PR antagonists.[8,9] Upon further investigation, we have discovered that the PR functional activity switched from an antagonist to a

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potent agonist when the 2-oxo atom of the benzoxazinone ring system was replaced by 2-thione moiety. For example, **1** (e.g., Ar = 3-chlorophenyl; R_1, $R_2 = CH_3$) was a potent PR antagonist in T47D cells ($IC_{50} = 9.3$ nM). Conversion to its corresponding benzoxazine-2-thione (**5**) resulted in a potent PR agonist ($EC_{50} = 1.7$ nM). Based on this finding, a number of novel 6-aryl benzoxazine-2-thiones (**2**–**47**) were synthesized and tested as PR modulators in various in vitro and in vivo assays. Synthesis, in vitro SAR, and in vivo activities of novel 6-aryl benzoxazine-2-thiones will be the subject of this report.

Chemistry

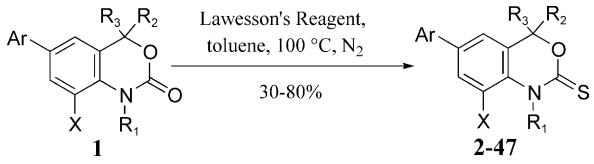
Synthesis of 6-aryl benzoxazine-2-thiones **2**–**47**[10] was readily effected in moderate to good yield by treatment of the corresponding 6-aryl benzoxazin-2-ones **1**[8,9] with Lawesson's reagent in toluene at 100 °C as described in Scheme 1.

Results and Discussion

The novel 6-aryl-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazine-2-thiones were evaluated in a alkaline phosphatase assay using a human T47D breast carcinoma cell line.[11,12] A few selected compounds were also tested in a PR competition binding assay using the cytosol from human T47D breast carcinoma cell line.[11,12] The results of their PR agonist activities are summarized in Tables 1–3. As illustrated in Table 1, the most potent compounds (e.g., **8**, **15**, and **29**) elicited activities in the subnanomolar range (EC_{50} 0.4–0.6 nM, Table 1) and were more potent than progesterone (EC_{50} 0.9 nM).

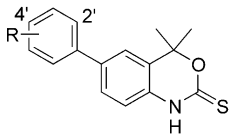
Various 6-(substituted phenyl) benzoxazine-2-thiones were prepared to examine the SAR of 6-aryl moiety. Compounds **3** and **5** with a meta-fluorine or chlorine substituent on the 6-aryl group were over one order of magnitude more potent than the corresponding *ortho*-substituted 6-aryl congeners **2** and **4**. Electron-withdrawing substituents in the *meta* position appeared to enhance potency over ones with electron-donating moiety (compare **3**, **5**, and **6**–**8** with **9**).

A number of 6-(3′, 5′-disubstituted phenyl) benzoxazine-2-thiones were evaluated. Compounds **12**–**17** with 3′-fluorine substitution on the 6-aryl group showed good PR agonist potency. However, when this 3′-fluorine atom was replaced by a larger group such as a chlorine or nitrile the resultant compounds **19**–**22** were, at best, 10-fold less potent. More interestingly, compounds **23**–**27** became weak PR antagonists when the 3′- and 5′-substituents appeared even larger (Br or alkoxy). 2′, 3′-Difluoro substituted compound **10** had similar potency to 3′-fluoro analogue **3** but was more potent than its 2′-fluoro analogue **2**. 3′, 4′-Disubstituted phenyl benzoxazine-2-thiones (**11** and **18**, EC_{50} 11.4 and 79.5 nM, respectively) were significantly less potent than their corresponding 3′, 5′-disubstituted regio isomers **12** and **13** (EC_{50}, 1.5 and 0.9 nM).

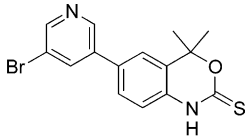


Scheme 1. 6-Aryl-1,4-dihydro-2H-benzo[*d*][1,3]oxazine-2-thiones.

Table 1. PR alkaline phosphatase activity of 6-aryl 4, 4-dimethyl-benzoxazine-2-thiones. SAR at the 6-position



2-27



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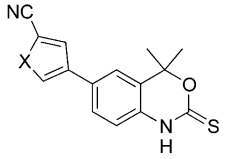
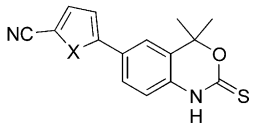


29-30

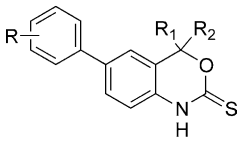


31-32

Compd	R	X	PR Alk. Phos. EC_{50} (nM)[a]
P4			0.9
MPA			0.1 (10.8)[b]
2	2′-F		11.6
3	3′-F		1.0
4	2′-Cl		49.8
5	3′-Cl		1.7
6	3′-Br		2.5
7	3′-NO_2		1.1
8	3′-CN		0.4
9	3′-Ome		7.2
10	2′-F, 3′-F		1.5
11	3′-F, 4′-F		11.4
12	3′-F, 5′-F		1.5
13	3′-F, 5′-Cl		0.9
14	3′-F, 5′-Br		1.7
15	3′-F, 5′-CN		0.6
16	3′-F, 5′-Ome		2.6
17	3′-F, 5′-CF_3		2.5
18	3′-Cl, 4′-F		79.5
19	3′-Cl, 5′-Cl		26.5
20	3′-Cl, 5′-CN		44.9
21	3′-CN, 5′-CN		42.0
22	3′-CN, 5′-Me		200.0
23	3′-Br, 5′-CN		> 10000.0 (100.0)[c]
24	3′-Br, 5′-Me		> 10000.0 (100.0)[c]
25	3′-Br, 5′-OCF_3		> 10000.0 (100.0)[c]
26	3′-CN, 5′-OCF_3		> 10000.0 (100.0)[c]
27	3′-CN, 5′-OMe		> 10000.0 (100.0)[c]
28			5.7
29		S	0.4 (3.0)[b]
30		O	1.2
31		S	0.7
32		O	1.25

[a]50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line and experimental values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ± 20% of mean or less.
[b]Value in parenthesis was 50% inhibitory concentration of tested compounds on the ^{3}H-P4 binding at PR using human T47D breast carcinoma cell line.
[c]Values in parenthesis were estimated IC_{50}s from alkaline phosphatase assay.

Table 2. PR alkaline phosphatase activity of 6-aryl benzoxazine-2-thiones. SAR at the 4-position

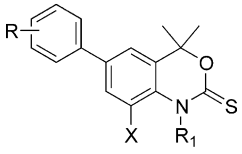


6

Compd	R	R_1	R_2	PR Alk. Phos. EC_{50} (nM)[a]
33	3′-F	Me	H	300.0
3	3′-F	Me	Me	1.0
5	3′-Cl	Me	Me	1.7
34	3′-Cl	Me	Allyl	0.3
35	3′-Cl	Me	Ph	5.7
36	3′-Cl	Me	Bn	88.5
37	3′-Cl	Et	Et	7.2
38	3′-Cl	Bn	Bn	1000.0
7	3′-NO_2	Me	Me	1.1
39	3′-NO_2	Et	Et	4.7
8	3′-CN	Me	Me	0.4
40	3′-CN	Spirohexyl		2.5

[a]50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line and experimental values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ± 20% of mean or less.

Table 3. PR alkaline phosphatase activity of other 6-aryl benzoxazine-2-thiones. SAR at the 1-and 8-positions



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Compd	R	R_1	X	PR Alk. Phos. EC_{50} (nM)[a]
41	3′-Br	Me	H	> 10000.0 (114.0)[b]
42	3′-F, 5′-Br	Me	H	> 10000.0 (100.0)[b]
43	3′-F, 5′-CN	Et	H	> 10000.0 (1000.0)[b]
44	3′-F, 4′-Cl	H	Br	1000.0
45	3′-F, 4′-CN	H	Br	> 10000.0
46	3′-F, 5′-CN	H	F	1000.0
47	3′-F, 5′-CN	H	Br	80.1

[a]50% effective concentration of tested compounds on alkaline phosphatase activity in the human T47D breast carcinoma cell line and experimental values represent the average of at least duplicate determinations. The standard deviations for these assays were typically ± 20% of mean or less.
[b]Values in parenthesis were estimated IC_{50}s from alkaline phosphatase assay.

Several compounds (**28**–**32**) with different heterocycles at the 6-position of benzoxazine-2-thiones were also potent PR agonists. 6-Thienyl benzoxazine-2-thiones **29** and **31** were slightly more potent than the corresponding 6-furyl analogues **30** and **32**. In the PR competition binding assay, cyano thiophene **29** showed good binding affinity (IC_{50} 3.0 nM) and was better than MPA (IC_{50} 10.8 nM).

Benzoxazine-2-thiones with different substituents at the 4-position were prepared and tested in the alkaline phosphatase assay (Table 2). The mono methyl substituted benzoxazine-2-thione (**33**) was 300 times less potent than its 4,4-dimethyl analogue **3**. Replacing one of the methyl groups at the 4-position of compound **5** with an allyl group to produce **34** resulted in a moderate increase in potency. However, replacement of one of the methyl groups at the 4-position of compound **5** with a phenyl or benzyl moiety decreased the potency of the corresponding compounds **35** and **36**. Congeners **37**, **38**, and **40** bearing larger symmetrical 4,4-substituted groups or spirohexyl moiety, were less potent than their corresponding 4,4-dimethyl analogues **5**, **7**, and **8**.

As illustrated in Table 3, replacing the 1-hydrogen atom by a methyl or ethyl group of **6**, **14**, and **15** led to weak PR antagonists **41**–**43**. This finding indicated that the nature of the substituent at the 1-position of the 6-aryl benzoxazine-2-thione system played a very critical role in their PR function and potency. 8-Substitution on the benzoxazine-2-thione nucleus with fluorine or bromine was not favorable as indicated by **44**–**47**. For example, 8-fluorobenzoxazine-2-thione **46** and 8-bromobenzoxazine-2-thione **47** were over two orders of magnitude less potent than their corresponding 8-hydrogen substituted analogue **15**.

Compounds **15**, **29**, and **31** were evaluated in the ovariectomized female rat decidualization model by either subcutaneous injection or oral administration (Table 4).[13] All three compounds showed good activity and were more potent than **P4**. Furthermore, compounds **15** and **29** had oral potency comparable to MPA administrated SC.

Since steroids such as MPA were known to cross-react with GR and AR,[14] the selectivity of **29** with GR and AR were examined using HRE-tk-luciferase assay in the human lung carcinoma cell line A549 for GR and in mouse fibroblast cell line L929 for AR.[12] As shown in Table 5, unlike MPA, **29** did not have any agonist cross-reactivity with GR or AR. Compound **29** showed very weak GR and AR antagonist activity compared to its PR agonist activity.

Table 4. Rat decidualization activities of P4, MPA, **15**, **29**, and **31**

Compd	P4	MPA	**15**	**29**	**31**
ED_{50} (mg/kg)[a]	5.62	0.40	(0.10)[b]	0.22 (0.62)[b]	(1.5)[b]

[a]Experimental values represent the average of at least duplicate determinations. The standard deviation for the decidualzation assay was typically ± 15% of mean or less.
[b]Values in parenthesis were obtained via oral administration while rest obtained via subcutaneous injection.

Table 5. Cross-reactivities of P4, MPA, and **29** with AR and GR

Compd	PR EC_{50} (nM)	AR EC_{50} (nM)	AR IC_{50} (nM)	GR EC_{50} (nM)	GR IC_{50} (nM)
P4[a]	0.92		37 (46%)		> 1000
MPA[a]	0.12	6.1 (159%)		10 (157%)	
29[b]	0.38	> 10000.0	379.0	> 10000.0	1316

[a]Data from reference 14.
[b]Experimental values represent the average of at least duplicate determinations. The standard deviation for these assays was typically ± 15% of mean or less.

In summary, we have discovered 6-aryl benzoxazine-2-thiones as potent, selective, and orally active PR agonists. The potency and selectivity of the most potent members were comparable to or better than MPA or progesterone. These compounds represent a novel class of nonsteroidal PR agonists that are potentially useful in contraception and hormone replacement therapy.

Acknowledgements

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10. A representative procedure and analytical data of the selected examples. A mixture of 6-(3-chlorophenyl)-4,4-dimethyl-1,4-dihydro-benzo[*d*][1,3]oxazin-2-one (0.15 g, 0.5 mmol) and Lawesson's reagent (0.24 g, 0.6 mmol) in anhydrous toluene was heated at reflux under nitrogen for 3 hrs. The solvent was removed and the residue was purified by a flash chromatography (silica gel, hexane:ethyl acetate/6:1) to afford **6-(3-chlorophenyl)-4,4-dimethyl-1,4-dihydro-benzo[*d*][1,3]oxazine-2-thione** (**5**) as a white solid (80 mg, 52%): mp 183–184 °C; ^{1}H NMR (DMSO-d_6) δ 12.25 (s, 1H, D_2O exchangeable), 7.78 (t, 1H, J = 1.7 Hz), 7.63–7.70 (m, 3H), 7.49 (t, 1H, J = 7.8 Hz), 7.42 (d, 1H, J = 8.1 Hz), 7.12 (d, 1H, J = 8.8 Hz), 1.72 (s, 6H); MS (EI) m/z 303 (M^+, 100%), 305 (M^+, 32%). Anal. calcd for $C_{16}H_{14}ClNOS$: C, 63.26; H, 4.64; N, 4.61. Found: C, 63.37; H, 4.62; N, 4.54. **3-(4,4-Dimethyl-2-thioxo-1,4-dihydro-2H-benzo[*d*][1,3]oxazin-6-yl)-benzonitrile** (**8**). A white solid: mp 236-237 °C; ^{1}H NMR (DMSO-d_6) δ 12.3 (s, 1H), 8.24 (s, 1H), 8.05 (d, 1H, J = 8.07 Hz), 7.82 (d, 1H, J = 7.68 Hz), 7.74–7.64 (m, 3H), 7.14 (d, 1H, J = 8.78 Hz), 1.71 (s, 6H); MS (APCI) m/z 295 ($[M+H]^+$, 100%). Anal. calcd for $C_{17}H_{14}N_2OS{\cdot}0.2H_2O$: C, 68.46; H, 4.83; N, 9.40. Found: C, 68.35; H, 4.91; N, 9.07. **3-(4,4-Dimethyl-2-thioxo-1,4-dihydro-2H-benzo[*d*][1,3]oxazin-6-yl)-5-fluorobenzonitrile** (**15**). A yellow solid: mp 248–249 °C; ^{1}H NMR (DMSO-d_6) δ 12.3 (s, 1H), 8.15 (bs, 1H), 8.02 (d, 1H, J = 10.48 Hz), 7.85–7.78 (m, 3H), 7.13 (d, 1H, J = 8.92 Hz), 1.71 (s, 6H); MS (APCI) m/z 313($[M+H]^+$, 100%). Anal. calcd for $C_{17}H_{13}FN_2OS$: C, 65.37; H, 4.19; N, 8.97. Found: C, 65.26; H, 4.31; N, 8.61. **4-(4,4-Dimethyl-2-thioxo-1,4-dihydro-2H-benzo[*d*][1,3]oxazin-6-yl)-thiophene-2-carbonitrile** (**29**). A yellow solid: mp 242–243 °C; ^{1}H NMR (DMSO-d_6) δ 12.22 (s, 1H, D_2O exchangeable), 8.50 (d, 1H, J = 1.2 Hz), 8.37 (d, 1H, J = 1.0 Hz), 7.71 (m, 2H), 7.09 (d, 1H, J = 8.0 Hz), 1.69 (s, 6H); MS (APCI) m/z 301 ($[M+H]^+$, 100%). Anal. calcd for $C_{15}H_{12}N_2OS_2$: C, 59.97; H, 4.03; N, 9.33. Found: C, 59.67; H, 3.85; N, 9.14.
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