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SAR studies of 6-(arylamino)-4,4-disubstituted-1-methyl-1,4-dihydro-benzo[*d*][1,3]oxazin-2-ones as progesterone receptor antagonists☆

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Abstract—We previously disclosed that 6-aryl benzoxazin-2-ones were PR modulators. In a continuation of this work we examined the SAR of new 6-arylamino benzoxazinones and found the targets **1**–**25**, with an extra amino linker between the pendent 6-aryl groups and benzoxazinone or benzoxazine-2-thione core, were PR antagonists. A series of compounds with substituents at the 1- and 4-positions as well as different 6-aryl groups were prepared and tested in the T47D cell alkaline phosphatase assay. Interestingly, the SAR unveiled from the 6-arylamino benzoxazinones was quite different from those of their parent compounds. For example, in contrast to the 6-aryl benzoxazinones, methyl substitution at the 1-position significantly increased the potency of 6-arylamino benzoxazinones. Several 6-arylamino benzoxazinones (e.g., **12**, IC_{50} = 5.0 nM) had low nanomolar in vitro potency as PR antagonists in the T47D cell alkaline phosphatase assay.

Progesterone receptor (PR) is a member of the superfamily of ligand-dependent transcription factors.[1] Progesterone is an endogenous hormone which plays an important role in female reproduction. PR agonists have been used widely in contraceptives and hormone therapy, often in combination with an estrogen. A selective PR antagonist may be potentially used in female contraception,[2] and for the treatment of various gynecological and obstetric diseases including hormone-dependent cancers and non-malignant chronic conditions such as fibroids and endometriosis.[3,4] Clinically successful PR antagonists, however, are limited and their therapeutic potential has not yet been fully realized. Mifepristone, the only marketed PR antagonist, demonstrated activity at other steroidal receptors such as glucocorticoid (GR) and androgen receptors and was nearly equipotent as an antagonist for both PR and GR. This potentially limits its chronic use.[5] To search for more selective PR antagonists, a number of non-steroidal PR antagonists have been investigated.[6–9] Recently, we have disclosed three series of novel PR antagonists including 6-aryl benzimidazolones (e.g., **31**), 5-aryl oxindoles (e.g., **32**), and 6-aryl benzoxazinones (e.g., **33**).[10–12] Similar SAR trends were observed for the benzimidazolone and oxindole scaffolds. However, the PR functional activities of the 6-aryl benzoxazinones were dependent on the nature of the 6-aryl group and a few analogs showed modest PR agonist activity in the T47D cell alkaline phosphatase assay.[11] To further explore the SAR of 6-substitution from the benzoxazinone template, our strategy was to place a linker between the 6-aryl groups and benzoxazinone core. A number of oxygen, nitrogen, carbon, and sulfur based linkers were evaluated. 6-Arylamino benzoxazinones (**1**–**25**) were among the most potent PR antagonists. The synthesis and in vitro SAR of a series of novel 6-arylamino benzoxazinones as PR antagonists are the subject of this report.

The preparation of target compounds **1**–**25** is shown in Scheme 1. Addition of the appropriate Grignard reagent to ketones **26** afforded amino carbinols **27**. Ring closure

Keywords: Progesterone receptor (PR); Non-steroidal PR modulator; PR antagonists.
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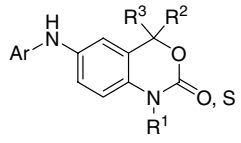
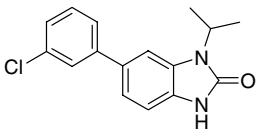
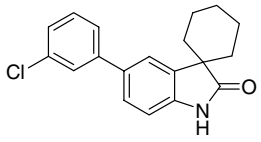


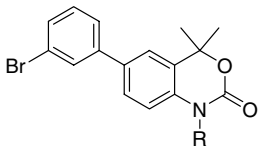
1-25



31, IC_{50} = 73.1 nM



32, IC_{50} = 56.0 nM



33a, R = H, IC_{50} = 3.3 nM
33b, R = CH_3, IC_{50} = 57.9 nM

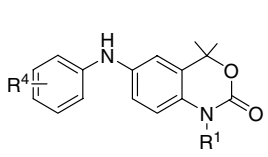
of alcohols **27** with carbonyldiimidazole provided the benzoxazinones **28**. Treatment of the cyclic carbamates **28** with 70% nitric acid yielded exclusive nitration at the 6-position resulting in **29**. Alkylation of **29** with various alkyl iodides followed by reduction of the nitro group afforded 1-alkylated intermediates **30** or direct reduction of **29** gave the 1-unsubstituted **30**. The benzoxazinone target compounds **1**–**8** and **11**–**25** were obtained by either coupling **30** to an appropriate boronic acid via a copper (II) acetate mediated protocol or by a palladium catalyzed reaction with an appropriate aryl iodide. The benzoxazine-2-thiones **9** and **10** were readily prepared by refluxing compounds **3** and **8** with Lawesson's reagent in toluene.

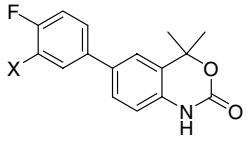
Among three series of PR antagonists we reported recently,[10–12] the 6-aryl benzoxazinone template (e.g., **33**) provided the most potent and selective PR antagonists as demonstrated in a number of in vitro and in vivo models. In addition, the PR functional activities of the 6-aryl benzoxazinones were dependent on the nature of the 6-aryl group.[11] Thus, the benzoxazinone template can provide, with a prudent choice of 6-aryl group, both PR agonists and antagonists. We decided to place a linker between the 6-aryl moiety and benzoxazinone core and examine the SAR of various linkers on the PR potency and functional activity of target compounds. From a handful of linkers that were screened, compounds with an NH linker were among the most potent PR antagonists. We therefore focused our effort on the 6-arylamino benzoxazinone template and prepared a number of compounds with various substitution patterns. These derivatives were evaluated in the T47D cell alkaline phosphatase assay, the results are detailed in Tables 1–4.

As shown in Table 1, 6-arylamino benzoxazinones methylated at the 1-position (**2**) resulted in about 4-fold improvement in PR antagonist potency over their des-methyl analogs **1**. This is interesting since the NH moiety at the 1-position has been demonstrated to play a critical role in the PR potency for the previously reported benzoxazinone scaffold (e.g., 6-(3-bromophenyl)-1,4,4-trimethyl-1*H*-benzo[*d*][1,3]oxazin-2(4*H*)-one **33b** is more than 10-fold less potent than its des-methyl analog **33a**). This finding suggested that 6-arylamino benzoxazinones and their parent compounds (e.g., **33**) may interact differently at the PR binding pocket.

Interestingly, alkylation of the 1-position with a group larger than the methyl group resulted in derivatives with reduced PR antagonist potency (Table 1). The 1-methyl benzoxazinone **3** exhibited moderate PR antagonist potency with an IC_{50} = 54.4 nM. Increasing the size of the 1-position substituent in compounds **4**–**7** caused a 17-fold or greater reduction in PR potency suggesting a size limitation at this position.

Table 1. Inhibition of progesterone induced alkaline phosphatase activity in T47D cells. SAR at the 1-position



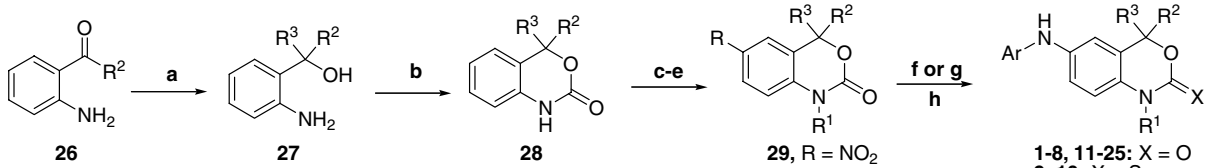


33c, R = Cl
33d, R = CN

Compound	R^1	R^4	PR Alk. Phos. IC_{50}[a] (nM)
1	H	2′-Cl, 3′-Cl	205.8
2	CH_3	2′-Cl, 3′-Cl	56.0
3	CH_3	4′-Br	54.4
4	C_2H_5	4′-Br	1000
5	*i*-C_3H_7	4′-Br	1000
6	Phenyl	4′-Br	3000
7	Benzyl	4′-Br	3000
33c			23.0
33d			15.1

[a] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviation for the assay was ±20% of the mean or less.

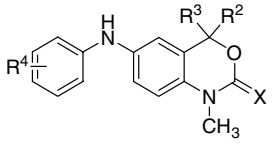
6-Arylamino benzoxazin-2-ones and benzoxazine-2-thiones



26 **27** **28** **29**, R = NO_2 **30**, R = NH_2 **1-8, 11-25:** X = O **9, 10:** X = S

Scheme 1. Reagents and conditions: (a) R^3MgBr, THF, 0 °C, N_2; (b) CDI, THF, rt, (c) HNO_3, H_2SO_4, AcOH; (d) NaH, THF, 0 °C, N_2, then R^1X; (e) $NaBH_4$, MeOH, Pd/C, 0 °C, N_2; (f) $Cu(OAC)_2$, Et_3N, aryl boronic acid, CH_2Cl_2, rt; (g) $Pd_2(dba)_3$, Cs_2CO_3, BINAP, 18-C-6, aryl iodide, THF, 40 °C; (h) Lawesson's reagent, toluene, reflux, N_2.

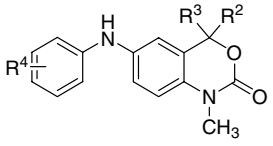
Table 2. Inhibition of progesterone induced alkaline phosphatase activity in T47D cells. SAR at the 2-position



Compound	R^2/R^3	R^4	X	PR Alk. Phos. IC_{50}[a] (nM)
3	CH_3	4′-Br	O	54.4
8	C_2H_5	3′-F, 4′-F	O	27.4
9	CH_3	4′-Br	S	30.0
10	C_2H_5	3′-F, 4′-F	S	42.2
33c				23.0
33d				15.1

[a] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviation for the assay was ±20% of the mean or less.

Table 3. Inhibition of progesterone induced alkaline phosphatase activity in T47D cells. SAR at the 4-position

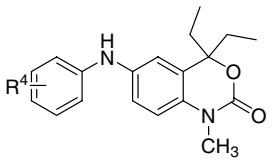


Compound	R^2	R^3	R^4	PR Alk. Phos. IC_{50}[a] (nM)
2	CH_3	CH_3	2′-Cl, 3′-Cl	56.0
11	CH_3	CH_3	3′-Cl, 4′-Cl	100.0
12	C_2H_5	C_2H_5	2′-Cl, 3′-Cl	5.0
13	C_2H_5	C_2H_5	3′-Cl, 4′-Cl	29.8
14	C_2H_5	C_2H_5	3′-Cl	13.1
15	C_2H_5	Thien-2-yl	3′-Cl	32.2
16	C_2H_5	Phenyl	3′-Cl	66.1
17	C_2H_5	4-Cl-phenyl	3′-Cl	71.8
18	4-Cl-phenyl	4-Cl-phenyl	3′-Cl	1000
33c				23.0
33d				15.1

[a] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviation for the assay was ±20% of the mean or less.

Substitution of a thio-carbonyl group at the 2-position for the carbonyl moiety, in general, switched PR antagonists to agonists for the oxindole (e.g., **32**) and benzoxazinone (e.g., **33**) templates.[13,14] However, as illustrated in Table 2, this functional activity switch did not take place in the 6-arylamino benzoxazinone scaffold. The benzoxazine-2-thiones **9** and **10** remained as PR antagonists (IC_{50} = 30.0, 42.2 nM) and had similar potency as their parent carbonyl compounds **3** and **8** (IC_{50} = 54.4, 27.4 nM).

Table 4. Inhibition of progesterone induced alkaline phosphatase activity in T47D cells. SAR of the 6-aryl group



Compound	R^4	PR Alk. Phos. IC_{50}[a] (nM)
12	2′-Cl, 3′-Cl	5.0
13	3′-Cl, 4′-Cl	29.8
14	3′-Cl	13.1
19	3′-F, 4′-F	27.4
20	3′-Cl, 4′-F	9.0
21	3′-F, 4′-CN	37.1
22	3′-CN, 4′-F	9.2
23	3′-NO_2, 5′-F	8.4
24	3′-Cl, 5′-Cl	8.1
25	3′-CN, 5′-F	14.5
33c		23.0
33d		15.1

[a] 50% inhibitory concentration of tested compounds on 1 nM progesterone induced alkaline phosphatase activity in the human T47D breast carcinoma cell line. Values represent the average of at least duplicate determinations. The standard deviation for the assay was ±20% of the mean or less.

As shown in Table 3, 6-arylamino benzoxazinones substituted at the 4-position by diethyl (**12** and **13**) showed modest improvement (3- to 10-fold) in PR antagonist potency over their dimethyl substituted counterparts (**2** and **11**). The 4-position was further explored by replacing one of the ethyl groups with various aryl moieties (**15**–**17**) shown in Table 3. In these examples no further improvement was observed. Instead, racemic compounds **15**–**17** were at least 2-fold less potent than their parent compound **14**. Replacement of both ethyl groups of compound **14** with the 4′-chlorophenyl group (**18**) resulted in a dramatic loss in antagonist potency.

Using the optimized 4,4-diethyl-1-methylbenzoxazinone core, a number of 6-aryl groups containing various electron-withdrawing substituents were examined as depicted in Table 4. Overall, these analogs had good to excellent PR antagonist potency (IC_{50} = 5.0–37.4 nM). Compounds **20** and **22** had IC_{50} of 9.0 and 9.2 nM and were more potent than their corresponding 6-aryl benzoxazinone analogs[11] **33c** and **33d** (IC_{50} = 23.0 and 15.1 nM, respectively).

In summary, we have demonstrated that 6-arylamino benzoxazinones are PR antagonists, which have a different SAR when compared to that of parent 6-aryl benzoxazinones. In contrast to their parent compounds, a methyl substitution at the 1-position significantly increased the potency. Diethyl substitution at the 4-position was found to be optimal. Several 6-arylamino benzoxazinones (e.g., **12**, IC_{50} = 5.0 nM) had low nanomolar in vitro potency and were potent PR antagonists in the T47D cell alkaline phosphatase assay.

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