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Potent Nonsteroidal Progesterone Receptor Agonists: Synthesis and SAR Study of 6-Aryl Benzoxazines†

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Abstract—Novel 6-aryl benzoxazines were prepared and examined as progesterone receptor (PR) modulators. In contrast to the structurally related 6-aryl dihydroquinoline PR antagonists, the 6-aryl benzoxazines were potent PR agonists. Compounds **4e**, **5b**, and **6a** with the 2,4,4-trimethyl-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazine core were the most potent PR agonists in the series with subnanomolar activities (EC_{50} 0.20–0.35 nM). Compound **6a** was more potent than progesterone (**P4**) in the in vivo decidualization assay in an ovariectomized female rat model by subcutaneous administration with an ED_{50} of 1.5 mg/kg (vs 5.62 mg/kg for **P4**).

The progesterone receptor (PR) is one of the intracellular gene regulators known as '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 in combination with estrogens. A selective PR antagonist is potentially useful for the treatment of hormone dependent cancers,[2] nonmalignant chronic conditions such as fibroids,[3] and endometriosis.[4] However, many of the steroidal PR modulators (e.g., mifepristone) have shown undesirable cross-reactivities with other steroid receptors such as the glucocorticoid and androgen receptors promoting a need for newer, more selective PR modulators.

Recently, several classes of the nonsteroidal PR modulators have been reported.[5] Among a number of interesting series, 6-aryl dihydroquinolines, such as 3-fluoro-5-(2,2,4-trimethyl-1,2-dihydro-quinolin-6-yl)-benzonitrile, have shown good PR antagonist activity.[5e] We prepared and evaluated a number of novel 6-aryl-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazines (**4a**–**6h**) as potential 6-aryl dihyroquinoline scaffold replacements. However, in contrast to the 6-aryl dihydroquinolines, most of our series were potent PR agonists. The synthesis and SAR of novel 6-aryl benzoxazines will be discussed in this report.

Synthesis

A general synthesis of 6-aryl benzoxazines is illustrated in Scheme 1. Treatment of compounds **1a**,**b** with an appropriate Grignard reagent formed *o*-amino carbinols (**2**) in good yield. Ring closure of **2** with an aldehyde or a ketone gave 6-bromo benzoxazines (**3**). A Suzuki cross-coupling of compound **3** with an arylboronic acid generated 6-aryl benzoxazines (**4a**–**6e**). In the case when an arylboronic acid was not available, **3** was converted to the arylborate following a literature procedure[6] and cross-coupled in situ with a suitable haloarene to generate compounds **4a**–**6e**.[7] Alkylation or acylation of **6c** at position 1 afforded 1-substituted 6-aryl benzoxazines (**6f**–**h**).

Results and Discussion

The novel 6-aryl-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazines were evaluated in the alkaline phosphatase[8] and PR competition binding assays using the human T47D breast carcinoma cell line.[8] The results of their PR

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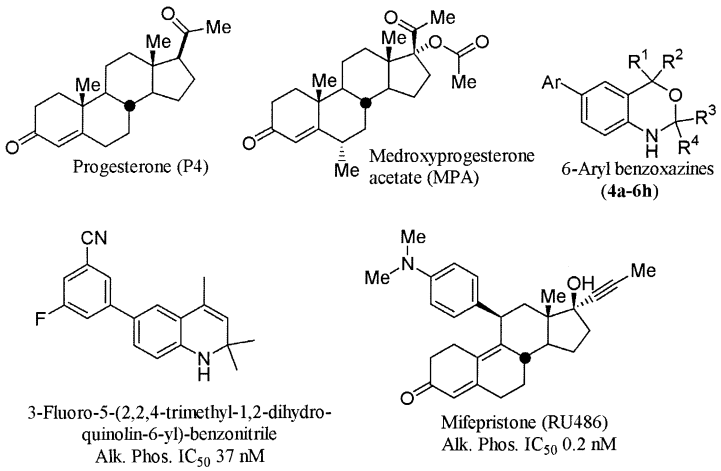


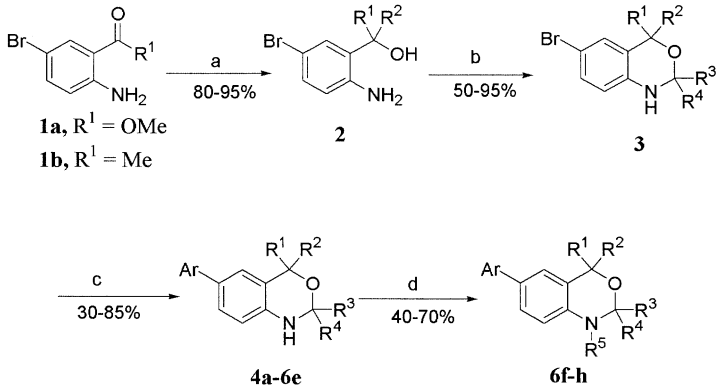
agonist, antagonist activities and relative binding affinities are summarized in Table 1. 6-Aryl benzoxazines with the 2,4,4-trimethyl-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazine core were the most potent PR agonists (Table 1). For example, compounds **4e**, **5b**, and **6a** elicited PR agonist activities in the alkaline phosphatase assay in the sub-nanomole range (EC_{50} 0.20–0.35 nM) and were more potent PR agonists than progesterone (EC_{50} 0.92 nM) and comparable to MPA (EC_{50} 0.12 nM, Table 1).

The nature and position of the substituents on the 6-aryl moieties in the compounds in Table 1 had a significant effect on the potency of the compounds. For instance, the *para*-fluoro substituent on **4n** (EC_{50} 23 nM and binding IC_{50} 166.0, respectively) significantly reduced the PR agonist activity and binding affinity compared to the *meta*-fluoro regio-isomer **4e** (EC_{50} 0.35 nM, binding IC_{50} 7.3 nM). The extra *para*-fluoro substitutent in **4o** reduced T47D alkaline phosphatase activity and binding affinity more than 10-fold compared to **4b**. Furthermore, the additional *meta*-nitrile substituent in **4e** compared to compounds **4a** caused about an order of magnitude increase in the T47D alkaline phosphatase potency.

Replacing one of the 4-methyl groups of **4e** with a phenyl moiety reduced activity of the resulting compound **4f** in the T47D alkaline phosphatase assay more than 20-fold. Furthermore, compounds **5c** and **6b** with a spiro-cyclohexyl group at position 4 were 10 times less potent than their 4,4-dimethyl congeners **5b** and **6a**.

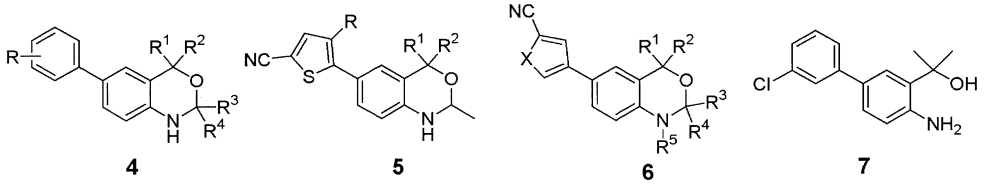
At position 2, substitution of methyl group for trifluoromethyl moiety marginally reduced the PR agonist activities in the alkaline phosphatase assay (Table 1). For example, 2-trifluromethylated compounds **4g** and **6c** were less active than their corresponding 2-methyl substituted analogues **4e** and **6a**. Introduction of an additional 2-methyl group to compounds **4b**, **4e**, and **6a** led to the dimethylated compounds **4d**, **4h**, and **6d** (alk. phos. EC_{50} 9.2–32.0 nM) which were much less active than their mono-methylated congeners. More surprisingly, when the size of the 2-substituent was increased by replacing the methyl group with an isopropyl, *t*-butyl, thien-2-yl, phenyl, and ethoxyl carbonyl moieties, the resulting compounds **4i–m** switched from PR agonists to PR antagonists (Table 1).

The NH moiety at position 1 was necessary for activity. Replacing the NH hydrogen atom of **6c** with an acetyl (**6f**) or a carbomethoxy group (**6g**) led to loss of activity at the highest concentration tested. Compound **6h** showed similar functional activity and binding affinity compared to its parent compound (**6c**). Presumably, **6h** was not stable and converted to compound **6c** under the conditions of assays used in our studies.



Scheme 1. Reagents and conditions: (a) R^2MgBr, ether, 0 °C; (b) R^3R^4CO, *p*-TsOH, toluene, rt; (c) $ArB(OH)_2$, $Pd(Ph_3P)_4$, Na_2CO_3, DME/H_2O, 85 °C or bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMF, 80 °C; ArBr, $Pd(dppf)Cl_2$, 2 N Na_2CO_3; (d) R^5Cl, NaH, DMF, rt.

Table 1. Alkaline phosphatase activities and binding affinities of **P4**, **MPA**, and 6-aryl benzoxazines



4 **5** **6** **7**

Compd	R	R^1	R^2	R^3	R^4	R^5	X	Alk. phos. EC_{50} (nM)[a]	Alk. phos. IC_{50} (nM)[a]	PR binding IC_{50} (nM)[a]
P4								0.92		3.5
MPA								0.12		10.8
4a	3-F	Me	Me	Me	H			2.60		23.0
4b	3-Cl	Me	Me	Me	H			2.40		9.0
4c	3-Cl	Me	Me	CF_3	H			2.9		41.3
4d	3-Cl	Me	Me	Me	Me			32.0		10.0
4e	3-CN, 5-F	Me	Me	Me	H			0.35		7.3
4f	3-CN, 5-F	Me	Ph	Me	H			8.20		24.7
4g	3-CN, 5-F	Me	Me	CF_3	H			1.7		7.1
4h	3-CN, 5-F	Me	Me	Me	Me			10.9		42.0
4i	3-CN, 5-F	Me	Me	$CH(CH_3)_2$	H			>10,000	47.0	ND[b]
4j	3-CN, 5-F	Me	Me	$C(CH_3)_3$	H			>10,000	126.6	3042
4k	3-CN, 5-F	Me	Me	Thien-2-yl	H			>10,000	51.2	1682
4l	3-CN, 5-F	Me	Me	Ph	H			>10,000	92.6	2060
4m	3-CN, 5-F	Me	Me	COOEt	H			>10,000	158.8	1793
4n	3-CN, 4-F	Me	Me	Me	H			23.0		166.0
4o	3-Cl, 4-F	Me	Me	Me	H			32.0		212.5
5a	H	Me	Me					1.05		27.0
5b	Me	Me	Me					0.20		8.1
5c	Me		Cyclohexyl					2.40		2.9
6a		Me	Me	Me	H	H	S	0.35		11.5
6b			Cyclohexyl	Me	H	H	S	3.50		12.0
6c		Me	Me	CF_3	H	H	S	0.8		4.9
6d		Me	Me	Me	Me	H	S	9.2		ND
6e		Me	Me	Me	H	H	O	4.55		113.4
6f		Me	Me	CF_3	H	COMe	S	>10,000		2087
6g		Me	Me	CF_3	H	COOMe	S	>10,000		ND
6h		Me	Me	CF_3	H	$CH_2OCOtBu$	S	0.9		7.9
7[c]								>10,000	103.0	301.0

[a]Experimental values represent the average of at least duplicate determinations.[8] The standard deviations for these assays were typically ±20% of mean or less.
[b]ND, not determined.
[c]Compound **7** was prepared by an acidic hydrolysis of compound **4b**.

The hemiaminal ring of our compounds could conceivably hydrolyze to an amino carbinol under appropriate conditions. This does not appear to be the case under the conditions of assays used in our studies for amino carbinol **7** was a weak PR antagonist when compared to the corresponding potent hemiaminal-containing agonists **4b–d**.

The in vivo decidualization activities of **6a** was evaluated and compared to **P4** and **MPA** in an ovariectomized mature female Sprague–Dawley rat model (~60-day old and 230 g) by subcutaneous injection (Table 2).[9] As illustrated from Table 2, **6a** showed potent in vivo decidualization activity and was more potent than **P4**.

In summary, novel 6-aryl benzoxazines as PR modulators were synthesized and evaluated in the in vitro and in vivo PR assays. The most potent compounds **4e**, **5b**, and **6a** in the series showed PR agonist activities comparable to MPA in the in vitro alkaline phosphatase assay and represented some of the most potent non-steroidal PR agonists to date. These compounds also demonstrated good binding activities in the PR competition binding assay. Compound **6a** was also active SC in the in vivo rat decidualization assay.

The structure–activity relationships of 6-aryl benzoxazines were examined at positions 1, 2, 4, and 6. While the nature of the substituent at positions 1, 4, and 6 played an important role in the potency and binding affinities of these compounds as PR agonists, the size of substituent at position 2 dictated the functional profile of the 6-aryl benzoxazines. The large groups at position 2 such as isopropyl or phenyl group switched the compounds from PR agonists to PR antagonists. This information was useful for the design of new PR

Table 2. Rat decidualization activities of **P4**, **MPA**, and **6a**

Compd	**P4**	**MPA**	**6a**
Decidualization ED_{50} (mg/kg)[a]	5.62	0.40	1.50

[a]Experimental values represent the average of at least duplicate determinations.[9] The standard deviation for the decidualzation assay was typically ±15% of mean or less.

agonists or antagonists using the 6-aryl benzoxazine scaffold.

Acknowledgement

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7. Analytical data of most potent 6-aryl benzoxazines. **3-Fluoro-5-(2,4,4-trimethyl-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl)benzonitrile** (**4e**). A white solid: mp 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 8.02 (t, 1H, J = 1.5 Hz), 7.87 (dt, 1H, J = 10.6, 2.2 Hz), 7.65 (m, 1H), 7.55 (d, 1H, J = 2.2 Hz), 7.44 (dd, 1H, J = 8.4, 2.2 Hz), 6.63 (d, 1H, J = 8.4 Hz), 6.58 (s, 1H), 4.82 (m, 1H), 1.52 (s, 3H), 1.50 (s, 3H), 1.28 (d, 3H, J = 5.1 Hz); MS (ESI) m/z 295 $[M-H]^-$. Anal. calcd for $C_{18}H_{17}FN_2O$: C, 72.96; H, 5.78; N, 9.45. Found: C, 72.63; H, 5.82; N, 9.27. **3-[4,4-Dimethyl-2-(trifluoromethyl)-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl]-5-fluorobenzonitrile** (**4g**). A white solid: mp 102–103 °C; ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1H), 7.91 (d, 1H, J = 10.7 Hz), 7.7 (d, 1H, J = 10.7 Hz) 7.62 (d, 1H, J = 1.98 Hz), 7.53 (dd, 1H, J = 8.33, 1.98 Hz), 7.05 (s, 1H), 6.87 (d, 1H, J = 8.33 Hz), 5.39–5.37 (m, 1H), 1.61 (s, 3H), 1.59 (s, 3H); MS (ES) m/z 349 $[M-H]^-$. Anal. calcd for $C_{18}H_{14}F_4N_2O$: C, 61.72; H, 4.03; N, 8.00. Found: C, 61.66; H, 3.94; N, 7.79. **5-(2,4,4-Trimethyl-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl)thiophene-2-carbonitrile** (**5a**). An off-white solid: mp 172–173 °C; ^{1}H NMR (DMSO-d_3) δ 7.88 (d, 1H, J = 4.0 Hz), 7.47 (d, 1H, J = 4.0 Hz), 7.43 (d, 1H, J = 2.0 Hz), 7.32 (dd, 1H, J = 8.36, 2.4 Hz), 6.77 (s, 1H), 6.60 (d, 1H, J = 8.4 Hz), 4.83 (m, 1H), 1.51 (s, 3H), 1.48 (s, 3H), 1.28 (d, 3H, J = 5.6 Hz); MS (ESI) m/z 283 $[M-H]^-$. Anal. calcd for $C_{16}H_{16}N_2OS{\cdot}0.2H_2O$: C, 66.73; H, 5.74; N, 9.73. Found: C, 66.82; H, 5.61; N, 9.77. **4-Methyl-5-(2,4,4-trimethyl-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl)thiophene-2-carbonitrile** (**5b**). A yellowish solid: mp 145–146 °C; ^{1}H NMR (DMSO-d_6) δ 7.79 (s, 1H), 7.18 (d, 1H, J = 2.0 Hz), 7.13 (dd, 1H, J = 8.4, 2.0 Hz), 6.68 (s, 1H), 6.54 (d, 1H, J = 8.3 Hz), 4.83 (m, 1H), 2.26 (s, 3H), 1.49 (s, 3H), 1.46 (s, 3H), 1.28 (d, 3H, J = 5.5 Hz); MS (ESI) m/z 299 $[M+H]^+$. Anal. calcd for $C_{17}H_{18}N_2OS$: C, 68.43; H, 6.08; N, 9.39. Found: C, 68.29; H, 6.11; N, 9.31. **4-(2,4,4-Trimethyl-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl)thiophene-2-carbonitrile** (**6a**). An off-white solid: mp 175–176 °C; ^{1}H NMR (DMSO-d_6) δ 8.39 (d, 1H, J = 1.5 Hz), 8.13 (d, 1H, J = 1.5 Hz), 7.47 (d, 1H, J = 1.9 Hz), 7.36 (dd, 1H, J = 8.4, 1.9 Hz), 6.59 (d, 1H, J = 8.4 Hz), 6.41 (s, 1H), 4.78 (m, 1H), 1.51 (s, 3H), 1.47 (s, 3H), 1.28 (d, 3H, J = 5.4 Hz); MS (ESI) m/z 285 $[M+H]^+$. Anal. calcd for $C_{16}H_{16}N_2OS{\cdot}0.2H_2O$: C, 66.73; H, 5.74; N, 9.73. Found: C, 66.82; H, 5.54; N, 9.87. **4-[4,4-Dimethyl-2-(trifluoromethyl)-1,4-dihydro-2*H*-3,1-benzoxazin-6-yl]thiophene-2-carbonitrile** (**6c**). A yellow solid: ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H), 8.19 (s, 1H), 7.54 (d, 1H, J = 1.98 Hz), 7.46 (dd, 1H, J = 8.33, 1.98 Hz), 6.90 (s, 1H), 6.83 (d, 1H, J = 8.33 Hz), 5.35 (d, 1H, J = 3.57 Hz), 1.59 (s, 3H), 1.57 (s, 3H); MS (ES) m/z 337 $[M-H]^-$.
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