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A NEW SERIES OF SELECTIVE DOPAMINE D_4 LIGANDS: 3-([4-ARYLPIPERAZIN-1-YL]ALKYLAMINO)-2*H*-1,4-BENZOXAZINES

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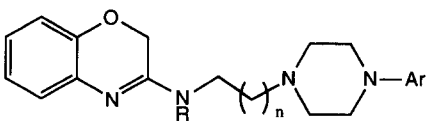
Abstract: A series of 3-[(4-arylpiperaz-1-yl)alkylamino]-2*H*-1,4-benzoxazines were prepared and their affinities for cloned human D_2, D_3, and D_4 dopamine receptor subtypes were measured. This led to the identification of **1a**, **1f**, and **1g** as high affinity selective antagonists at the D_4 receptor. © 1997 Elsevier Science Ltd.

From a survey of the recent literature, it is clear that the discovery of highly selective antagonists for the dopamine D_4 receptor is a priority for many research laboratories.[1] Interest in this area was initially spurred by the discovery that the atypical antipsychotic drug clozapine had a preference for this subclass of dopamine receptors[2] and also by the disputed[3] finding of increased concentrations of dopamine D_4 receptors in the post-mortem brains of schizophrenics.[4,5]

Recent reports of D_4 selective ligands from the scientific and patent literature show that the receptor is apparently receptive to a variety of classes of compounds.[1] As a further diversification of the family of heterocylic templates selective for this receptor subclass, we herein describe our results with the 3-[(4-arylpiperaz-1-yl)alkylamino]-2*H*-1,4-benzoxazines (Formula 1). The SAR of compounds of general Formula 1 for the D_2, D_3, and D_4 receptors was examined through the variation of three structural features, which are (a) the length the alkyl chain connecting the aryl piperazine and 3-aminobenzoxazine (b) the nature of the arylpiperazine moiety, and (c) the effect of an *N*-alkyl substituent (*R*) on the 3-amino nitrogen of the 1,4-benzoxazine. Alkyl chain lengths of 2 to 4 carbons were examined since the starting materials for these were commercially available.



Formula 1

Chemistry

Compounds of Formula 1 were prepared as shown in Scheme 1 via the condensation of 3-methoxy-2*H*-1,4-benzoxazine with the appropriate 1-aryl-4-aminoalkylpiperazine. 3-Methoxy-2*H*-1,4-benzoxazine (**3**) was prepared by the treatment of 2*H*-1,4-benzoxazin-3-one (**2**) with 1.1 equivalent Meerwein's salt in chloroform. The required 1-aryl-4-aminoalkylpiperazines (**6**) were obtained by the condensation of the appropriate

arylpiperazine (**4**) with the commercially available bromoalkylphthalimides (**5**) followed by treatment with hydrazine hydrate. The *N*-methyl derivative **7** was prepared from **1f** via deprotonation with NaH followed by treatment with iodomethane and purification using preparative TLC.

Results and Discussion

The affinities of the compounds for D_2, D_3, and D_4 dopamine receptor subtypes determined using standard displacement binding assays are shown in Table 1. Cloned human D_2, D_3, and D_4 receptors stably expressed in a CHO cell line were used as the binding substrate and [^{3}H] YM-09151 was used as the competitive ligand. All assays were carried out in triplicate.

An examination of the *N*-phenyl and *N*-pyrimidin-2-yl compounds (**1a** - **1c** and **1d** - **1f**, respectively) indicates that a chain length of 2 gave optimal D_4 binding in the former series, while a chain length of 4 was optimal in the latter. With the exception of **1c**, affinity at D_3 receptors was found to be minimal at all chain lengths. Using these preferred chain lengths as a guide, the nature of the aryl moiety was further examined through the use of 2-pyridyl (**1g** and **1h**), 2-quinolyl (**1i**), and the 2-methoxyphenyl (**1j**) piperazines. At equivalent chain lengths, the binding profiles of the pyridyl derivatives (**1g** and **1h**) more closely resemble those of the phenyl compounds (**1a** and**1c**) than those of the pyrimidine (**1d** and **1f**). Compound **1i** showed somewhat diminished D_4 binding relative to its pyridyl counterpart **1h**. The 2-methoxyphenyl derivative **1j** displayed much higher D_2 affinity than the equivalent unsubstituted compound **1a**. A similar effect of such 2-methoxy substitution has previously been noted in other series.[7]

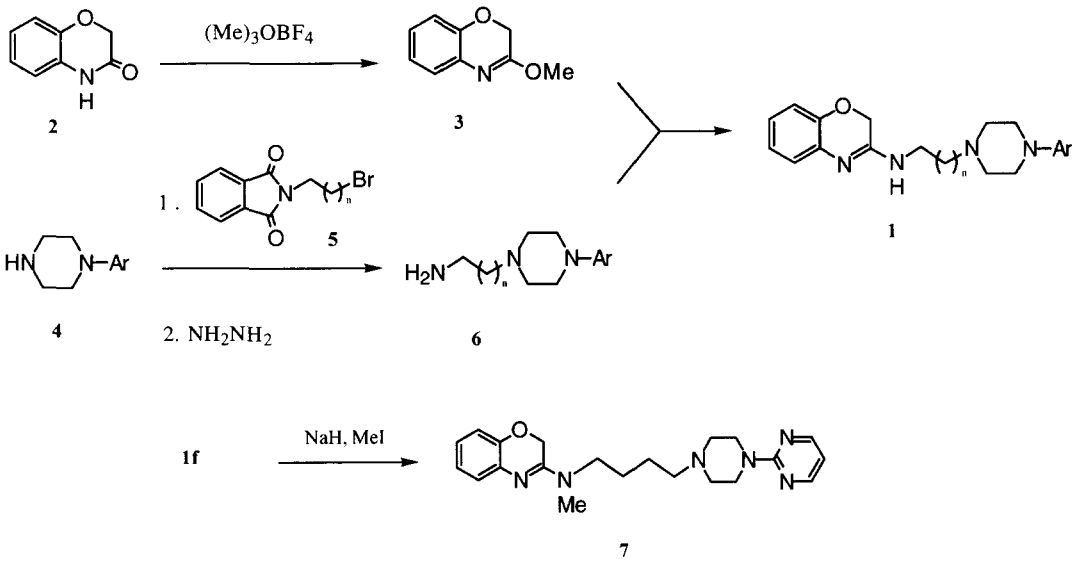
The effect a tertiary amine at the 2-amino position of the 2-aminobenzoxazines was examined through one example. Compound **7** demonstrated a highly diminished affinity for D_4 receptor sites (K_i = 558 ± 39) as compared to its secondary derivative **1f**.

Table 1. Binding Affinities (K_i in nM)[a] of 3-[(4-arylpiperaz-1-yl)alkylamino]-2H-1,4-benzoxazines[b] at Cloned Human Dopamine Receptor Subtypes.

cmpd	aryl	n	D_2	D_3	D_4	m.p °C	salt
1a	phenyl	2	2011 ± 477	3437 ± 615	18 ± 7	160-162	fum
1b	phenyl	3	2745 ± 801	>4000	188 ± 71	154-158	fum
1c	phenyl	4	94 ± 33	167 ± 8	52 ± 7	290-292	HBr
1d	pyrimidin-2-yl	2	>4000	>7900	107 ± 37	165-167	fum
1e	pyrimidin-2-yl	3	>4000	>7900	161 ± 31	175-178	fum
1f	pyrimidin-2-yl	4	2540 ± 460	1811 ± 611	19 ± 4	174-178	fum
1g	pyridin-2-yl	2	>4000	>7900	21 ± 9	168-172	fum
1h	pyridin-2-yl	4	543 ± 119	408 ± 112	50 ±13	116-120	fum
1i	quinolin-2-yl	4	nd	nd	125 ± 29	205-207	HBr
1j	2-methoxyphenyl	2	280 ± 12	>1000	3 ± 1.81	183-184	fum

[a] Binding data are the means of at least three independent experiments using standard displacement assays with [^{3}H]YM 09151 as the competitive ligand and the human dopamine receptor subtypes expressed in CHO cells. [b] For representative ^{1}H NMR see note 7.

Scheme 1. Preparation of 3-[(4-Arylpiperazin-1-yl)alkylamino] 2H-1,4-benzoxazines.



Agonist-stimulated GTPγ^{35}S binding has been widely used for many G protein coupled receptors and offers the possibility to distinguish agonists from antagonists.[8] The *in vitro* functional activities of compounds **1a**, **1f**, and **1g** were examined via the inhibition of 333 nM dopamine stimulated GTP binding in CHO cells expressing human D_4 receptors. Within this assay, compounds **1a**, **1f**, and **1g** were shown to be functional antagonists with IC_{50} values for inhibition of 95.0, 95.0 and 27.3 nm, respectively.

In conclusion, selected members of the 3-[(4-arylpiperaz-1-yl)alkylamino]-2*H*-1,4-benzoxazine series have proven themselves to be selective D_4 ligands. Most notable are the phenyl derivative **1a**, the pyridyl derivative **1g** and the pyrimidine derivative **1f** which display a greater than 60-fold selectivity for the D_4 receptor over D_2 and D_3. Although this study did not examine chain lengths longer than C-4, the determination that chain lengths of C-2 and C-4 were optimal for D_4 binding, combined with the necessity of a secondary amine at the 3-position of the benzoxazine suggests the possibility that some folding of the C-4 derivatives may occur within the D_4 receptor site. Preparation of extended chain derivatives and molecular modeling studies are currently under way to examine these possibilities.

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7. ^{1}H NMR: **1a** ($CDCl_3$) 7.25 (m, 2H), 7.13 (dd, J = 7.6, 2 Hz), 6.82 (m, 6H), 5.1 (b, NH), 4.4 (s, 1H), 3.59 (t, J = 5.2, 2H), 3.2 (bt, J = 5 Hz, 4H), 2.65 (m, 6H). **1f** ($CDCl_3$) 8.29 (d, J = 4 Hz, 2H), 7.1 (dd, J = 9, 3 Hz, 1H), 6.87 (m, 3H), 6.47 (d, J = 5 Hz, 1H) 4.35 (s, 2H), 3.8 (bt, J = 7 Hz, 4H), 3.48 (t, J = 7 Hz, 2H), 2.5 (t, J = 7 Hz, 4H), 2.4 (t, J = 7 Hz, 2H), 1.66 (m, 4H). **1g** ($CDCl_3$) 8.2 (dd, J = 6, 3 Hz, 1 H), 7.5 (dt, J = 8, 2 Hz, 1H), 7.1 (dd, J = 8, 2 Hz, 1H), 6.9 (m, 3H), 6.6 n, 2H), 5.2 (b, 1H, NH), 4.43 (s, 2H), 3.6 (t, J = 6 Hz, 2H), 3.36 (t, J = 6 Hz, 4H), 2.66 (t, J = 6 Hz, 2H), 2.60 (t, J = 6 Hz, 4H).

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