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1,2,4-Benzothiadiazine derivatives as α_1 and 5-HT_{1A} receptor ligands

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Abstract—A series of new 1,2,4-benzothiadiazine derivatives with an arylpiperazine mojety linked at position 3 of the heterocyclic ring were synthesized and assessed for their pharmacological profiles at α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}) by functional experiments and by in vitro binding studies at human cloned 5-HT_{1A} receptor. Compound **1** was identified as a novel α_{1D} antagonist ($pK_b\alpha_{1D} = 7.59$; $\alpha_{1D}/\alpha_{1A} > 389$; $\alpha_{1D}/\alpha_{1B} = 135$) with high selectivity over 5-HT_{1A} receptor (5-$HT_{1A}/\alpha_{1D} < 0.01$), while compound **6**, a 3,4-dihydro-derivative, was characterized as a novel 5-HT_{1A} receptor ligand, highly selective over α_{1D}-adrenoceptor subtype (pK_i5-$HT_{1A} = 8.04$; 5-$HT_{1A}/\alpha_{1D} = 1096$). Further pharmacological studies demonstrated that **6** is a partial agonist at 5-HT_{1A} receptor ($E_{max} = 23$, $pD_2 = 6.92$).

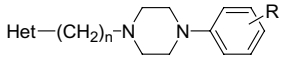
The existence of multiple adrenergic receptors is now firmly established and this has given new impulse to medicinal chemists for the search of new and more selective ligands.[1] The α_{1A} subtype has received much attention as a potential target for symptomatic treatment of benign prostatic hyperplasia (BPH) and several uroselective agents have been disclosed.[2] On the contrary a potential therapeutic use for α_{1B} and α_{1D} subtype selective ligands has not been found yet.

Receptor for serotonin (5-HT) represent also an heterogeneous population and seven distinct classes (5-HT_1 to 5-HT_7) have been identified according to structural diversity and preferred effector mechanism.[3] During the last decade, the 5-HT_{1A} subtype has been the most studied as a major target for neurological research and drug development.[4,5]

The α_1 and 5-HT_{1A} receptors belong to the class of G-protein coupled receptors, characterized by an heptahelical transmembrane structure which shows some common features in their binding sites.[6]

As a consequence, a large number of compounds show high affinity for both receptors and consequently poor selectivity.

A widely accepted model of adrenergic and serotoninergic ligands provides the presence of an arylpiperazine moiety, a linker and a heterocycle system.



The arylpiperazine represents the pharmacophore moiety, while the selectivity 5-HT_{1A}/α_1-adrenergic receptors is modulate by the type and the position of the substituent on the aromatic ring of the arylpiperazine moiety, by the length of the spacer and by the nature of the heterocycle system.[7]

On the bases of this model our research group has undertaken a research project aimed at developing new and selective ligands in order to get new insight into

Keywords: 5-HT_{1A} agonist; α_{1D} antagonist; Benzothiadiazine; Arylpiperazine.
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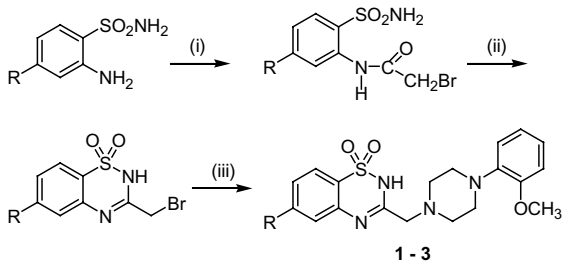
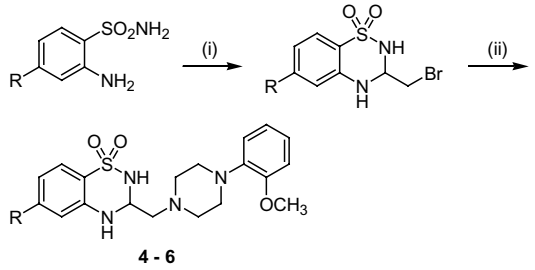


Scheme 1. Reagents and conditions: (i) bromoacethylbromide, THF anhydrous, room temperature; (ii) 1,4-dioxane, H_2SO_4 (few drops), reflux; (iii) 2-methoxyphenylpiperazine, acetonitrile, reflux.



Scheme 2. Reagents and conditions: (i) 2-bromomethyl-1,3-dioxolane, 1,4-dioxane, H_2SO_4 (few drops), reflux; (ii) 2-methoxyphenylpiperazine, acetonitrile, reflux.

the structural features that address selectivity toward one receptor or the other. In this work we present the synthesis and the pharmacological profile of a new series of 1,2,4-benzothiadiazine-arylpiperazine and the effect of some structural modifications on the affinity and selectivity for α_1-adrenergic and 5-HT_{1A} serotoninergic receptor subtypes.

The 2H-1,2,4-benzothiadiazine 1,1-dioxide derivatives **1**–**6** were synthesized, as outlined in Schemes 1 and 2, by nucleophilic substitution with 2-methoxyphenylpiperazine from the opportune 2*H*-3-bromomethyl-1,2,4-benzothiadiazines.

A detailed synthetic procedure and final characterization by ^{1}H NMR, infrared spectroscopy and C,H,N analysis are presented for 3-[4-(2-methoxy)phenylpiperazin-1-yl]methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**1**) and for the corresponding 3,4-dihydro-derivative (**4**).[8]

The compounds were converted into the corresponding di-hydrochloride salts for pharmacological tests.

The intermediates 2*H*- and 3,4-dihydro-2*H*-3-bromomethyl-1,2,4-benzothiadiazines were prepared from the corresponding 2-aminobenzenesolfonamides by condensation with bromoacetyl bromide and 2-bromomethyl-1,3-dioxolane, respectively.

Receptor subtype selectivity of compounds **1**–**6** was determined at α_1-adrenoceptors on different isolated tissues using BMY-7378 as standard compound. Blocking activity was assessed by antagonism of (−)-noradrenaline-induced contraction of rat prostatic vas deferens (α_{1A})[9] [pD_2 = 5.89 ± 0.01 (n = 30)] or thoracic aorta (α_{1D})[10] [pD_2 = 7.85 ± 0.01 (n = 30)] and by antagonism of (−)-phenylephrine-induced contraction of rat spleen (α_{1B})[11] [pD_2 = 4.95 ± 0.01 (n = 30)]. The same compounds were also assessed for in vitro binding affinity at 5-HT_{1A} subtype receptor in cell membranes of HeLa cells transfected with human cloned 5-HT_{1A} receptor.[12]

All compounds behave as competitive α_1-adrenoceptor antagonists, as they cause a parallel shift of agonist dose–response curves, and 5-HT_{1A}-ligands with varying degrees of potency and selectivity (Table 1). The exceptions are compounds **1** and **2** at α_{1A}-adrenoceptor for which any effect was seen at the maximum concentration tested (10^{-5} M). The best profile of activity at adrenergic system resides in compound **1**, which exhibits high α_{1D} affinity (pK_b = 7.59) and relevant α_{1D} selectivity (α_{1D}/α_{1A} > 389; α_{1D}/α_{1B} = 135). Moreover it shows weak affinity at 5-HT_{1A} receptor (pK_i < 6.0) and selectivity at α_{1D}-subtype of up to more than 30 folds (5-HT_{1A}/α_{1D} < 0.03). The introduction of chlorine atom in position 6 of benzothiadiazine ring (**3**) do not significantly change the affinity at α_1-adrenoceptor subtypes, while a more than 10-fold increase is observed at 5-HT_{1A} receptor (pK_i 5-HT_{1A} = 7.33) and, as a consequence, the selectivity 5-HT_{1A}/α_1 is strongly reduced. The introduction of a methyl group in position 6 of benzothiadiazine ring (**2**) is detrimental for α_{1D}-subtype affinity (pK_b = 5.50), with consequent loss of selectivity among α_1-adrenoceptors (α_{1D}/α_{1A} > 3; α_{1D}/α_{1B} = 0.28; $\alpha_{1B}/$

Table 1. Antagonist potency, expressed as pK_b ± SEM values,[a] and selectivities[b] of compounds **1**–**6** at α_1-adrenoceptors in isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}) and thoracic aorta (α_{1D})

Compd	R	p$K_b\alpha_{1A}$	p$K_b\alpha_{1B}$	p$K_b\alpha_{1D}$	α_{1D}/α_{1A}	α_{1D}/α_{1B}	α_{1B}/α_{1A}	pK_i5-HT_{1A}	5-HT_{1A}/α_{1D}
1	H	<5.0	5.46 ± 0.06	7.59 ± 0.15	>389	135	>3	<6.0	<0.03
2	CH_3	<5.0	6.05 ± 0.11	5.50 ± 0.07	>3	0.28	>11	6.97	29.5
3	Cl	5.28 ± 0.23	6.17 ± 0.16	7.75 ± 0.22	295	38	8	7.33	0.38
4	H	5.81 ± 0.09	5.97 ± 0.12	5.34 ± 0.34	0.35	0.23	1.5	7.78	275
5	CH_3	6.00 ± 0.10	6.24 ± 0.15	5.98 ± 0.17	0.95	0.55	2	<7	<10
6	Cl	5.05 ± 0.06	5.09 ± 0.03	5.00 ± 0.18	1	1	1	8.04	1096
BMY-7378[c]		(7.01 ± 0.08)	(7.48 ± 0.09)	(8.40 ± 0.09)	25	8	0.3	8.90	3.16

Binding data, expressed as pK_i values, of the same compounds at human cloned 5-HT_{1A} receptor and selectivity ratio 5-HT_{1A}/α_{1D}.

[a] pK_b values were calculated according to van Rossum[17] at one or two concentrations.

[b] Antilog of the difference between the pK_b values for α_{1A}, α_{1B} and α_{1D} adrenergic receptor subtypes.

[c] The antagonist potency of standard compound BMY-7378 is expressed as pA_2 values.

$\alpha_{1A} > 11$) and also respect to serotoninergic system (5-$HT_{1A}/\alpha_{1D} = 29.5$). The 3,4-dihydro derivatives (**4**–**6**) exhibit a weak affinity at α_1-adrenoceptor with a significant decrease of pK_b values at α_{1D} subtype, but they show an interesting affinity and selectivity at 5-HT_{1A} receptor. This finding could be explained considering the minor steric restriction of 5-HT_{1A} respects to α_1 receptor;[13,14] this might favour the interaction of more distorted structure of 3,4-dihydroderivatives (**4**–**6**) respects to unsaturated ones (**1**–**3**). Respect to its analogue **1**, the unsubstituted 3,4-dihydro-compound **4** presents lower affinity at α_{1D}-subtype ($pK_b = 5.34$) and higher affinity at 5-HT_{1A} receptor ($pK_i = 7.78$) with a selectivity ratio 5-HT_{1A}/α_{1D} of 275 folds.

Introduction of methyl group in position 6 of benzothiadiazine ring (**5**) causes a decrease of affinity and selectivity values at 5-HT_{1A} (pK_i5-$HT_{1A} < 7.0$; 5-$HT_{1A}/\alpha_{1D} < 10$), without an improved profile at α_1-adrenoceptors, while introduction of chlorine atom in the same position (**6**) contributes positively to the binding and selectivity at 5-HT_{1A} receptor (pK_i5-$HT_{1A} = 8.04$; 5-$HT_{1A}/\alpha_{1D} = 1096$).

Functional characterization of compound **6** at 5-HT_{1A} receptor was performed according to method of Stanton and Beer[15] with minor modifications, using [^{35}S]GTPγS binding, in cell membranes from HeLa cells transfected with human cloned 5-HT_{1A} receptor. Stimulation of [^{35}S]GTPγS binding induced by the tested compound was expressed as percent increase in binding above basal value, being the maximal stimulation observed with serotonin taken as 100%. The concentration–response curve of the agonistic activity was analyzed by nonlinear curve fitting of the logistic equation according to the method reported by De Lean et al.[16] using the ALLFIT program (from National Institutes of Health, Bethesda, MD) (Table 2).

The maximal stimulation of [^{35}S]GTPγS binding (E_{max}) achieved for **6** was 23 and the concentration required to obtain 50% of E_{max} (pD_2) was 6.92. The results obtained in the [^{35}S]GTPγS binding studies suggest that compound **6**, with a potency lower than that of the reference compound BMY 7378, reflecting therefore the moderate affinity values found on binding studies, can be considered a partial agonist at 5-HT_{1A} receptor.

In conclusion, we have discovered a new class of α_1-adrenoceptor and 5-HT_{1A} ligands bearing a benzothiadiazine-arylpiperazine structure. Adequate structural modifications address the selectivity toward the receptor subtype α_{1D} or 5-HT_{1A}. In particular compound **1** is outstanding for its affinity and selectivity at α_{1D} being one of the most selective antagonist for this subtype, at least in functional studies. Conversely compound **6** is outstanding for its affinity and selectivity for 5-HT_{1A} receptor. More extensive structure–activity relationship studies are in progress and will be reported in due course.

Table 2. Potency (pD_2) and relative effectiveness (E_{max})[a] values in the agonist-induced [^{35}S]GTPγ S-binding assay at human 5-HT_{1A} receptor

Compd	pD_2	E_{max}
6	6.92	23
BMY-7378	9.27	26

[a] Maximal stimulation expressed as a percentage of the maximal 5-HT response.

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References and notes

1. Ruffolo, R. R.; Bondinell, W.; Hieble, J. P. *J. Med. Chem.* **1995**, *38*, 3681.
2. Testa, R.; Taddei, C.; Poggesi, E.; Destefani, C.; Cotecchia, S.; Hieble, J. P.; Sulpizio, A. C.; Naselsky, D.; Bergsma, D.; Ellis, S.; Swift, A.; Ganguly, S.; Ruffolo, R. R.; Leonardi, A. *Pharmacol. Commun.* **1995**, *6*, 79.
3. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humpherey, P. P. A. *Pharmacol. Rev.* **1994**, *46*, 157.
4. Parks, C. L.; Robinson, P. S.; Sibille, E.; Shenk, T.; Toth, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10734.
5. Semkova, I.; Wolz, P.; Krieglstein, J. *Eur. J. Pharmacol.* **1998**, *359*, 251.
6. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. *J. Med. Chem.* **1992**, *35*, 3448.
7. López-Rodríguez, M. L.; Morcillo, M. J.; Fernández, E.; Porras, E.; Orensanz, L.; Beneytez, M. E.; Manzanares, J.; Fuentes, J. A. *J. Med. Chem.* **2001**, *44*, 186.
8. Selected compounds were prepared as follows.
3-4-(2-Methoxy)phenylpiperazin-1-ylmethyl-2*H*-1,2,4-benzothiadiazine-1,1-dioxide (**1**). To a solution of 3-bromomethyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (0.20 g, 0.7 mmol) in acetonitrile (5 mL) 2-methoxyphenylpiperazine (0.27 g, 1.4 mmol) was added under stirring at the temperature of 80 °C and the reaction mixture was refluxed for 1 h. After cooling at room temperature the residual 2-methoxyphenylpiperazine hydrobromide was removed and acetonitrile was evaporated in vacuo giving a residue which was purified by crystallization from acetone–petroleum ether 60–80 °C (0.24 g, yield 89% d.t.), mp 205–206 °C. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.8 (1H, s, D_2O changeable), 7.79 (1H, dd, $J = 7.5$, 1.4 Hz), 7.68 (1H, ddd, $J = 7.7$, 7.0, 1.4 Hz), 7.50 (1H, dd, $J = 7.7$, 1.2 Hz), 7.45 (1H, ddd, $J = 7.5$, 7.0, 1.2 Hz), 6.95–6.85 (4H, m), 3.76 (3H, s), 3.47 (2H, s), 3.02 (4H, m), 2.70 (4H, m); IR(Nujol): 3193, 1514, 1172, 1137, 759 (cm^{-1}). Anal. ($C_{19}H_{22}N_4O_3S$) C, H, N.
The free amine was transformed into the corresponding di-hydrochloride salt, which was crystallized from methanol/petroleum ether 60–80 °C; mp 230 °C.
3-[4-(2-Methoxy)phenylpiperazin-1-yl]methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-1,1-dioxide (**4**) was prepared with the same procedure of compound **1**, starting from 3-bromomethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-1,1-dioxide and it was purified by silica gel column chromatography using cyclohexane/ethyl acetate (1:1, v/v) and then chloroform/acetone (9.5:0.5, v/v) as eluant; (0.052 g, yield 19% d.t.), mp 132–135 °C (acetone–petroleum ether 60–80 °C), ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.51 (1H, s,

D_2O changeable), 7.44 (1H, dd, $J = 7.3$, 1.6 Hz), 7.28 (1H, ddd, $J = 8.0$, 7.8, 1.6 Hz), 7.15 (1H, s, D_2O changeable), 6.81–6.94 (4H, m), 6.73 (1H, dd, $J = 8.0$, 1.0 Hz), 6.71 (1H, ddd, $J = 8.0$, 7.3, 1.0 Hz), 4.87 (1H, m), 3.77 (3H, s), 3.00 (4H, m), 2.60 (6H, m); IR(Nujol): 3342, 3232, 1605, 1240, 1156, 750 (cm^{-1}). Anal. $C_{19}H_{24}N_4O_3S$ (C, H, N). The free amine was transformed into the corresponding di-hydrochloride salt, which was crystallized from methanol/petroleum ether 60–80 °C; mp 105 °C.

9. Eltze, M.; Boer, R.; Sanders, K. H.; Kolassa, N. *Eur. J. Pharmacol.* **1991**, *202*, 33.
10. Buckner, S. A.; Oheim, K. W.; Morse, P. A.; Knepper, S. M.; Hancock, A. A. *Eur. J. Pharmacol.* **1996**, *297*, 241.
11. Ko, F. N.; Guh, J. H.; Yu, S. M.; Hou, Y. S.; Wu, Y. C.; Teng, C. M. *Br. J. Pharmacol.* **1994**, *112*, 1174.
12. Testa, R.; Guarneri, L.; Poggesi, E.; Angelico, P.; Velasco, C.; Ibba, M.; Cilia, A.; Motta, G.; Riva, C.; Leonardi, A. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 1258.
13. López-Rodríguez, M. L.; Morcillo, M. J.; Rovat, T. K.; Fernández, E.; Vicente, B.; Sanz, A. M.; Hernández, M.; Orensanz, L. *J. Med. Chem.* **1999**, *42*, 36.
14. López-Rodríguez, M. L.; Morcillo, M. J.; Rovat, T. K.; Fernández, E.; Sanz, A. M.; Orensanz, L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 581.
15. Stanton, J. A.; Beer, M. S. *Eur. J. Pharmacol.* **1997**, *320*, 267.
16. De Lean, A.; Munson, P. J.; Rodbar, D. *Am. J. Physiol.* **1978**, *235*, E97–E102.
17. Van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299.