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ARYLPIPERAZIDE DERIVATIVES OF PHENYLPIPERAZINES AS A NEW CLASS OF POTENT AND SELECTIVE 5-HT$_{1B}$ RECEPTOR ANTAGONISTS

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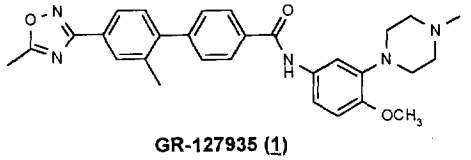
Abstract: A new series of arylpiperazide derivatives of phenylpiperazines of general formula **4** has been prepared and evaluated as 5-HT$_{1B}$ receptor antagonists. *In vitro* experiments at human cloned 5-HT$_{1B}$ receptors show that these derivatives are potent and selective 5-HT$_{1B}$ receptor antagonists. Among them, compound **4f** was found to be orally active, to gain access to the CNS and more importantly to induce an increase in extracellular brain 5-HT upon systemic administration. © 1997 Elsevier Science Ltd.

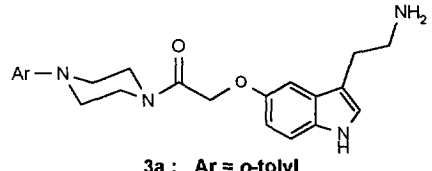
The blockade of terminal 5-HT$_{1B}$ receptors by selective antagonists has been proposed as a new approach toward the design of potentially efficient and/or fast-acting antidepressant drugs.[1] This hypothesis is based firstly on preclinical and clinical evidence indicating that an enhancement of 5-HT mediated neurotransmission might underlie the therapeutic effect of most antidepressant drugs.[2, 3] Secondly, pharmacological studies suggest that acute 5-HT$_{1B}$ receptor blockade should immediately elevate terminal 5-HT-release, since 5-HT$_{1B}$ autoreceptors in serotonin terminals suppress the synthesis and release of serotonin.[4, 5]

Thus, the design of potent and selective 5-HT$_{1B}$ antagonists represents a fascinating goal to reach in order to propose clinical candidates as tools to verify the above-mentioned hypothesis, and, ultimately to identify improved antidepressant drugs.

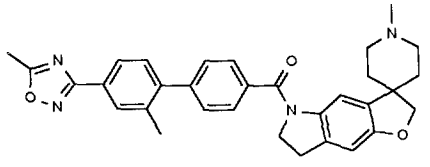
To date, few examples of such potent and selective 5-HT$_{1B}$ antagonists exist.[1] The first one to be described was GR-127935 (1).[6] However, this particular compound does not discriminate between 5-HT$_{1B}$ and 5-HT$_{1D}$ subtypes and shows agonist activity at both human cloned receptor subtypes. More recently, SB-224289 (2) has emerged as a potent and selective 5-HT$_{1B}$ inverse agonist[7] but preliminary pharmacological data indicate that this particular compound does not increase central 5-HT release in the guinea-pig frontal cortex upon systemic administration.[8]



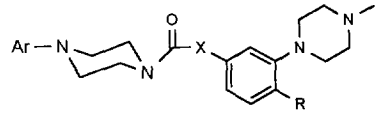
GR-127935 (1)



3a : Ar = o-tolyl
3b : Ar = p-(NHSO$_2$CH$_3$)C$_6$H$_4$



SB-224289 (2)



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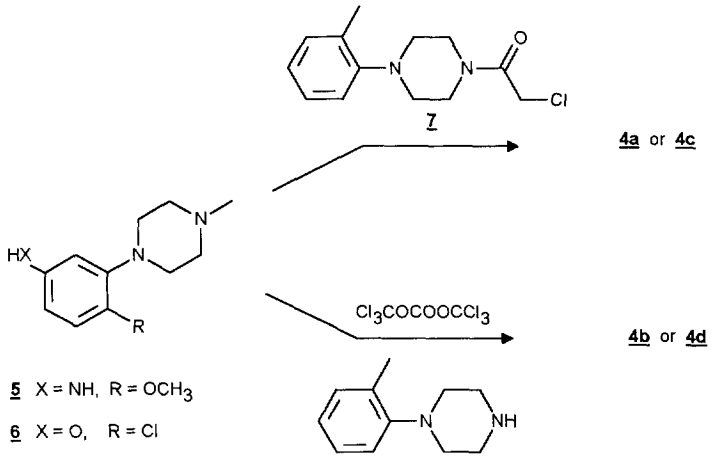
*Fax : (33).5.63.71.43.63

During the course of our program directed toward new drugs for the treatment of migraine, we have identified a series of arylpiperazide derivatives of serotonin (**3**) as potent 5-$HT_{1B/1D}$ agonists.[9, 10, 11] Structure-activity relationship studies with these types of compounds suggest that their intrinsic agonist activity could be due to the tryptamine part of the molecule. In other words, modification of the tryptamine moiety found in compounds **3** should modulate intrinsic activity and hopefully allow the design of antagonists.

This paper reports the synthesis and pharmacological evaluation of a new series of arylpiperazide derivatives of formula **4** which differ from compounds **3** by the replacement of the tryptamine residue by an arylpiperazine pharmacophore such as that found in GR-127935 (**1**).

The synthesis of the *o*-tolylpiperazide derivatives of phenylpiperazines **4** (see Table 1) can be easily achieved by two different routes depending on the nature of X. Compounds **4a** or **4c** (where X represents CH_2NH and CH_2O respectively) are prepared by condensation of the aniline derivative **5**[12] or the phenol analog **6**[12] with the α-chloro-methylamide **7**[10] (21 and 55 % yield, respectively).

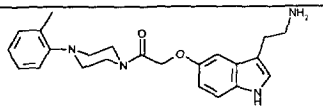
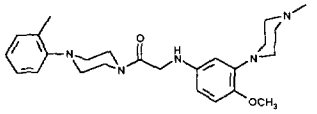
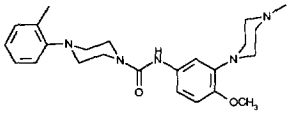
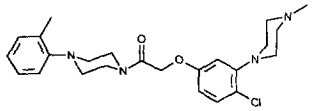
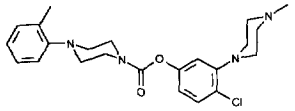


In the case of compound **4b**, the synthesis is achieved upon reaction of the aniline derivative **5** with 1/3 equivalent of triphosgene followed by condensation of the so formed carbamoylchloride with *o*-tolylpiperazine (26 % yield).[13] The carbamate **4d** is obtained by reacting phenol **6** successively with NaH and the carbamoylchloride prepared from *o*-tolylpiperazine and triphosgene (91 % yield).

Binding affinity of compounds **3a**, **4a-d** as well as GR-127935 and SB 224289 included for comparative purposes was evaluated at human cloned 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor subtypes according to previously published procedures.[14,15] Results reported in table 1 show that compounds **4a-4d** are potent ligands at human 5-HT_{1B} receptors. The binding affinity of these compounds is sensitive to the nature of the linker between the arylpiperazide moeity and the phenyl piperazine residue (compare **4b** with **4a** and **4d** with **4c**). It is interesting to note that, from this point of view, the results obtained here differ from what was observed previously[11] with agonists since compound **3a** was found to be a better ligand than its corresponding carbamate. However, replacement of the urea function or the methoxy group found in compound **4b** (Ki = 2.3 nM) respectively by a carbamate function and a chlorine atom as found in compound **4d** (Ki = 3.6 nM) does not modify their affinity for the 5-HT_{1B} receptor subtype. Contrary to the arylpiperazide derivative **3a** and GR-127935 which bind to both 5-HT_{1B} and 5-HT_{1D} receptor subtypes with approximately the same affinity, the newly reported *o*-tolylpiperazide derivatives of phenylpiperazine preferentially recognize h-5HT_{1B} binding sites with a selectivity

factor between 10 and 40. Interestingly enough, the 5-HT_{1B} ligands **4a-4d** also demonstrate a larger selectivity index with respect to 5-HT_{1A} receptors. This is another major difference with compound **3a** for which 5-HT_{1B}/5-HT_{1A} binding selectivity ratio is around 10.

Table 1*

		Binding (Ki, nM)			h5-HT_{1B}
		1A	1D	1B	Intrinsic activity
3a		8	2	0.8	full agonist (EC_{50} = 2.1 nM)
4a		3000	420	40	silent antagonist (K_B = 195 nM)
4b		390	48	2.3	silent antagonist (K_B = 12 nM)
4c		> 1000	240	11	silent antagonist (K_B = 38 nM)
4d		> 1000	150	3.6	silent antagonist
	GR 127935	72	0.7	0.14	partial agonist
	SB 224289	> 1000	260	10	N.D.**

* All compounds have been tested as their fumarate salts. Values are given as mean values of two or three experiments, each performed in duplicate, typically with individual values within 10-20 % of the mean.

** N.D.: not determined

In summary, compounds **4a-4d**, represent a new class of selective 5-HT_{1B} ligands which discriminate between 5-HT_{1B} and 5-HT_{1D} or 5-HT_{1A} receptors. In order to assess their intrinsic activity at the 5-HT_{1B} receptor subtype, these compounds together with the 5-HT_{1B} agonist **3a** have been evaluated for their ability to inhibit the forskolin-stimulated activity of adenylate cyclase coupled to human 5-HT_{1B} receptors in CHO-K_1 cells (table 1).[16] Interestingly enough, compounds **4a-4d** have no detectable agonist activity (EC_{50} > 1 000 nM) in this particular model. This is another major difference with the serotonin derivative **3a** which is characterized as a full agonist under the same conditions. Studies of antagonism of 5-CT-induced inhibition of cAMP formation in CHO-K_1 cell lines expressing human 5-HT_{1B} receptors (as expressed by the K_B values given in table 1) show that

all four derivatives **4a-4d** are potent 5-HT_{1B} antagonists. Within the series, compound **4b** emerged as the most potent antagonist having the highest affinity for the 5-HT_{1B} receptor.

Several analogs of the urea **4b** having various substituents on the aromatic ring of the arylpiperazide moeity were synthesized (compounds **4e-4k**, see table 2). They were prepared (27-94% yield) by the method reported above for the preparation of **4b** (condensation of the aniline derivative **5** with triphosgene followed by reaction of the so-formed carbamoylchloride with appropriate arylpiperazine). Biological results obtained with these compounds at human cloned 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor subtypes are reported in table 2.

Table 2*

		Binding (Ki, nM)			Antagonist potency (K_B, nM)
Cpd	Ar	5-HT_{1A}	5-HT_{1D}	5-HT_{1B}	5-HT_{1B}
4e	C_6H_5	450	340	18	27
4f	2-$MeOC_6H_4$	700	90	2.0	16
4g	3-$MeOC_6H_4$	79	70	8.0	(N.D.)**
4h	4-$MeOC_6H_4$	1000	230	8.4	12
4i	5,6,7,8-tetrahydronaphth-1-yl	257	26	0.5	39
4j	2,6-$Me_2C_6H_3$	393	37	1.3	10
4k	2,4-$Me_2C_6H_3$	571	88	1.8	1.9

* All compounds correspond to general formula **4** with X = NH and R = OCH_3 and have been tested as their fumarate salts. Values are given as mean values of two or three experiments, each performed in duplicate, typically with individual values within 10-20 % of the mean.

** Not determined

Analysis of 5-HT_{1B} receptor binding affinities (Ki) shows that the *ortho* aromatic substitution by an electron-donating group (compound **4f**) is better than *meta* or *para* substitution (compound **4g** and **4h**), while no substitution appears less interesting (compound **4e**). Based on these results, we prepared some disubstituted analogues having at least one *ortho* substituant (compound **4i-k**). These compounds are high affinity ligands at 5-HT_{1B} human cloned receptors, the tetrahydronaphthyle derivative **4i** being the most potent (Ki = 0.5nM). Almost all compounds exhibit good binding selectivity over $5HT_{1A}$ (greater than 100) and good binding selectivity over $5HT_{1D}$ (30 to 50) with the exception of compounds **4e** and **4g**. Analyses of the functional data (based on the 5-HT_{1B} mediated inhibition of cAMP formation stimulated by forskolin) reveals that compounds **4e-4k** have a similar profile to **4b** since all of them are potent antagonists with no detectable intrinsic activity, and can therefore be classified, in this particular model, as 5-HT_{1B} silent antagonists.

The *o*-methoxyphenylpiperazide derivative **4f** was selected for further pharmacological evaluations. At first, this antagonist was characterized as a very selective 5-HT_{1B} ligand since it has a low affinity (IC_{50} > 1 μM) at α_1, α_2, β_1, β_2, D_1, D_2, H_1, H_2,M (non-selective), 5-HT_{2A}, 5-HT_3, 5-HT_4 and 5-HT, NA or DA uptake sites.

Moreover, demonstration of the in vivo central activity of compound **4f** has been achieved by the reversal of hypothermia induced by a 5-$HT_{1B/1D}$ agonist.[17] Thus, the hypothermia induced by the agonist **3b**[18] (10 mg/kg, ip) was dose-dependently blocked by **4f** in vivo in the guinea-pig, (ED_{50} = 0.52 mg/kg, i.p., compared to 0.31 mg/kg i.p. for GR-127935). Compound **4f** was also active in the same model upon oral administration (ED_{50} = 0.31 mg/kg) suggesting a good bioavailability for that particular derivative. Finally, the ability of compound **4f** to induce an increase of terminal 5-HT release (which is the rationale for a potential antidepressant effect of 5-HT_{1B} antagonists) has been demonstrated in vivo. According to published procedures,[19] extracellular 5-HT was measured in hypothalamus of freely-moving guinea-pigs by use of brain microdialysis. Systemic administration of compound **4f** (0.16 and 0.63 mg/kg, ip) was found to increase dialysate levels of 5-HT (+ 56 and + 67 %, respectively, with respect to baseline values).[20] This result is particularly important since, under the same experimental conditions, neither GR-127935 nor SB-224289 were able to increase central 5-HT release. Similar data have been previously reported in the guinea-pig frontal cortex.[6,8] To our knowledge, this is the first demonstration that a selective 5-HT_{1B} receptor antagonist is able to increase brain, extracellular 5-HT levels following systemic administration.

In conclusion, a new class of highly potent and selective 5-HT_{1B} receptor antagonists of general formula **4** (X = NH) has been described. These urea derivatives can easily be prepared by coupling an arylpiperazine and 4-(methoxy)-3-(4-methylpiperazin-1-yl) aniline with triphosgene. Results obtained at human cloned 5-HT_{1B} receptors show that all reported examples of compounds of formula **4** are silent 5-HT_{1B} antagonists which differ mainly by their relative binding affinities and selectivities. Among them, the *o*-methoxyphenylpiperazide derivative **4f** appears to be a unique and promising new 5-HT_{1B} antagonist which is able to reverse the hypothermia induced by a 5-HT_{1B} agonist *in vivo* in the guinea-pig following oral administration, and to induce an increase of brain 5-HT release upon systemic administration.

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13 All new compounds have been fully characterized by CHN analysis, IR, MS and NMR spectra. A typical procedure for the preparation of ureas is illustrated by synthesis of compound **4f** : A solution of 4-methoxy-3-(4-methylpiperazin-1-yl)aniline **5** (2.1g; 9.4mmol) and triethylamine (1.3mL;9.4mmol) in CH_2Cl_2 (50mL) is added slowly to a cold (0°C) solution of triphosgene (935mg;3.2mmol) in CH_2Cl_2 (100mL) under nitrogen atmosphere. The mixture is then brought back to room temperature before addition of the *o*-methoxyphenylpiperazine (1.8g; 9.6mmol) and triethylamine (1.3mL; 9.4mmol) in CH_2Cl_2 (50mL). After 2h., it is poured into a saturated $NaHCO_3$ solution. The aqueous layer is extracted with CH_2Cl_2 (3X). Finally, mixed organic layers are washed with saturated NaCl solution, dried over $MgSO_4$ and evaporated. The crude is purified by flash-chromatography (CH_2Cl_2/MeOH/NH_4OH : 95/5/1) to give compound **4f** with 89% yield. The fumarate salt was prepared by treatment of **4f** dissolved in CH_2Cl_2 with stoechiometric amounts of fumaric acid followed by precipitation by addition of Et_2O. Anal. $C_{24}H_{33}N_5O_3$-$C_4H_4O_4$ (C,H,N); m.p. = 120-126°C (amorphous); MS (DCI) : 440 (MH+), 248, 193; IR (KBr) : 3600-3150, 2999, 2945, 2831, 1709, 1642, 1595, 1508; ^1H NMR (DMSO) : 2.33 (s, 3H); 2.63 (m, 4H); 2.96 (m, 8H) 3.55 (m, 4H); 3.72 (s, 3H); 3.79 (s, 3H); 6.57 (s, 2H); 6.78-7.10 (m, 7H); 8.34 (s, 1H). Spectral data and physico-chemical caracteristics of the other compounds described herein have been reported in the patent application WO-9602525.

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20 Values are given as mean values of 3 to 5 independent experiments, typically with individual values within 20 % of the mean.

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