A NEW ARYLPIPERAZINE DERIVATIVE PK47 – A DUAL SEROTONIN 5-HT$_{1A}$/5-HT$_{2A}$ RECEPTOR LIGAND WITH AN ANXIOLYTIC ACTIVITY

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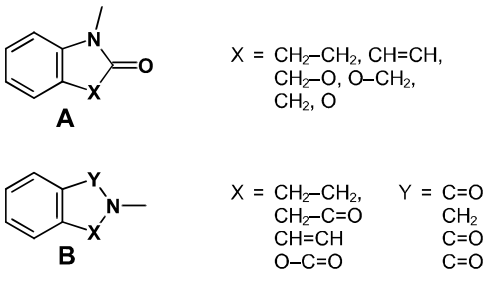
INTRODUCTION

The most thoroughly studied group of arylpiperazine derivatives, called long chain arylpiperazines (LCAPs), can be found as serotonin receptor ligands, especially 5-HT$_{1A}$ and 5-HT$_{2A}$ ones. Their general chemical structure consists of an alkyl chain (2–4 methylene units) attached to the N4 atom of the piperazine moiety, and a terminal amide or an imide fragment. The significance of the respective parts of LCAPs for 5-HT$_{1A}$ affinity, intrinsic activity and selectivity has been the subject of many structure-activity relationship studies.[1] Although the influence of the aryl group (typically a substituted phenyl or heteroaromatic moiety), as well as the length of the alkyl chain are rather well established, the function of the terminal fragment is still not clear. A great number of such fragments tested (even those without the amide group) suggest that different forces are engaged in stabilizing the ligand-receptor complex in this region. If we assume that an arylpiperazine fragment serves as an anchoring point, the highly flexible alkyl chain allows a terminal fragment of LCAPs to interact with different sites of a binding pocket.

During our systematic structure-affinity and structure-intrinsic activity studies within the LCAP group of 5-HT$_{1A}$ ligands different termini were used.[2–5] The main structural features explored included changes in the relative position of the amide group with regard to the aromatic ring, varied ring sizes (five- or six-membered) and introduction of an additional carbonyl group and/or the oxygen atom (Scheme 1). Although the studied compounds exhibited diversified 5-HT$_{1A}$ affinities and pharmacological profiles one generalization could be observed. Ligands with a nitrogen atom attached directly to the benzene ring (type **A**) even those showing a high 5-HT$_{1A}$ affinity were not active in vivo.[3] On the contrary, compounds of type **B** were usually highly potent in vivo 5-HT$_{1A}$ receptor ligands.[5]

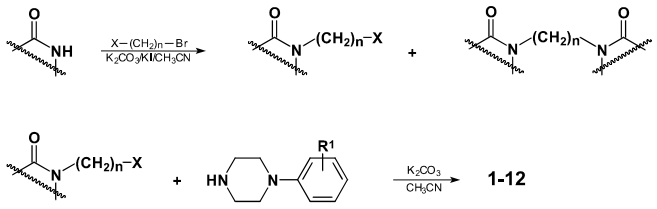


Scheme 1

In order to further extend diversity of terminal amides **B** we present the synthesis, 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor in vitro and in vivo studies of a new model arylpiperazines connected by three or four methylene group spacer with quinazolidin-4-one (**1**–**4**), 2-phenyl-2,3-dihydrophthalazine-1,4-dione (**5**–**8**) or 1-phenyl-1,2-dihydropyridazine-3,6-dione (**8**–**12**) moieties. On the basis of the obtained results, the most promising compound **2** was then tested in several animal models of anxiety and depression.

CHEMISTRY

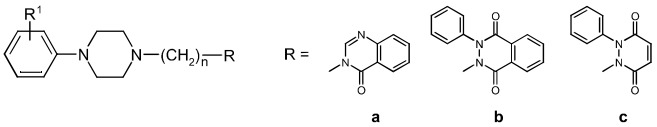
Final compounds **1**–**12** were obtained in a similar way (scheme 2) as their recently described phenylpiperazine and pyrimidylpiperazine analogues.[6] In short, the starting quinazolidin-4(3H)-one, 2-phenyl-2,3-dihydrophtalazine-1,4-dione and 1-phenyl-1,2-dihydropyridazine-3,6-dione were prepared from antranilic acid, ftalic anhydride and maleic anhydride, respectively, according to the published procedures.



Scheme 2

Subsequent alkylation with 1-bromo-3-chloropropane or 1,4-dibromobutane in the presence of K_2CO_3 in acetonitrile led to the formation of halogen intermediates and the symmetrically disubstituted by-products. Target compounds were obtained upon condensation of halogen derivatives with the respective 1-arylpiperazine. The structure of free bases **1**–**12** was confirmed by ^{1}H-NMR spectra, and by an elemental analysis after conversion to hydrochloride salts.

Table 1. Structure and 5-HT$_{1A}$ and 5-HT$_{2A}$ binding affinities of the compounds **1**–**12**.



No.	R^1	n	R	$K_i \pm$ SEM (nM) 5-HT$_{1A}$	$K_i \pm$ SEM (nM) 5-HT$_{2A}$
1	*m*-Cl	3	a	235 ± 13	16 ± 3
2	*m*-Cl	4	a	50 ± 9	68 ± 10
3	*o*-OCH$_3$	3	a	100 ± 3	460 ± 4
4	*o*-OCH$_3$	4	a	36 ± 1	566 ± 6
5	*m*-Cl	3	b	415 ± 13	657 ± 17
6	*m*-Cl	4	b	400 ± 20	580 ± 29
7	*o*-OCH$_3$	3	b	30 ± 1	300 ± 20
8	*o*-OCH$_3$	4	b	43 ± 9	375 ± 20
9	*m*-Cl	3	c	206 ± 9	270 ± 16
10	*m*-Cl	4	c	50 ± 8	1830 ± 26
11	*o*-OCH$_3$	3	c	54 ± 8	2120 ± 28
12	*o*-OCH$_3$	4	c	11 ± 2	1460 ± 24

RESULTS AND DISCUSSION

The affinity of the tested compounds for 5-HT_{1A} receptors varied from 11 nM (**12**) to 415 nM (**5**), whereas for 5-HT_{2A} receptors it ranged from 16 nM to 2100 for **1** and **11**, respectively (Table 1). Generally, *ortho*-methoxyphenyl-piperazine derivatives were always more active at 5-HT_{1A} receptors than the respective *meta*-chloro analogues. Moreover, compounds with a 4-membered alkyl chain spacer (even numbers) were more potent 5-HT_{1A} ligands than were those containing 3 methylene groups. Thus the in vitro results were in line with the general trends concerning affinities within the arylpiperazine group of ligands. However, a closer examination of the obtained 5-HT_{1A} binding data reveals that the influence of alkyl chain length, as well as the arylpiperazine used depend on the terminal fragment. In the case of series **a** and **c**, elongation of the spacer caused app. 4-fold enhancement of 5-HT_{1A} affinity, but in series **b** it was without effect. On the other hand, an increase in 5-HT_{1A} affinity, connected with the replacement of *m*-Cl by *o*-OCH3 in the phenyl ring, was the most significant for series **b** (10-fold) and less important for series **c** and **a** (4- and 2-fold, respectively).

Regarding 5-HT_{2A} receptors, only two relatively potent compounds were found (**1** and **2**, K_i = 16 and 68 nM, respectively), either containing a meta-chlorophenyl-piperazine and a quinazolidin-4-one (R = **a**) fragments. Other compounds displayed a low affinity for 5-HT_{2A} receptors. Derivatives **10**–**12** were the least potent 5-HT_{2A} ligands (K_i = 1450–2130 nM), but at the same time showing a high affinity for 5-HT_{1A} receptors, hence the highest 5-$HT_{2A}/_{1A}$ selectivity ($S_{2A}/_{1A}$ > 35).

The most in vitro active compounds (K_i < 70 nM) were further tested in several in vivo models to determine their functional profile at 5-HT_{1A} (**2**, **4**, **7**, **8**, **10**–**12**) and 5-HT_{2A} (**1**, **2**) receptors.

All the new 5-HT_{1A} ligands revealed an antagonistic activity at postsynaptic 5-HT_{1A} receptors, and three of them (**2**, **7** and **10**) behaved as agonists at presynaptic ones. Additionally, both the meta-chlorophenylpiperazine derivatives (**1** and **2**) containing quinazolidin-4-one fragment showed features of 5-HT_{2A} receptor antagonists.

On the basis of the results of a functional study, compound **2** (**PK47**) an agonist of presynaptic and an antagonist of postsynaptic 5-HT_{1A} receptors with a 5-HT_{2A} receptor antagonistic activity was selected for further in vivo preclinical studies as a potential psychotropic agent.

As a first step, the radioligand binding profile of compound **2** was completed by the determination of its affinity for dopamine D_2 receptors. Since the affinity found (K_i = 430 nM) was low, further in vivo examination in that direction was unfounded.

The succesive of our studies with compound **2** focused on its evaluation as a potential anxiolytic and/or antidepressant agent.

In the conflict drinking test in rats, compound **2** (0.3–12.5 mg/kg) dose-dependently increased the number of punished licks, but used in a higher dose (5 mg/kg) it induced sedation and other behavioral disturbances (e.g. abduction, weak tremor), so that dose was not tested. It seems that the observed effect was specifically anxiolytic, since when compound **2** was given in doses evoking an anticonflict activity, it affected neither the shock threshold nor the non-punished water consumption. It should be noted that the anticonflict effect of **2** was even more potent in terms of active dose than that produced by diazepam, used as a reference drug, and comparable to that of the partial 5-HT_{1A} receptor agonists buspirone and MM199, or of mixed 5-HT_{1A}/5-HT_{2A} ligands, eg. adatanserin.

On the other hand, compound **2** (0.31–1.25 mg/kg) was practically inactive in the plus-maze test in rats, in which diazepam (2.5–5 mg/kg) showed a marked anxiolytic-like activity. However, there also exist contradictory data about the effects of 5-HT_{1A} partial agonists (eg. buspirone) in this test, since anxiolytic-like, lack of effect or even anxiogenic effects were described.

The results of our experiments also showed that **2** (2.5–10 mg/kg) did not change immobility time in the Porsolt test in mice, while the typical antidepressant imipramine (30 mg/kg, a 70% decrease in immobility time, $p<0.01$), used as a reference drug, showed distinct activity in that model. However, such a result could be expected, since the 5-HT_{1A} receptor partial agonists buspirone and ipsapirone after systemic administration did not reduce immobility, either; they exhibited antidepressant-like activity in animals pretreated with an inhibitor of drug metabolism.

CONCLUSIONS

From among 12 new arylpiperazine derivatives compound **2** (a dual 5-HT_{1A}/5-HT_{2A} ligand) displayed distinct anxiolytic-like activity in the Vogel test, but was inactive in the plus-maze model, nor did it exert antidepressant-like properties in the forced swimming test in mice. Although compound **2** revealed remarkable anxiolytic-like properties already at low doses (from 0.31 mg/kg), the sedative effect at 5 mg/kg, excluded that ligand from being regarded as a potential drug. New derivatives of quinazolidin-4-one are currently being developed.

Acknowledgements

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