Med Chem Res (2012) 21:477–486
DOI 10.1007/s00044-011-9555-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, 5-HT_{1A} and 5-HT_{2A} receptor affinity and QSAR study of 1-benzhydryl-piperazine derivatives with xanthine moiety at N4

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Received: 5 July 2010 / Accepted: 6 January 2011 / Published online: 19 January 2011

Abstract Three novel 1-benzhydryl-piperazines with xanthine moiety at N4 were synthesized and tested for 5-HT_{1A} and 5-HT_{2A} receptor affinity. One of the compounds showed the highest affinity (58.6 nM) and selectivity (34 times) to 5-HT_{2A} receptor known for this class of compounds. A set of the three new and 31 previously synthesized 1-arylpiperazines with xanthine moiety at N4 was compiled and a QSAR study was performed in order to rationalize the further synthesis. It was found that the 5-HT_{1A} affinity depends on the shape of the molecules (ovality and number of circuits), the distribution of the electron density in the structures (partial charges at piperazine N1 and xanthine N1) and their charge transfer ability (HOMO energy). The 5-HT_{2A} affinity depends on the lipophilicity of the ligands and the distribution of the electron density in the structures (partial charges at piperazine N4 and xanthine O6). The QSAR results are in a good agreement with known pharmacophore models.

Keywords Piperazine · Xanthine · 5-HT_{1A} and 5-HT_{2A} · QSAR

Introduction

5-Hydroxytryptamine (5-HT, serotonin) is an indoleamine neurotransmitter found in both the central and peripheral nervous systems. It is important for a variety of physiological functions, including platelet aggregation, smooth muscle contraction, appetite, cognition, perception, mood, and other CNS functions (Hoyer *et al*., 1994; Roth, 1994). These diverse activities are mediated by large number of 5-HT receptor subtypes that are encoded by distinct genes. It now appears that there are at least 15 receptor subtypes that belong to seven families (Nichols and Nichols, 2008). Apart from 5-HT_3, all 5-HT receptors are coupled to G-proteins (GPCR). The 5-HT_1 subtypes decrease whereas 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 increase the cellular level of cAMP. 5-HT_2 increases the levels of inosine trisphosphate (IP3) and diglyceride (DAG). 5-HT_3 is a ligand-gated Na^+ and K^+ cation channel.

1-Arylpiperazines are well known 5-HT receptor ligands with 5-HT_{1A} and/or 5-HT_{2A} affinity (Oh *et al*., 2001; Lopez-Rodriguez *et al*., 2002). The inclusion of a complex heterocyclic fragment through a polymethylene spacer at piperazine position N4 often leads to more potent ligands in both 5-HT_{1A} and 5-HT_{2A} affinities (Nagamoto *et al*., 2004). In the present study we describe three new 1-benzhydryl-piperazine derivatives with xanthine moiety attached to N4 without any spacer or with a trimethylene one. Xanthines are well known mild CNS agents acting as antagonists of adenosine receptors (Daly, 2007). The new compounds have been synthesized initially as vasodilators and their 5-HT affinity was found by chance. They are weak 5-HT_{1A} binders but one of them is the best known 5-HT_{2A} binder in the class of N4-xanthine derivatives of 1-arylpiperazine. It shows strong 5-HT_{2A} selectivity being 34 times more affine to 5-HT_{2A} than to 5-HT_{1A}. Additionally, a set of similar 1-arylpiperazines with xanthine moiety at N4, tested previously (Chojnacka-Wojcik *et al*., 1995; Pawlowski *et al*., 1999; Pawlowski *et al*., 2000; Chlon *et al*., 2001; Kolaczkowski *et al*., 2005), was

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compiled and a QSAR study was performed in order to rationalize the further synthesis.

Materials and methods

Chemistry

Melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are not corrected. The IR spectra 400–4000 cm^{-1} were recorded on a Shimadzu FTIR 8101 M spectrophotometer (Shimadzu, Japan) in Nujol. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance DRX 250 (250 MHz) spectrometer (Germany). ^{1}H NMR spectra were measured for approximately 0.03 M while ^{13}C NMR spectra were measured for approximately 0.05 M solutions using DMSO-d_6 as solvent and chemical shifts were expressed as δ values in ppm against TMS as an internal standard. Mass spectra (MS) were recorded on an Esquire-LC-00075 spectrometer.TLC was performed on DC-Alufolien Kieselgel 60 F254, 0.20 mm (Merck, Germany) sheets with the mobile phase: chloroform/acetone/ethanol (3:3:4, *V/V*). The spots were detected at UV 254 nm. All names were generated by using structure-to-name and name-to-structure algorithms included with ChemBioDraw Ultra 11.0 (CambridgeSoft).

Synthetic grade chemicals procured from Merck, Germany, were used for the synthesis of the target compounds, as received. Non-commercially available intermediates required for the synthesis of novel derivatives of 1-benzhydrylpiperazine were prepared according to the literature procedures without modifications as follows: 8-bromocaffeine (**1**) (Gagausov *et al*., 1987); 8-(3-bromopropylamino)-1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**2**) (Peikov *et al*., 1990), 1-(3-iodoropropyl)-3,7-dimethylxanthine (**3**) (Peikov *et al*., 1988). The completion of reactions was monitored through TLC.

Microanalyses were performed on a Perkin-Elmer 2400-II Element analyzer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. All solutions were dried over anhydrous sodium sulfate and evaporated on a Büchi rotary evaporator at reduced pressure.

The given yields are those of analytically pure product. No efforts were made to optimize the yields.

*Synthesis of 1-benzhydryl-4-[1,3,7-trimethyl-2,6(3H,7H)-dioxo-1H-purine-8-yl]-piperazine (**5**)*

A mixture of 8-bromocaffeine (**1**) (2.73 g, 0.01 mol), 1-benzhydrylpiperazine (2.56 g, 0.01 mol) and triethylamine (1.01 g, 0.01 mol) was refluxed in DMF (20 ml) for 12 h. After the completion of reaction, the solvent was removed and the waxy residue was treated with cold chloroform to separate triethylamine hydrochloride. After filtration the solvent was removed and the residue was dissolved in acetone and was allowed to stand at 5°C overnight. The solid crystals obtained were filtered, washed with cold acetone, dried and recrystallized from, acetone. Yield 62% (2.75 g). M.p. 222–224°C. IR: 3075 (CH aromatic), 1705 (CO xanthine), 1664 (CO xanthine), 1648 with shoulders at 1608 and 1637 (C=N, C=C aromatic), 1558 and 1539 (C=C xanthine, C=C aromatic), 755 and 746 (CH aromatic); ^{1}H NMR: 2.53–2.49 m (4H, 2 × –CH_2–, piperazine), 2.36–3.29 m (4H, 2 × –CH_2–, piperazine), 3.61, 3.33 and 3.17 (s, 3 × 3*H*, CH_3), 4.51s (1*H*, –CH side chain), 7.49–7.19 (m, 10H, aromatic ring), ^{13}C NMR: 161.14 (C8xanth); 155.6 (C6xanth CO); 153.26 (C2xanth CO); 141.8, 128.67, 127.71, 127.18 (12 × C aromatic ring); 150.9 (C5xanth); 104.38 (C4xanth); 48.87 (2 × N4piperazine, CH_2), 50.94 (2 × N1piperazine, CH_2); 74.77 (CH–$(Ph)_2$ side chain), 32.31.2 (N7xanth CH_3), 29.35 (N1xant CH_3), 27.34 (N3xanth CH_3); MS *m/z*: 167 (100), 445 (M+1). Anal. $C_{25}H_{28}N_6O_2$ (C, H, N).

*Synthesis of 1-benzhydryl-4-[1,3,7-trimethyl-2,6(3H,7H)-dioxo-1H-purine-8-aminopropyl]-piperazine (**6**)*

A mixture of 8-(3-bromopropylamino)-1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**2**) (3.30 g, 0.01 mol), 1-benzhydrylpiperazine (2.56 g, 0.01 mol) and triethylamine (1.01 g, 0.01 mol) was refluxed in DMF (20 ml) for 13 h. After the completion of reaction, the reaction content was allowed to stand at 20°C overnight. The crude crystals obtained were filtered, washed with water to separate triethylamine hydrochloride, dried and recrystallized from isopropanol. Yield 55% (2.76 g). M.p. 187–188°C. IR: 3068 (CH aromatic), 1704 (CO xanthine), 1668 (CO xanthine), 1649 with shoulders at 1607 and 1635 (C=N, C=C aromatic), 1555 and 1540 (C=C xanthine, C=C aromatic), 754 and 744 (CH aromatic); ^{1}H NMR: 1.73–1.67 t (2*H*, –CH_2–, $J = 7$ Hz, side chain), 2.51–2.31 m (10*H*, 4 × –CH_2–, piperazine, –CH_2, N1 piperazine), 3.80–3.74 m (2*H*, –CH_2– side chain) 3.52, 3.30 and 3.16 (s, 3 × 3*H*, CH_3), 4.25 s (1*H*, –CH side chain), 4.34 s (1*H*, –NH side chain), 7.42–6.96 (m, 10H, aromatic ring), ^{13}C NMR: 154.2 (C6xanth CO); 152.9 (C2xanth CO); 150.9 (C8xanth); 142.9, 128.5, 127.6, 126.8 (12 × C aromatic ring); 148.4 (C5xanth); 101.8 (C4xanth); 52.9 (2 × N4piperazine, CH_2), 55.5 (2 × N1piperazine, CH_2); 75.16 (CH–$(Ph)_2$ side chain), 51.6 (side chain CH_2, N1piperazine), 41.3 (CH_2, –NH side chain), 29.18 (N7xanth CH_3), 27.14 (N1xant CH_3), 26.3 (side chain CH_2), 25.49 (N3xanth CH_3); MS *m/z*: 167 (100), 502 (M+1). Anal. $C_{28}H_{35}N_7O_2$ (C, H, N).

*Synthesis of 1-benzhydryl-4-[3-(3,7-dimethyl-2,6(3H,7H)-dioxo-1H-purine-1-yl)-propyl]-piperazine (**7**)*

A mixture of 1-(3-iodopropyl)-3,7-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**3**) (3.48 g, 0.01 mol), benzhydrylpiperazine (2.56 g, 0.01 mol) and triethylamine (1.01 g, 0.01 mol) was refluxed in DMF (20 ml) for 7 h. After the completion of reaction, the solvent was removed and the waxy residue was treated with isopropanol and the reaction content was allowed to stand at 20°C overnight. The crude crystals obtained were filtered, washed with water to separate triethylamine hydrochloride, dried, twofold recrystallized from isopropanol and once from ethanol. Yield 74% (3.5 g). M.p. 225–226°C. IR: 3074 (CH aromatic), 1706 (CO xanthine), 1669 (CO xanthine), 1645 with shoulders at 1606 and 1635 (C=N, C=C aromatic), 1555 and 1560 (C=C xanthine, C=C aromatic), 755 and 742 (CH aromatic); ^{1}H NMR: 2.05–1.85 t (2*H*, –CH_2–, $J = 7$ Hz, side chain), 2.81–2.21 m (10*H*, 4 × –CH_2–, piperazine, –CH_2, N1 piperazine), 3.95–3.90 t (2*H*, –CH_2–, $J = 7$ Hz, side chain), 3.88, 3.32 (s, 2 × 3*H*, CH_3), 4.49 s (1*H*, –CH side chain), 7.43–7.18 (m, 10H, aromatic ring), 8.05 s (1*H*, –C^8–H); ^{13}C NMR: 154.6 (C6xanth CO); 151.02 (C2xanth CO); 143.6 (C8xanth); 148.4, 128.7, 127.6, 127.2 (12 × C aromatic ring); 141.6 (C5xanth); 106.8 (C4xanth); 51.2 (2 × N4piperazine, CH_2), 53.2 (2 × N1piperazine CH_2); 73.4 (CH–$(Ph)_2$ side chain), 48.1 (side chain CH_2, N4piperazine), 48.1 (CH_2, N1piperazine), 33.2 (N7xanth CH_3), 37.7 (N1xant CH_2), 29.5 (side chain CH_2), 22.6 (N3xanth CH_3); MS *m/z*: 167 (100), 473 (M+1). Anal. $C_{27}H_{32}N_6O_2$ (C, H, N).

5-HT_{1A} and 5-HT_{2A} receptor binding assay

Radioligand studies with native 5-HT_{1A} and 5-HT_{2A} receptors were conducted according to the methods previously described (Bojarski *et al*., 1993). Briefly, the following were used: for 5-HT_{1A} assays, rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals), and 5-HT (10 μM) for non-specific binding; [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide (1 μM) for non-specific binding. K_i values were determined from at least three competition-binding experiments, in which 7–9 drug concentrations, run in triplicate, were used. The Cheng and Prusoff equation (Cheng and Prusoff, 1973) was used for K_i calculations.

QSAR study

Data set

The three novel compounds were added to a set of 31 1-arylpiperazines with xanthine moiety at N4, tested previously for 5-HT_{1A} and 5-HT_{2A} affinity (Chojnacka-Wojcik *et al*., 1995; Pawlowski *et al*., 1999; Pawlowski *et al*., 2000; Chlon *et al*., 2001; Kolaczkowski *et al*., 2005). The pK_i values range from 4.9 to 9.3 for 5-HT_{1A} and from 4.4 to 7.2 for 5-HT_{2A} (Table 1). Although the compounds originate from different papers, their affinities are comparable as they are tested by the same binding assay (Bojarski *et al*., 1993) in the same lab (Institute of Pharmacology, Polish Academy of Sciences).

Molecular descriptors

The structures were built in HyperChem 7.5 (HyperCube Ltd.) with extended polymethylene bridge and piperazine ring in a chair conformation. Their geometries were optimized by MM + force field (Hocquet and Langgard, 1998). The electron density distribution was calculated by semiempirical method AM1 (Dewar *et al*., 1985). The charges at all common heteroatoms, HOMO and LUMO energies form the set of electronic descriptors. Molecular properties, like molecular weight, number of rings, number of H-bond donors and acceptors, etc. and 3D descriptors, like volume, surface, ovality, dipole, etc. were calculated by MDL QSAR software, version 2.2 (Symyx). Log*P* values were calculated by ACD Labs software (ACD Inc.). The initial set of descriptors included around 50 ones.

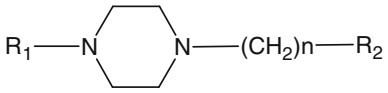
Variable selection

A genetic algorithm (GA) (Leardi *et al*., 1992) and stepwise regression, as implemented in the MDL QSAR package, were used as variable selection procedures. GA allows one to select a subset of the most significant predictors using two evolutionary operations: random mutation and genetic recombination (crossover). The parameters of the GA used in this study were initial population with size of 32, uniform crossover and one-point mutation. Friedman's lack-of-fit scoring function was used as a fitness function. The GA regression equations were generated on the basis of the selected variables by ordinary multiple linear regression (MLR). The stepwise regression was used in a forward mode with default value for F-to-enter (4.00) and F-to-remove (3.99). The final descriptor set was checked for intercorrelation.

Model assessment

Final models were assessed by explained variance r^2, standard error of estimate *SEE*, F-ratio, leave-one-out cross-validated (LOO-CV) correlation coefficient q^2_{LOO} and correlation coefficient q^2_{CV5} after cross validation in groups

Table 1 Structures and their affinities to 5-HT_{1A} and 5-HT_{2A} receptors, used in the QSAR study



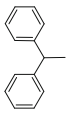
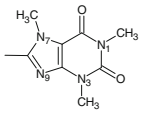
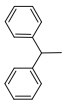
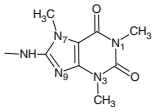
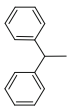
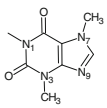
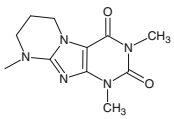
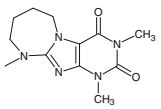
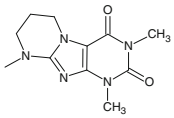
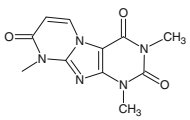
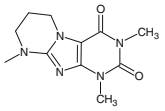
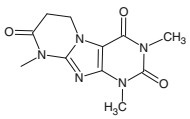
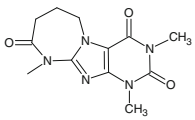
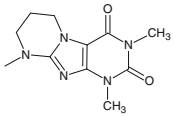
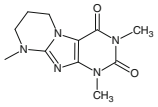
No	R_1	n	R_2	pK_i (5-HT_{1A})	pK_i (5-HT_{2A})	Reference
5		0		4.965	4.647	This study
6		3		5.804	6.512	This study
7		3		5.702	7.232	This study
8		3		7.796	6.375	Chojnacka-Wojcik *et al*., (1995)
9		3		7.509	6.588	Chojnacka-Wojcik *et al*., (1995)
10		4		8.678	6.614	Chojnacka-Wojcik *et al*., (1995)
11		3		7.602	5.330	Chojnacka-Wojcik *et al*., (1995)
12		3		7.444	5.089	Chojnacka-Wojcik *et al*., (1995)
13		3		7.377	4.553	Chojnacka-Wojcik *et al*., (1995)
14		3		6.324	4.411	Chojnacka-Wojcik *et al*., (1995)
15		3		7.590	6.900	Pawlowski *et al*., (1999)
16		3		8.553	6.313	Pawlowski *et al*., (1999)

Table 1 continued

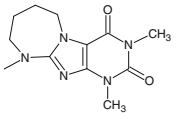
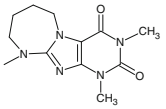
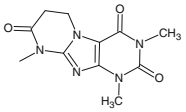
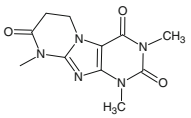
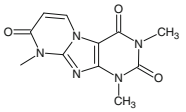
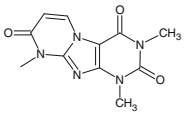
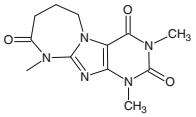
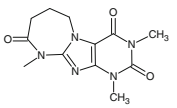
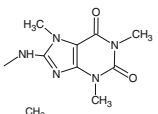
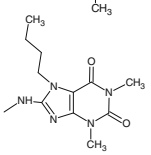
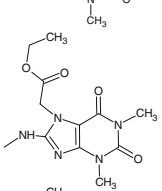
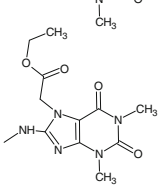
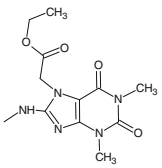
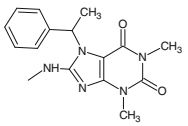
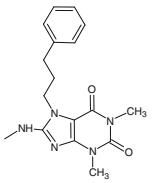
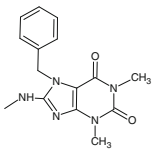
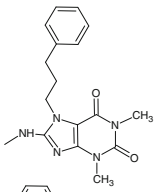
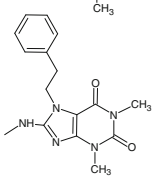
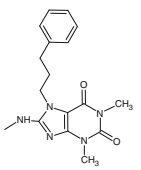
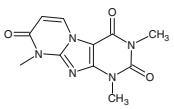
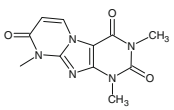
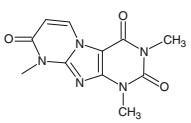
No	R_1	n	R_2	pK_i (5-HT_{1A})	pK_i (5-HT_{2A})	Reference
17		3		7.573	6.481	Pawlowski *et al*., (1999)
18		3		7.764	6.250	Pawlowski *et al*., (1999)
19		3		7.368	6.740	Pawlowski *et al*., (1999)
20		3		7.666	5.375	Pawlowski *et al*., (1999)
21		3		7.484	5.967	Pawlowski *et al*., (1999)
22		3		7.983	5.411	Pawlowski *et al*., (1999)
23		3		7.220	6.445	Pawlowski *et al*., (1999)
24		3		6.775	5.300	Pawlowski *et al*., (1999)
25		3		6.415	6.706	Pawlowski *et al*., (2000)
26		3		7.000	6.212	Pawlowski *et al*., (2000)
27		3		6.228	6.747	Pawlowski *et al*., (2000)
28		3		6.340	5.996	Pawlowski *et al*., (2000)
29		3		6.658	6.348	Chlon *et al*., (2001)

Table 1 continued

No	R_1	n	R_2	pK_i (5-HT_{1A})	pK_i (5-HT_{2A})	Reference
30		3		5.997	6.939	Chlon *et al*., (2001)
31		3		7.301	6.352	Chlon *et al*., (2001)
32		3		7.319	6.260	Chlon *et al*., (2001)
33		3		8.097	6.517	Chlon *et al*., (2001)
34		3		6.678	6.924	Chlon *et al*., (2001)
35		3		8.000	6.461	Chlon *et al*., (2001)
36		4		7.668	5.701	Kolaczkowski *et al*., (2005)
37		4		9.301	5.971	Kolaczkowski *et al*., (2005)
38		4		8.056	6.815	Kolaczkowski *et al*., (2005)

groups. To check the validity of the selected descriptor set, 100 randomizations of the dependent variable values among the compounds were carried out (*y* scrambling). Multiple r^2 were computed for each of corresponding regressions and the mean value is given as r^2_{scr}. Additionally, the newly synthesized compounds were separated into an external test set and their binding affinities were predicted by the derived models.

Results and discussion

Chemistry

The synthetic route for the preparation of the piperazine derivatives **5–7** are summarized in Scheme 1.

The readily available materials 8-bromocaffeine (**1**); 8-(3-bromopropylamino)-1,3,7-trimethyl-1*H*-purine-2,6 (3*H*,7*H*)-dione (**2**) and 1-(3-iodoropropyl)-3,7-dimethyl-xanthine (**3**) were aminated with benzhydrylpiperazine in the presence of triethylamine to give the target compounds **5–7** in high yield.

All the synthesized compounds were freely soluble in chloroform, dichloromethane, DMF and DMSO but insoluble in non-polar solvents like *n*-hexane. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, FABMS spectra and elemental analysis. The results were consistent with the assigned structures.

The ^{1}H NMR spectra of the compounds **5–7** showed the signals of the respective protons of the synthesized compounds, which were verified on the basis of their chemicals shifts, multiplicities and coupling constants.

The values of the chemical shifts of the protons and carbon atoms registered by ^{1}H NMR and ^{13}C-NMR spectra were compared with simulated values. A very good correlation between the registered and computed values was observed with ^{13}C-NMR spectra. We observed only small deviations of computed from experimental values. The deviations of registered in comparison with calculated values at ^{1}H NMR spectra are larger, due to an impossibility to render an account of influence of the solvent. Regardless, the simulated ^{1}H NMR spectra are in good correlation with experimental ones.

Pharmacology

The affinity constants of the three novel 1-arylpiperazines are given in Table 2. Compound **5** is very weak binder to both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (10.8 and 22.5 μM, respectively). Compound **6** is weak 5-HT$_{1A}$ binder (1.6 μM) and moderate 5-HT$_{2A}$ binder (307.5 nM) Thus, it is 5 times more selective on 5-HT$_{2A}$ than on 5-HT$_{1A}$. Compound **7** shows weak 5-HT$_{1A}$ affinity (2 μM) and high 5-HT$_{2A}$ affinity (58.6 nM). It is 34 times more selective on 5-HT$_{2A}$ than on 5-HT$_{1A}$ receptors.

QSAR study

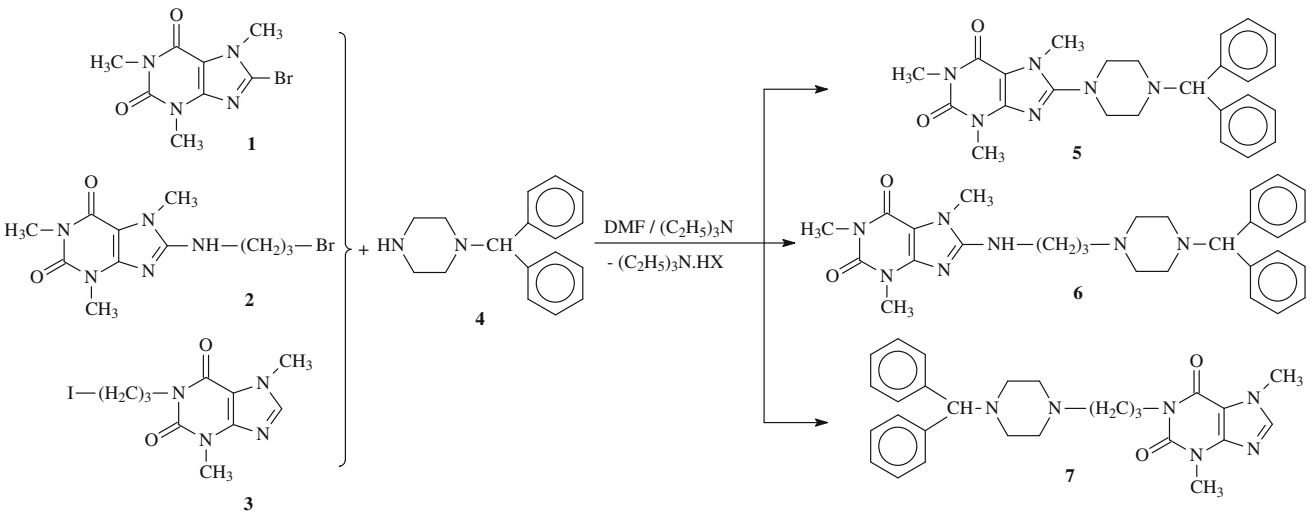
5-HT$_{1A}$ affinity model

Both variable selection procedures, GA and stepwise regression, led to an identical model for affinity to 5-HT$_{1A}$ receptors:

$$pK_i = -6.202(\pm 2.384)q_{N1pip} + 0.470(\pm 0.088)ncirc + 5.659(\pm 1.629)ovality + 2.107(\pm 0.491)HOMO + 222.1(\pm 66.83)q_{N1xant} + 80.597$$

Table 2 5-HT$_{1A}$/5-HT$_{2A}$ affinities of the novel 1-benzhydryl-piperazine derivatives

Compound	$K_i \pm$ SEM (nM)		5-HT$_{2A}$ selectivity 5-HT$_{1A}$/5-HT$_{2A}$
	5-HT$_{1A}$	5-HT$_{2A}$	
5	10830 ± 710	22540 ± 1860	0.5
6	1569 ± 90	307.5 ± 20	5.1
7	1988 ± 116	58.6 ± 4	33.9



Scheme 1

$n = 34$, $r^2 = 0.806$, SEE = 0.437, $F = 23.3$, $q^2_{LOO} = 0.713$, $q^2_{CV5} = 0.681$, $r^2_{scr} = 0.146$

No intercorrelation exists between the descriptors included in the model. The descriptor q_{N1pip} shows the partial atomic charge of piperazine N1 atom. Its values range from −0.266 (**6**) to −0.382 (**17**). The negative regression coefficient means that as more negative is the piperazine N1 atom as more affine is the compound. The charge of N1 depends on the aryl substituent. The inductive effect of the N1 substituents decrease in the order: phenyl > pyrimidinyl > benzhydryl. In the same order increases the 5-HT_{1A} binding affinity of the investigated piperazines.

The descriptor *ncirc* accounts for the number of graph circuits, the total number of all cycles in the molecular graph. For example, *ncirc* equals 2 for biphenyl, but 3 for naphthalene, that is two cycles of 6 edges and 1 cycle of 10 edges. The compounds in this study are divided into three groups according to the value of *ncirc*. Compounds **25**–**28** have 5 circuits in their structures. Compounds **5**–**7** and **29**–**35** have 6 circuits, while compounds **8**–**24** and **36**–**38** have 8 circuits. The positive regression coefficient of *ncirc* indicates that the more circuits in the structure of the ligand the higher is the affinity.

Ovality describes the overall size and shape of the molecules (Bodor *et al.*, 1989). It is defined as the ratio between the area of the surface of the molecule and that of the minimum surface corresponding to the volume of the molecule. For a sphere, ovality equals 1. As higher is the ovality value as far from a sphere is the shape of a molecule. Ovality of the investigated structures ranges from 1.76 for compound **5** to 1.99 for compound **35**. The positive correlation between ovality and affinity suggests that bulky substituents at piperazine N4 are favour for the 5-HT_{1A} affinity. Elongation of the spacer also increases ovality and should increase affinity.

The highest occupied molecular orbital (HOMO) energies of the investigated compounds correlates positively with their 5-HT_{1A} affinities, e.g. high HOMO energies correspond to high affinities. The HOMO energy reflects the compounds' ability to donate electrons in a charge-transfer type of interaction. The charge-transfer mechanism of action of hallucinogenic drugs, like LSD, is well known (Kang and Green, 1970; Kolb, 1987). Also there is empirical evidence that the hallucinogenic effects of LSD are mediated by a direct agonist effect at 5-HT_{2A} receptors (Ouagazzal *et al.*, 2001). Our result shows that a charge transfer is a possible mechanism also in the interaction between ligand and 5-HT_{1A} receptor.

The participation of the partial charge of xanthine N1 atom, expressed by the descriptor q_{N1xant}, in the 5-HT_{1A} model was surprising, because the substitutions in the set of investigated compounds are at positions far from N1. Even the 1-substituted xanthine derivative **7** does not show any significant difference in the charge of N1 in comparison with the 7- and 8-substituted xanthines. However, the removing of the descriptor q_{N1xant} from the model decreases both r^2 and q^2_{LOO} by 0.1. This subtle influence of the partial charge of xanthine N1 atom on 5-HT_{1A} receptor affinity will be explored in our further synthesis.

The correlation plot between predicted and experimental pK_i values for 5-HT_{1A} affinity is given in Fig. 1. The highest residual is 0.848 for compound **13**. Removing it led to a slightly improvement of r^2 and q^2 (data not shown).

The predictive ability of the model was tested on the newly synthesized compounds. They were excluded from the training set, the model was re-built and used to predict the pK_i (5-HT_{1A}) values of the three new compounds (Table 3). Compounds **6** and **7** were predicted well with residuals below 0.5 log unit, while compound **5** was overestimated by 0.7 log unit. The r^2_{pred} value is 0.834.

5-HT_{2A} affinity model

The initial model for 5-HT_{2A} affinity derived by GA and stepwise regression had low r^2 and q^2. The removing of two outliers with residuals >0.9 (compounds **25** and **35**) led to the following model:

$$pK_i = 0.464(\pm 0.073)logP + 175.2(\pm 49.72.)MaxNeg + 14.72(\pm 4.59)q_{N4pip} + 81.576$$

$n = 32$, $r^2 = 0.652$, SEE = 0.458, $F = 17.47$, $q^2_{LOO} = 0.569$, $q^2_{CV5} = 0.582$, $r^2_{scr} = 0.099$

No intercorrelation exists between the descriptors included in the model. The lipophilicity of compounds, expressed by the descriptor log*P*, correlates positively with the affinity, e.g. more lipophilic ligands bind more tightly. This result is in a good agreement with different 5-HT_{2A}

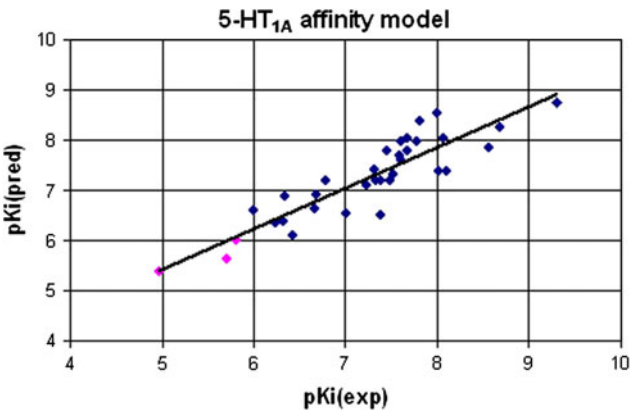


Fig. 1 Plot of predicted versus experimental pK_i values for 5-HT_{1A} affinity after LOO-CV. The new 1-benzhydryl-piperazine derivatives are given in *pink* (*gray*) *diamonds* (Color figure online)

Table 3 Predicted and experimental pK_i (5-HT_{1A}) and pK_i (5-HT_{2A}) values of the novel 1-benzhydryl-piperazine derivatives

Compound	pK_i (5-HT_{1A})			pK_i (5-HT_{2A})		
	Predicted	Experimental	Residual	Predicted	Experimental	Residual
5	5.698	4.965	−0.733	4.842	4.647	−0.195
6	6.268	5.804	−0.464	6.009	6.512	0.503
7	5.979	5.702	−0.277	7.383	7.232	−0.151

pharmacophore models, reviewed extensively by Bojarski (2006), which include one or two hydrophobic areas, necessary for receptor binding of agonists and antagonists.

Descriptor *MaxNeg* reflects the largest negative charge over the atoms in a molecule. In the studied molecules the largest negative charge is associated with xanthine O6 atom. The positive regression coefficient for *MaxNeg* means that less negative O6 atoms are more favorable for 5-HT_{2A} affinity. The partial charge of O6 depends on the substituent at N1 in the xanthine moiety. Compound **7** has the lowest negative charge of O6 due to the stronger positive inductive effect of the three-methylene bridge than that of the methyl group.

The descriptor q_{N4pip} shows the partial atomic charge of piperazine N4 atom. The xanthine moiety is bound to N4 directly in **5** or through a three- or four-methylene spacer in the rest of ligands. The positive correlation between this charge and the affinity means that less negative N4 atoms are more favorable for 5-HT_{2A} affinity. The charge of N4 depends on the length of methylene bridge, as longer is the chain as lower is the charge.

The correlation plot between predicted and experimental pK_i values for 5-HT_{2A} affinity is given in Fig. 2. Compounds **25** and **35** are outliers with residuals above |0.9|. Compound **25** has the highest positive residual, while compound **35** has the highest negative one. Both structures do not differ significantly from the rest of molecules in the set. Compound **35** is the most lipophilic (log$P$ = 5.82) not being the most affine (pK_i on 5-HT_{2A} = 6.461) as it is expected according to the 5-HT_{2A} model. Regarding compound **25** we do not have any reasonable explanation why it acts as an outlier.

The predictive ability of the model was tested on the newly synthesized compounds. They were excluded from the training set, the model was re-built and used to predict the pK_i (5-HT_{2A}) values of the three new compounds (Table 3). Compounds were predicted very well with residuals up to 0.5 log unit and $r^2_{pred} = 0.914$.

The structures of the new selective 5-HT_{2A} ligands are in a good agreement with the pharmacophore models, available in the literature (Bojarski, 2006). According to Glennon's model of a 5-HT_{2A} binding site (Glennon *et al.*, 1991) the pharmacophore consists of region of bulk tolerance at a distance from a basic nitrogen atom, area of

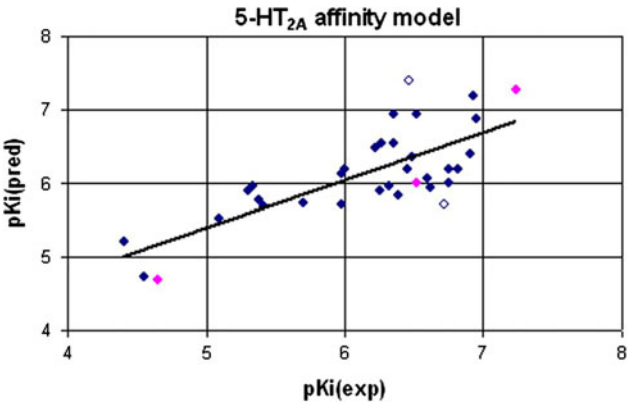


Fig. 2 Plot of predicted versus experimental pK_i values for 5-HT_{-2A} affinity after LOO-CV. Outliers are given in *blank diamonds*. New 1-benzhydryl-piperazine derivatives are given in *pink* (*gray*) *diamonds* (Color figure online)

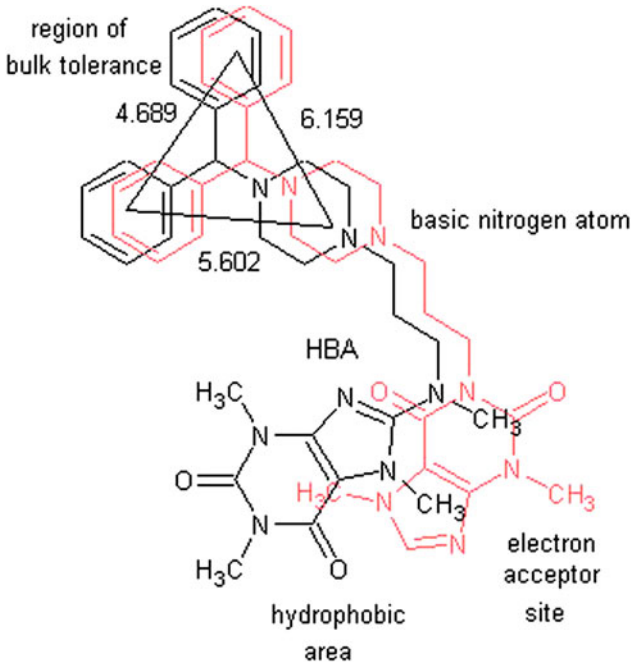


Fig. 3 5-HT_{2A} pharmacophore model according to Mokrosz *et al.* (1994), shown on compounds **6** and **7**

hydrogen bond acceptor (HBA), electron acceptor site and hydrophobic area (Fig. 3). Most of these elements, like region of bulk tolerance, basic nitrogen, HBA and

hydrophobic area, present in our structures, while the electron acceptor site is absent. Obviously, the next synthesis should explore the presence of this electron acceptor site. In the area of bulk tolerance, Mokrosz *et al*. (1994) suggest that the distances between the two aromatic centers are in the range 4.6–7.3 and these between the aromatic rings and the basic nitrogen are 5.2–8.4 and 5.7–8.5, respectively. The distances in the benzhydryl substituent at piperazine N1 fall in the suggested ranges.

In summary, the present study describes the synthesis and 5-HT_{1A}/5-HT_{2A} receptor affinity of novel 1-arylpiperazine derivatives with 5-HT_{2A} selectivity. The 2D-QSAR study reveals several structural features responsible for the affinity and selectivity of 5-HT ligands with benzhydryl substituent at N1 and xanthine moiety at N4. The results from the QSAR study and the analysis of 5-HT_{2A} pharmacophore elements directed to the next rational synthesis of new selective ligands.

Acknowledgments This work was supported by grant from the Medical Science Council of the Medical University of Sofia (project 5-I/2009).

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