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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1708–1712

Synthesis and QSAR studies on hypotensive 1-[3-(4-substituted phenylthio) propyl]-4-(substituted phenyl) piperazines☆

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Received 18 September 2006; revised 11 December 2006; accepted 21 December 2006
Available online 23 December 2006

Abstract—A series of 1-[3-(4-substituted phenylthio) propyl]-4-(substituted phenyl) piperazines has been synthesized and evaluated for hypotensive activity. The QSAR studies indicate that resonance and hydrophobic parameters of the aryl substituents are important for hypotensive activity. The similar role of resonance parameter in describing the variance of 5-HT_{2A} receptor binding affinities of these compounds suggests a possible role of 5-HT_{2A} receptors in mediating the hypotensive action of title compounds.

Hypertension is a state of abnormally high pressure in the arteries all the time. Between 85% and 90% of people suffer from primary hypertension (with no known cause), rest 10–15% of people have secondary hypertension (caused by renal, endocrine or pregnancy related diseases). The disease is usually symptom less but if untreated, it may result in heart enlargement and failure, renal dysfunction and cerebrovascular accidents.

Body mechanisms that control blood pressure are controlled by the sympathetic division of the autonomic nervous system and the kidneys. Drugs used, alone or in combination, for treatment of hypertension are diuretics, adrenergic blockers, centrally acting alpha-agonists, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II blockers, calcium channel blockers, direct vasodilators and more recently 5-HT antagonists.[1] One of the important vascular effects of 5-HT is its ability to act as a vasoconstrictor. The 5-HT_{2A}, 5-HT_{2B} and 5-HT_{1B} receptors have been implicated as mediators of 5-HT-induced contraction in vascular smooth muscle. 5-HT_{2A} receptors mediate contraction in many arteries including the rat thoracic aorta[2] and pulmonary arteries[3]. In our earlier work, 1-(aryloxy/thioaryloxy)-3-(*N*-arylpiperazinyl) propanes and propanols,[4–9] series of 1-[3-(4-substituted phenylthio) propyl]-4-(substituted phenyl) piperazines and related compounds[10–12] including 1-(aryl-piperazin-1-yl)-1-oxo-2-(thioaryloxy/aryloxy) propanes[13] have been investigated for their hypotensive activity. Among these compounds 1-[3-(4-acetamidophenylthio) propyl]-4-[3-methyl phenyl] piperazine[11] emerged as a potential antihypertensive agent. It showed activity comparable to centhaquin.[14] The QSAR studies on a set of the analogues of 1-[3-(4-acetamidophenylthio) propyl]-4-[3-methyl phenyl] piperazine indicated that the variance in activity is explained by the resonance and the hydrophobicity effects of the substituents on the phenyl rings. Based on the above observations and in order to further explore the effect of aromatic substitution on hypotensive activity, another series of 1-[3-(4-substituted phenylthio) propyl]-4-(substituted phenyl) piperazines was synthesized and evaluated for their hypotensive activity in vivo. In order to study the possible role of 5-HT_{2A} receptor affinity in the observed hypotensive activity in these molecules, some selected compounds including the least and most active ones were evaluated for their affinities to 5-HT_{2A} receptors. The quantitative structure–activity relationship (QSAR) studies have been carried out to analyze the observed variance in hypotensive activity in terms of the effect of physicochemical parameters (hydrophobic, steric and electronic) where the resonance and hydrophobic parameters for the substituents in the thioaryloxy and arylpiperazine part, respectively, were found to be important for explaining the variance in hypotensive activity and the former in 5-HT_{2A} receptor binding affinity. These studies are reported in this paper.

Keywords: Thioarylalkylpiperazines; Hypotensive; QSAR; 5-HT2A binding affinity; Hydrophobicity; Resonance.
☆ CDRI Communication No. 7077.
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doi:10.1016/j.bmcl.2006.12.072

The 3-chloropropyl 4-substituted-phenyl sulfides were prepared by the condensation of appropriate thiophenols with 1-bromo-3-chloropropane in the presence of sodium hydroxide in ethanol to give the key intermediate arylthiopropylchloride, which on condensation with the corresponding substituted phenyl piperazines in dry DMF in presence of sodium carbonate and sodium iodide afforded the desired compounds (**1**–**34**) in 70–85% yields[15] essentially according to our previously described method[11] (Scheme 1. and Table 1). All the compounds have been characterized by IR, FAB-MS, NMR spectroscopic techniques and elemental analysis.

Hypotensive activity. The compounds were tested[16] for their effect on blood pressure in anaesthetized cats by administration of 2 μmol/kg iv dose and the results are given in Table 1. These compounds in general are good hypotensive agents with % maximum blood pressure fall ranging from 12.49 to 61.02. The compounds **1** and **2** show high profile of hypotensive activity in terms of fall in blood pressure and longer duration of action. Two of these (**33** and **34**) compounds showed tachyphylaxis and one of them (**33**) with R = $-NO_2$ is a positional isomer of the compound **42** (Table 1) reported earlier.[12]

5-HT_{2A} receptor binding affinity. In vitro receptor binding studies for serotonergic 5-HT_{2A} (rat striatal membrane) were carried out[17] (Table 2). The radioligand and reference compound used for 5-HT_{2A} receptor were [^{3}H] Ketanserin (60–90 Ci/mmol) and Ketanserin (K_i = 0.4 nm), respectively.

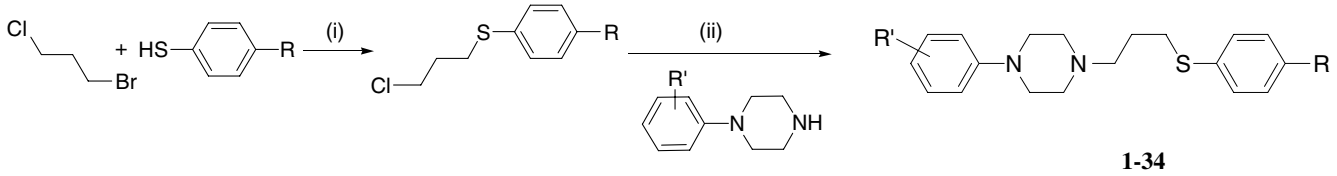
The QSAR studies have been carried out to find the influence of physicochemical factors on the hypotensive activity. In these studies the hypotensive activity was taken as dependent and different physicochemical parameters (hydrophobicity: π, electronic effect: σ-Hammett, resonance effect: $\mathcal{R}$, field effect: $\mathcal{F}$ and steric effect: MR) at positions R and R′ (Scheme 1) of the aromatic rings as independent variables. In addition to the newly synthesized 34 compounds (**1**–**34**), a set of 8 compounds (**35**–**42**, taken from our earlier[12] studies) showing >45% fall in blood pressure were also considered for these studies. Thus, a total set of 42 compounds was considered. Out of these compounds 3 compounds viz. **33**, **34** from the newly synthesized set and **42** from earlier reported compounds[12] were not considered for QSAR analysis as they showed tachyphylaxis. Thus, the remaining 39 compounds were divided into a training set of 28 compounds (**1**–**22**, **35**–**40**) (Table 1) and a test set containing 11 molecules (**23**–**32**, **41**[12]) (Table 1). The values for the physicochemical parameters were computed and taken from the literature[18] and the multi-parameter regression analysis was carried out using SYSTAT-7.1 software. Among different physicochemical parameters viz. σ (R′), σ (R), $\mathcal{R}$ (R), $\mathcal{F}$ (R), π (R), Σπ(R′), [Σπ (R′)]^2 and MR (R) and MR (R′), the parameters [Σπ (R′)]^2, MR (R) (r = 0.058) and π (R) (r = 0.037) showed the least correlation with activity while electronic σ (R′) (r = −0.306), σ (R) (r = −0.158); resonance $\mathcal{R}$ (R) (r = −0.177), $\mathcal{R}$ (R′) (r = 0.698); field effect $\mathcal{F}$ (R) (r = 0.140), $\mathcal{F}$ (R′) (r = −0.485); hydrophobic Σπ (R′) (r = 0.196) parameters showed considerable correlation with activity. Hence, different combinations of the independent parameters were used for correlating the activity using the classical Hansch regression analysis.

Although σ (R′), $\mathcal{F}$ (R′) and R (R′) had good correlations with hypotensive activity, but none of them alone or in combination with the other parameters could give statistically significant equation. Hence different sets of parameters viz. $\mathcal{R}$ (R), Σπ (R′), [Σπ (R′)]^2, with negligible intercorrelation between Σπ (R′) and $\mathcal{R}$ (R) (r = 0.1) and [Σπ (R′)]^2 and $\mathcal{R}$ (R) (r = 0.03), were tried to derive equations where a combination of $\mathcal{R}$ (R), Σπ (R′) and [Σπ (R′)]^2 led to the derivation of Eq. 1 where the figures in parentheses describe the standard error of the regression coefficient, n represents the number of data points, r is the correlation coefficient, s is the standard error from the regression and F is the measure of statistical significance of the regression model.

$$\log C = 0.599\mathcal{R}(\mathrm{R})(\pm 0.169) + 1.933\sum\pi(\mathrm{R}')(\pm 0.254) - 1.175[\sum\pi(\mathrm{R}')]^2(\pm 0.160) - 0.588(\pm 0.077)$$
$$n = 28,\ r = 0.846,\ s = 0.170,\ F = 20.21 \quad (1)$$

Eq. 1 obtained above is statistically significant as it has low standard error (0.170), moderate correlation coefficient (0.846) and high statistical significance ($F_{3,24\alpha 0.001}$ = 8.51; $F_{3,24}$ = 20.21) with >99.9% values for regression coefficients. It describes well the variation in the observed hypotensive activity as shown by comparison of observed and calculated hypotensive activity of these molecules (Table 1, Eq. 2, Fig. 1).

It is evident from the equation that resonance and hydrophobic effects play an important role in the variation of activity. The resonance effect at position 4 of the thioaryl ring positively contributes to the hypotensive activity. Thus, substituents with positive values of resonance parameter might show good activity. The relatively poorer correlation of hypotensive activity with σ and



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Scheme 1. Reagents and condition: (i) NaOH, C_2H_5OH; (ii) dry DMF, Na_2CO_3, NaI, 75 °C, 48 h.

Table 1. Observed '%' maximum blood pressure fall' in anaesthetized cats induced by 34 derivatives of the general structure shown in Scheme 1

Compound	R′	R	% Max. blood pressure fall[c] (2 μmol/kg)	Duration (min)	Mp (°C)	log C[e]		Values of Hansch parameters used		
						Found	Calcd/pred[f]	$\mathcal{R}$ (R)	Σπ (R′)	$[\Sigma\pi\ (R')]^2$
1	2-CH_3	CH_3	61.02	60	160	0.1946	0.0482	−0.13	0.56	0.3136
2	3-Cl	H	55.99	120	182	0.1046	0.1922	0.00	0.71	0.5041
3	3-CF_3	OCH_3	53.42	40	189[a]	0.0595	−0.1025	−0.51	0.88	0.7744
4	2-CH_3	NHC_2H_5	48.65	85	Oil	−0.0235	−0.1795	−0.51	0.56	0.3136
5	3,4-Di Cl	H	45.83	25	83	−0.0726	−0.2125	0.00	1.42	2.0164
6	3-Cl	OCH_3	42.62	30	Oil	−0.1291	−0.1135	−0.51	0.71	0.5041
7	4-F	Cl	42.31	65	95	−0.1347	−0.4300	−0.15	0.14	0.0196
8	2-CH_3	Cl	40.00	35	148	−0.1761	0.0362	−0.15	0.56	0.3136
9	3,4-Di Cl	F	38.30	6	Oil	−0.2071	−0.4163	−0.34	1.42	2.0164
10	3-CH_3	F	38.30	65	Oil	−0.2071	−0.0776	−0.34	0.56	0.3136
11	3,4-Di Cl	CH_3	36.36	10	Oil	−0.2431	−0.2904	−0.13	1.42	2.0164
12	3-F	F	34.00	60	184[a]	−0.2880	−0.5439	−0.34	0.14	0.0196
13	3,4-Di Cl	Cl	26.58	90	180	−0.4413	−0.3024	−0.15	1.42	2.0164
14	2-OCH_3	H	26.23	90	Oil	−0.4490	−0.6258	0.00	−0.02	−0.0004
15	4-F	CH_3	21.74	35	76	−0.5563	−0.4180	−0.13	0.14	0.0196
16	4-F	H	20.69	50	107	−0.5835	−0.3401	0.00	0.14	0.0196
17	3,4-Di Cl	OCH_3	21.05	11	Oil	−0.5741	−0.5182	−0.51	1.42	2.0164
18	3,4-Di Cl	$NHCOCH_3$	20.00	30	132	−0.6021	−0.3683	−0.26	1.42	2.0164
19	4-F	Br	19.86	0.30	97	−0.6059	−0.4420	−0.17	0.14	0.0196
20	4-F	OCH_3	17.46	0.30	202	−0.6747	−0.6458	−0.51	0.14	0.0196
21	3-F	OCH_3	17.02	30	152	−0.6880	−0.6458	−0.51	0.14	0.0196
22	4-OCH_3	H	15.38	30	95	−0.7404	−0.6258	0.000	−0.02	−0.0004
23[d]	4-OCH_3	F	12.49	60	97	−0.8452	−0.8299	−0.34	−0.02	−0.0004
24[d]	4-F	F	20.66	90	91	−0.5843	−0.5441	−0.34	0.14	0.0196
25[d]	3-CH_3	NHC_2H_5	41.14	10	Oil	−0.1556	−0.1795	−0.51	0.56	0.3136
26[d]	4-F	$NHCOCH_3$	21.21	17	138	−0.5699	−0.4964	−0.26	0.14	0.0196
27[d]	H	F	14.28	50	75	−0.7783	−0.7917	−0.34	0.00	0.0000
28[d]	2-OCH_3	Br	21.59	15	85	−0.5600	−0.7280	−0.17	−0.02	−0.0004
29[d]	4-F	NH_2	13.51	30	68	−0.8063	−0.7477	−0.68	0.14	0.0196
30[d]	4-F	NO_2	25.87	60	70	−0.4572	−0.2446	0.16	0.14	0.0196
31[d]	2-CH_3	F	37.59	30	40	−0.2200	−0.0777	−0.34	0.56	0.3136
32[d]	4-OCH_3	Br	23.06	40	114	−0.5233	−0.7280	−0.17	−0.02	−0.0004
33[b]	2-CH_3	NO_2	42.00	30	147	0.7241	—	0.16	0.56	0.3136
34[b]	H	H	41.07	30	60	0.6969	—	0.00	0.00	0.0000
35	3-CH_3	$NHCOCH_3$	55.0	>180	Oil	0.0871	−0.0297	−0.26	0.56	0.3136
36	3-CH_3	NH_2	24.28	47	148	−0.4939	−0.2814	−0.68	0.56	0.3136
37	3-CH_3	CH_3	55.74	84	160	0.1002	0.04820	−0.13	0.56	0.3136
38	3-CH_3	H	65.15	>86	Oil	0.2717	0.1261	0.00	0.56	0.3136
39	3-CH_3	Cl	43.80	92	Oil	−0.1082	0.03621	−0.15	0.56	0.3136
40	2-CH_3	$NHCOCH_3$	51.61	60	180	0.0279	−0.0297	0.26	0.56	0.3136
41[d]	3-CH_3	OCH_3	21.4	52	147	−0.5649	−0.1795	−0.51	0.56	0.3136
42[b]	3-CH_3	NO_2	84.41	120	190	0.7335	—	0.16	0.56	0.3136

[a] HCl salt.
[b] Compound showed tachyphylaxis.
[c] Each entry is mean of three experimentally determined values.
[d] Compounds included in the test set.
[e] Where C = % fall in BP/(100 − % fall in BP).
[f] Calcd (calculated for training set), pred (Predicted for test set).

$\mathcal{F}$ than $\mathcal{R}$ suggests that the phenyl ring containing the substituents R does not inhibit the conjugation between the π-electron clouds of the phenyl ring and the lone pair of the thio group and thus the major electronic effect is through resonance. It is interesting to note that the value of intercorrelation between the σ and $\mathcal{R}$ parameters in our earlier study[12] was 0.923 and hence it was difficult to decide between the role of $\mathcal{R}$ and σ for hypotensive activity while in this set of compounds with nonorthogonal relation of $\mathcal{R}$ and σ ($r = 0.077$) it is clear that the major contribution towards activity is through the resonance effect of the R substituents. The parabolic relationship between hypotensive activity and the hydrophobicity indicate the optimum hydrophobicity value for the substituent is $[\pi\ (R')]_0 = 0.823$ and that the transport of the drug to the active site is the rate-limiting step for hypotensive activity which is well expected as the hypotensive activity was measured in the whole animal system. These results also corroborate our earlier results where the similar dependence of hypotensive activity on resonance and hydrophobicity (in terms of molecular lipophilicity potentials) was observed.[12] The validity of the derived model was further tested on 11 molecules of the test set (indicated by * in Table 1)

Table 2. 5-HT_{2A} receptor binding affinity studies on selected compounds

Compound	% inhibition at 10^{-6} M[a]	K_i[a] (nm)	π (R)	log A[b] at 10^{-6} M	
				Found	Calcd
23	25.70	nd	0.14	−0.4610	−0.2059
24	32.90	nd	0.14	−0.3095	−0.2059
26	85.40	nd	−0.97	0.7671	0.7268
27	37.0	nd	0.14	−0.2311	−0.2059
32	37.10	nd	0.86	−0.2293	−0.5856
34	37.40	nd	0.0	−0.2237	0.2466
35	94.7	6.09	−0.97	1.2521	0.7268
38	75.58	13.6	0.0	0.4907	0.2466
40	72.65	21.1	−0.97	0.4243	0.7268
42	80.45	21.1	−0.28	0.6144	0.6238

[a] Values are means of two experiments, (nd, not done).
[b] Where A = % inhibition/(100 − % inhibition).

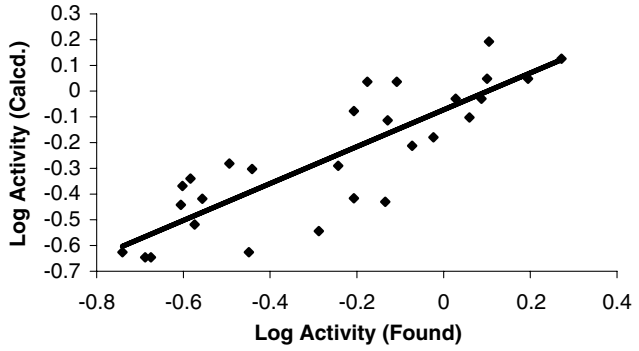

Figure 1. log $\text{Act.}_{\text{Calcd}} = 0.716 \log \text{Act.}_{\text{Found}}(\pm 0.88) - 0.072(\pm 0.035)$ $n = 28$, $r = 0.846$, $s = 0.138$, $F = 65.66$. (2)

where a good match between the observed and predicted hypotensive activity has been obtained (Fig. 2, Eq. 3).

As 10 of these compounds were studied for their 5-HT_{2A} binding affinities it appeared of interest to develop QSAR in these compounds. Among all the parameters considered, only π (R) ($r = -0.787$), π (R′) ($r = 0.784$), MR(R) ($r = 0.709$), $\mathcal{F}$ (R′) ($r = -0.336$) and $\mathcal{R}$ (R) ($r = 0.244$), $\mathcal{R}$ (R′) ($r = 0.304$) showed considerable correlation with biological activity. Among these MR(R) was highly correlated with π (R) ($r = -0.70$), and π (R) had a better correlation with log Affinity than MR (R) ($r = 0.709$) hence out of these two only π (R) was considered for regression analysis in combination with $\mathcal{R}$ (R), π (R′), $\mathcal{R}$ (R′).

Combination of π (R) and $\mathcal{R}$ (R′), π (R′) and $\mathcal{R}$ (R), π (R′) and $\mathcal{R}$ (R′) gave moderate values for correlation coefficient ($r = 0.787$, 0.788, 0.784, respectively), and also the regression coefficients with the independent parameters were statistically not significant (<90%).

The variation in 5-HT_{2A} binding affinities in this set of compounds was best described by the following Eq. 2 where a negligible intercorrelation between Σπ (R) and $\mathcal{R}$ (R) ($r = 0.08$) was observed.

$$\log C = 1.015\mathcal{R}(\text{R})(\pm 0.666) + 0.767\pi(\text{R})(\pm 0.192) + 0.247(\pm 0.165) \quad n = 10,\ r = 0.845,\ s = 0.34,\ F = 8.72 \tag{4}$$

Eq. 2 with good correlation coefficient value ($r = 0.845$), low standard error ($s = 0.34$) and good statistical significance ($F_{2,7\alpha 0.01} = 7.55$; $F_{2,7} = 8.72$, with >99.9% values for regression coefficients) well describes the variance in 5-HT_{2A} affinity as shown by the good match between observed and calculated log Affinity values (Fig. 3, Eq. 5. The dependence of hypotensive activity as well as 5-HT_{2A} receptor binding affinity positively on resonance parameter of the substituents of the thioaryl ring suggests that the 5-HT_{2A} receptors may be involved in mediating the hypotensive action of these compounds. The parabolic dependence of hypotensive activity on hydrophobicity of R′ substituents suggests that the rate limiting factor in the hypotensive action is the transport of the molecule at the active site which overweighs the role of hydrophobicity in the thioaryl part of the molecule for binding. It may be the reason for insignificant contribution of hydrophobicity of R substituents in the hypotensive activity while statistically significant negative contribution for binding affinity. These studies also support our earlier proposition[12] that the thioaryl part in these molecules is more important for binding at the receptor and the rest of the molecule is responsible for the transport of the molecule to the receptor site.

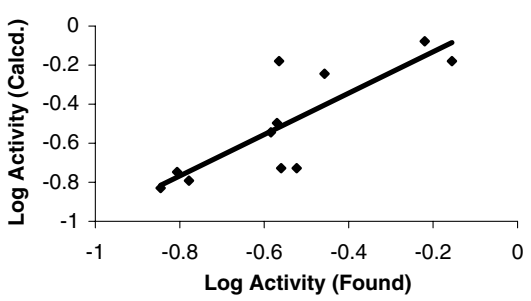

Figure 2. log $\text{Act.}_{\text{Calcd}} = 0.629 \log \text{Act.}_{\text{Found}}(\pm 0.148) - (\pm 0.085)$ $n = 11$, $r = 0.817$, $s = 0.133$, $F = 18.06$. (3)

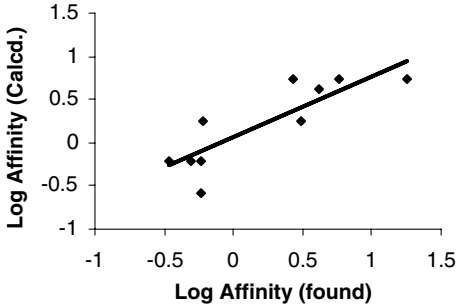

Figure 3. log $\text{Aff.}_{\text{Calcd}} = 0.714 \log \text{Aff}_{\text{Found}}(\pm 0.160) + 0.060(\pm 0.093)$ $n = 10$, $r = 0.845$, $s = 0.27$, $F = 19.94$. (5)

Acknowledgments

Authors are thankful to Messrs Z. Ali and A. S. Kushwaha for their excellent technical assistance; RSIC, Lucknow, for providing spectroscopic data and NIDA & NOVA Screen USA, for providing receptor binding data. One of the authors RC is thankful to CSIR, India, for the award of senior research fellowship.

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15. Experimental: All melting points were determined in an electrically heated apparatus (Tempo and Toshniwal) and are uncorrected. The IR spectra were recorded on Perkin-Elmer 137 spectrophotometers. Mass spectra were recorded on a JEOL SX 102/DA-6000 spectrometer. NMR spectra were recorded on Varian R-32 (90 MHz) instruments using tetramethylsilane as internal standard. Elemental analyses (C, H, N) of the synthesized compounds were found to be ±0.45 of the theoretical values. General Procedure: preparation of compound **10**: a mixture of 1-(3-methylphenyl) piperazine (0.352 g, 2 mmol), 1-[3-(4-fluorophenylthio) propylchloride (prepared according to literature method[1]) (0.429 g, 2.1 mmol) and sodium carbonate (0.22 g, 2.1 mmol), sodium iodide (0.002 g, 0.1 mmol) in dry dimethylformamide (2 mL) was stirred at 75 °C for 48 h. The reaction mixture was cooled and then poured into water. The separated solid was filtered and crystallized from ethanol–water to give 1-[3-(4-fluorophenylthio) propyl]-4-(methoxy-phenyl) piperazine. Yield: 0.60 g, 87.2%. FABMS *m/z*: 344 [M^+]. IR (KBr, cm^{-1}): 2900, 1520, 1500, 1445, 1230, 940, 840, 720. ^1H NMR ($CDCl_3$, 90 MHz): δ 1.4–1.9 (m, 2H), 2.2 (s, 3H), 2.3–2.8 (m, 8H), 2.9–3.2 (m, 4H), 6.4–6.7 (m, 6H), 6.9–7.2 (m, 2H). Anal. Calcd for $C_{20}H_{25}FN_2S$ (344.17): C, 69.73; H, 7.31; N, 8.13. Found: C, 70.44; H, 6.99; N, 8.0.
16. Hypotensive activity was studied in cats (2.5–3.5 kg) of either sex anaesthetized with either pentobarbitone sodium (35.0 mg/kg iv) or α-chlor-alose (80.0 mg/kg iv). Blood pressure from the left common carotid artery was recorded either on a kymograph through a mercury manometer or on a Grass model 7 polygraph through a Statham P23 dc transducer and 7P 1 low level DC preamplifier. A femoral vein was cannulated for injections and an endotracheal cannula was passed for recording of the respiration or for providing positive pressure ventilation.Contraction of the right nictitating membrane due to electric stimulation (10–20 Hz, 1 ms, 2–10 *v* for 5–10 s) of the cervical sympathetic nerve was recorded by means of frontal writing lever on a kymograph. Blood pressure responses to intravenous injections of epinephrine (1–2 μg), acetylcholine(1–3 μg), histamine (1–2 μg) and isoprenaline (0.5–1 μg) were obtained before and after the administration of the compound by iv route. Heart rate (HR) of the animals was either recorded on the Glass polygraph through a 7P4 tachograph preamplifier or displayed on a Tektronex physiograph.
17. 5-HT_{2A} Binding Assays. NIH-3T3-GF6 cells, which express cloned rat 5-HT_{2A} receptors, were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% Calf serum, 0.05% pen.-strep. and 200 μg/mL G418. The cells were scraped from the 100 × 20 mm plates and centrifuged at 1000*g* for 5 min. The pellet was homogenized (2 plates/mL) with a Polytron homogenizer in 50 mM Tris–HCl, pH 7.7 (25 °C). This homogenate was then stored in 1 mL aliquots at 70 °C. Before experiment the cells were thawed, resuspended in 50 mM Tris–HCl, pH 7.7 (25 °C) and centrifuged at 27000*g* for 12 min. The final pellet was resuspended in 25 nM Tris–HCl, pH 7.7, (25 °C), at a final concentration of 2 plates per 40 mL. For binding assays, the cell suspension (0.8 mL) was incubated in 25 mM Tris–HCl, pH 7.7 for 60 min at 25 °C with 100 μL of test compound and [^3H] Ketanserin each (0.3 nM final concentration).
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