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Synthesis and pharmacological evaluation of new arylpiperazines *N*-{4-[4-(aryl) piperazine-1-yl]-phenyl}-amine derivatives: Putative role of 5-HT$_{1A}$ receptors

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ABSTRACT

In an attempt to design novel 5-HT$_{1A}$ agonists/partial agonists, based on an arylpiperazine nucleus, a series of *N*-{4-[4-(aryl)piperazine-1-yl]-phenyl}-amine derivatives were synthesized and biologically tested. The anxiolytic effect of the compounds was investigated employing the Elevated plus Maze (EPM) task. On the basis of in vivo functional test, compound **1c** (3 mg/kg) and **4c** (3 mg/kg) induced significant increments in open arm entries and time on EPM as compared to Buspirone. The anxiolytic effects of compounds **1c** and **4c** were effectively antagonized by WAY-100635, a 5-HT$_{1A}$ receptor antagonist (0.5 mg/kg). Furthermore, we have also evaluated the concentration of 5-HT in the brain tissue using HPLC with fluorescent detection. Our result showed that serotonin levels were significantly decreased by ~38% ($p < 0.001$) and ~32% ($p < 0.001$) after acute administration of compounds **1c** and **4c**, respectively. These findings suggest that the anxiolytic like activity of these new arylpiperazines is mediated via 5-HT$_{1A}$ receptors in the brain.

1. Introduction

Anxiety is a common disorder affecting one eighth of the total population worldwide and has become an important area of research interest in psychopharmacology.[1] Now a days with increasing competition anxiety disorders have already become one of the most wide spread psychiatric diseases. Currently many reports suggest that the occurrence of anxiety is associated with the dysfunction of central monoamine neurotransmitter serotonin.

The serotonergic system has been consistently implicated in the pathophysiology of a number of psychiatric disorders including depression and anxiety.[2] 5-HT (serotonin) is found throughout the CNS in high levels and participates in modulating mood. According to the classic serotonin hypothesis, anxiety is usually associated with increased endogenous 5-HT, and anxiolytics tend to decrease endogenous 5-HT.[3] Among the 13 different serotonin receptors belonging to 'G' protein coupled receptor superfamily,[4] the 5-HT$_{1A}$ and 5-HT$_{2A}$ subtypes are most frequently considered to be the targets for anxiolytic and antidepressant drugs.[5] Indeed, 5-HT$_{1A}$ agonists and partial agonists (e.g., Buspirone (Fig. 1) and tandospirone)[6] demonstrate clinical effectiveness in the treatment of either disorder. However, development of agents of this type is still a topical subject of investigations and lies within the area of our interest.

Several structurally different compounds are known to bind 5-HT$_{1A}$ R sites. Among these arylpiperazine derivatives represent one of the most important classes of 5-HT$_{1A}$ R ligands.[7] Numerous studies have indicated that even minor modifications in the chemical structure of arylpiperazines, strongly affect the affinity and selectivity for the 5-HT$_{1A}$ receptor sites[8] and their effect on central nervous system.[9] The significance of the respective parts of arylpiperazines for 5-HT$_{1A}$ affinity, intrinsic activity and selectivity has been the subject of many structure–activity relationship studies.[10]

While searching for new agents for a possible treatment of anxiety and depressive disorder, we designed a novel class of arylpiper-

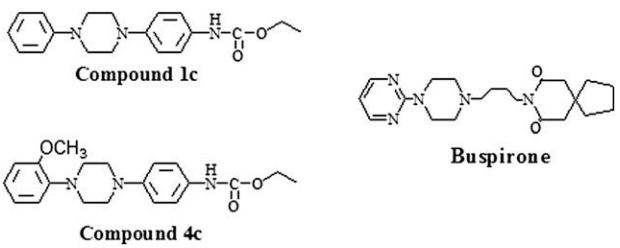

Figure 1. Structure of 5-HT$_{1A}$ partial agonist Buspirone and synthesized arylpiperazine derivatives **1c** and **4c**.

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azine derivatives containing different N-4 terminal amide and ester fragments. Further modifications involved diversifying the nature of the *N*-aryl substituent and the length and nature of an alkyl chain connecting the amide/ester fragment to the basic nitrogen of arylpiperazine. In the present paper, we described the synthesis of 12 compounds, the derivatives of *N*-{4-[4-(aryl) piperazine-1-yl]-phenyl}-amine and tested them for their anxiolytic like activity on Elevated plus maze in a rat model. On the basis of obtained results the two most promising compounds **1c** (Fig. 1) and **4c** (Fig. 1) were further tested for the activity of biochemical markers for anxiety glyoxalase1 and glutathione reductase. In addition, it was of interest to investigate the involvement of 5-HT receptor system through co-administration of the 5-HT_{1A} receptor antagonist WAY-100635. We have also investigated the effects of these synthesized compounds on serotonin levels in brain homogenates, using reverse phase high performance liquid chromatography (HPLC) with fluorescence detection The aim is to determine the neurochemical mechanisms of the antianxiety activity of these derivatives.

2. Results and discussion

2.1. Synthesis

The synthetic methodology employed to develop target compounds is summarized in Scheme 1. 1-Fluoro4-nitro benzene was reacted with *N*-arylpiperazine in dry DMSO to yield 1-(4-nitro phenyl)-4-arylpiperazine (**1a** to **4a**). The nitro group was reduced in the presence of Sn/HCl to afford the aniline, 4-[4-(aryl)-piperazine-1-yl]-phenyl amine (**1b** to **4b**). Then condensation of amine with acetic anhydride in methylene chloride gave amide with an approximate yield of ~75% (**1d** to **4d**). Alternatively, ethyl chloro formate was coupled with amine to yield the desired compound *N*-[4-(4-aryl-piperazine-1-yl)-phenyl]carbamic acid ethyl ester (**1c** to **4c**) in the presence of tri ethyl amine with an approximate yield of ~80%. Additionally, coupling of amine (**1b** to **4b**) with 2-(6-chloro)-nicotinic acid in acetonitrile at room temperature using the coupling agent, EDC/HCl in the presence of 1-hydroxybenzotriazole hydrate (HOBt) gave 6-chloro-*N*-{4-[4-(2-aryl)-piperazin-1-yl]-phenyl}-nicotinamide (**1e** to **4e**). All the compounds were fully characterized by IR, ^{1}H NMR and mass spectroscopy.

2.2. Effect of arylpiperazine derivatives on the rat behavior in elevated plus maze test

The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, (fear of novel space and fear of balancing on a relatively narrow raised platform) that can induce anxiety in humans.[11] An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in open arms of EPM. According to the main goal of our work, functional profile of all the derivatives was determined in vivo; to find out whether slight changes in the structure of the terminal amide/ester fragment and aryl fragment influence their anxiolytic activity.

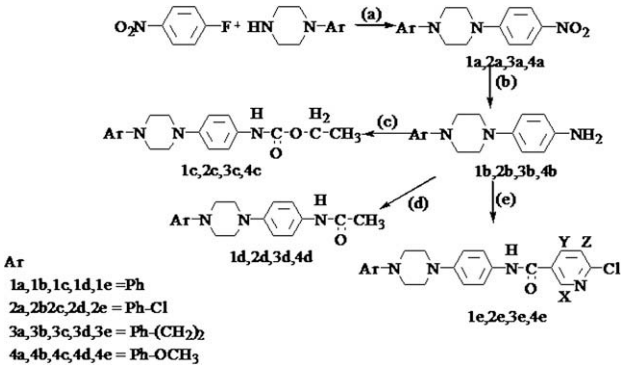
The effects of single administration of all the 12 aryl piperazine derivatives, Diazepam, Buspirone and vehicle on spent time and the number of entries into the open arms of EPM are shown in Table 1. One way analysis of variance revealed a significant increase in percentage time spent on the open arms after administration of both positive controls Diazepam and Buspirone as compared to control. The compounds were tested on EPM at different doses (data not shown) and the best results were found at the dose of 3 mg/kg. The majority of tested compounds produced a significant anxiolytic action displayed as a considerably increased number of open arm entries. Among all the 12 compounds, **1c** (3 mg/kg bwt) prolonged the percentage of entries in open arms with ~82% ($p < 0.001$) and **4c** (3 mg/kg bwt) with ~60% ($p < 0.001$) as compared to control. These results are found to be comparable with Buspirone. The total number of arm entries was not significantly different for all treatment groups.

The results presented in Table 1 have conclusively proven that the derivatives with terminal ester fragment significantly increased the entry as well as time spent in open arms (**1c** to **4c**) than the corresponding amide fragment (**1d** to **4e**). Within a set of respective structural analogs, unsubstituted phenyl piperazines displayed good anxiolytic activity. Introduction of electron withdrawing groups such as –Cl introduced at the ortho position into the phenyl piperazine moiety decreases the entry as well as time spent in open arms (**2c**, **2d**, **2e**). The same effect was observed with the introduction of linker alkyl between phenyl ring and piperazine (**3c**, **3d**, **3e**). While methoxy group (–OCH_3) introduced at the ortho position of phenyl piperazines showed anxiolytic activity (**4c**, **4d**, **4e**) comparable to unsubstitued phenyl piperazines (**1c**, **1d**, **1e**), irrespective of ester or amide terminal fragment. Compound **1c** containing unsubstituted phenyl ring and **4c** containing an *o*-OCH3 substituent turned out to be the most active in behavioral test. Hence, on the basis of results obtained from elevated plus maze, compounds **1c** and **4c** were analyzed further for their effect on biomarkers of anxiety.

2.3. Effect of arylpiperazine derivatives on glyoxalase1 and glutathione reductase activity

Two enzymes, glyoxalase 1 (Glo1) and glutathione reductase (GSHRd) regulate anxiety[12] and are considered as biomarkers for anxiety. Glo1 levels are found to be increased in anxiogenic conditions and low in anxiolytic conditions.[12] GSHRd is functionally related to Glo1 because reduced glutathione (GSH) is the cofactor for the enzymatic reaction that is catalyzed by Glo1 and levels of GSH are maintained by GSHRd.[13] Hovatta et al.[12] and Kromer et al.[14] have shown that there is an increased neuronal damage by methyl glyoxal (di carbonyl) glycation in anxiety.

In order to confirm the anxiolytic activities of compounds **1c** and **4c**, we tested the levels of these enzymes immediately after EPM experiment. Our results showed increased glyoxalase levels, induced by anxiety, were significantly decreased by both the compounds (Fig. 2A and B). Glo1 and GSHRd activity was found to be decreased by ~20% ($p < 0.05$) and ~15% ($p < 0.05$), respectively, with compound **1c** and by ~17% ($p < 0.05$) and ~12% ($p < 0.05$), respectively, with compound **4c**. Hence, our results further confirm that both these compounds are anxiolytic in nature, as the increased level of both the enzymes were significantly decreased after administration of compounds **1c** and **4c**.



Scheme 1. Reagents and conditions: (a) DMSO,rt,1 h (b) Sn/HCl, 60–70 °C, 3–4 h (c) ethyl chloro formate ($ClCOOC_2H_5$), DCM, Et_3 N, 2 h (d) acetic an hydride, DCM, Et_3 N, 2 h (e) 2-(6-chloro) nicotinic acid, EDC/HCl, HOBt, acetonitrile, 3–4 h.

Table 1
Effects of aryl piperazines (**1c** to **4e**) on the relevant anxiolytic parameters in the elevated plus maze test

Compound	Spent time into open arms	Number of entries into open arms	Number of entries into closed arms	Total number of entries	% Number of entries into open arms
1c	87.6 ± 21.40*	6.29 ± 2.4	9.19 ± 4.58	15.48 ± 5.28	40.64 ± 8.01*
2c	58.98 ± 23.97	4.23 ± 1.32	9.75 ± 2.98	13.98 ± 2.86	30.27 ± 13.45
3c	53.94 ± 19.26	3.94 ± 1.41	9.91 ± 3.65	13.85 ± 3.92	28.49 ± 12.68
4c	84.36 ± 23.68*	6.41 ± 2.12	11.80 ± 3.57	18.21 ± 4.8	35.23 ± 16.27*
1d	62.67 ± 15.24	4.20 ± 1.59	10.08 ± 4.2	14.28 ± 5.24	29.42 ± 9.89
2d	57.3 ± 20.28	4.52 ± 1.68	12.30 ± 3.4	16.82 ± 4.8	26.89 ± 13.67
3d	53.67 ± 18.47	3.60 ± 1.10	10.26 ± 3.87	13.86 ± 4.27	25.98 ± 10.2
4d	58.77 ± 21.26	4.44 ± 1.01	9.01 ± 2.86	15.45 ± 3.45	28.74 ± 11.12
1e	56.94 ± 24.40	4.52 ± 0.98	11.94 ± 4.12	16.46 ± 4.28	27.48 ± 12.26
2e	53.67 ± 18.68	3.18 ± 1.25	9.80 ± 3.18	12.98 ± 4.19	24.56 ± 8.79
3e	50.58 ± 14.86	3.31 ± 1.68	11.17 ± 3.65	14.48 ± 5.21	22.90 ± 11.28
4e	54.69 ± 17.28	4.33 ± 1.27	10.93 ± 3.87	15.26 ± 4.89	28.42 ± 12.82
Bus	85.56 ± 26.98*	5.35 ± 1.42	8.91 ± 4.20	14.26 ± 5.35	37.52 ± 12.38*
Dzp	105.36 ± 22.46*	8.34 ± 1.56	10.13 ± 3.76	18.47 ± 3.89	45.16 ± 14.7*
Veh	42.84 ± 17.51	3.03 ± 1.20	10.23 ± 3.40	13.26 ± 4.2	22.86 ± 10.28

Data represent the mean ± SEM; $n = 8$. Bus, Buspirone; Dzp, Diazepam; Veh, Tween 80 (1%), DMSO (1%).
* $p < 0.001$ compared with vehicle.

2.4. 5-HT$_{1A}$ Antagonism study using EPM test

Activation of pre-synaptic receptors by a 5-HT$_{1A}$ agonist reduces the firing rate of 5-HTergic neurones, leading to suppression of 5-HT synthesis, turnover and release in the diverse projection areas.[15] Forster et al. have reported that WAY-100635 is a highly selective 5-HT$_{1A}$ receptor antagonist that reverses the effects of 5-HT$_{1A}$ R agonist induced behavioral 5-HT syndrome in rodents.[16] The aim of this study was to determine whether any of the effects of Buspirone and compounds **1c** and **4c** on the elevated plus-maze were also reversed by WAY-100635.

After administration of Buspirone as well as both the compounds **1c** and **4c**, a significant increase in percentage time spent in the open arms compared to the control group has been noticed, while WAY-100635 alone was not significantly different with respect to control. (Fig. 3A and B). The percentage time spent in the open arms was significantly decreased, when WAY-100635 was injected intraperitoneally (ip) 15 min before administration of Buspirone and compounds **1c** and **4c**. In the case of compounds **1c** and **4c** the time spent in the open arms was decreased by ~35% ($p < 0.001$) and ~20% ($p < 0.01$), respectively. Similar effects were observed for the number of entries in open arms. An intraperitoneal injection of WAY-100635 (0.5 mg/kg) completely blocked the effects of compound **1c** (3 mg/kg).

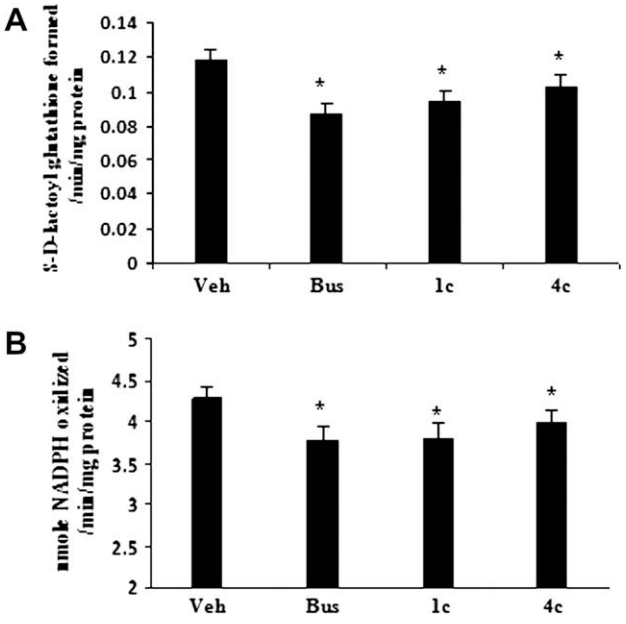


Figure 2. Effect of Buspirone, compounds **1c** and **4c** on glyoxalase activity (A) and glutathione reductase activity (B). Results are expressed as mean ± S.E.M, $n = 6$ each group * $p < 0.05$ compared with vehicle.

The anxiolytic effects of compounds **1c** and **4c** administration were shown to be attributable to actions on 5-HT$_{1A}$ receptors, because they were antagonized by the 5-HT$_{1A}$ receptor antagonist WAY-100635. These results suggest the possible involvement of the 5-HT$_{1A}$ receptor complex in the mechanism of action for these arylpiperazine derivatives.

2.5. Tissue concentration of 5-HT in the brain

In order to determine the neurochemical basis of anxiety, we have evaluated the extracellular serotonin (5-HT) levels in brain extracts of drug administered rats using HPLC with fluorescent detection. The neurotransmitter 5-HT plays a crucial role in anxiety related behavior in animals and humans.[17] Drugs acting at various serotonergic receptors and decreasing or increasing the activity of the central serotonergic system have 'anxiolytic' or 'anxiogenic' effects in animals, respectively.[18]

A representative chromatogram of 5-HT (serotonin) is shown in Figure 4A. The retention time obtained for 5-HT was 6.37 min. The peak identical to 5-HT in the chromatogram obtained from the brain extract of the drug administered rat was attributed on the basis of retention time. The difference between retention time for brain samples and that of authentic 5-HT was less than ~2%. Figure 4B shows a typical overlay of chromatograms obtained from brain homogenate of rats administered with vehicle and compounds **1c** and **4c**. It is evident from Figure 4B that compounds **1c** and **4c** decreased the extracellular serotonin levels as compared to control. It has been shown that exposure of animals to an animal test of anxiety, as the elevated plus maze test, causes an increase in 5-HT release.[19] This rise in extracellular 5-HT could be attenuated by administration of anxiolytic drugs,[20] indicating a central interaction of serotonergic transmission system. Our results have shown that the extracellular levels of 5-HT were significantly decreased by ~38% ($p < 0.001$) and ~32% ($p < 0.001$) following administration of compounds **1c** (3 mg/kg) and **4c** (3 mg/kg), respectively, as compared to control (Fig. 4C). These results also confirmed that compounds **1c** and **4c** have anxiolytic properties. This decrease in the extracellular 5-HT was also comparable with Buspirone.

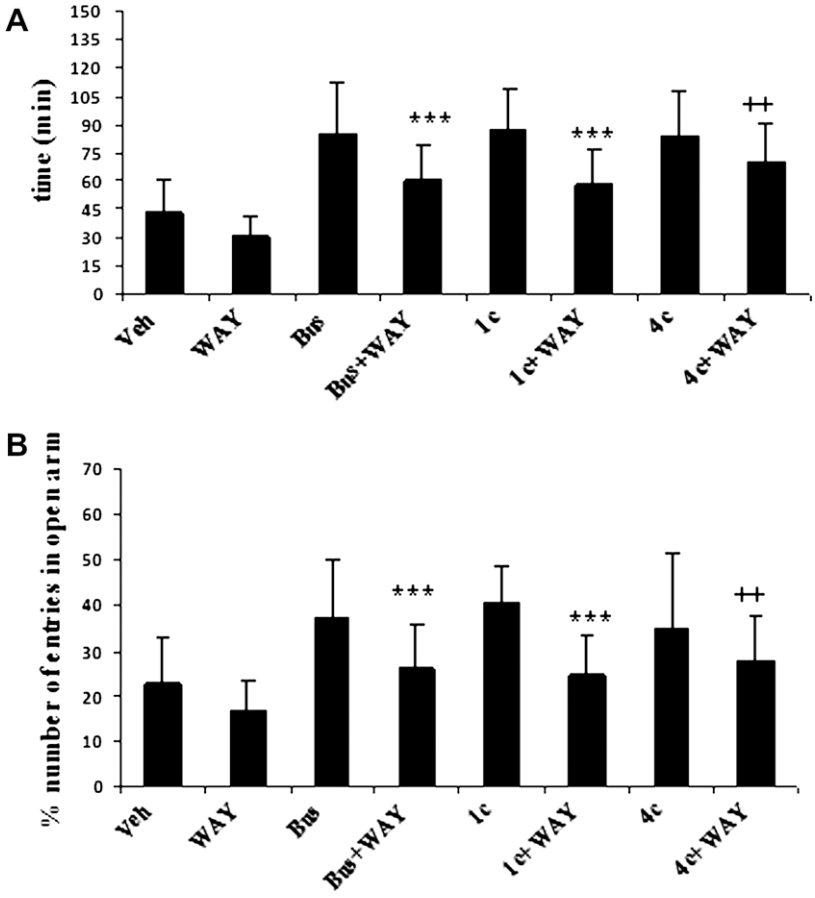


Figure 3. Influence of WAY-100635 on the anxiolytic effect of Buspirone and compounds **1c** and **4c**, expressed by the time spent in open arms (A), and the % number of open arm entries (B) in the elevated plus maze test. Results are expressed as mean ± S.E.M. $n = 8$, $^{***}p < 0.001$ compared with Bus, $^{***}p < 0.001$ compared with **1c**, $^{++}p < 0.01$ compared with **4c**.

Additionally, we have also analyzed the extracellular serotonin levels after co-administration of 5-HT_{1A} receptor antagonist viz WAY-100635. When rats were administered alone with WAY-100635, the serotonin levels were increased by ~28% ($p < 0.01$) as compared to control (Fig. 4D). The increased levels of extracellular serotonin were significantly brought down by ~30% ($p < 0.001$) and ~24% ($p < 0.01$) by compounds **1c** and **4c** with respect to WAY-100635 treated animals. These results suggest the involvement of 5-HT receptors, as the extracellular levels of 5-HT was significantly decreased after administration of compounds **1c** and **4c**. These results were also consistent with our findings of behavioral test data that compounds **1c** and **4c** are good anxiolytic.

3. Conclusion

Here we report the synthesis, structure–activity studies and discussion of pharmacological results of a series of 12 new derivatives of arylpiperazine, *N*-{4-[4-(aryl)-piperazine-1-yl]-phenyl}-amine. All the compounds were tested in the behavioral test-elevated plus maze and the differences in anxiolytic activity were explained on the basis of the substituents used on a phenyl piperazine fragment and the N-4 terminal fragment. Compound **1c** having an unsubstituted phenyl piperazine and **4c** having *o*-methoxy substituted phenyl piperazine were found to be the most active in the EPM test. Both compounds had ester functionality at their terminal N-4 fragment.

Since compounds **1c** and **4c** were found to be good anxiolytics; they were further examined for the activity of biochemical markers for anxiety, glyoxalase1 and glutathione reductase and found to decrease the levels of these two enzymes further confirming their anxiolytic activity. The study on the elevated plus maze using the antagonist WAY-100635 demonstrates that the anxiolytic effect of compounds **1c** and **4c** is mediated via 5-HT_{1A} receptor system. In addition to this, the extracellular serotonin levels were also found to be decreased after administration of these two compounds indicating that these compounds exhibit anxiolytic activity mediated by the serotonergic system. The effects observed on brain extracellular serotonin levels after co administration of compounds **1c** and **4c** with 5-HT_{1A} receptor antagonist WAY-100635, were also found to be consistent with our behavioral antagonism test data tentatively, confirming the involvement of 5-HT_{1A} receptors. The results discussed above are in line with the general view on the serotonin receptor affinity of arylpiperazine derivatives; however, additional information related to the activity and receptor specificity of these two compounds would be helpful in the elucidation of the mechanism of their anti anxiety activity, which will be reported at a later date.

4. Experimental

4.1. General

All organic solvents and common reagents were procured from the Merck India Ltd. All the arylpiperazines were procured from Aldrich Chemical Company, Inc. USA. TLC was carried out on commercially available flexible TLC silica gel (silica gel 60 F254)

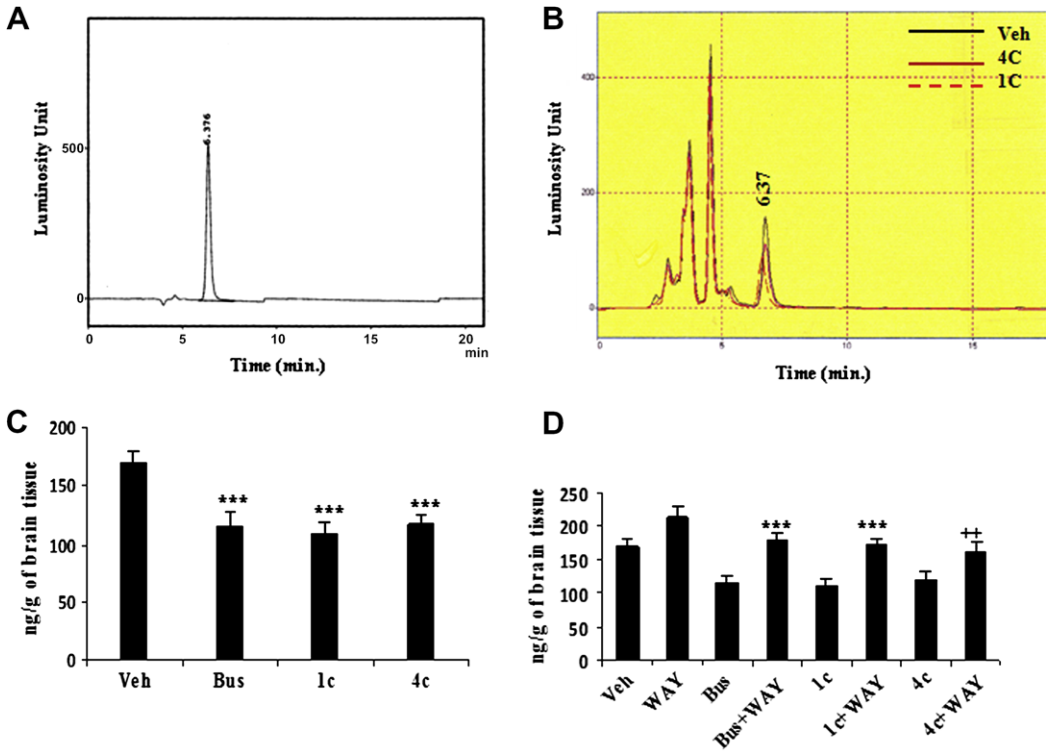


Figure 4. Chromatogram of serotonin standard (A) and an overlay chromatogram of brain extract of Vehicle, compounds **1c** and **4c** administered rats (B). Effect of Buspirone, compounds **1c** and **4c** on serotonin concentration in brain $^{***}p < 0.001$, $^{**}p < 0.01$ compared with Veh control (C) and influence of WAY-100635 on the effect of Buspirone, compounds **1c** and **4c** on serotonin concentration in brain (D). Results are expressed as mean ± S.E.M., $n = 6$, $^{***}p < 0.001$ compared with Bus, $^{***}p < 0.001$ compared with **1c**, $^{++}p < 0.01$ compared with **4c**.

plates (E Merck, Germany). The purity of all organic compounds was confirmed by TLC, ^{1}H NMR, IR, and mass spectroscopy. ^{1}H NMR spectra were recorded in Bruker Spectrospin Avance 300 instrument operating at 300 MHz in $CDCl_3$ or DMSO using TMS as an internal standard. The chemical shifts are reported in parts per million (δ) downfield from TMS and coupling constants are reported in Hertz (Hz). IR spectra (KBr) were recorded on a Perkin–Elmer BX FT-IR instrument. Melting points were determined on a Buchi melting point B-450 instrument. Mass spectra were recorded on a Qstar (Applied biosystem) ESI-MS mass spectrometer. Elemental analysis was performed on Heraeus CHN rapid analyzer.

4.2. General procedure for the synthesis of compounds 1a, 2a, 3a, 4a

A solution of 1-fluoro4-nitro benzene (2.120 ml, 20 mM) and *N*-arylpiperazine (20 mM) in 40 ml of dimethylsulfoxide was stirred at room temperature for 2 h. The resulting mixture was then washed with water and extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to obtain the product.

4.2.1. 1-(4-Nitro phenyl)-4-phenyl-piperazine (1a)

Yield: 80%; mp: 174–176 °C; $R_f = 0.77$ (SiO_2, $CHCl_3/CH_3OH = 98/02$); IR (KBr) ν cm^{-1}: 1328.20 (–NO_2 str, sym), 1590.51 (–NO_2 str, asym), 2831.85 (C–H str), 830.30 (C–N str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.36 (s, 4H, piperazine), 3.58–3.59 (d, 4H, piperazine), 6.86–8.1 (m, 9H, Ar–H); LC–MS: *m*/*e* 283 (M^+), 284 (M^++1); Anal. Calcd for $C_{16}H_{17}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 70.12; H, 7.36; N, 13.27.

4.2.2. 1-(2-Chloro-phenyl)-4-(nitro phenyl)-piperazine (2a)

Yield: 78%; mp: 204–206 °C; $R_f = 0.77$ (SiO_2, $CHCl_3/CH_3OH = 98/02$); IR (KBr) ν cm^{-1}: 1334 (NO_2 str, sym), 1579 (–NO_2 str, asym), 2829.85 (C–H str), 828.10 (C–N str); 798 (C–Cl str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.42 (s, 4H, piperazine), 3.58–3.61 (d, 4H, piperazine), 6.99–8.2 (m, 8H, Ar–H); LC–MS: *m*/*e* 317(M^+); Anal. Calcd for $C_{16}H_{16}ClN_3O_2$: C, 62.15; H, 6.37; N, 12.08. Found: C, 61.98; H, 6.39; N, 11.98.

4.2.3. 1-(4-Nitro phenyl)-4-phenethyl-piperazine (3a)

Yield: 80%; mp: 196–198 °C; $R_f = 0.77$ (SiO_2, $CHCl_3/CH_3OH = 98/02$); IR (KBr) ν cm^{-1}: 1332 (NO_2 str, sym), 1596.11 (–NO_2 str, asym), 2823.85 (C–H str), 836.30 (C–N str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ *2.52–2.59* (d, 2H, Ph–CH_2, $J = 8.0$ Hz), 2.79–2.81 (t, 2H, N–CH_2, $J = 8.1$ Hz), 3.23 (s, 4H, piperazine), 3.58 (s, 4H, piperazine), 6.8–8.15 (m, 9H, Ar–H); LC–MS: *m*/*e* 311(M^+); Anal. Calcd for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 7.40; N, 13.41. Found: C, 69.28; H, 7.35; N, 13.36.

4.2.4. 1-(2-Methoxy-phenyl)-4-(nitro phenyl)-piperazine (4a)

Yield: 85%; mp: 210–212 °C; $R_f = 0.77$ (SiO_2, $CHCl_3/CH_3OH = 98/02$); IR (KBr) ν cm^{-1}: 1329.20 (NO_2 str, sym), 1596 (–NO_2 str, asym), 2834.85 (C–H str), 830.30 (C–N str); ^{1}H NMR ($CDCl_3$, 300 MHz): δ *3.38–3.40* (d, 4H, piperazine), 3.59 (s, 4H, piperazine), 3.81 (s, 3H,OCH_3), 6.86–8.1 (m, 8H, Ar–H); LC–MS: *m*/*e* 313(M^+); Anal. Calcd for $C_{17}H_{19}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.35; H, 7.28; N, 12.16.

4.3. General procedure for the synthesis of compounds 1b, 2b, 3b, 4b

To a solution of 1-(4-nitro phenyl)-4-arylpiperazine (10 mM) in 20 ml of ethyl acetate was added tin (1.780 g, 15 mM) with 15 ml of concd HCl. The reaction mixture was refluxed for 2 h. After cooling the reaction mixture was alkalized by 10% NaOH and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated on rotary evaporator.

4.3.1. 4-(4-Phenyl piperazine-1-yl)-phenylamine (1b)

Yield: 80%; mp: 128–130 °C; R_f = 0.77 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3359.52 (N–H str), 1599.68 (N–H def), 1231.39 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ; 3.17–3.20 (m, 4H, piperazine), 3.32–3.35 (m, 4H, piperazine), 3.71 (s, 2H, NH_2), 6.66–7.31 (m, 9H, Ar–H); LC–MS: *m*/*e* 253 (M^+); Anal. Calcd for $C_{16}H_{19}N_3$: C, 75.85; H, 7.56; N, 16.59. Found: C, 75.68; H, 7.62; N, 16.70.

4.3.2. 4-[4-(2-Chloro phenyl)-piperazine-1-yl]-phenylamine (2b)

Yield: 78%; mp: 156–158 °C; R_f = 0.77 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3345.52 (N–H str), 1589.86 (N–H def), 1223.39 (C–N str), 798 (C–Cl str); ^1H NMR ($CDCl_3$, 300 MHz): δ *3.18–3.20* (m, 4H, piperazine), 3.32–3.35 (m, 4H, piperazine), 4.1 (s, 2H, NH_2), 6.56–7.25 (m, 8H, Ar–H); LC–MS: *m*/*e* 287 (M^+); Anal. Calcd for $C_{16}H_{18}ClN_3$: C, 66.78; H, 6.30; N, 14.60. Found: C, 66.92; H, 6.25; N, 14.52.

4.3.3. 4-(4-Phenyl piperazine-1-yl)-phenethylamine (3b)

Yield: 80%; mp: 149–151 °C; R_f = 0.77 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3345.72 (N–H str), 2831.85 (C–H str), 1587.54 (N–H def), 1230.39 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ *2.52–2.59* (d, 2H, Ph–CH_2, *J* = 8.0 Hz), 2.79–2.81 (t, 2H, N–CH_2, *J* = 8.1 Hz), 3.17(s, 4H, piperazine), 3.32–3.35 (m, 4H, piperazine), 3.79 (s, 2H, NH_2), 6.66–7.31 (m, 9H, Ar–H); LC–MS: *m*/*e* 281 (M^+); Anal. Calcd for $C_{18}H_{23}N_3$: C, 76.83; H, 8.24; N, 14.93. Found: C, 76.62; H, 8.45; N, 14.93.

4.3.4. 4-[4-(2-Methoxy phenyl)-piperazine-1-yl]-phenylamine (4b)

Yield: 80%; mp: 165–167 °C; R_f = 0.77 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3350.57 (N–H str), 1599.68 (N–H def), 1228.39 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ; 3.19 (s, 4H, piperazine), 3.32–3.35 (m, 4H, piperazine), 3.81 (s, 3H, OCH_3), 3.97 (s, 2H, NH_2), 6.66–7.31 (m, 8H, Ar–H); LC–MS: *m*/*e* 283 (M^+); Anal. Calcd for $C_{17}H_{21}N_3O$: C, 72.06; H, 7.47; N, 14.83. Found: C, 71.97; H, 7.34; N, 14.90.

4.4. General procedure for the synthesis of compounds 1c, 2c, 3c, 4c

A solution of 4-(4-aryl piperazine-1-yl)-phenylamine (10 mM) and ethylchloro formate (953 μl, 10 mM) in 40 ml dry dichloromethane was stirred at room temperature for 2 h in the presence of triethylamine (15 mM). The resulting mixture was then washed with 5% aq $NaHCO_3$ followed by water. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator.

4.4.1. *N*-[4-(4-Phenyl-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester (1c)

Yellowish solid. Yield: 80%; mp: 170–172 °C; R_f = 0.77 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3295.38 (N–H str), 1691.74 (C=O str), 1526.35 (N–H def), 2827.52 (C–H str); ^1H NMR ($CDCl_3$, 300 MHz): δ *1.007–1.055* (t, 3H, CH_2–CH_3, *J* = 7.2), 2.48–2.55 (q, 2H, –COO–CH_2, J = 7.2), 3.28–3.30 (d, 8H, piperazine), 6.83–7.25 (m, 10H, Ar–H, N–H); LC–MS: *m*/*e* 325 (M^+), 326 (M^++1); Anal. Calcd for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 70.21; H, 7.24; N, 13.02.

4.4.2. *N*-{4-[4-(2-Chloro-phenyl)-piperazin-1-yl]-phenyl}-carbamic acid ethyl ester (2c)

Colourless solid. Yield: 80%; mp: 225–227 °C; R_f = 0.73 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3253 (N–H str), 1684 (C=O str), 1501 (N–H def), 1316 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ *1.18–1.25* (q, 1 × 3H, CH_2–CH_3, *J* = 7.2 Hz), 3.14–3.15 (d, 4H, piperazine, *J* = 2.7), 3.23–3.24 (d, 4H, piperazine, *J* = 3.6), 4.10–4.17 (q, 2H, –COO–CH_2, *J* = 6.9 Hz), 6.38–7.32 (m, 9H, Ar–H, N–H); LC–MS: *m*/*e* 359(M^+); Anal. Calcd for $C_{19}H_{22}ClN_3O_2$: C, 63.42; H, 6.16; N, 11.68. Found: C, 63.48; H, 6.29; N, 11.82.

4.4.3. *N*-[4-(4-Phenethyl-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester (3c)

Colourless solid. Yield: 80%; mp: 210–212 °C; R_f = 0.69 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3313 (N–H str), 1689 (C=O str), 1520 (N–H def), 1314 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ *1.25–1.29* (t, 1 × 3H, CH_2–CH_3, *J* = 6.6 Hz), 2.55–2.62 (d, 2H, Ph–CH_2, *J* = 8.1 Hz), 2.79–2.81 (t, 2H, N–CH_2, *J* = 8.1 Hz), 3.09 (s, 4H, piperazine), 3.40 (s, 4H, piperazine), 4.10–4.17 (q, 2H, –COO–CH_2, *J* = 6.9 Hz), 6.89–7.33 (m, 10H, Ar–H, N–H); LC–MS: *m*/*e* 353(M^+); Anal. Calcd for $C_{21}H_{27}N_3O_2$: C, 71.36; H, 7.70; N, 11.89. Found: C, 71.46; H, 7.86; N, 11.82.

4.4.4. *N*-{4-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-phenyl}-carbamic acid ethyl ester (4c)

creamish solid. Yield: 85%; mp: 230–232 °C; R_f = 0.70 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}; 3253 (N–H str), 1685 (C=O str), 1535 (N–H def), 1316 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz); δ *1.18–1.25* (t, 3H, CH_2–CH_3, *J* = 6.9 Hz), 3.15 (s, 4H, piperazine), 3.23 (s, 4H, piperazine), 3.81 (s, 3H,OCH_3), 4.10–4.17 (q, 2H, –COO–CH_2, J = 6.9 Hz), 6.37–7.22 (m, 9H, Ar–H, N–H); LC–MS: *m*/*e* 355(M^+); Anal. Calcd for $C_{20}H_{25}N_3O_3$: C, 67.58; H, 7.09; N, 11.82. Found: C, 67.72; H, 7.19; N, 11.85.

4.5. General procedure for the synthesis of compounds 1d, 2d, 3d, 4d

To a solution of 4-(4-arylpiperazine-1-yl)-phenylamine (10 mM) and triethyl amine (15 mM) in 50 ml of dry dichloro methane was added acetic an hydride (945 μl, 10 mM) at room temperature. The resulting mixture was stirred at room temperature for 2 h. The mixture was then washed with 5% aq $NaHCO_3$ followed by water. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator.

4.5.1. *N*-[4-(4-Phenyl piperazin-1-yl)-phenyl]-acetamide (1d)

Yield: 90%; mp: 188–190 °C; R_f = 0.24 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3276.83 (N–H str), 1654.53 (C=O str), 1317.59 (C–N str), 1511.62 (N–H def); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.15 (s, 3H, CO-CH_3), 3.30–3.32 (d, 8H, piperazine), 6.89–7.41 (m, 10H, Ar–H, N–H); LC–MS: *m*/*e* 296 (M^++1); Anal. Calcd for $C_{18}H_{21}N_3O$: C, 73.19; H, 7.17; N, 14.23. Found: C, 73.30; H, 7.23; N, 14.37.

4.5.2. *N*-{4-[4-(2-Chloro-phenyl)-piperazin-1-yl]-phenyl}-acetamide (2d)

Yield: 85%; mp: 280–283 °C; R_f = 0.29 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3297 (N–H str), 1655 (C=O str), 1513 (N–H def), 1316 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.05 (s, 3H, $COCH_3$), 3.17(s,4H, piperazine), 3.27 (s, 4H, piperazine), 6.97–7.51(m, 9H, Ar–H, N–H); LC–MS: *m*/*e* 329(M^+); Anal. Calcd for $C_{18}H_{20}ClN_3O$: C, 65.55; H, 6.11; N, 12.74. Found: C, 65.68; H, 6.23; N, 12.87.

4.5.3. *N*-[4-(4-Phenethyl-piperazin-1-yl)-phenyl]-acetamide (3d)

Yield: 78%; mp: 267–270 °C; R_f = 0.23 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3297 (N–H str), 1654 (C=O str), 1524 (N–H def), 1370 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.07 (s, 3H, $COCH_3$), 2.76–2.81 (t, 2H, Ar-CH_2, *J* = 6.6), 3.12 (s, 2H, N–CH_2), 2.62 (s, 8H, piperazine), 6.81–7.31 (m, 10H, Ar–H, N–H); LC–MS:

m/*e* 323(M^+); Anal. Calcd for $C_{20}H_{25}N_3O$: C, 74.27; H, 7.79; N, 12.99. Found: C, 74.36; H, 7.56; N, 13.16.

4.5.4. *N*-{4-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-phenyl}-acetamide (4d)

Yield: 85%; mp: 290–292 °C; R_f = 0.32 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3262.55 (N–H str), 1642.22 (C=O str), 1517 (N–H def), 1315 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ 2.08 (s, 3H, $COCH_3$), 3.15(s,4H, piperazine), 3.18 (s, 3H, OCH_3), 3.25–3.26 (s, 4H, piperazine), 6.80–7.33(m, 9H, Ar–H, N–H); LC–MS: *m*/*e* 325(M^+); Anal. Calcd for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 70.25; H, 7.23; N, 12.74.

4.6. General procedure for the synthesis of compounds 1e, 2e, 3e, 4e

2-(6-Chloro)-nicotinic acid (1.575 g, 10 mM) and 1-Hydroxy benzotriazole hydrate (HOBt) (1.621 g, 12 mM) were slurried in dry acetonitrile (50 ml). Addition of EDC/HCl (2.108 g, 11 mM) to this mixture at room temperature generates the HOBt ester. Then 4-(4-arylpiperazine-1-yl)-phenylamine (2.53 g, 10 mM) was added and the mixture was stirred at room temperature for 3–4 h. The resulting mixture was then filtered and filtrate was concentrated on rotary evaporator. Then the compound was partitioned with ethyl acetate and aqueous bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. Finally after evaporation of the solvent the residue was purified by column chromatography and elution with hexane/ethyl acetate (70:30).

4.6.1. 6-Chloro-*N*-[4-(4-phenyl-piperazin-1-yl)-phenyl]-nicotinamide (1e)

Yield: 65%; mp: 430–432 °C; R_f = 0.56 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3313 (N–H str), 1637 (C=O str), 1522 (N–H def), 1324 (C–N str), 756 (C–Cl str); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.37 (m, 8H, piperazine), 6.92–7.55 (m, 10H, Ar–H, N–H), 8.16–8.19 [d, 2H(X + Y)], 8.85 [s, 1H(Z)]; LC–MS: *m*/*e* 392 (M^+); Anal. Calcd for $C_{22}H_{21}ClN_4O$: C, 67.26; H, 5.39; N, 14.26. Found: C, 67.37; H, 5.21; N, 14.32.

4.6.2. 6-Chloro-*N*-{4-[4-(2-chloro-phenyl)-piperazin-1-yl]-phenyl}-nicotinamide (2e)

Yield: 59%; mp: 412–415 °C; R_f = 0.55 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3334 (N–H str), 1623 (C=O str), 1513 (N–H def), 1319 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.06 (s, 4H, piperazine), 3.29 (s, 4H, piperazine), 6.9–7.8 (m, 9H, Ar–H, N–H), 8.09–8.12 [d, 1H(X), J = 8.4 Hz], 8.65 [s, 1H(Y)], 8.96 [s, 1H(Z)]; LC–MS: *m*/*e* 426 (M^+); Anal. Calcd for $C_{22}H_{20}Cl_2N_4O$: C, 61.83; H, 4.72; N, 13.11. Found: C, 61.67; H, 4.86; N, 13.02.

4.6.3. 6-Chloro-N-[4-(4-phenethyl-piperazin-1-yl)-phenyl]-nicotinamide (3e)

Yield:57%; mp: 395–398 °C; R_f = 0.51 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3280 (N–H str), 1642 (C=O str), 1534 (N–H def), 1373 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ *2.4–2.5* (d, 2H, Ph–CH_2), 2.71 (t, 2H, N–CH_2), 3.05 (s, 4H, piperazine), 3.82 (s, 4H, piperazine), 6.8–7.6 (m, 10H, Ar–H, N–H), 8.26 [s, 1 × H(X) + 1 × 1H(Y)], 8.85 [s, 1 × 1H(Z)]; LC–MS: *m*/*e* 420 (M^+); Anal. Calcd for $C_{24}H_{25}ClN_4O$: C, 68.48; H, 5.99; N, 13.31. Found: C, 68.29; H, 6.05; N, 13.22.

4.6.4. 6-Chloro-*N*-{4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-phenyl}-nicotinamide (4e)

Yield: 62%; mp: 380–382 °C; R_f = 0.54 (SiO_2, $CHCl_3$/CH_3OH = 98/02); IR (KBr) ν cm^{-1}: 3305 (N–H str), 1639 (C=O str), 1525 (N–H def), 1327 (C–N str); ^1H NMR ($CDCl_3$, 300 MHz): δ 3.21 (s, 4H, piperazine), 3.33 (s, 4H, piperazine), 3.79 (s, 3H, OCH_3), 6.8–8.2 (m, 9H, Ar–H, N–H), 8.39–8.42 [d, 1H(X), J = 9.1 Hz], 8.65 [s, 1H(Y)], 8.81 [s, 1H(Z)]; LC–MS: *m*/*e* 423 (M^+); Anal. Calcd for $C_{23}H_{23}ClN_4O_2$: C, 65.32; H, 5.48; N, 13.25. Found: C, 65.19; H, 5.32; N, 13.22.

4.7. Animals and drug treatment

Six to eight weeks old male Wistar rats, weighing 120–150 g each, were selected from the stock colony maintained in our animal facility with free access to food and water. Animals were maintained in an air-conditioned room. The room was maintained at 25 ± 2 °C with natural daytime and no light after 1900 h, until morning. All the experiments were performed during the light phase according to the guidelines of the Institutional Animal Ethics committee.

Diazepam ampoules (10 mg/2 ml; Ranbaxy Labs, India) and Buspirone hydrochloride (Sigma–Aldrich Co., St. Louis, MO, USA) were used as reference drugs. Diazepam and Buspirone were diluted in deionized water (Millipore quality). 5-HT_{1A} receptor antagonist WAY-100635, (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in 0.9% NaCl solution (saline). Six different concentrations (0.25, 0.5, 1, 2, 3, 4 mg/kg) of each compound were used in the form of freshly prepared suspensions in 1% tween 80 and 1% DMSO. All solutions were prepared freshly on test days and given intraperitoneally (ip) in a volume of 2 ml/kg body weight of rats.

The experimental animals were treated with Diazepam (1 mg/kg, n = 8), Buspirone (5 mg/kg, n = 8) or the compounds (0.25, 0.5, 1, 2, 3, 4 mg/kg) 60 min before evaluation in the maze. The control group was given saline with 1% tween 80 and 1% DMSO. The antagonist WAY-100635 (0.5 mg/kg, n = 8) was administered 15 min before the treatments.

4.8. Elevated plus maze test

The elevated plus maze was comprised of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 30 cm). The arms extend from a central platform (5 cm × 5 cm) that was elevated to a height of 50 cm above floor level. The experiments were performed between 9:00 h and 15:00 h. Each rat was placed in the central area of the maze with its head facing an open arm. The following behavioral parameters were recorded during 5 min exposure using FW: Camera O: Fire - iBBW 1.3 interface (Columbus Instruments): (1) time in open arms; (2) time in closed arms; (3) time in central area; (4) number of open arm entries; (5) number of closed arm entries; (6) number of central area entries. Any rat, which dropped of the plus maze, was excluded in the result.[21]

4.9. Assay of glyoxalase1 and glutathione reductase activity

After the elevated plus maze experiment the Wistar rats were dissected to remove their brain tissue, which was washed in ice cold saline (0.85% NaCl). The extraneous material was removed. The 20% homogenate of brain tissue in 0.1 M potassium phosphate buffer (pH 7.4) with 0.25 M sucrose was centrifuged at 800g for 5 min at 4 °C in an IEC-20 refrigerated centrifuge (Rotar no. 894) to separate the nuclear debris. The supernatant obtained was centrifuged at 10,500g for 20 min at 4 °C to obtain the post mitochondrial supernatant (PMS) which was used as a source for estimation of glutathione reductase. The PMS was centrifuged further at 36,000g for 1 h at 4 °C to obtain cytosolic fraction which was used as a source for estimation of glyoxalase 1. The activity of glyoxalase 1 was assayed by a slight modification of the technique described by Racker.[22] Glutathione reductase activity was assayed by the

method of Carlberg and Mannervik (1975).[23] The protein content was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.[24]

4.10. Determination of tissue concentration of 5-HT in brain

One hour after last administration, the experimental animals were decapitated by cervical dislocation, and the brains were removed immediately and placed on an ice-cold plate. The tissue samples were weighed and stored at −80 °C until homogenization.

Each frozen tissue sample was homogenized by ultrasonication in 500 μl of 0.4 M perchloric acid (solution A). The homogenates were kept on ice bath for 1 h and then centrifuged at 12,000*g* for 20 min at 4 °C. The pellets were discarded. An aliquot of 300 μl of supernatant was added to 150 μl of solution B (containing 0.2 M potassium citrate, 0.3 M di-potassium hydrogen phosphate and 0.2 M EDTA). The mixtures were kept on ice for 1 h and then centrifuged at 12,000*g* for 20 min at 4 °C again. Twenty microliters of the resultant supernatant were directly injected into high performance liquid chromatography (HPLC) system equipped with fluorescence detector. Fluorometric detection was accomplished using excitation and emission wavelengths of 280 and 340 nm, respectively. The extraction procedure was based on slightly modified method of Sperk and Nitta.[25]

The HPLC conditions: reversed phase C18 column (250 × 4.6 mm I.D., 5 um); mobile phase: 12.2 mM citric acid, 11.6 mM ammonium phosphate, 2.5 mM sodium octyl sulfate, 3.3 mM dibutyl amine phosphate, 1.1 mM disodium EDTA and 20% acetonitrile (V/V); Flow rate, 1.0 ml/min, pH of mobile phase-3.8.

Identification (by retention time) and measurement of the compounds (by peak area) in the samples was achieved by comparison to 0.1–10 ng/ml 5-HT standard solutions. Standard 5-HT was prepared as 0.5 mg/ml stock solution in distilled water.

4.11. Statistics

Calculation of the percentage time and number of entries on the open arms with 95% confidence limits and comparisons of the results were performed using computerized linear regression analysis, using GraphPad Prism (version 4.00, GraphPad Software Inc., San Diego, CA, USA). All the measurements were made in triplicate and all values are represented as mean ± S.E. The significance of difference between means of two groups was obtained with one-way analysis of variance (ANOVA), Tukey–Kramer multiple comparisons test, using Graph pad Prism 4.00 computer software, $p < 0.05$ was considered to be statistically significant.

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