Full Paper

1-Cinnamyl-4-(2-methoxyphenyl)piperazines: Synthesis, Binding Properties, and Docking to Dopamine (D_2) and Serotonin (5-HT_{1A}) Receptors

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Clinical properties of atypical antipsychotics are based on their interaction with D_2 dopamine receptor and serotonin 5-HT_{1A} and 5-HT_{2A} receptors. As a part of our research program on new antipsychotics, we synthesized various derivatives of 1-cinnamyl-4-(2-methoxyphenyl)piperazines, and evaluated their affinities for D_2, 5-HT_{1A}, 5-HT_{2A}, and adrenergic (α_1) receptors using radioligand-binding assays. In addition, we performed docking analysis using models for the D_2 and 5-HT_{1A} receptors. All compounds exhibited low to moderate affinity to 5-HT_{1A} and 5-HT_{2A} receptors, high affinity to the D_2 receptor and large variability in affinities for the α_1 receptor. Docking analysis indicated that the binding to D_2 and 5-HT_{1A} receptors is based on (i) interaction between protonated N1 of the piperazine ring and various aspartate residues, (ii) hydrogen bonds between various moieties of the ligand and the residues of threonine, serine, histidine or tryptophane, and (iii) edge-to-face interactions of the aromatic ring of the arylpiperazine moiety with phenylalanine or tyrosine residues. Docking data for the D_2 receptor can account for the binding properties obtained in binding assays, suggesting that the model is reliable and robust. However, docking data for the 5-HT_{1A} receptor cannot account for actual binding properties, suggesting that further refinement of the model is required.

Keywords: Dopamine D_2 receptor / Piperazines / Serotonin 5-HT_{1A} / 5-HT_{2A} receptor

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Introduction

More than fifty years ago, the discovery of drugs known today as "typical antipsychotics" significantly changed the pharmacological treatment of psychiatric disorders [1]. Although very useful, these drugs produce a host of adverse side effects, without having any effect on the negative symptoms of schizophrenia [1]. More hope came with introduction of "atypical antipsychotics" [2], which cause significantly less extrapyramidal side effects. On the molecular level, the difference between the two drug classes can be attributed to a different binding profile to the D_2 dopamine receptors and 5-HT_{2A} serotonin receptors. The second generation of atypical antipsychotics (also known as dopamine-serotonin system stabilizers) such as aripiprazole, offered further advantage in treatment due to an improved efficacy in dealin with negative symptoms of schizophrenia and a decreased incidence and severity of central and peripheral side effects [2]. Receptor-binding studies revealed that the second-generation atypical antipsychotics are partial agonists of D_2 and 5-HT_{1A} receptors, and antagonists of 5-HT_{2A} receptors [1, 2]. This promiscuity is based on the similarity in architecture of the binding sites among the aminergic receptors [3]. Therefore and in order to design more specific

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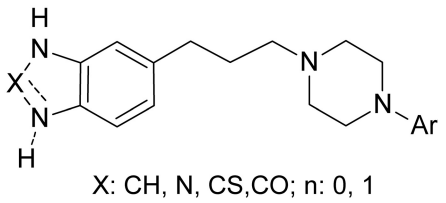


Figure 1. General structure of benzimidazole arylpiperazines with a mixed D_2 dopaminergic and 5-HT_{1A} serotonergic activity.

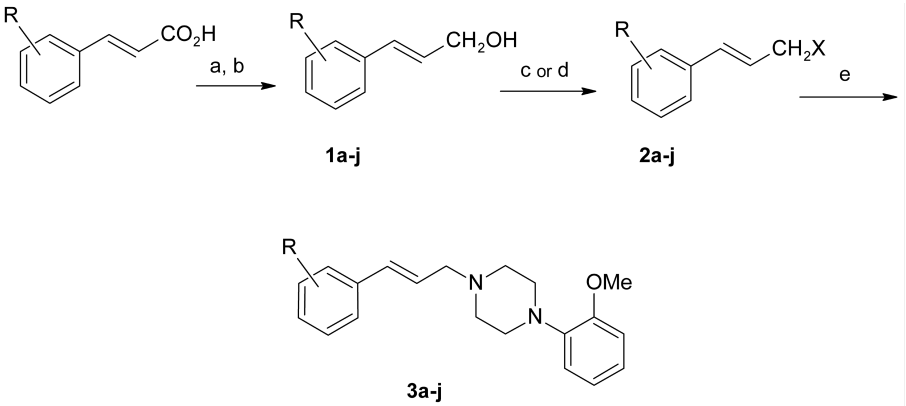
drugs (*e.g.* ones which could be used for individualized therapy), it is important to understand structural requirements for the interaction between the antipsychotics and their receptors.

Within a project aimed at the discovery of new antipsychotics, we have designed a series of heterocyclic arylpiperazines with both D_2 and 5-HT_{1A} receptor affinity. The general structure of these ligands is presented in Fig. 1 [4, 5]. Previously, we have been able to identify key interactions that may take place between these compounds and amino acid residues within the binding pockets of the D_2- and 5-HT_{1A} receptors using molecular docking analysis [6, 7]. In this study, our goal was to further scrutinize the methodology and the receptor models we used. Therefore, a series of new compounds was prepared, where the heterocyclic ring present in heterocyclic arylpiperazines (Fig. 1) has been replaced with the substituted phenyl moiety, and the usual flexible linkers with the more rigid (*E*)-prop-2-en-1-yl linker (Scheme 1). The new compounds were evaluated for their ability to bind at 5-HT_{1A}, 5-HT_{2A}, D_2, and α_1 adrenergic receptors, and further analyzed by means of *in silico* molecular docking to the computer models of the D_2 and 5-HT_{1A} receptors.

Results and discussion

The chemical structure and the synthetic route to novel nitro-, methoxy-, and chloro-cinnamylpiperazines **3a – j** is summarized in Scheme 1. These substituents were selected because they are regioisomeric, but with different properties: the nitro group is mesomeric electron-withdrawing, and a hydrogen-bond acceptor, the methoxy group is electron-releasing and a hydrogen-bond acceptor, and the chloro group is electron-releasing, with an inductive electron-withdrawing effect. Therefore, it was to be expected that they would influence the formation of the receptor-ligand complexes in a different manner.

Key intermediates were substituted cinnamyl halides **2a – j** that readily alkylate 1-(2-methoxyphenyl)piperazine (MPP) in acetonitrile at room temperature, to provide the final compounds **3a – j**. Cinnamyl halides are notoriously unstable, and special attention was paid to their preparation by using cinnamyl alcohols **1a – j** as starting material. Nitro- and chloro-cinnamyl alcohols **2b – d** and **2h – j**, and commercial cinnamyl alcohol **2a** were transformed to chlorides using thionylchloride in pyridine [8], with yields of 67 – 95%. Due to their instability, these intermediates were used immediately after the preparation and purification. Halogenation of methoxy cinnamyl alcohols **1e – g** under the same conditions gave a mixture of products. In our hands, the most efficient method was the conversion to halides **2e – g**, using 33% HBr in acetic acid [9]. The obtained bromides were very unstable and were used immediately after preparation without purification. Cinnamyl alcohols **1a – j** were obtained by reducing mixed anhydrides of cinnamic acids [10]. Cinnamic



(a) Ethyl chloroformate, Et_3N, THF, -7°C; (b) $NaBH_4$, MeOH; (c) $SOCl_2$, pyridine, rt; (d) HBr / AcOH, diethyl ether, rt; (e) 2-Methoxyphenyl-piperazine, Et_3N, acetonitrile, rt.
R: H (**1a**), *o*-NO_2 (**1b**), *m*-NO_2 (**1c**), *p*-NO_2 (**1d**), *o*-OMe (**1e**), *m*-OMe (**1f**), *p*-OMe (**1g**), *o*-Cl (**1h**), *m*-Cl (**1i**), *p*-Cl (**1j**).
X: Cl (**2a-d** and **2h-j**); Br (**2e-g**).

Scheme 1. Synthesis of (*2E*)-1-(3-phenylprop-2-enyl)-4-(2-methoxyphenyl)piperazines.

Table 1. Physical data for intermediates **1b–j** and **2a–j**.

Compd.	Mp (°C)	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	Yield (%)
1b	56–57	2.66 (s, 1H, OH), 4.37 (d, 2H, *J* = 5.4 Hz), 6.34 (dt, 1H, *J* = 15.8 Hz and *J* = 6 Hz), 7.07 (d, 1H, *J* = 15.8 Hz), 7.37 (t, 1H, *J* = 7.6 Hz), 7.50–7.62 (m, 2H), 7.89 (d, 1H, *J* = 8.6 Hz)	63.02; 124.37; 125.52; 128.00; 128.65; 132.42; 133.06; 134.15; 147.63	84
1c	53–54	2.91 (s,1H, OH), 4.39 (s, 2H), 6.48 (dt, 1H, *J* = 15.8 Hz and *J* = 5.0 Hz), 6.67 (d, 1H, *J* = 16.4 Hz), 7.44 (t, 1H, *J* = 7.8 Hz), 7.64 (d, 1H, *J* = 7.8 Hz), 8.03 (d, 1H, *J* = 8.0 Hz), 8.15 (s, 1H)	62.71; 120.72; 121.92; 127.780; 129.35; 131.89; 132.13; 138.41; 148.26	76
1d	107	1.79 (s, 1H, OH), 4.41 (d, 2H, *J* = 4.4 Hz), 6.54 (dt, 1H, *J* = 15.8 Hz and *J* = 5.0 Hz), 6.72 (d, 1H, *J* = 15.8 Hz), 7.51 (d, 2H, *J* = 8.6 Hz), 8.18 (d, 2H, *J* = 9 Hz)	63.05; 124.03; 126.91; 128.20; 133.59; 143.26	61
1e	oil	3.09 (s, 1H, OH), 3.83 (s, 3H, CH_3), 4.33 (d, 2H, *J* = 5.6 Hz), 6.39 (dt, 1H, *J* = 15.6 Hz and *J* = 6.2 Hz), 6.86-7.01 (m, 3H) 7.26 (t, 1H, *J* = 7.8 Hz), 7.45 (d, 1H, *J* = 7.2 Hz)	55.11; 63.62; 110.57; 120.44; 125.56; 126.72; 128.44; 129.22; 156.42	69
1f	oil	2.72 (s, 1H, OH), 3.76 (s, 3H, CH_3), 4.25 (d, 2H, *J* = 5.4 Hz), 6.30 (dt, 1H, *J* = 15.8 Hz and *J* = 5.4 Hz), 6.53 (d, 1H, *J* = 16.2 Hz), 6.78 (d, 1H, *J* = 8.2 Hz), 6.89 (s, 1H), 6.94 (d, 1H, *J* = 7.8 Hz), 7.20 (t, 1H, *J* = 7.8 Hz)	55.01; 63.22; 111.65; 113.05; 118.97; 128.89; 129.44; 130.55; 138.05; 159.59	78
1g	69–70	1.89 (s, 1H, OH), 3.81 (s, 3H, CH_3), 4.29 (d, 2H, *J* = 6 Hz), 6.23 (dt, 1H, *J* = 15.8 Hz and *J* = 5.8 Hz), 6.56 (d, 1H, *J* = 15.8 Hz), 6.85 (d, 2H, J = 8.8 Hz), 7.32 (d, 2H, *J* = 8.8 Hz)	55.24; 63,89; 113.98; 126.20; 127.65; 129.38; 130.95; 159.30	74
1h	oil	2.27 (s, 1H, OH), 4.33 (d, 2H, *J* = 6 Hz), 6.32 (dt, 1H, *J* = 16 Hz and *J* = 5.6 Hz), 6.99 (d, 1H, *J* = 16 Hz), 7.11-7.36 (m, 3H), 7.46-7.53 (m, 2H)	63.44; 126.81; 126.87; 126.94; 128.58; 129.62; 131.46; 133.06; 134.79	68
1i	oil	2.04 (s, 1H, OH), 4.32 (d, 2H, *J* = 5.2 Hz), 6.35(dt, 1H, *J* = 16 Hz and *J* = 5 Hz), 6.55 (d, 1H, *J* = 16 Hz), 7.19-7.26 (m, 3H) 7.35 (s,1H)	63.27; 124.61; 126.30; 127.54; 129.36; 129.78; 130.00; 134.44; 138.50	80
1j	51	1.74 (s, 1H, OH), 4.31 (d, 2H, *J* = 4.4 Hz), 6.32 (dt, 1H, *J* = 15.8 Hz and *J* = 5.6 Hz), 6.57 (d, 1H, *J* = 16 Hz), 7.29 (m, 4H)	63.45; 127.63; 128.74; 129.15; 129.71; 133.28; 135.15	80
2a	oil	4.23 (d, 2H, *J* =7.2 Hz), 6.29 (dt, 1H, *J* =15.6 Hz and *J* =7.2 Hz), 6.63 (d, 1H, *J* = 15.8 Hz), 7.21-7.41 (m, 5H)	45.41; 124.88; 126.68; 127.61; 128.61; 134.11; 135.86	91
2b	oil	4.26 (d, 2H, *J* = 7.0 Hz), 6.28 (dt, 1H, *J* = 15.4 Hz and *J* = 6.8 Hz), 7.17 (d, 1H, *J* = 15.6 Hz), 7.40-7.67 (m, 3H), 7.96 (d, 1H, *J* = 8 Hz)	44.48; 124.67; 128.75; 128.91; 129.18; 130.09; 133.28; 147.81	91
2c	oil	4.27 (d, 2H, *J* = 6.0 Hz), 6.46 (dt, 1H, *J* = 15.8 Hz and *J* = 6.6 Hz), 6.73 (d, 1H, *J* = 15.8 Hz), 8.09-8.29 (m, 5H)	44.43; 118.28; 122.35; 123.21; 128.07; 129.55; 129.69; 133.47; 142.00	68
2d	oil	4.27 (d, 2H, *J* = 7 Hz), 6.49 (dt, 1H, *J* = 15.8 Hz and *J* = 6.6 Hz), 6.74 (d, 1H, *J* = 15.8 Hz), 7.53 (d, 2H, *J* = 8.8 Hz), 8.19 (d, 2H, *J* = 8.8 Hz)	44.37; 123.80; 127.22; 129.55; 131.62; 142.25; 147.28	89
2e	oil	3.83 (s, 3H, CH_3), 4.17 (d, 2H, *J* = 7.8 Hz), 6.42 (dt, 1H, *J* = 15.6 Hz and *J* = 8 Hz), 6.91 (d, 1H, *J* = 15.8 Hz), 6.83-7.01 (m, 2H), 7.23 (t, 1H, *J* = 7.8 Hz), 7.41 (d, 1H, *J* = 7.8 Hz)	34.36; 55.35; 110.81; 120.61; 125.70; 127.23; 129.38; 129.49; 156.93	na[a]
2f	oil	3.79 (s, 3H, CH_3), 4.13 (d, 2H, *J* = 7.4 Hz), 6.27-6.42 (m, 1H), 6.59 (d, 1H, *J* = 15.8 Hz), 6.81 (d, 1H, *J* = 8.4 Hz), 6.90 (s, 1H), 6.96 (d, 1H, *J* = 7.8 Hz), 7.23 (t, 1H, *J* = 8.0 Hz)	33.26; 55.13; 111.90; 113.71; 119.20; 125.45; 129.58; 134.35; 137.16; 159.75	na[a]
2g	oil	3.79 (s, 3H, CH_3), 4.15 (d, 2H, *J* = 7.8 Hz), 6.17-6.33 (m, 1H), 6.58 (d, 1H, *J* = 15.4 Hz), 6.81-6.89 (m, 2H) 7.28-7.35 (m, 2H)	34.19; 55.22; 113.97; 122.86; 128.00; 128.44; 134.15; 159.73	na[a]
2h	oil	4.20 (d, 2H, *J* = 6.8 Hz), 6.29 (dt, 1H, *J* = 15.8 Hz and *J* = 6.8 Hz), 6.57 (d, 1H, *J* = 15.4 Hz), 7.11-7.39. (m, 4H)	44.90; 124.87; 126.36; 126.56; 128.14; 129.84; 132.59; 137.05; 137.70	95
2i	oil	4.21 (d, 2H, *J* = 7.0 Hz), 6.29 (dt, 1H, *J* = 15.8 Hz and *J* = 7.0 Hz), 6.57 (d, 1H, *J* = 15.6 Hz), 7.23-7.39 (m, 4H)	44.90; 124.87; 125.56; 126.54; 128.13; 129.84; 132.55; 137.01; 141.89	90
2j	oil	4.22 (d, 2H, *J* = 7.0 Hz), 6.28 (dt, 1H, *J* = 15.8 Hz and *J* = 7.0 Hz), 6.60 (d, 1H, *J* = 15.8 Hz), 7.15-7.30 (m, 4H)	45.15; 125.50; 127.89; 128.82; 132.82; 134.33	88

[a] Compounds **2e**, **2f**, and **2g** were used in unpurified form.

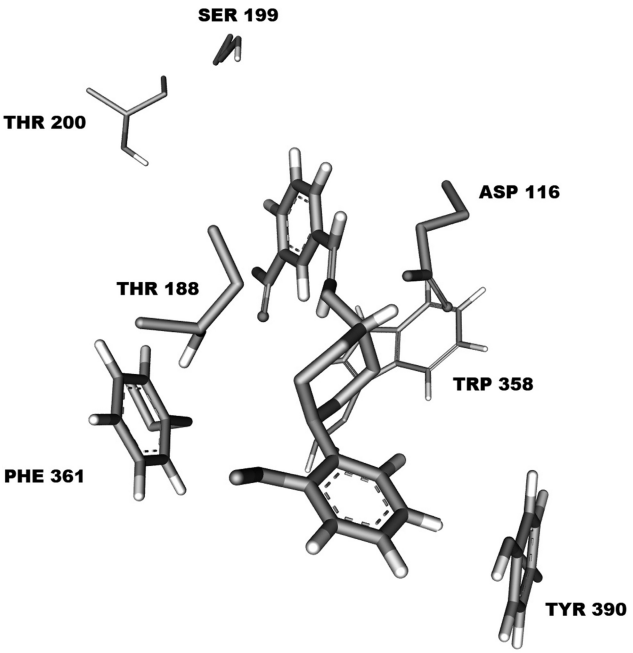
acids were transformed into mixed acyl anhydrides using ethylchloroformate in THF, and reduced thereafter using sodium borohydride/methanol at room temperature (overall yields: 61–84%). The physical properties of intermediates **1b–j** and **2a–j** prepared in this work are summarized in Table 1.

The obtained compounds were tested in radioligand binding assays for their affinity towards the D_2, 5-HT_{1A}, 5-

HT_{2A}, and α_1 receptors (Table 2). The cinnamyl compound with the highest affinity for the 5-HT_{1A} and 5-HT_{2A} receptors was the 2-methoxy derivate **3e** (Ki = 181 and 120 nM, respectively). All other compounds could be classified as weak 5-HT_{1A} and 5-HT_{2A} receptor ligands. Affinity constants for the D_2 receptor ranged between 10 – 50 nM, with the 2-methoxy derivate **3e** and the 3-nitro derivate **3c** being the strongest competitors (Ki of 14.2 and 16.9 nM, respectively). Small, but significantly higher affinities of these two ligands compared to the *para*-counterparts (**3d** and **3g**) and chlorinated analogues (**3h** and **3i**) suggest a existence of stabilizing interactions in compounds **3c** and **3e**. Small differences in affinity constants for the D_2 receptor suggest that the interactions accounting for those are week in nature. The greatest variability in affinities was seen for the α_1 receptor, with the 2-methoxy derivate **3e** being the strongest ligand (Ki = 33.2 nM) and the 3-chloro and 4-chloro derivatives **3i** and **3j** being as inactive as the [^{3}H]prazosin displacer. Therefore, 3-methoxy **3f**, 3-chloro **3i**, and 4-chloro **3j** 1-cinnamyl-4-(2-methoxyphenyl)piperazines can be regarded as rather specific D_2 receptor ligands, with an affinity at least 10-times higher than that for other receptors tested in this study.

Docking to D_2 and 5-HT_{1A} receptors was performed using the model of the ligand-binding pockets, as described in our previous publications [6, 7]. Amino acid residues that form the receptor binding site were selected based on the literature and our data obtained by molecular modelling. Interactions that stood out in the docking analysis comprised (i) a salt bridge between the protonated N1 of the piperazine ring and the negatively charged Asp 86 (D_2 receptor) or Asp 116 (5-HT_{1A} receptor), (ii) hydrogen bonds between the cinnamyl-moiety substituents and Ser 122 and His 189 of the D_2 receptor, (iii) hydrogen bonds between the methoxy group of the (2-methoxyphenyl)piperazine moiety and Trp 182 (D_2 receptor) or Thr 188 (5-HT_{1A} receptor), and (iv) edge-to-face interactions of the aromatic ring of the arylpiperazine part with Phe 178 and Tyr 216 of the D_2 receptor or with Phe 361 and Tyr 390 of the 5-HT_{1A} receptor (Fig. 2).

Docking analysis using the model for the binding pocket on the 5-HT_{1A} receptor indicated a significant number of stabilizing interactions between the receptor and the arylpiperazine part of the ligands (Fig. 2, Table 3). This is in accord with our previously published data [7]. However, introduction of the semi-rigid (*E*)-prop-2-en-1-yl linker seems to have a detrimental impact on the affinity towards the 5-HT_{1A} receptor. There is a significant decrease in affinity of (*E*)-prop-2-en-1-yl derivatives compared to the affinities of MPP itself, and the more flexible counterpart 1-(3-phenylprop-1-yl)-4-(2-methoxyphenyl)piperazine (Table 2). The introduction of a semi-rigid linker may restrict optimal fitting of the ligand into the narrow cavity of the receptor-binding site, but in our model of the 5-HT_{1A} receptor, we could not observe this so-called "steric bumping". Our model is also not able to explain the effects of the 2-methoxy **3e** and 3-nitro **3c** substitution on the affinity towards the 5-HT_{1A} receptor. Therefore, the molecular model of the 5-HT_{1A} receptor binding site that was used in this study requires further refinement, particularly in the region responsible for the binding of the cinnamyl part of the ligand.



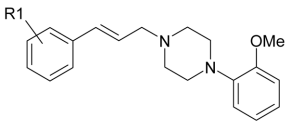
The 3D model describes a possible interaction of compound **3c** and the theoretical 5-HT_{1A} serotonin receptor model. A subset of residues involved in the ligand binding at the 5-HT_{1A} serotonin receptor and compound **3c** in its stable conformation are shown. Distances between ligand **3c** and the receptor atoms are given in Table 2.

Figure 2. Schematic representation of ligand **3c** interaction with the 5-HT_{1A} serotonin receptor.

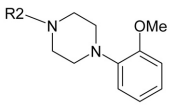
On the other hand, the (*E*)-3-phenylprop-2-en-1-yl linker (similar to the fully flexible propylene linker, compound **4**) fits well in the architecture of the ligand binding site of the D_2 receptor, suggesting nanomolar affinities for the ligands **3a – j**. Large increases in affinities of cinnamyl derivatives **3a – j** as well as the phenylpropyl derivative **4** compared to the parent MPP compound can be attributed to the aromatic-aromatic interactions between the phenyl residue of the ligand and the aromatic microdomain of the D_2 receptor binding site.

The higher affinities of compounds **3e** and **3c** towards the D_2 receptor can be attributed to a hydrogen bond between the nitro group of compound **3c** and Ser 122 (Fig. 3a, Table 4), and a hydrogen bond between the 2-

Table 2. Chemical structure of the examined arylpiperazine ligands and their binding constants for the 5-HT_{1A}, 5-HT_{2A}, D_2, and a_1 receptors.



Compound	R1	K_i ± S.E.M. (nM)			
		5-HT_{1A}	5-HT_{2A}	D_2	a_1
3a	H	583 ± 50	350 ± 28	24.3 ± 1.2	47.1 ± 4.0
3b	2-NO_2	564 ± 56	296 ± 28	22.4 ± 2.0	48.6 ± 4.6
3c	3-NO_2	225 ± 20	204 ± 18	16.9 ± 1.2	43.1 ± 4.6
3d	4-NO_2	651 ± 60	788 ± 80	66.5 ± 5.8	158 ± 16
3e	2-OCH_3	181 ± 16	120 ± 14	14.7 ± 1.2	33.2 ± 2.8
3f	3-OCH_3	708 ± 72	925 ± 96	36.0 ± 3.2	667 ± 7.0
3g	4-OCH_3	583 ± 56	943 ± 98	60.0 ± 5.6	80.0 ± 7.6
3h	2-Cl	343 ± 32	597 ± 65	32.0 ± 3.4	300 ± 36
3i	3-Cl	590 ± 64	922 ± 98	42.3 ± 4.8	>1000
3j	4-Cl	588 ± 64	990 ± 90	50.6 ± 5.8	>1000



	R2				
4	$PhCH_2CH_2CH_2$	3.7 ± 0.4	na	27 ± 2.2	7.8 ± 1.1
5	H	68 ± 4.2	na	1200 ± 37	na
		Reference Compounds			
8-OH-DPAT		2.0 ± 0.4	na	na	na
Ketanserin		na	3.1 ± 0.4	na	na
Haloperidol		na	na	12.0 ± 2.0	na
Prazosine		na	na	na	2.2 ± 0.3

K_i values represent the means of three independent experiments, done in triplicate using eight ligand concentrations (1.0 nM – 0.1 mM), according to the methods described in Experimental (section 4); S.E.M. = standard error of mean; na = not applicable.

Table 3. Distances in Å between ligand **3c** and the receptor atoms in the 5-HT_{1A} receptor-ligand complexes after docking.[a)]

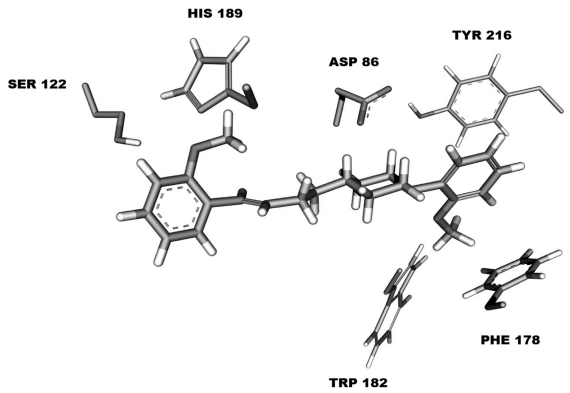
Receptor Residue/ (interacting atom)	Distance between ligand 3c – receptor (Å)	Ligand 3c (interacting atoms)
Asp 116 (O^-)	1.93	(NH^+)
Thr 188 (OH)	3.64	(OCH_3)
Ser 199 (OH)	>8.0	(NO_2)
Thr 200 (OH)	5.92	(NO_2)
Trp 358 (NH)	3.68	(NO_2)
Phe 361 (aromatic ring)	6.64	($CH^{Aromatic}$)
Tyr 390 (aromatic ring)	2.69	($CH^{Aromatic}$)

[a)] The measures are relative to the closest atom of the ligand, the center of the ring if the closest part of the ligand is an aromatic group and the heteroatom if it is involved in a hydrogen bond.

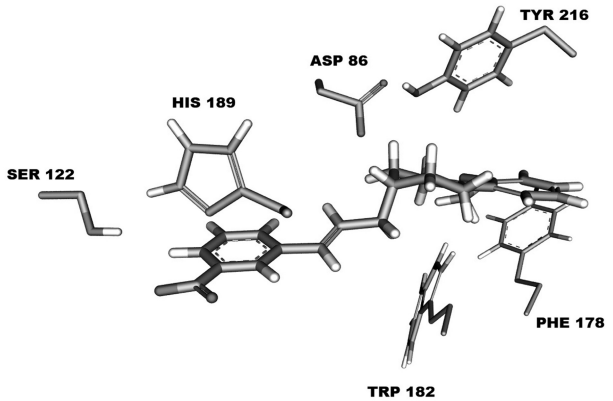
methoxy group of compound **3e** and Ser 122 and His 189 (Fig. 3b, Table 4). This effect is not seen in compounds **3h** and **3i** with chloro substituents in *ortho*- or *meta*- positions, due to the inability of chloro groups to build hydrogen bonds. Also, we could not detect the recently described attracting interactions of the [C-Halogen…N] and [C-Halogen…HN] type, most likely due to their strict spatial requirements [11, 12]. Taken together, it can be concluded that the ability to form hydrogen bonds – rather than the positive or negative inductive effect of the substituents – determines the affinity of these ligands to the D_2 receptor.

Aryl- or heteroaryl-substituted piperazines represent one of the largest categories of chemical structures with dopaminergic properties [13]. They are also a part of numerous high-affinity ligands for different G-protein coupled receptors [14]. In D_2-, 5-HT_{2A}-, and α_1-functional tests most of them behaves as antagonist [13, 15, 16] although few exceptions are reported in the literature [17]. The recently published *trans*-1-[(2-phenylcyclopropyl)methyl]-4-arylpiperazines [18] are close structural analogues of 1-cinnamyl-4-(2-methoxyphenyl)piperazines

a



b



The 3D model describes a possible interaction of compounds a: **3e** and b: **3c** and the theoretical dopamine D_2 receptor model. Subset of residues involved in the ligand binding at D_2 receptor and compounds **3c** and **3e** in their stable conformations are shown. Distances between ligand **3c** and **3e** and receptor atoms are given in Table 3.

Figure 3. Schematic representation of ligands **3c** and **3e** interaction with the D_2 dopamine receptor.

and a typical dopamine D_2 antagonists. The similarity in structure between these two groups of compounds make us believe that cinnamyl-4-(2-methoxyphenyl)piperazines share the same pharmacological properties with their cyclopropyl congeners. Substituted arylpiperazines cannot be simply classified as 5-HT_{1A}, agonists or antagonists; a number of them are partial agonists with a different degree of intrinsic activity [19]. Therefore, is difficult to predict the 5-HT_{1A} properties of cinnamyl-4-(2-methoxyphenyl)piperazines on the basis of literature data. Further functional studies on cinnamyl-4-(2-methoxyphenyl)piperazines are necessary for evaluating their full pharmacological properties.

Table 4. Distances in Å between ligands and receptor atoms in the D_2 receptor-ligand complexes after docking.[a)]

Receptor Residue/ (interacting atom)	Ligand	
	3c Distance (Å)/ (interacting atoms)	3e Distance (Å)/ (interacting atoms)
Asp 86/(O^-)	1.83/(NH^+)	1.79/(NH^+)
Ser 122/(OH)	2.77/(NO_2)	2.45/(OCH_3)
Ser 141/OH)	>8.0/(NO_2)	>8.0/(OCH_3)
Phe 178/(aromatic ring)	3.48/($CH^{Aromatic}$)	3.93/($CH^{Aromatic}$)
Trp 182/(N)	2.03/(OCH_3)	2.10/(OCH_3)
His 189	5.61/(NO_2)	3.18/(OCH_3)
Tyr 216/(aromatic ring)	2.99/($CH^{Aromatic}$)	3.22/($CH^{Aromatic}$)

[a)] The measures are relative to the closest atom of the ligand, the center of the ring, if the closest part of the ligand is an aromatic group and the heteroatom, if it is involved in a hydrogen bond.

Conclusions

The herein described 1-cinnamyl-4-(2-methoxyphenyl)piperazines show low to moderate affinity towards 5-HT_{1A} and 5-HT_{2A} receptors. Molecular docking analysis of their interactions with the 5-HT_{1A} receptor binding site cannot completely explain results obtained in binding studies, which implies that further refinement of this receptor model is necessary. The same compounds have high affinity towards the D_2 receptor. Docking studies using the D_2 receptor model revealed a series of interactions that effectively predict binding properties of the ligands, which also confirms the robustness of the receptor model used.

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Experimental

General

A Boetius PHMK apparatus (VEB Analytic, Dresden, Germany) was used to determine melting points, presented here as uncorrected. ^{1}H-NMR (at 200 MHz) and ^{13}C-NMR (at 50 MHz) spectra were recorded on a Gemini 2000 spectrometer (Varian, Palo Alto, CA, USA) with $CDCl_3$ as a solvent; unless otherwise stated, they are reported in ppm using tetramethylsilane as the internal standard. The IR spectra were run on a Perkin Elmer 457 Grating FT Infrared Spectrophotometer (Perkin Elmer, Beaconsfield, UK). The mass spectra were determined by time-of-flight mass spectrometry on Agilent Technologies LC MS TOF 6210 (Agilent, Palo

Alto, CA, USA). Samples were dissolved in a mixture of 50% water with 5 mM formic acid and 50% acetonitrile. For analytical thin-layer chromatography Merck F254 plastic-backed thin-layer silica gel plates were used (Merck, Darmstadt, Germany). Chromatographic purifications were performed on Merck-60 silica gel columns, 230400 mesh ASTM, under medium pressure (dry column flash chromatography). All reagents and solvents used in this work were obtained from Aldrich (Steinheim, Germany) and were used without further purification. Solutions were routinely dried over anhydrous Na_2SO_4 prior to evaporation.

Molecular modelling

Ligand-geometry optimization

All ligands used in the docking analysis were initially modelled using Accellrys Insight II molecular modelling software (www.accellrys.com) and inbuilt builder module routine for molecular geometry construction and optimization. It was postulated that the ligands are bound to the receptors in protonated form [20, 21], therefore, a formal charge of +1 was added to the piperazine nitrogen (1N).

Modelling of D_2-receptor binding site

Modelling of the ligand–D_2 receptor complexes was done as described earlier [6]. Shortly, the model of the D_2 receptor transmembrane helices was constructed directly from the bacteriorhodopsin coordinates derived from two-dimensional electron diffraction experiments, but the orientations of all TM domains were subsequently adjusted in order to mimic the topology of the TM domains of rhodopsin [22]. This model was tested for its ability to accommodate rigid agonist and semi-rigid antagonist molecules which were docked into the putative binding pocket with stabilizing interactions. The model is consistent with structure-activity relationships of agonists and antagonists that interact with the receptor [22] and with site-directed mutagenesis data [23–25].

Modelling of 5-HT_{1A} receptor binding site

The model of the human 5-HT_{1A} was built as described previously [7] using crystal structures of bovine rhodopsin (PDB codes 1F88, 1HZX, and 1L9H) [26] as a template. Comparative modelling by means of the MODELER program, which is part of the Insight II package from Accelrys, has been used. Our model includes seven transmembrane helices and second extracellular loops. The binding site of the ligand in the 5-HT_{1A} receptor was determined starting from the fact that for the ligand activity formation of the salt bridge between protonated piperazine nitrogen and Asp 116 is necessary [25], active site search procedure from Insight II binding site analysis module [27] was used to select all amino acid residues forming the cavity near Asp 116. The binding site defined in the previous step was further refined by manually excluding all amino acid residues that cannot come in direct contact with the inside of the cavity.

Docking

Docking of the selected ligands as presented in Table 1 was done by simulated annealing using the Affinity module from Insight on the SGI Octane2 workstation [28]. All ligands were docked as protonated, using the CFF91 force field. Amino acid residues charges were adjusted where needed. The protein binding site was determined by combining results from experimental data and the Insight II bind site analysis module. Initial position of the ligand in the binding site, was arbitrary, while protonated nitrogen on the ligand part was kept in close proximity of Asp 86 of the D_2 receptor or Asp 116 of the 5-HT_{1A} receptor. After initial ligand placement, no further constrains were applied and the docking procedure based on Monte-Carlo methodology was carried out. Up to 100 structures were produced in every run and each finally optimized in order to remove steric interaction with a gradient limit of 0.0042 kJ/mol or 4000 optimization steps.

Obtained docked structures were examined, and those with the lowest total energy were further filtered to obtain docking structures with the best ligand fit. We selected structures based on the following criteria: lowest total energy of the complex, shortest salt bridge formed between Asp 86 of the D_2 receptor or Asp 116 of the 5-HT_{1A} recepto, and proton on nitrogen, chair conformation of arylpiperazine ring, and aryl part of the molecule positioned in the rear hydrophobic pocket of the ligand. After an initial criterion was satisfied, the second step was examination of different interactions that can be formed between receptor and ligand (hydrogen bonds, aromatic–aromatic interactions, etc.). In that way, the best possible docking structures were selected. Structures were visualised using DS Visualise v1.6 [27] and obtained images were rendered using PovRay Raytracer v3.6 [29].

Chemistry

General procedure for the synthesis of (2E)-3-phenylprop-2-en-1-ols ***1b–j***

To a 250 mL round-bottom flask containing 19.9 mmol of substituted cinnamic acid, 30 mL of dry THF and 19.9 mmol triethylamine at −7°C was added dropwise 19.9 mmol of ethyl chloroformate. After being stirred for an additional 30 min, the reaction mixture was allowed to warm to 10°C and 2.7 g (76 mmol) of powdered sodium borohydride was added in one portion. To this suspension, 12 mL of absolute methanol was added dropwise over one hour at the same temperature. After being stirred at room temperature for additional one hour, the reaction mixture was poured into saturated ammonium chloride solution (200 mL) and extracted with 3×50 mL dichloromethane. The combined organic layer were dried over anhydrous Na_2SO_4 and concentrated. Crude product was purified by flash column chromatography (dichloromethane/methanol, 99 / 1).

General procedure for the synthesis of substituted [(1E)-3-chloroprop-1-enyl]benzene ***2a–d*** *and* ***2h–j***

To a chilled solution of thionyl chloride (32 mL) in diethylether was slowly added 26.2 mmol of cinnamyl alcohols (**1a–d** and **1h–j**) in portions. The mixture was stirred for 30 min at 0°C and allowed to warm to room temperature followed by stirring for an additional hour. The resulting mixture was poured into ice/water (500 mL) and extracted with dichloromethane (3×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by flash-column chromatography (dichloromethane) and used immediately.

*General procedure for the synthesis of 1-[(1E)-3-bromoprop-1-enyl]-methoxybenzenes **2e – 2g***

Hydrobromic acid (HBr) in acetic acid (33% solution, 2.24 g), 1.5 g (9.15 mmol) (*2E*)-3-(methoxyphenyl)prop-2-en-1-ol (**1e** or **1f** or **1g**) in 20 mL dry diethyl ether, was stirred for 120 min at room temperature under argon, and was then poured into a water/diethyl ether mixture (20 / 20 mL). The organic layer was separated, washed with saturated $NaHCO_3$ solution, then water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The product was used immediately without further purification.

*General procedure for the synthesis of (2E)-1-(3-arylprop-2-enyl)-4-(2-methoxyphenyl)piperazines **3a – j***

Cinnamyl halides **2a – j** (10.0 mmol), 1-(2-methoxyphenyl)piperazine (10.0 mmol) and 2.02 g (20.0 mmol) of triethylamine in 20 mL acetonitrile were stirred at room temperature for 24 h. The reaction mixture was poured into water and extracted with dichloromethane. After drying over Na_2SO_4 and concentration in vacuum, the products were purified by dry-flash chromatography using a gradient of methanol (0 – 5%) in dichloromethane as a solvent.

*(2E)-4-(2-Methoxyphenyl)-1-(3-phenylprop-2-enyl)piperazine **3a***

^{1}H-NMR (DMSO) 2.56 (m, 4H) 2.97 (m, 4H), 3.13 – 3.17 (m, 2H), 3.76 (s, 3H from OCH_3), 6.33 (dt, 1H, *J* = 16 Hz and *J* = 6.0 Hz), 6.57 (d, 1H, *J* = 16 Hz), 6.87 – 6.95 (m, 4H), 7.2 – 7.36 (m, 3H), 7.45 (d, 2H, *J* = 6.8 Hz); ^{13}C-NMR ($CDCl_3$) 50.28; 53.812; 55.50; 60.49; 112.13; 118.16; 121.05; 122.60; 126.48; 127.26, 127.66; 128.85; 132.43; 136.91; 141.50; 152.25; IR (cm^{-1}) 2830, 2812, 1499, 1454, 1241, 746; MS: m/e $[M+H]^+$ 308.4170; mp. 78 – 80°C; Yield 87%.

*(2E)-1-[4-(2-Methoxyphenyl)-3-(2-nitrophenyl)prop-2-enyl]-piperazine **3b***

^{1}H-NMR ($CDCl_3$) 2.75 (m, 4H) 3.14 (m, 4H), 3.29 (d, 2H, J = 6.6 Hz), 3.86 (s, 3H from OCH_3), 6.30 (dt, 1H, *J* = 15.8 Hz and *J* = 6.6 Hz), 6.84 – 6.98 (m, 4H), 7.04 (d, 1H, *J* = 16.2 Hz), 7.37 (t, 1H, *J* = 8.2 Hz), 7.55 (t, 1H, *J* = 7.6 Hz), 7.62 (d, 1H, *J* = 8.0 Hz), 7.90 (d, 1H, *J* = 7.8 Hz); ^{13}C-NMR ($CDCl_3$) 50.51; 53.35; 55.22; 60.67; 111.07; 118.11; 120.88; 122.86; 124.39; 127.93; 128.18; 128.60; 132.13; 132.97; 141.16; 147.66; 152.18; IR (cm^{-1}) 2938, 2814, 1523, 1501, 1347, 1241, 744; MS: m/e $[M+H]^+$ 354.1854; oil; Yield 56%.

*(2E)-4-(2-Methoxyphenyl)-1-[3-(3-nitrophenyl)prop-2-enyl]-piperazine **3c***

^{1}H-NMR ($CDCl_3$) 2.74 – 2.76 (m, 4H), 3.14 (m, 4H), 3.28 (d, 2H, *J* = 6.0 Hz), 3.86 (s, 3H from OCH_3), 6.55 (dt, 1H, *J* = 17 Hz and *J* = 6.2 Hz), 6.63 (d, 1H, *J* = 16 Hz), 6.85 – 7.05 (m, 4 H,), 7.47 (t, 1H, *J* = 8 Hz), 7.68 (d, 1H, *J* = 7.8 Hz), 8.07 (d, 1H, *J* = 8.2 Hz), 8.23 (s, 1H); ^{13}C-NMR ($CDCl_3$) 40.51; 53.37; 55.21; 60.59; 110.99; 118.09; 120.88; 121.94; 122.92; 129.36; 130.11; 130.60; 131.93; 138.61; 141.07; 148.46; 152.14; IR (cm^{-1}) 2972, 2810, 1529, 1499, 1456, 1351, 1238, 1132, 1018, 751; MS: m/e $[M+H]^+$ 354.1816; mp. 81°C; Yield 66%.

*(2E)-4-(2-Methoxyphenyl)-1-[3-(4-nitrophenyl)prop-2-enyl]-piperazine **3d***

^{1}H-NMR ($CDCl_3$) 2.74 (m, 4H), 3.14 (m, 4H), 3.28 (d, 2H, *J* = 6.0 Hz), 3.85 (s, 3H from OCH_3), 6.50 (dt, 1H, *J* = 16 Hz and *J* = 6.0 Hz), 6.64 (d, 1H, *J* = 16 Hz), 6.84 – 7.05 (m, 4H) 7.49 (d, 2H, *J* = 8.6 Hz), 8.15 (d, 2H, *J* = 9 Hz); ^{13}C-NMR ($CDCl_3$) 50.38; 53.29; 55.08; 60.58; 110.90; 117.94; 120.77; 122.81; 123.77; 126.60; 130.67; 131.88; 140.94; 143.16; 146.59; 152.01; IR (cm^{-1}) 2930, 2812, 1595, 1510, 1343, 1240, 751; MS: m/e $[M+H]^+$ 354.1843; mp. 117°C; Yield 92%.

*(2E)-4-(2-Methoxyphenyl)-1-[3-(2-methoxyphenyl)prop-2-enyl]-piperazine **3e***

^{1}H-NMR ($CDCl_3$) 2.72 (m, 4H), 3.13 (m, 4H), 3.25 (d, 2H, J = 6.8 Hz), 3.83 (s, 3H from OCH_3), 3.84 (s, 3H from OCH_3) 6.34 (dt, 1H, J = 16 Hz and J = 6.8 Hz), 6.83 – 7.04 (m, 7H), 7.24 (t, 1H, J = 7.6 Hz), 7.46 (d, 1H, J = 7.4 Hz); ^{13}C-NMR ($CDCl_3$) 50.51; 53.28; 55.17; 55.26; 61.52; 110.68; 110.97; 118.09; 120.21; 120.86; 122.77; 125.77; 126.69; 126.98; 127.76; 128.40; 141.20; 152.12; 156.44; IR (cm^{-1}) 2939, 2813, 1595, 1497, 1457, 1243, 1180, 1029, 750; MS: m/e $[M+H]^+$ 339.2067; mp 78°C; Yield 57%.

*(2E)-4-(2-Methoxyphenyl)-1-[3-(3-methoxyphenyl)prop-2-enyl]-piperazine **3f***

^{1}H-NMR ($CDCl_3$) 2.72 (m, 4H), 3.13 (m, 4H), 3.79 (s, 3H from OCH_3), 3.84 (s, 3H from OCH_3), 4.29 (d, 2H, *J* = 5.4 Hz) 6.32 (dt, 1H, *J* = 15.6 Hz and *J* = 5.4 Hz), 6.53 (d, 1H, *J* = 16 Hz), 6.76 – 7.04 (m, 7H), 7.22 (t, 1H, *J* = 8 Hz); ^{13}C-NMR ($CDCl_3$) 50.49; 53.29; 55.04; 55.19; 60.96; 110.96; 112.48; 113.23; 118.11; 118.95; 120.86; 122.86; 126.72; 129.45; 133.01; 138.23; 141.13; 152.12; 159.68; IR (cm^{-1}) 2939, 2831, 1590, 1498, 1454, 1242, 1036, 752; MS: m/e $[M+H]^+$ 339.2073; mp. 78°C; Yield 45%.

*(2E)-4-(2-Methoxyphenyl)-1-[3-(4-methoxyphenyl)prop-2-enyl]-piperazine **3g***

^{1}H-NMR ($CDCl_3$) 2.71 (m, 4H), 3.12 (m, 4H), 3.21 (d, 2H, J = 6.8 Hz), 3.78 (s, 3H from OCH_3), 3.84 (s, 3H from OCH_3), 6.17 (dt, 1H, *J* = 16 Hz and *J* = 6.8 Hz), 6.49 (d, 1H, *J* = 16 Hz), 6.82 – 7.04 (m, 6H), 7.32 (d, 2H, *J* = 8.8 Hz); ^{13}C-NMR ($CDCl_3$) 50.49; 53.26; 55.11; 55.15; 61.12; 110.96; 113.83; 118.08; 120.84, 122.79; 124.07; 127.36; 129.60; 132.55; 152.12; 159.01; IR (cm^{-1}) 2940, 2806, 1604, 1508, 1457, 1241, 1172, 1029, 752; MS: m/e $[M+H]^+$ 339.2093; mp. 75°C; Yield 95%.

*(2E)-1-[3-(2-Chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)piperazine **3h***

^{1}H-NMR ($CDCl_3$) 2.74 (m, 4H), 2.76 (m, 4H), 3.28 (d, 2H, *J* = 6.8 Hz), 3.86 (s, 3H from OCH_3), 6.31 (dt, 1H, *J* = 15.6 Hz and *J* = 6.8 Hz), 6.84 – 7.05 (m, 4H), 6.95 (d, 1H, *J* = 16 Hz), 7.15 – 7.26 (m, 1H) 7.18 (t, 1H, *J* = 5.2 Hz), 7.34 (d, 1H, *J* = 7 Hz), 7.55 (d, 1H, *J* = 7.4 Hz); ^{13}C-NMR ($CDCl_3$) 50.58; 53.37; 55.26; 61.04; 111.067; 118.17; 120.94; 122.91; 126.81; 128.45; 129.18; 129.47; 129.62; 132.82; 134.97; 141.22; 152.22; IR (cm^{-1}) 2940, 2815, 1591, 1500, 1452, 1240, 1137, 1030, 748; MS: m/e $[M+H]^+$ 342.1569; oil; Yield 71%.

*(2E)-1-[3-(3-Chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)piperazine **3i***

^{1}H-NMR ($CDCl_3$) 2.71 (m, 4H), 3.12 (m, 4H), 3.23 (d, 2H, *J* = 6.4 Hz), 3.85 (s, 3H from OCH_3), 6.32 (dt, 1H, *J* = 15.8 Hz and *J* = 6.4 Hz), 6.50 (d, 1H, *J* = 15.8 Hz), 6.83 – 7.04 (m, 4H), 7.15 – 7.25 (m, 3H), 7.36 (s, 1H); ^{13}C-NMR ($CDCl_3$) 50.53; 53.33; 55.21; 60.80; 111.05; 118.11; 120.90; 122.86; 124.36; 126.25; 127.30; 128.20; 129.69; 131.62; 134.41; 138.74; 141.16; 152.16; IR (cm^{-1}) 2939, 2815,

1591, 1500, 1454, 1240, 1138, 747; MS: m/e $[M+H]^+$ 343.1597; mp. 71°C; Yield 93%.

*(2E)-1-[3-(4-Chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)piperazine **3j***

^{1}H-NMR ($CDCl_3$) 2.71 (m, 4H), 3.12 (m, 4H), 3.22 (d, 2H, J = 6.6 Hz), 3.85 (s, 3H from OCH_3), 6.28 (dt, 1H, J = 15.6 Hz and J = 6.6 Hz), 6.50 (d, 1H, J = 16.2 Hz), 6.83–7.04 (m, 4H), 7.23–7.33 (m, 4H); ^{13}C-NMR ($CDCl_3$) 50.51; 53.31; 55.19; 60.88; 111.03; 118.09; 120.88; 122.85; 127.42; 128.60; 131.75; 132.95; 135.34; 141.16; 152.14. IR (cm^{-1}) 3477, 2938, 1588, 1498, 1450, 1241, 1122, 1025, 754; MS: m/e $[M+H]^+$ 343.1579; mp. 120°C; Yield 55%.

*4-(2-Methoxyphenyl)-1-(3-phenylpropyl)piperazine **5***

To 10.0 mmol solutions of 1-(2-methoxyphenyl)piperazine in 50 mL of DMF, 10.0 mmol of 1-chloro-3-phenylpropane, 6.0 g Na_2CO_3, and 0.2 g of KI were added. The mixture was stirred at 60°C for 18 h. After cooling, the precipitate was removed and the filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel using dichloromethane as the eluent. Product **5** was isolated as gummy substance which was converted to the hydrochloride with ethereal HCl. Recrystallization from EtOH gave pure **5** . 2 HCl salt.^{1}H-NMR (DMSO) 2.081 (m, 2H), 2.593 (t, 2H), 3.10–3.29 (m, 6H), 3.44–3.55 (m, 4H), 3.878 (s, 3H from OCH_3), 6.89 (t, 1H), 6.97–7.07 (m, 3H), 7.16–7.31 (m, 5H); ^{13}C-NMR (DMSO) 24.76, 32.22, 47.02, 50.88, 55.32, 55.57, 112.29, 118.74, 120.99, 124.30, 126.21, 128.36, 128.52, 138.49, 140.68, 151.94; MS: m/e $[M+H]^+$ 311.2047; mp. 195-196°C; Yield 83%.

Membrane preparation, radioligand binding assays, and data analysis

Synaptosomal membranes from fresh bovine caudate nuclei, hippocampi, and frontal cortex were prepared for radioligand binding assays as previously described [30]. [^{3}H]Spiperone (spec. act. 80.5 Ci $mmol^{-1}$) and 8-OH-[^{3}H]DPAT (spec. act. 223 Ci $mmol^{-1}$) and [^{3}H]prazosin (spec. act. 78.9 Ci $mmol^{-1}$) used to label D_2, 5-HT_{1A}, and α_1 adrenergic receptors, respectively, were purchased from Amersham Buchler GmbH (Braunschweig, Germany). For labeling of 5-HT_{2A} receptors, [^{3}H]ketanserin (spec. act. 72.2 Ci $mmol^{-1}$, Perkin Elmer LAS GmbH, Rodgau, Germany) was used.

[^{3}H]Spiperone-receptor binding assay

[^{3}H]Spiperone binding was assayed in 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl solution, pH 7.4, at a membrane protein concentration of 0.7 mg mL^{-1} at 37°C for 20 min in a total volume of 1.0 mL of the incubation mixture. Binding of the radioligand to the 5-HT_2 receptors was prevented by 50 μM ketanserin. The K_i values of the tested compounds were determined by competition binding at 0.2 nM of the radioligand and eight to ten different concentrations of each compound (10^{-5} to 10^{-10} M). Nonspecific binding was measured in the presence of 1.0 mM (+)-butaclamol. The reaction was terminated by rapid filtration through Whatman GF/C filters, which were further washed three times with 5.0 mL of ice-cold incubation buffer. Each point was determined in triplicate. Retained radioactivity was measured by introducing of dry filters into 10 mL of toluene-based scintillation liquid and counting in a 1219 Rackbeta Wallac scintillation counter (EG&G Wallac, Turku, Finland) at an efficiency of 51–55% for tritium. The K_d value determined for spiperone was 0.9 nM.

8-OH-[^{3}H]DPAT-receptor binding assay

Each tube contained 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl, pH 7.4, hippocampi synaptosomal membrane (prot. conc 0.7 mg mL^1), 0.6 nM 8-OH-[^{3}H]DPAT and various concentrations (10^{-5} to 10^{-9} M) of the tested compounds in a final volume of 0.5 mL. The tubes were incubated (20 min, 37°C) and the reaction terminated by vacuum filtration through Whatman GF/B filters. The filters were washed three times with 5 mL of ice-cold 25 mM Tris–HCl buffer, pH 7.4, and bound radioactivity was measured by liquid scintillation spectrometry. 5-HT_{1A}-specific binding was defined as the difference between the binding in the absence and in the presence of 10 μM 5hydroxytryptamine. The K_d value determined for 8-OH-DPAT was 1.6 nM.

[^{3}H]Ketanserin-receptor binding assay

Each tube contained 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl, pH 7.4, frontal cortex synaptosomal membranes (prot. conc 0.7 mg mL^{-1}), 1.0 nM [^{3}H]ketanserin, and various concentrations (10^{-5} to 10^{-9} M) of the tested compounds in a final volume of 0.5 mL. The tubes were incubated (20 min, 37°C) and the reaction terminated by vacuum filtration through Whatman GF/B filters. The filters were washed three times with 5 mL of ice-cold 25 mM Tris–HCl buffer, pH 7.4, and the bound radioactivity was measured by liquid scintillation spectrometry. Specific binding to 5-HT_{2A} receptors was defined as the difference between the binding in the absence and in the presence of cold 1 μM ketanserin. The K_d value determined for ketanserin was 2.2 nM.

[^{3}H]Prazosin-receptor binding assay

Each tube contained 1.0 mM EDTA, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, 120 mM NaCl, 25 mM Tris–HCl, pH 7.4, frontal cortex synaptosomal membranes (prot. conc 0.7 mg mL^{-1}), 1.0 nM [^{3}H]prazosin, and various concentrations (10^{-5} to 10^{-9} M) of the tested compounds in a final volume of 0.5 mL. The tubes were incubated (20 min, 37°C) and the reaction terminated by vacuum filtration through Whatman GF/B filters. The filters were washed three times with 5 mL of ice-cold 25 mM Tris–HCl buffer, pH 7.4, and bound radioactivity was measured by liquid scintillation spectrometry. Specific binding to α_1 was defined as the difference between the binding in the absence and in the presence of cold 1 μM prazosine. The K_d value determined for prazosine was 1.1 nM.

The inhibition curves on the different binding sites of the compounds reported in Table 2 were analyzed by nonlinear curve fitting utilizing the Graph-Pad Prism program [31]. Hill slope coefficients were fixed to unity during calculation.

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