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Searching for multi-target antipsychotics: Discovery of orally active heterocyclic *N*-phenylpiperazine ligands of D_2-*like* and 5-HT_{1A} receptors

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ABSTRACT

We described herein the design, synthesis, and pharmacological evaluation of *N*-phenylpiperazine heterocyclic derivatives as multi-target compounds potentially useful for the treatment of schizophrenia. The isosteric replacement of the heterocyclic ring at the biaryl motif generating pyrazole, 1,2,3-triazole, and 2-methylimidazole[1,2-*a*]pyridine derivatives resulted in 21 analogues with different substitutions at the *para*-biaryl and *para*-phenylpiperazine positions. Among the compounds prepared, **4** (LASSBio-579) and **10** (LASSBio-664) exhibited an adequate binding profile and a potential for schizophrenia positive symptoms treatment without cataleptogenic effects. Structural features of this molecular scaffold are discussed regarding binding affinity and selectivity for D_2-*like*, 5-HT_{1A}, and 5-HT_{2A} receptors.

1. Introduction

The centenary Ehrlich's magic bullet concept ('one disease, one target') has been the basis of drug design in the last decades. However, there is an increasing change in this paradigm and the modulation of a multiplicity of targets has been considered as the best option for treating a range of multifactorial diseases.[1–3] For example, most central nervous system disorders are highly complex in its pathophysiology and the most effective drugs present a complex pharmacology.[4] In this way, schizophrenia can be considered a challenging neuropsychiatric disease. It is characterized by the development of three distinct kinds of symptoms: positive (hyperactivity, delusions, hallucinations, disorganized speech), negative (avolition, anhedonia, social isolation), and cognitive (attentional impairment, memory deficits). The genesis of each symptom is not fully understood, but several brain structures and neurotransmitter systems are believed to be involved.[5]

Most of the multi-target drugs used clinically were not the result of a rational design but discovered by serendipity, such as clozapine (**1**) (Fig. 1), the first atypical antipsychotic.[1,6] Initially it was proposed that clozapine's atypical profile, mainly the lack of extrapyramidal symptoms at therapeutic doses, was triggered by its high affinity for D_4 dopamine receptors.[7,8] As a consequence, some D_4-selective compounds were developed but were not effective in clinical trials.[4,8] On the other hand, the role of 5-HT_{2A} receptors in ameliorating negative and cognitive symptoms of the disorder emerged and selective 5-HT_{2A} antagonists were designed.[9] In clinical trials, these compounds showed efficacy but no advance was demonstrated in comparison with current available agents.[4] Other attempts to develop 'magic bullets' for treating schizophrenia also failed and now most researches are back to the multi-target or 'intramolecular polypharmacy' approach.[10] The importance of multivalent ligands targeting G-protein-coupled receptors design to deal with multifaceted neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and schizophrenia has been pointed by many authors.[11–13]

Several targets are being explored for the development of new antipsychotics and most of them are dopamine or serotonin receptors.[14,15] In this context, a significant interest has emerged from the ability of aripiprazole (**2**) and bifeprunox (**3**) (Fig. 1) to activate the 5-HT_{1A} receptor and to improve the cognitive and negative symptoms of schizophrenia and to reduce the extrapyramidal effects

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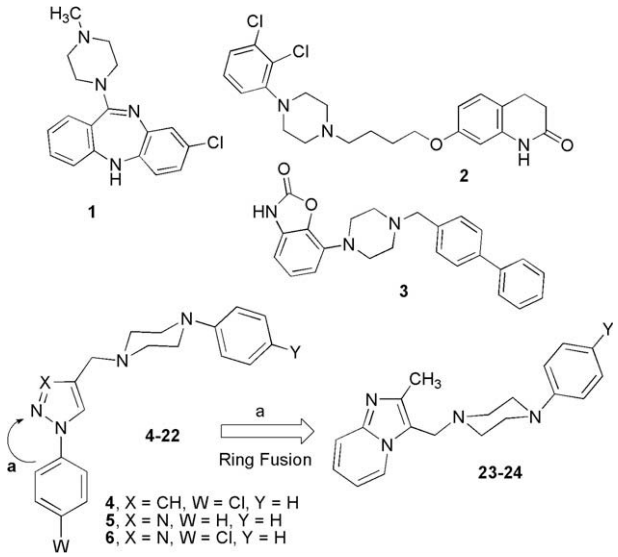


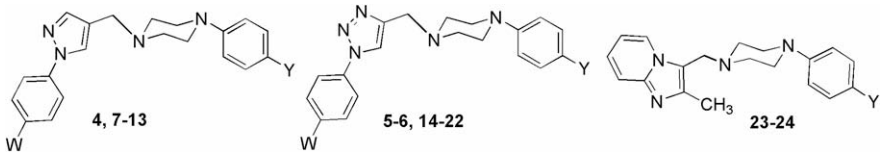
Figure 1. Molecular diversity of functionalized *N*-phenylpyrazole, *N*-phenyl-1,2,3-triazole, and imidazo[1,2-*a*]pyridine *N*-phenylpiperazine derivatives (**4**–**24**), including the structures of clozapine (**1**) and aripiprazole (**2**), two atypical antipsychotic drugs, and bifeprunox (**3**), in phase III clinical trials.

characteristics of the typical antipsychotics.[16,17] Studies with these drugs demonstrated that compounds with combined 5-HT_{1A} agonism and D_2 blockade might present an increased efficacy for all kinds of symptoms as well as an improved side-effect profile.[16,18,19]

Searching for new antipsychotic lead-compounds, our group described the design and synthesis of three *N*-phenylpiperazine derivatives—LASSBio-579 (**4**), LASSBio-580 (**5**) and LASSBio-581 (**6**) (Fig. 1)—which differ by the isosteric replacement between pyrazole and 1,2,3-triazole heterocyclic rings.[20] These compounds were originally proposed to be selective ligands for the D_2 or D_4 receptors. A preliminary pharmacological evaluation carried out by our group demonstrated that **4** and **6** act as agonists at pre-synaptic dopamine D_2-*like* receptors while **5** acts as antagonist at the same receptor.[20] We also showed that compound **4** (30 mg/kg ip) inhibits the induction of stereotyped behavior by amphetamine in rodents, an effect predictive of efficacy for treating the positive symptoms of schizophrenia.[21] Subsequently, Löber et al.[22] demonstrated that compound **5** has a high affinity for the D_4 receptor, presenting a K_i value of 9.9 nM and a selectivity ratio of 86–150 when compared to other D_2-*like* receptors. However, in vivo studies demonstrated that **4** and **6** modify behaviors and pharmacological effects mediated by dopaminergic and serotonergic neurotransmission in rodents, more specifically associated with D_2-*like*, 5-HT_{1A}, and 5-$HT_{2A/C}$ receptors[23] pointing to an interesting pharmacological profile characterized by the involvement of multiple receptors.

In this work, the molecular scaffold of compounds **4**–**6** was explored in the search of new antipsychotic lead-compounds with a multi-receptor profile. The molecular diversity of the compounds was achieved in three different subunits of the basic scaffold: (a) isosteric replacement of the heterocyclic ring at the biaryl motif generating pyrazole, 1,2,3-triazole and 2-methylimidazole[1,2-*a*]pyridine analogues, (b) addition of different substituents at the *para*-biaryl position (W), and (c) substitution at the *para*-phenylpiperazine position (Y) (Table 1). Substituents were selected

Table 1
Structures of the functionalized *N*-phenylpiperazine derivatives **4**–**24** and apparent affinities for D_2-*like*, 5-HT_{1A} and 5-HT_{2A} receptors



Compound	W	Y	Yield[a] (%)	Molecular formula[b]	K_i[c] (μM)			Ratio		
					D_2	5-HT_{1A}	5-HT_{2A}	D_2/5-HT_{1A}	D_2/5-HT_{2A}	5-HT_{1A}/5-HT_{2A}
1[d]	—	—	—	—	0.12	0.38	0.014	0.31	8.57	27.14
2[d]	—	—	—	—	0.001	0.0004	0.007	2.50	0.14	0.057
4	Cl	H	77	$C_{20}H_{21}ClN_4$	0.11	0.09	2.32	1.21	0.05	0.04
5[e]	H	H	72	$C_{19}H_{21}N_5$	0.73	0.48	5.66	1.54	0.13	0.08
6	Cl	H	77	$C_{19}H_{20}ClN_5$	0.95	1.22	10.91	0.78	0.09	0.11
7[e]	H	H	90	$C_{20}H_{22}N_4$	0.11	0.05	1.75	2.31	0.06	0.03
8[e]	H	F	83	$C_{20}H_{21}FN_4$	0.51	0.12	1.04	4.42	0.48	0.12
9[e]	H	Cl	79	$C_{20}H_{21}ClN_4$	3.74	0.38	0.92	9.89	4.04	0.41
10	F	H	79	$C_{20}H_{21}FN_4$	0.07	0.06	0.92	1.07	0.07	0.07
11	F	F	63	$C_{20}H_{20}F_2N_4$	0.48	0.24	0.53	2.05	0.91	0.44
12	Cl	Cl	86	$C_{20}H_{20}Cl_2N_4$	>2.65	0.62	>6.39			
13	Cl	OCH_3	85	$C_{21}H_{23}ClN_4O$	1.73	>4.44	>6.39			
14	H	F	78	$C_{19}H_{20}FN_5$	1.98	1.50	3.32	1.32	0.60	0.45
15	H	Cl	78	$C_{19}H_{20}ClN_5$	>7.94	4.25	2.06			2.07
16	F	H	82	$C_{19}H_{20}FN_5$	0.74	0.89	5.62	0.82	0.13	0.16
17	F	F	72	$C_{19}H_{19}F_2N_5$	>7.94	3.13	4.67			0.67
18	Cl	Cl	76	$C_{19}H_{19}Cl_2N_5$	>2.65	>4.44	3.22			
19	Cl	OH	38	$C_{19}H_{20}ClN_5O$	1.74	7.98	>19.17	0.22		
20	Cl	OCH_3	88	$C_{20}H_{22}ClN_5O$	>7.94	>13.31	>19.17			
21	NO_2	H	76	$C_{19}H_{20}N_6O_2$	3.34	2.78	>19.17	1.20		
22	NHAc	H	—	$C_{21}H_{24}N_6O$	>7.94	>13.31	>19.17			
23		H	48	$C_{19}H_{22}N_4$	>7.94	4.21	11.57			0.36
24[e]		Cl	45	$C_{19}H_{21}ClN_4$	>7.94	>13.31	4.92			

[a] Isolated yield from the reductive amination step described in Scheme 1.
[b] The analytical results for C, H, N were within ±0.4% of calculated values.
[c] For details about the protocol see Section 4.
[d] Controls: clozapine (**1**) and aripiprazole (**2**).
[e] Compounds previously described as high affinity D_4 ligands.[19,23]

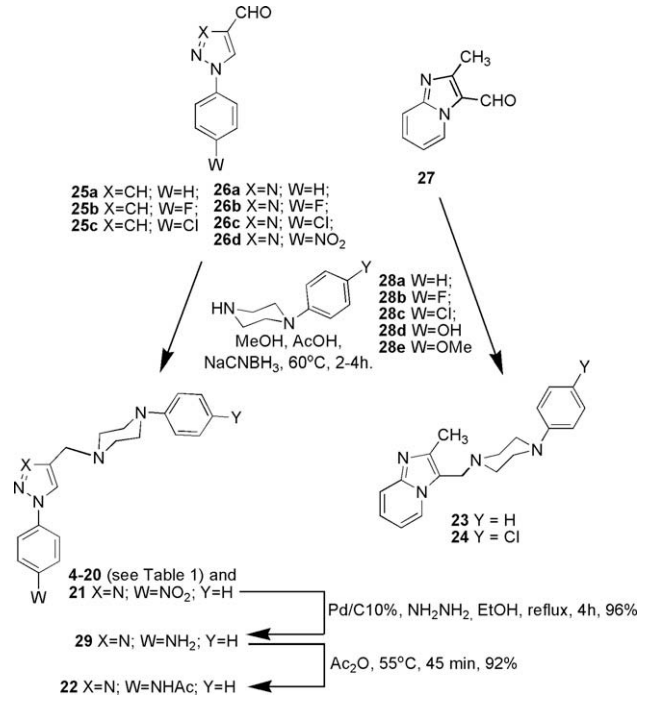
according to their electronic, lipophilic and steric properties. This strategy lead to the synthesis and pharmacological evaluation of 16 compounds: seven pyrazolic derivatives **7**–**13** and nine 1,2,3-triazole derivatives **14**–**22** (Fig. 1), including three compounds (**7**–**9**) already described as selective D_4 receptor ligands.[22] Furthermore, two imidazole[1,2-*a*]pyridine derivatives **23** and **24** were designed through fusion of phenyl and heteroarylazole rings (Fig. 1), conferring a planar rigid structure similar to other previously described dopamine ligands, such as indole,[24] benzimidazole,[25] pyrrolo[2,3-*b*]pyridine,[24] and pyrazolo[1,5-*a*]pyridine[26–29] derivatives. All these compounds, including **24**,[26] were described as selective D_4 receptor ligands, including D_4-selective imaging agents.[29]

2. Results and discussion

2.1. Chemistry

The synthetic route planned to achieve the *N*-phenylpiperazine derivatives **4–21**, **23**, and **24** explored the reductive amination of the corresponding substituted 1-aryl-pyrazole-4-carbaldehydes **25a–c**, 1-aryl-1,2,3-triazole-4-carbaldehydes **26a–d**, and 2-methylimidazole[1,2-*a*]pyridine-3-carbaldehyde (**27**), which were obtained from commercial sources or prepared as described previously.[20,30] The key step was performed through reduction of the imine adducts formed from treatment of the heterocyclic aldehydes **25a–c**, **26a–d**, and **27** with functionalized *N*-phenylpiperazines **28a–e** in dry methanol using sodium cyanoborohydride and catalytic amounts of acetic acid,[31] yielding the desired *N*-phenylpiperazine derivatives **4–21** and **23–24** as shown in Table 1. On the other hand, acetamide derivative **22** was prepared in 88% yield (two steps) from reduction of the nitro compound **21**,[32] followed by acetylation of the corresponding aniline intermediate **29** (Scheme 1).[33]

The structure of all 21 *N*-phenylpiperazine derivatives **4–24** were confirmed by the different analytical and spectroscopic techniques described in Section 4.



Scheme 1. Synthesis of functionalized *N*-phenylpiperazine derivatives **4–24** belonging to pyrazole, 1,2,3-triazole and imidazol [1,2-*a*] pyridine series.

2.2. Pharmacology

Affinity of the compounds for D_2-*like* receptors was determined via standard competition assays using rat striatum homogenate with [^{3}H]-YM-9151-2 (nemonapride) as the radioactive ligand. The affinities for 5-HT_{1A} and 5-HT_{2A} receptors were determined via competition assays using rat hippocampus homogenate with [^{3}H]-8-OH-DPAT and rat cortex homogenate with [^{3}H]-ketanserine, respectively. The results of these assays (Table 1) showed that the *N*-phenylpiperazine derivatives **4–24** have a broad range of affinities for these three receptors (K_i values between 0.07–7.94 μM, 0.05–13.31 μM, and 0.53–19.71 μM for D_2-*like*, 5-HT_{1A} and 5-HT_{2A} receptors, respectively) and that substituents had effect on the binding profile.

Evaluating the isosteric replacement of the heterocyclic ring at the biaryl motif, it is clear that pyrazole derivatives presented higher affinities than their 1,2,3-triazole and 2-methylimidazole[1,2-*a*] pyridine analogues for both dopamine and serotonin receptors (Table 1). The 1,2,3-triazole compounds presented K_i values of 4–17-fold higher for D_2, 9–15-fold higher for 5-HT_{1A} and 2–9-fold higher for 5-HT_{2A} receptors than the corresponding pyrazole analogues. Literature data demonstrated that substitution of the pyrazolic ring for a pyrrole in this same molecular scaffold is also prejudicial to D_2-*like* receptor binding.[22,34] Besides the previously described high affinity of **24** for D_4 receptors (K_i = 13 nM),[26] the 2-methylimidazole[1,2-*a*]-pyridine ring addition strongly reduced the affinity for serotonin receptors and abolished binding to D_2-*like* receptors. Thus, derivatives **23** and **24** failed to comply with the multi-receptor profile intended in this work. The observed differences between the affinity of 1-phenylpyrazole and 1-phenyl-1,2,3-triazole derivatives described herein for D_2-*like*, 5-HT_{1A} and 5-HT_{2A} receptors could be due to distinct conformational behavior of *N*-methylene-*N*-aryl-piperazine side chain resultant from different stereoelectronic effects of adjacent CH,CH versus CH,N groups, respectively, at pyrazole and 1,2,3-triazole rings.

The biarylmethylamine motif where the amine moiety is an arylpiperazine group has been successfully explored for developing dopamine receptor ligands as conformationally restricted analogues of benzamide antipsychotics, such as sulpiride. Derivatives presenting a biphenyl, 1-phenylpyrrole, 2-phenylpyrrole, 2-phenylimidazole, 4-phenylthiazole, and 3-phenylpiridine systems were successfully described as D_4-selective ligands with different intrinsic activity.[22,31,35–44] An important member of this series of compounds is the 2-phenylimidazole derivative NGD-94-1 (**30**) (Fig. 2), considered as a selective D_4 full antagonist (K_i = 3.6 nM) by some authors[44] although others reported that it was able to activate human recombinant dopamine $D_{4.4}$ receptors expressed in HEK293 cells.[45] With regard to 1-phenylpyrazole moiety explored in this work, compound FAUC 2020 (**31**) (Fig. 2) has been reported to be a selective D_4 partial agonist (K_i = 0.59 nM).[22]

Some of the studies cited above carried on with biarylmethylamine derivatives also describe compounds with considerable affinity for the 5-HT_{1A} receptors.[22,35,39,42,44] Both NGD-94-1 (**30**) and FAUC 2020 (**31**) bind with high affinity to this subtype of serotonin receptor (K_i = 180 and 37 nM, respectively).[22,44] Indeed, there is a high molecular similarity between the compounds assayed in present work, NGD-94-1 (**30**), FAUC 2020 (**31**), and bifeprunox (**3**), a mixed D_2 partial agonist (K_i = 2.2 nM) and 5-HT_{1A} agonist

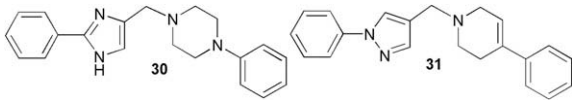


Figure 2. Structure of D_4-selective ligands NGD-94-1 (**30**) and FAUC 2020 (**31**).

(K_i = 9.3 nM)[42] currently in phase III clinical trials for schizophrenia treatment.[15] Differently from aripiprazole (**2**), bifeprunox (**3**) binds to $D_{4.4}$ receptor (K_i = 0.31 nM) acting as a partial agonist at this dopamine receptor.[46] Löber et al.[22] reported an affinity 75–650-fold higher for D_4 receptor than for other D_2-*like* receptors for the 1-phenyl-1,2,3-triazole derivative **5** (K_i $D_{2\text{-long}}$ = 1500 nM; D_3 = 1200 nM; D_4 = 9.9 nM) and for the 1-phenylpyrazole derivatives **7** (K_i $D_{2\text{-long}}$ = 140 nM; D_3 = 140 nM; D_4 = 1.0 nM), **8** (K_i $D_{2\text{-long}}$ = 450 nM; D_3 = 540 nM; D_4 = 2.9 nM) and **9** (K_i $D_{2\text{-long}}$ = 2900 nM; D_3 = 1900 nM; D_4 = 2.9 nM). Based on this evidence, we can propose that at least some of our compounds have a high affinity for the D4 receptor. If this could be confirmed through a direct binding assay for the D4 receptor, such compounds would combine properties presented by clozapine (**1**, high affinity for D4 receptors) and aripiprazole (**2**, high affinity for 5-HT_{1A} receptors) and thus provide a multi-receptor profile different from any antipsychotic drug.

In order to determine the effect of the substituents on binding, the K_i values were carefully analyzed. Considering substitution at *para*-biaryl position (W), neither Cl nor F altered significantly the affinity of the compounds for the three target receptors (e.g., see compounds **4** vs **7** vs **10**, or compounds **5** vs **6** vs **16**, Table 1), whereas acetamide group decreased significantly the affinity for D_2 and 5-HT_{1A} receptors (**5** vs **22**, Table 1), possibly due to a steric hindrance at this position. No significant effect of the substitution at this position could be noticed for 5-HT_{2A} binding.

On the other hand, addition of different substituents at *para*-phenylpiperazine position (Y) produced significant effects on the selectivity profile. A volume restriction for 5-HT_{1A} binding can also be observed at this position with significant reduction of affinity induced by addition of a methoxyl group (**12** vs **4**, **20** vs **6**, Table 1). A similar effect was reported with biphenyl derivatives, where a fluorine atom at the *para* position of the biaryl subunit slightly decreased the affinity for the 5-HT_{1A} receptor.[41] For binding to the D_2-*like* receptors, a decrease in affinity was achieved with the introduction of halogens at Y position (F—**5** vs **14**, **7** vs **8**, **10** vs **11**, **16** vs **17**; Cl—**4** vs **12**, **5** vs **15**, **6** vs **18**, **7** vs **9**; Table 1), an effect also reported by Lober et al.[22] for binding to all D_2-*like* receptors (D_2 short, D_2 long, D_3 and D_4) and by Feenstra et al.[42] with biphenyl derivatives. The presence of bulky groups at Y position (Cl—**4** vs **12**, **5** vs **15**, **6** vs **18**, **7** vs **9**; OCH_3—**4** vs **13**, **6** vs **20**, Table 1) also contributed to decrease the binding to D_2-*like* receptors while a small electron-donor substituent (OH—**6** vs **19**, Table 1) seems to promote discreet effect. 5-HT_{2A} receptor affinity is slightly affected by substituents introduced at the Y position. However, an electronic effect can modulate the compounds selectivity profile since electronegative substituents (F, Cl) improve (**5** vs **14**, **7** vs **8**, **10** vs **11**, **16** vs **17**, **5** vs **15**, **6** vs **18**, **7** vs **9**, Table 1) while electron-donating substituents (OH, OCH_3) impair (**4** vs **13**, **6** vs **19**, **6** vs **20**, Table 1) binding to the 5-HT_{2A} receptor. As a result, bulky electronegative substituents at the *para*-phenylpiperazine position may lead to compounds with higher affinity for 5-HT_{2A} than for 5-HT_{1A} or D_2-*like* receptors, such as compound **15**. This observation is in accordance with the finding that the increase of molecular volume is beneficial for 5-HT_{2A} receptors recognition.[47]

Based on both the magnitude of binding affinity and the selectivity profile of *N*-phenylpiperazine compounds so far studied (Table 1), five pyrazole derivatives were selected for in vivo evaluation. Compounds **4**, **7**, and **10** were chosen taking into account their highest affinity for D_2 and 5-HT_{1A} receptors. They have an equivalent selectivity profile, with similar affinities for 5-HT_{1A} and D_2-*like* and lower affinity for 5-HT_{2A} receptors (5-$HT_{1A} \cong D_2$-*like* > 5-HT_{2A}). Compound **8** was selected due to its approximately 10-fold higher affinity for 5-HT_{1A} receptor than for the other ones, a binding profile that could exhibit an in vivo differential effect regarding cognitive symptoms and extrapyramidal side effects. Finally, compound **11** was also selected as a representative of compounds with similar affinities for the three receptors, fitting with the multi-target approach aimed in this work.

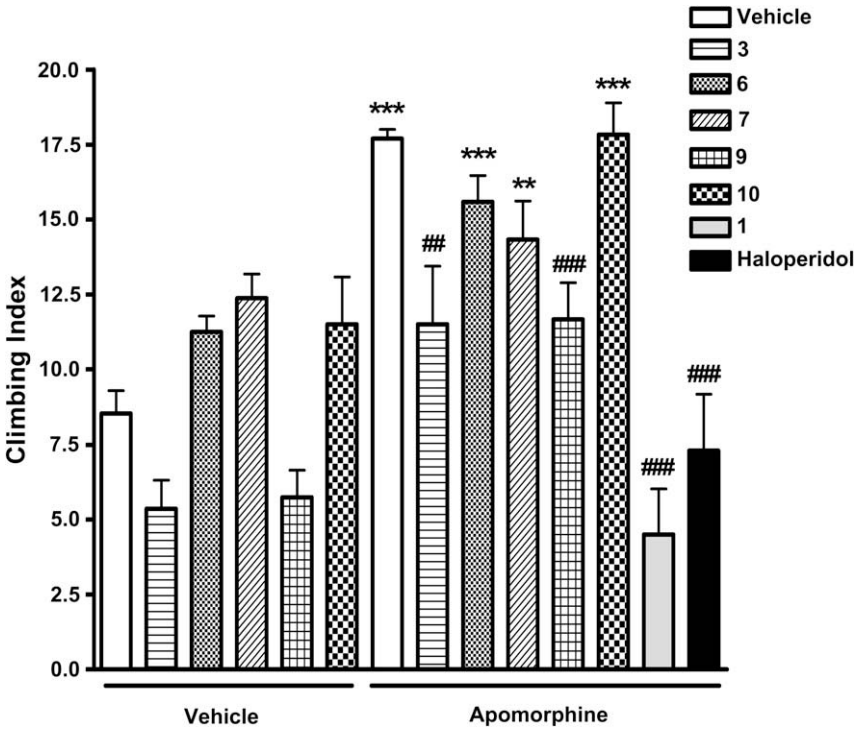


Figure 3. Effects of compounds **4**, **7**, **8**, **10**, and **11** (15 mg/kg po) in the apomorphine-induced climbing test. Clozapine (**1**) (15 mg/kg po) and haloperidol (0.5 mg/kg po) were used as reference drugs. Data are expressed as mean + SEM (n = 08–13). ANOVA ($F_{13,146}$ = 14.199, P <0.001): different from vehicle + vehicle group in post-hoc test $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001; different from vehicle + apomorphine group in post-hoc test $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001.

The animal model chosen for the initial evaluation of selected compounds was the apomorphine-induced climbing in mice. This model is based on the induction of a hyperdopaminergic state by apomorphine and has been classically linked to motor agitation, one of the schizophrenia positive symptoms.[47,48] *N*-Phenylpiperazine derivatives **4** and **10** (15 mg/kg, po) inhibited the apomorphine-induced climbing whereas none of the compounds had significant effect on the climbing index (Fig. 3).

The presence of a halogen atom at the *para*-biaryl position seems to be important in order to achieve an antidopaminergic activity in vivo since compound **7**, which has a binding affinity and selectivity profile similar to **4** and **10**, was not active in this animal model. A similar result was reported by Wijngaaden et al.[36] with 2-phenylpyrrole derivatives where the fluorine addition at the *para*-biaryl position reduced fourfold the potency for antagonizing the apomorphine-induced climbing. The lack of activity of compounds **8** and **11** at the tested dose is possibly due to their low affinity for the D_2-*like* receptor, approximately fivefold lower than for compounds **4** and **10**.

To further investigate their pharmacological profile, compounds **4** and **10** were also evaluated in the catalepsy test in mice, an animal model related to induction of extrapyramidal side effects, and in the rota-rod test in order to evaluate eventual motor impairments. Both derivatives did not induced catalepsy either at the dose active in the apomorphine-induced climbing test or at twice the dose (Table 2). Since 5-HT_{1A} agonists can attenuate the catalepsy induced by haloperidol and other antipsychotics,[49–52] the absence of catalepsy observed with **4** and **10** might be due to 5-HT_{1A} receptor stimulation. On the other hand, the two compounds produced impairment of the motor coordination, since they reduced the longest permanence time of the animals in the rota-rod apparatus (Table 2).

The potential of compound **4** (LASSBio-579) for treating the positive symptoms of schizophrenia had been previously supported by its ability to inhibit amphetamine-induced stereotypy in rats.[21] However, this substance has a limited oral bioavailability (0.6%) as well as low brain penetration (ratio between brain and plasma concentration = 6.3%).[53] In this work, we showed that the fluorine derivative compound **10** (LASSBio-664) also has a suitable pharmacological profile. Thus, further pharmacological evaluation of **10**, including functional in vitro assays, as well as determination of its pharmacokinetic profile are necessary to characterize this structurally-related prototype as an optimized analogue of *N*-phenylpiperazine derivative **4**.

3. Conclusions

In this work we describe the design, synthesis, and pharmacological evaluation of *N*-phenylpiperazine heteroarylazole derivatives as potential multi-target drugs and confirmed the potential usefulness of this molecular scaffold for the development of new second generation antipsychotic drugs.

Among the compounds prepared, **4** (LASSBio-579) and **10** (LASSBio-664) exhibited the highest affinity for binding to the D_2-*like* and 5-HT_{1A} receptors. In mice, these derivatives demonstrated a potential for treating positive symptoms of schizophrenia, once they inhibited apomorphine-induced climbing behavior and were devoid of cataleptogenic effects. Considering that compounds **4** and **10** produce some impairment of motor coordination and that **4** has a poor pharmacokinetic profile, further pharmacological investigation of compound **10** is needed to verify its potential advantages.

4. Experimental section

4.1. Chemistry

Melting points were determined with a Quimis Q340.M13 apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR), unless otherwise stated, was determined in deuterated chloroform containing ca. 1% tetramethylsilane as internal standard in a Bruker DPX-200 spectrometer at 200 MHz. Splitting patterns are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet; br, broad. Carbon magnetic resonance (^{13}C NMR) was determined with Bruker DPX-200 spectrometer at 50 MHz, using deuterated chloroform containing ca. 1% tetramethylsilane. Infrared (IR) spectra were obtained on a Nicolet-55a Magna spectrophotometer by using potassium bromide plates or liquid films. The ultraviolet spectra were obtained on a Hitachi U-2000 Spectrophotometer by using methanol as solvent and as internal standard.

Table 2
Effect of *N*-phenylpiperazine derivatives **4** and **10** in the catalepsy and rota-rod tests (n = 10). Clozapine (**1**) and haloperidol were used as reference drugs

Treatment	Catalepsy (s)[b]			Rota-rod test			
				Permanence time (s)[c]		Number of falls[d]	
	30 min	60 min	90 min	Before	After	Before	After
Vehicle	1.8 ± 1.1	2.7 ± 2.9	2.4 ± 2.6	233.3 ± 21.3	241.4 ± 18.0	1.3 ± 0.5	0.5 ± 0.2
4 (15 mg/kg)	5.6 ± 4.8	23.6 ± 27.5	32.2 ± 45.4	220.8 ± 27.8	149.3 ± 33.2*	1.9 ± 1.0	6.0 ± 1.8
4 (30 mg/kg)	3.9 ± 2.7	13.4 ± 19.2	10.5 ± 9.2				
10 (15 mg/kg)	7.9 ± 6.1	15.1 ± 8.4	25.2 ± 16.1	233.1 ± 22.9	160.6 ± 31.3*	2.4 ± 0.9	8.2 ± 2.6
10 (30 mg/kg)	13.1 ± 10.3	17.2 ± 11.2	36.6 ± 51.4				
1 (15 mg/kg)	23.4 ± 36.5	12.0 ± 8.1	3.5 ± 8.7	240.6 ± 24.9	95.3 ± 30.3***	1.3 ± 0.5	20.3 ± 18.3***
Halo[a] (4 mg/kg)	54.4 ± 68.2*	89.7 ± 79.1***	105.0 ± 75.9***	267.8 ± 16.9	73.5 ± 20.6***	0.7 ± 1.1	15.3 ± 3.8***

[a] Halo = haloperidol.

[b] Data are expressed as mean ± SD. Two-way repeated measure ANOVA (treatment factor: $F_{6,209}$ = 9.190, P <0.001; time factor: $F_{2,209}$ = 7.052, P = 0.001; treatment × time interaction: $F_{12,209}$ = 2.238. P = 0.008): different from vehicle group at the same time of measure in post-hoc test *P <0.05, **P <0.01, ***P <0.001.

[c] Data are expressed as mean ± SD. Two-way repeated measure ANOVA (treatment factor: $F_{4,91}$ = 2.957, P = 0.031; time factor: $F_{1,91}$ = 34.785, P <0.001; treatment × time interaction: $F_{4,91}$ = 4.981, P = 0.002): different from vehicle group at the same time of measure in post-hoc test *P <0.05, **P <0.01, ***P <0.001.

[d] Data are expressed as mean ± SD. Two-way repeated measure ANOVA (treatment factor: $F_{4,91}$ = 4.708, P = 0.003; time factor: $F_{1,91}$ = 27.711, P <0.001; treatment × time interaction: $F_{4,91}$ = 5.091, P = 0.002): different from vehicle group at the same time of measure in post-hoc test *P <0.05, **P <0.01, ***P <0.001.

Microanalysis data were obtained on a Thermofinnigan EA1112 analyzer, using a Metler MX5 electronic balance.

The progress of all reactions was monitored by TLC performed on 2.0 cm × 6.0 cm aluminum sheets precoated with Silica Gel 60 (F-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 and 365 nm. For column chromatography Merck Aluminum Oxide 60 (70–230 mesh) was used. The usual workup means that the organic extracts prior to concentration under reduced pressure had been treated with a saturated aqueous sodium chloride solution, referred to as brine, dried over anhydrous sodium sulfate and filtered.

4.2. General procedure for the preparation of *N*-phenylpiperazine derivatives 4–21 and 23–24

A solution of the corresponding heterocyclic aldehyde **25a–c**, **26a–d**, or **27** (0.7 mmol) and *N*-phenylpiperazine derivative **28a–e** (0.7 mmol) in dry methanol (2.5 mL) was adjusted to pH 6.0 by dropwise addition of concentrated acetic acid. Then, sodium cyanoborohydride (0.25 g, 4 mmol) was added and the resultant mixture stirred at 60 °C for 2–4 h. After removal of the solvent under reduced pressure, the residue was partitioned between dichloromethane and 10% aqueous potassium phosphate. The organic layer was separated and submitted to usual workup to yield a crude precipitate, which was purified by recrystallization in ethanol/water.

4.2.1. 1-[(1-(4-Chlorophenyl)-1*H*-pyrazol-4-yl)methyl]-4-phenylpiperazine (4)

Reductive amination between **25c** and **28a** afforded derivative **4** in 77% yield, as a white solid, mp 122 °C, R_f = 0.38 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3106–3022 (ν C–H), 1598–1497 (ν C=C and C=N), 1093 (ν C–Cl); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.67–2.71 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.21–3.26 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.59 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.83–6.95 (3H, m, H-2″, H-4″ and H-6″), 7.23–7.30 (2H, m, H-3″ and H-5″), 7.42 (2H, d, *J* = 8.9 Hz, H-3′ and H-5′), 7.64 (2H, d, *J* = 8.9 Hz, H-2′ and H-6′), 7.68 (1H, s, H-3), 7.90 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.1 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.3 (C-2″ and C-6″), 119.9 (C-4), 120.2 (C-2′ and C-6′), 126.6 (C-5), 129.3 (C-3′ and C-5′), 129.7 (C-3″ and C-5″), 132.0 (C-4′), 138.8 (C-1′), 142.3 (C-3), 151.4 (C-1″); UV (MeOH) λ_{max}: 255.0 nm. Anal. Calcd for $C_{20}H_{21}ClN_4$: C, 68.08; H, 6.00; N, 15.88. Found: C, 67.89; H, 5.98; N, 15.93.

4.2.2. 1-Phenyl-4-[(1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl]piperazine (5)

Reductive amination between **26a** and **28a** afforded derivative **5** in 72% yield, as a white solid, mp 150 °C, R_f = 0.48 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3083–3035 (ν C–H), 1601–1501 (ν C=C and C=N); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.73–2.78 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.21–3.26 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.84 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.82–6.95 (3H, m, H-2″, H-4″ and H-6″), 7.22–7.30 (2H, m, H-3″ and H-5″), 7.44–7.54 (3H, m, H-3′, H-4′ and H-5′), 7.73–7.77 (2H, m, H-2′ and H-6′), 7.98 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.4 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.3 (C-2″ and C-6″), 120.0 (C-4″), 120.6 (C-2′ and C-6′), 121.1 (C-5), 128.9 (C-4′), 129.3 (C-3′ and C-5′), 130.0 (C-3″ and C-5″), 137.2 (C-1′), 145.1 (C-4), 151.4 (C-1″); UV (MeOH) λ_{max}: 246 nm. Anal. Calcd for $C_{19}H_{21}N_5$: C, 71.45; H, 6.63; N, 21.93. Found: C, 71.56; H, 6.54; N, 21.77.

4.2.3. 1-[(1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl]-4-phenylpiperazine (6)

Reductive amination between **26c** and **28a** afforded derivative **6** in 77% yield, as a white solid, mp 151 °C, R_f = 0.48 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3094–3067 (λ C–H), 1599–1501 (λ C=C and C=N), 1097 (λ C–Cl); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.72–2.77 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.20–3.25 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.83 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.82–6.95 (3H, m, H-2″, H-4″ and H-6″), 7.22–7.30 (2H, m, H-3″ and H-5″), 7.50 (2H, d, *J* = 8.7 Hz, H-3′ and H-5′), 7.71 (2H, d, *J* = 8.7 Hz, H-2′ and H-6′), 7.95 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.4 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.3 (C-2″ and C-6″), 120.0 (C-4″), 121.0 (C-5), 121.8 (C-2′ and C-6′), 129.3 (C-3′ and C-5′), 130.1 (C-3″ and C-5″), 134.6 (C-4′), 135.7 (C-1′), 145.4 (C-4), 151.3 (C-1″); UV (MeOH) λ_{max}: 252 nm. Anal. Calcd for $C_{19}H_{20}ClN_5$: C, 64.49; H, 5.70; N, 19.79. Found: C, 64.61; H, 5.59; N, 19.75.

4.2.4. 1-Phenyl-4-[(1-phenyl-1*H*-pyrazol-4-yl)methyl]piperazine (7)

Reductive amination between **25a** and **28a** afforded derivative **7** in 90% yield, as a beige solid, mp 106 °C (literature 109 °C[22]), R_f = 0.34 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3096–3038 (ν C–H), 1600–1499 (ν C=C and C=N), 808 (ν C–H), 758 (ν C–H); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.64–2.66 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.20–3.23 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.56 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.88 (3H, m, H-2″, H-4″ and H-6″), 7.26 (3H, m, H-3′, H-4′ and H-5′), 7.44 (2H, m, H-3″ and H-5″), 7.67 (1H, s, H-3), 7.68 (2H, m, H-2′ and H-6′), 7.90 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.8 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.1 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.2 (C-2″ and C-6″), 119.1 (C-2′ and C-6′), 119.9 (C-4″), 126.5 (C-4′), 126.7 (C-5), 129.3 (C-3″ and C-5″), 129.6 (C-3′ and C-5′), 140.3 (C-1′), 142.0 (C-3), 151.5 (C-1″); UV (MeOH) λ_{max}: 252.0 nm. Anal. Calcd for $C_{20}H_{22}N_4$: C, 75.44; H, 6.96; N, 17.60. Found: C, 75.29; H, 6.93; N, 17.63.

4.2.5. 1-(4-Fluorophenyl)-4-[(1-phenyl-1*H*-pyrazol-4-yl)methyl]piperazine (8)

Reductive amination between **25a** and **28b** afforded derivative **8** in 83% yield, as a beige solid, mp 111–113 °C, R_f = 0.34 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3097–3050 (ν C–H), 1597–1499 (ν C=C and C=N), 1250–1241 (ν C–F), 814 (ν C–H), 755 (ν C–H); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.64–2.66 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.12–3.14 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.55 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.90 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.28 (1H, m, H-4′), 7.44 (2H, m, H-3 and H-5), 7.69 (2H, m, H-2′ and H-6′), 7.70 (1H, s, H-3), 7.89 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 115.6 (2C, d, *J* = 21.9 Hz, C-3″ and C-5″), 118.0 (2C, d, *J* = 7.5 Hz, C-2″ and C-6″), 119.0 (C-2′ and C-6′), 126.5 (C-4′), 126.6 (C-5), 129.6 (C-3′ and C-5′), 140.3 (C-1′), 142.0 (C-3), 148.1 (1C, d, *J* = 2.2 Hz, C-1″), 157.6 (1C, d, *J* = 237.3 Hz, C-4″); UV (MeOH) λ_{max}: 249.0 nm. Anal. Calcd for $C_{20}H_{21}FN_4$: C, 71.41; H, 6.29; N, 16.65. Found: C, 71.52; H, 6.32; N, 16.72.

4.2.6. 1-(4-Chlorophenyl)-4-[(1-phenyl-1*H*-pyrazol-4-yl)methyl]piperazine (9)

Reductive amination between **25a** and **28c** afforded derivative **9** in 79% yield, as a white solid, mp 125 °C, R_f = 0.36 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3099–3044 (ν C–H), 1641–1499 (ν C=C and C=N), 1117 (ν C–Cl), 808 (ν C–H), 751 (ν C–H); ^1H NMR (200 MHz, $CDCl_3$) δ: 2.62–2.65 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.16–3.18 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.55 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.83 (2H, d, *J* = 9.0 Hz, H-2″ and H-6″), 7.19 (2H, d, *J* = 9.0 Hz, H-3″ and H-5″), 7.28 (1H, m, H-4′), 7.44 (2H, m, H-3′ and H-5′), 7.68 (2H, s, H-2′ and H-6′), 7.70 (1H, s, H-3), 7.89 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.9 (Ar-CH_2N(CH_2–

$CH_2)_2$NPh), 117.4 (C-2″ and C-6″), 119.0 (C-2′ and C-6′), 124.7 (C-4″), 126.5 (C-4′), 126.6 (C-5), 129.1 (C-3″ and C-5″), 129.6 (C-3′ and C-5′), 140.3 (C-1′), 142.0 (C-3), 150.1 (C-1″); UV (MeOH) λ_{max}: 255.5 nm. Anal. Calcd for $C_{20}H_{21}ClN_4$: C, 68.08; H, 6.00; N, 15.88. Found: C, 67.85; H, 6.09; N, 15.75.

4.2.7. 1-[(1-(4-Fluorophenyl)-1*H*-pyrazol-4-yl)methyl)-4-phenylpiperazine (10)

Reductive amination between **25b** and **28a** afforded derivative **10** in 79% yield, as a white solid, mp 106 °C, R_f = 0.31 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3096–3081 (ν C–H), 1599–1513 (ν C=C and C=N), 1236 (ν C–F), 837 (ν C–H), 757 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.64–2.66 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.21–3.23 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.55 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.88 (3H, m, H-2″, H-4″ and H-6″), 7.14 (2H, m, H-3″ and H-5″), 7.26 (2H, m, H-3′ and H-5′), 7.62 (2H, m, H-2′ and H-6′), 7.66 (1H, s, H-3), 7.83 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.1 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.3 (C-2″ and C-6″), 116.4 (2C, d, J = 22.8 Hz, C-3′ and C-5′), 119.9 (C-4″), 120.8 (2C, d, J = 8.2 Hz, C-2′ and C-6′), 126.8 (C-5), 129.3 (C-3″ and C-5″), 136.6 (C-1′), 142.0 (C-3), 151.4 (C-1″), 161.2 (1C, d, J = 244.2 Hz, C-4′); UV (MeOH) λ_{max}: 250.0 nm. Anal. Calcd for $C_{20}H_{20}FN_4$: C, 71.41; H, 6.29; N, 16.65. Found: C, 71.48; H, 6.25; N, 16.62.

4.2.8. 1-(4-Fluorophenyl)-4-{[1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]methyl}piperazine (11)

Reductive amination between **25b** and **28b** afforded derivative **11** in 63% yield, as a white solid, mp 110 °C, R_f = 0.31 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3096–3083 (ν C–H), 1566–1516 (ν C=C and C=N), 1247 (ν C–F); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.66 (4H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.13 (4H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.55 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.94 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.13 (2H, m, H-3′ and H-5′), 7.63 (2H, m, H-2′ and H-6′), 7.66 (1H, s, H-3), 7.83 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.6 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 115.6 (2C, d, J = 21.9 Hz, C-3″ and C-5″), 116.4 (2C, d, J = 22.8 Hz, C-3′ and C-5′), 118.0 (2C, d, J = 7.6 Hz, C-2″ and C-6″), 120.8 (2C, d, J = 8.2 Hz, C-2′ and C-6′), 126.8 (C-5), 136.6 (1C, d, J = 2.8 Hz, C-1′), 142.0 (C-3), 148.1 (1C, d, J = 2.2 Hz, C-1″), 156.8 (1C, d, J = 189.6 Hz, C-4″), 161.7 (1C, d, J = 196.6 Hz, C-4′); UV (MeOH) λ_{max}: 248.0 nm. Anal. Calcd for $C_{20}H_{20}F_2N_4$: C, 67.78; H, 5.69; N, 15.81. Found: C, 67.55; H, 5.72; N, 15.72.

4.2.9. 1-(4-Chlorophenyl)-4-{[1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methyl}piperazine (12)

Reductive amination between **25c** and **28c** afforded derivative **12** in 86% yield, as a white solid, mp 130–132 °C, R_f = 0.37 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3106–3078 (ν C–H), 1596–1499 (ν C=C and C=N), 1096 (ν C–Cl); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.63–2.65 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.16–3.19 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.55 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.83 (2H, d, J = 8.9 Hz, H-2″ and H-6″), 7.20 (2H, d, J = 8.9 Hz, H-3″ and H-5″), 7.41 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.63 (2H, d, J = 8.9 Hz, H-2′ and H-6′), 7.68 (1H, s, H-3), 7.86 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.4 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 117.5 (C-2″ and C-6″), 120.2 (C-2′ and C-6′), 124.8 (C-4″), 126.6 (C-5), 129.2 (C-3″ and C-5″), 129.7 (C-3′ and C-5′), 132.0 (C-4′), 138.9 (C-1′), 142.3 (C-3), 150.1 (C-1″); UV (MeOH) λ_{max}: 258.0 nm. Anal. Calcd for $C_{20}H_{20}Cl_2N_4$: C, 62.02; H, 5.20; N, 14.47. Found: C, 62.14; H, 5.18; N, 14.52.

4.2.10. 1-{[1-(4-Chlorophenyl)-1*H*-pyrazol-4-yl]methyl}-4-(4-methoxyphenyl)piperazine (13)

Reductive amination between **25c** and **28e** afforded derivative **13** in 85% yield, as a white solid, mp 145 °C, R_f = 0.32 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3107–3078 (ν C–H), 1595–1498 (ν C=C and C=N), 1245 (ν C–O–C), 1093 (ν C–Cl), 1036 (ν C–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (4H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.15 (4H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.61 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.76 (3H, s, OCH_3), 6.82 (2H, d, J = 9.3 Hz, H-2″ and H-6″), 6.90 (2H, d, J = 9.3 Hz, H-3″, and H-5″), 7.41 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.63 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.67 (1H, s, H-3), 7.95 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.7 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 52.6 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.1 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 55.7 (OCH_3), 114.6 (C-2″ and C-6″), 118.4 (C-3″ and C-5″), 120.1 (C-2′ and C-6′), 126.7 (C-5), 129.6 (C-3′ and C-5′), 131.9 (C-4′), 138.8 (C-1′), 142.3 (C-3), 145.7 (C-1″), 154.0 (C-4″); UV (MeOH) λ_{max}: 255.0 nm. Anal. Calcd for $C_{21}H_{23}ClN_4O$: C, 65.87; H, 6.05; N, 14.63. Found: C, 65.95; H, 6.02; N, 14.71.

4.2.11. 1-(4-Fluorophenyl)-4-[(1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl]piperazine (14)

Reductive amination between **26a** and **28b** afforded derivative **14** in 78% yield, as a white solid, mp 150 °C, R_f = 0.39 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3082–3050 (ν C–H), 1595–1500 (ν C=C and C=N), 1241 (ν C–F), 813 (ν C–H), 757 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.73–2.76 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.13–3.16 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.83 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.92 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.48 (2H, m, H-3′, H-4′ and H-5′), 7.75 (2H, m, H-2′ and H-6′), 7.98 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.5 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 115.9 (2C, d, J = 22.0 Hz, C-3″ and C-5″), 118.0 (2C, d, J = 7.6 Hz, C-2″ and C-6″), 120.6 (C-2′ and C-6′), 121.0 (C-5), 129.9 (C-3′ and C-5′), 145.3 (C-4), 148.1 (C-1″); UV (MeOH) λ_{max}: 242.5 nm. Anal. Calcd for $C_{19}H_{20}FN_5$: C, 67.64; H, 5.97; N, 20.76. Found: C, 67.49; H, 6.01; N, 20.82.

4.2.12. 1-(4-Chlorophenyl)-4-[(1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl]piperazine (15)

Reductive amination between **26a** and **28c** afforded derivative **15** in 78% yield, as a white solid, mp 150 °C, R_f = 0.35 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3083 (ν C–H), 1598–1498 (ν C=C and C=N), 1044 (ν C–Cl), 811 (ν C–H), 759 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.65–2.67 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.09–3.12 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.75 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.74 (2H, d, J = 8.9 Hz, H-2″ and H-6″), 7.11 (2H, d, J = 8.9 Hz, H-3″ and H-5″), 7.39 (3H, m, H-3′, H-4′ and H-5′), 7.66 (2H, m, H-2′ and H-6′), 7.90 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.4 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 117.4 (C-2″ and C-6″), 120.6 (C-2′ and C-6′), 121.1 (C-5), 124.7 (C-4″), 128.9 (C-4′), 129.1 (C-3″ and C-5″), 129.9 (C-3′ and C-5′), 137.2 (C-1′), 145.0 (C-4), 150.0 (C-1″); UV (MeOH) λ_{max}: 252.0 nm. Anal. Calcd for $C_{19}H_{20}ClN_5$: C, 64.49; H, 5.70; N, 19.79. Found: C, 64.61; H, 5.72; N, 19.77.

4.2.13. 1-{[1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-4-phenylpiperazine (16)

Reductive amination between **26b** and **28a** afforded derivative **16** in 82% yield, as a white solid, mp 143 °C, R_f = 0.48 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3096–3011 (ν C–H), 1600–1503 (ν C=C and C=N), 1257 (ν C–F), 841 (ν C–H), 757 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.73–2.76 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.22–3.24 (4H, m, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 3.83 (2H, s, Ar-CH_2N(CH_2–CH_2)$_2$NPh), 6.88 (3H, m, H-2″, H-4″ and H-6″), 7.24

(2H, m, H-3″ and H-5″), 7.24 (2H, m, H-3′ and H-5′), 7.73 (2H, dd, J = 4.6 Hz and 8.7 Hz, H-2′ and H-6′), 7.93 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.0 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.1 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.3 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 116.5 (C-2″ and C-6″), 116.9 (2C, d, J = 23.0 Hz, C-3′ and C-5′), 120.3 (C-4″), 121.8 (C-5), 122.7 (2C, d, J = 8.6 Hz, C-2′ and C-6′), 129.4 (C-3″ and C-5″), 133.5 (C-1′), 144.4 (C-4), 162.6 (1C, d, J = 247.6 Hz, C-4′); UV (MeOH) λ_{max}: 245.0 nm. Anal. Calcd for $C_{19}H_{20}FN_5$: C, 67.64; H, 5.97; N, 20.76. Found: C, 67.55; H, 5.94; N, 20.71.

4.2.14. 1-(4-Fluorophenyl)-4-{[1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}piperazine (17)

Reductive amination between **26b** and **28b** afforded derivative **17** in 72% yield, as a white solid, mp 148 °C, R_f = 0.39 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3082–3059 (ν C–H), 1604–1518 (ν C=C and C=N), 1247 (ν C–F); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.73–2.75 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.13–3.15 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.82 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.89 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.20 (2H, d, J = 8.6 Hz, H-3′ and H-5′), 7.72 (2H, dd, J = 4.6 Hz and 8.6 Hz, H-2′ and H-6′), 7.92 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.3 (Ar-CH_2N(CH_2–*C*H_2)$_2$NPh), 53.2 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.4 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 115.7 (2C, d, J = 22.0 Hz, C-3″ and C-5″), 116.9 (2C, d, J = 23.1 Hz, C-3′ and C-5′), 118.0 (2C, d, J = 7.6 Hz, C-2″ and C-6″), 121.2 (C-5), 122.6 (2C, d, J = 8.6 Hz, C-2′ and C-6′), 133.6 (C-1′), 145.5 (C-4), 148.1 (C-1″), 157.4 (1C, d, J = 237.4 Hz, C-4″), 162.6 (1C, d, J = 247.5 Hz, C-4′); UV (MeOH) λ_{max}: 241.5 nm. Anal. Calcd for $C_{19}H_{19}F_2N_5$: C, 64.21; H, 5.39; N, 19.71. Found: C, 64.08; H, 5.44; N, 19.80.

4.2.15. 1-(4-Chlorophenyl)-4-{[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}piperazine (18)

Reductive amination between **26c** and **28c** afforded derivative **18** in 76% yield, as a white solid, mp 143 °C, R_f = 0.37 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3101–3085 (ν C–H), 1595–1496 (ν C=C and C=N), 1096 (ν C–Cl); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72–2.75 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.17–3.20 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.82 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.83 (2H, d, J = 8.9 Hz, H-2″ and H-6″), 7.19 (2H, d, J = 8.9 Hz, H-3″ and H-5″), 7.50 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.70 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.96 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.4 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 124.7 (C-4″), 129.1 (C-3″ and C-5″), 130.1 (C-3′ and C-5′), 134.6 (C-4′), 135.8 (C-1′), 145.5 (C-4), 150.0 (C-1″); UV (MeOH) λ_{max}: 253.5 nm. Anal. Calcd for $C_{19}H_{19}Cl_2N_5$: C, 58.77; H, 4.93; N, 18.04. Found: C, 58.55; H, 4.99; N, 18.06.

4.2.16. 1-(4-Hydroxyphenyl)-4-{[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}piperazine (19)

Reductive amination between **26c** and **28d** afforded derivative **19** in 38% yield, as a white solid, mp 228–230 °C, R_f = 0.16 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3530 (ν O-H), 1510–1498 (ν C=C and C=N), 1248–1216 (ν C–O–C), 1110 (ν C–Cl), 1048–1029 (ν C–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.18 (1H, br, O*H*), 2.98 (4H, s, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.10 (4H, s, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.83 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.80 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.50 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.69 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.94 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.9 (Ar-CH_2N(CH_2–*C*H_2)$_2$NPh), 52.2 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.8 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 115.0 (C-2″ and C-6″), 118.8 (C-3″ and C-5″), 121.3 (C-5), 121.7 (C-2′ and C-6′), 133.9 (C-4′), 135.9 (C-1′), 144.8 (C-4), 155.1 (C-4″); UV (MeOH) λ_{max}: 239.5 nm. Anal. Calcd for $C_{19}H_{20}ClN_5O$: C, 61.70; H, 5.45; N, 18.94. Found: C, 61.73; H, 5.47; N, 19.01.

4.2.17. 1-(4-Methoxyphenyl)-4-{[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}piperazine (20)

Reductive amination between **26c** and **28e** afforded derivative **20** in 88% yield, as a white solid, mp 153 °C, R_f = 0.48 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3092–3068 (ν C–H), 1514–1501 (ν C=C and C=N), 1252–1228 (ν C–O–C), 1097 (ν C–Cl), 1036–1022 (ν C–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.76 (4H, s, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.12 (4H, s, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.76 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 3.82 (3H, s, O*CH*$_3$), 6.88 (4H, m, H-2″, H-3″, H-5″ and H-6″), 7.49 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.70 (2H, d, J = 8.0 Hz, H-2′ and H-6′), 7.95 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 50.7 (Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 53.3 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.4 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 55.7 (O*C*H_3), 114.6 (C-2″ and C-6″), 118.3 (C-3″ and C-5″), 120.8 (C-5), 121.7 (C-2′ and C-6′), 130.0 (C-3′ and C-5′), 134.5 (C-4′), 135.7 (C-1′), 145.6 (C-4), 154.0 (C-4″); UV (MeOH) λ_{max}: 244.5 nm. Anal. Calcd for $C_{20}H_{22}ClN_5O$: C, 62.58; H, 5.78; N, 18.24. Found: C, 62.71; H, 5.83; N, 18.19.

4.2.18. 1-{[1-(4-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl}-4-phenylpiperazine (21)

Reductive amination between **26d** and **28a** afforded derivative **21** in 76% yield, as a yellow solid, mp 153 °C, R_f = 0.38 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3085 (ν C–H), 1598–1500 (ν C=C and C=N), 1345 (ν C–NO_2), 855 (ν C–H), 761 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.75–2.77 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.22–3.24 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.86 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.88 (3H, m, H-2″, H-4″ and H-6″), 7.26 (2H, m, H-3″ and H-5″), 7.99 (2H, d, J = 9.0 Hz, H-2′ and H-6′), 8.12 (1H, s, H-5), 8.41 (2H, d, J = 9.0 Hz, H-3′ and H-5′); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.22 (Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 53.2 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 53.3 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 116.3 (C-2″ and C-6″), 120.0 (C-4″), 120.5 (C-2′ and C-6′), 120.9 (C-5), 125.7 (C-3′ and C-5′), 129.3 (C-3″ and C-5″), 141.4 (C-4′), 146.3 (C-4), 147.3 (C-1′), 151.3 (C-1″); UV (MeOH) λ_{max}: 248.5 and 283.0 nm. Anal. Calcd for $C_{19}H_{20}N_6O_2$: C, 62.62; H, 5.53; N, 23.06. Found: C, 62.73; H, 5.48; N, 23.12.

4.2.19. 2-Methyl-3-(4-phenylpiperazinylmethyl)imidazo[1,2-*a*]-pyridine (23)

Reductive amination between **27** and **28a** afforded derivative **23** in 48% yield, as a yellow solid, mp 103–105 °C, R_f = 0.33 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3090–3062 (ν C–H), 1634–1504 (ν C=C and C=N), 754 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.46 (3H, s, *CH*$_3$), 2.59–2.61 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.15–3.17 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.81 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.84 (3H, m, H-2′, H-4′ and H-6′), 6.84 (1H, m, H-6), 7.20 (2H, m, H-3′ and H-5′), 7.20 (1H, m, H-7), 7.52 (1H, d, J = 8.9 Hz, H-8), 8.26 (1H, d, J = 6.6 Hz, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.6 (*CH*$_3$), 49.3 (Ar-CH_2N(CH_2–*C*H_2)$_2$NPh), 51.6 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 53.0 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 111.6 (C-6), 116.0 (C-2), 116.2 (C-2′ and C-6′), 116.6 (C-8), 119.9 (C-4′), 124.1 (C-7), 124.9 (C-5), 129.3 (C-3′ and C-5′), 142.4 (C-3), 144.9 (C-9), 151.4 (C-1′); UV (MeOH) λ_{max}: 228.0 and 246.5 nm. Anal. Calcd for $C_{19}H_{22}N_4$: C, 74.48; H, 7.24; N, 18.29. Found: C, 74.37; H, 7.29; N, 18.22.

4.2.20. 2-Methyl-3-[4-(4-chlorophenyl) piperazinylmethyl]imidazo-[1,2-*a*]-pyridine (24)

Reductive amination between **27** and **28b** afforded derivative **24** in 45% yield, as a yellow solid, mp 122–123 °C, R_f = 0.34 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.46 (3H, s, *CH*$_3$), 2.57–2.60 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.10–3.13 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.80 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 6.78 (1H, m, H-6), 6.81 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.16 (1H, m, H-7), 7.18 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.52 (1H, d,

J = 9.1 Hz, H-8), 8.24 (1H, d, J = 6.8 Hz, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.6 (*C*H_3), 49.3 (Ar-CH_2N(CH_2–*C*H_2)$_2$NPh), 51.5 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 52.8 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 111.6 (C-6), 115.7 (C-2), 116.7 (C-8), 117.4 (C-2′ and C-6′), 124.2 (C-7), 124.7 (C-4′), 124.8 (C-5), 129.1 (C-3′ and C-5′), 142.9 (C-3), 144.9 (C-9), 150.0 (C-1′). Anal. Calcd for $C_{19}H_{21}ClN_4$: C, 66.95; H, 6.21; N, 16.44. Found: C, 67.07; H, 6.26; N, 16.50.

4.2.21. 4-[4-(4-Phenylpiperazinilmetil)-1*H*-1,2,3-triazol-4-yl]aniline (29)

Derivative **21** (0.17 g, 0.47 mmol) was mixed with 95% ethanol (5.0 mL) and a catalytic amount of Pd/C10% under heating and reflux. When the mixture boiled, 80% aq hydrazine hydrate (0.38 mL, 11.5 mmol) was added dropwise. Four hours later, the reaction mixture was filtered in hot using Celite® and washed with CH_2Cl_2. 10% aq $NaHCO_3$ was added to the filtrate and afterwards it was extracted with CH_2Cl_2. The organic layer was separated and submitted to the usual workup. Compound **29** was obtained in 96% yield as a beige solid, mp 157 °C, R_f = 0.33 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3477–3417 (ν N–H), 3213–3148 (ν C–H), 1602–1521 (ν C=C and C=N), 757 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72–2.74 (4H, m, Ar-CH_2N(*CH*$_2$–CH_2)$_2$NPh), 3.21–3.23 (4H, m, Ar-CH_2N(CH_2–*CH*$_2$)$_2$NPh), 3.81 (2H, s, Ar-*CH*$_2$N(CH_2–CH_2)$_2$NPh), 3.89 (2H, s, N*H*$_2$), 6.77 (3H, m, H-2″, H-4″ and H-6″), 6.93 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 7.26 (2H, m, H-3″ and H-5″), 7.47 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 7.83 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 49.3 (Ar-CH_2N(CH_2–*C*H_2)$_2$NPh), 53.2 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.6 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 115.4 (C-3′ and C-5′), 116.2 (C-2″ and C-6″), 119.9 (C-4″), 121.1 (C-5), 122.4 (C-2′ and C-6′), 128.9 (C-1′), 129.3 (C-3″ and C-5″), 144.8 (C-4), 147.2 (C-4′), 151.4 (C-1″). UV (MeOH) λ_{max}: 251.6 and 277.8 nm.

4.2.22. 4-[4-(4-Phenylpiperazinilmetil)-1*H*-1,2,3-triazol-4-yl]acetamide (22)

Compound **29** (0.10 g, 0.30 mmol) was mixed with acetic anhydride (1.5 mL, 13.6 mmol) and the reaction mixture heated to 55 °C for 45 min. After neutralization with 10% aq solution of $NaHCO_3$ (80 mL), the mixture was extracted with CH_2Cl_2. The organic layer was separated and submitted to the usual workup. Compound **22** was obtained in 92% yield as a beige solid, mp 252 °C, R_f = 0.18 (CH_2Cl_2/MeOH 95:5). IR (KBr) cm^{-1}: 3444–3394 (ν N–H), 3311–3123 (ν C–H), 1665 (ν C=O), 825 (ν C–H), 753 (ν C–H); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.22 (3H, s, NHCOC*H*$_3$), 2.72–2.74 (4H, m, Ar-CH_2N(C*H*$_2$-CH_2)$_2$NPh), 3.20–3.22 (4H, m, Ar-CH_2N(CH_2–C*H*$_2$)$_2$NPh), 3.81 (2H, s, Ar-C*H*$_2$N(CH_2–CH_2)$_2$NPh), 6.88 (3H, m, H-2″, H-4″ and H-6″), 7.26 (2H, m, H-3″ and H-5″), 7.65 (2H, d, J = 9.1 Hz, H-2′ and H-6′), 7.72 (2H, d, J = 9.1 Hz, H-3′ and H-5′), 7.94 (1H, s, H-5), 8.04 (1H, s, N*H*COCH$_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 24.7 (NHCO*C*H$_3$), 49.2 (Ar-CH_2N(CH_2–CH_2)$_2$NPh), 53.2 (Ar-CH_2N(*C*H_2–CH_2)$_2$NPh), 53.5 (Ar-*C*H_2N(CH_2–CH_2)$_2$NPh), 120.8 (C-3′ and C-5′), 121.1 (C-5), 121.3 (C-2′ and C-6′), 129.3 (C-3″ and C-5″), 133.1 (C-1′), 138.8 (C-4′), 145.3 (C-4), 151.4 (C-1″), 169.0 (NH*C*OCH$_3$). UV (MeOH) λ_{max}: 257.0 nm. Anal. Calcd for $C_{21}H_{24}N_6O$: C, 67.00; H, 6.43; N, 22.32. Found: C, 67.19; H, 6.38; N, 22.41.

4.3. Pharmacology

4.3.1. In vitro binding assays

This procedure was approved by the Institutional Ethics Committee for Animal Care from the Federal University of Rio de Janeiro.

4.3.1.1. Tissue preparation. Adult male Wistar rats (200–300 g) were killed by decapitation. The brains were immediately removed on ice and hippocampus (for 5-HT_{1A} assay), striatum (for D_2-*like* assay) and cortex (for 5-HT_{2A}) were dissected and stored in liquid nitrogen until use. The structures were homogenized in ice-cold Tris–HCl 50 mM buffer (pH 7.4) containing different salts according to specific procedures for the three receptors. The resulting suspension was ultracentrifuged (48,000g_{av} at 4 °C), the pellet was resuspended and incubated at 37 °C for 10 min for removal of endogenous neurotransmitters. This suspension was cooled on ice and ultracentrifuged (48,000g_{av} at 4 °C) twice. The final pellet was resuspended in buffer yielding a proportion of 1.5 mL/g tissue and stored in liquid nitrogen until use.

4.3.1.2. D_2-*like* receptor. Different concentrations of test compounds were incubated at 37 °C for 60 min with striatal membranes (50 μg protein) and 0.1 nM [^{3}H]-YM-09151-2 ([^{3}H]-nemonapride, 82.5 Ci/mmol, New England Nuclear) in a buffer solution of Tris–HCl 50 mM (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$ and 1 mM EDTA, in a final volume of 500 μL. Nonspecific binding was estimated in the presence of 30 μM (−)sulpiride.

4.3.1.3. 5-HT_{1A} receptor. Different concentrations of test compounds were incubated at 37 °C for 15 min with hippocampal membranes (50 μg protein) and 1 nM [^{3}H]-8-OH-DPAT (170.2 Ci/mmol, New England Nuclear) in a buffer solution of Tris–HCl 50 mM (pH 7.4) containing 1 mM $CaCl_2$, 1 mM $MnCl_2$, 10 μM pargyline, in a final volume of 500 μL. Nonspecific binding was estimated in the presence of 10 μM serotonin. The K_d and B_{max} values obtained in a saturation experiment were 0.80 ± 0.22 nM and 166 ± 16 fmol/mg protein, respectively.

4.3.1.4. 5-HT_{2A} receptor. Different concentrations of test compounds were incubated at 37 °C for 15 min with cortex membranes (150 μg protein) and 1 nM [^{3}H]-ketanserin (67 Ci/mmol, New England Nuclear) in a buffer solution of Tris–HCl 50 mM (pH 7.4) containing 100 nM prazosin, in a final volume of 500 μL. Nonspecific binding was estimated in the presence of 1 μM unlabeled ketanserin. The K_d and B_{max} values obtained in a saturation experiment were 1.77 ± 0.67 nM and 348 ± 51 fmol/mg protein, respectively.

After incubation, samples were rapidly diluted with 3 × 4 mL of cold 5 mM Tris–HCl buffer and immediately filtered under vacuum on glass fiber filters (GMF 3, Filtrak, Germany) previously soaked in 0.5% polyethyleneimine. Filters were then dried and immersed in a scintillation mixture (POPOP (1,4-bis-[2-(5-phenyloxazolyl)]-benzene) 0.1 g/L and POP (2,5-diphenyloxazole) 4.0 g/L in toluene). The radioactivity retained in the filters was counted with a Packard Tri-Carb 1600 TR liquid scintillation analyzer. The assays were conducted to a maximum concentration of 30 μM or 10 μM according to the solubility of the compounds.

4.3.1.5. Statistical analysis. The median inhibitory concentrations (IC_{50}) for binding assays were estimated using a computerized nonlinear regression analysis of the untransformed data (Prism 4.0, GraphPad Software Inc.), assuming a single population of binding sites. The K_i values were calculated using the Cheng and Prusoff equation: $K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$. The K_d values used were obtained from saturation experiments performed in our tissue preparations for [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin (see details above) or from the literature[54] for [^{3}H]-YM-09151-2 (K_d = 0.036 nM).

4.4. Behavioral experiments

4.4.1. Animals

Adult male CF1 mice (25–35 g) from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS-RS) breeding colony were used. Animals were housed in groups of eight individuals in plastic

cages (17 × 28 × 13 cm) with free access to food (Nuvital®) and water. Mice were kept at constant room temperature (22 ± 2 °C) and humidity (60%), under a 12 h light–dark cycle (lights off at 7:00 pm) and were adapted to local conditions for at least 72 h before the experiments. All experimental protocols were approved by CONEP—Brazil (National Commission of Research Ethics, Protocol 2006541) and performed according to guidelines of the National Research Ethics Committee (published by National Heath Council, MS, 1998) and of the Brazilian law,[55] which are in compliance with the International Guiding Principles for Biomedical Research Involving Animals.[56]

4.4.2. Treatments

Compounds **4**, **10**, and haloperidol were suspended in saline with addition of 1% (v/v) polissorbate 80. Apomorphine was dissolved in saline with addition of 0.1% ascorbic acid and clozapine in saline with addition of 0.1% acetic acid 0.1 M. Vehicle groups received 1% (v/v) polissorbate 80 in saline. The drugs were administered by the oral route in a volume of 10 mL/kg body weight or subcutaneously in 5 mL/kg body weight. All doses are expressed as free base.

4.4.3. Apomorphine-induced climbing

Mice were treated with vehicle or one of the test substances and immediately put in cages (29 × 23 × 19 cm) with the floor, walls and top consisting of metal bars (2 mm diameter). Animals were allowed to freely explore the cages for 30 min. After that, they received a second treatment with apomorphine 4 mg/kg sc or vehicle sc. The climbing behavior score was evaluated as described by Park et al.[57]: normal behavior (0 point), increased activity and sniffing (1 point), occasional clinging to sides of cage with forepaws (2 points), intermittent clinging to sides of top of cage with all four paws (3 points) and uninterrupted climbing with all four paws (4 points). Climbing behavior was scored at 5, 10, 15, 20, 25, and 30 min after second treatment. The climbing index is calculated as the sum of all scores obtained by the same animal at each time interval.

4.4.4. Catalepsy test

Mice were gently placed by forepaws on a wood bar elevated 6.5 cm from the floor. The time spent by animals in this position (up to 3 min) was measured 30, 60, and 90 min after treating.

4.4.5. Rota-rod test

The apparatus consisted of a cylinder of 3 cm of diameter rotating at 5 rpm. One day before test the animals were trained once during five minutes. On the test day, mice that were able to stay 90 s balanced on the rotating rod were selected for testing. Mice performance was measured before and 60 min after drug administration. The integrity of motor coordination was assessed on the basis of the longest time of permanence and the number of falls in a 5 min period.

4.4.6. Statistical analysis

Apomorphine-induced climbing, catalepsy and rota-rod test results were analyzed by two-way ANOVA with repeated measures, where treatment was the first factor and time was the second one, followed by Student–Newman–Keuls post-hoc test. The analyses were performed using Sigma Stat 2.03 software (Jandel Scientific Corporation). Differences were considered statistically significant at $P < 0.05$.

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