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Novel imidazoline compounds as partial or full agonists of D_2-like dopamine receptors inspired by I_2-imidazoline binding sites ligand 2-BFI

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ABSTRACT

Based on the well known biological versatility of the imidazoline nucleus, we prepared the novel derivatives **3a**–**k** inspired by 2-BFI scaffold to assess imidazoline molecules as D_2-like dopamine receptor ligands. Conservative chemical modifications of the lead structure, such as the introduction of an hydroxy group in the aromatic ring alone or associated with *N*-benzyl substitution, provided partial (**3f**) or nearly full (**3e** and **3h**) agonists, all endowed with D_2-like potency comparable to that of dopamine.

1. Introduction

Dopamine (DA), a neurotransmitter distributed both centrally and peripherally, plays important neuroendocrine, cognitive, emotional and locomotor functions. These effects are mediated by five distinct receptor subtypes belonging to the superfamily of G-protein-coupled receptors (GPCRs). Based on their ability to activate or inhibit the enzyme adenylate cyclase, DA receptors are divided into two classes, D_1-like (D_1 and D_5) and D_2-like (D_2–D_4).[1] Selective drugs targeting these receptors show broad utility in the treatment of the diseases where the DA system is altered.[2] In particular, the D_2-like receptor family is considered the primary target of antiparkinson and antipsychotic drugs. In addition to L-DOPA, the immediate precursor of DA, D_2-like full agonists proved to be able to reduce bradykinesia, rigidity, and tremor typical of Parkinson's disease (PD).[2] In recent years, attention has been turned to D_2-like partial agonists as potential tools for the treatment of both the positive and negative symptoms of schizophrenia. Indeed, the normalization of dopaminergic activity induced by these agents does not seem to be associated with the extrapyramidal side effects (EPS), generally observed in the therapy with D_2 antagonists.[3]

Several studies of some of us have demonstrated the biological versatility of the imidazoline ring and the crucial role played by the bridge (X) and the aromatic area (Ar) forming the substituent in position 2 (Chart 1).[4] In particular, the peculiar chemical nature of the bridge determined preferential recognition by a specific biological system, whereas that of the aromatic region was responsible for the ligand functional behaviour. Based on these observations, the consequent rational design allowed us to obtain several novel imidazoline molecules, preferentially targeting imidazoline binding sites (I_1- and I_2-IBS) or α_2-adrenoreceptors (α_2-ARs).[4] Although the I_2-IBS identity still remains unknown, these binding proteins are widely distributed both centrally and peripherally and they are involved in psychiatric disorders, opiate withdrawal, PD and Alzheimer's diseases (AD).[5] In addition, brain dialysis revealed that I_2-IBS ligands induced an increase of extracellular monoamine concentration.[6] The imidazoline derivative

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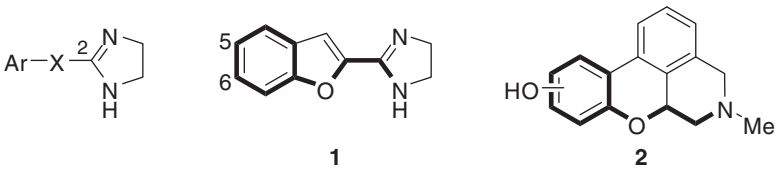


1 2

Chart 1. Common structural features of 2-BFI (**1**) and tetrahydrochromenoisoquinolines (**2**).

2-(2-benzofuranyl)-2-imidazoline (2-BFI, **1**, Chart 1), where Ar and X groups are represented by the benzofurane moiety, is currently considered a ligand of choice for the I_2-IBS study.[7]

Nevertheless, it also proved to be able to bind with some affinity (47 μM) to D_2-like receptors and to display central DA releasing/depleting property.[8] Moreover, our preliminary studies on porcine striatal membranes demonstrated that **1** was lacking of D_1-like affinity (K_i >100 μM). 2-BFI (**1**) shares a molecular framework with the tetrahydrochromenoisoquinolines (**2**, Chart 1), which we discovered to be endowed with D_3 affinity in micromolar range.[9] Based on the above considerations, we prepared the novel derivatives **3a**–**k** inspired by **1** scaffold (Chart 2) with the aim of assessing imidazoline molecules as DA receptor ligands.

The designed chemical modifications were performed both in the aromatic region and to the imidazoline nitrogen without changing the core structure of the lead, also to preserve a possible favourable synergism between I_2-IBS and DA systems. The choice of the alternative or simultaneous insertion of hydroxy groups in position 5 or 6 of the phenyl ring was suggested by the presence of these functions in the aromatic area of DA and numerous known D_2 agonists. Similarly, the presence of *N*-benzyl or *N*-propyl pendant groups in efficacious D_2-ligands,[2] suggested the N-substitution. The D_2-like affinities of compounds **3a**–**k** and the potencies of the most interesting derivatives (**3e**–**3h** and **3k**) and **1** were evaluated. In addition, I_2-IBS and α_2-AR affinities of all the novel derivatives were determined on rat whole brain membranes.

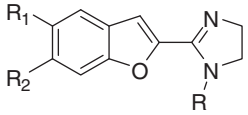
2. Chemistry

The imidazolines **5a** and **5f**–**k** were obtained by reaction of the esters **4a–c**[10] with the proper ethylendiamines (Scheme 1). The imidazolines **5b–e** were obtained by treatment of **5a** with the suitable alkyl iodide. The target compounds **3a**–**k** could be promptly obtained by O-demethylation carried out with a solution of BBr_3 in dichloromethane or with a solution of 48% HBr and acetic acid under reflux temperature.

3. Results and discussion

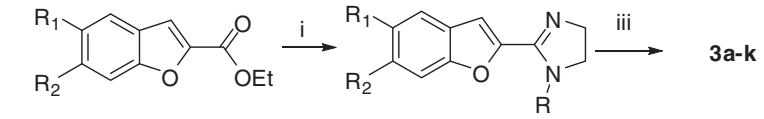
Affinity (K_i, μM), potency (EC_{50}, μM) and intrinsic activity (ia) values, of compounds **3a**–**k** are reported in Table 1 along with those of **1**. From our study it emerged that **1** partially activated the D_2-like receptor family (EC_{50} = 37.7; ia = 0.57), and conservative chemical modifications of its base structure modulated its I_2-IBS and dopaminergic properties. In particular, we observed that the introduction of one hydroxy group in position 6 of the aromatic ring (compound **3f**) enhanced the D_2-like profile of the lead. Indeed, **3f** showed a better affinity value (K_i = 14.45) compared to **1** (K_i = 47.0),[8] behaved as a partial agonist (ia = 0.48), and displayed a potency value (EC_{50} = 5.7) comparable to that of DA (EC_{50} = 4.8, K_i = 3.90). Moreover, **3f** retained a significant I_2-IBS affinity (K_i = 0.076) and high I_2-IBS/α_2-ARs selectivity. In contrast, the 5-hydroxy isomer **3a**, endowed with a similar good I_2-IBS affinity (K_i = 0.088) and I_2-IBS/α_2-ARs selectivity, failed to target the D_2-like receptors. The peculiar structure of the imidazoline nucleus allows the supposed dopaminergic pharmacophoric functions (basic amino and phenolic moieties) of both 5- and 6-hydroxy derivatives **3a** and **3f** be located at similar distances (7.96 Å and 7.82 Å, respectively).[11] Therefore, since only the 6-hydroxy derivative **3f** displayed significant D_2-like affinity, it appeared that the OH function triggered a productive hydrogen bond only if its spatial relationship with the oxygen bridge was comparable to that of the *meta* hydroxy function of DA.

As expected, the good I_2-IBS properties of **3a** and **3f** agreed with previous results obtained with ligands structurally related to **1**.[12] A negative influence of the 5,6-dihydroxy substitution was observed in the catechol derivatives **3i**, which reduced the I_2-IBS affinity and failed to interact with D_2-like receptors. A sharp, but profitable modulation of the biological profile of the lead was instead obtained with the N-substitution. Interestingly, the observed general decrease of the I_2-IBS affinity was associated, in some cases, with a significant D_2-like potency enhancement. In particular, the *N*-benzyl derivatives **3e** and **3h** behaved as a nearly D_2-like full agonists (for **3e**, ia = 0.85; for **3h**, ia = 0.82), with affinity (for **3e**, K_i = 5.0; for **3h**, K_i = 5.66), and potency values (for **3e**, EC_{50} = 6.3; for **3h**, EC_{50} = 5.8) comparable to those of DA. The presence of the *N*-benzyl substituent proved to be so beneficial that it was able to counterbalance the aforementioned negative effect of both hydroxy group in position 5 (compare **3e** vs **3a**) and catechol moiety (compare **3k** vs **3i**). This result might be conferred to a favourable hydrophobic interactions of the highly lipophilic *N*-benzyl group with the cluster of aromatic residues located in the sixth transmembrane helix, namely Phe6.44, Trp6.48, Phe6.51 and Phe6.52 according to the numbering proposed by Ballesteros and Weinstein.[13] Such a cluster, is involved in the ligand binding as



3a	R=H	R_1=OH	R_2=H	**3g**	R=*n*-C_3H_7	R_1=H	R_2=OH
3b	R=CH_3	R_1=OH	R_2=H	**3h**	R=CH_2Ph	R_1=H	R_2=OH
3c	R=C_2H_5	R_1=OH	R_2=H	**3i**	R=H	R_1=OH	R_2=OH
3d	R=*n*-C_3H_7	R_1=OH	R_2=H	**3j**	R=*n*-C_3H_7	R_1=OH	R_2=OH
3e	R=CH_2Ph	R_1=OH	R_2=H	**3k**	R=CH_2Ph	R_1=OH	R_2=OH
3f	R=H	R_1=H	R_2=OH				

Chart 2. Novel imidazoline molecules **3a**–**k** inspired by 2-BFI (**1**).



4a $R_1 = OCH_3$ $R_2 = H$
4b $R_1 = H$ $R_2 = OCH_3$
4c $R_1 = OCH_3$ $R_2 = OCH_3$

5a	R = H	$R_1 = OCH_3$	$R_2 = H$
5b[ii]	R = CH_3	$R_1 = OCH_3$	$R_2 = H$
5c[ii]	R = C_2H_5	$R_1 = OCH_3$	$R_2 = H$
5d[ii]	R = n-C_3H_7	$R_1 = OCH_3$	$R_2 = H$
5e[ii]	R = CH_2Ph	$R_1 = OCH_3$	$R_2 = H$
5f	R = H	$R_1 = H$	$R_2 = OCH_3$
5g	R = n-C_3H_7	$R_1 = H$	$R_2 = OCH_3$
5h	R = CH_2Ph	$R_1 = H$	$R_2 = OCH_3$
5i	R = H	$R_1 = OCH_3$	$R_2 = OCH_3$
5j	R = n-C_3H_7	$R_1 = OCH_3$	$R_2 = OCH_3$
5k	R = CH_2Ph	$R_1 = OCH_3$	$R_2 = OCH_3$

Scheme 1. Reagents: (i) $RNHCH_2CH_2NH_2$; Me_3Al; (ii) from **5a**: NaH, R-X; (iii) 48% HBr/CH_3COOH or BBr_3.

Table 1
Binding affinity, efficacy and intrinsic activity of compounds **3a–k**[a]

Compd	D_2-like receptors[b]			I_2-IBS[c]	α_2-ARs[c]
	K_i (μM)	EC_{50} (μM)	ia	K_i (μM)	K_i (μM)
1 (2-BFI)	47[d]	37.7 ± 5.1	0.57	0.0034 ± 0.0012	3.98 ± 0.60
3a	>100	nt	nt	0.0881 ± 0.005	2.95 ± 0.53
3b	>100	nt	nt	nt	nt
3c	>100	nt	nt	>100	25.5 ± 5.82
3d	15.81 ± 1.01	nt	nt	0.70 ± 0.20	3.64 ± 0.50
3e	5.0 ± 0.23	6.3 ± 0.8	0.85	1.10 ± 0.31	4.39 ± 0.51
3f	14.45 ± 1.10	5.7 ± 0.7	0.48	0.076 ± 0.02	11.0 ± 2.31
3g	13.84 ± 1.30	13.3 ± 1.6	0.64	2.10 ± 0.51	1.92 ± 0.32
3h	5.66 ± 1.00	5.8 ± 0.8	0.82	5.78 ± 0.48	2.08 ± 0.20
3i	>100	nt	nt	5.69 ± 0.52	24.21 ± 6.71
3j	>100	nt	nt	28.02 ± 2.33	10.16 ± 3.92
3k	18.49 ± 1.3	11.9 ± 1.2	0.52	10.39 ± 1.80	4.43 ± 2.80
DA	3.90 ± 0.59	4.8 ± 0.6	1.00	nt	nt

[a] The values are means ± SEM of at least three experiments; nt = not tested.
[b] Inhibition of [^{3}H] YM-09-151-2 (D_2-like) binding to porcine striatal membranes. EC_{50} and ia were measured by the [^{35}S]GTPγS binding experiments in striatal membranes.
[c] I_2-IBS and α_2-ARs binding were determined on rat whole brain membranes.
[d] Ref.[8].

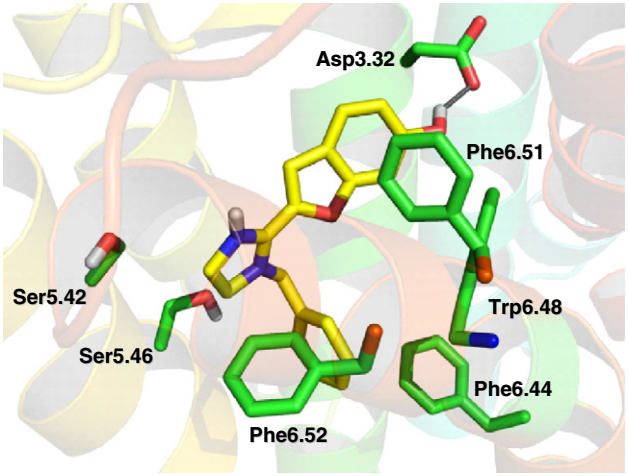


Figure 1. Docking of **3h** into the D_2 receptor binding. Side chains of Asp3.32, Ser5.42, Ser5.46, Phe6.44, Trp6.48, Phe6.51 and Phe6.52 are displayed to help interpretation of the proposed pose that was scored with a favourable free energy of binding of −3.93 kcal/mol.

well as in the structural rearrangements that participate in receptor activation.[14] It is also reported to be highly conserved among the whole family A of GPCRs.[15] However, taking also into account that the catechol derivatives **3i** and **3j** lack of D_2-like affinity, the possibility that the imidazoline molecules bind the receptor in an alternative mode compared to that of catecholamines can not be ruled out. On the other hand, Waugh et al. already proposed that imidazolines bind differently in the agonist pocket than phenethylamine agonists.[16,17]

To investigate this hypothesis, in the absence of any experimental structural data related to the biological target considered in this study, a theoretical model of the human D_2 receptor, based on the rhodopsine X-ray structure was considered.[18] On the basis of the obtained biological experimental data, we considered determinant for the binding to the receptor the hydrophobic interaction of the pendant benzyl group with the aforementioned cluster of aromatic residues. In our simulation (Fig. 1), a plausible correspondence of critical fragments of the ligand with the aminoacids crucial for D_2-like activity, can be achieved when **3h** interacts with the binding pocket in an almost flipped out way compared to that of the natural ligand DA. Such an unexpected observation, might be possible only if the key interaction, such as the ionic bridge towards Asp3.32 of TM3,[19] characterizing the docking of DA, might be played by a charge reinforced hydrogen bond given by the 6-hydroxy group of **3h** with the same aminoacid. Beside this polar interaction, the benzofuran ring might make hydrophobic interaction with Trp6.48 and Phe6.51, while the aromatic tail of *N*-benzyl group is packed against the benzene rings of Phe6.44 and Phe6.52, perhaps stabilizing, at the same time, the positive charge of the imidazoline ring through a charge transfer complex. Moreover, the docking of **5h**, the **3h** intermediate bearing a methoxy group in 6 position, did not produce any valuable pose in the receptor binding site. The proposed binding mode, might better rationalize the increased intrinsic activity observed for **3h** compared to **3f**. Deeper investigations are under progress to verify such a hypothesis.

Anyway, the degree of receptor activation, favoured by the N-substitution was affected by the feature of the pendant group (i.e., lipophilicity) and, sometimes, also by the structural characteristics of the parent compound. The 6-hydroxy-*N*-propyl derivative **3g**, for example, displayed affinity, potency and intrinsic activity values (K_i = 13.84; EC_{50} = 13.3, ia = 0.64) slightly lower than those of the corresponding *N*-benzyl derivative **3h**. Comparable D_2-like affinity was observed for the 5-hydroxy derivative **3d** (K_i = 15.81), whereas no D_2-like binding was shown by the 5,6-diydroxy-*N*-propyl (**3j**), 5-hydroxy-*N*-methyl (**3b**) and *N*-ethyl (**3c**) derivatives. Generally, the new compounds displayed α_2-AR affinity similar to that of the lead.

4. Conclusion

In conclusion, the present study: (i) demonstrated the validity of our design, directed to obtain novel imidazoline molecules targeting D_2-like receptors; (ii) confirmed the biological versatility of the imidazoline nucleus and the role of its substituent in position 2; (iii) highlighted that the N-substitution induced a decrease of I_2-IBS affinity, but might positively modulate the D_2-like activity; (iv) identified the D_2-like nearly full agonists **3h** and **3e**, and the partial agonist **3f**, all endowed with a potency value comparable to that of DA. **3f**, in addition, displayed a significant I_2-IBS affinity. To evaluate in vivo the behaviour of our compounds, the agonist **3h** was, preliminarily, assessed on open field test in rats. It is known that the performance in open field task may provide a good measure of the stimulant effects induced by DA agonists and L-DOPA.[20] We observed that intracerebroventricular injection with equimolar doses (0.0149 μM/kg body weight) of **3h** or L-DOPA induced a similar trend with increased locomotion and rearing in the exploratory behaviour (Table 2). The overall results justify the interest for this novel class of compounds and encourages the continuation of our study.

5. Experimental section

5.1. Chemistry

5.1.1. General procedures

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin–Elmer 297 and Varian EM-390 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Mass spectra were recorded on a HPLC-DAD-RID-MS (ion trap) 1100—LC MSD trap sl Agilent Technologies. IR spectra data, not shown because of the lack of unusual features, were obtained for all compounds reported and are consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory of the Department of Chemical Sciences. The elemental composition of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. The term 'dried' refers to the use of anhydrous sodium sulphate. Compounds were named following the IUPAC rules proposed by CSC ChemDraw (version 9.0).

5.1.2. 2-(4,5-Dihydro-1*H*-imidazol-2-yl)benzofuran-5-ol oxalate (3a)

The imidazoline **5a** (1.85 mmol) was dissolved in a mixture of acetic acid (3 mL) and 48% HBr solution (3 mL) and refluxed overnight. The solvent was removed and the solid residue was dissolved in a small amount of $NaHCO_3$ solution and extracted with ethyl acetate. The evaporation of the dried organic fraction gave a residue that was dissolved in a solution of oxalic acid in ethanol, stirred for 5 min then evaporated to dryness. The residue was recrystallized to give the product as oxalate salt (Table 3).

Similarly **3b–e** were prepared (Table 3). **3f** was characterized as hydrobromide after the first solvent evaporation.

5.1.3. 2-(1-Propyl-4,5-dihydro-1*H*-imidazol-2-yl)benzofuran-6-ol hydrobromide (3g)

To a solution of **5g** (1.16 mmol) in CH_2Cl_2 (7 mL) at −78 °C, a 2 M solution of BBr_3 (10.45 mmol) in CH_2Cl_2 was added dropwise. The mixture was left to warm to rt and stirred overnight. It was cooled again to −78 °C, and MeOH (3 mL) was carefully added. After stirring at rt for 15 min, the solvent was removed and the residue was recrystallized (Table 3).

Similarly **3h–k** were prepared. (Table 3).

5.1.4. 2-(5-Methoxybenzofuran-2-yl)-4,5-dihydro-1*H*-imidazole (5a)

Ethylendiamine (7.27 mmol) was added to a 2 M solution of Me_3Al (7.27 mmol) in toluene at a temperature below 10 °C (ice bath). When the gas formation was finished, ethyl 5-methoxybenzofuran-2-carboxylate (**4a**)[10] (4.54 mmol) was added portionwise at rt. The solution was refluxed for 3 h; after cooling, water (2 mL) was added and the mixture diluted with a 50% solution of CH_2Cl_2 in MeOH (15 mL), then refluxed for 15 min. After filtration and evaporation to dryness, ethyl acetate (25 mL) was added and the solution refluxed for 15 min. Evaporation of the dried mixture gave a residue that was recrystallized (Table 3).

Similarly **5f–k** were prepared (Table 3).

5.1.5. 2-(5-Methoxybenzofuran-2-yl)-1-methyl-4,5-dihydro-1*H*-imidazole (5b)

To a solution of **5a** (2.59 mmol) in dry THF (20 mL) a 60% suspension of NaH in mineral oil was added portionwise. The mixture was refluxed until the gas bubbling ceased, then cooled to rt. Methyl iodide (2.59 mmol) was added and the solution stirred at rt for 12 h, then poured onto ice and extracted with ethyl acetate. Evaporation of the dried solvent gave the crude product that was used in the next step without further purification (Table 3).

Similarly **5c–e** were prepared (Table 3).

5.2. Biological experiments

5.2.1. General procedures to estimate K_i values

The tested compounds were dissolved in DMSO. The level of DMSO did not exceed 1% and was maintained constant in all tubes. At least six different concentrations of each compound were used. The IC_{50} values, computer-generated using a nonlinear regression formula on the computer program GraphPad Prism, version 4.0 for Windows (San Diego, California, USA), were converted to K_i values according to the equation of Cheng and Prusoff.[21] Data represent the mean ± SEM of 3–5 separate experiments performed in triplicate. Protein concentration was assayed by the method of Bradford.[22]

5.2.1.1. I_2-IBS and $_2$-ARs binding assays. Rat whole brain membranes were prepared as previously described.[23] Briefly, brains were homogenized in 10 volumes (w/v) of Tris–sucrose buffer (50 mM Tris–HCl, 320 mM sucrose and 1 mM $MgCl_2$, pH 7.4) using a motor driven Teflon glass homogeniser. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the resulting supernatant was recentrifuged at 32,000*g* for 20 min at 4 °C. The resulting pellet was washed twice by resuspension in 10 volumes of assay buffer (50 mM Tris–HCl and 1 mM $MgCl_2$, pH 7.4) and centrifugation at 32,000*g* for 20 minutes at 4 °C. The membrane pellets were stored at −70 °C until use. Prior to binding studies, pellets were thawed and washed twice more by centrifugation as described

Table 2
Effects of icv injection of vehicle (control group), L-Dopa and **3h** on ambulatory and rearing episodes in rats

Groups	% Ambulatory episodes	% Rearing episodes
Control	3.2 ± 0.4	0.5 ± 0.3
L-Dopa	5.7 ± 0.9*	2.0 ± 0.3*
3h	6.3 ± 1.2*	2.4 ± 0.7*

Data are expressed as mean ± SE of 8 rats per group.
* P <0.05 versus control.

Table 3
Physicochemical characteristics of compounds **3a–k** and **5a–k**

Compd	Yield (%)	Anal. C, H, N mp (°C)	ESIMS *m/z* (MH^+)	NMR, $\delta^{\#}$ (DMSO-d_6), $\delta^{\&}$ ($CDCl_3$)
3a[a]	70	$C_{11}H_{10}N_2O_2 \cdot H_2C_2O_4 \cdot H_2O$ >300	203	[^{1}H]NMR $\delta^{\#}$ 10.81 (br s, 2H, NH_2^+); 9.65 (br s, 1H, OH); 7.88 (s, 1H, ArH); 7.55 (d, *J* = 8.96 Hz, 1H, ArH); 7.08–7.02 (m, 2H, ArH); 3.98 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 164.2 (2 × C=O); 155.6 (C imid); 154.6 (C, Ar); 149.7 (C, Ar); 139.4 (C, Ar), 127.2 (C, Ar); 118.8 (CH, Ar); 115.6 (CH, Ar); 112.4 (CH, Ar); 106.8 (CH, Ar); 44.1 (2 × CH_2)
3b[a]	68	$C_{12}H_{12}N_2O_2 \cdot H_2C_2O_4$ >300	217	[^{1}H]NMR $\delta^{\#}$ 10.46 (br s, 1H, NH^+); 9.70 (br s, 1H, OH); 8.00 (s, 1H, ArH); 7.60 (d, *J* = 8.89 Hz, 1H, ArH); 7.15–7.04 (m, 2H, ArH); 4.10–3.86 (m, 4H, $2CH_2$); 3.40 (s, 3H, CH_3). [^{13}C]NMR $\delta^{\#}$ 162.3 (2 × C=O); 155.0 (C imid); 154.6 (C, Ar); 150.2 (C, Ar); 138.8 (C, Ar), 127.6 (C, Ar); 119.7 (CH, Ar); 117.4 (CH, Ar); 113.3 (CH, Ar); 107.1 (CH, Ar); 50.7 (CH_2); 42.7 (CH_2); 42.2 (CH_3)
3c[a]	72	$C_{13}H_{14}N_2O_2 \cdot H_2C_2O_4$ >300	231	[^{1}H]NMR $\delta^{\#}$ 10.52 (br s, 1H, NH^+); 9.55 (br s, 1H, OH); 7.73 (s, 1H, ArH); 7.48 (d, *J* = 8.96 Hz, 1H, ArH); 7.11–7.02 (m, 2H, ArH); 3.88 (s, 4H, $2CH_2$); 3.69 (q, 2H, CH_2); 1.23 (t, *J* = 7.10 Hz, 3H, CH_3). [^{13}C]NMR $\delta^{\#}$ 162.3 (2 × C=O); 155.0 (C imid); 154.5 (C, Ar); 149.9 (C, Ar); 138.7 (C, Ar), 127.4 (C, Ar); 119.3 (CH, Ar); 117.2 (CH, Ar); 112.9 (CH, Ar); 106.9 (CH, Ar); 50.6 (CH_2); 43.0 (CH_2); 42.7 (CH_2); 12.3 (CH_3)
3d[a]	58	$C_{14}H_{16}N_2O_2 \cdot H_2C_2O_4 \cdot 4H_2O$ 235–238	245	[^{1}H]NMR $\delta^{\#}$ 10.58 (br s, 1H, NH^+); 9.45 (br s, 1H, OH); 7.96 (s, 1H, ArH); 7.57 (d, *J* = 9.00 Hz, 1H, ArH); 7.16–7.05 (m, 2H, ArH); 4.05–3.71 (m, 6H, $3CH_2$); 1.78–1.67 (m, 2H, CH_2); 0.95 (t, *J* = 7.46 Hz, 3H, CH_3). [^{13}C]NMR $\delta^{\#}$ 162.3 (2 × C=O); 154.6 (C imid); 154.5 (C, Ar); 149.5 (C, Ar); 138.4 (C, Ar), 127.0 (C, Ar); 118.9 (CH, Ar); 116.8 (CH, Ar); 112.5 (CH, Ar); 106.5 (CH, Ar); 50.8 (CH_2); 48.7 (CH_2); 42.7 (CH_2); 20.3 (CH_2); 10.8 (CH_3)
3e[a]	62	$C_{18}H_{16}N_2O_2 \cdot H_2C_2O_4 \cdot 2H_2O$ 260–263	293	[^{1}H]NMR $\delta^{\#}$ 10.82 (br s, 1H, NH^+); 9.61 (br s, 1H, OH); 7.96 (s, 1H, ArH); 7.60 (d, *J* = 8.94 Hz, 1H, ArH); 7.46–7.40 (m, 5H, ArH); 7.16–7.05 (m, 2H, ArH); 5.08 (s, 2H, CH_2); 3.99 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 162.3 (2 × C=O); 154.6 (2 × C, Ar); 149.5 (C, Ar); 138.4 (C, Ar), 134.3 (C, Ar); 128.9 (2 × CH, Ar); 128.2 (CH, Ar); 127.7 (2 × CH, Ar); 126.8 (C, Ar); 119.1 (CH, Ar); 117.2 (CH, Ar); 112.5 (CH, Ar); 106.5 (CH, Ar); 50.9 (CH_2); 50.7 (CH_2); 42.7 (CH_2)
3f[b]	76	$C_{11}H_{10}N_2O_2 \cdot HBr$ >300	202	[^{1}H]NMR $\delta^{\#}$ 10.61 (br s, 2H, NH_2^+); 10.35 (s, 1H, OH); 7.97 (s, 1H, ArH); 7.72 (d, *J* = 8.65 Hz,1H, ArH); 7.01–6.92 (m, 2H, ArH); 3.97 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 159.7 (C imid); 157.1 (C, Ar); 155.35 (C, Ar); 137.2 (C, Ar), 124.3 (CH, Ar); 118.4 (CH, Ar); 116.7 (C, Ar); 1115.1 (CH, Ar); 97.2 (CH, Ar); 44.4 (2 × CH_2)
3g[b]	53	$C_{14}H_{16}N_2O_2 \cdot HBr$ 273–275	245	[^{1}H]NMR $\delta^{\#}$ 10.36 (br s, 2H, NH^+ and OH); 8.04 (s, 1H, ArH); 7.72 (d, *J* = 8.60 Hz, 1H, ArH); 7.04–6.93 (m, 2H, ArH); 4.10–3.71 (m, 6H, $3CH_2$); 1.75–1.71 (m, 2H, CH_2); 0.95 (t, J = 7.41 Hz, 3H, CH_3). [^{13}C]NMR $\delta^{\#}$ 159.8 (C imid); 156.8 (C, Ar); 154.3 (C, Ar); 136.44 (C, Ar), 124.2 (C, Ar); 118.3 (CH, Ar); 117.9 (CH, Ar); 115.3 (CH, Ar); 97.3 (CH, Ar); 50.7 (CH_2); 48.7 (CH_2); 42.5 (CH_2); 20.3 (CH_2); 10.8 (CH_3)
3h[b]	69	$C_{18}H_{16}N_2O_2 \cdot HBr \cdot H_2O$ >300	293	[^{1}H]NMR $\delta^{\#}$ 10.40 (br s, 2H, NH^+ and OH); 8.02 (s, 1H, ArH); 7.70 (d, *J* = 8.66 Hz, 1H, ArH); 7.44–7.35 (m, 5H, ArH); 6.97–6.92 (m, 2H, ArH); 5.09 (s, 2H, CH_2); 3.97 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 159.9 (C imid); 156.9 (C, Ar); 154.5 (C, Ar); 136.4 (C, Ar), 134.4 (C, Ar); 128.9 (2 × CH, Ar); 128.1 (CH, Ar); 127.6 (2 × CH, Ar); 124.2 (C, Ar); 118.2 (C, Ar); 118.3 (CH, Ar); 115.3 (CH, Ar); 97.3 (CH, Ar); 50.9 (CH_2); 50.7 (CH_2); 42.7 (CH_3)
3i[b]	62	$C_{11}H_{10}N_2O_3 \cdot HBr \cdot H_2O$ >300	219	[^{1}H]NMR $\delta^{\#}$ 10.49 (br s, 2H, NH_2^+); 10.00 (br s, 1H, OH); 9.48 (br s, 1H, OH); 7.84 (m, 1H, ArH); 7.13–7.02 (m, 2H, ArH); 3.96 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 155.3 (C, Ar); 150.9 (C, Imid); 149.6 (C, Ar); 144.76 (C, Ar); 136.8 (C, Ar), 117.9 (C, Ar); 116.5 (CH, Ar); 106.2 (CH, Ar); 97.4 (CH, Ar); 44.31 (2 × CH_2)
3j[b]	42	$C_{14}H_{16}N_2O_3 \cdot HBr \cdot H_2O$ >300	261	[^{1}H]NMR $\delta^{\#}$ 10.24 (s, 1H, NH^+); 10.02 (br s, 1H, OH); 9.56 (br s, 1H, OH); 7.94 (s, 1H, ArH); 7.14 and 7.06 (2s, 2H, ArH); 4.07–3.89 (m, 4H, $2CH_2$); 3.73 (t, *J* = 7.45, 2H, CH_2); 1.74–1.70 (m, 2H, CH_2); 0.95 (t, *J* = 7.47 Hz, 3H, CH_3). [^{13}C]NMR $\delta^{\#}$ 154.1 (C, Ar); 150.6 (C imid); 149.7 (C, Ar); 144.9 (C, Ar), 135.9 (C, Ar); 117.8 (C, Ar); 117.7 (CH, Ar); 105.9 (CH, Ar); 97.4 (CH, Ar); 50.7 (CH_2); 48.6 (CH_2); 42.4 (CH_2); 20.3 (CH_2); 10.8 (CH_3)
3k[b]	38	$C_{18}H_{16}N_2O_3 \cdot HBr$ 258–260	308	[^{1}H]NMR $\delta^{\#}$ 10.46 (s, 1H, NH^+); 10.02 (br s, 1H, OH); 9.06 (br s, 1H, OH); 7.90 (s, 1H, ArH); 7.43–7.39 (m, 5H, ArH); 7.09–6.95 (m, 2H, ArH); 5.06 (s, 2H, CH_2); 3.95 (s, 4H, $2CH_2$). [^{13}C]NMR $\delta^{\#}$ 154.4 (C, Ar); 150.8 (C imid); 149.9 (C, Ar); 144.9 (C, Ar); 135.9 (C, Ar), 134.5 (C, Ar); 128.9 (2 × CH, Ar); 128.1 (CH, Ar); 127.6 (2 × CH, Ar); 118.1 (C, Ar); 117.8 (CH, Ar); 106.0 (CH, Ar); 97.4 (CH, Ar); 50.2 (CH_2); 50.6 (CH_2); 42.6 (CH_2)
5a[c]	45	140–144	217	[^{1}H]NMR $\delta^{\&}$ 7.38 (d, *J* = 8.98 Hz, 1H, ArH); 7.27 (s, 1H, ArH); 7.06–6.94 (m, 2H, ArH); 3.86 (s, 3H, OCH_3); 3.82 (br s, 4H, $2CH_2$)
5b	58	—	231	[^{1}H]NMR $\delta^{\&}$ 7.48 (d, *J* = 9.18 Hz, 1H, ArH); 7.20 (s, 1H, ArH); 7.10–6.85 (m, 2H, ArH); 4.00–3.80 (m, 5H, OCH_3 and CH_2); 3.45 (t, *J* = 10.15 Hz, 2H, CH_2); 3.18 (s, 3H, CH_3)
5c	51	—	245	[^{1}H]NMR $\delta^{\&}$ 7.42 (d, 1H, ArH); 7.27 (s, 1H, ArH); 7.18–7.10 (m, 2H, ArH); 4.20–3.95 (m, 6H, $3CH_2$); 3.80 (s, 3H, OCH_3); 1.40 (t, *J* = 7.14, 3H, CH_3)
5d	40	—	259	[^{1}H]NMR $\delta^{\#}$ 8.00 (s, 1H, ArH); 7.71 (d, *J* = 9.12 Hz, 1H, ArH); 7.38–7.18 (m, 2H, ArH); 4.13–3.66 (m, 9H, OCH_3 and $3CH_2$); 1.83–1.66 (m, 2H, CH_2); 0.95 (t, *J* = 7.43 Hz, 3H, CH_3)
5e	58		307	[^{1}H]NMR $\delta^{\#}$ 7.95 (s, 1H, ArH); 7.68 (d, 1H, ArH); 7.42–7.35 (m, 6H, ArH); 7.20 (m, 1H, ArH); 5.10 (s, 2H, CH_2); 4.02 (s, 4H, $2CH_2$); 3.80 (s, 3H, OCH_3)
5f[c]	43.5	139–141	217	[^{1}H]NMR $\delta^{\#}$ 7.61–7.56 (d, *J* = 8.62 Hz, 1H, ArH); 7.26–7.20 (m, 2H, ArH); 6.95–6.89 (m, 1H, ArH); 3.79 (s, 3H, OCH_3); 3.59 (br s, 4H, $2CH_2$)

(*continued on next page*)

Table 3 (*continued*)

Compd	Yield (%)	Anal. C, H, N mp (°C)	ESIMS *m/z* (MH^+)	NMR, $\delta^{\#}$ (DMSO-d_6), $\delta^{\&}$ ($CDCl_3$)
5g	51	—	259	[^{1}H]NMR $\delta^{\&}$ 7.50 (d, *J* = 8.65 Hz, 1H, ArH); 7.27 (s, 1H, ArH); 7.04–6.90 (m, 2H, ArH); 4.02–3.81 (m, 5H, OCH_3, CH_2); 3.61–3.39 (m, 4H, $2CH_2$); 1.73–1.58 (m, 2H, CH_2); 1.03–0.91 (t, *J* = 7.44 Hz, 3H, CH_3)
5h	50	—	307	[^{1}H]NMR $\delta^{\&}$7.48–6.87 (m, 9H, ArH); 4.69 (s, 2H, CH_2); 4.02–3.81 (m, 5H, OCH_3 and CH_2); 3.51–3.40 (m, 2H, CH_2)
5i[c]	50	140–142	247	[^{1}H]NMR $\delta^{\#}$ 7.41–7.36 (d, *J* = 8.93 Hz, 1H, ArH); 7.27 (s, 1H, ArH); 6.99–6.94 (m, 1H, ArH); 3.84–3.82 (m, 10H, 5,6-OCH_3 and $2CH_2$)
5j	46	—	289	[^{1}H]NMR $\delta^{\&}$ 7.27–7.01 (m, 3H, ArH); 3.99–3.90 (m, 8H, $2OCH_3$ and CH_2); 3.57–3.60 (m, 4H, $2CH_2$); 1.73–1.64 (m, 2H, CH_2); 1.03–0.95 (t, *J* = 7.46, 3H, CH_3)
5k	43	—	337	[^{1}H]NMR $\delta^{\&}$ 7.37–7.26 (m, 6H, ArH); 7.13–7.00 (m, 2H, ArH); 4.68 (s, 2H, CH_2); 3.97–3.91 (m, 8H, 5,6-OCH_3, CH_2); 3.45 (s, 2H, CH_2)

[a] Recrystallized from ethanol/diethyl ether.
[b] Recrystallized from methanol.
[c] Recrystallized from ethyl acetate.

above. Rat brain membranes (about 300 μg of proteins) were incubated to equilibrium (30 min, room temperature) with increasing concentrations of the tested ligands and fixed concentration (1 nM) of [^{3}H]2-BFI for I_2-IBS or [^{3}H]RX821002 for α_2-ARs. Incubations were terminated by rapid filtration through 0.5% polyethyleneimine pre-soaked Whatman GF/B filters using a Brandel M-24 cell harvester. Filters were washed twice with 5 mL of ice-cold assay buffer and the amount of radioactivity retained on them was determined by liquid scintillation counting. Nonspecific binding of [^{3}H]2-BFI or [^{3}H]RX821002 was defined in the presence, respectively of 10 μM BU224 for I_2-IBS or 10 μM rauwolscine for α_2-ARs.

5.2.1.2. D_2-like receptors binding assays. Porcine striatal membranes were prepared as previously described.[24] In brief, tissue was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.4 (buffer T) containing protease inhibitors (20 μg/mL soybean trypsin inhibitor, 200 μg/mL, and 160 μg/mL benzamidine), using an Ultra-Turrax TP-1810. The homogenate was centrifuged at 50,000*g* for 10 min at 4 °C. The resulting pellet was then washed once by resuspension in buffer T and recentrifuged. The final pellet was frozen at −20 °C until the time of assay. Striatal membranes (about 200 μg of proteins) were incubated to equilibrium (60 min, at 30 °C) with increasing concentrations of the tested ligands and fixed concentration (0.3 nM) of [^{3}H]YM-09-151-2. Incubation was terminated by dilution to 5 mL with ice cold buffer T, followed immediately by rapid filtration through glass fiber Whatman GF/C filters. The filters were then washed (3 × 5 mL) with buffer T and the amount of radioactivity retained on them was determined by liquid scintillation counting. Nonspecific binding was defined in the presence of 2.5 mM dopamine. In porcine striatal membranes, the equilibrium binding parameters of [^{3}H]YM-09-151-2 were: K_d = 0.40 ± 0.03 nM and B_{max} = 350 ± 39 fmol/mg of proteins.

5.2.1.3. D_1-like receptors binding assays. Porcine striatal membranes were prepared as previously described for D_2-like receptors binding assays. [^{3}H]SCH23390 binding to D_1-like receptors was assayed in a final incubation volume of 0.5 mL, which contained crude membranes (∼0.2 mg of protein), radioligand (∼0.5 nM), and the tested compound in the range 10^{-8}–10^{-4} M concentrations at 30 °C for 60 min. Incubation was terminated by dilution to 5 mL with ice cold buffer T, followed immediately by rapid filtration through glass fiber Whatman GF/C filters. The filters were then washed (3 × 5 mL) with buffer T and the amount of radioactivity retained on them was determined by liquid scintillation counting. Nonspecific binding was defined in the presence of 2.5 mM dopamine. In porcine striatal membranes, the equilibrium binding parameters of [^{3}H]SCH23390 were: K_d = 1.33 ± 0.12 nM and B_{max} = 320 ± 36 fmol/mg of proteins.

5.2.2. [^{35}S]GTPγS binding assays

[^{35}S]GTPγS binding experiments in striatal membranes were performed as previously described by Odagaki and Toyoshima.[25] This method allows to study G protein activation through D_2-like receptors. Briefly, aliquots of striatal membranes equivalent to 20 μg of protein were incubate at 30 °C for 60 min in 500 μl of 50 mM Tris–HCl buffer (pH 7.4) containing 100 μM GDP, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM EGTA, 0.2 mM DTT and 150 mM NaCl in the presence of 0.2 nM [^{35}S]GTPγS and six different concentrations of newly synthesized compounds. Non specific binding was determined in the presence of 100 μM unlabeled GTPγS. The reaction was terminated by rapid filtration through GF/C glass fiber filter washing twice with 4 ml of ice-cold 50 mM Tris/HCl buffer (pH 7.4). The bound radioactivity was measured by liquid scintillation spectrometry. The EC_{50} values were obtained by non linear regression method using the computer program GraphPad Prism, version 4.0 for Windows and are presented as mean ± SEM of three independent experiments. Intrinsic activity is relative to the full agonist dopamine where the percent maximal of increase above the basal binding (%E_{max}) was set to 1.

5.2.3. In vivo studies

5.2.3.1. Animals. Male Wistar rats (*n* = 24) (Harlan, UD, Italy) that weighed 200–225 g at the beginning of the experiments were used. The animals were individually housed in a room on a 12 h light/dark cycle (lights off at 7:00 A.M.) at constant temperature (20–22 °C) and humidity (45–55%). Rats were offered food pellets (4RF; Mucedola, Settimo Milanese, Italy) and tap water ad libitum. All procedures were conducted in adherence to the European Community Council Directive for Care and Use of Laboratory Animals.

5.2.3.2. Surgical procedure and treatments. After anesthesia with 10 mg/kg of a mixture of zolazepam and tiletamine (Zoletil 100, Italmed, Italy) by intraperitoneally injection, a stainless steel cannula was implanted in the animal's lateral cerebroventricle using a stereotaxic instrument into the right ventricle (1.0 mm posterior to the bregma, 1.8 mm lateral to the midline, and 3.5 mm ventral to the surface of the skull, according to the brain atlas of Paxinos and Watson.[26] Rats were divided in three groups: the control group received vehicle (physiological solution) into the right ventricle; the second group was administered by icv with 0.0149 μM/kg body weight of L-Dopa and the third group was treated by icv with equimolar dose of **3h**.

5.2.3.3. Locomotor activity. Immediately after treatment, automated locomotor activity boxes (MedAssociates, VT 05478) were used to quantify locomotor activity of all rats as previously described.[20] In brief, the ambulatory episodes and the rearing episodes recorded into the central square of the open field were expressed as percentage of total ambulatory episodes and rearing episodes, respectively. Ambulatory episodes were recorded when the low row of photocells was interrupted, while rearing counts were recorded by interruptions in the higher row of photocells. Low percentage of ambulatory and rearing episodes registered into the central area of the open field could represent behaviour correlated with anxiety.[27]

5.3. Molecular modelling

Docking of 3 h into the human D2 receptor was carried out with AutoDock ver 4.2.[28] Affinity maps were calculated on a $60 \times 60 \times 60$ cubic box centred on DA structure of the original model as supplied by Xhaard. A flexible binding cavity, comprising Asp3.32, Cys3.36, Ser5.42, Ser5.46, Phe6.44, Trp6.48, Phe6.51 and Phe6.52 and His 6.55 was defined. Docking was carried out with 100 run of Lamarckian genetic algorithm using AMBER and OPLS charges for receptor and agonist structure, respectively. The pose having the best estimated free energy of binding, among the most populated cluster, was selected as representative of agonist binding. Figures are rendered with Pymol.[29]

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