Bioorganic & Medicinal Chemistry 21 (2013) 1680–1684

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of dopamine D_4 receptor antagonists with planar chirality

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ARTICLE INFO

Article history:
Received 3 December 2012
Revised 18 January 2013
Accepted 24 January 2013
Available online 5 February 2013

Keywords:
GPCR
Dopamine
Subtype selectivity
Ligand efficacy
Paracyclophane
Planar chirality

ABSTRACT

Employing the D_4 selective phenylpiperazine **2** as a lead compound, planar chiral analogs with paracyclophane substructure were synthesized and evaluated for their ability to bind and activate dopamine receptors. The study revealed that the introduction of a [2.2]paracyclophane moiety is tolerated by dopamine receptors of the D_2 family. Subtype selectivity for D_4 and ligand efficacy depend on the absolute configuration of the test compounds. Whereas the achiral single-layered lead **2** and the double-layered paracyclophane (*R*)-**3** showed partial agonist properties, the enantiomer (*S*)-**3** behaved as a neutral antagonist.

1. Introduction

The dopamine D_4 receptor is a subtype of the D_2-like family belonging to the rhodopsin like G-protein coupled receptors (GPCRs).[1] D_4 receptor can be found in distinct areas of the central nervous system including the cerebral cortex, striatum, hippocampus, and amygdala.[2] Genetic studies have demonstrated that a variable number of tandem repeat polymorphisms, which has been detected for the D_4 receptor, is associated with an increased risk of developing attention deficit hyperactivity disorder (ADHD).[3] Moreover, an association between this genetic vulnerability for pathologic gambling and the presence of the seven-repeat allele of the D_4 receptor was shown.[4] D_4 antagonists might be also very useful for the treatment of psychotic diseases, although the theory that the D_4 receptor might play a crucial part in the etiology of schizophrenia remains controversial.[5] Because D_4 receptor activation causes penile erection in rodents, a promising approach to a therapeutic use of D_4 selective drug candidates results from the impact of the dopaminergic system on sexual stimulation.[6]

We have previously developed a number of 1,4-disubstituted arylpiperidines and piperazines (1,4-DAPs), displaying subtype selectivity for the dopamine receptor subtypes D_2, D_3 or D_4.[7] Employing a toolbox of head groups, amine moieties, linker elements and lipophilic appendages, the 1,4-DAP privileged structure proved to be a valuable starting point to fine-tune both GPCR subtype selectivity and intrinsic activity.[8] Thus, we could demonstrate that the attachment position of heteroarenes that served as lipophilic appendages strongly influenced the intrinsic activity of the test compounds at the D_4 receptor.[9] As an extension of our recent studies on [2.2]paracyclophanes as bioisosteres for arene derivatives including D_4 receptor ligands of type **1** (Fig. 1),[10] we envisaged to investigate if binding affinity and intrinsic activity of the promising lead compounds of type **2**[7d,11] could be varied by the introduction of a paracyclophane moiety. Because both partial agonists and neutral antagonists are of interest for D_4-related dysfunctions and paracyclophanes are useful molecular probes to investigate the size of binding sites, our results will be valuable for further work in GPCR based drug discovery. We were also intrigued by the question how ligand efficacy depends on the stereochemistry of the planar chiral unit. Thus, the target compound **3** should be synthesized in enantiomerically pure form. To learn more about the relationships between D_4 receptor binding and the position of the double-layered unit within the 1,4-DAP pharmacophore, we intended to prepare and biologically study also the cyclophanylpiperazine **4** as a regiosiomer of **3**.

2. Results and discussion

2.1. Synthesis

The synthesis of the enantiomerically pure cyclophanylpyrazoles **3** started from the racemic alkohol (±)-**5**, which was obtained from bromo substituted [2.2]paracyclophane by metallation and subsequent boronation (Scheme 1).[12] According to our previously

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http://dx.doi.org/10.1016/j.bmc.2013.01.065

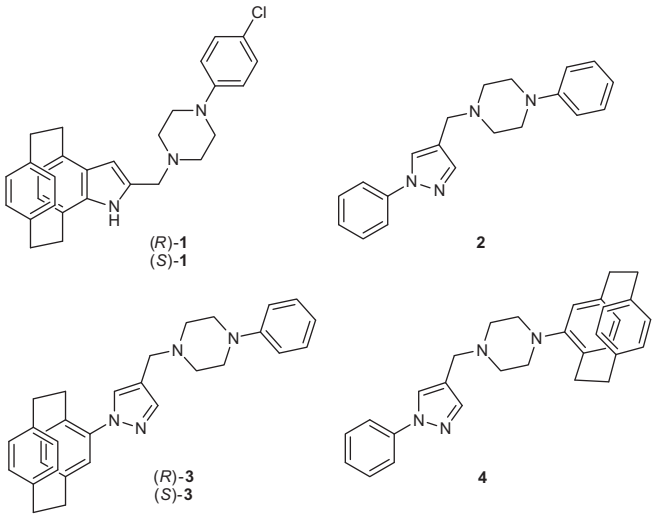
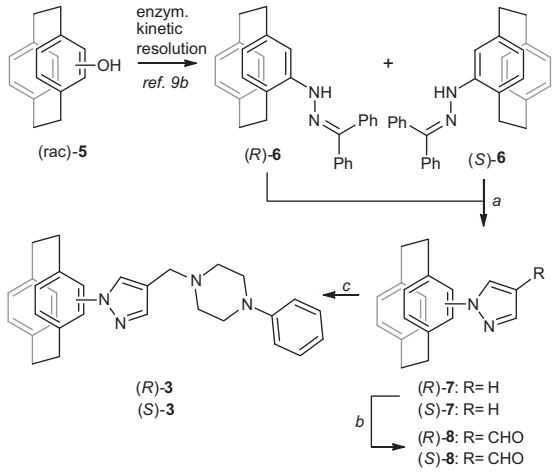


Figure 1. Dopamine D_4 receptor ligands.



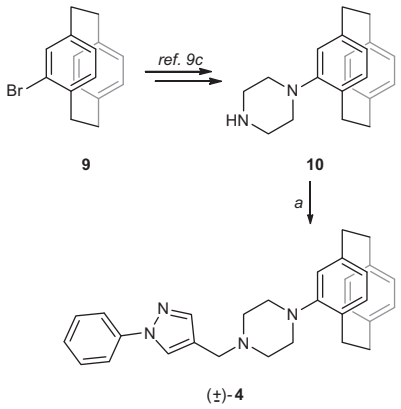
Scheme 1. Reagents and conditions: (a) (1) TosOH, HCl in EtOH, ethylene glycol, 80 °C, 18 h; (2) 1,1,3,3-tetramethoxypropane, HCl in EtOH, 80 °C, 1.5 h; (b) $POCl_3$, DMF, 1,2-dichloroethane, 0 °C to reflux, 2.5 h; (c) 1-phenylpiperazine, $Na(OAc)_3BH$, CH_2Cl_2, rt, 5 h.

published synthetic route,[10b] (±)-**5** was converted into the hydrazones (*R*)-**6** and (*S*)-**6**, whereas an enzymatic kinetic resolution of the respective 4-acetyloxy-[2.2]paracyclophane by *Candida cylindracea* lipase was the key step.[13] The pyrazoles (*R*)-**7** and (*S*)-**7** were obtained by treatment of (*R*)-**6** and (*S*)-**6** with HCl in ethanol in the presence of ethylene glycol and subsequent cyclocondensation with tetramethoxypropane. Finally, Vilsmeier–Haack formylation led to the pyrazole carbaldehydes (*R*)-**8** and (*S*)-**8**, which were converted into the desired target compounds (*R*)-**3** and (*S*)-**3** by reductive amination with 1-phenylpiperazine and $Na(OAc)_3BH$. Analysis by chiral HPLC gave ee values >98% for both enantiomers.

For the preparation of the reference ligand **4** we took advantage of our established synthesis of the piperazine derivative **10**,[10c] including a palladium free aryl-amine coupling reaction (Scheme 2).[14] Reductive alkylation with 1-phenylpyrazole-4-carbaldehyde[7d] gave the corresponding racemic tertiary amine **4**.

2.2. Receptor-ligand binding experiments

Receptor binding data were evaluated in radioligand displacement experiments using preparations of the human dopamine



Scheme 2. Reagents and conditions: (a) phenylpyrazole-4-carbaldehyde, $Na(OAc)_3BH$, CH_2Cl_2, rt, 24 h.

receptor subtypes D_{2long}, D_{2short},[15] D_3[16] and $D_{4.4}$[17] each stably expressed in Chinese hamster ovary (CHO) cells and the radioligand [^{3}H]spiperone.[18] Binding affinities to the dopamine D_1 receptor were determined when using membranes from bovine striatum and the radioligand [^{3}H]SCH23390.[18] As displayed in Table 1, all compounds show moderate binding to the D_1 subtype in the micromolar range. Interestingly, the planar chiral test compounds (*R*)-**3** and (*S*)-**3** incorporating a double-layered lipophilic appendage displayed substantial binding affinity to the GPCRs D_{2long}, D_{2short}, D_3 and $D_{4.4}$ with significant preference to the D_4 receptor. In detail, K_i values in the single digit nanomolar range (K_i = 4.5 nM and 2.5 nM for (*R*)-**3** and (*S*)-**3**, respectively) were slightly higher than the data of our lead compound **2** (K_i = 1.0 nM) and an enantiospecific difference in affinity could be observed. On the one hand, (*S*)-**3** showed a higher selectivity over D_3 than its (*R*)-enantiomer. On the other hand, the data for (*R*)-**3** clearly indicated stronger D_4 preference over D_{2long} and D_{2short} binding (53- and 78-fold). Compared to the endogenous ligand dopamine, the planar chiral test compounds (*R*)-**3** and (*S*)-**3** displayed superior D_4 binding affinity and subtype selectivity. Further SAR studies led us to the 1,4-DAP **4**, which represents a regioisomer of the test compound **3** that includes a paracyclophane unit within the head group. In fact, this structural modification induced a substantial reduction of D_4 affinity (K_i = 45 nM) while the binding properties for D_2 and D_3 were similar to (*S*)-**3**.

2.3. Functional experiments

The activation of D_4 receptors can be measured as a concentration-dependent inhibition of the forskolin-stimulated cyclic AMP

Table 1
Receptor binding data for the test compounds (*R*)-**3**, (*S*)-**3** and **4** at the bovine dopamine D_1 receptor and the human subtypes D_{2long}, D_{2short}, D_3 and $D_{4.4}$ in comparison to the reference agents dopamine and **2**

Compound	K_i value[a] (nM)				
	D_1	D_{2long}	D_{2short}	D_3	$D_{4.4}$
	[^{3}H]SCH23390			[^{3}H]spiperone	
(*R*)-**3**	1700 ± 63	350 ± 52	240 ± 40	23 ± 5.2	4.5 ± 0.19
(*S*)-**3**	1500 ± 75	55 ± 15	33 ± 7.3	20 ± 3.0	2.5 ± 0.29
4	2600 ± 920[b]	39 ± 13	28 ± 9.3	18 ± 1.8	45 ± 10
Dopamine	440 ± 72	190 ± 28	110 ± 49	21 ± 6.7	8.9 ± 2.2
2	1600 ± 570[b]	140 ± 7.8	75 ± 8.1	140 ± 13	1.0 ± 0.12

[a] K_i values are the means of 3–8 individual experiments ± SEM each done in triplicate.
[b] K_i values are the means of two individual experiments ± SD each done in triplicate.

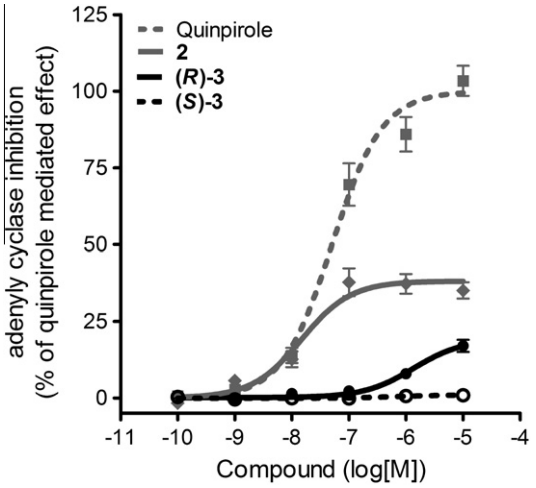


Figure 2. Adenylyl cyclase assay indicating the ability of the test compounds **2**, (*R*)-**3** and (*S*)-**3** to inhibit forskolin stimulated cAMP accumulation. The $D_{4.4}$ mediated inhibition of adenylyl cyclase was measured in the CHO cells expressing $D_{4.4}$. The compounds were tested in the presence of 10 μM forskolin. Quipirole served as a reference full agonist.

(cAMP) levels.[17] To evaluate the efficacy of the D_4 ligands (*R*)-**3** and (*S*)-**3** and the lead compound **2**, intrinsic activity of the test compounds was determined in a luminescence based adenylyl cyclase activity assay (Fig. 2). The assay was performed in the presence of 10 μM forskolin. Quinpriole was used as a reference compound yielding full agonist response. Whereas a 40% ligand efficacy clearly indicated partial agonist properties for the lead compound **2**, intrinsic activity was strongly reduced for the planar chiral paracyclophanes (*R*)-**3** and (*S*)-**3**. The (*R*)-enantiomer displayed 19% ligand efficacy and (*S*)-**3** proved to be a neutral antagonist. Thus, intrinsic activity depends on the chirality of the double-layered lipophilic appendage.

3. Conclusion

The intrinsic activity of a GPCR ligand determines whether it may be used to treat disorders that require stimulation or attenuation of specific signaling events, respectively. Planar chiral analogs of type **3** and **4** were synthesized and evaluated for their ability to bind and activate dopamine receptors. The study revealed that the introduction of a paracyclophane moiety is tolerated by dopamine receptors of the D_2 family. D_4 subtype selectivity and ligand efficacy depend on the absolute stereochemistry of the test compounds. Whereas the achiral single-layered lead **2** and the double-layered paracyclophane (*R*)-**3** showed partial agonist properties, the enantiomer (*S*)-**3** behaved as a neutral antagonist.

4. Experimental section

4.1. General

Melting points were determined on a Büchi 530 apparatus and are uncorrected. All ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with Bruker AC spectrometers at 360 MHz as well as 90 and 63 MHz. IR spectra were recorded with a Jasco FT/IR 410 spectrometer. Elemental analyses were performed by the Department of Organic Chemistry, University of Erlangen-Nürnberg. Optical rotations were measured with a Perkin–Elmer 241 spectropolarimeter. All reagents were of commercial quality and used as purchased. Flash chromatography was carried out with silica gel 60 (4.0–6.3 μm) eluting with the appropriate solution in the stated v/v proportions. Analytical thin-layer chromatography (TLC) was performed with silica gel plates on aluminium (Silica Gel 60 F254 from Merck). The ee values were determined by HPLC-analysis using the following HPLC equipment: Gilson 305 HPLC pump with pulsation suppressor (type 805); UV detector from Knauer; chiral CHIRALCEL OD column from Diacel (length 20 cm and diameter 0.64 cm); solvent system: petroleum ether/*i*-PrOH 6:4.

4.2. (*R*)-1-[2.2]Paracyclophane-4-yl-1H-pyrazole ((*R*)-7)

A solution of (*R*)-*N*-benzhydrylidene-*N*′-[2.2]paracyclophane-4-yl-hydrazine (50 mg, 0.13 mmol), toluene sulfonic acid hydrate (118 mg, 0.62 mmol) and ethylene glycol (0.1 mL) in ethanolic HCl (1 mL) are stirred in a pressure vial under nitrogen at 80 °C. After 18 h, a solution of tetramethoxypropane (20.4 mg, 0.13 mmol) in ethanol (0.1 mL) saturated with HCl was added. After stirring at 80 °C for 90 min, the solution was allowed to cool to room temperature, evaporated and the residue was added to an aqueous solution of saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 95:5) to give pure (*R*)-**7** (26.1 mg, 77%) as a colorless solid (mp 114 °C). EI-MS: *m/z* 274 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 2.55–2.63 (m, 1H), 2.83–3.22 (m, 6H), 3.45–3.53 (m, 1H), 6.48 (dd, *J* = 2.1 Hz, 2.1 Hz, 1H), 6.55 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 6.56 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 6.60 (d, *J* = 7.5 Hz, 1H), 6.61 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 6.62 (dd, *J* = 8.0 Hz, 1.9 Hz, 1H), 6.66 (d, *J* = 2.0 Hz, 1H), 6.67 (dd, *J* = 7.5 Hz, 2.0 Hz, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 7.79 (d, *J* = 2.1 Hz, 1H); IR: (KBr) ν (cm^{-1}): 1596. $[\alpha]_D^{20} = -98.8$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{19}H_{18}N_2$: C, 83.18; H, 6.61; N, 10.21. Found: C, 82.98; H, 6.52; N, 10.44. (*S*)-**7** was prepared under identical conditions. $[\alpha]_D^{20} = +100.2$ (*c* 1, $CHCl_3$).

4.3. (*R*)-1-[2.2]Paracyclophane-4-yl-1H-pyrazole-4-carbaldehyde ((*R*)-8)

To a pre-cooled solution of phosphoryl chloride (26 μL, 0.27 mmol) in 1,2-dichloroethane (dry, 0.5 mL) was added a solution of dimethylformamide (21 μL, 0.27 mmol) in 1,2-dichloroethane and the solution was stirred at 0 °C for 0.5 h. Then, a solution of (*R*)-**7** (50 mg, 0.18 mmol) in dry 1,2-dichloroethane (dry, 0.5 mL) was added and the mixture was refluxed under N_2 for 2 h. After addition of saturated aqueous sodium acetate at room temperature, stirring was continued for 0.5 h. After neutralization by addition of $NaHCO_3$, the mixture was extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 85:15) to give pure (*R*)-**8** (41 mg, 74%) as a colorless solid (mp 139 °C). EI-MS: *m/z* 302 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 2.61–2.70 (m, 1H), 2.86–3.23 (m, 6H), 3.35–3.54 (m, 1H), 6.55–6.70 (m, 7H), 8.20 (s, 1H), 8.24 (s, 1H), 10.01 (s, 1H); IR: (KBr) ν (cm^{-1}): 1681. $[\alpha]_D^{20} = -77.9$ (*c* 0.31, $CHCl_3$). Anal. Calcd for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.26. Found: C, 79.62; H, 5.80; N, 8.98. (*S*)-**8** was prepared under identical conditions. $[\alpha]_D^{20} = +78.8$ (*c* 0.75, $CHCl_3$).

4.4. (*R*)-1-(1-[2.2]Paracyclophane-4-yl-1H-pyrazol-4-ylmethyl)-4-phenyl-piperazine ((*R*)-3)

A suspension of (*R*)-**8** (21 mg, 0.07 mmol), sodium triacetoxyborohydride (18.6 mg, 0.10 mmol) and 1-phenylpiperazine (11.5 mg, 0.07 mmol) in dichloromethane (1.5 mL) was stirred for 5 h at room temperature. The mixture was added to a saturated aqueous solution of $NaHCO_3$ and was extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (ethyl ace-

tate/petroleum ether 7:3) to give pure (*R*)-**3** (25 mg, 80%) as a colorless solid (mp 131 °C). EI-MS: *m/z* 448 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 2.54–2.64 (m, 1H), 2.65–2.75 (m, 4H), 2.78–3.21 (m, 6H), 3.22–3.30 (m, 4H), 3.52 (ddd, *J* = 13.0 Hz, 9.9 Hz, 2.7 Hz, 1H), 3.60 (d, *J* = 13.7 Hz, 1H), 3.64 (d, *J* = 13.7 Hz, 1H), 6.55 (dd, *J* = 7.8 Hz, 1.8 Hz, 2H), 6.59 (d, *J* = 7.8 Hz, 1H), 6.62 (dd, *J* = 7.8 Hz, 1.8 Hz, 1H) 6.63 (dd, *J* = 7.8 Hz, 1.8 Hz, 1H), 6.66 (d, *J* = 1.8 Hz, 1H), 6.69 (dd, *J* = 7.8 Hz, 1.8 Hz, 1H), 6.69–6.89 (m, 1H), 6.91–6.98 (m, 2H), 7.23–7.30 (m, 2H), 7.67 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 2.1 Hz, 1H); IR: (KBr) ν (cm^{-1}): 1598. $[\alpha]_D^{20} = -75.8$ (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{30}H_{32}N_4$: C, 80.32; H, 7.19; N, 12.49. Found: C, 79.99; H, 7.08; N, 12.35. [ee] >98%. (*S*)-**3** was prepared under identical conditions. $[\alpha]_D^{20} = +74.8$ (*c* 1, $CHCl_3$). [ee] >98%.

4.5. 1-(1-Phenyl-1*H*-pyrazol-4-ylmethyl)-4-[2.2]paracyclophan-4-yl-piperazine (4)

A mixture of 1-phenylpyrazole-4-carbaldehyde (18.5 mg, 0.11 mmol), paracyclophanyl piperazine (31.4 mg, 0.11 mmol) and sodium triacetoxyborohydride (27.5 mg, 0.13 mmol) in dichloromethane (1.5 mL) was stirred at room temperature for 24 h. Then, the mixture was added to a saturated aqueous solution of $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether/ethyl acetate 4:6) to give pure **4** (35.2 mg, 72%) as a colorless solid (mp 78 °C). EI-MS: *m/z* 448 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 2.60–2.81 (m, 5H), 2.85–3.12 (m, 9H), 3.25 (ddd, *J* = 12.3 Hz, 9.0 Hz, 6.0 Hz, 1H), 3.37 (ddd, *J* = 12.7 Hz, 9.7 Hz, 2.4 Hz, 1H), 3.60 (s, 2H), 5.71 (d, *J* = 1.8 Hz, 1H), 6.27 (dd, *J* = 7.8 Hz, 1.8 Hz, 1H), 6.35 (dd, *J* = 7.9 Hz, 1.9 Hz, 1H), 6.40 (d, *J* = 7.8 Hz, 1H), 6.44 (dd, *J* = 7.9 Hz, 1.9 Hz, 1H), 6.52 (dd, *J* = 7.9 Hz, 1.9 Hz, 1H), 6.69 (dd, *J* = 7.9 Hz, 1.9 Hz, 1H), 7.24–7.31 (m, 1H), 7.41–7.48 (m, 2H), 7.67–7.73 (m, 3H), 7.92 (s, 1H); IR: (KBr) ν (cm^{-1}): 1596. Anal. Calcd for $C_{30}H_{32}N_4$: C, 80.32; H, 7.19; N, 12.49. Found: C, 80.63; H, 7.13; N, 12.32.

4.6. Radioligand binding studies

Receptor binding studies were carried out as described previously.[18] In brief, competition binding experiments with the human D_{2long}, D_{2short},[15] D_3[16] and $D_{4.4}$[17] receptors were run on membrane preparations from CHO cells stably expressing the corresponding receptor. Assays were run with membranes at protein concentrations per well of 14–20 μg/mL, 8 μg/mL, 6–20 μg/mL and 20 μg/mL for D_{2long}, D_{2short}, D_3 and $D_{4.4}$, respectively and with the radioligand [^{3}H]spiperone (specific activity = 84 Ci/mmol, PerkinElmer, Rodgau, Germany) at a final concentration of 0.5 nM in binding buffer (50 mM Tris, 1.0 mM EDTA, 5.0 mM $MgCl_2$, 100 μg/ml bacitracin and 5 μg/ml soybean trypsin inhibitor at pH 7.4). The K_D values of the membrane preparations used were 0.10–0.12 nM, 0.10 nM, 0.10–0.40 nM and 0.13 nM for D_{2long}, D_{2short}, D_3 and $D_{4.4}$, respectively at a receptor density expressed as B_{max} in the range of 220–1100 fmol/mg. For the determination of D1 affinity homogenate from bovine striatum was incubated with the radioligand [^{3}H]SCH 23390 (specific activity = 75 Ci/mmol, Biotrend, Cologne, Germany) and the test compounds as described above. Non-specific binding was determined in the presence of 10 μM haloperidol. Protein concentration was established by the method of Lowry using bovine serum albumin as a standard.[19]

4.7. Data analysis

The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM 5.0 (GraphPad Software, San Diego, CA). Competition curves were fitted to a sigmoid curve by non-linear regression analysis in which the log EC_{50} value and the Hill coefficient were free parameters. EC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[20]

4.8. Functional assays on the Inhibition of cAMP Accumulation

Bioluminescence based cAMP-Glo Promega assay was performed according to the manufacturer instructions (Promega Corporation, Madison, WI, USA). In this assay, the activation of cAMP-dependent protein kinase (PKA) is monitored by measuring ATP utilization in a kinase reaction by using a luciferase/luciferin luminescent reaction.[21] The $D_{4.4}$ receptor expressing CHO cells[17] were seeded into a white 96 well plate at the density of 10,000 cells/well 24 h before the assay. The cells were first washed once with Krebs Ringer Buffer, pH 7.4, and then incubated with different concentrations of the compounds under study in presence of 10 μM forskolin in Krebs Ringer Buffer containing 100 μM 3-isobutyl-1-methylxanthine (IBMX) and 100 μM 4-(3-butoxy-4-methoxybenzyl)imidazoline (Ro-20-1724), pH 7.4. After incubation for 20 min at 25 °C, cells were treated as previously described.[22] Luminescence was measured on a microplate reader Victor 3V (Perkin Elmer, Waltham, MA, USA).

The EC_{50} and E_{max} values were calculated as means ± SEM of data from at least three experiments with each concentration in triplicate.

Acknowledgments

F.S. is recipient of a Master and Back grant (Code No. PRR-MAB-A2011–19138) from ARS (Autonomous Region of Sardinia).

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