Full Paper

N-(4-(4-(2-Halogenophenyl)piperazin-1-yl)butyl) Substituted Cinnamoyl Amide Derivatives as Dopamine D_2 and D_3 Receptor Ligands

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A series of eight substituted *N*-(4-(4-(2-halogenophenyl)piperazin-1-yl)butyl)-3-phenylacryl amide derivatives have been synthesized and screened for binding affinities at dopamine hD_2 and hD_3 receptors. All compounds have shown high to remarkable receptor affinities and some have led to distinct selectivity for D_3 receptors. Highest D_3-receptor affinity has been observed for 3-(4-aminophenyl)-*N*-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide (hD_3 K_i 0.9 nM; hD_2 K_i 17.4 nM). Selectivity ratio has been best for 3-(4-chlorophenyl)-*N*-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)-acryl amide with a 56-fold preference for hD_3 versus hD_2. A functional activity test has been performed by a mitogenesis test for *N*-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)-3,3-diphenylacryl amide, which, surprisingly, has shown full agonist properties.

Keywords: Cinnamic acids / D_3 receptors / Dopamines / PET / Phenylpiperazines

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Introduction

Dopamine is an essential neurotransmitter in the central nervous system. Its corresponding receptors are divided into two receptor families: The D_1-like receptors including D_1 and D_5 receptor subtypes activate adenylyl cyclase. D_2, D_3, and D_4 receptor subtypes belonging to the D_2-like receptors inhibit adenylyl cyclase [1] Whereas dopamine D_2 receptors are located in highest density in the striatum, D_3 receptors are mainly located in the limbic system [2, 3]. Therefore, the dopamine D_3 receptor has become a promising target for antipsychotic drugs and the treatment of Parkinson's disease [3 – 5]. Moreover, dopamine D_3 receptors are implicated in the reinforcing effects of drugs of abuse [6]. A general structural pattern can be applied to many dopamine receptor ligands: An aryl amide is linked via a tetramethylene spacer to a basic residue with aryl substituent [7]. So, the partial dopamine D_3 receptor agonist BP 897, one of our lead compounds, follows these structural analogies [3]. The lead structure of new compounds based on BP 897 is drawn in bold (Fig. 1).

BP 897 shows a noteworthy pharmacological profile with potential for the treatment of Parkinson's disease and neuropsychiatric disorders [8, 9]. Replacement of the naphthylamide residue of BP 897 by a cinnamoyl group and choosing 1,2,3,4-tetrahydroisoquinoline as basic residue has lead to the full antagonist ST 198 [10, 11]. Antagonists also look promising in the treatment of Parkinson's disease and drug abuse [6, 12, 13]. The 1-(2-methoxyphenyl)piperazine derivatives ST 186 and ST 168 (Table 1) are partial agonists with even higher affinity at D_3 receptors than that of BP 897 [14]. Gmeiner *et al*. have reported about 1-(2,3-dichlorophenyl)piperazine derivatives with antagonist properties [15]. These dihalogenated phenylpiperazine derivatives have shown similar D_2 and D_3

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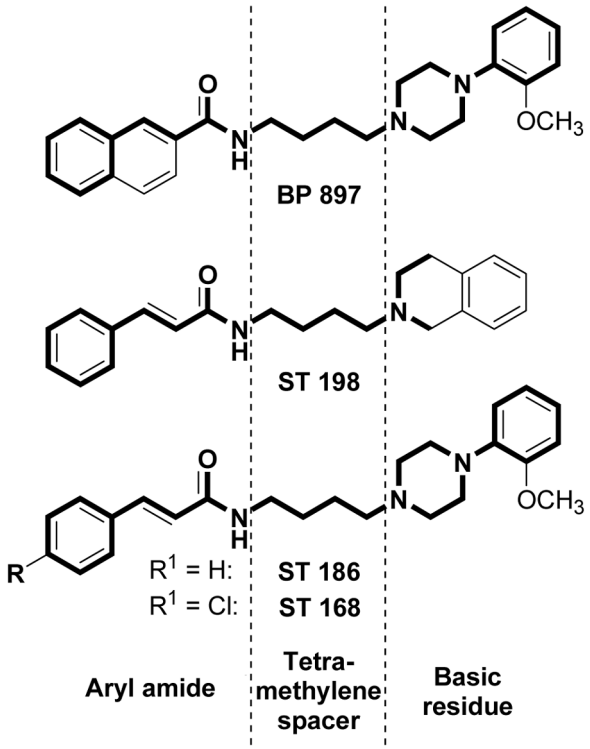
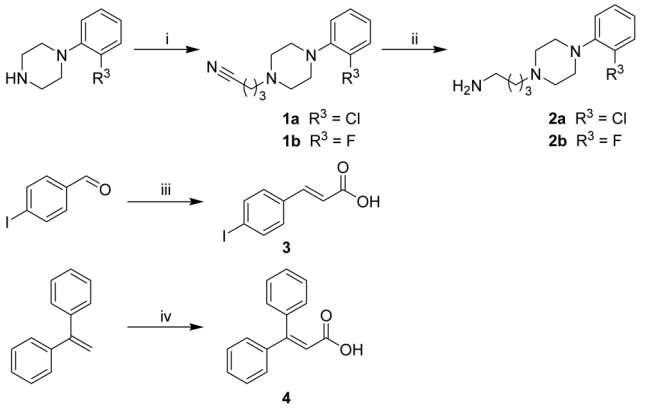


Figure 1. General structure pattern of dopamine D_3-receptor selective antagonists and partial agonists.



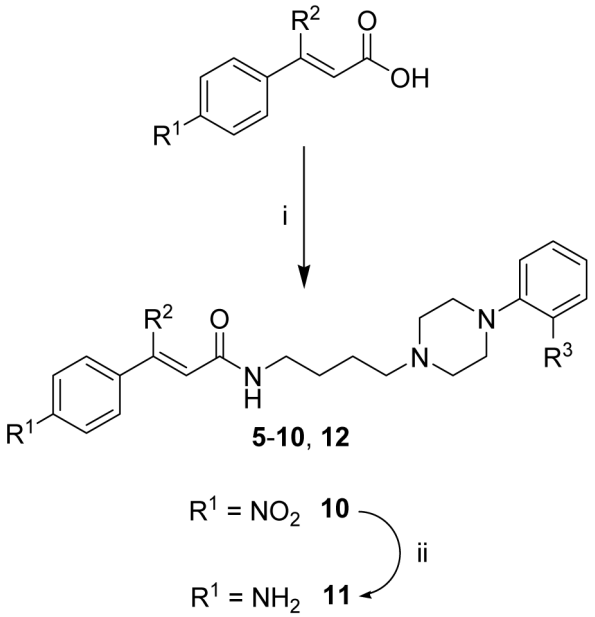
Reagents and conditions: (i) 4-bromobutyronitrile, acetonitrile, or acetone, K_2CO_3, reflux, 8 h; (ii) *Ra-Ni*, NH_3 aq., 25 bar H_2, 2 d; (iii) malonic acid, pyridine, piperidine, reflux, 3 h; (iv) oxalyl chloride, reflux, 2 h.

Scheme 1. Synthesis of compounds **1 – 4**.

receptor affinities to their 2-methoxyphenyl analogous [15]. Therefore, we have created a series of 3-phenylacryl amide derivatives with (2-halogenophenyl)piperazino residue. Most compounds of this series are (2-fluorophenyl)piperazine derivatives, which offer an advantage for the development of potential radioligands for positron emission tomography (PET) imaging of dopamine D_3 receptors. Radioiodination of *N*-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)-3-(4-iodophenyl)acryl amide might also provide a useful pharmacological tool for *in vivo* investigation of D_3 receptor distribution using single photon emission computed tomography (SPECT) imaging techniques. The newly designed compounds have been prepared and tested for their affinities at hD_2 and hD_3 receptors using [^{125}I]iodosulpiride as radioligand in displacement studies.

Chemistry

Alkylation of piperazine derivatives by 4-bromobutyronitrile led to the precursor compound **1a** (Scheme 1). Reduction of nitrile **1a** could be achieved by treatment with lithium aluminium hydride to obtain 4-(4-(2-chlorophenyl)piperazin-1-yl)butylamine **2a**. A more economic synthetic approach turned out to be the catalytic hydrogenation of the nitrile using Raney Nickel [16]. This method allowed scaling up to amounts of 20 g nitrile as educt and has been applied for synthesizing 4-(4-(2-fluorophenyl)piperazin-1-yl)butylamine **2b**. 4-Iodocinnamic acid **3** has been prepared by a classic Knoevenagel condensation of 4-iodobenzaldehyde with malonic acid in the presence of piperidine [17]. Decarboxylation of the product takes place *in situ* and the cinnamoyl structure of the product is clearly identified as *E*-isomer by its ^{1}HNMR spectrum (Scheme 1). The aryl amide moiety (Figure 1) of all compounds in this series is represented by a cinnamoyl residue. Cinnamic acid is a structural imitation of naphthalene-2-carboxylic acid of BP 897. The first connecting aryl ring of the 2-naphthyl residue has been replaced by a double bound, which provides the amide in a definite distance to the sterically fixed aryl structure. Formally, cinnamoyl is a ring-opened and truncated 2-naphthoyl imitate with slightly altered bond length and angle. Within this group, the compounds bear a variety of halide substituents as well as a nitro or amino group, each in 4-position (**5 – 11**). A different substitution pattern was chosen for compound **12**. An additional phenyl ring in the β-position of the double bond of the cinnamic acid basic structure has lead to considerably increased lipophilicity and bulkiness of this compound. The required cinnamoyl structure has been generated by a carboxylation reaction of oxalyl chloride with 1,1-diphenylethylene (Scheme 1) [18, 19]. Advantage has been taken from the unsubstituted phenyl structure as it disfavours an unwanted, possibly following additional decarboxylation reaction. The electronic influence of halogen substitution would have provoked the decarboxylation of the diphenylacrylic acid. Thus, for a smooth reaction the



Reagents and conditions: (i)a./thionyl chloride, CH_2Cl_2, Et_3N, 0°C, 10 min, rt, 1 h; b./ amine, CH_2Cl_2, Et_3N, 0°C, 10 min, rt, overnight. (ii) iron powder, $FeCl_3$, H_2O, reflux 15 h.

Scheme 2. Synthesis of compounds **5–12**.

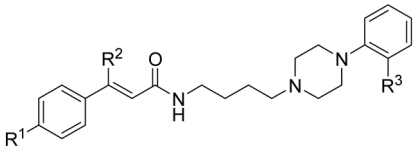
mixture could be heated to reflux without the product 3,3-diphenylacrylic acid **4** suffering from decomposition. Different pathways are possible for preparing the aminoalkyl spacer with alkanamino residue and aryl substituent, corresponding to the general structure pattern (Figure 1).

The cinnamic acid precursors have been converted into the corresponding acid chlorides by thionyl chloride and used directly without further purification for the final acylation of the primary amines **2** (Scheme 2). Yields have not been optimized on this reaction step. For the hydrogenation of compound **10** to the corresponding amino compound **11**, iron powder was taken in the presence of a catalytic amount of $FeCl_3$. Under these conditions, the nitro group is selectively reduced without affecting the double bound (Scheme 2) [20].

Pharmacological results and discussion

Following a molecular modeling study, which has been performed on analogous compounds (*e.g.* ST 168 and ST 186) *E*-cinnamide is established as a valuable structural basis [21]. These findings are confirmed by other potent *E*-cinnamoyl derivatives published [22]. Isomeric *Z*-derivatives have been reported to possess lower affinities [22]. ST 168 shows a similar D_3 receptor affinity but an increased selectivity compared to the unsubstituted cinnamoyl derivative ST 186 (Table 1). Therefore, we firstly had chosen the 4-chloro substituted cinnamoyl derivative ST 168 for further variations at the phenylpiperazino residue. Bioisosteric replacement of the 2-methoxy residue by 2-chloro substitution has lead to a decrease in D_3 receptor affinity and selectivity (Table 1). The 1-(2-fluorophenyl)piperazine derivative **8** has shown higher D_3 receptor affinity than the 2-chloro substituted compound **5** whereas D_2 receptor affinity has not been significantly different. Compound **8** has shown the best selectivity for D_3 receptors in this series. Methoxy- (of lead compound BP 897) and fluoro-substituted phenylpiperazines may show comparable physicochemical properties in aqueous environment. But, moreover, fluorine is expected to show higher metabolic stability and offers the development of a potential radioligand for PET imaging of dopamine D_3 receptors. Therefore, we have prepared a series of 4-substitued cinnamoyl derivatives (Table 1) containing compounds which have shown enhanced selectivity ratios and remarkable low to subnanomolar binding affinities at dopamine D_3 receptors. Within the 4-halogen substituted cinnamoyl derivatives, fluoro compound **9** has revealed the highest dopamine D_3 receptor affinity, whereas bromo derivative **7** has displayed an improved selectivity for the dopamine D_3 receptor. Due to its fluoro substitution of the cinnamoyl structure and the 2-fluoro-substituted phenylpiperazino residue, compound **9** offers two possibilities for ^{18}F radiolabeling. Best results for dopamine D_3 receptor selectivity have been obtained for iodo compound **6** and chloro compound **8**. Both compounds have shown a remarkable dopamine D_3 receptor affinity. Radioiodination of compound **6** might also provide a useful pharmacological tool for *in vivo* investigation of D_3 receptor distribution using SPECT imaging techniques. Nitro substitution (**10**) has also resulted in improved selectivity for the dopamine D_3 receptor, and has, additionally, led to increased dopamine D_3 receptor affinity. The amino-substituted analogue **11** has displayed a slightly deteriorated selectivity D_2/D_3 ratio, but has reached a superior binding affinity for the dopamine D_3 receptor. A different substitution pattern on the cinnamoyl structure has been performed on compound **12**. The phenyl substitution in β-position of the double bond has lead to a 3,3-diphenylacrylamido structure. Here, the lipophilic aryl residue is considerably increased and bulky. Spatial requirements are raised due to the voluminous diphenyl structure. When compared to the above discussed 4-substituted cinnamoyl derivatives, β-phenyl substitution has resulted in deteriorated dopamine D_3 receptor binding and consequently in deteriorated preference over the dopamine D_2 receptor.

Table 1. Chemical structures, physical data, and pharmacological screening results for human dopamine D_2 and D_3 receptor binding affinities.



No.	Formula	R^1	R^2	R^3	MW	MP (°C)	Yield (%)	K_i [nM][a]		hD_2/hD_3[b]
								hD_3	hD_2	
5	$C_{23}H_{27}Cl_2N_3O \times C_2H_2O_4 \times H_2O$	Cl	H	Cl	540.4	105.5[c]	19	18 ± 5	420 ± 240	23
6	$C_{23}H_{27}FIN_3O$	I	H	F	507.4	167.5[d]	9	3.2 ± 0.6	166.1 ± 27.6	52
7	$C_{23}H_{27}BrFN_3O \times 0.25H_2O$	Br	H	F	464.9	162.7[d]	10	7.9	170	22
8	$C_{23}H_{27}ClFN_3O \times 0.25H_2O$	Cl	H	F	420.4	158.7[d]	6	3.4	190	56
9	$C_{23}H_{27}F_2N_3O \times C_2H_2O_4$	F	H	F	489.5	174.8[e]	10	1.7 ± 0.4	25.6 ± 1.0	15
10	$C_{23}H_{27}FN_4O_3$	NO_2	H	F	426.5	164.8[d]	21	2.4	57	24
11	$C_{23}H_{29}FN_4O$	NH_2	H	F	396.5	151.0[d]	19	0.9	17.4	19
12	$C_{29}H_{32}FN_3O \times C_2H_2O_4$	H	Phenyl	F	547.6	164.8[f]	9	21.8	211	10
ST 186[g]		H	H	OCH_3				0.33 ± 0.1	13.54 ± 1.2	41
ST 168[g]		Cl	H	OCH_3				0.44 ± 0.09	22.6 ± 1.0	51
BP 897[h]								0.92 ± 0.2	61 ± 0.2	66

[a] Values (K_i) are mean of at least two independent determinations (±SEM of at least three independent determinations performed in triplicate).
[b] Ratio is calculated from corresponding K_i values.
[c] Crystallized from ethanol/diethylether.
[d] Crystallized from 2-propanol.
[e] Crystallized from methanol/diethylether.
[f] Crystallized from 2-propanol/diethylether.
[g] Ref. 14.
[h] Ref. 8.

In this study, compound **12** with its untypical structure has been functionally tested in a mitogenesis assay on [^{3}H]thymidine incorporation in NG 108-15 cells [23, 24]. Agonist-induced incorporation of [^{3}H]thymidine into growing cells has been used as basic principle. The intrinsic activity is quantified by determination of the effective concentration (EC_{50}) of the test compound and by comparing the maximum effect to that of the full agonist dopamine [25]. Results have surprisingly designated a full dopamine D_3 receptor agonist with an intrinsic activity of ~1 and an EC_{50} value of ~10 nM. 2,3-Chloro-disubstitution of the 4-phenylpiperazino residue would have lead to a typical antagonist structure [6]. As no typical agonist elements are given, the lack of antagonist properties complies with anticipation. The replacement of a 2-methoxy substituent, which is supposed to be indicative of partial agonism [6], by a 2-fluoro substituent in the 4-phenylpiperazino residue may have caused the change to full agonism. Since the intrinsic activity of this outstanding structure **12** could be attributed to its basic residue, no further mitogenesis tests have been performed within this series of compounds presented here.

Experimental

Chemistry

Melting points were determined on an Electrothermal IA 9000 digital or a Büchi (Germany) 512 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker (Germany) Avance DPX 400 (400 MHz) spectrometer. ^{1}H NMR data are reported in the following order: chemical shift (δ) in ppm downfield from tetramethylsilane as internal reference; multiplicity (br, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; quint, quintet; m, multiplet); approximate coupling constants (*J*) in Hertz (Hz); number and assignment of protons; *, exchangeable by D_2O; Pipera, piperazinyl. EI-MS was performed on a Finnigan (USA) Varian MAT CH7A or a MAT 212 Varian, FAB$^+$-MS on a Finnigan (USA) FAB MAT CH5DF (80 eV,) with xenon, DMSO as solvent, and a matrix of glycerol. Data are listed as mass number (m/z) and relative intensity (%). Elemental analyses (C, H, N) were measured on Perkin Elmer (USA) 240 B, Perkin Elmer (USA) 240 C, Vario-EL (Perkin Elmer, USA) or CHN-Rapid (Heraeus, Germany) and were within ± 0.4% of the theoretical values for all compounds. Hydrogenations were carried out on an autoclave model IV, 500 mL (Roth, Germany). Preparative, centrifugally accelerated, rotatory chromatography was performed using a Chromatotron 8924 (Harrison Research, USA) and glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck, Germany). Analytical thin layer chromatogra-

phy (TLC) was performed on silica gel 60 F_{254} plates (Merck, Germany). The spots were visualized with fast blue salt B, ninhydrin or by UV absorption at 254 nm. Chemical procedures are exemplary described for the fluoro compounds.

4-(4-(2-Fluorophenyl)piperazin-1-yl)butyronitrile ***1b***

A mixture of 1-(2-fluorophenyl)piperazine (40 mmol), 4-bromobutyrontrile (44 mmol), K_2CO_3 (60 mmol), and a catalytic amount of KI in 100 mL of acetone was heated to reflux for 8 h and then stirred at ambient temperature overnight. The suspension was filtrated and the residue washed with acetone. The filtrate was evaporated to dryness and dried under reduced pressure. The product suited well for the next reaction step without further purification. Yield 84%. $C_{14}H_{18}FN_3$ (247.32), colorless oil. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.13 – 7.08 (m, 2H, F-Ph-3H, -5H), 7.03 – 6.93 (m, 2H, F-Ph-4H, -6H), 3.01 (t, *J* = 4.7, 4H, Pipera-2H, -6H), 2.53 (t, *J* = 4.6, 4H, Pipera-3H, -5H), 2.49 (partially covered by DMSO peak, m, 2H, NC-CH_2), 2.42 (t, *J* = 6.9, 2H, CH_2-Pipera), 1.75 (2H, NC-CH_2-CH_2). EI-MS: 247 (M^+, 72), 207 (52), 193 (100).

4-(4-(2-Fluorophenyl)piperazin-1-yl)butylamine ***2b***

Reduction of the nitrile **1b** (30 mmol) was accomplished with freshly prepared Raney nickel (from 90 mmol aluminum-nickel-alloy) in 150 mL of aqueous ammonia 25% and 25 bar H_2 pressure at room temperature for 2 d. Cautious filtration and evaporation to dryness delivered an oily product, which crystallized as hydrogen dioxalate from 2-propanol. Yield 74%. $C_{14}H_{22}FN_3 \times 2C_2H_2O_4$ (431.42), m.p. 90-103°C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.99 (s*, br, 1H, H_2N), 7.19 – 7.00 (m, 4H, F-Ph), 3.22 (brs, 4H, Pipera-2H, -6H), 3.11 (brs, 4H, Pipera-3H, -5H), 2.91 (t, *J* = 7.0, 2H, H_2N-CH_2), 2.82 (t, *J* = 7.2, 2H, CH_2-Pipera), 1.69 – 1.67 (m, 2H, H_2N-CH_2-CH_2), 1.63 – 1.58 (m, 2H, CH_2-CH_2-Pipera). FAB$^+$-MS: 252 ([M+H]$^+$, 92), 72 (100).

4-Iodocinnamic acid ***3***

Malonic acid (10 mmol) was dissolved in 50 mL of pyridine and 0.5 mL of piperidine. The mixture was protected from light and stirred under argon. Then, 4-iodobenzaldehyde (8 mmol) was added and the mixture heated to reflux. After 3 h no more aldehyde was detected by TLC. The mixture was cooled down to ambient temperature and the acid liberated with 10 N HCl and ice under vigorous stirring. The solid product obtained was washed with water several times and dried extensively under reduced pressure. Yield 86%. $C_9H_7IO_2$ (274.05), m.p. 127.0°C. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 13.99 (brs*, 1H, COOH), 7.79 (d, *J* = 8.2, 2H, I-Ph-3H, -5H), 7.54 (d, *J* = 16.1, 1H, I-Ph-C*H*), 7.49 (d, *J* = 8.3, 2H, I-Ph-2H, -6H), 6.57 (d, *J* = 16.0, 2H, C*H*-COOH). EI-MS: 274 (M^+, 100).

3,3-Diphenylacrylic acid ***4***

Compound was prepared according to known methods [19]. Yield 44%. $C_{15}H_{12}O_2$ (224.26), m.p. 161.5°C (m.p. 167°C [19]). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 12.44 (s*, 1H, COOH), 7.41-7.36 (m, 3H, *Z*-Ph-3H, 4-H, 5-H, 3H, *E*-Ph-3H, -4H, -5H), 7.27-7.25 (m, 2H, *Z*-Ph-2H, -6H), 7.16-7.14 (m, 2H, *E*-Ph-2H, -6H). EI-MS: 224 (M^+, 100).

General procedure for preparation of 3-phenylacryl amides 5-10, ***12***

Thionyl chloride (30 mmol) was added to 2 mL of dry CH_2Cl_2 and a catalytic amount of triethylamine. This mixture was protected from light, cooled with ice, and stirred under argon. 3.0 mmol of the carboxylic acid was added carefully. The mixture was stirred for 10 min under ice and for 1 h without cooling. Then, the solvent and the excessive thionyl chloride were removed under reduced pressure. The crude acid chloride obtained was dissolved in 4 mL of dry CH_2Cl_2 and given to a mixture of amine **2** (2.7 mmol), triethylamine (8.1 mmol), and 7 mL of dry CH_2Cl_2, which had been stirred under argon and ice cooling for 10 min. Stirring continued under additional light protection overnight, until no more amine could be detected by TLC control. The solvent was removed under reduced pressure and the mixture stirred with water to give a light brown, solid product. Water was decanted and the product recrystallized from 2-propanol. Further purification using a chromatotron (PE, $CHCl_3$ 1 : 1, atm of NH_3) afforded the product.

3-(4-Chlorophenyl)-N-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide ***5***

^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.17 (m*, 1H, NH), 7.59 (d, *J* = 8.1, 4-Cl-Ph-2H, -6H), 7.44 (m, 4H, 4-Cl-Ph-3H, -5H, 4-Cl-Ph-CH, 2-Cl-Ph-3H), 7.31 (m, 1H, 2-Cl-Ph-4H), 7.18 (m, 1H, 2-Cl-Ph-6H), 7.09 (m, 1H, 2-Cl-Ph-5H), 6.73 (d, *J* = 15.8, 1H, CO-CH), 3.39-2.66 (partially covered by H_2O peak, 12H, CONH-CH_2, 8-Pipera-H, 1-Pipera-CH_2), 1.76-1.52 (m, 4H, CONH-CH_2-CH_2-CH_2). EI-MS: 430 (M^+, 7), 209 (100), 194 (20), 165 (88), 154 (27), 139 (28), 125 (13), 70 (91), 56 (31), 44 (23). Anal. calcd: C, 55.6; H, 5.78; N, 7.78; found: C, 55.2; H, 5.48; N, 7.38.

N-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)-3-(4-iodophenyl)acryl amide ***6***

^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.12 (t, *J* = 5.8, 1H, NH), 7.77 (d, *J* = 8.2, 2H, I-Ph-3H, -5H), 7.36 (d, *J* = 15.1, 1H, I-Ph-C*H*, d, *J* = 8.6, 2H, I-Ph-2H, -6H), 7.13-7.07 (m, 2H, F-Ph-3H, -5H), 7.03 – 6.93 (m, 2H, F-Ph-4H, -6H), 6.66 (d, *J* = 15.8, 1H, CH-CO), 3.20 (d, br, *J* = 5.5, 2H, HN-CH_2), 3.00 (t, *J* = 4.3, 4H, Pipera-2H, -6H), 2.51-2.50 (partially covered by DMSO peak, m, 4H, Pipera-3H, -5H), 2.34 (brs, 2H, CH_2-Pipera), 1.49 (brs, 4H, HN-CH_2-CH_2-CH_2). EI-MS: 507 ($M^{+\cdot}$, 5), 57 (16), 193 (100) FAB$^+$-MS: 508 ([M+H]$^+$, 100). Anal. calcd: C, 54.45; H, 5.36; N, 8.28; found: C, 54.24; H, 5.29; N, 8.23.

3-(4-Bromophenyl)-N-{4-[4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide ***7***

^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.12 (t, *J* = 5.4, 1H, NH), 7.61 (d, *J* = 8.4, 2H, Br-Ph-3H, -5H), 7.51 (d, *J* = 8.4, 2H, Br-Ph-2H, -6H), 7.39 (d, *J* = 15.8, 1H, Br-Ph-C*H*), 7.13-7.07 (m, 2H, F-Ph-3H, -5H), 7.03 – 6.93 (m, 2H, F-Ph-4H, -6H), 6.65 (d, *J* = 15.8, 1H, CH-CO), 3.21 – 3.19 (m, 2H, HN-CH_2), 3.00 – 2.99 (m, 4H, Pipera-2H, -6H), 2.51 (partially covered by DMSO peak, brs, 4H, Pipera-3H, -5H), 2.34 (brs, 2H, CH_2-Pipera), 1.49 (brs, 4H, HN-CH_2-CH_2-CH_2). FAB$^+$-MS: 463 (2), 462 (6), 461 (2), 460 ([M+H]$^+$, 6), 209 (4), 193 (6). Anal. calcd: C, 59.42; H, 5.96; N, 9.04; found: C, 59.42; H, 6.11; N, 8.85.

3-(4-Chlorophenyl)-N-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide ***8***

^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.12 (t, *J* = 5.5, 1H, NH), 7.58 (d, *J* = 8.5, 2H, Cl-Ph-2H, -6H), 7.47 (d, *J* = 8.5, 2H, Cl-Ph-3H, -5H), 7.40 (d, *J* = 15.8, 1H, Cl-Ph-C*H*), 7.13 – 7.07 (m, 2H, F-Ph-3H, -5H), 7.03-6.93 (m, 2H, F-Ph-4H, -6H), 6.63 (d, *J* = 15.8, 1H, CH-CO), 3.20 – 3.19 (m, 2H, HN-CH_2), 3.00 – 2.99 (m, 4H, Pipera-2H, -6H), 2.51 (partially covered by DMSO peak, brs, 4H, Pipera-3H, -5H), 2.34 (brs, 2H, CH_2-Pipera), 1.49 (brs, 4H, HN-CH$_2$-CH_2-CH_2). Anal. calcd: C, 65.71; H, 6.59; N, 9.99; found: C, 65.58; H, 6.71; N, 9.80.

3-(4-Fluorophenyl)-N-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide ***9***

^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.18 (t, *J* = 5.4, 1H, NH), 7.63 (dd, *J* = 5.6, 2H, F-Ph), 7.42 (d, *J* = 15.8, 1H, 4-F-Ph-C*H*), 7.25 (t, *J* = 8.8, 2H, F-Ph), 7.19 – 7.00 (m, 4H, F-Ph), 6.59 (d, *J* = 15.8, 1H, CH-CO), 3.22 – 3.21 (m, 2H, HN-CH_2, 4H, Pipera-2H, -6H), 3.15 (brs, 4H, Pipera-3H, -5H), 2.95 (t, *J* = 7.7, 2H, CH_2-Pipera), 1.66 – 1.62 (m, 2H, HN-CH$_2$-CH_2), 1.54-1.49 (m, 2H, CH_2-CH$_2$-Pipera). FAB$^+$-MS: 400 ([M+H]$^+$, 2), 149 (3). Anal. calcd: C, 61.34; H, 5.97; N, 8.58; found: C, 60.97; H, 5.92; N, 8.25.

N-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)-3-(4-nitrophenyl)acryl amide ***10***

^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.26 (d, *J* = 8.6, 1H, NH, 2H, O$_2$N-Ph-3H, -5H), 7.83 (d, *J* = 8.7, 2H, O$_2$N-Ph-2H, -6H), 7.53 (d, *J* = 15.8, 1H, O$_2$N-Ph-C*H*), 7.13-7.07 (m, 2H, F-Ph-3H, -5H), 7.03 – 6.93 (m, 2H, F-Ph-4H, -6H), 6.82 (d, *J* = 15.8, 1H, CH-C*O*), 3.23-3.22 (m, 2H, HN-CH_2), 3.00 – 2.99 (m, 4H, Pipera-2H, -6H), 2.51 (partially covered by DMSO peak, brs, 4H, Pipera-3H, -5H), 2.34 (brs, 2H, CH_2-Pipera), 1.50 (brs, 4H, HN-CH$_2$-CH_2-CH_2). FAB$^+$-MS: 427 ([M+H]$^+$, 80), 193 (89). Anal. calcd: C, 64.77; H, 6.38; N, 13.14; found: C, 64.56; H, 6.49; N, 13.16.

N-(4-(4-(2-Fluorophenyl)piperazin-1-yl)butyl)-3,3-diphenylacryl amide ***12***

^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.95 (t, *J* = 5.6, 1H, NH), 7.37 – 7.31 (m, 6H, Ph), 7.23 – 7.21 (m, 2H, Ph), 7.19-7.00 (m, 2H, Ph, 4H, F-Ph), 6.44 (s, 1H, CH-CO), 3.21 (brs, 4H, Pipera-2H, -6H), 3.10 (brs, 4H, Pipera-3H, -5H), 3.03 (dd, *J* = 6.6, 2H, HN-CH_2), 2.88 (t, *J* = 7.7, 2H, CH_2-Pipera), 1.56 – 1.49 (m, 2H, HN-CH$_2$-CH_2), 1.39 – 1.32 (m, 2H, CH_2-CH$_2$-Pipera). FAB$^+$-MS: 459 (18), 458 ([M+H]$^+$, 52), 207 (100). Anal. calcd: C, 67.99; H, 6.26; N, 7.67; found: C, 67.69; H, 6.05; N, 7.37.

3-(4-Aminophenyl)-N-(4-(4-(2-fluorophenyl)piperazin-1-yl)butyl)acryl amide ***11***

Iron powder (2.1 mmol), a catalytic amount of $FeCl_3$, and compound **10** (2 mmol) were suspended in 10 mL of H_2O. The mixture was heated to reflux for 15 h. During this time, catalytic amounts of iron powder (0.2 mmol) and $FeCl_3$ were added twice. Cooled down to ambient temperature, the mixture was quenched with 2 N NaOH and filtrated. The filtrate was extracted with CH_2Cl_2 three times. The combined organic layers were treated with brine and evaporated to dryness. The yellow oil obtained was purified using a chromatotron ($CHCl_3$, atm of NH_3). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.82 (t, *J* = 5.5, 1H, NH), 7.23 (d, *J* = 15.9, 1H, H$_2$N-Ph-C*H*), 7.21 (d, *J* = 8.4, 2H, H$_2$N-Ph-2H, -6H), 7.13 – 7.07 (m, 2H, F-Ph-3H, -5H), 7.03 – 6.92 (m, 2H, F-Ph-4H, -6H), 6.55 (d, *J* = 8.5, 2H, H$_2$N-Ph-3H, -5H), 6.28 (d, *J* = 15.7, 1H, CH-CO), 5.51 (s*, 2H, H$_2$N), 3.17 (d, *J* = 5.5, 2H, HN-CH_2), 3.00 – 2.99 (m, 4H, Pipera-2H, -6H), 2.51 (partially covered by DMSO peak, s, 4H, Pipera-3H, -5H), 2.33 (brs, 2H, CH_2-Pipera), 1.47 (brs, 4H, HN-CH$_2$-CH_2-CH_2). EI-MS: 396 (M$^{+\bullet}$, 5), 193 (36), 146 (100). Anal. calcd: C, 69.67; H, 7.37; N, 14.13; found: C, 69.67; H, 7.56; N, 13.96.

Pharmcological assays

Binding studies

For binding assays, Chinese hamster ovary (CHO) cells were stably transfected with cDNA of human D_{2L} and D_3 receptors and cloned [26, 27]. Binding to dopamine D_2 and D_3 receptors was evaluated by displacement studies with [^{125}I]iodosulpiride [28]. K_i values are calculated from the IC_{50} values according to the Cheng–Prusoff equation [29]. These cell lines were cultured in Dulbecco's Modified Eagle Medium, which was supplemented in 10% fetal calf serum, in an atmosphere of 5% CO_2. Cells were harvested from culture dishes in the presence of 0.2% trypsin, were centrifuged at 2000 *g* for 5 min and then homogenized in 10 mM Tris-HCl, pH 7.4, containing 5 mM $MgCl_2$, using a Polytron. The homogenate was centrifuged at 20 000 *g* for 15 min at 4°C and the pellet was resuspended by sonication in 50 mM of Tris-HCl, pH 7.4, containing: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; and $MgCl_2$, 8 mM (incubation buffer). Membranes were used either immediately or after storage at −70°C. A membrane volume of 200 µL, diluted in incubation buffer supplemented with 0.2% bovine serum albumin was added to polystyrene tubes containing (in 100 µL) 0.1 nM [^{125}I]iodosulpiride and drug, diluted in 100 µL of incubation buffer. Nonspecific binding was determined in the presence of 1 µM nemonapride. Incubations were run at 30°C for 30 min. Reactions were stopped by vacuum filtration through Whatman (Great Britain) GF/B glass-fiber filters coated in 0.3% polyethylenimine with automated cell harvester (Brandel-Beckman, Gaithersburg, MD, USA). Filters were rinsed three times with 5 mL of ice-cold incubation buffer and counted by liquid scintillation in 5 mL of ACS II (Amersham, USA).

Functional receptor tests

A mitogenesis test was performed on NG 108-15 cells expressing the dopamine D_3 receptor, measuring [^{3}H]thymidine incorporation [28]. NG 108-15 cells expressing the human D_3 receptor were cultured in Dulbecco's Modified Eagle Medium supplemented in 10% fetal calf serum in an atmosphere of 5% CO_2 and were plated in collagen-coated 96-well plates. After a 24 h culture, cells were washed twice with culture medium without feral calf serum and incubated for 16 h with 1 µM forskoline and quinpirole in increasing concentrations, in the absence or presence of compounds at 1.5, 3, 30, or 300 nM. Then, [^{3}H]thymidine (1 µCi/well) was added for 2 h and cells were harvested by vacuum filtration through Whatman GF/C glass-fiber filters using an automated cell harvester. The filters were rinsed 15 times with 200 µL of phosphate buffered saline. Radioactivity was counted by liquid scintigraphy in 5 mL of ACS II.

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