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Synthesis of novel lactam derivatives and their evaluation as ligands for the dopamine receptors, leading to a D_4-selective ligand

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Abstract—The preparation of some lactam (cyclic amide) derivatives bearing various phenylpiperazinylbutyl side chains attached to the amide nitrogen together with their dopamine receptor affinity study is described. The synthesis of the target compounds involved the preparation of the intermediate bromobutyl derivatives of the appropriate lactam followed by N-alkylation of the appropriate phenylpiperazines with these intermediates. Radioligand binding studies at D_2–D_5 receptor subtypes and a functional calcium assay of the target compounds at D_2 and D_5 receptor subtypes were performed. All compounds, except **12a** and **12b**, showed selectivity towards the D_2-like receptor subtypes. Selectivity of the indolinone derivatives **11a–d** at the D_4 receptors was observed. Compound **11b** exhibited a remarkable affinity to hD_4 receptors with K_i value of 0.04 ± 0.02 nm and was >43,000-fold selective over the hD_2 receptor. In the functional assay, all the active compounds were of antagonistic activity.

1. Introduction

Disturbances of the dopaminergic system account for many neurological and neuropsychiatric disorders including Parkinson's disease, Tourette's syndrome, schizophrenia, tardive dyskinesia and addiction to psychostimulants.[1,2] Five subtypes of dopamine receptors have been identified: D_1, D_2, D_3, D_4 and D_5. These subtypes are generally grouped into two families of dopamine receptors, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors, based on similar pharmacologic properties and primary structure homology.[2,3] Recent studies have revealed that the dopamine D_3 receptor subtype may be an important biological target for pharmacotherapeutic agents used in treatment of schizophrenia.[4] Essentially, all clinically useful neuroleptics are antagonists at the D_2 and D_3 receptors. Therefore, it has been concluded that the blockade of D_2 receptor in the caudate putamen region of the brain may be responsible for extrapyramidal side effects, while blockade of D_2/D_3 receptors in the limbic regions of the brain may be associated with antipsychotic effects.[5,6] This suggests that the D_3 receptor subtype may be a good pharmacological target for the development of antipsychotic agents with low risk of extrapyramidal side effects.

Several reports had shown molecules with a butylamide linking chain, an extended aromatic terminus on one end and an arylpiperazine on the other end are selective D_3 ligands with the type of intrinsic activity almost dependent on the phenyl substituent, for example, BP 897, NGB 2904 and GR 103691.[7–9] Structurally similar compounds made up of cyclic benzamides linked through an alkyl spacer to arylpiperazines were evaluated as mixed D_2/5-HT_2 receptor antagonists and atypical antipsychotic agents. In most of successful cases, the cyclic benzamide building blocks were linked to various arylpiperazines through a 4-carbon spacer that has been proved related to optimum activity.[10–12] As pointed out by Norman et al., the butyl spacer enabled the compound to adopt a folded conformation stabilized by an intramolecular hydrogen bond between the protonated piperazine and the amide carbonyl. This flexible butyl chain, in turn, allowed an optimum conformation and distance between the terminal pharmacophores resulting in optimum binding to the D_2 receptors[11] (Fig. 1).

Moreover, the dopaminergic activities of phenylpiperazines are not limited to D_3 receptors, thus phenylpiperazine derivatives linked to various heteroaryls by a

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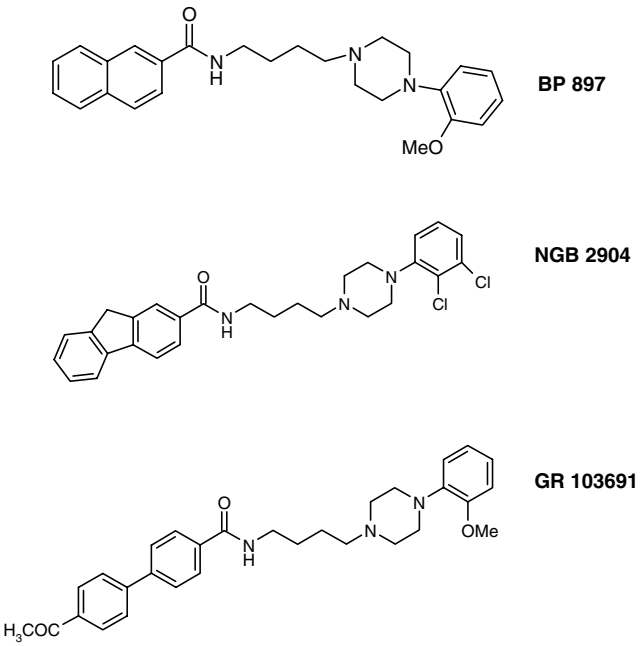


Figure 1. D_3 selective ligands with the general skeleton of an exocyclic amide butyl spacer, linking an extended aromatic terminus on one end and an arylpiperazine on the other.

methylene bridge looks like template for D_4 selectivity.[13,14]

It is deem of interest to test if the D_3 selectivity will be retained for structurally similar compounds with the amide in an endocyclic rather than an exocyclic form.

Thus, the goal of the current study is to explore the structure–activity/affinity relationships of various arylpiperazinylbutyllactams as candidates for dopamine receptors' ligands. Several heterocycles possessing cyclic amides particularly, saccharin, 4-quinazolinone, indolinone and isoindolinone linked through a butyl chain to various phenylpiperazines were prepared and evaluated in vitro for their binding affinities by radioligand-binding experiments and functional activities using a calcium fluorescence assay at different dopamine receptors. The protocol of these experiments has been described by us previously.[15–17]

2. Chemistry

Sixteen lactam derivatives **9**–**12**(**a**–**d**) bearing the butyl piperazinyl un- or substituted-phenyl moiety were prepared as outlined in Scheme 1. The general approach for the preparation of the target compounds involved alkylation of the appropriate phenylpiperazine with the appropriate bromobutyl lactam derivative. The required intermediate alkylating agents **5**–**8** were synthesized according to published procedures by the reaction of the precursor lactams **1**–**4** with 1,4-dibromobutane. The intermediate bromo derivatives **5**–**8** were then reacted with the appropriate phenylpiperazine in refluxing acetonitrile in the presence of triethylamine under nitrogen atmosphere to give the final compounds.

3. Results and discussion

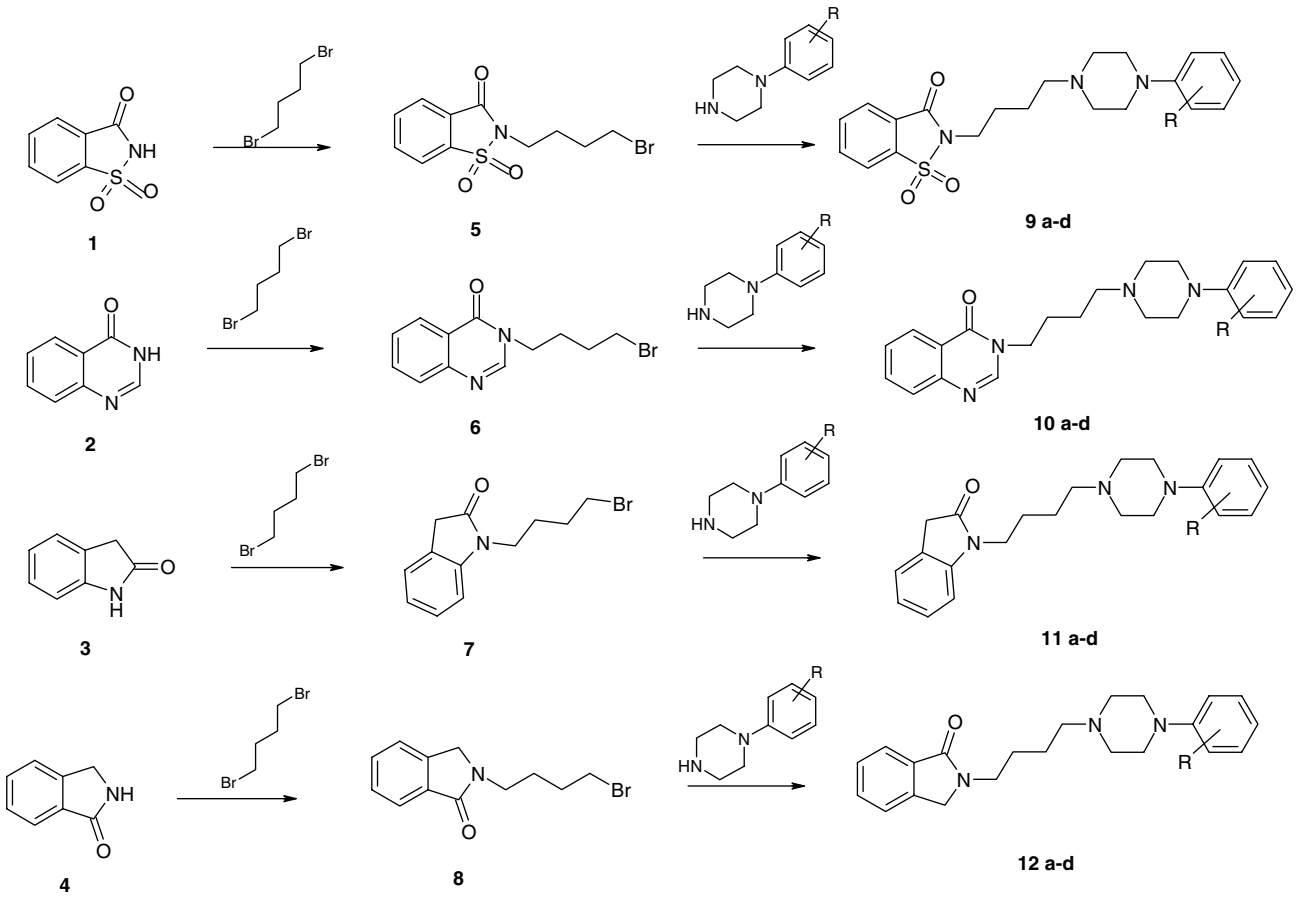
Table 1 shows binding data for the target compounds on hD_{2L}, hD_3, $hD_{4.4}$ and hD_5 receptors, stably expressed in CHO or HEK293 cells. [^{3}H]-SCH 23390 and [^{3}H]-Spiperone were used as radioligands for experiments at the D_1-like and D_2-like receptor family, respectively. The functional assay has been made upon hD_2 and hD_5 receptors.

Binding data reveal general affinity of the target compounds towards the D_2-like family of dopamine receptors with the exception of the isoindolinone derivatives **12a**,**b** that show D_5 dopamine receptor affinity. Affinities of compounds **9**,**10** (**a**–**d**) are almost distributed between the D_2 and D_4 receptors with compounds **9a** and **9d** being the most potent at D_2 receptors and **10d** being the most potent at D_4 antagonist as revealed by the functional assay, K_i = 1.2 nm. All the indolinone derivatives **11**(**a**–**d**) were selective to the D_4 receptors, this is regardless of the nature of the phenyl substituent. Compound **11b** is the most potent, with K_i = 0.04 nm for the D_4 receptors, thus it is >43,000-fold selective over the hD_2 receptor, >30,000-fold selective over the D_3 and >5000-fold more selective over the D_5 receptors, respectively.

Interestingly, compounds **12**(**a**–**d**), which are the positional isomers of **11**(**a**–**d**), exhibit a different affinity profile compared to all of the previous series. The affinity of these compounds is equally distributed between the two dopamine receptor families with compounds **12a**,**b** showing selectivity at D_5 receptors and **12c**,**d** showing selectivity at D_3 receptors.

The impact of the phenylpiperazine substituent viz. H, 2-OCH_3, 3-CF_3 and 4-Cl function upon activity is not absolute. However, compounds with the H substituent **9d**, **10d**, **11d**, **12d** were more biased towards the D_4 subtype selectivity; meanwhile compounds with the 3-CF_3 substituent **9c**, **10c**, **11c**, **12c** are more biased towards imparting D_3 subtype selectivity.

All the active compounds in the functional assay were of antagonistic activity and they were more active on the D_2 rather than D_5. The functional assay is dependent on the fact that stimulation of the dopamine receptors by agonists, for example, quinpirole for D_2-like receptors and SKF 38393 for hD_5 will cause an increase in intracellular Ca^{2+} which appears to represent a universal second messenger signal for a majority of recombinant GPCRs. Assay of the produced Ca^{2+} can be performed using Oregon Green 488 BAPTA-1/AM and a microplate reader. Dopamine agonists or antagonists are expected to alter the response to the standard agonists. The microplate reader based calcium assay is a very useful method for simply and quickly selecting the active compounds out of a considerable number of compounds. Comparing radioligand and calcium data, it can be seen that there are differences in the K_i values obtained, as the calcium-assay monitors a fast calcium-signal, these results represent non-equilibrium data, whereas radioligand binding studies were performed



Scheme 1.

under equilibrium conditions. The assay is fast, simple, and avoids the use of radioactivity.[16]

From the obtained results it seems that the D_3 selectivity is in great part dependent on the presence of the amide of the butylamido spacer in an exocyclic form, while its presence in an endocyclic form will lead to switch of affinities to other dopamine receptor subtypes.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Dimethylformamide (DMF) and acetonitrile were dried over molecular sieve 4Å and anhydrous sodium sulfate, respectively. Column chromatography was performed using silica gel (s.d. fine-chem. limited MUMBAI 400025, mesh size 200–400) using chloroform/ethanol (9:1) as eluent. TLC was performed on FLUKA silica gel TLC aluminium cards (0.2 mm thickness) with fluorescent indicator 254 nm. Melting points were determined in open capillaries on Electrothermal melting-point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ on Varian spectrometer at 300 MHz using tetramethylsilane (TMS) as internal reference. Chemical shift values are given in ppm. High resolution mass spectra (GC-HRMS) were recorded on an Agilent Mass spectrometer using positive ion electrospray ionization(ESI, purity >95%). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Elemental analysis of compound **11a** was made by the Microanalytical Unit, Faculty of Science, Cairo University, Egypt. IR spectra were recorded on Bruker FT-IR spectrophotometer as thin film for oils or as potassium bromide disc for solids. Compounds **2**, **4**, **5**–**8** were prepared according to the published procedures.[18–20]

4.2. General procedure for the preparation of the substitutedphenylpiperazine derivatives 9–12(a–d)

A mixture of **5**–**8** (0.5 mmol), the appropriate phenylpiperazine (0.55 mmol) and triethylamine (2 mL) in dry DMF (5 mL) was heated at 120 °C under nitrogen. The course of the reaction was followed by TLC. At the end of the reaction time, the reaction mixture was poured on to ice-water and extracted with chloroform. The extracts were dried over anhydrous sodium sulfate, filtered and evaporated untill dryness under reduced

Table 1. Radioligand binding study and functional calcium assay of compounds[a] **9–12**

Compound	Radioligand binding study		Calcium assay	
	Change % of receptor bound radioactivity by a 10 μM solution of the compound	K_i-values [nM] Average ± SD or SEM (number of experiments in triplicate)[b]	Change % of the agonist induced fluorescence by a 10 μM solution of the compound	K_i-values [nM] Average ± SD or SEM (number of experiments each including six values)[b]
9a	D_2 −100 D_3 −99 D_4 −101 D_5 −75	D_2 38 ± 2 (2) D_3 66 ± 4 (2) D_4 136 ± 75 (3) D_5 1629 ± 216 (2)	D_2 −62 D_5 −12	D_2 4.1 ± 3.1 (4 × 6) D_5 386 ± 131 (2 × 6)
9b	D_2 −99 D_3 −80 D_4 −101 D_5 −87	D_2 177 ± 58 (2) D_3 287 ± 43 (2) D_4 36 ± 13 (3) D_5 230 ± 15 (2)	D_2 −24 D_5 +4	D_2 >10,000 D_5 inactive
9c	D_2 −100 D_3 −98 D_4 −54 D_5 −89	D_2 113 ± 11 (2) D_3 107 ± 72 (3) D_4 >10,000 D_5 1756 ± 636 (2)	D_2 −44 D_5 +8	D_2 >10,000 D_5 inactive
9d	D_2 −100 D_3 −94 D_4 −83 D_5 −90	D_2 360 ± 31 (2) D_3 187 ± 33 (2) D_4 79 ± 18 (2) D_5 1132 ± 30 (2)	D_2 −56 D_5 +5	D_2 6.2 ± 4.2 (3 × 6) D_5 inactive
10a	D_2 −100 D_3 −89 D_4 −96 D_5 −5	D_2 46 ± 7 (2) D_3 527 ± 295 (3) D_4 81 ± 26 (2) D_5 >10,000	D_2 −37 D_5 +7	D_2 >10,000 D_5 inactive
10b	D_2 −100 D_3 −81 D_4 −102 D_5 −76	D_2 140 ± 92 (4) D_3 1780 ± 161 (2) D_4 158 ± 42 (3) D_5 507 ± 78 (3)	D_2 −44 D_5 +5	D_2 >10,000 D_5 inactive
10c	D_2 −107 D_3 −98 D_4 −98 D_5 −76	D_2 73 ± 9 (2) D_3 102 ± 13 (2) D_4 185 ± 79 (3) D_5 1703 ± 44 (2)	D_2 −38 D_5 +9	D_2 >10,000 D_5 inactive
10d	D_2 −105 D_3 −86 D_4 −91 D_5 −56	D_2 184 ± 14 (2) D_3 306 ± 8.5 (2) D_4 123 ± 66 (3) D_5 >10,000	D_2 −58 D_5 +2	D_2 1.2 ± 0.66 (2 × 6) D_5 inactive
11a	D_2 −106 D_3 −99 D_4 −100 D_5 −94	D_2 162 ± 14 (2) D_3 80 ± 27 (2) D_4 4.2 ± 1.8 (2) D_5 313 ± 47 (2)	D_2 −58 D_5 −2	D_2 153 ± 14 (2 × 6) D_5 >10,000
11b	D_2 −106 D_3 −83 D_4 −101 D_5 −93	D_2 1747 ± 118 (2) D_3 1204 ± 512 (3) D_4 0.04 ± 0.02 (3) D_5 202 ± 95 (2)	D_2 −28 D_5 +8	D_2 375 ± 23 (2 × 6) D_5 inactive
11c	D_2 −105 D_3 −96 D_4 −96 D_5 −93	D_2 323 ± 20 (2) D_3 247 ± 64 (3) D_4 28 ± 13 (2) D_5 190 ± 5 (5)	D_2 −55 D_5 +26	D_2 416 ± 108 (2 × 6) D_5 inactive
11d	D_2 −108 D_3 −97 D_4 −97 D_5 −95	D_2 405 ± 91 (2) D_3 195 ± 30 (2) D_4 9.6 ± 0.9 (2) D_5 330 ± 36 (2)	D_2 −54 D_5 +14	D_2 286 ± 3 (2 × 6) D_5 inactive
12a	D_2 −109 D_3 −100 D_4 −96 D_5 −80	D_2 189 ± 62 (3) D_3 35.8 ± 5.0 (2) D_4 216 ± 56 (2) D_5 80 ± 7.2 (2)	D_2 −54 D_5 −2	D_2 60 ± 42 (2 × 6) D_5 >10,000
12b	D_2 −105 D_3 −75	D_2 760 ± 118 (2) D_3 660 ± 206 (3)	D_2 −7 D_5 +21	D_2 >10,000 D_5 inactive

Table 1 (*continued*)

Compound	Radioligand binding study		Calcium assay	
	Change % of receptor bound radioactivity by a 10 μM solution of the compound	K_i-values [nM] Average ± SD or SEM (number of experiments in triplicate)[b]	Change % of the agonist induced fluorescence by a 10 μM solution of the compound	K_i-values [nM] Average ± SD or SEM (number of experiments each including six values)[b]
	D_4 −91 D_5 −100	D_4 142 ± 18 (2) D_5 33.6 ± 2.8 (2)		
12c	D_2 −106 D_3 −99 D_4 −95 D_5 −99	D_2 387 ± 2 (2) D_3 14 ± 4.2 (3) D_4 368 ± 176 (3) D_5 80.8 ± 5 (2)	D_2 −53 D_5 +41	D_2 26 ± 3.9 (2 × 6) D_5 inactive
12d	D_2 −107 D_3 −98 D_4 −94 D_5 −89	D_2 319 (1) D_3 30 ± 23 (3) D_4 134 ± 22 (2) D_5 75.4 ± 21 (2)	D_2 −52 D_5 −12	D_2 34 ± 19 (2 × 6) D_5 137 ± 59 (2 × 6)

[a] Binding assays using [^{3}H]-Spiperone for D_2, D_3 and D_4 and [^{3}H]-SCH 23390 for D_5.
[b] SD, standard deviation; SEM, standard error of the mean; the SEM is used when the number of values is less than three.

pressure. The crude compounds were purified using column chromatography.

4.3. 2-(4-(4-(2-Methoxyphenyl)-1-piperazinyl)butyl)-1,2-benzisothiazole-3(2*H*)-one 1,1-dioxide (9a)

Yield 45%; mp 107–109 °C; ^{1}H NMR δ 1.69 (m, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.916 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.48 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.664 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.09 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 3.80 (t, 2H, J = 7.2 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 3.85 (s, 3H, —OCH$_3$), 6.83–6.98 (m, 4H, aromatic H), 7.813–7.903 (m, 3H, aromatic H), 8.043 (d, 1H, J = 5 Hz, aromatic H). HRMS calculated for $C_{22}H_{28}N_3O_4S$ (M+H$^+$) 430.1801, found 430.1792.

4.4. 2-(4-(4-(4-Chlorophenyl)-1-piperazinyl)butyl)-1,2-benzisothiazole-3(2*H*)-one 1,1-dioxide (9b)

Yield 52%; mp 119–121 °C; ^{1}H NMR δ 1.66 (m, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.92 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.48 (t, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.61 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.17 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 3.83 (t, 2H, J = 7.2 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 6.84 (d, 2H, J = 9 Hz, aromatic H), 7.20 (d, 2H, J = 9 Hz, aromatic H), 7.82–7.90 (m, 3H, aromatic H), 8.07 (d, 1H, J = 5 Hz, aromatic H). HRMS calculated for $C_{21}H_{25}ClN_3O_3S$ (M+H$^+$) 434.1305, found 434.1284.

4.5. 2-(4-(4-(3-α,α,α-Trifluorotolyl)-1-piperazinyl)butyl)-1, 2-benzisothiazole-3(2*H*)-one 1,1-dioxide (9c)

Yield 40%; mp 111–113 °C; ^{1}H NMR δ 1.69 (m, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.93 (m, 2H, J = 7.2 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.51 (t, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.62 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.25 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 3.83 (t, 2H, J = 7.2 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 7.05–7.33 (m, 4H, aromatic H), 7.83–7.91 (m, 3H, aromatic H), 8.05 (d, 1H, J = 6 Hz, aromatic H). IR, Cm^{-1}: 3091 (aromatic CH), 2924 (aliphatic CH), 1734 (CO), 1337 and 1162 (SO$_2$). HRMS calculated for $C_{22}H_{25}F_3N_3O_3S$ (M+H$^+$) 468.1569, found 468.1510.

4.6. 2-(4-(4-Phenyl-1-piperazinyl)butyl)-1,2-benzisothiazole-3(2*H*)-one 1,1-dioxide (9d)

Yield 53%; mp 122–124 °C; ^{1}H NMR δ 1.66 (m, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.92 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.46 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.61 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.20 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 3.83 (t, 2H, J = 7.5 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 6.82–7.73 (m, 5H), 7.80–7.94 (m, 3H), 8.06 (d, 1H, J = 6 Hz). MS *m*/*z* (%): M$^+$ 400 (1.6), 399 (6.5), 175 (100.0). HRMS calculated for $C_{21}H_{26}N_3O_3S$ (M+H$^+$) 400.1695, found 400.1682.

4.7. 3-(4-(4-(2-Methoxyphenyl)-1-piperazinyl)butyl)quinazolin-4(3*H*)-one (10a)[18]

Yield 62%; Oil; ^{1}H NMR δ 1.67 (m, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.89 (m, 2H, J = 7.2 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.52 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.70 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.13 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 3.87 (s, 3H, OCH$_3$), 4.07 (t, 2H, J = 7.2 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 6.86–7.77 (m, 7H, aromatic H), 8.061 (s, 1H, —N—CH=N—), 8.34 (d, 1H, J = 7 Hz, aromatic H). HRMS calculated for $C_{23}H_{29}N_4O_2$ (M+H$^+$) 393.2291, found 393.2260.

4.8. 3-(4-(4-(4-Chlorophenyl)-1-piperazinyl)butyl)quinazolin-4(3*H*)-one (10b)

Yield 58%; mp 163–165 °C; ^{1}H NMR δ 1.66 (m, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$*CH$_2$*CH$_2$N—), 1.86 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH$_2$*CH$_2$CH$_2$N—), 2.47 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH$_2$*N—), 2.62 (br s, 4H, (CH$_2$)$_2$N^1 piperazinyl), 3.17 (br s, 4H, (CH$_2$)$_2$N^4 piperazinyl), 4.06 (t, 2H, J = 7.5 Hz, —CON*CH$_2$*CH$_2$CH$_2$CH$_2$N—), 6.82–7.78 (m, 7H, aromatic H), 8.05 (s,

1H, —N—CH=N—), 8.339 (d, 1H, J = 7 Hz, aromatic H). IR, Cm^{-1}: 3057 (aromatic CH), 2940 (aliphatic CH), 1660 (CO), 1612 (C=N). MS m/z (%): M^+ 397 (8.0), 396 (30.6), 209 (100.0). HRMS calculated for $C_{22}H_{26}ClN_4O$ ($M+H^+$) 397.1795, found 397.1791.

4.9. 3-(4-(4-(3-α,α,α-Trifluorotolyl)-1-piperazinyl)butyl)-quinazolin-4(3*H*)-one (10c)

Yield 46%; mp 77–79 °C; ^1H NMR δ 1.64 (m, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.89 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.47 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.61 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.24 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 4.07 (t, 2H, J = 7.5 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 7.04–7.78 (m, 7H, aromatic H), 8.06 (s, 1H, —N—CH=N—), 8.34 (d, 1H, J = 7 Hz, aromatic H). HRMS calculated for $C_{23}H_{26}F_3N_4O$ ($M+H^+$) 431.2059, found 431.2048.

4.10. 3-(4-(4-Phenyl-1-piperazinyl)butyl)quinazolin-4(3*H*)-one (10d)[19]

Yield 61%; mp 136–138 °C; ^1H NMR δ 1.61 (m, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.89 (m, 2H, J = 7.5 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.47 (t, 2H, J = 7.5 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.61 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.23 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 4.07 (t, 2H, J = 7.5 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 6.87–7.78 (m, 8H, aromatic H), 8.06 (s, 1H, —N—CH=N—), 8.35 (d, 1H, J = 7 Hz, aromatic H). MS m/z (%): M^+ 362 (44.2), 175 (100.0). HRMS calculated for $C_{22}H_{27}N_4O$ ($M+H^+$) 363.2185, found 363.2198.

4.11. 1-(4-(4-(2-Methoxyphenyl)-1-piperazinyl)butyl)-indolin-2-one (11a)

Yield 41%; Oil; ^1H NMR δ 1.65 (m, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.76 (m, 2H, J = 7.2 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.465 (t, 2H, J = 7.2 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.654 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.101 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.53 (s, 2H, CH$_2$CON indoline), 3.761 (t, 2H, J = 7.2 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 3.869 (s, 3H, OCH_3), 6.878–7.272 (m, 8H, aromatic H). IR, Cm^{-1}: 3056 (aromatic CH), 2924 (aliphatic CH), 1707 (CO). MS m/z (%): M^+ 379 (15.8), 205 (100.0). HRMS calculated for $C_{23}H_{30}N_3O_2$ ($M+H^+$) 380.2338, found. 380.2303.

4.12. 1-(4-(4-(4-Chlorophenyl)-1-piperazinyl)butyl)indolin-2-one (11b)

Yield 35%; Oil; ^1H NMR δ 1.65 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.74 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.46 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.60 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.16 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.53 (s, 2H, CH$_2$CON indoline), 3.76 (t, 2H, J = 7.2 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 6.82–7.27 (m, 8H, aromatic H). MS m/z (%): M^+ 384 (3.1), 383 (11.7), 209 (100.0). HRMS calculated for $C_{22}H_{27}N_3O$ ($M+H^+$) 384.1843, found 384.1810.

4.13. 1-(4-(4-(3-α,α,α-Trifluorotolyl)-1-piperazinyl)butyl)-indolin-2-one (11c)

Yield 37%; Oil; ^1H NMR δ 1.65 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.76 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.46 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.62 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.26 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.54 (s, 2H, CH$_2$CON indoline), 3.77 (t, 2H, J = 7.2 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 6.86–7.35 (m, 8H, aromatic H). MS m/z (%): M^+ 417 (5.0), 243 (100.0). HRMS calculated for $C_{23}H_{27}F_3N_3O$ ($M+H^+$) 418.2106, found 418.2119.

4.14. 1-(4-(4-Phenyl-1-piperazinyl)butyl)indolin-2-one (11d)

Yield 30%; Oil; ^1H NMR δ 1.649 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.758 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.456 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.611 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.209 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.532 (s, 2H, CH$_2$CON indoline), 3.786 (t, 2H, J = 7.2 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 6.858–7.292 (m, 9H, aromatic H). MS m/z (%): M^+ 349 (14.2), 175 (100.0). HRMS calculated for $C_{22}H_{28}N_3O$ ($M+H^+$) 350.2232, found 350.2249.

4.15. 2-(4-(4-(2-Methoxyphenyl)-1-piperazinyl)butyl)-1-isoindolinone (12a)

Yield 34%; Oil; ^1H NMR δ 1.65 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.76 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.51 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.70 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.09 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.67 (t, 2H, J = 7 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N—), 3.86 (s, 3H, OCH_3), 4.40 (s, 2H, CH$_2$NCO isoindoline), 6.87–7.80 (m, 8H, aromatic H). MS m/z (%): M^+ 379 (0.87), 216 (100.0). Analysis calculated for $C_{23}H_{29}N_3O_2$: C, 72.79; H, 7.70; N, 11.07. Found C, 72.39; H, 7.19; N, 11.20.

4.16. 2-(4-(4-(4-Chlorophenyl)-1-piperazinyl)butyl)-1-isoindolinone (12b)

Yield 42%; Oil; ^1H NMR δ 1.64 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.76 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.49 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.55 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.109 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.675 (t, 2H, J = 7 Hz, —CON*CH*$_2$CH$_2$CH$_2$CH$_2$N), 4.392 (s, 2H, CH$_2$NCO isoindoline), 6.788–7.577 (m, 8H, aromatic H). HRMS calculated for $C_{22}H_{27}ClN_3O$ 384.1843, found 384.1849.

4.17. 2-(4-(4-(3-α,α,α-Trifluorotolyl)-1-piperazinyl)butyl)-1-isoindolinone (12c)

Yield 40%; Oil; ^1H NMR δ 1.65 (m, 2H, J = 7 Hz, —CONCH$_2$CH$_2$*CH*$_2$CH$_2$N—), 1.75 (m, 2H, J = 7 Hz, —CONCH$_2$*CH*$_2$CH$_2$CH$_2$N—), 2.489 (t, 2H, J = 7 Hz, —CONCH$_2$CH$_2$CH$_2$*CH*$_2$N—), 2.614 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.146 (br s, 4H, $(CH_2)_2N^4$

piperazinyl), 3.665 (t, 2H, $J = 7$ Hz, —CON$CH_2$$CH_2$$CH_2$ CH_2N), 4.4 (s, 2H, CH_2NCO isoindoline), 7.041–7.81 (m, 8H, aromatic H). MS *m/z* (%): M^+ 417 (1.6), 217 (100.0). HRMS calculated for $C_{23}H_{27}F_3N_3O$ ($M+H^+$) 418.2116, found 418.2106.

4.18. 2-(4-(4-Phenyl-1-piperazinyl)butyl)-1-isoindolinone (12d)[20]

Yield 40%; Oil; ^{1}H NMR δ 1.645 (m, 2H, $J = 7$ Hz, —CONCH$_2$CH$_2$$CH_2CH_2$N—), 1.782 (m, 2H, $J = 7$ Hz, —CONCH$_2$$CH_2CH_2CH_2$N—), 2.496 (t, 2H, $J = 7$ Hz, —CONCH$_2$CH$_2$CH$_2$$CH_2$N—), 2.634 (br s, 4H, $(CH_2)_2N^1$ piperazinyl), 3.245 (br s, 4H, $(CH_2)_2N^4$ piperazinyl), 3.679 (t, 2H, $J = 7$ Hz, —CONCH_2CH$_2$CH$_2$CH$_2$N), 4.403 (s, 2H, CH_2NCO isoindoline), 6.859–7.8 (m, 9H, aromatic H). MS *m/z* (%): M^+ 349 (6.6), 217 (100.0). HRMS calculated for $C_{22}H_{28}N_3O$ ($M+H^+$) 350.2232, found 350.2243.

5. Pharmacology[15–17]

5.1. Dopamine receptor ligand activity

5.1.1. Cell culture. Human D_{2L}, D_3, $D_{4.4}$ and D_5 receptors were stably expressed in Chinese hamster ovary (CHO) cells and human embryonic kidney cells (HEK293), respectively. The density of receptors measured with [^{3}H]-SCH 23390 was 679.44 fmol/mg protein for D_5 receptor in HEK cells. The densities of receptors measured with [^{3}H]-Spiperone were 186.53 fmol/mg protein for D_2 receptor expressed in HEK cells; 6043 fmol/mg for the D_4 receptor and 14,474 fmol/mg for D_3 receptor, both expressed in CHO cells. Cells were grown at 37 °C under a humidified atmosphere of 5% CO_2: 95% air in HAM/F12-medium (Sigma–Aldrich) for CHO cells and Dulbecco's modified Eagle's medium Nutient mixture F-12 Ham for HEK293 cells, each supplemented with 10% foetal bovine serum, 1 mM L-glutamine, 20 U/mL penicillin G, 20 μg/L streptomycin and 0.2 μg/mL G 418 (all from Sigma–Aldrich).

5.1.2. Preparation of whole-cell-suspension.[17] Human D_2, D_3, D_4 and D_5 receptor cell lines (CHO) were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85% confluency, the medium was removed and the cells were incubated with 6 mL trypsin-EDTA-solution (Sigma–Aldrich) to remove the cells from the culture dish. After incubation, cells were suspended in 3–6 mL added medium in order to stop the effect of trypsin-EDTA solution.The resulting suspension was centrifuged (1800–2400 rpm/min, 4 °C, 4 min), the pellet resuspended in 10 mL PBS (ice-cooled, calcium- and magnesium-free), pelleted, and this procedure repeated. The resulting pellet was then resuspended in 12 mL buffer (5 mM magnesium chloride, 50 mM Tris–HCl, pH 7.4) and the resulting suspension was directly used for the radioligand binding assay.

5.1.3. Radioligand binding assay[17]. For the binding studies a procedure according to the protocol previously published but in 96-well format.[15] The assays with the whole-cell-suspension were carried out in triplicate in a volume of 550 μL (final concentration): Tris–Mg^{2+}-buffer (345 μL), [^{3}H]-ligand (50 μL), whole-cell-suspension (100 μL) and appropriate drugs (55 μL). Non-specific binding was determined using fluphenazine (100 μM) in D_5 test and haloperidol (10 μM) in D_2, D_3 and D_4 tests. For a fast screening the drugs were used in a concentration of 100 μM, and the percentage of removed radioligand determined. The incubation was initiated by addition of the radioligand. It was carried out in 96-deep well plates (Greiner bio-one Frickenhausen) using a Thermocycler (Thermocycler comfort, Eppendorf, Wessling) at 27 °C for 11/2 h, and stopped by rapid filtration with a Perkin-Elmer Mach III Harvester using a Perkin-Elmer Filtermat A., previously treated with 0.25% polyethyleneimine solution (Sigma–Aldrich), which was washed once with ice-cold water. The filtermat was dried for 3 min with 400 W microwave (WM21, Clatronic, Kemoen). The dry filtermat was placed on a filter paper (Omni filter plates, Perkin-Elmer Life Sciences) and each field of the filtermat moistened with 50 μL Microscint 20 scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT microplate scintillation counter (Packard, Ct, USA). For determining the K_i values at least two independent experiments in triplicate were performed. The competition binding data were analysed by the software GraphPad Prism™ using non-linear squares fit. For calculating the mean, SD and SEM the software Microsoft Excel was used. K_i values were calculated from IC_{50} values applying the equation of Cheng and Prusoff.[21]

6. Functional assay measuring intracellular Ca^{2+} with a fluorescence microplate reader[16,22]

6.1. Cell culture

Human D_{2L} and D_5 receptors were stably expressed in human embryonic kidney cells (HEK293) and cultured as above-mentioned.

6.2. Preparation of whole-cell-suspension

Human D_2 and D_5 receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85–90% confluency. The medium was removed and cells rinsed twice with 6 mL Krebs-Hepes buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 11.7 mM D-Glucose, 1.3 mM $CaCl_2$, 10 mM Hepes, pH 7.4) each time. After two washes, cells were loaded with 3 μL of a 0,5 M Oregon Green 488 BAPTA-1/AM-solution (Molecular Probes, Eugene, OR) (in DMSO) in 6 mL of the same buffer containing 3 μL of a 20% Pluronic F-127-solution (Sigma–Aldrich) (in DMSO) for 45 min at 37 °C. After 35-min incubation, the culture dish was rapped slightly in order to remove all cells from the dish for further incubation. Then 5 mL of Krebs-Hepes buffer was added and cells were suspended. The resulting suspension was separated in 10 vials (1.5 mL) and centrifuged (10,000 rot/min,

10 s), the pellets were resuspended in 1 mL Krebs-Hepes buffer twice per five pellets and centrifuged again. The pellets were resuspended in 16 mL (for screening of antagonistic activity) or 18 mL (for screening of agonistic activity) Krebs-Hepes buffer and plated into 96-well plates (OptiPlate HTRF-96, Packard, Meriden, CT; Cellstar, Tissue Culture Plate, 96W, Greiner bio-one, Frickenhausen). Microplates were kept at 37 °C under a humidified atmosphere of 5% CO_2: 95% air for 30 min.

6.3. Calcium assay[16]

Screening for agonistic and antagonistic activity was performed using a NOVOstar microplate reader (BMG LabTechnologies) with a pipettor system. Agonistic activity was tested after 30-min incubation of the plated cell suspension by injecting 20 μL buffer alone, standard agonist, or test compounds, respectively, dissolved in buffer sequentially into separate wells. Screening of compounds for antagonist activity or dose–response curves in presence of an antagonist was performed by preincubating the cells with 20 μL of the solutions of compounds (final concentrations: 100 μM, 50 μM, 10 μM, 5 μM, 1 μM, 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0,1 nM) at 37 °C for 30 min prior to injection of 20 μL standard agonist. Final concentration of test compounds for screening of agonist or antagonist activity was 10 μM, respectively. Quinpirole was used as standard agonist for hD_2 receptors and SKF 38393 for hD_5 receptors (final concentration: 1 μM). Fluorescence intensity was measured at 520 nm (bandwidth 25 nm) for 30 s at 0.4 s intervals. Excitation wavelength was 485 nm (bandwidth 20 nm). IC_{50} values were obtained by determination of the maximum fluorescence intensity of each data set and non-linear regression with sigmoidal dose–response equation using a four-parameter logistic equation on GraphPadPrism™ 3.0. K_i values were then calculated to account for different agonist concentrations and EC_{50} values applying a modified Cheng–Prusoff equation[21]:

$$K_i = \frac{IC_{50}}{1 + \frac{L}{EC_{50}}}$$

L: concentration of standard agonist (M); EC_{50}: effective concentration 50% of the standard agonists (M); IC_{50}: inhibitory concentration 50% of test compounds at the given experimental conditions, that is, standard agonist concentration.

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