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Dibenzazecine scaffold rebuilding—Is the flexibility always essential for high dopamine receptor affinities?

Maria Schulze [a], Franziska K. U. Müller [a], Jennifer M. Mason [b], Helmar Görls [c], Jochen Lehmann [a], Christoph Enzensperger [a,*]

[a] Institut für Pharmazie, Lehtuhl für Pharmazeutische/Medizinische Chemie, Friedrich-Schiller-Universität Jena, Philosophenweg 14, 07743 Jena, Germany
[b] Industrial Research Ltd, PO Box 31-310, Lower Hutt, New Zealand
[c] Institut für Anorganische und analytische Chemie, Friedrich-Schiller-Universität Jena, August-Bebel-Str. 2, 07743 Jena, Germany

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ABSTRACT

The moderately flexible 7-methyl-5,6,7,8,9,14-hexahydrodibenz[*d*,*g*]azecines are known to be potent dopamine receptor antagonists, whereas the corresponding rigid dibenzo[*d*,*g*]quinolizines are inactive. We built the scaffolds of dibenzo[*c*,*g*], [*c*,*f*]- and -[*d*,*f*]azecines and together with their ring closed, more rigid precursors, evaluated the affinities for the human D_1–D_5 receptors (radioligand binding) as well as the functionalities (calcium assay) and thus investigated the influence of annelation and conformative flexibility of these compounds on their affinity for human cloned dopamine receptors.

1. Introduction

The dopaminergic system plays an important role in regulating neuronal motor control, cognition, emotion, and vascular function. Dopamine antagonists are commonly used as antipsychotic drugs. It is now widely accepted for GPCRs of the amine cluster (i.e., dopamine, serotonin, norepinephrine, or histamine receptors) that antagonists mostly interact with two different hydrophobic areas and an anionic aspartate in the receptor's binding cavity.[1] This might explain why most of the antagonists have two aromatic moieties to interact with the abovementioned two hydrophobic sites, and a chargeable basic nitrogen which is crucial for the formation of an ionogenic salt-bridge to the aspartate anion in the binding pocket. It is not clarified yet, in what relative positions these three pharmacophores should be, to gain maximum affinity for the respective GPCRs. Changing the relative distances and angles of these structural elements may give more insights into receptor ligand interactions.

The rather flexible and symmetric dibenzo[*d*,*g*]azecines (e.g., **3**, **4**) are highly potent antagonists at the D_1-receptor subtype family (D_1 and D_5). For these azecines 3-hydroxy-derivatives show an advantage over 3-methoxy-substituted compounds. In contrast, the analogous, more rigid dibenzo[*d*,*g*]quinolizines (e.g., **1**, **2**) do not show any affinity.[2,3] So, obviously, the higher conformational mobility of the 10-membered dibenzo[*d*,*g*]azecines facilitates the binding to dopamine receptors. On the other hand, some dibenzoquinolizines such as stepholidine and chloroscoulerine, exhibit nanomolar affinities for the D_1 and D_{2L} receptors, and also the rather constrained dihydrexidine, a dibenzoquinoline derivative, is a highly potent D_1 agonist.[4–6] The scaffolds of the homologous dibenzazecines only differ in the position of the second annulated benzene moiety (Fig. 1). We therefore planned to synthesize dibenzazecines **7**, **8**, **10**, **11** and their tetracyclic precursors **5**, **6**, and **9**. To obtain meaningful information for SAR-studies on the changed scaffolds, dibenzo[*d*,*g*]-, -[*c*,*f*]- and -[*c*,*g*]azecines were synthesized with the same substitution pattern, which is based on the previously investigated potent dopamine antagonists 3-methoxy- and 3-hydroxy-7-methyl-5,6,7,8,9,14-hexahydrodibenzo[*d*,*g*]azecine (**3**, **4**).[2,3] The affinities of compounds **1–11** and the two enantiomers of dibenzo[*d*,*f*]azecine **12**[7] (Fig. 2) were measured for all dopamine receptor subtypes by radioligand binding experiments. The functionality of all compounds was determined by a functional calcium assay.[3]

2. Results

2.1. Chemistry

The synthesis of compounds **7–11** is described in Schemes 1 and 2. In order to obtain the dibenzo[*c*,*g*]azecines **7**, **8** and the dibenzo[*c*,*f*]azecines **10**, **11**, we first synthesized the appropriate

* Corresponding author. Tel.: +49 3641 949820; fax: +49 3641 949802.
E-mail address: ch.enzensperger@uni-jena.de (C. Enzensperger).

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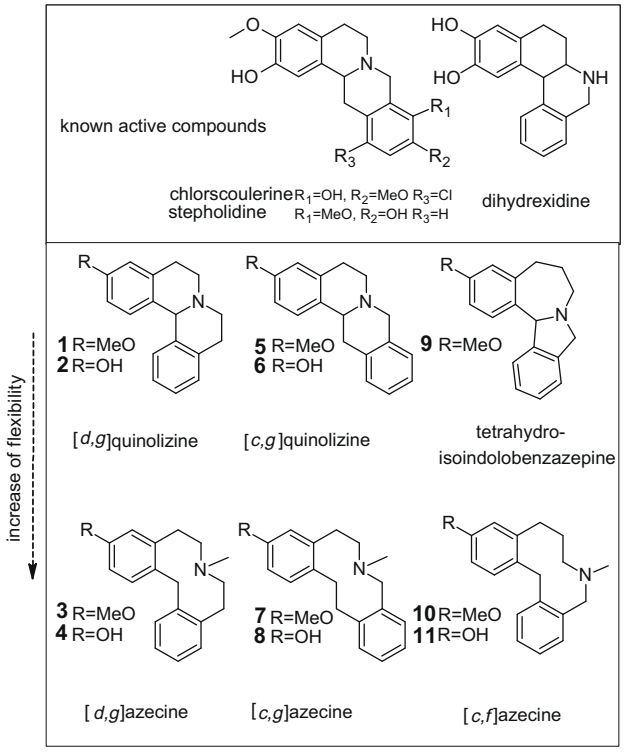


Figure 1. The underlying tetracyclic compounds and resulting azecines.

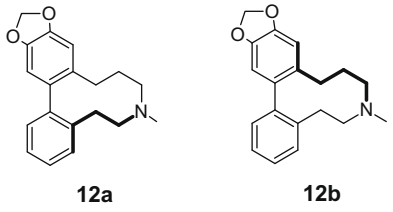
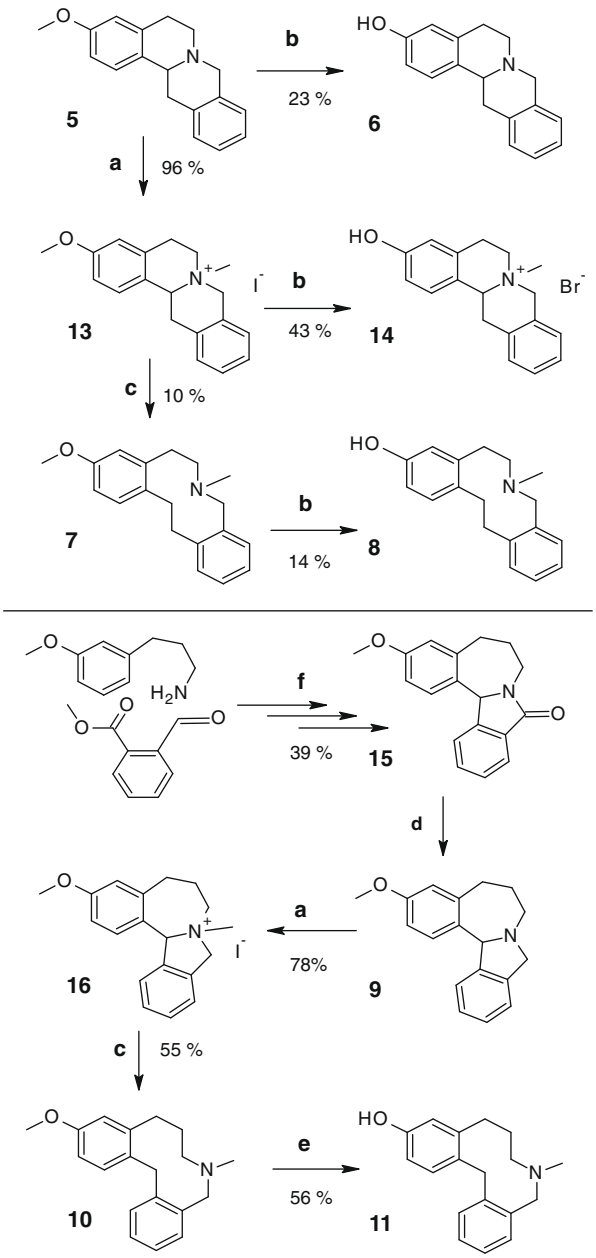


Figure 2. Dibenzo[*d*,*f*]azecines.

tetracyclic precursor molecules **5** and **9** and converted them to their quaternary salts **13** and **16** with methyl iodide in acetonitrile. Birch conditions (Na^0/NH_3^{liq}) were used to obtain **7** and **10**. Cleavage of the methyl-ether moieties of the quaternary salt **13**, the dibenzo[*c*,*g*]quinolizine **5** and the [*c*,*g*]azecine **7** was accomplished by using hydrobromic acid in glacial acetic acid. Borontribromide in chloroform was used to prepare the phenol **11** in excellent yields, but for the ether cleavage of compounds **5** and **7** with bortribromide, no product could be isolated. Compound **5** was prepared as described previously starting from 3-methoxy-phenylethylamine and 3-isochromanone.[8] Compound **9** was obtained by lithium aluminum hydride reduction of the tetracyclic lactam (**15**), which was synthesized in three steps starting from methyl-2-formylbenzoate and 3-(3-methoxyphenyl)-propylamine via $TiCl_4$ mediated acyliminium cyclization as described by Heaney and Shuhaibar.[9]

Synthesis of the dibenzo[*c*,*g*]azecines **7** and **8** under Birch conditions turned out to be more complicated than observed for the preparation of dibenzo[*d*,*g*]azecines.[2] The cleavage yielded a side product in high yields due to the second benzylamine structure, as outlined in Scheme 2. Thus, ring opening of the quaternary salt **14** yielded almost exclusively (>90%) 2-methyl-1-(2-methylbenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (**17**) instead of the desired azecine **8** and cleavage of the methoxy derivative **13** gave a mixture of 86% of 6-methoxy-2-methyl-1-(2-methylbenzyl)-

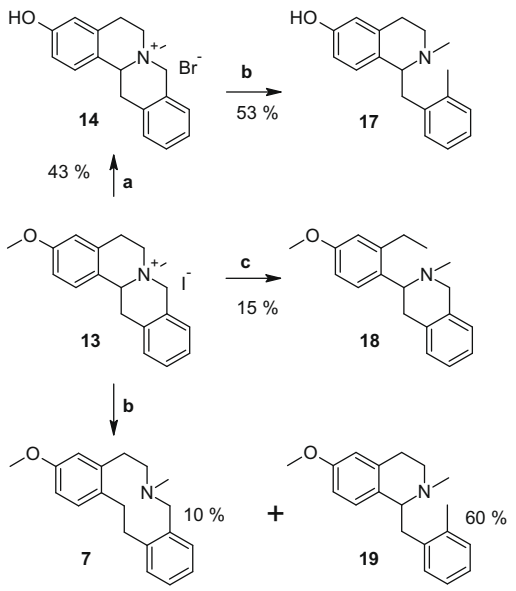


Scheme 1. Synthesis of the dibenzazecines **5**–**8**. Reagents and conditions: (a) methyl iodide, MeCN; (b) HBr, glacial acetic acid; (c) Na/NH_3^{liq}; (d) $LiAlH_4$, THF; (e) BBr_3, $CHCl_3$; (f) (i) toluene reflux; (ii) Na, methanol; (iii) $TiCl_4$, CH_2Cl_2.

1,2,3,4-tetra-hydroisoquinoline **19** and 14% of the desired azecine **7**. Both compounds were separated by column chromatography. In order to obtain better yields especially for the hydroxy derivative, we used a reaction sequence of Hofmann degradation followed by catalytic hydrogenation, which is described for known dibenzo[*c*,*g*]azecines.[10] Unfortunately, starting with the quaternary salt **13** we only obtained 3-(2-ethyl-4-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydro-isoquinoline (**18**). All by-products were characterized by NMR and GC–MS and screened for their dopamine receptor affinities.

2.2. Pharmacological investigations

Using radioligand displacement experiments, we screened all dibenzazecines and ring-closed analogues, as well as the mentioned synthetic by-products, for their affinities for all human



Scheme 2. Synthetic by-products during cleavage of quaternary salt **11**. Reagents and conditions: (a) HBr, glacial acetic acid; (b) Na/NH_3^{liq}; (c) (i) Ag_2O, Methanol; (ii) DMSO; (iii) H_2/Pt_2O.

cloned dopamine receptor subtypes D_1–D_5 and determined the functionality for the D_1 and D_{2L} receptors in an intracellular calcium assay. K_i values for the radioligand binding assay are given in nM units (Table 1). A detailed protocol is described in Section 5.

3. Discussion

Both the constrained tetracyclic dibenzo[*d,g*]quinolizine derivatives **1** and **2** and their ring opened tricyclic analogues **3** and **4** contain a dopamine-like phenylethylamine moiety, but only the tricyclic and more flexible azecines **3** and **4** display high affinities for dopamine receptors. The 3-methoxy quinolizine derivative **1** shows slight affinity for the D_1 receptor, but almost 100 times weaker than the analogous azecine **3**.

It is known from literature that the tetrahydroprotoberberines 12-chloroscoulerine and stepholidine, which are dibenzo[*c,g*]quinolizine derivatives, show high affinities for dopamine receptors.[5,6] We now synthesized 3-methoxy and 3-hydroxy dibenzo[*c,g*]quinolizines (**5**, **6**), which show good affinities as well. As already recognized for the dibenzo[*d,g*]azecines,[3] the hydroxy dibenzoquinolizine **6** exhibits a higher selectivity toward the D_1/D_5-receptors and higher affinities at these subtypes compared to the methoxy compound **5**. The modification in substitution pattern of stepholidine to compounds **5** and **6** lead to a change of functionality at D_1 receptors. While stepholidine is an agonist at this receptor subtype,[5] our tested tetrahydroprotoberberines (**5**, **6**) show antagonistic activities. It was now expected that ring opening of these potent tetracyclic compounds would increase affinities at all receptor subtypes. But surprisingly, both the hydroxy- and methoxy-substituted derivatives lost affinities nearly completely, despite the increased flexibility of the ring scaffold. Only at D_1 and D_5 receptors micromolar affinities can be detected for the methoxy compound (**7**). The hydroxy derivative does not show any advantage over the methoxy compound. The synthetic by-products **17**–**19** also show only weak micromolar or no affinities. Here, 1-benzyl-tetrahydroisoquinolines (**17**, **19**) turn out to be advantageous over the 3-phenyl-tetrahydroisoquinoline derivative (**18**), which shows very low affinities at all receptor subtypes. The selectivity of compounds **17** and **19** toward D_1 and D_2 receptors is an interesting observation and topic of our future investigations.

The dibenzo[*c,f*]azecines are of special interest since their scaffold can be seen as ring opened dihydrexidine derivative. Dihydrexidine is described as high-affinity dopamine agonist.[4] While the tetracyclic precursor **9** shows very low affinities for all receptor subtypes, the 11-methoxy- and 11-hydroxy[*c,f*]azecines (**10**, **11**) show slight affinities for the D_1 receptor with micromolar K_i values. In contrast to dibenzo[*d,g*]azecines, but similar to dibenzo[*c,g*]azecines, the hydroxy compound **11** shows significantly lower affinies for D_1 receptors than the methoxy compound **10**. Surprisingly, at D_5 receptors, only the hydroxy derivative has a weak affinity. Though dihydrexidine shows agonistic activities at D_1 receptors,[4] the investigated dibenzo[*c,g*]-azecines, which structurally resemble dihydrexidine, exhibit weak antagonistic affinities.

The dibenzo[*d,f*]azecines **12a**, **b** exhibit low affinities for dopamine receptors with micromolar K_i values, comparable to the binding affinities of the dibenzo[*c,g*]-homologs **7**, **8** and the dibenzo[*c,f*]azecines **10**, **11**. Interestingly, the (+)-enantiomer **12a** has slight affinities for D_1 and D_5 receptors, while the corresponding (−)-enantiomer **12b** exhibits no measureableable affinities. This correlates to observations made for 12-chloroscoulerine, where the two enantiomers show significant differences in their affinities for dopamine receptors. However, the racemic 12-chloroscoulerine displays almost the same activity as the active enantiomer.[12]

In the functional calcium assay all active compounds proved to be antagonists at D_1 and D_{2L} receptors. None of the tested compounds show agonistic activities. Hence, a change in the scaffold from dibenzo[*d,g*]- to other dibenzazecines seems to have no effect on functionality at dopamine receptors, while the substitution pattern, for example of dibenzo[*c,g*]quinolizines seems to have great impact on functionalities at dopamine receptors.

For the dibenzazecines **7** and **10** we could also obtain X-ray data (Fig. 3). Both compounds turned out to be angled in the crystal. Calculations of E-minimized conformations (*DS-visualizer*, *MOE*) gave for the dibenzo[*c,g*]azecine **7** a rather straight shape, which was also observed for E-minimized conformations of dibenzo[*d,g*]- and -[*c,g*]quinolizines (**1**, **2**, **5**, **6**). Measurement of distances between the aromatic centroids and the basic nitrogen in the crystal structure, as well as in the simulated E-minimized conformations, did not indicate a correlation between these distances and the observed affinities for dopamine receptors. Further molecular modeling studies are in progress.

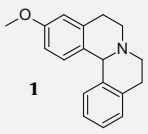
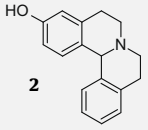
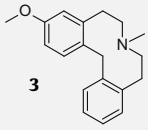
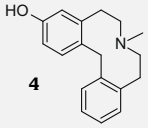
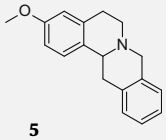
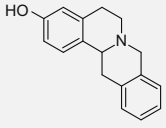
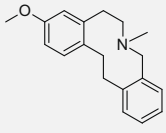
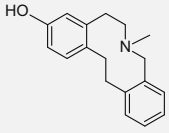
4. Conclusion

It can be summarized, that the annelation pattern in the tricyclic dibenzazecines and in the corresponding tetracyclic quinolizine and quinolizine-like derivatives highly influences the affinities, but not the functionalities at dopamine receptors. While dibenzo[*d,g*]azecines show nanomolar affinities for all dopamine receptors, dibenzo[*c,f*]-, -[*c,g*]- or -[*d,f*]azecines display micromolar K_i values for D_1 and D_5 receptor subtypes. All active test compounds turned out to act as antagonists.

Obviously, ring opening of dibenzo[*d,g*]quinolizines and the consequent increase in conformational flexibility, increases receptor affinities dramatically. The same effect occurs after ring opening of isoindoloazepine **9** to the dibenzo[*c,f*]azezines **10** and **11**, even though less pronounced. In spite of the close resemblance to the known dopamine receptor agonist dihydrexidine, these more flexible compounds, which represent a new tricyclic ring system, are antagonists with only moderate affinities.

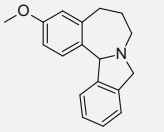
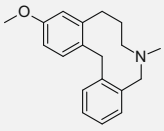
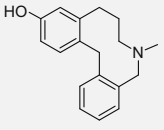
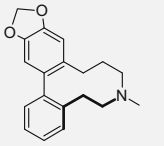
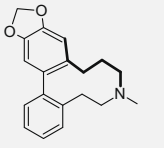
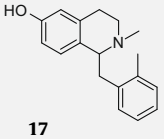
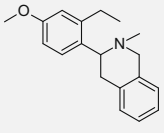
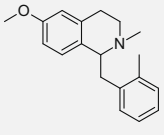
The constrained dibenzo[*c,g*]quinolizines **5**, **6** exhibit the highest receptor affinities among all investigated ring-closed compounds. In contrast to the positive influence of ring opening on

Table 1
Affinities (K_i) for dopamine D_1–D_5 receptor subtypes were determined by radioligand binding experiments

Compounds	Affinity for D_1, nM (K_i ± SEM)	Affinity for D_{2L}, nM (K_i ± SEM)	Affinity for D_3, nM (K_i ± SEM)	Affinity for D_4, nM (K_i ± SEM)	Affinity for D_5, nM (K_i ± SEM)
 **1**	2116 ± 484[b]	>10,000	>10,000	>10,000	>10,000
 **2**	>10,000	>10,000	>10,000	>10,000	>10,000
 **3**	28.5 ± 9.7[a]	13.0 ± 9.0[a]	75.7 ± 7.3[a]	43.4[a]	54 ± 20[a]
 **4**	0.39 ± 0.22[a]	17.5 ± 1.5[a]	47.5 ± 24[a]	11.3 ± 1[a]	1.5 ± 0.5[a]
Stepholidine[c]	5.9	974	30 (Rat)	3748	4.4
 **5**	183 ± 27.5[b]	320 ± 159[a]	251 ± 56[b]	2565 ± 1673[a]	185 ± 97[b]
 **6**	136 ± 42[a]	486 ± 106[b]	306.5 ± 61.5[b]	5092 ± 1589[b]	41.1 ± 24.3[a]
 **7**	1727 ± 238[a]	>10,000	>10,000	>10,000	1508 ± 136[b]
 **8**	>10,000	>10,000	>10,000	>10,000	>10,000
Dihydrexidine[c]	35.7	2607	170 (Rat)	13 (Rat)	16 (Rat)

(*continued on next page*)

Table 1 (*continued*)

Compounds	Affinity for D_1, nM (K_i ± SEM)	Affinity for D_{2L}, nM (K_i ± SEM)	Affinity for D_3, nM (K_i ± SEM)	Affinity for D_4, nM (K_i ± SEM)	Affinity for D_5, nM (K_i ± SEM)
 **9**	>10,000	>10,000	>10,000	>10,000	>10,000
 **10**	1264 ± 165.5[b]	>10,000	>10,000	>10,000	>10,000
 **11**	2070 ± 76[a]	>10,000	>10,000	>10,000	1810 ± 478[a]
 **12a**	4748 ± 500[a]	>10,000	>10,000	>10,000	1812 ± 164[b]
 **12b**	>10,000	>10,000	>10,000	>10,000	>10,000
 **17**	3019 ± 817[b]	2724 ± 411[b]	>10,000	>10,000	>10,000
 **18**	>10,000	>10,000	>10,000	>10,000	>10,000
 **19**	2349 ± 1414[b]	7371 ± 2018[b]	>10,000	6644 ± 2547[b]	>10,000

[a] K_i values are means of at least three experiments, performed in triplicate ±SEM.
[b] K_i values are means of two experiments, performed in triplicate ±SD.
[c] Values from the PDSP database 11.

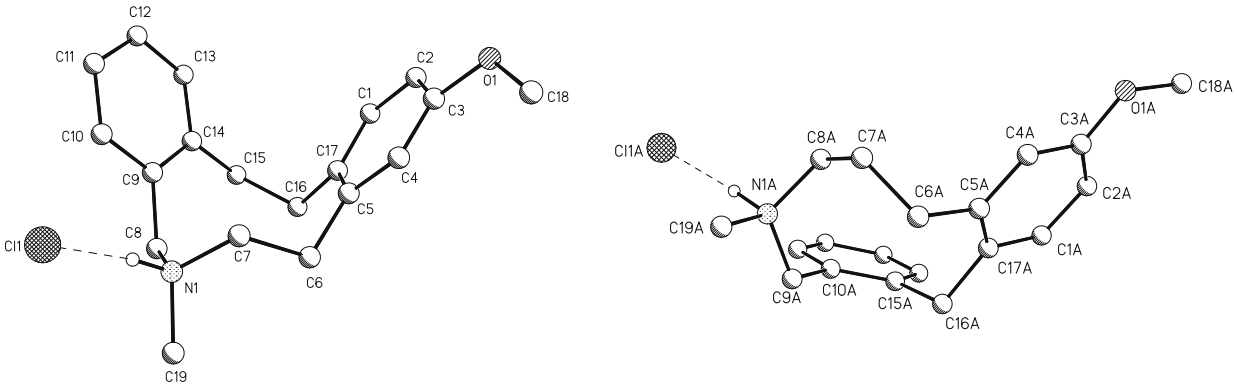


Figure 3. X-ray structures of dibenzo[*c*,*g*]azecine **7** and dibenzo[*c*,*f*]azecine **10**.

receptor affinities recognized for the other dibenzazecines, ring opening to the corresponding dibenzo[*c*,*g*]azecines **7**, **8** abolishes affinities. In this case, the dibenzo[*c*,*g*]quinolizines are more prone to interact with the receptor than the more flexible dibenzo-[*c*,*g*]azecines. Thus, higher flexibility and moderate increase in residual mobility are not always beneficial for receptor interaction.

Contrary to dibenzo[*d*,*g*]azecines, whose hydroxy derivative **4** has distinctly higher affinities than the methoxy congener **3**, the methoxy derivatives of dibenzo[*c*,*g*]- and -[*c*,*f*]azecines (**7** and **10**) unexpectedly display higher affinities. All investigated dibenzazecines show an explicit selectivity toward the D_1-receptor family. Our findings might contribute to essential insights for further molecular modeling studies on this topic.

5. Experimental

5.1. Synthesis and characterization of test compounds

Syntheses were performed under nitrogen with solvents and reagents of commercial availability with no further purification. Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz), respectively. Elemental analyses were performed on a Hereaus Vario EL apparatus for all test compounds. MS data were determined by GC–MS, using a Hewlett–Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). EI-MS-Spectra were recorded using LCQ Advantage by ThermoElectron and HRMS were recorded by a TSQ Quantum AM spectrometer (Therma Electron Corporation (Waltham, USA)).

5.1.1. Ring opening—general procedure

A 100-mL three-neck flask equipped with a balloon as an overflow tank was cooled in a liquid nitrogen bath. Ammonia was condensed into this flask until it was 3/4 filled. The cooling bath was removed and the ammonia was allowed to liquefy. After suspending 1 mmol of the quaternary salts (**13**, **16**) in the liquid ammonia, rice-grain-sized pieces of sodium were added to the stirred mixture until the developing blue color remained for 10–15 min. The mixture was quenched with 1–2 drops of saturated aqueous NH_4Cl. The ammonia was evaporated under nitrogen. The residue was portioned between ether and water and was stirred until two phases formed. The aqueous phase was extracted with ether (3×15 mL) and the pooled organic phases were dried over $MgSO_4$ and evaporated to yield the ring open compound (e.g., **7**, **10**). In case of dibenzo[*c*,*g*]azecines also tetrahydroisoquinoline derivatives (**17**, **19**) were obtained as by-products.

5.1.2. Synthesis of the quaternary salts (e.g., 13 and 16)—general procedure

A 10-fold molar excess of methyl iodide was added to a stirred solution of the respective quinolizine (e.g., **5**, **9**) in acetonitrile. Under nitrogen, the mixture was stirred for 48 h at room temperature. The precipitated solids were isolated by filtration, washed with little acetonitrile and dried in vacuo.

5.1.3. Ether cleavage of methoxylated compounds (5, 10, and 13)—general procedure

The methoxy compound was dissolved in a mixture of 20 mL of glacial acetic acid and 10 mL aqueous HBr (48%) and refluxed under nitrogen for 5 h. The solvents were removed in vacuo and the residue was crystallized from methanol/diethyl ether. Hydroxy compound **11** was obtained from the methoxy congener **10** by cleavage with a fivefold molar excess of BBr_3 in $CHCl_3$.

5.1.4. 3-Methoxy-5,8,13,13a-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinoline (5)

A solution of 3-methoxyphenethylamine (0.02 mol) and 3-isochromanone (0.022 mol) in toluene (100 mL) was refluxed under nitrogen for 6 h. After cooling down to room temperature, the amide can be filtered off as white-yellow solid. The amide (0.005 mol) was dissolved in a mixture of 40 mL acetonitrile and 5 mL $POCl_3$. The solution was refluxed for 18 h. The solvents were removed in vacuo and the resulting brown oil washed twice with 20 mL of petroleum ether. The residue was dissolved in 40 mL methanol and cooled to 0 °C in an ice bath. $NaBH_4$ (6 g) was added in small portions to the cooled reaction mixture. The cooling was removed and the suspension refluxed for 0.5 h. After evaporation of the solvent, the residue was dissolved in water and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The resulting yellow oil was crystallized from methanol to yield 62% of a yellow powder. Mp: 224 °C (hydrochloride), ^{1}H NMR: 250 MHz ($CDCl_3$): δ 2.59–3.42 (m, 6H); 3.59–3.86 (m, 2H); 3.82 (s, 3H, OMe); 4.03–4.08 (d, J = 14.95 Hz, 1H) 6.69–6.70 (d, J = 2.5 Hz, 1H, 4); 6.80–6.84 (dd, J = 2.4, 8.6 Hz, 1H, 2); 7.09–7.21 (m, 5H, 1, 9, 10, 11, 12); ^{13}C NMR: 250 MHz (DMSO-d_6): δ 29.77; 36.81; 51.31; 55.23; 58.60; 59.49; 76.56; 77.07; 77.58; 112.50; 113.18; 125.82; 126.14; 126.26; 126.56; 128.74; 130.27; 134.45; 134.49; 135.83; 157.82 GC–MS: m/z 264 (100%); 250 (11%); 234 (2%); 218 (6%); 204 (2%); 191 (2%); 178 (2%); 167 (1%); 160 (82%); 147 (10%); 139 (1%); 130 (4%); 117 (13%); 104 (54%); 91 (9%); 78 (18%); 65 (4%). Anal. ($C_{18}H_{19}NO$): C, H, N.

5.1.5. 5,8,13,13a-Tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolin-3-ol hydrobromide (6)

The synthesis was performed according to the general procedures in the manuscript from 0.4 g of the methyl-ether **5**. Recrystal-

lization from methanol/diethyl ether yielded 23% brown powder. Mp: 245 °C, ^{1}H NMR: 250 MHz (DMSO-d_6): δ 2.97–3.91 (m, 6H); 4.52–4.74 (m, 2H); 4.76–4.92 (m, 1H, 13*a*); 6.63–6.64 (d, *J* = 2,1 Hz, 1H, 4); 6.74–6.78 (dd, *J* = 1.9, 8.2 Hz, 1H, 2); 7.25–7.32 (m, 5H, 1, 9, 10, 11, 12); 10.52 (s, 1H, OH); ^{13}C NMR, dept: 250 MHz (DMSO-d_6): δ 23.97; 33.21; 50.45; 54.88; 59.56 (13*a*); 115.05; 115.19; 122.86; 126.51; 127.26; 127.35; 128.27; 128.84; 129.24; 132.15; 133.23; 157.22. Anal. ($C_{17}H_{18}BrNO \times 1/8$ HBr): C, H, N.

5.1.6. 10-Methoxy-6-methyl-5,6,7,8,13,14-hexahydrodibenzo[*c*,*g*] azecine hydrochloride (7)

The synthesis was performed according to the general procedures in the manuscript using 0.7 g of **13**. Flash chromatography on silica gel with methanol/chloroform (1:4) as eluent, gave a yellow oil. Addition of two drops of ethereal HCl and recrystallization from 2-propanol/diethyl ether yielded 9.7% of white crystals. Mp: 214 °C, ^{1}H NMR: 250 MHz (methanol-d_4): δ 2.80–2.94 (m, 1H, 4); 3.08–3.51 (m, 8H, 7, 8, 13, 14); 3.85–3.97 (m, 1H, 5); 4.42–4.48 (dd, *J* = 4.4, 10.7 Hz, 1H, 5); 5.95–5.99 (d, *J* = 8.4 Hz, 1H, 9); 6.35–6.39 (dd, *J* = 2.5, 8.4 Hz, 1H, 11); 6.68–6.69 (d, *J* = 2.3 Hz, 1H, 12); 7.10–7.22 (m, 4H, 1, 2, 3, 4). HRMS *m*/*z* calcd for $C_{19}H_{24}NO$ 282.1852 found 282.1850; EI-MS *m*/*z*: 281 (100%); 266 (12%); 250 (23%); 235 (13%); 223 (51%); 208 (17%); 192 (7%); 176 (56%); 165 (9%); 159 (7%); 146 (35%); 134 (23%); 115 (11%); 104 (18%); 91 (19%); 78 (10%); 65 (5%); 57 (9%); 44 (29%); 36 (17%); GC–MS: (base) *m*/*z*: 281 (3%); 266 (3%); 250 (5%); 235 (3%); 219 (2%); 176 (10%); 162 (8%); 144 (30%); 130 (11%); 118 (20%); 104 (100%); 91 (40%); 78 (80%); 65 (20%). Anal. ($C_{19}H_{24}ClNO \times 1/9$ HCl) C, N; for H calcd 7.55, found 6.92.

5.1.7. 6-Methyl-5,6,7,8,13,14-hexahydrodibenzo[*c*,*g*]azecin-10-ol hydrochloride (8)

The synthesis was performed according to the general procedures in the manuscript from 0.05 g of the methyl-ether **7**. The crude product was dissolved in a small amount of acetone. After adding two drops of ethereal HCl and some drops of ether, brown oil separates, which could be dried in vacuo to a brown powder. Mp: 198 °C. Yield 14.1%. ^{1}H NMR: 250 MHz (methanol-d_4): δ 2.78–2.91 (m, 1H,7); 2.99–3.09 (m, 7H, 13, 14, N–Me); 3.30–3.34 (m, 2H, 8); 3.50–3.60 (m, 1H, 7); 4.43–4.58 (q, *J* = 14.0 Hz, 2H, 5); 6.53–6.60 (m, 2H, 9, 11); 7.01–7.04 (d, *J* = 8.2 Hz, 1H, 12); 7.20–7.26 (m, 3H, 1,2,3,); 7.42–7.49 (d, *J* = 8.1 Hz, 4) ^{13}C NMR, dept: 250 MHz (methanol-d_4): δ 25.14; 31.74; 32.13; 42.82 (N–Me); 54.42; 55.66; 114.31; 115.46; 126.65; 127.74; 128.47; 128.66; 129.79; 130.55; 131.52; 132.29; 136.19; 142.02; 155.89. GC–MS: (base) *m*/*z*: 267 (2%); 252 (2%); 236 (2%); 223 (1%); 210 (2%); 146 (14%); 132 (2%); 120 (20%); 104 (30%); 91 (28%); 78 (33%); 65 (15%); 52 (18%); 44 (100%). Anal. ($C_{18}H_{22}ClNO \times 4/5H_2O \times 1/5$HCl) C, H, N.

5.1.8. 3-Methoxy-6,7,9,13b-tetrahydro-5*H*-isoindolo[1,2-*a*][2]benzazepine (9)

To a suspension of 5.0 g (0.13 mol) lithium aluminum hydride in 150 mL of dry THF there was added slowly under nitrogen and with cooling a solution of 5.0 g (0.18 mol) of the lactam **13** in 50 mL of dry THF. The mixture was refluxed for 1 h and stirred overnight. With cooling, the residual lithium aluminum hydride was decomposed by the careful addition of a saturated aqueous solution of potassium-sodium tartrate. After filtration of inorganic solids and washing of the filter cake with 50 mL of THF, the layers were separated and the aqueous layer was extracted with dichloromethane. After evaporation of the pooled organic extracts, the residue was dissolved in 50 mL of dichloromethane and dried over Na_2SO_4. Evaporation of the solvent yielded 2.2 g of **9** as yellow oil that slowly solidified after storage in the refrigerator. Work up from the lithium aluminum hydride reduction was accomplished as free base. Either strongly alkaline or acidic conditions should be avoided. This isoindole-derivative is very instable and attempts to convert it into an HCl-salt failed due the immediate formation of a dark green solution and no solids. The free base forms yellow oil which solidifies to a brick red solid upon storage in the refrigerator. Mp: 68–70 °C. Due to the instability, the pure compound shows three decomposition compounds in the GC–MS. The fragmentation of the amine is characteristic for quinolizine-like compounds and has a m−1 peak as the most stable fragment: *m*/*z*: 265 (34%); 264 (100%); 248 (32%); 236 (9%); 220 (8%); 204 (5%); 191 (6%); 156 (6%); 144 (13%); 131 (14%). ^{1}H NMR 250 MHz ($CDCl_3$): δ1.75–1.98 (m, 2H, 6); 2.74–3.40 (m, split in four separate signals, each matching 1H, 4H, 5, and 6); 3.83 (s, 3H, O–Me); 3.79–3.88 (d, *J* = 13 Hz, 1H, 8); 4.39–4.44 (d, *J* = 13 Hz, 1H, 8); 5.70 (s,1H, 13b); 6.62–6.67 (dd, *J* = 2.6, 8.5 Hz, 1H, 2); 6.77–6.78 (d, *J* = 2.6, 1H, 4); 7.17–7.20 (d, *J* = 8.5 Hz, 1H, 1); 7.25–7.30 (m, 4H, 9, 10, 11, 12). Anal. ($C_{18}H_{19}NO \times H_2O$): C, H, N.

5.1.9. 11-Methoxy-6-methyl-5,6,7,8,9,14-hexahydrodibenzo[*c*,*f*] azecine (10)

According the general procedure in the manuscript, 1.7 mmol (700 mg) of the mossy green crude quaternary salt **16** were ring opened with sodium in liq. ammonia. The amber colored oily residue was dissolved in 1 mL of 2-propanol and converted into an HCl-salt by the addition of three drops concd HCl acid. The greenish precipitation was recrystallized from acetone/2-propanol to give 300 mg of white needles with mp 208–210 °C. Yield: 55% after recrystallization. ^{1}H NMR 250 MHz (methanol-d_4): (HCl-salt) δ 1.9–2.2 (m, 1H, 5, 6 or 7); 2.4–3.0 (m, 4H, 5, 6 or 7); 3.09 (s, 3H, N-Me); 3.09–3.28 (m, 1H, 5, 6 or 7); 3.74 (s, 3H, O-Me); 3.91–3.97 (d, *J* = 15 Hz, 1H, 14); 4.21–4.27 (d, *J* = 15 Hz, 1H, 14); 4.41–4.46 (d, *J* = 13 Hz, 1H, 9); 4.76–4.80 (d, *J* = 13 Hz, 1H, 9); 6.72 (d, *J* = 2.5 Hz, 1H, 4); 6.79–6.81 (dd, *J* = 2.5, 8.5 Hz 1H, 2); 7.29–7.51 (m, 4H, 10, 11, 12, 1); 7.51–7.77 (dd, *J* = 8.0/1.0 Hz, 1H, 13). GC–MS: (base) *m*/*z*: 281 (37%); 266 (5%); 249 (10%); 235 (7%); 223 (19%); 209 (100%); 190 (84%); 177 (35%); 165 (32%); 152 (9%); 145 (21%); 115 (14%). Anal. ($C_{19}H_{23}NO \times$ HCl): C, H, N.

5.1.10. 6-Methyl-5,6,7,8,9,14-hexahydrodibenzo[*c*,*f*]azecin-11-ol (11)

An aqueous solution of 317 mg **10** HCl (1 mmol) was alkalized with some NaOH and extracted with $CHCl_3$. After drying over $MgSO_4$, the solvent was evaporated to approximately 50 mL. With an external cooling bath and under nitrogen atmosphere, 450 μL of BBr_3 (~5 mmol) were added through a septum. The cooling bath was removed and the reaction mixture was brought to reflux for 1 h. When the mixture returned to room temperature, it was poured onto chipped ice and the pH was brought to 9 with 2 N NaOH. Extraction with CH_2Cl_2, drying over $MgSO_4$ and evaporation yielded 151 mg of white foam. Yield 56%. Mp: 139–140 °C, ^{1}H NMR: 250 MHz ($CDCl_3$): (base): δ 1.48–1.51 (mc, 4H, 6, 10); 2.03–2.07 (mc, 4H, 7, 9); 2.17 (s, 3H, N–Me); 2.65–2.75 (mc, 4H, 5, 11); 4.06, (s, 2H, 16); 6.47–6.52 (dd, *J* = 2.6, 8.2, 1H, 2); 6.61 (d, *J* = 2.6, 1H, 4); 6.86–6.90 (d, *J* = 8.2, 1H, 1); 7.01–7.14 (m, 4H, 12, 13, 14, 15). GC–MS: (base) *m*/*z*: 267 (3%); 252 (2%); 236 (5%); 221 (3%); 209 (12%); 195 (39%); 176 (18%); 165 (45%); 152 (30%); 146 (30%); 132 (35%); 120 (46%); 115 (52%); 107 (48%); 104 (72%); 102 (71%); 91 (100%); 70 (100%); 57 (92%). Anal. ($C_{18}H_{21}NO \times 1/5$ H_2O): C, H, N.

5.1.11. 3-Methoxy-7-methyl-5,8,13,13a-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolinium iodide (13)

The synthesis was performed according to the general procedures in the manuscript using 2 g of **5**. The precipitate was filtered and washed with little ether to yield 96% of white crystals. Mp:

199 °C; ^{1}H NMR: 250 MHz (DMSO-d_6): δ 2.82 (s, 3H, N–Me); 2.98–3.45 (m, 3H); 3.78 (s, 3H, MeO) 3.82–4.16 (m, 3H); 4.76–4.98 (dd, J = 15.5, 39.75 Hz, 2H, 10); 5.15–5.22 (dd, J = 4.4, 12.5 Hz, 1H, 13*a*); 6.87–6.99 (m, 2H); 7.28–7.45 (m, 5H); ^{13}C NMR: 250 MHz (DMSO-d_6): δ 23.78; 28.96; 33.81; 55.76; 60.57; 64.70; 65.47; 113.77; 114.35; 122.58; 126.88; 127.25; 127.76; 128.51; 128.77; 130.16; 131.02; 132.39, 159.66. Anal. ($C_{19}H_{22}INO$): C, H, N.

5.1.12. 3-Hydroxy-7-methyl-5,8,13,13a-tetrahydro-6*H*-isoquino[3,2-*a*]isoquinolinium bromide (14)

The synthesis was performed according to the general procedures in the manuscript from 1 g of **13**. Recrystallization from methanol yielded 43% of a brown powder. Mp: 274 °C; ^{1}H NMR: 250 MHz (DMSO-d_6): δ 2.80 (s, 3H, N–Me); 2.96–3.41 (m, 2H); 3.84–4.07 (m, 4H); 4.70–4.49 (dd, J = 15.5, 39.75 Hz, 2H, 10); 5.08–5.14 (dd, J = 4.4, 12.5 Hz, 1H, 13*a*); 6.69 (d, J = 1.8 Hz, 1H, 4); 6.76–6.80 (dd, J = 1.9, 10.7 Hz, 1H, 2); 7.26–7.38 (m, 5H, 1.9, 10, 11, 12); 9.71 (s, 1H, OH); ^{13}C NMR, dept: 250 MHz (DMSO-d_6): δ 23.61; 28.99; 38.79 (N-Me); 60.62; 64.64; 65.50 (13*a*); 115.33; 115.44; 120.88; 127.18; 127.24; 127.74; 127.75; 128.66; 129.48; 130.90; 132.15; 157.72. Anal. ($C_{18}H_{20}BrNO \times 1/11$ HBr): C, H, N.

5.1.13. 3-Methoxy-5,6,7,13b-tetrahydro-9*H*-isoindolo[1,2-*a*][2]benzazepin-9-one (15)

According to the general procedure, described by Heaney and Shuhaibar[9], a mixture of 12.5 g (75.5 mmol) methyl-2-formylbenzoate and 12.35 g (75.5 mmol) of 3-(3-methoxyphenyl)-propylamine in 80 mL of toluene was refluxed for 4 h under Dean–Stark conditions. After evaporation of the solvent and recrystallization from ethyl acetate, 14 g of the imine were obtained as off-white powder with >99% GC–MS purity. Yield: 60%. GC–MS: 311 (1%); 296 (6%); 177 (100%); 145 (62%); 132 (25%); 121 (20%); 91 (16%). For the rearrangement, a pea-sized piece of metallic sodium was added to a solution of 1.5 g (4.8 mmol) of the imine in 50 mL of methanol and the mixture was stirred under an inert atmosphere for 2 days. After removal of the solvent, the residue was dissolved in dichloromethane and washed with water to yield after drying over Na_2SO_4 and evaporation of the solvent 1.2 g of a methoxy-isoindolone as oil. (Yield: 80%.) ^{1}H NMR: ($CDCl_3$) δ 1.90–1.99 (m, 2H, 9); 2.57–2.63 (t, J = 8.6 Hz, 2H, 10); 2.79 (s, 3H, 1-OMe); 3.18–3.29 (m, 1H, 8); 3.70 (s, 3H, 12-OMe); 3.73–3.82 (m, 1H, 8); 5.78 (s, 1H, 1); 6.62–6.73 (m, 3H, 11, 13, 15); 7.07–7.11 (t, J = 7.8 Hz, 1H, 14); 7.44–7.48 (m, 3H, 3, 4, 5); 7.73–7.77 (m, 1H, 2). GC–MS: 311 (38%); 297 (18%); 296 (88%); 188 (10%); 147 (100%); 133 (21%); 121 (32%); 91 (32%). For the acyliminium cyclization, 3.0 g (9.6 mmol) of the methoxy-isoindolone were dissolved in 50 mL of dichloromethane. The solution was cooled to −78 °C in a dry-ice/methanol bath under an inert atmosphere and 1.2 mL (9.6 mmol) of $TiCl_4$ were added through a septum. The solution was stirred in the ice bath and allowed to return to room temperature overnight. The reaction mixture was washed with satd solution of sodium hydrogen carbonate, the phases were separated, the organic layer dried over Na_2SO_4 and evaporated to leave the lactam as an oil which was purified by column chromatography ($CHCl_3$/MeOH) 9:1. Yield: 83%. ^{1}H NMR: 400 MHz ($CDCl_3$): δ 1.95–2.25 (mc, 2H, 6); 2.70–2.80 (mc, 2H, 5); 3.32–3.40 (mc, 2H, 7); 3.80 (s, 3H, OMe); 4.34–4.39 (mc, 2H, 7); 5.71 (s, 1H, 13b); 8.60 (s, 1H, 4); 6.73–6.76 (d, 1H, J = 7.5, 2) 7.17–7.20 (d, J = 7.5, 1H, 1); 7.46–7.58 (m, 3H, 10, 11, 12); 7.90–7.92 (d, J = 7.5, 13). GC–MS m/z: 279 (100%); 279 (87%); 264 (4%); 250 (53%); 220 (30%); 178 (11%); 165 (10%); 147 (14%) 130 (11%); 102 (6%).

5.1.14. 3-Methoxy-8-methyl-6,7,9,13b-tetrahydro-5*H*-isoindolo[1,2-*a*][2]benzazepinium iodide (16)

The solution of 2 g of compound **9** in acetonitrile and an excess of methyl iodide turns deeply green. After evaporation of the solvents, crude **16** was obtained as dark green oil. Attempts to induce crystallization from methanol/diethyl ether failed but the ether insoluble oil popped up under reduced pressure to mossy green foam which was successfully used for the ring opening procedure without further purification. Yield 72%.

5.1.15. 2-Methyl-1-(2-methylbenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol hydrochloride (17)

The synthesis was performed according to the general procedures in the manuscript using 0.5 g of **14**. Removing of ether in vacuo yielded white foam. Addition of two drops of etheric HCl and recrystallization from 2-propanol/diethyl ether yielded 53% of white crystals. Mp: 224 °C; ^{1}H NMR: 250 MHz (methanol-d_4): δ 1.95 (s, 3H, 2′-Me); 2.98 (s, 3H, N–Me); 3.08–3.51 (m, 5H); 3.85–3.97 (m, 1H); 4.42–4.48 (dd, J = 4.4, 10.7 Hz, 1H); 5.95–5.99 (d, J = 8.4 Hz, 1H, 8); 6.35–6.39 (dd, J = 2.5, 8.4 Hz, 1H, 7); 6.68–6.69 (d, J = 2.3 Hz, 1H, 5); 7.13–7.20 (m, 4H, 3′,4′,5′, 6′); ^{13}C NMR, dept: 250 MHz (methanol-d_4): δ 17.94 (2′-Me); 22.29; 36.13; 39.40; 44.90 (N–Me); 64.21; 113.36; 114.61; 120.04; 125.89; 127.15; 129.40; 130.17; 130.51; 130.88; 133.43; 137.21; 157.56. EI-MS m/z: 162 (100%); 147 (10%); 117 (2%); 105 (9%); 77 (5%); 65 (2%); 42 (11%); 36 (8%). Anal. ($C_{18}H_{22}ClNO \times 1/11$ HCl) C, H, N.

5.1.16. 3-(2-Ethyl-4-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydro-isoquinoline hydrochloride (18)

To a solution of 0.48 g (1.18 mmol) quaternary salt **11** in 20 mL methanol, 1 g of freshly prepared silver oxide was added and the mixture refluxed for 20 min. The suspension was cooled to room temperature, filtered and the solution treated with charcoal for 10 min at room temperature. After filtration the solvent was removed in vacuo. The residue was dissolved in 5 mL of DMSO and allowed to stand at room temperature for 15 min. Ice water (2.5 mL) was added, and the solution extracted with benzene. The organic layer was dried over potassium carbonate, passed through a column of alumina, and eluted with 100 mL of benzene to yield 208 mg of yellow oil, which was crystallized from acetone to obtain 125 mg of white crystals. The solid was dissolved in 20 mL of dioxane and stirred with platinum oxide in hydrogen atmosphere (balloon) at room temperature for 5 h. After filtration, the solvent was removed in vacuo. The resulting yellow oil crystallized after addition of two drops etheric HCl from 2-propanol/diethyl ether. Yield 15%. Mp: 229 °C; ^{1}H NMR: 250 MHz (methanol-d_4): δ 1.21–1.27 (t, J = 7.5 Hz, 3H, Ph–CH_2–CH_3); 2.70 (s, 3H, NMe); 2.61–2.93 (m, 2H, Ph–CH_2–CH_3); 3.24–3.65 (m, 2H); 3.84 (s, 3H, MeO); 4.65–4.92 (m, 3H); 6.74–6.99 (m, 2H); 7.28–7.33 (m, 4H); 7.51–7.59 (d; J = 8.5 Hz, 1H); ^{13}C NMR, dept: 250 MHz (methanol-d_4): δ 14.99 (Ph–CH_2–CH_3), 25.61; 35.03; 39.23 (N–Me); 54.47 (MeO); 56.52; 61.29 (3); 112.76; 114.78; 124.10; 125.91; 127.00; 127.86; 127.90; 127.96; 128.20; 131.69; 145.29; 160.65. GC–MS: (base) m/z: 281 (5%); 266 (3%); 250 (8%); 235 (5%); 219 (4%); 204 (2%); 191 (2%); 176 (20%); 162 (16%); 146 (58%); 132 (18%); 118 (23%); 104 (100%); 91 (44%); 78 (68%); 65 (19%). Anal. ($C_{19}H_{24}ClNO$) C, H, N.

5.1.17. 6-Methoxy-2-methyl-1-(2-methylbenzyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (19)

The synthesis was performed according to the general procedures in the manuscript using 0.7 g of **13**. Silica gel column with methanol/chloroform (1:4) as eluent yielded a yellow oil. Addition of two drops of etheric HCl and crystallization from 2-propanol/diethyl ether yielded 60% of white crystals. Mp: 192 °C; ^{1}H NMR: 250 MHz (methanol-d_4): δ 1.95 (s, 3H, 2′-Me); 3.00 (s, 3H, N–Me); 3.10–3.51 (m, 5H); 3.76 (s, 3H, MeO); 3.89–4.00 (m, 1H); 4.48–4.54 (dd, J = 4.3, 10.7 Hz, 1H, 1); 6.06–6.10 (d, J = 8.6 Hz, 1H, 8); 6.49–6.54 (dd, J = 2.4, 8.6 Hz, 1H, 7); 6.84–6.85 (d, J = 2.5 Hz, 1H, 5); 7.10–7.19 (m, 4H, 3′,4′,5′, 6′); ^{13}C NMR, dept: 250 MHz (metha-

nol-d_4): δ 19.38 (2′-Me); 22.01; 38.42; 40.10; 44.32 (N–Me); 55.28 (MeO); 64.32; 112.66; 113.54; 120.68; 126.15; 127.45; 129.97; 130.11; 130.48; 131.14; 133.43; 137.14; 159.70. GC–MS *m*/*z*: 176 (100%); 161 (23%); 144 (6%); 132 (54%); 117 (9%); 105 (91%); 91 (11%); 77 (70%); 65 (17%). Anal. ($C_{19}H_{24}ClNO$) C, H, N.

5.2. X-ray analyses of compounds 7 and 10

The intensity data for the compounds were collected on a Nonius KappaCCD diffractometer using graphite-monochromated Mo Kα radiation. Data were corrected for Lorentz and polarization effects but not for absorption effects.[13,14]

The structures were solved by direct methods (SHELXS[15]) and refined by full-matrix least squares techniques against F_o^2 (SHELXL-97[16]). For the whole compound **7** and for the amine-groups of **10** the hydrogen atoms were located by difference Fourier synthesis and refined isotropically. The other hydrogen atom positions were included at calculated positions with fixed thermal parameters. Compound **10** crystallized as a racemic twin. All non-hydrogen atoms were refined anisotropically.[16] XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

5.2.1. Crystal data for 7

$C_{19}H_{24}ClNO$, Mr = 317.84 g mol^{-1}, colorless prism, size 0.04 × 0.04 × 0.04 mm^3, monoclinic, space group $P2_1/c$, a = 14.4129(5), b = 11.1844(6), c = 11.4434(6) Å, β = 108.873(2)°, V = 1745.50(14) Å^3, T = −90 °C, Z = 4, ρ_{calcd} = 1.209 g cm^{-3}, μ (Mo Kα) = 2.21 cm^{-1}, F(0 0 0) = 680, 12,180 reflections in h(−18/18), k(−14/12), l(−14/14), measured in the range 2.62° ⩽ Θ ⩽ 27.46°, completeness Θ_{max} = 99.7%, 3987 independent reflections, R_{int} = 0.0569, 2797 reflections with $F_o > 4\sigma(F_o)$, 295 parameters, 0 restraints, $R1_{obs}$ = 0.0433, wR^2_{obs} = 0.0886, $R1_{all}$ = 0.0775, wR^2_{all} = 0.1024, GOOF = 1.010, largest difference peak and hole: 0.186/−0.229 e Å^{-3}.

5.2.2. Crystal data for 10

$C_{19}H_{25}ClNO_{1.50}$, Mr = 326.85 g mol^{-1}, colorless prism, size 0.04 × 0.04 × 0.04 mm^3, triclinic, space group $P1$, a = 7.1171(3), b = 14.7356(6), c = 17.1663(8) Å, α = 103.813(2)°, β = 95.077(3)°, γ = 90.005(3)°, V = 1740.97(13) Å^3, T = −90 °C, Z = 4, ρ_{calcd} = 1.247 g cm^{-3}, μ (Mo Kα) = 2.25 cm^{-1}, F(0 0 0) = 700, 12,481 reflections in h(−9/8), k(−19/19), l(−21/22), measured in the range 2.09° ⩽ Θ ⩽ 27.50°, completeness Θ_{max} = 98%, 12,481 independent reflections, R_{int} = 0.0000, 9649 reflections with $F_o > 4\sigma(F_o)$, 826 parameters, three restraints, $R1_{obs}$ = 0.0596, wR^2_{obs} = 0.1270, $R1_{all}$ = 0.0905, wR^2_{all} = 0.1453, GOOF = 1.039, Flack-parameter 0.47(6) (racemic twin), largest difference peak and hole: 0.395/−0.438 e Å^{-3}.

5.3. Pharmacological assays

5.3.1. Dopamine receptor affinity

5.3.1.1. Cell culture. Human D_1, D_{2L}, D_3, $D_{4.4}$, and D_5 receptors were stably expressed in Chinese hamster ovary (CHO) cells or human embryonic kidney cells (HEK293). D_1, D_{2L}, D_3, and D_5 were expressed in HEK cells and $D_{4.4}$ receptors were expressed in CHO cells, respectively. Cells were grown at 37 °C under an atmosphere of 5% CO_2: 95% air in HAM/F12-medium (Sigma–Aldrich) for CHO cells and Dulbecco's modified Eagles Medium Nutrient mixture F-12 Ham for HEK293 cells, each supplemented with 10% fetal bovine serum, 1 mM L-glutamine and 0.2 μg/mL of G 418 (all from Sigma–Aldrich).

5.3.1.2. Preparation of whole-cell-suspension[17]. Human D_1, D_{2L}, D_3, D_4, and D_5 receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85% confluence. The medium was removed and the cells were incubated with 3 mL trypsine–EDTA-solution (Sigma–Aldrich) to remove the cells from the culture dish. After incubation, cells were suspended in 6 mL medium in order to stop the effect of trypsine–EDTA-solution. The resulting suspension was centrifuged (483 RCF, 4 °C, 4 min). The pellet was re-suspended in 20 mL of ice-cooled PBS (phosphate-buffered saline: 137 mM NaCl, 2.7 mM, 8.1 mM Na_2HPO_4 and 1.4 mM KH_2PO_4) and again centrifuged to obtain a pellet. This procedure was repeated once before the resulting pellet was suspended in 12 mL of buffer (5 mM magnesium chloride, 50 mM TRIS–HCl, pH 7.4). This resulting suspension was directly used for the radioligand binding assay.

5.3.1.3. Radioligand binding assay. The binding studies were performed following the protocol previously described but in 96-well format.[3] 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). As radioligands, for the D_1 family (D_1 and D_5) [3H] SCH23390, and for the D_2 family (D_2, D_3, D_4) [3H] spiperone were used. Non-specific binding was determined using fluphenazine (100 μM) for D_1 and D_5 tests and haloperidol (10 μM) for D_2, D_3, and D_4 tests. The incubation was initiated by addition of the radioligand and was carried out in 96 deep well plates (Greiner bio-one, Frickenhausen) using a thermocycler (Thermocycler comfort®, Eppendorf, Wessling) at 27 °C. The incubation was terminated after 90 min by rapid filtration with a Perkin–Elmer Mach III Harvester™ using a Perkin–Elmer Filtermat A, previously treated with a 0.25% polyethyleneimine-solution (Sigma–Aldrich) and washed with water. The filtermat was dried for 3 min at 400 W using a microwave oven (MW 21, Clatronic, Kempen). The dry filtermat was placed in a filter plate (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 each in triplicate were performed.

The competition binding data were analyzed with GraphPad Prism™ software using nonlinear regression with sigmoidal dose response equation. For calculating of mean, standard deviation and standard error of the mean the software Microsoft Excel™ was used. K_i values were calculated from IC_{50} values applying the equation of Cheng and Prusoff.[18]

5.3.2. Functional assay: measuring intracellular Ca^{2+} with a fluorescence microplate reader[3,19]

5.3.2.1. Cell culture. Human D_1 and D_{2L} receptors were stably expressed in human embryonic kidney cells (HEK293) and cultured as mentioned above.

5.3.2.2. Preparation of whole-cell-suspension. Human D_1 and D_{2L} receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85–90% confluence. The medium was removed via a suction apparatus 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 these 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 Krebs-HEPES 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. To this cell suspension 5 mL of Krebs-HEPES buffer were added again to rinse all cells from the plate. Then the resulting suspension portioned in 10 1.5 mL Eppendorf caps and centrifuged at 10,640 RCF for 10 s.

The supernatant buffer was removed and the resulting 10 pellets are divided in two portions of five pellets, which are suspended, in 1 mL of Krebs-HEPES buffer, each. The two suspensions are centrifuged again for 10 s. After removing the buffer, the two pellets were combined and re-suspended in 1 mL buffer, diluted with 17 mL of Krebs-HEPES buffer and plated into 96-well plates (OptiPlate HTRF-96™, Packard, Meriden, CT; Cellstar, Tissue Culture Plate, 96 W, Greiner bio-one, Frickenhausen). Microplates were kept at 37 °C under an atmosphere including 5% CO_2 for 30 min before they were used for the assay.

5.3.2.3. Calcium assay[3]. Screening for agonistic and antagonistic activity was performed using a NOVOstar microplate reader™ (BMG LabTechnologies) with a pipettor system. Agonistic activities were tested by injecting 20 μL buffer alone as negative control, standard agonist in buffer as positive control, and test compounds in buffer in rising concentrations, respectively, each into separate wells. Fluorescence measurement started simultaneously to the automatic injection. SKF 38393 was used as standard agonist for D_1 receptors and quinpirole for D_2 receptors (final concentration: 1 μM).

Screening for antagonistic activities was performed by pre-incubating the cells with 20 μL of the test compound dilutions (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 30 min prior to injection of 20 μL standard agonist per well. As standard agonists we used, as described for the agonist screening, SKF38393 for D_1 and quinpirole for D_2 receptors, respectively. Fluorescence measurement also started simultaneously to the automatic injection. At least two independent experiments each in four or six replications were performed.

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). Agonistic or antagonistic activities were assessed by a dose response curve obtained by determination of the maximum fluorescence intensity of each data set and nonlinear regression with sigmoidal dose response equation using GraphPadPrism™ 3.0.

6. Supplementary data

Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 730823 for **7**, and CCDC 730824 for **10**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [E-mail: deposit@ccdc.cam.ac.uk].

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