Brief Articles

Dopamine/Serotonin Receptor Ligands. 13[1]: Homologization of a Benzindoloazecine-Type Dopamine Receptor Antagonist Modulates the Affinities for Dopamine D_1−D_5 Receptors

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Enlarging the 10-membered ring of 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**1**, LE 300) yielded two homologue antagonists. Their affinities and inhibitory activities at D_1−D_5 receptors were measured by radioligand binding experiments and a functional Ca^{2+} assay. Compared to **1**, phenylpropyl homologue **3** was superior in selectivity and affinity for the D_5 subtype (K_i = 0.6 nM), whereas the affinity of the indolylpropyl homologue **2** for all subtypes decreased. Compounds **2**, **3**, **10**, **11**, **17**, and **18** are derivatives of novel heterocyclic ring systems.

Dopaminergic transmission plays a key role in locomotion, emotion, cognition, and endocrinal secretion. "Azecine-type" dopamine receptor antagonists such as **1** (LE 300)[2−6] and its dibenzo-analogues[1,6] were found to show nanomolar, subnanomolar, and even picomolar[6] affinities predominantly for the D_1 and D_5 subtypes. The unique pharmacological profiles and the novelty of these midsized heterocycles as potential antidopaminergic drugs prompted us to conduct further SAR studies in which we expanded the central ring from 10 to 11.

Using the antidopaminergic compound **1** as the lead, we enlarged the size of the central heterocycle but maintained its basic features; two arylalkylamine moieties are made moderately rigid by being incorporated into a midsize heterocycle, with the two aromatic systems separated by a methylene group (Figure 1). As a result of the asymmetry of the resulting azacycloundecenes, the two possible isomers **2** and **3** had to be considered as target compounds.

Replacement of tryptamine in compound **1** with homotryptamine **5** leads to **2**. The synthesis was conducted similarly to that of **1**[5] by using **5** instead of tryptamine as the starting material. Compound **5** was synthesized from the chloride of 3-(1*H*-indol-3-yl)propanoic acid by treating the compound with concentrated aqueous ammonia[7] and reducing the resulting amide **4** by adding lithium aluminum hydride[8] (Scheme 1). The second starting material **6** was prepared by an ultrasound-assisted oxidation[9] of isochromane with potassium permanganate. Improving previous protocols, we activated **6** by converting it with PCl_5 into the corresponding ω-chloro acid chloride **7**.[10] The reaction of **5** and **7** yielded a mixture of chloroethylbenzamide **8** and lactam **9**. Because both compounds are expected to be cyclized with $POCl_3$, the mixture was subjected to ring closure and reduction of the cyclic iminium salt using $NaBH_4$ without prior separation, and the pentacyclic homoquinolizine derivative **10** was obtained (Scheme 1). Compound **10** was conclusively quaternized with methyl iodide and "opened" by treatment with sodium in liquid ammonia to yield the target molecule **2**.

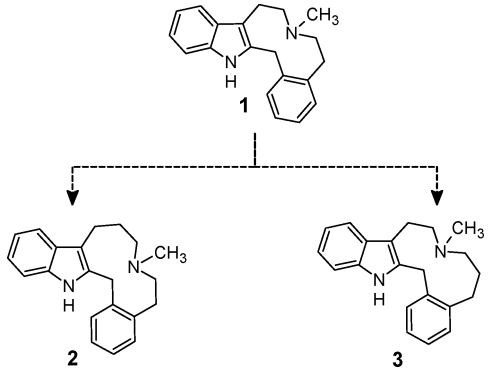

Figure 1. Lead compound **1** and its homologues **2** and **3**.

Lactone **15** served as the 3-carbon source for the preparation of homologue **3**. Ketone **12** was brominated to give the irritant and lacrimatory α-bromo ketone, which was directly treated with pyridine.[11] The resulting pyridinium salt **13** was heated with an aqueous solution of NaOH and subsequently neutralized to yield **14**. Vacuum pyrolysis of this betaine produced the desired lactone **15** (Scheme 2).[12]

Bischler Napieralski ring closure of compound **16**, obtained from lactone **15** and tryptamine, and subsequent reduction of the cyclic iminium salt by $NaBH_4$ yielded the pentacyclic precursor compound **17**. Methyl iodide in acetonitrile gave the quaternary salt **18**, which was cleaved to the target compound **3** by treatment with sodium in liquid ammonia (Scheme 2).

Both homologues **2** and **3** of **1** as well as their pentacyclic precursor molecules **10** and **17** were screened for their binding affinities for all human-cloned dopamine receptor subtypes by radioligand binding studies, following the protocol previously described,[13] but in a 96-well format. Additionally, their inhibitory activity was determined in a calcium fluorescence assay developed and established in our group.[14] Solubility problems did not occur. D_1, D_{2L}, D_3, $D_{4.4}$, and D_5 receptors were stably expressed in HEK 293 or CHO cells.

[^{3}H]SCH 23390 and [^{3}H]spiperone were used as radioligands at the D_1-like and D_2-like receptor family, respectively. Incubations at 27 °C were terminated after 90 min by rapid filtration

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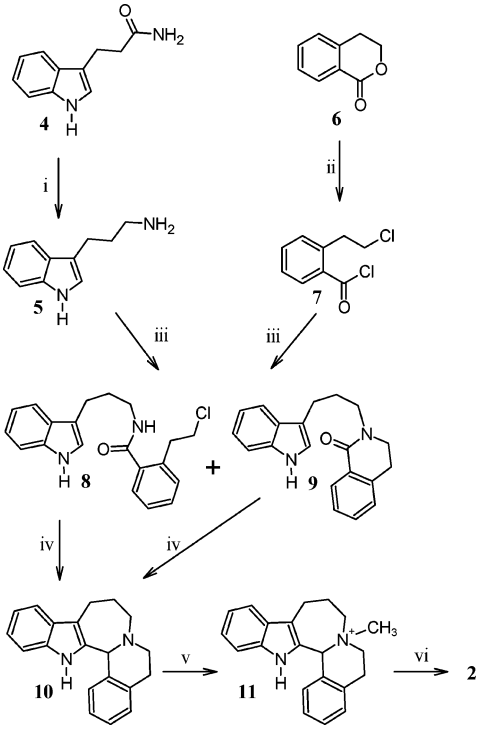
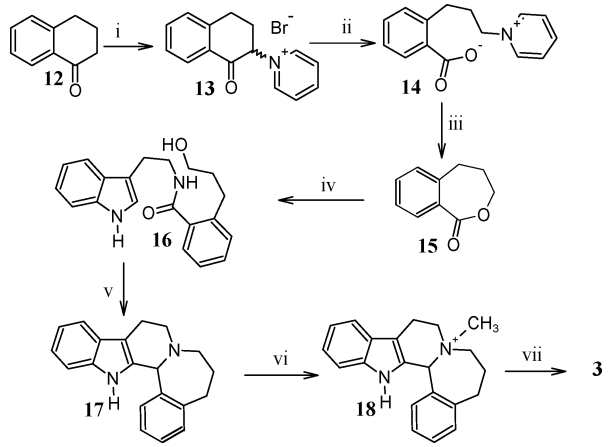
Scheme 1[a] Synthesis of 7-Methyl-5,6,7,8,9,10,15,16-octahydroindolo[3,2-*g*][3]benzazacycloundecine (**2**)



[a] Reagents and conditions: (i) $LiAlH_4$, dry THF, ice cooling, then reflux, 3 h; (ii) PCl_5, distillation; (iii) NEt_3, CH_2Cl_2, rt, 24 h; (iv) (1) $POCl_3$, MeCN, reflux, 72 h; (2) $NaBH_4$, ice cooling, MeOH, 1 h; (v) MeI, MeCN, rt, 5 days; (vi) Na^0, liq NH_3, −40 °C, 10 min.

Scheme 2[a] Synthesis of **3**



[a] Reagents and conditions: (i) (1) Br_2, Et_2O, −20 °C − rt, 3 h; (2) pyridine, rt, 2 weeks; (ii) (1) 2 N NaOH, reflux, 30 min; (2) HCl adj to pH 7; (iii) pyrolysis at 14 mbar; (iv) tryptamine, toluene, reflux, 24 h; (v) (1) $POCl_3$, MeCN, reflux 4 h; (2) $NaBH_4$, ice cooling, MeOH, 1 h; (vi) MeCN, MeI, 40 °C, 2 h; (vii) Na^0, liq NH_3, −40 °C, 10 min.

with a Perkin-Elmer Mach III harvester. At least two independent experiments were carried out, each in triplicate. K_i values were calculated from IC_{50} values by applying the equation of Cheng and Prusoff[15] and are given in nanomolar units (Table 1).

The 11-membered homologues **2** and **3** differ significantly with regard to their affinities for the dopamine receptor subtypes. Compared to the lead compound **1**, the compound with the ring enlargement next to the indole moiety (**2**) showed a decrease in affinities for all of the receptor subtypes, most predominantly for D_1 by a factor of 80, for D_3 by a factor of 20, for D_5 by a factor of 12, and for the D_2 receptor with only a 3-fold drop in affinity. (Table 1). Maintaining the tryptamine template and elongating phenylethyl to phenylpropyl (**3**) showed to be much more favorable. Apart from the D_3 subtype, **3** either showed the same affinity as or higher affinity than the lead compound **1**. Surprisingly, the affinity of the phenylpropyl homologue **3** for D_5 increased from $K_i = 7.5$ nM (lead compound **1**) to $K_i = 0.61$, displaying a 3.5-fold selectivity with regard to D_1.

Compounds **2** and **3**, as well as the precursor compounds **10** and **17**, include identical structural elements. In preliminary screening experiments, no affinities (radioligand displacement) or inhibitory activities (functional Ca^{2+} assay), were found ($K_i > 10\ \mu M$) for **10** and **17**, which confirms the hypothesis that only a very moderate rigidization, resulting from the incorporation of two arylalkylamine moieties into a midsized heterocycle, leads to high-affinity ligands. The functional calcium assay showed that compounds **1**, **2**, **3**, **10**, and **17** did not increase the intramolecular calcium concentration, which proves the absence of any agonistic activity. Concentration-dependent suppression of the calcium signal, induced by a standard agonist (SKF 38393 for D_1 and D_5 and quinpirole for the D_2 receptor) produced a sigmoidal curve that describes the inhibitory activity of the compounds.[14] Compounds **1**, **2**, and **3** are either inverse agonists or antagonists; compounds **10** and **17** are inactive.

The K_i values obtained from the calcium assay (Table 2) generally seem to be somewhat higher than those obtained from the radioligand displacement experiments. Only for the D_2 subtype were the K_i values derived from the functional calcium assay found to be significantly lower, indicating a higher inhibitory activity at this binding site than to be expected from the affinity measured by the radioligand experiment. Surprisingly, compounds **1** and **3** at D_1 are equal in affinity, whereas **3** is 20-fold more potent than **1** in the calcium data. It has to be pointed out that radio ligand binding is performed in a 90 min equilibrium, whereas the calcium signal induced by addition of the agonist is generated within seconds. No further explanation for discrepancies between both assays can be given at present.

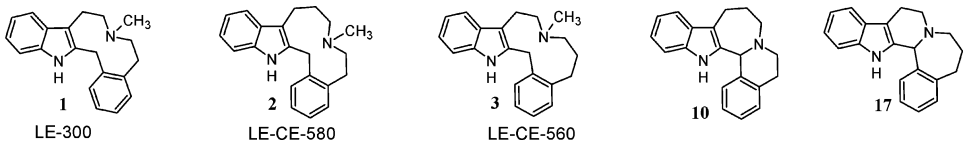
The results demonstrate that the biological activities of the 11-membered homologues are sensitive to the area of ring expansion. Replacing the 2-indolylethyl with a 3-indolylpropyl moiety generally decreases the affinities (D_1, D_3, D_5) by 1−2 orders of magnitude, whereas replacing the 2-phenylethyl part with 2-phenylpropyl enhances the affinities for all receptors, with the exception of D_3, but most remarkably for D_5, where compound **3** was shown to be a novel nanomolar to subnanomolar fairly selective ligand for the dopamine D_1/D_5 receptor family. Furthermore, compound **3** exhibits some selectivity toward D_5 in the radioligand binding studies, but not in the functional assay. These findings suggest that the optimal distance of the indole moiety to the nitrogen is measured by a two-carbon chain, which has to be confirmed in future investigations.

Experimental Section

2-(3-Hydroxypropyl)-*N*-[2-(1*H*-indol-3-yl)ethyl]benzamide (16): Under an inert atmosphere and protected from light, (20 mmol) **15** and (30 mmol) tryptamine in 6 mL of toluene were refluxed for 24 h. The solvent was removed and the residue portioned between CH_2Cl_2 and 2 N HCl. The organic layer was dried ($MgSO_4$) and decolorized with charcoal. Evaporation yielded **16**. (42%).

Condensation of Amine 5 with Acid Chloride 7: A solution of 25 mmol **7** in 50 mL CH_2Cl_2 is slowly added to a stirred solution of 25 mmol **5** and 75 mmol NEt_3 in 300 mL CH_2Cl_2 under nitrogen and protected from light. The solution is stirred overnight, concentrated to about 150 mL under reduced pressure, and washed

Table 1. Affinities(K_i, nM) for Dopamine Receptor D_1-D_5 Subtypes Determined by Radioligand Binding Experiments



	K_i (nM)				
compd	HEK D_1	CHO D_{2L}	CHO D_3	CHO $D_{4.4}$	HEK D_5
1 (LE-300)	1.9 ± 0.9^a	44.5 ± 15.8^a	25.9^b	108 ± 39^a	7.5 ± 0.3^a
2 (LE-CE-580)	163.5^b	143 ± 127^a	521 ± 239^a	184^b	92^b
3 (LE-CE-560)	2.2^b	14.5^b	277.5^b	98.4^b	0.61^b
10	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
17	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]

[a] K_i values are the means of three experiments, performed in triplicate ± SEM. [b] K_i values are the means of two experiments, performed in triplicate. [c] NA = no activity. Less than 70% radioligand displacement of a 10^{-5} M solution.

Table 2. Inhibitor Activities (K_i, NM) at Dopamine Receptor D1, D_2, and D_5 Subtypes Generated by a Functional Calcium Assay

	K_i (nM)		
compd	HEK D_1	HEK D_{2L}	HEK D_5
1	60.4 ± 20.4^a	19.0 ± 11.7^a	12.7 ± 6.35^a
2	304^b	38^b	71.6^b
3	2.8 ± 2.4^a	1.5^b	2.8^b
10	NA[c]	NA[c]	NA[c]
17	NA[c]	NA[c]	NA[c]

[a] K_i values are the means of three experiments, each including six values ± SEM. [b] K_i values are the means of two experiments, each including six values. [a] NA = no activity.

with 2 N HCl (3 × 50 mL) and 2 N NaOH (1×). The organic layer is separated, dried over $MgSO_4$, and evaporated to dryness. TLC from $CHCl_3$/MeOH 9:1 showed no starting materials but two new spots of **8** and **9**.

General Method for the Cyclization/Reduction Steps to 10 and 17: Under an inert atmosphere and protected from light, the benzamide **16** or the mixture of **8** and **9**, respectively, is refluxed in $POCl_3$/MeCN 1:13 v/v for 4 h (**17**) and 20:1 for 72 h (**10**). The solvents are removed under reduced pressure. The residue is purified by repeatedly leaching out the impurities and remaining $POCl_3$ first with boiling petroleum ether (40−60), next with diethyl ether, and last with toluene. The washings are discarded, and to the residue dissolved in methanol, $NaBH_4$ is added slowly. After refluxing for several hours, the solvent is evaporated to dryness, portioned between water and $CHCl_3$, and extracted several times with $CHCl_3$. After drying ($MgSO_4$) and evaporating, the bases are isolated as their hydrochloric salts (diethyl ether and ethereal HCl).

General Method for the Quaternization of the Tertiary Amines: A 10-fold molar excess of methyl iodide is added to a stirred solution of **10** or **17**, respectively, in acetonitrile or acetone. The mixture is stirred at room temperature for several days. Precipitated solids are removed by filtration and dried under vacuum. The yield is about 90%.

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 $^2/_3$ filled. The cooling bath was removed and the ammonia was allowed to liquefy and stir. To this solution, 300 mg (0.7 mmol) **11** or 120 mg (0.28 mmol) **18**, respectively, was added without any solvent. Pieces of sodium with the dimension of about a rice grain were added to this stirred reaction mixture until the developing blue color was maintained for 10−15 min. The blue color was quenched by the addition of a drop of satd NH_4Cl. The ammonia was evaporated under a stream of nitrogen and the residue was treated with 5 mL of water and 15 mL of diethyl ether and stirred until two clear phases formed. The ethereal phase was separated, dried over $MgSO_4$, and evaporated to dryness under reduced pressure to yield **2** as white foam and **3** as a colorless oil, respectively, which were converted into their HCl salt from diethyl ether with ethereal HCl. Yields: 82% for **3** and 93% for **2**, respectively.

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Supporting Information Available: Synthetic procedures and spectral characterization for compounds **2**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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