Tricyclic Azaergoline Analogues: Synthesis, Structural Modifications, and Pharmacological Studies

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As an extension of previous investigations on synthesis and dopamine autoreceptor activity of bicyclic ergoline analogues the tricyclic azaergoline analogues 9a and 9b were synthesized. Furthermore, the geometry of the aromatic β-ethylamine moiety of 9a,b was modified by stereoselective construction of the cycloheptenyl fused pyrazolopyridine derivative 7 and the aminomethyl substituted tricycle 10. Binding affinity of these compounds at dopamine (DA) receptor sites was investigated employing rat striatum homogenate: The compounds reveal modest to weak, but selective binding to a dopamine D-2 receptor when it is labelled with the OA-autoreceptor agonist [3H]-SND 919. In vivo studies with mice showed that 7, 9a,b, and 10 affect their CNS activity.

Since the fortuitous discovery of chlorpromazine1 a large number of neuroleptics have been developed2. It is postulated that the antipsychotic activity of the classical neuroleptics is due to a blocking effect at the postsynaptic D-2 receptors3. Since these D-2 receptor antagonists induce extrapyramidal side effects (i.e., pseudo-parkinsonism), alternative approaches for preventing the symptoms of schizophrenia attract considerable attention in recent years4. One of these novel approaches is to employ selective DA-autoreceptor agonists, which decrease synthesis and release of dopamine (DA)5,6. Thus the hyperactivity in the mesolimbic dopaminergic system (A 10 system) of schizophrenia patients should be reduced. Since it is assumed that the binding sites of the postsynaptic D-2 receptor and its prejunctional congener are very similar5, it seemed to be a valuable strategy to modify the molecular structure of known postsynaptic D-2 agonists, in order to develop DA autoreceptor agonists.

In fact, we and others have shown that this is possible, by applying the DA active bicyclic ergoline analogue 1 as a model compound. Structural modifications at the aromatic region resulted in selective autoreceptor activity of the derivatives 27, 3 (SND 919)8, and 4, 59.

As an extension of our work, we try to evaluate specific ligands for the DA autoreceptor by structural changes of the tricyclic ergoline analogue 6, which is also known to exhibit DA agonistic activity10. Thus, we are investigating aza analogues with pyrazolo[1,5-a]pyridine (8)11 or tetrahydropyrazolo[1,5-a]pyridine substructure (9). In this paper, we report on syntheses and pharmacological studies of both diastereomers of the hexahydropyrazoloquinoline derivative 9. As a further modification, the geometry of the aromatic ethylamine moiety of 9 was varied by construction of the tetrahydropyrazolopyridyl fused cycloheptenylamine 7 and the aminomethyl substituted pyran derivative 10.

Scheme 1
The synthesis of the target compounds 9a and 9b was planned by a stereospecific Curtius rearrangement starting from the carboxylates 14a and 14b, which we obtained from the pyrazolopyridine carboxylate 11 via the β-ketoester 12a and the iodide 13 as the key intermediates. The hydrolysis of the esters 14a and 14b was performed by NaOH in dioxane/H2O to yield the carboxylic acids 15a and 15b, respectively, both as pure diastereomers. Treatment of 15a,b with diphenyl phosphorazidate (DPPA) in acetonitrile, followed by acidic hydrolysis of the intermediately formed isocyanates, afforded the primary amines 16a and 16b with complete retention of configuration, indicated by NMR spectroscopy. This reaction is best monitored by IR-spectroscopy by observing the appearance/disappearance of the diagnostic bands at 2260 (N=C=O) and 1710 cm⁻¹ (O=C-OH). Finally the primary amines 16a and 16b were transformed into the dipropylamines 9a and 9b by use of propionic aldehyde and NaCNBH₃.

For the synthesis of the homologues cycloheptene fused heterocycles the hydroxyethyl substituted β-ketoester 12b, which can be efficiently derived from 11, according to previous studies, should serve as a suitable educt. Reduction of the aromatic ketone 12b was achieved by catalytic hydrogenation (Pd/C) at 60 bar pressure and 130°C to give 17 in 92% yield. Subsequently the primary alcohol 17 was converted into the mesylate 18. Treatment of 18 with NaI yielded the electrophile 19, which could be cyclized by a 7-(enolexo)-exo-tet ring closure using LDA as a base, at -78°C. Surprisingly, the ring closure proceeds stereoselectively to afford the trans diastereomer 20a exclusively. By contrast, the 6-(enolexo)-exo-tet cyclization of 13 affords 14a and 14b in a 1:1 ratio. We reason that the cycloheptene derivative 20a is formed selectively because - kinetically controlled - the reaction proceeds through a chair type transition state, leading to the trans configurated product (Figure 1). On the other hand, formation of the 6-membered ring requires much higher temp. (0°C). Thus, under the strongly basic conditions epimerization takes place and a thermodynamically controlled mixture of 14a and 14b is produced. Such an equilibration can be also observed for the 7-membered case, by treating 20a with LDA at 0°C, when a 1:1 mixture of diastereomers (20a and 20b) was isolated.

Our final product 7 was synthesized from 20a in 53% yield by hydrolysis, followed by Curtius rearrangement and reductive alkylation via the intermediates 21 and 22.
Previously, we have demonstrated that anionic cyclization of the iodide 12c results in O-alkylation to give the enol ether 23 exclusively\(^ {15}\). We regarded this compound as a useful precursor for the synthesis of the amine 10, which was planned to be investigated as a potential DA equivalent with a differently locked ethylamine conformation. Actually, catalytic hydrogenation (Pd/C) of the enol ether 23 afforded the pseudo-equatorially substituted pyran derivative 25a selectively. The nucleophilic approach from the pseudo-axial side might be explained by transition state stabilization due to interaction of the axially positioned oxygen lone-pair and the incipient low lying vacant CH-orbital \(\sigma^*\). In case of an equatorial approach, torsional strain between the axial O lone-pair and the incipient \(\sigma^*\) CH-orbital would destabilize the transition structure\(^ {16}\) (Fig. 2).

Employing rat striatal membranes, the novel tricyclic azaergoline analogues 7, 9a,b, and 10 were evaluated for their binding affinity to the dopamine D-1 receptor labelled with \(^{3}H\)-SCH 23390 and to the D-2 receptor sites labelled with \(^{3}H\)-spiroperidol and \(^{3}H\)-SND 919, a compound which in functional in vivo experiments pointed out to be an autoreceptor agonist\(^ {8}\). It turned out, that there exists a significant affinity to the DA receptor site labelled with \(^{3}H\)-SND 919, which is selective since an affinity to the postsynaptic D-1 and to the D-2 receptor labelled with \(^{3}H\)-spiroperidol could not be observed. However, the IC\(_{50}\) values demonstrate the ability of 7, 9a,b, and 10 to displace \(^{3}H\)-SND 919 is only modest (Table 1). The most active compound was the equatorially substituted cis isomer 9b, which gave an IC\(_{50}\) value of 4.4 \(\mu\)M. (By contrast, our previously developed bicyclic ergoline analogue (S)-3 showed a 147-fold higher affinity (IC\(_{50}\) = 0.03 \(\mu\)M)\(^ {9}\)). The trans configuration of the cycloheptene fused homologue 7 resulted only in a slight loss of affinity (IC\(_{50}\) = 6.3 \(\mu\)M), when compared to 9b. This is easy to understand because the dipropylamino group of 7 is also equatorially orientated. On the other hand, the cyclohexenylamine fused trans diastereomer 9a with an axially positioned dipropylamino group had a 4.3 fold lower affinity than 9b. The lowest IC\(_{50}\) value (23 \(\mu\)M) was measured for the pyran derivative 10, when also a conformational change of the ethylamine moiety resulted in a decrease of the affinity to the DA-receptor labelled with \(^{3}H\)-SND 919.

Table 1: Receptor Binding Data

<table>
<thead>
<tr>
<th>compd</th>
<th>D-1 (^ {1a})</th>
<th>D-2 (^ {1b})</th>
<th>DA-autoreceptor (^ {6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>6.3</td>
</tr>
<tr>
<td>9a</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>19</td>
</tr>
<tr>
<td>9b</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>23</td>
</tr>
</tbody>
</table>

\(^ {1a}\) 3H-ligand: SCH 23390, \(^ {1b}\) 3H-ligand: spiroperidol. \(^ {6}\) 3H-ligand: SND 919.

In accordance to the receptor binding data, 9b and 7 turned out to be the most active compounds in vivo. Our behavioural-activity screening with mice showed that all the test compounds (7, 9a,b, and 10) caused excitation including Straub tail, vocalization and convulsion, immediately after injection. After this period of excitation both cyclo-

![Scheme 4](image-url)

Fig 2

The structure of 25a was elucidated by \(^1H\)-NMR spectroscopy observing a significant dipolar exchange of magnetism between H-5\(_b\) and H-3, due to 1,3-diaxial interference. The position of H-5\(_a\) was determined by the \(^1\)H,\(^1\)H-COSY technique, a diagnostic vicinal coupling constant \(J_{5a,5b} = 10.7\) Hz, and a significant NOE with H-6\(_a\), (1,3-diaxial interaction).

The conversion of the ester 25a into the target compound 10 was performed by Curtius rearrangement through the intermediates 25c and 25d, as described for the syntheses of 7 and 9.

Alternatively, 25a can be synthesized directly from the primary alcohol 12a\(^ {13}\) - a synthetic precursor of 12c and 23 - by catalytic hydrogenation in acetic acid in 64% yield. This is an essential improvement of the synthesis since the pathway via 12c and 23 requires 3 additional steps and affords an overall yield of only 15%. We expect, that the reaction proceeds through the hemiacetal 24a. Then, 24a can either eliminate H\(_2\)O to give 23 which is subsequently hydrogenated, or hydrogenation takes place on an immediately formed stabilized carbenium ion. To investigate whether the formation of an enol ether by dehydration is essential for this one pot procedure the dimethyl substituted analogue 12d was heated using similar conditions (HOAc, 140°C, 50 bar H\(_2\), Pd/C). In fact, the reaction works very smoothly to give 25b as a single diastereomer, although - due to the quaternary C-atom - dehydration of the hemiacetal 24b is impossible.

The educt 12d was prepared from the aromatic ester 26\(^ {12}\) by ester condensation to give the \(\beta\)-keto ester 27. After deprotonation by LDA 27 was reacted with benzzyloxymethyl chloride to afford 12e, which could be debenzylated by hydrogenolysis to yield 12d.
hexenyl fused isomers 9a and 9b as well as the pyran 10 exhibited a decrease of locomotor activity at ≥ 50 mg/kg (for 9a), ≥ 25 mg/kg (for 9b), and ≥ 100 mg/kg (for 10). By contrast, 7 showed a locomotor stimulant action. Placing mice into an activity cage gave a 53% locomotor stimulant action. This work is supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Experimental Part

General

THF was distilled from LiAlH₄ immediately before use. CH₂Cl₂, acetone, and Et₂N were distilled from CaH₂. All liquid reagents were also purified by distillation. Unless otherwise noted reactions were conducted under dry N₂ atmosphere, and propionic aldehyde (116 mg, 2 mmol) in 5 ml of methanol was stirred at room temp. for 14 h. After the addition of 2 N HCl (1 ml) the reaction mixture was stirred for 15 min and worked up as described for 7 to give 50 mg (35%) of 9a as a colorless oil. C₁₇H₂₉N₃O (276.4) mol.-mass 276 (ms).- IR (NaCl): 2960; 2870 cm⁻¹.- ¹H-NMR (CDCl₃): δ (ppm) = 0.87 (t, J = 7.3 Hz, 6H, CH₃), 3.15-3.35 (m, 6H, CH₂CH₂), 4.32 (dd, J = 12.5, 5.5 Hz, 1H, H-C-3), 4.17 (dd, J = 12.5, 5.5 Hz, 1H, H-C-5), 3.93 (t, J = 7.3 Hz, 4H, CH₂CH₂), 2.13-2.17 (m, 1H, H-C-5), 2.39 (t, J = 4.14 Hz, 1H, H-C-9), 2.52-2.58 (m, 1H, H-C-8), 2.65-2.71 (m, 1H, H-C-5a), 2.79 (d, J = 13.9 Hz, 1H, H-C-8). 4.14 (dd, J = 12.5, 5.5 Hz, 1H, H-C-5), 3.42 (dd, J = 12.5, 5.5 Hz, 1H, H-C-8), 4.76 (t, J = 5.9 Hz, 1H, H-C-8), 7.28 (s, 1H, HC-2).

Elemental analysis was performed after conversion of 9b into its hydrochloride using methanolic HCl in ether. C₁₆H₂₇NO₃Cl (297.9) Calcd. C 64.9 H 9.48 N 14.1 Found C 64.9 H 9.48 N 13.9. (3RS,5aRS)-4-Dipropylaminol-4.5.6.7-8-hexahydro-3H-pyrazole [2.3.4-j]-quinoline (9b)

A mixture of 16b (40 mg, 0.23 mmol), NaCNBH₃ (29 mg, 0.46 mmol) and propionic aldehyde (133 mg, 2.3 mmol) in 5 ml of methanol was stirred at room temp. for 14 h. After the addition of 2 N HCl (1 ml) the reaction mixture was stirred for 15 min and worked up as described for 7 to give 37 mg (63%) of 9b as a colorless oil. C₁₆H₂₇NO₃Cl (261.4) mol.-mass 261 (ms).- IR (NaCl): 2960; 2870 cm⁻¹.- ¹H-NMR (CDCl₃): δ (ppm) = 0.89 (t, J = 7.3 Hz, 6H, CH₃), 1.22 (ddd, J = 13; 12; 3 Hz, 1H, H-C-6), 1.33 (q, J = 11.5 Hz, 1H, H-C-5), 1.52 (br-s, 4H, CH₂CH₂), 1.96-2.26 (m, 5H, H-C-3, H-C-5, H-C-6, H-C-7, H-C-8), 2.48 (br-s, 4H, CH₂CH₂), 2.70-2.78 (m, 2H, H-C-3, H-C-5a), 3.24 (br-s, 1H, H-C-4), 3.84 (dd, J = 12.5, 5.9 Hz, 1H, H-C-8), 4.26 (dd, J = 12.5, 5.9 Hz, 1H, H-C-8), 7.28 (s, 1H, HC-2).

Elemental analysis was performed after conversion of 9b into its hydrochloride using methanolic HCl in ether. C₁₆H₂₇NO₃Cl (297.9) Calcd. C 64.9 H 9.48 N 14.1 Found C 64.9 H 9.48 N 14.0.

Table 2: Locomotor Activity Measurements (0-60 min)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control Counts (±S.E.M.)</th>
<th>Treatment Counts (±S.E.M.)</th>
<th>Δ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (25 mg)</td>
<td>3046 ± 532</td>
<td>1992 ± 275</td>
<td>+53 %</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>d-Amph a (3 mg)</td>
<td>7754 ± 1797</td>
<td>4332 ± 1402</td>
<td>+79 %</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Halop b (3 mg)</td>
<td>1464 ± 305</td>
<td>3303 ± 534</td>
<td>-56 %</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

a d-Amphetamine sulfate; b Haloperidol HCl; c after injection of 0.9% NaCl; d increase (+) / decrease (-) of motor activity related to control counts.
was filtered (Cellite® AFA) and the filtrate was evaporated. The residue was purified by flash chromatography (CH₂Cl₂ - CH₂OH 97:3) to give 130 mg (86%) of 12d as a colorless solid. m.p. 161.5°C. IR (KBr): 3430; 2950; 1640 cm⁻¹. 1H-NMR (CDCl₃): δ (ppm) = 1.32 (s, 3H, CH₃). 1.77-2.04 (m, 4H, Hac-5, Hac-6). 2.60 (br-s, 1H, OH), 3.64-3.66 (m, 2H, CH₂O), 3.68 (s, 3H, OCH₃), 3.85-3.86 (m, 1H, HC-4). 4.00 (m, 1H, Hac-C-7), 4.27 (m, 1H, Hac-C-7), 7.22 (s, 1H, HC-2).

**Methyl (±)-3-(4-benzoyloxyethyl)-4,5,6,7-tetrahydropyrazolo [1,5-a]pyridine (12e)**

To a solution of 27 (250 mg, 1 mmol) in 10 ml of THF were added dropwise 3 ml of freshly prepared LDA (0.32 molar in THF) at -78°C. The reaction mixture was stirred at this temp, for 30 min, when it was added dropwise to a precooled solution (-78°C) of benzoxymethylchloride (BOM-Cl; 234 mg, 1.5 mmol) in 10 ml of THF. After 30 min it was added to a mixture of ether and satd. aq. solution of NaHCO₃. The org. layer was dried (MgSO₄) and evaporated and the residue was separated by flash chromatography (petroleum ether - EtOAc 1:1) to give 16G (250 mg) of pure 12e as a colorless solid; m.p. 83°C. C₁₈H₁₉N₂O₂ (370.5) Calcld. C 68.1 H 7.07 N 7.64 Found C 67.9 H 7.11 N 7.7 IR (KBr): 3040; 2950; 1740; 1660 cm⁻¹. 1H-NMR (CDCl₃): δ (ppm) = 1.46 (s, 3H, CCH₃). 1.73-2.29 (4H, Hac-C, Hbc-C-6). 3.55 (dd, J = 9.5, 8.8 Hz, 1H, PhCH₂OCD₂). 3.65 (s, 3H, OCH₃), 3.74 (dd, J = 9.5, 3.0 Hz, 1H, PhCH₂OCD₂). 3.83-3.85 (m, 1H, HC-4). 3.86 (dd, J = 13.2, 11.7, 5.1 Hz, Hac-C-7). 4.21 (br-d, J = 13.2, 3.6 Hz, 1H, Hbc-C-7). 4.46 (d, J = 11.7 Hz, 1H, PhCH₂). 4.46 (d, J = 11.7 Hz, 1H, PhCH₂). 7.25-7.36 (m, 5H, PhH), 7.69 (s, 1H, HC-2).

(4RS,5aRS)-4-amino-4,5,6,7,8-hexahydro-3H-pyrazolo[2,3,4-ij]quinoline (16b)

A mixture of 15b (43 mg, 0.21 mmol), diphenyl phosphorazidate (58 mg, 0.21 mmol) and triethylamine (21 mg, 0.21 mmol) in 15 ml of acetone-titrile was stirred at 60°C for 5 h. After the addition of 0.5 N HCl (3 ml) the reaction mixture was stirred at room temp. for 2.5 h and worked up as described for 16a to give 24 mg (65%) of 16b as a colorless oil. CI₃H₂N₂O₃ (177.3) mol.-mass 177 (ms). IR (NaCl): 3340; 2920; 2850 cm⁻¹. 1H-NMR (CDCl₃): δ (ppm) = 1.23 (dddd, J = 13.0, 12.5, 2.8 Hz, 1H, Hac-C-6). 1.27 (dddd, J = 11.8, 11.7, 11.6 Hz, 1H, Hbc-C-5). 1.97-2.04 (m, 2H, Hac-C-5, Hbc-C-6). 2.05-2.10 (m, 1H, Hac-C-7). 2.14-2.20 (m, 1H, Hbc-C-7). 2.19 (dd, J = 15.0; 2.3 Hz, 1H, Hbc-C-5). 2.74 (dddd, J = 12.5, 11.5, 5.5 Hz, 1H, Hbc-C-5a). 2.92 (dd, J = 15.0, 5.9 Hz, 1H, Hbc-C-3). 3.26 (dddd, J = 11.6, 10.5, 5.9 Hz, 1H, Hbc-C-3). 3.85 (dddd, J = 12.5, 12.5, 5.9 Hz, 1H, Hbc-C-8). 4.26 (dd, J = 12.5, 5.5 Hz, 1H, Hbc-C-8). 7.27 (s, 1H, HC-2).

**Methyl (±)-3-[4,5,6,7-tetrahydro-4-(2-hydroxyethyl)pyrazolo [1,5-a]pyridine (17)**

A mixture of 12b (1900 mg, 7.14 mmol) and 1 g of Pd/C (10%) in 60 ml of methanol was stirred at 130°C for 3 h under H₂ of 60 bar. Then it was filtered (Cellite® AFA) and the filtrate was evaporated to give 1650 mg (92%) of 17. Flash chromatography (CH₂Cl₂-CH₂OH 97:3) gave an analytically pure sample as a colorless oil. CI₃H₂N₂O₃ (252.3) Calcld. C 61.9 H 7.99 N 11.11 Found C 61.7 H 8.18 N 11.11 mol.-mass 252 (ms). IR (NaCl): 3340; 2950; 2860; 1740 cm⁻¹. 1H-NMR (CDCl₃): δ (ppm) = 1.71-1.23 (m, 6H, Hac-C-5, Hac-C-6, CH₂CH₂O). 2.57-2.60, 2.74-2.79 (2m, 4H, CH₃CH₂CO). 3.18-3.23 (m, 1H, HC-4). 3.68 (s, 3H, OCH₃). 3.72-3.77 (m, 2H, CH₂O). 3.98 (dd, J = 12.5, 9.5, 5.1 Hz, 1H, Hbc-C-7). 4.19 (dddd, J = 12.5, 5.1, 4.4 Hz, 1H, Hbc-C-7). 7.28 (s, 1H, HC-2).

**Methyl (±)-3-[4,5,6,7-tetrahydro-4-(2-mesylyl)pyrazolo [1,5-a]pyridine (18)**

To a solution of 17 (1500 mg, 5.95 mmol) in 100 ml of THF was added triethylamine (0.55 ml, 7 mmol) and methanesulfonyl chloride (0.97 ml, 7 mmol). After stirring at room temp. for 2 h the mixture was evaporated and the residue purified by flash chromatography (CH₂Cl₂-CH₂OH 94:6) to afford 1890 mg (96%) of 18 as a colorless oil. CI₃H₂N₂O₂S (330.4) Calcld. C 50.9 H 6.71 N 8.55 Found C 50.6 H 6.63 N 8.46 mol.-mass 330 (ms). IR (NaCl): 2950; 1740; 1350; 1170 cm⁻¹. 1H-NMR (CDCl₃): δ (ppm) = 1.82-2.16 (m, 6H, Hac-C-5, Hac-C-6, CH₂CH₂O). 2.55-2.61, 2.70-2.75 (2m, 4H, CH₃CH₂CO). 3.06 (s, 3H, OSO₂CH₃). 3.18-3.23 (m, 1H, HC-4). 3.68 (s, 3H, OCH₃). 3.96-4.04 (m, 1H, Hac-C-7). 4.17-4.22 (m, 1H, Hbc-C-7).
A mixture of 18 (1700 mg, 5.15 mmol) and NaI (7700 mg, 13.6 mmol) was stirred in 100 ml of boiling acetone for 4 h. After cooling to room temp. the solvent was removed and the residue extracted with ether. The extract was evaporated and the residue purified by flash chromatography (petroleum ether - EtOAc 4:6) to give 1230 mg (66%) of 19 as a colorless oil.

The org. layer was dried (MgSO4) and evaporated and the residue was separated by flash chromatography (petroleum ether - EtOAc 6:4) to give 140 mg (60%) of 21 as a colorless solid; m.p. 253°C.

Treatment of 20a (23.4 mg, 0.1 mmol) with freshly prepared LDA (0.32 molar in THF) at -78°C gave 25a as a colorless solid; m.p. 76°C.

A mixture of 12d (60 mg, 0.21 mmol) and 100 mg of Pd/C (10%) in 15 ml of acetic acid was stirred at 120°C for 3 h under H2 of 70 bar. Then it was filtered (Celite® AFA) and the filtrate was evaporated. The residue was dissolved in THF (0.3 ml), 0.32 molar in THF) at 0°C for 30 min gave a 1:1 mixture of 20a and 20b after usual work up.

A mixture of 20a (160 mg, 0.68 mmol) in 10 ml of dioxane and 10 ml of N NaOH was stirred at room temp. for 2.5 h. Then the solution was extracted with ether. The aqueous layer was neutralized and then acidified to pH 3-4 with citric acid (10% in H2O). After standing at room temp. for 2 h, filtration and drying of the residue gave 125 mg (83%) of 21 as a colorless solid; m.p. 114°C.

A mixture of 21 (300 mg, 1.36 mmol) and triethylamine (138 mg, 1.36 mmol) in 25 ml of acetonitrile was stirred at 60°C for 4 h. After addition of 0.5 N HCl (20 ml) the reaction mixture was stirred at room temp. for 2.5 h and worked up as described for 16a to give 234 mg (87%) of 22 as a colorless solid; m.p. 158.1-158.6°C (m, 1H, H-C-5), 1.84-1.96 (m, 2H, H-C-4, H-C-6), 2.03-2.11 (m, 3H, H-C-4, H-C-5, H-C-6), 2.41 (dd, J = 13.9, 11.0 Hz, H-C-9), 2.70-2.86 (m, 3H, H-C-5a, H-C-8, H-C-9), 3.93 (ddd, J = 12.5, 12.5, 4 Hz, 1H, H-C-3), 4.21 (dd, J = 12.5, 5.1 Hz, 1H, H-C-3), 7.21 (s, 1H, H-C-2).

Methyl (Sar,5aS)-5a,6,7,8,9-Octahydro-2,2a-diazabenzo[f,c]azulen-8-carboxylic acid (21)

A mixture of 12a (50 mg, 0.22 mmol) and 100 mg of Pd/C (10%) in 10 ml of acetic acid was stirred at 120°C for 3 h under H2 of 70 bar. Then it was filtered (Celite® AFA) and the filtrate was evaporated. A saturated aqueous solution of NaHCO3 was added and the mixture was extracted with EtOAc. The org. layer was dried (MgSO4) and evaporated and the residue was dissolved in tetrahydrofuran (petroleum ether - EtOAc 1:1) to give 30 mg (64%) of 25a as a colorless solid; m.p. 76°C.

A mixture of 12d (60 mg, 0.21 mmol) and 100 mg of Pd/C (10%) in 10 ml of acetic acid was stirred at 140°C for 5 h under H2 of 60 bar. Then it was filtered (Celite® AFA) and the filtrate was evaporated. The residue was dissolved in THF (0.3 ml), 0.32 molar in THF) at 0°C for 30 min gave a 1:1 mixture of 20a and 20b after usual work up.
with ether and the aqueous layer was concentrated, then acidified to pH 3-4 with citric acid (10% in H₂O) and extracted with CH₂Cl₂. The org. layer was dried (MgSO₄) and evaporated to give 330 mg (70%) of 25e as a colorless solid; m.p. 192°C. C₂₀H₂₂N₂O₂ (374.4) IR (KBr): 3080, 2920, 1575, 1450, 1370 cm⁻¹. 'H-NMR (CDCl₃): 1.02 (d, 3H, J = 6 Hz, CH₃), 1.18 (d, 3H, J = 6 Hz, CH₃), 1.91 (s, 3H, CH₃), 2.40 (t, 2H, J = 12 Hz, CH₂), 3.82 (s, 3H, OCH₃), 4.10 (dd, J = 12 Hz, 12 Hz, 2H, CH₂). 7.29 (s, 1H, CH₃). C₁₉H₂₀N₂O₂ (346.4) Calcd. C 76.4 H 6.93 N 10.4 Found C 76.2 H 7.06 N 10.5; mol.-mass (ms) 346 (ms). IR (KBr): 3080, 2920, 1575, 1450, 1370 cm⁻¹. 'H-NMR (CDCl₃): 1.02 (d, 3H, J = 6 Hz, CH₃), 1.23 (t, 3H, J = 6 Hz, CH₃), 2.35 (t, 2H, J = 12 Hz, CH₂), 3.75 (s, 3H, OCH₃), 4.00 (dd, J = 12 Hz, 12 Hz, 2H, CH₂). 7.79 (s, 1H, CH₃).

Receptor Binding Assay

DA receptor binding was performed as described using [³H]-SCH 23390 and [³H]-spiropiperidol as radioligands in concentrations of 0.3 nM and 0.5 nM, respectively. In the receptor binding assay for the characterization of the DA autoreceptor, [³H]-SND 919 (51 Ci/mmol specific activity) was used in a concentration of 0.5 nM. The experimental procedure was performed in analogy to the binding assay with [³H]-spiropiperidol as radioligand. For all receptor binding assays rat brain striatum was used.

Modified and Extended Behavioural-Activity Screening, based on Refs. 20, 21

All drugs were tested in a minimum of 3 doses (100/50/25 mg/kg) employing 3 mice (male NMRI mice, 20-33 g) per each dose. For the first 30 min after the test compound was injected i.p. (dose volume = 10 ml/kg, in 0.9 aqueous NaCl solution or 0.5 tragant suspension) only fundamental observations (e.g., convulsion, Straub tail, respiratory depression) were noted. Then the animals were investigated, including the following parameters: irregular/dyspnoic respiration, vocalization, restlessness, piloerection, retracted sides, abdominal cramps, exophthalmus, twitches, convulsion, pinna response, corneal response, palpebral closure, motor activity, spatial orientation, tail position, gait, hyperactivity/vocalization after stimulation, aggressivity, tremors, body position, righting reflex, hypothermia, hypothermia, lacrimation, dysphagia, salivation, sialorrhea, diarrhea, skin color, pupil size, light-pupil response, body tone, motor coordination, catalepsy, hot plate response, stereotype movements, death. Observations differing from saline treated control animals were observed again after 4 and 24 h. Each animal was used for one experiment only.

General Procedure for Locomotor Activity Measurement

Three mice (male NMRI mice, 20-33 g) in a macronol cage (type III) with free access to food and water were placed into a scanner box (RBM 3, MSE/INTRON, München/Müllingen, Germany) using electromagnetic field for the measurement at 3.45 pm. After 30 min aqueous 0.9% NaCl solution was injected i.p. Then the motor activity was recorded by a separate printer for 2 h. Following the same schedule at the following day the procedure was repeated after injection of the test compound. Totally, 8 groups of 3 mice were investigated using each animal only once. Data were examined using the t-test for pairs of observations according to Student and Wilcoxon's matched pair signed rank statistic.

References

11. Work is still in progress.