Synthesis, Pharmacological Investigation and Computational Studies on a Tricyclic Ergoline Analog with Selective Dopamine Autoreceptor Activity☆

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Summary

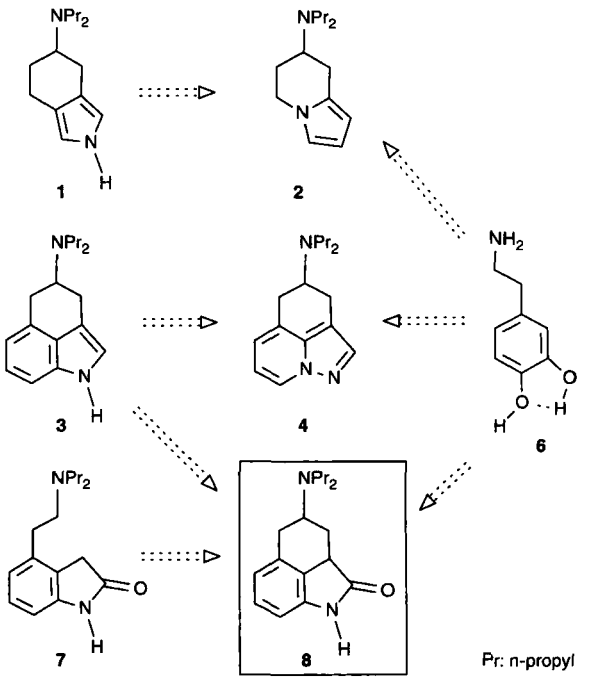
The novel aminobenzindolone **8** was prepared and evaluated as a potential antipsychotic agent. The target compound was synthesized in eight steps starting from the tetrahydrobenzindolone **9**. The key step of the synthesis was an electrophilic amination of the aromatic ketone **11** followed by reductive degradation when the diethoxymethyl group was employed for protection of the lactam nitrogen and also for the benzylic position 2a. Dopamine and serotonin receptor binding studies revealed **8** to be a potent and selective ligand at the D-2 autoreceptor (k_i = 4.0 nM). Further *in vivo* studies including the GBL-test and locomotor activity measurements indicated agonistic activity of **8** at the prejunctional binding sites. Comparison of *ab initio* based molecular electrostatic isopotential maps corroborates our hypothesis that the dopamine structure **6**, containing an intramolecular hydrogen bond donating effect of the *meta*-HO-group, represents the conformation which is active at the dopamine D-2 autoreceptor.

Introduction

Schizophrenia is known as a complex mental disorder including bizarre thought patterns, social impairment and hostility [1]. Unfortunately, the typical antipsychotic drugs, which are currently used to treat this devastating illness, suffer from limitations in efficacy and cause severe extrapyramidal side effects, e.g. tardive dyskinesia, dystonia, akathisia and Parkinsonism [2]. It is generally accepted that the activity of these classical neuroleptics is due to their ability to block dopamine receptors [3]. In recent years, the development of atypical neuroleptics that act by different mechanisms and do not induce adverse motor side effects has become a very active field of research [4,5].

Stimulation of presynaptically localized dopamine D-2 autoreceptors inhibits dopamine synthesis, release and neuronal firing [6]. Since schizophrenia is associated with increased mesolimbic dopaminergic activity [7] dopamine autoreceptor agonists are expected to be an interesting alternative to the classical dopamine D-2 antagonists [8]. As a consequence, several selective dopamine D-2 autoreceptor agonists have been reported, including pramipexole (SND 919) [9], talipexole (BHT 920) [10], EMD 49980 [11], 3-PPP [12], PD 118717 [13], and 7-HO-DPAT [14]. Since it is assumed that the binding site of the dopamine autoreceptor resembles that of the postsynaptic D-2 receptor [8], specific modification of D-2 agonistic model compounds seemed to be a valuable strategy for the discovery of novel autoreceptor selective ligands. In fact, this proved to be possible when we evaluated strong and selective activity of the novel (*S*)-aminoindolizine **2** [15] and the pyrazoloquinoline **4** (Scheme 1). [16,17] Both D-2 autoreceptor agonists can be seen as structural congeners of the BC-bicyclic and the ABC-tricyclic ergoline partial structures **1** and **3** [18]. Employing *ab initio* based computational studies we found that the molecular electrostatic potentials of the aromatic core structures of **2** and **4** strongly resemble those of the dopamine conformer **6** [16,17]. Besides the extended *anti*-conformation as well as a coplanar orientation of the side chain and the catechol ring an intramolecular H-bond donating effect of the meta HO-group is characteristic for **6**. Additionally, the *m*-OH is directed away from the amino group.

As a further candidate combining the structural similarity to the ergolines and the distribution of charge of the dopamine conformer **6** we envisioned to investigate the aminobenzindolone **8**. Furthermore, **8** was supposed to be of special interest since it can be seen as a conformationally restricted variant of the D-2 agonist ropinirole **7** [19] and as a carba analog of the very recently described mixed D-2/5HT-1a agonist U 86170F [20].



Scheme 1

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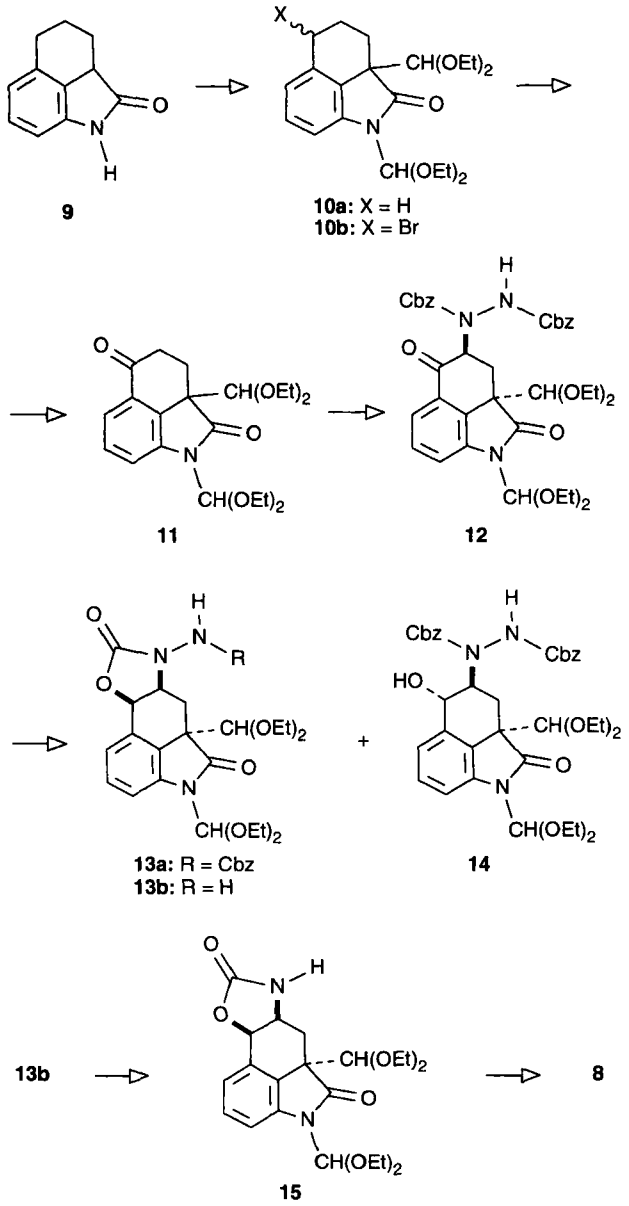
Synthesis

Chemical synthesis of the target molecule **8** was performed starting from the bis-diethoxymethyl protected tricycle **10a** [21] which can be easily prepared from the commercially available tetrahydrobenzindolone **9** [22]. After oxidation of the benzylic position 5, introduction of a nitrogen source in position 4 was envisioned by electrophilic amination. For this key step, our previously established methodology for the amination of ketone enolates employing dibenzyl azodicarboxylate should be applied [23].

Compound **10a** was reacted under the conditions for a radically induced bromination (NBS, dibenzoyl peroxide, CCl_4) to afford the bromide **10b** in 62 % yield as a 1:1 mixture of diastereomers. For analytical purposes, the isomers could be separated by flash chromatography and the relative configuration was established by ^1H NMR spectroscopy including NOE experiments. Since **10b** turned out to be unstable towards degradation reactions it was advantageous to proceed in the synthesis without isolation and purification of **10b**. Thus, elaboration of a one-pot procedure was fruitful affording the aromatic ketone **11** in a 60 % yield by bromination and subsequent Kornblum oxidation. Electrophilic amination was achieved by LDA induced enolate formation and subsequent treatment with dibenzyl azodicarboxylate to give the protected hydrazino ketone **12** in 85 % yield. Due to the shielding effect of the diethoxymethyl group in position 2a the *trans*-isomer was formed completely diastereoselectively. Analysis by ^1H NMR spectroscopy demonstrated that **12** predominantly exists in a half-chair conformation when the protected hydrazine substituent is dispositioned equatorially. The relative configuration was corroborated by a significant NOE between H-4_{ax} and the methine proton of the protecting group positioned at C-2a. Employing DDQ as an oxidating reagent we were not successful to prepare **11** in one step from **10a** [24]. During the following reaction steps reductive removal of the aromatic keto function, degradation of the protected hydrazine, introduction of the *N*-propyl substituents and deprotection had to be accomplished. Thus, *cis*-diasteoselective reduction of **12** by the sterically demanding $LiEt_3BH$ at –78 °C gave the respective hydrazino alcohol which showed transesterification when being stirred at room temperature. Besides the major product **13a** which could be isolated in 80 % yield formation of small amounts of the *trans*-hydrazino alcohol **14** (2 %) was observed. The oxazolidinone **13a** was subjected to catalytic hydrogenolysis (Pd/C, H_2) to yield **13b**. Subsequently, N,N-bond cleavage to give **15** was accomplished by Raney-Ni/H_2. Transformation of **15** to the final product **8** was performed by one-pot conditions including high pressure hydrogenolysis, reductive alkylation (propionic aldehyde, $NaCNBH_3$) and hydrolytic deprotection (HCl, H_2O).



Scheme 2

Pharmacology

In order to obtain information about the *in vitro* potency of the tricyclic ergoline analog **8**, dopamine and serotonin receptor binding studies were performed employing rat striatal membranes and homogenates of rat frontal cortex, respectively, when the selective dopamine autoreceptor agonist 7-HO-DPAT ((*R*)-7-hydroxy-2-*N*,*N*-dipropylaminotetralin) [14] was used as a reference. Thus, the ability of **8** to displace [^3H]-SCH 23390 [25] and [^3H]-spiperone [26] as specific radioligands labelling D-1 and D-2 sites was examined (Table 1). For evaluation of the affinity of **8** to the prejunctional dopamine receptor [^3H]-pramipexole was used, a compound which in functional *in vivo* experiments pointed out to be a selective D-2 autoreceptor agonist [9]. Serotonin receptor binding was measured employing the 5HT-1a receptor agonist [^3H]-8-HO-DPAT [27] and the 5HT-2 antagonist [^3H]-spiperone [28] as the respective radioligands. The test compound **8** exhibited strong affinity to the D-2 autoreceptor, labelled with [^3H]-pramipexole resulting in a k_i value of 4.0 nM. Furthermore, this binding turned out to be highly selective since the affinity indices for D-1, postsynaptic D-2, 5HT-1a and 5-HT-2 were 10500, 650, 225, and 2475 times higher, respectively.

Table 1: Receptor binding of **8** compared to 7-HO-DPAT.

Receptor (radioligand)	k_i [nM] ± S.E.M.	
	8	7-HO-DPAT
D-1 ([^{3}H]-SCH 23390)	42480 ± 2650	13000 (n = 2)
D-2 ([^{3}H]-spiperone)	4600 ± 600	450 (n = 2)
D-2 ([^{3}H]-pramipexole)	4.0 ± 0.6	0.3 (n = 2)
5-HT-1a ([^{3}H]-8-HO-DPAT)	910 ± 240	63 (n = 2)
5-HT-2 ([^{3}H]-spiperone)	9900 ± 3500	31000 (n = 2)
benzodiazepine ([^{3}H]-flunitrazepam)	> 10000	-----

Agonistic activity on the prejunctional dopamine receptor was determined by measuring the inhibition of γ-butyrolactone (GBL) induced acceleration of dopamine synthesis[10,29]. Treating rats with a dose of 10 mg/kg of **8** a 63 % lower L-Dopa level was measured indicating appreciable intrinsic activity (Table 2). In comparison, for 7-HO-DPAT a 90 % inhibition was determined employing identical conditions.

Table 2: Reversal of GBL induced Dopa accummulation by **8** compared to 7-OH-DPAT.

dose [mg/kg]	L-dopa ([µg/g] tissue weight ± S.E.M.)	number of exp.	Δ
control	6317 ± 183	8	
10 (**8**)	2346 ± 86	8	63 %
control	4396 ± 584	5	
10 (7-HO-DPAT)	424 ± 17	4	90 %

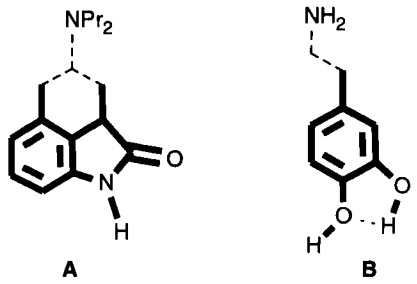
In vivo, the reduction of dopaminergic impulse flow is known to produce a reduction of locomotor activity [8]. Thus, placing mice in an activity cage gave a 97 % reduction of motor activity determined for the first 60 min after injection of 1 mg/kg of **8** (Table 3). For comparison haloperidol induced a 56 % inhibition at a dose of 3 mg/kg. The effect of **8** is not due to binding at the benzodiazepine receptor according to receptor binding studies when **8** was not able to displace [^{3}H]-flunitrazepam up to 10,000 nM. On the other hand, stereotyped behavior indicating postsynaptical striatal dopamine receptor stimulation or catalepsy, as a typical extrapyramidal effect of D2 antagonists, could not be observed, when up to 100 mg/kg were administered to mice.

Table 3: Inhibition of locomotor activity by **8** compared to haloperidol.

dose [mg/kg]	counts ± S.E.M.	number of exp.	Δ	p
control	2978 ± 1032	8		
1 (**8**)	67 ± 15	8	- 98 %	< 0.005
control	3303 ± 534	8		
3 (haloperidol)	1464 ± 305	8	- 56 %	< 0.005

Computational Studies

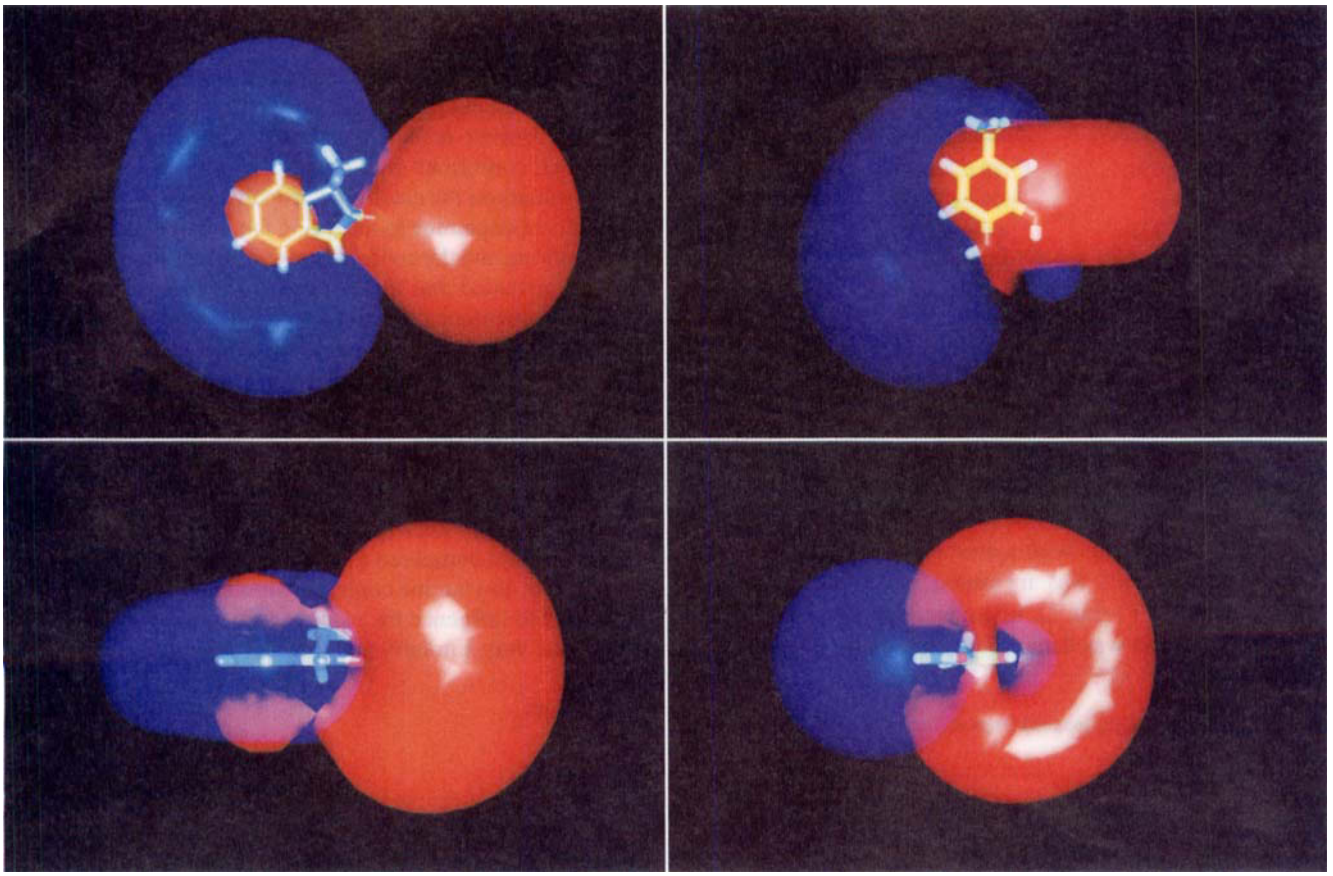
Superimposition and alignment of the previously reported dopamine autoreceptor agonists **2** and **4** with the natural neurotransmitter revealed that they represent an extended dopamine conformer. Subsequent *ab initio* based computational studies on the electronic properties of **2**, **4**, and various rotamers of dopamine (concerning the relative position of the catechol ring and the conformations of the aromatic hydroxyl groups) revealed that the respective three dimensional molecular electrostatic potential maps of the aromatic core structures correspond well for the dopamine conformation **6** with the *meta*-hydroxy group as an intramolecular hydrogen bond donor [16]. Thus, we assume that the natural neurotransmitter adopts a conformation, which is similar to **6** when interacting with the D-2 autoreceptor. In order to verify our model, the charge distribution and the resultant electrostatic environment of the indolinone fragment **A** (Scheme 3) needed to be calculated and compared to the relevant dopamine substructure **B**. The alkylamine containing moieties (dotted lines) are supposed to influence the electrostatic properties of the core fragments very similarly and thus have been neglected.



Scheme 3

For the calculation of three-dimensional molecular electrostatic potential maps (MEPs) the structure of **A** and **B** was minimized employing the *cvff* force field of the program DISCOVER and subsequent optimization of the geometries by use of the MOPAC program system choosing the MNDO parameter set. Based on the thus obtained geometries *ab initio* electronic structure computations were performed at the SCF level of theory of the program package TURBOMOL. The single-point calculations were done using the 6-31G* basis set. Due to the computed wave functions a grid was constructed surrounding the fragments of interest and, subsequently, isopotential maps were created. Figure 1 shows the MEP maps of **A** (left) and **B** (right) from the front and from the bottom side. The positive values (+0.003 Hartrees, blue) and the negative isopotential surfaces (–0.003 Hartrees, red) represent a model for the interaction of a positive test charge (probe) and the minimized core fragments. Comparison of the MEP maps indicates an analogues shape and location for **A** and **B** and thus corroborates our hypothesis that **6** represents the conformation which is active at the dopamine D-2 autoreceptor.

Further efforts including the synthesis and biological investigation of **8** in enantiomerically pure form are underway.



A B

Figure 1: Molecular electrostatic isopotential maps for the core fragments **A** (left) and **B** (right) contoured at –0.003 Hartrees (red) and +0.003 Hartrees (blue). The molecules are viewed from the front side (top) and from the bottom side (below).

Acknowledgment

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Experimental Section

General

THF was distilled from Na/benzophenone immediately before use. All liquid reagents were also purified by distillation. Unless otherwise noted reactions were conducted under dry N_2. Evaporations of final product solutions were done under vacuo with a rotatory evaporator. Flash chromatography was carried out with 230–400 mesh silica gel.– Melting points: Büchi melting point apparatus, uncorrected.– IR spectra: Perkin Elmer 881 spectrometer.– Mass spectra: Varian CH7 instrument.– NMR spectra: Jeol JNM-GX 400 spectrometer at 400 MHz, spectra were measured as $CDCl_3$ solutions using tetramethylsilane as internal standard.– Elemental analyses: Heraeus CHN Rapid instrument.

(2aRS,4SR)-4-Dipropylamino-2a,3,4,5-tetrahydrobenz[c,d]indole-2(1H)-one (8)

A mixture of **15** (217 mg, 0.5 mmol) and 10 % Pd/C (50 mg) in MeOH (20 ml) was stirred at 80 °C for 2 h under H_2 pressure (60 bar). Then it was filtered (Celite^R AFA). $NaCNBH_3$ (31 mg, 0.5 mmol) and propionaldehyde (0.27 ml, 3.5 mmol) were added to the filtrate. After being stirred for 14 h at room temp., sat. aqueous $NaHCO_3$ was added and the mixture was concentrated. It was then extracted with Et_2O. The org. layer was dried ($MgSO_4$) and evaporated and the residue was dissolved in MeOH (6 ml). After addition of 1N HCl (10 ml) the mixture was stirred for 45 min at 70 °C. Then, the solution was extracted with Et_2O. The aqueous layer was render basic to pH 8–9 with 6N NaOH and sat. aqueous $NaHCO_3$ and subsequently extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (hexane–isopropanol 9:1) to give **8** (46 mg, 34 %) as a yellowish solid.– $C_{17}H_{24}N_2O$ (272.3); mol. mass 272 (ms).– IR (NaCl): 3220, 2960, 1705.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, J = 7.3 Hz, 6H, CH_3), 1.37 (ddd, J = 12.5, 11.8, 11.8 Hz, 1H, 3-H_{ax}), 1.43–1.52 (m, 4H, $\underline{CH_2}CH_3$), 2.38–2.48 (m, 4H, NCH_2), 2.48–2.53 (m, 1H, 3-H_{eq}), 2.60 (dd, J = 17.6, 9.5 Hz, 1H, 5-H_a), 3.05 (dd, J = 17.6, 7.3 Hz, 1H, 5-H_b), 3.37 (m, 2H, 2a-H, 4-H), 6.64 (d, J = 8.1 Hz, 1H, 6-H or 8-H), 7.11 (t, J = 8.1 Hz, 1H, 7-H). Preparation of a hydrochloride salt: To a solution of **8** (46 mg) in Et_2O (3 ml) was added HCl (sat. solution in Et_2O) until precipitation was complete. The mixture was filtered and the remaining crystals were dried.– $C_{17}H_{25}ClN_2O$ (308.9) Calcd. C 66.1 H 8.16 N 9.1 Found C 66.2 H 8.46 N 8.7; mol. mass 272 (M–HCl, ms).– IR (KBr): 3220; 2960; 1705 cm^{-1}.

*(±)-5-Bromo-1,2,2a,3,4,5-hexahydro-2-oxobenz[c,d]indole-1,2a-dicarbaldehyde tetraethylacetal (**10b**)*

A mixture of **10a** [21] (162 mg, 0.43 mmol), dibenzoyl peroxide (7 mg, 0.03 mmol), and NBS (82 mg, 0.46 mmol) in CCl_4 (5 ml) was stirred for 40 min at 90 °C. Then, the mixture was filtered and the filtrate was evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc 7:3) to give **10b** (123 mg, 62 %, 1:1 diastereomeric mixture) as a colorless oil which was not stable enough for microanalysis. ^{1}H-NMR ($CDCl_3$) for the *cis*-isomer: δ (ppm) = 0.89 (t, J = 7 Hz, 3H, CH_3), 1.16 (t, J = 7 Hz, 3H, CH_3), 1.24 (t, J = 7 Hz, 3H, CH_3), 1.30 (t, J = 7 Hz, 3H, CH_3) 1.57–1.62 (m, 1H,

3-H_a), 2.49–2.54 (m, 1H, 4-H_a), 2.59–2.65 (m, 2H, 3-H_b, 4-H_b), 3.17–3.21 (m, 1H, OCH_2), 3.42–3.48 (m, 2H, OCH_2), 3.58–3.76 (m, 4H, OCH_2), 3.92–3.96 (m, 1H, OCH_2), 5.12 (s, 1H, CH($OEt)_2$), 5.34 (t, J = 7 Hz, 1H, 5-H), 6.15 (s, 1H, NCH), 7.00 (dd, J = 6.4, 2.1 Hz, 1H, 6-H), 7.20–7.23 (m, 2H, 7-H, 8-H).– ^{1}H-NMR ($CDCl_3$) for the *trans*-isomer: δ (ppm) = 1.09 (t, J = 7 Hz, 3H, CH_3), 1.18 (t, J = 7 Hz, 3H, CH_3), 1.19 (t, J = 7 Hz, 3H, CH_3), 1.26 (t, J = 7 Hz, 3H, CH_3), 1.74 (ddd, J = 13.7, 9.0, 6.4 Hz, 1H, 3-H_a), 2.06 (dddd, J = 14.1, 9.0, 8.1, 5.6 Hz, 1H, 4-H_a), 2.32 (ddd, J = 13.3, 7.7, 5.6 Hz, 1H, 3-H_b), 2.77 (dddd, J = 14.1, 7.7, 6.8, 6.4 Hz, 1H, 4-H_b), 3.40–3.51 (m, 3H, OCH_2), 3.61–3.79 (m, 5H, OCH_2), 4.64 (s, 1H, CH($OEt)_2$), 5.67 (dd, J = 7.7, 6.8 Hz, 1H, 5-H), 6.15 (s, 1H, NCH), 7.20–7.26 (m, 3H, arom.).

(±)-1,2,2a,3,4,5-Hexahydro-2,5-dioxo-benz[c,d]indole-1,2a-dicarbaldehyde tetraethylacetal (**11**)

To a mixture of NBS (3 g, 16.8 mmol) and dibenzoyl peroxide (0.23 g, 0.095 mmol) in CCl_4 (30 ml) was added a solution of **10a** [21] (6.03 g, 16 mmol) in CCl_4 (20 ml) at 80 °C. Subsequently, the reaction mixture was heated at 95 °Cbath temperature and stirred for 45 min. Then it was filtered and triethylamine (8.8 ml) and DMSO (50 ml) were added to the filtrate. After being stirred at room temp. for 4 h, satd. aqueous $NaHCO_3$ was added and the mixture was extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether–EtOAc 3:1) to give **11** (3.69 g, 60 %) as a yellowish solid, mp 85 °C.– $C_{21}H_{29}NO_6$ (391.5) Calcd. C 64.4 H 7.47 N 3.6 Found C 64.3 H 7.35 N 3.7; mol. mass 391 (ms).– IR (NaCl): 2980; 1720; 1690 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.09 (t, J = 7.0 Hz, 3H, CH_3), 1.13 (t, J = 7.0 Hz, 3H, CH_3), 1.20 (t, J = 7.0 Hz, 3H, CH_3), 1.27 (t, J = 7.0 Hz, 3H, CH_3), 1.92 (ddd, J = 13.5, 12.0, 7.7 Hz, 1H, 3-H_a), 2.52 (dd, J = 19.0, 7.7 Hz, 1H, 4-H_a), 2.64 (dd, J = 13.5, 6.8 Hz, 1H, 3-H_b), 2.93 (ddd, 19.0, 12.0, 6.8 Hz, 1H, 4-H_b), 3.38–3.77 (m, 8H, OCH_2), 4.76 (s, 1H, CCH($OC_2H_5)_2$), 6,17 (s, 1H, NCH), 7.35 (dd, J = 8.1, 7.7 Hz, 1H, 7-H), 7.44 (d, J = 8.1 Hz, 1H, 6-H or 8-H), 7.48 (d, J = 7.7 Hz, 1H, 6-H or 8-H).– ^{13}C-NMR ($CDCl_3$) δ = 14.7 (CH_3), 14.7 (CH_3), 14.8 (CH_3), 14.9 (CH_3), 25.2 (H_2C-3), 34.8 (H_2C-4), 51.9 (C-2a), 62,3 (OCH_2), 62.7 (OCH_2), 65.8 (OCH_2), 66.2 (OCH_2), 99.6 (NCH), 106.7 (CCH($OC_2H_5)_2$), 115.9 (HC-6 or HC-8), 118.4 (HC-6 or HC-8), 129.0 (HC-7), 130.5 (C-8b), 133.6 (C-5a), 139.4 (C-8a), 177.1 (C-2), 196.4 (C-5).

Dibenzyl 1-[(2aRS,4SR)-1,2a-Bis(diethoxymethyl)-1,2,2a,3,4,5-hexahydro-2,5-dioxobenz[c,d]indole-4-yl]-1,2-hydrazine dicarboxylate (**12**)

To a solution of diisopropylamine (2.6 ml, 18.5 mmol) in THF (37 ml) was added *n*-BuLi (10.1 ml, 1.6 molar in hexane) at –78 °C. The mixture was allowed to warm up to 0 °C. After 30 min it was added to a solution of **11** (5.87 g, 15 mmol) in THF (170 ml) at –78 °C. After 1 h a precooled solution (–78 °C) of dibenzyl azodicarboxylate (5.37 g, 18 mmol) in THF (23 ml) was added and the reaction mixture was stirred for 3 min. After addition of 10 % aqueous NH_4Cl and extraction with Et_2O, the organic layer was dried, evaporated and purified by flash chromatography (petroleum ether–EtOAc 7:3) to give **12** (7.72 g, 75 %) as a colorless solid, mp 53 °C besides recovered **11** (0.47 g, 8 %).– $C_{37}H_{43}N_3O_{10}$ (689.8) Calcd. C 64.4 H 6.28 N 6.1 Found C 64.4 H 6.16 N 6.2.– IR (KBr): 3390; 2980; 1730 (br) cm^{-1}.– ^{1}H-NMR (DMSO-d_6, 100 °C): δ (ppm) = 0.97 (t, J = 7.1 Hz, 6H, CH_3), 1.10 (t, J = 7.1 Hz, 3H, CH_3), 1.20 (t, 3H, J = 7.1 Hz, CH_3), 2.02 (dd, J = 13.0, 13.0 Hz, 1H, 3-H_a), 2.84 (dd, J = 13.0, 5.8 Hz, 1H, 3-H_b), 3.37–3.74 (m, 8H, O$CH_2$$CH_3$), 4.82 (s, 1H, CCH($OC_2H_5)_2$), 4.99–5.08 m, 4H, O$CH_2$-phenyl), 5.22–5.24 (m, 1H, 4-H), 6.09 (s, 1H, NCH), 7.26–7.37 (m, 13H, aromat.), 9.23 (br. s, 1H, NH).

Benzyl N-[(5aRS,6aSR,9aRS)-4,5a-Bis(diethoxymethyl)-4,5,6,6 a,7,8, 9a-octahydro-5,8-dioxoxazolo[4',5':1,2]benz[5,4,3,-c,d]indole-7-yl]-carbamate (**13a**), *Dibenzyl 1-[(2aRS,4SR,5SR)-1,2a-bis(diethoxymethyl)-1,2,2a,3,4,5-hexahydro-5-hydroxy-2-oxobenz[c,d]indol-4-yl]-1,2-hydrazine dicarboxylate* (**14**)

To a solution of **12** (158 mg, 0.2 mmol) in THF (12 ml) was slowly added $LiBH(Et)_3$ (0.22 ml, 1 molar in THF) at –78 °C. After 1.5 h at –78 °C stirring was continued for 1 h at room temp. Then Et_2O and sat. aqueous $NaHCO_3$ were added. The organic layer was dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (petroleum ether–EtOAc 65:35) to give **13a** (93 mg, 80 %) and **14** (2.5 mg, 2 %). **13a** (colorless solid, mp 70 °C): $C_{30}H_{37}N_3O_9$ (583.6) Calcd. C 61.7 H 6.39 N 7.2 Found C 61.7 H 6.43 N 7.3; mol. mass 584 (ms).– IR (KBr): 3300; 2980; 1780; 1725 cm^{-1}.– ^{1}H-NMR (DMSO-d_6, 100 °C): δ (ppm) = 0.99 (t, J = 7.0 Hz, 3H, CH_3), 1.10 (t, J = 7.1 Hz, 3H, CH_3), 1.11 (t, J = 7.1 Hz, 3H, CH_3), 1.20 (t, J = 7.1 Hz, 3H, CH_3), 1.18–1.24 (m, 1H, 6-H_a), 2.56–2.61 (dd, J = 13.3, 7.7 Hz, 1H, 6-H_b), 3.39–3.73 (m, 8H, O$CH_2$$CH_3$), 4.69 (s, 1H, CCH($OC_2H_5)_2$), 4.69–4.75 (q, J = 8 Hz, 1H, 6a-H), 5.08–5.15 (m, 2H, OCH_2-Phenyl), 5.67 (d, J = 8 Hz, 1H, 9a-H), 6.09 (s, 1H, NCH), 7.07 (d, J = 8.1 Hz, 1H, 1-H or 3-H), 7.19 (d, J = 7.7 Hz, 1H, 1-H or 3-H), 7.27–7.39 (m, 6H, 2-H, Phenyl), 9.24 (br. s, 1H, NH). **14** (colorless solid, mp 66 °C): $C_{37}H_{45}N_3O_{10}$ (691.8) Calcd. C 64.2 H 6.56 N 6.1 Found C 64.0 H 6.55 N 6.1.– IR (KBr): 3460; 3300; 1720 cm^{-1}.– ^{1}H-NMR (DMSO-d_6, 100 °C): δ (ppm) = 0.85 (t, J = 6.8 Hz, 3H, CH_3), 1.04–1.10 (m, 6H, 2CH_3), 1.18 (t, J = 6.8 Hz, 3H, CH_3), 1.61 (t, J = 11.7 Hz, 1H, 3-H_{ax}), 2.53–2.56 (m, 1H, 3-H_{eq}), 3.16–3.19 (m, 1H, OCH_2), 3.47–3.69 (m, 7H, OCH_2), 4.77 (s, 1H, CCH($OC_2H_5)_2$), 4.92–4.94 (m, 2H, 4-H, 5-H), 5.03 (s, 2H, CH_2–Phenyl), 5.11 (s, 2H, CH_2-Phenyl), 6.02 (s, 1H, NCH), 6.91 (d, J = 7.7 Hz, 1H, 6-H or 8-H), 7.03 (d, J = 8.1 Hz, 1H, 6-H or 8-H), 7.15 (t, J = 8.1 Hz, 1H, 7-H), 7.26–7.32 (m, 10H, ar), 9.03 (br-s, 1H, NH).

(5aRS,6aSR,9aRS)-7-Amino-4,5,5a,6,6a,7,8,9a-octahydro-5,8-dioxo-oxazolo[4',5':1,2]benz[5,4,3-c,d]indole-4,5a-dicarbaldehyde tetraethylacetal (**13b**)

A mixture of **13a** (408 mg, 0.7 mmol) and Pd/C (74 mg, 10%) in EtOH (40 ml) was stirred for 1 h at room temp. under a balloon of H_2. Then, it was filtered (Celite^R AFA) and the filtrate was evaporated. The residue was purified by flash chromatography (CH_2Cl_2–CH_3OH 98:2) to give **13b** (261 mg, 83 %) of **13a** as a colorless solid, mp 57 °C.– $C_{22}H_{31}N_3O_7$ (449.5) Calcd. C 58.8 H 6.95 N 9.3 Found C 59.0 H 7.17 N 9.0; mol. mass 449 (ms).– IR (KBr): 3350, 2980; 1760; 1720 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.07 (t, J = 7.0 Hz, 3H, CH_3), 1.20 (t, J = 7.0 Hz, 3H, CH_3), 1.26 (t, J = 7.0 Hz, 3H, CH_3), 1.27 (t, J = 7.0 Hz, 3H, CH_3), 1.27–1.32 (m, 1H, 3-H_a), 2.79 (dd, J = 13.3, 7.7 Hz, 1H, 3-H_b), 3.44–3.85 (m, 8H, OCH_2), 3.80 (br. s, 2H, NH_2, D_2O-exchange), 4.61 (ddd, J = 9.0, 7.7, 7.3 Hz, 1H, 6a-H), 4.75 (s, 1H, CCH($OC_2H_5)_2$), 5.67 (d, J = 9.0 Hz, 1H, 9a-H), 6.16 (s, 1H, NCH), 7.21 (t, J = 4.3 Hz, 1H, 2-H), 7.28–7.33 (m, 2H, 1-H, 3-H).

(5aRS,6aSR,9aRS)-4,5,5a,6,6a,7,8,9a-Octahydro-5,8-dioxo-oxazolo-[4',5':1,2]benz[5,4,3-c,d]indole-4,5a-dicarbaldehyde tetraethylacetal (**15**)

A mixture of **13b** (1.39g, 3.09 mmol) and Raney-Ni (0.1 g) in EtOH (20 ml) was stirred at for 18 h at room temp. under H_2 pressure (60 bar). Then it was filtered and the filtrate was evaporated. The residue was purified by flash chromatography (hexane–isopropanol 4:1) to give **15** (1.06 g, 79 %) as a colorless solid, mp 120 °C.– $C_{22}H_{30}N_2O_7$ (434.5) Calcd. C 60.8 H 6.96 N 6.5 Found C 60.9 H 7.18 N 6.2; mol. mass 434 (ms).– IR (KBr): 3300; 2980; 1750; 1720 cm^{-1}.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.07 (t, J = 7.1 Hz, 3H, CH_3), 1.19 (t, J = 7.1 Hz, 3H, CH_3), 1.25 (t, J = 7.1 Hz, 3H, CH_3), 1.27 (t, J = 7.1 Hz, 3H, CH_3), 1.23–1.28 (m, 1H, 6-H_a), 2.74 (dd, J = 13.5, 7.5 Hz, 1H, 6-H_b), 3.40–3.83 (m, 8H, OCH_2), 4.67 (s, 1H, CCH($OC_2H_5)_2$), 4.77 (ddd, J = 8.8, 8.1, 7.7 Hz, 1H, 6a-H), 5.66 (br. s, 1H, NH, D_2O-exchange), 5.74 (d, J = 8.8 Hz, 1H, 9a-H), 6.15 (s, 1H, NCH), 7.22–7.25 (m, 1H, H-2), 7.30–7.35 (m, 2H, H-1, H-3).

Receptor Binding Assay

a) Dopamine Receptor Binding

DA receptor binding was performed as previously described using [^{3}H]-SCH 23390 [25] and [^{3}H]-spiperone [26] 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, [^{3}H]-pramipexole (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 [^{3}H]-spiperone as a radioligand. For all receptor binding tests rat brain striatum was used.

b) Serotonin Receptor Binding

5HT-1a and 5-HT2 receptor binding was performed as described using [^{3}H]-8-HO-DPAT [27] (240 Ci/mmol) and [^{3}H]-spiperone [28] (240 Ci/mmol) as the respective radioligands in a concentration of 0.2 nM for both cases. Non-specific binding was estimated using serotonin (100 µM) and ketanserin

(10 µM). For the serotonin receptor binding tests rat frontal cortex homogenate was used.

c) Benzodiazepine Receptor Binding

For these studies the *Benzodiazepine Receptor Preparation for Radioreceptor Assays* (RBI Inc.) was employed. [^{3}H]-flunitrazepam was used as the radioligand in a concentration of 2 nM.

GBL Induced L-Dopa Accummulation

Male rats of 180–200 g body weight (Chbb: THOM, Dr. Karl Thomae GmbH, Biberach, Germany) were used. They were kept under standard laboratory conditions, with food and water available *ad libidum*. The rats were injected with saline (0.9 % NaCl, 2.5 ml /kg body weight s.c.) or the respective test compound in a volume of 2.5 ml/kg s.c.; γ-butyrolactone (GBL, 750 mg/kg) and the decarboxylase inhibitor NSD 1015 (100 mg/kg) were injected i.p. 35 and 30 min, respectively, before decapitation [10]. After decaptitation, the corpus striatum was quickly dissected on ice. The preparation of the corresponding brain tissue and the determination of the dopa content by means of HPLC with electrochemical detection were carried out as described in refs. [10,30].

Locomotor Activity Measurement

Three mice (male NMRI mice, 20–33 g) in a macrolon cage (type III) with free access to food and water were placed into a scanner box (RBM 3, MSE/INTRON, München/Münsing, Germany) using an 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 on 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.[31,32].

Computational Studies:

Calculations were performed on a Silicon Graphics Indigo 2 Extreme R4400 workstation computer.

Ab initio calculations were performed using the TURBOMOL 2.300 program system (BIOSYM Tech. Inc., San Diego). For molecular mechanics calculations the *cvff* force field of the program system DISCOVER 94.0 was used (BIOSYM Tech. Inc., San Diego). Semiempirical calculations were performed using the MOPAC 6.0 [33] program system choosing the MNDO parameter set. The above mentioned software packages are implemented into the program INSIGHT II Release 2.3.5 (BIOSYM Tech. Inc., San Diego).

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