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Dopamine/serotonin receptor ligands. Part 15: Oxygenation of the benz-indolo-azecine LE 300 leads to novel subnanomolar dopamine D_1/D_5 antagonists☆

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Abstract—Relying on the high affinities of the benz-indolo-azecine LE 300 (**1**) and the hydroxylated dibenz-azecine LE 404 (**2b**) for the D_1/D_5 receptor subtypes, we synthesized methoxylated, hydroxylated and an indole-N methylated derivatives of **1** (Fig. 1). Hydroxylation of azecine derivatives is beneficial with regard to the affinities and selectivities for all the dopamine receptor subtypes. The 'serotonin-derived' 3-oxygenated target compounds but not the 11-oxygenated analogues were superior to the unsubstituted LE 300. 11-Methoxy-7,14-dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**3e**) was found to be the most potent antagonist at $D_2/D_3/D_4$ and D_5 receptor subtypes (K_i for D_5 = 0.23 nmol) of all known benz-indolo-azecines.

Locomotion, emotion, cognition and endocrinal secretion are linked with dopaminergic transmission. Most of the dopamine receptor antagonists used as antipsychotics inhibit the D_2-family (D_2, D_3 and D_4), 'azecine-styled' dopamine antagonists like **1** (LE 300)[5,2–4] or its dibenzo-analogues[4] **2** showed selectivity primarily for the D_1 family (D_1 and D_5) (Fig. 1).

Previous investigations of this novel class of antidopaminergic drugs revealed that replacing the indole moiety in the lead **1** with benzene is tolerated without a loss in affinity for most of the dopamine receptor subtypes.[3] (compare **1** and **2c** in Table 1). But the affinities of **2c** were increased by substituents in the aromatic system (compare **2c**–**b** in Table 1). The phenolic **2b** showed a 73-fold higher affinity for the D_1 receptor compared to the MeO-congener **2a**, and its affinity for D_5 was also higher (Table 1). On the other hand, the affinity for D_2 was slightly higher for the MeO-compound **2a** than for **2b** (Table 1).

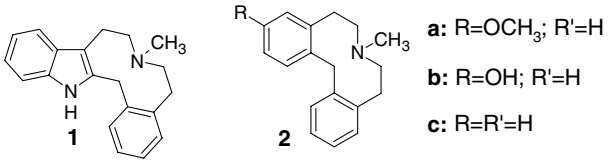

Figure 1. The lead compounds LE 300 (**1**) and dibenzazecines **2a**–**d**.

The lead compound **1** of all azecine-type dopamine receptor ligands has not been substituted up to now in the aromatic rings or in the indole nitrogen. To investigate if the effects of methoxylation and hydroxylation of azecine derivatives with regard to the affinities and selectivities for all of the dopamine receptor subtypes are beneficial, we attached MeO-/HO-groups to the benz-indolo-azecine scaffold of **1** at the indole moiety (**3a** and **b**) and at the benzene part (**3c** and **d**), respectively (Fig. 2). Furthermore, we methylated the indole-N of **3a**, which yielded **3e**. Finally, we characterized the interaction of these target compounds with the human-cloned D_1–D_5 receptor subtypes by radioligand-binding experiments and by a functional calcium assay.

To synthesize the 11-methoxy-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine **3a**, the corresponding indolo-benzazecin-11-ol **3b** and the 7,14-dimethyl derivative **3e**, a

Keywords: Dopamine receptor ligands; Azecines; LE 300; Oxygenation; N-methylation.
☆ See Ref. 1.
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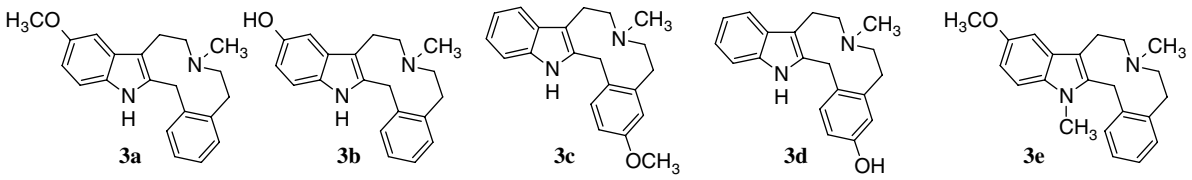
Table 1. Affinities (K_i, nM) for dopamine receptor D_1–D_5 subtypes determined by radioligand-binding experiments



Compound	K_i (nM)				
	HEK D_1	CHO D_{2L}	CHO D_3	CHO $D_{4.4}$	HEK D_5
1 (LE-300)[7]	1.9 ± 0,9	44.5 ± 15.8	40.3 ± 14.4[a]	109 ± 39	7.5 ± 0.3
2a	28.5 ± 9.7	13 ± 9	75.7 ± 7.3[b]	43.4 ± 13.2[b]	38.3 ± 24[a]
2b[3]	0.39 ± 0.22	17.5 ± 2.1	47 ± 24	11.3 ± 1	1.5 ± 0.2
2c[3]	4.5 ± 2.1	56.5 ± 9.0	52.5 ± 6.4	134 ± 15	11.2 ± 1.8
3a	0.82 ± 0.056[b]	11.9 ± 5.6[a]	475 ± 48.5[b]	266 ± 22[b]	3.6 ± 0.6[a]
3b	0.56 ± 0.06[b]	38.4 ± 14[a]	944 ± 171[b]	398 ± 288[a]	0.39 ± 0.16[a]
3c	19.0 ± 2.3[b]	22.8 ± 11.1[a]	1135 ± 237[b]	92.6 ± 5.3[a]	31.5 ± 14.7[b]
3d	3.7 ± 1.1[b]	74.7 ± 34[a]	2070 ± 776[b]	1359 ± 926[a]	5.4 ± 3.6[a]
3e	2.00 ± 1.6[a]	1.70 ± 0.6[a]	3.78 ± 2.4[a]	21.55 ± 3.5[b]	0.23 ± 0.06[a]

[a] K_i values are means of three experiments, performed in triplicate ± SEM.
[b] K_i values are means of two experiments, performed in triplicate ± SEM.

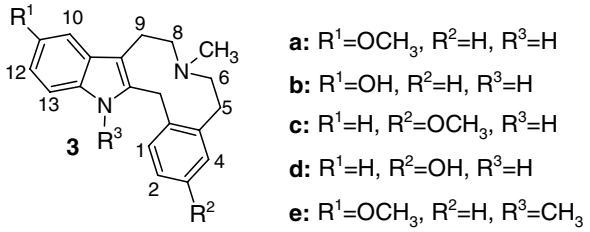
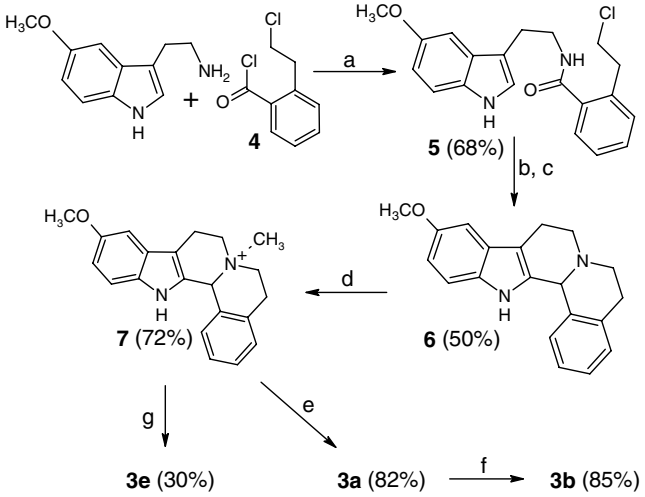


Figure 2. Novel derivatives of LE 300 (**1**) with numbering of the ring system.

mixture of equimolar amounts of 5-methoxy-tryptamine and chloroethylbenzoylchloride[6] (**4**) was reacted together with 3 mol of triethylamine in dichloromethane. The resulting benzamide derivative **5** was cyclized with $POCl_3$ in acetonitrile. $NaBH_4$ reduction of the intermediate in methanol yielded the pentacyclic benzindolo-quinolizine **6**. After quaternization with methyl iodide in acetone, the resulting compound **7** was cleaved using elemental sodium in liquid ammonia to yield **3a**.[11] The phenolic analogue **3b**[12] was obtained from **3a** by ether cleavage with BBr_3 in dichloromethane.



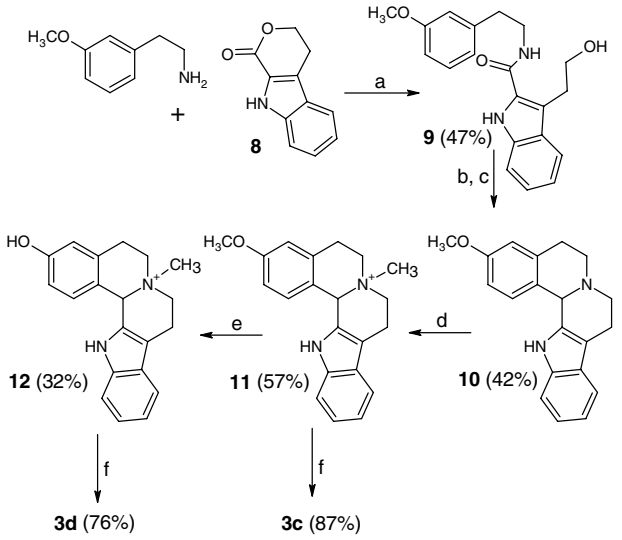
Scheme 1. Synthesis of benz-indolo-azecines **3a** and **3b**. Reagents and conditions: (a) 5-methoxy-tryptamine, 3 mol NEt_3, CH_2Cl_2, 0 °C–rt; (b) $POCl_3$/acetonitrile 1:20, 5 h, 30 °C, under nitrogen; (c) $NaBH_4$, MeOH, 0 °C, 1 h, reflux; (d) methyl iodide, acetone; (e) Na, liq NH_3; (f) BBr_3, CH_2Cl_2, 5 h, reflux; (g) Na, liq NH_3, methyl iodide.

Interestingly, the dimethylated benz-indolo-acezine **3e** could be obtained in a one-pot reaction out of **7** just by adding 1.5 mol of methyl iodide to the solution of **7** in liquid ammonia after adding the sodium which is needed to cleave the central C–N bond. Purification was performed by preparing the hydrochloride salt of **3e**[15] and recrystallization from isopropanole (Scheme 1).

3-Methoxyphenethylamine and the indololactone **8**[7] were used for synthesizing the 3-methoxy-7-methyl-hexahydro-indolo[3,2-*f*][3]-benzazecine **3c** and the corresponding indolo[3,2-*f*][3]-benzazecin-3-ol **3d**. Applying the cyclization/reduction sequence with $POCl_3$ in acetonitrile and $NaBH_4$ in methanol to the hydroxyethyl-benzamide **9** yielded the corresponding quinolizine derivative **10**. Quaternization with methyl iodide and the subsequent ring cleavage of the quaternary salt **11** resulted in **3c**.[13] The O-demethylation was conducted with HBr in acetic acid and produced the phenolic quaternary salt **12**. The target compound **3d**[14] was obtained by ring cleavage (Scheme 2).

All of the target compounds were screened for their binding affinities for human-cloned D_1, D_{2L}, D_3, $D_{4.4}$ and D_5 receptors, stably expressed in HEK 293 or CHO cells, by radioligand-binding experiments. The protocol has been described.[8,16]

Additionally, the functionality of the target compounds was investigated for the D_1, D_2 and D_5 receptors using a



Scheme 2. Synthesis of benz-indolo-azecines **3c** and **3d**. Reagents and conditions: (a) 1.3 mol 3-methoxyphenethylamine, toluene, 24 h, reflux; (b) $POCl_3$/acetonitrile 1:1, 24 h, reflux, under nitrogen; (c) $NaBH_4$, MeOH, 0°, 1 h, reflux; (d) methyl iodide, acetonitrile; (e) HBr/HOAc, 3 h, reflux; (f) Na, liq NH_3.

calcium fluorescence assay which has been developed and established in our group.[9]

None of the reported compounds increased the intramolecular calcium concentration of any type of cell. This excludes agonistic activity. Instead, the compounds suppressed the calcium signal which was induced by standard dopamine agonists (D_1/D_5 receptors: SKF 38393, D_2: quinpirol) in a concentration-dependent manner and produced sigmoidal curves which characterize the inhibition of the respective agonists' action (data not shown). These findings confirmed that the compounds are either antagonists or inverse agonists in both of the dopamine receptor families. Affinity data (Table 1) were generated from radioligand-binding experiments.

SAR with regard to the influence of the HO-/MeO-substitution of the benz-indolo-azecines on the affinity for the D_1 receptor are similar to those found for the dibenz-azecines: Of all of the new indolic compounds **3a**–**d**, the phenolic derivatives **3b**, **d** are superior to their methoxy analogues **3a**, **c** in binding to the D_1 receptor. The same was found for the D_5 receptor. In the affinity for the D_2 receptor, we observed a marginally higher affinity for the methoxy-substituted **2a** in the dibenzo-azecines **2a**, **b** (Table 1). This tendency could be seen in former investigations,[4] is observed for several unpublished compounds and was affirmed for the new benz-indolo-azecines: in both series of compounds—the ones with an oxygenated indole moiety (**3a** and **b**) and also the ones with the, respectively, substituted benzene moiety (**3c** and **d**)—the methoxy-derivatives **3a**, **c** exhibited a 3-fold higher affinity for the D_2 receptor compared to their phenolic congeners **3b** and **d** (Table 1).

In addition to these general relations, it is noteworthy that the structural area where oxygenation takes place is quite relevant. The compounds **3c** and **d**, which may be considered a combination of **1** and **2a**, **b** rather than **3a**, **b**, exhibited lower affinities than their indole-substituted counterparts **3a**, **b** and **e**. Compounds **3c** and **d** do maintain the oxygenated benzene ring of **2a** and **b**, consequently the introduction of the indole for the unsubstituted benzene ring is disfavoured. Perhaps the indole—rather than the benzene-moiety is involved in receptor binding, and hydroxylation or—to a lesser extent—methoxylation at the indole, yielding 'serotonin-derived' structures, facilitates the receptor binding, whereas these additional substituents in the benzene part do not enhance or lower the protein binding significantly. The substituents on benzene ring effect steric or electronic interaction, that causes a decrease in affinity. The selectivity profile is characterized by a preference for the D_1/D_5 family and for **3e** there is a remarkable 9-fold D_5 selectivity over the D_1 receptor.

Selecting one of the more attractive 11-oxygenated derivatives (**3a**), we methylated the indole nitrogen, and the resulting compound **3e** showed an increase in all of the affinities compared to the desmethyl derivative **3a** with the exception of that for D_1. The affinity for the D_1 receptor decreased by a factor of 2.4, whereas the affinity for D_5 increased by factor 15 yielding a 9-fold D_5-selectivity. Furthermore, a surprising increase was observed for D_2 and D_3: the affinity rose from 12 to 1.7 nM and from 474 to 3.8 nM, respectively. For D_4 the affinity improved more than 10-fold. Thus, compound **3e**, showing the same high affinity for the D_1 and D_2 receptors, a very high affinity for D_5 and considerable affinities for D_3 and D_4, features a receptor profile which is quite unusual for the azecine-like dopamine receptor antagonists and different from those being investigated so far by our group.

Since the affinity of **3e** is not reduced in comparison to **3a**, it can be excluded that the indole NH of the benz-indolo-azecines interacts in the same way as the OH in **2b**. The influence on the affinity profile caused by the indole *N*-methyl group must rather be a steric than an electronic effect.

In general the SAR regarding the interaction of the HO- and the MeO-compounds with the D_1 receptor are similar for the dibenzo- and the benzindolo-derivatives. But the significant loss in affinity that occurs in the unsubstituted dibenzazecine **2c** by introducing a MeO-substituent (**2a**) was not found for the benz-indolo-azecine. Here the compound with a MeO-substitution at the indole moiety (**3a**) shows higher affinity than the unsubstituted **1**.

In conclusion: to investigate the influence of methoxylation, hydroxylation and indole-N-methylation, we synthesized five appropriate derivatives of the dopamine antagonist LE 300, measured the affinities for all human-cloned dopamine receptors and characterized them as antagonists or inverse agonists in a fluorescence calcium assay. These structural modifications partially improved affinities and changed selectivities. 'Serotonin-derived' compounds were superior to their

'tryptamine-derived' isomers. The one-pot reaction for both the indole-N-methylation and the ring expansion procedure under Birch conditions represents a novel synthetic strategy that might be useful for the preparation of further N-alkylated indolo-azecines. It might also be used for alkylating phenolic groups simultaneously.

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11. 11-Methoxy-7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**3a**): white foam, mp 160 °C. ^{1}H NMR (250 MHz; $CDCl_3$): δ 2.6 (s, 3H, N–CH_3) 2.9–2.6 (m, 8H, H-5, 6, 8, 9), 3.8 (s, 3H, –OCH_3), 4.4 (s, 2H, H-15), 6.8 (dd, 1H, J = 2.5, 8.7 Hz, H-12), 6.95 (d, 1H, J = 2.5 Hz, H-10), 7.2 (m, 4H, H-2,3,4,13), 7.3 (m_c, 1H, H-1), 7.7 (s, 1H, –NH) ppm. GC/MS: M = 320 (79%), M = 275, 276 (32%), M = 262 (27%), M = 248 (32%), M = 173 (16%), M = 160 (53%), M = 58 (100%). Anal. ($C_{21}H_{24}N_2O$): C, N, H.
12. 7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecin-11-ol (**3b**): from isopropanol grey powder, mp 175–180 °C. ^{1}H NMR (250 MHz; methanol d_4): δ 2.2 (s, 3H, N–CH_3) 2.9–2.6 (m, 8H, H-5, 6, 8, 9), 4.2 (s, 2H, H-15), 6.65 (dd, 1H, J = 2.3, 8.6 Hz, H-12), 6.8 (d, 1H, J = 2.3 Hz, H-10), 7.1 (d, 1H, J = 8.6 Hz, H-13), 7.25 (m, 4H, H-2,3,4), 7.3 (m_c, 1H, H-1). ppm. GC/MS: M = 306 (85%), M = 262, 261 (37%), M = 234 (50%), M = 207 (30%), M = 160 (29%), M = 146 (29%), M = 58 (100%). Anal. ($C_{20}H_{22}N_2O$): C, N, H.
13. 3-Methoxy-7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**3c**): white powder, mp 252 °C (HCl salt). ^{1}H NMR (250 MHz; $CDCl_3$): (free base) δ 2.6 (s, 3H, N–CH_3) 2.5 (m_c, 2H, H-5 or H-9), 2.6 (m_c, 4H, H-6 or H-8 and 5 or 9), 2.7 (m_c, 2H, H-6 or H-8), 2.8 (s, 3H, –OCH_3), 4.2 (s, 2H, H-15), 6.5 (d, 1H, J = 2.7 Hz, H-4), 6.6 (dd, 1H, J = 2.9; 8.4 Hz, H-2), 7.0 (m_c, 2H, H-11 and H-12), 7.1 (d, 1H, J = 2.7 Hz, H-10), 7.2 (d, 1H, J = 1.5 Hz, H-13),7.3 (m_c, 1H, H-1), 7.6 (s, 1H, –NH) ppm. GC/MS: M = 320 (100%); M = 275 (35%); M = 262 (31%) M = 248 (35%); M = 217 (25%); M = 190 (28%); M = 143 (26%). Anal. ($C_{21}H_{25}N_2OCl$): C, N, H.
14. 7-Methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecin-3-ol (**3d**): off-white powder, mp 135 °C. ^{1}H NMR (250 MHz; $CDCl_3$): δ 2.3 (s, 3H, N–CH_3), 2.5 (dd, 2H, J = 5.4 Hz, H-5 or H-9), 2.6 (dd, 2H, J = 5.2 Hz, H-5 or H-9), 2.7 (m_c, 2H, H-6 or H-8), 2.8 (m_c, 2H, H-6 or H-8), 4.2 (s, 2H, H-15), 6.5 (d, 1H, J = 2.7 Hz, H-4), 6.6 (dd, 1H, J = 2.9; 8.2 Hz, H-2), 7.0 (m_c, 2H, H-11 and H-12), 7.05 (m_c, 1H, H-10 or H-13), 7.1 (m_c, 1H, H-10 or H-13), 7.3 (m_c, 1H, H-1), 7.7 (s, 1H, –NH) ppm. GC/MS: M = 306 (100%); M = 261 (45%); M = 248 (41%); M = 234 (60%); M = 176 (48%); M = 143 (53%). Anal. ($C_{20}H_{22}N_2O$): C, N, H.
15. 11-Methoxy-7,14-dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine hydrochloride (**3e**): off-white solid, mp 226–242 °C (slow decomposition). ^{1}H NMR (250 MHz; $CDCl_3$): δ 2.36 (s, 3H, N–CH_3), 2.53–2.46 (m, 4H, H-6 and H-8), 2.8–2.73 (m, 4H, H-5 and H-9), 3.51 (s, 3H, indole-N–CH_3), 3.87 (s, 3H, –OCH_3), 4.4 (s, 2H, H-15), 6.85–6.8 (dd, 1H, J = 8.7, 2.4 Hz, H-12), 6.93–6.94 (d, 1H, J = 2.4 Hz, H-10), 7.02–7.33 (m, 5H, H-1, H-4), 7.1 (d, 1H, J = 8.7 Hz, H-13) ppm. GC/MS: M = 334 (100%); M = 290 (18%); M = 276 (57%); M = 262 (82%); M = 229 (80%); M = 187 (34%); M = 174 (65%); M = 160 (34%). Anal. ($C_{22}H_{27}N_2OCl$): C, N, H.
16. [3H]SCH 23390 and [3H]spiperone were used as radioligands at the D_1-like and D_2-like receptor family, respectively. Incubation at 27 °C was terminated after 90 min by rapid filtration 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, applying the equation of Cheng and Prusoff,[10] and are given in nanomolar units (Table 1).