Hussein El-Subbagh[a, b], Thomas Wittig[b], Michael Decker[b], Sigurd Elz[c], Martin Nieger[d], Jochen Lehmann[b]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt
[b] Institute of Pharmacy, Pharmaceutical/Medicinal Chemistry, Friedrich-Schiller-University Jena, Jena, Germany
[c] Institute of Pharmacy, Regensburg University, Regensburg, Germany
[d] Institute of Inorganic Chemistry, University of Bonn, Bonn, Germany

Dopamine/Serotonin Receptor Ligands. Part IV [1]: Synthesis and Pharmacology of Novel 3-Benzazecines and 3-Benzazonines as Potential 5-HT_{2A} and Dopamine Receptor Ligands

LE 300 represents a structurally novel type of antagonist acting preferentially at the dopamine D_1/D_5 receptors and the serotonin 5-HT_{2A} receptor. The compound consists of a 10-membered central azecine ring fused to indole on one and to benzene on the other side. To estimate the importance of the indole moiety in this highly active benz-indolo-azepine, the indole has to be removed and the "de-indolised" analog reinvestigated pharmacologically. Accordingly, we synthesized 3-benzazecines and in addition some homologuous 3-benzazonines. Methoxylated β-phenylethylamines were treated with ethyl ω-bromo-butanoates and -pentanoates, respectively, to give the corresponding lactams which were cyclized ($POCl_3$) and reduced ($NaBH_4$), yielding the *cis*-annelated (X-ray) benzindolizines and -quinolizines. The 10- and 9-membered rings were obtained by cleavage of the central C–N bond, which was performed in the following two ways: Quarternisaion with methyl iodide and cleavage with sodium in liquid ammonia gave the NCH_3 derivatives, reaction with benzyloxycarbonyl chloride/$NaBH_4$ followed by catalytic debenzylation yielded a corresponding NH compound. Functional experiments on rat artery segments precontracted with ketanserin and radioligand binding experiments using human cloned dopamine receptor subtypes were conducted with all of the benzazecine and benzazonine derivatives. In contrast to the benz-indolo-compound LE 300 they did not show any significant affinity towards the D_1, D_2, D_4, and D_5 receptors and only moderate antagonistic activity at the 5-HT_{2A} receptor. It can be concluded from our study that an indole moiety or at least another second aromatic system at the central azecine ring is part of the pharmacophore and thus essential for high biological activity.

Keywords: 3-Benzazonine; 3-Benzazecine; 5-HT_{2A} ligand; Dopamine receptor ligand; LE 300

Received: March 12, 2002 [FP682]

Full Paper

Introduction

Serotonin (5-HT) plays a major role in the regulation of numerous physiological functions including affective behavior, memory, and thermo-regulation [2], through the interaction with a number of serotonin receptor subtypes [3]. The 5-HT_{2A} receptor subtype received particular attention because it is well characterized in the human body with respect to distribution and function. Stimulation of 5-HT_{2A} receptor would result in psychiatric symptoms, vasoconstriction, and platelet aggregation; consequently, selective 5-HT_{2A} receptor antagonists showed a therapeutic efficacy for the treatment of mental and cardiovascular illness [4]. On the other hand, various dopamine subtype antagonists appear to offer promising approaches for the development of neuroleptic type drugs [5–7]. Several 5-HT_{2A} blockers or antagonists such as ketanserin and sarpogrelate [8] proved to be clinically useful in treating hypertension and ischemia. Meanwhile, mixed D_2/5-HT_{2A} receptor antagonists such as risperidone and clozapine proved to be atypical neuroleptics.

Recently, compound LE 300 was prepared in our laboratory [9]. LE 300 is a hybrid of tryptamine and phenethylamine linked through the semi-rigid azecine ring, and showed a subnanomolar affinity towards the rat

Correspondence: Jochen Lehmann, Institute of Pharmacy, Pharmaceutical/Medicinal Chemistry, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany. Phone: +49 3641 949803, Fax: +49 3641 949802, e-mail: j.lehmann@uni-jena.de

© 2002 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 0365-6233/09/0443 $ 17.50+.50/0

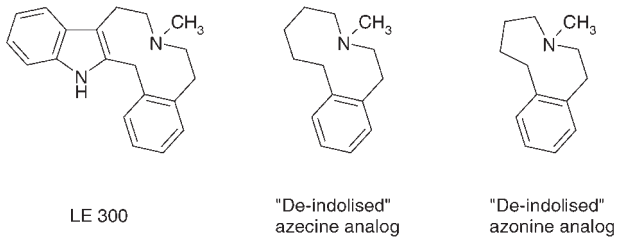

Figure 1

striatal D_1 receptor [9] and high functional activity at the 5-HT_{2A} receptor [10]. In the present study, some new analogs of LE 300 were synthesized from which the indole moiety was omitted, in order to estimate its necessity for biological activity. In addition, methoxylation of the remaining benzene part and ring constriction to azonine analogs were performed.

Results and discussion

Chemistry

The synthesis of the target compounds **20**–**24** is depicted in Figure 2. The lactam **3** was prepared by refluxing 3-methoxyphenethylamine (**1**) and ethyl 4-bromobutyrate in dioxane. The same procedure was adopted to produce the reported compound **4** [11] in nearly quantitative yield, using 3,4-dimethoxyphenethylamine (**2**) and ethyl 4-bromobutyrate instead of butyrolactone [11]. **3** was cyclized using $POCl_3$ in refluxing toluene to yield the salt **7**, which was subsequently reduced into its corresponding hexahydropyrrolo[2,1-*a*]isoquinoline **11**. Treatment of **11** with CH_3I/acetone afforded the quarternary salt **15** which was subjected to ring opening using Na/NH_3 to produce the target compound **20**. In order to synthesize the 9,10-dimethoxy-3-benzazonines **21** and

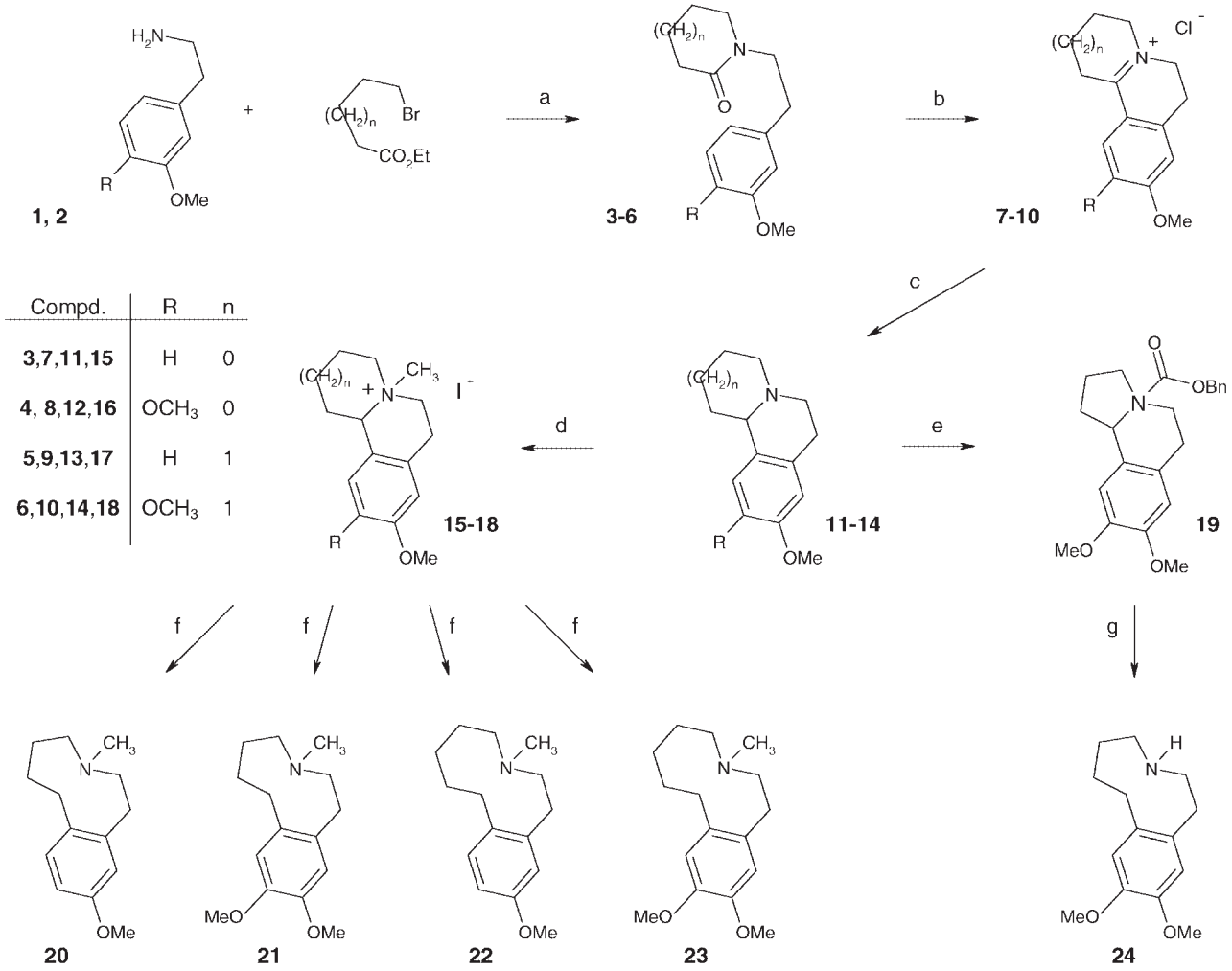

Figure 2. Synthesis of target compounds.

[a] (a) Dioxane, 120 °C, 12 h; (b) $POCl_3$/toluene, reflux, 4 h; (c) $NaBH_4$/MeOH; (d) CH_3I/acetone; (f) Na,NH_3(liq.); (e) BnOCOCl, $NaCNBH_3$; (g) H_2,10 % Pd/C.

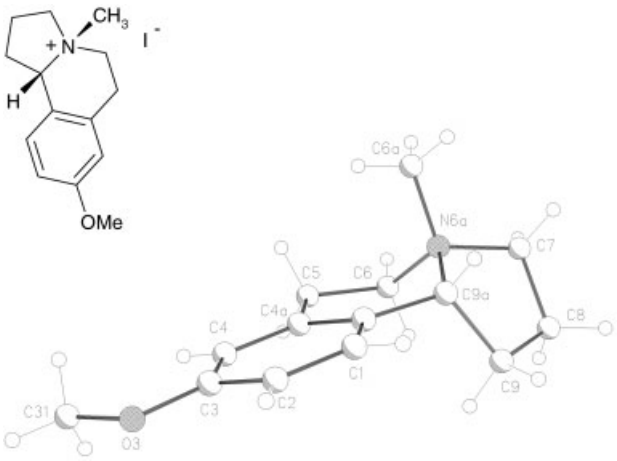

Figure 3. Stick and ball drawing obtained by X-ray from crystals of the *cis*-annelated indolizinium compound **15**.

24, reported procedures were adopted to prepare the required intermediates **8**, **12**, and **16** [11, 12]. Compound **16** was subjected to ring opening yielding the target **21**. Meanwhile, the dimethoxy intermediate **12** was reacted with benzyl chloroformate and sodium cyanoborohydride to give the 3-benzyloxycarbonyl-3-benzazonine analog **19**. Catalytic hydrogenolysis (H_2, Pd/C) and decarboxylation produced the required secondary amine **24**.

In order to produce the homologous azecine series the starting piperidones **5** and **6** [13] were prepared by refluxing **1** and **2**, respectively, with ethyl 5-bromovalerate in dioxane, which gave better yields than the reported procedure using δ-valerolactone [13]. **5** was cyclized using $POCl_3$ in refluxing toluene to yield **9**, which was subsequently reduced into its quinolizine **13**. Treatment of **13** with CH_3I/acetone afforded **17** which was subjected to

Table 1. Percentage decrease of dopamine receptor (D_1, D_{2L}, D_4, and D_5) bound radioactivity by 10 µM solutions of compounds **11**–**14**, **20**–**24**, and **LE 300.**

Compound[a]	D_1	D_{2L}	D_4	D_5
11	–3	–2	n.d.	n.d.
12	–14	–6	0	–14
13	–21	–7	n.d.	n.d.
14	–8	–3	n.d.	n.d.
20	–24	–8	n.d.	n.d.
21	–8	0	–9	–13
22	–9	–5	n.d.	n.d.
23	–0	–5	n.d.	n.d.
24	–4	–4	0	–7
LE 300	–96	–91	–95	–100

n.d.: not determined

C,N cleavage using Na/NH_3 to produce the target compound **22**. The synthesis of **23** necessitated the preparation of some reported intermediates such as **10**, **14**, and **18** [13]. Quinolizines and indolizines can be *cis*- or *trans*-annelated. X-ray analysis of the quarternary salt **15** showed a cis-annelated indolizine (Figure 3). In earlier studies we have confirmed by X-ray investigations the same *cis*-annelated structure for benz-indolo-quinolizines [14].

Pharmacology

All of the "de-indolised" benzazonine and benzazecine target compounds together with their precursors **11**–**14**

Table 2. Affinity of compounds **19**–**21** and **23** towards 5-HT_{2A} receptor at arbitrary concentration of 100 µM, using the rat tail artery assay.

Compound	*n*[a]	PA_2[b] ± *SEM*	E_{max}(5-HT)[c] ± *SEM* (%)	*c* (µM)[d]
19	5	<4	98 ± 2	100
20	5	5.09 ± 0.02	85 ± 5	100
21	5	5.48 ± 0.05	98 ± 4	100
23	5	4.13 ± 0.07	97 ± 1	100
LE 300 [8]	22	8.32 ± 0.02	89–98	0.01–1
Ketanserin [13]	36	9.55 ± 0.02	88–91	0.00047–0.047

[a] Number of experiments. [b] Absolute affinity of the tested compounds and positive controls. [c] Relative maximum effect of 5-HT in presence of antagonist. [d] Micromolar concentrations of the tested compounds and positive controls.

and the "indolised" reference LE 300 were screened as ligands for the human dopamine receptor family (D_1, D_{2L}, D_4, and D_5) adopting the radioligand binding studies described by Mierau et al. [15]. The results are given in Table 1 and they clearly demonstrate the absence of any affinity, with the expected exception of LE 300. In addition the compounds were subjected to rat tail artery assay [16–18] to evaluate their potency as 5-HT_{2A} receptor ligands. Only the benzazonine derivatives **20**, **21** and the benzazecine **23** showed moderate antagonistic activities toward 5-HT_{2A} receptor with PA_2 values of 5.09, 5.48, and 4.13, respectively (Table 2). None of the tested compounds showed activity comparable to the lead LE 300 (PA_2 = 8.32) or the positive control ketanserin (PA_2 = 9.55) [16]. Thus the replacement of the indole moiety in LE 300 decreased the binding affinity not only for dopamine receptors but also toward the 5-HT_{2A} dramatically. The results also show that the existence of either a monomethoxy or a dimethoxy groups on the benzene ring did not really contribute to the binding potential (**20**, PA_2 5.09 versus **21**, PA_2 5.48). The benzazonine ring system in **20** and **21** favors the activity rather than the benzazecine ring (**23**, PA_2 4.13). The obtained results will set the parameters for the future derivatization and modification of the lead compound LE 300.

Acknowledgements

The Alexander von Humboldt foundation fellowship awarded to Professor Dr. Hussein El-Subbagh is greatly appreciated.

Experimental

Chemistry

Melting points were determined in open capillaries on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC 400 (400 MHz) spectrometer, chemical shifts are given in δ (ppm) values downfield from Me_4Si as an internal standard. Elemental analyses (C, H, N) were carried out at the Institute of Pharmacy, University of Bonn, Germany, results were within –0.4 % of the theoretical values. Mass spectrometry were carried on MS-30 and MS-50 instruments of A.E.I., Manchester, England. Thin layer chromatography (TLC) was performed on Merck 5 × 10 cm plates, precoated with silica gel GF_{254}. Chromatographic separations were carried out either on gravity column or on a Chromatotron model No. 7924T, Harrison Research, Palo Alto, CA, USA. Compounds **4** [9], **8**, **12** [9, 10], **16** [10], **6**, **10**, **14**, **18** [11] were previously reported. [^{3}H]-Spiperone was purchased from Amersham, UK, with a specific activity of 97.0 Ci/mmol. [^{3}H]-SCH 23390 was also purchased from Amersham, UK, with a specific activity of 83.0 Ci/mmol. Prazosin, ketanserin, haloperidol, and fluphenazine were purchased from Sigma-Aldrich, Germany.

N-[2-(3-Methoxyphenyl)ethyl]-2-pyrrolidone (**3**)

A mixture of 3-methoxyphenethylamine (**1**, 9.83 g, 65 mmol) and ethyl 4-bromobutyrate (5.85 g, 30 mmol) in dioxane (100 mL) was heated under reflux for 36 h. The reaction mixture was cooled and evaporated in vacuo. EtOAc (50 mL) was added and the precipitated amine hydrobromide (6.6 g) was filtered off. The filtrate was evaporated under reduced pressure and the obtained residue was chromatographed on a silica gel column (20 % hexane/EtOAc) to produce 6.4 g (97 % yield) of **3**. ^{1}H NMR ($CDCl_3$): δ 1.9–2.15 (m, 2 H, CH_2), 2.32–3.5 (m, 2 H, CH_2), 2.72–2.95 (t, *J* Hz, 2 H, CH_2), 3.24–3.41 (t, *J* Hz, 2 H, CH_2), 3.47–3.69 (m, 2 H, CH_2), 3.85 (s, 3 H, OCH_3), 6.72–6.96 (m, 3 H, ArH), 7.42 (s, 1 H, ArH). *m/z* (219.1; 45 %). Anal. ($C_{13}H_{17}NO_2$) C, H, N.

8-Methoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-a]isoquinoline (**11**)

A solution of **3** (3 g, 13.7 mmol) and $POCl_3$ (4 mL) in dry toluene (50 mL) was heated under reflux for 4 h, and then allowed to cool. Dry ether (100 mL) was added and the obtained oily product (**7**) was separated and used for the next reaction without further purification. The oily salt was dissolved in methanol (50 mL), then 7 g of $NaBH_4$ was added in portions with stirring for 2 h at room temperature. Methanol was evaporated under reduced pressure and water (50 mL) was added. The resulted emulsion was extracted with ether, separated, dried, and evaporated to give 2.4 g (86 % yield) of **11**. ^{1}H NMR ($CDCl_3$): δ 0.8–3.5 (m, 11 H, $5CH_2$ + CH), 3.85 (s, 3 H, OCH_3), 6.5–7.3 (m, 3 H, ArH). *m/z* (203.1; 51 %). Anal. ($C_{13}H_{17}NO$) C, H, N.

4-Methyl-8-methoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-a]-isoquinolinium iodide (**15**)

A solution of **11** (2.4 g, 11.8 mmol) in dry acetone (30 mL) was treated with iodomethane (4 mL) and allowed to stand at room temperature for 12 h. The precipitate obtained was recrystallized from ethanol to afford **15**, the methiodide salt of **11** (3 g, 74 % yield), mp 234–235 °C. ^{1}H NMR (DMSO-d_6): δ 1.9–4.0 (m, 10 H $5CH_2$), 3.3 (s, 3 H, NCH_3), 3.88 (s, 3 H, OCH_3), 4.6–4.9 (m, 1 H, CH), 6.85–7.3 (m, 3 H, ArH). Anal. ($C_{14}H_{20}INO$) C, H, N.

10-Methoxy-3-methyl-2,3,4,5,6,7-hexahydro-1H-3-benzazonine (**20**)

A mixture of **15** (1.0 g, 2.9 mmol) and liquified NH_3 (30 mL) was stirred at –40 °C for 1 h during which Na metal (2.0 g) was added portionwise. The reaction was finally quenched by the addition of a saturated aqueous solution of NH_4Cl and stirring was continued at room temperature for 12 h. H_2O (20 mL) was then added and the resulting emulsion was extracted with ether (100 mL). The ethereal extract was washed with 5 % NaOH solution (30 mL) and then H_2O (30 mL), separated, dried, and evaporated to give **20** (0.5 g, 78 % yield). ^{1}H NMR ($CDCl_3$): δ 0.9–1.3 (m, 2 H, CH_2), 1.5–1.9 (m, 2 H, CH_2), 2.1–3.2 (m, 8 H, CH_2), 2.3 (s, 3 H, NCH_3), 3.85 (s, 3 H, OCH_3), 6.5–7.1 (m, 3 H, ArH). *m/z* (219.2, 83 %). Anal. ($C_{14}H_{21}NO$) C, H, N.

9,10-Dimethoxy-3-methyl-2,3,4,5,6,7-hexahydro-1H-3-benzazonine (**21**)

A mixture of **16** (1.0 g, 2.6 mmol) and liquified NH_3 (g) (30 mL) was stirred at –40 °C for 1 h during which Na metal (2.0 g) was added in portions and the procedure continued as mentioned under **20** to give **21** (0.4 g, 62 %). ^{1}H-NMR ($CDCl_3$): δ 1.05–1.12 (m, 2 H, CH_2), 1.64–1.71 (m, 2 H, CH_2), 2.30 (s, 3 H, N-CH_3), 2.35–2.4 (m, 2 H, CH_2), 2.44–2.47 (m, 2 H, CH_2), 2.61–2.64 (m, 2 H, CH_2), 2.90–2.94 (m, 2 H, CH_2), 3.78 (s, 6 H, OCH_3), 6.47 (s, 1 H, ArH), 6.56 (s, 1 H, ArH). *m/z* (249.2, 50 %). Anal. ($C_{15}H_{23}NO_2$) C, H, N.

3-Benzyloxycarbonyl-9,10-dimethoxy-2,3,4,5,6,7-hexahydro-1H-3-benzazonine (**19**)

A solution of **12** (2.3 g, 10 mmol) in dry THF (30 mL) was cooled to –70 °C. Benzyl chloroformate (3.4 g, 2.8 mL, 20 mmol) was added dropwise and stirring continued for 1 h at –70 °C, then $NaCNBH_3$ (0.94 g, 15 mmol) was added at the same temperature and stirring continued for another 1 h. The reaction mixture warmed up to room temperature and stirred for 12 h, then poured into NaOH solution (20 %, 30 mL) and extracted with EtOAc (100 mL). The organic layer was separated, dried, and evaporated. The oily product was chromatographed on a silica gel column (1 : 1 CH_2Cl_2/hexane) to give **19** (1.2 g, 32 % yield). ^{1}H NMR ($CDCl_3$): δ 0.88–0.95 (m, 2 H, CH_2), 1.02–1.16 (m, 2 H, CH_2), 1.25–1.45 (m, 2 H, CH_2), 1.64–1.85 (m, 2 H, CH_2), 2.55–2.65 (m, 2 H, CH_2), 2.80–2.95 (m, 2 H, CH_2), 3.84 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 5.20 (d, 2 H, *J* Hz, CH_2-Ph), 6.59 (s, 1 H, ArH), 6.62 (s, 1 H, ArH), 7.30–7.41 (m, 5 H, ArH). Anal. ($C_{22}H_{27}NO_4$) C, H, N.

9,10-Dimethoxy-2,3,4,5,6,7-hexahydro-1H-3-benzazonine (**24**)

A slurry of **19** (0.5 g, 1.4 mmol) and 250 mg of 10 % Pd/C in methanol (200 mL) was subjected to hydrogenation using a Parr hydrogenator at 50 psi for 3 h. The catalyst was then removed by filtration and the filtrate was evaporated in vacuo to give crude **24**. The oily product was chromatographed on a silica gel column (2 : 1 CH_2Cl_2/hexane) to give **24** as a colourless oil (0.2 g, 63 % yield). ^{1}H NMR ($CDCl_3$): δ 0.88–0.97 (m, 2 H, CH_2), 1.0–1.2 (m, 2 H, CH_2), 1.25–1.5 (m, 2 H, CH_2), 1.65–1.76 (m, 2 H, CH_2), 2.45–2.72 (m, 4 H, CH_2), 3.0–3.15 (m, 1 H, NH), 3.85 (s, 6 H, OCH_3), 6.52 (s, 1 H, ArH), 6.62 (s, 1 H, ArH), Anal. ($C_{14}H_{21}NO_2$) C, H, N.

N-[2-(3-Methoxyphenyl)ethyl]-2-piperidone (**5**)

A mixture of 3-methoxyphenethylamine (**1**, 9.8 g, 65 mmol) and ethyl 5-bromovalerate (6.3 g, 30 mmol) in dioxane (100 mL) was heated under reflux for 36 h and continued as mentioned under **3**. The crude product was chromatographed on a silica gel column (20 % hexane/EtOAc) to produce 6.8 g (97 % yield) of oily **5**. ^{1}H NMR ($CDCl_3$): δ 1.6–1.95 (m, 4 H, CH_2), 2.25–2.5 (m, 2 H, CH_2), 2.65–3.34 (m, 4 H, CH_2), 3.43–3.7 (m, 2 H, CH_2), 3.85 (s, 3 H, OCH_3), 6.62–6.87 (m, 3 H, ArH), 7.13–7.38 (m, 1 H, ArH). *m/z* (233.2, 31 %). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

2-Methoxy-1,3,4,6,7,11b-hexahydro-2H-benzo[a]quinolizine (**13**)

A solution of **5** (6.9 g, 29.6 mmol) and $POCl_3$ (10 mL) in dry toluene (100 mL) was heated under reflux for 4 h and continued as mentioned under **11** to produce **9** which was used for next reaction without further purification. The oily salt **9** was dissolved in methanol (100 mL), then 14 g of $NaBH_4$ were added in portions with stirring over a period of 2 h at room temperature and continued as mentioned under **11** to produce 5.5 g (84 % yield) of **13**. ^{1}H NMR ($CDCl_3$): δ 1.20–3.30 (m, 13 H, CH_2 & CH), 3.85 (s, 3 H, OCH_3), 6.56–6.94 (m, 2 H, ArH), 7.15 (m, 1 H, ArH). *m/z* (217.3, 58 %). Anal. ($C_{14}H_{19}NO$) C, H, N.

9-Methoxy-5-methyl-1,3,4,6,7,11b-hexahydro-2H-benzo[a]-quinolizinium iodide (**17**)

A solution of **13** (4.8 g, 22.0 mmol) in dry acetone (20 mL) was treated with iodomethane (5 mL) and continued as mentioned under **15** to produce 6.0 g (75 % yield) of **17**. ^{1}H-NMR (DMSO-d_6): δ 1.52–2.1 (m, 2 H, CH_2), 2.96–4.3 (m, 10 H, CH_2), 3.42 (s, 3 H, NCH_3), 3.80 (s, 3 H, OCH_3), 4.35–4.66 (m, 1 H, CH), 6.8–7.05 (m, 2 H, ArH), 7.3 (m, 1 H, ArH). Anal. ($C_{15}H_{22}INO$) C, H, N.

11-Methoxy-3-methyl-1,2,3,4,5,6,7,8-octahydro-3-benzazecine (**22**)

A mixture of **17** (1.0 g, 2.8 mmol) and liquified NH_3 (30 mL) was stirred at –40 °C for 1h during which Na metal (2.0 g) was added portionwise and the procedure continued as mentioned under **20** to give **22** (0.4 g, 61 %). ^{1}H NMR ($CDCl_3$): δ 1.18–2.23 (m, 10 H, CH_2), 2.05 (s, 3 H, N-CH_3), 2.6–2.86 (m, 4 H, CH_2), 3.78 (s, 3 H, OCH_3), 6.64–6.78 (m, 2 H, ArH), 7.02–7.1 (m, 1 H, ArH). *m/z* (233.2, 100 %). Anal. ($C_{15}H_{23}NO$) C, H, N.

10,11-Dimethoxy-3-methyl-1,2,3,4,5,6,7,8-octahydro-3-benzazecine (**23**)

A mixture of **18** (1.0 g, 2.7 mmol) and liquified NH_3 (30 mL) was stirred at –40 °C for 1h during which Na metal (2.0 g) was added in portions and the procedure continued as mentioned under **20** to give **23** (0.4 g, 56.2 %). ^{1}H-NMR ($CDCl_3$): δ 0.84–2.03 (m, 10 H, CH_2), 2.10 (s, 3 H, N-CH_3), 2.30–2.85 (m, 4 H, CH_2), 3.85 (s, 6 H, OCH_3), 6.68 (s, 2 H, ArH). *m/z* (263.3, 88 %). Anal. ($C_{16}H_{25}NO_2$) C, H, N.

X-Ray structure analysis of compound **15**

Compound **15** ($C_{14}H_{20}INO$), colorless crystals, crystal dimension 0.20 × 0.35 × 0.45 mm^3; *M* = 663.5; monoclinic, space group $P2_1/c$ (no. 14), *a* = 7.5156(2), *b* = 15.7478(5), *c* = 12.5268(3) Å, β = 105.342(2)°, *V* = 1.42978(7) nm^3, *Z* = 4, μ($Mo_{K\alpha}$) = 2.226 mm^{-1}, *T* = 123(2) K, *F*(000) = 688. 16347 reflection up to $2\theta_{max.}$ = 56.6° were measured on an Nonius-Kappa CCD diffractometer with $Mo_{K\alpha}$ radiation, 3315 of which were independent and used for all calculations. The structure was solved by direct methods and refined to F^2 anisotropically, the H atoms were refined with a riding model. The final quality coefficient *wR*2(F^2) was 0.0475, with a conventional *R*(*F*) = 0.0192 for 156. An empirical absorption correction was applied. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 178049. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223336033; e-mail: deposit@ccdc.cam.ac.uk).

Biological testing

A) 5-HT$_{2A}$ receptor ligand activity

In brief, cylindrical segments of 3–4 mm length were mounted isometrically (initial tension 5 mN) by means of two stainless L-shaped steel hooks (diameter 0.15 mm) in a modified Krebs-Henseleit solution (37 °C) of composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and D-glucose 10.0. The solution was aerated with 95 % O_2/5 % CO_2 and contained prazosin (30 nM) to block α_1 adrenoceptors, and cocaine (6 μM) to block neuronal uptake of amines. During an equilibration period of 120 min the preparations were primed once (after 60 min) with 5-HT (1 and 10 μM) to monitor tissue viability. Two cumulative concentration-effect curves for 5-HT (0.01–30 μM for the first curve) were determined in the absence and presence of potential antagonists which were usually incubated for 30 min or 120 min (LE 300, ketanserin at 0.47 and 1 nM, respectively). Control experiments in the absence of antagonist revealed that for the 30-min protocol, two successive concentration-effect curves for 5-HT were superimposable (data not shown). When the 120-min protocol was applied, a leftward shift of the control curves of approximately 0.1–0.3 logarithmic units was usually observed. The daily mean sensitisation was used to correct the dextral shift measured for treated organs [13–15]. Results are expressed as

mean ± standard error (*SEM* or *SE*) unless otherwise indicated. Antagonist affinity was calculated as apparent pA_2 value according to equation (1) when only one or two antagonist concentrations were used [14]. ([*c*] = mol/L, *r* is the ratio of agonist concentrations in the presence and absence of antagonist that elicit 50 % of the respective maximum effect.)

$$pA_2 = -\log_{10} c(\text{antagonist}) + \log_{10} (r - 1) \quad (1)$$

Full pA_2 values were calculated according to the method of Schild [15] when a set of different antagonist concentrations over at least 1.5 logarithmic units was studied. Single organ preparations were from at least three ($5\text{-}HT_{2A}$) animals.

B) Dopamine receptor ligand activity

i) Cell culture

Human D_1, D_{2L}, D_4, and D_5 receptors were stably expressed in Chinese hamster ovary (CHO) cells as previously described [5]. The density of receptors measured with [^{3}H]-SCH 23390 was 307.15 fmol/mg protein for D_1 and 679.44 fmol/mg for D_5. The density of receptors measured with [^{3}H]-Spiperone was 2021 fmol/mg protein for D_{2L} and 137.21 fmol/mg for D_4. These cells were grown at 37 °C under a humidified atmosphere of 5 % CO_2: 95 % air in HAM/F12-medium (Sigma-Aldrich), supplemented with 10 % fetal bovine serum, 1 mM l-glutamine, 20 U/mL penicillinG, 20 µg/mL streptomycin, and 0.2 µg/mL G 418 (all from Sigma-Aldrich).

ii) Preparation of whole-cell-suspension

Human D_1, D_{2L}, D_4, and D_5 receptor cell lines (CHO) were grown on T 175 culture dishes (Nunc) to 85 % confluency, the medium was removed and the cells were incubated with 6 mL trypsine-EDTA-solution (Sigma-Aldrich) to remove the cells from the culture dish. The resulting suspension was centrifuged (1000 rot/min, 4 °C, 4 min), the pellet resuspended in 10 mL PBS (ice-cooled, calcium- and magnesium-free), pelleted, and this procedure repeated. The resulting pellet was then resuspended in 12 mL buffer (5 mM magnesium chloride, 50 mM TRIS-HCl pH = 7.4) and the resulting suspension was directly used for the radioligand binding assay.

iii) Radioligand binding assay

For the binding studies a procedure according to Mierau et al. was used [16]. The binding assays with the whole-cell-suspension were carried out in triplicate in a volume of 1.1 mL (final concentration): TRIS-Mg^{2+}-buffer (690 µL), [^{3}H]-ligand (100 µL), whole-cell-suspension (200 µL) and appropriate drugs (110 µL). Non-specific binding was determined using fluphenazine (100 µM) in D_1 and D_5 tests and haloperidol (10 µM) in D_2 and D_4 tests. For a fast screening' the drugs were used in a concentration of 100 µM, and the percentage of removed radioligand determined. The incubation was initiated by addition of the radioligand. The incubations were carried out at 27 °C for 2 h, and stopped by rapid filtration through a glass fiber filter (Schleicher and Schüll, Germany), previously treated with 0.25 % polyethyleneimine solution (Sigma-Aldrich), which was washed twice with ice-cold water. The radioactivity retained on the filters was counted using a Beckman LS 6000 SC scintillation counter. The competition binding data was analysed by the software GraphPad Prism™ using non-linear least squares fit.

References

[1] Part III: A. H. Abadi, S. Lankow, B. Höfgen, M. Decker, M. U. Kassack, J. Lehmann, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 367–373.

[2] E. Zifa, G. Fillion, *Pharmacol. Rev.* **1992**, *44*, 401–457.

[3] L. J. Siever, R. S. Khan, B. A. Lawlor, R. L. Trestman, T. L. Lawrence, E. F. Corraco, *Pharmacol. Rev.* **1991**, *43*, 509–525.

[4] D. Hoyer, D. E. Clarke, J. R. Fozard, P. R. Hartig, G. R. Martin, E. J. Mylecharane, P. R. Saxena, P. P. A. Humphrey, *Pharmacol. Rev.* **1994**, *46*, 157–203.

[5] H. Anderson, B. Nielsen, *Antipsychotics. DN&P* **1991**, *4*, 150–157.

[6] P. Sokoloff, B. Giros, M. Martres, M. Bouthenet, J. Schwartz, *Nature* **1990**, *347*, 146–151.

[7] H. VanTol, J. Bunzow, H. Guan, R. Sunahara, P. Seemann, H. Niznik, O. Civelli, *Nature* **1991**, *350*, 610–614.

[8] H. Hara, M. Osakabe, A. Kitajima, Y. Tamao, R. Kikumoto, *Thromb. Haemost.* **1991**, *65*, 415–420.

[9] T. Witt, F. J. Hock, J. Lehmann, *J. Med. Chem.* **2000**, *43*, 2079–2081.

[10] S. A. F. Rostom, A. M. Farghaly, F. S. G. Solimann, M. M. El-Semary, S. Elz, J. Lehmann, *Arch. Pharm. Pharm. Med. Chem.* **2001**, *334*, 241–247.

[11] K. Wiesner, Z. Valenta, A. J. Manson, F. W. Stonner, *J. Am. Chem. Soc.* **1955**, *77*, 675–83.

[12] J. B. Bremner, K. N. Winzenberg, *Austr. J. Chem.* **1984**, *37*, 1203–15.

[13] J. B. Bremner, K. N. Winzenberg, *Austr. J. Chem.* **1985**, *38*, 1591–612.

[14] J. Lehmann, M. Nieger, T. Witt, *Heterocycles* **1994**, *38*, 511–528.

[15] J. Mierau, F. J. Schneider, H. A. Ensinger, C. L. Chio, M. E. Lajiness, R. M. Huff, *Mol. Pharmacol.* **1995**, *290*, 29–36.

[16] H. Pertz, S. Elz, *J. Pharm. Pharmacol.* **1995**, *47*, 310–316.

[17] R. F. Furchgott in *Catecholamines, Handbook of Experimental Pharmacology, Vol. 33* (Eds.: H. Blaschko, E. Muscholl), Springer-Verlag, Berlin, **1972**, 283–335.

[18] O. Arunlakshana, H. O. Schild, *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48–58.