Full Paper

Novel Fused Pyrrole Heterocyclic Ring Systems as Structure Analogs of LE 300: Synthesis and Pharmacological Evaluation as Serotonin 5-HT_{2A}, Dopamine and Histamine H_1 Receptor Ligands*

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LE 300 represents a structurally novel type of antagonists acting preferentially at the dopamine D_1/D_5 receptors and the serotonin 5-HT_{2A} receptor. This compound consists of a ten-membered central azecine ring fused to an indole ring on one side and a benzene moiety on the other side. To estimate the importance of the indole and / or phenyl moieties in this highly active benzindolo-azecine, both rings were removed and replaced with a 1*H*-pyrrole counterpart. Accordingly, some new analogs of **LE 300** namely, pyrrolo[2,3-*g*]indolizine, pyrrolo[3,2-*a*]quinolizine rings and their corresponding dimethylpyrrolo[2,3-*d*]azonine, and dimethylpyrrolo[2,3-*d*]azecine were synthesized to be evaluated for their activity at the 5-HT_{2A} and dopamine D_1, D_{2L}, D_4, D_5 receptors in relation to **LE 300**. In addition, their activity at the H_1-histamine receptors was also determined. The results suggested that the rigid pyrrolo[2,3-*g*]indolizine **7** and pyrrolo[3,2-*a*]quinolizine **8** analogs lacked biological activity in the adopted three bioassays. However, their corresponding flexible pyrrolo[2,3-*d*]azonine **11** and pyrrolo[2,3-*d*]azecine **12** derivatives revealed weak partial agonistic activity and weak antagonistic potency at the serotonin 5-HT_{2A} and histamine H_1 receptors, respectively. Meanwhile, they showed no affinity to any of the four utilized dopamine receptors. Variation in ring size did not contribute to a significant influence on the three tested bioactivities. Removal of the hydrophobic moiety (phenyl ring) and replacement of the indole moiety with a 1*H*-pyrrole counterpart led to a dramatic alteration in the profile of activity of such azecine-type compounds.

Keywords: Azecine / Azonine / Indolizine / 1*H*-Pyrrole / Quinolizine

Received: September 12, 2009; accepted: November 3, 2009

DOI 10.1002/ardp.200900219

Introduction

Over the past two decades, much evidence has been accumulated on the biological importance of serotonin (5-hydroxytryptamine, 5-HT) as an important neurotransmitter in the central and peripheral nervous systems (CNS and PNS, respectively). 5-HT has been reported to modulate the regulation of a variety of important physiological functions including affective behavior, memory, and thermo-regulation via the interaction at serotonin receptor subtypes [1]. At least 14 distinct serotonin receptor subtypes are expressed in the mammalian CNS, each of which is assigned to one of seven families, namely 5-

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Abbreviation: 5-Hydroxytryptamine (5-HT)

* Dedicated to Prof. Dr. Jochen Lehmann, Institute of Pharmacy, Pharmaceutical Medicinal Chemistry, Friedrich-Schiller-University Jena, Germany, on the occasion of his 65th birthday.

HT_1 to 5-HT_7 [2]. Among these, the 5-HT_{2A} receptor subtype is a member of the G-protein-coupled receptor (GPCR) superfamily, and has been implicated in working memory, cognitive processes, affective disorders such as schizophrenia, and mediates the primary effects of hallucinogenic drugs [3–5]. In addition, many peripheral tissues also express the 5-HT_{2A} receptor as they are involved in platelet function, and induce contraction within the vasculature [6]. 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 [7].

On the other hand, dopamine-receptor-mediated neurotransmission is known to play an important role in regulating neuronal motor control, cognition, event prediction, emotion, and vascular function. Neuropsychiatric diseases such as schizophrenia, Parkinson's disease, or addiction are strongly related to a disregulation of the dopaminergic signal transduction [8, 9]. There are five dopamine receptor subtypes that may be divided into two subfamilies: the G_s-coupled D_1-like receptors (D_1, D_5) and the G_i-coupled D_2-like receptors (D_2, D_3, D_4) [10]. Because of a lack of highly selective D_1- and D_5-receptor ligands, knowledge about the physiological impact of activation or antagonism of these single receptors, especially the D_5 receptor, is strictly limited or nonexistent [11]. The D_5 receptor is of special interest, because it is, in contrast to the D_1 receptor, directly coupled to the $GABA_A$ receptor, enabling inhibitory functional interactions [12].

The observation that risperidone and clozapine can act as mixed D_2/5-HT_{2A} receptor antagonists has led to the so-called "atypical neuroleptics" which are distinguished from the old traditional neuroleptics in their enhanced efficacy in treating the negative symptoms of schizophrenia and related psychoses, and their decreased liability for causing extra-pyramidal system (EPS) side effects [13, 14].

The lead compound LE 300 (7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (Fig. 1) introduced by Lehmann *et al*. [15], revealed a subnanomolar affinity towards the rat striatal D_1 receptor and high functional activity at the 5-HT_{2A} receptor [15–17]. The idea behind the synthesis of such a heterocyclic system was to incorporate the substructures of tryptamine and β-phenylethylamine moieties into a moderately constrained ten-membered semi-rigid azecine ring [15]. Intensive SAR studies were performed within this novel class of dopamine receptor ligands, including variations in ring size [18], de-indolization of the structure [19], the insertion of an additional oxygen atom into the alicyclus

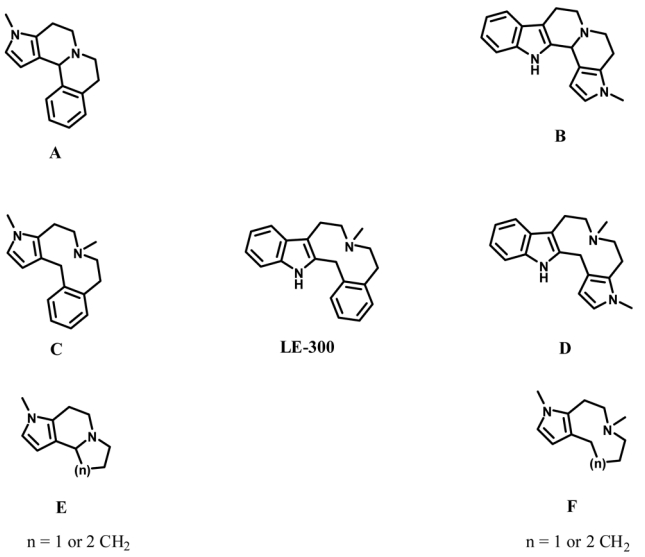


Figure 1. Structures of **LE-300**, relevant compounds **A–D**, and targeted analogs **E** and **F**.

[20, 21] and changing one of the aromatic moieties (indole replaced by benzene, thiophene, and 1-methyl-1*H*-pyrrole, respectively) and its location with respect to each other at the central alicyclic ring [22]. Such "azecine-type" dopamine receptor antagonists derived from LE 300 and its dibenzo-analogues were found to show nanomolar, subnanomolar, and even picomolar [23] affinities predominantly for the D_1 and D_5 subtypes.

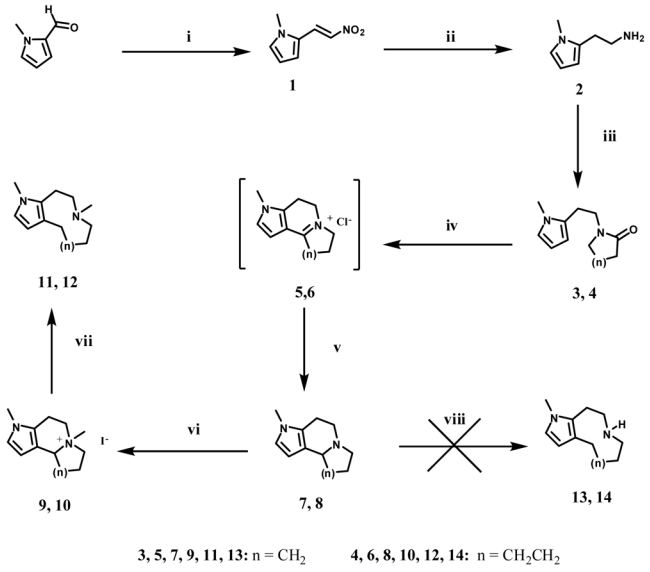
In the present study, and in continuation of the interest in azecine-type compounds [20, 24], some new analogs of LE 300, namely pyrrolo[2,3-*g*]indolizine, pyrrolo[3,2-*a*]quinolizine ring systems and their corresponding dimethylpyrrolo[2,3-*d*]azonine, and dimethylpyrrolo[2,3-*d*]azecine with general structures **E** and **F**, respectively, were synthesized to be evaluated for their activity at the 5-HT_{2A} and dopamine D_1, D_{2L}, D_4, D_5 receptors. The newly designed compounds were deprived of both the indole and aryl moieties present in LE 300, while replaced with a 1-methyl-1*H*-pyrrole counterpart together with a constriction of the ten-membered azecine to the nine-membered azonine ring, in an attempt to estimate the influence of such a structure variation on the anticipated biological activities. The activities of the newly synthesized intermediate and target compounds at the 5-HT_{2A} and dopamine D_1, D_{2L}, D_4, D_5 receptors were compared with the previously reported structure analogs **A–D** [24] (Fig. 1), whose dopaminergic binding profile was not reported before. Furthermore, owing to the structure similarity of such β-phenylethylamine-like compounds with histamine, and the reported H_1-antihistaminic activity of the structure analogs **A–D** [24], it was considered

worthwhile to investigate and compare the activity of the target compounds at the H_1-histamine receptors with those of their analogs **A–D** (Fig. 1).

Results and discussion

Chemistry

The synthetic strategies adopted to obtain the intermediate and target compounds are depicted in Scheme 1. The key intermediate amine **2**, namely 2-(2-aminoethyl)-1-methyl-1*H*-pyrrole, was prepared according to a previously described procedure [24]. It involved condensation of 1-methyl-1*H*-pyrrol-2-carbaldehyde with nitromethane in the presence of piperidinium acetate to yield the 1-methyl-2-(2-nitoethenyl)-1*H*-pyrrole **1**, which, in turn, was reduced to the amine **2** using lithium aluminium hydride. The amine **2** was condensed under nitrogen with either ethyl 4-bromobutanoate or ethyl 5-bromopentanoate in the presence of potassium carbonate and traces of potassium iodide to produce the lactams **3** and **4**, respectively. Cyclization of these lactams to the corresponding pyrrolo[2,3-*g*]indolizine **7** and pyrrolo[3,2-*a*]quinolizine **8** ring systems was performed according to a modified procedure of Lehmann *et al*. [25]. It comprised internal cyclization of the lactams **3** and **4** with phosphorous oxychloride according to Bischler–Napieralski reaction conditions [26–28] followed by *in-situ* reduction of the resulting indolizinium **5** and quinolizinium **6** salts with sodium borohydride. The targeted dimethylpyrrolo[2,3-*d*]azonine **11** and dimethylpyrrolo[2,3-*d*]azecine **12** were obtained by initial quaternization of **7** and **8** using methyl iodide, followed by the reductive cleavage of the corresponding methiodides **9** and **10**, with sodium metal in liquid ammonia at −78°C, according to Birch-reduction conditions for the cleavage of internal C-N bonds [29]. At this stage, it was considered worthwhile to synthesize the secondary amine analogues **13** and **14** as interesting structure variants via quaternization of compounds **7** and **8** with benzyl chloroformate in the presence of sodium cyanoborohydride followed by catalytic hydrogenolysis and decarboxylation according to a previously described procedure [19]. Unfortunately, trials to isolate the expected intermediate carbamate salts were unsuccessful due to their high instability.



Reagents and reaction conditions: i: CH_3NO_2, piperidine, reflux, 30 min; **ii:** $LiAlH_4$, THF, NaOH, reflux, 48h; **iii:** ethyl 4-bromobutanoate or ethyl 5-bromopentanoate, K_2CO_3, ethanol, reflux, 48h; **iv:** $POCl_3$, toluene, reflux, 4h; **v:** $NaBH_4$, MeOH, r. t., 18h; **vi:** CH_3I, K_2CO_3, acetone, r. t., 18h; **vii:** Na metal, liquid NH_3, -78 °C; **viii**: a) BnOCOCl, $NaCNBH_3$, THF, -70 °C, b) H_2, 10% Pd/C.

Scheme 1. Synthesis of compounds **1–12**.

Pharmacology

In-vitro serotonin 5-HT$_{2A}$ receptor-activity screening using the rat-tail artery model

Owing to the wide distribution of the serotonin 5-HT_{2A} receptor subtype in many peripheral tissues mediating contractile responses of various vascular, gastrointestinal tract (GIT), and uterine smooth muscles, the tested compounds were subjected to the rat-tail artery bioassay [30–32] to evaluate their potency as 5-HT_{2A} receptor ligands. The experiment aimed to assess the activity of the newly synthesized compounds (competitive antagonists, full or partial agonists), in relation to the previously reported analogs **A–D** [24], the structure lead LE-300 (Fig. 1), and ketanserin (a reference 5-HT_{2A} antagonist). The results depicted in Table 1 revealed that the pyrrolo[2,3-*g*]indolizine **7** and pyrrolo[3,2-*a*]quinolizine **8** derivatives were devoid of any activity at this receptor site (pA_2 values < 4.00). On the other hand, the corresponding pyrrolo[2,3-*d*]azonine **11** and pyrrolo[2,3-*d*]azecine **12** compounds showed partial agonistic activity (pA_2 values 4.08 and 4.01, respectively), when compared with their structurally relevant analogs **C** and **D** which showed remarkable antagonistic activity (pA_2 values 8.01 and 6.13, respectively), the lead LE 300, and ketanserin (the positive control) with pA_2 values of 8.32 and 9.55, respectively.

Dopamine-receptor ligand-binding activity

The newly synthesized target compounds **7**, **8** and **11**, **12** together with their structure analogs **A–D** (which were not previously investigated for this particular activity) and the reference LE 300 were preliminarily screened as ligands for the human dopamine receptor family (D_1, D_{2L},

Table 1. Affinity of the tested compounds towards serotonin 5-HT_{2A} receptor using the rat-tail artery assay.

Compound	c (μM)[a]	N[b]	pA_2[c] ± SEM	E_{max}(5-HT)[d] ± SEM (%)	Description of activity
7	100	3	<4.00	n.d.[e]	inactive
8	100	3	<4.00	n.d.[e]	inactive
11	100	4	4.08 ± 0.06	94 ± 4	partial agonist
12	100	4	4.01 ± 0.10	99 ± 7	partial agonist
A*	100	4	4.49 ± 0.10	70 ± 4	partial agonist
B*	1.0	6	6.74 ± 0.06	93 ± 2	antagonist
C*	0.01–1.0	20	8.01 ± 0.03	98 ± 2	antagonist
D*	10	6	6.13 ± 0.05	99 ± 7	antagonist
LE-300**	0.1–1.0	22	8.32 ± 0.02	89–98	antagonist
Ketanserin**	$4.7 \cdot 10^{-4}$ to $4.7 \cdot 10^{-2}$	36	9.55 ± 0.02	88–91	reference antagonist

[a] Micromolar concentrations of the tested compounds and positive controls.
[b] Number of experiments.
[c] Absolute affinity of the tested compounds and positive controls.
[d] Relative maximum effect of 5-HT in the presence of the antagonist.
[e] Not determined.
* Reference [24].
** Reference [19].

Table 2. Percentage decrease of dopamine receptor (D_1, D_{2L}, D_4 and D_5)-bound radioactivity by 10 μM solutions of the tested compounds.

Compound	D_1	D_{2L}	D_4	D_5
7	−5	−7	0	−10
8	−6	−5	−8	−7
11	−6	−7	−17	−9
12	0	−4	−19	−11
A	−14	−14	−11	−11
B	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]
C	−95	−74	−61	−93
D	−87	−46	−51	−56
LE-300*	−96	−91	−95	−100

[a] Not determined.
* Reference [19].

D_4, and D_5) adopting the radioligand binding assay described by Mierau *et al*. [33].

The results are given in Table 2 and they clearly demonstrate the absence of affinity of the target compounds **7**, **8**, **11**, and **12** to all the four tested dopamine receptor types, when compared with the reference lead compound LE 300. However, structurally relevant, fused azecine analogs **C** and **D** revealed moderate to high affinity towards dopamine D_1 and D_5 receptors, whereas their fused quinolizine precursors **A** and **B** lacked affinity to all the tested dopamine receptor types. Out of the tested compounds **A**–**D**, a potential high-activity profile was exhibited by the benzo[*d*]pyrrolo[3,2-*g*]azecine **C** which showed a remarkable high-affinity profile comparable with that of the lead compound LE 300, particularly at the D_1 and D_5 receptors (Table 2).

Table 3. Affinity of the tested compounds towards histamine H_1 receptor using the guinea-pig ileum assay.

Compound	c (μM)[a]	N[b]	pA_2[c] ± SEM	Description of activity
7	100	3	<4.00	inactive
8	100	3	<4.00	inactive
11	100	4	4.01 ± 0.10	weak antagonist
12	100	4	4.03 ± 0.07	weak antagonist
A*	10–100	8	5.49 ± 0.04	antagonist
B*	10	4	5.31 ± 0.09	antagonist
C*	0.1–10	9	8.01 ± 0.03	antagonist
D*	10	5	5.46 ± 0.16	antagonist
LE-300*	0.1–10	25	7.24 ± 0.02	antagonist
Ketanserin*	0.01–1.0	23	8.85 ± 0.08	antagonist
Mepyramine*	$3.0 \cdot 10^{-4}$ to 0.1	29	9.07 ± 0.04	reference antagonist

[a] Micromolar concentrations of the tested compounds and positive controls.
[b] Number of experiments.
[c] Absolute affinity of the tested compounds and positive controls.
* Reference [24].

In-vitro histamine H_1 receptor activity

The newly synthesized target compounds **7**, **8** and **11**, **12** were further evaluated for their activity as histamine H_1 receptor ligands using the guinea-pig ileum segments bioassay [32]. The experiment contributes to the contractile response caused by histamine on the smooth muscles of the ileum and aimed to assess the activity of the newly synthesized compounds (competitive antagonists, full or partial agonists), in relation to the previously reported analogs **A**–**D**, the structure lead LE-300, and mepyramine, a reference H_1-histamine antagonist.

The results recorded in Table 3 declared that the pyrrolo[2,3-*g*]indolizine **7** and pyrrolo[3,2-*a*]quinolizine **8**

derivatives proved to be totally inactive at this receptor site (pA_2 value < 4.00). The target azonine **11** and azecine **12** analogs, instead, showed weak antagonistic activity (pA_2 values 4.01 and 4.03, respectively), when compared with their structurally relevant analogs **C** and **D** which showed remarkable antagonistic activity (pA_2 values 8.01 and 5.46, respectively). It should be noted that the benzo[*d*]pyrrolo[3,2-*g*]azecine **C** showed distinctive antagonistic activity (pA_2: 8.01), which was more potent than the reference lead compound LE 300 (pA_2: 7.24) and comparable with that of mepyramine (pA_2: 9.07), the reference H_1-histamine antagonist used in this assay.

Collectively, the obtained data showed that the rigid pyrrolo[2,3-*g*]indolizine **7** and pyrrolo[3,2-*a*]quinolizine **8** analogs lacked biological activity in the adopted three bioassays. However, their corresponding flexible pyrrolo[2,3-*d*]azonine **11** and pyrrolo[2,3-*d*]azecine **12** derivatives revealed weak partial agonistic activity and weak antagonistic potency at the serotonin 5-HT_{2A} and histamine H_1 receptors, respectively. Meanwhile, they showed no affinity to any of the four utilized dopamine receptors.

Compared with the previously described analogs **A**–**D** (Fig. 1), while the target rigid structures **7** and **8** were deprived of any biological activity, their structure congeners benzo[*a*]pyrrolo[2,3-*h*]quinolizine **A** and indolo[2,3-*a*]pyrrolo[2,3-*h*]quinolizine **B** exhibited an observable profile of bioactivities at the serotonin 5-HT_{2A} and histamine H_1 receptors. Meanwhile, the flexible analogs **11** and **12** exhibited a weaker activity profile at the same receptor sites when compared with their structure analogs benzo[*d*]pyrrolo[3,2-*g*]azecine **C** and indolo[3,2-*d*]pyrrolo[3,2-*g*]azecine **D** and the structure lead LE 300 (Tables 1 and 3). On the other hand, none of the tested compounds was able to show affinity to any of the utilized dopamine receptors (D_1, D_{2L}, D_4, and D_5) in the radioligand binding assay, except for the analog **C** which showed a significant dopamine receptor binding affinity comparable with that of the reference lead compound LE 300, particularly at the D_1 and D_5 receptor sites (Table 2).

Based on the above-mentioned results, it could be clearly concluded that variation in ring size (9- or 10-membered rings) did not contribute to a significant influence on the three tested bioactivities. Moreover, removal of the hydrophobic moiety (phenyl ring) and replacement of the indole moiety with a 1*H*-pyrrole counterpart led to a dramatic decrease in the profile of activity of such azecine-type compounds. These results are concordant with the previous findings of Lehmann *et al.*, who stated that the presence of two aromatic rings annulated to a central 9- or 10-membered azonine or azecine ring systems are indispensable for high affinities at the dopamine and/or serotonin receptors [19]. Consequently, the obtained results will set the parameters for the future derivatization and modification of the lead compound LE 300.

Experimental

Chemistry

Melting points were determined in open capillaries on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK) and are uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer FTIR "Paragor 1000" infrared spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) using the KBr disc technique. ^{1}H-NMR spectra were recorded on Bruker AC400 (400 MHz) spectrometer (Bruker Bioscience, USA), chemical shifts are given in δ (ppm) using tetramethylsilane as an internal standard and $CDCl_3$ as a solvent. Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. 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. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck, Germany) and the spots were detected by exposure to UV-lamp at λ 254. Chromatographic separations were carried out on gravity column using silica gel S (0.2–0.5 mm) for column chromatography. The synthesis of the starting 1-methyl-2-(2-nitoethenyl)-1*H*-pyrrole **1** and 2-(2-aminoethyl)-1-methyl-1*H*-pyrrole **2**, was performed according to a reported literature procedure [24].

*N-[2-(1-Methyl-1H-pyrrol-2-yl)ethyl]-2-pyrrolidone and N-[2-(1-methyl-1H-pyrrol-2-yl)ethyl]-2-piperidone **3, 4***

Under nitrogen atmosphere, a mixture of 2-(2-aminoethyl)-1-methyl-1*H*-pyrrole (**2**, 3.73 g, 30 mmol) and ethyl 4-bromobutanoate or ethyl 5-bromopentanoate (20 mmol), anhydrous potassium carbonate (4.1 g, 30 mmol) and few crystals of potassium iodide in absolute ethanol (100 mL) was heated under reflux for 48 h. After evaporating the solvent to dryness *in vacuo*, the residue was treated with water and extracted several times with chloroform. The organic extract was washed three times with a saturated solution of sodium chloride, then thoroughly washed with water, dried over anhydrous sodium sulphate, and filtered. Rotary evaporation of chloroform afforded a creamy white solid residue which was crystallized from toluene/petroleum ether (60:80) (3:1).

*Compound **3***

M.p.: 92–94°C; yield: 2.15 g (56%); IR (cm^{-1}) ν: 1630 (C=O); ^{1}H-NMR δ: 1.84–2.16 (m, 2H, CH_2), 2.36 (t, *J* = 7 Hz, 2H, CH_2), 2.80 (t, *J* = 7 Hz, 2H, CH_2), 3.20–3.50 (m, 4H, 2 CH_2), 3.60 (s, 3H, CH_3), 5.92–6.12 (m, 2H, pyrrole C_3-H and C_4-H), 6.60 (d, *J* = 7 Hz, 1H, pyrrole C_5-H). Anal. calculated for $C_{11}H_{16}N_2O$ (192.26): C, 68.72; H, 8.39; N, 14.57. Found: C, 68.59; H, 8.47; N, 14.41.

*Compound **4***

M.p.: 105–107°C; yield: 3.5 g (85%); IR (cm^{-1}) ν: 1630 (C=O); ^{1}H-NMR δ: 1.60–2.04 (m, 4H, 2 CH_2), 2.20–2.50 (m, 2H, CH_2), 2.84 (t, *J* = 7 Hz, 2H, CH_2), 3.04–3.28 (m, 2H, CH_2), 3.36–3.72 (m, 5H, CH_2 and CH_3), 5.84–6.12 (m, 2H, pyrrole C_3-H and C_4-H), 6.50 (d, *J* = 7

Hz, 1H, pyrrole C_5-H). Anal. calculated for ($C_{12}H_{18}N_2O$) (206.28): C, 69.87; H, 8.80; N, 13.58. Found: C, 70.04; H, 8.67; N, 13.42.

4,5,7,8,9,9a-Hexahydro-3-methyl-3H-pyrrolo[2,3-g] indolizine and 3,4,5,7,8,9,10,10a-octahydro-3-methylpyrrolo[3,2-a]quinolizine ***7, 8***

Under anhydrous conditions, a mixture of the appropriate lactam **3** or **4** (20 mmol) and phosphorous oxychloride (10 mL) was heated under reflux for 4 h and then allowed to cool. Dry ether (100 mL) was added where an oily product was separated. Excess solvent was evaporated under reduced pressure to afford the intermediate oily salts (**5**, **6**) which were used for the next reaction *in situ* without further purification. The residue was dissolved in methanol (50 mL) and then 7 g of sodium borohydride were added in portions with stirring for 2 h while keeping cool at 0°C. Stirring was continued for further 18 h at room temperature, then, methanol was evaporated *in vacuo* and the residue was treated with water (100 mL). The resulting emulsion was extracted thoroughly with ether, and the ethereal extract was washed with a saturated solution of sodium chloride, then thoroughly washed with water, dried over anhydrous sodium sulphate, and filtered. Evaporation of excess ether under reduced pressure afforded a yellow solid residue which was crystallized from ethanol/petroleum ether (60:80) (4:1).

Compound ***7***

M.p.: 134–136°C; yield: 1.62 g (46%); ^{1}H-NMR δ: 1.60–2.00 (m, 4H, 2 CH_2), 2.60–3.0 (m, 6H, 3 CH_2), 3.44 (s, 3H, CH_3), 3.72 (t, *J* = 7 Hz, 1H, CH), 5.88 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.50 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{11}H_{16}N_2$ (176.26): C, 74.96; H, 9.15; N, 15.89. Found: C, 75.09; H, 9.02; N, 15.67.

Compound ***8***

M.p.: 151–153°C; yield: 2.1 g (55%); ^{1}H-NMR δ: 1.24–1.96 (m, 4H, 2 CH_2), 2.20–2.50 (m, 4H, 2 CH_2), 2.72–3.12 (m, 5H, 2 CH_2 and CH), 3.44 (s, 3H, CH_3), 5.88 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.44 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{12}H_{18}N_2$ (190.28): C, 75.74; H, 9.53; N, 14.72. Found: C, 75.81; H, 9.48; N, 14.81.

Methyl 4,5,7,8,9,9a-hexahydro-3-methyl-3H-pyrrolo[2,3-g] indolizinim iodide and methyl 3,4,5,7,8,9,10,10a-octahydro-3-methylpyrrolo[3,2-a]quinolizinium iodide ***9, 10***

Under anhydrous conditions, a solution of the appropriate cyclized compound **7** or **8** (10 mmol) in dry acetone (30 mL) was treated with iodomethane (4 mL) while stirring for 2 h and then allowed to stand at room temperature overnight. The precipitate obtained was filtered, washed with cold acetone, dried, and recrystallized from ethanol.

Compound ***9***

M.p.: 175–177°C; yield: 1.52 g (48%); ^{1}H-NMR δ: 1.72–1.92 (m, 2H, CH_2), 2.96–3.20 (m, 4H, 2 CH_2), 3.48 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 4.08–4.32 (m, 4H, 2 CH_2), 4.88 (t, *J* = 7 Hz, 1H, CH), 5.94 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.60 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{12}H_{19}IN_2$ (318.2): C, 45.30; H, 6.02; N, 8.80. Found: C, 45.18; H, 6.17; N, 8.64.

Compound ***10***

M.p.: 183–185°C; yield: 1.56 g (47%); ^{1}H-NMR δ: 1.72–1.92 (m, 4H, 2 CH_2), 2.24–2.48 (m, 2H, CH_2), 2.68–2.92 (m, 2H, CH_2), 3.08–3.28 (m, 2H, CH_2), 3.40–3.68 (m, 5H, CH_2 and CH_3), 3.76 (s, 3H, CH_3), 4.08 (m, 1H, CH), 6.04 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.56 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{13}H_{21}IN_2$ (332.22): C, 47.00; H, 6.37; N, 8.43. Found: C, 46.89; H, 6.44; N, 8.29.

1,4,5,6,7,8,9,10-Octahydro-1,8-dimethylpyrrolo[2,3-d] azonine and 4,5,6,7,8,9,10,11-octahydro-1,9-dimethyl-1H-pyrrolo[2,3-d]azecine ***11, 12***

To a stirred suspension of the appropriate methiodide salt **9** or **10** (5 mmol) in absolute ethanol (5 mL) in a three-necked flask immersed in a liquified nitrogen bath, liquified ammonia (30 mL) was introduced. To the reaction mixture (maintained at −40°C with methanol-dry ice), sodium metal (2 g) was added portionwise over a period of 45 min and the resulting deep blue solution was stirred for further 45 min. The reaction was finally quenched by the addition of a few crystals of ammonium chloride, and stirring was continued at room temperature for further 18 h. Water (20 mL) was then added and the resulting emulsion was extracted thoroughly with methylene chloride (5 × 25 mL). The organic extract was washed twice with 5% sodium hydroxide solution, then thoroughly washed with water, dried over anhydrous sodium sulphate, filtered, and evaporated to dryness under reduced pressure. The remaining light orange oil was chromatographed on a silica gel column using a mixture ethyl acetate/methanol (8:2) as eluent to yield a deep yellow oily product.

Compound ***11***

Yield: 0.54 g (56%); ^{1}H-NMR δ: 1.0–1.50 (m, 4H, 2 CH_2), 1.72–2.0 (m, 2H, CH_2), 2.16 (s, 3H, CH_3), 2.36–2.80 (m, 4H, 2 CH_2), 2.96–3.32 (m, 2H, CH_2), 3.50 (s, 3H, CH_3), 6.12 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.50 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{12}H_{20}N_2$ (192.3): C, 74.95; H, 10.48; N, 14.57. Found: C, 74.81; H, 10.62; N, 14.38.

Compound ***12***

Yield: 0.51 g (49%); ^{1}H-NMR δ: 1.24–1.88 (2 m, 6H, 3 CH_2), 2.08 (s, 3H, CH_3), 2.28–3.16 (4 m, 8H, 4 CH_2), 3.50 (s, 3H, CH_3), 5.88 (d, *J* = 7 Hz, 1H, pyrrol-H), 6.08 (d, *J* = 7 Hz, 1H, pyrrol-H). Anal. calculated for $C_{13}H_{22}N_2$ (206.33): C, 75.68; H, 10.75; N, 13.58. Found: C, 75.52; H, 10.88; N, 13.41.

Biological testing

In-vitro serotonin 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 a1 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 to 30 μM for the first curve) were determined in the absence and presence of potential antagonists which were usually incubated for

30 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 to 0.3 logarithmic units was usually observed. The daily mean sensitization was used to correct the dextral shift measured for treated organs. 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. ([*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 [30] when a set of different antagonist concentrations over at least 1.5 logarithmic units was studied. Single organ preparations were obtained from at least three animals.

Dopamine receptor ligand activity

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 [34]. 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, Germany), supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 20 U/mL penicillin G, 20 μg/mL streptomycin, and 0.2 μg/mL G 418 (all from Sigma-Aldrich).

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, Denmark) 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.

Radioligand binding assay

For the binding studies a procedure according to Mierau *et al.* was used [33]. 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 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 glass-fiber filter (Schleicher and Schüll, Germany), previously treated with a 0.25% polyethylene imine 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 (Beckman Instruments, USA). The competition binding data was analyzed by the software GraphPad Prism™ (GraphPad Prism™, San Diega, CA, USA) using non-linear least squares fit.

In-vitro histamine H_1 receptor activity

Whole segments of guinea-pig ileum (1.5 to 2.0 cm in length) were mounted isotonically (preload 0.5 g) in Tyrode solution (37°C) of the composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, KH_2PO_4 0.4, $NaHCO_3$ 11.9, and D-glucose 5.1. The solution was aerated with 95% O_2/5% CO_2 and contained atropine (0.1 μM) to block ileal M_3 receptors. During an equilibrium period of 80 min, the organs were primed three times with histamine (1 and 10 μM every time). Up to four cumulative concentration-effect curves for histamine (0.1 to 30 μM for first curve) were determined in the absence and presence of potential antagonists which were incubated for 10 min. Control experiments in the absence of antagonist revealed that four successive concentration-effect curves for histamine were superimposable.

Results are expressed as mean ± standard error (*SEM* or *SE*) unless otherwise indicated. Antagonist affinity was calculated as apparent pA_2 value as previously described under equation (1). Full pA_2 values were calculated according to the method of Schild [30] when a set of different antagonist concentrations over at least 1.5 logarithmic units was studied. Single organ preparations were from at least two animals.

The author is extremely grateful to Prof. Dr. Jochen Lehmann, Institute of Pharmacy, Pharmaceutical Medicinal Chemistry, Friedrich-Schiller-University Jena, Jena, Germany, for his generous guidance during the course of this investigation. His great efforts and sincere assistance in managing the biological studies in this work are highly appreciated.

The author has declared no conflict of interest.

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