Eur J Med Chem (1997) 32, 253–261

Synthesis, pharmacology and therapeutic potential of 10-methoxypyrazino[1,2-*a*]indoles, partial agonists at the $5HT_{2C}$ receptor

M Bös*, F Jenck, JR Martin, JL Moreau, V Mutel, AJ Sleight, U Widmer

Pharma Division, Preclinical CNS Research, F Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland

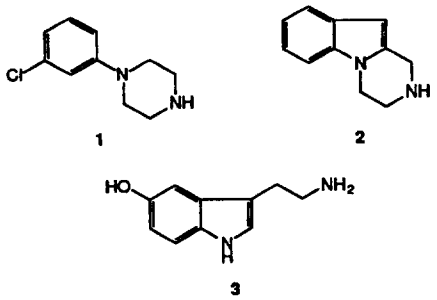
(Received 1 July 1996; accepted 23 September 1996)

Summary — A series of new 10-methoxypyrazino[1,2-*a*]indoles has been prepared and shown to be $5HT_{2C}$ receptor ligands. The studied compounds **10a–j** were found to act as partial agonists at the $5HT_{2C}$ receptor, binding with high affinity and moderate selectivity versus $5HT_{1A}$ and $5HT_{2A}$ receptors, but inducing only a submaximal increase in phosphoinositol formation. Compound **10j** was demonstrated to be active in animal models of obsessive-compulsive disorder, depression and panic anxiety.

pyrazinoindole / serotonin / $5HT_{2C}$ receptor / partial agonist / psychiatric disorder

Introduction

The diverse effects of serotonin (5HT) are mediated by a variety of 5HT receptor subtypes [1]. Due to their relative brain specificity and pattern of regional mRNA distribution [2], $5HT_{2C}$ receptors offer a promising target for designing novel drugs for the therapy of neuropsychiatric disorders in which serotonergic neurotransmission plays an important role. 1-(*m*-Chlorophenyl)piperazine **1** (mCPP), a metabolite of the antidepressant trazodone [3], is a preferential $5HT_{2C}$ receptor agonist [2] (see table I). Pyrazino[1,2-*a*]-indoles **2** [4, 5], bridged derivates of phenylpiperazines, combine the structural features of mCPP (piperazine moiety) (**1**) and the natural ligand 5HT (indole moiety) (**3**). The parent compound **2** displays only low affinity to $5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$ receptors [5] (cf table I). We have found that the introduction of a methoxy substituent in position 10 increases the affinity of this ring system to 5HT receptor subtypes and in particular increases the selectivity for the $5HT_{2C}$ receptor subtype. We report here the synthesis of new derivatives of pyrazino[1,2-*a*]indoles, their pharmacology in vitro and in vivo, as well as the therapeutic potential of a representative compound (**10j**) in several animal models of common psychiatric disorders.



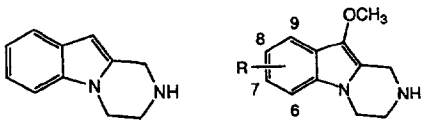
Results

Chemistry

Scheme 1 summarizes the synthetic procedures used to obtain 10-methoxypyrazino[1,2-*a*]indoles **10**. The compounds **10a–j** were prepared starting from the appropriate anthranilates **4**. After *N*-alkylation with bromoacetate in the presence of K_2CO_3 intramolecular ester condensation of the phenylglycine esters **6** yielded the 3-hydroxyindole-2-carboxylates **7** (62–90%). The phenylglycine esters **6** could also be prepared by alkylation of isatoic anhydrides **5** with bromoacetate in DMSO/KOH followed by alcoholysis. Subsequently, the hydroxy group was methylated with diazomethane or dimethylsulfate to give quantitatively the ethers **8**. The pyrazine ring was built up in two steps in moderate to good yields (51–96%) by alkylation with

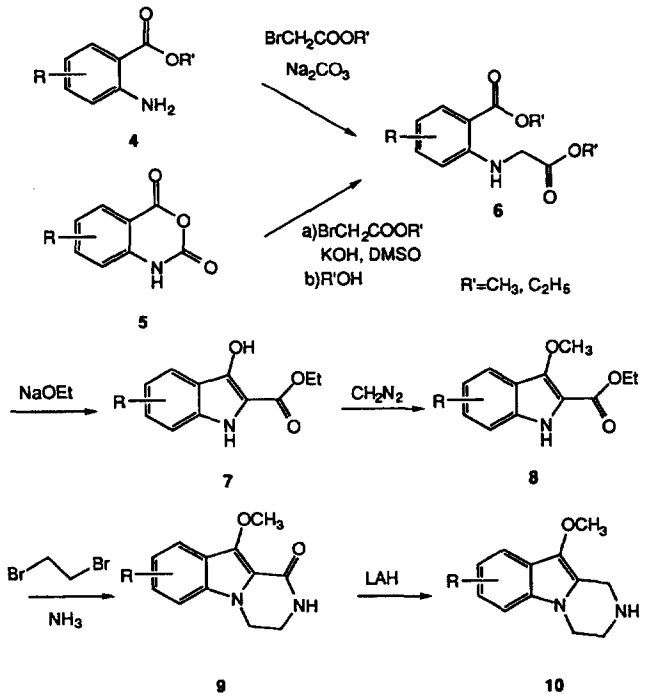
*Correspondence and reprints

Table I. Pyrazino[1,2-*a*]indoles: binding affinities (pK_i) for $5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$ receptors and efficacy (pEC_{50}) in inducing IP_3 formation in vitro.



2 **10**

Compound	*R*	*pK_i*			*IP_3 formation*	
		$5HT_{1A}$	*$5HT_{2A}$*	*$5HT_{2C}$*	*pEC_{50}*	*Intrinsic activity*
1		7.4	7.1	7.9	6.9	1
2		5.9	5.1	5.7		
10a	H	5.7	5.9	7.1	5.5	0.5
10b	6-Br	6.7	7.2	8.3	5.8	0.5
10c	7-Cl	6.0	6.5	7.4	5.8	0.26
10d	8-F	6.0	6.6	7.8	5.7	0.95
10e	8-Cl	5.7	7.1	8.0	6.1	0.7
10f	8-Br	5.7	7.4	8.3	5.6	0.96
10g	9-F	5.6	6.1	7.2	5.5	0.4
10h	9-Br	6.3	7.4	8.1	5.9	0.3
10i	9-*i*-Propyl	7.0	7.4	8.0	5.1	0.4
10j	9-CH_3	7.1	6.4	7.6	6.1	0.6

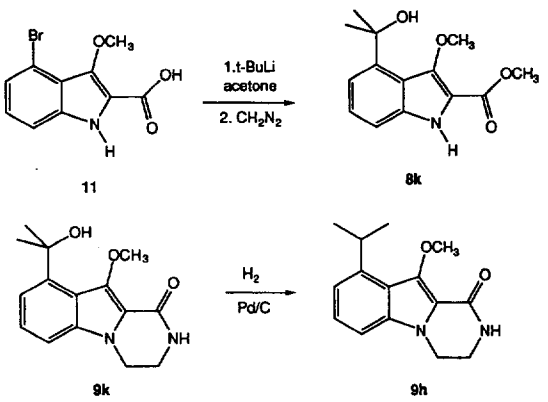


Scheme 1.

dibromoethane and sodium hydroxide followed by cyclization with ammonia. Reduction of the lactames **9** was accomplished with $LiAlH_4$ or B_2H_6. The isopropyl substituent of compound **10i** was introduced by halogen–lithium exchange of the 4-bromo-3-methoxy indole carboxylic acid **11** followed by the addition of acetone and subsequent hydrogenation of the hydroxy group (scheme 2).

Pharmacology

Serotonin receptor binding experiments (table I) in human cell lines with [^{3}H]-5HT ($5HT_{1A}$ and $5HT_{2C}$) and [^{3}H]-DOB ($5HT_{2A}$) revealed higher affinity of the compounds **10a–j** for $5HT_{2C}$ binding sites as compared to the $5HT_{1A}$ and $5HT_{2A}$ receptors. The compounds described here were found to be partial agonists at the $5HT_{2C}$ receptor subtype, binding with high affinity but inducing only a submaximal increase in phosphoinositol formation (cf IP_3 intrinsic activity, table I). The affinity of the 10-methoxypyrazino[1,2-*a*]indoles to the $5HT_{2C}$ binding site increases with increasing size of the substituents in position 6, 7, 8 or 9 (F < CH_3 < Cl < Br). In order to quantify the in vivo effect, the compounds were tested for their capacity to antagonize a $5HT_{2C}$ receptor-mediated function namely mCPP-induced penile erection [6] (table II).



Scheme 2.

Based on these data, **10j** was found to combine intermediate intrinsic activity and potent in vivo antagonistic properties at the $5HT_{2C}$ receptor. Therefore, it was selected as the best partial agonist for evaluation in animal models of psychiatric disorders. These tests have been developed to simulate important aspects of psychiatric disorders. For example, chronic mild stress-induced anhedonia in rats simulates important characteristics of depression [7], whereas intracerebral stimulation-induced escape behavior has been validated as a model of certain aspects of panic anxiety [8]. Compulsive drinking in rats [9] and compulsive whole body scratching in squirrel monkeys [10] have been proposed as models of obsessive-compulsive disorder (OCD).

In the anhedonia model of depression in rats, the hedonic state of the animals is assessed using the intracranial self-stimulation (ICSS) technique in which a rat can turn on brain stimulation eliciting appetitive/rewarding properties. The ICSS procedure has proved to be a useful technique for the study of reward, motivation and anhedonia (lack of sensitivity to pleasurable stimuli) because it reflects the direct activation of brain reward systems. Thus, ICSS threshold may be used as an index of the hedonic/anhedonic state of an animal. A 'depression-like' state can be induced in rats by submitting them to a chronic mild unpredictable stress procedure. This results in a progressive increase in ICSS thresholds, meaning a progressive decrease in sensitivity to reward (anhedonia). Experimental compounds are tested in this model for their capacity to prevent or reverse the development of such a stress-induced anhedonia, ie, for their antidepressant-like properties. The effects of **10j** (3 mg/kg ip given twice daily) in this anhedonia model of depression are shown in figure 1 (top panel). The statistical analysis revealed a significant difference between vehicle- and drug-treated stressed groups ($F(10,180) = 4.46$, $P < 0.001$). In vehicle-treated stressed animals, there was a significant stress-induced increase in self-stimulation threshold (anhedonia index, ($F(10,99) = 7.82$, $P < 0.001$). This 'anhedonic or depressed state' gradually developed over the duration of the stress period. When the stress regime was terminated on day 19, the anhedonia index progressively returned to the pre-stress baseline level. In contrast, in drug-treated stressed rats, **10j** prevented the development of such an 'anhedonic state'. The anhedonia index did not appreciably vary throughout the entire experiment ($F(10,99) = 2.34$, $P > 0.01$). Self-stimulation thresholds remained at about control levels until the end of the experiment.

In the model of panic anxiety in rats, animals are given aversive brain stimulation eliciting intense emotional and motor responses reflected by abrupt escape reactions together with stress-characteristic autonomic changes. This behavioral response is similar to a panic attack in humans and can be shaped into operant self-interruption behavior. Experimental compounds are tested in this model for their capacity to increase or decrease escape threshold, ie, for their capacity to exhibit antipanic- or propanic-like properties, respectively. In this model of panic anxiety, three doses of **10j** (1, 3.2 and 10 mg/kg ip) administered versus vehicle induced a dose-dependent and statistically significant increase in frequency threshold for escape behavior (see figure 1, bottom panel). Comparison of individual doses versus vehicle shows that the doses 3.2 and 10 mg/kg induced statistically significant antipanic-like effects (1 mg/kg: 32.9% increase, $t = 1.65$, $P > 0.05$; 3.2 mg/kg: 54.8% increase, $t = 3.44$, $P < 0.01$; 10 mg/kg: 94.3% increase, $t = 4.38$, $P < 0.001$). This antipanic-like effect is comparable in amplitude to the effect of the clinically established antipanic drug clonazepam [8].

Table II. Antagonism of $5HT_{2C}$ receptor-mediated functions: inhibition of mCPP-induced penile erections [6].

Compound	*ID_{50} (mg/kg sc)*
10a	3.6
10b	10
10c	>10
10d	4.2
10e	>10
10f	14.3
10g	10
10h	4.3
10i	4
10j	2.7

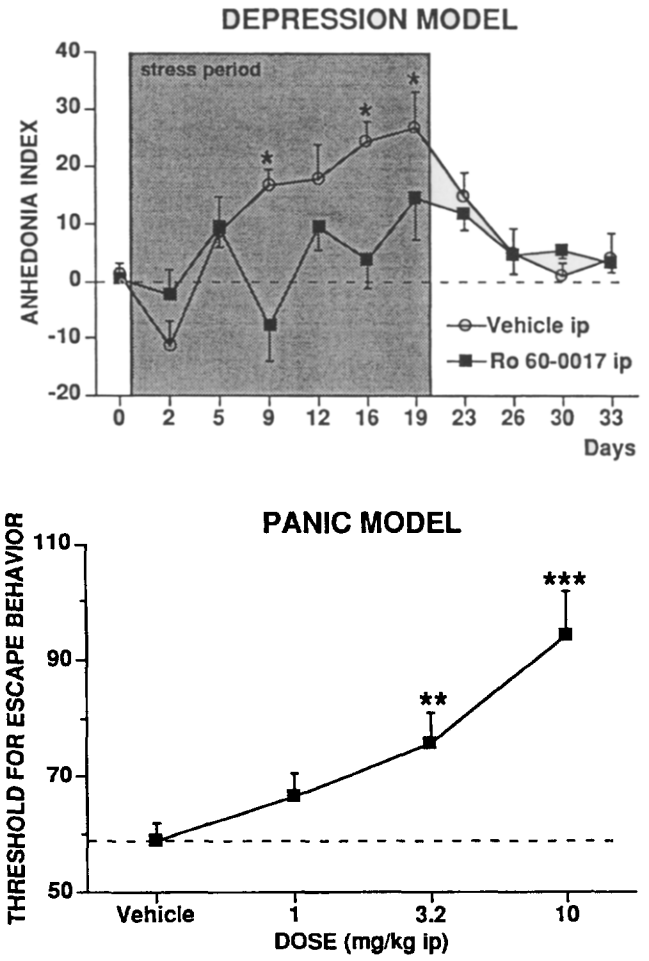


Fig 1. Antidepressant and antipanic properties of **10j** in rats. Top panel: antidepressant-like effects of **10j** in stress-induced anhedonic rats. Anhedonia index (% change in self-stimulation threshold) in stressed animals treated twice a day with vehicle (open circles) or 3 mg/kg ip **10j** (solid squares). The chronic mild stress procedure was applied from day 0 to day 19 (shaded area). Results (means ± SEM) are expressed as percentage change from pre-stress baseline thresholds. An asterisk indicates a statistically significant difference (Dunnett's *t*-test, $P < 0.01$) between vehicle- and drug-treated groups. Bottom panel: Effects of acute administration (ip, 30 min pretreatment time) of **10j** on frequency thresholds for escape behavior. Increases in threshold reflect antiaversive action indicative of antipanic potential (**$P < 0.01$, ***$P < 0.001$, unpaired Student's *t*-test, comparisons vehicle vs doses).

In the polydipsia model of OCD in rats, when food-deprived animals are intermittently delivered food pellets at a fixed time interval, they typically develop a pattern of excessive drinking, ie, polydipsia. This compulsive drinking behavior, similar to the compulsive aspects of OCD, can be prevented by administrating compounds efficacious in treating OCD in humans. Experimental compounds are tested in this model for their ability to antagonize polydipsic behavior, ie, for their potential anti-OCD properties. In this polydipsia model, **10j** reduced compulsive drinking induced by a stress condition (fig 2, top panel). Pretest oral administration of **10j** at the dose 30 mg/kg resulted

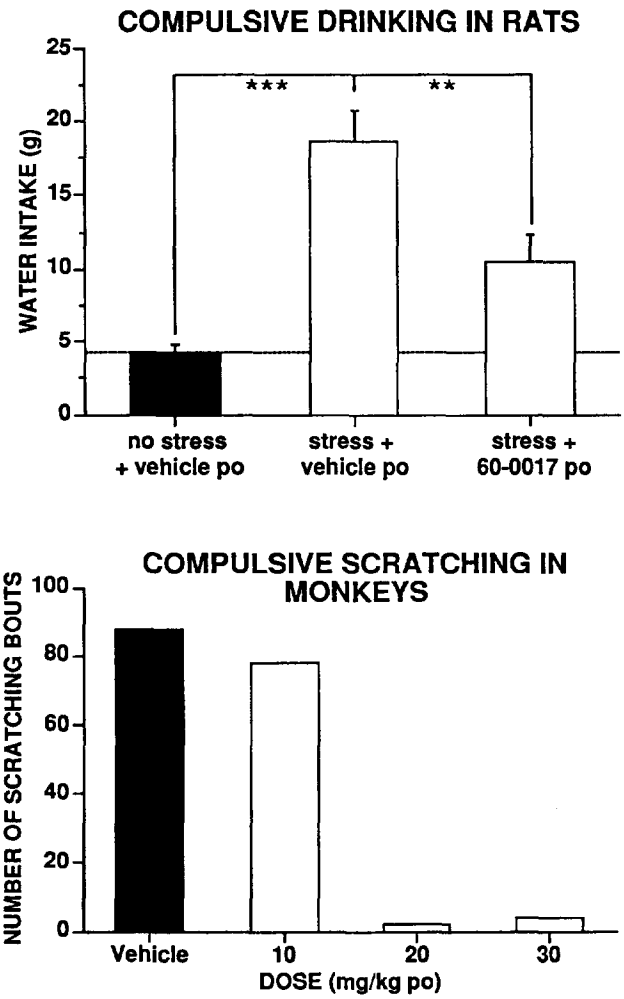


Fig 2. Anticompulsive properties of **10j** in rats and monkeys. Top panel: water intake by non-stressed vehicle-treated animals and by stressed rats treated with **10j** (30 mg/kg po) or vehicle. **10j** significantly decreased compulsive drinking (Wilcoxon test, **$P < 0.01$, ***$P < 0.001$). Bottom panel: antagonism of 8-OH-DPAT-induced whole-body scratching behavior in squirrel monkeys. **10j** or vehicle was orally administered 15 min prior to challenge with 8-OH-DPAT. The mean number of scratching bouts occurring during the next 2 h was recorded.

in intake of only 10.5 g versus 18.6 g observed under the stress condition after vehicle treatment (Wilcoxon signed rank test, $P = 0.01$). In comparison following vehicle administration only 4.3 g of water were consumed demonstrating the effectiveness of the stress condition in generating compulsive drinking.

In the model of compulsive behavior in squirrel monkeys, drug-induced scratching behavior is investigated. Monkeys tend to display this kind of behavior apparently out of context when they experience situations of danger, conflict, or tension. These displacement activities show a resemblance to symptoms of OCD. Experimental compounds are tested in this model for their ability to reduce compulsive scratching behavior. Three doses of **10j** (10, 20 and 30 mg/kg po) were evaluated. The doses 20 and 30 mg/kg decreased the number of scratching bouts recorded over the 2 h observation period versus that observed under the vehicle condition (fig 2, bottom panel).

These in vivo results show that **10j**, a representative partial agonist at the $5HT_{2C}$ receptor, exhibits therapeutic potential for treating several psychiatric disorders. In animals, **10j** exhibits a 30-fold selectivity for $5HT_{2C}$ ($pK_i = 7.6$) vs $5HT_{2A}$ ($pK_i = 6.1$) receptor, but only a three-fold selectivity for $5HT_{2C}$ ($pK_i = 7.6$) vs $5HT_{1A}$ receptor ($pK_i = 7.1$). Therefore the effects of **10j** could as well be ascribed to the activation of $5HT_{1A}$ receptors. When evaluated in vivo, however, **10j** (up to 10 mg/kg sc) was found to be completely inactive in either inducing or antagonizing behavioral signs (lower lip retraction and flat body posture) which reflect $5HT_{1A}$ receptor-mediated functions ([17], unpublished results). In addition, when tested for affinity at other G-protein-coupled receptors ($5HT_{1D}$, D_1, D_2, α_1, α_2, M_1, M_2) **10j** always exhibited a pK_i smaller than 5.8. Altogether, these results suggest a likely role for $5HT_{2C}$ receptors in some aspects of OCD, panic anxiety and depression. Compounds modulating $5HT_{2C}$-receptor-mediated functions, such as **10j**, may offer an innovative approach to the treatment of these psychiatric disorders.

Experimental protocols

Chemistry

General

Melting points were determined in capillary tubes (Büchi 530 apparatus) and are uncorrected. Column chromatography was carried out by using silica gel (230–400 mesh; Merck) and 0.3–1.0 bar pressure. Spectra were recorded with the following instruments: ^{1}H-NMR (δ values in ppm relative to internal TMS, coupling constants J in Hz): Bruker AC-250 (250 MHz); MS: MS9 updated with a Finnigan MAT data system SS 200. Elementary analyses (C, H, N) for novel compounds were within 0.4% of the theoretical values.

General procedure for the preparation of N-(2-alkoxycarbonyl)phenyl glycine esters ***6c–h***

A mixture of anthranilate **4** (0.05 mol) and sodium carbonate (0.05 mol) in bromoacetate (40 mL) was stirred for 18 h at 80 °C. After concentration, water (150 mL), ethanol (15 mL) and 25% ammonia (15 mL) were added and the mixture was stirred for 2 h. The resulting precipitate was filtered and dried. In case the product did not crystallize the mixture was extracted with diethylether or ethyl acetate and the solvent was removed.

Ethyl N-[5-chloro-2-(ethoxycarbonyl)phenyl]glycinate ***6c***. Yield 70%. Mp 45.5–46.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (t, J = 7.2 Hz, 3H), 1.35 (t, J = 7.2 Hz, 3H), 3.96 (d, J = 5.2 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 4.33 (q, J = 7.2 Hz, 2H), 6.50 (d, J = 2 Hz, 1H), 6.61 (dd, J = 2 Hz, J = 8.5 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H).

Ethyl N-[4-fluoro-2-(ethoxycarbonyl)phenyl]glycinate ***6d***. Yield 59%. Mp 66–67 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7 Hz, 3H), 4.09 (d, J = 5.7 Hz, 2H), 4.16 (q, J = 7 Hz, 2H), 4.29 (q, J = 7.2 Hz, 2H), 6.66 (dd, J = 9.3 Hz, J = 4.5 Hz, 1H), 7.31 (ddd, J = 9.5 Hz, J = 9.3 Hz, J = 3.2 Hz, 1H), 7.55 (dd, J = 9.7 Hz, J = 3.2 Hz, 1H), 7.82 (t, J = 5.7 Hz, 1H).

Ethyl N-[4-chloro-2-(methoxycarbonyl)phenyl]glycinate ***6e***. Yield 64%. Mp 82–83 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, J = 7.1 Hz, 3H), 3.88 (s, 3H), 3.97 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 6.47 (d, J = 8.9 Hz, 1H), 7.30 (dd, J = 9 Hz, J = 2.6 Hz, 1H), 7.90 (d, J = 2.6 Hz, 1H).

Ethyl N-[4-bromo-2-(ethoxycarbonyl)phenyl]glycinate ***6f***. Yield 74%. Mp 95–96 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, J = 7.2 Hz, 3H), 1.39 (t, J = 7.2 Hz, 3H), 3.96 (d, J = 5.5 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 4.34 (q, J = 7.2 Hz, 2H), 6.41 (d, J = 8.7 Hz, 1H), 7.41 (dd, J = 8,7 Hz, J = 2.5 Hz, 1H), 8.03 (d, J = 2.5 Hz, 1H).

Methyl N-[3-fluoro-2-(methoxycarbonyl)phenyl]glycinate ***6g***. Yield 91%. Mp 69–70 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.80 (s, 3H), 3.92 (s, 3H), 3.98 (d, J = 5 Hz, 2H), 6.27 (d, J = 7.5 Hz, 1H), 6.40 (dd, J = 10 Hz, J = 7.5 Hz, 1H), 7.25 (AB, J = 15 Hz, J = 7.5 Hz, 1H), 8.15 (m, 1H).

Ethyl N-[3-bromo-2-(ethoxycarbonyl)phenyl]glycinate ***6h***. Yield 96%, orange oil, ^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, J = 7.1 Hz, 3H), 1.43 (t, J = 7.1 Hz, 3H), 3.88 (d, J = 5 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.44 (q, J = 7.1 Hz, 2H), 6.46 (dd, J = 8 Hz, J = 2 Hz, 1H), 6.95 (dd, J = 8 Hz, J = 2 Hz, 1H), 7.06 (t, J = 8 Hz, 1H).

Ethyl N-[2-(ethoxycarbonyl)-3-methylphenyl]glycinate ***6j***. A mixture of isatoic anhydride (10 mmol), powdered potassium hydroxide (1 g) and ethyl bromoacetate (11 mmol) was stirred for 3 h at room temperature. After the addition of ethanol (30 mL) stirring was continued for an additional 30 min and extracted with water and ether. The organic layer was dried and the solvent was removed. The residue was subjected to Kugelrohr destillation (0.4 mbar, 190 °C) to yield 88% of a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 1.29 (t, J = 7.5 Hz, 3H), 1.40 (t, J = 7 Hz, 3H), 2.44 (s, 3H), 3.92 (s, 2H), 4.24 (q, J = 7 Hz, 2H), 4.39 (q, J = 7 Hz, 2H), 6.37 (d, J = 8 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 7.15 (d, J = 8 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H).

General procedure for the preparation of 3-hydroxyindole-2-carboxylic acid ethylesters ***7***
A solution of sodium (0.2 mol) in ethanol (70 mL) was combined with a solution of *N*-(2-alkoxycarbonyl)phenyl glycine ester **6** (0.1 mol) in diethylether (200 mL). The mixture was heated to reflux for 2 h. After cooling, water was added and the mixture was extracted with ether. The aqueous layer was adjusted to pH 8 by addition of dry ice and the resulting precipitate was filtered, washed with water, dried and recrystallized.

Methyl 7-bromo-3-hydroxyindole-2-carboxylate ***7b***. Yield 86% from **6b** [12]. Mp 255–258 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.82 (s, 3H), 6.89 (t, J = 7.5 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 1.7 Hz, 1H).

Ethyl 6-chloro-3-hydroxyindole-2-carboxylate ***7c***. Yield 62%. Mp 168–170 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, J = 7.2 Hz, 3H), 4.30 (q, J = 7.2 Hz, 2H), 6.96 (dd, J = 8.7 Hz, J = 1.7 Hz, 1H), 7.28 (d, J = 1.7 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H).

Ethyl 5-fluoro-3-hydroxyindole-2-carboxylate ***7d***. Yield 67% (toluene). Mp 152–154 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, J = 7 Hz, 3H), 4.31 (q, J = 7 Hz, 2H), 7.09 (dt, J = 9.2 Hz, J = 2.5 Hz, 1H), 7.30 (dd, J = 9 Hz, J = 4.5 Hz, 1H), 7.43 (dd, J = 9.5 Hz, J = 2.5 Hz, 1H).

Ethyl 5-chloro-3-hydroxyindole-2-carboxylate ***7e***. Yield 79% (toluene). Mp 172–174 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (t, J = 7.5 Hz, 3H), 4.43 (q, J = 7.5 Hz, 2H), 7.18 (AB, J = 7.5 Hz, 1H), 7.27 (AB, d, J = 7.5 Hz, J = 2.5 Hz, 1H), 7.70 (d, J = 2.5 Hz, 1H).

Ethyl 5-bromo-3-hydroxyindole-2-carboxylate ***7f***. Yield 74%. Mp 185 °C. ^{1}H-NMR ($CDCl_3$) δ: 142 (t, J = 7.2 Hz, 3H), 4.44 (q, J = 7.5 Hz, 2H), 7.15 (d, J = 9 Hz, 1H), 7.40 (dd, J = 9 Hz, J = 2 Hz, 1H), 7.87 (d, J = 2 Hz, 1H).

Methyl 4-fluoro-3-hydroxyindole-2-carboxylate ***7g***. Yield 71%. Mp 207–208 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.97 (s, 3H), 6.72 (dd, J = 10 Hz, J = 7.5 Hz, 1H), 7.03 (d, J = 8 Hz, 1H), 7.23 (ddd, J = 8 Hz, J = 7.5 Hz, J = 5 Hz, 1H).

Ethyl 4-bromo-3-hydroxyindole-2-carboxylate ***7h***. Yield 90%. Mp 157–180 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (t, J = 7.5 Hz, 3H), 4.34 (q, J = 7.5 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 2 Hz, 1H), 7.3 (dd, J = 7.5 Hz, J = 2 Hz), 8.72 (s, 1H), 11.2 (s, 1H).

Ethyl 3-hydroxy-4-methylindole-2-carboxylate ***7j***. Yield 77% (ethanol). Mp 124–125 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (t, J = 7.5 Hz, 3H), 2.71 (s, 3H), 4.42 (q, J = 7.5 Hz, 2H), 6.79 (d, J = 7 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 7 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H).

General procedure for the preparation of 3-methoxyindole-2-carboxylates ***8***
A mixture of 3-hydroxyindole-2-carboxylate **7** (0.05 mol) and methanol (500 mL) was treated with diazomethane (60% in diethylether, 200 mL). After 0.5 h the solvent was removed and the product was recrystallized.

Methyl 7-bromo-3-methoxyindole-2-carboxylate ***8b***. Oil (quantitative). ^{1}H-NMR ($CDCl_3$) δ: 3.98 (s, 1H), 4.13 (s, 1H), 6.99 (t, J = 7.5 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H).

Ethyl 6-chloro-3-methoxyindole-2-carboxylate ***8c***. Yield (cyclohexane) 85%. Mp 145–145.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (t, J = 7.12 Hz, 3H), 4.11 (s, 3H), 4.43 (q, J = 7.12 Hz, 2H), 7.07 (dd, J = 8.6 Hz, J = 1.7 Hz, 1H), 7.31 (d, J = 1.7 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 8.36 (s, 1H).

Ethyl 5-fluoro-3-methoxyindole-2-carboxylate ***8d***. Yield 99% (toluene/cyclohexane). Mp 96–99 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (t, J = 7 Hz, 3H), 4.08 (s, 3H), 4.43 (q, J = 7 Hz, 2H), 7.07 (dt, J = 10 Hz, J = 2.5 Hz, 1H), 7.25 (dd, J = 5 Hz, J = 2.5Hz, 1H), 7.37 (dd, J = 7.5 Hz, J = 2.5 Hz, 1H), 8.5 (s, 1H).

Ethyl 5-chloro-3-methoxyindole-2-carboxylate ***8e***. Quantitative yield (ethyl acetate/hexane). Mp 125–127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (t, J = 8 Hz, 3H), 4.09 (s, 3H), 4.43 (q, J = 8 Hz, 2H), 7.25 (AB, 1H), 7.73 (s, 1H).

Ethyl 5-bromo-3-methoxyindole-2-carboxylate ***8f***. Quantitative yield. Mp 139 °C (toluene). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (t, J = 7 Hz, 3H), 4.09 (s, 3H), 4.43 (q, J = 7 Hz, 3H), 7.20 (d, J = 8.7 Hz, 1H), 7.38 (dd, J = 8.7 Hz, J = 1.7 Hz, 1H), 7.89 (d, J = 1.7 Hz, 1H).

Methyl 4-fluoro-3-methoxyindole-2-carboxylate ***8g***. Quantitative yield. Mp 137–139 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.98 (s, 3H), 4.05 (d, J = 0.95 Hz, 3H), 6.75 (dd, J = 7.6 Hz, J =10.7 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.19 (ddd, J = 8.3 Hz, J = 7.6 Hz, J = 4.7 Hz, 1H), 8.62 (s, 1H).

Ethyl 4-bromo-3-methoxyindole-2-carboxylate ***8h***. Yield (hexane) 79%. Mp 137–139 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (t, J = 7.5 Hz, 3H), 4.02 (s, 3H), 4.45 (q, J = 7.5 Hz, 2H), 7.11 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 8.05 (s, 1H).

Ethyl 3-methoxy-4-methylindole-2-carboxylate ***8j***. Yield (ethanol) 60%. Mp 109–110 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (t, J = 7 Hz, 3H), 2.69 (s, 3H), 4.00 (s, 3H), 4.43 (q, J = 7 Hz, 2H), 6.84 (d, J = 6.5 Hz, 1H), 7.15 (AB, J = 7.5 Hz, J = 6.5 Hz, 2H), 8.50 (s, 1H).

General procedure for the preparation of 10-methoxy-1,2,3,4-tetrahydropyrazino[1,2-a]indol-1-on ***9***
A mixture of 3-methoxyindole-2-carboxylic acid ethyl ester **8** (0.035 mol), 1,2-dibromoethane (100 mL) 28% sodium hydroxide (100 mL) and tetrabutylammonium bromide (1.7 mmol) was stirred at ambient temperature. After 18 h the layers were separated and the aqueous layer was extracted with methylenechloride. The combined organic layers were dried and the solvent was removed. The residue was taken up in ammonia (330 mL) and the mixture was heated to 80 °C in an autoclave. After 18 h ammonia was evaporated and the residue was taken up in water. The crystals were isolated, dried and recrystallized.

1,2,3,4-Tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9a***. Yield 96% from **8a** [13]. Mp 172 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.56 (m, 2H), 4.00 (s, 3H), 4.18 (dd, J = 5.5 Hz, J = 5.5 Hz, 2H), 7.06 (t, J = 8 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 7.64 (d, J = 8 Hz, 1H), 7.97 (s, 1H).

6-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9b***. Yield 90%. Mp 148–150 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (m, 2H), 3.98 (s, 1H), 4.73 (dd, J = 5 Hz, J = 5 Hz, 2H), 6.98 (t, J = 7.5 Hz, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H).

7-Chloro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9c***. Yield 75% (ethanol). Mp 218–220 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.56 (m, 2H), 4.00 (s, 3H), 4.19 (dd, J = 5 Hz, J = 5 Hz, 2H), 7.06 (dd, J = 8.6 Hz, J = 1.6 Hz, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.67 (d, J = 1.6 Hz, 1H).

8-Fluoro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9d***. Yield 60% (ethylacetate). Mp 220–223 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (m, 2H), 3.98 (s, 3H), 4.19 (dd, J = 5.7 Hz, J = 5.5 Hz, 2H), 7.18 (ddd, J = 9.2 Hz, J = 9 Hz, J = 2.5 Hz, 1H), 7.38 (dd, J = 10 Hz, J = 2.5 Hz, 1H), 7.54 (dd, J = 9 Hz, J = 4.2 Hz, 1H).

8-Chloro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9e***. Yield 55% (ethylacetate). Mp 218–220 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (m, 2H), 3.99 (s, 3H), 4.20 (t, J = 5.2 Hz, 2H), 7.06 (dd, J = 8.2 Hz, J = 1.5 Hz ,1H), 7.55 (d, J = 8.2 Hz, 1H), 7.67 (d, J = 2.5 Hz, 1H), 8.09 (s, 1H).

8-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9f***. Yield 74%. Mp 211 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.77 (m, 2H), 4.16 (s, 3H), 1.16 (t, J = 6 Hz, 2H), 6.73 (s, 1H), 7.14 (d, J = 8.7 Hz, 1H), 7.40 (dd, J = 8.7 Hz, J = 1.7 Hz, 1H), 7.92 (d, J = 1.7 Hz, 1H).

9-Fluoro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9g***. Yield 51% (ethanol). Mp 190–193 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.57 (m, 2H), 3.93 (s, 3H), 4.21 (t, J = 5Hz, 2H), 6.81 (dd, J = 7.5 Hz, J = 5 Hz, 1H), 7.26 (ddd, J = 7.5 Hz, J = 5 Hz, J = 5 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 8.10 (s, 1H).

9-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9h***. Yield 92% (ethanol). Mp 205–206 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.58 (m, 2H), 3.90 (s, 3H), 4.22 (t, J = 5Hz, 2H), 7.21 (m, 2H), 7.53 (d, J = 7.5Hz), 8.13 (s, 1H).

1,2,3,4-Tetrahydro-10-methoxy-9-methylpyrazino[1,2-a]indol-1-on ***9j***. Yield 91%. Mp 201–202 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.71 (s, 3H), 3.75 (m, 2H), 4.08 (s, 3H), 4.15 (dd, J = 5 Hz, J = 5 Hz, 2H), 6.60 (s, 1H), 6.84 (d, J = 7.5 Hz, 1H), 7.07 (d, J = 8.5 Hz, 1H), 7.21 (dd, J = 8.5 Hz, J = 7.5 Hz, 1H).

9-(1'-Hydroxy-1'-methylethyl)-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol-1-on ***9k***. Yield 82%. Mp 201–202 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.59 (s, 6H), 3.58 (m, 2H), 4.07 (s (3H), 4.19 (t, J = 5 Hz, 2H), 5.13 (s, 1H), 7.22 (m, 2H), 7.36 (m, 1H), 8.06 (s, 1H).

General procedure for the preparation of 1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol ***10a, c–e, g, h***

A solution of 1,2,3,4-tetrahydro-10-methoxypyrazino-indol-1-on **9** (3 mmol) and $LiAlH_4$ (6 mmol) in dry THF (40 mL) was heated under reflux for 2 h. The excess hydride was quenched by adding water dropwise. After the addition of sodium sulfate (5 g) the reaction mixture was filtered and the solvent was removed. The residue was dissolved in ethanol (50 mL) and ethanolic HCl solution (20 mL) was added to yield the corresponding hydrochloride. **10i** was crystallized as the fumarate.

1,2,3,4-Tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10a***. Yield 67%. Mp 208 °C (decomp). ^{1}H-NMR (DMSO-d_6) δ: 3.62 (t, J = 5 Hz, 2H), 3.88 (s, 3H), 4.23 (t, J = 5 Hz, 2H), 4.46 (s, 2H), 7.03 (dt, J = 2.5 Hz, J = 9.2 Hz, 1H), 7.35 (dd, J = 10 Hz, J = 2.5 Hz, 1H), 7.47 (dd, J = 9.2 Hz, J = 4.2 Hz, 1H). Anal: $C_{12}H_{14}N_2O$•HCl (C, H, N).

7-Chloro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10c***. Yield 34%. Mp 228–230 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.62 (t, J = 7.5 Hz, 2H), 3.87 (s, 3H), 4.25 (t, J = 7.5 Hz, 2H), 4.46 (s, 2H), 7.08 (dd, J = 7.5 Hz, J = 2 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 2 Hz, 1H), 10.06 (s, 2H). Anal: $C_{12}H_{13}ClN_2O$•HCl (C, H, N).

8-Fluoro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10d***. Yield 73%. Mp 219–221 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.64 (t, J = 5 Hz, 2H), 3.85 (s, 3H), 4.24 (t, J = 5 Hz, 2H), 4.46 (s, 2H), 7.03 (dt, J = 9.2 Hz, J = 2.5 Hz, 1H), 7.35 (dd, J = 10 Hz, J = 2.5 Hz, 1H), 7.47 (dd, J = 4.2 Hz, J = 9.2 Hz, 1H).

8-Chloro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10e***. Yield 73%. Mp 234–235 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.63 (t, J = 7.5 Hz, 2H), 3.87 (s, 3H), 4.25 (t, J = 7.5 Hz, 2H), 4.47 (s, 2H), 7.18 (dd, J = 10 Hz, J = 2.5 Hz, 1H), 7.49 (d, J = 10 Hz, 1H), 7.62 (d, J = 2.5 Hz, 1H). Anal: $C_{12}H_{13}ClN_2O$•HCl (C, H, N).

9-Fluoro-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10g***. Yield 79%. Mp 227–231 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.63 (t, J = 7.5 Hz, 2H), 3.81 (s, 3H), 4.26 (t, J = 7.5 Hz, 2H), 4.46 (s, 2H), 6.86 (dd, J = 12.5 Hz, J = 7.5 Hz, 1H), 7.14 (ddd, J = 12.5 Hz, J = 7.5 Hz, J = 5 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H). Anal: $C_{12}H_{13}FN_2O$•HCl (C, H, N).

9-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10h***. Yield 36%. Mp 236–238 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.65 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 4.27 (t, J = 7.5 Hz, 2H), 4.5 (s, 2H), 7.08 (dd, J = 7.5 Hz, J = 7.5 Hz, 1H), 7.26 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 7.5 Hz, 1H), 10.03 (s, 2H). Anal: $C_{12}H_{13}BrN_2O$•HCl (C, H, N).

1,2,3,4-Tetrahydro-10-methoxy-9-isopropylpyrazino[1,2-a]-indole•$C_4H_4O_4$ ***10i***. Yield 81%. Mp 176–177 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (d, J = 7 Hz, 6H), 3.25 (t, J = 7.5 Hz, 2H), 3.68 (sept, J = 7 Hz, 1H), 3.72 (s, 3H), 3.92 (t, J = 7.5 Hz, 2H), 4.15 (s, 2H), 6.57 (s, 2H), 6.90 (d, J = 7 Hz, 1H), 7.02 (t, J = 7 Hz, 1H), 7.13 (d, J = 7 Hz, 1H). Anal: $C_{13}H_{16}N_2O$•$C_4H_4O_4$ (C, H, N)

1,2,3,4-Tetrahydro-10-methoxy-9-methylpyrazino[1,2-a]indole HCl ***10j***. Yield 56%. Mp 231–233 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.58 (s, 3H), 3.62 (t, J = 7.5 Hz, 2H), 3.76 (s, 3H), 4.19 (t, J = 7.5 Hz, 2H), 4.48 (s, 2H), 6.81 (d, J = 7.5 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 9.9 (s, 2H). Anal: $C_{13}H_{16}N_2O$•HCl (C, H, N).

General procedure for the preparation of 1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indol ***10b,f***

A solution of 1,2,3,4-tetrahydro-10-methoxypyrazinoindol-1-on **9** (3 mmol) and B_2H_6 (15 mmol) in dry THF (60 mL) was heated under reflux for 3 h. A saturated ethanolic HCl solution was added and the mixture was refluxed for 1 h, cooled to room temperature and basified with NaOH (28%). After extraction with ethyl acetate the organic layer was dried with Na_2SO_4 and the solvent was removed. The residue was dissolved in ethanol (50 mL) and ethanolic HCl solution (20 mL) was added to yield the corresponding hydrochloride.

6-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10b***. Yield 39%. ^{1}H-NMR (DMSO-d_6) δ: 3.61 (t, J = 5 Hz, 2H), 3.86 (s, 3H), 4.47 (s, 2H), 4.74 (t, J = 5 Hz, 2H), 7.00 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H). Anal: $C_{12}H_{13}BrN_2O$•HCl (C, H, N).

8-Bromo-1,2,3,4-tetrahydro-10-methoxypyrazino[1,2-a]indole HCl ***10f***. Yield 51%. Mp 239 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.63 (t, J = 5 Hz, 2H), 3.87 (s, 3H), 4.23 (t, J = 5 Hz, 2H), 4.48 (s, 2H), 7.30 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 2.5 Hz, 1H). Anal: $C_{12}H_{13}BrN_2O$•HCl (C, H, N).

4-Bromo-3-methoxyindole-2-carboxylic acid ***11***
A mixture of **8h** (450 mg, 1.5 mmol) and sodium hydroxide (2N, 1.5 mL) in ethanol (5 mL) was stirred at 80 °C for 30 min. Ethanol was removed and the residue was acidified with HCl and the precipitate filtered. White crystals, yield 86%. Mp 195–199 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.87 (s, 3H), 7.13 (dd, J = 8 Hz, J = 5 Hz, 1H), 7.23 (d, J = 5 Hz, 1H), 7.36 (d, J = 8 Hz, 1H).

Methyl 4-(1'-hydroxy-1'-methylethyl)-3-methoxyindole-2-carboxylate ***8k***
To a solution of **11** (270 mg, 1 mmol) in dry THF was added *t*-butyllithium (2.5 mL, 1.5 M solution) at –78 °C. After 1 h at this temperature acetone (2.7 mL, 37 mmol) was added and the reaction mixture was warmed to room temperature. After extraction with 2 N NaOH the aqueous phase was acidified with HCl and extracted with ethyl acetate. The solvent was removed and the residue was dissolved in methanol (10 mL) and treated with diazomethane (60% in diethylether, 10 mL). The solution was then evaporated in vacuo and the residue purified by column chromatography (*n*-hexane/ethyl acetate 3:1 as eluent, slower eluted band). White crystals, yield 32%. Mp 131–132 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.70 (s, 6H), 3.98 (s, 3H), 4.24 (s, 3H), 5.41 (s, 1H), 7.08 (dd, J = 5 Hz, J = 2 Hz, 1H), 7.22 (d, J = 5 Hz, 1H), 7.24 (dd, J = 5 Hz, J = 5 Hz, J = 2 Hz, 1H).

1,2,3,4-Tetrahydro-9-isopropyl-10-methoxypyrazino[1,2-a]-indol-1-on ***9i***
A solution of **9k** (315 mg, 1.1 mmol) in saturated ethanolic HCl (12 mL) was hydrogenated over Pd on carbon. The catalyst was filtered off and the solvent was removed to yield **9i** (97%). Yellow crystals. Mp 192–193 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (d, J = 7.5 Hz, 6H), 3.75 (m, 2H), 3.91 (sept, J = 7.5 Hz, 1H), 4.12 (s, 3H), 4.15 (dd, J = 5 Hz, 2H), 7.03 (d, J = 6 Hz, 1H), 7.09 (d, J = 9 Hz, 1H), 7.30 (dd, J = 9 Hz, J = 6 Hz, 1H).

Cell culture and membrane preparation

Membranes obtained from NIH 3T3 cells lines expressing either human $5HT_{1A}$, human $5HT_{2A}$ or human $5HT_{2C}$ receptors were kindly donated by N Stam (NV Organon). For each receptor subtype, a single batch of membranes were grown using fermentation techniques described previously [13].

Radioligand binding assays

Radioligand binding assays were as previously described for the human $5HT_{2A}$ receptor with minor modifications for the labeling of human $5HT_{1A}$ and human $5HT_{2C}$ receptors. Briefly, on the day of the experiment, membranes were thawed and resuspended in 10x the original volume of assay buffer. This gives a concentration of approximately 4 x 10^5 cells per assay tube. This assay buffer consisted of Tris-HCl 50 mM, pargyline 10^{-5} M, $MgCl_2$ 5 mM and ascorbic acid 0.1% pH 7.4. All compounds were dissolved in 10% DMSO and diluted in assay buffer. Assays were similar for each receptor and consisted of 100 µL of membrane preparation (depending on the assay), 50 µL of radioligand ([^{3}H]-5HT 1 nM final concentration for labeling human $5HT_{1A}$ and human $5HT_{2C}$ receptor binding sites and [^{3}H]-DOB 1 nM final concentration for labeling human $5HT_{2A}$ receptors). Non-specific binding was defined in the presence of 10 µM 5HT for human $5HT_{1A}$ and $5HT_{2C}$ receptor and 10 µM methysergide for human $5HT_{2A}$ receptor. All incubations were performed at room temperature for 1 h and the reactions stopped by rapid filtration through Whatmann GF/B filters. The filters were washed with 3 x 2 mL of Tris-HCl (50 mM, pH 7.4) and the radioactivity retained on the filters was measured by scintillation spectroscopy in 2 mL of scintillation fluid. All experiments were performed in triplicate and repeated at least three times.

Saturation analyses were performed for each receptor using at least eight concentrations of each radioligand (concentrations ranging from 0.05 to 10 nM). Dissociation constants (K_d) were calculated using the EBDA/LIGAND program [14, 15]

Displacement curves were constructed for each compound at each receptor using seven concentrations of the displacing agents (one data point per log unit of concentration: 10^{-11} to 10^{-5} M). Displacement curves were analyzed using EBDA/LIGAND to calculate pK_i values.

Radioligands

Radioligands were purchased from New England Nuclear. The specific activities of [^{3}H]-5HT and [^{3}H]-DOB were 29.7 and 15.0 Ci/mmol.

Tissue preparation for measurement of IP_3 production

$5HT_{2C}$ receptor-mediated stimulation of IP_3 production was measured in the choroid plexus of the rat. The choroid plexus was removed, placed in 200 µL of oxygenated Krebs solution and incubated with 0.35 nmol myoinositol and 0.35 nmol [^{3}H]myoinositol for 1 h at 37 °C. During this incubation, the tubes were gassed with 95% oxygen/5% CO_2 every 20 min. A mixture of LiCl and pargyline was then added (final concentration: LiCl = 10 mM, pargyline = 10 µM) and 10 min later the test compounds (final incubation volume = 250 µl). Dose–response curves were constructed from data obtained from three separate measures per data point. The mixture was incubated for a further 30 min at 37 °C. The assays were stopped by the addition of 25 µL of a stopping solution ($HClO_4$ 2.64 N + EDTA 40 mM). Assay tubes were frozen on dry ice for 15 min, thawed and then kept on ice for 1 h. The tubes were then centrifuged for 20 min at 24 000 *g*. Then, 250 µL of the supernatant was removed and placed in Eppendorf tubes together with 25 µL 4 M KOH. The sample were mixed well and kept on ice for 15 min. These samples were then recentrifuged for 15 min at 14 000 rpm. We removed 230 µL of supernatant and added 30 µL of phytic acid. The isolation of IP3 was as described previously [16].

A concentration–response curve was constructed for 5HT, mCPP and the synthesized compounds. Six concentrations were used per test compound with the highest concentration tested being 0.1 mM. The maximal effect produced by each compound was compared to the stimulation induced by 10 µM 5HT in order to calculate the relative intrinsic activity.

Depression model

Male albino Wistar rats (300–400 g) were implanted with intracranial electrodes into the ventral tegmental area. Rewarding and motivational properties induced by electrical stimulation of this area are used to train the rats for self-stimulation behavior. This behavior allows measurement of the animals' sensitivity to reward. After recovery from surgery, they were trained to

poke their nose into a hole in the side-wall of the test chamber for interrupting a light beam to trigger brain stimulation. Self-stimulation training was continued until stable responding was achieved. Subsequently, the threshold for self-stimulation behavior was determined as previously described [11]. This threshold is considered to reflect the motivational level of each animal and is therefore taken as an anhedonia index (the higher this index, the more 'depressed' the rats are). Animals were then submitted to a three-week stress regimen consisting of a variety of unpredictable, mild stressors. During this period, different groups of stressed and nonstressed animals were injected ip twice daily (around 7 am and 3 pm) with either physiological saline (5 mL/kg) or **10j** (3 mg/kg). The dose was calculated as the salt. The anhedonia index was determined twice weekly throughout the experiment. Results were analyzed by analysis of variance followed by Dunnett's *t*-test. A *P*-value equal or less than 0.01 was taken as statistically significant.

Panic anxiety model

Male albino Wistar rats (300 g) were implanted with intracranial electrodes into the dorsal periaqueductal gray. Electrical stimulation of this area elicits strong aversive reactions and escape attempts which can be shaped into operant self-interruption behavior. Rats were trained to terminate the stimulation by escaping from one compartment into the opposite compartment of the test chamber. Escape performance (time to turn off the stimulation) was recorded by a computerized sytem. Threshold for self-interruption behavior (sensitivity to aversion) was then determined just before and 30 min after ip drug administration. The drug was dissolved in 0.3% v/v Tween 80 in physiological saline and injected in a volume of 5 mL/kg. Doses refer to the weight of the salt. Drug effects were evaluated by comparing postinjection values of individual dose versus vehicle effects using an unpaired Student's *t*-test, with a *P*-value of equal or less than 0.05 accepted as statistically significant.

Obsessive-compulsive disorder models

Compulsive drinking in rats
Prior to testing, female albino Wistar rats (200–300 g) were maintained on a restricted diet at 80% of their free feeding body weight. To induce compulsive excessive drinking, rats were placed in an operant chamber where a pellet dispenser delivered one 45 mg food pellet on a fixed time schedule of 1 min throughout the 1 h daily session (stress condition). In a control condition (no stress), all 60 pellets were placed in the dispenser cup prior to the start of testing. Water was available at all times in the test chamber. Test sessions under treatment alternated with sessions without treatment in order to maintain stable responding. Rats were given orally either **10j** (30 mg/kg po) or vehicle 30 min prior to the start of testing. The dose was calculated as the salt. Water intake was measured to the nearest gram. Data were analyzed by a two-tailed Wilcoxon test, with a *P*-value of equal or less than 0.01 accepted as statistically significant.

Compulsive scratching in monkeys
Adult male squirrel monkeys (about 1 kg body weight) received oral administration of vehicle (1 mL/kg of 0.3% v/v Tween 80 in water; $N = 11$) or **10j** (10, 20 or 30 mg/kg; $N = 2$) followed 15 min later by sc administration of 0.1 mg/kg 8-OH-DPAT. This dose of 8-OH-DPAT was selected as the lowest to reliably induce compulsive whole-body scratching behavior in squirrel monkeys. Doses refer to the weight of the salt. The number of scratching bouts was then scored during the 2 h following treatment. Due to the small number of monkeys used in this observation experiment, descriptive statistics are used in presenting the results.

References

1 Zifa E, Fillion G (1992) *Pharmacol Rev* 44, 401–458
2 Kennett GA (1993) *Curr Opin Invest Drugs* 2, 317–362
3 Caccia S, Ballabio M, Samanin R, Zanini MG, Garattini S (1981) *J Pharm Pharmacol* 33, 477–478
4 Rajur SB, Merwade AY, Hendi SB, Basanagoudar LD (1989) *Indian J Chem* 28B, 1065–1068
5 Mokrosz JL, Boksa J, Bojarski AJ, Charakchieva-Minol S (1993) *Med Chem Res* 3, 240–248
6 Berendsen HHG, Jenck F, Broekkamp CLE (1990) *Psychopharmacology* 101, 57–61
7 Moreau JL, Scherschlicht R, Jenck F, Martin JR (1995) *Behav Pharmacol* 6, 682–687
8 Jenck F, Moreau JL, Martin JR (1995) *Psychiat Res* 57, 181–191
9 Woods A, Smith C, Szewczak M, Dunn RW, Cornfelt M, Corbett R (1993) *Psychopharmacology* 112, 195–198
10 Moreau JL, Griebel G, Jenck F, Martin JR, Widmer U, Haefely WE (1992) *Brain Res Bull* 29, 901–904
11 Moreau JL, Bourson A, Jenck F, Martin JR, Mortas P (1994) *J Psychiatr Neurosci* 19, 51–56
12 von Auwers K (1912) *Liebigs Ann Chem* 393, 338–383
13 Sleight AJ, Stam NJ, Mutel V, Vanderheyden PML (1996) *Biochem Pharmacol* 51, 71–76
14 McPherson GA (1985) Biosoft, Cambridge
15 Munson P, Rodbard D (1980) *Anal Biochem* 107, 220–239
16 Bourson A, Wanner D, Wyler R et al (1995) *Pharmacol Biochem Behav* 53, 107–114
17 Berendsen HHG, Jenck F, Broekkamp CLE (1989) *Pharmacol Biochem Behav* 33, 821–827