Novel Agonists of 5HT$_{2C}$ Receptors. Synthesis and Biological Evaluation of Substituted 2-(Indol-1-yl)-1-methylethylamines and 2-(Indeno[1,2-b]pyrrol-1-yl)-1-methylethylamines. Improved Therapeutics for Obsessive Compulsive Disorder†

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Received January 14, 1997

The syntheses of a series of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno[1,2-b]pyrrol-1-yl)-1-methylethylamines are reported. The binding affinities of the compounds at 5HT$_{2C}$ and 5HT$_{2A}$ receptors (79% homology in the transmembrane domain) were determined. The ligands displayed selectivity for 5HT$_{2C}$ receptors relative to 5HT$_{2A}$ receptors. Compounds were functionally characterized both in vitro and in vivo as 5HT$_{2C}$ receptor agonists. 5f, 5l, 5n, 5o, 5q, 14c, 14f, 14k, and 14m exhibited anticomulsive activity in an animal model of obsessive compulsive disorder.

Introduction

Selective serotonin reuptake inhibitors (SSRIs) increase extracellular levels of serotonin (5HT) and thereby nonselectively cause stimulation of all postsynaptic 5HT receptor subtypes. SSRIs have become standard therapy for neuropsychiatric disorders such as obsessive compulsive disorder (OCD), depression, and panic anxiety. There is accumulating evidence for the involvement of 5HT$_{2C}$ receptor-mediated functions in the therapeutic efficacy of SSRIs. The increased 5HT synaptic content resulting from the reuptake inhibition also allows 5HT to act on the other 5HT receptor subtypes, possibly explaining some of the side effects associated with SSRI treatment. Selective 5HT$_{2C}$ receptor agonists, therefore, may represent a direct means to produce the beneficial therapeutic effects of SSRIs without concomitant side effects.

Our goal was to find 5HT$_{2C}$ receptor agonists which (i) display at least 10-fold selectivity versus the 5HT$_{2A}$ receptor subtype, for which sequence homology of the transmembrane region is high, (ii) show in vivo activity after oral administration in functional models of 5HT$_{2C}$ receptor activation, and (iii) demonstrate therapeutic potential in an animal model of obsessive compulsive disorder.

Gliennon et al. have shown that N,N-dimethylisotryptamines, i.e. derivatives of N,N-dimethyl-2-(indol-1-yl)ethylamines are isosteric with the corresponding N,N-dimethyltryptamines with respect to serotonin receptor affinity. Such compounds are readily available via N-alkylation. We therefore screened isotryptamines for 5HT$_{2C}$ receptor affinity and extended our study to the methylene homologues 1,4-dihydroindeno[1,2-b]pyrroles.

In this paper we report on the synthesis and the pharmacology of indoles and 1,4-dihydroindeno[1,2-b]pyrroles in which a 2-aminopropyl side chain is attached to the N atom of the heterocycle. In analogy to phenylalkylamines, the α-methyl group was incorporated in order to suppress metabolic side chain deamination and to increase the lipophilicity of the compounds, allowing better CNS penetration. Within these series of compounds we have identified agonists to the 5HT$_{2C}$ receptor binding with high affinity and selectivity versus the 5HT$_{2A}$ receptor. Some of these new ligands were evaluated in rats in the schedule-induced polydipsia paradigm, an animal model of obsessive compulsive disorder. As a comparison we have included 5-fluoroα-tryptamine (15) and fluoxetine in our study.

Chemistry

Substituted 2-(indol-1-yl)-1-methylethylamines 5 were prepared according to Scheme 1. Deprotonation of indoles 1 followed by alkylation with propylene oxide led to the secondary alcohols 2. S$_{N}$2 reaction of the corresponding mesylates 3 with sodium azide and reduction of the azides 4 with either hydrogen or LiAlH$_4$ produced the amines 5 with excellent yields. The enantiomerically pure compounds 5k–q were prepared from the (R)- or (S)-epoxide with inversion of configuration at the stereogenic center. The monosubstituted indoles are commercially available.

The dihalogenated building blocks can be prepared from the corresponding dihalogenated nitrotoluenes as described in the patent literature. For the synthesis of 5-chloro-6-fluorindole 1k we have adopted a protocol developed by Wender and White (Scheme 2). 2-Bromo-4-fluorophenylamine (6) was acylated with trifluoroacetic anhydride to give 7. Upon treatment with methyllithium and tert-butyllithium, a dilithium

† Dedicated to Prof. Dr. Dieter Seebach on the occasion of his 60th birthday.
reagent was formed which underwent cyclization with chloroacetaldehyde to the hydroxymide. Dehydration followed by hydrolysis gave 1k in nine steps and an overall yield of 15%. The preparation of the substituted 1,4-dihydroindeno [1,2-b]pyroles is shown in Scheme 3. Alkylation of the indan-1-ones 9 was performed by Claisen rearrangement of an in situ formed allyl vinyl ether system. Ozonolysis of 10 and subsequent cleavage of the acetal with TFA led to the 1,4-dicarbonyl compounds 11, which were then reacted with commercially available 1-amino-2-propanol ([S], (R), (RS)) to yield the 1,4-dihydroindeno [1,2-b]pyroles 12. The secondary amines were transformed into the amines 14 via the azides 13 as described for the synthesis of the indole derivatives 5.

Pharmacology

The affinity of the compounds for 5HT₂C and 5HT₂A human receptors was assessed using displacement of [³H]5HT and [³H]DOB, respectively. To assess functional efficacy at 5HT₂C receptors, the ligands were evaluated for stimulation of phosphoinositide turnover in the choroid plexus of the rat. The compounds were also assessed for induction of penile erection in rats which is a symptom of the serotonin syndrome reflecting 5HT₂C receptor activation in rodents. Finally, compounds which displayed interesting in vivo activity were further tested in the schedule-induced polydipsia model of OCD in rats for potential anticomulsive effects.

Results

The radioligand binding experiments (Table 1) showed higher affinity of the indoles 5 and the inden[1,2-b]-pyrroles 14 for 5HT₂C binding sites than for the structurally (79% homology between the transmembrane regions) very similar 5HT₂A receptor. Compounds with halogen substituents in position 4, 5, and 6 of the indole ring possess higher affinities for this receptor subtype as compared to derivatives bearing electron-donating substituents such as methoxy and methyl groups. The dihalogenated indoles showed the highest 5HT₂C receptor affinities. The (S)-enantiomers display higher af-
The effect of the 5HT$_2C$ receptor ligands in stimulating phosphoinositol formation (cf. IP$_3$ formation, pEC$_{50}$ intrinsic activity, Table 1) was studied in rat choroid plexus. Compounds 5a–d, 14a, and 14d induced only a submaximal increase whereas the maximum responses of the other derivatives 5e–q, 14b, 14c, 14e–m, and 15 were the same as that produced by 5HT (10$^{-5}$ M, intrinsic activity = 1), suggesting that these ligands are full agonists at the 5HT$_2C$ receptor (cf. 5n, 14m, and 15, Figure 1). In vivo results, i.e. induction of penile erections, are presented in Table 2 (although not shown here, the reference compound fluoxetine was found to induce penile erection with ED$_{50}$ = 4.3 mg/kg sc).

SSRIs such as fluoxetine are currently in use for the treatment of OCD. These drugs, however, exhibit a delayed onset of action and less than optimal therapeutic efficacy. Schedule-induced polydipsia in rats has been proposed as a model of OCD.$^5$ In this model, food-deprived rats which receive intermittently delivered food pellets on a fixed-time schedule typically develop a pattern of excessive drinking, i.e. polydipsia. This paradigm has been pharmacologically validated as a model of OCD. Experimental compounds are tested in this model for their ability to attenuate polydipsic behavior, i.e. for their potential anti-OCD effects. The selected 5HT$_2C$ receptor agonists evaluated in the schedule-induced polydipsia model all significantly reduced the excessive drinking with MED values (minimal effective dose; i.e. the lowest dose tested which was found to statistically significantly reduce water intake relative to vehicle treatment) within the dose range 1–30 mg/kg (ip) with doses selected at half-logarithmic units (cf. Table 3). The magnitude of the suppression of polydipsia was compared among all of these compounds for the doses up to 10 mg/kg and was found to be 75% or more. In comparison, fluoxetine was much less potent, first achieving a statistically significant reduction in water intake of only 11% at 30 mg/kg (ip) (with no appreciable effect at doses up to 10 mg/kg ip).

Conclusions

Compounds were identified which exhibited high-affinity binding to human 5HT$_2C$ receptors with selectivity versus 5HT$_2A$ receptors. Such compounds were characterized in vitro and in vivo as 5HT$_2C$ agonists. Two of these compounds underwent a broad binding evaluation: 5l and 14k exhibited affinity for several other 5HT receptor subtypes (1A, 3, 4, 6, 7) which was at least 2 logarithmic units lower than for 5HT$_2C$ receptors and had IC$_{50}$ ≥ 1 µM for 26 other receptors across numerous different neurotransmitter systems (unpublished results). In the isolated rat fundus strip assay, both 5l and 14k act as agonists at the 5HT$_2B$ receptor (pD$_2$ = 8.0 and 6.1, respectively; unpublished results). At present little is known concerning the physiological function of 5HT$_2B$ receptors, due in part to the lack of highly selective ligands; interestingly a 5HT$_2C$ receptor agonist which is structurally different from those described in this report and which exhibited antagonistic activity at the 5HT$_2B$ receptor was also found to reduce schedule-induced polydipsia (unpublished results). Therefore the 5HT$_2B$ receptor is unlikely to play a major role in the functional effects described here.

These 5HT$_2C$ receptor agonists were found to significantly suppress schedule-induced polydipsia in rats, even at doses lacking any appreciable effects on spontaneous behavior. These results suggest that 5HT$_2C$ receptor agonists may be of therapeutic value in OCD. In this respect, it is interesting to note that although fluoxetine was found to be active in this animal model of OCD, its potency was low when compared to the dose range producing adverse effects. 5HT$_2C$ receptor agonists may, thus, potentially offer improved therapy of OCD.

**Experimental Section**

**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
Preparation of (R)-1-(6-Chloro-5-fluorindol-1-yl)propan-2-ol (2l) (Standard Procedure A).

To a mixture of sodium hydride (0.09 g, 3.7 mmol) in THF (15 mL) was added 6-chloro-5-fluorindole (1k) (0.5 g, 3 mmol) at 0°C. After 1 h (R)-propylene oxide (0.42 mL, 6 mmol) was added, and the mixture was stirred at room temperature for 30 min. The reaction was quenched with water, and the mixture was extracted with diethyl ether and washed with brine. The organic layer was dried, and the solvent was removed. The residue was subjected to chromatography (toluene/ethyl acetate, 1:1, as eluant) to yield 2l (0.51 g, 74%) as white crystals: mp 104–105°C; [α]D398 = –60.4° (c = 0.25, CHCl₃); ¹H NMR (CDCl₃) δ 1.61 (d, J = 5 Hz, 1 H), 2.56 (dd, J = 3.0, 2.5 Hz, 1 H), 3.74 (d, J = 5 Hz, 1 H), 4.20 (m, 1 H), 6.45 (m, 1 H), 6.14 (d, J = 3.2 Hz, 1 H), 7.17 (d, J = 3.2 Hz, 1 H), 7.07 (d, J = 9.5 Hz, 1 H), 7.38 (d, J = 7.5 Hz, 1 H); MS (EI) m/z 227 (M⁺), 182 (100). Anal. (C₁₁H₁₃FN₂ClO) C, H, N.

Preparation of (S)-1-(2-Azidopropyl)-6-chloro-5-fluorindole (3l) (Standard Procedure B).

To a solution of 2l (0.28 g, 1.2 mmol) in dichloromethane (6 mL) and triethylamine (0.5 mL) was added methanesulfonyl chloride (0.2 mL, 2.5 mmol) at 0°C. After 1 h, the mixture was added and the mixture was extracted with 1 M sodium carbonate and washed with ether. The organic layer was dried, and the solvent was removed. The residue was subjected to chromatography (toluene as eluant) to yield 3l as yellow oil (0.26 g, 1 mmol): mp 185–186°C; [α]D398 = –28.8° (c = 0.25, MeOH); ¹H NMR (CDCl₃) δ 1.49 (d, J = 14.5 Hz, 1 H), 1.30 (d, J = 14.5, 7.5 Hz, 3 H), 6.37 (d, J = 14.5 Hz, 1 H), 6.49 (s, 1 H), 4.28 (d, J = 3 Hz, 1 H), 6.43 (d, J = 7 Hz, 1 H), 7.05 (t, J = 7.5 Hz, 1 H), 7.13 (d, J = 7.5 Hz, 1 H), 7.25 (d, J = 3 Hz, 1 H); MS (EI) m/z 204 (M⁺), 161, 44 (100). Anal. (C₁₂H₁₂ClF₂N₂O₂) C, H, N.
in 2,2-dimethoxypropane (200 mL) was boiled under reflux for 64 h on a Dean–Stark trap filled with molecular sieves (0.4 mm, 2 nm pearl shaped). The solvents were evaporated, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 6:1) to give 10d (12.7 g, 51%) as a yellow oil: \[\text{H NMR (CDCl}_3\text{)} \delta 1.18 (s, 3 H), 1.45 (s, 3 H), 1.69 (d, \text{J} = 2 Hz, 3 H), 2.18 (s, 1 H), 2.39 (s, 1 H), 2.43 (m, 1 H), 2.65 (m, 1 H), 5.58 (m, 2 H), 7.41 (m, 2 H), 7.50 (s, 1 H); MS (EI) m/z 228 (M+), 213 (M+ − Me), 173 (100), 158, 115, 55.

Compounds 10a–h and 10j–k were prepared in the same way from 4-methoxyindan-1-one (9a), 5-methoxyindan-1-one (9b), 6-methoxyindan-1-one (9c), 7-methoxyindan-1-one (9d), 6-fluorooindan-1-one (9e), 6-bromoindan-1-one (9f), 6-fluoro-3,3-dimethylindan-1-one (9g), 6-fluoro-3,3-dimethylindan-1-one (9k), 12–13, and 6-methoxy-3,3-dimethylindan-1-one (9k), 12–15 respectively.

Preparation of (R,S)-2-(2-Oxooethyl)-3,3,6-trimethyl-indan-1-one (11i) (Standard Procedure E). An oxygen stream (2.5 g of ozone) was conducted for 1 h while stirring through a solution of 10l (12.7 g, 55.6 mmol) in dichloromethane (200 mL) and methanol (40 mL) cooled to −70 °C. Subsequently, the solution was washed with oxygen for 5 min and with argon for 10 min. After the addition of dimethyl sulfoxide (6.12 mL, 83.4 mmol), the mixture was stirred at room temperature for 18 h. The reaction mixture was evaporated, the residue was treated with dichloromethane (150 mL) and after the addition of water (25 mL) and trifluoroacetic acid (25 mL), the mixture was stirred at room temperature for 2.5 h. The mixture was subsequently poured into water (150 mL) and neutralized while stirring by addition of hydrogen carbonate. Water (100 mL) was added, the phases were separated, and the aqueous phase was extracted twice with dichloromethane (150 mL each time). The combined organic phases were dried (magnesium sulfate) and concentrated to give 11i (11.3 g, 94%) as light yellow oil: \[\text{H NMR (CDCl}_3\text{)} \delta 1.11 (s, 3 H), 1.51 (s, 3 H), 2.41 (s, 3 H), 2.61 (m, 1 H), 3.04 (m, 2 H), 7.40 (d, \text{J} = 7 Hz, 1 H), 7.46 (d, \text{J} = 7 Hz, 1 H), 7.52 (s, 1 H), 9.99 (s, 1 H); MS (EI) m/z 186 (M+ − CO), 173 (100), 159, 145, 128, 43.

Preparation of (R)-1-(4,4,7-Trimethyl-1,4-dihydroindeno[1,2-b]pyrrolo-1-yl)propan-2-ol (12k) (Standard Procedure F). A solution of 11j (2.16 g, 10 mmol) and p-toluene sulfonic acid (80 mg) in toluene (90 mL) was heated on a Dean–Stark trap. A solution of (R)-1-amino-2-propanol (3.0 g, 40 mmol) in toluene (20 mL) was added dropwise over a period of 5 min. Subsequently, the mixture was boiled for an additional 45 min, during which the solvent was reduced to a volume of 20 mL. The cooled reaction mixture was purified by column chromatography (ethyl acetate/hexane, 1:2) to yield 12k (1.5 g, 59%) as a brown oil: \[\text{H NMR (CDCl}_3\text{)} \delta 0.29 (d, \text{J} = 3 Hz, 2 H), 1.41 (s, 3 H), 2.34 (s, 3 H), 3.99 (m, 1 H), 4.18 (d, \text{J} = 7 Hz, 1 H), 6.11 (d, \text{J} = 7 Hz, 1 H), 6.90 (d, \text{J} = 7 Hz, 1 H), 7.07 (s, 1 H), 7.21 (d, \text{J} = 7 Hz, 1 H); MS (EI) m/z 255 (M+), 240 (100), 194.

Preparation of (S)-1-(2-Azidopropyl)-4,4,7-trimethyl-1,4-dihydroindeno[1,2-b]pyrrole (13k) (Standard Procedure G). Methylene sulfoxide chloride (0.91 mL, 11.7 mmol) was added dropwise while stirring to a solution, cooled to 0 °C, of 12k (1.5 g, 5.87 mmol) and triethylamine (3.27 mL, 23.5 mmol) in dichloromethane (50 mL), and the mixture was stirred at this temperature for an additional 1.5 h. The reaction mixture was subsequently diluted with dichloromethane (150 mL), washed twice with saturated sodium hydrogen carbonate solution (70 mL each time) and once with brine (70 mL), dried (magnesium sulfate), and evaporated. The residue was dissolved in DMF (50 mL) and treated with sodium azide (0.76 g, 11.7 mmol) and the mixture was heated to 60 °C for 15 h while stirring. After cooling the solution was poured into water (100 mL) and extracted twice with ethyl acetate (100 mL each time). The combined organic phases were washed once with water (100 mL), and the organic phase was dried (magnesium sulfate) and evaporated. The residue was purified by column chromatography (hexane/ethyl acetate, 1:1) to give 13k (1.13 g, 68%) as a reddish oil: \[\text{H NMR (CDCl}_3\text{)} \delta 1.30 (d, \text{J} = 5 Hz, 3 H), 1.41 (s, 3 H), 2.39 (s, 3 H), 3.92 (m, 1 H), 4.09 (m, 2 H), 6.12 (d, \text{J} = 2 Hz, 1 H), 6.65 (d, \text{J} = 2 Hz, 1 H), 6.90
Preparation of (S)-(2,4,4-Trimethyl-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:1) (14k) (Standard Procedure H). 13k (1.1 g, 3.92 mmol) dissolved in ethanol (50 mL) was hydrogenated over platinum oxide (10 mg) for 4 h at room temperature. The catalyst was subsequently filtered off and rinsed with ethanol, and the solvent was evaporated. The residue was dissolved in ether (80 mL), filtered, and treated while stirring with a solution of fumaric acid (455 mg, 3.92 mmol) in methanol (15 mL). The mixture was stirred at room temperature for 24 h, and the crystals were subsequently filtered off to give 14k (805 mg, 77%) as a white solid: mp 196 °C; [α]D = +11.2° (c = 0.25, MeOH); 1H NMR (DMSO-d6) δ 1.01 (d, J = 5 Hz, 3 H), 1.32 (s, 6 H), 2.32 (s, 3 H), 3.44 (m, 1 H), 4.14 (dd, J = 10, 7 Hz, 1 H), 4.41 (dd, J = 10, 4 Hz, 1 H), 6.06 (d, J = 2 Hz, 1 H), 6.71 (d, J = 2 Hz, 1 H), 7.23 (d, J = 7 Hz, 1 H), 7.33 (s, 1 H); MS (EI) m/z 254 (M+), 211, 196, 44 (100). Anal. (C17H21N2O4) C, H, N.

Compounds 14a–j and 14l,m were synthesized according to standard procedures D, E, F, and G.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.5) (14a): 83%; mp 194 °C; 1H NMR (DMSO-d6) δ 1.01 (d, J = 5 Hz, 3 H), 3.30 (s, 3 H), 3.31 (m, 1 H), 3.63 (s, 3 H), 4.08 (dd, J = 10, 7 Hz, 1 H), 4.21 (dd, J = 10, 4 Hz, 1 H), 6.08 (d, J = 2 Hz, 1 H), 6.61 (d, J = 2 Hz, 1 H), 6.65 (s, 1 H), 6.76 (d, J = 7 Hz, 1 H), 6.85 (d, J = 2 Hz, 1 H), 7.22 (m, 2 H); MS (EI) m/z 242 (M+), 199, 44 (100). Anal. (C16H19FN2O4) C, H, N.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.5) (14b): 81%; mp 189 °C; 1H NMR (DMSO-d6) δ 1.02 (d, J = 5 Hz, 3 H), 3.33 (m, 1 H), 3.39 (s, 3 H), 3.75 (s, 3 H), 4.05 (dd, J = 10, 7 Hz, 1 H), 4.23 (dd, J = 10, 4 Hz, 1 H), 6.09 (d, J = 2 Hz, 1 H), 6.46 (s, 1 H), 6.77 (d, J = 2 Hz, 1 H), 6.79 (dd, J = 7 Hz, 2 H), 7.08 (d, J = 2 Hz, 1 H), 7.44 (d, J = 7 Hz, 1 H); MS (EI) m/z 242 (M+), 199, 44 (100). Anal. (C16H19FN2O4) C, H, N.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.52) (14c): 74%; mp 203 °C; 1H NMR (DMSO-d6) δ 1.01 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.11 (d, J = 2 Hz, 1 H), 6.46 (s, 1 H), 6.63 (dd, J = 7, 2 Hz, 1 H), 6.85 (d, J = 2 Hz, 1 H), 7.08 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) m/z 242 (M+), 199, 44 (100). Anal. (C16H19FN2O4) C, H, N.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.5) (14e): 68%; mp 207 °C; [α]D = −21.6° (c = 0.25, MeOH); 1H NMR (DMSO-d6) δ 1.02 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.11 (d, J = 2 Hz, 1 H), 6.46 (s, 1 H), 6.63 (dd, J = 7, 2 Hz, 1 H), 6.86 (d, J = 2 Hz, 1 H), 7.09 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) m/z 242 (M+), 199, 44 (100). Anal. (C16H19FN2O4) C, H, N.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.5) (14f): 77%; mp 206 °C; [α]D = +23.2° (c = 0.25, MeOH); 1H NMR (DMSO-d6) δ 1.01 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.11 (d, J = 2 Hz, 1 H), 6.44 (s, 1 H), 6.45 (d, J = 2 Hz, 1 H), 6.86 (d, J = 2 Hz, 1 H), 7.09 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) m/z 242 (M+), 199, 44 (100). Anal. (C16H19FN2O4) C, H, N.

(RS)-2-(5-Methoxy-1,4-dihydropyridino[1,2-b]pyrrol-1-yl)-1-methylthelylamine fumarate (1:0.5) (14g): 54% mp 194 °C; [α]D = +16.8° (c = 0.25, MeOH); 1H NMR (DMSO-d6) δ 1.02 (d, J = 5 Hz, 3 H), 3.29 (m, 1 H), 3.40 (s, 3 H), 4.09 (dd, J = 10, 7 Hz, 1 H), 4.23 (dd, J = 10, 4 Hz, 1 H), 6.14 (d, J = 2 Hz, 1 H), 6.45 (d, J = 2 Hz, 1 H), 6.83 (d, J = 7, 1 Hz, 1 H), 6.91 (d, J = 2 Hz, 1 H), 7.40 (m, 2 H); MS (EI) m/z 230 (M+), 187, 44 (100). Anal. (C12H13FN2O4) C, H, F, N.


Radioligand Binding Assays. Radioligand binding assays were as previously described for the human 5HT2A receptor with minor modifications for the labeling of human 5HT2C receptors. Briefly, on the day of the experiment, membranes were thawed and resuspended in 10 times the original volume of assay buffer. This gives a concentration of approximately 4 × 10−8 M per 1 mL assay tube. This assay buffer consisted of Tris-HCl 50 mM, pargyline 10−5 M, MgCl2 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 ([3H]-5HT 1 nM final concentration for labeling human 5HT2C receptor binding sites, and [3H]DOB 1 nM final concentration for labeling human 5HT2A receptors). Nonspecific binding was defined in the presence of 100 μM of the selective 5HT2A receptor antagonist mianserin and 10 μM methyleneisergide in the case of the human 5HT2A receptor. All incubations were performed at room temperature for 1 h and the reactions stopped by rapid filtration through Whatman GF/B filters. The filters were washed with 3 × 2 mL of Tris-HCl (50 mM, pH 7.4), and the
radioactivity retained on the filters was measured by scintillation spectrophotometry 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.\(^{11,22} \)

Displacement curves were constructed from data obtained from three separate measures per each compound at each receptor using seven concentrations of the displacing agents (one data point per log unit of concentration: \( 10^{-11} - 10^{-3} \) M). Displacement curves were analyzed using EBDA/LIGAND to calculate \( K_i \) values.

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

**Tissue Preparation and Incubation for Measurement of IP\(_3\) Production.** \(5HT_{EC}\) receptor-mediated stimulation of IP\(_3\) production was measured in the chori-plexus of the rat. The choroid plexus was removed, placed in 200 \( \mu \)L of oxygenated Krebs solution, and incubated with 0.35 nmol of \([^{3H}]\)-myo-inositol for 1 h at 37 °C. During this incubation, the tissues 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 \( \mu \)M) and 10 min later the test compounds (final incubation volume = 250 \( \mu \)L). Dose–response curves were constructed from data obtained from three separate measures per data point. The mixture was incubated for a further 0.5 h at 37 °C. The assays were stopped by the addition of 25 \( \mu \)L 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 2400g. Then, 250 \( \mu \)L of the supernatant was removed and placed in Eppendorf tubes together with 25 \( \mu \)L of 4 M KO\(_2\)H. The samples were then kept on ice for 15 min. These samples were then reconstituted for 15 min at 14 000 rpm. We removed 230 \( \mu \)L of supernatant and added 30 \( \mu \)L of phytic acid. The isolation of IP\(_3\) was described in a previous report.\(^23\)

A concentration response curve was constructed for \(5HT_{EC}\) receptor agonism in vivo, elicitation of penile erection was determined in RORO rats (Biological Research Laboratories, CH-4144 Füllinsdorf, Switzerland). All drugs were dissolved or microsuspended in 0.3% v/v Tween-80 in physiological saline. All drug solutions were freshly prepared and injected subcutaneously (sc) in a volume of 5 mL/kg body weight or administered i.p. in a volume of 10 mL/kg body weight. Control animals were injected with an equivalent volume of vehicle. When drug solutions were prepared from store bottles, they were always available during test sessions with intake measured to the nearest 1 g. Evaluation was done to compare the effect of each dose to that obtained for the vehicle condition using a two-tailed Wilcoxon test with a p-value of ≤ 0.05 accepted as statistically significant. The lowest dose tested which yielded a statistically significant difference to vehicle treatment (MED, minimum effective dose) was determined.

**Acknowledgment.** We would like to express our thanks to Serge Burner, Rolf Canesso, Annick Gruschwitz, Benedikt Hofstetter, and Philipp Oberli for their skillful technical assistance, Dr. Wolf Arnold for the NMR spectra, Walter Meister for the mass spectra, and Dr. Stephan Müller for the microanalyses.

**References.**


J M970030L