Articles

(+)-*cis*-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]-naphthyridine: A 5-$HT_{2C/2B}$ Receptor Antagonist with Low 5-HT_{2A} Receptor Affinity

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The indolonaphthyridine **8** is described as a selective 5-$HT_{2C/2B}$ vs 5-HT_{2A} receptor antagonist. The compound was synthesized in seven steps starting from indoline and isonicotinic acid chloride. The key step is a photocyclization of the indolinyl tetrahydropyridinocarbamic acid ethyl ester **4** to the *cis*-octahydroindolo[1,7-*bc*][2,6]naphthyridinecarbamic acid ethyl ester **5**. The synthesis was accomplished by reduction with aluminum hydride and racemic resolution. The indolonaphthyridine **8** exerted the binding profile of a selective 5-HT_{2C} receptor ligand (pK_D 7.8) and behaved as an antagonist on the 5-HT-induced accumulation of inositol phosphates in pig choroid plexus cells (pK_B 7.13). Compound **8** dose-dependently inhibited the ACTH response to MK-212 in rats and the MK-212-induced hypophagic effect with an ID_{50} value of 0.3 mg/kg sc. Compound **8** acted as a 5-HT_{2B} receptor antagonist at the rat stomach fundus with a pK_B value of 7.34.

Introduction

The 5-HT_2 receptor family comprises currently three members, i.e., the 2A, 2B, and 2C subtypes.[1] Sequence analysis revealed an >80% homology between their putative transmembrane regions.[2-6] Therefore, it is not surprising that many compounds once believed to be selective for 5-HT_{2A} receptors (former classical 5-HT_2 receptors) also bind with high affinity to 5-HT_{2C} and 5-HT_{2B} sites. The 5-HT_{2B} receptor has only recently been cloned, and consequently few pharmacological data are available.[5,7,8] Of the various 5-HT_2 receptor subtypes, the 5-HT_{2A} receptor is best characterized with respect to distribution and function. Its presence in the brain and periphery has been widely reported, and the receptor has been functionally characterized in the gastrointestinal tract, cardiovascular system, and brain.[9]

The 5-HT_{2B} receptor was initially characterized in the rat stomach fundus, but at present little is known about its presence and function in other regions including the brain.[5,8] The 5-HT_{2C} receptor was initially discovered in the choroid plexus and later throughout the brain, but no convincing evidence is available with respect to its presence in the periphery. The lack of selective 5-HT_{2C} receptor antagonists is still severely hampering the investigation of the (patho)physiological role of these receptors. Due to the very close structural and also pharmacological similarities between 5-HT_{2A} and 5-HT_{2C} receptors, it would not be surprising to discover that some of the functional effects once attributed to 5-HT_{2A} receptor activation may indeed be mediated by 5-HT_{2C} or, as far as the periphery is concerned, 5-HT_{2B} receptors. 5-HT_{2C} receptors have been suggested to play a role in a variety of processes such as feeding, locomotion, CSF production, ACTH release, and diseases like eating disorders, migraine, obsessive compulsive disorders, and anxiety.[10-16] These suggestions are mainly based on preclinical and clinical studies with experimental drugs such as mCPP, MK-212, and TFMPP which are relatively selective 5-HT_{2C} receptor agonists.

We now have discovered in a random screening program for new monoaminergic receptor ligands a 5-HT_{2C} receptor antagonist, **8**, which discriminates between 5-HT_{2A} and 5-HT_{2C} receptor sites. As functional in vitro tests revealed, the compound concomitantly acts as antagonist at peripheral 5-HT_{2B} receptors. The compound is a more potent alternative to the most recently described 5-$HT_{2C/2B}$ receptor antagonist SB 200646A.[17,18] Our paper describes the synthesis and pharmacological characterization of SDZ SER-082 (**8**).

Chemistry

The indolo[1,7-*bc*][2,6]naphthyridine ring system has never been described in the literature. The active principle **8** was prepared via the reaction sequence depicted in Scheme 1. The indoline was condensed with isonicotinic acid chloride to form the amide **1**. The pyridine nitrogen of **1** was methylated by methyl iodide to give **2**, and the quaternary pyridinium salt was subsequently reduced by sodium borohydride according to Ferles[19] to yield the *N*-methyltetrahydropyridine **3**. The molecule **3** possesses an open intramolecular [4 + 2] system ranging from the indoline C_7 to the tetrahydropyridine $C_{3'}$. Therefore, a thermal-induced or photoinduced pericyclic ring closure to the target five-ring system was deemed to be possible. However, all trials to cyclize **3** by UV irradiation or thermally to form the indolonaphthyridine ring system failed. When the

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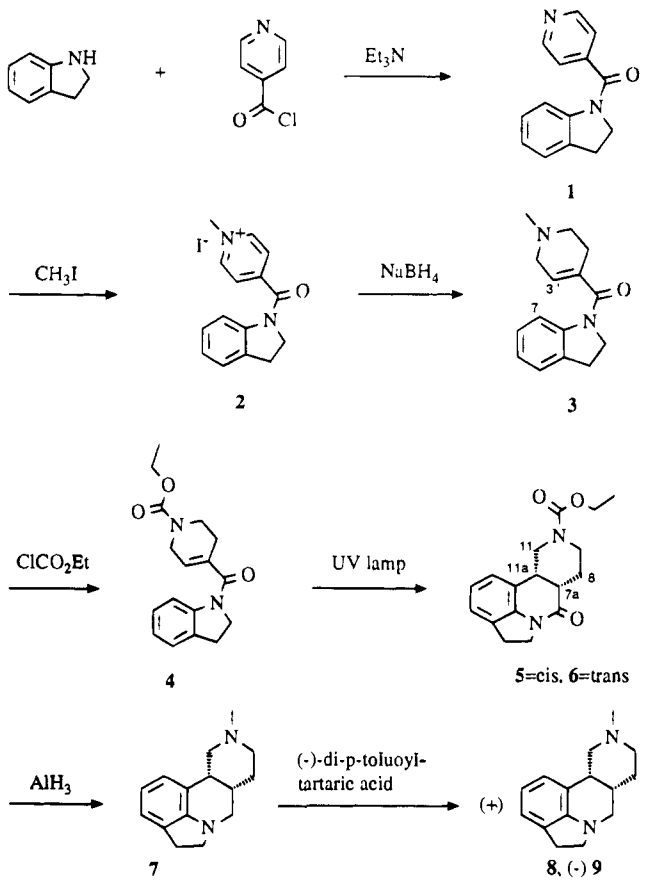
Scheme 1



compound **3** was converted with ethyl chloroformate into the carbamate **4**, the photocyclization ran smoothly. The carbamate **4** thus reacted by irradiation with a broad-spectrum mercury lamp to the desired indolonaphthyridinone **5**. The photoreaction afforded the *cis*- and *trans*-naphthyridinones **5** and **6** in a 2:1 mixture.

An analysis of a 360 MHz ^{1}H NMR spectrum ($CDCl_3$) of **5** confirmed the cis ring junction. Proton H_{7a} appears as a quartet with a chemical shift of 2.94 ppm. An expansion of the data—supported by homonuclear double-resonance experiments (NOE)—indicated H_{7a} coupled to H_8 axial (5.5 Hz) and H_8 equatorial (5.5 Hz). Proton H_{7a} was also coupled to H_{11a} (5.5 Hz). This pattern confirmed that H_{7a} and H_{11a} are in a cis relationship. The same analysis of a ^{1}H NMR spectrum of **6** revealed a coupling of H_{7a} to H_8 axial (12.5 Hz) and H_8 equatorial (3.5 Hz). The H_{7a}/H_{11a} coupling was found to be 12.5 Hz, indicating the trans nature of the compound **6**.

The *cis*-carbamate **5** was recovered by column chromatography and reduced by aluminum hydride to give rise to the racemic indolonaphthyridine **7**. The racemate **7** was resolved with (−)-di-*O*,*O*′-p-toluoyl-L-(+)-tartaric acid to obtain the enantiomerically pure indolonaphthyridine **8**. Applying the antipode acid, **9** was obtained in analogous fashion (Scheme 1). The corresponding trans isomer of **7** can be derived from the reduction of compound **6**. As binding experiments revealed, the trans isomer showed little 5-HT$_{2A/2C}$ selectivity and is therefore not further described in this paper.

Pharmacology

From the radioligand binding experiments listed in Table 1, it is evident that **8**, although not highly potent,

Table 1. Receptor Binding Profile of **8** and **9**[a]

receptor	ligand	affinity[b]
5-HT$_{1A}$	[^{3}H]-8-OH-DPAT	6.1 (<5)
5-HT$_{1B}$	[^{125}I]ICYP	4.6 (nd)
5-HT$_{1D}$	[^{3}H]-5-HT	5.4 (nd)
5-HT$_{2A}$	[^{3}H]ketanserin	6.2 (5.7)
5-HT$_{2C}$	[^{3}H]mesulergine	7.8 (6.7)
5-HT$_3$[c]	[^{3}H]ICS 205-930	4.7 (nd)
adrenergic α_1	[^{3}H]prazosin	5.6 (6.0)
adrenergic α_2	[^{3}H]clonidine	6.5 (6.0)
dopaminergic D_1	[^{3}H]SCH-23390	<5 (<5)
dopaminergic D_2	[^{3}H]spiperone	<5 (<5)

[a] Affinities of the (−)-antipode are given in brackets. [b] Receptor affinities are given as pK_D values (−log, M) ± standard error of the mean (SEM): ±0.1−0.2; the values are of $n = 4$−6 independent determinations performed in triplicate. [c] $n = 2$.

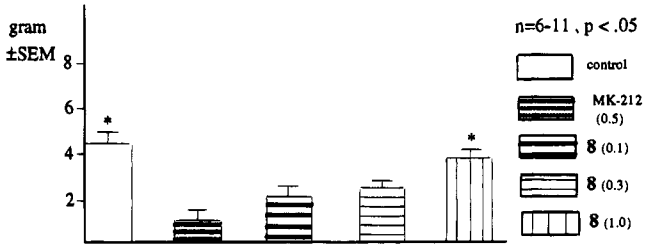


Figure 1. Inhibition of the MK-212-induced hypophagia by **8**. Doses are given in mg/kg sc. **8** dose-dependently antagonized the MK-212 effect on food intake.

shows clear selectivity for 5-HT$_{2C}$ binding sites. Of special note is the selectivity toward the analogous 5-HT$_{2A}$ receptor (i.e., factor of 40). The compound displayed affinity at concentrations up to around 1 μM at 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{2A}$, and adrenergic α_1 and α_2 sites, whereas its affinities for the 5-HT$_{1B}$, 5-HT$_3$, and dopamine D_1 and D_2 receptors were in the 10−50 μM range. The (−)-antipode **9** still showed some selectivity for the 5-HT$_{2C}$ binding site. However, the compound was 10 times less potent (Table 1 in brackets).

The (+)-enantiomer **8** had affinity and selectivity for human recombinant 5-HT$_{2C}$ receptors with a pK_D of 7.38 ± 0.11 (mean ± SEM, $n = 3$).[20] The compound was shown in autoradiographic studies to displace 5-HT$_{2C}$ binding from human choroid plexus at 100-fold lower concentration than from human claustrum 5-HT$_{2A}$ sites.[21]

The naphthyridine **8** was functionally characterized in an in vitro model for 5-HT$_{2C}$ receptor activation, namely the phosphatidylinositol accumulation in pig choroid plexus cells. Compound **8** displayed a low intrinsic activity, which (at 30 μmol/L) amounted to 10% of the effect of 5-HT. The compound **8** (300 nmol/L) antagonized the 5-HT-induced response with a pK_B value of 7.13 ± 0.10 (mean ± SEM, $n = 3$). Also, in vivo **8** behaved as a 5-HT$_{2C}$ receptor antagonist. Ritanserin,[22] a potent 5-HT$_{2A/2C}$ receptor antagonist, was investigated for comparative purposes. In a first model, hypophagia in rats was induced by the selective 5-HT$_{2C}$ receptor agonist MK-212. Ritanserin and **8** dose-dependently antagonized the hypophagic effect of MK-212. Both compounds were approximately equipotent (ID_{50} values of 0.3 mg/kg sc) as shown in Figures 1 and 2. The 5-HT$_{2C}$ receptor agonist also caused an increase in plasma ACTH levels. Similarly, the MK-212-induced rise in plasma ACTH was dose-dependently blocked by **8** and ritanserin (Tables 2 and 3). Compound **8** per se (1 mg/kg sc) did not alter ACTH levels. These results

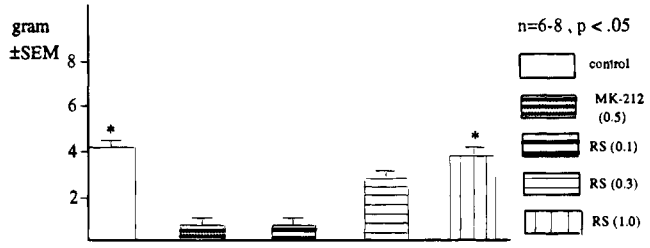


Figure 2. Inhibition of the MK-212-induced hypophagia by ritanserin (RS). Doses given in mg/kg sc. Ritanserin dose-dependently antagonized the MK-212 effect on food intake.

Table 2. Inhibition of ACTH Response to MK-212 by **8** in Rats[a]

pretreatment	treatment	ACTH after 30 min
solvent	solvent	35.8 ± 7.3
solvent	**8** (1.0)	38.5 ± 8.3
solvent	MK-212	321.5 ± 51.0
8 (0.1)	MK-212	165.8 ± 36.6
8 (1.0)	MK-212	95.1 ± 33.6

[a] Data (ng/mL) are means ± SEM of eight experiments. **8** (dose in mg/kg sc) was administered 20 min prior to MK-212 (1 mg/kg sc). Blood sample was taken 30 min after MK-212 application. **8** significantly inhibited MK-212-induced ACTH increase (0.1 mg/kg, $p < 0.05$; 1.0 mg/kg, $p < 0.001$).

Table 3. Inhibition of ACTH Response to MK-212 by Ritanserin in Rats[a]

pretreatment	treatment	ACTH after 30 min
solvent	solvent	30.3 ± 3.8
solvent	ritanserin (1.0)	37.2 ± 6.1
solvent	MK-212	199.3 ± 26.8
ritanserin (0.1)	MK-212	204.3 ± 46.5
ritanserin (1.0)	MK-212	95.4 ± 25.5

[a] Data (ng/mL) are means ± SEM of 8–16 experiments. Ritanserin (dose in mg/kg sc) was administered 60 min prior to MK-212 (1.0 mg/kg sc). Blood sample was taken 30 min after MK-212 application. Ritanserin (1.0 mg/kg) significantly inhibited the ACTH response induced by MK-212 ($p < 0.01$).

clearly establish **8** as a centrally active 5-HT_{2C} receptor antagonist.

Compound **8** was further evaluated in a functional model of 5-HT_{2B} receptor activation, namely the rat stomach fundus.[7,8] The naphthyridine **8** contracted rat fundic strips via stimulation of 5-HT_{2B} receptors in concentrations from 3×10^{-9} to 3×10^{-7} M. A pD_2 value of 7.23 ± 0.09 (mean ± SEM, $p < 0.05$) was calculated with an efficacy of 32%. Compound **8** was also tested as an antagonist of DOI-induced contractions. DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], a 5-HT_{2B} agonist, contracted the rat fundus in concentrations from 10^{-9} to 10^{-7} M. Compound **8** (10^{-7} M) shifted the concentration–response curve of DOI to the right, and from the difference in pD_2 values, a pK_B value of 7.34 ± 0.10 (mean ± SEM, $n = 7$) was calculated. The above findings prove the naphthyridine **8** as a selective 5-$HT_{2C/2B}$ receptor antagonist with low intrinsic activity.

Conclusion

The naphthyridine **8** was found to be a selective and potent 5-$HT_{2C/2B}$ receptor antagonist with low partial agonistic properties in two in vitro tests with a high receptor–effector coupling. The compound is especially devoid of high 5-HT_{2A} receptor affinity. This ligand may therefore serve as a useful tool to elaborate the (patho)-physiological role of the 5-HT_{2C} receptor in the central nervous system without the bias accompanying the classical palette of 5-$HT_{2A/2C}$ ligands.

Experimental Section

Chemistry. ^{1}H NMR spectra were measured on a Bruker Spectrospin 360 MHz (WH-360) 90 MHz (HX-90) or a Varian Gemini 200 MHz spectrometer using Me_4Si as an internal standard. IR spectra were recorded on a Perkin-Elmer 297 spectrophotometer and mass spectra on a Finigan MAT 212. Melting points were determined on a Buchi 512 apparatus and are not corrected. Elemental analyses were within ±0.4% of theoretical values. All reactions were followed by TLC carried out on Merck 60 F254 silica gel plates. Solutions were dried over Na_2SO_4 and concentrated with a Buchi rotary evaporator at low pressure (Milt PVK 600 vacuum controller). Column chromatography was performed with silica gel (Merck 60, 230–400 mesh ASTM) applying a medium performance solvent pump (Matkemi RP). A more polar solvent (usually MeOH) was added during the second part of the chromatography. The determination of the enantiomeric excess (ee) was performed by means of ^{1}H NMR spectroscopy using a solution of (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol (Aldrich) in $CDCl_3$. Salts of bases were generally prepared by adding the particular acid to the base dissolved in acetone or MeOH.

2,3-Dihydro-1-(4-pyridylcarbonyl)-1*H*-indole (1). A suspension of (123.0 g, 1.0 mol) isonicotinic acid in dry EtOH (500 mL) was cooled to 0 °C. To the stirred mixture was added slowly 8 N ethanolic HCl (125 mL, 1.0 mol), and the mixture was kept at 0 °C for 1 h. The precipitated product was filtered off, washed with dry Et_2O, and dried. The obtained isonicotinic acid hydrochloride (150.0 g, 0.94 mol) was solved in $SOCl_2$ (350 mL), refluxed, and stirred vigorously for 7 h. The mixture was stored overnight at room temperature without stirring. The solution was concentrated by evaporation, the residue was taken up three times in dry toluene, and the solvent was evaporated to remove excessive $SOCl_2$. The crude product was recrystallized from dry toluene/Et_2O and dried at 45 °C to yield 166.0 g (93%, based on isonicotinic acid) of isonicotinic acid chloride hydrochloride as colorless crystals. The obtained acid chloride (166.0 g, 0.93 mol) was dissolved in CH_2Cl_2 (800 mL). At 20–25 °C, Et_3N (270 mL, 1.87 mol) was added and, keeping the same temperature, a solution of indoline (104.7 mL, 0.93 mol) in CH_2Cl_2 (250 mL) was slowly dropped into the reaction mixture. The solution was stirred at room temperature for 24 h, stored without stirring for 12 h, and extracted with CH_2Cl_2/H_2O. The organic fraction was dried and evaporated. The dark crude product was recrystallized from CH_2Cl_2/EtOH/hexane to yield 151.0 g (72 %) of **1** as gray-brown crystals: mp 127–128 °C; MS *m/e* 224 (M^+); IR (KBr) ν 1635 cm^{-1} (C=O); ^{1}H NMR (360 MHz, DMSO-d_6) δ 3.12 (t, 2 H, $J = 8.5$ Hz), 3.98 (t, 2 H, $J = 8.5$ Hz), 7.04 (t, 1 H, $J = 7.0$ Hz), 7.15 (t, 1 H, $J = 7.0$ Hz), 7.26 (d, 1 H, $J = 7.0$ Hz), 7.49 (dd, 2 H, $J = 5.0$, 6.0 Hz), 7.72 (br d, 1 H, $J = 7.0$ Hz), 8.70 (dd, 2 H, $J = 5.0$, 6.0 Hz). Anal. ($C_{14}H_{12}N_2O$), C, H, N, O.

4-[(2,3-Dihydro-1*H*-indol-1-yl)carbonyl]-1-methylpyridinium Iodide (2). A solution of **1** (151.0 g, 0.67 mol) in acetone (1500 mL) was heated up to reflux temperature, and within 15 min methyl iodide (92.9 mL, 1.49 mol) was added. Yellow crystals were precipitating from the reaction mixture. After 45 min, additional methyl iodide (46.5 mL, 0.75 mol) was added. The mixture was kept refluxing for 3 h and then cooled to 0 °C. The product was filtered off, washed with ether, recrystallized from acetone/Et_2O, and dried to give 233.0 g (94%) of **2** as a yellow powder: mp 242–243 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 3.15 (t, 2 H, $J = 8.0$ Hz), 3.96 (t, 2 H, $J = 8.0$ Hz), 4.41 (s, 3 H), 7.16 (t, 1 H, $J = 7.0$ Hz), 7.29 (t, 1 H, $J = 7.0$ Hz), 7.35 (d, 1 H, $J = 7.0$ Hz), 8.12 (d, 1 H, $J = 7.0$ Hz), 8.36 (d, 2 H, $J = 6.5$ Hz), 9.17 (d, 2 H, $J = 6.5$ Hz). Anal. ($C_{15}H_{15}IN_2O$) C, H, N, O.

2,3-Dihydro-1-[(1,2,3,6-tetrahydro-1-methyl-4-pyridyl)carbonyl]-1*H*-indole (3). A suspension of **2** (233.0 g, 0.64 mol) in EtOH (2300 mL) was cooled to 10 °C. The temperature

was kept between 10 and 20 °C while a mixture of $NaBH_4$ (72.5 g, 1.92 mol) in H_2O (715 mL) and concentrated NaOH (71.4 mL) was added slowly. *The reaction was exothermic!* After the addition was completed, the reaction mixture was stirred for 3 h at 20 °C. The solution was concentrated by evaporation, and the remaining H_2O was extracted three times with CH_2Cl_2. The combined organic fractions were dried and concentrated by evaporation. The purification by crystallization from Et_2O/petroleum ether yielded 104.0 g (67%) of **3** as light beige crystals: mp 70–72 °C; MS *m/e* 242 (M^+); ^1H NMR (360 MHz, $CDCl_3$) δ 2.42 (s, 3H), sharp multiplet 2.54 (m, 2 H), 2.64 (t, 2 H, J = 6.0 Hz), overlapped doublet 3.08 (d, 2 H, J = 3.0 Hz), partly overlapped triplet 3.10 (t, 2 H, J = 7.5 Hz), 4.12 (t, 2 H, J = 7.5 Hz), sharp multiplet 6.03 (m, 1 H), 7.01 (t, 1 H, J = 7.0 Hz), overlapped triplet 7.19 (t, 1 H, J = 7.0 Hz), overlapped doublet 7.20 (d, 1 H, J = 7.0 Hz), 7.89 (br s, 1H). Anal. ($C_{15}H_{18}N_2O$) C, H, N, O.

[4-[(2,3-Dihydro-1*H*-indol-1-yl)carbonyl]-1,2,3,6-tetrahydro-1-pyridino]carbamic Acid Ethyl Ester (4). To a solution of **3** (104.0 g, 0.43 mol) in dry toluene (1700 mL) was added Et(iPr)$_2$N (112.6 mL, 0.65 mol), and the reaction mixture was heated up to 80 °C. Subsequently a solution of ethyl chloroformate (141.1 mL, 1.42 mol) in dry toluene (450 mL) was added. The mixture was stirred for 17 h at 80 °C and then cooled to room temperature and poured on ice/2 N HCl. The mixture was extracted with toluene. The organic fraction was dried and concentrated by evaporation. The yellow oily residue was purified by column chromatography (methyl *tert*-butyl ether/hexane, 1:1) and crystallized from methyl *tert*-butyl ether/Et_2O to yield 87.8 g (68%) of **4**: mp 112–113 °C; MS *m/e* 300 (M^+); IR (CH_2Cl_2) ν 1630, 1690 cm^{-1} (C=O); ^1H NMR (360 MHz, $CDCl_3$) δ 1.30 (t, 3 H, J = 7.0 Hz), 2.48 (m, 2 H), 3.12 (t, 2 H, J = 7.5 Hz), 3.66 (m, 2 H), partly overlapped doublet 4.09 (d, 2 H, J = 7.0 Hz), partly overlapped triplet 4.10 (t, 2H, J = 7.5 Hz), 4.19 (q, 2 H, J = 7.0 Hz), 6.05 (br s, 1 H), triplet 7.04 (t, 1 H, J = 6.5 Hz), partly overlapped triplet 7.20 (t, 1 H, J = 6.5 Hz), partly overlapped doublet 7.21 (d, 1 H, J = 6.5 Hz), 7.82 (br s, 1 H). Anal. ($C_{17}H_{20}N_2O_3$) C, H, N, O.

***trans*- and *cis*-4,5,7a,8,9,10,11,11a-Octahydro-7-oxo-7*H*-indolo[1,7-*bc*][2,6]naphthyridine-10-carbamic Acid Ethyl Ester (6 and 5).** A solution of **4** (7.8 g, 0.026 mol) in toluene (1000 mL) was irradiated using a mercury high-pressure multispectral lamp (400 W). In a 12 h interval, the lamp was cleaned and the reaction mixture cleared with active coal and filtrated through hyflo. After 48 h, the solution was concentrated by evaporation. The isomers were separated by column chromatography (methyl *tert*-butyl ether/hexane, 1:1) to yield 1.6 g (21%) of the trans isomer (**6**) as colorless crystals: mp 132–133 °C; MS *m/e* 300 (M^+); and 3.4 g (44%) of the cis isomer (**5**) as a yellow oil: MS *m/e* 300 (M^+). ^1H NMR (360 MHz, $CDCl_3$) of *trans*-**6**: δ 1.31 (t, 3 H, J = 7.0 Hz), 1.58 (dq, 1 H, J = 3.5, 12.5 Hz), 2.24 (dt, 1 H, J = 3.5, 12.5 Hz), 2.42 (dd, 1 H, J = 3.5, 12.5 Hz), 2.80 (m, 3 H), 3.12–3.32 (m, 2 H), 3.99 (sextet, 1 H, J = 3.0, 7.0 Hz), partly overlapped quartet 4.19 (q, 2 H, J = 7.0 Hz), partly overlapped multiplet 4.20 (m, 1 H), 4.40 (brs, 1 H), 4.98 (br s, 1 H), 6.99 (t, 1 H, J = 7.0 Hz), 7.05 (d, 1 H, J = 7.0 Hz), 7.14 (d, 1 H, J = 7.0 Hz). ^1H NMR (360 MHz, DMSO-d_6, 150 °C) of *cis*-**5**: δ 1.20 (t, 3 H, J = 6.5 Hz), 1.64 (octet, 1 H, J = 5.5 Hz), 2.03 (m, 1 H), 2.84 (q, 1 H, J = 5.5 Hz), 3.10–3.22 (m, 4 H), 3.28 (dd, 1 H, J = 9.0 Hz), 3.54 (dt, 1 H, J = 5.5, 6.0 Hz), 3.62 (dd, 1 H, J = 5.5 Hz), 3.95 (m, 2 H), 4.07 (q, 2 H, J = 6.5 Hz), 6.90 (t, 1 H, J = 7.0 Hz), overlapping doublets 7.08 (2d, 2 H, J = 7.0 Hz). Anal. ($C_{17}H_{20}N_2O_3$) C, H, N, O.

***cis*-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo-[1,7-*bc*][2,6]naphthyridine Hydromalonate (7).** A suspension of $LiAlH_4$ (8.80 g, 0.24 mol) in dry THF (120 mL) was cooled to −50 °C. Keeping this temperature, a solution of concentrated H_2SO_4 (6.63 mL, 0.12 mol) in THF (120 mL) was added within 50 min. The reaction mixture was stirred for 30 min and the temperature adjusted to 20 °C. To the reaction mixture was added a solution of **5** (17.70 g, 0.059 mol) in THF (250 mL) while the mixture was kept between 20 and 30 °C. After 2 h of stirring at room temperature, the reaction mixture was cooled to −20 °C and the excessive hydride was quenched by adding water dropwise. The mixture was transposed with aqueous NaOH (30%, 40 mL) and diluted with toluene. After stirring for 30 min, the organic layer was separated and the water fraction was extracted three times with toluene. The combined organic fractions were dried and evaporated. The crude product was obtained as yellow crystals and converted into the malonate salt for further purification. Recrystallization from MeOH/acetone afforded 16.2 g (81%) of **7**: mp 158–159 °C; MS *m/e* 228 (M^+); ^1H NMR (360 MHz, $CDCl_3$, of the free base) δ 1.77 (m, 1 H), 2.06 (br s, 1 H), 2.15–2.42 (m, 3 H), 2.27 (s, 3 H), 2.55 (br, s, 1 H), 2.70 (br d, 1 H, J = 10.0 Hz), 2.85–3.17 (m, 6 H), 3.41 (m, 1 H), 6.62 (t, 1 H, J = 7.0 Hz), 6.88 (d, 1 H, J = 7.0 Hz), 6.94 (d, 1 H, J = 7.0 Hz). Anal. ($C_{15}H_{20}N_2 \cdot C_3H_4O_4$) C, H, N, O.

(+)-cis-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]naphthyridine Hydrofumarate (8). The racemate **7** (13.6 g, 0.06 mol) was dissolved in acetone (350 mL). To this was added a solution of (−)-di-*O*,*O*′-*p*-toluoyl-L-(+)-tartaric acid (24.0 g, 0.06 mol) in acetone (300 mL). The solution was concentrated by evaporation until crystallization started. The precipitating salt was stirred, and Et_2O was added to complete crystallization. The salt was filtered off, washed with acetone/Et_2O, and recrystallized five times from EtOH/acetone. The procedure afforded an optical rotation of the salt: $[\alpha]_D$ −46.0° (c = 0.5, MeOH); mp 158–159 °C. The salt was subsequently taken up in a mixture of 300 mL of ice/50 mL of concentrated NH_3/100 mL of CH_2Cl_2. The organic layer was separated, and the H_2O fraction was reextracted twice with CH_2Cl_2. The combined organic fractions were dried and concentrated by evaporation to yield 1.4 g (10%) of **8** as free base: $[\alpha]_D$ + 132.0° (c = 0.5, MeOH); ee >99%. **8** as hydrofumarate salt: mp 177–178 °C; $[\alpha]_D$ +85.6° (c = 0.5, MeOH).

(−)-cis-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]naphthyridine Hydrofumarate (9). The (−)-cis enantiomer **9** was obtained following the same procedure, using the (+)-antipode acid.

Pharmacology. Radioligand Binding Studies. A detailed description of the methods used in our laboratories to label 5-HT receptor subtypes has been given before (Pazos,[23] Hoyer,[24] Waeber[25]). The 5-HT$_{1A}$ binding was carried out in pig frontal cortex membranes using [^{3}H]-8-OH-DPAT. [^{125}I]I-CYP was used to label 5-HT$_{1B}$ sites in membranes of rat frontal cortex: experiments were carried out in the presence of 30 μM isoprenaline in order to avoid binding to β-adrenoceptors. 5-HT$_{1D}$ binding was carried out in calf caudate membranes with [^{3}H]-5-HT in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine (in order to prevent binding to 5-HT$_{1A}$ and 5-HT$_{2C}$ sites). [^{3}H]Ketanserin was the radioligand used for 5-HT$_{2A}$ binding studies using rat cortex preparations, while 5-HT$_{2C}$ binding was performed in preparations of porcine choroid plexus using [^{3}H]mesulergine. Nonspecific binding was defined in the presence of 10 μM 5-HT (5-HT$_1$) or 10 μM mianserin (5-HT$_2$). 5-HT$_3$ binding was carried out in membranes of N1E-115 neuroblastoma cells using [^{3}H]ICS 205-930 as previously described (Hoyer and Neijt[26]). Nonspecific binding was determined with MDL 72222 (10 μM).

The binding assays for α_1 ([^{3}H]prazosin) and α_2 ([^{3}H]clonidine) adrenoceptors were based on the methods by Greengrass and Bremner[27] and U'Prichard,[28] respectively, with slight modifications as described by Ruedeberg et al.[29] The membranes were prepared from fresh calf brain cortex. Nonspecific binding was determined with 10 μM phentolamine and 1 μM unlabeled clonidine, respectively. For dopamine receptor binding assays, membranes from calf striatal tissue were prepared. [^{3}H]SCH-23390 (Billard[30]) was used as the D$_1$ ligand, and nonspecific binding was defined with 1 μM unlabeled SCH-23390. The dopamine D$_2$ assay with [^{3}H]spiperone was essentially performed as described by Urwyler and Coward.[31] Cinanserin (0.5 μM) was used to mask binding to 5-HT$_{2A}$ receptors, while nonspecific binding was assessed with haloperidol (5 μM). In all experiments, IC$_{50}$ values (concentration of the test compound which inhibits 50% of the specific binding of the radioligand) obtained from the displacement curves were converted to K_D values with the Cheng–Prusoff equation. All data are expressed as pK_D values (−log, M).

Phosphatidylinositol Turnover. The effects of **8** on accumulation of inositol phosphates were measured in pig choroid plexus according to Hoyer et al.[32] A suspension of pig choroid plexus cells was prepared and incubated with 0.64 μM [^{3}H]-*myo*-inositol (3 μCi/mL) for 60 min. Suspensions were then transferred to Beckman biovials containing test drugs and incubated for a further 60 min at 37 °C. Subsequently, the reaction was stopped by addition of 1.5 mL of chloroform/methanol. After workup, 1 mL of the aqueous phase was added to **1** mL of a slurry of AG 1X8 resin (Biorad) and filtered with 5 mL water. The [^{3}H]inositol phosphate product was removed from the resin by 3 mL of 1 M HCl and the radioactivity counted.

Inhibition of MK-212-Induced Hypophagia. Male Wistar–Kyoto rats (200–270 g) were fasted for 2 days and placed during the second day on walking grids. In the morning of the third day, the animals received MK-212 (0.5 mg/kg sc) or solvent and were placed in individual cages. Five minutes later, a weighed amount of food (normal rat chow) was added and animals were allowed to eat for 45 min. At the end of this period, the remaining amount of food was determined. Solvent or **8** was given 25 min prior to MK-212.

Inhibition of MK-212-Induced ACTH Increase. Male Sprague–Dawley rats (200–250 g) were decapitated 30 min after a high but submaximal dose of MK-212 (0.1 mg/kg sc), and trunk blood was collected to prepare plasma. The naphthyridine **8** (0, 0.1, or 1 mg/kg sc) was administrated 20 min prior to MK-212. ACTH was measured by radioimmunoassay, using a kit specially developed to determine rat ACTH (Eurodiagnostics BV, Apeldoorn, The Netherlands).

Rat Stomach Fundus Studies. Experiments were carried out on stomach fundus strips from male Wistar–Kyoto rats weighing 190–250 g, sacrificed by CO_2. The stomach was dissected, and four strips of 2 mm width and 2 cm length were cut parallel to the greater curvature. Strips were set up in organ baths of 20 mL containing Krebs solution constantly bubbled with 5% CO_2 in oxygen. The composition of the Krebs solution was (in mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 1.25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; and $NaHCO_3$, 25. Contractions were measured isotonically under a resting tension of 1 g. Prior to testing, the strips were allowed to equilibrate for 1.5–2.5 h, during which the bath fluid was replaced every 15 min. A single contraction to carbachol (10^{-6} M) was established which causes a near maximal effect and used as reference contraction (100%). The 5-HT agonist, DOI, was added according to a cumulative concentration schedule. Only a single agonist response curve was established on each preparation. Compound **8** was added to the bath fluid 15 min before the first dose of DOI.

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