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O-Methylasparvenone, a Nitrogen-free Serotonin Antagonist†

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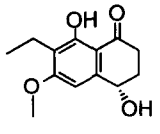
Abstract—*O*-Methylasparvenone (**1**) and asparvenone (**2**) were isolated from an *Aspergillus parvulus* Smith broth in a microbial screening for 5-HT_{2C} ligands and found to be 5-HT_{2C} antagonists. They represent the first nitrogen-free serotonin ligands. The absolute configuration of **1** was determined to be *S* by X-ray analysis of the corresponding Mosher-ester. A short and efficient synthesis of **rac-1** was developed. This protocol was applied to the synthesis of derivatives of **1** and a structure–affinity relationship was established. © 1997 Elsevier Science Ltd.

Introduction

Serotonin (5-HT) is an important modulatory neurotransmitter that generally exhibits inhibitory activity. In 1979 the existence of several 5-HT binding sites was recognized for the first time. Subsequently, the receptors through which serotonin produces its effects have been the subject of intense investigation.[1]

There is considerable interest in the development of 5-HT_{2C} receptor agonists for depression and obsessive–compulsive disorder[2,3] as well as 5-HT_{2C} receptor antagonists for anxiety disorders.[4] In a microbial screening program for 5-HT_{2C} ligands we have discovered the antagonists **1** and **2**, which bind with moderate affinity (Table 1). Based on the spectral analysis, the molecular structures were determined to be identical with that of the known compounds *O*-methylasparvenone (**1**)[5,6] and asparvenone (**2**).[7] To the best of our knowledge, **1** and **2** represent the first nitrogen-free 5-HT ligands. We report here a short and efficient protocol for the synthesis of **rac-1**, the separation of the enantiomers **1** and **ent-1** via Mosher-esters and the determination of the absolute configuration. Furthermore, derivatives **10**, **11**, and **12** were prepared and a structure–affinity relationship was established.



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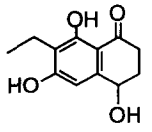

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†Dedicated to Professor A. I. Meyers on the occasion of his 65th birthday.

Isolation and structure elucidation

The microorganism FB6036 originated from a soil sample collected in Kyoto, Japan. This strain was identified as *Aspergillus parvulus* Smith according to its morphological characteristics[8] and is known as a producer of asparvenone **2**, which was isolated along with **1**. The isolation of the active compounds was carried out by monitoring the affinity for the 5-HT_{2C} rat receptor.

The molecular formula of **1** was found to be $C_{13}H_{16}O_4$ and based on ^1H NMR, ^{13}C NMR spectra, and C–H long-range couplings obtained from the HMBC experiments the structure was determined. The spectra are in every respect identical with that of the known natural product *O*-methylasparvenone.[5,6] The absolute configuration at the C-4 position of **1** was reported to be *R* based on the X-ray analysis of the corresponding monoacetate.[5] We obtained suitable crystals for X-ray analysis from the (*S*)-Mosher ester of **1** (vide infra). The structure is shown in Figure 1. This analysis affords the

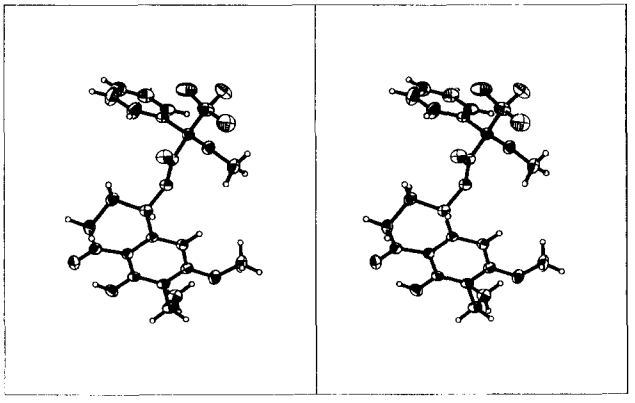


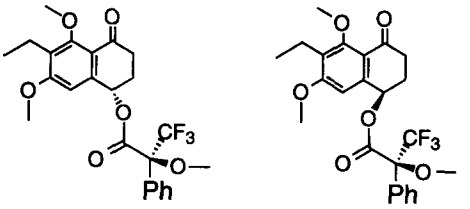
Figure 1.

assignment of the absolute configuration of **1** as the *S*-configuration.

Chemistry

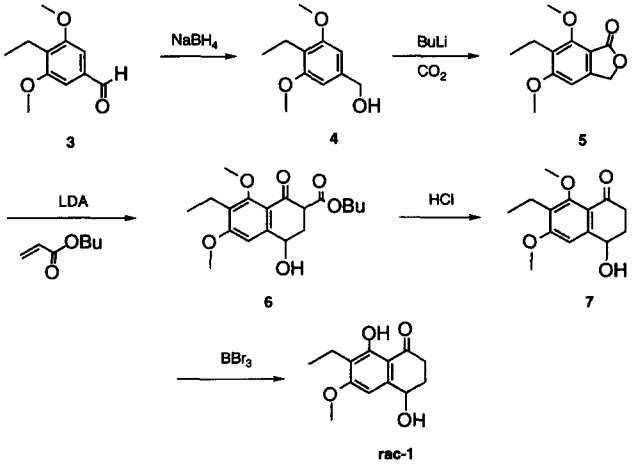
The isolation of **1** from the broth is rather difficult and time consuming. Therefore, a practical synthesis was developed. The key step of the synthesis of **rac-1** (Scheme 1) is the tandem Michael–Dieckmann reaction of the carbanion of the isobenzofuranone **5** with acrylate.[9] 6-Ethyl-5,7-dimethoxy-3*H*-isobenzofuran-1-one (**5**) was easily accessible from the known aldehyde **3**[10] in a two step sequence. Reduction of **3** with sodium borohydride provided the benzyl alcohol **4**. Metalation of **4** with *n*-butyllithium in the presence of *N,N,N',N'*-tetramethylethylenediamine followed by reaction with carbon dioxide[11] afforded **5** in 50% yield. The two-carbon ring expansion was effected by carbanion formation with LDA and addition of the anion to butyl acrylate followed by cyclization of the intermediate Michael-product to the β-ketoester **6**. Hydrolysis of the ester **6** followed by decarboxylation generated **7**. Selective demethylation with BBr_3[12] yielded *rac-O*-methylasparvenone (**rac-1**).

The resolution of **rac-1** via the corresponding Mosher-esters **8** and **9** yielded after ester hydrolysis and ether cleavage the natural product **1** and the enantiomer **ent-1**.



8 9

In order to establish a structure–affinity relationship (Table 1) we prepared analogues of **1** using this protocol. The building blocks for compounds **10** and **12** were prepared as shown in Schemes 2 and 3.



Scheme 1.

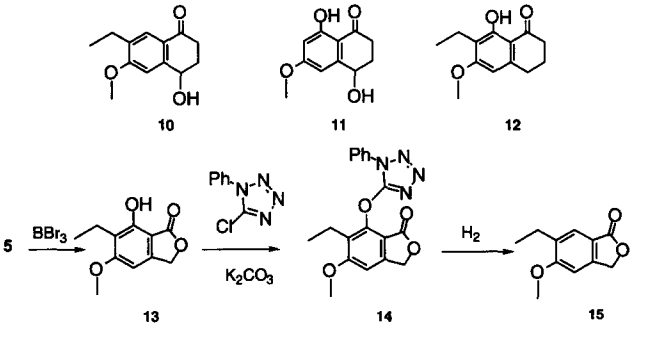
In Scheme 2 the synthesis of 6-ethyl-5-methoxy-3*H*-isobenzofuran-1-one (**15**) is reported. Demethylation of **5** with BBr_3 yielded the phenol **13** in 97%. Arylation with 5-chloro-1-phenyl-1*H*-tetrazole in the presence of K_2CO_3 followed by hydrogenation afforded **15**. The preparation of methyl 3-ethyl-2,4-dimethoxy-6-methyl-benzoate **19** is outlined in Scheme 3. Bromination of 4-ethyl-3,5-dimethoxy-toluene **16** was followed by halogen-lithium exchange and carboxylation with CO_2 to give **18**. Esterformation with diazomethane produced **19** which was subjected to the precedent tandem Michael–Dieckmann reaction.

Pharmacology

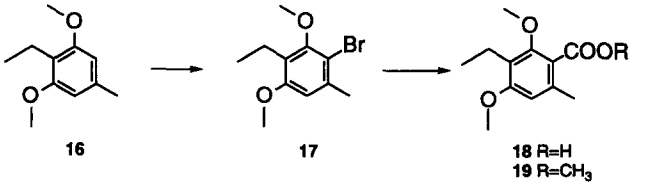
The affinity of the compounds **1**, **ent-1**, **2**, **7**, **10**, **11**, and **12** for 5-HT_{2A} and 5-HT_{2C} human receptors was assessed using displacement of [^{3}H]-5-HT and [^{3}H]-DOB, respectively. 5-HT_{2C} receptor binding affinities are listed in Table 1. All compounds failed to displace [^{3}H]-DOB from the 5-HT_{2A} receptor binding site at concentrations of up to 10 mM even though the sequence homology between the 5-HT_{2A} and 5-HT_{2C} receptors is high especially in the putative membrane spanning domain (79%).[1]

The phenolic hydroxy group and the lipophilic alkyl side chain are important for binding, since the methyl ether **7**, the desoxy derivative **10** and the desethyl compound **11** display virtually no affinity for the 5-HT_{2C} receptor. On the other hand, the benzylic OH seems not to be implicated in receptor binding. The affinity of **12** is about the same as that of **1**.

O-methylasparvenone **1** was functionally characterized in an in vitro model for 5-HT_{2C} receptor activation, namely the phosphoinositol turnover in the choroid plexus of the rat. **1** displayed no intrinsic activity and the 5-HT-induced response was fully antagonized (Fig. 2).



Scheme 2.



Scheme 3.

Table 1. $5HT_{2C}$ receptor binding affinities (pK_i)

Compound	pK_i
1	6.7
ent-1	6.4
2	6.4
7	<5
10	5.3
11	<5
12	6.6

We have also caused parallel rightward shifts in the dose response curve of 5-HT with increasing concentrations of **1** (results not shown). Schild analysis of these data yield a curve with a slope of 1.1 and a pA2 value of 6.7 which are indicative of competitive antagonism. Consequently it could be suggested that binding of **1** to the $5\text{-}HT_{2C}$ receptor is a reversible competitive interaction.

In summary, we have identified the first nitrogen-free 5-HT ligands, namely *O*-methylasparvenone (**1**) and asparvenone (**2**). They bind preferentially to the $5\text{-}HT_{2C}$ receptor subtype and display antagonistic properties.

Experimental

Isolation

A portion of the stock culture (100 mL) of FB6036 preserved at −80 °C was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of seed medium consisting of 2% glucose, 2% potato starch, 2% toast soya, 0.5% yeast extract, 0.25% NaCl, 0.32% $CaCO_3$, 0.005% $ZnSO_4 \cdot 7H_2O$, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.0005% $MnSO_4 \cdot 4H_2O$ and 0.03% Nissan disfoam CA-123. The pH of the medium was adjusted to 7.0 prior to the addition of calcium bicarbonate. This seed culture was shaken on a rotary shaker at 220 rpm at 27 °C for 3 days. Then 2 mL aliquots were transferred into ten 500 mL flasks, each containing 100 mL of fermentation medium which consisted of 3% glucose, 1% corn steep liquor, 0.5% Pharmamedia, 0.5% gluten, 0.5% S-3 meat, 0.5% dried yeast, 0.2% $CaCO_3$ and 0.03% Nissan disfoam CA-123 (pH 6.5). The flasks were incubated for four days under the same conditions described above. From the whole broth (1 L), the mycelia were removed by centrifugation and the broth (690 mL) was extracted with ethyl acetate (1.3 L). The organic layer was concentrated under reduced pressure and the residue (181 mg) was subjected to a column of silica gel (Merck Kieselgel 60, 10 g, id 5 × 35 cm), prepacked with *n*-hexane. The column was developed with *n*-hexane: EtOAc (5:1, 3:1, 2:1, 1:1, 1:2, v/v). Two portions of active fractions were combined and concentrated under reduced pressure independently (fraction A 23.5 mg, fraction B 5 mg). Each fraction was further purified by a preparative silica gel HPLC (YMC packed column R-SIL-5, 120A, id 6 × 250 mm) developed with dichloromethane–ethanol at a flow rate of 1 mL/min. Fraction A yielded 17 mg of **1** (40:1, retention time = 7.3 min), colorless needles from petroleum ether-ethylacetate. **2** (2.5 mg) was isolated from fraction B (30:1, retention time = 14.3 min) as a colorless powder.

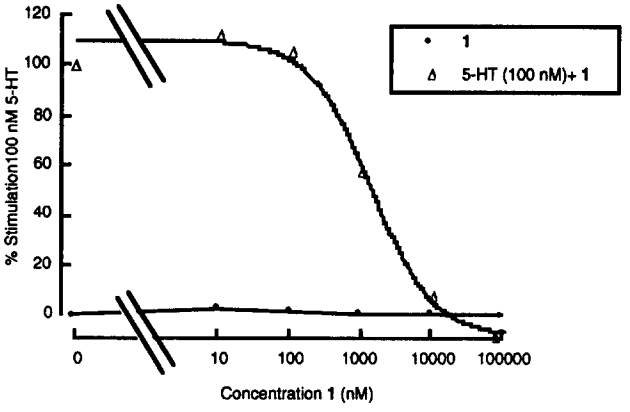


Figure 2. Effects of *O*-methylasparvenone (**1**) on the IP_3 formation in the rat choroid plexus.

Chemistry

General. All laboratory glassware was dried at 130 °C and purged with dry argon. Tetrahydrofuran was distilled from sodium benzophenone ketyl and was then transferred via syringe. 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: IR (cm^{-1}): Nicolet-7199-FT-IR. ^1H 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.

4-Ethyl-3,5-dimethoxybenzyl alcohol (4). To a suspension of 4-ethyl-3,5-dimethoxybenzaldehyde[10] (6.99 g, 36 mmol) in ethanol (180 mL) was added sodium borohydride (6.81 g, 180 mmol) at 20–30 °C in portions. The reaction mixture was stirred for 1 h and quenched with water (36 mL). The suspension was stirred for 16 h and evaported in vacuo. The residue was suspended in diethyl ether (360 mL) and washed with water (2 × 180 mL) and brine (45 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude material (6.90 g white crystals) was purified by Kugelrohr-distillation yielding **4** (6.05 g, 86%) as white crystals (bp ≈ 130 °C/0.3 mbar, mp 56–58 °C. ^1H NMR ($CDCl_3$) δ 6.56 (s, 2H), 4.64 (d, *J* = 5 Hz, 2H), 3.82 (s, 6H), 2.64 (q, *J* = 7.4 Hz, 2H), 1.68 (t, *J* = 5 Hz, 1H), 1.07 (t, *J* = 7.4 Hz, 3H). Anal. calcd ($C_{11}H_{16}O_3$) C, H.

6-Ethyl-5,7-dimethoxy-3*H*-isobenzofuran-1-one (5). To a mixture of **4** (7.85 g, 40 mmol) and *n*-hexane

(200 mL) was added *N,N,N',N'*-tetramethylethylendiamine (12 mL, 80 mmol) and subsequently *n*-butyllithium (80 mmol in hexane). The resulting suspension was heated at reflux for 5 h. After cooling to −65 °C dry carbondioxide was bubbled through the reaction mixture. The yellow suspension was warmed to room temperature over night and quenched with 3 N HCl (250 mL). Ether (400 mL) was added and the layers were separated. The organic layer was washed with water (200 mL) and brine (50 mL). The aqueous phase was extracted with ether (200 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed and the oily residue was dissolved in toluene (200 mL) and heated under reflux for 1 h using a Dean–Stark trap. The toluene was removed under reduced pressure and the crude crystals were suspended in ether (30 mL) and filtered to give white crystals of **5** (4.9 g, 55.3%) with a melting point of 105–106 °C. ^{1}H NMR ($CDCl_3$) δ 6.64 (s, 1H) 5.18 (s, 2H) 4.07 (s, 3H) 3.91 (s, 3H) 2.69 (q, *J* = 7.4 Hz, 2H) 1.09 (t, *J* = 7.4 Hz, 3H). Anal. calcd ($C_{12}H_{14}O_4$) C, H.

Butyl (2*RS*,4*RS*)- and (2*RS*,4*SR*)-7-ethyl-4-hydroxy-6,8-dimethoxy-1-oxo-3,4-dihydro-1(2*H*)-naphthalene-2-carboxylate (6). To a solution of freshly prepared LDA (53.3 mmol) in THF:hexane 9:1 (530 mL) was added **5** (10.8 g, 48.5 mmol) at −75 °C. After stirring for 75 min at −75 °C, butyl acrylate (7.6 mL, 53.3 mmol) was added. The mixture was stirred at −75 °C for 1 h, warmed to −40 °C and stirred for an additional 3 h at that temperature. The reaction was quenched with AcOH (25 mL), diluted with Et_2O (2400 mL) and washed twice with H_2O (1000 mL) and brine (250 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by chromatography on silica gel (920 g) by gradient elution using hexane:AcOEt 4:1 and 2:1 to give (*R,S*)-3-(6-ethyl-5,7-dimethoxy-1-oxo-3*H*-isobenzofuran-3-yl)-propionic acid butyl ester (7 g, 41%) as yellow crystals and **6** as yellow oil (8.45 g, 50%). ^{1}H NMR ($CDCl_3$) δ 6.93, 6.83, 6.77 (s, 1H) 5.0, 4.88, 4.55 (m, 1H) 4.13 (m, 2H) 3,91, 3.98 (s, 3H) 3.79, 3.78 (s, 3H) 2.76–2.25 (m, 4H) 1.64 (m, 2H) 1.39 (m, 2H) 1.03 (m, 3H) 0.95 (m, 3H).

(*RS*)-7-ethyl-4-hydroxy-6,8-dimethoxy-3,4-dihydro-1-(2*H*)-naphthalenone (7). To a solution of **6** (10.2 g, 29 mmol) in ethanol (290 mL) was added 1 N Na_2CO_3 (290 mL). The mixture was stirred for 15 h at room temperature. After the addition of 1 N HCl (640 mL) the cloudy solution was heated at 60 °C for 15 min, cooled and extracted with ethyl acetate (580 mL, 3 × 290 mL). The organic layer was washed twice with water (290 mL) and with brine (145 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by chromatography on silica gel (420 g) using ethyl acetate to give yellowish crystals of **7** (5.1 g, 70.8%) mp of 102–104 °C. ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 1H) 4.86 (m, 1H) 3.91 (s, 3H) 3.78 (s, 3H) 2.85 (ddd, *J* = 4.7 Hz, *J* = 7 Hz, *J* = 17 Hz, 1H) 2.54 (ddd, *J* = 4.9 Hz, *J* = 9.7 Hz, *J* = 17 Hz, 1H) 2.63 (q, *J* = 16.5 Hz, 2H) 2.34 (m, 1H) 2.10 (m, 1H) 1.95 (d, *J* = 6.2 Hz, 1H) 1.10 (t, *J* = 16.5 Hz, 2H). Anal. calcd ($C_{14}H_{18}O_4$) C, H.

(*RS*)-7-ethyl-4,8-dihydroxy-6-methoxy-3,4-dihydro-1-(2*H*)-naphthalenone (rac-1). To a solution of **7** (125 mg, 0.5 mmol) in dichloromethane (10 mL) was added 0.5 M boron tribromide (1.1 mL, dichloromethane) at −75 °C. After 5 min the cooling bath was removed and the reaction mixture was warmed to room temperature and stirred at ambient temperature for 30 min. The reaction was quenched with water (10 mL), dichloromethane was removed and the residue was heated at reflux for 30 min. The mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layer was washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4) filtered and evaporated. The residue was purified on silica gel (2.5 g) with AcOEt:hexane (1:1) as eluent. The crude product was recrystallised from cyclohexane. Yield 67 mg (57%) of **rac-1**, mp 108–111 °C. ^{1}H NMR ($CDCl_3$) δ 6.64 (s, 1H) 4.85 (m, 1H) 3.92 (s, 3H) 2.90 (ddd, *J* = 4.7 Hz, *J* = 7.2 Hz, *J* = 17.7 Hz, 1H) 2.64 (q, *J* = 7.5 Hz, 2H) 2.60 (ddd, *J* = 4.7 Hz, *J* = 9.2 Hz, *J* = 17.7 Hz, 1H) 2.32 (m, 1H) 2.11 (m, 1H) 1.88 (d, *J* = 6 Hz, 1H) 1.08 (t, *J* = 7.5 Hz, 3H). Anal. calcd ($C_{13}H_{16}O_4$) C, H.

Mosher ester preparation (8 and 9). To a solution of (*R*)-α-trifluormethyl-α-methoxy-phenylacetic acid (4.22 g, 18 mmol) and diethyl azodicarboxylate (6.18 mL, 34 mmol) in ether (450 mL) was added a solution of triphenylphosphin (8.87 g, 34 mmol) and **7** (4.5 g, 18 mmol) in ether (450 mL). The mixture was stirred for 2 h at room temperature, the solvent was removed and the residue was dissolved in toluene (50 mL) and subjected to column chromatography on silica gel (240 g) using hexane:*t*-butyl-methyl-ether 2:1 as eluent to give 6.68 g of the mixtures of diastereomers. The residue was further purified by chromatography on silica gel (920 g) with hexane:*t*-butyl-methyl-ether 2:1 to give (*R*)-3,3,3-trifluoro-2-methoxy-2-phenyl-propionic acid (*R*)-6-ethyl-5,7-dimethoxy-4-oxo-1,2,3,4-tetrahydro-naphtalen-1-yl ester (**8**) (2.5 g), a mixed fraction of (*R*)-3,3,3-trifluoro-2-methoxy-2-phenyl-propionic acid (*R*)- and (*S*)-6-ethyl-5,7-dimethoxy-4-oxo-1,2,3,4-tetrahydro-naphtalen-1-yl ester (3.45 g), and (*R*)-3,3,3-trifluoro-2-methoxy-2-phenyl-propionic acid (*S*)-6-ethyl-5,7-dimethoxy-4-oxo-1,2,3,4-tetrahydro-naphtalen-1-yl ester (**9**) (0.75 g). The mixed fraction was chromatographed to give an additional 0.62 g of **8** and 2.08 g of **9**. The total yield of **8** was 3.12 g (51%). ^{1}H NMR ($CDCl_3$) δ 7.52–7.49 (m, 2H) 7.40–7.34 (m, 3H) 6.48 (s, 1H) 6.21 (dd, *J* = 3.8Hz, *J* = 3.8Hz) 3.76 (s, 3H) 3.65 (s, 3H) 3.56 (m, 3H) 2.63 (q, *J* = 7.5 Hz, 2H) 1.07 (t, *J* = 7.5 Hz, 3H). The total yield of **9** was 2.83 g (46%). ^{1}H NMR ($CDCl_3$) δ 7.43–7.24 (m, 5H) 6.71 (s, 1H) 6.24 (dd, *J* = 3.37 Hz, *J* = 3.33Hz, 1H) 3.80 (s, 3H) 3.78 (s, 3H) 3.47 (m, 3H) 2.66 (q, *J* = 7.4 Hz, 2H) 1.10 (t, *J* = 7.4 Hz, 3H)

(*S*)-7-ethyl-4,8-dihydroxy-6-methoxy-3,4-dihydro-1-(2*H*)-naphthalenone (1). A solution of **7** (2.55 g, 5.47 mmol) and lithiumhydroxide mono hydrate (0.9 g, 21.9 mmol) in dioxane (55 mL) and H_2O (55 mL) was stirred for 20 h at ambient temperature. The reaction mixture was diluted with water (275 mL) and the precipitation was filtered off. The aqueous phase was saturated with NaCl and extracted with ethyl acetate (3 × 300 mL). The combined organic layers were washed with 1 N Na_2CO_3 (140 mL) and brine (140 mL), dried (Na_2SO_4), and the solvent was removed. The residue was dissolved in dichloromethane (80 mL) and BBr_3 (0.43 mL, 4.48 mmol) was added at −75 °C. After 5 min the cooling bath was removed and the reaction mixture was warmed to room temperature and stirred at ambient temperature for 30 min. The reaction was quenched with water (80 mL), the organic solvent was removed and the residue was heated at reflux for 30 min. The mixture was extracted with ethyl acetate (3 × 80 mL) and the organic layer was washed with water (80 mL) and brine (40 mL), dried (Na_2SO_4) filtered and evaporated. The residue was purified on silica gel (60 g) with toluene:AcOEt (4:1) as eluent. The crude product was recrystallized from cyclohexane. Yield 0.83 g (86 %) **1**, mp 125–126 °C. Anal. calcd ($C_{13}H_{16}O_4$) C, H. $[\alpha]_D$ = +27.2° (*c* 0.25; $CHCl_3$)

(*R*)-7-ethyl-4,8-dihydroxy-6-methoxy-3,4-dihydro-1-(2*H*)-naphthalenone (ent-1). A solution of **8** (3 g, 6.47 mmol) and lithiumhydroxide mono hydrate (1.1 g, 25.9 mmol) in dioxane (65 mL) and H_2O (65 mL) was stirred for 20 h at ambient temperature. The reaction mixture was diluted with water (325 mL) and the precipitation was filtered off. The aqueous phase was saturated with NaCl and extracted with ethyl acetate (3 × 320 mL). The combined organic layers were washed with 1 N Na_2CO_3 (160 mL) and brine (160 mL), dried (Na_2SO_4) and the solvent was removed. The residue was dissolved in dichloromethane (87 mL) and BBr_3 (0.46 mL, 4.79 mmol) was added at −75 °C. After 5 min the cooling bath was removed and the reaction mixture was warmed to room temperature and stirred at ambient temperature for 30 min. The reaction was quenched with water (87 mL), the organic solvent was removed and the residue was heated at reflux for 30 min. The mixture was extracted with ethyl acetate (3 × 87 mL) and the organic layer was washed with water (87 mL) and brine (43 mL), dried (Na_2SO_4) filtered and evaporated. The residue was purified on silica gel (60 g) with toluene:AcOEt (4:1) as eluent. The crude product was recrystallized from cyclohexane. Yield 0.8 g (77 %) **ent-1**, mp 126–127 °C. Anal. calcd ($C_{13}H_{16}O_4$) C, H. $[\alpha]_D$ = −34.4° (*c* 0.25; $CHCl_3$).

(*RS*)-7-ethyl-4-hydroxy-6-methoxy-3,4-dihydro-1(2*H*)-naphthalenone (10). Using the precedent procedure and starting from **15** (330 mg, 1.72 mmol), 57 mg (15%) of **10** as white crystals were obtained. mp 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H) 7.03 (s, 1H) 4.93 (dd, *J* = 4.2 Hz, *J* = 8.5 Hz, 1H) 3.92 (s, 1H) 2.81 (ddd, *J* = 4.5 Hz, *J* = 6.7 Hz, *J* = 11.2 Hz) 2.63 (q, *J* = 7.5 Hz, 2H) 2.58 (ddd, *J* = 4.6 Hz, *J* = 10.3 Hz, *J* = 17.3 Hz,1H) 2.47 (m, 1H) 2.16 (m, 1H) 1.19 (t, *J* = 7.5 Hz, 3H).

(*RS*)-4,8-dihydroxy-6-methoxy-3,4-dihydro-1(2*H*)-naphthalenone (11). Using the precedent procedure and starting from 5,7-dimethoxy-3*H*-isobenzofuran-1-one (1.04 g, 5mmol),[13] 130 mg (13%) of **9** were obtained, mp 93–94 °C (*c*-hexane). ^{1}H NMR ($CDCl_3$) δ 6.59 (d, *J* = 2.5 Hz, 1H) 6.35 (d, *J* = 2.5 Hz, 1H) 4.82, (dd, *J* = 3.9 Hz, *J* = 8.2 Hz, 1H) 3.84 (s, 1H) 2.91 (ddd, *J* = 4.8 Hz, *J* = 7.3 Hz, *J* = 17.8 Hz, 1H) 2.61 (ddd, *J* = 4.8 Hz, *J* = 7.3 Hz, *J* = 17.8 Hz, 1H) 2.13 (m, 1H) 2.03 (m, 1H). Anal. calcd ($C_{11}H_{12}O_4$) C, H.

7-Ethyl-8-hydroxy-6-methoxy-3,4-dihydro-1(2*H*)-naphthalenone (12). Using the precedent procedure and starting from **19** (132 mg, 0.55 mmol) 27 mg, (22%) of **12** were obtained, mp 71–73 °C. ^{1}H NMR ($CDCl_3$) δ 12.86 (s, 1H) 6.26 (s, 1H) 3.87 (s, 3H) 2.87 (t, *J* = 5 Hz, 2H) 2.63 (t, *J* = 5 Hz, 2H) 2.62 (q, *J* = 7.5 Hz, 2H) 2.06 (p, *J* = 5 Hz, 2H) 1.08 (t, *J* = 7.5 Hz, 3H). Anal. calcd ($C_{13}H_{16}O_3$) C, H.

6-Ethyl-7-hydroxy-5-methoxy-3*H*-isobenzofuran-1-one (13). To a solution of **5** (222 mg, 1 mmol) in dichloromethane (10 mL) a 1 M solution of boron tribromide in dichloromethane (1.1 mL) at −75 °C was added. After 5 min the cooling bath was removed and the reaction mixture was warmed to room temperature and stirred at ambient temperature for 48 h. The reaction was quenched with water (10 mL), the organic solvent was removed and the residue was heated at reflux for 30 min. Upon cooling **13** precipitated as brownish crystals (202 mg, 97%). A sample was recrystallized from ethanol and showed a mp of 168–169 °C. ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 1H) 6.48 (s, 1H) 5.24 (s, 2H) 3.89 (s, 3H) 2.67 (q, *J* = 7.5 Hz, 2H) 1.10 (t, *J* = 7.5 Hz, 3H). Anal. calcd ($C_{13}H_{16}O_4$) C, H.

6-Ethyl-5-methoxy-7-(1-phenyl-1*H*-tetrazol-5-yloxy)-3*H*-isobenzofuran-1-one (14). A mixture of **11** (208 mg, 1 mmol), 5-chlor-1-phenyl-1*H*-tetrazol (190 mg, 1.04 mmol), and potassium carbonate (260 mg, 1,88 mmol) in dioxane (10 mL) was refluxed for 24 h. The solvent was removed, water was added (910 mL) and the solution was acidified with aqueous HCl (25%) and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and the solvent was removed. The residue was recrystallized from ethanol. White crystals of **14** (75 mg, 23%), mp 209–210 °C. ^{1}H NMR ($CDCl_3$) δ 7.92 (m, 2H) 7.55 (m, 2H) 6.86 (s, 1H) 5.23 (s, 2H) 3.97 (s, 3H) 2.71 (q, *J* = 7.5 Hz, 2H) 1.12 (t, *J* = 7.5 Hz, 3H). Anal. calcd ($C_{18}H_{16}N_4O_4$) C, H, N.

6-Ethyl-5-methoxy-3*H*-isobenzofuran-1-one (15). A solution of **14** (35 mg, 0.1 mmol) in ethanol (10 mL) was hydrogenated over Pd on carbon (10 mg, 10%) at 60 °C and 10 bar. After 24 h the catalyst was filtered off and the solvent was removed. The residue was

subjected to chromatography with ethyl acetate the give **15** (17 mg, 93%). White crystals, mp 95–96 °C. ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H) 6.85 (s, 1H) 5.23 (s, 2H) 3.92 (s, 3H) 2.68 (q, J = 7.5 Hz, 2H) 1.21 (t, J = 7.5 Hz, 3H). Anal. calcd ($C_{11}H_{12}O_3$) C, H.

2-Bromo-4-ethyl-3,5-dimethoxytoluene (17). To a solution of 4-ethyl-3,5-dimethoxytoluene (**16**) (4.4 g, 24.6 mmol) in tetrachloromethane (125 mL) was added a 1 M solution of bromine in tetrachloromethane (24.6 mL) at 0 °C. After 2 h the solvent was removed and the residue was distilled at 0.4 mbar. Colorless oil of **17** (6.3 g, 99%) bp 130 °C. ^{1}H NMR ($CDCl_3$) δ 6.56 (s, 1H) 3.81 (s, 3H) 3.80 (s, 3H) 2.66 (q, J = 7.5 Hz, 2H) 2.38 (s, 3H) 1.12 (t, J = 7.5 Hz, 3H).

3-Ethyl-2,4-dimethoxy-6-methylbenzoic acid (18). To a solution of **17** (2.6 g, 10 mmol) in dry THF (100 mL) was added a 1.6 N n-butyllithium solution in hexane (12.5 mmol) at −50 °C. After 30 min at this temperature the yellow solution was cooled to −78 °C and carbondioxide was introduced. The reaction was acidified with 1 N HCl (50 mL) warmed to room temperature and diluted with ether (200 mL). The organic layer was extracted with water (50 mL) and brine (25 mL) and dried (Na_2SO_4). The solvent was removed and the residue was purified by column chromatography (n-hexane:AcOEt, 4:1) to yield white crystals of **18** (1.58 g, 70%), mp 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 6.57 (s, 1H) 3.86 (s, 6H) 2.64 (q, J = 7.5 Hz, 2H) 2.55 (s, 3H) 1.14 (t, J = 7.5 Hz, 3H). Anal. calcd ($C_{12}H_{16}O_4$) C, H.

Methyl 3-ethyl-2,4-dimethoxy-6-methylbenzoate (19). A solution of **18** (0.9 g, 4 mmol) in ether (40 mL) was treated with diazomethane (60% in diethylether, 20 mL). After 0.5 h the solvent was removed and the residue was distilled at 0.4 mbar. Colorless oil of **19** (730 mg, 76%). Bp 115 °C. ^{1}H NMR ($CDCl_3$) δ 6.47 (s, 1H) 3.90 (s, 3H) 3.81 (s, 3H) 3.77 (s, 3H) 2.30 (s, 3H) 2.61 (q, J = 7.5 Hz, 2H) 1.12 (t, J = 7.5 Hz, 3H).

X-ray crystallographic analysis

Preparation of the (*S*)-Mosher ester of (1). To a solution of (*S*)-α-trifluormethyl-α-methoxy-phenylacetic acidchloride (24.5 mL, 1.4 equiv) in dry pyridine (300 mL), was slowly added **1** (23.5 mg) in 0.75 mL of dry dichloromethane, and the mixture was stirred for 30 min at 0 °C. After stirred at room temperature for 18 h, 3-dimethylamino-1-propylamine (24 mL) was added and the mixture was stirred for 5 min. After dilution with diethylether and washing with cooled 1 N HCl, cooled saturated Na_2CO_3 aq and brine, the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give 35.1 mg crude ester. The crude powder was purified by preparative TLC (Merck Kieselgel 60 F_{254}, n-hexane:EtOAc (7:3, v/v), and gave 31.3 mg (R_f = 0.6, 57%) colorless prismatic crystal from MeOH; mp 100.8–101.9 °C; $[\alpha]^{22}_D$ −70.36° (c 0.0132; MeOH); HRFAB-MS (m/z); calcd 453.1525, found 453.1519 $[M+H]^+$; IR (KBr) cm^{-1} 2974, 1739 (C=O), 1639, 1280, 1168; ^{1}H NMR ($CDCl_3$) δ 1.09 (t, J = 7.5 Hz, 3H), 2.28 (dddd, J = 5.0, 5.0, 5.5, 14.0 Hz, 1H), 2.38 (dddd, J = 3.0, 4.5, 9.0, 14.0 Hz, 1H), 2.61 (ddd, J = 4.5, 5.0, 18.0 Hz, 1H), 2.66 (q, J = 7.5 Hz, 2H), 2.76 (ddd, J = 5.0, 9.0, 18.0 Hz, 1H), 6.24 (dd, J = 3.0, 5.5 Hz, 1H), 6.51 (s, 1H), 12.75(s, 1H). Anal. calcd ($C_{23}H_{23}O_6F_3$) C, H.

The colorless prismatic crystal belonged to the orthorhombic system, space group $P2_12_12_1$, with a dimension of a = 7.835(2) Å, b = 10.110(3) Å, c = 26.397(6) Å, and V = 2091.0(9) Å^3, Z = 4, D_{calcd} = 1.437 Mg/m^3. Data were collected using a Siemens RSm/V diffractometer (MoKα radiation (l = 0.71073 Å), with a graphite crystal monochromator, ω scans, $2\theta_{max}$ = 56°, and index ranges ($0 \leq h \leq 10$, $0 \leq k \leq 13$, $0 \leq l \leq 34$) to observe 2174 reflections (F > 5.0s (F)). The molecular structure was solved and refined by Siemens SHELXTL[14] using direct methods, and full-matrix least-square calculation. Atomic coordinates, bond lenghts, bond angles, and torsion angles were deposited at the Cambridge Crystallographic Data Centre.

Pharmacology

Cell culture and membrane preparation. Membranes obtained from NIH 3T3 cells lines expressing either human 5-HT_{2A} or human 5-HT_{2C} receptors were kindly donated by Dr Nico Stam (N.V. Organon) For each receptor subtype, a single batch of membranes were grown using fermentation techniques previously described.[15]

Radioligand binding assays. Radioligand binding assays were as previously described for the human 5-HT_{2A} receptor[15] with minor modifications for the labelling of human 5-HT_{2C} 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 4×10^5 cells per assay tube. The 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 at a concentration of 10^{-3} M 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 5-HT_{2C} receptor binding sites and [^{3}H]-DOB 1 nM final concentration for labeling human 5-HT_{2A} receptors). Non-specific binding was defined in the presence of 10 μM 5-HT for human 5-HT_{2C} receptor and 10 μM methysergide for human 5-HT_{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 × 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 concns of each radioligand (concn ranging from 0.05 to 10 nM). Dissociation constants (K_d) were calculated using the EBDA/LIGAND program.

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}–10^{-5} M). Displacement curves were analysed using EBDA/LIGAND to calculate pK_i values.[16,17]

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

Tissue preparation and incubation for measurement of IP_3 production. 5-HT_{2C}-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 60 min 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 60 min. 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 IP_3 was as described in a previous report.[18]

A concentration response curve was constructed for 5-HT, 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 5-HT in order to calculate the relative intrinsic activity.

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References

1. Boess, F. G.; Martin, I. L. *Neuropharmacol.* **1994**, *33*, 275.

2. Moreau, J.-L.; Bös, M.; Jenck, F.; Martin, J. R.; Mortas, P.; Wichmann, J. *Eur. Neuropharmacol.* **1996**, *6*, 169.

3. Martin, J. R.; Bös, M.; Jenck, F.; Köhler, C.; Moreau, J.-L.; Sleight, A. J.; Stadler, H.; Wichmann, J. *Eur. Neuropsychopharmacol.* **1995**, *5*, 209.

4. Weinhardt, K. K.; Bonhaus, D. W.; De Souza A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2687.

5. Chao, P. D.; Schiff, P. L. J.; Slatkin, D. J.; Knapp, J. E.; Chao, M.; Rosenstein, R. D. *Lloydia* **1975**, *38*, 213.

6. Chao, M.; Rosenstein, R. D.; Chao, P. D.; Schiff, P. L. J.; Slatkin, D. J.; Knapp, J. E. *Acta Cryst.* **1975**, *B31*, 2937.

7. Chao, P. D.; Schiff, P. L. J.; Slatkin, D. J.; Knapp, J. E. *J. Chem. Research (S)* **1979**, 236.

8. Raper, K. B.; Fennell, D. I. *The genus Aspergillus*; Robert E. Krieger: New York, 1973; Chap. XII.

9. Broom, N. J. P.; Sammes, P. G. *J. C. S. Chem. Comm.* **1978**, 162.

10. Azzena, U.; Cossu, S.; Denurra, T.; Melloni, G.; Piroddi, A. M. *Synthesis* **1990**, *21*, 313.

11. Uemura, M.; Tokuyama S.; Sakan, T. *Chem. Lett.* **1975**, 1195.

12. Schäfer, W.; Frank, B. *Chem. Ber.* **1966**, *99*, 160.

13. Noire P. D.; Franck R. W. *Synthesis* **1980**, *11*, 882.

14. Sheldrick, G. M. *Acta Crystallogr.* Sect. A, **1990**, *46*, 467.

15. Sleight, A. J.; Stam, N. J.; Mutel, V.; Vanderheyden, P. M. L. *Biochem. Pharmacol.* **1996**, *51*, 71.

16. McPherson, G. A. KINETIC, EBDA, LOWRY. A collection of radioligand binding analysis programs. **1985**, Biosoft, Cambridge

17. Munson, P.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

18. Bourson, A.; Wanner, D.; Wyler, R.; Petit, N.; Zwingelstein, C.; Rudler, A.; Sleight, A. J. *Pharmacol. Biochem. Behav.* **1995**, *53*, 107