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# Studies on 1-arylpiperazine derivatives with affinity for rat 5-HT$_7$ and 5-HT$_{1A}$ receptors

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## Abstract

Several 1-aryl-4-(2-arylethyl)piperazine derivatives were synthesized and tested in-vitro for their binding affinity for 5-HT$_7$ and 5-HT$_{1A}$ receptors. These compounds displayed 5-HT$_7$ receptor affinity ranging between $K_i = 474$ nM and $K_i = 8.2$ nM, besides high affinity for the 5-HT$_{1A}$ receptor. Intrinsic activity of the most potent compounds was assessed. 4-[2-(3-Methoxyphenyl)ethyl]-1-(2-methoxyphenyl)piperazine (**16**) and 1-(1,2-benzisoxazol-3-yl)-4-[2-(3-methoxyphenyl)ethyl]piperazine (**20**) ($K_i = 24.5$ and 8.2 nM, respectively) behaved as partial agonist and full agonist, respectively, when tested for 5-HT$_7$ receptor-mediated relaxation of substance P-induced guinea-pig ileum contraction.

## Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is involved in various physiological and pathological processes by interaction with 14 distinct 5-HT receptors that have been grouped on the basis of operational, structural and transductional information (Hoyer et al 2002): 5-HT$_1$ (comprising 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ subtypes), 5-HT$_2$ (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$), 5-HT$_3$, 5-HT$_4$, 5-HT$_5$ (5-HT$_{5A}$, 5-HT$_{5B}$), 5-HT$_6$, and 5-HT$_7$.

The 5-HT$_7$ receptor has been identified in rat (Lovenberg et al 1993; Meyerhof et al 1993; Ruat et al 1993; Shen et al 1993), mouse (Plassat et al 1993), man (Bard et al 1993), guinea-pig (Tsou et al 1994) and pig (Bhalla et al 2002) by the application of molecular cloning.

Earlier studies (Bard et al 1993; Meyerhof et al 1993; Shen et al 1993) found evidence of the presence of mRNA encoding for 5-HT$_7$ receptor in either the central nervous system (thalamus, hippocampus, mesencephalon, cortex) or in peripheral tissues (pancreas, spleen, coronary artery, ileum). Recent studies (Belenky & Pickard 2001; Neumaier et al 2001) have confirmed the presence of the 5-HT$_7$ receptor protein in these tissues. On the basis of such anatomical distribution it has been proposed that 5-HT$_7$ receptors may be involved in depression (Sleight et al 1995; Shimizu et al 1996; Mullins et al 1999), control of circadian rhythms (Lovenberg et al 1993; Kawahara et al 1994; Quintero & McMahon 1999; Smith et al 2001), relaxation of vascular smooth muscles (Leung et al 1996; Terron 1996; Villalon et al 1997; Centurion et al 2000), and migraine pathogenesis (Terron 2002).

Clearly, the 5-HT$_7$ receptor might be a valuable novel drug target. To date, the search for selective 5-HT$_7$-receptor antagonists has led to the discovery of SB-269970 (Lovell et al 2000), SB-258719 (Forbes et al 1998), SB-656104 (Forbes et al 2002), DR4004 (Kikuchi et al 1999), and LY215840 (Cushing et al 1996). However, these compounds present several limits because of their low potency (SB-258719), modest selectivity (SB-656104, LY215840, DR4004), and low metabolic stability (SB-269970). Therefore, the search for selectively acting 5-HT$_7$ receptor ligands as useful pharmacological tools or potential drugs is still open.

With this aim, we initiated a research programme by screening at rat cloned 5-HT$_7$ receptor a number of 1-(2-methoxyphenyl)piperazine derivatives, previously prepared

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in our laboratory as potential 5-$HT_{1A}$ ligands, because some authors (Adachi et al 1999; Kikuchi et al 1999; López-Rodríguez et al 2000) reported that compounds having this structure type were capable of binding at the 5-$HT_7$ receptor (compounds **1**–**3**, Figure 1). In this way, we have identified compounds **4**–**6** (Table 1) that possessed moderate 5-$HT_7$ receptor affinity. Therefore, we modified the structure of such compounds, having as our primary goal the improvement of the 5-$HT_7$ receptor affinity.

## Materials and Methods

### Synthesis

Column chromatography was performed with 1:30 ICN silica gel 60A (63–200 $\mu$m) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus and are uncorrected. Elemental analyses (C, H, N) were performed on an Eurovector Euro EA 3000 analyser; the analytical results were within $\pm$ 0.4% of the theoretical values for the formula given. $^1$H NMR spectra were recorded on a Varian Mercury-VX instrument (300 MHz) with $CDCl_3$ as solvent. Chemical shift values were reported in ppm ($\delta$). An HP6890-5973 MSD gas chromatograph/mass spectrometer was used to record mass spectra; only significant *m/z* peaks, with their percentage of relative intensity in parentheses, were reported. All spectra were in accordance with the assigned structures. A standard procedure was used to transform final compounds into their hydrochloride salts. The following intermediates have been prepared following literature methods: 5-methoxy-2-tetralone (Cornforth & Robinson 1949), 3-(1-piperazinyl)-1,2-benzisoxazole (Yevich et al 1986), 1-(2-chloroethyl)-3-methoxybenzene (Kato et al 1993), 1-(2-chloroethyl)-2-methoxybenzene (Kato et al 1993), 1-(2-chloroethyl)-2,6-dimethoxybenzene (Boltze & Dell 1967), 2,3,4-trimethoxyphenylacetic acid (Arndts et al 1994). All reactions were carried out under an atmosphere of nitrogen.

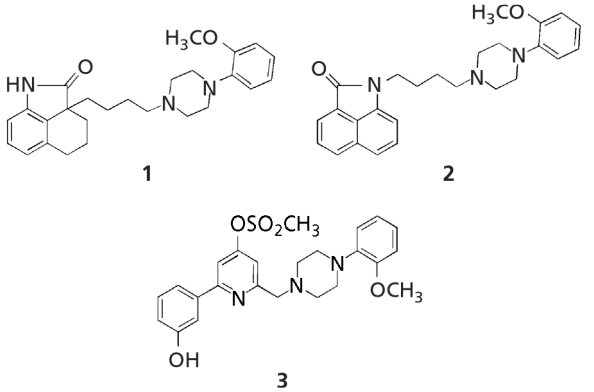



**Figure 1** Structures of 5-$HT_7$ receptor ligands with 1-(2-methoxyphenyl)piperazine structure (Adachi et al 1999; Kikuchi et al 1999; Lopez-Rodriguez et al 2000).

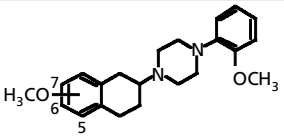
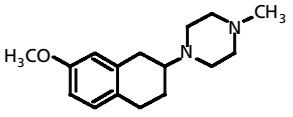
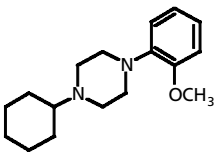
### General procedure for preparation of compounds 4–8

A mixture of ketone (6.0 mmol) and the appropriate 1-substituted piperazine (24.0 mmol) in anhydrous toluene (60 mL) was refluxed overnight in the presence of a catalytic amount of *p*-toluenesulfonic acid and the formed water was azeotropically distilled off and collected by a Dean-Stark trap. After cooling, the solvent was evaporated, the crude enamine was solubilized in ethanol (60 mL) and hydrogenated at normal pressure and room temperature in the presence of $PtO_2$ (0.1 g) until the uptake ceased. Then, the solvent was evaporated in-vacuo to give a crude residue which was chromatographed as detailed below to provide pure compounds **4–8** as pale yellow oils.

*4-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-1-(2-methoxyphenyl)piperazine (**4**)*
Eluted with $CHCl_3$/AcOEt, 3:2, in 21% yield. $^1$H NMR: $\delta$ 1.53–1.67 (m, 1H, CHHCHC*H*HCHH), 2.17–2.24 (m, 1H, CHHCHCHHC*H*H), 2.48–2.60 (m, 1H, CHHCHCH *H*CHH), 2.69–2.78 (m, 2H, CH*H*CHCHHCH*H*), 2.86 (br t, 4H, $(CH_2)_2$N), 2.96–3.04 (m, 2H, C*H*HC*H*CHHCHH), 3.13 (br s, 4H, $(CH_2)_2$NAr), 3.80, 3.86 (2 s, 6H, $CH_3$), 6.63–7.11 (m, 7H, aromatic). GC-MS *m/z* 353 ($M^+ + 1$, 25), 352 ($M^+$, 100), 192 (45), 150 (39), 149 (38). The hydrochloride salt melted at 243–244 °C (from $CH_3OH$). Anal. ($C_{22}H_{28}N_2O_2 \cdot 2HCl$) C, H, N.

*4-(6-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-1-(2-methoxyphenyl)piperazine (**5**)*
Eluted with $CH_2Cl_2$/AcOEt, 9:1, in 43% yield. $^1$H NMR: $\delta$ 1.59–1.67 (m, 1H, CHHCHC*H*HCHH), 2.13–2.17 (m, 1H,

**Table 1** Binding affinities of target compounds **4**–**8**.

| Compound | Structure | $K_i$ s.e.m. (nM) 5-$HT_7$ |
|---|---|---|
| **4** (5-$OCH_3$)<br>**5** (6-$OCH_3$)<br>**6** (7-$OCH_3$) |  | 616 ± 20<br>563 ± 35<br>115 ± 18 |
| **7** |  | > 1000 (16%)[a] |
| **8** |  | > 1000 (39%) |

[a]Full $K_i$ not obtained, percentage inhibition at the concentration shown given in parentheses.

CHHCHCHHC*H*H), 2.70–2.99 (m, 9H, C*HH*C*H*C*H* H*C*H*H*, $(CH_2)_2$N), 3.12 (br t, 4H, $(CH_2)_2$NAr), 3.76, 3.85 (2 s, 6H, $CH_3$), 6.61–7.18 (m, 7H, aromatic). GC-MS *m/z* 353 ($M^+ + 1$, 11), 352 ($M^+$, 45), 192 (100), 150 (48). The hydrochloride salt melted at 208–211 °C (from $CH_3OH$/$Et_2O$). Anal. ($C_{22}H_{28}N_2O_2$·2HCl) C, H, N.

*4-(7-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-1-(2-methoxyphenyl)piperazine (**6**)*
Eluted with $CHCl_3$/AcOEt, 3:2 , in 76% yield. $^1$H NMR: $\delta$ 1.59–1.71 (m, 1H, CHHCHC*H*HCHH), 2.13–2.19 (m, 1H, CHHCHCHHC*H*H), 2.70–2.98 (m, 9H, C*HH*C*H*C*H* H*C*H*H*, $(CH_2)_2$N), 3.13 (br t, 4H, $(CH_2)_2$NAr), 3.76, 3.86 (2 s, 6H, $CH_3$), 6.62–7.02 (m, 7H, aromatic). GC-MS *m/z* 353 ($M^+ + 1$, 26), 352 ($M^+$, 100), 192 (56), 150 (48), 149 (34). The hydrochloride salt melted at 249–250 °C (from $CH_3OH$). Anal. ($C_{22}H_{28}N_2O_2$·2HCl) C, H, N.

*1-(7-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-1-methylpiperazine (**7**)*
Eluted with $CHCl_3$/$CH_3OH$, 19:1, in 45% yield. $^1$H NMR: $\delta$ 1.55–1.67 (m, 1H, CHHCHC*H*HCHH), 2.08–2.14 (m, 1H, CHHCHCHHC*H*H), 2.31 (s, 3H, $NCH_3$), 2.50 and 2.69 (2 br s, 8H, piperazine), 2.70–2.89 (m, 5H, C*HH*C*H*CH*H*CH*H*), 3.76 (s, 3H, $OCH_3$), 6.61–6.69 (m, 3H, aromatic). GC-MS *m/z* 261 ($M^+ + 1$, 20), 260 ($M^+$, 100), 189 (28), 160 (63). The hydrochloride salt melted at 240 °C dec. (from $CH_3OH$/$Et_2O$). Anal. ($C_{16}H_{24}N_2O$·2HCl) C, H, N.

*4-Cyclohexyl-1-(2-methoxyphenyl)piperazine (**8**)*
Eluted with $CHCl_3$/AcOEt, 1:1, in 47% yield. $^1$H NMR: $\delta$ 1.06–1.32 (m, 5H, C*H*H$(CH_2CH_2)_2$), 1.60–1.64 (m, 1H, CH*H*$(CH_2CH_2)_2$), 1.74–1.81 and 1.91–1.97 (m, 4H, CHH$(CH_2CH_2)_2$), 2.26–2.33 (m, 1H, CHN), 2.76 (br t, 4H, $(CH_2)_2$N), 3.08 (br s, 4H, $(CH_2)_2$NAr), 3.84 (s, 3H, $CH_3$), 6.82–7.00 (m, 3H, aromatic). GC-MS *m/z* 275 ($M^+ + 1$, 27), 274 ($M^+$, 95), 232 (27), 231 (100), 149 (43). The hydrochloride salt melted at 260 °C, dec. (from $CH_3OH$/$Et_2O$). Anal. ($C_{17}H_{26}N_2O$·2HCl) C, H, N.

*General procedure for preparation of amides **15a–d***
A mixture of carboxylic acid **9a–d** (0.48 mmol) and 1,1′-carbonyldiimidazole (0.50 mmol) in 10 mL anhydrous tetrahydrofuran (THF) was stirred for 8 h. A solution of 1-(2-methoxyphenyl)piperazine (0.5 mmol) in 10 mL anhydrous THF was added and the resulting mixture was stirred for 1 h. The reaction mixture was partitioned between AcOEt and $H_2O$. The organic layer was washed with 5% aqueous $NaHCO_3$, dried ($Na_2SO_4$) and concentrated in-vacuo. The crude residue was chromatographed ($CHCl_3$/AcOEt, 1:1, as eluent) to give pure target amides as pale yellow semisolids in 73–78% yield.

*4-(2,5-Dimethoxyphenylacetyl)-1-(2-methoxyphenyl)piperazine (**15a**)* $^1$H NMR: $\delta$ 2.90 and 3.00 (2 br t, 4H, $(CH_2)_2$NAr), 3.64 and 3.83 (2 br t, 4H, N$(CH_2)_2$), 3.72 (s, 2H, $CH_2CO$), 3.75, 3.79, 3.86 (3 s, 9H, $CH_3$), 6.73–7.05 (m, 7H, aromatic). GC-MS *m/z* 371 ($M^+ + 1$, 19), 370 ($M^+$, 76), 191 (23), 162 (100).

*4-(3,4-Dimethoxyphenylacetyl)-1-(2-methoxyphenyl)piperazine (**15b**)* $^1$H NMR: $\delta$ 2.86 and 2.99 (2 br t, 4H, $(CH_2)_2$NAr), 3.60–3.64 and 3.80–3.84 (m, 4H, N$(CH_2)_2$), 3.72 (s, 2H, $CH_2CO$), 3.85, 3.86, 3.87 (3 s, 9H, $CH_3$), 6.75–7.04 (m, 7H, aromatic). GC-MS *m/z* 371 ($M^+ + 1$, 21), 370 ($M^+$, 81), 162 (100), 151 (45).

*1-(2-Methoxyphenyl)-4-(2,3,4-trimethoxyphenylacetyl)piperazine (**15c**)* $^1$H NMR: $\delta$ 2.90 and 3.00 (2 br t, 4H, $(CH_2)_2$NAr), 3.64–3.67 and 3.80–3.85 (m, 4H, N$(CH_2)_2$), 3.69 (s, 2H, $CH_2CO$), 3.84, 3.86, 3.88, 3.90 (4 s, 12H, $CH_3$), 6.87–7.53 (m, 6H, aromatic). GC-MS *m/z* 401 ($M^+ + 1$, 26), 400 ($M^+$, 100), 181 (35), 162 (90), 149 (36).

*1-(2-Methoxyphenyl)-4-(2,4,6-trimethoxyphenylacetyl)piperazine (**15d**)* $^1$H NMR: $\delta$ 3.02 (br t, 4H, $(CH_2)_2$NAr), 3.65 (s, 2H, $CH_2CO$), 3.72–3.76 and 3.78–3.82 (m, 4H, N$(CH_2)_2$), 3.78, 3.81, 3.88 (3 s, 12H, $CH_3$), 6.87–7.53 (m, 6H, aromatic). GC-MS *m/z* 401 ($M^+ + 1$, 11), 400 ($M^+$, 42), 181 (100), 162 (36).

*General procedure for preparation of compounds **16–22***
A stirred mixture of alkylating agent **10–14** (8.0 mmol), 1-substituted piperazine (9.6 mmol) and $Na_2CO_3$ (8.0 mmol) in acetonitrile was refluxed overnight. After the mixture was cooled, the mixture was evaporated to dryness and $H_2O$ (20 mL) was added to the residue. The aqueous phase was extracted with AcOEt (2 × 30 mL). The collected organic layers were dried over $Na_2SO_4$ and evaporated under reduced pressure. The crude residue was chromatographed, as indicated below, to yield pure compounds **16–22** as pale yellow oils.

*4-[2-(3-Methoxyphenyl)ethyl]-1-(2-methoxyphenyl)-piperazine (**16**)* Eluted with $CHCl_3$/AcOEt, 7:3, in 10% yield. $^1$H NMR: $\delta$ 2.66–2.87 (m, 8H, benzylic, $CH_2N(CH_2)_2$), 3.14 (br s, 4H, $(CH_2)_2$NAr), 3.80 and 3.87 (2 s, 6H, $CH_3$), 6.73–7.24 (m, 8H, aromatic). GC-MS *m/z* 327 ($M^+ + 1$, 2), 326 ($M^+$, 8), 205 (100), 190 (31). The hydrochloride salt melted at 220 °C (from $CH_3OH$/$Et_2O$). Anal. ($C_{20}H_{26}N_2O_2$·2HCl) C, H, N.

*4-[2-(3-Hydroxyphenyl)ethyl]-1-(2-methoxyphenyl)-piperazine (**17**)* Eluted with $CHCl_3$/AcOEt, 1:1, in 17% yield. $^1$H NMR: $\delta$ 2.69–2.87 (m, 8H, benzylic, $CH_2N(CH_2)_2$), 3.16 (br s, 4H, $(CH_2)_2$NAr), 3.86 (s, 3H, $CH_3$), 6.66–7.18 (m, 8H, aromatic), 9.11 (s, 1H, OH, $D_2O$ exchanged). GC-MS *m/z* 313 ($M^+ + 1$, 2), 312 ($M^+$, 8), 205 (100), 190 (29). The hydrochloride salt melted at 200–205 °C (from $CH_3OH$/$Et_2O$). Anal. ($C_{19}H_{24}N_2O_2$· 2HCl· $H_2O$) C, H, N.

*4-[2-(2-Methoxyphenyl)ethyl]-1-(2-methoxyphenyl)-piperazine (**18**)* Eluted with $CHCl_3$/AcOEt, 7:3, in 30%

yield. $^1$H NMR: $\delta$ 2.61–2.67 (m, 2H, benzylic), 2.76 (br s, 2H, C$H_2$N(CH$_2$)$_2$), 2.85–2.90 (m, 4H, CH$_2$N(C$H_2$)$_2$), 3.14 (br s, 4H, (C$H_2$)$_2$NAr), 3.83, 3.87 (2 s, 6H, CH$_3$), 6.84–7.26 (m, 8H, aromatic). GC-MS $m/z$ 327 ($M^+ + 1$, 1), 326 ($M^+$, 6), 205 (100), 190 (26). The hydrochloride salt melted at 210–212 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·2HCl) C, H, N.

*4-[2-(4-Methoxyphenyl)ethyl]-1-(2-methoxyphenyl)-piperazine (**19**)* Eluted with CHCl$_3$/AcOEt, 7:3, in 18% yield. $^1$H NMR: $\delta$ 2.61–2.67 (m, 2H, benzylic), 2.74 (br s, 2H, C$H_2$N(CH$_2$)$_2$), 2.77–2.83 (m, 4H, CH$_2$N(C$H_2$)$_2$), 3.13 (br s, 4H, (C$H_2$)$_2$NAr), 3.79, 3.89 (2 s, 6H, CH$_3$), 6.82–7.16 (m, 8H, aromatic). GC-MS $m/z$ 327 ($M^+ + 1$, 1), 326 ($M^+$, 6), 205 (100), 190 (30). The hydrochloride salt melted at 230–232 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·2HCl) C, H, N.

*1-(1,2-Benzisoxazol-3-yl)-4-[2-(3-methoxyphenyl)ethyl] piperazine (**20**)* Eluted with CHCl$_3$/AcOEt, 1:1, in 22% yield. $^1$H NMR: $\delta$ 2.66–2.86 (m, 8H, benzylic CH$_2$N(CH$_2$)$_2$), 3.62 (br t, 4H, (C$H_2$)$_2$NAr), 3.80 (s, 3H, CH$_3$), 6.74–7.71 (m, 8H, aromatic). GC-MS $m/z$ 337 ($M^+$, 1), 216 (100), 97 (72). The hydrochloride salt melted at 198–200 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·2HCl) C, H, N.

*1-(2-Hydroxyphenyl)-4-[2-(3-methoxyphenyl)ethyl]-piperazine (**21**)* Eluted with CHCl$_3$/AcOEt, 1:1, in 10% yield. $^1$H NMR: $\delta$ 2.66–2.86 (m, 8H, benzylic CH$_2$N(CH$_2$)$_2$), 2.95 (br s, 4H, (C$H_2$)$_2$NAr), 3.81 (s, 3H, CH$_3$), 6.74–7.25 (m, 9H, aromatic, OH, 1H, D$_2$O exchanged). GC-MS $m/z$ 313 ($M^+ + 1$, 1), 312 ($M^+$, 3), 191 (100). The hydrochloride salt melted at 234–236 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·2HCl·$^1/_3$H$_2$O) C, H, N.

*4-[2-(2,6-Dimethoxyphenyl)ethyl]-1-(2-methoxyphenyl) piperazine (**22**)* Eluted with CHCl$_3$/AcOEt, 1:1, in 41% yield. $^1$H NMR: $\delta$ 2.51–2.56 (m, 2H, benzylic), 2.78 (br s, 2H, C$H_2$N(CH$_2$)$_2$), 2.90–2.95 (m, 4H, CH$_2$N(C$H_2$)$_2$), 3.15 (br s, 4H, (C$H_2$)$_2$NAr), 3.81, 3.87 (2 s, 9H, CH$_3$), 6.53–7.16 (m, 7H, aromatic). GC-MS $m/z$ 357 ($M^+ + 1$, 3), 356 ($M^+$, 12), 205 (100), 190 (27). The hydrochloride salt melted at 188–190 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·2HCl·H$_2$O) C, H, N.

*General procedure for preparation of compounds **23–26*** Borane-methyl sulfide complex, as 10.0 M BH$_3$ in excess methyl sulfide (0.6 mL, 6.0 mmol) was dropped into an ice-cooled solution of amide **15a–d** (2.0 mmol) in anhydrous THF (10 mL), under stirring. After being refluxed for 4 h, the reaction mixture was cooled at −10 °C and CH$_3$OH was added very carefully dropwise until gas evolution ceased. The mixture was treated with 3 M HCl (5 mL) and was refluxed for 1 h. After cooling, the mixture was basified with 3 M NaOH and extracted with AcOEt (3 × 30 mL). The collected organic layers were dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/AcOEt, 1:1, as eluent) to give compounds **23–26** as pale yellow liquids.

*4-[2-(2,5-Dimethoxyphenyl)ethyl]-1-(2-methoxyphenyl) piperazine (**23**)* Yield 83%. $^1$H NMR: $\delta$ 2.62–2.68 (m, 2H, benzylic), 2.77 (br s, 4H, CH$_2$N(C$H_2$)$_2$), 2.83–2.88 (m, 2H, C$H_2$N(CH$_2$)$_2$), 3.15 (br s, 4H, (C$H_2$)$_2$NAr), 3.76, 3.78, 3.87 (3 s, 9H, CH$_3$), 6.69–7.03 (m, 7H, aromatic). GC-MS $m/z$ 357 ($M^+ + 1$, 3), 356 ($M^+$, 11), 206 (20), 205 (100), 190 (31). The hydrochloride salt melted at 195–196 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·HCl·H$_2$O) C, H, N.

*4-[2-(3,4-Dimethoxyphenyl)ethyl]-1-(2-methoxyphenyl) piperazine (**24**)* $^1$H NMR: $\delta$ 2.63–2.68 (m, 2H, benzylic), 2.75–2.83 (m, 6H, CH$_2$N(CH$_2$)$_2$), 3.14 (br s, 4H, (C$H_2$)$_2$NAr), 3.86, 3.87, 3.88 (3 s, 9H, CH$_3$), 6.75–7.04 (m, 7H, aromatic). GC-MS $m/z$ 357 ($M^+ + 1$, 1), 356 ($M^+$, 3), 205 (100), 190 (23). The hydrochloride salt melted at 234–236 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{21}$H$_{28}$N$_2$O$_3$·HCl) C, H, N.

*4-[2-(2,3,4-Trimethoxyphenyl)ethyl]-1-(2-methoxyphenyl)piperazine (**25**)* $^1$H NMR: $\delta$ 2.59–2.65 (m, 2H, benzylic), 2.76–2.83 (m, 6H, CH$_2$N(CH$_2$)$_2$), 3.14 (br s, 4H, (C$H_2$)$_2$NAr), 3.84, 3.87, 3.88, 3.90 (4 s, 12H, CH$_3$), 6.60–7.20 (m, 6H, aromatic). GC-MS $m/z$ 387 ($M^+ + 1$, 2), 386 ($M^+$, 9), 205 (100), 190 (25). The hydrochloride salt melted at 240 °C, dec. (from CH$_3$OH/Et$_2$O). Anal. (C$_{22}$H$_{30}$N$_2$O$_4$·HCl) C, H, N.

*4-[2-(2,4,6-Trimethoxyphenyl)ethyl]-1-(2-methoxyphenyl)piperazine (**26**)* $^1$H NMR: $\delta$ 2.46–2.57 (m, 2H, benzylic), 2.76–2.86 (m, 6H, CH$_2$N(CH$_2$)$_2$), 3.14 (br s, 4H, (C$H_2$)$_2$NAr), 3.79, 3.80, 3.87 (3 s, 12H, CH$_3$), 6.85–7.02 (m, 6H, aromatic). GC-MS $m/z$ 387 ($M^+ + 1$, 3), 386 ($M^+$, 14), 206 (20), 205 (100), 190 (27). The hydrochloride salt melted at 121–123 °C (from CH$_3$OH/Et$_2$O). Anal. (C$_{22}$H$_{30}$N$_2$O$_4$) C, H, N.

## Pharmacology

Male Wistar Hannover rats (200–250 g) and male albino Dunkin-Hartley guinea-pigs (300–350 g) were from Harlan (S. Pietro al Natisone, Italy). The animals were handled according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, O.J. No. L358, December 18, 1986).

Rat recombinant serotonin 5-HT$_7$ receptor expressed in HEK-293 cells were purchased from PerkinElmer-NEN (Betsville, MD).

[$^3$H]Lysergide and [$^3$H]-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) were obtained from NEN Life Science Products (Zaventem, Belgium). 5-Carboxamidotryptamine (5-CT), ketanserin, and substance P were purchased from Tocris Cookson Ltd. (Bristol, UK). 8-OH-DPAT hydrobromide was from RBI, Milan, Italy.

SB-269970, atropine, and pyrilamine were from Sigma-Aldrich (Milan, Italy).

For receptor binding studies, compounds **16**–**26** were dissolved in absolute ethanol. For isolated guinea-pig ileum assay, compounds **16** and **20** were dissolved in dressed Krebs–Henseleit solution, pH 7.4.

*Radioligand binding assay at rat cloned 5-HT$_7$ receptor*

Binding of [$^3$H]lysergide at rat cloned 5-HT$_7$ receptor was performed according to Jasper et al (1997) with minor modifications. In 1 mL incubation buffer (50 mM Tris, 10 mM $MgCl_2$ and 0.5 mM EDTA, pH 7.4) were suspended 30 $\mu$g of membranes, 2.5 nM [$^3$H]lysergide, the drugs or reference compound (six to nine concentrations). The samples were incubated for 60 min at 37 °C. The incubation was stopped by rapid filtration on GF/A glass fibre filters (pre-soaked in 0.5% polyethylenimine for 30 min). The filters were washed with 3 × 3 mL ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was determined in the presence of 10 $\mu$M 5-CT. Approximately 90% of specific binding was determined under these conditions.

*Radioligand binding assay at rat hippocampal membrane 5-HT$_{1A}$ receptors*

Binding experiments were performed according to Borsini et al (1995) with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the hippocampus was dissected. The hippocampus (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL 50 mM Tris buffer, pH 7.6. The homogenate was centrifuged at 48 000 *g* for 15 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 25 mL buffer, then pre-incubated for 10 min at 37 °C. The homogenate was centrifuged at 48 000 *g* for 15 min at 4 °C. The supernatant was discarded, and the final pellet was stored at −80 °C until used. Each tube received in a final volume of 1 mL 50 mM Tris (pH 7.6) hippocampus membranes suspension and 1 nM [$^3$H]-8-OH-DPAT. For competitive inhibition experiments various concentrations of drugs studied were incubated. Nonspecific binding was defined using 1 $\mu$M 8-OH-DPAT. Samples were incubated at 37 °C for 20 min and then filtered on Whatman GF/B glass microfibre filters. The $K_d$ value determined for 8-OH-DPAT was 8.8 nM.

*Isolated guinea-pig ileum assay*

Guinea-pigs were anaesthetized and then decapitated and the proximal ileum removed. The intestine was carefully flushed several times with warm Krebs–Henseleit solution (in mM: 118 NaCl, 25 $NaHCO_3$, 4.7 KCl, 0.6 $MgSO_4$, 1.2 $KH_2PO_4$, 1.2 $CaCl_2$, 11.2 glucose, pH 7.4). Whole ileal segments, approximately 3 cm in length, were suspended under 1.0 g tension in Krebs solution gassed with 95% $O_2$ and 5% $CO_2$ and maintained at 37 °C. According to Carter et al (1995) with minor modification, the bathing medium contained 1 $\mu$M atropine to antagonize cholinergically mediated contractions due to activation of 5-HT$_3$ and 5-HT$_4$ receptors, 1 $\mu$M ketanserin to block 5-HT$_{2A}$ receptors, 1 $\mu$M pyrilamine to block $H_1$ receptors. Changes in tension of the tissue were recorded by a Fort 10 Original WPI isometric transducer (2Biological Instruments, Italy) connected to a PowerLab/400 workstation (PowerLab/400, ADInstruments Pty Ltd, Castle Hill, Australia). Tissue was contracted by 100 nM substance P. This value was preliminarily determined by concentration–response curves (1–200 nM). Substance P (100 nM) elicited 80% of maximum contraction. The reference agonist 5-CT or **16** or **20** was added 3 min before addition of substance P and non-cumulative concentration–response curves were constructed (0.001–10 $\mu$M). We determined that 5-CT induced relaxation with maximal response (39%) at a concentration of 3 $\mu$M, and so 5-HT$_7$ desensitization was achieved by equilibrating for 1 h in the presence of 3 $\mu$M 5-CT, changing the bathing solution every 15 min. 5-CT, **16** or **20** was added 3 min before substance P addition.

Agonist 5-CT, **16** and partial agonist **20** were tested also in the presence of the antagonist SB-269970 (0.1–3 $\mu$M). The isolated guinea-pig ileum was equilibrated for 75 min with antagonist before constructing concentration–response curves of 5-CT, **16**, and **20**.

Tissue responses were recorded as gram changes in isometric tension and expressed as percentage of reduction in the height of the contraction.

### Statistical methods

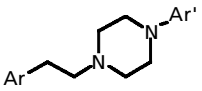
The inhibition curves on the different binding sites of the compounds reported in Table 1 and Table 2 were analysed by non-linear curve fitting utilizing the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA). Values are means ± s.e.m. from three experiments in triplicate. The value for the inhibition constant, $K_i$, was calculated by using the Cheng & Prusoff (1973) equation. Agonist potencies, expressed as EC50, were obtained from non-linear iterative curve fitting by GraphPad Prism. One-way analysis of variance was used to estimate the significance of difference. A difference with $P < 0.005$ was considered statistically significant.

## Results and Discussion

The synthesis of compounds **4**–**8** (Figure 2) paralleled a procedure reported in the literature for the synthesis of structurally related 2-aminotetralins (McDermed et al 1975). Condensation of the appropriate ketone with 1-(2-methoxyphenyl)piperazine or 1-methylpiperazine in the presence of a catalytic amount of *p*-toluenesulfonic acid gave the intermediate enamines, which were catalytically hydrogenated in the presence of $PtO_2$ to give target compounds **4**–**8**.

*N*-Arylethylpiperazine derivatives **16**–**26** were prepared (Figure 3) from the appropriate 1-arylpiperazine by alkylation with 2-chloroethyl derivatives **10**–**14** or by condensation with carboxylic acids **9a**–**d** and subsequent reduction of the formed amides **15a**–**d** with borane methyl sulfide complex (BMS) (Brown et al 1982).

**Table 2** Binding affinities of final compounds **16–26**.




| Compound | Ar | Ar′ | $K_i$ s.e.m. (nM) | |
|---|---|---|---|---|
| | | | 5-$HT_7$ | 5-$HT_{1A}$ |
| **16** | 3-$OCH_3$-Ph | 2-$OCH_3$-Ph | 24.5 ± 3.0 | 2.37 ± 0.30 |
| **17** | 3-OH-Ph | 2-$OCH_3$-Ph | 35.4 ± 2.50 | 8.12 ± 1.50 |
| **18** | 2-$OCH_3$-Ph | 2-$OCH_3$-Ph | 160 ± 15 | NT |
| **19** | 4-$OCH_3$-Ph | 2-$OCH_3$-Ph | 126 ± 12 | NT |
| **20** | 3-$OCH_3$-Ph | 3-(1,2-benzisoxazolyl)- | 8.2 ± 0.8 | 3.63 ± 0.25 |
| **21** | 3-$OCH_3$-Ph | 2-OH-Ph | 51.5 ± 8.3 | 3.50 ± 0.30 |
| **22** | 2,6-di-$OCH_3$-Ph | 2-$OCH_3$-Ph | 747 ± 50 | NT |
| **23** | 2,5-di-$OCH_3$-Ph | 2-$OCH_3$-Ph | 31.9 ± 5.4 | 21.9 ± 3.20 |
| **24** | 3,4-di-$OCH_3$-Ph | 2-$OCH_3$-Ph | 48.3 ± 6.5 | 8.36 ± 0.25 |
| **25** | 2,3,4,-tri-$OCH_3$-Ph | 2-$OCH_3$-Ph | 71 ± 8.1 | 4.50 ± 0.22 |
| **26** | 2,4,6-tri-$OCH_3$-Ph | 2-$OCH_3$-Ph | 300 ± 25 | NT |

NT, not tested.

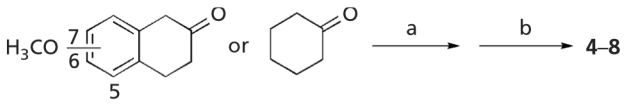



**Figure 2** Synthesis of compounds **4–8**. Reagents and conditions: a, 1-(2-methoxyphenyl)piperazine or 1-methylpiperazine, *p*-toluenesulfonic acid, toluene, reflux, 18 h; b, $H_2$, $PtO_2$, ethanol, 1 atm, room temperature.

Affinity values of the target compounds **4–8** and **16–26** for 5-$HT_7$ receptors are listed in Tables 1 and 2. Although our study was aimed at identifying new 5-$HT_7$ receptor ligands, we investigated the 5-$HT_{1A}$ receptor affinity of only those compounds displaying $K_i$ values at 5-$HT_7$ receptor lower than 100 nM (Table 2), because it is well documented that 1-(2-methoxyphenyl)piperazine derivatives are capable of binding at 5-$HT_{1A}$ receptors (Lopez-Rodriguez et al 2002) as well as other receptors. Considering the starting compounds **4–6**, it can be noted that they showed moderate 5-$HT_7$ receptor affinity. In

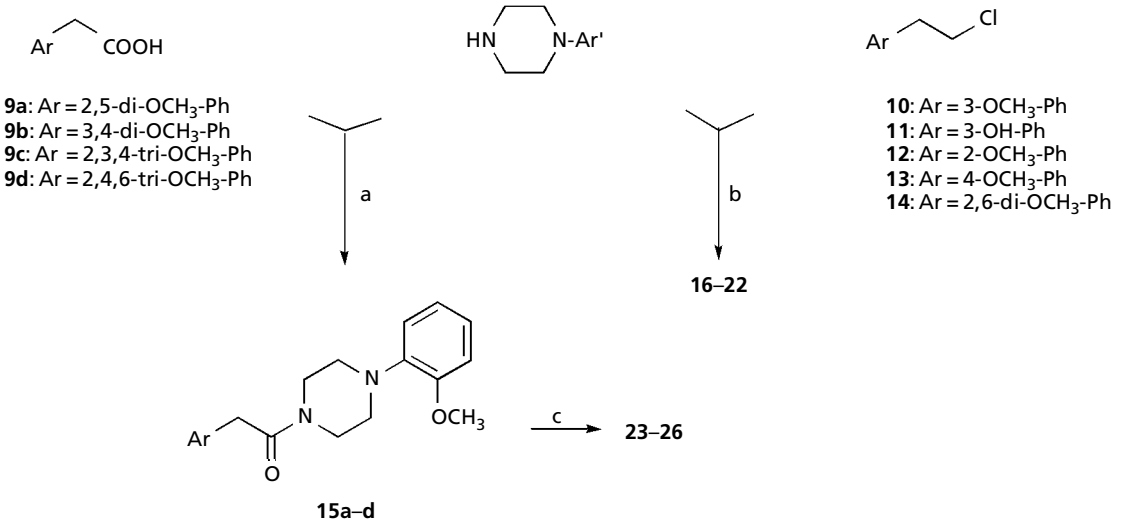



**Figure 3** Synthesis of compounds **16–26**. Reagents and conditions: a, 1,1′-carbonyldiimidazole, anhydrous THF, 8 h, room temperature; b, $K_2CO_3$, $CH_3CN$, reflux, 18 h; c, BMS, anhydrous THF, reflux, 4 h.

view of its relatively high affinity, compound **6** underwent structural modifications. Removal of the aromatic ring linked to the piperazine ring or of the aromatic ring of the tetralin system gave rise to compounds **7** and **8** that were devoid of 5-$HT_7$ receptor affinity. Therefore, it resulted that both aromatic rings were necessary for 5-$HT_7$ receptor affinity of derivative **6**. As far as compounds **4**–**6** can be considered rigid congeners of 4-(2-arylethyl)-1-(2-methoxyphenyl)piperazine derivatives, the opening of the saturated ring of the tetralin system was accomplished to obtain an easily accessible series of compounds. Derivative **16** showed higher 5-$HT_7$ receptor affinity ($K_i = 24.5$ nM) than the corresponding tetralin derivatives **4** and **6**, indicating that the constrained structure of the ethyl chain such as in **4** and **6** was detrimental for 5-$HT_7$ receptor affinity. However, compound **16** presented high 5-$HT_{1A}$ receptor affinity. Therefore, we modified **16** with the aim of increasing the affinity and specificity for 5-$HT_7$ receptor. In particular, we evaluated the corresponding 3-hydroxy derivative **17** and also the 2- and 4- methoxy substituted isomers of **16** (compounds **18** and **19**). Demethylation of **16** did not vary the 5-$HT_7$ receptor affinity, whereas shifting of the methoxy group from 3-position to 2- or 4-position caused a drop in 5-$HT_7$ receptor affinity of one order of magnitude. Moreover, we considered analogues of **16** having more than one methoxyl substituent on the aromatic ring (compounds **22**–**26**). It can be noted that derivatives **23**–**25** that maintained a methoxyl substituent in the *meta* position retained 5-$HT_7$ receptor affinity in the same range as **16** ($K_i = 31.9$–$71$ nM), whereas **22** and **26** showed a marked decrease in 5-$HT_7$ receptor affinity.

At this point, as the modifications of **16** on the aromatic ring linked to the ethyl chain had minimal effect on 5-$HT_7$ receptor affinity, we replaced the 2-methoxyphenyl group linked to the *N*-1 piperazine with a 1,2-benzisoxazol-3-yl group or with a 2-hydroxyphenyl group (compounds **20** and **21**, respectively). The first modification was performed because in a previous study we found that this replacement lowered the 5-$HT_{1A}$ affinity, leaving unchanged that for 5-$HT_7$ receptor (Perrone et al 2003). 1,2-Benzisoxazol-3-yl derivative **20** demonstrated higher 5-$HT_7$ receptor affinity than **16**, but, in this case, no improvement in selectivity over 5-$HT_{1A}$ receptor was achieved. The second modification was performed because it had been reported that the replacement of the 2-methoxyphenyl group linked to the *N*-1 piperazine with the 2-hydroxyphenyl group caused a drop in 5-$HT_{1A}$ receptor affinity (Oficialdegui et al 2000; Martinez-Esparza et al 2001). Unfortunately, compound **21** displayed a two-times lower 5-$HT_7$ receptor affinity than **16** and no selectivity for the 5-$HT_{1A}$ receptor. Taken together, affinity data of compounds **17**–**26** showed that the modifications of **16** might have had only a moderate effect on 5-$HT_7$ receptor affinity and that more wide-ranging structural modifications were probably necessary to achieve higher selectivity over 5-$HT_{1A}$ receptors.

Compounds **16** and **20**, showing the highest 5-$HT_7$ receptor affinities among the studied compounds, were tested for 5-$HT_7$ intrinsic activity in isolated guinea-pig ileum preparation along with 5-$HT_7$ receptor agonist 5-CT. It has been reported that 5-$HT_7$ agonists can produce dose-dependent guinea-pig ileum relaxation of substance P-induced contraction (Carter et al 1995). EC50 values of 5-CT, **16** and **20** were found to be $0.63 \pm 0.02$, $3.28 \pm 0.46$ and $3.63 \pm 0.50$ $\mu$M, respectively. Compound **20** behaved as a 5-$HT_7$ full agonist like 5-CT (Figure 4). Compound SB-269970, reported to be a potent and selective 5-$HT_7$ receptor antagonist (Hagan et al 2000; Lovell et al 2000), was able to revert those effects by shifting to the right the corresponding concentration–response curve in a surmountable manner (Figure 5). On the other hand, derivative **16** behaved as a partial agonist because it elicited only 28%

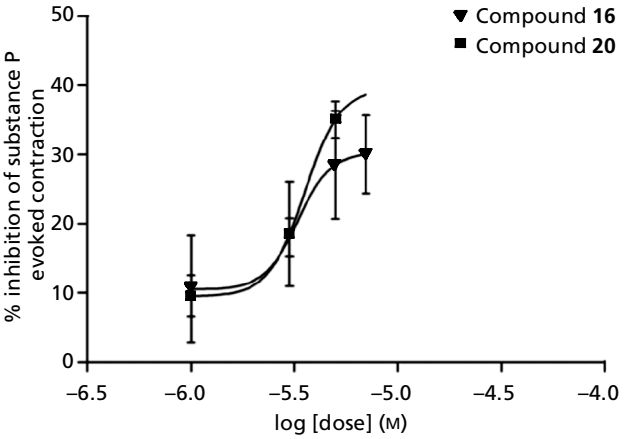



**Figure 4** Concentration–response curves of inhibition of substance P-inducedl contracture in guinea-pig ileum elicited by derivatives **16** and **20**. Data represent the mean ± s.e. from four to seven separate experiments.

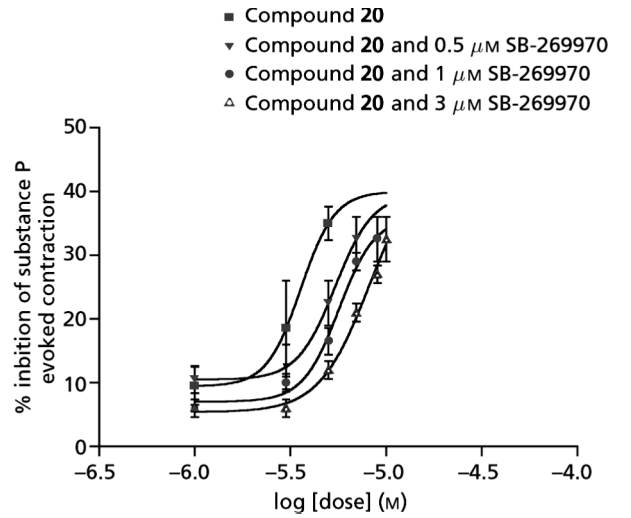



**Figure 5** Effect of SB-269970 on concentration–response curves of inhibition of substance P-induced contracture in guinea-pig ileum elicited by compound **20**. Data represent the mean ± s.e. from four to seven separate experiments.

of the maximal response (Figure 4). The slope of Schild plots (95% confidence intervals) of 5-CT, **16**, and **20** were 1.2 (1.0–1.4), 1.0 (0.9–1.1), and 1.1 (0.9–1.3), respectively. $pA_2$ values of 5-CT, **16**, and **20** were $7.48 \pm 0.12$ ($n = 12$), $7.80 \pm 0.20$ ($n = 14$), and $7.62 \pm 0.50$ ($n = 22$), respectively.

## Conclusions

Structural modifications on 4-(7-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-1-(2-methoxyphenyl)piperazine (**6**) led to the identification of several 1-aryl-4-(2-arylethyl)piperazine derivatives having moderate to good 5-$HT_7$ receptor affinity, but no selectivity over 5-$HT_{1A}$ receptor. The most potent compounds of this series (i.e. 4-[2-(3-methoxyphenyl)ethyl]-1-(2-methoxyphenyl)piperazine (**16**) and 4-[2-(3-methoxyphenyl)ethyl]-1-(1,2-benzisoxazol-3-yl)piperazine (**20**) displayed $K_i$ values of 24.5 and 8.2 nM, respectively, at the 5-$HT_7$ receptor. Moreover, derivative **20** displayed agonist properties like 5-CT when tested for 5-$HT_7$ receptor mediated relaxation of substance P-induced guinea-pig ileum contraction, whereas compound **16** behaved as a partial agonist.

## References


Adachi, M., Sasatani, T., Chomei, N., Fukui, Y., Yasui, M. (1999) Preparation of pyridine derivatives as 5-$HT_7$ receptor binding agents. PCT Int. Appl. WO 9931062. *Chem. Abstr.* **131**: 58848

Arndts, D., Loesel, W., Roos, O. (1994) Preparation of annelated dihydropyridines as drugs. Ger. Offen. DE 4220312. *Chem. Abstr.* **120**: 217299

Bard, J. A., Zgombick, J., Adham, N., Vaysse, P., Branchek, T. A., Weinshank, R. L. (1993) Cloning of a novel human serotonin receptor (5-$HT_7$) positively linked to adenylate cyclase. *J. Biol. Chem.* **268**: 23422–23426

Belenky, M. A., Pickard, G. E. (2001) Subcellular distribution of 5-$HT_{1B}$ and 5-$HT_7$ receptors in the mouse suprachiasmatic nucleus. *J. Comp. Neurol.* **432**: 371–388

Bhalla, P., Saxena, P. R., Sharma, H. S. (2002) Molecular cloning and tissue distribution of mRNA encoding porcine 5-$HT_7$ receptor and its comparison with the structure of other species. *Mol. Cell. Biochem.* **238**: 81–88

Boltze, K. H., Dell, H. D. (1967) 2-Substituted resorcinol dimethyl ether. *Justus Liebigs Annalen der Chemie* **709**: 63–69

Borsini, F., Giraldo, E., Monferini, E., Antonini, G., Parenti, M., Bietti, G., Donetti, A. (1995) BIMT 17, a 5-$HT_{2A}$ receptor antagonist and 5-$HT_{1A}$ receptor full agonist in rat cerebral cortex. *Naunyn Schmiedebergs Arch. Pharmacol.* **352**: 276–282

Brown, H. C., Choi, Y. M., Narasimhan, S. (1982) Selective reductions. 29. A simple technique to achieve an enhanced rate of reduction of representative organic compounds by borane-dimethyl sulfide. *J. Org. Chem.* **47**: 3153–3163

Carter, D., Champney, M., Hwang, B., Eglen, R. M. (1995) Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum. *Eur. J. Pharmacol.* **280**: 243–250

Centurion, D., Sanchez-Lopez, A., Ortiz, M. I., De Vries, P., Saxena, P. R., Villalon, C. M. (2000) Mediation of 5-HT-induced internal carotid vasodilatation in GR127935- and ritanserin-pretreated dogs by 5-$HT_7$ receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **362**: 169–176

Cheng, Y. C., Prusoff, W. H. (1973) Relationship between the inhibition constant (*Ki*) and the concentration of inhibitor which causes 50 per cent inhibition ($IC_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **22**: 3099–3108

Cornforth, J. W., Robinson, R. (1949) Experiments on the synthesis of substances related to the sterols. Part XLVIII. Synthesis of a tricyclic degradation product of cholesterol. *J. Chem. Soc.* 1855–1865

Cushing, D. J., Zgombick, J. M., Nelson, D. L., Cohen, M. L. (1996) LY215840, a high-affinity 5-$HT_7$ receptor ligand, blocks serotonin-induced relaxation in canine coronary artery. *J. Pharmacol. Exp. Ther.* **277**: 1560–1566

Forbes, I. T., Dabbs, S., Duckworth, D. M., Jennings, A. J., King, F. D., Lovell, P. J., Brown, A. M., Collin, L., Hagan, J. J., Middlemiss, D. N., Riley, G. J., Thomas, D. R., Upton, N. (1998) (R)-3,N-dimethyl-N-[1-methyl-3-(4-methyl-piperidin-1-yl) propyl]benzenesulfonamide: the first selective 5-$HT_7$ receptor antagonist. *J. Med. Chem.* **41**: 655–657

Forbes, I. T., Douglas, S., Gribble, A. D., Ife, R. J., Lightfoot, A. P., Garner, A. E., Riley, G. J., Jeffrey, P., Stevens, A. J., Stean, T. O., Thomas, D. R. (2002) SB-656104-A: a novel 5-$HT_7$ receptor antagonist with improved in vivo properties. *Bioorg. Med. Chem. Lett.* **12**: 3341–3344

Hagan, J. J., Price, G. W., Jeffrey, P., Deeks, N. J., Stean, T., Piper, D., Smith, M. I., Upton, N., Medhurst, A. D., Middlemiss, D. N., Riley, G. J., Lovell, P. J., Bromidge, S. M., Thomas, D. R. (2000) Characterization of SB-269970-A, a selective 5-$HT_7$ receptor antagonist. *Br. J. Pharmacol.* **130**: 539–548

Hoyer, D., Hannon, J. P., Martin, G. R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **71**: 533–554

Jasper, J. R., Kosaka, A., To, Z. P., Chang, D. J., Eglen, R. M. (1997) Cloning, expression and pharmacology of a truncated splice variant of the human 5-$HT_7$ receptor (h5-$HT_{7b}$). *Br. J. Pharmacol.* **122**: 126–132

Kato, Y., Takemoto, M., Achiwa, K. (1993) Prostanoids and related compounds. IV. Synthesis of isoindolinone derivatives possessing inhibitory activity for thromboxane A2 analog (U-46619)-induced vasoconstriction. *Chem. Pharm. Bull.* **41**: 2003–2006

Kawahara, F., Saito, H., Katsuki, H. (1994) Inhibition by 5-$HT_7$ receptor stimulation of GABAA receptor-activated current in cultured rat suprachiasmatic neurons. *J. Physiol. (London)* **478**: 67–73

Kikuchi, C., Nagaso, H., Hiranuma, T., Koyama, M. (1999) Tetrahydrobenzindoles: selective antagonists of the 5-$HT_7$ receptor. *J. Med. Chem.* **42**: 533–535

Leung, E., Walsh, L. K. M., Pulido-Rios, M. T., Eglen, R. M. (1996) Characterization of putative 5-$HT_7$ receptors mediating direct relaxation in cynomolgus monkey isolated jugular vein. *Br. J. Pharmacol.* **117**: 926–930

López-Rodríguez, M. L., Porras, E., Benhamú, B., Ramos, J. A., Morcillo, M. J., Lavandera, J. L. (2000) First pharmacophoric hypothesis for 5-$HT_7$ antagonism. *Bioorg. Med. Chem. Lett.* **10**: 1097–1100

López-Rodríguez, M. L., Ayala, D., Benhamu, B., Morcillo, M. J., Viso, A. (2002) Arylpiperazine derivatives acting at 5-$HT_{1A}$ receptors. *Curr. Med. Chem.* **9**: 443–469

Lovell, P. J., Bromidge, S. M., Dabbs, S., Duckworth, D. M., Forbes, I. T., Jennings, A. J., King, F. D., Middlemiss, D. N., Rahman, S. K., Saunders, D. V., Collin, L. L., Hagan, J. J., Riley, G. J., Thomas, D. R. (2000) A novel, potent, and selective 5-$HT_7$ antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl) phenol (SB-269970). *J. Med. Chem.* **43**: 342–345

Lovenberg, T. W., Baron, B. M., de Lecea, L., Miller, J. D., Prosser, R. A., Rea, M. A., Foye, P. E., Racke, M., Slone, A. L., Siegel, B. W., Danielson, P. E., Sutcliffe, J. G., Erlander, M. G. (1993) A novel adenylyl cyclase-activating serotonin receptor ($5\text{-HT}_7$) implicated in the regulation of mammalian circadian rhythms. *Neuron* **11**: 449–458

Martinez-Esparza, J., Oficialdegui, A. M., Perez-Silanes, S., Heras, B., Orus, L., Palop, J. A., Lasheras, B., Roca, J., Mourelle, M., Bosch, A., Del Castillo, J. C., Tordera, R., Del Rio, J., Monge, A. (2001) New 1-aryl-3-(4-arylpiperazin-1-yl)propane derivatives, with dual action at $5\text{-HT}_{1A}$ serotonin receptors and serotonin transporter, as a new class of antidepressants. *J. Med. Chem.* **44**: 418–428

McDermed, J. D., McKenzie, G. M., Phillips, A. P. (1975) Synthesis and pharmacology of some 2-aminotetralins. Dopamine receptor agonists. *J. Med. Chem.* **18**: 362–366

Meyerhof, W., Obermuller, F., Fehr, S., Richter, D. (1993) A novel rat serotonin receptor: primary structure, pharmacology, and expression pattern in distinct brain regions. *DNA Cell Biol.* **12**: 401–409

Mullins, U. L., Gianutsos, G., Eison, A. S. (1999) Effects of antidepressants on $5\text{-HT}_7$ receptor regulation in the rat hypothalamus. *Neuropsychopharmacology* **21**: 352–367

Neumaier, J. F., Sexton, T. J., Yracheta, J., Diaz, A. M., Brownfield, M. (2001) Localization of $5\text{-HT}_7$ receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. *J. Chem. Neuroanat.* **21**: 63–73

Oficialdegui, A. A., Martinez, J., Perez, S., Heras, B., Irurzun, M., Palop, J. A., Tordera, R., Lasheras, B., del Rio, J., Monge, A. (2000) Design, synthesis and biological evaluation of new 3-[(4-aryl)piperazin-1-yl]-1-arylpropane derivatives as potential antidepressants with a dual mode of action: serotonin reuptake inhibition and $5\text{-HT}_{1A}$ receptor antagonism. *Farmaco* **55**: 345–353

Perrone, R., Berardi, F., Colabufo, N. A., Lacivita, E., Leopoldo, M., Tortorella, V. (2003) Synthesis and structure-affinity relationships of 1-[$\omega$-(4-aryl-1-piperazinyl)alkyl]-1-arylketones as $5\text{-HT}_7$ receptor ligands. *J. Med. Chem.* **46**: 646–649

Plassat, J.-L., Amlaiky, N., Hen, R. (1993) Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.* **44**: 229–236

Quintero, J. E., McMahon, D. G. (1999) Serotonin modulates glutamate responses in isolated suprachiasmatic nucleus neurons. *J. Neurophysiol.* **83**: 533–539

Ruat, M., Traiffort, E., Leurs, R., Tardivel-Lacombe, J., Diaz, J., Arrang, J. M., Schwartz, J. C. (1993) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor ($5\text{-HT}_7$) activating cAMP formation. *Proc. Natl. Acad. Sci. USA* **90**: 8547–5851

Shen, Y., Monsma, F. J., Metcalf, M. A., Jose, P. A., Hamblin, M. W., Sibley, D. R. (1993) Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem.* **268**: 18200–18204

Shimizu, M., Nishida, A., Zensho, H., Yamawaki, S. (1996) Chronic antidepressant exposure enhances 5-hydroxytryptamine$_7$ receptor-mediated cyclic adenosine monophosphate accumulation in rat frontocortical astrocytes. *J. Pharmacol. Exp. Ther.* **279**: 1551–1558

Sleight, A. J., Carolo, C., Petit, N., Zwingelstein, C., Bourson, A. (1995) Identification of 5-hydroxytryptamine$_7$ receptor binding sites in rat hypothalamus: sensitivity to chronic antidepressant treatment. *Mol. Pharmacol.* **47**: 99–103

Smith, B. N., Sollars, P. J., Dudek, F. E., Pickard, G. E. (2001) Serotonergic modulation of retinal input to the mouse suprachiasmatic nucleus mediated by $5\text{-HT}_{1B}$ and $5\text{-HT}_7$ receptors. *J. Biol. Rhythms* **16**: 25–39

Terron, J. A. (1996) The relaxant 5-HT receptor in the dog coronary artery smooth muscle: pharmacological resemblance to the cloned $5\text{-HT}_7$ receptor subtype. *Br. J. Pharm.* **118**: 1421–1429

Terron, J. A. (2002) Is the $5\text{-HT}_7$ receptor involved in the pathogenesis and prophylactic treatment of migraine? *Eur. J. Pharmacol.* **439**: 1–11

Tsou, A., Kosaka, A., Bach, C., Zuppan, P., Yee, C., Tom, L., Alvarez, R., Ramsey, S., Bonhaus, D. W., Stefanich, E., Jakeman, L., Eglen, R. M., Chan, H. W. (1994) Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase. *J. Neurochem.* **63**: 456–464

Villalon, C. M., Centurion, D., Lujan-Estrada, M., Terron, J. A., Sanchez-Lopez, A. (1997) Mediation of 5-HT-induced external carotid vasodilation in GR 127935-pretreated vagosympathectomized dogs by the putative $5\text{-HT}_7$ receptor. *Br. J. Pharmacol.* **120**: 1319–1327

Yevich, J. P., New, J. S., Smith, D. W., Lobeck, W. G., Catt, J. D., Minielli, J. L., Eison, M. S., Taylor, D. P., Riblet, L. A., Temple, D. L. (1986) Synthesis and biological evaluation of 1-(1,2-benzisothiazol-3-yl)- and (1,2-benzisoxazol-3-yl)piperazine derivatives as potential antipsychotic agents. *J. Med. Chem.* **29**: 359–369