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Design and synthesis of long-chain arylpiperazines with mixed affinity for serotonin transporter (SERT) and 5-HT$_{1A}$ receptor

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Abstract

A new generation of antidepressant agents could be represented by compounds with mixed activity as serotonin transporter (SERT) inhibitors and 5-HT$_{1A}$ receptor antagonists. We report here on the synthesis and evaluation of SERT and 5-HT$_{1A}$ receptor affinity of long-chain arylpiperazines obtained either by modifying 6-nitroquipazine into a long-chain arylpiperazine or by inserting a modified 6-nitroquipazine moiety or other structures endowed with SERT affinity into a long-chain arylpiperazine with 5-HT$_{1A}$ affinity. Among the compounds studied, 2-[4-(2-methoxyphenyl)piperazin-1-yl]-*N*-(6-nitro-2-quinolyl)ethylamine (21) and 1-(5-bromo-1,2,3,4-tetrahydronaphthalen-1-yl)-3-[4-(2-methoxyphenyl)-piperazin-1-yl]-1-propanone (24) showed good affinity values for SERT and 5-HT$_{1A}$ receptors (SERT: K_i (inhibition constant) = 71.8 and 62.8 nM; 5-HT$_{1A}$ K_i = 14.2 and 0.82 nM, respectively).

Introduction

Major depression is a mental illness that has been estimated to be the second leading cause of premature death or disability in the US population, surpassed only by heart disease (Musselman et al 1998). About 50 years ago, the introduction of tricyclic antidepressants and monoamine oxidase inhibitors dramatically changed the treatment of depression. Unfortunately, the broad mechanism of action of tricyclic antidepressants causes many unwanted effects, poor tolerability and poor risk profile (Feighner 1999). The introduction of selective serotonin reuptake inhibitors (SSRIs) over 20 years ago was the next major step in the evolution of second generation drugs with better tolerability and safety profile (Pinder & Wieringa 1993). However, the main drawback of SSRIs is their delayed onset of action (2–4 weeks). It has been proposed that 5-HT$_{1A}$ (5-hydroxytryptamine$_{1A}$, serotonin$_{1A}$) autoreceptors are involved in this delay. Inhibition of the serotonin transporter (SERT) raises the synaptic concentration of 5-HT, which activates presynaptic 5-HT$_{1A}$ autoreceptors, causing a reduction of neuronal firing. Approximately 2–4 weeks after initiation of treatment, the presynaptic 5-HT$_{1A}$ autoreceptors become desensitized, the release of serotonin from nerve terminals is increased and serotonergic neurotransmission potentiated (Spinks & Spinks 2002). It has been suggested that a faster onset of antidepressant activity could be achieved by inhibition of SERT combined with blockade of presynaptic 5-HT$_{1A}$ receptors. In fact, co-administration of the SSRI paroxetine with the 5-HT$_{1A}$ receptor antagonist WAY-100635 increased extracellular serotonin levels in terminal regions of the serotonergic system (Artigas et al 1996). Based on these observations, much research effort has been made to optimize a single ligand with mixed SSRI/5-HT$_{1A}$ activity. The overlapping type approach has been extensively used for this purpose (Perez et al 1998). By use of a common basic nitrogen, the 5-HT$_{1A}$ and SERT pharmacophore components were merged. Several chemical entities have been used as SERT moieties, including the cyclohexyl indole (Meagher et al 2001) fluoxetine (Figure 1) and related γ-phenoxypropylamine (Martinez-Esparza et al 2001). However, the use of the well-known SSRI 6-nitroquipazine (Vaatstra et al 1981) (Figure 1) has not been reported yet. Because we have extensively

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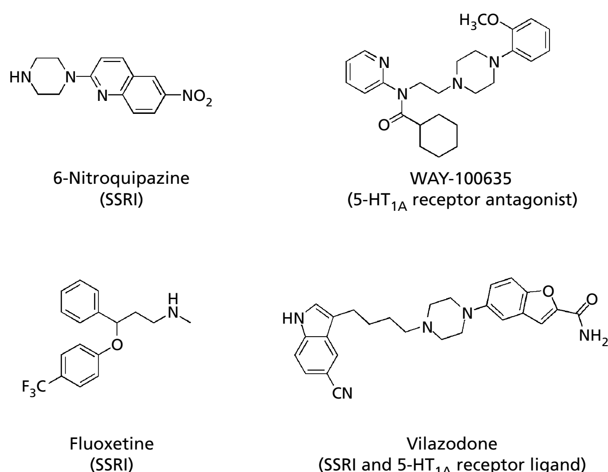

Figure 1 Molecular structure of 6-nitroquipazine, WAY-100635, fluoxetine and vilazodone.

studied a class of long-chain arylpiperazines with high 5-HT_{1A} receptor affinity, exemplified by derivative **1** (Table 1) (Perrone et al 2000), we modified such a framework to obtain mixed SERT/5-HT_{1A} receptor ligands. In particular, the aim of this study was to find an appropriate way to incorporate the 6-nitroquipazine moiety into a long-chain arylpiperazine. During the course of this study, other research groups have reported several long-chain arylpiperazines with mixed SSRI/5-HT_{1A} affinity. Among these, vilazodone represents the most relevant outcome (Heinrich et al 2004). Initially, we modified 6-nitroquipazine into a long-chain arylpiperazine (**A** model, Figure 2A). This approach resulted in compounds with modest affinity for the 5-HT_{1A} receptor and SERT. Subsequently, we linked the arylpiperazine moiety responsible for 5-HT_{1A} receptor affinity to structures endowed with SERT affinity through an alkyl chain (**B** model, Figure 2B).

Materials and Methods

Synthesis

Column chromatography was performed with 1:30 ICN silica gel 60A (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on a Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded on a Varian Mercury-VX spectrometer, with $CDCl_3$ as solvent. Chemical shifts were denoted in δ units (ppm) relative to the solvent (^{1}H NMR peaks: 7.26 for $CDCl_3$). Recording of mass spectra was done on an HP6890–5973 MSD gas chromatograph/mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, were

Table 1 Physical properties and binding affinities of compounds **1**, **10–14**

Compound	n	Ar	Formula[a]	mp (°C)	K_i (nM)[b]	
					SERT	5-HT_{1A}
1	3	2-pyridyl	—	—	>1000 (30%)[c]	0.48 ± 0.07[d]
10	3	6-NO_2-2-quinolyl	$C_{27}H_{32}N_4O_3$·2HCl	242–245	11 ± 0.7	2500 ± 120
11	3	2-quinolyl	—	—	100 ± 4.50	80 ± 6.0[d]
12	3	2-naphthyl	$C_{28}H_{34}N_2O$·2HCl	214–216	169 ± 15.0	335 ± 20
13	2	2-quinolyl	$C_{26}H_{31}N_3O$·3HCl	264–266	35.3 ± 7.00	1350 ± 130
14	4	2-quinolyl	$C_{28}H_{35}N_3O$·2HCl·0.3H_2O	248–250	88.9 ± 6.20	>1000 (44%)
6-Nitroquipazine					0.41 ± 0.12	>1000 (40%)
Fluoxetine					4.36 ± 0.25	>1000 (6%)

[a]All compounds were recrystallized from CH_3OH/Et_2O. Analysis for C,H,N; results were within ±0.4% of the theoretical values for the formulas given. [b]The values are the means ± s.e.m. from three independent experiments in triplicate ($P < 0.0001$). Individual difference between the various compounds have been examined using Tukey's post-hoc test ($P < 0.0001$). Difference in the K_i values between the receptors for each compound have been analysed using the Mann–Whitney *U*-test ($P = 0.005$, $U = 13.50$). [c]Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses. [d]See Perrone et al (2000).

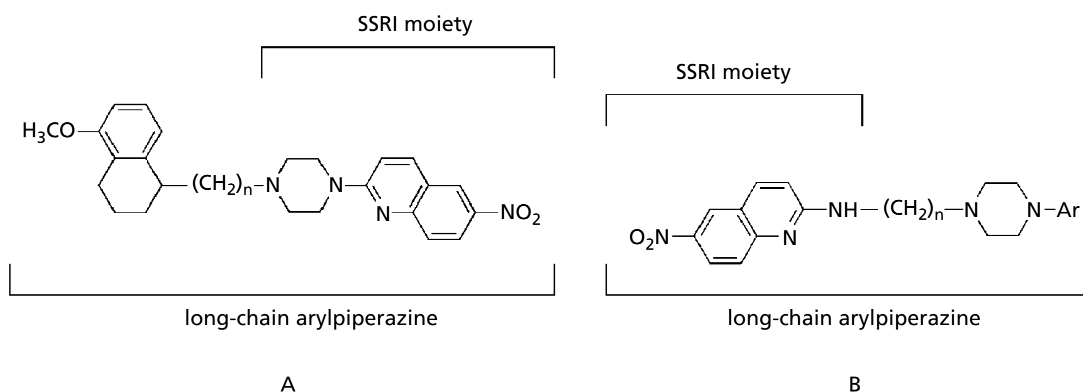

Figure 2 Proposed models to obtain long-chain arylpiperazines with mixed SSRI/5-HT$_{1A}$ affinity.

reported. Compounds **10**, **14**, **24**, and **25** were characterized by ESI$^+$/MS/MS with an Agilent 1100 Series LC-MSD trap System VL workstation. All spectra were in accordance with the assigned structures. All target compounds were transformed into their hydrochloride salts in the usual manner. The following compounds were synthesized by published procedures: 1-(2-bromoethyl)-5-methoxy-1,2,3,4-tetrahydronaphthalene (**2a**) (Perrone et al 1996), 1-(3-bromopropyl)-5-methoxy-1,2,3,4-tetrahydronaphthalene (**2b**) (Perrone et al 1995b), 1-(4-chlorobutyl)-5-methoxy-1,2,3,4-tetrahydronaphthalene (**2c**) (Perrone et al 1995b), 1-(3-chloro-1-phenylpropoxy)-4-(trifluoromethyl)benzene (**3**) (Wirth et al 2000), 6-bromo-2-chloroquinoline (**4a**) (Lee et al 2000), 2-chloro-6-nitroquinoline (**4b**) (Lee et al 2000), 2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine (**5a**) (Perrone et al 1995a), 2-[4-(2-pyridyl)piperazin-1-yl]ethylamine (**5b**) (Perrone et al 1994), 2-(4-phenylpiperazin-1-yl)ethylamine (**5c**) (Perrone et al 1994), 5-bromo-3,4-dihydro-1(2*H*)-naphthalenone (**6a**) (Chini et al 1988), 7-bromo-3,4-dihydro-1(2*H*)-naphthalenone (**6b**) (Chini et al 1988), 1-(2-naphthyl)piperazine (Glennon et al 1986), 1-(2-quinolyl)piperazine (Fourneau & Barrelet 1929) and 1-(6-nitro-2-quinolyl)piperazine (Lee et al 2000).

*(E)-5-Bromo-1-(3-chloropropylidene)-1,2,3,4-tetrahydronaphthalene (7**a**)*

To a stirred solution of Grignard reagent, prepared from Mg turnings (0.35 g, 14.4 mmol) and cyclopropyl bromide (1.1 mL, 13.7 mmol) in anhydrous tetrahydrofuran (THF, 30 mL) under dry N_2, 5-bromo-3,4-dihydro-1(2*H*)-naphthalenone (**6a**) (1.51 g, 6.7 mmol) was added dropwise in the same solvent (15 mL). The mixture was refluxed overnight and cooled on an ice-water bath. A cooled saturated solution of NH_4Cl (40 mL) was then added and the separated organic phase was evaporated and the residue was immediately stirred with 3 M HCl–acetic acid (2:1, v/v, 15 mL) for 1 h. The mixture was then diluted with H_2O (30 mL) and extracted with Et_2O (70 mL). The separated organic layer was washed several times with a 20% aqueous Na_2CO_3 solution until the aqueous phase remained basic. Evaporation of the dried (Na_2SO_4) organic layer gave a crude residue that was purified by column chromatography with petroleum ether–$CHCl_3$ (9:1 v/v), as eluent (0.40 g, 21% yield). ^{1}H NMR: δ 1.83–1.96 (m, 2H), 2.46 (br t, 2H), 2.65–2.72 (m, 2H), 2.83 (t, 2H, $J = 6.3$ Hz), 3.58–3.63 (m, 2H), 5.97 (br t, 1H), 7.01 (t, 1H, $J = 7.8$ Hz), 7.43 (dd, 1H, $J = 7.8$, 1.0 Hz), 7.50 (d, 1H, $J = 8.0$ Hz). GC-MS *m*/*z* 286 (M^++2, 40) 284 (M^+, 31), 237 (25), 235 (26), 156 (100).

*(E)-7-Bromo-1-(3-chloropropylidene)-1,2,3,4-tetrahydronaphthalene (7**b**)*

Following the procedure reported for **7a**, this compound was prepared from 7-bromo-3,4-dihydro-1(2*H*)-naphthalenone (**6b**) in 37% yield. ^{1}H NMR: δ 1.77–1.86 (m, 2H), 2.46–2.51 (m, 2H), 2.66–2.73 (m, 4H), 3.61 (t, 2H, $J = 7.0$ Hz), 5.94–5.99 (m, 1H), 6.95 (d, 1H, $J = 8.2$ Hz), 7.24 (dd, 1H, J = 8.4, 2.1 Hz), 7.68 (d, 1H, $J = 1.9$ Hz). GC-MS *m*/*z* 286 (M^++2, 32) 284 (M^+, 25), 237 (22), 235 (22), 156 (100).

*1-(5-Bromo-1,2,3,4-tetrahydronaphthalen-1-yl)-3-chloro-1-propanone (9**a**)*

A dry 100 mL flask equipped with a magnetic stirrer, a pressure-equalizing dropping funnel, and a reflux condenser was flushed with dry N_2. The flask was charged with the alkene **7a** (1.03 g, 3.6 mmol) in anhydrous THF (20 mL) and cooled to 0–5°C with an ice-water bath. Hydroboration was achieved by the drop-wise addition of borane methyl sulfide complex (0.2 mL, 2.0 mmol). Following the addition of the hydride, the cooling bath was removed and the solution was stirred for 6 h at room temperature. Ethanol (1.4 mL) was then added, followed by 3 M NaOH (2.4 mL). After cooling to 0–5°C in an ice-water bath, 35% H_2O_2 (0.7 mL) was added drop-wise. Immediately following the addition of the peroxide, the reaction mixture was refluxed for 1 h. The reaction mixture was then poured into ice-water and extracted with Et_2O (20 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated in-vacuo. The crude residue was chromatographed (petroleum ether–AcOEt, 9:1, as eluent) to give 0.45 g of alcohol **8a** (60% yield). GC-MS analysis revealed that a mixture of diastereoisomers was obtained (GC/MS *m*/*z* 302 (M^+, 1)). This mixture was used for the next step without any attempt at separation.

A 100-mL flask was charged with a solution of **8a** (0.36 g, 1.2 mmol), TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical; 3.75 mg, 0.024 mmol) and of

Bu_4NBr (15.5 mg, 0.048 mmol) in 25 mL of CH_2Cl_2 and cooled to 0°C. A solution of *m*-CPBA (*m*-chloroperbenzoic acid; 0.50 g, 2.9 mmol) in 20 mL of CH_2Cl_2 was added drop-wise. The reaction mixture turned bright orange upon addition of *m*-CPBA. The reaction was stirred at 0°C for 10 min and at room temperature for 3 h, by which time the orange colour had faded away. The reaction was quenched by the addition of 1 M NaOH (5 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was chromatographed (petroleum ether–AcOEt (9:1 v/v)) to afford pure ketone **9a** (0.19 g, 52% yield). GC-MS *m/z* 302 (M^++2, 1) 300 (M^+, 1), 211 (67), 209 (69), 130 (100).

*1-(7-Bromo-1,2,3,4-tetrahydronaphthalen-1-yl)-3-chloro-1-propanone (**9b**)*

Following the procedure reported for **9a,** this derivative was obtained from alkene **7b** in 30% overall yield. GC-MS *m/z* 302 (M^++2, 6) 300 (M^+, 5), 211 (91), 209 (91), 130 (100).

*General procedure for the synthesis of derivatives **10, 12–14, 17***

A stirred mixture of the appropriate alkylating agent (1.0 mmol), 1-arylpiperazine (1.2 mmol) and potassium carbonate (1.0 mmol) in acetonitrile (40 mL) was refluxed overnight. After cooling, the mixture was evaporated to dryness and water 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 purified by chromatography, yielding the expected compound as pale yellow oil.

*1-(6-Nitro-2-quinolyl)-4-[3-(1,2,3,4-tetrahydro-5-methoxy-1-naphthyl)propyl]piperazine (**10**).* Compound **10** was eluted with $CHCl_3$–AcOEt (1:1 v/v) in 15% yield. ^{1}H NMR: δ 1.63–1.82 (m, 8H), 2.43–2.46 (m, 2H), 2.59–2.72 (m, 6H), 2.80 (br s, 1H), 3.81 (s, 3H), 3.82–3.88 (m, 4H), 6.68 (t, 1H, J = 8.2 Hz), 6.82 (t, 1H, J = 7.7 Hz), 7.04–7.15 (m, 2H), 7.63–7.70 (m, 1H), 7.93–7.98 (m, 1H), 8.27–8.32 (m, 1H), 8.52–8.56 (m, 1H). ESI$^+$/MS *m/z* 461 (MH^+). ESI$^+$/MS/MS *m/z* 216 (100), 170 (23).

*1-(2-Naphthyl)-4-[3-(1,2,3,4-tetrahydro-5-methoxy-1-naphthyl)propyl]piperazine (**12**).* Compound **12** was eluted with $CHCl_3$–AcOEt (4:1 v/v) in 67% yield. ^{1}H NMR: δ 1.60–1.86 (m, 8H), 2.49 (br s, 2H), 2.55–2.72 (m, 6H), 2.79 (br s, 1H), 3.35 (br t, 4H), 3.81 (s, 3H), 6.66 (d, 1H, J = 8.0 Hz), 6.81 (d, 1H, J = 7.7 Hz), 7.08–7.13 (m, 2H), 7.25–7.32 (m, 2H), 7.37–7.43 (m, 1H), 7.68–7.74 (m, 3H). GC-MS *m/z* 415 (M^++1, 23) 414 (M^+, 75), 399 (23), 225 (100).

*1-(2-Quinolyl)-4-[2-(1,2,3,4-tetrahydro-5-methoxy-1-naphthyl)ethyl]piperazine (**13**).* Compound **13** was eluted with $CHCl_3$–AcOEt (1:1 v.v) in 71% yield. ^{1}H NMR: δ 1.76–1.85 (m, 6H), 2.48–2.74 (m, 8H), 2.87–2.90 (m, 1H), 3.77 (app t, 4H), 3.81 (s, 3H), 6.67 (d, 1H, J = 8.1 Hz), 6.83 (d, 1H, J = 7.6 Hz), 6.98 (d, 1H, J = 9.2 Hz), 7.12 (t, 1H, J = 7.9 Hz), 7.20–7.26 (m, 1H), 7.51–7.61 (m, 2H), 7.71 (d, 1H, J = 7.8 Hz), 7.89 (d, 1H, J = 8.9 Hz). GC-MS *m/z* 402 (M^++1, 1) 401 (M^+, 3), 257 (45), 157 (100).

*1-(2-Quinolyl)-4-[4-(1,2,3,4-tetrahydro-5-methoxy-1-naphthyl)butyl]piperazine (**14**).* Compound **14** was eluted with $CHCl_3$–AcOEt (1:1 v/v) in 11% yield. ^{1}H NMR: δ 1.29–1.75 (m, 8H), 2.33 (t, 2H, J = 7.6 Hz), 2.45–2.68 (m, 7H), 3.68 (br t, 4H), 3.71 (s, 3H), 6.56 (d, 1H, J = 7.8 Hz), 6.71 (d, 1H, J = 7.8 Hz), 6.89 (d, 1H, J = 9.2 Hz), 7.02 (t, 1H, J = 8.0 Hz), 7.10–7.17 (m, 1H), 7.41–7.51 (m, 2H), 7.61 (d, 1H, J = 8.4 Hz), 7.79 (d, 1H, J = 9.2 Hz). ESI$^+$/MS *m/z* 430 (MH^+). ESI$^+$/MS/MS *m/z* 171 (100).

*1-Phenyl-4-[3-phenyl-3-[4-(trifluoromethyl)phenoxy]-propyl]piperazine (**17**).* Compound **17** was eluted with $CHCl_3$–AcOEt (9:1 v/v) in 10% yield. ^{1}H NMR: δ 2.11–2.17, 2.25–2.37 (m, 2H), 2.65 (br t, 2H), 2.71 (br s, 4H), 3.27 (br t, 4H), 5.31–5.36 (m, 1H), 6.85–6.94 (m, 5H), 7.20–7.44 (m, 9H). GC-MS *m/z* 441 (M^++1, 17) 440 (M^+, 54), 189 (20), 175 (100).

*General procedure for the preparation of derivatives **18–23***

A mixture of the chloro derivative **4a** or **4b** (3.6 mmol) and an amine **5a** or **5b** (3.6 mmol) was heated in a closed glass tube at 150°C for 5 h. After cooling, it was diluted with CH_2Cl_2 and washed with a 20% aqueous solution of Na_2CO_3. The separated organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by chromatography, yielding the expected compound as pale yellow oil.

*N-(6-Bromo-2-quinolyl)-2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine (**18**).* Compound **18** was eluted with $CHCl_3$–AcOEt (1:1 v/v) in 13% yield. ^{1}H NMR: δ 2.78 (app t, 6H), 3.15 (br s, 4H), 3.65 (q, 2H, J = 7.4 Hz), 3.87 (s, 3H), 5.59 (br s, 1H, D_2O exchanged), 6.69 (d, 1H, J = 9.1 Hz), 6.85–7.05 (m, 4H), 7.55–7.56 (m, 2H), 7.68–7.71 (m, 2H). GC-MS *m/z* 442 (M^++2, 3) 440 (M^+, 4), 218 (87), 205 (100), 190 (40).

*N-(6-Bromo-2-quinolyl)-2-[4-(2-pyridyl)piperazin-1-yl]ethylamine (**19**).* Compound **19** was eluted with $CHCl_3$–CH_3OH (19:1 v/v) in 16% yield. ^{1}H NMR: δ 2.67 (app t, 4H), 2.74 (t, 2H, J = 5.8 Hz), 3.60 (app t, 4H), 3.65 (q, 2H, J = 7.4 Hz), 5.55 (br s, 1H, D_2O exchanged), 6.61–6.71 (m, 3H), 7.45–7.60 (m, 3H), 7.69–7.71 (m, 2H), 8.18–8.21 (m, 1H). GC-MS *m/z* 413 (M^++2, 0.5) 411 (M^+, 1), 189 (100), 176 (77), 147 (37).

*N-(6-Bromo-2-quinolyl)-2-(4-phenylpiperazin-1-yl) ethylamine (**20**).* Compound **20** was eluted with $CHCl_3$–AcOEt (1:1 v/v) in 38% yield. ^{1}H NMR: δ 2.57 (t, 2H, J = 5.9 Hz), 2.64 (app t, 4H), 3.20 (app t, 4H), 3.41–

3.48 (m, 2H), 6.24 (br s, 1H, D_2O exchanged), 6.85–6.95 (m, 5H), 7.24–7.31 (m, 4H), 8.20 (br s, 1H). GC-MS *m/z* 412 (M^++2, 4) 410 (M^+, 5), 188 (100), 175 (91), 132 (53).

*2-[4-(2-Methoxyphenyl)piperazin-1-yl]-N-(6-nitro-2-quinolyl)ethylamine (**21**)*. Compound **21** was eluted with $CHCl_3$–CH_3OH (19:1 v/v) in 30% yield. ^{1}H NMR: δ 2.67–2.77 (m, 6H), 3.12 (br s, 4H), 3.62–3.69 (m, 2H), 3.86 (s, 3H), 5.83 (br s, 1H, D_2O exchanged), 6.77 (d, 1H, $J = 9.0$ Hz), 6.86–7.05 (m, 4H), 7.67 (d, 1H, $J = 9.3$ Hz), 7.87 (d, 1H, $J = 9.1$ Hz), 8.30 (dd, 1H, $J = 9.2$, 2.6 Hz), 8.51 (d, 1H, $J = 2.5$ Hz). GC-MS *m/z* 408 (M^++1, 1), 407 (M^+, 4), 218 (44), 205 (100), 190 (36).

*N-(6-Nitro-2-quinolyl)-2-[4-(2-pyridyl) piperazin-1-yl]ethylamine (**22**)*. Compound **22** was eluted with $CHCl_3$–CH_3OH (19:1 v/v) in 42% yield. ^{1}H NMR: δ 2.68 (app t, 4H), 2.75 (t, 2H, $J = 5.9$ Hz), 3.60 (app t, 4H), 3.69–3.78 (m, 2H), 5.84 (br s, 1H, D_2O exchanged), 6.62–6.68 (m, 2H), 6.68 (d, 1H, $J = 9.1$ Hz), 7.46–7.52 (m, 1H), 7.67 (d, 1H, $J = 9.2$ Hz), 7.88 (d, 1H, $J = 8.9$ Hz), 8.19–8.32 (m, 1H), 8.30 (dd, 1H, $J = 9.2$, 2.6 Hz), 8.51 (d, 1H, $J = 2.6$ Hz). GC-MS *m/z* 378 (M^+, 6), 189 (68), 176 (100), 147 (42).

*N-(6-Nitro-2-quinolyl)-2-(4-phenylpiperazin-1-yl)ethylamine (**23**)*. Compound **23** was eluted with $CHCl_3$–CH_3OH (19:1 v/v) in 14% yield. ^{1}H NMR: δ 2.52–2.82 (m, 6H), 3.28 (br t, 4H), 3.72 (br d, 2H), 5.93 (br s, 1H, D_2O exchanged), 6.79 (d, 1H, $J = 8.8$ Hz), 6.86–6.96 (m, 4H), 7.28–7.30 (m, 1H), 7.67 (d, 1H, $J = 9.1$ Hz), 7.87 (d, 1H, $J = 9.1$ Hz), 8.30 (dd, 1H, $J = 9.4$, 2.5 Hz), 8.52 (d, 1H, $J = 2.5$ Hz). GC-MS *m/z* 377 (M^+, 6), 188 (58), 175 (100), 132 (49).

*1-(5-Bromo-1,2,3,4-tetrahydronaphthalen-1-yl)-3-[4-(2-methoxyphenyl)-piperazin-1-yl]-1-propanone (**24**)*. A stirred solution of **9a** (0.06 g, 0.2 mmol), 1-(2-methoxyphenyl)piperazine (0.07 g, 0.4 mmol) and a few drops of triethylamine in toluene (20 mL) was refluxed for 24 h. After cooling, the mixture was evaporated to dryness and the residue was treated with 20% Na_2CO_3 aqueous solution (20 mL). The mixture was extracted with CH_2Cl_2 (2×30 mL). The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed ($CHCl_3$–AcOEt, 1:1 v/v) to yield pure **24** as a pale yellow oil (0.06 g, 65% yield). ^{1}H NMR: δ 1.65–1.79, 1.81–2.09 (m, 4H), 2.64–2.82 (m, 10H), 3.07 (m, 4H), 3.85–3.92 (s + m, 4H), 6.84–7.02 (m, 6H), 7.46 (dd, 1H, $J = 7.4$, 1.7 Hz). ESI^+/MS *m/z* 457 (MH^+). ESI^+/MS/MS *m/z* 205 (100).

*1-(7-Bromo-1,2,3,4-tetrahydronaphthalen-1-yl)-3-[4-(2-methoxyphenyl)-piperazin-1-yl]-1-propanone (**25**)*. Following the procedure for the preparation of **24**, compound **25** was obtained in the same yield starting from **9b**. ^{1}H NMR: δ 1.73–1.89, 1.92–2.02 (m, 4H), 2.63–2.80 (m, 10H), 3.07 (m, 4H), 3.85–3.94 (s + m, 4H), 6.85 (d, 1H, J = 7.7 Hz), 6.91–6.94 (m, 2H), 6.96–7.03 (m, 2H), 7.10–7.17 (m, 2H). ESI^+/MS *m/z* 457 (MH^+). ESI^+/MS/MS *m/z* 205 (100).

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) and were handled according to internationally accepted principles for care of laboratory animals (EEC Council Directive 86/609, O. J. No. L358, December 18, 1986). Human recombinant serotonin transporter (SERT) expressed in HEK293 cells, [^{3}H]-5-HT, tritiated 8-hydroxy-*N*,*N*-dipropylaminotetralyn ([^{3}H]-8-OH-DPAT), and [^{3}H]-imipramine were purchased from PerkinElmer Life Science (Zaventem, Belgium). 6-Nitroquipazine maleate was obtained from Tocris Cookson Ltd (Bristol, UK). Paroxetine hydrochloride was purchased from Kemprotec Ltd 11 (Middlesbrough, UK). 8-OH-DPAT hydrobromide was from RBI (Milan, Italy). Ficoll and iproniazid were from Sigma-Aldrich (Milan, Italy). For receptor binding studies, all compounds were dissolved in absolute ethanol. For the isolated guinea-pig ileum assay, compounds **21** and **24** were dissolved in Krebs–Henseleit solution, pH 7.4.

Radioligand binding assay at rat hippocampal membranes 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 of 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 of buffer and 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 of 50 mM Tris (pH 7.6) hippocampus membrane 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 μM 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 (dissociation constant) determined for 8-OH-DPAT was 8.8 nM.

Radioligand binding assay at human cloned SERT

Binding experiments were performed according to Blakely et al (1991) with minor modifications. To a total 0.5 mL of incubation buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4) was added 7.4 μg of human serotonin transporter membranes, [^{3}H]imipramine (3.8 nM, $K_d = 2.6$ nM), and unlabelled ligand. Each tube was equilibrated for 30 min

at 25°C and filtered over GF/C filter pre-soaked in 0.5% polyethyleneimine (PEI) and washed twice with 0.5 mL of ice-cold buffer (0.9% NaCl). Nonspecific binding was determined in the presence of 10 μM paroxetine. Specific binding was 85% of total binding.

Isolated guinea-pig ileum assay

Sections of guinea-pig ileum were prepared according to Forster et al (1995) with minor modifications. Briefly, guinea-pigs (250–300 g) were killed by decapitation. The intestine was removed and washed quickly in a Krebs solution (in mM: 118 NaCl, 4.8 KCl, 25 $NaHCO_3$, 0.6 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 1.25 $CaCl_2$). Sections (2 cm long) were placed in 20 mL organ baths containing Krebs solution dressed with 1 μM ketanserin and 1 μM pyrilamine at 37°C and bubbled with 5% CO_2–95% O_2. These compounds were used to block the serotonergic 5-HT_{2A} and histaminergic H_1 receptor-mediated muscle relaxant effect (Bill et al 1990; Colabufo et al 2003). The strips were placed under a 1 g load and contractility was measured using an appropriate transducer (Fort 10, WPI, Sarasota, USA), connected to a PowerLab recorder (ADInstrument, Caste Hill, NSW, Australia). The segments were stimulated at 0.05 Hz using a digital stimulator (Letica 12106 Panlab) at 150 mA, 1 ms duration, with platinum electrodes positioned longitudinally in the organ bath. Following a 90–120-min equilibrium period, during which the Krebs solution was changed several times, 8-OH-DPAT as agonist reference compound and test compounds **21** or **24** were added cumulatively. All compounds were tested at 0.50–50 mM. The effectiveness of a given compound to inhibit electrically induced contraction was measured as the percentage change from baseline. The concentration of a given test compound eliciting half-maximal inhibition of the electrically induced contraction (EC50) was determined by non-linear curve fitting using the mean response of at least three separate trials as the given response for a single concentration. Compound **21** displayed full agonist activity, therefore it was tested in the presence of the antagonist WAY-100635 (0.1–5 μM). The isolated guinea-pig ileum was equilibrated for 75 min with antagonist before constructing concentration–response curves of agonist. Tissue responses were recorded as gram changes in isometric tension and expressed as percentage of reduction in the height of the contraction.

5-HT re-uptake in-vitro assay

The preparation of synaptosomal fraction from rat brain was performed as described by Wong et al (1993) with minor modifications. Rats were killed by decapitation and cerebral cortex was quickly removed. The crude cortex (1 g) homogenized in buffer containing 0.32 M sucrose (10 mL) was centrifuged at 1000 *g* for 10 min at 4°C. Then the supernatant was centrifuged at 15 000 *g* for 12 min at 4°C. The supernatant was discarded and the pellet was suspended in sucrose 0.32 M (5 mL) applying a gradient prepared for SW 25.1 rotor, consisting of 7 mL each of 4% Ficoll, 6% Ficoll and 13% w/v Ficoll in 0.32 M sucrose. The preparation was centrifuged at 64 000 *g* for 45 min at 4°C. The collected synaptosomal fraction at the 6–13% interface was diluted with 4 mL of 0.32 M sucrose and then centrifuged at 50 000 *g* for 20 min at 4°C. The pellet was suspended in buffer and stored at −80°C until use. The protein content was determined by the Lowry method. Synaptosomal uptake of [^{3}H]-5-HT was determined as reported by Wong et al (1993) with minor modifications. The synaptosomal preparation (500 μg of protein) in a total volume of 1 mL was incubated at 37°C for 5 min in Krebs-bicarbonate medium (containing in mM: 10 glucose, 0.1 iproniazid, 1 ascorbic acid and 0.17 EDTA), 50 nM [^{3}H]-5-HT and compounds at five different concentrations. The suspension was diluted with 2 mL of 0.9% saline solution and filtered on GF/B filters. These filters were rinsed twice with 5 mL 0.9% saline solution. Total [^{3}H]-5-HT re-uptake inhibition was obtained in the presence of 10 μM paroxetine.

Statistical methods

The inhibition curves on the different binding sites of the compounds reported in Tables 1 and 2 were analysed by non-linear curve fitting utilizing the GraphPad Prism program. The value for the inhibition constant, K_i, was calculated by using the Cheng–Prusoff equation (Cheng & Prusoff 1973). 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. $P < 0.05$ was considered statistically significant.

Results and Discussion

The target compounds were prepared by several synthetic routes. Compounds **10** and **12**–**14** were prepared by alkylating the appropriate 1-arylpiperazine with alkyl halide derivatives **2a**–**c**. These latter intermediates were prepared according to published literature methods, as detailed in Material and Methods. Compound **17** was prepared by reacting 1-(3-chloro-1-phenylpropoxy)-4-(trifluoromethyl)-benzene (**3**) with 1-phenylpiperazine. Compounds **18**–**23** were prepared as follows: nucleophilic aromatic substitution of 4-aryl-1-piperazinoethanamines **5a**–**c** on 2-chloroquinolines **4a**, **b** gave the expected target compounds. Also in this case the required intermediates were prepared according to literature methods. Target compounds **24** and **25** (Figure 3) were prepared as follows: 5- or 7-bromo substituted 3,4-dihydro-1(2*H*)-naphthalenone (**6a** or **6b**, respectively) (Chini et al 1988) were reacted with cyclopropyl magnesium bromide to afford an intermediate tertiary alcohol that was dehydrated with HCl/CH_3COOH to give alkene **7a** or **7b** (Perrone et al 1995b). Hydroboration-oxidation (Lane 1973) of the latter afforded alcohol **8a** or **8b** that underwent TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) catalysed oxidation using *m*-CPBA (*m*-chloroperbenzoic acid) (Rychnovsky & Vaidyanathan 1999) to give the key ketone **9a** or **9b**. Reactions of **9a** or **9b**

Table 2 Physical properties and binding affinities of compounds **15**–**25**

Compound	R	Ar	Formula[a]	mp (°C)	K_i (nM)[b]	
					SERT	5-HT$_{1A}$
15	I	2-OCH$_3$-Ph	—	—	650[c]	450[c]
16	I	2-Py	—	—	1500[c]	1600[c]
17	I	Ph	$C_{26}H_{27}F_3N_2O{\cdot}2HCl{\cdot}H_2O$	186–189	>1000 (31%)[d]	878–70.0
18	II	2-OCH$_3$-Ph	$C_{22}H_{25}BrN_4O{\cdot}3HCl{\cdot}H_2O$	249–251	2212 ± 120	93.3 ± 7.52
19	II	2-Py	$C_{20}H_{22}BrN_5{\cdot}4HCl$	231–232	>6000 (19%)	62.4 ± 8.10
20	II	Ph	$C_{21}H_{23}BrN_4{\cdot}2HCl{\cdot}0.5H_2O$	266–268	>6000 (48%)	896 ± 35.0
21	III	2-OCH$_3$-Ph	$C_{22}H_{25}N_5O_3{\cdot}3HCl{\cdot}H_2O$	214–217	71.8 ± 8.20	14.2 ± 3.00
22	III	2-Py	$C_{20}H_{22}N_6O_2{\cdot}3HCl{\cdot}H_2O$	260 dec	>6000 (35%)	22.0 ± 4.10
23	III	Ph	$C_{21}H_{23}N_5O_2{\cdot}2HCl{\cdot}0.4H_2O$	250 dec	>6000 (40%)	85.4 ± 7.33
24	IV	2-OCH$_3$-Ph	$C_{24}H_{29}BrN_2O_2{\cdot}2HCl$	165–167	62.8 ± 2.20	0.82 ± 0.04
25	V	2-OCH$_3$-Ph	$C_{24}H_{29}BrN_2O_2{\cdot}HCl$	167–169	>1000 (44%)	9.7 ± 0.5

[a]All compounds were recrystallized from CH_3OH/Et_2O. Analysis for C,H,N; results were within ±0.4% of the theoretical values for the formulas given. [b]The values are the means ± s.e.m. from three independent experiments in triplicate ($P < 0.0001$). Individual difference between the various compounds have been examined using Tukey's post-hoc test ($P < 0.0001$). Difference in the K_i values between the receptors for each compound have been analysed using the Mann-Whitney *U*-test ($P = 0.0058$, $U = 18$). [c]See Martinez-Esparza et al. (2001). [d]Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.

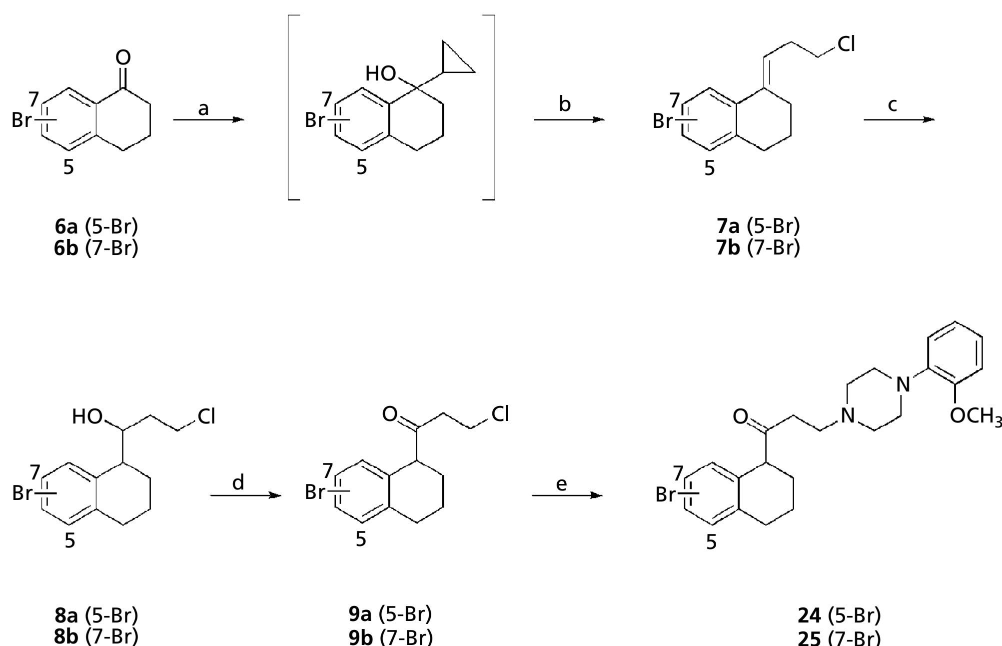

Figure 3 Synthesis of compounds **24** and **25**. Reagents: a, cyclopropyl magnesium bromide; b, HCl/CH_3COOH; c, i: borane methyl sulfide complex, ii: H_2O_2; d, TEMPO, *m*-CPBA; e, 1-(2-methoxyphenyl)piperazine.

with 1-(2-methoxyphenyl)piperazine gave target compounds **24** and **25**.

As a starting point, we assessed that 6-nitroquipazine did not bind at the 5-HT_{1A} receptor and that the arylpiperazine **1** was devoid of SERT affinity ($K_i > 1000$ nM, Table 1). By substituting the 2-pyridyl ring of **1** with the 6-NO_2-2-quinolyl nucleus, we obtained the derivative **10**, which showed good SERT affinity ($K_i = 11$ nM) but no affinity for the 5-HT_{1A} receptor ($K_i = 2500$ nM). Next, we studied the substitution of the 2-pyridyl ring with other bicyclic systems (i.e., 2-quinolyl and 2-naphthyl). This replacement lowered the 5-HT_{1A} receptor affinity. The presence of a nitro group in compound **10** is greatly detrimental for the 5-HT_{1A} receptor affinity. In contrast, the 6-nitro group seems to be beneficial for SERT affinity (i.e., **10** vs **11**). In an attempt to optimize the dual affinity profile of **11** (SERT: $K_i = 100$ nM; 5-HT_{1A}: $K_i = 80$ nM), we prepared its shorter and longer homologues, **13** and **14**, respectively. No significant improvement in SERT affinity was achieved and there was a significant loss in 5-HT_{1A} affinity. These results indicated that incorporation of the potent SSRI 6-nitroquipazine and related structures into a long-chain arylpiperazine, according to the **A** model (Figure 2), led to compounds with moderate to low 5-HT_{1A}/SERT affinity. In fact, the structural features that are necessary for 5-HT_{1A} affinity are detrimental for SERT affinity and vice-versa. Therefore, we changed our strategy, according to the **B** model (Figure 2). In particular, we substituted the tetralin moiety linked to the 3-position of propyl chain of **1** with the following groups that were proved to possess SERT affinity (Lee et al 2000): a 6-nitro-2-aminoquinoline moiety (substituent type III, Table 2); a fluoxetine-related structure type I; and 6-bromo-2-aminoquinoline (type II). We also studied 1-(2-methoxyphenyl)piperazine and 1-phenylpiperazine derivatives because various 5-HT_{1A} receptor ligands contained those structures. During the course of this study, Martinez-Esparza et al (2001) published syntheses of compounds **15** and **16**, which were designed for the same purpose. This second strategy originated compounds **15**–**23**, which displayed a wide range of SERT and 5-HT_{1A} receptor affinity. Only 1-(2-methoxyphenyl)piperazine derivatives **15** and **21** showed mixed SERT/5-HT_{1A} affinity. Of these, **21** was the only compound endowed with good affinity values (SERT: $K_i = 71.8$ nM; 5-HT_{1A} $K_i = 14.2$ nM). We also designed a modified tetralin moiety that shared with the other SSRI moieties the presence of an electron-withdrawing group on the bicyclic system and a heteroatom in the proximity of the linker (structure type IV and V). These new moieties were attached to 1-(2-methoxyphenyl)piperazine through an ethyl chain. We obtained compounds **24** and **25**, which showed a remarkable difference in SERT affinity. In fact, compound **25** was devoid of SERT affinity, whereas the derivative **24** displayed SERT affinity ($K_i = 62.8$ nM). Both compounds were high-affinity 5-HT_{1A} receptor ligands. The 5-HT_{1A} affinity data of arylpiperazine derivatives **15**–**25** showed that variations of the R structure (Table 2) were better tolerated by the 5-HT_{1A} receptor. In contrast, minor modification of the R structure resulted in great differences in the SERT affinity (i.e., **18** vs **21**; **24** vs **25**). Moreover, as evidenced by compounds **15** and **21**, also the arylpiperazine part of the molecule modulated binding to SERT. A general trend on this aspect cannot be drawn due to the limited number of the compounds studied. Therefore, further studies will be needed. Compounds **21** and **24** underwent further biological evaluations. Both derivatives **21** and **24** weakly inhibited re-uptake of [^{3}H]-5-HT in rat synaptosomes in-vitro (Wong et al 1993) ($EC50 = 621 \pm 15$ nM and 593 ± 24 nM, respectively; $P < 0.0001$), in agreement with their modest binding affinity values. The intrinsic activity of **21** and **24** at the 5-HT_{1A} receptor was assessed in an isolated guinea-pig ileum assay (Forster et al 1995). Unfortunately, neither compound displayed antagonistic properties at the 5-HT_{1A} receptor. In fact, derivative **21** acted as a full agonist ($EC50 = 3.93 \pm 0.08$ μM; $P < 0.0001$) and its activity was reverted by the 5-HT_{1A} receptor antagonist WAY-100635 in a dose-dependent manner (pA_2 ($-$log antagonist constant, K_b) $= 7.4 \pm 0.3$), whereas derivative **24** behaved as a partial agonist ($EC50 = 6.25 \pm 0.65$ μM, $P < 0.0001$).

Conclusion

We have followed two strategies to obtain new compounds with affinity at both the 5-HT_{1A} receptor and SERT (**A** and **B** models, Figure 2). The best results were obtained within the **B** series when the 1-(2-methoxyphenyl)piperazine is linked through a propyl chain to a structure endowed with SERT affinity. Compounds **21** and **24** showed good affinity values for SERT and 5-HT_{1A} receptor. They also weakly inhibited re-uptake of [^{3}H]-5-HT in rat synaptosomes in vitro and they acted as 5-HT_{1A} receptor agonist or partial agonist, respectively.

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