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3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives: high-affinity ligands for the serotonin transporter

Susanna Nencetti, Gian Carlo Demontis, Maria Rosa Mazzoni, Laura Betti, Irene Banti, Armando Rossello and Annalina Lapucci

Abstract

The structural requirements for high-affinity binding at the serotonin transporter (SERT) have been investigated through the preparation of some 3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives. The affinity of synthesised piperidinic compounds (**1–4**) at the SERT was evaluated by displacement of [^{3}H]-paroxetine binding. Derived inhibition constant (K_i) values were in the same order of magnitude as that of fluoxetine, ranging between 2 and 400 nM. To better define the profiles of these compounds as potential antidepressants, binding affinity for 5-HT_{1A} receptors and α_2-adrenoceptors was also investigated by competition experiments using [^{3}H]8-hydroxy-2-(dipropylamino)tetralin ([^{3}H]8-OH-DPAT) and [^{3}H]rauwolscine as radiolabelled ligands, respectively. Inhibition data indicate that compounds **1–4** possess a very weak affinity for these receptors. The high affinity of compound **1** for SERT indicates that it is worth investigating further.

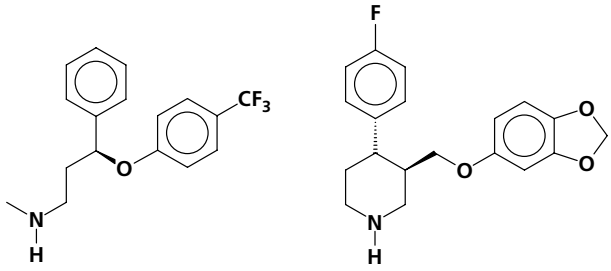
Introduction

Clinical depression is a common illness that affects people of all ages and is now considered a major public health problem (Sartorius 2001). Until the 1980s, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) were the most important drugs used for the treatment of depression (Pacher & Kecskemeti 2004). The antidepressant effect of the TCAs and MAOIs was explained by the 'monoamine hypothesis', which assumes that depression is caused by a dysfunction in the monoaminergic neurotransmitter systems (Schildkraut 1965; Coppen 1967). On the basis of this hypothesis, the TCAs and MAOIs are thought to exert their therapeutic effect by improving synaptic levels of norepinephrine and serotonin, thus restoring physiological functionality (Hindmarch 2001). Both classes of drugs show low selectivity, which is responsible for their severe side-effects (Stahl 1998; Feighner 1999; Spinks & Spinks 2002). Moreover TCAs are relatively toxic, and overdose with these antidepressants is one of the commonest causes of drug poisoning and death (Henry et al 1995; Kerr et al 2001). These negative properties provided the impetus for the research and development of new generations of antidepressant agents that possessed better tolerability and safety profiles. Among these new agents were the selective serotonin reuptake inhibitors (SSRIs) (Delgado et al 1999), such as fluoxetine and paroxetine (Fuller 1995), shown in Figure 1, which possess a more favourable side-effect profile than the TCAs and MAOIs, and safety over a wide dose range. These drugs are currently major pharmacological tools for the treatment of depressive illnesses. The SSRIs act by selectively blocking the reuptake of serotonin from the synaptic cleft, thereby increasing serotoninergic transmission. In common with the TCAs, the SSRIs have a slow onset of pharmacological action, typically taking 2–4 weeks to exert an antidepressant effect. This delay may be attributed to the time required to desensitise 5-HT_{1A} autoreceptors located in cell bodies (Gelenberg and Chesen 2000; Blier 2001a, b; Spinks & Spinks 2002; Schloss & Henn 2004). The relatively benign side-effect profile of the SSRIs compared with the TCAs allows their use in a much wider range of indications. In fact, they are successfully used to treat panic disorders, post-traumatic stress disorders, social phobia, obsessive–compulsive disorders, premenstrual dysphoric disorder, bulimia nervosa and anorexia (Schatzberg

Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy

Susanna Nencetti, Gian Carlo Demontis, Maria Rosa Mazzoni, Laura Betti, Irene Banti, Armando Rossello, Annalina Lapucci

Correspondence: Susanna Nencetti, Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy. E-mail: nencettis@farm.unipi.it



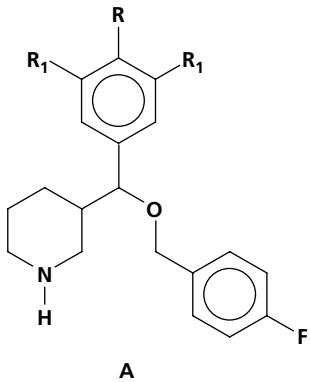
(*S*)-Fluoxetine (3*S*,4*R*)-Paroxetine

Figure 1 Structures of (*S*)-fluoxetine (left) and (3*S*,4*R*)-paroxetine.

2000; Spinks & Spinks 2002). More recently, the SSRIs have been shown to be strong activators of carbonic anhydrase and may therefore be beneficial in the treatment of Alzheimer's disease (Casini et al 2003).

There is a continuing interest in the monoamine transporters and in compounds able to interact with them (Ghorai et al 2003; Kolhatkar et al 2003; Loland et al 2003; Orjales et al 2003; Rothman & Bauman 2003; Kolhatkar et al 2004; Zhou et al 2004; Zheng et al 2005; Moltzen & Bang-Andersen 2006), and in particular in compounds that act as selective serotonin reuptake inhibitors (Plisson et al 2004; Jarkas et al 2005), as promising agents for the treatment of central nervous system disorders (Spinks & Spinks 2002).

The aim of this study was to synthesise some 3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives of type **A** (**1–4**; Figure 2) and to evaluate their biological properties. These compounds possess a piperidine nucleus and a lipophilic side chain containing two aryl portions as fluoxetine. The substituent at the 4-position of the benzyloxy group is a fluorine atom, while the substituents R and R_1 of the aryl group are a hydrogen or fluorine atom or a trifluoromethyl group. Such substituents were chosen because these atoms or groups are present in some active antidepressants. Binding affinities for the serotonin transporter (SERT), 5-HT_{1A} and α_2-adrenoceptors of pure (*RR/SS*)-diastereoisomers **1–4** were evaluated on the basis of their ability to inhibit radioligand binding.



A

Figure 2 General structure of the 3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives (**1–4**). The functional groups are given in Table 1.

Material and Methods

Animals

Cerebral tissue was obtained from adult Wistar rats (250–300 g) and New Zealand White rabbits (4–5 kg) obtained from a commercial source (Charles River Laboratories, Inc., Wilmington, MA, USA). Animals were maintained in standard laboratory conditions and feeding in sawdust-lined cages and a 12-h light/dark cycle. Rats were killed by cervical dislocation; rabbits were killed by intravenous injection of a lethal dose of pentobarbital. All procedures conformed to the guidelines of the International European ethical standards for the care and use of laboratory animals and all protocols were approved by the Ethical Deontological Committee for animal experimentation of the University of Pisa.

Synthesis

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. IR spectra were recorded as Nujol mulls, or as liquid films on a Mattson 1000 FTIR spectrometer (Madison, WI, USA). ^{1}H NMR spectra were recorded with a Varian Gemini-200 spectrometer (Varian, Palo Alto, CA, USA) operating at 200 mHz, in ca. 2% $CDCl_3$ solution. Mass spectra were detected with a Hewlett Packard 5988A spectrometer (Hewlett Packard, Mississauga, Canada) (EI, 70 eV). Analytical thin-layer chromatography (TLC) was carried out using 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Preparative TLC was carried out on 1 or 2 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 70–230 mesh silica gel. Evaporation was performed in vacuo (rotating evaporator). Na_2SO_4 was used as the drying agent. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ± 0.4%.

General procedure for the synthesis of N-benzylpiperidin-3-yl(aryl)-methanol (10–13)

A solution of the appropriate aryl bromide (0.025 mol) in anhydrous tehtrahydrofuran (THF) (25 mL) was added dropwise under nitrogen to a stirred suspension of magnesium (0.608 g, 0.025 gatom) in dry THF (50 mL). The reaction mixture was refluxed for 30 min, then a solution of 1-benzyl-3-formylpiperidine (**5**) (Balsamo et al 1987; Gilligan et al 1992) (2.5 g, 0.0123 mol) in anhydrous THF (25 mL) was added dropwise to the arylmagnesium bromide **6–9**. The resulting mixture was stirred and refluxed for 14 h then poured into iced water and extracted with ethyl acetate. Evaporation of the dried extracts gave a crude solid consisting of an almost 1:1 mixture of diastereoisomers, which was subjected to silica-gel column chromatography. Elution with 1:1 ethyl acetate/hexane mixture followed by recrystallisation (hexane) yielded pure (*RR/SS*) **10–13** (^{1}H NMR).

10: yield 60%; mp 117–119 °C; IR ν 3500 cm^{-1}; ^{1}H NMR δ 7.36–7.15 (m, 10H), 4.92 (m, 1H), 3.55 (m, 2H), 2.80–1.41 (m, 9H); MS (EI, 70 eV) *m/e* 281 (M^+) Anal. $C_{19}H_{23}NO$ (C, H, N). **11**: yield 57.6%; mp 133–135 °C; IR: ν 3500 cm^{-1}; ^{1}H NMR δ 7.32–7.27 (m, 7H), 7.06–6.97 (m, 2H), 4.94 (m, 1H), 3.51 (m, 2H), 2.53–1.38 (m, 9H); MS (EI, 70 eV) *m/e* 299 (M^+); Anal. $C_{19}H_{22}FNO$ (C, H, N). **12**: yield: 65%; mp 137–140 °C; IR: ν 3500 cm^{-1}; ^{1}H NMR: δ 7.60–7.26 (m, 9H), 5.06 (m, 1H); 3.50 (m, 2H); 2.80–1.32 (m, 9H); MS (EI, 70 eV) *m/e* 349 (M^+) Anal. $C_{20}H_{22}F_3NO$ (C, H, N). **13**: yield 53%; mp 135–136 °C; IR: ν 3500 cm^{-1}; ^{1}H NMR δ 7.80–7.62 (m, 3H), 7.31–7.21 (m, 5H), 5.01 (m, 1H), 3.48 (m, 2H), 2.81–1.32 (m, 9H); MS (EI, 70 eV) *m/e* 417 (M^+); Anal. $C_{21}H_{21}F_6NO$ (C, H, N).

General procedure for the preparation of 3-[(aryl)(4-fluorobenzyloxy)methyl]-N-benzyl piperidines (14–17)

To an ice-cooled stirred solution of the opportune alcohol **10–13** (1.34 mmol) in anhydrous THF (8 mL) 60% NaH (1.36 mmol) was added gradually in portions under nitrogen atmosphere. To the mixture was then added $(Bu)_4NI$ (0.0134 mmol) and dropwise a solution of 4-fluorobenzylchloride (1.34 mmol) in anhydrous THF. After stirring for 1 h, the reaction mixture was extracted with ethyl acetate and the extracts washed with water. The combined organic extracts were dried and evaporated to give an oily residue, which was purified by silica-gel column chromatography eluting with 1:1 ethyl acetate/hexane mixture to yield pure **14–17**.

14: yield 76%; ^{1}H NMR δ 7.29–6.89 (m, 14H), 4.30–4.04 (m, 3H), 3.31 (m, 2H), 2.78–1.26 (m, 9H); Anal. $C_{26}H_{28}FNO$ (C, H, N). **15:** yield 33%; ^{1}H NMR δ 7.26–6.95 (m, 13H), 4.28–4.03 (m, 3H), 3.31 (m, 2H), 2.80–1.26 (m, 9H); Anal. $C_{26}H_{27}F_2NO$ (C, H, N). **16:** yield 60%; ^{1}H NMR δ 7.57–6.85 (m, 13H), 4.29–4.05 (m, 3H), 3.33 (m, 2H), 2.82–1.25 (m, 9H); Anal. $C_{27}H_{27}F_4NO$ (C, H, N). **17:** yield 79% ^{1}H NMR δ 7.75–6.92 (m, 12H), 4.54–4.17 (m, 3H), 3.41 (m, 2H), 2.80–1.26 (m, 9H); Anal. $C_{28}H_{26}F_7NO$ (C, H, N).

General procedure for the preparation of 3-[(aryl)(4-fluorobenzyloxy)methyl]-piperidine hydrochlorides (1–4·HCl)

To a solution of **14–17** (0.6 mmol) in anhydrous ethanol (20 mL) was added a solution of ethanol/hydrochloric acid, pH ≈ 3. The mixture was shaken under hydrogen at room temperature and atmospheric pressure for 24 h in the presence of 10% palladium on charcoal (250 mg), then the catalyst was filtered off and the solution was evaporated to yield a crude solid. Pure piperidine hydrochlorides **1–4** were obtained by crystallisation.

1·HCl: yield 46%; mp ($CHCl_3$-Et_2O) 143–145°C. ^{1}H NMR δ 9.56–9.20 (brs, 1H), 7.37–7.18 (m, 7H), 7.06–6.98 (m, 2H), 4.42 (d, J = 11.3 Hz, 1H), 4.24 (m, 1H), 4.16 (d, J = 11.3 Hz, 1H), 3.28 (m, 2H), 2.67 (m, 2H), 2.31 (m, 1H), 1.91–1.17 (m, 4H); Anal. $C_{19}H_{23}ClFNO$ (C, H, N). **2·HCl**: yield 50%; mp ($CHCl_3$-Et_2O) 185–187 °C. ^{1}H NMR δ 9.59–9.26 (brs, 1H), 7.24–7.17 (m, 4H), 7.11–6.98 (m, 4H), 4.32 (d, J = 11.5 Hz, 1H), 4.20 (m, 1H), 4.15 (d, J = 11.5 Hz, 1H) 3.24 (m, 2H), 2.68 (m, 2H), 2.29 (m, 1H), 1.84–1.16 (m, 4H); Anal. $C_{19}H_{22}ClF_2NO$ (C, H, N). **3·HCl**: yield: 61%; mp ($CHCl_3$-Et_2O) 188–190°C. ^{1}H NMR δ 9.47–9.13 (brs, 1H), 7.64 (d, J = 7.7 Hz, 2H) 7.41 (d, J = 7.7 Hz, 2H), 7.23–7.19 (m, 2H), 7.10–6.99 (m, 2H), 4.42 (d, J = 11.5 Hz, 1H), 4.30 (m, 1H), 4.18 (d, J = 11.5 Hz, 1H), 3.32 (m, 2H), 2.74 (m, 2H), 2.34 (m, 1H), 1.81–1.21 (m, 4H); Anal. $C_{20}H_{22}ClF_4NO$ (C, H, N). **4·HCl**: yield 25%; mp ($CHCl_3$-Et_2O) 195–197 °C. ^{1}H NMR δ 9.67–9.26 (brs, 1H), 7.85–7.69 (m, 3H), 7.22–7.15 (m, 2H), 7.10–6.92 (m, 2H), 4.47 (d, J = 12.1 Hz, 1H), 4.35 (m, 1H), 4.23 (d, J = 12.1 Hz, 1H), 3.37 (m, 2H), 2.78 (m, 2H), 2.36–1.21 (m, 5H); Anal. $C_{21}H_{21}ClF_7NO$ (C, H, N).

Binding assays

For SERT and 5-HT_{1A} receptor binding assays, tissue from rabbit frontal cortex was collected and stored at –80°C until used.

Membranes used in SERT binding assays were prepared from frozen tissue using a polytron homogeniser, in Tris-buffer saline (composition: 120 mM NaCl, 5 mM KCl, 50 mM Tris-HCl, pH 7.4) at 4°C; 1:30 (w:v; 1 g homogenised tissue added to 30 mL buffer). The homogenate was centrifuged at 46 000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet was resuspended by diluting 1:30 (w:v) in homogenisation buffer. The pellet was recentrifuged at 46 000 *g* for 10 min. The resulting pellet was resuspended 1:30 (w:v) in buffer containing 0.32 M sucrose, divided into 1 mL aliquots and stored at –80°C.

On the day of the experiment, one aliquot was quickly thawed at 37°C, centrifuged at 46 000 *g* for 10 min at 4°C and the pellet resuspended in homogenisation buffer at a dilution of 1:30 (w:v). [^{3}H]Paroxetine (specific activity, 19.7 Ci mmol^{-1}) binding assays were performed in duplicate in glass tubes in a total volume of 2 mL Tris-saline with a concentration of [^{3}H]paroxetine of 200 pM, incubated for 2 h at room temperature. In the experimental conditions used, specific binding (see below) reached equilibrium after 90 min. Ligand bound to the transporter was separated from free ligand by filtration using a 30-well manifold (Brandel Harvester, Brandel, Gaithersburg, MD, USA), with glass-fibre filters (GF/C) pre-soaked for 2 h at room temperature with 0.6% (w:v) polyethylenimine. Filters were quickly washed four times with ice-cold 50 mM Tris-HCl, pH 7.4, placed into scintillation vials and soaked overnight in 3 mL Cytoscint ES (MP Biomedicals Solon, OH, USA). The following day, samples were read by scintillation spectroscopy in a β-counter (PerkinElmer, Waltham, MA, USA). Non-specific binding defined in the presence of 2 mM serotonin was subtracted from total binding (in the absence of competitors) to obtain specific binding. In a typical assay, specific binding was ~80% of total binding.

For saturation studies, membranes were incubated in Tris-saline buffer with six different concentrations of [^{3}H]paroxetine ranging from 10 pM to 1 nM.

Competition experiments were carried out by incubating membranes and 200 pM [^{3}H]paroxetine with seven different concentrations of the test compounds (0.5 nM to 1 μM). Stock solutions (1 mM) of test compounds were prepared in ethanol and then diluted in Tris-saline buffer to the required concentration.

Membranes used in 5-HT_{1A} receptor binding assays were prepared by homogenisation of frozen rabbit frontal cortex in

10 vol ice-cold 50 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose, 160 μg mL^{-1} benzamidine, 200 μg mL^{-1} bacitracin and 20 μg mL^{-1} trypsin inhibitor, using an ultraturrax homogeniser. The homogenate was centrifuged at 500 *g* for 5 min at 4°C. The supernatant was collected, and centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was resuspended in 10 vol 50 mM Tris-HCl, pH 7.4, containing protease inhibitors as above and incubated at 37°C for 15 min with constant shaking to remove endogenous serotonin. The membrane homogenate was then centrifuged as above and stored in aliquots at −80°C until the time of assay. On the day of the experiment, one aliquot was resuspended in 50 mM Tris-HCl, pH 7.4, at a dilution of 1:10 (w:v). [^{3}H]8-hydroxy-2-(dipropylamino)tetralin ([^{3}H]8-OH-DPAT; specific activity, 129 Ci/mmol) binding assays were performed by incubating rabbit cortical membranes (0.3–0.4 mg protein) with 0.4 nM [^{3}H]8-OH-DPAT in 1 mL 50 mM Tris-HCl, pH 7.4, at 37°C for 15 min. Binding reactions were terminated by rapid filtration through Whatman GF/C fibre filters under reduced pressure using the apparatus described above. Filters were washed three times with 5 mL ice-cold 50 mM Tris-HCl, pH 7.4, placed into scintillation vials and soaked overnight in 3 mL Cytoscint ES (ICN). Sample radioactivity was counted by scintillation spectroscopy in a β-counter. Non-specific binding, defined in the presence of 10 μM 8-OH-DPAT, was 20% of total binding for all experiments.

For saturation studies, membranes (0.3 mg protein) were incubated in 50 mM Tris-HCl, pH 7.4, with five different concentrations of [^{3}H]8-OH-DPAT ranging from 0.3 to 20 nM. Competition experiments were carried out by incubating membranes (0.3 mg protein) and 0.4 nM [^{3}H]8-OH-DPAT with seven different concentrations of the test compounds (50 nM to 100 μM). Stock solutions (5 mM) of test compounds were prepared in ethanol and then diluted in 50 mM Tris-HCl, pH 7.4, to the required concentrations.

For α_2-adrenoceptor binding assays, cerebral cortex was dissected from rat brain. The tissue was homogenised in 20 vol ice-cold 50 mM Tris-HCl buffer, pH 7.7, containing 5 mM EDTA. The homogenate was centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was diluted in 20 vol 50 mM Tris-HCl buffer, pH 7.7, for use in binding assays. [^{3}H]Rauwolscine (specific activity, 83 Ci mmol^{-1}) binding assay was performed in duplicate by incubating aliquots of membrane preparation (0.2–0.3 mg protein) in 50 mM Tris-HCl buffer, pH 7.7, with 2 nM [^{3}H]rauwolscine in a final volume of 1 mL. Incubation was carried out at 25°C for 60 min. Non-specific binding was defined in the presence of 10 μM rauwolscine. Binding reactions were terminated by rapid filtration through Whatman GF/C fibre filters under reduced pressure using the apparatus described above. Filters were washed four times with 5 mL ice-cold 50 mM Tris-HCl, pH 7.7, placed into scintillation vials and soaked in scintillation liquid as described above. Sample radioactivity was measured as described above. Specific binding was obtained by subtracting non-specific binding from total binding, and was ~90% of total binding. For saturation studies, membranes (0.2 mg protein) were incubated in 50 mM Tris-HCl buffer, pH 7.7, with five different concentrations of [^{3}H]rauwolscine ranging from 0.5 to 15 nM. Competition experiments were carried out by incubating membranes and 2 nM [^{3}H]rauwolscine with seven different concentrations of the test compounds (50 nM to 50 μM). Compounds were dissolved in DMSO at stock solution concentration of 5 mM and then diluted in 50 mM Tris-HCl buffer, pH 7.7 to the required concentrations. Blank experiments were carried out to determine the effect of DMSO (~2% of reaction volume) on binding.

Protein content was determined by the method of Lowry (1951) using bovine serum albumin as a standard.

Data analysis

Saturation data were analysed and fitted by non-linear regression analysis using the GraphPad Prism (Version 3.00) computer program (GraphPad Prism, Inc., San Diego, CA, USA). The dissociation constants (K_D values) calculated for [^{3}H]-paroxetine and [^{3}H]8-OH-DPAT binding to rabbit cortical membranes were 56.0 ± 8.0 pM (n=3) and 5.1 ± 1.0 nM (n=3), respectively. The K_D value for [^{3}H]rauwolscine binding to rat cortical membranes was 4.3 ± 0.8 nM (n=3).

The inhibition curves of compounds reported in Table 1 were analysed and fitted by non-linear regression analysis using GraphPad Prism. The inhibition constant (K_i) value was calculated using the Cheng–Prusoff equation (Cheng & Prusoff 1973). K_i values are presented as mean ± s.e.m. of three independent experiments performed in duplicate.

The Kruskal–Wallis test followed by Dunn's multiple comparison post hoc test was used to evaluate the statistical significance of differences between K_i values.

Chemistry

Diastereoisomeric pure 3-[(aryl)(4-fluorobenzyloxy)methyl] piperidines (**1–4**) (^{1}H NMR) were prepared as racemic mixtures, as outlined in Figure 3. Treatment of aldehyde **5**

Table 1 Inhibition of specific radioligand binding to the serotonin transporter (SERT), 5-HT$_{1A}$ receptors and α_2-adrenoceptors by ether derivatives **1–4** compared with reference compounds fluoxetine, 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) and rauwolscine, respectively. The general structure of compounds **1–4** is given in Figure 2

Compound	R	R_1	K_i (nM)[a]		
			SERT	5-HT$_{1A}$	α_2-adrenoceptors
1	H	H	2.1 ± 1.1	2900 ± 180	7800 ± 750
2	F	H	17.0 ± 7.2	6400 ± 850	2200 ± 250
3	CF_3	H	6.0 ± 1.3	29500 ± 1500	6900 ± 700
4	H	CF_3	400.0 ± 230	–[b]	–[b]
Fluoxetine			5.8 ± 2.9	nd	nd
8-OH-DPAT			nd	4.2 ± 0.8	nd
Rauwolscine			nd	nd	3.8 ± 0.3

[a]K_i values are the mean ± s.e.m. for three experiments.
[b]K_i value was not calculated because the inhibition percentage at 100 μM was too low.
nd, not determined.
K_i values of compounds **1–3** for [^{3}H]paroxetine, [^{3}H]8-OH-DPAT and [^{3}H]rauwolscine binding inhibition were significantly different, $P < 0.05$.

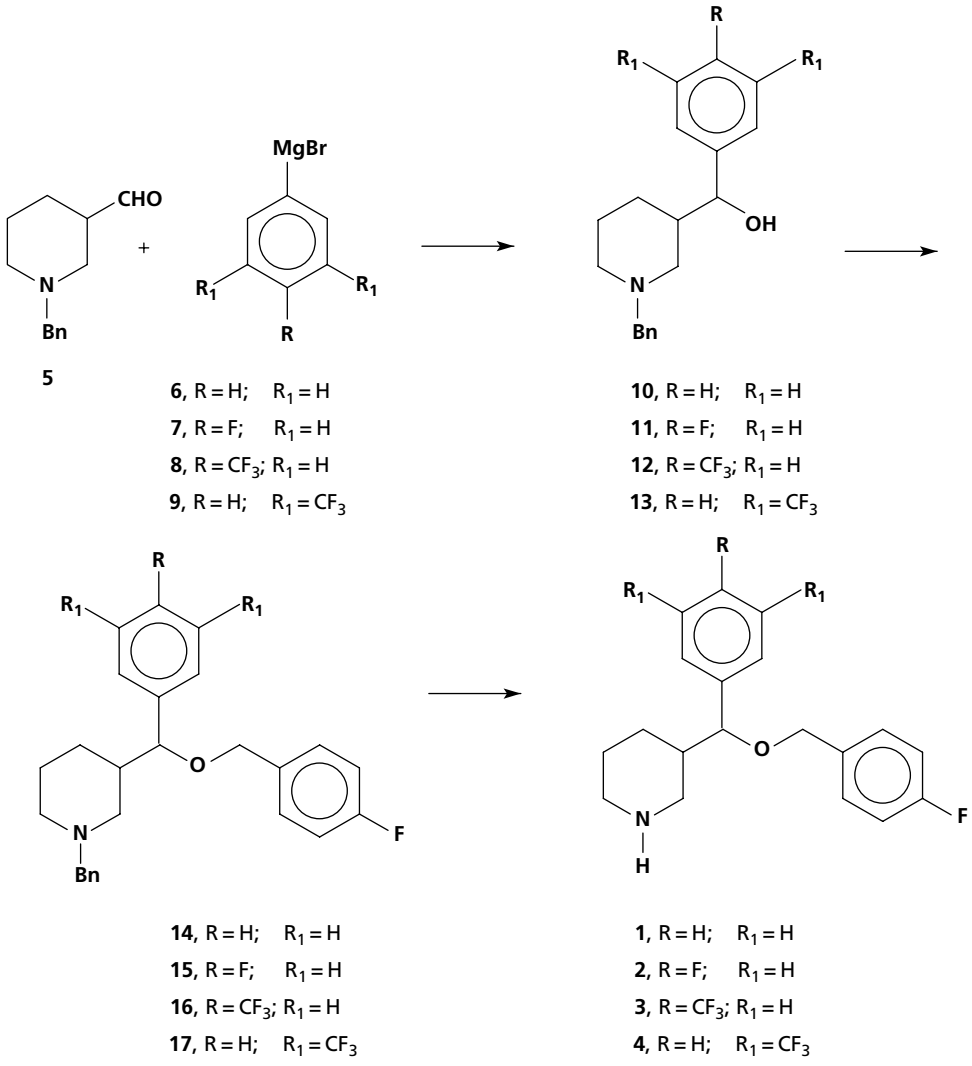


Figure 3 Synthesis of 3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives **1–4**.

(Balsamo et al 1987; Gilligan et al 1992) with the appropriate arylmagnesium bromide (**6–9**) yielded the diastereoisomeric alcohols **10–13**. Pure compounds (*RR/SS*) **10–13** were obtained by column chromatography of the crude product consisting of an almost 1:1 mixture of corresponding diastereoisomers followed by recrystallisation. The exact relative configuration of (*RR/SS*) **10–13** was unequivocally established by an appropriate ^{1}H NMR study (unpublished results). The other diastereoisomers (relative configuration *RS/SR*) were isolated in a mixture with the (*RR/SS*). The reaction of **10–13** as sodium salts with 4-fluorobenzylchloride in the presence of tetrabutylammonium iodide gave the ether derivatives **14–17**. Catalytic hydrogenolysis in acidic medium (HCl) of **14–17** yielded the racemic mixture of the final products **1–4** as hydrochlorides.

Because no stereocentres are involved in the transformation of (*RR/SS*) **10–13** into the corresponding compounds **1–4,** ethers **1–4** and alcohols **10–13** possess the same relative configurations.

Results and Discussion

Compounds **1–4** were tested as racemic mixtures for their ability to interfere with serotoninergic transmission by evaluating their binding affinity for SERT expressed at the level of the rabbit cerebral cortex. [^{3}H]Paroxetine was used as specific radiolabelled ligand for SERT. To define the specificity of these new potential antidepressants as SERT inhibitors, their ability to inhibit [^{3}H]8-OH-DPAT and [^{3}H]rauwolscine binding to 5-HT$_{1A}$ and α_2-adrenoceptors, respectively, was also evaluated.

The K_i values for 3-piperidine ethers **1–4** are shown in Table 1, together with those obtained in the same tests for fluoxetine, 8-OH-DPAT and rauwolscine. Table 1 shows that compounds **1–3** are potent inhibitors of [^{3}H]paroxetine binding to SERT, with K_i values ranging from 2.1 nM (**1**) to 17.0 nM (**2**). K_i values for compounds **1–3**, unsubstituted at the R_1 position, are in the same range as that for fluoxetine

($K_i = 5.8$ nM). It is immediately evident that compound **4**, which possesses an R_1 substituent, is a weaker inhibitor of [^{3}H]paroxetine binding than compounds **1–3**. The bis-trifluoromethyl substitution of compound **4** negatively affects its activity as an inhibitor of [^{3}H]paroxetine binding ($K_i = 400$ nM), while compounds with no substitution in R_1 position exhibit potent interaction with SERT and possess a narrow range of K_i values ($K_i = 2–17$ nM). Compound **1** which has hydrogen atoms at R and R_1 positions is the most potent inhibitor, while the K_i values for compounds **2** (R = F, R_1 = H) and **3** (R = CF_3, R_1 = H) are 8- and 3-fold higher than that of **1**, respectively. This result suggests that the R substituent on this aromatic ring does not play an essential role in the interaction with the SERT. Statistical analysis did not show any significant difference between K_i values of compounds **1–4**.

With respect to the ability of these compounds to interact with 5-HT_{1A} and α_2-adrenoceptors, data in Table 1 show that compounds **1–4** are very weak inhibitors of both [^{3}H]8-OH-DPAT and [^{3}H]rauwolscine binding, with K_i values in the μM range for compounds **1–3** and mM range for compound **4**. The differences between K_i values of compounds **1–3** for [^{3}H]paroxetine, [^{3}H]8-OH-DPAT and [^{3}H]-Rauwolscine binding inhibition were significant ($P < 0.05$).

Conclusions

In summary, new 3-[(aryl)(4-fluorobenzyloxy)methyl]piperidine derivatives were synthesised. SERT inhibition studies showed that compounds **1–3** are potent inhibitors of [^{3}H]-paroxetine binding to SERT, with K_i values in the nanomolar range, but they display a very weak affinity for 5-HT_{1A} receptors and α_2-adreneroceptors. In particular, compound **1** is the most interesting of this series, exhibiting a K_i value at SERT similar to that of fluoxetine (K_i values 2.1 ± 1.1 nM vs 5.8 ± 2.9 nM). Since these new piperidine derivatives are potent inhibitors of [^{3}H]paroxetine binding to SERT, it is reasonable to assume that they possess high affinity for this amine transporter. On the basis of their affinity profile, these compounds may provide useful information for the design of new SSRIs. The interesting result obtained with compound **1** suggests that it may be a likely candidate for further pharmacological characterisation. Separation and evaluation of the activity of its enantiomers is in progress.

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