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Synthesis and affinity for serotonin and dopamine transporters of some benzophenone oxime ether derivatives

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Abstract

In a previous study, we reported the synthesis of several 3-(methylenaminoxymethyl)-substituted piperidine derivatives and their ability to interfere with the transmission mediated by biogenic amines. The present study describes the preparation of some new oxime derivatives and their capacity to inhibit serotonin (SERT) and dopamine (DAT) transporters expressed at the level of the rabbit cortex and the striatal membranes, respectively. All the compounds showed K_i values in the micromolar range on both transporters.

Keywords: Dopamine; Serotonin; Oxime derivatives

1. Introduction

According to the monoamine theory, depression is a disease of the central nervous system associated with a dysfunction of the metabolism and/or of the synaptic transmission mediated by the biogenic amines dopamine (DA), serotonin (5-HT), noradrenaline (NA) [1,2]. The therapeutic treatment of this pathology is linked with the use of drugs that enhance the synaptic levels of these amines such as biogenic amines reuptake inhibitors. In this class of antidepressant agents, a particular importance is attached to selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline, paroxetine, citalopran and fluvoxamine, which have been used with success in depressed patients in view of their favourable side-effect profile with respect to classic tricyclic antidepressants (TCAs) [1,3].

In a previous work [4], we evaluated the inhibitory activity of some 3-(methylenaminoxy)methyl piperidines of type **1** (Fig. 1) on biogenic amine reuptake (NA, DA, 5-HT) in rabbit brain synaptosomal fractions. This study showed that all compounds possess an appreciable activity, and the one in which R and R_1 are two phenyls (**1a**) is the most active in this series of compounds, with K_i values in the micromolar range. On the basis of these data, with the aim to gain further knowledge about the influence of certain molecular portions of **1a** on its biological properties, we synthesised compounds **2a**, **2b**, **3a** and **3b**, in which the benzophenone oximic portion of **1a** is still present, while the aminic side-chain, ethylpiperidinic (**2a** and **2b**) or ethyldimethylaminic (**3a** and **3b**), is different.

2. Chemistry

The oxime ethers **2a**, **2b**, **3a** and **3b** were synthesised as indicated in Scheme 1. Reaction of benzophenone or 4-

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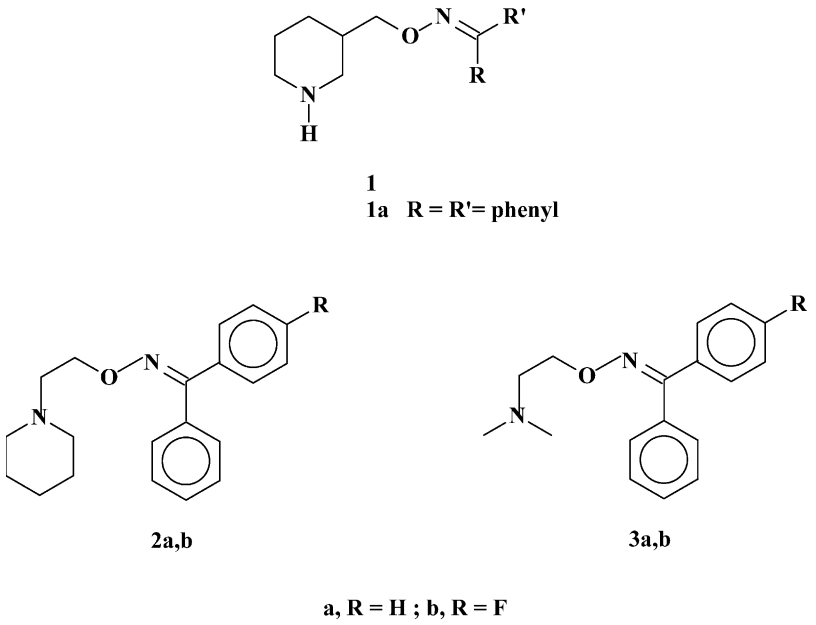


1
1a R = R'= phenyl

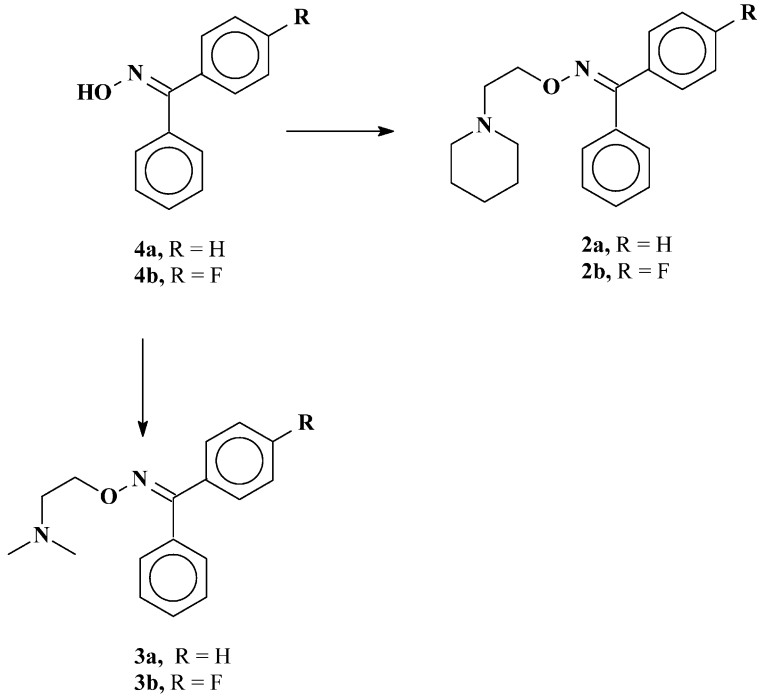
2a,b **3a,b**

a, R = H ; b, R = F

Fig. 1.

fluorobenzophenone with hydroxylamine hydrochloride afforded the oximes **4a** and **4b** [5,6]. The reaction of **4a** and **4b** with the appropriate alkyl chloride in DMSO and sodium hydroxide, in the presence of 18-Crown-6, yielded the crude oxime ethers **2a**, **2b**, **3a** and **3b**, which were purified by column chromatography and then transformed into the corresponding hydrochloride salts.

The structure of compounds **2** and **3** was confirmed by their spectral data. The *E* configuration around the double bond of the methylenaminoxy group of com-



4a, R = H
4b, R = F

2a, R = H
2b, R = F

3a, R = H
3b, R = F

a, R = H; b, R = F

Scheme 1.

pounds **2b** and **3b** was assumed on the basis of the configuration of the starting oxime **4b** [6] which have been proved to be configurationally stable in the reaction conditions employed.

3. Pharmacology

Compounds **2a**, **2b**, **3a** and **3b** were tested for their ability to interfere with the systems of transmission of biogenic amines, by directly evaluating the degree to which they bind to the serotonin (SERT) and dopamine (DAT) transporters expressed at the level of the rabbit cerebral cortex and the striatal membranes, respectively. In order to allow a more homogeneous comparison of all biological data, the same type of test was also used for compound **1a**, which was previously tested [4] by means of the direct uptake method. [^{3}H]-paroxetine was used as the specific tritiated ligand for SERT, while [^{3}H]WIN 35,428 was used to label DAT. The binding affinity indices of oxime ethers **2a**, **2b**, **3a** and **3b** are reported in Table 1, together with those obtained in the same tests with the previously studied compound **1a** and fluoxetine.

4. Results and discussion

As regard affinity for SERT, the data in Table 1 show that all the synthesised compounds **2a**, **2b**, **3a** and **3b** exhibit very low affinity values with K_i in the micromolar range, in the same way as the previously studied compound **1a** whereas the affinity value of the reference compound, fluoxetine is in the nanomolar range.

Also, the affinity for DAT of the compounds **2a** and **3a**, in which R = H, is low with K_i values in the micromolar range, so the DAT/SERT affinity ratio is very low, whereas for the fluoxetine is 689.

Furthermore, it may be seen from Table 1 that the compounds in which one of the aromatic rings is substituted in the *para* position with a fluorine atom (**2b** and **3b**) exhibit a SERT affinity index slightly lower than that of the corresponding compounds unsubstituted on the aromatic ring, without any appreciable change in their affinity for DAT, with a ratio of 4.1 for compound **3b**.

In conclusion, it may be said that the changes made in the structure of compound **1a** lead to a slight increase in the affinity for SERT, in particular in compounds in which R = F (**2b** and **3b**). Overall results indicate that compounds **2a**, **2b**, **3a** and **3b** exhibit a certain selectivity for DAT compared with SERT, but this tendency is attenuated by the insertion of a fluorine atom on one of the aromatic rings.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra for comparison between compounds were recorded with a Perkin–Elmer model 1310, as nujol mulls in the case of solid substances, or as liquid film in the case of liquids. ^{1}H NMR spectra were routinely recorded with a Varian CFT 20 (80 MHz) instrument in ca. 5% solution of $CDCl_3$ (for neutral compounds or the free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The oximes **4a** and (*E*)-**4b** were prepared by the method described in literature. [5,6]. Analytical TLC were carried out on 0.25 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. $MgSO_4$ was always used as the drying agent. Evaporation was carried out in vacuo (rotating evaporator). Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within $\pm$0.4%.

5.1.1. General procedure for the synthesis of oximethers *2a, 2b, 3a and 3b*

N-(2-Chloroethyl) piperidine or 2-(dimethylamino)-ethyl chloride hydrochloride (23 mmol), NaOH (46 mmol) and 18-Crown-6 (23 mmol) were added to a stirred solution of the appropriate oxime **4a** and **4b** [5,6] (23 mmol) in anhydrous DMSO (20 ml). After comple-

Table 1
Affinity for SERT and DAT of compounds **1a**, **2a**, **2b**, **3a** and **3b**

Comp.	R	R_1	5-HT-T K_i (μM)	DAT K_i (μM)	Ratio
2a	H	bdc–$C_5H_{10}N$	11.4±2.7	3.6±1.2	0.3
2b	F	bdc–$C_5H_{10}N$	4.2±1.3	3.9±1.1	0.9
3a	H	$N(Me)_2$	9.0±3.1	7.2±2.6	0.8
3b	F	$N(Me)_2$	1.2±0.3	4.9±1.4	4.1
1a			25.0±6.1	n.t.	
Fluoxetine			$5.8 \times 10^{-3} \pm 2.9 \times 10^{-3}$	4.0±1.7	689

tion of the addition the reaction mixture was stirred at 40 °C for 48 h, diluted with water and extracted with $CHCl_3$. The evaporation of the washed (H_2O) and dried $CHCl_3$ extracts give an oily residue that was purified by column chromatography ($CHCl_3$/5% MeOH) to yield pure **2a**, **2b**, **3a** and **3b**.

2a: (39%) ^{1}H NMR δ 1.25–2.40 (m, 10H), 2.58–2.73 (m, 2H), 4.22–4.37 (m, 2H), 7.06–7.89 (m, 10H). **2b:** (96%) ^{1}H NMR δ 1.43–2.34 (m, 10H), 2.52–2.65 (m, 2H), 4.18–4.40 (m, 2H), 7.01–7.43 (m, 9H). **3a:** (59%) ^{1}H NMR δ 2.24 (m, 6H), 2.57–2.72 (m, 2H), 4.20–4.36 (m, 2H), 7.29–7.39 (m, 10H). **3b:** (37%) ^{1}H NMR δ 2.25 (m, 6H), 2.48–2.72 (m, 2H), 4.19–4.36 (m, 2H), 6.96–7.37 (m, 9H).

Compounds **2a**, **2b**, **3a** and **3b** were converted to the corresponding hydrochloride salts by dissolving the bases in Et_2O and subsequent treatment with an excess of Et_2O·HCl. The solid precipitate was filtered and crystallised from MeOH/Et_2O to yield pure **2a·HCl**, **2b·HCl**, **3a·HCl**, **3b·HCl**. Melting points are reported in Table 2.

5.2. Binding studies

5.2.1. Affinity for 5-HT transporter (SERT)

The affinity of compounds (**2a**, **2b**, **3a**, and **3b**) for the serotonin transporter was assayed in rabbit cortex membranes, labelled with [^{3}H]-paroxetine, whose specific activity ranged from 15 to 20 Ci/mmol.

Tissue from the anterior pole of the rabbit frontal cortex was collected and stored at −35 °C until used. Membranes were prepared from frozen tissue using an ultraturrax homogeniser, in a saline solution whose composition was (mM): NaCl (120); KCl (5), Tris–HCl (50), pH 7.4 at 4 °C, using a dilution factor of 1:30 (w:v). The homogenate was centrifuged for 10 min at a relative centrifugal force of 46,000 × g, keeping the temperature at 4 °C. The supernatant was discarded and the pellet was resuspended by diluting 1:30 (w:v) in the homogenisation buffer. The pellet was recentrifuged at 46,000 × g for 10 min and the pellet resuspended 1:30 in buffer without sucrose, splitted in 1 ml aliquots and stored frozen at −35 °C. On the day of the experiment, one aliquot was quickly thawed at 37 °C, centrifuged for 10 min at 46,000 × g at 4 °C and the pellet resuspended in homogenisation buffer at a dilution of 1:30 (w:v).

Competition assays were performed in duplicate glass tubes in a total volume of 2000 μl, with a concentration of [^{3}H]-paroxetine of 100–200 pM, using an incubation time of 2 h at room temperature. In the experimental conditions used, specific binding (see below) reached equilibrium after 90 min. Separation of ligand bound to the transporter from free ligand was performed by filtration using a 30 well manyfold (Brandel), with glass fiber filters (GF/C) pre-soaked for 2 h at room temperature with 0.6% (w:v) polyethylenimine. Filters were quickly washed four times with cold (4 °C) Tris–HCl 50 mM. Filters were removed, put into scintillation vials and let to stay overnight in 3 ml of Cytoscint ES (ICN). The following day, samples were read by scintillation spectroscopy in a β-counter (Packard). Unspecific 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, total binding was 900 d.p.m., unspecific was 200 d.p.m., and specific binding was 700 d.p.m.

5.2.2. Affinity for DA transporter (DAT)

The affinity of compounds (**2a**, **2b**, **3a**, and **3b**) for the DAT was assayed in rabbit striatal membranes, labelled with the cocaine analogue [^{3}H]-WIN 35,428, whose specific activity was 84.5 Ci/mmol.

Tissue was collected and stored at −35 °C until used. Membranes were prepared from frozen tissue using an ultraturrax homogeniser, in a saline solution whose composition was (mM): NaCl (48); sucrose (320), pH 7.7 at 4 °C, by mixing Na_2HPO_4/NaH_2PO_4 (25). The homogenate was centrifuged for 10 min at 48,000 relative centrifugal force of 46,000 × g, keeping the temperature at 4 °C. The supernatant was discarded and the pellet resuspended by diluting 1:100 (w:v) in the homogenisation buffer. The pellet was recentrifuged at 46,000 × g for 10 min and the pellet was resuspended 1:100 in buffer without sucrose, splitted in 3.5 ml aliquots and stored frozen at −35 °C. On the day of the experiment, one 3.5 ml aliquot was quickly thawed at 37 °C, centrifuged for 10 min at 46,000 × g at 4 °C and the pellet was resuspended in buffer without sucrose at a dilution of 1:200 (w:v).

Competition assays were done in duplicate glass tubes in a total volume of 500 μl, with a concentration of [^{3}H]-WIN 35,428 of 0.5–2 nM, using an incubation time of 2

Table 2
Affinity for SERT and DAT of compounds **1a**, **2a**, **2b**, **3a** and **3b**

Comp.	R	R_1	m.p. (°C)	Formula	Analysis
2a·HCl	H	bdc–$C_5H_{10}N$	182–184	$C_{20}H_{25}ClN_2O$	C, H, N
2b·HCl	F	bdc–$C_5H_{10}N$	165–166	$C_{20}H_{24}ClFN_2O$	C, H, N
3a·HCl	H	$N(Me)_2$	185–186	$C_{17}H_{21}ClN_2O$	C, H, N
3b·HCl	F	$N(Me)_2$	154–155	$C_{17}H_{20}ClFN_2O$	C, H, N

h at 4 °C. In the experimental conditions used, specific binding (see below) reached equilibrium after 60 min. Separation of ligand bound to the transporter from free ligand was by filtration using a 30 well manyfold (Brandel), with glass fiber filters (GF/C) pre-soaked for 2 h at room temperature with 0.6% (w:v) polyethylenimine. Filters were quickly washed three times with cold (4 °C) Tris–HCl 50 mM. Filters were removed, put in scintillation vials and let to stay overnight in 3 ml of Cytoscint ES (ICN). The following day, samples were read by scintillation spectroscopy in a β-counter (Packard). Unspecific binding, defined in the presence of 10 mM dopamine, was subtracted from total binding (in the absence of competitors) to obtain specific binding. In a typical assay, total binding was 600 d.p.m., unspecific was 150 d.p.m., and specific binding was 450 d.p.m.

The affinity of [^{3}H]-WIN 35,428, for the dopamine transporter was assessed in separate saturation experiments, and gave an average dissociation constant (K_d) of 6.1 nM.

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