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**Original article**

# Synthesis by microwave irradiation and binding properties of novel 5-$HT_{1A}$ receptor ligands

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**Abstract** – This work reports the synthesis by microwave irradiation and the binding tests on the 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors of new substituted piperazines in order to identify selective ligands for 5-$HT_{1A}$ subtype receptor. Conventional heating and microwave irradiation of the reactions was compared. Synthesis by microwave irradiation gave the desired compounds in better yields than those obtained by conventional heating. The overall times for the syntheses were considerably reduced. Some resulting active compounds (**29** and **39**) were characterised by a good selectivity profile for the 5-$HT_{1A}$ subtype receptor. The more active compounds were selected and further evaluated for their binding affinities on $D_1$, $D_2$ dopaminergic and $\alpha_1$, $\alpha_2$ adrenergic receptors. The compound with higher affinity and selectivity for the 5-$HT_{1A}$ over all the considered receptors was the 3-{4-[4-(1,2,3,4-tetrahydronaphthyl)-1-piperazinyl]butan}-benzotriazinone (−)**29** (5-$HT_{1A}$ $K_i = 36$ nM, other receptors not active). © 2001 Éditions scientifiques et médicales Elsevier SAS

**microwave / serotonin / 5-HT receptors / benzotriazinone**

## 1. Introduction

Serotonin is a neuromediator well known for its implication in mood regulation, anxiety, depression, and insomnia. The discovery of several classes of receptors for serotonin and the search for their implications in the mechanism of mood and anxiety have been widely studied [1–4]. One of these receptor subtypes, the 5-$HT_{1A}$ one, is found in high concentration in the limbic system in which it is thought to play a role in emotional processes. The activation of the 5-$HT_{1A}$ receptor leads to a number of physiological changes that can be easily quantified. The synthesis of a specific 5-$HT_{1A}$ receptor ligand, the 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH–DPAT), and the study of its pharmacological properties showed that agonists inhibited 5-HT function and release, which is a potentially important event for the treatment of anxiety states. Several structurally different compounds possessed high affinity and selectivity for 5-$HT_{1A}$ receptors [5]. Among these some piperazine compounds such buspirone [6], gepirone [7], or ipsapirone [8] showed selective agonist or partial agonist activity for 5-$HT_{1A}$ receptors and has proved to be effective for the treatment of anxiety states or depression. The search for new selective 5-$HT_{1A}$ receptor agonists has yielded active compounds. During recent years, serotoninergic 5-$HT_{1A}$ ligands have been suggested as useful therapies for anxiety [9, 10] depression [11], nausea and vomiting [12, 13], Alzheimer's disease [14], prostate cancer [15], hypertension, pain [16,17] or alcoholism [18].

In the past, we studied new arylpiperazine derivatives [19–23] and binding studies showed that several of these compounds had a good affinity for serotoninergic 5-$HT_{1A}$ receptors and low affinity for serotonin-

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ergic 5-$HT_{2A}$,5-$HT_{2C}$, dopaminergic $D_1$, $D_2$ and adrenergic $\alpha_1$, $\alpha_2$ receptors.

To try to obtain selective 5-$HT_{1A}$ ligands we, therefore, decided to introduce some structural modification such as the nature of the aromatic heterocycle, the length of the aliphatic chain (*n*), and the nature of the substituents on the piperazine moiety.

In both lead identification and lead optimisation processes, there is an acute need for new organic small molecules. Traditional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for these compounds. The fields of combinatorial and automated medicinal chemistry (i.e. the simultaneous or parallel synthesis of libraries) [24–26] have been developed to meet the increasing requirement of new compounds for drug discovery; within these fields, speed is of the essence. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates is an emerging technique. In recent years, microwaves have become popular among synthetic organic chemists both to improve classical organic reactions, [27] shortening reaction times and/or improving yields, as well as to promote new reactions [28].

Moreover, when carrying out a reaction in a microwave oven, the use of a solvent can sometimes be avoided which is important in order to make the synthesis more environmentally friendly ('green chemistry'). The commercially available instrument used herein features a built-in magnetic stirrer, direct temperature control of the reaction mixture with the aid of fluoroptic probes and software that enables online temperature control by regulation of microwave power output (see Section 5).

This article reports the synthesis, by microwave irradiation, of new arylpiperazine derivatives and pharmacological evaluations as selective serotonin receptor ligands.

Conventional heating (oil bath) and microwave irradiation of the reactions were compared.

## 2. Chemistry

The synthesis of some intermediates 4-X-substituted piperazines (*figures 1 and 2*) and final 1,2,3-benzotriazinone (**11–30**) and 3-hydroxy-1,2,3-benzotriazinone (**33–42**) derivatives (*figure 3*) were performed using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis. The experimental conditions used in our work were similar to those used by conventional heating, with the same concentration of starting material and volume of solvent. All reactions were performed in closed vessels. All the reactions were performed by microwave program, which was composed by appropriate ramping and holding steps. Identification of the optimum profile power/time and temperature for every considered compound was reported in *table III*.

All the 4-X-substituted-piperazines were commercially available except the 1-(2-thienylmethyl 5-X)-piperazines (**4a–b**) and the intermediates 1-(1,2,3,4-tetrahydronaphth-1-yl)piperazine (**8**). The synthetic approach employed for the preparation of **4a–b** is summarised in *figure 1*. Treatment of 2-thiophenemethanol (**1**) with thionyl chloride in toluene gives the corresponding 2-chloromethyl thiophene **2a**. The

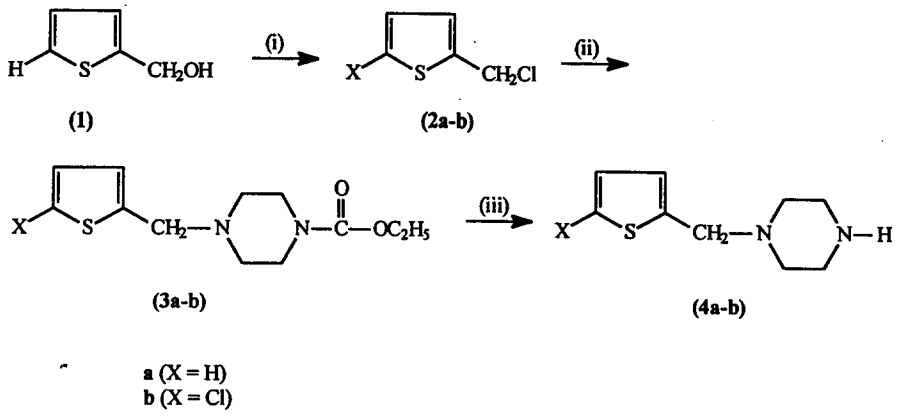



**Figure 1.** Reagents and conditions: (i) $SOCl_2$, toluene, 55 °C, 20′, μv; (ii) ethyl piperazine-1-carboxylate, $K_2CO_3$, NaI, DMF, reflux, 1 h, μv; (iii) KOH, water, methanol, reflux, 1 h, μv.

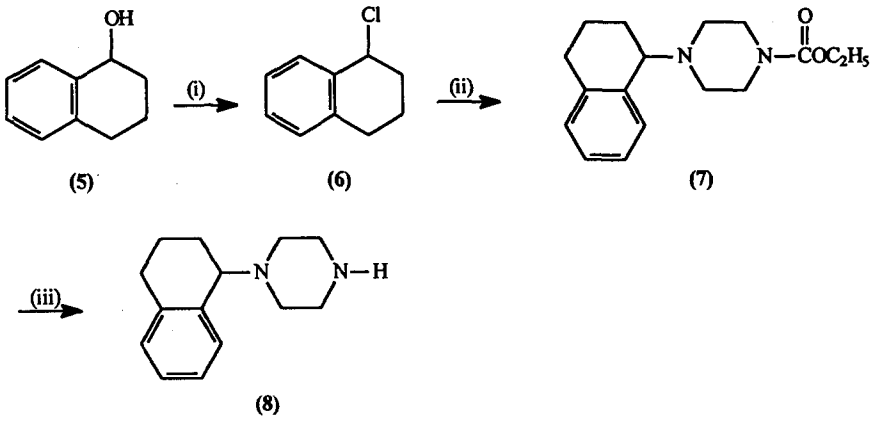



**Figure 2.** Reagents and conditions: (i) $SOCl_2$, toluene; (ii) ethyl piperazine-1-carboxylate, $K_2CO_3$, NaI, DMF, reflux, 70′, μv; (iii) KOH, water, methanol, reflux, 1 h, μv.

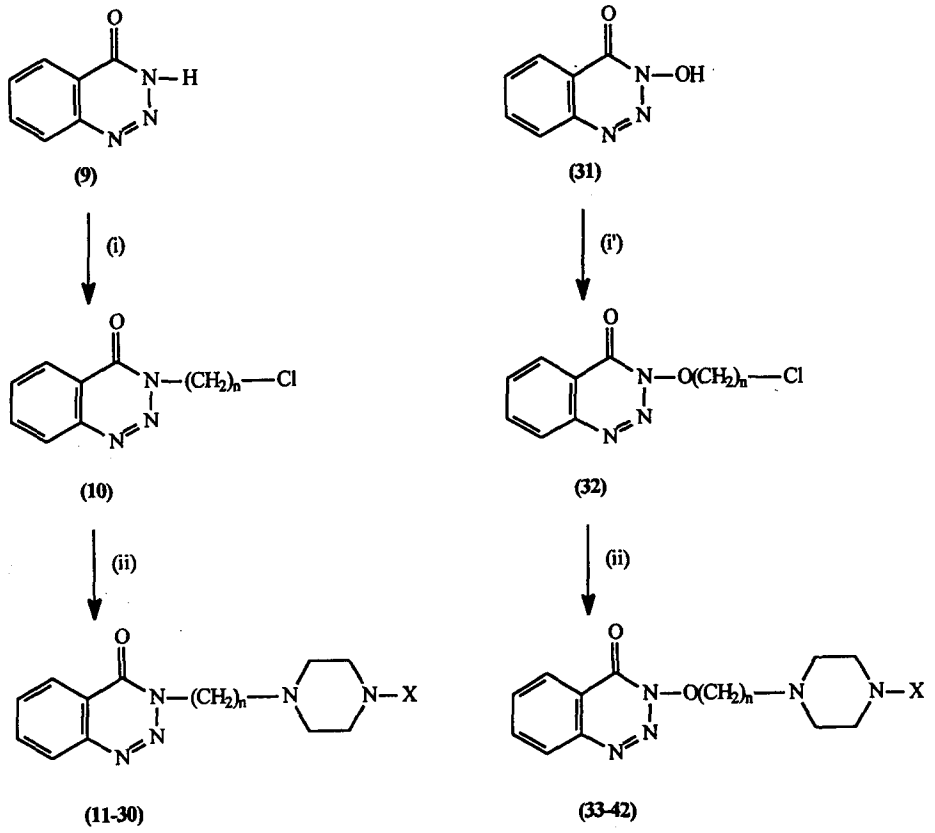



**Figure 3.** Reagents and conditions: (i) $Br(CH_2)_nCl$, $K_2CO_3$, DMF, 30′, μv; (i′) $Br(CH_2)_nCl$, NaOH, absolute ethanol, 30′, μv; (ii) 4-X-substituted-piperazine, $K_2CO_3$, NaI, DMF, 70′, μv.

intermediate **2b** was commercially available. The condensation of ethyl piperazine-1-carboxylate with the corresponding **2a** or **2b** gives, respectively, the intermediates **3a** and **3b**. Saponification and decarboxylation provided the desired intermediates **4a** and **4b**.

Intermediate 1-(1,2,3,4-tetrahydronaphth-1-yl)-piperazine (**8**) was obtained by using the same condition, which was employed for the preparation of

compounds **4a–b** starting from 1,2,3,4-tetrahydro-1-naphthol (**5**) with thionyl chloride in toluene to give the corresponding 1,2,3,4-tetrahydro-1-chloronaphthyl (**6**). The condensation of ethyl piperazine-1-carboxylate with **6**, followed by saponification and decarboxylation, provided desired compound **8** (*figure 2*). The two enantiomers were obtained as reported in literature [29] from the (+/−)1-(1,2,3,4-tetrahydronaphthyl) piperazine (**8**). The synthesis of final 1,2,3-benzotriazinone (**11–30**) and 3-hydroxy-1,2,3-benzotriazinone (**33–42**) derivatives was summarised in *figure 3*. The general procedure is as follows: alkylation of starting heterocycle 1,2,3-benzotriazin-4(3H)-one (**9**) with 1-bromo-2-chloroetane, 1-bromo-3-chloropropane, or 1-bromo-4-chlorobutane and $K_2CO_3$ in DMF or 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (**31**) with 1-bromo-2-chloroetane or 1-bromo-3-chloropropane in presence of NaOH in absolute ethanol gave the general corresponding cloroalkyl benzotriazinone derivatives **10** and **32**.

Condensation reaction of the obtained compounds **10** or **32** with desired 4-X-substituted-piperazines was performed in DMF in the presence of $K_2CO_3$, NaI, under reflux to give, respectively, the final compounds **11–30** and **33–42**.

The synthesised products were transformed into the corresponding hydrochloride salts using dry HCl in anhydrous diethyl ether. The associated physical data of final compounds were reported in *tables I and II*. The parameters of time, power, temperature used for the irradiation and conditions used by conventional heating were as reported in *table III*. Analytical purification of each final product was obtained by chromatography on silica gel column and further by crystallisation from the appropriate solvent. All new compounds gave satisfactory elemental analyses (C, H, Cl, N, S) and were characterised by $^1$H-NMR and mass spectrometry (LCQ–MS Thermoquest-Ion trap). $^1$H-NMR and MS data for all final compounds were consistent with the proposed structures.

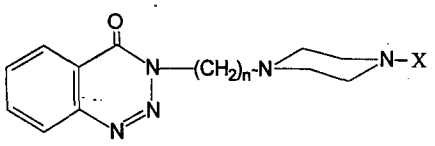
## 3. Pharmacology

The compounds (**11–30**) and (**33–42**) reported in *tables I and II* were tested for their in vitro affinity on serotonin 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors by radioligand binding assays. The more active compounds on serotonin receptors have been selected and evaluated for their affinity on dopaminergic ($D_1$ and $D_2$) and adrenergic ($\alpha_1$ and $\alpha_2$) receptors. All the compounds were tested as water-soluble, hydrochloride salts. The following specific radioligands and tissue sources were used, (a) serotonin 5-$HT_{1A}$ receptors, [$^3$H]-8-OH–DPAT, rat cerebral cortex membranes; (b) serotonin 5-$HT_{2A}$ receptors, [$^3$H]ketanserin, rat frontal cortex membranes; (c) serotonin 5-$HT_{2C}$ receptors, [$^3$H]mesulergine, rat frontal cortex membranes; (d) dopamine $D_1$ receptors [$^3$H]SCH-23390, rat strial membranes; (e) dopamine $D_2$ receptors [$^3$H]spiperone, rat strial membranes; (f) $\alpha_1$ adrenergic receptors [$^3$H]prazosin, rat brain cortex membranes; (g) $\alpha_2$ adrenergic receptors [$^3$H]-UK, rat brain cortex membranes. Concentrations required to inhibit 50% of radioligand specific binding ($IC_{50}$) were determined through four independent experiments with samples in triplicate using seven to nine different concentrations of the titled compound. Specific binding, defined as described in the Section 5, represented more than 75% of total binding in all three assays. The $K_i$ (nM) values were calculated from the Cheng–Prusoff equation [30] $K_i = IC_{50}/1+(\text{ligand}/K_d)$ and listed in *table IV*. ($K_d$ for [$^3$H]-8-OH-DPAT, [$^3$H]ketanserin, [$^3$H]SCH-23390, [$^3$H]spiperone, [$^3$H]-Prazosin and [$^3$H]-UK were respectively 0.8, 0.85, 0.20, 0.25, 0.12 and 0.91 nM).

## 4. Results and discussion

Thirty benzotriazinone derivatives (**11–30** and **33–42**) were synthesised under microwave irradiation and were obtained in higher yields (80–94%) and cleaner reactions than those obtained by conventional heating. The overall reaction times were dramatically reduced from 24 h to 70 min. Analogous results were obtained for all intermediates (*table III*). The prepared benzotriazinone derivatives were then evaluated in vitro, in a preliminary binding screening, for their affinity for 5-$HT_{1A}$ receptors and their selectivities were compared with those of two other serotonin receptors, 5-$HT_{2A}$ and 5-$HT_{2C}$. Affinity constants ($K_i$ (nM)) for three subtypes receptors 5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2C}$, have been determined and compared with those for ligands 8-OH–DPAT, Ketanserine and Mesulergine. Products showing a $K_i$ values $<10^5$ for at least one of the three examined receptors in the preliminary binding screening are reported in *table IV*, whereas products exhibiting $K_i$ (nM) values $>10^5$ for all receptors were considered inactive and were not reported in the *table IV*.

**Table I.** Physicochemical properties of 1,2,3-benzotriazinone derivatives (**11–30**).



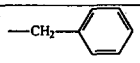
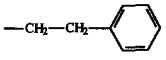
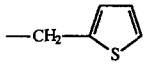
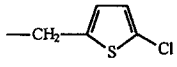
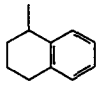
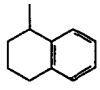
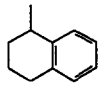
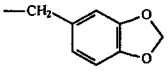
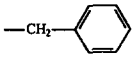
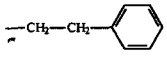
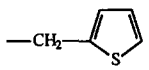
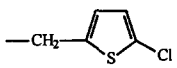
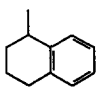
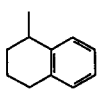
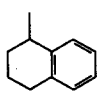
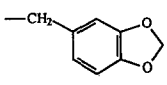
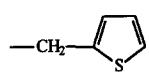
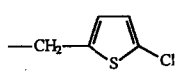
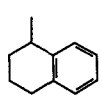
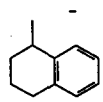
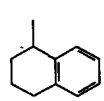
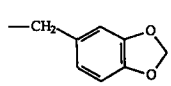
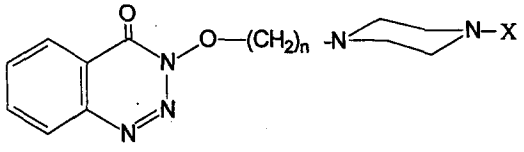
| Compound | X | n | Formula [a] | M.p. (°C) | Cryst. solvent [b] | Yield [c] % |
|---|---|---|---|---|---|---|
| **11** |  | 2 | $C_{19}H_{24}N_5O \cdot HCl$ | 248-252 | a+b | 92 |
| **12** |  | 2 | $C_{21}H_{25}N_5O \cdot 2HCl$ | 251-252 | a+b | 90 |
| **13** |  | 2 | $C_{18}H_{20}N_6O \cdot HCl$ | 243-244 | a+b | 80 |
| **14** |  | 2 | $C_{17}H_{19}N_7O \cdot HCl$ | 247-248 | c | 88 |
| **15** |  | 2 | $C_{18}H_{21}N_5OS \cdot HCl$ | 235-237 | a+b | 82 |
| **16** |  | 2 | $C_{18}H_{20}ClN_5OS \cdot HCl$ | 201-203 | a+b | 80 |
| **(±) 17** |  | 2 | $C_{23}H_{27}N_5O \cdot HCl$ | 204-206 | a+b | 84 |
| **(-) 17** [d] |  | 2 | $C_{23}H_{27}N_5O \cdot HCl$ | 208-210 | a+b | 82 |
| **(+) 17** [d] |  | 2 | $C_{23}H_{27}N_5O \cdot HCl$ | 206-208 | a+b | 80 |
| **18** |  | 2 | $C_{21}H_{23}N_7O_3 \cdot HCl$ | 109-110 | a+b | 80 |
| **19** |  | 3 | $C_{21}H_{26}ClN_5O \cdot HCl$ | 201-202 | a+b | 84 |
| **20** |  | 3 | $C_{22}H_{27}N_5O \cdot 2HCl$ | 210-211 | a+b | 82 |
| **21** |  | 3 | $C_{19}H_{22}N_6O \cdot HCl$ | 224-225 | a+b | 86 |

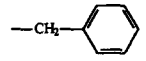
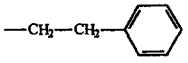
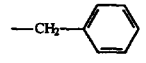
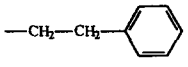
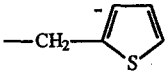
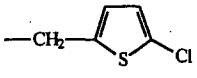
**Table I.** (*Continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| **22** |  | 3 | $C_{18}H_{21}N_7O \cdot HCl$ | 220-221 | c | 82 |
| **23** |  | 3 | $C_{19}H_{23}N_5OS \cdot HCl$ | 199-201 | a+b | 85 |
| **24** |  | 3 | $C_{19}H_{22}ClN_5OS \cdot HCl$ | 213-215 | a+b | 84 |
| **(±) 25** |  | 3 | $C_{24}H_{29}N_5O \cdot HCl$ | 200-205 | a+b | 80 |
| **(-) 25[d]** |  | 3 | $C_{24}H_{29}N_5O \cdot HCl$ | 207-208 | a+b | 82 |
| **(+) 25[d]** |  | 3 | $C_{24}H_{29}N_5O \cdot HCl$ | 202-204 | a+b | 85 |
| **26** |  | 3 | $C_{22}H_{25}N_5O_3 \cdot HCl$ | 200-205 | a+b | 80 |
| **27** |  | 4 | $C_{20}H_{25}N_5OS \cdot HCl$ | 256-258 | a+b | 80 |
| **28** |  | 4 | $C_{20}H_{24}ClN_5OS \cdot HCl$ | 230-2323 | a+b | 90 |
| **(±) 29** |  | 4 | $C_{25}H_{31}N_5O \cdot HCl$ | 213-215 | a+b | 88 |
| **(-) 29[d]** |  | 4 | $C_{25}H_{31}N_5O \cdot HCl$ | 213-214 | a+b | 90 |
| **(+) 29[d]** |  | 4 | $C_{25}H_{31}N_5O \cdot HCl$ | 208-210 | a+b | 85 |
| **30** |  | 4 | $C_{23}H_{27}N_5O_3 \cdot HCl$ | 261-262 | a+b | 90 |

[a] Satisfactory microanalyses obtained: C, H, Cl, N,S values are within ±0.4% of the theoretical values.
[b] Cryst. solvent: a=dietyl ether; b=ethyl alcohol; c=methyl alcohol.
[c] Yields related to last step are referred to microwave irradiation.
[d] (-) **17** :$[\alpha]^{25}_D$ = - 5.80 ° (c = 0.01 , MeOH); (+) **17**:$[\alpha]^{25}_D$ = + 4.12° (c = 0.01, MeOH);
(-) **25**:$[\alpha]^{25}_D$ = - 6.69° (c = 0.01, MeOH); (+) **25**:$[\alpha]^{25}_D$ = + 4.19° (c = 0.01, MeOH);
(-) **29**:$[\alpha]^{25}_D$ = - 8.31° (c = 0.01 , MeOH); (+) **29**:$[\alpha]^{25}_D$ = + 6.11° (c = 0.01, MeOH).

**Table II.** Physicochemical properties of 3-hydroxy-1,2,3-benzotriazinone derivatives (**33-42**).



| Compound | X | n | Formula[a] | M.p. (°C) | Cryst. solvent[b] | Yield[c] % |
|---|---|---|---|---|---|---|
| **33** |  | 2 | $C_{20}H_{24}N_5O_2 \cdot HCl$ | 212-213 | a+b | 92 |
| **34** |  | 2 | $C_{21}H_{27}N_5O_2 \cdot HCl$ | 185-186 | a+b | 90 |
| **35** |  | 2 | $C_{18}H_{20}N_6O_2 \cdot HCl$ | 194-195 | a+b | 94 |
| **36** |  | 2 | $C_{17}H_{19}N_7O_2 \cdot HCl$ | 214-215 | c | 85 |
| **37** |  | 3 | $C_{21}H_{25}N_5O_2 \cdot 2HCl$ | 219-220 | a+b | 85 |
| **38** |  | 3 | $C_{22}H_{27}N_5O_2 \cdot 2HCl$ | 230-231 | a+b | 87 |
| **39** |  | 3 | $C_{19}H_{22}N_6O_2 \cdot HCl$ | 189-190 | a+b | 90 |
| **40** |  | 3 | $C_{18}H_{21}N_7O_2 \cdot HCl$ | 184-185 | c | 92 |
| **41** |  | 3 | $C_{19}H_{23}N_5O_2S \cdot HCl$ | 203-205 | a+b | 87 |
| **42** |  | 3 | $C_{19}H_{22}ClN_5O_2S \cdot HCl$ | 198-200 | a+b | 88 |

[a] Satisfactory microanalyses obtained: C, H, Cl, N values are within ±0.4% of the theoretical values.
[b] Cryst. solvent: a=dietyl ether; b=ethyl alcohol; c=methyl alcohol.
[c] Yields related to last step are referred to microwave irradiation.

In this work, we studied (a) the influence of the modification of the aromatic heterocycle; (b) the length of the alkyl side chain ($n$); (c) the substitutions on the piperazine moiety (X); and (d) the stereochemistry of the tetrahydronaphthyl compounds.

(a) Regarding the modification of the aromatic heterocycle, the replacement of 1,2,3-benzotriazinone (compounds **11–30**) with its analogue 3-hydroxy-1,2,3-benzotriazinone (compounds **33–42**) has led to the same binding profile. The 5-$HT_{1A}$ affinities obtained are quite similar.

(b) The influence of the alkyl side chain ($n$) on the results reported in *table IV* showed that generally highest affinity for 5-$HT_{1A}$ receptors appeared when $n = 4$ for the serie of 1,2,3-benzotriazinone and $n = 3$ for the serie of 3-hydroxy-1,2,3-benzotriazinone. These results agreed with those obtained for the compound in a previous work [25]

in which more activity was showed by compounds in which the distance between the nitrogen of the 3-hydroxy-1,2,3-benzotriazinone ring and the nitrogen of the piperazine ring was $n = 3$. The analogues $n = 2$ for the both series present a low affinity.

(c) Concerning the influence of the substituent on the N-4 of piperazine moiety, the highest affinity were obtained for the series **11–30** with the presence of the tetrahydronaphthyl nucleus ($n = 4$ compounds (±) **29**, (−) **29**, (+) **29**) and for the series **33–42** with the presence of the pyridine ring ($n = 3$ compound **39**). The results showed that the compounds supporting on N-4 of the piperazine moiety a 2-thienyl methyl-5-X ring did not present any affinity for the selected receptors ex-

**Table III.** Conventional heating versus microwave irradiation for intermediates (**2a**, **3a–b**, **4a–b**, **6**, **7**, **8**, **10**, **32**) and final compounds (**11–30** and **33–42**).

| Compound | Conventional heating [a] | | | Microwave irradiation | | | |
|---|---|---|---|---|---|---|---|
| | Yield (%) | Time (min) | Temperature (°C) | Yield (%) | Time (min) | Power (W) | Temperature (°C) |
| **2** | 85 | 60 | 55 | 96 | 5 | 80 | 55 |
| | | | | | 10 | 400 | |
| | | | | | 5 | 200 | |
| **3a** | 54 | 24 h | reflux | 80 | 5 | 80 | 80 |
| | | | | | 50 | 400 | 120 |
| | | | | | 5 | 200 | 110 |
| **3b** | 63 | 24 h | reflux | 92 | 5 | 80 | 80 |
| | | | | | 50 | 400 | 120 |
| | | | | | 5 | 200 | 110 |
| **4a** | 74 | 12 h | reflux | 95 | 5 | 80 | 70 |
| | | | | | 50 | 400 | 90 |
| | | | | | 5 | 200 | 80 |
| **4b** | 63 | 12 h | reflux | 90 | 5 | 80 | 70 |
| | | | | | 50 | 400 | 90 |
| | | | | | 5 | 200 | 80 |
| **6** | 87 | 60 | 55 | 98 | 5 | 80 | 55 |
| | | | | | 10 | 400 | |
| | | | | | 5 | 200 | |
| **7** | 63 | 24 h | reflux | 90 | 5 | 80 | 80 |
| | | | | | 60 | 400 | 120 |
| | | | | | 5 | 200 | 110 |
| **8** | 84 | 12 h | reflux | 95 | 5 | 80 | 70 |
| | | | | | 50 | 400 | 90 |
| | | | | | 5 | 200 | 90 |
| **10** | 72–85 [b] | 180 | 80 | 90–96 [b] | 5 | 80 | 80 |
| | | | | | 20 | 400 | 120 |
| | | | | | 5 | 200 | 110 |
| **11–30** | 44–65 [b] | 24 h | reflux | 80–92 [b] | 5 | 80 | 80 |
| | | | | | 60 | 400 | 120 |
| | | | | | 5 | 200 | 110 |
| **32** | 60–70 [b] | 180 | 80–94 [b] | 5 | 80 | | |
| | | | | | 20 | 400 | 55 |
| | | | | | 5 | 200 | |
| **33–42** | 35–60 [b] | 24 h | reflux | 85–94 [b] | 5 | 80 | 80 |
| | | | | | 60 | 400 | 120 |
| | | | | | 5 | 200 | 110 |

The experimental conditions used on microwave irradiation were similar to those used by conventional heating, with the same amount of starting reagent and volume of solvent. More details on the experimental conditions of the reactions are reported in Section 5.

[a] Oil bath.

[b] Ranging between the reported percentage.

**Table IV.** Binding affinities of active 1,2,3-benzotriazinone (**11–30**) and 3-hydroxy-1,2,3-benzotroazinone (**33–42**) derivatives.

| Compound | Receptor affinity $K_i$ (nM) [a] | | |
|---|---|---|---|
| | 5-$HT_{1A}$ [$^3$H]8–OH–DPAT | 5-$HT_{2A}$ [$^3$H]ketanserin | 5-$HT_{2C}$ [$^3$H]mesulergine |
| **12** | $>10^5$ | 1300 ± 150 | $>10^5$ |
| **13** | 1600 ± 158 | $>10^5$ | $>10^5$ |
| **15** | 4000 ± 350 | $>10^5$ | $>10^5$ |
| (±) **17** | 380 ± 26 | $>10^5$ | $>10^5$ |
| (−) **17** | 500 ± 42 | $>10^5$ | $>10^5$ |
| (+) **17** | 1000 ± 90 | $>10^5$ | $>10^5$ |
| **19** | 2500 ± 270 | $>10^5$ | $>10^5$ |
| **20** | $>10^5$ | 2500 ± 260 | $>10^5$ |
| **21** | 350 ± 38 | 850 ± 90 | 1700 ± 160 |
| **22** | 680 ± 63 | 5000 ± 580 | 4000 ± 520 |
| **23** | 6000 ± 500 | $>10^5$ | $>10^5$ |
| (±) **25** | 300 ± 27 | 2750 ± 250 | $>10^5$ |
| (−) **25** | 450 ± 30 | $>10^5$ | $>10^5$ |
| (+) **25** | 980 ± 70 | 2000 ± 180 | 5000 ± 480 |
| **27** | 450 ± 38 | $>10^5$ | $>10^5$ |
| **28** | 900 ± 78 | $>10^5$ | $>10^5$ |
| (±) **29** | 60 ± 5 | 3000 ± 270 | $>10^5$ |
| (−) **29** | 36 ± 4 | $>10^5$ | $>10^5$ |
| (+) **29** | 103 ± 6 | 3000 ± 240 | $>10^5$ |
| **30** | 330 ± 35 | 2000 ± 220 | $>10^5$ |
| **34** | $>10^5$ | 2800 ± 255 | 7500 ± 660 |
| **35** | 3300 ± 290 | $>10^5$ | $>10^5$ |
| **37** | 650 ± 62 | 6000 ± 560 | $>10^5$ |
| **38** | 1300 ± 110 | 2000 ± 190 | 5000 ± 520 |
| **39** | 55 ± 4 | 2500 ± 240 | 2000 ± 180 |
| **40** | 660 ± 52 | $>10^5$ | $>10^5$ |
| **41** | 2200 ± 200 | $>10^5$ | $>10^5$ |
| **42** | 1500 ± 120 | $>10^5$ | $>10^5$ |

[a] $K_i$ value followed by S.E.M. For purpose of comparison, 8-OH–DPAT, Ketanserine and Mesulergine binds at 5-$HT_{1A}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors with $K_i$ values of 0.80, 0.85 and 1.90 nM, respectively, under these assay conditions.

cept **27** and **28** ($n = 4$) which showed a moderate affinity but high selectivity versus 5-$HT_{1A}$ receptor subtype. The tested 1,2,3-benzotriazinone derivatives **11**, **14**, **16**, **18**, **24**, **26** and the tested 3-hydroxy-1,2,3-benzotriazinone derivatives **33** and **36** were inactive ($K_i > 10^5$ nM).

(d) Regarding the influence of the stereochemistry of the tetrahydronaphthyl compounds, the chiral resolution of the compound **17**, **25** and **29** was performed and enantiomers were tested on the binding screening. The racemate (+/−) and levo enantiomers(−) showed affinity for 5-$HT_{1A}$ receptor better than that of their dextro isomers.

The compound with higher affinity and selectivity for the 5-$HT_{1A}$ over all the considered receptors was the 3-{4-[4-(1,2,3,4-tetrahydronaphthyl)-1-piperazinyl]butan}-benzotriazinone (−)**29** (5-$HT_{1A}$ $K_i = 36$ nM, other receptors not active).

Only compounds **12**, **20** and **34**, supported on the N-4 piperazine moiety a phenylethyl residue showed no affinity binding on the 5-$HT_{1A}$ receptor subtype but exhibited a moderate affinity binding on the 5-$HT_{2A}$ subtype. The two classes of compounds (**11–30**) and (**33–42**) retained a good and similar selectivity for 5-$HT_{1A}$ in regard to 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors.

As mentioned in Section 1, the most active compounds of the serie 1,2,3-benzotriazinone (**11–30**) and 3-hydroxy-1,2,3-benzotriazinone (**33–42**) on 5-$HT_{1A}$ (**21**, **27**, **29**, **30** and **39**), were further evaluated for their affinity as dopaminergic ($D_1$ and $D_2$) and adrenergic ($\alpha_1$ and $\alpha_2$) receptors. As far as the dopaminergic system was concerned, the $D_1$ and $D_2$ receptor affinity, all the compounds showed $K_i$ (nM) values of above $10^5$ except compound **21**, which exhibited a $K_i$ value of 91 nM at the $D_2$ receptor. The affinity for adrenergic $\alpha_1$ and $\alpha_2$ receptors were quite moderate

only in two cases (**21** and **27**). In particular, compound **21** showed for only $\alpha_1$ receptor a $K_i$ value of 212 nM; compound **27** showed moderate $K_i$ values both for $\alpha_1$ and $\alpha_2$ receptor, respectively, of 240 and 375 nM. These preliminary structure–activity relationship studies showed that new structural modification led to new derivatives characterised by minor affinity and a high selectivity for 5-$HT_{1A}$ receptor.

## 5. Experimental protocols

### *5.1. Chemistry*

Synthesis was performed using a microwave oven ETHOS 1600, Milestone. The experiments were carried out at atmospheric pressure in standard Pyrex glassware with a reflux condenser fitted through the roof of the microwave cavity. The temperature of the stirred reaction mixture was monitored directly by a microwave-transparent fluoroptic probe inserted into the solution. Melting points (m.p.) were determined using a Kofler hot-stage apparatus and are uncorrected. Thin layer chromatography was performed on Merck Silica Gel 60 plates with fluorescent indicator and the plates were visualised with UV light (254 nm). Kieselgel 60 was used for column chromatography. Where analyses are indicated only by the symbols of the elements, results obtained are within ±0.4% of the theoretical values. The structure was verified spectroscopically by proton $^1$H-NMR. Spectra were recorded on Bruker AM-500 instruments. Chemical shifts are given as $\delta$ with references to $Me_4Si$ as internal standard. Mass spectra of the final products were performed on LCQ thermoquest-ion trap mass spectrometry. Optical rotation ($\alpha$) of the pure enantiomers was measured by a Perkin–Elmer 141 optical activity polarimeter. Anhydrous $Na_2SO_4$ was used as a drying agent for organic extraction throughout. Analytical data, m.p. and crystallisation solvents are reported in *tables I and II*. Compounds (+) **8** and (−) **8** were obtained from (+/−)**8** using the same procedure as reported in [29].

#### *5.1.1. 2-Chloromethyl thiophene (**2a**)*

Thionylchloride (26 mL, 0.35 mol) was added at 15 °C to a solution of 2-thiophenmethanol (**1**) (0.24 mol) in toluene (200 mL). The mixture was stirred at room temperature (r.t.) for 30 min and then introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. The mixture was cooled, washed twice with ice-water, dried, and evaporated to give **2a** as an oil (yield 96%), which was used without further purification in the next step.

#### *5.1.2. Ethyl 4-[(5-X-thien-2-yl) methyl]-piperazine-1-carboxylate (**3a–b**)*

A mixture of 2-chloromethyl-5-X-thiophene (**2a** or **2b**) (0.65 mol), ethyl piperazine-1-carboxylate (0.79 mol), potassium carbonate (1.43 mol), and sodium iodide (0.06 mol) in 600 mL of DMF was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling, the mixture was filtered and the solvent was evaporated off. The residual oil was taken up with ice water, and the product was extracted three times with ethyl acetate. A solution of 2 N hydrogen chloride was then added. The hydrochloride formed was filtered off. The base was displaced from its salt with sodium carbonate in a dichloromethane–water mixture. The organic layer was separated, dried, and evaporated under vacuum to give respectively **3a** (yield 80%) or **3b** (yield 92%) as an oil; (**3a**) $^1$H-NMR ($CDCl_3$) $\delta$ 1.23 (t, 3H, $J = 7$ Hz), 2.38 (s, NH), 2.42 (m, 4H), 2.80 (m, 4H), 3.52 (d, 2H, $J = 7.5$ Hz), 4.10 (q, 2H, $J = 7$Hz), 6.81 (d, 1H, $J = 7.5$ Hz), 6.84 (t, 1H, $J = 7.5$ Hz), 7.12 (t, 1H, $J = 7.5$ Hz). Similar $^1$H-NMR occur in intermediate **3b**.

#### *5.1.3. 1-[(5-Thien-2-yl) methyl]piperazine (**4a–b**)*

Potassium hydroxide (5.36 mol) was slowly added into the reaction vessel containing a solution of 1-(2-thienylmethyl-5-X)-4-carbetoxypiperazine (**3a** or **3b**) (0.46 mol), water (100 mL), and methanol (400 mL). The desired parameters (microwave power, temperature and time) were set as reported in *table III*. The reaction was monitored by thin layer chromatography. After the starting material had disappeared, the mixture was cooled, filtered, and extracted with dichloromethane. The organic layer was separated, dried, and evaporated to give respectively **4a** (yield 95%) or **4b** (yield 90%) as an oil; (**4a**) $^1$H-NMR($CDCl_3$) $\delta$ 2.41(s, NH), 2.48 (m, 4H), 2.84 (m, 4H), 3.60 (d, 2H, $J = 7.5$ Hz), 6.83 (d, 1H, $J = 7.5$ Hz), 6.86 (t, 1H, $J = 7.5$ Hz), 7.15 (t, 1H, $J = 7.5$ Hz). Similar $^1$H-NMR occur in intermediate **4b**.

#### *5.1.4. 1-Chloro-1,2,3,4-tetrahydronaphthalene (**6**)*

Thionylchloride (26 mL, 0.50 mol) was added at 15 °C to a solution of 1,2,3,4-tetrahydro-1-naphthol (**5**) (0.36 mol) in toluene (300 mL). The mixture was stirred

at r.t. for 30 min and then introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. The mixture was cooled, washed twice with ice water, dried, and evaporated to give **6** as an oil (yield 98%), which was used without further purification in the next step.

*5.1.5. Ethyl 4-(1,2,3,4-tetrahydro-1-naphth-1-yl)-piperazine-1-carboxylate (7)*

A mixture of 1,2,3,4-tetrahydro-1-chloronaphthyl (**6**) (0.85 mol), ethyl piperazine-1-carboxylate (1.0 mol), potassium carbonate (1.87 mol), and sodium iodide (0.06 mol) in 600 mL of DMF was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling, the mixture was filtered and the solvent was evaporated off. The residual oil was taken up with ice water, and the product was extracted three times with ethyl acetate. A solution of 2 N hydrogen chloride was then added. The hydrochloride formed was filtered off. The base was displaced from its salt with sodium carbonate in a dichloromethane–water mixture. The organic layer was separated, dried, and evaporated under vacuum to give **7** as an oil (yield 90%); $^1$H-NMR ($CDCl_3$) $\delta$ 1.25 (t, 3H, $J = 7$ Hz), 1.60–2.10 (m, 4H), 2.31–2.90 (m, 6H), 3.50 (t, 4H, $J = 6$ Hz), 3.82 (t, 1H, $J = 7$ Hz), 4.15 (q, 2H, $J = 7$ Hz), 7.00–7.75 (m, 4H).

*5.1.6. (±) 1-(1,2,3,4-Tetrahydronaphth-1-yl) piperazine (8)*

Potassium hydroxide (7.00 mol) was slowly added into the reaction vessel containing a solution of ethyl 4-(1,2,3,4-tetrahydronaphth-1-yl)piperazine-1-carboxylate (**7**) (0.60 mol), water (100 mL), and methanol (400 mL). The desired parameters (microwave power, temperature and time) were set as reported in *table III*. The reaction was monitored by thin layer chromatography. After the starting material had disappeared, the mixture was cooled, filtered, and extracted with dichloromethane. The organic layer was separated, dried, and evaporated. The residual oil was taken up with ethanol, and oxalic acid (0.57 mol) dissolved in ethanol was added. The oxalate formed was filtered off, and the base was displaced from its salt with sodium carbonate in a dichloromethane–water mixture. The organic layer was dried, and the solvent was evaporated under vacuum to give an oil **8** (yield 95%); $^1$H-NMR ($CDCl_3$) $\delta$ 1.50–2.10 (m, 4H), 2.00 (s, NH), 2.41–2.80 (m, 6H), 2.90 (t, 4H, $J = 6$ Hz), 3,75 (t, 1H, $J = 7$ Hz), 7.02–7.80 (m, 4H).

*5.1.7. 3-(n-Chloroalkyl)-1,2,3-benzotriazin-4(3H)-one (10)*

A mixture of 1-bromo-2-chloroethane or 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane (0.09 mol), 1,2,3-benzotriazin-4H-one (**9**) (0.03 mol), $K_2CO_3$ (0.05 mol) in DMF (70 mL) was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling the mixture was concentrated to dryness and the residue was dissolved in water (50 mL); the solution was extracted several times with $CH_2Cl_2$. The organic phase was dried, concentrated and chromatographed by column of silica gel (ether/methanol, 9:1 v/v) to give the general compound **10** as a solid (yields ranging between 90 and 96%). $^1$H-NMR spectra for all intermediates were consistent with the proposed structures.

*5.1.8. General procedure for compounds 11–30*

The synthesis of 3-{2-[4-(benzyl)-1-piperazinyl]ethyl]}-benzotriazinone (**11**) is typical; a mixture of **11** (0.03 mol), *N*-benzylpiperazine (0.03 mol) and NaI (0.05 mol), $K_2CO_3$ (0.05 mol) in DMF (50 mL) was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling, the mixture was concentrated to dryness and the residue was dissolved in water (50 mL). The solution was extracted several times with $CH_2Cl_2$. The organic phase was dried, concentrated and chromatographed by column of silica gel (ether–ethanol 9:1 v/v) to give the final compound **11** as a white crystalline solid (yield 92%). $^1$H-NMR ($CDCl_3$) $\delta$ 2.40 (m, 4H), 2.70 (m, 4H), 2.90 (t, 2H, $J = 6.8$ Hz), 3.49 (s, 2H), ?4.60 (t, 2H, $J = 6.8$ Hz), 7.19–7.29 (m, 5H), 7.80 (t, 1H, $J = 7.6$ Hz), 7.95 (t, 1H, $J = 7.6$ Hz), 8.19 (d, 1H, $J = 8.1$ Hz), 8.37 (d, 1H, $J = 7.9$ Hz). $^1$H-NMR spectra for all final compounds were consistent with proposed structures.

*5.1.9. 3-(n-Chloroalkoxy-1,2,3-benzotriazin-4(3H)-one (32)*

A mixture of 1-bromo-2-chloroethane or 1-bromo-3-chloropropane (0.023 mol), 3-hydroxy-1,2,3-benzotriazin-4H-one (**31**) (0.023 mol), NaOH (0.023 mol) in absolute ethanol (50 mL) was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling, the mixture was concentrated to dryness and the residue was dissolved in water (40 mL). The solution was extracted several times with $CH_2Cl_2$.

The organic phase was dried, concentrated and chromatographed by column of silica gel (ether–methanol, 9:1 v/v) to give the general compound **32** as a solid (yields ranging between 80 and 94%).$^1$H-NMR spectra for all intermediates were consistent with the proposed structures.

*5.1.10. General procedure for compounds **33–42***

The synthesis of 3-{2-[4-(benzyl)-1-piperazinyl]-ethyloxy]}benzotriazinone (**33**) is typical; a mixture of **32** (0.03 mol), *N*-benzylpiperazine (0.03 mol), NaI (0.010 mol) and $K_2CO_3$ (0.05 mol) in DMF (50 mL) was introduced into the reaction vessel and the desired parameters (microwave power, temperature and time) were set as reported in *table III*. After cooling, the mixture was concentrated to dryness and the residue was dissolved in water (50 mL). The solution was extracted several times with $CH_2Cl_2$. The organic phase was dried, concentrated and chromatographed by column of silica gel (ether–ethanol 9:1 v/v) to give the final compound **33** as a white crystalline solid (yield 92%) $^1$H-NMR ($CDCl_3$) $\delta$ 2.33 (m, 4H), 2.56 (m, 4H), 2.91 (t, 2H, $J = 6.8$ Hz), 3.39 (s, 2H), 4.57 (t, 2H, $J = 6.8$ Hz), 7.23–7.30 (m, 5H), 7.97 (t, 1H, $J = 7.6$ Hz), 7.81 (t, 1H, $J = 7.6$ Hz), 8.20 (d, 1H, $J = 8.1$ Hz), 8.38 (d, 1H, $J = 7.9$ Hz). $^1$H-NMR spectra for all final compounds (**33–42**) were consistent with the proposed structures.

*5.2. Pharmacology*

*5.2.1. 5-HT$_{1A}$ binding assay*

Radioligand binding assay was performed following a published procedure [31]. Cerebral cortex from male Wistar rats (150–200 g) was homogenised in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) with a Brinkmann Polytron (setting 5 for 15 s), and the homogenate was centrifuged at 48 000×*g* for 10 min. The resulting pellet was then resuspended in the same buffer, incubated for 10 min at 37 °C, and centrifuged at 48 000×*g* for 10 min. The final pellet was resuspended (8 mg wet weight) in 250 μL of 50 mM Tris–HCl, pH 7.7 containing 1 nM [$^3$H]-8-OH–DPAT ([$^3$H]-8-OH–DPAT), membranes from 8 mg (wet weight) of tissue and the compounds to be tested. The tubes were incubated for 30 min at 25 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters. The filters were washed twice with 5 mL of ice-cold Tris–HCl buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [$^3$H]-8-OH–DPAT binding was defined as the difference between binding in the absence and presence of 5-HT (1 μM).

*5.2.2. 5-HT$_{2A}$ and 5-HT$_{2C}$ binding assays*

Radioligand binding assays were performed as previously reported by Claudi et al. [32]. Briefly, frontal cortical regions of male Wistar rats (150–200 g, Charles River) were dissected on ice, homogenised in ice-cold buffer solution (50 mM Tris–HCl, pH 7.7, at 25 °C) and centrifuged at 40 000×*g* for 10 min. The supernatant was discarded and the pellet was resuspended in the same volume of Tris–HCl buffer and incubated at 37 °C for 10 min prior to a second centrifugation. Binding experiments with [$^3$H]ketanserin (76 Ci mmol$^{-1}$; New England Nuclear) and [$^3$H]mesulergine (75.8 Ci mmol$^{-1}$; Amersham) were performed in 250 μL of buffer, which contained 1 nmol [$^3$H]ketanserin or [$^3$H]mesulergine, membranes from 10 mg (wet weight) of tissue and the compounds to be tested. After 30 min of incubation at 25 °C, separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B glass fibre filters, which were washed three times with ice-cold buffer, dried and counted in 5 mL of Aquassure (Packard, Downers Grove, USA). Non specific binding was measured in the presence of 10 μM 5-HT for 5-HT$_{2A}$ sites and 10 μM cinanserin for 5-HT$_{2C}$ sites with specific binding defined as the total binding minus the non-specific binding.

*5.2.3. D$_1$ and D$_2$ dopaminergic binding assay*

The binding assay for $D_1$ dopaminergic receptors was that described by Trampus et al. [33]. Corpora striate were homogenised in 100 vol. (w/v) ice cold 50 mM Tris–HCl buffer (pH 7.4 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 10 min at 20 000×*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice cold Tris–HCl pH 7.4. containing (mM): 120 NaCl, 5 KCl, 2 $CaCl_2$ and 1 $MgCl_2$. Each assay tube contained [$^3$H]SCH-23390 and [$^3$H]spiperone to achieve a final concentration of 1 nM, and resuspended membranes (3 mg fresh tissue). The tubes were incubated for 45 min at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl buffer (pH 7.4 at 25 °C).The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [$^3$H]SCH-23390 and [$^3$H]spiperone binding was defined as the difference between binding in the absence or in the

presence of 10 μM SCH-23390 and 1 μM (+)-butaclamol, respectively.

*5.2.4. $\alpha_1$ adrenergic binding assay*

The procedure used in the radioligand binding assay has been reported in detail by Varani et al. [34]. Brain cortex was homogenised in 30 vol. (w/v) ice-cold 50 mM Tris–HCl buffer, (pH 7.4 at 25 °C) using a Polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged twice for 20 min at 40 000×*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl, 140 mM NaCl (pH 7.4 at 25 °C) containing [$^3$H]prazosin to achieve a final concentration of 1 nM, resuspended membranes from 5 mg fresh tissue and the compounds to be tested. The tubes were incubated for 1 h at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl, buffer (pH 7.4 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [$^3$H]prazosin binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

*5.2.5. $\alpha_2$ adrenergic binding assay*

Binding assays were performed essentially according to Varani et al [35]. Brain cortex was homogenised in 30 vol. (w/v) ice-cold 50 mM Tris–HCl, using a polytron PT10 (setting 5 for 20 s). Homogenates were centrifuged 20 min at 40 000×*g* with resuspension of the pellet in fresh buffer. The final pellet was resuspended in 50 mM ice-cold Tris–HCl, 0.5 mM EDTA, 10 mM $MgCl_2$ (pH 7.5 at 25 °C). Each assay tube contained [$^3$H]-UK to achieve a final concentration of 1 nM, resuspended membranes from 5 mg fresh tissue and the compounds to be tested. The tubes were incubated for 1 h at 25 °C and the incubation was terminated by rapid filtration under vacuum through Whatman GF/B glass fibre filters. The filters were washed three times with 5 mL ice-cold 50 mM Tris–HCl, 0.5 mM EDTA buffer (pH 7.5 at 25 °C). The radioactivity bound to the filters was measured by a liquid scintillation counter. Specific [$^3$H]-UK binding was defined as the difference between binding in the absence or in the presence of 10 μM phentolamine.

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