Full Paper

Synthesis and 5-HT$_{2A}$, 5-HT$_{1A}$ and α_1-Binding Affinities of 2-[2-Hydroxy-3-(pyridin-3-yl-methyl)amino]-, 2-[2-Hydroxy-3-(2-pyridin-2-yl-ethyl)amino]- and 2-[2-Hydroxy-3-(4-*N*-methyl-piperazin-1-yl)-amino]propoxybenzaldehyde-*O*-(substituted) Benzyl Oximes

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Some oxime ether-substituted aryloxypropanolamines **3**-**5**, structurally related to the active metabolite **2** of sarpogrelate **1**, were synthesized and tested for their affinities at 5-HT$_{2A}$ and 5-HT$_{1A}$ serotoninergic receptors as well as at the α_1-adrenoceptor. The results show that the compounds possess, at least partially, the ability of the model compounds **1** and **2** to interact with the 5-HT$_{2A}$-receptors; they have the same selectivity towards 5-HT$_{2A}$ receptors *vs* α_1-adrenoceptors.

Keywords: Benzyl oximes derivatives / Oximes ether-substituted aryloxypropanolamines / Sarpogrelate analogues / Serotoninergic and adrenergic activity

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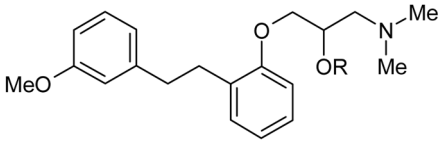
Introduction

Serotonin (5-HT) is a biogenic amine involved in different physiological functions, including that of inducing platelet activation and aggregation [1]. This effect is mediated by the activation of 5-HT$_{2A}$-receptors which are located on platelets, as well as centrally. Consequently, the antagonists of this 5-HT-receptor subtype may be considered to be good tools in the treatment of thrombosis [2] and cardiovascular disease [3, 4].

Sarpogrelate **1** (Fig. 1) is a 5-HT$_{2A}$-receptor antagonist now clinically available in Japan for the treatment of the peripheral arterial disease [5 – 7]. Starting from the observation that also the hydrolysis product of sarpogrelate (M-1, **2**; Fig. 1) is a potent 5-HT$_{2A}$-receptor antagonist, some compounds structurally related to M-1 were developed [8, 9], in which different substituents were tested on the aminic nitrogen and the aryloxy group. As a result, it was shown that these molecular portions play important, albeit at times unexpected, roles in determining the 5-HT$_{2A}$ affinity level of the new compounds. Furthermore, despite the broad variety of substituents tested, to our knowledge, less attention has been dedicated to the effects on the affinity of substituents on the aminic nitrogen bearing a hetero aromatic portion; analogously, from literature data no studies appear to have been carried out on analogues of M-1 in which its 3-methoxyphenylethyl group is replaced by a more complex molecular



Sarpogrelate 1 : R = $CO(CH_2)_2COOH$
M-1 2 : R = H

Figure 1. Structure of compounds **1** and **2**.

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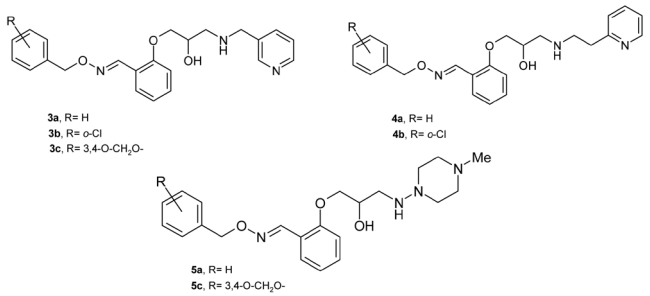
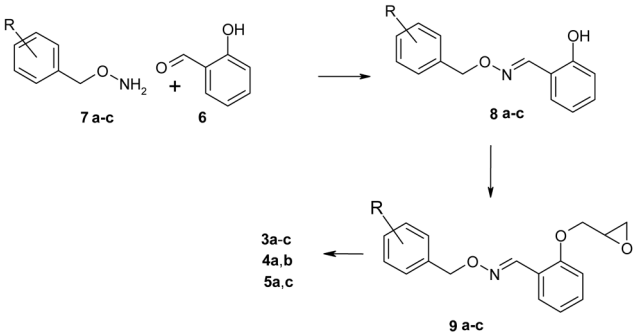


Figure 2. Structure of compounds **3–5**.



a: R= H; **b**: R= *o*-Cl; **c**: R= 3,4-OCH_2O-,

Scheme 1. Synthesis of amino alcohols **3a–c**, **4a, b** and **5a, c**.

portion possessing conformational freedom different from that of an aliphatic chain.

On the basis of these considerations and with the aim of getting further information about the structural requirements for 5-HT_{2A} affinity, we designed and synthesized some compounds of types **3** and **4** which can be viewed as analogues of M-1 **2**, in which the substituent on amino nitrogen presents a hetero aromatic portion such as that 3-methylpyridinylic or 2-ethylpyridinylic, respectively. In addition, in order to explore the effects on the 5-HT_{2A} affinity of a new type of *N*-substitution, also **5a** and **5c** were synthesized, in which the alkylpyridine of **3a**, **c** and **4a, b** are replaced by a 1-amino-4-methyl piperazine moiety.

Moreover, in these new compounds **3–5** (Fig. 2) the ethylene linker between the two aromatic portion of **1** and **2** is substituted by a hetero atomic spacer C–O–N=C– with the *E* configuration around the oxime double bond. This molecular portion has been successfully used in the design of drugs of other classes [10–13] and allowed us to obtain new compounds endowed with a good biological activity. This new linker which differs from the starting one in the steric and electronic characteristics might interact with the receptor through a hydrogen bond or might direct the aryl group in a different manner with respect to the linker present in the model compounds **1** and **2**.

All new compounds were tested for their binding affinity to 5-$HT_{2A,}$ $5HT_{1A}$ and α_1 receptors, to verify how the structural modifications can modulate the affinity towards these receptors.

Chemistry

Aminoalcohols **3a–c**, **4a, b** and **5a, c** were synthesized as shown in Scheme 1. Reaction of salicylaldehyde **6** with the appropriate *O*-arylmethyloxyamines hydrochlorides **7a–c** in a $CHCl_3$:H_2O (1:1) mixture yielded the corresponding oxime ethers **8a–c**, which were transformed into their potassium salts, and were then allowed to react with epibromohydrine to yield the corresponding epoxides **9a–c**. Treatment of the appropriate epoxide **9** with 3-aminomethylpyridine, 2-aminoethylpyridine or 1-amino-4-methylpiperazine afforded the compounds **3a–c**, **4a, b** and **5a, c**, respectively.

Results and conclusions

The affinity of the new compounds **3a–c**, **4a**, **b** and **5a, c** for 5-$HT_{2A,}$ $5HT_{1A}$ and α_1 receptors was determined by radioligand-binding assays carried out on rat cerebral cortex, using [^{3}H] ketanserin, [^{3}H] 8-OH-DPAT, [^{3}H] prazosin as the specific radioligands, respectively.

Results, expressed as K_i (nM), are reported in Table 1, together with those obtained in the same tests with sarpogrelate **1** and M-1 **2**, taken as reference drugs.

All the new compounds showed a determinable affinity for 5-HT_{2A} receptors, with the K_i values ranging from 9700 nM of **3b** to 97.7 nM of **4a**. In the same tests, the sarpogrelate **1** and M-1 **2** exhibited K_i values of 22 and 9.7 nM, respectively. In particular, among the 3-methylpyridinyl compounds **3a–c**, the *o*-chloro-substituted **3b** and the phenyl unsubstituted **3a** exhibited an affinity, with K_i values of 9700 nM and 1990 nM, respectively; the 3,4-methylendioxy compound **3c** showed an intermediate affinity (K_i = 5620 nM). An affinity comparable to that of **3a** and **3c** was shown by their corresponding piperazinyl analogues **5a** and **5c** (K_i values of 2000 and 6450 nM, respectively).

Among the new compounds, **4a** and **4b** showed the highest affinity, with K_i values of 97.7 and 182 nM, respectively. Moreover, the more active compound **4a** showed binding value appreciably higher than those of M-1 **2** (9.7 nM) and sarpogrelate **1** (22 nM).

Table 1. Affinity of compounds **3a–c**, **4a, b** and **5a, c** for 5-HT_{2A}, 5-HT_{1A} and α_1 receptors.

Compound	K_i (nM)[a]			K_i Ratio	
	5-HT_{2A}	5-HT_{1A}	α_1	5-HT_{1A}/5-HT_{2A}	α_1/5-HT_{2A}
3a	1990 ± 80	>10 000[b]	>10 000[b]	>5.0	>5.0
3b	9700 ± 430	>10 000[b]	>10 000[b]	>1.0	>1.0
3c	5620 ± 250	8910 ± 400	>10 000[b]	1.5	>1.1
4a	97.7 ± 4.2	6300 ±280	>10 000[b]	64.4	>102.3
4b	182 ± 8.0	7580 ± 340	>10 000[b]	41.6	>54.9
5a	2000 ± 85	>10 000[b]	>10 000[b]	>5.0	>5.0
5c	6450 ± 290	9500 ± 420	>10 000[b]	1.4	>1.5
Sarpogrelat**e 1**	22 ± 1.0	>10 000[b]	2000 ± 90	>454.5	90.9
M-1 **2**	9.7 ± 0.4	3160 ± 140	500 ± 20	325.7	51.5

[a] K_i values are taken from three experiments, expressed as means ± SEM.
[b] The K_i values were not calculated because the inhibition percentages at 10 μM were too low.

At the 5HT_{1A} subtype receptor the new compounds **3a-c**, **4a, b** and **5a, c** showed high K_i values (ranging from 7580 – 10 000). The K_i ratio between 5-HT_{1A} and 5-HT_{2A} was not significantly different to the one for compounds **3** and **5**, but is higher for compounds **4a** and **4b**.

The new compounds are devoid of any affinity towards the α_1-adrenergic receptor. The reference drug sarpogrelate **1** and its metabolite **2** show, at this receptor, K_i values of 2000 and 500 nM, respectively. The K_i ratio between α_1/5HT_{2A} for the most active compounds **4a** and **4b** show values comparable to those of the reference drugs, (102.0, 54.9 for **4a** and **4b** and 90.9, 51.5 for **1** and **2**, respectively).

The results indicate that with the newly synthesized compounds **3 – 5**, only the 2-ethylpyridinyl moiety of **4** is the one that better addresses the affinity towards the serotoninergic receptors. This indicates that the presence of this substituent, on the amino moiety of the aminopropanol chain, does not prevent the interaction of this group with the receptor.

The introduction of the more rigid amino-etheral sequence as in the **4a** and **4b** derivatives, with respect to the more flexible ethylenic linker of **1** and **2**, is able to maintain an appreciable affinity on 5-HT_{2A}-receptor for these molecules.

In conclusion, given that this work has not obtained compounds possessing a greater affinity for the 5-HT_{2A} receptor than that of the model compound M-1 **2**, however, it allowed us to identify in the compound **4a** a new lead, that could be a good start point to design new ligands endowed with high affinity and selectivity towards 5-HT_{2A} receptor.

Experimental

General

Melting points were determined on a Kofler hot-stage apparatus (C. Reichert, Vienna, Austria) and are uncorrected. ^{1}H-NMR spectra of all compounds were obtained with a Gemini 200 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 200 MHz, in a approx. 2% solution of $CDCl_3$. Analytical TLCs 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. Mass spectra were detected with a Hewlett Packard 5988A spectrometer (Hewlett-Packard, Palo Alto, CA, USA). Evaporation was performed *in vacuo* (rotating evaporator); the *O*-arylmethyloxyamines to be used for the preparation of the desired compounds **3 – 5**, not commercially available, were prepared following the synthetic method described in the literature [14]. Na_2SO_4 was always used as the drying agent. Elemental analyses(C, H, N) were performed in our analytical laboratory and agreed with theoretical values to within ± 0.4% (cf. Supplemental Material).

Chemistry

*General procedure for the synthesis of 2-Hydroxy-benzaldehyde-O-benzyl oxime **8a**, 2-Hydroxy-benzaldehyde-O-(o-chlorobenzyl) oxime **8b** and 2-Hydroxy-benzaldehyde-O-(3,4-methylenedioxy-benzyl) oxime **8c***

A mixture of the appropriate *O*-aryloxyamine **7a – c** (0.02 mol) in H_2O (10 mL) and the salicylaldehyde **6** (2.44 g, 0.02 mol) in $CHCl_3$ (30 mL) was stirred at room temperature for 24 h. The organic phase was separated, and the aqueous solution was extracted twice with $CHCl_3$. Evaporation of the dried and filtered organic extracts afforded a residue consisting almost exclusively of the corresponding phenols **8a – c**.

8a: (81%) 1H-NMR: δ 5.20 (s, 2H); 6.91 – 7.43 (m, 9H); 8.23 (s, 1H); 9.76 (brs, 1H). **8b:** (50%); 1H-NMR: δ 5.26 (s, 2H); 6.91 – 7.45 (m, 8H); 8.19 (s, 1H); 9.60 (brs, 1H). **8c:** (67%); 1H-NMR: δ 5.08 (s, 2H); 5.98 (s, 2H); 6.80 – 7.00 (m, 5H); 7.12 – 7.29 (m, 2H); 8.20 (s, 1H); 9.80 (brs, 1H).

*General procedure for the synthesis of 2-(Glycidoxy)benzaldehyde **O**-substituted benzyl oxime **9a – c***

A solution of the appropriate 2-hydroxy-benzaldehyde-*O*-substituted benzyl oxime **8a – c** (3.52 mmol) in *t*-BuOH (5 mL) was added to a solution of KOH (0.40 g, 7.18 mmol) in *t*-BuOH (5 mL).

The resulting mixture was added dropwise to a stirred and cooled (0°C) solution of epibromohydrine (0.62 mL, 7.18 mmol) in *t*-BuOH (1 mL). After stirring at room temperature for 48 h, the mixture was evaporated, taken up with Et_2O and washed with H_2O. The organic phase was then dried and evaporated to give the desired products **9a–c**. **9a**: (40%); 1H-NMR: δ 2.74–2.94 (m, 2H), 3.25–3.40 (m, 1H); 3.96–4.04 (m, 1H); 4.15–4.27 (m, 1H); 5.23 (s, 2H); 6.86–7.02 (m, 4H); 7.27–7.84 (m, 5H); 8.58 (s, 1H).MS (m/z): M^+ 283 (1.1).**9b**: (55%); 1H-NMR: δ 2.74–2.94 (m, 2H), 3.37–3.43 (m, 1H); 3.98–4.04 (m, 1H); 4.18–4.22 (m, 1H); 5,35 (s, 2H); 6.86-7.83 (m, 8H, Ar); 8.63 (s, 1H). **9c**: (55%); 1H-NMR: δ 2.73–2.76 (m, 1H), 2.90 (t, 1H, J = 5 Hz); 3.30–3.43 (m, 1H); 3.94–4.02 (m, 1H); 4.18–4.26 (m, 1H); 5.11 (s, 2H); 5.96 (s, 2H); 6.90–7.00 (m, 5H); 7.22–7.40 (m, 2H); 8.56 (s, 1H).

*Synthesis of 2-[2-Hydroxy-3-(pyridin-3-yl-methyl)amino] propoxy benzaldehyde-O-benzyl- (**3a**), –O-(o-chlorobenzyl)- (**3b**) and –O-3,4-methylendioxy-benzyl- (**3c**) oximes, 2-[2-Hydroxy-3-(2-pyridin-2-yl-ethyl)amino]propoxy benzaldehyde **O**-benzyl- (**4a**), –O-(o-chlorobenzyl)- (**4b**) -oximes and 2-[2-Hydroxy-3-(4-methyl-piperazin-1-yl)amino] propoxy benzaldehyde O-benzyl- (**5a**) and –O-3,4-methylendioxy-benzyl- (**5c**) oximes*

$LiClO_4$ (3.76 g, 35.35 mmol) and the appropriate substituted amine (3.9 mmol) were added to a stirred solution of the appropriate glycidoxy derivatives **9a–c** (3.5 mmol) in anhydrous CH_3CN (15 mL). After stirring at room temperature for 24 h, the solution was diluted with Et_2O and washed with a solution of NaCl. The organic phase was then dried, filtered and evaporated, to give an oily residue, which was purified by transformation of the corresponding oxalate salt, and crystallised from the proper solvent, to give the desired amines **3a–c**, **4a**, **b**, **5a**, **c** as oxalate salts. **3a**: MeOH/Et_2O; ^{1}H-NMR: (DMSO) δ 2.98–3.18 (m, 2H); 4.00 (brs, 2H); 4.23 (s, 2H); 5.17 (s, 2H); 6.95–7.08 (m, 2H); 7.35–7.42 (m, 6H); 7.66–7.69 (m, 1H); 7.92–7.96 (m, 1H); 7.78 (dt, 1H, J = 7.7, 1.8 Hz); 8.50 (brs, 1H); 8.56 (d, 1H, J = 4.5Hz); 8.68 (s, 1H). Anal. Calc.: $C_{23}H_{25}N_3O_3 \times H_2C_2O_4$ (C, H, N). **3b**: (MeOH): ^{1}H-NMR free base: ($CDCl_3$) δ 2.80 –2.92 (m, 2H); 3.86 (s, 2H); 4.03–4.13 (m, 5H); 5.33 (s, 2H); 6.88–7.01 (m, 2H); 7.27–7.53 (m, 6H); 7.68–7.72 (m, 2H); 8.44–8.58 (m, 3H). Anal Calc.: $C_{23}H_{24}N_3O_3Cl \times 2H_2C_2O_4$ (C, H, N). **3c**: (MeOH): ^{1}H-NMR free base: ($CDCl_3$) δ 2.25 –2.90 (brs, 2H); 3.86 (s, 2H); 4.05–4.12 (m, 5H); 5.10 (s, 2H);5.96 (s, 2H); 6.78–7.01 (m, 5H); 7.27–7.33 (m, 3H); 7.68–7.72 (m, 2H); 8.41 (s, 1H); 8.49–8.58 (m, 2H); Anal Calc.: $C_{24}H_{25}N_3O_5 \times 2H_2C_2O_4$ (C, H, N). **4a**: (EtOH): ^{1}H-NMR: (DMSO) δ 3.10–3.38 (m, 6H); 4.06 (brs, 2H); 5.16 (s, 2H); 6.98–7.09 (m, 2H); 7.27–7.40 (m, 6H); 7.65–7.77 (m, 2H); 8.48–8.57 (m, 3H); Anal Calc.: $C_{24}H_{27}N_3O_3 \times 2H_2C_2O_4$ (C, H, N). **4b**: (MeOH): ^{1}H-NMR: (DMSO) δ 3.04–3.40 (brs, 6H); 4.03 (brs, 2H); 5.25 (s, 2H); 6.93–7.10 (m, 2H); 7.20–7.58 (m, 6H); 7.62–7.80 (m, 2H); 8.42–8.54 (m, 2H); 8.60 (m, 1H) Anal Calc.: $C_{24}H_{26}N_3O_3Cl \times 2H_2C_2O_4$ (C, H, N). **5a**: (*i*-PrOH): ^{1}H-NMR: (DMSO) δ 2.26 (s, 3H); 2.67–2.75 (brs, 2H); 3.16–3.27 (brs, 4H); 3.3–4.10 (brs); 5.17 (s, 2H); 5.84 (brs); 6.01 (s, 2H); 6.96–7.11 (m, 3H); 7.36–7.48 (m, 5H); 7.68-7.72 (m, 1H); 8.59 (s, 1H). Anal Calc.: $C_{22}H_{30}N_4O_3 \times 2H_2C_2O_4$ (C, H, N). **5c** (EtOH): ^{1}H-NMR: (DMSO) δ 2.26 (s, 3H); 2.67–2.75 (brs, 2H); 2.90–3.16 (brs, 4H); 3.5–4.12 (brs); 4.50–4.70 (brs, 2H); 5.06 (s, 2H); 6.01 (s, 2H); 6.90–7.10 (m, 5H); 7.36–7.48 (m, 1H); 7.68–7.72 (m, 2H); 8.56 (s, 1H). Anal. Calc.: $C_{23}H_{30}N_4O_5 \times 2H_2C_2O_4$ (C, H, N).

Biological assays

5-HT_{2A}-Receptor binding

Cerebral cortex was dissected from rat brain and the tissue was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.4. The homogenate was centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was suspended in 35 volumes of 50 mM Tris-HCl buffer, incubated at 37°C for 10 min to remove endogenous 5-HT, and centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was frozen at –80°C until the time of assay.

The binding assay was performed according to the method reported in the literature [15] incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) in Tris-HCl buffer at pH 7.4 with approximately 0.48 nM [^{3}H] ketanserin (Perkin-Elmer Life Science, Milano, Italy: 77.2 Ci/mmol) in a final volume of 1 mL. Incubation was carried out at 37°C for 15 min. Non-specific binding was defined in the presence of 10 μM spiperone. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filtrates were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. The receptor-bound radioactivity was measured as described above.

5-HT_{1A}-Receptor binding

Cerebral cortex was dissected from rat brain and the tissue was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.4. The homogenate was centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was suspended in 35 volumes of 50 mM Tris-HCl buffer, incubated at 37°C for 10 min to remove endogenous 5-HT, and centrifuged at 48 000 g for 15 min at 4°C. The resulting pellet was frozen at –80°C until the time of assay.

The binding assay was performed according to the method reported in the literature [16] incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) in Tris-HCl buffer at pH 7.4 with approximately 0.5 nM [^{3}H] 8-OH-DPAT (Perkin-Elmer Life Science: 106 Ci/mmol) in a final volume of 1 mL. Incubation was carried out at 37°C for 15 min. Non-specific binding was defined in the presence of 10 μM 8-OH-DPAT. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filtrates were washed four times with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting non-specific binding from total binding and approximated to 85-90% of total binding.

α_1-Receptor binding

Rat cerebral cortex was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.7 containing 5 mM EDTA (buffer T1) in an ultraturrax homogenizer. The homogenate was centrifuged at 48 000 *g* for 15 min at 4°C. The pellet was suspended in 20 volumes of ice-cold buffer T1. It was then homogenized and centrifuged at 48 000 *g* for 15 min at 4°C. The resulting pellet was frozen at –80°C until the time of assay.

The binding assay was performed according to the method reported in the literature [17] incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) in 50 mM Tris-HCl buffer at pH 7.7 with approximately 0.2 nM [^{3}H] prazosin (Perkin-Elmer Life Science: 81 Ci/mmol) 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 1 μM prazosin. The binding reaction was concluded by filtration through Whatman GF/C glass fiber filters under reduced pressure. Filtrates were washed four times

with 5 mL aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting nonspecific binding from total binding and approximated to 85 – 90% of total binding.

Compounds were dissolved in buffer and added to the assay mixture. The inhibition of specific binding was determined in the presence of a single concentration (10 μM) of the potential displacing agent. The concentration of tested compounds that produces 50% inhibition of specific radioligand binding (IC_{50}) was determined by log-probit analysis with increasing concentrations of the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to Cheng and Prusoff [18] equation K_d of [^{3}H] ketanserin binding to cortex membranes was 0.48 nM (5-HT_{2A}); K_d of [^{3}H] 8-OH-DPAT binding to cortex membranes was 2 nM (5-HT_{1A}) and K_d of [^{3}H] prazosin binding to cortex membranes was 0.24 nM (α_1). The protein concentration was determined according to the method of Lowry [19], using bovine serum albumin as a standard.

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