Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 22, pp. 2857-2862, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10089-0

NOVEL PYRIDAZINO[4,5-*b*][1,5]OXAZEPINES AND -THIAZEPINES AS 5-HT$_{1A}$ RECEPTOR LIGANDS

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The role of 5-HT$_{1A}$ receptors in psychiatric disorders such as anxiety and depression, has been well established. Arylpiperazines, aralkylamines, and aryloxyalkylamines are among the agents that have been the most extensively studied as drug candidates.[1-4] Although many of them possess nanomolar affinity, and a proper intrinsic activity for the 5-HT$_{1A}$ receptors they show variable receptor selectivity with a consequence of untoward side effects. We herein report that pyridazino[4,5-*b*][1,5]oxazepines and pyridazino[4,5-*b*][1,5]thiazepines, when substituted with a phenoxyalkylaminoacyl group, have high affinity for 5-HT$_{1A}$ receptors with remarkable selectivities with respect to α and D receptors.

Syntheses of the novel bicyclic pyridazines were carried out as outlined in Scheme 1. Reaction of the 4*H*-oxazepine **3** or thiazepine **4**[5] with haloacyl chlorides took place smoothly to afford intermediates **5-12**, which upon treatment with amines under various conditions gave the expected products **13-34** in acceptable yields (Table 1).[6] The aminoalkyl analogue **35** was obtained by reduction of the aminoacyl derivative **21** with diborane. Compound **38**, the 'ring-open' analogue of **30**, was prepared in a straightforward way starting from **36**[7] as shown in Scheme 2.

All new compounds were tested in a receptor binding assay for 5-HT$_{1A}$ receptors, and the most active compounds were also investigated in α_1 and α_2 as well as 5-HT$_2$ and D$_2$ receptor binding assays.[8] A summary of the results is shown in Table 1. None of the active compounds showed significant activities for 5-HT$_2$ or D$_2$ receptors (not shown).

In the oxazepine series (**13-25**), **20** and **25** had at least a full order of magnitude higher affinity than any of the other derivatives indicating that a three or four atom tether between the two nitrogens, and an *ortho*-methoxyphenoxyethyl moiety at the amino nitrogen provide a very advantageous arrangement for fitting to the receptor. Interestingly enough, in the thiazepine series (**26-34**), the distance between the basic amino and the

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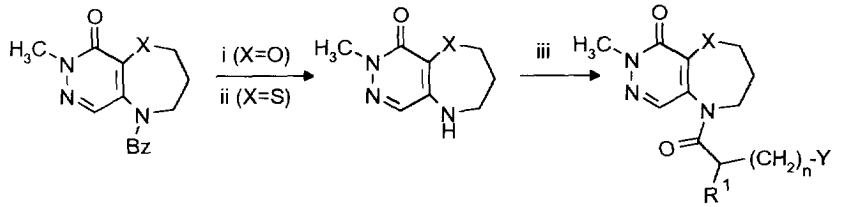
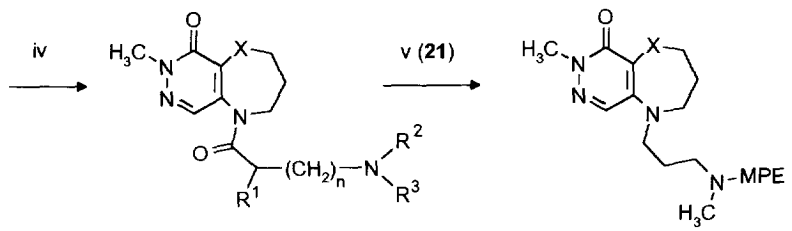
Scheme 1



1 X=O
2 X=S

3 X=O
4 X=S

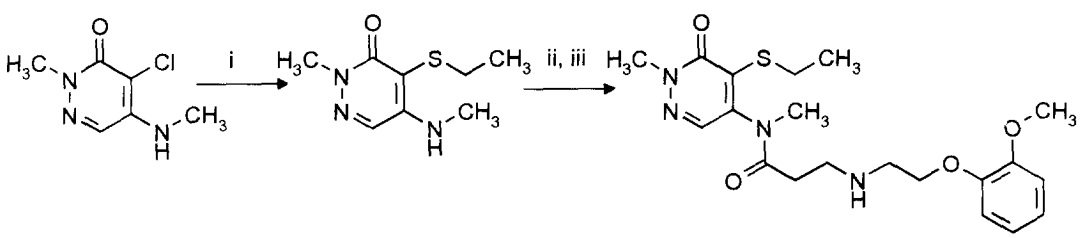
5-7 X=O, R^1=H, Y=Cl, n=0,1,2
8-10 X=S, R^1=H, Y=Cl, n=0,1,2
11 X=O, R^1=CH_3, Y=Br, n=0
12 X=S, R^1=CH_3, Y=Br, n=0



13-34 **35**

Reagents and conditions[a]:
i: Pd/C, cyclohexene, EtOH, reflux, 1 h; ii: PhOH (1.15 eq), H_3PO_3, 150 °C, 3 h; iii: $Y(CH_2)_nCH(R^1)COCl$, DMAP, DMF, 40-45 °C, 4 h; iv: R^2R^3NH (2.1 eq), 70 °C, 3 h (Meth. A), 120 °C, 3 h (Meth. B), or R^2R^3NH (1 eq), Et_3N, CH_3CN, rt, 5-48 h (Meth. C) or R^2R^3NH (1.3 eq), isopropanol, (Et_3N), reflux, 5 h (Meth. D); v: THF, B_2H_6, rt, 6 h.

Scheme 2



36 **37** **38**

Reagents and conditions:
i: NaH, EtSH, PhH, reflux, 24 h; ii: $Cl(CH_2)_2COCl$, DMF, DMAP, 45-50 °C 2 h; iii: $(MPE)NH_2$, DMAP, Et_3N , i-PrOH, 60 °C, 2 h

[a] **Abbreviations:** DMAP: 4-(*N,N*-dimethylamino)pyridine; MPE: 2-(2-methoxyphenoxy)ethyl; THF: tetrahydrofurane; rt: room temperature

Table 1. The 5-HT_{1A}, α_1, and α_2 receptor binding data of compounds **12-34** and reference compounds.

Compound	X	n	R^1	R^{2a}	R^{3a}	Method (yield%)	mp °C	Salt and/or solvate	IC_{50} (nM) 5-HT_{1A}	IC_{50}(μM) α_1	IC_{50}(μM) α_2
13	O	0	H	H	PE	C (80)	121-122	HCl.2H_2O	230±45	-	-
14	O	0	H	H	MPE	C (61)	179-181	HCl.H_2O	163±18	12.5±1.0	21.0±9.0
15	O	0	CH_3	H	MPE	C (54)	117-121	HCl	>1000	-	-
16	O	0	H	H	BDM	A (62)	102-104	fum. H_2O	299±72	> 10	4.6±1.5
17	O	0	H	MPP		A(31)	233-235	H_2O	>1000	-	-
18	O	0	H	MPHPP		C (45)	58	-	>1000	-	-
19	O	1	H	H	PE	D (60)	82-84	fum.	524±90	8.9±1.4	0.54±0.09
20	O	1	H	H	MPE	D (58)	207-208	HCl	19.5±3.4	1.7±0.6	1.8±0.9
21	O	1	H	CH_3	MPE	D (50)	126-128	fum.	134±36	16.1±1.0	9.5±6.3
22	O	1	H	Bz	MPE	D (59)	78-80	fum.	1003±180	-	-
23	O	1	H	H	CPE	B (16)	hygroscopic	HCl.H_2O	312±54	-	-
24	O	1	H	MPHPP		C (58)	149	-	>1000	> 10	47.0±10
25	O	2	H	H	MPE	B (35)	110-111	HCl.H_2O	17.8±1.6	0.69±0.24	3.9±0.7
26	S	0	H	H	MPE	D (43)	210	HCl.H_2O	37.0±10.8	> 10	3.1±0.6
27	S	0	CH_3	H	MPE	B (40)	120-122	HCl.H_2O	>1000	-	-
28	S	0	H	H	BDM	B (65)	194-196	HCl.H_2O	44.9±4.1	19.0±7.0	2.33±0.97
29	S	1	H	H	PE	D (70)	98-100	HCl.2H_2O	150±21	-	-
30	S	1	H	H	MPE	D (37)	97-100	HCl.H_2O	11.4±0.8	1.7±0.9	0.27±0.03
31	S	1	H	Bz	MPE	D (31)	101-102	-	>1000	-	-
32	S	1	H	H	CPE	D (18)	hygroscopic	HCl.H_2O	181±44	-	-
33	S	1	H	MPP		C (78)	187-188	-	118±8	-	-
34	S	2	H	H	MPE	D (30)	122-124	H_2O	9.2±0.11	0.93±0.08	0.86±0.028
35									>1000	-	-
38									32.7±1.5	-	-
Buspiron									7.13±0.25	-	-
8-OH-DPAT									8.06±1.61	-	-

[a]Abbreviations: Bz: benzyl; CPE: 2-(3-chlorophenoxy)ethyl; BDM: [1,4]benzodioxanyl-2-methyl, fum: fumaric acid; MPE: 2-(2-methoxyphenoxy)ethyl; MPHPP: 4-(3-(2-methoxyphenoxy)-2-hydroxy-1-propyl)-1-piperazinyl; MPP: 4-(2-methoxyphenyl)-1-piperazinyl; PE: 2-phenoxyethyl

thiazepine nitrogen has not proven to be so critical, as examplefied by the similar affinities of **26**, **30** and **34**. A somewhat higher affinities of the thiazepines over the oxazepines might be explained by the more lipophilic character of the former compounds, which might lead to additional hydrophobic interactions.

The modest affinity of the *N*-methyl derivative **21**, and the lack of activity of **22, 24** and **31** indicate that the interaction of the hydrogen-bond donor ammonium nitrogen with the receptor may be sterically sensitive.

For comparative purposes, the 'acyclic thiazepine' **38**, and a reduced compound, **35** were also evaluated. The former compound exhibited a fairly high affinity, whereas **35** was inactive.

In summary, we developed two novel series of *d*-fused pyridazines as potential ligands for 5-HT_{1A} receptor. Some of these agents bind with high affinities.

Acknowledgement: We thank to OMFB for support. Two of us, P. M. and N. H. thank the 'Stiftung Aktion Österreich-Ungarn' for the possibility of personal discussions.

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6. **General procedure for the preparation of compounds 13-35.**
Step I. Synthesis of intermediates **5-12:** To a stirred solution of the oxazepinone (**3**) or thiazepinone (**4**) (41.1 mmoles) and 4-(*N,N*-dimethylamino)pyridine (41.1 mmoles) in DMF (60 ml), a solution of the appropriate haloacyl chloride (65.6 mmoles) in DMF (23 ml) was dropped, while the temperature was kept below 45 °C. After stirring the mixture at 40-45 °C for 2 h, a solution of the appropriate haloacyl chloride (32.8 mmoles) in DMF (12 ml) was added, and stirring was continued at 40-45 °C for further 3 h. Then the solvent was distilled off *in vacuo*. The residue was treated with water (50 ml) and extracted with ethyl acetate (4x150 ml). The organic solution was evaporated to dryness. The residue was triturated with diethyl ether to give the haloacyl intermediate which was used without further purification.
Step II. Reaction of the haloacyl intermediates with amines, methods A-D:
Method A: A mixture of the appropriate haloacyl compound (2.5 mmoles) and the amine (5.2 mmoles) was stirred at 70 °C for 3 h. Then it was treated with water (10 ml), and extracted with ethyl acetate (4x20 ml). The organic solution was evaporated to dryness. The residue was subjected to column-chromatography on silica gel using a mixture of chloroform - methanol as eluting agent. The crude product was crystallized or a salt was prepared.
Method B: The same as above except that the reaction mixture was kept at 120 °C for 3 h.
Method C: To a stirred suspension of the haloacyl compound (2.5 mmoles) in acetonitrile (5 ml), the amine (2.5 mmoles) and triethyl amine (2.5 mmoles) were added. The reaction mixture was stirred at room temperature for 5-48 h (tlc). The residue was treated with water (10 ml) and extracted with ethyl acetate (4x20 ml). The organic solution was evaporated to dryness. The residue was crystallized or a salt was prepared.
Method D: To a stirred solution of the haloacyl compound (2.9 mmoles) in isopropanol (10 ml) and the appropriately substituted amine (4.0 mmoles) were added. For compounds **26, 29, 31, 33** and **34,** triethyl amine (3.2 mmoles) was also added. The reaction mixture was refluxed (or kept at 60 °C for compounds **19-22, 26, 29, 31, 33** and **34**) for 5 h, then worked-up according to Method A.
Preparation of 35: To a stirred solution of **21** (2.5 mmoles) in THF (23 ml) a 1.8 M solution of diborane in THF (10.0 mmoles, 5.55 ml) was dropped (10 min) at 0 °C. The reaction mixture was stirred at rt for 6 h, then a solution of ethanol (4.5 ml) and water (0.3 ml) was added dropwise while keeping it below 10 °C. The mixture was stirred at rt for 1.5 h, then HCl/ethanol (8 ml, 20%) was added. The resulting mixture was stirred for 3 h, then refluxed for 2.5 h. The solvent was distilled off *in*

vacuo. and the residue was subjected to column-chromatography on silica gel using a mixture of ethyl acetate - methanol to give **35** (0.7 g, 40%) as hygroscopic foam.

Preparation of compound 38: Compound **37** was obtained by treatment of **36**[7] with NaH and EtSH in benzene at reflux temperature for 24 h. Yield: 78 %, mp: 104-105 °C. Then it was treated with 3-chloropropionyl chloride as described above in Step I. The crude product was allowed to react with 2-(2-methoxyphenoxy)ethylamine as described above in method D to give **38** as yellow oil. Yield: 36 %.

All compounds prepared gave satisfactory analytical data (C,H,N). ^{1}H NMR (250 MHz, rt) data are as follows.

5: δ ($CDCl_3$) 2.08 (m, 2H), 3.31 (t, 2H), 3.62 (s, 3H), 4.32 (t, 2H), 4.58 (s, 2H), 7.52-8.03 (brs, 1H).

6: δ ($CDCl_3$) 2.20 (m, 2H), 2.80; 2.97 (d (t, s): hindered rotation, 2H), 3.72 (s, 3H), 3.20-4.10 (m, 2x2H), 4.43 (m, 2H), 7.53; 7.77 (2xs, hindered rotation, 1H)

7: δ ($CDCl_3$) 2.20 (m, 2x2H), 2.59, 2.73 (d: hindered rotation, 2H), 3.52-3.84 (m, 2x2H), 3.78 (s, 3H), 4.47 (t, 2H), 7.60; 7.77 (2xs, hindered rotation, 1H).

8: δ ($CDCl_3$) 2.00-3.22 (m, 2x2H), 3.33 (t, 2H), 3.68 (s, 3H), 4.26 (s, 2H), 7.68 (s, 1H).

10: δ ($CDCl_3$) 2.48 (m, 4H), 2.87 (t, 2H), 3.37 (m, 2H), 3.87 (t, 2H), 4.00 (s, 3H), 4.13-4.79 (m, 2H), 7.75 (s, 1H).

12: δ ($CDCl_3$) 1.88 (d, 3H), 2.17 (m, 2H), 2.68 (m, 2H), 3.75 (s, 3H), 4.45 (q, 2H), 4.69 (t, 2H), 7.34 (s, 1H).

13: δ (DMSO-d_6, 353 K) 2.18 (m, 2H), 3.26-3.60 (m, 6H), 3.65 (d, 3H), 3.92 (t, 2H), 4.12-4.56 (m, 4H), 6.99 (m, 3H), 7.30 (m, 2H), 7.67,7.97 (2xs, hindered rotation, 1H), 9.25 (s, 2H, salt).

14: δ ($CDCl_3$) 2.16 (q, 2H), 2.42 (s, 2H), 3.00 (t, 2H), 3.60 (m, 2H), 3.69 (s, 3H), 3.80 (s, 3H), 4.07 (t, 2H), 4.33 (t, 2H), 6.85 (m, 4H), 7.65 (s, 1H).

15: δ (DMSO-d_6) 1.59 (d, 3H), 1.97-2.30 (m, 2H), 3.38 (m, 2+1H), 3.60 (d, 3H), 3.72; 3.94 (2xm, 2x1H), 3.77 (s, 3H), 4.36 (m, 4H), 6.97 (m, 4H), 7.72; 8.16 (2xs, hindered rotation, 2x0,5H), 9.67 (brs, salt).

16: δ (DMSO-d_6) 2.12 (m, 2H), 2.84 (m, 4H), 3.63 (s, 3H), 3.73 (m, 1H), 3.92 (m, 2H), 4.10 (m, 2x2H), 6.66 (s, 2H, fum.), 6.86 (m, 4H), 7.76; 7.97 (2xs, hindered rotation, 1H).

17: δ ($CDCl_3$) 2.26 (m, 2H), 2.72 (d, 4H), 3.08 (d, 4H), 3.29 (s, 2H), 3.43 (s, 1H), 4.10 (s, 1H), 3.91 (s, 3H), 3.99 (s, 3H), 4.48 (m, 2H), 6.94 (m, 4H), 7.82 (s, 1H).

18: δ ($CDCl_3$) 2.22 (m, 2H), 2.53 (m, 12H), 3.18; 3.32 (m, 2H), 3.76 (s, 3H), 3.85 (s, 3H), 4.02 (m, 2H), 4.12 (m, 1H), 4.43 (m, 2H), 6.92 (m, 4H), 7.28; 7.75 (2xs, hindered rotation, 1H).

19: δ (DMSO-d_6) 2.07 (m, 2H), 2.62-3.37 (m, 6H), 3.64 (d, 3H), 3.92 (t, 2H), 4.08-4.42 (m, 4H), 6.53 (s, 2H, fum.), 6.97 (m, 3H), 7.28 (m, 2H), 7.76; 7.94 (2xs, hindered rotation, 1H).

20: δ (DMSO-d_6) 2.05; 2.19 (2m, 2H), 3.12; 3.90 (2m, 2x1H, 4-CH_2), 3.38 (m, 6H), 3.61 (d: hindered rotation, 3H), 3.78 (s, 3H), 4.21 (m, 4H), 6.96 (m, 4H), 7.80; 7.97 (2xs, hindered rotation, 1H), 9.22 (d, 2H, salt).

21: δ (DMSO-d_6, 333 K) 2.09 (m, 2H), 2.33 (s, 3H), 2.62 (m, 2H), 2.80 (m, 4H), 3.60 (s, 3H), 3.76 (s, 3H), 3.85 (m, 2H), 4.01 (t, 2H), 4.27 (t, 2H), 6.61 (s, 2H, fum.), 6.89 (m, 4H), 7.75 (s, 1H).

22: δ (DMSO-d_6, 333 K) 2.06 (m, 2H), 2.62 (t, 2H), 2.86 (t, 2H), 2.95 (t, 2H), 3.60 (s, 3H), 3.71 (m, 2H), 4.03 (t, 2H), 4.25 (t, 2H), 6.10 (brs, 2H), 6.65 (s, 2H, fum.), 6.87 (m, 4H,), 7.28 (m, 5H), 7.70 (s, 1H).

23: δ (DMSO-d_6, 353 K) 2.16 (q, 2H), 3.06 (t, 2H), 3.46 (m, 4H), 3.63 (s, 3H), 3.89 (m, 2H), 4.38 (m, 4H), 7.06 (m, 3H), 7.33 (t, 1H), 7.85 (s, 1H), 9.39 (brs, 2H, salt).

24: δ ($CDCl_3$) 2.24 (m, 2H), 2.24-2.92 (m, 16H), 3.78 (s, 3H), 3.86 (s, 3H), 4.00 (d, 2H), 4.12 (m, 1H), 4.46 (m, 2H), 6.94 (m, 4H), 7.28; 7.58 (2xs, hindered rotation, 1H).

25: δ ($CDCl_3$) 1.98-2.18 (m, 4H), 2.61 (t, 2H), 3.18 (t, 2H), 3.31 (t, 2H), 3.72 (s, 3H), 4.03 (s, 3H), 4.18 (m, 4H), 6.92 (m, 4H), 7.76 (s, 1H), 9.89 (s, 2H, salt).

26: δ ($CDCl_3$) 1.01-1.34 (m, 4H,), 2.77-3.19 (m, 4H), 3.30 (s, 2H), 3.54 (s, 3H), 3.68 (s, 3H), 4.23 (t, 2H), 6.90 (m, 4H), 7.65; 7.85 (2xs, hindered rotation, 1H), 9.50 (brs, 1H).

27: δ (DMSO-d_6 + TFA, 363 K) δ1.55 (br. d, 3H), 2.10-2.40 (m, 2H), 2.98 (m,1H) and 3.16 (m, 1H), 3.42 (t, 2H), 3.55-3.3.85 (ovl. m, 2H), 3.62 (s, 3H), 3.78 (s, 3H), 4.30 (t, 2H), 4.48 (m, 1H,), 6.80-7.05 (ovl. m, 4H), 7.78 (s, 1H), 9.40 (br, 2H)

28: δ ($CDCl_3$) 2.17 (m, 2H), 3.18 (m, 6H), 3.63 (d, 3H), 4.08 (m, 2H), 4.39 (d, 2H), 4.77 (m, 1H), 6.98 (m, 4H), 7.68; 7.99 (2xs, hindered rotation, 1H), 9.82 (brs, 2H, salt).

29: δ (DMSO-d_6, 348 K) 2.28 (q, 2H), 3.03 (m, 4H), 3.39 (m, 4H), 3.68 (s, 3H), 3.99 (t, 2H), 4.40 (t, 2H), 7.05 (m, 3H), 7.36 (m, 1H), 7.84 (s, 1H), 9.39 (brs, 2H, salt).

30: δ (DMSO-d_6, 333 K) 2.37 (m, 2H), 3.13 (m, 4H), 3.36 (t, 4H), 3.62 (s, 3H), 3.81 (s, 3H), 4.31 (t, 2H), 6.98 (m, 4H), 7.73 (s, 1H), 9.24 (brs, 2H, salt).

31: δ (DMSO-d_6) 1.80-2.40 (m, 2H), 2.60-3.15 (m, 4H), 3.34 and 3.36 (2xs, 2H), 3.65 and 3.68 (2xbr, s, 3H), 3.75 (s, 3H), 3.75-4.40 (ovl. m, 10H), 6.80-7.00 (ovl. m, 4H), 7.15-7.45 (ovl. m, 5H), 7.60, 7.82 (2xs, hindered rotation, 1H)

32: δ (DMSO-d_6, 353 K) 2.24 (q, 2H), 2.87-3.21 (m, 4H), 3.38-3.52 (m, 4H), 3.62 (s, 3H,), 3.96 (m, 2H), 4.35 (t, 2H), 7.02 (m, 3H), 7.34 (t, 1H), 7.80 (s, 1H), 8.63 (brs, 2H, salt).

33: δ ($CDCl_3$) 1.77 (m, 2H), 2.09 (m, 2H), 2.62 (m, 8H), 2.89 (m, 2H), 3.17 (m, 4H), 3.78 (s, 3H), 3.86 (s, 3H), 6.92 (m, 4H), 7.52 (s, 1H).

34: δ ($CDCl_3$) 1.74 (m, 4H), 2.01 (m, 4H), 2.58 (m, 2H), 2.81 (m, 2H), 2.97 (t, 2H), 3.76 (s, 3H), 3.84 (s, 3H), 4.08 (t, 2H), 6.90 (m, 4H), 7.52 (s, 1H).

35: δ (DMSO-d_6, 353 K) 1.96-2.19 (m, 4H), 2.87 (s, 3H), 3.33-3.62 (m, 8H), 3.52 (s, 3H), 3.79 (s, 3H), 4.16 (t, 4H), 6.98 (m, 4H), 7.23 (s, 1H), 11.8 (br s, 1H).

38: δ (DMSO-d_6 + TFA, 363 K) 1.22 (t, 3H), 2.72 (br, 2H), 3.13 (s, 3H), 3.15 (ovl. m, 6H), 3.70 (s,3H), 3.80 (s,3H), 4.25 (t, 2H), 6.80-7.05 (ovl. m, 4H), 7.86 (s, 1H), 8.75 (br, 2H).

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8. **Radioligand Binding Assays**. For all receptor binding assays, organs were prepared from male SPRD (Charles River, Hungary) rats weighing 200-230 g, as follows.

5-HT$_{1A}$ receptor: Hippocampus was separated, frozen on solid CO_2, weighed and homogenized in 0.32 mM saccharose (1:10, wt/vol.) at 4 °C, and then centrifuged at 750 g for 10 minutes. The supernatant was recentrifuged at 10,000 g for 30 minutes. The pellet is suspended in 10 volumes of distilled water and shaken for 30 minutes at 4 °C, followed by centrifugation at 35,000 g for 20 minutes. The pellet was dispersed in 40 volumes of 0.05 M TRIS-HCl buffer (pH=7.4), incubated for 10 minutes at 37°C and centrifuged at 35,000 g for 20 minutes. The final pellet was suspended in 40 volumes of measuring buffer (0.05 M TRIS-HCl buffer with 5.7 mM ascorbic acid, 10 mM pargyline and 4 mM $CaCl_2$ in it pH=7.4) and stored in 14 ml aliquots at -20 °C for 2 weeks, as a maximum period. The aliquot was incubated for 15 minutes at 37 °C with 0.5 nM [^{3}H]-8-hydroxy-(di-*n*-propylamino)tetraline (8-OH-DPAT) (specific activity: 950.9 TBq/mmol) in the presence or absence of several concentrations of the competing drug in a final volume of 1 ml of measuring buffer. Non-specific binding was determined with 10 mM 8-OH-DPAT. Separation was carried out by filtering through a Whatman GF/B filter. The filter was pre-soaked with a 0.3 % PEI solution for 0.5 hours.

α_1 Adrenoceptor: Heart was collected and placed in 0.9% ice-cold saline, then homogenized in 6 ml of ice-cold preparing buffer (0.25 M saccharose, 50 mM Tris-HCl, pH=7.4) by Ultra-Turax Tp 18-10 3x10 min. The homogenate was diluted to 30 ml with the same buffer, filtered over 4 layers of gauze, then centrifuged at 4.000 g for 20 min. The pellet was suspended in 30 ml of assay buffer (50 mM Tris-HCl, 1 mM EDTA, pH=7.4) and centrifuged as before. The pellet was then homogenized in 6-fold volume (wet weight/vol.) assay buffer, protein content was measured by Bio-Rad, diluted if necessary to reach 1.0 mg/ml of protein concentration. The membrane was aliquoted and stored at -20°C for 4 weeks as a maximum period. The aliquot was incubated for 30 minutes at 30 °C with [^{3}H]-prazosin (888 GBq/mmol specific activity) at a concentration of 0.8 nM in the presence or absence of several concentrations of the competing drug in a final volume of 1 ml of buffer (50 mM TRIS -HCl, 1 mM EDTA, pH=7.4). Non-specific binding was determined with 1 mM prazosin. Separation was carried out by filtering through a Whatman GF/B filter. The filter was pre-soaked with a 0.3 % PEI solution for 2 hours.

α_2 Adrenoceptor: Freshly collected human blood from healthy donors was mixed with 3.8 % Na-citrate at 1:9 proportion (citrate : blood) and kept at room temperature for 20 min. Then the mixture was centrifuged at 1,000 g for 5 min. The pellet was discarded and the supernatant was recentrifuged at 16,000 g for 10 min. The pellet was then washed twice with the measuring buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM EDTA, pH=7.2) and resuspended. The thrombocyte-number was determined by counting in Bürker-chamber and diluted if necessary to reach 2-4x10^9 trombocytes/ml. The trombocyte preparation was always made freshly. Fractions of the final cell suspensions were incubated at 25 °C for 30 min with [^{3}H]-yohimbine (3089 GBq/mmol specific activity) used at a concentration of 2 nM in the presence or absence of several concentrations of the competing drug in a final volume of 0.25 ml of buffer (50 mM TRIS-HCl, 5 mM EDTA, 100 mM NaCl pH=7.2). Non-specific binding was determined with 1 mM yohimbine. Separation was carried out by filtering through a Whatman GF/C filter.

(*Received in Belgium* 4 *August* 1997; *accepted* 6 *October* 1997)