Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 7, pp. 667-672, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00092-5

SYNTHESIS, BIOLOGICAL *IN VITRO* EVALUATION AND STEREOSELECTIVITY OF ONDANSETRON ANALOGUES: NOVEL 5-HT_{2A} RECEPTOR ANTAGONISTS§

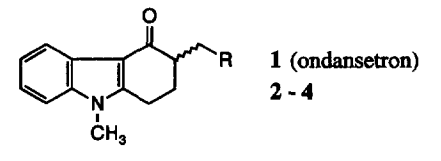
Sigurd Elz* and Wolfgang L. Heil

Institute of Pharmacy, Freie Universität Berlin,
Königin-Luise-Strasse 2 + 4, D-14195 Berlin (Dahlem), Germany

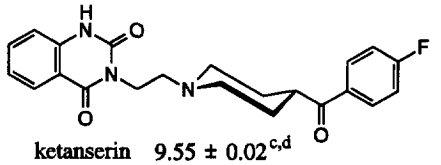
Abstract. The tetrahydrocarbazolone moiety of the 5-HT_3 receptor antagonist ondansetron has been combined with molecular fragments of typical 5-HT_{2A} receptor ligands. Several of the resulting compounds are potent 5-HT_{2A} antagonists. The antipodes of the most potent compound, a *N*-substituted 4-(4-fluorobenzoyl)piperidine, are analogues of ketanserin which display a high degree of stereoselectivity at 5-HT_{2A} receptors (148:1).

The 1-imidazolylmethyl substituted tetrahydrocarbazolone ondansetron (**1**)[1,2] is one of the prototypic potent 5-HT_3 receptor antagonists which are used to prevent severe vomiting caused by cancer chemotherapy and radiotherapy.[3] During a structure-activity study dealing with analogues of **1**[4] we became aware of the moderate micromolar affinity of some derivatives (Chart 1, **2** - **4**) in a vascular 5-HT_{2A} receptor assay.[5] Affinity for 5-HT_{2A} receptors is atypical of 5-HT_3 receptor antagonists. The antagonist potency of **2** - **4** was comparable with that of 4-(4-fluorobenzoyl)piperidine (**5**),[6,7] a structural fragment of the classical 5-HT_{2A} receptor blocking agent ketanserin[8] (Chart 1). In a first approach this observation led us to the synthesis and

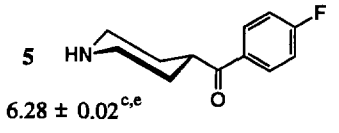
Chart 1. 5-HT_{2A} receptor affinity[a] of ondansetron (**1**), ketanserin and derivatives.



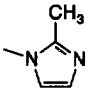
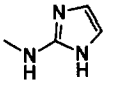
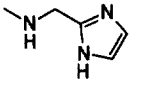
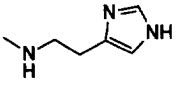
1 (ondansetron)
2 - **4**



ketanserin 9.55 ± 0.02[c,d]



6.28 ± 0.02[c,e]

R	compound	5-HT_{2A} affinity[a,b]
	**1**	4.66 ± 0.05
	**2**	6.73 ± 0.08
	**3**	6.59 ± 0.04
	**4**	6.75 ± 0.04

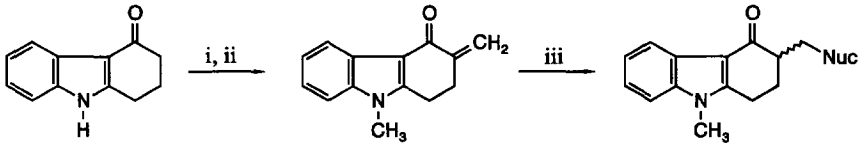
[a] Rat tail artery; pK_B ± s.e.m.; N = 4 - 6. [b] Ref. 4. [c] pA_2 ± s.e.; slope constrained to unity; N = 12 - 36. [d] pK_i = 8.46 *vs.* [^{3}H]ketanserin (rat frontal cortex); ref. 6. [e] pK_i = 6.40; ref. 6.

§ Dedicated to Prof. Dr. Dr. Walter Schunack, Berlin, on the occasion of his 60th birthday.

evaluation of potent 'hybrids' associating the tricyclic moiety of **1** with well-known 5-HT_{2A} affinity contributing molecular fragments, *e. g.*, substituted piperidines, piperazines and tetrahydropyridines.

1,2,3,9-Tetrahydro-4*H*-carbazol-4-one (**6**, Chart 2) was available by the reaction of phenylhydrazine with cyclohexane-1,3-dione.[9] The synthesis of the racemic final compounds **8** - **18** is outlined in Chart 2. The secondary amines HNuc used for the last step (for structures see Table 1) were either commercially available or prepared according to the literature, *viz.* HNuc for the preparation of **14**,[10] **15**,[11] and **16** - **18**.[12] The most potent compound (**13**)[13] was resolved *via* diastereomeric ditoluoyl hydrogentartrates.[14] HPLC analysis revealed that the enantiomeric excess of (+)- and (–)- **13** was ≥ 98 %.[15] The absolute configuration has not yet been assigned.

Chart 2. Synthesis of ondansetron analogous 5-HT_{2A} receptor antagonists.[a]



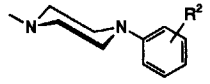
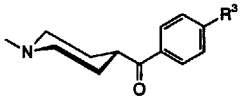
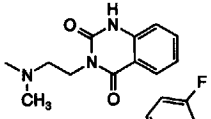
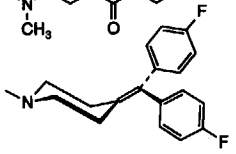
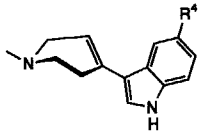
6 **7** **8 - 18**

[a] Experimental conditions: (i) $(MeO)_2SO_2$, KOH in Me_2CO/H_2O; 90 min reflux, yield 86 % (from MeOH) (ref. 30); (ii) $(H_2CO)_x$, conc. HCl in DMF, 6 h reflux, column chromatography (silica gel, CH_2Cl_2/EtOAc 9+1), yield 78 % (from $MeOH/H_2O$) (ref. 31); (iii) 1.33 eq HNuc (secondary amine, see Table 1) in dioxane/H_2O, 60 - 80 °C, yields 71 - 86 % (**8** - **15**) and 62 - 65 % (**16** - **18**); for isolation see note 32.

The functional antagonist affinity pattern of **8** - **18** was established with regard to the prominent affinities of ketanserin for 5-HT_{2A},[16] α_1,[17] and H_1[18] receptors, and of ondansetron for 5-HT_3 receptors[19] which trigger the neuronal release of enteric acetylcholine resulting in a M_3 receptor mediated contraction of ileal segments.[20] Affinity data are expressed as pK_B,[21] pA_2,[22,23] or pD'_2[21] values (Table 1). The new compounds display higher affinity for 5-HT_{2A}, α_1, and H_1 receptors than **1**. They represent only weak muscarinic M_3 antagonists, and except for **15**, 5-HT_3 antagonism is not measurable in the guinea-pig ileum assay.[20]

The interaction with (*R*)-phenylephrine at α_1 receptors (details not shown) and with 5-HT at 5-HT_{2A} receptors is of competitive nature for nearly all compounds, as depicted in Figure 1 for **12** and enantiomers of **13**. Replacement of the imidazole moiety of **1** by piperidine results in a significant increase of 5-HT_{2A} antagonism (**8**). This effect is enhanced by the additional introduction of aryl or aroyl substituents (**9**, **12**, **13**, **16**, **17**). Piperidine and piperazine ring are apparently bioisosteric (**9** ≈ **11**). Concerning the phenyl ring, a 4-fluoro atom is more favourable than 4-hydrogen or 2-fluoro substitution, the latter intensifying α_1 antagonist properties (**10**). Similar observations have been reported for quinazolinediones[7] related to ketanserin and naphthalenesultams[24] but not for silacyclopentane derivatives.[25] It is evident that the *quinazolinedione* but *not* the fluorobenzoylpiperidine moiety of ketanserin can be effectively mimicked by the tetrahydrocarbazolone part of **1**. The most potent derivative, (–)-**13**, and ketanserin display similar 5-HT_{2A}/α_1 receptor selectivity (19/1 *vs.* 29/1) while (–)-**13** is more favourable with regard to the interference with histamine H_1 receptors (5-HT_{2A}/H_1: 417/1 for (–)-**13** *vs.* 5/1 for ketanserin). In addition, the interaction of (–)-**13** and (+)-**13** with 5-HT_{2A} receptors is highly stereoselective (eudismic ratio ≥ 148/1). Comparable enantiomeric potency ratios have been reported previously for irindalone,[26] CV-5197 (a 1,5-benzoxathiepin)[27] and chain branched

Table 1. Physicochemical and *in vitro* pharmacological properties of racemic **8** - **18** and of enantiomers of compound **13**.

Nuc	compound	mp [°C]	antagonist affinities (mean ± s.e.m.)				
			5-HT_{2A}[a]	α_1[b]	H_1[c]	M_3[d]	5-HT_3[e]
 R^1 = H	**8**	139	5.98 ± 0.08	6.11 ± 0.11	5.69 ± 0.05	4.98 ± 0.03	<5.3[f]
R^1 = C_6H_5	**9**	159	8.43 ± 0.07	7.58 ± 0.07	6.40 ± 0.04	4.97 ± 0.05	<5.2
 R^2 = 2-F	**10**	162	7.80 ± 0.05	8.75 ± 0.05[g]	6.19 ± 0.08	4.72 ± 0.04	<5.2
R^2 = 4-F	**11**	181	8.60 ± 0.03[g,h]	7.80 ± 0.08	6.74 ± 0.06	5.55 ± 0.06	<5.5
 R^3 = H	**12**	185	8.34 ± 0.03[g]	7.36 ± 0.06	6.53 ± 0.04	5.01 ± 0.03	<5.5
R^3 = F	(±)-**13**	171	9.21 ± 0.05[g]	7.96 ± 0.02	6.92 ± 0.04	5.19 ± 0.06	<5.7
	(−)-**13**	171	9.36 ± 0.03[g]	8.08 ± 0.04	6.74 ± 0.05	n. d	n. d.
	(+)-**13**	171	7.19 ± 0.05[g]	7.28 ± 0.05	6.82 ± 0.06	n. d.	n. d.
	**14**	175-6	5.50 ± 0.05	6.45 ± 0.03	5.90 ± 0.02	4.68 ± 0.04	<5.7
	**15**	199	6.30 ± 0.11	5.81 ± 0.04	7.41 ± 0.07	5.70 ± 0.13	6.08 ± 0.04[i]
 R^4 = H	**16**	179-80[j]	8.20 ± 0.06[g]	7.58 ± 0.05	6.62 ± 0.05	n. d.	n. d.
R^4 = F	**17**	178[j]	7.22 ± 0.05	6.49 ± 0.08	6.60 ± 0.04	n. d.	n. d.
R^4 = OCH_3	**18**	155[j]	6.09 ± 0.09	6.59 ± 0.05	6.31 ± 0.05	n. d.	n. d.
	ketanserin		9.55 ± 0.02[g]	8.09 ± 0.03[g]	8.85 ± 0.08[g,k]	4.18 ± 0.08	n. d.
	ondansetron		4.66 ± 0.05	5.29 ± 0.05	4.37 ± 0.07	4.72 ± 0.06	7.01 ± 0.04[g,l]

[a] Rat tail artery; pK_B; N = 4 - 6. [b] Rat aorta; agonist (*R*)-phenylephrine; pK_B; N = 3 - 5; reference antagonist prazosine: pA_2 = 10.38. [c] Guinea-pig ileum; agonist histamine; pK_B; N = 4 - 12; reference antagonist mepyramine: pA_2 = 9.07. [d] Guinea-pig ileum; agonist carbachol; pK_B; N = 3 - 12; reference antagonist atropine: pA_2 = 9.11. [e] Guinea-pig ileum; pK_B; N = 3 - 5. [f] Partial agonist, see Figure 2. [g] pA_2 ± s.e.; slope constrained to unity (P > 0.05; $N \geq 12$) unless otherwise indicated. [h] Slope 1.08 ± 0.02. [i] pD'_2 value. [j] Decomposition. [k] Slope 0.82 ± 0.03. [l] Slope 0.87 ± 0.03. n. d. not determined.

derivatives of ketanserin.[28] The 5-HT_{2A} receptor affinity is attenuated when the aryl substituted 4-methylenepiperidine moiety of ritanserin,[11] a very potent 5-$HT_{2A/2C}$ blocker,[5] is introduced (**15**). Therefore structure-activity relationships for ritanserin-like compounds may differ from those observed for ketanserin-type 5-HT_{2A} antagonists with regard to the heteroaromatic moieties of the molecules. Derivatives of the mixed 5-HT_1/5-HT_2 receptor agonist RU 24969[12] (**18** and analogues **16**, **17**) are devoid of 5-HT_{2A} receptor stimulating properties and display structure-affinity relationships similar to recently communicated naphthalenesultam derivatives of RU 24969.[29]

Surprisingly, piperidine **8** turned out to be a partial 5-HT_3 receptor agonist. The contractile effect was susceptible to blockade by ondansetron,[2] a selective 5-HT_3 receptor antagonist (Figure 2).

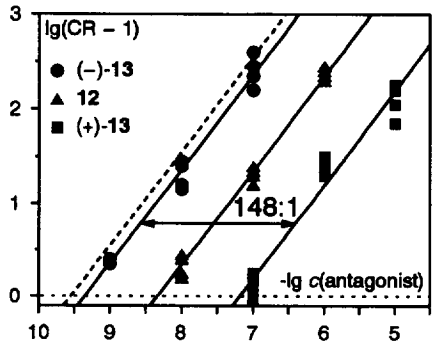


Figure 1. Competitive 5-HT_{2A} receptor antagonism of ketanserin (dashed line), **12** and enantiomers of **13** on rat tail artery[16] ($N \geq 12$) analyzed according to Schild.[22] Slopes of regression lines were not significantly different from unity and were therefore constrained to unity.[23] The large eudismic potency ratio of (−)-**13** and (+)-**13** is indicated by the arrow.

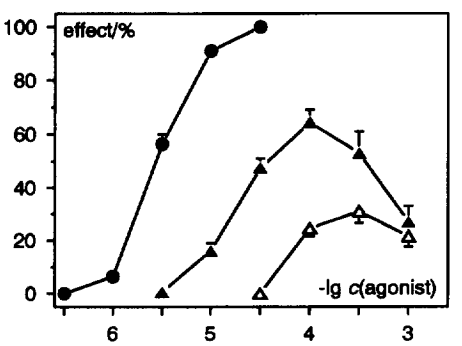


Figure 2. Contraction of isolated guinea-pig ileal longitudinal muscle strips by 5-HT (●, $N = 7$) and piperidine **8** in the absence (▲, $N = 7$) and presence of 0.5 μM ondansetron (Δ, $N = 7$). $pD_2 \pm$ s.e.m. was 5.56 ± 0.03 for 5-HT and 4.75 ± 0.04 for **8** (intrinsic activity 0.64 ± 0.05). Results for **8** may be underestimated due to the antimuscarinic properties of **8** (Table 1)[20] $pK_B \pm$ s.e.m. for ondansetron was estimated to be 6.77 ± 0.09.

Acknowledgement. We are indebted to W. Schunack, Berlin, for generous support and continued encouragement. H. Beer and K. T. Wanner, Munich, are thanked for their introduction into the HPLC technique. S. E. received a grant by Fonds der Chemie, Frankfort/Main.

References and Notes

1. Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. *Nature (London)* **1987**, *330*, 746.
2. Butler, A.; Hill, J. M.; Ireland, S. J.; Jordan, C. C.; Tyers, M. B. *Br. J. Pharmacol.* **1988**, *94*, 397.
3. For review see (a) Peroutka, S. J. *Pharmacol. Rev.* **1991**, *43*, 579, (b) *5-Hydroxytryptamine-3 Receptor Antagonists*; King, F. D.; Jones, B. J.; Sanger, G. J.; Eds.; CRC Press, Boca Raton, 1994.
4. Heil, W. L.; Elz, S.; Pertz, H. *Arch. Pharm. (Weinheim)* **1993**, *326*, 670.
5. For nomenclature of 5-HT receptor subtypes see Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A. *Pharmacol. Rev.* **1994**, *46*, 157.

6. Herndon, J. L.; Ismaiel, A.; Ingher, S. P.; Teitler, M.; Glennon, R. A. *J. Med. Chem.* **1992**, *35*, 4903.

7. Elz, S. *Synthesis and Functional In Vitro Pharmacology of Antiserotoninergic$_2$ Ketanserin Analogues and N-Imidazolylalkyl Substituted 4-(4-Fluorobenzoyl)piperidine-1-carboxamidines* XIIth International Symposium on Medicinal Chemistry, Sept. 13 - 17, 1992, Basle, Switzerland, poster P-128.C.

8. Van Nueten, J. M.; Janssen, P. A. J.; Van Beek, J.; Xhonneux, R.; Verbeuren, T. J.; Vanhoutte, P. M. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 217.

9. Clemo, G. R.; Felton, D. G. I. *J. Chem. Soc.* **1951**, 700.

10. Radwansky, A.; Elz, S.; Lehmann, J. *Arch. Pharm. (Weinheim)* **1993**, *326*, 243.

11. (a) Janssen Pharmaceutica N. V. (Kennis, L. E. J.; Vandenberk, J.; Mertens, J. C.; Invs.) E.P. 110,435 (29.09.1983) (b) Leysen, J. E.; Gommeren, W.; Van Gompel, P.; Wynants, J.; Janssen, P. F. M.; Laduron, P. M. *Mol. Pharmacol.* **1985**, *27*, 600.

12. Guillaume, J.; Durmont, C.; Laurent, J.; Nédélec, L. *Eur. J. Med. Chem.* **1987**, *22*, 33.

13. Prepared from **7** (3 mmol) and **5** (4 mmol) in dioxane/H_2O (1+1) (8 mL, 80 °C, 2 h); crystallization from MeOH; yield 83 % $C_{26}H_{27}FN_2O_2$ (anal.: C, H, N); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ [ppm] 8.10-7.98 (m, 3 H, carb-5-H, 2 phe-H), 7.53 (d, J = 8 Hz, 1 H, carb-8-H), 7.40-7.33 (m, 2 H, 2 phe-H), 7.28-7.18 (m, 2 H, carb-6-H, carb-7-H), 3.73, (s, 3 H, N-CH_3), 3.46-3.35 (m, partially superimposed, 1 H, pip-4-H), 3.11-2.92 (m, 3 H, ind-C<u>H</u>$_2$-CH_2-CH-CO, 1 pip-H), 2.86-2.81 (m, 1 H, 1 pip-H), 2.72-2.64 (m, 2 H, CO-C<u>H</u>-<u>H</u>CH-N), 2.55-2.45 (m, superimposed, 1 H, CO-CH-HC<u>H</u>-N), 2.28-2.20 (m, 2 H, ind-CH_2-<u>H</u>CH-CH-CO, 1 pip-H), 2.03-1.95 (m, 2 H, ind-CH_2-HC<u>H</u>-CH-CO, 1 pip-H), 1.82-1.72 (m, 2 H, 2 pip-H), 1.65-1.55 (m, 2 H, 2 pip-H); MS (EI, 70 eV): *m/z* 418 ($M^{\bullet+}$, 21 %), 56 (100 %).

14. A hot solution of (+)-(2*S*,3*S*)-*O,O'*-ditoluoyltartaric acid (2.4 mmol) in EtOH (30 mL) was added to a hot solution of **13** (2.3 mmol) in EtOH (150 mL) and left to crystallize. After 2 further crystallizations from DMF/H_2O (2+1) (60 mL, 45 mL) the 1:1 salt $C_{46}H_{45}FN_2O_{10}$ (anal.: C, H, N) was isolated (yield 29 %, mp 166 °C (dec.), $[\alpha]^{20}_D$ +43° (β = 0.5 g/100 mL; DMF)). Treatment with CH_2Cl_2/aqueous $NaHCO_3$ (w = 0.05) gave the free base of (−)-**13** (anal.: C, H, N, yield 96 %, mp 171 °C, $[\alpha]^{20}_D$ −25° (β = 0.5 g/100 mL; MeOH)). From the combined mother liquors the enantiomeric salt was similarly obtained with (−)-(2*R*,3*R*)-*O,O'*-ditoluoyltartaric acid (yield 39 %, mp 166 °C (dec.), $[\alpha]^{20}_D$ −43°). Liberation of the base afforded (+)-**13** (97 %, mp 171 °C, $[\alpha]^{20}_D$ +25°).

15. HPLC conditions: ChiraDex™ column (5 µm 250-4, Merck no. 51333), eluent MeOH/H_2O/triethylamine (40+58+2 (*V/V*)), 0.6 mL/min, UV detection 300 nm, injection of 20 µL (0.2 mg base/mL MeOH); retention times 18.6 min ((+)-**13**) and 24.7 min ((−)-**13**), *ee* ≥ 98 % for both. Stock solutions (0.01 M) of the hydrochlorides in EtOH/H_2O (1+1) used for the bioassays were enantiomerically stable for at least 4 weeks when stored in a freezer.

16. In brief, cylindrical segments (4 - 6 mm) of rat tail artery were suspended horizontally between L-shaped stainless steel hooks (isometric, resting force 5 mN, modified Krebs-Henseleit solution, 37 °C). During an equilibration period of 120 min tissue viability was checked by a priming dose of 5-HT (1 µM). Up to 3 concentration-effect curves were obtained in one preparation (cumulative technique) in the continuous presence of cocaine (6 µM) and prazosine (30 nM). Antagonists were incubated for 30 or 60 min. For further details see ref. 17.

17. Pertz, H.; Eich, E. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, *345*, 394.

18. Isolated guinea-pig ileal whole segments, isotonic (preload 5 mN), Tyrode solution containing 0.1 µM atropine, 37 °C, cumulative technique, up to 4 concentration-effect curves in one preparation, incubation of antagonists 10 min.

19. The method of Buchheit, K.-H.; Engel, G.; Mutschler, E.; Richardson, B. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *329*, 36 was modified as follows: Tyrode solution with 1 µM methysergide, 1 µM cho-

line, and 10 μM 5-methoxytryptamine for the desensitization of 5-HT_4 receptors, 2 initial priming doses of 5-HT (3 μM), 15 min dose cycle (non-cumulative), 2 concentration-effect curves, incubation of antagonists 30 min.

20. M_3 receptor affinity was determined as described under note 18 in the absence of atropine (agonist: carbamoylcholine). Concentrations of 5-HT_3 agonists and antagonists that block ileal muscarinic M_3 receptors on the smooth muscle cell, mask their interference with neuronal 5-HT_3 receptors and therefore render a precise 5-HT_3 affinity determination impossible in the range of 10 - 100 μM. A 5-HT_3 radioligand binding assay is not yet available in our laboratory.

21. Van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299.

22. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48.

23. Miesch, F.; Turlot, J.-C.; Ehrhardt, J.-D.; Schwartz, J. *J. Pharmacol. (Paris)* **1977**, *8*, 27.

24. Malleron, J.-L.; Comte, M.-T.; Guérémy, C.; Peyronel, J.-F.; Truchon, A.; Blanchard, J.-C.; Doble, A.; Piot, O.; Zundel, J.-L.; Huon, C.; Martin, B.; Mouton, P.; Viroulaud, A.; Allam, D.; Betschart, J. *J. Med. Chem.* **1991**, *34*, 2477.

25. Damour, D.; Barreau, M.; Dutruc-Rosset, G.; Doble, A.; Piot, O.; Mignani, S. *BioMed. Chem. Lett.* **1994**, *4*, 415.

26. Bøgesø, K. P.; Arnt, J.; Boeck, V.; Christensen, A. V.; Hyttel, J.; Gundertofte Jensen, K. *J. Med. Chem.* **1988**, *31*, 2247.

27. Kori, M.; Kamiya, K.; Kurihara, E.; Sugihara, H. *Chem. Pharm. Bull.* **1991**, *39*, 922.

28. Elz, S. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, R100.

29. Malleron, J.-L.; Guérémy, C.; Mignani, S.; Peyronel, J.-F.; Truchon, A.; Blanchard, J.-C.; Doble, A.; Laduron, P.; Piot, O.; Zundel, J.-L.; Betschart, J.; Canard, H.; Chaillou, P.; Ferris, O.; Huon, C.; Just, B.; Kerphirique, R.; Martin, B.; Mouton, P.; Renaudon, A. *J. Med. Chem.* **1993**, *36*, 1194.

30. Ballantine, J. A.; Barrett, C. B.; Beer, R. J. S.; Boggiano, B. G.; Eardley, S.; Jennings, B. E.; Robertson, A. *J. Chem. Soc.* **1957**, 2227.

31. Glaxo Group Ltd. (Coates, I. H.; Bell, J. A.; Humber, D. C.; Ewan, G. B.; Invs.) G.B. 2,153,821 (29.08.1985).

32. Isolation by column chromatography on silica gel (Merck) (**14**: CH_2Cl_2/MeOH/aqueous NH_3 (w = 0.25) (90+9.5+0.5 (*V/V*)); **16** - **18**: CH_2Cl_2/MeOH/aqueous NH_3 (w = 0.25) (94.5+5+0.5 (*V/V*))) and/or by crystallization. Recrystallization from MeOH/H_2O (**8**), MeOH (**9** - **13**), absol. EtOH (**14**), CH_2Cl_2/Et_2O (**15**).

(*Received in Belgium* 28 *November* 1994; *accepted* 2 *February* 1995)