Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2457–2462

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

FUNCTIONAL CHARACTERISTICS OF A SERIES OF N4-SUBSTITUTED 1-(2,3-DIHYDRO-1,4-BENZODIOXIN-5-YL)PIPERAZINES AS 5-HT$_{1A}$ RECEPTOR LIGANDS.

STRUCTURE-ACTIVITY RELATIONSHIPS.

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Received 20 May 1998; accepted 22 July 1998

Abstract: The agonistic/antagonistic profile of a series of 10 N4-substituted1-(2,3-dihydro-1,4-benzodioxin -5-yl)piperazines is evaluated in the *in vitro* adenylyl cyclase assay. The profile is severely affected by the characteristics of the N4-substituents ranging from full agonism (benzamidoethyl derivative **1**), mixed agonism/antagonism (phthalimidobutyl derivative **7**) to predominantly antagonism (saccharinbutyl derivate **9**). A novel full antagonist **10**, as potent as WAY 100635, is obtained by substitution of Cl at C-7 of the benzodioxinyl moiety in **9**.

Introduction: For the 5-HT$_{1A}$ receptor many potent ligands are available. Most of these ligands are agonists or partial agonists. Full antagonists are rare (for review see[1]). For the 5-HT$_{1A}$ receptor ligands a great number of structure-affinity relationships have been published. But there is a paucity of published structure-activity relationships studies[2]. In this paper the structural features for agonism, partial agonism and antagonism are presented of a series of N4-substituted benzodioxinyl piperazines using the adenylyl cyclase assay in CHO cells stably expressing the human 5-HT$_{1A}$ receptor. The reference compounds flesinoxan, a full agonist, ipsapirone, a partial agonist and WAY 100635, a pure antagonist are included in the study.

The affinity of the compounds for the 5-HT$_{1A}$ receptor is assessed in rat frontal cortex membranes.

Chemistry: The synthesis of the N4-substituted arylpiperazines **1, 2, 3, 4, 6** and **10** was published[3-5]. The synthesis of the novel arylpiperazines **5, 7, 8, 9** was carried out as outlined in the scheme. Physicochemical data of **5, 7, 8, 9**[6].

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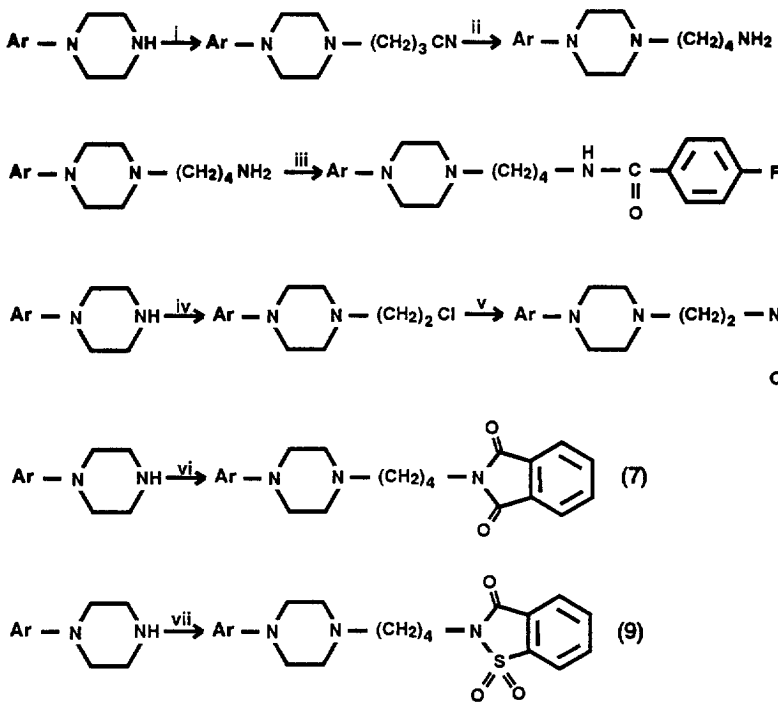
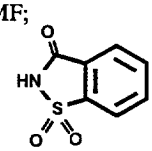
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00406-5



Scheme. reagents i.$Cl(CH_2)_3CN/(iPr)_2NEt/CH_3CN$); ii $LiAlH_4$ /THF;
iii. p-fluorobenzoylchloride /CH_2Cl_2; iv. $Br(CH_2)_2Cl/NaOH/CH_2Cl_2$;
v. NaH/DMF/saccharin; vi. N-(4-bromobutyl) phthalimide /K_2CO_3 /DMF;
vii. N-(4-bromobutyl) saccharin /K_2CO_3 /MEK.
Ar = 1-(2,3-dihydro-1,4-benzodioxin. 5-yl) piperazine; saccharin =

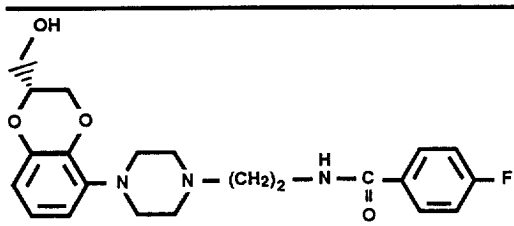
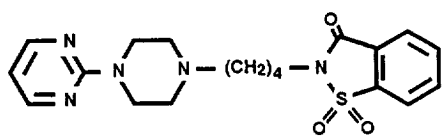
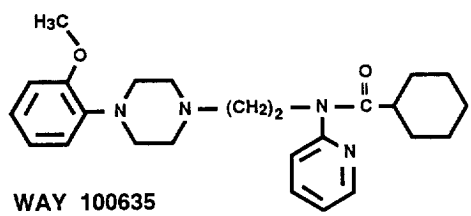


Pharmacology: The agonistic / antagonistic profile of the compounds were evaluated by the adenylyl cyclase assay in CHO cells stably expressing the human 5-HT_{1A} receptor[7]. The radioligand binding studies were carried out on rat frontal cortex membranes using [^{3}H]-8-OH-DPAT as radioligand[8].

Results: The intrinsic activity and potency of the reference 5-HT_{1A} receptor ligands are summarized in Table 1`. In the adenylyl cyclase assay flesinoxan acts as a full agonist. Ipsapirone is a partial agonist, displaying no antagonistic properties at concentrations up to 10^{-6} M. WAY 100635 is a potent full antagonist. The results of the N4-substituted benzodioxinylpiperazines **1-10** are shown in Table 2. Compound **1**, a close analogue of flesinoxan, is a potent full agonist. The corresponding butyrophenone derivatives **2** and **3**, however are only partial agonists with intrinsic activities of 50%, 48% and 71%, 67% respectively. The compounds display low efficacies as antagonists. Replacement of the carbonyl group of **3** by methylene (**4**) changes the pharmacological profile from predominantly agonistic (**3**) to predominantly

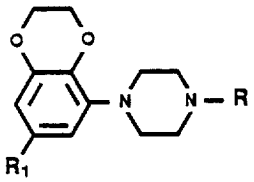
antagonistic (4). Compound 4 is a potent mixed agonist /antagonist. These results show that in this series the presence of a secondary amide function is necessary for full agonism. Lengthening the side chain of 1 by two carbon atoms (5) reduces the intrinsic activity to 81%, 85%. The potency decreases by a factor of 24. The phthalimidoethyl derivative 6 displays neither consistent agonistic nor antagonistic activity at the maximal concentration of 10^{-6} M.

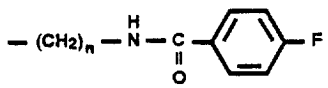
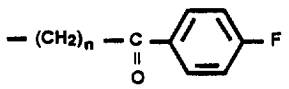
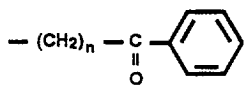
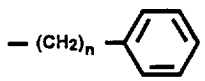
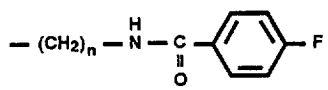
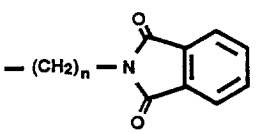
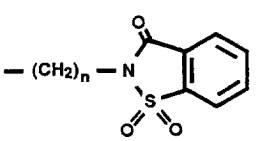
Table 1: Potency (pEC_{50}, pA_2), Agonistic and Antagonistic efficacy and Inhibition Constants (pKi) of 5-HT_{1A} Receptor Reference Compounds. ± SEM between brackets.

	pEC_{50}	%ago	pA_2	%anta	pK_i
 FLESINOXAN	8.18 (0.19)	100		0	8.77 (0.05)
 IPSAPIRONE	7.17 (0.20)	69		0	8.26 (0.06)
 WAY 100635		0	9.69 (0.05)	100	9.30 (0.07)

Lengthening of the alkyl chain of 6 by two carbon atoms results in 7, a partial agonist with high potency as agonist as well as antagonist. The saccharinethyl derivative 8, is a full agonist with low potency. Lengthening of the alkyl chain of 8 by two carbon atoms changes the profile completely as 9 is a highly

Table 2: Potency (pEC_{50}, pA_2), Agonistic and Antagonistic Efficacy and Inhibition Constants (pKi) of N_4 - Substituted benzodioxinylpiperazines for 5-HT_{1A} receptors. ± SEM between brackets.



R	n	R_1	no	pEC_{50}	%ago	pA_2	%anta	pK_i
	2	H	1	9.02 9.07	97 97		0 0	9.53 (0.12)
	3	H	2	8.28 8.13	50 48		22 42	8.87 (0.26)
	3	H	3	8.54 8.55	71 67		33 18	9.17 (0.05)
	4	H	4	8.17 8.22	45 50	7.82 7.60	74 70	9.49 (0.00)
	4	H	5	7.90 7.50	81 85		17 0	8.98 (0.24)
	2	H	6		0 30		0 2	7.50 (0.11)
	4	H	7	8.87 8.24	44 49	9.18 9.17	55 62	9.64 (0.11)
	2	H	8	6.60 6.20	97 86		0 0	8.91 (0.28)
	4	H	9		11 23	10.1 9.44	68 65	9.54 (0.21)
	4	Cl	10		0 0	10.08 9.54	100 100	9.15 (0.08)

potent antagonist (pA_2=10.1, 9.44) with an efficacy of 68%,65% and without significant agonistic properties.

Full antagonism is obtained by substitution in **9** with a chloro atom at the 7-position of the benzodioxinyl moiety (**10**). Compound **10** is as potent as the reference compound WAY 100635.

The linear chain analogues **1** - **5** and the cyclic imides **7** - **10** display a high affinity for the rat 5-HT_{1A} receptor(Table 2). Agonists, mixed agonists/antagonists and antagonists bind equally well. The presence of a secondary amide function is not a prerequisite for high affinity. The high potency of the saccharinethyl derivative **8** in the binding studies is in contrast to the low potency in the adenylyl cyclase test. This discrepancy can not be explained easily. It is possible that **8** has a higher affinity for the rat 5-HT_{1A} receptor than for the human 5-HT_{1A} receptor.

Summarizing, the characteristics of the N4-substituent at the benzodioxinylpiperazine have a large effect on the agonistic/antagonistic profile of the compounds. The N4-benzamidoethyl derivative **1** is a potent full agonist, the N4-phenylbutyl **4** and N4-phthalimidobutyl **7** derivatives are potent mixed agonists / antagonists and the N4-saccharinbutyl derivative **9** is predominantly antagonistic.

A novel potent full 5-HT_{1A} receptor antagonist is the 7-chlorobenzodioxinyl piperazine analogue of **9** compound **10**.

References and Notes:

1. van Wijngaarden, I.; Soudijn, W.; Tulp, M.Th.M. *Serotonin Receptors and their Ligands*; Olivier, B., van Wijngaarden I, Soudijn, W., Eds.; Elsevier Science Publisher; Amsterdam **1997**, Pharmacochemistry Library Vol.. 27, pp 17-44.
2. Yasunaga, T.; Naito, R.; Kontani, T.; Tsukamoto, S.; Nomura, T.; Yamaguchi, T., Mase, T. *J. Med. Chem.* **1997**, 40, 1252.
3. van Steen, B.J.; van Wijngaarden, I.; Tulp, M.Th.M.; Soudijn, W. *J.Med. Chem.* **1994**, 37, 2761.
4. van Steen, B.J.; van Wijngaarden, I.; Tulp, M.Th.M.; Soudijn, W. *J. Med. Chem.* **1995**, 38, 4303.
5. Hartog, J.; van Steen, B.J.; Mos, J.; Schipper, J. **1995**, EP 0633260 A1.
6. Compound **5**: $C_{23}H_{28}F_1N_3O_3.HCl.0.25H_2O$, m.p.201-2°C; compound **7**: $C_{24}H_{27}N_3O_4$, m.p.128-30°C; compound **8**: $C_{21}H_{23}N_3O_5S_1.HCl$, m.p.289-92°C; compound **9**:$C_{23}H_{27}N_3O_5S_1$. $0.10H_2O$, m.p. 146-9°C.
7. Adenylyl cyclase assay: Chinese hamster ovary cells (CHO cells) in which the human 5-HT_{1A} receptor was stably expressed were grown in α-Delbucco's modified Eagle medium (α-DMEM) supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 1 muM pyruvate 5000 units/mL penicillin and 5000 mg/ml streptomycin at 37°C in 93% air/7% CO_2. Cells grown to confluency in 24 wells plates were loaded with 1 muCi [^{3}H]-adenine in 0.5 mL culture medium per well and incubated for 2h at 37°C. After incubation the cells were washed with 0.5 mL phosphate buffered saline (PBS) containing 1 mM isobutylmethylxanthine (IBMX, phosphodiesterase inhibitor) and reincubated for 10 min with 0.5 mL PBS, containing 1 mM IBMX, 0.1 muM forskolin in the presence or absence of the test compound. Each test was performed in four fold. The reaction was stopped after aspiration by 1 mL of ice cold 5% trichloroacetic acid solution containing 1.1 mM of ATP and 1.0 mM c-AMP. The plates are centrifuged for 10 min at 250xg. Sequential chromotography on a 1 mL Dowex (50WX4 200-400 mesh cationic exchange resin) column and a 600 mg aluminumoxyde column permitted the separation of [^{3}H]ATP and [^{3}H]c-AMP. 0.8 mL supernatant followed by 2 mL ultrapure water (UW) was

percolated through the Dowex column and collected in 6 mL Instagel for liquid scintillation counting ([^{3}H]ATP fraction). Thereafter the Dowex column was coupled to the aluminumoxyde column and the columns were rinsed with 4x 1 mL of UW. After uncoupling, the aluminumoxyde column was eluted with 0.1 M imidazole.HCl solution, pH=7.5. The eluate was collected in 6 mL Instagel for liquid scintillation counting ([^{3}H]c-AMP fraction). The recovery of [^{3}H]c-AMP from the sequential column chromatography was determined by parallel chromatography of a stock solution of 0.8 mL stopping mixture spiked wit 0.1 muM [^{3}H]c-AMP.

Data handling. The [^{3}H]c-AMP produced by forskolin stimulation of adenlylyl cyclase is calculated as the ratio corrected for basal production of [^{3}H]c-AMP and fixed at 100%.

$$\%\text{conversion} = \frac{[^3\text{H}]\text{cAMP}}{[^3\text{H}]\text{cAMP} + [^3\text{H}]\text{ATP}} * 100\%$$

Agonistic potency. Inhibition of the forskolin stimulated production of c-AMP by the compounds was assessed from concentration-effect relationship curves using the non-linear curve fitting program INPLOT (GraphPad Software, San Diego, Ca.) and expressed as pEC_{50} values. The mean value for four observations per data point was used for the construction of the curves and the pEC_{50} values were obtained from two independent experiments. The intrinsic activity (=efficacy) of a compound is expressed as a percentage of its maximal effect relative to the maximal effect of 8-OH-DPAT.

Antagonist potency. Antagonist potency of a compound was assessed by its ability to concentration dependently antagonize the inhibition of forskolin stimulated c-AMP production by 10^{-6} M 8-OH-DPAT.

The IC_{50} values obtained were converted to pA_2 values using the Cheng-Prusoff equation for competitive interactions:

$$pA_2 = -\log IC_{50} + \log (1 + L / EC_{50})$$

were L is the concentration and EC_{50} is the median effective dose of the ligand used. Full antagonists block the effect of 8-OH-DPAT for 100%. The efficacy of a compound is expressed as a percentage ranging from 0-100%.

8. Gozlan, H.; El Mestikawy, S.; Pichet, L.; Glowinski, J.; Hamon, M. Nature **1983**, 305, 140.