Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 243–248

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Structure–Activity Relationship of 2-(Aminoalkyl)-2,3,3a,8-tetrahydrodibenzo[*c*,*f*]isoxazolo[2,3-*a*]azepine Derivatives: A Novel Series of 5-$HT_{2A/2C}$ Receptor Antagonists. Part 1

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Received 26 July 2001; revised 18 October 2001; accepted 30 October 2001

Abstract—The synthesis of a series of novel 2-(aminoalkyl)-2,3,3a,8-tetrahydrodibenzo[*c*,*f*]isoxazolo[2,3-*a*]azepine derivatives as well as their 5-$HT_{2A/2C}$ and H_1 receptor binding affinities are described. The in vivo activity as potential anxiolytics of the synthesised compounds was measured in a mCPP challenge test. One of the compounds, **2a**, proved to be a potent 5-$HT_{2A/2C}$ receptor antagonist showing as well oral activity and therefore could be considered as a potential anxiolytic/antidepressant agent.

Introduction

The neurotransmitter serotonin (5-HT) induces a variety of effects that are mediated by specific receptors. These receptors can be subdivided in seven families, 5-$HT_{1–7}$, comprising a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes.[1] The 5-HT_2 receptor family currently accommodates three receptor types, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which are similar in terms of their molecular structure, pharmacology and signal transduction pathways.[2–4] The 5-HT_{2A} receptor is widely distributed, both in peripheral tissues and in the CNS. In contrast, the 5-HT_{2C} receptor has been found only in the CNS, being highly expressed in many regions of the mammalian brain including the choroid plexus and the limbic and basal ganglia structures.[1]

The 5-HT agonist *m*-chlorophenylpiperazine (mCPP) is often used as a probe in clinical studies to challenge brain 5-HT functions in humans.[5] The compound decreases slow-wave sleep[6] and induces symptoms of anxiety, arousal, nervousness, distress and headache in volunteers.[7–12] mCPP has been given as a challenge to patients with obsessive–compulsive disorder,[13,14] panic disorder,[15,16] seasonal affective disorder[17] and major depression.[18,19] In animals, mCPP also induces symptoms of anxiety in various animal models.[20–24] As this mCPP-induced anxiety seems to be mediated via the 5-HT_{2C} receptor, 5-HT_{2C} antagonists thus might have potential value in the treatment of anxiety and depression.[25,26]

In recent years, we started a programme at Janssen Research Foundation searching for potent, centrally active 5-HT_{2C} receptor antagonists that are able to antagonise mCPP-induced anxiety in rats. As a first result of our synthesis programme, R95292 (**1**) was found to display high affinity in vitro for both 5-HT_{2A} and 5-HT_{2C} receptors.[27a] Unfortunately, R95292 was not active in vivo in our mCPP challenge test. Nevertheless, we decided to proceed with a systematic chemical modification programme of this lead, in order to find potent and selective 5-$HT_{2A/2C}$ antagonists that were active against mCPP-induced anxiety in rats. We now report on the synthesis and structure–activity relationship of a

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0960-894X/02/$ - see front matter
PII: S0960-894X(01)00721-1

series of 2-(aminoalkyl)-2,3,3a,8-tetrahydrodibenzo[*c*,*f*]-isoxazolo[2,3-*a*]azepine derivatives (**2**) as novel 5-$HT_{2A/2C}$ antagonists, and therefore potential anxiolytics/antidepressants.[27b] These compounds are structurally related to mianserin (**3**),[28] a well known 5-$HT_{2A/2C}$ and α_2-adrenoceptor antagonist as well as histamine-H_1 antagonist, which shows also activity in our mCPP challenge test (Fig. 1).[22]

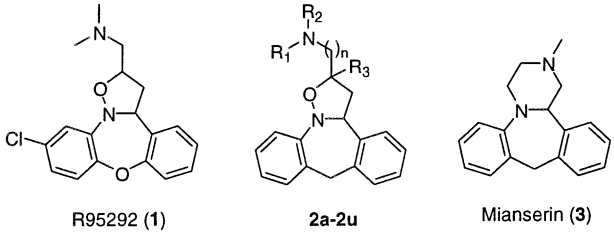


Figure 1.

Chemistry

2,3,3a,8-Tetrahydrodibenzo[*c*,*f*]isoxazolo[2,3-*a*]azepines **2**, bearing different aminoalkyl chains in the 2-position, were synthesised by cycloaddition of 11*H*-dibenz-[*b*,*e*]azepine-5-oxide (morphanthridine-5-oxide, **6**) with various alkenylamines, as illustrated in Scheme 1. This *N*-oxide had been previously described in literature by oxidation of 11*H*-dibenz[*b*,*e*]azepine (morphanthridine, **4**; **CAUTION**: skin irritant) with *m*-chloroperbenzoic acid using chloroform and diethyl ether as solvents, in 24% yield.[29] We could increase the yield up to 78% by oxidation of its dihydro-analogue, 5,6-dihydromorphanthridine (**5**),[30] using *m*-chloroperbenzoic acid in dichloromethane at room temperature. We decided to explore this reaction using other known reagents for the oxidation of secondary amines to nitrones. The highest yield and mildest reaction conditions resulted when we used 3-phenyl-2-(phenylsulfonyl)oxaziridine (Davis' reagent) as oxidant.[31] Thus, reaction of **5** with Davis' reagent in dichloromethane at room temperature afforded **6** in 87% yield, after chromatographic purification.

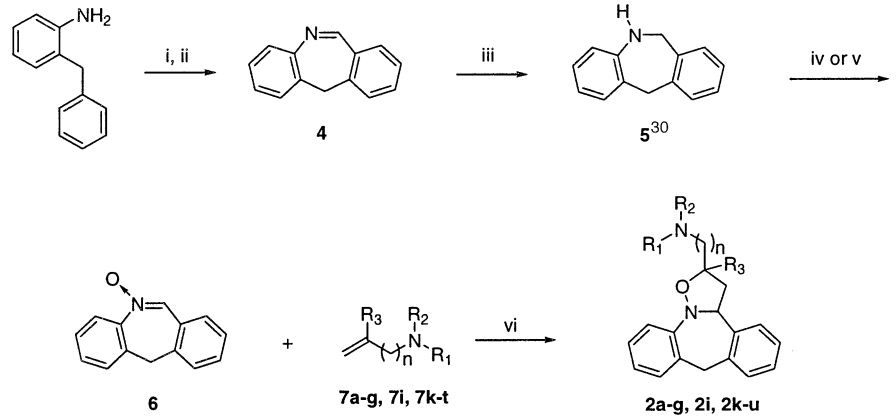
The synthesis of the tricyclic precursor 6,11-dihydro-5*H*-dibenz[*b*,*e*]azepine (5,6-dihydromorphanthridine, **5**) was initially achieved using one of the methods described in literature.[30] Formylation of 2-benzylaniline with formic acid, followed by the Bischler–Napieralski reaction in the presence of polyphosphoric acid and phosphorus oxychloride, afforded morphanthridine (**4**), which after catalytic hydrogenation gave compound **5** in 52% overall yield (Scheme 1). We have developed a new and more efficient synthesis of **5**, which is illustrated in Scheme 2. Condensation of *o*-aminobenzylalcohol with benzaldehyde gave 2-phenyl-1,4-dihydro-*2H*-benz[*d*][1,3]-oxazine (**8**) in quantitative yield. Reductive cleavage with $NaBH_4$ in ethanol afforded [2-benzylaminophenyl]methanol (**9**), which was cyclised to the desired 5,6-dihydromorphanthridine (**5**) with sulphuric acid using dichloromethane as co-solvent, the overall yield of these three steps being 85%.

Cycloaddition of **6** with different alkenylamines **7** gave compounds **2a**–**g**, **2i**, and **2k**–**u**, as it is depicted in Scheme 1. It is noteworthy that only in the case of the cycloaddition of **6** with **7k** ($R_3 = CH_3$) both stereoisomers, **2k** (*cis*) and **2l** (*trans*), were obtained in a reasonable yield. In the rest of the reactions only the *cis* isomers were isolated and traces of the *trans* isomers were detected occasionally. The relative configuration of the chiral centres was determined by means of NOE difference experiments on the protons of the isoxazolidine ring. When compound **2a** was resynthesised in large scale for further biological testing, the corresponding *trans* isomer **2u** could be also isolated in less than 1% yield.

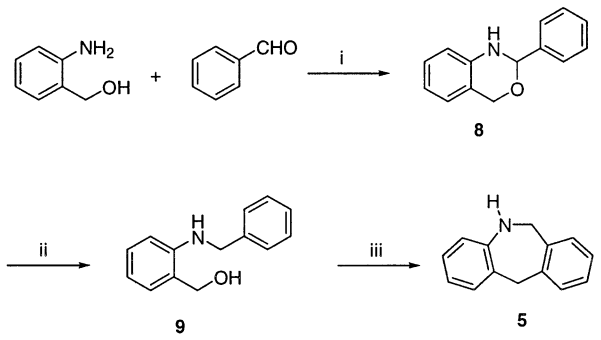
Compounds **2h** and **2j** were prepared from **2g** and **2i**, by deprotection of the nitrogen atom using hydrazine hydrate in ethanol in the former case, and sodium hydroxide in a mixture water/methanol, in the latter (Scheme 3).

Biological Results and Discussion

The affinities of the compounds for the 5-HT_2 receptors were measured by means of radioligand binding studies



Scheme 1. Reagents and conditions: (i) HCOOH, reflux, 2 h, 82%; (ii) PPA, $POCl_3$, 120 °C, 12 h, 70%; (iii) H_2, Pd/C (10%), CH_3OH, rt, 91%; (iv) mCPBA, CH_2Cl_2, rt, 2 h, 78%; (v) 3-phenyl-2-(phenylsulfonyl)oxaziridine, CH_2Cl_2, rt, 2 h, 87%; (vi) toluene, 100–120 °C, sealed tube, 16 h, 28–79%.

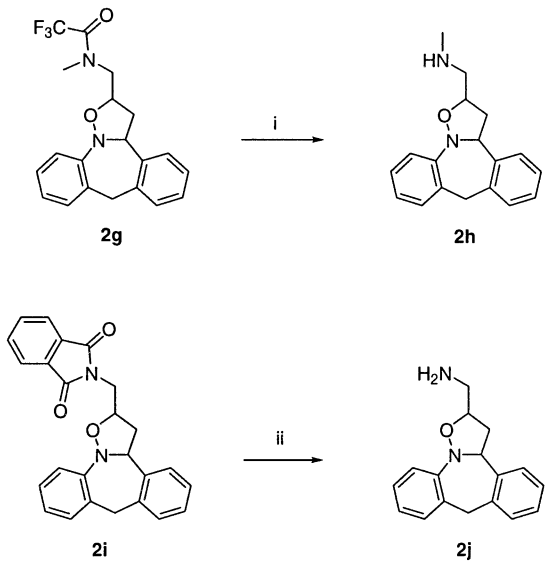


Scheme 2. Reagents and conditions: (i) $ClCH_2COOH$ (cat), $(CH_3)_2CHOH$, rt, 16 h, quant; (ii) $NaBH_4$, CH_3CH_2OH, reflux, 1 h, quant; (iii) H_2SO_4, CH_2Cl_2, −40 °C to rt, 85%.

conducted with: (a) human cloned 5-HT_{2A} receptor, expressed in L929 cells using [^{125}I]R91150 as radioligand[32] and (b) human cloned 5-HT_{2C} receptor, expressed in CHO cells using [^{3}H]mesulergine as radioligand.[33] The experiments to measure the affinities for the H_1 receptor were conducted with human cloned H_1 receptors expressed in CHO cells, using [^{3}H]pyrilamine as radioligand.[32] The affinities of the compounds for other serotonergic as well as dopaminergic and adrenergic receptors were also measured. All these compounds interact most predominantly with the 5-HT_2 and the H_1 receptors. The experiments to measure the in vivo activity of the compounds in our mCPP challenge test were performed in male Wistar rats, weighing between 200 and 220 g, following the method described by Meert and co-workers.[22] The test compounds were administered subcutaneously or orally or both, depending on the availability and solubility of the compounds as well as on the preliminary activity found.

Antagonistic effects on the H_1 receptor are not associated with therapeutic efficacy in depression or anxiety, but may contribute to potential sedative effects of those drugs.[34] Therefore, this possibility should be taken into account for the future development of any of these compounds. Table 1 shows the affinities for the 5-HT_{2A}, 5-HT_{2C} and H_1 receptors of the synthesised compounds as well as their activity in our mCPP challenge test.

Replacement of the oxygen atom present in the 8-position of the tricyclic system of our initial lead R95292 (**1**), by the methylene group present in the dimethylaminomethyl analogue **2a**, resulted in a dramatic increase of in vivo activity. This improvement was not related to a better in vitro affinity for the 5-HT_2 receptors, as can be deduced from the values shown on the table. Only a significant increase of affinity for H_1 receptor was observed when comparing **2a** with R95292. The receptor binding profile of **2a** was very similar to that of mianserin (**3**), but our compound was much more potent in the mCPP challenge test, both subcutaneously and orally. Elongation of the spacer between the dimethylamino moiety and the isoxazolidine ring (**2b–d**) was detrimental for in vivo activity, although compounds **2b** and **2c** did not lose so dramatically the in vitro affinities for the 5-HT_2 receptors. The basic nature of the nitrogen atom at that particular distance from the



Scheme 3. Reagents and conditions: (i) NaOH, CH_3OH/H_2O (5:1), 60 °C, 3 h, 82%; (ii) NH_2–NH2·H_2O, CH_3CH_2OH, reflux, 4 h, 22%.

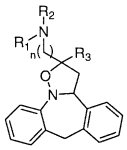
tetracyclic system was proved to be essential for activity: the amide analogue **2o** was completely inactive.

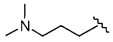
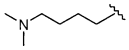
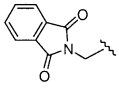
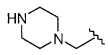
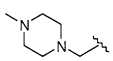
The methylaminomethyl- and aminomethyl-analogues **2h** and **2j**, respectively, showed a quite similar in vitro profile, although affinity for H_1 receptor in the case of **2j** was much lower. However, neither of these two compounds showed significant activity in the mCPP test. The introduction of bulkier substituents on the nitrogen was detrimental for both in vitro as well as in vivo activities. Only the diethylamino derivative **2e** kept the levels of receptor binding affinity while retaining some activity in the mCPP test as well. Even its pyrrolidine analogue **2m** lost the in vivo activity. Also, the piperazine derivatives prepared, with a second basic nitrogen, were inactive.

As was described in the Chemistry section, two stereoisomers were obtained having an additional exocyclic methyl group in the 2-position of the dibenzoisoxazoloazepine system. The *cis* isomer **2k** showed affinity for receptors comparable to that of compound **2a**, but was inactive in the mCPP test. On the other hand, the *trans* isomer **2l** was devoid of in vitro activity as well. Finally compound **2u**, the *trans* isomer of the most active compound **2a**, proved also to be completely inactive.

There are substantial species differences for 5-HT_2 receptors, which might be up to two orders of magnitude for K_i values at 5-HT_{2A} between human and rat receptor. This fact may well explain the apparent discrepancies between the high binding affinity of some compounds and their lack of in vivo activity. Table 2 shows the binding affinity for several other serotonergic, dopaminergic and adrenergic receptors of mianserin and compounds **2a**, **2b**, **2h**, which were the only ones showing some in vivo activity. As all these compounds interact predominantly with 5-HT_2 and H_1 receptors, and having in mind that selective H_1 antagonists were completely inactive in our mCPP challenge test, it seems

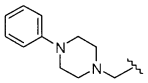
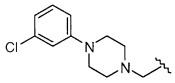
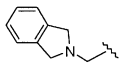
Table 1. 5-$HT_{2A/2C}$ and H_1 affinities and activity in mCPP challenge test of 2-(aminoalkyl)-2,3,3a,8-tetrahydrodibenzo[*c*,*f*]isoxazolo[2,3-*a*]azepine derivatives



Compd		$5HT_{2A}$ pIC_{50}	$5HT_{2C}$ pIC_{50}	H_1 pIC_{50}	mCPP (sc) ED_{50} (mg/kg)	mCPP (po) ED_{50} (mg/kg)
1	Ref	8.72	8.42	6.99	> 2.5	> 2.5
3		7.98	8.13	8.47	0.16	2.5
2a		7.64	7.91	8.17	0.04	0.63
2b		7.04	7.43	7.08	2.5	2.5
2c		8.41	7.73	6.17	n.t.	> 2.5
2d		< 6	< 6	< 6	n.t.	> 2.5
2e		7.37	7.84	7.87	n.t.	2.5
2f		< 6	6.49	6.82	n.t.	> 2.5
2g		< 6	6.16	< 6	n.t.	> 2.5
2h		7.88	8.15	7.97	0.16	> 2.5
2i		< 6	< 6	< 6	n.t.	> 2.5
2j		7.80	8.25	6.7	n.t.	> 2.5
2k *cis*		7.24	7.86	8.78	n.t.	> 2.5
2l *trans*		< 6	6.03	7.21	> 2.5	> 2.5
2m		7.60	7.79	8.52	> 2.5	> 2.5
2n		6.85	< 6	7.58	2.5	n.t.
2o		< 6	< 6	< 6	n.t.	> 2.5
2p		< 6	< 6	< 6	n.t.	> 2.5
2q		6.55	< 6	n.t.	> 2.5	> 2.5

(*Continued on next page*)

Table 1 (*continued*)

Compd		$5HT_{2A}$ pIC_{50}	$5HT_{2C}$ pIC_{50}	H_1 pIC_{50}	mCPP (sc) ED_{50} (mg/kg)	mCPP (po) ED_{50} (mg/kg)
2r		<6	<6	<6	n.t.	n.t.
2s		<6	<6	<6	n.t.	>2.5
2t		<6	<6	<6	n.t.	>2.5
2u (*trans*)		<6	<6	<6	>2.5	n.t.

n.t., not tested.

Table 2. Other receptor binding affinities of compounds **2a**, **2b**, **2h** and mianserin (pIC_{50} values); all assays were performed with human cloned receptors (D_2 also with rat receptor) following standard procedures

Compd	α_{1A}	α_{2A}	α_{2C}	5-HT_{1A}	5-HT_{1D}	5-HT_3	5-HT_7	D_2 (rat)	D_{2L}	D_3	D_4
Mianserin	6.39	7.68	7.37	6.40	6.42	5.85	6.94	5.45	5.15	5.41	5.48
2a	6.27	6.43	5.73	6.01	6.18	5.92	n.t.	6.71	6.06	6.77	6.88
2b	5.72	<6	<6	<6	<6	n.t.	n.t.	<6	5.02	<6	n.t.
2h	6.72	6.36	<6	<6	<6	<6	7.73	n.t.	5.77	6.47	6.52

n.t., not tested.

evident that the anxiolytic effects should be mediated by one of the 5-HT_2 receptors or by both of them. Those data reinforce the hypothesis that mCPP-induced anxiety symptoms are most likely mediated via the 5-HT_{2C} receptor.

In summary, we have discovered a new series of 2-(aminoalkyl)-2,3,3a,8-tetrahydrodibenzo[*c*,*f*]isoxazolo [2,3-*a*]azepine derivatives as novel 5-$HT_{2A/2C}$ antagonists. One of the compounds, the dimethylaminomethyl derivative **2a**, was an orally potent mCPP antagonist as shown in our in vivo mCPP challenge test, and therefore could be considered as a potential anxiolytic/antidepressant agent. Separation of compound **2a** into its enantiomers is currently in progress. Pharmacological results of those pure enantiomers will be the subject for further publications.

Acknowledgements

The authors would like to thank Ms. Valle Ancos, Alcira Del Cerro, Rosario Díaz, Celia Pérez and Victoria Pérez for their experimental work performed. They would also like to thank Mr. Willy Van Gorp for his help in management and coordination of all data generated.

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