Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1149–1152

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Pharmacological Evaluation of 6-Piperidino- and 6-Piperazinoalkyl-2(3*H*)-benzothiazolones as Mixed σ/5-HT$_{1A}$ Ligands

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Received 2 November 2001; revised 1 February 2002; accepted 18 February 2002

Abstract—In an effort to produce new pharmacological probes with mixed σ/5-HT$_{1A}$ affinity, we have synthesized a series of 12 original 6-piperidino- or piperazino-alkyl-2(3*H*)-benzothiazolones and their receptor binding profile (σ, 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_3$, D$_2$, H$_1$, and M$_1$) was determined. The best mixed σ/5-HT$_{1A}$ affinity profile was found within the piperidine series with 4-benzyl substitution associated to linker methylene chain $n=2$ (K_i 5 and 4 nM, respectively). Moreover, a highly selective σ2 ligand was obtained with a 3,4-dichlorobenzyl substitution associated to $n=4$ (K_i 2 nM, selectivity ratio σ1/σ2 = 70).

The serotonin 5-HT$_{1A}$ receptor has been implicated in a lot of physio-pathogenic processes including the regulation of cognition, psychosis, feeding/satiety, temperature regulation, anxiety, depression, sleep, pain perception, and sexual activity.[1] The development of non-benzodiazepine anxiolytics such as buspirone (**1**) (Fig. 1), a partial agonist at 5-HT$_{1A}$ receptors, has substantiated the correlation between serotonin and anxiety.[2] In addition, there has been a recent report that flesinoxan (**2**), a 5-HT$_{1A}$ full agonist, is effective in generalized anxiety disorder in humans.[3] On the contrary, the 5-HT$_{1A}$ receptor antagonists, (+)-WAY-100135 (**3**) and WAY-100635 (**4**), show limited anxiolytic activity.[4] In contrast, **3** and **4** have shown robust activity in a number of preclinical models of cognitive impairment associated with Alzheimer's disease.[5] Harder et al.[6] reported that **4** alleviates impairments caused by dizocilipine (MK-801), a non-competitive NMDA antagonist, in monkeys. Similarly, Boast et al.[7] found that **4** significantly reduced the cognitive impairment caused by MK-801, in a delayed non-match to sample radial arm maze task in rats. Furthermore, Carli et al.[8] showed that post-training administration of WAY-100635 was able to reverse a learning deficit induced in rats by administration of

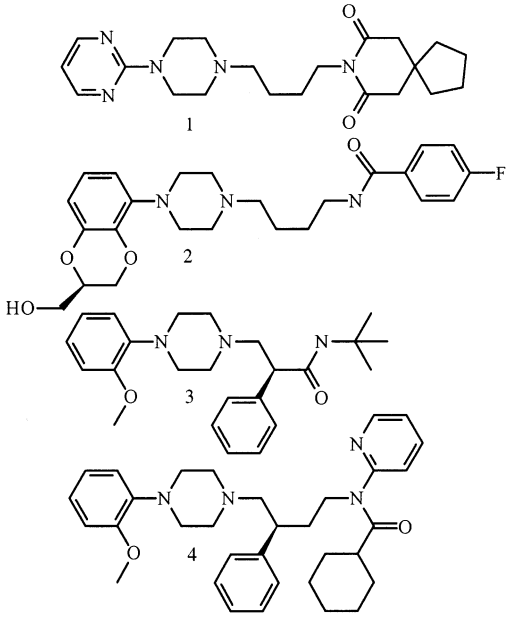

Figure 1. Structure of some 5-HT$_{1A}$ ligands.

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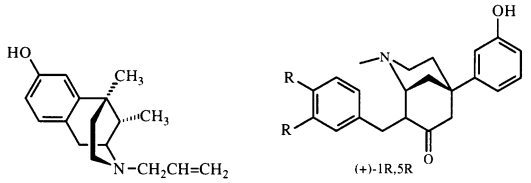


Figure 2. Some examples of sigma **1** and **2** ligands.

scopolamine, a cholinergic antagonist, in an auto-shaping learning task.

It is noteworthy that **1**–**4** all have in common a *N*-arylpiperazine moiety in their structure (Fig. 1).

On the other hand, sigma receptors are involved in a number of physio-pathological functions such as modulation and biosynthesis of several neurotransmitters, motor control, and cell growth and proliferation.[9] The lack of endogenous ligands (progesterone, neuropeptide Y, and substance P fragments remain debatable candidates)[10–13] and the existence of at least two sigma receptor subtypes, termed σ1 and σ2,[14] have hampered so far major clinical applications. Recently, a mammalian protein which binds σ1 ligands has been cloned and showed no homology to known mammalian proteins.[15]

The sustained interest for σ ligands stems from the possibility of developing clinical agents for the treatment of psychiatric and motor disorders,[16] tumor diagnosis,[17] cocaine,[18] neuroprotection,[19] and other neurological diseases. Several classes of structurally unrelated compounds interact with these receptors and unfortunately σ ligands in general display low selectivity.[20,21] Among potent and selective σ1 ligands are the cis-(+)-*N*-substituted normetazocine derivatives, that is (+)-pentazocine, (+)-*N*-allyl-normetazocine (SKF 10,047) (Fig. 2), and (+)-*N*-benzyl-*N*-normetazocine.[22] The list of σ2 selective ligands is more limited (Fig. 2). Examples of σ2 selective ligands are (+)-1*R*,5*R*-(E)-8-benzylidene-5-(3-hydroxyphenyl)-2-methylmorphan-7-one and its 3,4-dichloro derivative (CB64D and CB184, respectively).[23] A decade ago attempts were made to separate sigma binding from 5-HT_{1A} binding in order to achieve greater selectivity; one of the first papers to address this issue in a series of arylalkylamines was published by Glennon et al.[24]

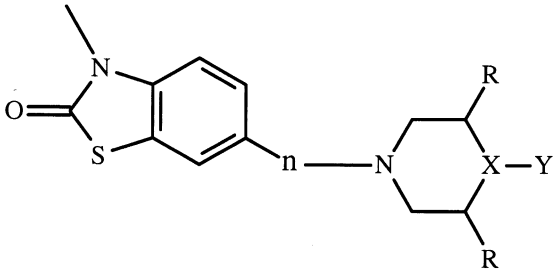


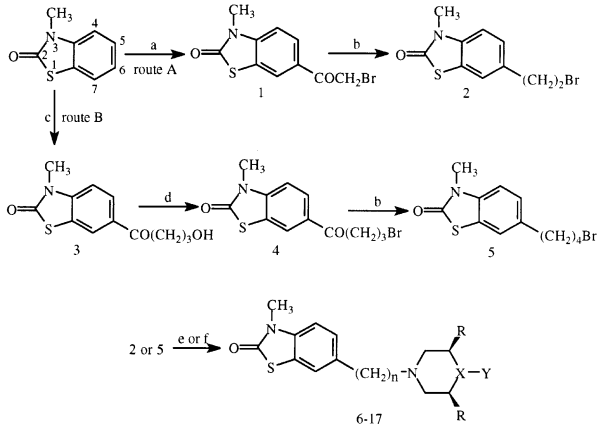
Figure 3. General structure of the mixed sigma/5-HT_{1A} ligands (*n* = number of methylenes, R = H or CH_3, X = N or CH, Y = phenyl, benzyl, 2,4- or 3,4-dichlorobenzyl).

Table 1. Preparation of 6-piperidino and 6-piperazinoalkyl-2(3*H*)-benzothiazolones according to Scheme 1

Compd	n	R	X	Y	Yield (%)
6	2	H	CH	Phenyl	60
7	4	H	CH	Phenyl	43
8	2	H	CH	Benzyl	56
9	4	H	CH	Benzyl	50
10	2	H	N	Benzyl	45
11	4	H	N	Benzyl	55
12	2	H	N	2,4-$Cl_2C_6H_3CH_2$–	60
13	4	H	N	2,4-$Cl_2C_6H_3CH_2$–	42
14	2	H	N	3,4-$Cl_2C_6H_3CH_2$–	56
15	4	H	N	3,4-$Cl_2C_6H_3CH_2$–	45
16	2	CH_3	N	H	60
17	4	CH_3	N	H	40

Several recent publications support the hypothesis that sigma and 5-HT_{1A} ligands may serve as atypical antipsychotic agents with minimal extra-pyramidal side effects.[25–27] Consequently, in an effort to produce new pharmacological probes with mixed σ/5-HT_{1A} affinity, we have synthesized a series of 12 original 6-piperidino- or piperazino-alkyl-2(3*H*)-benzothiazolones (Fig. 3) and their receptor binding profile (σ, 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, D_2, H_1, and M_1) was determined. The design of these ligands was based on the following arguments: (i) CoMFA investigations showed that almost all sigma ligands are characterized by a central basic nitrogen flanked by two aromatic hydrophobic groups;[20] (ii) it is well established that the *N*-phenylpiperazine moiety are good 5-HT_{1A} and σ pharmacophores;[28] (iii) 2(3*H*)-benzothiazolones have been successfully used as template both in the 5-HT_{1A} and sigma axis.[29,30] The nature of substituents and the reaction yield of these ligands are reported in the Table 1 and their corresponding binding affinities are listed in the Table 2. Their synthetic approach is shown in the Scheme 1.

Most of these compounds exhibited rather high affinity (in the nanomolar range) at sigma and/or 5-HT_{1A} receptors. Within the piperidine series, binding studies showed that for sigma affinity 4-phenyl substitution was



Scheme 1. Synthesis pathway of 6-piperidino or 6-piperazinoalkyl-2(3*H*)benzothiazolones (**6**–**17**). Reagents and conditions: (a) $BrCH_2COCl$, $AlCl_3$–DMF; (b) Et_3SiH, TFA; (c) $Cl(CH_2)_3COCl$, PPA; (d) HBr (gas), acetone, 20 °C; (e) secondary cyclic amine, Et_3N, acetone reflux, for 24 h; (f) heterocyclic amine, K_2CO_3, DMF, for 5 h.

Table 2. Binding affinities of 6-piperidino and 6-piperazinoalkyl-2(3*H*)-benzothiazolones. Binding measurements were done in triplicate and sigma binding refers to displacement of [^{3}H]-DTG

Compd	K_i (nM)							
	Sigma	5-HT_{1A}	5-HT_{2A}	5-HT_3	D_2	H_1	M_1	Sigma/5-HT_{1A}
6	2	4	100	20,000	1,000	20	1000	0.5
7	3	10	400	10,000	30	6000	NT[a]	0.30
8	5	4	400	2000	>100	3	100	1.25
9	5	30	700	2000	100	9	200	0.17
10	3	30	3000	20,000	>10,000	7	500	0.10
11	2	40	900	8000	10,000	<100	10,000	0.05
12	8	100	1000	20,000	100	10	NT[a]	0.08
13	9	200	900	2000	100	<100	<10,000	0.04
14	5	30	5000	3000	200	40	1,000	0.17
15	2	300	10,000	30,000	100	9	500	0.006
16	2000	>10,000	>10,000	>10,000	>10,000	20,000	>10,000	0.20
17	100	>100,000	>100,000	>100,000	>100,000	>100,000	>100,000	0.001

[a]NT not determined.

superior to 4-benzyl irrespective of the length (*n*) of the methylene linker between the benzothiazolinone and piperidine moiety while for 5-HT_{1A} affinity 4-phenyl and 4-benzyl substitution gave nearly equal scores, with however a superiority for $n=2$.

Within the piperazine series, binding studies indicated that for sigma affinity 4-benzyl and 3,4-dichlorobenzyl substitutions were superior to 2,4-dichlorobenzyl with low incidence of the methylene number *n*; for 5-HT_{1A} affinity, scores ranked benzyl > 3,4-dichlorobenzyl > 2,4-dichlorobenzyl with $n=2>n=4$. Conclusively, the best mixed σ/5-HT_{1A} affinity profile was found within the piperidine series with 4-benzyl substitution associated to $n=2$ (K_i 5 and 4 nM, respectively). In addition, this study also shows that some of these compounds bind at H_1 receptor with nanomolar affinity (e.g., compounds **8**, **9**, **10** and **15** with K_i values of 3, 9, 7 and 9, respectively). Furthermore, **8** was found to be the best mixed σ/5-HT_{1A}/H_1 ligand while **9** and **10** exhibited a mixed σ/5-HT_{1A}/H_1 affinity profile less pronounced. Incidentally, a selective sigma (σ2 vs σ1) ligand (**15**) was obtained with 3,4-dichlorobenzyl associated to $n=4$ (K_i 2 nM). This compound was also found to be a potent H_1 receptor ligand ($K_i=9$ nM), thus suggesting that **15** has an interesting profile for both sigma and H_1 receptors. For compounds having high affinity for sigma sites, the selectivity ratio σ1/σ2 was measured. Sigma-1 affinity was measured using (+)-pentazocine as previously reported.[30] Sigma-2 affinity was measured using DTG as reported by Mach et al.[31] The results indicate that compound **15** has much higher affinity at σ2 than at σ1 receptors (selectivity ratio = 70).

This situation is reminiscent of CB64D, CB184 and ibogaine, which are σ2-selective ligands reported by Bowen et al.[23] Compound **15**, however has much higher σ2 affinity when compared to these σ2 ligands.

Further work is now being done to delineate the structure–activity relationship of selective sigma-2 ligands using compound **15** as lead structure.

Experimental

Chemistry

The synthesis strategy to afford the desired 6-aminoalkyl-2(3*H*)benzothiazolones (**6**–**17**) was based on the initial elaboration of 6-bromoalkyl side chain on 3-methyl-(3*H*)benzothiazolone. Two different approaches (route A and B) were established depending upon the number of methylene units ($n=2$ or 4) as previously described.[29] Access to **2** and **5** was made possible by selective reduction of the ketone group (80–90% yield) using the couple triethylsilane-trifluoroacetic acid (Et_3SiH-TFA) according to the procedure described above.[29] Final products were obtained by treatment of compounds **2** or **5** by an appropriate heterocyclic amine (Scheme 1).

Pharmacology

Final compounds **6**–**17** were assessed for in vitro affinity at σ sites and 5-HT_{1A}, 5-HT_{2A}, M_1, H_1 and D_2 receptors (Table 2). The σ radioligand binding assays were carried out using [^{3}H] (+)-pentazocine for σ_1 subtype receptors[30] and [^{3}H] DTG in the presence of 1 μM (+)-pentazocine for σ_2 receptors (Table 3).[31]

The other receptor binding assays were done by ADIR & Cie (1, rue Carle Hébert, 92415 Corbevoie, France) and were performed using the following radioligands on rat brain membranes: [^{3}H]prazosin (cortex), [^{3}H]-8-OH-DPAT (hyppocampus), [^{3}H] ketanserin (cortex) for 5-HT_{1A}, 5HT_{2A} and 5-HT_3 receptors, respectively,

Table 3. σ1/σ2 selectivity ratio for compounds **6**, **11** and **15**. Binding measurements were done in triplicate

Compd	K_i (nM)		n_H	σ1/σ2
	σ1	σ2		
6	20.0	2	1.05	10
11	37.2	2	0.94	19
15	139	2	0.74	70

[^{3}H]pyrilamine for H_1 receptor, [^{3}H]telenzepine for M_1, and [^{3}H]raclopride (striatum) for D_2-dopamine receptors.

Acknowledgements

We thank ADIR et Cie Company (92415 Courbevoie, France) for financial support during this study and for collecting some of binding data.

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