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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2217–2220
Bioorganic & Medicinal Chemistry Letters

Thioxanthene-derived analogs as σ_1 receptor ligands

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Received 7 January 2004; accepted 4 February 2004

Abstract—An investigation of the structure–affinity relationships for the binding of thioxanthene-related structures indicates that an intact thioxanthene ring is not required for binding at σ_1 receptors, and that with the appropriate structural modifications, affinity can be enhanced to the subnanomolar level. Certain of the analogs displayed >180-fold selectivity for σ_1 versus σ_2 receptors.

Sigma receptors remain nearly as enigmatic today as they were immediately following their discovery more than 20 years ago (reviewed[1,2]). Sigma (σ) receptors, originally thought to represent a type of opioid receptor, are now classified as belonging to one of two major types: σ_1 and σ_2 receptors.[3,4] Although certain opioid receptor ligands (e.g., cyclazocine, pentazocine) bind at σ_1 receptors,[1] the receptors are clearly distinct from other opioid receptors both in pharmacology and structure. Only within the past decade were σ_1 receptors cloned from guinea pig liver,[5] mouse,[6] rat,[7] and human[8] sources. Sigma$_1$ knock-out mice also have been generated to explore σ receptor pharmacology.[9]

Currently, σ_1 ligands are being explored for their neuroprotective actions, for example,[10] as a novel strategy against cocaine addiction and toxicity, for example,[11] and for their potential in treating various cardiovascular disorders.[12] However, one of the first therapeutic claims for sigma ligands was as potential antipsychotics because various antipsychotic agents were shown to possess moderate to very high affinity.[13,14] Research on a possible role for σ_1 receptors in psychotic states has not been abandoned.[15–17] Certain butyrophenones (e.g., haloperidol) and tricyclic antipsychotics (e.g., phenothiazines, thioxanthenes) bind at σ receptors with K_i values ranging from about 3 to 500 nM.[14] We have previously examined haloperidol analogs[18] and have shown that they likely bind at σ_1 receptors due to their close structural relationship to a proposed σ_1 pharmacophore. Indeed, structural modification of haloperidol analogs to more closely agree with σ_1 pharmacophoric requirements resulted in ligands with enhanced affinity for σ_1 receptors.[18] In other words, rather than representing a novel type of antipsychotic mechanism, σ_1 receptors might simply accommodate haloperidol-like agents because they approximate the σ_1 binding pharmacophore.

One of the proposed pharmacophores for σ_1 binding includes an amine site, flanked by two hydrophobic domains 'A' and 'B'.[19,20] Hydrophobic site 'A' can accommodate an aryl ring and is situated about four to six atoms distant from the amine binding site; a five-atom chain is seemingly optimal.[21] A secondary hydrophobic binding site 'B' is also nearby the amine; the site is closer to the amine than site 'A' and is associated with a region of bulk tolerance.[20] N-Substituted 5-(phenyl)pentylamines, for example, bind with low nanomolar affinity at σ_1 receptors.[20,21] The purpose of the present investigation was to examine analogs of the thioxanthene antipsychotics to determine if they conform to the same pharmacophoric requirements. It has been reported, for example, that *cis*-clopenthixol (**1**; $IC_{50} = 152$ nM) binds at σ receptors with an affinity nearly identical to that of its *trans*-isomer ($IC_{50} = 145$ nM),[13] suggesting that the aromatic chloro group plays a minimal role in binding.

Keywords: Phenylpentylamines; Antipsychotics; σ Receptors.
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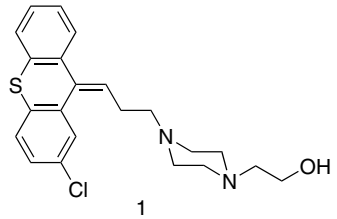
doi:10.1016/j.bmcl.2004.02.018



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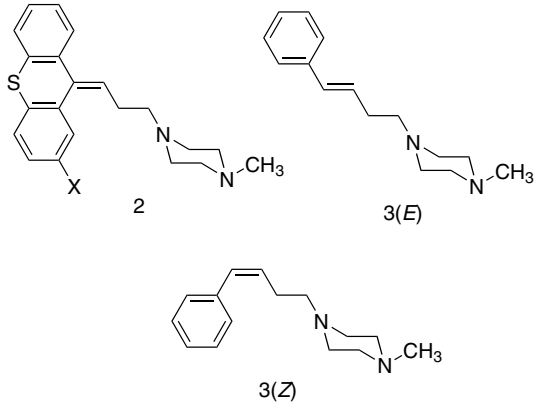
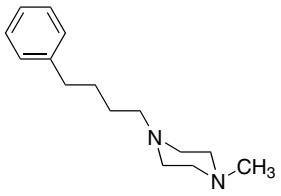
Perphenazine (σ, $IC_{50} = 26$ nM),[13] the phenothiazine counterpart of **1**, binds with enhanced affinity indicating either that the phenothiazine nitrogen atom contributes somewhat to its higher affinity, or that the presence of unsaturation in **1** detracts from affinity. It might be noted, however, that these studies were performed prior to the discovery of σ_1 and σ_2 receptors.

The present investigation began with a simplified version of the generalized thioxanthene **2** as a starting point so as to determine the role of the double bond. As with **1**, the two isomers of **3** were found to bind with identical affinity at σ_1 receptors ($K_i = 29$ nM; Table 1).



Evidently, stereochemistry is unimportant; furthermore, **3** binds with an affinity comparable to its reduced analog **4** ($K_i = 20$ nM). The results suggest there is considerable bulk tolerance in the region of the receptor that accommodates the aromatic ring(s). Because we have found that replacement of the piperazine ring 4-position nitrogen atom by CH is not only tolerated in structurally-related phenylalkylpiperazines, but also enhances their σ_1 affinity,[22] compound **5** was prepared and evaluated.



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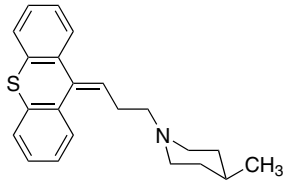

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Thioxanthene **5** ($K_i = 0.8$ nM) displayed 40 times the affinity of **3**. Features examined next were the necessity of the sulfur atom and the role of the alkenyl bond in **5**.

Compound **6** ($K_i = 1.9$ nM) is a ring-opened analog of **5** and compound **7** ($K_i = 1.0$ nM) represents the same compound with a reduced double bond. The affinities of **5**–**7** indicate that neither the sulfur atom nor the alkenyl double bond makes a significant contribution to σ_1 binding.

We have already shown that a phenylpentyl moiety is optimal for σ_1 receptor binding.[22] In addition, we have demonstrated that chain lengthening of butyrophenones

Table 1. Physicochemical and σ receptor binding properties of compounds examined[a]

	Recrystallized solvent	Percent yield (%)	Melting point (°C)	Empirical formula	σ_1 K_i (nM)	(±SEM)	σ_2 K_i (nM)	(±SEM)	Selectivity (σ_2 K_i/σ_1 K_i)
3a	—	—	—	—	29	(6)	1300	(210)	45
3b	—	—	—	—	29	(4)	2540	(180)	88
4	—	—	—	—	20	(3)	900	(80)	45
5	EtOAc/H_2O	15	168–169	$C_{22}H_{25}NS \cdot C_2H_2O_4$[b]	0.8	(0.1)	26	(8)	33
6	2-PrOH	62	184–185	$C_{22}H_{27}N \cdot C_2H_2O_4$	1.9	(0.2)	28	(2)	15
7	MeOH	61	170–171	$C_{22}H_{29}N \cdot C_2H_2O_4$	1.1	(0.1)	28	(5)	25
8	EtOH/Et_2O	60	165–167	$C_{23}H_{29}N \cdot C_2H_2O_4$	0.13	(0.06)	25	(4)	192
9	95% EtOH	92	190–192	$C_{23}H_{31}N \cdot C_2H_2O_4$	0.09	(0.01)	17	(3)	189
11	—	—	—	—	17	(1)	1,100	(390)	65
12	—	—	—	—	> 1000		—[c]		
13	MeOH/Et_2O	66	121–123	$C_{21}H_{29}NO \cdot C_2H_2O_4$	12	(3)	800	(110)	67
14	MeOH/Et_2O	58	132–134	$C_{21}H_{29}N \cdot C_2H_2O_4$	1.4	(0.2)	51	(8)	36
15a	EtOH/Et_2O	45	159–161	$C_{26}H_{29}N \cdot C_2H_2O_4$[d]	0.89	(0.10)	41	(11)	46
15b	95% EtOH	35	216–218	$C_{25}H_{27}N \cdot C_2H_2O_4$[b]	1.3	(0.4)	142	(43)	110
16a	EtOH/Et_2O	95	116–118	$C_{26}H_{31}N \cdot C_2H_2O_4$	0.48	(0.06)	32	(4)	67
16b	EtOH/Et_2O	96	189–191	$C_{25}H_{29}N \cdot C_2H_2O_4$[e]	0.66	(0.21)	100	(42)	152

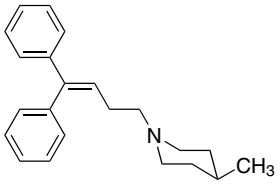
[a] Radioligand binding assays were performed as previously reported[24] and K_i values represent a minimum of three determinations. Compounds **3a**, **3b**, and **4** were prepared as previously described,[26] and **11** (Aldrich) and **12** (Research Biochemicals, Inc.) were obtained from commercial sources as their HCl salts. The remaining compounds, as their oxalate salts, analyzed within 0.4% of theory for C, H, N.

[b] Crystallized with 0.5 mol of H_2O.

[c] K_i value not determined.

[d] Crystallized with 0.75 mol of H_2O.

[e] Crystallized with 0.25 mol of H_2O.



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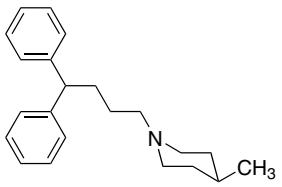
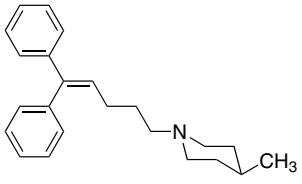


7

to their corresponding valerophenones results in enhanced affinity.[18] Likewise, we expected that chain lengthening of **6** and **7** to their phenylpentylamine counterparts **8** and **9**, respectively, would also result in enhanced affinity. This was found to be the case. Compound **8** ($K_i = 0.13$ nM) displayed 15 times the affinity of **6**, and **9** ($K_i = 0.09$ nM) displayed 11 times the affinity of **7**. The results further support the concept that the alkenyl double bond plays a negligible role in σ_1 binding and, because the affinity of **9** is comparable to what we had earlier reported for **10** ($K_i = 0.07$ nM),[22] that there must exist a region of bulk tolerance on σ_1 receptors to accommodate the 'extra' benzylic phenyl ring of **9**.



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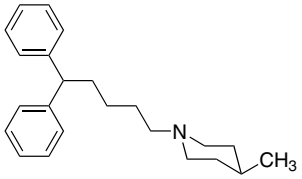

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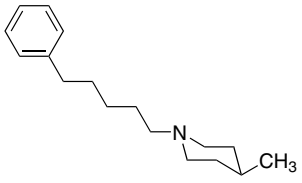

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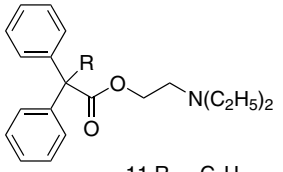
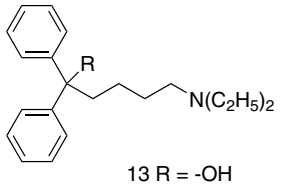
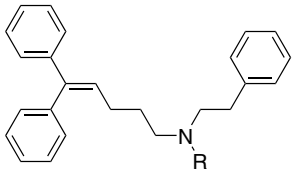
Having now determined that there may be a region of bulk tolerance associated with the 'A' site, and already aware that a five-membered chain separating the aryl portion from a basic amine is optimal, we examined several structurally-related agents to determine the generality of this finding. Klein and Musacchio[23] have reported that SKF-525-A (**11**), an inhibitor of liver cytochrome P-450, binds at σ_1 receptors with high affinity ($K_i = 3$ nM). We examined **11** and found it to bind with $K_i = 17$ nM. The structurally-related benactyzine (**12**; $K_i > 1000$ nM) binds with reduced affinity. The results imply that one (or more) of the polar substituents might be responsible for reduced affinity, and that a cyclic amine is not necessary for binding. Accordingly, we prepared and examined **13**–**16**. Compound **13** ($K_i = 14$ nM) binds with higher affinity than **12**, and removal of the hydroxyl group (i.e., **14**, $K_i = 1.4$ nM) resulted in 10-fold enhanced affinity. Compound **15a** ($K_i = 0.89$ nM) displayed high affinity, as did its secondary amine counterpart **15b** ($K_i = 1.3$ nM). Here, too, the role of the alkenyl double bond is minimal, but its reduction basically doubled affinity (**16a** and **16b**, $K_i = 0.48$ and 0.66 nM, respectively). It might be noted that the affinities of **16a** and **16b** are similar to that of the previously reported **17a** and **17b** ($K_i = 0.25$ and 0.17 nM, respectively).[19] Hence, it seems that N-substituted aryl-X-amines (where X is a five-membered chain) can bind at σ_1 receptors with high affinity, and that introduction of a second aryl moiety at the benzylic position is tolerated.



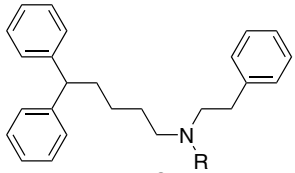
11 R = -C_3H_7
12 R = -OH



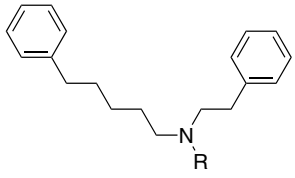
13 R = -OH
14 R = -H



15a R = CH_3
15b R = H



16a R = CH_3
16b R = H



17a R = CH_3
17b R = H

Compounds **3**–**9** were examined at σ_2 receptors to determine their σ_1 selectivity. Compound **3**(***E***), **3**(***Z***), and **4** displayed low affinity ($K_i \geqslant 900$ nM) for σ_2 receptors (Table 1). Compounds **5**–**9** ($K_i = 17$–28 nM) displayed higher affinity σ_2 receptors, but due to the very high affinity of the five-membered chain analogs **8** and **9** for σ_1 receptors, they possessed about 190-fold σ_1 selectivity. Compounds **13**–**16** displayed varying affinity for σ_2 receptors but lower affinity than they displayed at σ_1 receptors; only **16b** possessed >150-fold σ_1 selectivity.

In summary, the results of this investigation show (i) that an intact thioxanthene ring (as found in, e.g., **5**) is not required for high affinity σ_1 binding and that ring-open analogs (e.g., **6** and **7**) retain affinity, (ii) that extension of the aryl-to-amine chain from four to five atoms increases σ_1 affinity (comparing **6** and **7**, with **8** and **9**, respectively), (iii) that the longer-chain compounds display several-fold enhanced selectivity for σ_1 versus σ_2 receptors, and (iv) that there appears to exist a previously unrecognized region of bulk tolerance associated with hydrophobic site 'A' as evidenced by the similarity in σ_1 affinity of **9** and **10**. Although a role for σ_1 receptors in the actions of certain antipsychotics is not precluded, it is quite likely that the thioxanthenes, like the butyrophenones, bind at σ_1 receptors because they approximate the σ_1 binding pharmacophore; indeed, structural modification in both series such that

they more closely resemble the pharmacophore resulted in enhanced affinity. Structurally-related agents that bear a second benzylic aryl group, such as SKF-525-A (**11**) and compounds **13**–**16**, also bind at σ_1 receptors. One of the hallmarks of σ_1 receptors is the diversity of structure types that are accommodated. N-Substituted aryl-X-amines (where X is, optimally, a five-membered chain) bind at σ_1 receptors. Given that there now is evidence for a region of bulk tolerance associated both with the 'A' and 'B' hydrophobic sites, diverse structure types *should* be tolerated.

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