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Oxo-bridged isomers of aza-trishomocubane sigma (σ) receptor ligands: Synthesis, in vitro binding, and molecular modeling

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ABSTRACT

Isomeric oxo-bridged analogs of aza-trishomocubane sigma (σ) receptor ligands were synthesized and shown to display a reduced affinity for the σ receptor. In the case of phenethyl derivative **4**, there was a concomitant introduction of high-affinity for the α_{2C} adrenergic receptor, and moderate affinity for the dopamine transporter. Molecular modeling was undertaken to rationalize these results.

Sigma (σ) receptors were first proposed in 1976 as an opioid receptor subtype.[1,2] Intensive study over the past three decades has identified the σ receptor as a unique receptor distinct from all other mammalian proteins.[3,4] Of the two σ receptor subtypes, σ_1 and σ_2, only the σ_1 receptor has been cloned.[5,6] The identification of an endogenous ligand for these sites remains controversial. Sigma receptors exert a potent neuromodulatory effect over the glutamatergic system, a role consistent with their ubiquitous CNS distribution, and have been implicated in a diverse range of CNS disorders including depression,[7] psychosis,[8] and drug addiction.[9]

Many clinically-used antipsychotics, such as haloperidol, as well as anti-depressants like fluoxetine (Prozac) and sertraline (Zoloft), also display a high-affinity for the σ receptor.[10–12] The rational design of highly subtype-selective ligands is complicated by the extreme heterogeneity of known ligands, and a lack of structural information regarding the σ binding site(s).

We have previously demonstrated that trishomocubane-derived hemiaminals, such as **1** and **2** (Fig. 1), represent a scaffold for the development of highly selective σ ligands.[13–16] Within this class of compounds, the distance between the polycyclic hemiaminal and the aryl group is the primary determinant of subtype selectivity; benzyl derivatives display a preference for the σ_1 receptor, and phenethyl derivatives prefer the σ_2 receptor.[14–16] Halogen substitution of the phenyl ring, particularly in the 3-position, further enhances both σ receptor affinity, and subtype selectivity.[14–16]

Compounds **1** and **2** (Fig. 1) were previously shown to display a high-affinity for the σ_2 (K_i = 20 nM, σ_1/σ_2 = 7.6) and σ_1 (K_i = 10 nM, σ_2/σ_1 = 37) receptors respectively, with negligible affinity for other CNS receptors.[14–16] Compounds **1** and **2** have also

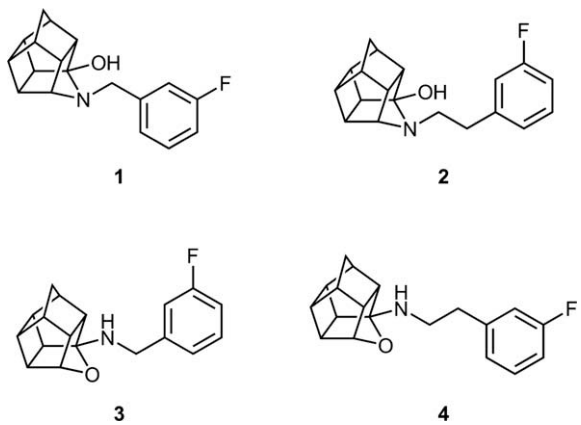

Figure 1. Polycyclic hemiaminals and their corresponding hemiaminal ethers.

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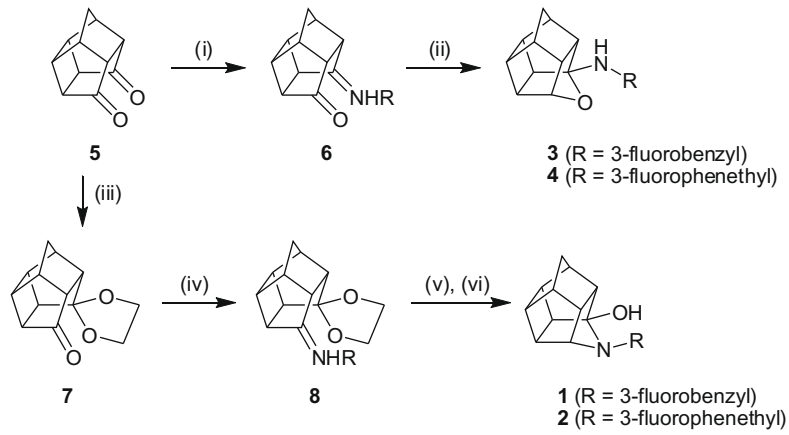

Scheme 1. Reagents and conditions: (i) 3-Fluorophenylalkylamine, toluene, 110 °C, 3 h; (ii) $NaBH_4$, MeOH, THF, rt, 24 h, 24–46% over two steps; (iii) ethylene glycol, *p*-toluenesulfonic acid (cat.), toluene, 110 °C, 5 h, 93%; (iv) 3-fluorophenylalkylamine, EtOH, 100 °C, 18 h; (v) $NaBH_4$, EtOH, rt, 8 h; (vi) aq 4 M HCl, acetone, rt, 12 h, 34–52% over three steps.

been shown to enhance amphetamine-induced dopamine release in vitro,[17] and modulate cocaine-induced behavioral effects in vivo.[18]

Replacement of the aza-bridge of hemiaminals **1** and **2** with an oxo-bridge gives the isomeric hemiaminal ethers **3** and **4**, respectively. It was anticipated that **3** and **4** might retain the σ receptor affinity of **1** and **2**, owing to their structural consistency with the σ_1 receptor pharmacophore proposed by Glennon et al.[19] To investigate this possibility, **1**–**4** were synthesized (Scheme 1), and subjected to CNS receptor assays.

Commercially available Cookson's diketone (pentacyclo [5.4.0.0.^{2,6}0.^{3,10}0^{5,9}]undecane-8,11-dione, **5**), was condensed with the appropriate primary amine to give imine **6**, which, upon treatment with $NaBH_4$, underwent transannular cyclization to furnish racemic hemiaminal ethers **3** and **4**. Protection of **5** as the ethylene ketal (**7**) and condensation with the appropriate primary amine gave imine **8**. Reduction of **8** with $NaBH_4$, followed by hydrolysis of the ketal and subsequent transannular cyclization gave racemic hemiaminals **1** and **2**.

All compounds were screened against a diverse panel of CNS receptors (see Table S1). As expected, **1** (Table 1) showed a selectivity for the σ_2 site (K_i = 31 nM, σ_1/σ_2 = 4.94), and **2** showed a preference for the σ_1 site (K_i = 12 nM, σ_1/σ_2 = 0.250). Surprisingly, substitution of the aza-bridge of **1** with an oxo-bridge in **3** significantly reduced affinity at both σ_1 (K_i = 2280 nM) and σ_2 (K_i = 1642 nM), suggesting that either the hemiaminal functionality, or the stereochemistry of the nitrogen atom relative to the polycyclic group, is important for σ receptor binding. However, for the oxo-bridged phenethylamine derivative **4**, the reduction in σ receptor affinity was less pronounced. An order of magnitude reduction in binding affinity was observed at both σ_1 (K_i = 149 nM) and σ_2 (K_i = 363 nM). Additionally, high-affinity for the α_{2C} adrenergic receptor (K_i = 20 nM), and moderate affinity for the dopamine transporter (DAT, K_i = 137 nM) was introduced. Molecular modeling was undertaken to rationalize the reduction of σ affinity observed for **3**, and the loss of selectivity for the σ receptor in the case of **4**.

Table 1
Selected CNS receptor affinity for compounds **1**–**4**

Compound	K_i[a] (nM)				
	σ_1	σ_2	α_{2C}	DAT	σ_1/σ_2
1	153 (±17)	31 (±7)	>10,000	>10,000	4.94
2	12 (±1)	48 (±10)	>10,000	>10,000	0.250
3	2280 (±792)	1642 (±182)	>10,000	>10,000	1.39
4	149 (±42)	363 (±37)	20 (±3)	137 (±27)	0.410

[a] Each value represents the mean of four experiments (standard error in parentheses).

Gas-phase structures of **1**–**4**, were optimized using the Becke-3-Lee-Yang-Parr (B3LYP)[20,21] hybrid density functional. The 6-31+G(d) basis set,[22–24] used in all calculations, describes the polarization of the C, N, O and F atomic orbitals and includes diffuse functions that can model the increased electron density that occurs on the N, O, F, and bridging C atoms. Energies obtained from B3LYP/6-31+G(d) calculations are accurate to approximately ±10 kJ/mol[25] although the errors in the relative energies of closely related species are likely to be much less than this.

The minimum energy conformers of compounds **1**–**4** were obtained by first performing constrained geometry optimizations, using loose convergence criteria, whilst scanning the rotatable C–C and C–N bonds in 60° or 120° steps (depending on the molecule and the given dihedral angle). The lowest energy structures thus obtained were then fully optimized using tight convergence criteria for the Kohn–Sham orbitals and an ultrafine DFT integration grid. The OH dihedral angle in aza-analogues **1** and **2** was then scanned in 30° steps to ensure the lowest energy structure was obtained. In all cases the nature of the final structures were confirmed by calculating the Hessian and ensuring there were no negative Eigenvalues. The details of all fully optimized structures are available as Supplementary data. All calculations were performed using the GAUSSIAN03 program package,[26] on computing facilities at the Australian National Computational Infrastructure Facility (NCI).

The global minimum energy structures obtained for molecules **1**–**4** are illustrated in Figure 2 (a–d, respectively). The relative energies, dipole moments, selected intramolecular distances, and selected natural atomic charges[27,28] are provided in Table 2. The hemiaminal carbon atom (C1), the substituted carbon on the fluorobenzene ring (C2), and carbon atoms in the alkyl bridge (C3, and C4 for molecules **2** and **4**) are labeled in Figure 2. It can be seen from Table 2 that the oxo-bridged compounds, **3** and **4**, are slightly more stable than the aza-bridged compounds **1** and **2**. The fluorobenzene group is 'extended' in compounds **1**, **2**, and **3**, whereas **4** has a folded conformation. The NCCC dihedral angle along the alkyl bridge in **4** is approximately 64° and in **1**, **2**, and **3** the equivalent angle is approximately 180°. The folded conformation has a significantly smaller dipole moment than the other structures.

For each molecule a number of low energy conformers were identified, including an 'extended' conformer of **4** with an electronic energy only 0.6 kJ/mol higher than that of the structure shown in Figure 1. The four local minimum energy structures obtained for **4**, as described in the Supplementary data, all had

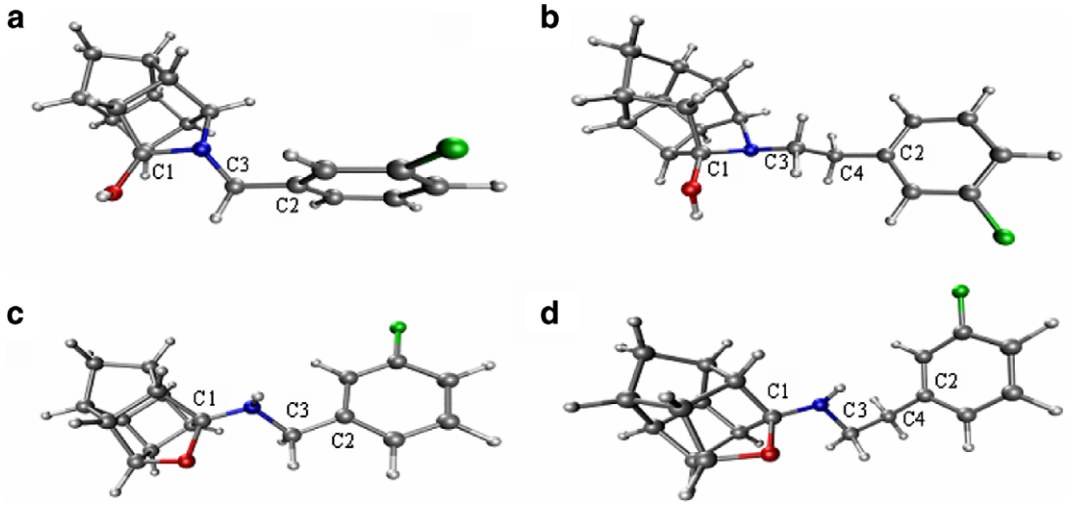

Figure 2. B3LYP/6-31+G(d) optimized global minimum geometries for **1**, panel (a); **2**, panel (b); **3**, panel (c); and **4**, panel (d). Carbon atoms are shown in gray, hydrogen atoms in white, and the nitrogen, oxygen and fluorine atoms as blue, red and green, respectively.

Table 2
Characteristics of the global minimum energy structures of compounds **1**–**4**, with atoms as labeled in Figure 2

Compound	Relative energy[a] (kJ/mol)	Total dipole moment (Debye)	Distance (Å)			Natural atomic charges (e)					
			R_{C1-C2}	R_{C1-F}	R_{N-F}	F	N	O	C1	C3	C4
1	12.6	2.14	3.83	6.47	5.11	−0.35	−0.55	−0.75	+0.43	−0.27	–
2	15.9	3.15	5.02	7.69	6.85	−0.35	−0.55	−0.75	+0.43	−0.26	−0.48
3	0.0	2.10	3.78	6.42	6.01	−0.35	−0.69	−0.60	+0.41	−0.27	–
4	0.0	1.89	4.41	6.51	5.35	−0.35	−0.70	−0.60	+0.41	−0.26	−0.48

[a] Relative to the aza-bridged structure.

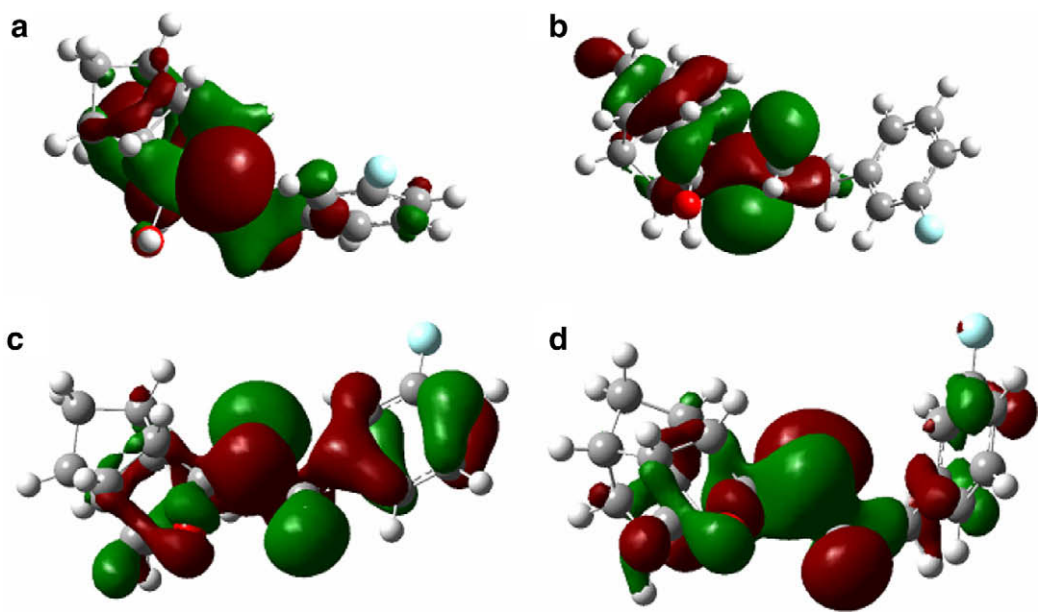

Figure 3. Highest occupied Kohn–Sham orbitals for **1**, panel (a), **2**, panel (b), **3**, panel (c), and **4**, panel (d). The orbitals are represented as 0.02 a.u. contours, drawn using a coarse grid.

energies within 1 kJ/mol. This relative conformational freedom, when compared to **1**, **2**, and **3**, may explain the loss of selectivity for the σ receptor, as evidenced by the introduction of affinity for the α_{2C} adrenergic receptor and the dopamine transporter.

One of the most marked differences between the oxo- and aza-bridged compounds are the differing natural atomic charges on the N and O atoms. In the aza-bridged compounds **1** and **2** the O atom has a larger negative charge than the N atom, whereas the oxo-bridged compounds **3** and **4** have a larger negative charge on the N atom than the O atom. The natural atomic charge on the hemiaminal carbon (C1) was approximately +0.42 in all cases, and the fluorobenzene bridging carbon (C2) was effectively uncharged. In all four compounds there is significant negative charge on the carbon atoms in the alkyl bridge (C3 and C4), suggesting that binding

to the σ receptor may occur via this motif. The natural atomic charges were consistent for all of the optimized conformers obtained for each molecule (see Table S6).

The nature of ligand binding was further investigated by examining the Kohn–Sham orbitals of each compound. Although the Kohn–Sham orbitals are a theoretical construct with which to expand the molecular electron density, the occupied orbitals have been shown to behave in the same way as molecular orbitals. They are considered to be a good basis for qualitative the interpretation of molecular orbitals,[29–31] and have been widely used for this purpose. The highest occupied Kohn–Sham orbital (HOKSO) is thus representative of the highest occupied molecular orbital. The HOKSOs of each of the optimized structures in Figure 2 are shown in Figure 3. HOKSOs for the other optimized geometries obtained are illustrated in the Supplementary data.

In the case of compounds **1**, **2**, and **4**, the HOKSO is relatively confined to the alkyl bridge, particularly the bridging carbon atom C3, with some electron density also on the polycyclic hemiaminal group. However, **3**, and to a lesser extent **4**, show significant HOKSO density from the π orbitals of the fluorobenzene group. Affinity for the σ receptor was severely reduced in **3**, and significantly decreased in **4**, suggesting that HOKSO density on the aromatic ring reduces affinity for the σ receptor.

Aza-trishomocubane hemiaminals, but not their isomeric oxo-bridged hemiaminal ethers, represent excellent scaffolds for the further development of selective σ receptor ligands. Additionally, oxo-bridged isomer **4** represents a novel lead molecule for the development of selective α_{2C} adrenergic ligands.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.019.

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