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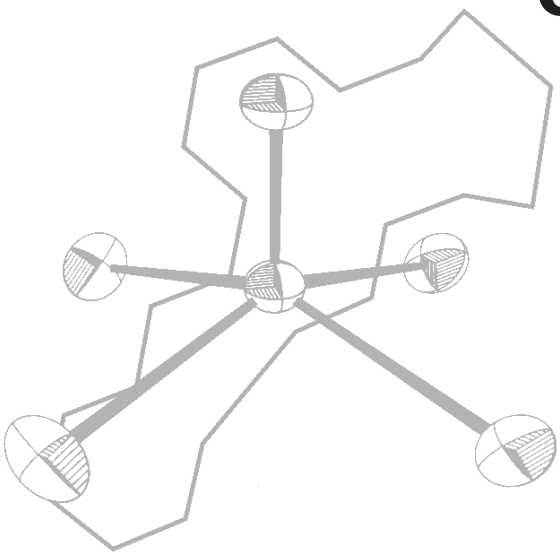

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Synthesis and Binding Studies of Trishomocubanes: Novel Ligands for σ Binding Sites

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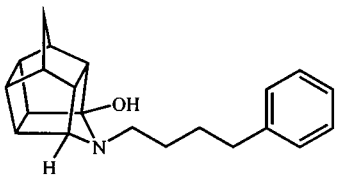
Five new analogues of the σ_2-selective trishomocubane *N*-(3′-fluorophenyl)methyl-4-azahexacyclo-[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecan-3-ol (2) have been synthesized and assessed for their affinities at both σ_1 and σ_2 sites. The structural requirements which influence σ binding of functionalized trishomocubanes were investigated with a view to develop more potent σ ligands, with particular emphasis on the σ_2 subtype. All synthesized compounds displayed moderate affinity to σ binding sites. Binding at the σ_1 site ranged from 107 to 1100 nM while binding at the σ_2 site ranged from 135 to 250 nM. The subtype selectivity was influenced by the nature and position of substitution on the aromatic ring. Fluorine substitution in the *meta* position, as in (2), is favoured over *ortho*, or *para* or *meta* and *para* difluoro substitution for σ_2 selectivity. Compounds (3)–(5) not only demonstrated lower affinity at the σ_2 site but a reversal of subtype selectivity with preferential binding at the σ_1 site (σ_1/σ_2: 0·8 for (3); 0·4 for (4); 0·8 for (6)). Introduction of CF_3 and NO_2 groups into the *meta* position in compounds (6) and (7) retained σ_2 selectivity although to a lesser extent when compared to (2) (σ_1/σ_2: 7·6 for (2); 2·0 for (6); 4·5 for (7)).

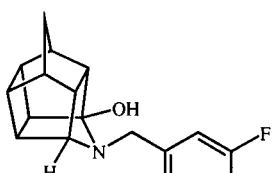
Introduction

Following two decades of intensive study, sigma (σ) binding sites have been defined as a class of high affinity and saturable binding sites present in both central nervous system and peripheral tissues.[1,2] There are at least two identified subtypes, namely σ_1 and σ_2 sites.[3,4] Sigma binding sites have been implicated in many pharmacological functions, such as motor effects,[5,6] sterol biosynthesis,[7,8] neuroprotection and tumour biology.[9] Recently, σ_2 binding sites have been described as biomarkers of cellular proliferation in breast cancer.[10] However, the functional importance of σ sites still remains elusive due to the lack of selective σ ligands as specific tools. A number of high affinity and selective σ_1 ligands have been synthesized, whereas the σ_2 ligands to date have either low affinity or poor subtype selectivity or cross reactivity for other binding sites.[11]

Our interest in exploring the bioactivity of polycyclic molecules, particularly trishomocubanes, was initiated by the observation that several D_3 trishomocubane derivatives demonstrated promising anticataleptic activities *in vivo* presumably through an effect on dopamine neurotransmission.[12,13] In our earlier study,[14] 20 trishomocubane analogues were synthesized and evaluated in *in vitro* binding experiments for their neuroreceptor selectivity and affinity. Most of the members in this series unexpectedly displayed moderate to high affinity for σ binding sites with no cross reactivity to other screened receptors and subtypes, including dopamine, cholinergic, opioid, *N*-methyl-D-aspartate (NMDA), phencyclidine and serotonin. Particularly, the aza hexacyclo compound (1)* showed the highest affinity and subtype selectivity for the σ_1 site (K_i = 9·4 nM, $\sigma_1/\sigma_2 = 19$), whereas compound (2)† displayed the highest affinity for the σ_2 site (K_i = 19·6 nM, $\sigma_2/\sigma_1 = 8$).[14,15]



(1)



(2)

Herein we report the synthesis and binding studies of new analogues of (2). This work aims at developing more potent and selective σ_2 ligands.

* *N*-(4′-Phenylbutyl)-4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecan-3-ol.
† *N*-(3′-Fluorophenyl)methyl-4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecan-3-ol.

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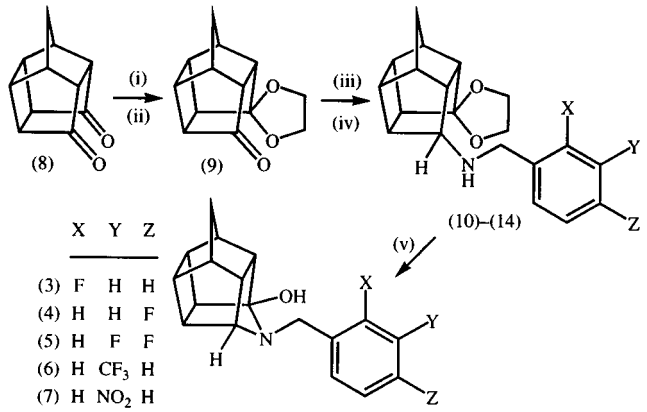
Results and Discussion

The general synthetic route used for the preparation of functionalized trishomocubane analogues is outlined in Scheme 1. The Cookson diketone (8) was reacted with ethylene glycol in the presence of catalytic amount of *p*-toluenesulfonic acid to yield the monoketal (9). Compound (9) was used as the general starting material for all new compounds. When the monoketal (9) was heated with appropriately substituted benzylamines in ethanol at 100°C overnight, it afforded the intermediate imines which were then treated with sodium borohydride without isolation to give the pentacycloundecylamines (10)–(14). Hydrolysis of the ketal function with aqueous HCl yielded the corresponding azahexacyclododecanes (3)–(7) as a result of transannular cyclization.[15] All compounds were characterized by ^{1}H n.m.r. spectroscopy, mass spectrometry and elemental analysis. Evaluation of the newly prepared compounds for their affinity to σ_1 and σ_2 binding sites was measured in guinea pig brain membranes according to the binding procedure given in the Experimental section.

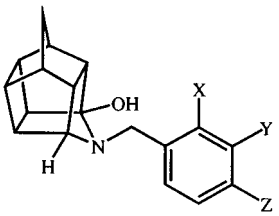


Scheme 1. (i) Ethylene glycol; (ii) *p*-toluenesulfonic acid, reflux in benzene; (iii) substituted benzylamine, 100°C for 14 h; (iv) sodium borohydride, room temperature for 6 h; (v) 2 M HCl, room temperature overnight.

Our earlier work with functionalized trishomocubanes revealed that a one-carbon alkyl chain between the trishomocubane moiety and the aromatic ring was essential for σ_2 binding. Increasing the alkyl chain to two carbons resulted in a reversal of subtype selectivity. In addition, fluorine substitution in the *meta* position on the benzene ring enhanced selectivity and affinity for σ_2 binding sites.[14] As a result, (2) displayed the highest affinity as well as the best selectivity among all other compounds and was chosen as a potential lead for development of σ_2-selective ligands. In order to develop more potent σ_2 ligands the one-carbon alkyl chain was retained and the effect of fluorine substitution around the aromatic ring was investigated, along with other *meta* substituents.

All newly synthesized compounds (3)–(7) displayed moderate affinities and varying σ subtype selectivity. The results are presented in Table 1 as inhibition constants (K_i). Compounds (3) and (4) with fluorine substitution in the *ortho* and *para* position respectively resulted in weaker σ_2 binding and a reversal of subtype selectivity when compared to (2) with preferential binding at the σ_1 site (σ_1/σ_2: 7·6 for (2); 0·8 for (3); 0·4 for (4)). Similarly the *m,p*-difluoro-substituted compound (5) negated the σ_2 selectivity afforded by a single *meta* fluorine substitution as seen with compound (2). Interestingly, all fluorinated compounds (3)–(5) showed a similar 10-fold decrease in affinity for the σ_2 site while their affinity for the σ_1 site was retained at the same order of magnitude when compared with compound (2).

Table 1. ***In vitro*** **binding results of trishomocubane analogues at σ_1 and σ_2 sites**



Compound	Substituents X	Y	Z	K_i (nM) σ_1	σ_2	σ_1/σ_2 ratio
(2)	H	F	H	152	20	7·6
(3)	F	H	H	182	230	0·8
(4)	H	H	F	107	250	0·4
(5)	H	F	F	198	239	0·8
(6)	H	CF_3	H	270	135	2·0
(7)	H	NO_2	H	1100	242	4·5

To determine the influence of functional groups other than halogens on σ binding and subtype selectivity, we introduced CF_3 and NO_2 groups in the *meta* position on the aromatic ring, and obtained compounds (6) and (7) respectively. These two compounds retained σ_2 selectivity (σ_1/σ_2: 7·6 for (2); 2·0 for (6); 4·5 for (7)) even though their affinity for the σ_2 binding site was considerably less than that of (2), 7 times lower for (6) and 12 times lower for (7) (σ_2: K_i = 20 nM for (2); K_i = 135 nM for (6); K_i = 242 nM for (7)) (Table 1). A comparison of the data for σ_1 binding revealed that, although (7) has lower affinity for σ_2 binding sites when compared with (6), the nitro compound (7) displayed a better σ_2 selectivity due to its markedly lower affinity for σ_1 sites. Compound (2) still remains as the most potent and selective trishomocubane analogue for binding at the σ_2 site.

Therefore, *meta* substitution in these compounds has been found to be essential for enhanced binding and selectivity at σ_2 sites. Also, fluorine substitution in the *meta* position affords markedly higher affinity and subtype selectivity for σ_2 binding when compared with other halogens including chlorine, bromine and iodine as we have described earlier.[15] Fluorine substitution is found to be more favourable when compared to CF_3 and NO_2 groups for σ_2 binding. Compound

(2) still appears as the most promising lead for the development of more potent and selective σ_2 binding ligands. Detailed structure–activity analyses are in progress to evaluate parameters such as changes in molecular conformation, steric influences and effects of substitution electronegativity on σ_2 binding.

Experimental

The reagents, including the Cookson diketone (8), ethylene glycol, *p*-toluenesulfonic acid, sodium borohydride, 2-fluorobenzylamine, 4-fluorobenzylamine, 3,4-difluorobenzylamine, 3-trifluoromethylbenzylamine, and 3-nitrobenzylamine hydrochloride salt, were purchased from Aldrich and used with no further purification. All synthesized compounds were examined by thin-layer chromatography on silica and alumina. Melting points were performed in a sealed capillary and are uncorrected. Unless specified otherwise ^{1}H n.m.r. spectra were recorded as solutions in $CDCl_3$ at 300 MHz on a Varian instrument. Elemental analyses were performed by the Department of Chemical Engineering, The University of Sydney. Mass spectra were recorded on a Finnigan/MAT TSQ46 system, with chemical ionization (c.i.) being used, by the Department of Pharmacy, The University of Sydney.

[^{3}H](+)-Pentazocine, [Ring-1,3-^{3}H] (58 Ci/mmol),* and [^{3}H]DTG, [5-^{3}H](1,3-di-*o*-tolylguanidine di-[*p*-Ring-^{3}H]) (35 Ci/mmol), were purchased from Dupont/New England Nuclear (Boston, MA, U.S.A.). (+)-Pentazocine and 1,3-di-*o*-tolylguanidine (DTG) were purchased from Research Biochemicals Inc. (Natick, MA, U.S.A.). Haloperidol was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The scintillant used was Emulsifier-Safe and was purchased from Packard Instruments B.V.–Chemical Operations, Groningen, The Netherlands. Whatman GF/B filters were purchased from Whatman. Binding assays were performed in polycarbon P3 tubes purchased from John's Medical Supplies. Liquid scintillation spectrometry was carried out by using a Packard 1500 Tri-Carb liquid scintillation analyser (Packard Instrument Co., Downers Grove, IL, U.S.A.).

General Method for Preparation of 4-Azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecanes

A mixture of the Cookson diketone (8) (1 g, 5·7 mmol), ethylene glycol (0·32 ml, 5·7 mmol) and *p*-toluenesulfonic acid (0·01 g) were reacted in boiling benzene (5 ml) for 5 h, allowed to cool and basified with a saturated solution of sodium bicarbonate (25 ml). The benzene layer was separated and the aqueous layer extracted with dichloromethane (10 ml×2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and the solvent was removed by rotary evaporation. Recrystallization of the residue by using diethyl ether (30 ml) gave the monoketal (9) as a white solid (86%). The monoketal (9) (2 g, 9·2 mmol) was added to appropriately substituted benzylamines (9·2 mmol) in a sealed tube with ethanol (20 ml) and the mixture was heated to 100°C for 14 h. The mixture was allowed to cool and treated with excess sodium borohydride (0·4 g) with stirring at room temperature for 6 h. Ethanol was removed by rotary evaporation and water (30 ml) was added to the residue. The mixture was extracted with dichloromethane (20 ml×3) and the extracts were dried over anhydrous sodium sulfate and filtered; the solvent was removed by rotary evaporation. The resulting pentacyclo[6.3.0.0^{2,6}.0^{3,10}.0^{5,9}]undecylamines (10)–(14) were hydrolysed with 2 M HCl (40 ml) at room temperature overnight. The reaction mixture was then basified with 1 M sodium hydroxide to pH 14 and extracted with dichloromethane (20 ml×3). The extracts were dried over anhydrous sodium sulfate, filtered and the solvent was removed by rotary evaporation. The crude products were crystallized and recrystallized from isopropyl alcohol (approx. 20 ml); this resulted in the desired azahexacyclododecanes (3)–(7) (30–40%).[15]

N-*(2′-Fluorophenyl)methyl-4-azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (3)*

Treating the monoketal (9) with 2-fluorobenzylamine according to the method described above afforded the title compound as a white *solid* (30%), m.p. 164–165°C (Found: C, 75·9; H, 6·2; N, 4·9. $C_{18}H_{18}FNO$ requires C, 76·3; H, 6·4; N, 4·9%). Mass spectrum: c.i. *m/z* 284 (99%, M+1). ^{1}H n.m.r. (300 MHz): δ 1·5, 1H, d, *J* 10·5 Hz, CHC**H**$_2$CH; 1·8, d, *J* 10·2 Hz, CHC**H**$_2$CH; 2·4–3·0, 8H, m, CH; 3·3, 1H, t, *J* 4·7 Hz, NCH; 3·5, 1H, d, *J* 14 Hz, NCH_2; 3·8, 1H, d, *J* 13·7 Hz, NCH_2; 7·0, 1H, m, ArH; 7·1, 1H, m, ArH; 7·2, 1H, m, ArH; 7·4, 1H, m, ArH.

N-*(4′-Fluorophenyl)methyl-4-azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (4)*

Treating the monoketal (9) with 4-fluorobenzylamine according to the method described above afforded the title compound as a white *solid* (30%), m.p. 166–167°C (Found: C, 75·9; H, 6·4; N, 5·0. $C_{18}H_{18}FNO$ requires C, 76·3; H, 6·4; N, 4·9%). Mass spectrum: c.i. *m/z* 284 (99%, M+1). ^{1}H n.m.r. (300 MHz): δ 1·5, 1H, d, *J* 10·4 Hz, CHC**H**$_2$CH; 1·8, 1H, d, *J* 10·4 Hz, CHC**H**$_2$CH; 2·4–3·0, 8H, m, CH; 3·3, 1H, t, *J* 4·8 Hz, NCH; 3·4, 1H, d, *J* 13·5 Hz, NCH_2; 3·75, 1H, d, *J* 13·5 Hz, NCH_2; 7·0, 2H, m, ArH; 7·3, 2H, m, ArH.

N-*(3′,4′-Difluorophenyl)methyl-4-azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (5)*

Treating the monoketal (9) with 3,4-difluorobenzylamine according to the method described above afforded the title compound as a white *solid* (30%), m.p. 161–162°C (Found: C, 71·3; H, 5·6; N, 4·7. $C_{18}H_{17}F_2NO$ requires C, 71·7; H, 5·7; N, 4·7%). Mass spectrum: c.i. *m/z* 302 (99%, M+1). ^{1}H n.m.r. (300 MHz): δ 1·5, 1H, d, *J* 10·4 Hz, CHC**H**$_2$CH; 1·8, d, *J* 10·4 Hz, CHC**H**$_2$CH; 2·4–3·0, 8H, m, CH; 3·3, 1H, t, *J* 4·7 Hz, NCH; 3·4, 1H, d, *J* 14 Hz, NCH_2; 3·8, 1H, d, *J* 13·7 Hz, NCH_2; 7·0–7·3, 3H, m, ArH.

N-*(3′-Trifluoromethylphenyl)methyl-4-azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (6)*

Treating the monoketal (9) with 3-trifluoromethylbenzylamine according to the method described above afforded the title compound as a white *solid* (30%), m.p. 163–164°C (Found: C, 68·1; H, 5·4; N, 4·2. $C_{19}H_{18}F_3NO$ requires C, 68·5; H, 5·4; N, 4·2%). Mass spectrum: c.i. *m/z* 334 (99%, M+1). ^{1}H n.m.r. (300 MHz): δ 1·5, 1H, d, *J* 10·2 Hz, CHC**H**$_2$CH; 1·8, d, *J* 10·7 Hz, CHC**H**$_2$CH; 2·4–3·0, 8H, m, CH; 3·3, 1H, t, *J* 4·8 Hz, NCH; 3·5, 1H, d, *J* 14 Hz, NCH_2; 3·8, 1H, d, *J* 14 Hz, NCH_2; 7·4–7·6, 4H, m, ArH.

N-*(3′-Nitrophenyl)methyl-4-azahexacyclo-[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (7)*

3-Nitrobenzylamine hydrochloride (2·5 g) was mixed with 1M NaOH until pH 14 and extracted with dichloromethane to give the free base, 3-nitrobenzylamine, as a brown oil (1·8 g, 89%). Treating the monoketal (9) with 3-nitrobenzylamine according to the method described above afforded the title compound as a brown *solid* (30%), m.p. 169–170°C (Found: C, 69·7; H, 5·9; N, 9·1. $C_{18}H_{18}N_2O_3$ requires C, 69·7; H, 5·9; N, 9·0%). Mass spectrum: c.i. *m/z* 311 (99%, M+1). ^{1}H n.m.r. (300 MHz): δ 1·5, 1H, d, *J* 10·7 Hz, CHC**H**$_2$CH; 1·8, d, *J* 10·4 Hz, CHC**H**$_2$CH; 2·4–3·0, 8H, m, CH; 3·3, 1H, t, *J*

* 1 Ci = 3·7×10^{10} Bq = 3·7×10^{10} s^{-1}.

5 Hz, NCH; 3·5, 1H, d, *J* 14·6 Hz, NCH_2; 3·9, 1H, d, *J* 14·6 Hz, NCH_2; 7·5, 1H, t, *J* 7·8 Hz, ArH; 7·7, 1H, d, *J* 7·8 Hz, ArH; 8·1, 1H, d, *J* 8·1 Hz, ArH; 8·2, 1H, s, ArH.

In Vitro *Binding Procedures*

Receptor source. Membranes of guinea pig whole brain plus cerebellum were prepared as the source of receptors.

σ_1 Binding assay. [^{3}H](+)-Pentazocine was used to label σ_1 sites.[16] Non-specific binding was determined by using 10 μM of the high affinity σ binding drug haloperidol. Briefly, each assay tube contained radioligand at a final concentration of 2 nM [^{3}H](+)-pentazocine, tissue suspension (approximately 18 mg fresh weight per tube), various concentrations of test compounds (1 nM–100 μM) and the assay buffer in a final volume of 1 ml. After incubation at 37°C for 150 min, the reaction was terminated by rapid filtration through a Brandel 24-well cell harvester over Whatman GF/B glass fibre filters that were presoaked in a solution of 0·5% polyethylenimine at room temperature for at least 2 h prior to use. Following addition of scintillation cocktail, samples were allowed to equilibrate overnight. The amount of bound radioactivity was determined by liquid scintillation spectrometry with a Packard liquid scintillation analyser. Each concentration of test compounds was tested in quadruplicate. Binding data were fitted by using KaleidaGraph, Version 3.0, Abelbeck software. [^{3}H](+)-Pentazocine saturation binding data were best described by a one-site model.

σ_2 Binding assay. The selectivity for σ_2 sites of the test compounds was measured by labelling with [^{3}H]DTG. The [^{3}H]DTG saturation binding data were best described by a two-site model.[14] Binding was therefore carried out in the presence of (+)-pentazocine to mask σ_1 sites.[17] Similarly, each assay tube contained 10 nM [^{3}H]DTG, tissue suspension, various concentrations of test compounds and the assay buffer in a final volume of 1 ml. Following incubation at 25°C for 90 min in the [^{3}H]DTG assay, the workup procedure was as described in the σ_1 assay.

Acknowledgment

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