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TRISHOMOCUBANES, A NEW CLASS OF SELECTIVE AND HIGH AFFINITY LIGANDS FOR THE SIGMA BINDING SITE

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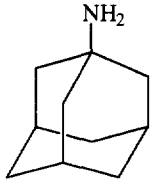
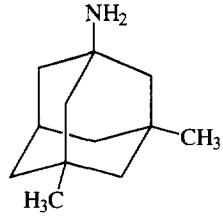
Abstract. The synthesis, receptor binding, and preliminary structural characterisation of trishomocubanes of types pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecylamines and 4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecanes are described. These ligands demonstrated high selectivity and affinity for the sigma binding site.

The chemistry of polycyclic molecules has been of interest over the past five decades but only recently have their pharmacological properties been investigated. The tricyclic amantadine **1** and memantine **2** are clinically used in the treatment of Parkinson's disease and dementia.[1,2] There is considerable evidence that suggest both amantadine and memantine act as antagonists at the NMDA receptor by binding to the PCP binding site inside the channel of the NMDA receptor complex.[3-5] Recently it has been reported that amatadine at therapeutic concentrations also interacts with the sigma binding site (Ki = 20.25 μM).[6]



Amantadine (**1**)

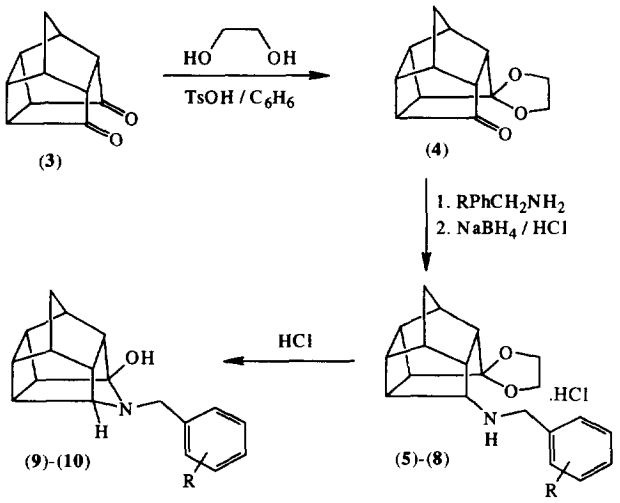


Memantine (**2**)

The sigma binding site has been the focus of intense study ever since it was originally proposed as an opiate receptor in 1976 based on the action of SKF-10,047. Subsequent investigations using radioligand binding techniques demonstrated the unique distribution of these sites.[7] Interest in the sigma binding site is largely motivated by the observation that the sigma site is a high affinity binding site for psychoactive drugs including many of the atypical antipsychotic drugs, such as BMY 14,802 that lack affinity for the dopamine and serotonin sites.[8,9] Many dissimilar types of drugs bind to the sigma site including steroids such as progesterone.[10] Although considerable progress has been made in the development of selective ligands, and in the identification of structural requirements of ligands for sigma binding, progress in the characterisation of the biological role of these sites has been slow and remains controversial.

Recently the synthesis and pharmacological properties of trishomocubanes consisting of pentacyclo[6.3.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines and pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines were reported. These molecules displayed antagonism of reserpine induced catatonia, which compared favourably to that of amantadine, and reduction of oxotremorine induced tremor and salivation in rats.[11,12] Such promising anticataleptic and mild to weak anticholinergic activities suggest that these polycyclic amines are a potential new class of anti-Parkinson agents. It was suggested that on the basis of these in vivo studies that the anti-Parkinson properties of these compounds could be attributed to their effects on the dopaminergic system.[12] Herein we report the synthesis, in vitro receptor binding and preliminary structural characterisation of trishomocubanes consisting of pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines and 4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecanes.

Chemistry. The general synthetic route for the preparation of the described trishomocubanes is outlined in Scheme 1.[12,13]



Scheme 1

Reaction of the Cookson diketone **3** (5.7 mmol) with ethylene glycol (5.7 mmol) and toluenesulfonic acid (0.05 mmol) in boiling benzene (5 mL) for 5 hours yielded the monoketone **4** in 85% yield. Pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines **5-8** were prepared by treating the monoketone **4** (4.6 mmol) with the appropriately substituted benzylamines (4.6 mmol) in a sealed tube with ethanol (10 mL) as solvent at 100°C for 14 h. On cooling the reaction mixture, the intermediate imines were treated with excess sodium borohydride (5.3 mmol) at room temperature for 4 hours. Following isolation of the products by extraction, they were converted to their hydrochloride salts using gaseous hydrochloric acid. The HCl salts were recrystallised from isopropanol to obtain the pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines **5-8** in 40-60% yield. Hydrolysis of mono-ketals of the type **5-8** (4.2 mmol) using dilute HCl (20 mL, 2M) resulted in the formation of 4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]dodecane derivatives **9** and **10** in 70% yield after recrystallisation from isopropanol. The ^{1}H NMR spectra of all compounds in the syntheses were used in the

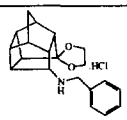
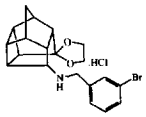
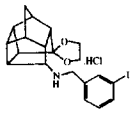
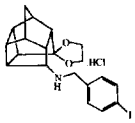
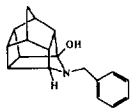
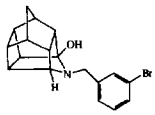
identification of essential structural characteristics. Further characterisation involved the use of mass spectrometry. All compounds gave satisfactory elemental analysis.

Radioligand Binding Assays. Compounds **5-10** were tested through the NIMH/NOVASCREEN Drug Discovery & Development Program (Contract No. NIMH-2003). Briefly, competitive binding assays were performed in either 250 or 500 µL volumes containing, by volume, 80% receptor preparations, 10% radioligand and 10% test compound/cold ligand (non-specific binding determinant)/4% DMSO (total binding determinant). All compounds were solubilised in neat DMSO which was diluted to a final concentration of 0.4% in the assay. Assays were terminated by rapid vacuum filtration over Whatman glass fiber filters followed by rapid washing with cold buffer. Radioactivity was determined by liquid scintillation or gamma spectrometry. Enzyme activity assays were similarly performed. Data were reduced by a software program proprietary to NOVASCREEN. Sigma binding experiments were performed using [^{3}H]DTG and guinea pig brain membranes as described by Weber et al.[14]

Results and Discussion.

Trishomocubanes used in this study consisted of four pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecylamines **5-8** and two 4-azahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecanes **9-10** which were synthesised and evaluated in in vitro experiments to determine their neuroreceptor selectivity and affinity.

Table 1. Binding affinities of trishomocubanes for the sigma and PCP binding sites.

Compd	Structure	mp °C	Sigma-binding (Ki = nM)	PCP-binding (Ki = µM)
(**5**)		182-184	78.7	>10
(**6**)		186-188	6.9	>10
(**7**)		182-184	58.2	>10
(**8**)		192-194	34.5	>10
(**9**)		150-152	30.1	>10
(**10**)		158-160	109.0	>10

All compounds displayed selectivity and high affinity for the sigma binding site when compared with other receptors screened including subtypes of the dopaminergic, muscarinic and serotonergic systems (data not shown). In contrast to reports that some sigma binding ligands exhibit cross-reactivity with the NMDA receptor ion-channel (PCP site),[15] this was not evident with the above trishomocubanes.

The data shown in Table 1 revealed that sigma binding of trishomocubanes is affected by various structural modifications. In compounds **5-8** the type and position of aromatic substitution had an influence on sigma affinity with Ki values ranging from 6.9 to 78.7 nM. The introduction of a halogen in the meta-position of the aromatic ring in compound **5** (Ki = 78.7 nM) resulted in a 10-fold increase in affinity in the case of bromine, compound **6** (Ki = 6.9 nM), and only a slight increase in the case of iodine, compound **7** (Ki = 58.2 nM). In the latter case para-substitution resulted in an almost 2-fold increase in affinity, compound **8** (Ki = 34.5 nM). The hydrolysis of ketals **5** and **6** resulted in the formation of **9** and **10** respectively. The transformation of **5** to **9** saw a significant rise in sigma affinity from 78.7 nM to 30.1 nM. The same trend however was not evident with the hydrolysis of **6** (Ki = 6.9 nM) in the formation of **10** (Ki = 109 nM).

Structural studies have been undertaken to elucidate some of the pharmacological observations. The synthesis of compounds **5-8** will result in mixture of diastereomers and enantiomers. At present there is no experimental evidence to quantitate the relative yields of isomers in a given preparation, or the abundance after purification by recrystallisation. Further work is in progress. It was argued that in the light of the current model for the sigma binding site,[16] the pair of enantiomers generated by reflection through the cubane moiety mirror plane are structurally indistinguishable. Diastereomers are formed from two different configurations on the chiral carbon bearing the amine group: the axial and equatorial isomers have the amine group axial and equatorial to the cubane moiety, respectively. These two diastereomers would be expected to interact differently with the sigma binding site.

Single crystals were grown from an as-prepared mixture in isopropanol solvent of the compound with the highest affinity for the sigma binding site, and one crystal was selected for structure determination. Figure 1 is the X-ray single crystal structure of the axial isomer of compound **6**.

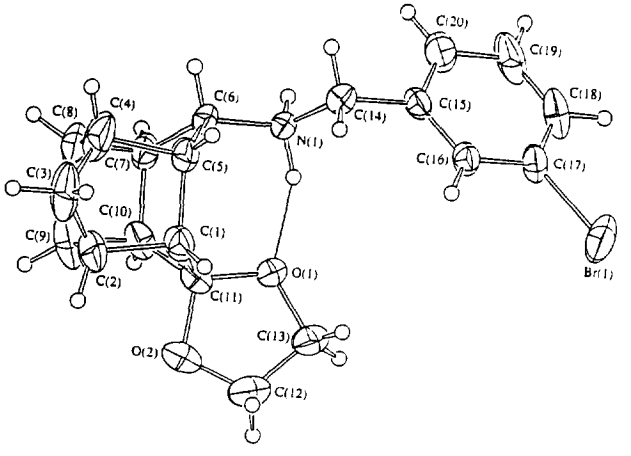


Figure 1.

Energy minimisation of the X-ray structure using Sybyl 6.2 software displaced hydrogen atoms by less than 0.1Å with the exception of the two hydrogen atoms on the ketal group which moved 0.5Å. A conformational analysis of the energy minimised structure using Sybyl 6.2 (gas phase analysis) indicated the minimum energy conformation correlated well with the extended chain conformation observed in the X-ray structure. This conformation is stabilised by a hydrogen bond between an amine hydrogen atom and an oxygen atom in the ketal group. The lowest energy conformation calculated with the chain in the folded configuration was approximately 4kJ/mol higher in total energy with the hydrogen bond still providing structural stability. Figure 2 is a schematic of (a) the X-ray single crystal structure, (b) the minimised energy conformation of the axial isomer, and (c) the minimum energy conformation for the equatorial isomer. The results of a conformation search on the equatorial isomer indicated that the extended and folded chain conformations had almost equal probability within the range 4kJ/mol. The calculated difference in total energy for the axial and equatorial isomers is approximately 24kJ/mol providing further evidence that the axial isomer is energetically more favourable and probably predominates.

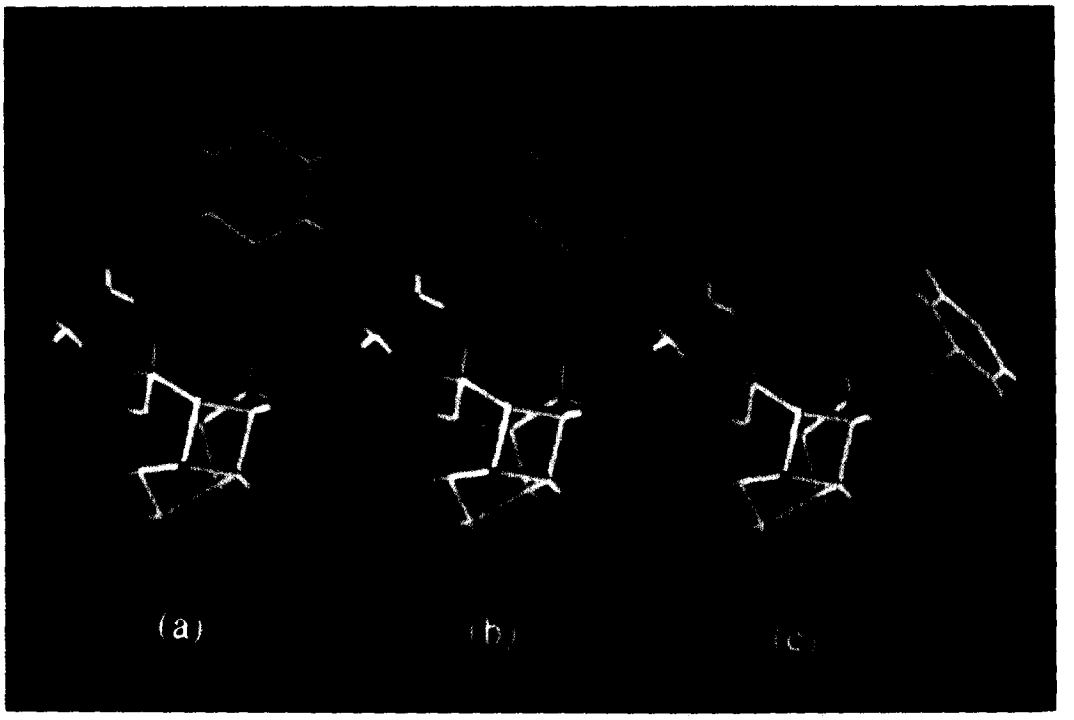


Figure 2.

The spatial relationship between the amine nitrogen atom and hydrophobic regions of molecules with affinity for the sigma binding site are highlighted in studies on structure-function.[16] There are two hydrophobic regions in these molecules. The distance from the nitrogen atom to the cubane moiety is 2.6 - 4.9 Å and to the aromatic ring is 3.2 - 6.0 Å. For the axial isomer the distance between the two

hydrophobic regions is 4.5 - 9.8; for the folded chain equatorial isomer conformation is 5.2 - 8.5 Å; and for the extended chain equatorial isomer is 3.8 - 9.4 Å. These distances are consistent with putative models for the sigma binding site.

In summary trishomocubanes of the types pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecylamines and 4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$] dodecanes can be said to represent a new novel class of compounds exhibiting selectivity and high affinity for the sigma binding site. Several lines of study suggest that the sigma binding site and sigma ligands play a role in the modulation of nigrostriatal dopamine neurotransmission[17] which may account for the anti-Parkinson properties mentioned earlier with related trishomocubanes.[12] Current investigations include the extension of compound series and binding selectivity of trishomocubanes for sigma subtypes, sigma-1 and sigma-2, as well as further pharmacological and structural evaluation.

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