Chapter 2

Review 1: Polycyclic cage structures as lipophilic scaffolds for neuro-active drugs

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Polycyclic cage structures as lipophilic scaffolds for neuro-active drugs

Jacques Joubert[1,5], Dr. Werner J Geldenhuys[2], Prof. Cornelis J Van der Schyf[2], Prof. Douglas W Oliver[3], Prof. H Gert Kruger[4], Dr. Thavendran Govender[4], Prof. Sarel F Malan[1,5*]

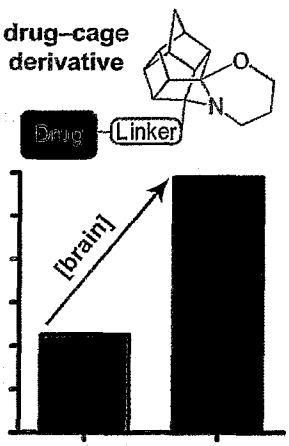
[1] *School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.*

[2]*Department of Pharmaceutical Sciences, Northeastern Ohio Universities Colleges of Medicine and Pharmacy, Rootstown OH, 44272, USA*

[3]*Pharmacology and* [5]*Pharmaceutical Chemistry, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa.*[4]*Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban 4001, South Africa*

*Corresponding author at present address: School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa. Tel: +27 21959 3190; fax: +27 21959 1588; e-mail: sfmalan@uwc.ac.za

Graphical Abstract



Scaffold for neuroprotection: Less than five per cent of small-molecule drugs are able to cross the blood--brain barrier (BBB). Developments in the design of compounds incorporating lipophilic, polycyclic structures have been under intense investigation in recent years. Conjugation of privileged moieties and known drug structures to these polycyclic structures has enhanced the delivery of neuroactive drugs across the BBB with subsequent neuroprotective and/or neurorestorative activity.

Abstract

Polycyclic cage scaffolds have been successfully used in the development of numerous lead compounds demonstrating activity in the central nervous system (CNS). Several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia, and stroke, as well as drug abuse, can be modulated with polycyclic cage derivatives. These cage moieties, including adamantane and pentacycloundecane derivatives, improve the pharmacokinetic and pharmacodynamic properties of conjugated parent drugs and serve as an important scaffold in the design of therapeutically active agents for the treatment of neurological disorders. In this Minireview, we focus on the recent developments in the field of polycyclic cage compounds, as well as the relationship between the lipophilic character of these cage-derived drugs and the ability of such compounds to target and reach the CNS and improve the pharmacodynamic properties of compounds conjugated to it.

Keywords: Apoptosis, Biological Activity, Cage Compounds, Drug Design, Neuroprotection

2.1. Introduction

Neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia and stroke, are increasingly becoming a burden on society, both in terms of the quality of life of patients, as well as that of their caregivers and families. The challenge that underlies treatment of neurodegenerative diseases, is that although we understand the basis of many of these diseases, few compounds exist which have been shown to unequivocally prevent or restore neuronal damage. The complexity of the diseases,[1-4] as well as the location of the brain behind physiological barriers, such as the selectively permeable blood-brain barrier, decreases the number of candidates that may be evaluated as therapeutically active drugs.[5]

In our efforts to develop neuroprotective or neurorestorative compounds, we have focused our attention on the polycyclic cage derivatives pentacycloundecane and amantadine, which have been used successfully to develop lead compounds for a multitude of neurodegenerative diseases.[6,7] The diamonoid amantadine (**1**, Symmetrel®) with its proven anti-viral activity,[8-10] and serendipitous discovery of anti-Parkinsonian activity,[11] led to the study of the structurally related pentacycloundecane (**3**, **4**, **5**) and adamantane (**1**, **2**) moieties as possible

scaffolds in drug discovery (Figure 1). These polycyclic cage compounds may be used to modify and improve the pharmacokinetic and pharmacodynamic properties of drugs, i.e. as a scaffold for side-chain attachment or for improvement of the drug's lipophilicity.[12]. The hydrophobicity of the hydrocarbon "cage" of amantadine (**1**), although the amino group is protonated at physiological pH, enables it to cross the blood-brain barrier and to enter the central nervous system (CNS). It has long been recognised that hydrocarbon moieties promote the transport of drugs across cell membranes and increase their affinity for lipophilic regions in receptor molecules.[12] The incorporation of cage frameworks into drugs should have the added advantage of reduced metabolic degradation by the inherent stability and the steric bulk of the cage skeleton, thus prolonging the drug's activity and reducing the frequency of administration to the patient.[12] Initial areas in which the introduction of an adamantane substituent have shown to result in increased longevity of drug action, drug potency and speed of action, as well as receptor site specificity includes antibacterial activity,[13] anabolic action[14] and analgesic activity.[15]

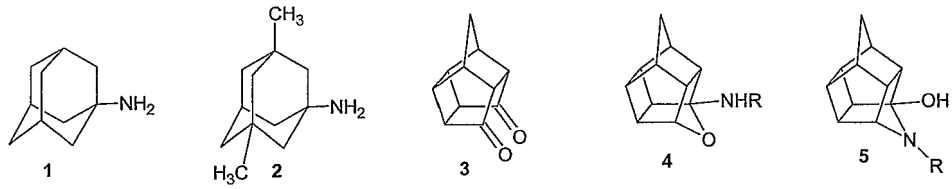


Figure 1: Representative polycyclic moieties

These compounds have a rich history in the chemistry field, which has recently transitioned to medicinal chemistry where several papers have been published describing their affinities for various drug targets in the CNS.[16-18] In this review, we focus on the adamantane (**1**,**2**) and pentacycloundecane (**3**-**5**) scaffolds in its use for developing neuro-active compounds. The use of these derivatives as scaffolds for drug design, their intrinsic lipophilic characteristics, which may aid in increasing a compound's therapeutic index and ability to reach the CNS[19] will be discussed.

2.2. Ion channels

Prior to 1986, little was published on the biological activity profiles of the pentacyclic polycyclic compounds. That year, the first account of biological activity for pentacycloundecanes was published, showing *L*-type calcium channel inhibition by 8-benzylamino-8,11-oxapentacycloundecane (NGP1-01, **6**, Figure 2).[20] Interestingly enough, early on in the synthesis of NGP1-01, it was first assumed that the aza-derivative (**5**) was

formed. Later on, two independent studies based on X-ray crystallographic data, indicated that when the imine (dione **3** + H_2NR) was reduced with addition of sodium borohydrate, the oxa-derivative (**4**) was formed, whereas utilizing sodium cyanoborohydrate lead to the aza-derivative (**5**).[21,22] Following up on the work done with NGP1-01, Malan *et al.* investigated the structure-activity relationships of a series of pentacycloundecane compounds related to NGP1-01 (**6-11**) as *L*-type calcium channel inhibitors (Figure 2).[23,24] Two main features were investigated: the effect of the steric and electrostatic properties of substituents on the benzene ring, and the effect of increased molecular volumw of the cage scaffold. From the electrophysiological experiments, it was evident that substitution on the aromatic ring increases the inhibition of calcium current, in the order meta>ortho>para. Additionally, it was shown that aromatic side chains with methoxy groups, represented by compound **9**, demonstrated better activity than compounds **7** and **8** bearing nitro-groups, suggesting an important electronic contribution of the substituents on the aromatic structure. It was also established that increasing the size of the cage from a five-membered to a six-membered (**10**) ring led to an increase in *L*-type calcium channel inhibition. These findings were corroborated with regression analysis using molecular descriptors, indicating that increased molecular volume and surface area led to increased activity.

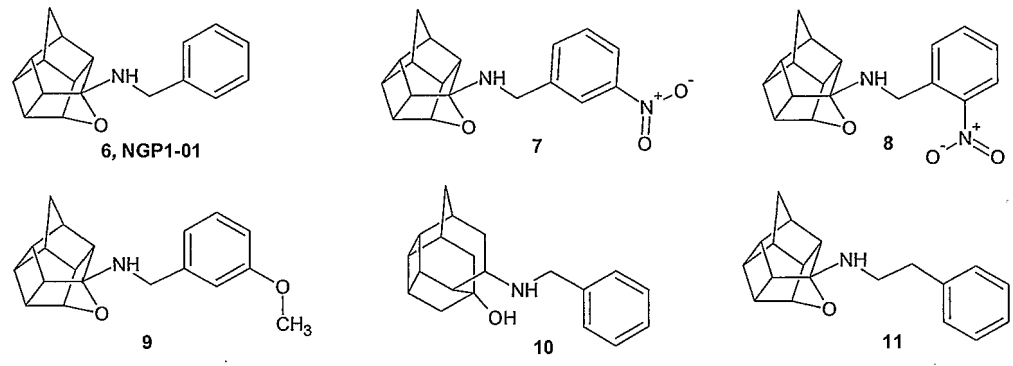


Figure 2. NGP1-01 (**6**) and other pentacycloundecane derivatives (**7-11**) evaluated for their ability to block calcium-flux through ion channels.

As the adamantane-amine compounds amantadine (**1**, Symmetrel®) and memantine (**2**, Namenda®) were proven to be non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists,[25] a logical step was to evaluate the pentacycloundecane compounds for similar activity. Comparing the structure of amantadine (**1**) and memantine (**2**) with the pentacycloundecane moieties (**3**, **4**, **5**), it is clear that these compounds share similar molecular volumes.[7] Recently, NGP1-01 (**6**) and other pentacycloundecane derivatives (**7-11**) were investigated for their ability to block calcium flow through the NMDA receptor channel (Figure 2). These studies showed that NGP1-01 was able to block NMDA receptor

induced calcium flux into synaptoneurosomes with an IC_{50} of 2.98 μM. This was comparable to that seen for memantine, 3.05 μM, in the same assay. The major structure-activity relationship seen from this study, was that an increase in cage size to a six-membered ring (**10**, IC_{50} = 36.22 μM) led to a decrease in NMDA receptor activity, indicating a possible limitation in the size of the NMDA receptor binding pocket. Radio-active binding studies with known NMDA receptor radio-ligands, [^{3}H]MK-801 and [^{3}H]PCP, indicated that these compounds bind to a site distant from the phencyclidine (PCP) binding site where amantadine (**1**) and memantine (**2**) interacts with the NMDA receptor.[7] Furthermore, NGP1-01 was able to antagonize NMDA induced calcium uptake in an uncompetitive manner. Compound **11** with an additional carbon between the cage and aromatic benzene (when compared to NGP1-01) led to a decrease in activity (IC_{50} = 23.5 μM), indicating the importance of chain length for NMDA receptor activity.[7]

The positive in vitro results with NGP1-01 as a voltage gated *L*-type calcium channel blocker[26] and NMDA receptor antagonist,[7] prompted its evaluation in an acute neurodegeneration model. Mdzinarishvili *et al.* evaluated NGP1-01 in a permanent occlusion stroke model.[27] Here, the middle cerebral artery occlusion model allowed for the induction of an ischemic stroke, in which NGP1-01 was tested. Calcium overload of neurons during ischemic attack is suggested to trigger the apoptotic events which occur due to ischemia.[28,29] When NGP1-01 was injected at a concentration of 20 mg/kg, 30 minutes before the stroke induction in female CD-1 mice, a resultant reduction in infarct area by more than 40% was evident. A reduction in brain swelling of 80% was also reported. The ability of NGP1-01 to show neuroprotection in this model, corroborates previous studies which suggested that utilizing a combination of NMDA receptor antagonists and voltage gated calcium channel antagonists in stroke yields superior results, as compared to using individual agents.[30-33] Similarly, NGP1-01 was able to show protection equal to that of memantine (**2**), in a transient version of the stroke model, as well as improved sensorimotor deficits in the animal subjects.[34]

Another avenue of investigation for ion channel inhibition was the development of the lead compound nitro-memantine (**12**) and its analogues. This compound does not only address the excitotoxicity damage caused by excessive calcium influx *via* uncompetitive antagonism of the NMDA receptor, but also initiates *S*-nitrosylation of crucial cysteine residues in the NMDA receptor channel which may lead to enhanced activity (Figure 3).[35] This concept was

recently employed to evaluate the neuroprotective potential of novel pentacycloundecane derivatives (**13-18**, Figure 3) that were able to act as multimodal *L*-type calcium channel antagonists and *S*-nitrosylation agents for the NMDA receptor.[36] In general, the nitrate compounds **16**, **17** and **18** exhibited greater *S*-nitrosylation capacity (20-64%) than the unsaturated nitro compounds **13** and **14** (11% and 15%, respectively). Compound **15** however exhibited *S*-nitrosylation comparable to that of the nitrate compounds (39%). At a concentration of 10 μM, all these compounds (**13-18**) exhibited better *L*-type calcium channel blocking activity than NGP1-01 (30%), with **13** (96.3%), **14** (88%) and **15** (92.2%) showing the most significant inhibition. The results for NMDA receptor inhibition indicated that at a 100 μM concentration **13** (25.4%), **14** (20.24%), **15** (33.14%) and **18** (24.55%) showed significant inhibition of the NMDA receptor and attenuated calcium flux into the cells to a greater extent than the reference NGP1-01 (14.01%). From this data it is evident that compound **15** is a potent NMDA receptor inhibitor and calcium channel blocker, and also serves as an effective nitrosylation agent of the NMDA receptor. This data suggests that it is possible to develop nitrosylating calcium channel inhibitors using the pentacycloundecane moiety as scaffold.

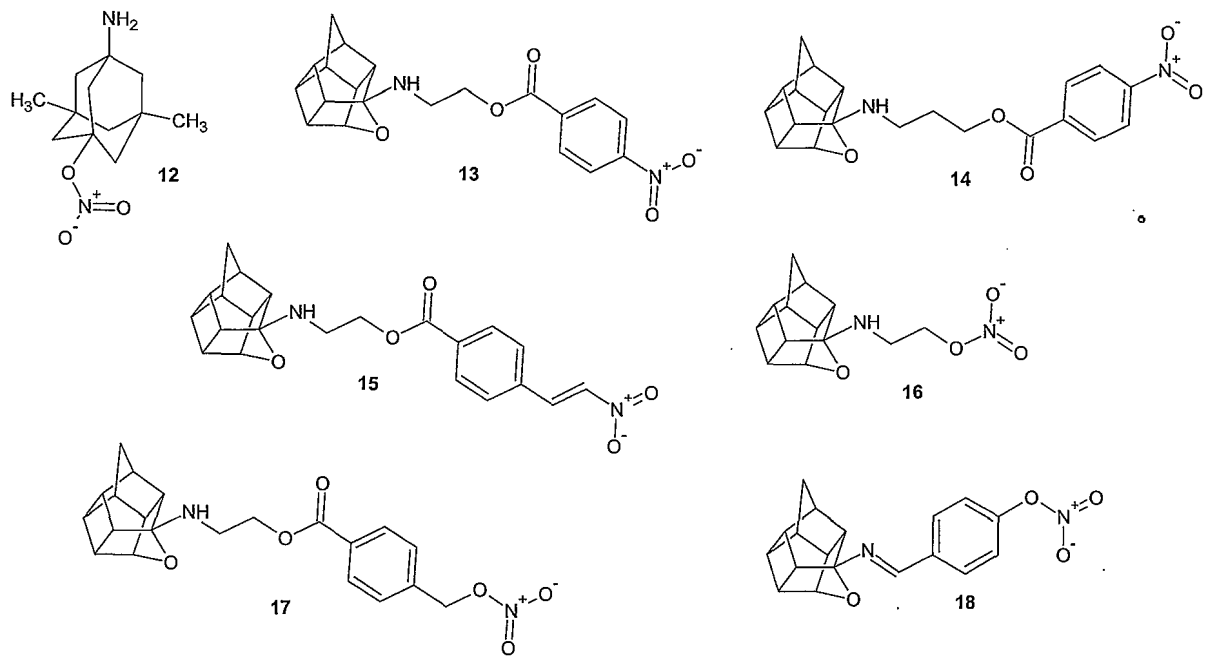


Figure 3. Pentacycloundecane nitrosylating derivatives evaluated for their ability to block calcium flux.

2.3. Monoamine oxidase B and apoptosis

The monoamine oxidase B (MAO-B) enzyme belongs to a group of enzymes which contain a flavin moiety. This enzyme is located on the outer surface of mitochondria, and is responsible for the metabolism of the neurotransmitter dopamine among others. Its importance is underscored in neurodegenerative diseases, where MAO-B inhibitors are currently used as symptomatic treatment in Parkinson's disease.[37-42] By inhibiting MAO-B, the endogenous level of dopamine is increased, thereby normalizing the dopamine level that was decreased due to the destruction of dopaminergic neurons in the substantia nigra. It was also suggested that inhibition of MAO-B may be neuroprotective. This is largely based on findings that the mitochondrial complex I toxin, MPP^+, is converted from MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to MPP^+ by the MAO-B enzyme.[43] The MPTP Parkinsonian mouse model has also been applied in the development of MAO-B inhibitors which can be used to prevent, as well as to treat Parkinson's disease. Although not FDA approved for neuroprotection, the MAO-B inhibitors rasagiline (Agilect®) and selegiline (Eldepryl®) which are not cage related have shown great promise in both in vitro and in vivo models as neuroprotective and possibly neurorestorative agents.[44-54]

In an earlier pilot study, it was shown that the pentacycloundecane structure also exhibits neuroprotective activity.[55] When C57BL/6 mice were treated with 35 mg/kg MPTP, 50-60% loss of striatal dopamine was observed after one week. This loss of dopamine levels in these mice was attenuated by pretreatment with the phenylethylamine pentacycloundecane derivative (**11**, Figure 2), albeit at a high dose of 300 mg/kg. This high dose of the drug suggests either a significant degree of clearance of the compound *via* metabolism or that the compound may be a substrate for efflux pumps such as *p*-glycoprotein. Although the protection instituted by compound **11** was marginal, it must be pointed out that this was a single dose injection of compound **11** which probably is not an irreversible inhibitor of MAO-B. Daily injections of the lead compound for this study, NGP1-01 (100 mg/kg/day), did not show any protection against MPTP, suggesting the mechanism of action by which these pentacycloundecane compounds act was more likely to protect the striatum pre-MPTP injection than as a restorative agent after MPTP injection.

Follow-up studies on the possible interaction of pentacycloundecane derivatives with MAO-B led to the realization that these compounds (Figure 2) were largely inactive as MAO-B inhibitors.[56] At 300 μM, these compounds were weak MAO-B inhibitors, with inhibition of

50% or less at this concentration. The compound that displayed possible neuroprotection in the MPTP model, compound **11**, inhibited MAO-B only moderately, with 50% reduction at 300 μM. As the propargylamine moiety was implicated in the neuroprotective activity of rasagiline and selegiline, an expansion of the polycyclic moieties set to include propargylamine derivatives (**19-26**, figure 4) was investigated and tested for MAO-B inhibition at a concentration of 300 μM.[57] Compounds **19** and **21** were inactive as MAO-B enzyme inhibitors and compounds **22-26** had modest activity (5.83%-20.13%). Only compound **20** was able to significantly inhibit MAO-B activity (73%) in this study. It can be speculated that the compounds containing a phenyl side chain (**20**, **26** and selegiline) were able to effectively block the entrance cavity of MAO-B. This trend may also be attributed to the extended planar character of **20**. It has been reported in literature that planar compounds frequently act as potent inhibitors of MAO-B.[58,59] Further development of these studies is necessary to obtain a complete picture of the structure activity relationships of these compounds.

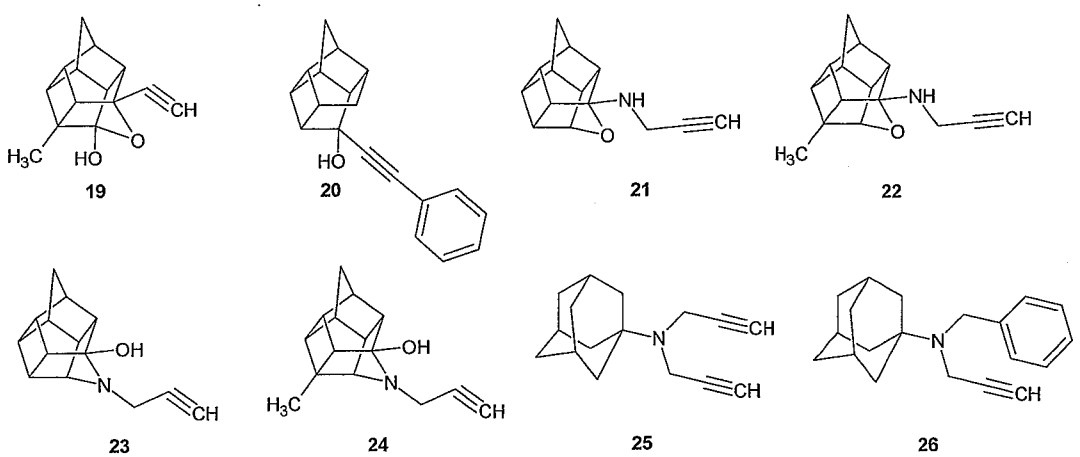


Figure 4: Propargylamine and related polycyclic derivatives evaluated for MOA-B inhibition and anti-apoptotic activity

Propargylamine derivatives of pentacycloundecane (**19-24**) and adamantane (**25,26**) propargylamine derivatives were also evaluated for in vitro anti-apoptotic activity using the Desphiper™ kit, which marks changes in the mitochondrial membrane potential that takes place during apoptosis (Figure 4).[57] All these compounds (**19-26**) exhibited significant anti-apoptotic activity, and improved cell health between 9 and 41%. The compound with the most significant anti-apoptotic activity was **19** (41%). This compound was twice as potent as the positive control, and cultures treated with **19** had only 0.5% apoptotic cells in the analysed samples. Compound **20** (39.2% anti-apoptotic activity) was slightly less active than **19**, with only 2.8% of the cells being apoptotic. The results indicated that the anti-apoptotic activity of

propargylamine can most probably be attributed to the acetylene group. Also, for a drug to be able to reach the mitochondria it requires quite remarkable trans-membrane delivery which in this case may be facilitated by the pentacycloundecane cage moiety resulting in increase in anti-apoptotic activity. Whether this activity is due to the acetylene group, cage moiety, or the acetylene group in conjunction with a nearby electronegative atom/group and the cage moiety, needs to be investigated further. The oxa- and aza-compounds have comparable activity, with the exception of compound **22**, where the oxa-derivative of the methylated cage demonstrated increased activity. A very interesting observation is that compounds **19** - **22** were all able to inhibit even baseline apoptotic processes. Based on these results it can be concluded that 8-phenylethynyl-8-hydroxy-pentacycloundecane (**20**) exhibited dual action in both inhibiting MAO-B and apoptosis. Having interesting anti-apoptotic activity, these polycyclic propragylamine derivatives can be used as lead compounds in the development and design of potent inhibitors of apoptosis.

2.4. Dopamine Transporter

Other mechanisms may also have played a role in the neuroprotection observed for compound **11** (figure 2) in the MPTP mouse model.[55] Since MPP^+ uses the neuronal dopamine transporter (DAT) to enter the neuronal terminal, the pentacycloundecane derivatives (**1**-**5**, Figure 1) were evaluated for their ability to block DAT as well as for their effect on the release of dopamine from the neurons. The lead compound, NGP1-01 (**6**) and the pheylethylamine derivative **11** were able to block dopamine uptake with an IC_{50} of 57 μM and 23 μM, respectively. Taking this into account, it is likely that the neuroprotection seen in the MPTP model is probably due to DAT inhibition, rather than MAO-B inhibition. Additionally, the pentacycloundecane derivatives (Figure 2) did not show any significant ability to release dopamine from pre-loaded synaptosomes. Structure-activity relationship studies indicated that for DAT inhibition, the meta position on the aromatic ring was favored over ortho and para.[55]

Further investigation of the ability of compound **11** to interact with the DAT system was initiated using superfused striatal tissue.[60] It was shown that **11** was able to increase the concentration of dopamine in the superfusate, while decreasing methamphetamine-stimulated dopamine release and increase dopamine levels in the superfusate when the tissue was stimulated with 30 mM KCl. Since the increase in dopamine levels when stimulated by KCl in the superfusate was decreased when calcium was omitted in the buffer, and **11** only weakly

blocked voltage gated *L*-type calcium channels in previous studies (IC_{50} of 23 μM),[20,21] it was concluded that **11** interacted with DAT in a calcium-dependent manner, although with a mechanism other than that of methamphetamine.[60]

2.5. Sigma Receptor

The sigma receptor has recently received increasing attention as it was established that it belongs to a different class than the opiate receptors. Furthermore, sigma receptors are now being recognized as potential drug targets in a variety of CNS disorders, including schizophrenia, depression and drug addiction.[61-65] It is also used as a target for neurological imaging probes.[63,64] In the late 1990's, amantadine (**1**) was found to bind to sigma receptors (K*i* = 20 μM), which sparked the interest of the group of Kassiou *et al.* to develop pentacycloundecane derivatives as possible sigma antagonists.[66-73] It was found that the aza-pentacycloundecane derivatives were potent sigma-1 and sigma-2 receptor ligands, with *K*i values well below 500 nM for most of the derivatives (**27-31**, Figure 5). Structure-activity relationships of these compounds, indicated that optimal sigma-1 selectivity required a 2-carbon linker between the cage and the aromatic ring, whereas sigma-2 selectivity required only a 1-carbon linker.[74] Furthermore, the meta-position on the aromatic ring was the most influential in terms of activity enhancement due to different conjugated electronegative functional groups. Newer evidence also suggests that oxa-pentacycloundecane derivatives are not as potent sigma receptor inhibitors as the aza-pentacycloundecane derivatives, as observed when comparing **28** (sigma-1: *K*i = 12 nM; sigma-2: *K*i = 48 nM) to **30** (sigma-1: *K*i = 149 nM; sigma-2: *K*i = 363 nM) and comparing compound **27** (sigma-1: *K*i = 153 nM and sigma-2: *K*i = 31 nM) to **29** (sigma-1: *K*i = 2280 nM and sigma-2: *K*i = 1642 nM).[75] Additionally, it was found that **30** was a potent adrenergic alpha-2c receptor antagonist (*K*i = 20 nM), suggesting that the mechanisms of action for many of the in vivo activities observed for these compounds are still to be elucidated. With sigma receptors highly expressed in the dopaminergic system, studies were also initiated to demonstrate the effect of these compounds on dopamine.[68,70,71,75,76] In contrast to the compounds discussed in the previous section, it was shown that compounds **27-31** enhanced methamphetamine-stimulated dopamine release in the striatum and effect locomotor activity in animal models. Further investigations into these structures led to the development of compounds where the inclusion of a benzyl-piperidine moiety (represented by **31**) between the aza-pentacycloundecane and the aromatic

group resulted in significant sigma receptor antagonistic activity and selectivity for sigma-1 over sigma-2 (sigma-1: *K*i = 9 nM and sigma-2: *K*i = 223 nM).[70]

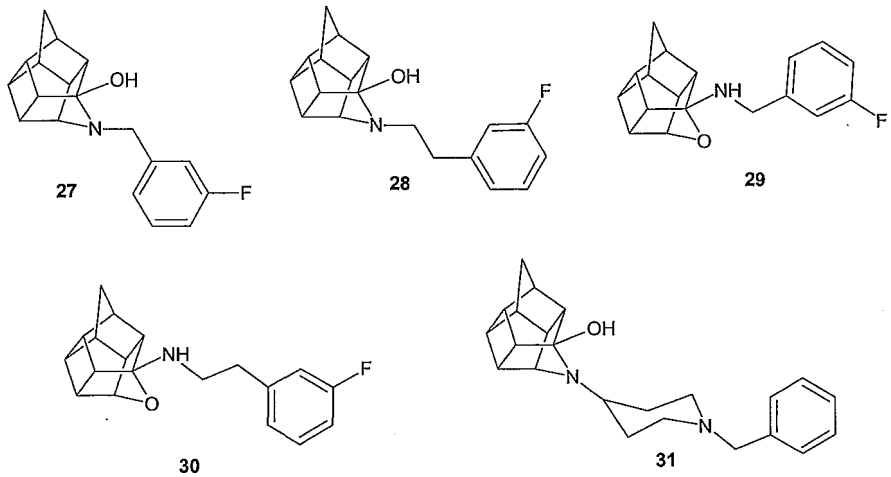


Figure 5: Pentacycloundecane derivatives evaluated for sigma receptor binding affinity

Banister *et al*, synthesized and evaluated a series of aza-pentacycloundecane derivatives (**32-34**), incorporating subunits from several known sigma receptor ligands, including haloperidol, NE-100 and RHM-2 for affinity against sigma receptors and dopamine receptors (Figure 6).[77] These hybrid aza-pentacycloundecane derivatives (**32-34**) exhibited good selectivity for sigma-1 and sigma-2 receptors over multiple dopamine receptors. The molecular hybrid obtained from haloperidol (**32**, sigma-1 *K*i = 27 nM, sigma-2 *K*i = 55 nM) had reduced affinity for D1–D5 dopamine receptors when compared to the parent drug haloperidol. The compound with the greatest sigma-1 affinity in the series, namely compound **34** (sigma-1 *K*i = 7.6 nM, sigma-2 *K*i = 225 nM) demonstrated a complete reversal of sigma receptor subtype selectivity, as displayed by the highly sigma-2 selective parent drug benzamide, (RHM-2 with sigma-1 *K*i = 10412 nM, sigma-2 *K*i = 13.3 nM). These results confirm the utility of the aza-pentacycloundecane scaffold for the development of highly selective sigma receptor ligands.

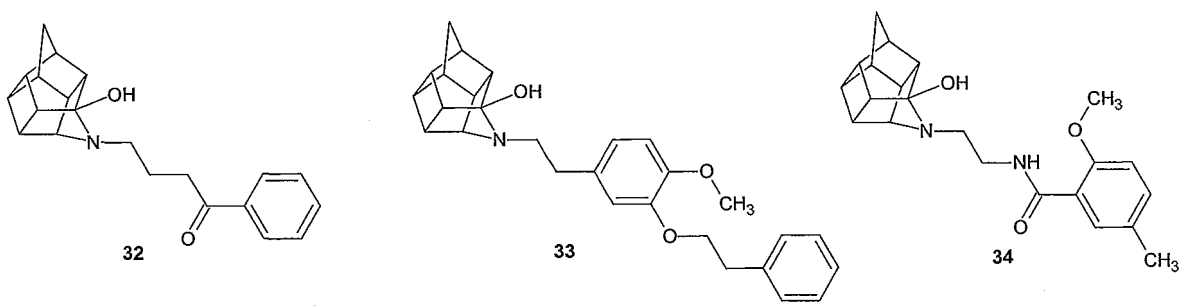


Figure 6: The drug-hybrid aza-pentacycloundecane analogs evaluated for sigma receptor inhibitory activity.

Recently Banister *et al.* also developed a series of aza-adamantanols and aza-adamantanes with significant sigma receptor activity (Figure 7).[78] The aza-adamantanols (represented by **35** and **36**) generally display low affinity for both sigma-1 (*Ki* values = 234–1950 nM) and sigma-2 receptors (*Ki* values = 201–1020 nM). Deoxygenation of the azaadamantols to give the corresponding achiral aza-adamantanes (represented by **37** and **38**) greatly improves affinity for sigma-1 (*Ki* values = 8.3–239 nM) as well as sigma-2 receptors (*Ki* values = 34–132 nM). A slight preference for sigma-1 receptors was observed (ration of sigma-1/sigma-2 = 2.7–7.5). These adamantane derivatives represents lead novel compounds for the development of potent and selective sigma-1 receptor ligands.

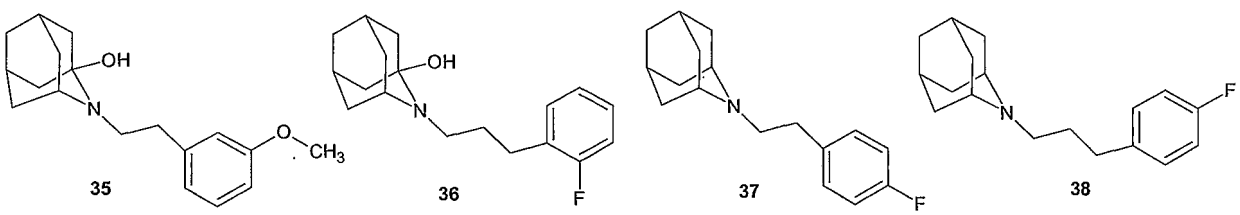


Figure 7: Examples of aza-adamantanols (**35, 36**) and aza-adamantanes (**37, 38**) with significant sigma receptor binding affinity.

2.6. Nitric Oxide Synthase Inhibitors

The pentacycloundecane scaffold was also investigated in the development of novel nitric oxide synthase (NOS) inhibitors. NOS has been considered a novel target for the treatment of several neurodegenerative disorders, including Alzheimer's and Parkinson's disease.[79-83] Wilkes *et al.* hypothesized that introducing a pentacycloundecane moiety on the NOS inhibitors aminoguanidine (IC_{50} = 2306 μM) and tryptamine (IC_{50} = 164 μM) could lead to derivatives (**39-44**, Figure 8) with increased NOS inhibition due to the additional bulky substituent.[84] An additional advantage to using the pentacycloundecane moiety, as described in later paragraphs, is their ability of the compound to cross the blood-brain barrier (BBB). Using rat brain homogenate, Wilkes *et al.* measured the ability of compounds **39-44** to inhibit the reaction of nitric oxide with oxyhemoglobin and the formation of methemoglobin. This gives an indication of NOS inhibitory activity.[84] Aminoguanidine derivatives **39** (IC_{50} = 905.1 μM) and **42** (IC_{50} = 88.03 μM) are markedly more potent than aminoguanidine. The indole derivatives of tryptamine (**40, 41, 43, 44**) have comparable IC_{50} values, all ranging between 200 and 300 μM. Compound **44** was shown to be the most potent inhibitor of maximal NOS activity, with 83% inhibition, and in comparison to its imine precursor (**41**), it appears that a saturated bond is necessary for improved activity. Although not discussed by

the authors, this could indicate that the increase in rotational freedom of **44** perhaps facilitate a better fit of these compounds into the active site of NOS. Additionally, the aza bridgehead was slightly more active than the corresponding oxa bridgehead analogue (c.f. **43** and **40**)

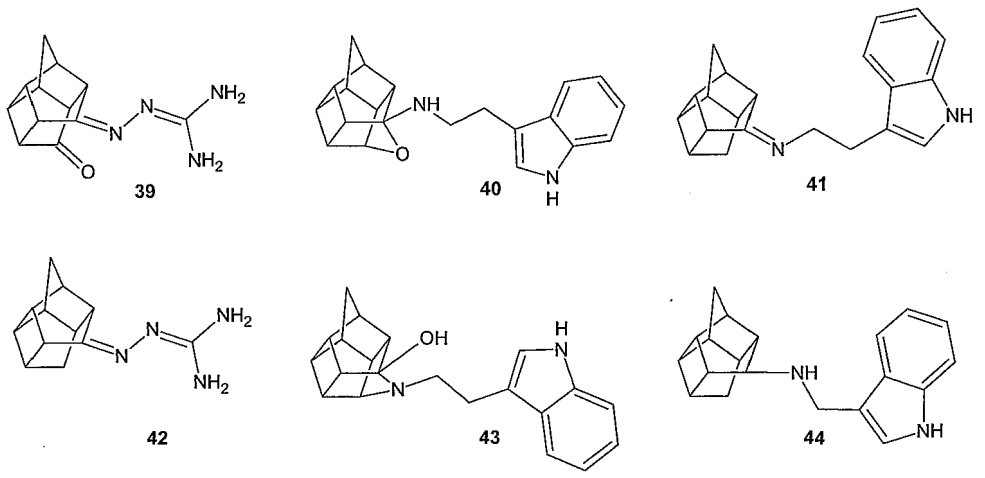


Figure 8: Pentacycloundecane aminoguanidine and tryptamine derivatives evaluated for NOS inhibition

Joubert *et al.* described the development of fluorescent molecular probes to be used as NOS inhibitors (Figure 9).[85,86] The goal in these studies was to develop fluorescent probes which exhibited similar inhibitory activity on NOS as the well-known NOS inhibitor 7-nitroindazole (7-NI).[83] Polycyclic compounds (amantadine and pentacycloundecane) were conjugated to the fluorescent ligands resembling the structure of 7-NI. The rational was that the lipophylic nature of the polycyclic compounds would increase the activity of the fluorescent probes by facilitating increased blood brain barrier permeability and penetration through cell membranes. The results from the inhibition studies showed that compounds **45**, **46**, **47**, **49**, **51**, **53**, **55**, **57** (IC_{50} values between 0.29 μM to 9.5 μM) displayed comparable potencies to that of 7-NI (IC_{50} = 0.11 μM). Although none of the compounds were found to be more potent than 7-NI, the coumarin adamantane (**47**), dansyl adamantane (**49**), indazole pentacycloundecane (**46**) and cyanoisoindole adamantane (**55**) exhibited IC_{50} values of 0.85 μM, 0.41 μM, 0.35 μM, and 0.29 μM, respectively. These compounds could possibly be used as molecular probes in the development of high-throughput screening NOS inhibition displacement assays. Further studies on isoform selectivity will elaborate on the potential of these compounds as fluorescent molecular probes. The aforementioned fluorescent derivatives were further evaluated for their potential as multifunctional neuroprotective agents[86] and it was revealed that compounds **47**, **49**, **53** and **55** exhibited favourable inhibitory activities against NOS, the NMDA receptor channel and *L*-type calcium channels. These derivatives were also able to

scavenge detrimental neurodegenerative free radicals.[86] *In silico* studies also indicated that these derivatives have a high degree of oral bioavailability and might be effectively transported across the BBB.[86]

2.7. Polycyclic Fluorescent Ligands in Neurodegeneration

Radioligand binding techniques have been widely used to study receptor pharmacology and physiology.[87,88] Despite the usefulness and sensitivity of radioligand binding techniques, the use of alternative methods, for instance fluorescent techniques, to study receptor-ligand binding interactions may provide information not readily accessible by conventional radioreceptor techniques. This will also circumvent some of the drawbacks, such as high cost, regulated disposal, health hazard and potential technical implications, associated with this methodology.[89,90] Techniques to visualize physiological or pathophysiological changes in living cells have become increasingly important in biomedical sciences. Fluorescent probes are excellent tools to analyze and clarify the roles of biomolecules in living cells, affording high spatial and temporal resolution *via* microscopic imaging. The development of tools for probing biological events has thus become an area of intense interest.[91,92]

With the above in mind, we focused on the expansion of the set of fluorescent derivatives described earlier.[85,86] This resulted in the synthesis of compounds **48**, **50**, **52**, **54**, **56** and **58** utilising adamantane-3-aminopropanol as an intermediate to extend the chain length between the adamantyl and fluorescent moieties (Figure 9).[93] These derivatives were evaluated as potential fluorescent ligands for the NMDA receptor and *L*-type calcium channels in murine synaptoneurosomes utilising the fluorescent ratiometric calcium indicator Mag-Fura-2/AM. Compounds **48**, **50** and **56** display significant *L*-type calcium channel inhibition with compounds **50** and **58** exhibiting NMDA receptor antagonistic activity. All these compounds show improved activity when compared to NGP1-01 (**6**) and amantadine (**1**) in both the NMDA and *L*-type calcium channel assays. Generally it was observed that increased chain length improves *L*-type calcium channel inhibition and NMDA receptor activity. This led to the hypothesis that an increase in chain length might indicate deeper immersion into the NMDA receptor and *L*-type calcium channels, and may be necessary for stronger interaction with their respective putative binding sites. Compound **50** was further used as a fluorescent NMDA receptor ligand in a fluorescent competition assay utilizing known NMDA receptor inhibitors to demonstrate the possible applications of these novel fluorescent analogues.[93] Further investigation on the application of these derivatives (especially on the NOS enzyme

and the NMDA receptor) will develop their potential as fluorescent ligands in the study of neurodegeneration.

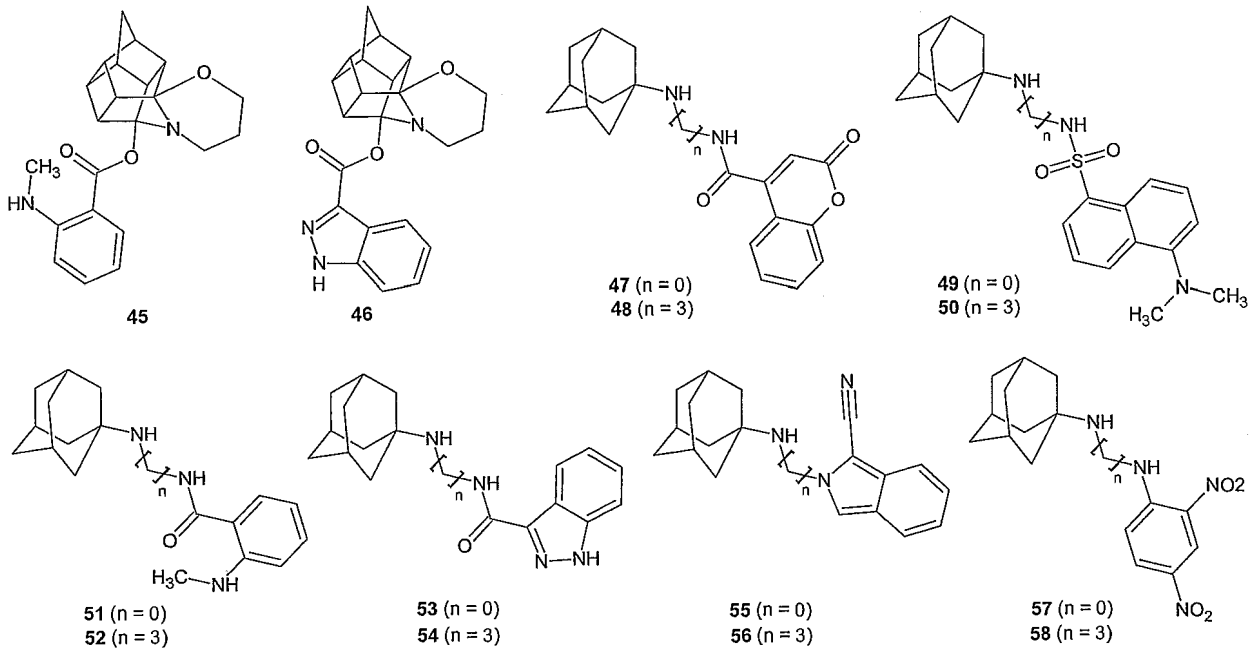


Figure 9: Fluorescent polycyclic analogues evaluated as fluorescent ligands.

2.8. Blood-Brain Barrier

When treating diseased of the CNS, the blood-brain barrier (BBB) is a major hurdle in targeting the brain. As Partridge pointed out, 98% of the CNS drugs fail to reach the clinic, due to low or inadequate permeability.[5,94] The BBB is a physiological barrier, made up of a selectively permeable system in the brain vasculature. Compared to the peripheral vasculature which is considerably more permeable, the BBB contains tight junctions, which allows for selective control over the type of compounds that are able to penetrate the brain.[95] Since inflammation has been connected to a multitude of neurodegenerative diseases, there has been interest in treating these deleterious diseases with anti-inflammatory drugs, for instance cyclooxygenase (COX) inhibitors or other similar non-steroidal anti-inflammatory drugs (NSAIDs).[96,97] Two separate studies have shown the utility of the pentacycloundecane derivatives in acting as lipophilic carriers for compounds to cross the BBB. Zah *et al.* first evaluated BBB permeability characteristics of a series of pentacyloundecane structures in an animal study.[19] The series largely consisted of benzylamine-type side chains attached to the pentacycloundecane scaffold (Figure 2, represented by compounds **6**, **7** and **11**) and was found to readily penetrate the CNS. Literature suggests a log*BB* (log of ratio of [brain]/[blood]) of 0.3 to be optimal for drugs which are used to treat CNS diseases.[98] Zah *et*

al. suggested that the pentacycloundecane derivatives reached maximal concentration in the brain at 1 hour post intra-peritoneal injection.[19] Correlation with *in silico* descriptors proved to be more difficult because of the small number of compounds in this series. The Log P_{oct}, molecular refractivity (MR) and solvent accessible molecular volume (SV) shows the best correlation to the log*BB* suggesting that the BBB permeability of the pentacycloundecane compound is largely driven by hydrophobic interactions. This study unambiguously indicated that the pentacycloundecane compounds reach the CNS in concentrations that could be therapeutically relevant.[19]

Using the results from the first study as background, Prins *et al.* was able to use pentacycloundecane derivatives and amantadine as carrier molecules to increase the BBB permeability of NSAIDs (Figure 10).[99] In this study, they used acetylsalicylic acid (log*BB* = -0.229) and ibuprofen (log*BB* = -0.0457) as parent NSAIDs with which they developed prodrugs using the polycyclic moieties as carriers (**59**-**62**). The acetylsalicylic acid prodrugs **59** and **60** substantially increase the log*BB* (0.127 and 0.143), considerably more than the un-conjugated drug. For instance, compound **60** increase the ratio of brain/blood from 0.9 to 1.39. The same was seen for the other ester prodrug, compound **59**. In both cases, the amide prodrugs, **61** and **62**, exhibit lower ratios (log*BB* = -0.309 and -0.4089), which were attributed by the authors to the slower hydrolysis when compared to the ester bonds of compounds **56** and **57**. These studies confirm the ability of pentacycoundecane and the adamantane moieties to significantly increase the CNS levels of poorly permeable NSAIDs.

As an addition to this study a series of pentacycloundecane NSAIDs was synthesized which included a 2-carbon spacer between the pentacycloundecane and the respective ester-NSAIDs (**63**, **64**, Figure 10).[100] It was thought that the increase in chain length may lead to less structural hindrance and make the compound more liable for enzymatic hydrolysis once inside the CNS and possibly lead to higher concentrations of the free NSAIDs. The BBB permeability of test compounds **63** and **64**, and more importantly, the extent to which the prodrug conjugates improved the CNS delivery of the free drugs, were calculated. The free acetylsalicylic acid has a blood and brain concentration of 80.77 μg/ml and 23.21 μg/ml respectively. After administration of prodrug **63**, acetylsalicylic acid is observed in the blood and brain as concentrations of 66.49 μg/ml and 54.52 μg/ml respectively. The salicylate is present at about twice the concentration in the brain after prodrug administration in comparison to the free drug administration. Initial absorption also seems to be enhanced for

the prodrug. A free Ibuprofen dosage yields a blood concentration of 1.82 μg/ml and a brain concentration of 1.72 μg/ml. Conjugation of ibuprofen to the pentacycloundecane structure to produce the prodrug **64**, leads to increased blood and brain concentrations for ibuprofen 168.79 μg/ml and 110.62 ug/ml respectively. This result indicates much higher concentrations of ibuprofen in both the CNS and in blood. This is indicative of significantly enhanced absorption from the intestines after intraperitoneal administration and thus enhanced concentrations of the free ibuprofen in the CNS. Further studies are presently underway where the NSAIDs are conjugated to an adamantane structure with a 3-carbon spacer to potentially reduce the structural hindrance presented by direct conjugation (represented by compounds **61** and **62**) and thus increase yields of free NSAIDs concentration after enzymatic hydrolysis in the CNS. The novel adamantane compounds also include NSAIDs such as naproxen and ketoprofen in addition to acetylsalicylic acid and ibuprofen. Preliminary *in silico* and in vitro studies indicate favourable neuroprotective activity and improved BBB permeability of the NSAIDs with this strategy.

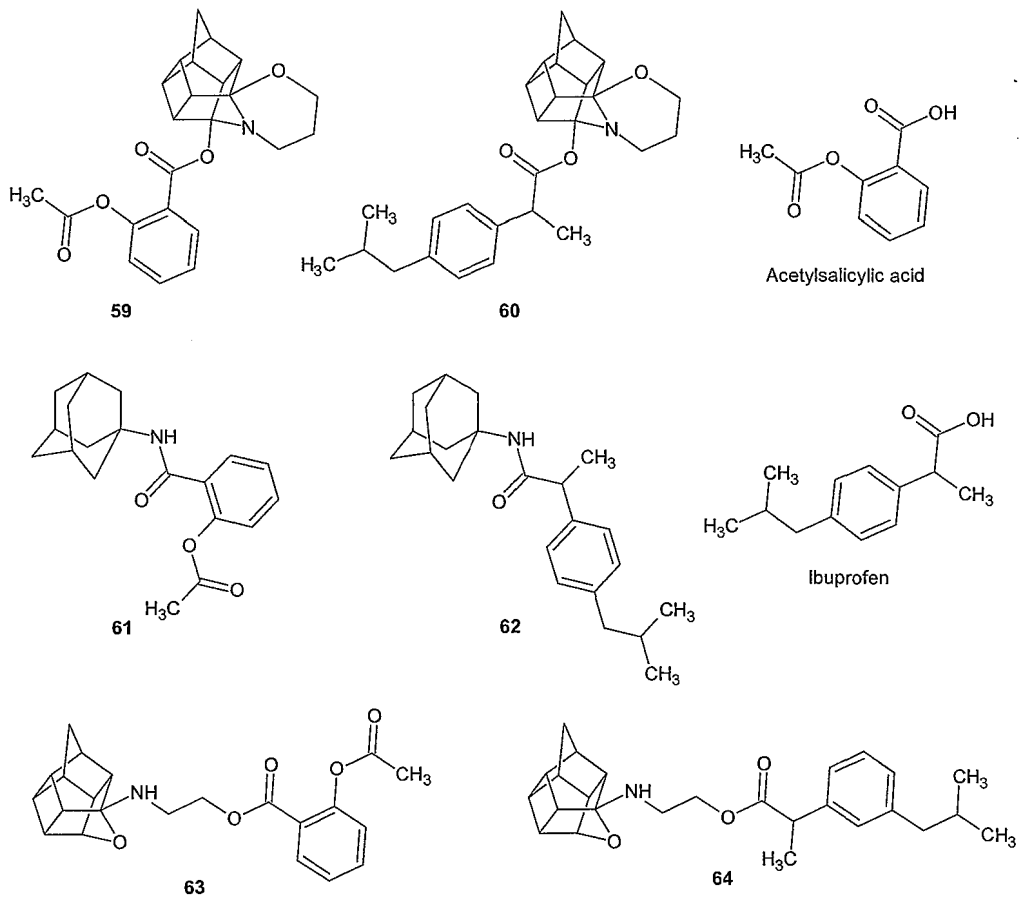
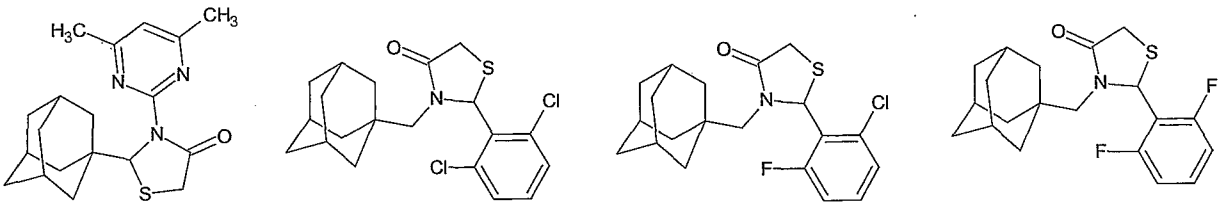


Figure 10: Polycyclic nonsteroidal anti-inflammatory drugs (NSAIDs) conjugated and evaluated for BBB permeability.

The adamantane structure has been used extensively as a lipophilic carrier to enhance transport to the CNS. Amantadine was used as a lipophilic scaffold to develop ester-bond azidothymidine (AZT) prodrugs to improve the transport of AZT into the CNS. In studies where the prodrugs were administered intravenously to rats, the prodrugs were detected in brain tissue at 7 to 18 times higher concentrations than AZT in spite of the negligible amount of the prodrug in the cerebrospinal fluid.[101] More compounds with similar results were later reported where a series of thiazolidin-4-ones bearing the lipophilic adamantly moiety were evaluated for anti-HIV-1 activity.[102] The majority of compounds where amantadine is conjugated at the 2 position showed modest anti-HIV-1 activity, while 2-adamantan-1-yl-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one (**65**) exhibit remarkable antiviral potency (EC_{50} = 0.67 mM). An additional series of compounds were synthesised (**65**, **67**, **68**) with the adamantyl moiety at the 3-position of the thiazolidinone ring. These compounds exhibit high to modest anti-HIV-1 activity (EC_{50} = 1.0-2.0 μM) but also present pronounced cytostatic activity (Figure 11). Adamantane was also used as a lipophilic scaffold attached to a leucine-encephalin derived analgesic peptide that was found to be delivered effectively into the CNS using animal models.[103,104] A methamine derived 1-amino-3-[18F]fluoromethyl-5-adamantane was prepared for evaluation as a positron emission tomography (PET) tracer. In vitro studies revealed that the compound is effectively delivered through the BBB and it shows potential as a ligand for mapping the NMDA receptor-ligand complex.[105]



65 66 67 68

Figure 11: Lipophilic amantadine prodrugs evaluated for BBB permeability and anti-HIV-1 activity

Lipophilic derivatives incorporating the adamantane structure as a carrier, such as 5-adamantylcarboxamido-1,3,4-thiadiazole-2-sulfonamide (**69**, Figure 12), exhibit promising in vivo anticonvulsant properties when tested in a animal model.[106] This result confirms that penetration through the blood-brain barrier is an important factor influencing bioavailability of this CNS drug. Similar observations with related molecules were also reported (**70**, **71**, Figure 12).[107]

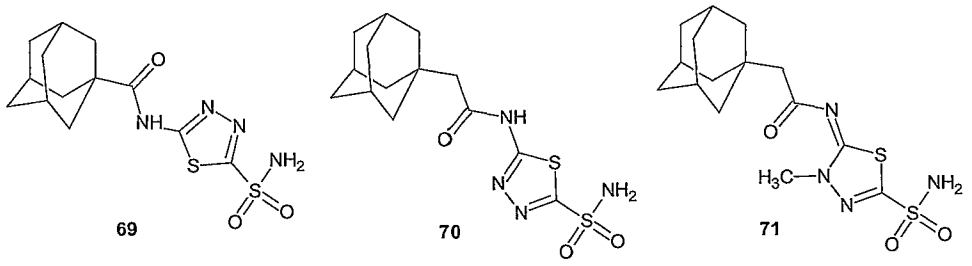


Figure 12: Adamantylcarboxamido-thiadiazole-sulfonamide compounds evaluated for anticonvulsant activity

2.9. Conclusion

From the examples in literature, it can be deduced that pentacycloundecane and adamantane moieties serve as versatile scaffolds for multiple drug targets in the CNS and importantly, simple side chain modification may affect the activity profile significantly. From receptors and ion channels to enzymes and imaging agents, it appears that these compounds are indeed able to provide a stable lipophillic scaffold for the development of neuro-active drugs. These scaffolds can also be used effectively to increase blood-brain barrier permeability of privileged structures. The effects that the polycyclic cage moieties have at ion channels, may have secondary positive effects in the brain, especially with regard to neurodegenerative diseases and this will always have to be factored into the strategy of employing these structures as scaffolds in drug design. It is clear that more in vivo studies are needed to follow up the numerous *in vitro* studies reported so far to advance drug discovery and development in this field. The next few years will unquestionably lead to significant development in these fields of research.

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