
Chapter 1

Introduction & Rational

1.1. Neurodegeneration

Neurodegeneration can be described as the process by which certain neurons in the central nervous system (CNS), and especially the brain, are damaged by a variety of mechanisms (Hibbs *et al.*, 1987). Neurodegeneration and the development of neuroprotective agents have in recent years become an increasingly important focus of research. Much time and effort have gone into establishing the cause of neuropsychiatric disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) as they negatively influence the quality of life of millions of people around the world. These disorders are increasingly becoming a burden to society as the world's population is growing older and the need has therefore increased to discover and develop drugs to prevent these diseases from occurring or developing further (Rang *et al.*, 2007). Currently, there is relatively good symptomatic therapy for both PD and AD, but no proven therapy that prevents cell death, or restores damaged neurons to a normal state (Dawson & Dawson, 2002). There are three key mechanisms of neuronal cell death and they may act separately, or co-operatively to cause neurodegeneration (Araki *et al.*, 2001). This "lethal triplet" includes metabolic compromise, oxidative stress and excitotoxicity, the combined effect of which causes neuronal cell death that is both necrotic and apoptotic in nature. Aspects of these three mechanisms are believed to play a role in the neurodegeneration that occurs in both acute conditions, such as stroke and epilepsy, and in chronic neurodegenerative disorders like Parkinson's disease, Alzheimer's disease and Huntington's disease (Alexi *et al.*, 2000).

Metabolic compromise may be caused by ischemic stroke, asphyxiation, hypoglycaemia and certain toxic products of respiration. Neurotoxic toxins are usually mitochondrial poisons and include amongst others cyanide, carbon monoxide, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 3-nitropropionic acid (3-NP). These mitochondrial toxins result in loss of mitochondrial function and therefore depletion in cellular ATP. Due to the high concentration of mitochondria in the basal ganglia, there is a preponderance of neurodegeneration in these parts following toxic assault. Dysfunction of the mitochondria leads

to loss of intracellular calcium buffering capacity and an increase in the production of damaging oxygen and nitrogen based free radicals, that lead to oxidative stress and the activation of a host of calcium dependant proteases and lipases which result in neurodegeneration (Alexi *et al.*, 2000; Meldrum & Garthwaite, 1990).

In the process of aging the efficiency of the mitochondrial electron transport chain gradually decreases resulting in increased production of highly reactive free radicals, namely reactive oxygen species such as the superoxide anion (O_2^-), and hydroxyl radical ($\cdot OH$) and the reactive nitrogen species (RNS), i.e. peroxynitrate ($ONOO^-$) (Beal, 1995; Hagen *et al.*, 1997). The oxidizing reactions caused by these reactive species destroy membrane lipids, proteins, and DNA and could thus cause neurodegeneration at excessive concentrations. The production of free radicals may be enhanced by certain physiological substances including intracellular calcium, dopamine and nitric oxide synthase (NOS) (Alexi *et al.*, 2000; Mattson *et al.*, 2003).

Excitotoxicity is a term used to describe neuronal death due to the over stimulation of *N*-methyl-D-aspartate (NMDA) receptors, an event that causes an excessive influx of calcium into neuronal cells (Kemp & McKernan, 2002). Calcium overloading of neuronal cells can cause the activation of calcium-dependant signals to enzymes such as phospholipases and proteases, as well as oxidative stress through ROS and RNS, leading to cell death (Mattson, 2003; Meldrum & Garthmaite, 1990; Alexi *et al.*, 2000). Calcium may also enter through voltage-dependant calcium channels (VDCC) and are implicated in calcium overload and mitochondrial disruption (CanoAbad *et al.*, 2001; Mattson, 2003).

Calcium overload stimulates the production of the calcium dependant neuronal nitric oxide synthase (nNOS), an enzyme responsible for catalysing the formation of nitric oxide (NO) in the central nervous system (CNS). Overstimulation of nNOS and subsequent overproduction of NO is known to lead to the development of neurodegenerative processes and cell death (Moncada *et al.*, 1989). Harmful effects caused by an overproduction of NO are thought to be mediated by peroxynitrate ($ONOO^-$), the product obtained when NO and the superoxide anion (O_2^-) react during cellular respiration. Peroxynitrate ($ONOO^-$) causes injury to the mitochondrial electron transport chain resulting in damage and eventual neurodegeneration and death of neurons through the oxidative processes described (Moncada *et al.*, 1989; Hibbs *et al.*, 1987).

1.2. Polycyclic cage compounds and their use as neuro-active drugs

Investigations into the synthesis and chemistry of novel saturated polycyclic hydrocarbon 'cage' compounds have been the aim of several research groups. Interest in the pharmacology and medicinal potential of these compounds was realised with the discovery that the 1-amino-adamantane or amantadine (**1**, Figure 1.1) had antiviral activity against the influenza virus (Davies *et al.*, 1964). Subsequent to this discovery, anti-parkinsonian activity of amantadine (**1**) was found through the serendipitous observation of improvement of Parkinson's disease in patients treated for influenza with the drug (Schwab *et al.*, 1972). Amantadine's pharmacological mechanism as an anti-parkinsonian agent has generally been attributed to its action on nigrostriatal dopaminergic neurons. The anti-Parkinsonian activity of amantadine is expressed by increasing extracellular dopamine (DA) levels *via* DA re-uptake inhibition (Mizoguchi *et al.*, 1994) or DA release and NMDA receptor antagonism (Danysz *et al.*, 1997). Electrophysiological studies further indicated that amantadine acted *via* the phencyclidine (PCP) or MK-801 (dizocilpine) binding site located within the NMDA receptor/ion channel complex (Parsons *et al.* 1999). Interest in the pharmacology of polycyclic cage amines was further stimulated when the 1-amino-3,5-dimethyladamantane derivative of amantadine, memantine (**2**, Figure 1.1), was found to be a clinically well tolerated NMDA receptor antagonist (Parsons *et al.*, 1999). Both amantadine and memantine are low-affinity non-competitive NMDA receptor antagonists and show promise as neuroprotective drugs by preventing excessive influx of calcium into neuronal cells (Parsons *et al.*, 1999). These adamantane cage structures and other potent unnon-competitive NMDA receptor antagonists such as the non-cage compounds MK-801 and phencyclidine (PCP) bind to the PCP binding site located within the ion-channel pore of the NMDA receptor (Dingledine *et al.*, 1999). This antagonism is use dependant as the PCP site is only accessible when the ion channel pore is in an open or activated state. Blocking is accelerated by increased open-channel probability and this suggests that compounds having pronounced use-dependency will have greater affinity for brain regions where over stimulation occur (Grauert *et al.*, 1997). Once bound, the blocker can be trapped by channel closure. Recovery from the trapped, blocked state is generally slow. Hence blockage produced by MK-801 or PCP is difficult to reverse, especially for the long acting MK-801 (Dingledine *et al.*, 1999). As a result, the use of these long duration antagonists is associated with adverse CNS side effects including hallucinations (Carter *et al.*, 1994), memory impairment and neuronal vacuolisation (Brigge, 1993). The low-affinity, use-dependant channel blockers such

as amantadine and memantine are generally not associated with these unacceptable side effects and are well tolerated. The favourable clinical toleration of amantadine (**1**) and memantine (**2**) as opposed to MK-801 also lies in the fact that these polycyclic adamantanes exhibit rapid and strong voltage-dependant blocking kinetics of the NMDA receptor channel (Parsons *et al.*, 1999). From the above discussion it is clear that both amantadine and memantine are ideal lead compounds for the development of novel compounds to treat NMDA-mediated neurodegenerative disorders.

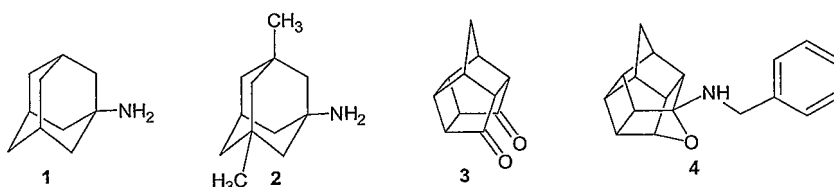


Figure 1.1: Structural correlations of the pentacycloundecane derivatives (**3**, **4**) with amantadine (**1**) and memantine (**2**; Oliver *et al.*, 1991).

A structural similarity (Figure 1.1) exists between the polycyclic cage structure of the adamantane amines (**1,2**) and that of the polycyclic pentacycloundecaneamines (**3, 4**; Oliver *et al.*, 1991a; Marchand, 1995). Pentacycloundecylamines (represented by **4**) are derived from Cookson's diketone (**3**; pentacyclo[5.4.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione), the so called "bird cage" compound, obtained from the intramolecular photocyclisation of the Diels Alder adduct of *p*-benzoquinone and cyclopentadiene (Cooksen *et al.*, 1958). The pentacycloundecane structures and derivatives thereof, like the adamantanes, have a variety of pharmacological activities (Geldenhuys *et al.*, 2005) including antiviral (Oliver *et al.*, 1991c), anti-parkinsonian (Oliver *et al.*, 1991 a,b,c), calcium channel and sodium channel antagonism (Liebenberg *et al.*, 2000; Malan *et al.*, 2000; Van der Schyf *et al.*, 1986) and sigma receptor antagonism (Lui *et al.*, 2001; Nguyen *et al.*, 1999, Kassiou *et al.*, 1996).

The observed anti-parkinsonian activity of amantadine (**1**) and neuroprotective activity of both amantadine (**1**) and memantine (**2**) have led to the evaluation of the pentacycloundecylamines (represented by **4**) as possible therapeutic agents for neurodegenerative disorders (Figure 1.1; Oliver *et al.*, 1991). The calcium channel and sodium channel antagonism described for some of these pentacycloundecylamines also indicate that these structures might act as potential neuroprotective agents (Malan *et al.*, 2000 & 2003). Van der Schyf *et al.*, (1986) first characterized NGP1-01 (**4**; 8-benzylamino-8,11-oxapentacycloundecane) as a novel calcium channel antagonist. Electrophysical studies on NGP1-01 (**4**) and its derivatives indicated *L*-type calcium channel antagonism for this class of

compounds (Malan *et al.*, 2000). Structure activity relationships for NGP1-01 and its derivatives as a putative calcium channel antagonist found that there was a domination of steric and geometric constraints rather than electronic considerations (Malan *et al.*, 2000). Aromatic substitution of NGP1-01 influenced activity significantly while increasing the cage size also led to better inhibition of calcium current (Malan *et al.*, 2000). In the late 1990's a series of azapentacycloundecylamines and pentacycloundecanes were evaluated for sigma receptor (a calcium channel modulator) activity (Kassiou *et al.*, 1996). This might further contribute to the calcium channel activity of the pentacycloundecylamines. Recently, NGP1-01 and other pentacycloundecane derivatives were investigated for their ability to block calcium flow through the NMDA receptor channel (Figure 1.2) (Geldenhuis *et al.*, 2003). In these studies NGP1-01 was able to block NMDA receptor induced calcium flux in an uncompetitive manner, comparable to memantine in the same assay. *In vivo* evaluation of a small series of pentacycloundecylamines in the MPTP parkinsonian mouse model indicated possible neuroprotective activity of these compounds (Geldenhuis *et al.*, 2003). Based on this information it is evident that polycyclic cage compounds have the ability to modulate various neurodegenerative targets and might act as effective neuroprotective drugs, especially in combination with specific functional moieties which could effect neuroprotection through dual or multiple mechanisms.

Another benefit of the adamantane and pentacycloundecylamine polycyclic compounds is their ability to enter the CNS (Prins *et al.*, 2008; Zah *et al.*, 2003). For drugs to exert meaningful effects in the CNS they have to cross the blood brain barrier (BBB) and it was found that the adamantanes and pentacycloundecylamines penetrate the CNS in sufficient concentration to exert pharmacological effects (Prins *et al.*, 2008; Zah *et al.*, 2003). These polycyclic structures also show modification and improvement of the pharmacokinetic and pharmacodynamic properties of known drugs and privileged structures conjugated to them (Oliver *et al.*, 1991a; Geldenhuis *et al.*, 2005). Polycyclic cage structures therefore appear to be useful lipophilic scaffolds to explore the design of potential pharmacologically active compounds with BBB permeability.

1.3. Fluorescent ligands

Radioligand binding techniques have been widely used to study receptor and enzyme pharmacology and physiology. Despite the usefulness and sensitivity of radioligand binding techniques, the use of alternative methods, such as fluorescent ligands, to study ligand-

receptor/enzyme binding interactions may provide information not readily accessible with the use of conventional radioligand binding techniques. This will also circumvent some of the problems associated with radioligand binding assays, such as high cost, difficulty of disposal, health hazard and potential technical implications, associated with radiobinding assays. (McCabe *et al.*, 1992; Auer *et al.*, 1998 & McGrath *et al.*, 1996).

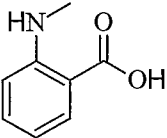
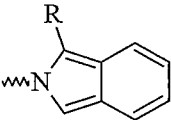
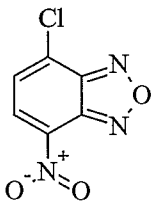
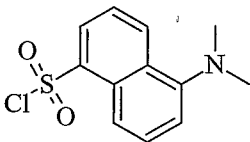
Fluorescent ligands have been used since the mid-1970s and offer several advantages over traditional radioligand binding techniques (McGrath *et al.*, 1996). Some of these advantages include: determining the properties of receptor internalization and sub cellular localization, the study of the thermodynamics and kinetics of ligand binding and the ability to assess the nature of the micro-environment of the ligand binding site (Middleton *et al.*, 2005). These ligands also provide high sensitivity, great versatility while minimally perturbing the cell under investigation and offer an attractive alternative to the application of radioligands without the problems associated with this methodology (Daily *et al.*, 2003).

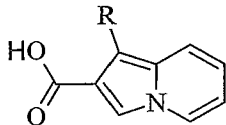
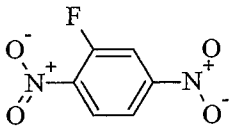
The use of fluorescent ligands is growing rapidly within the pharmacological community and other disciplines. In recent years the use of fluorescent detection methods, that is, fluorescent microscopy, confocal laser scanning microscopy, flow cytometry, multiphoton fluorescent microscopy and image analysis, in nonradioactive assays have found widespread applicability in receptor and enzyme pharmacology (Leipoldo *et al.*, 2009; Daily *et al.*, 2003 & McGrath *et al.*, 1996). Techniques employed to visualize physiological or pathophysiological changes in living cells have become increasingly important in biomedical sciences. Fluorescent ligands with high affinity for receptors and enzymes are one of the cornerstones of real-time imaging of live cells and are powerful tools in various scientific fields (Middleton *et al.*, 2005). Small molecule fluorescent ligands are excellent tools to analyze and clarify the roles of biomolecules in proteins and living cells, affording high spatial and temporal resolution *via* microscopic imaging (Leipoldo *et al.*, 2009; McGrath *et al.*, 1996 & Auer *et al.*, 1998). The development of ligands for probing biological events has thus become an area of intense interest (Leipoldo *et al.*, 2009).

It has therefore been the objective of various research groups to design fluorescent ligands for use in directly quantifying ligand-receptor and/or ligand-enzyme interactions within proteins and cells and to facilitate the development of high-throughput screening methods as alternative to radioligand binding techniques (Middleton *et al.*, 2005; Leipoldo *et al.*, 2009 & Auer *et al.*, 1998). The greatest challenge in the design of fluorometric assays lies in the

development of an appropriate fluorescent ligand while maintaining potency, selectivity, site specificity and accommodating the steric demands of the fluorophore, especially where the fluorescent moiety is conjugated to a small biologically active molecule (Leipoldo *et al.*, 2009; McGrath *et al.*, 1996). Nonetheless, there have been several reports of successful fluorophore-conjugation with small molecule ligands, and advances in fluorometric assays utilising fluorescent ligands have encouraged pursuit of this approach (Leipoldo *et al.*, 2009; Middleton *et al.*, 2005; Hovius *et al.*, 2000). Numerous fluorophores with the required spectrofluorometric properties have been described which may be conjugated to active small molecules, some of which are depicted in table 1.1.

Table 1.1: Fluorescent properties of selected fluorophores

Fluorophore	Description	Excitation/Emission
 N-methylantranilic acid	One of the smallest reactive fluorophores. Small size reduces the likelihood that it will interfere with the function of the active biomolecule, an important advantage when designing site-selective probes (Angelides & Brown, 1984; Angelides & Nutter, 1983)	368/437 nm in MeOH (Haugland <i>et al.</i> , 2002).
 R = CN (Cyanoisoidole); NO ₂ (Nitroisoidole); SCN (Thiocyanoisoidole)	The aromatic dialdehydes, o-phthalaldehyde (OPA) and naphthalene-2,3-dicarboxaldehyde are essentially not fluorescent until reacted with a primary amine in the presence of excess cyanide or thiols (Benson & Hare, 1975).	334/455 nm for the reaction product with glycine in the presence of cyanide, measured in pH 7.0 buffer/MeCN (Haugland <i>et al.</i> , 2002).
 Nitrobenz-2-oxa-1,3-diazole-4-yl (NBD)	NBD is a suitable fluorophore since it is relatively small and is not expected to sterically hinder interactions when coupled to the parent molecule with an appropriate spacer molecule. NBD has a relatively small dipole moment, reducing the possibility of an electrostatic interaction, that could result in a loss of affinity compared to the parent group (Ghosh & Whitehouse, 1968; Birkett <i>et al.</i> , 1970).	485/540 nm for the primary aliphatic amine derivatives of NBD chloride in MeOH and secondary amines in MeOH (Haugland <i>et al.</i> , 2002).
 Dansyl chloride	Dansyl chloride is non-fluorescent until reacted with amines to form amides that exhibit large Stokes shifts, along with environmentally sensitive fluorescent quantum yields and emission maxima (Haugland <i>et al.</i> , 2002).	337/492 nm for the primary aliphatic amine derivatives of dansyl chloride in CHCl ₃ . Emission and quantum yield are highly solvent dependant (Haugland <i>et al.</i> , 2002).

 <p>R = CN (Cyanoindolizine); EtCOC (Ethylindolizine)</p>	<p>The indolizine structures are suitable fluorophores since they are relatively small and are not expected to sterically hinder interactions when coupled to the parent molecule with an appropriate spacer molecule (Weber, 1952).</p>	<p>340/400 nm in EtOH (Haugland <i>et al.</i>, 2002).</p>
 <p>1-Fluoro-2,4-dinitrobenzene</p>	<p>A relatively small fluorophore that reduces the likelihood to interference with the function of the biomolecule (Haugland <i>et al.</i>, 2002).</p>	<p>396/449 nm in EtOH (Haugland <i>et al.</i>, 2002).</p>

1.4. Study Aim

This study was aimed at developing fluorescent ligands, which can be used to study receptor-ligand and/or enzyme-ligand interactions using fluorescent techniques. Conjugates of fluorophores that possess high affinity for the NMDA receptor, voltage gated calcium channels (VGCC) and/or the NOS enzyme was designed and synthesised with the aim to directly measure binding of these novel molecules to receptors and/or enzymes utilizing fluorescent techniques (compounds **5 – 20**, Figure 1.2). These compounds could be particularly useful in competitive binding studies to determine the specificity and affinity of uncharacterized compounds (Leipoldo *et al.*, 2009; Middleton *et al.*, 2005; McGrath *et al.*, 1996). Novel fluorescent polycyclic ligands found with the sought-after potency, sensitivity and selectivity for a specific receptor or enzyme, could be used to screen potential test compounds for specific binding to a receptor or enzyme, either intracellular or extracellular, by competitive binding with this fluorescent polycyclic ligand. The fluorescence of the displaced ligand could be used to determine and quantify the binding of the compound(s) screened.

For this study the primary objectives were to design and synthesise a series of fluorescent polycyclic ligands and to evaluate these compounds as fluorescent ligands for CNS enzyme and receptor targets directly pertaining to the development of neurodegeneration (compounds **5 – 20**, Figure 1.2). These targets include the NOS enzyme, VGCC and/or the NMDA receptor. The polycyclic structures, including adamantane (**1**, Figure 1.1) and pentacycloundecane derivatives (represented by **4**, Figure 1.1), were selected to be conjugated to known fluorophores (Table 1.1), because of the uncompetitive manner of binding of these polycyclic structures to the VGCC and NMDA receptor and the additional benefit of these

molecules to facilitate CNS penetration (Geldenhuis *et al.*, 2005). This may aid the selective binding of the fluorescent ligands to their intended target(s).

The second objective was to write and publish a series of review papers directly pertaining to this research. The first review describes polycyclic structures as lipophilic scaffolds for neuroactive drugs (Chapter 2), while the second review focuses on the design of fluorescent ligands for central nervous system imaging (Chapter 3). A third review focuses on recent developments in the patented literature on NOS inhibitors (Chapter 4).

A third objective was to evaluate the biological profiles of compounds selected from the synthesised series as multifunctional neuroprotective agents, possibly displaying activity against the NOS enzyme, VGCC, NMDAR and for oxidative free radicals.

The final findings from this study were compiled into three research articles published in peer-reviewed journals. The first article (Chapter 5) describes the design and synthesis of novel fluorescent-polycyclic conjugates that display micromolar inhibition of the NOS enzyme (compounds **5**, **6**, **9-14**; Figure 1.2). This study is based on the design of fluorescent ligands resembling the structure of 7-nitroindazole (7-NI), a selective nNOS inhibitor (Handy *et al.*, 1995), conjugated to polycyclic structures for effective BBB permeability and increased selectivity. These novel fluorescent ligands may be used to develop high-throughput direct binding nNOS competition assays. (Data from a M.Sc. degree, Joubert *et al.*, 2007, was used for this paper and additional data was included such as; improved synthetic methods, additional biological data and fluorescent measurements, which were compiled during the first year of this Ph.D. study to elaborate on the potential value of these fluorescent compounds). The second paper (Chapter 6) describes the expansion of the initial set of fluorescent polycyclic derivatives and their potential as multifunctional neuroprotective agents (compounds **7-11**, **14**; Figure 1.2). It was found that the compounds designed in the first paper exhibited dual or multifunctional activity against various CNS targets that play a role in neurodegeneration, which sparked further investigation of these novel compounds. The third and final article included (Chapter 7) describes the synthesis and design of fluorescent polycyclic ligands (compounds **5-11**, **14**, **19**, **20**; Figure 1.2.) for the NMDA receptor and/or VGCC and includes a direct binding assay as proof of concept, portraying the potential application of these novel fluorescent ligands as imaging probes for the development of direct binding competition assays for CNS disorders.

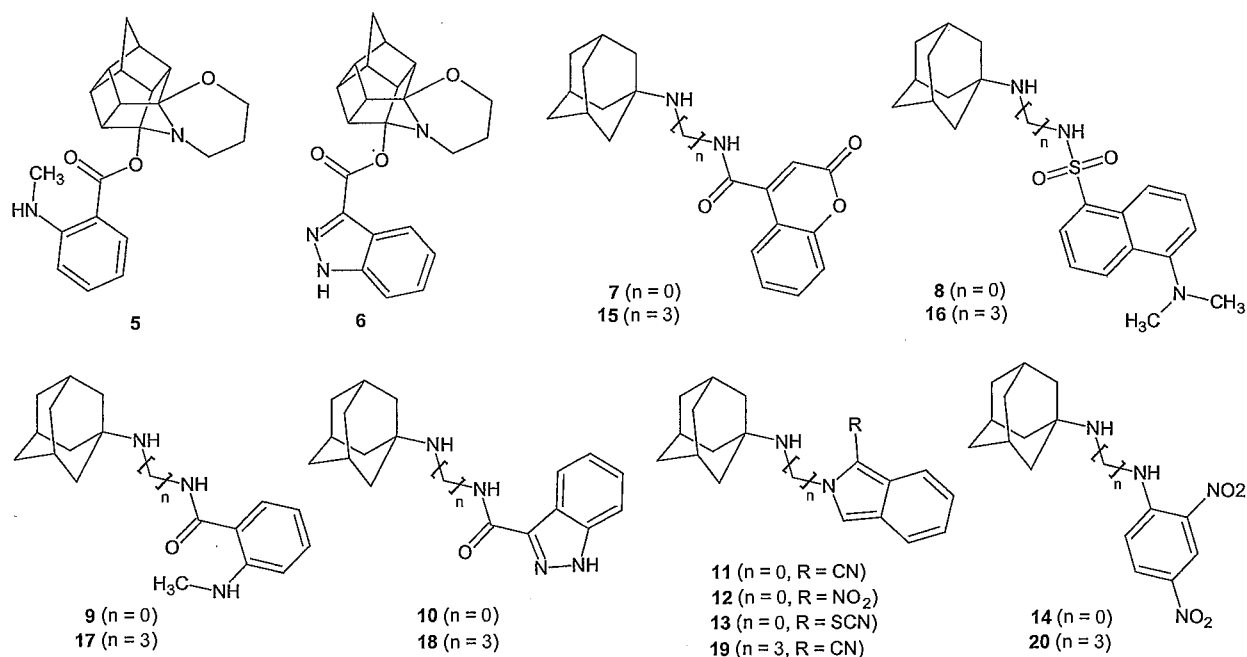


Figure 1.2: Fluorescent polycyclic analogues synthesised and evaluated as potential fluorescent ligands.

References

- ALEXI, T., BORLOGAN, C.V., FAULL R.L.M., WILLIAMS, C.E., CLARK., R.G., GLUCKMAN, P.D. & HUGES, P.E. 2000. Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. *Progress in Neurobiology*, 60(5):409-470.
- ANGELIDES, K.J., & BROWN, G.B. 1984. Fluorescence resonance energy transfer on the voltage-dependent sodium channel. Spatial relationship and site coupling between the batrachotoxin and *Leiurus quinquestriatus quinquestriatus* alpha-scorpion toxin receptors. *Journal of Biological Chemistry*, 259:6117-6126.
- ANGELIDES, K.J., & NUTTER, T.J. 1983. Preparation and characterization of fluorescent scorpion toxins from *Leiurus quinquestriatus quinquestriatus* as probes of the sodium channel of excitable cells. *Journal of Biological Chemistry*, 258:11948-11957.
- ARAKI, T., KUMAGAI, T., TANAKE, K., MATSUBARA, M., KATO, H., ITOYAMA, Y., IMAI, Y. 2001. Neuroprotective effect of riluzole in MPTP-treated mice. *Brain research*, 918(1-2):176-181.

AUER, M., MOORE, K. J., MYERS-ALMES, F. J., GUENTHER, R., POPE, A. J. & STOEKLI, K. A. 1998. Fluorescence Correlation Spectroscopy: Lead Discovery by Miniaturisation HTS. *Drug Discovery Today*, 3:457-465.

BEAL, M.F. 1995. Aging, energy, and oxidative stress in neurodegenerative diseases. *Annals of neurology*, 38(3)357-366.

BENSON, J.R. & HARE, P.E. 1975. o-Phthalaldehyde: Fluorogenic Detection of Primary Amines in the Picomole Range. Comparison with Fluorescamine and Ninhydrin. *Proceedings of the National Academy of Science*, 72:619-622.

BIRKETT, D.J., PRICE, N.C., RADDA, G.K. & SALMON, A.G. 1970. The reactivity of SH groups with a fluorogenic reagent. *The Journal of the Federation of American Societies for Experimental Biology Letters*, 6:46-348.

BRIGGE, C.F. 1993. Structural requirements for the development of potent N-methyl-D-aspartic acid (NMDA) receptor antagonists. *Biochemical Pharmacology*, 45(8):1547-1561.

CANO-ABAD, M.F., VILLARROYA, M., GARCIA, A.G., GABILAN, N.H. & LOPEZ, M.G. 2001. Calcium entry through L-type calcium channels causes mitochondrial disruption and chromaffin cell death. *Journal of Biological Chemistry*, 276(43):39695-39704.

CARTER, A.J. 1994. Many agents that antagonize the NMDA receptor-channel complex *in vivo* also cause disturbances of motor coordination. *Journal of Pharmacology and Experimental Therapeutics*, 269(2):573-580.

COOKSEN, R.C., GRUNDWELL, E. & HUDEC, J. 1958. Synthesis of cage-like molecules by irradiation of Diels-Alder adducts. *Chemistry and Industry*, 39:1003-1004.

DALY, C.J. & McGRATH, J.C. Fluorescent ligands, antibodies, and proteins for the study of receptors. 2003. *Pharmacology & Therapeutics*, 100:101-118.

DANYSZ, W., PARSONS, C.G., KORNHUBER, J., SCHMIDT, W.J. & QUACK, G. 1997. Aminoadamantanes as NMDA receptor antagonists and antiparkinsonian agents - preclinical studies. *Neuroscience & Biobehavioral Reviews*, 21(4):455-468.

DAVIES, W.L., GRUNERT, R.R., HAFF, R.F., MCGAHEN, J.W., NEUMAYER, E.M., PAULSHOCK, M., WATTS, J.C., WOOD, T.R., HERMANN, E.C. & HOFFMANN, C.E. 1964. Antiviral activity of 1-adamantanamine (amantadine). *Science*, 144:862-863.

DAWSON, T.M. & DAWSON, V.L. 2002. Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nature Neuroscience*, 5:1058-1061.

DINGLEDINE, R., BORGES, K., BOWIE, D. & TRYANELIS, S.F. 1999. The glutamate receptor ion channels. *Pharmacological Reviews*, 51(1):7-61.

GELDENHUYS, W.J., MALAN, S.F., BLOOMQUIST, J.R. MARCHAND, A.P. & VAN DER SCHYF, C. J. 2005. Pharmacology and structure-activity relationships of bioactive polycyclic cage compounds: a focus on pentacycloundecane derivatives. *Medical Research Reviews*, 25(1):21-48.

GHOSH, P.B. & WHITEHOUSE, M.W. 1968. 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole: a New Fluorogenic Reagent for Amino Acids and other Amines. *Biochemistry Journal*, 108:155-156.

GRAUERT, M., BECHTEL, W.D., ENSIGER, H.A., MERSZ, H. & CARTER, A.J. 1997. Synthesis and structure-activity relationships of 6,7-benzomorphan derivatives as antagonists of the NMDA receptor-channel complex. *Journal of Medicinal Chemistry*, 40(18):2922-2930.

HAGEN, T.M., YOWE, D.L., BARTHOLOMEW, J.C., WEHR, C.M., DO, K.L., PARK, J. & AMES, B.N. 1997. Mitochondrial decay in hepatocytes from old rats: Membrane potential declines, heterogeneity and antioxidants increase. *Proceedings of the national academy of sciences of the United States of America*, 94(7):3064-3069.

HAUGLAND, J.G., GREGORY, J., SPENCE, M.T.Z. JOHNSON, I. & MILLER, E. 2002. Handbook of fluorescent probes and research products. USA: Molecular probes.

HIBBS, J.B., VAVRIN, Z. & TAINTOR, R.R. 1987. L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *Journal of Immunology*, 138:550-565.

HOVIUS, R., VALLOTTON, P., WOHLAND, T. & VOGEL, H. 2000. Fluorescence techniques: shedding light on ligand–receptor interactions. *Trends in Pharmacological Sciences*, 21(7):266-273.

JOUBERT, J., VAN DYK, S., MALAN, S.F. 2007. NOS inhibition of novel fluorescent polycyclic ligands. North-West University: Potchefstroom Campus. (Dissertation – M.Sc) 106 p.

KASSIOU, M., NGUYEN, V.H. KNOTT, R., CHRISTIE, M. & HAMBLEY, T.W. 1996. Trishomocubanes, a new class of selective and high affinity ligands for the sigma binding site. *Bioorganic and Medicinal Chemistry Letters*, 6:595-600.

KEMP, J.A., & McKERNAN, R.M. 2002. NMDA receptor pathways as drug targets. *Nature Neuroscience*, 5:1039-1042.

LEOPOLDO, M. LACIVITA, E., BERARDI, F. & PERRONE, R. 2009. Developments in fluorescent probes for receptor research. *Drug Discovery Today*, 14(13-14):709-712.

LIEBENBERG, W., VAN DER WALT, J.J. & VAN DER SCHYF, C.J. 2000. Effects of derivatives of NGP 1-01, a putative calcium channel antagonist, on electrically stimulated guinea-pig papillary muscle. *Pharmazie*, 55(11):833-836.

LIU, X., NUWAYHID, S., CHRISTIE, M.J. KASSIOU, M. & WERLING, L.L. 2001. Trishomocubanes: novel sigma-receptor ligands modulate amphetamine-stimulated [³H]dopamine release. *European Journal of Pharmacology*, 422(1-3):39-45.

MALAN, S. F., DYASON, K., WAGENAAR, B., VAN DER WATT, J.J. & VAN DER SCHYF, C.J. 2003. The structure and ion channel activity of 6-benzylamino-3-hydroxyhexacyclo[6.5.0.0(3,7).0(4,12).0(5,10).0(9,13)]tridecane. *Archiv der Pharmazie*, 336(2):127-133.

MALAN, S.F. VAN DER WALT, J.J. & VAN DER SCHYF, C.J. 2000. Structure-activity relationships of polycyclic aromatic amines with calcium channel blocking activity. *Archiv der Pharmazie*, 333(1):10-16.

MARCHAND, A.P., ARNEY, B.E., DAVE, P.R., SATYANARAYANAN., WATSON, W.H. & NAGL, A. 1988. Transannular cyclizations in the

- pentacyclo[5.4.0.0²,6.0³,10.0⁵,9]undecane-8,11-dione system. A reinvestigation. *Journal of Organic Chemistry*, 53(11):2644–2647.
- MATTSON, M.P. 2003. Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. *Neuromolecular Medicine*, 3(2):65-94.
- McCABE, R.T., NEWMAN, A.H. & SKOLNICK, P. 1992. AHN 682 a fluorescent ligand for periheral-type benzodiazepine receptors. *Journal of Pharmacology and Experimental Therapeutics*, 262 (2):734–740.
- McGRATH, J.C., ARRIBAS, S. & DALY, C.J., 1996. Fluorescent Ligands for the Study of Receptors. *Trends in Pharmacological Sciences*, 17:393-399.
- MELDRUM, B. & GARTHWAITE, J. 1990. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends in Pharmacological Sciences*, 11(9):379-387.
- MIDDLETON, R.J. & KELLAM, B. 2005. Fluorophore-tagged GPCR ligands. *Current Opinion in Chemical Biology*, 9(5):517-525.
- MIZOGUCI, K., YOKOO, H., YOSHIDA, M., TANAKA, T. & TANAKE, M. 1994. Amantadine increases the extracellular dopamine levels in the striatum by re-uptake inhibition and by *N*-methyl-D-aspartate antagonism. *Brain Research*, 662(1-2):255-258.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. 1989. Biosynthesis of nitric oxide from *L*-arginine. A pathway for the regulation of cell function and communication. *Biochemical Pharmacology*, 38(11):1709-1715.
- NGUYEN, V.H., KASSIOU, M., JOHNSON, G.A., & CHRISTIE, M.J. 1996. Comparison of binding parameters of sigma 1 and sigma 2 binding sites in rat and guinea pig brain membranes: novel subtype-selective trishomocubanes. *European Journal of Pharmacology*, 311(2-3):233-240.
- OLIVER, D. W., DEKKER, T. G. & SNYCKERS, F. O. 1991a. Pentacycloundecylamines Synthesis and Pharmacology. *European Journal of Medicinal Chemistry*, 1991a, 26:375-379.

- OLIVER, D.W., DEKKER, T.G. & SNYCKERS, F.O. 1991c. Antiviral properties of 4-amino-(D3)-trishomocubanes. *Arzneimittelforschung*, 41(5):549-552.
- OLIVER, D.W., DEKKER, T.G. SNYCKERS, F.O. & FOURIE, T.G. 1991b. Synthesis and biological activity of D3-trishomocubyl-4-amines. *Journal of Medicinal Chemistry*, 34(2):851-854.
- PARSONS, C.G., DANYSZ, W. & QUACK, G. 1999. Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist - a review of preclinical data. *Neuropharmacology*, 38(6):735-776.
- PRINS, L.H., DU PREEZ, J.L., VAN DYK, S. & MALAN, S.F. 2009. Polycyclic cage structures as carrier molecules for neuroprotective non-steroidal anti-inflammatory drugs. *European Journal of Medicinal Chemistry*, 44(6):2577-2582.
- RANG, H.P., DALE, M.M. & RITTER, J.M. Rang and Dale's Pharmacology, Elsevier, Philadelphia. 2007; chapter. 35, pp. 508.
- SCHWAB, R. S. POSKANZER, D. C. ENGLAND, A. C. & YOUNG, R.R. 1972. Amantadine in Parkinson's disease. Review of more than two years' experience. *Journal of the American Medical Association*, 222(7):792-795.
- VAN DER SCHYF, C. J., SQUIER, G. J. & COETZEE, W. A. 1986. Characterization of NGP 1-01, an aromatic polycyclic amine, as a calcium antagonist. *Pharmacological Research Community*, 18(5):407-417.
- WEBER, G. 1952. Polarization of the Fluorescence of Macromolecules. *Biochemistry Journal*, 51:155-167.
- ZAH, J., TERRE'BLANCHE, G., ERASMUS, E. & MALAN, S. F. 2003. Physicochemical prediction of a brain-blood distribution profile in polycyclic amines. *Bioorganic and Medicinal Chemistry*, 11(17):3569-3578.