CHAPTER 2
RATIONALE AND SELECTION OF COMPOUNDS

Considering the need for improved strategies for the treatment of neurodegenerative diseases, the development of new neuroprotective agents is an active field of research. Alzheimer's disease (AD) and Parkinson's disease (PD) are two of many devastating neuronal breakdown diseases, with a curative strategy not known, although symptomatic treatment regimes are in practice. Since the underlying mechanisms of neurodegeneration are largely unknown, creating effective multifunctional neuroprotective drugs, is not an easily attainable goal. This study focused on the development of multifunctional drugs, which firstly may halt the neuronal breakdown process, and secondly may also be functional in treating the symptoms. The drugs should therefore act as symptomatic as well as curative treatment.

1 Propargylamine

1.1 Introduction

Rasagiline [N-propargyl-(R)-aminoindan] is a second generation propargylamine that irreversibly inhibits brain MAO-B, and has promising neuroprotective activity. Currently it is registered in several countries as monotherapy and adjunctive therapy for the management of PD (Chen *et al.*, 2007). After several years of study and research, it has been established that the neuroprotective effects of rasagiline [N-propargyl-l(R)-aminoindan] can be attributed to its propargyl moiety. The propargylamine functional group is therefore the pharmacophore responsible for the neuroprotective activity. This observation was made on the grounds that propargylamine itself exerts the same neuroprotective effects as offered by rasagiline. It has also been established that the MAO-B inhibiting activity is not a prerequisite for the neuroprotection provided by rasagiline (Bar-Am *et al.*, 2005).

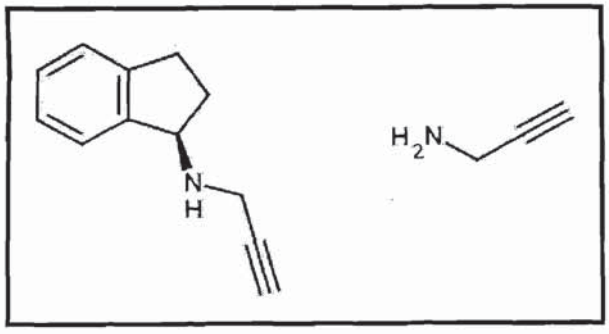


Fig. 2.1 The structures of rasagiline (left) and propargylamine (right)

Propargylamines have been reported to inhibit apoptosis (Bar-Am *et al.*, 2005). As described in the previous section, apoptosis is a cell death process by which nerve cells in certain areas of the brain are destroyed. This leads to the characteristic signs and symptoms of neurodegenerative diseases as seen in Parkinson's disease (PD) and Alzheimer's disease (AD). A well-known hypothesis suggests that this process is the predominant mode of neuronal death in neurodegenerative disorders (Bar-Am *et al.*, 2005). For this reason it is of significant importance to develop and study compounds, such as propargylamine derivatives (propargylamines), that may possibly attenuate the progressive neurodegeneration found in these diseases.

Also of significance is the observation that propargylamine is a MAO-inhibitor. Although this inhibition is very poor (Bar-Am *et al.*, 2005), it may contribute to its observed neuroprotective activity (Yu *et al.*, 1992). Other propargylamine derivatives are reported to act as more potent MAO-inhibitors. Since MAO activity is increased in both Alzheimer's and Parkinson's disease (Mandel *et al.*, 2005) MAO-inhibitors may be of further therapeutic benefit.

1.2 Neuroprotective properties of propargylamine

The neuroprotective properties of propargylamine (PA) are very diverse and include the following:

- Propargylamine significantly increases mRNA expression levels of glial cell-line derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF). GDNF's increased level is mediated through activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Maruyama *et al.*, 2004).
- Propargylamine may inhibit p53 dependent apoptosis, which is mediated by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Berry *et al.*, 1998; Berry, 1999; Zhang *et al.*, 1999).
- Propargylamine prevents the activation and translocation of GAPDH from the cytoplasm to the nucleus (Mandel *et al.*, 2005), possibly through binding to GAPDH (Kragten *et al.*, 1998), and thereby immobilising it.
- Propargylamine reverses the apoptotic effect, by preventing the activation of caspase-3, and the cleavage of poly(ADP-ribose)polymerase (PARP) (Bar-Am *et al.*, 2005).
- Propargylamine significantly increases Bcl-2 mRNA levels, while markedly reducing Bax gene expression. It increases the Bcl-2/Bax mRNA ratio, with an increase in Bcl-2 levels and a decrease in Bax levels (Bar-Am *et al.*, 2005; Mandel *et al.*, 2005). Bad- and Bim-protein (BH3-only proteins

of the Bcl-2 family) levels (Bar-Am *et al.*, 2005; Weinreb *et al.*, 2004; Youdim *et al.*, 2003) are reduced, while the anti-apoptotic Bcl-2 family member, Bcl-xL is induced (Weinreb *et al.*, 2004; Youdim *et al.*, 2003).

- Even though propargylamine does not affect holo-APP mRNA levels, it significantly down-regulates holo-APP protein levels. This indicates a post-transcriptional regulatory mechanism (Rodgers *et al.*, 2002; Shaw *et al.*, 2001).
- Propargylamine increases sAPPα levels (Bar-Am *et al.*, 2005).
- Propargylamine stimulates sAPPα release via the protein kinase C/mitogen-activated protein kinase (PKC/MAPK) signalling pathway (Yogev-Falach *et al.*, 2002, 2003).
- Propargylamine down-regulates PKCγ levels, whilst increasing PKCα and PKCϵ levels (Weinreb *et al.*, 2004). It stimulates PKC phosphorylation and induces translocation of the isoforms PKCα and PKCϵ to the hippocampal membrane department, and down-regulates PKCδ levels (Mandel *et al.*, 2005).
- Propargylamine dose dependently induces MAPK phosphorylation, with MEK and PKC-signalling being of significant importance (Yogev-Falach *et al.*, 2003). It activates ERK1/2 pathways resulting in increased processing of APP via activation of zinc-dependent metalloprotease (super oxide dismutase, SOD) and α-secretase, to release sAPPα (Mandel *et al.*, 2005).
- Propargylamine activates the PKC-MAPK pathway (Bar-Am *et al.*, 2005).
- Propargylamine inhibits monoamine oxidase, even though its neuroprotective activity is independent of its MAO-inhibitory activity (Maruyama *et al.*, 2004).

2 Polycyclic cage structure compounds

2.1 Introduction

Polycyclic cage compounds, such as amantadine and memantine, have various pharmaceutical applications, with special interest in the symptomatic and proposed curative treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The polycyclic cage can be used to modify and improve the pharmacokinetic and pharmacodynamic properties of drugs, and from literature it is apparent that the polycyclic cage is useful as both a scaffold for side-chain attachment as well as for improving a drug's lipophilicity. This lipophilicity enhances a drug's transport across cellular membranes, and

increases its affinity for lypophilic regions in receptors (Zah *et al.*, 2003). In addition, these polycyclic moieties afford metabolic stability, thereby prolonging the pharmacological effect of a drug, leading to a reduction of dosing frequency of medicine and helping to improve patient compliance (Brookes *et al.*, 1992). The novel NMDA channel antagonism of these compounds, combined with DA uptake inhibition and L-type calcium channel blocking activity, suggests that pentacyclo-undecane derived compounds may have exciting potential as therapeutic agents for neurodegenerative diseases, such as traumatic brain injury, stroke, Parkinson's disease and Alzheimer's disease (Geldenhuys *et al.*, 2004).

With the focus being on the development of multifunctional drugs, it was thus a rational decision to incorporate the polycyclic cage structures into the novel drugs envisaged for synthesis. In the current study the focus will therefore be on cage compounds that have potential to be therapeutic in the treatment of neurodegenerative diseases, especially Parkinson's disease and Alzheimer's disease. The cage structures will include adamantylamines (e.g. amantadine and memantine) and pentacyclo-undecylamines.

2.2 Biological activity

The adamantylamines, amantadine and memantine, offer therapeutic benefit by increasing extra cellular dopamine levels and preventing excessive influx of calcium into neuronal cells. Amantadine possesses anti-parkinsonian activity since it increases extra cellular dopamine (DA) levels through DA re-uptake inhibition (Mizoguchi *et al.*, 1994) and DA release (Danysz *et al.*, 1997). Electrophysiological studies further indicated that amantadine acts through the phencyclidine (PCP) binding site located within the N-methyl-D-aspartate (NMDA) receptor/ion channel complex, blocking uptake of calcium ions into neurons (Parsons *et al.*, 1999). This antagonism is use-dependent in that the PCP site is only accessible when the ion channel pore is in an open or activated state. The channel block is accelerated by increases in open channel probability (Geldenhuys *et al.*, 2004). Memantine is used clinically in Alzheimer's disease (Kroemer *et al.*, 1998), and is a low-affinity non-competitive NMDA antagonist. Due to this classification it shows definite potential as a neuroprotective drug, by preventing excessive influx of calcium into neuronal cells (Turski *et al.*, 1991; Zuddas *et al.*, 1992; Brouillet & Beal, 1993). It exhibits rapid and strong voltage-dependent blocking kinetics. Partial trapping and release of memantine from NMDA channels, favour the occurrence of strong block only during sustained receptor stimulation (such as may occur in excitatory neurotoxic events including ischemia and brain trauma), with more limited effects expected during normal synaptic transmission (Parsons *et al.*, 1999).

Low-affinity use-dependent channel blockers (i.e. antagonists with a K_i above 200 nM) such as amantadine and memantine are generally not associated with the unacceptable side-effects, such as hallucinations (Carter, 1994), memory impairment (Trist, 2000) and neuronal vacuolisation (Bigge, 1993), and are well tolerated (Parsons *et al.*, 1999; Kroemer *et al.*, 1998). Amantadine and memantine are thus ideal lead compounds for the development of other agents with favourable indices to treat NMDA/ion channel-mediated neurodegenerative diseases (Gendenhuys *et al.*, 2004).

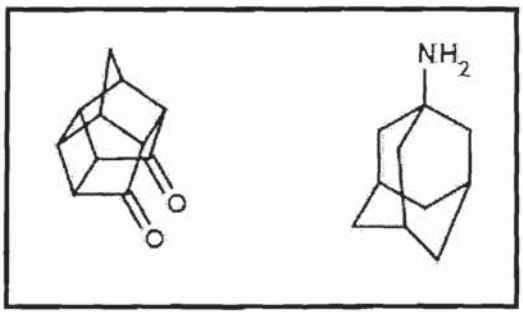


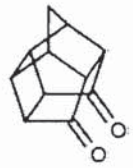
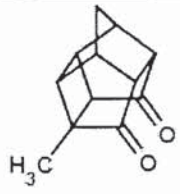
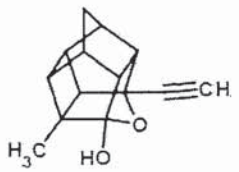
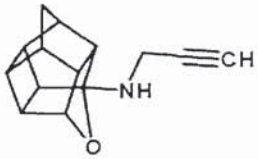
Fig. 2.2 The structures of pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8,11-dione (left) and amantadine (right)

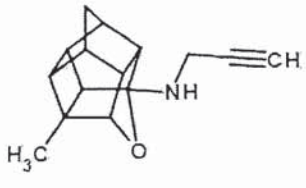
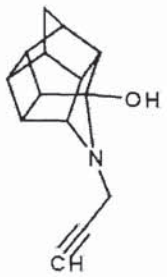
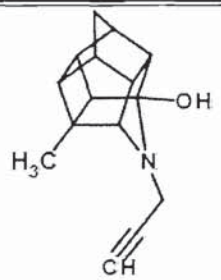
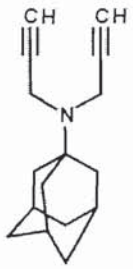
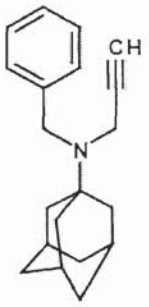
Pentacyclo-undecylamines are derived from Cookson's diketone (pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8,11-dione), the so-called "bird cage" compound, that is obtained from the intramolecular [2 + 2] photocyclisation of the Diels-Alder adduct of p-benzoquinone and cyclopentadiene (Cookson *et al.*, 1964). The pentacyclo-undecylamines are beneficial due to their broad spectrum of therapeutic activity. They have been shown to increase cellular dopamine levels, by inhibiting DA uptake and stimulating DA release (Geldenhuys *et al.*, 2003; Geldenhuys *et al.*, 2004; Geldenhuys *et al.*, 2005). They also have very weak MAO-B inhibiting activity, and apparent neuroprotective activity. It is thus clear that these compounds can definitely contribute to the design of neuroprotective agents. The spectrum of activity within the pentacyclo-undecylamines make them ideal candidates for exploration as possible lead compounds for dual symptomatic and neuroprotective therapies against PD (Geldenhuys *et al.*, 2004) and possibly AD as well.

3 Relevant compounds to be synthesised

The compounds that were selected for this study are illustrated in Table 2.1. With the exception of **1a-c**, all the compounds were polycyclic cage compounds conjugated to propargylamine or a derivative thereof.

Table 2.1. Compounds to be synthesised

COMPOUND	STRUCTURE	NAME
1a		Pentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8-11-dione
1b		1-Methyl-pentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8-11-dione
1c		Pentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-11-one
2		1-Methyl-8-ethynyl-11-hydroxy-8,11-oxapentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane
3		8-Phenylethynyl-8-hydroxy-pentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane
4a		8-(N)-Propargylamino-8,11-oxapentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane

4b		1-Methyl-8-(N)-propargylamino-8,11-oxapentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane
5a		8-Hydroxy-(N)-propargyl-8,11-azapentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane
5b		1-Methyl-8-hydroxy-(N)-propargyl-8,11-azapentacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane
6		*N,N*-Propargyl-adamantan-1-amine
7		N-Propargyl-N-benzyl-adamantan-1-amine

3.1 Rationale of compounds to be synthesised

Compounds **1a**, **1b** and **1c** will be synthesised as precursors to test compounds **2-5**. Compounds **4a** and **5a** will be synthesised from precursor **1a,** compounds **2**, **4b** and **5b** from precursor **1b** and compound **3** from precursor **1c**. Compounds **6** and **7** will be synthesised from amantadine.

Compounds **2** and **3** will be synthesised to evaluate the activity of an acetylene group, with compound **2** synthesised to evaluate the necessity and the activity of a terminal acetylene group, and compound **3** producing insight into the activity of an acetylene group between two non-polar groups, which is not terminal.

Compounds **4a**, **4b**, **5a**, **5b**, **6** and **7** will be synthesised to evaluate the effect of different polycyclic cage structures on the activity of the propargylamine group when combined in one structure. They will also be synthesised as an approach to combine the neuroprotective activity of propargylamine with that of the polycyclic cage structures. Compounds **5a**, **5b** and **7** will also be synthesised to evaluate the activity of the tertiary propargylamine. Compound **6** will give insight into the effect of a second propargyl group on the nitrogen.

4 Concluding remarks

Developing a compound that can offer symptomatic relief as well as change the course of a disease (i.e. an anti-apoptotic drug), can be of significant importance. Since propargylamine and propargylamine derivatives are anti-apoptotic agents as well as MAO inhibitors, they could serve as possible therapeutic agents with potential. In this study a series of propargylamine derivatives will be synthesised and evaluated as anti-apoptotic agents and MAO-B inhibitors. Such multifunctional drugs may have enhanced value in the treatment of neurodegenerative disorders such as PD and AD.