Chapter 8

Conclusion

The use of small molecule fluorescent ligands have in recent years become an area of intense interest, especially in the study of neurological disorders (Leopoldo *et al.*, 2009), yielding mechanistic insights into central nervous system (CNS) receptor and enzyme binding and assisting in the design of high throughput screening (HTS) assays and prodromal diagnostic tools/methods.

This study focused on the development of novel fluorescent ligands, using polycyclic structures as scaffolds with inherent activity and the ability to promote favourable pharmacokinetic and pharmacodynamic properties of the fluorescent moieties conjugated to these scaffolds. This study set out to design and synthesise a series of fluorescent polycyclic structures (**5-6**, **9-14**, Figure 8.1), resembling the structure of the selective neuronal nitric oxide synthase (NOS) inhibitor 7-nitroindazole (7-NI) and other privileged structures. These compounds exhibited moderate to high affinity and inhibition of the NOS enzyme and favourable spectroscopic properties for fluorescent imaging (Joubert *et al.,* 2008). The compounds identified, especially **5** and **11** ($IC_{50} < 1$ μM), may be further utilised in the design of HTS fluorescent assays and *in vivo* imaging studies using modern spectroscopic techniques, including fluorescent microscopy, confocal laser scanning microscopy, flow cytometry and multiphoton microscopy. These compounds thus have potential as pharmacological tools to investigate NOS enzyme-ligand interactions in the quest for effective neuroprotective strategies, while at the same time, circumventing the drawbacks associated with traditional radioligand binding assays, which are still the preferred method utilised in the study of ligand-binding interactions with NOS isoforms (Joubert *et al.*, 2011a). The potential of these novel fluorescent polycyclic structures as NOS inhibitors and the documented calcium channel modulation observed for selected cage structures (Van der Schyf *et al.*, 1986; Malan *et al.*, 2000; Malan *et al.*, 2003), indicate that these novel compounds may also, in addition to their fluorescent application(s), find use as multipotent therapeutic agents in neuroprotection.

Excess accumulation of calcium in neuronal cells rapidly leads to cell death through a variety of mechanisms, including activation of proteases, nucleases, phospholipases, NOS, and other degenerative enzymes that not only lead to the activation of cell death cascades, but also to free radical formation (Lipton, 1999; Van der Schyf *et al.*, 2009). The adamantane fluorescent compounds described above (**9-11**, **14**; Figure 8.1) and structurally related novel fluorescent adamantanes (**7**, **8**; Figure 8.1) were evaluated for VGCC-, NMDA receptor- and NOS inhibition and their ability to scavenge detrimental oxygen and nitrogen free radicals (Joubert *et al.*, 2011b).

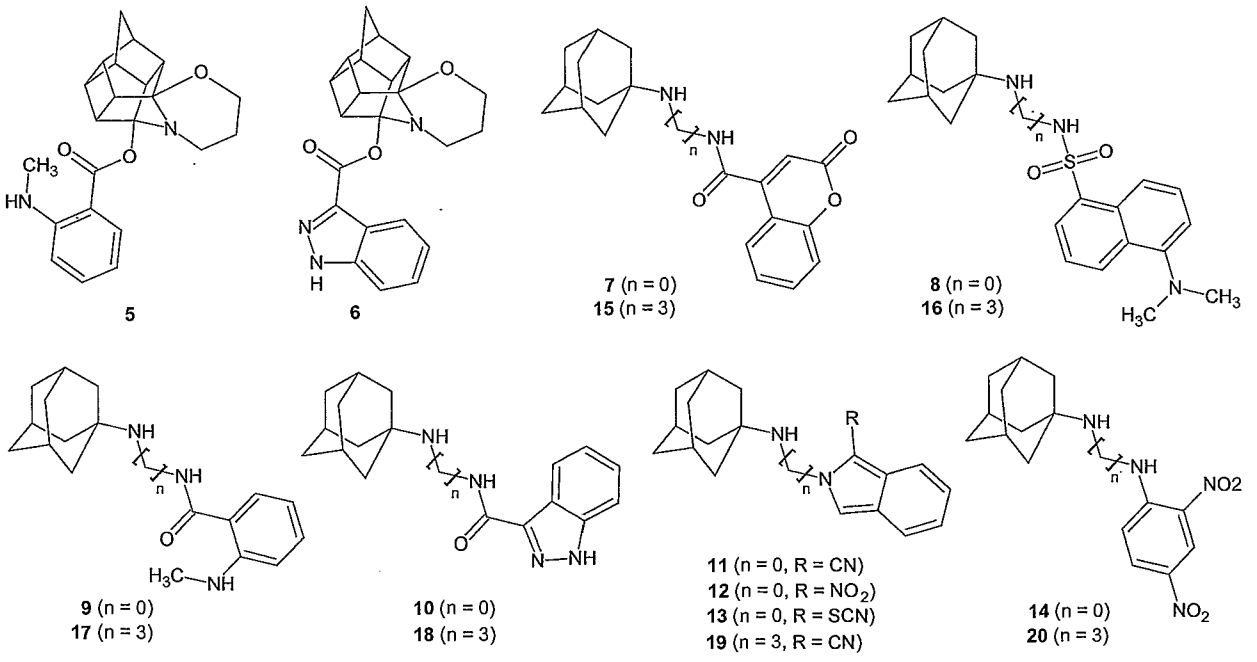

Figure 8.1: The fluorescent polycyclic series designed, synthesised and evaluated in this study

The results indicated that the heterocyclic adamantane-amine compounds (**7-11**, **14**) have a high degree of inhibitory activity on VGCC, NMDA receptors and NOS and demonstrated free radical scavenging activity in the $DPPH^{\cdot+}$ and $ABTS^{+}$ assays. This reiterates the theory that these fluorescent adamantane compounds have the ability to act as multifunctional neuroprotective agents. In addition, the potential of increased dopamine neuro-transmission is postulated due to the amantadine moiety present in all these structures. The fluorescent adamantane compounds containing polyaromatic functionalities (**7**, **8**, **10**, **11**) exhibited an increased degree of multifunctionality when compared to those with mono-aromatic structures (**9**, **14**). The coumarin (**7**) and dansyl (**8**) adamantanes in general had the best multifunctional neuroprotective activities in the series. Using these privileged structures and employing bioisosteric substitution of the amine and amide functions, an extended series of compounds

could be synthesised to further develop structure activity relationships in this series of fluorescent compounds. *In silico* studies also indicated that all the novel fluorescent adamantane compounds should have a high degree of oral bioavailability and would be transported effectively across the blood brain barrier. *In vivo* studies will further elaborate on these findings. These compounds can therefore be considered as potential multifunctional neuroprotective agents and may serve as new lead structures in the search for active therapeutics. An additional benefit of these derivatives is that they may also be utilised in the development of fluorescent displacement and other pharmacological studies. This application could provide critical information about the neurodegenerative processes present in neurodegenerative disorders and replace the use of traditional and hazardous radioligand binding studies. The potential of these fluorescent heterocyclic compounds should therefore be further explored as multifunctional neuroprotective drugs with favourable physicochemical properties and/or as fluorescent ligands in biological assay development.

Taking the above positive findings regarding the inhibition of the NMDA receptor and VGCC of the novel fluorescent polycyclic series into account, the next logical step was to develop an additional series of fluorescent polycyclic ligands to expand on the existing set. The synthesis of a series of fluorescent derivatives followed utilising a propanol linker to extend the chain length between the adamantyl and fluorescent moieties (**15-20**; Figure 8.1; Joubert *et al.*, 2011c). The chain length was increased to potentially reduce structural hindrance and allow for deeper immersion into the receptor channel and calcium channel binding pockets. These derivatives were evaluated as potential fluorescent ligands for the NMDA receptor and VGCC in murine synaptoneurosomes utilising the fluorescent ratiometric calcium indicator Fura-2/AM. The coumarin (**15**), dansyl (**16**) and cyanoisoindole (**19**) fluorescent moieties conjugated to adamantane-3-aminopropanol displayed significant VGCC blocking activity with the dansyl (**16**) and di-nitrobenzene (**20**) adamantane-3-aminopropanol fluorescent derivatives exhibiting NMDA receptor antagonistic activity. All these compounds showed improved activity when compared to known NMDA receptor and VGCC inhibitors in the series. Generally it was also observed that the increased chain length analogues (**15-20**) had improved VGCC and NMDA receptor inhibitory activities when compared to their directly conjugated counterparts (**7-11**, **14**), i.e. compound **8** had a 22% inhibition of NMDA receptor calcium flux compared to the three-carbon chain analogue (**16**) that showed 47% inhibition. This indicated that increased chain length might facilitate stronger interaction with the respective putative binding site(s) or cause less steric hinderance to binding at this site. The

dansyl analogue, *N*-[3-(1-adamantylamino)propyl]-5-dimethylamino-naphthalene-1-sulfonamide (**16**) was further used as a NMDA receptor ligand in a fluorescent competition assay, and was displaced by known NMDA receptor inhibitors such as MK-801, NGP1-01 and amantadine by up to 17 - 46%. This demonstrated the potential application of these novel analogues in fluorescent imaging techniques and illustrated their benefit over the use of hazardous and expensive radioligands. The novel adamantane-3-aminopropanol fluorescent compounds (**15-20**) may also be utilised as potential multifunctional neuroprotective agents as indicated by their NMDA receptor, VGCC and NOS inhibition, antioxidant activity and improved dopamine transmission (Joubert *et al.*, 2008).

In conclusion, this study describes the synthesis, NOS inhibition, VGCC- and NMDAR-mediated calcium influx inhibition and multifunctional neuroprotective potential of a novel series of fluorescent compounds possessing long wavelength fluorescence and high Stokes shifts. The fluorescent applications of these compounds have also been demonstrated with the design of a novel fluorescent competition assay. Further investigations need to be conducted into the development of fluorescent displacement assays utilising the novel fluorescent compounds discovered in these studies. Additional fluorescent studies utilising a fluorescent microscope or confocal laser scanning microscopy will also elaborate on the potential value of these fluorescent ligands as pharmacological tools. Ultimately, the use of these ligands might provide an attractive alternative to radioligand binding studies and offer methods to directly study the kinetics of ligand-receptor or ligand-enzyme interactions and thereby facilitate the development of novel imaging techniques for the study and treatment of neurodegenerative disorders.

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