Chapter 9
Additional Research Outputs

The following is a depiction of research outputs directly and indirectly pertaining to the previous chapters that serves to further illustrate the potential of fluorescent polycyclic compounds and other compounds in the study of neurodegeneration. Part one of this chapter presents a series of posters while part two consists of additional papers accepted for publication not included in this thesis.

The poster presentations represent additional research into the design and development of fluorescent polycyclic ligands and therapeutic agents for neurodegenerative disorders.

Part 1 - List of posters and presentation details:

- Poster 2: Joubert, J., Van Dyk, S., Malan, S. F. Novel fluorescent polycyclic ligands for mechanistic insights into neurodegeneration and neuroprotection (2009), iThemba Pharmaceuticals Launch Symposium, Hilton Hotel, Santon, Gauteng (Poster session, May 2011).

Poster 1 describes the synthesis and evaluation of fluorescent ligands that may be used in the study of the NOS enzyme (expansion of Article 1, Chapter 5). Additional synthetic routes summarised in this poster, include the design and synthesis of additional pentacycloundecane fluorescent compounds (Table 3, Poster 1) as well as a pilot molecular modelling study into the NOS enzyme isoforms (Figures 7 and 8, Poster 1) that revealed interesting results, indicating that the novel compounds, especially the longer linkage compounds, may have the ability to inhibit nNOS selectively over iNOS and eNOS. This assumption was made based on the dock scores of the test compounds compared to the respective isoforms and their binding interactions with the NOS protein structure.

Poster 2 elaborated on the research done in articles 2 and 3 (Chapters 6 and 7). The poster includes additional synthetic routes of the adamantane and pentacycloundecane fluorescent...
compounds (Figures 6, 7 and 8, Poster 2) and the design and development of the NMDA receptor and VGCC assays. Further development of the pentacycloundecane fluorescent derivatives in particular are currently underway. Both the improvement of synthetic procedures and biological evaluations are explored to establish the potential of these novel compounds as fluorescent ligands, in order to gain mechanistic insights into neurodegenerative disorders and aid in the design of novel neuroprotective therapeutic agents.

Part two of this chapter includes two further research papers that were submitted and accepted for publication during the course of this PhD study period.

**Part 2 - List of additional articles accepted for publication** (Both these papers will be made available upon request):


Article A describes the synthesis of novel nitro- and nitrate-pentacycloundecane polycyclic compounds evaluated for VGCC blocking activity and the ability to S-nitrosylate the NMDA receptor resulting in enhanced NMDA receptor antagonistic activity. The direct involvement in this study was the design and conduction of VGCC and NMDA receptor assays and structure elucidation of the synthesised compounds. The article was accepted for publication in Medicinal Chemistry (Bentham Publishers).

Article B was accepted for publication in Bioorganic and Medicinal Chemistry (Elservier). This study investigated the antioxidant activity of a series of quinolones and structurally related flavones as potential therapeutic agents for neurodegenerative disorders. The major contribution made to this paper was *in silico* molecular modeling to determine the bioavailability, blood-brain barrier permeability, physical-chemical properties and toxicity profiles of the series of neuroprotective antioxidants.
Synthesis and Evaluation of Fluorescent Polycyclic Nitric Oxide Synthase (NOS) Enzyme Ligands

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Introduction

Background

The medicinal potential of polyyclic compounds was realized with the discovery that amantadine (1) inhibited viral activity. Subsequently to this discovery, it was found that amantadine could be identical to patients with Parkinson's disease. Polyyclic compounds are a class of organic compounds that contain multiple cycles, or ring structures. They are known for their structural rigidity and are often used as building blocks in drug design. The nitric oxide synthase (NOS) enzyme is involved in the production of nitric oxide, a molecule that plays a crucial role in various biological processes. The polyyclic compounds (1) that were synthesized in this study were expected to have antioxidant properties.

NOS is an endothelial protein consisting of a homologous catalytic oxygenase domain, a catalytic protein, and a membrane binding region. The polyyclic compounds (1) have been shown to exhibit antioxidant activity in vivo and in vitro.

Nitric oxide synthases (NOS)

NOS is a flavin-dependent enzyme that catalyzes the production of NO from L-arginine. The enzyme is involved in various physiological processes, including inflammation, vascular function, and neurotransmission. The synthesis and evaluation of NOS inhibitors are of considerable interest due to their potential therapeutic applications.

Aim and objectives

The aim of the study was to synthesize polyyclic derivatives as potential inhibitors of nitric oxide synthase (NOS). Polyyclic structures were selected for their potential antioxidant and pharmacological properties. The compounds were synthesized and evaluated for their ability to inhibit NOS activity in vitro.

Synthesis

The compounds were synthesized using conventional synthetic methods. The chemistry involved the synthesis of polyyclic compounds, followed by purification and characterization. The compounds were then evaluated for their inhibitory activity against NOS.

Biological evaluation

The oxygenated prolyl hydroxylation assay was employed to determine the activity of the novel compounds. The inhibition of the enzyme by the compounds was determined using NOS as the enzyme substrate.

Molecular Modelling

The high level of amino acid conservation and structural similarity in the conserved, catalytic residues of the aromatic ring and aromatic residues in the catalytic site of NOS was noted. The aromatization process of NOS inhibitors was studied by using the Ligplot module of Discovery Spectra 1.3 software to identify the specific aromatic residues in the catalytic site of the NOS enzymes.

Conclusion

We have identified a number of fluorescent polyyclic compounds with moderate to high affinity for the NOS enzyme, which may be useful for further studies using molecular mapping techniques such as confocal microscopy, flow cytometry, or multiphoton microscopy. These compounds have potential as useful pharmacological tools to investigate enzyme ligand interactions in the quest for effective neuroprotective or anti-inflammatory agents.

References

SYNTHESIS, EVALUATION AND APPLICATION OF NOVEL FLUORESCENT POLYCYCLIC NMDA RECEPTOR AND CALCIUM CHANNEL LIGANDS

Introduction

The N-methyl-D-aspartate receptor (NMDAR) has been suggested as a drug target through its involvement in neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. Overstimulation of the NMDAR by an excess of the endogenous neurotransmitter glutamate during pathological conditions leads to excessive influx of calcium (Ca²⁺) into neuronal cells resulting in cell death, a process known as excitotoxicity. Ca²⁺ entry through L-type Ca²⁺ channels also contributes to Ca²⁺ overload and mitochondrial disruption that lead to the recruitment or release of mediators responsible for the activation of an apoptotic cascade and ultimately, in cell death (Fig. 1). Excitotoxicity also leads to the activation of nitric oxide synthase (NOS). Neuronal/NOS (nNOS) is physiologically activated by astrocytes and neurons, whereas nitric oxide (NO), glutamate and glycine that increase intracellular Ca²⁺ concentrations and leads to the formation of NO (a free radical) and cell death (Fig. 1). It does so by synthesis of NO and L-citrulline from the terminal nitrogen atom of L-arginine via the intermediate NG-hydroxy-L-citrulline (Fig. 2). Overproduction of NO has been implicated in neurodegenerative diseases, convulsions and parkinsonism.

Biological activity

Ca²⁺ and NMDA array: The fluorescent ratiometric indicator, Fluo-3/AM (a UV excited Ca²⁺ indicator), and a fluorescence spectrophotometer were used to evaluate the presence of intracellular free calcium through L-type Ca²⁺ channels and the NMDA receptor utilizing murine sympathetic neurones. The NMDA receptor blockers evaluate whether the compounds are selective for the NMDA receptor or Ca²⁺ channels or has a dual Ca²⁺ blocking activity.

Synthesis and Characterisation

The fluorescent molecules chosen for synthesis included N-methylanthranilate, Indole-1, 1-fluoro-2,4-dinitrobenzene, 1-cyanocinnamidine, Coumarin, Dan酰 and NBD. These fluorescent structures were conjugated to the respective cage moieties, directly or by means of appropriate arino-linkers to provide the fluorescent poly cyclic ligands with desired spectroscopic properties.

Aim and Objectives

The aim of this investigation is to provide fluorescent poly cyclic ligands structurally related to known NMDA receptor and L-type Ca²⁺ channel ligands (Fig. 3) for a better understanding of the NMDA receptor and L-type Ca²⁺ channel interactions (nNOS). It is a further object to provide a method and reagents for use in determining neurological interactions, intracellularly and extracellularly. We aim to achieve greater insights into the neuroprotective mechanisms of these compounds, by means of fluorescent imaging.

Figure 1: Excessive Ca²⁺ influx leading to cell death

Figure 2: Reaction catalysed by NO from L-citrulline

Figure 3: Similarities between poly cyclic compounds

Figure 4: Synthesis of fluorescent water (9, 9) and azo (7, 7) derivatives of tetra azoctane cage moieties

Figure 5: Synthesis of fluorescent amide (9, 9) and amine (13, 11) derivatives

Figure 6: Synthesis of fluorescent amine (13, 14) and amide (16, 17, 18) azo derivatives

Figure 7: Synthesis of fluorescent pentacyclic amine derivatives by amidation

Figure 8: Synthesis of fluorescent pentacyclic amine derivatives by amidation

Figure 9: Supersensitive inhibition curves of compounds with significant NO inhibition activity

Figure 10: Supervised inhibition curves of test compounds with low NO inhibition activity

Figure 11: Experiments demonstrating the effect of NO-generated reagent (500 mM) and Ca²⁺ influx, in the absence and presence of test compound 100 μM and control 100 μM

Figure 12: Test compounds (100 μM) for NMDA/pentacyclic Ca²⁺ channel inhibition and L-type Ca²⁺ channel inhibition

Conclusion

We have synthesised a series of fluorescent poly cyclic structures which may be utilised for further in vitro and in vivo studies using modern imaging techniques (r.i.e. confocal laser scanning microscopy, flow cytometry or multiphoton microscopy). The potential of these novel fluorescent poly cyclic structures may find application as fluorescent probes to better understand neurodegenerative and neuroprotective mechanisms. Additional assays on these compounds will be performed on the radioligand binding assay, GTPγS, calcium channel, NOS enzyme assay and ischaemia brain barrier permeability, which will facilitate further elaboration on these compounds' potential value.

References