Fluorescent polycyclic ligands: Strategies towards the synthesis and evaluation of fluorescently labelled receptor and enzyme ligands

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Dedicated to my parents, André and Lina Joubert

Abstract

Neurodegenerative disorders, including Alzheimer's and Parkinson's disease, and the development of neuroprotective agents have received significant research attention in recent years. Development of novel imaging techniques to study the biological mechanisms involved in the progression of these disorders have become an area of research interest. The design of novel small molecule imaging probes in combination with modern imaging techniques may provide information on neuroprotective binding site interactions and would assist in the design of novel biological assay methods. Techniques to visualize physiological or pathophysiological changes in proteins and living cells have become increasingly important in biomedical sciences, especially fluorescent techniques. Fluorescent ligands in combination with sophisticated fluorescent imaging technologies are useful tools to analyze and clarify the roles of biomolecules in living cells, affording high spatial and temporal resolution.

This study is based on the development of polycyclic fluorescent ligands, which may be used in the study of receptor-ligand and/or enzyme-ligand interactions, utilizing these fluorescently labeled ligands in combination with fluorescent imaging techniques. Fluorescent conjugates with high affinity for the *N*-methyl-D-aspartate (NMDA) receptor, voltage gated calcium channels (VGCC) and/or the nitric oxide synthase (NOS) enzyme were designed and synthesised with the aim to directly measure binding of these novel molecules to receptors and/or enzymes.

The first goal was to develop fluorescent ligands that exhibit similar inhibitory activity on NOS compared to the well-known selective neuronal NOS inhibitor 7-nitroindazole (7-NI). Polycyclic compounds, including amantadine and pentacycloundecane derivatives, were conjugated to fluorescent moieties that resemble the structure of 7-NI. It was thought that the lipophilic nature of the polycyclic compounds would increase the activity of the fluorescent moieties by facilitating increased blood brain barrier permeability and penetration through cell membranes. This would also potentially increase the selectivity of the novel conjugated compounds as selective neuronal NOS inhibitors, similar to 7-NI. The results from the NOS inhibition studies indicated that the novel fluorescent conjugates (**5-14**) inhibited the NOS enzyme at micromolar concentrations. Although none of the novel fluorescent polycyclic compounds were found to be more potent than 7-NI (IC_{50} = 0.11 μM), the indazole-

pentacycloundecane (**5**), the coumarin-adamantane (**7**), the dansyl-adamantane (**8**), and the cyanoisoindole-adamantane (**11**) conjugates, exhibited IC_{50} values below 1 μM. These compounds could possibly be used as molecular probes in the development of high-throughput screening or competitive NOS 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 developed resulting in a series of novel fluorescent polycyclic conjugates with potent NOS inhibition indicating the potential of these compounds as neuroprotective agents. Due to the polycyclic structure's inherent inhibitory activity towards the NMDA receptor and VGCC we evaluated these derivatives as possible multifunctional neuroprotective agents acting on various neuroprotective targets. In the biological studies it was observed that four adamantane fluorescent compounds (**7**, **8**, **10**, **11**) exhibited a high degree of inhibitory activity against the NOS enzyme and NMDA receptor and blocked VGCC. The fluorescent compounds were further able to scavange detrimental neurodegenerative free radicals. *In silico* studies also predicted a high degree of oral bioavailability and that these novel compounds should be effectively transported across the blood brain barrier.

Taking the positive findings on the inhibition of the NMDA receptor and VGCC activity of the novel fluorescent polycyclic ligands into account we focused on the expansion of this series. This resulted in the synthesis of a series of fluorescent derivatives utilizing adamantane-3-aminopropanol as an intermediate to extend the chain length between the adamantyl and fluorescent moieties, to potentially reduce sterical hindrance and increase activity. These novel adamantane-3-aminopropanol fluorescent ligands were also evaluated for inhibition of the NMDA receptor and VGCC. The coumarin-, dansyl- and cyanoisoindole adamantane-3-aminopropanol fluorescent conjugates (**15**, **16**, **19**) displayed significant VGCC inhibition, with the dansyl (**16**) and di-nitrobenzene (**20**) fluorescent derivatives exhibiting NMDA receptor antagonistic activity. All these compounds showed improved activity when compared to known NMDA receptor and VGCC inhibitors in this class. Generally it was observed that the increased chain length analogues had improved VGCC inhibition and NMDA receptor activity when compared to their directly conjugated counterparts. This led to the conclusion that an increase in chain length might indicate deeper immersion into the NMDA receptor and VGCC which may be necessary for stronger interaction with their putative binding sites. The dansyl analogue, *N*-[3-(1-adamantylamino)propyl]-5-dimethylaminonaphthalene-1-sulfonamide (**16**), 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 and their benefit over the use of hazardous and expensive radioligand binding studies.

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 and may also yield novel therapeutic agents against neurodegenerative disorders.

Keywords: Drug Design, Cage Compounds, Neuroprotection, Biological Activity, Fluorescence, Fluorescent Ligands.

Uittreksel

Neurodegeneratiewe siektes, insluitend Alzheimer- en Parkinson se siektes, en die ontwikkeling van neurobeskermende middels het in die afgelope jare baie aandag geniet. Nuwe beelingstegnieke wat die meganisme van biologiese sisteme betrokke by die ontwikkeling van neurodegeneratiewe siektes bepaal, is 'n area waarop baie navorsing gedoen word. Die ontwikkeling van nuwe klein-molekuul beeldingsligande in kombinasie met moderne tegnieke kan inligting verskaf oor ligandinteraksies met neurodegeneratiewe promotors. Tegnieke om die uitbeelding van fisiologiese of patofisiologiese veranderinge in proteiene en lewende selle uit te beeld het baie belangrik geword in biologiese wetenskappe. Fluoressensietegnieke is so 'n voorbeeld. Fluoresserende ligande in kombinasie met nuwe beeldingstegnieke lewer uitstekende resolusie en is veral bruikbaar in die analise, en om die rol te bepaal, van biologiese molekules in selle.

Hierdie studie is gebaseer op die ontwikkeling van polisikliese fluoresserende ligande en fluoressensie beeldingstegnieke wat gebruik kan word in die analise en uitbeelding van reseptor-ligandinteraksies en/of ensiem-ligandinteraksies. Fluoresserende konjugate met 'n hoë affiniteit vir *N*-metiel-D-aspartaat (NMDA) reseptors, ladingafhanklike kalsiumkanale (VGCC) en/of die stikstofoksiedsintetase (NOS) ensiem, is ontwerp en gesintetiseer met die doel om die direkte binding van die nuwe verbindings aan reseptore en ensieme te analiseer en uit te beeld.

Die eerste doelwit was om polisikliese fluoresserende ligande te ontwikkel wat die NOS-ensiem inhibeer en vergelykbaar is met die aktiwiteit van die bekende NOS-inhibeerder, 7-nitroindasool (7-NI). Die lipofiele karakter van die polisikliese strukture wat gekonjugeer is aan die fluoresesserende ligande het die potensiaal om die aktiwiteit van die fluoresserende verbinding te verbeter deur die bloedbreinskans- en selmembraan-deurlaatbaarheid te verhoog. Die resultate van die NOS-inhibisie studies het getoon dat die nuwe fluoresserende konjugate (**5-14**) die NOS-ensiem by mikromolêre konsentrasies inhibeer. Alhoewel geen van die nuwe polisikliese fluoresserende verbindings meer potent was as 7-NI (IC_{50} = 0.11 μM) nie, het die indasool-pentasikloundekaan (**5**), die kumarien-adamantaan (**7**), die dansiel-

adamantaan (**8**) en die sianoisoindool-adamantaan (**11**) IC_{50} waardes onder 1 μM getoon. Die verbindings het dus die potensiaal om gebruik te word as molukulêre ligande in die ontwikkeling van vinnige en kompeterende NOS-verplasende biologiese toetse. Verdere studies op die verskillende NOS-isoforme sal die potensiaal van die verbindings as fluoresserende ligande verder beskryf.

Die bogenoemde fluoresserende derivate is verder ontwikkel om 'n nuwe reeks fluoresserende polisikliese konjugate te lewer met potente NOS-inhiberende eienskappe, wat oor die potensiaal beskik om as neurobeskermde verbindings op te tree. As gevolg van die polisikliese verbindings se aktiwiteit op die NMDA-reseptor en VGCC is die derivate getoets vir moontlike multipotente neuroberskermde eienskappe wat op verskeie neurobeskermende teikens inwerk. Vanuit die biologiese studies is bevestig dat vier amantadaan-konjugate (**7**, **8**, **10**, **11**) goeie inhiberende aktiwiteit getoon teen die NOS-ensiem en die NMDA-reseptor en verder ook die VGCC effektief blokkeer. Die fluoresserende verbindings het ook oor die vermoë beskik om skadelike neurodegeneratiewe vryradikale te neutraliseer. *In silico* studies het getoon dat die verbindings oor die vermoë beskik om oraal biobeskikbaar behoort te wees en effektief afgelewer sal word oor die bloedbreinskans.

Na aanleiding van die positiewe bevindings rakende die inhibisie van die NMDA-reseptor en VGCC aktiwiteit van die nuwe fluoresserende polisikliese ligande, is besluit om te fokus op die uitbreiding van die reeks. Dit het gelei tot die sintese van 'n nuwe reeks verbindings, waaronder fluoresserende derivate waarby adamantaan-3-aminopropanol gebruik is as intermediêr en die kettinglengte tussen die adamantaan en die fluoresserende verbinding is verleng om sodoende steriese hindernis te verminder en die aktiwiteit te verhoog. Die nuwe adamantaan-3-aminopropanol fluoresserende ligande is geëvalueer vir inhibisie van die NMDA-reseptor en die VGCC. Die kumarien-, dansiel- en sianoisoindool-adamantaan-3-aminopropanol fluoresserende konjugate (**15**, **16**, **19**) het VGCC inhibisie getoon, terwyl die dansiel- (**16**) en dinitrobenseen- (**20**) derivate NMDA-antagonistiese aktiwiteit getoon het. Al die verbindings toon hoër aktiwiteit as die bekende NMDA-reseptor en VGCC-inhibeerders in hierdie klas. Oor die algemeen het die langkettinganaloë verhoogde VGCC-inhibisie en NMDA-reseptor aktiwiteit in vergelyking met die direk-gekonjugeerde derivate getoon het.

Dit het tot die gevolgtrekking gelei dat die verlenging in kettinglengte die verbinding dieper laat bind in die NMDA-reseptor en VGCC, wat moonltik nodig is vir sterker interaksie met die binding setel. Die dansiel-analoog, *N*-[3-(1-adamataanamino)propiel]-5-

dimetielaminonaftaleen-1-sulfoonamied (**16**), is gebruik as 'n fluoresserende NMDA-reseptorligand in 'n fluoresserende kompetisiestudie, waarin bekende NMDA-reseptor-inhibeerders gebruik is om die potensieële gebruik van die nuwe fluoresserende analoë te demonstreer en hul voordele bo die gebruik van gevaarlike en duur radioligand binding studies te illustreer.

Addisionele studies aangaande die gebruik van hierdie ligande word benodig, veral studies rakende die NOS-ensiem en NMDA-reseptor. Dit sal die potensiaal van die verbindings as fluoresserende ligande verder illustreer en dit moontlik maak om nuwe terapeutiese middels teen neurodegeneratiewe siektes te ontdek.

Sleutelwoorde: Geneesmiddelontwerp, Hokkie Verbindings, Neurobeskerming, Biologiese Aktiwiteit, Fluoressensie, Fluoresserende Ligande.

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