From a cage to a model –
From the heart to the brain:
Novel concepts in neuroprotection

Prof SF Malan

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From a cage to a model – From the heart to the brain: Novel concepts in neuroprotection

Inaugural lecture:

*Sarel F. Malan (Ph.D., M.Sc, B.Pharm, DTE)*

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Abstract

Neurodegenerative diseases, such as Parkinson's (PD) and Alzheimer's diseases, are increasingly becoming a burden to society as the world's population is growing older. Neurodegeneration and the development of neuroprotective agents have thus in recent years become an increasingly important focus of research. Currently, relatively good symptomatic therapy for PD exists, but no proven therapy that prevents cell death (neuroprotection), or restores damaged neurons to a normal state (neurorescue) are available. Intracellular calcium homeostasis, or rather the lack thereof, have in many studies been implicated in neuronal degeneration and is currently believed to be one of its main causes. An intracellular calcium overload leads to the activation of various enzyme systems, as well as accumulation of free radicals intracellularly. These and other calcium related processes ultimately lead to cell death and neurodegeneration. Previous studies evaluating the biological activity of the polycyclic cage amines indicate activity that includes amongst others, neuroprotective activity and selectivity and high affinity for the sigma-binding site. The pentacycloundecylamine derivatives show structural similarities to known NMDA antagonists and its peripheral L-type calcium channel antagonism as observed in cardiac myocytes is well described. Although little is known about the mechanisms of CNS activity of the compounds, it is postulated that these derivatives could yet be of therapeutic value in the treatment of neurodegenerative disorders like Parkinson's and Alzheimer's disease.

Introduction

General Principles in Drug Design and Development

The cornerstone of drug design and development lies in the effective combination of chemistry, biology and pharmacology – to identify clinically validated drug targets, synthesise the relevant compounds for interaction with it, and effectively evaluate the pharmacological effects thereof. Effective use of such a system increases the potential of obtaining preclinical lead compounds that could be further developed into therapeutic drugs.
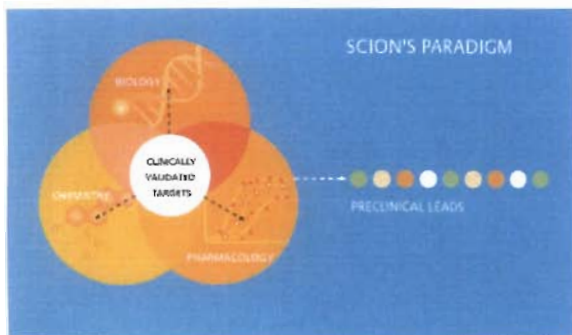

Figure 1:   Scion applies an innovative drug discovery strategy that integrates functional biology, medicinal chemistry and pharmacology. This approach allows the Company to evaluate target function and drug activity at the earliest stages of the discovery process.

Developments in molecular biology and especially the human genome project (started in 1990 and completed in 2000) has had an immense impact on the current paradigm of drug design. The genetic contribution to disease and drug response can now be studied and an understanding of genes and subsequent pathways for protein synthesis has led to the development of powerful new therapeutic approaches to disease. One of the first successes in this area was the development of imatinib mesylate (Gleevec), an inhibitor of tyrosine kinase, for the treatment of chronic myelogenous leukaemia. The contribution of minor gene variants to metabolism (especially drug metabolism), general good health and resistance to disease was also clarified in recent years.
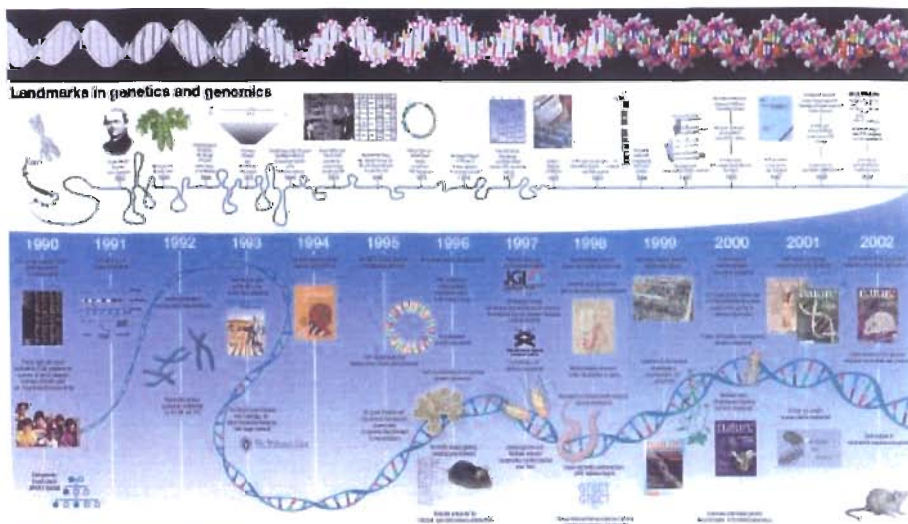
Figure 2: Landmarks in genetics and genomics: 1865, Gregor Mendel describes the laws of genetics; 1953, James Watson and Francis Crick describes the double helix structure of DNA; 1983, First human disease gene, for Huntington's disease, is mapped; 1990, Human Genome Project is initiated and the gene for breast cancer is described; 1995, First bacterial genome, that of *Haemophilus influenzae* is mapped; 1999, Sequence of the first human chromosome, chromosome 22, is completed and full scale mapping of the human genome starts; 2000, First version of the human genome sequence is completed.
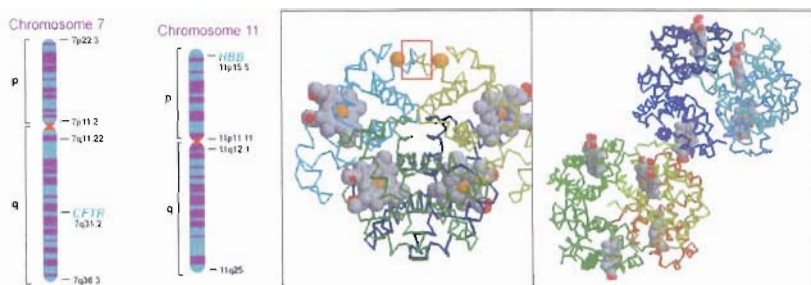
Figure 3: Identifying the gene and protein structure responsible for cystic fibrosis and sickle cell anaemia respectively.

Knowledge gained from the genome project and similar studies in combination with developments in molecular modelling and simulation will without a doubt take an even more prominent position in drug design and development strategies in the future. New drugs can

now be developed according to the lock and key principal where a specific ligand for a receptor or enzyme can be designed computationally to fit into the active site or cavity of the targeted protein.
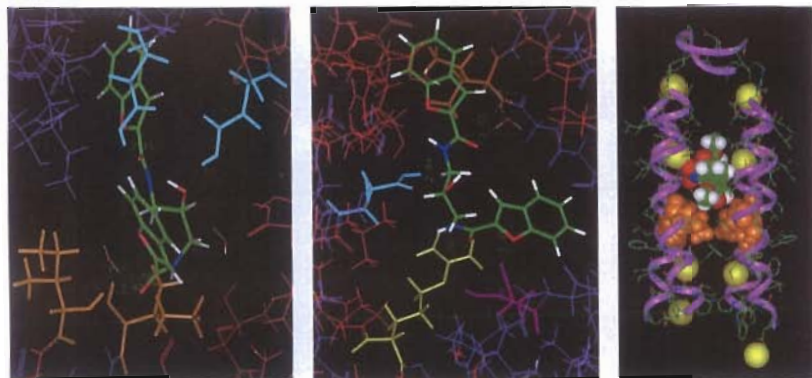
Figure 4: Docking and fitting of ligands in modelled enzyme, receptor or channel

Although we have only started to 'scratch the surface' of these new developments and technologies, they have markedly improved the medicinal chemist's chances of success. The limits of our knowledge have however also been exposed and many more complicated questions in the search for new effective and safe drugs will have to be answered. To move forward we will have to critically apply all of our existing knowledge, effectively use the state of the art technologies and always keep an open mind.
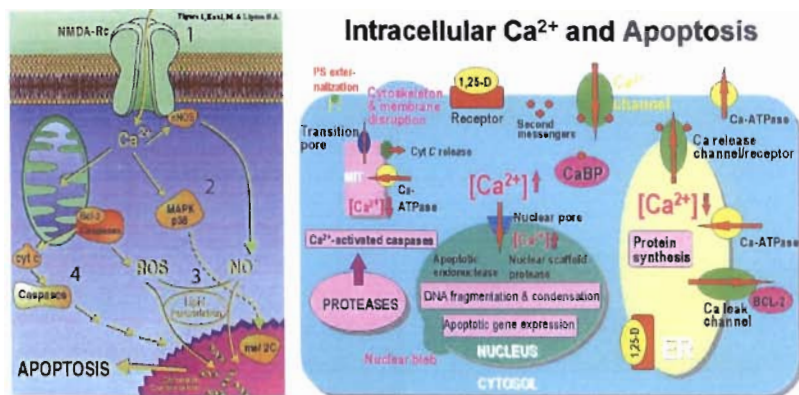
Ion Channels and Disease.

Ion channels are critical components of all living cells and regulates various essential biological processes by controlling intracellular ion concentrations. Ineffective functioning of ion channels contribute to and, in many cases, cause a number of human diseases such as arrhythmias, ischemia, hypertension, pain, epilepsy, anxiety and diabetes.

Ion channel diseases may arise due to any one or a combination of the following:

- mutations in the promoter region of the gene may cause expression level changes
- mutations in the coding region may lead to gain or loss of function
- defective regulation of channel activity by endogenous ligands
- autoantibodies to channels leading to downregulation or enhancement of function
- ion channels being secreted by cells and their subsequent insertion into cell membranes leading to large nonselective pores causing cell lysis and death
- toxins enhancing or inhibiting ion channel function.

Ion channels represent a well-established class of pharmaceutical targets in the treatment of a wide-variety of diseases with an annual market value of $100 million. The developments in genomics and molecular biology have however dramatically increased the number of ion channels and subtypes providing a major opportunity to design novel, more selective modulators with better therapeutic profiles.


Figure 5: Role of calcium in exitotoxicity and apoptosis.

Several cardiovascular and CNS disorders and disease states already have established ion channel modulator therapies. They include: hypertension, angina and cardiac ischemia, cardiac arrhythmias and inotropic agents, epilepsy, migraine, anxiety and panic disorders. Furthermore, there are several disorders and disease states that have ion channel modulators in clinical development. These include: stroke and cerebrovascular disease, traumatic brain injury, high risk cardiovascular surgery (such as cardiopulmonary bypass graft, CABG), Alzheimer's disease, dementia (vascular and AIDS-related), amyotrophic lateral sclerosis (ALS), Parkinson's disease, cystic fibrosis, spinal cord injury, multiple sclerosis (MS), and chronic (including neuropathic) pain.

Neurodegeneration and Calcium Homeostasis

Although it is not the sole mechanism mediating neuronal cell death, calcium plays an integral role in the pathology underlying neurodegeneration. Disregulation of calcium influx through voltage operated calcium channels (e.g. L-type calcium channels) as well as N-methyl-D-aspartate (NMDA) receptor operated channels is a key contributor to intracellular accumulation of excessive calcium. Although the NMDA receptor complex can be modulated by many endogenous compounds, the activation state of both aforementioned channels is regulated through changes in membrane potential.

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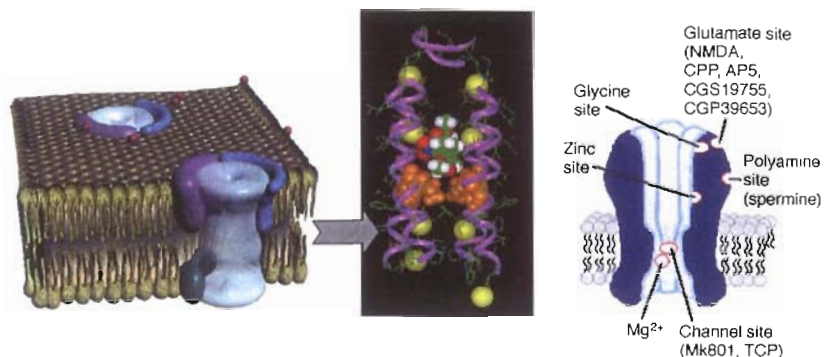
Figure 6: Schematic and molecular modelling representation of a calcium (left and centre) and NMDA (right) channel

Neuronal calcium concentrations are maintained through a multifaceted process consisting of Ca^{2+} influx and efflux, intracellular Ca^{2+} storage and an intracellular Ca^{2+} buffering system. Calcium influx is gated by voltage operated calcium channels as well as by glutamate-controlled NMDA receptor operated channels. Efflux is controlled through calcium/sodium exchanger pumps as well as energy dependent calcium-ATPase pumps. Despite existing homeostatic mechanisms, pathological elevations in intracellular Ca^{2+} do occur and lead to the inappropriate activation of normally dormant (or low level) calcium-dependent processes which in turn result in metabolic disturbances and eventual neurodegeneration as observed in traumatic brain injury and diseases like epilepsy, Parkinson's, Alzheimer's, AIDS dementia and ALS.

# Polycyclic Amines, Calcium Channel Modulation and Application thereof in Neuroprotection

Polycyclic cage amines

The biological activity of adamantane derivatives has been well described in the last decades. Interest in these compounds was stimulated by the observed ion channel and anti-viral activity of 1-amino-adamantane or amantadine (**1**, fig. 7) against a range of viruses, including influenza. More recently, through serendipitous observation, the anti-parkinsonian activity of amantadine became known. The activity was attributed to the fact that these compounds led to increased extra cellular dopamine (DA) levels via DA re-uptake inhibition or DA release. Electrophysiological studies confirmed the NMDA receptor/ion channel interaction of these compounds that lead to a block of calcium ion uptake into neurons. Memantine (**2**, fig. 7) was recently approved by FDA for Alzheimer's disease.

(1) Amantadine R_1 = H, R_2 = H   (3) Rimantadine
(2) Memantine R_1 = CH_3, R_2 = CH_3
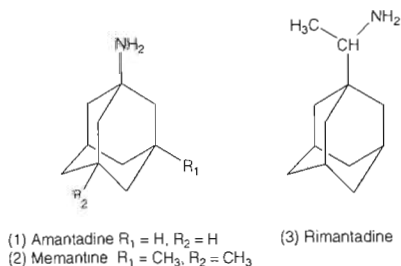
Figure 7:   Adamantane amine derivatives.

With the above structures as lead compounds, the D3-trishomocubanes was synthesised by rearrangement reactions from the original Cooksons cage diketone (a, fig. 10). These compounds (fig. 8) not only showed *in vivo* activity against Herpes simplex II and Influenza A2/Taiwan, but also had promising anti cataleptic activity with weak to mild anti cholinergic activities - comparable to amantadine. SAR studies indicated a preference for hydrophobic structures and the presence of aromatic moieties.
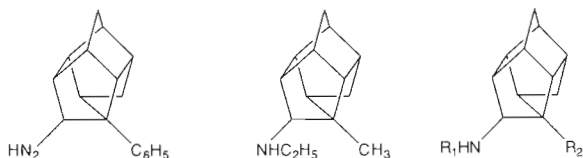
Figure 8:   D3-trishomocubanes with vivo activity against Herpes simplex II and Influenza A2/Taiwan and promising anti cataleptic activity

The channel effects of the adamantanes also lead to the synthesis of a large number of Petacycloundecylamine derivatives. Since the synthesis of the prototype of these polycyclic amines in 1971, various biological activities have been described for these structures. Especially the calcium channel blocking effects of the cage amines were extensively studied in our labs.
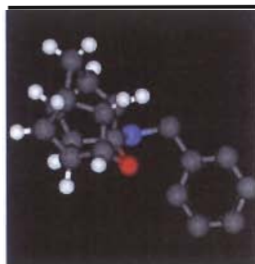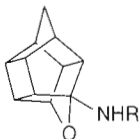
Figure 9:   Polycyclic cage structure of pentacycloundecane amines

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Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane–8,11-dione was obtained by photocyclisation of the Diels-Alder adduct resulting from the reaction between p-benzoquinone and cyclopentadiene. This polycyclic diketone was then further reacted (fig 10) to afford the required amine.

(a)

(i) Dean-Strark
(ii) NaBH$_4$/methanol-THF

(iii) HO(CH$_2$)$_2$OH
(iv) Huang-Minlon
(v) NH$_2$OH, NaBH$_4$

(vi) N$_2$CHCO$_2$Et
(vii) F$_3$B.OEt
(viii) NaCl, DMSO H$_2$O, N$_2$

(ix) 30% SeO$_2$, H$_2$O$_2$, t-Butanol

1; R = benzylamine   8
2; R = phenyhydrazyne

3a; R = 4-nitrobenzylamine
3b; R = 3-nitrobenzylamine
3c; R = 2-nitrobenzylamine

4a; R = 4-methoxybenzylamine
4b; R = 3-methoxybenzylamine
4c; R = 2-methoxybenzylamine

5a; R = 3-methylpyridine
5b; R = 4-methylpyridine

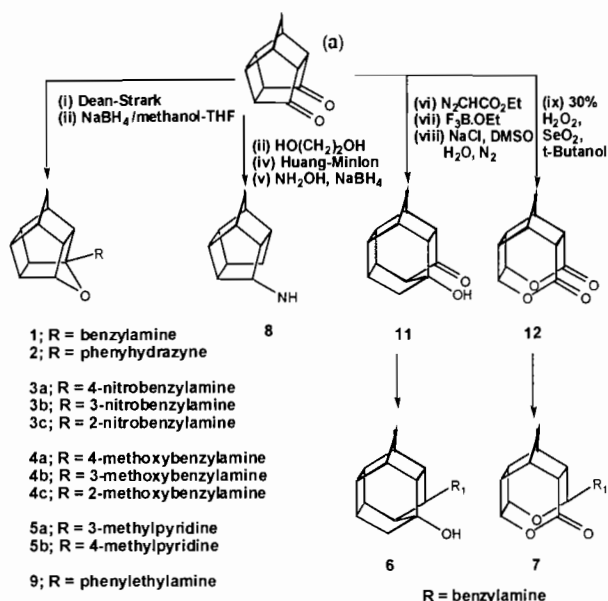9; R = phenylethylamine

11   12

6   7

R = benzylamine

Figure 10: General route of synthesis for pentacycloundecane derivatives

Calcium Channel Modulation of Pentacycloundecyl Amines

The experimental data show that these compounds voltage dependently inhibited ion currents in L-type calcium channels (fig. 11). Effects on sodium channels and the fast component of the delayed rectifier potassium channel was also observed. No effects were observed for T-type calcium channels or for the inward rectifier and slow component of the delayed rectifier potassium channels. The slope factors obtained from curve fitting suggested a stoichiometric relationship of compound to receptor and exclude the possibility of a non-selective interaction with the membrane, an event that is likely to change the function of all channels. The activity profiles on ion channels elicited by these polycyclic amines can be effectively manipulated through structural modification and structure-related modulation of the action of these derivatives on the L-type calcium channel was observed.
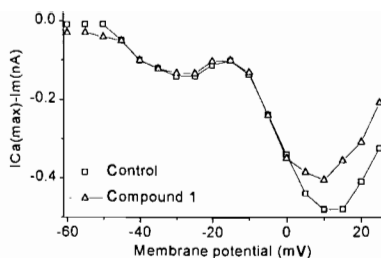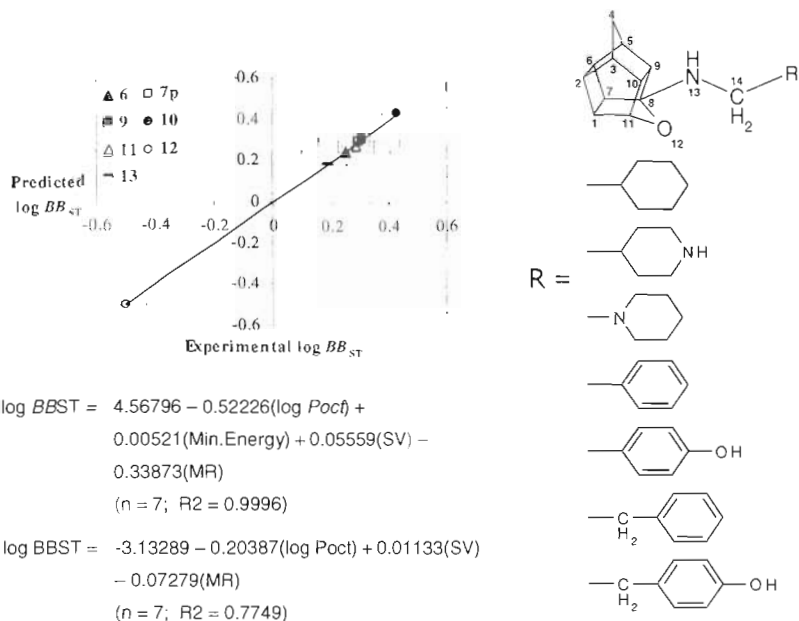
Figure 11: Voltage dependent block of L-type calcium channels as observed in cardiac myocytes

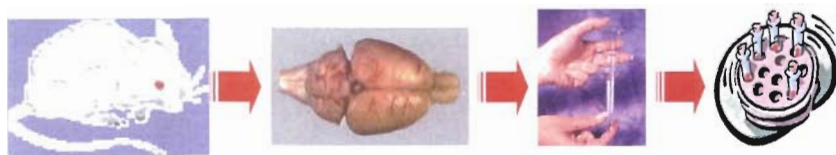## Neuroprotection of Pentacycloundecyl Amines

As intracellular calcium overload is indicated as one of the main contributing factors in neurodegenerative diseases, the ion channel activities of these compounds observed in cardiac myocytes indicated promising application of the possible channel effects in the CNS. This and the structural similarities with the adamantane amines prompted further investigation of the neuroprotective effects of the pentacyloundecane derivatives.

The first step in this study was to determine whether the compounds crossed the blood-brain barrier to reach sufficient concentrations in the brain for biological effect. A series of pentacycloundecyl amines (fig. 12) were characterised by both experimental and calculative methods, followed by biological assessment and statistical manipulation of the results obtained. In doing so, a simple biological model was established for the comparative evaluation of brain-blood permeability within the class. A highly sensitive ESI-MS.MS analytical procedure was developed for the detection of these compounds in biological tissues, indicating significant drug concentrations in the brain after intraperitoneal administration to C57Bl/6 mice. Stepwise multiple linear regression analysis of all data yielded two meaningful models (R^2 = 0.9996 & R^2 = 0.7749) depicting lipophylicity (log P_{oct}), solvent accessible molecular volume (SV), molar refractivity (MR) and system energy as the prime determinants of the brain-blood profile for these amines.

$$\log BB_{ST} = 4.56796 - 0.52226(\log P_{oct}) + 0.00521(Min.Energy) + 0.05559(SV) - 0.33873(MR)$$
$$(n = 7;\ R^2 = 0.9996)$$

$$\log BB_{ST} = -3.13289 - 0.20387(\log P_{oct}) + 0.01133(SV) - 0.07279(MR)$$
$$(n = 7;\ R^2 = 0.7749)$$

Figure 12: Correlation between blood-brain barrier permeability and physicochemical properties of pentacycloundecane derivatives.

Initial screening for neuroprotection was done using the MPTP mouse model and determining nigro-striatal dopamine levels (dopamine levels are depleted in parkinsonian models). As for memantine, the protection observed for most compounds in the study was not significant in this model. Binding and functional studies on the NMDA receptor/channel and DA transporter (release and uptake) however showed significant effects and the structure-activity relationships observed indicated specific binding.


Figure 13: Isolation of synaptoneurosomes from the mouse brain for functional and binding studies.
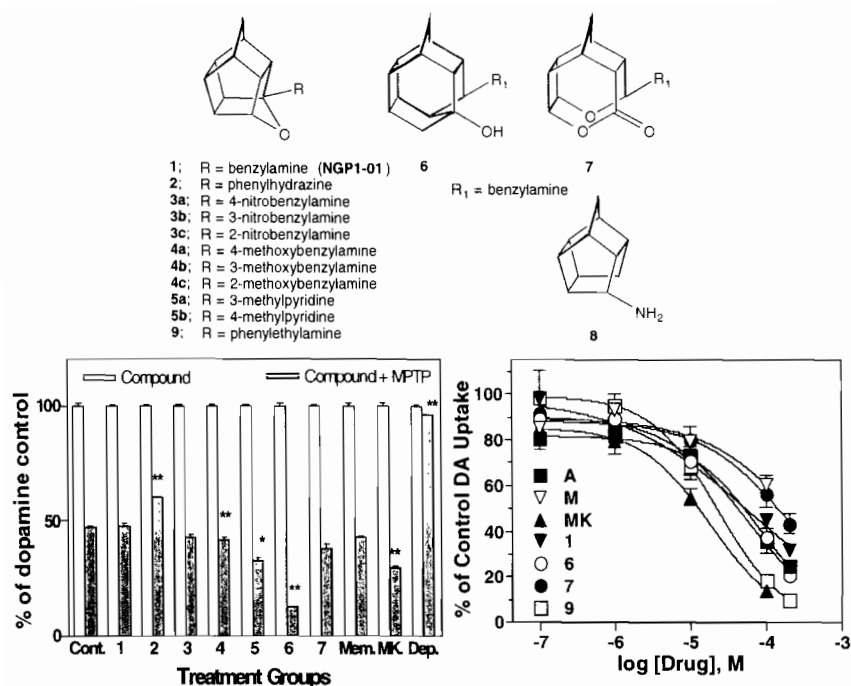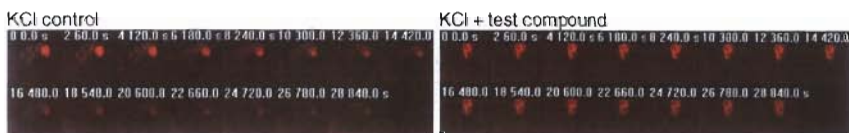
Figure 14: Pentacycloundecane derivatives used in neuroprotective study, inhibition of dopamine depletion in the MPTP mouse model (left) and inhibition of the dopamine transporter by selected compounds (right).

The cage compounds proved to be effective inhibitors of dopamine uptake, with IC_{50} values comparable to that of amantadine. The most active compound (**9**, fig. 14) had an IC_{50} value of 23 µM. All of the polycyclic cage amines showed greater activity for blocking DA uptake than for causing release of DA. NGP1-01 (**1**, fig. 14) proved to be the most potent compound in the NMDA mediated $^{45}Ca^{2+}$ flux assay with an IC_{50} of 2.98 µM, while 8-amino-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**8**, fig. 14) had an IC_{50} of 4.06 µM. Increasing the polycyclic cage size of NGP1-01 from a pentacycloundecane to a tridecane cage structure but retaining the N-benzyl moiety decreased potency 10 fold, indicating a limitation on the volume of the cage that can be accommodated in the presumed channel binding site. The results are consistent with noncompetitive antagonism for this group of compounds. Radioligand binding studies with [³H]MK-801 or [³H]TCP showed little or no displacement by the pentacycloundecyl amines, suggesting that these compounds bind to a unique site in the NMDA channel.
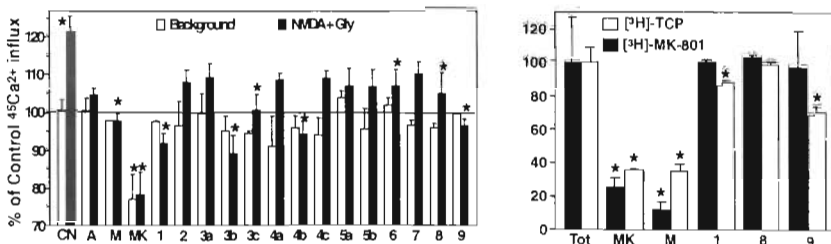
Figure 15: Inhibition of NMDA receptor mediated calcium flux (left) and displacement of ^{3}H TCP and MK-801.

Calcium is mainly gated through voltage dependent calcium channels and N-Methyl-D-Aspartate (NMDA) receptor operated channels in the CNS. The activation state of both these channels is however regulated through changes in membrane potential. Recent studies in our laboratory has shown that the pentacycloundecane derivatives caused an overall but structure related reduction in KCl-induced membrane depolarisation.


Figure 16: Inhibition of KCl-induced membrane depolarisation by a pentacycloundecane derivative.

In summary it is thus clear that the polycyclic cage compounds might be useful as neuroprotective therapeutic agents by:

- Preventing calcium overload through
  - inhibition of membrane depolarisation;
  - dual NMDA and L-type calcium channel block;
- Attenuating DA imbalances through DA reuptake inhibition and
- Inhibiting active transport of toxins into neuronal cell by DAT

Molecular Modelling

Developments in molecular modelling has impacted significantly on the paradigm of drug design and development. In our polycyclic amine research it is also contributing hugely to our efforts to understand and predict the biological activity of the synthesised chemical structures. New structures can now be designed using pharmacophore design, Conformational Molecular Field Analysis (CoMFA) and Conformational Molecular Similarity Index Analysis (CoMSIA) while the biological and physicochemical properties of planned structures can be predicted using the above and QSAR techniques.
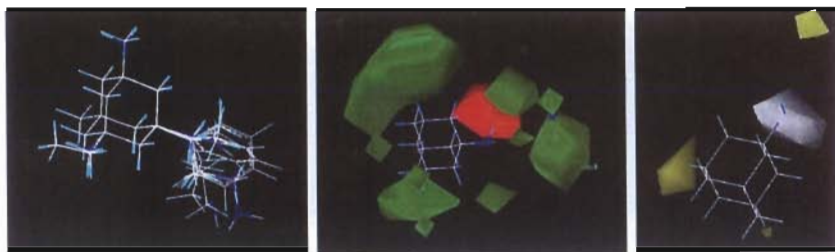
Figure 17: Application of CoMFA and CoMSIA in prediction of binding to the PCP site of the NMDA receptor channel from the molecular overlay of the memantine training set. CoMFA contour map where sterically favoured/disfavoured areas are shown in green/yellow and electrostatic favoured/disfavoured areas are shown in blue/red. CoMSIA contour map with yellow contours where hydrophobic substituents are favoured and white contours where hydrophilic substituents are favoured

An example of how QSAR is applied can be seen in the prediction of blood-brain barrier permeability for the polycyclic compounds (fig. 12). CoMFA and CoMSIA was used to predict the binding of the pentacycloundecane derivatives to the PCP binding site using memantine derivatives as the training set (fig. 17).

Future perspectives

Neuroprotection

Further biological evaluation targeting various other points in the lethal cascade of neurodegeneration could prove to be invaluable in fully describing the biological profile of the polycyclic cage compounds. The option of targeting more than one step in the cascade would also be beneficial in the design of dual or multiple acting drugs. Combination of entities known to be active on specific enzyme systems with the polycyclic cage amine (ion channel activity) could render molecules with neuroprotection through more than one mechanism. Application of techniques like microdialysis and fluorescence could also greatly enhance the biological screening process and shed light on the mechanism of neuroprotection.

HIV protease

With the reported antiviral activity of the trishomocubanes, the evaluation of other polycyclic derivatives for this activity could yet yield active structures for the treatment of viral diseases. Initial modelling studies in this area showed great promise for inhibition of HIV protease inhibition (fig. 18).
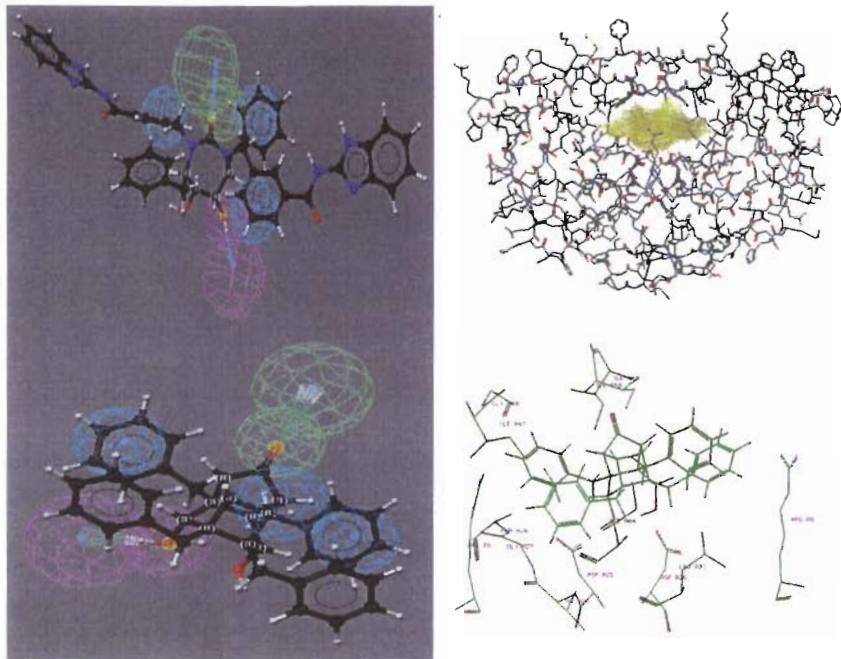
Figure 18: Generation of a pharmacophore hypothesis (top left) and fitting of a proposed polycyclic inhibitor (bottom left) using Catalyst®. Docking of a proposed ligand in the active site (top right) using Cerius2® and InsightII® (bottom right).

Conclusion

From the above it is clear that developments in molecular modelling and genomics have greatly enhanced the drug design process. Application of these and other techniques thus holds tremendous promise for the future of medicinal chemistry. These developments, the availability of new techniques and a better understanding of the mechanism of disease has revitalised the research in polycyclic amines.

The well described channel activity of these compounds on heart cells gave a good indication of the possible neuroprotective activity of the polycyclic cage compounds. Following onto this research, novel compounds with application in neuroprotective disorders were thus developed. Molecular modelling has further aided in our understanding of the mode and mechanism of action of these compounds and new structures with predicted activity can now be designed.

What was learned from studies on the heart was thus successfully applied for diseases of the brain. Evolving from a two dimensional drawing of a cage to the three dimensional modelling of active neuroprotective compounds in their protein binding site has further enhanced our drug design process. The modelling, design, synthesis and biological evaluation of diverse polycyclic cage derivatives therefore holds tremendous potential in the search for neuroprotective and other therapies.