UNIVERSITY OF KWAZULU-NATAL

DESIGN, SYNTHESIS AND PHARMACEUTICAL APPLICATION OF NOVEL POLYCYCLIC ‘CAGE’ DIAMINES

2010

OLUSEYE KEHINDE ONAJOLE
DESIGN, SYNTHESIS AND PHARMACEUTICAL APPLICATION OF NOVEL POLYCYCLIC ‘CAGE’ DIAMINES

OLUSEYE KEHINDE ONAJOLE

2010

A thesis submitted to the School of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal, Westville, for the degree of Doctor of Philosophy.

This thesis has been prepared according to Format 4 as outlined in the guidelines from the Faculty of Science and Agriculture which states:

This is a thesis in which the chapters are written as a set of discrete research papers, with an Overall Introduction and Final Discussion. Where one (or all) of the chapters has already been published. Typically these chapters will have been published in internationally-recognised, peer-reviewed journals.

As the candidate’s supervisor, I have approved this thesis for submission.

Supervisor:

Signed: .................................... Name: .................................. Date:..............................

Co-Supervisor:

Signed: .............................. Name: .............................. Date:.........................
Despite over 50 centuries of living with the disease, tuberculosis (TB) still remains one of the oldest and deadliest diseases known to man and is gradually becoming a serious threat to the human race. According to the 2009 Global tuberculosis control report of the World Health Organisation (WHO), it is estimated that about 9.4 million incident cases of TB occurred globally. Of these cases an estimated 1.4 million were HIV positive of which 78% were in the African region while 13% are located in the South-East Asia Region. An estimate of 1.3 million deaths was reportedly caused by TB among HIV negative people. South Africa has the highest percentage of HIV patients living with tuberculosis. The design, synthesis and evaluation of novel polycyclic ‘cage’ amines for their pharmaceutical profiles are presented in this thesis. In this project a total of 12 novel intermediates and 31 novel products were synthesised. A thorough NMR elucidation of the various structures was also pursued.

This study was motivated by the reported discovery of SQ109 by Sequella. SQ109 (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine) shares the same 1,2 ethylenediamine pharmacophore with ethambutol (EMB), a commercial TB-drug. SQ109 also possess remarkable activity against MDR-TB which includes the EMB resistant strain suggesting that SQ109 is a new anti-TB drug and not an EMB analogue. SQ109 comprises of a polycyclic adamantane moiety, an isoprenyl moiety and a diamine.

This study had three main aims, namely (a) the design and synthesis of novel polycyclic ‘cage’ amines derivatives; the polycyclic ‘cage’ moieties investigated in this study includes adamantane, trishomocubane, oxa-pentacycloundecane, aza-pentacycloundecane, pentacyclodecane and pentacycloundecane, (b) structural elucidation (using 2D NMR techniques) of synthesized novel polycyclic ‘cage’ amine derivatives (c) the anti-mycobacterial screening of novel polycyclic ‘cage’ amines derivatives against H37Rv, MDR (multi-drug resistant) and XDR (extensively-drug resistant) strains of Mycobacteria tuberculosis and (d) the anti-bacterial and anti-fungal screening of selected novel polycyclic ‘cage’ amine derivatives.

Furthermore, the design, synthesis and NMR elucidation of a family of similar novel PCU diamine ligands are also reported herein. The aim of these ligands is to complex and transport copper ions to the sites of inflammation caused by arthritis. The known pharmaceutical properties of polycyclic ‘cage’ compounds such as their ability to cross membranes due to improved drug lipophilicilty makes them suitable candidates for such a study. This project stems from a logic collaboration between UKZN, UCT (University of Cape Town) and CPUT (Cape
Peninsula University of Technology) to test some of these cage diamines for activity against arthritis. Experimental work in this aspect is performed by the group of Prof. Graham E. Jackson (UCT) and Dr. Sebusi Odisitse (UPUT).
DECLARATIONS
DECLARATION 1 - PLAGIARISM

I, …………………………………………………………………………………., declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons’ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed

……………………………………………………………………………….
DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication)

**Publication 1**


Contributions: A continuation from my MSc degree. I synthesized three novel final compounds and wrote the paper. All anti-mycobacterial analysis was carried out by Prof. I. Wiid, Prof. van Helden PD and Mrs K. Govender while Ms. K. Muthausamy carried out cytotoxicity analysis. All other authors are supervisors.

**Publication 2**


Contributions: I synthesized all the compounds, carried out NMR elucidations and wrote the paper. All other authors are supervisors.

**Publication 3**


Contributions: I synthesized all compounds and wrote the paper. All anti-mycobacterial analysis was carried out by Prof. I. Wiid and Prof. P. D. van Helden. All other authors are supervisors.

**Publication 4**


Contributions: I synthesized all compounds, carried out NMR elucidations with the assistance of Ms. M.M. makatini. I wrote the paper. All other authors are supervisors.

**Publication 5**


Contributions: I carried out NMR elucidations and wrote the paper. All other authors are supervisors.
Publication 6


Contributions: I synthesized the compounds, carried out all anti-microbial analysis under the supervision of Prof. Y. Coovadia, Mrs. D. Naidu and Dr. N. Singh and wrote the paper. All other authors are supervisors.

Publication 7


Contributions: I synthesized all compounds, carried out anti-microbial analysis under the supervision of Prof. Y. Coovadia, Mrs. D. Naidu and Dr. N. Singh and wrote the paper. Dr. B. Somai and Ms X. V. Belewa ran the anti-fungal analysis of all synthesized compounds against a plant fungus. All other authors are supervisors.

Publication 8


Contributions: I synthesized all compounds with some assistance from Mr. S. Sosibo. I carried out the NMR elucidation of the compounds and wrote the paper. All anti-TB analysis was carried out by Prof. I. Wiid and Prof P. D. van Helden. All other authors are supervisors.

Publication 9

Onajole, O. K.; Coovadia, Y.; Govender, P.; Kruger, H. G.; Maguire, G. E. M; Pillay, M.; Govender, T.; *Novel polycyclic ‘cage’-1,2-diamines as potential anti-tuberculosis agents;* manuscript submission pending, due to possible patent.

Contributions: I synthesized all compounds, carried out NMR elucidations and wrote the paper. All anti-TB analysis was carried out by Prof. Y. Coovadia and M. Pillay. All other authors are supervisors.

Signed:

__________________________
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I would like to express my heartfelt appreciation and gratitude to the four muskatereers, the “GGKM” (Dr. Thavi Govender, Dr. Patrick Govender, Prof Hendrik G. Kruger and Dr. Glenn E.M. Maguire) researchers. I took a life changing decision in 2006 to join the group and I have never regretted making that choice, you guys are the best. You are four great researchers and made me what I am today; you helped to build the passion I have for chemistry. You provided a home far away from home for me and made me part of your lifes and created a place for me in your group. I say thank you for everything most especially for the support, care, understanding and financial assistance you rendered to me during the burial of my mother.

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Much thanks to the University of KwaZulu-Natal, ASPEN pharmaceutical company and NRF (National Research Foundation) for their generous financial support.

I say thank you to my Lord and saviour Jesus Christ for keeping me safe in South Africa from the beginning of this project in 2007 even till now; without you I am nothing, you are my source, my everything, the pillar that holds my life, my all in all. Thank you, the Lord of my life.
DEDICATION

This work is dedicated to the memory of my loving mother late Mrs. Olabisi Mercy Olufunmilayo Onajole. “Iya ibeji” you are the best mother and woman I have ever known, a VIRTOUS woman.

I count it a great privilege and honour to be called your son, the fruit of your womb.

Mummy, words can express how much I miss you.
LIST OF PUBLICATIONS

1. Onajole, OK.; Sosibo, S.; Govender, P.; Govender, T.; van Helden, PD.; Maguire, GEM.; Majerski, M.; Wiid, I.; Kruger, HG.; Novel linear diamine disubstituted polycyclic cage derivatives as potential anti-mycobacterial candidates; manuscript submitted to Chemical Biology and Drug Design 2010.

2. Onajole, OK.; Belewa, XV.; Coovadia, Y.; Govender, T.; Kruger, HG.; Maguire, GEM.; Naidu, D.; Somai, B.; Singh, N.; Govender. P. SQ109 analogues as potential antimicrobial candidates; Medicinal Chemistry Research accepted for publication 2010.

3. Onajole, OK.; Coovadia, Y.; Govender, T.; Kruger, HG.; Maguire, GEM.; Naidu, D.; Singh, N.; Govender P. In-vitro antifungal and antibacterial activities of pentacycloundecane tetra- amines; Chemical Biology and Drug Design accepted for publication 2010.

4. Onajole, OK.; Govender, P.; Govender, T.; Maguire, GEM.; Kruger, HG. NMR elucidation of novel SQ109 derivatives; Structural Chemistry accepted 2010 doi: 10.1007/s11224-010-9661-3

5. Chakka, SK.; Onajole, OK.; Govender, T.; Maguire, GEM.; Su, H.; Kruger, HG. 1,7-Dimethylpentacyclo[5.4.0.02,6.03,10.05,9]undecane-8,11-dione; Acta Cryst 2010. E66, o1901

6. Altaib, MS.; Arvidsson, PI.; Govender, T.; Maguire, GEM.; Makatini, M.; Onajole, OK.; Kruger, HG.; Synthesis and NMR elucidation of novel pentacycloundecane based peptides; Magnetic Resonance in Chemistry, 2010, 48, 6, 435.


8. Onajole, OK.; Govender, P.; van Helden, PD.; Kruger, HG.; Maguire, GEM.; Wiid, I.; Govender, T. Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates; European Journal of Medicinal Chemistry, 2010, 45, 2075.

9. Onajole, OK.; Govender, P.; Govender, T.; Maguire, GEM.; and Kruger, HG. Synthesis and NMR elucidation of novel pentacyclo-undecane diamine ligands; Structural Chemistry 2009, 20, 6, 1067.


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CHAPTER 1
INTRODUCTION

1.1 Background

Tuberculosis (TB) is a highly contagious and infectious disease mainly of the lungs but can easily spread to other organs in the body. Pulmonary tuberculosis is primarily caused by a bacterium called Mycobacteria tuberculosis (M. tuberculosis). Pulmonary tuberculosis has been in existence for hundreds of centuries with scientific evidence of tuberculosis residues in Egyptian mummies.\(^1\)\(^-\)\(^5\) Despite the fact that we have been living for more than 50 centuries with the disease, tuberculosis remains one of the deadliest diseases known to man and is gradually becoming a threat to the human race. The World Health Organisation (WHO) 2010 Global Tuberculosis report, estimated that about 9.4 million cases of TB occurred in 2009 of which 35 % occurred in South-East Asia, 30 % in Africa and 20 % in Western Pacific region. HIV-positive incident cases was estimated at 11-13 %; Africa has the highest percentage (80 %) of HIV-positive people living with tuberculosis.\(^6\)

Tuberculosis can be easily managed, prevented and controlled using currently available drugs. The World Health Organisation introduced a drug regimen called DOTS (Directly Observed Treatment Short course) in the mid-90s. This regimen employs a six month treatment with known anti-mycobacterial drugs which includes ethambutol, isoniazid, pyrazinamide, and rifampicin also known as rifampin (Figure 1). These four drugs are prescribed on a daily basis for a two month period. Isonaizid and rifampicin are continued daily for an additional four months; these are referred to as first line drugs.

However, inconsistent treatment, partial intake, in-availability of drugs and negligence during these six month therapy period can easily result in a situation where M. tuberculosis becomes resistant to these first line drugs. This appears to be the major cause for the emergence of multi drug-resistant (MDR) strain of tuberculosis. MDR-TB is classified as being resistant to at least isoniazid and rifampicin, the two most powerful existing anti-TB drugs.\(^7\)

MDR-TB can be cured using second line drugs which include streptomycin, p-aminosalicyclic acid, ethionamide cycloserine and fluoroquinolone based drugs such as ciprofloxacin, sparfloxacin, sitafloxacin, moxifloxacin etc (Figure 2).
The second line drugs are very toxic, expensive and possess numerous side effects. Inconsistency in the administration of these drugs renders them less effective against MDR-TB resulting into extensively drug-resistant TB (XDR-TB). At this stage there are little or no therapies that can be offered to XDR-TB patients. The emergence of HIV/AIDS has caused a major setback in the treatment of tuberculosis globally. Tuberculosis infection is very rampant in HIV positive patients due to a drop in their immune system which encourages dormant or growing mycobacteria to grow well and flourish in their host. HIV and TB form a lethal combination and frequently leads to premature death if not attended to on time.
In the context of the current global TB public health disaster, the urgent development of new anti-TB drugs cannot be overemphasized. Researchers have explored the possibility of improving the therapeutic effect of already existing anti-TB drugs such as ethambutol, isonazid etc. in order to further enhance/improve the half life and potency of such drugs in the host and
also to lessen any existing side effects. Over the years, several promising drug candidates emerged, each one possessing different site and mode of action on the mycobacterium.

### 1.2 Site and mode of action of current TB drugs

Much research has been undertaken in order to gain more insight to the site and mode of action of current and potential anti-TB drugs. This knowledge has helped a great deal in the design of potent drugs which target specific sites or biosynthesis pathway in the organism or bacillus. The cell wall of mycobacterium spp. is essential for its survival and growth in the host. The cell wall comprises mainly of arabinogalactan, peptidoglycan, lipoarabinomannan and mycolic acid.

The disruption of the biosynthesis pathway of these molecules most especially peptidoglycan and mycolic acid, should easily damage the assembly of the macromolecules, which leads to the death of the cell.

Ethambutol was initially believed to inhibit the biosynthesis of arabinogalactan and lipoarabinomannan of *M. tuberculosis* and other mycobacteria. However, it was subsequently established that ethambutol inhibits the polymerization step of arabinan biosynthesis of arabinogalactan. Isoniazid and ethionamide are prodrugs* and are activated within the mycobacterial cell. Isoniazid primarily inhibits the biosynthesis of mycolic acid in the organism. Ethionamide is believed to possess a similar mechanism of action as isoniazid. Isoniazid and ethionamide are structurally related thus explaining the similarity observed in their mode of action. Streptomycin is a broad-spectrum antibiotic possessing antibacterial activity against most Gram-positive, Gram-negative and mycobacterial species. Streptomycin inhibits the protein synthesis of the bacilli by interacting with the 30S ribosomal subunit.

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* A prodrug is a pharmacological substance (drug) which is administered in an inactive (less active) form. Once administered, the prodrug is metabolised *in vivo* into an active metabolite.
Figure 3: First-Line Treatment of Tuberculosis (TB) for Drug-Sensitive TB

Known anti-TB drugs could either be bacteriostatic or bacteriocidal. Bacteriostatic anti-TB drugs such as ethambutol, ethionamide etc. stop the growth or flourishing of the bacilli in its host without necessarily killing it and re-surgence can occur if the treatment is discontinued. Bacteriocidal anti-TB drugs such as isoniazid and rifampicin kill the bacilli and in most cases prove to be more effective than static drugs against *M. tuberculosis*. A list of known anti-TB drugs with their mechanisms/site of action, effect on the bacterial cell wall and targets are presented in Table 1.
<table>
<thead>
<tr>
<th>Drug (year of Discovery)</th>
<th>MIC (g/ml)</th>
<th>Effect on bacterial cell</th>
<th>Mechanisms of action</th>
<th>Targets</th>
<th>Genes involved in resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (1952)</td>
<td>0.01-0.2</td>
<td>Bactericidal</td>
<td>Inhibition of cell wall mycolic acid synthesis and other multiple effects on DNA, lipids, carbohydrates, and NAD metabolism</td>
<td>Multiple targets including acyl carrier protein reductase (InhA)</td>
<td>katG, inhA, ndh</td>
</tr>
<tr>
<td>Rifampicin (1966)</td>
<td>0.05-0.5</td>
<td>Bactericidal</td>
<td>Inhibition of RNA synthesis</td>
<td>RNA polymerase β subunit</td>
<td>rpoB</td>
</tr>
<tr>
<td>Pyrazinamide (1952)</td>
<td>20-100 pH 5.5 or 6.0</td>
<td>Bacteriostatic/bactericidal</td>
<td>Disruption of membrane transport and energy depletion</td>
<td>Membrane energy metabolism</td>
<td>pncA</td>
</tr>
<tr>
<td>Ethambutol (1961)</td>
<td>1-5</td>
<td>Bacteriostatic</td>
<td>Inhibition of cell wall arabinogalactan synthesis</td>
<td>Arabinosyl transferase</td>
<td>embCAB</td>
</tr>
<tr>
<td>Streptomycin (1944)</td>
<td>2-8</td>
<td>Bactericidal</td>
<td>Inhibition of protein synthesis</td>
<td>Ribosomal S12 protein and 16S rRNA</td>
<td>rpsL, rrs</td>
</tr>
<tr>
<td>Kanamycin (1957)</td>
<td>1-8</td>
<td>Bactericidal</td>
<td>Inhibition of protein synthesis</td>
<td>16S rRNA</td>
<td>Rrs</td>
</tr>
<tr>
<td>Quinolones (1963)</td>
<td>0.2-4</td>
<td>Bactericidal</td>
<td>Inhibition of DNA synthesis</td>
<td>DNA gyrase</td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td>Ethionamide (1956)</td>
<td>0.6-2.5</td>
<td>Bacteriostatic</td>
<td>Inhibition of mycolic acid synthesis</td>
<td>Acyl carrier protein reductase (InhA)</td>
<td>inhA, etaA/ethA</td>
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<tr>
<td>p-Aminosalicylic acid PAS (1946)</td>
<td>1-8</td>
<td>Bacteriostatic</td>
<td>Inhibition of folic acid and iron metabolism?</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cycloserine (1952)</td>
<td>5-20</td>
<td>Bacteriostatic</td>
<td>Inhibition of peptidoglycan synthesis</td>
<td>D-alanine racemase</td>
<td>alrA, Ddl</td>
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1.3 Development of new generation anti-TB drug candidates

The Stop TB Strategy and Global plan of the World Health Organisation to eliminate TB form part of an on-going campaign to reduce the burden of TB and deaths resulting from TB by the year 2015. The Global Plan to Stop TB ‘2006-2015’ Action for life, was launched in January 2006 at the World Economic Forum in Davos, Switzerland. This campaign has led to the fast tracking in the clinical trials of identified anti-TB drug candidates for the past four years. It should be noted that no new anti-TB drug has been approved for use in the past four decades and this shortcoming has resulted in numerous deaths due to the emergence of drug resistant TB.

In order for this set goal to be achieved by the year 2015, there is an urgent need for highly potent chemotherapeutics that are fast acting, have fewer side effects and are also cost effective. Researchers over the last decade have indentified numerous potent anti-tuberculosis candidates some of which are presently undergoing advanced stages of clinical trials; these compounds include derivatives containing diamine (SQ109), diaryl quinoline (TMC207), nitroimidazooxazine (PA-824), pyrrole (LL-3858) and nitrodiiodhro-imidazooxazole (OPC-67683) functional groups. Table 2, shows a list of potential anti-TB candidates at discovery, pre-clinical and clinical stages of development.

TMC207 (a diaryquinoline)

TMC207, a diaryquinoline derivative, (also known as R207910, Figure 5) was first reported in 2005 by Andries et al. TMC207 is a pure enantiomer with two chiral centers, its chemical name is 1-(6-bromo-2-methoxyl-quinolin-3-yl)-4-dimethylamino-2-naphthalen-1-yl-1-phenyl-butan-2-ol (C_{12}H_{13}BrN_{2}O_{2}). The absolute configuration of the two asymmetric centers was identified as R,S i.e. the carbon bearing the phenyl substituent is of (R)-form and the carbon bearing the hydroxyl substituent is of (S)-form. Diarylquinolines has a unique structure and mode of action which differs from fluoroquinolones (specific to type II topoisomerases inhibition) and quinolines such as mefloquine. TMC207 possesses anti-TB activity against the H_{37}Rv reference susceptible strain and drug-resistant strains of tuberculosis at a MIC range of 0.030 to 0.120 µg mL\(^{-1}\) compared to 0.500 µg/mL for rifampin and 0.120 µg mL\(^{-1}\) for isoniazid. TMC207 appears to be mycobacterium specific as significant MIC values were reported for M. bovis, M. kansasii and M. ulcerans including those that are naturally resistant to many known anti-TB agents such as the M. avium complex, M. abscessus, M. fortuitum and M. marinum. An increase in MIC values were observed for Gram-positive and Gram-negative bacteria. The target for TMC207 was proposed to be the mycobacterial F1F0 proton ATP synthase. A drug combination of TMC207 with first line anti-TB drugs (rifampin, isonazid and pyrazinamide) carried out in an animal model (mice). When TMC207 is used as a monotherapy, it exhibited similar activity as the combination of rifampin, isoniazid and pyrazinamide. Due to drug resistance, the development of clinical use of TMC207 as monotherapy is not encouraged. TMC207 however displays remarkable bactericidal activities when used in combination
with isonazid and pyrazinamide or rifampin and pyrazinamide proving to be more active than the DOTS regimen. Further study shows that TMC207 is rapidly metabolised by cytochrome P<sub>450</sub>, which is increased with the use of rifampin thus resulting into an unwanted interaction between TMC207 and rifampin. A recent study also showed that TMC207 posseses excellent activity against Mycobacterium leprae, the causal organism of leprosy using a mouse model.

As mentioned earlier the organism *M. tuberculosis* can remain dormant in its host for a long period of time, all known anti-TB drugs including isoniazid and rifampin have no effect on dormant mycobacteria. However, treatment of a culture of anaerobic cells (deprived of oxygen) with TMC207 causes a dose-dependent reduction of the ATP synthesis level which results in a reduction in the colony forming units (CFUs) after introducing oxygen. TMC207 also gave impressive results in various mouse models of TB, whilst initial Phase 1 safety and Phase 2 efficacy studies continue to yield very promising results.

**LL3858 (Pyrrole derivative)**

LL-3858 is a pyrrole derivative (Figure 5) which was developed by Lupin Limited in India. It exhibited potent anti-TB activity against both drug sensitive and resistant strain (MDR) of tuberculosis at a MIC of 0.06-0.5 µg mL<sup>-1</sup>. In-vitro anti-TB screening of LL-3858, shows dose dependent mycobactericidal activity (monotherapy) and a synergistic effect when used in combination with rifampin. In murine models, LL-3858 demonstrates superior monotherapeutic activity in comparison to isoniazid. Combination therapy of LL-3858 with other known anti-TB drugs such as isoniazid, rifampin, pyrizinamide and ethambutol showed increased activity. There is little published information available about this compound and its mode or site of action in the mycobacterium is still unknown. LL-3858 is currently undergoing advanced phase 1 clinical trials.
SQ109 (an adamantane diamine derivative)

SQ109 (N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine is an analogue of ethambutol which was first reported by Lee et al. in 2003. They have used a combinatorial approach where a library of 63,238 1,2-diamine analogues of ethambutol were synthesized and screened for their anti-TB activities. SQ109 was identified from this library as the most active with a 14-35 fold activity over ethambutol as the control drug. Further studies, however showed only a fivefold increase in activity over that of ethambutol. SQ109 inhibits the biosynthesis of the cell wall but its specific target is still unknown. Recent studies showed that SQ109 exhibits activity against numerous multi-drug resistant TB strains which includes an ethambutol resistant strain; thus suggesting that SQ109 is a new anti-TB drug and not an ethambutol analogue. In vivo activity of SQ109 in animal models also showed excellent activity against infected mice.
Figure 5: Structures of TMC207, LL3858, OPC-67683, PA-824 and SQ109

Pharmacokinetics and pharmacodynamics of SQ109 in animal models displayed good bioavailability with the highest concentration found in the lungs (>MIC).\textsuperscript{43,44} Further \textit{in vitro} drug combination analysis using the BACTEC 460 system shows that SQ109 has a synergistic effect when used in combination with isoniazid and rifampicin, two of the most important first-line anti-TB drugs and an additive effect was observed when used in combination with streptomycin.\textsuperscript{45} No synergy or additive effects were observed for the combination of SQ109 with ethambutol or pyrazinamide.\textsuperscript{45} SQ109 recently completed phase 1 clinical trials.\textsuperscript{46}
Table 2: Compounds targeting tuberculosis in different stages of development

<table>
<thead>
<tr>
<th>Source or organization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLINICAL STUDIES</strong></td>
</tr>
<tr>
<td>Diamine SQ109 Sequella</td>
</tr>
<tr>
<td>Diarylquinoline TMC207 Tibotec Inc.</td>
</tr>
<tr>
<td>Gatifloxacin OFLOTUB consortium, others</td>
</tr>
<tr>
<td>Moxifloxacin Bayer, TB Alliance, others</td>
</tr>
<tr>
<td>Nitrodihydroimidazo-oxazole OPC-67683 Otsuka Pharmaceutical</td>
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<tr>
<td>Nitroimidazole PA-824 TB Alliance</td>
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<tr>
<td>Pyrrole LL3858 Lupin Pharmaceuticals</td>
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<tr>
<td>Rifapentine Hoechst</td>
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<tr>
<td><strong>PRECLINICAL TESTING</strong></td>
</tr>
<tr>
<td>Dipiperidine SQ609 Sequella</td>
</tr>
<tr>
<td>Nitroimidazole backup compounds Otsuka Pharmaceutical</td>
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<tr>
<td>Nonfluorinated quinolones TaiGen</td>
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<td>Oxazolidinones Pfizer</td>
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<td>Synthase inhibitor FAS20013 FASgen</td>
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<td><strong>DISCOVERY STAGE</strong></td>
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<tr>
<td>Cell-wall synthesis inhibitors Colorado State University, NIAID</td>
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<td>Dihydrolipoamide acyltransferase inhibitors Cornell University, NIAID</td>
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<tr>
<td>Enoyl reductase (InhA) inhibitors GlaxoSmithKline, TB Alliance</td>
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<tr>
<td>Malate synthase inhibitors GlaxoSmithKline, others</td>
</tr>
<tr>
<td>Multifunctional molecules TB Alliance, Cumbre Pharmaceuticals</td>
</tr>
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<td>Mycobacterial gyrase inhibitors TB Alliance, GlaxoSmithKline</td>
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<td>Nitrofuranylamides University of Tennessee, NIAID</td>
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<td>Nitroimidazole analogs TB Alliance, University of Auckland, Uni. of Illinois</td>
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<td>Picolinamide imidazoles NIAID</td>
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<td>Pleuromutins GlaxoSmithKline, TB Alliance</td>
</tr>
<tr>
<td>Promazine analogs Salisbury University</td>
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<tr>
<td>Protease inhibitors Medivir</td>
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<td>Proteasome inhibitors Cornell University, NIAID</td>
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<tr>
<td>Quinolones TB Alliance, KRICT, Yonsei University</td>
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<tr>
<td>Riminophenazines Institutes of Materia Medica</td>
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<tr>
<td>Thiolactomycin analogs NIAID, NIH</td>
</tr>
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</table>

KRICT = Korea Research Institute of Chemical Technology; NIAID = National Institute of Allergy and Infectious Diseases; NIH = National Institutes of Health; TB Alliance = Global Alliance for TB Drug Development; SOURCES: Stop TB, NIAID, TB Alliance, Doctors Without Borders
Polycyclic ‘cage’ chemistry

Polycyclic ‘cage’ chemistry has been of great interest to both medicinal and organic chemists for over four decades. These compounds include adamantane, trishomocubane, pentacycloundecane, pentacyclodecane, basketane, cubane etc. Of all these compounds, adamantane is the most studied. The chemistry and medicinal value of polycyclic compounds gained popularity with the discovery of amantadine or 1-amino adamantane which exhibited anti-viral activities against several species which includes hepatitis C and the influenza virus. The incorporation of ‘cage’ compounds into pharmaceutical drugs is an interesting application which is fast growing and has not been fully explored yet. Such compounds increases the drug lipophilicity thus serving as a transport aid to carry such drug across cellular membranes including the Blood Brain Barrier (BBB) and the Central Nervous System (CNS). They also increase the drug’s affinity for lipophilic regions in receptor molecules. It helps to decrease the drug metabolism thus prolonging the pharmaceutical effect of such drug in the body consequently reducing the frequency of dosage intake of such drugs.

This project was motivated by the reported anti-TB activity of SQ109. The structure of SQ109 [N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine] can be divided into 3 main moieties; which are the adamantyl moiety, ethane-1,2-diamine moiety (EMB pharmacophore) and the isoprenyl (geranyl) moiety. The aim of this project is to evaluate the importance of these moieties and how it contributes to activity. This was achieved by substituting the linear 1,2-diamine with cyclic diamines such as piperazine and homopiperazine. Also the length of the hydrocarbons side chains (saturated or unsaturated) were varied to investigate the effect of these elements on the bioactivity of the compound. Finally, the polycyclic ‘cage’ moiety was also varied by substituting adamantane with other known polycyclic moieties such as pentacycloundecane, trishomocubane, pentacyclodecane, oxapentacycloundecane etc.

Figure 6: Examples of polycyclic ‘cage’ compounds
Research carried out in this thesis

This thesis focuses on the synthesis of novel polycyclic ‘cage’ amine derivatives, the structural elucidation thereof and their evaluation against *M. tuberculosis*, fungi [yeast and filamentous fungi (moulds)] and bacteria. This work consists of a series of papers (1-9). Based on the format adopted for this thesis, the numbering of structures, figures, schemes and tables in each of the chapters 2-10 will vary. The referencing style will also vary according to the style specified by the journal.

Chapter 2 (paper 1)\(^5\) is a completion of a study carried in my MSc dissertation titled “Design, synthesis and screening of novel pentacycloundecane tetra-amine derivatives as potential anti-tuberculosis agents”. In this paper, compounds 9a, 9b and 9c were synthesized as part of my PhD studies. The most active compounds (6a, 6b, 8a, 8b and 9a) were re-synthesized and further screened against H\textsubscript{37}Rv and X194 (an extensively drug-resistant) strains of tuberculosis using the BACTEC 460TB system. In order to complete this study, IC\textsubscript{50} (inhibition concentration) experiments were also carried out on the most active compounds using the MDBK (Miadin Darby Bovine Kidney epithelium) cell line from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville. The structural activity relationship (SAR) of compounds 8b and 9a (paper 1)\(^5\) helped in understanding the different and type of functionalities essential for the activity of these classes of compounds as anti-TB agents. The BACTEC analysis was carried by Prof. I. Wiid at Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

Chapter 3 (paper 2)\(^5\) was motivated by reports that SQ109 possesses antifungal activity against *Candida albicans* and fluconazole resistant strains of *Candida albicans*.\(^6\) As a result compounds 8a (GKM8), 8b (GKM9) and 9a (GKM11) were selected for further antimicrobial studies. In this study a total of twenty pathogenic fungi strains and 18 ATCC bacteria (nine Gram-positive and nine Gram-negative) strains were used and the minimum inhibitory concentration (MIC) was determined using the Clinical and Laboratory Standards Institute (CLSI) guidelines. The biological screening was performed by me under the guidance of Dr N. Singh of the School of Biological and Conservation Science (UKZN) and Mrs D. Naidu and Prof. Y. Coovadia of the Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

Chapter 4 (paper 3)\(^5\) focuses on the synthesis and evaluation of five novel SQ109 analogues against H\textsubscript{37}Rv and X174 (an extensively drug-resistant) strains of tuberculosis. Further studies were carried out on these analogues which included the structural elucidation using 2D NMR techniques (Chapter 5, paper 4)\(^\text{a}\) and anti-microbial screening of these analogues against fungi and bacteria (Chapter 6, paper 5)\(^\text{a}\). The NMR elucidation was solely done by me. The biological screening was performed by me under the guidance of Dr N. Singh in the School of Biological and Conservation Science (UKZN).
and Mrs D. Naidu and Prof. Y. Coovadia of the Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

Chapter 7 (paper 6) evaluates the effect of different polycyclic ‘cage’ moieties on the anti-TB activity of these class of compounds. Herein, the 2-adamantyl moiety of SQ109 was substituted with pentacycloundecyl, aza-pentacycloundecyl, oxa-pentacycloundecyl and trishomocubyl moieties and screened for anti-TB activity. The anti-TB analysis was carried out by Mr. M. Pillay and Prof. Y. Coovadia of Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

It was postulated that disubstitution on the polycyclic ‘cage’ moieties compared to the mono-substituted in this case SQ109 (adamantyl moiety), might improve the activity as an anti-TB agent. Three polycyclic ‘cage’ moieties namely pentacycloundecane, tricyclodecane and pentacyclodecane were employed in this study (Chapter 8, paper 7). The synthesis and structural elucidation of some of the PCU precursors were also reported (Chapter 9, paper 8).

In chapter 10 (paper 9) the synthesis and NMR elucidation of novel PCU diamine ligands were reported. Collaboration established with Prof. Graham Jackson of the School of Chemistry, University of Cape Town and Dr. Sebusi Odistse of the School of Chemistry, Cape Peninsula University of Technology, investigates the application of polycyclic cage compounds as ligands in pharmaceutical applications. Odistse et al. recently reported the application of copper (II) diaminodiamide derivatives of pentacycloundecane as a potential anti-inflammatory agent. Based on the success of this project, new PCU diamine ligands were designed and proposed. The synthesis and NMR studies are reported herein. Similar anti-inflammatory studies with these compounds are currently being performed at the other institutions.
References

9. National Institute of Allergy and Infectious Diseases (NIAID); webpage: www.niaid.nih.gov/topics/tuberculosis/Understanding/Pages/tbHIV.aspx accessed on 03-11-2010.


60. *Tuberculosis (Edinb)* **2008**, *88*, 159-61.


CHAPTER 2
PENTACYCLOUNDECANE DERIVED CYCLIC TETRA-AMINES: SYNTHESIS AND EVALUATION AS POTENT ANTI-TUBERCULOSIS AGENTS

Oluseye K. Onajole,a Karnishree Govender,b Patrick Govender, c Paul D. van Helden, d Hendrik G. Kruger,a Glenn E. M. Maguire,a Karen Muthusamy, c Manormoney Pillay, b Ian Wiid, d and Thavendran Govender.e

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b Department of Medical Microbiology Nelson R Mandela School of Medicine, Durban, University of KwaZulu-Natal, South Africa.
c School of Biochemistry, University of KwaZulu-Natal, Durban, South Africa
d Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.
e School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa.

Corresponding author. Tel.: +27-31-2608212; Fax: +27-31-2603091 Email address: govenderthav@ukzn.ac.za (T. Govender)

Abstract
As part of an ongoing effort to develop highly potent anti-tuberculosis agents, fourteen pentacycloundecane (PCU) tetra-amine compounds were synthesized and screened for their in vitro anti-mycobacterial activity against two TB strains, H37Rv and XDR 194 [an extensively drug-resistant strain of tuberculosis (TB)]. Using the broth macrodilution method, nitrofuranylamide based compounds (6a and 6b) showed almost similar activities against the H37Rv strain of Mycobacterium tuberculosis when compared with the control drug, ethambutol. N-geranyl piperazine PCU (8a) and trans-trans farnesyl piperazine PCU (8b) were 3.2 and 3.7 times more potent than commercially available ethambutol. Both isoprenyl PCU tetra-amine derivatives and N-decyl piperazine PCU (9a) were highly active against the XDR 194 strain of tuberculosis with MICs in the range of 0.63 - 3.02 µM. Cytotoxicities (IC50) of isoprenyl based compounds (8a, 8b) and compound 9a were tested on a mammalian cell line [MDBK (Madin Darby bovine kidney epithelium)] with values of 30, 24 and 25 µM respectively.

Keywords: XDR-tuberculosis (TB), Pentacycloundecane, Isoprenyl, SQ109
1. Introduction

The WHO estimate of 9.2 million new tuberculosis cases with 1.7 million deaths in 2006, indicates that tuberculosis (TB) still accounts for a significantly large proportion of the global disease burden and mortality [1]. India, China, Indonesia, South Africa and Nigeria featured as the top five of the highest affected countries. The highest incidence rates were found in Africa, with twelve of the fifteen countries in this continent having the most severe infection rates.

The worldwide increase in prevalence of *Mycobacterium tuberculosis* and the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has imposed a serious setback on global TB control [2]. There were approximately 0.5 million reported cases of MDR-TB throughout the world in 2006 [2]. Aside from protracted treatment periods [2] and the use of more toxic and expensive second line drugs [3], MDR-TB is associated with low cure rates and high mortality [4]. XDR-TB found in most parts of the world [3] is almost untreatable and is accompanied by higher mortality rates than MDR-TB [5].

The difficulties associated with the long duration of therapy, such as patient non-compliance following improvement after the intensive phase and ingestion of multiple doses consisting of many tablets have also led to a need to develop simple drug regimen. The ensuing limited treatment options for MDR and XDR-TB have created a renewed interest in the development of novel anti-TB drug candidates. These new drugs must effectively shorten treatment time and act against the subpopulation of slowly metabolising bacilli.

Among the promising potential anti-TB drugs currently undergoing human clinical trials are the third generation fluoroquinolones, gatifloxacin and moxifloxacin, diarylquinolone TMC 207, nitroimidazole PA-824 and nitroimidazo-oxazole OPC-67683 [6]. Other drugs that are in the preclinical phase include the diamine SQ109 (2), dipiperidines SQ609, nitroimidazo-oxazole back-up, synthase inhibitor FAS20013, translocase I inhibitors and non-fluorinated quinolones [6].

SQ109 (N-geranyl-N‘-(2-adamantyl)ethane-1,2-diamine), developed by Sequella Inc. was first synthesized by Lee et al.[7] using solid phase synthesis of 1,2 diamine analogues of ethambutol (EMB) (1) (Fig. 1). From the several 1,2 diamine derivatives obtained using a combinatorial approach, SQ109 (2) was found to be the most potent new anti-TB lead. SQ109 (2) is an effective compound against EMB resistant strains [8]. Its activity against XDR-TB strains and a 25 % reduction in the time to cure mice by enhancing the activity of isoniazid and rifampicin was also reported. SQ 775 (3) is an example of a cyclic diamine compound with an activity similar to the control drug EMB [9].
Non-TB related research showed that polycyclic “cage” compounds (such as adamantane and pentacycloundecane) have been reported to improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across the Blood Brain Barrier (BBB) or Central Nervous System (CNS) [10,11]. It can also reduce the bio-degradation of the drug thereby prolonging the pharmaceutical effect of such agents in the body [11-14]. SQ109 and SQ775 both have an adamantane moiety in common suggesting that this skeleton might be contributing to its lipophilicity thereby enhancing their anti-TB activities.

In a similar development, Tangallapally et al. [15, 16] recently reported novel nitrofuranylamide compounds with promising in vitro anti-tuberculosis activities. However, this activity could not be duplicated in in vivo analysis due to a short serum half life and rapid elimination of the compound. This led to the introduction of a bicyclic (tetrahydroisoquinoline) moiety in order to make it more resistant to proteolysis [17]. This particular modification showed an improvement in serum half life while improving its MIC activity for compound 4 [17].

For our work it was proposed that the replacement of the bicyclic tetrahydroisoquinoline with a more rigid lipophilic compound such as the pentacycloundecane (PCU) moiety, a polycyclic “cage”, might further enhance the activity by facilitating the movement of these molecules across the lipid-enriched bacterial cell membrane.

Inspired by the work of Lee et al. [7] and Tangallapally et al. [15-17] a series of novel amine based compounds (5-13) bearing the lipophilic PCU molecule were designed and synthesized (Figure 3).
Figure 3: PCU amine derivatives 5-13.
2. Chemistry

The starting material, PCU ditosylate [18] was synthesized from pentacycloundecane-8,11-dione as illustrated in Scheme 1. The starting material, Cookson’s dione (14) [19] was reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to afford the endo-8,endo-11 diol (15) which upon dehydration under Dean-Stark conditions, yielded the corresponding 3,5-dially-4-oxahexacyclo[5.4.1.0\(_{2,6}\).0\(_{3,10}\).0\(_{5,9}\).0\(_{8,11}\)]dodecane (16) [20]. Ozonolysis of the hexacyclic ether (16) followed by reductive work up yielded the PCU diol (17) [18] which upon tosylation gave the PCU ditosylate (18) [18]. The overall yield of (18) from (14) was 60 %.

Scheme 1: Reagents and conditions: (a) H\(_2\)C=CHCH\(_2\)MgBr, dry THF; (b) Dean-Stark apparatus, H\(_2\)SO\(_4\), benzene, reflux; (c) O\(_3\), dry CH\(_3\)OH, NaBH\(_4\); (d) p-TsCl, powdered KOH, THF.

PCU ditosylate (18) was reacted with excess piperazine/homopiperazine in dry dichloromethane at -78°C to afford the tetra-amines 5\(_a\) and 5\(_b\) respectively (62 %) and these were reacted with 5-nitrofuran-2-carbonyl chloride (19) to obtain compounds 6\(_a\) and 6\(_b\) respectively (65 %). PCU ditosylate (18) was reacted with N-benzyl homopiperazine (20) at a 1:2.2 ratio with triethylamine under reflux conditions to obtain compound 7 (Scheme 2).
Scheme 2: Reagents and conditions: (a) piperazine/homopiperazine, DCM, -78°C, rt, 24 hrs; (b) DCM, Et₃N, reflux 16 hrs; (c) CH₃CN, Et₃N, reflux, N₂ atm.

Compound 5a was successfully reacted with geranyl bromide (21a) to afford compound 8a but its purification proved difficult via column chromatography. To solve this problem, a new synthetic route was introduced. Applying the methodology employed to synthesize compound 5a, geranyl bromide (21a) was reacted with excess piperazine/homopiperazine to obtain N-geranyl piperazine (22a) and N-geranyl homopiperazine (23a) while the reaction of trans-trans farnesyl bromide (21b) with piperazine/homopiperazine afforded compounds 22b and 23b. Piperazine was also reacted with three linear alkane chains (C10, C15 and C20) to obtain compounds 25a, 25b and 25c (Scheme 3).
Reaction of PCU ditosylate (18) with compound 22a and 22b in the presence of K₂CO₃ with reflux resulted in compounds 8a and 8b respectively (yield 71 %) which were easily purified via column chromatography. Attempts to react PCU ditosylate (19) with 23a and 23b using a similar methodology employed in the synthesis of compounds 8a and 8b were unsuccessful. PCU ditosylate (18) was reacted with 25a, 25b and 25c to yield linear alkane derivatives of PCU dipiperazine 9a, 9b and 9c respectively. Reaction of the PCU ditosylate (18) with N-benzoyl piperazine (26) yielded compound 10, followed by the reduction of the carbonyl group using lithium aluminium hydride to obtain the corresponding benzyl tetra-amine 11 in 55 % yield (Scheme 4).
Scheme 4: Reagents and conditions: (a) 22 or 25 or 26, K$_2$CO$_3$, CH$_3$CN, reflux under N$_2$ atm; (b) LiAlH$_4$, dry THF and reflux under N$_2$ atm, 36 hours.

2-(aminomethyl) pyridine (27) and ethanolamine (28) were reacted with benzaldehyde (29) via reductive amination to obtain their corresponding secondary amines, N-benzyl-N-{(pyridin-2-yl)methyl}amine (30) [21,22] and 2-(Benzylamino)ethanol (31) [23] (Scheme 5).
Scheme 5: Reagents and conditions: (a) CH$_3$CH$_2$OH, rt, 1 hr, NaBH$_4$, reflux, stir overnight, N$_2$ atm; (b) CH$_3$OH, rt, 2 hr, NaBH$_4$, stir overnight, N$_2$ atm.

N-Benzyl-$N$-{(pyridin-2-yl)methyl}amine (30) was reacted with PCU ditosylate (18) to obtain compound (32) after which the benzyl protecting groups were removed by catalytic hydrogenation using ammonium formate with 10 % palladium on carbon to obtain compound 12 (yield 67 %). 2-(benzylamino) ethanol (31) was treated with PCU ditosylate (18) to obtain compound 13 in an overall yield from 18 of 70 % (Scheme 6).

Scheme 6: Reagents and conditions: (a) 30 or 31, CH$_3$CN, Et$_3$N, reflux, N$_2$ atm; (b) 10 % Pd/C, ammonium formate, dry MeOH, reflux, 16 hrs, N$_2$ atm.
3. Results and Discussion

All synthesized novel PCU amine derivatives 5-13 excluding 9 were screened in vitro against *M. tuberculosis* H$_{37}$Rv (ATCC 25618) using a broth macrodilution method (BMM). The ClogP of each novel compound was obtained using the ACDLABS 11.0 program.* The results are depicted in Table 1.

MIC results were obtained after 21 days of incubation. Compounds 5a and 5b differ in structure only by the ring sizes of the diamines with 5a being a six membered and 5b a seven membered ring. Compound 5b shows low activity against the H$_{37}$Rv strain, while 5a did not show any activity at the highest concentration tested. Modification to these compounds with the introduction of a nitrofuran group to form compounds 6a and 6b gave promising activity with MIC values of 24.1 and 23.1 μM respectively which are similar to the control drug, EMB. Compounds 6a and 6b did not show any significant difference in calculated ClogP values when compared to 5a and 5b respectively.

Compound 7 (the benzyl derivative of 5b) exhibits similar activity to 5b (μg/mL) while compound 11 (the benzyl derivative of 5a) demonstrates an improved activity against H$_{37}$Rv, whilst 5a did not show comparable activity. The benzoyl derivative 10 (of 5a) showed a weaker potency compared to 11. Compound 12 and 13 did not show any significant activity.

Based on results obtained by Lee et al. [7, 8] in the development of SQ109 (2), it was decided to synthesize the geranyl and *trans-trans* farnesyl derivative of 5a and 5b. As discussed above, the isoprenyl derivatives of 5b proved to be elusive. Compounds 8a and 8b had MIC values of 6.09 and 5.04 μM respectively. The activity of these lipophilic alkene bearing compounds (8a and 8b) were similar (expressed in μg/mL) to the commercially available analogue, EMB, when screened against H$_{37}$Rv.

Table 1: ClogP values and in vitro anti-mycobacterial activity on day 21 of novel PCU amine compounds (5-13) against *M. tuberculosis* H$_{37}$Rv.

<table>
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<tr>
<th>Compound</th>
<th>R</th>
<th>MW</th>
<th>ClogP$^a$</th>
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<td></td>
<td></td>
<td></td>
<td>µg/mL µM</td>
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</tr>
<tr>
<td>1</td>
<td>Ethambutol</td>
<td>204</td>
<td>NA</td>
<td>4 19.6</td>
</tr>
<tr>
<td>5a</td>
<td>N'(CH$_2$)$_4$NH</td>
<td>385</td>
<td>0.41 ± 0.55</td>
<td>&gt;128 ND</td>
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<tr>
<td>5b</td>
<td>N'(CH$_2$)$_5$NH</td>
<td>413</td>
<td>1.18 ± 0.54</td>
<td>64 155</td>
</tr>
<tr>
<td>6a</td>
<td>N'(CH$_2$)$_4$NCO(C$_4$H$_2$O)NO$_2$</td>
<td>663</td>
<td>0.57 ± 0.83</td>
<td>16 24.1</td>
</tr>
<tr>
<td>6b</td>
<td>N'(CH$_2$)$_5$NCO(C$_4$H$_2$O)NO$_2$</td>
<td>691</td>
<td>1.32 ± 0.81</td>
<td>16 23.2</td>
</tr>
<tr>
<td>7</td>
<td>N'(CH$_2$)$_5$NCH$_2$C$_6$H$_5$</td>
<td>592</td>
<td>4.11 ± 0.73</td>
<td>64 108.1</td>
</tr>
<tr>
<td>8a</td>
<td>N'(CH$<em>2$)$<em>4$NC$</em>{10}$H$</em>{17}$</td>
<td>657</td>
<td>7.35 ± 0.73</td>
<td>4 6.09</td>
</tr>
<tr>
<td>8b</td>
<td>N'(CH$<em>2$)$<em>4$NC$</em>{15}$H$</em>{25}$</td>
<td>793</td>
<td>11.42 ± 0.77</td>
<td>4 5.04</td>
</tr>
<tr>
<td>10</td>
<td>N'(CH$_2$)$_4$NCO$_2$C$_4$H$_5$</td>
<td>592</td>
<td>1.47 ± 0.67</td>
<td>128 216.2</td>
</tr>
<tr>
<td>11</td>
<td>N'(CH$_2$)$_4$NCH$_2$C$_6$H$_5$</td>
<td>565</td>
<td>2.61 ± 0.83</td>
<td>64 113.3</td>
</tr>
<tr>
<td>12</td>
<td>2-pyridylmethylamino$^b$</td>
<td>429</td>
<td>0.82 ± 0.40</td>
<td>128 298</td>
</tr>
<tr>
<td>13</td>
<td>N[(CH$_2$)$_2$OH]CH$_2$C$_6$H$_5$</td>
<td>515</td>
<td>4.22 ± 0.54</td>
<td>128 248</td>
</tr>
</tbody>
</table>

$^a$ClogP was calculated using ACD/labs software v11.0; MIC: Minimal Inhibitory Concentration; ND: Not determined. MW: Molecular weight. NA: Not applicable.

Based on the results for compounds 6a, 6b, 8a and 8b we decided to test the molecules with a more accurate method (BACTEC 460 TB system) against the H$_{37}$Rv strain. Compounds 6a and 6b (Table 2) showed little difference when compared to the results obtained from the BMM method. Further screening was not carried out, although it is known that MICs of drugs differ slightly depending on the assay method used [25]. Due to the activity of 8a and 8b we decided to investigate the effect of using long alkane chains in place of the alkene derivatives to determine whether this functionality is essential for efficacy. This led to the synthesis of compounds 9a (ten carbon alkane), 9b (fifteen carbon alkane) and 9c (twenty carbon alkane) (Scheme 4). Anti-mycobacterial screening of compounds 9b and 9c was not possible however; due to their insolubility at biological pH presumably due to their highly lipophilic nature (ClogP values are 14.43 ± 0.52 and 19.74 ± 0.52 respectively). Compounds 8a, 8b and 9a were tested against an XDR strain of *M. tuberculosis* [strain
X194, resistant to first line drugs (Isoniazid and Rifampicin) and second line drugs (Kanamycin, Ofloxacin and Amikacin) [BACTEC 460 TB system]. These compounds were further analyzed for cytotoxicity on mammalian cells from the MDBK (Madin Darby bovine kidney epithelium) line (Table 2).

Table 2: BACTEC and IC₅₀ results using MDBK cell line for selected compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ClogP</th>
<th>H₃⁷Rv (MIC, µM)</th>
<th>H₃⁷Rv (MIC, µg/mL)</th>
<th>XDR 194 (MIC, µM)</th>
<th>XDR 194 (MIC, µg/mL)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>0.57±0.83</td>
<td>MIC&gt;12.07 (SD±7.47)</td>
<td>MIC&gt;8 (SD±4.24)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>6b</td>
<td>1.32±0.81</td>
<td>MIC&gt;11.58 (SD±7.16)</td>
<td>MIC&gt;8 (SD±4.24)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>8a</td>
<td>7.35 ± 0.73</td>
<td>1.52 &gt; MIC &gt; 0.76 (SD ± 0.54)</td>
<td>&gt; MIC &gt; 0.5 (SD ± 0.35)</td>
<td>3.04 &gt; MIC &gt; 1.52 (SD ± 0.54)</td>
<td>2.0 &gt; MIC &gt; 1.0 (SD ± 0.71)</td>
<td>30</td>
</tr>
<tr>
<td>8b</td>
<td>11.42 ± 0.77</td>
<td>0.63 &gt; MIC &gt; 0.32 (SD ± 0.22)</td>
<td>0.5 &gt; MIC &gt; 0.25 (SD ± 0.18)</td>
<td>1.26 &gt; MIC &gt; 0.63 (SD ± 0.45)</td>
<td>1 &gt; MIC &gt; 0.5 (SD ± 0.35)</td>
<td>24</td>
</tr>
<tr>
<td>9a</td>
<td>9.12 ± 0.52</td>
<td>NT</td>
<td>NT</td>
<td>1.5 &gt; MIC &gt; 0.75 (SD ± 0.53)</td>
<td>1 &gt; MIC &gt; 0.5 (SD ± 0.35)</td>
<td>25</td>
</tr>
<tr>
<td>SQ109*</td>
<td>6.04 ± 0.45</td>
<td>0.63</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>26</td>
</tr>
</tbody>
</table>

*Literature value [8]; NT: Not Tested.

Compounds 8a and 8b show potent activity against the H₃⁷Rv strain with 8b being approximately two-fold more active than 8a against both H₃⁷Rv and XDR 194 strains respectively. The MIC for EMB in H₃⁷Rv is 0.94 µg/mL [25] the MIC for 8b against H₃⁷Rv was lower (0.5 - 0.25 µg/mL, Table 2). The XDR strain used was resistant to EMB at the breakpoint concentration of 2.5 µg/mL in BACTEC [24]. Therefore, the MIC for the XDR strain lies beyond 2.5 µg/mL. This means that the efficacy of compound 8b is between 2.5 to 5 times more effective in inhibiting the XDR strain than EMB (8b killed the strain between 1 µg/mL and 0.5 µg/mL, Table 2). Also, the sensitivity for 8b between H₃⁷Rv and X194 was approximately two fold (Table 2). The results indicate that our compounds properly have a different cellular target to that of EMB.

9a proved to be twice as active as compound 8a against the XDR strain of M. tuberculosis. It was not considered necessary to test 9a using the BMM method since the BACTEC system is much more accurate. Compound 9a has a higher ClogP value compared to 8a (Table 2), suggesting that lipophilicity could play an important role in the activity of this class of compounds. However, this assumption could not be further evaluated due to insolubility problems encountered with compounds 9b and 9c. It should be noted that the MICs for 9a and 8b fall in the same range (1 > MIC > 0.5), however conversion to µM makes quite a significant difference as compound 8b is more active than 9a (8b has a larger molecular weight).
Compounds 8a and 8b are structurally related to SQ109, they all have polycyclic “cage” moieties, diamines (linear or cyclic) and long alkene chains, which form the basis of this comparison. As illustrated in Table 2, 8a is only half as active as SQ109 whereas 8b is twice as active as SQ109 [8]. This suggests that the length of the alkene chain (lipophilicity again) may be playing an important role in the activity of these compounds. The cytotoxocities (IC₅₀, Table 2) of these compounds (8a, 8b and 9a) and that of SQ109 fall in the same range between 20 – 30 µM with compound 8a being the least toxic.

A potential anti-TB candidate needs to be evaluated as a drug combination, i.e. the possibility of incorporating such an anti-TB candidate with other existing anti-TB drugs. Based on this, further studies were carried out to investigate the interaction of compound 8b (the most active compound in this series) with two known anti-TB drugs (Isoniazid and Rifampicin) by means of in vitro testing against the H₃₇Rv strain of M. tuberculosis. The interaction of compound 8b with these two anti-TB drugs could be antagonist, additive, synergistic or have no effect at all. Results obtained using once again the BACTEC 460 TB system showed that compound 8b (0.5 µg/mL) had no antagonist or synergistic effect; it did however have an additive effect.

4. Conclusion

A series of novel PCU tetra-amines (fourteen compounds) were synthesized and screened for their anti-TB activity of which five compounds (6a, 6b, 8a, 8b and 9a) displayed the highest anti-TB activity against M. tuberculosis H₃₇Rv. Compounds 8a, 8b and 9a were the most potent with excellent activities giving MICs of 3.04, 1.26 and 1.50 µM respectively against an XDR-TB strain (X194). These results indicate that lipophilicity is an important component of their efficacy (having larger ClogP values than SQ109). This does however limit their potential application due to solubility problems (as seen for molecules 9b and 9c). We are examining the design of new derivatives in terms of their structure-activity relationship. It is hoped that new more soluble active species can be obtained. The first generation of these active compounds however, is currently undergoing further in vitro and in vivo analysis.

5. Experimental

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using CDCl₃ as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and coupling constant (J) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment recorded in cm⁻¹. All reactions were monitored using Thin Layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70 – 230 mesh) and CH₂Cl₂:CH₃OH:NH₄OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds
was judged to be >95% based upon $^1$H NMR and LC-MS analysis. Mass Spectra were obtained using a Waters LCT Premier Time of Flight mass spectrometer. Tetrahydrofuran was freshly distilled before use from a sodium benzophenone under N$_2$ atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use. The syntheses of the precursors are described in their corresponding references.

Clog P gives an indication of the lipophilicity of the drug with reference to its pharmacological importance (pharmacokinetics and pharmacodynamics) [15, 26].

5.1.1. Synthesis of PCU tetra-amine 5a & 5b

To a vigorously stirred solution of the cyclic diamines (piperazine/homopiperazine) (22 mmol) in DCM (400 mL) at -78 °C (dry ice, 2-propanol) under N$_2$ atmosphere was added dropwise PCU ditosylate (18, 1.2 g, 2.2 mmol) in DCM (100 mL) over 45 minutes. The reaction mixture was left to attain room temperature with stirring for 24 hours. The solution was washed with water to remove excess diamines, the obtained organic extract was dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via column chromatography using CH$_3$Cl: MeOH: NH$_4$OH (88:10:2) to obtain a pure product.

5.1.1a. PCU dipiperazine (5a)

A yellow oil ($R_f = 0.2, 0.56$ g, 68 % yield). IR $\nu_{max}$: broad absorption (N-H) 3367, 2949, 1655, 1463, 1125 and 746 cm$^{-1}$. HRMS calculated for C$_{23}$H$_{36}$N$_4$O (M + H$^+$) 385.2955, found 385.2967. $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta_H$ 1.52 (AB, $J_{AB} =$ 10.1 Hz, 1H), 1.87 (AB, $J_{AB} =$ 10.1 Hz, 1H), 1.99 (t, $J =$ 7.92 Hz, 2H), 2.38-2.60 (m, 8H), 2.95 (2H). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta_C$ 29.7 (t), 41.8 (d), 43.4 (t), 44.5 (d), 45.6 (t), 48.0 (d), 53.7 (t), 55.1 (t), 58.8 (d), 94.8 (s).

5.1.1b. PCU dihomopiperazine (5b)

A light yellow oil, ($R_f = 0.2, 0.55$ g, 62 %). IR $\nu_{max}$: broad absorption (N-H) 3386, 2818, 1465, 1108, 927, 918 and 748 cm$^{-1}$. HRMS calculated for C$_{25}$H$_{40}$N$_4$O (M + H$^+$) 413.3280 found 413.3287. $^1$H NMR [CDCl$_3$, 400 MHz] $\delta_H$ 1.48 (AB, $J_{AB} =$ 10.2 Hz, 1H), 1.84 (AB, $J_{AB} =$ 10.2 Hz, 1H), 1.76 (2H), 1.98 (2H), 2.35-2.69 (m, 10H), 2.89-2.94 (m, 4H). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta_C$ 30.0 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 47.2 (t), 48.0 (d), 48.6 (d), 54.3-55.0 (t), 57.8 (t), 58.8 (d), 94.8 (s).

5.1.2. Synthesis of 5-nitrofuran-2-carbonyl diamine PCU 6a & 6b

To a stirred mixture of PCU tetra-amines (5a and 5b, 1.2 mmol) in DCM (2 mL) and Et$_3$N (670 µL, 4.8 mmol) under N$_2$ atmosphere was added freshly prepared 5-nitrofuran-2-carbonyl chloride (19 [15] 3.6 mmol) in DCM (3 mL) and stirred for 18 hours at reflux. The reaction mixture was cooled and diluted with 60 mL of ethyl acetate and washed sequentially with (2 x 50 mL) 10 % NaHCO$_3$, (2 x 50 mL) water and (2 x 50 mL) brine. The organic layer was dried over anhydrous Na$_2$SO$_4$ and
concentrated in vacuo. The crude residue was purified via column chromatography using CH$_3$Cl: MeOH: NH$_4$OH (88:10:2).

**5.1.2a. 5-nitrofuran-2-carbonyl piperazine PCU (6a)**

A brown oil (R$_f$ = 0.7, 0.56 g, 65 %). IR $\nu$$_{max}$: 2927, 1630, 1352, 1021 and 752 cm$^{-1}$. HRMS calculated for C$_{33}$H$_{38}$N$_6$O$_9$ (M + H$^+$) 663.2779, found 663.2758. $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta$H 1.52 ($J_{AB}$ = 10.1 Hz, 1H), 1.87 ($J_{AB}$ = 10.1 Hz, 1H), 1.99 (t, $J$ = 7.36 Hz, 2H), 2.38-2.61 (m, 10H), 3.76 (s, 2H), 3.85 (s, 2H), 7.15 (1H), 7.32 (1H). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta$C 29.9 (t), 41.8 (d), 43.1 (t), 43.5 (t), 44.5 (d), 46.6 (t), 48.1 (d), 53.4 (t), 54.4 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.1 (d), 148.7 (s), 151.2 (s), 156.6 (s).

**5.1.2b. 5-nitrofuran-2-carbonyl homopiperazine PCU (6b)**

A brown oil (R$_f$ = 0.51 g, 61 %). IR $\nu$$_{max}$: 3479, 2951, 2859, 1627, 1530, 1352, 810 and 729 cm$^{-1}$. HRMS calculated for C$_{35}$H$_{42}$N$_6$O$_9$ (M + H$^+$) 691.3092, found 691.3105. $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta$H 1.52 ($J_{AB}$ = 10.1 Hz, 1H), 1.87 ($J_{AB}$ = 10.1 Hz, 1H), 1.96-2.03 (m, 4H) 2.37-2.88 (m, 10H), 3.72-3.77 (m, 2H), 3.84 (2H), 7.19 (1H), 7.34 (1H). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta$C 27.0 (t), 28.7 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.4 (d), 46.2 (t), 47.3 (t), 47.9 (t), 48.0 (d), 48.8 (t), 54.2-54.9 (t), 54.7 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.0 (d), 149.2 (s), 151.2 (s), 157.9 (s).

**5.1.3. Synthesis of N-benzyl homopiperazine PCU (7)**

A mixture of N-benzyl homopiperazine (20, 0.7 g, 3.68 mmol) and PCU ditosylate (18, 0.93 g, 1.67 mmol) and triethylamine (350 µL, 2.5 mmol) in CH$_3$CN (20 mL) was refluxed for four days under N$_2$ atmosphere. The reaction mixture was cooled, filtered and concentrated in vacuo. The crude residue was purified via column chromatography on silica gel using CH$_3$Cl: MeOH: NH$_4$OH (88:10:2, R$_f$ = 0.65) as eluent to give the product as a yellow oil (0.84 g, 85 %). IR $\nu$$_{max}$: 2935, 2811, 1452, 1351, 1111, 729 and 695 cm$^{-1}$. HRMS calculated for C$_{39}$H$_{52}$N$_4$O (M + H$^+$) 593.4219 found 593.4230. $^1$H NMR [CDCl$_3$, 400 MHz] $\delta$H 1.48 ($J_{AB}$ = 10.2 Hz, 1H), 1.84 ($J_{AB}$ = 10.2 Hz, 1H), 1.77 (2H), 1.96 (t, $J$ = 8.0 Hz, 2H), 2.35-2.73 (m, 14H), 3.60 (s, 2H), 7.26-7.31 (m, 5H). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta$C 27.5 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.4-55.1 (t), 58.8 (d) 62.8 (t), 94.9 (s), 126.8 (d), 128.2 (d), 128.9 (d), 139.5 (s).

**5.1.4. Synthesis of N-isoprenyl/linear alkane diamines**

To a vigorously stirred solution of diamine (piperazine/homopiperazine) (10 mmol) in DCM (750 mL) at -78 ºC (dry ice, 2-propanol) under N$_2$ atmosphere was added dropwise a solution of isoprenyl bromide (21a or 21b, 2 mmol)/ linear alkane bromide (24a, 24b or 24c, 2 mmol) in DCM (250 mL) over 45 minutes. The reaction mixture was left to attain room temperature with stirring for 24 hours. The solution was washed with water to remove excess piperazine, the organic extract was dried over
Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified via column chromatography using CH$_3$Cl: MeOH: NH$_2$OH (88:10:2).

5.1.4a. N-geranyl piperazine (22a)

A yellow oil ($R_f = 0.5$, 0.52 g, 68%). $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta_H$ 1.56 (s, 3H), 1.60 (s, 3H), 1.64 (s, 3H), 1.98-2.08 (m, 4H), 2.52 (NH proton), 2.98 (d, 2H), 5.04 (t, $J = 7.12$ Hz, 1H), 5.22 (m, 1H). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta_C$ 16.4 (q), 17.7 (q), 25.7 (q), 26.4 (t), 39.8 (t), 45.1 (t), 52.8 (t), 56.2 (t), 120.1 (d), 124.0 (d), 131.6 (s), 139.7 (s).

5.1.4b. N-trans-trans farnesyl piperazine (22b)

A yellow oil ($R_f = 0.6$, 0.5 g, 65%). $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta_H$ 1.51 (s, 3H), 1.52 (s, 3H), 1.56 (s, 3H), 1.59 (s, 3H), 1.86-2.01 (m, 8H), 2.40 (NH), 2.90 (m, 2H), 4.98-5.02 (m, 2H), 5.17 (t, 1H, $J = 6.6$ Hz). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta_C$ 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.7 (t), 39.7 (t), 39.7 (t), 45.2 (t), 53.1 (t), 56.0 (t), 56.3 (t), 120.2 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.0 (s), 139.2 (s).

5.1.4c. N-geranyl homopiperazine (23a)

A yellow oil ($R_f = 0.5$, 0.5 g, 64%). $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta_H$ 1.47 (s, 3H), 1.50 (s, 3H), 1.55 (s, 3H), 1.69 (sxt, 2H), 1.88-1.98 (m, 4H), 2.52-2.55 (m, 4H), 2.82-2.88 (m, 4H), 2.98 (d, 2H), 3.51 (NH), 4.93-4.97 (m, 1H), 5.11-5.13 (m, 1H). [CDCl$_3$, 100 MHz]; $\delta_C$ 16.1 (q), 17.6 (q), 25.5 (q), 25.7 (q), 26.5 (t), 26.8 (t), 29.2 (t), 29.5 (t), 3.51 (NH), 4.93-4.97 (m, 1H), 5.11-5.13 (m, 1H). [CDCl$_3$, 100 MHz]; $\delta_C$ 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.7 (t), 29.9 (t), 39.7 (t), 39.7 (t), 46.9 (t), 48.3 (t), 54.5 (t), 56.2 (t), 57.7 (t), 121.5 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.1 (s), 138.5 (s).

5.1.4d. N-trans-trans farnesyl homopiperazine (23b)

A yellow oil ($R_f = 0.6$, 0.52 g, 66%). $^1$H NMR [CDCl$_3$, 400 MHz]; $\delta_H$ 1.58 (s, 3H), 1.59 (s, 3H), 1.63 (s, 3H), 1.68 (s, 3H), 1.80 (sxt, 2H), 1.95-1.98 (m, 2H), 2.04-2.12 (m, 6H), 2.59 (NH), 2.63-2.68 (m, 4H), 2.92-2.98 (m, 4H), 3.11 (d, 2H), 5.07-5.12 (m, 2H), 5.27 (t, 1H). $^{13}$C NMR [CDCl$_3$, 100 MHz]; $\delta_C$ 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.7 (t), 29.9 (t), 39.7 (t), 39.7 (t), 46.9 (t), 48.3 (t), 54.5 (t), 56.2 (t), 57.7 (t), 121.5 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.1 (s), 138.5 (s).

5.1.4e. N-C10 piperazine (25a)

A white solid ($R_f = 0.4$, 0.46 g, 58%). $^1$H NMR [CDCl$_3$, 600 MHz]; $\delta_H$ 0.83 (t, 3H), 1.22 (m, 14H), 1.44 (s, 2H), 1.93 (m, 1H), 2.30 (m, 2H), 2.45 (s, 4H), 2.92 (s, 4H). $^{13}$C NMR [CDCl$_3$, 150 MHz]; $\delta_C$ 14.1 (q), 22.6 (t), 26.5 (t), 27.5 (t), 29.3 (t), 29.5 (t), 31.9 (t), 45.1 (t), 53.2 (t), 59.1 (t).

5.1.4f. N-C15 piperazine (25b)

A white solid ($R_f = 0.4$, 0.45 g, 58%). $^1$H NMR [CDCl$_3$, 600 MHz]; $\delta_H$ 0.89 (t, 3H), 1.26-1.28 (m, 24H), 1.49 (s, 2H), 1.81 (s, 1H), 2.30 (m, 2H), 2.41 (s, 3H), 2.91 (s, 4H). $^{13}$C NMR [CDCl$_3$, 150 MHz].
5.1.5. Synthesis of PCU piperazine isoprenyl/linear alkane

A mixture of \( N \)-isoprenyl piperazine/linear alkane piperazine (1.9 mmol) was reacted with (0.47 g, 0.84 mmol) PCU ditosylate (18) and \( K_2CO_3 \) (0.175 g, 1.27 mmol) in \( CH_3CN \) (10 mL) with reflux under nitrogen atmosphere for four days. The reaction was cooled, filtered and concentrated in vacuo to obtain a crude product. The residue was purified via column chromatography on silica gel using \( CHCl_3/MeOH/NH_4OH: 88:10:2 \) as eluent to give the product as yellow oil.

5.1.5a. \( N \)-geranyl piperazine PCU (8a)

A yellow oil (\( R_f = 0.7, 0.40 \) g, 71 %). IR \( \nu_{\text{max}} \): 2929, 1672, 1448, 1375, 1294, 1006 and 821 cm\(^{-1}\). HRMS calculated for \( C_{43}H_{68}N_4O \) (M + H\(^+\)) 657.5471, found 657.5446; \(^1\)H NMR [\( CDCl_3, 400 \) MHz] \( \delta_H 1.51 \) (\( J_{AB} = 10.2 \) Hz, 1H), 1.84 (\( J_{AB} = 10.2 \) Hz, 1H), 1.95-2.06 (m, 8H), 2.35-2.57 (m, 12H), 2.95 (d, 2H), 5.04 (t, \( J = 6.72 \) Hz, 1H), 5.22 (t, \( J = 6.4 \) Hz, 1H). \(^{13}\)C NMR [\( CDCl_3, 100 \) MHz]: \( \delta_C 16.4 \) (q), 17.7 (q), 25.7 (q), 26.4 (t), 30.1 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.0 (t), 53.2 (t), 54.6 (t), 55.8 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.7 (d), 124.1 (d), 131.5 (s), 138.9 (s).

5.1.5b. \( N\)-trans trans farnesyl piperazine PCU (8b)

A yellow oil (\( R_f = 0.8, 0.42 \) g, 63 %). IR \( \nu_{\text{max}} \): 2929, 1670, 1448, 1375, 1294, 1153, 1006 and 822 cm\(^{-1}\). HRMS calculated for \( C_{53}H_{84}N_4O \) (M + H\(^+\)) 793.6723, found 793.6714. \(^1\)H NMR [\( CDCl_3, 400 \) MHz] \( \delta_H 1.48 \) (\( J_{AB} = 10.2 \) Hz, 1H), 1.84 (\( J_{AB} = 10.2 \) Hz, 1H), 1.56 (s, 3H), 1.57 (s, 3H), 1.60 (s, 3H), 1.65 (s, 3H), 1.91-2.08 (m, 10H), 2.35-2.60 (m, 12H), 2.95 (d, 2H), 5.07 (m, 2H), 5.23 (m, 1H). \(^{13}\)C NMR [\( CDCl_3, 100 \) MHz]: \( \delta_C 16.0 \) (q), 16.5 (q), 17.7 (q), 25.7 (q), 26.4 (t), 26.7 (t), 30.1 (t), 39.7 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.6 (d), 124.0 (d), 124.2 (d), 131.3 (s), 135.2 (s), 138.9 (s).

5.1.5c. C10-piperazine PCU (9a)

A dark brown oil (\( R_f = 0.8, 0.41 \) g, 73 %). IR \( \nu_{\text{max}} \): 2852, 2807, 1464, 1.295, 1161, 826 and 721 cm\(^{-1}\). HRMS calculated for \( C_{48}H_{56}N_4O \) (M + H\(^+\)) 665.6092, found 665.6114; \(^1\)H NMR [\( CDCl_3, 400 \) MHz] \( \delta_H 0.79 \) (\( J = 6.44 \) Hz, 3H), 1.17-1.19 (m, 14H), 1.40 (\( J_{AB} = 9.2 \) Hz, 1H), 1.42 (s, 2H), 1.77
(J_{AB} = 10.2 Hz, 1H), 1.91 (m, 2H), 2.28-2.32 (m, 3H), 2.37-2.52 (m, 11H). ^{13}\text{C} \text{ NMR} [\text{CDCl}_3, 100 MHz]: \delta_C 14.1 (q), 22.6 (t), 26.4 (t), 27.5 (t), 29.3 (t), 29.5 (t), 29.5 (t), 31.8 (t), 41.7 (d), 43.4 (t), 44.4 (d), 47.9 (d), 52.6 (t), 54.5 (t), 58.6 (t), 58.7 (d), 94.6 (s).

5.1.5d. C15-piperazine PCU (9b)

A light brown oil (R_f = 0.8, 0.48 g, 71 %). IR \nu_{\text{max}}: 2915, 2850, 2808, 1466, 1375, 1163, 1120, 826 and 720 cm^{-1}. MS (TOF) calculated for C_{53}H_{96}N_{4}O (M + H^+) 805.7657, found 805.7698; ^{1}\text{H} \text{ NMR} [\text{CDCl}_3, 400 MHz] \delta_H 0.77 (m, 3H), 1.15 (s, br, 24H), 1.40 (m, 3H), 1.76 (J_{AB} = 10.0 Hz, 1H), 1.89 (3H), 2.20 (m, 2H), 2.27-2.40 (m, 11H), 2.49 (s, 2H). ^{13}\text{C} \text{ NMR} [\text{CDCl}_3, 100 MHz]: \delta_C 14.1 (q), 22.6 (t), 26.9 (t), 27.6 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.6 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t), 44.2 (d), 47.9 (d), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.7 (s).

5.1.5e. C20- piperazine PCU (9c)

A yellow solid (R_f = 0.8, 0.58 g, 73 %). IR \nu_{\text{max}}: 2915, 2849, 2809, 1469, 1375, 1163, 1119, 827 and 718 cm^{-1}. MS (TOF) calculated for C_{63}H_{116}N_{4}O (M + H^+) 945.9222, found 945.9210; ^{1}\text{H} \text{ NMR} [\text{CDCl}_3, 400 MHz] \delta_H 0.86 (m, 3H), 1.22 (s, br, 30H), 1.45 (m, 3H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.96 (t, 2H), 2.27-2.56 (m, 15H). ^{13}\text{C} \text{ NMR} [\text{CDCl}_3, 100 MHz]: \delta_C 14.1 (t), 22.7 (t), 26.8 (t), 27.6 (t), 29.4 (t), 29.4 (t), 29.6 (t), 29.7 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.8 (s).

5.1.6. N-benzoyl piperazine PCU (10)

A mixture of N-benzoyl piperazine (26, 0.7 g, 3.68 mmol) and PCU ditosylate (18, 0.93 g, 1.67 mmol) and K_2CO_3 (0.28 g, 2.0 mmol) in CH_3CN (20 mL) was refluxed for four days under N_2 atmosphere. The reaction mixture was cooled, filtered and concentrated \textit{in vacuo}. The crude residue was purified via column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.75) as eluent to give the product as a yellow oil (0.84 g, 85 %). IR \nu_{\text{max}}: 3463, 2952, 1623, 1430, 1292 and 723 cm^{-1}. HRMS calculated for C_{37}H_{44}N_{4}O_{3} (M + H^+) 593.3492, found 593.3505. ^{1}\text{H} \text{ NMR} [\text{CDCl}_3, 400 MHz]; \delta_H 1.50 (J_{AB} = 10.3 Hz, 1H), 1.85 (J_{AB} = 10.3 Hz, 1H), 2.36-2.59 (m, 10H), 3.42 (s, 2H), 3.78 (s, 2H) and 7.37 (s, 5H). ^{13}\text{C} \text{ NMR} [\text{CDCl}_3, 100 MHz]; \delta_C 29.8 (t), 41.8 (d), 42.0 (t), 43.5 (t), 44.4 (d), 47.6 (t), 48.0 (d), 52.9 (t), 94.7 (s), 127.0 (d), 128.1 (d), 128.5 (d), 135.7 (s), 170.3 (s).

5.1.7. N-benzyl piperazine PCU (11)

N-benzoyl piperazine PCU (10, 1 g, 1.7 mmol) was added to a stirred suspension of lithium aluminium hydride (0.25 g, 6.8 mmol) in dry THF under nitrogen. The solution was refluxed for 36 hours under N_2. The solution was diluted with diethyl ether and the excess LAH was quenched by the
dropwise addition of saturated aqueous Na₂SO₄. The solution was filtered, dried over anhydrous Na₂SO₄ and the solvent was removed in vacuo to afford a residue which was purified via column chromatography on silica gel using CH₃Cl: MeOH: NH₄OH (88:10:2, Rₐ = 0.62) as eluent to give the product as a yellow oil (55 %). IR νmax: 2948, 1656, 1149 and 734 cm⁻¹. HRMS calculated for C₂₇H₄₆N₄O (M + H⁺) 565.3906, found 565.3911. ¹H NMR [CDCl₃, 400 MHz]; δH 1.48 (Jₐₐ = 10.3 Hz, 1H), 1.84 (Jₐₐ = 10.3 Hz, 1H), 1.96 (t, J = 7.92 Hz, 2H) 2.34-2.56 (m, 10H), 3.47 (s, 2H) and 7.2-7.28 (m, 5H). ¹³C NMR [CDCl₃, 100 MHz]; δC 29.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 54.6 (t), 58.8 (d), 63.0 (t), 94.8 (s), 127.0 (d), 128.2 (d), 129.3 (d),138.0 (s).

5.1.8. Synthesis of 2-(aminomethyl) pyridine PCU (12)

A mixture of 2-(aminomethyl) pyridine (27, 1 g, 9.2 mmol) and benzaldehyde (29, 0.98 g, 9.2 mmol) in ethanol (15 mL) was stirred for one hour at room temperature under nitrogen atmosphere, the corresponding imine was reduced with solid NaBH₄ (0.7 g, 18 mmol) which was added slowly over 30 minutes, the mixture was further stirred for an additional 30 minutes, the mixture was then refluxed overnight. The mixture was allowed to cool to RT, an additional ethanol (15 mL) was added to the reaction vessel after which 10 % HCl was added to quench excess NaBH₄. The acidic mixture was basified with 25 % aqueous ammonia solution. The desired product was extracted with dichloromethane (2 x 50 mL) and the solution was dried over Na₂SO₄ and concentrated in vacuo. The crude product was distilled under vacuum to afford (1.2 g, 65 %) N-benzyl-N-{(pyridin-2-yl)methyl}amine (30) [21,22]. ¹H NMR [CDCl₃, 400 MHz]; δH: 2.25 (NH), 3.84 (s, 2H), 3.92 (s, 2H), 7.14 (t, 1H), 7.26 (d, 1H), 7.30-7.37 (m, 5H), 7.62 (t, 1H), 8.56 (d, 1H). ¹³C NMR [CDCl₃, 100 MHz]; δC 53.4 (t), 54.4 (t), 121.8 (d), 122.2 (d), 126.9 (d), 128.1 (d), 128.3 (d), 136.3 (d), 140.0 (d), 149.2 (s), 156.7 (s).

In the next step, A mixture of N-benzyl-N-{(pyridin-2-yl)methyl}amine (30, 1.5 g, 7.6 mmol), PCU ditosylate (18, 1.9 g, 3.4 mmol) and Et₃N (710 µL, 5.1 mmol) in CH₃CN (20 mL) was refluxed under nitrogen for four days. The reaction was monitored on TLC, after completion the reaction was filtered and concentrated in vacuo. The crude product was purified via column chromatography on silica gel using CH₃Cl: MeOH: NH₄OH (88:10:2, Rₐ = 0.8) as eluent to give N-benzyl-N-(2-methylpyridine) PCU (32) (1.55 g, 75 %). ¹H NMR [CDCl₃, 400 MHz]; δH 1.39 (Jₐₐ = 10.2Hz, 1H), 1.73 (Jₐₐ = 10.2Hz, 1H), 2.01 (t, J = 7.8 Hz, 2H), 2.18-2.58 (m, 8H), 3.61 (s, 2H), 3.72 (s, 2H), 7.10 (t, J = 5.2Hz, 1H), 7.19-7.33 (m, 5H), 7.52 (d, J = 7.7Hz, 1H), 7.60 (t, J = 7.5Hz, 1H), 8.47 (d, J = 4.2Hz, 1H). ¹³C NMR [CDCl₃, 100 MHz]; δC 30.0 (t), 41.6 (d), 43.4 (t), 44.3 (d), 47.8 (d),50.1 (t), 58.4 (t), 58.7 (d), 60.1 (t), 94.8 (s), 121.8 (d), 122.8 (d), 126.9 (d), 128.2 (d), 128.8 (d), 136.4 (d), 139.4 (s), 148.8 (d), 160.4 (s).
A mixture of (1.8 g, 1.7 mmol) N-benzyl-N-\{(pyridin-2-yl)methyl\}amine PCU (32), ammonium formate (0.54 g, 8.5 mmol) and 150 mg of 10 % Pd/C in methanol was refluxed under nitrogen atmosphere for 15 hours [27]. The mixture was cooled to RT, filtered and solution concentrated. The residue obtained was made alkaline with NaHCO₃ and extracted with CHCl₃. The was mixture dried over Na₂SO₄, concentrated in vacuo to afford pure 2 (aminomethyl) pyridine PCU (12) (CHCl₃: MeOH: NH₄OH, 88:10:2, Rᵣ = 0.65, 0.85 g, 67 %). IR νmax: 3314, 2955, 1665, 1590, 1433 and 753 cm⁻¹. HRMS calculated for C₂₇H₃₂N₄O (M + H⁺) 429.2654, found 429.2667. ¹H NMR [CDCl₃, 400 MHz]: δH 1.48 (Jₚ = 10.2Hz, 1H), 1.83 (Jₚ = 10.2Hz, 1H), 2.01 (t, J = 7.28 Hz, 2H), 2.3-2.56 (m, 4H), 2.76 (m, 2H), 3.89 (s, 2H), 7.12 (t, 1H), 7.30 (d, 1H), 7.60 (t, 1H), 8.51 (d, 1H). ¹³C NMR [CDCl₃, 100 MHz]: δC: 32.5 (t), 41.5 (d), 43.5 (t), 44.2 (d), 46.1 (t), 47.9 (d), 55.1 (t), 58.5 (d), 95.4 (s), 122.0 (d), 122.2 (d), 136.5 (d), 149.2 (d), 159.3 (s).

**5.1.9. Synthesis of N-benzyl ethanolamine PCU (13)**

A mixture of benzaldehyde (29, 2.1 g, 20 mmol) and N-ethanolamine (28, 1.2 g, 20 mmol) in methanol (20 mL) was stirred at 25 ºC under N₂ atmosphere for two hours. The mixture was cooled to 0 ºC using an external ice-salt bath after which NaBH₄ (1.5 g, 40 mmol) was added slowly. The mixture was stirred overnight at RT. Excess NaBH₄ was quenched by adding 10 % HCl (20 mL), the mixture was basified with 25 % NH₄OH and the product was extracted from the mixture with dichloromethane (2 x 30 mL). The solvent was dried over Na₂SO₄ and concentrated in vacuo to afford pure 2-(Benzylamino) ethanol (31) (2.2 g, 72 %). ¹H NMR [CDCl₃, 400 MHz]: δH 2.71 (t, 2H), 3.04 (NH), 3.60 (t, 2H), 3.75 (s, 2H), 7.23-7.33 (m, 5H). ¹³C NMR [CDCl₃, 100 MHz]: δC: 50.6 (t), 53.4 (t), 60.6 (t), 127.0 (d), 128.1 (d), 128.3 (d), 139.6 (s).

A mixture of 2-(Benzylamino) ethanol (31, 1.2 g, 7.9 mmol), PCU ditosylate (18, 2 g, 3.6 mmol) and K₂CO₃ (0.745 g, 5.4 mmol) in CH₃CN (50 mL) was refluxed under nitrogen for four days. The reaction was monitored using TLC. After completion the reaction was filtered and concentrated in vacuo. The crude product was purified via column chromatography on silica gel using CH₃Cl: MeOH: NH₄OH (88:10:2, Rᵣ = 0.7) as eluent to give 2-(Benzylamino)ethanol PCU (13, 1.3 g, 70 %). IR νmax: 3371, 2951, 1601, 1452, 1043, 732 and 697 cm⁻¹. HRMS calculated for C₃₃H₄₂N₂O₃ (M + H⁺) 515.3274, found 515.3288. ¹H NMR [CDCl₃, 400 MHz]: δH 1.44 (Jₚ = 10.2Hz, 1H), 1.77 (Jₚ = 10.2Hz, 1H), 1.96 (t, J = 7.36 Hz, 2H), 2.25-2.64 (m, 8H) 3.54 (t, 2H), 3.61 (s, 2H), 7.27-7.28 (m, 5H). ¹³C NMR [CDCl₃, 100 MHz]: δC: 29.3 (t), 41.5 (d), 43.4 (t), 44.1 (d), 47.7 (d), 49.6 (t), 55.0 (t), 58.4 (t), 58.4 (d), 58.7 (t), 95.1 (s), 127.1 (d), 128.3 (s), 129.0 (s).
5.2. Biological testing

5.2.1. Broth Macrodilution Method

All mycobacterial work was carried out in a Level III Biosafety laboratory. All synthesized novel “cage” compounds were evaluated in triplicate against *M. tuberculosis* H37Rv. Each PCU amine compound was dissolved in methanol to prepare a final concentration of 12.8 mg/mL and dilution a 100 fold with 7H9 broth medium to give a stock concentration of 128 µg/mL. EMB was dissolved in DMSO and water similarly to serve as a standard. These were diluted two fold in sterile 30 mL universal tubes containing Middlebrook 7H9 broth supplemented with casitone, glycerol and 10% OADC enrichment to give a concentration range of 128 µg/mL to 0.125 µg/mL. Tubes containing Middlebrook 7H9 broth without compounds served as the compound free controls while Middlebrook 7H9 broth containing solvents alone served as controls to monitor their inhibitory effect.

A standardized inoculum was prepared by vortexing a log phase culture of *M. tuberculosis* H37Rv in a sterile tube containing 4.5 mL phosphate buffer, 0.05 % Tween 80 and 4 to 6 glass beads (5 mm diameter). After allowing the clumps to settle, the supernatant was aspirated and adjusted to a MacFarland number 1 standard, equivalent to 1x10^7 colony forming units (CFU)/mL. This was diluted in 7H9 broth to obtain a concentration of 1x10^5 CFU/mL. Of this, 500 µL was added into each tube of broth containing the diluted test compound concentrations, the compound free controls and the solvent controls. Colony counts of the test inoculum were prepared by plating out 20 µL onto Middlebrook 7H11 agar plates. Plates and tubes were incubated aerobically at 37 °C for twenty one days. Macroscopic readings of the tubes were documented every seven days until twenty one days.

5.2.2. BACTEC 460 TB Analysis

*Mycobacterium tuberculosis* reference strain H37Rv (ATCC 25618) and XDR strain 194 (drug sensitivity: Isoniazid > 0.2 µg/mL; Rifampicin > 1.0 µg/mL; EMB > 2.5 µg/mL; Kanamycin > 5.0 µg/mL; Ofloxacin > 2.0 µg/mL; Amikacin > 5.0 µg/mL) were cultured in Middlebrook 7H9 medium[28], enriched with OADC (0.005 %, v/v, oleic acid; 0.5 %, 171 w/v, BSA; 0.2 %, w/v, glucose; 0.02 %, v/v, catalase and 0.085 %, w/v, NaCl)[24]. Incubation was with continuous stirring at 37 °C. Reproducible growth (< 1.0 % difference) was recorded under standardized conditions. Cultures with an optical density between 0.4 and 0.6 (at 600 nm) were in exponential growth. At an optical density of approximately 0.16, 0.1 mL of culture was inoculated into a BACTEC vial (Becton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37 °C to a growth index (GI) of 500 (+/- 50). This culture was used as the primary culture for drug testing. The growth index is a quantitative determination of radioactive CO₂ on a scale from 0 to 999. Drug compounds were diluted with methanol (filter sterilized through Millex LG syringe driven filters, Millipore). Compound
concentrations in BACTEC vials ranged between 8 µg/mL to 0.03125 µg/mL (final concentration). Growth index results were recorded every 24 hours. Growth rates of the mycobacterial strains were calculated as daily growth index difference (ΔGI) where a ΔGI > 10 was considered positive growth. Purity of *M. tuberculosis* cultures was monitored by plating onto 7H11 mycobacterium agar plates[24].

5.3. Cytotoxicity Analysis

Materials

The RPMI 1640 (with 25 mM HEPES and L-glutamine), penicillin/streptomycin solution, and trypsin-versene mixture were purchased from Lonza and the heat-inactivated foetal bovine serum (FBS) was from Invitrogen. Cytotoxicity was assessed using the Promega CellTiter 96 non-radioactive cell proliferation assay and an EL x 800 automated microplate reader from Bio-Tek Instruments, Inc.

Methods

The toxicities of the compounds were determined on the MDBK (Madin Darby bovine kidney epithelium) cell line (supplied by the Department of Biochemistry, University of Kwa-Zulu Natal, South Africa). The cells were grown in a 37 °C incubator as a monolayer in RPMI 1640 supplemented with 10 % (v/v) heat-inactivated FBS and penicillin/streptomycin at a final concentration of 0.1 mg/mL. Cells were then trypsinised and plated at a density of 5 × 10^4 cells per well into a 96-well plate and incubated for 6 hours at 37 °C. Media was removed from all wells and replaced with fresh media. The test compounds were dissolved in 1 % methanol with the aid of sonication. Dilutions of the test compounds were then prepared using fresh media before addition to the cells. The control untreated cell culture was incubated in fresh media containing an appropriate volume of the 1 % methanol solution. This was included as a 100 % viability internal control. All test samples were done in quintuplicate and repeated twice on different days. Cells were incubated with the test samples for 42 hours at 37 °C. Cell viability was assessed on the basis of the conversion of the tetrazolium salt {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide} (MTT) into purple formazan crystals which can be dissolved using acidified isopropanol and then spectrophotometrically analyzed. The Promega CellTiter 96 non-radioactive cell proliferation assay was performed as per manufacturer’s instructions. Briefly, MTT was added to each well and the plate incubated at 37 °C for 3 hours before the formazan crystals were dissolved and the absorbance read at 570 nm. Percent cytotoxicity was calculated as follows: percent cytotoxicity = 100 − [(OD of sample/OD of control) × 100 %]. All standard deviations were below 10 %.
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Supplementary data

The $^1$H, $^{13}$C NMR and MS spectra are provided as supplementary information.
References


CHAPTER 3

IN-VITRO ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF PENTACYCLOUNDECANE TETRA-AMINES


1 School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
2 Microbiology, National Health Laboratory Services (N HLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa
3 School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa
4 School of Biological and Conservation Sciences, Durban, University of KwaZulu-Natal, South Africa.
5 School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

Abstract

The antifungal and antimicrobial activities of three pentacycloundecane (PCU) tetra-amines derivatives are reported herein. The in vitro activity of these PCU derivatives against yeasts (Candida albicans and non-albicans species) and filamentous fungi was evaluated using the Clinical and Laboratory Standards Institute (CLSI) M27-A2 and M38-A2 guidelines and the 2H-terazolium salt, (MTS) colorimetric method. The MIC against most of the tested clinical fungal strains for GKM8 and GKM9 derivatives range from 15.6-62.5 µg ml⁻¹ while GKM11 ranged from 3.9-7.8 µg ml⁻¹. The GKM11 derivative was also active against fluconazole resistant strains of fungi. The GKM11 derivative also exhibited promising activity against filamentous fungi in that it was 2.5 times more active than amphotericin B against Sporothrix schenckii. Antibacterial activity was determined using the broth microdilution method (BMM) and the iodonitrotetrazolium chloride (INT) colorimetric method. The GKM11 derivative was mainly active against Gram-positive bacteria with MIC ranging from 3.9-7.8 µg ml⁻¹. Activity against Gram-negative bacteria tested was limited to Escherichia coli and Elizabethkingia meningoseptica (MIC of 31 µg ml⁻¹).

Key words: Polycyclic “cage”, pentacycloundecane, antibacterial, antifungal

Introduction

Studies over the past two decades have shown an alarming increase in the number of infections caused by opportunistic fungal pathogens in immuno-compromised/suppressed patients, which are gradually becoming a threat to public health (1-3). These pathogens pose a major setback to effective HIV treatment and management. Candida albicans is a recognised opportunistic pathogen, while...
other Candida species are increasingly emerging as potential pathogens associated with opportunistic infections (4). Without timely intervention, infections by Aspergillus species in immunocompromised/suppressed patients can easily spread from the site of infection to other parts of the body such as the kidneys, liver, spleen, brain and gut (5, 6). A 40% prevalence of invasive aspergillosis with a mortality rate of 78 to 100% is reported in high-risk liver transplant recipients (7, 8).

Presently, a limited drug arsenal that primarily includes polyene- and azole-type compounds are available for treatment of fungal infection. Significant drawbacks are associated with the use of some of these compounds, for example, amphotericin B is reported to be highly nephrotoxic and less effective against Candida species (9, 10). The emergence of drug-resistant fungal strains coupled with the increasing persistence of mycoses in immunocompromised patients creates an urgent need to design new antifungal drugs. Optimally, these drugs should have a good safety profile and different modes of action that promote a broader antimicrobial spectrum of activity to target multiple infections such as fungi, bacteria and/or tuberculosis, especially in the empirical treatment of hospitalised patients with sepsis of unknown origin.

The incorporation of polycyclic ‘cage’ compounds into pharmaceutical drugs has enjoyed much attention from researchers for several decades now (11). Polycyclic “cage” compounds attached to drug groups improves the drug lipophilicity hence enabling it to cross the blood-brain barrier and to enter the central nervous system (12, 13). It can also reduce metabolic degradation of the drug thereby prolonging its pharmaceutical effect in the body (14, 15). Many researchers have reported the discovery of polycyclic based compounds that possess a broader antimicrobial spectrum against viral, bacterial and fungal infections (16-22). SQ109 (N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine) an anti-TB agent (23) was recently reported to inhibit Candida albicans including fluconazole resistant strain at a MIC of 4-8 µg ml⁻¹ (24).

We recently reported the synthesis of fourteen PCU tetra-amine derivatives that were screened against the H₃⁷Rv strain of Mycobacterium tuberculosis (25). The GKM8, GKM9 and GKM11 derivatives were screened further against the extensively drug resistance M. tuberculosis strain, XDR 194, (drug sensitivity: Isoniazid > 0.2 µg ml⁻¹; Rifampicin > 1.0 µg ml⁻¹; EMB > 2.5 µg ml⁻¹; Kanamycin > 5.0 µg ml⁻¹; Ofloxacin > 2.0 µg ml⁻¹; Amikacin > 5.0 µg ml⁻¹) with MICs of 3.04, 1.26 and 1.50 μM. Motivated by this finding, the three most active pentacycloundecane (PCU) tetra-amines derivatives (Figure 1) were selected for further study. In this paper, we describe the in vitro activity of these PCU tetra-amine derivatives against yeast, filamentous fungi and bacteria. This is the first time that PCU-based derivatives are being reported to possess both antifungal and antibacterial activities.
Materials and methods

Antifungal assay

Twenty clinical isolates, ten of yeasts and ten of filamentous fungi (moulds) were used in this study (Table 1) while two ATCC reference strains [(Candida parapsilosis (ATCC 22019) and Candida albicans (ATCC 90028)] served as controls as recommended by Clinical and Laboratory Standards Institute, CLSI (formerly the National Committee for Clinical Laboratory Standards). The clinical isolates were obtained from the National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Hospital, Durban, South Africa. The isolates were recovered from the respiratory tracts, tissue samples and blood specimens of patients managed by the Hospital. Yeast isolates were identified at the species level using carbohydrate assimilation tests (API 32 ID, bioMérieux, Marcy l’Etoile, France) while the filamentous fungi were identified by macroscopic and microscopic morphological features.

Antifungal/antibacterial agents: PCU tetra-amine derivatives (GKM8, GKM9 and GKM11) were synthesized as described previously (25). The hydrochloride salt of each compound was dissolved in 10 % methanol and diluted with sterile double distilled water. Amphotericin B (Sigma-Aldrich) was used as reference drug. Freshly prepared solutions for all PCU derivatives and reference drugs were used in this study. The final concentrations of the antimicrobial agents ranged from 0.0012 to 80 µg ml⁻¹ amphotericin B, 0.0015 to 100 µg ml⁻¹ neomycin and 0.0076 to 500 µg ml⁻¹ of GKM 8, 9 or 11. All drug dilutions were carried out in a 96-well flat-bottom microtitre plates; each well contained 100 µL of twofold serial dilutions of the drugs tested (2 x final concentrations) and the plates were stored at -70 ºC until the day of testing.

Figure 1: Free salts of PCU tetra-amine derivatives: GKM8, GKM9 and GKM11 (25)
Antifungal susceptibility tests: All microbial work was carried out in a Level II Bio-safety cabinet while following Good Laboratory Practice (GLP) procedures. Evaluation of the susceptibility of *Candida albicans*, non-*albicans* species and filamentous fungi was performed using the broth microdilution method according to CLSI M38-A2 (for filamentous fungi) and M27-A2 (for yeast) guidelines (26, 27). Yeast strains were grown aerobically overnight at 35 °C on Sabouraud dextrose agar (Merck) plates. Yeasts were harvested and suspended in 1 % sterile saline and the turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08-0.1 equivalent to the No. 0.5 McFarland standard following the NCCLS M27-A2 guideline (26). The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium (with L-glutamine, without bicarbonate, Cambrex Bio Science Verviers, Belgium) and 0.165 M morpholinepropanesulmonic acid (MOPS, Sigma-Aldrich) buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculum (1-5 x 10³ CFU ml⁻¹). The microtitre plates were allowed to thaw and equilibrate to room temperature under aseptic conditions after which 100 µl aliquots of the working inoculum suspension were dispensed into each well and the plates incubated in an aerobic environment at 35 °C for 24 h. Growth was observed visually with the aid of a concave mirror; MICs were taken on a growth or no-growth (100% visible-growth inhibition) scale.

MTS method: The MICs were also determined by colorimetric method using the dye, 2H-terazolium salt (MTS, Promega Corporation, Madison, USA). After MICs were determined visually on each micotitre plates, 20 µl of 2H-terazolium salt (28) was added directly to each well, incubated at 37 °C for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (Biotek, Powerwave XS2). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

The activity of the PCU tetra-amine derivatives against filamentous fungi was also determined using the broth microdilution method according to the CLSI M38-A2 guideline (27). Cultures were grown on Potato dextrose agar at 35 °C until sporulation (48 h to 7 days). Spores were harvested and suspended in 1 % sterile saline, allowed to settle and the upper layer aspirated. The turbidity was measured spectrophotometrically at 625 nm and optical density was adjusted to yield a stock suspension of 0.4-5 x 10⁶ sporangiospores per millilitre. A working suspension was prepared by diluting 1:50 of the conidia stock suspension in a standard medium (RPMI 1640, MOPS). The fungal inocula (100 µl) were added to each well of 96-well flat-bottom microdilution plates containing 100 µl of drug dilution and incubated for 48 h in an aerobic incubator at 35 °C. After incubation, potential antimicrobial activity (MICs) was assessed as described previously.
**Antibacterial susceptibility assay:** All microbial work was carried out in a Level II Bio-safety cabinet while following Good Laboratory Practice (GLP) procedures. Bacterial susceptibility was determined using the broth microdilution method (29, 30). Eighteen ATCC bacterial cultures (identified in Table 2) obtained from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville, South Africa were freshly cultured in Mueller-Hinton broth. The overnight cultures after 16-18 h of incubation at 37 ºC were adjusted to a turbidity of No. 0.5 McFarland standard. At a wavelength of 625 nm, the absorbance was adjusted between 0.08-0.1 to yield a stock suspension of 0.4-5 x 10⁸ CFU ml⁻¹, which was diluted one hundred fold to obtain a working suspension of 10⁶ CFU ml⁻¹.

The microtitre plates were placed in the laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots (100 µl) of bacterial inocula were added to prepared drug samples and incubated aerobically for 16-18 h at 37 ºC. Following incubation, 50 µL of freshly prepared iodonitrotetrazolium chloride (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride, INT, 200 µg ml⁻¹) solution was added to each well and the plate was further incubated for 45 minutes at 37 ºC in the dark. When the colourless INT is reduced to red after incubation, persistent growth of the bacteria is indicated, while no colour change denotes the lack of bacterial growth. Neomycin was used as a control drug in this study. All analyses were performed in triplicates.

**Results and discussion**

**Antifungal activity**

Ten clinical strains of yeast and ten clinical strains of filamentous fungi were employed in evaluating the antifungal potency of the novel PCU tetra-amine derivatives. MICs values obtained from the CLSI and dye (terazolium salt) methods were in agreement (31, 32). The control drug, amphotericin B presented a MIC ranging from 0.02-1.25 µg ml⁻¹ (Table 1). The GKM8 and GKM9 derivatives displayed MIC in the concentration range of 15.6-62.5 µg ml⁻¹ against some of the fungal strains while GKM11 was found to possess a MIC in a concentration range of 1.96-62.5 µg ml⁻¹ against most of the tested fungal strains. The GKM11 derivative showed MIC in a range of 3.9-7.8 µg ml⁻¹ against all Candida species and Trichosporon asahii. Of the three derivatives employed in this study, GKM11 was the most potent against majority of fungal strains. Against filamentous fungi strains, GKM11 displays a MIC that is 1.3 and 2.5 times more active than amphotericin B against Scopularopsis spp. and Sporothrix schenckii. Two Aspergillus species namely A. fumigatus and A. flavus were used in this study. Interestingly, none of the test compounds were active against A. flavus (two strains). However, GKM8 and GKM9 both recorded a MIC of 31-62.5 µg ml⁻¹ while GKM11 possessed a MIC of 3.9-7.8 µg ml⁻¹ against the three A. fumigatus strains examined. Cytotoxicity analysis (IC₅₀) of GKM8, GKM9 and GKM11 were carried using mammalian cell line [MDBK
(Madin Darby bovine kidney epithelium)] with IC$_{50}$ values of 30, 24 and 25 µM obtained respectively (25). It is observed that GKM8 and GKM9 only show significant activity as an antifungal agent at MIC: 15.6 µg ml$^{-1}$ (19.4 and 16.6 µM) beyond which it becomes toxic (IC$_{50}$: 30 and 24 µM). GKM11 on the other hand shows promising antifungal activity at MIC of 1.96-7.8 µg ml$^{-1}$ (2.4-9.6 µM) against most fungal strains used. These values fall below the concentration at which it is toxic to healthy cells (IC$_{50}$: 25 µM).

Table 1: Antifungal activity of PCU tetra-amine derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg ml$^{-1}$)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GKM8</td>
<td>GKM9</td>
<td>GKM11</td>
<td>Ampho. B</td>
</tr>
<tr>
<td>Candida utilis</td>
<td>31</td>
<td>15.6</td>
<td>7.8</td>
<td>0.31</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>15.6</td>
<td>15.6</td>
<td>3.9</td>
<td>1.25</td>
</tr>
<tr>
<td>Candida tropicalis (2)</td>
<td>15.6-31</td>
<td>15.6-31</td>
<td>3.9-7.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Candida parapsilosis (2)</td>
<td>15.6-31</td>
<td>15.6-62.5</td>
<td>3.9</td>
<td>0.04-1.25</td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td>62.5</td>
<td>500</td>
<td>7.8</td>
<td>0.31</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>15.6</td>
<td>15.6</td>
<td>3.9</td>
<td>0.62</td>
</tr>
<tr>
<td>Candida krusei*</td>
<td>31</td>
<td>31</td>
<td>7.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Candida parapsilosis*</td>
<td>31</td>
<td>31</td>
<td>7.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Aspergillus fumigatus  (3)</td>
<td>31-62.5</td>
<td>31-62.5</td>
<td>3.9-7.8</td>
<td>0.63-1.25</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>125</td>
<td>500</td>
<td>62.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Aspergillus flavus (2)</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>1.25</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>15.6</td>
<td>15.6</td>
<td>1.96</td>
<td>5</td>
</tr>
<tr>
<td>Scopularopsis spp.</td>
<td>31</td>
<td>31</td>
<td>7.8</td>
<td>10</td>
</tr>
<tr>
<td>Rhizomucor spp.</td>
<td>31</td>
<td>250</td>
<td>62.5</td>
<td>0.32</td>
</tr>
<tr>
<td>Pencillium spp.</td>
<td>62.5</td>
<td>31</td>
<td>15.6</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Values in brackets indicate number of isolates that were screened if more than one. * Fluconazole resistant strains; Ampho. B; Amphotericin B. The data are reported as the mean ± standard error of the mean of ≤ 5.

**Antibacterial activity**

A total of 18 ATCC bacterial strains (nine Gram-positive and nine Gram-negative) were used in this study. Commercially available neomycin was active against all Gram-positive and Gram-negative bacterial strains used in this study. The tested compounds (GKM8, GKM9 and GKM11) showed no activity against the following Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 35032 and ATCC 27853), *Proteus mirabilis* (ATCC 29906), *Proteus vulgaris* (ATCC 13315), *Klebsiella pneumoniae* (ATCC 700603), *Klebsiella oxytoca* (ATCC 13812) and *Serratia rubidaea* (ATCC
However, activity was recorded against *Escherichia coli* (ATCC 35218) and *Elizabethkingia meningoseptica* (ATCC 13253) where a MIC ranging between 125-250 µg ml\(^{-1}\) was recorded for GKM8 and GKM9 while that of GKM11 was 31 µg ml\(^{-1}\) (38.2µM) against both strains (Table 2).

Interestingly, the three PCU tetra-amine derivatives showed promising activity against most of the Gram-positive bacteria except *Staphylococcus saprophyticus* (ATCC 35552), *Enterococcus faecium* (ATCC 19434) and *Streptococcus aerogenes* (ATCC 13048). GKM8 and GKM9 displayed a MIC of 15.6-62.5 µg ml\(^{-1}\) whilst the MIC activity of GKM11 ranged from 3.9-7.8 µg ml\(^{-1}\) (4.8-9.6 µM) against *Staphylococcus aureus* (ATCC 43300), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus xylosus* (ATCC 35033), *Bacillus subtilis* (ATCC 6051), *Rhodococcus equi* (ATCC 6939) and *Streptococcus agalactiae* (ATCC 13813). Importantly, GKM9 and GKM11 were 1.6 and 6.4 times more active than the control drug (neomycin) against *Staphylococcus aureus* (ATCC 43300) only (Table 2).

Table 2: Antibacterial activity of PCU tetra-amine derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GKM8</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>250</td>
</tr>
<tr>
<td><em>Elizabethkingia meningoseptica</em></td>
<td>250</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>31</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>62.5</td>
</tr>
<tr>
<td><em>Staphylococcus xylosus</em></td>
<td>62.5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>31</td>
</tr>
<tr>
<td><em>Rhodococcus equi</em></td>
<td>31</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>31</td>
</tr>
</tbody>
</table>

In conclusion, the PCU tetra-amine derivatives displayed antimicrobial activities against yeast, filamentous fungi and bacteria. GKM8 and GKM9 were least active with an MIC of 15.6-125 µg ml\(^{-1}\) against some of the tested fungal strains. GKM11 [MIC: 1.95-7.8 µg ml\(^{-1}\) (2.4-9.6 µM)] proved to be most potent against 17 clinical strains of fungi *i.e.* isolates of *C. albicans*, *C. krusei*, *C. tropicalis*, *Trichosporon asahii*, *A. fumigatus*, *Sporothrix schenckii*, *Scopularopsis* spp. with the exception of two *A. flavus* strains and *Rhizomucor* spp.; Antibacterial analysis evaluation shows that GKM8 and GKM9 possess a MIC of 15.6-62.5 µg ml\(^{-1}\) against most Gram-positive bacterial strains while GKM11 possess a MIC 3.9-125 µg ml\(^{-1}\). The GKM8 and GKM9 (a ten and fifteen carbon alkene moiety) derivatives proved to be less active compared to GKM11 (a ten carbon alkane moiety) against a variety of yeasts, filamentous fungi and bacteria species, suggesting that the saturated
hydrocarbon alkane moiety plays a key chemical function. It would be logical to screen the fifteen carbon alkane derivative of GKM11 for its antifungal/bacterial activities however this was not feasible due to its insolubility at biological pH (25). The three derivatives, however, did not show promising activity against Gram-negative bacteria. The GKM11 derivative thus possesses moderate antifungal activity and antibacterial activity that is seemingly restricted to Gram-positive strains. The low anti-fungal and bacterial activities possessed by GKM11 against some fungi and bacteria at MIC range of 1.95-7.8µg ml\(^{-1}\) (2.4-9.6 µM) which is below its IC\(_{50}\) value (25 µM) suggests that GKM11 could be an potential therapeutic candidate against a broad range of infections.

**Acknowledgements**

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References


(14) Brookes, K. B., Hickmott, P. W., Jutle, K. K., and Schreyer, C. A. (1992) Introduction of pharmacophoric groups into polycyclic systems .4. aziridine, oxiran, and tertiary beta-


CHAPTER 4
SYNTHESIS AND EVALUATION OF SQ109 ANALOGUES AS POTENTIAL ANTI-TUBERCULOSIS CANDIDATES

Oluseye K. Onajole, a Patrick Govender, b Paul D. van Helden, c Hendrik G. Kruger, a* Glenn E. M. Maguire, a Ian Wiid, c and Thavendran Govender, d*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa
c Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.
d School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa.

Abstract

As part of an ongoing project to develop highly potent anti-tuberculosis therapeutics, six SQ109 derivatives were synthesized and screened in vitro for their anti-tuberculosis activity against the ATCC strain H₃₇Rv and the extensively drug-resistant clinical strain XDR 173. Compound 16 with an extended alkene chain was the most active against both strains of Mycobacterium tuberculosis within a MIC range of 0.5 to 0.25 µM. Compound 12 and SQ109 were potent within a MIC range of 1 to 0.5 µM, whilst compound 18 displayed an activity within the MIC range of 0.5 – 2 µM against both Mycobacterium tuberculosis strains.

Keywords: anti-tuberculosis (TB), SQ109, 1,2 cage diamine, XDR

1.1 Introduction

Despite thousands of years of living with the disease, tuberculosis (TB) remains one of the most deadly diseases known to man [1,2]. Recently, there has been an alarming increase in the number of TB patients all over the world and an estimated eight million people developed active TB in 2004 with two million dying as a result [1]. The recent emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis resulted in a major setback in the global fight against TB. The prevailing situation is made worse by the continuous increase in the number of immune-
compromised patients living with HIV who are more prone to TB and other bacterial infections [1,3,4].

The urgent need for highly potent and effective drugs to combat the increasing TB pandemic cannot be overemphasized. Presently, SQ109 (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine) which is a second generation agent developed from the first line drug ethambutol is one of the most promising anti-TB drug candidates at the clinical trials stage [5,6]. SQ109 displays high potency against the H37Rv reference strain and MDR strains of *Mycobacterium tuberculosis* [7,8]. SQ109 is relatively potent against *M. tuberculosis, M. bovis, M. marinum,* and less active against *M. avium* and *M. smegmatis* [9]. SQ109 also shows promising activity against *Candida albicans* (4-8 µg/ml) [9]. SQ109 in combination with other first line drugs such as isoniazid and rifampicin have been reported to be synergistic and an additive effect was observed when used with streptomycin [10].

We recently reported the anti-TB activities of three PCU tetra-amine derivatives (Figure 1, compounds 1-3) [11]. Against both the H37Rv and XDR 194 (drug sensitivity: Isoniazid > 0.2 µg/mL; Rifampicin > 1.0 µg/mL; EMB > 2.5 µg/mL; Kanamycin > 5.0 µg/mL; Ofloxacin > 2.0 µg/mL; Amikacin > 5.0 µg/mL) strains of *M. tuberculosis,* it was discovered that the longer alkene side chain (15 carbons) cyclic tetra-amine of compound 2 rendered it two-fold more active than compound 1 (10 carbons in the side chains). Compound 3 has aliphatic side chains (10 carbons) and exhibits similar activity as compound 2 against the XDR 194 *M. tuberculosis* strain [11]. Encouraged by these observations, a series of novel SQ109 derivatives were synthesized and investigated for their anti-TB activities. In particular we want to examine the importance of chain length and saturation of these SQ109 derivatives.
2.1 Chemistry

The starting material geraniol 4 (Scheme 1) was converted to geranyl bromide 5 in 95-96 % yield by treatment with phosphorus tribromide. Compound 5 was reacted with phthalimide in the presence of K$_2$CO$_3$ to obtain the corresponding compound 6 (60 % yield), which was converted with hydrazine hydrate to geranylamine 7 with a 34% yield. The free salt of amantadine was reacted with chloroacetyl chloride to obtain compound 9 in 94-96 % yield (Scheme 1). Reaction of 9 with geranylamine 7 resulted in compound 10 in a 40 % yield. The latter compound was subjected to reduction using LiAlH$_4$ to obtain the 1, 2 diamine intermediate, which was converted to the hydrochloride salt 11 in 71 % yield. Compound 11 was subjected to catalytic hydrogenation using 10% Pd/C to obtain derivative 12 in 52 % yield.
Scheme 1: Reagents and conditions: (a) PBr₃, THF, rt, 30 minutes; (b) phthalimide, K₂CO₃, DMF, rt, overnight; (c) MeOH, H₂NNH₂.H₂O, rt, overnight; (d) NH₃/CHCl₃, chloroacetyl chloride, CHCl₃, 2 h; (e) geranylamine (7), Et₃N, DCM, overnight, rt; (f) LiAlH₄, THF, N₂, reflux for16 h; then HCl, MeOH; (g) 10 % Pd/C, NH₄HCO₂, MeOH, reflux, 16 h, N₂, then HCl, MeOH.

A different synthetic route from that reported in literature [7] was employed in the synthesis of SQ109 and its longer branched alkene chain derivative 16 (see Scheme 2). Geranyl bromide 5 and trans-trans farnesyl bromide 13 were reacted with excess ethylene diamine in dry dichloromethane at – 78 ºC to afford the 1,2 diamines 14 and 15 respectively (86 %) and these were reacted with 2-adamantanone via reductive amination to obtain the desired 2-adamantyldiamines; the diamines were treated with hydrochloride to obtain SQ109 and 16 (57 % and 47 % yield). SQ109 and 16 were subjected to catalytic hydrogenation using 10 % Pd/C, the obtained products were converted to its HCl salts to obtain compounds 17 and 18 with a 57 % and 54 % yield (Scheme 2).
The successful synthesis of compounds SQ109, 11, 12, 16, 17 and 18 and were confirmed using $^1$H, $^{13}$C and HR-MS. All compounds were confirmed to be greater than 95% pure via LC-MS prior to their use in the evaluation of their biological efficacies. All of these compounds were readily soluble in aqueous media in the HCl salt form at a stock concentration of 100 µM.

### 3.1 Results and Discussion

Employing the BACTEC 460 TB system, all compounds underwent an initial in vitro screening to determine their activities against the *M. tuberculosis* H₃₇Rv strain [12]. As expected SQ109 proved to be most potent with a MIC of 0.5-1 µM while its reduced derivative 17 was not as active with an MIC range of 2 > MIC > 1 µM, suggesting that the alkene moiety is essential for anti-TB activity. The MIC values obtained for compounds 11 and 12 (reduced form of 11) seemingly contradicts this statement as 12 (MIC: 0.5 - 1 µM) proved to be more active than its precursor 11 (10 > MIC > 1 µM). Compound 11 (1-adamantyl moiety) did not show much improvement over the 2-adamantyl moiety (SQ109), whilst compound 12 (reduced derivative of 11) possess similar activity as SQ109.

Compound 16 showed the highest activity with a MIC value of 0.25 µM whereas its reduced analogue 18 showed an activity of 0.5 µM against the H₃₇Rv strain. The most active compounds were selected for further analysis against an extensively drug-resistant (XDR 173 clinical isolate) strain of tuberculosis [resistant to the following drugs at breakpoint concentrations on 7H11 agar plates: first line drugs, Rifampicin (1 µg/mL), Ethambutol (7.5 µg/mL), Isoniazid (0.2 µg/mL). Second line
drugs, Streptomycin (2 µg/mL), Kapriomycin (10 µg/mL), Amikacin (5 µg/mL), Kanamycin (5 µg/mL)]

Table 1: The MICs (µM) of the compounds against bacteria and *M. tuberculosis* (H37Rv and XDR 173 strains)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H37Rv</td>
</tr>
<tr>
<td>11</td>
<td>![Structure Image]</td>
<td>10&gt;MIC&gt;1</td>
</tr>
<tr>
<td>12</td>
<td>![Structure Image]</td>
<td>1&gt;MIC&gt;0.5</td>
</tr>
<tr>
<td>16</td>
<td>![Structure Image]</td>
<td>0.5&gt;MIC&gt;0.25</td>
</tr>
<tr>
<td>17</td>
<td>![Structure Image]</td>
<td>2&gt;MIC&gt;1</td>
</tr>
<tr>
<td>18</td>
<td>![Structure Image]</td>
<td>1&gt;MIC&gt;0.5</td>
</tr>
</tbody>
</table>
Nd; Not determined;

In tests against the XDR 173 strain, compound 16 was the most active (MIC-0.25 µM) and was twice as active as SQ109. Compound 12 which displayed similar activities (MIC-0.5 µM) and was four-fold more active than compound 18.

Our previous studies [11] showed that derivatives of the fifteen carbon alkene chain possessed higher anti-TB activity as compared with the ten carbon alkene analogue. This trend was also observed in this study.

4.1 Conclusion

Novel SQ109 analogues with potent anti TB activities were identified with compound 16 being the most potent against H37Rv and XDR strains of TB. Further application of compound 18 (ClogP†; 9.05 ± 0.39) may be hindered due to a solubility problem experienced in biological conditions however compound 16 (ClogP; 8.08 ± 0.48) did not possess such problem. These compounds are presently undergoing further in-vitro and in-vivo studies against pathogenic and non-pathogenic strains of bacteria and fungi.

5.1 Experimental

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using CDCl3 as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and the coupling constants (J) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. All reactions were monitored using Thin Layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH3Cl:CH3OH:NH4OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds was judged to be >95% based upon 1H NMR and LC-MS analysis. Mass Spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled before use from a sodium benzophenone under N2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use. The syntheses of the precursors are described in their corresponding references. Clog P

† ClogP was calculated using ACD/labs software v11.0
gives an indication of the lipophilicity of the drug with reference to its pharmacological importance (pharmacokinetics and pharmacodynamics). The values were calculated using ACD/Labs LogP software v11.0.

**Synthesis of compound 11 (N-Geranyl-N’-(1-adamantyl)ethane-1,2-diamine)**

To a stirring solution of 10 (0.82 g, 2.4 mmol) in dry THF (15 mL) was added lithium aluminium hydride LAH (0.27 g, 7.2 mmol) and refluxed under nitrogen, reaction was monitored via TLC until no starting material is present. The solution was cooled and THF (10 mL) added after which saturated aqueous Na₂SO₄ was added dropwise to quench excess LAH. The solution was filtered, dried over anhydrous Na₂SO₄ and the solvent was removed in vacuo to afford a residue which was purified via column chromatography on silica gel using CHCl₃:CH₃OH:NH₄OH (88:10:2) as eluent to give a oil (71%) and converted to its HCl salt. A yellowish solid (HCl salt); 71 % yield; Melting point: 135-138 ºC, HR-MS calculated for C₂₂H₃₉N₂ ([M+H]⁺ of free base) 331.3108, found 331.3104; ¹H NMR (CDCl₃, 600 MHz): δH 1.51 (s, 5H), 1.55-1.56 (m, 11H), 1.59 (s, 4H), 1.90 – 2.01 (m, 8H), 2.33-2.35 (m, 3H), 2.63 (s, 4H), 3.14 (d, J = 6.72 Hz, 2H), 5.01 (m, 1H), 5.17 (t, J = 6.6 Hz, 1H). ¹³C NMR (CDCl₃, 150 MHz); δC 16.1, 17.5, 25.5, 26.3, 29.4, 36.5, 39.5, 39.6, 42.4, 46.8, 49.4, 122.5, 124.0, 131.3, 137.7

**Synthesis of SQ109 (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine) and compound 16 (N-trans-trans farnesyl -N’-(2-adamantyl)ethane-1,2-diamine)**

A mixture of N - isoprenyl ethylene diamine (15 or 16, 4.0 mmol) and 2-adamantanone (0.72 g, 4.8 mmol) in methanol (15 mL) was stirred for 2 h at room temperature under nitrogen atmosphere. The resulting imine was reduced with solid NaBH₄ (0.27 g, 7.2 mmol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench excess NaBH₄. The solution was extracted with ethyl acetate (2 x 50 mL) and the solution dried over Na₂SO₄ and concentration in vacuo. The crude product was purified via column chromatography on silica gel using CHCl₃:CH₃OH:NH₄OH (88:10:2) as eluent to give a yellow oil and converted to its HCl salt.

**Data for SQ109 (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine)**

A white solid; 57% yield, Melting point: 180-184 ºC, HR-MS calculated for C₂₂H₃₉N₂ ([M+H]⁺ of free base) 331.3108, found 331.3135; ¹H NMR (CDCl₃, 600 MHz): δH 1.39 – 1.41 (m, 2H), 1.50 (s, 3H), 1.55 (s, 3H), 1.57 – 1.61 (m, 10H), 1.74 -1.78 (m, 5H), 1.84 – 1.93 (m, 5H), 1.96 – 2.00 (m, 2H), 2.63 (s, 1H), 2.68 (s, 4H), 3.20 (2H, d, J = 6.06 Hz), 4.99 (m, 1H), 5.18 (m, 1H). ¹³C NMR (CDCl₃, 150 MHz); δC 16.1, 17.5, 25.5, 26.3, 27.4, 27.6, 31.1, 31.8, 37.4, 37.7, 39.5, 45.6, 46.3, 48.3, 61.7, 121.4, 123.9, 131.3, 138.6.
Compound 16 (N-trans-trans farnesyl-N’-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 47 % yield, Melting point: 146–150 ºC, HR-MS calculated for C_{27}H_{47}N_{2} ([M+H]^+ of free base) 399.3734, found 399.3740; 1H NMR (CDCl₃, 600 MHz): δH 1.45 – 1.47 (m, 3H), 1.57 (s, 6H), 1.62 (s, 3H), 1.65 (s, 3H), 1.68 (s, 4H), 1.80 – 1.82 (m, 5H), 1.92 – 2.08 (m, 10H), 2.67 (s, 1H), 2.70 (s, 4H), 3.22 (d, J = 6.72), 5.05 – 5.10 (m, 2H), 5.25 (t, J = 6.5, 1H); 13C NMR (CDCl₃, 150 MHz); δC 16.0, 16.3, 17.7, 25.7, 26.4, 26.7, 27.6, 27.8, 31.3, 32.2, 37.6, 38.0, 39.6, 39.7, 46.6, 47.1, 49.6, 61.9, 123.0, 124.0, 124.3, 135.1, 137.6.

Synthesis of Compound 12, 17 and 18

A mixture of compound 11, SQ109 and 16 (2.4mmol), ammonium formate (0.76 g, 12 mmol) and 350 mg of 10% Pd/C in dry methanol (20 mL) was refluxed under a nitrogen atmosphere for 16 hours. The mixture was cooled to room temperature, filtered through a sintered glass and concentrated in vacuo. The residue obtained was made slightly alkaline with NaHCO₃ and extracted with chloroform (2 x 50 mL). The solution was dried over anhydrous Na₂SO₄ and concentrated in vacuo, purified via column chromatography on silica gel using CHCl₃:CH₃OH:NH₄OH (88:10:2) as eluent to give the product as a oil and converted to its HCl salt.

Compound 12 (N-(3,7-dimethyloctyl)-N’-(1-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 52 % yield, Melting point: 210 – 214 ºC, HR-MS calculated for C_{22}H_{43}N_{2} ([M+H]^+ of free base) 335.3421, found 335.3418; 1H NMR (CDCl₃, 400 MHz): δH 0.74 – 0.77 (m, 9H), 1.03 (m, 2H), 1.11 – 1.21 (m, 4H), 1.38 – 1.41 (m, 2H), 1.48 – 1.57 (m, 14H), 1.96 (s, 3H), 2.50 (s, 2H), 2.63 (s, 4H). 13C NMR (CDCl₃, 100 MHz); δC 19.7, 22.5, 22.6, 24.6, 27.9, 29.5, 30.9, 36.6, 37.3, 39.2, 39.7, 42.5, 47.7, 50.1, 50.7.

Compound 17 (N-(3,7-dimethyloctyl)-N’-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 57 % yield, Melting point: 182-185 ºC, HR-MS calculated for C_{22}H_{43}N_{2} ([M+H]^+ of free base) 335.3421, found 335.3415; 1H NMR (CDCl₃, 400 MHz): δH 0.79 – 0.83 (m, 12H), 1.00 – 1.11 (m, 5H), 1.14 – 1.33 (m, 8H), 1.42 1.50 (m, 5H), 1.66 (br s, 6H), 1.78 – 1.80 (m, 5H), 1.90 – 1.93 (m, 2H), 2.54 – 2.60 (m, 2H), 2.66 (s, 1H), 2.68 (s, 4H). 13C NMR (CDCl₃, 100 MHz); δC 19.6, 22.5, 22.6, 24.6, 27.5, 27.7, 27.9, 30.9, 31.2, 32.1, 37.2, 37.3, 37.5, 37.9, 39.2, 46.3, 47.7, 50.0, 61.8.

Compound 18 (N-(3,7,11-trimethylidodecyl)-N’-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 54 % yield, Melting point: 114-118 ºC, HR-MS calculated for C_{27}H_{53}N_{2} ([M+H]^+ of free base) 405.4203, found 405.4186; 1H NMR (CDCl₃, 400 MHz): δH 0.79 – 0.83 (m, 12H), 1.00 – 1.11 (m, 5H), 1.14 – 1.33 (m, 8H), 1.42 1.50 (m, 5H), 1.66 (br s, 6H), 1.78 – 1.80 (m, 5H), 1.90 – 1.93 (m, 2H), 2.54 – 2.60 (m, 2H), 2.66 (s, 1H), 2.68 (s, 4H). 13C NMR (CDCl₃, 100 MHz); δC 19.6, 22.5, 22.6, 24.6, 27.5, 27.7, 27.9, 30.9, 31.2, 32.1, 37.2, 37.3, 37.5, 37.9, 39.2, 46.3, 47.7, 50.0, 61.8.
\( \delta_C \) 19.8, 22.6, 22.7, 24.4, 24.8, 27.6, 27.8, 27.9, 31.0, 31.3, 32.2, 32.8, 37.3, 37.4, 27.5, 37.6, 38.0, 39.3, 46.5, 47.9, 50.1, 61.9.

Acknowledgements

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References

3. Tuberculosis (TB) WHO Extensively drug-resistant tuberculosis, 
9. Tuberculosis (Edinb) **2008**, *88*, 159-61.
    **2006**, *58*, 332-337.
    4297 - 4305.
    Dickinson Microbiology System, Sparks, Md, **1995**.
CHAPTER 5

NMR ELUCIDATION OF NOVEL SQ109 DERIVATIVES

Oluseye K. Onajole, a Patrick Govender, b Thavendran Govender, c Glenn E. M. Maguire, a and Hendrik G. Kruger a*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa
c School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa

Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091

Abstract

The NMR elucidation of five novel SQ109 analogues including SQ109 is reported herein. These compounds were synthesized as potential anti-tuberculosis candidates. One dimensional NMR (1H and 13C) techniques show a series of overlapping signals from the methine and methylene groups of these compounds, thereby making it extremely difficult to assign all NMR signals. Two dimensional (2D) NMR techniques were instrumental in overcoming these difficulties. This paper appears to be a rare report on the complete structure elucidation of mono-substituted adamantane moieties.

Introduction

Polycyclic “cage” chemistry has been attracting the interest of medicinal and organic chemists since the early 1960s. These compounds include pentacycloundecane, trishomocubane, adamantane and basketane. Adamantane is the most studied which has led to the discovery of numerous pharmaceutical candidates [1-6]. The polycyclic “cage” has been reported to improve the pharmacokinetic and pharmacological properties of known drug candidates [6-10]. However, its pharmaceutical importance only drew increased attention after the discovery of 1-amino adamantane or amantadine. This molecule possesses anti-viral activity against several species including hepatitis C[11] and the influenza virus[12,13]. Memantine is another important amino-adamantane used in the clinical treatment of Alzheimer’s disease (Figure 1) [14-16]. One of such discoveries is SQ109 (1) (N-geranyl-N’-(2-adamantyl)ethane-1,2 diamine) first reported by Lee et al.[17] SQ109 is an ethambutol cage derivative and possesses excellent activity against MDR (multi drug-resistant) strains of tuberculosis [18]. It also exhibits a synergistic effect when used in combination with rifampicin and isonazid while an additive effect is observed when combined with streptomycin [19]. SQ109 is currently in clinical trials.
The use of 1D NMR techniques and computational studies has been utilized in the elucidation of the adamantane skeleton,[20-24] however 2D NMR studies of mono-substituted adamantane derivatives has not enjoyed much attention. NMR and computational study of “cage” molecules such as pentacycloundecane and trishomocubane based compounds has been a major focus of our group for the past five years [25-36]. We recently reported the NMR elucidation of pentacycloundecane based tetra-amines[37,38] which were screened for their anti-tuberculosis activities [39]. Herein we report the full NMR studies of SQ109 and analogues (2-6) (Figure 2) recently synthesized and reported as part of an ongoing project aimed at developing novel molecules with potency against tuberculosis including XDR (extensively drug-resistant) strains of tuberculosis [40].
Figure 2: Structure of compounds 1-6

The numbering of the structures was chosen to differ from the IUPAC rules to assist with comparison of NMR data of the same atoms in different compounds. The starting material for SQ109 and compound 5 is 2-adamantanone while amantadine hydrochloride was the starting material for compound 3. The syntheses of these compounds were reported in literature [40].

**Results and discussion**

We chose the ten carbon alkene chain as the starting point for the NMR elucidation of SQ109. Two alkene proton signals are observed at $\delta = 4.99$ and 5.18 ppm and represents H-4’ or H-8’. H-4’ is assigned to the more deshielded peak at 5.18 ppm. A methylene proton (doublet, $J = 6.06$ Hz) at $\delta = 3.20$ ppm shows a COSY correlation to H-4’ (5.18 ppm) and was assigned to H-3’. The remaining alkene proton (H-8’ at 4.99 ppm) displays a COSY correlation to H-7’ at 2.00 ppm. H-7’ exhibits a HMBC correlation to C-8’ ($\delta = 123.9$ ppm), two quarternary carbons ($\delta = 131.3$ and 138.6 ppm) which were assigned to C-5’ or C-9’ and a methylene carbon at $\delta = 39.5$ ppm which was assigned to
C-6’. The corresponding protons of the assigned carbon signals were determined using HSQC spectrum. H-6’ (δ = 1.94 ppm) displays a HMBC correlation to a quartenary carbon at δ = 138.6 ppm thus confirming its assignment to C-5’. HMBC correlation of H-6’ is also observed with C-4’ and C-8’ respectively. C-4’ and C-5’ both show HMBC correlation to a methyl proton (δ = 1.55 ppm) which was assigned to H-12’ while C-8’ and C-9’ also display HMBC correlations to two methyl protons (δ = 1.50 and 1.58 ppm), the former was assigned to H-10’ while the latter was assigned to H-11’ respectively, based on literature [41]. The H-11’ methyl proton displays ROESY interaction with H-8’ while H-10’ displays ROESY interaction with H-7’, confirming this assignment. Also observed on the ROESY spectrum, is the interaction of H-12’ with H-3’, H-6’ and H-7’ respectively. Two methylene protons appearing as a singlet at δ = 2.68 ppm were assigned to H-1’ and H-2’. These two methylene carbons (δ = 45.6 and 48.3 ppm) were confirmed to correspond to the proton signal at δ = 2.68 ppm using the HSQC spectrum. H-3’ (δ = 3.20 ppm) displays HMBC correlation to the methylene carbon at δ = 48.3 ppm which was assigned to C-2’ while the other carbon (45.6 ppm) was assigned to C-1’.

The adamantane skeleton was elucidated next. ROESY and HMBC techniques can potentially be used to determine the connection between the side chain and the cage moiety. The adamantyl skeleton has a plane of symmetry (through C-2, C-5, C-6, C-7). This simplifies the NMR spectrum of the cage significantly. Due to the meso nature of the 2-adamantyl moiety C-1/3, C-4/9 and C-8/10 appear as single carbon and proton resonances. A methine carbon (δ = 61.7 ppm) exhibits a HMBC correlation to H-1’/H-2’ (δ = 2.68 ppm), this was assigned to C-2. H-2 (δ = 2.64 ppm) shows a COSY correlation to a methine signal at δ = 1.77 ppm (integrating to two protons). The HSQC spectrum shows that this signal corresponds to a methine carbon, thus it was assigned to C-1 and C-3 (δ = 31.8 ppm). The protons attached to these carbon atoms are also identical and register at 1.77 ppm. The remaining two methine protons at δ = 1.68 and 1.74 ppm belongs to H-5 or H-7.

The most convenient handle to identify the NMR signals of the adamantane moiety is by recognizing the through space deshielding experienced by H-4b/H-9b. Since the C-2 to NH bond rotates freely, protons H-1 (δ = 1.77 ppm), H-2 (δ = 2.64 ppm), H-3 (δ = 1.77 ppm) and H-4b/H-9b should experience through space deshielding from the electronegative nitrogen atom. Two pairs of doublet protons at δ = 1.41 and 1.89 ppm (JHH = 12.5 Hz) corresponds to a carbon signal at δ = 31.1 ppm which integrates to two methylene carbons, the former was assigned to H-4a/H-9a while the latter was assigned to H-4b/H-9b. These assignments were confirmed via COSY and ROESY spectra. Both pairs of the doublets (H-4/9) displayed COSY correlations with H-1/3 and a methyl proton appearing at δ = 1.68 ppm, which was assigned to H-5. H-5 shows a COSY correlation with another signal at δ = 1.62 ppm, this was assigned to H-6. The other methine proton
appearing at $\delta = 1.74$ ppm was assigned to H-7. H-2 exhibits HMBC correlations to C-4/9 and another methylene carbon appearing at $\delta = 37.4$ ppm (integrating to two methylene carbons); this was assigned to C-8/10. The HSQC spectrum confirms that this carbon correlates to two methylene signals at $\delta = 1.75$ and 1.62 ppm respectively (H-8/10). Distinction between H-8a and H-8b (and H-10a/H-10b) is yet to be made.

Table 1: $^1$H NMR chemical shifts of compounds 1-6

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*Solvent CDCl$_3$.

**600 MHz for $^1$H.

*400 MHz for $^1$H.

H-2 also exhibits a HMBC correlation with C-1' ($\delta = 45.6$ ppm). H-7 displays a COSY correlation with H-6 and H-8/10 respectively. H-2 displays a ROESY interaction with H-1/3 ($\delta = 1.77$ ppm) and H-8a/10a ($\delta = 1.62$ ppm) while H-4a/9a show a ROESY interaction with H-6 ($\delta = 1.62$ ppm), H-4b/9b
(δ = 1.89 ppm) and H-8b/10b (δ = 1.75 ppm). H-1’/2’ (δ = 2.68 ppm) also display a ROESY interaction with H-1/3 (δ = 1.77 ppm) and H-4b/9b (δ = 1.89 ppm) further confirming the positioning of these protons in a different environment. C-4/9 displays a HMBC correlation with H-8a/10a, H-6 H-4a/9a. C-8/10 exhibits a HMBC correlation with H-1/3, H-6, H-7, H-4a/9a and H-4b/9b respectively. A HMBC correlation was also observed for C-7 with H-1/3, H-6, H-8/10 (δ = 1.62 and 1.75 ppm). All assignments were further confirmed using the HSQC spectrum and is presented in Tables 1 and 2.

Table 2: 13C NMR chemical shifts of compounds 1-6

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a Solvent CDCl3.
b 150 MHz for 13C.
c 100 MHz for 13C.

The same methodology was utilized for the elucidation of compound 2 (and the remaining molecules); similar details will be omitted for the sake of brevity in the rest of the manuscript. The
only variation to the original structure is the hydrogenation of the alkene bonds to alkanes. The absence of two methylene protons is observed at $\delta = 4.99$ and 5.18 ppm and confirms the successful reduction of compound 1. The proton signal at $\delta = 2.54$ ppm is assigned to H-3’ and it displays a HMBC correlation to a methine carbon ($\delta = 30.9$ ppm) and a methylene carbon ($\delta = 37.3$ ppm), these signals were assigned to C-5’ and C-4’ respectively. H-3’ also shows a HMBC correlation to C-2’ ($\delta = 49.9$ ppm) while C-1’ was assigned to $\delta = 46.4$ ppm. C-5’ displays a HMBC correlation to H-12’ ($\delta = 0.81$ ppm). The only remaining methine carbon appears at $\delta = 27.9$ ppm and was assigned to C-9’. H-9’ ($\delta = 1.44$ ppm) displays both NOESY and COSY correlations to a methylene proton, H-8’ ($\delta = 1.05$ ppm) and methyl protons ($\delta = 0.78$-0.79 ppm) which should be H-10’ and/or H-11’. H-8’ shows HMBC correlations to C-10’ and C-11’ ($\delta = 22.5$-$22.6$ ppm), C-9’ ($\delta = 27.9$ ppm) and two other methylene carbons appearing at $\delta = 24.6$ and 37.3 ppm. The confirmation of the H-10’/H-11’ assignment is obtained through the HSQC spectrum using the assigned carbon signals. Distinction between the two methyl groups (C-11’ and C-10’) seems impossible. The methylene carbon ($\delta = 24.6$ ppm) displays a HMBC correlation to H-8’ and H-9’; thus it was assigned to C-7’ and the remaining carbon signal at $\delta = 37.3$ ppm was assigned to C-6’ through elimination. H-8’ also displays NOESY and COSY correlations with H-7’ and H-9’. The remaining signals were identified using the HSQC spectrum. It is clear from Tables 1 and 2 that there is good correlation of the proton and carbon signals for the 2-adamantyl moiety of SQ109 and compound 2. This gives some reassurance about the correctness of the assignments. Major differences are observed for the long carbon chain which is as expected.

Unique aspects for the structure elucidation of compound 3 and 4; will be discussed next. Due to the change in the positioning of the R group from position 2 to 1, there is a change in the plane of symmetry for the adamantyl skeleton. Three planes of symmetry are possible. Due to free rotation of the C-1 to N bond, the following groups are therefore equivalent: C-2/C-8/C-9 (CH$_2$), C-4/C-6/C-10 (CH$_2$) and C-3/C-5/C-7 (CH). The $^{13}$C APT spectrum displays three methine carbon signals appearing at $\delta = 29.7$, 123.0 and 124.1 ppm. The methine resonances 123.0 and 124.1 ppm were assigned to C-4’ and C-8’ following the previous elucidation of the alkene chain of SQ109. The remaining methine resonance at $\delta = 29.7$ ppm is quite intense suggesting that there is more than one carbon registered to this signal. The proton signal associated with that (1.99 ppm) integrates to three protons, suggesting three equivalent CH-groups. This signal was assigned to C-3, C-5 and C-7 being the only remaining methine carbons present in the molecule. C-3/5/7 show a HMBC correlation to a signal at $\delta = 1.56$-1.61 ppm, this signal could belong to any of H-2/8/9 or H-4/6/10. The HSQC spectrum shows that two intense methylene carbon signals ($\delta = 36.8$ and 42.8 ppm) integrating to three carbons each correlates to this proton signal ($\delta = 1.56$-1.61 ppm). A quarternary carbon at $\delta = 50.2$ ppm (the only one in the adamantane region) was assigned to C-1. C-1 also shows a HMBC correlation to this signal ($\delta = 1.56$-1.61 ppm). C-1 can only exhibit a HMBC correlation to protons H-
2/8/9 and not to H-4/6/10, hence confirming the location of the remaining adamantyl protons. The position of the carbon resonance for C-2/8/9 at a higher frequency ($\delta = 42.8$ ppm) is in line with the expected negative inductive effect induced by the C-1-NH-R group. The NMR spectra for compound 4 are very similar to that of compound 3 (adamantyl part) and compound 2 (side chain part). These assignments are presented in Tables 1 and 2.

For compound 5 only the discussion on the elucidation of the side chain will be presented. The $^1$H NMR spectrum of compound 5 shows an intense overlapping of methylene signals at 2.70 ppm, a doublet methylene signal at 3.22 ppm ($J = 6.72$ Hz) and a methine signal at 5.25 ppm ($J = 6.60$ Hz) and two overlapping methine signals at 5.06-5.09 ppm. The two overlapping methylene resonances appearing at 2.70 ppm was assigned to H-1'/H-2' as before. The methine proton (5.25 ppm) appearing at a higher frequency was assigned to H-4’, this proton displays both COSY and NOESY interactions with the methylene proton appearing at 3.22 ppm (H-3’). The two remaining methine proton signals appearing at 5.06-5.09 ppm should be H-8’ or H-14’. These methine protons (5.06-5.09 ppm) display COSY correlations to two methylene signals at $\delta = 2.03$ and 2.07 ppm which should then be H-7’ or H-13’. Due to overlap, clarification of these signals follows later.

The $^{13}$C spectrum of compound 5 shows three quarternary carbon resonances appearing at 131.3, 135.1 and 137.6 ppm. H-3’ displays a HMBC correlation with C-2’ (49.6 ppm), C-4’ (123.0 ppm) and a quarternary carbon signal appearing at 137.6 ppm; this was assigned to C-5’. C-5’ exhibits HMBC correlations to a methylene proton ($\delta = 1.99$ ppm) and methyl proton ($\delta = 1.62$ ppm); these were assigned to H-6’ and H-12’ respectively. The quarternary carbon signal at 131.3 ppm shows HMBC correlations to a methylene proton at $\delta = 2.03$ ppm and two methyl protons ($\delta = 1.57$ and 1.65 ppm), this quarternary carbon was assigned to C-15’, being the only quarternary carbon that can show HMBC correlation with two methyl protons [H-16’ ($\delta = 1.57$ ppm) and H-17’ ($\delta = 1.65$ ppm) respectively]. The methylene proton at 2.03 ppm was assigned to H-13’. Through elimination the remaining quarternary carbon signal at 135.1 ppm was assigned to C-9’. The signal appearing at 2.07 ppm was confirmed through COSY interaction with H-8’ ($\delta = 5.08$ ppm) to be H-7’. C-9’ ($\delta = 135.1$ ppm) displays a HMBC correlation with H-7’, a methylene proton ($\delta = 1.94$ ppm) and a methyl proton ($\delta = 1.56$ ppm), these were assigned to H-11’ and H-10’ respectively. H-10’ ($\delta = 1.56$ ppm) shows NOESY interaction with H-7’ and H-11’ while H-12’ ($\delta = 1.62$ ppm) shows NOESY interaction with H-3’, H-6’ and H-7’ respectively. H-17’ ($\delta = 1.65$ ppm) also displays NOESY interactions with H-14’ ($\delta = 5.06$ ppm) while H-16’ ($\delta = 1.57$ ppm) shows NOESY interaction with H-13’. The methine proton, H-2 ($\delta = 2.67$ ppm) displays NOESY interactions with H-1/3 ($\delta = 1.83$ ppm) and H-8a/10a ($\delta = 1.68$ ppm). H-4b/9b ($\delta = 1.93$ ppm) show NOESY interactions with H-1/3 ($\delta = 1.83$ ppm), H-5 ($\delta = 1.74$ ppm) and H-1’/2’ ($\delta = 2.70$ ppm) while H-4a/9a display NOESY interactions with H-1/3, H-5, H-6 ($\delta = 1.68$ ppm) and H-8b/10b ($\delta = 1.81$ ppm). The NMR data of compound 5 is presented in
Tables 1 and 2. Compound 6 was elucidated following similar methodology as carried out for compound 2; the NMR data for compound 6 is also presented in Tables 1 and 2.

Conclusion

The full NMR elucidation of SQ109 and its derivatives was successfully carried out. Although considerable overlapping of proton and carbon signals was observed, 2D NMR techniques proved to be a useful tool in their elucidation. The adamantyl moiety is meso depending on the positioning of the R group, hence the similarities in the cage atom signals for all carbon and protons signals (Table 1 and 2) which are almost identical for compounds 1, 2, 5 and 6 (R-group on position 2) and compounds 3 and 4 (R-group on position 1). These studies supplement a small number of reports in literature on the complete elucidation of the mono-substituted adamantane skeleton.

Experimental

The NMR spectra were recorded on a Bruker AVANCE III 400 and 600 MHz NMR spectrometers using Topspin 2.1 (Bruker, Karlsruhe, Germany). The chemical shifts were referenced to the solvent peak $\delta = 7.24$ ppm ($^1$H) and 77.0 ppm ($^{13}$C) for CDCl$_3$ at ambient temperature. The $^1$H NMR spectra were recorded at a transmitter frequency of 600.103 MHz (spectral width 11.38 ppm; acquisition time of 2.398 s; the 30° pulse width equals 10 $\mu$s and 16 scans were recorded with a relaxation delay of 1s) while the $^1$H NMR spectra were recorded at a transmitter frequency of 400.222 MHz (spectral width, 20.547 ppm; acquisition time, 1.99 s; pulse width, 10$\mu$s; scans, 16; relaxation delay, 1.0 s) for the AVANCE III 400 MHz spectrometer. The $^{13}$C NMR spectra were recorded at 150.910 MHz (spectral width 238.9 ppm; acquisition time 0.908 s; 30° pulse width, 9.00 $\mu$s; 4800 scans; relaxation delay 2.00 s) for the AVANCE III 600 MHz spectrometer while the $^{13}$C NMR spectra were recorded at 100.645 MHz (spectral width, 238.843 ppm, acquisition time 1.363 s, 30° pulse width, 8.40 $\mu$s; 1024 scans, relaxation delay 2.00 s) for the AVANCE III 400 MHz spectrometer.

The 2D experimental data parameters obtained on the Bruker AVANCE III 400 MHz were as follows: 90° pulse width, 10 $\mu$s for all spectra; spectral width for $^1$H, 3084.42, 3289.47 and 1851.85 Hz for 2, 4 and 6 respectively (NOESY, COSY, HSQC and HMBC); spectral width for $^{13}$C, 16670.416, and 22352.855 Hz (HSQC and HMBC) for 2, 4 and 6; number of data points per spectrum in F2 were 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) for compounds 2, 4 and 6; number of time-increments in F1 were 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for compounds 2, 4 and 6; relaxation delay for compounds 2, 4 and 6 were 1.35, 1.37 and 1.13 s (COSY experiments), 1.92, 1.94 and 1.70 s (NOESY experiments), 1.22, 1.26 and 0.78 s (HMBC experiments) 1.43, 1.44 and 1.32 s (HSQC experiments).
All 2D experimental data obtained on the Bruker AVANCE III 600 MHz for SQ109, 3 and 5 are as follows; 90° pulse width, 15.1 μs for all spectra; spectral width for 1 H are 6830.60, 4261.36 and 2495.01 Hz for SQ109, 3 and 5 (NOESY, COSY, HSQC and HMBC) and 7211.54 Hz for SQ109 (ROESY); spectral width for 13C, 25000 and 36057. 691 Hz (HSQC and HMBC) for SQ109, 3 and 5, number of data points per spectrum in F2 were 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) while number of time – increments in F1 were 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for SQ109, 3 and 5. The relaxation delay for compound SQ109, 3 and 5 are 1.0 s for COSY, NOESY, HSQC and HMBC respectively and a relaxation delay of 1.50 s was recorded for SQ109 (ROESY); spectra acquired in phase-sensitive mode for all compounds are NOESY and HSQC; spectra for all compounds were acquired in absolute value mode. Gradients were used for the COSY, HSQC and HMBC spectra of SQ109 and 2-6. All NMR spectra are available as supplementary material.

Acknowledgement

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References

CHAPTER 6
SQ109 ANALOGUES AS POTENTIAL ANTIMICROBIAL CANDIDATES

Oluseye K. Onajole, a Xoliswa V. Belewa, b Yacoob Coovadia, c Thavendran Govender, d Hendrik G. Kruger, a Glenn E. M. Maguire, a Dianithi Naidu, c Benesh Somai, b Nisha Singh, e and Patrick Govender, f*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, South Africa
c Microbiology, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa
d School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa
e School of Biological and Conservation Sciences, Durban, University of KwaZulu-Natal, South Africa
f School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

Abstract

The antimicrobial activity of five novel SQ109 derivatives including SQ109 against bacteria, yeast and filamentous fungi is reported herein. Using broth microdilution techniques, compounds 2 and 3 were found to be active against most tested fungi and bacteria, with minimum inhibitory concentrations (MICs) ranging from 0.98-31 µg mL⁻¹, except for Klebsiella pneumonia where the MIC was 250 µg mL⁻¹. SQ109 and derivative 4 did not show any significant activity against most of the organisms used. However, their reduced derivatives 1 and 5 showed promising activity with MICs between 0.49 and 62.5 µg mL⁻¹ against most of the microorganisms used.

Keywords: Antimicrobial activity; structural activity relationship; SQ109; 1,2 diamine

Introduction

Fungal infection in humans is commonly caused by Candida species mainly Candida albicans. Opportunistic fungal pathogens are increasingly becoming a global epidemic coupled with the emergence of drug resistant fungi. Fungal infection caused by non-Candida species is also becoming rampant especially in immuno-compromised/suppressed patients (Castroagudin et al., 2005; Netsvyetayeva et al., 2009). Fungal and bacterial infections are most prominent in transplant patients

* Tel.: +27-31-2607814; Fax: +27-31-2607942 Email address: govenderpt@ukzn.ac.za (P. Govender)
resulting in high mortality rates (Shi et al., 2009; Singh et al., 2003; Zeglen et al., 2009). The emergence of multidrug resistant fungal and bacterial strains has narrowed down possible treatment options; hence the need to design new antimicrobial therapeutics cannot be over emphasized. Preferably these new classes of drugs should have a broader antimicrobial spectrum of activity with little or no side effects.

SQ109 (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine), a second generation agent developed from the first line drug ethambutol (Lee et al., 2003) is a promising anti-tuberculosis (TB) candidate. SQ109 is specific for mycobacteria showing high activity against Mycobacterium tuberculosis, M. bovis and M. marinum, but shows reduced activity against M. avium and M. smegmatis (SQ109, 2008).

We recently reported the anti-mycobacterial activities of SQ109 and its analogues (1-5). It was found that the longer 15 carbon alkene side chain diamine [compound 2] was twofold more active than SQ109 (which has a ten carbon side chain) against both H37Rv and XDR 173 (an extensively resistant) strains of M. tuberculosis (Onajole et al., 2010). The anti-mycobacterial activity of these compounds was lost when their alkene chain was reduced to a branched alkane chain. However, compound 5 demonstrated better activity than compound 4 against both H37Rv and X173 strains of M. tuberculosis (Onajole et al., 2010). SQ109 was also reported to possess promising activity against susceptible and fluconazole resistant strains of Candida albicans at a MIC of 4-8 µg mL⁻¹ (SQ109, 2008). Inspired by this report, all SQ109 analogues (1-5) were screened in this study for other potential antimicrobial (fungal and bacterial) activities.
Results and discussion

Geranyl bromide and trans, trans farnesyl bromide were reacted with excess ethylene diamine in dry dichloromethane at −78 ºC to afford (E)-N'-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (6) and N'-(2(E),6(E)-3,7,11-trimethyldodeca-2,6,10-trienyl)ethane-1,2-diamine (7) in a 86 % yield. Isoprenyl diamines 6 and 7 were reacted with 2-adamantanone (1.2:1) via reductive amination to obtain the desired substituted diamines; the diamines were treated with hydrochloride to obtain SQ109 and 2 (57 % and 47 % yield). SQ109 and 2 were subjected to catalytic hydrogenation using 10 % Pd/C, the obtained products were converted to their HCl salts to yield compounds 1 and 3 with a 57 % and 54 % yield (Scheme 1). The free salt of amantadine (8) was reacted with chloroacetyl chloride to obtain compound 9 in 96 % yield. Reaction of 9 with geranylamine resulted in compound 10 (40 % yield). Compound 10 was subjected to reduction using LiAlH₄ to obtain the 1,2 diamine intermediate, which was converted to its hydrochloride salt 4 in 71 % yield. Compound 4 was subjected to catalytic hydrogenation using 10% Pd/C to obtain derivative 5 (52 % yield).

Figure 1: Free salts of SQ109 and its derivatives 1-5
Twenty pathogenic isolates, nine yeast, ten filamentous fungi (moulds) and one plant filamentous fungus were employed in this study (Table 1). For amphotericin B, sixteen of the nineteen (84%) tested clinical fungal strains exhibited an MIC ranging between 0.02 and 1.25 µg mL^{-1} (Table 1). SQ109 and compound 4 show slight activity between 62.5 and 125 µg mL^{-1} against most of the fungal strains used with the exception of Sporothrix schenckii where a MIC of 1.95 and 3.9 µg mL^{-1} were obtained. Interestingly, compounds 1 and 5 (reduced derivatives of SQ109 and 4) displayed higher activities (MIC ranging between 0.49-31 µg mL^{-1}) against all fungal strains when compared to SQ109 and 4. A MIC value of 25 µg mL^{-1} was also obtained for compounds 1, 2 and 5 against Aspergillus flavus (a plant filamentous fungus) while SQ109, 3 and 4 did not show any activity against this organism even at the highest concentration tested (100 µg mL^{-1}). Compound 2 (a fifteen carbon alkene chain derivative of SQ109) and 3 (reduced derivative of 2) possessed MIC values ranging between 0.98-31 µg mL^{-1}. Compound 2 (fifteen carbon alkene chain derivative) exhibited greater activity than SQ109 (a ten carbon alkene chain), suggesting that the longer alkene chain contributes to
higher antifungal activity. Although compound 3 were expected to be more active than compound 2, this however was not the case especially with the plant filamentous fungus. It is worth noting that all tested compounds (SQ109, analogues 1-5) exhibited greater antifungal activity than amphotericin B (control drug) for Sporothrix schenckii. Compounds 1 and 5 were five times more active while compounds 2 and 3 were approximately three times more active than amphotericin B against Scopularopsis spp. (Table 1). Interestingly, all tested compounds showed good activity against fluconazole-sensitive strain of Candida krusei, more importantly their MIC improved against the fluconazole-resistant strain of Candida krusei. Compounds 2 and 3 displayed slightly lower activity than amphotericin B while compounds 1 and 5 possess similar MIC values as the control drug against fluconazole-resistant strain of Candida krusei.

Table 1: Antifungal activity of SQ109 and its derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SQ109</td>
</tr>
<tr>
<td>Candida utilis</td>
<td>15.6</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>250</td>
</tr>
<tr>
<td>Candida tropicalis (2)</td>
<td>62.5-125</td>
</tr>
<tr>
<td>Candida parapsilosis (2)</td>
<td>250-500</td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td>125</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>125</td>
</tr>
<tr>
<td>Candida krusei*</td>
<td>15.6</td>
</tr>
<tr>
<td>Candida parapsilosis*</td>
<td>250</td>
</tr>
<tr>
<td>Aspergillus fumigatus (2)</td>
<td>250</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>125</td>
</tr>
<tr>
<td>Aspergillus flavus (2)</td>
<td>500</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>1.95</td>
</tr>
<tr>
<td>Scopularopsis spp.</td>
<td>62.5</td>
</tr>
<tr>
<td>Rhizomucor spp.</td>
<td>125</td>
</tr>
<tr>
<td>Pencillium spp.</td>
<td>125</td>
</tr>
<tr>
<td>Aspergillus flavus **</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* Fluconazole-resistant strains; ** Plant fungus (MRC2527); Values in brackets indicate number of isolates that were screened if more than one. Amph. B = Amphotericin B; Nd = Not done. The data are reported as the mean ± standard error of the mean of ≤ 5.
Four Gram-positive and four Gram-negative ATCC bacterial strains were employed in this study. Commercially available neomycin (Sigma) was active against bacterial strains used in this study (Table 2). SQ109 and derivative 4 did not show any significant activity against any of the bacterial strains tested. Interestingly compound 1 and 5 showed activity against all the strains which seems to indicate that activity is enhanced by the reduction of the branched alkene chain to a branched alkane chain. Compound 2 and 3 proved to be approximately two- and three-fold more active than neomycin respectively against Staphylococcus aureus (ATCC 43300). Generally compound 3 exhibited two-fold greater activity than compound 2 except for E. Coli (same MIC) and Klebsiella spp. This trend is reversed for Klebsiella spp. It can be tentatively suggested that compound 2 may penetrate the capsule layer surrounding Klebsiella spp. with greater ease than compound 3.

Table 2: Antibacterial activity of SQ109 and its derivatives

<table>
<thead>
<tr>
<th>Strains (ATCC number)</th>
<th>MIC (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SQ109 1 2 3 4 5 Neomycin</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis (6051)</td>
<td>500 15.6 7.8 3.9 125 15.6 0.78</td>
</tr>
<tr>
<td>Rhodococcus equi (6939)</td>
<td>125 7.8 7.8 3.9 125 15.6 1.56</td>
</tr>
<tr>
<td>Staphylococcus xylosus (35033)</td>
<td>500 15.6 7.8 3.9 250 15.6 0.20</td>
</tr>
<tr>
<td>Staphylococcus aureus (43300)</td>
<td>1000 31.25 15.6 7.8 500 62.5 25</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli (35218)</td>
<td>500 31.25 7.8 7.8 500 31.25 3.13</td>
</tr>
<tr>
<td>Klebsiella oxytoca (13182)</td>
<td>500 62.5 15.6 31.25 500 62.5 6.25</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (700603)</td>
<td>1000 125 125 250 1000 62.5 6.25</td>
</tr>
<tr>
<td>Elizabethkingia meningoseptica (13253)</td>
<td>500 31.25 15.6 7.8 500 31.25 12.5</td>
</tr>
</tbody>
</table>

Conclusion

The susceptibility data obtained in this study strongly suggests that compound 2 could serve as an effective therapeutic agent against a broad range of infections. Compound 2 was reported (Onajole et al., 2010) to be highly potent against an extensively resistant strain of mycobacterium (XDR-TB), and the same compound has further shown promising antifungal and antibacterial activity in this study. In our previous study (Onajole et al., 2010), it was observed that SQ109 and compound 2 lost activity against Mycobacterium spp. when reduced to compounds 1 and 3, but compound 4 gained activity when reduced to 5. This is believed to be due to the positioning of the long carbon chains i.e. position 1 (compound 5) on the adamantyl moiety compared to position 2 (compound 1). However in the present study, the positioning of the long alkene/alkane chain either on position 1 or 2 on the
adamantyl moiety had little effect on either the antibacterial or antifungal activities, as the alkane chain derivatives (1 and 5) were more favoured as antifungal and antibacterial agents compared to their alkene chain derivatives (SQ109 and 4).

Experimental
Chemistry

All necessary chemicals were purchased from Sigma-aldrich, Merck and Fluka. Reactions were monitored using thin layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH$_3$Cl:CH$_3$OH:NH$_4$OH (88:10:2) as the eluent (solvent mixture). The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments in CDCl$_3$ using trimethylsilane (TMS) as internal standard: Mass spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3, melting point results were un-corrected. Level of purity for all compounds was judged to be ≥95% based upon $^1$H NMR prior to their use in the evaluation for biological efficacies.

(E)-N’-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (6)

A light yellow oil (86 %, R$_f$ = 0.45). $^1$H NMR [CDCl$_3$, 400 MHz]: δ H 1.36 (s, NH), 1.56 (3H, s, CH$_3$), 1.61 (3H, s, CH$_3$), 1.64 (3H, s, CH$_3$), 1.97 (2H, m, CH$_2$), 2.05 (2H, m, CH$_2$), 2.63 (2H, t, $J$ = 6.1, 5.6 Hz, CH$_2$), 2.78 (2H, t, $J$ = 5.7, 6.1 Hz, CH$_2$), 3.21 (2H, d, $J$ = 6.8 Hz, CH$_2$), 5.06 (1H, t, $J$ = 6.8 Hz, CH), 5.23 (1H, t, $J$ = 6.8 Hz, CH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: δ C 16.3 (CH$_3$), 17.6 (CH$_3$), 25.7 (CH$_3$), 26.5 (CH$_2$), 39.6 (CH$_2$), 41.8 (CH$_2$), 47.1 (CH$_2$), 52.1 (CH$_2$), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

N’-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl]ethane-1,2-diamine (7)

A light yellow oil (86 %, R$_f$ = 0.50). $^1$H NMR [CDCl$_3$, 400 MHz]: δ H 1.32 (s, NH), 1.60 (6H, s, 2xCH$_3$), 1.65 (3H, s, CH$_3$), 1.68 (3H, s, CH$_3$), 1.95-2.11 (8H, m, 4xCH$_2$), 2.67 (2H, t, $J$ = 6.0, 5.7 Hz, CH$_2$), 2.81 (2H, t, $J$ = 5.7 Hz, 6.0 Hz, CH$_2$), 3.24 (2H, d, $J$ = 6.7 Hz, CH$_2$), 5.09 (2H, m, 2xCH), 5.27 (1H, m, CH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: δ C 16.0 (CH$_3$), 16.3 (CH$_3$), 17.7 (CH$_3$), 25.7 (CH$_3$), 26.4 (CH$_2$), 26.7 (CH$_2$), 39.6 (CH$_2$), 39.7 (CH$_2$), 41.9 (CH$_2$), 47.1 (CH$_2$), 52.2 (CH$_2$), 122.9 (CH), 124.0 (CH), 124.3 (CH), 131.3 (C), 135.1 (C), 137.7 (C).

N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine (SQ109)

A white solid; 57% yield, Melting point: 180-184 ºC, HR-MS calculated for C$_{22}$H$_{39}$N$_2$ ([M+H]$^+$ of free base) 331.3108, found 331.3135; $^1$H NMR (CDCl$_3$, 600 MHz): δ H 1.41 (2H, d, $J$ = 12.5 Hz, H-
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4a/9a), 1.50 (3H, s, CH₃), 1.55 (3H, s, CH₃), 1.58 (3H, s, CH₃), 1.59-1.61 (4H, m, H-6, H-8a/10a), 1.68 (1H, s, H-5), 1.74-1.78 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.89 (2H, d, J = 12.5 Hz, H-4b/9b), 1.93 (2H, m, CH₂), 2.00 (2H, m, CH₂), 2.64 (1H, s, H-2), 2.68 (4H, s, 2xCH₂), 3.20 (2H, d, J = 12.5 Hz, H-4b/9b), 3.20 (2H, d, J = 6.06 Hz, CH₂), 4.99 (1H, m, CH), 5.18 (1H, m, CH). ¹³C NMR (CDCl₃, 150 MHz); δC 16.1 (CH₃), 17.5 (CH₃), 25.5 (CH₃), 26.3 (CH₂), 27.4 (C-5), 27.6 (C-7), 31.1 (C-4, 9), 31.8 (C-1, 3), 37.4 (C-8, 10), 37.7 (C-6), 39.5 (CH₂), 45.6 (CH₂), 46.3 (CH₂), 48.3 (CH₂), 61.7 (C-2), 121.4 (CH), 123.9 (CH), 131.3 (C), 138.6 (C).

N-(3,7-dimethyloctyl)-N´-(2-adamantyl)ethane-1,2-diamine (1)

A white solid (HCl salt); 57 % yield, Melting point: 182-185 ºC, HR-MS calculated for C₂₂H₄₃N₂ ([M+H]+ of free base) 335.3421, found 335.3415; ¹H NMR (CDCl₃, 400 MHz): δH 0.78–0.81 (9H, m, 3xCH₃), 1.01–1.09 (m, 3H, 2xCH₂), 1.14–1.26 (4H, m, 2xCH₂), 1.40-1.48 (m, 4H (H-4a/9a, 2xCH), 1H (CH₂)), 1.64 (4H, s, H-6, H-8a/10a), 1.70 (1H, s, H-5), 1.75–1.78 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.91 (2H, d, J = 12.2 Hz, H-4b/9b), 2.52–2.54 (2H, m, CH₂), 2.63 (1H, s, H-2), 2.66 (4H, s, 2xCH₂). ¹³C NMR (CDCl₃, 100 MHz); δC 19.6 (CH₃), 22.6 (CH₃), 22.6 (CH₃), 24.4 (CH₂), 24.6 (CH₂), 27.5 (C-5), 27.7 (C-7), 27.9 (CH), 30.9 (CH), 31.2 (C-4, 9), 32.1 (C-1, 3), 37.2 (CH₂), 37.3 (CH₂), 37.5 (C-8, 10), 37.9 (C-6), 39.2 (CH₂), 46.3 (CH₂), 47.7 (CH₂), 50.0 (CH₂), 61.8 (C-2).

N-trans-trans-farnesyl-N´-(2-adamantyl)ethane-1,2-diamine (2)

A white solid (HCl salt); 47 % yield, Melting point: 146–150 ºC, HR-MS calculated for C₂₇H₄₇N₂ ([M+H]+ of free base) 399.3734, found 399.3740; ¹H NMR (CDCl₃, 600 MHz): δH 1.47 (2H, d, J = 12.2 Hz, H-4a/9a), 1.57 (6H, s, 2xCH₃), 1.62 (3H, s, CH₃), 1.65 (3H, s, CH₃), 1.68 (4H, s, H-6, H-8a/10a), 1.74 (1H, s, H-5), 1.80–1.82 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.93-1.96 (4H, m, H-4b/9b, CH₂), 5.05–5.10 (2H, m, 2xCH₂), 5.25 (1H, t, J = 6.5 Hz, CH); ¹³C NMR (CDCl₃, 150 MHz); δC 16.0 (CH₃), 16.3 (CH₃), 17.7 (CH₃), 25.7 (CH₃), 26.4 (CH₂), 26.7 (CH₂), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4, 9), 32.2 (C-1, 3), 37.6 (C-8, 10), 38.0 (C-6), 39.6 (CH₂), 39.7 (CH₂), 46.6 (CH₂), 47.1 (CH₂), 49.6 (CH₂), 61.9 (C-2), 123.0 (CH), 124.0 (CH), 124.3 (CH), 131.3 (C), 135.1 (C), 137.6 (C).

N-(3,7,11-trimethyldodecyl)-N´-(2-adamantyl)ethane-1,2-diamine (3)

A white solid (HCl salt); 54 % yield, Melting point: 114-118 ºC, HR-MS calculated for C₂₇H₅₃N₂ ([M+H]+ of free base) 405.4203, found 405.4186; ¹H NMR (CDCl₃, 400 MHz): δH 0.79–0.83 (12H, m, 4xCH₃), 0.98–1.11 (m, 4H, 2xCH₂), 1H (CH₂), 1.66 (4H, br s, H-6, H-8a/10a), 1.72 (1H, s, H-5), 1.78–1.81 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.93 (2H, d, J = 12.2 Hz, H-4b/9b), 2.54–2.60 (2H, m, CH₂), 2.66 (1H, s, H-2), 2.68 (4H, s, 2xCH₂). ¹³C NMR (CDCl₃, 100 MHz); δC 19.6 (CH₃), 19.7 (CH₃), 22.6 (CH₂), 22.7 (CH₂), 24.4 (CH₂), 24.8 (CH₂), 27.6 (C-5), 27.8 (C-7), 27.9 (CH), 31.0 (CH), 31.3 (C-4, 9), 32.2 (C-1,
3), 32.8 (CH), 37.3 (CH2), 37.4 (CH2), 37.5 (C-8, 10), 37.6 (CH2), 38.0 (C-6), 39.3 (CH2), 46.5 (CH2), 47.9 (CH2), 50.1 (CH2), 61.9 (C-2).

**N-Geranyl-N’-(1-adamantyl)acetamide (10)**

A light yellow oil, 40 % yield; \(^1\)H NMR (CDCl\(_3\), 600 MHz): 1.57 (3H, s, CH\(_3\)), 1.60 (CH\(_3\)), 1.65 (9H, s, H-4, H-6, H-10, CH\(_3\)), 1.98 (8H, s, H-2, H-8, H-9, CH\(_2\)), 2.04-2.06 (5H, m, H-3, H-5, H-7, CH\(_2\)), 3.08 (2H, s, CH\(_2\)), 3.15 (2H, d, \(J = 6.9\) Hz, CH\(_2\)), 5.05 (1H, t, \(J = 7.0\) Hz, CH), 5.16 (1H, t, \(J = 6.6\) Hz, CH), 6.94 (CONH). \(^{13}\)C NMR (CDCl\(_3\), 150 MHz); \(\delta_{C}\) 16.3 (CH\(_3\)), 17.7 (CH\(_3\)), 25.7 (CH\(_3\)), 26.4 (CH\(_2\)), 29.4 (C-3, 5, 7), 36.3 (C-4, 6, 10), 39.6 (CH\(_2\)), 41.6 (C-2, 8, 9), 47.0 (CH\(_2\)), 51.0 (C-1), 52.6 (CH\(_2\)), 121.9 (CH), 123.9 (CH), 131.6 (C), 138.8 (C), 170.6 (C=O).

**N-Geranyl-N’-(1-adamantyl)ethane-1,2-diamine (4)**

A yellowish solid (HCl salt); 71 % yield; Melting point: 135-138 °C, HR-MS calculated for C\(_{22}\)H\(_{39}\)N\(_2\) ([M+H]+ of free base) 331.3108, found 331.3104; \(^1\)H NMR (CDCl\(_3\), 600 MHz): 1.53 (3H, s, CH\(_3\)), 1.57 (CH\(_3\)), 1.58 (6H, s, H-2, H-8, H-9), 1.56-1.61 (6H, m, H-4, H-6, H-10), 1.64 (3H, s, CH\(_3\)), 1.94 (2H, m, CH\(_2\)), 1.99 (3H, s, H-3, H-5, H-7), 2.01 (2H, m, CH\(_2\)), 2.63 (4H, s, 2xCH\(_2\)), 3.14 (2H, d, \(J = 6.72\) Hz, CH\(_2\)), 5.01 (1H, m, CH), 5.17 (1H, t, \(J = 6.6\) Hz, CH). \(^{13}\)C NMR (CDCl\(_3\), 150 MHz); \(\delta_{C}\) 16.3 (CH\(_3\)), 17.6 (CH\(_3\)), 25.6 (CH\(_3\)), 26.3 (CH\(_2\)), 29.7 (C-3, 5, 7), 36.8 (C-4, 6, 10), 39.6 (CH\(_2\)), 39.9 (CH\(_2\)), 42.8 (C-2, 8, 9), 47.1 (CH\(_3\)), 50.0 (CH\(_2\)), 50.2 (C-1), 123.0 (CH), 124.1 (CH), 131.4 (C), 137.4 (C).

**N-(3,7-dimethyloctyl)-N’-(1-adamantyl)ethane-1,2-diamine (5)**

A white solid (HCl salt); 52 % yield, Melting point: 210 – 214 °C, HR-MS calculated for C\(_{22}\)H\(_{43}\)N\(_2\) ([M+H]+ of free base) 335.3421, found 335.3418; \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta_{H}\) 0.74–0.77 (9H, m, 3xCH\(_3\)), 0.98–1.05 (m, 3H, 2xCH\(_2\)), 1.11–1.21 (4H, m, 2xCH\(_2\)), 1.35–1.44 (m, 2H (2xCH), 1H (CH\(_3\))), 1.48–1.57 (12H, m, H-2, H-4, H-6, H-8, H-9, H-10), 1.96 (3H, s, H-3, H-5, H-7), 2.50 (2H, s, CH\(_2\)), 2.63 (4H, s, 2xCH\(_2\)). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz); \(\delta_{C}\) 19.7 (CH\(_3\)), 22.5 (CH\(_3\)), 22.6 (CH\(_3\)), 24.6 (CH\(_2\)), 27.9 (CH), 29.5 (C-3, 5, 7), 30.9 (CH), 36.6 (C-4, 6, 10), 37.2 (CH\(_2\)), 37.3 (CH\(_2\)), 39.2 (CH\(_2\)), 39.7 (CH\(_2\)), 42.5 (C-2, 8, 9), 47.7 (CH\(_3\)), 50.1 (CH\(_2\)), 50.7 (C-1).

**Antimicrobial evaluation**

**Antifungal assay**

Twenty pathogenic isolates, nine human yeast, ten human filamentous fungi and one plant filamentous fungus were used in this study while two reference strains [Candida parapsilosis (ATCC 22019) and Candida albicans (ATCC 90028)] served as controls as recommended by Clinical and Laboratory Standards Institute, (CLSI, formerly the National Committee for Clinical Laboratory Standards). The clinical isolates were obtained from the National Health Laboratory Services
(NHLS), Inkosi Albert Luthuli Hospital, Durban, South Africa. The fungal isolates were recovered from the respiratory tracts, tissue samples and blood specimens of patients managed by the Hospital. Yeast isolates were identified at the species level using carbohydrate assimilation tests (API 32 ID, bioMérieux, Marcy l’Etoile, France) while the filamentous fungi were identified by macroscopic and microscopic morphological features.

**Antifungal/antibacterial agents**

The hydrochloride salt of each compound [SQ109 and its derivatives (1-5)] was dissolved in 10% methanol and diluted with sterile double distilled water. Amphotericin B (Sigma-Aldrich) and neomycin (Sigma) were used as reference drugs. Freshly prepared solutions of SQ109, its derivatives and the reference drugs were used in this study. The final concentrations of the antimicrobial agents ranged from 0.0012 to 80 µg mL⁻¹ amphotericin B, 0.0015 to 100 µg mL⁻¹ neomycin and derivative 3, 0.0076 to 1000 µg mL⁻¹ for SQ109 and derivatives 1, 2, 4 and 5. All drug dilutions were performed in 96-well flat-bottom microtitre plates. Each well contained 100 µL of twofold serial dilutions of the tested drugs (2 x final concentrations) and were stored at -70 ºC until the day of testing.

**Antifungal susceptibility assay**

Evaluation of the susceptibility of *Candida albicans*, non-*Candida albicans* species and filamentous fungi (moulds) was performed using the broth microdilution method according to CLSI (formerly NCCLS) M27-A2 (for yeast) and M38-A2 (for filamentous fungi) guidelines (CLSI, 2008; NCCLS, 2002). Yeast strains were grown aerobically overnight at 35 ºC on Sabouraud dextrose agar (Merck) plates. Each yeast strain was harvested and suspended in 1% sterile saline and the turbidity of the supernatants measured spectrophotometrically at 625 nm to achieve an absorbance of 0.08-0.10 units equivalent to the No. 0.5 McFarland standard of the NCCLS M27-A2 guidelines (NCCLS, 2002). The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium (with L-glutamine, without bicarbonate, Cambrex Bio Science Verviers, Belgium) and 0.165 M morpholinepropanesulfonic acid (MOPS, Sigma-Aldrich) buffered to pH 7.0. The working suspension was further diluted (1:50) with the medium to obtain the final test inoculum concentration of 1-5 x 10³ CFU mL⁻¹. Microtitre plates containing frozen serial dilutions of the antibiotics and compounds 1-5 were allowed to thaw and equilibrate to room temperature under aseptic conditions after which 100 µL aliquots of the working inoculum suspension were dispensed into each well and the cultures were aerobically incubated at 35ºC for 24 h. Growth was observed visually with the aid of a concave mirror; MICs were taken on a growth or no-growth (100% visible-growth inhibition) scale.
MTS reduction Analysis

The MICs were also determined by colorimetric method using the dye, 2H-terazolium salt (MTS, Promega Corporation, Madison, USA). After MICs were determined visually on each micotitre plates, 20 µL of 2H-terazolium salt (Promega, 2005) was added directly to each well, incubated at 37 °C for 4 h and the absorbance was spectrophotometrically determined at 490 nm using a microplate reader (PowerWave XS2, BioTek). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

The anti-fungal activities of SQ109 and its derivatives were also determined against human pathogenic filamentous fungi using the broth microdilution method according to the CLSI M38-A2 guidelines (CLSI, 2008). Cultures were grown on potato dextrose agar at 35°C until sporulation (48 h to 7 days). Spores were harvested and suspended in 1 % sterile saline, allowed to settle and the upper layer aspirated. The turbidity was measured spectrophotometrically and the optical density was adjusted to yield a stock suspension of 0.4-5.0 x 10^6 sporangiospores per millilitre. A working suspension was prepared by making a 1:50 dilution of the conidia stock suspension in a standard medium (RPMI 1640, MOPS). Each fungal inoculum (100 µL) was added to individual wells of 96-well flat-bottom microdilution plates containing 100 µL of the appropriate dilution of the drug and aerobically incubated for 48 h at 35°C. After incubation, potential antimicrobial activity (MICs) was assessed as described in the yeast section. All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

XTT reduction assay

Plant pathogenic Aspergillus flavus (MRC2527) was routinely grown on glucose minimal agar (pH 6.5) (50 mL/L of 20x nitrate salts, 1 mL/L trace elements, 10 g/L glucose) plates in an incubator at 28°C for 4 to 5 days prior to use. When required, spores were harvested by flooding the surface of the plates with sterile water containing 0.1% Tween 20 and gently scraping off the spores with a sterile glass rod. Spore counts were done using a haemocytometer. Therefore the metabolic activity of A. flavus against SQ109 and its derivatives was investigated using a modified procedure of Antachopoulos et al. (Antachopoulos et al., 2006; Antachopoulos et al., 2007). A concentration of 1.0 x 10^5 A. flavus spores was first inoculated into RPMI 1640 medium (with L-glutamine; without bicarbonate) (Gibco BRL, Life Technologies) in flat-bottomed 96-well microtiter plates. For each compound, the required concentrations were prepared by diluting each to attain a final volume of 200 µL per well. The concentrations of each tested compound ranged between 25 µg mL\(^{-1}\) to 100 µg mL\(^{-1}\). A positive control, containing only fungal spores and no antibiotic was prepared for examination of spore viability. Negative controls, containing each compound with no spores were also prepared. These negative control wells served as a measure of the background absorbance (blank wells) contributed by the reagents used in the study. The plate was incubated at 28°C for 24 h. After the
incubation period, 50 µL of 1.25 mM XTT/menadione solution was added to each well, resulting in a final concentration of 100 µg mL\(^{-1}\) XTT and 25 µM menadione. The reactions were incubated for a further 2 h at 37 °C to allow for the conversion of the XTT into its formazan derivative and subsequently shaken for two minutes to mix the reagents. Thereafter, the absorbance was determined at 450 nm (PowerWave XS2, BioTek). The specific amount of XTT conversion was determined by subtracting the background absorbance (blank) from each experimental well as described by Antachopoulos et al. (Antachopoulos et al., 2007). Each experiment was done in triplicate and repeated at least once.

**Antibacterial susceptibility assay**

Bacterial susceptibility was determined using the broth microdilution method (Eloff, 1998; Schwalbe et al., 2007). Eight ATCC bacterial cultures obtained from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville, South Africa were freshly cultured in Mueller-Hinton broth. The turbidity of overnight cultures that were grown for 16-18 h at 37ºC was adjusted to that of No. 0.5 McFarland standard. At a wavelength of 625 nm, the absorbance was adjusted between 0.08-0.10 units to yield a stock suspension of 0.4-5.0 x 10^8 CFU mL\(^{-1}\), which was diluted one hundred fold to obtain a working suspension of 10^6 CFU mL\(^{-1}\).

The microtitre plates containing frozen dilutions of compounds were allowed to equilibrate to room temperature under aseptic conditions. Aliquots (100 µL) of bacterial inocula were added to the prepared drug samples and incubated aerobically for 16-18 h at 37ºC. Following incubation, 50 µL of freshly prepared iodonitrotetrazolium chloride (2-(4-iodophenyl)-3-(4-nitrophanyl)-5-phenyl-2H-tetrazolium chloride), (INT) solution (200 µg mL\(^{-1}\)) was added to each well and the plate was further incubated for 45 minutes at 37ºC in the dark. When the colourless INT is reduced to red after incubation, persistent growth of the bacteria is indicated, while no colour change denotes a lack of bacterial growth. Neomycin was used as a control drug in this study and all analyses were performed in triplicate.

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References


CHAPTER 7

NOVEL POLYCYCLIC ‘CAGE’-1,2-DIAMINES AS POTENTIAL ANTI-TUBERCULOSIS AGENTS

Oluseye K. Onajole,a Yacoob Coovadia,b Patrick Govender,c Hendrik G. Kruger,a* Glenn E.M. Maguire,a Melendhran Pillay,b and Thavendran Govender d†

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b Microbiology, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa
c School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa
d School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa

ABSTRACT

SQ109 (N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine) is a novel anti-tuberculosis candidate presently in clinical trials. In this report, a series of polycyclic ‘cage’ derivatives of N-geranyl-1,2 diamines were synthesized and screened for their anti-mycobacterial activity against H37Rv, multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of tuberculosis. By substituting the adamantyl skeleton of SQ109 with trishomocubanyl (9), oxa-pentacycloundecyl (14, 16), pentacycloundecyl, PCU, (10, 15) and aza-pentacycloundecyl (22, 23), the effect of other polycyclic “cage” skeletons could be investigated. Using the BACTEC MGIT 960 system, compound 9 (trishomocubanyl moiety) proved to be the most active (MICs: 0.5 -2 µg/mL) while PCU hydroxyl derivatives (15 and 23), oxa-pentacycloundecyl and aza-pentacycloundecyl derivatives displayed similar activity to SQ109 (MICs: 0.5-4 µg/mL) against all three strains of TB used in this study. Compounds 10, 14 and 21 displayed similar activities (MICs: 1-8 µg/mL) against all three strains of tuberculosis.

Keywords: SQ109, polycyclic “cage”, tuberculosis, anti-tuberculosis

INTRODUCTION

Tuberculosis (TB) is a highly contagious and insidious disease with a high infection rate that has been present in humans since antiquity. TB is predominantly caused by Mycobacterium tuberculosis which is a slow growing bacterium. The causal organism can remain dormant in the host (human) for a very long time and may only become active when the person falls sick or has a back drop in his/her
immune system. This event in particular has become most prominent in immuno-compromised patients such as those living with HIV (Human Immunodeficiency Virus). The 2009 global tuberculosis control report of the World Health Organisation (WHO), estimated that about 9.4 million incident cases of TB occurred globally. An estimated 1.4 million were HIV positive, of which, 78% were in Africa while 13% are located in South-East Asia [1].

There is an urgent need for highly potent, more effective drugs with fewer or no side effects and shorter treatment periods to combat the increasing TB pandemic. Present studies, identify molecules such as diarylquinolone (TMC207), nitroimidazole (OPC67683 and PA824), pyrrole (LL3858), diamine (SQ109) are in different stages of clinical trials [2-5].

SQ109 (2) first reported by Lee et al. [6] shares the same 1,2 ethylenediamine pharmacophore with ethambutol (1). SQ109 proved to be 14-35 fold more active than ethambutol against the H37Rv strain of tuberculosis [6]. SQ109 also possesses remarkable activity against MDR-TB which includes the EMB resistant strain suggesting that SQ109 is a new anti-TB drug with new mechanism and not an EMB analogue [7]. SQ109 induces a synergistic effect when used in combination with other first line drugs such as isoniazid and rifampicin, however an additive effect is observed when used with streptomycin [8].

The incorporation of polycyclic “cage” compounds (such as adamantane and pentacyclo-undecane) in potential pharmaceutical application has enjoyed much attention from researchers for four decades starting with the discovery of amantadine an anti-viral drug [9-11]. Polycyclic “cage” compounds possess the ability to improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across cell membranes. It’s been reported that ‘cage’ moieties such as adamantane and PCU are able to cross the Blood Brain Barrier (BBB) and the Central Nervous System (CNS) [9,12,13]. It also helps to reduce the bio-degradation of drugs in biological systems thus prolonging their pharmaceutical effect in the body [9,14,15]. SQ109 (2) and SQ117 (3) both have a lipophilic moiety, namely the 2-adamantly moiety in SQ109 (log P; 5.26) and a diphenyl moiety in SQ117 (log P; 5.50). However, different MIC values were reported for each [7]. SQ109 was five-fold more active than SQ117 suggesting that the nature of the lipophilic group does play a role in their activities even though SQ117 possesses a higher log P value [7].
In the course of our research for novel anti-tubercular compounds,[16] we recently demonstrated the importance of chain length and saturation of some SQ109 derivatives [17]. This present study, aims at investigating the possibility of further enhancing/improving the anti-TB activity of the diamine via substitution of the adamantyl moiety with other polycyclic compounds such as trishomocubane and pentacycloundecane. Based on this; seven novel diamine based compounds bearing the lipophilic pentacycloundecane and trishomocubane cages were synthesized and screened for activity against drug sensitive (H$_{37}$Rv) and drug resistant strains of tuberculosis.
Scheme 1: Synthetic route for the synthesis of PCU monoketone and trishomocubanone

**Results and discussion**

**Chemistry**

Cookson’s dione 4 [18,19] was the starting material in the synthesis of trishomocubanone (5) and the PCU-monoketone (6). Trishomocubanone was synthesized via a six step reaction pathway as reported in literature [20,21] and PCU monoketone was synthesized via a five step reaction pathway as reported in literature [20-22] (Scheme 1). Geranyl bromide 7 was reacted with excess ethane-1,2-diamine in dry dichloromethane at –78 ºC to afford (E)-N’-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine 8 (86 % yield). Isoprenyl ethane-1,2-diamine 8 was reacted with trishomocubanone, PCU monoketone and 2-adamantanone via reductive amination; the resulting imines were reduced with NaBH4 to obtain polycyclic-diamines; the diamines were converted to their HCl salts to obtain compounds 9 [N-geranyl-N’-(11-trishomocubanyl)ethane-1,2-diamine hydrochloride], 10 [N-geranyl-N’-(8-pentacycloundecyl)ethane-1,2-diamine hydrochloride] and SQ109 [N-geranyl-N’-(2-adamantyl)ethane-1,2-diamine hydrochloride] (55-57 % yield). The expected orientation of the nitrogen atom on the PCU skeleton upon reductive amination with NaBH4 is the endo-form [23,24]. This orientation was confirmed by a NOESY experiment as H-8 (2.67 ppm) displayed through space interaction with H-5 and H-9 (2.16-2.18 ppm).
Scheme 2: Reagents and conditions: (a) ethylene diamine (100:1), -78 °C, dry DCM; (b) MeOH, polycyclic ‘cage’ monoketone (1.2:1), N₂ atmosphere, 2 h, NaBH₄, overnight; then HCl, MeOH

It was anticipated that the presence of a hydroxyl group (endo/exo positioning) on the cage moiety might contribute significantly to its activity; this led to the design and successful synthesis of compound 15 and 23. 8-Benzylamino-8,11-oxapentacyclo-[5.4.0.0²,6.0³,10.0⁵,9]undecane (11) was synthesized as reported in literature [13,25]. Compound 11 was debenzylated using 10% Pd/C under hydrogen gas at atmospheric pressure to obtain 12 in 60.8 % yield [26]. Compound 12 was reacted with chloroacetyl chloride to obtain compound 13 (62 % yield), which was reacted with geranylamine[17] to obtain compound 14 (58.5 % yield). Compound 14 was reduced using a strong reducing agent (LAH) at a 1:4 ratio in dry THF with refluxing to obtain a compound with m/z of 357.2897 (M+H⁺, 33.7 % yield). Compound 15 was proposed and this structure was confirmed using 2D NMR experiments. A through space interaction of H-11 (3.86 ppm) with H-1 (2.68 ppm), H-3 (2.30 ppm) and H-10 (2.36 ppm) was observed thus proving that the hydroxyl group was at an endo position. H-8 also displayed a NOESY interaction with H-5 (2.34 ppm), H-7 (2.80 ppm), H-9 (2.51 ppm) and H-1'/2' (ethylene protons at 3.18 ppm) while the methylene protons, H-1'/2', displayed NOESY interactions with methyl protons (1.61 ppm), H-7, H-8 and H-9 respectively. These assignments confirm the endo positioning of the isoprenyl diamine moiety on the PCU cage as a result of the breakage of the ether bridge.

Attempts to synthesize the H-8 endo-orientated compound 10 prove unsuccessful; however an exo positioning of the R-group would be obtained if an oxa-pentacycloundecyl moiety is used, this led to the design and successful synthesis of compound 16. This was achieved by using a milder reducing reagent (65 % Red-Al in toluene) on compound 14 to obtain 16 (45.2 % yield). NMR spectroscopy of compound 14 showed the successful synthesis as no extra methine carbon was observed at position 57.4 ppm and a quarternary carbon (C-8) at position 110.1 ppm was observed. C-8 displays a HMBC correlation with methylene protons (H-1') at 3.02 ppm while H-11 (4.58 ppm) also displayed NOESY interactions with H-1, H-3 and H-10 respectively, thus confirming the successful synthesis of compound 16.
Replacement of the oxo-bridge of 16 with an aza-bridge to give the isomeric hemiaminal 23 was also achieved (Scheme 4) and this compound was screened for anti-TB activity. Mono-protection of Cookson’s diketone 4 was carried out to obtain the ethylene ketal 17 in 74 % yield and condensation with benzylamine gave the imine 18. Reduction with NaBH₄, followed by hydrolysis resulted in the formation of the racemic hexacyclic cage amine 19. Benzyl deprotection of 19 led to compound 20 (44 % yield). Geranylamine was reacted with chloroacetyl chloride to afford (E)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (21, 81 % yield) which was reacted with 4-azahexacyclo[5.4.1.0²,⁶.0⁵,⁹.0⁸,¹¹]dodecan-3-ol (20) to obtain 22 (81 % yield). Reduction of 22 to yield the desired compound 23 (72 % yield) was achieved using 65 % Red-Al in toluene.
The successful syntheses of all novel compounds were confirmed using $^1$H, $^{13}$C, 2D NMR experiments (COSY, HSQC, HMBC, ROESY and NOE SY), IR and HR-MS. All compounds were tested as racemate and confirmed to be greater than 95% pure via NMR spectroscopy prior to their evaluation for biological activities.

Anti-tubercular activity

The in vitro anti-mycobacterial activity of all the compounds against M. tuberculosis strain was carried out using the Mycobacteria Growth Indicator Tube system (MGIT). The minimum inhibitory concentration (MIC, $\mu$g/mL) was detected by BACTEC 960TB (Becton Dickinson). All compounds were screened against H$_{37}$Rv (ATCC No: 25177), MDR and XDR strains of tuberculosis and the results are summarized in Table 1. Compounds 14 and 22 did not show any promising anti-TB activity implied that the carbonyl group contributes negatively to their inhibitory effect. Compounds 15 (endo-positioned hydroxyl group) and 23 (exo-positioned hydroxyl group) displayed similar activity as SQ109 against both MDR and XDR strains of TB at a MIC of 2 and 4$\mu$g/mL respectively. On the other hand, compound 10 (similar to 15) without the hydroxyl group displayed reduced activity against all tested TB stains; this appears that the hydroxyl group notwithstanding its positioning is essential for anti-tubercular activity. The oxa-pentacycloundecyl derivative 16 proves to be more active (two-fold) than 10 suggesting that the exo positioning of the isoprenyl diamine moiety on the pencycloundecanyl moiety is also essential for the anti-TB activity of this class of compounds.
compounds. Compound 9 proves to be the most active in this series; with a twofold increase in activity against MDR and XDR-TB when compared to 10, 15, 16, 23 and SQ109. The D3-trishomocubyl derivative 9 displayed significantly higher activity than its pentacycloundecyl and adamantyl counterparts.

Table 1: The MICs of the target compounds against M. tuberculosis (H37Rv, MDR and XDR) strains

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MIC (µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>H37Rv</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Structure 9" /></td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Structure 10" /></td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td><img src="image" alt="Structure 14" /></td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td><img src="image" alt="Structure 15" /></td>
<td>0.5</td>
</tr>
<tr>
<td>16</td>
<td><img src="image" alt="Structure 16" /></td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td><img src="image" alt="Structure 22" /></td>
<td>1</td>
</tr>
</tbody>
</table>
This result suggests that the nature or type of polycyclic “cage” compounds is important for activity. As observed by many researchers in the field [9,11] the substitution of the polycyclic “cage” moiety (adamantyl) with similar moieties such as trishomocubane, pentacycloundecane, oxapentacycloundecane etc. in most cases maintains the activity of such compounds. Of all reported polycyclic ‘cage’ compounds only trishomocubane possess a unique D3 stereochemistry which could have contributed to its activity in this series.

Conclusion

We have synthesized a series of novel polycyclic ‘cage’ diamine derivatives with potent anti-TB activity. As observed, the positioning of the isoprenyl diamine on the PCU moiety either endo or exo does influence its anti-TB activity while the hydroxyl group is also essential for activity. Compound 9 (trishomocubyl moiety) was identified as the most potent against MDR and XDR strains of TB used with a two-fold increase in activity than 10, 15 (pentacycloundecyl), 16 (oxapentacycloundecyl), 23 (azapentacycloundecyl), and SQ109 (2-adamantyl). This suggests that the type of polycyclic ‘cage’ moiety used has an influence on the activity of such compound. Further studies are ongoing in our laboratory to derivatise the lead compound with the possibility of enhancing its anti-TB activity.

Experimental

The NMR spectroscopic data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using CDCl₃ as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and the coupling constants (J) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Reactions were
monitored using thin layer chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by column chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH₂Cl₂:CH₃OH:NH₄OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds was judged to be >95% based upon ¹H NMR analysis. Purification of compound 15 were done via semi-preparative HPLC on a Shimadzu, LC-6AD instrument using water (solvent A) and acetonitrile (solvent B) while methanol (as Solvent B) was used for compound 23. An ACE 5C18 150 x 21.2 mm column was used. A gradient elution system of 95 % solution A and 5 % solution B which changes linearly over 25 min to 20 % solution A and 80 % solution B at 15 mL/min, detected on a UV-VIS detector at 215 and 254 nm. Mass Spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Melting points results were uncorrected. Tetrahydrofuran was freshly distilled before use from a flask containing sodium benzophenone under N₂ atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use.

Synthesis of (E)-N'-{(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (8) [17]

To a vigorously stirred solution of ethane-1,2-diamine (55.4 g, 0.92 mol) in dichloromethane (400 mL) at -78 °C under N₂ atmosphere was dropwise added geranyl bromide 7 (2 g, 9.2 mmol) in 1200 mL of dichloromethane over 4 hours. The reaction mixture was left to attain room temperature with stirring over night. The solution mixture was reduced in vacuo to about 500 mL and washed with water to remove excess ethane-1,2 diamine, the organic extract was dried over Mg₂SO₄ and concentrated in vacuo. The crude residue was purified using column chromatography [eluent; CHCl₃:CH₃OH:NH₄OH (88:10:2)] to give a light yellow oil (1.56 g, 86 %, R_f = 0.45). ¹H NMR [CDCl₃, 400 MHz]: δH 1.36 (NH), 1.56 (3H, s, CH₃), 1.61 (3H, s, CH₃), 1.64 (3H, s, CH₃), 1.97 (2H, m, CH₂), 2.05 (2H, m, CH₂), 2.63 (2H, t, J = 5.6 Hz, CH₂), 2.78 (2H, t, J = 5.7, 6.1 Hz, CH₂), 3.21 (2H, d, J = 6.8 Hz, CH₂), 5.06 (1H, J = 6.9 Hz, CH), 5.23 (1H, J = 6.8 Hz, CH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.3 (CH₃), 17.6 (CH₃), 25.7 (CH₃), 26.5 (CH₂), 39.6 (CH₂), 41.8 (CH₂), 47.1 (CH₂), 52.1 (CH₂), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

Synthesis of N-Geranyl-N’-(trishomocubanyl)ethane-1,2-diamine (9), N-Geranyl-N’-(pentacycloundecyl)ethane-1,2-diamine (10), N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine (SQ109)

A mixture of isoprenyl diamine 8 (1.2 mol) and polycyclic ‘cage’ mono ketone (1 mol) in methanol (15 mL) was stirred for 2 h at room temperature under a nitrogen atmosphere. The resulting imine was reduced with solid NaBH₄ (1.2 mol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench excess NaBH₄. The solution was extracted with ethyl acetate (2 x 50 mL) and
the solution dried over Na₂SO₄ and concentration in vacuo. The crude product was purified via column chromatography on silica gel using CHCl₃:CH₃OH:NH₄OH (88:10:2) as eluent to give a yellow oil and converted to its HCl salt.

**Data for N-Geranyl-N’-(11-trishomocubyl)ethane-1,2-diamine dihydrochloride (9)**

A white solid (Mp; 174-178 ºC, 0.65 g, yield 57%, Rₜ = 0.64). IR vₓₜₐₜₐ: 3388, 2949, 2871, 2736, 1585, 1443, 1034, 773 and 555 cm⁻¹. MS (TOF) calculated for C₂₃H₃₇N₂ (M + H⁺ of free base) 341.2951, found 341.2940. ¹H NMR [CDCl₃, 400 MHz]: δH 1.21-1.28 (3H, m, H-4a, H-7a, H-7s), 1.37 (1H, AB, d, J = 10.2 Hz, H-4s), 1.53 (3H, s, CH₃), 1.57 (3H, s, CH₃), 1.61 (3H, s, CH₃), 1.85-1.88 (2H, m, H-1, 2), 1.91-1.96 (4H, m, H-8, 10, CH₂), 1.97-2.03 (5H, m, H-5, 6, 9, CH₂), 2.37 (1H, s, H-3), 2.63-2.69 (4H, m, 2xCH₂), 2.91 (1H, s, H-11), 3.17 (2H, d, J = 6.8 Hz, CH₂), 5.03 (1H, m, CH), 5.19 (1H, m, CH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.2 (CH₃), 17.6 (CH₃), 25.6 (CH₃), 26.4 (CH₂), 32.8 (C-4), 33.4 (C-7), 39.5 (CH₂), 40.6 (C-8), 41.4 (C-2), 44.2 (C-3), 44.7 (C-9), 46.9 (CH₂), 47.0 (C-5), 47.4 (C-6), 48.0 (CH₂), 49.2 (CH₂), 50.4 (C-10), 51.6 (C-1), 64.2 (C-11), 122.8 (CH), 124.0 (CH), 131.3 (C), 137.5 (C).

**Data for N-Geranyl-N’-(8-pentacycloundecyl)ethane-1,2-diamine dihydrochloride (10)**

A white solid (Mp; 158-160 ºC, 0.59 g, yield 55 %, Rₜ = 0.62). IR vₓₜₐₐ: 3140, 3048, 2948, 2694, 1444, 1405, 1034, 793 and 557 cm⁻¹. MS (TOF) calculated for C₂₃H₃₇N₂ (M + H⁺ of free base) 341.2951, found 341.2928. ¹H NMR [CDCl₃, 400 MHz]: δH 0.94 (1H, AB, J = 11.9 Hz, H-11a), 1.13 (1H, AB, J = 10.3 Hz, H-4a), 1.55 (3H, s, CH₃), 1.59 (3H, s, CH₃), 1.62 (3H, s, CH₃), 1.64 (1H, s, H-4s), 1.83 (2H, br s, NH), 1.91-1.98 (2H, m, CH₂), 2.02-2.07 (2H, m, CH₂), 2.16-2.18 (3H, m, H-3, 5, 9), 2.26 (1H, s, H-10), 2.31 (1H, AB, J = 11.7 Hz, H-11s), 2.43 (1H, m, H-6), 2.49-2.54 (2H, m, H-2, 7), 2.60-2.62 (1H, m, H-1), 2.63-2.70 (5H, m, H-8, 2xCH₂), 3.19 (2H, d, J = 6.8 Hz, CH₂), 5.05 (1H, t, J = 5.6 Hz, CH), 5.21 (1H, t, J = 5.9 Hz, CH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.2 (CH₃), 17.6 (CH₃), 25.7
(CH₃), 26.5 (CH₂), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4/9), 37.6 (C-8/10), 37.9 (C-6), 39.6 (CH₂), 46.5 (CH₂), 47.1 (CH₂), 49.5 (CH₂), 61.9 (C-2), 122.9 (CH), 124.1 (CH), 131.5 (C), 137.6 (C).

8-Benzylamino-8,11-oxapentacyclo[5.4.0.0²,6.0³,10.0⁵,9]undecane (11)[13]

PCU-8,11-dione (Cookson’s dione) was synthesized via photocyclization of Diels-Alder adducts obtained from reacting freshly cracked cyclopentadiene with p-benzoquinone [18,19]. PCU-dione (5g, 28.7 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 50 mL) and cooled with stirring to 5°C with an external ice bath. Benzylamine (3.39 g, 31.6 mmol) was added slowly with continuous stirring while maintaining the temperature. The reaction mixture was stirred over 30 minutes and the resulting hydroxylamine (a white precipitate) was filtered and washed with cold THF. Dehydration of the hydroxylamine in dry benzene was achieved under Dean-Stark condition for 1 h or until no more water collected in the trap. The resulting solution was concentrated in vacuo to obtain the Schiff base (a yellow oil) which was reduced with NaBH₄ (1.63 g, 43.05 mmol) in dry methanol (30 mL) and dry THF (150 mL) with stirring for 24 hr at room temperature. The solution was concentration in vacuo and water (2 x 100 mL) was added and the resulting mixture extracted with CH₂Cl₂ (4 x 50 mL) and the combined organic solution dried over MgSO₄ and concentrated in vacuo to yield a yellow oil. Purification was carried using column chromatography with solvent system; Hexane:CH₂Cl₂ (1:1) to obtain 11 as a colourless solid. Melting point: 79-80 °C, 2.73 g, 36 % yield, Rf = 0.20. ¹H NMR [CDCl₃, 400 MHz]: δH 1.55 (AB, J = 10.44 Hz, 1H), 1.91 (AB, J = 10.48 Hz, 1H), 2.14 (br s, 1H, NH), 2.42 (t, J = 4.9 Hz, 1H), 2.51-2.62 (m, 4H), 2.71-2.84 (m, 3H), 3.98 (AB, J = 13.36 Hz, 1H), 4.03 (AB, J = 13.36 Hz, 1H), 4.66 (t, J = 5.3 Hz, 1H), 7.21-7.37 (m, 5H). ¹³C NMR [CDCl₃, 100 MHz]: δC 41.5 (CH), 42.0 (CH), 43.1 (CH), 43.2 (CH₂), 44.5 (CH), 44.8 (2xCH), 47.8 (CH₂), 54.7 (CH), 55.2 (CH), 82.5 (CH), 109.5 (C), 126.8 (CH), 127.8 (CH), 128.3 (CH), 140.8 (C).

Synthesis of 8-amino-8,11-oxapentacyclo[5.4.0.0²,6.0³,10.0⁵,9]undecane (12)

To a solution of compound 11 (2.73 g, 10.3 mmol) dissolved in dry methanol (100 mL) was added 10% Pd/C (1.37 g) and stirred under hydrogen gas at atmospheric pressure for 16 h or until no starting material was observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated in vacuo and the crude product purified on silica using CH₂Cl₂:CH₃OH (95:5) to obtain 12 in pure form. A white solid (Melting point: 143-145 °C, 1.66 g, yield 60.8 % yield, Rf = 0.52). ¹H NMR [CDCl₃, 400 MHz]: δH 1.50 (AB, J = 10.44 Hz, 1H), 1.86 (AB, J = 10.48 Hz, 1H), 2.09 (NH₂), 2.30-2.37 (m, 3H), 2.51-2.56 (m, 2H), 2.69-2.80 (m, 3H), 4.56 (t, 1H). ¹³C NMR [CDCl₃, 100 MHz]: δC 41.4 (CH), 42.4 (CH), 43.3 (CH), 43.4 (CH₂), 44.8 (CH), 47.0 (CH), 55.1 (CH), 57.4 (CH), 83.0 (CH), 106.3 (C).
Synthesis of compound 8-chloroacetylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (13)

A mixture of compound 12 (1.62 g, 9.2 mmol), chloroacetyl chloride (0.807 mL, 10.1 mmol) and K₂CO₃ (2.54 g, 18.4 mmol) in dry DCM (20 mL) was stirred and heated gently at 40 ºC for 1 h and allowed to stir overnight without heat. Purification was carried on silica, CH₂Cl₂:CH₃OH (95:5) to obtain pale yellow oil which solidified on standing at room temperature to afford a yellowish solid. Melting point; 152-154 ºC, 1.44 g, 62 %, Rᵣ = 0.67. ¹H NMR [CDCl₃, 400 MHz]: δ別 1.53 (AB, J = 10.6 Hz, 1H), 1.91 (AB, 10.6 Hz, 1H), 2.42 (s, 1H), 2.59-2.62 (m, 2H), 2.74-2.79 (m, 1H), 3.06-4.07 (m, 4H), 4.02 (s, 2H), 4.73 (t, 1H). ¹³C NMR [CDCl₃, 100 MHz]: δC 41.4 (CH), 41.9 (CH), 42.6 (CH₂), 43.2 (CH₂), 43.6 (CH), 44.6 (CH), 44.8 (CH), 46.1 (CH), 55.0 (CH), 56.7 (CH), 83.7 (CH), 102.9 (C), 166.2 (C).

Synthesis of 8-[(E)-N-3,7-dimethylocta-2,6-dienylamino]acetamide-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane (14)

To a solution of geranylamine (0.33 g, 3.57 mmol) in dry THF (15 mL) was added K₂CO₃ (0.37 g) and compound 13 (0.45 g, 1.8 mmol), the mixture was stirred with reflux, and the reaction was monitored by TLC until no starting material was observed. The reaction mixture was cooled, filtered and concentrated in vacuo, the crude product was purified on silica gel; CHCl₃:CH₃OH (95:5) to obtain 14. A yellow oil (380 mg, 57.5 % yield, Rᵣ = 0.34.). IR vₘₐₓ: 3308, 2966, 1672, 1514, 1002 and 747 cm⁻¹. MS (TOF) calculated for C₂₃H₃₃N₂O₂ (M + H⁺) 369.2537, found 369.2537. ¹H NMR [CDCl₃, 400 MHz]: δ別 1.54 (1H, AB, J = 10.5 Hz, H-4a), 1.57 (3H, s, CH₃), 1.58 (3H, s, CH₃), 1.59 (3H, s, CH₃), 1.90 (1H, AB, J = 10.5 Hz, H-4s), 1.95-2.06 (4H, m, 2xCH₂), 2.41 (1H, t, J = 4.76 Hz, H-3), 2.53 (1H, m, H-5), 2.57-2.60 (1H, m, H-2), 2.69-2.73 (1H, m, H-6), 2.85-3.00 (4H, m, H-1, 7, 9, 10), 3.18 (2H, d, J = 6.88 Hz, CH₂), 3.19 (2H, s, CH₂), 4.69 (1H, t, J = 5.16 Hz, H-11), 5.02-5.06 (1H, m, CH), 5.13-5.17 (1H, m, CH), 7.94 (CONH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.2 (CH₃), 17.7 (CH₃), 25.6 (CH₂), 26.4 (CH₂), 39.5 (CH₂), 41.4 (C-2), 41.8 (C-2), 43.4 (C-4), 43.5 (C-5), 44.6 (C-1), 44.8 (C-3), 45.9 (C-7), 47.1 (CH₂), 52.2 (CH₂), 54.8 (C-10), 56.4 (C-9), 83.5 (C-11), 102.2 (C-8), 121.7 (CH), 123.9 (CH), 131.6 (C), 139.0 (C), 172.1 (C=O).

Synthesis of 11-hydroxypentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]-undecane-8-amoenoethyl[(E)-N-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (15)

Compound 14 (460 mg, 1.25 mmol) dissolved in dry THF (15 mL) was added LAH (0.24 g, 6.25 mmol) gently; the mixture was refluxed overnight under nitrogen atmosphere. The reaction vessel was cooled and the mixture quenched with aqueous Na₂SO₄, the obtained precipitate was filtered off and the filtrate concentrated to obtain the crude product. The crude product was purified via preparative HPLC (as specified above, retention time: 9.1 min) sample was lyophilized to obtain a
(150 mg, 33.7 % yield) and converted to its HCl salt to obtain a yellowish slurry. IR \( \nu_{\text{max}} \): 3149, 3048, 2960, 2809, 1452, 1071 and 568 cm\(^{-1}\) MS (TOF) calculated for C\(_{23}\)H\(_{37}\)N\(_2\)O (M + H\(^+\) of free base) 357.2900, found 357.2897. \(^1\)H NMR [CDCl\(_3\), 400 MHz]: \( \delta_{\text{H}} \) 1.08 (1H, AB, \( J = 10.8 \) Hz, H-4a), 1.53 (3H, s, CH\(_3\)), 1.61-1.63 (7H, m, H-4s, 2xCH\(_2\)), 1.98-2.01 (4H, m, 2xCH\(_2\)), 2.30-2.38 (3H, m, H-3, 5, 10), 2.50-2.55 (2H, m, H-2, 9), 2.64-2.69 (2H, m, H-1, 6), 2.80 (1H, s, H-7), 2.94 (s, 1H, H-8), 3.18 (4H, s, 2xCH\(_2\)), 3.49 (2H, d, \( J = 7.2 \) Hz, CH\(_2\)), 3.86 (1H, t, \( J = 3.2 \) Hz, H-11), 4.98 (1H, t, \( J = 5.2 \) Hz, CH), 5.22 (1H, t, \( J = 6.7 \) Hz, CH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: \( \delta_{\text{C}} \) 16.4 (CH\(_3\)), 17.6 (CH\(_3\)), 25.6 (CH\(_3\)), 26.1 (CH\(_2\)), 34.0 (C-4), 34.9 (C-7), 37.8 (C-1), 39.5 (CH\(_2\)), 39.7 (C-2), 40.2 (C-6), 42.1 (C-9), 43.0 (C-3), 43.1 (CH\(_2\)), 43.2 (CH\(_2\)), 44.7 (C-5/10), 44.8 (C-5/10), 45.3 (CH\(_2\)), 57.4 (C-8), 70.4 (C-11), 114.2 (CH), 123.3 (CH), 131.9 (C), 145.2 (C).

**Synthesis of 8,11-oxapentacyclo[5.4.0.0\(^2\)6\(^4\)0\(^3\)10\(^5\)9\]undecane-8-aminoethyl[(E)-N-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (16)**

To a solution of compound 14 (0.38 g, 1.03 mmol) in dry THF (10 mL) at 0 °C was added slowly 65 % Red Al in toluene (1.02 mL, 5.16 mmol) and kept at this temperature for 10 minutes. The reaction was kept at 35 °C for an hour and refluxed. The reaction was monitored until no starting material was observed on TLC. THF (20 mL) was added to the reaction mixture and quenched with 5N NaOH (10 mL), the organic layer was separated, dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo}. The crude product was purified on silica gel; the column was first flushed with 100 mL of CHCl\(_3\):CH\(_3\)OH (95:5) after which a CHCl\(_3\):CH\(_3\)OH:NH\(_2\)OH (88:10:2) as eluent mixture was introduced to obtain 16 (165 mg, 45.2 %, \( R_f = 0.57 \)) in pure form and converted to its HCl salt to obtain a yellow slurry. IR \( \nu_{\text{max}} \): 3359, 2960, 1449, 1370, 1009 and 556 cm\(^{-1}\). MS (TOF) calculated for C\(_{23}\)H\(_{35}\)N\(_2\)O (M + H\(^+\) of free base) 355.2744, found 355.2744. \(^1\)H NMR [CDCl\(_3\), 400 MHz]: \( \delta_{\text{H}} \) 1.52 (1H, AB, \( J = 10.5 \) Hz, H-4a), 1.57 (3H, s, CH\(_3\)), 1.62 (3H, s, CH\(_3\)), 1.65 (3H, s, CH\(_3\)), 1.88 (1H, AB, \( J = 10.5 \) Hz, H-4s), 1.97-2.07 (4H, m, 2xCH\(_2\)), 2.39 (1H, t, H-3), 2.46 (1H, t, \( J = 4.7 \) Hz, H-5), 2.50-2.59 (3H, m, H-2, 7, 9), 2.66-2.69 (1H, m, H-6), 2.73-2.81 (4H, m, H-1, 10, CH\(_2\)), 3.00 (2H, t, \( J = 5.4 \), 5.9 Hz, CH\(_2\)), 3.29 (2H, d, \( J = 6.96 \) Hz, CH\(_2\)), 4.58 (1H, t, \( J = 5.2 \) Hz, H-11), 5.03-5.07 (1H, m, CH), 5.22-5.26 (1H, m, CH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: \( \delta_{\text{C}} \) 16.4 (CH\(_3\)), 17.7 (CH\(_3\)), 25.7 (CH\(_3\)), 26.5 (CH\(_2\)), 39.6 (CH\(_2\)), 41.5 (C-2), 41.9 (C-6), 42.3 (CH\(_2\)), 43.1 (C-5), 43.2 (C-4), 44.5 (C-1, 7), 44.8 (C-3), 46.3 (CH\(_2\)), 49.3 (CH\(_2\)), 54.7 (C-10), 55.1 (C-9), 82.5 (C-11), 110.1 (C-8), 120.2 (CH), 124.0 (CH), 131.8 (C), 140.4 (C).
Synthesis of pentacyclo[5.4.0.0^2,6.0^3,10.0^5,9]undecane-8,11-dione ethylene acetal (17)[27-29]

A mixture of PCU dione 4 (10g, 57 mmol), ethylene glycol (4.5 mL, 80 mmol) and p-toluenesulfonic acid (0.33g, 1.9 mmol) was dissolved in toluene (45 mL) and refluxed using a Dean-Stark apparatus for three days. The reaction mixture was allowed to cool down followed by addition of cold aqueous solution of 10% (m/v) Na₂CO₃ (60 mL) and extracted with dichloromethane (3 x 25 mL). The organic layer was dried using anhydrous Na₂SO₄. The mixture was filtered and the solvent evaporated in vacuo. The crude product was purified via silica gel column chromatography (40:60; EtOAc: Hexane) and obtained as a white solid (Melting point = 71 ºC; 10.3 g, 80 %, R_f = 0.55). ¹H NMR [CDCl₃, 400 MHz]: δH 1.55 (AB, J = 11.0 Hz, 1H), 1.84 (AB, J = 11.0 Hz, 1H), 2.37-2.38 (m, 1H), 2.44-2.47 (m, 1H), 2.51-2.63 (m, 3H), 2.74-2.79 (m, 2H), 2.89-2.94 (m, 1H), 3.80-3.91 (m, 4H).

¹³C NMR [CDCl₃, 100 MHz] δC 36.3 (CH), 38.7 (CH₂), 41.3 (CH), 41.4 (CH), 42.2 (CH), 42.8 (CH), 45.8 (CH), 50.7 (CH), 52.9 (CH), 64.4 (CH₂), 65.6 (CH₂), 113.8 (C), 215.2 (C).

Synthesis of 4-benzyl-4-azahexacyclo[5.4.1.0^2,6.0^3,10.0^5,9.0^8,11]dodecan-3-ol (19)

A mixture of compound 17 (1g, 4.59 mmol) and benzylamine (0.59g, 5.5 mmol) in EtOH (10 mL) was heated at 100 ºC using a sealed high pressure glass tube for 16 h. The solution was cooled and NaBH₄ (0.35g, 9.17 mmol) was added gradually and the mixture was stirred at room temperature for 8 h. The solution was concentrated in vacuo, water (20 mL) was added and the intermediate was extracted twice with a 3x15 mL portion of CH₂Cl₂. The combined organic solution was washed with brine (15 mL) dried (MgSO₄) and concentrated in vacuo. To the crude product was added acetone (30 mL) and aqueous 4M HCl (20 mL) with stirring at room temperature for 12 hours. Water was added (250 mL) and the solution was basified to pH 14 with aqueous 1M NaOH and extracted with CH₂Cl₂ (3x25 mL). The combined organic extract was dried over MgSO₄ and concentrated to obtain crude product, which was recrystallised from isopropanol to yield the desired product 19 (0.35 g, 29 % yield). Melting point; 158-159 ºC; ¹H NMR [DMSO-d₆, 400 MHz]: δH 1.43 (AB, J = 10.2 Hz, 1H), 1.76 (AB, J = 10.2 Hz, 1H), 2.27 (t, J = 4.7 Hz, 1H), 2.46-2.51 (m, 4H), 2.60-2.69 (m, 2H), 2.77-2.80 (m, 1H), 3.17 (t, J = 5.0 Hz, 1H), 3.23 (br, 1H), 3.61 (d, J = 13.3 Hz, 1H), 5.76 (s, OH), 7.17-7.31 (m, 5H). ¹³C NMR [DMSO-d₆, 100 MHz] δC 41.2 (CH), 41.5 (CH), 41.6 (CH), 41.8 (CH₂), 42.5 (CH), 44.7 (CH), 45.1 (CH), 50.4 (CH), 50.8 (CH₂), 55.3 (CH), 65.2 (CH), 105.9 (C), 126.2 (CH), 127.9 (CH), 128.4 (CH), 140.6 (C).

Synthesis of 4-azahexacyclo[5.4.1.0^2,6.0^3,10.0^5,9.0^8,11]dodecan-3-ol (20)

To a solution of compound 18 (2.2 g, 8.3 mmol) in of dry CH₃OH (30 mL) was added gently 10 % Pd/C (1.1 g) and stirred under H₂ atmospheric pressure for 16 h or until no starting material was
observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated in vacuo and the crude product purified on silica using CHCl₃:CH₃OH:NH₄OH (88:10:2) to obtain 19 in pure form as a white solid (M.p: 81-83 °C, 0.64 g, yield 44.1 %, Rₜ = 0.30). ¹H NMR [CDCl₃, 400 MHz]: δH 1.48 (AB, J = 10.5 Hz, 1H), 1.83 (AB, J = 10.4 Hz, 1H), 2.37-2.46 (m, 3H), 2.57-2.62 (m, 2H), 2.67-2.82 (m, 3H), 3.53 (t, J = 5.0 Hz, 1H). ¹³C NMR [CDCl₃, 100 MHz]: δC 41.6 (CH), 41.8 (CH₂), 42.6 (CH), 43.4 (CH), 44.9 (CH), 45.8 (CH), 46.1 (CH), 54.5 (CH), 55.2 (CH), 60.5 (CH) 105.9 (C).

Synthesis of (E)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (21)

A mixture of chloroacetyl chloride (0.88 g, 7.8 mmol), geranylamine (1 g, 6.5 mmol) and K₂CO₃ (1.08 g, 7.8 mmol,) in dry THF (30 mL) was refluxed for 16 h. The solution was filtered and concentrated in vacuo and purified on silica using CHCl₃:EtOAc (70:30) to afford (E)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide [20, 1.20 g, 81 % yield, Rₜ = 0.80]. ¹H NMR [CDCl₃, 400 MHz]: δH 1.56 (s, 3H) 1.64 (s, 6H), 1.97-2.08 (m, 4H), 3.86 (t, J = 6.24 Hz, 2H), 4.00 (s, 2H), 5.03 (t, J = 6.72 Hz, 1H), 5.16 (t, J = 6.96 Hz, 1H). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.2 (CH₃), 17.6 (CH₃), 25.6 (CH₃), 26.2 (CH₂), 37.7 (CH₂), 39.3 (CH₂), 42.5 (CH₂), 118.9 (CH), 123.6 (CH), 131.8 (C), 140.7 (C), 165.5 (C=O).

Synthesis of 4-[(E)-N-3,7-dimethylocta-2,6-dienyl]acetamide-4-azahexacyclo[5.4.1.0.0.2,6 .0³.⁰⁵.⁰⁸.¹¹]dodecan-3-ol (22)

To a stirred solution of compound 20 (0.3 g, 1.7 mmol) in dry THF (15 mL) was added (E)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (21, 0.39 g, 1.7 mmol) and K₂CO₃ (0.35 g, 2.55 mmol). The mixture was then refluxed overnight under N₂ atmosphere. The reaction was allowed to cool to room temperature, filtered and concentrated in vacuo. The crude product was purified on silica using CHCl₃:CH₃OH (90:10,) to afford the product 22 as light yellow oil. (445 mg, 72 % yield, Rₜ = 0.53) IR vmax: 3230, 2966, 2868, 1661, 1348 and 728 cm⁻¹. MS (TOF) calculated for C₂₃H₃₃N₂O₂ (M + H⁺) 369.2537, found 369.2539. ¹H NMR [CDCl₃, 400 MHz]: δH 1.49 (3H, s, CH₃), 1.52 (1H, m, H-4a), 1.56 (3H, s, CH₃), 1.57 (3H, s, CH₃), 1.84 (1H, m, H-4s), 1.87-1.98 (4H, m, 2xCH₂), 2.56 (1H, s, H-3), 2.66-2.70 (2H, m, H-2, 5), 2.78-2.84 (3H, m, H-6, 7, 9), 2.95 (1H, s, H-10), 3.06 (1H, s, H-1), 3.39 (br s, 1H), 3.71 (d, J = 15.4 Hz, 1H), 3.75 (2H, m, CH₂), 4.05 (1H, t, J = 4.8 Hz, H-11), 4.97 (1H, t, J = 5.6 Hz, CH), 5.08 (1H, t, J = 6.3 Hz, CH), 7.93 (s, CONH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.1 (CH₃), 17.5 (CH₃), 25.4 (CH₃), 26.2 (CH₂), 37.5 (CH₂), 39.3 (CH₂) 40.4 (C-1), 41.1 (C-4), 41.4 (C-2, 6), 43.2 (C-7), 43.8 (C-5), 46.2 (C-3), 47.5 (CH₂), 49.6 (C-10), 52.8 (C-9), 65.9 (C-11), 111.8 (C-8), 119.1 (CH), 123.6 (CH), 131.4 (C), 139.5 (C), 164.5 (C=O).
Synthesis of 4-[(E)-N-ethyl-3,7-dimethyl octa-2,6-dienyl]amine-4-azahexacyclo[5.4.1.0^{.2}.0^{3}.0^{5}.0^{8}.0^{11}]dodecan-3-ol dihydrochloride (23)

To a solution of compound 22 (0.36 g, 0.97 mmol) in dry THF (10 mL) at 0 ºC was added slowly 65 % Red Al in toluene (0.58 mL, 2.9 mmol) and the mixture was kept at this temperature for 10 minutes. The reaction was kept at 35 ºC for an hour and refluxed; the reaction was monitored until no starting material was observed on LC-MS. 20 mL of THF was added to the reaction mixture and quenched with 5N NaOH (10 mL), the organic layer was separated, dried over Na2SO4 and concentrated in vacuo. The crude product was dissolved in methanol (5 mL) and purified using preparative HPLC (as specified above, retention time: 13.6 mins). The sample was lyophilized to obtain 23 (200 mg, 55 % yield) and converted to its HCl salt to obtain a light yellow slurry. IR νmax: 2967, 2866, 1668, 1198, 1129, 831 and 719 cm⁻¹. MS (TOF) calculated for C₂₃H₃₅N₂O (M + H⁺ of free base) 355.2744, found 355.2748. ¹H NMR [CDCl3, 400 MHz]: δH 1.57 (3H, s, CH₃), 1.60 (1H, H-4a), 1.65 (6H, s, 2xCH₃), 1.93 (1H, d, J = 10.92 Hz, H-4s), 2.00-2.07 (4H, m, 2xCH₂), 2.60 (1H, m, H-3), 2.73 (1H, m, H-2), 2.78 (1H, m, H-5), 2.83-2.87 (3H, m, H-6, 7, 9), 2.96-2.98 (1H, m, H-10), 3.01-3.24 (5H, m, CH₂, 2xCH₂), 3.41 (2H, d, J = 7.16 Hz, CH₂), 3.90 (1H, t, J = 5.1 Hz, H-11), 5.03 (1H, m, CH), 5.21 (1H, t, J = 6.5 Hz, CH), 5.61 (OH, NH). ¹³C NMR [CDCl₃, 100 MHz]: δC 16.4 (CH₃), 17.7 (CH₃), 25.7 (CH₃), 26.3 (CH₂), 39.6 (CH₂), 41.1 (C-1), 41.2 (C-4), 41.6 (C-2), 41.7 (C-6), 43.5 (C-7), 44.1 (C-5), 44.3 (CH₃), 44.5 (CH₂), 45.9 (CH₂), 46.3 (C-3), 50.1 (C-10), 53.5 (C-9), 67.3 (C-11), 113.5 (C-8), 117.0 (CH), 123.7 (CH), 132.1 (C), 143.4 (C).

Biological testing

Compounds 9, 10, 14, 15, 16, 22, 23 and SQ109 were first dissolved in 100 % Methanol, sonicated and filter sterilized using 0.22 µm polycarbonate sterile filters to obtain a stock concentration of 10 mg/mL. This was diluted in sterile water and twofold serial dilutions were made to give working concentration ranges of 8 µg/mL to 0.125 µg/mL. 1mL volume of each concentration was aliquoted in cryovials and stored at -70 ºC.

Bactec MGIT 960 analysis

Susceptibility testing with the BACTEC MGIT 960 system (Becton Dickinson) was performed according to the manufacturer’s recommendations. Mycobacterial work was carried out in a level III biosafety laboratory. M.tuberculosis reference strain H₃₇Rv (ATCC No. 25177), MDR (drug sensitivity: isoniazid > 0.2 µg/mL, rifampicin > 1.0 µg/mL and EMB > 5 µg/mL) and XDR strains (drug sensitivity: isoniazid > 0.2 µg/mL, rifampicin > 1.0 µg/mL, EMB > 5 µg/mL, streptomycin > 2.0 µg/mL, ofloxacin > 2.0 µg/mL and kanamycin > 5.0 µg/mL) were cultured in Middlebrook 7H9 medium [30], enriched with OADC (0.00 5%, v/v, oleic acid; 0.5 %, 171 w/v, BSA; 0.2 %, w/v,
glucose; 0.02 %, v/v, catalase and 0.085 %, w/v, NaCl) and incubated at 37 ºC. Freshly grown cultures were used to prepare a standardised inoculum in a sterile tube containing 4.5 mL phosphate buffer, 0.05 % tween 80 with glass beads (5mm diameter) by vortexing. Once the clumps were allowed to settle for 45 min, the supernatant was aspirated and adjusted to a McFarland No. 1 standard, equivalent to a $10^7$ colony forming units CFU/mL. 0.5 mL of the standardised inoculum was diluted tenfold to obtain a final concentration of $10^5$ CFU/mL, after which 0.5 mL of the standardised inoculum was added to each of the MGIT containing the compounds. A 1:100 inoculum dilution was used to inoculate the drug free control tubes. This represents 1 % of the bacterial population. The MGITs were loaded in the BACTEC 960 drawers and the MIC was determined to be the lowest dilutions that were negative by the automated system in the compound containing tubes when the control tube showed positive. Antimycobacterial analysis of all compounds was done in triplicate.

All compounds containing tubes belonging to the positive drug free control were unloaded from the BACTEC 960 system and a Ziehl Neelson stain was performed to confirm the presence of *M. tuberculosis*[31]. Colony counts of the test inoculum were also prepared by plating out 20 µL onto Middelbrook 7H11 agar plates. Plates were incubated aerobically at 37 ºC for 21 days.

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**References**

CHAPTER 8
NOVEL LINEAR DIAMINE DISUBSTITUTED POLYCYCLIC ‘CAGE’ DERIVATIVES AS POTENTIAL ANTI-MYCOBACTERIAL CANDIDATES

Oluseye K. Onajole,a Sphelele Sosibo,a Patrick Govender,b Thavendran Govender,c Paul D. van Helden,d Glenn E. M. Maguire,a Kata Mlinarić-Majerski,e Ian Wiid,d and Hendrik G. Kruger,a*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa
c School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa
d Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa
e Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia

Abstract

As part of an ongoing project to develop highly potent anti-tuberculosis therapeutics, a series novel polycyclic ‘cage’ tetra-amines were synthesized and screened for in-vitro anti-tuberculosis activities against the H37Rv strain of tuberculosis. Three disubstituted polycyclic moieties, namely pentacyclodecane, pentacycloundecane (PCU), and tricyclodecane were used in this study. Compounds 5 and 7 showed similar activity to SQ109 at a MIC of 1 µM while compounds 4, 6 and 8 displayed MIC activity at 1<MIC<10 µM. This study demonstrates the first reported analysis of pentacyclo[5.3.0.02,5.03,9.04,8]decane as a potential therapeutic agent.

Keywords: Anti-tuberculosis activity, SQ109, pentacyclodecane, pentacycloundecane and tricyclodecane, isoprenyl diamine

Introduction

Tuberculosis is a curable deadly contagious disease and has been in existence for over 5 centuries. According to the 2009 Global tuberculosis control report of the World Health Organisation, it is estimated that about 9.4 million incident cases of TB occurred globally. Of these cases an estimated 1.4 million were HIV positive of which 78 % were in Africa while 13 % are located in South-East

* Corresponding author. Tel.: +27-31-2602181; Fax: +27-31-2603091 Email address: kruger@ukzn.ac.za (H. G. Kruger); Homepage: http://ggkm.ukzn.ac.za
Asia. An estimate of 1.3 million deaths was reportedly caused by TB among HIV negative people. South Africa has the highest percentage of HIV patients living with tuberculosis (1).

The need for the development of new, faster-acting therapeutics with reduced side effects and which are active against emerging drug resistant TB strains cannot be overemphasized. Researchers over the last decade have indentified numerous potent anti-tuberculosis candidates some of which are presently undergoing advanced stages of clinical trials; these compounds include derivatives containing diamine (SQ109), diaryl quinoline (TMC 207), nitroimidazooxazine (PA-824), pyrrole (LL-3858) and nitrodihydro-imidazooxazole (OPC-67683) functional groups (2-4).

SQ109 ($N$-Geranyl-$N'$-(2-adamantyl)ethane-1,2-diamine) first reported in 2003 by Lee et al(5) is an analogue of ethambutol possessing remarkable activity against MDR (multi-drug resistant)(6) and XDR (extensively-drug resistant) TB (7). SQ109 also has excellent distribution into various tissues, with the highest concentration in the lung followed by the spleen and kidney tissues (8). SQ109 recently completed phase one human clinical trials (9).

As part of our ongoing studies aimed at developing new polycyclic ‘cage’ amines with anti-TB properties,(7, 10) we recently reported the activity of novel SQ109 derivatives. Compound 1 ($N$-trans-trans farnesyl -$N'$-(2-adamantyl)ethane-1,2-diamine) was discovered to be more active than SQ109 suggesting that the length of the isoprenyl group plays a significant role in its activity (7). We also reported novel pentacycloundecane (PCU) cyclic tetra-amine derivatives (Figure 1, compounds 2 and 3) with excellent activity against susceptible and resistant (XDR) strains of tuberculosis (10).

![Figure 1: Structure of SQ109 and compounds 1-3](image)

Inspired by this, we synthesized a series of novel disubstituted polycyclic ‘cage’ bearing linear diamine isoprenyl moieties and screened against *M. tuberculosis*. It was postulated that disubstitution
on the polycyclic ‘cage’ moieties compared to the mono-substituted **SQ109** (adamantyl moiety), might improve the activity as an anti-TB agent. Three polycyclic ‘cage’ moieties pentacyclo[5.4.0.0²,6.3,10.0⁵,9]undecane, tricyclo[3.3.1.1³,7]decane and pentacyclo[5.3.0.0²,5.0³,9.0⁴,8]decane were employed in this study. Over a century ago, Thiele(11) reported the carbonation of cyclopentadienylpotassium to obtain a Diels-Alder dimer of cyclopentadiene carboxylic acid (“Thiele’s acid”). Pentacyclo[5.3.0.0²,5.0³,9.0⁴,8]decane-2,5-dicarboxylic acid which was used in this study was synthesized from Thiele’s acid (12). Although this 1,3-bishomocubane (pentacyclodecane) has been extensively studied with regards to its geometry (C₂ symmetry) and chirality (12-17); its use in medicinal chemistry has not yet been investigated. This is the first time that a pentacyclodecane based derivative has been evaluated for potential pharmaceutical properties.

![Diagram of polycyclic 'cage' moieties](image)

Figure 2: Structure of polycyclic ‘cage’ tetra-amine derivatives (4-8)
Results and discussion

Chemistry

Following similar methodology as reported in literature,(7) (E)-N’-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (10) was reacted with 2-adamantanone (1.2:1) (reductive amination) to obtain SQ109 (57%). The polycyclic diacid compounds were synthesized as reported in literature. PCU diol diacid 11,(18) PCU ether diacid 12,(19, 20) 1,3-adamantanecarboxylic acid (13),(21) 1,3-adamantane-1,2-dicarboxylic acid (14)(22) and pentacyclodecane-2,5-diacid (15)(12, 15, 16) were reacted with glycine methyl ester using coupling reagents hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-methylmorphine (NMM) to obtain compounds 16 (51.5 %), 17 (68.7 %), 18, 19 (quantitative), and 20 (42 %) respectively.

Scheme 1:  Reagents and conditions: (a) ethylene diamine (100:1), -78 °C, dry DCM; (b) MeOH, 2-adamantanone (1.2:1), N2 atmosphere, 2 h, NaBH4, overnight.

PCU dimethoxyl diamide 16 and 17, adamantyl dimethoxyl diamide 18 and 19, and pentacyclodecyl dimethoxyl diamide 20 were hydrolyzed using lithium hydroxide in THF to obtain the corresponding polycyclic glycine derivatives 21, 22, 23, 24 and 25 in quantitative yields. Polycyclic ‘cage’ glycine derivatives 21-25 were coupled with geranylamine(7) using HOBT, EDC and NMM as coupling reagents to obtain compounds 26 (28 %), 27 (59 %), 28 (69 %), 29 (72 %) and 30 (67 %). Compounds 27 and 30 were purified on preparative HPLC while compounds 26, 28 and 29 were re-crystallized from acetonitrile to obtain white crystalline solids. Reduction of the tetra-carbonyl groups of PCU derivatives 26 and 27 were achieved using LiAlH4 at a 1:20 ratio to yield compounds 4 and 5 (30 and 34 %). Interestingly, the reduction of 26 yielded both 4 and 5 as products, suggesting that LAH dehydrated the diol to form an ether bridge. Separation was achieved using preparative HPLC. Following similar reduction conditions, compounds 28, 29 and 30 were reduced to their corresponding tetra-amines 6 (41.8 %), 7 (33.0 %) and 8 (28.1 %) respectively (Scheme 2). Purification of compounds 4, 5, 6, 7 and 8 was achieved via preparative HPLC using acetonitrile and water as the eluent; it should be noted that compound 8 was obtained as a racemate. All compounds were characterized by 1H, 13C (APT), COSY, NOESY/ROESY, HSQC, HMBC, IR
and HR-MS experiments. Compounds 4, 5, 6 and 7 with the exception of 8 are meso compounds thus simplifying their elucidation using 2D NMR techniques.

Scheme 2: (a) DMF, glycine methyl ester, EDC, HOBt and NMM, stirring, 16 hr; (b) THF, 1M LiOH solution, stirring overnight; (c) DMF, geranylamine, EDC, HOBt and NMM, stirring, 16 hr; (d) dry THF, LiAlH₄ (1:20), reflux 5 days, N₂ atm.

**Anti-tubercular activity**

The anti-TB activity of the novel polycyclic compounds 26-30 were assayed against *M. tuberculosis* (H₃7Rv). The inhibitory activity of all these compounds is summarized in Table 1, together with the results obtained for SQ109. No anti-TB activity was observed for precursors 26-30 even at the highest tested concentration (50 µM), suggesting that the carbonyl group contributes negatively to the activity of these compounds. Compound 8 was less active than SQ109; this compound has a carbon atom spacer between the pentacyclocdecyl moiety and the bis-isoprenyl diamine moiety suggesting that spacer length maybe critical to activity.

Compound 6 having one carbon atom spacer between the ‘cage’ and bis-isoprenyl diamine was not as active as its two atom spacer analogue 7, once again suggesting that spacer length contributes to the
efficacy of these molecules. Compounds 4, 5 (pentacycloundecyl) and 7 (adamantyl) are quite similar as they have a two carbon atom spacers. Diol 4, however was not as active, whereas compound 5 with an ether bridge was as effective as 7 both being in the same range as SQ109. We have previously reported a study of PCU ether cyclic tetra-amines (1-3) (two “armed” ether compounds similar to 6) that displayed excellent activity against both susceptible and resistant strains of tuberculosis (10).

Table 1: In-vitro activity of compounds 4-8, 27-30 against M. tuberculosis H37Rv strain

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. formula</th>
<th>MW (gmol⁻¹)</th>
<th>MIC (µM, H37Rv)</th>
</tr>
</thead>
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<tr>
<td>4</td>
<td>C₃₉H₆₆N₄O₂</td>
<td>622.5</td>
<td>1&lt;MIC&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>C₃₉H₆₄N₄O</td>
<td>604.5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>C₃₆H₆₄N₄</td>
<td>552.5</td>
<td>1&lt;MIC&lt;10</td>
</tr>
<tr>
<td>7</td>
<td>C₃₈H₆₈N₄</td>
<td>580.5</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>C₃₈H₆₀N₄</td>
<td>548.5</td>
<td>1&lt;MIC&lt;10</td>
</tr>
<tr>
<td>26</td>
<td>C₃₉H₅₈N₄O₆</td>
<td>678.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>27</td>
<td>C₃₉H₅₆N₄O₅</td>
<td>660.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>28</td>
<td>C₃₆H₅₆N₄O₄</td>
<td>608.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>29</td>
<td>C₃₈H₆₀N₄O₄</td>
<td>636.5</td>
<td>&gt;50</td>
</tr>
<tr>
<td>30</td>
<td>C₃₆H₅₂N₄O₄</td>
<td>604.4</td>
<td>&gt;50</td>
</tr>
<tr>
<td>SQ109</td>
<td>C₂₂H₃₈N₂</td>
<td>330.3</td>
<td>1</td>
</tr>
</tbody>
</table>

The MICs are reported as the Standard derivation of ≤ 6.36 with the exception of compounds 26-30.

Conclusion

We have synthesized and evaluated a series of novel polycyclic linear tetra-amines as inhibitors of M. tuberculosis H37Rv. This study has investigated the use of a number of disubstituted polycyclic ‘cages’ with isoprenyl diamines side arms. Compounds 6 and 8 (one carbon atom spacers) gave encouraging inhibitory results. This is the first example of the pentacyclodecane being employed in medicinal chemistry. Compounds 5 and 7 (two carbon atom spacer) were the most efficacious. As in our previous studies an ether functional group tends to improve overall activity.
Experimental Section

General

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments NMR spectrometer using Topspin 2.1 (Bruker, Karlsruhe, Germany). The chemical shifts were referenced to the solvent peak for CDCl$_3$ ($^1$H: 7.24 ppm, $^{13}$C: 77.0 ppm), D$_2$O ($^1$H: 4.79 ppm), CD$_3$OD ($^1$H: 3.32 ppm, $^{13}$C: 49.3 ppm) at 25 ºC. All chemical shifts ($\delta$) were quoted in parts per million downfield from TMS and the coupling constants ($J$) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. All reactions were monitored using thin layer chromatography (TLC, Merck Kieselgel 60, F254). Purifications were carried by column chromatography using Fluka Kieselgel 60 (70–230 mesh). Purification of final compounds was done via semi-preparative HPLC on a Shimadzu, LC-6AD instrument using water (solvent A) and Acetronitrile (solvent B). An ACE 5C18 150 x 21.2 mm column was used. A gradient elution system of 95 % solvent A and 5 % solvent B which changes linearly over 25 min to 20 % solvent A and 80 % solvent B and stays at 95 % solvent B (5 % solvent A) for 5 extra minutes at 15 ml/min was used and detected on a UV-VIS detector at 215 and 254 nm. High Resolution Mass Spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode with an internal calibration while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3 and melting points are uncorrected. Tetrahydrofuran was freshly distilled from a flask containing sodium benzophenone under N$_2$ atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use.

Synthesis of (E)-N’-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (10)

To a vigorously stirred solution of ethylene diamine (55.4 g, 0.92 mol) in dichloromethane (400 mL) at -78 ºC under N$_2$ atmosphere was dropwise added geranyl bromide 9 (2 g, 9.2 mmol) in 1200 mL of dichloromethane over 4 hours. The reaction mixture was left to attain room with stirring over night. The solution mixture was reduced in vacuo to about 500 mL and washed with water to remove excess ethane-1,2 diamine, the organic extract was dried over Mg$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified using column chromatography [eluent; CHCl$_3$:CH$_3$OH:NH$_4$OH (88:10:2)] to give a light yellow oil (1.56 g, 86 %, R$_f = 0.45$). $^1$H NMR [CDCl$_3$, 400 MHz]: $\delta$H 1.16 (NH), 1.56 (3H, s, CH$_3$), 1.61 (3H, s, CH$_3$), 1.64 (3H, s, CH$_3$), 1.97 (2H, m, CH$_2$), 2.05 (2H, m, CH$_2$), 2.63 (2H, t, $J = 5.6$ Hz, CH$_2$), 2.78 (2H, t, $J = 5.7$, 6.1 Hz, CH$_2$), 3.21 (2H, $d, J = 6.8$ Hz, CH$_2$), 5.06 (1H, $J = 6.9$ Hz, CH), 5.23 (1H, $J = 6.8$ Hz, CH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta$C 16.3 (CH$_3$), 17.6 (CH$_3$), 25.7 (CH$_3$), 26.5 (CH$_2$), 39.6 (CH$_2$), 41.8 (CH$_2$), 47.1 (CH$_2$), 52.1 (CH$_2$), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).
Data for SQ109 [N-Geranyl-N′-(2-adamantyl)]ethane-1,2-diamine

A mixture of isoprenyl diamine 10 (0.35 g, 1.78 mmol) and 2-adamantane (0.29 g, 1.96 mmol) in methanol (10 mL) was stirred for 2 h at room temperature under a nitrogen atmosphere. The resulting imine was reduced with solid NaBH₄ (0.13 g, 3.56 mmol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench the excess NaBH₄. The solution was extracted with ethyl acetate (2 x 50 mL), the combined organic extracts dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified via column chromatography on silica gel using CHCl₃:CH₃OH:NH₄OH (88:10:2, Rₜ = 0.6) as eluent to obtain a colourless oil (57 % yield). HR-MS calculated for C₂₂H₃₉N₂ (M+H)⁺ 331.3108, found 331.3135; ¹H NMR (CDCl₃, 400 MHz): δH 1.47 (2H, d, J = 12.5 Hz, H-4a/9a), 1.57 (3H, s, CH₃), 1.61 (3H, s, CH₃), 1.65 (3H, s, CH₃), 1.66-1.68 (4H, m, H-6, 8a/10a), 1.74 (1H, s, H-5), 1.80-1.82 (5H, m, H-1, 3, 7, 8b/10b), 1.95 (2H, d, J = 12.6 Hz, H-4b/9b), 1.98-2.00 (2H, m, CH₂), 2.03-2.09 (2H, m, CH₂), 2.67 (1H, s, H-2), 2.70 (4H, s, 2CH₂), 3.22 (2H, d, J = 6.8 Hz, CH₂), 5.06 (1H, m, CH), 5.24 (1H, t, J = 6.7 Hz, CH). ¹³C NMR (CDCl₃, 100 MHz); δC 16.3 (CH₃), 17.6 (CH₃), 25.7 (CH₃), 26.5 (CH₂), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4/9), 32.1 (C-1/3), 37.6 (C-8/10), 37.9 (C-6), 39.6 (CH₂), 46.5 (CH₂), 47.1 (CH₂), 49.5 (CH₂), 61.9 (C-2), 122.9 (CH), 124.1 (CH), 131.5 (C), 137.6 (C).

General synthesis of polycyclic dimethoxyl diamide

A mixture of the polycyclic diacid (11-15) (1 mol), glycine methyl ester (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated in vacuo. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % NaHCO₃ (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified via column chromatography using silica to obtain the desired products 16-20 respectively.

Data for N′,N′-bis(2-oxo-2-methoxyethyl)-8,11-dihydroxy-pentacyclo[5.4.0.0₂,₆.₃,₁₀.0₅,₉]undecane-8,11-diacetylcarboxamide (16)

A yellow slurry (CHCl₃; CH₃OH: NH₄OH (88:10:2), Rₜ = 0.6, yield 51.5 %). IR νmax: 3266, 2953, 1744, 1639, 1533, 1435 and 1204 cm⁻¹. MS (TOF) calculated for C₃₁H₃₅N₂O₈ (M + H⁺) 437.1918, found 437.1893. ¹H NMR [CDCl₃, 400 MHz]; δH 1.06 (1H, d, J = 10.8 Hz, H-4a), 1.52 (1H, d, J = 10.8 Hz, H-4s), 2.25 (1H, d, J = 15.0 Hz, CH₂), 2.31 (1H, s, H-9/10), 2.38 (1H, s, H-3/5), 2.40 (1H, d, J = 15.0 Hz, CH₂), 2.46 (1H, s, H-1/7), 2.49 (1H, s, H-2/6), 3.67 (3H, s, CH₃), 3.94 (2H, m, CH₂),
7.61 (t, 15.5 Hz, NH).  \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: δ\(_{C}\) 34.0 (C-4), 39.3 (C-2/6), 40.9 (CH\(_2\)), 42.9 (C-1/7), 44.3 (C-3/5), 44.3 (CH\(_2\)), 49.5 (C-9/10), 52.3 (CH\(_3\)), 77.3 (C-8/11), 170.2 (C=O), 172.9 (C=O).

Data for \(N'\),\(N'\)-bis(2-oxo-2-methoxyethyl)-8,11-oxapentacyclo[5.4.0.0\(^2\),6.3,10.0\(^5\),9]undecane-8,11-diacetylcarboxamide (17)

A yellow slurry [CHCl\(_3\): CH\(_3\)OH: NH\(_4\)OH (88:10:2), \(R_f = 0.8\), yield 68.7\%]. IR \(\nu_{\text{max}}\): 3320, 2954, 1741, 1649, 1534 and 1201 cm\(^{-1}\). MS (TOF) calculated for C\(_{21}\)H\(_{27}\)N\(_2\)O\(_7\) (M + H\(^+\)) 419.1813, found 419.1803. \(^{1}\)H NMR [CDCl\(_3\), 400 MHz]: δ\(_{H}\) 1.49 (1H, d, \(J = 10.5\) Hz, H-4a), 1.85 (1H, d, \(J = 10.5\) Hz, H-4s), 2.43 (1H, s, H-3/5), 2.62 (2H, m, H-2/6, 9/10), 2.65 (1H, s, H-1/7), 2.68-2.80 (2H, t, \(J = 15.7\) Hz, CH\(_2\)), 3.65 (3H, s, CH\(_3\)), 3.98 (2H, t, \(J = 5.5\) Hz, CH\(_2\)), 7.07 (t, \(J = 5.2\) Hz, NH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: δ\(_{C}\) 39.4 (CH\(_2\)), 41.0 (CH\(_2\)), 41.4 (C-2/6), 43.4 (C-4), 44.0 (C-3/5), 48.1 (C-1/7), 52.1 (CH\(_3\)), 58.6 (C-9/10), 94.0 (C-8/11), 170.3 (C=O), 170.9 (C=O).

Data for \(N',N'\)-bis(2-oxo-2-methoxyethyl)tricyclo[3.3.1.1\(^3\),7]decane-1,3-dicarboxamide (18)

A yellow slurry [CHCl\(_3\): CH\(_3\)OH; (9:1), \(R_f = 0.5\), quantitative yield]. IR \(\nu_{\text{max}}\): 3350, 2907, 1741, 1639, 1517, 1201 and 1180 cm\(^{-1}\). MS (TOF) calculated for C\(_{18}\)H\(_{27}\)N\(_2\)O\(_6\) (M + H\(^+\)) 367.1864, found 367.1864. \(^{1}\)H NMR [CDCl\(_3\), 400 MHz]: δ\(_{H}\) 1.65 (1H, s, H-6), 1.78-1.86 (4H, m, H-4/9, 8/10), 1.97 (1H, s, H-2), 2.17 (1H, s, H-5/7), 3.70 (3H, s, CH\(_3\)), 3.96 (2H, m, CH\(_2\)), 6.29 (br s, NH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: δ\(_{C}\) 28.0 (C-5/7), 35.3 (C-6), 38.1 (C-4/9, 8/10), 40.2 (C-2) 41.0 (C-1/3), 41.1 (CH\(_2\)), 52.2 (CH\(_3\)), 170.5 (C=O), 177.1 (C=O).

Data for \(N',N'\)-bis(2-oxo-2-methoxyethyl)tricyclo[3.3.1.1\(^3\),7]decane-1,3-diacetylcarboxamide (19)

A yellow slurry [CHCl\(_3\): CH\(_3\)OH; (9:1), \(R_f = 0.4\) quantitative yield]. IR \(\nu_{\text{max}}\): 3300, 2901, 1743, 1644, 1535, 1203 and 1181 cm\(^{-1}\). MS (TOF) calculated for C\(_{20}\)H\(_{31}\)N\(_2\)O\(_6\) (M + H\(^+\)) 395.2177, found 395.2178. \(^{1}\)H NMR [CDCl\(_3\), 400 MHz]: δ\(_{H}\) 1.45-1.51 (6H, complex m, H-2, 4/9, 6, 8/10), 1.97-1.98 (3H, m, H-5/7, CH\(_2\)), 3.68 (3H, s, CH\(_3\)), 3.97 (2H, d, \(J = 5.5\) Hz, CH\(_2\)), 6.34 (t, \(J = 5.4\) Hz, NH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: δ\(_{C}\) 28.8 (C-5/7), 33.4 (C-1/3), 35.7 (C-6), 40.8 (CH\(_2\)), 41.6 (C-4/9, 8/10), 46.9 (C-2), 50.7 (CH\(_2\)), 52.1 (CH\(_3\)), 170.7 (C=O), 171.1 (C=O).

Data for \(N',N'\)-bis(2-oxo-2-methoxyethyl)pentacyclo[5.3.0.0\(^2\),5.0\(^3\),9.0\(^4\),8]decane-2,5-dicarboxamide (20)

A whitish yellow solid [CHCl\(_3\):CH\(_3\)OH:NH\(_4\)OH; (88:10:2), \(R_f = 0.5\) 42\% yield]. Melting point: 156-158 ⁰C. IR \(\nu_{\text{max}}\): 3313, 2984, 1755, 1652, 1638, 1521, 1197, 1173 and 1156 cm\(^{-1}\). MS (TOF) calculated for C\(_{18}\)H\(_{23}\)N\(_2\)O\(_6\) (M + H\(^+\)) 363.1551, found 363.1589. \(^{1}\)H NMR [CDCl\(_3\), 400 MHz]: δ\(_{H}\) 1.48
(1H, AB, J = 11.4 Hz, H-10a), 1.64 (1H, AB, J = 11.4 Hz, H-10b), 1.70 (1H, AB, J = 11 Hz, H-6a), 2.18 (1H, AB, J = 10.8 Hz, H-6b), 2.56 (1H, m, H-7), 2.75-2.83 (2H, m, H-4, 8), 2.87-2.90 (2H, m, H-1, 9), 2.99 (1H, q, J = 9.0 Hz, H-3), 3.67 (6H, s, 2CH3), 3.86-4.02 (4H, m, 2CH2), 6.55 (s, NH), 6.77 (s, NH). 13C NMR [CDCl3, 100 MHz]: δC 37.7 (C-10), 39.5 (C-4), 40.8 (C-9), 41.0 (CH2), 41.1 (C-6), 41.2 (CH2), 43.4 (C-8), 43.8 (C-3), 47.2 (C-7), 52.0 (CH3), 52.1 (CH3), 54.9 (C-1), 61.6 (C-2), 62.3 (C-5), 170.2 (C=O), 170.3 (C=O), 172.9 (C=O), 173.4 (C=O).

**General synthesis of polycyclic diamide diacid 21-25**

Polycyclic ‘cage’ dimethoxyl diamide (16-20) dissolved in THF (20 mL) was added 1 M LiOH solution (12.4 mL) with stirring overnight. The resulting solution was acidified to pH 7. The desired product was extracted with ethyl acetate (3 x 100 mL) and the combined organic layer was dried with anhydrous Na2SO4 and concentrated in vacuo. Quantitative yields were obtained for all steps.

**Data for 8,11-dihydroxypentacyclo[5.4.0.02,6.3,10.05,9]undecane-8,11-diacetylcarboxamido diacetic acid (21)**

A white solid. IR νmax: 3249, 2934, 1599, 1390, 1290 and 1101 cm⁻¹. MS (TOF) calculated for C19H25N2O8 (M + H+) 409.1605, found 409.1589. 1H NMR [CD3OD, 400 MHz]: δH 1.20 (1H, d, J = 10.7 Hz, H-4a), 1.65 (1H, d, J = 10.7 Hz, H-4s), 2.36 (1H, d, J = 14.6 Hz, CH2), 2.37 (1H, s, H-9/10), 2.48 (1H, d, J = 14.6 Hz, CH2), 2.56 (1H, s, H-3/5), 2.60 (1H, s, H-1/7), 2.64 (1H, s, H-2/6), 3.80 (2H, t, J = 5.3 Hz, CH2). 13C NMR [CD3OD, 100 MHz]: δC 34.8 (C-4), 40.7 (C-2/6), 44.4 (C-1/7), 44.6 (CH2), 45.7 (CH2), 45.8 (C-3/5), 51.0 (C-9/10), 78.6 (C-8/11), 174.0 (C=O), 176.4 (C=O).

**Data for 8,11-oxapentacyclo[5.4.0.02,6.3,10.05,9]undecane-8,11-diacetylcarboxamido diacetic acid (22)**

A cream white solid obtained. IR νmax: 3284, 2960, 1593, 1397 and 1297 cm⁻¹. MS (TOF) calculated for C19H23N2O7 (M + H+) 397.1605, found 397.1631. 1H NMR [CD3OD, 400 MHz]: δH 1.59 (1H, d, J = 10.4 Hz, H-4a), 1.97 (1H, d, J = 10.4 Hz, H-4s), 2.50 (1H, s, H-3/5), 2.73-2.75 (2H, s, H-2/6, 9/10), 2.77 (1H, s, H-1/7), 2.81 (2H, s, CH2), 3.78 (2H, s, CH2). 13C NMR [CD3OD, 100 MHz]: δC 40.1 (CH2), 42.8 (C-2/6), 44.3 (C-4), 44.6 (CH2), 45.6 (C-3/5), 49.5 (C-1/7), 59.9 (C-9/10), 95.2 (C-8/11), 172.4 (C=O), 176.2 (C=O).

**Data for tricyclo[3.3.1.13,7]decane-1,3-dicarboxamidodiacetic acid (23)**

A white solid obtained. IR νmax: 3341, 2909, 1590, 1533, and 1397 cm⁻¹. MS (TOF) calculated for C16H23N2O6 (M + H+) 339.1551, found 339.1551. 1H NMR [CD3OD, 400 MHz]: δH 1.74 (1H, s, H-6), 1.85-1.93 (4H, m, H-4/9, 8/10), 1.99 (1H, s, H-2), 2.19 (1H, s, H-5/7), 3.72 (2H, s, CH2), 8.51 (s, NH). 13C NMR [CD3OD, 100 MHz]: δC 30.0 (C-5/7), 36.8 (C-6), 39.5 (C-4/9, 8/10), 41.7 (C-2), 42.5 (C-1/3), 44.8 (CH2), 176.6 (C=O), 179.7 (C=O).
Data for tricyclo[3.3.1.1^3,7]decane-1,3-diacetylcarboxamidodiacetic acid (24)

A white solid obtained. IR $v_{\text{max}}$: 3291, 2901, 1589, 1396 and 1314 cm$^{-1}$. MS (TOF) calculated for C$_{18}$H$_{27}$N$_2$O$_6$ (M + H$^+$) 367.1864, found 367.1855. $^1$H NMR [CD$_3$OD, 400 MHz]: $\delta$H 1.54-1.64 (6H, complex m, H-2, 4/9, 6, 8/10), 2.01-2.04 (3H, m, H-5/7, CH$_2$), 3.74 (2H, s, CH$_2$), 8.54 (s, NH). $^{13}$C NMR [CD$_3$OD, 100 MHz]: $\delta$C 30.9 (C-5/7), 34.9 (C-1/3), 37.3 (C-6), 43.1 (C-4/9, 8/10), 48.9 (C-2), 52.0 (CH$_2$), 173.7 (C=O), 176.8 (C=O).

Data for pentacyclo[5.3.0.0^2,5.0^3,9.0^4,8]decane-2,5-dicarboxamido diacetic acid (25)

A whitish yellow slurry obtained. IR $v_{\text{max}}$: 3289, 2968, 1593, 1533, 1428, 1396 and 1310 cm$^{-1}$. MS (TOF) calculated for C$_{16}$H$_{19}$N$_2$O$_6$ (M + H$^+$) 335.1238, found 335.1237. $^1$H NMR [D$_2$O, 400 MHz]: $\delta$H 1.50 (1H, AB, $J$ = 11.5 Hz, H-10a), 1.72 (1H, AB, $J$ = 11.5 Hz, H-10b), 1.79 (1H, AB, $J$ = 10.6 Hz, H-6a), 2.20 (1H, AB, $J$ = 10.7 Hz, H-6b), 2.67 (1H, m, H-7), 2.78-2.81 (1H, m, H-4), 2.84-2.86 (2H, m, H-1, 8), 2.97 (1H, m, H-9), 3.12 (1H, q, $J$ = 9.1 Hz, H-3), 3.68-3.86 (4H, m, 2CH$_2$). $^{13}$C NMR [D$_2$O, 100 MHz]: $\delta$C 37.0 (C-10), 38.3 (C-6), 39.9 (C-4), 40.4 (C-9), 43.1 (CH$_2$), 43.2 (CH$_2$), 43.2 (C-8), 43.7 (C-3), 47.1 (C-7), 54.6 (C-1), 61.0 (C-2), 62.8 (C-5), 175.4 (C=O), 175.7 (C=O), 176.4 (C=O), 176.6 (C=O).

General synthesis of compounds 26-30

A mixture of the polycyclic diamide diacid (21-25) (1 mol), geranylamine(7) (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated in vacuo. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % NaHCO$_3$ (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na$_2$SO$_4$ and concentrated in vacuo.

Data for $N^\prime,N^\prime$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0^2,6.3,10.0^5,9]undecane-8,11-dimethylenecarboxamide (26)

The crude product was recrystallized from acetonitrile to obtain 26 as a white solid (0.57 g, 38 %). Melting point: 149-152 ºC. IR $v_{\text{max}}$: 3267, 3143, 2967, 2922, 1636, 1522, 1222 and 769 cm$^{-1}$. MS (TOF) calculated for C$_{39}$H$_{59}$N$_4$O$_6$ (M + H$^+$) 679.4429, found 679.4428. $^1$H NMR [CDCl$_3$, 600 MHz]: $\delta$H 1.11 (1H, AB, $J$ = 10.7 Hz, H-4a), 1.53 (1H, H-4s), 1.56 (3H, s, CH$_3$), 1.62 (3H, s, CH$_3$), 1.64 (3H, s, CH$_3$), 1.97 (2H, m, CH$_2$), 2.04 (2H, m, CH$_2$), 2.24 (1H, s, H-9/10), 2.29 (1H, AB, $J$ = 14.6 Hz, CH$_2$), 2.39 (1H, AB, $J$ = 14.6 Hz, CH$_2$), 2.43 (1H, s, H-3/5), 2.52-2.55 (2H, m, H-1/7, 2/6), 3.81 (2H, t, $J$ = 6.08 Hz, CH$_2$), 3.89 (2H, t, $J$ = 5.46 Hz, CH$_2$), 5.04 (1H, t, $J$ = 6.54 Hz, CH), 5.13 (1H, t, $J$ = 6.78 Hz, CH), 6.51 (NH), 7.29 (NH). $^{13}$C NMR [CDCl$_3$, 150 MHz]: $\delta$C 16.3 (CH$_3$), 17.7 (CH$_3$), 25.7 (CH$_3$), 26.4 (CH$_3$), 34.0 (C-4), 37.5 (CH$_2$), 39.4 (C-2/6), 39.5 (CH$_3$), 42.7 (C-1/7), 43.0 (CH$_2$), 44.4 (CH$_3$), 46.1 (CH$_3$), 57.0 (2CH$_2$), 61.6 (CH$_2$), 129.9 (C), 131.8 (C), 173.7 (C=O), 176.4 (C=O), 176.6 (C=O).
Data for \( \text{N',N'-bis}[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0^2,6.3.10.0^5,9]undecane-8,11-dimethylenecarboxamide} \ (27) \)

The crude product was purified using preparative HPLC to obtain yellow oil, retention time: 19.3 min (0.83 g, 59 %). IR \( \nu_{\text{max}} \): 3288, 2965, 2927, 2862, 1642, 1529, 1440 and 1234 cm\(^{-1} \). MS (TOF) calculated for C\(_{39}\)H\(_{57}\)N\(_4\)O\(_5\) (M + H\(^+\)) 661.4323, found 661.4307. \(^1\)H NMR [CDCl\(_3\), 400 MHz]: \( \delta \) 1.51 (1H, H-4a), 1.55 (3H, s, CH\(_3\)), 1.61 (3H, s, CH\(_3\)), 1.64 (3H, s, CH\(_3\)), 1.88 (1H, d, \( J = 10.64 \) Hz, H-4s), 1.94 (2H, m, CH\(_2\)), 2.02 (2H, m, CH\(_2\)), 2.45 (1H, s, H-3/5), 2.60 (1H, s, H-9/10), 2.63 (2H, s, H-1/7, H-2/6), 2.66-2.78 (2H, m, CH\(_2\)), 3.79 (2H, t, \( J = 6.08 \) Hz, CH\(_2\)), 3.87 (2H, d, \( J = 5.4 \) Hz, CH\(_2\)), 5.02 (1H, m, CH), 5.09 (1H, m, CH), 6.33 (NH), 7.54 (NH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: \( \delta \) C 16.3 (CH\(_3\)), 17.6 (CH\(_3\)), 25.6 (CH\(_3\)), 26.4 (CH\(_2\)), 37.5 (CH\(_2\)), 39.4 (CH\(_2\)), 39.5 (CH\(_2\)), 41.5 (C-2/6), 43.2 (CH\(_2\)), 43.4 (C-4), 44.0 (C-3/5), 48.2 (C-1/7), 58.7 (C-9/10), 93.9 (C-8/11), 119.5 (CH), 123.7 (CH), 131.8 (C), 140.0 (C), 168.9 (C=O), 170.3 (C=O).

Data for \( \text{N',N'-bis}[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide} \ (28) \)

A white crystalline solid, re-crystallized from acetonitrile was obtained (69 % yield). Melting point: 106-108 °C. IR \( \nu_{\text{max}} \): 3288, 3075, 2908, 2856, 1668, 1643, 1529, 1448, and 1236 cm\(^{-1} \). MS (TOF) calculated for C\(_{36}\)H\(_{57}\)N\(_4\)O\(_4\) (M + H\(^+\)) 609.4374, found 609.4364. \(^1\)H NMR [CDCl\(_3\), 400 MHz]: \( \delta \) H 1.56 (3H, s, CH\(_3\)), 1.63 (3H, s, CH\(_3\)), 1.65 (3H, s, CH\(_3\)), 1.66 (1H, s, H-6), 1.80-1.87 (4H, m, H-4/9, 8/10), 1.96-2.06 (5H, m, H-2, 2CH\(_2\)), 2.18 (1H, s, H-5/7), 3.81 (2H, t, \( J = 6.0 \) Hz, CH\(_2\)), 3.86 (2H, d, \( J = 4.96 \) Hz, CH\(_2\)), 5.04 (1H, t, \( J = 6.5 \) Hz, CH), 5.14 (1H, t, \( J = 6.8 \) Hz, CH), 6.58 (br s, NH), 6.96 (br s, NH). \(^{13}\)C NMR [CDCl\(_3\), 100 MHz]: \( \delta \) C 16.3 (CH\(_3\)), 17.7 (CH\(_3\)), 25.7 (CH\(_3\)), 26.4 (CH\(_2\)), 28.1 (C-5/7), 35.3 (C-6), 37.5 (CH\(_2\)), 38.1 (C-4/9, 8/10), 39.5 (CH\(_2\)), 40.4 (C-2), 41.0 (C-1/3), 43.4 (CH\(_2\)), 119.5 (CH), 123.8 (CH), 131.7 (C), 139.9 (C), 168.9 (C=O), 177.7 (C=O).

Data for \( \text{N',N'-bis}[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylenecarboxamide} \ (29) \)

A white crystalline solid, re-crystallized from acetonitrile was obtained (72 % yield). Melting point: 115-117 °C. IR \( \nu_{\text{max}} \): 3290, 2905, 2851, 1628, 1534, 1445 and 1267 cm\(^{-1} \). MS (TOF) calculated for C\(_{38}\)H\(_{61}\)N\(_4\)O\(_4\) (M + H\(^+\)) 637.4687, found 637.4686. \(^1\)H NMR [CDCl\(_3\), 400 MHz]: \( \delta \) H 1.46-1.53 (6H, m, H-2, 6, 4/9, 8/10), 1.56 (3H, s, CH\(_3\)), 1.63 (3H, s, CH\(_3\)), 1.64 (3H, s, CH\(_3\)), 1.94-2.06 (7H, m, H-5/7, 3CH\(_2\)), 3.81 (2H, t, \( J = 6.0 \) Hz, CH\(_2\)), 3.85 (2H, d, \( J = 5.4 \) Hz, CH\(_2\)), 5.03 (1H, t, \( J = 6.6 \) Hz, CH), 5.14 (1H, t, \( J = 6.0 \) Hz, CH), 6.53 (t, \( J = 4.8 \) Hz, NH), 6.94 (t, \( J = 5.2 \) Hz, NH). \(^{13}\)C NMR [CDCl\(_3\), 100
MHz: $\delta_C$ 16.3 (CH$_3$), 17.7 (CH$_3$), 25.6 (CH$_3$), 26.4 (CH$_2$), 28.9 (C-5/7), 33.5 (C-1/3), 35.8 (C-6), 37.5 (CH$_2$), 39.5 (CH$_2$), 41.7 (C-4/9, 8/10), 43.3 (CH$_2$), 47.4 (C-2), 50.7 (CH$_2$), 119.5 (CH), 123.7 (CH), 131.7 (C), 139.9 (C), 169.1 (C=O), 171.7 (C=O).

Data for $N',N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0$^{2,5}$,0$^{3,9}$,0$^{4,8}$]decane-2,5-dicarboxamide (30)

The crude product was purified using preparative HPLC to obtain yellowish oil, retention time: 22.5 min (67 % yield). IR $\nu_{\text{max}}$: 3314, 2968, 2927, 2875, 1636, 1521, 1266 and 729 cm$^{-1}$. MS (TOF) calculated for C$_{36}$H$_{53}$N$_4$O$_4$ (M + H$^+$) 605.4061, found 605.4077. $^1$H NMR [CDCl$_3$, 400 MHz]: $\delta_H$ 1.42 (1H, AB, $J = 11.4$ Hz, H-10a), 1.56 (6H, s, 2CH$_3$), 1.61-1.67 (14H, m, H-6a, 10b, 4CH$_3$), 1.94-1.97 (4H, m, 2CH$_2$), 2.01-2.04 (4H, m, 2CH$_2$), 2.20 (1H, AB, $J = 10.4$ Hz, H-6b), 2.60 (1H, s, H-7), 2.78-2.84 (2H, m, H-4, 8), 2.88 (1H, s, H-1), 2.93 (1H, m, H-9), 3.08 (1H, q, $J = 8.9$ Hz, H-3), 3.60-3.66 (2H, m, CH$_2$), 3.69-3.74 (2H, m, CH$_2$), 3.82-3.89 (2H, m, CH$_2$), 4.03-4.13 (2H, m, CH$_2$), 5.05 (2H, t, $J = 6.6$ Hz, 2CH), 5.18 (2H, t, $J = 6.6$ Hz, 2CH), 6.44 (br s, NH), 6.59 (br s, NH), 6.88 (br s, NH), 6.93 (br s, NH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta_C$ 16.2 (CH$_3$), 17.7 (CH$_3$), 25.6 (CH$_3$), 26.5 (CH$_2$), 37.6 (C-10), 37.6 (CH$_2$), 38.8 (C-6), 39.5 (CH$_2$), 39.6 (C-4), 40.7 (C-9), 43.1 (CH$_2$), 43.2 (CH$_2$), 43.5 (C-8), 44.0 (C-3), 47.4 (C-7), 53.6 (C-1), 61.9 (C-2), 62.9 (C-5), 120.0 (CH), 120.1 (CH), 124.0 (CH), 131.5 (C), 138.8 (C), 138.9 (C), 168.4 (C=O), 173.7 (C=O), 173.8 (C=O).

General synthesis of Compounds 4-8

To a stirring solution of 26-30 (1 mol) in dry THF as added LiAlH$_4$ (20 mol) and reflux under N$_2$ atm., reaction was monitored via LC-MS. After completion, excess LiAlH$_4$ was quenched by dropwise addition of aqueous Na$_2$SO$_4$, the resulting white precipitate was filtered and the filtrate concentrated in vacuo. The crude products were dissolved in acetonitrile (5-8 mL) and purified using preparative HPLC.

Data for $[N',N'-(8,11-dihydroxypentacyclo[5.4.0.0$^{2,6}$,3$^{10}$,0$^{5,9}$]undecane-8,11-diethylene)]-bis[(N''-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)

A yellow oil, retention time: 14.7 min (50 mg, 30 %). IR $\nu_{\text{max}}$: 2962, 2859, 2775, 2685, 1575, 1449, 1342 and 758 cm$^{-1}$. MS (TOF) calculated for C$_{39}$H$_{67}$N$_4$O$_2$ (M + H$^+$) 623.5259, found 623.5235. $^1$H NMR [CDCl$_3$, 400 MHz]: $\delta_H$ 1.09 (1H, d, $J = 10.0$ Hz, H-4a), 1.48 (1H, H-4s), 1.54 (3H, s, CH$_3$), 1.62 (3H, s, CH$_3$), 1.65 (3H, s, CH$_3$), 1.73 (2H, s, CH$_2$), 2.02 (4H, s, 2CH$_2$), 2.19 (1H, s, H-9/10), 2.28 (1H, s, H-3/5), 2.44 (1H, s, H-1/7), 2.48 (1H, s, H-2/6), 3.04-3.08 (2H, m, CH$_2$), 3.25 (2H, m, CH$_2$), 3.32 (2H, m, CH$_2$), 3.52 (2H, m, CH$_2$), 4.99 (1H, m, CH), 5.23 (1H, m, CH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta_C$ 16.5 (CH$_3$), 17.7 (CH$_3$), 25.6 (CH$_3$), 26.2 (CH$_2$), 34.0 (C-4), 34.6 (CH$_2$), 39.5 (C-2/6), 39.6
Data for \([N',N'-(8,11-oxapentacyclo[5.4.0.0^2,6.3.1,0^5,9]undecane-8,11-diethylene)]-\)
\([N''-(E)-3,7-dimethylocta-2,6-dienyl]ethane-1,2-diamine\) (5)

A yellow slurry oil, retention time: 16.3 min (0.12 g, 34 %). IR ν_max: 2965, 2858, 2765, 2683, 1560, 1453, 1342 and 763 cm⁻¹. MS (TOF) calculated for C_{39}H_{65}N_{4}O (M + H⁺) 605.5153, found 605.5137. 

\[^1^H\ NMR\ [CDCl\textsubscript{3}, 600 MHz]: δ_H 1.49 (1H, d, J = 10.4 Hz, H-4a), 1.53 (3H, s, CH\textsubscript{3}), 1.61 (3H, s, CH\textsubscript{3}), 1.63 (3H, s, CH\textsubscript{3}), 1.85 (1H, d, J = 10.4 Hz, H-4s), 2.01-2.03 (4H, m, 2CH\textsubscript{2}), 2.10 (2H, m, CH\textsubscript{2}), 2.34 (1H, s, H-3/5), 2.51 (2H, s, H-1/7, 9/10), 2.56 (1H, s, H-2/6), 3.02 (2H, m, CH\textsubscript{2}), 3.13 (2H, m, CH\textsubscript{2}), 3.21 (2H, m, CH\textsubscript{2}), 3.43-3.46 (2H, m, CH\textsubscript{2}), 4.99 (1H, m, CH), 5.21 (1H, m, CH). \n
\[^{13}C\ NMR\ [CDCl\textsubscript{3}, 150 MHz]: δ_C 16.5 (CH\textsubscript{3}), 17.7 (CH\textsubscript{3}), 25.7 (CH\textsubscript{3}), 26.3 (CH\textsubscript{2}), 28.7 (CH\textsubscript{2}), 39.7 (CH\textsubscript{2}), 41.2 (C-2/6), 43.1 (CH\textsubscript{2}), 43.6 (C-4), 43.8 (C-3/5), 45.0 (CH\textsubscript{2}), 45.1 (CH\textsubscript{2}), 45.2 (CH\textsubscript{2}), 47.5 (C-1/7), 58.0 (C-9/10), 95.5 (C-8/11), 115.9 (CH), 123.5 (CH), 132.0 (C), 143.9 (C).

Data for \([N',N'-(tricyclo[3.3.1.1^3,7]decane-1,3-dimethylene)]-\)
\([N''-(E)-3,7-dimethylocta-2,6-dienyl]ethane-1,2-diamine\) (6)

A yellow slurry oil, retention time: 11.1 min (0.56 g, 41.8 %). IR ν_max: 2907, 2849, 2763, 2678, 1563, 1449, 1343 and 763 cm⁻¹. MS (TOF) calculated for C_{36}H_{65}N_{4} (M + H⁺) 553.5204, found 553.5207. 

\[^1^H\ NMR\ [CDCl\textsubscript{3}, 400 MHz]: δ_H 1.35 (2H, d, J = 11.5 Hz, H-4/9 or H-8/10), 1.55 (3H, s, CH\textsubscript{3}), 1.52-1.56 (2H, m, H-4/9 or H-8/10), 1.58 (2H, s, H-2, 6), 1.63 (3H, s, CH\textsubscript{3}), 1.64 (3H, s, CH\textsubscript{3}), 2.01-2.06 (5H, br s, H-5/7, 2CH\textsubscript{2}), 2.49 (2H, s, CH\textsubscript{2}), 3.12 (2H, m, CH\textsubscript{2}), 3.18 (2H, m, CH\textsubscript{2}), 3.51 (2H, d, J = 7.3 Hz, CH\textsubscript{2}), 5.00 (1H, m, CH), 5.25 (1H, t, J = 6.8 Hz, CH). \n
\[^{13}C\ NMR\ [CDCl\textsubscript{3}, 100 MHz]: δ_C 16.4 (CH\textsubscript{3}), 17.7 (CH\textsubscript{3}), 25.6 (CH\textsubscript{3}), 26.2 (CH\textsubscript{2}), 28.0 (C-5/7), 33.6 (C-1/3), 36.1 (C-6), 39.5 (C-4/9, 8/10), 39.6 (CH\textsubscript{2}), 40.4 (C-2), 44.0 (CH\textsubscript{2}), 44.8 (CH\textsubscript{2}), 46.5 (CH\textsubscript{2}), 60.4 (CH\textsubscript{2}), 114.6 (CH), 123.3 (CH), 132.0 (C), 143.9 (C).

Data for \([N',N'-(tricyclo[3.3.1.1^3,7]decane-1,3-diethylene)]-\)
\([N''-(E)-3,7-dimethylocta-2,6-dienyl]ethane-1,2-diamine\) (7)

A yellow slurry oil, retention time: 11.2 min (0.29 g, 33 %). IR ν_max: 2911, 2847, 1765, 2682, 1567, 1449, 1340 and 763 cm⁻¹. MS (TOF) calculated for C_{38}H_{69}N_{4} (M + H⁺) 581.5517, found 581.5506. 

\[^1^H\ NMR\ [CDCl\textsubscript{3}, 400 MHz]: δ_H 1.25 (1H, s, H-2), 1.35-1.43 (4H, m, H-4/9, 8/10), 1.47-1.50 (2H, m, CH\textsubscript{2}), 1.54 (1H, s, H-6), 1.56 (3H, s, CH\textsubscript{3}), 1.64 (3H, s, CH\textsubscript{3}), 1.67 (3H, s, CH\textsubscript{3}), 1.99 (1H, s, H-5/7), 2.02-2.05 (4H, m, 2CH\textsubscript{2}), 2.89 (2H, s, CH\textsubscript{2}), 3.22 (2H, m, CH\textsubscript{2}), 3.26 (2H, m, CH\textsubscript{2}), 3.52 (2H, d, J = 7.2 Hz, CH\textsubscript{2}), 5.01 (1H, m, CH), 5.26 (1H, t, J = 6.7 Hz, CH). \n
\[^{13}C\ NMR\ [CDCl\textsubscript{3}, 100 MHz]: δ_C 16.5 (CH\textsubscript{3}), 17.7 (CH\textsubscript{3}), 25.7 (CH\textsubscript{3}), 26.2 (CH\textsubscript{2}), 28.5 (C-5/7), 32.4 (C-1/3), 36.0 (C-6), 39.5 (CH\textsubscript{2}), 39.6 (CH\textsubscript{2}).
Data for \([N’,N’’-(pentacyclo[5.3.0.0^2,5.0^3,9.0^4,8]decane-2,5-dimethylene)]-bis[(N’’-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine]\) (8)

A yellow slurry oil, retention time: 11.8 min (0.52 g, 28.1 %). IR $\nu_{\text{max}}$: 2966, 2925, 2855, 2774, 1578, 1446, 1342 and 756 cm$^{-1}$. MS (TOF) calculated for C$_{36}$H$_{61}$N$_4$ (M + H$^+$) 549.4891, found 549.4929.

$^1$H NMR [CDCl$_3$, 400 MHz]: $\delta$H 1.24 (1H, AB, $J = 12.4$ Hz, H-6a), 1.27 (1H, AB, $J = 12.9$ Hz, H-10a), 1.59 (1H, H-10b), 1.65 (6H, s, 2CH$_3$), 1.86 (1H, AB, $J = 10.7$ Hz, H-6b), 2.01-2.07 (8H, m, 4CH$_2$), 2.35 (2H, m, H-3, 4), 2.49 (1H, s, H-7), 2.59 (1H, s, H-1), 2.64 (1H, AB, $J = 13.1$ Hz, CH$_2$), 2.66 (1H, m, H-8), 2.76 (1H, m, H-9), 2.82 (1H, AB, $J = 12.8$ Hz, CH$_2$), 2.96 (1H, AB, $J = 13.1$ Hz, CH$_2$), 3.07-3.29 (8H, m, 4CH$_2$), 3.25 (1H, AB, $J = 13.1$ Hz, CH$_2$), 3.49 (4H, d, $J = 7.1$ Hz, 2CH$_2$), 5.00 (2H, m, 2CH), 5.26 (2H, t, $J = 6.9$ Hz, 2CH). $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta$C 16.5 (CH$_3$), 17.7 (CH$_3$), 25.6 (CH$_3$), 26.2 (CH$_2$), 37.6 (C-10), 39.6 (CH$_2$), 40.2 (C-4), 40.3 (C-6), 40.5 (C-9), 42.3 (C-3), 43.2 (C-8), 43.9 (CH$_3$), 44.3 (CH$_3$), 45.3 (CH$_2$), 46.1 (CH$_2$), 46.2 (CH$_2$), 46.9 (C-7), 48.9 (CH$_2$), 50.4 (CH$_2$), 50.8 (C-1), 53.0 (C-2), 54.2 (C-5), 114.6 (CH), 114.7 (CH), 123.4 (CH$_2$), 132.0 (C), 144.9 (C), 145.0 (C).

Anti-mycobacterial assay

The BACTEC system has been devised to monitor mycobacterial growth of the slow growing species. *M. tuberculosis* H$_{37}$Rv reference strain (ATCC 25618) was used to evaluate the compounds for their anti-tuberculosis activity. The strain is sensitive to Isoniazid with an MIC for INH at 0.03 µg/mL in BACTEC 12B medium. All work was carried out in the BSL3 laboratory of the Division of Molecular Biology of the University of Stellenbosch. Bacterial colonies were cultured and selected from Loewenstein-Jensen slant cultures. A colony of *M. tuberculosis* reference strain H$_{37}$Rv was cultured in 7H9 mycobacterial medium (Difco) enriched with ADC (Biolab art. C70) with continuous stirring at 37 °C. When cultures reached a density of approximately 0.16 at A$_{600}$ nm (one McFarland), 0.1 mL was inoculated into a Bactec vial (23). These primary cultures were incubated at 37 °C until a growth index of 500 (+/-50) was reached. These primary cultures were used for drug testing of anti-tuberculosis compounds. Cultures were regularly stained by acid-fast staining (ZN staining) to control for contamination (24).

The compounds 4-8, 26-30 and SQ109 were dissolved in methanol to make a stock solution of 10 mM concentration. 0.1 mL of primary culture and 0.1 mL drug compound were added to a BACTEC vial, the vials incubated at 37 °C, and the growth monitored every 24 hours. The final concentrations in BACTEC 12B medium were 50 µM, 10 µM, and 1 µM and the final methanol concentration in the
12B medium was 2.5%. Read-out values are expressed as growth index (GI). Controls included cultures with and without solvent (2.5 % Methanol). GI readings were continued until the controls reached the maximum GI value approximately 500 or more. Control GI values between 50 and 800 are normally used to evaluate the efficacy of compounds with possible anti-tuberculosis activity (23).

Acknowledgements

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Supplementary data

Supplementary data (1H, 13C NMR and HRMS spectra) associated with this article can be found in the online version at XXXXXX

References


CHAPTER 9
SYNTHESIS AND NMR ASSIGNMENT OF PENTACYCLOUNDECANE PRECURSORS OF POTENTIAL PHARMACEUTICAL AGENTS

Oluseye K. Onajole, a Maya M. Makatini, a Patrick Govender, b Thavendran Govender, c Glenn E. M. Maguire, a and Hendrik G. Kruger a*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa
b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa
c School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa

Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091

Abstract

The synthesis and complete NMR elucidation of eight novel pentacycloundecane (PCU) derivatives are reported. These compounds are precursors in the synthesis of PCU based anti-tuberculosis (TB) agents and potential HIV protease inhibitors. Two dimensional NMR techniques were used to assign the NMR spectra for these compounds. Substitution of the cage molecule at (C-8/11) further complicates the assignment, since some of the substituted alkyl chain groups overlap with the cage proton signals. The side chain heteroatoms also introduce rare through-space deshielding effect to some of the carbon atoms of the cage skeleton. Ring strain in the rigid cage skeleton appears to induce drastic electronic changes some parts of the cage framework. This observation is more dramatic for the C-4 methylene group of the cage diols and the cage ethers.

Introduction

Polycyclic “cage” molecules have been of interest to scientists for many years. The chemistry and applications of “cage” compounds (such as pentacycloundecane and trishomocubane) were the focus of a number of South African scientists over the years. The value of 2D NMR techniques to determine the structure of cage compounds has been reported by several authors. Our group has successfully utilized these techniques in the elucidation of numerous PCU derivatives synthesized in our laboratory. As part of our ongoing programme in this field, the NMR assignment of eight novel PCU derivatives (1-8) is reported (Figure 1).
Onajole et al. \cite{25} recently reported novel PCU tetra amine compounds as potential anti-TB agents. The eight compounds communicated herein are all precursors in the synthesis of these series of potential anti-TB and HIV protease agents presently being studied in our laboratory.

The starting material for these molecules is Cookson’s dione 9\cite{26}. Cookson’s dione is reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to obtain endo-8,endo-11 diol 10.\cite{10} Ozonolysis of the diol 10 followed by an oxidative workup yielded the PCU diol dicarboxylic acid 11. Compound 11 is reacted with ammonium chloride using coupling reagents hydroxybenzotriazole (HOBT), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-methylmorpholine (NMM) to obtain compound 1 (Scheme 1).

Figure 1: PCU derivatives (1-8)
Scheme 1: Synthesis of PCU derivative 1

The starting material in the synthesis of compounds 2-5 is the PCU ether dicarboxylic acid 12.\[^{10,21}\] Compound 12 was converted to its corresponding diacid chloride\[^{10}\] and reacted with ammonia-saturated chloroform at 0 ºC to obtain 2 in good yield. Compound 2 was subjected to a Hofmann rearrangement reaction using sodium hypochlorite and sodium hydroxide to obtain compound 3. Reduction of compound 2 using lithium aluminium hydride yielded the corresponding derivative 4 (scheme 2).

* The synthesis of compound 11 was first achieved by Mr. R. Karpoormath in our group (unpublished results).
Scheme 2: Synthesis of PCU derivatives 2-4

Compounds 12 and 11 were converted to 5 and 7 respectively using glycine methyl, HOBT, EDC and NMM. Compounds 5 and 7 were hydrolyzed using lithium hydroxide to obtain their corresponding acid 6 and 8.

Results and discussions

All PCU compounds (1-8) was analogous to those described before[19,21] and the results are presented in Tables 1 and 2.

The C-N bond in compound 1 and 2 experiences partial double bond character which hinders “free” rotation of the sp² amide nitrogen. Thus for compound 1, the cis-amide proton (δ=7.33 ppm) to the carboxamide oxygen appears at a higher frequency compared to the trans-amide proton (δ=7.08 ppm) due to a through-space deshielding effect of the cis-proton.[24,27] A similar observation was noted in the elucidation of compound 2.

An interesting observation in Table 1 begs an explanation. Why would H-1/7 and H-9/10, H-1’ and C-8/11, in compound 2 be more deshielded than the corresponding signals in compound 1? Protons on the cage are normally deshielded if a hetero atom of a side chain is positioned such that the electron rich hetero atom is in close proximity of the cage proton(s).[4,19,28,29] It is possible that the OH and NH₂ groups in 1 cause intramolecular hydrogen bond interactions. This will largely “tie” the two groups together at room temperature. However, for compound 2 much less intramolecular hydrogen bond interactions are possible. The result is that the side arms are “free” to rotate around C-8/11 and
C-1’ at room temperature, spending on average more time in close proximity to H-1/7 and H-9/10. This will cause the through-space deshielding effect observed for protons H-1/7 and H-9/10.

The tertiary carbon signals of a tertiary alcohol and that of tertiary ether normally appears quite close together between 60 - 70 ppm, however the corresponding values for 1 and 2 are quite far apart. The tertiary alcohol carbon of compound 1 (C-8/11) appears at 76.6 ppm while the C-8/11 for compound 2 appears at $\delta=93.6$ ppm. This observation suggests that the intramolecular hydrogen bond interaction experienced in compound 1 contributes to the shielding effect observed for C-8/11. The hydrogen bond is absent in 2 and it appears that a through-space deshielding effect from the side chain heteroatoms on the exo-side of C-8/11 causes that this carbon atom is registered at a much higher frequency. This through-space deshielding of carbon atoms has been observed before for two similar compounds in our group, as well as by other researchers for different molecules.

Another large chemical shift to a higher frequency is observed for C-4 when the cage diol (such as 1) is converted to the cage ether (such as 2). The side arms are too short to induce a through-space deshielding effect to C-4. To obtain some insight in this interesting phenomenon, it was decided to optimize the two structures with a Density Functional Theory calculation [B3LYP32-34 with the 6-31+G(d) basis set]. The PCU skeleton resembles a rigid cyclohexane boat structure (C-1, C-7, C-8, C-9, C-10 and C-11). It was shown in previous studies that a reduction in distance (of ~0.1 Å) between C-8 and C-11 is responsible for large ring strain in the molecule. The calculated distance between C-8 and C-11 in the cage diol 1 is 2.94 Å and the cage ether 2 is 2.21 Å; a difference of about 0.7 Å. A deviation in the angles between C-11, C-10 and C-3 for these two compounds was also observed (101.4º for compound 1 and 109.0º for compound 2). These deviations appear to somehow influence the electron densities in the two compounds for C-4.

The $^{13}$C NMR spectrum of the two molecules were calculated (GIAO)36-40 and it was found that C-4 for compound 1 appears at ~ 45 ppm and for compound 2 at ~ 54 ppm. This theoretical result coincides with the experimental observation in which C-4 for compound 2 registers at higher frequency of about 9 ppm more than for compound 1. Since this is also observed in the theoretical case, it is expected that C-4 for compound 2 experience larger electron density than the corresponding carbon for 1. The molecular orbitals for the two molecules were calculated and the 10 highest occupied molecular orbitals (MOs) were compared. For compound 1, only the fifth highest MO showed electron density from C-3 through C-4 to C-5. All the other nine MOs showed either no electron density in that part of the molecule or showed a node (non bonding interaction) at C-4. For compound 2, both the fifth and tenth highest MOs showed electron density involving C-3 through C4

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† Results generated using Chemdraw Ultra 10.
to C-5. The increased electron density of C-4 for compound 2 should explain the difference in carbon shifts for C-4 in these two molecules.

The signal for C-2’ (δ=173.0 ppm) is absent in the $^{13}$C and HMBC spectra of 3, thus confirming the success of the Hofmann rearrangement reaction. The $^{13}$C spectrum shows two methylene carbons appearing at δ=43.4 and 43.7 ppm. The assignment of the former carbon was made to C-4 and the latter to C-1’ since the positions of H-4a and H-4s are known. The H-1’ protons display NOESY interactions with the NH$_2$ protons (δ=1.39 ppm), H-1/7 (δ=2.58 ppm) and H-9/10 (δ=2.57 ppm) respectively. Carbons C-9/10 (δ=56.4 ppm) show HMBC correlations with H-1’, H-1/7, H-4a and H-4s respectively. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table 1 and 2.

The loss of the carbonyl signal at 173.0 ppm ($^{13}$C NMR spectrum of 4) and the presence of methylene protons at 2.62 – 2.74 ppm (1H NMR spectrum) confirm the successful reduction of compound 1 to 4. The APT $^{13}$C spectrum shows three methylene carbons at 36.2, 38.8 and 43.4 ppm, the signal at δ=43.4 ppm was assigned to C-4 (due to HSQC correlation with the H-4a and H-4s protons) while the remaining two signals should belong to C-1’ and C-2’. COSY and NOESY spectra show correlations between the two methylene protons appearing at δ=1.81 and 2.62 – 2.74 ppm which belongs to H-1’ and H-2’ respectively. H-1’ (δ=1.81 ppm) displays HMBC spectrum correlations with C-1/7 (δ=47.7 ppm), C-9/10 (δ=58.3 ppm), C-8/11 (δ=95.3 ppm) and C-2’ (δ=38.8 ppm) respectively. The H-2’ protons show HMBC correlations with C-1’ (δ=36.2 ppm) and C-8/11. H-1’ exhibit a NOESY interactions with H-1/7 and H-9/10 respectively. These assignments are presented in Table 1 and 2.

The APT $^{13}$C spectrum of compound 5 shows a quaternary carbon at δ=94.0 ppm which was assigned to C-8/11. C-8/11 displays a HMBC correlation with a methylene proton at δ=2.68 – 2.80 ppm this was assigned to H-1’. The $^{13}$C spectrum shows two carbonyl carbons at δ=170.3 and 170.9 ppm. HMBC correlation was observed between H-1’ and a carbonyl carbon (170.3 ppm) which was then assigned to C-2’, thus the second carbonyl carbon (δ=170.9 ppm) was assigned to C-4’. C-4’ display a HMBC correlation to H-3’ (δ=3.98 ppm) and H-5’ (δ=3.65 ppm). The NH protons at δ=7.07 ppm show a HMBC correlation to C-2’ (δ=170.3 ppm), C-3’ (δ=41.0 ppm) and C-4’ (δ=170.9 ppm). The NH proton also displays a NOESY interaction with H-1’ and H-3’ respectively and a COSY correlation with H-3’ only. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table 1 and 2.
Table 1: $^1$H NMR chemical shifts of compounds 1 - 8

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* Solvent (CD$_3$)SO; $^*$ 400 MHz for $^1$H; $^\text{a}$ Solvent CDCl$_3$; $^\text{b}$ 600 MHz for $^1$H; $^\text{c}$ Solvent CD$_3$OD
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</table>

$^a$ Solvent (CD$_3$)$_2$SO; $^b$ 100 MHz for $^{13}$C; $^c$ Solvent CDCl$_3$; $^d$ 150 MHz for $^{13}$C; $^e$ Solvent CD$_3$OD
The \(^{13}\text{C}\) spectrum of 6 shows two carboxylic carbon signals appearing at \(\delta=172.4\) and 176.2 ppm. The carbon at 172.4 ppm exhibits HMBC correlations with two proton signals at \(\delta=2.81\) and 3.78 ppm which should be protons H-1' and H-3'. This carbon (\(\delta=172.4\) ppm) was therefore assigned to C-2' while \(\delta=176.2\) ppm was assigned to C-4'. The carboxylic carbon (C-4') shows HMBC correlation to protons at \(\delta=3.78\) ppm which was assigned to H-3' while \(\delta=2.81\) ppm was assigned to H-1'. H-1' displays HMBC correlations with C-1/7 (\(\delta=49.5\) ppm), C-9/10 (\(\delta=59.9\) ppm), C-8/11 (\(\delta=95.2\) ppm) and C-2' (\(\delta=172.4\) ppm) respectively while H-3' shows HMBC correlations to C-2' (\(\delta=172.4\) ppm) and C-4' (\(\delta=176.2\) ppm) respectively. As was the case with the previous NMR signals, a similar trend is observed between the two related structures. The assigned signals are presented in Table 1 and 2.

A similar route, employed in the synthesis of 5 and 6 was used to obtain compounds 7 and 8. The \(^{13}\text{C}\) spectrum shows two carbonyl carbons at \(\delta=170.2\) and 172.9 ppm. The signal at \(\delta=170.2\) ppm shows an HMBC correlation with the methyl (\(\delta=3.67\) ppm) and methylene (\(\delta=3.94\) ppm) protons. This carbonyl was therefore assigned to C-4' and the methylene and methyl groups to H-3' and H-5' respectively. The remaining carbonyl carbon was assigned to C-2' (\(\delta=172.9\) ppm). The C-2' carbon resonance displays a HMBC correlation to a set of doublets at \([\delta=2.25\) and 2.40 ppm (\(J=15\) Hz)] and to the H-3’ protons. The set of doublets were assigned to H-1’. The signal at 7.61 ppm was assigned to the NH proton, this proton displayed COSY and NOESY interactions with H-3’ and H-1’, thus confirming the assignment of the nitrogen proton. The assignment of compound 7 is presented in Table 1 and 2.

The absence of a methyl signal both in the \(^1\text{H}\) and \(^{13}\text{C}\) spectrum of compound 8 suggests the successful hydrolysis of compound 7 to its corresponding diacid 8. A methylene proton appearing at 2.36 and 2.48 ppm (\(J=14.6\) Hz) shows a HMBC correlation to the quaternary carbon C-8/11 (\(\delta=78.6\) ppm) and was thus assigned to H-1’. The H-1’ signal displays a HMBC correlation with C-1/7 (\(\delta=44.4\) ppm), C-9/10 (\(\delta=51.0\) ppm) and a carbonyl signal at \(\delta=174.0\) ppm which was assigned to C-2’. The remaining carbonyl was assigned to C-4’. C-4’ shows a HMBC correlation with a methylene proton (\(\delta=3.80\) ppm) which was assigned to H-3’. The assignments are presented in Table 1 and 2.

**Conclusion**

The synthesis and complete elucidation of eight novel PCU derivatives was successfully carried out. The synthesis was achieved utilizing known protocols from literature. Compounds 1 and 2 exhibit interesting conformation effects of the ‘cage’ side chains due to the presence of intramolecular hydrogen bonding in compound 1. Similar through-space deshielding of the cage protons when in proximity to a side chain hetero atom was reported previously by our research group. A rare through-space deshielding effect on some of the cage carbons is observed. It also appears that ring strain
experienced by different PCU skeletons is responsible for large electronic differences of certain groups in the cage framework.

**Experimental**

Infrared spectra were obtained from a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment. Accurate mass spectra were measured with a Bruker Micro TOF-QII instrument while melting point analysis (uncorrected) was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled using sodium wire/benzophenone under a N₂ atmosphere. Dichloromethane was dried using phosphorus pentoxide. The NMR data were recorded on a Bruker AVANCE III 400 and 600 instruments; the chemical shifts were referenced to the solvent peak, namely 7.24 ppm for CDCl₃, 2.50 ppm for (CD₃)₂SO and 3.34 ppm for CD₃OD at ambient temperature. The ¹H NMR spectra were recorded at a transmitter frequency of 600.103 MHz (spectral width, 12335.526 Hz; acquisition time, 1.328 s; 90° pulse width, 15μs; scans, 16; relaxation delay, 1.0 s) for the Bruker AVANCE III 600 instrument while the ¹H NMR spectra were recorded at a transmitter frequency of 400.222 MHz (spectral width, 8223.685 Hz; acquisition time, 3.98 s; 90° pulse width, 10μs; scans, 16; relaxation delay, 1.0 s) for the Bruker AVANCE III 400 instrument. The ¹³C NMR spectra were recorded at 150.910 MHz (spectral width, 36057.69 Hz; acquisition time, 9.08 s; 90° pulse width, 9.00 μs; scans, 4800; relaxation delay, 2.00 s) for the Bruker AVANCE III 600 instrument while the ¹³C NMR spectra were recorded at 100.645 MHz (spectral width, 24038.461 Hz; acquisition time, 1.363 s; 90° pulse width, 8.40 μs; scans, 3200; relaxation delay, 2.00 s) for the Bruker AVANCE III 400 instrument.

The 2D experimental data parameters obtained on the Bruker AVANCE III 400 were as follows: 90° pulse width, 10 μs for all spectra; spectral width for ¹H, 3355.705, 2403.846, 2577.320, 3144.654, 3875.969 and 3623.188 Hz for 1, 2, 4, 5, 6, 7 and 8 respectively (NOESY, COSY, HSQC and HMBC); spectral width for ¹³C, 1666.666 Hz (HSQC and HMBC) for 1, 2, 5, 7 and 8, 16670.416 Hz (HSQC and HMBC) for 4; number of data points per spectrum, 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) for compounds 1 - 8; number of time-incremented spectra, 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for compounds 1 - 8; relaxation delay for compounds 1, 2, 5 - 8 was 1.4 s and 4 was 1.3 s for COSY experiments, the relaxation delay for NOESY experiments are 2.0 s for compounds 1, 6, 7 and 8, 1.8 s for 2 and 1.9 s for 4 and 5 respectively. The relaxation delay for HMBC experiments are 1.3 s for 1, 6 and 8, 1.0 s for 2, 1.1 s for 4 and 1.2 s for 5 while HSQC experiments had 1.4 s for 1, 2, 4 and 5 and 1.5 s for 6 – 8 respectively. All 2D experimental data obtained on the Bruker AVANCE III 600 for compound 3 are as follows; 90° pulse width, 15.1 μs for all spectra; spectral width for ¹H is 6009.615 Hz (NOESY, COSY, HSQC and HMBC); spectral width for ¹³C, 25000 and 36057.691 Hz (HSQC and HMBC) for 3, number of data points per spectrum, 2048 (COSY), 2048 (NOESY), 4096
Synthesis of PCU diol diacid 11

A solution of the diene 10\(^{[10]}\) (15.0 g, 58.0 mmol) in dry methanol (100 mL) was purged with nitrogen for six hours while cooling in a dry ice-isopropanol bath (-78 °C). Ozone was bubbled into the reaction mixture until a blue-purple colour persisted indicating the presence of excess ozone in the system and hence the completion of the reaction. The excess ozone gas was purge with nitrogen and the solvent (MeOH) removed \textit{in vacuo}. Formic acid (100 mL) was added to the ozonide and the mixture was cooled in an ice bath with stirring. Hydrogen peroxide (150 mL, 30%) was then added drop-wise to the stirring cooled reaction mixture. The reaction was left to attain ambient temperature for one hour and refluxed gently for twelve hours, the resulting mixture was concentrated \textit{in vacuo} to yield the diol diacid 11 (15.6 g, 92 % yield) as a white solid. M.p: 153 – 157 °C, \(^1\)H NMR [(CD\(_3\))\(_2\)SO, 400 MHz]: \(\delta\)H 1.03 (AB, \(J_{AB} = 10.4\) Hz, 1H), 1.51 (AB, \(J_{AB} = 10.4\) Hz, 1H), 2.23 (s, 3H), 2.26 (s, 2H), 2.37 (s, 2H), 2.48 – 2.50 (m, 3H), 2.57 – 2.59 (m, 2H). \(^{13}\)C NMR [(CD\(_3\))\(_2\)SO, 100 MHz]: \(\delta\)C 33.5 (t), 38.8 (d), 42.2 (d), 43.9 (d), 44.1 (t), 49.9 (d), 76.5 (s), 172.4 (s).

Synthesis of PCU diol diamide 1

A mixture of the PCU diol diacid 11 (5.0 g, 17.0 mmol), NH\(_4\)Cl (2.4 g, 44.2 mmol), HOBt (5.7 g, 37.4 mmol), EDC (7.1 g, 37.4 mmol) and NMM (4.9 mL, 44.2 mmol) was dissolved in DMF (30 mL) and stirred at room temperature for 36 hours. The reaction mixture was concentrated \textit{in vacuo}. The crude product was dissolved in 1M HCl (50 mL) and extracted with DCM (2 x 50 mL). The combined organic solution was washed successively with 10% NaHCO\(_3\) (2 x 50 mL) and brine (2 x 50 mL). The resulting organic solution was dried over Na\(_2\)SO\(_4\) and concentrated \textit{in vacuo}. The crude product was purified \textit{via} column chromatography on silica gel using CHCl\(_3\): MeOH: 25 % NH\(_4\)OH (88:10:2, \(R_f = 0.3\), 72 % yield) to obtain a white solid. M.p.; 207 – 209 °C, IR \(\nu_{max}\): 3172, 2961, 1656, 1408, 1287, 728, 634, and 436 cm\(^{-1}\). MS (TOF) calculated for C\(_{15}\)H\(_{21}\)N\(_2\)O\(_4\) (M + H\(^+\)) 293.1496, found 293.1484. The NMR data for 1 are presented in Table 1 and 2.

Synthesis of PCU ether diamide 2

To a solution of the PCU ether diacid 12\(^{[10]}\) (1.5 g, 5.4 mmol) in DCM (10 mL) was added oxalyl chloride in DCM (10 mL) dropwise over ten minutes. Two drops of DMF was added and stirred for two hours under nitrogen. The resulting mixture was concentrated \textit{in vacuo} to obtain 13.\(^{10}\) Compound 13 was re-dissolved in dry DCM (20 mL) and added drop wise to a stirring solution of
NH\textsubscript{3}/CHCl\textsubscript{3} at 0 °C over 20 minutes. The reaction was stirred for two hours, filtered and the filtrate concentrated \textit{in vacuo}. Purification was carried out on silica gel using CHCl\textsubscript{3}: MeOH: 25 % NH\textsubscript{4}OH (88:10:2, R\textsubscript{f} = 0.5) to obtain a yellow slurry (1.1 g, 74 % yield). IR \nu\textsubscript{max}: 3343, 3193, 2961, 1653, 1397, 643 and 587 cm\textsuperscript{-1}. MS (TOF) calculated for C\textsubscript{13}H\textsubscript{19}N\textsubscript{2}O\textsubscript{3} (M + H\textsuperscript{+}) 275.1396, found 275.1397. The NMR data for 2 are listed in Table 1 and 2.

**Synthesis of PCU ether derivative 3**

A mixture of crude PCU ether diamide 2 (3 g, 10.9 mmol), sodium hypochlorite (45 mL) and 4M sodium hydroxide (45 mL) in a 250 round bottom flask was heated to 80 °C overnight. The mixture was concentrated \textit{in vacuo} and purified via column chromatography on silica gel using CHCl\textsubscript{3}: MeOH: 25 % NH\textsubscript{4}OH (88:10:2, R\textsubscript{f} = 0.6) to obtain a compound 3 as a yellow slurry (1.2 g, 50 %). IR \nu\textsubscript{max}: 3355, 2949, 2925, 2857, 1563, 1468, 1381, 1304 and 523 cm\textsuperscript{-1}. MS (TOF) calculated for C\textsubscript{13}H\textsubscript{19}N\textsubscript{2}O (M + H\textsuperscript{+}) 219.1492, found 219.1487. The NMR data for 3 are presented in Table 1 and 2.

**Synthesis of PCU ether derivative 4**

To a solution of the PCU ether diamide 2 (7 g, 25.0 mmol) in THF (40 mL) in an external ice cold bath was added slowly ten equivalence of LAH (9.6 g, 25.3 mmol), the resulting mixture was refluxed for 24 hours under nitrogen. The reaction mixture was allowed to cool to room temperature after which aqueous Na\textsubscript{2}SO\textsubscript{4} was added drop wise over one hour. The resulting precipitate was filter off and the filtrate concentrated \textit{in vacuo}. The crude product was purified via column chromatography on silica gel using CHCl\textsubscript{3}: MeOH: 25 % NH\textsubscript{4}OH (88:10:2, R\textsubscript{f} = 0.5) to obtain compound 4 as a yellow slurry (2.48 g, 39.5 %). IR \nu\textsubscript{max}: 3358, 2949, 2859, 1562, 1467, 1330 and 527 cm\textsuperscript{-1}. MS (TOF) calculated for C\textsubscript{13}H\textsubscript{19}O\textsubscript{2}N (M + H\textsuperscript{+}) 247.1805, found 247.1827. The NMR data for 4 are listed in Table 1 and 2.

**Synthesis of PCU dimethoxyl diamide 5 and 7**

A mixture of the PCU diacid (11/12) (1 mol), glycine methyl ester (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated \textit{in vacuo}. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % NaHCO\textsubscript{3} (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated \textit{in vacuo}. The crude product was purified via column chromatography on silica gel using CHCl\textsubscript{3}: MeOH: 25 % NH\textsubscript{4}OH (88:10:2) to obtain the desired products 5 and 7 respectively.
Data for PCU ether dimethoxyl diamide 5

A yellow oil (2.59 g, Rf = 0.8, yield 68.7 %). IR νmax: 3320, 2954, 1741, 1649, 1534 and 1201 cm⁻¹. MS (TOF) calculated for C₂₁H₂₇N₂O₇ (M + H⁺) 419.1813, found 419.1803. The NMR data for 5 are presented in Table 1 and 2.

Data for PCU diol dimethoxyl diamide 7

A yellow oil (2.3 g, Rf = 0.6, yield 51.5 %). IR νmax: 3266, 2953, 1744, 1639, 1533, 1435 and 1204 cm⁻¹. MS (TOF) calculated for C₂₁H₂₉N₂O₈ (M + H⁺) 437.1918, found 437.1893. The NMR data for 7 are listed in Table 1 and 2.

Synthesis of PCU diamide diacid 6 and 8

PCU diacid dimethoxyl (5 and 7) (2.59 g) dissolved in THF (20 mL) was added 1 M LiOH solution (12.4 mL) with stirring overnight. THF was removed in vacuo and the aqueous solution was neutralized using 12M HCl to pH 7. The desired product was extracted with ethyl acetate (3 x 100 mL) and the combined organic layer was dried with anhydrous Na₂SO₄ and concentrated in vacuo.

Data for PCU ether diamide diacid 6

A yellow white solid (2.32 g). IR νmax: 3284, 2960, 1593, 1397 and 1297 cm⁻¹. MS (TOF) calculated for C₁₉H₂₃N₂O₇ (M + H⁺) 397.1605, found 397.1631. The NMR data for 6 are presented in Table 1 and 2.

Data for PCU diol diamide diacid 8

A yellow white solid (2.0 g). IR νmax: 3249, 2934, 1599, 1390, 1290 and 1101 cm⁻¹. MS (TOF) calculated for C₁₉H₂₅N₂O₈ (M + H⁺) 409.1605, found 409.1589. The NMR data for 8 are listed in Table 1 and 2.

Computational details

DFT gas phase calculations were executed by using GAUSSIAN 09. The density functional hybrid method B3LYP was employed in combination with the 6-31+G(d) basis set. In the present case, diffuse functions on heavy atoms only is justified because the negative charge is mainly located on oxygen and nitrogen atoms. Polarization functions remove some limitations of the basis set by expansion of the virtual space. Solvation and catalytic effects were not considered in order to simplify the model. The side chains were manually rotated to find the lowest energy structure for each compound (1 and 2). NMR shifts for the optimized structures were calculated at the same level using the GIAO method. The molecular orbitals of the optimized structures were calculated with
the “pop=full” command. The Cartesian coordinates of these two low energy structures are available as supplementary material.

Acknowledgements

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Reference

27. E. Breitmaier, *Structure elucidation by NMR in Organic Chemistry*; Wiley and Sons Ltd:
    770, 221-228.
41. Gaussian 09, Revision A.1, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A.
    Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji,
    M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg,
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CHAPTER 10
SYNTHESIS AND NMR ELUCIDATION OF NOVEL PENTACYCLO-UNDECANE DIAMINE LIGANDS

Oluseye K. Onajole,a Patrick Govender,b Thavendran Govender,c Glenn E. M. Maguire,a and Hendrik G. Krugera*

aSchool of Chemistry, University of KwaZulu-Natal, Durban 4001, South Africa
bSchool of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban 4001, South Africa
cSchool of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban 4001, South Africa

Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091

Abstract
The synthesis and NMR elucidation of eight novel pentacyclo-undecane (PCU) diamine compounds are reported. These ligands are potential anti-inflammatory agents to be used against rheumatoid arthritis (RA). One dimensional NMR techniques (1H and 13C spectra) show major overlapping of methine resonances of the “cage” (PCU) thereby making it extremely difficult to assign all NMR signals. This overlapping occurs as a result of the substitutions made at the quaternary carbons (C-8/C-11) of the cage. Two dimensional NMR techniques proved to be a useful tool in overcoming this problem.

Keywords: 2D NMR, Pentacyclo-undecane diamine, Ligands.

Introduction
The chemistry of polycyclic “cage” compounds have been of major interest to organic chemists for decades.[1-4] As part of an ongoing programme to utilize NMR spectroscopy for the structure elucidation of PCU derivatives, the NMR assignments of eight PCU diamine derivatives (1-8) was attempted (Figure 1).

Many authors over the years have attributed the difficulties encountered in the elucidation of cage based compounds.[1,5-11] Geminal/vicinal proton-proton coupling and long-range proton-proton interactions result in broad unresolved resonances. However, the emergence of a range of standard 2D NMR techniques has helped a great deal in overcoming these problems. Our group has successfully used 2D NMR techniques to elucidate numerous PCU derivatives.[12-21] Odisitse et al.[22] recently reported potent anti-inflammatory activity for the PCU diamine 9; this discovery led
to the design and synthesis of compounds 3-8 which are currently being investigated as potential inflammatory agents while compounds 1 and 2 are precursors to 3 and 4.

Figure 1: PCU diamine compounds 1-8
The starting material for the synthesis of PCU diamine derivatives 1-4 is the PCU ditosylate 10\[23\]

Di-tert-butyl dicarbonate 11 is reacted with excess diamine 12/13 (1:10 ratio) to yield N-tert-butoxycarbonyl diamines 14 and 15 in quantitative yields.[24]

To a solution of N-tert-butoxycarbonyl diamine in methanol was added 1.1 mole equivalent of benzaldehyde to yield compounds 16 and 17.[24]

A mixture of PCU ditosylate 10 and N’-benzyl-N”-tert-butoxycarbonyl diamine (16 and 17) in acetonitrile and triethylamine was refluxed under nitrogen for 36 hours to obtain the novel compounds 18 and 19. The benzyl groups of compounds 18 and 19 were deprotected using 10% Pd/C and ammonium formate to yield compounds 1 and 2 respectively. Compounds 3 and 4 were obtained after removal of the Boc protecting groups.

Scheme 1: Synthesis of N’-benzyl-N”-tert-butoxycarbonyl diamine (16 and 17)

A mixture of PCU ditosylate 10 and N’-benzyl-N”-tert-butoxycarbonyl diamine (16 and 17) in acetonitrile and triethylamine was refluxed under nitrogen for 36 hours to obtain the novel compounds 18 and 19. The benzyl groups of compounds 18 and 19 were deprotected using 10% Pd/C and ammonium formate to yield compounds 1 and 2 respectively. Compounds 3 and 4 were obtained after removal of the Boc protecting groups.
Scheme 2: Synthesis of novel PCU diamine compounds (1-4)

The starting material for the synthesis of PCU carbonyl diamine derivatives (5-8) is the PCU ether diacid 20[25]. PCU ether diacid 20 was converted to its corresponding acid chloride 21 as described in literature.[25] Compound 21 was reacted with N-substituted diamines 22 and 23 under a nitrogen atmosphere to obtain compounds 5 and 6 (Scheme 3). Compounds 5 and 6 were subjected to reduction using lithium aluminium hydride to obtain the corresponding diamine compounds 7 and 8.
Scheme 3: Synthesis of PCU diamine compounds 5 - 8

Results and discussion

All pentacyclo-undecane diamine compounds (1-8) reported are meso compounds thereby simplifying the NMR elucidations, since all groups on the cage, except the methylene group at C-4, exit as pairs. From literature,[16,17] it is known that the H-4 protons appear as a pair of doublets (geminal protons) one for each of H-4a and H-4s with AB spin resonances of approximately 1.50 and 1.80 ppm ($J \sim 10$Hz) respectively.

In the $^1$H NMR spectrum of compound 1 the geminal protons H-4a and H-4s appear at 1.49 and 1.85 ppm respectively with a coupling constant of 10.4 Hz. This pair of doublets shows both COSY and NOESY correlations with resonances registering at 2.34 ppm which was assigned to H-3/H-5. H-3/H-5 exhibits COSY correlations to two signals at 2.46 and 2.57 ppm which should be H-2/H-6 and H-9/H-10. Since only H-2/H-6 should show COSY interaction with one more set of protons (H-1/H-7) the signal at 2.57 ppm was assigned to H-2/H-6; these protons exhibit a COSY correlation with an additional signal at 2.47 ppm, which was assigned to H-1/H-7. On the other hand, H-9/H-10 is only
supposed to show one COSY correlation, namely with H-3/H-5. By way of elimination the signal at 2.46 ppm was assigned to H-9/H-10. H-4a exhibits a NOESY interaction with H-2/H-6 (2.57 ppm) and H-3/H-5 (2.34 ppm) while H-4s shows a NOESY interaction with H-3/H-5 (2.34 ppm) and H-9/H-10 (2.46 ppm) respectively. This reinforced the assignments made before.

The methylene protons, H-1’ (1.93 ppm) exhibit a NOESY interaction with H-9/H-10 (2.46 ppm) and a signal at 2.68 ppm (2H) which was assigned to H-2’. The $^1$H spectrum of compound 1 shows the absence of methylene benzyl protons at 3.55 ppm and its ring protons (7.17 – 7.26 ppm) indicating the successful deprotection of the precursor 18. A NOESY interaction between the methylene protons of H-1’ (1.93 ppm) and that of H-3’ (2.68 ppm) was observed, the same unique through space effect was reported for similar cage compounds [17]. Distinction between H-2’ and H-3’ is possible by looking at their COSY correlations. H-1’ shows a COSY correlation with H-2’ (2.68 ppm) while H-3’ exhibits COSY correlations to H-4’ (3.18 ppm). The two NH protons register at 2.13 and 5.34 ppm show NOESY interactions with H-3’ and H-4’ respectively. All corresponding carbons of the assigned protons were identified using the HSQC spectrum. H-4’ exhibits a HMBC correlation with the Boc carbonyl carbon (C-5’) registering at 156.1 ppm and also with C-3’ (49.0 ppm). The methyl protons (H-7’) exhibit a HMBC interaction with the quaternary carbon, C-6’ (79.0 ppm). Also evident on the HMBC spectrum, is the correlation of C-8/11 (95.6 ppm) with H-1’ (1.93 ppm) and H-2’ (2.68 ppm) while C-1’ (32.1 ppm) shows an interaction with H-2’ (2.68 ppm) and H-1/H-7 (2.47 ppm) respectively. C-9/C-10 (58.4 ppm) exhibit a HMBC correlation with H-2/H-6, H-1/H-7, H-3/H-5, H-1’ and H-4a while H-4’ (3.18 ppm) interacts with C-3’ (49.0 ppm). Further confirmation of these assignments was performed using the HSQC spectrum. The NMR assignments of compound 1 are presented in Table 1.

A similar methodology as applied in the elucidation of compound 1 was used to elucidate the cage protons and carbons of the remaining compounds. Details of the subsequent NMR assignments of the PCU skeleton will be omitted in further discussions.

The $^1$H spectrum of compound 2, confirms the successful removal of the benzyl protecting group when compared to that of compound 19. H-4’ (1.47 ppm) exhibits NOESY and COSY correlations with H-3’ (2.47 ppm) and H-5’ (2.99 ppm) while NOESY interactions between H-5’ (2.99 ppm) and the H-8’ methyl protons (1.25 ppm) were also observed. H-5’ shows HMBC correlations with C-4’ (29.8 ppm), C-3’ (47.4 ppm) and the C-6’ carbonyl carbon registering at 156.1 ppm. A HMBC correlation was also observed between the C-7’ quaternary carbon (78.6 ppm) and H-8’ (1.25 ppm). C-8/11 shows HMBC correlations with H-2’ (2.51 ppm) and H-1’ (1.77 ppm) while C-9/10 interacts with H-2/6 (2.43 ppm), H-1/H-7 (2.33 ppm), H-3/H-5 (2.20 ppm), H-1’ (1.77 ppm), H-4s (1.70 ppm) and H-4a (1.35 ppm) respectively. These assignments are presented in Table 1.
Table 1: NMR data for PCU derivatives 1 and 2

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\(^{a}\) Solvent CDCl₃

\(^{b}\) 600MHz for \(^1H\) and 150 MHz for \(^{13}C\)

It is clear from Table 1 that the proton and carbon signals for compounds 1 and 2 correlate. This gives some reassurance about the correctness of the assignments.

The PCU diethylene diamine was obtained as its HCl salt and basified to obtain compound 3 which was dissolved in CDCl₃. The \(^1H\) spectrum confirms the absence of the Boc methyl protons positioned at 1.40 ppm in the precursor, compound 1. It also appears that H-4' in compound 3 registers at a
lower frequency compared to that for compound 1. COSY interaction between H-1’ (1.93 ppm) and H-2’ (2.67 ppm) was observed while H-3’ shows both COSY and NOESY correlations with H-4’ (2.75 ppm). The NOESY spectrum displays an interaction of H-1’ (1.93 ppm) with H-9/H-10 (2.45 ppm), H-1/H-7 (2.47 ppm), H-2’ (2.67 ppm) and also like the precursor (1), with H-3’ (2.63 ppm). H-3’ shows a HMBC correlation with C-4’ (41.6 ppm) and C-2’ (46.3 ppm) while C-8/11 correlates with H-2’ and H-1’ respectively. C-9/10 (58.6 ppm) show HMBC correlations with H-1’ and H-4a (1.48 ppm) while C-1/C-7 (47.9 ppm) correlate with H-1’. Comparison of the $^{13}$C data for compound 3 with that of the precursor 1 again display remarkable agreement. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table 2.

A similar rational was carried out for compound 4 but unlike 3, the NMR spectra of 4 were unresolved suggesting that the compound is unstable, hence the HCl salt of compound 4 was used, requiring D$_2$O as the NMR solvent. As expected, no NH protons were observed for the $^1$H NMR spectrum (as observed in compound 3) due to deuterium exchange of the NH protons. A methylene proton registering at 2.13 ppm was assigned to H-4’. H-4’ shows both NOESY and COSY correlations to two methylene protons registered at 3.14 and 3.20 ppm (H-5’ and/or H-3’) further confirmation for the assignment of H-4’. H-1’ shows a COSY interaction to a signal registering at 3.16 – 3.24 ppm; this was assigned to H-2’, H-1’ also exhibits a NOESY correlation to H-9/H-10 (2.67 ppm), H-1/H-7 (2.69 ppm) and an overlapping signal at 3.16 – 3.24 ppm; this signal was assigned to H-2 and H-3. Carbon signals were assigned using the HSQC spectrum. C-5’ (36.6 ppm) shows a HMBC interaction with H-3’ (3.20 ppm) and H-4’ (2.13 ppm) while C-3’ (44.5 ppm) correlates with H-2’ (2.16 - 2.24 ppm) and H-4’ (2.13 ppm) respectively. C-8/C-11 show a HMBC correlation with H-2’ and H-1’. These assignments are presented in Table 2.


Table 2: NMR data for PCU derivatives 3 and 4

<table>
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<th>Atom</th>
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<th>J(Hz)</th>
<th>δ(^{13}C)(^{a,b})</th>
<th>Atom</th>
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\(^{a}\) Solvent CDCl₃; \(^{b}\) 600MHz for \(^{1}H\) and 150 MHz for \(^{13}C\)

\(^{c}\) Solvent D₂O; \(^{d}\) 400MHz for \(^{1}H\) and 100 MHz for \(^{13}C\)

Compound 5 shows a NOESY interaction between H-9/H-10 (2.54 ppm) and H-1’ (2.65 ppm) while H-3’ (3.25 ppm) interacts with H-4’ (2.33 ppm) and H-5’ (2.15 ppm) respectively. The \(^{1}H\) NMR spectrum displays an amide proton registering at 7.08 ppm; the amide signal exhibits a NOESY interaction with H-1’, H-3’, H-4’ and H-5’ respectively. Each corresponding carbon signal was confirmed using the HSQC spectrum. H-3’ exhibits HMBC correlations with C-4’ (57.9 ppm) and the carbonyl carbon (C-2’) registering at 170 ppm. H-1’ also shows HMBC correlations with C-1/7 (48.2 ppm), C-9/10 (58.6 ppm), C-8/11 (93.8 ppm) and C-2’ (170.0 ppm) respectively. The assigned signals are presented in Table 3.
The $^1$H NMR spectrum of compound 6 displays a similar pattern when compared to that of 5. Major differences were observed for the region from 2.0 – 2.40 ppm. This is due to the methyl protons H-6’ (0.95 ppm) in compound 6 which are further removed from the nitrogen atom. The methylene protons (H-5’) are overlapping with the methylene protons of H-4’ registering at 2.47 – 2.49 ppm. H-3’ and the amide N-H’ proton shows NOESY interactions with H-4’, H-5’ (2.47 – 2.49 ppm) and H-6’ (0.95 ppm) respectively. Due to the overlapping of the proton peaks for H-4’ and H-5’, the HSQC spectrum was as useful as before. The HMBC spectrum was thus vital in assigning the carbon signals of H-4’ and H-5’. H-3’ exhibits a HMBC correlation to a carbon signal registering at 51.5 ppm; this was assigned to C-4’ while H-6’ shows correlation to a carbon signal registering at 11.7 ppm; this was assigned to C-5’. The carbonyl carbon (C-2’) registering at 169.8 ppm shows a HMBC correlation with H-3’ and H-1’ respectively. Further assignments and confirmation of all other proton signals were done using the HSQC spectrum. The assignments are presented in Table 3.
Table 3: NMR data for PCU derivatives 5 and 6

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<th>$\delta^{13}C^\text{a,b}$</th>
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*a Solvent CDCl$_3$, b 600MHz for $^1H$ and 150 MHz for $^{13}C$.

For compound 7, the absence of a carbonyl signal registering at 170 ppm ($^{13}C$ spectrum) and the presence of a methylene proton at 3.23 ppm ($^1H$ NMR spectrum) confirms the successful reduction of compound 5. H-1' (2.22 ppm) shows NOESY and COSY interactions with H-2' ($\delta$ 3.23). Two methylene protons registering at 3.50 – 3.59 ppm were assigned to H-3' and H-4' while the methyl protons registering at $\delta$ 2.97 was assigned to H-5'. Each corresponding carbon signal was confirmed.
using the HSQC spectrum. The HMBC spectrum was vital in assigning C-3’ and C-4’. The HMBC spectrum exhibits a correlation of a carbon signal registering at 52.4 ppm with H-3’ and H-5’; this carbon was assigned to C-4’ while C-3’ was assigned to a signal registering at 41.8 ppm. All assignments were further confirmed using the HSQC spectrum and are presented in Table 4.

For compound 8, once again the absence of a carbonyl signal at 169.8 ppm (\(^{13}\)C NMR spectrum) and the presence of a methylene proton at 3.22 ppm (\(^{1}\)H NMR spectrum) confirmed the successful synthesis of 8 from 6. H-1’ (2.22 ppm) shows a COSY correlation with H-2’ (3.22 ppm) and a NOESY interaction with H-1/H-7 (2.65 ppm) and H-9/H-10 (2.63 ppm) respectively. The methyl proton registering at 1.30 ppm was assigned to H-6’, H-6’ exhibits a COSY and NOESY interaction with a signal at 3.28 ppm which was assigned to H-5’. H-6’ also shows a NOESY interaction with a signal registering at 3.52 ppm (4H) which was assigned to H-3’ and H-4’. All carbon signals were assigned to their corresponding protons using the HSQC spectrum. C-6’ exhibits a HMBC correlation to H-5’ while a carbon signal registering at 46.7 ppm shows a HMBC interaction to H-5’ while another carbon signal registering at 41.4 ppm shows a correlation with H-2’; the latter was assigned to C-4’ and the latter to C-3’. Further assignments were confirmed using the HSQC spectrum and are presented in Table 4.
Table 4: NMR data for PCU derivatives 7 and 8

| Atom | Compound 7 | | | | Compound 8 | | | |
|------|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|      | δ\(^1\)H\(^a,b\) | J(Hz) | δ\(^13\)C\(^a,b\) | Atom | δ\(^1\)H\(^a,b\) | J(Hz) | δ\(^13\)C\(^a,b\) |
| 1/7  | 2.65       | 47.2          | 1/7  | 2.65       | 47.2          |
| 2/6  | 2.69       | 41.1          | 2/6  | 2.68       | 41.1          |
| 3/5  | 2.48       | 44.0          | 3/5  | 2.47       | 44.1          |
| 4a   | 1.55       | 10.48         | 4a   | 1.55       | 7.04          | 43.0          |
| 4s   | 1.93       | 10.48         | 4s   | 1.93       | 7.04          | 43.0          |
| 8    | -          | 94.9          | 8    | -          | 94.9          |
| 9/10 | 2.63       | 57.9          | 8    | -          | 94.8          |
| 11   | -          | 94.9          | 11   | -          | 94.8          |
| 1'   | 2.22       | 8.24, 7.72    | 1'   | 2.22       | 8.04, 7.92    | 28.1          |
| 2'   | 3.23       | 45.2          | 2'   | 3.22       | 45.1          |
| 3'   | 3.50-3.59  | 41.8          | 3'   | 3.52       | 41.4          |
| 4'   | 2.50-3.59  | 52.4          | 4'   | 3.52       | 46.7          |
| 5'   | 2.97       | 43.4          | 5'   | 3.28       | 48.1          |

* Solvent D\(_2\)O, \(^a\) 400MHz for \(^1\)H and 100 MHz for \(^{13}\)C

**Conclusion**

The full NMR elucidation of eight novel PCU diamine analogues was successfully carried out. Although considerable overlap of proton and carbon signals occurs, 2D NMR techniques again proved to be a useful and convenient tool in the elucidation of these PCU cage ligands. These compounds are currently being tested as potential anti-inflammatory agents.
Supporting information

All NMR spectra mentioned in the text are available as supportive information.

Experimental

The NMR data were recorded on Bruker AVANCE III 400 and 600 MHz instruments; the chemical shifts were referenced to the solvent peak 4.79 ppm for D$_2$O and 7.24 ppm for CDCl$_3$ at ambient temperature. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment. Accurate mass spectra were carried out on aBruker Micro TOF-QII instrument while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled from a sodium benzophenone ketyl solution under N$_2$ atmosphere while dichloromethane was dried using phosphorus pentoxide as the drying agent.

General synthesis of $N$-tert-butoxycarbonyl diamine[24] (14 and 15)

Di-$n$-tert-butyldicarbonate (50.0 mmol, 1 eq) was dissolved in CH$_2$Cl$_2$ (450 mL) and added dropwise to a solution of diamine (0.50 mol, 10 eq) in CH$_2$Cl$_2$ (450 mL) over a period of 3 h, whilst the reaction flask was kept in an ice bath. The reaction mixture was stirred overnight at room temperature after which it was washed with water (4 x 250 mL). The CH$_2$Cl$_2$ solution was separated then dried over MgSO$_4$, removed in vacuo to give the product.

Data for $N$-tert-butoxycarbonylethylene diamine (14)

A colorless oil. [4.8 g, yield 60 %, $R_f = 0.30$-0.40 (solvent system; CH$_3$Cl: MeOH: NH$_4$OH: 88:10:2)]. $^1$H NMR [CDCl$_3$, 400 MHz]: $\delta$H 1.37 (s, 9H), 2.04 (s, 2H, NH$_2$), 2.68 (t, 2H), 3.07 (q, 2H), 5.22 (s, 1H, NH), $^{13}$C NMR [CDCl$_3$, 100 MHz]: $\delta$ 28.2 (q), 41.5 (t), 43.0 (t), 79.0 (s), 156.2 (s).

Data for $N$-tert-butoxycarbonylpropane 1, 3- diamine (15)

A colourless oil [Yield 70 %, 5.7 g, $R_f = 0.30$-0.40 (solvent system; CHCl$_3$: MeOH: NH$_4$OH: 88:10:2)]. $^1$H NMR [CDCl$_3$, 600 MHz]: $\delta$H 1.15 (s, 9H), 1.33 (m, 2H), 2.47 (t, 2H), 2.91 (2H), 5.41 (s, 1H, NH). $^{13}$C NMR [CDCl$_3$, 150 MHz]: $\delta$ 27.9 (q), 32.9 (t), 37.6 (t), 39.0 (t), 78.1 (s), 155.8 (s).

General synthesis of $N'$-benzyl-$N$-tert-butoxycarbonyl diamine[24] (16 and 17)

A mixture of benzaldehyde (39.2 mmol, 1.1 eq) and $N$-tert-Butoxycarbonyl diamine (35.6 mmol, 1 eq) with 1g of freshly dried 3Å molecular sieve in methanol (60 mL) was stirred at 25 °C under N$_2$ atmosphere for 4 hours. The mixture was cooled to 0°C using an external ice-salt bath after which solid NaBH$_4$ (4 eq.) was added slowly over 30 min and stirred overnight at RT. The reaction mixture was concentrated in vacuo and the residue partitioned between ethyl acetate and water (100 mL). The organic solution was extracted with a solution of 0.5 N HCl (3 x 100 mL). The aqueous solution was cooled to 0°C and basified with ammonia solution. The result solution was extracted with
dichloromethane (2 x 200 mL), separated then dried over MgSO₄ and concentrated in vacuo to afford the product.

**Data for N'-benzyl-N'-tert-butoxycarbonylethylene diamine (16)**

A colourless oil. [5.0 g, yield 56%, Rf = 0.60 (solvent system; CHCl₃: MeOH: NH₄OH: 88:10:2)].

1H NMR [CDCl₃, 400 MHz]: δH 7.21-7.26 (m, 5H), 5.07 (s, 1H, NH), 3.77 (s, 2H), 3.23 (q, 2H), 2.73 (t, 2H), 1.43 (s, 9H). 13C NMR [CDCl₃, 100 MHz]: δC 28.3 (q), 40.1 (t), 48.4 (t), 53.4 (t), 79.1 (s), 127.0 (d), 128.0 (d), 128.4 (d), 140.0 (s), 156.1 (s).

**Data for N'-benzyl-N'-tert-butoxycarbonylpropane 1, 3-diamine (17)**

A colourless oil. [5.0 g, yield 67 %, Rf = 0.62 (solvent system; CHCl₃: MeOH: NH₄OH: 88:10:2)].

1H NMR [CDCl₃, 600 MHz]: δH 1.43 (s, 9H), 1.64 (m, 2H), 2.67 (t, 2H), 3.19 (2H), 3.74 (s, 2H), 5.47 (s, 1H, NH), 7.23-7.34 (m, 5H). 13C NMR [CDCl₃, 150 MHz]: δC 28.3 (q), 29.5 (t), 39.3 (t), 47.0 (t), 53.8 (t), 78.6 (s), 126.7 (d), 127.9 (d), 128.2 (d), 140.0 (s), 156.0 (s).

**General synthesis of N'-benzyl-N'-tert-butoxycarbonyl diamine PCU (18 and 19)**

A mixture of N'-benzyl-N'-tert-butoxycarbonyl diamine (9.90 mmol) and PCU ditosylate[23] (4.50 mmol) and triethylamine (9.9 mmol) in CH₃CN (30 mL) was refluxed for two days under N₂ atmosphere. The reaction mixture was cooled and concentrated in vacuo. The crude residue was dissolved in CH₂Cl₂ (150 mL) and washed successively with water (3 x 50 mL) after which the CH₂Cl₂ solution was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified via column chromatography on silica gel using CHCl₃: MeOH: NH₄OH (88:10:2).

**Data for N'-benzyl-N'-tert-butoxycarbonylethylene diamine PCU (18)**

A yellow oil (2.61 g, 82 %, Rf = 0.68). IR νmax: 3136 cm⁻¹, 2964 cm⁻¹, 1702 cm⁻¹, 1364 cm⁻¹, 1163 cm⁻¹, 735 cm⁻¹, 698 cm⁻¹. MS (TOF) calculated for C₄₃H₆₀N₄O₅ (M + H⁺) 713.4590, found 713.4714. 1H NMR [CDCl₃, 400 MHz]: δH 1.41 (s, 9H), 1.45 (AB, JAB = 10.28 Hz, 1H), 1.80 (AB, JAB = 10.28 Hz, 1H), 2.77 (t, 2H), 2.27 (s, 1H), 2.38-2.40 (m, 2H), 2.47-2.58 (m, 5H), 3.14 (m, 2H), 3.55 (s, 2H), 4.78 (br, NH), 7.17-7.27 (m, 5H). 13C NMR (CDCl₃, 100 MHz): δC 28.2 (q), 29.4 (t), 39.4 (t), 41.3 (d), 43.2 (t), 44.0 (d), 47.6 (d), 49.5 (t), 52.6 (t), 58.1 (t), 58.4 (d), 78.5 (s), 94.6 (s), 126.7 (d), 128.0 (d), 128.5 (d), 155.7 (s).

**Data for N'-benzyl-N'-tert-butoxycarbonylpropane 1, 3-diamine PCU (19)**

A yellow oil (2.63 g, 79 %, Rf = 0.60). IR νmax: 3345 cm⁻¹, 2962 cm⁻¹, 2863 cm⁻¹, 1694 cm⁻¹, 1508 cm⁻¹, 1165 cm⁻¹, 732 cm⁻¹, 698 cm⁻¹. MS (TOF) calculated for C₄₅H₆₄N₄O₅ (M + H⁺) 741.4949, found 741.4899. 1H NMR [CDCl₃, 600 MHz]: δH 1.43 (s, 9H), 1.64 (1H), 1.46 (1H), 1.61 (s, 2H), 1.81 (AB, JAB = 10.14 Hz, 1H), 1.97 (t, 2H), 2.28 (s, 1H), 2.37-2.39 (m, 2H), 2.42-2.55 (m, 5H), 3.13 (2H), 3.53 (s,
2H), 5.53 (br, NH), 7.21-7.30 (m, 5H). 13C NMR [CDCl3, 150 MHz]: δC 26.5 (t), 28.5 (q), 29.4 (t), 39.7 (t), 41.6 (d), 43.4 (t), 44.3 (d), 47.8 (d), 49.7 (t), 52.0 (t), 58.7 (t), 58.7 (d), 78.5 (s), 94.9 (s), 126.9 (d), 128.4 (d), 128.9 (d), 139.4 (s), 156.0 (s).

**General synthesis of N-tert-butoxycarbonyldiamine PCU (1 and 2)**

A mixture of (4.9 mmol) N′-benzyl-N′-tert-butoxycarbonyl diamine PCU, ammonium formate (24.5 mmol) and 350 mg of 10% Pd/C in methanol (30 mL) was refluxed under a nitrogen atmosphere for 15 hours. The mixture was cooled to room temperature, filtered and concentrated. The residue obtained was made slightly alkaline with NaHCO3 and extracted with CHCl3 (2 x 100mL). The mixture was dried over anhydrous Na2SO4, concentrated in vacuo to afford pure N-tert-butoxycarbonyl diamine PCU.

**Data for N-tert-butoxycarbonylethylene diamine PCU (1)**

A light yellow oil (CHCl3: MeOH: NH4OH - 88:10:2, Rf = 0.42; 1.9 g, 73 %). IR υmax: 2965 cm−1, 1692 cm−1, 1513 cm−1, 1248 cm−1, 1163 cm−1,753 cm−1. MS (TOF) calculated for C29H48N4O5 (M + H+) 533.3651, found 533.3654. 1H NMR [CDCl3, 600 MHz]: δH 1.40 (br, 9H), 1.49 (AB, JAB = 10.4Hz, 1H), 1.84 (AB, JAB = 10.3Hz, 1H), 1.93 (t, 2H), 2.34 (s, 1H), 2.46-2.48 (m, 2H), 2.57 (s, 1H), 2.62-2.71 (m, 4H), 3.18 (t, 2H), 5.34 (br, NH). 13C NMR (CDCl3, 150 MHz): δC 28.4 (q), 31.1 (t), 41.5 (d), 43.5 (t), 44.2 (d), 46.0 (t), 47.8 (d), 49.9 (t), 58.4 (d), 79.0 (s), 95.6 (s), 156.1 (s).

**Data for N-tert-butoxycarbonylpropane 1,3-diamine PCU (2)**

A light yellow oil (CHCl3: MeOH: NH4OH - 88:10:2, Rf = 0.42; 1.7 g, 68 %). IR υmax: 3320 cm−1, 2962 cm−1, 1690 cm−1, 1517 cm−1, 1166 cm−1,751 cm−1. MS (TOF) calculated for C31H52N4O5 (M + H+) 561.3963, found 561.3971. 1H NMR [CDCl3, 600 MHz]: δH 1.47 (br, 9H), 1.47 (t, 2H), 1.70 (AB, JAB = 10.3 Hz, 1H), 1.77 (t, 2H), 2.20 (s, 1H), 2.31-2.33 (m, 2H), 2.43 (s, 1H), 2.46-2.52 (m, 4H), 2.99 (t, 2H), 5.30 (br, NH). 13C NMR [CDCl3, 150 MHz]: δC 28.3 (q), 29.8 (t), 32.5 (t), 38.9 (t), 41.5 (d), 43.4 (t), 44.1 (d), 46.2 (t), 47.4 (t), 47.8 (d), 58.5 (d), 78.6 (s), 95.2 (s), 156.1 (s).

**General synthesis of PCU diamine.HCl (3 and 4)**

N-tert-butoxycarbonyl diamine PCU (1 or 2, 3.9 mmol) was dissolved in MeOH (30 mL) and a concentrated HCl solution (12 M, 5 mL) was added. The reaction was stirred for 16 h after which the solvent was evaporated under reduced pressure; the obtained residue was filtered and washed with petroleum ether and diethyl ether to obtain PCU diamine hydrochloric salt.

**Data for PCU ethylene diamine.HCl (3)**

A white solid (1.12 g). Melting point: 192-196 °C. IR υmax: 3394 cm−1, 2955 cm−1, 2791 cm−1, 2677 cm−1, 2438 cm−1, 1637 cm−1, 1159 cm−1, 1025 cm−1, 915 cm−1. MS (TOF) calculated for C19H32N4O (M + H+) 333.2649, found 333.2649. 1H NMR [CDCl3, 600 MHz]: δH 1.48 (AB, JAB = 10.3 Hz, 1H), 1.84
(AB, J<sub>AB</sub> = 10.4 Hz, 1H), 1.93 (t, 2H), 2.34 (s, 1H), 2.46 (s, 1H), 2.47 (s, 1H), 2.57 (s, 1H), 2.63 (m, 2H), 2.68 (m, 2H), 2.75 (t, 2H).  \(^{13}\)C NMR (CDCl<sub>3</sub>, 150 MHz): δ<sub>C</sub> 32.6 (t), 41.5 (d), 41.6 (t), 43.4 (t), 44.2 (d), 46.2 (t), 47.8 (d), 52.4 (t), 58.5 (d), 95.4 (s).

Data for PCU propane 1, 3-diamine.HCl (4)
A white solid (1.0 g). Melting point: 276-284°C. IR ν<sub>max</sub>: 3389 cm<sup>-1</sup>, 2954 cm<sup>-1</sup>, 2837 cm<sup>-1</sup>, 2696 cm<sup>-1</sup>, 1520 cm<sup>-1</sup>, 1467 cm<sup>-1</sup>, 1179 cm<sup>-1</sup>. MS (TOF) calculated for C<sub>22</sub>H<sub>36</sub>N<sub>4</sub>O (M + H<sup>+</sup>) 361.2961, found 361.2940. \(^{1}\)H NMR [CDCl<sub>3</sub>, 400 MHz]: δ<sub>H</sub> 1.60 (AB, J<sub>AB</sub> = 10.48 Hz, 1H), 1.97 (AB, J<sub>AB</sub> = 10.48 Hz, 1H), 2.13 (m, 2H), 2.34 (m, 2H), 2.52 (s, 1H), 2.67 (s, 1H), 2.69 (s, 1H), 2.73 (s, 1H), 3.14 (t, 2H), 3.12-3.24 (m, 4H). \(^{13}\)C NMR (CDCl<sub>3</sub>, 100 MHz): δ<sub>C</sub> 23.8 (t), 28.2 (t), 36.6 (t), 41.2 (d), 43.0 (t), 44.1 (d), 44.5 (t), 44.5 (t), 47.3 (d), 57.9 (d), 94.9 (s).

General synthesis of N'-substituted diamine carbonyl PCU (5 and 6)
To a vigorously stirring solution of N'-substituted diamine (4 mole equivalence) in CH<sub>2</sub>Cl<sub>2</sub> is added dropwise a solution of freshly prepared PCU ether acid chloride 21[25] in CH<sub>2</sub>Cl<sub>2</sub> for 30 minutes under inert atmosphere. The reaction was allowed to stir for 4 hours at room temperature and dried in vacuo. The resulting brown oil was dissolved in water and the product extracted with CH<sub>2</sub>Cl<sub>2</sub>, the organic solution was dried over MgSO<sub>4</sub> and concentrated in vacuo. The crude product was purified via column chromatography on silica gel using CHCl<sub>3</sub>: MeOH: NH<sub>4</sub>OH (88:10:2).

Data for N', N' dimethylamino ethylamine carbonyl PCU (5)
A yellow slurry (CHCl<sub>3</sub>: MeOH: NH<sub>4</sub>OH - 88:10:2, R<sub>f</sub> = 0.52; 73 %). IR ν<sub>max</sub>: 3288 cm<sup>-1</sup>, 2951 cm<sup>-1</sup>, 2863 cm<sup>-1</sup>, 2828 cm<sup>-1</sup>, 2783 cm<sup>-1</sup>, 1642 cm<sup>-1</sup>, 1544 cm<sup>-1</sup>, 1460 cm<sup>-1</sup>, 1036 cm<sup>-1</sup>, 847 cm<sup>-1</sup>. MS (TOF) calculated for C<sub>23</sub>H<sub>36</sub>N<sub>4</sub>O<sub>3</sub> (M + H<sup>+</sup>) 417.2860, found 417.2848. \(^{1}\)H NMR [CDCl<sub>3</sub>, 600 MHz]: δ<sub>H</sub> 1.49 (AB, J<sub>AB</sub> = 10.56 Hz, 1H), 1.83 (AB, J<sub>AB</sub> = 10.56 Hz, 1H), 2.15 (s, 6H), 2.33 (t, 2H), 2.40 (s, 1H), 2.54 (s, 1H), 2.57 (s, 1H), 2.59 (s, 1H), 2.65 (t, 2H), 3.25 (m, 2H). \(^{13}\)C NMR [CDCl<sub>3</sub>, 150 MHz]: δ<sub>C</sub> 36.8 (t), 39.7 (t), 41.5 (d), 43.4 (t), 44.1 (d), 45.1 (q), 48.3 (d), 57.9 (t), 58.6 (d), 93.8 (s), 170.0 (s).

Data for N', N' diethylamino ethylamine carbonyl PCU (6)
A yellow slurry (CHCl<sub>3</sub>: MeOH: NH<sub>4</sub>OH - 88:10:2, R<sub>f</sub> = 0.57; 60 %). IR ν<sub>max</sub>: 3300 cm<sup>-1</sup>, 2967 cm<sup>-1</sup>, 2864 cm<sup>-1</sup>, 2816 cm<sup>-1</sup>, 1642 cm<sup>-1</sup>, 1542 cm<sup>-1</sup>, 1448 cm<sup>-1</sup>, 1179 cm<sup>-1</sup>, 1067 cm<sup>-1</sup>, 730 cm<sup>-1</sup>. MS (TOF) calculated for C<sub>27</sub>H<sub>44</sub>N<sub>4</sub>O<sub>3</sub> (M + H<sup>+</sup>) 473.3486, found 473.3468. \(^{1}\)H NMR [CDCl<sub>3</sub>, 600 MHz]: δ<sub>H</sub> 0.95 (t, 6H), 1.50 (AB, J<sub>AB</sub> = 10.56Hz, 1 H), 1.85 (AB, J<sub>AB</sub> = 10.56Hz, 1H), 2.42 (s, 1H), 2.47 – 2.49 (m, 6H), 2.56 (s, 1H), 2.59 (s, 1H), 2.61 (s, 1H), 2.70 (t, 2H), 3.26 (m, 2H). \(^{13}\)C NMR [CDCl<sub>3</sub>, 150 MHz]: δ<sub>C</sub> 11.7 (q), 36.9 (t), 39.7 (t), 41.4 (d), 43.4 (t), 44.1 (d), 46.5 (q), 48.2 (d), 51.5 (t), 58.5 (d), 93.9 (s), 169.8 (s).

General synthesis of N'-substituted diamine PCU (7 and 8)
To a stirring solution of N'-substituted diamine carbonyl PCU (5 or 6, 1 mole) in dry THF as added LiAlH<sub>4</sub> (5 mol) and refluxed under N<sub>2</sub>, reaction was monitored via TLC. After completion, excess
LiAlH₄ was quenched by drop-wise addition of aqueous Na₂SO₄, the resulting precipitate was filtered and the filtrate concentrated in vacuo. The crude product was purified via column chromatography on alumina using CHCl₃:MeOH (90:10). The resulting pure product was converted to its corresponding hydrochloric salt.

**Data for PCU \(N',N'\)dimethylamino ethylamine HCl (7)**

A grey solid (CHCl₃: MeOH - 90:10, \(R_f = 0.56\); 24.7 %), melting point: 269-272°C. IR \(\nu_{\text{max}}\): 3312 cm⁻¹, 2959 cm⁻¹, 2743 cm⁻¹, 2433 cm⁻¹, 1586 cm⁻¹, 1472 cm⁻¹, 1294 cm⁻¹, 1134 cm⁻¹, 994 cm⁻¹, 529 cm⁻¹. MS (TOF) calculated for C₂₂H₄₈N₄O (M + H⁺) 389.3275, found 389.3269. ¹H NMR [CDCl₃, 400 MHz]: \(\delta_H\) 1.55 (AB, \(J_{AB} = 10.48\) Hz, 1H), 1.93 (AB, \(J_{AB} = 10.48\) Hz, 1H), 2.22 (t, 2H), 2.48 (s, 1H), 2.63 (s, 1H), 2.65 (s, 1H), 2.69 (s, 1H), 2.97 (s, 6H), 3.23 (m, 2H), 3.50 – 3.59 (m, 4H). ¹³C NMR [CDCl₃, 100 MHz]: \(\delta_C\) 28.1 (t), 41.1 (d), 41.8 (t), 43.0 (t), 43.4 (q), 44.0 (d), 45.2 (t), 47.2 (d), 52.4 (t), 57.9 (d), 94.9 (s).

**Data for PCU \(N',N'\)diethylamino ethylamine HCl (8)**

A viscous brown oil (CHCl₃:MeOH - 90:10, \(R_f = 0.59\); 25.8 %). IR \(\nu_{\text{max}}\): 3488 cm⁻¹, 3417 cm⁻¹, 2976 cm⁻¹, 2585 cm⁻¹, 2426 cm⁻¹, 1461 cm⁻¹, 1388 cm⁻¹, 1388 cm⁻¹, 1009 cm⁻¹, 531 cm⁻¹. MS (TOF) calculated for C₂₇H₄₈N₄O (M + H⁺) 445.3900, found 445.3907. ¹H NMR [CDCl₃, 400 MHz]: \(\delta_H\) 1.30 (t, 6H), 1.55 (AB, \(J_{AB} = 7.04\) Hz, 1H), 1.93 (AB, \(J_{AB} = 7.04\) Hz, 1H), 2.22 (t, 2H), 2.47 (s, 1H), 2.63 (s, 1H), 2.65 (s, 1H), 2.68 (s, 1H), 3.22 (m, 2H), 3.28 (q, 4H), 3.52 (s, 4H). ¹³C NMR [CDCl₃, 100 MHz]: \(\delta_C\) 8.2 (q), 28.1 (t), 41.4 (t), 43.0 (t), 44.1 (d), 45.1 (t), 46.7 (t), 47.2 (d), 48.1 (t), 57.9 (d), 94.8 (s).

**Acknowledgements**

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References

CHAPTER 11
SUMMARY AND CONCLUSIONS

SUMMARY

The chemistry of polycyclic ‘cage’ compounds remains an interesting and attractive field to many organic and medicinal chemists. The unique chemical and biochemical characteristics of these polycyclic moieties, since the discovery of adamantane, have led to the exploration of their medicinal and pharmaceutical properties.

This project focused mainly on the design, synthesis and screening of polycyclic ‘cage’ analogues as potential anti-tuberculosis and anti-microbial (anti-fungal and bacterial) agents. A range of polycyclic ‘cage’ moieties such as adamantane, pentacycloundecane, pentacyclodecane, trishomocubane, oxapentacycloundecane and aza-pentacycloundecane were utilised in this study. In this project a total of 12 novel intermediates and 31 novel products were synthesised. A thorough NMR elucidation of the various structures was also pursued.

Compounds GKM8, GKM9 and GKM11 (Figure 1) showed similar anti-TB activities as SQ109. These three PCU cyclic tetra-amine derivatives were screened against selected clinical pathogenic strains of fungi (yeasts and moulds) and ATCC strains of bacteria (Gram-positive and Gram-negative strains). GKM11 (a 10 carbon linear alkane chain derivative) however showed promising anti-microbial activities against most of the strains used while GKM8 (10 carbon branched alkene chain) and GKM9 (15 carbon branched alkene chain) showed reasonable activity against similar strains of microorganisms.

![Figure 1: Free salts of PCU tetra-amine derivatives: GKM8, GKM9 and GKM11](image)

This discovery led to the synthesis of several SQ109 analogues where the type of chain and the positioning of the chain on the adamantyl moiety were varied. Compound 3 (a 15 carbon branched
alkene chain) proved to be most active against TB with a twofold increase activity over SQ109 (a 10 carbon alkene chain). This finding supports an earlier report where it was shown that GKM9 was twice as active than GKM8 against tuberculosis. However, the reduction of the alkene chain to branched alkane chains resulted in a loss of anti-TB activity with the exception of compound 2 where anti-TB activity was gained over its alkene version 1. This anomaly is believed to be due the positioning of the hydrocarbon chain diamine on the adamantyl moiety. Synthesis of the 1-adamantyl version of compounds 3 and 5 would proof this conclusion. Attempts to achieved that failed due inability to synthesis the trans-trans farnesyl amine required.

![Figure 2: Structure of SQ109 and its analogues](image)

The next step was to investigate the anti-microbial activity of these compounds (Figure 2). This result showed that the branched alkane derivatives 2, 4 and 5 possessed promising anti-fungal and antibacterial (mainly against Gram-positive strains). The branched alkene derivatives (SQ109 and 1) did not show any promising anti-microbial activities with the exception of 3 which displayed significant anti-microbial activity against most of the strains used. It should be noted that compound GKM11 (linear alkane chain derivative) possess higher antimicrobial activities than GKM8 and GKM9 (alkene chain derivatives); compounds 2, 4 and 5 (branched alkane chain derivatives) also possess
higher antimicrobial activities than its branched alkene chain counterparts 1, 3 and SQ109. This observation shows that antimicrobial activity is enhanced with the alkane chain.

In a related study, Bogatcheva et al.\textsuperscript{1} of Sequella Inc reported the discovery of dipiperidines as new antitubercular agents. Using combinatorial chemistry a library of 10,358 compounds were synthesized and screened for activity against \textit{Mycobacterium tuberculosis}. This led to the discovery of SQ609 with a MIC of 6.25 µM while its counterpart SQ611 possesses a MIC of 31.25 µM.\textsuperscript{1} These two compounds have in common the adamantyl and dipiperidine moiety however the dipiperidine moiety is on position 1 of the adamantane for SQ609 and on position 2 of the adamantane for SQ611 thus supporting the hypothesis that different positioning of the R-group could influence the activities of such compounds (Figure 3).

![Figure 3: Structure of SQ609 and SQ611](image)

Figure 3: Structure of SQ609 and SQ611

Figure 4 shows monosubstituted polycyclic ‘cage’ compounds with the isoprenyl diamine moiety maintained while varying the polycyclic ‘cage’ group (such as trishomocubane, pentacycloundecane, aza-pentacycloundecane and oxa-pentacycloundecane). In this series, trishomocubanyl diamine derivative 6 exhibited a twofold activity over that of compounds 7, 8, 9, 10, 11, 12 and SQ109 against MDR and XDR strains of tuberculosis.
It was postulated that disubstitution on the polycyclic ‘cage’ moieties compared to the mono-substituted SQ109 (adamantyl moiety), might improve the activity as an anti-TB agent. This was not observed as the disubstituted polycyclic ‘cage’ derivatives 14 and 16 showed similar activity than SQ109 while 13, 15 and 17 showed reduced activities (Figure 5). This study produced the first report of the pentacyclodecane ‘cage’ moiety 17 for medicinal use.
Figure 5: Structure of disubstituted polycyclic ‘cage’ diamine derivatives

An interesting observation in the structural elucidation of compounds 18 and 19 using NMR techniques showed an unusual deshielding of H-1/7, H-9/10, H-1’ and C-8/11 in compound 18 than the corresponding signals in compound 19 (Figure 6). This is due to the ability of the side arms to freely rotate around C-8/11 and C-1’ at room temperature, spending on average more time in close proximity to H-1/7 and H-9/10 while factors such as intramolecular hydrogen bonding and thorough space deshielding effects contributed to the C-8/11 of compound 19 to appear at a higher frequency compared to 18. Computational chemistry was also instrumental in explaining the large chemical shift to a higher frequency observed for the C-4 of cage diol 18 and cage ether 19. A similar observation was noted for compounds 22/23 and 24/25.
Figure 6: Structure of PCU diamine precursors

A series of PCU based diamine ligands were also synthesized and elucidated. The full structural elucidation of these compounds adds to an impressive library utilising 2D NMR experiments in overcoming severe overlapping proton and carbon NMR signals. A family of the PCU-diamine compounds (28-33, Figure 7) are currently being investigated by collaborators at UCT and CPUT for activity against arthritus.
Figure 7: Structure of PCU diamines (26-33)
CONCLUSION

This study has led to the identification of a series of novel polycyclic based diamines with potent activities against tuberculosis, bacteria and fungi. A total of 22 novel polycyclic amine derivatives were screened against tuberculosis while nine novel PCU diamine ligands are being investigated for complexation and transport of copper ions for potential activity against arthritus.
Reference

SUPPORTING INFORMATION

Supporting information includes 1D NMR, 2D NMR, IR and HR-MS experiments.

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CHAPTER 3

PENTACYCLOUNDECANE DERIVED CYCLIC TETRA-AMINES: SYNTHESIS AND EVALUATION AS POTENT ANTI-TUBERCULOSIS AGENTS

Oluseye K. Onajole,a Karnishree Govender,b Patrick Govender,c Paul D. van Helden,d Hendrik G. Kruger,a Glenn E. M. Maguire,a Karen Muthusamy,c Manormoney Pillay,b Ian Wiid,d and Thavendran Govender.e*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa.
b Department of Medical Microbiology Nelson R Mandela School of Medicine, Durban, University of KwaZulu-Natal, South Africa.
c School of Biochemistry, University of KwaZulu-Natal, Durban, South Africa.
d Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.
e School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa.
$^1$H NMR spectrum of C10 piperazine PCU (9a)
$^{13}$C NMR spectrum of C10 piperazine PCU (9a)
IR spectrum of C10 piperazine PCU (9a)

$\text{1H NMR spectrum of C15 piperazine PCU (9b)}$
$^{13}$C NMR spectrum of C15 piperazine PCU (9b)
IR spectrum of C15 piperazine PCU (9b)

\[ \text{Proton nmr of C20Pip. PCU in } d_{cdcl3} \]

$\text{^1H NMR spectrum of C20 piperazine PCU (9c)}$
$^{13}$C NMR spectrum of C20 piperazine PCU (9c)
IR spectrum of C20 piperazine PCU (9c)
Mass spectrum of C10 piperazine PCU (9a)
Mass spectrum of C15 piperazine PCU (9b)
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Mass spectrum of C20 piperazine PCU (9c)
CHAPTER 4
SYNTHESIS AND EVALUATION OF SQ109 ANALOGUES AS POTENTIAL ANTI-TUBERCULOSIS CANDIDATES

Oluseye K. Onajole, a Patrick Govender, b Paul D. van Helden, c Hendrik G. Kruger, a* Glenn E. M. Maguire, a Ian Wiid, c and Thavendran Govender, d*

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

c Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

d School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa.

* Corresponding authors. Tel.: +27-31-2608212; Fax: +27-31-2603091 Email address: govenderthav@ukzn.ac.za (T. Govender), Tel.: +27-31-2602181; Fax: +27-31-2603091 Email address: Kruger@ukzn.ac.za (H. G. Kruger)
$^1$H NMR spectrum of compound 11 in CDCl$_3$
$^{13}$C NMR spectrum of compound 11 in CDCl$_3$
IR spectrum of compound 11
$^1$H NMR of Compound 12 in CDCl$_3$
$^{13}$C NMR of Compound 12 in CDCl$_3$
\(^1\)H NMR spectrum of compound 16 in CDCl\(_3\)
$^{13}$C NMR spectrum of compound 16 in CDCl$_3$
IR spectrum of compound 16
$^1$H NMR spectrum of compound 17 in CDCl$_3$
$^{13}$C NMR spectrum of compound 17 in CDCl$_3$
IR spectrum of compound 17
$^1$H NMR spectrum of compound 18 in CDCl$_3$
$^{13}$C NMR spectrum of compound 18 in CDCl$_3$
IR spectrum of compound 18
$^1$H NMR spectrum of SQ109 in CDCl$_3$
$^{13}$C NMR spectrum of SQ109 in CDCl$_3$
HRMS spectrum of compound 11
HRMS spectrum of compound 12
HRMS spectrum of SQ109
HRMS spectrum of compound 16
HRMS spectrum of compound 17
HRMS spectrum of compound 18
CHAPTER 5

NMR ELUCIDATION OF NOVEL SQ109 DERIVATIVES

Oluseye K. Onajole, Patrick Govender, Thavendran Govender, Glenn E. M. Maguire, and Hendrik G. Kruger

\(^a\) School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

\(^b\) School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

\(^c\) School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa
$^1$H spectrum of SQ109
$^{13}$C APT spectrum of SQ109
COSY spectrum of SQ109
SQ109 (1)
Expanded COSY spectrum of SQ109
HSQC spectrum of SQ109
SQ109 (1)
Expanded ROESY spectrum of SQ109
HMBC spectrum of SQ109
\(^1\text{H}\) spectrum of Compound 2
$^{13}$C APT spectrum of Compound 2
COSY spectrum of Compound 2
NOESY spectrum of Compound 2
HSQC spectrum of Compound 2
HMBC spectrum of Compound 2
$^1$H spectrum of Compound 3
$^{13}$C APT spectrum of Compound 3
COSY spectrum of Compound 3
NOESY spectrum of Compound 3
HSQC spectrum of Compound 3
HMBC spectrum of Compound 3
$^1$H spectrum of Compound 4
$^{13}$C APT spectrum of Compound 4
COSY spectrum of Compound 4
NOESY spectrum of Compound 4
HSQC spectrum of Compound 4
HMBC spectrum of Compound 4
$^1$H spectrum of Compound 5
$^{13}\text{C}$ APT spectrum of Compound 5
COSY spectrum of Compound 5
NOESY spectrum of Compound 5
Expanded NOESY spectrum of Compound 5
HSQC spectrum of Compound 5
HMBC spectrum of Compound 5
$^1$H spectrum of Compound 6
$^{13}$C APT spectrum of Compound 6
COSY spectrum of Compound 6
NOESY spectrum of Compound 6
HSQC spectrum of Compound 6
CHAPTER 7

NOVEL POLYCYCLIC ‘CAGE’-1,2-DIAMINES AS POTENTIAL ANTI-TUBERCULOSIS AGENTS

Oluseye K. Onajole, a Yacoob Coovadia, b Patrick Govender, c Hendrik G. Kruger, a * Glenn E. M. Maguire, a Melendhran Pillay, b and Thavendran Govender a,†

a School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

b Microbiology, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa

c School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

d School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa

* Corresponding authors. Tel.: +27-31-2602181; Fax: +27-31-2603091 Email address: Kruger@ukzn.ac.za (H. G. Kruger).
† Tel.: +27-31-2608212; Fax: +27-31-2603091 Email address: govenderthav@ukzn.ac.za (T. Govender).
proton nmr of geranyl ethylene diamine in cdcl3
$^1$H NMR of $N$-geranyl ethane-1,2-diamine (8) in CDCl$_3$
C13 nmr of geranyl ethylene diamine in CDCl3
$^{13}$C (APT) NMR of $N$-geranyl ethane-1,2-diamine (8) in CDCl$_3$
$^1$H NMR of $N$-Geranyl-$N'$-(trishomocubanyl)ethane-1,2-diamine (9) in CDCl$_3$
$^{13}$C (APT) NMR of $N$-Geranyl-$N'$-(trishomocubanyl)ethane-1,2-diamine (9) in CDCl$_3$
HSQC spectrum of N-Geranyl-N'-(trishomocubanyl)ethane-1,2-diamine (9) in CDCl$_3$. 
IR spectrum of $N$-Geranyl-$N'$-(trishomocubanyl)ethane-1,2-diamine (9)
$^1$H NMR of $N$-Geranyl-$N'$-(pentacycloundecyl)ethane-1,2-diamine (10) in CDCl$_3$
apt 13C nmr of GKM6P on 400MHz
$^{13}$C (APT) NMR of $N$-Geranyl-$N'$-(pentacycloundecyl)ethane-1,2-diamine (10) in CDCl$_3$
HSQC spectrum of $N$-Geranyl-$N'$-(pentacycloundecyl)ethane-1,2-diamine (10) in CDCl$_3$
IR spectrum of $N$-Geranyl-$N'$(pentacycloundecyl)ethane-1,2-diamine (10)
$^{1}H$ NMR of $N$-Geranyl-$N'$-(2-adamantyl)ethane-1,2-diamine (SQ109) in CDCl$_3$
$^{13}$C (APT) NMR of $N$-Geranyl-$N'$-(2-adamantyl)ethane-1,2-diamine (SQ109) in CDCl$_3$
HSQC spectrum of $N$-Geranyl-$N'$-(2-adamantyl)ethane-1,2-diamine (SQ109) in CDCl$_3$
IR spectrum of $N$-Geranyl-$N'$-(2-adamantyl)ethane-1,2-diamine (SQ109)
$^1$H NMR spectrum of compound 14 in CDCl$_3$
$^{13}$C APT NMR spectrum of compound 14 in CDCl$_3$
HSQC spectrum of compound 14 in CDCl$_3$
IR spectrum of compound 14
$^1$H NMR spectrum of compound 15 in CDCl$_3$
$^{13}$C (APT) NMR spectrum of compound 15 in CDCl$_3$
HSQC spectrum of compound 15 in CDCl$_3$
IR spectrum of compound 15
$^1$H NMR spectrum of compound 16 in CDCl$_3$
$^{13}$C (APT) NMR spectrum of compound 16 in CDCl$_3$. 
HSQC spectrum of compound 16 in CDCl₃
IR spectrum of compound 16
\(^1\)H NMR spectrum of compound 22 in CDCl\(_3\)
$^{13}$C (APT) NMR spectrum of compound 22 in CDCl$_3$
HSQC spectrum of compound 22 in CDCl$_3$
IR spectrum of compound 22
$^1$H NMR spectrum of compound 23 in CDCl$_3$
$^{13}$C (APT) NMR spectrum of compound 23 in CDCl$_3$
HSQC spectrum of compound 23 in CDCl₃
IR spectrum of compound 23

%T

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2866.34
1668.24
1430.50
1346.80
1198.19
1178.22
890.43
831.69
798.99
719.85
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HRMS spectrum of Compound 9
HRMS spectrum of Compound 10

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- Method: tune_wide_expert.m
- Sample Name: SQ109
- Comment
- Acquisition Date: 9/10/2009 6:43:51 PM
- Operator: BDAL@DE
- Instrument / Ser#: micrOTOF-Q 10139

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![HRMS spectrum of Compound 10](image)

### Intens. x10^6

![HRMS spectrum of Compound 10](image)

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2. C 17 H 39 N 4 O 2: -5.7, -7.9, 0.5, ok, even, 67.58, 0.1055, 0.0027, 0.0424, 0.0009, 0.9392

SQ109
HRMS spectrum of SQ109
HRMS spectrum of Compound 14

Analysis Info
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Sample Name: PCU OH GERANYL DIAMINE
Comment:

Acquisition Parameter
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Focus: Not active
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HRMS spectrum of Compound 15
HRMS spectrum of Compound 16

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**Operator**: BDAL@DE
**Instrument / Ser#**: microTOF-Q 10139

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**Page**: 1 of 1
HRMS spectrum of Compound 22
HRMS spectrum of Compound 23
CHAPTER 8

NOVEL LINEAR DIAMINE DISUBSTITUTED POLYCYCLIC ‘CAGE’ DERIVATIVES AS POTENTIAL ANTI-MYCOBACTERIAL CANDIDATES

Oluseye K. Onajole,\textsuperscript{a} Sphelele Sosibo,\textsuperscript{a} Patrick Govender,\textsuperscript{b} Thavendran Govender,\textsuperscript{c} Paul D. van Helden,\textsuperscript{d} Glenn E. M. Maguire,\textsuperscript{a} Kata Mlinarić-Majerski,\textsuperscript{e} Ian Wiid,\textsuperscript{c} and Hendrik G. Kruger,\textsuperscript{a}\textsuperscript{*}

\textsuperscript{a} School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

\textsuperscript{b} School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa

\textsuperscript{c} School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa

\textsuperscript{d} Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

\textsuperscript{e} Department of Organic Chemistry and Biochemistry, Rudjer Bošković Institute, Zagreb, Croatia

\textsuperscript{*} Corresponding author. Tel.: +27-31-2602181; Fax: +27-31-2603091 Email address: kruger@ukzn.ac.za (H. G. Kruger); Homepage: http://ggkm.ukzn.ac.za
$^1$H NMR spectrum of (N-Geranyl-N'-(2-adamantyl)ethane-1,2-diamine) SQ109
$^{13}$C NMR spectrum of (N-Geranyl-N’-(2-adamantyl)ethane-1,2-diamine) SQ109
$^1$H NMR spectrum of \(N',N'\)-bis[2-\((E)-3,7\)-dimethyl-8-oxoethyl]oxy-2,6-dienylamino]-2-oxoethyl\]-8,11-dihydroxy pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (26)
$^{13}$C NMR spectrum of N,N'-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0$^2$6.3,10.$^0$9]undecane-8,11-dimethylenecarboxamide (26)
IR spectrum of $N', N'\text{-bis}[2-((E)-3,7\text{-dimethyl}2,6\text{-dienylamino})\text{-2-oxoethyl}]\text{-8,11\text{-dihydroxypentacyclo}[5.4.0.0^{2,6}.3^{10}.0^{5,8}]undecane-8,11\text{-dimethylene}\text{carboxamide}}$ (26)
$^1$H NMR spectrum of $N,N\text{-}bis[2-((E)-3,7\text{-}dimethyl\text{oct}a-2,6\text{-}dienylamino)\text{-}2\text{-}oxoethyl]}\text{-}8,11\text{-}oxapentacycl[5.4.0.0^{2,6}.3^{10}.0^{5,9}]\text{undecane-8,11-dimethylenecarboxamide}$ (27)
$^{13}$C NMR spectrum of $N^\prime,N^\prime$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0$^{2,6}$,3$^{10}$,0$^5,9$]undecane-8,11-dimethylenecarboxamide (27)
IR spectrum of $N',N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienlamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0$^{2,6}$,3$^{10}$,0$^{5,9}$]undecane-8,11-dimethylenecarboxamide (27)
$^1$H NMR spectrum of $N',N''$-bis[2-$((E)$-3,7-dimethyl-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dicarboxamide (28)
$^{13}$C NMR spectrum of $N',N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dicarboxamide (28)

IR spectrum of $N',N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dicarboxamide (28)
$^1$H NMR spectrum $N', N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dimethylene-carboxamide (29)
$^{13}$C NMR spectrum of $N,N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dimethylenecarboxamide (29)

IR spectrum of $N,N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1$^{3,7}$]decane-1,3-dimethylenecarboxamide (29)
$^1$H NMR spectrum of $N',N'$-bis[2-((E)-3,7-dimethylocta-2,6-dienlamino)-2-oxoethyl]-pentacyclo[5.3.0.0$^{2,5}$.0$^{3,9}$.0$^{6,8}$]decane-2,5-dicarboxamide (30)
$^{13}$C NMR spectrum of $N^{'},N^{'-}$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0$_2$.5.0$_3$.9.0$_4$.8]decane-2,5-dicarboxamide (30)

IR spectrum of $N^{'},N^{'-}$-bis[2-((E)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0$_2$.5.0$_3$.9.0$_4$.8]decane-2,5-dicarboxamide (30)
\(^1\)H NMR spectrum of \([N,N'-(8,11-dihydroxypentacyclo[5.4.0.0^2.6.3.10.0^5.9]undecane-8,11-diethylene)]-bis([N'-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)
$^{13}$C NMR spectrum of spectrum of $[N',N'-(8,11$-dihydroxypentacyclo[5.4.0.0$^{2,6}.3^{10}.0^{6,9}]$undecane-8,11-diethylene)]-bis[(N''-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)
IR spectrum of spectrum of \([N',N'-(8,11\text{-dihydroxypentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,8}]undecane-8,11\text{-diethylene}]\text{-bis\[\(N'-(E)\text{-3,7-dimethylocta-2,6-dienyl}\text{ethane-1,2-diamine}\]}\text{)}(4)\text{)}\)
$^1$H NMR spectrum of \( [N',N'-(8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]\text{undecane-8,11-diethylene})\text{-bis}(N''-(E)-3,7\text{-dimethylocta-2,6-dienyl})\text{ethane-1,2-diamine}] \ (5) \)
$^{13}$C NMR spectrum of $[\text{N}^\prime,\text{N}^\prime-(8,11\text{-oxapentacyclo}[5.4.0.0^{2,6}.3^{10}.0^{5,9}]\text{undecane-8,11-diethylene})\text{-bis[(N}^\prime\text{'-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine]} $ (5)
IR spectrum of \([N,N^\prime-(8,11\text{-oxapentacyclo}[5.4.0.0^{2,6}.3.10.0^{5,9}]\text{undecane}-8,11\text{-diethylene})\text{-bis}([N^\prime-(E)-3,7\text{-dimethylocta}-2,6\text{-dienyl})\text{ethane}-1,2\text{-diamine}]\ (5)\)
$^1$H NMR spectrum of [N',N'-(tricyclo[3.3.1.1\textsuperscript{3,7}]decane-1,3-dimethylene)]-bis[(N'-\textsuperscript{(E)}-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (6)
$^{13}$C NMR spectrum of $[N', N'-(\text{tricyclo}[3.3.1.1^{3,7}]\text{decane-1,3-dimethylene})]-\text{bis}([N''- (E)-3,7\text{-dimethylocta-2,6-dienyl})\text{ethane-1,2-diamine}]$ (6)

IR spectrum of $[N'', N''-(\text{tricyclo}[3.3.1.1^{3,7}]\text{decane-1,3-dimethylene})]-\text{bis}([N''- (E)-3,7\text{-dimethylocta-2,6-dienyl})\text{ethane-1,2-diamine}]$ (6)
$^1$H NMR spectrum of [N',N'-(tricyclo[3.3.1.1$^3$7]decane-1,3-diethylene)]-bis[(N'-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (7)
$^{13}$C NMR spectrum of \([N',N'-(\text{tricyclo}[3.3.1.1^{3,7}]\text{decane-1,3-diethylene})-\text{bis}[(N'-(E)-3,7\text{dimethylocta-2,6-dienyl})\text{ethane-1,2-diamine}][7)

IR spectrum of \([N',N'-(\text{tricyclo}[3.3.1.1^{3,7}]\text{decane-1,3-diethylene})-\text{bis}[(N'-(E)-3,7\text{dimethylocta-2,6-dienyl})\text{ethane-1,2-diamine}][7)
$^1$H NMR spectrum of [N',N'-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]-bis[(N''-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)
$^{13}$C NMR spectrum of $[N',N'-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]$-bis$[(N'-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine]$ (8)
IR spectrum of [N’,N’-(pentacyclo[5.3.0.0^2,5.0^3,9.0^4,8]decane-2,5-dimethylene)]-bis[(N’-(E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)
HRMS spectrum of SQ109

HRMS spectrum compound 26
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![Mass Spectra Diagram]

*MS, 0.4 min #23*

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<th>c-Conf</th>
<th>mSig ma</th>
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![Mass Spectra Diagram]

*MS, 2.1 min #124*

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HRMS spectrum compound 27
HRMS spectrum compound 28
HRMS spectrum compound 29
Analysis Info

Analysis Name: D:\Data\kenney\GKM-K1000001.d
Method: tune_wide_expert.m
Sample Name: THIELE TETRACARBONYL EDGERANYL
Comment: MASS = 603

Acquisition Parameter

Source Type: ESI
Ion Polarity: Positive
Focus: Not active
Scan Begin: 100 m/z
Scan End: 2000 m/z
Set Nebulizer: 0.4 Bar
Set Capillary: 4500 V
Set End Plate Offset: -500 V
Set Collision Cell RF: 500.0 Vpp
Set Dry Heater: 200 °C
Set Dry Gas: 4.0 l/min
Set Divert Valve: Source

Intens. x10^2

EPC 98.9946-2001.0439 +All MS

Intens. x10^4

+MS, 0.5min #62

Intens. x10^2

+MS, 1.9min #160

Meas. m/z

# Formula

m/z err mean [ppm]

db N- e- Conf mSig ma

Std I I Std m I Std m I Std m I Std m I Comb Dev

605.4077

1 C36 H53 N4 O4 605.4061 -2.6 -2.0 12.5 ok even 6.65 0.0086 0.0013 0.0037 0.0011 0.8312
2 C35 H57 O8 605.4048 -4.8 -4.1 7.5 ok even 7.86 0.0124 0.0026 0.0041 0.0012 0.9103
3 C37 H49 N8 605.4075 -0.4 0.2 17.5 ok even 16.54 0.0230 0.0066 0.0080 0.0011 0.8519

Bruker Compass DataAnalysis 4.0
printed: 8/26/2010 8:13:56 PM
HRMS spectrum compound 30

Analysis Info
Analysis Name: D:\Data\kenny\623-GKM9PD.d
Method: tune-wide.expert.m
Sample Name: 623-GKM9PD
Comment:

Acquisition Info
Analysis Date: 8/12/2010 1:57:03 AM
Operator: BOAL@DE
Instrument/Ser#: micrOTOF-Q 10139

Acquisition Parameter
Source Type: ESI
Ionization: Positive
Set Neutralizer: 0.4 Bar
Set Dry Gas: 4.0 l/min
Scan Begin: 500 m/z
Scan End: 2000 m/z
Set End Plate Offset: -500 V
Set Collision Cell RF: 1000.0 Vpp
Set Divert Valve: Source

Bruker Compass DataAnalysis 4.0 printed: 8/26/2010 8:58:35 PM Page 1 of 2
HRMS spectrum compound 4
HRMS spectrum compound 5

Acquisition info
Analysis Name: D:\Data\kenny\GKM-KA-553000001.d
Method: tune\_wide\_expert.m
Sample Name: GKM-KA-553
Comment: 1,3 ADAMATYL EDA GERANYL

Acquisition Parameter
- Source Type: ESI
- Ion Polarity: Positive
- Focus: Not active
- Scan Begin: 100 m/z
- Scan End: 3000 m/z
- Set Capillary: 4500 V
- Set End Plate Offset: -500 V
- Set Collision Cell RF: 500.0 Vpp
- Set Nebulizer: 1.0 Bar
- Set Dry Heater: 200 °C
- Set Dry Gas: 4.0 l/min
- Set Divert Valve: Source

Intens x10^4 vs Time [min]

Intens x10^4 vs m/z

Measure # Formula m/z err [ppm] Mean err [ppm] mSig ma Std I Mean m/z Std I Van Nor m Std m/z Diff Std Comb Dev
553.5207
1 C 35 H 68 O 4 553.5160 -0.9 -2.5 1.5 ok even 0.0117 0.0014 0.0053 0.0009 0.9653
2 C 36 H 65 N 4 553.5204 -0.5 -0.1 6.5 ok even 12.48 0.0209 0.0004 0.0071 0.0029 0.7371

Bruker Compass DataAnalysis 4.0
printed: 8/25/2010 8:54:31 PM Page 1 of 1
HRMS spectrum compound 6

Analysis Info
Analysis Name: D:\Details\mkm\GKM-KB (581).d
Method: tune_wide_expert.m
Sample Name: GKM-KB
Comment: MASS = 581, 1.3 ADAMANTYL (CH2)2 DIEA GERANYL

Acquisition Info
Source Type: ESI
Ion Polarity: Positive
Scan Begin: 100 m/z
Scan End: 3000 m/z

Intensities

- BPC 99.0000-3001.0000 = All MS
- nMS, 0.9min #60

- m/z 1521.9591
- m/z 2121.9157
- m/z 2721.8884
- m/z 561.5508

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HRMS spectrum compound 7
HRMS spectrum compound 8
CHAPTER 9

SYNTHESIS AND NMR ASSIGNMENT OF PENTACYCLOUNDECANE PRECURSORS OF POTENTIAL PHARMACEUTICAL AGENTS

Oluseye K. Onajole,\textsuperscript{a} Maya M. Makatini,\textsuperscript{a} Patrick Govender,\textsuperscript{b} Thavendran Govender,\textsuperscript{c} Glenn E. M. Maguire,\textsuperscript{a} and Hendrik G. Krugera*  

\textsuperscript{a} School of Chemistry, University of KwaZulu-Natal, Durban, South Africa  
\textsuperscript{b} School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, South Africa  
\textsuperscript{c} School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa  

Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091
Proton NMR of FCU diol diamide in DMSO-d6 on 400MHz

$^1$H spectrum of compound 1 in (CD$_3$)$_2$SO
$^{13}$C spectrum of compound 1 in (CD$_3$)$_2$SO
COSY spectrum of compound 1 in (CD$_3$)$_2$SO
NOESY spectrum of compound 1 in (CD$_3$)$_2$SO
HSQC spectrum of compound 1 in (CD$_3$)$_2$SO
HMBC spectrum of compound 1 in (CD$_3$)$_2$SO
IR spectrum of compound 1
proton nmr of PCU ether diamide in CDCl₃ on 400MHz

1H spectrum of compound 2 in CDCl₃
Apt nmr of FCU ether diamide in CDCl$_3$ on 400MHz

\[ \text{13C spectrum of compound 2 in CDCl}_3 \]
COSY spectrum of compound 2 in CDCl₃
NOESY spectrum of compound 2 in CDCl₃
HSQC spectrum of compound 2 in CDCl₃
HMBC spectrum of compound 2 in CDCl₃
IR spectrum of compound 2
$^1$H spectrum of compound 3 in CDCl$_3$
$^{13}$C spectrum of compound 3 in CDCl$_3$
COSY spectrum of compound 3 in CDCl$_3$
NOESY spectrum of compound 3 in CDCl$_3$
HSQC spectrum of compound 3 in CDCl₃
HMBC spectrum of compound 3 in CDCl₃
IR spectrum of compound 3
$^1$H spectrum of compound 4 in CDCl$_3$
$^{13}$C spectrum of compound 4 in CDCl$_3$
COSY spectrum of compound 4 in CDCl$_3$
NOESY spectrum of compound 4 in CDCl₃
HSQC spectrum of compound 4 in CDCl₃
HMBC spectrum of compound 4 in CDCl₃
IR spectrum of compound 4

%T vs. cm⁻¹

- 3358.93
- 2859.62
- 2949.15
- 1562.51
- 1467.78
- 1394.10
- 1330.58
- 1230.11
- 1135.98
- 1049.99
- 952.49
- 912.05
- 870.38
- 819.53
- 790.41
- 780.41
- 770.41
- 691.86
- 635.94
- 561.12
- 527.86

NH₂ H₂N

4
$^1$H spectrum of compound 5 in CDCl$_3$
APT nmr of PCU ether glycine methyl in CDCl$_3$ 400MHz

$^{13}$C spectrum of compound 5 in CDCl$_3$
COSY spectrum of compound 5 in CDCl₃
NOESY spectrum of compound 5 in CDCl₃
HSQC spectrum of compound 5 in CDCl₃

Hsqc nmr of PCU ether glycine methyl in cdcl3 400MHz
HMBC spectrum of compound 5 in CDCl₃
IR spectrum of compound 5
$^1$H spectrum of compound 6 in CD$_2$OD
$^{13}$C spectrum of compound 6 in CD$_3$OD
COSY spectrum of compound 6 in CD$_3$OD
NOESY spectrum of compound 6 in CD$_3$OD
HSQC spectrum of compound 6 in CD$_3$OD
HMBC spectrum of compound 6 in CD$_2$OD
IR spectrum of compound 6
$^1$H NMR spectrum of compound 7 in CDCl$_3$
13C NMR spectrum of compound 7 in CDCl₃

apt nmr of PCU diol glycine methoxyl in cdcl3 400MHz
Cosy nmr of PCU diol glycin methoxyl in cdcl3 400MHz

COSY spectrum of compound 7 in CDCl3
NOESY spectrum of compound 7 in CDCl₃
HSQC spectrum of compound 7 in CDCl₃
HMBC spectrum of compound 7 in CDCl₃
IR spectrum of compound 7
$^{1}H$ NMR spectrum of compound 8 in CD$_3$OD

$^{1}H$ NMR spectrum of PCU diol glycine in MeOD 400MHz
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COSY spectrum of compound 8 in CD$_3$OD
NOESY spectrum of compound 8 in CD$_3$OD
HMBC spectrum of compound 8 in CD$_3$OD
IR spectrum of compound 8
HRMS spectrum of compound 1
HRMS spectrum of compound 2
HRMS spectrum of compound 3
HRMS spectrum of compound 4
HRMS spectrum of compound 5
HRMS spectrum of compound 6
HRMS spectrum of compound 7
HRMS spectrum of compound 8
Cartesian coordinates of optimised structures for compound 1 and 2

[B3LYP/6-31+G(d)] opt freq

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CHAPTER 10
SYNTHESIS AND NMR ELUCIDATION OF NOVEL PENTACYCLO-UNDECANE DIAMINE LIGANDS

Oluseye K. Onajole, a Patrick Govender, b Thavendran Govender, c Glenn E. M. Maguire, a and Hendrik G. Kruger a*

a School of Chemistry, University of KwaZulu-Natal, Durban 4001, South Africa
b School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban 4001, South Africa
c School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban 4001, South Africa

Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091
$^1$H NMR spectrum of N-tert-butoxycarbonylethylene diamine PCU (1)
$^{13}$C NMR spectrum of $N$-tert-butoxycarbonylethylene diamine PCU (1)
COSY spectrum of N-tert-butoxycarbonylethylene diamine PCU (1)
NOESY spectrum of N-tert-butoxycarbonyl ethylene diamine PCU (1)
HSQC spectrum of N-tert-butoxycarbonylethylene diamine PCU (1)
HMBC spectrum of N-tert-butoxycarbonylethylene diamine PCU (1)
IR spectrum of \( N \)-tert-butoxycarbonylethylene diamine PCU (1)
$^1$H NMR spectrum of $N$-tert-butoxycarbonylpropane diamine PCU (2)
$^{13}$C NMR spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
COSY spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
NOESY spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
HSQC spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
HMBC spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
IR spectrum of N-tert-butoxycarbonylpropane diamine PCU (2)
$^1$H NMR spectrum of PCU ethylene diamine (3)
$^{13}C$ (APT) NMR spectrum of PCU ethylene diamine (3)
Proton NMR of PCU ethylene diamine in cdcl3
probe=5mmBBQ2/av600

COSY spectrum of PCU ethylene diamine (3)
NOESY spectrum of PCU ethylene diamine (3)

Proton nmr of PCU ethylene diamine
in cdcl3
probe=9mmBBC2av600
HSQC spectrum of PCU ethylene diamine (3)

Proton nmr of PCU ethylene diamine
in cdcl3
probe=9mmBB2av600
HMBC spectrum of PCU ethylene diamine (3)
spectrum of PCU ethylene diamine (3)
$^1$H NMR spectrum of PCU propane 1,3 diamine HCl (4)
$^{13}$C NMR spectrum of PCU propane 1,3 diamine HCl (4)
COSY NMR spectrum of PCU propane 1,3-diamine HCl (4)
NOESY NMR spectrum of PCU propane 1,3-diamine HCl (4)
HSQC NMR spectrum of PCU propane 1,3-diamine HCl (4)
HMBC spectrum of PCU propane 1,3- diamine HCl (4)
IR spectrum of PCU propane 1,3- diamine HCl (4)
$^1$H NMR spectrum of PCU carbonyl-N,N dimethyl amino ethylamine (5)
$^{13}$C NMR spectrum of PCU carbonyl-\(\text{N,N dimethyl amino ethylamine}\) (5)
COSY spectrum of PCU carbonyl-N,N dimethyl amino ethylamine (5)
NOESY spectrum of PCU carbonyl-N,N-dimethyl amino ethylamine (5)
HSQC spectrum of PCU carbonyl- $N,N$ dimethyl amino ethylamine (5)
HMBC spectrum of PCU carbonyl- N,N dimethyl amino ethylamine (5)
IR spectrum of PCU carbonyl- \( N,N \) dimethyl amino ethylamine (5)
$^1$H NMR spectrum of PCU carbonyl-$N,N$ diethyl amino ethylamine (6)
$^{13}$C NMR spectrum of PCU carbonyl-N,N diethyl amino ethylamine (6)
COSY spectrum of PCU carbonyl- N,N diethyl amino ethylamine (6)
NOESY spectrum of PCU carbonyl- $N,N$ diethyl amino ethylamine (6)
HSQC spectrum of PCU carbonyl- $N,N$ diethyl amino ethylamine (6)
HMBC spectrum of PCU carbonyl- N,N diethyl amino ethylamine (6)
IR spectrum of PCU carbonyl- $N$,$N$ diethyl amino ethylamine (6)
$^1$H NMR spectrum of PCU - $N,N$ dimethyl amino ethylamine HCl (7)
13C NMR spectrum of PCU - $N,N$-dimethyl amino ethylamine HCl (7)
COSY spectrum of PCU - $N,N$ dimethyl amino ethylamine HCl (7)
NOESY spectrum of PCU - N,N dimethyl amino ethylamine HCl (7)
HSQC spectrum of PCU - N,N dimethyl amino ethylamine HCl (7)
HMBC spectrum of PCU - N,N dimethyl amino ethylamine HCl (7)
IR spectrum of PCU - $N,N$ dimethyl amino ethylamine HCl (7)
$^1$H NMR spectrum of PCU - $N,N$ diethyl amino ethylamine HCl (8)
$^{13}$C NMR spectrum of PCU - $N,N$ diethyl amino ethylamine HCl (8)
COSY spectrum of PCU - N,N-diethyl amino ethylamine HCl (8)
NOESY spectrum of PCU - N,N diethyl amino ethylamine HCl (8)
HSQC spectrum of PCU - N,N diethyl amino ethylamine HCl (8)
HMBC spectrum of PCU - N,N diethyl amino ethylamine HCl (8)
IR spectrum of PCU - $N,N$ diethyl amino ethylamine (8)
HRMS spectrum of compound 1
HRMS spectrum of compound 2
HRMS spectrum of compound 3
HRMS spectrum of compound 4
HRMS spectrum of compound 5
HRMS spectrum of compound 6
HRMS spectrum of compound 7
HRMS spectrum of compound 8