Development of potential dual-action antibacterial agents

Joseph Imre Ambrus
University of Wollongong

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Development of Potential Dual-Action Antibacterial Agents

A thesis submitted in partial fulfillment of the requirements for the award of the degree of

Doctor of Philosophy

From

The University of Wollongong

By

Joseph Imre Ambrus

BACHELOR OF MEDICINAL CHEMISTRY (HONOURS)

Supervisor: Prof. John B. Bremner
School of Chemistry
May, 2008
CERTIFICATION

I, Joseph Imre Ambrus, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, Faculty of Science, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Joseph I. Ambrus

30th May, 2008
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>5′-MHC-D</td>
<td>5′-Methoxyhydnoocarpin-d</td>
</tr>
<tr>
<td>13-CPTC</td>
<td>13-Cyclopentylthio-5-hydroxytetracycline</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>Abq</td>
<td>AB quartet</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption, distribution, metabolism, excretion</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
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<tr>
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<td>boc</td>
<td>tert-Butoxycarbonyl</td>
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<tr>
<td>bs</td>
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<tr>
<td><em>B. subtilis</em></td>
<td><em>Bacillus subtilis</em></td>
</tr>
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<td>calcd</td>
<td>Calculated</td>
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<td>CAN</td>
<td>Ceric ammonium nitrate</td>
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</tr>
<tr>
<td>coe</td>
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</tr>
<tr>
<td>conc</td>
<td>Concentrated</td>
</tr>
<tr>
<td>d</td>
<td>Days or doublet (when used during NMR assignments)</td>
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<tr>
<td>dd</td>
<td>Doublet of doublets</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>dd</td>
<td>doublet of doublets</td>
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<td>DEPT</td>
<td>Distortionless enhancement by polarisation transfer</td>
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<td>DIBAL</td>
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<td>Efflux pump inhibitor</td>
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<tr>
<td>eq</td>
<td>Equivalents</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionisation</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography – mass spectrometry</td>
</tr>
<tr>
<td>gCOSY</td>
<td>Gradient correlation spectroscopy</td>
</tr>
<tr>
<td>gHMBC</td>
<td>Gradient heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>gHSQC</td>
<td>Gradient heteronuclear single quantum correlation</td>
</tr>
<tr>
<td>glu</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen/proton</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>HOBT</td>
<td>1-Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>lac</td>
<td>Lactate</td>
</tr>
<tr>
<td>LG</td>
<td>Leaving group</td>
</tr>
<tr>
<td>Lit.</td>
<td>Literature</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low resolution mass spectrometry</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MATE</td>
<td>Multidrug and toxic compound extrusion</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance (or resistant)</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MFS</td>
<td>Major facilitator superfamily</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrum</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>n-BuLi</td>
<td>n-Butyllithium</td>
</tr>
<tr>
<td>NMP</td>
<td>N-Methylpyrrolidone</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PABA</td>
<td>p-Aminobenzoic acid</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>pH</td>
<td>Potential of hydrogen</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>piv</td>
<td>Pivalate</td>
</tr>
<tr>
<td>PNB</td>
<td>p-Nitrobenzyl</td>
</tr>
<tr>
<td>PPA</td>
<td>Polyphosphoric acid</td>
</tr>
</tbody>
</table>
ppm  Parts per million
Rf   Retention factor
RNA  Ribonucleic acid
RND  Resistance-nodulation division
rRNA Ribosomal ribonucleic acid
r.t. Room temperature
s    Singlet
SAR  Structure-activity relationship
*S. aureus* *Staphylococcus aureus*
SMR  Small multidrug resistance
t    Triplet
TBAB Tetrabutylammonium bromide
TBAI Tetrabutylammonium iodide
td   Triplet of doublets
TEA  Triethylamine
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
TLC  Thin layer chromatography
TMS  Tetramethylsilane
tRNA Transfer ribonucleic acid
tyr  Tyrosine
UV   Ultraviolet
VLC  Vacuum liquid chromatography
WT   Wild-type (bacterial strain)
δ    Chemical shift (in parts per million, downfield from TMS)
Publications arising from this work to date:

Journal articles:


Selected Conference Abstracts:

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ABSTRACT

“Development of Potential Dual-Action Antibacterial Agents.”

Joseph I. Ambrus
University of Wollongong, 2008

With the ever-present threat of bacteria becoming resistant to all known antibacterial drugs comes a pressing need to develop new antibacterial agents which circumvent this resistance. One of the major mechanisms of resistance that bacteria employ to compromise the activity of antibacterials is through efflux pumps. These pumps, such as the NorA pump in Staphylococcus aureus, have the ability to extrude a wide range of structurally dissimilar antibiotics, hence conferring multidrug resistance to the bacteria. To date, there have been no therapeutically useful inhibitors of efflux pumps developed and thus there is great scope to develop agents which address this clinically relevant problem.

This dissertation focused on two main strands of research. The first addressed the need for new inhibitors of bacterial efflux pumps. A structure-activity based approach to drug design was utilised, centering on the lead NorA inhibitor 2-phenyl-5-nitro-1H-indole. These synthetic efforts led to the discovery of 2-phenyl-1H-indole-5-carbonitrile (70) as a potential new inhibitor of the NorA pump, with an MIC of 3.6 µM (in the presence of the antibacterial berberine at 100 µg/mL; 269.0 µM) against the NorA wild-type strain of S. aureus. A serendipitous discovery of a novel antibacterial agent, the alcohol (2-phenyl-1H-indol-5-yl)-methanol (75), was made during these studies. This alcohol was found to have a direct antibacterial MIC of 13.4 µM against a NorA pump knockout strain of S. aureus and 28.0 µM against the NorA wild-type and overexpressing strains of S. aureus. This new
compound offers a simple, heterocyclic lead compound for future development as an antibacterial agent.

The inter-related second strand of research took advantage of dual action-based approaches to drug design. Several dual action drugs were synthesised which combined an efflux pump-sensitive antibiotic (ciprofloxacin) and efflux pump inhibitor analogue. Of these compounds, 90, 91 and 96 showed promising antibacterial activities with MIC’s of 0.6, 3.9 and 1.5 μM respectively against all three strains of S. aureus (NorA knockout, NorA wild-type and NorA overexpressing). This data confirmed that these dual action drugs were evading this particular resistance mechanism and helped to validate this principle of dual action drug design.

A novel dual action prodrug was also designed and a protected version synthesised. This prodrug contained the antibiotic (ciprofloxacin) linked to an efflux pump inhibitor analogue through a β-lactam nucleus, which was planned to act as a bacterially-specific triggering mechanism. Future work will involve the complete synthesis and testing of this prodrug 122 to assess its antibacterial activity and to determine if it is acting as a dual action prodrug.

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