

# **Alkaloid-Like Molecules as AChE Inhibitors and Anticancer Agents for Therapeutic Relief of Alzheimer's Disease and Cancer**

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for the award of the degree of

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**Steven Gareth Williams**

B. Sci Applied Chemistry (Hons)

Supervisor: Associate Professor Alison T. Ung

Co-supervisors: Dr Jason Ashmore and Dr Ronald Shimmon

School of Mathematical and Physical Sciences

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### **Declaration / Certificate of authorship and originality**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

Steven Williams

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## List of Abbreviations

Å	Angstrom
$\delta$	Chemical Shift (NMR)
$\lambda$	Wavelength
$\nu_{\max}$	Maximum absorbance
$[\alpha]_D^{25}$	Specific rotation for a Na lamp at 589 nm at 25°C
[L]	Ligand concentration
$[M]^{+}$	Molecular ion
$[M + H]^{+}$	Protonated molecular ion
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChR	Acetylcholine receptor
AD	Alzheimer's disease
Ar	Aromatic
Asp	Aspartic acid
ATCh	Acetylthiocholine
BChE	Butyrylcholinesterase
br	Broad (NMR)
c	Concentration in g/100 mL
calc	Calculated
CAS	Catalytic Active Site
d	Doublet (NMR)
Da	Dalton
DCM	Dichloromethane
dd	Doublet of doublets (NMR)
ddd	Doublet of doublets of doublets (NMR)
DEPT	Distortionless enhancement by polarization transfer
DMAD	Dimethyl acetylenedicarboxylate
DMSO	Dimethyl sulfoxide
dq	Doublet of quartets (NMR)
dsep	Doublet of septets (NMR)
dsex	Doublet of sextets (NMR)
dt	Doublet of triplets (NMR)
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)

eeAChE	Electric eel acetylcholinesterase (EC 3.1.1.7)
EtOAc	Ethyl Acetate
Equiv.	Equivalents
F <sub>254</sub>	Fluorescent at 254nm
FDA	Food and Drug Authority
FTIR	Fourier transform infrared spectroscopy
g	Gram
GC-MS	Gas chromatography-mass spectroscopy
Glu	Glutamic acid
Gly	Glycine
COSY	Correlation spectroscopy
HSQC	Heteronuclear single quantum correlation
hAChE	Human acetylcholinesterase
His	Histidine
HRMS	High-resolution mass spectroscopy
HTS	High throughput screening
Hz	Hertz
IC <sub>50</sub>	The half maximal inhibitory concentration
IR	Infrared
<i>J</i>	Coupling constant (NMR)
K <sub>D</sub>	Ligand dissociation constant
LRMS	Low-resolution mass spectroscopy
m	Multiplet (NMR)
<i>m/z</i>	Mass to charge ratio
mg	Milligrams
mL	Millilitre
mmol	Millimole
m.p.	Melting point
NCE	New Chemical Entity
ng	Nanogram
nmol	Nanomole
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
non	nonet (NMR)

PAS	Peripheral Active Site
PDB	Protein database
Ph	Phenyl
Phe	Phenylalanine
ppm	Part per million
Pet. spir.	Petroleum spirits
PSA	Polar surface area
q	Quartet (NMR)
qin	Quintet (NMR)
Q-TOF	Quadrupole Time-of-Flight
R <sub>f</sub>	Retention factor
R <sub>t</sub>	Retention time
r.t.	Room temperature
s	Singlet (NMR)
SAR	Structure-activity relationship
s	Second
Ser	Serine
sp.	Species
STD	Saturation Transfer Difference
t	Triplet (NMR)
TcAChE	<i>Torpedo californica</i> acetylcholinesterase (EC 3.1.1.7)
td	Triplet of doublets (NMR)
TLC	Thin layer chromatography
TMS	Trimethylsilane
Try	Tryptophan
Tyr	Tyrosine
U	The enzyme unit

#### **Publications from this Thesis**

1. An alkaloid-like 3-azabicyclo[3.3.1]non-3-ene library obtained from the bridged Ritter reaction, Steven Gareth Williams, Mohan Bhadbhade, Roger Bishop and Alison Thavary Ung, *Tetrahedron* **2017**, *73*, 116-128.
2. Synthesis and Crystal Structure of Unexpected (1S,4R,5R,6S)-4-cyano-2,2,6-trimethyl-3-azabicyclo[3.3.1]nonan-6-yl acetate, Steven Gareth Williams, Mohan Bhadbhade, Roger Bishop and Alison Thavary Ung, *Aus J Chem*, **submitted**.

#### **Presented Conference Posters with Accepted Abstracts from This Thesis**

1. Alkaloid-like molecules as AChE inhibitors. Steven Gareth Williams, Alison Thavary Ung. Presented at 'RACI Medicinal Chemistry and Chemical Biology NSW Symposium', 28 September 2015, The University of Sydney, NSW, Australia.
2. Alkaloid-like Molecules for Drug Discovery. Steven Gareth Williams, Tristan Rawling and Alison Thavary Ung. Presented at 'RACI Medicinal Chemistry and Chemical Biology Meeting', 6-9 November 2016, Crowne Plaza Coogee Beach, Australia.



## Abstract

Due to the prevalence of alkaloids in the chemical drug space and the broad range of biological properties held by the *Aristolelia* alkaloids, including anticancer properties, a library of structurally similar alkaloid-like compounds has been synthesised, containing the 3-aza-bicyclo[3.3.1]nonane architecture, in order to explore the cytotoxicity of it and its derivatives.

The 3-aza-bicyclo[3.3.1]nonane core was obtained *via* the bridged Ritter reaction with (-)- $\beta$ -pinene and various nitriles to afford 18 compounds (Chapter 2). Several of the compounds obtained from the bridged Ritter reaction were derivatised to give an additional 17 compounds (Chapter 3). The information obtained from these reaction outcomes, were used to further understand the bridged Ritter reaction mechanism. X-ray crystallography was used for analysis of the projection of the scaffold and substituents within the 3D space of the crystal lattice to further understand the reactivity of the synthesised scaffolds.

The library of alkaloid-like compounds was tested for their biological properties. The breast cancer cell lines MDA-MB-231 and MCF-7 were investigated due to in-house data that showed activity for a related series of compounds (Chapter 4). The MDA-MB-231 cell line was tested in-house and 3 of 28 compounds showed significant activity in the reduction of cell viability, however, it is believed that they possess general toxicity, as opposed to having a cytotoxic nature. This library was deemed not viable for developing as cytotoxic agents within this project.

Acetylcholine esterase (AChE) was chosen as an alternative target to be screened against (Chapter 5). Two complementary assays were used to determine the activity were 9 of the 27 tested compounds showed weak activity. SAR data and molecular modeling was used to develop a rational drug design approach to synthesise an improved inhibitor. Two of the designed compounds were synthesised and evaluated for their AChE inhibition properties and both showed relative increase in activity compared to their precursors. In addition to the docking studies used to guide the design of improved AChE inhibitors, molecular modeling was utilised to assess the drug-like properties and ADMET descriptors for each of the synthesised compounds.

Lastly, broad screening of the biological properties of a selection of the synthesised compounds is currently being investigated by the services of the Lily OIDD program with 16 of the 31 submitted compounds are currently undergoing screening and results from five compounds have been returned so far.