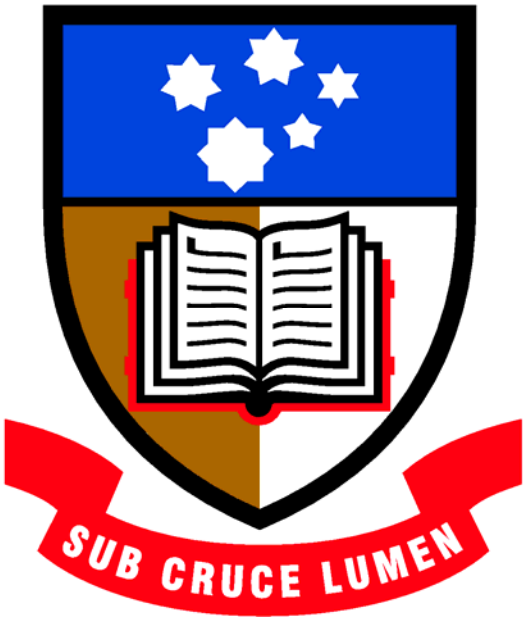
Synthesis of Glutamate Mimics as Neuropathic Pain Modulating Agents

A thesis submitted for the

Degree of Doctor of Philosophy

Nathan John Stanley

B.Sc. (Hons)



Department of Chemistry

The University of Adelaide

December 2009

Table of Contents

Abstract

As part of the vital search towards improved therapeutic agents for the treatment of neuropathic pain, the central nervous system ubiquitous glutamate receptors have become a major focus of research. As such, the discovery of glutamate receptor ligands with improved potency and selectivity has been an important area of study for many decades, though there is still much knowledge to be gained.

Outlined herein are the syntheses towards a series of potentially biologically active 3'-cycloalkyl-substituted carboxycyclopropylglycine analogues. These syntheses utilize novel synthetic chemistry to construct the cyclopropane core with all required stereochemistry. As a consequence of this work, two new cycloalkylcarboxycycloproplyglycine analogues were successfully synthesized, utilizing the reaction of 1,2-dioxines with protected phosphonates in a 20% overall yield for one diastereoisomer.

Secondly, the syntheses of a series of 1,4- and 1,5-substituted 1,2,3-triazole amino acids as a new class of potential glutamate receptor ligands. Briefly, a series of six 1,4- and 1,5-triazole amino acids were successfully synthesized utilizing both copper (I) and ruthenium-catalysed cycloaddition of functionalized azides and alkynes.

Furthermore, contained within Chapter 4 are the details and results of *in vitro* binding assays used in screening for possible active compounds. As an example, *in vitro* drug screening at NMDA, kainate and AMPA ionotropic glutamate receptor subtypes revealed activity of triazole amino acid **48** with an EC_{50} value of 49 μM at AMPA receptors. Also, drug screening at metabotropic glutamate receptor subtypes 1, 2 and 4 revealed potent agonist activity of cyclopropane amino acid **44a** at mGluR2 with an EC_{50} value of 0.05 μM. Cyclopropane amino acid **44a** was thus selected for further testing *in vivo* in a rodent model of neuropathic pain. The results indicated that cyclopropane amino acid **44a** significantly and dose-dependently decreased mechanical allodynia, one of the symptoms of neuropathic pain. It was suggested that this effect was due to activation of mGlu2 and 3 receptors located on both neuronal and glial cells within the dorsal horn of the spinal cord.

Lastly, in an effort to rationalize the *in vitro* binding data, the newly synthesized cyclopropane and triazole amino acids were docked *in silico* into the NMDA, AMPA, mGluR1 and mGluR3 receptors available as x-ray crystal structures. Only limited data was obtained regarding the mGluR1 and mGluR3 dockings. However, AMPA receptor docking of the new *in vitro* active triazole amino acids **45** and **48** revealed positive docking interactions in agreement with those seen for the endogenous ligand, glutamate and the selective agonist AMPA. The docking of these new compounds was also computed to be highly energetically favourable, thus suggesting plausible binding modes.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Nathan Stanley and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Acknowledgements

I would like to extend sincere thanks to my supervisors Professor Dennis Taylor, Professor Andrew Abell and Associate Professor Rod Irvine who have encouraged me to keep pushing forward even when things were turning pear-shaped.

Special thanks goes out to Dr Thomas Avery and Dr Daniel Pedersen for all their invaluable help and practical tips in the lab. Without Dr Mark Hutchinson, the *in vivo* experiments and *in silico* docking work would not have happened; thankyou. Thanks also to Peter Grace for helping with the *in vivo* testing.

Many thanks to Birgitte Nielsen, Trine Kvist and especially Professor Hans Bräuner-Osborne at the University of Copenhagen in Denmark for kindly doing the *in vitro* receptor binding assays.

Gratitude is expressed to the Faculty of Sciences for providing the financial support necessary for this research to be undertaken.

Finally, to my beautiful wife, Penelope; I could not have made it through this without your support. Thankyou for putting up with me over the past four years throughout my PhD.

Abbreviations

Ac	acetyl
AcOH	acetic acid
Anal. Calcd.	analysis calculated
Bn	benzyl
Boc	*tertiary*-butoxycarbonyl
Cbz	carboxybenzyl
CCG	carboxy cyclopropyl glycine
CNS	central nervous system
COSY	correlated spectroscopy
Cp*	pentamethylcyclopentadiene
Δ	heat
DCM	dichloromethane
DCVC	dry column vacuum chromatography
DIAD	diisopropyl azodicarboxylate
DMSO	dimethyl sulfoxide
DPPA	diphenyl phosphoryl azide
EC_{50}	concentration which elicits a 50% maximal effect
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ee	enantiomeric excess
EI	electron impact
ESI	electrospray ionisation
Et	ethyl
equiv.	equivalent(s)
de	diastereomeric excess
g	gram(s)
HOBt	*N*-Hydroxybenzotriazole
HRMS	high resolution mass spectrometry
h	hour(s)
hv	light
Hz	hertz
IC_{50}	concentration which elicits 50% maximum inhibition

iGluR	ionotropic glutamate receptor
IR	infrared
i.t.	intrathecal
J	coupling constant
lit.	literature
m	meta
M	moles per litre
m-CPBA	*meta*-chloroperbenzoic acid
m/z	mass to charge ratio
Me	methyl
MeOH	methanol
mGluR	metabotropic glutamate receptor
MIRC	Michael initiated ring closure
mol	mole(s)
mp	melting point
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PDC	pyridinium dichromate
Pd/C	palladium on carbon
Ph	phenyl
ppm	parts per million
R_f	retention factor
ROESY	Rotating Frame Overhauser Effect Spectroscopy
rt	room temperature
t-Bu, Bu^t	*tertiary*-butyl
TEA	triethylamine
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TPP	triphenylphosphine
TPPO	triphenylphosphine oxide
UV	ultraviolet