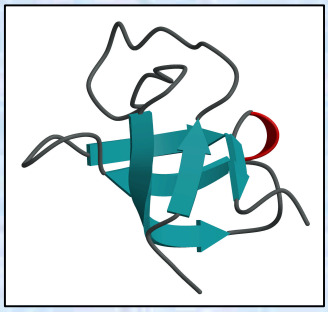
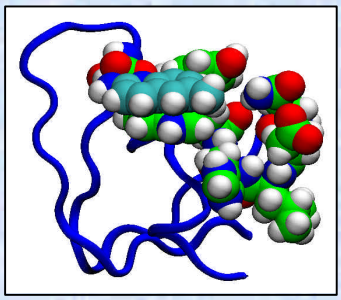




Development of Small-Molecule Ligands for SH3 Protein Domains

by Steven Robert Inglis
B.Sc. (Hons)

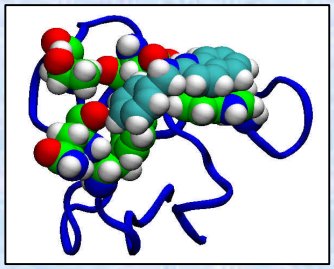
A thesis submitted for the degree of
Doctor of Philosophy

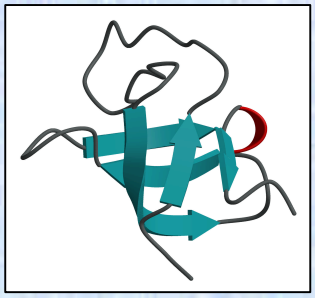
THE UNIVERSITY OF ADELAIDE
AUSTRALIA

SUB CRUCE LUMEN

December 2004

A joint venture between the School of Molecular & Biomedical Science (Discipline of Biochemistry) and the School of Chemistry & Physics (Discipline of Chemistry) at the University of Adelaide, South Australia





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Inglis et al., *J. Med. Chem.* **2004**, *47*, 5405-5417 ..... A-8

Summary

Src Homology 3 (SH3) domains are small protein-protein interaction domains that bind to proline-rich peptides, mediating a range of important biological processes. Because the deregulation of events involving SH3 domains forms the basis of many human diseases, the SH3 domains are appealing targets for the development of potential therapeutics. Previously in the field, no examples of entirely small-molecule ligands for the SH3 domains have been identified. However, in our research group, we have discovered a class of heterocyclic compounds that bind to the Tec SH3 domain at conserved residues in the proline-rich peptide binding site, with weak to moderate affinity. The highest affinity of these was 2-aminoquinoline (K_d = 125 μM).

In this thesis, a range of approaches are described, that were intended to contribute towards development of higher affinity small-molecule ligands for the Tec SH3 domain. Preliminary experiments, involving testing a variety of compounds structurally related to 2-aminoquinoline, provided new structure activity information, and led to a better understanding of the 2-aminoquinoline/SH3 domain binding event. The major component of this thesis is a thorough investigation into the synthesis of a range of 2-aminoquinoline derivatives. *N*-Substituted-2-aminoquinolines were synthesised, however these compounds bound the SH3 domain with slightly lower affinity than 2-aminoquinoline. 6-Substituted-2-aminoquinolines were subsequently prepared, and ligands were identified with up to six-fold improved affinity relative to 2-aminoquinoline, and enhanced selectivity for the Tec SH3 domain.

The techniques used for the ligand binding studies were Nuclear Magnetic Resonance (NMR) chemical shift perturbation and Fluorescence Polarisation (FP) peptide displacement assays. As part of the ligand binding studies, it was intended that the 3D structure of a 2-aminoquinoline ligand/SH3 complex would be obtained using NMR methods, provided that a ligand was identified that bound the SH3 domain in slow exchange on the NMR timescale. However, this goal was not fulfilled. Despite this, the work presented in this thesis provides a solid foundation for the development of potent 2-aminoquinoline ligands for SH3 domains, with engineered specificity.

Statement

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference has been made in the text. In addition, no work performed by another person has been presented, without due reference in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Steven R Inglis, December 2004.

Acknowledgements

The completion of this PhD has been a long, and challenging journey. Therefore, I must now acknowledge many people who have assisted with making the journey a most satisfying one.

Firstly, I must offer sincere thanks to my principal supervisor Dr. Grant Booker, for giving me the opportunity to undertake a PhD, in a field of research that I have grown to enjoy greatly. Without your contribution to ensuring I could receive a scholarship, who knows where I would be now! Many thanks also for your belief in me, your support and enthusiasm, kind nature, and open door policy. All of these little things have helped greatly to make the PhD experience more enjoyable.

I would like to also offer equally sincere thanks to my co-supervisor (or perhaps not so co-!!) Dr. Simon Pyke. You too have routinely given up significant amounts of your time to assist me with various problems. You have also made a great contribution to my scientific development and understanding of Chemistry. Your level of enthusiasm for the project, and support for me has been outstanding and I am most greatful.

Thanks to all members of the Booker group. Special mention should go to, Cvetan Stojkoski for your assistance with protein work, IT support, and for being a good mate to have a beer with. Thanks also to Kasper Kowalski for assistance with protein NMR experiments, and Anita Merkel, simply for providing a happy face around the place, and making the Booker lab a most pleasant place to work. Thanks to other Booker group members past and present, who have helped with various things or been good lab mates: eg. Kim Branson, Sharon Pursglove, Sue Fowler, Bec Bilton, Innes Atmosukarto, Carlie Delaine, Filomena Occhiodoro, Eric Bonython, Iain Murchland, Lucky Tran and Lisa Biggs.

Many thanks to all members of the Pyke group. Special thanks to Dr. David Armitt. Dave, you have been a great asset to our lab, and have always happy to help with chemical problems, however large or small. Your knowledge of organic synthesis is far and wide, and this was very helpful, on many occasions. Thanks to Lab 3 and other chemistry department members, past and present who have helped with various things, or who have simply been good people to work with: eg. Ben Greatrex, Sally Plush, Marcus Cole, John Carver, Jacquie Cawthray, Rhiannon Jones, Daniel Fritz, Penny Kerr, Daniel Bilusich, Suresh Dua, Sam Peppe, Monique Jensen, Sean Alexander and Emma Wiadrowski. Special thanks also to John Cameron, for doing such a fine job running the chemistry store, and to Phil Clements for

maintenance of the NMR spectrometers. These two features are critical for the effective running of a chemistry department.

I must also give a HUGE thankyou to my family, particularly my parents, Nicole and Norm for tremendous love and support throughout the PhD journey. Mum and Dad, I feel greatly indebted to you both for (still) providing me with a good home, and all of the creature comforts that help so much when you take on a huge job, like a PhD. Hopefully I can repay you, in someway one of these days. Thanks also to my sister Michèle and brother in law Nige, for your love and support over the years. Thanks too Nige for all the tennis games, and I am hoping we can start to play regularly again soon. (I am also planning to win for once.) Thanks also to my brother Paul and partner Nicole, for additional love and support, and for allowing me to come and stay with you in Melbourne at call.

Last, but definitely not least, thanks to my loving girl friend, and best friend, Rebecca. You have been very patient, caring, and understanding of my needs throughout my PhD, and you were always keen to listen during testing times. Thankyou for all you have done for me. I love you very much. May we share some more relaxing times together, beyond this thesis!!

Abbreviations

DMAP	*N*,*N*-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
FP	Fluorescence Polarisation
GST	glutathione-*S*-transferase
HSQC	Heteronuclear Single Quantum Coherence
mP	millipolarisation (units)
NBS	*N*-bromosuccinimide
SAR	structure activity relationship
SH2	Src Homology 2
SH3	Src Homology 3
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography