Investigating 2-Aminoquinoline Derivatives as Small Molecule Ligands for the Tec SH3 Domain

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PhD Thesis

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SUMMARY

SH3 domains are small non-catalytic protein domains of around 50-70 amino acids in length. They are found in a variety of proteins and have a number of different functions including roles within cellular signalling pathways, that when deregulated, may lead to diseases such as cancer and osteoporosis. Therefore SH3 domains provide an attractive target for drug design studies.

Many SH3 domains, in their native state, have been found to bind to proline rich peptides. The murine Tec protein contains an SH3 domain that binds to a native proline rich peptide sequence. The structure of the Tec SH3 domain has been solved by NMR methods and is therefore a good starting point for research into small molecule ligand design for the SH3 domain. Within our research group, studies have been undertaken in order to find a small molecule ligand for the SH3 domain. The lead compound for these investigations, 2-aminoquinoline, was found to bind to the Tec SH3 domain in the same region as the native proline rich peptide with a K_d of 125 μM.

Previous structure activity relationship (SAR) studies within our research group have focused on including substituents at the 4-, 5-, 6-, 7- and 8-positions around the quinoline ring as well as substitution at the amino nitrogen. Introducing a substituent at the 6-position has improved the binding affinity of the 2-aminoquinoline ligand and has yielded the best ligand to date with a K_d of 25 μM. Preliminary results have shown that substituents at the 5- and 7-positions did not improve the affinity of the ligand for the SH3 domain.

This thesis describes a number of ways in which SAR studies of 2-aminoquinoline ligands have been further investigated. Substituents have been introduced into a number of positions around the quinoline ring with the aim of improving upon the best ligand to date and also investigating the possibility of finding another region of potential protein-ligand interaction on the protein surface.

A large number of ligands of three general classes have been synthesised in order to achieve the aims of this project. The first class of ligands contain a substituent in either the 5- or 7-position of 2-aminoquinoline. These were synthesised in order to extend and complete the research into the effect of introducing a substituent in these positions. The second class of

ligands included a variety of 2-aminoquinoline derivatives that all contained a substituent at the 6-position. The goal of synthesising these 2-aminoquinoline derivatives is to extend the research that has already been done in this area and improve the binding affinity of the ligand for the Tec SH3 domain. The final class of ligand involves substitution at the 3-position of 2-aminoquinoline, in order to study the potential for the ligand to make a further contact with the protein surface in that region.

The research presented within this thesis conclusively shows that introducing a substituent at either 5- or 7-positions of 2-aminoquinoline does not improve the affinity of the small molecule for the Tec SH3 domain. All of the 6-substituted ligands do however bind to the Tec SH3 domain with far greater affinity, relative to 2-aminoquinoline, and some of these derivatives bind with greater affinity than any previously prepared. The studies performed have also shown that there is the potential to make further interactions with the protein surface by introducing a substituent in the 3-position of the 2-aminoquinoline ligand. The research contained within this thesis will potentially allow for the synthesis of a ligand with a higher affinity for the Tec SH3 domain. This in turn may allow for the unambiguous characterisation of the mode of binding of the ligand to the protein surface by either NMR methods or X-ray crystallography.

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.

Rhiannon Jones, October 2007

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LIST OF ABBREVIATIONS

DMAP	*N*,*N*-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
HSQC	Heteronuclear Single Quantum Coherence
LDA	Lithium diisopropyl amide
MPM	Methoxyphenylmethyl (as protecting group)
NBS	*N*-Bromosuccinimide
PH	Plekstrin Homology
SAR	Structure Activity Relationship
SH2	Src Homology 2
SH3	Src Homology 3
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography