Development of 2-Aminoquinoline Derivatives as Ligands for Tec SH3 Domain

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SUMMARY

Src Homology 3 (SH3) domains are small, non-catalytic protein-protein interaction domains comprising of approximately 50-70 amino acids. Protein complexes containing SH3 domains are found in a variety of cell signalling pathways controlling processes such as cell proliferation, apoptosis and gene expression. SH3 domains mediate these pathways by binding to proline-rich sequences on partner proteins thereby controlling the assembly of large multiprotein complexes. Many of these pathways, when deregulated, lead to diseases such as osteoporosis and cancer, therefore making SH3 domains appealing targets for the development of potential therapeutics.

Numerous SH3 domain structures have been determined by NMR or X-ray crystallography, including the solution structure of the murine Tec kinase SH3 domain, which was determined by NMR spectroscopy. The binding site for the native proline-rich peptide to the Tec SH3 domain was determined to be a shallow indentation on the surface of the protein. The development of high affinity small molecule ligands for the SH3 domain is of particular interest within our research group and has involved the murine Tec SH3 domain as a model system for structure based drug design. Initial studies determined that 2-aminoquinoline binds to the same shallow indentation on the SH3 domain surface as the native peptide with a K_d of 125 μM. 2-Aminoquinoline therefore serves as the lead compound for our investigations.

Previous studies in the development of small molecule ligands with improved affinity for the Tec SH3 domain have involved substitution at all positions of the 2-aminoquinoline core structure, including substitution at the amino nitrogen. Substituents in the 6-position appear to make favourable contacts with the protein surface and these have provided some of the highest affinity ligands to date, with the highest affinity ligand displaying a K_d of 9 μM.

This thesis describes the further development of small molecule ligands with substituents at the 6-position of 2-aminoquinoline and relevant structure activity relationship studies. Over 40 6-substituted ligands of three general classes have been prepared with the overall aim of improving the affinity of these ligands for the SH3 domain and gaining further insight into the binding mode of these ligands with the SH3 domain.

Previous studies of 6-substituted 2-aminoquinolines have involved ligands substituted with an acetal group at the 6-position of 2-aminoquinoline. This functionality is however, unstable under physiological conditions. The first class of ligands were synthesised in order to extend on this previous acetal-substituted work with the aim of increasing the stability of the ligands in addition to improving the affinity for the SH3 domain. This class of ligand contains a saturated *N*-heterocyclic substituent at the 6-position. These ligands were prepared using palladium catalysed Buchwald-Hartwig chemistry and required significant investigation into the optimal reaction conditions to effect the desired transformations. The binding affinity studies of the newly prepared ligands is also discussed.

The second class of ligands contain an aryloxymethyl or arylthiomethyl substituent at the 6-position. These ligands were prepared to complete previous studies involving simple 6-phenoxymethyl substituents. The final class of ligands combines both concepts of the first two classes of ligands and links a phenoxymethyl substituent with a saturated *N*-heterocycle in an extended 6-substituted ligand. The preparation of these ligands involved further investigations into Buchwald-Hartwig aminations.

The research presented in this thesis demonstrates that a 6-heterocyclic substituent can improve the binding affinity of 2-aminoquinoline for the Tec SH3 domain with some of the ligands prepared displaying equal or similar affinity to the highest affinity ligands previously reported. Similarly, 6-aryloxymethyl and 6-arylthiomethyl substitution has provided ligands with improved binding affinity relative to 2-aminoquinoline. Furthermore, a few of these ligands displayed the highest affinity for the Tec SH3 domain of all the ligands prepared to date. Some of these ligands, and also a number of the extended 6-substituted ligands, however, displayed unusual behaviour in the NMR assay and appear to bind through a different mode to other 2-aminoquinolines with more simple substitution. It is proposed that these ligands bind to the Tec SH3 domain through a 'see-saw' type mechanism; however, further studies of the binding interaction of these types of compounds are required to confirm this. These additional studies would also provide valuable information for the design of additional 2-aminoquinoline ligands with improved affinity for the Tec SH3 domain.

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 subject to the provisions of the Copyright Act of 1968.

Jessica Smith, February 2009.

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LIST OF ABBREVIATIONS

COSY	Correlation Spectroscopy
DABCO	1,4-Diazabicyclo[2,2,2]octane
DMAP	*N*,*N*-Dimethylaminopyridine
DME	Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
FP	Fluorescence Polarisation
Grb2	Growth factor receptor-bound protein 2
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Coherence
LDA	Lithium diisopropylamide
LHMDS	Lithium bis(trimethylsilyl)amide
m-CPBA	*meta*-Chloroperbenzoic acid
NBS	*N*-Bromosuccinimide
PH	Plekstrin Homology
PMB	*para*-Methoxybenzyl (as protecting group)
ROESY	Rotating Frame Overhauser Enhanced Spectroscopy
SAR	Structure Activity Relationship
SH2	Src Homology 2
SH3	Src Homology 3
SOS	Son of Sevenless protein
TBAI	Tetra-(*n*-butyl)ammonium iodide
TBDMS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
TH	Tec Homology
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography