Development of Small-Molecule Ligands for SH3 Protein Domains

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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 ($K_d = 125 \mu M$).

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.
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### Abbreviations

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