

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

by **Rhiannon Jones**



**PhD Thesis**

**School of Chemistry and Physics**

**The University of Adelaide**

# TABLE OF CONTENTS

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<b>LIST OF FIGURES .....</b>	<b>IV</b>
<b>LIST OF TABLES.....</b>	<b>VIII</b>
<b>SUMMARY .....</b>	<b>X</b>
<b>STATEMENT .....</b>	<b>XII</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>XIII</b>
<b>LIST OF ABBREVIATIONS .....</b>	<b>XIV</b>
<b>INTRODUCTION.....</b>	<b>1</b>
1.1    The SH3 Domain: Structure and Function .....	1
1.1.1.    The SH3 Domain .....	1
1.1.2.    Roles of SH3 Domains .....	2
1.2    The Tec Family of Protein Tyrosine Kinases .....	4
1.3    Ligands for the SH3 Domain .....	5
1.3.1.    Naturally Occurring Ligands .....	5
1.3.2.    Novel Peptide-Peptoid Ligands .....	6
1.4    The Development of Small Molecule Ligands for the Tec SH3 Domain .....	9
1.4.1.    Strategies Involved in Structure Based Drug Design .....	9
1.4.2.    Computational Screening and the Identification of a Lead Compound.....	10
1.4.3.    Assay Methods.....	11
1.4.4.    Lead Optimisation and Structure Activity Relationship Studies .....	14
1.4.5.    The Binding Model.....	15
1.4.6.    Substitution at the 6-position of 2-Aminoquinoline .....	16
1.4.7.    Investigating <i>N</i> -Substitution .....	18
1.4.8.    Substitution at the 5- and 7-Positions of 2-Aminoquinoline .....	19
1.5    Aims.....	20
1.5.1.    General Aims .....	20
1.5.2.    Synthetic Targets .....	20
<b>COMPLETING STUDIES OF 5- AND 7- SUBSTITUTED LIGANDS.....</b>	<b>23</b>

2.1	Introduction.....	23
2.2	Synthesis of 5- and 7- Substituted 2- Aminoquinolines .....	24
2.2.1.	General Synthetic Pathway .....	24
2.2.2.	Synthesis of Hydroxymethyl Substituted 2-Aminoquinolines .....	24
2.3	Binding Data for the 5- and 7-Substituted -2-Aminoquinolines.....	29
<b>6-SUBSTITUTED 2-AMINOQUINOLINES .....</b>		<b>32</b>
3.1	Introduction.....	32
3.2	Synthesis of 6-Substituted-2-Aminoquinolines .....	32
3.2.1.	General Synthetic Pathway .....	32
3.2.2.	Synthesis of 6-Phenoxymethyl-2-aminoquinoline Derivatives .....	33
3.3	Binding Data for the 6-Phenoxymethyl-2-aminoquinoline Derivatives.....	41
3.3.1.	Chemical Shift Mapping .....	42
3.3.2.	Potential Problems with $K_d$ Determination.....	43
3.3.3.	Revised Binding Constants .....	53
3.3.4.	Conclusions From The Binding Data for the 6-Substituted 2- Aminoquinolines.....	54
<b>3-SUBSTITUTED 2-AMINOQUINOLINES .....</b>		<b>56</b>
4.1	Introduction.....	56
4.2	Synthesis of Simple 3-Substituted-2-Aminoquinoline Ligands .....	56
4.2.1.	General Synthetic Pathway .....	56
4.2.2.	Synthesis of Simple 3-Substituted-2-Aminoquinolines.....	57
4.3	Binding Data for the Simple 3-Substituted-2-Aminoquinoline Ligands.....	58
4.4	Introducing More Complex Substituents in the 3-Position of 2-Aminquinoline....	59
4.4.1.	General Synthetic Pathway .....	59
4.4.2.	Synthesis of 3-Phenoxymethyl-2-aminoquinolines .....	60
4.5	Alternative Method for Preparing Ligands with Complex Substitution in the 3- Position .....	68
4.5.1.	General Synthetic Pathway .....	68
4.5.2.	Synthesis of 2-Chloro-3-[( <i>E</i> )-2-phenylvinyl]quinoline and 2-Chloro-3- [( <i>Z</i> )-2-phenylvinyl]quinoline .....	68
4.5.3.	Synthesis of 3-(2-Phenylethyl)quinolin-2-amine.....	71
4.5.4.	Synthesis of Further 3-(2-Phenylethyl)quinolin-2-amine Derivatives ..	76
4.6	Binding Data for the More Complex 3-Substituted 2-Aminoquinoline Ligands ...	83
4.6.1.	Chemical Shift Mapping .....	85

4.7	Synthesis of Further Complex 3-Substituted Ligands .....	86
4.8	Binding Data for Remaining 3-Substituted Ligand .....	87
<b>CONCLUSIONS AND FUTURE DIRECTIONS .....</b>		<b>89</b>
5.1	The 5- and 7- Positions of 2-Aminoquinoline .....	89
5.1.1.	Summary and Conclusions .....	89
5.2	The 6-Position of 2-Aminoquinoline .....	90
5.2.1.	Summary and Conclusions .....	90
5.2.2.	Proposed Future Work .....	91
5.3	The 3-Position of 2-Aminoquinoline .....	93
5.3.1.	Summary and Conclusions .....	93
5.3.2.	Proposed Future Work .....	94
5.4	Proposed Further Studies .....	96
5.4.1.	Solubility .....	96
5.4.2.	Combining Substituents .....	97
5.4.3.	Forcing the Ligand and SH3 domain into Slow Exchange .....	97
<b>EXPERIMENTAL .....</b>		<b>99</b>
6.1	General Methods .....	99
6.2	Synthetic Procedures .....	100
6.2.1.	5- and 7-Substituted 2-Aminoquinolines .....	100
6.2.2.	6-Substituted 2-Aminoquinolines .....	106
6.2.3.	3-Substituted 2-Aminoquinolines .....	130
6.2.4.	Complex 3-Substituted-2-Aminoquinolines .....	132
6.3	Protein Methods .....	163
6.3.1.	Solutions .....	163
6.3.2.	Protein Preparation .....	164
6.3.3.	The [ <sup>1</sup> H, <sup>15</sup> N] HSQC NMR Chemical Shift Perturbation Assay <sup>46</sup> .....	165
<b>REFERENCES .....</b>		<b>169</b>
<b>Appendix 1. X-Ray Crystal Structure Data for 3-Methyl-2-phenoxy-4-aminoquinoline (83) .....</b>		<b>175</b>

# LIST OF FIGURES

---

<b>Figure 1.</b> Structure of the <i>murine</i> Tec SH3 Domain <sup>3</sup> .....	1
<b>Figure 2.</b> Cartoon representation of the role of the SH2 and SH3 domains in the deactivation of c-Src. ....	2
<b>Figure 3.</b> A cartoon representation of the Ras pathway and the involvement of the SH3 domain within this pathway. <sup>9</sup> .....	3
<b>Figure 4.</b> Cartoon representation of A. Class I PPII helix ligands, and B. Class II PPII helix ligands binding to the SH3 domain binding site. Figure adapted from Feng <i>et al.</i> <sup>38</sup> .....	6
<b>Figure 5.</b> Two of the novel ligands with a PLPPLP core that were found to bind to the Src SH3 domain. <sup>40, 41</sup> .....	7
<b>Figure 6.</b> The Crk specific peptide-peptoid ligand compared with the Crk-specific wild-type peptide ligand (left). <sup>42</sup> .....	8
<b>Figure 7.</b> The ligands designed by Nguyen <i>et al.</i> <sup>34</sup> as compared with the C <sup>β</sup> -substituted ligands. <sup>43</sup> .....	8
<b>Figure 8.</b> Schematic diagram of the method used for structure based drug design. ....	10
<b>Figure 9.</b> 2-Aminoquinazoline, <b>1</b> which was predicted to bind to the Tec SH3 domain by <i>in-silico</i> screening and structurally similar 2-aminoquinoline, <b>2</b> . ....	11
<b>Figure 10.</b> Structure of the tracer FLUORO-PRS-2. ....	11
<b>Figure 11.</b> Schematic representation of the fluorescence polarisation assay. ....	12
<b>Figure 12.</b> A. An expansion of several peaks in overlaid HSQC spectra from the assay of <b>2</b> . B. Chemical shift mapping of the residues that move in the assay of <b>2</b> . C. The binding isotherm derived from the HSQC NMR assay of <b>2</b> . <sup>46</sup> .....	13
<b>Figure 13.</b> Schematic representation of slow exchange (A), intermediate exchange (B) and fast exchange (C). ....	14
<b>Figure 14.</b> The lead compound, <b>2</b> and its $K_d$ and $EC_{50}$ values. ....	14
<b>Figure 15.</b> Proposed binding model of 2-aminoquinoline with the Tec SH3 domain and the lead compound. <sup>46</sup> .....	16
<b>Figure 16.</b> The charged amino acids that may form a contact with a substituent in the 6-position of <b>2</b> . ....	16
<b>Figure 17.</b> The structure of the 6-phoxymethyl-2-aminoquinoline and its binding affinity. ....	18
<b>Figure 18.</b> The reason for the reduction in binding for the <i>N</i> -substituted ligands. ....	19
<b>Figure 19.</b> Ligands with substituents in the 5- or 7-position. ....	20
<b>Figure 20.</b> The 5- and 7-hydroxymethyl-2-aminoquinoline target ligands. ....	21

<b>Figure 21.</b> The 6-phenoxyethyl-2-aminoquinoline target ligands. ....	21
<b>Figure 22.</b> The 3-substituted-2-aminoquinoline target ligands. ....	22
<b>Figure 23.</b> The previously prepared 7-substituted ligands. ....	23
<b>Figure 24.</b> The retro-synthetic pathway for the synthesis of 5- and 7-substituted 2-aminoquinolines. ....	24
<b>Figure 25.</b> A. The <sup>1</sup> H spectrum of <b>28</b> with H(3) and H(8) inset B. The <sup>13</sup> C NMR spectrum of <b>28</b> with C(3) and C(8) inset. ....	28
<b>Figure 26.</b> The tautomerism between amino and imino forms of <b>28</b> that occurs to cause the line broadening in the NMR spectra. ....	29
<b>Figure 27.</b> The resonance contributors that shield the nuclei at C(3) and C(8) of the imino form of <b>28</b> . ....	29
<b>Figure 28.</b> The retro-synthetic pathway for the synthesis of 6-substituted phenoxyethyl-2-aminoquinolines. ....	32
<b>Figure 29.</b> The resonance contributors that bring about the broadened signal due to H(3) in the <sup>1</sup> H NMR spectrum of <b>44</b> . ....	35
<b>Figure 30.</b> Cleavages from the mass spectra of <b>46-60</b> . ....	37
<b>Figure 31.</b> The resonance donation of electron density from the phenolic oxygen to C(4').	38
<b>Figure 32.</b> The fragmentation that gives rise to the ion corresponding to <i>m/z</i> 157 in the mass spectra of <b>61-75</b> . ....	39
<b>Figure 33.</b> The resonance contributor that shields the proton at H(3) to cause the upfield shift in the <sup>1</sup> H NMR spectra of <b>61-75</b> . ....	40
<b>Figure 34.</b> Chemical shift mapping of the backbone of the Tec SH3 domain with the 6-substituted ligands where $\delta_H > \sim 0.1$ ppm for a number of the ligands. Downfield shifts are marked in green and upfield shifts are marked in red. ....	42
<b>Figure 35.</b> The equilibrium dissociation binding isotherm for: A. <b>65</b> and B. <b>70</b> . ....	43
<b>Figure 36.</b> The equilibrium binding dissociation isotherm for: A. <b>67</b> ; B. <b>68</b> ; C. <b>72</b> ; D. <b>73</b> . ....	45
<b>Figure 37.</b> The binding isotherm for <b>73</b> with the first data point removed. The original curve is shown in pink for comparison. ....	46
<b>Figure 38.</b> Overlay of NMR spectra from the HSQC NMR assay of <b>73</b> at varying equilibration times. A. <b>73</b> at 0.013 mM; B. <b>73</b> at 0.040 mM. ....	48
<b>Figure 39.</b> Cartoon representation of A. one site binding and B. two site binding. ....	49
<b>Figure 40.</b> Binding isotherm of <b>73</b> showing the two possible curves that can be drawn through the data. The original curve is shown in pink. The curve that can be drawn through the earlier data points is shown in purple and the curve that can be drawn through the later data points is shown in blue. ....	50

<b>Figure 41.</b> Overlay of NMR spectra from the HSQC NMR assay of <b>67</b> , with an enlargement of the peak due to Y227 and the <sup>1</sup> H 1D slices for the same peak at different ligand concentrations. ....	51
<b>Figure 42.</b> Overlay of 1D <sup>1</sup> H traces for Y227 signals from the HSQC NMR assay of <b>73</b> A. at room temperature and B. at 35°C. ....	52
<b>Figure 43.</b> The retro-synthetic pathway for the synthesis of simple 3-substituted 2-aminoquinolines. ....	56
<b>Figure 44.</b> The resonance structures that reduce the ability of the ligands to form a salt bridge. ....	59
<b>Figure 45.</b> The retrosynthetic pathway for the formation of novel 3-substituted ligands. ....	60
<b>Figure 46.</b> The characteristic loss of the phenoxy radical in the mass spectrum of <b>80</b> . ....	62
<b>Figure 47.</b> The <sup>1</sup> H and <sup>13</sup> C NMR spectra of <b>80</b> for comparison with those of <b>83</b> , below. ....	63
<b>Figure 48.</b> A. The <sup>1</sup> H NMR spectrum and B. the <sup>13</sup> C NMR spectrum of the unknown, <b>81</b> , produced from the Kóródi Amination reaction on <b>80</b> . ....	64
<b>Figure 49.</b> <b>83</b> , the product obtained from the amination reaction of <b>80</b> . ....	65
<b>Figure 50.</b> Proposed mechanism for the formation of <b>83</b> . ....	66
<b>Figure 51.</b> The electron donating effect of the phenoxy group on C(3) and C(4a). ....	67
<b>Figure 52.</b> The electron donating effect of the amino group on C(3) and C(4a). ....	67
<b>Figure 53.</b> An alternative pathway for the synthesis of novel 3-substituted ligands. ....	68
<b>Figure 54.</b> The mechanism of <i>para</i> -methoxybenzylamine attack that catalyses the isomerisation of the <i>E</i> isomer to <i>Z</i> isomer. ....	74
<b>Figure 55.</b> The tautomerism that occurs to cause the line broadening of C(8a) in the <sup>13</sup> C spectrum of <b>92</b> . ....	75
<b>Figure 56.</b> Chemical shift mapping of the backbone of the Tec SH3 domain with A. the 6-substituted ligands and B. the complex 3-substituted ligands where $\delta_H > \sim 0.1$ ppm for a number of the ligands. Downfield shifts are marked in green and upfield shifts are marked in red. ....	85
<b>Figure 57.</b> A. A graphical representation of the protein surface showing the indentation below W215; B. <b>115</b> superimposed on the protein surface to illustrate a possible method of binding. ....	86
<b>Figure 58.</b> The two conformations that the <i>E</i> isomer of <b>90</b> may take. ....	88
<b>Figure 59.</b> The 5- and 7-substituted ligands that were assayed for binding affinity to the Tec SH3 domain. ....	89
<b>Figure 60.</b> The highest affinity 6-phenoxy methyl ligands. ....	91

<b>Figure 61.</b> Proposed future ligands that contain bulkier substituents in the 4-position of the phenoxyethyl group. ....	91
<b>Figure 62.</b> Proposed future ligands containing electron withdrawing substituents on the phenoxyethyl group. ....	92
<b>Figure 63.</b> Proposed future ligands combining 4-substitution with an electron withdrawing substituent on the phenoxyethyl group. ....	92
<b>Figure 64.</b> The simple 3-substituted ligands that were assayed for binding affinity to the Tec SH3 domain. ....	93
<b>Figure 65.</b> The highest affinity 3-substituted ligand, <b>115</b> . ....	94
<b>Figure 66.</b> Proposed future ligand that involves a bulky substituent in the 3-position of 2-aminoquinoline. ....	94
<b>Figure 67.</b> Proposed ligands with resonance electron donating substituents on the phenyl group. ....	95
<b>Figure 68.</b> Proposed ligand with a constrained ring system. $n = 0$ or $1$ , $R^1$ and $R^2 = H$ or Ar. ....	95
<b>Figure 69.</b> Proposed future ligand with an additional polar group to improve solubility.....	96
<b>Figure 70.</b> Proposed future ligands containing heteroaromatic rings to improve solubility.	96
<b>Figure 71.</b> Proposed pathway for combining substitution in both the 3- and 6-position of 2-aminoquinoline. PG = Protecting Group. ....	97
<b>Figure 72.</b> The overlay of the $[^1H, ^{15}N]$ HSQC NMR spectra from the chemical shift perturbation assay of <b>23</b> . ....	166
<b>Figure 73.</b> The binding isotherm created from the data in Table 29 that was used to calculate the equilibrium dissociation binding constant of <b>23</b> . Error bars are set at 1 standard deviation.....	168



# LIST OF TABLES

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<b>Table 1.</b> A number of pathologies associated with SH3 domains and their potential targets for drug design. Table adapted from Dalgarno <i>et al.</i> <sup>2</sup> .....	4
<b>Table 2.</b> Analogues of 2-aminoquinoline and their binding affinity in comparison with 2-aminoquinoline. ....	15
<b>Table 3.</b> A number of 6-substituted-2-aminoquinoline ligands found to bind to the Tec SH3 domain, and their binding affinities. <sup>46, 50</sup> .....	17
<b>Table 4.</b> <i>N</i> -substituted analogues of 2-aminoquinoline along with their binding activities..	18
<b>Table 5.</b> Comparison of chemical shifts ( $\delta$ , ppm) for the methylene protons of the methyl and bromomethyl substituted compounds <b>35-38</b> . ....	26
<b>Table 6.</b> Comparison of chemical shifts ( $\delta$ , ppm) for the CH <sub>2</sub> protons of the bromomethyl and acetoxymethyl substituted compounds <b>37-40</b> . ....	26
<b>Table 7.</b> Comparison of chemical shifts ( $\delta$ , ppm) for H(3) of 2-chloroquinolines <b>33</b> and <b>34</b> , (quinoline-2-yl)acetamides <b>39</b> and <b>40</b> and 2-aminoquinolines <b>27</b> and <b>28</b> . ....	27
<b>Table 8.</b> The binding data for the 5- and 7-substituted 2-aminoquinoline ligands. ....	30
Note: $K_d$ was determined by NMR, $EC_{50}$ was determined by fluorescence polarization spectroscopy.....	30
<b>Table 9.</b> Comparison of chemical shifts ( $\delta$ , ppm) for the benzylic protons of <b>45-60</b> . ....	37
<b>Table 10.</b> Comparison of chemical shifts ( $\delta$ , ppm) for C(4') of each of the 4'-substituted (phoxymethyl)quinoline derivatives. ....	38
<b>Table 11.</b> Comparison of chemical shifts ( $\delta$ , ppm) for H(3) and H(4) of <b>46-60</b> and <b>61-75</b> ..	40
<b>Table 12.</b> The binding affinities of 6-substituted phoxymethyl ligands.....	41
<b>Table 13.</b> The 6-substituted ligands that did not reach saturation during the binding assay.	44
<b>Table 14.</b> The binding affinities and statistical data for the binding curves of some 6-substituted ligands.....	46
<b>Table 15.</b> The revised equilibrium dissociation binding constants, and statistical data.....	54
*2nd data point deleted; †2nd and 3rd data points deleted.....	54
<b>Table 16.</b> The equilibrium dissociation binding constants of simple 3-substituted ligands. ....	59
<b>Table 17.</b> Significant changes in chemical shift ( $\delta$ , ppm) in the <sup>13</sup> C NMR spectrum upon conversion of <b>80</b> to <b>83</b> . ....	67
<b>Table 18.</b> Yield of <b>86</b> obtained using different bases in the Horner-Emmons modification to the Wittig reaction. ....	71
<b>Table 19.</b> Significant changes of chemical shift ( $\delta$ , ppm) in the <sup>13</sup> C NMR spectrum upon conversion of <b>92</b> to <b>93</b> . ....	76

<b>Table 20.</b> The details of the benzylic protons from the $^1\text{H}$ NMR spectra of the substituted benzylphosphonates <b>94-102</b> .....	77
<b>Table 21.</b> The $\text{M}^+$ and first fragmentation from the low resolution mass spectra of <b>104-111</b> . .....	78
<b>Table 22.</b> Comparison of chemical shifts for H(4) ( $\delta$ , ppm) between <b>77</b> and the 2-chloro-3-(2-phenylvinyl)quinolines <b>86</b> and <b>103-111</b> .....	79
<b>Table 23.</b> Comparison of chemical shifts for H(4) ( $\delta$ , ppm) between the phenylvinyl compounds, <b>103-109</b> and the phenylethyl compounds, <b>112-118</b> . ....	80
<b>Table 24.</b> Comparison of chemical shifts for the phenyl carbons in the <i>para</i> substituted products, <b>103</b> , <b>106</b> and <b>107</b> and the parent compound, <b>86</b> . ....	80
<b>Table 25.</b> Comparison of the chemical shifts ( $\delta$ , ppm) of the phenyl protons in the $^1\text{H}$ NMR spectra of <b>86</b> and unknowns <b>121</b> and <b>122</b> .....	82
<b>Table 26.</b> Comparison of the chemical shifts ( $\delta$ , ppm) of the phenyl carbons in the $^{13}\text{C}$ NMR spectra of <b>86</b> and unknowns <b>121</b> and <b>122</b> .....	82
<b>Table 27.</b> Comparison of chemical shifts for the quinolyl carbons in the complex 3-substituted ligands.....	83
<b>Table 28.</b> The binding data for the complex 3-substituted 2-aminoquinolines.....	84
<b>Table 29.</b> The change in chemical shifts for all the residues whose corresponding signals shift significantly ( $> \sim 0.1$ ppm) during the assay of <b>23</b> (above) and the normalised values and averages for the same assay (below).....	167

# SUMMARY

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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  $\mu$ M.

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  $\mu$ M. 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

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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

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DMAP	<i>N,N</i> -Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
HSQC	Heteronuclear Single Quantum Coherence
LDA	Lithium diisopropyl amide
MPM	Methoxyphenylmethyl (as protecting group)
NBS	<i>N</i> -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