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Structure-Based Design of Peptidomimetic Antagonists of P56^{lck} SH2 Domain

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Abstract—Starting from the tetrapeptide Ac-pYEEI-NHMe and using a structure-based approach, we have designed and synthesised a peptidomimetic ligand for p56^{lck} SH2 domain containing a conformationally restricted replacement for the two glutamate residues. We have explored replacments for the isoleucine residue in the pY + 3 pocket and thus identified 1-(*R*)-amino-3-(*S*)-indaneacetic acid as the most potent replacement. We also report the X-ray crystal structures of two of the antagonists.

p56^{lck} is a key non-receptor tyrosine kinase involved in signal transduction through the T-cell receptor (TcR) in T-helper (T_H) cells. This signal is essential for the activation and proliferation of T_H cells. p56^{lck} consists of a kinase domain which is responsible for phosphorylating tyrosine-containing proteins, an SH3 domain which binds proline-rich peptide motifs and an SH2 domain which binds phosphotyrosine-containing proteins. As part of our autoimmune disease programme, we are interested in preventing T_H cell proliferation by designing p56^{lck} SH2 domain antagonists.

SH2 domains are modules of approximately 100 amino acids. They bind phosphotyrosine containing sequences with high affinity and specificity. The optimum binding sequence for p56^{lck} SH2 domain was determined to be pTyr-Glu-Glu-Ile from a phosphopeptide library screen.[1] The crystal structure of p56^{lck} SH2 domain bound to a peptide (hamster middle-T antigen **1**) containing this sequence has been determined and this was our starting point for the design of p56^{lck} SH2 domain antagonists.[2,3] The hamster middle-T antigen **1** is an 11-mer peptide and the structure revealed that of the 11 amino acids only four made hydrogen bond contact with the SH2 domain. These contacts were bounded by the backbone amide carbonyl C-terminal to the pY and the backbone amide carbonyl C-terminal to the isoleucine at pY + 3. A truncation analysis (Table 1) using a series of peptides capped at the N-terminus with an acetyl group and capped at the C-terminus with a methylamide group indicated that the tetrapeptide **4**, which was only 5-fold less potent than the HmT antigen, was the optimal length to be used as a lead for the design of a p56^{lck} SH2 antagonist. A crystal structure of the related tetrapeptide with a carboxylic acid at the C-terminus has now been reported[4] and this binds in a similar conformation to the corresponding part of the middle-T antigen.

The design of a therapeutically useful compound which could reach its target inside T_H cells requires four principal issues to be addressed: (1) the peptidic nature of the compounds resulting in their instability to proteolysis, (2) an improvement in the affinity of the compound for the SH2 domain, (3) the propensity of the phenylphosphate group to undergo hydrolysis, (4) the

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Table 1. Truncation analysis on hamster middle-T antigen

Compd	Amino acid sequence	IC_{50} (μM)[a]
1	EPQpYEEIPIYL	0.36
2	AcpYENHMe	350
3	AcpYEENHMe	17
4	AcpYEEINHMe	2.0
5	AcpYEEIPNHMe	1.6

[a]Compounds were tested using a scintillation proximity assay.[5]

low likelihood of cell penetration due to the charged nature of the phosphotyrosine containing peptides. In this paper, we describe the identification of an uncharged replacement for the doubly charged Glu-Glu sequence and the preparation of compounds containing potent replacements for the isoleucine substituent in the pY + 3 pocket.[6]

A search was carried out of internal angles in N–C(C=O)–N–C–C=O substructures found in the Cambridge crystallographic database, which identified the 9-aminopyridazinodiazepine carboxamide **6**[7] as a potential Glu-Glu mimetic. This substructure is shown overlaid on the tetrapeptide in Figure 2. It can be seen that both of the β carbon atoms of the glutamic acid side-chains are overlaid by ring carbons of the 9-aminopyridazinodiazepine. Synthesis of the tetrapeptide mimetic containing the bicycle **7** confirmed it was a suitable replacement for the Glu-Glu linker since **7** was essentially equipotent with the tetrapeptide (Fig. 1). We obtained co-crystals of **7** bound to lck SH2 domain (Fig. 3).[8] This structure shows that the tyrosine phosphate adopts a similar conformation to that of the peptide structure reported by Eck[2], and indeed the hydrogen bond contacts for the acetamide and the phosphotyrosine side-chain correspond to those observed in the Eck structure. However, the 9-aminopyridazinodiazepine is considerably displaced from the peptide backbone in Eck's structure and as a result the isoleucine does not penetrate as deeply into the pY + 3 pocket. ^{1}H NMR experiments showed that the side-chain of isoleucine was relatively mobile in the pY + 3 pocket. The bicyclic ring system makes a number of contacts with the SH2 domain. There is a hydrogen bond from the side-chain NH of Lys 57 to the C6 carbonyl of the 9-aminopyridazinodiazepine. The C8 and C9 carbons of the 9-aminopyridazinodiazepine make Van der Waals contacts with the aromatic ring of tyrosine 59, which may explain why the isoleucine does not penetrate the pY + 3 pocket very deeply, or it may be due to crystal packing effects. There are water mediated hydrogen bonds from the carbonyl of the acetamide to the backbone NH of histidine 58 and from the C10 carbonyl of the 9-aminopyridazinodiazepine to the backbone NH of lysine 60. More detailed analysis of this structure will be published shortly.

We subsequently turned our attention towards improving the activity of the lead series by finding improved ligands for the pY + 3 pocket. The pocket is bounded predominantly by hydrophobic residues with a tyrosine located at the bottom of the pocket and also a water

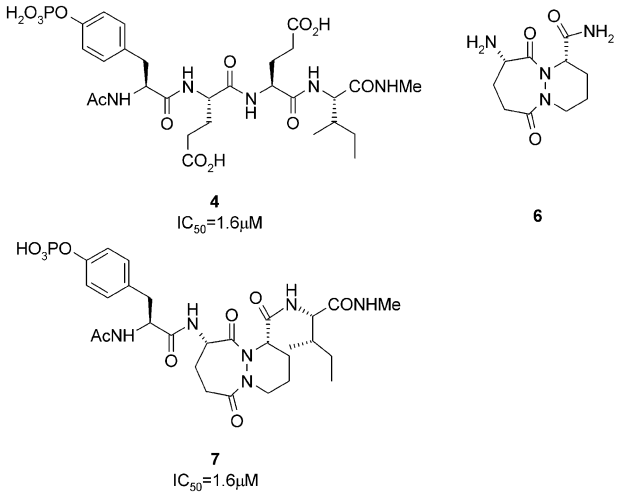


Figure 1. Conformational restriction of the Glu-Glu linker with 9-aminopyridazinodiazepine.

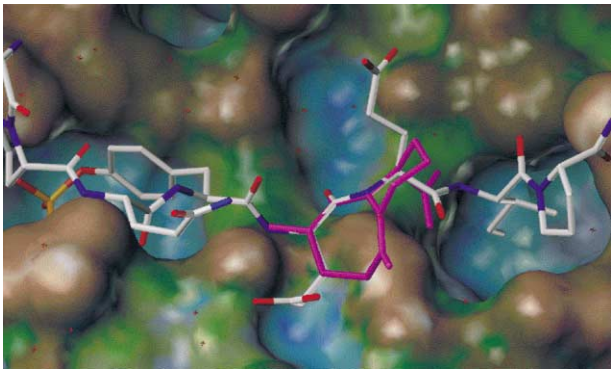


Figure 2. Molecular modelling showing the the 9-aminopyridazinodiazepine **6** (magenta) overlaid on the peptide backbone of the hmT antigen (multicolour) from Eck's structure.[2]

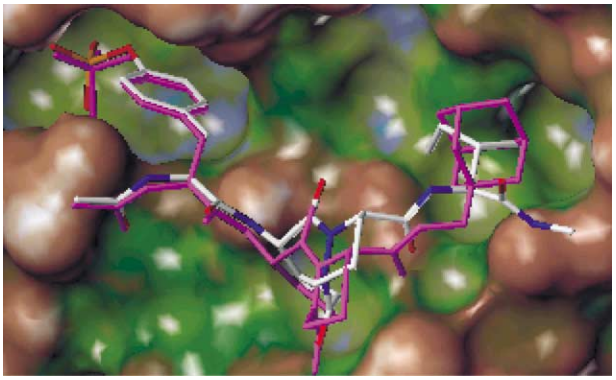
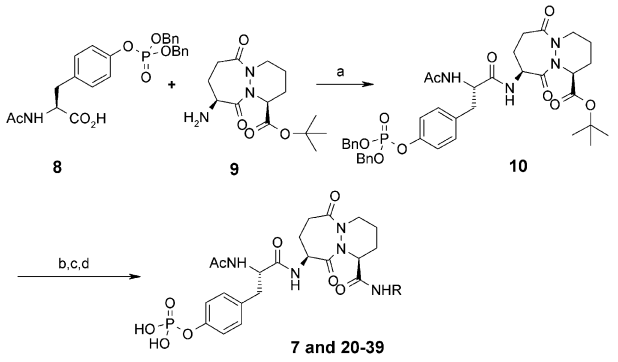


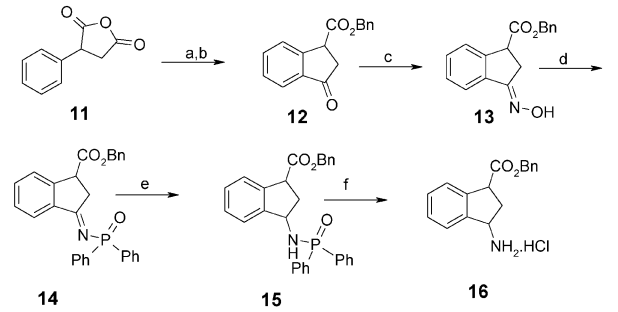
Figure 3. Comparison of the crystal structures of lck SH2/isoleucinemethylamide **7** (multicolour) and lck SH2/adamantylmethylamide **31** (magenta).

molecule close to the base of the pocket. Our initial strategy was to more completely occupy the pocket by incorporating replacements for isoleucine methylamide into the structure.

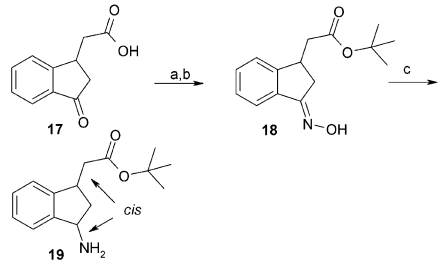
The hmT antigen was prepared by Fmoc-SPPS on a Milligen 9050 peptide synthesiser using the solid-phase



Scheme 1. Reagents and conditions: (a) IBCF, NMM, THF, −20 °C; (b) 20% TFA/CH_2Cl_2; (c) IBCF, NMM, RNH_2; (d) 95% aq TFA (examples **7**, **20**–**33**, **38**, **39**) or 10% Pd/C, AcOH (examples **34**–**37**).



Scheme 2. Reagents and conditions: (a) $AlCl_3$, $ClCH_2CH_2Cl$; (b) BnOH, H_2SO_4; (c) $NH_2OH{\cdot}HCl$, NaOH, EtOH; (d) $ClPPh_2$, NEt_3, CH_2Cl_2, ether, −78 °C; (e) $NaBH_4$, THF; (f) 4 M HCl, dioxan.



Scheme 3. Reagents and conditions: (a) *N*,*N*-diisopropyl-*O*-*t*-butylisourea, CH_2Cl_2; (b) $NH_2OH{\cdot}HCl$, NaOH, EtOH; (c) H_2, Pd/C, MeOH.

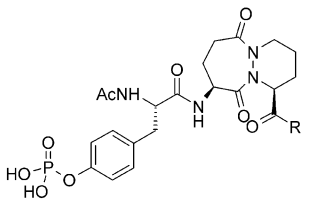
phosphorylation protocol described by Kitas et al.[9] Peptides (**2**–**5**) were prepared by sequentially coupling methylamine, the appropriate *N*-BOC amino acid and either *N*-BOC–Tyr–(PO_3Bn)–OH (for the tetra and pentapeptides) or Ac–Tyr–(PO_3Bn)–OH (for the di and tripeptides) from C- to N-terminus using isobutylchloroformate as the coupling agent. Removal of the *N*-BOC protective group was effected with 4 N HCl in ethyl acetate. Removal of the *N*-BOC protective group from phosphotyrosine containing peptides was effected with HCl in acetic acid or formic acid. Acetylation of the N-terminus was effected with acetylchloride and *N*-methylmorpholine. Finally, hydrogenation of the protecting groups with 10% Pd/C in either methanol or formic acid afforded the desired peptides.

Target compounds were prepared as described in Scheme 1. The dibenzylphosphotyrosine **8** was coupled to the 9-aminopyridazinodiazepine **9** using a mixed anhydride procedure to give products **10**. The *tert*-butylester was removed using TFA and the resulting acid was coupled to the appropriate amine using the mixed anhydride procedure. Deprotection of the dibenzyl phosphate and *tert*-butyl or benzyl esters present on the amine was carried out with either TFA or by catalytic hydrogenation using 10% Pd/C to afford target compounds **7** and **20**–**39**. Mixtures of diastereoisomers were separated by RP-HPLC.

Amines were commercially available except for the benzyl ester of 3-aminoindan-1-carboxylate **16** and the *tert*-butyl ester of 3-aminoindan-1-acetic acid **19**. The synthesis of compound **16** is described in Scheme 2. Phenylsuccinic anhydride **11** was treated with $AlCl_3$ to give 3-oxoindan-1-acetic acid which was protected as a benzyl ester to give compound **12**. Treatment with hydroxylamine hydrochloride and base afforded the oxime **13**. Treatment of **13** with diphenylchloro phosphine gave the iminophosphine oxide **14** which was reduced to the aminophosphine oxide **15** with $NaBH_4$. Treatment of **15** with HCl in dioxan gave the benzyl ester of 3-aminoindan-1-carboxylic acid **16** as a mixture of all four diastereomers. Compound **19** was prepared as summarised in Scheme 3. 3-Oxoindan-1-acetic acid **17** was treated with *N*,*N*,-diisopropyl-*O*-*t*-butylisourea followed by hydroxylamine hydrochloride and base to afford the oxime **18**. Catalytic hydrogenation of **18** gave the *tert*-butylester of 3-aminoindan-1-acetic acid **19** as a mixture of the two *cis* diastereoisomers.

Inspection of the crystal structure of **7** showed that the C-terminal methylamide did not make any hydrogen bond contacts with the SH2 domain. In support of this, incorporation of (*S*)-2-methylbutylamine into the structure afforded **27** which was actually slightly more active than the parent amino acid derivative. Hence, the first series of replacements targeted were commercially available amines which might occupy the pocket more fully. However, neither benzylamine (**20**, 1.5 μM) nor cyclohexylmethylamine (**29**, 1.6 μM) showed any improvement in activity compared to the isobutyl group. A wide range of substituted benzylamines were incorporated (a selection of these replacements are shown in Table 2) which had very similar activity to the benzylamide. One exception was the *p*-chlorobenzylamide (**21**, 0.4 μM) which was 4-fold more active than the benzylamide. In fact most of the replacements (Table 2) showed an activity of between 1 and 10 μM. Even the adamantylmethyl group (**30**, 1.1 μM) was of roughly equal potency to the methylbutylamide. A crystal structure was obtained for **30** which showed the adamantyl group bound to the mouth of the pocket, and the 9-aminopyridazinodiazepine displaced even further from the peptide backbone of Eck's structure.[10] Inspection of the crystal structures shows there are a number of water mediated interactions between the pyridazinodiazepine bicycle and the SH2 domain and this may allow sufficient movement of the bicycle to accommodate a wide range of substituents in the pY + 3

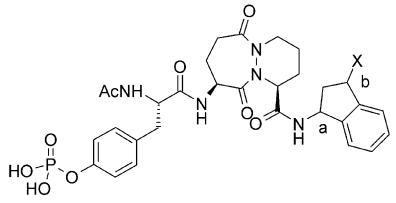
Table 2. Lck SH2 domain inhibitory activity of peptidomimetics containing alkylamides and conformationally restricted benzylamides in the pY + 3 pocket



Compd	R	IC_{50} (μM)[a]
20	$PhCH_2NH$	1.5
21	*p*$ClPhCH_2NH$	0.4
22	*p*$HOPhCH_2NH$	10
23	EtNH	8.5
24	PrNH	6.0
25	BuNH	1.9
26	PnNH	1.8
27	(*S*)-2-Methylbutylamine	1.2
28	NeopentylNH	1.8
29	Cyclohexylmethylamine	1.6
30	Adamantylmethylamine	1.1
31	1-Aminomethylnaphthalene	1.9
32	1-Aminonaphthalene	1.1
33	1-Aminoindane	1.2

[a]Compounds were tested using a scintillation proximity assay.[5]

Table 3. Lck SH2 domain inhibitory activity of peptidomimetics containing aminoindanes in the pY + 3 pocket



Compd	a*	b*	X	IC_{50} (μM)[a]
34	Trans diast. A		CO_2H	7.7
35	Trans diast. B		CO_2H	7.3
36	R	S	CO_2H	0.1
37	S	R	CO_2H	2.0
38	R	R	CH_2CO_2H	0.03
39	S	S	CH_2CO_2H	2.8

[a]Compounds were tested using a scintillation proximity assay.[5]

pocket. A limited correlation between structure and activity was observed in certain series of compounds. For instance, in the alkylamides there was a gradual increase in activity on going from the ethylamide (**23**, 8.5 μM) to the butylamide (**25**, 1.9 μM) but no further increase in activity for the pentylamide (**26**, 1.8 μM).

A series of benzylamides was prepared in an attempt to improve the potency of the series, including the conformationally restricted 1-aminoindanylamide (**33**, 1.2 μM) and 1-aminonaphthylamide (**32**, 1.1 μM) and the benzfused derivative 1-aminomethylnaphthylamide (**31**, 1.9 μM). All were essentially equipotent with the benzylamide.

Inspection of the crystal structure of **7** indicated that a carboxylic acid attached to one of the conformationally restricted benzylamines might be able to form a hydrogen bond to arginine 67 on the rim of the pY + 3 pocket. The 3-aminoindan-1-carboxylic acid was prepared as a mixture of all four possible diastereomers which was separated into the four constituent diastereomers by preparative RP-HPLC. The 3(*R*)aminoindan-1(*S*)-carboxylic acid (**37**, 0.1 μM, Table 3) was the most potent diastereoisomer binding 16-fold more tightly than the benzylamide. Modelling suggested that substitution of the indane with an acetic acid should also allow interaction with arginine 67. The aminoindaneacetic acid was prepared as a mixture of *cis* diastereomers and separated by RP-HPLC. The 3(*R*)-aminoindan-1(*R*)-acetic acid (**39**, 0.033 μM) was the most potent isomer and indeed the most potent pY + 3 replacement prepared in this series. We were unable to obtain a crystal structure of the aminoindane acetic acid to confirm the interaction of the carboxylic acid with arginine 67 or indeed the binding of the phenyl ring in the pY + 3 pocket.

Starting from the crystal structure of $p56^{lck}$ SH2 domain reported by Eck et al.[2] we have determined the minimum peptide sequence needed for optimal binding to be the tetrapeptide Ac-pY-E-E-I-NHMe. We have designed an uncharged replacement for the Glu-Glu linker and explored potential replacements for the isoleucine moiety in the pY + 3 pocket. The majority of replacements were of similar activity to the isoleucinemethylamide. However, we did identify a potent replacement containing a carboxylic acid. Having addressed the issues of peptide hydrolysis and potency, we will describe the identification of phosphotyrosine replacements and antagonists of reduced charge in a subsequent paper.

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References and Notes

1. Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Hase, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J.; Neel, B. G.; Birge, R. B.; Fajardo, J. E.; Chou, M. M.; Hanafusa, H.; Schaffhausen, B.; Cantley, L. C. *Cell* **1993**, *72*, 767.
2. Eck, M. J.; Schoelson, S. E.; Harrison, S. C. *Nature* **1993**, *362*, 87.
3. A number of structures of $p56^{lck}$ SH2 domain have subsequently appeared in the literature. (a) Eck, M. J.; Atwell, S. K.; Shoelson, S. E.; Harrison, S. C. *Nature* **1994**, *368*, 764. (b) Mikol, V.; Baumann, G.; Keller, T. H.; Manning, U.; Zurini, M. G. M. *J. Mol. Biol.* **1995**, *246*, 344, as well as a number of structures of the closely related $p60^{src}$ SH2 domain. (c) Shakespeare, W.; Yang, M.; Bohacek, R.; Carasoli, F.; Stebbins, K.; Sundaramoorthi, R.; Azimioara, M.; Yu, C.; Pradeepan, S.; Metcalf III, C.; Haraldson, C.; Merry, T.; Dalgarno, D.; Narula, S.; Hatada, M.; Lu, X.; van Schravendijk, M.-R.; Adams, S.;

Violette, S.; Smith, J.; Guan, W.; Bartlett, C.; Herson, J.; Iuliucci, J.; Weigele, M.; Sawyer, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9373. (d) Buchanan, J. L.; Bohacek, R. S.; Luke, G. P.; Hatada, M.; Lu, X.; Dalgarno, D. C.; Narula, S. S.; Yuan, R.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2353. Plummer, M. S.; Holland, D. R.; Shahripour, A.; Lunney, E. A.; Fergus, J. H.; Marks, J. S.; McConnell, P.; Mueller, W. T.; Sawyer, T. K. *J. Med. Chem.* **1997**, *40*, 3719.

4. Tong, L.; Warren, T. C.; King, J.; Betageri, R.; Rose, J.; Jakes, S. *J. Mol. Biol.* **1996**, *256*, 601.

5. The SH2 domain from amino acid residues 127 to 229 of $p56^{lck}$ was expressed as a GST fusion protein by subcloning into the vector pGEX-3X (Amersham Pharmacia Biotech.). After induction with isopropylthiogalactoside, soluble GST-SH2 protein was initially purified on a Glutathione Sepharose column (Amersham Pharmacia Biotech) followed by phospho-Tyrosine Agarose affinity chromatography (Sigma Ltd.). A final buffer exchange was carried out on a PD10 column (Amersham Pharmacia Biotech).

The assay employed a scintillation proximity readout and monitored the binding of the SH2 fusion protein to the pY containing peptide LEDFFTATEGQpYQPQP. This peptide corresponds to the region around the autoregulatory site at Y 505 on $p56^{lck}$. The assay was run on a microtitre format and used a biotinylated version of the above peptide at a final concn of 7 nM and 100 μg of streptavidin coated SPA beads (Amersham Pharmacia Biotech) per well. The buffer used to dilute beads and protein reagents was 100 mM Tris–HCl pH 8.0, 100 mM NaCl, 4 mM DTT, 100 μg/mL BSA. Titration curves of inhibitors or control compounds were diluted in DMSO and added to the assay so that the final concn of diluant was 5%. Finally the assay was initiated by adding 50 ng of GST-lck SH_2 fusion protein, 150 ng of anti-GST IgG (Molecular Probes, Inc.) and 50 nCi of ^{125}I anti rabbit IgG, antibody. Binding was allowed to reach equilibrium for a minimum of 4 h prior to counting on a Wallac Microbeta counter.

6. A number of groups have addressed these issues in recent years. For a recent review on the identification of SH2 domain antagonists including $p56^{lck}$, see; Garcia-Echeverria, C. *Curr. Med. Chem.* **2001**, *8*, 1589.

7. Attwood, M. R.; Hassall, C. H.; Krohn, A.; Lawton, G.; Redshaw, S. *J. Chem. Soc., Perkin Trans. 1* **1986**, *6*, 1011.

8. The atomic coordinates of compound **7** bound to $p56^{lck}$ SH2 domain have been deposited to the Protein Data Bank and the EBI deposition code is EBI-8436.

9. Kitas, E. A.; Knorr, R.; Trzeciak, A.; Bannwarth, W. *Helv. Chim. Acta* **1991**, *74*, 1314.

10. The atomic coordinates of compound **30** bound to $p56^{lck}$ SH2 domain have been deposited to the Protein Data Bank and the EBI deposition code is EBI-8445.