Src Homology-2 Inhibitors: Peptidomimetic and Nonpeptide

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Abstract: The structural and functional characterization of Src homology-2 (SH2) domains and their relationship to catalytic proteins (e.g., kinases, phosphatases, and lipases) or non-catalytic proteins (e.g., upstream adapters, and downstream transcription factors) has significantly impacted our understanding of signal transduction pathways and the identification of promising therapeutic targets for drug discovery. Such SH2-containing proteins are known to be intimately involved in the regulation of a number of cellular processes, including growth, mitogenesis, motility, metabolism, and gene transcription. Molecular recognition and biochemical selectivity exists for various SH2 domains based on their binding to phosphotyrosine (pTyr) and contiguous C-terminal amino acids of cognate protein 'partners' in a sequence-dependent manner (i.e., ~pTyr-AA_1-AA_2-AA_3~) which result in the formation of signal transduction protein complexes in cells. In recent years, drug discovery efforts have advanced peptidomimetic and nonpeptide inhibitors of such protein-protein interactions based on mimicking pTyr-containing peptide ligands as well as SH2 structure-based *de novo* design of nonpeptide templates that can capture key binding sites on the target protein. Noteworthy are peptidomimetic and nonpeptide inhibitors of Src, Lck, Grb2, PI-3K, and Zap70 from pioneering efforts that led to the first examples of cellularly and *in vivo* active SH2 inhibitors. This mini-review highlights key achievements in SH2 inhibitor drug discovery with an emphasis on peptidomimetic and nonpeptide lead compounds in terms of structure-based design, key chemical and biological properties, and proof-of-concept studies relative to further defining the role(s) of SH2 domains in signal transduction processes, cellular functions, and *in vivo* disease models.

Keywords: signal transduction, Src homology domain, Src homology-2, SH2, Src, Lck, PI-3K, Grb2, Zap70, phosphopeptide, phosphotyrosine, pTyr mimics, protein-protein interactions, peptidomimetic, nonpeptide, structure-based drug design.

INTRODUCTION

Signal transduction processes are complex in nature and include numerous mechanisms to provide time-ordered, generally reversible, and dynamic regulation of intracellular pathways that ultimately are manifest within the scope of growth, mitogenesis, motility, metabolism, and gene transcription. Many of the major types of signal transduction pathways which have been rigorously studied over recent years are mediated by Tyr phosphorylation by protein tyrosine kinases (PTKs) and/or pTyr dephosphorylation by protein tyrosine phosphatases (PTPs) of specific substrate proteins [1,2]. These changes in phosphorylation state are then responsible for activation, inactivation and/or localization of these proteins, thus causing a signal to be propagated, amplified, interrupted, and/or compartmentalized within a cell. Intimately involved in such temporal and spatial regulation of signal transduction pathways are Src

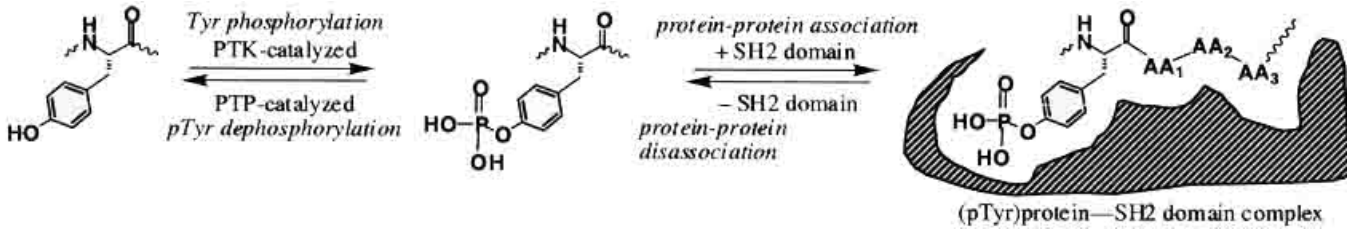

Fig. (1). Phosphotyrosine (pTyr) formation (via PTKs), degradation (via PTPs), and molecular recogntion in protein-protein interactions (via SH2 domains).

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Table I. Src homology-2 (SH2) Proteins as Therapeutic Targets

Therapeutic target	Domain substructure	Disease state(s)
Src	SH3–SH2–Kinase	Cancer, osteoporosis
Hck	SH3–SH2–Kinase	Immune disease (AIDS)
Lck	SH3–SH2–Kinase	Immune disease
Syk	SH2–SH2–Kinase	Allergy, asthma
Zap70	SH2–SH2–Kinase	Autoimmune disease
Syp	SH2–SH2–Phosphatase	Anemia
STATs	DNA-binding–SH3–SH2	Inflammatory diseases
Grb2	SH3–SH2–SH3	Cancer, chronic mylogenous leukemia
Grb7	PH–SH2	Breast cancer
Shc	SH2–PTB	Cancer, erythroleukemia
PI-3K	SH3–SH2–SH2 (p85 subunit)	Cancer
Blk	PH–SH3–SH2–Kinase	Pre-B-cell leukemia
Bcr/Abl	SH3–SH2–Kinase	Chronic mylogenous leukemia
Tec	PH–SH3–SH2–Kinase	Myelodysplastic syndrome

homology-2 (SH2) domain-containing proteins [3] which provide dynamic and reversible binding of cognate pTyr-containing proteins. Such non-catalytic, protein-protein interactions exemplify a third dimensionality to the orhestration of signal transduction mechanisms [4] as depicted very simply in Fig. (**1**) to integrate SH2-pTyr molecular recognition to pTyr formation (via PTKs) and degradation (via PTPs).

The blockade of SH2 domain-dependent, protein-protein interactions has emerged as a new strategy in drug discovery to identify novel therapeutic agents [5] which selectively inhibit signal transduction pathways in cells as related to cancer, osteoporosis, allergy, asthma, inflammatory diseases, and a plethora of other disease states (Table 1). Herein, this mini-review highlights several key research achievements related to the phosphopeptide-SH2 domain interactions and drug discovery efforts that have been focused on pTyr mimicry and the development of peptidomimetic as well as nonpeptide inhibitors of key SH2 therapeutic targets (e.g., Src, Lck, Grb2, PI-3K, Zap70). It is noted that several comprehensive reviews and commentaries on SH2 inhibitor design and structure-activity relationships have been previously reported [6-14].

3D STRUCTURE OF SH2 DOMAINS AND PHOSPHOPEPTIDE MOLECULAR RECOGNITION

SH2 domains are non-catalytic motifs of approximately 100 amino acids which have been determined to be one of the top twenty-five most frequently occurring protein structural types that have been identified from the human genome [15,16]. Numerous X-ray and/or NMR structures have been determined for SH2 domains (e.g., Src, Grb2,and Zap70) and complexes thereof with phosphopeptide, peptidomimetic or nonpeptide inhibitors [17-29]. In retrospect, the prototypic SH2 domain was that of Src, the first discovered oncogenic PTK which has been investigated by numerous functional genomic, structural biology, molecular biochemistry and cellular mechanistic studies [30,31]. High resolution X-ray structures [18,19] of the Src SH2 domain complexed with high affinity pTyr-containing peptide ligands of the pTyr-Glu-Glu-Ile type have provided detailed molecular maps revealing that most of the peptide is quite solvent exposed, with the only significantly buried portions being the pTyr and Ile sidechains as schematically illustrated in Fig. (**2**). The only critical and direct protein-ligand contact is that of the pY+1 Glu backbone NH that H-bonds to the backbone C=O of His βD4 (His-204 in Src) of the protein. The pTyr binding pocket of Src SH2 domain contains four positively charged residues: Arg αA2 (Arg-158), Arg βB5 (Arg-178), His βD4, and Lys βD6 (Lys-206). The most critical of these residues is Arg βB5 (of the FLVRES sequence) which forms two H-bonds with the phosphate oxygens of the pTyr sidechain. In Src, the mutation of Arg βB5 essentially abolishes all binding of pTyr containing ligands to the SH2 domain [32]. Nevertheless, it is noted that beyond Arg βB5 the phosphate moiety of pTyr is involved in numerous other H-bonding interactions with ThrBC2 (Thr-182), GluBC1 (Glu-181), and Ser βB7 (Ser-180), as well as a hydrophobic contact with the alkyl sidechain of Lys βD6. Again, relative to the pTyr-Glu-Glu-Ile type ligands, the pY+3 binding pocket of Src SH2 provides hydrophobic contacts with the Ile sidechain of the ligand. The pY+3 pocket of the Src SH2 domain is formed by two loop regions and is comprised of several key residues, including Tyr βD5 (Tyr-305), Ile βE4 (Ile-217), ThrEF1 (Thr-218) and Gly9 βG3 (Gly-239). Recently, a more complete understanding of the role of Src SH2 to the self-regulation of Src activity has been revealed by X-ray structures of near full-length Src [33-35].

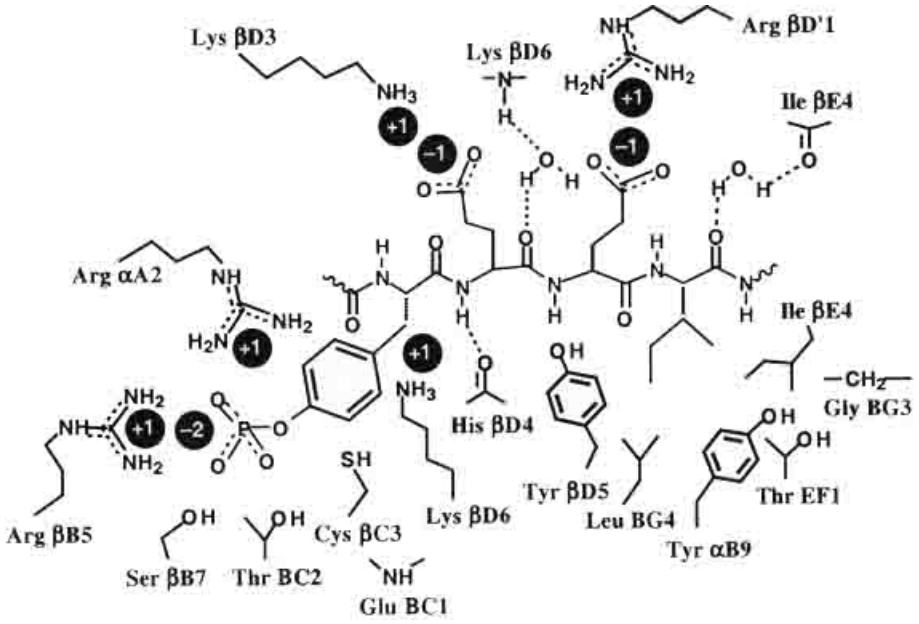

Fig. (2). A model of ~pTyr-Glu-Glu-Ile~ complexed with Src SH2 domain is shown as extrapolated from X-ray structural studies[18,19]. The two major binding pockets for the pTyr and Ile residues of the phosphopeptide as well as key intermolecular H-bonding contacts (two via structural water) are shown (see text for details).

SH2 SPECIFICITY AND PHOSPHOPEPTIDE STRUCTURE-ACTIVITY RELATIONSHIPS

The prevalence of SH2 domain-containing proteins in various cells illustrates the importance of molecular recognition with cognate pTyr-containing proteins to specifically modulate signal transduction pathways. Such molecular recognition between SH2 domains and their preferred pTyr-containing ligands may be conceptually simplified to pTyr- AA_1- AA_2- AA_3 wherein AA_1, AA_2, and AA_3 refer to the first (pY+1), second (pY+2), and third (pY+3) residues within the phosphotyrosine sequence. Significant affinity between pTyr and the phosphotyrosine binding site of the SH2 domain is critical to such molecular recognition. Furthermore, the C-terminal pY+1, pY+2, and pY+3 residues contribute to SH2 domain binding affinity and biochemical specificity, in a sequence-dependent manner, as determined for both cognate pTyr-proteins as well as synthetic pTyr-peptide libraries [36,37]. In the latter case, the preferred amino acids at the pY+1, pY+2 and pY+3

Table 2. Specificities of SH2 Domain Binding of Phosphopeptide Libraries

Library Design: **Gly-Asp-Gly-pTyr-AA_1-AA_2-AA_3-Ser-Pro-Leu-Leu-Leu**

SH2 Domain	pY+1 (AA_1)	pY+2 (AA_2)	pY+3 (AA_3)
Src	Glu, Asp, Thr	Glu, Asn, Tyr	Ile, Met, Leu
Lck	Glu, Thr, Gln	Glu, Asp	Ile, Val, Met
Abl	Glu, Ile, Val	Asn, Glu, Asp	Pro, Val, Leu
Grb2	Gln, Tyr, Val	Asn	Tyr, Gln, Phe
Shc	Ile, Glu, Tyr	(Any)	Ile, Leu, Met
PLCγ(N-SH2)	Leu, Ile, Val	Glu, Asp	Leu, Ile,Val
PLCγ(C-SH2)	Val, Ile, Leu	Ile, Leu	Pro, Val, Ile
Syk (N-SH2)	Gln, Thr, Glu	Glu, Gln, Thr	Thr
Syk (C-SH2)	Thr	Thr	Ile, Leu, Met
p85 (N-SH2)	Met, Ile, Val, Glu	(Any)	Met

positions of a pTyr-peptide sequence have been determined for several SH2 domains (Table 2). Such phosphopeptide library studies have confirmed and extended the known specifities of SH2 domains to bind cognate proteins in cells. For example, for Src SH2 the preferred amino acids at the pY+1, pY+2 and pY+3 positions include the known Glu-Glu-Ile sequence as found in the cognate pTyr containing sequence from middle T antigen. Also, the known extraordinary specificity of Grb2 SH2 binding to pY+2 Asn containing cognate protein sequences was determined by the aforementioned phosphopeptide library investigations.

A noteworthy systematic structure-activity investigation of phosphopeptide binding to a SH2 domain was that performed on Ac-pTyr-Glu-Glu-Ile-Glu and its binding to Src SH2 [19]. In this study, numerous L- and D-amino acid substitutions were examined at the pY+1, pY+2 and pY+3 positions, respectively (*cf.* compounds **1-20**, Table 3). Briefly, this Src SH2–phosphopeptide binding analysis showed that both the pY+1 and pY+2 positions were well-tolerated by several L-amino acids, including pTyr, Gln, Ser, Asp, His, Trp, Phe, and Tyr. In contrast, D-amino acid substitution at the pY+1 position resulted in markedly decreased Src SH2 binding potencies to Src SH2. Interestingly, at the pY+2 position it was determined that hydrophobic D-amino acid substitution such as by D-1-naphthylalanine (D-1-Nal) provided effective Src SH2 binding affinity. Specifically, it was found that Ac-pTyr-Glu-D-1-Nal-Ile-Glu (compound **16**) was only 2-fold less potent than the parent phosphopeptide to bind Src SH2. This study also identified the pentapeptide **18**, Ac-pTyr-pTyr-pTyr-Ile-Glu, and its C-truncated tripeptide analog **20**, Ac-pTyr-pTyr-pTyr, as highly potent Src SH2 inhibitors.

Further exploitation of phosphopeptide chemical diversity and SH2 structure-based design strategies were independently reported [38,39] in the discovery of potent tripeptide inhibitors **21** and **22** of the Src SH2 domain as

Table 3. SAR of Phosphopeptide Inhibitors of Src SH2.

Lead Compound: **Ac-pTyr-Glu-Glu-Ile-Glu**

Compound	Peptide Structure	Src SH2 Binding Relative Potency
1	Ac-pTyr-Glu-Glu-Ile-Glu	1.0 (IC50 = 0.7 μM)
2	Ac-Tyr-Glu-Glu-Ile-Glu	<0.001
3	Ac-pTyr-Ala-Glu-Ile-Glu	0.07
4	Ac-pTyr-Gln-Glu-Ile-Glu	0.2
5	Ac-pTyr-Asp-Glu-Ile-Glu	0.15
6	Ac-pTyr-His-Glu-Ile-Glu	0.37
7	Ac-pTyr-pTyr-Glu-Ile-Glu	1.0
8	Ac-pTyr-D-Glu-Glu Ile-Glu	0.004
9	Ac-pTyr-Glu-Ala-Ile-Glu	0.07
10	Ac-pTyr-Glu-Gln-Ile-Glu	0.13
11	Ac-pTyr-Glu-Asp-Ile-Glu	0.7
12	Ac-pTyr-Glu-His-Ile-Glu	0.14
13	Ac-pTyr-Glu-pTyr-Ile-Glu	1.9
14	Ac-pTyr-Glu-D-Glu-Ile-Glu	0.05
15	Ac-pTyr-Glu-D-Ala-Ile-Glu	0.05
16	Ac-pTyr-Glu-D-1-Nal-Ile-Glu	0.45
17	Ac-pTyr-Glu-Glu-Ala-Glu	0.05
18	Ac-pTyr-pTyr-pTyr-Ile-Glu	16.7
19	Ac-pTyr-Glu-Glu	0.06
20	Ac-pTyr-pTyr-pTyr	4.1

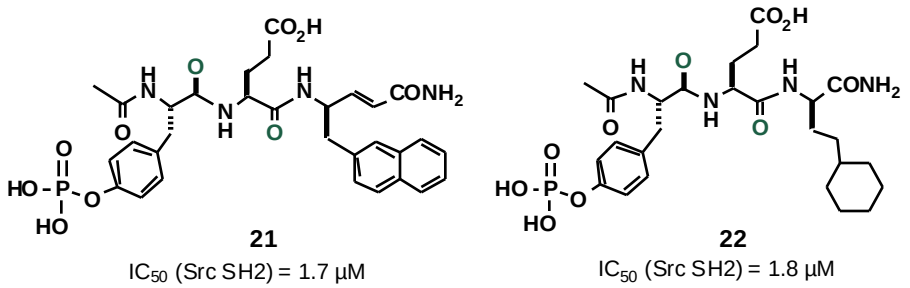

Fig. (3). Chemical structures of pY+2 D-amino acid modified tripeptide inhibitors of Src SH2 (see text for details).

shown in Fig. (**3**). Such tripeptides incorporated hydrophobic D-amino acids at the pY+2 position that were predicted to extend their sidechains to bind the pY+3 site of Src SH2. In the specific case of tripeptide **22**, Ac-pTyr-Glu-D-Hcy-NH_2 (wherein D-Hcy refers to D-homocyclohexylalanine), the determination of an X-ray structure of Src SH2 complexed with it confirmed the predicted 3D model of phosphopeptide binding with respect to D-Hcy sidechain interaction with the pY+3 site of the protein [39]. The X-ray structure of Src SH2 complexed with tripeptide **22** also revealed that the C-terminal carboxamide moiety did not interact with the protein, but was projected away towards the solvent. The implication of this finding was further realized by the fact that the $CONH_2$ group could be deleted without significant decrease in Src SH2 binding affinity as shown in a first-generation series of peptidomimetic inhibitors *(vide infra).*

PEPTIDOMIMETIC AND NONPEPTIDE INHIBITORS OF SH2 DOMAINS

The aforementioned Src SH2-phosphopeptide complexes were the first to provide insights into molecular recognition of pTyr with the pY site of a SH2 domain as well as to guide the structure-based design of peptidomimetic and nonpeptide inhibitors with particular focus on the pY+1, pY+2, and pY+3 site interactions. In the latter case, SH2 structure-based design strategies provided the opportunity to seek additional binding interactions beyond those existing for a cognate phosphopeptide. As exemplified by the first-generation of phosphopeptide inhibitors of Src SH2 which culminated in potent tripeptide analogs, it became obvious that the binding of phosphopeptides to SH2 domains was more complicated than a simplistic "two-pronged plug" concept. As detailed below, key advances in SH2 inhibitor drug discovery have been focused on pTyr mimicry as well as the development of potent, selective, cellularly active and *in vivo* effective peptidomimetic or nonpeptide second-generation lead compounds.

pTyr Mimicry and Prodrug Strategies

A major challenge of SH2 inhibitor drug discovery has been the pTyr moiety in terms of developing metabolically-stable pTyr mimics that exhibit high affinity to a SH2 domain. In this context, pTyr mimics **23-37** as shown in Fig. (**4**) have been investigated in phosphopeptides as well as in second-generation peptidomimetic and nonpeptide lead compounds. One of the most promising and earliest pTyr mimic was that of F_2Pmp (Phe[*p*-CF_2-PO_3H_2]) in which a non-hydrolyzable difluoromethylphosphonate replaced the phosphate ester [40, 41]. Also noteworthy as one of the first designed, non-phosphorous containing pTyr mimics, Phe(*p*-$OCH[CO_2H]_2$) showed potential for the development of Src SH2 inhibitors [42]. As the result of significant efforts over recent years, a variety of novel pTyr mimics incorporating phosphonate [25,26], phosphinate [43,44], carboxylate [27, 45, 46], and other mono- or non-charged groups [47] as phosphate replacements have been been developed in the design of peptide, peptidomimetic and nonpeptide inhibitors of the SH2 domains of Src, Lck, Grb2 and Zap70 *(vide infra).*

The pTyr moiety has been challenging in that SH2 binding is extraordinarily dependent on effective interactions with the pY site. There has been little, if any, success in solving the pTyr mimicry problem or identifying novel lead compounds using high-throughput screening. Hence, pTyr mimics *(vide supra)* have provided an opportunity for creativity in the design of metabolically-stable, potent and cell-penetrating SH2 inhibitors. Overall, the pTyr issue has been addressed by quite different approaches: (i) exploiting phosphonate groups to gain cellular and tissue selectivity as exemplified by the bone-targeted Dmp, Dpp, and Cpp moieties of recently described Src SH2 inhibitors [25-27]; (ii) reduction of the charged nature of a phosphate or phosphonate group by replacement with carboxylate or phosphinate moieties as exemplified in Src, Lck and Grb2 SH2 inhibitors [42-44, 47]; (iii) exploiting chemically-reactive groups in the pY site as exemplified by the Cys residue in Src SH2 with pTyr mimics incorporating aldehyde moieties [22, 46]; and (iv) masking the charged nature of a phosphonate or carboxylate group by prodrug moieties as exemplified by bis-acyloxymethylester modified F_2Pmp [48]. Relative to any of these approaches, the ultimate objective is to develop cell-penetrating SH2 inhibitors that block signal transduction pathways and cellular activities that correlate with such SH2-containing protein mechanisms. Of potential utility to address the pTyr issue and cell-penetration is the adaptation of the two-hybrid system to function in a surrogate mammalian cell line which enables an approach to monitor SH2 domain binding to pTyr-containing protein sequences in a regulated mechanism-based cellular assay [49]. Exemplifying the use of this SH2 mechanism-driven, two-hybrid cell model was the identification of an effective F_2Pmp-containing

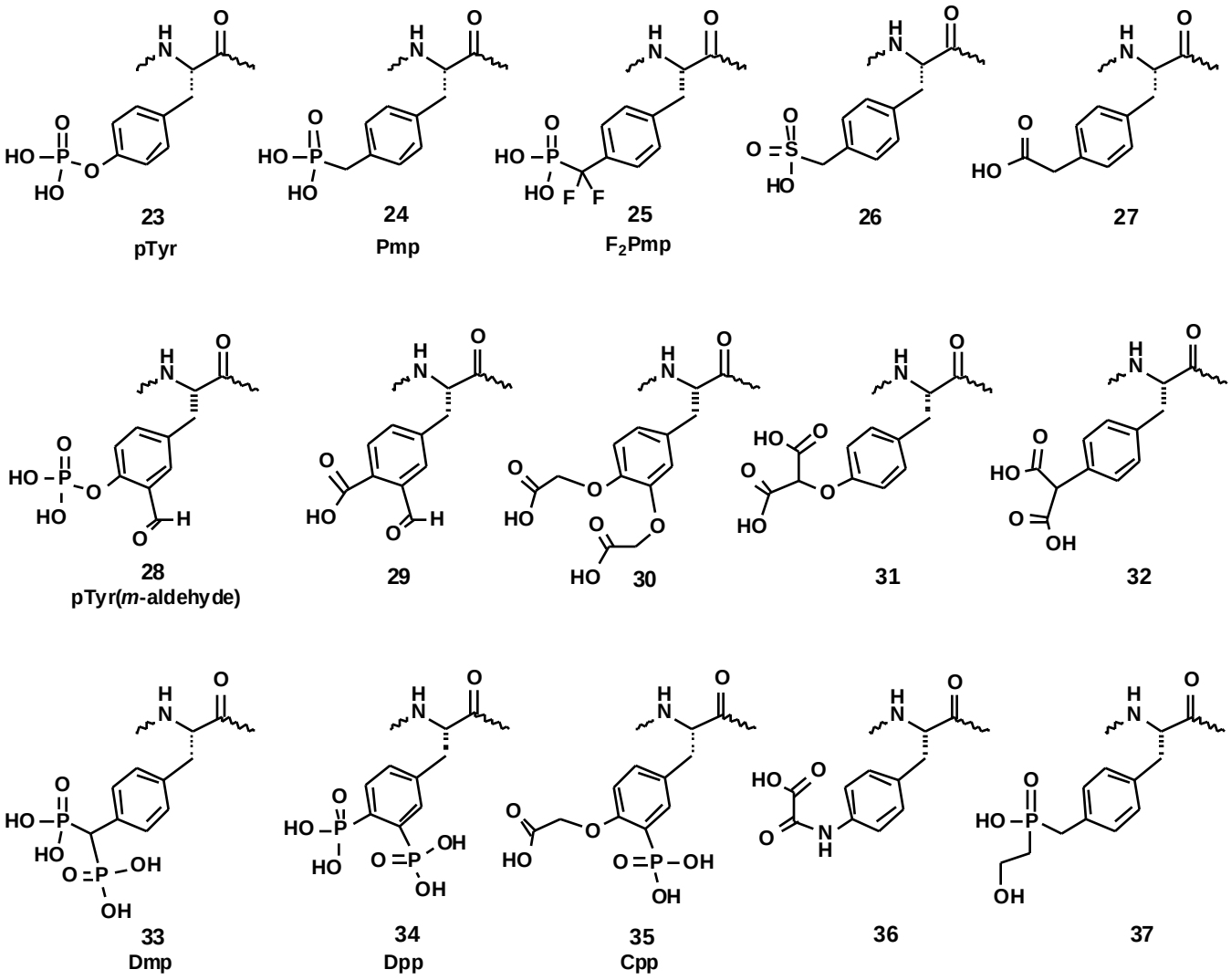

Fig. (4). Chemical structures of pTyr mimics exemplifying chemical diversity and, in a few cases, multiple functional group replacement of the phosphate group (see text for details).

peptidomimetic Src SH2 inhibitor incorporating acyloxymethyl prodrug modification versus an essentially inactive parent compound [49].

Peptidomimetic Inhibitors of SH2 Domains

Several examples of the structure-based design of peptidomimetic inhibitors of SH2 domains are noteworthy to highlight within the scope of this mini-review, including recent drug discovery efforts that have been focused on Src SH2, Lck SH2, Grb2 SH2 and Zap70 SH2.

Src SH2 Inhibitors

Of no surprise, the logical extension of the previously described pY+2 hydrophobic D-amino acid modified tripeptide inhibitors of Src SH2 was further transformation at the C-terminus to advance dipeptide and peptidomimetic analogs by structure-based design. Peptidomimetic inhibitors **38-43** as shown in Fig. (**5**) illustrate minimization of the chemical structure of the cognate phosphopeptide to the most critically required groups to bind to the pY and pY+3 sites as well as to match key H-bonding interactions afforded by the phosphopeptide backbone with Src SH2 [20, 50]. The convergence of pTyr mimics (*e.g.*, F_2Pmp) with pY+1 amino acids having disubstituted carboxamides (*e.g.*, Glu-NRR' wherein R and R' represented independent alkyl groups or were cyclized to form a pyrrolidine ring) was achieved to advance this series of promising peptidomimetic inhibitors of Src SH2 [50]. Related to this series, the most potent C-terminal amide modifications were NMe-$(CH_2)_3$-cyclohexyl and 2-(cyclohexylethyl)-pyrrolidine. A noteworthy pTyr replacement to this series was the ureido-linked phosphophenylmethyl-N(CH_2-CO_2H)-CO moiety that yielded a relatively potent Src SH2 inhibitor **41**, but more importantly illustrated the potential of structure-based design of nonpeptide inhibitors [20]. Specifically, the X-ray structure of this ureido-type peptidomimetic complexed to Src SH2 revealed that the compound bound with a *cis*-conformation at the C-terminal amide linkage and that the structural water typically observed in Src SH2-

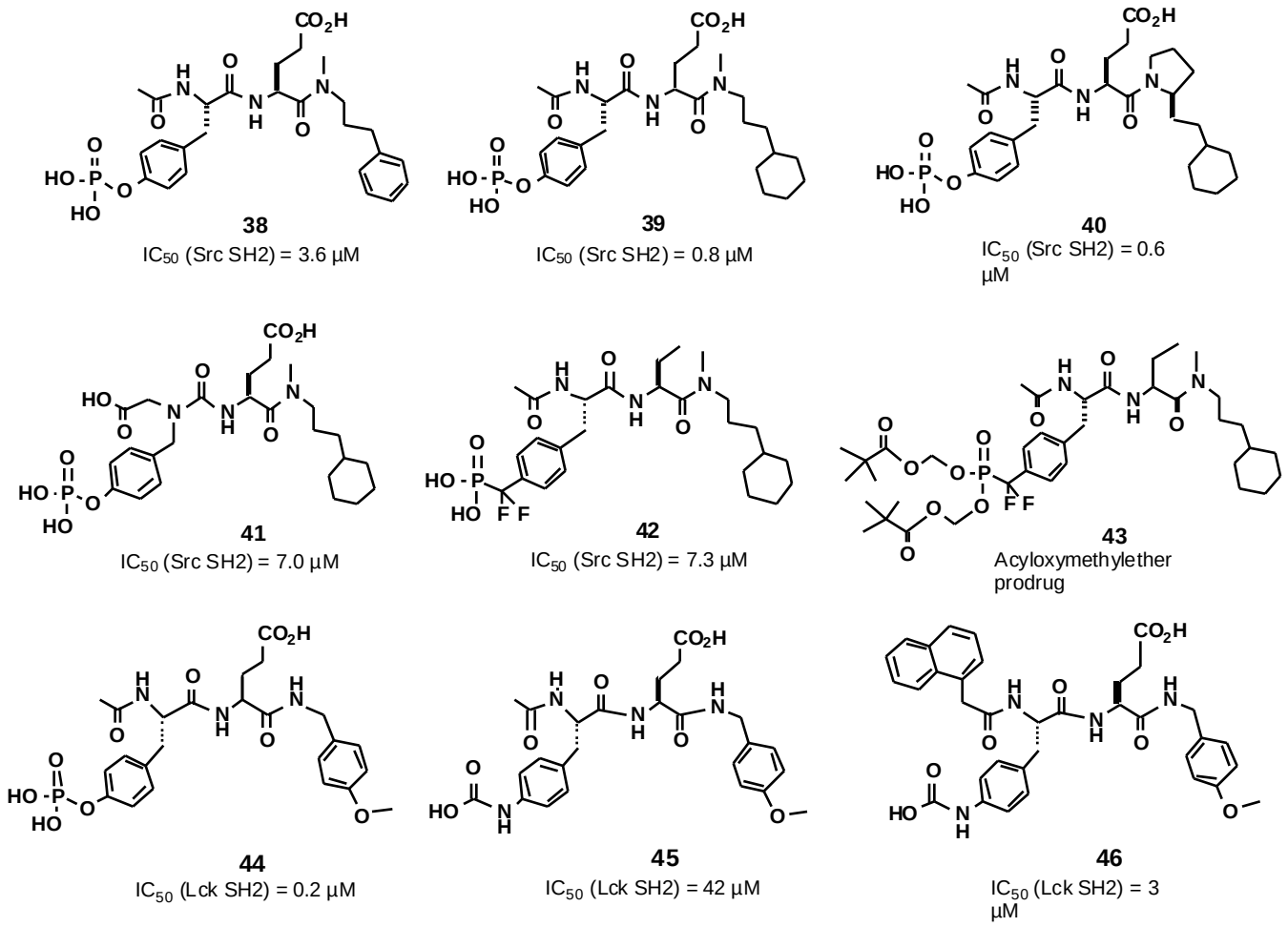

Fig. (5). Chemical structures of peptidomimetic inhibitors of Src SH2 and Lck SH2 (see text for details).

phosphopeptide complexes (*i.e.,* pY+1 backbone carbonyl-H_2O-protein) was displaced by the *cis*-amide group. Finally, an aldehyde-modified pTyr moiety has been shown to effectively inhibit Src SH2 by virtue of targeting a Cys residue that is unique to the pY site of this SH2 domain, and a X-ray structure of the complex revealed a hemithioacetal type covalent bond formed between the pY site Cys sidechain thiol functionality and peptidomimetic aldehyde group [22].

Lck SH2 Inhibitors

Similar to the phosphopeptide structure-activity campaign focused on Src SH2 *(vide supra)*, the development of dipeptide and peptidomimetic inhibitors of Lck SH2 [47, 51, 52] has been successful to advance an initial series of lead compounds **44-46** as shown in Fig. (**5**). Such Lck SH2 inhibitors also illustrate a focus on pTyr mimics such as the mono-charged oxamic acid as a replacement of the phosphate group.

Grb2 SH2 Inhibitors

The structure-based design of peptidomimetic inhibitors of Grb2 SH2, exemplified by compounds **47-55** as shown in Fig. (**6**) exploit a type-I β-turn conformation centered about the pY+1 and pY+2 residues (including the fact that the carboxamide sidechain of the highly Grb2 SH2-specific pY+2 Asn residue H-bonds directly to the protein) based on X-ray structural studies [28, 43, 44, 53]. In particular, a series of pY+1 α,α-disubstituted amino acids (*e.g.,* 1-amino-cyclohexyl-carboxylic acid or Aca) were incorporated into peptidomimetic inhibitors of Grb2 SH2 to stabilize a β-turn conformation in addition to further modifications by pTyr mimics, and both N- and C-terminal hydrophobic functionalities to interact with key complementary sites on the protein. In a few cases, X-ray structures have confirmed molecular modeling predictions of such compounds designed to bind to the Grb2 SH2 domain with respect to key inhibitor functionalities, including N-terminal capping groups (*e.g.,* 3-aminobenzyloxycarbonyl), the β-turn stabilizing pY+1 Aca residue, pY+2 Asn and its replacement by cyclic β-amino acid carboxamides [54-56], and C-terminal hydrophobic groups as shown in Fig. (**5**). Noteworthy in this series was the discovery of the cellularly active peptidomimetic Grb2 SH2 inhibitor **52** that provided proof-of-concept to this drug discovery strategy to block the Grb2–Ras pathway in terms of novel anti-cancer drug development [55]. Further expanding the scope of pTyr mimics, the design and evaluation of a series of non-phosphorous replacements (*i.e.,* carboxylate-based) have also been advanced [45, 57] as exemplified by compounds **56-61** shown in Fig. (**7**).

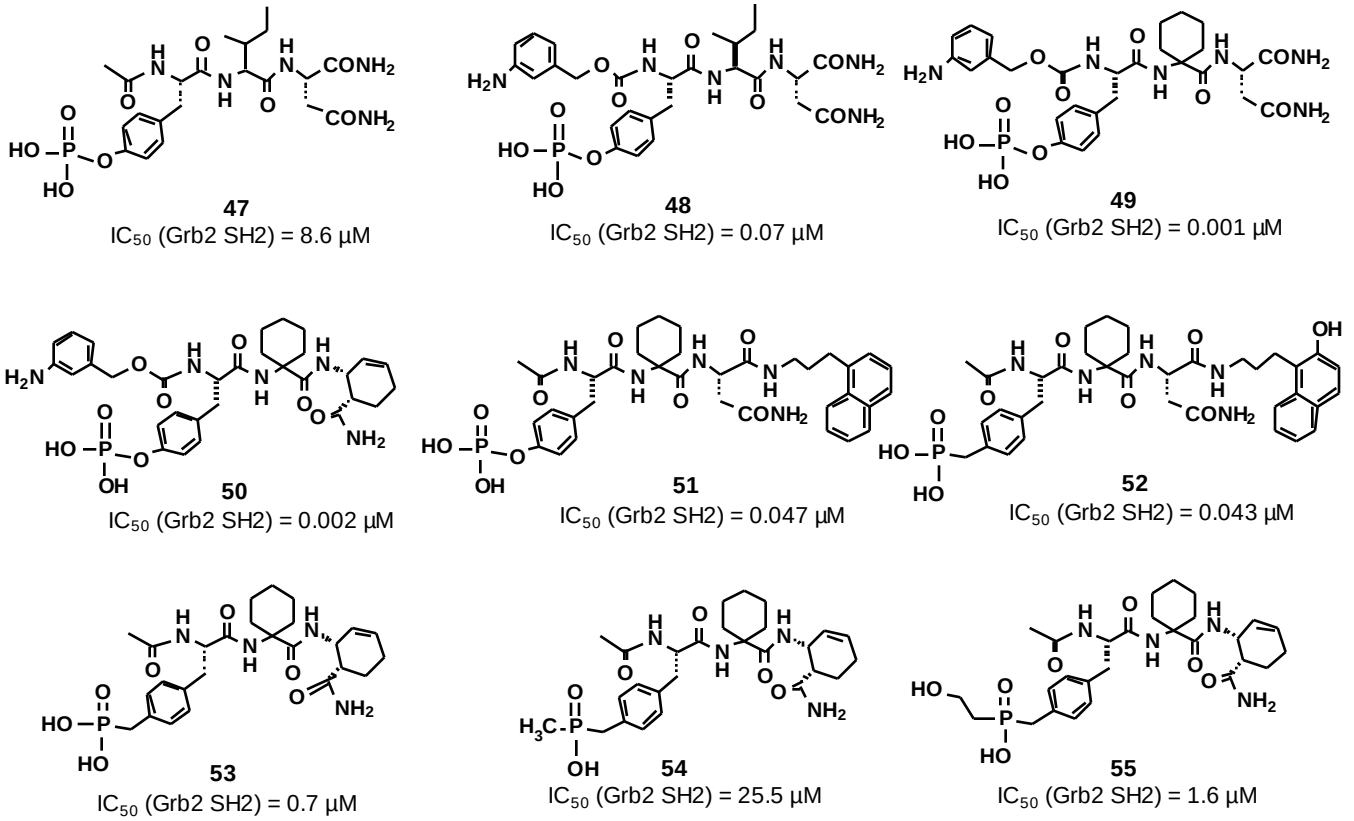

Fig. (6). Chemical structures of peptidomimetic inhibitors of Grb2 SH2 (see text for details).

Zap70 SH2 Inhibitors

Relative to Zap70 SH2 drug discovery, the structure-based design of Zap70 SH2 inhibitors has been significantly more challenging relative to the aforementioned studies on Src, Lck and Grb2 SH2. The potential for structure-based design was first recognized upon the successful determination [29] of a X-ray structure of the tandem N- and C-terminal SH2 domains of Zap70 complexed with a cognate bis-pTyr containing phosphopeptide, Asn-Gln-Leu-pTyr-Asn-Glu-Leu-Asn-Glu-Gly-Arg-Arg-Glu-Glu-pTyr-Asp-Val-Leu-Asp, correlating to the ζ-subunit sequence of the T-cell receptor ITAM. Interestingly, the X-ray structure of the Zap70 N/C-SH2–bis-pTyr phosphopeptide complex showed that the pY pocket corresponding to the N-terminal SH2 domain was actually formed at the interface of the two SH2 domains. Furthermore, the high affinity of the above bis-pTyr phosphopeptide (IC_{50} = 0.038 μM) versus its constituent mono-pTyr containing tetrapeptides, Ac-pTyr-Asn-Glu-Leu-NH_2 (IC_{50} = 422 μM) and Ac-pTyr-Asp-Val-Leu-NH_2 (IC_{50} = 328 μM), to bind Zap70 N/C-SH2 correlates well with a predictable entropic advantage from bidentate interactions. A peptidomimetic derived from a combinatorial library-based approach [58] led to the

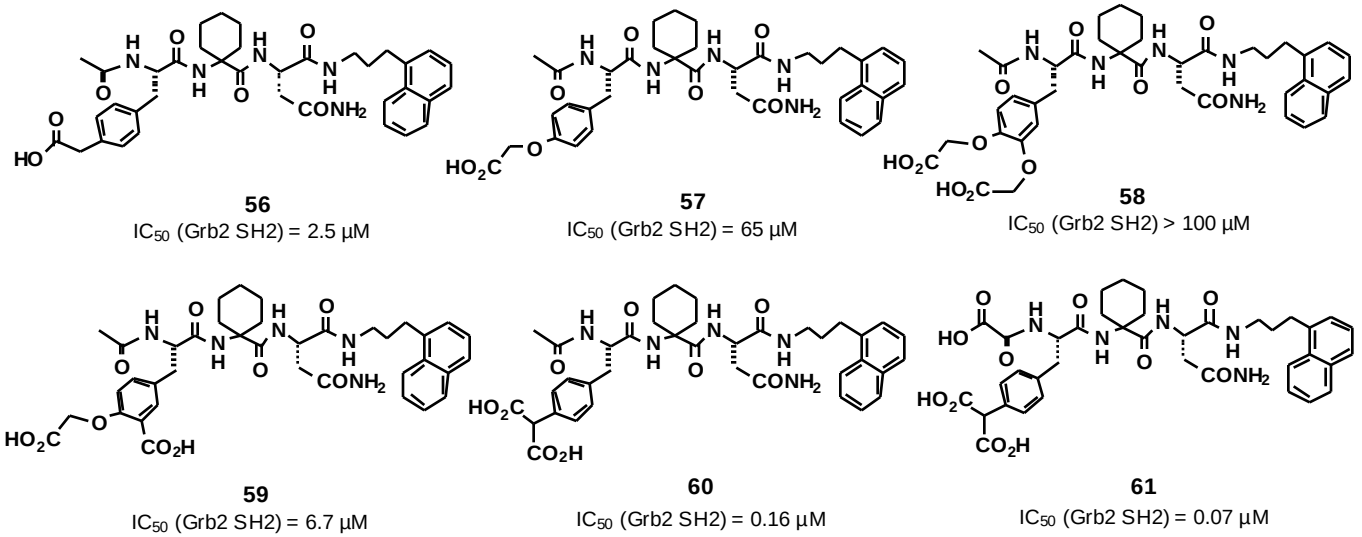

Fig. (7). Chemical structures of peptidomimetic inhibitors of Grb2 SH2 (see text for details).

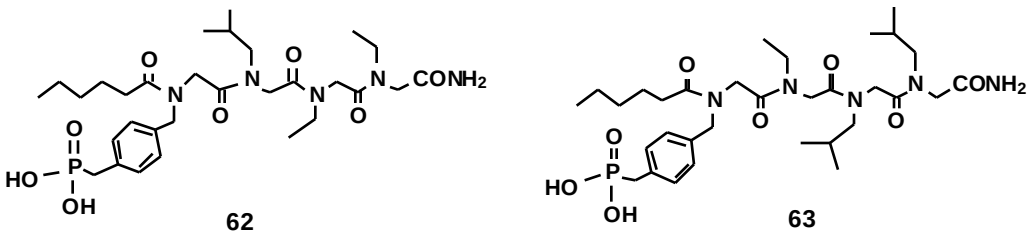

Fig. (8). Chemical structures of peptidomimetic inhibitors of Zap70 SH2 (see text for details).

identification of achiral multi-N-substituted-Gly lead compounds of the generic structure Ac-NR_1-Gly-NR_2-Gly-NR_3-Gly-NR_4-Gly-NH_2, exemplified by compounds **62** and **63** as shown in Fig. (**8**), that exhibited Zap70 N/C-SH2 binding affinities in the 25-100 µM range.

Nonpeptide Inhibitors of SH2 Domains

Several examples of the structure-based design of nonpeptide inhibitors of SH2 domains are noteworthy to highlight within the scope of this mini-review, including recent drug discovery efforts that have been focused on Src SH2, Lck SH2, Grb2 SH2 and Zap70 SH2.

Src SH2 Inhibitors

The structure-based design of a series of nonpeptides utilizing a *m*-aminomethyl-benzamide template led to a relatively potent Src SH2 inhibitor (compound **64**, IC_{50} = 6.6 µM), and a X-ray structure of the Src SH2–nonpeptide complex confirmed its predicted interactions with the target protein [21] as schematically depicted in Fig. (**9**). The benzamide $CONH_2$ moiety was determined to displace the same structural water as the aforementioned ureido-type peptidomimetic Src SH2 inhibitor (compound **41**) to further support the *de novo* design strategy that led to this nonpeptide inhibitor series. Structure-activity studies [21] of this nonpeptide revealed that both a pY+3 group and the benzamide $CONH_2$ moiety were critical for Src SH2 binding as exemplified by compounds **65** and **66**, respectively *(vide infra).*

Increased potency and Src SH2 selectivity was subsequently reported [26, 27, 59] for a series of nonpeptide inhibitors incorporating both the benzamide template and key pTyr mimics, including F_2Pmp, Pmp, Cpp, and Dmp as exemplifed by compounds **67-72** shown in Fig. (**10**). These nonpeptides illustrate further modification of the benzamide template by a pY+1 methyl group which was designed to effect increased Src SH2 binding affinity. A novel series of bicyclic benzamide templates have been recently reported [24, 25, 46] to provide highly potent nonpeptide lead compounds **73-75** as shown in Fig. (**10**) that exhibit cellular activity and, in one case, *in vivo* efficacy. Noteworthy was the bicyclic benzamide template

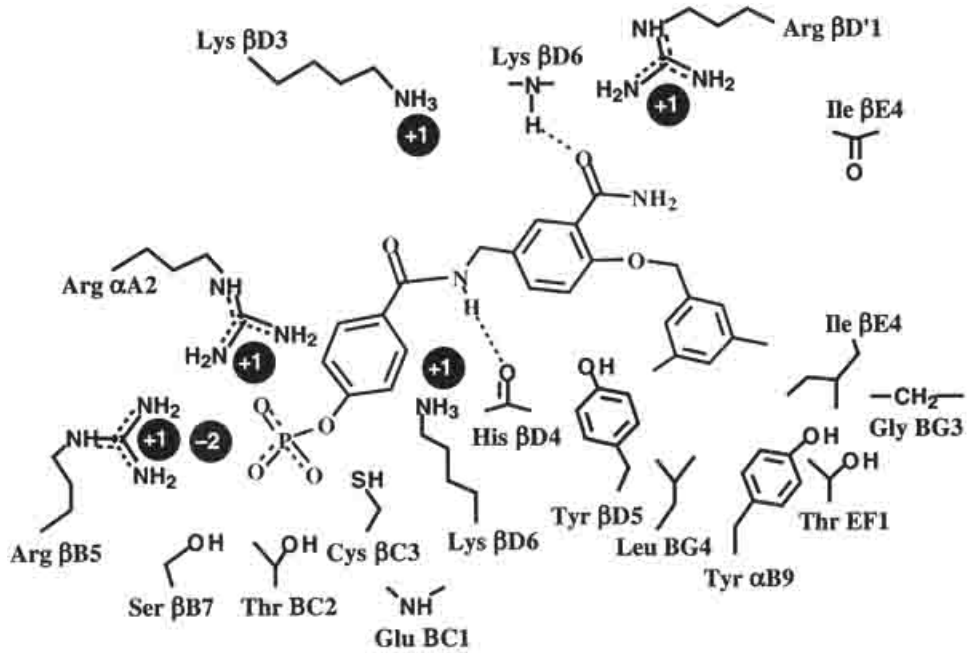

Fig. (9). A model of a *de novo* structure-based designed nonpeptide complexed with Src SH2 illustrating functional group interactions with the pY and pY+3 binding pockets as well as the displacement of structural water proximate to the pY+2 site by the benzamide $CONH_2$ group (see text for details).

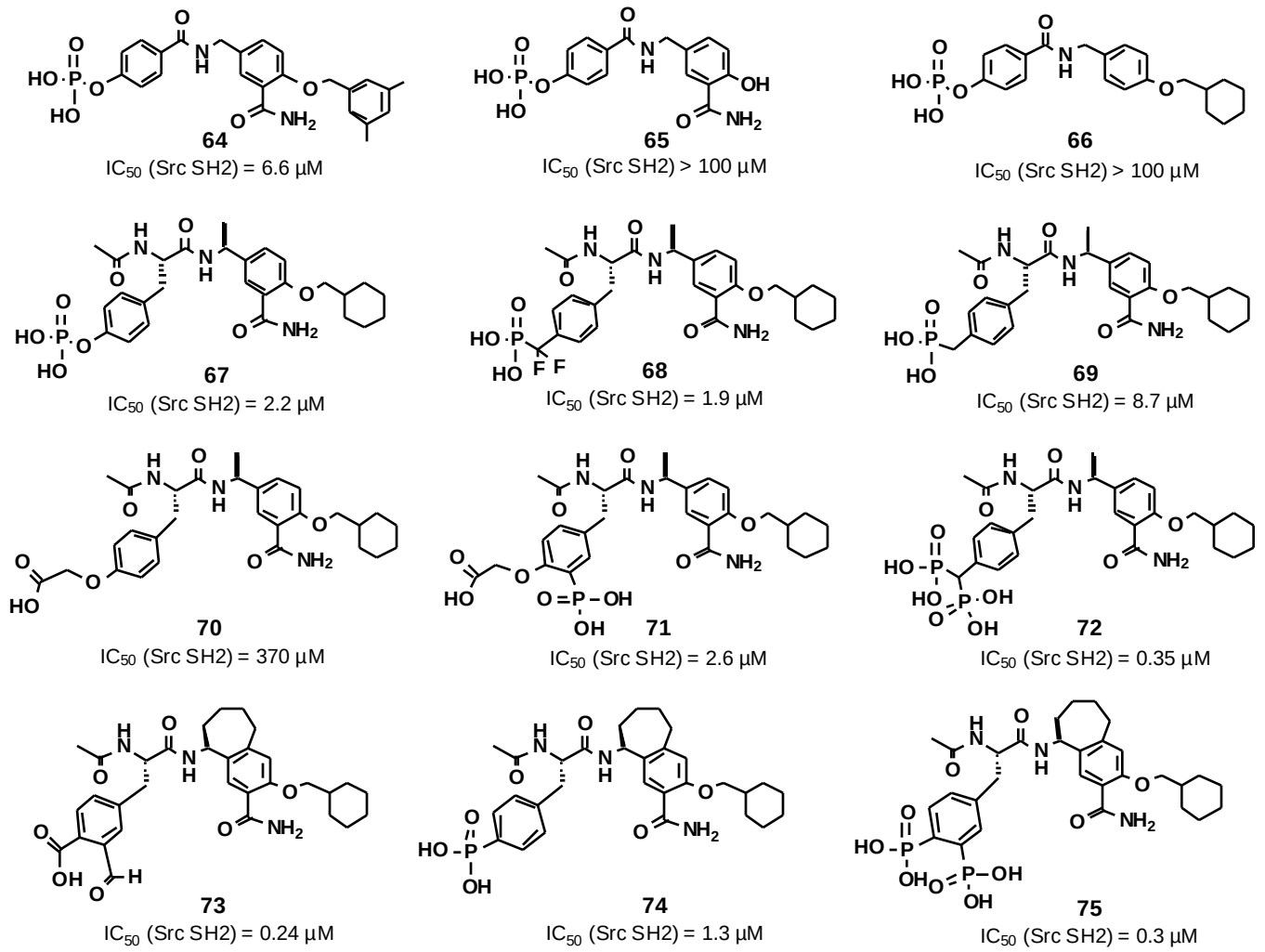

Fig. (10). Chemical structures of nonpeptide inhibitors of Src SH2 (see text for details).

that was designed to effect both increased hydrophobic binding interactions with Src SH2 and entropic advantage of locking the conformation of the benzamide by virtue of the fused cycloaliphatic ring system [24, 25]. The cellular and *in vivo* efficacy of the nonpeptide **75** correlates with the bone-targeting properties conferred by its pTyr mimic, Dpp, relative to biological testing in osteoporosis and related disease models [25, 46]. The design of Dpp as well as Dmp and Cpp as novel pTyr mimics were developed relative to the determination of an X-ray structure of Src SH2 complexed with the tricarboxylic acid, citrate, which was found to effect multiple H-bonding and ionic interactions of in the pY site. Comparative analysis of Dmp, Cpp, and Dpp versus their mono-functionalized pTyr parent analogs (*e.g.*, Pmp, Phe[4-OCH_2CO_2H], and Phe[4-PO_3H_2] also showed that introduction of the second phosphonate group significantly increased Src SH2 binding affinity (*cf.*, **69** vs **72**, **70** vs **71**, and **74** vs **75**). Therefore, such multi-functionalized pTyr mimics possessed both enhanced Src SH2 potency and bone-targeting properties [25, 26], of which the latter property has been determined by measurement of binding to hydroxyapatite and bone tissue [60].

Recently, a novel series of cyclic lactam-based, nonpeptide inhibitors of Src SH2 have been reported [61-63]. Specifically, compounds **76-81** as shown in Fig. (**11**) exemplify a caprolactam template in which the carbonyl moiety was designed, and confirmed by X-ray structure, to displace the same structural water as the aforementioned ureido-type peptidomimetic **41** and benzamide-based nonpeptide **64**. Functionalization of the caprolactam template by pTyr mimics and pY+3 hydrophobic groups provided highly potent Src SH2 inhibitors (*cf.*, compounds **78-81**). Particularly noteworthy was the tricarboxy-modified pTyr mimic incorporated in the nonpeptide **81** relative to exhibiting high affinity to Src SH2.

Zap70 SH2 Inhibitors

A novel series of heterocyclic (*e.g.*, oxadiazole) template-based nonpeptide inhibitors of Zap70 SH2 have been advanced [22, 64]. Specifically, compounds **82-87** as shown in Fig. (**12**) illustrate the structure-activity relationships of modifications at the pY, pY+2, and pY+3 sites of an oxadiazole template that led to a potent and specific nonpeptide inhibitor of Zap70 SH2 (compound **87**). Noteworthy was the determination that these nonpeptide

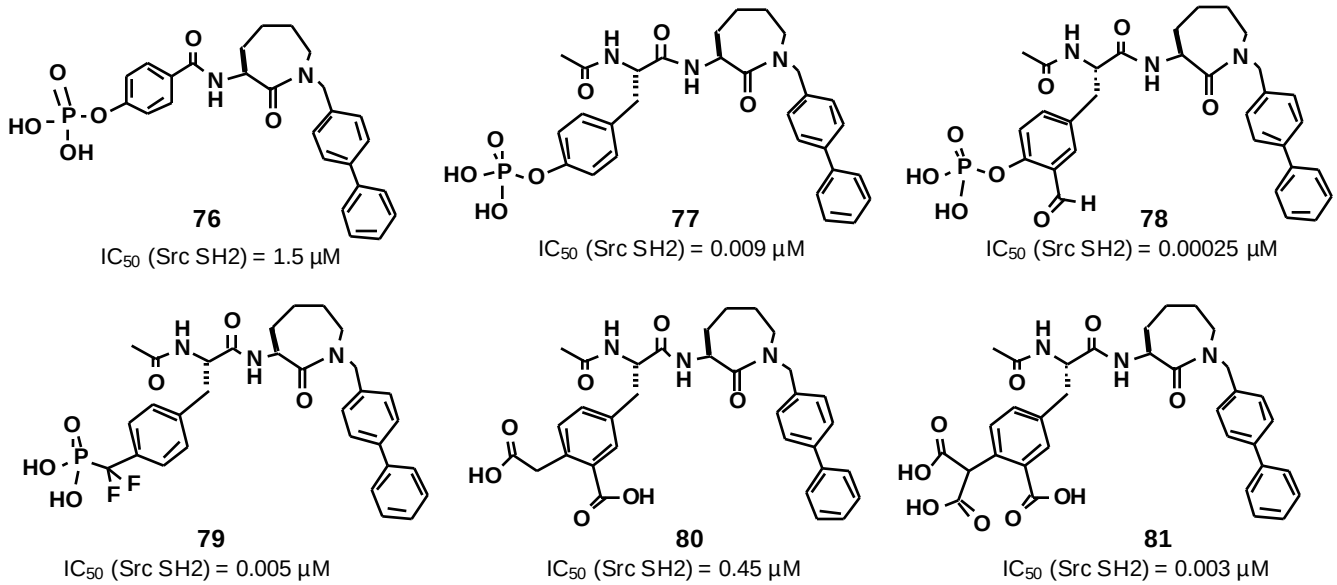

Fig. (11). Chemical structures of nonpeptide inhibitors of Src SH2 (see text for details).

inhibitors were effective to bind to both the Zap70 tandem N/C-SH2 domains of Zap70 as well as an isolated C-terminal SH2 protein construct by gel shift studies [64]. Another reported [65] series of novel nonpeptides have illustrated the use of a steroid-based template to develop potent monodentate and bidentate inhibitors **88** and **89** as shown in Fig. (**12**). These nonpeptides were also described to effect cellular activity.

Lck SH2 Inhibitors

Several recent approaches [66-68] have led to the structure-based design of nonpeptide inhibitors of Lck SH2 as exemplified by compounds **90-92** shown in Fig. (**13**). A chemical library approach [66] was used to discover the potent Lck SH2 inhibitor **90**. A pyridone-based template has been been developed [67] with the inclusion of pTyr mimics

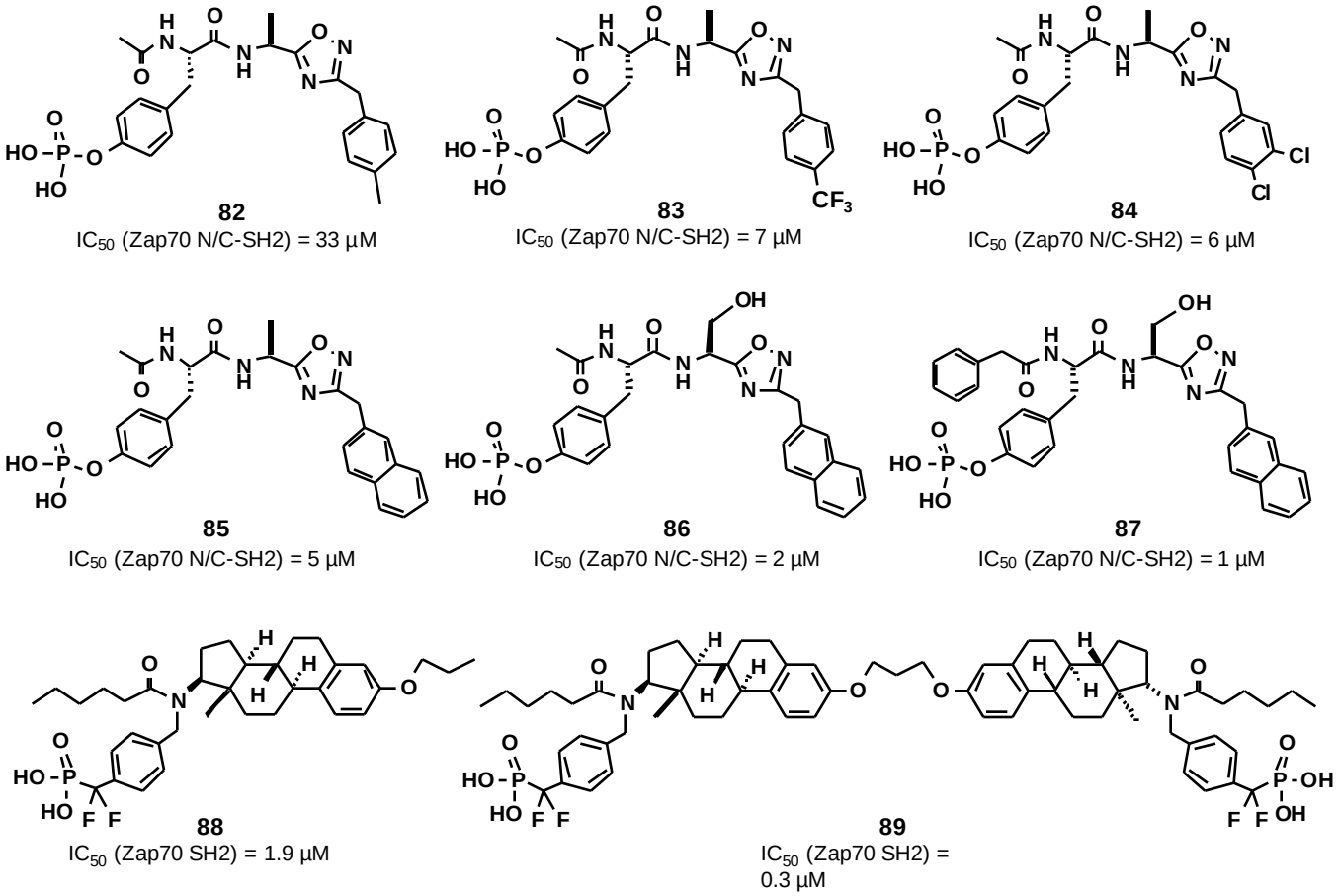

Fig. (12). Chemical structures of nonpeptide inhibitors of Zap70 SH2 (see text for details).

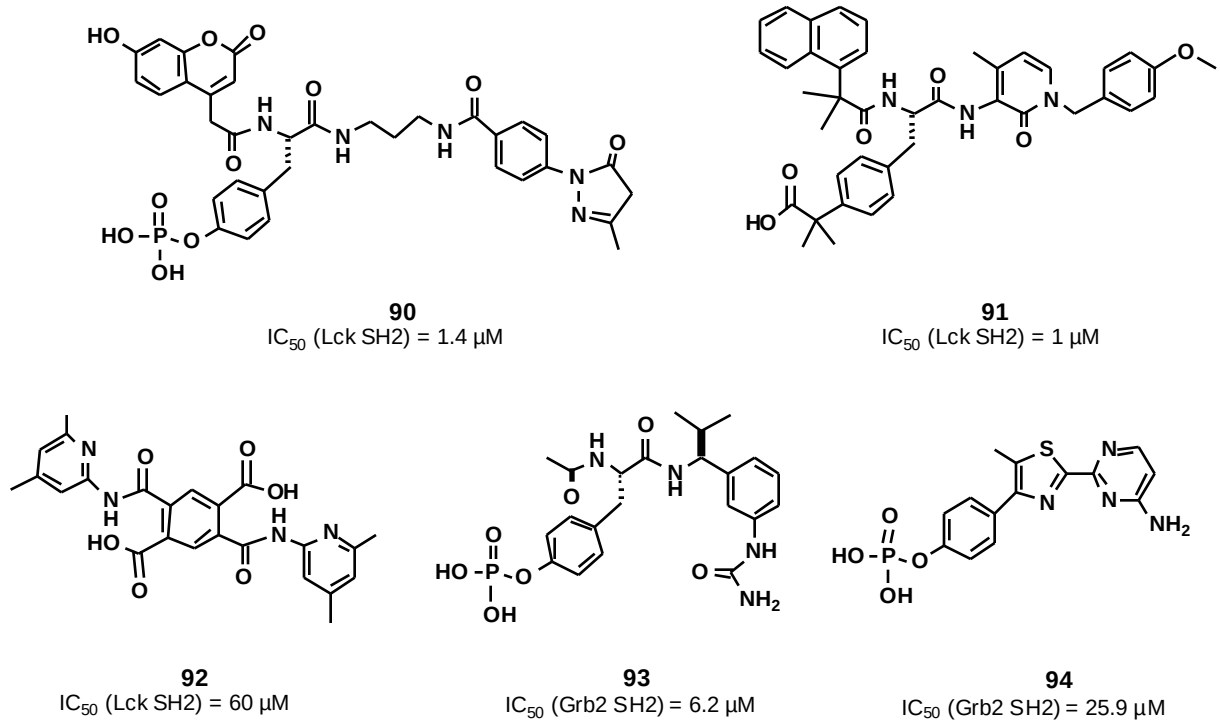

Fig. (13). Chemical structures of nonpeptide inhibitors of Lck SH2 and Grb2 SH2 (see text for details).

to provide the potent nonpeptide Lck SH2 inhibitor **91**. Finally, a NMR strategy [68] has shown utility in the structure-based identification of a bisphthalamic acid-based nonpeptide **92** as a weakly potent inhibitor of Lck SH2.

Grb2 SH2 Inhibitors

The structure-based design of nonpeptide inhibitors of Grb2 SH2 have recently been described [69, 70] as exemplified by compounds **93** and **94** shown in Fig. (**13**). Albeit these two compounds are only weakly potent, they exemplify very interesting lead compounds for possible functional group optimization.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

This mini-review describes several key achievements in drug discovery efforts focused on SH2 inhibitors with a particular emphasis on peptidomimetic and nonpeptide lead compounds. Without question, there has been tremendous progress made relative to the first determinations of the mode of binding and structure-activity properties of phosphopeptide interactions with various SH2 domains. In particular, the development of first- and second-generation SH2 inhibitors targeting Src, Grb2, Lck and Zap70 have shown creativity and determination in the stepwise evolution of peptidomimetic and/or nonpeptide lead compounds. Such compounds have also addressed replacement of the pTyr or, more specifically, the phosphate group, by multiple functional group modification to advance novel pTyr mimics. Similarly, the SH2 binding sites complementary for the pY+1, pY+2 and pY+3 amino acids of cognate phosphopeptides have been captured and, in many cases, transcended by use of nonpeptide templates which provide auxiliary hydrophobic interactions, structural water replacement and/or other H-bonding interactions relative to phosphopeptides. The first proof-of-concept studies for Grb2 and Src have been advanced to validate SH2-dependent signal transduction pathways in cells as well as *in vivo* studies in established disease models to hallmark such SH2 inhibitor drug discovery. Future studies are expected to further build upon the foundation of structure-based design and integration of chemical diversity to promote the development of potent, selective and metabolically-stable SH2 inhibitors relative to both drug discovery and our increased understanding of cellular mechanisms involving SH2-containing proteins.

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