
2 Inhibitors of Signaling Interfaces

Targeting Src Homology 2 Domains in Drug Discovery

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1. INTRODUCTION

The Src homology 2 (SH2) domain is a noncatalytic module of approx 100 amino acids that is mainly involved in directing interactions with cellular substrates (1,2). The SH2 domain recognizes short phosphotyrosine-containing sequences in which specificity is conveyed by the residues immediately C-terminal to the phosphotyrosine amino acid (3–5). The specific association of an SH2 domain with a phosphotyrosine-containing sequence of another protein precipitates a cascade of intracellular protein–protein interactions that result in signal propagation (1,6,7). Antagonists of critical SH2-binding events can be reasonably result in the inactivation of undesirable signal transduction networks and can represent a targeted treatment of a broad range of human diseases (e.g., cancer, osteoporosis, disorders of the immune and cardiovascular systems) (8).

Highly potent antagonists of specific SH2 domains have been identified largely by structure-based drug design efforts, but these compounds had several undesirable features (9–12). In addition to the intrinsic problems associated with the peptidic nature of some of these molecules (e.g., degradation, rapid elimination from plasma, high first-pass metabolism, and very low oral bioavailability),

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the phosphate group of phosphotyrosine, which is an essential element for binding to the SH2 domain, is metabolically unstable to phosphatases present in cells and further limits the ability of these compounds to reach efficacious concentrations inside the cell (13). Attempts to address these issues have appeared in the literature for a variety of SH2 domains and, recently, significant advances have been accomplished in the identification of antagonist of the SH2 domains of Src, Lck, and Grb2 that showed activity in cell-based assays and animal models.

2. ANTAGONISTS OF THE PP60^{C-SRC} SH2 DOMAIN

The nonreceptor tyrosine kinase pp60^{C-SRC} contains a myristoylated *N*-terminal domain, a catalytic kinase region, an SH2 domain, an SH3, and a short C-terminal regulatory peptide sequence, often called the tail (14–15). Overexpression or hyperactivation of this protein has been implicated in the development of human breast adenocarcinomas and colon carcinoma (16–17). Furthermore, pp60^{C-SRC}, which is normally expressed at high levels in osteoclasts, has also been implicated in regulating osteoclast-mediated resorption of bone (18–19). The kinase domain of Src can activate intracellular signaling networks by phosphorylating docking proteins that can then cause the relocation of SH2 domain-containing signaling proteins. The SH2 domain of Src has been postulated to play an important role in regulating these intracellular signaling events by acting as an adaptor that brings specific protein substrates into the signaling complex (e.g., truncation of the SH2 domain in Src affects its transforming abilities without affecting its kinase activity) (20). Compounds that modulate pp60^{C-SRC} regulated signal transduction pathways by blocking its SH2 domain offer potential value as antiproliferative agents or in the treatment of osteoporosis.

Extensive nuclear magnetic resonance (NMR) and X-ray crystallographic studies of the Src SH2 protein complexed with peptides containing the Tyr(PO₃H₂)-Glu-Glu-Ile motif (4–5) provided a detailed molecular map of the binding pockets of this therapeutic target (21–24). Examination of the three-dimensional structures revealed the presence of two major binding pockets, one interacting with phosphotyrosine and the other with the side chain of isoleucine at the X₊₃ position. The two glutamic acid residues do not make a strong interaction with the protein and mainly serve to orient the phosphotyrosine and isoleucine side chains at their respective binding pockets (21,25). This structural information has been instrumental in the design of potent and selective Src SH2 antagonist and representative compounds have been selected to illustrate the approaches used by different groups.

Using a dipeptide framework (26), compound **1**¹ (Fig. 1) was designed with the intent of replacing the three C-terminal residues in Ac-Tyr(PO₃H₂)-

¹To normalize data from different experiments, results for individual antagonists were presented in this paper as a ratio, IC₅₀(test) = IC₅₀(standard).

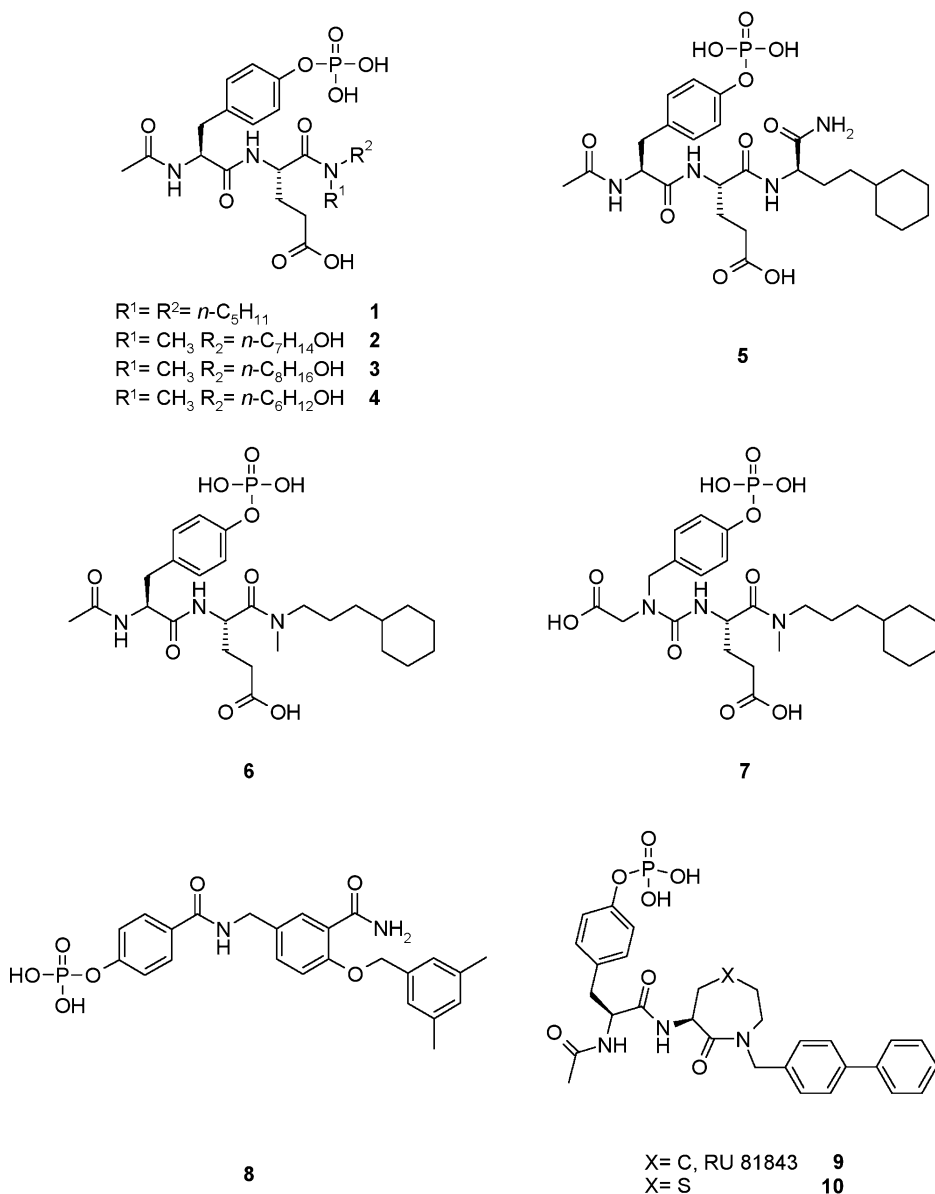


Fig. 1. Antagonists of the Src SH2 domain.

Glu-Glu-Ile-Glu-OH with a fragment that would access the hydrophobic binding pocket at the X_{+3} position without compromising interactions within the phosphotyrosine binding pocket (26,27). Following this approach, the C-terminal heptanol (compound **2**, $\text{IC}_{50}(\mathbf{2})/\text{IC}_{50}(\text{standard}) = 3.6$; Fig. 1) and octanol

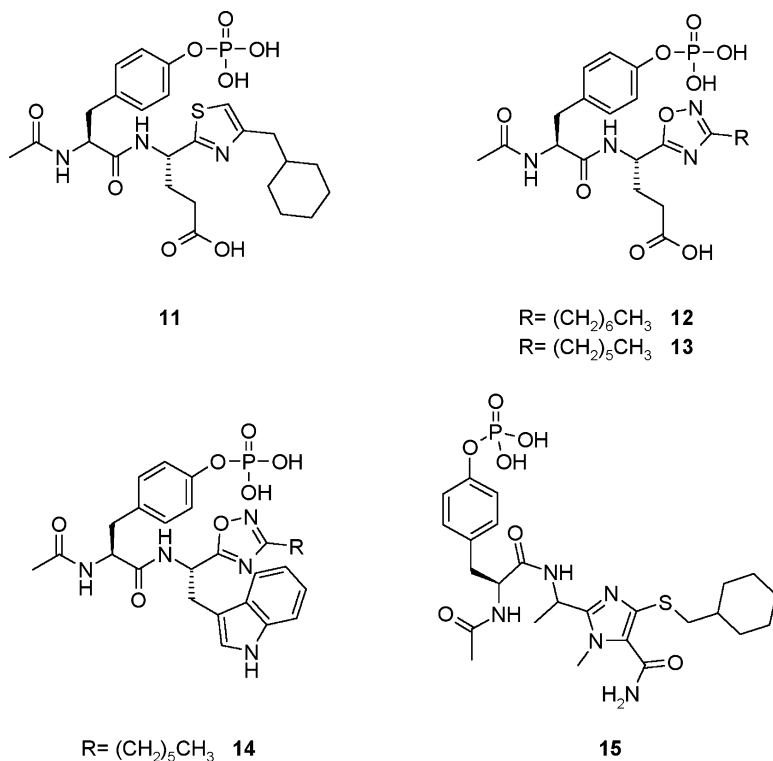


Fig. 2. Antagonists of the Src SH2 domain based on the use of heterocyclic scaffolds.

(compound **3**, $IC_{50}(\mathbf{3})/IC_{50}[\text{standard}] = 2.2$; Fig. 2) analog were synthesized to form a hydrogen-bonding interaction with amino acid residues lining the X_{+3} pocket. This interaction was confirmed by solving the X-ray structure of the Src SH2 domain co-crystallized with compound **4** ($IC_{50}(\mathbf{4})/IC_{50}[\text{standard}] = 6.4$; Fig. 1). Efforts to enhance binding affinity by increasing intramolecular association and filling or shielding the phosphotyrosine binding site with a set of different N-terminal groups were unsuccessful.

A further step in the dipeptidation of a previously reported antagonist (compound **5**, Fig. 1; $IC_{50} = 8.5 \mu\text{M}$) (**28**) was obtained by removing the C-terminal carboxamide. This moiety was previously thought to be involved in an intramolecular hydrogen bond with the oxygen of the backbone carbonyl group of phosphotyrosine. Compounds with binding affinities in the low micromolar range were obtained by changing the rotational freedom and hydrophobicity of the C-terminus (e.g., compound **6**, Fig. 1; $IC_{50} = 0.79 \mu\text{M}$). Modeling studies with these new ligands provided the basis for the design of nonpeptide Src antagonists containing urea linkage (compound **7**, Fig. 1; $IC_{50} = 7.0 \mu\text{M}$) (**29**)

The X-ray crystal structure of compound **7** complexed with the Src SH2 domain revealed a conformation and binding interactions quite different from the initial predictions: (1) the orientation of the phenyl phosphate ring is nearly orthogonal to that exhibited by phosphotyrosine-containing compounds in other X-ray structures; (2) a *cis*-amide bond between glutamic acid and the C-terminal groups; and (3) a lack of interaction between the side chain of Arg¹² and both the N-terminal carboxyl moiety and the phosphate group of the ligand. These factors can account for the low binding affinity observed for these urea-containing compounds, but, more important, they illustrate some of the difficulties associated with the prediction of ligand binding by computational methods. This issue was also encountered in another *de novo* series of nonpeptide antagonists (30). In this case, the X-ray structure of compound **8** (Fig. 1; IC₅₀ = 6.6 μM) with the Src SH2 domain revealed that the phenylphosphate group is capable of a binding mode at the phosphotyrosine site substantially different from that observed for the phosphotyrosine side chain in peptides bound to the Src SH2 domain.

In a modular approach to identify nonpeptidic Src SH2 antagonists, different scaffolds have been utilized to mimic the Glu–Glu dipeptide. Structure–activity relationship (SAR) studies with caprolactam/thioazepinone derivatives led to the identification of potent Src SH2 inhibitors (compounds **9** and **10**, IC₅₀ = 9 nM and IC₅₀ = 87 nM, respectively; Fig. 1) (31,32). The X-ray structures of some of these compounds complexed with the Src SH2 domain revealed that these templates deliver their substituents into the phosphotyrosine and X₊₃ pockets.

Optimization of 2,4- and 2,5-substituted thiazole, and 1,2,4-oxadiazole derivatives resulted in Src SH2 inhibitors (e.g., compounds **11**, IC₅₀ = 26 μM; **12**, IC₅₀ = 7 μM; **13**, IC₅₀ = 8 μM; and **14**, IC₅₀ = 16 μM; Fig. 2) that showed binding affinities similar to the reference tetrapeptide (33,34). In a very similar approach, tetrasubstituted imidazoles (e.g., compound **15**, IC₅₀ = 8.6 μM; Fig. 2) were used to mimic the interactions made by the corresponding amino acid side chains of the peptide antagonist, but these compounds showed weaker activity than the reference peptide (35).

Recently, phosphate, phosphonate, or phosphonic acid derivatives, which were prepared by parallel synthesis, were screened (BIAcore technology) to identify potential phosphotyrosine mimetics that bind to the Src SH2 domain. Naphthyl, tetrahydroquinoline, and amido phosphonate derivatives with binding affinities in the mM range were discovered (36). Parallel to this strategy, small fragments with low binding affinity for Src SH2 were screened using crystal soaking (37). Structure determination of more than 20 of these compounds bound into the phosphotyrosine pocket of Src SH2 domain allowed a selection of the best fragments to incorporate into antagonist platforms. Malonate-type

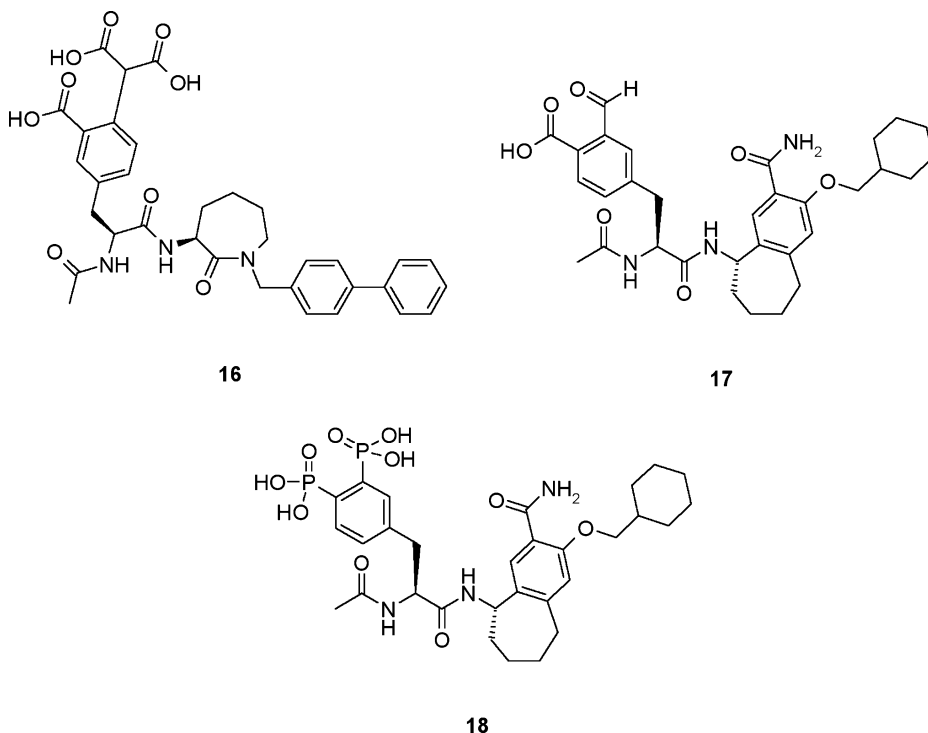


Fig. 3. Antagonists of the Src SH2 domain containing phosphotyrosine mimetics.

inhibitors (compound **16**, Fig. 3), which combine a previously published antagonist scaffold (31,32) and a phosphotyrosine mimetic identified by this X-ray technique, showed potent *in vitro* activity ($IC_{50} = 5$ nM) and good stability in rat and human plasma.

In addition to the preceding screening approaches, extensive synthetic efforts have been devoted to the identification and preparation of phosphotyrosine mimics with improved stability and cellular permeability (38–44). Replacement of phosphotyrosine by some of these nonhydrolyzable analogs has advanced the discovery of compounds for cellular and *in vivo* studies (40–42). Some of these antagonist are briefly described in the following.

Compound **17** (AP22161, Fig. 3) binds *in vitro* with high affinity to the Src SH2 domain ($IC_{50} = 0.24$ μM) and inhibits Src cellular activity (41). The formyl group was introduced to form a hemithioacetal with the side chain of cysteine-188 when the compound is bound to the protein (45–47). NMR experiments and *in vitro* studies with wild-type Src and mutant cysteine-188 SH2 domains demonstrated that compounds containing the 3-formyl-4-carboxy-substituted phenylalanine building block target cysteine-188 and that this binding is

reversible (41). Cysteine-188 is unique for the Src SH2 domain providing selectivity against other members of the Src family of tyrosine kinases (e.g., IC_{50} = 29.4 μM and 421.9 μM for the SH2 domains of Yes and ZAP 70, respectively). Compound **17** showed partial morphological reversion of the transformed phenotype of cSrcY527F cells at 100 μM and inhibited osteoclast-mediated resorption of dentine with an IC_{50} value of 42.9 μM . A better in vivo inhibitory profile was obtained with compounds containing 3',4'-diphosphonophenylalanine. This phosphotyrosine mimetic exhibits bone-targeting properties that confer osteoclast selectivity, hence reducing undesired effects in other cell types. Compound **18** (AP22408, Fig. 3) inhibited Src SH2 binding with an IC_{50} of 0.30 μM (42,48) and blocks rabbit osteoclast-mediated resorption of dentine slices (IC_{50} = 1.6 μM). Furthermore, this compound showed a statistically significant (p = 0.0379) antiresorptive activity in an in vivo thyroparathyroidectomized model of parathyroid hormone-induced bone resorption when administered intravenously (50 mg/kg, twice daily). Although these type of derivatives allowed to validate Src as a therapeutic target for the treatment of osteoporosis in preclinical models, they did not progress into clinical trials.

3. ANTAGONISTS OF THE LCK SH2 DOMAIN

The Src family tyrosine kinase p56^{lck} (Lck: lymphoid T-cell tyrosine kinase) is predominantly expressed in T-lymphocytes and natural killers (49–51). p56^{lck} is absolutely required in the early phase of T-cell antigen receptor (TCR) activation and plays a critical role in T-cell-mediated immune responses (52). Like all Src homologs, it comprises a unique amino-terminal region, followed by Src-homology domains SH3 and SH2, and a tyrosine kinase domain (53). Lck is localized to the plasma membrane through myristoylation and palmitoylation, and is associated through its unique amino-terminal segment with the cytoplasmic tails of the T-cell co-receptor glycoprotein CD4 or CD8 (54,55). Upon kinase activation (56), p56^{lck} phosphorylates specific tyrosine residues of the ξ -chain of TCR within a motif termed immunoglobulin receptor family tyrosine-based activation motives (ITAMs). The phosphorylated residues recruit a second cytoplasmic protein tyrosine kinase called ZAP-70 to promote T-cell activation (57,58). The SH2 domain of Lck regulates the kinase activity of the protein (59) and may mediate protein–protein interactions with ZAP-70 and/or the ξ subunit of the T-cell receptor (60). Several lines of evidence have established a critical role for p56^{lck} in antigen-induced T-cell responses (61–63). Consequently, there has been much effort in developing kinase inhibitors and SH2 antagonists for p56^{lck} with a view for therapeutic use in a number of diseases such as multiple sclerosis, asthma, inflammatory bowel disease, rheumatoid arthritis, and T-cell-based leukemias and lymphomas (64,65).

The SH2 domain of Lck exhibits a marked preference for the sequence Tyr(PO₃H₂)-Glu-Glu-Ile (5) and this motif served as the starting point for the design of antagonist for this protein. This effort was guided by the early report of an X-ray structure of Lck SH2 in complex with a phosphopeptide (EPQpYEEIPYL) (66). Analysis of this structure revealed the presence of a large hydrophobic pocket for the residue at the X₊₃ position (isoleucine) and the lack of grooves or pockets for the X₊₁ and X₊₂ (glutamic acids) residues. The interactions made by the side chains of phosphotyrosine and isoleucine are complemented by a network of hydrogen bonds to the peptide backbone. As for the design of Src SH2 antagonists, the identification of an uncharged replacement for the doubly charged Glu-Glu sequence has been an additional challenge for this target. Even though relatively limited work has been reported to date on antagonist of Lck SH2 domain, significant progress has been made over the past few years.

SAR studies and computational chemistry were initially utilized to reduce the overall charge of Ac-Tyr(PO₃H₂)-Glu-Glu-Ile-OH ($K_d = 0.1 \mu M$) while maintaining Lck SH2 inhibitory activity (67). The most potent compound was obtained by replacing the C-terminal dipeptide Glu-Ile-OH with (*S*)-1-(4-isopropylphenyl)ethylamine and the glutamic acid at the X₊₁ position with leucine (compound **19**, $K_d = 0.2 \mu M$; Fig. 4). This last modification was guided by early SAR studies that indicated that a wide range of amino acids with uncharged side chains were well tolerated at X₊₁ (68). In an attempt to identify a replacement of the phosphotyrosine residue and using the skeleton of compound **20** ($K_d = 0.18 \mu M$; Fig. 4), a series of compounds that contain monocharged, non-hydrolyzable phosphate groups were synthesized (e.g., difluoroacetic acid, (*R/S*)-hydroxyacetic, sulfonic acid, oxamic acid) (69). The drop in activity observed for these derivatives (75- to 920-fold decrease in potency relative to compound **20**) was partially compensated by incorporating lipophilic substituents, particularly naphthylacetyl groups, at the N-terminus (e.g., compound **21**, $K_d = 5.0 \mu M$; Fig. 4). Recently, this type of derivatives was elaborated to an Lck SH2 antagonist (compound **22**, $K_d = 1 \mu M$; Fig. 4) that shows relatively good cell permeability (70). Compound **22** is the result of a design effort to rigidify the backbone of the molecule (introduction of the pyridone ring), enforce a favorable conformation for binding (incorporation of a methyl group at the 4-position of the pyridine ring), and increase cell permeability by enhancing desolvation (dimethyl substitution adjacent to the carboxylate group). The preceding properties allowed the effect of Lck SH2 inhibition in a cellular setting, to be demonstrated for the first time. Calcium mobilization is a very early event in T-cell activation and an antagonist of Lck SH2 should inhibit receptor-mediated increase in cytosolic calcium. Inhibition of intracellular calcium concentration was observed ($ED_{50} = 10 \mu M$) when compound **22** was tested in Jurkat T-cells activated with mouse antihuman CD3 antibody. Additional

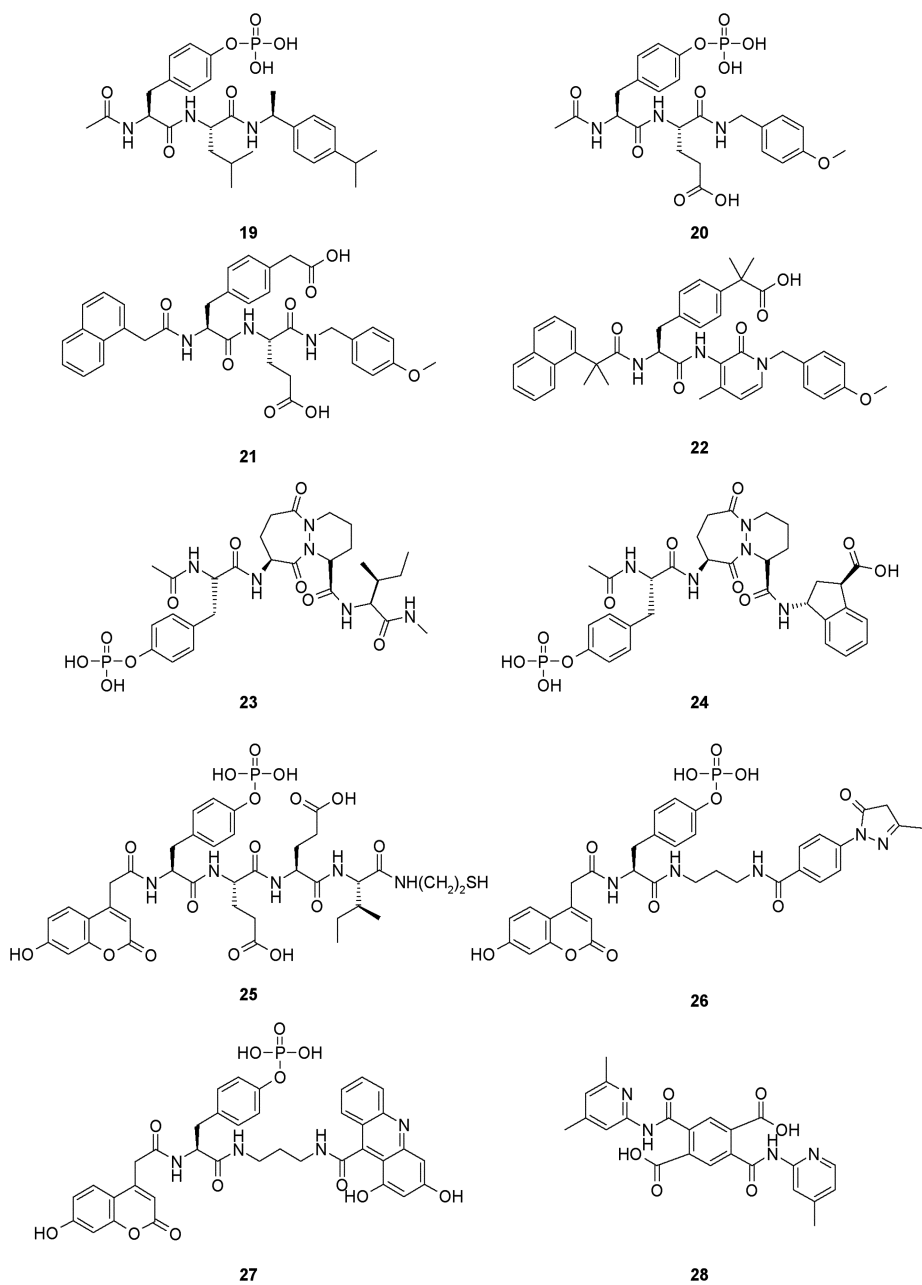


Fig. 4. Antagonists of the Lck SH2 domain.

experiments show that this effect was enantiomer and TCR-signaling dependent, confirming that this antagonist function via the intended mechanism.

Recently, a new class of structure-based Lck SH2 antagonists incorporating 9-aminopyridazinodiazepine as a Glu–Glu mimetic has met with success (71). After confirming that the diazepine moiety was a suitable Glu–Glu replacement, the X-ray structure of compound **23** ($IC_{50} = 1.6 \mu M$; Fig. 4) bound to Lck SH2 was utilized to improve the potency of this type of derivatives by optimizing the interactions with the X_{+3} pocket. The best result was obtained when the C-terminal isoleucine methylamide was replaced with (1R,3R)-3-amino-indan-1-carboxylic acid (compound **24**, $IC_{50} = 0.03 \mu M$). This moiety was introduced to favor a hydrogen bond between the C-terminal carboxylic acid and the side chain of an arginine (Arg-67) on the rim of the X_{+3} pocket.

Synthetic combinatorial libraries have also been exploited to identify Lck SH2 antagonists (72). A focused library was prepared by coupling 900 carboxylic acids to the N-terminus of Tyr(PO_3H_2)-Glu-Glu-Ile (73). Despite previous observations on the lack of SH2 selectivity or affinity for modifications introduced at the N-terminus of phosphotyrosine in most of the SH2 consensus sequences (74), highly potent and selective phosphopeptides were identified by screening this library. For example, compound **25** (Fig. 4) exhibits a nearly five fold preference for the Lck SH2 domain vs that of Fyn ($K_d^{Lck} = 35 \text{ nM}$ vs $K_d^{Fyn} = 150 \text{ nM}$) and good general SH2 selectivity (e.g., $K_d^{PLC\gamma 1} = 4.9 \mu M$, $K_d^{p85} = 9.3 \mu M$ and $K_d^{Grb2} = 11.3 \mu M$). In a continuation of this strategy (72), two separate libraries were constructed to identify a Glu–Glu surrogate (84-member library) and a replacement of isoleucine (900-member library). This approach ultimately afforded compounds **26** ($IC_{50} = 1.4 \mu M$, $K_d = 2.9 \mu M$; Fig. 4) and **27** ($IC_{50} = 2.4 \mu M$; Fig. 4), which exhibit binding affinities for p56^{lck} SH2 that are comparable to the activity observed for Ac-Tyr(PO_3H_2)-Glu-Glu-Ile-NH₂ ($IC_{50} = 0.66 \mu M$; $K_d = 1.3 \mu M$).

As an alternative to conventional screening assays, an NMR-based screening approach called SAR by NMRTM (75,76) was used to identify novel phosphotyrosine mimetics that bind to Lck SH2 (77). This NMR method allows building blocks that bind to a target protein with low affinity (mM to μM range) to be reliably identified. A number of phosphonates, multiply charged aromatic acids, and phthalamate analogs with binding affinities in the millimolar were identified (e.g., compound **28**, $IC_{50} = 0.06 \text{ mM}$; Fig. 4), but no Lck SH2 antagonists containing these molecules have so far been disclosed.

4. ANTAGONISTS OF THE SH2 DOMAIN OF Grb2

Growth factor receptor-bound protein 2 (Grb2) is an adapter protein composed of two SH3 domains flanking a single SH2 domain (78,79). This protein plays a key role in the Ras signal-transduction pathway by linking a variety of tyrosine kinase receptors (e.g., EGFR, erbB-2, c-MET), receptor-associated

proteins (e.g. Syp, IRS-1), and oncogenic proteins (e.g. BCR-Abl) to the mitogen-activated protein (MAP) kinase cascade (80,81). In unstimulated cells, Grb2 is located in the cytosol in complex with the guanine nucleotide exchange factor for Ras, son of sevenless (Sos), through SH3-mediated protein–protein interactions. Upon activation of receptor tyrosine kinases by growth factors, the Grb2–Sos complex translocates to the plasma membrane, and converts the inactive Ras·GDP to active Ras·GTP (82–86). Activated Ras triggers the MAP kinase cascade that is essential for cell growth and differentiation. The interaction between Grb2 and the activated kinases or the phosphorylated receptor-associated proteins is mediated by the SH2 domain of the signaling protein that recognizes specific phosphotyrosine sequences. Agents that specifically disrupt the protein–protein interactions mediated by Grb2 SH2 could potentially shut down the Ras signaling pathway and present an intervention point for the treatment of hyperproliferative diseases (8,87–89).

Phosphotyrosyl peptide libraries have shown that the consensus sequence for Grb2 SH2 is Tyr(PO₃H₂)-X₊₁-Asn-X₊₃ and the residue that determines specificity is asparagine (4,5,90–92). Numerous X-ray and NMR structures of the Grb2 SH2 domain and complexed thereof with phosphotyrosyl peptides containing the preceding consensus sequence or peptidomimetic inhibitors have been determined (for representative examples, *see* refs. 93 and 94). The folding of the ligand-bound SH2 domain of Grb2 shows a general pattern consisting of a central antiparallel β -sheet flanked by two α -helices, and the phosphopeptide ligand binds in a type I β -turn conformation centered around the X₊₁ and X₊₂ residues. The folded conformation of the ligand, the exclusive selectivity of Grb2 SH2 for asparagine at the X₊₂ position, and specific protein–ligand interactions identified in structural studies have been extensively exploited in the design and identification of potent and selective Grb2 SH2 antagonists (12). Representative examples have been selected to illustrate this structure-based design approach.

Starting from the tripeptide Ac-Tyr-(PO₃H₂)-Ile-Asn-NH₂, which is the minimal recognition motif retaining μ M affinity for the Grb2 SH2 domain (IC₅₀ = 8.64 μ M), the N-terminal acetyl group was replaced with 3-aminobenzyloxy-carbonyl to form a stacking interaction with the side chain of an arginine residue (Arg α A2) and a hydrogen bond interaction with the phosphate group of phosphotyrosine (95). This single replacement resulted in a 133-fold increase in binding activity (IC₅₀ = 0.065 μ M), and was the first breakthrough in the identification of potent antagonists of the Grb2-SH2 domain. A further improvement in activity was obtained when isoleucine was replaced by 1-aminocyclohexanecarboxylic acid (compound **29**, IC₅₀ = 1 nM; Fig. 5) (96). This α,α -disubstituted cyclic α -amino acids was selected by molecular modeling to induce a local right-handed 3₁₀ helical conformation and to establish multiple van der Waals interactions with the amino acids forming the Grb2 SH2 X₊₁

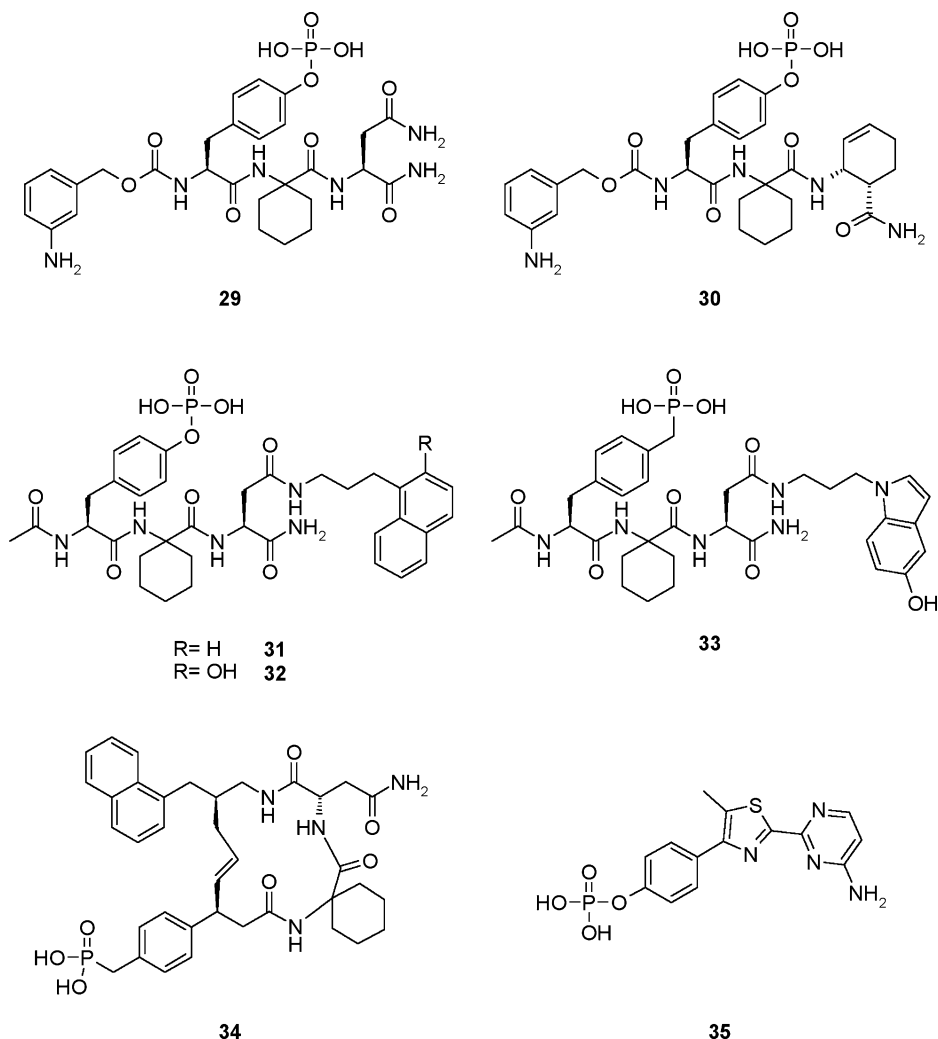


Fig. 5. Antagonists of the Grb2 SH2 domain.

binding pocket. This molecular prediction was confirmed later by X-ray crystallography (97). Incorporation of Ac₆c had a positive impact not only in the Grb2 SH2 binding affinity of the modified antagonist, but also in its specificity profile against other SH2 domains. Thus, compound **29** shows 240- and 1500-fold preferential binding to Grb2 SH2 over p85 N-terminal SH2 and Lck SH2, respectively, and at least 1200-fold selectivity to Grb2 SH2 over Shp2 SH2 in competitive binding phosphopeptide assays (96).

The core sequence of compound **29** has been extensively utilized as a “molecular platform” to identify asparagine mimetics, increase the number of

interactions with the protein, or introduce conformational constraints. An overview of some of these modifications is covered in the following.

Experiments with degenerate phosphopeptide libraries (4,5,91,92) and structural studies (94) have identified and confirmed the exclusive selectivity of Grb2 SH2 for asparagine at the X₊₂ position. Asparagine occupies the *i* + 2 position of a type-I β -turn and its side chain forms hydrogen bond interactions with the backbone carbonyl groups of Lys β D6 and Leu β E4, and the NH of Lys β D6. Interactive molecular modeling showed that (1*S*,2*R*)-cyclic β -amino acids with different ring sizes were able to preserve the orientation of the carboxamide group of asparagine without clashing with the side chain of Trp EF1 (97,98). Replacement of asparagine by (1*S*,2*R*)-2-amino-cyclohex-3-ene carboxylic acid (Achec) resulted in compound **30** (Fig. 5) which has a binding affinity almost identical to that observed for compound **29** (IC₅₀ = 1.6 nM vs IC₅₀ = 1 nM). The X-ray crystal structure of Grb2 SH2 bound to compound **30** revealed the expected (1*S*,2*R*) configuration for Achec and showed that this β -amino acid perfectly mimics the intermolecular hydrogen bond interactions of the side chain of asparagine (97).

Several reports have described the design of inhibitors containing C-terminal substituted carboxamides (99,100). These modifications were introduced in the parent compound to create additional van der Waals contacts with an extended hydrophobic region on the surface of Grb2 SH2 (100). Noteworthy in this series is the high potency achieved with the naphthyl (e.g., compounds **31** and **32**, IC₅₀ = 47 nM and 11 nM, respectively; Fig. 5) and 3-indol-1-yl-propyl (e.g., compound **33**, IC₅₀ = 0.3 nM; Fig. 5) derivatives. Macrocyclic variants of these derivatives have also been reported (e.g., compound **34**, IC₅₀ = 0.02 μ M) (101,102). The conformational constraints imposed by the cyclization were expected to increase binding affinity by favoring a β -bend conformation and reducing entropy penalties. Recently, *trans*- and *cis*-cyclopropanes have also been used to enforce locally extended and reversed turn peptide conformations in Grb2 SH2 antagonists (103).

Structure-based design efforts have also advanced nonpeptidic Grb2 SH2 antagonists. Following a minimal pharmacophore strategy, a rigid thiazole spacer was used to link two moieties that mimic the main pharmacophores of the natural ligand, the phenylphosphate of the phosphotyrosine residue, and the β -carboxamide of asparagine. The Grb2-SH2 binding affinity of the mimetic (compound **35**, IC₅₀ = 26 μ M; Fig. 5) was found to be in the same range as that measured for two reference phosphopeptides (Ac-Tyr(PO₃H₂)-Xxx-Asn-NH₂, IC₅₀ = 67 μ M, Xxx = Gly; IC₅₀ = 4 μ M, Xxx = Val) (104).

Until very recently, the investigation of intracellular signal transduction pathways triggered by the interaction of Grb2 SH2 with phosphoproteins was compromised by the paucity of reported antagonists of Grb2 SH2 that are effective in cell-based assays. Phosphotyrosine mimetics (12,101,105–108), prodrug

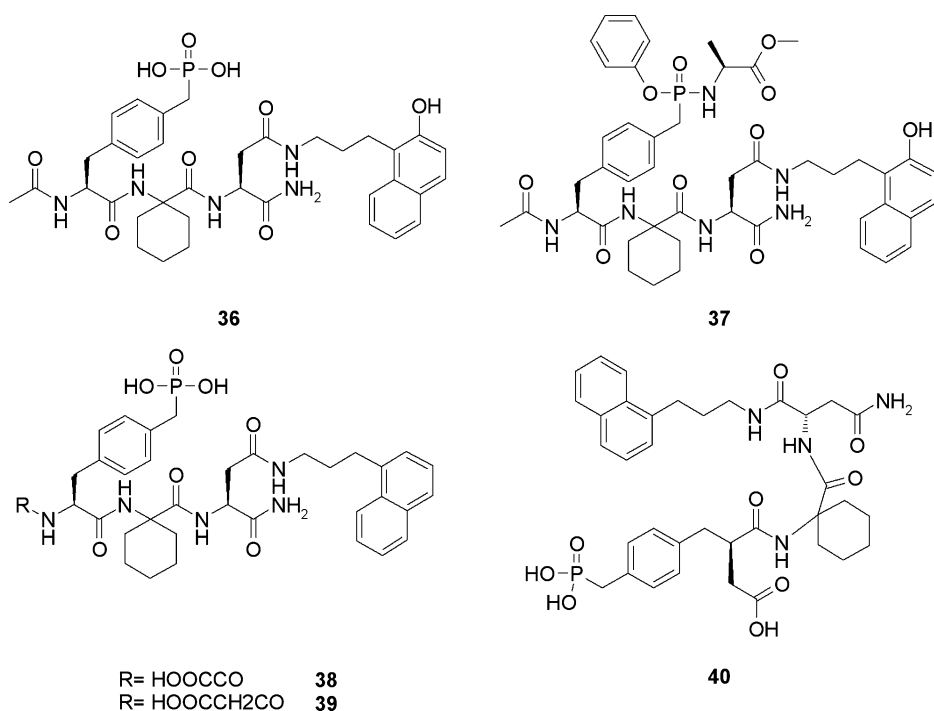


Fig. 6. Representative examples of Grb2 SH2 antagonists active in cell-based assays.

systems (109), and cell-permeable vectors (110) have been used to improve the cellular permeability and intracellular stability of Grb2 SH2 antagonist (109,111–113). These approaches have allowed the identification of tool compounds to validate the Grb2 target in cellular settings. Thus, compound **36** (Fig. 6) blocks EGFR- and Shc-Grb2 protein–protein interactions in human mammary carcinoma MDA-MB-468 cell and inhibits the anchorage-independent growth of these cells (112). Grb2 SH2 inhibition by compound **37** (Fig. 6), a prodrug derivative of compound **36** with improved cell permeability, induced expression of the cell cycle inhibitors p21^{Waf1/Cip1/CAPI} and p27^{Kip1}, reverse transformation (112), and inhibit hepatocyte growth factor– induced motility (113). All together these findings provide experimental evidence that targeting Grb2 SH2 is sufficient to block normal Ras function and mitogenesis, and to reverse transformation in a cellular setting. Inhibition of Grb2 SH2 binding in MDA-MB-453 cell-based systems has also been reported for analog of compound **36** containing several phosphotyrosine mimetics and N-terminal capping groups (113,116). These derivatives achieved intracellular inhibition of Grb2 SH2 binding at micromolar to submicromolar concentrations (e.g., compound **38**, IC₅₀ = 0.5 μ M; Fig. 6). Of particular note in this study is the usefulness of the

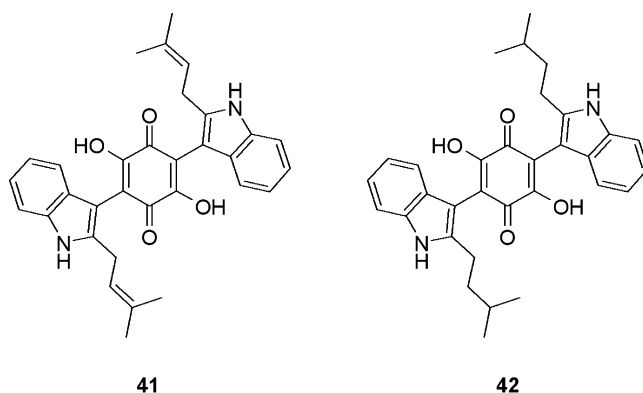


Fig. 7. Dihydroxyquinoline derivatives that inhibit Grb2 SH2-b mediated protein–protein interactions.

N-terminal oxalyl group to enhance the binding potency of both phosphorous and non-phosphorous-containing phosphotyrosine mimetics. Molecular modeling dynamics simulations of compound **38** in the phosphotyrosine-binding pocket of the Grb2 SH2 domain identified new interactions between the positively charged arginine-67 residue and elements of the N $^{\alpha}$ -oxalyl group. These interactions can account for the positive binding effect observed for the oxalyl-containing phosphotyrosine mimetics. The potential binding enhancements afforded by the oxalyl group have been further explored using other acidic N $^{\alpha}$ -terminal substituents (116). N $^{\alpha}$ -oxalyl can be replaced by N $^{\alpha}$ -malonyl without affecting the ex-cellular Grb2 binding inhibition activity of the antagonist. Consistent with this result, compound **39** was equipotent to compound **38** in inhibiting MDA-MB-453 cell growth. Recently, antimetogenic activity ($IC_{50} = 8 \mu M$) has also been reported for a Grb2 SH2 antagonist containing a new phosphotyrosyl mimetic (105). Compound **40** (Fig. 6) exhibits potent in vitro Grb2 SH2 inhibitory activity ($IC_{50} = 8 nM$) and is able to inhibit intracellular association of Grb2 protein with phosphorylated p185^{erbB-2} at concentrations equivalent to its antimetogenic activity. Similar results have also been obtained with derivatives of compound **34** (101).

In addition to the previous synthetic compounds, several natural products and derivatives thereof have also been described to interfere with the protein–protein interactions mediated by Grb2. A series of naturally occurring bis(indolyl)dihydroxyquinolines have been shown to inhibit the binding of Grb2 to the tyrosine-phosphorylated form of the EGFR tyrosine kinase (e.g., compound **41**, Asterriquinone E, $IC_{50} = 2.9 \mu M$; Fig. 7) (117). Promising inhibitory properties have also been observed with a synthetic analog of the above natural product (e.g., compound **42**, $IC_{50} = 1.2 \mu M$; Fig. 7) (118). Other

natural products (e.g., actinomycin D, C₂ and VII, lutein, 8-*O*-methylsclerotioramine, sclerotiorin, and isochromophilone iv) have also been reported to inhibit the Shc/Grb2 protein–protein interaction in a dose-dependent manner (119–124). Although the published results suggest that the preceding natural products could be nonphosphorylated Grb2 antagonists, so far no evidence has been given to confirm that these compounds interact with the pockets that exist on the phosphotyrosine-binding surface of Grb2 SH2.

5. CONCLUSIONS

Although some of the SH2 antagonists reviewed and discussed herein have been valuable tool compounds for improving our understanding of several intracellular signaling pathways, the identification of potential therapeutic agents for the treatment of diseases associated with Src, Lck, or Grb2 molecular interactions has met with limited success. Structure-based design approaches have been used to reduce the size, charge, and peptide character of SH2 antagonists while increasing their potency, selectivity, and ability to penetrate and reach the intracellular target. These medicinal chemistry efforts have led to true mechanism-based agents that have validated the concept of SH2 domain inhibition in cellular settings and animal models, but, unfortunately, none of these antagonists has so far entered clinical trials.

REFERENCES

1. Koch CA, Anderson D, Moran MF, Ellis C, Pawson T. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* 1991; 252:668–674.
2. Sadowski I, Stone JC, Pawson T. A noncatalytic domain conserved among cytoplasmic protein tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus p130^{gag-fps}. *Mol Cell Biol* 1986; 6:4396.
3. Ladbury JE, Arold S. Searching for specificity in SH domains. *Chem Biol* 2000; 7:R3–R8.
4. Songyang Z, Shoelson SE, McGlade J, et al. Specific motifs recognized by the SH2 domain of Csk, 3BP2, fps/fes, Grb-2, HCP, SHC, Syk, and Vav. *Mol Cell Biol* 1994; 14:2777–2785.
5. Songyang Z, Shoelson SE, Chaudhuri M, et al. SH2 domains recognize specific phosphopeptide sequences. *Cell* 1993; 72:767–778.
6. Pawson T, Gish GD, Nash P. SH2 domains, interaction modules and cellular wiring. *Trends Cell Biol* 2001; 11:504–511.
7. Pawson T. Protein modules and signalling networks. *Nature* 1995; 373:573–580.
8. Botfield MC, Green J. SH2 and SH3 domains: choreographers of multiple signaling pathways. *Annu Rev Med Chem* 1995; 30:227–237.
9. García-Echeverría C. Antagonist of Src homology 2 (SH2) domains of Grb2, Src, Lck and ZAP-70. *Curr Med Chem* 2001; 8:1715–1730.
10. Shakespeare WC. SH2 domain inhibition: a problem solved? *Curr Opin Chem Biol* 2001; 5:409–415.
11. Vu CB. Recent advances in the design and synthesis of SH2 inhibitors of Src, Grb2, and ZAP-70. *Curr Med Chem* 2000; 7:1081–1100.

12. Fretz H, Furet P, García-Echeverría C, Rahuel J, Schoepfer J. Structure based design of compounds inhibiting Grb2 SH2 mediated protein–protein interactions. *Curr Pharm Des* 2000; 6:1777–1796.
13. Gilmer T, Rodriguez M, Jordan S, et al. Peptide inhibitors of src SH3-SH2-phosphoprotein interactions. *J Biol Chem* 1994; 269:31711–31719.
14. Resh MD. Myristylation and palmitylation of Src family members: the fats of the matter. *Cell* 1994; 76:411–413.
15. Superti-Furga G. Regulation of the Src protein tyrosine kinase. *FEBS Lett* 1995; 369:62–66.
16. Boerner RJ, Kassel DB, Barker SC, Ellis B, DeLacy P, Knight WB. Correlation of the phosphorylation states of pp60c-src with tyrosine kinase activity: the intramolecular pY530-SH2 complex retains significant activity if Y419 is phosphorylated. *Biochemistry* 1996; 35:9519–9525.
17. Luttrell DK, Lee A, Lansing TJ, et al. Involvement of pp60^{src} with the major signaling pathways in human breast cancer. *Proc Nat Acad Sci USA* 1994; 91:83–87.
18. Boyce BF, Yoneda T, Lowe C, Soriano P, Mundy GR. Requirement of pp60^{csrc} expression for osteoclasts to form ruffled borders and resorb bone in mice. *J Clin Invest* 1992; 90:1622–1627.
19. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src protooncogene leads to osteopetrosis in mice. *Cell* 1991; 64:693–702.
20. Courtneidge SA, Fumagalli S, Koegl M, Superti-Furga G, Twamley-Stein GM. The Src family of protein tyrosine kinases: regulation and functions. *Dev Suppl* 1993; 57–64.
21. Davidson JP, Lubman O, Rose T, Waksman G, Martin SF. Calorimetric and structural studies of 1,2,3-trisubstituted cyclopropanes as conformational constrained peptide inhibitors of Src SH2 domain binding. *J Am Chem Soc* 2002; 124:205–215.
22. Xu W, Harrison SC, Eck MJ. Three dimensional structure of the tyrosine kinase c-Src. *Nature* 1997; 385:595–602.
23. Waksman G, Shoelson SE, Pant N, Cowburn D, Kuriyan J. Binding of a high affinity phosphotyrosyl peptide to the Src SH2 domain: Crystal structures of the complexed and peptide-free forms. *Cell* 1993; 72: 779–790.
24. Waksman G, Kominos D, Robertson SC, et al. Crystal structure of the phosphotyrosine recognition domain SH2 of v-src complexed with tyrosine-phosphorylated peptides. *Nature* 1992; 358:646–653.
25. Verkhivker GM, Bouzida D, Gehlhaar DK, et al. Hierarchy of simulation models in predicting structures and energetics of the Src SH2 domains binding to tyrosyl phosphopeptides. *J Med Chem* 2002; 45:72–89.
26. Charifson PS, Shewchuk LM, Rocque W, et al. Peptide ligands of pp60c-src SH2 domains: a thermodynamic and structural study. *Biochemistry* 1997; 36:6283–6293.
27. Pacofsky GJ, Lackey K, Alligood KJ, et al. Potent dipeptide inhibitors of the pp60c-src SH2 domain. *J Med Chem* 1998; 41:1894–1908.
28. Plummer MS, Prasad JV, Para KS, et al. Hydrophobic D-amino acids in the design of peptide ligands for the pp60^{src} SH2 domain. *Drug Des Discov* 1996; 13:75–81.
29. Plummer MS, Holland DR, Shahripour A, et al. Design, synthesis, and cocrystal structure of a nonpeptide Src SH2 domain ligand. *J Med Chem* 1997; 40:3719–3725.
30. Lunney EA, Para KS, Rubin JR, et al. Structure based design of novel series of nonpeptide ligands that bind to the pp60^{src} SH2 domain. *J Am Chem Soc* 1997; 119:12471–12476.
31. Deprez P, Baholet I, Burlet S, et al. Discovery of highly potent Src SH₂ binders: structure-activity studies and X-ray structures. *Bioorg Med Chem Lett* 2002; 12:1291–1294.
32. Lesuisse D, Deprez P, Albert E, et al. Discovery of thioazepinone ligands for the Src SH₂: from non-specific to specific binding. *Bioorg Med Chem Lett* 2001; 11:2127–2131.

33. Buchanan JL, Vu ChB, Merry TJ, et al. Structure activity relationships of a novel class of Src SH2 inhibitors. *Bioorg Med Chem Lett* 1999; 9:2359–2364.
34. Buchanan JL, Bohacek RS, Luke GP, et al. Structure based design and synthesis of a novel class of Src SH2 inhibitors. *Bioorg Med Chem Lett* 1999; 9:2353–2358.
35. Deprez P, Mandine E, Vermond A, Lesuisse D. Imidazole-based ligands of the Src SH₂ protein. *Bioorg Med Chem Lett* 2002; 12:1287–1289.
36. Deprez P, Mandine E, Gofflo D, Meunier S, Lesuisse D. Small ligands interacting with the phosphotyrosine binding pocket of the Src SH₂ protein. *Bioorg Med Chem Lett* 2002; 12:1295–1298.
37. Lesuisse D, Lange G, Deprez P, et al. SAR and X-ray. A new approach combining fragment-based screening and rational drug design: application to the discovery of nanomolar inhibitors of Src SH2. *J Med Chem* 2002; 45:2379–2387.
38. Kawahata N, Yang MG, Luke GP, et al. A novel phosphotyrosine mimetic 4'-carboxymethoxy-3'-phosphonophenylalanine (Cpp): exploitation in the design of nonpeptide inhibitors of pp60^{Src} SH2 domain. *Bioorg Med Chem Lett* 2001; 11:2319–2323.
39. Sundaramoorthi R, Siedem C, Vu CB, et al. Selective inhibition of Src SH2 by a novel thiol-targeting tricarbonyl-modified inhibitor and mechanistic analysis by ¹H/¹³C NMR spectroscopy. *Bioorg Med Chem Lett* 2001; 11:1665–1669.
40. Bohacek RS, Dalgarno DC, Hatada M, et al. X-ray structure of citrate bound to Src SH2 leads to a high-affinity, bone-targeted Src SH2 inhibitor. *J Med Chem* 2001; 44:660–663.
41. Violette SM, Shakespeare WC, Barlett C, et al. A Src SH2 selective binding compound inhibits osteoclast mediated resorption. *Chem Biol* 2000; 7:225–235.
42. Shakespeare W, Yang M, Bohacek R, et al. Structure-based design of an osteoclast selective, nonpeptide Src homology 2 inhibitor with in vivo antiresorptive activity. *Proc Nat Acad Sci USA* 2000; 97:9373–9378.
43. Rickles RJ, Henry PA, Guan W, et al. A novel mechanism-based mammalian cell assay for the identification of SH2-domain-specific protein–protein inhibitors. *Chem Biol* 1998; 5:529–538.
44. Stankovic CJ, Surendran N, Lunney EA, et al. The role of 4-phosphonodifluoromethyl- and 4-phosphono-phenylalanine in the selectivity and cellular uptake of SH2 domain ligands. *Bioorg Med Chem Lett* 1997; 7:1909–1914.
45. Shakespeare WC, Bohacek RS, Narula SS, et al. An efficient synthesis of a 4'-phosphonodifluoromethyl-3'-formylphenylalanine containing Src SH2 ligand. *Bioorg Med Chem Lett* 1999; 9:3109–3112.
46. Allgood KJ, Charifson PS, Crosby R, et al. The formation of a covalent complex between a dipeptide ligand and the Src SH2 domain. *Bioorg Med Chem Lett* 1998; 8:1189–1194.
47. Machida K, Mayer BJ. The SH2 domain: versatile Signaling module and pharmaceutical target. *Biochem Biophys Act.* 2003; 1747:4–25.
48. Violette SM, Guan W, Barlett C, et al. Bone-targeted Src SH2 inhibitors block Src cellular activity and osteoclast-mediated resorption. *Bone* 2001; 28:54–64.
49. Chan AC, Desia DM, Weiss A. The role of protein-tyrosine kinases and protein-tyrosine phosphatases in T-cell antigen receptor signal-transduction. *Ann Rev Immunol* 1994; 12:555–592.
50. Weiss A, Littman D. Signal transduction by lymphocyte antigen receptors. *Cell* 1994; 76:263–266.
51. Cooper JA, Howell B. The when and how of Src regulation. *Cell* 1993; 73:1051–1054.
52. Perlmutter RM, Marth JD, Lewis DB, Peet R, Ziegler SF, Wilson CB. Structure and expression of lck transcripts in human lymphoid cells. *J Cell Biochem* 1988; 38:117–126.
53. Eck MJ, Atwell SK, Shoelson SE, Harrison SC. Structure of the regulatory domains of the Src-family tyrosine kinase Lck. *Nature* 1994; 368:764–769.

54. Turner JM, Brodsky MH, Irving BA, Levin SD, Perlmutter RM, Littman DR. Interaction of the unique N-terminal region of the tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. *Cell* 1990; 60:755–765.
55. Shaw AS, Amrein KE, Hammond C, Stern DF, Sefton BM, Rose JK. The lck tyrosine protein kinase interacts with the cytoplasmic tail of CD4 glycoprotein through its unique amino terminal domain. *Cell* 1989; 59:627–636.
56. Veillette A, Fournel M. The CD4 associated tyrosine protein kinase p56lck is positively regulated through its site of autophosphorylation. *Oncogene* 1990; 5:1455–1462.
57. Denny MF, Patai B, Straus DB. Differential T-cell antigen receptor signaling mediated by the Src family kinases Lck and Fyn. *Mol Cell Biol* 2000; 20:1426–1435.
58. Iwashima M, Irving BA, van Oers NSC, Chan AC, Weiss A. Sequential interactions of the TCR with two distinct cytoplasmic tyrosine. *Science* 1994; 263:1136–1139.
59. Couture C, Songyang Z, Jascur T, et al. Regulation of the Lck SH2 domain by tyrosine phosphorylation. *J Biol Chem* 1996; 271:24880–24884.
60. Strauss DB, Chan AC, Patai B, Weiss A. SH2 domain function is essential for the role of the Lck tyrosine kinase in T cell receptor signal transduction. *J Biol Chem* 1996; 271:9976–9981.
61. Sundvold V, Torgersen KM, Post NH, et al. Cutting edge: T cell-specific adapter protein inhibits T cell activation by modulating lck activity. *J Immunol* 2000; 165:2927–2931.
62. van Oers NSC, Lowinkropf B, Finlay D, Connolly K, Weiss A. $\alpha\beta$ T cell development is abolished in mice lacking both Lck and Fyn protein tyrosine kinases. *Immunity* 1996; 5:429–436.
63. Straus DB, Weiss A. Genetic evidence for the involvement of the Lck tyrosine kinase in signal transduction through the T-cell antigen receptor. *Cell* 1992; 70:585–593.
64. Dowden J, Ward SG. Inhibitors of p56^{lck}: assessing their potential as tools as manipulating T-lymphocyte activation. *Expert Opin Ther Patents* 2001; 11:295–306.
65. Broadbridge RJ, Sharma RP. The Src homology-2 domains (SH2 domains) of the protein tyrosine kinase p56^{lck}: structure, mechanism and drug design. *Curr Drug Targets* 2000; 1:365–386.
66. Eck MJ, Shoelson SE, Harrison SC. Recognition of a high-affinity phosphotyrosyl peptide by the Src homology-2 domain of p56lck. *Nature* 1993; 362:87–91.
67. Llinas-Brunet M, Beaulieu PL, Cameron DR, et al. Phosphotyrosine-containing dipeptides as high-affinity ligands for the p56^{lck} SH2 domain. *J Med Chem* 1999; 42:722–729.
68. Morelock MM, Ingraham RH, Betageri R, Jakes S. Determination of receptor-ligand kinetics and equilibrium binding constants using surface plasmon resonance: application to the Lck SH2 domain. *J Med Chem* 1995; 38:1309–1318.
69. Beaulieu PL, Cameron DR, Ferland JM, et al. Ligands for the tyrosine kinase p56lck SH2 domain: discovery of potent dipeptide derivatives with monocharged, nonhydrolyzable phosphate replacements. *J Med Chem* 1999; 42:1757–1766.
70. Proudfoot JR, Betageri R, Cardozo M, et al. Nonpeptidic, monocharged, cell permeable ligands for the p56lck SH2 domain. *J Med Chem* 2001; 44:2421–2431.
71. Hobbs CJ, Bit RA, Cansfield AD, et al. Structure-based design of peptidomimetic antagonists of p56^{lck} SH2 domain. *Bioorg Med Chem Lett* 2002; 12:1365–1369.
72. Lee TR, Lawrence DS. Acquisition of highaffinity, SH2 targeted ligands via a spatially focused library. *J Med Chem* 1999; 42:784–787.
73. Lee TR, Lawrence DS. SH2-directed ligands for the Lck tyrosine kinase. *J Med Chem* 2000; 43:1173–1179.
74. Kuriyan J, Cowburn D. Modular peptide recognition domains in eukaryotic signaling. *Annu Rev Biophys Biomol Struct* 1997; 26:259–288.

75. Shuker SB, Hajduk PJ, Meadows RP, Fesik SW. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* 1996; 274:1531–1534.
76. Hajduk PJ, Meadows RP, Fesik SW. Drug design: discovering high-affinity ligands for proteins. *Science* 1997; 278:497–499.
77. Hajduk PJ, Zhou MM, Fesik SW. NMR-based discovery of phosphotyrosine mimetics that bind to the Lck SH2 domain. *Bioorg Med Chem Lett* 1999; 9:2403–2406.
78. Lowenstein EJ, Daly RJ, Batzer AG, et al. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 1992; 70:431–442.
79. Chardin P, Cussac D, Maignan S, Ducruix A. The Grb2 adaptor. *FEBS Lett* 1995; 369: 47–51.
80. Downward J. Control of ras activation. *Cancer Surv* 1996; 27:87–100.
81. Downward J. The GRB2/Sem-5 adaptor protein. *FEBS Lett* 1994; 338:113–117.
82. Buday L, Egan SE, Rodriguez V, Cantrell DA, Downward J. A complex of Grb2 adaptor protein, Sos exchange factor, and a 36-kDa membrane-bound tyrosine phosphoprotein is implicated in Ras activation in T cells. *J Biol Chem* 1994; 269:9019–9023.
83. Reif K, Buday L, Downward J, Cantrell DA. SH3 domains of the adapter molecule Grb2 complex with two proteins in T cells: the guanine nucleotide exchange protein Sos and a 75-kDa protein that is a substrate for T cell antigen receptor-activated tyrosine kinases. *J Biol Chem* 1994; 269:14081–14087.
84. Li N, Batzer A, Daly R, et al. Guanine-nucleotide-releasing factor hSos1 binds to Grb2 and links receptor tyrosine kinases to Ras signalling. *Nature* 1993; 363:85–88.
85. Gale NW, Kaplan S, Lowenstein EJ, Schlessinger J, Bar-Sagi D. Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras. *Nature* 1993; 363:88–92.
86. Buday L, Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* 1993; 73:611–620.
87. Verbeek BS, Adriaansen-Slot SS, Rijksen G, Vroom TM. Grb2 overexpression in nuclei and cytoplasm of human breast cells: a histochemical and biochemical study of normal and neoplastic mammary tissue specimens. *J Pathol* 1997; 183:195–203.
88. Lowe PN, Skinner R. Regulation of Ras signal transduction in normal and transformed cells. *Cell Signalling* 1994; 6:109–123.
89. Dati C, Muraca R, Tazartes O, et al. CerbB2 and Ras expression levels in breast cancer are correlated and show a cooperative association with unfavorable clinical outcome. *Int J Cancer* 1991; 65:45–50.
90. Kessels HWHG, Ward AC, Schumacher TNM. Specificity and affinity motifs for Grb2 SH2-ligand interactions. *Proc Natl Acad Sci USA* 2002; 99:8524–8529.
91. Gram H, Schmitz R, Zuber JF, Baumann G. Identification of phosphopeptide ligands for the Src-homology 2 (SH2) domain of Grb2 by phage display. *Eur J Biochem* 1997; 246: 633–637.
92. Mueller K, Gombert FO, Manning U, et al. Rapid identification of phosphopeptide ligands for SH2 domains. Screening of peptide libraries by fluorescence-activated bead sorting. *J Biol Chem* 1996; 271:16500–16505.
93. Ogura K, Tsuchiya S, Terasawa H, et al. Solution structure of the SH2 domain of Grb2 complexed with the Shc-derived phosphotyrosine-containing peptide. *J Mol Biol* 1999; 289: 439–445.
94. Rahuel J, Gay B, Erdmann D, et al. Structural basis for specificity of Grb2SH2 revealed by a novel ligand binding mode. *Nat Struct Biol* 1996; 3:586–589.
95. Furet P, Gay B, Garcia-Echeverria C, et al. Discovery of 3-Aminobenzoyloxycarbonyl as an N-Terminal group conferring high affinity to the minimal phosphopeptide sequence recognized by the Grb2-SH2 domain. *J Med Chem* 1997; 40:3551–3556.
96. Garcia-Echeverria C, Furet P, Gay B, et al. Potent antagonists of the SH2 domain of Grb2: optimization of the X+1 position of 3-Amino-Z-Tyr(PO₃H₂)-X_{n+1}-Asn-NH₂. *J Med Chem* 1998; 41:1741–1744.

97. Furet P, Garcia-Echeverria C, Gay B, Schoepfer J, Zeller M, Rahuel J. Structure-based design, synthesis, and X-ray crystallography of a high-affinity antagonist of the Grb2-SH2 domain containing an asparagine mimetic. *J Med Chem* 1999; 42:2358–2363.
98. Furet P, Gay B, Schoepfer J, et al. Potent Grb2 SH2 antagonists containing asparagine mimetics. *Proc Am Pept Symp* 2000; 17:573–575.
99. Schoepfer J, Fretz H, Gay B, et al. Highly potent inhibitors of the Grb2-SH2 domain. *Bioorg Med Chem Lett* 1999; 9:221–226.
100. Furet P, Gay B, Caravatti G, et al. Structure-based design and synthesis of high affinity tripeptide ligands of the Grb2-SH2 domain. *J Med Chem* 1998; 41:3442–3449.
101. Wei C-Q, Gao Y, Lee K, et al. Macrocyclization in the design of Grb2 SH2 domain-binding ligands exhibiting high potency in whole-cell systems. *J Med Chem* 2003; 46:244–254.
102. Gao Y, Voigt J, Wu JX, Yang D, Burke TR, Jr. Macrocyclization in the design of a conformationally constrained Grb2 SH2 domain inhibitor. *Bioorg Med Chem Lett* 2001; 11:1889–1892.
103. Plake HR, Sundberg TB, Woodward AR, Martin SF. Design and synthesis of conformationally constrained, extended and reverse turn pseudopeptides as Grb2-SH2 domain antagonists. *Tetrahedron Lett* 2003; 44:1571–1574.
104. Caravatti G, Rahuel J, Gay B, Furet P. Structure-based design of a non-peptidic antagonist of the SH2 domain of Grb2. *Bioorg Med Chem Lett* 1999; 9:1973–1978.
105. Wei C-Q, Li B, Guo R, Yang D, Burke TR, Jr. Development of a phosphatase-stable phosphotyrosyl mimetic suitably protected for the synthesis of high-affinity Grb2 SH2 domain-binding ligands. *Bioorg Med Chem Lett* 2002; 12:2781–2784.
106. Cody WL, Lin Z, Panek RL, Rose DW, Rubin JR. Progress in the development of inhibitors of SH2 domains. *Curr Pharm Des* 2000; 6:59–98.
107. Garcia-Echeverria C. Preparation of glyco-, phospho-, and sulfopeptides. In: Kates SA, Albericio F, eds. *Solid-Phase Synthesis: A Practical Guide*. New York Basel: Marcel Dekker, 2000:419–473.
108. Burke TR, Jr., Gao Y, Yao Z-J, Voigt J, Luo J, Yang D. Potent non phosphate containing Grb2 SH2 domain inhibitors. *Pept Sci* 2000;49–52.
109. Liu Q-W, Vidal M, Mathé C, Périgaud C, Garbay C. Inhibition of Ras-dependent mitogenic pathway by phosphopeptide prodrugs with antiproliferative properties. *Bioorg Med Chem Lett* 2000; 10:669–672.
110. Dunican DJ, Doherty P. Designing cell-permeant phosphopeptides to modulate intracellular signaling pathways. *Biopolymers* 2001; 60:45–60.
111. Gay B, Suarez S, Caravatti G, Furet P, Meyer Thomas, Schoepfer J. Selective Grb2 SH2 inhibitors as anti-ras therapy. *Int J Cancer* 1999; 83:235–241.
112. Gay B, Suarez S, Weber C, et al. Effect of potent and selective inhibitors of the Grb2 SH2 domain on cell motility. *J Biol Chem* 1999; 274: 23311–23315.
113. Yao ZJ, King CR, Cao T, et al. Potent inhibition of Grb2 SH2 domain binding by non-phosphate-containing ligands. *J Med Chem* 1999; 42:25–35.
114. Burke TR, Jr., Luo J, Yao ZJ, et al. Monocarboxylic-based phosphotyrosyl mimetics in the design of Grb2 SH2 domain inhibitors. *Bioorg Med Chem Lett* 1999; 9:347–352.
115. Ye B, Akamatsu M, Shoelson SE, et al. L-O-(2-Malonyl)tyrosine: a new phosphotyrosyl mimetic for the preparation of Src homology 2 domain inhibitory peptides. *J Med Chem* 1995; 38:4270–4270.
116. Burke TR, Jr., Yao Z-J, Gao Y, et al. N-terminal carboxyl and tetrazole-containing amides as adjuvants to Grb2 SH2 domain ligand binding. *Bioorg Med Chem Lett* 2001; 9:1439–1445.
117. Alvi KA, Pu H, Luche M, et al. Asterriquinones produced by *Aspergillus candidus* inhibit binding of the Grb-2 adapter to phosphorylated EGF receptor tyrosine kinase. *J Antibiot* 1999; 52:215–223.

118. Harris GD, Jr., Nguyen A, App H, Hirth P, McMahon G, Tang C. A One-pot, two-step synthesis of tetrahydro Asterriquinone E. *Org Lett* 1999; 1:431–433.
119. Nam J-Y, Son K-H, Kim H-K, et al. Sclerotiorin and isochromophilone IV: inhibitors of the Grb2-Shc interaction, isolated from *Penicillium multicolor* F1753. *J Microbiol Biotechnol* 2000; 10:544–546.
120. Kim H-K, Nam J-Y, Han MY, et al. Natural and synthetic analogues of Actinomycin D as Grb2-SH2 domain blockers. *Bioorg Med Chem Lett* 2000; 10:1455–1457.
121. Nam J-Y, Kim H-K, Son K-H, et al. 8-O-Methylsclerotiorinamine, antagonists of the Grb2-SH2 domain, isolated from *Penicillium multicolor*. *J Nat Prod* 2000; 63:1303–1305.
122. Lee E-S, Ahn B-T, Lee S-B, Kim H-K, Bok S-H, Jeong T-S. Isolation of Grb2-Shc domain binding inhibition component from *Agastache rugosa*. *Saengyak Hakoechi* 2000; 30:404–408.
123. Kim HK, Nam JY, Han MY, et al. Actinomycin D as a novel SH2 domain ligand inhibits Shc/Grb2 interaction in B104-1-1 (neu-transformed NIH3T3) and SAA (hEGFR-overexpressed NIH3T3) cells. *FEBS Lett* 1999; 453:174–178.
124. Nam JY, Kim HK, Son KH, et al. Actinomycin D, C2 and VII, inhibitors of GRB2-SHC interaction produced by *Streptomyces*. *Bioorg Med Chem Lett* 1998; 8:2001–2002.



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