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Brief Article

Development of Non-Peptide Ligands of Growth Factor Receptor-Bound Protein 2-Src Homology 2 Domain Using Molecular Modeling and NMR Spectroscopy†

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We report a novel series of non-peptide ligands that inhibit the growth factor receptor-bound protein 2 (Grb2)-Src homology 2 (SH2) domain binding, designed using a combined computational and NMR-driven approach. We have identified a new lead compound, **1n** (IC_{50} = 56 μM), which is cytotoxic in HER2-positive breast cancer cells and disrupts the interaction between HER2 and Grb2. Thus, **1n** can be used as a scaffold for the development of efficient Grb2-SH2 domain binding inhibitors.

Introduction

The human epidermal growth factor receptor 2 (HER2[a] or ErbB-2) is an important therapeutic target in breast cancer, which is overexpressed in around 25% of the invasive breast cancers and associated with poor disease-free survival and resistance to chemotherapeutic drugs.[1−3] Blockage of HER2 activation by inhibiting its interaction with the Src homology 2 (SH2) domain of the growth factor receptor-bound protein 2 (Grb2) has emerged as a new drug discovery strategy to identify novel therapeutic agents that selectively block the HER2 signaling pathway.[4] Oligopeptides have been reported as strong inhibitors of the HER2-Grb2 interaction by blocking the Grb2-SH2 domain.[5] However, features such as limited stability or bioavailability have compromised their use in vivo and subsequently the definitive validation of Grb2-SH2 domain binding inhibition as a useful therapeutic approach. In this regard and in terms of finding non-phosphopeptide compounds as Grb2-SH2 ligands, very little progress has been reported.[6,7] Therefore, the development of new non-peptide small molecules capable of disrupting binding between HER2 and Grb2 might bolster research in this field and overcome the limitations of the currently available inhibitors.

In this paper we describe a structurally novel series of small molecules that bind to the Grb2-SH2 domain. These compounds have been designed using molecular modeling techniques and evaluated by an ELISA-type assay. NMR chemical shift perturbation studies with the ligand **1k** enabled the identification of its binding site in the SH2 domain of Grb2 and to confirm that this family of molecules reproduces the key interactions predicted by the computational models. These results allowed the design of lead compound **1n** (IC_{50} = 56 μM), which represents a new scaffold to develop more efficient Grb2-SH2 domain binding inhibitors as tools for validation of anticancer therapies based on the blockage of the Grb2-mediated downstream pathways.

Results and Discussion

In the search for Grb2-SH2 ligands with a structurally novel core, we first identified the residues involved in key interactions in the crystal structure of the Grb2-SH2 domain in complex with the high-affinity 2-Abz-EpYINQ-NH_2 pentapeptide.[8] This structure reveals the pivotal roles of two protein sites in the interaction with the inhibitor. The first site is a hydrophilic pocket highly conserved among SH2 domains, which is responsible for pTyr binding and is defined by a cluster of serine residues, Ser88 (βB7), Ser90 (βC2), and Ser96 (βC3) (the positions in parentheses indicate the topology of the Grb2-SH2 domain, following the commonly used notation of Eck and collaborators[9]), and the positively charged amino acids Arg67 (αA2) and Arg86 (αB5) (Figure 1A). The residue Arg67 (αA2) is especially important, since it is involved in the interactions with the N-terminal domain of the peptide and forms a hydrogen bond with the backbone carbonyl group of the pY − 1 amino acid. The second site includes the backbone atoms of the amino acids His107 (βD4), Lys109 (βD6), and Leu120 (βE4), which form four hydrogen bonds with the pY + 1 and pY + 2 positions of the inhibitor (Figure 1A). According to these interactions, we designed non-peptide compounds with a novel structural core (Table 1): (i) the W substituent mimics the backbone carbonyl group of the amino acid in position pY − 1 that interacts with Arg67 (αA2); (ii) the pTyr is linked to the spacer by an amide group, which preserves the hydrogen bond with the backbone carbonyl group of His107 (βD4) observed for the N−H group of the pY + 1 residue in 2-Abz-EpYINQ-NH_2; (iii) the terminal group is designed to contact the backbone carbonyl and the N−H groups of Lys109 (βD6), mimicking the role of Asn in position pY + 2 (Figure 1B); (iv) spacers of two and three methylene units were chosen to provide enough distance and flexibility to enable concerted interactions with the two binding sites.

†Dedicated to Professor Antonio García Martínez on the occasion of his retirement.

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[a] Abbreviations: Grb2, growth factor receptor-bound protein 2; HER2, human epidermal growth factor receptor 2; SH2, Src homology 2.

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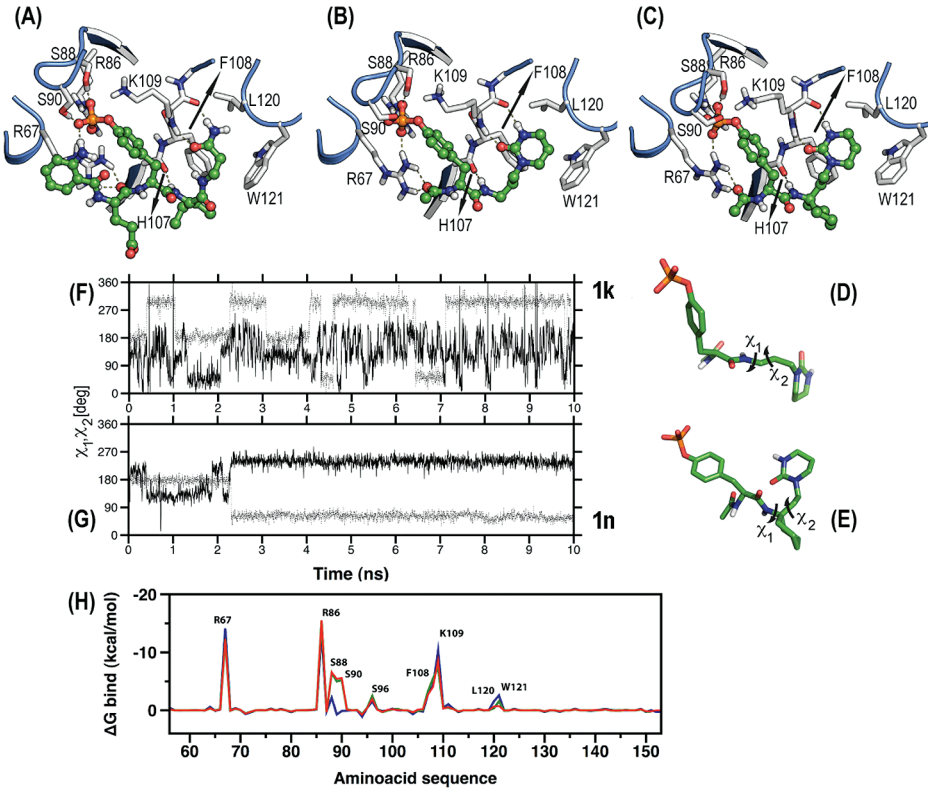


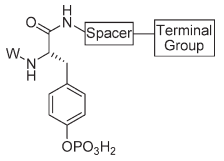
Figure 1. Representative structures obtained in the molecular dynamics (MD) simulations of Grb2-SH2 in complex with (A) the 2-Abz-EpYINQ-NH_2 pentapeptide (PDB code 1ZFP) and with (B) **1k** and (C) **1n**. (D−G) Evolution of dihedral angles χ_1 (black) and χ_2 (gray) in the vicinity of the cyclohexyl ring of **1n** (E, G) and the analogous angles in **1k** (D, F) obtained during the MD simulations of the ligands in bulk water. Representative structures of **1k** (D) and **1n** (E) obtained during the simulations in water are shown. (H) Binding free energy decomposition on a per residue basis for Grb2-SH2 in complexes with 2-Abz-EpYINQ-NH_2 (blue), **1k** (red), and **1n** (green).

The interactions between the Grb2-SH2 domain and **1k** relative to the 2-Abz-EpYINQ-NH2 pentapeptide were evaluated by comparing molecular dynamics (MD) simulations for the two complexes performed with MM-GBSA methodology.[10] In both cases equilibration was achieved during the first 5 ns of simulation, as monitored by the all-atoms root-mean-square deviation (rmsd) and from key interatomic ionic and hydrogen bond distances between Grb2-SH2 and either 2-Abz-EpYINQ-NH_2 or **1k** as a function of time. It is worth mentioning that for **1k** (Figure 1B) and all other designed molecules (data not shown) the key interactions with Arg67 (αA2), His107 (βD4), and Lys109 (βD6) were maintained during the full simulation time, similar to what was observed for 2-Abz-EpYINQ-NH_2 (Figure 1A).

The synthesis of **1a**−**j** was accomplished by phosphorylation of commercial *N*-acetyl- or *N*-benzoyl-L-tyrosine or of the readily available *N*-butyryl-L-tyrosine **2**, which was followed by condensation with the appropriate amine and subsequent deprotection of the phosphate group (Scheme 1). Intermediates **3a**−**c** were prepared by a one-pot procedure, which involved the temporary protection of the carboxyl group of *N*-acyl-L-tyrosine as a silyl ester followed by in situ phosphitylation of the tyrosyl hydroxyl group and oxidation of the resultant phosphite triester. Derivatives **4a**−**j** were obtained by condensation of **3a**−**c** with the corresponding amine in the presence of EDC as a coupling reagent and HOBt as an additive to minimize racemization.[11] Final removal of the benzyl groups by catalytic hydrogenation of protected amides **4a**−**j** yielded the target compounds **1a**−**j**. Noncommercial amines 1-(3-aminopropyl)tetrahydropyrimidin-2(1*H*)-one (**5a**) and 1-(2-aminoethyl)imidazolin-2-one (**5b**) were obtained by treatment of *N*-(3-aminopropyl)propane-1,3-diamine or *N*-(2-aminoethyl)ethane-1,2-diamine, respectively, with urea. The amount of racemization following this synthetic protocol was estimated by coupling *N*-acetyl-L-tyrosine with (1*S*)-1-phenylethylamine and comparing the area of the peaks of methyl groups of 1-phenylethylamine in the ^{1}H NMR spectrum. The ratio between the two diastereomers [*S*/*S* (1.38 ppm); *S*/*R* (1.49 ppm)] was 5:1 (data not shown). Therefore, we decided to synthesize the enantiopure isomers of the most potent compound in the series (**1a**; see Table 1), i.e., **1k** and **1l**, which were obtained using the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group (Scheme 2) to prevent racemization. After phosphorylation, Fmoc derivative **6a** or **6b** was condensed with amine **5a**, following the above-mentioned procedure. The acetyl group was introduced after Fmoc removal, and amides **8a**,**b** were finally debenzylated to yield the (*R*)- and (*S*)-*N*-acetyl-*N*′-[3-(2-oxoperhydropyrimidin-1-yl)propyl]-*O*-phosphonotyrosinamides **1l** and **1k** (Scheme 2). Racemic **1m** was obtained from *N*-acetyl-D,L-tyrosine following the procedure in Scheme 1.

The ability of the synthetic compounds to bind to the Grb2-SH2 domain was determined by an ELISA competition test using Grb2-SH2 and the phosphopeptide biotin-Ahx-PSpYV-NVQN[12] (Table 1). Among this series of ligands, **1k** showed the highest affinity and was selected to study its binding to Grb2-SH2 by NMR chemical shift perturbation experiments. The data were recorded using the 98-residue Grb2-SH2 construct described by Waugh and colleagues, which comprises the polypeptide segment 55−153,[13] and the assignments of the backbone amide resonances were taken from the work by Thanabal and colleagues.[14] The resonance assignments of Grb2-SH2 in complex with **1k** were obtained by analyzing a series of [^{15}N,^{1}H]HSQC spectra of samples containing ^{15}N-labeled

Table 1. Affinity of Compounds **1a**−**n** for the Grb2-SH2 Domain



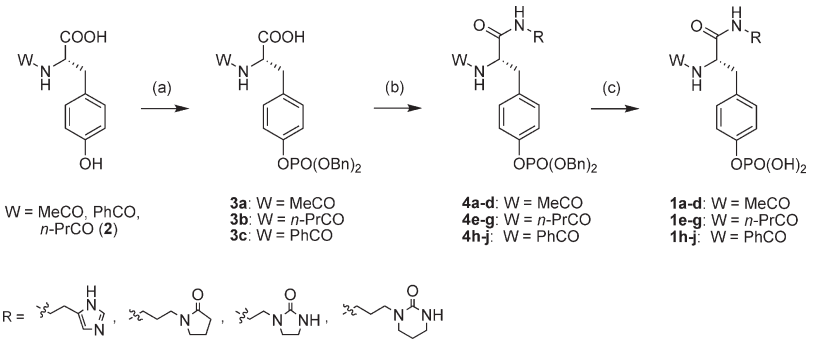
Compound	W	Spacer	Terminal Group	IC_{50} (μM)[a,b]
1a	Me-CO-	-$(CH_2)_3$-		190±25
1b	Me-CO-	-$(CH_2)_2$-		Inactive[c]
1c	Me-CO-	-$(CH_2)_2$-		Inactive[c]
1d	Me-CO-	-$(CH_2)_3$-		Inactive[c]
1e	*n*Pr-CO-	-$(CH_2)_3$-		314±5
1f	*n*Pr-CO-	-$(CH_2)_2$-		1330±98
1g	*n*Pr-CO-	-$(CH_2)_3$-		Inactive[c]
1h	Ph-CO-	-$(CH_2)_3$-		Inactive[c]
1i	Ph-CO-	-$(CH_2)_2$-		Inactive[c]
1j	Ph-CO-	-$(CH_2)_3$-		Inactive[c]
1k	Me-CO-	-$(CH_2)_3$-		174±22
1l				Inactive[c]
1m				2307±644
1n	Me-CO-			56±7

[a] Competition binding assays were conducted as described in the Supporting Information. Dose-response relationships were generated by nonlinear regression fits of the competition curves with the software Prism. Data are expressed as the mean ± SEM of at least three independent experiments carried out in duplicate. [b] For N^2-{[1-({(2*R*)-2-carboxymethyl-3-[4-phosphonomethyl)phenyl]propanoyl}amino)cyclohexyl]carbonyl}-N^1-[3-(1-naphthyl)propyl]-L-aspartamide (122A-130, **9**), kindly donated by Prof. T. R. Burke, which was used as a control of the ELISA test, we obtained IC_{50} values comparable to those previously reported (see **8a**, ref 21). [c] Inactive compounds displace less than 10% of the phosphopeptide at the maximal concentration used (5 mM).

Grb2-SH2 and different ratios of compound **1k** (5:1 → 1:5). Figure 2A shows a superposition of two 2D [^{15}N,^{1}H]HSQC spectra of Grb2-SH2 acquired in the absence and presence (1:1 molar protein/compound ratio) of **1k**, from which the residues with large chemical shift changes were identified. As a control, to rule out that these shifts arise solely from interactions due to the presence of pTyr, which is a common moiety in all the Grb2-SH2 inhibitors reported so far, [^{15}N,^{1}H]HSQC spectra of the protein in the presence of a 2-fold excess of *N*-acetylphosphotyrosine were obtained under identical conditions as the spectra of Figure 2A. It was found that this compound induces chemical shifts exclusively in a small number of Grb2 amino acid residues, which are all located in the pTyr binding pocket (Supporting Information Figure S1). A plot of the combined ^{1}H and ^{15}N chemical shifts for each residue, $\Delta\delta_{av}$, versus the Grb2-SH2 amino acid sequence shows five regions, I−V, that undergo significant chemical shift differences, which are indicative of structural rearrangements upon binding of **1k** (Figure 2B). The most strongly affected residue in region I is Arg67 (αA2), which interacts with the N-terminal carbonyl and phosphate groups of **1k**, similar to previous observations with different ligands.[7,15−17] Sequence regions II and III correspond to a hydrophilic, positively charged surface area involved in the stabilization of the phosphate group by means of electrostatic and hydrogen bond interactions. The chemical shift differences observed for Arg86 (βB5), Ser88 (βB7), and Ser96 (βC3) are particularly relevant, since these residues have previously been reported to be fundamental for the interaction with pTyr.[13,15−17] In regions IV and V, large chemical shift differences are seen for His107 (βD4) and Lys109 (βD6), which are engaged in hydrogen bond interactions with **1k** through their backbone amide groups. The neighboring residues Phe108, Val110, Leu111, and Trp121 (EF1) form a lipophilic pocket that stabilizes the protein−inhibitor complex by van der Waals interactions with the terminal group of **1k**.[15,16] The strong effect on Trp121 (EF1) is of special interest, since this residue is responsible for the specificity of ligand binding to Grb2 when compared to other SH2 domain-containing proteins.[7,15−17] Overall these results indicate that the residues with significant chemical shift perturbations due to the presence of bound **1k** (Figure 2C) coincide with those found to form a binding pocket in several crystal or solution structures of Grb2-SH2[7,15−17] and validate the assumptions used to design this new family of Grb2-SH2 binding blockers. Hence, we attributed the moderate affinity of **1k** to a high degree of conformational freedom due to the methylene spacer so that improved binding might be achieved by introducing conformational restrictions in this part of the molecule. Thus, a new compound, **1n**, was designed using **1k** as a scaffold and introducing in the spacer a conformationally constrained cyclohexyl ring. MD simulations in an explicit water environment with **1k** and **1n** were performed to evaluate the rigidity introduced by the cyclohexyl group in terms of the χ_1 and χ_2 angles (Figure 1D−G). Molecule **1k** is highly flexible, adopting different conformations during the simulation time, while the cyclohexyl ring of **1n** constrains the backbone, thus favoring the β-turn conformation required for Grb2 binding and the interactions with Phe108 (βD5) and Trp121 (EF1) (Figure 1C). The stability of **1n** in complex with Grb2-SH2 was also evaluated using the MM-GBSA methodology[10] and compared with the results obtained previously for the pentapeptide 2-Abz-EpYINQ-NH_2 and **1k**. The binding free energy decomposition on a per residue basis is shown for the three complexes in Figure 1H, where **1k** and **1n** exhibit similar interaction profiles as 2-Abz-EpYINQ-NH_2, with the only exceptions of Ser90 (βC2) and Leu120 (βE4). The absence of the anthranyl moiety of the peptide in **1k** and **1n** seems to facilitate the interaction between Ser90 and pTyr, whereas the terminal groups of **1k** and **1n** cannot interact with the backbone amide of Leu120 (βE4) (see Figure 1).

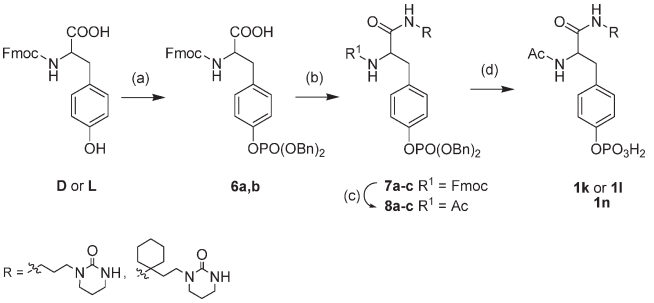
To evaluate the biological effect of the cyclohexyl group in the spacer, **1n** was prepared following the procedure indicated in Scheme 2 starting from L-tyrosine and 1-[2-(1-aminocyclohexyl)ethyl]tetrahydropyrimidin-2(1*H*)-one (**5c**). The affinity of **1n** for Grb2-SH2 was found to be 3-fold higher than for **1k**, inhibiting Grb2-SH2 domain binding with an IC_{50} of 56 μM (Table 1) in a competitive manner (Supporting Information Figure S2). We further assessed the in vitro potential of the new inhibitor in a HER2-positive MCF7 human breast cancer cell line. **1n** inhibited proliferation of these cells with an IC_{50} of 100 μM. In addition, at this concentration, no statistically

Scheme 1. Synthesis of Compounds **1a**−**j**[a]



[a] Reagents: (a) (i) NMM, ^{t}BuMe$_2$SiCl, CH$_3$CN; (ii) 1*H*-tetrazole, ^{i}Pr$_2$NP(OBn)$_2$; (iii) ^{t}BuOOH (aq); (b) (i) HOBt, EDC, CH$_2$Cl$_2$, R-NH$_2$; (c) H$_2$/Pd(C)/EtOH.

Scheme 2. Synthesis of Enantiopure Derivatives **1k**, **1l**, and **1n**[a]



[a] Reagents: (a) (i) NMM, ^{t}BuMe$_2$SiCl, CH$_3$CN; (ii) 1*H*-tetrazole, ^{i}Pr$_2$NP(OBn)$_2$; (iii) ^{t}BuOOH (aq); (b) HOBt, EDC, CH$_2$Cl$_2$, **5a** or HOBt, DIC, THF, **5c**; (c) Et$_2$NH, AcCl, CH$_3$CN; (d) H$_2$/Pd(C)/EtOH.

significant effect was observed on fibroblasts, ruling out a general cytotoxicity of the compound (Figure 3A). To further confirm the involvement of HER2-Grb2 interaction in these effects, we assessed the cytotoxicity of **1n** in two different HER2-negative breast cancer cells, HER2-negative MCF7 and MDA-231 cell lines. In both cases, **1n** did not show any significant effect (Figure 3B). In addition, we studied the direct interaction between HER2 and Grb2 by immunoprecipitation experiments. HER2-positive MCF7 cells were treated with **1n** or vehicle, washed, and lysed. HER2 was immunoprecipitated and HER2 and Grb2 immunoblotted using specific antibodies (Figure 3C), and the bands were densitometered. Our results showed a 30% decrease of the interaction between HER2 and Grb2 in the presence of **1n**. Taken together, these results clearly support that the cytotoxic effect of **1n** can be at least partially ascribed to its binding to the Grb2-SH2 domain and the concomitant inhibition of the HER2-Grb2 interaction. It is conceivable that interaction of the ligand with residue Trp121 (EF1, responsible for the specificity of ligand binding to Grb2 vs other SH2-containing proteins[7,15−17]) could confer some degree of selectivity for the recognition of Grb2-SH2 domain.

In summary, using a computation- and NMR-guided approach, we designed a structurally novel non-peptide inhibitor of the Grb2-SH2 binding, **1n**, which binds to the Grb2-SH2 domain with an IC$_{50}$ of 56 μM. This compound selectively inhibits proliferation of the HER2-positive MCF7 breast cancer cell line with no apparent toxicity toward fibroblasts and no effect on two different HER2- negative breast cancer cell lines. Furthermore, **1n** is able to disrupt the interaction HER2-Grb2. Therefore, this compound is a promising scaffold for the development of efficient Grb2-SH2 domain binding inhibitors able to block the Grb2-associated proliferative downstream pathways.

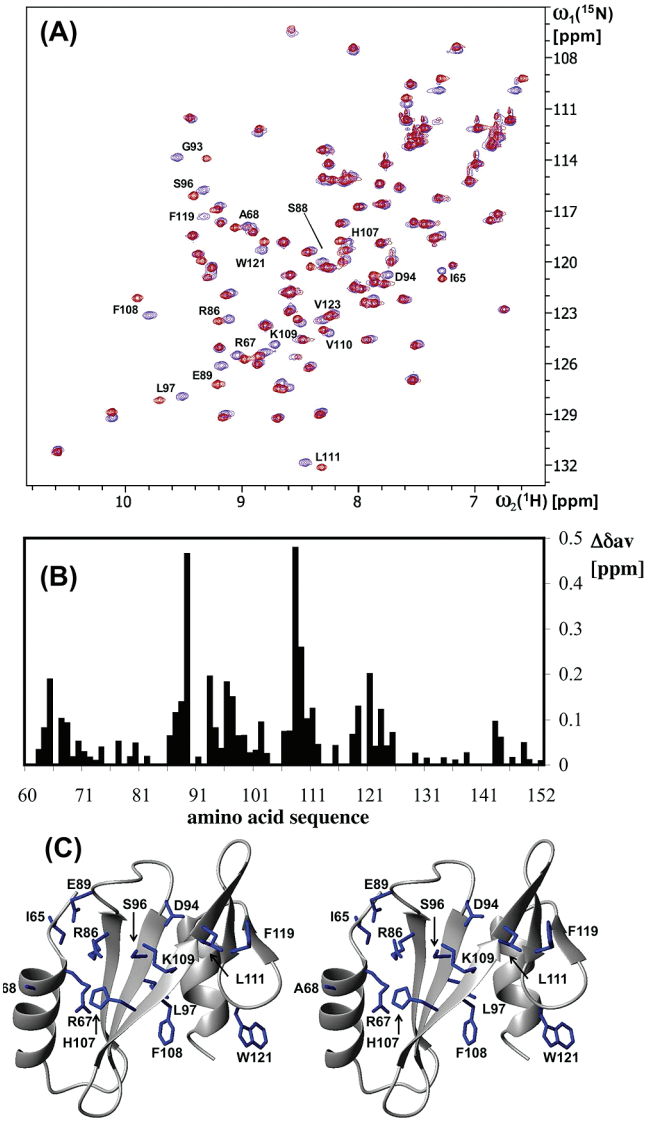


Figure 2. NMR chemical shift mapping studies. (A) 2D [^{15}N,^{1}H]-HSQC spectra of the Grb2-SH2 domain free (blue) and in complex with **1k** (red) (molar ratio protein/compound 1:1). (B) Plot of the amino acid sequence vs the chemical shift changes in the ^{1}H−^{15}N backbone moieties of the Grb2-SH2 domain due to **1k** binding. (C) Stereoribbon presentation of the Grb2-SH2 domain. The side chains of the residues that exhibit chemical shift differences larger than 0.1 ppm (B) are identified.

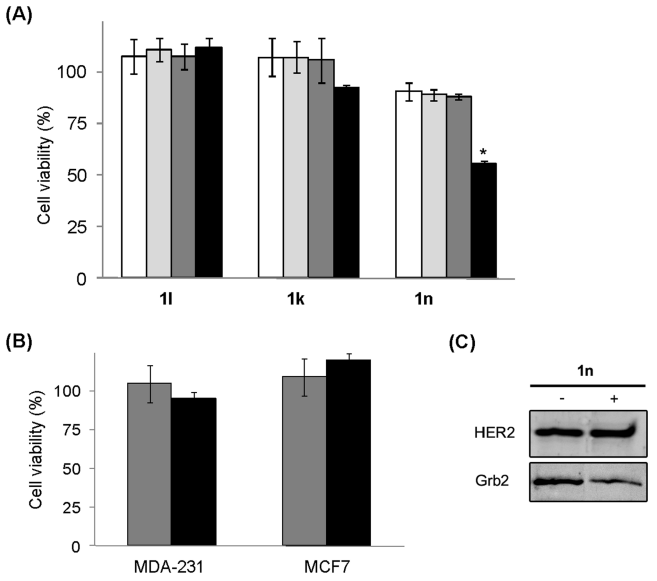


Figure 3. In vitro cytotoxic activity of (A) **1l**, **1k**, and **1n** toward N1 fibroblasts at 50 μM (white) and 100 μM (light gray) and toward HER2 positive-MCF7 human breast cancer cells at 50 μM (dark gray) and 100 μM (black) and (B) **1n** toward HER2 negative MDA-231 and MCF7 human breast cancer cells at 50 μM (gray) and 100 μM (black). (C) Inhibition of the HER2-Grb2 interaction by **1n**. HER2-positive MCF7 cells were treated with **1n** or vehicle as in (A), washed, and lysed. HER2 was immunoprecipitated, and HER2 and Grb2 were immunoblotted using specific antibodies: $*$, $p < 0.01$ vs control.

Experimental Section

Full synthetic details and characterization data, including elemental analysis results, are given in the Supporting Information. All compounds tested are >95% pure by elemental analysis. *N*-Butyryl-L-tyrosine (**2**),[18] *N*-acetyl-*O*-[bis(benzoyloxy)phosphoryl]-L-tyrosine (**3a**),[19] and amines **5a**[20] and **5b**[20] were synthesized according to previously described procedures. Detailed synthetic procedures for the intermediates **3a**−**c**, **4a**−**j**, **5c**, **6a**,**b**, **7a**−**c**, and **8a**−**c** are described in the Supporting Information.

Synthesis of *N*-Acyl-*O*-phosphonotyrosinamides (1a−n). To a solution of the corresponding amides **4a**−**j**, **8a**−**c** (1 equiv) in absolute EtOH (10 mL), 10% Pd(C) was added (200 mg), and the suspension was hydrogenated at room temperature at an initial pressure of 45 psi until the disappearance of the starting benzylated amide (TLC). The mixture was filtered over Celite and the solvent was removed under reduced pressure to yield the pure debenzylated amides **1a**−**n** (34−93%), which were then recrystallized from the appropriate solvent(s).

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Supporting Information Available: Synthetic procedures for the intermediates **3a**−**c**, **4a**−**j**, **5c**, **6a**,**b**, **7a**−**c**, and **8a**−**c**; analytical and spectral characterization data of the synthesized compounds; experimental details for determination of binding affinity, protein sample preparation, NMR methods, in vitro cell cytotoxicity, and immunoprecipitation experiments; superposition of the [^{15}N,^{1}H]HSQC spectra of Grb2-SH2 in the presence and absence of *N*-acetylphosphotyrosine; competition binding curves for **9** and **1n**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Slamon, D. J.; Clark, G. M.; Wong, S. G.; Levin, W. J.; Ullrich, A.; McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **1987**, *235*, 177–182.
(2) Nahta, R.; Yu, D.; Hung, M.-C.; Hortobagyi, G. N.; Esteva, F. J. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat. Clin. Pract. Oncol.* **2006**, *3*, 269–280.
(3) Baselga, J.; Swain, S. M. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat. Rev. Cancer* **2009**, *9*, 463–475.
(4) Dharmawardana, P. G.; Peruzzi, B.; Giubellino, A.; Burke, T. R.; Bottaro, D. P. Molecular targeting of growth factor receptor-bound 2 (Grb2) as an anti-cancer strategy. *Anti-Cancer Drugs* **2006**, *17*, 13–20.
(5) Constantino, L.; Barlocco, D. Inhibitors for proteins endowed with catalytic and non-catalytic activity which recognize pTyr. *Curr. Med. Chem.* **2004**, *11*, 2725–2747.
(6) Jiang, S.; Liao, C.; Bindu, L.; Yin, B.; Worthy, K. W.; Fisher, R. J.; Burke, T. R., Jr.; Nicklaus, M. C.; Roller, P. P. Discovery of thioether-bridged cyclic pentapeptides binding to Grb2-SH2 domain with high affinity. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2693–2698.
(7) Vu, C. B. Recent advances in the design and synthesis of SH2 inhibitors of Src, Grb2 and ZAP-70. *Curr. Med. Chem.* **2000**, *7*, 1081–1100.
(8) Rahuel, J.; García-Echeverría, C.; Furet, P.; Strauss, A.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Gay, B. Structural basis for the high affinity of amino-aromatic SH2 phosphopeptide ligands. *J. Mol. Biol.* **1998**, *279*, 1013–1022.
(9) Eck, M. J.; Shoelson, S. E.; Harrison, S. C. Recognition of a high-affinity phosphotyrosyl peptide by the Src homology-2 domain of p56lck. *Nature* **1993**, *362*, 87–91.
(10) Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D. A.; Cheatham, T. E., 3rd. Calculating structures and free energies of complex molecules: combining molecular mechanisms and continuum models. *Acc. Chem. Res.* **2000**, *33*, 889–897.
(11) Albericio, F.; Kates, S. A. In *Solid-Phase Synthesis: A Practical Guide*; Kates, S. A., Albericio, F., Eds.; Marcel Dekker: New York, 2000; p 278.
(12) Liu, W.-Q.; Vidal, M.; Gresh, N.; Roques, B. P.; Garbay, C. Small peptides containing phosphotyrosine and adjacent alphaMe-phosphotyrosine or its mimetics as highly potent inhibitors of Grb2 SH2 domain. *J. Med. Chem.* **1999**, *42*, 3737–3741.
(13) Phan, J.; Shi, Z.-D.; Burke, T. R.; Waugh, D. S. Crystal structures of a high-affinity macrocyclic peptide mimetic in complex with the Grb2 SH2 domain. *J. Mol. Biol.* **2005**, *353*, 104–115.
(14) Thornton, K. H.; Mueller, W. T.; McConnell, P.; Zhu, G.; Saltiel, A. R.; Thanabal, V. Nuclear magnetic resonance solution structure of the growth factor receptor-bound protein 2 Src homology 2 domain. *Biochemistry* **1996**, *35*, 11852–11864.
(15) Ogura, K.; Tsuchiya, S.; Terasawa, H.; Yuzawa, S.; Hatanaka, H.; Mandiyan, V.; Schlessinger, J.; Inagaki, F. Solution structure of the SH2 domain of Grb2 complexed with the Shc-derived phosphotyrosine-containing peptide. *J. Mol. Biol.* **1999**, *289*, 439–445.
(16) Ogura, K.; Shiga, T.; Yokochi, M.; Yuzawa, S.; Burke, T. R., Jr.; Inagaki, F. Solution structure of the Grb2 SH2 domain complexed with a high-affinity inhibitor. *J. Biomol. NMR* **2008**, *42*, 197–207.
(17) Nioche, P.; Liu, W.-Q.; Broutin, I.; Charbonnier, F.; Latreille, M.-T.; Vidal, M.; Roques, B.; Garbay, C.; Ducruix, A. Crystal structures of the SH2 domain of Grb2: highlight on the binding of a new high-affinity inhibitor. *J. Mol. Biol.* **2002**, *315*, 1167–1177.
(18) Moya, E.; Blagbrough, I. S. Total synthesis of polyamine amides PhTX-4.3.3 and PhTX-3.4.3: reductive alkylation is a rapid, practical route to philanthotoxins. *Tetrahedron Lett.* **1995**, *36*, 9401–9404.
(19) Llinas-Brunet, M.; Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Betageri, R.; Cardozo, M.; Jakes, S.; Lukas, S.; Patel, U.; Proudfoot, J.; Moss, N. Phosphotyrosine-containing dipeptides as high-affinity ligands for the p56lck SH2 domain. *J. Med. Chem.* **1999**, *42*, 722–729.
(20) Hurwitz, M. D.; Auten, J. P. US2613212, 1952.
(21) Wei, C.-Q.; Li, B.; Guo, R.; Yang, D.; Burke, T. R., 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.