Bioorganic & Medicinal Chemistry Letters 19 (2009) 2693–2698

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of thioether-bridged cyclic pentapeptides binding to Grb2-SH2 domain with high affinity

Sheng Jiang [a,*], Chenzhong Liao [b], Lakshman Bindu [c], Biaolin Yin [a], Karen W. Worthy [c], Robert J. Fisher [c], Terrence R. Burke Jr. [b], Marc C. Nicklaus [b], Peter P. Roller [b]

[a] Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510663, PR China
[b] Laboratory of Medicinal Chemistry, National Cancer Instistute, NIH, Frederick, MD 21702, USA
[c] Laboratory of Protein Chemistry, SAIC-Frederick, Frederick, MD 21702, USA

ARTICLE INFO

Article history:
Received 15 December 2008
Revised 6 March 2009
Accepted 26 March 2009
Available online 29 March 2009

Keywords:
Grb2-SH2 domain
Cyclic peptides
G1TE analogs
Phosphotyrosine
Grb2-SH2 antagonist

ABSTRACT

Blocking the interaction between phosphotyrosine (pTyr)-containing activated receptors and the Src homology 2 (SH2) domain of the growth factor receptor-bound protein 2 (Grb 2) is considered to be an effective and non-cytotoxic strategy to develop new anti-proliferate agents due to its potential to shut down the Ras activation pathway. In this study, a series of phosphotyrosine containing cyclic pentapeptides were designed and synthesized based upon the phage library derived cyclopeptide, **G1TE**. A comprehensive SAR study was also carried out to develop potent Grb2-SH2 domain antagonists based upon this novel template. With both the peptidomimetic optimization of the amino acid side-chains and the constraint of the backbone conformation guided by molecular modeling, we developed several potent antagonists with low micromolar range binding affinity, such as cyclic peptide **15** with an K_d = 0.359 μM, which is providing a novel template for the development of Grb2-SH2 domain antagonists as potential therapeutics for certain cancers.

© 2009 Elsevier Ltd. All rights reserved.

Growth factor receptor-bound protein 2 (Grb2) is an essential adapter protein, possessing a single Src homology 2 (SH2) domain flanked by two SH3 domains.[1] Grb2-SH2 domain directly mediates erbB-2 (HER-2/neu) signaling by binding to pTyr motifs and the downstream activation of mitogenic Ras pathways, which have been implicated in the etiology of certain breast cancer.[2,3] Grb2-SH2 domain is an ideal target for drug development,[4,5] in that the phosphopeptide ligands that bind it are required to adopt a β-turn conformation, which has been demonstrated by both NMR and X-ray analysis.[6–8] Molecules that can selectively antagonize the Grb2-SH2 should interrupt these signaling pathways. These agents may act as therapeutic leads for diseases, such as cancer, in which the Ras signaling pathway plays a major role.[9–12]

In our previous studies, we discovered a novel disulfide-bridged cyclic peptide G1, generated by phage library.[13] This peptide was comprised of the sequence -Glu-Leu-Tyr-Glu-Asn-Val-Gly-Met-Tyr-, bracketed by two terminal cysteines that formed the cyclic peptide through a disulfide linkage. Subsequently, we further designed and synthesized its redox stable thioether-bridged analogue, cyclo (CH_2CO-Glu^1-Leu^2-Tyr^3-Glu^4-Asn^5-Val^6-Gly^7-Met^8-Tyr^9-Cys^{10})-amide, termed **G1TE** (Fig. 1), which has shown comparable binding affinities (IC_{50} = 15–20 μM) to the Grb2-SH2 domain protein, in Biacore binding assays.[13] Based on the **G1TE**, a number of redox-stable thioether-cyclized analogs were developed, some of which exhibited potent binding affinity.[14–19] **G1TE** and its analogues may form a largest circle-like binding surface when they bind to Grb2-SH2 domain, as suggested by molecular modeling and NMR studies.[20,21] Molecular modeling of the complex structure of Grb2-SH2 protein with **G1TE** indicated that the side chains of the amino acid residues in the 1, 3 and 5 positions of **G1TE** form strong hydrogen-bond interactions with the Arg 67 (αA-helix) and Arg 86 (βC-strand) residues and the three Ser residues 88, 90, and 96, and Lys 109 and Leu 120 within the binding pocket. However, the amino acid residues in the 2, 7, 8, and 9 positions of **G1TE** do not interact with the Grb2-SH2 protein. To further improve the affinity and reduce the peptide character of the novel Grb2-SH2 domain antagonists, we were interested in developing a new scaffold with smaller ring size but higher affinity. We assumed that keeping essential amino acids and deleting unnecessary amino acids of **G1TE** would increase the binding affinity and selectivity. Replacement of Glu1, Leu2 and Tyr3 of **G1TE** with p-Tyr would increase hydrogen-bond interaction with the Arg 67 and Arg 86 of the SH2 domain binding cavity; on the other hand, the phosphoryl group itself would undergo additional intramolecular hydrogen-bond interactions, which stabilizes the binding conformation of cyclic peptide. This is a brand new strategy distinct from any approaches reported previously to improve the selectivity and affinity. This article reports our recent discovery of potent tyrosine-phosphorylated **G1TE** analogues by overall side-chain and backbone modifications and conformational constraint (Fig. 1).

* Corresponding author. Tel.: +86 20 32290439; fax: +86 20 32290606.
E-mail addresses: jiang_sheng@gibh.ac.cn, jiangsh9@gmail.com (S. Jiang).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.134

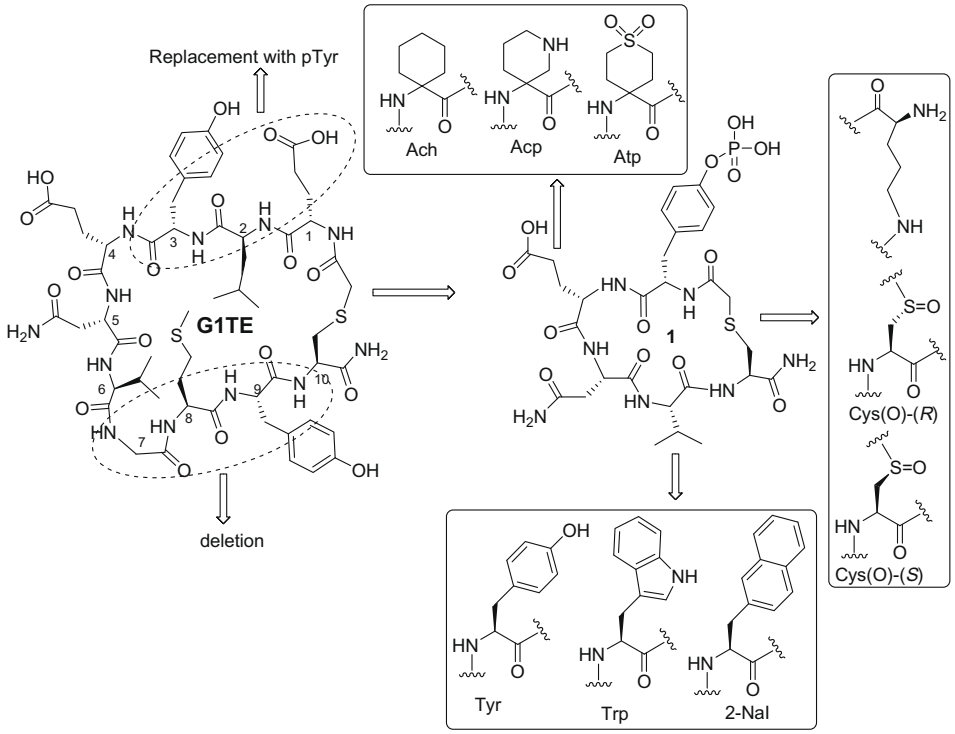

Figure 1. Molecular design of Grb2-SH2 domain antagonists based upon thioether-bridged cyclic peptide **G1TE**.

The cyclic peptides **1**–**15** in Table 1 were synthesized by using the procedure described in Fig. 2. The linear peptide precursors were synthesized on an ABI 433A peptide synthesizer, starting with Rink Amide resin for establishing the C-terminal carboxamide, and using the chemical protocols based on the Fmoc chemistry. The incorporation of *N*-Fmoc-Tyr[PO(OH, OBzl)-OH was carried out manually and coupled twice using HATU as the coupling reagent. After completion of peptide elongation, the Mmt protecting group on Cys was removed by treating with 1% TFA in DCM to afford the resin bound peptide **19**. Subsequently, cyclic peptides were formed by using NMM as a base. The resulting cyclopeptide bound resins were washed six times with NMP, DCM, and MeOH, and then dried under reduced pressure overnight. The cyclopeptides were cleaved from the resin and fully deprotected by treating with 2.5% TIS and 2.5% H_2O in TFA (10 mL/g resin) for 2 h. The oxidation of the thioether functionality into sulfoxide was accomplished using a 5% H_2O_2 aqueous solution. The resulting two isomers were easily separated by RP-HPLC. All peptides were purified to homogeneity by preparative RP-HPLC (Vydac C18 column), and their structures were confirmed by MALDITOF-MS mass spectrometry. The absolute configuration of the sulfoxide diastereoisomers was determined by CD spectra using documented method.[22–26]

Table 1
Grb2-SH2 domain inhibitory activity of the **G1TE** analogs **1–16** with the general structure cyclo-(pTyr^1–AA^2–Asn^3–AA^4–AA^5)-amide[a]

Compds	Modifications of amino acid residues			Activity K_d[b] (μM)
	AA^2	AA^4	AA^5	
G1TE	–	–	–	25
1	Glu	Val	Cys-CH_2CO-	3.6 (±0.0.01)
2	Ach	Val	Cys-CH_2CO-	1.42 (±0.04)
3	Ach	Tyr	Cys-CH_2CO-	1.06 (±0.03)
4	Ach	Trp	Cys-CH_2CO-	1.05 (±0.03)
5	Ach	2-Nal	Cys-CH_2CO-	2.71 (±0.09)
6	Ach	Val	Cys(O)-(*R*)-CH_3CO-	1.56 (±0.05)
7	Ach	Val	Cys(O)-(*S*)-CH_2CO-	1.76 (±0.04)
8	Ach	Tyr	Cys(O)-(*R*)-CH_3CO-	1.36 (±0.04)
9	Ach	Tyr	Cys(O)-(*S*)-CH_2CO-	1.72 (±0.05)
10	Ach	Trp	Cys(O)-(*R*)-CH_3CO-	2.14 (±0.06)
11	Ach	Trp	Cys(O)-(*S*)-CH_2CO-	1.29 (±0.03)
12	Ach	2-Nal	Cys(O)-(*R*)-CH_3CO-	4.8 (±0.0.03)
13	Ach	2-Nal	Cys(O)-(*S*)-CH_2CO-	nd[c]
14	Atp	Val	Cys-CH_2CO-	0.88 (±0.20)
15	Acp	Val	Cys-CH_2CO-	0.359 (±0.06)
16	Ach	Val	Lys	7.55

[a] The binding affinity of these compounds was evaluated by Biacore assays.
[b] Values are means of two experiments, standard deviation is given in parentheses.
[c] nd = not determined.

The synthesis of cyclic peptide **16** was described in Fig 3. The linear peptide precursors H-(Boc)Lys-pTyr(OBzl)-Ach-Asn-Val-2-Cl-Trt resin were synthesized by Fmoc SPPS. After the peptides were cleaved from resin, the head-to-tail macrocyclization of the linear peptide was achieved successfully using HATU as the coupling reagent and HOAt as the accelerator[27] and then deprotected with the TFA–TIS–H_2O cocktail. After side-chain deprotection with the TFA–TIS–H_2O cocktail, the cyclic peptide **16** was obtained in 30% overall yield.

Docking studies of these 16 compounds were performed on the crystal structure of Grb2-SH2 domain (PDBID: 1TZE),[6] to which the KPFpYVNV peptide bound, using the program Glide 4.5,[28–31] part of the First Discovery suite (Maestro). The starting structure for the protein was prepared and fixed by the Protein Preparation Wizard, a comprehensive protein preparation facility. All water molecules were deleted; the bound ligand (KPFpYVNV) was adjusted manually; bond orders were assigned, and then hydrogen was added to all atoms in the structure. The orientation of hydroxyl groups, amide groups of Asn and Gln, and charge state of His residues were optimized using the utility protassign. The final step was a restrained minimization by the impref utility until the average root-mean-square-deviation (rmsd) of the hydrogen atoms reached 0.3 Å, leaving heavy atoms in place. In order to study the binding modes of the antagonists, grids files representing shape and properties of

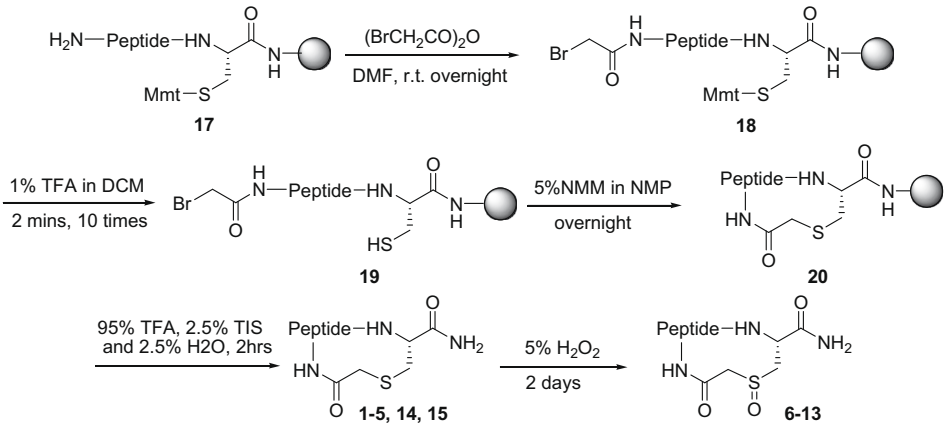

Figure 2. General procedure for the synthesis of thioether and sulfoxide bridged cyclic pentapeptides.

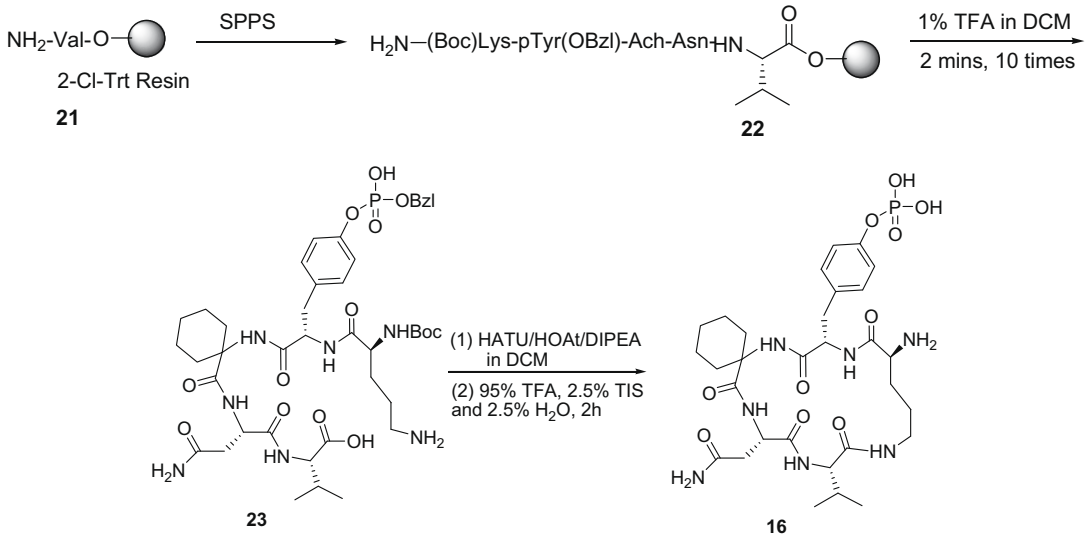

Figure 3. Synthetic route of cyclic pentapeptide **16**.

the receptor were generated. The three-dimensional structures of the compounds **1**–**16** were constructed using the builder tool available through Maestro interface by modifying the known peptide bound in 1TZE. The initial geometries of the structures were optimized using the OPLS_2005 force field, performing 5000 steps of conjugate gradient minimization. The compounds were subjected to flexible docking using the pre-computed grid files. Glide 4.5 has three docking precision mode: high-throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP). Here, to try to get the closet binding conformations, we used XP mode.

To try to explain the biological activity differences between cyclic peptides, conformational searches were adopted. The software used was MACROMODEL 9.5,[32] and the method utilized was Torsional sampling (Monte Carlo Multiple Minimum, MCMM), with the OPLS_2005 force field. Most experimental studies are carried out in solvent, and the solvent medium can have a major effect on molecular structures and energies, especially for molecules having several polar functional groups, like those we study here. So when doing conformation searching, we used a called GB/SA model to treat the solvent as a fully equilibrated analytical continuum starting the near the van der Walls surface of the solute. The parameters were set according to the following: the energy window for saving structures was 50 kJ/mol; the maximum iterations for minimizing the molecules were 5000; the convergence threshold was 0.05; and the others adopted the default values.

It has been well documented that phosphotyrosine is important for linear peptide ligand binding to Grb2-SH2 domain by forming extensive hydrogen bonds and hydrophobic interactions.[6,33] Deleting unnecessary amino acids of **G1TE**, and phosphorylation of Tyr3 of **G1TE** provided for us peptide **1** that competitively bound to the Grb2-SH2 domain protein with an K_d of 3.6 μM. The inhibitory activity of pTyr-containing cyclic pentapeptide **1** is at least 7 times more potent than the corresponding **G1TE**. Molecular modeling indicates that the negatively charged pTyr oxygens of cyclic pentapeptide **1** form key binding interactions with the Arg 67 and Arg 86 and the three Ser residues 88, 90 and 96 of the protein, and consensus amino acid Glu 2 of the cyclic pentapeptide **1** form two extra hydrogen bonds with residue Arg 67 because of the strong positive-negative charges attraction (Fig. 4A).

The X-ray structure of Grb2-SH2 domain complexed with pTyr-containing peptide ligand has shown that the phosphopeptide binds to Grb2-SH2 domain in a β-turn conformation due to the existence of Trp121 around the asparagine binding pocket.[6] Therefore, we intentionally introduced a turn-inducing amino acid, such as 1-amino-1-cyclohexylic acid (Ach), in the position 2. When replacing Glu 4 with Ach 4, the resulting peptide **2** loses charge

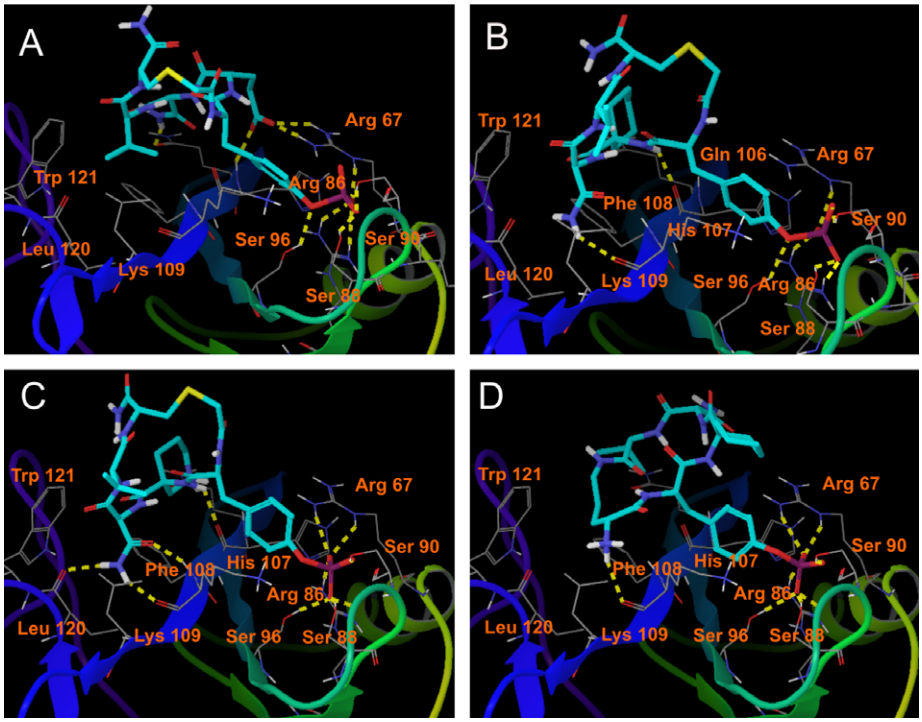

Figure 4. Docking poses of cyclic peptides **1** (A), **2** (B), **15** (C, R enantiomer) and **16** (D) with Grb2-SH2 domain (PDB ID 1TZE). Hydrogen bonds between ligands and protein are represented by yellow dot lines. For clarity, only polar hydrogen atoms are shown here.

interactions with Arg 67, but gains hydrophobic Van der Waals interactions with Gln 106 and Phe 108 of the Grb2-SH2 domain, as shown in Figure 4B. In addition, the 3_{10} helix turn, with the torsion angles $\varphi = -62.1^{o}$, $\psi = -34.3^{o}$ and $\omega = 170.4^{o}$, induced by Ach is also favored for the peptide binding to the Grb2-SH2 domain. The overall effect makes peptide **2** ($K_d = 1.42$ μM) approximately 2.5-fold more potent than peptide **1**.

By analyzing the model of the complex structure of **G1TE** with Grb2-SH2 domain, we found that Val 4 has van der Waals interactions with the side chains of Lys 109 and Leu 120 of the Grb2-SH2 domain. In addition, Val 4 localizes in a hydrophobic region of the Grb2 protein, which is composed of Phe 108, Gln 106, Trp 121, Lys 109, and Leu 120. Therefore, introducing a more hydrophobic amino acid residue into position 4 of cyclic pentapeptide **2** would improve this kind of van der Waals interactions. Considering these characteristic interactions, we incorporated a series of hydrophobic amino acids in position 4, as shown in Table 1. The binding affinity of the modified peptides **3** ($K_d = 1.06$ μM) and **4** ($K_d = 1.05$ μM) increases approximately 1.5-fold when replacing Val 4 with tyrosine (Tyr) or tryptophan (Trp). Compared to valine, these amino acids are more hydrophobic, which results in favorable and well defined Van der Waals interactions. However, these improved interactions can be overweighed by increasing exposed hydrophobic surface area under some circumstances. For instance, the binding affinity of the resulting peptides **5** ($K_d = 2.71$ μM) was not improved, and even decreased when Val 4 was substituted with L-2-naphthylalanine (2-Nal).

To further constrain the backbone conformation of cyclic pentapeptides, we also tried to modify the thioether bridge of these peptides, since this $-CH_2-S-CH_2-$ motif is conformational flexible and may contribute to an entropic penalty for the thioether-bridged cyclic pentapeptides binding to Grb2 protein. When the thioether-cyclized peptide **2** was oxidized into the relatively more rigid R-configured sulfoxide **6** and S-configured sulfoxide **7**, the binding affinity was slightly decreased (**6**, $K_d = 1.56$ μM; **7**, $K_d = 1.76$ μM) than its precursor thioether analog **2**, probably due to the disfavored change of the peptide backbone conformation. This trend was also observed in the Tyr 4, Trp 4, and Nal 4-containing peptides **3**, **4**, **5**, **8**, **9**, **10**, **11** and **12**. Compared to thioether-bridged analogues, the activities of these sulfoxide bridged cyclopeptides are not improved, due to the steric hindrance caused by the bulky side chain of tyrosine, tryptophan, and 2-naphthylalanine, respectively. We also incorporated Polyamide linkage into cyclic pentapeptides **2**, and examined the potency to determine the optimal linkage which favors forming the β-turn like conformation. The binding affinity of the resulting peptide **16** ($K_d = 7.55$ μM) was 5.3 times less than its precursor thioether analog **2**. This indicates that the rigid cyclization constraint conferred by the backbone cyclization disfavors either the formation of β-turn conformation or the optimal orientation of the side chains of essential residues. These results indicate that the linkage in cyclic pentapeptides, which are not involved in the direct interactions with the binding pocket of the protein, still has a function to induce the required conformation via its backbone.

4-Amino-1,1-dioxo-hexahydro-thiopyran-4-carboxylic acid (Atp) and 3-amino-piperidine-4-carboxylic acid (Acp) are turn-inducing amino acids, similar to Ach. We replaced Ach 2 with Atp and Acp, respectively, whereby the resulting cyclic peptides **14** and **15** showed 1.6 and 4 times more inhibitory activity than the corresponding unmodified peptide **2**, respectively (**14**, $K_d = 0.88$ μM; **15**, $K_d = 0.359$ μM). Molecular modeling shows that that the replacing would induce more hydrogen-bond interactions with Leu 120 and Lys 109 of the Grb2-SH2 domain (Fig. 4C).

There are two intramolecular hydrogen bonds, which are formed by a same oxygen atom with two same hydrogen atoms in the 18 member ring, in almost all these 16 compounds' docking conformations (Fig. 5E is as an example), except that cyclic peptide **1** has three (Fig. 5B). This extra hydrogen bond is created by the propanoic acid group with the amide group sitting next to it. But for the global minimum conformations we searched, the situations are different. Cyclic peptide **15**, actually, is a racemic mixture. For its R enantiomer, the global minimum conformation has only one intramolecular

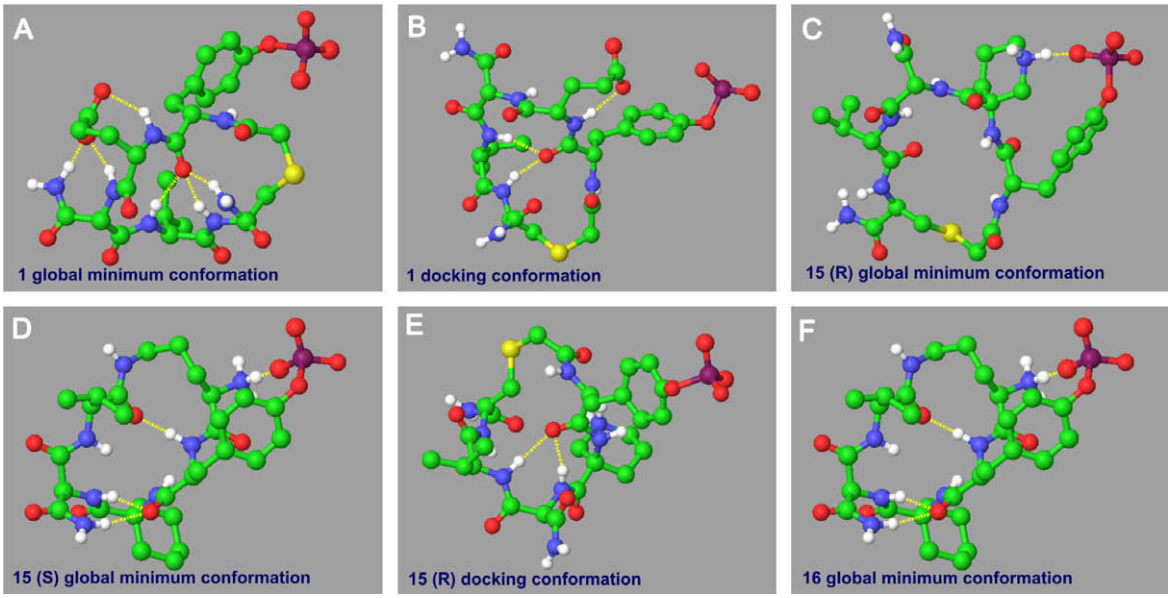

Figure 5. Conformation analyses of compounds **1**, **15** and **16**. Intramolecular hydrogen bonds are represented by yellow dot lines. For clarity, only polar hydrogen atoms are shown here.

hydrogen bond, produced by the phosphoric acid group and the charged amino atom in the rigid six-member ring (Fig. 5C). Whereas, its *S* enantiomer's global minimum conformation has four hydrogen bonds (Fig. 5D), which makes it much more difficult to change its global minimum conformation to binding conformation than *R* enantiomer. So we can conclude that *R* enantiomer of cyclic peptide **15** has stronger binding affinity than *S* enantiomer just from conformational point. Cyclic peptide **1** can even produce six intramolecular hydrogen bonds (Fig. 5A), among which three are through the flexible propanoic acid group; Cyclic peptide **16** can produce four (Fig. 5F), one of which is formed by the negative charged phosphoric acid group with positive charged amino group and therefore is much stronger than other hydrogen bonds. So, for the same reason, for cyclic peptides **1** and **16**, it is also much harder to change their global minimum conformations to binding conformations than other analogues. This, from the other side, explains why cyclic peptide **15** has best and cyclic peptides **1** and **16** have worst biological activities toward Grb2-SH2 domain.

In summary, we have designed and synthesized a series of phosphotyrosine containing cyclic pentapeptides through deleting unnecessary amino acids, based upon molecular modeling of the complex structure of Grb2-SH2 protein with **G1TE**. By introducing a turn-inducing Acp in 2th position, a hydrophobic and less flexible valine in the 4th position, and keeping thioether linkage respectively, we developed a globally and locally optimized inhibitor **15** with a $K_d = 0.359$ μM, as evaluated by Biacore assays. This fully elaborated small peptidomimetic provides us a novel template for the development of pharmacokinetically improved peptidomimetics that can selectively bind to Grb2-SH2 domain, and may act as potential therapeutic agents for the treatment of erbB2-related cancers.

Acknowledgments

This project was supported by the National Natural Science Foundation (Grant no. 20802078), National Basic Research Program of China (973 Program, Grant no. 2009CB940900), the Guangdong Natural Science Foundation (Grant no. 8151066302000008) and the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.134.

References and notes

1. Margolis, B. *Prog. Biophys. Mol. Biol.* **1994**, *62*, 223.
2. Yip, S. S.; Crew, A. J.; Gee, J. M.; Hui, R.; Blamey, R. W.; Robertson, J. F.; Nicholson, R. I.; Sutherland, R. L.; Daly, R. J. *Int. J. Cancer* **2000**, *88*, 363.
3. Lim, S. J.; Lopez-Berestein, G.; Hung, M. C.; Lupu, R.; Tari, A. M. *Oncogene* **2000**, *19*, 6271.
4. Caravatti, G.; Rahuel, J.; Gay, B.; Furet, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1973.
5. Long, Y.-Q.; Yao, Z.-J.; Voigt, J. H.; Lung, F.-D. T.; Luo, J. H.; Burke, T. R.; King, C. R.; Yang, D.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 902.
6. Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; Garcia-Echeverria, C.; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Grütter, M. J. *Nat. Struct. Biol.* **1996**, *3*, 586.
7. Ogura, K.; Tsuchiya, S.; Terasawa, H.; Yuzawa, S.; Hatanaka, H.; Mandiyan, V.; Schlessinger, J.; Inagaki, F. J. *J. Biomol. NMR* **1997**, *10*, 273.
8. Ogura, K.; Tsuchiya, S.; Terasawa, H.; Yuzawa, S.; Hatanaka, H.; Mandiyan, V.; Schlessinger, J.; Inagaki, F. *J. Mol. Biol.* **1999**, *289*, 439.
9. Shakespeare, W. C. *Curr. Opin. Chem. Biol.* **2001**, *5*, 409.
10. Machida, K.; Mayer, B. J. *BBA-Proteins Proteomics* **2005**, *1747*, 1.
11. Muller, G. *Top. Curr. Chem.* **2001**, *211*, 17.
12. Fretz, H.; Furet, P.; Garcia-Echeverria, C.; Schoepfer, J.; Rahuel, J. *Curr. Pharm. Des.* **2000**, *6*, 1777.
13. Oligino, L.; Lung, F.-D. T.; Sastry, L.; Bigelow, J.; Cao, T.; Curran, M.; Burke, T. R.; Wang, S.-M.; Krag, D.; Roller, P. P.; King, C. R. *J. Biol. Chem.* **1997**, *272*, 29046.
14. Li, P.; Peach, M. L.; Zhang, M.; Liu, H.; Yang, D.; Nicklaus, M.; Roller, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 895.
15. Jiang, S.; Li, P.; Lai, C. C.; Kelley, J. A.; Roller, P. P. *J. Org. Chem.* **2006**, *71*, 7307.
16. Jiang, S.; Li, P.; Peach, M. L.; Bindu, L.; Worthy, K. W.; Fisher, R. J.; Burker, T. R., Jr.; Nicklaus, M.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **2006**, *349*, 497.
17. Song, Y.-L.; Roller, P. P.; Long, Y.-Q. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3205.
18. Song, Y.-L.; Peach, M. L.; Roller, P. P.; Qiu, S.; Wang, S.; Long, Y.-Q. *J. Med. Chem.* **2006**, *49*, 1585.
19. Li, P.; Zhang, M.; Peach, M. L.; Zhang, X.; Liu, H.; Nicklaus, M.; Yang, D.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **2003**, *307*, 1038.
20. Lung, F.-D. T.; Long, Y.-Q.; King, C. R.; Varady, J.; Wu, X.-W.; Wang, S.; Roller, P. P. *J. Peptide Res.* **2001**, *57*, 447.
21. Lou, Y.-C.; Lung, F.-D. T.; Pai, M.-T.; Tzeng, S.-R.; Wei, S.-Y.; Roller, P. P.; Cheng, J.-W. *Arch. Biochem. Biophys.* **1999**, *372*, 309.
22. Mislow, K.; Green, M. M.; Laur, P.; Melillo, J. T.; Simmons, T.; Ternay, A. L., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 1958.
23. Ottenheijm, H. C. J.; Liskamp, R. M. J.; Helquist, P.; Lauher, J. W.; Shekhani, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 1720.
24. Kubec, R.; Musah, R. A. *Phytochemistry* **2001**, *58*, 981.
25. Liskamp, R. M. J.; Zeegers, H. J. M.; Ottenheijm, H. C. J. *J. Org. Chem.* **1981**, *46*, 5408.

26. Van den Broek, L. A. G. M.; Breuer, M. L.; Liskamp, R. M. J.; Ottenheijm, H. C. J. *J. Org. Chem.* **1987**, *52*, 1511.
27. (a) Li, P.; Jiang, S.; Lee, S.-L.; Lin, C. Y.; Johnson, M. D.; Dickson, R. B.; Michejda, C. J.; Roller, P. P. *J. Med. Chem.* **2007**, *50*, 5976; (b) Kamenecka, T. M.; Danishefsky, S. J. *Chem.-Eur. J.* **2001**, *7*, 41; (c) Kamenecka, T. M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2993; (d) Kamenecka, T. M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2995; (e) Jou, G.; González, I.; Albericio, F.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **1997**, *62*, 354; (f) Jiang, S.; Li, P.; Lee, S.-L.; Lin, C. Y.; Long, Y.-Q.; Johnson, M. D.; Dickson, R. B.; Roller, P. P. *Org. Lett.* **2007**, *9*, 9.
28. GLIDE, *Version 4.5*; Schrodinger, LLC: New York, NY, 2007.
29. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
30. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750.
31. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177.
32. MACROMODEL *9.5*; Schrodinger, LLC: New York, NY, 2007.
33. Nioche, P.; Liu, W.-Q.; Broutin, I.; Charbonnier, F.; Latreille, M.-T.; Vidal, M.; Roques, B.; Garbay, C.; Ducruix, A. *J. Mol. Biol.* **2002**, *315*, 1167.