Bioorganic & Medicinal Chemistry Letters 19 (2009) 3305–3309

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Natural product inhibitors of protein–protein interactions mediated by Src-family SH2 domains

Bianca Sperl [a], Markus H. J. Seifert [b], Thorsten Berg [a,*]

[a] Department of Molecular Biology, Max Planck Institute of Biochemistry, and Center for Integrated Protein Science Munich (CIPSM), Am Klopferspitz 18, 82152 Martinsried, Germany
[b] 4SC AG, Am Klopferspitz 19a, 82152 Martinsried, Germany

ARTICLE INFO

Article history:
Received 11 February 2009
Revised 15 April 2009
Accepted 17 April 2009
Available online 23 April 2009

ABSTRACT

In this Letter, we report the natural products salvianolic acid A, salvianolic acid B, and caftaric acid as inhibitors of the protein–protein interactions mediated by the SH2 domains of the Src-family kinases Src and Lck, two established disease targets. Moreover, we propose a binding mode for the inhibitors based on molecular modeling, which will facilitate chemical optimization efforts of these important lead structures for drug discovery.

Protein–protein interactions form the basis for virtually all aspects of molecular signaling.[1] The development or discovery of highly potent and selective antagonists of protein–protein interactions,[2,3] some of which are even undergoing clinical trials,[4,5] has impressively demonstrated the principal feasibility of targeting protein–protein interactions.[6–8] Although rational approaches starting from native peptide sequences have yielded excellent inhibitors of protein–protein interactions in some cases,[9–12] there is a general lack of drug-like lead structures for inhibitors of protein–protein interactions. This is particularly the case for intracellular protein–protein interactions which are mediated by phosphorylated amino acids, such as the interactions between phosphotyrosine-containing sequences and Src homology (SH) 2 domains or phosphotyrosine binding (PTB) domains,[13] or between phosphoserine/phosphothreonine-containing motifs and 14-3-3 proteins, WW domains, FHA domains, WD40 repeats, or polo-box domains (PBD).[14] Rationally designed inhibitors against such interactions which bear the side chain of the respective phosphorylated amino acid tend to display unfavorable activity and stability profiles in tissue culture, presumably due to poor cellular uptake across the hydrophobic cell membrane, and poor intracellular stability due to cleavage of the phosphate group by intracellular phosphatases. Non-cleavable and/or non-ionic phosphate mimetics tend to display significantly lower affinities for their targets than their natural counterparts. Therefore, small-molecule lead structures which target protein–protein interactions mediated by phosphorylated amino acids are highly sought after.

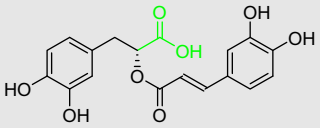
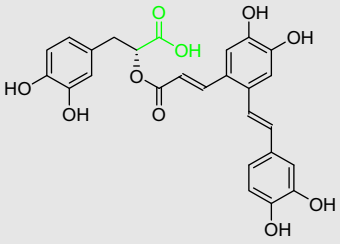
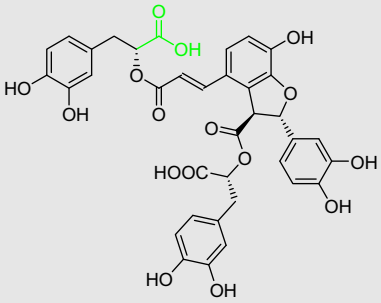
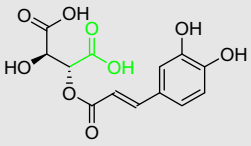
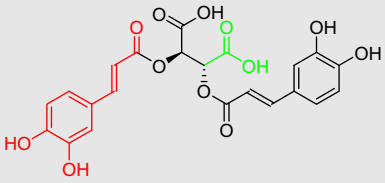
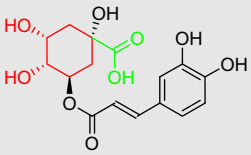
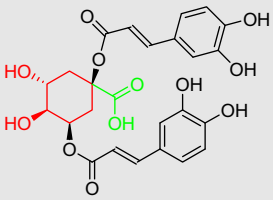
In view of the vast size of chemical space, natural products have been suggested as biologically privileged starting points for drug discovery efforts.[15,16] Unfortunately, only a limited number of natural products have been reported as inhibitors of protein–protein interactions to date.[17–19] Natural product inhibitors of phosphorylation-dependent protein–protein interactions are particularly rare. Examples include thymoquinone as an inhibitor of several interactions mediated by phosphotyrosine, phosphoserine and phosphothreonine;[20] 8-*O*-methylsclerotiorinamine,[21] the RNA polymerase inhibitor actinomycin D and its derivatives actinomycin C2 and VII,[22,23] as well as asterriquinones[24] as antagonists of the Grb2-SH2 domain; and finally, rosmarinic acid, which has been reported to inhibit the interaction between the SH2 domain of the Src-family tyrosine kinase Lck and proteins bearing a pYEEI-motif.[25–28] Since Lck is a crucial mediator of signaling in immune cells, this biological activity provides one possible rationale for the experimental use of rosmarinic acid as an immunosuppressive agent.[27,29,30] Rosmarinic acid has also been reported to inhibit the SH2 domain of Src itself,[27] which has been the focus of numerous drug discovery efforts as a consequence of its role as target for cancer therapy.[31] Several highly potent antagonists of the SH2 domains of Src and Lck derived from the proteins' shared phosphotyrosine binding motif have been developed in the recent past.[32–39]

In an effort to discover additional natural products which inhibit protein–protein interactions mediated by SH2 domains, we tested compounds which bear similarity to rosmarinic acid (**1**) against a small panel of SH2 domains in fluorescence polarization assays.[40–42] Rosmarinic acid (**1**) inhibited the interaction between the SH2 domain of Lck and the peptide 5-carboxyfluorescein-GpYEEIP with an IC_{50} of 55 ± 2 μM (Table 1 and Fig. 1A). Binding of the SH2 domain of c-Src to the same phosphotyrosine-containing peptide was inhibited more potently (IC_{50} = 26 ± 2 μM). In contrast, the SH2 domains of the STAT family members STAT1, STAT3, and STAT5b were inhibited only at significantly higher concentrations. Salvianolic acid A (**2**) turned out to be a more potent antagonist of the Lck SH2 domain (IC_{50} = 23.5 ± 0.4 μM) than rosmarinic acid. Similarly to rosmarinic acid, it also inhibited the Src SH2 domain (IC_{50} = 36.2 ± 0.9 μM), and, to a much lesser extent, the STAT

* Corresponding author. Tel.: + 49 89 8578 2535; fax: + 49 89 8578 2454.
E-mail address: berg@biochem.mpg.de (T. Berg).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.083

Table 1
Activities of compounds **1**–**7** against the function of the SH2 domains of Lck, Src, STAT1, STAT3, and STAT5b

Compound	Structure	Lck apparent IC_{50}[a] (μM) or inhibition[a] (%)	Src apparent IC_{50}[a] (μM) or inhibition[a] (%)	STAT1 apparent IC_{50}[a] (μM) or inhibition[a] (%)	STAT3 apparent IC_{50}[a] (μM) or inhibition[a] (%)	STAT5b apparent IC_{50}[a] (μM) or inhibition[a] (%)
1		55 ± 2	26 ± 2	38.0 ± 0.6% inhibition at 200 μM	34 ± 1% inhibition at 200 μM	32.6 ± 0.6% inhibition at 200 μM
2		23.5 ± 0.4	36.2 ± 0.9	41 ± 1% inhibition at 200 μM	32 ± 2% inhibition at 200 μM	30.2 ± 0.6% inhibition at 200 μM
3		41 ± 2	90 ± 27	15 ± 5% inhibition at 200 μM	19 ± 3% inhibition at 200 μM	3.6 ± 0.5% inhibition at 200 μM
4		118 ± 16	74 ± 5	19 ± 2% inhibition at 200 μM	30 ± 3% inhibition at 200 μM	31.2 ± 0.7% inhibition at 200 μM
5		9 ± 4% inhibition at 200 μM	12 ± 3% inhibition at 200 μM	0 ± 1% inhibition at 200 μM	12 ± 1% inhibition at 200 μM	11 ± 1% inhibition at 200 μM
6		12 ± 4% inhibition at 200 μM	3 ± 2% inhibition at 200 μM	−3 ± 1% inhibition at 200 μM	5 ± 2% inhibition at 200 μM	−1 ± 1% inhibition at 200 μM
7		12 ± 3% inhibition at 200 μM	7 ± 1% inhibition at 200 μM	4 ± 1% inhibition at 200 μM	7 ± 4% inhibition at 200 μM	−1 ± 2% inhibition at 200 μM

The carboxylic acid moiety which is considered to be the phosphate mimetic as indicated by the docking studies is depicted in green. The chemical entities depicted in red are assumed to be the underlying cause for the inactivity of compounds **5**–**7** as indicated by the docking experiments. The IC_{50} values of the positive control peptide GpYEEIP against Lck and Src are 257 ± 14 nM and 1626 ± 65 nM, respectively.

[a] Values are means of three experiments ± standard error of the mean.

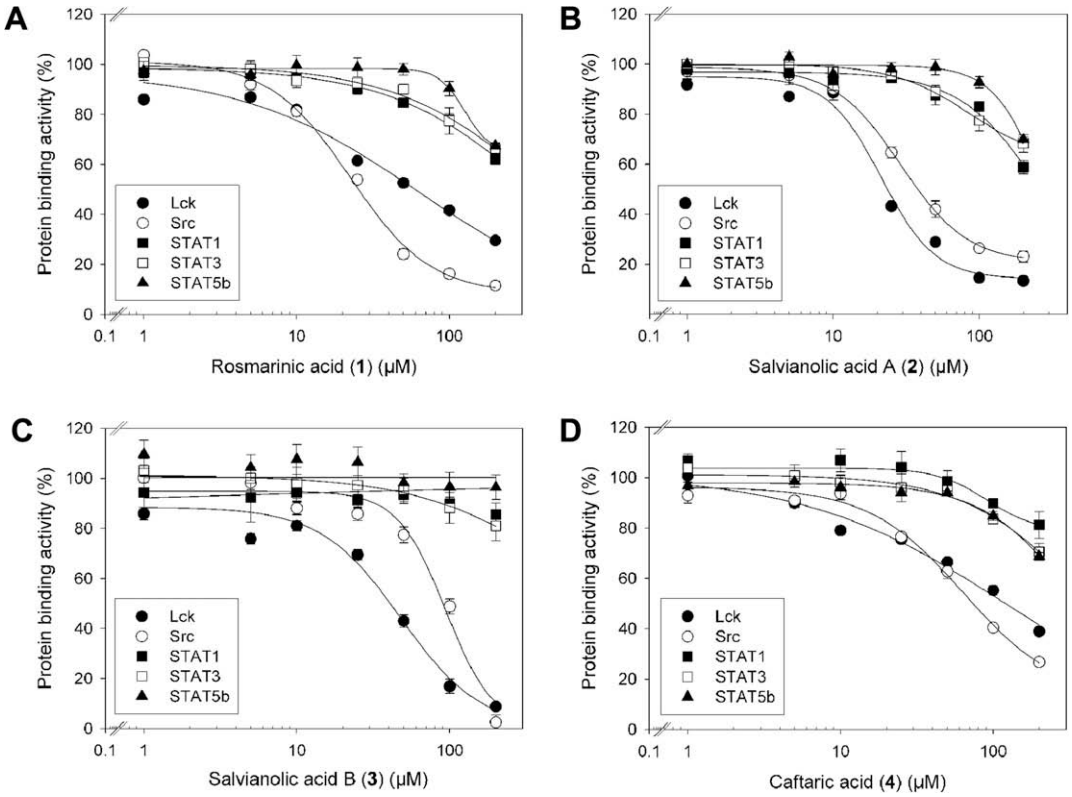


Figure 1. Activity profiles of compounds **1**–**4** against the function of the SH2 domains of Lck, Src, STAT1, STAT3, and STAT5b.

SH2 domains (Table 1 and Fig. 1B). The activity of salvianolic acid B (**3**) against the Lck SH2 domain (IC_{50} = 41 ± 2 μM) was somewhat lower than that of salvianolic acid A, but still higher than that of rosmarinic acid (Table 1 and Fig. 1C). With IC_{50} values of 118 μM and 74 μM against the SH2 domains of Lck and Src, respectively, caftaric acid (**4**) displays 2–3-fold lower activity than rosmarinic acid (Table 1 and Fig. 1D). In contrast to compounds **1**–**4**, cichoric acid (**5**), chlorogenic acid (**6**), and cynarin (**7**) did not show significant activities against any of the investigated SH2 domains.

In order to rationalize the activity of rosmarinic acid, we performed molecular modeling studies based on the published crystal structure of the Lck SH2 domain bound to the peptide motif pYEEI.[43] Its phosphotyrosine residue makes key interactions with the Lck amino acids Arg134, Arg154, Glu157, Ser158, and Lys182 (Fig. 2A). Two of the oxygen atoms of the phosphate group form bidentate interactions with the guanidinium group of Arg154. One of these oxygens also interacts with Arg134; the other one forms an additional hydrogen bond with the main chain amide proton of Glu157. The third oxygen forms a hydrogen bond with the hydroxyl group of Ser158. The aromatic ring of the phosphotyrosine interacts with the side chains of Lck Arg134 and Lys182. The amide group of glutamate at the pY+1 position forms a strong interaction with the carbonyl oxygen of His180, and the side chain of glutamate at the pY+2 position is located in the vicinity of Arg184. The isoleucine in the pY+3 position is located in a binding pocket in part formed by Arg196.

Molecular modeling predicted that the carboxylic acid moiety of rosmarinic acid forms hydrogen bonds with Arg154, Ser164, and Lys182 (Fig. 2B). The carboxyl group involved in the linkage between the two caffeic acid subunits may bind to Arg134, depending on the conformational state of Arg134 (the X-ray structure of the pYEEI-bound Lck SH2 domain shows two conformational states for Arg134).[43] However, it is more likely that one of the catechol subunits forms hydrogen bonds with Arg134 and Glu157 (Fig. 2B and Supplementary Fig. S1); the other catechol is predicted to interact with Arg184. Thus, rosmarinic acid is likely to bind to the Lck SH2 domain by forming interactions which are critically important for binding of the preferred binding motif pYEEI.

In order to test the validity of the binding pose, we subjected the rosmarinic acid-derived natural products **2**–**7** to the docking experiments. Both salvianolic acids A (**2**) and B (**3**), which share the core structure of rosmarinic acid (**1**), were predicted to bind to the Lck SH2 domain based on the same interactions as rosmarinic acid, only with additional hydrophobic and hydrophilic contacts providing increased affinity. One of the hydroxyl groups of the additional catechol unit present in salvianolic acid A (**2**) can form a hydrogen bond with Lys179 of Lck (Fig. 2C). Salvianolic acid B can additionally interact with Glu174. However, these surface-exposed hydrophilic interactions do not necessarily increase the binding affinity. Furthermore, the large number of additional rotatable bonds in salvianolic acid B leads to an increased entropy penalty upon binding to Lck (Fig. 2D).[44] While both salvianolic acids A and B address additional solvent-exposed hydrophilic interactions compared to rosmarinic acid, our modeling revealed that neither of them fill the hydrophobic pocket usually occupied by the isoleucine side chain of the pYEEI motif, suggesting the possibility of improvement of compound activities by chemical modification. The additional carboxylate of caftaric acid is tightly coordinated to Arg134, Ser156, and Ser158, which partially compensates for the missing interactions conferred by one of the additional catechol groups in rosmarinic acid (Fig. 2E and Supplementary Fig. S1).

Molecular modeling suggested that the natural products cichoric acid (**5**), chlorogenic acid (**6**), and cynarin (**7**), which were found to be inactive in the fluorescence polarization assay (Table 1), are unable to bind to the phosphate binding pocket without either causing significant distortions of the protein structure [in case of cichoric acid (**5**)] or forming geometrically sub-optimal hydrogen

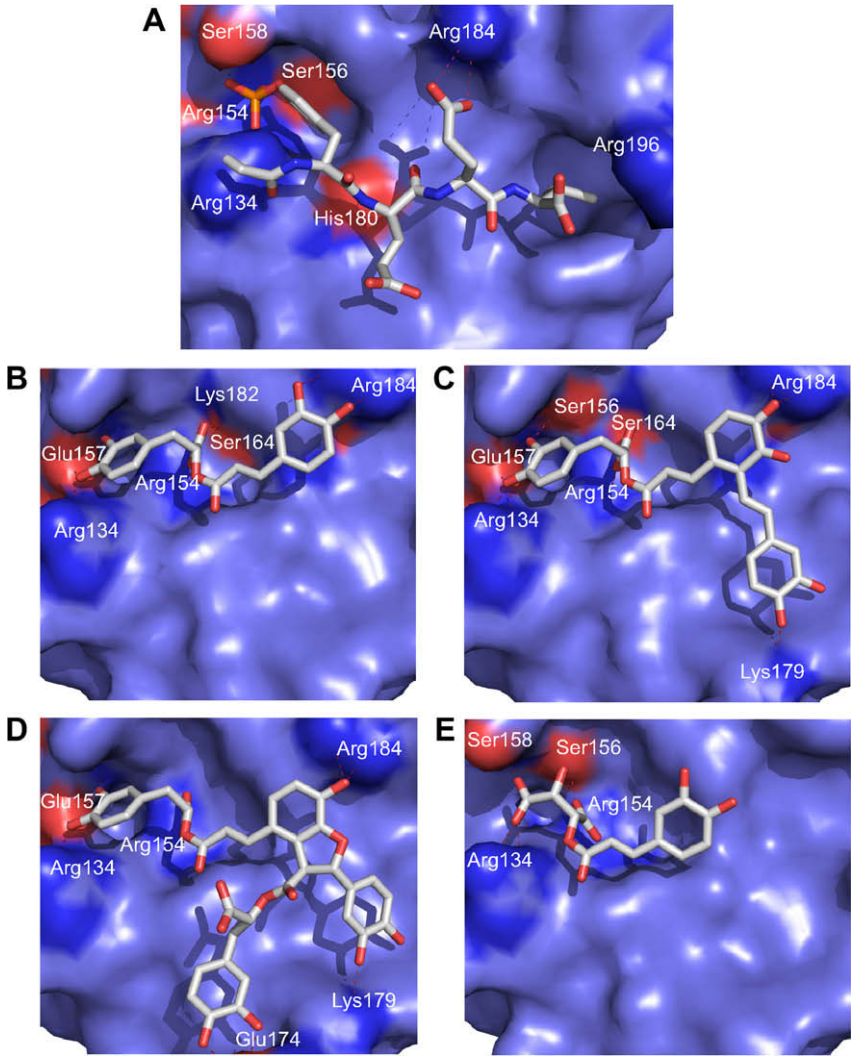


Figure 2. Model for binding of organic ligands to the Lck SH2 domain. (A) pYEEI (taken from PBD entry 1LKK;[43] (B) predicted binding mode of rosmarinic acid (**1**); (C) salvianolic acid A (**2**); (D) salvianolic acid B (**3**); and (E) caftaric acid (**4**). The figure was generated using PyMOL.[49]

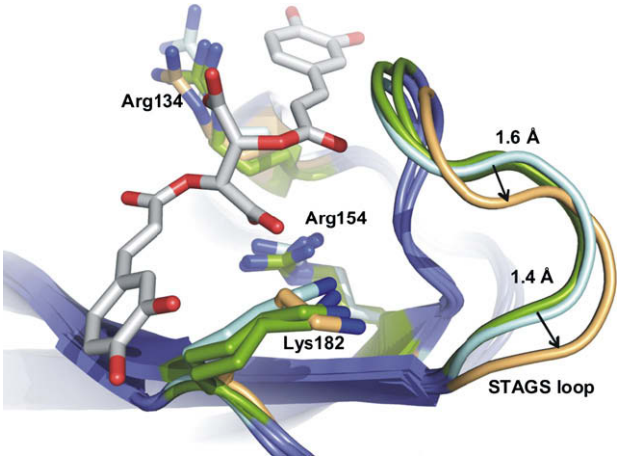


Figure 3. Binding of cichoric acid (**5**) to the Lck SH2 domain as predicted by molecular modeling would require a significant shift of the loop formed by amino acids 158–162 (the STAGS loop). The conformation of the STAGS loop of Lck when bound to various ligands is depicted in the following color code: cyan: Lck bound to pYEEI; green: Lck bound to rosmarinic acid (**1**) or caftaric acid (**4**); gold: Lck bound to cichoric acid (**5**). Cichoric acid itself is shown in silver. The other ligands are not depicted for clarity. The figure was generated using PyMOL.[49]

bonds [in case of chlorogenic acid (**6**) and cynarin (**7**)]. For example, while the flexibility of the phosphate binding pocket allows for an accommodation of cichoric acid (**5**), this would require a shift of the ^{158}STAGS^{162} motif (STAGS loop) covering the pocket by 1.4–1.6 Å as compared to pYEEI, rosmarinic acid (**1**), and caftaric acid (**4**) (Fig. 3). This distortion of the structure leads to imperfect protein–ligand hydrogen bonding as indicated by the hydrogen bonding interaction energies (MAB force field)[45] of −168.1, −70.6, −65.1, and −36.4 for pYEEI, rosmarinic acid, caftaric acid, and cichoric acid, respectively. This decrease in interaction energies cannot be attributed solely to the differences in the number of hydrogen bond donors and acceptors between the ligands: for example, cichoric acid (**5**) clearly has more hydrogen bonding possibilities than rosmarinic acid (**1**), but is not able to exploit this potential due to its steric demand.

In summary, we have discovered three natural product inhibitors of protein–protein interaction relevant for human diseases. Salvianolic acid A, salvianolic acid B, and to a lesser extent, caftaric acid, were demonstrated to inhibit binding between the SH2 domains of Lck and Src and their phosphotyrosine-containing binding motif. Rosmarinic acid and salvianolic acids A and B are components of the roots of *Salvia miltiorrhiza* (Danshen), extracts of which have long documented use in Asian countries for the treatment of

cardiovascular and cerebrovascular diseases.[46,47] Since inhibition of the Lck SH2 domain has been suggested as a possible underlying molecular cause for the immunosuppressant activity of rosmarinic acid,[27] and salvianolic acid A is at least twofold more active than rosmarinic acid against this target, it could also be tested for its suitability as an immunosuppressive agent.[29,30] However, it should be noted that despite the increased activity of salvianolic acid A (**2**) against Lck as compared to rosmarinic acid (**1**), the ligand binding efficiencies of the compounds are approximately equal because of the higher molecular weight of salvianolic acid A.[48] Moreover, we have proposed a potential binding model for the inhibitor–drug interaction which is consistent with the observed structure–activity relationships, and which therefore will be instrumental for the development of more potent natural product-inspired lead compounds against Src-family SH2 domains.

Acknowledgments

This work was generously supported by the Department of Molecular Biology (Director: Professor Axel Ullrich) at the Max Planck Institute of Biochemistry, and the Bundesministerium für Bildung und Forschung (NGFN-2, Grant 01GS0451 to T.B.). We extend our thanks to Martin Gräber for experimental support, Dr. Brigitte Biesinger (University of Erlangen, Germany) for providing the pGEX-LckSH2 expression plasmid, and to Angela Hollis for critical reading of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.083.

References and notes

1. Alberts, B. *Cell* **1998**, *92*, 291.
2. Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844.
3. Oltersdorf, T.; Elmore, S. W.; Shoemaker, A. R.; Armstrong, R. C.; Augeri, D. J.; Belli, B. A.; Bruncko, M.; Deckwerth, T. L.; Dinges, J.; Hajduk, P. J.; Joseph, M. K.; Kitada, S.; Korsmeyer, S. J.; Kunzer, A. R.; Letai, A.; Li, C.; Mitten, M. J.; Nettesheim, D. G.; Ng, S.; Nimmer, P. M.; O'Connor, J. M.; Oleksijew, A.; Petros, A. M.; Reed, J. C.; Shen, W.; Tahir, S. K.; Thompson, C. B.; Tomaselli, K. J.; Wang, B.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H. *Nature* **2005**, *435*, 677.
4. Dechantsreiter, M. A.; Planker, E.; Matha, B.; Lohof, E.; Holzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.
5. Tse, C.; Shoemaker, A. R.; Adickes, J.; Anderson, M. G.; Chen, J.; Jin, S.; Johnson, E. F.; Marsh, K. C.; Mitten, M. J.; Nimmer, P.; Roberts, L.; Tahir, S. K.; Xiao, Y.; Yang, X.; Zhang, H.; Fesik, S.; Rosenberg, S. H.; Elmore, S. W. *Cancer Res.* **2008**, *68*, 3421.
6. Berg, T. *Curr. Opin. Drug. Discov. Dev.* **2008**, *11*, 666.
7. Wells, J. A.; McClendon, C. L. *Nature* **2007**, *450*, 1001.
8. Gonzalez-Ruiz, D.; Gohlke, H. *Curr. Med. Chem.* **2006**, *13*, 2607.
9. Heckmann, D.; Meyer, A.; Marinelli, L.; Zahn, G.; Stragies, R.; Kessler, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 3571.
10. Heckmann, D.; Meyer, A.; Laufer, B.; Zahn, G.; Stragies, R.; Kessler, H. *Chembiochem.* **2008**, *9*, 1397.
11. Goodman, S. L.; Holzemann, G.; Sulyok, G. A.; Kessler, H. *J. Med. Chem.* **2002**, *45*, 1045.
12. Gibson, C.; Sulyok, G. A.; Hahn, D.; Goodman, S. L.; Holzemann, G.; Kessler, H. *Angew. Chem., Int. Ed.* **2001**, *40*, 165.
13. Schlessinger, J.; Lemmon, M. A. *Sci. STKE* **2003**, *2003*, RE12.
14. Yaffe, M. B.; Smerdon, S. J. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 225.
15. Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2879.
16. Dekker, F. J.; Koch, M. A.; Waldmann, H. *Curr. Opin. Chem. Biol.* **2005**, *9*, 232.
17. Duncan, S. J.; Gruschow, S.; Williams, D. H.; McNicholas, C.; Purewal, R.; Hajek, M.; Gerlitz, M.; Martin, S.; Wrigley, S. K.; Moore, M. *J. Am. Chem. Soc.* **2001**, *123*, 554.
18. Nikolovska-Coleska, Z.; Xu, L.; Hu, Z.; Tomita, Y.; Li, P.; Roller, P. P.; Wang, R.; Fang, X.; Guo, R.; Zhang, M.; Lippman, M. E.; Yang, D.; Wang, S. *J. Med. Chem.* **2004**, *47*, 2430.
19. Lepourcelet, M.; Chen, Y. N.; France, D. S.; Wang, H.; Crews, P.; Petersen, F.; Bruseo, C.; Wood, A. W.; Shivdasani, R. A. *Cancer Cell* **2004**, *5*, 91.
20. Reindl, W.; Yuan, J.; Kramer, A.; Strebhardt, K.; Berg, T. *Chem. Biol.* **2008**, *15*, 459.
21. Nam, J. Y.; Kim, H. K.; Kwon, J. Y.; Han, M. Y.; Son, K. H.; Lee, U. C.; Choi, J. D.; Kwon, B. M. *J. Nat. Prod.* **2000**, *63*, 1303.
22. Kim, H. K.; Nam, J. Y.; Han, M. Y.; Lee, E. K.; Choi, J. D.; Bok, S. H.; Kwon, B. M. *FEBS Lett.* **1999**, *453*, 174.
23. Kim, H. K.; Nam, J. Y.; Han, M. Y.; Son, K. H.; Choi, J. D.; Kwon, B. M.; Takusagawa, H. L.; Huang, Y.; Takusagawa, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1455.
24. Alvi, K. A.; Pu, H.; Luche, M.; Rice, A.; App, H.; McMahon, G.; Dare, H.; Margolis, B. *J. Antibiot.* **1999**, *52*, 215.
25. Ahn, S. C.; Oh, W. K.; Kim, B. Y.; Kang, D. O.; Kim, M. S.; Heo, G. Y.; Ahn, J. S. *Planta Med.* **2003**, *69*, 642.
26. Park, S. H.; Kang, S. H.; Lim, S. H.; Oh, H. S.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3455.
27. Won, J.; Hur, Y. G.; Hur, E. M.; Park, S. H.; Kang, M. A.; Choi, Y.; Park, C.; Lee, K. H.; Yun, Y. *Eur. J. Immunol.* **2003**, *33*, 870.
28. Park, S. H.; Oh, H. S.; Kang, M. A.; Cho, H.; Prasad, J. B.; Won, J.; Lee, K. H. *Bioorg. Med. Chem.* **2007**, *15*, 3938.
29. Jung da, Y.; Kim, E. Y.; Joo, S. Y.; Park, J. B.; Moon, C.; Kim, S. H.; Sim, E. Y.; Joh, J. W.; Kwon, C. H.; Kwon, G. Y.; Kim, S. J. *Exp. Mol. Med.* **2008**, *40*, 1.
30. Yun, S. Y.; Hur, Y. G.; Kang, M. A.; Lee, J.; Ahn, C.; Won, J. *Transplantation* **2003**, *75*, 1758.
31. Shakespeare, W. C. *Curr. Opin. Chem. Biol.* **2001**, *5*, 409.
32. Sundaramoorthi, R.; Shakespeare, W. C.; Keenan, T. P.; Metcalf, C. A., 3rd; Wang, Y.; Mani, U.; Taylor, M.; Liu, S.; Bohacek, R. S.; Narula, S. S.; Dalgarno, D. C.; van Schravandijk, M. R.; Violette, S. M.; Liou, S.; Adams, S.; Ram, M. K.; Keats, J. A.; Weigle, M.; Sawyer, T. K.; Weigele, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3063.
33. Shakespeare, W. C.; Bohacek, R. S.; Azimioara, M. D.; Macek, K. J.; Luke, G. P.; Dalgarno, D. C.; Hatada, M. H.; Lu, X.; Violette, S. M.; Bartlett, C.; Sawyer, T. K. *J. Med. Chem.* **2000**, *43*, 3815.
34. Shakespeare, W.; Yang, M.; Bohacek, R.; Cerasoli, F.; Stebbins, K.; Sundaramoorthi, R.; Azimioara, M.; Vu, C.; Pradeepan, S.; Metcalf, C., 3rd; Haraldson, C.; Merry, T.; Dalgarno, D.; Narula, S.; Hatada, M.; Lu, X.; van Schravendijk, M. R.; Adams, S.; Violette, S.; Smith, J.; Guan, W.; Bartlett, C.; Herson, J.; Iuliucci, J.; Weigele, M.; Sawyer, T. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9373.
35. Violette, S. M.; Shakespeare, W. C.; Bartlett, C.; Guan, W.; Smith, J. A.; Rickles, R. J.; Bohacek, R. S.; Holt, D. A.; Baron, R.; Sawyer, T. K. *Chem. Biol.* **2000**, *7*, 225.
36. Shakespeare, W. C.; Wang, Y.; Bohacek, R.; Keenan, T.; Sundaramoorthi, R.; Metcalf, C., 3rd; Dilauro, A.; Roeloffzen, S.; Liu, S.; Saltmarsh, J.; Paramanathan, G.; Dalgarno, D.; Narula, S.; Pradeepan, S.; van Schravendijk, M. R.; Keats, J.; Ram, M.; Liou, S.; Adams, S.; Wardwell, S.; Bogus, J.; Iuliucci, J.; Weigele, M.; Xing, L.; Boyce, B.; Sawyer, T. K. *Chem. Biol. Drug Des.* **2008**, *71*, 97.
37. Boyce, B. F.; Xing, L.; Yao, Z.; Shakespeare, W. C.; Wang, Y.; Metcalf, C. A., 3rd; Sundaramoorthi, R.; Dalgarno, D. C.; Iuliucci, J. D.; Sawyer, T. K. *Ann. NY Acad. Sci.* **2006**, *1068*, 447.
38. Shakespeare, W. C.; Metcalf, C. A., 3rd; Wang, Y.; Sundaramoorthi, R.; Keenan, T.; Weigele, M.; Bohacek, R. S.; Dalgarno, D. C.; Sawyer, T. K. *Curr. Opin. Drug Discov. Dev.* **2003**, *6*, 729.
39. Costantino, L.; Barlocco, D. *Curr. Med. Chem.* **2004**, *11*, 2725.
40. Lynch, B. A.; Loiacono, K. A.; Tiong, C. L.; Adams, S. E.; MacNeil, I. A. *Anal. Biochem.* **1997**, *247*, 77.
41. Schust, J.; Berg, T. *Anal. Biochem.* **2004**, *330*, 114.
42. Muller, J.; Schust, J.; Berg, T. *Anal. Biochem.* **2008**, *375*, 249.
43. Tong, L.; Warren, T. C.; King, J.; Betageri, R.; Rose, J.; Jakes, S. *J. Mol. Biol.* **1996**, *256*, 601.
44. Gohlke, H.; Klebe, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 2644.
45. Gerber, P. R.; Muller, K. *J. Comput. Aided Mol. Des.* **1995**, *9*, 251.
46. Han, J. Y.; Fan, J. Y.; Horie, Y.; Miura, S.; Cui, D. H.; Ishii, H.; Hibi, T.; Tsuneki, H.; Kimura, I. *Pharmacol. Ther.* **2008**, *117*, 280.
47. Zhou, L.; Zuo, Z.; Chow, M. S. *J. Clin. Pharmacol.* **2005**, *45*, 1345.
48. Reynolds, C. H.; Tounge, B. A.; Bembenek, S. D. *J. Med. Chem.* **2008**, *51*, 2432.
49. DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientifc: Palo Alto, CA, USA, 2002.