Bioorganic & Medicinal Chemistry Letters 19 (2009) 5613–5616

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure-based design of novel human Pin1 inhibitors (I)

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ARTICLE INFO

Article history:
Received 18 June 2009
Revised 7 August 2009
Accepted 7 August 2009
Available online 13 August 2009

Keywords:
Pin1
PPIase
Anticancer
SBDD
Pin1 inhibitor

ABSTRACT

Pin1 is a member of the *cis–trans* peptidyl-prolyl isomerase family with potential anti-cancer therapeutic value. Here we report structure-based de novo design and optimization of novel Pin1 inhibitors. Without a viable lead from internal screenings, we designed a series of novel Pin1 inhibitors by interrogating and exploring a protein crystal structure of Pin1. The ligand efficiency of the initial concept molecule was optimized with integrated SBDD and parallel chemistry approaches, resulting in a more attractive lead series.

Originally identified in a yeast two-hybrid screen, Pin1 (Protein Interacting with NIMA) interacts physically and functionally with the mitotic kinase NIMA (Never In Mitosis gene A).[1] Pin1 is a member of peptidyl-prolyl *cis–trans* isomerase (PPIase) family.[1] Pin1 substrate proteins are important in cell-cycle regulation and are commonly deregulated in human cancer cells. The enzyme plays a key role in negatively regulating entry into mitosis and is also required for proper progression through mitosis.[2,3] Depletion of Pin1 causes mitotic arrest and apoptosis in budding yeast and tumor cell lines.[1] Pin1 was found to be up-regulated in a wide variety of human tumors and its over-expression correlates with tumor grade.[4] Inhibition of Pin1 presents a new opportunity for anticancer therapy.

Pin1 consists of two structural domains: a catalytic C-terminal PPIase domain that performs the rotamase function and an N-terminal WW domain[5] presumably for substrate recognition. Its catalytic site is unique among other PPIase's because it recognizes an unusual phosphorylated Ser/Thr-Pro motif in its substrates. The first crystal structures of Pin1 have been reported where an Ala-Pro dipeptide fragment[5] was observed in the PPIase catalytic site.

Pin1 inhibitor development began with an irreversible, small molecule inhibitor, Juglone (**1**, Fig. 1),[6] that was useful in probing Pin1 biology. Reversible inhibitor design was initially based on a substrate inhibitor, Pintide (**2**, Fig. 1), which achieved low μM potency.[7] Subsequent studies led to reversible phosphorylated inhibitors with low nM inhibitions of Pin1, including peptidomimetic series (**3**, Fig. 1)[8] and non-peptidomimetic series.[9,10] The binding conformation of these phosphorylated series have been characterized crystallographically.[10,11] Other novel phosphorylated Pin1 inhibitors were also reported.[12,13] Recently, moderately potent (K_i μM-range) non-phosphorylated small molecule inhibitors[14–17] such as thiazolinediones (**4**, Fig. 1)[17] have been reported. We, herein describe our design and synthesis of potent, reversible, non-peptidic small molecular Pin1 inhibitors.

The initial goal of our Pin1 program was to discover a Pin1 small molecular inhibitor that can be used to investigate and exploit potential oncology indications. In order to identify inhibitory hits, more than one million compounds were screened twice with a Pin1 fluorescence polarization binding assay[18] and a Pin1 enzymatic assay.[18,19] While there were apparent hits from the high-throughput screening (HTS), none of these could be confirmed in secondary assays such as iso-thermal calorimetric titration[20] and NMR study[21] on ligand-protein interaction.

To seek a potential starting point for design, we evaluated inhibitors of other PPIase family members. FKBP-12 is a known PPIase with well-characterized small molecular inhibitors.[22] Since both Pin1 and FKBP recognize proline residues as part of the substrate epitope, a collection of more than 200 inhibitors of FKBP-12 with diverse structures were screened against Pin1.

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doi:10.1016/j.bmcl.2009.08.034

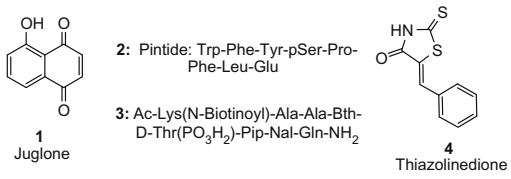


Figure 1. Representative known Pin1 inhibitors.

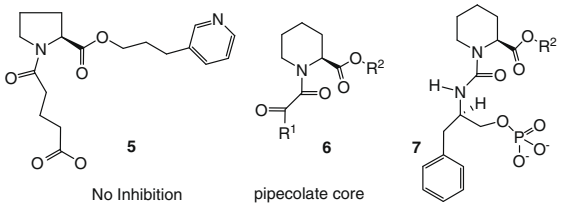


Figure 3. Design based on binding mode of FKBP inhibitors.

Unfortunately, no hit was discovered from this effort. Although the screening campaign failed, a concurrent protein crystallization effort provided the necessary starting point to launch a lead discovery effort. A genetically engineered Pin1 catalytic domain, construct K7782Q,[10] was found to crystallize readily,[18] producing X-ray apo structure (Fig. 2) and co-crystal structure with citric acid (data not shown). In comparison with the published Pin1 crystal structure (Fig. 2, orange),[5] our apo structure does not contain the N-terminal WW domain, but aligned well with published structure in the PPIase domain.

The apo Pin1 K7782Q catalytic domain structure provided a starting point for inhibitor design. While the apo structure does not contain sulfate ion, the positively charged recognition pocket formed by Lys-63, Arg-68, and Arg-69 was occupied by crystal water molecules or a citric acid molecule. A crystal water molecule was also located in a canyon region shaped by Arg-69, Ser-114, and Trp-73. The prolyl binding pocket formed by His-59, Phe-134, and His-157 is filled with water molecules. In comparison with FKBP-12,[23] the Pin1 catalytic site presents a rich network of H-bond potential with less hydrophobic surface and a somewhat larger binding site.

Based on the similarities of Pin1 to the FKBP-12 active site, a proline ester core was investigated. The existence of a positively charged pocket in the Pin1 active site inspired a simple inhibitor (**5**, Fig. 3) which was designed to fill the proline-binding site and the adjacent charged pocket. It showed no inhibition of the Pin1 catalytic function, suggesting that additional binding interactions are necessary. For example, the carboxylate may not bind with affinity comparable to the phosphate of natural substrate. Alternatively, the carboxylate may not be positioned optimally to satisfy the Pin1 phosphate pocket.

Pipecolate core **6** is a well-known scaffold for FKBP inhibitors. Compared with FKBP-12's binding pocket (PDB ID: 1FKG),[23] Pin1 active site has additional Ser-154 and Gln-131 residues, which provides two hydrogen bond donors to the putative ketoamide moiety in classic FKBP inhibitors. We modeled[24] the pipecolate core (**7**) into Pin1 catalytic site based on its binding conformation in FKBP co-crystal structure as a guide. Additional binding interaction was targeted by building a phenyl fragment to interact with the canyon formed by Arg-69 and Ser-114 (Fig. 4). This phenyl group not only could potentially form a hydrophobic stacking interaction[25] with the side chain of Arg-69, it could also provide a vector to reach the hydrophobic pocket formed by Trp-73 and Ala-116. As a result, compound **11** (Scheme 1) was designed to reach the charged pocket, the proline-binding site and the Trp-73 canyon to maximize potential interactions with the R-beta-amino alcohol appears to fit better.

Compound **11** was synthesized readily from known intermediate **8** in five steps (Scheme 1). The yields reported here for urea formation (from **8** to **9**) and phosphate ester formation (from **9** to **10**) are un-optimized. The five step sequence is mild and robust,[9] allowing a number of analogs with various central cores (i.e., pipecolate replacements) to be synthesized via a similar route. Compound **11** was determined to be a low micromolar Pin1 inhibitor. The positive result provided the initial validation for further structure-based design and optimization of the de novo lead. Analogs of **11** were designed and synthesized, targeting the optimal ester and proline-derived inhibitor components. Among them, pipecolate analogs appeared to be most active, with low micromolar to submicromolar K_i in the Pin1 enzymatic assay.

We found that the charged interactions are crucial. In comparison to the phosphate, the corresponding sulfate **12a** was significantly

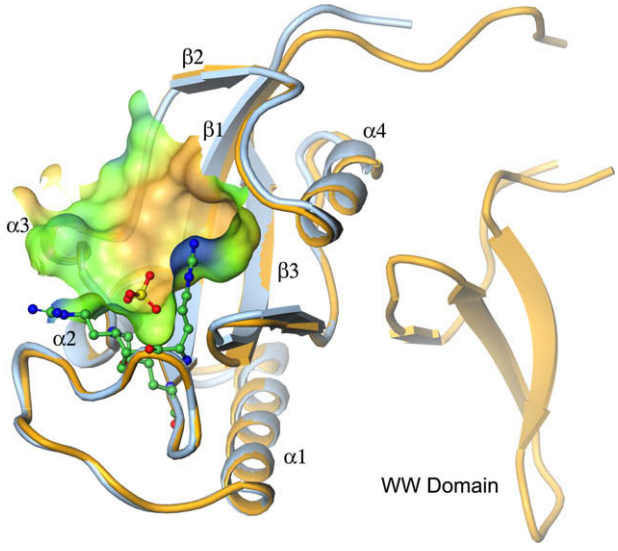


Figure 2. X-ray apo structure of Pin1 K7782Q (light blue, PDB ID: 3IK8) versus full length Pin1 (orange, PDB ID: 1PIN, Ref. 5). Binding site surface, Lys-63, Arg-68, and Arg-69 are also shown. The surface is colored by hydrophobicity (brown = hydrophobic area).

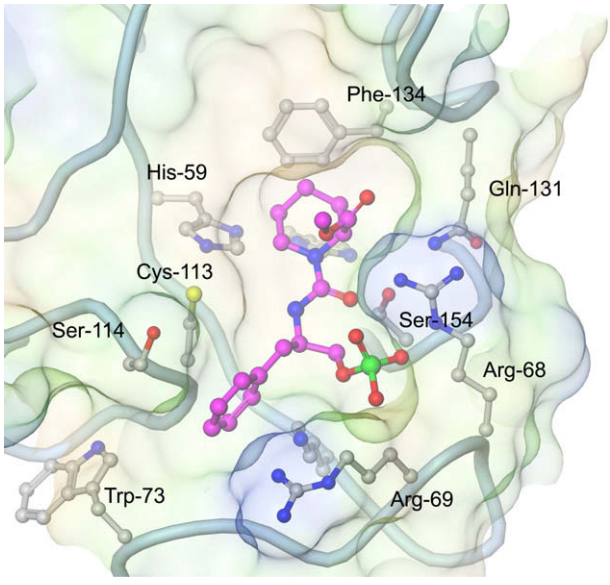
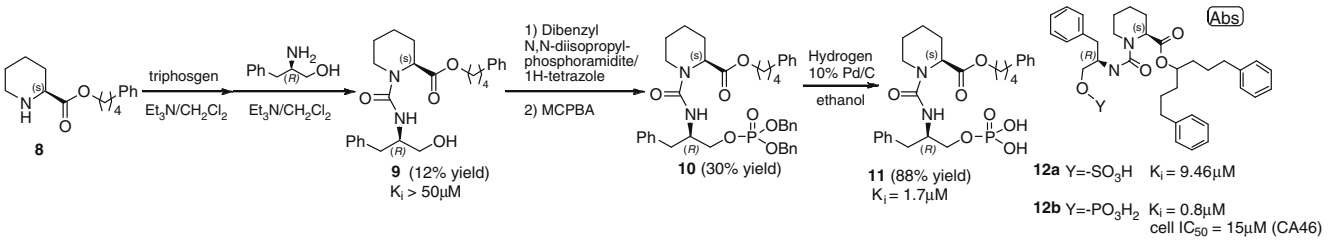


Figure 4. Predicted binding mode of pipecolate-based Pin1 inhibitors.



Scheme 1.

less potent (>10× loss) and the alcohol core **9** was inactive. The binding of these compounds was further confirmed by both iso-thermal calorimetric titration and NMR study. Compound **12b** is the most potent compound in the series with an affinity (K_i) of 0.825 μM. It also inhibited the growth of cancer cells with Pin1 mechanism-liked phenotypical cell-cycle effects (IC_{50} = 15 μM). As expected, this series of compounds demonstrated significant selectivity over FKBP despite containing a pipecolate fragment, a putative FKBP binding motif. For example, compound **12b** had a much weaker binding affinity to FKBP-12 (K_i = 137 μM). Compound **12b** was synthesized in a route similar to the synthesis of compound **11**.

Optimization of potency and ligand efficiency. Despite achieving modest cellular activity, the phosphate containing pipecolate Pin1 inhibitors demonstrated shallow SAR and their potency were limited to low micromolar range. Additionally, the design was focused on potency, resulting in compound **12b** being neither efficient (LE = 0.19),[26] nor drug-like (MW = 636, and Clog *P* = 7.5). We suspected that the pipecolate core or its ester linkage to the lipophilic side-chain was not optimal. Attempts to obtain a co-crystal structures of **11** or analogs were not fruitful. To further improve potency and drug-like properties, a focused combinatorial library (~200 compounds) was designed and synthesized (Scheme 2) using the phenylalaninol phosphate as an 'anchor' to search for a pipecolate replacement. The library core **16** was easily prepared in a three-step sequence from commercially available amino alcohol **13**. The divergent step (from **16** to **17**) for library synthesis was conducted in aqueous 1 M sodium carbonate solution, which was designed to scavenge any un-reacted acylating (or sulfonylating) reagents and prevent potential interference with the Pin1 screening assay.

Several potent hits were identified with the high-throughput PPIase enzymatic assay. The hits were resynthesized and confirmed by iso-thermal calorimetric titration. Amide was generally the best among the three tested linkers-amide, sulfonamide and urea (**17c** vs **17a/b**, Scheme 2). Compounds **18a** and **18b**, the fused bi-aryl amides, are about 10–30-fold more potent than their corresponding pipecolate. The 'S' enantiomer of **18a** was also synthesized but found to be less active (K_i = 5.6 μM, 56x vs **18a**). With good affinity and solubility, compound **18b** was crystallized with the Pin1 PPIase K7782Q mutant and its structure was solved. A high-resolution protein structure of Pin1 with a potent small molecular inhibitor (Fig. 5)[18] was available to guide our drug design.

The crystal structure of **18b** revealed a new binding mode in the PPIase site, far different from our initial model. As shown in Figure 5, the binding appears to be dominated by three factors. As expected, the phosphate group fits well in to the charged pocket formed by Lys-63, Arg-68, and Arg-69 to form hydrogen bonds to all three residues. The phenyl group binds to the 'prolyl pocket' formed by His-59, His-157, Met-130, and Phe-134. The benzene ring forms an edge-on interaction with His-157. The thiophenyl group binds to the hydrophobic area formed by Phe-134, Met-130 and Leu-122. The amide NH makes a weak H-bond to Cys-113 (3.3 Å), while the amide oxygen is exposed to solvent. This binding mode shows that while there is a rich hydrogen bonding potential in the pocket,

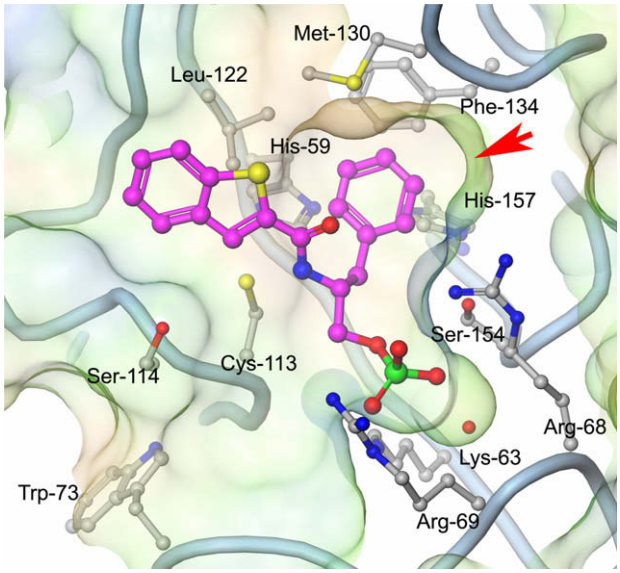
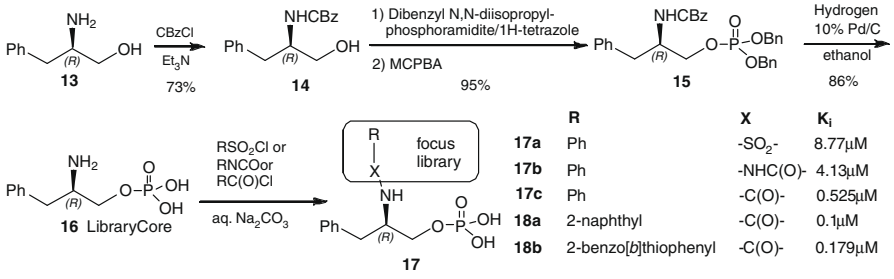
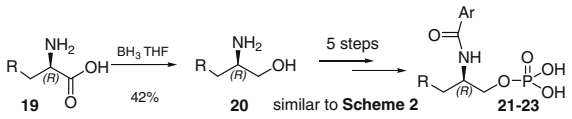


Figure 5. X-ray co-crystal structure of **18b** and Pin1 K7782Q construct. (PDB ID: 3IKD).



Scheme 2.



Compound	R	Ar	K_i (μM)
21a	3-F-phenyl	2-naphthyl	0.008
21b	3-F-phenyl	2-benzothiophenyl	0.006
22a	3-methylphenyl	2-naphthyl	0.032
22b	3-methylphenyl	2-benzothiophenyl	0.057
23a	2,3-diF-phenyl	2-naphthyl	0.078
23b	2,3-diF-phenyl	2-benzothiophenyl	0.089

Scheme 3.

hydrophobic interaction dominates the binding affinity for these compounds. The channel formed by Ser-114, Arg-69 into the Trp-73 pocket is harder to access than what we had anticipated.

An analysis of the **18b** co-crystal structure revealed a small cavity between to the phenyl C-3 position of the inhibitors and the Pin1 Phe-134 residue, as shown in Figure 5. Since the cavity appeared hydrophobic, analogs with small substitutions on C-3 such as F and CH_3 were synthesized and tested (Scheme 3). Both analogs **21–22** bound tighter than the corresponding phenyl analogs **18a/b**, with the fluoro analogs being most active. The fluoro atom added to C-3 position produced a dramatic boost in inhibitory activity (**21b** vs **18b**, 30x), corresponding to a highly efficient contribution to binding ($\Delta\Delta G = -2.0$ kcal/mol).[27] The X-ray co-crystal structure of **22b** (Fig. 6) confirmed our hypothesis. Further substitution to the phenyl ring reduced binding affinity (**23a–b**).

Compound **21b**, a 3-fluorophenylalanine derivative, is one of the most potent inhibitors known with a phosphate group ($K_i = 6$ nM). Although it is an efficient ligand with desirable properties (LE = 0.43, MW = 409, Clog $P = 3.1$, Clog $D = -0.76$), compound **21b** was inactive in whole cell assay, likely due to the poor permeability conferred by the polar phosphate group.

In conclusion, a series of potent, none peptide-based Pin1 inhibitors was designed without a viable lead from HTS. We discovered the aminophenylpropanol core by utilizing structure-based de novo design. After further optimization with an integrated SBDD and combinatorial library approach, the initial micromolar lead (**11**) was developed into a series of potent, non-peptidic, small molecular Pin1 inhibitors with attractive properties (**21a/b**). The discovery improved our confidence in pursuing drug-like Pin1 inhibitors. However, the whole cell activity, presumably due to poor permeability, remained to be an unsolved problem even for this series of Pin1 inhibitors. The simplified pharmacophore and the three Pin1 crystal structures reported herein set the stage for other drug-like Pin-1 inhibitor designs. In the following communications, progress toward improving cell permeability of Pin1 inhibitors will be reported.

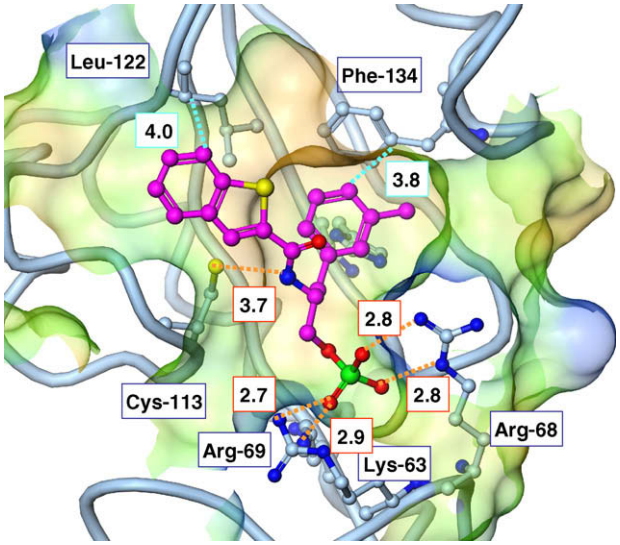


Figure 6. X-ray co-crystal structure of **22b** and Pin1 K7782Q construct (distance in Å, PDB ID: 3IKG).

Acknowledgment

We thank Dr. Robert Kania for his input and insightful discussion while preparing this Letter.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.034.

References and notes

1. Lu, K. P.; Hanes, S. D.; Hunter, T. *Nature* **1996**, *380*, 544.
2. Lu, K. P. *Prog. Cell Cycle Res.* **2000**, *4*, 83.
3. Winkler, K. E.; Swenson, K. I.; Kornbluth, S.; Means, A. R. *Science* **2000**, *287*, 1644.
4. Lu, K.P.; Wulf, G.; Zhou, X. Z. PCT Int. Appl. WO 2001038878, 2001.
5. Ranganathan, R.; Lu, K. P.; Hunter, T. *Cell* **1997**, *89*, 875.
6. Hennig, L.; Christner, C.; Kipping, M.; Schelbert, B.; Rucknagel, K. P.; Grabley, S.; Kullertz, G.; Fischer, G. *Biochemistry* **1998**, *37*, 5953.
7. Lu, P. J.; Zhou, X. Z.; Shen, M.; Lu, K. P. *Science* **1999**, *283*, 1325.
8. Wildemann, D.; Erdmann, F.; Alvarez, B. H.; Stoller, G.; Zhou, X. Z.; Fanghanel, J.; Schutkowski, M.; Lu, K. P.; Fischer, G. *J. Med. Chem.* **2006**, *49*, 2147.
9. Guo, C.; Dagostino, E. F.; Dong, L.; Hou, X.; Margosiak, S. A. PCT Int. Appl. WO 2004087720, 2004.
10. Matthews, D. A.; Dagostino, E. F.; Ferre, R. A.; Gaur, S.; Guo, C.; Hou, X.; Margosiak, S.; Mroczkowski, B.; Nakayama, G. R.; Parge, H. E.; Zhu, J. X. PCT Int. Appl. WO 2004005315, 2004.
11. Zhang, Y.; Daum, S.; Wildemann, D.; Zhou, X. Z.; Verdecia, M. A.; Bowman, M. E.; Lucke, C.; Hunter, T.; Lu, K. P.; Fischer, G.; Noel, J. P. *ACS Chem. Biol.* **2007**, *2*, 320.
12. Wang, X. J.; Xu, B.; Mullins, A. B.; Neiler, F. K.; Etzkorn, F. A. *J. Am. Chem. Soc.* **2004**, *126*, 15533.
13. Siegrist, R.; Zurcher, M.; Baumgartner, C.; Seiler, P.; Diederich, F.; Daum, S.; Fischer, G.; Klein, C.; Dangl, M.; Schwaiger, M. *Helv. Chim. Acta* **2007**, *90*, 217.
14. Do, Q.-Q. T.; Guo, C.; Humphries, P. S.; Marakovits, J. T.; Dong, L.; Hou, X.; Johnson, M. C. PCT Int. Appl. WO 2006040646, 2006.
15. Bayer, E.; Thutewohl, M.; Christner, C.; Tradler, T.; Osterkamp, F.; Waldmann, H.; Bayer, P. *Chem. Commun. (Camb)* **2005**, 516.
16. Daum, S.; Erdmann, F.; Fischer, G.; Feaux de Lacroix, B.; Hessamian-Alinejad, A.; Houben, S.; Frank, W.; Braun, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 7454.
17. Bao, L.; Kimzey, A. PCT Int. Appl. WO 2004093803, 2004.
18. See Supplementary data.
19. Zhu, J. X.; Dagostino, E.; Rejto, P. A.; Mroczkowski, B.; Murray, B. *Biochem. Biophys. Res. Commun.* **2007**, *359*, 529.
20. Daum, S.; Fanghanel, J.; Wildemann, D.; Schiene-Fischer, C. *Biochemistry* **2006**, *45*, 12125.
21. Namanja, A. T.; Peng, T.; Zintsmaster, J. S.; Elson, A. C.; Shakour, M. G.; Peng, J. W. *Structure* **2007**, *15*, 313. In our NMR studies (unpublished), we observed ligand-protein interaction similar to this report.
22. Babine, R. E. V. J. E.; Gold, B. G. *Exp. Opin. Ther. Patents* **2005**, *15*, 555.
23. Holt, D. A.; Luengo, J. I.; Yamashita, D. S.; Oh, H. J.; Konialian, A. L.; Yen, H. K.; Rozamus, L. W.; Brandt, M.; Bossard, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 9925.
24. For flexible docking method used herein, see: Gehlhaar, D. K.; Verkhivker, G. M.; Rejto, P. A.; Sherman, C. J.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. *Chem. Biol.* **1995**, *2*, 317.
25. Varghese, J. N.; Smith, P. W.; Sollis, S. L.; Blick, T. J.; Sahasrabudhe, A.; McKimm-Breschkin, J. L.; Colman, P. M. *Structure* **1998**, *6*, 735.
26. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
27. $G = -RT\ \ln(K_a) = RT\ \ln(K_d) \approx 1.36\ \log(K_i)$ [kcal/mol], at $T = 298$ K. And $\Delta\Delta G = \Delta G(21b) - \Delta G(18b)$.