Bioorganic & Medicinal Chemistry Letters 19 (2009) 4839–4842

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of new Hsp90 inhibitors by structure-based virtual screening

Tae-Joon Hong [a], Hwangseo Park [b], Yun-Jung Kim [a], Jae-Hoon Jeong [c], Ji-Sook Hahn [a,*]

[a] School of Chemical and Biological Engineering, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-744, Republic of Korea
[b] Department of Bioscience and Biotechnology, Sejong University, 98 Kunja-dong, Gwangjin-gu, Seoul 143-747, Republic of Korea
[c] Research Center for Molecular and Cellular Biology, Inha University, 253 Yonghyun-dong, Namgu, Incheon 402-751, Republic of Korea

ARTICLE INFO

Article history:
Received 10 February 2009
Revised 27 May 2009
Accepted 10 June 2009
Available online 13 June 2009

Keywords:
Hsp90 inhibitors
Anti-cancer drug
Virtual screening
Pyrimidine-2,4,6-trione
4*H*-1,2,4-triazole-3-thiol

ABSTRACT

Structure-based virtual screening identified pyrimidine-2,4,6-trione and 4*H*-1,2,4-triazole-3-thiol as novel scaffolds of Hsp90 ATPase inhibitors. Their binding modes in the ATP-binding pocket of Hsp90 were analyzed using AutoDoc program combined with molecular dynamics (MD) simulations.

Hsp90 is a ubiquitous and abundant molecular chaperone involved in folding, stabilization, and activation of 'client' proteins.[1,2] The Hsp90 client proteins include many oncogenic proteins such as Her2/ErbB2, Akt, Raf-1, Cdk4, Hif-1α, MET, hTERT, hormone receptors, mutant p53, survivin, IP6K2, which are related to the development and progression of cancer.[3–6] Inhibition of Hsp90 results in simultaneous degradation of the multiple oncogenic client proteins by ubiquitin–proteasome pathway. For this reason, Hsp90 has emerged as a promising target for anti-cancer drug development.[3,7–10]

The Hsp90 chaperone machinery is a dynamic multi-chaperone complex consisting of an Hsp90 homodimer and several co-chaperones.[1,2] Binding and hydrolysis of ATP in the N-terminal ATPase domain drives the conformational change of Hsp90, leading to the progression of chaperone cycle.[11,12] Most of the currently developed Hsp90 inhibitors, including the natural products geldanamycin (GA) and radicicol, inhibit Hsp90 ATPase activity by docking to the N-terminal ATP-binding pocket.[3,7–10] Hsp90 in cancer cells mainly exists as an active chaperone complex, reflecting a high demand for Hsp90-dependent protein folding to maintain malignancy.[13] The active Hsp90 in cancer cells has higher affinity to Hsp90 inhibitors than the latent form in normal cells, which enables selective targeting of Hsp90 inhibitors to cancer cells.[13] The therapeutic potential of Hsp90 inhibitors has been verified by the initial success of 17-allylamino-17-demethoxygeldanamycin (17-AAG), one of the less toxic derivatives of GA, in several Phase I and Phase II clinical trials in cancers.[14–18] Other synthetic Hsp90 inhibitors such as purine-based drug BIIB021 and isoxazole-based drug NVP-AUY922/VER-52296 also have entered clinical trials,[18–20] accelerating the continuing effort to develop novel Hsp90 inhibitors with reduced toxicity and enhanced efficacy.

In the previous study, we identified a novel class of 3-phenyl-2-styryl-3*H*-quinazolin-4-one Hsp90 inhibitors by means of a drug-design protocol involving a newly developed structure-based virtual screening tool.[21] We used the modified version of the AutoDoc program to carry out docking simulations of test compounds in the ATP-binding pocket of Hsp90.[21–23] The 3-D coordinates in the crystal structure of human Hsp90α complexed with a benzenesulfonamide inhibitor (PDB code: 2BZ5)[24] were selected for the virtual screening with docking simulations. The reason for this selection lies in that the crystal structure was determined at high resoltion (1.9 Å) in complex with the inhibitor with submicromolar activity that had been identified with a computer-aided drug design method. After removing the ligand and solvent molecules, hydrogen atoms were added to each protein atom. Considering the significant role of the solvent-mediated interaction in protein–ligand docking, the structural water molecules found within 3.5 Å from the ligand in the original X-ray structure were also included in the receptor model. The docking library of about 85,000 compounds for Hsp90 was constructed from the latest version of InterBioScreen DB (Interbioscreen Database, Moscow, http://www.ibscreen.com) containing approximately 30,000 natural and 290,000 synthetic compounds. This selection was based on drug-like filters that adopt only the compounds with physicochemical properties of potential drug candidates and without reactive functional group(s). Docking simulations with

* Corresponding author. Tel.: +82 2 880 9228; fax: +82 2 888 1604.
E-mail address: hahnjs@snu.ac.kr (J.-S. Hahn).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.06.040

AutoDoc were then carried out in the ATP-binding site of Hsp90 to score and rank the compounds in docking library according to the binding affinity for Hsp90.

After in vitro ATPase activity assay and Her2 degradation assay, we were able to identify five Hsp90 inhibitors, among which three compounds share a same scaffold of 3-phenyl-2-styryl-3*H*-quinazolin-4-one.[21] In this report, we further characterized two remaining compounds which have different structures (Fig. 1, **1b** and **2b**). Inhibitory effect of each compound on *Saccharomyces cerevisiae* Hsp90 ATPase activity was examined by ATPase assay using colorimetric detection of the released inorganic phosphate complexed with malachite green reagent (Bioassay systems co.) in 96-well plate format.[21,25] Compounds **1b** and **2b** showed the IC_{50} values of 24.6 μM and 15.4 μM, respectively, while geldanamycin showed the IC_{50} value of 2.3 μM (Table 1). The anti-proliferation activities of **1b** and **2b** were measured by SRB (sulforhodamine B) assay with MCF-7 breast cancer cell line.[26] The GI_{50} values of **1b** and **2b** were 18.1 μM and 44.6 μM, respectively. The GI_{50} of geldanamycin was 9.6 μM (Table 1). Although **1b** was less effective than **2b** in inhibition of Hsp90 ATPase activity**, 1b** was more effective in growth inhibition of MCF-7 cells, which might be due to the differences in cell permeability or stability of the compounds.

In an attempt to obtain some structural insights into the mechanisms of inhibition by the identified inhibitors for Hsp90, their binding modes in the ATP-binding pocket of Hsp90 were calculated using AutoDoc program with the procedure described previously.[21] An improved binding configuration of Hsp90–inhibitor complex was then obtained with molecular dynamics (MD) simulations of the complex in aqueous solution based on the structure of the protein–inhibitor complex obtained with the precedent docking simulations. The most stable structures of Hsp90–inhibitor complexes obtained from docking simulations were equilibrated in solution through 1 ns MD simulation with the AMBER program of version 7, which had been successful in modeling the structures of proteins[27]

Table 1
Inhibitory effects of **1b** and **2b** on yeast Hsp90 ATPase and proliferation of MCF-7 human breast cancer cell line

Compound	Geldanamycin	**1b**	**2b**
IC_{50} (μM)[a]	2.3	24.6	15.4
GI_{50} (μM)[a]	9.6	18.1	44.6

IC_{50}, the concentration inhibiting Hsp90 ATPase activity by 50%.
GI_{50}, the concentration inhibiting cell growth by 50%.
[a] Values correspond to $n = 2$.

and nucleic acids[28] in solution. We used the force field parameters for proteins reported by Cornell and coworkers.[29] Atomic partial charges for ligand atoms were calculated through the RESP method[30] to be consistent with the standard AMBER force field. The equilibration procedure started with the addition of sodium ions as the six sodium ions to neutralize the total charge of the all-atom model of Hsp90. The system was then immersed in a rectangular solvent box containing about 8000 TIP3P water molecules. After 1000 cycles of energy minimization to remove bad van der Waals contacts, we equilibrated the system beginning with 20 ps equilibration dynamics of the solvent molecules at 300 K. The next step involved equilibration of the solute with a fixed configuration of the solvent molecules for 10 ps at 10, 50, 100, 150, 200, 250, and 300 K. Then, the equilibration dynamics of the entire system was performed at 300 K for 500 ps using the periodic boundary condition. The SHAKE algorithm[31] was applied to fix all bond lengths involving hydrogen atom. We used a time step of 1.5 fs and a nonbond-interaction cutoff radius of 12 Å. The equilibrium of the system was monitored by the convergent behavior of the root-mean-square deviation of the backbone C_α atoms from the starting structure.

Figure 2A shows the representative MD trajectory snapshot for Hsp90–**1b** complex in aqueous solution. It should be noted that due to the structural flexibility of Hsp90, the calculated binding

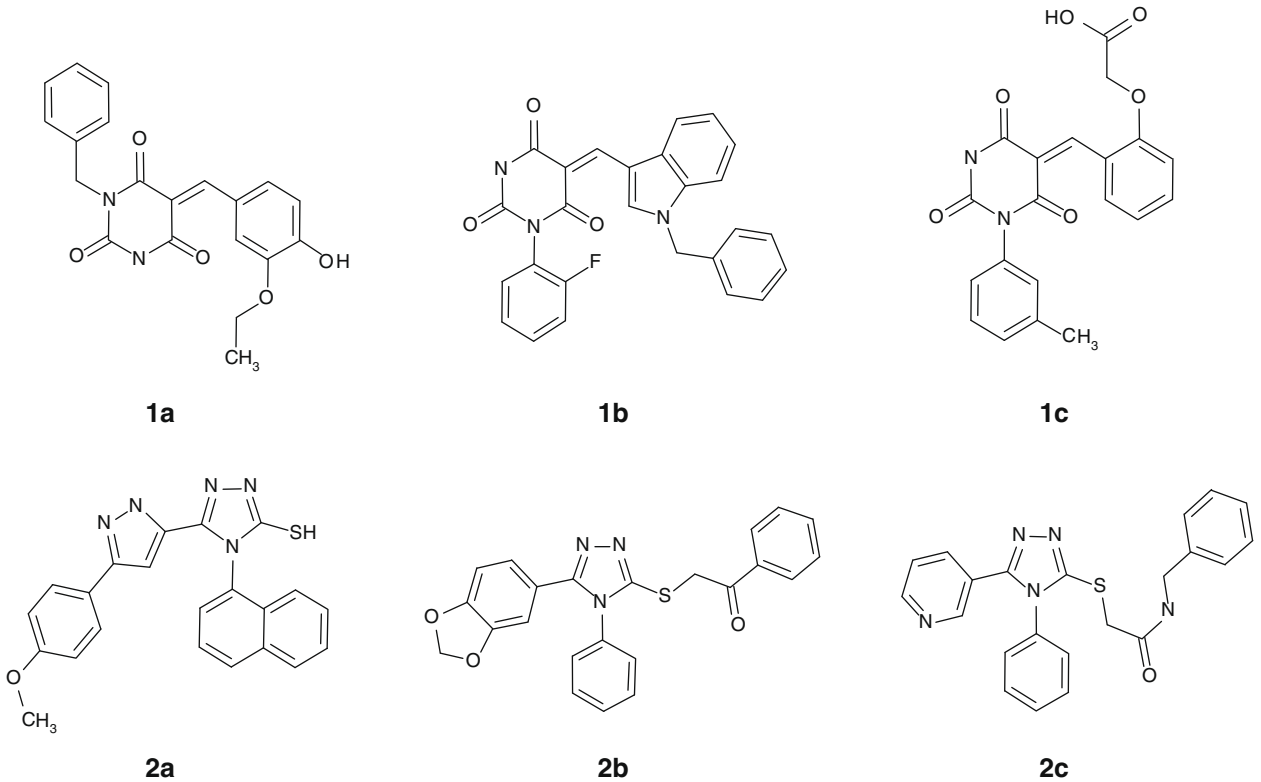

Figure 1. Six compounds used in this study. Compounds **1a**, **1b**, and **1c** have pyrimidine-2,4,6-trione scaffold, while **2a**, **2b**, and **2c** have 4*H*-1,2,4-triazole-3-thiol scaffold.

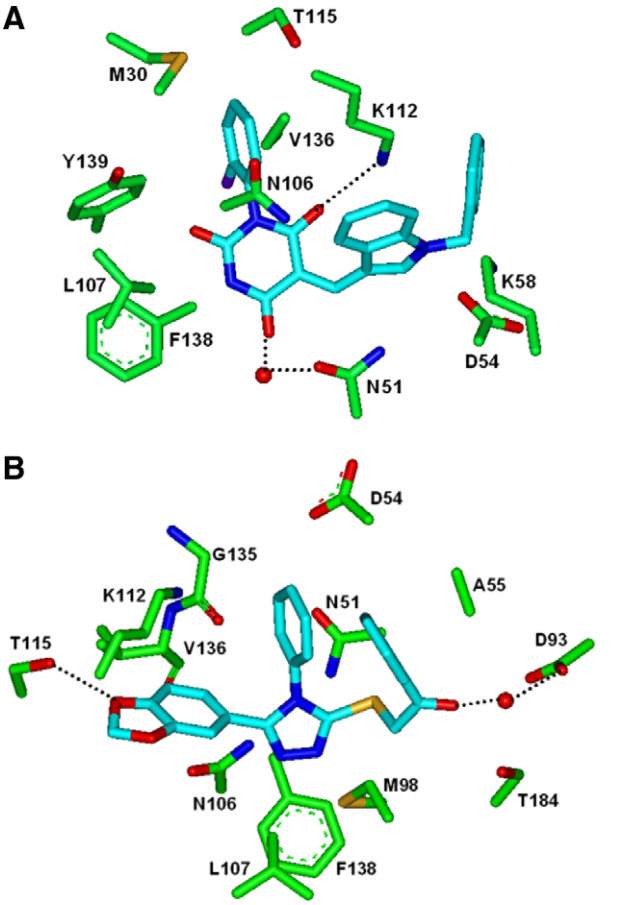

Figure 2. Representative MD trajectory snapshots of (A) **1b** and (B) **2b** binding to ATP-binding pocket of Hsp90. Carbon atoms of the protein and the ligand are indicated in green and cyan, respectively. Each dotted line indicates a hydrogen bond.

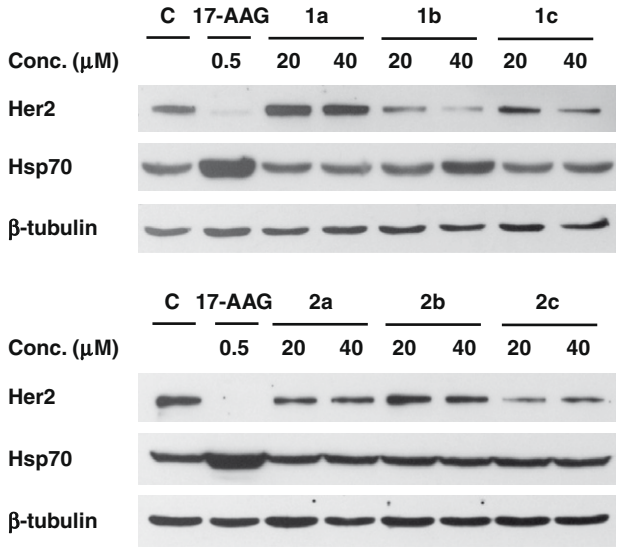

Figure 3. Effects of Hsp90 inhibitors on Her2 degradation. MCF-7 cells were treated with indicated concentrations of 17-AAG or each three of pyrimidine-2,4,6-trione (**1a**, **1b**, and **1c**) and 4*H*-1,2,4-triazole-3-thiol (**2a**, **2b**, and **2c**) compounds. DMSO was treated as a negative control (C). The protein levels of Her2 and Hsp70 were detected by Western blotting. β-Tubulin was used as a loading control.

mode should be dependent on the selected conformation of the protein. This problem can be overcome by choosing the most probable structure obtained from the molecular dynamics simulations that involve the motions of protein atoms as well as the ligand. We note that a stable hydrogen bond is established between one of the carbonyl oxygens of **1b** and the side chain of Lys112. The inhibitors can be further stabilized in the ATP-binding site by the establishment of an additional hydrogen bond between one of the carbonyl oxygens and the structural water molecule that is in turn hydrogen-bonded to the side chain aminocarbonyl group of Asn51. This exemplifies the involvement of a solvent-mediated hydrogen bond, which has been considered as one of the significant binding forces in protein–ligand interactions in aqueous solution,[32] in the stabilization of the inhibitors in the ATP-binding site of Hsp90. The hydrophobic interactions between the nonpolar groups of **1b** and the side chains of Met30, Leu107, Val136, and Phe138 seem to be also a significant binding force stabilizing **1b** in the ATP-binding site.

The representative MD trajectory snapshot for Hsp90–**2b** complex is shown in Figure 2B. It is noted that one of the oxygen atoms on the terminal dioxolane ring forms a hydrogen bond with the side chain hydroxyl group of Thr115. It is a common feature in binding modes of **2b** and **1b** that a solvent-mediated hydrogen bond is involved in the stabilization of the inhibitor in the ATP-binding site of Hsp90. The presence of such a solvent-mediated hydrogen bond in the Hsp90–inhibitor complexes indicates that the role of structural water molecules should be taken into account for designing of new potent Hsp90 inhibitors. As in the Hsp90–**1b** complex, hydrophobic interactions should also be a significant binding force for stabilizing **2b** in the ATP-binding site because its nonpolar groups form van der Waals contacts with the side chains of Ala55, Met98, Leu107, Val136, and Phe138.

In order to test the potential of these inhibitors as starting points to develop novel Hsp90 inhibitors, we selected two compounds (**1a** and **1c)** sharing pyrimidine-2,4,6-trione scaffold with **1b,** and two compounds (**2a** and **2c**) sharing 4*H*-1,2,4-triazole-3-thiol scaffold with **2b** (Fig. 1), among the chemicals we obtained from the original virtual screening and examined their effects toward inhibition of Hsp90 by Her2 degradation assay. MCF-7 cells were treated with each compound or 17-AAG for 24 h, and the levels of Her2, an Hsp90 client protein, were detected by Western blotting analysis. All compounds except **1a** led to degradation of Her2 to some extent, confirming their functions as Hsp90 inhibitors (Fig. 3). Compound **1b** showing the most potent effect on Her2 degradation also induced expression of Hsp70, in agreement with its effect on inhibition of Hsp90. Since heat shock transcription factor (Hsf1) is inactivated by Hsp90, most of Hsp90 inhibitors have been shown to induce expression of Hsf1 target genes including HSP70.[33] Other compounds did not show obvious effect on Hsp70 induction, probably reflecting their relatively low efficacy. Taken together, these results suggest the possibility that the two newly identified scaffolds could contribute to developing novel Hsp90 inhibitors with improved properties.

In conclusion, we have been able to identify Hsp90 inhibitors with new scaffolds, pyrimidine-2,4,6-trione and 4*H*-1,2,4-triazole-3-thiol, by utilizing the structure-based virtual screening, empathizing the usefulness of virtual screening in the development of Hsp90 inhibitors with novel structures. Their binding modes in the ATP-binding pocket of Hsp90, which were obtained using AutoDoc program combined with molecular dynamics (MD) simulations of the complexes in aqueous solution, would provied useful information to design various effective derivatives based on these Hsp90 inhibitors.

Acknowledgments

This work was supported by grants from Korea Research Foundation (2005-005-J16002) and the Basic Research Program of the

Korea Science & Engineering Foundation (R01-2006-000-10255-0), and Korea Research Foundation and The Korean Federation of Science and Technology Socities Grant (MOEHRD, Basic Research Promotion Fund).

References and notes

1. Csermely, P.; Schnaider, T.; Soti, C.; Prohaszka, Z.; Nardai, G. *Pharmacol. Ther.* **1998**, *79*, 129.
2. Wandinger, S. K.; Richter, K.; Buchner, J. J. *Biol. Chem.* **2008**, *283*, 18473.
3. Sharp, S.; Workman, P. *Adv. Cancer. Res.* **2006**, *95*, 323.
4. Neckers, L.; Ivy, S. P. *Curr. Opin. Oncol.* **2003**, *15*, 419.
5. Fortugno, P.; Beltrami, E.; Plescia, J.; Fontana, J.; Pradhan, D.; Marchisio, P. C.; Sessa, W. C.; Altieri, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13791.
6. Chakraborty, A.; Koldobskiy, M. A.; Sixt, K. M.; Juluri, K. R.; Mustafa, A. K.; Snowman, A. M.; van Rossum, D. B.; Patterson, R. L.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1134.
7. Neckers, L. *Curr. Top. Med. Chem.* **2006**, *6*, 1163.
8. Powers, M. V.; Workman, P. *FEBS Lett.* **2007**, *581*, 3758.
9. Burger, A. M. *Cancer. Lett.* **2007**, *245*, 11.
10. Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D. *Drug. Discovery Today* **2004**, *9*, 881.
11. Panaretou, B.; Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *EMBO J.* **1998**, *17*, 4829.
12. Pearl, L. H.; Prodromou, C. *Annu. Rev. Biochem.* **2006**, *75*, 271.
13. Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature* **2003**, *425*, 407.
14. Banerji, U. *Clin. Cancer. Res.* **2009**, *15*, 9.
15. Heath, E. I.; Hillman, D. W.; Vaishampayan, U.; Sheng, S.; Sarkar, F.; Harper, F.; Gaskins, M.; Pitot, H. C.; Tan, W.; Ivy, S. P.; Pili, R.; Carducci, M. A.; Erlichman, C.; Liu, G. *Clin. Cancer. Res.* **2008**, *14*, 7940.
16. Solit, D. B.; Osman, I.; Polsky, D.; Panageas, K. S.; Daud, A.; Goydos, J. S.; Teitcher, J.; Wolchok, J. D.; Germino, F. J.; Krown, S. E.; Coit, D.; Rosen, N.; Chapman, P. B. *Clin. Cancer. Res.* **2008**, *14*, 8302.
17. Tse, A. N.; Klimstra, D. S.; Gonen, M.; Shah, M.; Sheikh, T.; Sikorski, R.; Carvajal, R.; Mui, J.; Tipian, C.; O'Reilly, E.; Chung, K.; Maki, R.; Lefkowitz, R.; Brown, K.; Manova-Todorova, K.; Wu, N.; Egorin, M. J.; Kelsen, D.; Schwartz, G. K. *Clin. Cancer. Res.* **2008**, *14*, 6704.
18. Taldone, T.; Gozman, A.; Maharaj, R.; Chiosis, G. *Curr. Opin. Pharmacol.* **2008**, *8*, 370.
19. Pearl, L. H.; Prodromou, C.; Workman, P. *Biochem. J.* **2008**, *410*, 439.
20. Brough, P. A.; Aherne, W.; Barril, X.; Borgognoni, J.; Boxall, K.; Cansfield, J. E.; Cheung, K.-M. J.; Collins, I.; Davies, N. G. M.; Drysdale, M. J.; Dymock, B.; Eccles, S. A.; Finch, H.; Fink, A.; Hayes, A.; Howes, R.; Hubbard, R. E.; James, K.; Jordan, A. M.; Lockie, A.; Martins, V.; Massey, A.; Matthews, T. P.; McDonald, E.; Northfield, C. J.; Pearl, L. H.; Prodromou, C.; Ray, S.; Raynaud, F. I.; Roughley, S. D.; Sharp, S. Y.; Surgenor, A.; Walmsley, D. L.; Webb, P.; Wood, M.; Workman, P.; Wright, L. *J. Med. Chem.* **2008**, *51*, 196.
21. Park, H.; Kim, Y. J.; Hahn, J. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6345.
22. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
23. Hwangseo Park, J. L. S. L. *Proteins: Struct. Funct. Bioinform.* **2006**, *65*, 549.
24. Barril, X.; Brough, P.; Drysdale, M.; Hubbard, R. E.; Massey, A.; Surgenor, A.; Wright, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5187.
25. Rowlands, M. G.; Newbatt, Y. M.; Prodromou, C.; Pearl, L. H.; Workman, P.; Aherne, W. *Anal. Biochem.* **2004**, *327*, 176.
26. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. J. *Natl. Cancer. Inst.* **1990**, *82*, 1107.
27. Case, D. A. C.; Cheatham, T. E., III; Darden, T.; Gohlke, H.; Luo, R.; Merz, K. M., Jr.; Onufriev, A.; Simmerling, C.; Wang, B.; Woods, R. J. *J. Comput. Chem.* **2005**, *26*, 1668.
28. Park, H.; Lee, S. *J. Chem. Theor. Comput.* **2006**, *2*, 858.
29. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
30. Bayly, C. I.; Cieplak, P.; Cornell, W.; Kollman, P. A. *J. Phys. Chem.* **1993**, *97*, 10269.
31. Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.
32. Verdonk, M. L.; Chessari, G.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Nissink, J. W.; Taylor, R. D.; Taylor, R. *J. Med. Chem.* **2005**, *48*, 6504.
33. Zou, J.; Guo, Y.; Guettouche, T.; Smith, D. F.; Voellmy, R. *Cell* **1998**, *94*, 471.