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Syntheses of triazole-modified zanamivir analogues via click chemistry and anti-AIV activities

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Abstract—Sixteen novel 4-triazole-modified zanamivir (**1**) analogues were synthesized using the click reactions, and their inhibitory activities against avian influenza virus (AIV, H5N1) were determined. Compound **3b** exerts promising inhibitory activity with EC_{50} of 6.4 μM, which is very close to that of zanamivir (EC_{50} = 2.8 μM). Molecular modeling provided the information about the binding model between inhibitors and neuraminidase, which are in good agreement with inhibitory activities.

Recently, the H5N1 subtype of avian influenza virus (AIV) has been spreading in Southern China, Africa, and European Union mainly imputing to migratory birds.[1] More than 200 human cases of AIV infection mainly as a result of poultry-to-human transmission have been reported with a >50% case fatality rate for H5N1 infections.[2] A mutant virus capable of efficient human-to-human transmission could trigger another influenza pandemic.[2,3] Whereas H5N1 vaccines have been significantly hampered by the high mutability of the virus.[4] Accordingly, discovering effective drugs, which are not susceptible to mutation, is an urgent need.

There are currently only a few drugs available for AIV treatment. The licensed existing drugs are the zanamivir[5] (**1**) and oseltamivir phosphate[6] (**2**) (Fig. 1) as specific neuraminidase (NA) inhibitors. However, these drugs are not effectively used owing to the rapid emergence of resistant virus during treatment. Many groups have designed and synthesized some zanamivir analogues aimed at developing more potent, long-lasting therapeutic drugs for anti-AIV infection.[7] For example, Woodhall et al.[8] have synthesized a series of zanamivir mimetics based on sialic acid aldolase.

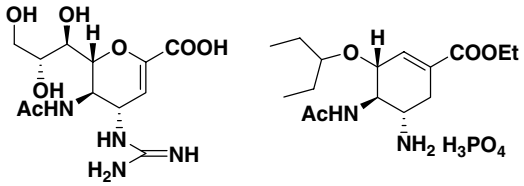

Figure 1. The structures of zanamivir (**1**) and oseltamivir phosphate (**2**).

Click chemistry, coined by Barry K. Sharpless,[9] is experiencing a growing popularity.[10] The process of click chemistry is modular, wide in scope, high yielding, and requires only simple reaction and purification conditions. Such kind of chemistry is possible to generate a plethora of new compounds reliably and thereby has been applied in lead discovery and optimization. The Huisgen 1,3-dipolar cycloaddition of azides and alkynes[11] is regarded as the 'cream of the crop' of concerted

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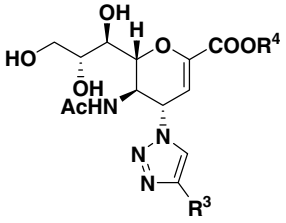
reactions, therefore it has been a general and standard reaction of click chemistry.[9,10]

Compound **9**, a key intermediate for synthesizing zanamivir (Scheme 1), contains an azide group. Accordingly, this compound is a good starting material to synthesize zanamivir analogues by using click reactions. Thus, we designed and synthesized a series of zanamivir analogues (**3a**–**p**) with different substituted triazoles and tested their preliminary anti-AIV activities. This reaction produces a unique substituted position for R^3. To increase the structural diversity of the analogues, we selected R^3 with different size. Scheme 1 depicts the sequence of reactions that led to the preparation of compounds **3a**–**p** using *N*-acetylneuraminic acid (NANA, **4)** as the starting material. Meanwhile, taking compounds **3b** and **3i** as examples, the interaction models between inhibitors and neuraminidase were studied.

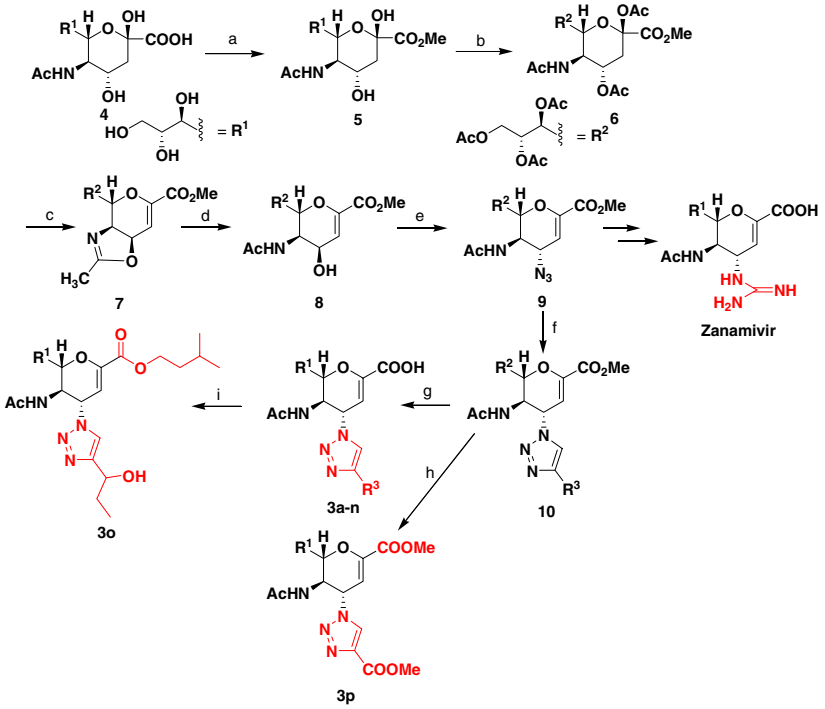
As shown in Scheme 1, compound **4** was esterified with methanol in the presence of an acidic ion exchange resin.[12] Oxazoline **7** was prepared using a modification of the procedure described by Chandler et al.[13] Compound **6** was obtained using acetic anhydride in pyridine with 4-(dimethylamino)pyridine (DMAP) catalysis, then was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in ethyl acetate at 52 °C to give **7** in high yield. Compound **7** was hydrolyzed without purification. Reacted with diphenylphosphoryl azide (DPPA) and 1,8-diazabicyclo[5,4,0]undecen-7-ene (DBU), the alcohol **8** was converted to the inverted azide **9**, the key intermediate, applying Scheigetz methods.[14] Compound **10** was obtained by a click reaction between azide **9** and

Table 1. Chemical Structures of Compounds **3a**–**p** and their anti-AIV activities



Compound	R^3	R^4	Protective rate[a] at 50 μM
3a	C_6H_5	H	18.7
3b	$CH(OH)C_2H_5$	H	> 61.4
3c	Cyclopropyl	H	8.9
3d	CH_2CH_2OH	H	14.5
3e	$(CH_2)_3OH$	H	6.3
3f	Pyridin-2-yl	H	NA[b]
3g	C_6H_4–4-F	H	NA[b]
3h	C_6H_4–4-C_2H_5	H	34.4
3i	C_6H_4–4-OC_3H_7	H	40.2
3j	C_6H_4–4-C_3H_7	H	22.1
3k	C_6H_4–4-C_6H_5	H	19.1
3l	$CH_2OC_6H_5$	H	34.8
3m	$CH_2N(C_2H_4)_2O$	H	17.3
3n	CH_2NH_2	H	NA[b]
3o	$CH(OH)C_2H_5$	$(CH_2)_2CH(CH_3)_2$	8.9
3p	$COOCH_3$	CH_3	9.9

[a] Antiviral efficacy of test compounds was assessed in vitro by Neutral Red (NR) uptake assay and expressed as percent protective rate against virus infection.[15] Data are means of three independent experiments. The protective rate for zanamivir at 50 μM is 86.1%.
[b] No activity at 50 μM.



Scheme 1. Reagents and conditions: (a) H^+ Resin, CH_3OH, 25 °C (91%); (b) Ac_2O, pyridine, DMAP (85%); (c) TMSOTf, EtOAc, N_2 (84%); (d) AcOH, EtOAc, H_2O (42%); (e) DPPA, DBU, benzene (85%); (f) $R^3C \equiv CH$, sodium ascorbate, $CuSO_4$, C_2H_5OH/H_2O = 1:1, 25 °C (60–80%); (g) (1) NaOH, CH_3OH, 25 °C, (2) H^+ Resin (95%); (h) (1) $NaOCH_3$, CH_3OH, 25 °C, (2) H^+ Resin (90%); (i) 3-methyl-1-butanol, H^+ Resin, THF (50%).

alkyne in 60–80% yield, and then hydrolyzed with NaOH/methanol and $NaOCH_3$/methanol to give **3a**–**n** and **3p**, respectively. **3o** was synthesized by the corresponding acid reacting with 3-methyl-1-butanol in the presence of an acidic ion exchange resin.

Protective rates[15] of compounds **3a**–**p** against infected cell (MDCK cell) at 50 μM are summarized in Table 1. EC_{50} values (the concentration of a compound in the media which reduced virus-infected cell numbers by 50% of the untreated control value) of compound **3b** and zanamivir (**1**) were determined as 6.4 and 2.8 μM, respectively. Although general structure–activity relationship of those compounds is not evident, the bioassay results still provide some information for further structural modification. Replacement of carboxyl acid group with esters cannot be tolerated (compounds **3b** and **3o**), leading to a remarkable loss of activity. Comparing compound **3a** with compounds **3g**–**k**, the substitutions on the phenyl ring substantially play an important role in the potency activity of compounds. Generally, alkyl (**3h** and **3j**) and alkoxy (**3i**) substituents are favorable to the activities of these compounds, however, introductions of large aromatic (**3k**) or halogen (**3g**) substituents are improper. Among non-aromatic substituents of R^3, propyl alcohol of **3b** is favorable to increase activity.

To better understand the activity discrepancy of compounds **3a**–**h** with zanamivir, we compare the 3D binding models of zanamivir[16] to NA (Fig. 2A) obtained from the crystal structure with that of inhibitors **3b** and **3i** to NA derived from docking simulation. Molecular docking was performed by using the AutoDock3.05 program.[17] The crystal structure of a wild-type NA in complex with zanamivir was retrieved from Protein

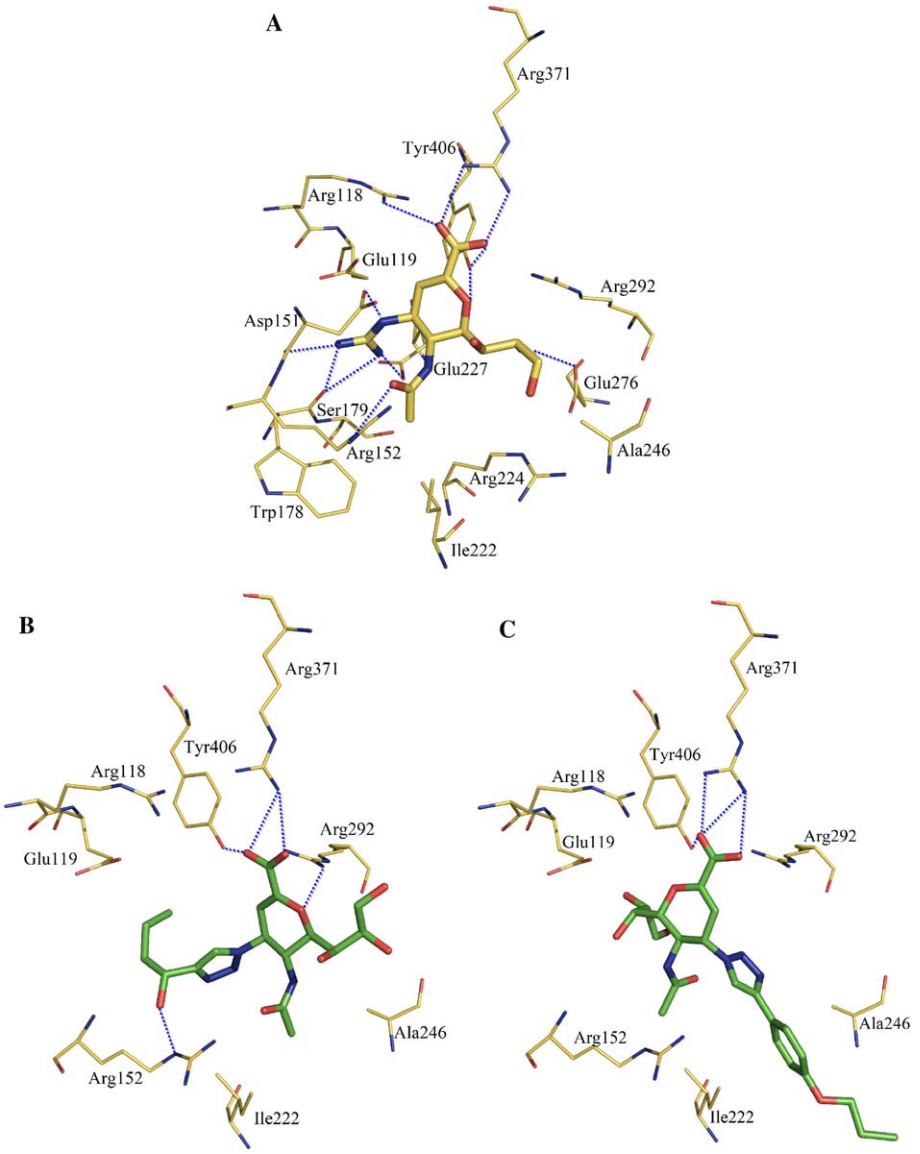


Figure 2. Detailed interactions of zanamivir (A), **3b** (B), and **3i** (C) to the binding sites of NA. Hydrogen-bonds are represented by *blue dotted lines*; Docking models of **3b** (D) and **3i** (E) overlaid with the binding conformation of zanamivir (**1**) into the active sites of NA. The NA surface was colored by electrostatic potential. These images were generated using the PyMol program (http://www.pymol.org/). Compounds **3b**, **3i**, and zanamivir (**1**) are indicated by green, green, and yellow thick sticks, respectively.

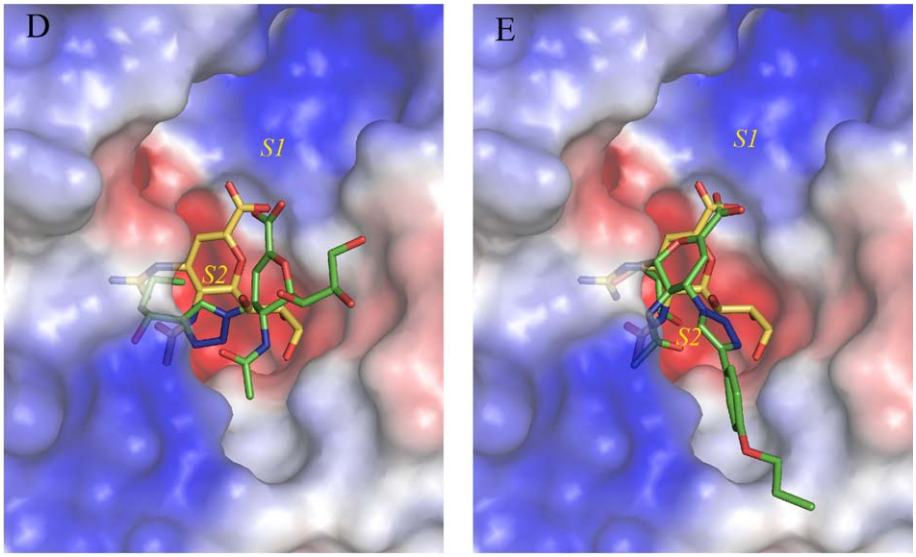


Figure 2. (*continued*)

Data Bank (PDB entry 1NNC).[16] During the docking simulation, the protonation states of the residues of NA at pH 7.0 were used, and the docking parameters in our previous study[18] were adopted. For each inhibitor, conformation with the lowest predicted binding free energy to NA was selected for further analysis. Figures 2B–E show the binding models of compounds **3b** and **3i** with NA.

The binding site of NA has been characterized as highly polar by virtue of the 10 charged (Arg 118, 152, 224, 292, 371, Glu 119, 227, 276, 277, and Asp 151) and only four hydrophobic (Trp 178, Ile 222, Ala 246, and Tyr 406) constitutional amino acids.[19] Figure 2A indicates that the carboxylic acid of zanamivir (**1**) forms strong hydrogen-bonds with two arginine residues (Arg118 and Arg371) in the S1 region (Fig. 2D). Around the guanidinyl moiety of zanamivir, there is a spacially restricted region (S2, Fig. 2D) with negatively charged electrostatic environment caused by three acidic residues, Glu119, Asp151, and Glu227 (Fig. 2A). Actually, two different orientations of R^3 group are observed when the guanidinyl is replaced by a bulky and less polar triazole ring, which more or less decreased the interaction between inhibitors and NA, and hence resulted in a drop of inhibitory activity. In Figures 2B and D, the binding conformation of compound **3b,** which has a smaller propyl alcohol group at R^3 moiety, moves up and only forms a hydrogen-bond with residue Arg152 in the S2 region (guanidinyl moiety of zanamivir forms five hydrogen-bonds in S2 region, Fig. 2A). The R^3 of **3i** is too large to be accommodated in the subsite S2, and hence the six-membered ring flips over and takes a reversed orientation of zanamivir (Figs. 2C and E). Accordingly, being devoid of hydrogen-bond interaction with S2 (Fig. 2C), this series of compounds are generally less potent than **3b** and zanamivir. This alternative binding model (Fig. 2E) can also be seen in the X-ray crystal structures of the complexes of NA with some inhibitors discovered by Stoll et al.[20]

In summary, 16 novel 4-triazole-modified zanamivir analogues were designed and synthesized by using the click reactions. Some compounds exert moderate inhibition against AIV (H5N1), compound **3b** is almost as active as zanamivir (**1**). Molecular modeling result provides the information about the binding model between inhibitors and neuraminidase, and is helpful for future inhibitors' design.

Acknowledgments

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References and notes

1. Enserink, M. *Science* **2006**, *311*, 932.
2. Wong, S. S. Y.; Yuen, K. Y. *Chest* **2006**, *129*, 156.
3. Ungchusak, K.; Auewarakul, P.; Dowell, S. F.; Kitphati, R.; Auwanit, W.; Puthavathana, P.; Uiprasertkul, M.; Boonnak, K.; Pittayawonganon, C.; Cox, N. J.; Zaki, S. R.; Thawatsupha, P.; Chittaganpitch, M.; Khontong, R.; Simmerman, J. M.; Chunsutthiwat, S. *N. Eng. J. Med.* **2005**, *352*, 333.
4. Hayden, F. G.; Couch, R. B. *Rev. Med. Virol.* **1992**, *2*, 89.
5. (a) von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418; (b)

Woods, J. M.; Bethell, R. C.; Coates, J. A.; Healy, N.; Hiscox, S. A.; Pearson, B. A.; Ryan, D. M.; Tilling, J.; Walcott, S. M.; Penn, C. R. *Antimicrob. Agents Chemother.* **1993**, *37*, 1473.

6. (a) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* **1998**, *41*, 2451; (b) Mendel, D. B.; Tai, C. Y.; Escarpe, P. A.; Li, W.; Sidwell, R. W.; Huffman, J. H.; Sweet, C.; Jakeman, K. J.; Merson, J.; Lacy, S. A.; Lew, W.; Williams, M. A.; Zhang, L.; Chen, M. S.; Bischofberger, N.; Kim, C. U. *Antimicrob. Agents Chemother.* **1998**, *42*, 640.
7. (a) Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Starkey, I. D.; Cobley, K. N.; Weston, H.; Scicinski, J.; Merritt, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R. J.; Morley, P. J.; Pateman, T.; Beresford, A. *J. Med. Chem.* **1998**, *41*, 787; (b) Taylor, N. R.; Cleasby, A.; Singh, O.; Skarzynski, T.; Wonacott, A. J.; Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Bethell, R.; Colman, P.; Varghese, J. *J. Med. Chem.* **1998**, *41*, 798; (c) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921; (d) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1925; (e) Macdonald, S. J.; Cameron, R.; Demaine, D. A.; Fenton, R. J.; Foster, G.; Gower, D.; Hamblin, J. N.; Hamilton, S.; Hart, G. J.; Hill, A. P.; Inglis, G. G.; Jin, B.; Jones, H. T.; McConnell, D. B.; McKimm-Breschkin, J.; Mills, G.; Nguyen, V.; Owens, I. J.; Parry, N.; Shanahan, S. E.; Smith, D.; Watson, K. G.; Wu, W. Y.; Tucker, S. P. *J. Med. Chem.* **2005**, *48*, 2964.
8. (a) Woodhall, T.; Williams, G.; Berry, A.; Nelson, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 2109; (b) Woodhall, T.; Williams, G.; Berry, A.; Nelson, A. *Org. Biomol. Chem.* **2005**, *3*, 1795.
9. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004.
10. (a) Kolb, H. C.; Sharpless, K. B. *Drug Discov. Today* **2003**, *8*, 1128; (b) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603; (c) Best, M. D.; Brik, A.; Chapman, E.; Lee, L. V.; Cheng, W. C.; Wong, C. H. *Chem. BioChem.* **2004**, *5*, 811; (d) Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *Chem. BioChem.* **2005**, *6*, 1167.
11. Huisgen, R.. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: Pergamon, 1984; vol. 1, pp 1–176.
12. Martin, R.; Witte, K. L.; Wong, C. H. *Bioorg. Med. Chem.* **1998**, *6*, 1283.
13. Chandler, M.; Bamford, M. J.; Conroy, R.; Lamont, B.; Patel, B.; Patel, V. K.; Steeples, I. P.; Storer, R.; Weir, N. G.; Wright, M.; Williamson, C. *J. Chem. Soc., Perkin Trans. 1* **1995**, *9*, 1173.
14. Scheigetz, J.; Zamboni, R.; Bernstein, M. A.; Roy, B. *Org. Prep. Proced. Int.* **1995**, *27*, 637.
15. Confluent MDCK cells were cultured in 96-well flat-bottomed microplates at a density of 2×10^4 cells/well for 24 h at 37 °C in 5% CO_2. After the cells were infected with 100 times of $TCID_{50}$ A/H5N1 virus which are dilutions of viral stocks, various concentrations of compounds and trypsin were added. At the same time, uninfected cells and infected cells without compound were performed as controls. Antiviral efficacy of test compounds was assessed in vitro by Neutral Red (NR) uptake assay. After 2 days post-infection, cells were incubated with NR dye for 2–3 h at 37 °C. Free dye was washed from the wells and the uptake dye was quantified using a microplate reader at absorbance 540 nm. Absorbance values were expressed as percentages of controls.
16. Varghese, J. N.; Epa, V. C.; Colman, P. M. *Protein Sci.* **1995**, *4*, 1081.
17. 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.
18. Liu, H.; Huang, X.; Shen, J.; Luo, X.; Li, M.; Xiong, B.; Chen, G.; Shen, J.; Yang, Y.; Jiang, H.; Chen, K. *J. Med. Chem.* **2002**, *45*, 4816.
19. Maring, C. J.; Stoll, V. S.; Zhao, C.; Sun, M.; Krueger, A. C.; Stewart, K. D.; Madigan, D. L.; Kati, W. M.; Xu, Y.; Carrick, R. J.; Montgomery, D. A.; Kempf-Grote, A.; Marsh, K. C.; Molla, A.; Steffy, K. R.; Sham, H. L.; Laver, W. G.; Gu, Y. G.; Kempf, D. J.; Kohlbrenner, W. E. *J. Med. Chem.* **2005**, *48*, 3980.
20. Stoll, V.; Stewart, K. D.; Maring, C. J.; Muchmore, S.; Giranda, V.; Gu, Y. G.; Wang, G.; Chen, Y.; Sun, M.; Zhao, C.; Kennedy, A. L.; Madigan, D. L.; Xu, Y.; Saldivar, A.; Kati, W.; Laver, G.; Sowin, T.; Sham, H. L.; Greer, J.; Kempf, D. *Biochemistry* **2003**, *42*, 718.