Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3693–3699

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Identification of Novel Inhibitors of BCR-ABL Tyrosine Kinase via Virtual Screening

Hui Peng,[a,†] Niu Huang,[a,†] Jing Qi,[a] Ping Xie,[b] Chen Xu,[a] Jianxiang Wang[a] and Chunzheng Yang[a,*]

[a]*State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences, Peking Union Medical College, 288 Nanjing Road, Tianjin 300020, PR China*

[b]*Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China*

Received 26 June 2003; revised 7 August 2003; accepted 7 August 2003

**Abstract**—Inhibition of BCR-ABL tyrosine kinase activity has shown to be essential for the treatment of chronic myelogenous leukemia (CML). However, drug resistance has quickly arisen in recent clinical trials for STI571 (Gleevec), which is the first approved drug of CML by inhibiting ABL tyrosine kinase. It is desirable to develop new types of ABL tyrosine kinase inhibitors that may overcome this drug resistance problem. Here we present the discovery of novel inhibitors targeted at the catalytic domain of ABL tyrosine kinase by using three-dimensional database searching techniques. From a database containing 200,000 commercially available compounds, the top 1000 compounds with the best DOCK energy score were selected and subjected to structural diversity and drug likeness analysis, 15 compounds were submitted for biological assay. Eight out of the 15 showed inhibitory activity against K562 cells with $IC_{50}$ value ranging from 10 to 200 μM. Two promising compounds showed inhibition in further ABL tyrosine phosphorylation assay. It is anticipated that those two compounds can serve as lead compounds for further drug design and optimization.


Over 90% of cases of chronic myelogenous leukemia (CML) and 10–25% of cases of adult acute lymphoblastic leukemia (ALL) are associated with Philadelphia chromosome (Ph) which involves a fusion of the breakpoint cluster region (BCR) gene on chromosome 22 at band q11 with the ABL proto-oncogene on chromosome 9 at band q34.[1] This BCR-ABL fusion protein acts as an onco-protein by activating several signaling pathways that lead to malignant transformations, which are known as Myc, Ras, c-Raf, MAPK/ERK, SAPK/JNK, Stat, NFKB, PI3K kinase, and c-Jun.[2] The oncogenic ability of BCR-ABL requires deregulated tyrosine kinase activity which leads to the recruitment of adaptor molecule, the phosphorylation of signaling molecules, and the activation of downstream signaling events.[3–6] Expression of the BCR-ABL kinases upregulates cell proliferation, decreases apoptosis, increases cytokine-independent growth, decreases adhesion to the bone marrow stroma, and produces cytoskeletal abnormalities.[7–10] Therefore, inhibition of BCR-ABL tyrosine kinase activity is essential for the treatment of CML.

STI571 (also known as its brand name Gleevec, Novartis Pharmaceutical Inc.), a FDA recently approved drug for CML, is the first tyrosine kinase inhibitor that has shown to be effective in inducing clinical, hematological and molecular remissions for CML patients.[11] The discovery of STI571 is based on the observation of inhibitory effect on the intracellular BCR-ABL tyrosine kinase. The success of this drug development represents a new direction in the drug design and discovery process by targeting specific tyrosine kinases important for intracellular signal transduction pathways. However, recent clinical trials of STI571 in CML demonstrated that many patients with advanced stage disease respond initially but relapse. This drug resistance was suggested to associate with a single amino acid substitution of Thr315 with Ile in the ABL kinase catalytic domain or a progressive BCR-ABL gene amplification.[12] Although

*Corresponding author. Tel./fax: +86-22-2723-0740; e-mail: czyang@public.tpt.tj.cn
†H. Peng and N. Huang contributed equally to this work.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.014

the development of STI571 resistance presents a new therapeutic challenge, the fact that BCR-ABL remains active in STI571-resistant cells suggests that this enzyme remains to be a promising molecular target to develop the second-generation ABL-targeted tyrosine kinase inhibitors which could be used alone or in combination with STI571 to prevent the development of resistance.[2,10] The crystal complex structure of a variant of STI571 bound to the catalytic domain of ABL provided important insights on the molecular mechanism of STI571 that it can achieve high specificity by recognizing a distinctive inactive conformation of the activation loop of ABL.[13] The ability of the catalytic domain of ABL tyrosine kinases to adopt characteristic inactive conformations is suggested to be a critical event of target proteins for the development of specific tyrosine kinase inhibitors.

Traditionally, random testing of large numbers of compounds from either natural sources or synthetic combinatorial libraries was both costly and time-consuming. Computer screening approach can filter out undesirable compounds on the basis of a wide variety of criteria, including calculated receptor binding affinity and chemico-physical properties. The structure-based database search program DOCK enable the best-scored compounds to be selected based on molecular complementarity to the target receptor.[14] Recently, the DOCK program has successfully been applied to find lead compounds for HIV-1 protease, growth hormone antagonists and thrombin inhibitors. Therefore, it is desirable to identify novel small compounds to inhibit ABL tyrosine kinase activity, and ideally overcome the drug resistance problem of STI571, via a computer screening study.

Database searching was performed on a database containing ~200,000 commercially available compounds (www.chemdiv.com), which was converted into 3-D MOL2 format via in-house procedures. The PDB entry 1FPU[13] of BCR-ABL kinase domain in complex with a variant of STI571 was retrieved from the Protein Data Bank. The same binding site as STI571 was chosen for docking. Waters and complexed ligand were deleted; hydrogens and charges were added by SYBYL6.6. All docking calculations were carried out with DOCK4.0.1 using flexible ligands based on the anchored search method.[15] The standard docking protocol was followed as (1) target preparation, (2) sphere set generation, (3) force-field grid calculation and (4) docking and scoring. Based on docking results, 1000 compounds with the top energy score were selected for further analysis. This was done by clustering these compounds into structurally diverse sets based on their molecular fingerprints and choosing compounds from individual groups. Based on the well-known 'Rule-of-5' criteria[16] and commercial availability, samples of 15 compounds were subjected to biological testing.

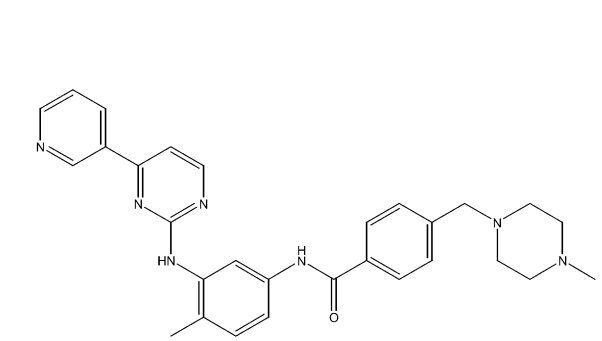
The inhibitory assay of the growth of Ph-positive K562 cells was tested for all 15 compounds. The MTT assays were performed to assess the viability of the tumor cell treated with these compounds. Table 1 presents $IC_{50}$ values for inhibition of K562 cell line. It was shown that compounds **3** and **8** inhibited the growth of K562 cells in a dose dependent manner. The concentration of compound **3** to inhibit 50% cell growth ($IC_{50}$) was 24.03 μM, and it was at a higher concentration (29.54 μM) for compound **8**. Compounds **3** and **8** (Fig. 1) were selected for further biological experiments due to their potent cellular activity and distinct structural features.

Since the above inhibitory activity was mediated via the inhibition of BCR-ABL tyrosine kinase, the following assay was performed to determine the expression levels of BCR-ABL in K562 cell lysates by Western blotting analysis.[17] The cultured K562 cells were lysed and the whole cell lysates protein were separated on 7.5% SDS-PAGE. The proteins were transferred onto nitrocellulose membrane, and the amount of BCR-ABL protein and protein tyrosine kinase activity were detected with anti-ABL and antiphosphotyrosine antibodies respectively. Representative western blots for two compounds are illustrated in Figures 2 and 3. These results indicated that along with increased concentration of these compounds, the expression levels of tyrosine phosphorylation tend to decrease in amount. It seems to us that compound **3** was at least 2-fold more potent than compound **8** at inducing down-regulation of BCR-ABL tyrosine kinase in intact cells. However, compounds **3** and **8** did not appear to affect expression levels of BCR-ABL after 5-h incubation. This study provided important in vitro data to support the notion that no significant differences in the amount of ABL protein were observed on the ABL immunoblot assay. However, there was a significant reduction in the amount of phosphotyrosine detected in the presence of these two compounds. Therefore, compounds **3** and **8** can inhibit ABL kinase activity without affecting the amount of ABL protein. It is notable that these two active compounds belong to different chemical classes and their structures are also different to those previously reported BCR-ABL tyrosine kinase inhibitors. These results strongly suggest that the computer screening strategy

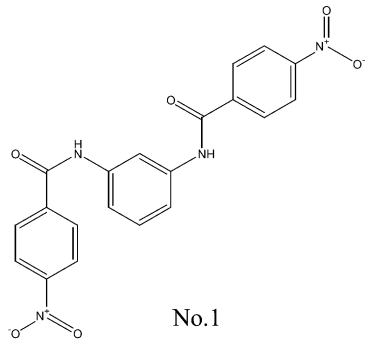
**Table 1.** K562 cells growth inhibition values for selected compounds

| Compd | Inhibition of K562 cells growth ($IC_{50}$, μM)[a] |
|---|---|
| STI571 | 0.40 |
| **1** | >246 |
| **2** | 129.61 |
| **3** | 24.03 |
| **4** | 60.56 |
| **5** | >215 |
| **6** | >215.8 |
| **7** | 108.15 |
| **8** | 29.54 |
| **9** | >230.3 |
| **10** | 108.1 |
| **11** | 58.22 |
| **12** | >271.9 |
| **13** | 160.25 |
| **14** | >241.9 |
| **15** | >247.2 |

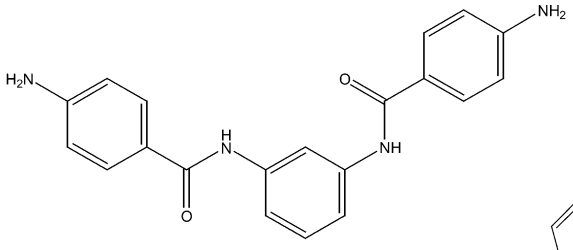
[a]Values are means of three experiments. Inhibition of growth of K562 was determined by MTT assay.



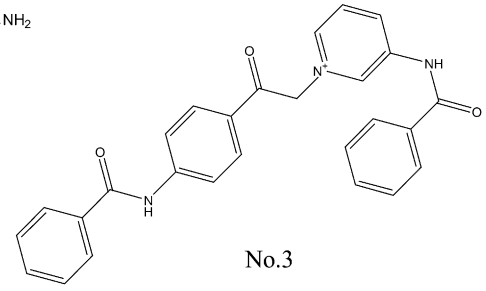
STI571



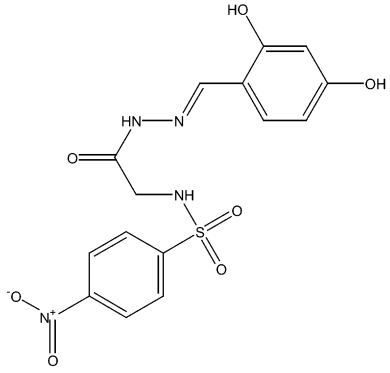
No.1



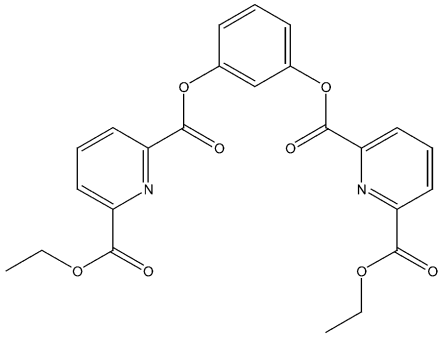
No.2



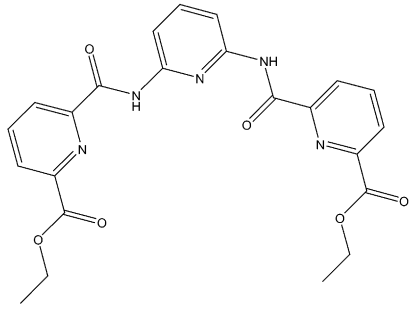
No.3



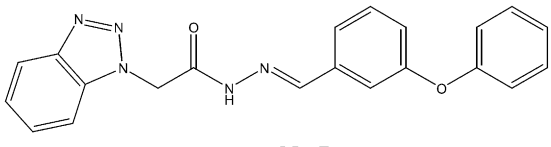
No.4



No.5



No.6



No.7

**Figure 1.** Chemical structures of STI571 and selected compounds.

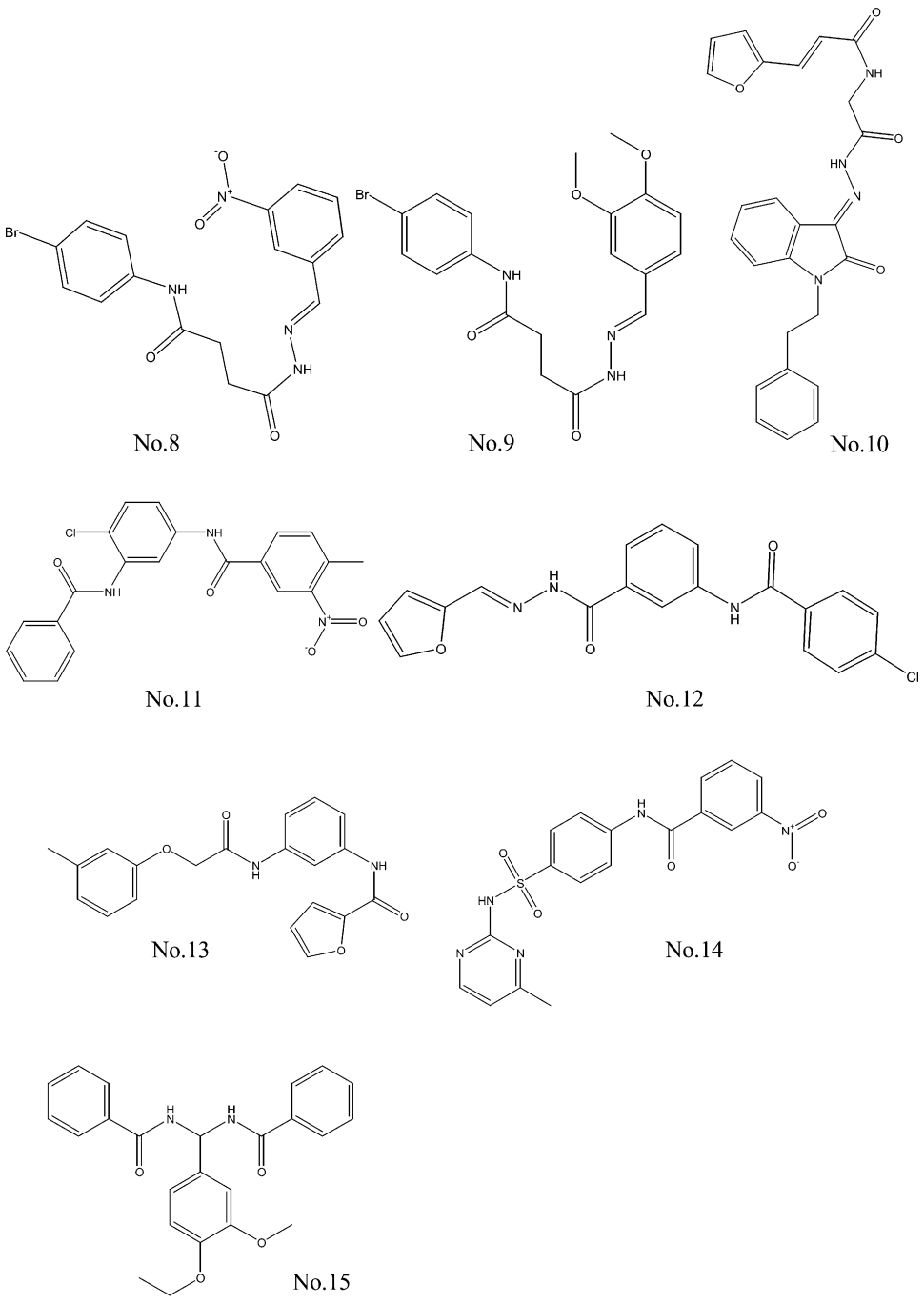



**Figure 1.** *(continued)*

employed in this study was effective in identifying novel small molecule inhibitors of BCR-ABL tyrosine kinase.

The docking model of compound **3** with BCR-ABL kinase domain may shed light into its inhibition activity. The docked complex structure shown in Figure 4 suggests different binding characteristics of compound **3** comparing with that of STI571. Compound **3** does not reach as deeply into the kinase domain. The benzamido and and benzoylmethyl groups at N-1 position of pyridinyl moiety occupy the place of the core phenyl ring of STI571. The majority of the interaction between compound **3** and the protein are also mediated by van der Waals interaction. The phenyl-moiety of compound **3** makes more extensive van der Waals and hydrophobic contacts with the nonpolar side chains of several residues deep in the binding cleft, including Val289, Val299 and Ala380. Moreover, the side chains of Asp381 and Glu286 form two hydrogen bonds with the secondary amino group of pyridinyl moiety of compound **3** (Fig. 5) where STI571 can form five hydrogen bonds in the same binding site of BCR-ABL. Experimental studies[13] have suggested that the mutation of Thr315 result in the drug resistance of STI571, which can be rationalized by the hydrogen bond between STI571 and the side chain of Thr315 (Fig. 6). However, no hydrogen bond could be formed between compound **3** and the side chain of Thr315. It may indicate that compound **3** can exert its

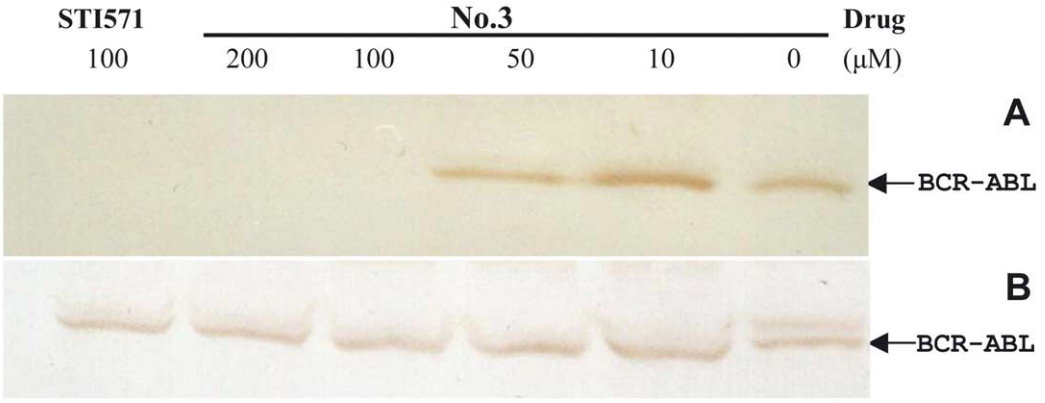



**Figure 2.** Effect of No. 3 on BCR-ABL kinase. K562 cells were incubated for 5 h in the presence of the indicated concentration of No. 3. Cells were lysed and equal amounts of lysate were analyzed by immunoblotting with antiphosphotyrosine (A) or anti-ABL antibodies (B). The migration of BCR-ABL is marked with an arrow on the right of the panel.

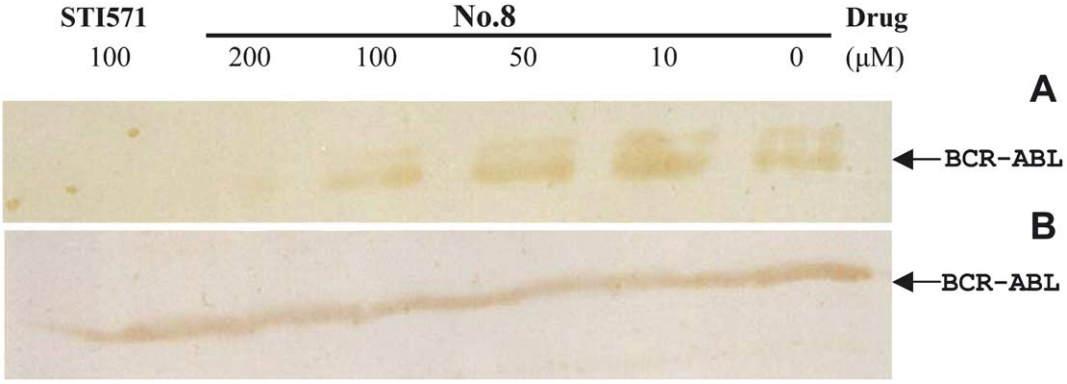



**Figure 3.** Effect of No. 8 on BCR-ABL kinase. K562 cells were incubated for 5 h in the presence of the indicated concentration of No. 8. Cells were lysed and equal amounts of lysate were analyzed by immunoblotting with antiphosphotyrosine (A) or anti-ABL antibodies (B). The migration of BCR-ABL is marked with an arrow on the right of the panel.

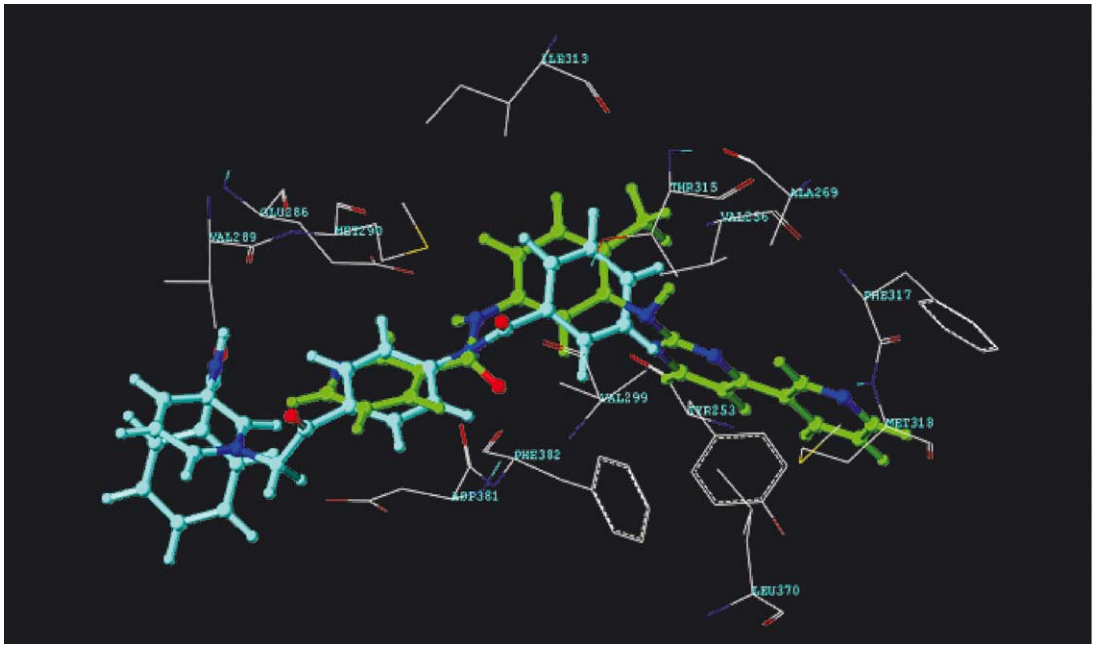



**Figure 4.** Comparison of a variant of STI571 (green) and compound **3** (cyan) bind to ABL. The principal interactions between the inhibitor and the active site residues were shown.

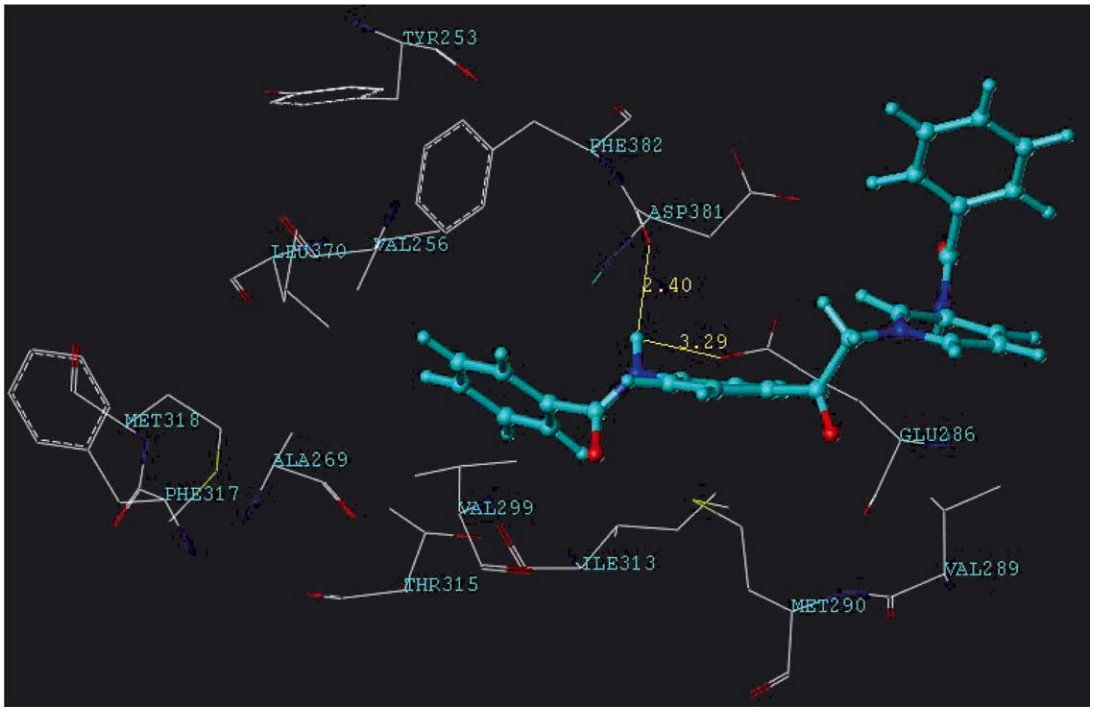



**Figure 5.** The binding mode of inhibitor compound **3** (cyan) in ABL active site. Residues whose side chains are shown and labeled are those that make H-bond and hydrophobic interaction with compound **3**. Also shown are the two hydrogen bonds (yellow line) made by carbonyl atoms of Asp381 and Glu286 with compound **3**.

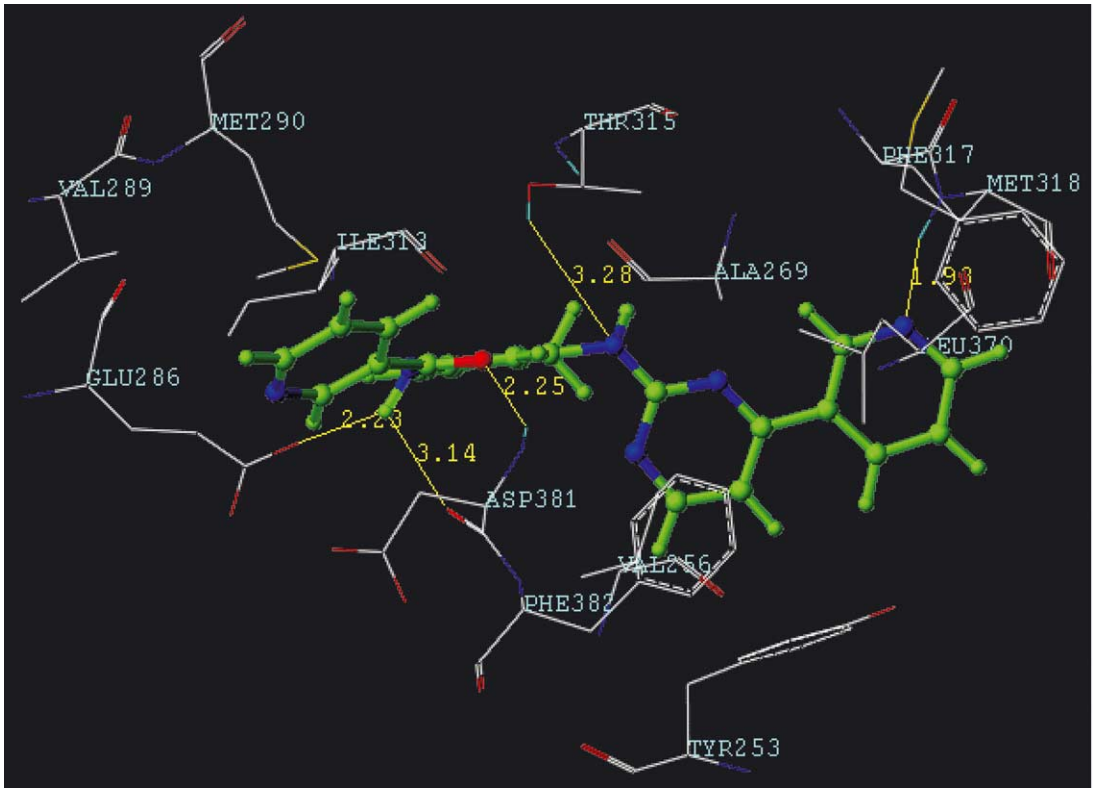



**Figure 6.** The binding mode of inhibitor a variant of STI571 (green) in ABL active site. Residues whose side chains are shown and labeled are those that make H-bond and hydrophobic interaction with a variant of STI571. Hydrogen bonds are depicted as yellow line with the donor–acceptor distance given in Å.

inhibitory effect on STI571-resistant human leukemia cells through inhibiting ABL kinase function. It should be noted that study of establishing a cell line with resistance to STI571 are required to determine potential role of compound **3** as the lead compound to develop second-generation ABL kinase inhibitor. These results will provide useful information in understanding the structural and chemical features of BCR-ABL tyrosine kinase inhibitor and in designing new potential compounds.

In our study, compounds **3** and **8** showed notable inhibitory activity against Ph-positive human K562 cells in agreement with its potency of BCR-ABL tyrosine kinase activity. These initial screened hits (10–100 μM) could be modified to yield better binding affinities. The required improvement in ligand binding affinity is equivalent to the energy by forming a couple of additional hydrogen bonds. Extensive efforts to further optimize the small molecule inhibitors of BCR-ABL tyrosine kinase by using structure-based drug design techniques are currently ongoing in our laboratory. Although the limitations in current computer screening methods, our initial results with targeting BCR-ABL tyrosine kinase are encouraging. Optimization of these compounds to enhance their affinity and decrease their side effects is expected to lead to new classes of chemical agents for the treatment of CML.

In conclusion, virtual screening against the catalytic domain of BCR-ABL kinase has been used to identify novel small molecule inhibitors. Of these inhibitors, compounds **3** and **8** display inhibition of cell viability and cell growth in the K562 cell line. We further demonstrated that compounds **3** and **8** can inhibit ABL kinase activity but have no effect in the amount of ABL protein. Therefore, compounds **3** and **8** represent two novel small molecule inhibitors of BCR-ABL tyrosine kinase for the further optimization which may lead to the second-generation of drugs with the capability of overcoming the STI571 resistance in clinical trials.

## Acknowledgements

This work was supported by grants from National '863' High Technology R&D Project Foundation in China, Grant No. 2001AA234021-9.

## References and Notes

1. Rowley, J. D. *Nature* **1973**, *243*, 290.
2. Advani, A. S.; Pendergast, A. M. *Leuk. Res.* **2002**, *26*, 713.
3. Dai, Z.; Quackenbush, R. C.; Courtney, K. D.; Grove, M.; Cortez, D.; Reuther, G. W.; Pendergast, A. M. *Genes Dev.* **1998**, *12*, 1415.
4. Evans, C. A.; Owen-Lynch, P. J.; Whetton, A. D.; Dive, C. *Cancer Res.* **1993**, *53*, 1735.
5. Daley, G. Q.; Van Etten, R. A. *Science* **1990**, *247*, 824.
6. Lugo, T. G.; Pendergast, A. M.; Muller, A. J.; Witte, O. N. *Science* **1990**, *247*, 1079.
7. Sawyers, C. L.; Callahan, W.; Witte, O. N. *Cell* **1992**, *70*, 901.
8. Afar, D. E.; Goga, A.; McLaughlin, J.; Witte, O. N.; Sawyers, C. L. *Science* **1994**, *264*, 424.
9. Sawyers, C. L. *N. Engl. J. Med.* **1999**, *340*, 1330.
10. Jahagirdar, B. N.; Miller, J. S.; Shet, A.; Verfaillie, C. M. *Exp. Hematol.* **2001**, *29*, 543.
11. Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. *N. Engl. J. Med.* **2001**, *344*, 1031.
12. Gorre, M. E.; Mohammed, M.; Ellwood, K.; Hsu, N.; Paquette, R.; Rao, P. N.; Sawyers, C. L. *Science* **2001**, *293*, 876.
13. Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W. J.; Clarkson, B.; Kuriyan, J. *Science* **2000**, *289*, 1938.
14. Kuntz, I. D. *Science* **1992**, *257*, 1078.
15. Ewing, T. J. A.; Kuntz, I. D. *J. Comput. Chem.* **1997**, *18*, 1175.
16. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Res.* **1997**, *23*, 3.
17. Druker, B.; Okuda, K.; Matulonis, U.; Salgia, R.; Roberts, T.; Griffin, J. D. *Blood* **1992**, *79*, 2215.