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Hybrid compounds as new Bcr/Abl inhibitors

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ABSTRACT

A series of 2,4-disubstituted thiazole derivatives were designed and synthesized as new Bcr/Abl inhibitors by hybriding the structural moieties from FDA approved imatinib, nilotinib and dasatinib. The new inhibitors strongly suppressed the activity of Bcr/Abl kinase and potently inhibited the proliferation of K562 and KU812 leukemia cancer cells. Compound **4i** displayed comparable potency with that of nilotinib in both biochemical kinase assay and cancer cell growth inhibition assay. These inhibitors might serve as lead compounds for further developing new anticancer drugs.

Chronic myelogenous leukemia (CML) is a hematological malignancy representing about 20% of adult leukemia and being characterized by the occurrence of the Philadelphia (Ph) chromosome. The Philadelphia (Ph) chromosome is a truncated version of chromosome 22 resulting from the reciprocal translocation between chromosome 9 and 22. The chimeric Bcr/Abl gene generated by the translocation encodes a fusion protein with constitutively activated kinase activity.[1–3] Bcr/Abl kinase is a well validated target for development of small molecular inhibitors to treat CML. The first generation Bcr/Abl inhibitor imatinib (STI571 or Gleevec) has achieved tremendous clinical success and become the first-line drug in conventional treatment of CML.[4,5] However, emerging acquired resistance to imatinib is becoming a major challenge. Three–four percent resistance rates were reported in newly diagnosed chronic phase CML patients. For imatinib treated CML patients in accelerated or blastic phases, the acquired resistance percentages were up to 40–50% or over 80%, respectively.[6] The point mutations in the kinase domain of Bcr/Abl are the primary mechanism for the imatinib resistance, and about 100 point mutations have been identified to date.[7]

To overcome the drug resistance to imatinib, several classes of second generation kinase inhibitors have been designed and synthesized.[8] Type-II Bcr/Abl inhibitor nilotinib[9] and type-I Bcr/Abl inhibitor dasatinib[10,11] have been approved as the second-line drugs to treat adult patients in all phases of CML with resistance to imatinib. However, neither nilotinib nor dasatinib could suppress the proliferation of leukemia cells harboring the Bcr/Abl T315I mutant.[12–14] Only recently, a few small molecules were reported to show good efficacy against the Bcr/Abl T315I mutation.[15] One molecule AP24534 was advanced into phase II clinical trial in US[15g].

In this Letter, we report the design, synthesis and biological evaluation of a new kind of Bcr/Abl inhibitors by hybriding the structural moieties from FDA approved imatinib, nilotinib and dasatinib (Fig. 1).

The synthesis of compounds **4a–m** was outlined in Scheme 1. Briefly, substituted methyl 3-aminobenzoate **1** was slowly added to a solution of acetyl chloride and NH_4SCN in acetone to produce the methyl 3-(3-acetylthioureido)benzoate **2**. 3-(4-(Pyridin-3-yl)thiazol-2-ylamino)-benzoate methyl ester **3** was synthesized by reaction of compounds **2** with 2-bromo-1-(pyridin-3-yl)-ethanone. Finally, the designed compounds **4a–4m** were readily prepared by direct aminolysis of intermediate **3** (for **4a–4f**, **4j–4m**) or condensation of the hydrolyzed **3** (acids. for **4g–4i**) with different anilines.

The kinase inhibitory activities of compounds **4a–4m** were evaluated via a well established FRET-based Z'-Lyte assay.[16] Imatinib and nilotinib were utilized as the positive controls to validate the screening assay. Under the screening conditions, Imatinib and nilotinib displayed IC_{50} values of 309 and 39.3 nM against Bcr/Abl fused kinase, which was highly consistent to the reported data.[9] As expected, the designed hybrid compound **4a** also potently inhibited the kinase activity of Bcr/Abl with an IC_{50} value of 0.67 μM.

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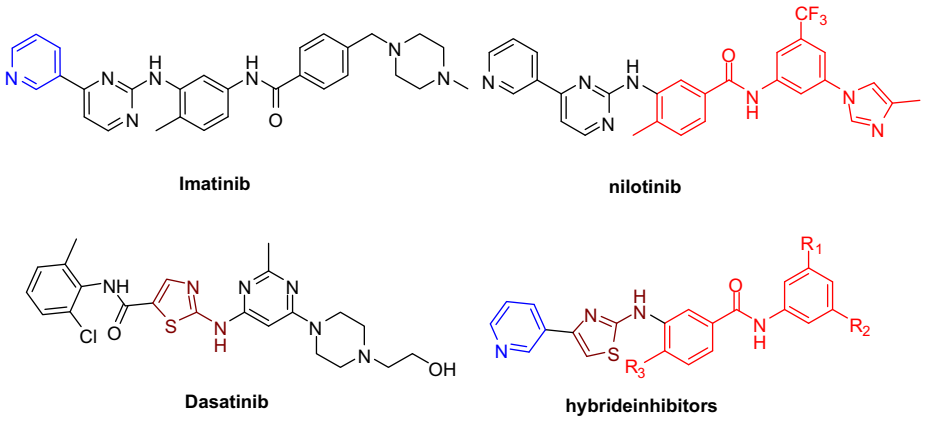
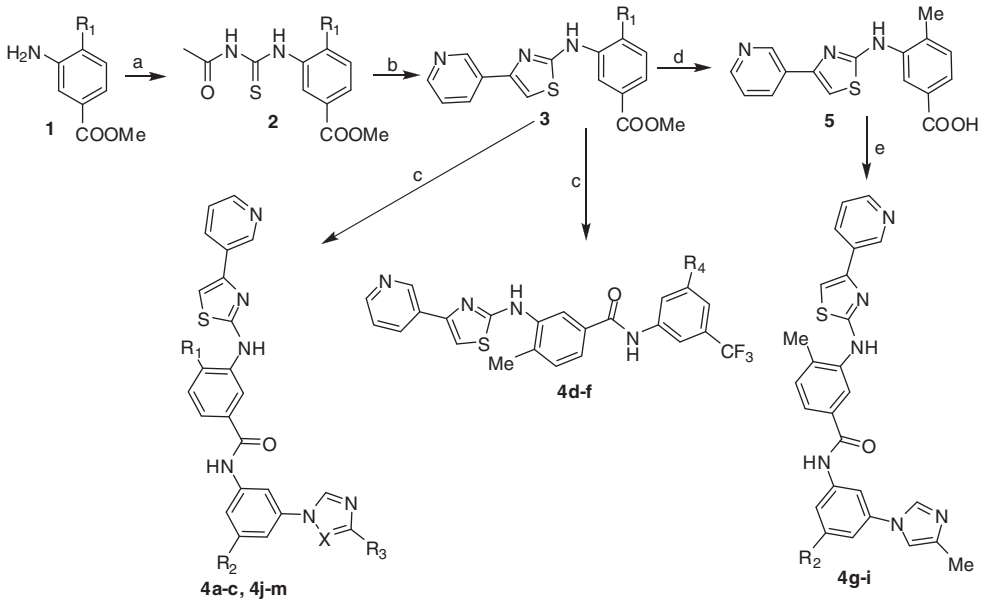


Figure 1. Chemical structures of FDA approved Bcr/Abl inhibitors and the designed hybrid compounds.



Scheme 1. Reagents and conditions: (a) acetyl chloride, NH_4SCN, acetone, 80–86%; (b) 2-bromo-1-(pyridin-3-yl)ethanone, K_2CO_3, MeOH, 78–88%; (c) aniline, *t*-BuOK, THF, 79–85%; (d) NaOH, MeOH, 83%; (e) diethyl cyanophosphonate, Et_3N, DMF, 50–60%.

Molecular docking study was performed to investigate the binding modes of compound **4a** with Bcr/Abl (PDB code: 3CS9) using GOLD 3.01.[17] It was shown that compound **4a** bind to ATP pocket of Abl in a similar fashion to that of nilotinib (Fig. 2a). Specifically, the 4-(pyridin-3-yl)thiazole core occupied the adenine pocket of the kinase, the amide formed two hydrogen bonds with Glu286 and Asp381, and the trifluoromethylphenyl-imidazole moiety bound deeply into the DFG-out hydrophobic pocket.

A structure–activity study revealed that the 4-methyl-1*H*-imidazol-1-yl group in **4a** could be replaced by other five-member heterocyclic substituents (**4b–4d**) without significantly interfering the inhibitory activity against Bcr/Abl kinase. However, when the 4-methyl-1*H*-imidazol-1-yl group was removed (**4f**), the kinase inhibitory activity was almost totally abolished (Table 1). This might be due to the fact that the removal of the 4-methyl-1*H*-imidazol-1-yl group could reduce the hydrophobic interaction with DFG-out pocket. The molecular modeling studies also suggested that the CF_3 group in compound 4a could be replaced with a slightly larger hydrophobic group to achieve a better fit to the DFG-out pocket of Abl kinase (Fig. 2b). Compounds **4h** and **4i** indeed showed better bioactivity than that of **4b**. Especially, compound **4i** possessed an IC_{50} of 20.1 nM against Bcr/Ab. However, when the CF_3 group was replaced by an adamantyl group, compound **4j** became totally inactive, which might be due to the spatial collision with the DFG-out pocket of Abl. When the CF_3 group in compound **4a** was replaced by a relatively small Cl group (**4k**), the potency decreased. It was clear that the methyl group at R^1 position restricted the compound's conformation to fit into the ATP binding site of Abl protein, it might also form additional hydrophobic interactions with Ala269, Val270 and Lys271. Not surprisingly, the removal or replacing with slightly bigger ethyl group of this group (**4l** and **4m**) led to a dramatic activity decrease consistent to their potent Bcr/Abl kinase inhibitory activities, the compounds also displayed strong suppression on the growth of human chronic myelogenous leukaemia K562 and Ku812 cells with express high level of Bcr/Abl protein. For instance, compound **4i** potently inhibited the growth of K562 and Ku812 cells with IC_{50} values of 10 and 11 nM, respectively, which were equally potent to nilotinib. Furthermore, the compounds also potently inhibited the proliferation of imatinib-resistant CML cells (K562-R) (Table 1).

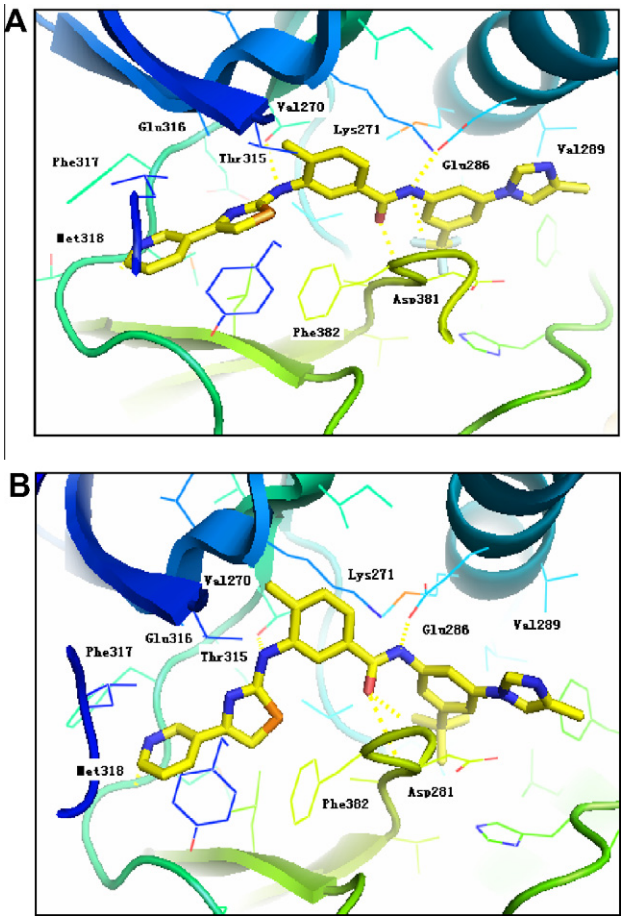
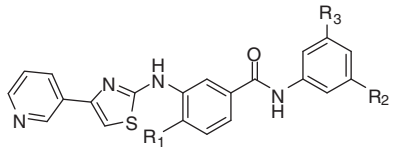


Figure 2. The predicted binding modes of compounds **4a** and **4i** with Abl kinase. Blue: nitrogen; red: oxygen ;. Doteed lines indicate H-bond.

Table 1
Structure–activity relationship of pyridin-thiazol-2-ylamino-benzamide derivatives.[a]



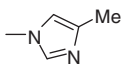
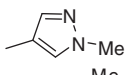
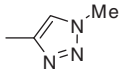
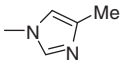
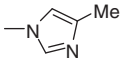
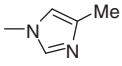
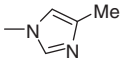
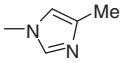
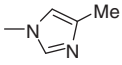
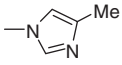
Compd	R^1	R^2	R^3	Bcr/Abl	Cell Growth inhibition IC_{50} (nM)		
				Kinase inhibition IC_{50} (nM)	K562	KU812	K562-R[b]
Imatinib				309	380	337	6.05
Nilotinib				39.3	6.5	3.4	260
4a	Me	CF_3		671	28	30	1830
4b	Me	CF_3		507	28	36	2140
4c	Me	CF_3		480	26	38	1630
4d	Me	CF_3		491	27	27.1	5380
4e	Me	CF_3		277	57	90	3510

Table 1 (*continued*)

Compd	R^1	R^2	R^3	Bcr/Abl	Cell Growth inhibition IC_{50} (nM)		
				Kinase inhibition IC_{50} (nM)	K562	KU812	K562-R[b]
4f	Me	CF3	H	>10000	3005	1235	1440
4g	Me	Me		774	449	871	3290
4h	Me	*i*-Pr		177	31	51	2910
4i	Me	*t*-Bu		20.1	10	11	640
4j	Me			>2000	1061	2805	11,850
4k	Me	Cl		1300	812	518	2360
4l	H	CF_3		3780	3193	2733	1720
4m	Et	CF_3		5510	1603	945	2720

[a] ABL activity assays were performed using the FRET-based Z'-Lyte assay according to the manufacturer's instructions. The anti-proliferative activities of the compounds were evaluated by CCK-8 kit. The data were reported as the means of at least three independent experiments.
[b] Imatinib induced resistant CML K562 cells.

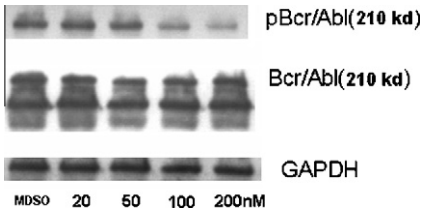


Figure 3. Compound **4i** dose-dependently inhibits the phosphorylation of Bcr/Abl in K562 cells. Results are representative of at least three independent experiments.

Taking compound **4i** as an example, the Bcr/Abl kinase inhibitory activity was further validated by using western-blot analysis. As shown in Figure 3, compound **4i** strongly inhibited the autophosphrylation of Bcr/Abl fused protein in a dose-dependent manner after a 4 h treatment in K562 CML cells. Flow cytometric analysis revealed that compound **4i** also dose-dependently induced the G0/G1 phase arrest and apoptosis of K562 cancer cells, which might be a consequent response of Bcr/Abl kinase inhibition (Fig. 4)

In summary, a series of 2,4-disubstituted thiazole derivatives were designed and synthesized as new Bcr/Abl inhibitors by hybriding the structural moieties from FDA approved imatinib, nilotinib and dasatinib. The resulting compounds strongly suppressed the activity of Bcr/Abl kinase and potently inhibited the proliferation of K562 and KU812 leukemia cancer cells. Furthermore, the compounds also potently inhibited the proliferation of imatinib-resistant CML cells (K562-R). One of the most potent compound **4i** displayed comparable potency with that of nilotinib in both biochemical kinase assay and cancer cell growth inhibition assay. As a consequent response of Bcr/Abl kinase inhibition, compound **4i** also dose-dependently induced the G0/G1 phase arrest and apoptosis of K562 cancer cells. These inhibitors might serve as lead compounds for further developing new anticancer drugs.

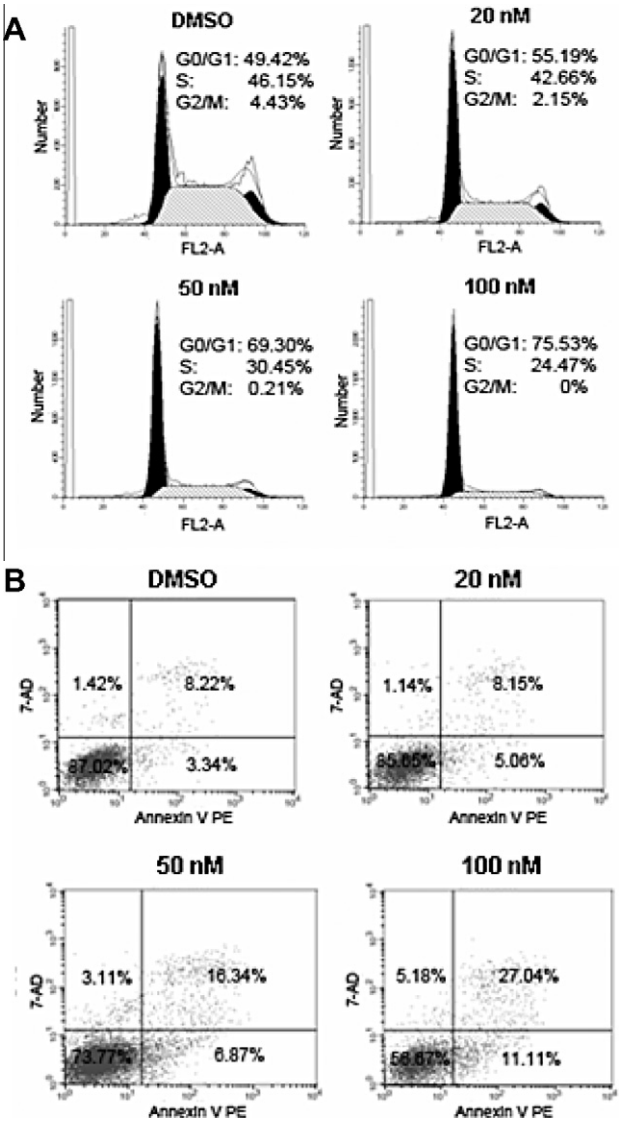


Figure 4. A. Compound **4i** dose-dependently induces G0/G1 arrest (A) and apoptosis of K562 cancer cells. Results are representative of at least three independent experiments.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.029.

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