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Design, Synthesis and Cytotoxic Evaluation of Novel Imatinib Amide Derivatives that Target Abl Kinase

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Abstract: Novel imatinib amide derivatives (**a1-28**, **b1-9**) were synthesized and evaluated for their biological activities. All compounds were characterized by ^{1}H NMR, MS and elemental analysis. Among all the derivatives, compounds **a4**, **a10**, **a21**, **b1** and **b2** displayed the most significant ability of inhibiting K562 cell proliferation with the IC_{50} values of 0.67, 0.66, 0.65, 0.59 and 0.62 μM, respectively, indicating that these compounds were potent inhibitors of Bcr-Abl in leukemic K562 cells, comparable to the reference compound imatinib. Molecular docking study was performed to position compounds **a21** and **b1** into the active site of Abl to determine the probable binding modes.

Keywords: Amide derivatives, Bcr-Abl inhibitors, chronic myeloid leukemia, imatinib, inhibiting activity, molecular docking, SAR.

INTRODUCTION

The Ph chromosome, discovered in 1960 by Nowell and Hungerford [1], is a truncated chromosome 22 that results from a reciprocal exchange of genetic material between the long arms of chromosomes 9 and 22. The translocation results in the juxtaposition of 3' DNA sequences derived from the Abelson (Abl) proto-oncogene normally located on chromosome 9 with 5' sequences of the breakpoint cluster region (Bcr) gene on chromosome 22 and creates the Bcr-Abl oncogene [2-4]. The tyrosine kinase activity of Bcr-Abl leads to the chronic phase of chronic myeloid leukemia (CML) [5-7].

Therefore, screening small molecules that inhibit Bcr-Abl kinase activity of leukemic cells without adversely affecting the normal cell population has been the mainstream concept of curing leukemia patients. Imatinib (STI571, Gleevec) inhibits the activity of Bcr-Abl by binding to the kinase domain of Bcr-Abl when the protein is in its closed, inactive conformation [8]. Thus, imatinib is considered as a potent selective Bcr-Abl tyrosine kinase inhibitor and a first-line therapy for the majority of CML cases because of its high efficacy and relatively mild side effects [9, 10]. Although the majority of diagnosed patients achieve durable responses to STI-571 therapy at both the hematological and cytogenetic levels, relapse and resistance are observed in a large percentage of patients [11]. Results from crystallographic studies indicate that mutations in the kinase domain of Bcr-Abl itself account for the main reasons of resistance to STI-571 [12, 13].

The frequency of relapse and resistance in leukemia cases undergoing STI-571 therapy has paved the way for the development of second generation Bcr-Abl inhibitors such as dasatinib [14] and nilotinib [15]. Due to the challenge of more and more leukemia patients emerging every year, efforts are now focused on the synthesis of novel molecules that inhibit the Bcr-Abl to provide more opportunity for the endangered patients.

In recent years, it was reported that the benzene acrylamide and benzamide derivatives have potent anti-tumor activity [16-18]. Since X-ray crystal structure shows that most of the interactions of imatinib with the protein is from the moiety of 2-[*N*-(2-methyl-5-amino-phenyl) amino]-4-(3-pyridyl) pyrimidine, we would like to hybridize the moieties of imatinib and benzene acrylamide or benzamide, make them into one molecule by the principle of pharmacophore combination and design a series of novel imatinib derivatives with the expectation of obtaining compounds with potent inhibition activity of Bcr-Abl in leukemic K562 cells. Biological assays have proved that our strategy is successful by yielding several compounds with IC_{50} values around 0.59 μM to inhibit the K562 cell proliferation.

MATERIALS AND METHODS

1. Instruments

^{1}H NMR spectra were recorded with a Bruker DRX 300 model spectrometer in $CDCl_3$ and $(CD_3)_2SO$ solutions with TMS as an internal standard. Melting points were measured on a Buchi micro melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Carbon, hydrogen and nitrogen assays were obtained with a CHN–O-Rapid instrument and were within ± 0.4% of the theoretical values.

2. Syntheses

Thirty-eight compounds were synthesized via the route outlined in Scheme (**1**). Compounds **2** were synthesized by the known procedure [19], and compounds **3** were obtained as reported [20]. The target compounds **a1-26** were prepared

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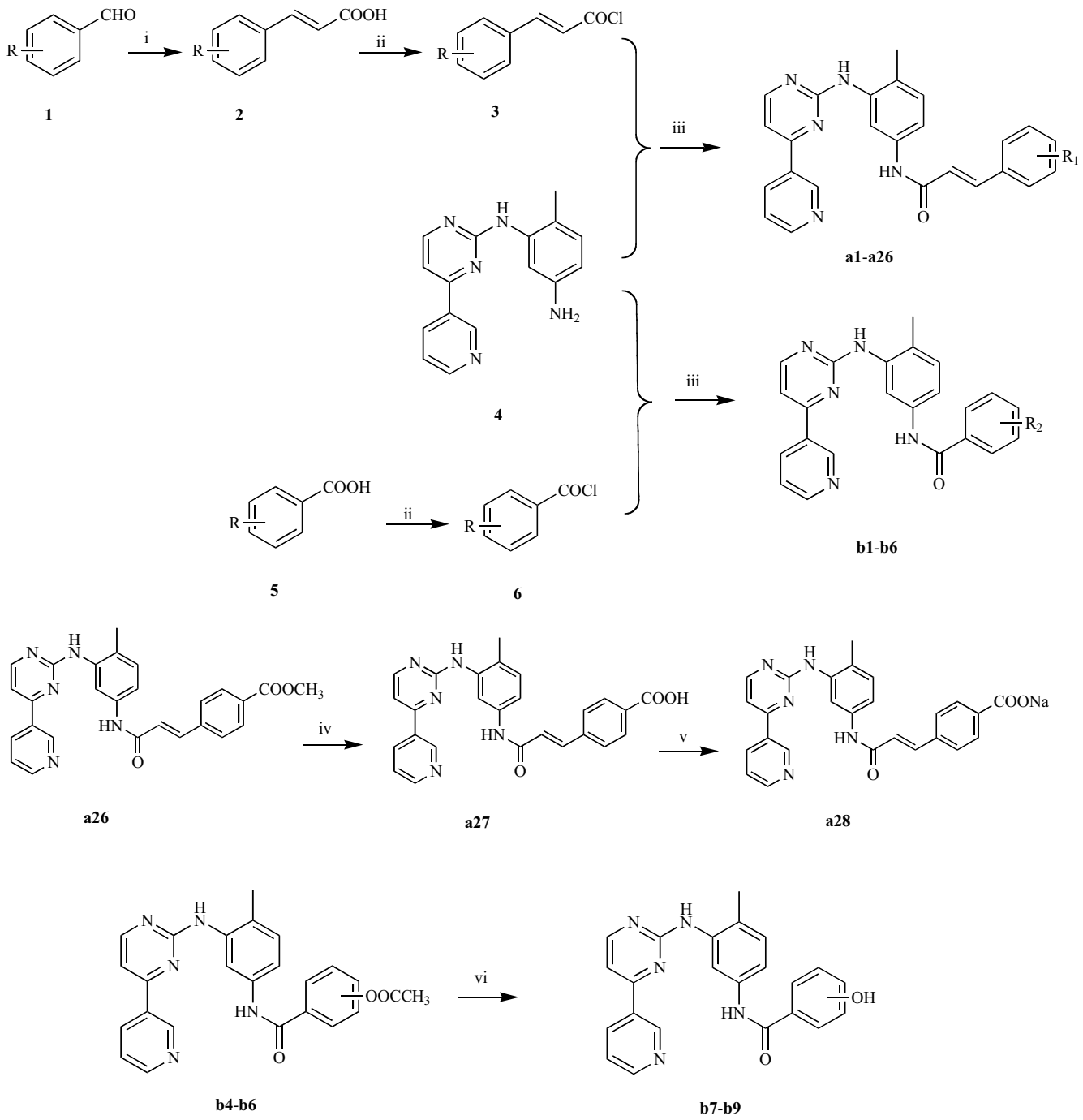


Scheme (1). Reagents and conditions: (i) malonic, pyridine, piperidine, 90 ¡ãC; (ii) (COCl)2, CH2Cl2, rt; (iii) CH2Cl2, *N,N*-diisopropylethylamine, rt; (iv) KOH, methanol, reflux; (v) NaOH, methanol, rt (vi) NH3.H2O, methanol, rt .

by the reaction of compounds **3** and **4**, while compounds **b1-6** were prepared by the reaction of compounds **4** and **6**. Furthermore, **a27** was synthesized by hydrolysis reaction of **a26**, and **a27** interacted with NaOH to obtain **a28**. Finally, compounds **b7-9** were synthesized by hydrolysis reaction of **b4-6**.

General Procedure for the Preparation of Compounds a1-26

To a solution of **4** (4.5 mmol) and 1.5 mL of *N, N*-diisopropylethylamine in 30 mL of CH_2Cl_2 was added **3** (5.4 mmol) at 0 - 5 °C, and the reaction mixture was stirred for 12 h at room temperature, the solid that formed was collected by filtration. The product was obtained and purified by silica gel chromatography to afford the target product.

a1: (E)-N-(4-methyl-3-(4-(pyridin-3-yl) Pyrimidin-2-ylamino) phenyl) Cinnamamide

White solid; Yield: 87.3%; m.p. 187-189 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.21 (s, 3H), 6.80 (d, 1H, J = 1.8 Hz), 7.22 (d, 1H, J = 2.4 Hz), 7.41-7.44 (m, 5H), 7.56-7.69 (m, 4H), 8.04 (s, 1H), 8.43-8.57 (m, 2H), 8.71 (d, 1H, J = 1.8

Hz), 9.33 (s, 1H), 10.21 (s, 1H). MS (ESI): 409.0 ($C_{25}H_{21}N_5O$, $[M+H]^+$); Anal. Calcd for $C_{25}H_{21}N_5O$: C, 73.68; H, 5.21; N, 17.22%; Found: C, 73.69; H, 5.19; N, 17.19%.

a2:(E)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-3-(2-(trifluoromethyl) phenyl) Acrylamide

White solid; Yield: 84.5%; m.p. 284-286 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.23 (s, 3H), 6.89-6.93 (d, 1H, J = 12.0 Hz), 7.21-7.19 (d, 1H, J = 5.4 Hz), 7.41-7.55 (m, 4H), 7.63-7.90 (m, 4H), 8.03 (d, 1H, J = 1.8 Hz), 8.48-8.66 (m, 2H), 8.70 (d, 1H, J = 2.4 Hz), 8.98 (s, 1H), 9.27 (s, 1H), 10.32 (s, 1H). MS (ESI): 476.5 ($C_{26}H_{20}F_3N_5O$, $[M+H]^+$); Anal. Calcd for $C_{26}H_{20}F_3N_5O$:C, 65.66; H, 4.29; N, 14.75%; Found: C, 65.68; H, 4.24; N, 14.73%.

a3:(E)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-3-(3-(trifluoromethyl) phenyl) Acrylamide

White solid; Yield: 80.5%; m.p. 281-282 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.23 (s, 3H), 6.89-6.93 (d, 1H, J = 12.0 Hz), 7.21-7.19 (d, 1H, J = 6.0 Hz), 7.41-7.55 (m, 4H), 7.63-7.90 (m, 4H), 8.03 (d, 1H, J = 1.8 Hz), 8.58-8.66 (m, 2H), 8.80-8.81 (d, 1H, J = 3.6 Hz), 8.98 (s, 1H), 9.21 (s, 1H), 10.15 (s, 1H). MS (ESI): 476.4 ($C_{26}H_{20}F_3N_5O$, $[M+H]^+$); Anal. Calcd for $C_{26}H_{20}F_3N_5O$: C,65.67; H, 4.25; N, 14.76%; Found: C, 65.68; H, 4.24; N, 14.75%.

a4:(E)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-3-(4-(trifluoromethyl) phenyl) Acrylamide

Yellow solid; Yield: 81.9%; m.p. 288-291 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.18 (s, 3H), 6.91-6.95 (d, 1H, J = 12.3 Hz), 7.14-7.17 (d, 1H, J = 8.4 Hz), 7.44-7.51 (m, 3H), 7.58-7.63 (d, 1H, J = 15.6 Hz), 7.73-7.82 (m, 4H), 7.96 (s, 1H), 8.44-8.48 (t, 2H, J = 12.3 Hz), 8.64-8.65 (d, 1H, J = 3.6 Hz), 8.97 (s, 1H), 9.24 (s, 1H), 10.25 (s, 1H). MS (ESI): 476.5 ($C_{26}H_{20}F_3N_5O$, $[M+H]^+$); Anal. Calcd for $C_{26}H_{20}F_3N_5O$: C, 65.68; H, 4.27; N, 14.74%; Found: C, 65.70; H, 4.27; N, 14.73%.

a5:(E)-3-(2-chlorophenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Champagne solid ; Yield: 82.6% ; m.p. 255-256 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.29 (s, 3H), 6.88-6.90 (d, 1H, J = 9.0 Hz), 7.19-7.21 (d, 1H, J = 6.3 Hz), 7.39-7.42 (t, 4H, J = 8.1 Hz), 7.50-7.59 (m, 2H), 7.76-7.78 (m, 1H), 7.84-7.88 (d, 1H, J = 12.0 Hz), 8.00 (s, 1H), 8.47-8.54 (m, 2H), 8.69-8.70 (d, 1H, J = 2.4 Hz), 8.98 (s, 1H), 9.27 (d, 1H, J = 1.2 Hz), 10.21 (s, 1H). MS (ESI): 443.7 ($C_{25}H_{20}ClN_5O$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{20}ClN_5O$: C, 68.11; H, 4.55; N, 15.91%; Found: C, 68.05; H, 4.56; N, 15.85%.

a6:(E)-3-(3-chlorophenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

White solid; Yield: 82.9%; m.p. 228-229 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.22 (s, 3H), 6.88-6.92 (d, 1H, J = 12.0 Hz), 7.19-7.21 (d, 1H, J = 6.3 Hz), 7.39-7.42 (t, 4H, J = 8.4 Hz), 7.50-7.59 (m, 2H), 7.76-7.88 (m, 1H), 7.84-7.88 (d, 1H, J = 12.3 Hz), 8.01 (s, 1H), 8.47-8.53 (m, 2H), 8.69-8.70 (d, 1H, J = 2.4 Hz), 8.97 (s, 1H), 9.27-9.28 (d, 1H, J = 3.0 Hz), 10.27 (s, 1H). MS (ESI): 443.3 ($C_{25}H_{20}ClN_5O$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{20}ClN_5O$: C, 67.99; H, 4.55; N, 15.88%; Found: C, 67.95; H, 4.56; N, 15.85%.

a7:(E)-3-(4-chlorophenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)-pyrimidin-2-ylamino)phenyl) Acrylamide

Light yellow solid; Yield: 85.7%; m.p. 273-275 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.22 (s, 3H), 7.99-7.81 (d, 1H, J = 8.4 Hz), 7.20-7.21 (d, 1H, J = 3.6 Hz), 7.40-7.49 (m, 2H), 7.56-7.58 (m, 4H), 7.74-7.76 (d, 2H, J = 4.8 Hz), 8.00 (s, 1H), 8.47-8.53 (m, 2H), 8.69-8.70 (d, 1H, J = 2.4 Hz), 9.02 (s, 1H), 9.30 (s, 1H), 10.21 (s, 1H). MS (ESI): 443.0 ($C_{25}H_{20}ClN_5O$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{20}ClN_5O$: C, 67.92; H, 4.58; N, 15.85%; Found: C, 67.95; H, 4.56; N, 15.84%.

a8:(E)-3-(2-chloro-5-(trifluoromethyl)phenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Light brown solid; Yield: 80.8%; m.p. 267-270 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 7.04 (d, 1H, J = 1.2 Hz), 7.21 (d, 1H, J = 1.8 Hz), 7.50-7.57 (m, 3H), 7.75-7.84 (m, 3H), 8.03 (m, 2H), 8.47-8.53 (m, 2H), 8.67-8.69 (m, 1H), 8.95 (s, 1H), 9.27 (d, 1H, J = 1.2 Hz), 10.27 (s, 1H). MS (ESI): 510.9 ($C_{26}H_{19}ClF_3N_5O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{19}ClF_3N_5O$: C, 61.27; H, 3.76; N, 13.78%; Found: C, 61.24; H, 3.76; N, 13.73%.

a9:(E)-3-(3,5-dichlorophenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Light yellow solid; Yield: 82.8%; m.p. 252-253 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 6.91-6.95 (d, 1H, J = 12.3 Hz), 7.19-7.21 (d, 1H, J = 6.3 Hz), 7.35-7.70 (m, 7H), 7.98-7.99 (d, 1H, J = 1.8 Hz), 8.45-8.53 (m, 2H), 8.68-8.70 (m, 1H), 8.95 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.16 (s, 1H). MS (ESI): 477.4 ($C_{25}H_{19}Cl_2N_5O$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{19}Cl_2N_5O$: C, 63.10; H, 4.04; N, 14.75%; Found: C, 63.03; H, 4.02; N, 14.70%.

a10:(E)-3-(2-methoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Light yellow solid; Yield: 85.2%; m.p. 245-247 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.89 (s, 3H), 6.87 (d, 1H, J = 1.2 Hz), 7.01-7.03 (t, 1H, J = 8.4 Hz), 7.09-7.11 (d, 1H, J = 6.3 Hz), 7.18-7.19 (d, 1H, J = 4.8 Hz), 7.40-7.45 (m, 3H), 7.52-7.57 (m, 2H), 7.78-7.80 (d, 1H, J = 8.4 Hz), 7.99 (s, 1H), 8.48-8.53 (m, 2H), 8.69-8.70 (d, 1H, J = 3.3 Hz), 8.97 (s, 1H), 9.27 (d, 1H, J = 1.2 Hz), 10.12 (s, 1H). MS (ESI): 438.5 ($C_{26}H_{23}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{23}N_5O_2$: C, 71.41; H, 5.33; N, 16.06%; Found: C, 71.38; H, 5.30; N, 16.01%.

a11:(E)-3-(3-methoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

White solid; Yield: 84.7%; m.p. 234-236 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.80 (s, 3H), 6.81-6.85 (d, 1H, J = 12.3 Hz), 6.69-7.00 (m, 1H), 7.18-7.38 (m, 3H), 7.40-7.44 (m, 3H), 7.51-7.57 (m, 2H), 7.98-8.00 (t, 1H, J = 6.3 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.11 (s, 1H). MS (ESI): 438.8 ($C_{26}H_{23}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{23}N_5O_2$: C,

71.40; H, 5.36; N, 16.01%; Found: C, 71.38; H, 5.30; N, 16.02%.

a12:(E)-3-(4-methoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Light yellow solid; Yield: 87.5%; m.p. 202-204 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.83(s, 3H), 6.70-6.71 (d, 1H, J = 5.4 Hz), 6.00-7.03 (d, 1H, J = 9.0 Hz), 7.19-7.22 (m, 3H), 7.51-7.55 (m, 4H), 8.00 (s, 1H), 8.53-8.55 (m, 2H), 8.69 (s, 1H), 9.02 (s, 1H), 9.29 (s, 1H), 10.01 (s, 1H). MS (ESI): 438.2 ($C_{26}H_{23}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{23}N_5O_2$: C, 71.38; H, 5.32; N, 16.00%; Found: C, 71.37; H, 5.30; N, 16.01%.

a13:(E)-3-(2,5-dimethoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Gray brown solid; Yield: 82.3%; m.p. 232-233 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.76 (s, 3H), 3.83 (s, 3H), 6.85-6.90 (d, 1H, J = 15.0 Hz), 6.95-6.99 (m, 2H), 7.12-7.20 (m, 2H), 7.40-7.44 (m, 2H), 7.51-7.55 (m, 1H), 7.74-7.78 (d, 1H, J = 11.7 Hz), 7.99 (d, 1H, J = 1.5 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.2 Hz), 10.11 (s, 1H). MS (ESI): 468.5 ($C_{27}H_{25}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}N_5O_3$: C, 69.40; H, 5.40 N, 14.98%; Found: C, 69.36; H, 5.39; N, 14.98%.

a14:(E)-3-(3,4-dimethoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

White solid; Yield: 84.9%; m.p. 236-240 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.83 (s, 6H), 6.70-6.72 (d, 1H, J = 6.3 Hz), 6.99-6.02 (d, 1H, J = 8.4 Hz), 7.18-7.22 (m, 3H), 7.50-7.55 (m, 4H), 8.00 (s, 1H), 8.49-8.54 (m, 2H), 8.70-8.73 (d, 1H, J = 9.6 Hz), 9.03 (s, 1H), 9.28 (s, 1H), 10.12 (s, 1H). MS (ESI): 469.0 ($C_{27}H_{25}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}N_5O_3$: C, 69.35; H, 5.38; N, 15.04%; Found: C, 69.36; H, 5.39; N, 15.00%.

a15:(E)-3-(3,5-dimethoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Yellow solid; Yield: 81.5%; m.p. 244-245 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.76 (s, 6H), 6.54-6.55 (t, 1H, J = 3.6 Hz), 6.79-6.89 (m, 3H), 7.18-7.20 (d, 1H, J = 6.3 Hz), 7.40-7.56 (m, 4H), 7.99 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.95 (s, 1H), 9.26-9.27 (d, 1H, J = 1.8 Hz), 10.19 (s, 1H). MS (ESI): 468.7 ($C_{27}H_{25}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}N_5O_3$: C, 69.41; H, 5.39; N, 15.01%; Found: C, 69.37; H, 5.39; N, 14.98%.

a16:(E)-3-(3,4,5-trimethoxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Coffee solid; Yield: 82.3%; m.p. 232-233 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.76 (s, 3H), 3.83 (s, 6H), 6.85-6.90 (d, 1H, J = 15.0 Hz), 6.95-6.99 (m, 2H), 7.12-7.20 (m, 2H), 7.40-7.44 (m, 2H), 7.51-7.55 (m, 1H), 7.74-7.78 (d, 1H, J = 11.7 Hz), 7.99 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.2 Hz), 10.11 (s, 1H). MS (ESI): 498.6 ($C_{28}H_{27}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{28}H_{27}N_5O_4$: C, 67.62; H, 5.49; N, 18.07%; Found: C, 67.59; H, 5.47; N, 14.08%.

a17:(E)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-3-p-tolylacrylamide

Light brown solid; Yield: 87.4%; m.p. 257-258 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.88 (s, 3H), 6.81-6.86 (d, 1H, J = 15.3 Hz), 6.96-7.00 (m, 1H), 7.18-7.38 (m, 3H), 7.40-7.44 (m, 3H), 7.51-7.57 (m, 2H), 7.98-8.00 (t, 1H, J = 6.3 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.11 (s, 1H). MS (ESI): 423.4 ($C_{26}H_{23}N_5O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{23}N_5O$: C, 70.10; H, 5.56; N, 16.60%; Found: C, 74.09; H, 5.50; N, 16.62%.

a18:(E)-3-(4-fluorophenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

Grown solid; Yield: 80.5%; m.p. 273-275 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.22 (s, 3H), 6.81-6.86 (d, 1H, J = 15.3 Hz), 6.96-7.00 (m, 1H), 7.18-7.38 (m, 3H), 7.40-7.44 (m, 3H), 7.51-7.57 (m, 2H), 7.98-8.00 (t, 1H, J = 6.3 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.11 (s, 1H).. MS (ESI): 426.4 ($C_{25}H_{20}FN_5O$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{20}FN_5O$: C, 70.60; H, 4.73; N, 16.49%; Found: C, 70.58; H, 4.74; N, 16.46%.

a19:(E)-3-(3-fluoro-2-methylphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) phenyl) Acrylamide

Light yellow solid; Yield: 82.0%; m.p. 238-239 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 2.30 (s, 3H), 6.76-6.81 (d, 1H, J = 15.3 Hz), 7.17-7.55 (m, 7H), 7.75-7.80 (d, 1H, J = 15.6 Hz), 7.98-7.99 (d, 1H, J = 1.8 Hz), 8.45-8.53 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.19 (s, 1H). MS (ESI): 441.1 ($C_{26}H_{22}FN_5O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{22}FN_5O$: C, 71.01; H, 5.10; N, 15.98%; Found: C, 71.06; H, 5.06; N, 15.94%.

a20:(E)-3-(5-fluoro-2-methylphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) phenyl) Acrylamide

Coffee solid; Yield: 84.9%; m.p. 226-228 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 2.38 (s, 3H), 6.75-6.80 (d, 1H, J = 15.0 Hz), 7.11-7.21 (m, 2H), 7.29-7.45 (m, 4H), 7.51-7.56 (m, 1H), 7.75 (d, 1H, J = 1.5 Hz), 8.00-8.01 (d, 1H, J = 1.8 Hz), 8.46-8.50 (m, 2H), 8.53-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.8 Hz), 10.16 (s, 1H). MS (ESI): 440.9 ($C_{26}H_{22}FN_5O$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{22}FN_5O$: C, 71.09; H, 5.06; N, 15.98%; Found: C, 71.06; H, 5.05; N, 15.94%.

a21:(E)-3-(3,4-dimethylphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

White solid; Yield: 85.5%; m.p. 283-284 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 2.26 (s, 6H), 6.75-6.80 (d, 1H, J = 15.3 Hz), 7.17-7.21 (m, 2H), 7.33-7.45 (m, 6H), 7.98 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.5 Hz), 10.08 (s, 1H). MS (ESI): 436.9 ($C_{27}H_{25}N_5O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{25}N_5O$: C, 74.48; H, 5.76; N, 16.11%; Found: C, 74.46; H, 5.79; N, 16.08%.

a22:(E)-3-(4-(diphenylamino)phenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) phenyl) Acrylamide

Yellow solid; Yield: 89.8%; m.p. 295-296 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 10.10 (s , 1H); 9.28 (s , 1H); 8.97(s, 1H); 8.70-8.69 (d, 1H, J = 4.0Hz); 8.55-8.49 (m, 2H); 7.98 (s, 1H); 7.59-7.52 (m, 1H); 7.51-7.49 (t, 3H, J = 8.0Hz); 7.44-7.41(t, 2H, J = 12.4Hz); 7.37-7.34 (t, 4H, J = 12.0Hz); 7.19-7.17(d, 1H, J = 8.4Hz); 7.14-7.12 (d, 2H, J = 7.2Hz); 7.10-7.08 (d, 4H, J = 8.0Hz); 6.95-6.93 (d, 2H, J = 8.4Hz), 6.70-6.67 (d, 1H, J = 10.8Hz); 2.21(s, 3H). MS (ESI): 575.0 ($C_{37}H_{30}N_6O$, $[M+H]^+$). Anal. Calcd for $C_{37}H_{30}N_6O$: C, 77.34; H, 5.26; N, 14.62%; Found: C, 77.33; H, 5.26; N, 14.62%.

a23:(E)-3-(4-(dimethylamino)phenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) phenyl) Acrylamide

Yellow solid; Yield: 89.5%; m.p. 258-259 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 2.26 (s, 6H), 6.75-6.80 (d, 1H, J = 15.3 Hz), 7.17-7.21 (m, 2H), 7.33-7.45 (m, 6H), 7.98 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.5 Hz), 10.11 (s, 1H). MS (ESI): 449.0 ($C_{27}H_{26}N_6O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{26}N_6O$: C, 71.96; H, 5.86; N, 18.66%; Found: C, 71.98; H, 5.82; N, 18.65%.

a24:(E)-4-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylamino)-3-oxoprop-1-enyl)phenyl Acetate

Yellow solid; Yield: 83.2%; m.p. 252-253 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 3.83 (s, 3H), 6.75-6.80 (d, 1H, J = 15.3 Hz), 7.17-7.21 (m, 2H), 7.33-7.45 (m, 6H), 7.98 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.5 Hz), 10.08 (s, 1H). MS (ESI): 467.0 ($C_{27}H_{23}N_5O$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{23}N_5O$: C, 69.66; H, 4.98; N, 15.04%; Found: C, 69.66; H, 4.98; N, 15.04%.

a25:(E)-3-(4-hydroxyphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl) Acrylamide

White solid; Yield: 82.8%; m.p. 220-221 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.21 (s, 3H), 6.75-6.80 (d, 1H, J = 15.3 Hz), 7.17-7.21 (m, 2H), 7.33-7.45 (m, 6H), 7.98 (d, 1H, J = 1.8 Hz), 8.45-8.52 (m, 2H), 8.68-8.70 (m, 1H), 8.94 (s, 1H), 9.27 (d, 1H, J = 1.5 Hz), 10.08 (s, 1H). MS (ESI): 425.0 ($C_{25}H_{21}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{21}N_5O_2$: C, 70.91; H, 5.00; N, 16.56%; Found: C, 70.91; H, 5.00; N, 16.54%.

a26:(E)-4-methyl-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylamino)-3-oxoprop-1-enyl)benzoate

Yellow solid; Yield: 87.9%; m.p. 267-271 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.18 (s, 3H), 3.83 (s, 3H), 6.65-6.76 (m, 1H), 7.15-7.17 (d, 1H, J = 8.1 Hz), 7.46-7.57 (m, 2H), 7.61-7.63 (d, 2H, J = 6.9 Hz), 7.70-7.75 (t, 2H, J = 15.6 Hz), 7.77-7.89 (m, 3H), 7.96 (s, 1H), 8.38-8.49 (m, 2H), 8.64 (s, 1H), 8.96 (s, 1H), 9.23 (s, 1H), 10.23 (s, 1H). MS (ESI): 466.5 ($C_{27}H_{23}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{27}H_{23}N_5O_3$: C, 69.66; H, 4.97; N, 15.04%; Found: C, 69.66; H, 4.98; N, 15.04%.

a27:(E)-4-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylamino)-3-oxoprop-1-enyl)benzoic Acid

To a solution of KOH (0.25mol) and 10 mL of H_2O in 50 mL of methanol **a26** (0.05mol) was added slowly, and the reaction mixture was refluxed for 2 h. 50 mL of H_2O the reaction solution, followed by adjustment of pH = 3 with HCl solution. A yellow solid was obtained and recrystallizated from methanol to afford **a27**.

Yellow solid; Yield: 94.6%; m.p. 293-296 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.22 (s, 3H), 6.97-7.00 (d, 1H, J = 9.0 Hz), 7.20-7.24 (d, 1H, J = 12.3 Hz), 7.43-7.45 (t, 2H, J = 6.0 Hz), 7.55-7.64 (m, 2H), 7.73-7.75 (d, 2H, J = 5.4 Hz), 7.99-8.00 (d, 3H, J = 3.6 Hz), 8.49-8.55 (m, 2H), 8.70-8.71 (d, 1H, J = 2.4 Hz), 8.99 (s, 1H), 9.29 (s, 1H), 10.31 (s, 1H) , 13.10 (s, 1H). MS (ESI): 452.4 ($C_{26}H_{21}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{21}N_5O_3$: C, 69.19; H, 4.69; N, 15.50%; Found: C, 69.17; H, 4.69; N, 15.51%.

a28:sodium(E)-4-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylamino)-3-oxoprop-1-enyl)benzoate

To a solution of NaOH (0.05mol) in 30 mL of methanol compound **a27** (0.05mol) at 0-5 °C was added, and the reaction mixture was stirred for 1 h at room temperature. Then, the solvent was concentrated to afford **a28**.

Yellow solid; Yield: 97.0%; m.p. > 300 °C. ^{1}H NMR (DMSO, 300 MHz) δ: 2.22 (s, 3H), 6.97-7.00 (d, 1H, J = 12.4Hz), 7.21-7.24 (d, 1H, J = 13.2Hz), 7.43-7.44 (t, 2H, J = 6.4Hz), 7.55-7.63 (m, 2H), 7.73-7.75 (d, 2H, J = 8.4Hz), 7.99-8.00 (d, 3H, J = 5.2Hz), 8.49-8.55 (m, 2H), 8.70-8.71 (d, 1H, J = 4.4Hz), 8.99 (s, 1H), 9.29 (s, 1H), 10.31(s, 1H). MS (ESI): 474.9 ($C_{26}H_{20}N_5NaO_3$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{20}N_5NaO_3$: C, 65.96; H, 4.26; N, 14.80%; Found: C, 65.96; H, 4.26; N, 14.79%.

General Procedure for the Preparation of Compounds b1-6

Compounds **b1-6** were synthesized by the procedure as compounds **a1-26**.

b1: N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)-phenyl)-4-(trifluoromethyl)benzamide

White solid; Yield: 84.2%; m.p. 218-220 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.24 (s, 3H), 7.22-7.24 (d, 1H, J = 8.4Hz), 7.43-7.45 (d, 1H, J = 5.2Hz), 7.50-7.55 (m, 2H), 7.90-7.92 (d, 2H, J = 8.4Hz), 8.12-8.16 (t, 3H, J = 16.4Hz), 8.45-8.54 (m, 2H), 8.68-8.69 (t, 1H, J = 4.4Hz), 9.00 (s, 1H), 9.28 (s, 1H), 10.49 (s, 1H). MS (ESI): 451.0 ($C_{24}H_{18}F_3N_5O$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{18}F_3N_5O$: C, 64.16; H, 4.05; N, 15.57%; Found: C, 64.14; H, 4.04; N, 15.58%.

b2:4-methyl-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide

Light yellow solid; Yield: 84.7%; m.p. 211-213 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.23 (s, 3H), 2.38 (s, 3H), 7.20-7.23 (d, 1H, J = 10.4Hz), 7.32-8.53 (m, 5H), 7.85-7.88 (t, 1H, J = 12.0Hz), 7.01-8.10 (m, 2H), 8.49-8.54 (m, 2H), 8.73 (s, 1H), 8.94-9.07 (m, 1H), 9.27 (s, 1H), 10.14 (s, 1H). MS

(ESI): 398.0 ($C_{24}H_{21}N_5O$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{21}N_5O$: C, 72.89; H, 5.35; N, 17.71%; Found: C, 72.90; H, 5.33; N, 17.69%.

b3:3,4,5-trimethoxy-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide

Yellow solid; Yield: 84.0%; m.p. 236-238 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.30 (s, 3H), 3.32 (s, 9H), 6.72-6.87 (m, 1H), 7.22-7.24 (d, 1H, J = 8.0Hz), 7.41-8.61 (m, 3H), 8.14 (s, 1H), 8.40-8.73 (m, 4H), 9.00 (s, 1H), 9.27 (s, 1H), 10.75 (s, 1H). MS (ESI): 472.2 ($C_{26}H_{25}N_5O_4$, $[M+H]^+$). Anal. Calcd for $C_{26}H_{25}N_5O_4$: C, 66.23; H, 5.34; N, 14.85%; Found: C, 66.23; H, 5.33; N, 14.87%.

b4:2-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylcarbamoyl)phenyl Acetate

Light yellow solid; Yield: 80. 5%; m.p. 165-167 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.23 (s, 3H), 2.41 (s, 3H), 7.32 (m, 4H), 7.44 (m, 1H), 7.53-7.55 (m, 1H), 7.61-7.63 (m, 1H), 7.90-7.93 (m, 1H), 8.22 (s, 1H), 8.51-8.54 (m, 2H), 8.61 (s, 1H), 8.82 (s, 1H), 9.21 (s, 1H). MS (ESI): 441.0 ($C_{25}H_{21}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{21}N_5O_3$: C, 68.33; H, 4.82; N, 15.94%; Found: C, 68.33; H, 4.83; N, 15.94%.

b5: 3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylcarbamoyl)phenyl Acetate

Light yellow solid; Yield: 86. 5%; m.p. 204-206 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.32 (s, 3H), 7.02 (s, 1H), 7.13-7.25 (m, 4H), 7.27 (d, 1H, J = 1.8Hz), 7.12-7.56 (m, 1H), 7.86-7.91 (t, 3H, J = 15.6Hz), 8.47-8.49 (d, 2H, J = 5.4Hz), 8.57 (s, 1H), 8.66-8.67 (t, 1H, J = 4.5Hz), 9.21 (d, 1H, J = 1.5Hz). MS (ESI): 441.0 ($C_{25}H_{21}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{21}N_5O_3$: C, 68.33; H, 4.82; N, 15.94%; Found: C, 68.33; H, 4.83; N, 15.94%.

b6:3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylcarbamoyl)phenyl Acetate

Yellow solid; Yield: 87.7%; m.p. 210-212 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.32 (s, 3H), 7.02 (s, 1H), 7.15-7.24 (m, 2H), 7.33-7.48 (m, 2H), 7.46-7.48 (d, 2H, J = 7.8Hz), 7.59 (s, 1H), 7.69-7.72 (d, 1H, J = 7.5Hz), 7.93 (s, 1H), 8.47-8.49 (d, 2H, J = 5.4Hz), 8.56 (s, 1H), 8.66-8.67 (d, 1H, J = 3.6Hz), 9.20 (s, 1H). MS (ESI): 440.9 ($C_{25}H_{21}N_5O_3$, $[M+H]^+$). Anal. Calcd for $C_{25}H_{21}N_5O_3$: C, 68.32; H, 4.82; N, 15.94%; Found: C, 68.33; H, 4.82; N, 15.94%.

General Procedure for the Preparation of Compounds b7-9

To a solution of **b4-6** (5 mmol) in 10 mL of methanol 10 mLof $NH_3.H_2O$ was added, and the reaction mixture was stirred for 12 h at room temperature. Then, the solid that formed was collected by filtration and recrystallizated from methanol to afford **b7-9**.

b7: 2-hydroxy-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide

Green solid; Yield: 87.2%; m.p. 247-248 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.18 (s, 3H), 6.90-6.95 (t, 2H, J = 16.2Hz), 7.19-7.22 (d, 1H, J = 8.4Hz), 7.34-7.51 (m, 4H), 7.92-8.08 (m, 2H), 8.31-8.49 (m, 2H), 8.62-8.64 (d, 1H, J = 4.5Hz), 8.97 (s, 1H), 9.26 (s, 1H), 10.31 (s, 1H), 10.94 (s, 1H). MS (ESI): 399.0 ($C_{23}H_{19}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.82; N, 17.62%; Found: C, 69.53; H, 4.83; N, 17.62%.

b8: 2-hydroxy-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide

White solid; Yield: 88.7%; m.p. 222-224 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.16 (s, 3H), 6.80-6,82 (d, 2H, J = 6.9Hz), 7.12-7.14 (d, 1H, J = 7.2Hz), 7.32- 7.48 (m, 3H), 7.81-7.89 (d, 2H, J = 7.2Hz), 8.00 (s, 2H), 8.45 (s, 2H), 8.63 (s, 1H), 8.94 (s, 1H), 9.22 (s, 1H), 9.91 (s, 1H), 10.05 (s, 1H). MS (ESI): 399.0 ($C_{23}H_{19}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.82; N, 17.62%; Found: C, 69.53; H, 4.83; N, 17.62%.

b9: 2-hydroxy-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide

Green solid; Yield: 87.9%; m.p. 261-262 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.18 (s, 3H), 6.90-6.95 (t, 2H, J = 16.2Hz), 7.19-7.22 (d, 1H, J = 8.4Hz), 7.34-7.51 (m, 4H), 7.92-8.08 (m, 2H), 8.31-8.49 (m, 2H), 8.62-8.64 (d, 1H, J = 4.5Hz), 8.97 (s, 1H), 9.26 (s, 1H), 10.31 (s, 1H), 10.94 (s, 1H). MS (ESI): 398.9 ($C_{23}H_{19}N_5O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{19}N_5O_2$: C, 69.51; H, 4.81; N, 17.62%; Found: C, 69.53; H, 4.82; N, 17.62%.

3. Biological Activity

K562 cells were incubated at 37 °C under 5% CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. For growth inhibition studies, K562 cells were grown to log phase and diluted to 1×10^5 cells mL^{-1} with the complete medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. The subsequent incubation was permitted at 37 °C, 5% CO_2 atmosphere for 24 h before the cytotoxicity assessments and varying concentrations of each compound were added with imatinib as positive reference and DMSO as negative control. After 96 h of treatment, 20 μL of PBS containing 5.0 mg mL^{-1} of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)) was added to each well. 4 h later, 100 μL extraction solution (10% SDS - 5% isobutyl alcohol - 0.01 M HCl) was added. After an overnight incubation at 37 °C, the optical density was measured at a wavelength of 572 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times [21]. The results were presented in Table **1**.

The structure-activity relationship studies were analyzed to determine how the substituents affected the anti-proliferative activity subsequently. Among the first series of compounds **a1-28**, **a4** (R = 4-CF_3), **a10** (R = 2-OCH_3) and **a21** (R = 3,4-*di*-CH_3) displayed the most potent activity with the IC_{50} values of 0.67, 0.66 and 0.65 μM, respectively, more effective than imatinib in inhibiting K562 cell growth. It can be seen that compounds with small substituents at position 2 and at position 4 were more potent

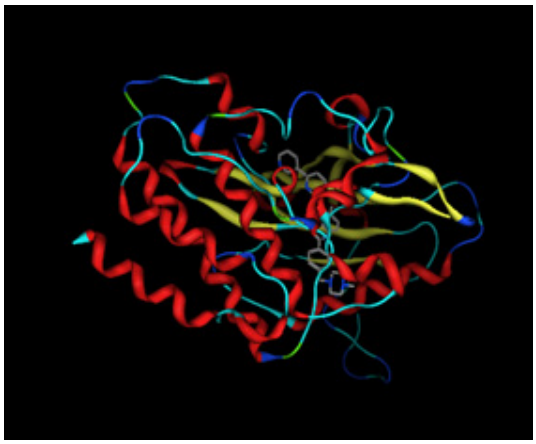
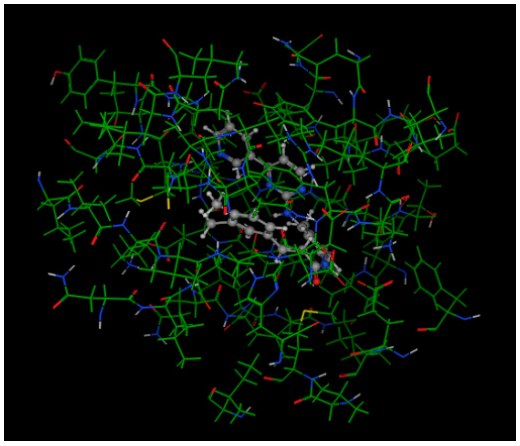
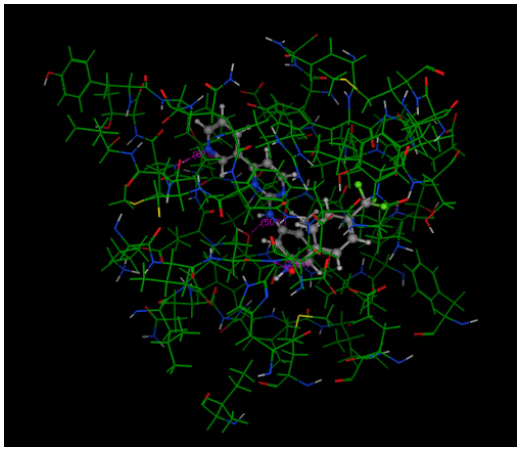


Fig. (1). The tertiary structure, active region and ligand of 1IEP.



(**a**)



(**b**)

Fig. (2). (**a**) The 3D binding mode of compound **a21**. (**b**) The 3D binding mode of compound **b1**.

than the corresponding substituents at position 3, from the compounds substituted with whether a electron donating group (-OCH_3) or a electron withdrawing group (-CF_3, -Cl). The sequence of activity of the compounds substituted with the methoxy group was 2-OCH_3 > 2,5-*di*-OCH_3 > 4-OCH_3 > 3,4-*di*-OCH_3> 3-OCH3 > 3,5-*di*-OCH_3> 3,4,5-*tri*-OCH_3. This demonstrated that the methoxy group substitution of position 2 had much effect on the activities, and the increase in the number of methoxy group had an adverse effect on the molecule, **a16** showing at least a fourfold reduction in cytotoxicity compared with only one methoxy substituted compounds **a10**, **a11** and **a12**. Compound **a8** had both chlorine and trifluoromethyl groups, whereas displaying less activity than compounds substituted with the groups separately. The similar case also emerged when **a19** and **a20** with double substituents of fluorine and methyl groups. In order to increase the solubility of the molecule, the methyl ester group of compound **a26** was converted into carboxyl group (**a27**) and sodium salt (**a28**) further, but the anti-proliferative activity was not synchronized with the increase of solubility. All of these above suggested a possible critical role for these groups in the distribution of the electron density on the right hand benzene ring, thereby affecting the binding site and binding strength of the molecular target.

Compounds **b1-9** showed almost the same moderate anti-leukemia activities, particularly **b1** and **b2** with IC_{50} value of 0.59 and 0.62 μM, respectively. Compound **b7** was obtained by removing the acetyl group of **b4** with ammonia, displaying higher activity. Compounds **b5** and **b8**, **b6** and **b9** revealed the same regularity, because these replacements of groups increased the hydrophobic interaction between compounds and receptor.

4. Molecular Docking

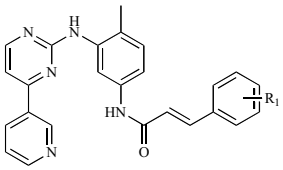
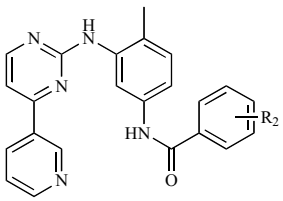
Molecular docking studies were performed to get a better insight into the binding affinity and guide further SAR studies, using Molecular Operating Environment (MOE). 2008. 10 software with crystal structure of Abl enzyme given in PDB code 1IEP, of which ligand is imatinib (Fig. **1**). All the compounds were docked over the ligand atoms in the active binding site and they occupied nearly the same space in the binding pocket. The 3D binding modes of **a21** and **b1** were depicted in Fig. (**2**). From 2D-presentation for the binding interactions of compound **a21** with receptor (Fig. **2a**), it can be seen that compound **a21** interacts with Met 318, Thr 315 and Glu 286 of receptor active site. The introduction of the new fragment benzene propylene increases the flexibility of the molecule, but as the substitution number in the benzene ring of benzene propylene group increases, the binding interaction between compound and receptor decreases. Compound **b1** (Fig. **2b**) as well as **a21**, formed well binding affinity to the active pocket via hydrophobic interactions, and had one more interaction.

These results of molecular docking studies proved that compounds **a21** and **b1** had high binding potency of receptor.

CONCLUSION

In the present study, two series of imatinib derivatives, 37 compounds were designed, synthesized and evaluated for their biological activities. All these compounds showed remarkable anti-proliferative activities against K562 leukemia cell lines, and compounds **a4**, **a10**, **a21**, **b1** and **b2** exhibited the most potent activities, even better than imatinib. Docking simulation were performed to position compounds **a21** and

Table 1. Antiproliferative activity (IC_{50}) against leukemia K562 cells of compounds a1-28 and b1-9.

Structure	Comp.no	R_1	IC_{50}(μM)	Comp.no	R_1	IC_{50}(μM)
 a1-a28	a1	H	1.27±0.05	a15	3,5-2OCH_3	2.87±0.19
	a2	2-CF_3	0.84±0.11	a16	3,4,5-3OCH_3	7.82±0.08
	a3	3-CF_3	1.04±0.04	a17	4-CH_3	0.83±0.28
	a4	4-CF_3	0.67±0.03	a18	4-F	0.89±0.02
	a5	2-Cl	0.89±0.04	a19	2-CH_3-3-F	0.99±0.02
	a6	3-Cl	5.41±0.10	a20	2-CH_3-5-F	4.34±0.18
	a7	4-Cl	1.76±0.17	a21	3,4-2CH_3	0.65±0.01
	a8	2-Cl-5-CF_3	4.13±0.04	a22	4-N(Ph)$_2$	6.10±0.06
	a9	3,5-2Cl	6.12±0.23	a23	4-N(CH_3)$_2$	5.45±0.21
	a10	2-OCH_3	0.66±0.01	a24	4-$OOCCH_3$	3.94±0.19
	a11	3-OCH_3	1.69±0.01	a25	4-OH	2.10±0.05
	a12	4-OCH_3	1.13±0.08	a26	4-$COOCH_3$	2.28±0.31
	a13	2,5-2OCH_3	0.91±0.01	a27	4-COOH	2.96±0.11
	a14	3,4-2OCH_3	1.48±0.15	a28	4-COONa	3.23±0.15
 b1-b9	Comp. no	R_2	IC_{50}(μM)	Comp. no	R_2	IC_{50}(μM)
	b1	4- CF_3	0.59±0.01	b6	4-$OOCCH_3$	1.38±0.03
	b2	4-CH_3	0.62±0.03	b7	2-OH	0.80±0.05
	b3	3,4,5-3OCH_3	6.01±0.33	b8	3-OH	0.99±0.27
	b4	2-$OOCCH_3$	1.83±0.18	b9	4-OH	1.23±0.04
	b5	3-$OOCCH_3$	1.02±0.12	Imatinib		0.73±0.02

b1 into the active site of Abl, the result shows the two compounds can bind well with the active pocket.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Nowell, P. C.; Hungerford, D. A. A minute chromosome in human chronic granulocytic leukemia. *Science*, **1960**, *132*, 1497-1501.

[2] Shtivelman, E.; Lifshitz, B.; Gale, R. P.; Canaani, E. Fused transcript of abl and bcr genes in chronic myelogenous leukemia. *Nature*, **1985**, *315*, 550-554.

[3] Groffen, J.; Stephenson, J. R.; Heisterkamp, N.; de Klein, A.; Bartram, C. R.; Grosveld, G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*, **1984**, *36*, 93-99.

[4] Thomas, O'Hare.; Michael, W. N. Deininger.; Christopher, A. E.; Clackson, T.; Druker, B. J. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. *Clin. Cancer Res*., **2011**, *17*, 212-221.

[5] Lugo, T. G.; Pendergast, A. M.; Muller, A. J.; Witte, O. N. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science*, **1990**, *247*, 1079-1082.

[6] Cilloni, D.; Saglio, G. Molecular Pathways: BCR-ABL. *Clin. Cancer Res*., **2012**, *18*, 930-937.

[7] Helgason, G. V.; Karvela, M.; Holyoake, T. L. Kill one bird with two stones: potential efficacy of BCR-ABL and autophagy inhibition in CML. *Blood*, **2011**, *118*, 2035-2043.

[8] Druker, B. J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G. M.; Fanning, S.; Zimmermann, J.; Lydon, N. B. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.*, **1996**, *2*, 561-566.

[9] Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantariian, H.; Capedeville, R.; Ohno-Jones, S.; Sawyer, C. L. Efficacy and safety of a specific inhibitor of Bcr-Abl tyrosine kinase in chronic myeloid leukemia. *N. Eng. J. Med*., **2001**, *344*, 1031-1037.

[10] Roche-Lestienne, C.; Soenen-Cornu, V.; Grardel-Duflos, N.; Laï , J. L.; Philippe, N.; Facon, T.; Fenaux, P.; Preudhomme, C. Several types of mutations of the Abl can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood*, **2002**, *100*, 1014-1018.

[11] Shah, N. P.; Sawyers, C. L. Mechanisms of resistance to STI571 in Philadelphia chromosome-associated leukemias. *Oncogene*, **2003**, *22*, 7389-7395.

[12] Le, C. P.; Tassi, E.; Varella-Garcia, M.; Barni, R.; Mologni, L.; Cabrita, G.; Marchesi, E.; Supino, R.; Gambacorti-Passerini, C. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood*, **2000**, *95*, 1758-1766.

[13] Mahon, F. X.; Deininger, M. W.; Schultheis, B.; Chabrol, J.; Reiffers, J.; Goldman, J. M.; Melo, J. V. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood*, **2000**, *96*, 1070-1079.

[14] Copland, M.; Hamilton, A.; Eirick, L. J.; Baird, J. W.; Allan, E. K.; Jordanides, N.; Barow, M.; Mountford, J. C.; Holyoake, T. L. Dasatinib (BMS2354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*, **2006**, *107*, 4532-4539.

[15] Weisberg, E.; Manley, P.; Mestan, J.; Cowan-Jacob, S.; Ray, A.; Griffin, J. D. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br. J. Cancer*, **2006**, *94*, 1765-1769.

[16] Welch D. R.; Harper D. E.; Yohem K.H. U-77863: a novel cinnamide isolated from streptomyces griseolutens that inhibits cancer invasion and metastasis. Clin Exp Metastas, **1993**, *11*, 201-212.

[17] Vitaliy N.; Karson S.P.; Paul J.H. Identification from a Combinatorial Library of a Small Molecule that Selectively Induces Apoptosis in Cancer Cells. *J Am Chem Soc*, **2003**, *125*, 14672-14673.

[18] Bai, H.; Zhao, X. Y.; Gong, Y. X.; Zhong, J. Q.; Zhu, Q. F.; Liu, X. Y.; Liu, L. F.; Zhou, Q. X. Benzamide derivative with anticancer activity and preparation method and use thereof. US: 20130225810 A1.

[19] Li, F. N.; Kim, N. J.; Paek, S. M.; Kwon, D.Y.; Min, K. H.; Jeong, Y. S.; Kim, S.Y.; Park, Y. H.; Kim, H. D.; Park, H. G.; Suh, Y. G. Design, synthesis, and biological evaluation of novel diarylalkyl amides as TRPV1 antagonists. *Bioorg. Med. Chem*., **2009**, *17*, 3557-3567.

[20] Yao, R. S.; Li, T. T.; Xu, J.; Jiang, L. E.; Ruan, B. F. Design, Synthesis and Anti-itch Activity Evaluation of Aromatic Amino Acid Derivatives as Gastrin-Releasing Peptide Receptor Antagonists. *Medicinal Chemistry*, **2012**, *8*, 865-873.

[21] Yao, R. S.; Lu, X. Q.; Guan, Q. X.; Zheng, L.; Lu, X.; Ruan, B. F. Synthesis and biological evaluation of some novel resveratrol amide derivatives as potential anti-tumor agents. *European Journal of Medicinal Chemistry*, **2013**, *62*, 222-231.

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