Design, Synthesis and Bioactivities of Phenylamino-Pyrimidine Derivatives as Novel Protein Tyrosine Kinase Inhibitors

Xinfu Hong[1,2], Xianzhao Kuang[1,2], Guohua Ding[1,2], Yilin Liu[1,2], Jinlin Liu[1,2], Yanjin Zhao[1] and Shuxin Li*[,1,2]

[1]*Institute of Radiation and Irradiation Medicine, Academy of Military Medical Science, Beijing 100850, China;* [2]*Department of Medicinal Chemistry, Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China*

Received January 02, 2011: Revised April 26, 2011: Accepted April 29, 2011

Abstract: A series of novel phenylamino-pyrimidine derivatives were designed and synthesized as antitumor agent based on the lead compound of Imatinib by application of the principle of bioisosterism, hybridization and structural optimization. The bioactivities of the new target compounds were tested against human KU812 cells *in vitro*, some target compounds show promising activities and can be considered for further development.

Keywords: Protein tyrosine kinase, Phenylamino-pyrimidine, Synthesis, Bioactivity.

INTRODUCTION

Protein kinases play an important role in signal transduction pathways, regulating a number of cellular functions such as cell proliferation, differentiation, migration and cell death [1-3]. So the inhibition of protein kinases, especially protein tyrosine kinases (PTKs), have been recognized as attractive cell-signaling targets for drug discovery in the treatment of cancer and other diseases [4-7]. During the past few years, progress has been made aiming at finding selective PTKs inhibitors. A number of small molecule compounds, especially the class of 2-phenylaminopyrimidine compounds, such as Imatinib (STI571) [8-12] and Nilotinib (AMN107) [13-14], functioning at the molecular level with high specificity, shows promise as a therapeutic agent for the treatment of chronic myelogenous leukemia (CML). But as STI571 is widely used, drug-resistance seriously hampers therapeutic efficacy [15-16]. Hence, our study was to design and synthesize a series of novel phenylamino-pyrimidine derivatives based on the lead compound of Imatinib by application of the principle of bioisosterism, hybriddization and structural optimization (Fig. **1**).

CHEMISTRY

The strategy for the synthesis of the target compounds began with the 4,6-dichloro-2-methyl-pyrimidine which was aminated with intermediates (**1a-b**) to afford intermediates (**2a-b**) in high yields. Amination of intermediates (**2a-b**) with morpholine in 1,4-dioxane gave intermediates (**3a-b**), which were hydrolyzed with LiOH in the solution of water and methanol to afford intermediates (**4a-b**). Amidation of the intermediates (**4a-b**) with substituented phenylamino through CDI gave the target compounds (**5a-d**, **6a-d**) [17]; but when we tried to synthesize the target compounds (**5a-d**, **6a-d**) by amidation of the intermediates (**4a-b**) with substituented phenylamino through thionyl chloride, the original target compounds (**5a-d**, **6a-d**) were not obtained (Scheme **1**). To our astonishment, ^{1}HNMR and LC/MS showed that chloro atom was introduced in the pyrimidine ring and the new compounds (**7a-g**, **8a-g**) were obtained. So in this paper, besides the original target compounds (**5a-d**, **6a-d**), we also synthesized the new compounds (**7a-g**, **8a-g**) and tested their bioactivities.

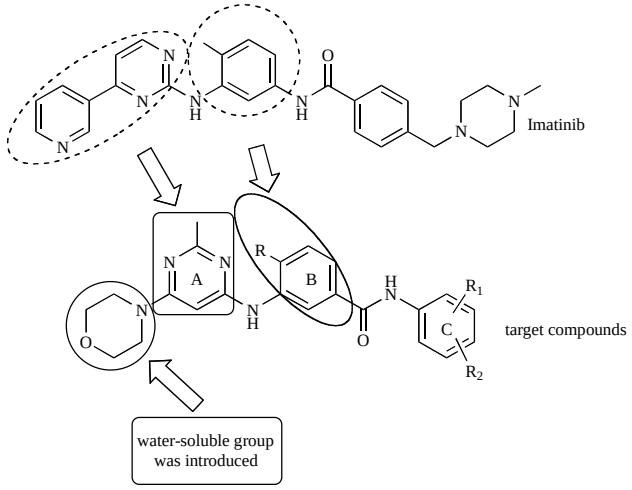


Fig. (1). structure of the target compounds.

GENERAL

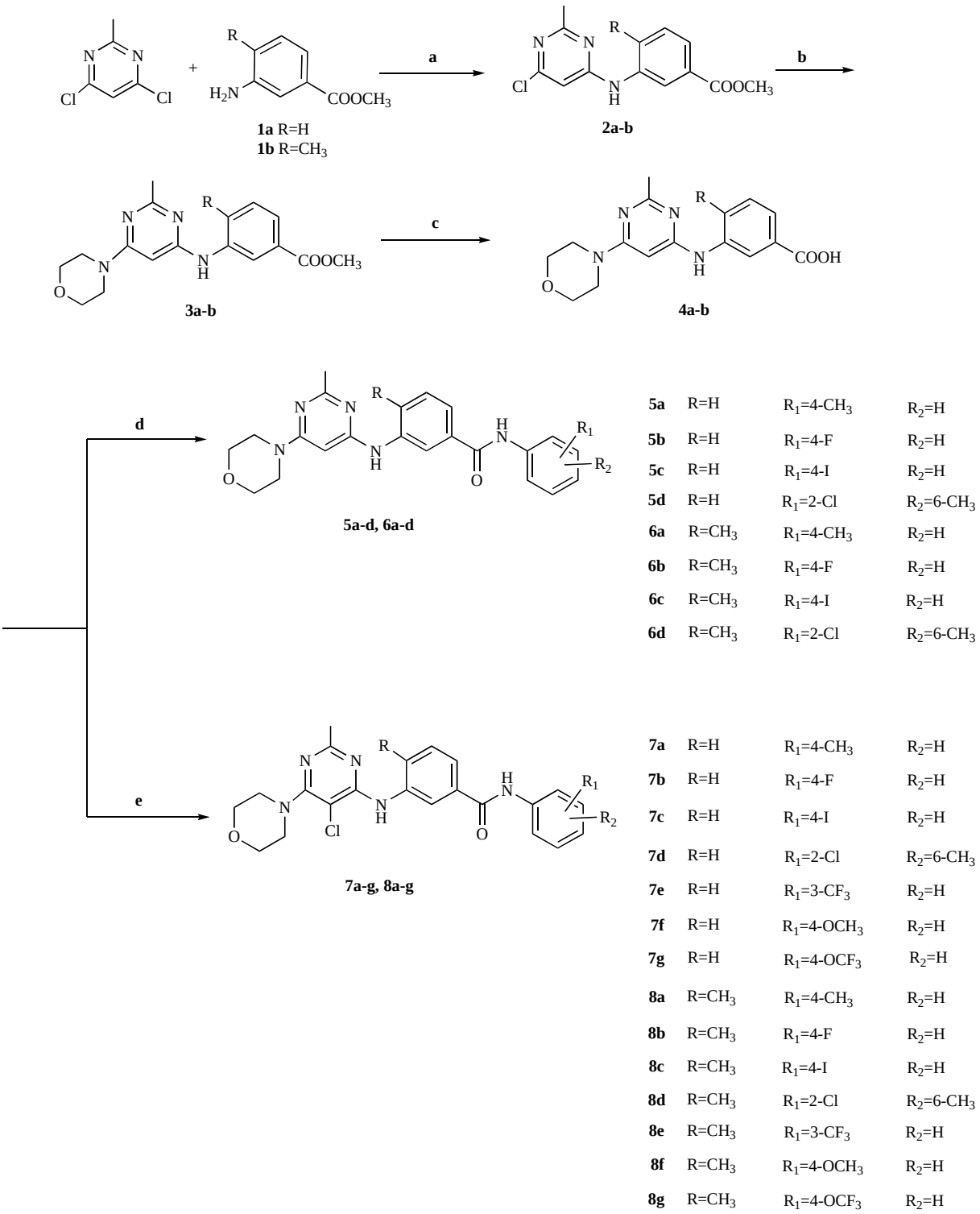
All reagents were purchased from commercial sources and used without further purification. Melting points were measured in open capillaries and are uncorrected. ^{1}H-NMR was recorded in DMSO-d_6 on a Bruker Avance 400 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were obtained from Agilent 1100 LC/MS Spectrometry Services.

RESULTS

In our attempts to obtain compounds with more potent bioactivities and lower drug-reisistance, a series of novel

*Address correspondence to this author at the Institute of Radiation and Irradiation Medicine, Academy of Military Medical Science Beijing 100850, China; Tel/Fax: +86-010-66932289; E-mail: lisx28@163.com

1570-1808/11 $58.00+.00



Scheme 1. Synthesis of the target compounds; Reagents and condition: a) con.HCl, isopropanol; b) morpholine, 1,4-dioxane; c) LiOH, H_2O, methanol, 2mol/LHCl; d) CDI, substituented phenylamino; e) thionyl chloride; substituented phenylamino.

phenylamino-pyrimidine derivatives were synthesized and evaluated as potent PTKs inhibitors. The antitumor activity of the new target compounds were determined against human KU812 cells *in vitro*, and the results were shown in Table **1**. Compared with STI-571, some of the target compounds obviously showed excellent bioactivities, especially the compounds **7b** and **7e**. Compounds **7d** and **7g** showed equivalent bioactivities with STI-571. Analyzing the bioactivities and their structures, the following structure-activity relationship was gained. Simply through introduction of a methyl in the pyrimidine ring(A ring), the inhibition against PTKs was enhanced, but the methyl(when R=CH_3) group in B ring had little effect on their bioactivities. In these phenylamino-pyrimidine derivatives, the introduction of chloro

Table 1. Bioactivities of the Target Compounds Against KU812 Cell *In Vitro*

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
STI-571	0.86	-	-
5a	17.61	**6a**	>50.0
5b	1.65	**6b**	15.80
5c	>100.0	**6c**	>100.0
5d	15 .60	**6d**	11.42
7a	5.12	**8a**	>50.0
7b	0.51	**8b**	20.71
7c	30.86	**8c**	>100.0
7d	0.90	**8d**	2.69
7e	0.41	**8e**	10.31
7f	>50.0	**8f**	>100.0
7g	0.78	**8g**	8.7

KU812 cells (purchased from American Type Culture Collection) cultured in a RPMI-1640 medium (manufactured by Sigma) containing 10% (v/v) fetal calf serum (FCS) (manufactured by Sigma) (RPMI-1640/FCS). KU812 cells were seeded at density of 5000 cells/100 μl/well and 4000 cells/100μl/well in each of 96-hole-plate (manufactured by costar), respectively. The plate was incubated in a CO_2 incubator overnight. A test drug was prepared with dimethylsulfoxide (DMSO) (manufactured by Nacalai Tesque) in the concentration 1000 times higher than the test concentration (0, 0.00001 to 1μM). The resulting solution was diluted 500 times in a RPMI-1640/FCS medium and then 100 μl of the diluent was added in a well. The plate was incubated in a CO_2 incubator. After 72 hours, 20μl of Cell counting Kit-8 (5 mmol/l WST-8, 0.2 mmol/l 1-Methoxy PMS, 150 mmol/l NaCl) (manufactured by Dojindo) was added to each well. After reaction for color development in a CO_2 incubator for 3 hours, an absorbance offermazan, generated by reduction of WST-8 was determined at 450 nm using Multilevel counter ARVOsx (manufactured by Wallac). In the RPMI-1640/FCS medium containing 0.1% DMSO, when absorbance of a region in which cells after culturing in the CO_2 incubator for 72 hours were seeded is defined as a cell growth inhibition rate of 0% and absorbance of a region in which cells were not seeded is defined as a cell growth inhibition rate of 100%, a log conc value in terms of log (inhibition rate/(100-inhibition rate)) and a plotted IC_{50} value (μM) were calculated. The results are shown in Table **1** [18]. As a control drug, STI571 was used.

atom in the pyrimidine ring also played an important role in antitumor activities to some extent. So in our next study, we will keep these pharmacophores and select **7b**, and **7e** as candidate compounds for futher development.

EXPERIMENTAL SECTION

Synthesis of the Intermediates (4a, 4b)

3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzoic acid (4a)

Step1: methyl 3-(6-chloro-2-methylpyrimidin-4-ylamino) benzoate

Compound **1a** (10.0 g, 66.0 mmol), 4,6-dichloro-2-methylpyrimidine (13.0 g, 80.0 mmol) and 2 mL con.HCl (36%) were added to 200 mL isopropanol. When the mixture was refluxed for 3h, the solution was cooled and placed in the refrigerator overnight. The precipitate was colleted by filtration, washed with cold isopropanol (2×50 mL) to afford white powder (16.8 g, 92% yield), mp: 192-195 °C.

Step2: 3-(6-chloro-2-methylpyrimidin-4-ylamino)benzoic acid (4a)

Methyl 3-(6-chloro-2-methylpyrimidin-4-ylamino) benzoate (15.0 g, 54 mmol) and morpholine (14 mL, 162 mmol) were dissolved in 1,4-dioxane (150mL). The reaction mixture was held at reflux for 15h, then the solvent was removed under vaccum affording the expected product (**3a**). A solution of 50ml methanol, 30 mL H_2O and 4.5 g LiOH was added to the compound (**3a**), and the reaction was stirred at room temperature for 5h. Methanol was evaporated and the residue was then brought into PH(2~3) with 2 mol/L HCl. The precipitate was filtrated, washed with ethanol and dried to afford white powder (14.8 g, 87.3 % yield), mp: >250 °C.

4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino) benzoic acid (4b)

The intermediate 4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino) benzoic acid (**4b**) was prepared from methyl 3-amino-4-methylbenzoate and 4,6-dichloro-2-methylpyrimidine by using the general procedure in **4a** described above. Yield: 89.7 %, mp: >250 °C.

Synthesis of the Target Compounds

The General Procedure for the Synthesis of N-(substituentedphenyl)-3-(2-methyl-6-morpholinopyrimidin-4-ylamino) benzamides (5a-5d)

Compound (**4a**) (1 mmol) and CDI (1 mmol) were dissolved in 15mL of DMSO, then the reaction mixture was maintained at the temperature of 50 °C under nitrogen atmosphere for 3h. The reaction mixture was cooled to reach room temperature, substituented phenylamino (1 mmol) were added and the solution was stirred overnight. The mixture was poured into water, extracted two times with ethyl acetate, the combined organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum. The crude product was purified by chromatographic colum to afford white powder.

N-p-tolyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (5a)

Yield 68%, mp: 185-188 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.30 (d, 6H), 3.46 (t, 4H), 3.66 (t, 4H), 5.84 (s, 1H), δ 7.15 (d, 2H), 7.44 (m, 2H), 7.65 (d, 2H), 7.98 (m,

2H), 9.23 (s, 1H), 10.13 (s, 1H); *M/z*: 404.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 68.47; H, 6.25; N, 17.36. Found C, 68.33; H, 6.27; N, 17.39.

N-(4-fluorophenyl)-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (5b)

Yield 72%, mp: 191-194 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.45 (t, 4H), 3.66 (t, 4H), 5.84 (s, 1H), 7.19 (t, 2H), 7.45 (m, 2H), 7.92 (m, 4H), 9.26 (s, 1H), 10.13 (s, 1H); *M/z*: 408.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 64.85; H, 5.44; N, 17.19. Found C, 64.98; H, 5.47; N, 17.17.

N-(4-iodophenyl)-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (5c)

Yield 67%, mp: 197-200 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.45 (t, 4H), 3.66 (t, 4H), 5.84 (s, 1H), 7.56 (m, 6H), 7.99 (m, 2H), 9.25 (s, 1H), 10.22 (s, 1H); *M/z*: 516.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 51.27; H, 4.30; N, 13.59. Found C, 51.53; H, 4.31; N, 13.55.

N-(2-chloro-4-methylphenyl)-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (5d)

Yield 71%, mp: 197-200 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.30 (d, 6H), 3.45 (t, 4H), 3.65 (t, 4H), 5.84 (s,1H), 7.15 (d, 2H), 7.44 (m, 2H), 7.65 (d, 1H), 7.98 (m, 2H), 9.23 (s, 1H), 10.13 (s, 1H); *M/z*: 438.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 63.08; H, 5.52; N, 15.99. Found C, 63.02; H, 5.54; N, 15.97.

The General Procedure for the Synthesis of N-(substituentedphenyl)-4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamides (6a-6d)

Compound (**4b**) (1 mmol) and CDI (1 mmol) were dissolved in 15mL of DMSO, then the reaction mixture was maintained at the temperature of 50 °C under nitrogen atmosphere for 3h. The reaction mixture was cooled to reach room temperature, substituented phenylamino (1 mmol) were added and the solution was stirred overnight. The mixture was poured into water, extracted two times with ethyl acetate, the combined organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum, crude product was purified by chromatographic colum to afford white powder.

N-p-tolyl-4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamides (6a)

Yield 70%, mp: 179-182 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.26 (t, 9H), 3.38 (t, 4H), 3.62 (t, 4H), 5.58 (s, 1H), 7.14 (d, 2H), 7.37 (d, 1H), 7.66 (m, 3H), 8.01 (s, 1H), 8.46 (s, 1H), 10.06 (s, 1H); *M/z*: 418.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 69.04; H, 6.52; N, 16.77. Found C, 69.11; H, 6.53; N, 16.79.

N-(4-fluorophenyl)-4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (6b)

Yield 74%, mp: 188-191 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.25 (d, 6H), 3.38 (t, 4H), 3.62 (t, 4H), 5.58 (s, 1H), 7.18 (t, 2H), 7.38 (d, 1H), 7.68 (dd, 1H), 7.78 (m, 2H), 8.02 (s, 1H), 8.46 (s, 1H), 10.20 (s, 1H); *M/z*: 422.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 65.54; H, 5.74; N, 16.62. Found C, 65.93; H, 5.69; N, 16.63.

N-(4-iodophenyl)-4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (6c)

Yield 65%, mp: 193-196 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.25 (d, 6H), 3.38 (t, 4H), 3.62 (t, 4H), 5.59 (s, 1H), 7.10 (t, 1H), 7.36 (m, 2H), 7.65 (m, 2H), 7.76 (dd, 1H), 8.02 (d, 1H), 8.46 (s, 1H), 10.14 (s, 1H); *M/z*: 530.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 52.18; H, 4.57; N, 13.23. Found C, 52.23; H, 4.56; N, 13.26.

N-(2-chloro-4-methylphenyl)-4-methyl-3-(2-methyl-6-morpholinopyrimidin-4-ylamino)benzamide (6d)

Yield 68%, mp: 188-191 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.25 (t, 9H), 3.38 (t, 4H), 3.62 (t, 4H), 5.58 (s, 1H), 7.14 (d, 2H), 7.37 (d, 1H), 7.66 (m, 2H), 8.01 (s, 1H), 8.46 (s, 1H), 10.06 (s, 1H); *M/z*: 452.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 63.78; H, 5.80; N, 15.50. Found C, 63.91; H, 5.81; N, 15.48.

The General Procedure for the Synthesis of N-(substituentedphenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamides (7a-7g)

Compound (**4a**) (1 mmol) was dissolved in 20 mL thionyl chloride and 1 mL DMF, the solution was held at reflux for 4h, then the thionyl chloride was removed under vaccum affording the expected acyl chloride. To a suspension of substituented phenylamino (1 mmol) and Et_3N (3.5 mL) in CH_2Cl_2 (15 mL) was added a solution of the expected acyl chloride in CH_2Cl_2 at dropwise with the temperature under 10 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solution was poured into 100 mL CH_2Cl_2, washed with water (2×100 mL), saturated sodium carbonate (2×100 mL) and brine (2×100 mL). The organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum. The crude product was purified by chromatographic colum to afford white powder.

N-p-tolyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (7a)

Yield 74%, mp: 198-201 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.30 (d, 6H), 3.45 (t, 4H), 3.71 (t, 4H), 7.15 (d, 2H), 7.44 (t, 1H), 7.64 (m, 3H), 7.90 (dd, 1H), 8.16 (t, 1H), 8.84 (s, 1H), 10.12 (s, 1H); *M/z*: 438.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 63.08; H, 5.52; N, 15.99. Found C, 62.89; H, 5.23; N, 15.96.

N-(4-fluorophenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)- benzamide (7b)

Yield 76%, mp: 211-213 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.50 (s, 3H), 3.44 (t, 4H), 3.71 (t, 4H), 7.20 (t, 2H), 7.46 (t, 1H), 7.62 (d, 1H), 7.85 (m, 3H), 8.18 (s, 1H), 8.89 (s, 1H), 10.30 (s, 1H); *M/z*: 442.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 59.80; H, 4.79; N, 15.85. Found C, 59.86; H, 4.80; N, 15.83.

N-(4-iodophenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)- benzamide (7c)

Yield 70%, mp: 195-198 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.46 (t, 4H), 3.70 (t, 4H), 7.46 (t, 1H), 7.66 (m, 5H), 7.91 (dd, 1H), 8.18 (s, 1H), 8.86 (s, 1H), 10.30 (s, 1H); *M/z*: 550.0 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 48.06; H, 3.85; N, 12.74. Found C, 48.20; H, 3.85; N, 12.77.

N-(2-chloro-6-methylphenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (7d)

Yield 75%, mp: 198-201 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ:2.30 (d, 6H), 3.45 (t, 4H), 3.70 (t, 4H), 7.15 (d, 2H), 7.45 (m, 1H), 7.64 (m, 3H), 7.90 (dd, 1H), 8.17 (m, 1H), 8.84 (s, 1H), 10.12 (s, 1H); *M/z*: 472.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 58.48; H, 4.91; N, 14.83. Found C, 58.36; H, 4.90; N, 14.82.

N-(3-(trifluoromethyl)phenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (7e)

Yield 69%, mp: 214-217 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.45 (t, 4H), 3.71(t, 4H), 7.56 (m, 4H), 7.92 (d, 1H), 8.06 (d, 1H), 8.23 (d, 2H), 8.87 (s, 1H), 10.52 (s, 1H); *M/z*: 492.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 56.16; H, 4.30; N, 14.24. Found C, 56.10; H, 4.31; N, 14.27.

N-(4-methoxyphenyl)-3-(5-chloro-2-methyl-6-morpholino-pyrimidin-4-ylamino)- benzamide (7f)

Yield 78%, mp: 193-196 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.45 (t, 4H), δ 3.70 (t, 4H), 3.75 (s, 3H), 6.93 (d, 2H), 7.44 (m, 1H), 7.64 (m, 3H), 7.89 (d, 1H), 8.16 (s, 1H), 8.88 (s, 1H), 10.11 (s, 1H); *M/z*: 454.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 60.86; H, 5.33; N, 15.43. Found C, 61.04; H, 5.32; N, 15.40.

N-(4-(trifluoromethoxy)phenyl)-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (7g)

Yield 67%, mp: 192-195 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.32 (s, 3H), 3.46 (t, 4H), 3.70 (t, 4H), 7.43 (m, 3H), 7.63 (d, 1H), 7.91 (m, 3H), 8.20 (s, 1H), 8.87 (s, 1H), 10.40 (s, 1H); *M/z*: 508.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 54.39; H, 4.17; N, 13.79**.** Found C, 54.23; H, 4.17; N, 13.83.

The General Procedure for the Synthesis of N-(substituentedphenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8a-8g)

Compound (**4b**) (1 mmol) was dissolved in 20mL thionyl chloride and 1mL DMF, the solution was held at reflux for 4h, then the thionyl chloride was removed under vaccum affording the expected acyl chloride. To a suspension of substituented phenylamino (1 mmol) and Et_3N (3.5 mL) in CH_2Cl_2 (15 mL) was added a solution of the expected acyl chloride in CH_2Cl_2 at dropwise with the temperature under 10 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solution was poured into 100 mL CH_2Cl_2, washed with water (2×100 mL), saturated sodium carbonate (2×100 mL) and brine (2×100 mL). The organic layer was dried over anhydrous $MgSO_4$. The solvent was removed under vacuum. The crude product was purified by chromatographic colum to afford white powder.

N-p-tolyl-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)- benzamide (8a)

Yield 72%, mp: 190-193 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.19 (t, 9H), 3.44 (t, 4H), 3.70 (t, 4H), 7.14 (d, 2H), 7.39 (d, 1H), 7.76 (m, 4H), 8.57 (s, 1H), 10.40 (s, 1H); *M/z*: 452.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 63.78; H, 5.80; N, 15.50. Found C, 63.72; H, 5.81; N, 15.52.

N-(4-fluorophenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8b)

Yield 75%, mp: 205-207 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.19 (d, 6H), 3.43 (t,4H), 3.71 (t, 4H), 7.18 (t, 2H), 7.41 (d, 1H), 7.82 (m, 4H), 8.63 (s, 1H), 10.25 (s, 1H); *M/z*: 456.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 60.59; H, 5.08; N, 15.36. Found C, 60.41; H, 5.09; N, 15.31.

N-(4-iodophenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8c)

Yield 71%, mp:194-197 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.19 (d, 6H), 3.44 (t, 4H), 3.70 (t, 4H), 7.41 (d, 1H), 7.66 (m, 4H), 7.77 (dd, 1H), 7.88 (d, 1H), 8.58 (s, 1H), 10.25 (s, 1H); *M/z*: 564.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 49.00; H, 4.11; N, 12.42. Found C, 49.15; H, 4.10; N, 12.43.

N-(2-chloro-6-methylphenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8d)

Yield 73%, mp: 192-195 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.20 (t, 9H), 3.44 (t, 4H), 3.70 (t, 4H), 7.36 (m, 4H), 7.86 (m, 2H), 8.59 (s, 1H), 9.94 (s, 1H); *M/z*: 486.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 59.26; H, 5.18; N, 14.40. Found C, 59.32; H, 5.17; N, 14.36.

N-(3-(trifluoromethyl)phenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8e)

Yield 68%, mp: 212-215 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.20 (d, 6H), 3.44 (t, 4H), 3.71 (t, 4H), 7.44 (t, 2H), 7.59 (t, 1H), 7.80 (dd, 1H), 7.92 (s, 1H), 8.07 (d, 1H), 8.24 (s, 1H), 8.60 (s, 1H), 10.46 (s, 1H); *M/z*: 506.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 56.98; H, 4.58; N, 13.84. Found C, 56.80; H, 4.58; N, 13.79.

N-(4-methoxyphenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8f)

Yield 76%, mp: 186-189 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.18 (d, 6H), 3.43 (t, 4H), 3.71(t, 4H), 6.92 (d, 2H), 7.40 (d, 1H), 7.76 (m, 4H), 8.62 (s, 1H), 10.07 (s, 1H); *M/z*: 468.2 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 61.60; H, 5.60; N, 14.97. Found C, 61.41; H, 5.61; N, 15.01.

N-(4-(trifluoromethoxy)phenyl)-4-methyl-3-(5-chloro-2-methyl-6-morpholinopyrimidin-4-ylamino)-benzamide (8g)

Yield 69%, mp: 187-190 °C; ^{1}HNMR (DMSO-d_6, 400MHz) δ: 2.19 (d, 6H), 3.45 (t, 4H), 3.70 (t, 4H), 7.39 (m, 3H), 7.84 (m, 4H), 8.59 (s, 1H), 10.35 (s, 1H); *M/z*: 522.1 ($[M+H]^+$, 100%). Elem. Anal. Calcd: C, 55.23; H, 4.44; N, 13.42. Found C, 55.17; H, 4.43; N, 13.43.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant No.30973616).

REFERENCES

[1] Peter, T.; Pascal, F. Strategies toward the Design of Novel and Selective Protein Tyrosine Kinase Inhibitors. *Pharmacol. Ther*., **1999**, *82*, 195-206.

[2] Doriano, F.; Stephan, R.; Elisabeth, B.; Sandra, W. C.; Gabriele, F.; Janis, L.; Jurgen, M.; Terence, O.; Peter, T.; Bhabatosh, C.; Heinz, F.; Jurg, Z.; Thomas, M.; Giorgio, C.; Pascal, F.; Paul, W. M. Pro-

tein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol. Ther*., **2002**, *93*, 79- 98.

[3] Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; Reilly, T.O.; Wood, J.; Zimmermann, J. Tyrosine kinase inhibitors: From rational design to clinical trials. *Med. Res. Rev*., **2001**, *21*, 499-512.

[4] Doriano, F.; David, P.; Alex, M. Protein tyrosine kinase inhibitors: new treatment modalities?. *Curr. Opin. Pharmachol*., **2002**, *2*, 374-381.

[5] Alexander, L. Protein Tyrosine Kinase Inhibitors as Novel Therapeutic Agents. *Pharmacol. Ther*., **1999**, *82*, 231-239.

[6] Keykavous, P.; Philip, A.C. Designing bisubstrate analog inhibitors for protein kinases. *Pharmacol. Ther*., **2002**, *93*, 145-157.

[7] Ron, B.; Marc, A. H.; Kerry, A.P.; Philip, A.C. Protein tyrosine kinase substrate interactions. *Curr. Opin. Struc. Biol*., **2006**, *16*, 668-675

[8] Bhushan, N.; Williamm G.B.; Patricia, P.; Thomas, S.; Darren, R.V.; Todd, M.W.; Bayard, C.; John, K. Crystal Structures of the Kinase Domain of c-Abl in Complex with the Small Molecule Inhibitors PD173955 and Imatinib (STI-571)[1]. *Cancer Res*., **2002**, *62*, 4236-4243.

[9] 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.

[10] Kantarjian, H.; Sawyers, C.; Hochhaus, A.; Guilhot, F.; Schiffer, C.; Gambacorti-Passerini, C.; Niederwieser, D.; Resta, D.; Capdeville, R.; Zoellner, U.; Talpaz, M.; Druker, B. Hematologic andcytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N. Engl. J. Med*., **2002**, *346*, 645-652.

[11] Bakhtiar, R.; Khemani, L.; Hayes, M.; Bedman, T.; Tse, F. Quantification of the anti-leukemia drug STI571 (Gleevec™) and its metabolite (CGP 74588) in monkey plasma using a semi-automated solid phase extraction procedure and liquid chromatography-tandem mass spectrometry. *J. Pharmaceut. Biomed*., **2002**, *28*, 1183-1194.

[12] Drummond, M.W.; Holyoake, T.L. Tyrosine kinase inhibitors in the treatment of chronic myeloid leukaemia: so far so good?. *Blood Rev*., **2001**, *15*, 85-95.

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

[14] Elias, J.; Samih, E.A.; Jorge, C.; Hagop, K. Nilotinib: a novel Bcr-Abl tyrosine kinase inhibitor for the treatment of leukemias. *Expert. Opin. Inv. Drug*, **2008**, *17*(7), 1127-1136.

[15] Mercedes, E.G.; Mansoor, M.; Katharine, E.; Nicholas, H.; Ron, P.; Nagesh, R.P.; Charles, L.S. Clinical Resistance to STI-571 Cancer Therapy Caused by BCR-ABL Gene Mutation or Amplification. *Science*, **2001**, *29*, 876-880.

[16] Carlo, B.G.; Rosalind, H.G.; Rocco Piazza.; Annamaria, G.; Roberta, R.; Leonardo, S. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet. Oncol*., **2003**, *4*, 75-85.

[17] Lalji, K.G.; Aashvini, B.; Aakanksha, K.; Puranik, P.; Vincent, C.O. Improved synthesis of histone deacetylase inhibitors (HDIs) (MS-275 and CI-994) and inhibitory effects of HDIs alone or in combination with RAMBAs or retinoids on growth of human LNCaP prostate cancer cells and tumor xenografts. *Bioorgan. Med. Chem*., **2008**, *16*, 3352-3360.

[18] Hye Won Chung, M.D.; Jing wen, M.D.; Jong-Baeck Lim, M.D.; Seungmin Bang, M.D.; Seung Woo Park, M.D.; Si Young Song, M.D. Radiosensitization effect of STI-571 on pancreatic cancer *in vitro*. *Int. J. Radiat. Oncol*., **2009**, *75*(3), 862–869.