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Design and Synthesis of Novel Dasatinib Analogues

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Design, synthesis, characterization and *in vitro* biological assay of a series of novel carboxylic acid and amino acid analogs of dasatinib (**1**) as anticancer agents are reported. Some of the synthesized analogs were identified as potent Src/Abl kinase inhibitors with greater antiproliferative activity against K652 and T315I cancer cell lines. The synthetic process involves condensation of N-(2-chloro-6-methylphenyl)-2-[[6-[4(-1-pipearazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide with carboxylic acids in the presence of dicyclohexylcarbodiimide and oxyma in organic solvent medium. Compounds were characterized and tested for anticancer activity on leukemia cancer cell lines K562 and Baf3/T315. Analogues of lactic acid, mandalic acid, leucine and proline have shown promising antiproliferative activity compared to dasatinib.

Keywords: Dasatinib derivatives, Antiproliferative activity, Anticancer.

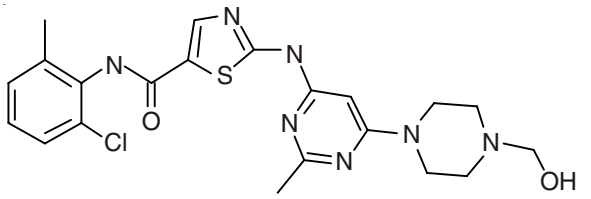
INTRODUCTION

Dasatinib is an orally available, small-molecule and is a multi-targeted tyrosine kinase inhibitor that potently inhibits multi-Bcr-Abl and Src family tyrosine kinases inhibitor approved for first line therapy in patients with chronic myelogenous leukemia (CML) and philadelphia chromosome-positive acute lymphoblastic leukemia [1]. It is being evaluated for use in numerous other cancers. Due to increasing resistance and intolerance to imatinib, efforts were made to develop new drugs candidates that could inhibit the Bcr-Abl tyrosine kinase. This led to the discovery of second generation drugs for chronic myelogenous leukemia. The second generation tyrosine kinase inhibitors (TKI's) was developed with rational drug design approach due to increased knowledge in structural biology of the Bcr-Abl tyrosine kinase [2]. Dasatinib has excellent anticancer activity, specifically against chronic myelogenous leukemia and several imatinib resistant cells.

Molecules containing a thiazole amine-moiety exhibit interesting biological activities depending on the substitution pattern at the thiazole ring [3]. 2-Aminothiazole nucleus is found to be well known pharmacophore for a broad spectrum of activities, like antibacterial [4], antifungal [5], antitubercular [6], anti-HIV [7], anti-inflammatory [8], antiprotozoal [9], hypertension [10], schizophrenia [11] and estrogen receptors [12] as well as novel class of adenosine receptor antagonists [13], whereas other analogues are used as fungicides, inhibiting *in vivo* growth of *Xanthomonas* and as an ingredient of herbicides or as schistosomicidal and anthelmintic drugs [14]. It also reported in the literature that 2-aminothiazole-5-aromatic carboxamides are useful kinase inhibitors of protein tyrosine kinase and p38 kinase [15]. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [16]. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. By considering the above kinase inhibitor activities of 2-aminothiazole-5-aromatic caroboxamides, we have designed and synthesized several novel 2-aminothiazole-5-aromatic carboxamide derivatives containing pyrimidine, piperazine ring extensions and evaluated their anticancer activity through molecular docking studies by using 2GQG as their PDBID and antiproliferative activity on different leukemia cell lines K562and Baf3/T315I with dasatinib as a reference standard. Dasatinib is marketed under the brand name SPRYCEL®. The general structures of dasatinib (N-(2-chloro-6-methylphenyl)-2-[6-[4-(2-hydroxyethyl)piperazin-1-yl)-2-methyl pyrimidin-4-yl]amino]-5-thiazolecarboxamide) and its novel derivatives are shown in Fig. 1.

EXPERIMENTAL

All reactions were monitored by thin layer chromatography and carried out on 0.2 mm E. Merck silica gel plates (60F-



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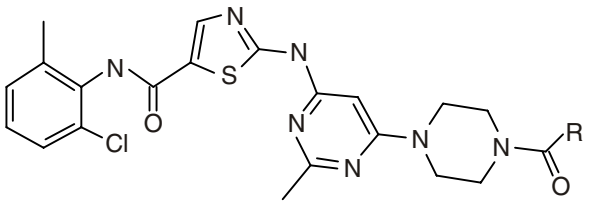


2(a-k)

Fig. 1. Dasatinib (**1**) and its analogues **2(a-k)**

254) using UV light as a visualizing agent. All solvents were obtained from commercial source and freshly distilled before use. Analytical HPLC was performed on a waters system equipped with UV detector set at 320 nm. Compounds were dissolved in mobile phase and injected through a 100 mL loop. The following gradient system was used as eluent mobile phase A: 10 mM KH_2PO_4 buffer pH 3.5 with H_3PO_4 and mobile phase B: acetonitrile, methanol in the ratio of 1:1. HPLC retention times (t_R) were obtained, at flow rate 1.0 mL/min, using the following conditions. Inert sill ODS-4 (250 × 4.6 mm, 5 µm) column a gradient run up to 45 min. The IR spectral data of all the compounds were recorded on Perkin-Elmer FT-IR spectrometer (MODEL No; PARAGON-1000). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer. The chemical shifts are reported in δ parts per million (ppm) relative to TMS as standard. The mass spectra were recorded on Waters LC/MS/MS. All melting points are uncorrected

General procedure

Synthesis of N-(2-chloro-6-methylphenyl)-2-(6-chloro-2-methylpyrimidine-4-yl-amino)-1,3-thiazole-5-carboxamide (5): 2-Amino-N-(2-chloro-6-methylphenyl)-1,3-thiazole-5-carboxamide (**3**) (5 g, 18.67 mmol) and 4,6-dichloro-2-methylpyrimidine (**4**) (3.65 g, 22.4 mmol), tetrahydrofuran (65 mL) were charged into a 250 mL four necked round bottomed flask. Stirred the mass for 15 min. To this a 30 % w/w solution of sodium *t*-butoxide in tetrahydrofuran (21 g, 65.36 mmol) was added slowly with cooling to keep the temperature at 10-20 °C. The mixture was stirred for 1.5 h and cooled to 0-5 °C. Then 21.5 mL of 2 N HCl was added slowly and the compound was collected by vacuum filtration. Then the compound was washed with water (15 mL) and drying of the filtered wet compound to give 6.63 g (86.4 %) of a cream coloured solid. m.p.: 282-286.5 °C; HPLC: 99.0 %; ^{1}H NMR (DMSO-d_6): δ 2.249 (s, 3H), 2.594 (s, 3H), 6.952 (s, 2H), 7.25-7.31 (m, 2H), 7.40-7.42 (dd, 1H, *J* = 6.4), 8.32 (s, 1H), 10.02 (s, 1H), 12.24 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 167.37; 161.22, 159.52, 158.47, 157.5, 140.77, 138.74, 133.28, 132.35, 129.02, 128.24, 126.99-127.18, 103.38, 66.97, 25.12, 18.23; MS: (ESI), *m/z* 394.0 (M), 396.1 (M+2); IR (KBr, ν_{max}, cm^{-1}): 3421.93 (OH), 3241.69 (-NH), 2876.91 (aliphatic C-H stretching), 1639.68 (C=O), 770.66 (Cl).

Synthesis of N-(2-chloro-6-methylphenyl)-2-{[6-[4(1-piperazinyl)-2-methyl-4-pyrimidinyl-]amino]-5-thiazole-carboxamide (6): N-(2-Chloro-6-methylphenyl) 2-(6-chloro-2-methylpyrimidine-4-ylamino)-1,3-thiazolecarbox amide (**5**) (5 g, 0.01 mol) piperazine, (5.45 g, 0.06 mol) and *n*-butanol (40 mL) were charged into a 250 mL four necked round bottomed flask. Slowly added diisopropylethylamine (4.42 mL, 0.02 mol) to the reaction mass.The reaction mixture was heated at 120 °C for 4-5 h. Then the mixture was cooled slowly to room temperature. The obtained solid was collected by vacuum filtration, washed with *n*-butanol and dried the wet compound to give 8.1 g (81.2 %) of a cream coloured solid. m.p.: 256-260 °C; HPLC: 95.2 %; ^{1}H NMR (DMSO-d_6): δ 2.249 (s, 3H), 2.40 (s, 3H), 2.3-2.75 (t, 4H), 3.44 (t, 4H), 6.02 (s, 1H,), 7.237-2.3 (m, 2H), 7.39-7.41 (m, 1H), 8.22 (s, 1H), 11.17 (b NH); ^{13}C NMR (DMSO-d_6): δ 165.04, 162.46-162.59, 159.9, 156.86, 140.79, 138.78, 133.50, 132.40, 128.96, 128.0, 126.94, 125.60, 82.42, 45.20, 44.73, 25.53, 18.25; MS; (ESI), *m/z* 444.3 (M), 446.2 (M+2); IR (KBr, ν_{max}, cm^{-1}): 3421.93 (OH), 3241.69 (-NH), 2876.91 (aliphatic-H stretching), 1639.68 (C=O), 770.66 (Cl).

General procedure for the synthesis of novel dasatinib analogues 2(a), 2(b), 2(f), 2(g) and 2(h): For the synthesis of dasatinib analogues **2(a), 2(b), 2(f), 2(g)** and **2(h)** the corresponding carboxylic acid L(-) lactic acid, R(+) mandalic acid, 3-(trifluoro methane sulfonic acid), 4,4-difluorocyclo-hexanoic acid and 3-iodo-4-methyl benzoic acid were employed as substrates to couple with compound **6**.

To mixture of compound N-(2-chloro-6-methylphenyl)-2-{[6-[4(1-piperazinyl)-2-methyl -4-pyrimidinyl]amino]-5-thiazolecarboxamide (**6**) (5.1 mol equivalent), the corresponding carboxylic acid (1:1 mol equivalents), DCC (1:1 mol equivalents) and oxyma (1:1 mol equivalents) were charged into 4-necked round bottomed flask along with tetrahydrofuran. The slurry was slowly heated at 63-65 °C for 4-5 h. Then cooled slowly to room temperature.The reaction mixture was quenched into demineralized water. The obtained solid was collected by vacuum filtration, washed with demineralized water. Suck dried thoroughly to afford crude product. The above obtained crude product was dissolved in hot dimethyl sulfoxide (DMSO) (5 volumes to compound weight) and charcoal treatment was given. The hot solution was slowly diluted with water (6 volumes to DMSO quantity) and cooled slowly to room temperature. The solid was colected by vacuum filtration and washed with 10 mL of demineralized water. Drying of the wet compound to get a white coloured product.

N-(2-Chloro-6-methylphenyl)-2-[[6-[4-[(2S)-2-hydroxy-propanoyl]-1-piperazinyl]-2-methyl-4-pyrimidinyl]-amino]-5-thiazolecarboxamide [2(b)]: The obtained crude compound was purified from column chromatography by elution of 5 % methanol in chloroform. m.p.: 258 °C; HPLC: 85.5 %; ^{1}H NMR (DMSO-d_6): δ 1.20 (d, 3H, *J* = 4 Hz), 2.24 (s, 3H), 2.42 (s, 3H), 3.5-3.6 (bs, 8H), 4.43-4.5.45 (p, 1H), 4.98 (d, 1H, *J* = 7.2 Hz), 6.07 (s, 1H), 7.23-7.30 (p, 2H), 7.39-7.41 (d, 1H, *J* = 6.8 Hz), 8.22 (s, 1H), 9.8 (s, 1H), 11.5 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 172.3, 165.2-162.5, 159.8, 156.9, 140.8, 138.7, 133.5, 132.4, 128.9, 128.1, 126.9, 125.7, 82.8,

64.2, 43.73-43.8, 43.0, 41.0 25.5, 20.5 18.2; Mass: 516.4; IR (KBr, ν_{max}, cm^{-1}): 3405.2, 3255.9, 3063.4, 2979.2, 2923.1, 1632.5, 1577.6, 771.9.

N-(2-Chloro-6-methylphenyl)-2-[[2-methyl-6-[4-[3-(trifluoromethylsulfonyl)benzo yl]-1-piperazinyl]-4-pyrimidinyl]amino]-5- thiazolecarboxamide [2(h)]: m.p.: 195 °C (MeOH cryst.); HPLC: 97.2 %; ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H), 2.42 (s, 3H), 3.43-3.74 (t, 8H), 6.075 (s, 1H), 7.23-7.2 (q, 2H, *J* = 7.6 Hz), 7.39-7.41 (d, 1H, *J* = 6.8 Hz), 7.94-7.98 (t, 1H), 8.11-8.136 (d, 1H7.6 Hz), 8.20-8.26 (q, 3H), 9.891 (s, 1H) 11.54 (s, NH); ^{13}C NMR (DMSO-d_6): δ 166.4, 165.2, 162.4, 162.2, 159.9, 156.9, 140.8, 138.8, 138.1, 136.0, 133.4, 132.4, 1316, 131.2-131.6, 129.9, 129.0, 128.1, 126.9, 125.7, 120.9, 117.7, 82.9, 46.3, 43.3, 42.8, 41.4, 25.5, 18.2; Mass; 680.27; IR (KBr, ν_{max}, cm^{-1}): 3404.72, 3190.92, 2922.51, 1665.02 1618.07, 1578.07, 766.78.

N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(4,4-difluorocyclohexanecarbonyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide [2(f)]: Recrystallization from methanol. m.p.: 277.1 °C; HPLC: 97.97.6 %; ^{1}H NMR (DMSO-d_6): δ 1.57-1.63 (q, 2H), 1.75-1.93 (m, 3H), 2.04-2.06 (t, 2H *J* = 6.8 Hz), 2.240 (s, 3H) 2.42 (s, 3H), 2.80-2.85 (m, 1H), 3.55-3.64 (t, 8H), 6.074 (s, 1H), 7.23-7.25 (d, 1H, *J* = 7.6 Hz), 7.27-7.3 (t, 1H *J* = 7.2 Hz), 8.22 (s, 1H), 9.89 (s, 1H) 11.46 (s, NH); ^{13}C NMR (DMSO-d_6): δ 172.5, 165.2, 162.23-162.55, 159.9, 156.9, 140.8, 138.8, 133.5, 132.4, 129.0, 128.1, 126.9 125.74-126.10, 123.7, 121.3, 82.8, 43.8-44.12, 43.1, 40.6, 36.4, 32.00-32.4, 25.4-25.5, 18.26; Mass: 590.42; IR (KBr, ν_{max}, cm^{-1}): 3415.12, 3215.13, 2940.57, 1665.02 1620.54, 1578.41, 773.58.

N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(3-iodo-4-methyl-benzoyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]-amino]-5-thiazolecarboxamide [2(g)]: Recrystallization from methanol. m.p.: 275.6 °C; HPLC: 97.2 %; ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H), 2.41 (s, 6H), 3.57-3.6 (t, 8H), 6.070 (s, 1H), 7.26-7.28 (d, 2H, *J* = 8.4 Hz), 7.40 (s, 3H), 7.87 (s, 1H), 8.22 (s, 1H), 9.894 (s, 1H), 11.53 (s, NH); ^{13}C NMR (DMSO-d_6): δ 167.4, 165.2, 162.5, 162.2, 159.9, 156.9, 142.5, 140.8, 138.7, 137.0, 135.0, 133.5, 132.4, 129.6, 128.9, 128.1, 126.9, 125.7, 101.0, 82.8, 46.4, 43.3, 27.4, 25.5 18.2; Mass: 688.42; IR (KBr, ν_{max}, cm^{-1}): 3412.03, 3245.36, 2973.01, 2920.82, 1662.04, 1610.39, 1572.88, 774.4.

General procedure for the synthesis of dasatinib analogues 2(c), 2(d), 2(e), 2(i), 2(j) and 2(k): A mixture of compound N-(2-chloro-6-methylphenyl)-2-{[6-(-1-piperazinyl)-2-methyl-4-pyrimidin]amino}-5-thiazole-5-carboxamide (**6**) (5.1 mol equivalent), the corresponding fmoc protected amino acid (1:1 mol equivalents), DCC (1:1 mol equivalents) and oxyma (1:1 mol equivalents) were charged into 4-necked round bottomed flask along with tetrahydrofuran. The slurry was slowly heated at 63-65 °C for 4-5 h. Then cooled slowly to room temperature. The reaction mixture was quenched into demineralized water. The obtained solid was collected by vacuum filtration, washed with demineralized water. Suck dried thoroughly to afford crude product. The product obtained was dissolved in 20 % piperidine solution and stirred at ambiant temperature for 1-2 h. The compound was extracted into methylenedichloride solvent. The solid was collected by concentration of the organic layer.

2-[[6-[4-[(2S)-2-amino-4-methyl-pentanoyl]-1-piperazin]-2-methyl-4-pyrimidin]amino]-N-(2-chloro-6-methylphenyl)-5-thiazolecarboxamide [2(c)]: The obtained crude compound was purified from column chromatography by elution of 5 % methanol in chloroform, m.p.: 160.4 °C; HPLC: 99.3 %; ^{1}H NMR (DMSO-d_6): δ 0.878-0.91 (d, 6H, *J* = 6.4 Hz), 1.23-1.290 (m, 3H), 1.75-1.82 (m, 1H), 2.243 (s, 3H), 2.430 (s, 3H), 3.49-3.69 (m, 8H) 6.068 (s, 1H), 7.14-7.3 (m, 2H *J* = 6.4 Hz), 7.39-7.41 (d, 1H, *J* = 7.6), 8.22 (s, 1H), 9.9 (s, 1H), 11.5 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 173.9, 165.1, 162.2-162.5, 159.8, 156.9, 140.7, 138.7, 133.4, 132.4, 128.9, 128.1, 126.9, 125.7, 82.7, 54.4, 44.1, 43.7 43.2, 40.8, 38.3, 25.5, 23.118.2, 16.0, 1 1.4; Mass: 557.38; IR (KBr, ν_{max}, cm^{-1}): 3383.53, 3252.17, 2954.94, 2926.14, 2867.48, 1614.54, 1577.76, 771.15.

2-[[6-[4-[(2S,3S)-2-amino-3-methyl-pentanoyl]-1-piperazinyl]-methyl4pyrimidinyl]amino]-N-(2-chloro-6-methylphenyl)-5-thiazolecarboxamide [2(d)]: The obtained crude compound was purified from column chromatography by elution of 5 % MeOH in $CHCl_3$ m.p.: 160.4 °C; HPLC: 99.3 %; ^{1}H NMR (DMSO-d_6): δ 0.828-0.866 (q, 6H), 1.016-1.090 (m, 1H), 1.44-1.53 (m, 2H), 2.24 (s, 3H), 2.42 (s, 3H), 3.47-3.48 (d, 2H, *J* = 6 Hz), 3.62-3.64 (m, 8H, *J* = 6.8 Hz), 6.2 (s, 1H), 7.23-7.3 (m, 2H, *J* = 6.4 Hz), 7.39-7.41 (d, 1H, *J* = 7.6), 9.89 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 173.9, 165.1, 162.2-162.5, 159.8, 156.9, 140.7, 138.7, 133.4, 132.4, 128.9, 128.1, 126.9, 125.7, 82.7, 54.4, 44.1, 43.7 43.2, 40.8, 38.3, 25.5, 23.118.2, 16.0, 11.4: Mass: 557.38; IR (KBr, ν_{max}, cm^{-1}): 3387.65, 3252.43, 2962.19, 2927.72, 2874.12, 1613.58, 1577.93, 773.19.

2-[[6-[4-[(2S)-2-amino-3-phenylpropanoyl]-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-N-(2-chloro-6-methylphenyl)-5-thiazolecarboxamide [2(e)]: The obtained crude compound was purified from column chromatography by elution of 5 % methanol in chloroform. m.p.: 156 °C; HPLC: 99.3 %; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H), 2.41 (s, 3H), 2.65 (q, 1H), 2.77 (q, 1H), 3.04 (brs, 1H), 3.48-3.55 (m, 6H), 3.93 (t, 1H), 6.0 (s, 1H), 7.18-7.40 (m, 8H), 8.22 (s, 1H), 9.8 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 173.2, 165.1, 162.4, 162.0, 159.8, 156.9, 140.7, 138.7, 138.3, 133.4, 132.4, 129.4, 128.9, 128.0, 126.9, 126.1, 125.7, 82.6, 51.6, 43.9, 43.2, 43.0, 42.1, 40.7, 25.5, 18.2; Mass: 591.3; IR (KBr, ν_{max}, cm^{-1}): 3374.4, 3249.7, 3061.1, 2922.5, 1617.9, 1577.6, 1536.8, 1503.8, 770.7

2-[[6-[4-[(2S)-2-Aminopropanoyl]-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-N-(2-chloro-6-methylphenyl)-5-thiazolecarboxamide [2(i)]: The obtained crude compound was purified from column chromatography by elution of 5 % methanol in chloroform; HPLC: 98.7 %; ^{1}H NMR (DMSO-d_6): δ 1.12 (d, 3H), 2.24 (s, 3H), 2.43 (s, 3H), 3.51-3.63 (m, 8H), 3.83 (q, 1H), 6.0 (s, 1H), 7.23 (m, 2H), 7.39 (d, 1H), 8.23 (s, 1H), 9.9 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 173.5, 165.1, 162.2-162.4, 159.8, 156.9, 140.8, 138.7, 138.3, 133.4, 132.4, 128.9, 128.1, 126.9, 125.7, 82.7, 79.14, 45.9, 43.12-43.8, 40.9, 25.5, 20.8 18.2; Mass: 515; IR (KBr, ν_{max}, cm^{-1}): 3405.0, 2921.7, 1611.9, 1577.9, 1539.0, 1500.3, 775.3.

N-(2-Chloro-6-methyl phenyl)-2-[[2-methyl-6-[4-[(2S)-pyrrolidine-2-carbonyl]-1-piperazin-yl]-4-pyrimidinyl]-amino]-5-thiazolecarboxamide [2(j)]: The obtained crude

compound was purified from column chromatography by elution of 5 % methanol in chloroform; HPLC: 98.7 %; ^{1}H NMR (DMSO-d_6): δ 1.587-1.668 (m, 3H), 2.012 (s, 1H), 2.242 (s, 3H), 2.429 (s, 3H), 2.632-2.691 (m, 1H), 2.976-3.031 (m, 1H), 3.523-3.617 (m, 8H), 3.867-3.902 (t, 1H), 6.077 (s, 1H), 7.239-7.303 (dt, 2H), 7.393-7.412 (d, 1H), 8.233 (s, 1H), 8.321 (s, 1H), 9.902 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 173.5, 165.1, 162.2-162.4, 159.8, 156.9, 140.8, 138.7, 138.3, 133.4, 132.4, 128.9, 128.1, 126.9, 125.7, 82.7, 79.14, 45.9, 43.12- 43.8, 40.9, 25.5, 20.8 18.2; Mass: 541.43; IR (KBr, ν_{max}, cm^{-1}): 3406.5, 3251.0, 2924.8, 1610.6, 1577.3, 1539.0, 1500.7, 775.6.

(4S)-4-amino-5-[4-[6-[[5-[(2-chloro-6-methylphenyl)-carbamoyl]-2-thiazolyl]amino]-2-methyl-4-pyrimidinyl]-1-piperazinyl]-5-oxo-pentanoic acid [2(k)]: The obtained crude compound was purified from column chromatography by elution of 5 % methanol in chloroform; HPLC: 98.5 %; ^{1}H NMR (DMSO-d_6): δ 1.587-1.668 (m, 3H), 1.40-1.45 (m, 2H), 1.746-1.765 (m, 1H), 2.130-2.282 (m, 4H), 2.431 (s, 1H), 3.564-3.66 (m, 8H), 6.083 (s, 1H), 7.258-7.306 (dt, 2H), 7.390-7.410 (d, 1H), 8.231 (s, 1H), 9.894 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 174.2, 173.5, 165.2, 162.2-162.2, 159.9, 156.9, 140.8, 138.8, 133.5, 132.4, 129.0, 128.1, 127.0, 125.7, 82.8, 49.6, 43.8, 43.2, 41.0, 30.8, 30.3, 25.5, 18.3; Mass: 572.3; IR (KBr, ν_{max}, cm^{-1}): 3406.5, 3251.0, 2924.8, 1610.6, 1577.3, 1539.0, 1500.7, 775.6.

RESULTS AND DISCUSSION

Molecular docking studies: We have studied *in silico* activity to screen the designed analogs of dasatinib (Table-1).

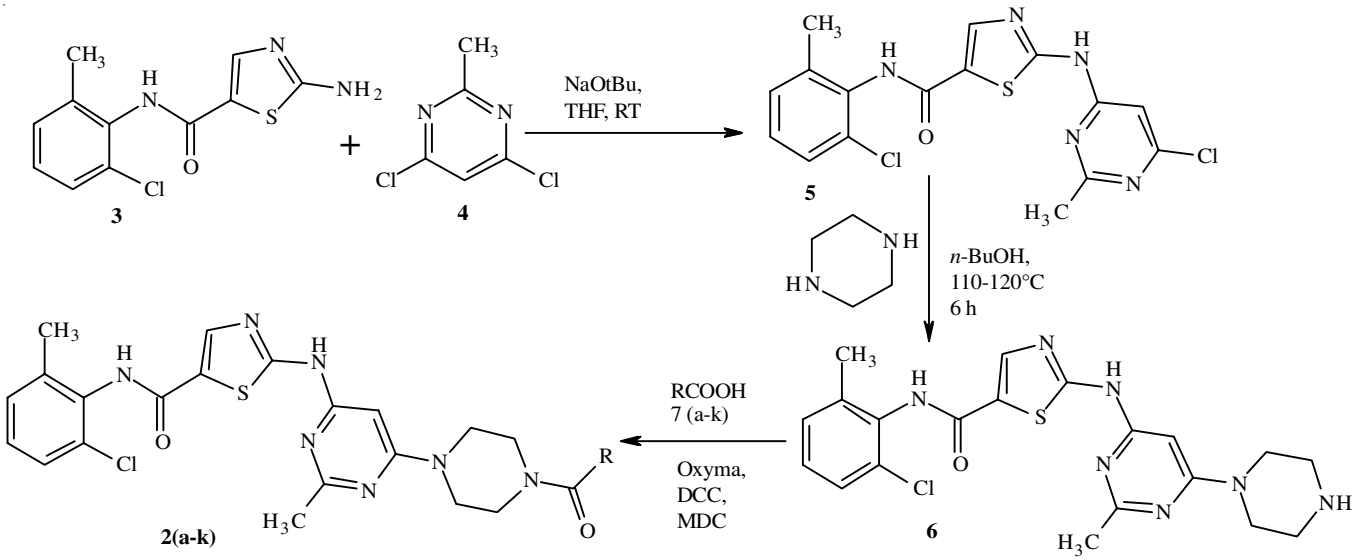
According to *in silico* studies, it is observed that, five novel analogs **2(a), 2(b), 2(c), 2(f) and 2(j)** among eleven docked structures were found to be potent compared to dasatinib. Molecules **2(d), 2(e) and 2(i)** have been identified to have moderate activity and compounds **2(g), 2(h) and 2(k)** are expected to be inactive. To test for anticancer activity against leukemia K562 and mutated leukemia Baf3/T315I cancer lines the designed analogues were synthesized and tested for their anti-cancer activity against K562 and Baf3/T315I.

TABLE-1
MOLECULAR DOCKING RESULTS OF DASATINIB ANALOGUES

Molecule	H-Bond residue	Binding affinity
Dasatinib	THR 315, MET 318, HOH 623	-9.6
2a	THR 315, MET 318, HOH 623	-9.9
2b	THR 315, MET 318, HOH 623	-10.0
2c	THR 315, MET 318, HOH 623	-9.8
2d	THR 315, MET 318, HOH 623	-8.9
2e	THR 315, MET 318, HOH 623	-9.5
2f	THR 315, MET 318, HOH 623	-9.9
2g	THR 315, MET 318, HOH 623	-8.6
2h	THR 315, MET 318, HOH 623	-3.3
2i	THR 315, MET 318, HOH 623	-9.3
2j	THR 315, MET 318, HOH 623	-9.9

The compounds described are fall into two sub-classes that differ by the acid group appended to the amino group of the piperizine. Analogs of dasatinib were studied involving introduction of different acid and amino acid moieties in place of hydroxyethyl function on piperazine ring to test their anti-cancer activity. According to **Scheme-I**, novel analogues of dasatinib are synthesized by replacing the hydroxyethyl moiety on piperizine ring with different carboxylic acid groups like mandalic acid, lactic acid, 3-iodo-4-methyl benzoic acid, 3-(triflurometh yl)thiobenzoic acid and 4,4-difluoro cyclohexane carboxylic acid residues and different chiral amino acid groups like leucine, isoleucine, proline, alanine, phenyl alanine, glutamine, *etc*. As indicated by the docking studies, analogues containing mandalic acid, lactic acid, 4,4-difluoro cyclohexane carboxylic acid and l-proline substitutions are proven to be more potent and analogues with 3-iodo-4-methyl benzoic acid, 3-(trifluoromethyl)thiobenzoic acid residues are least active and the remaining analogues are found to be moderately active.

According to **Scheme-I**, the starting material N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino]-5-thiazolecarboxamide (**6**) was prepared following literature methodology [17] by reacting 2-amino-N-(2-chloro-6-methyl phenyl)-1,3-thiazole-5-carboxamide (**3**) with 4,6-dichloro-2 methylpyrimidin (**4**) in tetrahydrofuran



Scheme-I: Synthesis of novel Dasatinib analogues

in the presence of potassium *tert*-butoxide to afford N-(2-chloro-6-methylphenyl)-2-(6-chloro-2-methylpyrimidine-4-yl-amino)-1,3-thiazolecarboxamide (**5**). The intermediate (**5**) was reacted with piperazine in *n*-butanol at 115-120 °C to yield N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino]-5-thiazolecarboxamide (**6**).

Synthesis of analogues **2(a-k)**, of dasatinib involves coupling of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino] -5-thiazolecarboxamide (**6**) with carboxylic acids as listed in Table-4 in the presence of dicyclohexylcarbodiimide (DCC), ethyl 2-cyano-2-hydroxylaminoacetate (oxyma) in tetrahydrofuran as solvent. The analogues were isolated as crystalline solids in 85-95 % yield with > 97 purity by HPLC.

During the synthesis of analogues of dasatinib, for the coupling of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino]-5-thiazolecarboxamide (**6**) with carboxylic acids, we studied the reaction with various amide coupling reagents like DCC-oxyma, EDC·HCl, HOBT, HATU (Table-2). It is observed that only 40 % conversion observed when reaction was carried out with DCC, EDC·HCl, HATU and HOBT in THF solvent at 55-60 °C. Whereas optimum conversion (> 90 %) was observed when coupling carried out in the presence of DCC-oxyma reagent. In dichloromethane and acetonitrile reaction did not proceeded (Table-2).

TABLE-2
EXPERIMENTAL STUDY OF DASATINIB ANALOGUES WITH DIFFERENT REAGENTS

Comp. **6g**	Lactic acid	Solvent	Reagent	Yield, g (%)
10.0	2.2	THF	EDC·HCl + HOBT	5.0 g (43)
10.0	2.2	THF	EDC·HCl + HATU	7.2 g (61.9)
100.0	2.2	THF	DCC + Oxyma	10.4 g (89.44)

During the synthesis of analogues of dasatinib, for the coupling of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino]-5-thiazolecarboxamide (**6**) with carboxylic acids, we also studied the reaction with various solvents and reaction temperature conditions (Table-3).

TABLE-3
EXPERIMENTAL STUDY OF DASATINIB ANALOGUES WITH DIFFERENT SOLVENTS & DIFFERENT TEMPERATURE CONDITIONS

Comp. **6g**	Lactic acid	Solvent	Reaction temp. (°C)	Yield g (%)
10.0	2.2	MDC	35-40	8.5 g (73.1)
10.0	2.2	ACN	55-60	10.0 g (86.0)
10.0	2.2	THF	55-60	10.7 g (90.6)

From all the experimental data it was concluded that combination of DCC/oxyma in tetrahydrofuran solvent system at 55-60 °C found to be optimum condition for the coupling of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(piperazin-1-yl)-2-methylpyrimidin-4-yl]amino] -5-thiazolecarboxamide (**6**) with carboxylic acids. It is observed from the experimental results that more than 95 % conversion and > 90 % isolated yields achieved with lactic acid, mandalic acid, leucine, 4,4-difluorocyclohexane carboxylic acid and alanine. Whereas moderate conversion (about 75 %) was achieved with isoleucine, phenylalanine, 3-iodo-4-methyl benzoic acid, 3-(trifluoromethyl) thiobenzoic acid, proline and glutamine. The experimental results are tabulated in Table-4.

TABLE-4
PHYSICAL CHARACTERISTICS AND PURITIES OF ANALOGUES OF DASATINIB

Product	R = COOH	Yield* (%)	m.p. (°C)	HPLC purity
2(a)	Lactic acid	92.5	195.0	97.20
2(b)	Mandalic acid	90.5	258.0	85.50
2(c)	Leucine	94.0	160.4	99.30
2(d)	Isoleucine	71.0	160.5	99.20
2(e)	Phenyl alanine	76.0	156.0	99.30
2(f)	4,4-Difluorocyclo hexanecarboxylic acid	96.0	277.1	97.97
2(g)	3-Iodo-4-methyl benzoic acid	85.0	275.6	97.20
2(h)	3-(Trifluoromethyl) thio benzoic acid	88.0	195.0	97.20
2(i)	Alanine	65.0	> 250	98.70
2(j)	Proline	70.0	> 250	98.70
2(k)	Glutamine	67.0	> 250	98.50

*Purification by column chromatography

Based on the experimental design, the following analogues of dasatinib (**2a-k**) were synthesized and characterized.

***in vitro* activity:** Anti-proliferative activity was carried out to determine the activity of synthesized analogs. Tests were conducted on leukemia cancer cell lines. The standard drug taken was dasatinib for K562 and mutated leukemia (Baf3/T315) cancer cell lines.

MTT assay procedure: Briefly, cells were plated at a density of 1000 to 10,000 cells/well in 96 well plates and incubated overnight at 37 °C in 5 % CO_2 incubator. The following day, the cells were treated with the test compound and proto drug dasatinib allowed to incubate for various time points up to 96 h. MTT reagent was then added to the treated cells for an additional 4 h and then processed according to the standard protocol. All activities of the 9 hits, as calculated against these cell lines. Among all these hits, molecules **2(a), 2(b), 2(c), 2(f)** and **2(j)** were showing activity (IC_{50} of 1 nM) on K562 leukemia cell lines and molecules **2(a), 2(b), 2(c)** were showing activity (IC_{50} of 1 nM) on mutated (Baf3/T315I) leukemia cell lines (Table-5).

Conclusion

A total of eleven novel analogues of dasatinib were designed by substituting the ethanol side chain of piperazine moiety with various carboxylic acids and amino acids. These were subjected to molecular modeling study to estimate the binding affinity using PDB ID 2GQG imported from protein data bank (PDB). The highly potent analogues **2(a), 2(b), 2(c), 2(f)** and **2(j)**, the moderate potent analogues **2(d), 2(e)** and **2(i)** and the least potent analogues **2(g), 2(h)** and **2(k)** were selected from docking studies to synthesize. Evaluated the biological activity on K562 cell lines were employed for cytotoxicity studies and the IC_{50} values were calculated. The results obtained from the activity study revealed that cytotoxicity of dasatinib analogues

TABLE-5
in vitro ACTIVITY STUDY OF NOVEL DASATINIB ANALOGUES ON LEUKEMIA CANCER CELL LINE (K562) AND MUTATED LEUKEMIA CANCER CELL LINE (Baf3/T315i)

in vitro activity on leukemia (K562) cancer cell line		*in vitro* activity on mutated Leukemia (Baf3/T315I) cancer cell line
Molecule	IC_{50}	IC_{50}
Dasatinib	1.500	7963
2a	0.410	4228
2b	0.150	1842
2c	0.620	5184
2d	5.000	–
2e	6.000	–
2f	0.580	9699
2g	11.000	–
2h	10.000	–
2i	7.000	>10000
2j	0.210	–
2k	125.000	–

2(a), 2(b), 2(c), 2(f) and **2(j)** are better than dasatinib. **2(g), 2(h)** and **2(k)** had shown less activity. The results obtained from docking studies are in concordant with biological testing results. Further, *in vivo* studies of **2(a), 2(b), 2(c), 2(f)** and **2(j)** molecules are in progress to evaluate the lead molecule, which will be further advanced to complete preclinical study.

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