Research Article

Pyrimidines: Synthesis and Antiproliferative Activity on Human Leukemia Cells

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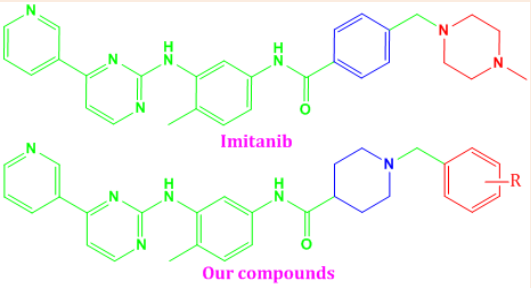
Abstract

A novel series of building blocks consisting of 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine have been synthesized as potential antiproliferative agents. Compounds **10c, 12d** and **14a** with an electron releasing substituent at the ortho and para position on the phenyl ring showed excellent *in vitro* potency against tested human leukemia cells (K562 and Reh) with IC_{50} values range from 2.3 to 5.3 µM.

Keywords: Piperidine-4-carboxylic acid, Stannous chloride, Anticancer agents, Leukemia, MTT assay



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Introduction

Leukemia which comprises of different types of blood cancer cells is common in all ages. Among, acute lymphoblastic leukemia (ALL) is the most common type of leukemia in young children and Chronic myelogenous leukemia (CML) is most common in adults. The number of children affected by ALL has doubled in the last 20 years in India and about 25,000 Indian children diagnosed with ALL every year [1]. Imatinib, an anti-cancer agent used to treat CML is prepared by an intermediate *N*-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine, and it has also been used to treat for gastrointestinal stromal tumors (GISTs) [2]. It selectively inhibits Bcr-Abl kinase and results in cytogenetic response in a very high proportion of chronic myeloid leukemia [3]. It has high efficacy and mild side effects and is now a frontline treatment for CML [4]. However, resistance to medications is common in leukemia. Therefore, improved chemotherapy regimens and other strategies are needed. In this regards, the use of combinatorial approaches toward the synthesis of drug-like scaffolds helps to speed up drug discovery.

Pyrimidines and their analogues represent an important class of nitrogen heterocycles, which is found in both various biologically active natural compounds and designed medicinal agents [5-7]. The pyrimidine derivatives comprise a diverse and interesting group of drugs [8-10]. Pyrimidine, being an integral part of DNA and RNA, have imparts diverse biological activity viz. anticancer [11], antiviral [12], antiprotozoal [13], antihypertensive [14], antihistaminic [15], anti-inflammatory [16], central nervous activities [17], antibacterial [18], antifungal [19] and, in particular antiangiogenic agents [20]. Specifically, disubstituted pyrimidines have shown potent anticancer activity as CDK inhibitors [21], TNF-α inhibitors [22], Abl tyrosine protein kinase inhibitors [23], PI-3 kinase inhibitors [24], Akt kinase inhibitors [25] and cytokines inhibitors [26].

The literature has recorded several instances, in which incorporation of two to three structural features required for activity in a single molecule has given rise to significant enhancement in activity [27,28]. In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents [29-31], we have

synthesized some novel *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide by combining two biolabile components together, targeting potent molecules possessing antileukemic activity. These compounds showed efficient anti-proliferative activity.

Experimental section

Chemistry

Melting points were determined using SELACO-650 hot stage melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as internal standard (chemical shift in δ ppm). Spin multiplets are given as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass and purity were recorded on a LCMSD-Trap-XCT. Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck made TLC plates.

Synthesis of 3-dimethylamino-1-(pyridin-3-yl)prop-2-en-1-one (3)

A mixture of 3-acetylpyridine **(1)** (25 g, 20.63 mmol) and *N*,*N*-dimethylformamide dimethyl acetal **(2)** (31.95 g, 26.82 mmol) was refluxed for 16 h under nitrogen. Upon completion of the reaction, the mixture was concentrated under reduced pressure. To the residue, cyclohexane was added, and the mixture was cooled to 0 °C. The precipitate was collected by filtration to afford the product as yellow crystals (90 %). MP: 80 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.0 (d, 1H, Py-H), 8.62 (dd, 1H, Py-H), 8.25 (dt, 1H, Py-H), 7.81 (d, 1H, COCH=CH) , 7.35 (dd, 1H, Py-H), 5.75 (d, 1H, COCH=CH), 3.25 (s, 3H, CH_3), 3.02 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3080, 1685, 1620, 1448, 1354, 748. MS (ESI) m/z: 177.09.

Synthesis of *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine (5)

To a mixture of 3-dimethylamino-1-(pyridin-3-yl)propenone **(3)** (25 g, 11.34 mmol) and *N*-(2-methyl-5-nitrophenyl)guanidinium nitrate **(4)** (47.66 g, 14.74 mmol) in *n*-butanol (200 mL), sodium hydroxide (8.63 g, 216 mmol) was added. The mixture was refluxed for 16 h and then cooled to 0 °C. The precipitate was collected by filtration and washed with methanol and diethyl ether, and dried to get the product (92 %) as a yellow solid. MP: 197 °C. ^{1}H-NMR δ: 8.93 (d, 1H, Py-H), 8.71 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.45 (d, 1H, pyrimidyl-H), 8.30 (d, 1H, Py-H), 7.45 (dd, 1H, Py-H), 7.30 (d, 1H, pyrimidyl-H), 6.75 (d, 1H, Ar-H), 6.70 (d, 1H, Ar-H), 6.38 (dd, 1H, Ar-H), 2.08 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3076, 1655, 1521, 1476, 870. MS (ESI) m/z: 308.11.

General procedure for the synthesis of 6-methyl-*N*^{1}-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (6)

To a solution of stannous chloride dihydrate in hydrochloric acid (30 mL) at 0 °C, *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **(5)** was added in portions and stirred for 6 h. Progress of reaction was monitored by TLC. Upon completion, the mixture was poured into crushed ice, made alkaline with solid sodium hydroxide, and extracted with ethyl acetate. The combined organic layer was washed two to three times with water and dried over anhydrous sodium sulfate. The solvent was evaporated to get crude product, which was purified by recrystallization from methylene chloride to get the compound as a yellow solid.

Synthesis of 6-methyl-*N*^{1}-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (6)

The product obtained was yellow solid (75%) from *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine **(5)** (10 g, 3.254 mmol), and stannous chloride dihydrate (29 g, 12.974 mmol) in 35 ml hydrochloric acid. MP: 144 °C. ^{1}H-NMR δ: 8.98 (d, 1H, Py-H), 8.65 (dd, 1H, Py-H), 8.58 (s, 1H, NH), 8.42 (d, 1H, pyrimidyl-H), 8.34 (d, 1H, Py-H), 7.48 (dd, 1H, Py-H), 7.30 (d, 1H, pyrimidyl-H), 6.82 (d, 1H, Ar-H), 6.75 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 4.80 (br, 2H, NH_2), 2.05 (s, 3H, CH_3). MS (ESI) m/z: 278.13.

General procedure for the synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (8)

Piperidine-4-carboxylic acid **(7)** was taken in dry *N*,*N*-dimethyl formamide and cooled to 0-5 ^{0}C in ice bath. Then isobutyl chloroformate and *N*-methyl morpholine were added to the reaction mixture. The reaction mixture was allowed to stir for 10-15 min. After that 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine **(6)** was added, then reaction mixture was allowed to room temperature under stirring for 5-6 h. Progress of reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using MDC and methanol (1:1) to get *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)**.

Synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (8)

The product obtained was pale yellow color from piperidine-4-carboxylic acid **(7)** (0.046 g, 0.36 mmol), 6-methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine **(6)** (0.1 g, 0.36 mmol), isobutyl chloroformate (0.078 g, 0.772 mmol) and *N*-methyl morpholine (0.078 g, 0.772 mmol). MP: 142 ^{o}C. ^{1}H-NMR δ: 9.20 (s, 1H, CO-NH), 8.95 (d, 1H, Py-H), 8.70 (dd, 1H, Py-H), 8.61 (s, 1H, NH), 8.46 (d, 1H, pyrimidyl-H), 8.32 (d, 1H, Py-H), 7.40 (dd, 1H, Py-H), 7.33 (d, 1H, pyrimidyl-H), 6.76 (d, 1H, Ar-H), 6.69 (d, 1H, Ar-H), 6.32 (dd, 1H, Ar-H), 3.53 (t, 2H, CH_2), 3.28 (s, 1H, NH), 3.20 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.35 (t, 2H, CH_2), 2.10 (t, 2H, CH_2) 2.01 (s, 3H, CH_3). MS (ESI) m/z: 389.2 (100.0%). Anal. calcd. for $C_{22}H_{24}N_6O$ (in %): C-68.02, H-6.23, N-21.63. Found: C-67.96, H-6.17, N-21.65.

General procedure for the synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl) piperidine-4-carboxamide derivatives 10(a-d)

The *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** was dissolved in dry dichloromethane. To this reaction mixture triethylamine was added and cooled to 0-5 ^{0}C in ice bath. Then different sulfonyl chlorides **9(a-d)** are added. The reaction mixture was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using dichloromethane and methanol (1:1).

***N*-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-((4-nitrophenyl)sulfonyl)piperidine-4-carboxamide (10a)**

The product obtained was pale yellow color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (**8**) (0.1 g, 0.257 mmol), 4-nitrobenzene sulphonyl chloride **(9a)** (0.055 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.23 (s, 1H, CO-NH), 8.92 (d, 1H, Py-H), 8.75 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.47 (d, 1H, pyrimidyl-H), 8.40 (d, 2H, Ar-H), 8.30 (d, 1H, Py-H), 8.15 (d, 2H, Ar-H), 7.43 (dd, 1H, Py-H), 7.30 (d, 1H, pyrimidyl-H), 6.71 (d, 1H, Ar-H), 6.69 (d, 1H, Ar-H), 6.35 (dd, 1H, Ar-H), 3.50 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 2.80-2.88 (bs, 1H, CH), 2.38 (t, 2H, CH_2), 2.12 (t, 2H, CH_2) 2.03 (s, 3H, CH_3). MS (ESI) m/z: 574.78 (100.0%). Anal. calcd. for $C_{28}H_{27}N_7O_5S$ (in %): C-58.63, H-4.74, N-17.09. Found: C-58.66, H-4.71, N-17.05.

***N*-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-(o-tolylsulfonyl)piperidine-4-carboxamide (10b)**

The product obtained was pale yellow color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (**8**) (0.1 g, 0.257 mmol), 2-methylbenzene sulphonyl chloride (**9b)** (0.049 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.23 (s, 1H, CO-NH), 8.91 (d, 1H, Py-H), 8.73 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.49 (d, 1H, pyrimidyl-H), 8.40 (d, 1H, Py-H), 7.78 (d, 1H, Ar-H), 7.53 (d, 1H, Ar-H), 7.45 (t, 2H, Ar-H), 7.37 (dd, 1H, Py-H), 7.28 (d, 1H, pyrimidyl-H), 6.73 (d, 1H, Ar-H), 6.60 (d, 1H, Ar-H), 6.35

(dd, 1H, Ar-H), 3.50 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 2.78-2.85 (bs, 1H, CH), 2.70 (s, 3H, CH_3), 2.32 (t, 2H, CH_2), 2.15 (t, 2H, CH_2), 2.05 (s, 3H, CH_3). MS (ESI) m/z: 543.61 (100.0%). Anal. calcd. for $C_{29}H_{30}N_6O_3S$ (in %): C-64.19, H-5.57, N-15.49. Found: C-64.16, H-5.51, N-15.45.

1-((4-Methoxyphenyl)sulfonyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (10c)

The product obtained was pale yellow color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol), 4-methoxybenzene sulphonyl chloride **(9c)** (0.053 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.18 (s, 1H, CO-NH), 8.90 (d, 1H, Py-H), 8.74 (dd, 1H, Py-H), 8.65 (s, 1H, NH), 8.40 (d, 1H, pyrimidyl-H), 8.35 (d, 1H, Py-H), 7.63 (dd, 2H, Ar-H), 7.35 (dd, 1H, Py-H), 7.30 (d, 1H, pyrimidyl-H), 7.10 (d, 2H, Ar-H), 6.70 (d, 1H, Ar-H), 6.63 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 3.84 (s, 3H, OCH_3), 3.50 (t, 2H, CH_2), 3.24 (t, 2H, CH_2), 2.80-2.88 (bs, 1H, CH), 2.34 (t, 2H, CH_2), 2.11 (t, 2H, CH_2) 2.03 (s, 3H, CH_3). MS (ESI) m/z: 559.59 (100.0%), Anal. calcd. for $C_{29}H_{30}N_6O_4S$ (in %): C-62.35, H-5.41, N-15.04. Found: C-62.29, H-5.37, N-15.05.

1-((3-Chlorophenyl)sulfonyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (10d)

The product obtained was pale yellow color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3-chlorobenzene Sulphonyl chloride **(9d)** (0.054 g, 0.257 mmol) and triethylamine(0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.21 (s, 1H, CO-NH), 8.95 (d, 1H, Py-H), 8.74 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.51 (d, 1H, pyrimidyl-H), 8.36 (d, 1H, Py-H), 8.21 (s, 1H, Ar-H), 7.75 (d, 1H, Ar-H), 7.66 (t, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.42 (dd, 1H, Py-H), 7.31 (d, 1H, pyrimidyl-H), 6.74 (d, 1H, Ar-H), 6.67 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 3.55 (t, 2H, CH_2), 3.22 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.36 (t, 2H, CH_2), 2.12 (t, 2H, CH_2) 2.05 (s, 3H, CH_3). MS (ESI) m/z: 564.106 (100.0%). Anal. calcd. for $C_{28}H_{27}ClN_6O_3S$ (in %): C-59.73, H-4.83, N-14.93. Found: C- C-59.70, H-4.81, N-14.90.

General procedure for the synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl) piperidine-4-carboxamide derivatives 12(a-f)

The *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** was dissolved in dry dichloromethane. To this reaction mixture triethylamine was added and cooled to 0-5 ^{0}C in ice bath. Then different benzyl chlorides **11(a-f)** are added. The reaction mixture was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using dichloromethane and methanol (1:1).

1-Benzyl-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (12a)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and benzyl chloride **(11a)** (0.032 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.19 (s, 1H, CO-NH), 8.92 (d, 1H, Py-H), 8.73 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.48 (d, 1H, pyrimidyl-H), 8.35 (d, 1H, Py-H), 7.41 (dd, 1H, Py-H), 7.31 (d, 1H, pyrimidyl-H), 7.23-7.27 (m, 5H, Ar-H), 6.75 (d, 1H, Ar-H), 6.66 (d, 1H, Ar-H), 6.31 (dd, 1H, Ar-H), 3.60 (s, 2H, CH_2-), 3.52 (t, 2H, CH_2), 3.21 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.32 (t, 2H, CH_2), 2.11 (t, 2H, CH_2) 2.03 (s, 3H, CH_3). MS (ESI) m/z: 479.508. Anal. calcd. For $C_{29}H_{30}N_6O$ (in %): C- 72.78; H- 6.32; N- 17.56. Found: C- 72.82; H- 6.36; N- 17.59.

1-(3-Methoxybenzyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (12b)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3-methoxybenzyl chloride **(11b)** (0.040 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.20 (s, 1H, CO-NH), 8.95 (d, 1H, Py-H), 8.70 (dd, 1H, Py-H), 8.61 (s, 1H, NH), 8.46 (d, 1H, pyrimidyl-H), 8.32 (d, 1H, Py-H), 7.40 (dd, 1H, Py-H), 7.33 (d, 1H, pyrimidyl-H), 7.25 (t, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 6.76 (d, 2H, Ar-H), 6.69 (d, 1H, Ar-H), 6.32 (dd, 1H, Ar-H), 3.75 (s, 3H, OCH_3), 3.62 (s, 2H, CH_2-), 3.53 (t, 2H, CH_2), 3.20 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.35 (t, 2H, CH_2), 2.10 (t, 2H, CH_2) 2.01 (s, 3H, CH_3). MS (ESI) m/z: 509.682. Anal. calcd. For $C_{30}H_{32}N_6O_2$ (in %): C- 70.84; H- 6.34; N- 16.52. Found: C- 70.89; H- 6.39; N- 16.58.

1-(4-Methoxybenzyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (12c)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 4-methoxybenzyl chloride **(11c)** (0.040 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.18 (s, 1H, CO-NH), 8.92 (d, 1H, Py-H), 8.75 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.40 (d, 1H, pyrimidyl-H), 8.32 (d, 1H, Py-H), 7.43 (dd, 1H, Py-H), 7.35 (d, 1H, pyrimidyl-H), 6.83 (dd, 2H, Ar-H), 6.75 (d, 1H, Ar-H), 6.64 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 3.85 (s, 3H, OCH_3), 3.60 (s, 2H, CH_2), 3.51 (t, 2H, CH_2), 3.18 (t, 2H, CH_2), 2.80-2.87 (bs, 1H, CH), 2.33 (t, 2H, CH_2), 2.12 (t, 2H, CH_2) 2.04 (s, 3H, CH_3). MS (ESI) m/z: 509.680. Anal. calcd. For $C_{30}H_{32}N_6O_2$ (in %): C- 70.84; H- 6.34; N- 16.52. Found: C- 70.89; H- 6.38; N- 16.57.

***N*-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-(3-methylbenzyl)piperidine-4-carboxamide (12d)**

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3-methylbenzyl chloride **(11d)** (0.036 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.22 (s, 1H, CO-NH), 8.96 (d, 1H, Py-H), 8.73 (dd, 1H, Py-H), 8.60 (s, 1H, NH), 8.43 (d, 1H, pyrimidyl-H), 8.30 (d, 1H, Py-H), 7.40 (dd, 1H, Py-H), 7.32 (d, 1H, pyrimidyl-H), 7.27 (t, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 6.82 (d, 1H, Ar-H), 6.71 (d, 2H, Ar-H), 6.65 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 3.62 (s, 2H, CH_2-), 3.50 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.33 (t, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.10 (t, 2H, CH_2) 2.05 (s, 3H, CH_3). MS (ESI) m/z: 493.515. Anal. calcd. For $C_{30}H_{32}N_6O$ (in %): C- 73.14; H- 6.55; N- 17.06. Found: C- 73.17; H- 6.61; N- 17.11.

1-(3,4-Difluorobenzyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (12e)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3,4-difluorobenzyl chloride **(11e)** (0.041 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.20 (s, 1H, CO-NH), 8.93 (d, 1H, Py-H), 8.77 (dd, 1H, Py-H), 8.63 (s, 1H, NH), 8.45 (d, 1H, pyrimidyl-H), 8.32 (d, 1H, Py-H), 7.40 (dd, 1H, Py-H), 7.31 (d, 1H, pyrimidyl-H), 7.20 (dd, 1H, Ar-H), 7.12 (dd, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 6.78 (d, 1H, Ar-H), 6.63 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 3.64 (s, 2H, CH_2-), 3.51 (t, 2H, CH_2), 3.20 (t, 2H, CH_2), 2.75-2.84 (bs, 1H, CH), 2.30 (t, 2H, CH_2), 2.13 (t, 2H, CH_2) 2.01 (s, 3H, CH_3). MS (ESI) m/z: 515.589. Anal. calcd. For $C_{29}H_{28}F_2N_6O$ (in %): C- 67.69; H- 5.48; N- 16.33. Found: C- 67.76; H- 5.54; N- 16.41.

***N*-(4-Methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-(3-nitrobenzyl)piperidine-4-carboxamide (12f)**

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3-nitrobenzyl chloride **(11f)** (0.044 g, 0.257

mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.23 (s, 1H, CO-NH), 8.94 (d, 1H, Py-H), 8.75 (dd, 1H, Py-H), 8.62 (s, 1H, NH), 8.40 (d, 1H, pyrimidyl-H), 8.32 (d, 1H, Py-H), 7.45 (dd, 1H, Py-H), 7.35 (d, 1H, pyrimidyl-H), 7.23 (t, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 6.80 (d, 1H, Ar-H), 6.71 (d, 2H, Ar-H), 6.62 (d, 1H, Ar-H), 6.33 (dd, 1H, Ar-H), 3.63 (s, 2H, CH_2-), 3.54 (t, 2H, CH_2), 3.24 (t, 2H, CH_2), 2.77-2.86 (bs, 1H, CH), 2.35 (t, 2H, CH_2), 2.12 (t, 2H, CH_2) 2.02 (s, 3H, CH_3). MS (ESI) m/z: 524.593. Anal. calcd. For $C_{29}H_{29}N_7O_3$ (in %): C- 66.52; H- 5.58; N- 18.73. Found: C- 66.59; H- 5.63; N- 18.78.

General procedure for the synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl) piperidine-4-carboxamide derivatives 14(a-c)

The *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** was dissolved in dry dichloromethane. To this reaction mixture triethylamine was added and cooled to 0-5 ^{0}C in ice bath. Then different aromatic isothiocyanates **13(a-c)** are added. The reaction mixture was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by column chromatography over silica gel (60-120 mesh) using dichloromethane and methanol (1:1).

1-((4-Methylphenyl)carbamothioyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (14a)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 4-methylphenyl isothiocyanate **(13a)** (0.043 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.22 (s, 1H, CO-NH), 9.16 (s, 1H, CS-NH), 8.93 (d, 1H, Py-H), 8.74 (dd, 1H, Py-H), 8.59 (s, 1H, NH), 8.42 (d, 1H, pyrimidyl-H), 8.30 (d, 1H, Py-H), 7.46 (dd, 1H, Py-H), 7.35 (d, 1H, pyrimidyl-H), 7.29 (dd, 2H, Ar-H), 6.60 (dd, 2H, Ar-H), 6.71 (d, 1H, Ar-H), 6.62 (d, 1H, Ar-H), 6.35 (dd, 1H, Ar-H), 3.50 (t, 2H, CH_2), 3.23 (t, 2H, CH_2), 2.79-2.89 (bs, 1H, CH), 2.33 (t, 2H, CH_2), 2.89 (s, 3H, -CH_3), 2.11 (t, 2H, CH_2) 2.03 (s, 3H, CH_3). MS (ESI) m/z: 538.66 (100.0%), Anal. calcd. for $C_{30}H_{31}N_7OS$ (in %): C- 67.01, H-5.81, N-18.24. Found: C-67.19, H-5.93, N-18.32.

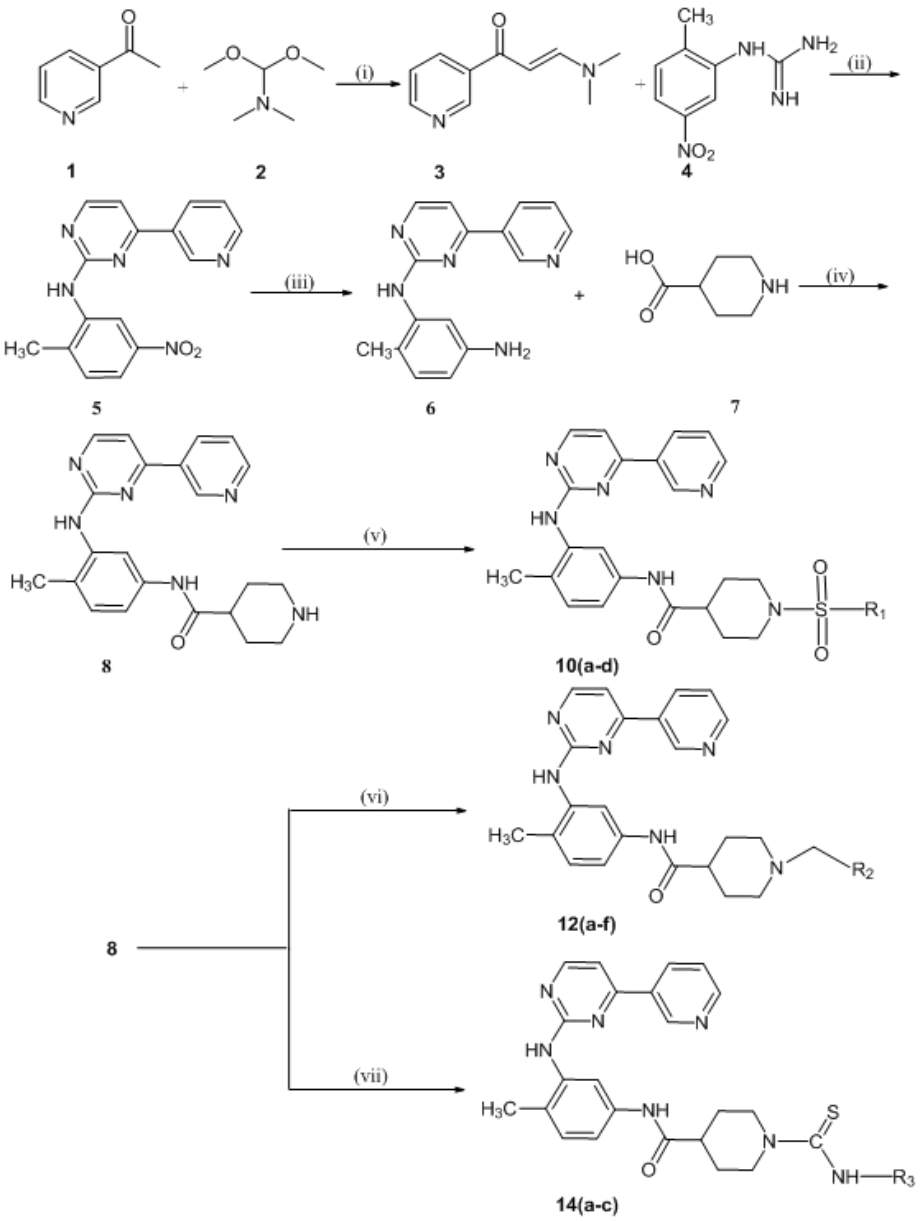
1-((2-Methoxyphenyl)carbamothioyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (14b)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 2-methoxyphenyl isothiocyanate **(13b)** (0.042 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.20 (s, 1H, CO-NH), 9.14 (s, 1H, CS-NH), 8.92 (d, 1H, Py-H), 8.75 (dd, 1H, Py-H), 8.63 (s, 1H, NH), 8.40 (d, 1H, pyrimidyl-H), 8.35 (d, 1H, Py-H), 7.42 (dd, 1H, Py-H), 7.31 (d, 1H, pyrimidyl-H), 6.86 (d, 1H, Ar-H), 6.79 (d, 1H, Ar-H), 6.70 (dd, 2H, Ar-H), 6.68 (d, 1H, Ar-H), 6.59 (d, 1H, Ar-H), 6.34 (dd, 1H, Ar-H), 3.85 (s, 3H, OCH_3), 3.56 (t, 2H, CH_2), 3.24 (t, 2H, CH_2), 2.75-2.86 (bs, 1H, CH), 2.37 (t, 2H, CH_2), 2.14 (t, 2H, CH_2) 2.05 (s, 3H, CH_3). MS (ESI) m/z: 554.69 (100.0%), Anal. calcd. for $C_{30}H_{31}N_7O_2S$ (in %): C-65.08, H-5.64, N-17.71. Found: C-65.02, H-5.60, N-17.66.

1-((3-Methoxyphenyl)carbamothioyl)-*N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (14c)

The product obtained was dark brown color from *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide **(8)** (0.1 g, 0.257 mmol) and 3-methoxyphenyl isothiocyanate **(13c)** (0.042 g, 0.257 mmol) and triethylamine (0.078 g, 0.772 mmol). ^{1}H-NMR δ: 9.21 (s, 1H, CO-NH), 9.15 (s, 1H, CS-NH), 8.90 (d, 1H, Py-H), 8.75 (dd, 1H, Py-H), 8.64 (s, 1H, NH), 8.42 (d, 1H, pyrimidyl-H), 8.36 (d, 1H, Py-H), 7.48 (dd, 1H, Py-H), 7.34 (d, 1H, pyrimidyl-H), 7.10 (t, 1H, Ar-H), 6.78 (d, 1H, Ar-H), 6.62 (d, 1H, Ar-H), 6.38 (d, 1H, Ar-H), 6.30 (dd, 1H, Ar-H), 6.25 (bs, 1H, Ar-H), 6.08 (d, 1H, Ar-H), 3.83 (s, 3H, OCH_3), 3.54 (t, 2H, CH_2), 3.25 (t, 2H,

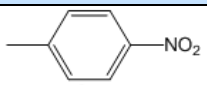
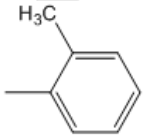
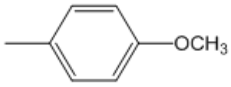
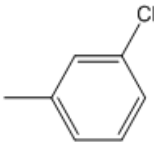
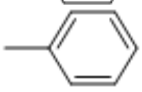
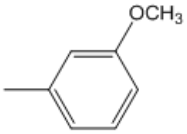
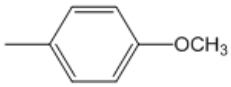
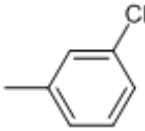
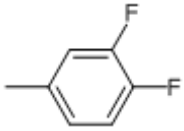
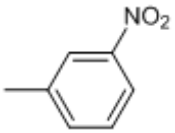
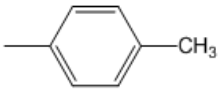
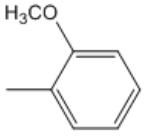
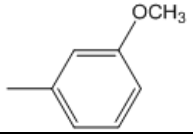
CH_2), 2.79-2.89 (bs, 1H, CH), 2.38 (t, 2H, CH_2), 2.15 (t, 2H, CH_2), 2.01 (s, 3H, CH_3). MS (ESI) m/z: 554.69 (100.0%), Anal. calcd. for $C_{30}H_{31}N_7O_2S$ (in %): C-65.08, H-5.64, N-17.71. Found: C-65.03, H-5.60, N-17.73.



Scheme 1

Reagents and Conditions: (i) 100 ^{0}C (ii) NaOH, *n*-butanol, 110 ^{0}C (iii) $SnCl_2 \cdot 2H_2O$, hydrochloric acid, 0 ^{0}C-rt (iv) IBCF, NMP, DMF (v) MDC, TEA, 0 ^{0}C-r.t. **9a** = 4-nitrobenzene sulphonyl chloride; **9b** = 2-methylbenzene sulphonyl chloride; **9c** = 4-methoxybenzene sulphonyl chloride; **9d** = 3-chlorobenzene sulphonyl chloride; (vi) MDC, TEA, 0 ^{0}C-r.t. **11a** = benzyl chloride; **11b** = 3-methoxybenzyl chloride; **11c** = 4-methoxybenzyl chloride; **11d** = 3-methylbenzyl chloride; **11e** = 3,4-difluorobenzyl chloride; **11f** = 3-nitrobenzyl chloride; (vii) MDC, TEA, 0 ^{0}C-r.t. **13a** = 4-methylphenyl isothiocyanate; **13b** = 3-methoxyphenyl isothiocyanate; **13c** = 2-methoxyphenyl isothiocyanate.

Table 1 Chemical structure, yield, melting point and IC_{50} values of the synthesized compounds **10(a–d), 12(a-f)** and **14(a–c)** as determined based on MTT assay

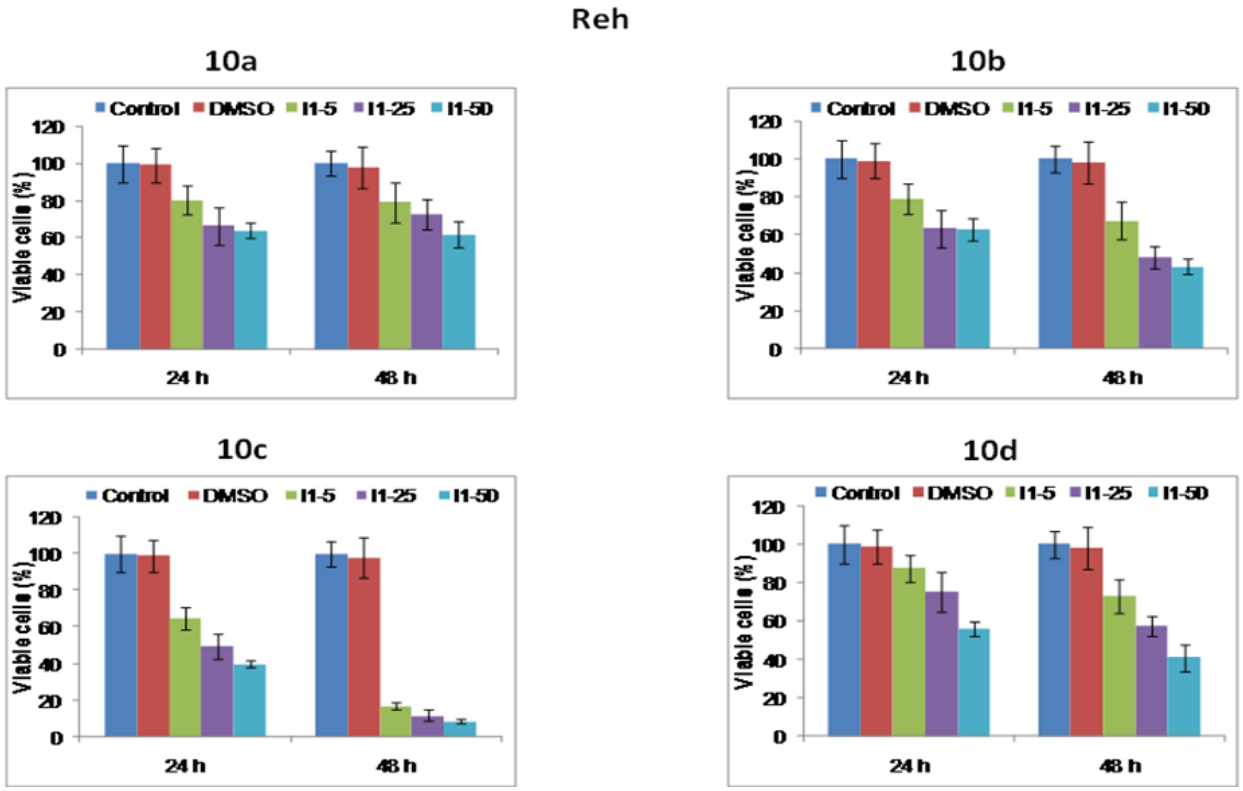
Compound	R1/R2/R3	Yield	MP (°C)	IC_{50} at 48 h (K562)	IC_{50} at 48 h (Reh)
10a		84	124	>50	>50
10b		79	135	48.2	24.1
10c		76	134	4.1	2.3
10d		85	145	>50	35.2
12a		82	138	>50	>50
12b		84	146	17.1	11.2
12c		81	149	21.4	8.1
12d		77	136	3.2	3.3
12e		79	145	19.4	10.1
12f		82	142	48.7	33.3
14a		86	143	5.3	4.1
14b		78	135	18.3	8.4
14c		77	136	14.1	9.2

***In vitro* anti-proliferative activity**

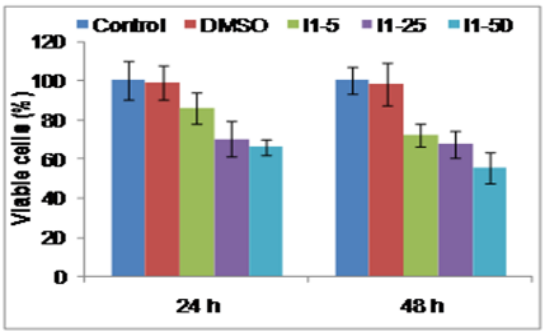
Cell lines and culture Human cell line, **K562** (chronic myelogenous leukemia) was purchased from National Center for Cell Science, Pune, India and **Reh** was a kind gift from Michael Lieber, USA. Cells were grown in RPMI 1640 containing 10% FBS, 100 U of Penicillin G/mL, and 100 µg of streptomycin/mL at 37 ^{0}C in a humidified atmosphere containing 5% CO_2.

MTT assay

The effect of piperidine-4-carboxamide derivatives **10(a-d), 12(a-f)** and **14(a-c)** on the proliferation of leukemia cells **(K562** and **Reh)** was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet formazan. Exponentially growing **K562** or **Reh** cells (1 x 10^4 cells /well) were plated in triplicates and incubated with 5, 20, and 50 µM of **10(a-d), 12(a-f)** and **14(a-c).** Cells were harvested after 24 and 48 h of treatment and incubated with MTT (5 mg/mL) at 37 ^{0}C. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of *N*,*N*-dimethylformamide and 10% of sodium dodecyl sulfate). Absorbance was measured at 570 nm using ELISA plate reader. The mean absorbance of culture medium was used as the blank and was subtracted. IC_{50} values (concentration of compound causing 50% inhibition of cell growth) were estimated after 48 h of compound treatment. The absorbance of vehicle cells was taken as 100% viability, and the values of treated cells were calculated as a percentage of control and presented as histograms (**Figures 1** and **2**).



12a



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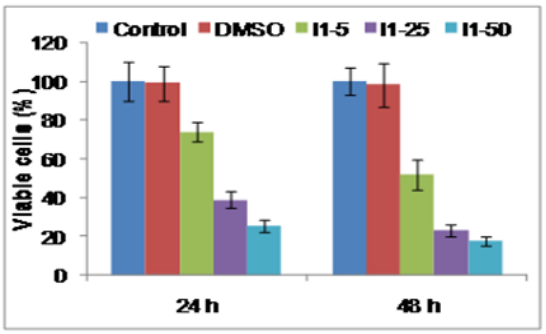

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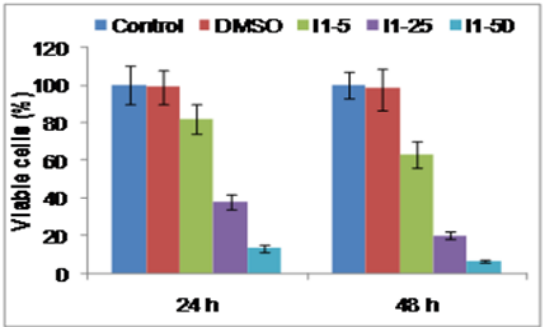

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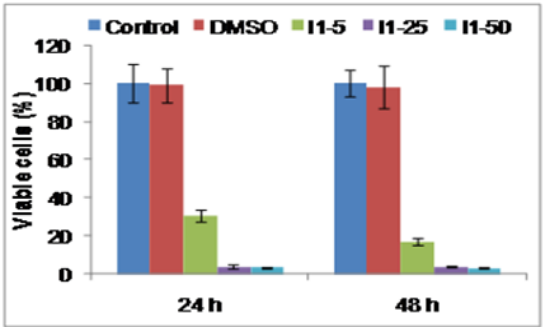

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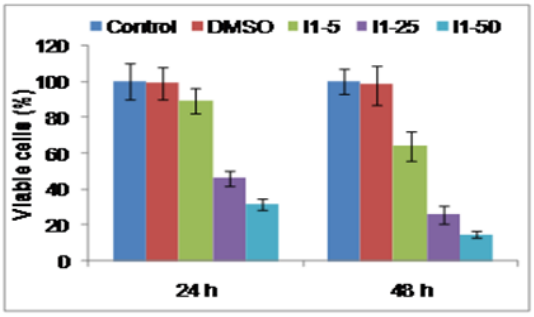

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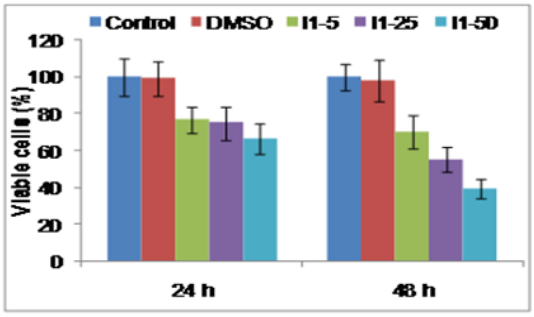

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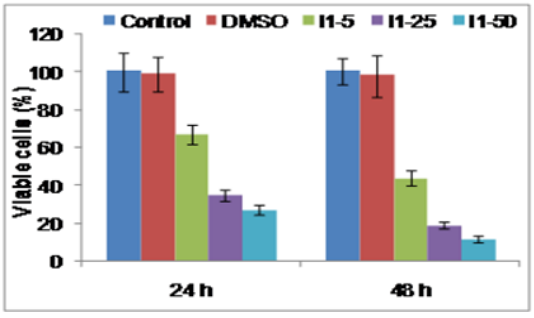

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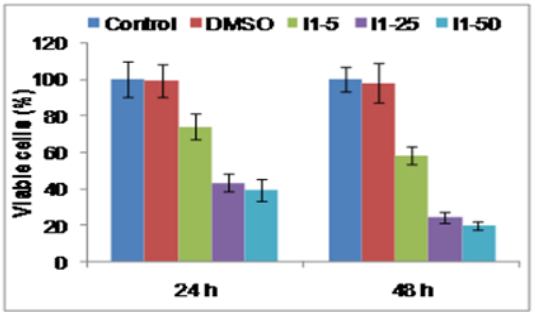


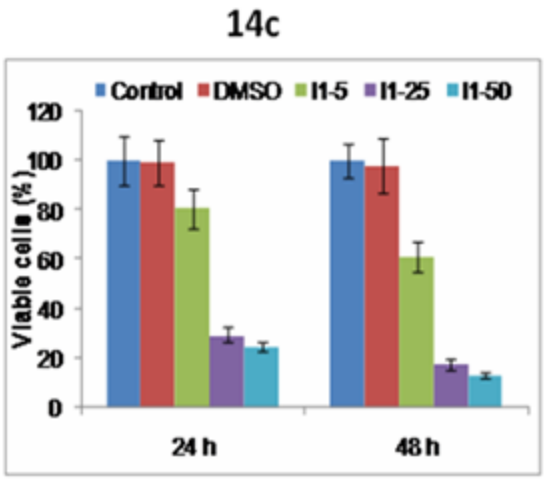
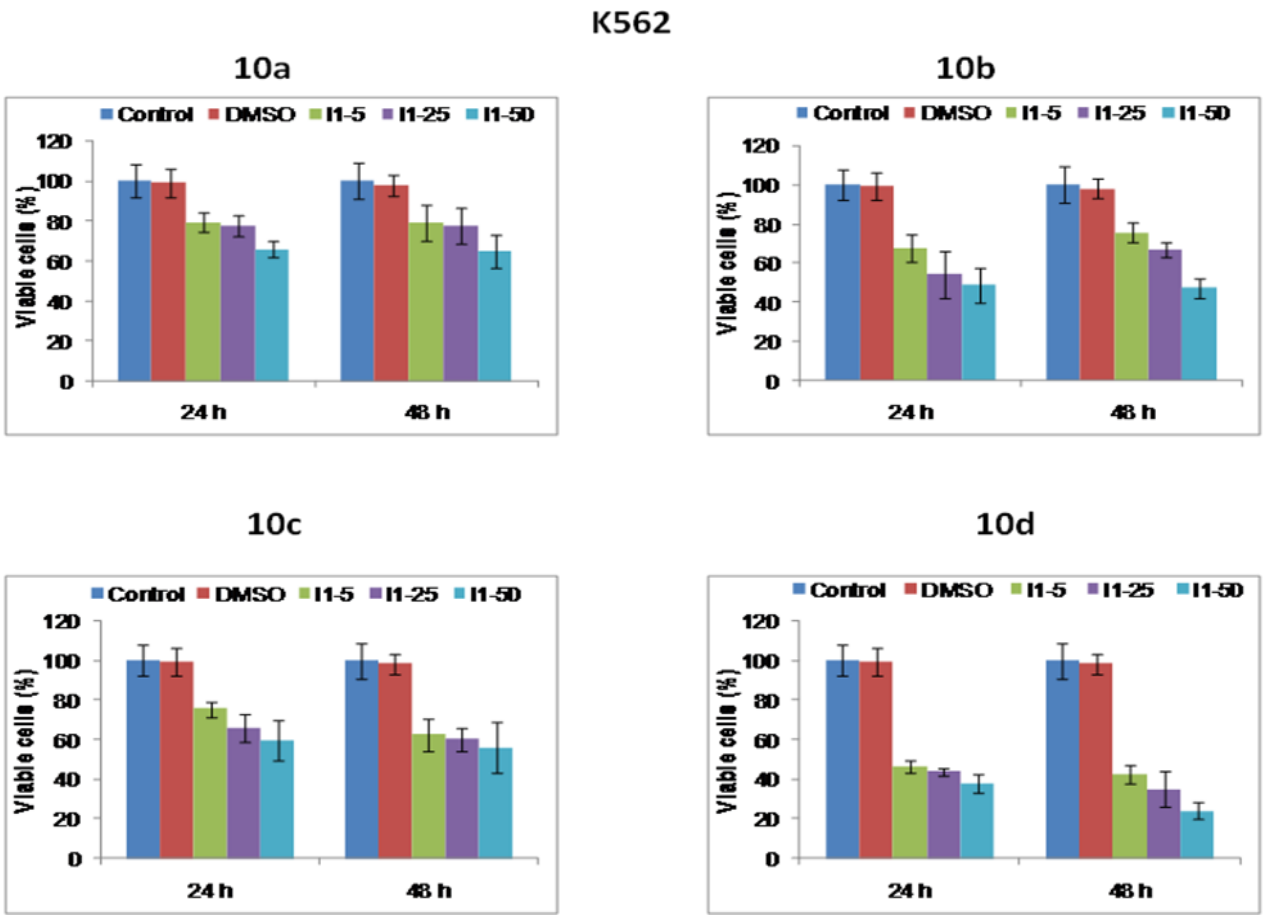


Figure 1 Determination of the effect of piperidine-4-carboxamide derivatives **10(a-d), 12(a-f)** and **14(a-c)** on cell proliferation of Reh by MTT assay. After 24 and 48 h of exposure, Reh cells with **10(a-d), 12(a-f)** and **14(a-c)** (5, 25 and 50 µM) were incubated with MTT (5 mg / mL) in duplicates, and resulting blue formazan precipitate was dissolved in detergent, and absorbance was measured at 570 nm. Results are presented as percentage of viable cells (the cell viability of vehicle cells were considered as 100%)



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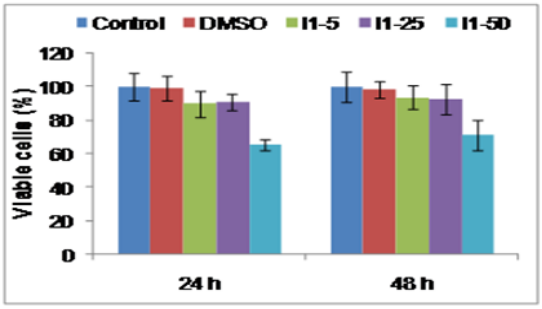

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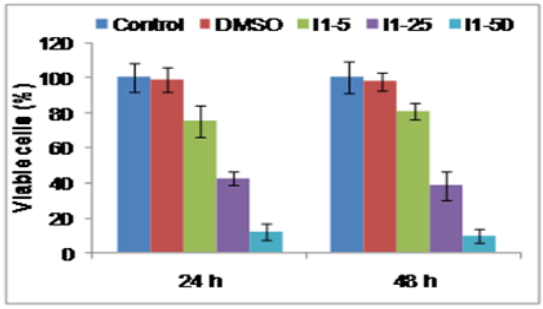
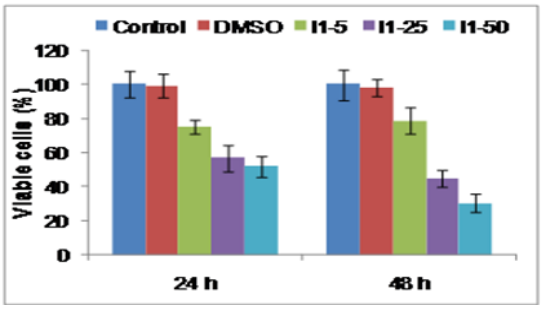


12c



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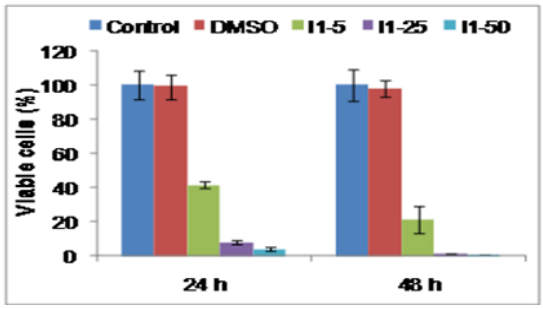

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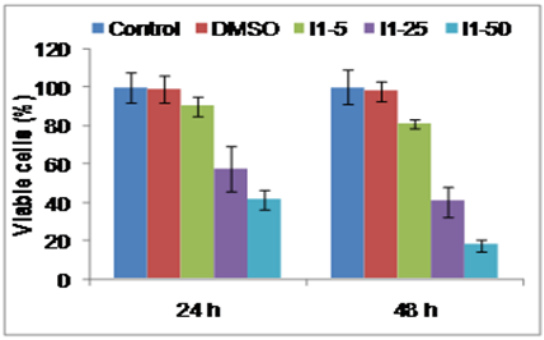

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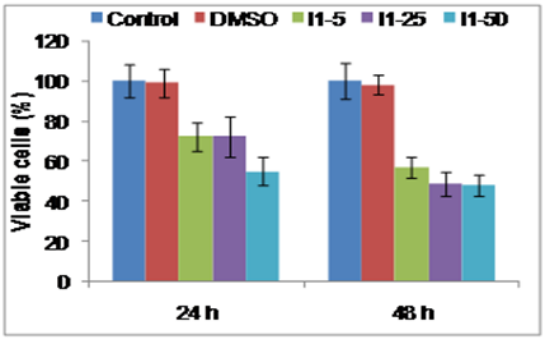
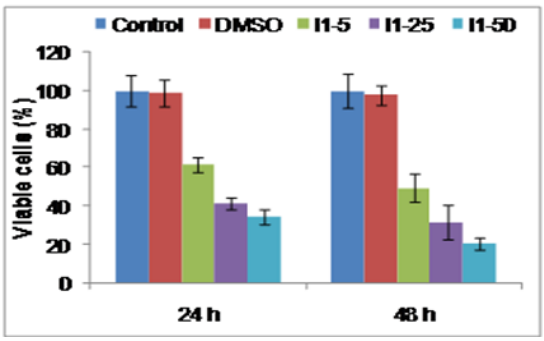


14a



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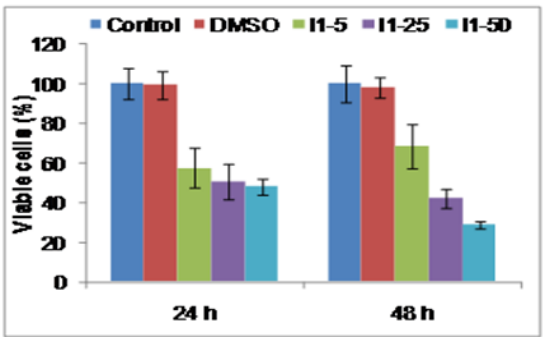

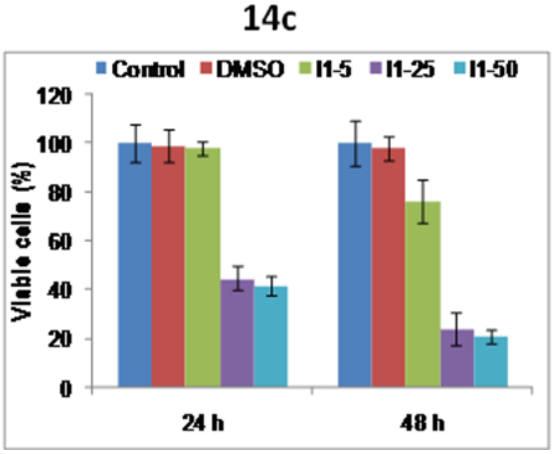

Figure 2 Determination of the effect of piperidine-4-carboxamide derivatives **10(a-d), 12(a-f)** and **14(a-c)** on cell proliferation of K562 by MTT assay. After 24 and 48 h of exposure, K562 cells with **10(a-d), 12(a-f)** and **14(a-c)** (5, 25 and 50 µM) were incubated with MTT (5 mg / mL) in duplicates, and resulting blue formazan precipitate was dissolved in detergent, and absorbance was measured at 570 nm. Results are presented as percentage of viable cells (the cell viability of vehicle cells were considered as 100%)

Results and discussion

Chemistry

Synthesis of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide

Synthesis of the key intermediate *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (**8**) is outlined in **Scheme 1**. To prepare the pyrimidine ring system, the general method was used [32], which involved reacting 3-acetylpyridine (**1**) with *N*,*N*-dimethylformamide dimethyl acetal (**2**) to give the 3-dimethylamino-1-(pyridin-3-yl)prop-2-en-1-one (**3**) in 90% yield. The enaminone **3** reacts with 1-(2-methyl-5-nitrophenyl)guanidine (**4**) in presence of base to give *N*-(2-methyl-5-nitrophenyl)-4-(pyridin-3-yl)pyrimidin-2-amine (**5**). Reduction of compound (**5**) with $SnCl_2 \cdot 2H_2O$ afforded 6-methyl-N^1-(4-pyridin-3-yl-pyrimidin-2-yl)benzene-1,3-diamine (**6**) in 75% yield. 6-Methyl-N^1-(4-(pyridin-3-yl)pyrimidin-2-yl)benzene-1,3-diamine (**6**) (1.0 eq) and piperidine-4-carboxylic acid (**7**) (1.0 eq) in *N*,*N*-dimethyl formamide in the presence of base *N*-methyl morpholine, isobutyl chloroformate, and reaction mixture was stirred for 5-6 h at room temperature, which gave target key intermediate **8**. The absence of –COOH proton peak and presence of –NH proton peak confirmed the formation of compound **8** with a good yield 90%.

The nucleophilic substitution reaction of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide (**8**) with different substituted aromatic sulfonyl chlorides **9(a–d)** (R–SO_2Cl)/benzyl chlorides **11(a–f)** (R–CH_2-Cl)/aromatic isothiocyanates **13(a–c)** (R-N=C=S) was carried out in the presence of triethylamine and dichloromethane as solvent with a good yield of 74–80%. The absence of –NH and presence of –CS–NH proton peak in synthesized derivatives **10(a-d), 12(a-f)** and **14(a-c)** in ^{1}H NMR spectra confirmed the identity of the products. It is also confirmed by IR data, for sulfonamide series **10(a-d)** which showed asymmetric stretching frequency of O=S=O in the range 1350-1370 cm^{-1} and symmetric stretching frequency at 1270–1290 cm^{-1}. For carboxamide series **12(a-f)**, IR data showed stretching frequency of –C=O at 1630–1670 cm^{-1} and similarly for **14(a-c)**, stretching frequency at 3350–3360 cm^{-1} for –NH and 1640–1660 cm^{-1} for –C=O group. The chemical structures of all the synthesized compounds are given in **Table 1**.

Biology

Some of the derivatives of pyrimidines showed potent antiproliferative activity against two human cancer cell lines (A549 and HL60) [33]. Some representatives of pyrrolo[2,3-d]pyrimidines have been investigated for antiproliferative activity against human melanoma cell line A375 [34]. B. Singh et al., tested for antiproliferative activity of thiazolo[5,4-*d*]pyrimidines against 8 cancer cell lines [35]. In view of the above findings, to investigate the cytotoxic effect of compounds **10(a-d), 12(a-f)** and **14(a-c)**, we used MTT assay against human leukemia cells. For this K562 and Reh cells growing in the log phase were treated with different concentrations (5, 25 and 50 µM) of *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide derivatives **10(a-d), 12(a-f)** and **14(a-c)**. To rule out the effect of solvent on the cell growth, negative control tests were performed using DMSO at the concentration used for highest concentration of the compound tested. The effects of the compounds were expressed as percentage of viable cells. IC_{50} value was determined for 48 h, and the results are tabulated in **Table 1**. Interestingly, our preliminary results showed that *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide derivatives **10(a-d), 12(a-f)** and **14(a-c)** are more sensitive towards acute lymphocytic leukemia cells (Reh) compared to chronic myelogenous leukemia cells (K562). Studies are required to understand the underlying mechanism of sensitivity towards Reh cells. As shown in **Table 1**, most of the compounds inhibited the cell growth with less than 10 µM concentration in Reh cells.

From the **Figures 1** and **2**, it was found that incorporation of 4-methoxyphenyl **(10c)**, 3-methylphenyl **(12d)** and 4-methylphenyl **(14a)** resulted interesting inhibition with IC_{50} values of 4.1, 3.2 and 5.3 µM, respectively, for K562 cells and 2.3, 3.3 and 4.1 µM, respectively, for Reh cells. Within the few variations considered in this study, the presence of electron-releasing groups afforded a clear beneficial effect with regard to antiproliferative properties. However, the replacement of methoxy with a nitro group **(10c** and **10a)** resulted in twelve- and twenty five fold reduced activity with IC_{50} values >50 and >50 µM, respectively. Among benzyl derivatives **12(a-f)** compounds, **12d** showed good inhibition with IC_{50} value of 3.2 and 3.3 µM, against K562 and Reh cells respectively. The remaining compounds in the series, **12a** and **12f** showed seventeen and sixteen fold less inhibitory activity. The inhibition by compound **10c, 12d** and **14a** could be attributed to the electron-releasing methoxy and methyl groups present on the substituted phenyl ring at the para and meta positions, respectively. Compounds **10c, 12d** and **14a** having electron releasing groups enhances the activity, whereas **10a, 10d, 12e** and **12f** having electron withdrawing groups (NO_2, Cl, F) decreases the activity. Interestingly, compounds **10b, 12b, 12c, 14b** and **14c** having electron releasing methyl (ortho), methoxy (ortho, meta, para) groups, respectively showed less inhibitory activity.

We have briefly investigated the importance of *N*-terminal functional group (sulfonamide, benzylamine, thioamide) in the *N*-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)piperidine-4-carboxamide derivatives, first with different groups added on the phenyl ring. These modifications results in change in antiproliferative activity profile of the synthesized compounds. In this contrast, changing the substituent on the phenyl ring in the same position in **(10c, 12c** and **14a)** and **(10d, 12b, 12d, 12f** and **14c)** reveals that *N*-terminal functional group is not affects the activity. Secondly, by comparing the substituent on phenyl ring **12(b-f)** with without substitution on the phenyl ring **(12a)**, reveals that the substituent on the substituted phenyl ring plays a key role in the activity. Finally, from the above studies, it reveals that the substitution at *N*-terminal of the phenyl ring play a key role in its antiproliferative activity.

Conclusion

Our study demonstrates that the title compounds showed potent antileukemic properties against human leukemia cells. Interestingly from the cytotoxic assays we noted that the compounds with electron releasing groups at *N*-terminal functional of the phenyl ring resulted in an increase in the activity by inducing cell death. They exhibit IC_{50} values ranging from 2.3 µM to >50 µM. Further studies are required to know the mechanism of action of these molecules. These studies are currently under progress.

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