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Novel Thiadiazole Derivatives as Bcr-Abl Tyrosine Kinase Inhibitors

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ABSTRACT

The present work mainly aims to discover novel small molecular inhibitors against important molecular target T3151 Abl mutant involved in leukemia. Five heterocyclic compounds **1-5** with N and S atoms (thiosemicarbazone, thiadiazole and thiazolidinoyl derivatives) were synthesised and characterised using spectral data. Docking study was carried out for **1-5** against the T315I Bcr-Abl mutant. The compounds **3-5** with phenothiazine pharmacophore showed promising docking score than with the derivatives having the coumarin pharmacophore **1** and **2.** So the compounds **3-5** were tested for their anticancer activity against leukemic K562 cell line by trypan blue, MTT and LDH assays. Compound **5** showed marked anticancer activity and exhibited an IC_{50} value of 11.12 and 50.66 µg/ml against trypan blue and MTT assay respectively. Further a dose-dependent increase in LDH release was observed, confirming the antiproliferative potential of the compounds.

INTRODUCTION

In the post-genomic period, rational anti-cancer drug discovery aims to discover small molecules that change the activity of key therapeutic targets responsible for carcinogenesis (William and Kaelin 1999). Computer- aided or *in silico* design is being used to expedite and facilitate the lead molecule identification (Ali Muhammad *et al.,* 2015). It reduces the volume of chemical space and allows to focus on more promising candidates for lead discovery and optimization (Kapetanovic, 2008). Chronic myelogenous leukemia (CML) is a hematological stem cell disorder caused by deregulated growth of myeloid cells in the bone marrow and the accumulation of excessive white blood cells. Abelson tyrosine kinase (Abl) is involved in cell growth and proliferation and is usually under tight control (Noronha *et al.,* 2008). However, a large number of CML patients have the Philadelphia chromosome in which ABL gene from chromosome 9 fused with the breakpoint cluster (BCR) gene from chromosome 22. This Philadelphia chromosome is responsible for the production of Bcr-Abl, a constitutively active tyrosine kinase that causes uncontrolled cellular proliferation. Imatinib is an Abl inhibitor and is currently used as first line therapy (Desogus *et al.,* 2015; Mughal *et al.,* 2013). However, during the long term treatment with imatinib a high percentage of clinical relapse has been observed (Noronha *et al.,* 2008). A majority of these relapsed patients has several point mutations in and around the ATP binding pocket of the ABL kinase domain in Bcr-Abel (Michele *et al.,* 2007; Jorge *et al.,* 2011). In order to address the resistance of mutated Bcr-Abl to imatinib, 2(nd) generation inhibitors like dasatinib, and nilotinib were developed and used for the treatment of CML patients who are resistant to imatinib (Karthigai Priya *et al.,* 2015; Muhammad *et al.,* 2015; 2016; Noronha *et al.,* 2008). All of the Bcr-Abl mutants are inhibited by the 2(nd) generation inhibitors with the exception of the T315I mutant. Several 3(rd) generation inhibitors are currently in progress to target the T315I mutation. 1,3,4-thiadiazolines nucleus is a biologically active heterocyclic ring, which is associated with a wide range of pharmacological activities (Katritzky and Rees, 1981). 1,3,4-thiadiazolines/thiadiazoles are shown to exhibit antibacterial, diuretic, antifungal, anti-inflammatory, herbicidal and antiviral activities.

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In our previous studies, we have reported phenothiazine derivatives as Bcr-Abl inhibitors Karthigai Priya *et al.,* 2015, Ali Muhammad *et al.,* 2015). Now in the present work we used thiadiazole derivatives as anticancer agents for leukemia. A series of substituted 1,3,4-thiadiazoles has been reported as potent Abl tyrosine kinase inhibitors (Radi *et al.,* 2008).

If the present work is encouraging, it is anticipated that people will have additional drugs for the treatment of patients with the mutated Bcr-Abl-T315I. The success of these inhibitors will have a greater implication not only in CML, but also other diseases caused due to kinases where the mutated gatekeeper residue plays a major role.

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EXPERIMENTAL

General

All compounds synthesized were characterized by IR, ^{1}H-NMR and ^{13}C-NMR elemental analyses and are described in the experimental section. Melting points were determined in a BUNA melting point instrument and are uncorrected. IR spectra were recorded on a Shimadzu Affinity-I FT-IR spectrometer. NMR spectra were measured at 400 MHz on a Bruker-400 spectrometer using TMS as internal standard and DMSO-d_6 as solvent.

Elemental analyses were determined using a Perkin Elmer 240C Elemental Analyzer. 2-acetyl phenothiazine has been purchased from Sigma Aldrich and all the chemicals used in this study were of analytical grade and purchased from M/s High Media, India. The parameters of the Lipinski's rule were determined using DruLi software.

General method of preparation of thiosemicarbazone 1 and 3

To a solution of 3-acetyl coumarin or 2-acetyl phenothiazine (0.001 mol) and thiosemicarbazide (0.001 mol) in methanol (40ml), few drops of concentrated hydrochloric acid was added and the reaction mixture was heated under reflux for 2 h. The reaction mixture was cooled and then it was poured into crushed ice. The separated solid was filtered, dried but could not be induced to crystallize. So it was purified by column chromatography using silica gel (60-120 mesh) with benzene-ethyl acetate mixture 9.5:0.5) as eluent to yield **1** and **3** respectively.

Preparation of 1, 3, 4-thiadiazole 2 and 4

Thiosemicarbazone **1** or **3** (0.025 mol) was treated with freshly distilled acetic anhydride and the mixture was refluxed for 3 h in a water bath. The removal of solvent from the cooled reaction mixture in vacuum affords compounds **2** and **4** respectively**.** The compounds were recrystallised from ethanol.

(*E*)-2-((Z)-(1-(10H-phenothiazin-2-yl)ethylidene)hydrazono)thiazolidin-4-one 5

A mixture of **3** (0.005 mol), ethyl chloroacetate (0.005 mol) in ethanol and anhydrous sodium acetate (0.01 mol) was heated under reflux for 6 h. After cooling, the reaction mixture was poured into ice water. The product was filtered and dried. The compound was recrystallised from ethanol to yield **5.**

2-(1-(2-oxo-2H-chromen-3-yl)ethylidene) hydrazinecarbothioamide (1)

Yield 80%. m. p. 148 ^{o}C. IR (KBr): 3155, 3236, 3390, 1720, 1604, 1072 cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.40 (br.s, 1H), 8.41 (m, 1H), 8.35 (br.s, 1H), 7.95 (br.s,1H), 7.76 (m, 1H), 7.61(m, 1H), 7.36 (m, 2H), 2.24 (s, 3H). ^{13}C-NMR: (100 MHz, DMSO-d_6): δ 179.17(C=S), 159.10 (C=O), 153.29(C=N), 146.02, 141.95, 132.40, 129.09, 125.71, 124.74, 118.82, 115.90, 15.92(CH_3). m/z: 261.12, Anal. Calc. C, 55.16; H, 4.24; N, 16.08; found C, 54.33; H, 4.64; N, 17.68.

N-(4-acetyl-5-methyl-5-(2-oxo-2H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2yl)acetamide (2)

Yield 78%. m.p. 178 ^{o}C. IR (KBr, cm^{-1}): 3250, 1720, 1620. ^{1}H-NMR ((DMSO-d_6 400 MHz): δ 11.63 (br.s, NH), 7.95 (m, 1H), 7.84 (m, 1H), 7.62 (m, 1H), 7.38 (m, 2H), 2.42 (s,3H), 2.17 (s,3H), 2.24 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 169.26, 167.64, 143.27, 143.13, 126.71, 125.06, 123.44, 121.27, 119.99, 119.72, 78.93, 26.82, 23.67, 22.41. m/z: 345.1 . Anal. Calc. C, 55.64; H, 4.38; N, 12.17; found C, 56.24; H, 5.33; N, 13.44.

2-(1-(10H-phenothiazin-2-yl)ethylidene) hydrazinecarbothioamide (3)

yield: 83% m.p. 173 ^{o}C. IR (KBr, cm^{-1}): 3460, 3338, 3280, 3167, 1571, 1452, 1274, 740. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.16(d,*J*=2Hz, 1H, H-1), 6.65(dd, *J*= 1.2 &8Hz, 1H, H-3), 6.89, (d, *J*= 8Hz, 1H, H-4), 6.91, (d, *J*= 8Hz, 1H, H-6), 6.74 (dt, *J*= 8 & 1.6Hz, 1H, H-7), 6.98(dt, *J*= 8& 1.4Hz, 1H, H-8), 7.30 (dd, *J*=1.2 & 8 Hz, 1H, H-9), 2.22(s, 3H, CH_3),10.24 (br.S, NH), 7.67 (br.S, NH), 8.35 (br.S, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ118.27(C-3), 120.20(C-1), 147.13(C-2), 127.66(C-4),115.86(C-4a), 114.70(C-5a), 136.95(C-6), 111.84(C-7), 125.83(C-8), 126.20(C-9), 141.97(C-9a), 141.72(C-10a), 13.75(CH_3). m/z: 314.07. Anal. Calc. C, 57.30; H, 4.49; N, 17.82; found C, 56.80; H, 5.06; N, 16.98.

N-(4-acetyl-5-methyl-5-(10H-phenothiazin-2-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (4)

yield: 79% m.p. 178 ^{o}C IR(KBr, cm^{-1}): 3437, 2953, 1700, 1625, 1392, 1294, 1246, 731. ^{1}H-NMR (400 MHz, DMSO-d_6): δ: 11.66 (br.s,1H, NH), 7.63 (m, 1H, H-1), 7.61 (m, 1H, H-8), 7.55(m, 1H, H-9), 7.50 (d, *J*=8Hz, 1H, H-4), 7.37 (m, 1H, H-7), 7.30 (d, *J* =8Hz, 1H, H-2), 7.28 (m, 1H, H-6), 2.27 (s, 3H,CH_3). 2.19 (s, 3H,CH_3) 2.0 (s, 3H, CH_3), ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.71 (C=N), 143.29 (C-2), 138.56 (C-9a), 138.42 (C-10a), 131.58 (C-4), 131.08 (C-6), 127.73 (C-8), 127.53 (C-9), 126.92 (C-1), 123.90 (C-5a), 123.75 (C-3), 123.30 (C-4a), 111.98 (C-7), 77.95 (SCN). 26.47 ($COCH_3$), 23.52 ($COCH_3$), 22.40 (CH_3), m/z: 398.09. Anal. Calc. C, 57.27; H, 4.55; N, 14.06; found C, 56.97; H, 4.35; N, 15.13.

(*E*)-2-((Z)-(1-(10H-phenothiazin-2-yl)ethylidene)hydrazono)thiazolidin-4-one (5)

m.p. 208 ^{o}C Yield: 76% IR (KBr,cm^{-1}): 3446, 2994, 1707, 1621, 1575, 1436, 1313, 1246, 1026, 742, 638, 521. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.71(br.s, 1H, NH), 7.20 (m, 1H, H-1), 7.18(m, 1H, H-9), 6.99 (m, 1H, H-4), 6.89(m, 1H, H-8), 6.75 (d, *J*=8 Hz, 1H, H-7), 6.69 (d, *J*= 8Hz, 1H, H-2), 2.28(s, 3H, CH3), 3.84 (br.s, 2H, CH_2). ^{13}C-NMR (400 MHz, DMSO-d_6): δ 174.47 (C=O), 164.00 (C=N), 159.17 (C=N), 149.00 (C-2), 141.77 (C-10a), 141.65 (C-9a), 137.20 (C-6), 127.66 (C-4), 126.71 (C-9), 125.91 (C-8), 120.11(C-1), 118.66(C-3), 115.81 (C-5a), 114.49 (C-4a), 111.84 (C-7), 14.40 (CH_3), 32.91 (-S-CH_2), m/z: 398.09. Anal. Calc. C, 57.61; H, 3.98; N, 15.81; found C, 58.21; H, 4.17; N, 15.21.

Molecular docking

Crystal structure of T315I mutant Bcr-Abl kinase (pdb entry: 2V7A) containing ATP as a ligand was used in modeling experiments without further processing. Compound **1-5** were constructed on ChemDraw 8.0. Structures were copied to Discovery Studio v. 2.5.5 and saved as Mol file. The structure was imported to Autodock and subjected to geometry optimization by running energy minimization. Docking was carried out with Autodock 4.2. Version and the visualization of docked result carried using chimera 1.10 and Discoverystudio 4.5 (Trott and Olson, 2010)

Determination of *In vitro* antiproliferative effect of the compounds on cultured K562 cells

K562 cells were purchased from NCCS Pune was maintained in RPMI-1640 (life) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO_2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO_2 incubator. Extracts were added to grown cells at concentrations of 10, 50, 100 and 200 µg/ml from a stock of 1mg/ml 0.1% DMSO and incubated for 24 hours. The % Difference in viability was determined by standard MTT assay after 24 hours of incubation.

Trypan Blue Exclusion Assay

Trypan blue is a vital stain used to selectively colour dead tissues or cells blue. The live cells possess intact cell membranes that exclude trypan blue, whereas dead cells do not. So a viablecell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. A 1:1 dilution of the culture cell suspension with a 0.4% trypan blue solution (1;1 dilution in PBS) was charged on the counting chamber of a heamocytometer and counted at 40x (Olympus CH 20).

Stained cells and total cells were counted to percentage of viable cells was calculated. Control was also treated in the same manner (Korzeniewski and Callewaert 2000).

MTT cell viability assay

Determination of *In vitro* antiproliferative effect of the compounds on cultured K562 cells

K562 cells were purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37 °C in 5 % CO_2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO_2 incubator. Extracts were added to grown cells at concentrations of 10, 50, 100 and 200 µg/ml from a stock of 1mg/ml 0.1% DMSO and incubated for 24 hours. The % Difference in viability was determined by standard MTT assay after 24 hours of incubation (Masters, 2000, Muhammad *et al*., 2015; 2016).

MTT assay

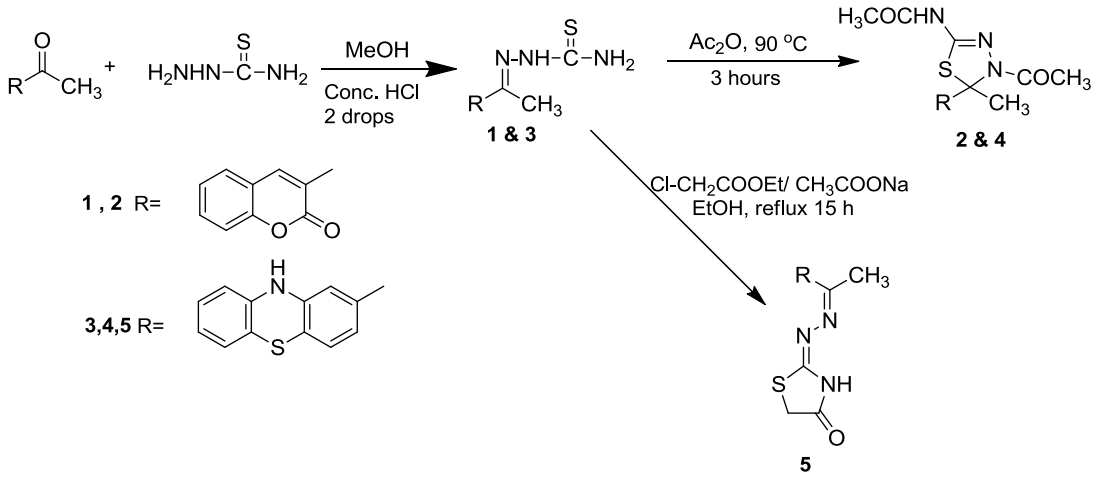
MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (5 mg/ml dissolved in PBS). It was then incubated at 37 ^{o}C for 3 hours.MTT was removed by washing with 1x PBS and 200µl of DMSO was added to the culture. Incubation was done at room temperature for 30 min until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in an ELISA reader (LISASCAN, Erba) (Mosmann, 1989, Arung *et al*., 2000).

Lactate Dehydrogenase Leakage Assay

Lactate dehydrogenase (LDH) release is an indicator of membrane integrity and thus resulting in cell injury. LDH assay was performed to evaluate the LDH release to the media following treatment with the **3, 4** and **5** (10, 50, 100 and 200 µg/ml) on K562 for 24 h and it was measured using standard protocols. The intracellular LDH was determined after lysing the cells by rapid freezing and thawing in liquid nitrogen. The LDH release was measured at an absorbance of 340 nm.

The percentage of LDH release was calculated as: (LDH activity in media) / (LDH activity in media þ intracellular LDH activity) X100%. Results are presented as percentage of LDH release subtracting the control values from treated ones. All the experiments were performed in triplicate and yielded similar results (Korzeniewski and Callewaert 2000).



Scheme 1: Synthesis of compounds **1-5.**

RESULT AND DISCUSSION

3-acetyl coumarin is treated with thiosemicarbazide to form the thiocarbazone **1** which further refluxed with acetic anhydride to form the thiadiazole **2**. The same procedure was followed for the preparation of the **3** and **4.** Compound **3** on refluxing with ethylchloroacetate in presence of anhydrous sodium acetate to form **5** (**Scheme 1**). All the compounds were characterised by their IR, NMR and mass spectral data. Molecular docking studies were carried out with the synthesized compounds **1-5** against cancer target proteins T5131 Bcr-Abl mutant aura kinase. The drug likeliness of the molecules was analyzed by Lipinski's rule. The molecules with good scoring function were further studied with *in vitro* studies against K562 cell lines using Trypan blue, MTT and LDH assay.

Characterisation of the compounds 1-5

The IR spectrum of **1** showed characteristic bands at 3155, 3236 and 3390 cm^{-1} due to NH and NH_2 groups at 1720 cm^{-1} is due to coumarin ring carbonyl at 1604 cm^{-1} due to the >C=N and at 1072 cm^{-1} for >C=S group.

In the ^{1}H NMR spectrum **t**he methyl protons appeared as a singlet δ 2.24 ppm. Three singlets at δ 10.40, 8.35 and 7.95 are observed for the NH and NH_2 protons. The bunches of signals between δ 7.36 - 8.41 are due to aromatic protons. The ^{13}C NMR spectrum showed the presence of aromatic carbons could be readily distinguished by their characteristic absorption around δ 120. The signals at δ 179.17, 159.10 and 153.29 are due to >C=S, >C=O and >C=N carbons. The upfield signal at δ 15.92 is due to methyl carbon.

In the IR spectrum of **2** the stretching frequency at 1720 and 1620 cm^{-1} are due to the carbonyl and amide carbonyl groups respectively. The bands at 3250 cm^{-1} is due to the NH group. In the ^{1}H NMR spectrum a multiplets appeared between δ 7.38-7.84 for four protons revealed the presence of aromatic protons. The sharp singlet at δ 7.95 is assigned for the (H-4) proton. The sharp singlet's at δ 2.24, 2.17 and 2.03 each with three protons are assigned for two amide methyl protons and one methyl protons, respectively. Further a singlet at δ 11.63 is assigned to NH proton. ^{13}C NMR spectrum revealed the presence of carbons around δ 120. The signals at δ169.26 and 167.64 are due to the secondary amide carbonyl and tertiary amide carbonyl respectively. The upfield signals at δ 26.82, 23.67 and 22.41 are due to secondary amide methyl, tertiary amide methyl and methyl carbon atoms respectively.

IR spectrum of **3** it exhibited absorption bands at 3167, 3280, 3368, 3460 cm^{-1} for N-H stretching and at 1600 and 1571 cm^{-1} for C=C and C=N groups respectively. The methyl group appeared as a singlet at δ 2.22. The aromatic protons of the phenothiazine nucleus appeared at δ 7.16, 6.65, 6.89, 6.91, 6.74, 6.98 and 7.30 each integrated for one proton. The NH protons appeared between δ 7.67 and 10.24. In the ^{13}C-NMR the signal at δ13.75 is due to methyl carbon, at 178.95 is due to C=S and at δ 147.13 is due to C=N. The rest of the signals between δ111.84 and 141.72 were attributed to the phenothiazine ring carbon atoms

In **4** the IR spectrum exhibited a broad bond at 3437cm^{-1} for NH group,1700cm^{-1} for C=O group and 1625 cm^{-1} for C=C. In the ^{1}HNMR methyl proton appeared as a singlet at δ 2.0.The singlets at δ2.19 and 2.27 were attributed to acetyl protons ($COCH_3$).The broad singlet at δ 11.66 is due to NH proton. The signals at δ 7.63,7.30,7.50,7.28,7.61,7.55 are due to phenothiazine ring protons.In the ^{13} C NMR the signals at δ 22.40 is due to methyl carbon, at δ 23.52 due to –$COCH_3$ methyl carbon. The signals at δ 168.41 and 161.42 were attributed to acyl carbonyl carbon and at δ 167.71 to C=N.The remaining carbon signals at δ 111.98 and 138.56 due to phenothiazine ring system. The ipso carbonsignal appeared at δ 77.95.

In compound **5** the IR spectrum exhibited bonds at δ 3446cm^{-1} for NH group,1707 cm^{-1} for C=O group and at 1622cm^{-1} for C=C group.^{1}H NMR exhibited signals at δ 2.28 for methyl group, δ 3.84 for S-CH_2 protons at δ 8.71 for NH protons. The phenothiazine ring proton appeared between δ 6.69 and 7.20.In the ^{13}C-NMR is exhibited signals at δ 14.90 for methyl carbon and at δ 32.91 for S-CH_2 carbon. The carbonyl carbon appeared at δ 174.47

and the C=N carbons appeared at δ 159.17 and 164.00.The phenothiazine carbon signals appeared between δ 111.64 and 141.77.

Molecular Docking studies

The efforts made in the recent years in the field of medicinal chemistry to find new Bcr-Abl tyrosine kinase inhibitors resulted in the design and synthesis of new generation compounds. In this respect, the application of computational structure and ligand based drug design approaches could aid the identification of new class of compounds as Bcr-Abl inhbitors. In the present work, we have synthesised few compounds having a chemical structure based on a central 1,3,4-thiadiazole core bearing a acetamido group at position 2 and an pharmacophore (phenothiazine or coumarin) moiety at position 5. In addition to that the precursors of the thiadiazole ring system, the thiosemi carbazones were also used for docking.The binding affinity of the T315I mutant Bcr-Abl kinase with the compounds **1-5** were measured by kcal/mol. The docking scores, H-bonding interactions and surrounding amino acids in the binding pocket for the following phenothiazine and coumarin derivatives were given in the **Table 1**.

Molecular docking studies

Molecular docking studies have been done using the PyRx. Version 0.8 docking program.

Protein preparation

The T3151 Abl mutant protein structures was obtained from the Protein Data Bank(PDB ID was 2V7A). The co-crystallized ligand (desatnib) in the T3151 Abl mutant structure was removed. Then the polar hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT. Further ADT was used to remove crystal water, Gasteiger charges were added to each atom, and merged the non-polar hydrogen atom to the protein structure. The distance between donor and acceptor atom that forms a hydrogen bond was defined as 1.9A with a tolerance of 0.5A and the acceptor-hydrogen-doner angle was not less than 120^{o} . The structures were then saved in PDB file format, for further studies in Autodock vina under PyRx 0.8 Platform.

Ligand preparation

Ligand 2D structures were drawn using ChemDraw Ultra 8.0 (ChemOffice 2002). Chem3D Ultra 8.0 was used to convert 2D structure in to 3D and the energy minimized using semi-empirical AM1 method. Minimise energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as pdb file format for input to ADT. All the ligand structures were then saved in SDF file format, to carry out docking in Autodock vina.

Grid formation

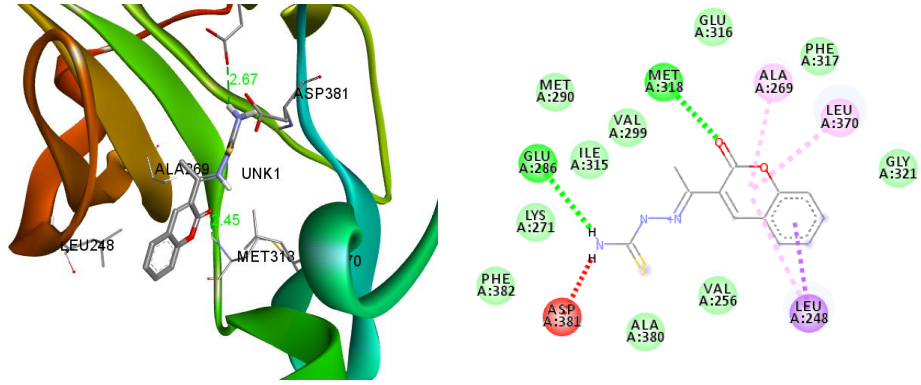
A grid box with dimension of 40 x 40 x $40A^3$ with 0.375A spacing and centered on 29.470, 47.997, 8.863 was created around the binding site of desatinib on T3151 Abl mutant protein using ADT. The centre of the box was set at crizotinib ligand centre and grid energy calculations were carried out.

Docking protocol

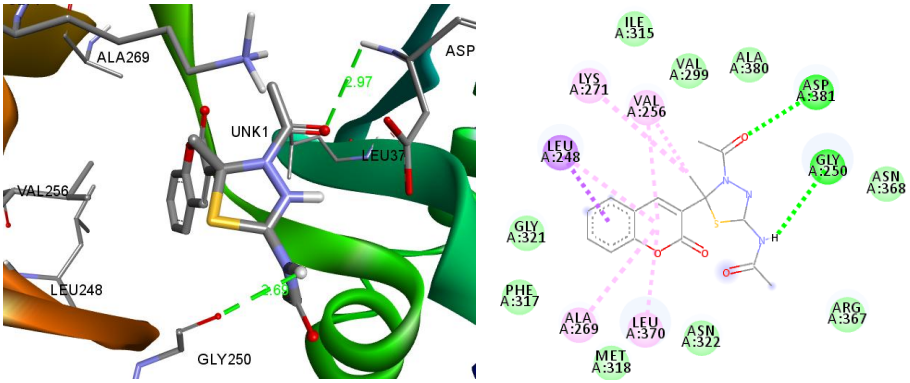
For the AutoDock docking calculation, default parameters were used and 10 docked conformations were generated for each compound. The energy calculations were done using genetic algorithms. The outputs were exported to Chimera 1.10 and Discovery studio 4.5 for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active site.

The ligands 3-5 have shown good scores in the docking studies when compared with the corresponding coumarin derivatives 1 and 2. In compound 1 the Carbonyl group of the coumarin nucleus forms an hydrogen bond with the NH group of Met318. Additional hydrogen bonds occured between the amido NH group and carbonyl group of Glu286 as well as the carbonyl group of Asp381. The coumarin moiety was well placed inside the hydrophobic region having the residues Ala269, Leu370 and Leu248. In 2 hydrogen bonds occurred between the acetyl C=O group of the inhibitor and NH group of Asp381. The amido NH group forms hydrogen bond with the carbonyl group of Gly250. The coumarin moiety was located in the hydrophobic region covered by Val256, Leu248, Ala269, Leu370 and Lys 271. Lys 271 is the ATP binding site. In compound 3 the NH group of phenothiazine forms hydrogen bonds with Met318. The phenothiazine moiety was inside the hydrophobic pocket containing amino acids Leu248, Ala269, Leu370 and Ile315. The compound 4 forms a dipolar ion by undergoing a rearrangement to form hydrogen bonds with the receptor protein. It forms hydrogen bonds between NH group of the thiadiazole ring and the carbonyl group of Tyr 320. The amido NH group forms hydrogen bond with the carbonyl group of Thr319. Further the acetyl carbonyl group forms hydrogen bonds with the NH group of ASN322. Also one more hydrogen bond was observed between the NH group of phenothiazine ring and the carbonyl group of Gly249. Apart from that the phenothiazine ring moiety was well placed inside the hydrophobic region containing the mino acids Lys271, Ala269, Val256 and Leu248. For compound 5 only hydrophobic interactions were observed without any hydrogen bonds. The phenothiazine was in the hydrophobic region conyaining the amino acid Ala269, Leu248, Leu370, Lys 271, Ile315 and Ala380. All the above binding modes were equivalent to the binding mode previously found for 1,3,4-thiadiazole derivatives (Fabrizio *et al*.,. 2008).

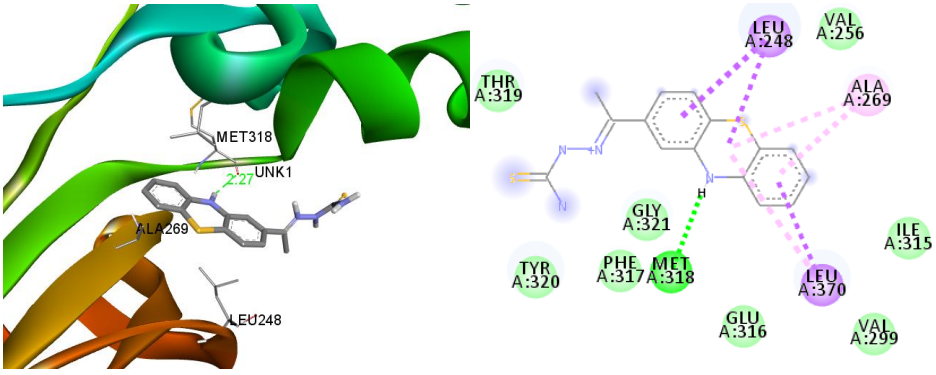
For all the above compounds profitable interactions between the aromatic moieties with the hydrophobic region and hydrogen bond contacts with residues of the protein were identified to be important activity. The hydrogen bond donor-acceptor motif seems to be a structural requirement for activity of inhibitors. Comparatively the ligands 3-5 indicates a good affinity towards the T3151 Abl mutant and thereby suggests that they can behave as third generation tyrosinase inhibitors for the CML.



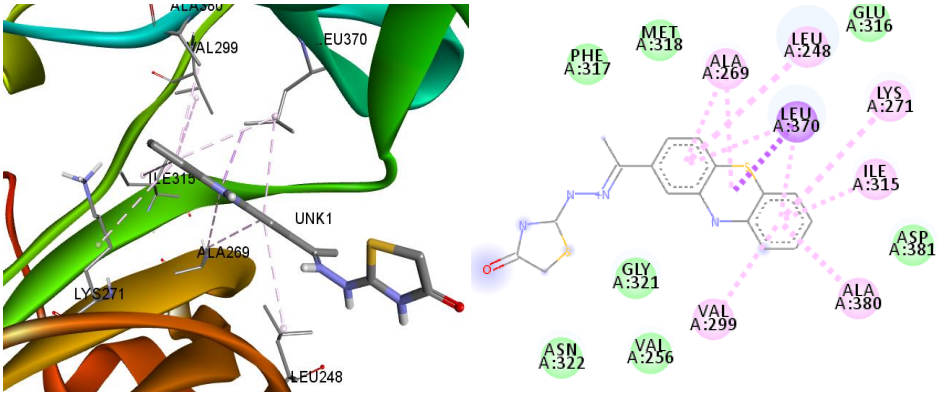
Compound 1



Compound 2



Compound 3



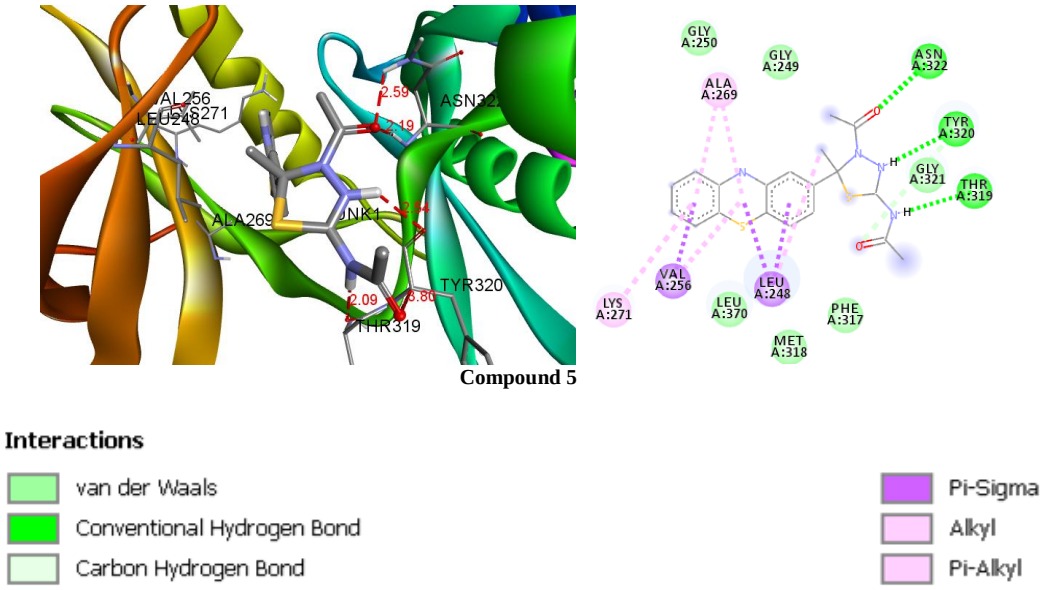


Fig. 1: 3D and 2D binding mode of the compounds 1-5 with T313I Bcr-Abl mutant.

Table 1: The docking scores for the Compounds **1-5** against T315I Bcr-Abl mutant.

Ligand	Binding Affinity	RMSD/ub	RMSD/lb	Ligand	Binding Affinity	RMSD/ub	RMSD/lb
1	-7.2	0	0	**4**	-7.7	4.381	3.548
	-7.2	6.719	4.427		-7.6	10.025	7.198
	-7.1	6.872	5.482		-7.5	3.329	2.141
	-6.9	4.497	3.932		-7.3	7.704	5.863
	-6.9	5.232	4.658		-7.1	2.867	2.378
	-6.8	3.782	3.32		-9.1	0	0
	-6.8	6.968	4.738		-9	8.744	5.596
	-6.8	2.988	1.938		-8.9	8.131	3.985
	-6.6	7.017	4.489		-8.5	3.391	2.327
2	-8.9	0	0		-8.4	4.361	2.573
	-8.1	2.81	2.146		-8.1	8.536	5.244
	-7.9	2.89	2.003		-7.9	3.978	2.668
	-7.8	6.43	3.041		-7.9	9.616	4.55
	-7.7	4.838	3.064		-7.8	9.357	6.889
	-7.7	8.202	5.914		-8.6	0	0
	-7.6	3.317	2.243		-8.4	2.532	1.935
	-7.4	5.1	3.056		-8.3	9.275	3.91
	-7.3	8.518	5.909		-8.2	3.305	2.535
3	-7.9	0	0	**5**	-8.2	7.934	4.986
	-7.9	4.448	3.177		-8.2	8.69	3.523
	-7.8	3.98	3.224		-8.2	8.546	6.03
	-7.8	2.349	2.051		-8	8.786	6.767
					-8	2.601	1.81

Table 2: Lipinski's rule for the compounds **1-5.**

Compound	Molecular weight	Molecular formula	No. of H-donar	No. of H-acceptor	Rotatable bonds	Log p
1	261.06	C12H11N3O2S	1	1	3	4.84
2	345.08	C16H15N3O4S	1	1	3	5.38
3	314.07	C15H14N4S2	0	2	2	4.84
4	398.09	C19H18N4O2S2	0	2	2	5.33
5	354.06	C17H14N4OS2	0	2	2	5.40

Taking in to consideration of all the docking scores of the compounds **1-5** with the T3151 Bcr-Abl kinase proteins and **3-5** exhibited reasonably good scores and appears to be more favorable when compared with **1** and **2.** To identify whether the above selected ligands can act like drug molecules the Lipinski's rule of five parameters like molecular weight, molecular formulae, number of H-bond donor, Number of H-bond acceptors, Number of rotatable bonds and the Log P value were calculated for the compounds **1-5**. All the parameters are well within the rule except a slight variation in the Log P value of **2,4** and **5**. Based on the majority of the acceptable parameters all the five compounds may be used as drug like molecules **(Table 2)**.

In Vitro Anticancer Activities

Trypan Blue Exclusion assay

Compounds **1-5** induces cytotoxicity in K562 leukemic cell line in a dose dependent manner. In order to evaluate the cytotoxic effect of **1-5** on K562 leukemic cell line, we used trypan blue assay. K562 cells were treated with 10, 50, 100 or 200µg/ml of **1-5**. Since the compounds were dissolved in DMSO, the cells with DMSO were used as vehicle control. Following addition of the compound, cells were counted after 24 h. The IC_{50} values were calculated and tabulated (**Table 3.**). Results showed that cell growth was affected with increase in concentration of the compound (**Figure 3**).The effect was limited in case of **1,2** and **4** whereas in case of **3** and **5,** the effect was appreciable.

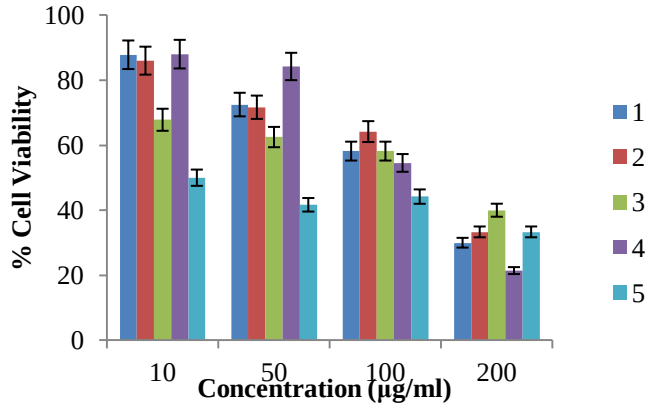


Fig. 3: Trypan Blue assay, showing the percentage of cell viability in a dose dependent manner.

MTT Assay

In order to evaluate the cytotoxic effect of **1-5** on K562 leukemic cell line, we also used the MTT assay. K562 cells were treated with 10, 50, 100 and 200 mg/ml of **1-5**. Since the compounds were dissolved in DMSO,the cells with DMSO were used as a vehicle control. IC_{50} values were calculated and tabulated **(Table 3)**. Compounds **1** and **2** showed an IC_{50} value of more than 200 µg/ml and are less active. But compounds **3, 4** and **5** induce cytotoxicity in K562 leukemic cell line in a dose dependent manner.

Table 3: IC_{50} Values of **3-5** Trypan Blue and MTT assay.

Compound	IC_{50}(µg/ml)	
	Trypan Blue assay	MTT assay
1	111.6 ±1.4	123.6 ±6.8
2	125.6 ±3.1	122.3 ±4.2
3	108.7 ±3.8	119.8 ± 2.1
4	133.01 ± 2.6	130 ± 3.3
5	11.14 ± 1.4	50.66 ± 2.3
Cisplatin	4.24 ± 3.2	8.96 ± 3.3

Lactate Dehydrogenase Leakage assay (LDH)

LDH release assay was performed to test the cell membrane damage induced by the compounds **1-5**. For this, K562 cells were cultured with 10, 50,100 and 200 µg/ml of **1-5** and LDH released was measured at 48 h. Consistent with above results, the LDH release was not appreciable in case of compounds **1** and **2**. we observed a dose- dependent increase in LDH release in compounds **3, 4** and **5**, further confirming the cytotoxic potential of these compounds. The results of the compounds **3, 4** and **5** were given in the **Figure 4**.

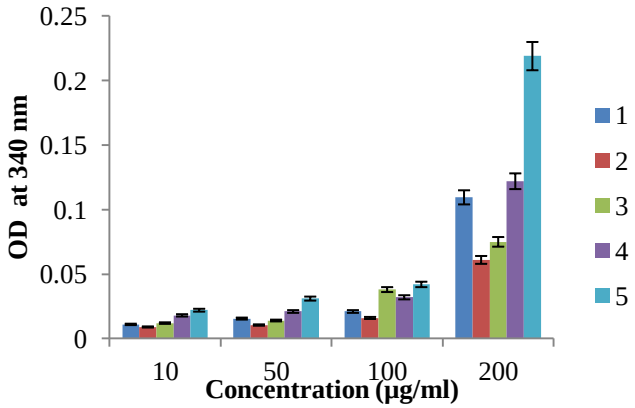


Fig. 4: Lactate Dehydrogenase Leakage Assay by dose dependent manner.

CONCLUSIONS

This work mainly aims to discover novel small molecular inhibitors against important molecular targets involved in leukemia. The T3151 Abl mutant was selected as the receptor protein in the Abl kinase domain in Bcr-Abl and is present in the K562 leukemic cancer lines. Five heterocyclic compounds **1-5** were synthesised, characterised and docking study was carried out against the T315I Bcr-Abl mutant. The compounds **3-5** with phenothiazine pharmacophore showed promising docking score than with the derivatives having the coumarin pharmacophore **1** and **2.** So the compounds **3-5** were tested for their anticancer activity against leukemic K562 cell line by trypan blue, MTT and LDH assays.

Compound **5** showed marked anticancer activity and exhibited an IC_{50} value of 11.12 and 50.66 µg/ml against trypan blue and MTT assay respectively. Further a dose-dependent increase in LDH release was observed, confirming the antiproliferative potential of the compounds. The hydrogen bond donor-acceptor motif seems to be a structural requirement for activity of inhibitors **3-5**. Comparatively the ligands **3-5** indicates a good affinity towards the T3151 Abl mutant and thereby suggests that they can behave as third generation tyrosinase inhibitors for the CML and may be taken as the basic template for further work up.

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REFERENCES

Arung, ET, Wicaksono, BD, Handoko, YA, Kusuma, IW, Yulia, D, Ferrry, S. Anti- cancer properties of diethylether extract of wood from sukun (antocarpus altilis) in human breast cancer (T47D) cells). J Immunol Methods 2009; 8:217-324.

Desogus A, Schenone S, Brullo C, Tintori C, Musumeci F, Bcr-Abl tyrosine kinase inhibitors:a patent review, Expert Opin. Ther. Patents 2015 25(4)

Farbrizio, M, Federico, F, Emmanuele C, Silvia, S, Giovanni, M, Maurizio, B. N-(thiazol-2-yl)-2-thiophene caroxamide derivatives as Abl inhibitors identified by a pharmacophore-based data base screening of commercially available compounds, 2008;18:4328-4331.

Jorge, C, Elias, J, Hagop, K, C. Cameron Yin, Jianqin Shan, Susan O'Brien,Guillermo Garcia-Manero, Francis Giles, Megan Breeden, Nubia Reeves, William G. Wierda and Dan Jones., Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors, blood journal, 2011

Kapetanovic, IM. Computer-aided drug discovery and development (CADDD): *in silico*-chemico-biological approach, Chem Biol Interact. 2008; 171:165–176.

Karthigai Priya, M, Ali Muhammad, S, Ravi, S, Novel phenothiazine compounds as third generation Bcr-Abl Tyrosine kinase inhibitors, Int J Pharm Bio Sci 2015; 6: 707 – 716;

Katritzky, AR, Rees, CW. In Comprehensive Heterocyclic Chemistry, The structure, Reactions, Synthesis and Uses of Heterocyclic Compounds, ed. By Potts, K.T. Pergamon Prees. 1981; 6 :548.

Korzeniewski, C, Callewaert, DM. An enzyme-release assay for natural cytotoxicity. J. Immunol Methods. 1983; 64:313–320.

Masters, RW. 2000, Animal cell culture, Trypan Blue Assay sop, 3rd ed. 2000:p1 – 3.

Michele, M, Elena, C, Chiara, S. Crystal Structure of the T315I Abl Mutant in Complex with the Aurora Kinases Inhibitor PHA-739358, Cancer Res 2007; 67: 17.

Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65:55-63.

Mughal A, Aslam H M, Khan A M H, Saleem S, Umah R, Saleem M, Bcr-Abl tyrosine kinase inhibitors- current status, Infectious Agents and Cancer 2013:8;23

Muhammad, SA, Ravi, S, Thangamani, A, Synthesis and evaluation of some Novel *N*-substituted rhodanines for their anticancer activity, Med Chem Res, 2016, 1-11.

Muhammad, SA, Suganya, S, Ravi, S, Venkatachalapathi, S, Synthesis of novel cyclohexanone derivatives as Bcr-Abl T1351 Inhibitors, Int J Pharm Pharm Sci, Vol 7, Issue 2015:12:95-199.

Noronha, G, Cao, J, Chow, CP, Dneprovskaia, E, Fine, RM, Hood, J, Kang X, Klebansky, B, Lohse, D, Mak, CC, McPherson, A, Palanki, MS, Pathak, VP, Renick, J, Soll, R, Zeng, B. Inhibitors of ABL and the ABL-T315I mutation, Curr Top Med Chem. 2008;8:905-21

Radi M, Crespan E, Botta G, Falchi F, Maga G, Manetti F, Corradi V, Mancini M, Santucci M A, Schenone S, Botta M, Discovery and SAR of 1,3,4-thiadiazole derivatives as potent Abl tyrosine kinase inhibitors and cytodifferentiating agents, Bioorg Med Chem Lett 2008:18;1207–1211.

Trott, O, Olson, AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31: 455–461

William, G. Kaelin, Jr, Choosing anticancer drug targets in the postgenomic era, J Clin Invest. 1999;104: 1503–1506.

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