Research Paper

Design Synthesis and Biological Evaluation of Dithiocarbamate Substituted 2-Aminobenzothiazole Derivatives as Proviral Integration Site of Moloney Murine Leukaemia Virus 1 Kinase Inhibitors

P. SAI HARSHITA, CH.SUPRIYA, SELINA SRAVANTHI, V. JYOTHI, S. R. PEDDI[1], VIJJULATHA MANGA[1], V. SADDANAPU[2] AND T. SARITHA JYOSTNA*

Department of Pharmaceutical Chemistry, Sarojini Naidu Vanita Pharmacy MahaVidyalaya, Secunderabad, Telangana-500001, [1]Department of Chemistry, Osmania University, Hyderabad, Telangana-500007, [2]Department of Applied biology, CSIR-Indian Institute of Chemical Technology, Hyderabad, Telangana-500007, India

In the present study, we intended to synthesize novel derivatives against Proviral Integration site of Moloney murine leukaemia virus 1 kinase, a biomarker over expressed in numerous malignancies. A novel series of derivatives containing dithiocarbamate moiety as a side chain at the second position of 2-amino benzothiazole nucleus were synthesized and characterized by spectral analysis. From the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide Assay performed, compounds 4a, 4c and 4h of the series emerged as potent anticancer agents against SK-OV-3 cell lines with half-maximal inhibitory concentration value of 34.52±0.5 µM, 34.28±0.06 µM and 29.17±0.66 µM, respectively ($p<0.05$) compared to standard drug doxorubicin's half-maximal inhibitory concentration value 17.07±0.05 µM. From the anti-mitotic activity studies all the compounds showed good to moderate activity with half-maximal inhibitory concentration values ranging from 1.88±0.66 to 20.91±0.34 µM, ($p<0.05$). The evaluation of *in silico* absorption, distribution, metabolism, excretion, and toxicity and molecular descriptors, proved that the synthesized compounds posses drug like properties and are safer to the normal cells. From the molecular docking studies, compounds 4a, 4c, 4h showed good binding affinity with PIM 1 Kinase protein and retained required amino acid interaction similar to the co-crystal. Hence, this study proves 2 amino benzothiazole dithiocarbamate derivatives can be used as encouraging leads as PIM 1 Kinase inhibitors.).

Key words: PIM1 Kinase, dithiocarbamate, SK-OV-3, ovarian cancer, anti-mitotic activity

Cancer encompasses a collection of diseases in which normal cells progressively transform into malignant cells accompanied by an augmented proliferation, invasiveness and metastasis. Cancer treatment and prevention remains to be an unmet medical need despite the massive developments and advances for their therapeutic intervention[1]. Targeted therapy of cancer is the foundation of precision medicine that targets proteins, genes and biomarkers that control how cancer cells grow, divide, and spread. Targeted and specific inhibition of a molecular oncogenic targets is theorized to have a significant role in a hindering the progression of a specific tumor and is an effective strategy to combat cancer[2]. PIM (Proviral Integration site of Moloney murine leukaemia virus) family of proto-oncogenes are serine/threonine kinases, calcium/calmodulin dependent[3]. The three constituents, PIM 1, PIM 2 and PIM 3 show high homology amongst each other and their discovery dates back to 1980's when they were identified in transgenic mouse models by cloning of retroviral integration site in Moloney murine leukaemia Virus (M-MuLV) generated lymphomas[4]. PIM 1 kinase kinase is highly conserved, constitutively active. B-lymphoid, myeloid cell lines, haematopoietic malignancies, prostate, ovarian and uropeithelial cell carcinomas show a high expression

***Address for correspondence**
E-mail: sarithajyostna13@gmail.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms

Accepted 22 December 2020
Revised 15 September 2020
Received 29 August 2020
Indian J Pharm Sci 2020;82(6):1015-1024

of this unusual biomarker[5]. Cell cycle, cell survival epigenetic dynamics regulation, cellular and replicative senescence and apoptosis are the key cellular functions that are regulated by this proto-oncogene[6]. According to the crystal structure analysis experiments[7,8], PIM 1 kinase showed a conventional kinase fold arrangement with two lobes and a cleft intervening between the C-terminus and N-terminus. The N-terminus lobe constitutes of two beta sheets (βH1 and βH2) anti-parallel to each other. The C-terminus lobe, on the other hand has alpha sheets parallel to each other. A hinge region contained in the residues 121-126 joins these two domains. Accordingly, targeting PIM 1 kinase is an intriguing approach for suppressing tumor proliferation both at apoptosis induction level and proliferation suppression. Despite the avalanche of trials to discover potent PIM 1 kinase inhibitors, none of the disclosed compounds was approved in the market and continuous research is being conducted to cover this unmet medical need[9,10,11]. Imidazopyridazine[12], substituted benzylidiene-1, 3- thiazolidne-2, 4 diones[13] and quinones[14] have been previously reported as PIM 1 Kinase inhibitors. In another approach to synthesise PIM 1 Kinase inhibitors, heterocyclization of steroids was performed to yield biologically active products[15]. Endeavoured by the previous findings, we designed and synthesized novel scaffolds by incorporating dithiocarbamate as a side chain on amino group at 2nd position of benzothiazole as PIM 1 kinase inhibitors. Benzothiazoles and their derivatives have invited immense attention from medicinal chemistry researchers due to their wide variety of pharmacological activities like antimicrobial, antitubercular, antitumor, antimalarial, anticonvulsant, antihelmintic, analgesic and anti-inflammatory activities[16-20]. Literature survey emphasized the importance of benzothiazole derivatives having a conjugated system composed of donor and acceptor end groups (push-pull structure) as pharmaceutical substances, nonlinear optical materials, molecular dyads and chemosensors. This interesting scaffold has been found alone or incorporated into diverse therapeutic agents of known antineoplastic activity such as tiazofurin, dasatinib and bleomycin. 2-(4-aminophenyl) benzothiazole was found to possess significant anti-tumor activity. In a study conducted by Leyla *et al.*, the lead compound 2-(4-aminophenyl) benzothiazole originally synthesized as an intermediate for screening of tyrosine-kinase inhibitors showed in vitro cytotoxic activity against MCF-7 breast carcinoma cell lines[21]. Brassinin, chemically a dithiocarbamicester and an indolephytoalexin, was first isolated from Chinese cabbage which demonstrated anti-proliferative activity in human acute T-lymphoblastic leukaemia cells[22]. Gaspari et al reported brassinin and its derivatives as inhibitors of indolamine 2, 3-dioxygenase (IDO), a new cancer immunosuppression target and SAR studies showed, the dithiocarbamate portion of the brassinin as a crucial for activity, which was found to bind to the heme iron of IDO[23]. Numerous applications of dithiocarbamates were identified in anticancer, antifungal, antibacterial, rodent repelling and growth depressing studies[24-27]. Although many new benzothiazole derivatives have been synthesized as potential antitumor agents, there is very scarce literature data on antitumor potentials of dithiocarbamate substituted benzothiazoles. Hence this combination would provide favourable structural properties of both dithiocarbamate and benzothiazole moiety. In the present study we report the synthesis of a new series of benzo[d]thiazol-2-ylcarbamoyl carbamodithioate derivatives, their cytotoxicity on ovarian cancer cell lines, anti-mitotic activity on Bengal gram seeds, drug likeliness, absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties and molecular docking studies against the PIM 1 kinase.

MATERIALS AND METHODS

General:

All the chemicals used were procured from Merck, Sigma-Aldrich and SD fine Company and were of analytical grade. Melting point (MP) of the all the newly synthesized compounds were recorded on Metler Fp-51 instrument. Fourier-transform infrared spectroscopy (FT-IR) absorption spectra were obtained in the range 4000-400 cm-1 on Alpha Bruker FT-IR instrument. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on BrukerUx-NMR instrument and the samples was made in $CDCl_3$ using methyl silane (Me4Si) as the internal standard and chemical shifts were expressed in parts per million (PPM). High-resolution mass spectrometry (HRMS) was recorded on Bruker maXis 10138. Reactions were monitored by Thin Layer Chromatography (TLC) using precoated silica plates (0.25 mm silica gel) obtained from E.Merck and visualization was done by Ultraviolet (UV) light at 254 nm. All the solvents used for silica gel column chromatography was distilled prior to use. Silica gel 60-120 mesh (Merck) was used as an adsorbent for column chromatography.

Chemistry:

General procedure for the synthesis of ditiocarbamate substituted 2-amino benzothiazolederivatives (4a-h); N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide (2) was synthesized by modification of a previously reported method[28]. An equimolar mixture of appropriate amine (3) and anhydrous potassium carbonate in dimethyl formamide was stirred at room temperature for 5 min, and then carbon disulphide (3 equiv) was added. The reaction mixture was stirred for additional 20 min, and then N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide (1 equiv) (2) was added. Stirring was continued at room temperature until the reaction was completed as monitored by TLC. The mixture was poured into cold water, extracted with ethyl acetate (3×30 ml), the organic phase was washed once more with water and dried with sodium sulfate and filtered. The solvent was evaporated under reduced pressure and the resultant residue was purified by chromatography over silica gel using a mixture of petroleum ether and ethyl acetate as solvent to give the desired compounds 4a-h. The obtained compounds were subjected to analytical characterization.

(Benzo[d]thiazol-2-ylcarbamoyl) methyl ethyl carbamodithioate (4a); Yield: 83 %; MP 108-110°; $C_{12}H_{15}N_3S_3O$; Infra-Red (IR) (KBR) cm^{-1}: 3389.86 (N-H, amine), 3053.42 (Aromatic, C-H), 2913.46 (Aliphatic, C-H), 1688.51(C=O), 1226.13 (C=S); ^{1}H NMR (400 MHz, Dimethyl sulfoxide (DMSO)): δ 1.16-1.23 (t, 3H, CH_3), 2.22-2.51 (q, 2H, CH_2CH_3), 3.46 (s, 2H, CH_2S), 5.36 (bs, 1H, NH), 6.98-7.02 (t, 1H, Ar-H),77.18-7.22 (t, 1H, Ar-H), 7.32-7.34 (d, 1H, Ar-H), 7.64-7.66 (d 1H, Ar-H); HRMS (m/z) (%) 313.06 (M+2) (Calculated 311.02).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl propyl carbamodithioate (4b); Yield: 79 %; MP 116-118°; $C_{13}H_{17}N_3S_3O$; IR (FT-IR-cm^{-1}): 3390.72 (N-H, amine), 3052.80 (Aromatic, C-H), 2917.47(Aliphatic, C-H), 1687.66 (C=O), 1279.95(C=S); 1H NMR (400 MHz, $CDCl_3$): δ 0.95-0.98 (t, 3H, CH_3), 1.25-1.29 (t, 2H, CH_2), 1.42-1.75 (m, 2H, CH_3), 3.65 (s, 2H, CH_2S), 5.32 (bs, 1H, NH), 7.12- 7.16 (t, 1H, Ar-H), 7.30-7.34 (t, 1H, Ar-H), 7.52-7.56 (d, 1H, Ar-H), 7.59-7.61 (d, 1H, Ar H); HRMS (m/z) % 327.18 (M+2) (Calculated 325.46).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl butyl carbamodithioate (4c); Yield: 77 %; MP 120-122°; $C_{14}H_{19}N_3S_3O$; IR (FT-IR-cm^{-1}): 3389.56 (N-H, amine), 3053.53 (Aromatic, C-H), 2915.96 (Aliphatic, C-H), 1697.64 (C=O), 1248.8(C=S); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88-0.95 (t, 3H, CH_3), 1.25-1.34 (t, 2H, CH_2), 1.44-2.04-175 (m, 2H, CH_3), 4.06 (s, 2H, CH_2S), 5.75 (bs, 1H, NH), 7.12 –7.59 (m, 6H of benzothiazole); HRMS (m/z) % 342.06 (M+2) (Calculated 340. 06).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl morpholine-4-carbodithioate (4d); Yield; 72 %, MP 124-126°; $C_{14}H_{17}N_3S_3O_2$; IR (FT-IR-cm^{-1}): 3219.01 (N-H, amine), 3053.50 (Aromatic, C-H), 2915.50 (Aliphatic, C-H), 1691.43 (C=O), 1236.34 (C=S); 1H NMR (400 MHz, CDCl3): δ3.73-4.37 (m, 8H, morpholine), 4.41 (s, 2H, CH2S), 7.32-7.34 (t, 1H, Ar H), 7.41-7.46 (t, 1H, Ar H), 7.79-7.82 (q, 2H, Ar H), 10.46 (bs, 1H, NH); HRMS (m/z) % 354.04 (M+1) (Calculated 353.47).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl piperidine-1-carbodithioate (4e); Yield; 74 %, MP 142-144°; $C_{15}H_{19}N_3S_3O$; IR (FT-IR-cm^{-1}): 3275.65 (N-H, amine), 3068.92 (Aromatic, C-H), 2852.49 (Aliphatic, C-H), 1697.83 (C=O), 1242.29(C=S); ^{1}H NMR (400 MHz, $CDCl_3$) : δ0.86-0.90 (m, 6H, $(CH_2)_3$ of piperidine), 3.90-4.32 (m, 4H, $N(CH_2)_2$ of piperidine), 4.40 (s, 2H, CH_2S), 7.28-7.32 (t, 1H, ArH), 7.40-7.44 (t, 1H, ArH), 7.79-7.81((d, 2H, ArH), 10.62 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 24.03, 25.47, 26.08, 29.70, 39.35, 52.06, 54.48, 121.32, 123.92, 126.18, 132.36, 148.62, 157.33, 167.40, 193.17; HRMS (m/z) % 351.29 (M+1) (Calculated 351.05).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl 4-methyl piperazine-1-carbodithioate (4f); Yield; 86 %, MP 146-148°; $C_{15}H_{20}N_4S_3O$; IR (FT-IR-cm^{-1}): 3354.81 (N-H, amine), 3051.61 (Aromatic, C-H), 2919.68 (Aliphatic, C-H), 1713.98 (C=O), 1230.23 (C=S); ^{1}H NMR (400 MHz, CDCl3): δ 1.25 (s, 3H, CH3), 2.35-3.97 (m, 8H, N $(CH)_2$ of piperazine), 4.40 (s, 2H, CH2S), 7.28-7.33 (t, 1H, Ar H), 7.41-7.45 (t, 1H, Ar H), 7.79-7.82 (m, 2H, Ar H), 10.58 (bs, 1H, NH); HRMS (m/z) % 367.07 (M+1) (Calculated 366.06).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl 4-ethyl piperazine-1-carbodithioate (4g): Yield; 76 %, MP 148-150°; $C_{16}H_{22}N_4S_3O$; IR (FT-IR-cm^{-1}):3276.60 (N-H, amine), 3055.96 (Aromatic, C-H), 2959.91 (Aliphatic, C-H), 1694.89 (C=O), 1030.41 (C=S); ^{1}H NMR (400 MHz, CDCl3): δ 1.09-1.12 (t, 3H, CH3), 2.44-2.50 (q, 2H, CH2CH3),-2.57-4.38 (m, 8H, $N(CH_2)_2$ of piperazine), 4.40 (s, 2H, CH_2S), 7.27-7.30 (t, 1H, Ar H), 7.40-7.45 (t, 1H, Ar H), 7.79-7.82 (d, 2H, Ar H), 10.64 (s, 1H, NH); ^{13}C NMR (100 MHz, CDCl3): δ (ppm) = 11.95, 39.34, 50.50, 51.78, 52.01, 52.62, 76.73, 121.18, 121.34, 123.98, 126.23, 132.31, 148.53, 157.42, 167.15, 194.27; HRMS (m/z) % 381.06 (M+1) (Calculated 380.08).

(Benzo[d]thiazol-2-ylcarbamoyl)methylbenzylcarbamodithioate (4h); Yield; 81 %, MP 196-198°; $C_{17}H_{17}N_3S_3O$; IR (FT-IRcm^{-1}): 3398.42 (N-H, amine), 3052.98 (Aromatic, C-H), 2050.82 (Aliphatic, C-H), 1694.55 (C=O), 1281.24 (C=S); ^{1}H NMR (400 MHz, CDCl3): δ3.63 (s, 2H, CH_2ArH), 4.36 (s, 2H, CH_2S), 5.42 (bs, 1H, NH), 7.11-7.60 (m, 9H, ArH); HRMS (m/z) % 373.00 (M+1) (Calculated 373.04).

MTT Assay:

The human ovarian cancer cell line (SK-OV-3) were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % heat-inactivated fetal bovine serum, 2 mmol/l glutamine, 1 % antibiotics solution (100 U/ml penicillin G, 100 mg/ml streptomycin and 5 µg/ml amphotericin-B). Cells were grown in 25 cm^2 tissue culture flasks in a humidified atmosphere of 5 % CO_2 at 37°. All cytotoxicity experiments were carried out in 96 micro titre well plates. All the consumables and plastic-wares were procured from Himedia, India unless stated otherwise. In vitro cytotoxicity was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazoliumbromide (MTT) Assay[29]. When the density reached approximately 70 % confluency, the cell culture was trypsinised with 0.2 % trypsin solution, 0.02 % Ethylenediaminetetraacetic acid (EDTA) in phosphate buffer saline solution and re-suspended in complete culture media. Both normal and cancer cells were pre incubated at a concentration of 2×10^4 cells/ml in culture medium for 3 h at 37° and 5 % CO_2, 75 % relative Humidity. Morphological changes of drug treated cells were examined using an inverted microscope at different time intervals and compared with the cells serving as control. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and various concentrations of the compounds were added into micro plates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated for 4 hr at 37° and 5 % CO_2. 10 µl of MTT labelling mixture was added and incubated for 4 hr. 100 µl of DMSO was added to each well and incubate for overnight. Untreated cells represented a control group and doxorubicin treated as a positive control. Absorbance of the samples was measured using a micro plate (Enzyme-linked immunosorbent assay (ELISA)), a uniform shaking (300 rpm for 5 min) was applied to dissolve formazan crystals and the absorbance was recorded at 570 nm. Three independent experiments were performed. Cell viability was calculated using the following equation: (test-sample OD-blank control OD)/ (untreated control OD-blank control OD)×100 %. A non-linear regression graph was plotted between cell survival (%) and log10 drug/compound concentration and the 50 % minimum inhibitory concentration (IC50) was determined using Prism software version 7 (GraphPad Software Inc., San Diego, CA, USA).

Anti-mitotic activity:

Synthesized derivatives were tested for anti-mitotic using chick pea seeds (Cicerarietinum)[30]. Bengal gram seeds was soaked overnight and taken in the petridish with a moisten filter paper in order to hasten the germination process. Drug solutions were prepared in methanol to get various concentrations. After germination of seeds, a group of 10 seeds were distributed in each petri dish. For every 24 h, 1ml each concentration of the test drug was added to petri dish and labelled accordingly. Treatment with test compounds and control was continued for 7 d. Length of radicals was measured in cm at the end of the 7th d. Lengths of radicals, treated with test compounds were calculated. The results were expressed as IC50 values and % inhibition was calculated.

Molecular descriptors, bioactivity prediction and ADMET Properties:

All the newly synthesized compounds were subjected to molinspiration (https://www.molinspiration.com/), an online server was used to predict the drug likeness character and bioactivity parameters of all the selected ligand molecules. These descriptors are useful in the general understanding of the chemical interactions between the interested compound with its target, and help in ascertaining the drug properties. ADMET properties of the synthesised compounds were predicted using the preADMET online software (https://preadmet.bmdrc.kr/). This prediction includes the evaluation and estimation of the percentage human intestinal absorption (% HIA), *in vitro* Caco-2 cell permeability, *in vitro* Maden Darby Canine Kidney (MDCK) cell permeability, *in vitro* plasma protein binding and *in vivo* blood brain barrier (BBB) penetration ability. The server was also used to predict the possible interaction of drug molecule with various isoenzymes. The prediction was based on a vector machine classification algorithm and in-house substructure pattern recognition method, which were built via regression methods[31]. The percentage HIA data includes the sum of bioavailability and absorption values evaluated from the ratio of cumulative excretion in urine, bile and

feces. The following is the interpretation of the data: 0-20 % of HIA is poorly absorbed compounds, 20-70 % of HIA are moderately absorbed compounds and 70-100 % of HIA are compounds with good absorption. The Caco-2 cells are derived from human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. Compounds are said to be low permeable if the cell permeability is less than 4, while compounds in the range of 4-70 are moderately permeable and compounds with the cell permeability greater than 70 are highly permeable. MDCK cell system is a significant tool for screening rapid permeability and the *in vitro* MDCK value<25 indicates low permeability compounds, 25-500 depicts moderately permeable compounds and>500 indicates compounds that are highly permeable. Blood brain barrier (BBB) penetration helps in predicting if the compounds have the ability to pass the blood brain barrier or not. It is an essential tool for evaluating the efficacy of the Central nervous system (CNS)- active compounds and CNS inactive compounds must not pass through it. BBB penetration rate<0.40 are CNS inactive (-) compounds and BBB penetration rate>0.40 are CNS active (+) compounds. Hence the Molecular descriptors, bioactivity aspects and ADMET properties of the synthesised derivatives were evaluated. The values were compared with those of two reported PIM 1 kinase inhibitors SG1 1776 and AZ 1208[32].

Docking studies:

The three dimensional (3D) co-ordinates of crystallographic structure of PIM 1 kinase (PDB ID: 3BGQ) was downloaded from Brookheaven protein DataBank (https://www.rcsb.org). GLIDE 5.6 was used for molecular docking. The protein was prepared using protein preparation Wizard applying the default parameters[33]. The minimization of the complex was continued using OPLS-2005 (OptimizedPotential for Liquid Simulations) force field until the root mean square deviation (RMSD) reached the value of 0.3Å[34]. The ligands for docking studies were prepared using LigPrepSchrödinger. GLIDE XP (extraprecision) method was followed for docking calculations. A grid was generated around the co-crystallized ligand. Receptor vander Waals scaling for the non-polar atoms was kept at 0.9. The lower energy conformation of the ligands were selected and docked into the grid generated from the protein structures. The docking results were analysed for the interactions with the active site amino acids.

RESULTS AND DISCUSSION:

The synthesis of 2-amino benzothiazole dithiocarbamate derivatives was performed in 2 steps (fig. 1). In the first step, N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide (2) was synthesized by a nucleophilic attack of 2-amino benzothiazole on chloroacetyl chloride with dichloromethane as the solvent and triethyl amine as the base. The progress of the reaction was monitored by TLC. The precipitate obtained was filtered and dried over anhydrous sodium sulfate and was recrystallized from ethanol. Following which, the reaction consisted of an initial nucleophilic addition of primary and secondary amines to carbon disulphide in the presence of the base anhydrous K_2CO_3 to give dithiocarbamate. This was first undertaken by Hofmann more than 130 years ago[35]. S-acyl dithiocarbamates were obtained by acylation of dithiocarbamate with N-(1, 3-benzothiazol-2-yl)-2-chloroacetamide. Reaction mixture was monitored by TLC. The obtained derivatives were then extracted from ethyl acetate, washed with water and dried over sodium sulphate. They were later subjected to purification by passing through silica gel using mixture of Dichloromethane (DCM) and ethyl acetate as eluents. All new compounds were characterized using IR, ^{1}H NMR and HRMS analysis and their spectral analyses were consistent with the assigned structures and listed in the experimental section.

All the synthesized compounds 4a-h were screened for their anticancer activity against SKOV3 cell line using MTT Assay in comparison with the standard drug doxorubicin. From the results (Table 1), it is clear

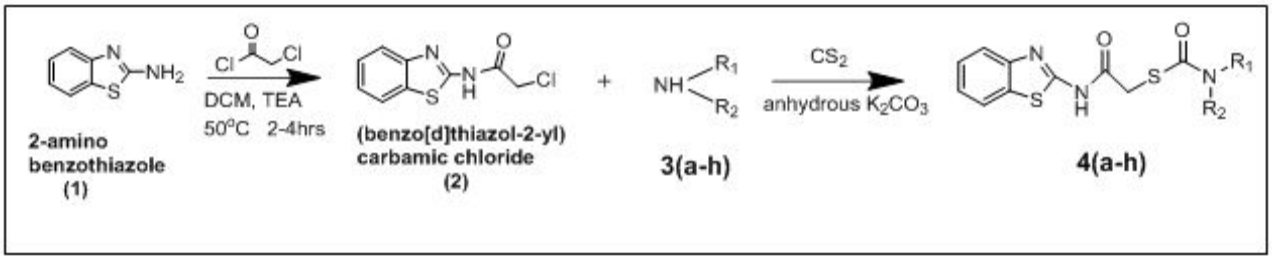

Fig. 1: Representative scheme for the Synthesis of 2-amino benzothiazole dithiocarbamate drivatives
3a-h: 3a-ethylamine, 3b-propylamine, 3c-butylamine, 3d-morpholine, 3e-piperidine, 3f-methyl piperazine, 3g- ethyl piperazine, 3h- benzyl amine

that all the compounds showed good activity against the cancer cell line. Compound 4h (benzyl amine) exhibited prominent activity with IC50 29.17±0.5 µM. Compounds 4a (ethylamine) and 4c (butylamine) showed almost equivalent potency of IC50 34.52±0.06 µg/ml and 34.28±0.66 µg/ml respectively. Further, compound 4d (morpholine) showed little decrease in activity with IC50 value 35.20±0.07 µg/ml. Compound 4e (five membered heterocyclic moiety piperidine) and 4f (N-methyl substituted piperazine ring) moiety exhibited same potency of IC50 values 37.80±1.3 µg/ml and 36.50±0.43 µg/ml respectively. Whereas 4g (N-substituted ethyl piperazine) decreased in activity with IC50 38.39±0.67 µg/ml. Hence, the presence of benzyl amine, ethylamine and butylamine moieties contributed to a better activity.

Synthesized compounds 4a-h were futher evaluated for their anti-mitotic activity using chick pea seeds (Cicerarietinum). Synthesized compounds were found to inhibit the growth of the germinating roots from the seeds (Table 2). Among the series compound 4c (butyl amine) showed highest potency with IC50 of 1.88±0.66 µM. The compounds 4a, 4b, 4d, 4e and 4h showed the inhibition range between 4.85±0.7 µM to 5.71±0.98 µM. Other compounds 4f (1-methyl piperazine) and 4g (1-ethyl piperazine) showed less activities with IC50 of 20.27±1.7 µM and 20.91±0.34 µM respectively. Butyl amine, ethylamine, propylamine, morpholine, piperidine and benzylamine derivatives showed good anti-mitotic activity.

The molecular properties of the synthesised compounds 4a-h were predicted (Table 3). It is observed that all the compounds have significant HIA capacity as the value ranges from 93.54 to 98.57. It is shown that the compounds are moderately permeable to Caco-2 cells with the range of values between 12.44-48.65 nm/sec. The values for the in vitro plasma protein binding are in the range of 75.61-100 % indicating their strong binding capacity with plasma proteins. Hence there is high probability that these compounds can reach the desired target. The *in vivo* blood brain

TABLE 1: ANTICANCER ACTIVITY OF COMPOUNDS 4a-h

Sample code	IC_{50} (µM)[a]
4a	34.52±0.5
4b	42.12±0.89
4c	34.28± 0.06
4d	35.20±0.7
4e	37.80±1.3
4f	36.50±0.43
4g	38.39±0.67
4h	29.17±0.66
Doxorubicin[b]	17.07±0.05

[a] IC50 is the concentration of compound required for 50% inhibition of cell viability determined using MTT assay and each value represents the mean±SEM. [b] Doxorubicin is used as the standard drug

TABLE 2: RESULTS OF ANTI MITOTIC ASSAY OF COMPOUNDS 4a-h

Sample code	$IC_{50\ (\mu M)a}$
4a	5.71±0.98
4b	4.85±0.7
4c	1.88±0.66
4d	4.89±0.78
4e	5.20±1.1
4f	20.27±1.7
4g	20.91±0.34
4h	5.56±0.7

[a] IC50 values are average of three replicate Assays and each value represents the mean±SEM

TABLE 3: PREDICTED ADMET PROPERTIES OF COMPOUNDS 4a-h

Sample code	Human intestinal absorption (HIA, %)a	In vitro Caco-2 Cell Permeability (nm/sec)b	In vitro MDCK Cell Permeability (nm/sec)c	In vitro Plasma Protein binding (%)d	In vivo Blood-brain Barrier Penetration (C.brain/C.blood)b	Toxicity
4a	96.65	12.44	2.69	87.72	0.29	Mutagenicity
4b	93.54	16.29	0.65	88.48	0.67	Negative
4c	96.66	28.71	0.71	93.37	0.23	Negative
4d	97.24	29.63	44.43	98.16	0.03	Negative
4e	98.38	42.73	0.28	95.6	0.51	Negative
4f	98.57	46.56	0.2	75.61	0.29	Mutagenicity
4g	98.49	48.65	0.31	81.3	0.2	Reproductive toxicity
4h	97.43	47.75	0.37	100	0.18	Negative
SGI 1776	96.08	24.35	0.07	83.9	1.18	Mutagenicity
AZ 1208	96.02	3.11	44.524	81	0.82	Mutagenicity

[a]Human intestinal absorption is the sum of bioavailability and absorption values evaluated from the ratio of cumulative excretion in urine, bile and feces; [b] Caco-2 cells are derived from human colon adenocarcinoma and posses multiple drug transport pathways through the intestinal epithelium; [c] MDCK cell system can be used as a good tool for screening of rapid permeability; [d] The percent of drug that binds to plasma proteins; [e] Blood-Brain Barrier (BBB) penetration is represented as BB = [Brain]/[Blood]

barrier penetration values are ranged from 0.03-0.67 confirming them as moderately CNS active compounds. The toxicity evaluation revealed that compounds 4b, 4c, 4d, 4e and 4h proved to be non-toxic in terms of mutagenecity, tumorigeicity, irritancy, hepatotoxicity and reproductive toxicity. The other compounds showed mutagenic and tumerogenic toxicity. This over all evaluation has proved that the synthesised compounds are safer to the normal cells are suitable anti-tumor agents. Additionally, we have compared the above properties with those of reported PIM 1 Kinase inhibitors: SGI 1776 and AZ 1208 with the newly synthesized derivatives. This comparison revealed that the reported compounds possessed mutagenicity while the most potent synthesised derivatives, 4c and 4h lacked toxicity.

The bioactivity parameters of compounds 4a-h (Table 4) like G Protein-coupled Receptor (GPCR) Ligand property, ion channel modulator, kinase inhibitor, nuclear receptor ligand interactions, protease inhibitor and enzyme inhibitor were predicted for the synthesized compounds and compared with the values of SGI 1776 and AZ 1208. The results support them as safer compounds with good binding capacities and cell growth inhibitors. The understanding of the chemical interactions between the synthesised compounds under study and their pharmacological target cell is as important process in establishing their drug-like properties. The predicted Lipinski properties of 4a-h (Table 5) with valid scorings bolster their safer drug behaviour. An orally available drug is elected to be in agreement with Lipinki's rule if the molecular weight is less than 500 Daltons, the number of hydrogen bond donors is less than 5, the number of hydrogen bond acceptors is less than 10, the partition co-efficient (log P) value is less than 5 and the molecular refractivity is within the range of. The results exhibit that compounds stratify to Lipinski's, so they should theoretically manifest good oral absorption. As the Lipinski rule violation is zero for all the compounds the total polar surface area was calculated and is observed that the value is less than 140 for all compounds except 4d.

TABLE 4: BIOACTIVITY OF COMPOUNDS 4a-h

	Bioactivity					
Sample code	**GPCRL**	**ICM**	**KI**	**NRL**	**PI**	**EI**
4a	-0.39	-0.58	-0.64	-0.88	-0.47	-0.15
4b	-0.61	-0.95	-0.94	-1.28	-0.78	-0.45
4c	-0.54	-0.9	-0.87	-1.17	-0.67	-0.4
4d	-0.62	-1.1	-0.88	-1.27	-0.82	-0.5
4e	-0.52	-0.99	-0.91	-1.23	-0.77	-0.44
4f	-0.48	-0.94	-0.78	-1.22	-0.75	-0.44
4g	-0.47	-0.95	-0.82	-1.16	-0.75	-0.45
4h	-0.48	-0.8	-0.69	-0.98	-0.55	-0.38
SGI1776	-0.2	-0.27	-0.13	-0.2	-0.19	-0.21
AZ 1208	-1.26	-1.22	-0.75	-1.01	-1.33	-0.46

Abbreviations: GPCRL: G protein-coupled receptor Ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor.
For organic compounds, If the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (<-5.0) then inactive

TABLE 5: LIPINSKI'S RULE OF FIVE FACTORS FOR THE COMPOUNDS 4a-h

Sample	**Lipinski parameters**						**Veber parameters**			**Other parameters**	
Code	**M. W. g/ mol**	**HB Don**	**HB Acc**	**log P (o/w)**	**M.R. cm^3/mol**	**Lip. Vio.**	**T.P.S.A.**	**No. of R.B.**	**Veb. Vio.**	**Volume**	Solubility
4a	311.46	2	5	2.96	86.54	0	139.65	6	0	284.61	-3.74
4b	325.48	2	5	3.44	91.34	0	139.65	7	0	302.75	-4.064
4c	339.51	2	5	3.92	96.15	0	139.65	8	0	320.65	-4.404
4d	353.49	1	6	2.42	99.13	0	140.09	5	1	324.93	-4.168
4e	351.52	1	5	3.74	102.85	0	130.86	5	0	334.69	-4.741
4f	366.54	1	6	2.47	109.66	0	134.1	5	0	350.11	-3.527
4g	380.56	1	6	2.96	114.47	0	134.1	6	0	369.19	-3.693
4h	373.53	2	5	4.12	106.22	0	139.65	7	0	337.85	-4.423
SG1 1776	405.42	1	7	4.27	107.95	0	54.68	7	0	348.51	-5.03
AZ 1208	221.28	1	2	2.42	65.26	0	71.47	0	0	169.12	-3.39

Abbreviations: M.W.: Molecular weight; H.B. Don.: Hydrogen bond donors; H. B. Acc.: Hydrogen bond acceptors; log P: octanol to water partition coefficient; M.R.: Molecular refractivity; Lip. Vio.: Lipinski Violations; T.P.S.A.: Total Polar surface area; No. of R.B: Number of rotatable bonds; Veb. Vio.: Veber Violations; No. of S.C.: Number of stereo centers; V.Volume: Van der Waals volume.

The number of rotatable bonds is used to represent molecular flexibility which plays a vital role in drug bioavailability. This value is less than 10 for all the compounds and confirms to the Veber violations. This complementary acceptability with respect to Lipinski and Veber rules proved them as safe administrable drugs and establishes their pharmacological activity. The results are in compliance with the values of SGI 1776 and AZ 1208.

Docking studies were carried out to evaluate the binding affinity and the interaction between the synthesized compounds with the PIM 1 Kinase protein. PIM 1 Kinase protein binding with a small molecule N-cyclohexyl-3-[3-(trifluoromethyl)phenyl][1,2,4] triazolo[4,3-b]pyridazin-6-amine as the co-crystal (PBD ID : 3BGQ, resolution 2.00 Å) was used. The docking score and Amino acid interactions of the compounds 4a-h (Table 6) varies from -4.674 to -6.020. The compounds showed purely hydrophobic and hydrogen bond interactions with the protein active site. Compound 4a (fig. 2) showed the highest binding affinity. The docked pose of the compound 4c (fig.3) also depicted good binding affinity and showed that the nitrogen of the butyl amine formed hydrogen bond with GLU 171 and amide oxygen on benzothiazole moiety formed hydrogen bond with LYS 67. Compound 4h, showing good binding affinity (fig. 4), also showed that its phenyl rings and dithiocarbamate moiety occupied hydrophobic groove making significant vander Waals contacts with the hydrophobic surface having side chain residues of LYS 67 and PHE 49. The other compounds showed only one interaction or failed to show interactions with the target. The interacting compounds were deeply embedded into the hydrophobic pocket. From the above observation, it can be proposed that these compounds had better interaction with the receptor active site due to the presence of hydrophobic groups. According to the docking scores and interactions, we suggest that compounds 4a, 4c and 4h are significantly active and this correlates with the anticancer and anti-mitotic activities. Based on the results, the synthesised derivatives can serve as promising leads for inhibition of PIM 1 kinase enzyme.

TABLE 6: MOLECULAR DOCKING RESULTS OF COMPOUNDS 4a-h WITH PIM1 KINASE PROTEIN (3BGQ)

Compound	Docking score	Glide E energy	Interacting residues
		(Kcal/mol)	
4a	-6.02	-44.055	GLU 171
4b	-5.245	-59.868	-
4c	-5.816	-52.361	GLU 171, LYS 67
4d	-5.424	-40.418	-
4e	-4.674	-42.549	PHE 49, LYS 67
4f	-4.974	-50.887	LYS 169
4g	-4.95	-47.846	PHE 49, LYS 67
4h	-5.961	-51.83	PHE 49, LYS 67

PIM 1 kinase plays a crucial role as oncoprotein in various cancers and is significantly overexpressed in haematological and solid tumors. Thus PIM 1 kinase emerges as an executional target for cancer. However, currently the major challenge that comes with PIM 1 target therapy are the toxicity of the synthesized lead compounds. Hence, based on the literature we have designed and synthesized, dithiocarbamate substituted benzothiazole derivatives as PIM 1 inhibitors. While previous studies evaluated the cytotoxicity of synthesised heterocycles in HT-29 cell line[36], we have selected SK-OV-3 cell line based on the recent studies that have highlighted the role of PIM 1 kinase in ovarian cancer[37]. Overall, from our docking, *in silico*, anticancer and anti mitotic activites 4a (ethylamine), 4c (butylamine) and 4h (benzylamine) compounds proved to be promising compounds. These results indicate that simple aliphatic amines, alicyclic amines and aromatic amines are important for activity whereas substituted alicyclic amines with alkyl group

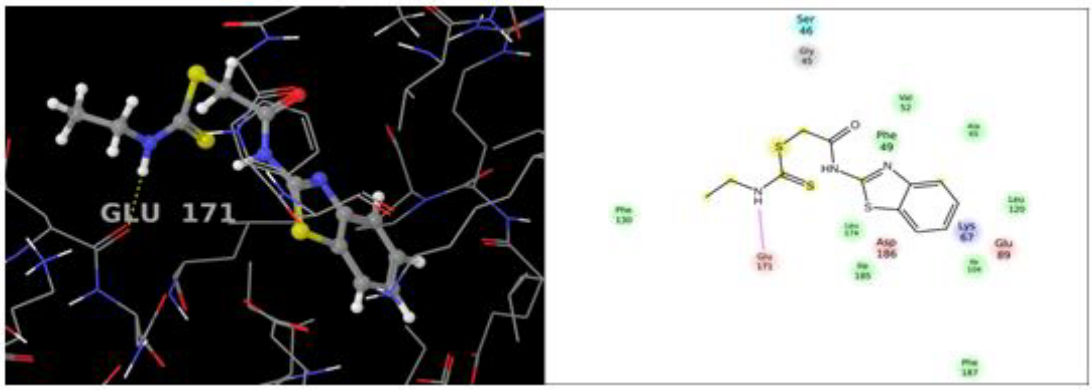

Fig. 2: Docking position of the compound 4a with PIM 1 Kinase protein (3BGQ)

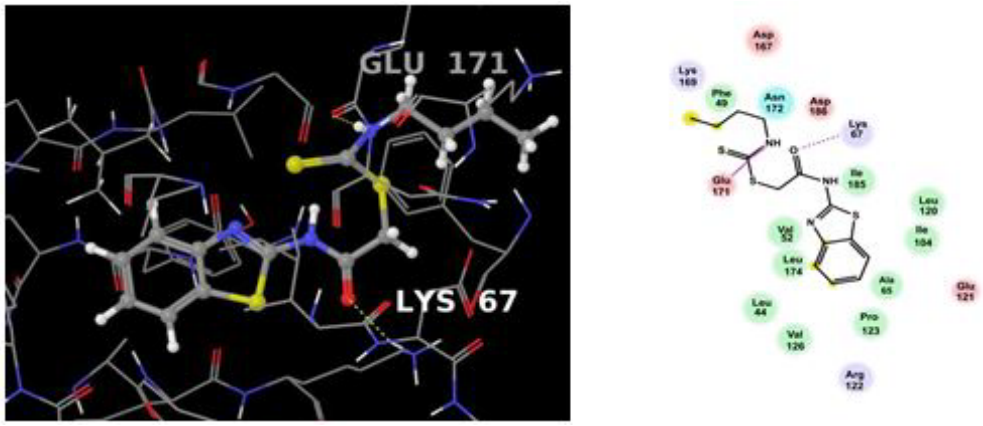

Fig. 3: Docking position of the compound 4c with PIM 1 Kinase protein (3BGQ)

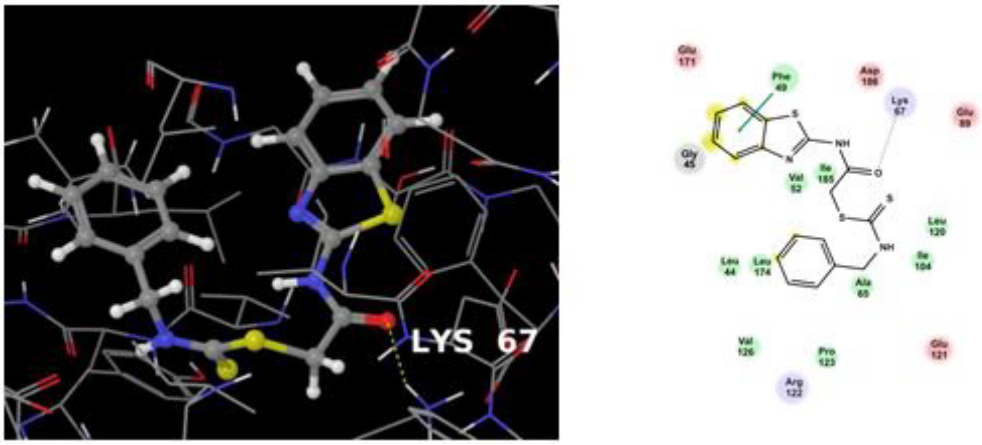

Fig. 4: Docking position of the compound 4h with PIM 1 Kinase protein (3BGQ)

decreased the activity. Envisaged from docking studies, all the compounds have mainly showed hydrophobic interactions through vander Waals contacts with the hydrophobic surface which has increased the receptor-ligand interaction, hydrogen bonding interactions and π–π interactions. With respect to the docking studies of the previously reported C-3 functionalised oxindole derivatives[38] interactions with a common amino acid LYS 67 has been seen. To the best of our knowledge, this is the first study exploring dithiocarbamate substituted derivatives as PIM 1 kinase inhibitors and to improve potency further structural optimization is required. Based on the obtained results, further studies on structural modifications, in vitro toxicity studies and PIM 1 Kinase inhibitory assay is underway.

Acknowledgements:

The authors are thankful to Telangana State Council of Science and Technology (TSCOST), Telangana, for funding this project. We are also thankful to Dr.M.Vijjulatha, Professor, Molecular modelling and Medicinal Chemistry lab, Osmania University for molecular docking studies. We are grateful to Dr. A. Anthony, Senior scientist, IICT, Hyderabad for carrying out anticancer screening studies. Finally we are thankful to the Principal and Management, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Hyderabad for providing the laboratory facilities for carrying out this work successfully.

Conflict of interest:

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Bertram JS. The molecular biology of cancer. Mol Aspects Med 2000;21(6):167-223.
2. Tsimberidou AM. Targeted therapy in cancer. Cancer Chemother Pharmacol 2015;76(6):1113-32.
3. Zhang X, Song M, Kundu JK, Lee MH, Liu ZZ. PIM Kinase as an Executional Target in Cancer. J Cancer Prev 2018;23(3):109-16.
4. Nawijn MC, Alendar A, Berns A. For better or for worse: the role of PIM oncogenes in tumorigenesis. Nat Rev Cancer 2011;11(1):23-34.
5. Arrouchi H, Lakhlili W, Ibrahimi A. A review on PIM kinases in tumors. Bioinformation 2019;15(1):40-5.
6. Tursynbay Y, Zhang J, Li Z, Tokay T, Zhumadilov Z, Wu D,

et al. PIM kinases as cancer drug target: An update (Review). Biomed Rep 2016;4(2):140-6.
7. Asati V, Mahapatra DK, Bharti, SK. PIM kinase inhibitors: Structural and pharmacological perspectives. Eur J Med Chem 2019;172:95-108.
8. Kumar A, Mandiyan V, Suzuki Y, Zhang C, Rice J, Tsai J *et al.* Crystal Structures of Proto-oncogene Kinase PIM 1: A Target of Aberrant Somatic Hypermutations in Diffuse Large Cell Lymphoma. J Mol Biol 2005;348(1):183-93.
9. Jeyapal GP, Chandrasekar MJN, Krishnasamy R, Selvaraj J, Mohammad M, Nanjan MJ. Potential Pharmacological Inhibitors of Pim Kinase Under Clinical Trials. Anticancer Agents Med Chem 2018;18(8):1100-14.
10. Arrouchi H, Lakhlili W, Ibrahimi A. A review on PIM kinases in tumors. Bioinformation 2019;15(1):40-5.
11. Foulks JM, Carpenter KJ, Luo B, Xu Y, Senina A, Nix R, *et al.* A Small-Compound Inhibitor of PIM Kinases as a Potential Treatment for Urothelial Carcinomas. Neoplasia 2014;16(5):403-12.
12. Chen LS, Redkar S, Taverna P, Cortes JE, Gandhi V. Mechanisms of cytotoxicity to Pim kinase inhibitor, SGI-1776, in acute myeloid leukemia. Blood 2011;118(3):693-702.
13. Cervantes-Gomez F, Stellrecht CM, Ayres ML, Keating MJ, Wierda WG, Gandhi V. PIM kinase inhibitor, AZD1208, inhibits protein translation and induces autophagy in primary chronic lymphocytic leukemia cells. Oncotarget 2019;10(29):2793-809.
14. Schroeder RL, Goyal N, Bratton M, Townley I, Pham NA, Tram P, *et al.* Identification of quinones as novel PIM1 kinase inhibitors. Bioorg Med Chem Lett 2016;26(13):3187-91.
15. Mohareb RM, Samir EM, Halim PA. Synthesis, and anti-proliferative, Pim-1 kinase inhibitors and molecular docking of thiophenes derived from estrone. Bioorg Chem 2019;83:402-13.
16. Noolvi MN, Patel HM, Kaur M. Benzothiazoles: search for anticancer agents. Eur J Med Chem 2012;54:447-62.
17. Zhao S, Zhao L, Zhang X, Liu C, Hao C, Xie H, *et al.* Design, synthesis, and structure-activity relationship studies of benzothiazole derivatives as antifungal agents. Eur J Med Chem 2016;123:514-22.
18. Ugale VG, Patel HM, Wadodkar SG, Bari SB, Shirkhedkar AA, Surana SJ. Quinazolino-benzothiazoles: fused pharmacophores as anticonvulsant agents. Eur J Med Chem 2012;53:107-13.
19. Singh MK, Tilak R, Nath G, Awasthi SK, Agarwal A. Design, synthesis and antimicrobial activity of novel benzothiazole analogs. Eur J Med Chem 2013;63:635-44.
20. Zhu XY, Etukala JR, Eyunni SVK, Setola V, Roth BL, Ablordeppey SY. Benzothiazoles as probes for the 5HT1A receptor and the serotonin transporter (SERT): A search for new dual-acting agents as potential antidepressants. Eur J Med Chem 2012;53:124-32.
21. Yurttas L, Tay F, Demirayak S. Synthesis and antitumor activity evaluation of new 2-(4-aminophenyl)benzothiazole derivatives bearing different heterocyclic rings. J Enzyme Inhib Med Chem 2015;30(3):458-65.
22. Csomós P, Zupkó I, Réthy B, Fodor L, Falkay G, Bernáth G. Isobrassinin and its analogues: novel types of antiproliferative agents. Bioorg Med Chem Lett 2006;16(24):6273-76.
23. Gaspar P, Banerjee T, Malachowski WP, Muller AJ, Prendergast GC, DuHadaway, J.; Bennett, S.; Donovan, A.M. Structure-Activity Study of Brassinin Derivatives as Indoleamine 2,3-Dioxygenase Inhibitors. J Med Chem 2006;49(2):684-92.
24. Duan Y, Ma Y, Zhang E, Shi X, Wang M, Ye X, et al. Design and synthesis of novel 1,2,3-triazole-dithiocarbamate hybrids as potential anticancer agents. Eur J Med Chem 2013;62:11-19.
25. Zou Y, Yu S, Li R, Zhao Q, Li X, Wu M. Synthesis, antifungal activities and molecular Docking studies of novel2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl) propyl Dithiocarbamates. Eur J Med Chem 2014;73:366-74.
26. Ding P, Gao M, Mao B, Cao S, Liu C, *et al.* Synthesis and biological evaluation of quinazolin-4(3H)-one derivatives bearing dithiocarbamate side chain at C2-position as potential antitumor agents. Eur J Med Chem 2016;10:364-73.
27. Altıntop MD, Sever B, Çiftçi GA, Kucukoglu K, Özdemir, A, Soleimani SS, *et al.* Synthesis and evaluation of new benzodioxole-based dithiocarbamate derivatives as potential anticancer agents and hCA-I and hCA-II inhibitors. Eur J Med Chem 2016;125:190-96.
28. Lafta SJ, Abass SJ. Synthesis and characterization of some 2-Aminobenzothiazole derivatives. Al-Mustansiriyah J Sci 2012;23(5):105-18.
29. Nethi SK, Rico-Oller B, Rodríguez-Diéguez A, GómezRuiz S, Patra CR. Design, synthesis and characterization of doped-titanium oxide nanomaterials with environmental and angiogenic applications. Sci Total Environ 2017:599:1263.
30. Bacharaju K, Jambula SR, Sivan S, Tangeda JS, Manga V. Design, synthesis, molecular docking and biological evaluation of new dithiocarbamates substituted benzimidazole and chalcones as possible chemotherapeutic agents. Bioorg Med Chem Lett 2012;22(9):3274-77.
31. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, *et al.* admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. J Chem Inf Model 2010;52(11):3099-105.
32. Zhang X, Song M, Kundu JK, Lee MH, Liu ZZ. PIM Kinase as an Executional Target in Cancer. J Cancer Prev 2018;23(3):109-16.
33. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA et al. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes. J Med Chem 2006;49(21):6177-96.
34. Jorgensen WL, Maxwell DS, Tirado-Rives J. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am 1996;118(45):11225-36.
35. Rudorf W. Reactions of Carbon disulfide with N-nucleophiles. J. Sulphur Chem 2007;28(3):295-339.
36. Abnous K, Manavi H, Mehri S, Alibolandi M, Kamali H, Ghandadi M, *et al.* *In vitro* evaluation of dihydropyridine-3-carbonitriles as potential cytotoxic agents through PIM-1 protein kinase inhibition. Res Pharm Sci 2017;12(3):196-20.
37. Wu Y, Deng Y, Zhu Y, Duan Y, Weng W, Wu X. Pim1 promotes cell proliferation and regulates glycolysis via interaction with MYC in ovarian cancer. Onco Targets Ther 2018;11:6647–56.
38. Sun H, Wang X, Li G, Zhang L, Liu J, Zhao L. Design, synthesis and biological evaluation of novel C3-functionalized oxindoles as potential Pim-1 kinase inhibitors. RSC Adv 2015;5(37):29456-66.