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

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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 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 uroepithelial cell carcinomas show a high expression

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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 benzylidine-1, 3- thiazolidine-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, antihelminthic, 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 benz[d]thiazol-2-ylcarbamoyl carbamodithioate derivatives, their cytotoxicity on ovarian cancer cell lines, anti-mitotic activity on Bengal gram seeds, drug likeness, 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 CDCl3 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 benzothiazole derivatives (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°; CDCl3: δ 0.95-0.98 (t, 3H, CH3), 2.44-2.51 (q, 2H, CH2CH3),-2.57-4.38 (m, 8H, N(CH2)2) (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 piperidine-1-carbodithioate (4e); Yield: 74 %, MP 142-144°; 15N3S3O2; IR (FT-IR-cm-1): 3275.65 (N-H, amine), 1697.83 (C=O), 1242.29 (C=S); 1H NMR (400 MHz, CDCl3): δ 0.80-0.90 (m, 6H, (CH2)2 of piperazine), 4.40 (s, 2H, CH2S), 7.28-7.32 (t, 1H, ArH), 7.40-7.44 (t, 1H, ArH), 7.79-7.81 (q, 2H, ArH), 10.62 (s, 1H, NH); 13C NMR (100 MHz, CDCl3, 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) % 367.07 (M+1) (Calculated 366.06).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl 4-ethyl pipеразин-1-carбодитиоат (4f); Yield: 86 %, MP 146-148°; 15N3S3O2; IR (FT-IR-cm-1): 3275.65 (N-H, amine), 1697.83 (C=O), 1242.29 (C=S); 1H NMR (400 MHz, CDCl3): δ 0.80-0.90 (m, 6H, (CH2)2 of piperazine), 4.40 (s, 2H, CH2S), 7.28-7.33 (t, 1H, ArH), 7.41-7.45 (t, 1H, ArH), 7.79-7.82 (q, 2H, ArH), 10.58 (bs, 1H, NH); HRMS (m/z) % 367.07 (M+1) (Calculated 366.06).

(Benzo[d]thiazol-2-ylcarbamoyl) methyl 4-ethyl pipеразин-1-carбодитиоат (4g); Yield: 76 %, MP 148-150°; 15N3S3O2; IR (FT-IR-cm-1): 3275.65 (N-H, amine), 1694.89 (C=O), 1030.41 (C=S); 1H NMR (400 MHz, CDCl3): δ 0.90-1.12 (t, 3H, CH3), 2.44-2.50 (q, 2H, CH2CH3),-2.57-4.38 (m, 8H, N(CH2)2 of piperazine), 4.40 (s, 2H, CH2S), 7.27-7.30 (t, 1H, ArH), 7.40-7.45 (t, 1H, ArH), 7.79-7.82 (d, 2H, ArH), 10.64 (s, 1H, NH); 13C 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).
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 cm² tissue culture flasks in a humidified atmosphere of 5% CO₂ at 37°C. All cytotoxicity experiments were carried out in 96 micro titre well plates. All the consumables and plastic-ware 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.

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 5×10⁴ cells/ml in culture medium for 3 h at 37°C and 5% CO₂, 75% relative Humidity. 

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, 1H 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

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*Fig. 1: Representative scheme for the Synthesis of 2-amino benzothiazole dithiocarbamate derivatives*

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 further 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, 4c 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 | IC50 (µM) |
|-------------|-----------|
| 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 |

### Table 2: Results of Anti Mitotic Assay of Compounds 4a-h

| Sample code | IC50 (µM) |
|-------------|-----------|
| 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 |

### Table 3: Predicted ADMET Properties of Compounds 4a-h

| Sample code | Human intestinal absorption (HIA, %) | In vitro Caco-2 Cell Permeability | In vitro MDCK Cell Permeability | In vitro Plasma Protein binding (%d) | In vivo Blood-barrier Penetration | Toxicity |
|-------------|-------------------------------------|----------------------------------|---------------------------------|-------------------------------------|----------------------------------|----------|
|             | (nm/sec)                            | (nm/sec)                         |                                |                                    |                                  |          |
| 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                            | 95.66                               | 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 |

*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, tumorigecity, irritancy, hepatotoxicity and reproductive toxicity. The other compounds showed mutagenic and tumorogenic 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 mutagenic activity while the most potent synthesized 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 cells 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 Lipinski’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**

| Sample code | Bioactivity |  |
|-------------|-------------|---|
| 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 |

**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. (cm³/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 | -4.06 |
| 4c     | 339.51 | 2 | 5 | 3.92 | 96.15 | 0 | 139.65 | 8 | 0 | 320.65 | -4.40 |
| 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 | -3.8 |
| SGI1776 | 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 (PDB 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 synthesized derivatives can serve as promising leads for inhibition of PIM 1 kinase enzyme.

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 activities 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

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

| Compound | Docking score | Glide E energy (Kcal/mol) | Interacting residues |
|----------|---------------|---------------------------|---------------------|
| 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      |

Fig. 2: Docking position of the compound 4a 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.

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

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