*In–Silico d*esign, synthesis, characterization and biological evaluation of novel 2-azetidinone derivatives for anti–Leukemic activity:

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Abstract: 2-Azetidinone shows biological activities like anti-bacterial, anti-microbial activity, anti-tubercular activity, and anti-cancer activity. 2-azetdinone derivatives were synthesized by simple procedures. The first step is synthesis of benzohydrazide through nucleophilic substitution reaction between methyl benzoate and hydrazine hydrate. The above formed compound is then treated with substituted aromatic aldehydes in the presence of catalytic amount of concentrated hydrochloric acid with stirring for one hour to give benzohydrazone which results in the formation of Schiff bases.Schiff bases undergone cyclisation in the presence of chloroacetylchloride and diethylenediamine by using ethanol as a solvent upon stirring for 4 hour's yielded 2-azetidinone derivatives. The *in-silico* anti-leukemic activity was determined by using the computational tools i.e. "PASS Online", "AutoDock4.2" and "ADMET" properties by online software's. Among these six derivatives compounds (AZT-6) was shown more activity when compared with the other five compounds.

Keywords: 2-azetidinone, *In-Silico* drug design, Anti-leukemic, TPK-BCR-ABL-1, Docking.

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I. INTRODUCTION

Tyrosine Protein Kinase BCR-ABL- 1 gene is a chimeric protein which is necessary to play a central role in the pathogenesis of Philadelphia (Ph) chromosome-positive leukemias, notably chronic myeloid leukemia (CML) [1]. Chronic myeloid leukemia (CML) contains primitive hematopoietic progenitor cells which is a clonal myeloproliferative disorder. The occurrence of chronic myeloid leukemia results by the Philadelphia translocation t (9;22) which fuses the long arm parts of chromosome 9 to chromosome 22 results in the formation of the hybrid gene, BCR-ABL-1. This protein product will be found in more than 95% of chronic myeloid leukemia patients which is a major cause of the disease [2].

Most significant areas of research in the field of medicinal chemistry were carried out on the heterocyclic compounds. Heterocyclic 2-azetidinones are considered as an important contribution to science and humanity, because they are the constituents of living organisms, natural products, drugs and many more substances which will be more useful to the mankind and society in all walks of life. The attention of the chemists has always drawn over the years for the synthesis of heterocyclic compounds because of their biological properties. Depending upon their physiological and industrial significances they are equally interesting for its theoretical implication for the diversity. Heterocyclic compounds are the large number of drugs introduced in pharmacopoeias every year. Synthesis and evaluation of the heterocyclic compounds has drawn more attention for the chemists and biologists over the years [3-10]. Azetidinones are four membered nitrogen containing heterocyclic's which are useful substrates in organic chemistry for the design and preparation of biologically active compounds by the adequate fictionalization in the different positions of the ring. The azetidinones are a part of antibiotic structure, figure 1 illustrates the general mechanism of its synthesis. Azetidinones posses various biological activities such as antibacterial [11-13], antifungal [14], anti-inflammatory [15-16], anti-

convulsant [17], anti-tubercular [18], anti-cancer and antibiotic activities [19-20].

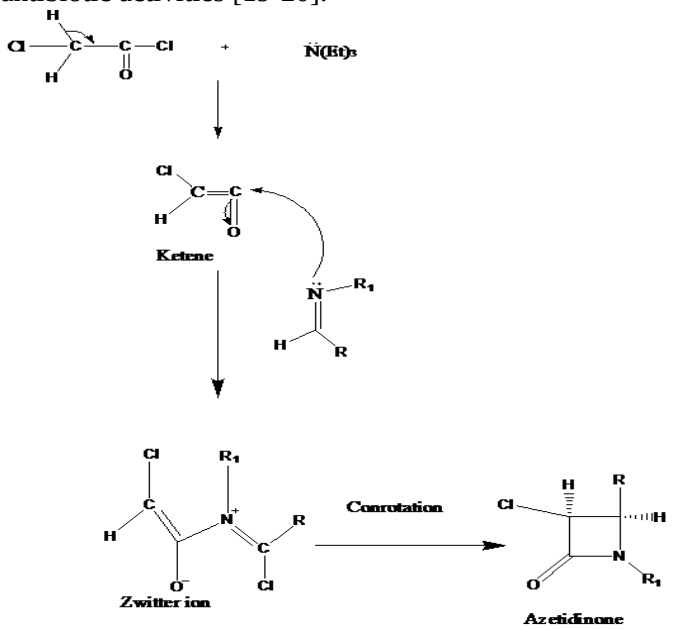


Figure 1: General mechanism of azetidinones synthesis.

These can also function as enzyme inhibitors and are effective against the central nervous system. These are carbonyl derivatives of azetidines which contain carbonyl group at position-2. So, these are called as 2-azetidinones or more commonly β-lactams. The very first β- lactam was synthesized by H. Straudinger in 1907 via [2+2] cycloaddition reaction of katane and imine and this reaction is termed as the "Staudinger synthesis". The importance of the β- lactams has been establisted after the discovery of the penicillin by "Alexander Flemming" in 1928 observed bacteriolysis in a nutrient broth at St. Mary's Hospital in London [11].

According to the kubinyi most of the drugs in the past were discovered by serendipitously where serendipity plays an important role in finding new drugs [21-23]. At present, the drug discovery is shifted towards drug design, where results depends on understanding the biochemistry of the disease, identifying disease causative proteins, pathways and then designing compounds that are capable of modulating the role of these proteins. In this approach, both experimental and computational methods play an important in the drug discovery and development process via reducing the cost, time and failure chances at late stage of drug discovery pipeline. Some of the benefits of invovling CADD at early stage of drug development are: **a.** More efficient drug discovery and development process can be achieved by computer based methods. **b.** Information related to the chemical and biological databases will help in identifying the ligands and targets to optimize novel drugs. **c.** Drug likeness or pharmacokinetic properties for the chemical compounds should be screened in order to enable early detection of the compounds which are likely to fail in clinical trials and further to enhance detection of promising entities [24-25]. Typical workflow of early stage drug discovery using CADD is shown in figure 2.

II. Results and Discussion

In this present research work, six derivatives of 2-azetidinones were synthesized in three step facile procedure with good yields. All the reactions were monitored by TLC and purification was done by recrystallization process. All the derivatives were characterized using spectral studies like FT-IR spectroscopy, ^{1}H-NMR spectroscopy and Mass spectrometry. Six derivatives were screened for *In- silico* Anti-leukemic activity, based on the *in- silico* results the title compounds were screened for *In- vitro* anti-leukemic activity.

In- silico anti-leukemic screening:

In order to prove the alternative hypothesis, *In Silico* estimation of activity for the synthesized derivatives had been performed using PASS online web resource. The results showed that the studied compounds were having good anti-leukemic activity compared to that of current marketed drugs. This had given the assurance to take the research to further level in future for screening the anti-leukemic activity using other targets.

In- vitro Anti-leukemic activity:

The anti-leukemic activity of the title compounds were done by the MTT assay, the target is Tyrosine protein kinase BCR-ABL-1 Gene which was performed by using Bosutinib as reference standard. Among all the six derivatives the compound AZT-6 exhibited potent anti-leukemic activity when compared with the other derivatives.

In-silico anti-leukemic screening :

Table 1: Estimation of probability of activity by PAAS. (TPKI = Tyrosine protein kinase inhibitor | AL = Anti-leukemic activity)

Compound	Pa	Pi	Activity
Bosutinib	0.786	0.003	TPKI
AZT-5	0.957	0.003	AL
AZT-6	0.962	0.003	AL
AZT-1	0.979	0.002	AL
AZT-3	0.964	0.003	AL
AZT-2	0.959	0.003	AL
AZT-4	0.958	0.003	AL

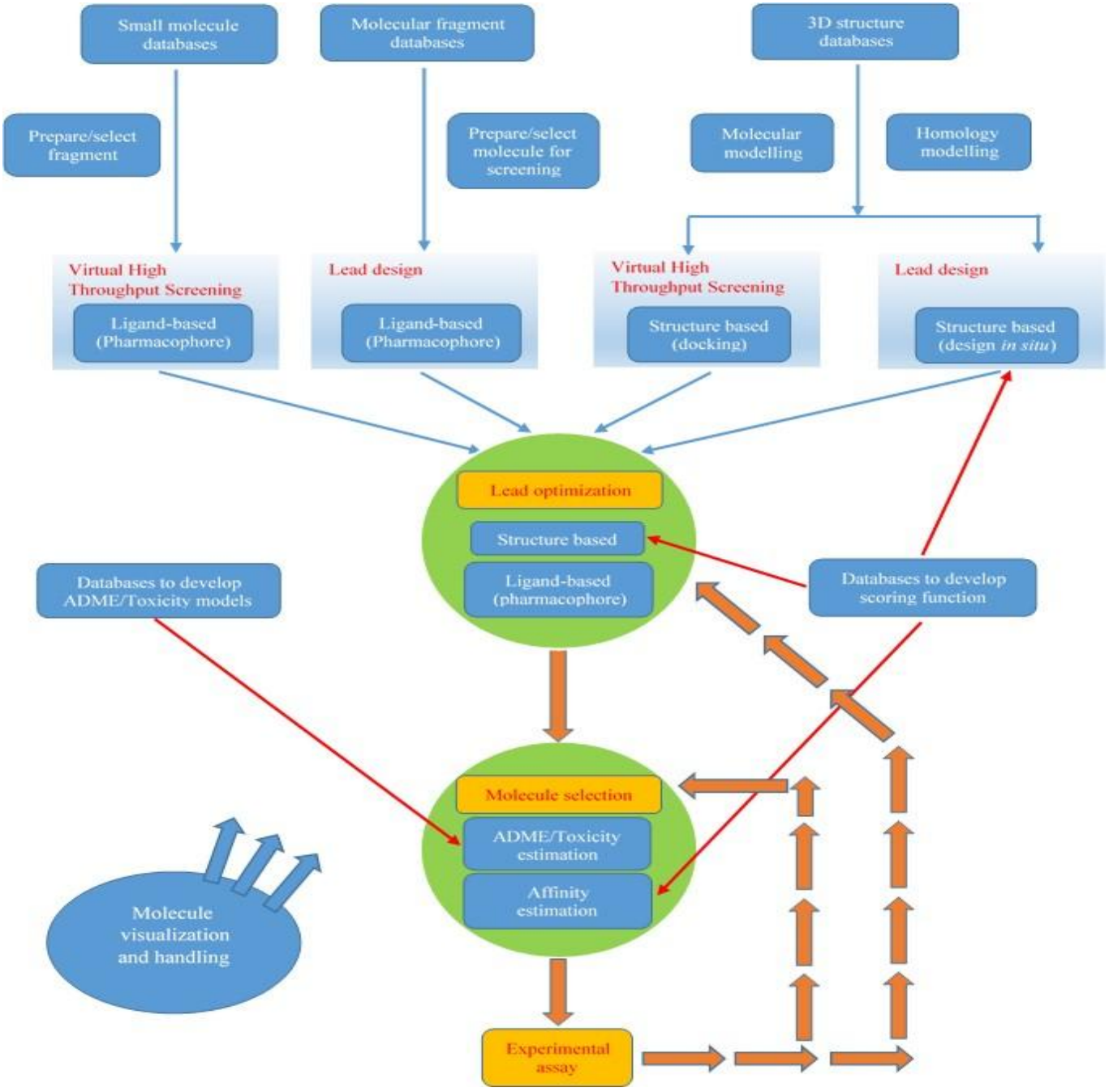


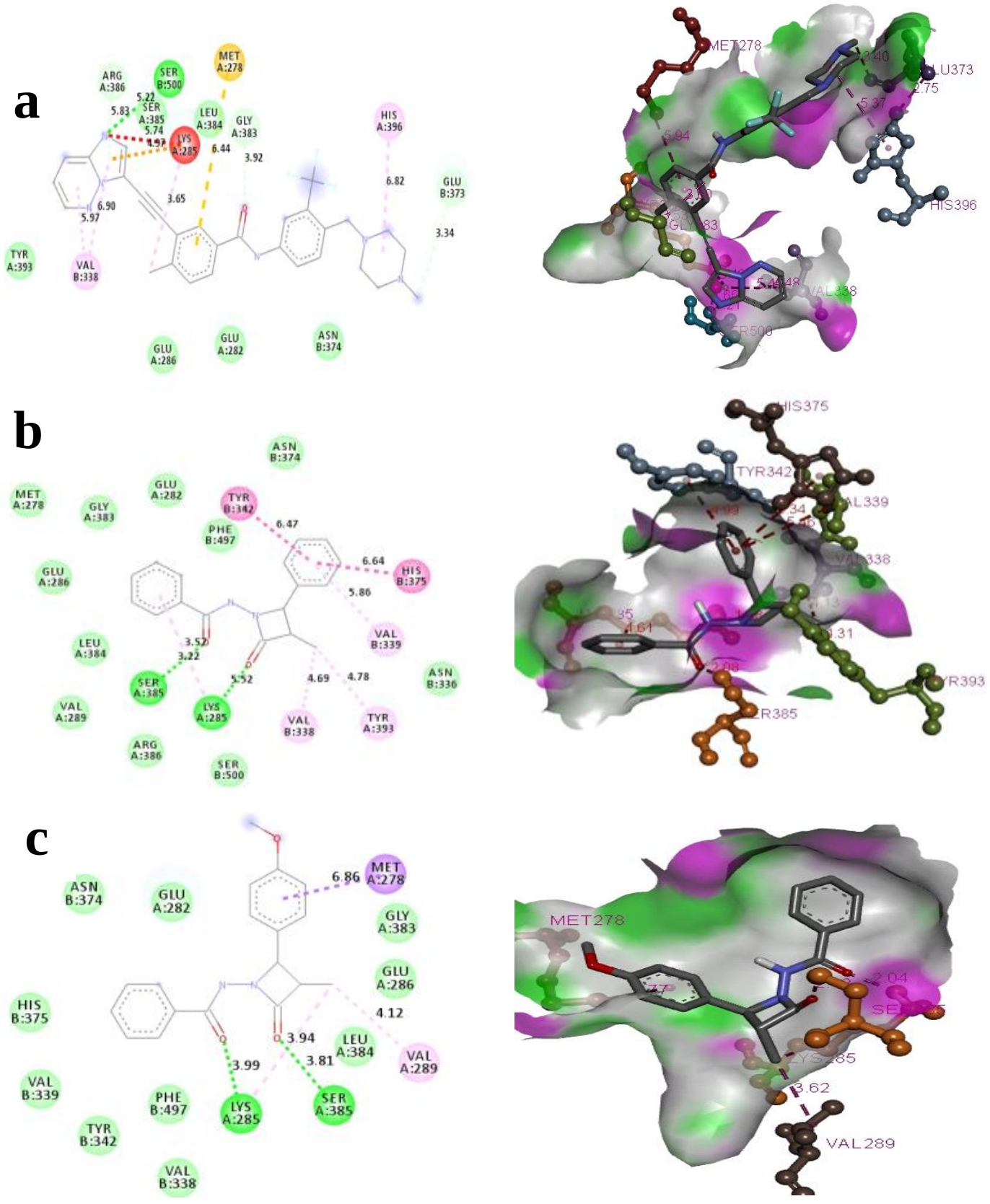
Figure 2: Typical workflow of Computer Aided Drug Discovery (CADD):

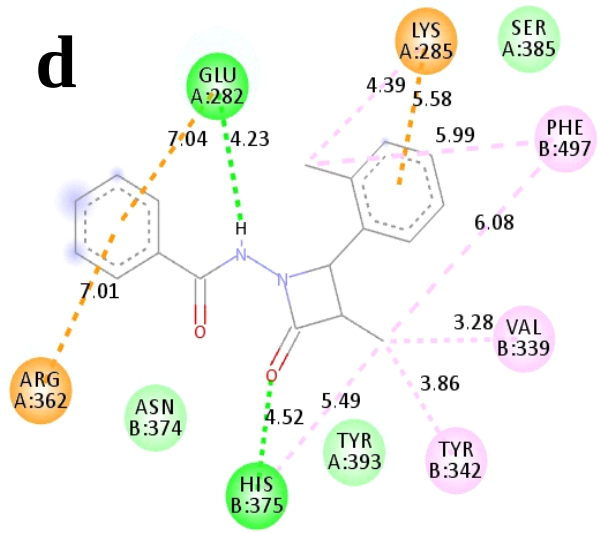
QSAR, Drug Likeness and Docking results:

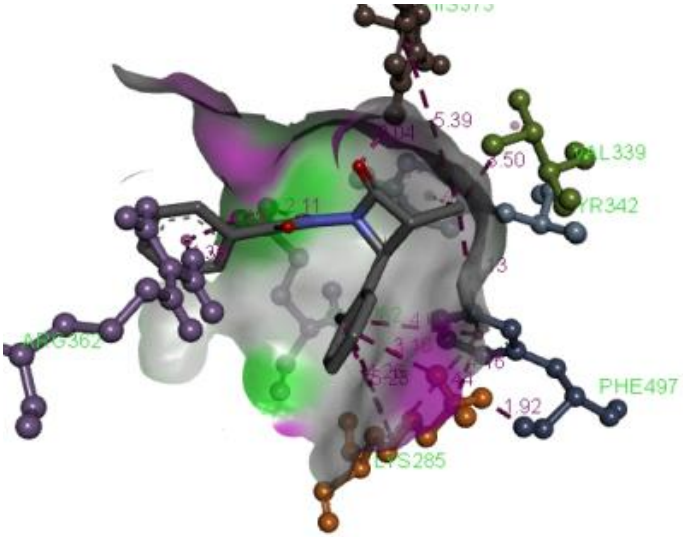
Molecular docking studies of TPK-BCR-ABL-1 (PDB ID: 2HZI) with designed potential inhibitors was carried out by using Auto dock 4.2. And the results has been tabulated in table 2. 2D and 3D snapshots depicting the docking poses along with molecular level interactions responsible for the binding have been shown in figure 3 for the standard control drug Bosutinib and six novel 2-Azetidinone derivatives respectively.

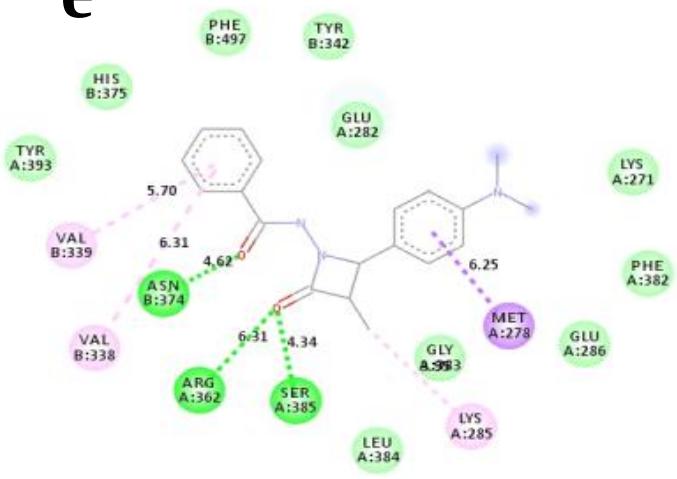
Table 2. Docking scores for standard drug and samples

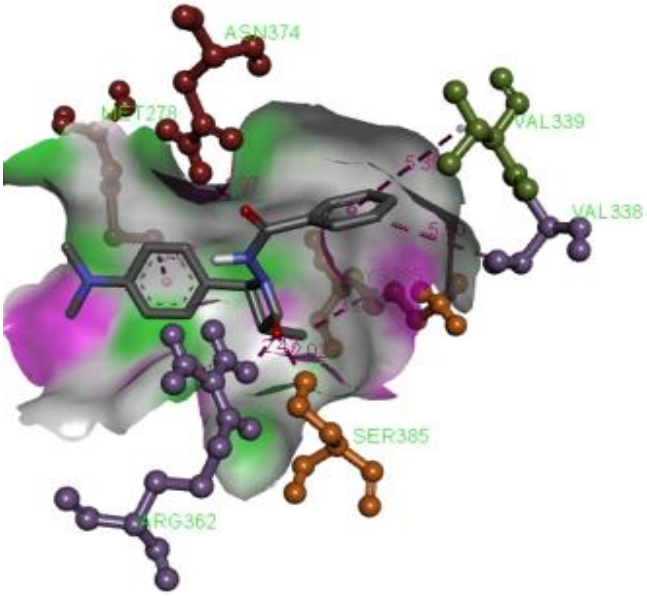
S.No	Compound	Binding Energy (Kcal/mol)
1	Bosutinib	-7.19
2	AZT-1	-6.57
3	AZT-2	-6.88
4	AZT-3	-4.37
5	AZT-4	-6.08
6	AZT-5	-6.51
7	AZT-6	-7.24

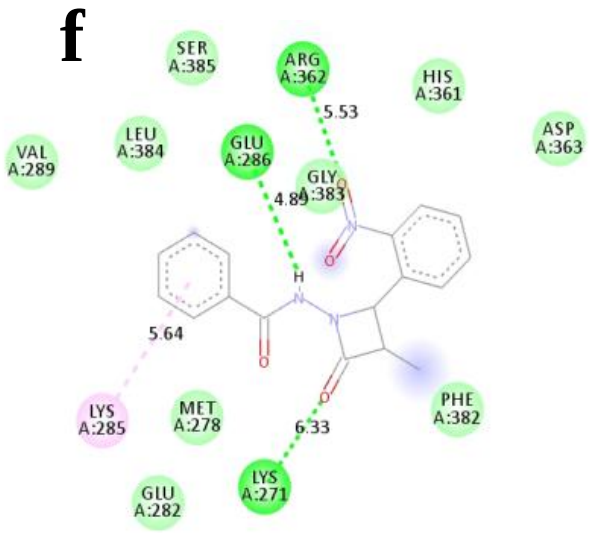


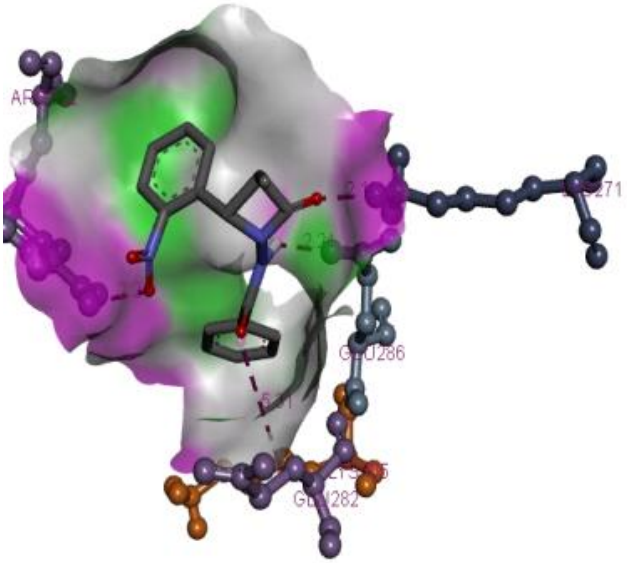












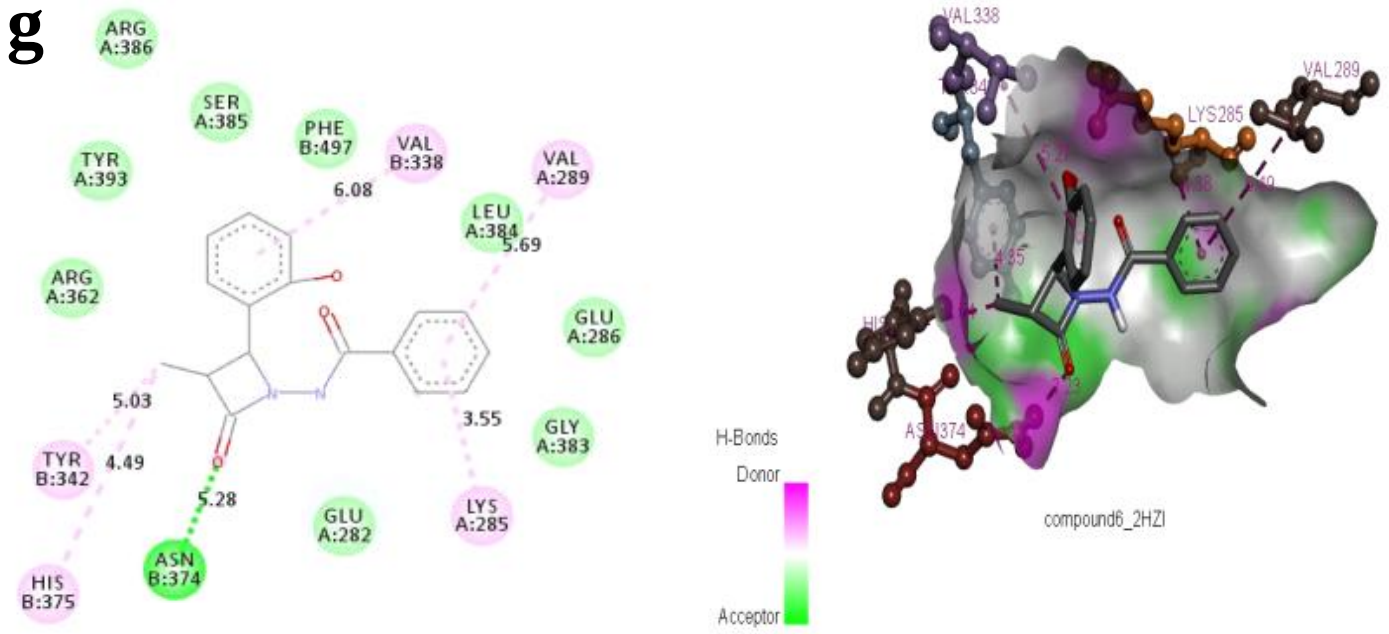


Figure 3: 2D and 3D docking snapshots of **a)** Bosutinib, **b)** AZT-1, **c)** AZT-2, **d)** AZT-3, **e)** AZT-4, **f)** AZT-5 and **g)** AZT-6 showing molecular level interactions.

Evaluation of pharmacokinetics, drug likeness and medicinal chemistry friendliness of molecules – Swiss ADME

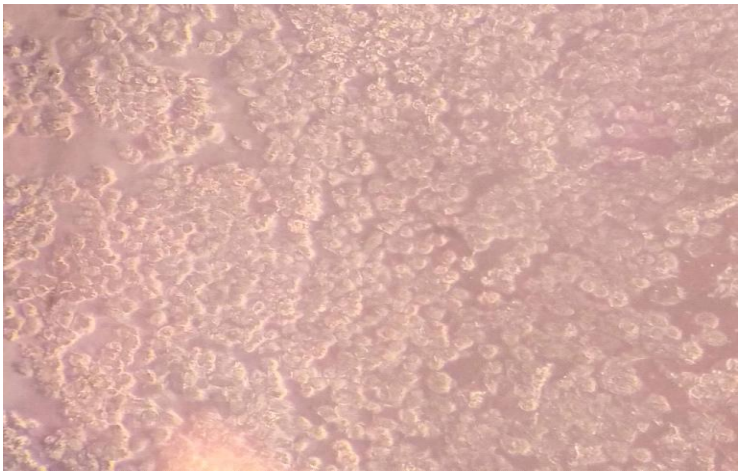
To be effective as a potent drug, a molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited. In that context, computer models constitute valid alternatives to experiments. The Swiss ADME web tool that gives easy efficient input, free access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILED Egg, iLOGP and Bioavailability Radar to support drug discovery endeavors. Class: <10 - Insoluble, 10 - Poorly, 6-Moderetly, 4 - soluble, 2 - very, 0 highly. During the time- and resource-consuming processes of drug discovery and development, a large number of molecular structures are evaluated according to very diverse parameters in order to steer the selection of which chemicals to synthesize, test and promote, with the final goal to identify those with the best chance to become an effective medicine for the patients. The molecules must show high biological activity together with low toxicity. Equally important is the access to and concentration at the therapeutic target in the organism. It has been demonstr-ated that early estimation of ADME in the discovery phase reduces drastically the fraction of pharmacokinetics-related failure in the clinical phases 1. As per the Swiss ADME predictions, results of which were tabulated in tables 2 and 3; all the synthesized 2-Azetidinones are as per Lipinski's rule. Hence all the synthesized compounds have potential drug likeness, leadlikeness, skin permeation and synthetic accessibility.

***In-vitro* Antileukemic Activity:**

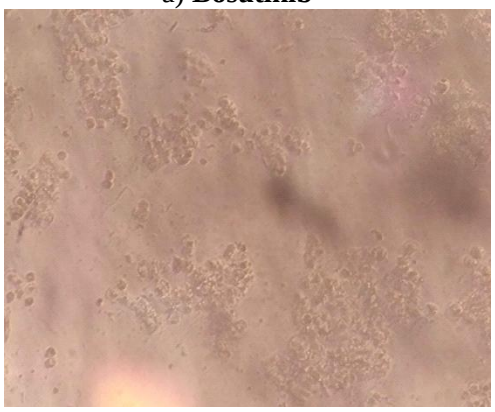
Antileukemic activity of the synthesized derivatives was performed by the MTT assay and the results were shown below. As per the results shown in tables 4 and 5 along with the photomicrographs shown in figure 4, among the six derivatives compound six have shown potent activity when compared to other derivatives.

III. CONCLUSION

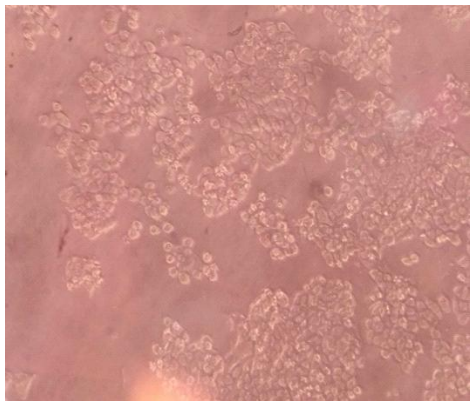
2-azetidinones derivatives were synthesized, characterized and screened for *in-silico* antileukemic and *in-vitro* antileukemic activities using respective standards. *In-silico* antileukemic screening was performed by PASS online web resource. The results of *in-vitro* antileukemic screening revealed that compound AZT-6 shown good activity when compared to standard among all the derivatives. This is may be due to the fact that the target TPK-BCR- ABL-1 is hydrophilic in nature and highly polar, but the designed titled compounds are lipophilic in nature and are less polar this may be the one of the reason but not the only reason for rejecting null hypothesis.



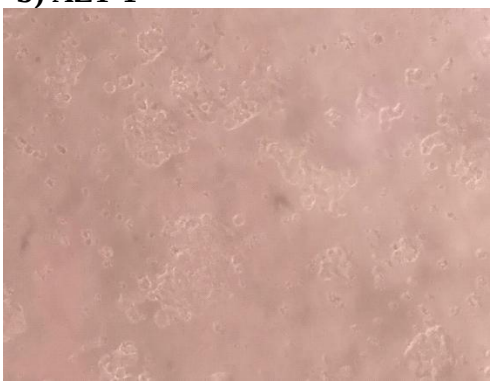
a) **Bosutinib**



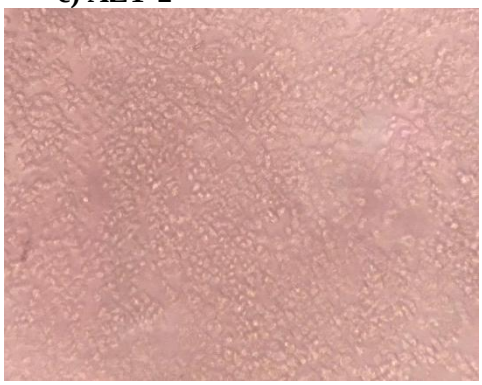
b) AZT-1



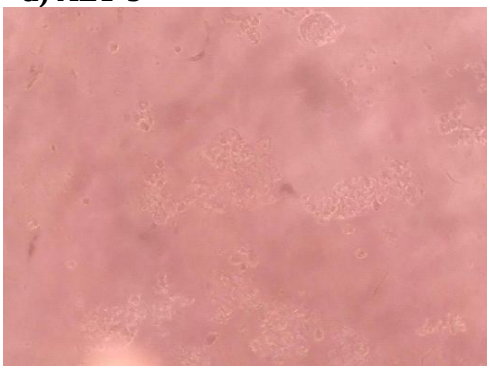
c) AZT-2



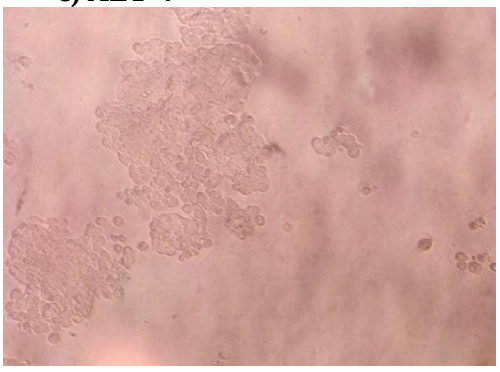
d) AZT-3



e) AZT-4



f) AZT-5



g) AZT-6

Figure 4: Photomicrograph of MTT assay of the cells treated with a) Bosutinib, b) AZT-1, c) AZT-2, d) AZT-3, e) AZT-4, f) AZT-5 and g) AZT-6.

Table 2: ADME properties of the studied compounds

Compound Code	Log P	Total polar surface area	No. of H-bond	No. of H-bond donors	No. of violations	No. of rotatable bonds	Molar refractivity	GI Absorption	BBB Permeant	Log Kp cm/s	Bioavailability score	Synthetic accessibility
AZT-1	2.14	49.41	2	1	0	4	83.23	High	No	-6.11	0.55	2.95
AZT-2	2.40	49.41	2	1	0	4	88.20	High	No	-5.72	0.55	3.05
AZT- 3	2.17	49.41	2	1	0	4	88.24	High	No	-5.87	0.55	3.19
AZT-4	2.60	52.65	2	1	0	5	97.44	High	No	-6.29	0.55	3.19
AZT-5	1.67	95.43	4	1	0	5	92.05	High	No	-6.51	0.55	3.11
AZT-6	1.80	69.64	3	2	0	4	85.26	High	No	-6.46	0.55	3.40
Bosutinib	4.58	82.88	7	1	3	9	150.6	High	No	-5.72	0.55	3.76

Table 3: Toxicology profiling of the studied compounds

Compound Code	Class	LD_{50} mg/kg	Hepatoxicity Prediction & Probability	Carcinogenicity Prediction & Probability	Immunotoxcity Prediction & Probability	Mutagenicity Prediction & Probability	Cytotoxcity Prediction & Probability
AZT-6	4	2000	Active & 0.58	Inactive & 0.53	Inactive & 0.99	Inactive & 0.69	Inactive & 0.73
AZT-5	4	1500	Active & 0.52	Inactive & 0.61	Inactive & 0.99	Active & 0.52	Inactive & 0.75
AZT-4	4	1500	Active & 0.52	Inactive & 0.57	Inactive & 0.99	Inactive & 0.64	Inactive & 0.62
ZT- 3	4	1500	Active & 0.53	Active & 0.54	Inactive & 0.99	Inactive & 0.73	Inactive & 0.77
AZT-2	4	1500	Active & 0.58	Inactive & 0.57	Inactive & 0.99	Inactive & 0.67	Inactive & 0.66
AZT-1	4	1500	Active & 0.53	Active & 0.56	Inactive & 0.99	Inactive & 0.71	Inactive& 0.73
Bosutinib	4	849	Inactive & 0.60	Inactive & 0.54	Active & 0.97	Active & 0.58	Inactive & 0.70

Table. 4: IC_{50} value of coumpounds with K562 cell lines in µM/mL:

S.No	Compound	IC_{50} value (µM/mL)
1	AZT-1	15.27
2	AZT-2	9.05
3	AZT-3	627.45
4	AZT-4	34.7
5	AZT-5	16.78
6	AZT-6	4.89
7	Bosutinib	5.38

The results of *in - vitro* antileukemic screening revealed that the synthesized derivatives have good antileukemic activity when compared that of current marketed drugs. However, further research work need to be carried out to know the relationship between structure and biological activity. The further scope of present research work is to establish the antileukemic activity of the synthesized derivatives on other targets, especially on *in-vivo* antileukemic activity and QSAR studies.

IV. Materials & Methods

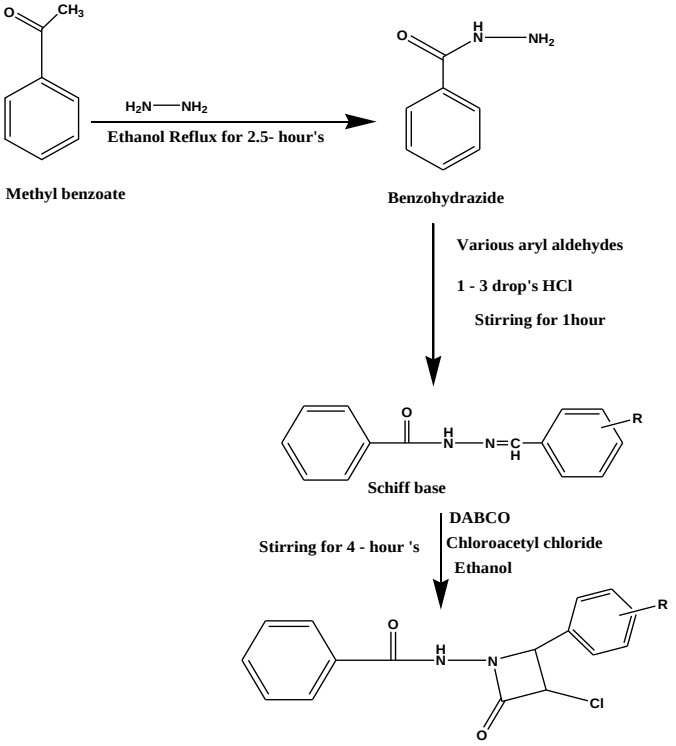
All solvents and chemicals were used as purchased without further purification. In the present work methyl benzoate and hydrazine hydrate have been chosen as starting materials. The progress of all reactions was monitored on Merck precoated silica gel plates (with fluorescence indicator UV 254) using ethyl acetate/n-hexane as solvent system. Spots were visualized by irradiation with ultraviolet light (254 nm). Melting points (mp) were taken in open capillaries on a Ana lab melting

point apparatus. Proton (1 H) NMR spectra were recorded on a Bruker Avance 500 (500.13 MHz for 1 H) using $CDCl_3$ as solvent. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for 1 H). IR spectra were recorded on a Varian 800 FT-IR Bruker series. GC- MS mass spectrometry (Apex) has been used for determining the molecular weight of the compounds.

Materials:

Diethylene diamine was purchased from Avra and Hydrazine hydrate from Molychem and other chemicals were purchased from SD FINE Chemicals. All the other chemicals are of AR grade. Purity of the compound was checked by using Pre coated aluminum sheets [n-hexane: Ethylacetate (8:2)] were detected by UV chamber.

Scheme of work:



N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)benzamide

Compound	R
AZT-1	H
AZT-2	4-OCH_3
AZT-3	2-Cl
AZT-4	4- $N(CH_3)_2$
AZT-5	2 - NO_2
AZT-6	2 - OH

General method for the synthesis of studied compounds :

The procedure for the synthesis of compounds consists of three steps.

Step-1*: *Synthesis of Benzohydrazide:

The methyl benzoate (0.1 mol) in 10 ml of ethanol and hydrazine hydrate (80% 0.1mol) was refluxed for 2.5 hour's. The completion of the reaction was checked by a thin layer chromatography. Then the mixture was cooled and then filtered. The solid mass was crystallized from ethanol to give hydrazides.

Step-2: Synthesis of benzhydrazone:

To the above mixture of benzohydrazide (0.01 mol) and various aryl aldehyde (0.01mol) dissolved in 20 ml of water. To the above mixture three drops of concentrated Hydrochloric acid was added thoroughly with continuous stirring for 1hour at room temperature and insoluble solid was generated & washed with water, dried and recrystallized from ethanol.

Step-3: Synthesis of azetidinones:

0.02mol of substituted Schiff base and 0.04mol of chloroacetyl chloride in the presence 0.04 mol of diethylene diamine by taking the 30 ml ethanol as a solvent. The reaction mixture was undergone for continuous stirred for 4 hours. After the completion of reaction the reaction mixture was kept for 48 hours at room temperature. Then concentrated, mixture was cooled, with ice cold water and filtered the mixture. Then the obtained solid was then dried and recrystallised from hot ethanol.

N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)benzamide:

Yellow Solid, Yeild-90%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, 10(H) Ar-H=7.4. IR (Kbr): (C-Cl -Stretch) 805, (Primary and secondary amine (NH_2) Wagging) 850.13, (C-C aromatic Stretch) 1475, (C=O amide and C=O azt (Stretch) 1563 and 1606, (C-H aromatic (Stretch) 2746, (Primary and secondary amine (NH_2) Stretch) 2936. GC-MS spectrometry results for $C_{16}H_{13}ClN_2O_2$: 304.74. Melting point-148-150°c.

N-(3-chloro-2-(4-methyoxyphenyl)-4-oxoazetidin-1-yl)benzamide:

Yellow, Yeild-92%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, 1H-CH= 3.73, S, 9(H) Ar-H=7.4. IR (Kbr): (C-Cl -Stretch) 805, (Primary and secondary amine (NH_2) Wagging) 850, (C-C aromatic Stretch) 1430, (C=O amide and C=O azt (Stretch)

1475.36 and 1913, (C-H aromatic (Stretch) 2748.03, (Primary and secondary amine (NH_2) Stretch) 2937.29. GC-MS spectrometry results for $C_{17}H_{15}ClN_2O_2$: 314.77. Melting point-148-149°c.

N-(3-chloro-2-(2-chlorophenyl)-4-oxaazetidin-1-yl)benzamide:

Light Yellow, Yeild-89%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, S, 10(H) Ar-H-7.4. IR (Kbr): (C-Cl -Stretch) 805, (Primary and secondary amine (NH_2) Wagging) 850, (C-C aromatic Stretch) 1440, (C=O amide and C=O azt (Stretch) 1475.55 and 1913.17, (C-H aromatic (Stretch) 2748.22, (Primary and secondary amine (NH_2) Stretch) 2937. GC-MS spectrometry results for $C_{16}H_{12}Cl_2N_2O_2$.: 335.18. Melting point-149-150°c.

N-(3-chloro-2-(4-dimethylamino)phenyl)-4-oxoazetidin-1-yl)benzamide:

Dark Red , Yeild-88%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, S, 9(H) Ar-H=7-8. IR (Kbr): (C-Cl -Stretch) 730, (Primary and secondary amine (NH_2) Wagging) 812, (C=O amide and C=O azt (Stretch) 1649 and 1705, (C-H aromatic (Stretch) 2935, (Primary and secondary amine (NH_2) Stretch) 3405. GC-MS spectrometry results for $C_{18}H_{18}ClN_3O_2$.: 343.81. Melting point-148-150°c.

N-(3-chloro-2-(nitrophenyl)-4-oxoazetidin-1-yl)benzamide:

White, Yeild-85%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, S, 1H,-CH-=4-5, S, 9(H) Ar-H=7-8. IR (Kbr): (C-Cl -Stretch) 849.44, (Primary and secondary amine (NH_2) Wagging) 896.96, (Nitro compound(N-O) assymetric(Stretch) 1293.91, (C=O amide and C=O azt (Stretch) 1566.65 and 1624.72, (C-H aromatic (Stretch) 2973.43, (Primary and secondary amine (NH_2) Stretch) 3387.06. GC-MS spectrometry results for $C_{16}H_{12}ClN_3O_4$.: 345.74. Melting point-148-150°c.

N-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide:

Yellow, Yeild-90%, ^{1}H-NMR: Chemical shift (δ), ppm (500.13 MHz, $CDCl_3$): δ =3, S, S, 1H,-OH-=4, S, 9(H) Ar-H=7-8.4. IR (Kbr): (C-Cl -Stretch) 804.63, (Primary and secondary amine (NH_2) Wagging) 850.77,(C=O amide and C=O azt (Stretch) 1521.91 and 1612.12, (C-H aromatic (Stretch) 2748.36, (Primary and secondary amine (NH_2) Stretch) 2937. GC-MS spectrometry results for $C_{16}H_{13}ClN_2O_3$: 316.74, Melting point-147-150°c.

In-Silico activity screening:

PASS Online server [26]:

This can be performed through an online web resource called PASS (Prediction of Activity Spectrum of Substances), which is a novel theoretical approach used to screen the novel pharmacological activities of the title compounds. PASS is an online program, which compare the structure of the novel compound with the well known biologically active compounds and predicts the activity of the formulated compounds. By using this thousand's of compounds can be screened for their novel pharmacological activities. For the prediction of compounds for their novel pharmacological activities the chemical formula was necessary and can be predicted by drawing in chemsketch and submitted into PASS online for the possible mechanism of actions.

Docking studies:

Auto Dock is an automated procedure for predicting the interaction of ligands with biomacromolecular targets [27]. The motivation for this work arises from problems in the design of bioactive compounds, and in particular the field of computer-aided drug design.

Docking Protocol:

AutoDock4.2 is parameterized to use a model of the protein and ligand that includes polar hydrogen atoms, but not hydrogen atoms bonded to carbon atoms. An extended PDB format, termed PDBQT, is used for coordinate files, which includes atomic partial charges and atom types. The current Auto Dock force field uses several atom types for the most common atoms, including separate types for aliphatic and aromatic carbon atoms, and separate types for polar atoms that form hydrogen bonds and those that do not. PDBQT files also include information on the tensional degrees of freedom. In cases where specific side chains in the protein are treated as flexible, a separate PDBQT file is also created for the side chain coordinates. Auto Dock Tools, the Graphical User Interface for Auto Dock, may be used for creating PDBQT files from traditional PDB files. Auto Dock Tools includes a number of methods for analyzing the results of docking simulations, including tools for clustering results by conformational similarity, visualizing conformations, visualizing interactions between ligands and proteins, and visualizing the affinity potentials

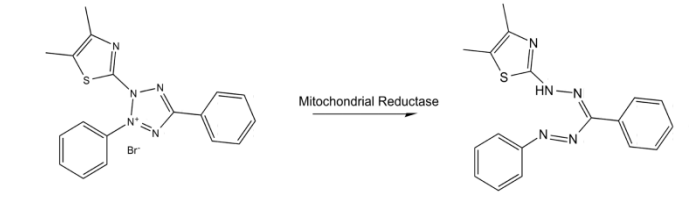
created by Auto Grid details of which has been explained elsewhere in various instances [28-31]. All the docking studies are done using AUTODOCK 4.2 version and the images are rendered using Accelry's Discovery studio visualizer v4.0 interface.

In-Vitro biological activity Screening:

Antileukemic activity of the synthesized derivatives was performed by the MTT assay and the results were shown below.

Principle of assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-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 solubilised with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan (Formazan)

Materials:

FBS (Gibco, Invitrogen)Cat No -10270106, Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062, 96- well plates.

Cell lines	Media
K562 (Leukaemia)	RPMI1640 with low glucose (Cat No-11875-093)

Procedure:

The cells were seeded at a density of approximately 5×10^3cells/well in a 96-well flat-bottom micro plate and maintained at 37ºC in 95% humidity and 5% CO2 for overnight. Different concentration (400, 200,100,50,25,12.5 µg/mL) of samples was treated. The cells were incubated for another 48 hours. The cells in well were washed twice with phosphate buffer solution, and 20 µL of the MTT staining solution (5mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37°C.After 4h, 100 µL of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader [32-33].

Formula:

Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100.

Using graph Pad Prism Version5.1, we calculate the IC 50 of compounds. Note – DMSO Concentration is less 1.5% in experiments. Concentrations are in duplicates.

V. AUTHORS CONTRIBUTION

MY, PN and MD designed the study. MY executed the work. SB supported MY in executing the designed work. MY, PN, MD and SB analyzed the data. MY wrote the manuscript. MY, PN and MD edited the manuscript. All authors have read and approved the final manuscript.

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