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Characterization, Molecular Docking, and *in vitro* Evaluation of Triazole Derivatives as Potential Anti-Cancer Agents

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Abstract

Background: Cancer is defined as the uncontrolled growth and spread of abnormal cells. Mercaptopurine is used to treat many cancers such as-pancreatic, breast, colon, head& neck etc. Structure based and target-based drug innovation are two main approaches in the new molecule development process. In structure-based discovery, if the identified compound shows any significant pharmacological activity, then it is subjected to further development process and studies. Triazoles are heterocyclic five membered ring structures with 3 nitrogens and 2 carbon atoms. Triazoles are having wide spectrum of therapeutic activities such as anti-microbial, anti-convulsant etc. The aim of the present study is to analyze the targeting efficiency of the drug through molecular docking and synthesize and to perform In-Vitro anti-cancer studies for the justification of the docking results. **Objective:** To perform in sillico molecular docking and in-vitro anticancer studies of proposed 1,2,4-triazole derivatives for the determination of their anti-cancer activity. **Methodology:** A series of ten triazole compounds with different substituents were drawn in ACD Lab Chem. Sketch software. Molecular and Biological properties were identified using Molinspiration software. The compounds that obeyed Lipinski rule of five are subjected for pharmacokinetic parameters prediction and docking analysis. Swiss dock ADME software is used for the prediction of Absorption, Distribution, Metabolism, Elimination. Then the compounds are docked with target enzymes in Chimera software1.14 version. The molecular docking studies revealed favorable molecular interactions and binding energies. The compounds that showed good docking results were synthesized through wet lab synthesis and further preceded for *in vitro* anti-cancer studies. **Results:** Three compounds are selected for wet lab synthesis due to their good docking results compared to other compounds. The synthesized compounds are subjected to different In-vitro anticancer studies and found to be having potential anti-cancer activity. **Conclusion:** The pharmacokinetic and docking studies conclude that the triazole compounds have potential as anti-cancer agents. The In-vitro anticancer studies revealed that the triazole derivatives are having high potency of anticancer activity against pancreatic cell lines.

Keywords
Molinspiration, Swiss dock, ADME and Chimera, Neutral red reuptake assay, Cell lines etc.

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INTRODUCTION:

Cancer is a group of diseases with abnormal proliferation of cells which results in the alterations in the genetic information of cells that have the potential to invade to other parts of the body and damage the subsequent cells [1,2]. Cancer starts

when genes which usually control cell division, growth, and apoptosis in normal cells are damaged due to mutation/exposure to toxic chemicals.[3] These altered genes cause the cells to proliferate abnormally causing damage to the neighboring healthy cells [4]. Pancreatic cancer is the seventh leading cause of cancer-related deaths worldwide. It caused 432,242 new deaths are caused in 2018 due to pancreatic cancer. Although the exact cause of pancreatic cancer is unknown, certain risk factors have been identified, such as ethnicity, family history and genetic factors, Helicobacter pylori infection, non-O blood group tobacco smoking, diabetes mellitus, obesity, dietary factors, alcohol abuse, age, and chronic pancreatitis. [5,6]

Enzymatic pathways involved in Pathogenesis of Cancer: Mitogen activated pathway (MAP-Kinase) involves a series of proteins that interfaces a signal from receptor that is present on the surface of the cell to the DNA present in the nucleus, which regulates the gene expression, cellular growth and apoptosis etc.[7] Any alterations in the MAP Kinase pathway lead to tumorogenicity of cells and resistance to apoptosis.[8] Protein kinases are the enzymes that catalyses protein phosphorylation through ATP and converts inactive protein into active protein regulating the biological activity of protein. Deregulated kinases are found to be central for survival and spread of cancer cells. Tyrosine kinase is the subclass of protein kinases. [9,10]

Heat shock proteins play an important role in protein interactions such as folding and assisting in the protein conformation and prevention of protein aggregation. These are released during stress conditions. Their distinctive interaction with the oncogenes excels the progression of individual cancer. [11,12]

Tubulin is a protein which polymerizes into microtubules that act as a skeletal system of the living cells. Microtubules undergo various formations that enable the cell to undergo mitosis and intracellular transport.[12] Tubulin binding chemotherapy drugs suppress the dynamics of mitotic spindle to cause mitotic arrest and cell death.[13]

In silico drug design:

In-silico drug designing is a uttering used to mean performed on a computer. Miramontes coined the term In-silico to characterize the experiments that are carried in a computer. Ligand based drug design & Structure based drug design are the two methods involved in the in-silico drug design.[14] Ligand based drug design grasps on the molecules that bind to the target of interest. It is mainly used to derive a pharmacophores. Structure based drug design relies on the knowledge of three-dimensional structure retrieved through X-ray crystallography, NMR spectroscopy, homology modelling.

Molecular docking is a process that predicts the binding orientation of ligand to the target molecule and estimates the binding affinity and composed of

1. Pose Generation: The ligand is incorporated into the binding site by altering its rotational and translational degrees of freedom.
2. Scoring: The binding energy is estimated on the generated pose.

Triazoles are heterocyclic compounds having five membered ring structures with two carbon atoms and three nitrogen atoms. Molecular formula is $C_2H_2N_3$. The two isomers of triazoles are differed with respect to the positions of three nitrogen atoms. Triazoles are having wide spectrum biological activity such as, antimicrobial, anticonvulsant, anti-inflammatory, antimalarial, antiulcer, antiprotozoal, insecticidal etc.

Due to its stability towards metabolism, it acts as an important pharmacophore by interacting at the active site of receptor as a hydrogen donor and acceptor. Also due to its polar nature the triazoles increases the solubility of the drug resulting in the improvement of pharmacological profile of the drug. [15,16] A large number of triazole derivatives are reported for possessing anti-cancer activity.[17] Hence the present study was conducted to determine the anticancer activity of triazole compounds.

In-vitro Anti-Cancer Studies:

MTT assay: The MTT assay is a colorimetric assay for measuring cell metabolic activity. It is based on the ability of Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent cellular oxidoreductase enzymes to reduce the tetrazolium dye MTT to its insoluble formazan, which has a purple color. [18] In the MTT assay a solubilization solution (dimethyl sulfoxide or acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. MTT method is one of the most widely used methods to analyze cell proliferation and viability.[20]

Neutral red Uptake Assay: The neutral red uptake assay provides a quantitative estimation of the number of viable cells in a culture. It is based on the ability of viable cells to incorporate and bind the supravital dye neutral red in the lysosomes. Most primary cells and cell lines from diverse origin may

be successfully used. Cells are seeded in 96-well tissue culture plates and are treated for the appropriate period. The plates are then incubated for 2 h with a medium containing neutral red. The cells are subsequently washed, the dye is extracted in each well and the absorbance is read using a spectrophotometer.

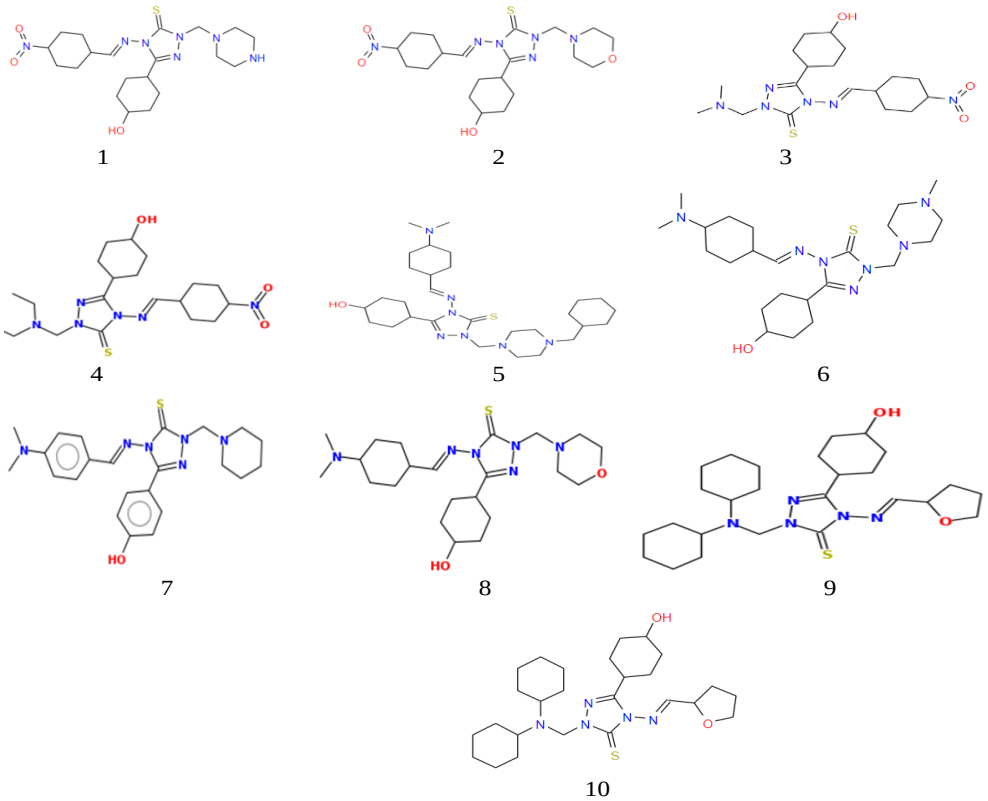
MATERIALS AND METHODS:

Preparation of Triazole structure: Ten number of triazole derivatives were prepared In-silico molecular modifications at R1 & R2 positions by using ACD Lab Chem. sketch software figure 1. 3D-drawing, optimizing, and calculating various molecular descriptors of proposed derivatives were done by using ACD Lab Chem. sketch software Table 1.

Table 1: Molecular descriptors of proposed 1,2,4-triazole derivatives.

S.NO	Molecular Formula	Molar Refractivity Cm^3 (±0.5)	Molecular Weight	Surface Tension Dyne/cm (±7.0)	Polarizability $10^{-24}cm^3$ (± 0.5)
1	$C_{20}H_{21}N_7O_3S$	119.39	439.49	65.75	47.33
2	$C_{20}H_{20}N_6O_4S$	117.72	440.47	64.01	46.68
3	$C_{18}H_{18}N_6O_3S$	109.29	398.88	57.32	43.32
4	$C_{20}H_{22}N_6O_3S$	118.51	426.49	54.72	46.98
5	$C_{29}H_{33}N_7OS$	157.15	527.68	50.04	62.30
6	$C_{23}H_{29}N_7OS$	131.23	451.35	50.15	52.37
7	$C_{23}H_{28}N_6OS$	128.09	436.57	50.06	50.77
8	$C_{22}H_{26}N_5OS$	124.87	438.54	51.35	49.50
9	$C_{18}H_{21}N_5O_2S$	1o5.02	371.45	41.63	41.63
10	$C_{19}H_{21}N_5O_2S$	107.47	383.46	56.62	42.59

Figure 1. Chemical structures of proposed triazole derivatives



Calculation of Biological Properties: The Molinspiration software was used to study the LogP values, violation of Lipinski's rule of five and drug likeness etc. [21,22] The smiles are used to calculate the biological properties. The results are shown in table no 2 and 3.

Prediction of ADME Properties: Swiss Dock ADME software is used for the prediction of pharmacodynamic properties like Absorption, Dissolution, Metabolism, and Elimination. The prediction of ADME properties helps to eliminate the less potential drugs before the start of synthesis and

trials of drug.[23] The results are shown in table no 4.

Glide Score: The glide score of the selected 5 compound is obtained through Schrodinger trial version software. The results are displayed in table no.5.

Docking Studies: The docking studies were done by using Mcule docking software. This software has been developed by Robert kriss, Mark Sandor, Zoltanszalai, Ferenc Szalai, Lazlo Havancsak. It offers database and modelling tools.

Protein/Target preparation: Nearly 10,000 automatically prepared target structures integrated from the PDB Database. All these structures are presented in pdb form and can be used ata any for docking process. We can also draw the structures as drawing tool is available or we can upload the files in pdb/mol2. The target molecules are selected from the database and their 3d structure appears.

Ligand Preparation: If the ligand molecule is available in software database, then we can select that structure. If the ligand molecule is new molecule, then we can draw our structure or we can upload the file in mol2. format. This software uses the auto dock Vina algorithm, the auto dock tools are utilised automatically for structure editing such as addition of hydrogen charges if none exists, add gasteiger charges, merge charges and remove lone-pairs non-polar hydrogens, non-standard residues water molecules etc.

Docking: The docking was performed through Auto dock vina and results were displayed after docking. The results are shown in Table no 6.

Wet Lab Synthesis: The samples that showed good docking scores are further synthesized through wet lab synthesis and analysed through IR spectra for their molecular groups.

Characterization of Compounds by IR Spectral Study: The FTIR is used for the identification of organic and inorganic molecular components and structure. Fourier Transform-Infrared Spectroscopy (FTIR) is an analytical technique used to identify organic (and in some cases inorganic) materials. This technique measures the absorption of infrared radiation by the sample material versus wavelength. All the IR spectra are measured using a FTIR-4100 type. The spectral resolution of the instrument is 0.25cm^{-1}, and the spectral data are stored in the database at the intervals of 0.5cm^{-1} at 4000 – 2000cm^{-1} and of 0.25cm^{-1} in 2000–400cm^{-1}.

Assessment of *in vitro* anticancer activity

Cell proliferation–MTT Assay: The metabolic activity of growing cells was assessed by means of the MTT assay. In the test, yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide–Sigma) is metabolized by viable cells to purple formazan crystals. HT-29 and A549 cells were plated on flat-bottom 96-well microplates at a density of 3 x 104 cells/ml (HT-29) and 1 x 104 (A549) cells/ml in 100 µl of a complete growth medium.

The next day, the culture medium was removed, and the cells exposed to serial dilutions of HWE and HJE at concentrations ranging from 0.1–5 mg/ml. After 96hour incubation, the cells were incubated for 3 h with an MTT solution (5 mg⁄ml), and formazan crystals then solubilized overnight by adding SDS buffer (10% SDS in 0.01 N HCl).

The colour product of the reaction was quantified by measuring absorbance at a 570 nm wavelength using an Emax Miocroplate Reader (Menlo Park, CA, USA). IC50 was calculated using the computerized linear regression analysis of quantal log dose-probit functions, according to the method of Litchfield and Wilcoxon. Cell viability (%) was expressed as a percentage relative to the untreated control cells.

Cell viability–Neutral Red (NR) Assay: The neutral red assay determines the accumulation of neutral red dye in the lysosomes of viable, uninjured cells. The HT-29, CCD 841 CoTr, A549, and HSF cells were plated on 96-well microplates at a density of 1 x 105 cells/ml in a complete growth medium. After 24-hour incubation, the growth medium was replaced by a fresh medium (containing 2% FBS) and the cells exposed to serial dilutions of HWE and HJE (0.1–5 mg/ml). After 24 h, the cells were incubated with the NR reagent for 3 h, fixed with the NR fixative solution (1% CaCl2 in 0.5% formalin) for 3 min at room temperature, and solubilized in 1% acetic acid in 50% ethanol under shaking for 20 min. Absorbance was measured at 550 nm using an E_{max} Miocroplate Reader.

Calculation of IC50 Values: According to the FDA, IC50 represents the concentration of a drug that is required for 50% inhibition in vitro. EC50 also represents the plasma concentration required for obtaining 50% of a maximum effect in vivo. It is comparable to an EC50 for agonist drugs. The values were calculated by using Graph Pad Prism**.**

IC50 = (Top + Baseline) / 2Y = Bottom + (Top-Bottom) / (1+10^ ((LogIC50X) * HillSlope + log ((Top-Bottom) / (Fifty-Bottom)-1).

Note the distinction between the parameter Bottom and Baseline. Bottom is the Y value of the bottom plateau of the curve itself. Baseline is the Y value that defines 0% maximal inhibition by a standard drug. You will want to constrain Baseline to be a constant value based on controls. You may also want to constrain Top.

RESULTS AND DISCUSSION

Molinspiration: Molinspiration software is used to calculate the biological properties and to analyse the Lipinski rule of five. The results are shown in tables no 2 and 3.

Table 2: Lipinski's rule analysis of proposed 1,2,4-triazole derivatives.

S.NO	Molecular Formula	Mi Log P	Mol. Wt	HAC	HBDO	Rot bonds	Violation
1.	$C_{20}H_{21}N_7O_3S$	0.13	451.60	10	2	6	0
2.	$C_{20}H_{20}N_6O_4S^*$	2.41	44.49	10	1	6	0
3.	$C_{18}H_{18}N_6O_3S$	0.83	410.54	9	1	6	1
4.	$C_{20}H_{22}N_6O_3S^*$	1.58	438.60	9	1	8	0
5.	$C_{29}H_{33}N_7OS$	3.26	545.84	8	1	6	1
6.	$C_{23}H_{29}N_7OS$	0.92	463.70	8	1	6	1
7.	$C_{23}H_{28}N_6OS^*$	3.61	436.58	7	1	6	0
8.	$C_{22}H_{26}N_5OS^*$	0.92	450.65	8	1	6	0
9.	$C_{18}H_{21}N_5O_2S^*$	3.93	489.73	7	1	7	0
10.	$C_{19}H_{21}N_5O_2S$	1.03	393.56	7	1	5	1

M.W-molecular weight; HAC-number of hydrogen bond acceptor; HDO-number of Hydrogen bond donor; rot b-number of rotatable bonds.

Table 3: Molinspiration Bioactivity Score

S.NO	Molecular Formula	GPCR Ligand (-)	Ion Channel Modulator (-)	Kinase Inhibitor (-)	Nuclear Receptor Ligand (-)	Protease Inhibitor (-)	Enzyme Inhibitor (-)
1.	$C_{20}H_{21}N_7O_3S$	0.55	0.56	0.75	0.93	0.61	0.37
2.	$C_{20}H_{20}N_6O^*$	0.85	0.87	0.82	0.90	0.89	0.65
3.	$C_{18}H_{18}N_6OS$	0.67	0.61	0.88	0.93	0.69	0.45
4.	$C_{20}H_{22}N_6O^*$	0.64	0.65	0.85	0.93	0.72	0.42
5.	$C_{29}H_{33}N_7OS$	0.27	0.54	0.52	O.78	0.41	0.28
6.	$C_{23}H_{29}N_7OS$	0.37	0.53	0.88	0.90	0.52	0.31
7.	$C_{23}H_{28}N_6OS^*$	0.81	0.95	0.85	1.02	1.06	0.60
8.	$C_{22}H_{26}N_5OS^*$	0.43	0.62	0.61	0.92	0.53	0.54
9	$C_{18}H_{21}N_5O_2^*$	0.46	0.57	0.64	0.73	0.48	0.37
10.	$C_{19}H_{21}N_5O_2S$	0.64	0.81	0.84	0.99	0.73	0.51

NOTE: The compounds with star mark have shown better properties and obeyed the Lipinski rule of five than the remaining compounds. So, these five compounds were docked in Chimera software and analysed for their ADME properties.

Prediction of ADME Properties: The selected compounds were analysed for their ADME properties through Swiss Dock ADME Software.

Table 4: ADME Prediction Scores

Molecular Formula	Lipophilicity (log Pα/w)	Water solubility (Log S)	GI Absorption	CYP_3A_4 Inhibitor	Synthetic Accessibility
$C_{20}H_{20}N_6O_4S$ (Sample 1)	-2.26	Soluble	Low	No	5.77
$C_{20}H_{22}N_6O_3S$ (Sample 2)	-2.00	Moderately soluble	Low	Yes	5.93
$C_{23}H_{28}N_6OS$ (Sample 3)	-4.72	Soluble	High	Yes	5.84
$C_{22}H_{26}N_5OS$ (Sample 4)	-4.45	Soluble	High	Yes	5.75
$C_{18}H_{21}N_5O_2S$ (Sample 5)	-4.06	Moderately soluble	High	No	5.02

Glide Score: The glide score results are shown below.

Table 5: Glide Scores of the compounds

S.NO	Compounds	Glide Score
1	Sample 1	-7.63
2	Sample 2	-7.19
3	Sample 3	-8.28
4	Sample 4	-9.68
5	Sample 5	-7.52

Docking Results: The docking analysis is performed through Chimera software 1.14 version and the results are shown in table 6 and the figures are shown in figure no 2.

Table 6: Docking Results: Binding Energy of samples with target enzymes

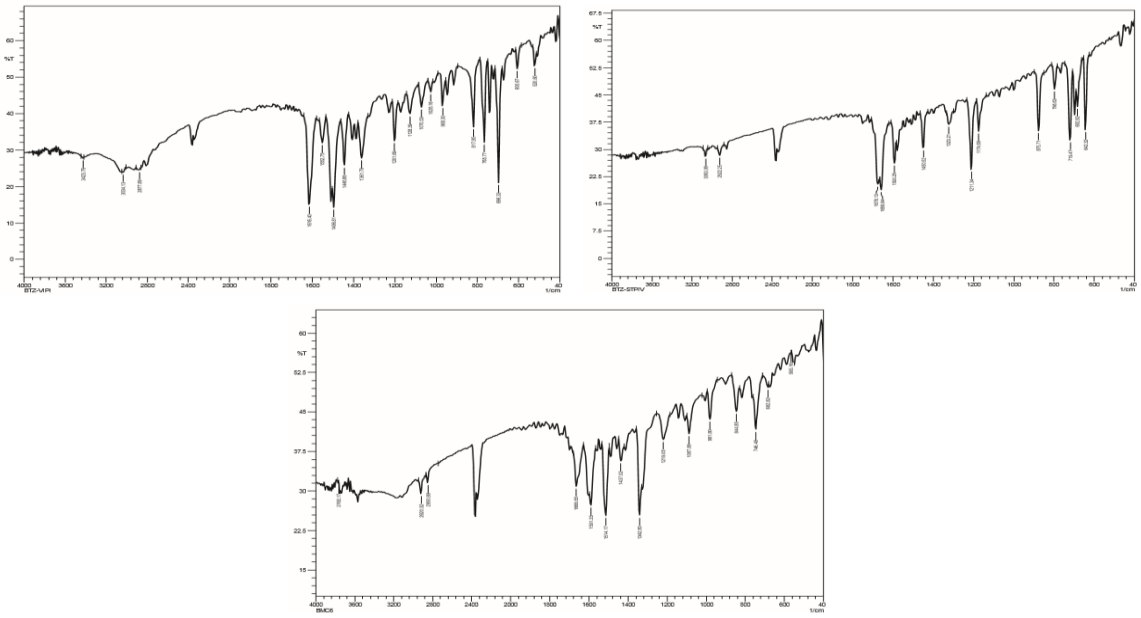
Target enzyme	Sample 1 (-)	Sample 2 (-)	Sample 3 (-)	Sample 4 (-)	Sample 5 (-)
Protein Kinase c	8.0	7.9	7.5	7.3	7.8
Tyrosine Kinase	7.9	7.8	7.2	7.9	7.4
HSP90	7.4	7.4	7.4	7.2	8.2
Tubulin	8.0	6.3	7.9	7.3	7.4
MAP kinase	7.5	7.8	7.5	7.2	8.0

Note: Based on the docking results, the samples 2,3,4 were subjected to wet lab synthesis and further proceeded to molecular characterization and in-vitro anti-cancer studies.

Table 7: FTIR Studies for the synthesized compounds:

S.NO	Compound	IR Spectra
1.	Sample 2	O-H str (3423), C-H str of CH3 in DEA (3034), C-H str of Mannich base (2877, 2840), C=N str of triazole (1616), N=O str (1552), C=S (1201) (Figure 5.4.1).
2	Sample 3	O-H str (3634), C-H str of Mannich base (2922, 2827), C=N str of triazole (1678), C=S (1174), C-H str DMAB (3063) (Figure 5.4.2).
3	Sample 4	O-H str (3765), C-H str of Mannich base (2920, 2850), C=N str of triazole (1666), C-O-C str (1087), C=S str(1219), C-H str DMAB (3068) (Figure 5.4.3).

Figure 4 FTIR spectral images of sample 2,3,4 respectively



Compound 4

EVALUATION OF IN VITRO ANTICANCER ACTIVITY

Table 8: IC50 values of selected compounds on PANC-1 (µg/mL)

S.NO	DRUG	IC50 Value
1	*Paclitaxel (standard)*	547.55 ± 33.52
2	Triazole derivative – A (sample 2)	557.66±50.24
3	Triazole derivative –B (Sample 3)	631.99 ± 50.24
4	Triazole Derivative –C (sample 4)	523.88±50.24

Table 9: MTT Assay- % viability of PANC-1 (Pancreatic Cancer Cell Line)

S.NO	DRUG	% Viability						
	Concentration (µg/ml)	**3.1**	**6.3**	**12.5**	**25.0**	**50.0**	**100.0**	**200.0**
1	Negative control	96	96	96	96	96	96	96
2	*Paclitaxel (standard)*	83	81	77	67	57	52	47
3	Triazole derivative –A (sample 2)	69	63	65	62	60	54	46
4	Triazole derivative –B (sample 3)	75	73	71	61	54	50	43
5	Triazolederivative –C (sample 4)	72	68	66	60	58	53	47

Note: From the results of MTT Assay and IC50 values, the Triazole derivative -B compound (sample3) has shown the highest results. So, the sample 3 is further preceded for the cell line testing through Neutral Red uptake Assay Method. The results are shown in figure 5.

Figure 3: MORPHOLOGICAL CHANGES IN PANC-1 (PANCREATIC CANCER CELL LINE)
Figure 3a: Before treatment with Triazole derivative sample

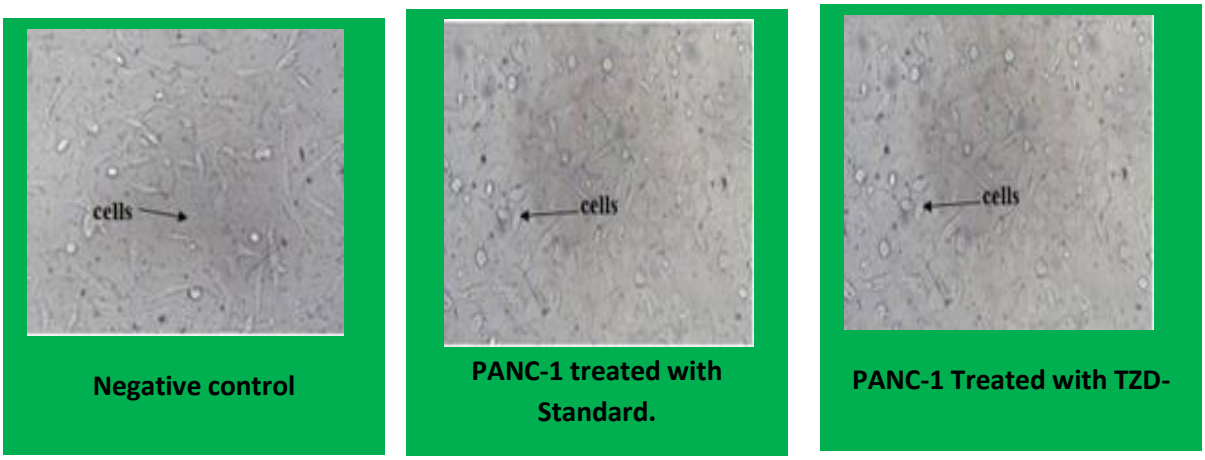


Figure 3b: After treatment with triazole derivative sample

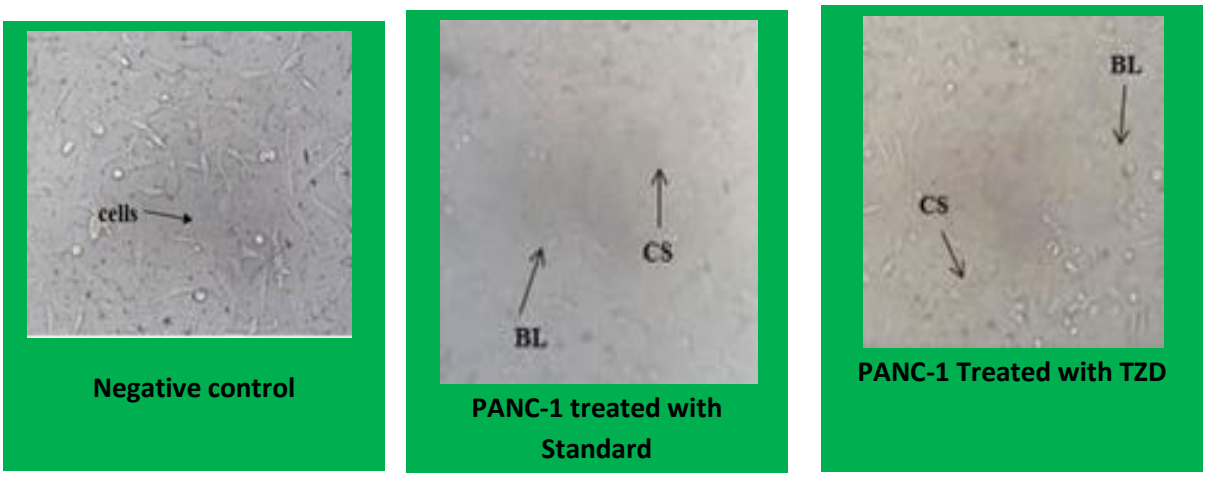
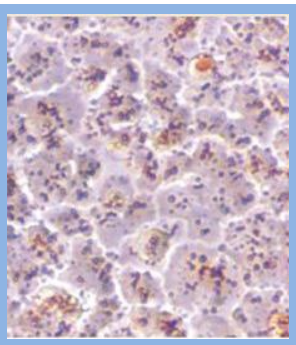
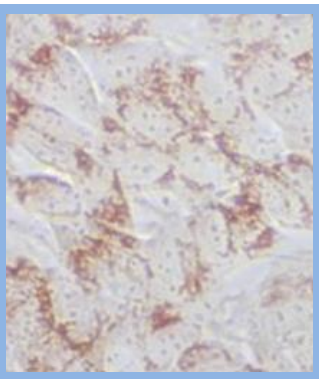


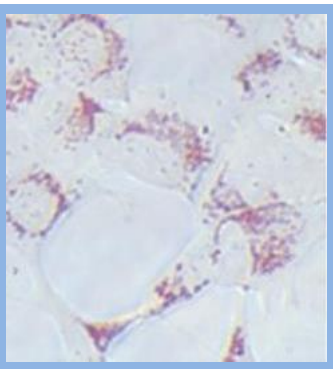
Figure 4: NEUTRAL RED UPTAKE TEST FOR CELL VIABILITY CHANGES IN PANC-1



Negative control



PANC-1 treated with Standard.



PANC-1 Treated with Test

DISCUSSION:

By using ACD Lab Chem, Sketch, we have drawn 10 triazole derivatives and their molecular properties such as molar refractivity, surface tension, polarizability, molecular weight etc. These all properties plays an important role in the pharmacotherapy of the drug. Molecular weight is the total mass of the molecule or the sum of all the atoms present in the molecule. The molecular weight of any drug/biologics should be within the limits, of below 1000 daltons.as it effects the absorption and distribution of drug. Smaller the size of the molecule, the absorption is fast, and it can freely travel to any destination in the body without causing any obstructions. Also, if the molecular weight is high means the size will also be increased, and it causes the disturbance in ADME properties and during manufacturing process. So as seen in table 1, all the compounds are in and around 400-500 range which is below 1000 Daltons. Molar Refractivity is the total polarizability of the molecule and is dependent on temperature, pressure, and the index of refraction. It is the real volume of molecule. It plays a role in the London forces that act in the drug receptor interaction. The molar refractive values are also within the range of 40-200 table 1.etc.

Surface Tension is the force exerted by the molecules that are present underneath on the surface layer which results in the shrinking of surface into the bulk of the compound. This results in the decreased surface area. There is a range of surface tension values for every dosage form and for oral, it is 36.6-65.7 dyne /cm and all the triazzole derivatives are within the range. It mainly affects the solubility of the drug. So, as seen in table 1 all these molecular properties are within the range of standards and so these triazole derivatives can be developed into dosage forms.

The Lipinski rule and its violation is verified in Molinspiration software as mentioned in table 2. Lipinski rule states that there should not be more than 10 hydrogen acceptors and 5 hydrogen bond donors. All the compound that violated the rule are eliminated from the further proceedings as they are unfit for docking process. The Lipinski rule plays major role in pharmacokinetics (ADME) rather than in pharmacodynamics of the drug. The biological activity such as enzyme inhibition, GPCR ligand inhibition were also calculated in this software shown in table no 3. GPCR ligand is the surface binding receptor that is involved in the signaling pathways of various disease. So GPCR inhibition is especially important for the drug molecule. Tyrosine kinases are enzymes responsible for so-called cascade activation of many proteins through signal transduction. Proteaase and kinase enzymes enhance the metabolism of foreign substances, so kinase inhibition is necessary for the drugs to avoid metabolism and elimination. Proteins are activated by phosphorylation, a step that TKIs inhibit. Almost all the derivatives are having good enzyme inhibitory activity.

As shown in table no 4, the ADME properties are calculated through Swiss dock software. Lipophilicity is the ability of a compound to dissolve in the in the lipids, fats etc. As our body is both made up of both lipid and water, the lipophicity should be less than >5.if it exceeds , the drug crosses blood brain barrier and causes CNS damage. Water solubility property helps in understanding the solubility of drug in water[46]. As the blood and other body parts are made up of water, it will be easy for the drug to get absorbed and distributed. As shown in table 3 the compounds are having the capacity to inhibit metabolic enzymes which results in the early metabolism of drug followed by elimination. GLIDE score function is mainly to separate the compounds

that bind actively form those that do not as shown in table no 5. The table no 6 shows the docking results is the binding energies of the ligand compound and target molecule. The higher the binding value, the higher the affinity between ligand and the target molecule which allows the ligand molecule binding to the target molecule and inhibiting or altering its functions or causing cell death etc. Due to the good binding energies shown by the samples 2,3,4 than the other samples, these samples were further proceeding for wet lab synthesis. The synthesized compounds were analysed through FTIR to identify the molecular structures. The IC50 values are estimated through glass prism method. It is the minimum concentration required for in vitro assays. The MTT assay is performed for the samples2,3,4 and the values are shown in table 9. The MTT assay is used to identify the cell viability in a culture medium. The sample 3 has shown higher value than the other two compounds. So it is further preceded for Neutral red uptake assay. The neutral red uptake assay is performed, and the results showed that the sample 3 has successfully inhibited the cancer cell lines as shown in figure 3.

CONCLUSION:

The present study scientifically established the *In–Sillico* design, synthesis, characterization, ADME Prediction and docking studies to predict anti-cancer activity. We have selected 10 triazole derivatives and all the compounds shown good molecular properties. But based on the analysis of Lipinski rule of five, only five compounds were passed the rule and so those compounds were further preceded to pharmacokinetic and docking studies. ADME studies have resulted with the 3 compounds having good solubility, lipophilicity, enzyme inhibition. From docking scores, we can conclude that these 3 compounds possess anticancer activity and can be further preceded to wet lab synthesis. The synthesized compounds were identified for their chemical structures through FTIR. The IC50 values are determined through glass prism method. The MTT assay and Neutral red uptake assay were performed, and the results showed that the triazole derivative has anticancer activity. These can be further preceded for clinical studies.

Conflict of Interest statement:

The authors hereby declare that we have no conflict of interest.

Abbreviation	Expansion
MAP	Mitogen activated protein
ADME	Absorption, Distribution, Metabolism, Elimination
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

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