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Molecular Docking Studies and *In Silico* Drug Design of Certain Novel Benzothiazole Derivatives That Targets MTHFD2 and PARP-2 Inhibitors for Triple-Negative Breast Cancer

Madhumithra M[*1], Dr.Niraimathi V[2], Dr. Sunitha P.G[3], Dr. Deattu N[4], Jawaharsamuvel R[5]

College of Pharmacy, Madras Medical College, Chennai-600003 India.

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ABSTRACT

OBJECTIVE: When certain body cells proliferate uncontrollably and infiltrate other bodily regions, it can lead to cancer. Bicyclic chemicals of the heterocyclic class include benzothizole (BTA). Broad range biological activities are exhibited by BTA derivatives, including anti-tubercular, anti- malarial, anti-leishamanial, anti-histamine, anti-fungal, anti- caner, anti- oxidant, anti-inflammation. The objective of the study is too carried out the docking studies of 2- mercapto benzothiazole derivatives with known anticancer targets MTHFD2 and PARP-2 Inhibitors by using Autodock programmes. **METHODS:** Docking studies were carried out using Autodock version 4.2 (1.5.6) for all 3 compounds and docking scores were compared with the scores of standard drug Tamoxifen. Validation of ligands was carried out by using Lipinski rule of five. **RESULTS:** 3 ligands show higher docking scores and shows better drug-likeness properties as compared to the reference drugs. The compounds show lowest docking energy ad hydrogen bondings stabilize the interactions. The most promising compounds were determined by analyzing the docking findings. **CONCLUSION**: Benzothiazole derivatives may be potential inhibitors of MTHFD2 and PARP-2 inhibtor, providing a new therapeutic option for cancer, according to molecular docking studies. To confirm these results and investigate these chemicals' potential for therapeutic use, more research is necessary.

Keywords: Cancer treatment, Molecular docking, MTHFD2, PARP-2 Inhibitor, and Benzothiazole derivatives.

INTRODUCTION

These day's cancer is the most pervasive life threatening disease which is spreading because of the lifestyle we are living. An conjectured 12.66 million people were diagnosed estimated with cancer around the world in 2008. In 2018, 18 million new cases were diagnosed, with the most prevalent cancer types being lung (2.09 million), breast (2.09 million), and prostate (1.28 million) [1].In 2018, 18 million new cases were diagnosed, with the most prevalent cancer types being lung (2.09 million), breast (2.09 million), and prostate (1.28 million) [1]. In research, benzothiazole is utilized as a building block to synthesize different kinds of bioactive compounds. It is comparatively stable due to its aromaticity. A great deal of interest is in the synthesis of derivative chemicals because of their medicinal applications[2]. It was not until recently recognized how the mitochondrial one-carbon folate metabolic enzyme, bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase (MTHFD2), contributes to cancer. While MTHFD2 expression is either nonexistent or very low in the majority of adult differentiated tissues, it is strongly expressed in embryos and a variety of malignancies. MTHFD2's correlation with cancer patient outcome and its elevated expression in tumor cells demonstrate the protein's significance in malignancies. Furthermore, gene knockdown research has demonstrated the significant influence of MTHFD2 deficiency on malignancies[3]. PARP inhibitors, often known as PARPi, were the first approved cancer drugs that specifically targeted the DNA damage response in breast and ovarian cancers with BRCA1/2 mutations. Since then, there have been significant advancements in our understanding of the mechanisms behind tumor sensitivity to PARP inhibitors and the application of PARPi in the management of diverse cancer types. For individuals with HER2- negative, locally progress (or) metastatic gBRCA -mutated breast cancer, PARP inhibitor treatments are a welcome addition to the therapeutic toolbox[4].

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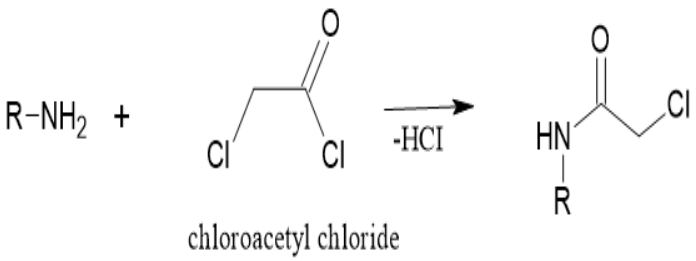
MATERIALS AND METHODS

Group of compounds were designed by based on the scheme.

STEP-1



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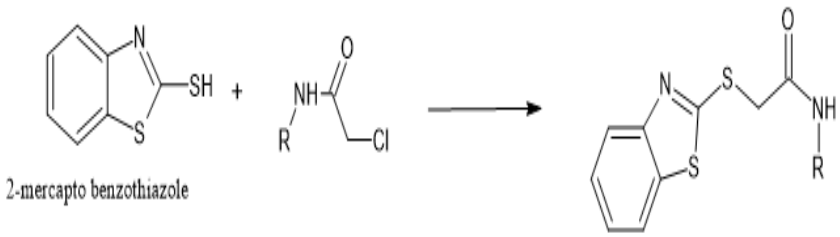
STEP-2



(4) (5) (6)

Based on the scheme 57 compounds were designed and the structures are displayed in the table no.1

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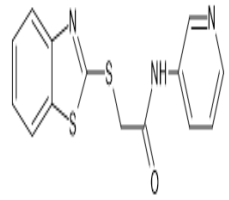
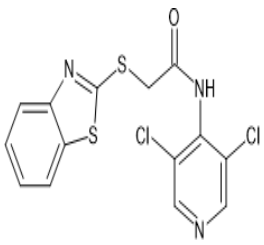
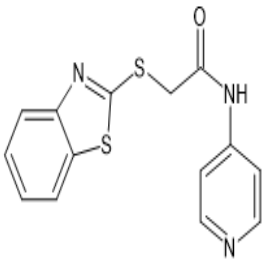
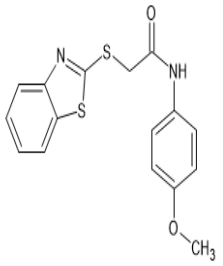
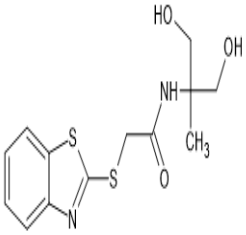
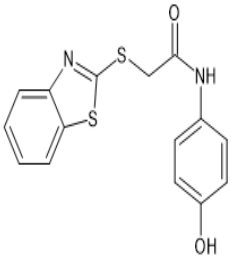
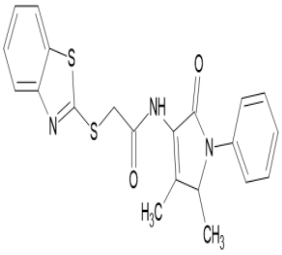
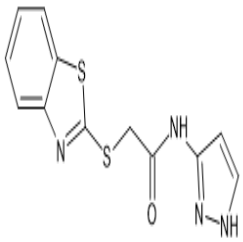
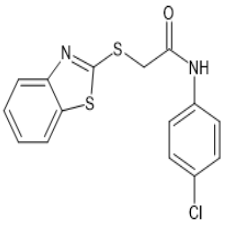
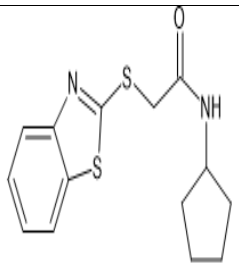
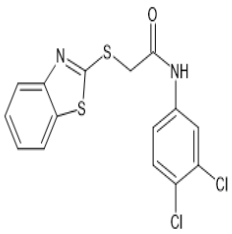
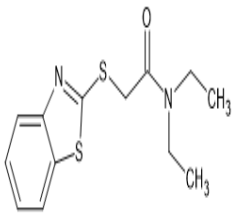
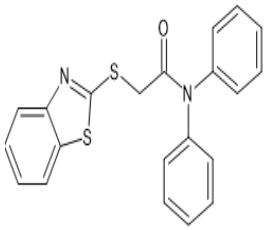
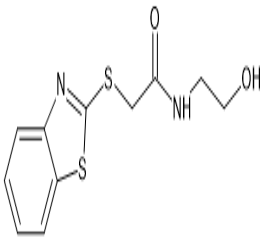
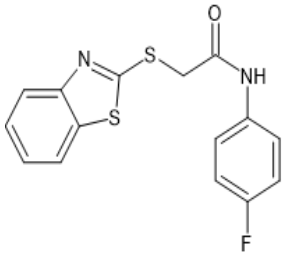
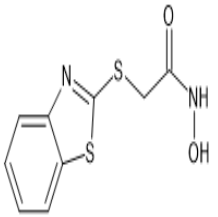
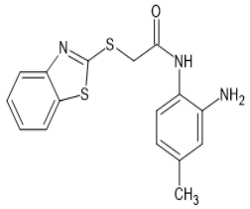
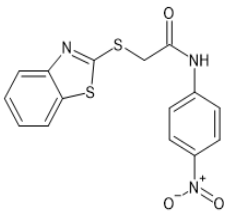
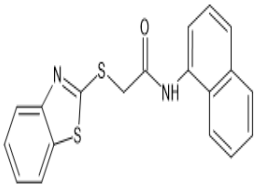
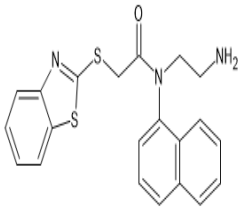
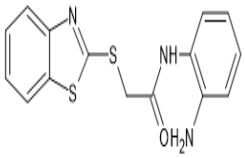
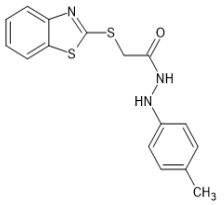
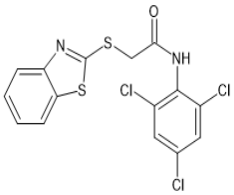
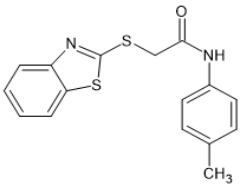
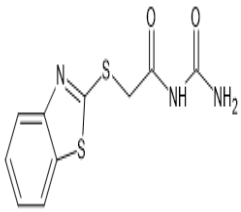
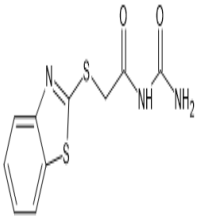
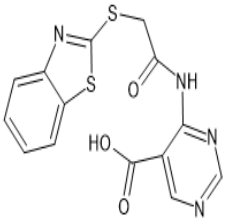
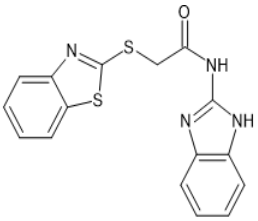
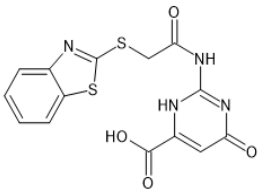
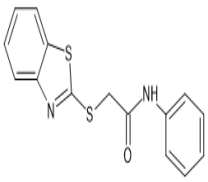
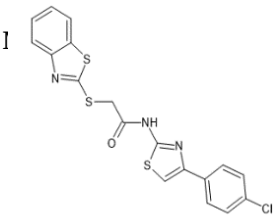
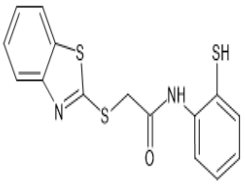
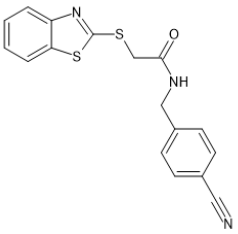
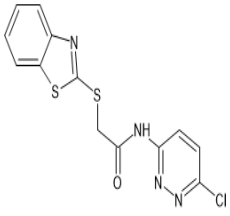
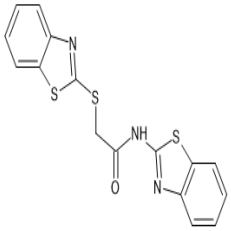
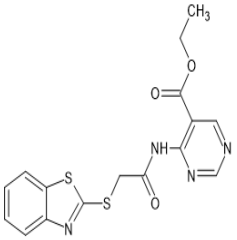
Table No.1: Structures of the newly designed ligands

 **MM01**	 **MM02**	 **MM03**	 **MM04**
 **MM05**	 **MM06**	 **MM07**	 **MM08**
 **MM09**	 **MM10**	 **MM11**	 **MM12**
 **MM13**	 **MM14**	 **MM15**	 **MM16**

 **MM17**	 **MM18**	 **MM19**	 **MM20**
 **MM21**	 **MM22**	 **MM23**	 **MM24**
 **MM25**	 **MM26**	 **MM27**	
 **MM29**	 **MM30**		 **MM32**
 **MM33**	 **MM34**	 **MM35**	 **MM36**

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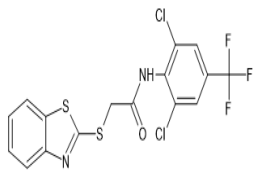
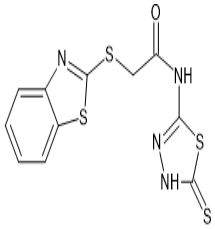
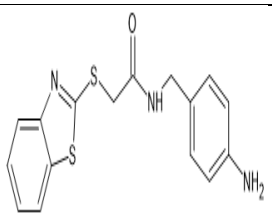
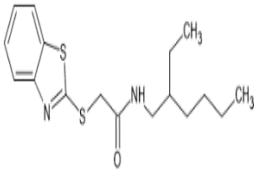
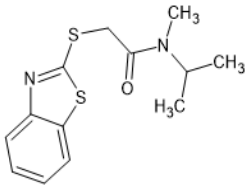
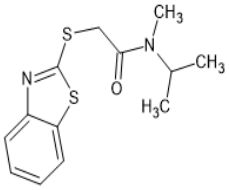
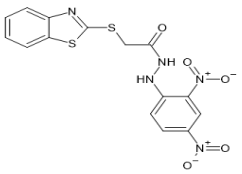
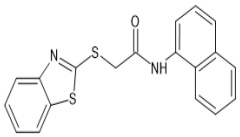
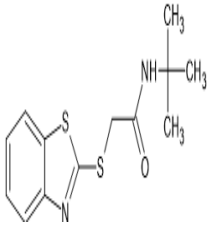
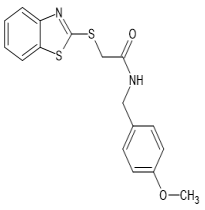
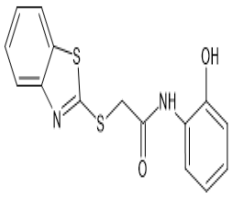
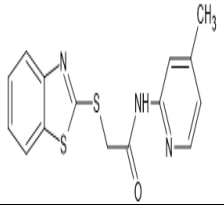
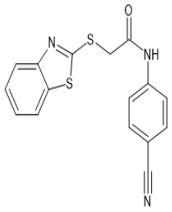
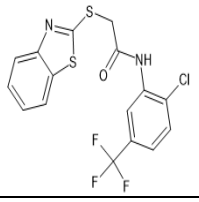
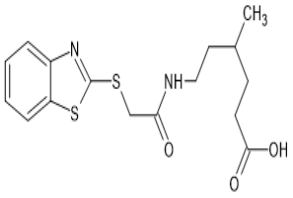
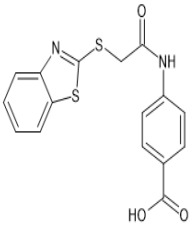
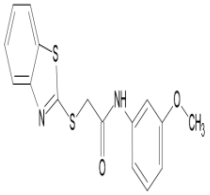
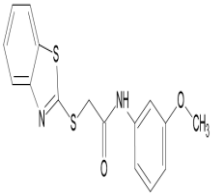
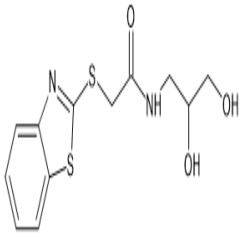
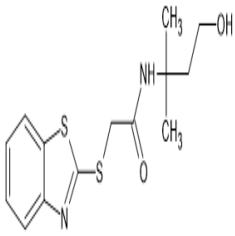
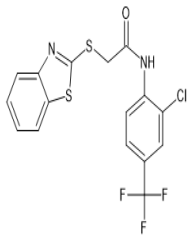
 **MM37**	 **MM38**	 **MM39**	 **MM40**
 **MM41**	 **MM42**	 **MM43**	 **MM44**

MM45 	**MM46** 	**MM47** 	**MM48** 
MM49 	**MM50** 	**MM51** 	**MM52** 
MM53 	**MM54** 	**MM55** 	**MM56** 

MM57 			

The novelty of the compounds was analyzed through the PUBCHEM and ZINC 15 databases. By using the Molinspiration database, druglikeness screening based on the Lipinski rule of five is carried out for the novel compounds, and the toxicity of the compounds is evaluated using OSIRIS toxicity explorer software. The outcomes are shown in the table no.2.

Table No.2: Novelty, Druglikeness, and Toxicity Report

S.NO	LIGANDS ID	NOVELTY OF THE COMPOUNDS	VIOLATION ABSENCE	NON-TOXIC
1	MM01	NO	NO	NO
2	MM02	NO	NO	NO
3	MM03	NO	NO	NO
4	MM04	NO	NO	NO
5	MM05	NO	YES	NO
6	MM06	YES	NO	NO
7	MM07	NO	NO	NO
8	MM08	NO	NO	NO
9	MM09	NO	NO	NO
10	MM10	NO	NO	NO
11	MM11	NO	YES	NO
12	MM12	NO	NO	NO
13	MM13	NO	YES	NO
14	MM14	NO	NO	NO
15	MM15	NO	NO	NO
16	MM16	NO	NO	NO
17	MM17	NO	NO	YES
18	MM18	NO	YES	NO
19	MM19	NO	YES	YES

20	MM20	YES	NO	YES
21	MM21	NO	NO	NO
22	MM22	YES	NO	NO
23	MM23	NO	NO	YES
24	MM24	NO	NO	NO
25	MM25	NO	YES	YES
26	MM26	NO	YES	YES
27	MM27	YES	YES	NO
28	MM28	NO	NO	YES
29	MM29	YES	YES	NO
30	MM30	NO	NO	NO
31	MM31	NO	YES	NO
32	MM32	YES	YES	YES
33	MM33	NO	NO	NO
34	MM34	NO	NO	NO
35	MM35	NO	YES	NO
36	MM36	YES	YES	NO
37	MM37	YES	YES	YES
38	MM38	NO	YES	NO
39	MM39	YES	NO	NO
40	MM40	YES	YES	NO
41	MM41	NO	NO	NO
42	MM42	NO	YES	YES
43	MM43	YES	YES	NO
44	MM44	NO	NO	YES
45	MM45	NO	NO	NO
46	MM46	NO	NO	NO
47	MM47	NO	NO	NO
48	MM48	NO	NO	NO

49	MM49	NO	NO	NO
50	MM50	NO	YES	YES
51	MM51	NO	YES	NO
52	MM52	NO	YES	NO
53	MM53	NO	YES	NO
54	MM54	NO	YES	NO
55	MM55	NO	YES	NO
56	MM56	NO	NO	YES
57	MM57	NO	YES	YES

PHARMACOPHORE MODELLING

In the late 1800s, Paul Ehrlich created the first pharmacophore concept. The term "pharmacophore" refers to a molecular structure that contains the key components that give a medicine its biological activity. Pharmacophore, according to IUPAC, is the collection of steric and electronic characteristics required to guarantee the best supramolecular interactions with a particular biological target structure and to initiate (or inhibit) its biological response. It is predicated on the idea that biological action on the same target results from shared chemical functions and spatial organization.

- Hydrogen bond acceptors (HBAs)
- Hydrogen bond donors (HBDs)
- Hydrophobic regions (H)
- Positively and negatively ionizable groups (PI/NI)
- Aromatic regions (AR) and
- Metal coordinating regions are the most crucial pharmacophore characteristics[5].

MOLECULAR DOCKING STUDY[6]

In the current molecular simulation study, Autodock tools 4.2(1.5.6) software was used for the prediction of binding energy of ligands with MTHFD2 and PARP-2 Inhibitors.

a. Preparation of target protein

The three- dimensional structure of MTHFD2 and PARP-2 Inhibitors was acquired from the Protein data bank as PDB format (PDB ID: 5TC4- Homosapien, Resolution 1.89Å) and (PDB ID: 7R59- Homosapien, Resolution 2.00Å) (www.rcsb.org/pdb). Co-crystalized ligands, Cofactors and water molecules are removed from the crystal structure using *Molegro Molecular Viewer*. The protein of the target enzyme was exported from the *Molegro Molecular Viewer* in PDB format and saved as Protein.db in a repository work folder- destination folder.

b. Ligand preparation

The two- dimensional chemical structures of the ligand molecules were sketched by using ChemSketch and saved in MDL Mol format. The energy minimization of the ligands were carried out with Chem 3D Pro 12.0. The energy minimized ligand molecules were saved as ligand.pdb in the same repository work folder.

c. Docking studies

teps involved in Docking

Step 1: Get the input file ready

➢ A protein database provided the generated protein structure file in PDB format. Charges were introduced and water molecules were extracted. Both polar and non-polar hydrogens were included and stored in the AutoDock folder as.pdb files.

➢ The generated ligand structure was saved in the AutoDock folder as pdbqt format after being added with charges in PDB format.

Step 2: Use AutoGrid to prepare the grid parameter file

➢ The space and number of points along the grid box's x, y, and z dimensions were set in a new grid parameter file (gpf) via the Autogrid graphical user interface.

➢ The grid file was then stored in the AutoDock folder in gpf format.

Step 3: Use AutoDock to prepare the docking parameter file

➢ A new docking parameter file (dpf) was made in the Autodock graphical user interface.

➢ A genetic algorithm was used to set the input parameters, including the number of runs, evaluations, and ligand conformal search parameters.

➢ The dock file was then stored in the dock.dpf format.

Step 4: Run AutoDock with the ready-made files

➢ AutoDock was done via the command-line interface. Typically, the command is written as "autodock4.exep dock.dpf –l dock.dlg."

➢ The docking computations were tracked and the outcomes examined.

Step 5: Examine the outcome

The results showed the binding energy.The outcome follows.Molegro Molecular Viewer was used to view the pdb file, and binding interactions between the protein and ligand such hydrophobic and electrostatic interactions were anticipated as hydrogen bonds.

RESULTS AND DISCUSSION

A. *INSILICO* STUDIES

The process of identifying and creating potential medication candidates with computer tools is known as "*in-silico* drug discovery." In addition to computer-aided drug design (CADD) techniques, including virtual ligand screening and profiling, in silico structure prediction, optimization, and refinement, this approach also uses other molecular modeling tools.

The structure and IUPAC name of selected compounds are given in below table.

Table No.3: Structure And IUPAC Name Of Selected Compounds

S.NO	LIGANDS ID	IUPAC NAME
1	MM6	2-[(1,3-benzothiazol-2-yl)sulfanyl]-*N*-(4-hydroxyphenyl)acetamide
2	MM22	2-[(1,3-benzothiazol-2-yl)sulfanyl]-*N*'-(4-methylphenyl)acetohydrazide
3	MM39	2-(benzo[d]thiazol-2-ylthio)-N-(5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide

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B. DOCKING SCORES OF SELECTED LIGANDS

For molecular docking investigations against two distinct proteins, such as MTHFD2 (PDB ID: 5TC4) and PARP-2 inhibitor (PDB ID: 7R59), ligands exhibiting druglikeness properties without toxicity were selected. The ligands' binding energy versus two proteins and also the docking score of standard drug is also displayed in the table No.3.

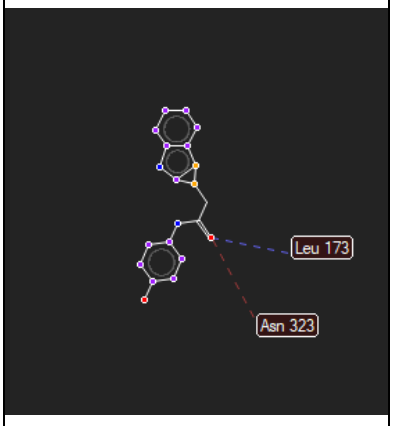
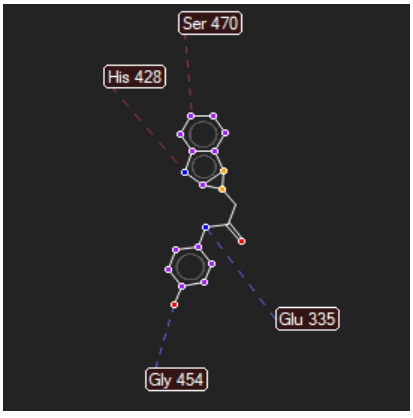
Table No.4: Docking Scores of the ligands

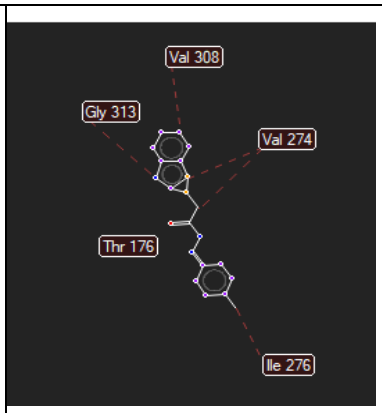
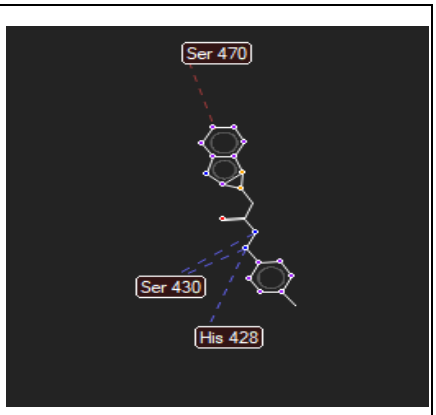
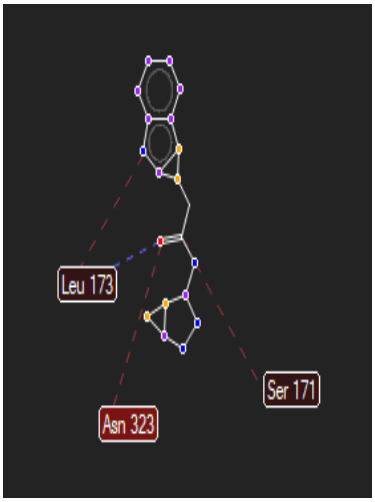
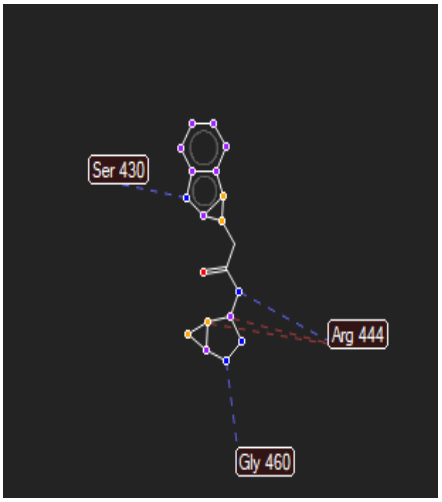
S.NO	LIGANDS	DOCKING SCORE	
		MTHFD2	PARP-2 INHIBITORS
1	MM6	-7.38	-8.4
2	MM22	-7.77	-9.4
3	MM39	-7.45	-8.57
4	Tamoxifen	-5.6	-4.11

C. LIGANDS INTERACTION

Of the high docking scores ligand- target complexes with different hydrogen bonding interactions generated by Autodock Tools 4.2(1.5.6) software were shown below

Table No.5: Ligand Interaction With MTHFD2 Receptor And PARP-2 Inhibitors

S.NO	LIGANDS ID	LIGAND INTERACTION WITH MTHFD2 RECEPTOR	LIGAND INTERACTION WITH PARP-2 INHIBITORS
1	MM6		

2	MM22		
\ 3	MM39		

D. PREDICTION OF TOXICITY

Osiris Property Explorer an online cheminformatics application called Osiris Property Explorer is used to assess the potential for toxicity of created molecular compounds. Green or red are the color codes assigned to the virtual toxicity results. Green in this case denotes compounds that are non-toxic and safe, whereas red denotes molecules that are toxic and have undesirable effects, such as mutagenicity, tumorigenicity, irritability, and reproductive impacts. By drawing the structures using an online tool, the suggested compounds' in-silico toxicity was ascertained.

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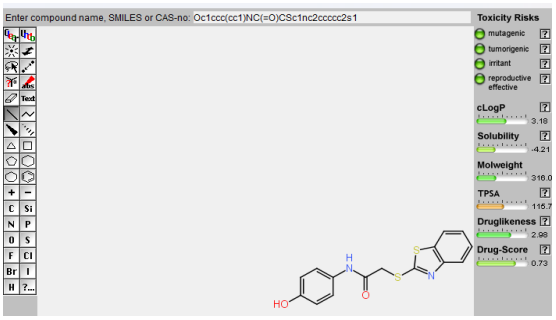
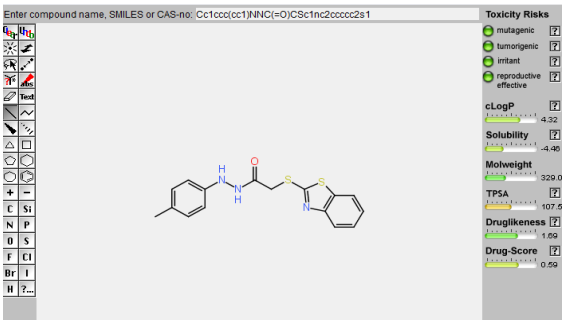
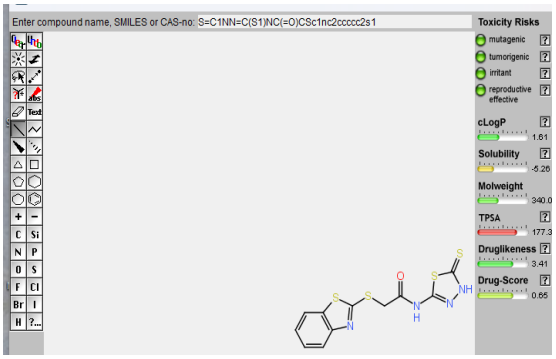
Table No.6: *Insilico* toxicity prediction by OSIRIS PROPERTY EXPLORER

S.NO	LIGANDS ID	TOXICITY PREDICTION
1	MM6	
2	MM22	
3	MM39	

E. PREDICTION OF DRUGLIKENESS

The chemical characteristics that influence a compound's absorption, distribution, metabolism, excretion, and toxicity (ADMET) are a qualitative indicator of drug similarity. A complicated balance of several molecular characteristics and structural elements determines whether a given molecule is similar to recognized medications. This is known as drug likeness. qualities such as molecule size, flexibility, electrical dispersion, hydrogen bonding, and hydrophobicity. Using several online tools, such as Molinspiration and Osiris Property Explorer, the drug-likeness characteristics of the recently created ligands were ascertained.

Lipinski's five rules

Lipinski's rule of five is a general guideline for assessing drug similarity, or if a chemical compound with a particular pharmacological or biological activity possesses the characteristics that would likely make it an oral medicine that works in humans. Christopher A. Lipinski developed the rule in 1997 after seeing that the majority of pharmaceuticals are lipophilic and relatively tiny molecules.

According to Lipinski's rule, an oral medication does not violate any of the following requirements:

- Not more than 5 Hydrogen bond donors (Nitrogen or Oxygen atoms with one or more Hydrogen atoms)
- Not more than15 rotatable bonds.
- Not more than 10 Hydrogen bond acceptors (Nitrogen or Oxygen atoms)
- Molecular weight under 500 Daltons
- Partition coefficient of log P less than 5[7].

Table No.7: Prediction of druglikeness

S.No	Ligands Id	Molecular Weight	No. Of Hba	No. Of Hbd	C log p	No. Of Rot.b	n violation
1	MM6	330.43	4	1	3.66	4	0
2	MM22	253.35	4	2	1.83	3	0
3	MM39	340.48	5	2	2.40	4	0

The drug similarity properties captured by Molinspiration are shown in the screenshots below.

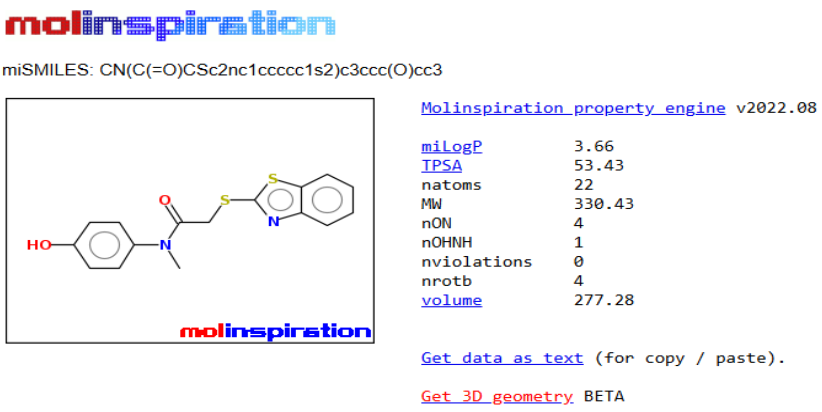


Figure 1: Molecular properties of ligand MM6

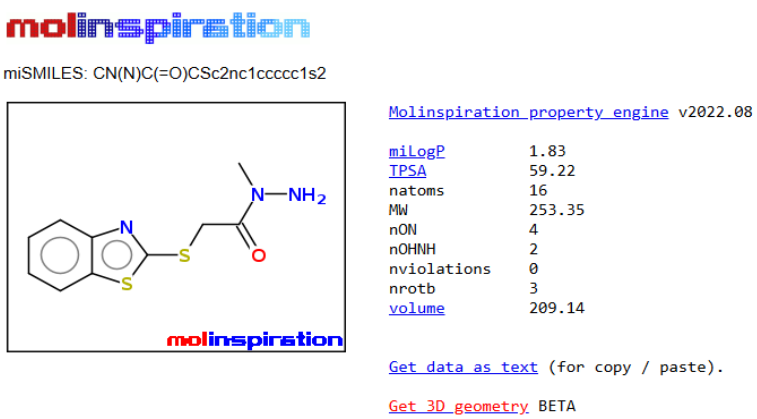


Figure 2: Molecular properties of ligand MM22

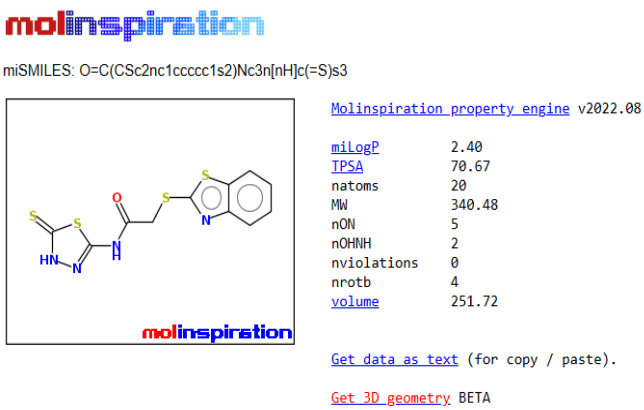


Figure 3: Molecular properties of ligand MM39

CONCLUSION

A new method that is widely utilized to cut costs and time in drug discovery is flexible docking of ligands to receptor molecules. This work successfully identifies strong inhibitors of MTHFD2 and PARP. The contacts are stabilized by hydrogen bonding, and all of the compounds exhibit the lowest docking energy. The compounds exhibited good docking scores with the range of (-7 to 9) when compared with the standard drug (tamoxifen) with the range of (-4 to -5). The heterocyclic benzothiazole possesses anticancer properties. So this study concluded that all 3 benzothiazole derivatives will be significant leads for further investigation of anticancer study.

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CONFLICTS OF INTEREST

The author declares there is no conflict of interest.

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