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MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATION STUDIES OF CHALCONE DERIVATIVES AS TOPOISOMERASE II INHIBITORS

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ABSTRACT: Cancer is the uncontrolled growth of abnormal cells in the body. It is the second leading cause of death globally, accounting for an estimated 9.6 million deaths in 2018. Topoisomerases are important cellular targets especially in the treatment of human cancers. They are of two types mainly topoisomerase II alpha and beta. Some of the most powerful anticancer drugs used clinically such as etoposide, teniposide, doxorubicin, daunorubicin, mitoxanrone, Amascarine etc act by causing DNA disorders. Topoisomerase II alpha is the target of action selected in this present study. Doxorubicin, one of the potent anticancer drugs that can be used to treat many cancers by acting on topoisomerase II alpha. Benzimidazole and pyrazole is an organic compound that is heterocyclic in nature. These are important pharmacophores and privileged structures in medicinal chemistry. It possess pharmacological activities such as antimicrobial, antiviral, anticancer, antiinflammatory, analgesic, antifungal, antitubercular, anti-convulsant, ACE-inhibitory etc. This study evaluates the anticancer activity of benzimidazole and pyrazole hybrid derivatives on 4FM9 using docking and molecular dynamic studies.

INTRODUCTION: Cancer is the uncontrolled growth of abnormal cells in the body. It is the second leading cause of death globally, accounting for an estimated 9.6 million deaths in 2018. Cancer develops when the body's normal control mechanism stops working. Old cells do not die and instead grow out of control, forming new, abnormal cells. These extra cells may form a mass of tissue, called a tumour. Most cancers form tumours, but not all tumours are cancerous. Benign, or noncancerous, tumours do not spread to other parts of the body, and do not create new tumours.

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Malignant, or cancerous, tumours crowd out healthy cells, interfere with body functions, and draw nutrients from body tissues. Cancers continue to grow and spread by direct extension or through a process called metastasis, whereby the malignant cells travel through the lymphatic or blood vessels eventually forming new tumours in other parts of the body. The major types of cancer are carcinoma, sarcoma, melanoma, lymphoma, and leukaemia.

Hormonal changes, environmental factors and inherited mutation in gene cause damage and failure of repair DNA. It results in mutation of genome. This may lead to activation of growth promoting oncogenes or inactivation of tumour suppresser gene causing unregulated cell proliferation. Mutation in genome also causes alteration in gene that regulates apoptosis and reduced apoptosis occur, finally leading to tumour

progression and malignant neoplasm [1, 2, 3, 4]. Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs", "molecularly targeted therapies", "precision medicines", or similar names.

Targeted therapies differ from standard chemotherapy in several ways:

One approach to identify potential targets is to compare the amounts of individual proteins in cancer cells with those in normal cells. Proteins that are present in cancer cells but not normal cells or that are more abundant in cancer cells would be potential targets, especially if they are known to be involved in cell growth survival. Another approach to identify potential targets is to determine whether cancer cells produce mutant (altered) proteins that drive cancer progression. Researchers also look for abnormalities in chromosomes that are present in cancer cells but not in normal cells. Sometimes these chromosome abnormalities result in the creation of a fusion gene (a gene that incorporates parts of two different genes) whose product, called a fusion protein, may drive cancer development. Such fusion proteins are potential targets for targeted cancer therapies.

Once a candidate target has been identified, the next step is to develop a therapy that affects the target in a way that interferes with its ability to promote cancer cell growth or survival. Many different targeted therapies have been approved for use in cancer treatment. These therapies include hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers angiogenesis inhibitors, immunotherapies, and toxin delivery [5, 6, 7].

Topoisomerases are important cellular targets especially in the treatment of human cancers. They are of two types mainly topoisomerase II alpha and beta. Topoisomerase II alpha is the target of action selected in this present study. Some of the most powerful anticancer drugs used clinically such as etoposide, teniposide, doxorubicin, daunorubicin, mitoxanrone, Amascarine etc act by causing DNA disorders. Doxorubicin one of the potent anticancer drugs that can be used to treat many cancers act on topoisomerase II alpha. It is the standard drug used in this work [8, 9].

Benzimidazole is an organic compound that is heterocyclic and aromatic in nature. It is a bicyclic compound formed by the fusion of the benzene and imidazole ring systems. It is an important pharmacophore and a privileged structure in medicinal chemistry. It possess pharmacological activities such as antimicrobial, antiviral, anticancer, anti-inflammatory, analgesic *etc* [10].

Pyrazole ring is a prominent structural motif found in pharmaceutically active compound. This is because of its ease of preparation and pharmacological activity. Pyrazoles are reported to possess wide range of pharmacological activity such as antimicrobial, antifungal, anticancer, antitubercular, anti-inflammatory, anti-convulsant, ACE-inhibitory *etc* [11].

In this present study we are developing benzimidazoles and pyrazole hybrid derivatives which act efficiently on topoisomerase II alpha with ensured superior safety and low toxicity.

Experimental Section:

Potential Target: Protein Structure and Protein Receptor Preparation: DNA topoisomerases enzymes control DNA topology by cleaving and re-joining DNA strands and play an important role in the regulation of the physiological function of the genome as well as DNA processes such as replication, transcription, recombination, Repair, chromosome decondensation and sister chromatid. Beyond their normal functions, topoisomerases are important cellular targets especially in the treatment of human cancers. Some of the most powerful anticancer drugs used clinically act by causing DNA disorders. Topoisomerase inhibitors block the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome and leading to apoptosis in proliferating cells and cell death. Our main aim is to block the topoisomerase II enzyme resulting in death of tumour cells [12]. The crystal structure of Human Topoisomerase II alpha bound with DNA (PDB ID: 4fm9) and resolution 2.90Å **Fig. 1** is

downloaded from the "Protein Data Bank" (https://www.rcsb.org/structure/4fm9) [13] and synthesized with the help of the software Auto dock tools. The first step of the protein preparation is the removal of the water molecules from the protein. The reason for deleting the water molecules is that if we have water molecules present around the protein's pocket region, the ligand will not comfortably set in the pocket region giving inaccurate results in docking. Polar hydrogens have been added followed by energy minimization in the torsional space and Kollman charges have also been added to the protein. All the heta-atoms present in the protein are also removed as they are unusual residues of DNA, RNA, proteins, and other atoms which can inhibit the binding sites and create trouble in protein–ligand binding. The output structure of the macromolecule is then saved in pdbqt format [14].

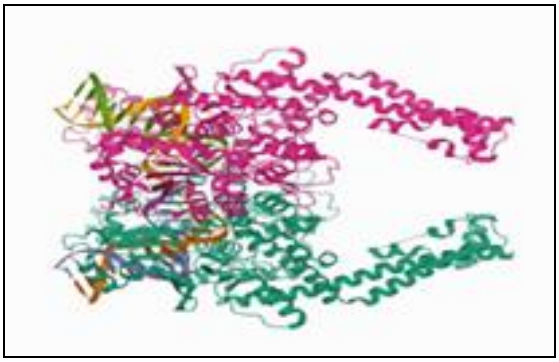
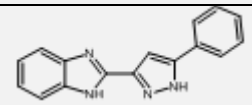
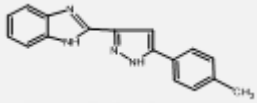
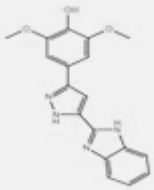
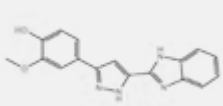
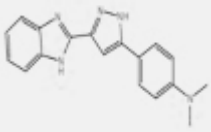
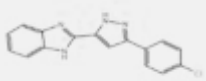
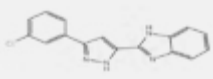
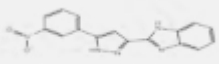
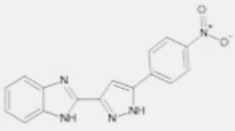
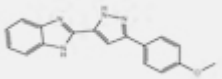
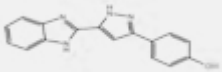
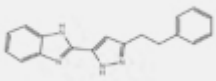


FIG. 1: CRYSTAL STRUCTURE OF PROTEIN TOPOISOMERASE II (PDB ID: 4FM9)

Potential Inhibitor:

TABLE 1: LIST OF PROPOSED DERIVATIVES

Compound Code	Structure
MT1	
MT2	
MT3	
MT4	
MT5	
MT6	
MT7	
MT8	
MT9	

MT10	
MT11	
MT12	

Biological Activity Prediction: The biological activity of the compounds was predicted by using PASS ONLINE software. The approach used in PASS is based on the suggestion that activity = f (structure). The result of prediction is represented as list of activities with appropriate Pa and Pi. The tool will interpret the biological activity spectra using 2D structure of molecules. The structure of derivatives was drawn using ACD ChemSketch. Login to the website using the ID and password provided. The structure of the molecule or the smiles notation can be directly loaded to PASS prediction website.

If 0.5< Pa<0, the compound is likely to reveal its activity in experiments but this probability is less and the compound is not so similar to the known pharmaceutical agents. If Pa<0.5, the compound is unlikely its activity in experiments but if the presence of activity is confirmed in the compound, it might be a new chemical entity [15, 16].

Drug-likeness Properties and ADMET properties: Drug-like properties have become an integrated part of the drug discovery process. They are playing a critical role in the successful development of drug candidates. A set of rules and guidelines for determining the structural properties is preferred for initial screening of drug-likeness of compound. Some of them are Lipinski's rule, MDDR-like rule, Veber's rule, Ghose filter, Egan rule, Muegge rule, Lipophilicity (iLOGP, WLOGP, XLOGP3, MLOGP, Log Po/w), water solubility (Log S (SILICOSIT)), etc. According to Lipinski's rule (Pfizer's rule or simply the rule of five (RO5)), any chemical compound can be used as an orally active drug if and only if it will not violate that set of rules. The mentioned rules preliminarily justify that whether the compound is ideal for drug synthesis or not. Some of the rules like molecular weight < 500, hydrogen-bond donors < 5, hydrogen-bond acceptor < 10, MLOGP (noctanol–water partition coefficient) < 4.15, number of rotatable bonds <5, molar refractivity should be between 40 and 130, log P ranging between − 0.4 to + 5.6, solubility (log S) > − 5.7, also help us to preliminary test the suitable drug molecule . All these in silico studies help in differentiating between druglike and non-drug-like structures.

All these properties are studied with the help of the online software molinspiration and chemsketch. This software facilitates us to analyse all the physiochemical properties, drug-likeness properties, pharmacokinetics, lipophilicity, etc.

Along with the RO5 and other pharmacokinetic rules, the designed inhibitor molecules must follow the ADMET properties. "Absorption" is the journey of the drug throughout our body, "Distribution" is about the transfer of drug from one location (organ) to another, "Metabolism", is a set of chemical reactions which drug undergoes. After metabolism is done, the drug should be eliminated from any part of the body in any form like sweat, urine, excrete, etc., called "Excretion" and "Toxicity" is the degree to which a drug can damage an organism

The ADMET properties of designed molecules were determined by pre-ADMET open-source tool [17, 18, 19, 20].

Molecular Docking and Visualization: Molecular docking is used to predict the structure of the intermolecular complex formed between two molecules. The small molecule called ligand usually interact with protein binding sites. Binding site are areas of protein known to be active in forming of compounds. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes.

It also predicts the strength of the binding, the energy of the complex the types of signals produced and calculate the binding affinity between two molecules using scoring functions. The most interesting case is the type protein-ligand interaction, which has its applications in medicine.

Types of docking:

1. Lock and Key\Rigid Docking

2. Induced fit\Flexible Docking.

Lock and key/ Rigid Docking: In rigid docking, both the internal geometry of the receptor and ligands is kept fixed and docking is performed.

Induced fit/ Flexible Docking: An enumeration on the rotations of one of the molecules is performed. Every rotation the surface cell occupancy and energy are calculated later the most optimum pose is selected.

Docking is a method which predicts the preferred orientation of one molecule to second when bounded to form a stable complex and done by using Auto dock 4.2-under PyRx virtual screening tool.

Stages of docking:

1. Ligand preparation

2. Protein selection

3. Protein preparation

4. Docking

5. Visualizing docking results

Ligand Preparation: The structures that are docked must be good representation of the actual ligand structures as they are docked in a protein-ligand complex in order to give the best result. For this the structures must show following conditions,

Must be prepared in PDB format and must have all hydrogen's. Must consist of a single molecule that has no covalent bonds to the receptor, with no accompanying fragments such as counter ions and solvents. Must have realistic bond lengths and bond angles.

In Auto dock Tool,

- Ligand molecule is converted into pdb.
- Detect root of ligand and Set no of Torsions
- Finally, Ligand is saved as pdbqt format.

Protein Selection: The selected protein 4fm9 which has the specific biological activity was downloaded in the PDB format using respective PDB ID from protein data bank (www.pdb.org).

Protein Preparation: By the protein preparation utility, crystallographic water molecules and ligands are removed from protein. The chemistry of proteins is corrected for missing hydrogen atoms and saved in PDB format.

For the preparation of the protein (receptor) molecule:

- Download the required protein 4fm9 molecule in pdb format from rcsb.org
- Open the downloaded pdb file of the molecule in Auto dock tools
- Prepare the protein by deleting water and selected atoms, adding both Kollman Charges and Compute Gasteiger charges.
- Finally save the file as pdbqt.

Grid Preparation:

- Prepare the grid by assigning XYZ parameters [X = 17.245, Y = 39.350, Z = 25.275]
- Save the file in gpf format and run the command prompt.

D:\project> "autogrid4.exe" -p 1.gpf -l 1.glg

Docking in Autodock 4: After making the protein 4fm9 and ligands MT1-MT12 to pdbqt format, the grid was made to maximum. Then docking was done to obtain the docking score.

- Assign the GA runs and Population size in search parameters.

  Number of GA Runs = 100

  Population size = 500

- Save the file in dpf format and run the command prompt.

D:\project > "autodock4.exe" -p 1.dpf -l 1.dlg.

Analysis of Auto Dock Result:

- Open the dlg file and play the conformation
- The parameters like binding energy, RMSD value, inhibitory constant was obtained from the dlg file.

Visualization of Docking Results: Visualization was performed using Protein Ligand Interaction Profiler and Protein plus Server from where we obtained the hydrogen bond details, amino acids involved and 2D image of protein- ligand complex was obtained respectively [21].

Molecular Dynamics: Molecular dynamics simulations are important tools for understanding the physical basis of the structure and function of biological macromolecules. The early view of proteins as relatively rigid structures has been replaced by a dynamic model in which the internal motions and resulting conformational changes play an essential role in their function. Molecular dynamics can be used to explore conformational space, and is often the method of choice for large molecules such as proteins. Dynamic study was done on Maestro version 12.3.013, MM share version 4.9.013, and the platform is Linux -x86-64. The software used is Schrondinger [22].

RESULTS AND DISCUSSION: *In-silico* design was successfully carried out with the aid different softwares such as chemsketch, Molinspiration, pre-ADMET, CORINA, Autodock. A series of derivatives were designed using these softwares.

Prediction of Biological Activity of Compounds: Prediction of biological activity spectra of derivatives (PASS) PASS is a software designed for the evaluation of biological activity of drug like molecules in terms of Pa and Pi values. It can be used for the determination of biological activity prior to synthesis. Pa and Pi of derivatives are given in the **Table 2.**

TABLE 2: PREDICTION OF BIOLOGICAL ACTIVITY SPECTRA

Compound Code	Pa	Pi
MT1	0.561	0.054
MT2	0.557	0.061
MT3	0.673	0.031
MT4	0.619	0.041
MT5	0.537	0.007
MT6	0.515	0.008
MT7	0.597	0.008
MT8	0.581	0.004
MT9	0.499	0.066
MT10	0.574	0.005
MT11	0.544	0.007
MT12	0.527	0.007

Analysis of Lipinski's Rule of Five: Lipinski's rule of five is a thumb rule to determine whether a chemical compound with certain biological or pharmacological activity has physicochemical properties that would make it an orally active drug. The analysis was performed by using molinspiration software and the results are shown in the **Table 3.**

TABLE 3: ANALYSIS OF LIPINSKI'S RULE OF FIVE

Compound	log p	molecular weight	NoN	Nohnh	Nrotb	n violation
MT1	3.48	260.3	4	2	2	0
MT2	3.93	274.33	4	2	2	0
MT3	2.83	336.35	7	3	4	0
MT4	2.82	306.32	6	3	3	0
MT5	3.58	303.37	5	2	3	0
MT6	4.16	294.75	4	2	2	0
MT7	4.13	294.75	4	2	2	0
MT8	3.44	305.3	7	2	3	0
MT9	3.41	305.3	7	2	3	0
MT10	3.53	290.33	5	2	3	0

MT11	3	276.3	5	3	2	0
MT12	4.03	288.35	4	2	4	0

Prediction of Drug Likeness: Drug likeness is a concept used in drug design for how "drug like" a substance is. It is estimated from the molecular structure before the compound is even synthesised and tested. Table shows the analysis of drug likeness of the proposed molecules.

TABLE 4: PREDICTION OF DRUG LIKENESS

Compound	GPCR Lgand	Ion channel modulator	Kinase inhibitors	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
MT1	0.05	0.03	0.55	-0.32	-0.32	0.12
MT2	0.04	-0.06	0.5	-0.3	-0.33	0.04
MT3	0.06	-0.03	0.53	-0.19	-0.22	0.12
MT4	0.09	-0.02	0.58	-0.17	-0.28	0.12
MT5	0.12	-0.01	0.58	-0.2	-0.22	0.07
MT6	0.08	0.02	0.54	-0.3	-0.32	0.07
MT7	0.08	0.01	0.54	-0.27	-0.34	0.09
MT8	-0.06	-0.04	0.38	-0.32	-0.34	-0.03
MT9	-0.05	-0.05	0.41	-0.32	-0.35	-0.01
MT10	0.05	-0.07	0.51	-0.25	-0.28	0.05
MT11	0.14	0.08	0.61	-0.11	-0.25	0.18
MT12	0.28	0.02	0.6	-0.22	-0.1	0.2

TABLE 5: PREDICTION OF DRUG LIKENESS

Code.	CMC like Rule	CMC like Rule Violations	Lead-like Rule Violation Fields	Lead like Rule	Lead like Rule Violations	MDDR like Rule	MDDR like Rule Violation Fields	MDDR like Rule Violations	Rule of Five	Rule of Five Violations	WDI like Rule	WDI like Rule Violations
MT1	Qualified	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT2	Qualified	0	AlopP98_value	AlopP98_value	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT3	Qualified	0	AlopP98_value	AlopP98_value	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT4	Qualified	0	AlopP98_value	AlopP98_value	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT5	Qualified	0	AlopP98_value	AlopP98_value	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT6	Qualified	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT7	Qualified	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT8	Failed	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	failed	0
MT9	Failed	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	failed	0
MT10	Qualified	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT11	Qualified	0	AlopP98_value	Violated	1	Mid-structure	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0
MT12	Qualified	0	AlopP98_value	Violated	1	Mid-structur	No_Rotatable_ bonds	1	Suitable	0	In 90% cutoff	0

ADMET Profile: ADMET of the derivatives were determined by pre-ADMET software. Table shows the ADMET of proposed analogues.

TABLE 6: ADMET PROFILE

Compound Code	BBB	Buffer solubility mg L	Caco2	CYP_2C19 _inhibition	CYP_2C9_i nhibition	CYP_2D6_i nhibition	CYP_2D6_ substrate	CYP_3A4_i nhibition	CYP_3 A4_sub strate	HIA
MT1	6.32599	500.933	19.1898	Inhibitor	Inhibitor	Non	Non	Inhibitor	Inhibitor	90.95160
MT2	7.48157	360.956	22.161	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	91.2002
MT3	1.90259	2236.15	26.0753	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	85.32792
MT4	2.54099	704.838	20.2204	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	86.75986
MT5	4.33208	193.516	22.3558	Non	Non	Non	Non	Non	Non	91.35946
MT6	7.79687	308.3	23.4958	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	92.13043
MT7	7.82104	183.943	26.4746	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	92.13043
MT8	1.59263	54939.1	17.9623	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	79.368794
MT9	2.90811	32778.7	14.8564	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	79.368791
MT10	3.29685	485.582	17.824	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	90.230077

MT11	3.3725	609.798	2.17495	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	88.058232
MT12	7.11458	195.286	19.6104	Inhibitor	Inhibitor	Non	Non	Inhibitor	Non	98.1827

TABLE 7: ADMET PROFILE

Compound Code	MDCK	Pgp inhibition	Plasma Protein Binding	Pure water solubility mg L	Skin Permeability	Solvation Free Energy	AlogP98 value	A Mol Ref
MT1	186.059	Non	93.519368	22.1401	-3.74854	-10.130000**	3.9529	77.911
MT2	76.551	Non	89.498998	6.59757	-3.66683	-9.660000**	4.4391	82.9522
MT3	0.096162	Non	81.86157	48.5669	-4.43183	-18.550000**	3.6527	92.5315
MT4	0.883235	Non	83.341276	23.1862	-4.29044	-17.130000**	3.6691	86.0683
MT5	62.5163	Non	87.989493	15.8791	-3.77532	-17.270000**	4.1151	92.3396
MT6	68.9504	Non	89.756733	3.22919	-3.82326	-10.410000**	4.6173	82.7158
MT7	108.986	non	92.000487	3.39839	-3.82327	-10.410000**	4.6173	82.7158
MT8	1.94129	non	93.520777	49.6811	-4.01394	-14.240000**	4.3583	82.0173
MT9	0.0955542	non	92.571624	52.2843	-4.01251	-14.240000**	4.3583	82.017300**
MT10	22.0762	Non	85.621553	15.3011	-4.07527	-11.550000**	3.9365	84.3742
MT11	8.98155	Non	86.99386	64.1987	-4.11196	-15.710000**	3.6855	79.6051
MT12	98.1827	Non	86.710909	37.0878	-3.47051	-10.070000**	4.4439	87.0612

TABLE 8: ADMET PROFILE

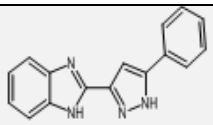
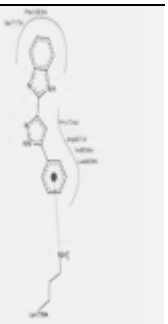
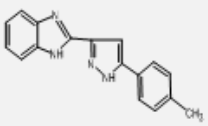
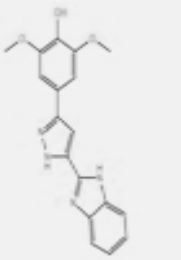
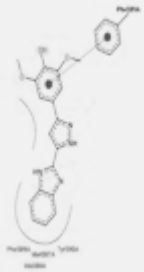
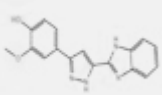
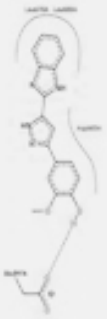
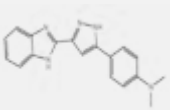
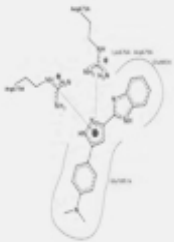
Compound Code	Algae at	Ames test	Carcino Mouse	Carcino Rat	Daphnia at	hERG inhibition	Medaka at	Minnow at	TA100_10RLI	TA100_NA	TA1535_10RLI	TA1535_NA
MT1	0.0289818	Mutagen	Positive	negative	0.024756	medium_risk	0.0012 4613	0.0019 7833	Negative	negative	positive	positive
MT2	0.0159876	Mutagen	Positive	negative	0.0163793	medium_risk	0.0005 65604	0.0010 1265	Negative	negative	Negative	positive
MT3	0.0101065	Mutagen	Negative	positive	0.025185	medium_risk	0.0013 9607	0.0021 8033	Negative	negative	Negative	negative
MT4	0.0146522	Mutagen	Negative	negative	0.0257642	medium_risk	0.0014 2324	0.0021 4891	Negative	negative	Negative	negative
MT5	0.0151634	Mutagen	Positive	negative	0.0201504	medium_risk	0.0008 78512	0.0018 928	Negative	negative	Negative	negative
MT6	0.0117851	Mutagen	Positive	negative	0.0117042	medium_risk	0.0003 17502	0.0005 90531	Positive	negative	Negative	Positive
MT7	0.0129257	mutagen	Positive	negative	0.0108377	medium_risk	0.0002 74796	0.0005 89209	Positive	negative	Negative	negative
MT8	0.0163861	mutagen	Negative	negative	0.0122541	medium_risk	0.0003 41574	0.0004 63306	Positive	negative	Negative	negative
MT9	0.0157583	mutagen	Negative	negative	0.0149206	medium_risk	0.0004 94384	0.0004 57816	Positive	negative	Negative	positive
MT10	0.0209063	mutagen	Negative	negative	0.0236424	medium_risk	0.0011 7154	0.0020 4128	Positive	Negative	Negative	negative
MT11	0.021623	mutagen	Negative	negative	0.0264012	medium_risk	0.0014 5582	0.0020 0107	Negative	Negative	Negative	negative
MT12	0.0124085	mutagen	Positive	negative	0.0164723	medium_risk	0.0005 79894	0.0010 9751	Negative	Negative	Negative	negative

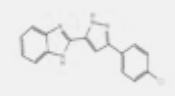
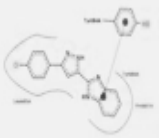
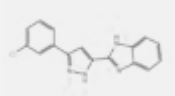
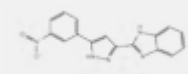
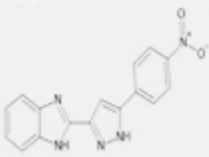
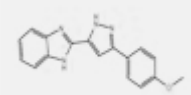
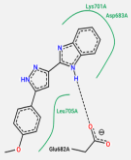
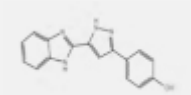
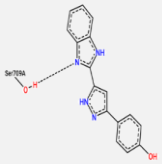
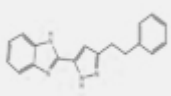
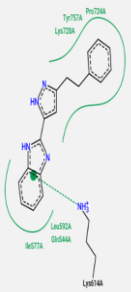
A series of novel chemical entities tethered with pyrazole and benzimidazole structural motifs were designed with a view to produce potent biological anticancer agents.

Molecular Docking: The flexible docking studies of MT1 to MT12 molecules were carried out. The amino acid residues selected for the docking studies are ASP541, ASP543, GLU461, TYR684, LYS662, ILE856. The results gained via flexible docking studies of ligands (MT1 to MT12) are furnished below.

TABLE 9: RESULTS OF FLEXIBLE DOCKING OF LIGANDS

Compound	RMSD	Binding energy (kcal/mol)	Estimated inhibitory constant (mu)	No. of hydrogren bonds	Hydrogen bond details	Amino acid involved in interaction	2D structure

 MT1	49.91	-8.36	749.77 nM	1	ARG727A H-A: 2.42 D-A: 3.18	Hydrophobic interaction LEU829A: 3.62, LEU829A: 3.94, ASP831A: 3.11 VAL836A: 3.03 PHE1003A: 3.73 π- Stacking: PHE1003A: 4.98 π- Cation Interaction: LYS728A: 3.26	
 MT2	64.14	-6.70	12.35 uM	3	GLU594A H-A: 1.93 D-A: 2.72 ARG633A H-A: 2.04 D-A: 3.02 ARG633A H-A: 2.93 D-A: 3.63	Hydrophobic interaction ALA588A: 3.30 TYR590A: 3.63	
 MT3	53.18	-6.92	8.53 uM	5	ALA588A H-A: 2.50 D-A: 3.49 GLU597A H-A: 2.23 D-A: 3.07 TRP598A: H-A: 3.39 D-A: 3.78 ARG633A H-A: 2.46 D-A: 3.44 ARG633A H-A: 2.86 D-A: 3.74	Hydrophobic interaction ALA588A: 3.34 PHE589A: 3.06 TYR590A: 3.36 ARG633A: 3.47	
 MT4	51.06	-7.48	3.30 uM	4	GLU597A H-A: 2.02 D-A: 2.88 ARG675A H-A: 2.86 D-A: 3.26 ASP683A: H-A: 3.25 D-A: 3.96 TYR684A H-A: 3.65 D-A: 4.05	Hydrophobic interaction ARG675A: 3.71 LEU678A: 3.57 LEU680A: 3.49 PRO681A: 3.27 ASP683A: 3.37	
 MT5	54.56	-8.49	597.32 nM	1	LYS676A: H-A: 3.22 D-A: 3.59	Hydrophobic interaction ARG675A: 3.16 LYS676A: 3.62 GLU682A: 3.60 π- Cation interactions ARG672A: 3.98	

 MT6	58.06	-6.81	10.20 uM	3	ASP683A: H-A: 2.15 D-A: 2.95 ASP683A: H-A: 2.50 D-A: 2.95 TYR684A: H-A: 2.87 D-A: 3.88	Hydrophobic interaction PRO681A: 3.24 TYR684A: 3.94 TYR684A: 3.67 LEU685A: 3.14 THR690A: 3.25	
 MT7	52.999	-5.43	1.79 uM	1	592A LEU H-A: 2.35 D-A: 3.41	Hydrophobic interaction 702A GLU 705A LEU Halogen Bond 712A GLU	No Interactions
 MT8	53.784	-5.64 kcal/mol	7.11 uM	-	-	Hydrophobic Interaction: 592A LEU 593A PRO 668A PHE 705A LEU 705A LEU	No Interactions
 MT9	52.911	-6.56	31.35 uM	1	ARG672A H-A:2.69 D-A:3.35	Hydrophobic Interaction: 592A LEU 593A PRO 705A LEU 831A ASP	No Interactions
 MT10	53.180	-5.38	1.58 uM	2	682A GLU H-A :2.28 D-A:3.10 709A SER H-A: 2.99 D-A:3.90	Hydrophobic Interaction: 701A LYS 701A LYS 705A LEU	
 MT11	50.956	-6.26	2.92 uM	3	592A LEU H-A: 2.98 D-A:3.94 672A ARG H-A: 3.02 D-A: 3.84 709A SER H-A: 2.22 D-A: 3.19	Hydrophobic Interaction: 592A LEU 593A PRO 705A LEU	
 MT12	52.602	-8.39	502.25 nM	3	614A LYS H-A: 2.90 D-A: 3.82 756ASER H-A: 3.26 D-A: 3.99 758A HIS H-A: 2.90 D-A: 3.70	Hydrophobic Interaction: 577A ILE 592A LEU 713A ARG 724A PRO 757A TYR 757A TYR 758A HIS [π-Cation Interaction 759A HIS]	

By analysing the results of flexible docking studies MT12 molecule was found to be the better molecule among all other molecules. The MT12 molecule exhibited strong binding affinity with 4FM9 protein with binding energy of -8.39 kcal/mol and thus turned out to be the most active benzimidazole and pyrazole derivative against Human Topo II protein. The rigid molecular docking of MT12 was done by assigning GA run 100 and population size 500.

Binding energy of MT12: -8.74 kcal/mol

RMSD: 53.751A

Estimated Inhibition constant, Ki: 395.19 nm

This MT12 molecular was selected for molecular dynamic simulation studies.

Molecular Dynamic Study of MT12:
Ligand Information:
Smiles: c1ccccc1CCc2cc ([nH] n2)-c (n3) [nH]c(c34)cccc4

No. of Atoms: 38 (total) 22 (heavy)

Atomic Mass: 288.355 au

Charge: 0

Mol. Formula: C18H16N4

No. of Fragments: 2 No. of Rot. Bonds: 4

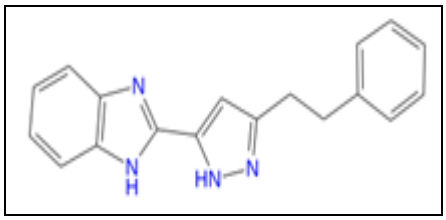


FIG. 2: STRUCTURE OF MT12 MOLECULE

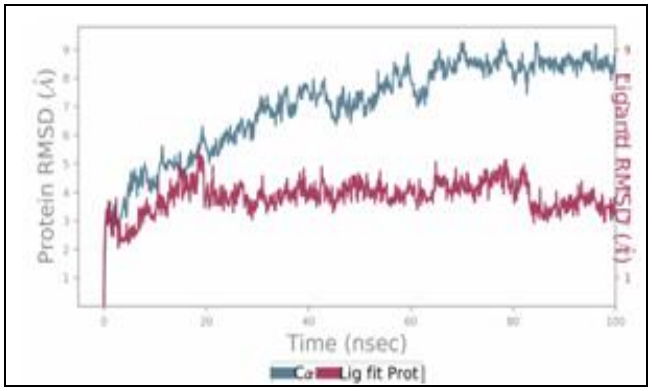


FIG. 3: PROTEIN LIGAND RMSD

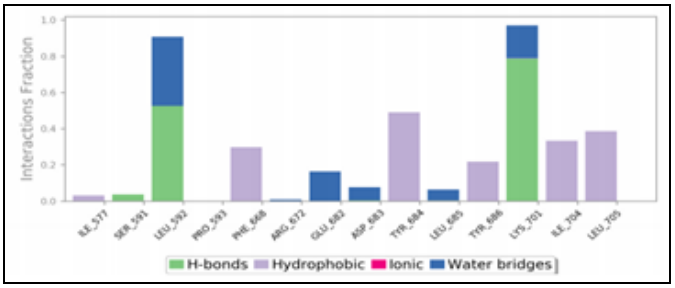


FIG. 4: PROTEIN LIGAND CONTACTS

Protein-ligand Interactions (or 'contacts') are Categorized into four Types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. Each interaction type contains more specific subtypes, which can be explored through the 'Simulation Interactions Diagram' panel. The stacked bar charts are normalized over the course of the trajectory: for example, a value of 0.7 suggests that 70% of the simulation time the specific interaction is maintained. Values over 1.0 are possible as some protein residue may make multiple contacts of same subtype with the ligand.

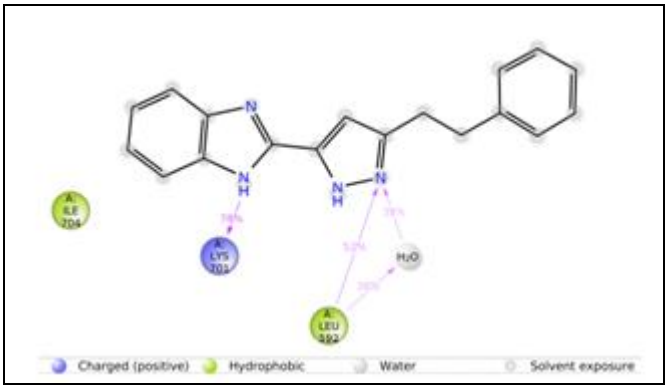


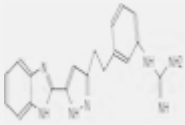
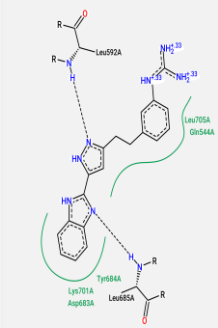
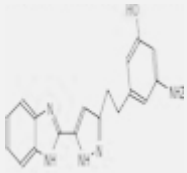
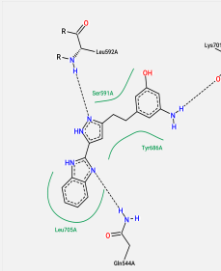
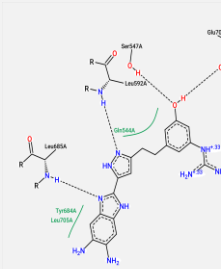
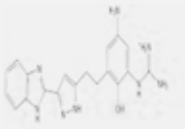
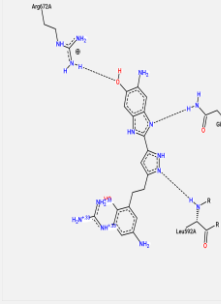
FIG. 5: LIGAND ATOM INTERACTIONS WITH THE PROTEIN RESIDUES

A schematic diagram of detailed ligand atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown. It is possible to have interactions with >100% as some residues may have multiple interactions of a single type with the same ligand atom. For example, the ARG side chain has four H-bond donors that can all hydrogen-bond to a single H-bond acceptor.

As the interaction of MT12 ligand molecule with the protein was found to be inefficient **Fig. 5**, certain modifications were done on MT12 to enhance its binding properties. Hydrogen containing groups such as NH2, OH and guanidine

groups were introduced at the different positions in pyrazole ring and benzimidazole ring respectively. As a result, 4 modified structures MT12a, MT12b, MT12c, MT12d, were designed and flexible molecular docking was carried out. The results obtained are as follows:

TABLE 10: RESULTS OF FLEXIBLE DOCKING OF MT12A-MT12D

Compound	RMSD	Binding energy (kcal/mol)	Estimated inhibitory constant	No. of hydrogren bonds	Hydrogen bond details	Amino acid involved in interaction	2D structure
MT12a 	53.445	-9.55	100.19nM	3	GLN 544A H-A: 2.18 D-A: 3.07 LEU 592A H-A:2.06 D-A:3.01 LEU 685A H-A:2.04 D-A: 2.99	Hydrophobic Interactions GLN544A 3.17 ILE577A 3.78 LEU685A 3.15 LYS701A 3.24 GLU702A 3.62 LEU705A 3.77 LEU705A 3.31	
MT12b 	52.414	-9.48	112.72 nM	4	GLN544A, H-A:2.13, D-A:3.07 LEU592A, H-A: 2.11, D-A: 3.08 LEU685A, H-A: 3.23, D-A: 4.05 LYS701A, H-A: 2.28, D-A: 3.14	LEU685A 3.73 TYR686A 3.45 TYR686A 3.12 LEU705A 3.35 LEU705A 3.62	
MT12c 	53.336	-9.85	60.45 nM	5	GLN 544A, H-A: 2.39, D-A: 3.27 SER 547A, A:1.88, D-A: 2.66, LEU 592A, H-A: 2.17, D-A: 3.08, ARG 675A, H-A:3.18, D-A: 4.10, LEU 685A H-A: 2.34, D-A: 3.31	Hydrophobic Interactions GLN542A 3.51 LEU 685A 3.09 LYS 701A 3.79	
MT12d 	53.450	-9.25	165.89 nM	8	GLN544A H-A: 2.04, D-A: 2.91, TYR 590A, H-A: 3.32, D-A: 4.09, LEU 592A, H-A: 1.90, D-A: 2.89, ARG 672A, H-A: 2.10, D-A: 3.03, LEU 685A, H-A: 2.08, D-A: 2.96, GLU702A, H-A: 2.62, D-A: 3.43, PHE706A, H-A: 3.16, D-A: 3.52, GLU712A, H-A: 3.44, D-A: 4.06	Hydrophobic Interactions GLN542A 3.45 GLN544A 3.56 GLU702A 2.99 LEU705A 2.98	

After the analysis of above docking result MT12d was proceeded for the dynamic study.

Molecular Dynamic Study of MT12d:

Ligand Information:

Smiles: c1c(N)cc (NC(N) =[NH2+]) c(O)c1CCc2cc (n[nH]2)-c(n3)[nH]c(c34)cccc4

Num. of Atoms: 49 (total) 28 (heavy)

Atomic Mass: 377.432 au

Charge: +1

Mol. Formula: C19H21N8O

No. of Fragments: 4 No. of Rot. Bonds: 9

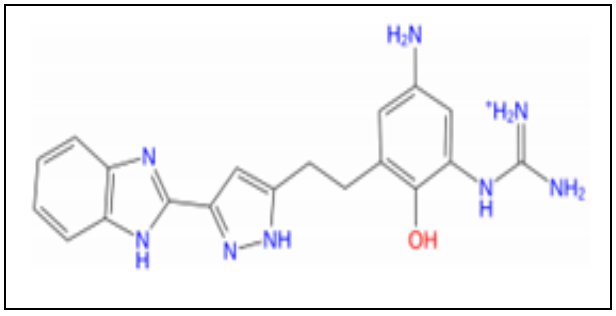


FIG. 6: STRUCTURE OF MT12D MOLECULE

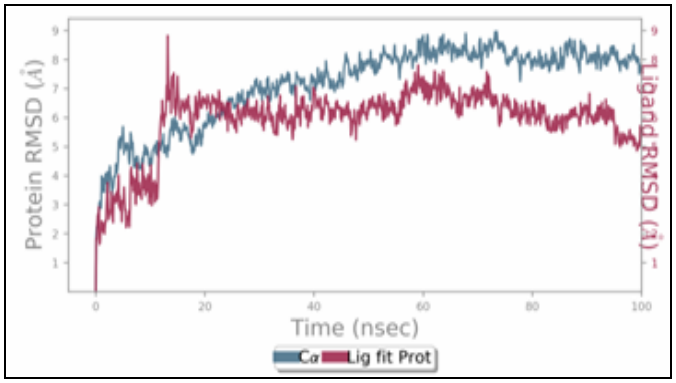


FIG. 7: PROTEIN LIGAND RMSD OF MT12D

Compared to MT12, the protein ligand RMSD of MT12d is better because there is more interaction between the protein and the ligand.

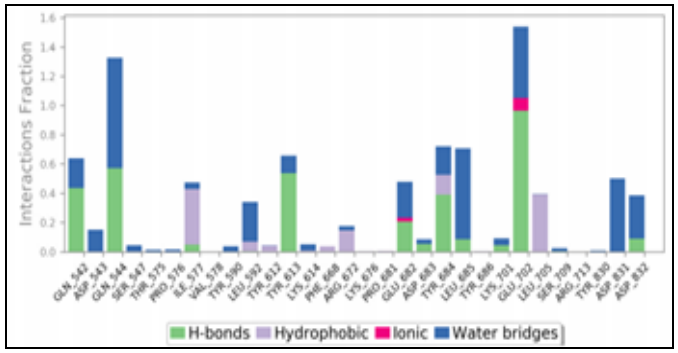


FIG. 8: PROTEIN LIGAND CONTACTS OF MT12D

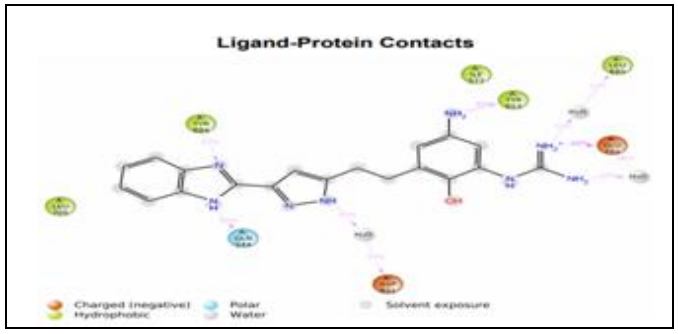
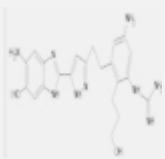
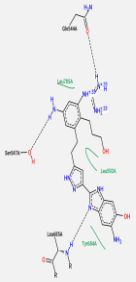
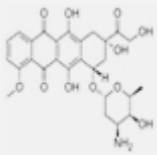
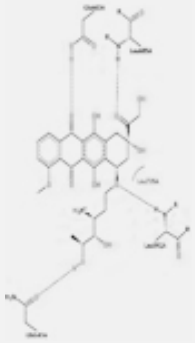


FIG. 9: MT12D INTERACTIONS WITH THE PROTEIN RESIDUES

Even though the docking and dynamics result of MT12d was better compared to the MT12, with an aim to improve the interactions MT12d molecule was again redesigned. The OH group at the substituted pyrazole ring was replaced with a butanol chain. The new molecule has given the name MT12e and docking studies were done. To know how good our designed molecules is, we compared the docking results with the standard drug Doxorubicin. The results obtained are given below.

TABLE 12: DOCKING RESULTS OF MT12E AND DOXORUBICIN

Compound	RMSD	Binding energy (kcal/mol)	Estimated inhibitory constant	No. of hydrogen bonds	Hydrogen bond details	Amino acid involved in interaction	2D image
MT12e 	51.273 A	-9.64	85.75 nM	7	GLN542A, H-A:3.31, D-A:3.76, GLN544A, H-A:3.04, D, A:4.04, TYR590A, H-A:3.17, D-A:3.55, ARG675A, H-A:3.11, D-A:4.05 LEU685A, H-A:2.20, D-A:3.17, GLU702A, H-A:3.01 D-A:3.92, SER709A,	Hydrophobic Interaction GLN544A3.47, ILE 577A 2.91, LEU 592A, 3.48, LEU 592A 3.23, LEU 685A 2.95, LYS 701A 3.83, LEU 705A 3.55, LEU	

Reference drug (Doxorubicin) 	53.31	-7.88	23.62nM	12	H-A:2.69, D-A:3.06 GLN 542A, H-A:2.39, D, A:2.97, GLN 542A, H-A 3.24, D-A3.77, GLN 542A, H-A 3.05, D-A3.76, GLN 544A, H-A 3.11, D-A 3.55 LYS550A, H-A 3.29, D-A3.88, TYR 590A, H-A 203, D-A2.64 LEU 592A, H-A 1.89, D-A 2.88 ARG 672A, H-A 3.02, D-A 3.86 GLU 682A, H-A: 2.87, D-A: 3.43, ASP 683A H-A 3.16, D-A 3.50, LEU 685A H-A 1.71, D-A 2.69, TYR 686A H-A 3.59, D-A 4.05	705A, 3.00 Hydrophobic Interaction ILE 577A 3.75 PRO 593A 3.49 TYR 686A 3 .12 GLU 702A 3.28 LEU 705A 3.04	

The docking score of standard drugs (Doxorubicin) is -7.88. The docking score of MT12e is -9.64, which is better than the standard drug

CONCLUSION: A new series of benzimidazole pyrazole hybrid derivatives MT1 to MT12 were designed by using various softwares and docked using 4FM9 protein for their evaluation as anti-proliferative agents against human topoisomerase II. Among them MT12 molecule was found to be the better one and molecular dynamic study of MT12 molecule was carried out. The results obtained from the dynamic study suggest that the interaction of the ligand molecule with the protein was inefficient. This study revealed that substituting pyrazole ring with a hydrogen containing groups like OH, NH2, Guanidine and butyl moiety will increase the anti-proliferative activity comparing with unsubstituted derivatives. The amino acids and the hydrogen bonding involved in the interaction were a few. So, modifications were done in the MT12 molecule to increase its binding properties. For this hydrogen containing groups like OH, NH2, Guanidine was substituted at the pyrazole and benzimidazole moieties and proceeded for rigid docking studies. The modified molecule MT12d has given best docking results and the dynamic study was done. Even though the results obtained was better compared to MT12, the interactions seemed to be less. We found out that the OH group in the substituted pyrazole ring was not involved in any interactions with the amino acids. So there came a need to substitute the OH group with more hydrogen containing long chain groups. A butanol group was substituted by replacing the OH group of MT12 and MT12e was developed and the docking studies was done

The docking results obtained was positive and hope giving, because among all other designed benzimidazole and pyrazole hybrid derivatives MT12e molecule has given the best binding energy around -10 Kcal/mol. The hydrogen bond involved was also more and the inhibitory constant was also good. Further studies on MT12e will be carried out in future and we also plan to conduct molecular dynamic study. We hope this molecule can be modified to form a better, promising anticancer agent among all other benzimidazole and pyrazole hybrid derivatives.

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