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Research Article

# *IN SILICO* AND *IN VITRO SCREENING* OF FEW SUBSTITUTED BENZIMIDAZOLE AS ANTICANCER AGENT

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**ABSTRACT**

Thymidylate synthase inhibitors have potential as an anticancer chemotherapyby inhibiting the enzyme thymidylate synthase.Thymidylate synthase converts deoxyuridine mono phosphate to deoxythymidine mono phosphate by getting methyl group from 5, 10 methylene tetrahydrofolate. This results in imbalance in levels of deoxyuridine triphosphate which cause DNA damage. Two substituted benzimidazole play a vital role in the field of medicinal chemistry as antiviral, antimicrobial, anticancer etc. The three Dimension structure of the protein is retrieved from the PDB and its active sites are predicted from Qsite Finder. All the 10 structures of the ligands were drawn using Chemsketch12 and they are converted to PDB format. The Pdb file of protein is downloaded from the protein data bank [2BBQ].The active site is predicted from the Q site finder online software. The docking was carried out using auto dock 4.2 software.2-(4-nitrophenyl)-1*H*-benzimidazole shows good binding energy -12.84 kcal/mol with 3 hydrogen bonds.It was synthesised and in vitro MTT assay were carried out for that compounds.In future in vitro activities with different cancer cell lines can be carried out.

**Keywords:** Thymidylatesynthetase, 2 substituted benzimidazole, MTT assay, insilico.

## INTRODUCTION

Molecular Modelling has become a well-established discipline in pharmaceutical research. It has created unprecedented opportunities for assisting the medicinal chemist in the rational design of new therapeutic agents. The central aim is to provide a comprehensive overview of the strategies currently used in computer assisted drug design[1,2].In DNA synthesis and repair thymidine triphosphate is used. It is obtained by phosphorylation of thymidine monophosphate generated by thymidylatesynthetase.

5,10-methylenetetrahydrofolate + dUMP ⇌ dihydrofolate + dTMP

This provides the sole de novo pathway for production of dTMP and is the only enzyme in folate metabolism in which the 5,10-methylenetetrahydrofolate is oxidised during one-carbon transfer. The enzyme's activity is a two-stage process. First, deoxyuridine monophosphate (dUMP) binds to a receptor site; this induces a configurational change which opens an adjacent binding site for N-5,10-methylene-tetrahydrofolate (CH2FH4). The folate's one carbon group is then transferred to the uridine ring, yielding deoxythymidine monophosphate (dTMP) and dihydrofolate .

The enzyme is essential for regulating the balanced supply of the 4 DNA precursors in normal DNA replication: defects in the enzyme activity affecting the regulation process cause various biological and genetic abnormalities, such as thymineless

death.Thymidylatesynthetase can be an anti-cancer chemotherapy target.The second process be**,** it gets methyl group from 5,10 methylene tetrahydrofolate. One notable exception to this is thymidylatesynthetase, a "bottleneck" enzyme which provides the only means of adding a methyl group to the 5-position of the pyrimidine ring in the de novo synthesis of thymidine.Since thymidine is the only nucleotide precursor specific to DNA, TS is an obvious target for cytotoxic agents.

Two substitutedbenzimidazole play a vital role in the field of medicinal chemistry as antiviral, antimicrobial,anticancer etc.The MTT assay's main application allows to assess the viability (cell counting) and the proliferation of cells (cell culture assays). It can also be used to determine cytotoxicity of potential medicinal agents and toxic materials, since those agents would stimulate or inhibit cell viability. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death, or changing metabolism of cells, can be deduced through the production of a dose-response curve. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

## MATERIALS AND METHODS

### Softwares and database

Protein Data Bank, Acd/ Chemsketch, Q-Site Finder, Auto Dock and Pymol

All chemical structures were drawn using chem sketch software and all the files were converted to PDB file format using online smiles translator. Protein target was downloaded from the PDB(Protein Data Bank). The protein PDB I.D is 2BBQ.Active site in protein target were determined from the online software Q-site finder. Docking of protein with 2-substituted benzimidazole derivatives were carried out using Autodock 4.0.The results were analysed using Pymol.

### General procedure for synthesis of 2 -sub-benzimidazoles:

In a round bottom flask, the powdered form of 0.02 moles of ortho-phenlyenediamine (2.16 gms) ,0.02 moles of respective acid ,2-3 drops of Concentrated HCl as a catalyst and 25 ml of ethyl alcohol as a solvent were added. To this few pieces of porcelain was also added to prevent bumping. The above mixture was refluxed in a water bath for about 4 hours at a temperature of $95^0$C.This reaction mixture was cooled to room temperature and poured little by little into crushed ice. The separated solid was filtered and dried to obtain the desired product[3,4,5.]The scheme is mentioned in figure 1.

### MTT Assay Procedure

Plate cells (104 – 106 cells) in 200 ml PBS in 96-well (flat bottom) were prepared.

20 ml of MTT solution was added and mixed well. it was incubated for 4h in 37C in dark. The aliquot was removed for analysis and 200ml of acidic iso propyl alcohol was added and mixed well. Additionally it was incubated for one hour in dark at 37C. The plate was read in ELISA Reader. The Optical density was measured at 570nm (background wavelength is 630nm)[6,7,8.]

## RESULTS AND DISCUSSION

Three hydrogen bonds are formed between 2-(4-nitrophenyl)-1H-benzimidazole the ligand and the protein.The hydrogen of guanidine ring present in arginine [ARG 35] forms hydrogen bond with oxygen of nitro group in the ligand. The bond length is $1.32^0$A.The NH of lysine [LYS 10] form hydrogen bond with nitro group of the ligand. The bond length is $1.998^0$A. The carbonyl oxygen($CONH_2$) of glutamine[GLN 33] forms hydrogen bond with NH of benzimidazole. The bond length is $2.218^0$A.Formation of two hydrogen bonds between 4-(1H-benzimidazole-2-yl)anilinethe ligand and the protein.Hydrogens of amino group present in ligand is interacted with oxygen of carboxylate of glutamic acid[GLU 6] amino acid residue of protein .The length of the hydrogen bond formed is $2.13^0$A.The second hydrogen bond is formed between amino group of ligand and oxygen of carbonyl in amide of glutamine[GLN 3] amino acid residue. The hydrogen bond length is $2.056^0$A.

Formation of one hydrogen bond between 4-(1H-benzimidazole-2-yl)N,N-dimethyl aniline the

ligand and the protein.The NH of benzimidazole forms hydrogen bond with carbonyl amide of glutamine [GLN 33] aminoacid. The bond length is 2.106$^0$A.There is a formation of one hydrogen bonds between2-phenyl-1H-benzimidazole the ligand and the protein.The NH of benzimidazole interact with carbonyl oxygen(COOH) of arginine [ARG 127] residue. The bond length is 2.179$^0$A. one hydrogen bonds between the 2-(4-chlorophenyl)-1H-benzimidazole ligand and the protein.The NH hydrogen of benzimidazole forms hydrogen bond with oxygen of carboxylate of glutamic acid[GLN 33].The hydrogen bond length is 1.827$^0$A.

2-(2-chloro phenyl)-1H-benzimidazole forms one hydrogen bonds with the protein.The NH of benzimidazole interact with carbonyl oxygen($CONH_2$) of glutamine [GLN 33] residue. The bond length is 1.93$^0$A. 2-(furan-2-yl)-1H-benzimidazole forms two hydrogen bonds with the protein.The oxygen of carboxylate of glutamine[GLN 33] forms interaction with NH of benzimidazole. The bond length is 1.949$^0$A.The oxygen of furan forms hydrogen bond interaction with $NH_2$ of glutamine[GLN 33].The bond length is 2.188$^0$A. 2-(3-H-indol-2-yl)-1H-benzimidazole forms two hydrogen bonds between the ligand and the protein.The carbonyl oxygen of arginine [ARG 127] forms hydrogen bond with NH of benzimidazole. The bond length is 1.928$^0$A. 100µM concentration of 2-(4-nitrophenyl)-1*H*-benzimidazole have a growth inhibition of 41.1% which shows that it has a moderate anticancer activity against the breast cancer cell lines(MCF7).

The selected Active Site residues for docking are GLU58, ILE79, TRP80, CYS146, and PHE176.

2-substituted benzimidazole compounds shown good affinity towards Thymidine kinaseenzyme, out of which 2-(4-nitrophenyl)-1*H*-benzimidazole give the best results. 2-(4-nitrophenyl)-1*H*-benzimidazole form 3 hydrogen bond with the residue LYS10,ARG35,GLN33 with distance 2.068$^0$A, 1.933$^0$A, 2.218$^0$A. 2-(4-nitrophenyl)-1*H*-benzimidazole possess good binding affinities as well as more number of hydrogen bonds hence it can be targeted as Thymidine kinaseenzyme inhibitor.

The free 1-NH benzimidazole is needed for the anticancer activity .The nitro substitution favours the ligands towards the target .The predicted site for thymidylate synthase protein is GLU6, GLN3, GLN33, ARG35, LYS10 and ARF127. In MTT assay,2-(4-nitrophenyl)-1*H*-benzimidazole shows moderate anticancer activity against the breast cancer cell lines(MCF7). If it is tested against the colon cancer cell lines it would have exhibit very good activity.

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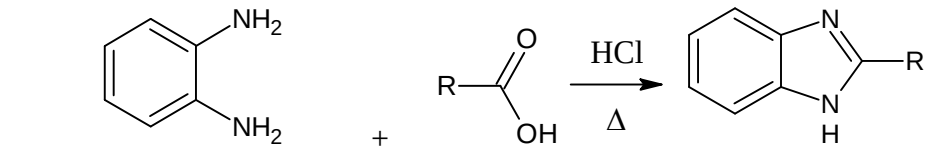
## Scheme




o-phenylenediamine R-acid 2-sub- Benzimidazole

**Fig.1: Scheme for preparing 2 substituted benzimidazole**

**Table 1: Structures of ligands used for docking**

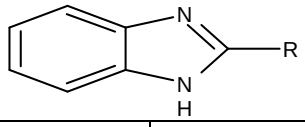
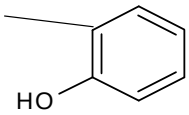
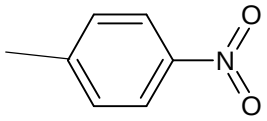
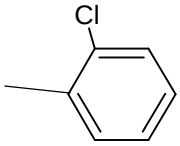
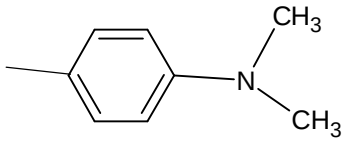
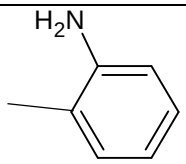
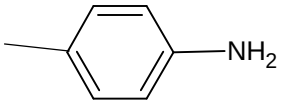
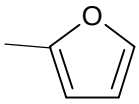
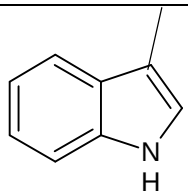
| | | |
|---|---|---|
| **General structure of ligand:** |  | |
| R |  |  |
| R |  |  |
| R |  |  |
| R |  |  |
| R |  | |

**Table 2: In silicoPhysico-Chemical Properties of the Ligands**

| S.NO | LIGANDS | SMILES NOTATION | LOG P | MOLECULAR WEIGHT(gm) | MOLECULAR FORMULA |
|---|---|---|---|---|---|
| 1. | 2-(1H-benzimidazoles-2-yl)phenol | Oc3ccccc3c1nc2ccccc2n1 | 3.25+/-0.34 | 210.2313 | $C_{10}H_{10}N_2O$ |
| 2. | 2-(4-nitrophenyl)-1-H-benzimidazole | O=N(=O)c1ccc(cc1)c2nc3ccccc3n2 | 3.21+/-0.30 | 239.22946 | $C_{13}H_9N_3O_2$ |
| 3. | 2-(4-chlorophenyl)-1H-benzimidazole | Clc1ccc(cc1)c2nc3ccccc3n2 | 3.84+/-0.32 | 228.67696 | $C_{13}H_9N_2Cl$ |
| 4. | 2-(2-chlorophenyl)-1-H-benzimidazole | Clc3ccccc3c1nc2ccccc2n1 | 3.42+/-0.31 | 228.67696 | $C_{13}H_9N_2Cl$ |
| 5. | 4-(1H-benzimidazol-2-yl)-N,N-dimethyl aniline | CN(C)c1ccc(cc1)c2nc3ccccc3n2 | 3.66+/-0.31 | 237.2997 | $C_{15}H_{15}N_3$ |
| 6. | 2-(1H-benzimidazol-2-yl)aniline | Nc3ccccc3c1nc2ccccc2n1 | 3.00+/-0.34 | 209.24654 | $C_{13}H_{11}N_3$ |
| 7. | 4-(1H-benzimidazol-2-yl)aniline | Nc1ccc(cc1)C2Nc3ccccc3N2 | 2.49+/-0.30 | 209.24654 | $C_{13}H_{11}N_3$ |
| 8. | 2-(furan-2-yl)-1H-benzimidazole | n1c3ccccc3nc1c2ccco2 | 2.67+/-0.52 | 184.19402 | $C_{11}H_8N_2O$ |
| 9. | 2-(3H-indol-2-yl)-1H-benzimidazole | c1cccc2nc(nc12)C=3Cc4ccccc4N=3 | 2.48+/-0.60 | 233.267 | $C_{15}H_{11}N_3$ |
| 10. | 2-phenyl-1H-benzimidazole | c1cccc2nc(nc12)c3ccccc3 | 3.25+/-0.28 | 194.2319 | $C_{13}H_{10}N_2$ |

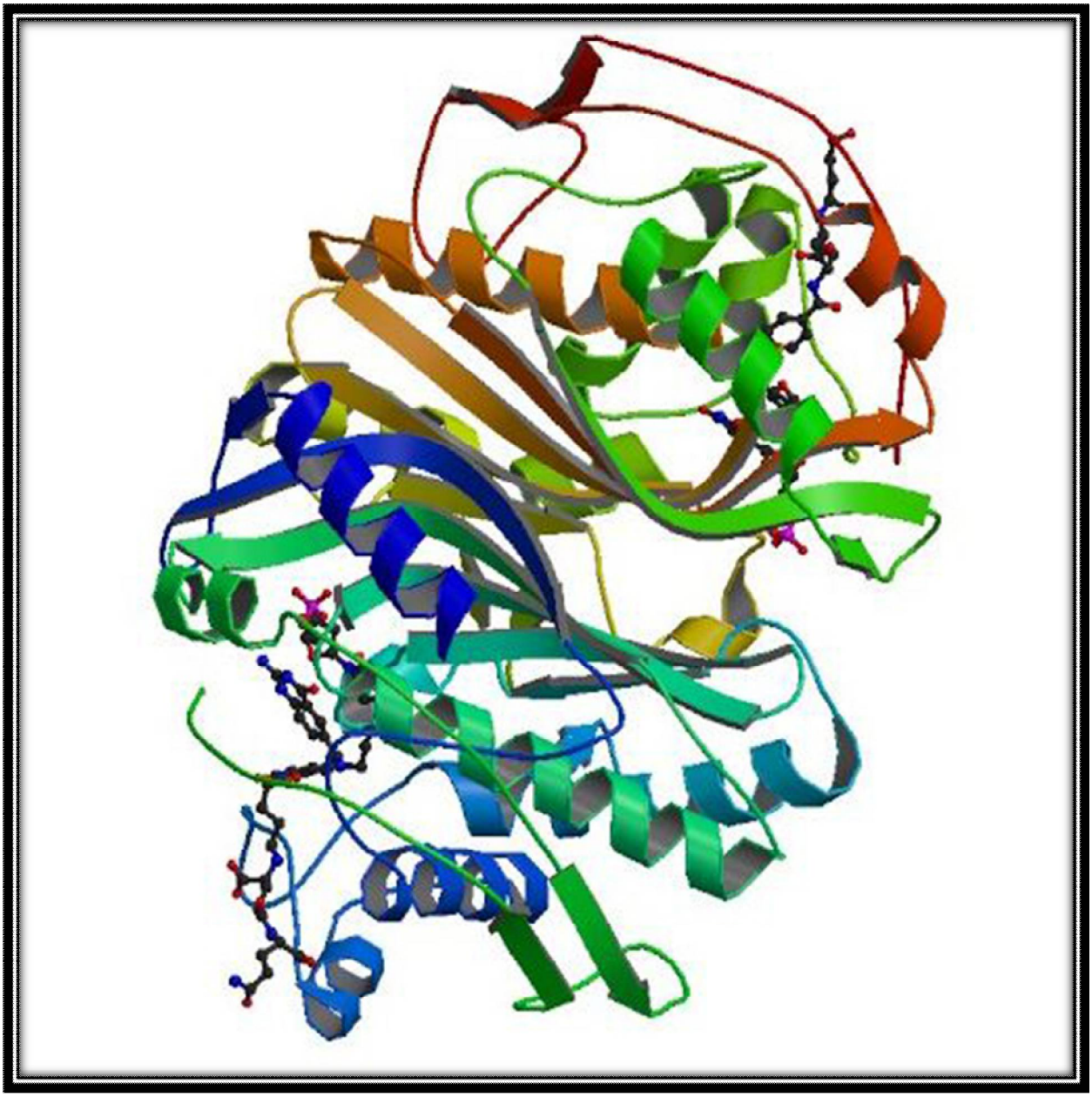


**Fig.2: Image of ThymidylateSynthetase downloaded from protein data bank**

**Table 3: Physicochemical Characterisation Data fortheSynthesised 2sub-Benzimidazoles**

| S.No. | Name of the Compound | Melting Point in ◦C | $R_f$ | % Yield | Appearance | Solubilty |
|---|---|---|---|---|---|---|
| 1 | 2-(4-nitrophenyl)-1*H*-benzimidazole | 240 | 0.75 | 69.7% | Amorphous | Soluble in ethanol |

**Table 4: List of Binding Energies and Hydrogen Bonding Interactions between 2-Substituted BenzimidazolesandThymidylateSynthetase**

| S.NO | LIGANDS | BINDING ENERGY | HYDROGEN BONDS | DISTANCE IN $^0$ A | AMINOACIDS INVOVLEDI IN H-BOND |
|---|---|---|---|---|---|
| 1 | 2-(1*H*-benzimidazol-2-yl)aniline | Infinite | - | - | - |
| 2 | 4-(1*H*-benzimidazol-2-yl)aniline | -11.09 Kcal/mol | 2 | 2.13 | GLU6 |
| | | | | 2.056 | GLU3 |
| 3 | 4-(1*H*-benzimidazol-2-yl)-*N*,*N*-dimethyl aniline | -10.66 Kcal/mol | 1 | 2.106 | GLN33 |
| 4 | 2-(4-nitrophenyl)-1*H*-benzimidazole | -12.84 Kcal/mol | 3 | 2.218 | GLN33 |
| | | | | 1.933 | ARG35 |
| | | | | 2.068 | LYS10 |
| 5 | 2-phenyl-1*H*-benzimidazole | -11.68 Kcal/mol | 1 | 2.179 | ARG127 |
| 6 | 2-(4-chlorophenyl)-1*H*-benzimidazole | -11.02 Kcal/mol | 1 | 1.827 | GLN33 |
| 7 | 2-(2-chlorophenyl)-1*H*-benzimidazole | -11.03 Kcal/mol | 1 | 1.93 | GLN33 |
| 8 | 2-(furan-2-yl)-1*H*-benzimidazole | -10.77 Kcal/mol | 2 | 1.949 | GLN33 |
| | | | | 2.188 | GLN33 |
| 9 | 2-(3*H*-indol-2-yl)-1*H*-benzimidazole | -12.01 Kcal/mol | 1 | 1.928 | ARG127 |
| 10 | 2-(1*H*-benzimidazol-2-yl)phenol | Infinite | - | - | - |

**Table 5: MTT Assay of 2-(4-Nitrophenyl)-1*H*-Benzimidazole**

| S. No. | Compound concentration(µM) | Cell viability (%) | Percentage growth inhibition (%) |
|---|---|---|---|
| 1. | 100 | 58.9 | 41.1 |
| 2. | 10 | 62.5 | 37.5 |
| 3. | 1 | 71.1 | 28.9 |
| 4. | 0.1 | 68.1 | 31.9 |
| 5. | 0.01 | 83.3 | 16.7 |

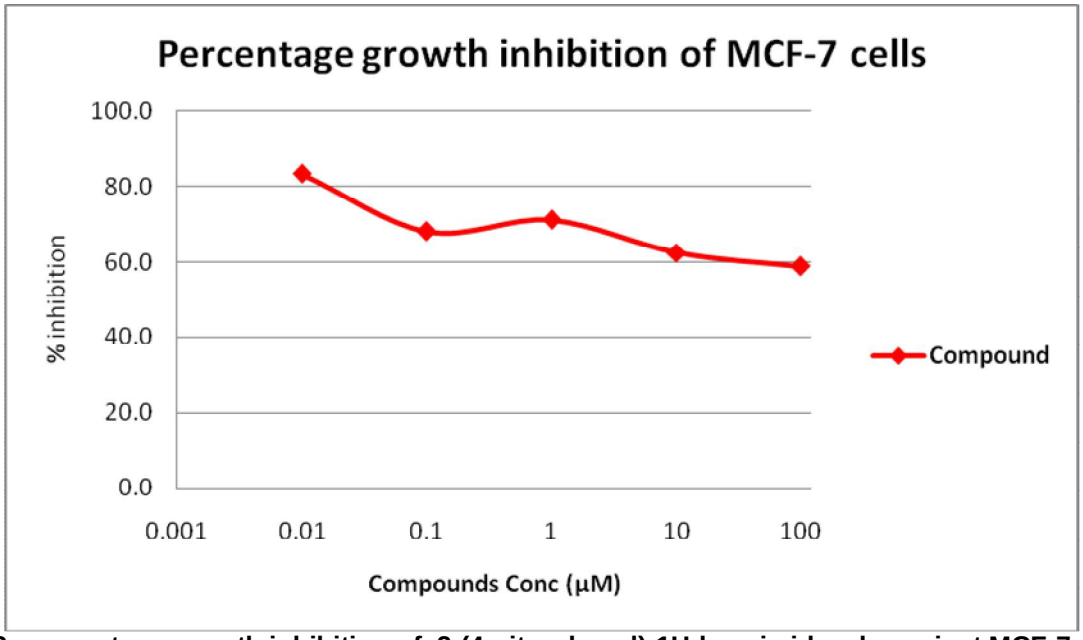



**Fig.3: percentage growth inhibition of 2-(4-nitrophenyl)-1H-benzimidazole against MCF-7 cells**

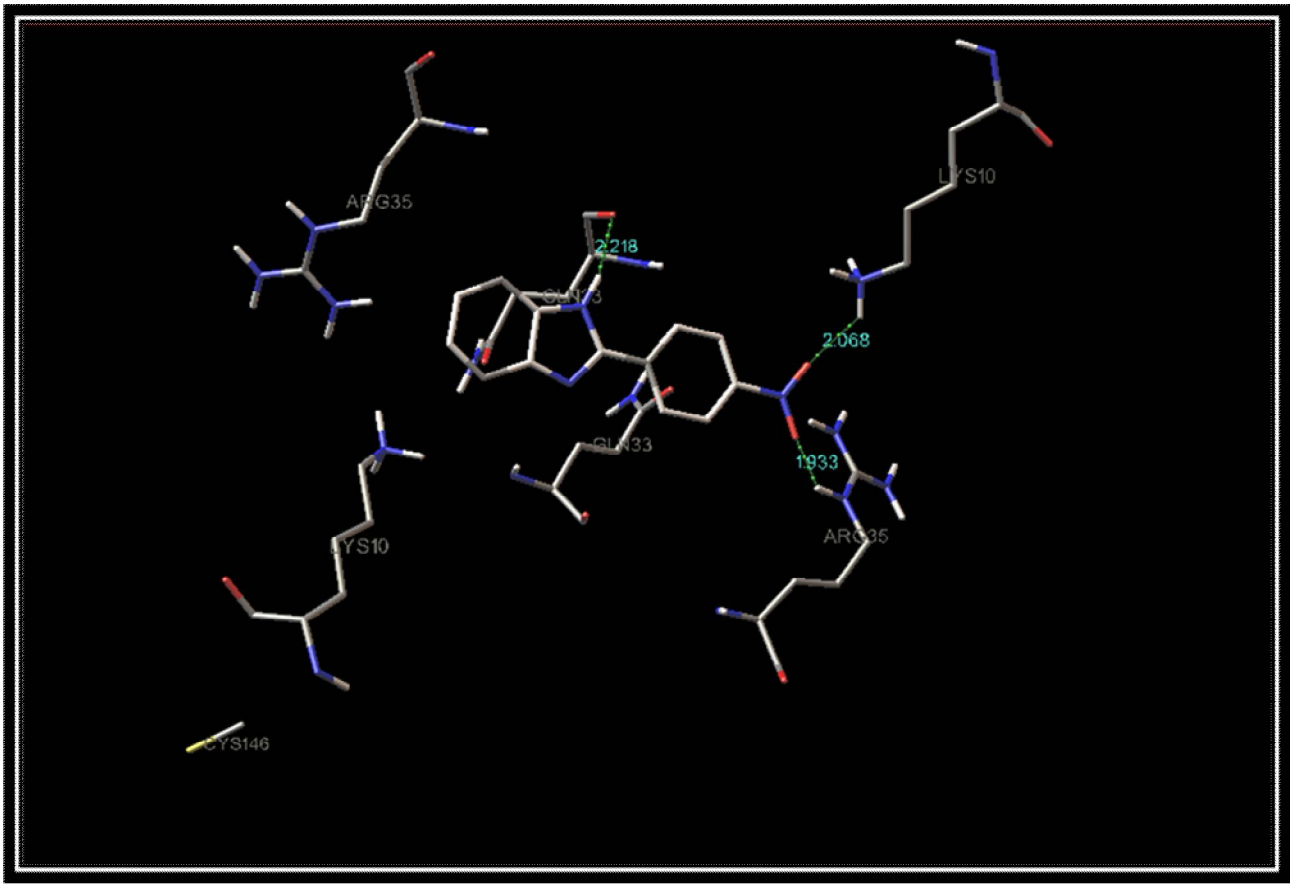



**Fig.4: Docking Complex Of 2-(4-nitrophenyl)-1H-Benzimidazole With 2BBQ**

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