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Molecular Docking Studies of Substituted Biguanides Against AMP activated Protein Kinase.

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ABSTRACT

Type 2 diabetes is characterized by abnormal metabolism of glucose and fat, due in part to resistance to the actions of insulin in peripheral tissues. The benefit of exercise in diabetic patients is well known and recent research indicates that AMP activated protein kinase [AMPK] plays a major role in this exercise related effect. AMPK is considered as a master switch regulating glucose and lipid metabolism. In this paper we studied the interactions of substituted biguanide compounds with AMPK receptor in *Insilico* model. The various conformations of the substituted biguanide compounds were docked with the target protein AMPK using the software Discovery Studio version 3.5. The most effective ones were identified based on interaction energy and docking score. Further investigations into the antidiabetic potential of the identified compounds may open new avenues for the design more potent inhibitors.

Keywords: Discovery Studio 3.5, AMP activated protein kinase, Molecular docking, substituted biguanides.

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INTRODUCTION

Obesity is a said to greatest public challenge around the world by WHO [1, 2]. It is one of the serious health problems due to high incidence of metabolic syndromes like type 2 diabetes, cardiovascular disease, and osteoarthritis seen in obese individuals [3, 4]. Biguanides are a class of compounds known for more than 100 years [5]. Bigunides have broad applications. Preparation of biguanide salts were reported by Bamberger and Dieckmann. They have applications like antiseptics [6], antimalarials [7, 8], serotoninergic antagonists [9], antitumoral agents [10] and hypoglycemic agents [11].

The metabolic effects of metformin are mediated by the target AMP-activated protein kinase [AMPK]. The enzyme AMPK is the major regulator of lipid biosynthetic pathways by phosphorylation and inactivation of key enzymes such as acetyl-CoA carboxylase [ACC]. AMPK also regulates the fatty acid oxidation, muscle glucose uptake [12-14], expression of cAMP-stimulated gluconeogenic genes such as PEPCK and G6Pase [15], and glucose-stimulated genes associated with hepatic lipogenesis, including fatty acid synthase [FAS], Spot-14 [S14], and L-type pyruvate kinase [16]. The DM2 can be treated by the activation of AMPK as it express the muscle hexokinase and glucose transporters [Glut4] mimicking the effects of extensive exercise training [17]

Molecular Docking studies are useful in analyzing the orientation of the molecule [ligand/inhibitor], and predicting the affinity of a given molecule to a protein-binding site [18]. Virtual screening of chemical moieties is one of the main techniques currently used in Drug Discovery which is useful in testing natural and synthesized compounds [19, 20]. The aim of this study is to screen new bigunide AMP-activated protein kinase using molecular docking approach.

MATERIALS AND METHODS

A typical docking study requires three computational steps before running the docking program: [1] preparation of the receptor, [2] preparation of the ligand, and [3] setup of the parameters of the docking program.

Receptor preparation

The protein data bank is a valuable source of 3D structures of proteins and nucleic acids. The X-ray crystallography or NMR spectroscopy data of proteins can be obtained from the ebsites like RCSB, PDBE and PDBj. The three dimensional structure of AMP-activated protein kinase [AMPK] was retrieved from protein data bank [http://www.rcsb.org/pdb/home/home.do].

AMPK

The crystal structures of the human 4EAICo-crystal structure of an AMPK core with AMP have been determined to 2.20 Å resolutions. AMPK protein was prepared by retaining its crystal ligand. The water molecules and heteroatoms were deleted. Then hydrogen atoms were added and by keeping fixed atom constraints on side chain and backbone of the receptor molecule, only the hydrogen's were minimized.

Ligand preparation

Structures of the compounds were drawn by using Chemsketch and then the ligands were loaded in Discovery Studio 3.5 The compound datasets were screened effectively for CDOCKER energy to decrease cost and clinical failures of new drugs.

Target Protein and Active Site Prediction

The most important or favored regions of the proteins were evaluated by means of various literature survey and the site was selected with the presence of most active amino acids within different active sites of protein

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Molecular docking

Docking was mainly carried out by using Discovery Studio 3.5. The required structure of proteins and ligands were prepared. The prepared proteins were defined as receptor molecule by clicking on define selected molecule as receptor under define and edit binding site and by selecting only the ligand part and clicking on define sphere from receptor site. By means of this the crystal ligand defined the binding site of 9Å on the receptor molecule. Now the prepared receptor molecule can be input for input receptor molecule parameter in the CDOCKER protocol parameter explorer. Each of the molecules were given as input in other parameter meant for input ligands and the protocol were run as many times as the number of inhibitors selected. The CDOCKER ENERGY of best poses docked into the receptor of all derivatives was calculated.

RESULTS AND DISCUSSION

Docking studies of the designed compounds were carried out to find out the best fit orientation of the molecule with the specified target. The designed compounds were docked into with 4EAI structure. Docking was performed using Discovery Studio 3.5. From the results obtained it was observed that all the designed compounds exhibited good binding with the targets. Dock scores of biguanide derivatives with the AMPK Target Proteins are given in the Table No: 2. Interaction of Active Site Residues of AMPK with biguanide derivatives are given in the Table No: 3. Docking model of active site residues of AMPK with MET117 and MET119 are given in the figure no 1 and 2. In the present study comparing to other compounds MET117 showed three hydrogen bonding and CDOCKER energy was found to be 22.5011. MET119 showed three hydrogen bonding interaction with AMPK on docking and CDOCKER energy was found to be 16.6198.

Table No: 1 Structures of biguanide derivatives and Structure code

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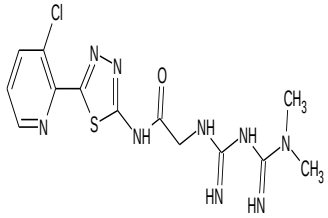
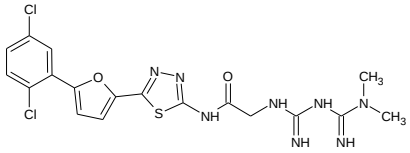
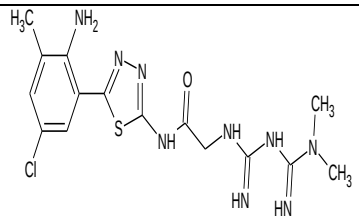
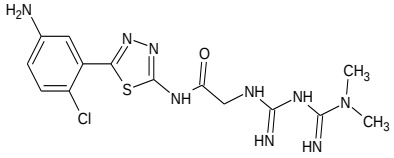
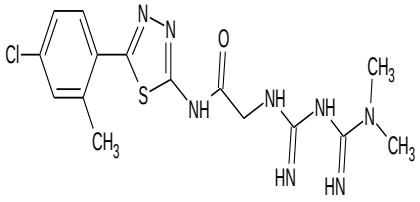
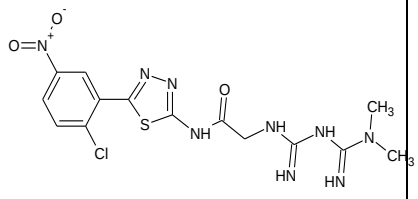
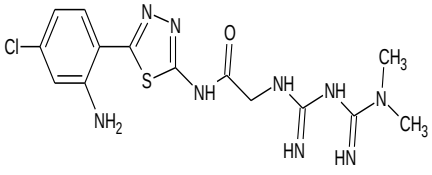
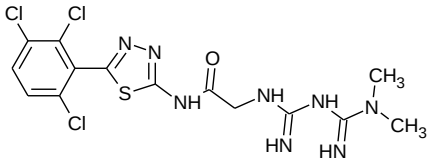
MET101		MET120	
MET102		MET121	
MET103		MET122	
MET104		MET123	

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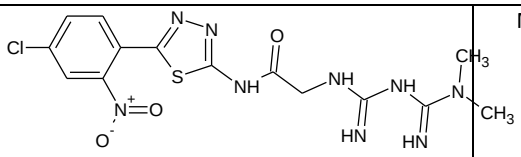
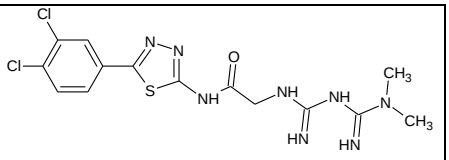
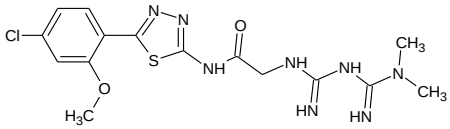
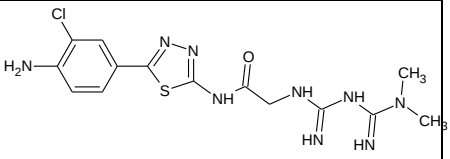
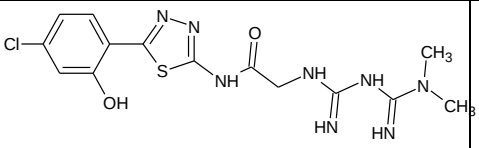
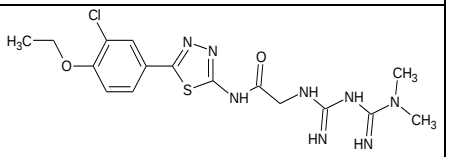
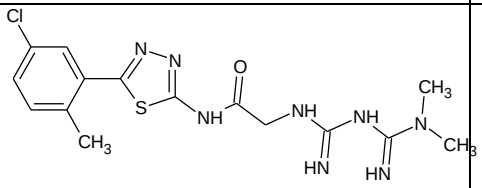
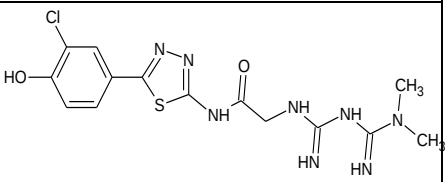
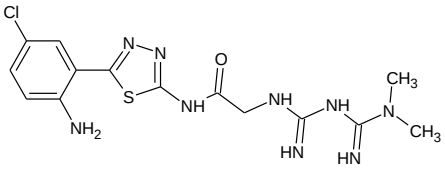
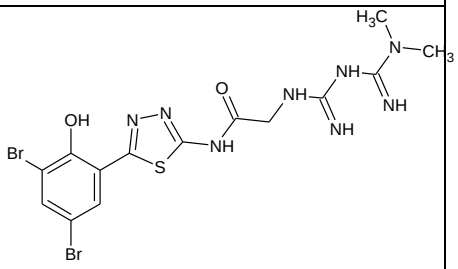
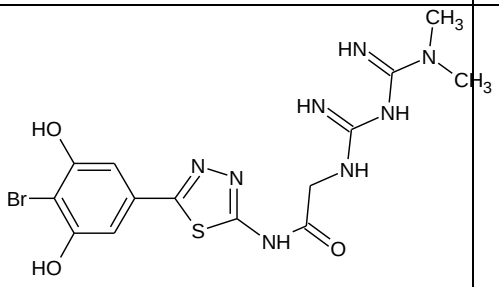
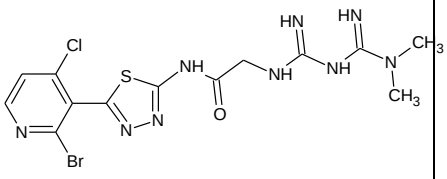
MET105		MET124	
MET106		MET125	
MET107		MET126	
MET108		MET127	
MET110		MET128	
MET117		MET119	

Table no: 2 Dock scores of biguanide derivatives with the AMPK Target Proteins

S. No	C docker energy	Interaction ligand residue	H bond distance	Amino acids
MET1 01	-780.083	N of Thiadiazole	2.3687	SER402
MET102	-531.195	NH	1.9931	MET410
MET103	-591.708	NH	1.9791	MET410
MET104	-539.708	NH	1.9879	MET410
MET105	-613.646	NH	1.972	MET410
MET106	9.8632	NH	2.3657	ALA493

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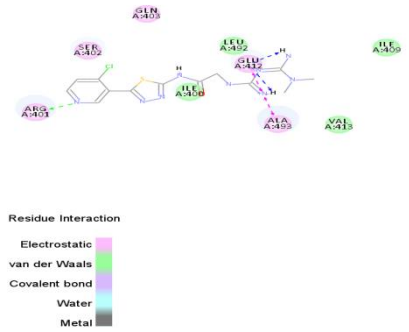
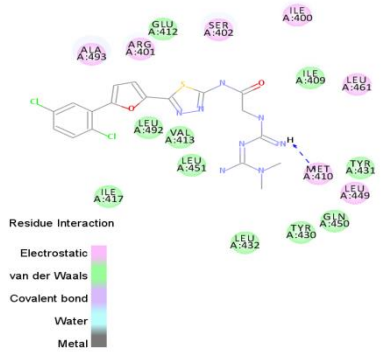
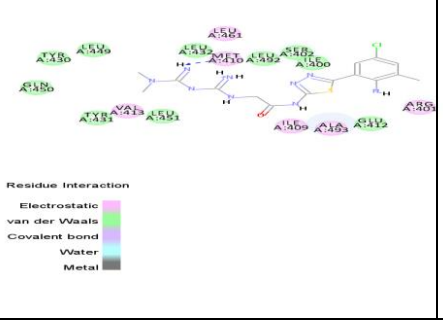
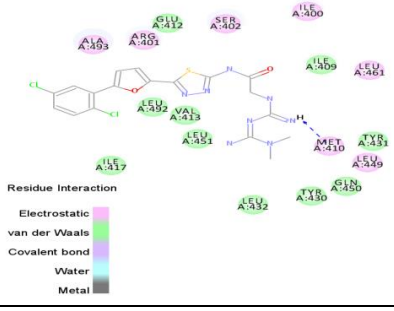
MET107	-10.0701	N of Thiadiazole	2.412	ALA493
MET108	-606.987	NH	2.0143	ARG401
MET110	-642.055	NH	2.0613	MET410
MET117	22.5011	attached to Oxygen, NH, NH	1.8094,2.1917,1.9423	ARG401,ALA493,ALA493
MET120	-474.916	NH	1.8797	MET410
MET121	4.59653	NH	2.0732	GLU412
MET122	6.10499	NH	2.3463	ALA493
MET123	2.89902	NH	2.2126	GLU412
MET124	-554.025	NH	1.9337	MET410
MET125	-611.821	NH	2.1757	MET410
MET126	-5.03452	NH	2.4437	GLN403
MET127	-571.218	NH	1.9948	MET410
MET128	-631.673	NH	1.9561	MET410
MET119	16.6198	OH,NH,OH	2.1284,1.9283,1.9784	ARG401,GLU412,ARG401

Table: 3 Interaction of Active Site Residues of AMPK with biguanide derivatives

S.NO	COMPOUND CODE	**SNO**	
MET1 01		MET120	
MET102		MET121	

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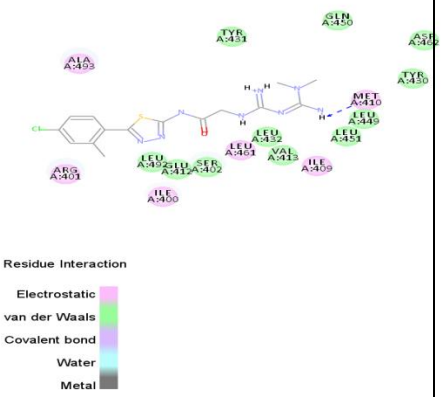
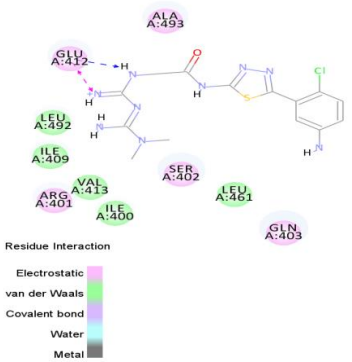
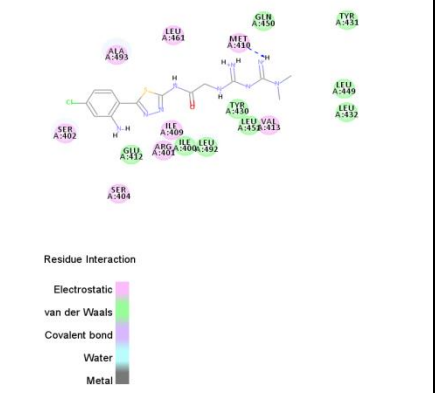
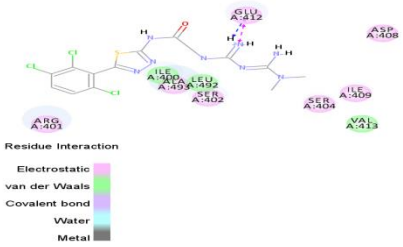
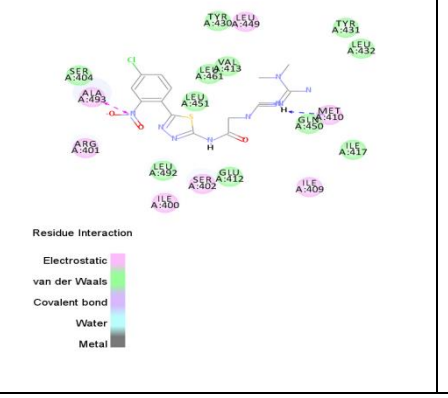
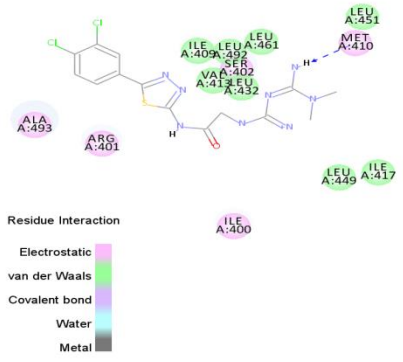
MET103		MET122	
MET104		MET123	
MET105		MET124	

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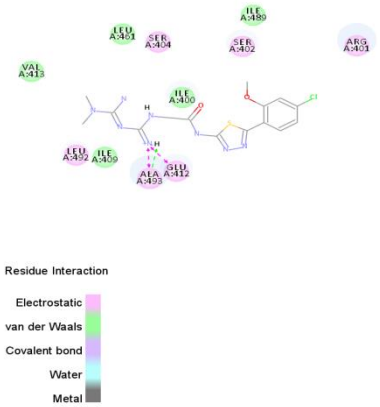
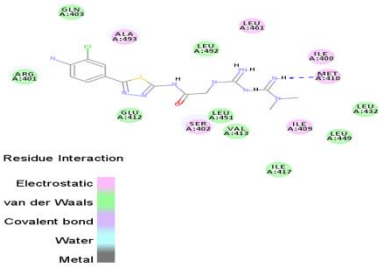
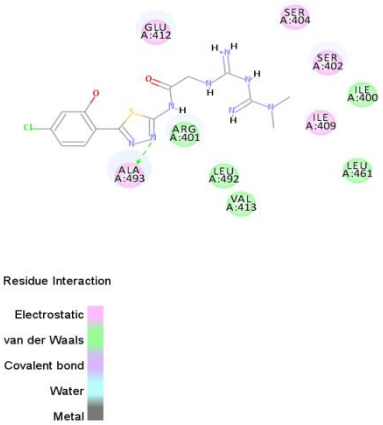
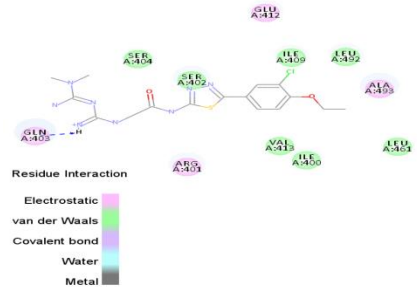
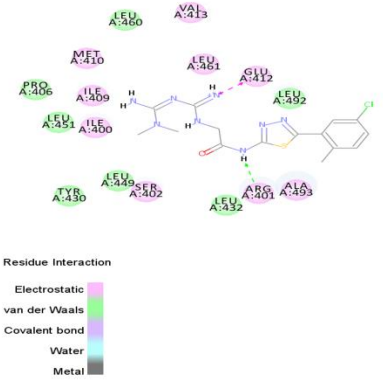
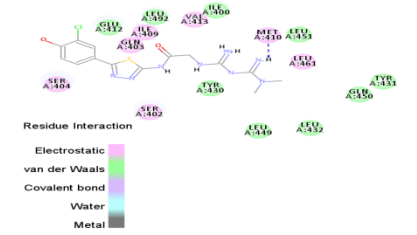
MET106		MET125	
MET107		MET126	
MET108		MET127	

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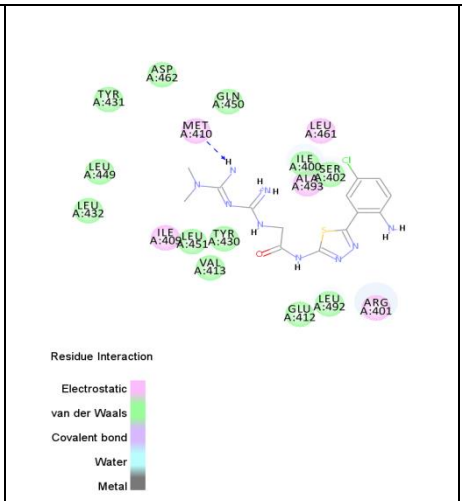
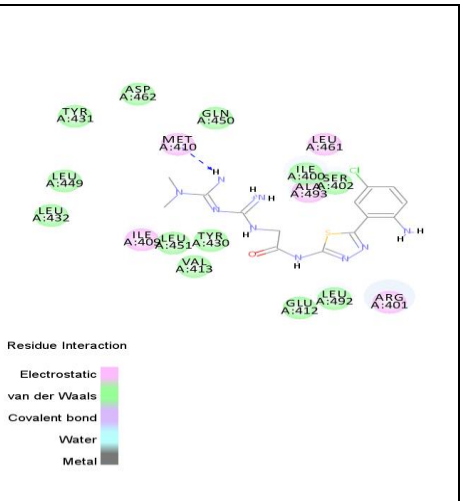
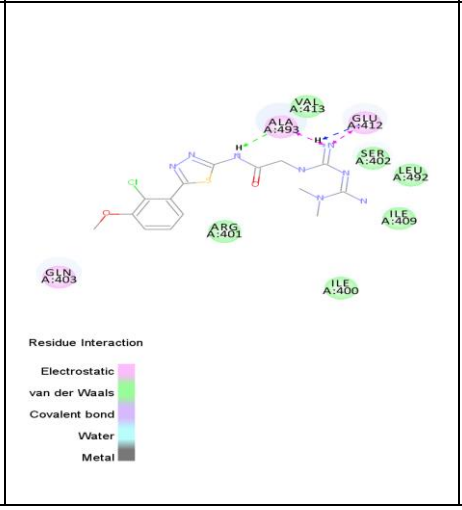
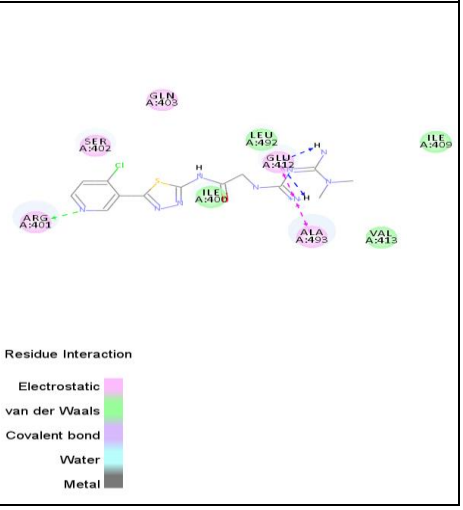
MET110		MET128	
MET117		MET119	

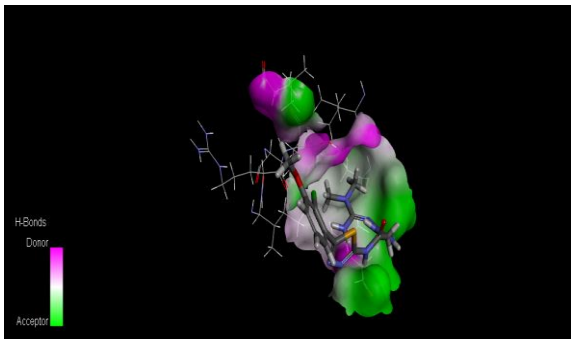


Figure no 1: Docking model of active site residues of AMPK with Met117

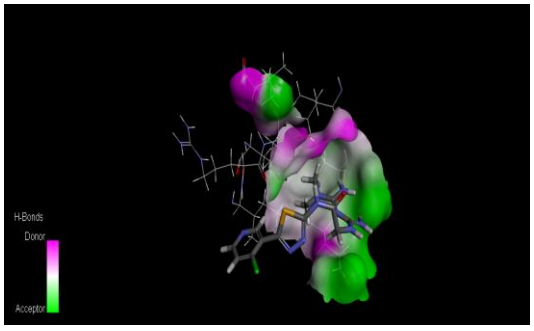


Figure no 2: Docking model of active site residues of AMPK with Met119

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CONCLUSION

The binding affinities of the ligands can be are predicted by docking protocols. Our aim is to analyze the docking score of substituted bigunides with an existing protein AMPK under study. In the present study 20 derivatives of biguanide linked with 1, 3, 4-thiadiazoles were designed. Molecular docking was performed in target using Discovery Studio 3.5. From the above *insilico* studies it was concluded that the compounds MET117 and MET119 possess good characteristics feature for the lead molecule. In addition, we suggest these compounds to be tested *invivo* against antidiabetic activity.

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