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STRUCTURAL OPTIMIZATION AND DOCKING STUDIES OF IMIDAZO [2,1-B][1,3,4]THIADIAZOLE DERIVATIVES AS FTSZ CELL DIVISION PROTEIN INHIBITORS IN MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Filamentous temperature-sensitive protein Z (FtsZ) is recently considerable as attractive target for anti-bacterial drug discovery. The inhibition action of Filamenting temperature-sensitive mutant Z, an indispensable and highly conserved bacterial cytokinesis protein, is a favourable perspective for the development of a new class of antibacterial agents The series of imidazo[2,1-b][1,3,4]thiadiazole derivatives has been reported as an antitubercular activity. In view of antimycobacterial activity, it is targeted to FtsZ protein. Molecular mechanics studies of imidazo[2,1-b][1,3,4]thiadiazole derivatives were performed according to the Hartree-Fock (HF) calculation method by Argus Lab 4.0.1 software. Our docking studies revealed that all the compounds (1-10) have the potential to inhibit FtsZ protein with a binding energy in a range of -4.78 to -6.08 Kcal/mol.

KEYWORDS: FtsZ, Argus Lab 4.0.1, conformational analysis, HOMO, LUMO.

1. INTRODUCTION

The emergence of multi-drug resistant Mycobacterium tuberculosis (Mtb) strains has made many of the currently available anti-TB (Tuberculosis) drugs ineffective. Multi-drug resistant TB (MDR-TB) is a form of TB that does not respond to the standard treatments using first line drugs. Poor chemotherapeutics and the inadequate administration of drugs have led to the development of MDR-TB, the treatment of which requires administration of more expensive,

second line antibiotics for up to two years.[1,2] It has been estimated that 90% of humans who are exposed to or infected with the pathogen have latent- TB, which has a 10% chance of progressing to an active-TB diseased state during their lifetime.[3] Now- a days one-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb), the etiological agent of TB, resulting in 9.2 million new cases and 1.7 million deaths in 2006[4] So, there is urgent need for the development of novel TB drugs that are effective against both drug sensitive and resistant Mtb strains. In this context, Filamenting temperature-sensitive protein Z (FtsZ), an essential bacterial cytokinesis protein, is a highly promising therapeutic target.[5,6] FtsZ (Filamentous temperature sensitive protein Z) is a bacterial GTPase that is structurally similar to eukaryotic cytoskeletal protein tubulin.[7] FtsZ proteins is highly conserved in most bacteria but absent in higher eukaryotes, which proposes that FtsZ inhibitors should not be toxic to human cells. FtsZ undergoes GTP-dependent polymerization into filaments, which assemble into a highly dynamic Z-ring to form a cell-division complex called a divisome on the inner membrane of the mid-cell.[8] The divisome constricts to induce septum formation, followed by splitting of the daughter cells.[9] So inactivation of FtsZ or alteration of FtsZ assembly results in the inhibition of cell division. It is a very promising target for new antimicrobial drug development. Patel et al reported synthesis, spectral studies, antimycobacterial evaluation and cytotoxic activity of various imidazo[2,1-b][1,3,4]thiadiazole derivatives with minimum inhibitory concentration values (MIC) in the range of 3.14 t0 6.25 (µg/ml).[10] In view of antimycobacterial activity of imidazo [2,1-b][1,3,4]thiadiazole derivatives, we have taken those series of compounds (1-10) FtsZ target to study its binding affinity by computational conformational analysis. The present work describes the computer aided geometry optimization (active conformation) and excited state properties of these compounds 1-10 by using Argus Lab software (V: 4.0.1). The protein binding pattern of imidazo[2,1-b][1,3,4]thiadiazole derivatives to X-ray crystallized structure of *Mycobacterium tuberculosis* FtsZ (PDB ID: 2Q1Y) was investigated using Autodock version 4.0.

2. MATERIALS AND METHODS

2.1. Argus lab

The structure of imidazo [2,1-b][1,3,4]thiadiazole derivatives (1-10) were drawn with ACD Lab Chem Sketch software and saved as MDL molfiles (.mol). All conformational analysis (geometry optimization) study was performed on a window based computer using Argus lab software (V: 4.0.1). Conformational analysis (geometry optimization) was carried out using

PM3 semi-empirical QM parameterization according to Hartree-Fock calculation method by Argus Lab software.[11] Geometry of the molecule was converged after the molecule was drawn and cleaned in Argus lab and the program computed the energy until the maximum cycles reached for the convergence (stopping point) of the molecule. The electronic excited-state calculations were carried out by ZINDO semi-empirical method which is parameterized for low energy excited-states of organic and organometallic molecules.[12] The minimum potential energy is calculated by using geometry convergence function in Argus lab software. Surfaces created to visualize the excited state properties such as orbital, electron densities, electrostatic potentials (ESP) mapped density. The minimum potential energy was calculated through the geometry convergence map,[13-16] Mulliken Atomic Charges and ZDO Atomic Charges were determined using PM3 method.

2.2. Autodock

Autodock version 4.0[17] is used to predict the binding energy and IC_{50} values for the imidazo[2,1-b][1,3,4]thiadiazole derivatives (1-10) with drug target FtsZ . The X-ray crystal structure of the *Mycobacterium tuberculosis* FtsZ with bound sulphate ion (PDB code: 2Q1Y) was resolved using X-ray diffraction method with a resolution factor of 2.3 Å was retrieved from the RCSB Protein Data Bank (http: //www. rcsb.org). The energy scoring grid box was set to 90, 90 and 90 Å (x, y, and z) centered at X = - 6.307; Y = 53.272; and Z = 0.296 with 0.375 angstroms grid points spacing assigned with default atomic salvation parameters. Lamarckian Genetic Algorithm (LGA) was selected as a docking engine, with all the docking parameters set to default. After LGA run, autodock reports the best docking solution along with IC_{50} values for docked complex, and the results are reported based on the cluster analysis.[18] From a total of 10 docking modes represented by LGA cluster analysis, the lowest energy docking mode with respective IC_{50} prediction was selected from the docking simulation. The autodock calculation was run thrice to check the convergence of the results.

3. RESULTS AND DISCUSSION

The molecule 2-(1-methyl-1*H*-imidazol-2-yl)-6-(4-nitrophenyl) imidazo[2,1-*b*][1,3,4]thiadiazole (compound **6**) is build using molecule builder of Argus lab. The Molecule Settings of compound **6** are atoms 32, Net charge zero and Valence electrons 114. Active conformation of compound **6** with labeled atoms is illustrated in figure 1 by Argus Lab software**.** The active conformation with labeled atoms of compound **6** was found to be

245.49732239 kcal/mol which is the minimum potential energy calculated by geometry convergence as calculated by RHF/ PM3method, Argus Lab 4.0.1 suite.

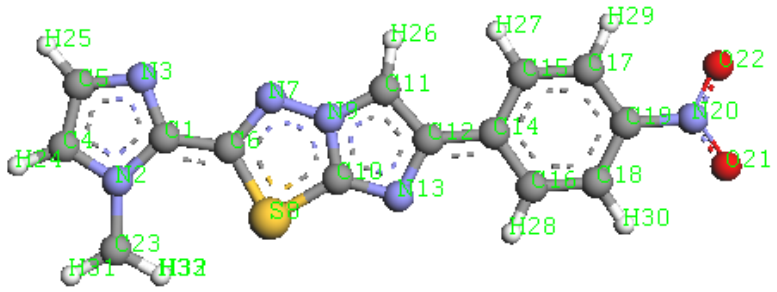


Figure 1: Active conformation of compound 6 with optimized geometry by Argus Lab 4.0.1 software.

3.1. Molecular Docking Simulation

The molecular docking studies was performed in order to study the detailed molecular basis of interactions and to estimate the binding affinity of the present studied compounds imidazo[2,1-b][1,3,4]thiadiazole derivatives (compound 1-10) with FtsZ protein active site. In order to understand the plausible experimental activity of the present studied compounds, the half maximal inhibitory concentration (IC_{50}) value was also performed. IC_{50} value is a useful parameter to quantitatively measure the effectiveness of compound to inhibit a given biological process by half and is universally used to symbolize the inhibitory effect of the compounds.[19] The predicted IC_{50} values along with associated binding energies for the compounds 1-10 are shown in Table 1. In silico protein ligand studies of 2Q1Y with test molecules showed docking scores in the range of -4.78 kcal / mol to -6.08 kcal /mol. Taken the best result out of three runs; compound **6** with binding energy-6.08 kcal/mol and IC_{50} value of 35.03 micro molar is quite promising FtsZ inhibitor. The hydrogen bond interactions of compound **6** with 2Q1Y showed two hydrogen bond interaction with Arg140: HH12, Arg140: HH22 with the distance of 2.039 Å and 2.087 Å respectively (Figure 2).

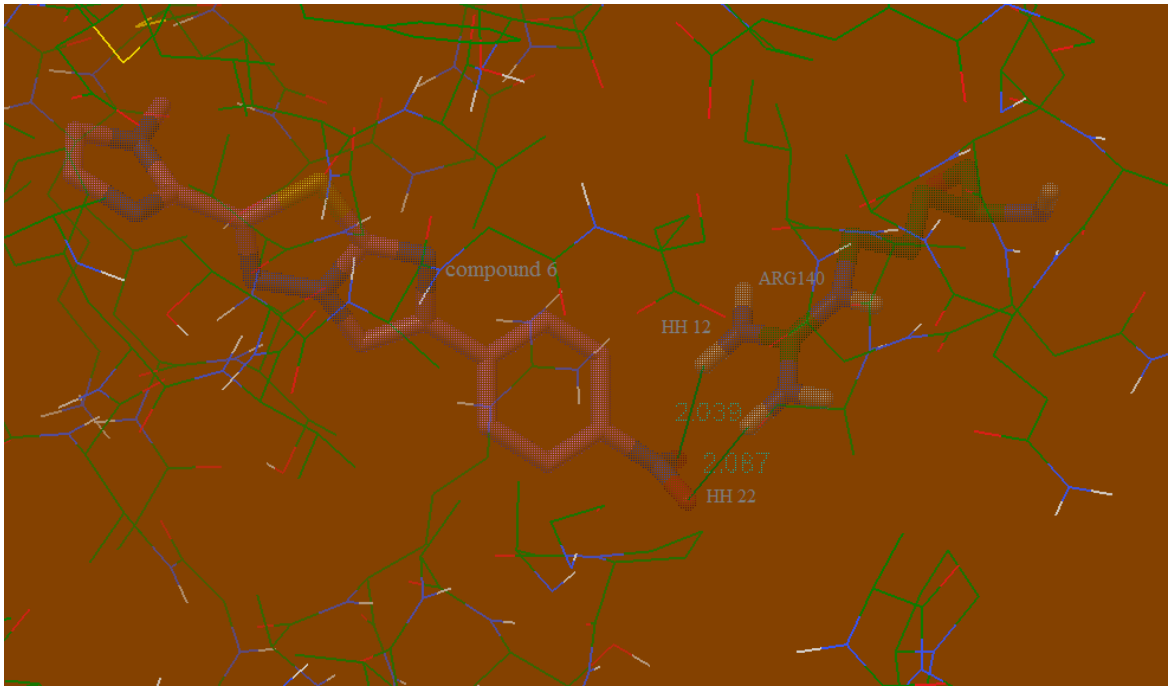
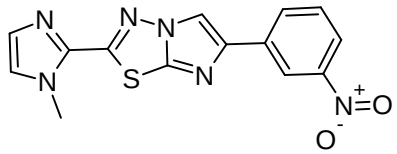
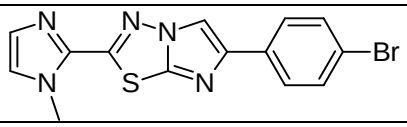
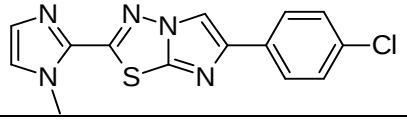
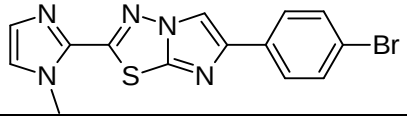
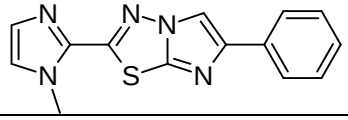
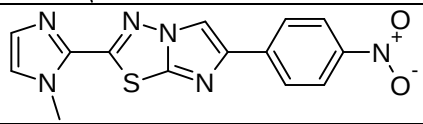
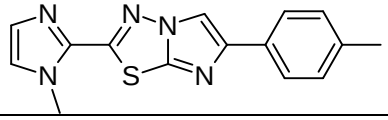
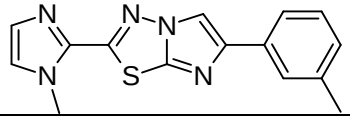
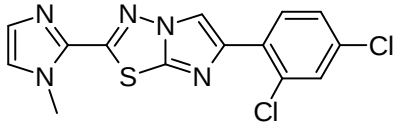
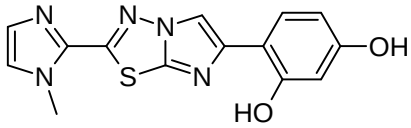


Figure 2: Docking poses of 2Q1Y with compound 6 forming hydrogen bond at Arg140.

Table 1: Quantum mechanical calculation data of compounds 1-10.

S. no	Structure of Compounds	E_{HOMO} (eV)	E_{LUMO} (eV)	GAP (eV)	Docking score (kcal / mol)	IC_{50} (µM)
1		-0.148438	0.038140	0.186578	-5.95	43.68
2		-0.143865	0.068982	0.212847	-4.93	257.4
3		-0.143488	0.073427	0.216915	-4.9	254.73
4		-0.143374	0.073795	0.217169	-4.78	315.08
5		-0.128426	0.077177	0.205603	-4.88	263.12
6		**-0.152309**	**0.033702**	**0.186011**	**-6.08**	**35.03**
7		-0.247400	-0.045248	0.202152	-4.96	230.58

8		-0.220672	-0.031357	0.189315	-4.81	297.57
9		-0.144347	0.066755	0.211102	-4.9	257.66
10		-0.145780	0.051520	0.1973	-5.2	154.76

3.2. Electronic properties

It is important to examine the frontier molecular orbital energies (E_{HOMO} and E_{LUMO}) so as to explain the qualitative prediction of electronic properties of the complex. This was done theoretically using PM3. The positive and negative phases of the orbital are represented by the two colors, the blue regions represent an increase in electron density and the red regions a decrease in electron density. E_{HOMO} is a quantum chemical parameter which is correlated well with the electron donating ability of the molecule. High value of E_{HOMO} indicates the tendency of the molecule to donate electrons to appropriate acceptor molecule of low empty molecular orbital.[20] The energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$) is an important parameter as a function of reactivity of the imidazo[2,1-b][1,3,4]thiadiazole derivatives (compound 1-10) towards antibacterial activity. As ΔE decreases the reactivity of the molecule increases were leading to increase in the electron donating efficiency of the molecule[21] Lower values of the energy difference will render good antibacterial efficiency, because the energy to remove an electron from the last occupied orbital will be low. The calculated E_{HOMO} and E_{LUMO} and band gap are recorded in Table 1. The calculated energy of HOMO, LUMO and the energy gap were **-0.152309**, **0.033702** and **0.186011** eV, respectively for compound **6**. It has the lowest ΔE value than the other investigated thiadiazole molecules that indicates better towards antibacterial activity.

3.3. HOMO and LUMO orbitals

The Frontier molecular Orbital, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were found to be extremely useful in describing electron density clouds around the molecule. Among the molecular orbitals, HOMO is a non bonding type while the LUMO is a π molecular orbital. Electrophilic attacks were influenced very well with atomic sites having high density of the HOMO orbital, whereas nucleophilic attacks on atomic sites having high density of the LUMO orbital (Kunichi Fukui was awarded

the Nobel prize in chemistry in 1981 for developing this concept). The active conformation and electron density clouds of compound **6** represents the arrangement of electrons around the atom which determines the energy level of compound **6**. The positive and negative charges are indicated by blue and red color, respectively. The frontier molecular orbitals i.e. Highest energy occupied molecular orbital (HOMO) and the lowest unoccupied (LUMO) molecular orbital were shown in Figure 3 and Figure 4.

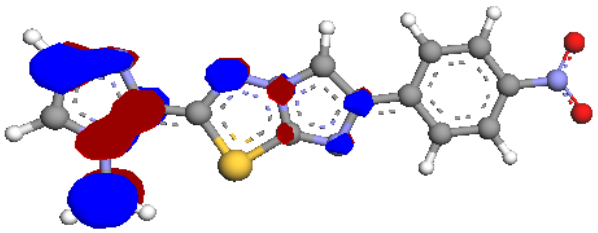


Figure 3: Visualize the HOMO (Highest Occupied Molecular Orbitals) (MO 57) of compound 6, blue shows positive and red shows negative.

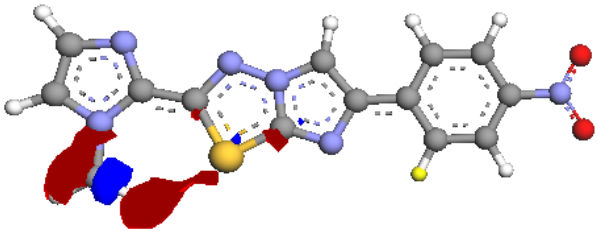
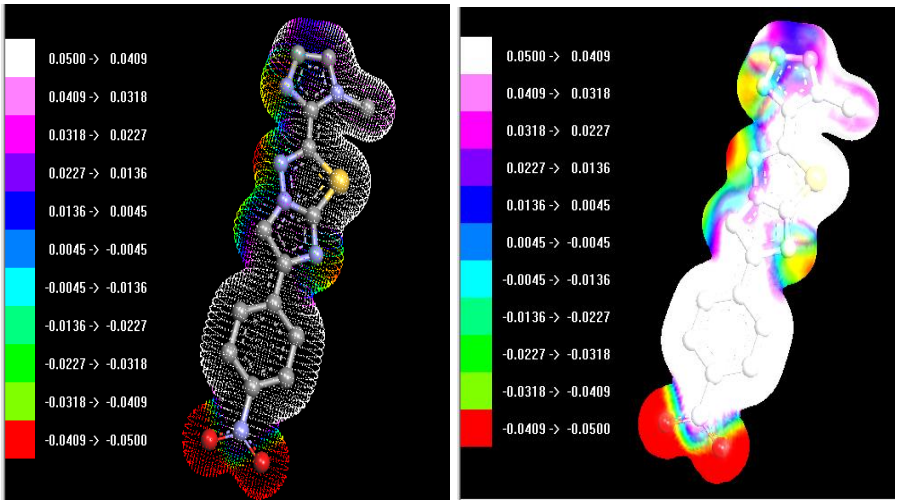


Figure 4: Visualize the LUMO (Lowest Unoccupied Molecular Orbitals) (MO 58) of compound **6**, blue shows positive and red shows negative.

3.4. Electrostatic Potential

The electrostatic potential is a physical property of a distribution of electric charge creates an electric potential in the surrounding space.[22] A positive electric potential means that a positive charge will be repelled in that region of space. A negative electric potential means that a positive charge will be attracted. The Electrostatic Potential (ESP) of compound **6** ground state mapped onto the electron density surface for the ground state rendered as mesh and translucent surface to reveal the underlying structure (Figure **5a** and Figure **5b).** The colors are the values of the ESP energy (in Hartrees) at the points on the electron density surface. The red color indicates the enhanced electron density around the oxygen-ends of nitro group of the molecule representing the most negative regions of the ESP (region of highest stability) for a positive test charge where it would have favourable interaction energy.

On the other hand the hydrogen-ends of the molecule and aromatic region, seen in white/blue color, shows the region of least stability for the positive test charge indicating the unfavourable interaction energy. Thus an ESP mapped density surface can be used to show the regions of a molecule that might be more favourable to nucleophilic or electrophilic attack, making these types of surfaces useful for the qualitative interpretations.



(a) mesh **(b) translucent**

Figure 5: Electrostatic potential (ESP) mapped electron density surface of compound 6.

4. CONCLUSION

The present work indicates that the best conformation of compound **6** was found to be -245.497 kcal/mol which is the minimum potential energy calculated by Argus Lab software. At this point compound **6** will be more active to interact with the receptors. Such types of interactions are significant for drug- receptor interactions. The Electrostatic Potential (ESP) of compound **6** shows the oxygen end of nitro group of the molecule is more favourable region of interaction. Docking simulations of compound **6** with FtsZ Mycobacterium tuberculosis suggested that – (O=N=O) end of the molecule is the key structural features that interacting with Arg140 with inhibition of -6.08 kcal/ mol. The results showed that among the analogues 1-10, compound **6** is potentially more effective target of FtsZ from computational studies as well as exhibited significant MIC value that found from literature. Experimental validation of our predictions is certainly encouraging in view of the particular significance of FtsZ target in curbing tuberculosis proliferation.

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