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In-Silico studies on molecular orbitals, geometry optimization and molecular docking of Curcumin as an anti-bacterial drug targets FtsZ protein

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Abstract: Resistance to many of the current antibacterial agents is now an alarming health problem worldwide, necessitating a proactive effort to identify new targets and for the development of entirely different antibacterial drugs. Filamenting temperature-sensitive mutant Z (FtsZ), an analogue of eukaryotic tubulin, is an essential and highly conserved bacterial cytokinesis protein considered as attractive target for anti-bacterial drug discovery. Curcumin, a naturally occurring dietary polyphenolic compound is found to bind to FtsZ in vitro with a dissociation constant of 7.3±1.8 μM. It induced filamentation in the Bacillus subtilis 168, suggesting that it inhibited the assembly of FtsZ protofilaments and also increased the GTPase activity of FtsZ. Conformational analysis and geometry optimization of curcumin was performed according to the Hartree- Fock (HF) calculation method by ArgusLab 4.0.1 software. The protein binding energy calculation and docking simulations were carried out by in silico studies using the tool AutoDock 4.0. The three dimensional (3D) structures of the Bacillus subtilis FtsZ with bound potassium ion (PDB code: 2VXY) with a resolution factor of 1.7 Å was retrieved from the RCSB Protein Data Bank. The observed docking result predicts high binding affinity of the curcumin to the receptor with up to −6.16 kcal/mol of energy.

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

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I. INTRODUCTION

The continuous need and rapid spread of new multidrug resistant (MDR) strains of bacterial pathogens (e. g. *E. Coli, S. Aureus, B. Subtilis, S. Pyogenes*) demands for development of new antibacterial. The bacterial cell comprises of several essential proteins that are preserved throughout the bacterial cell but are absent from humans. So, influence of proteins in mechanism of bacterial cell division has been regarded as attractive target against the multi drug resistant strains of pathogenic bacteria [1-3]. FtsZ (Filamentous temperature sensitive protein Z), is structurally prokaryotic homologue of eukaryotic cytoskeletal protein tubulin, polymerizes to form a Z-ring [4-5]. It is a GTPase that undergoes GTP-dependent polymerization in a nucleotide-dependent manner 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 [6]. The recruitment of other cell-division proteins leads to contraction of Z-ring and invagination of the cell membrane as well as cell wall. That causes septation of the cell and formation of two new daughter cells, as well as the depolymerization of the Z-ring [7]. Hence inactivation and alteration FtsZ assembly obstructs the formation of Z-ring and formation of daughter cell. In this context, a new approach for division of most bacterial cells, FtsZ protein is selected as an attractive antibacterial target. Rai. D et al reported Curcumin, a naturally occurring dietary polyphenolic compound inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring. The in vitro study showed that curcumin interacted to *B. subtilis* 168 FtsZ with a dissociation constant of 7.3+1.8 μM and also perturbed the secondary structure of FtsZ [8]. In an attempt to exhibit the antibacterial activity of curcumin, the interaction of curcumin with *B. subtilis* FtsZ was computed. The present work describes the

computer aided molecular orbitals, excited state properties geometry Optimization (active conformation) of curcumin by ArgusLab4.0.1 software. The geometry of a molecule influence its energy level and physical and chemical properties. On rotation of a molecule, it forms different conformation and spatial arrangement to attain lowest energy configuration of most stable state [9].The total molecular energy can be calculated in terms of potential energy surface as a sum of energies associated with each type of bonded interactions i.e. bond length, bond angle and dihedral angle as well as non-bonded interactions (Van der Waals and electrostatic) taking place in a molecule and on atomic properties of a molecule [10]. The protein binding pattern of curcumin to X-ray crystallized structure of *Bacillus subtilis* FtsZ (PDB ID: 2VXY) was investigated using Autodock version 4.0 to confirm its antibacterial activity.

II. Results and Discussion

The molecule curcumin is build using molecule builder of Argus lab. The Molecule Settings of curcumin are atoms 51, Net charge zero and Valence electrons 144. Active conformation of curcumin with labeled atoms is illustrated in figure 1 by Argus Lab software**.** The active conformation with labeled atoms of curcumin was found to be -111981.41 kcal/mol which is the minimum potential energy calculated by geometry convergence as calculated by RHF/ PM3method, Argus Lab 4.0.1 suite. This is known as self-consistent field (SCF) energy required which for the interaction of drug with the receptor. In terms of quantum-mechanical system, the SCF energy is the average interaction between a given particle and other particles that a system consisting of many particles.

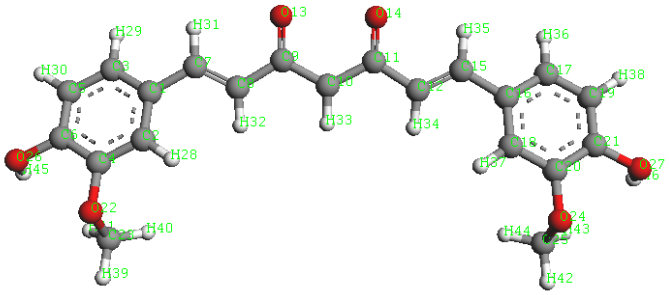

Figure 1: Active conformation of curcumin with optimized geometry by Argus Lab 4.0.1 software.

Heat of Formation

At standard conditions the atomic heat of formation is the heat released during the formation of the stable form of the element from individual atoms. As these are implicitly included by the parameterization, thermo dynamical corrections (e.g., zero-point energies) should not be added to the formation energy, as these are completely included by the parameterization. The most energetically favorable conformation of curcumin is found to have a heat of formation of 18185.1495 kcal/mol as performed by the Argus Lab software.

Molecular docking simulation

Molecular docking studies was performed in order to study the detailed molecular basis of interactions and to predict the binding affinity of the present studied compound curcumin with FtsZ protein active site. Fuchs and co-workers were designed and synthesized analogues of curcumin were tested against prostate and breast cancer lines [20]. The result of these analogues showed twenty times greater inhibitory activity than that of curcumin. By considering the multiple actions exhibited by the parent compound curcumin, we are interested to check these compounds could potentially inhibit FtsZ protein as well. 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 [21]. The binding Energies (kcal/mol) and IC_{50} value of curcumin, its analogues and PC190723 (known inhibitor of FtsZ) are calculated (Table 1).

Table 1: Calculated Binding Energies (kcal/mol) and IC_{50} value of curcumin, its analogues and PC190723 (known inhibitor of FtsZ):

S.No	Ligand Name	IC_{50} value in µM	Docking energies in Kcal/mol
1.	Analogue 3	32.4	-6.12
2.	**Analogue 12**	**18.59**	**-6.45**
3.	Analogue 16	62.02	-5.74
4.	Analogue 18	96.44	-5.48
5.	Analogue 22	55.21	-5.52
6.	Analogue 23	328.33	-4.75
7.	Curcumin	32.02	-6.13
8.	PC190723	35.42	-5.96

The predicted IC_{50} values along with associated binding energies and hydrogen bonds for the curcumin and its analogue 12 is shown in Table 2. Taken the best result out of three runs; docking score of curcumin with FtsZ protein 2VXY showed binding energy -6.13 Kcal/mol and IC_{50} value of 32.02 micro molar. In Figure 2(a) and (b), the residue SER223 and LYS155 exhibited H-bond interaction with hydroxyl group of C21 phenyl

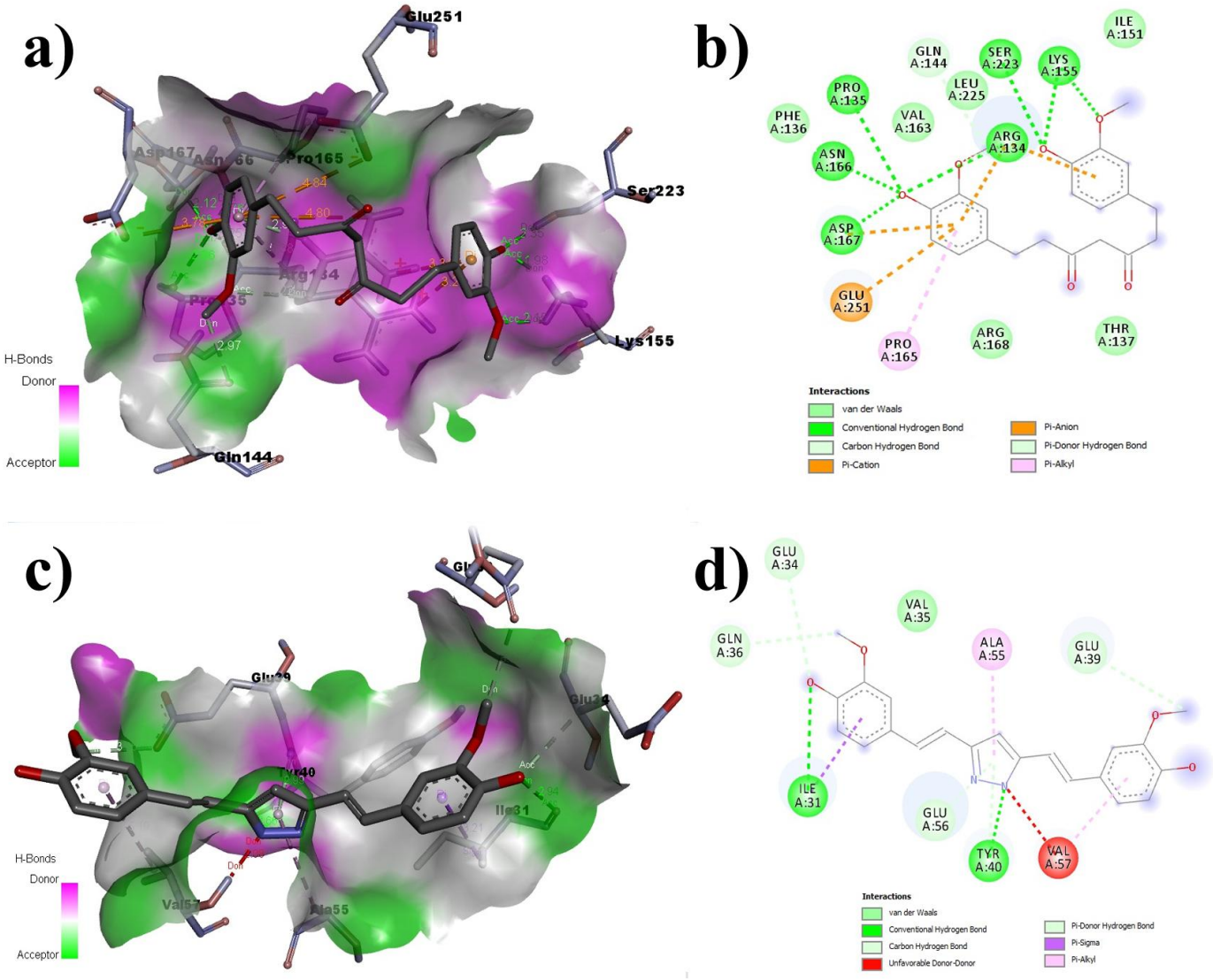

Figure 2. Docking poses of 2VXY with curcumin: **(a)** showing H-bond and contacts formed by curcumin with FtsZ protein residues ARG134; PRO135; LYS155; ASN166; ASP167 and SER223. **(b)** 2D representation snapshot showing various interactions formed by curcumin at the catalytical active site of 2VXY. : **(c)** Showing H-bond and interactions formed by Analogue12 with FtsZ protein residues ILE31; GLU34; GLN36; GLU39; TYR40; ALA55 and VAL57. **(d)** 2D representation snapshot showing various interactions formed by Analogue 12 at the catalytical active site of 2VXY.

group of curcumin with a distance of 2.35 and 1.98Å respectively. The residue LYS155 is also forming H-bond with methoxy group of C21 phenyl group of curcumin with a distance of 2.15Å. It implied that the curcumin was strongly bound within the active site of the protein molecule and can be considered as a promising lead compound. In addition, hydroxyl group of C6 phenyl group of curcumin was found to be forming H-bond interaction with ARG134, PRO135, ASN166, ASP167 with a distance of 3.25, 2.86, 2.76 and 2.12 Å respectively and methoxy group with the residue GLN144 (distance of 2.97Å) represented in supplementary figure S1. Those H-bonds were observed to be crucial, leading to stabilization of the complex. Apart from hydrogen bonds, curcumin was also found to be involving π-cation and π-anion interactions with the residues ARG134, 1SP167, GLU251 and π-alkyl with PRO165 and give more impact on stability of the molecule. However among curcumin and its analogues, analogue 12 was the most promising FtsZ inhibitor with a binding energy of -6.45 kcal/mol and IC_{50} value of 18.59 micro molar (Table 2). The favourable binding of analogue 12 can also be explained on the basis of the position of its =N-NH- fragments of pyrazole ring with TYR40 (distance of 2.66Å) and hydroxyl group of phenyl ring with ILE31 (distance of 2.94Å). In addition, ILE31

and ALA55 were also forming pi-sigma and Pi-alkyl interaction with phenyl pyrazole ring of analogue 12.

Table 2: Docking energies, IC50 value and interaction profile of curcumin and analogue 12 with 2VXY reported by Autodock version 4.0:

Ligand name	Docking energies (kcal/mol)	IC_{50} (µM)	H-Bond Acceptor residues	H-Bond Donor residues
Curcumin	-6.13	32.02	Arg134; Pro135;	Lys155; Asn166; Asp167; Ser223
Analogue12	**-6.45**	**18.59**	**Ile31; Gln36; Gln39**	**Glu34; Tyr40**

There is an unfavorable donor-donor interaction of VAL57 with =N-NH- fragments of pyrazole ring and one pi-alkyl interaction with phenyl ring. As shown in the docked pose of most active analogue 12 within the active site of FtsZ protein, methoxy and hydroxyl group of phenyl ring formed pi-donor hydrogen bond with GLU34, GLU39 and GLN36 (Supplementary Figure S2). The best result out of three runs of docking score with respect to its IC_{50} value of 2VXY with curcumin and analogue 12 were done by Autodock version 4.0, represented in Supplementary table S1.

Electronic properties

The frontier molecular orbital energies (E_{HOMO} and E_{LUMO}) are remarkable guidelines to explain the qualitative prediction of electronic properties and reactivity of a chemical species. 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 energy and empty molecular orbital [22]. The energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$) is an important parameter as a function of reactivity of curcumin towards antibacterial activity. As ΔE decreases the reactivity of the molecule increases were leading to increase in the electron donating efficiency of the molecule [23] 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.

Owing to biological activities, curcumin and its analogues were also taken to study their electronic properties. The calculated E_{HOMO} and E_{LUMO} and energy gap of curcumin and its analogues are recorded in Table 3. The calculated energy of HOMO, LUMO and the energy gap were -0.282574, -0.276647 and 0.006 eV, respectively for curcumin. But the analogue 12 has the lowest energy gap value than the other investigated analogues in this study. It can be clearly seen that HOMO, LUMO and the energy gap of analogue 12 were -0.221237, -0.215810 and 0.005 eV, respectively. The value of energy gap can use to get information by comparing them with similar compounds. The Lower values of the energy difference will show more potency than others, because the energy required to remove an electron from the last occupied orbital will be low [24]. Here analogue 12 is showing lowest energy gap, thus better antibacterial property which also satisfied by its best binding energy among the docked compounds.

Table 3: Calculated Binding Energies (kcal/mol) and IC_{50} value of curcumin, its analogues and PC190723 (known inhibitor of FtsZ):

Ligand Name	HOMO	LUMO	Energy gap
Curcumin	-0.282574	-0.276647	0.006
Analogue 3	-0.20744	-0.1942	0.01324
Analogue 12	**-0.221237**	**-0.215810**	**0.005**
Analogue 16	-0.341342	-0.035232	0.30611
Analogue 18	0.105267	0.125575	0.020308
Analogue 22	0.105175	0.124764	0.019589
Analogue 23	-0.340884	-0.326881	0.014

HOMO and LUMO orbitals

The Frontier molecular Orbital, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were found to be extremely applicable in narrating 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 curcumin represent the arrangement of electrons around the atom which determines the energy level of curcumin. 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 of curcumin and its analogues were shown in Figure 3.

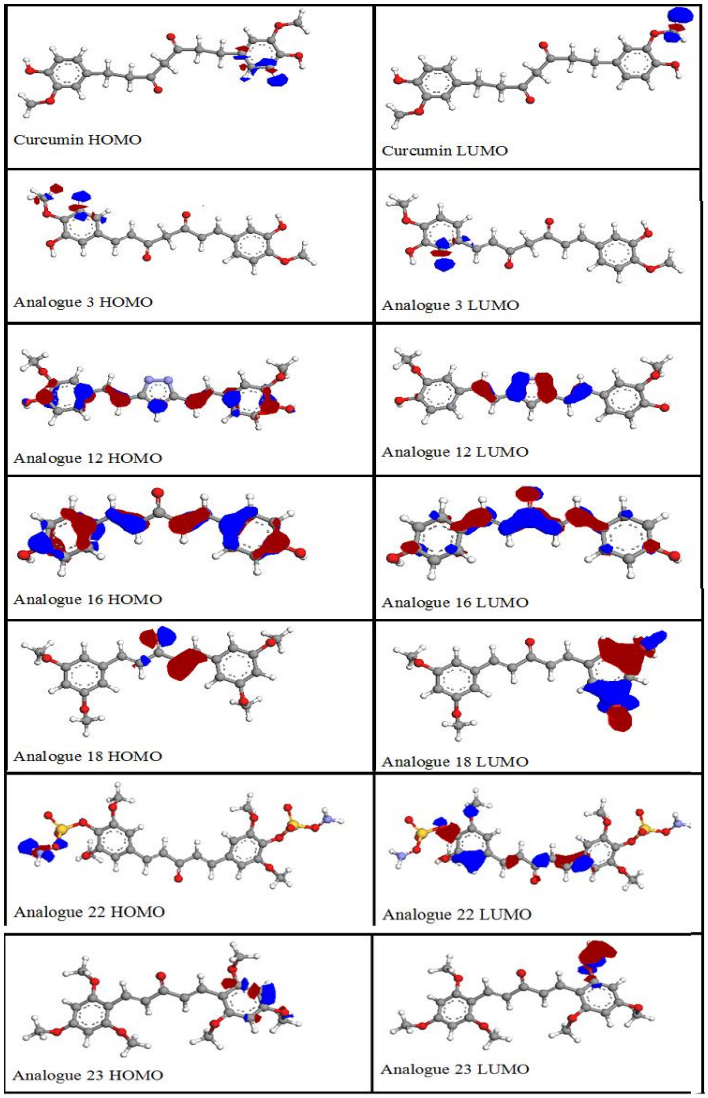

Figure 3: Charge distribution of the HOMO and LUMO in the optimized molecules **(**blue shows positive and red shows negative).

Electrostatic potential

The electrostatic potential is a physical property of a distribution of electric charge creates an electric potential in the surrounding space [25]. 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 curcumin and analogue 12 ground state mapped onto the electron density surface for the ground state rendered translucent and mesh surface to reveal the underlying structure Figure 4(a), (b), (c) and (d). 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 one keto groups, one methoxy groups and one hydroxyl groups at phenyl ring of the curcumin representing the most negative regions of the ESP (region of highest stability) for a positive test charge where it would have favorable interaction energy. On the other hand blue color is representing the hydrogen-ends of the curcumin blue color, shows the region of least stability for the positive test charge indicating the unfavorable interaction energy [Figure 4(a) and (b)].. But in case of analogue 12, the red region is showing most favorable part of interaction and the blue region at second nitrogen of pyrazole ring is indicating less favorable part of molecular surface [Figure 4(c) and (d)]. In this way, an ESP mapped density surface can be helped full to analyze the attack of nucleophile or electrophile in a molecule which lead to qualitative interpretations of molecular surface.

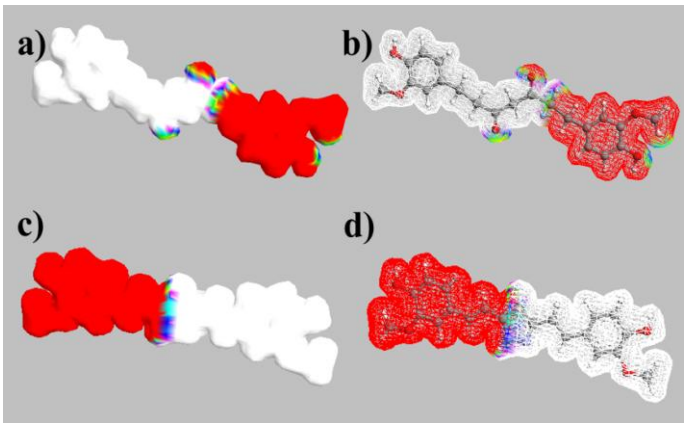

Figure 4: Electrostatic potential (ESP) mapped electron density surface of (a) curcumin (translucent); (b) curcumin (mesh); (c) analogue 12 (translucent) and (d) analogue 12 (mesh).

III. CONCLUSION

A systematic Quantum chemical parameters such as EHOMO, ELUMO, energy gap (ΔE), electro static potential and binding interaction of curcumin and its analogues with its receptor have been carried out to a deeper insight into their molecular properties. Among the different ligands, curcumin analogue **12** displayed the best interaction with the FtsZ protein of *Bacillus Subtilis* (PDB ID: 2VXY). Its binding energy of -6.45 kcal/mol is remarkably better than that of PC190723 (-5.96 kcal/mol), a known inhibitor of the FtsZ. The present work denotes that the best conformation of analogue **12** is built to be at -345.142 kcal/mol which is the minimum potential energy by using Argus Lab software. At this point curcumin is considered as more active with its antibacterial properties. The HOMO and LUMO analysis are used to establish the charge transfer within the molecule. Curcumin analogue **12** with a low energy gap of 0.005eV is found to associate with the high chemical activity and low kinetic stability. Experimental validation of our predictions is certainly encouraging in view of the particular significance of FtsZ target of their antibacterial properties.

IV. Material and Methods:

Arguslab

The structure of curcumin was 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, Mulliken Atomic Charges and ZDO Atomic Charges were determined using PM3 method [13-16].

Autodock

Autodock version 4.0 [17] is used to estimate the binding energy and IC_{50} values for the curcumin with drug target FtsZ. The X-ray crystal structure of the *Bacillus subtilis* FtsZ with bound potassium ion (PDB code: 2VXY) was resolved using X-ray diffraction method with a resolution factor of 1.7 Å was retrieved from the RCSB Protein Data Bank (http: //www. rcsb.org). In the protein, the presence of water molecules were removed and polar hydrogen added. Automatically the root of each ligand molecule is detected and torsions were selected. All torsions of the ligand were allowed to rotate and checked for the selected residues. Blind docking was done to determine where the ligands would preferentially bind. Pre-calculated grid maps were required for running the program, which were calculated using the Autogrid program [18]. The energy scoring grid box was set to 104, 104 and 104Å (x, y, and z) centered at X = - 6.974; Y = 29.698; and Z = 13.232 with 0.139 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 [19]. 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.

Authors Contribution: ST and SKS conceived the idea. ST performed the experiments. ST and SKS interpreted the data and wrote the manuscript.

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