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Synthesis, molecular modeling and structural characterization of vanillin derivatives as antimicrobial agents

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HIGHLIGHTS

- The synthesis, crystal structure and antibacterial activities of these compounds have not been reported so far.
- Our current findings are completely new.
- Their biological activities are also evaluated for FtsZ inhibitory activity.

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ABSTRACT

Two vanillin derivatives have been designed and synthesized and their biological activities were also evaluated for antimicrobial activity. Their chemical structures are characterized by single crystal X-ray diffraction studies, ^{1}H NMR, MS, and elemental analysis. Structural stabilization of them followed by intramolecular as well as intermolecular H-bonds makes these molecules as perfect examples in molecular recognition with self-complementary donor and acceptor units within a single molecule. Docking simulations have been performed to position compounds into the FtsZ active site to determine their probable binding model. Compound **3a** shows the most potent biological activity, which may be a promising antimicrobial leading compound for the further research.

1. Introduction

Although several classes of antibacterial agents are presently available, resistance in most of the pathogenic bacteria to these drugs constantly emerges. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents is a very important task [1]. Vanillin (4-hydroxy-3-methoxybenzaldehyde), a dietary flavoring agent, has been reported to show antioxidant and anti-mutagenic activities, and has also been proved to be an anticarcinogen against a variety of chemical and physical agents [2–6]. Vanillin is considered to be one of the most widely appreciated flavor compounds, with an odor threshold for humans equal to 11.8×10^{-14} M, and has the unique characteristic that, even at high doses, the flavor is still pleasant [7]. Besides its flavor qualities, vanillin exhibits the antimicrobial potential and has been used as a natural food preservative [8]. In old medicinal literature, vanilla was described as a remedy for fevers [9,10]. Schiff bases, named after Hugo Schiff [11], have also been shown to exhibit a broad range of biological activities [12–18]. FtsZ, an essential protein for bacterial viability [19–21], is considered to be the most critical component of the division machinery. It is a highly conserved and potentially broad-spectrum antibacterial target, it does have structural and functional homology, suggesting that FtsZ may also be amenable to inhibitor development. The growing efforts to discover hybrid drugs resulting from the combination of pharmacophoric moieties of different known lead. Hence, the impressive results of Vanillin derivatives and various phenylamine fueled our interest in combining two scaffolds and exploring their possibilities as potential anti-FtsZ agents.

2. Experimental

2.1. Chemistry

All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melt-

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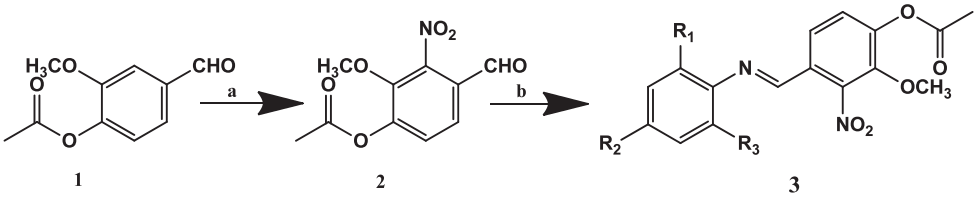


3a: R_1=R_3=H, R_2=CH_3O;
3b: R_2=H, R_1=R_3=$(CH_3)_2CH_2$

Reagents: (a) HNO_3 fuming; (b) 4-methoxyaniline or 2,6-diisopropylaniline in methanol or acetonitrile

Scheme 1. Synthesis route of the title compounds.

ing points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were collected on a Bruker DPX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

The synthetic route to target compounds (**3a** and **3b**) is shown in Scheme 1.

*2.1.1. Synthesis of 4-formyl-2-methoxyphenyl acetate (**2**)*

To a stirred and cooled fuming nitric acid (12 mL) was added slowly compound 4-formyl-2-methoxyphenyl acetate (2.4 g) over 2 h. The dark brown reaction mixture was stirred for 5 h and then poured into crushed ice. The yellow precipitate was filtered off and washed with cold water (300 mL), to form compound **2**.

*2.1.2. General method of synthesis of vanillin derivatives (**3a** and **3b**)*

An equivocal compound **2** (1 mmol) and substituted aromatic amine (1 mmol) in methanol or acetonitrile was stirred for 4–6 h at room temperature, then reaction mixture was concentrated under reduced pressure, recrystallized from ethanol to give vanillin derivatives **3a** and **3b.**

*2.1.2.1. (E)-2-Methoxy-4-(((4-methoxyphenyl)imino)methyl)-3-nitrophenyl acetate (**3a**).* Mp: 114–115 °C. Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$): 2.39–2.40 (m, 3H), 3.83 (s, 3H), 3.94 (s, 3H), 6.91–6.94 (m, 2H), 7.22–7.26 (m, 2H), 7.32–7.34 (m, 1H), 7.95 (s, 1H), 8.34 (s, 1H). MS (ESI): 345($C_{17}H_{17}N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.14; Found: C, 59.52; H, 4.79; N, 8.02.

*2.1.2.2. (E)-4-(((2,6-Diisopropylphenyl)imino)methyl)-2-methoxy-3-nitrophenyl acetate (**3b**).* Mp: 147–149 °C. Yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$): 1.16–1.17 (m, 12H), 2.41(s, 3H), 3.2.85–2.90 (m, 2H), 3.96 (s, 3H), 7.71–7.76 (m, 3H), 7.26 (s, 1H), 7.37–7.39 (d, J = 6.0 Hz, 1H), 8.08 (s, 1H). MS (ESI): 399 ($C_{22}H_{27}N_2O_5$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{264}N_2O_5$: C, 66.32; H, 6.58; N, 7.03; Found: C, 66.19; H, 6.61; N, 7.17.

2.2. X-ray crystallography

Single crystal X-ray diffraction data was collected on a Bruker *D*-8 venture diffractometer at room temperature (293 K). The X-ray generator was operated at 50 kV and 35 mA using Mo Kα radiation (λ = 0.71073 Å). The data was collected using SMART software package. The data were reduced by SAINT-PLUS, an empirical absorption correction was applied using the package SADABS and XPREP were used to determine the space group. The crystal structure was solved by direct methods using SIR92 and refined by full-matrix least-squares method using SHELXL97 [22,23]. All non-hydrogen atoms were refined anisotropically and hydrogen atoms have been refined in the riding mode on their carrier atoms wherever applicable.

2.3. Molecular docking study

Automated docking studies were carried out using Discovery Studio (version 3.1) as implemented through the graphical user interface DS-CDocker protocal.

The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of EGFR protein (PDB code: 2VAM) [24] complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/

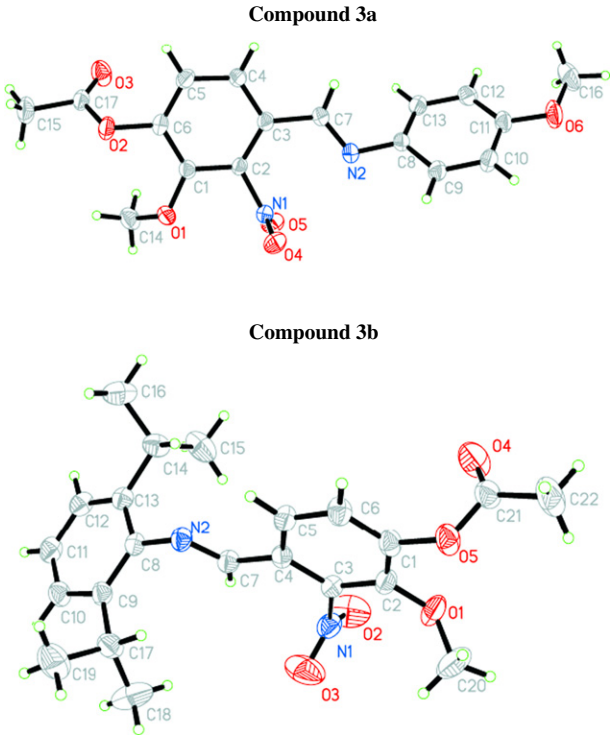


Fig. 1. Molecular structures of the title compounds with atomic numbering scheme.

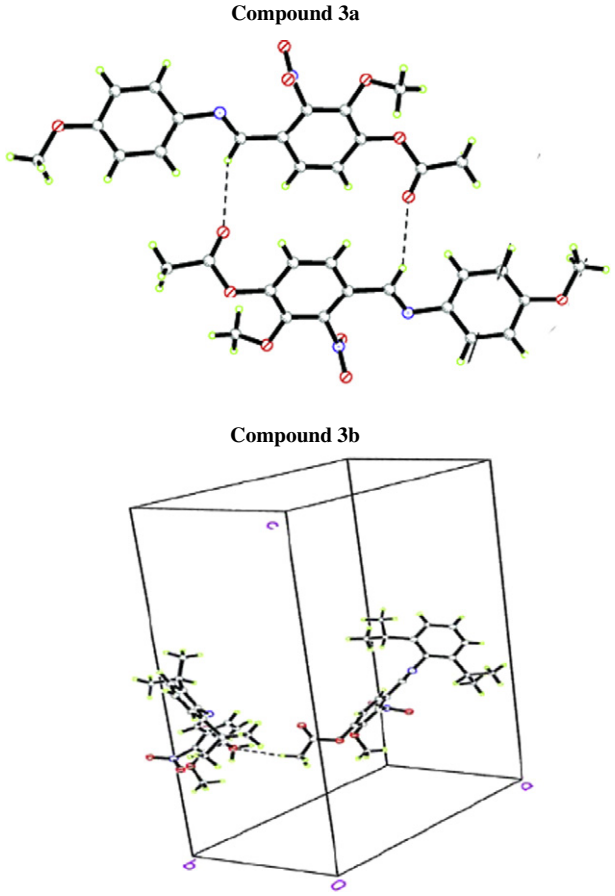


Fig. 2. Crystal packing of the title compounds.

pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins.

2.4. Antibacterial assay

The antibacterial activity of the synthesized compounds was tested against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylth-iazol-2-yl)-2,5-diphenyl tetrazoliumbromide). A stock solution of the synthesized compound (100 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol/L, pH 7.4, $Na_2HPO_4{\cdot}12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

Table 1
Crystallographic data, details of data collection and structure refinement parameters.

Compound	**3a**	**3b**
Empirical formula	$C_{17}H_{16}N_2O_6$	$C_{22}H_{26}N_2O_5$
Formula weight	344.32	398.45
Crystal system	Monoclinic	Orthorhombic
Space group	P21/c	Pbca
a (Å)	7.1969(7)	19.0075(15)
b (Å)	13.6667(13)	8.7528(7)
c (Å)	16.9664(15)	26.320(2)
α (°)	90	90
β (°)	98.621(3)	90
γ (°)	90	90
V (Å)	1649.9(3)	4378.8(6)
Z	4	8
D calc/g cm^{-3}	1.386	1.209
θ Range (°)	2.8, 34.3	2.6–26.5
F (000)	720.0	1696
Reflections collected/unique	19195/4562	45684/4490
Data/restraints/parameters	3236/0/229	3438/
Absorption coefficient (mm^{-1})	0.107	0.086
$R_1/_wR_2$ [$I > 2\sigma$ (I)]	0.0461/0.1271	0.0610/0.1708
$R_1/_wR_2$ (all date)	0.0732/0.1271	0.0806/0.1708
GOOF	1.043	1.053

2.5. Inhibition of FtsZ polymerization

The FtsZ was expressed and purified with the following modification [25]. A polymerization/depolymerization step was added before preparative gel filtration. This improved purification gave a protein preparation that was >99% pure with a 1:1 M ratio of GDP to FtsZ.

The polymerization and depolymerization of purified FtsZ at 0.5 mg/mL. Compounds were initially evaluated at 100 μM. If inhibition was observed, the compounds were retested at several concentrations. The percentage control activity was calculated by comparison with an assay without compound. Three independent curves were run. Quantitative analysis of the FtsZ GTPase enzymatic reaction was done by monitoring the oxidation of NADH as a decrease in absorbance at 340 nm.

3. Results and discussion

3.1. Crystal structures of compounds *3a* and *3b*

Crystals of three compounds were obtained from methanol solution. Fig. 1 shows a perspective view of the monomeric unit with the atomic numbering scheme, and Fig. 2 depicts the intramolecular and intermolecular hydrogen bonds. Crystallographic data, details of data collection and structure refinement parameters are listed in Table 1. The hydrogen bond lengths and bond angles are given in Table 2.

Single crystals of **3a** (0.32 mm × 0.27 mm × 0.25 mm) and **3b** (0.38 mm × 0.26 mm × 0.25 mm) were mounted on a *D*-8 venture

Table 2
Hydrogen bond lengths (Å) and bond angles (°).

Compounds	D–H…A	d(D–H)	d(H…A)	d(D…A)	∠DHA
3a	C(7)–H(7)…N(1)	0.93	2.56	3.4092(18)	153
3b	C(7)–H(7)…N(1)	0.93	2.57	2.887(2)	100
	C(14)–H(14)…N(2)	0.98	2.38	2.838(3)	108
	C(17)–H(17)…N(2)	0.98	2.43	2.931(3)	111
	C(20)–H(20A)…O(5)	0.96	2.59	3.146(4)	117
	C(22)–H(22A)…O(5)	0.96	2.53	3.484(4)	171

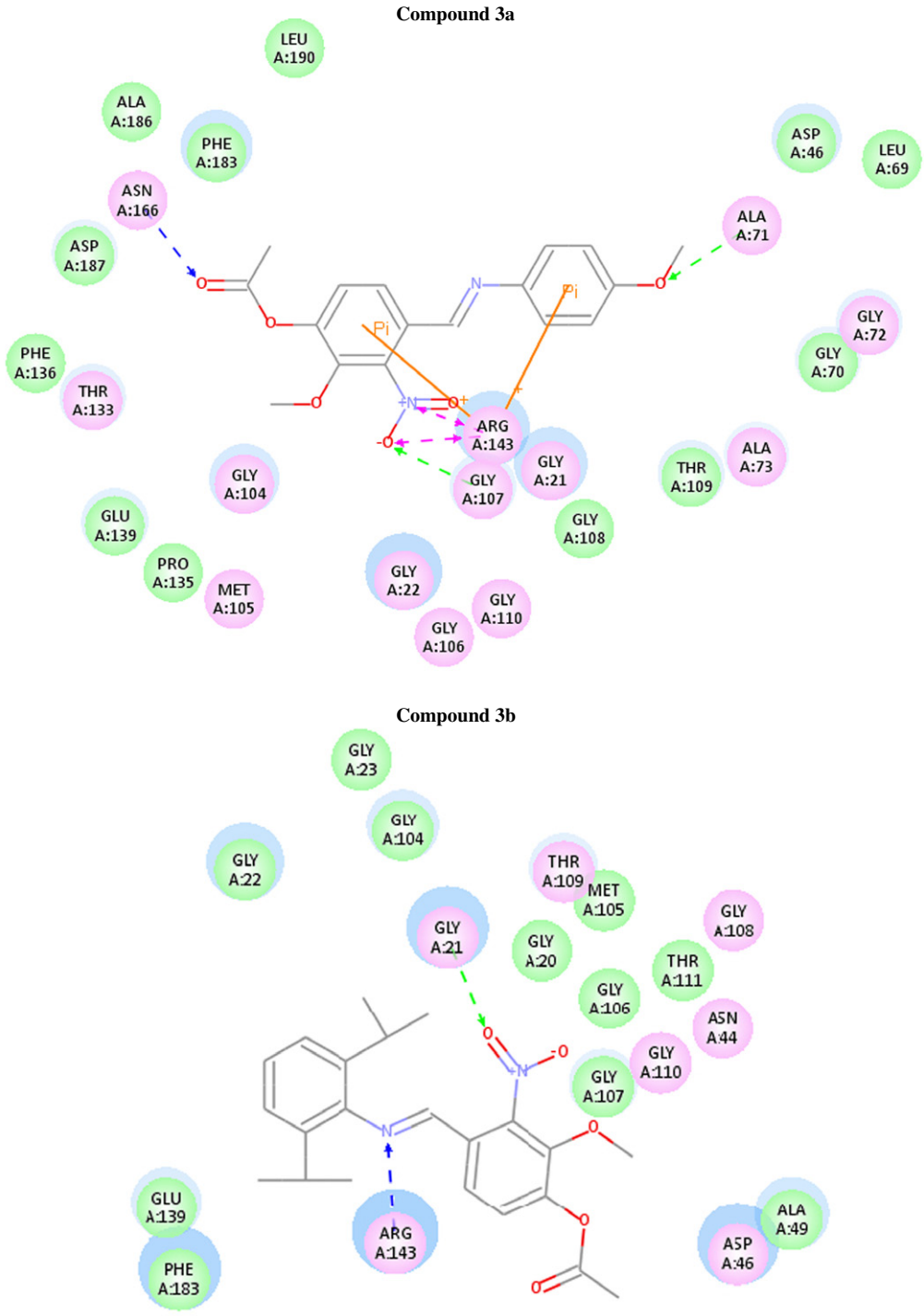
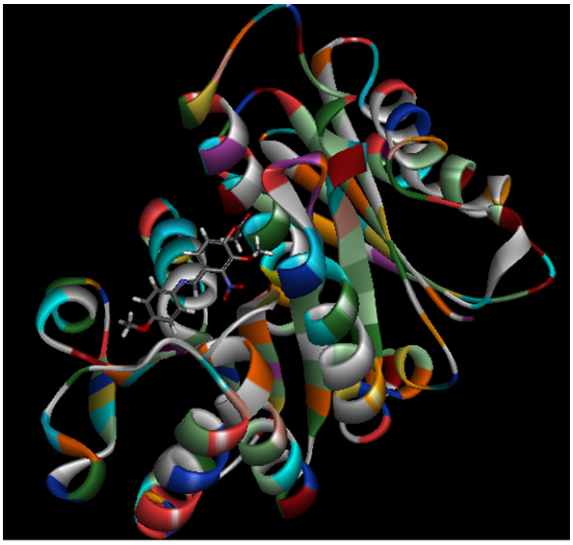


Fig. 3. 2D molecular docking modeling with 2VAM.

diffractometer equipped with graphite-monochromated MoKa (λ = 0.71073 Å) radiation. For **3a**, a total of 19195 reflections were collected, of which 4562 were unique with R_{int} = 0.020 and 3236 observed reflections with $I > 2\sigma$ (I) were used in the succeeding structure calculations. The final cycle of refinement of fullmatrix least-squares was converged to R = 0.0461 and wR = 0.1271. The highest and lowest residual peaks in the final difference Fourier map are 0.22 and −0.16 e/Å3, respectively. For **3b**, a total of 45,684 reflections were collected, of which 4490 were unique with R_{int} = 0.033 and 3438 observed reflections with $I > 2\sigma$ (I) were used in the succeeding structure calculations. The final cycle of refinement of fullmatrix least-squares was converged to R = 0.0610 and wR = 0.1708. The highest and lowest residual peaks in the final difference Fourier map are 0.32 and −0.30 e/Å3, respectively.

In the crystal structure of compound **3a**, there are two benzene rings in the molecule. C(1), C(2), C(3), C(4), C(5) and C(6) form the first plane with the mean deviation of 0.0059 Å, defined as plane I; Similarly, C(8), C(9), C(10), C(11), C(12) and C(13) forms the second plane with the mean deviation of 0.0110 Å, defined as plane II. The dihedral angle between plane I and plane II is 150.9°. In the crystal structure of compound **3b**, there are two benzene rings in the molecule. C(1), C(2), C(3), C(4), C(5) and C(6) form the first plane with the mean deviation of 0.0026 Å, defined as plane I; Similarly, C(8), C(9), C(10), C(11), C(12) and C(13) forms the second plane with the

Compound 3a



Compound 3b

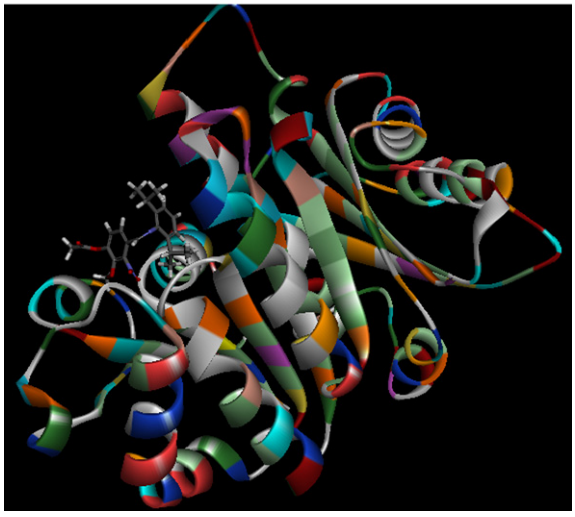


Fig. 4. Binding poses of compounds **3a** and **3b** in the active site of *B. Subtilis* FtsZ.

mean deviation of 0.0127 Å, defined as plane II. The dihedral angle between planes I and II is 170.8°.

3.2. Antibacterial activities

The MICs of compounds **3a** and **3b** against these bacterial strains are tested by MTT method. Based on the data obtained, we found that compound **3a** exhibits better inhibitory activities (MICs: 1.56–6.25 μg/mL) than **3b** (MICs: 6.25–12.5 μg/mL), maybe a potential antibacterial agent.

3.3. Inhibition of FtsZ polymerization

The GTPase FtsZ inhibitory potency of the selected compounds was examined and among the tested compounds, compounds **3a** showed potent inhibitory activities with polymerization ID_{50} of 2.1 μM than **3b** (ID_{50} = 7.6 μM).

3.4. Experimental protocol of docking study

Molecular docking of the synthesized compounds and FtsZ was performed on the binding model based on the FtsZ protein complex structure (2VAM.pdb). The binding poses of active compounds are selected through CDOCKER INTERACTION ENERGY [26]. Docking algorithm utilized: CDOCKER algorithm; definition of binding site: 39.172, −4.4254, 8.9296; radius: 9; scoring function: CDOCKER interaction energy; rigid receptor: PDB code 2VAM; flexible ligand docking: YES; cluster analysis of docking poses: ten optimal poses were retained.

The binding model of compounds and FtsZ was depicted in Figs. 3 and 4. In the binding model, compound **3a** was nicely bound to the FtsZ with five hydrogen interaction bonds. Moreover, the π-cation interactions were existed between benzene-ring and amino acids Arg 143. Besides, compound **3b** was nicely bound to the FtsZ with two hydrogen interaction bonds.

4. Conclusion

In summary, two vanillin derivatives **3a** and **3b** were synthesized and tested for their inhibitory activities against *E. coli*, *Pseudomonas aeruginosa fluorescence*, *B. subtilis* and *S. aureus*. Both of them exhibited potent antibacterial and FtsZ inhibitory activities. Particularly, Compound **3a** was proved to be the more potent compound. Molecular modeling study provided further insight into interactions between the enzyme and its ligand.

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