Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

© 2011-18, publisher and licensee JDDT, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open Access Research Article

Evaluation of Antimicrobial Profile of Some Novel 1, 3, 4-Oxadiazole Derivatives Followed by Molecular Docking Against 3G7E Bacterial DNA Gyrase

Asish Bhaumik*[1]; M. Chinna Eswaraiah[2]; Raja Chakraborty[3]

[1]* Research Scholar, Faculty of Pharmaceutical Science, Assam down town University, Gandhi Nagar, Panikhaiti, Guwahati- 781026, Assam, India.

[2] Professor, Department of Pharmacognosy, Anurag Pharmacy College, Ananthagiri, Kodad-508206, Suryapet (Dist.), Telangana, India.

[3] Professor, Faculty of Pharmaceutical Science, Assam down town University, Gandhi Nagar, Panikhaiti, Guwahati- 781026, Assam, India.

ABSTRACT

The main aim and objective of the present research work was the design, synthesis, spectral characterization and evaluation of *in vitro* antimicrobial profile of some novel oxadiazole derivatives followed by molecular docking studies against bacterial DNA gyrase. The molecular structures of the synthesized compounds were assigned by IR, NMR and Mass spectral analysis. Molecular docking studies were carried out by AUTO DOCK programme. The *in vitro* antibacterial and antifungal activities were done by paper disk diffusion and agar streak dilution technique. In silico molecular docking studies the binding energy of synthesized compounds (AB1-AB8) were found to be -7.66, -7.67, -7.12, -7.12, -6.59, -6.46, -7.35, -5.09 which indicated that the compound had the high binding affinity towards the bacterial DNA gyrase with PDB id 3G7E and inhibit the function topoisomerase in comparison with standard drug ciprofloxacin (-7.44). The preliminary antimicrobial screening displayed that most of the synthesized compounds were executed moderate to good antimicrobial activity against following bacteria: *S. aureus (ATCC 9144), B. subtilis (ATCC 6633), S. epidermidis (ATCC 12228), P. Aeruginosa (ATCC27853), E.coli (ATCC25922), V. cholerrae (ATCC14035)* and fungi: *A. Niger (ATCC 9029), A.flavus (ATCC204304), C. albicans (ATCC10231)* and *B. dermatitis (ATCC 26199)* etc. All the synthesized compounds exhibited moderate to good antibacterial and antifungal activity with an MIC range of 12-37µg/ml. Among these eight synthesized oxadiazole derivatives, compound AB1; AB2 and AB7 were found to be very good antibacterial as well as antifungal potentiality with an MIC range of 13-12 µg/ml; 7-10 µg/ml and 15-18 µg/ml.

Keywords: Molecular docking; NMR; disk diffusion; antibacterial; antifungal and MIC etc.

Article Info: Received 14 May 2019; Review Completed 27 June 2019; Accepted 04 July 2019; Available online 15 July 2019

Cite this article as:

Bhaumik A, Chinna Eswaraiah M; Chakraborty R, Evaluation of Antimicrobial Profile of Some Novel 1, 3, 4-Oxadiazole Derivatives Followed by Molecular Docking Against 3G7E Bacterial DNA Gyrase, Journal of Drug Delivery and Therapeutics. 2019; 9(4):438-453 **http://dx.doi.org/10.22270/jddt.v9i4.3081**

***Address for Correspondence:**

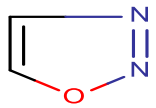
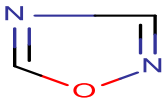
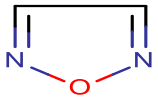
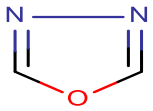
Asish Bhaumik, Research Scholar, Faculty of Pharmaceutical Science, Assam down town University, Gandhi Nagar, Panikhaiti, Guwahati- 781026, Assam, India.

1. INTRODUCTION

1. 1. Structural features of Oxadiazole

Oxadiazoles are a class of heterocyclic aromatic chemical compound of the azoles family; with the molecular formula $C_2H_2N_2O$. There are four isomers of oxadiazole depending on the position of nitrogen atom in the ring [1]. In chemistry, methine is a trivalent functional group =CH−, derived formally from methane. It consists of a carbon atom bound by two single bonds and one double bond, where one of the single bonds is to hydrogen. The group is also called methyne or methene; its IUPAC systematic name is methylylidene or methanylylidene. Oxadiazole is derived from furan by replacement of two methine (-CH=) group by two pyridine type nitrogen (-N=) [2]. 1, 3, 4-oxadiazole is a five member heterocyclic aromatic compound containing two nitrogen atom at position three and four and one oxygen atom present at position one. 1, 3, 4 oxadiazole is thermally stable than other oxadiazoles, these oxadiazole are very important compound in medicinal chemistry due to their potential therapeutic efficacy.

Table-1: Structure of different isomers of Oxadiazole

 1,2,3-oxadiazole	 1,2,4-oxadiazole
 1,2,5-oxadiazole	 1,3,4-oxadiazole

1. 2. Physico-chemical properties of 1, 3, 4-Oxadiazole

1, 3, 4-oxadiazole is a liquid having boiling point 150ºC. 2, 5-disubstituted-1, 3, 4-oxadiazole derivatives are colourless substances. The lower alkyl derivatives are liquids which distil without decomposition. Replacement of an alkyl residue by an aryl radical considerably raises the melting and boiling points. Usually the asymmetrical 1, 3, 4-oxadiazole derivatives melt and boil at lower temperature than the symmetrical compounds. The solubility of oxadiazoles in water varies with the substituent's present: 2, 5-dimethyl-1, 3, 4-oxadiazole is miscible with water in all proportions whereas the solubility of 2, 5-diphenyl-1, 3, 4-oxadiazole in water is less. Electrophilic introduction of functional groups (for example nitro or sulphuric acid groups) into the nucleus is unusual. Electrophilic substitution occurs in aryl substituent. Halogenations are also difficult, but 2, 5-diaryl-1, 3, 4-oxadiazoles, afford complexes with halogens. A range of acylation and alkylation reactions of hydroxyl, thio and amino-1, 3, 4-oxadiazoles occur at the ring nitrogen [3].

1. 3. Chemical properties of 1, 3, 4-Oxadiazole

1, 3, 4-oxadiazole is a five member heterocyclic aromatic compound containing two nitrogen atom at position three and four and one oxygen atom present at position one. 1, 3, 4-oxadiazole is thermally stable than other oxadiazoles, these oxadiazoles are very important compound in medicinal chemistry due to their potential biological activities. Oxadiazole, a very weak base due to inductive effect of the extra heteroatom. The replace of two -CH= groups in furan by two pyridine type (-N=) lowers aromaticity of resulting oxadiazole ring to an extent that the oxadiazole ring exhibit character of conjugated diene. The electrophillic substitutions in oxadiazole ring are extremely difficult at the carbon atom because, the relatively low electron density on the carbon atom which can be attributed to electron withdrawal effect of the pyridine type nitrogen atom. If oxadiazole ring is substituted with electron-releasing groups, the attack of electrophiles occurs at nitrogen. The ring is generally resistant to nucleophiles attack [4].

1. 4. Biological activity

The extensive literature survey revealed that 1, 3, 4-oxadiazoles were reported to possess a wide range of biological activities such as anti inflammatory, anticancer, antibacterial, antifungal, anti tubercular, anti-HIV, anthelmentic (antimicrobial), anti oxidant, analgesic and anticonvulsant [5-8].

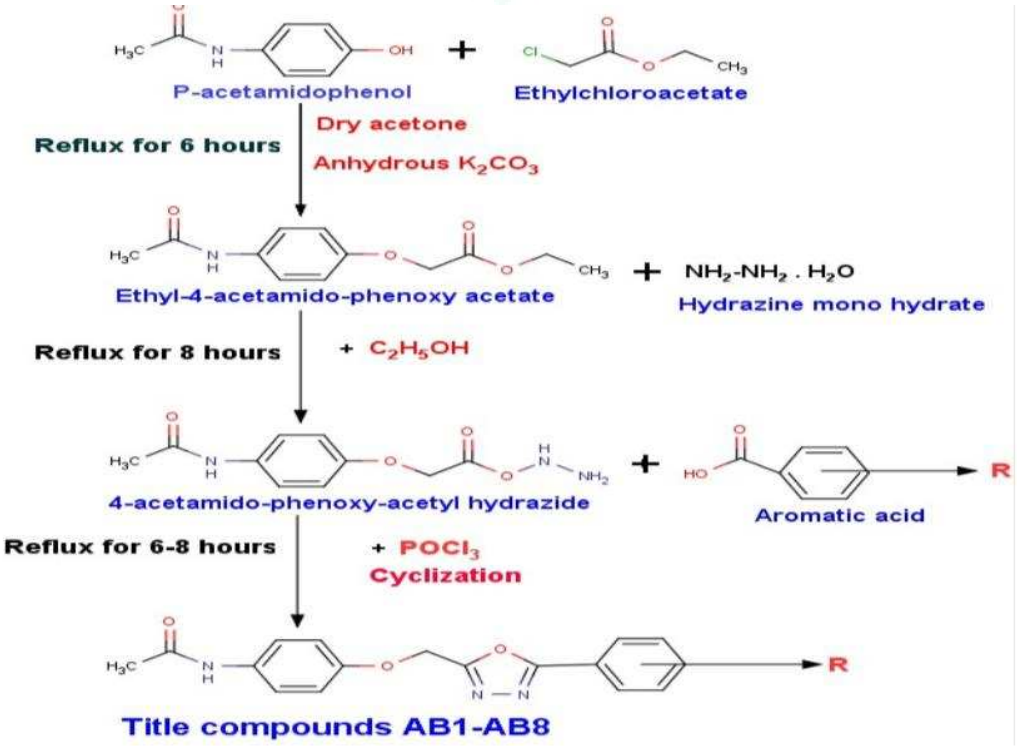
2. EXPERIMENTAL CHEMISTRY

2. 1. Materials and method

2. 1. 1. Chemicals

The solvents and other chemicals which were used for the synthesis and purification of target compounds provided by institutional store and were of LR and AR grade.

2. 1. 2. Synthetic scheme



Steps involved in the synthesis of target compounds [9-10]

Step 1: Ethyl-4-acetamido phenoxy acetate: A mixture of p-acetamido phenol (0.01 mol) and ethyl chloroacetate (0.01 mol) was refluxed by using dry acetone in presence of anhydrous potassium carbonate (K_2CO_3) for 6hrs. The reaction mixture was cooled and then poured in to crushed ice. The solid product obtained, these product was filtered, dried and recrystallized using ethanol.

Step 2: 4-Acetamido phenoxy acetyl hydrazide: A mixture of ethyl-4-acetamido phenoxy acetate (0.01 mol), hydrazine hydrate (0.01 mol) in ethanol (15 ml) was refluxed for 5-8 hrs. The reaction mixture was cooled and then poured in to crushed ice. The solid product was obtained; this product was filtered, dried and recrystallized from ethanol.

Step 3: 2-(4-Acetamidophenoxy methyl) -5-aryl substituted - 1, 3, 4-oxadiazole: A mixture of 4-Acetamido phenoxy acetyl hydrazide (0.01 mol) and various aromatic acids (0.01 mol) in phosphorus oxychloride (10 ml) was refluxed for 6-8 hours. The completion of the reaction process was monitored by TLC plates. The contents were cooled and poured into the crushed ice and then neutralized the reaction mixture with sodium bicarbonate solution and the solid product was obtained, the product was filtered, dried and recrystallized from ethanol .

2. 1. 3. Determination of Physicochemical properties

Melting point: The melting point (or, rarely, liquefaction point) of a substance is the temperature at which it changes state from solid to liquid. At the melting point the solid and liquid phase exists in equilibrium. The melting points of the synthesized compounds were determined by open capillary tube method.

R_f value: The retardation factor, R_f is commonly used in paper chromatography and thin layer chromatography for analyzing and comparing different substances. It can be mathematically described by the following ratio. TLC method was used to determine the progress of the reaction. TLC plates were pre-coated Silica gel (HF254-200 mesh) aluminium plates using ethyl acetate: n-hexane are used as solvent and visualized under UV- chamber.

$$\mathbf{R_f} = \frac{\textbf{Migration distance of substance}}{\textbf{Migration distance of solvent front}}$$

Solubility profile: Solubility is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid, liquid or gaseous solvent. The solubility of a substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as on temperature, pressure and presence of other chemicals (including changes to the pH) of the solution.

Percentage yield (%): The theoretical yield is an organic reaction is the weight of the product which would be obtained if the reaction had proceeded to completion according to the chemical equation. The yield is the weight of pure product which is isolated from the reaction. The percentage yield may be expressed as:

$$\textbf{Yield}\ (\%) = \frac{\textbf{Weight of the product}}{\textbf{Theoritical yield}} \times \mathbf{100}$$

2. 1. 4. Spectral analysis

Functional group determination

The IR spectra of the synthesized compounds were recorded on ABB Bomen FT-IR spectrometer MB 104 IR spectra with potassium bromide pellets.

Determination of number of protons

The number of protons present in each synthesized compounds were determined by BRUKER NMR spectrometer (^{1}H-NMR) in DMSO. TMS was used as an internal standard.

Determination of molecular weight

The Mass spectra of synthesized compounds were recorded by JEOL GCmate. Mass spectroscopy mainly used to determine the molecular weight of the synthesized compounds. The IR, ^{1}H-NMR and MASS spectra are used to assign the structure of synthesized compounds.

3. COMPUTATIONAL CHEMISTRY

3. 1. Molecular docking [11]

Computational chemistry is a branch of chemistry that uses computer simulation to assist in solving chemical problems. It uses methods of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of molecules and solids. Molecular docking is defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustment resulting in the overall binding is referred to as "induced fit. The aim of the molecular docking to achieve an optimized conformation for both the protein and the ligand and to achieve relative orientation between protein and ligand such that free energy of overall system is minimized. The application of docking are the hit identification docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest and the lead optimization docking can be used to predict in where and in which relative orientation a ligand binds to a protein. This information may in turn be used to design more potent and selective analogues.

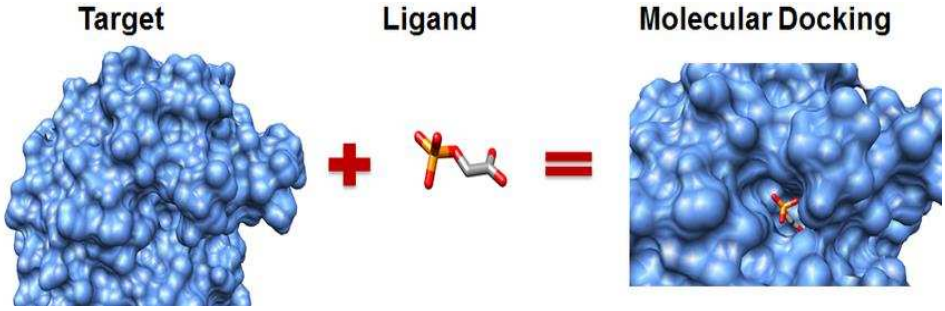


Fig-1-A: Elements in molecular docking

3. 2. Posing and scoring function of docking [12]

Posing the process of determining whether a given conformation and orientation of a ligand fits the active site. This is usually a fuzzy procedure that returns many alternative results. pose score is a measure of the fit of a ligand into the active site. Scoring during the posing phase usually involves simple energy calculations (Vdw + H-bond + dE = Vender walls + Hydrogen bond + Dessolve energy. EE= Electrostatic Energy. TIME = Total Intermolecular Energy). One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

$$\Delta G_{bind} = \Delta G_0 + \Delta G_{hb} \sum n - bonds + \Delta G_{ionic} \sum_{ionic-int} + \Delta G \text{ lipophilic } |A| + \Delta G_{rot} NROT$$

A more general thermodynamic "master" equation is as follows:

$$G_{bind} = -RTlnK_d$$

$$K_d = \frac{[Ligand][Receptor]}{[Complex]}$$

$$\Delta G_{bind} = \Delta G_{desolvation} + \Delta G_{motion} + \Delta G_{configuration} + \Delta G_{interaction}$$

3. 3. Requirements

Purpose: Computational Analysis. **Job Id:** NRS/008/10/2016. **PDB Code:** 3G7E. **Soft ware used:** Auto Dock 4.

Structure of bacterial DNA gyrase (E. coli gyrase B)

Crystalline structure of the target protein Topoisomerase bacterial DNA gyrase with PDB id 3G7E was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were been added. Different orientation of the lead molecules AB1 to AB8 along with standard drug ciprofloxacin with respect to the target protein was evaluated by Auto dock program and the best dock pose was selected based on the interaction study analysis.

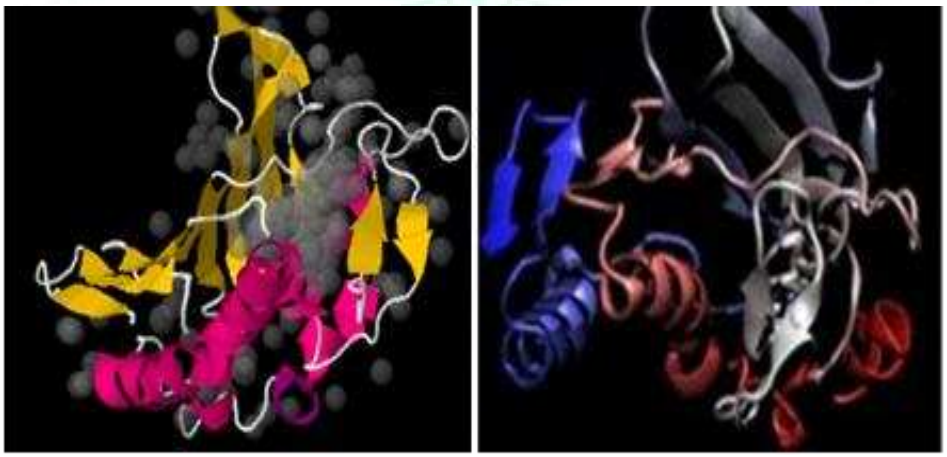


Fig-1-B: Structure of bacterial DNA gyrase

4. EXPERIMENTAL MICROBIOLOGY

4. 1. Microorganisms

The standard strains (American type culture collection, ATCC, USA, Rockville) and the pathological strains were supplied from the department of Pharmaceutical Microbiology & Biotechnology, Anurag Pharmacy College, Kodad, Nalgonda, T. S, India. The antimicrobial activity of the synthesized compounds was screened by using against the following bacteria and fungi.

A. Bacteria: Gram positive: *S. aureus (ATCC 9144), B. subtilis (ATCC 6633), S. epidermidis (ATCC 12228).* **Gram negative:** *P. Aeruginosa (ATCC27853), E.coli (ATCC25922), V. cholerrae (ATCC14035)*

B. Fungi: *A. Niger (ATCC 9029), A. flavus (ATCC204304), C. albicans (ATCC10231)* and *B. dermatitis (ATCC 26199)* etc.

4. 2. Chemicals and drugs

DMF was used as solvent and Ciprofloxacin (antibacterial and Ketaconazole (antifungal) were used as standard antimicrobial drugs.

4. 3. Procedure

Preparation of nutrient agar medium [13-14]

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Table-2-A: Composition of Nutrient agar medium for bacterial growth

S. l No.	Ingredients	Grams/Litter
1.	Peptone	10 gm
2.	Beef extract	10 gm
3.	Agar	20 gm
4.	Sodium chloride	5.0 gm
5.	Distilled water	1000 ml
6.	PH	7.0

Table-2-B: Composition of Sabouraud dextrose agar medium for fungal growth

S. l No.	Ingredients	Grams/Litter
1.	Glucose	20 gm
2.	Peptone	10 gm
3.	Agar	20 gm
4.	Distilled water	1000 ml
5.	PH	5.4

All the ingredients were added to distilled water and boiled to dissolve all the ingredients in medium completely. The medium sterilized by autoclaving at 15 lbs pressure. Temperature was maintained at 121^{0}C for 15 minutes.

Preliminary screening of anti-bacterial activity by paper disc diffusion method [15]

The sterilized (autoclaved at 120^o C for 30 min) medium was inoculated (1mL/100 ml of medium) with the suspension [10^5 cfu m/l (colony forming unit per millilitre)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petri dish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hour at RT and incubated at 37 ^{o}C for 24 hours for anti-bacterial and antifungal activities respectively. Ciprofloxacin (100 µg/disc) and ketoconazoloe (100 µg/disc) was used as a standard. The observed zone of inhibition was compared with standard drug.

Determination of MIC [14 and 16]

MIC of the synthesized compounds was determined by agar streak dilution method. A stock solution of the synthesized compounds (100 µg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately105 cfu m/l and applied or spread on surface of agar medium. All plates were incubated at 37o C 2 to 3 days for bacterial and fungal growth. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

5. RESULTS

Table-3: Represents the list of synthesized oxadiazole derivatives

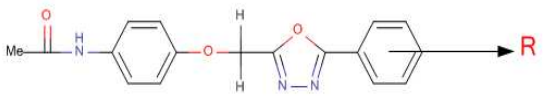
Sl. No.	Compound codes.	
		R
1.	AB1	4-NH_2
2.	AB2	2, 4-di-Cl
3.	AB3	4-F
4.	AB4	2-Br
5.	AB5	2-Br, 4-NO_2
6.	AB6	4-NO_2
7.	AB7	3, 5-di-NO_2
8.	AB8	2-OH, 3, 5-di-NO_2

Table 4: Physicochemical properties of synthesized compounds

Sl. No.	Compounds code	M. F	M. Wt	R_f value	m. p	Yield
1.	AB1	$C_{17}H_{16}N_4O_3$	324.33	0.77	116-119 ^{0}C	74.5 %.
2.	AB2	$C_{17}H_{13}Cl_2N_3O_3$	378.209	0.74	180-183 ^{0}C	69.9%
3.	AB3	$C_{17}H_{14}FN_3O_3$	327.309	0.75	189-193 ^{0}C	74%
4.	AB4	$C_{17}H_{14}BrN_3O_3$	388.215	0.65	183-186 ^{0}C	69%
5.	AB5	$C_{17}H_{13}BrN_4O_5$	433.213	0.64	166-163 ^{0}C	60%
6.	AB6	$C_{17}H_{14}N_4O_5$	354.31	0.72	171-175 ^{0}C	64%
7.	AB7	$C_{17}H_{13}N_5O_7$	399.31	0.68	199-204 ^{0}C	78%
8.	AB8	$C_{17}H_{13}N_5O_8$	415.31	0.72	212-215^{0}C	68%

Spectral data of synthesized compounds

Compound AB1

N-(4-{[5-(4-aminophenyl)-1, 3, 4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3393.16 cm^{-1} (Ar-NH), 1633.67 cm^{-1} (C=N), 1575.88 cm^{-1} (C=C), 1069.05 cm^{-1} (-C-O-C-), 3132.54 cm^{-1} (Ar-CH), 1249.43 cm^{-1}($Ar-NH_2$), ^{1}H-NMR δ (ppm): 6.45-7.4 (s, 8H, Ar-H), 5.17 (s, 2H,$-CH_2$), 4.1(s, 2H, $-NH_2$), 2.05 (s,1H, $-CH_3$), 8.05 (s, 1H, -NH), Mass (m/e value) % relative abundance: 324.12 (M^+) (5.1), 310.87 (4) , 296.22 (8.25), 282.76 (2.2), 272.38(2.32), 262.6432 (7.3), 248.34 (11), 217.12 (15), 207.14 (7), 116.67 (18), 58.33(B).

Compound AB2

N-(4-{[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide.. IR (KBr) ν (cm^{-1}): 3381.92 cm^{-1} (Ar-NH), 1673.42 cm^{-1} (C=N), 1545.03 cm^{-1} (C=C), 1085.04 cm^{-1} (-C-O-C-), 687.47 cm^{-1}(C-Cl), 3115.62 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.6-7.82(s, 8H, Ar-CH), 2.5 (s, 3H, $-CH_3$), 8.03(s, 1H, -NH), 5.22(s, 2H, $-CH_2$), Mass (m/e value) % relative abundance: 377.03 (M^+) (2.8), 333.16 (1.5), 325.42 (2.7), 286.43 (2.6), 183.26 (6), 160.62 (7), 140.65 (16), 115.64 (33), 95.53 (B).

Compound AB3

N-(4-{[5-(4-flurophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3392.09 cm^{-1} (Ar-NH), 1617.53 cm^{-1} (C=N), 1528.16 cm^{-1} (C=C), 1093.52 cm^{-1} (-C-O-C-), 1371.78 cm^{-1} (C-F), 3114.61 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 2.21 (s, 1H, $-CH_3$), 8.09 (s, 1H, -NH), 5.21(s, 1H, $-CH_2$), 6.7-8.01(m, 8H, Ar-CH), Mass (m/e value) % relative abundance: 327.10 (M^+) (6.3), 310.37 (2.3), 299.57 (3), 282.87 (3.9), 266.22 (5), 249.61 (1.2), 232.72 (4), 104.86 (8.1), 75.50 (B).

Compound AB4

N-(4-{[5-(2-bromophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3286.82 cm^{-1} (Ar-NH), 1617.53 cm^{-1} (C=N), 1528.16 cm^{-1} (C=C), 1093.52 cm^{-1} (-C-O-C-), 687.47 cm^{-1} (C-Br), 3114.61 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 2.21 (s, 1H, $-CH_3$), 8.09(s, 1H, -NH), 5.21(s, 1H, $-CH_2$), 6.7-8.01(m, 8H, Ar-CH), Mass (m/e value) % relative abundance: 387.02(M^+) (6.3), 310.37 (2.3), 299.57 (3), 282.87 (3.9), 266.22 (5), 249.61 (1.2), 232.72 (4), 104.86 (8.1), 75.60 (B).

Compound AB5

N-(4-{[5-(2-bromo,4-nitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3381.95 cm^{-1} (Ar-NH), 1684.44 cm^{-1} (C=N), 1586.2 cm^{-1} (C=C), 1064.25 cm^{-1} (-C-O-C-), 1365.57 cm^{-1}(N=O), 619.89 cm^{-1} (C-Br), 3130.43 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.74-8.36(m, 7H, Ar-CH) ,5.31(s, 2H, $-CH_2$),2.31 (s, 1H, $-CH_3$), 8.16(s, 1H, -NH), Mass (m/e value) % relative abundance: 432.00 (M^+) (4), 388.71 (8.1), 362.27 (4.2), 233.28 (5), 217.31 (8.9), 182.52 (5), 96.79 (7), 78.82(B).

Compound AB6

N-(4-{[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl)acetamide. IR (KBr) ν (cm^{-1}): 3382.43 cm^{-1} (Ar-NH), 1703.01 cm^{-1} (C=N), 1592.32 cm^{-1} (C=C), 1088.54 cm^{-1} (-C-O-C-), 1378.11 cm^{-1} (N=O), 3112.69 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.41-7.8(m, 8H, Ar-CH), 2.42 (s, 3H, $-CH_3$), 8.13(s, 1H, -NH), 5.21(s, 2H, CH_2), Mass (m/e value) % relative abundance: 354.09 (M^+) (3.8), 335.16 (4.8), 302.39 (3.1), 287.43 (3.7), 249.58 (7.1), 226.00 (5.8), 204.96 (6.7), 127.56 (13.1), 103.69 (9), 89.93 (B).

Compound AB7

N-(4-{[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3382.02 cm^{-1} (Ar-NH), 1677.79 cm^{-1} (C=N), 1530.6 cm^{-1} (C=C), 1089.68 cm^{-1} (-C-O-C-), 1372.45 cm^{-1} (N=O), 1523.12 asym cm^{-1} (N=O), 3117.5 cm^{-1} (Ar-CH), ^{1}H-NMR δ (ppm): 6.83-8.42(m, 8H, Ar-CH), 5.35(s, 2H,$-CH_2$), 2.07 (s, 1H,- CH_3), 8.24 (s, 1H, -NH), Mass (m/e value) % relative abundance: 399.08 (M^+) (5), 388.76 (13), 380.25 (8), 261.63 (8), 182.52 (5), 167.62 (17), 156.56 (19), 81.97(B).

Compound AB8

N-(4-{[5-(2-hydroxy-3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]methoxy}phenyl) acetamide. IR (KBr) ν (cm^{-1}): 3118.84 cm^{-1} (Ar-NH), 1654.42 cm^{-1} (C=N), 1541.89. cm^{-1} (C=C), 1368.45 cm-1 (N=O), 1528.45 asym. cm-1 (N=O),1090.01 cm^{-1} (-C-O-C-), 3118.84 cm^{-1} (Ar-CH), 3382.83 cm^{-1}(Ar-OH), ^{1}H-NMR δ (ppm): 6.7-7.6(s, 6H, Ar-CH), 2.11 (s, H, $-CH_3$), 8.00(s, 1H, -NH), 5.12(s, 1H, $-CH_2$), Mass (m/e value) % relative abundance: 415.07(M) (11.1), 318.68 (16), 292.76 (7), 276.89 (20), 249.99 (8.2), 236.0277 (28.1), 203.2266 (76), 182.2587 (8), 134.4966 (32), 116.55 (B)

Table-5: Docking results analysis

C. C	EFEB (k.cal/mol)	EIC (Ki) uM	vdw+H-bond+de (k.cal/mol)	EE (k.cal/mlo)	TIME (k.cal /mol)	Fr (%)	IS
AB1	**-7.66**	**2.43**	**-9.16**	**-0.22**	**-9.37**	**50**	**859.57**
AB2	**-7.67**	**2.40**	**-9.08**	**+0.05**	**-9.04**	**50**	**937.679**
AB3	**-7.12**	**6.03**	**-8.71**	**-0.20**	**-8.91**	**100**	**907.998**
AB4	**-7.12**	**6.00**	**-8.54**	**+0.13**	**-8.41**	**50**	**824.232**
AB5	-6.59	14.77	-8.70	+0.10	-8.60	50	920.18
AB6	-6.46	18.24	-7.89	-0.21	-8.10	50	917.303
AB7	**-7.35**	**4.10**	**9.70**	**-0.04**	**-9.74**	**50**	**928.775**
AB8	-5.09	187.23	-7.79	+0.46	-7.32	100	909.889
Ciprofloxacin	**-7.44**	**-3.51**	**7.26**	**-1.21**	**-8.47**	**50**	**850.017**

C. C = Compounds code. EFEB = Est. Free Energy of Binding. EIC = Est. Inhibition Constant. Vdw + H-bond + dE = Vender walls + Hydrogen bond + Dessolve energy. EE = Electrostatic Energy. TIME = Total Intermolecular Energy. Fr = Frequency. IS = Interaction Surface.

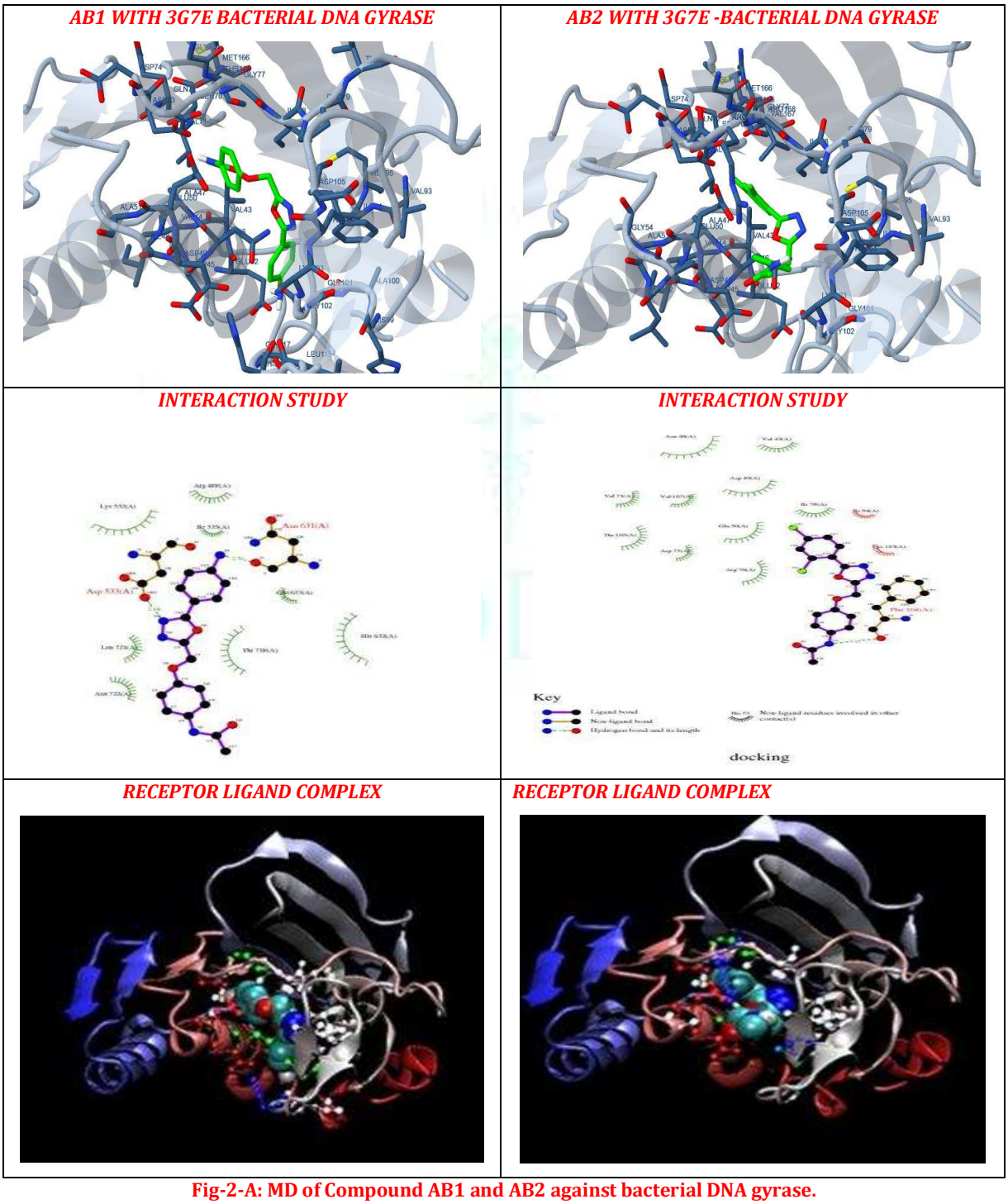


Fig-2-A: MD of Compound AB1 and AB2 against bacterial DNA gyrase.

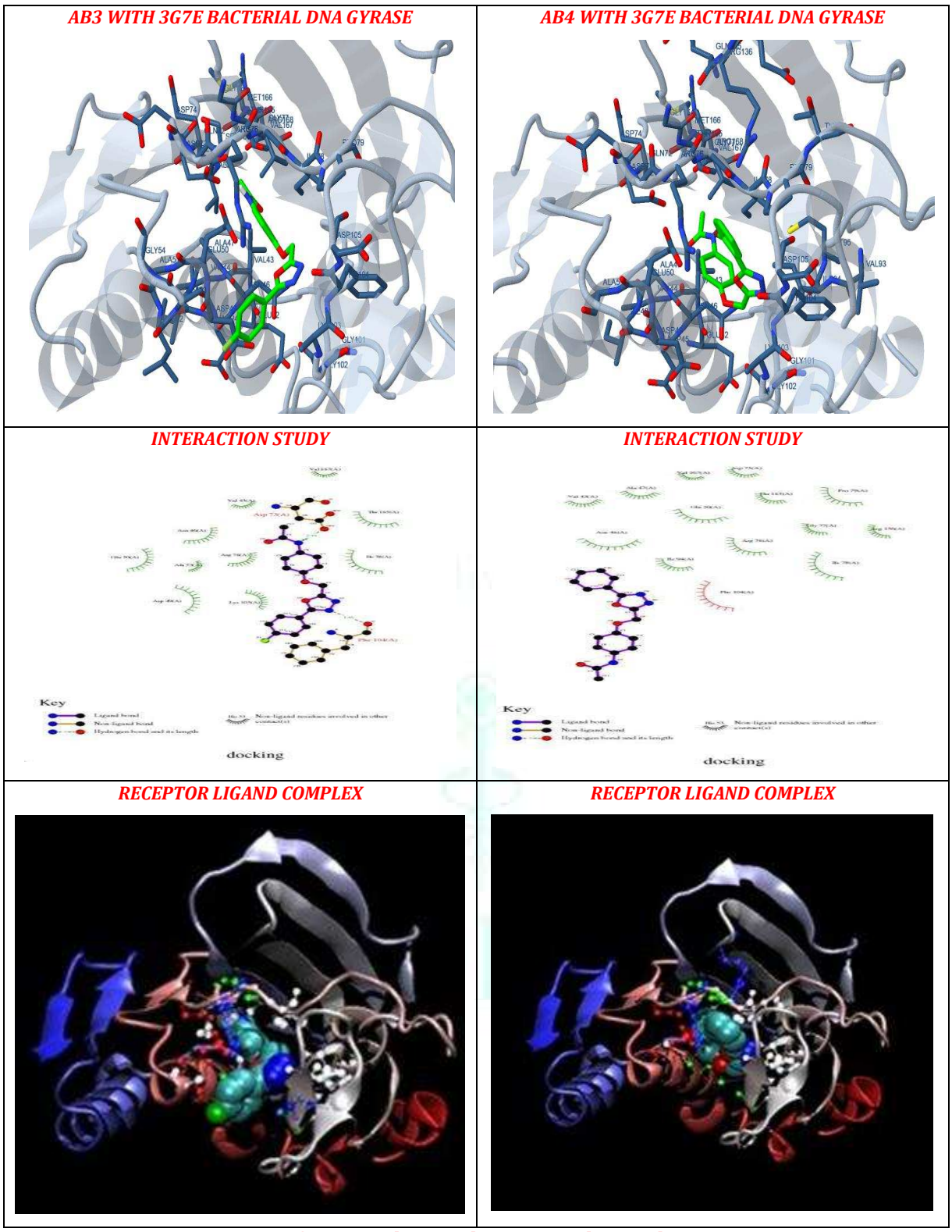


Fig-2-B: MD of Compound AB3 and AB4 against bacterial DNA gyrase.

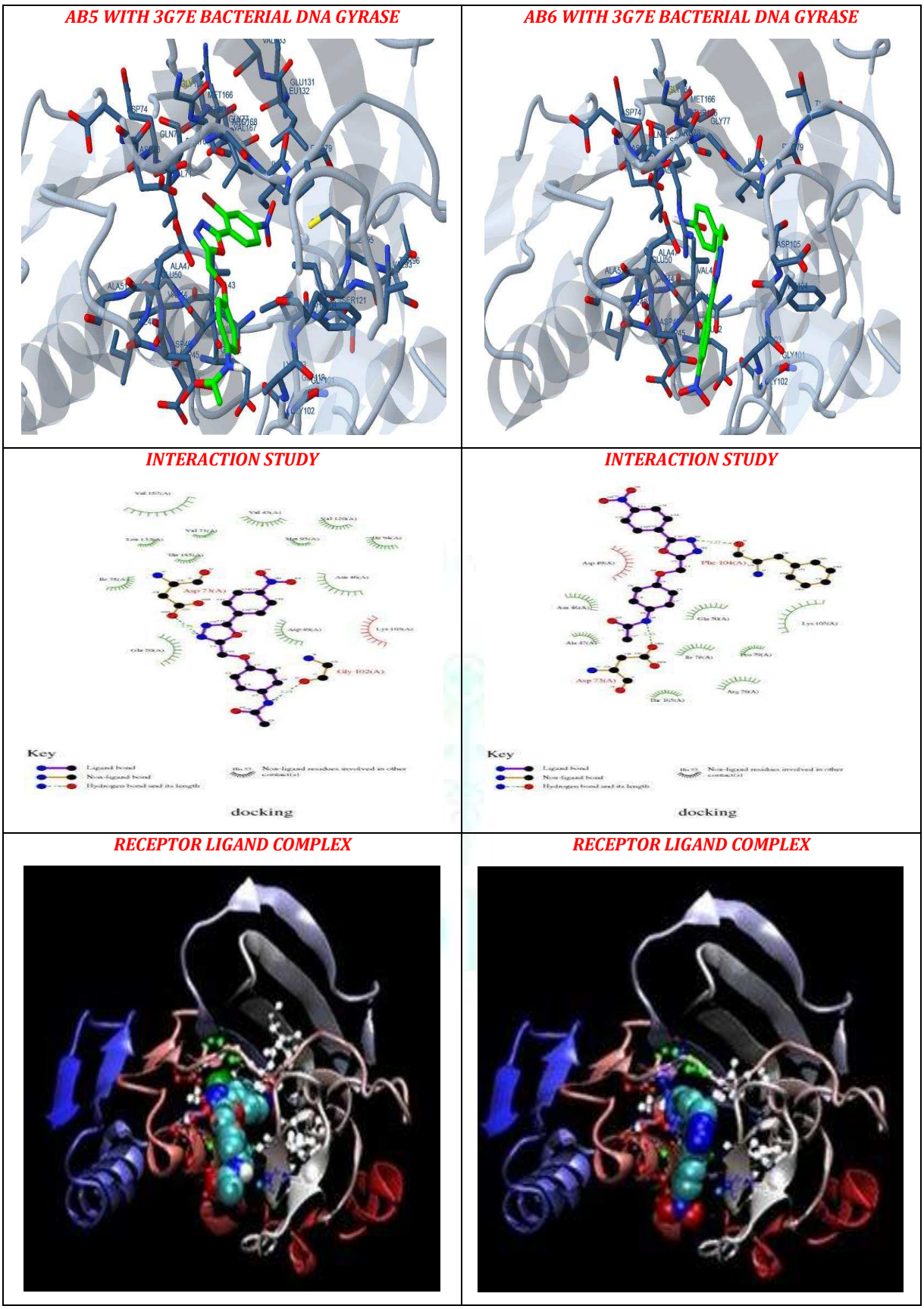


Fig-2-C: MD of Compound AB5 and AB6 against bacterial DNA gyrase.

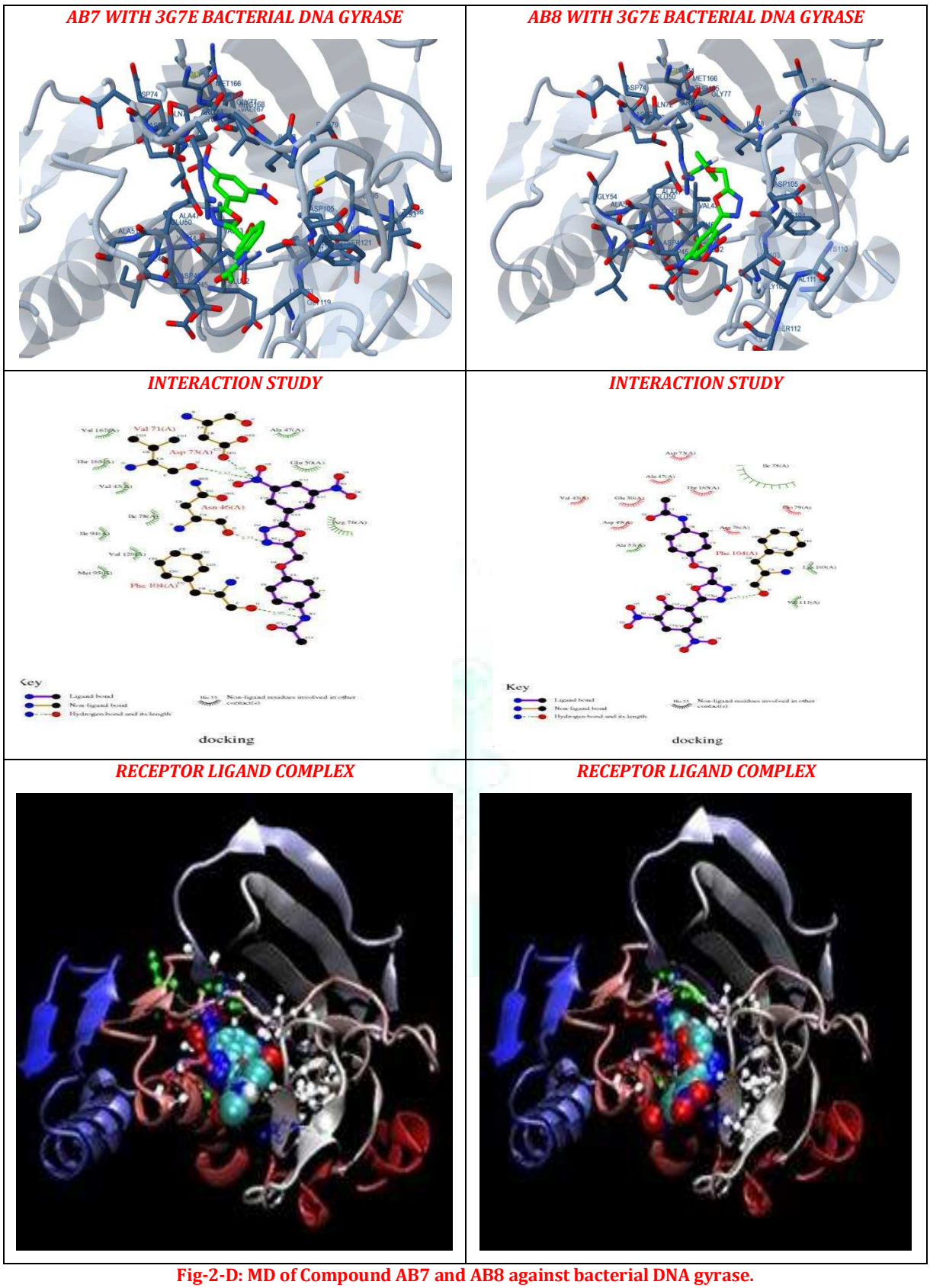


Fig-2-D: MD of Compound AB7 and AB8 against bacterial DNA gyrase.

Table-6-A: Zone of inhibition of synthesized compounds (AB1-AB8) against Gram positive bacteria

C. C	BACTERIA								
	S. aureus			*B. subtilis*			*S. epidermidis*		
	Concentration (µg/ml)								
	50	**100**	150	50	**100**	150	50	**100**	150
	Zone of inhibition in mm								
AB1	7.4	**12.3**	10.1	9.1	**10.9**	9.1	7.5	**11.6**	8.7
AB2	7.6	**12.8**	8.4	7.1	**11.4**	9.4	7.9	**13.1**	10.4
AB3	6.7	**11.2**	8.1	6.5	**10.4**	8.1	6.3	**10.4**	7.9
AB4	6.2	**10.2**	6.9	5.0	**9.5**	6.9	6.1	**8.8**	7.1
AB5	5.6	**8.6**	6.5	5.2	**8.2**	6.7	5.2	**8.0**	6.3
AB6	5.4	**7.8**	9.5	5.2	**8.0**	6.3	5.0	**7.1**	6.3
AB7	7.2	**11.9**	6.4	6.5	**10.6**	8.4	7.0	**11.0**	8.3
AB8	5.3	**7.8**	7.1	5.2	**7.5**	6.2	5.0	**8.1**	6.1
Ciprofloxacin	Concentration (100 µgm/ml)								
	28.9			**24.4**			**26.8**		

Table-6-B: Zone of inhibition of synthesized compounds (AB1-AB8) against Gram negative bacteria

C. C	BACTERIA								
	E. coli			*P. aeruginosa*			*V. cholerae*		
	Concentration (µg/ml)								
	50	100	150	50	100	150	50	100	150
	Zone of inhibition in mm								
AB1	7.2	**11.4**	10.4	6.4	**10.7**	8.8	7.3	**11.3**	8.4
AB2	7.3	**11.6**	10.8	6.9	**11.0**	9.0	7.5	**12.8**	10.0
AB3	6.5	**10.9**	10.2	6.0	**10.0**	7.8	6.3	**10.4**	7.5
AB4	6.2	**10.5**	9.2	5.0	**9.5**	6.9	6.1	**8.8**	7.1
AB5	5.5	**7.5**	6.9	5.3	8.3	6.8	5.3	**8.2**	6.4
AB6	5.3	**7.6**	6.2	5.2	7.9	6.1	5.1	**7.2**	5.7
AB7	7.0	**11.0**	10.9	6.1	**10.3**	8.1	6.9	**11**	8.0
AB8	5.1	**7.4**	6.0	5.0	7.5	6.0	5.0	**8.0**	6.0
Ciprofloxacin	Concentration (100 µgm/ml)								
	28.5			**24**			**26.4**		

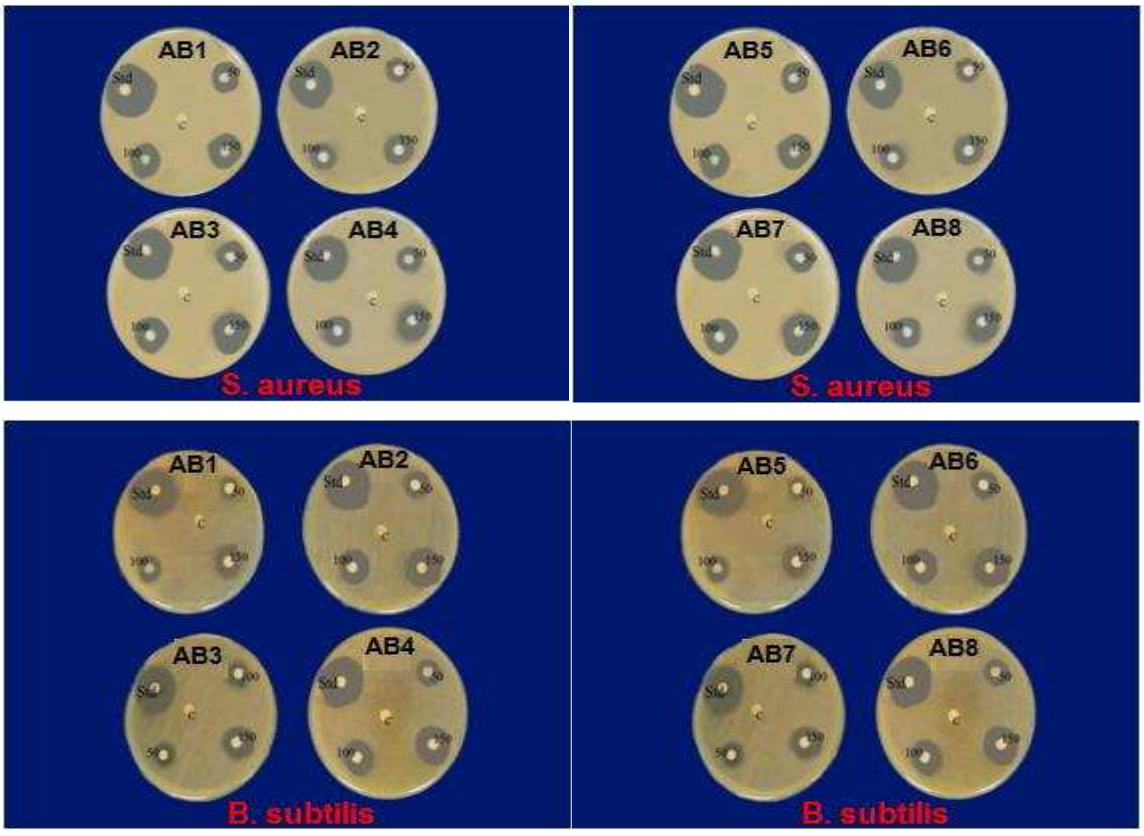


Fig 3-A(1): Zone of inhibition of bacteria by test compounds.

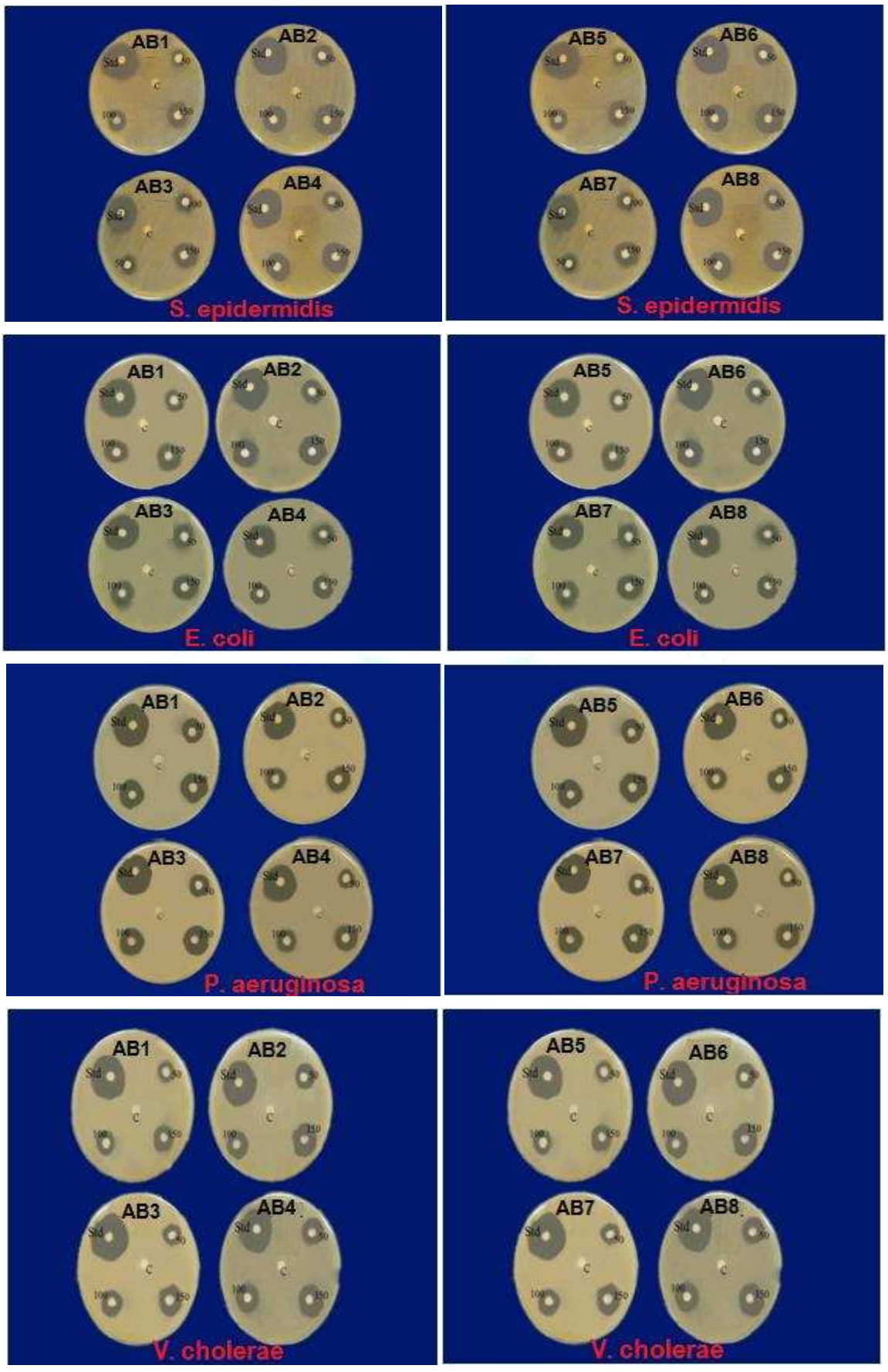


Fig 3-A(2): Zone of inhibition of bacteria by test compounds.

Table-6-C: Zone of inhibition of synthesized compounds (AB1-AB8) against fungi.

C. C	FUNGI								
	A. niger			*C. albicans*			*B. dermatitis*		
	Concentration (µg/ml)								
	50	100	150	50	100	150	50	100	150
	Zone of inhibition in mm								
AB1	7.1	**11.4**	9.4	6.3	**9.9**	8.2	6.9	**11.3**	8.7
AB2	7.3	**11.9**	9.7	6.8	**10.2**	8.4	7.1	**11.9**	9.2
AB3	6.5	**10.0**	8.2	5.6	**9.0**	7.2	5.5	**9.3**	7.5
AB4	6.0	**9.6**	7.8	5.0	**8.8**	6.4	5.2	**8.7**	7.2
AB5	5.5	**8.7**	6.9	5.3	**8.3**	6.3	5.3	**8.2**	6.8
AB6	5.3	**7.6**	6.2	5.2	**7.9**	6.1	5.1	**7.2**	5.9
AB7	6.9	**10.6**	8.8	5.8	**9.3**	7.5	5.8	**9.8**	7.9
AB8	5.1	**7.4**	6.4	5.0	**7.5**	6.2	5.0	**8.3**	6.4
Ketoconazole	**Concentration (100 µgm/ml)**								
	28			**29**			**27**		

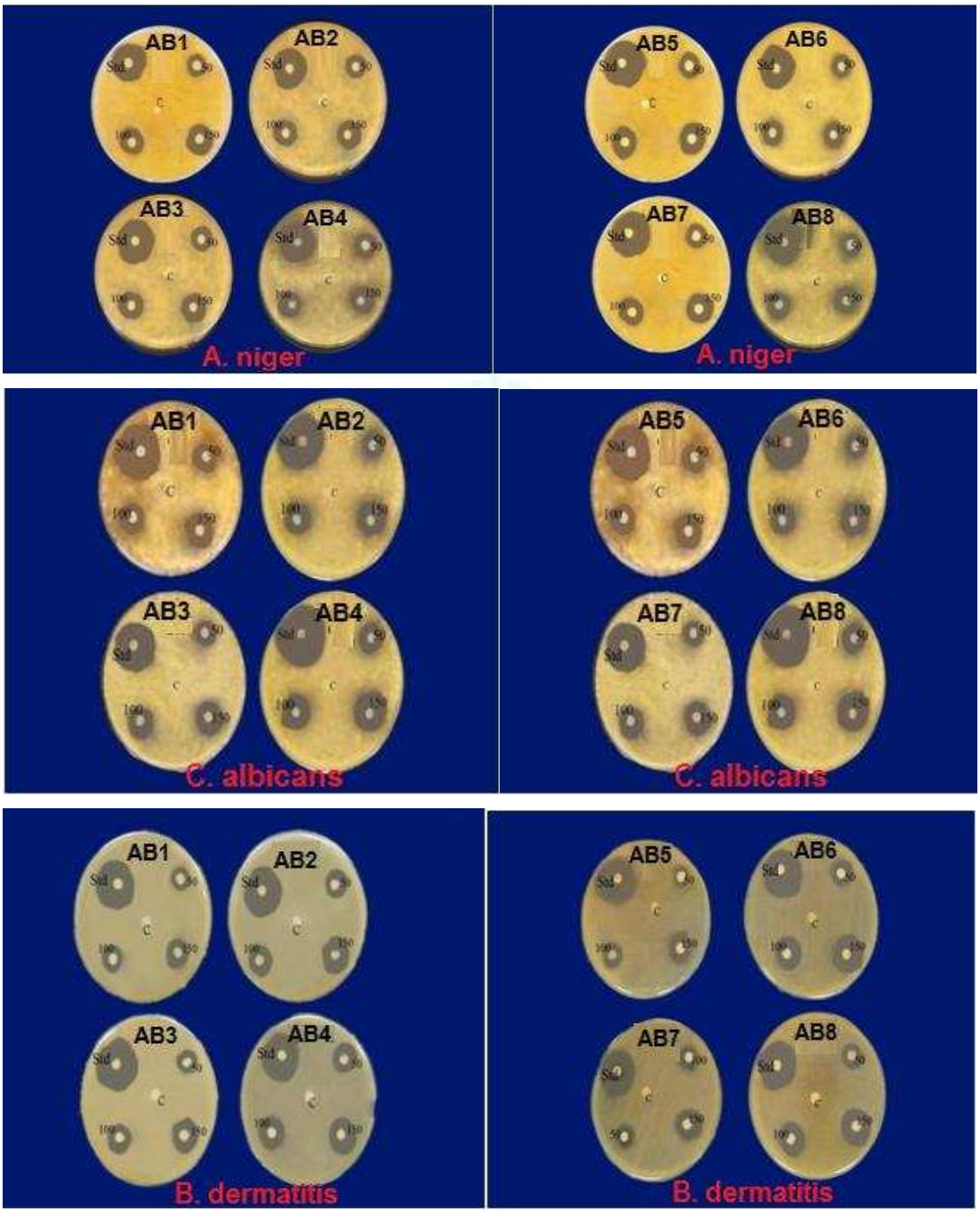


Fig 3-B: Zone of inhibition of fungi by test compounds

Table-7-A Minimum inhibitory Concentration (MIC) of Synthesized compounds against Gram positive bacteria

C.C	BACTERIA		
	S. aureus	*B. subtilis*	*S. epidermidis*
	Concentration (µgm/ml)		
AB1	**12**	**13**	**12**
AB2	**7**	**8**	**10**
AB3	22	23	20
AB4	25	26	22
AB5	27	28	30
AB6	30	32	33
AB7	**17**	**18**	**17**
AB8	33	37	37
Ciprofloxacin	**0.2**	**0.2**	**0.2**

Table-7-B: Minimum inhibitory Concentration (MIC) of Synthesized Compounds against Gram negative bacteria

C.C	BACTERIA		
	E. coli	*P. aeruginosa*	*V. cholerae*
	Concentration (µg/ml)		
AB1	**13**	**12**	**12**
AB2	**8**	**7**	**10**
AB3	23	22	20
AB4	22	26	22
AB5	28	27	30
AB6	30	32	33
AB7	**15**	**18**	**17**
AB8	37	33	37
Ciprofloxacin	**0.3**	**0.3**	**0.3**

Table-7-C: Minimum Inhibitory Concentration (MIC) of Synthesized Compounds against fungi

C.C	FUNGI		
	A. niger	*C. albicans*	*B. dermatitis*
	Concentration (µgm/ml)		
AB1	**12**	**12**	**13**
AB2	**10**	**7**	**8**
AB3	20	22	23
AB4	25	26	26
AB5	30	27	28
AB6	33	32	35
AB7	**17**	**18**	**18**
AB8	35	33	37
Ketoconazole	**5**	**5**	**5**

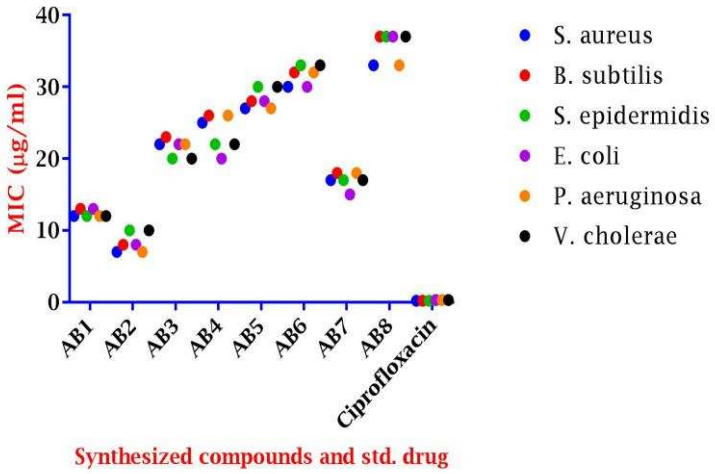


Fig-4A: MIC of synthesized compounds against different bacteria.

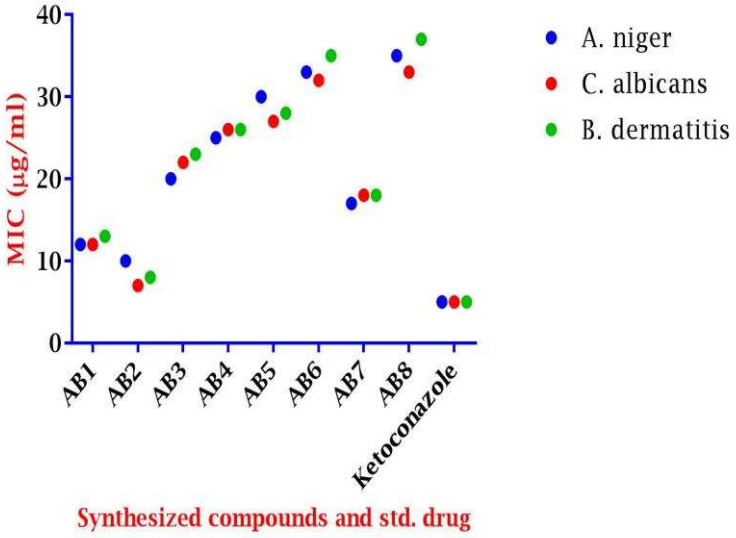


Fig-4-B: MIC of synthesized compounds against different fungi

6. DISCUSSION

6. 1. Experimental chemistry

An R_f value was characteristic for any given compound (provided that the same stationary and mobile phases are used). It provided corroborative evidence as to the identity of the new synthetic compound. R_f values of synthesized compounds indicated the reaction was proceeded in right direction and purity of new synthetic derivatives (AB1-AB8). The synthetic procedure of oxadiazole derivatives was found to have satisfactory yield. All the synthesized compounds were found to be insoluble in water, slightly soluble in Chloroform, ethanol and freely soluble in DMF, DMSO. The spectral analysis confirmed the successful synthesis of desired compounds.

6. 2. Computational chemistry

Most of the scoring functions in molecular docking are physics based molecular mechanics force fields that estimate the energy of the binding pose; a low (negative) energy indicates a stable system and thus a likely binding interaction. Molecular docking is performed to find out the binding affinity or molecular interaction energy (kcal/mol) of docked compounds. Lowest (negative value) energy of docked molecule indicates high binding affinity with the target protein/compound. In silico molecular docking studies the binding energy of synthesized compounds (AB1-AB8) were found to be -7.66, -7.67, -7.12, -7.12, -6.59, -6.46, -7.35, -5.09 which indicated that the compound had high binding affinity towards the bacterial DNA gyrase (topoisomerase) with PDB id 3G7E and inhibit the function topoisomerase in comparison with standard drug ciprofloxacin (-7.44).

6. 3. Experimental microbiology

(i) Synthesized compounds were (50, 100 and 150 µg/ml) screened for antimicrobial activity by paper disc diffusion method. From the data shown in table the observations were made as followed:

- Most of the synthesized compounds executed moderate to good antimicrobial activity against the tested microorganisms.
- When compared to standard drug ciprofloxacin, compounds AB2, AB1, AB7, AB3, AB4 were found to exhibit good Antibacterial activity.
- When compared to standard drug ketoconazole, compounds AB2, AB1, AB7, AB3, AB4 and AB8 were found to exhibit good antifungal activity.

(ii) The MIC of synthesized compounds was screened by agar streak dilution method and from the experimental data the following observation was made:

- All the synthesized compounds exhibited moderate to good antibacterial and antifungal activity with an MIC range of 12-37µg/ml.
- Among these eight synthesized oxadiazole derivatives, compound AB1; AB2 and AB7 were found to be very good antibacterial as well as antifungal potentiality with an MIC range of 13-12 µg/ml; 7-10 µg/ml and 15-18 µg/ml.

7. CONCLUSION

Here we concluded that the compound AB2, AB1, AB7, AB3, AB4 and AB8 possessed potential antimicrobial activity against following bacteria: *S. aureus (ATCC 9144), B. subtilis (ATCC 6633), S. epidermidis (ATCC 12228), P. Aeruginosa (ATCC27853), E.coli (ATCC25922), V. cholerrae (ATCC14035)* and fungi: *A. Niger (ATCC 9029), A.flavus (ATCC204304), C. albicans (ATCC10231)* and *B. dermatitis (ATCC 26199)* etc. The antibacterial potentiality of synthesized compounds were correlated with in silico molecular docking studies which was also proved molecular interaction of the compounds with the target protein bacterial DNA gyrase for their binding affinity and known to be inhibitors for bacterial DNA gyrase.

ACKNOWLEDGEMENT

We would like to thank the Department of Pharmaceutical Microbiology and Biotechnology, Anurag Pharmacy College, Suryapet, Kodad, Telangana. We would like to express our heartful thank to Indian Nobel Research Solution, Chennai, Tamil Nadu.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

[1] R. R. Gupta, M. Kumar, V. Gupta. Heterocyclic Chemistry: *Five member Heterocycles.* India: Springer; 2005; 1st Ed: 525-546.

[2] Clapp LB, Katritzky AR, Ress CW. (Eds.), "*1, 2, 3-oxadiazoles and 1, 2, 4-oxadiazoles". Comprehensive Heterocyclic Chemistry*, Pergamon Press, Oxford, 1984; Vol.6:365.

[3] Kharb R, Sharma PC, Bhandari A, Shaharyar M. *Synthesis, spectral characterization and anthelmintic evaluation of some novel imidazole bearing triazole derivatives*. Der Pharmacia Letter 2012; 4(2): 652-657.

[4] Hetzheim A., Mockel K., *"1, 3, 4-oxadiazole". Adv. Heterocyclic. Chem.* 1966; 7:183.

[5] Dewangan Dhansay, Pandey Alok. *Synthesis of some Novel 2, 5-DiSubstituted 1, 3, 4-Oxadiazole and its Analgesic, Anti-Inflammatory, Anti-Bacterial and Anti-Tubercular Activity*. International journal of chemistry; 2: 1397-1412.

[6] Sun J, Zhu H, Yang ZM, Zhu HL: *Synthesis, molecular modelling and biological evaluation of 2- aminomethyl-5-(quinolin-2-yl)-1, 3, 4-oxadiazole-2(3H)-thione quinolone derivatives as novel anticancer agent*. European Journal of Medicinal Chemistry 2013; 60: 23-28.

[7] Zhang XM, Qiu M, Sun J, Zhang YB, Yang YS, Wang XL, Tang JF, Zhu HL: *Synthesis, biological evaluation, and molecular docking studies of 1, 3, 4-oxadiazole derivatives possessing 1, 4-benzodioxan moiety as potential anticancer agents.* Bioorganic & Medicinal Chemistry 2011; 19: 6518–6524.

[8] Ravichandran V, Shalini S, Sundram K and Sokkalingam A D*: QSAR study of substituted 1, 3, 4-oxadiazole naphthyridines as HIV-1 integrase inhibitors.* European Journal of Medicinal Chemistry 2010; 45:2791-2797.

[9] K. Ilango, P. Valentina, N. Umarani, T Kumar. *"Synthesis and characterization of 2, 5-disubstituted-1, 3, 4-oxadiazoles as potential inflammatory agents*". Indian J. Chemistry., 2009; 1:72-76.

[10] K. M. Khan, M. Rani, S. M. Haider. *"Microwave-Assisted Synthesis of 2, 5-Disubstituted-1, 3, 4-Oxadiazoles".* Letter Organic Chemistry. 2004; 1:151.

[11] Pelletier JC et al. Preparation of highly substituted gamma lactam FSH receptor agonists, Bioorg. Med. Chem 2005; 13 : 5986-5 995.

[12] (a) Gregory L. Warren, Stephen D. *"Chapter 16: Scoring Drug-Receptor Interactions".* WarrenPublished 2011 doi: 10.1039/9781849733410-00440.

(b) Gramatica P, Livingstone DJ, Davis AM. Drug Design Strategies: Quantitative Approaches. Royal Society of Chemistry. pp. 440–457. doi:10.1039/9781849733410-00440. ISBN 978-1849731669.

[13] Vibhor K Jain, Sudeep Mandal, Dibyajyoti Saha,, Bindu Jain."*Synthesis, characterization and evaluation of antibacterial and antifungal activity of triazole derivatives of gallic acid"* Ijabpt 2010; 1 (3): 1300-1311.

[14] Chandrakant R. Kokare. Pharmaceutical *Microbiology Experiments and Technique,* Second Edition, pp 63, 138-139.

[15] R. S. Gaud, G. D. Gupta "*Practical microbiology"*. Nirali prakashan. Mumbai 3rd ed. 2004: 41.

[16] Hawkey BY, Lewis DA, *Medical microbiology: a practical approach*. United Kingdom: Oxford university press; 1994. 181-194.