An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

MOLECULAR DOCKING STUDIES OF 4-BENZYLIDENE-2-(4-HYDROXY-3-METHOXYSTYRYL) OXAZOL-5(4H)-ONES; INTERACTIONS WITH ARG-136 AND ASP-73 OF DNA GYRASE

Manjusha RK*, Arifa Begum, Swapna B, Shaheen Begum, Bharathi K, Sujatha D

Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati 517502, Andhra Pradesh, India

*Corresponding author E-mail**: manjurk9393@gmail.com**

ARTICLE INFO

Key Words

Oxazolone, Azalactones, DNA Gyrase, Molecular docking, Ferulic acid

ABSTRACT

The structure of DNA gyrase is well established and recent studies suggest the utility of this structure in the antibacterial drug development process. A series of 4-substituted benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones were designed where the benzylidene ring is substituted with various electron releasing (OH,OCH_3,CH_3) or withdrawing (NO_2) groups or halogen atoms (F, Cl) and molecular docking studies were performed to probe their binding affinity towards DNA Gyrase enzyme. To study the effect of substitution (3-OCH_3, 4-OH) on styryl ring, binding affinities were compared using 4-substituted benzylidene-2-(styryl) oxazol-5(4H)-ones. Results showed that presence of 3-methoxy and 4-hydroxy groups on styryl ring lead to the molecules with good interactions (Gibbs free energy: -8.08 to -8.98 kcal/mol) when compared to that of compounds containing unsubstituted styryl (Gibbs free energy: -7.75to -8.18 kcal/mol) ring with the target enzyme. In both the series, substitution at benzylidene ring seems to significantly enhance the binding interactions indicating that it is a critical scaffold for binding with the enzyme. Moreover, most of the compounds were able to establish hydrogen bonding with Asp-73 and Arg-136 aminoacids present in the active site of enzyme.

INTRODUCTION:

DNA gyrase (DNA topoisomerase II), which plays a critical role in controlling the DNA topology and bacterial vitality, is a well explored target for antibacterial agents. DNA gyrase contains two subunits (GyrA and GyrB) and forms a tetramer (A2B2) when the enzyme is activated. GyrA contains the catalytic tyrosine, which can cleave the DNA and GyrB contains the ATP binding pocket Arg-136 and Gly-164 (1-3). Aminocoumarins such as Novobiocin, cyclothialidines and fluoroquinolones like ciprofloxacin are conventional DNA gyrase inhibitors (3). Heterocyclic compounds including pyrimidine indoles, pyrrolopyrimidines, N-phenyl pyrrolamides and benzimidazole urea derivatives are extensively studied for their DNA gyrase

inhibitory activity (4). Bacterial resistance is high in case of the antibacterial agents used in the treatment of infections caused by *Streptococcus aureus*, *Enterococcus faecalis, Klebsiella pneumonia, Escherichia coli,Proteus vulgaris and Streptococcus typhi*. Mutations which are responsible to coumarin drugs in Escherichia coli strains (Arg-136, Cys, His or Her and Gly-164) are located at the ATP-binding site of the GyrB proteins. Combating the mutations at Arg-136 and Gly-164, the most important contact points of GyrB, can be envisioned to overcome bacterial resistance (5-7). Oxazolones and their derivatives are associated with diverse set of biological activities including antioxidant, antimicrobial, anticancer, antidiabetic, analgesic and antiniflammatory activities (8-15). Cinnamic acid derivatives possessing phenolic hydroxyl group such as ferulic acid (3-methoxy, 4-hydroxy cinnamic acid) exhibit significant antibacterial activities (16-18).

Ferulic acid and its derivatives display interesting pharmacological activities viz., hepatoprotective, anti-diabetic, antiinflammatory and neuroprotective activities (19-20). Arylidene ring is known to enhance the lipophilicity and thereby bioavailability of the parent structure (21). Owing to the importance of oxazolone, arylidene and ferulic acid moieties, these scaffolds were incorporated into a single structural entity to give 4-benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones (**Fig.1 F1-F8**). Molecular docking studies were performed to predict their binding poses and their binding interactions with DNA gyrase enzyme. To study the effect of substitution on styryl ring, few oxazolones were designed possessing unsubstituted styryl ring i.e. 4-substituted benzylidene-2-(styryl) oxazol-5(4H)-ones (**Fig.1 C1-C8**).

Benzylidene ring was substituted with different groups (OH, OCH_3, NO_2, Cl, and Br) so as to observe the impact of electron releasing/withdrawing/lipophilicity on binding affinity with the target enzyme.

MATERIALS AND METHODS

SwissDock:

SwissDock(http://swissdock.vital-it.ch) was used for performing molecular docking studies. It is based on the docking software EADock dihedral space sampling DSS (fast docking) by using the CHARMM ((Chemistry at HARvard Macromolecular Mechanics) force field. Docking involves step by step process in SwissDock, which includes, generation of binding modes of ligands either in local docking (user-defined box) or in blind docking (in the vicinity of entire protein surface) and estimation of CHARMM energies; Ranking of binding modes by using FACTS (fast analytical continuum treatment of salvation) model. Evaluation of Gibbs frees energy (ΔG) and fullfitness; Cluster ranking based on fullfitness values and best binding modes (21).

Preparation of protein

Target structure can be determined either by specifying its identifier (PDBID) from the Protein Data Bank (PDB) or user can upload the structure file. The crystal structure of DNA Gyrase (PDB ID: 1KZN; resolution 2.30 Aº) was retrieved from the Brookhaven PDB and submitted for the docking studies. Details of the protein are given in Table.1. Clorobiocin was used as reference compound to compare the binding modes of the studied ligands. In the crystal structure, in built ligand Clorobiocin (**Fig.2**) was found to establish hydrogen bonding interactions with Asp-73, Thr-165, Asn-46 and Arg-136 (22).

Preparation of ligand

In SwissDock protocol, ligand can be directly selected from ZINC database or by uploading the structure file in the Mol2 format (structure must contain all hydrogens and 3D co-ordinates). The ligand molecules (F1-F8 and C1-C8) were built using Chem Draw 12 version and saved in mol2 format before submitting in Swiss Dock.

Docking parameters

In the available three docking parameters (very fast, fast and accurate), fast parameter was selected for the studies. The whole protein structure was considered for the docking and the results were viewed in UCSF Chimera.

RESULTS & DISCUSSION

4- Substituted benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones

In the series of 4-benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones, highest binding affinity was observed with 3,4,5-trimethoxy derivative (F6) which showed ΔG= -8.98 kcal/mol and fullfitness of -1274.50 kcal/mol when compared to unsubstituted derivative (F1) which showed ΔG= -8.08 kcal/mol and fullfitness of -1302.15 kcal/mol. The results of the molecular docking studies are presented in table.2. Moderate binding affinity was observed with derivatives possessing 3,4-dimethoxy (F5), 4-methoxy (F4), 4-Bromo (F7), 4-Nitro (F8) and 4-hydroxy (F3) derivatives in the order of -8.73>-8.50>-8.37>-8.36>-8.33 kcal/mol. When 4-chloro derivative (F2) was docked into the binding site, poor binding interaction was observed (ΔG=-8.20 kcal/mol).

It can be concluded that binding affinity was enhanced with the presence of either electron withdrawing or donating groups on benzylidene ring suggesting that pivotal role of benzylidene ring for the binding affinity with DNA gyrase enzyme. It can be observed that, good binding interaction is elicited as the number of methoxy groups increases on the benzylidene ring. The Gibbs free energy of the compounds was ranged from -8.08 to -8.98 kcal/mol suggesting good binding affinity of 4-substituted benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones. The inbuilt ligand, clorobiocin [(3R,4S,5R,6S)-6-[8-chloro-4-hydroxy-3-[[4- hydroxy- 3- (3-methylbut-2-enyl)phenyl] carbonylamino] -2 - oxo-chromen-7-yl] oxy-5-hydroxy-3-methoxy-2,2-dimethyl-oxan-4-yl] 5-methyl-1H-pyrrole-2-carboxylate] showed highest binding interaction with the ΔG of -9.67 kcal/mol and fullfitness of -1202.57 kcal/mol.

4-Substituted benzylidene-2-(styryl) oxazol-5(4H)-ones

In 4-substituted benzylidene-2-(styryl) oxazol-5(4H)-ones, significant binding interaction was observed with 3,4,5-trimethoxy derivative (C6) which showed ΔG of -8.18 kcal/mol and fullfitness of -1261.03 kcal/mol when compared to unsubstituted derivative (C1) which showed poor binding affinity with ΔG -7.75 kcal/mol and fullfitness of -1299.95 kcal/mol. Compound with 3, 4-dimethoxy substitution (C5) (-8.12 kcal/mol) also exhibited good binding affinity with the target. Moderate binding interaction was exhibited by the compounds bearing 4-chloro (C2), 4-Bromo (C7),4-hydroxy (C3), 4-methoxy (C4) and 4-Nitro (C8) in the ΔG values order of -7.92>-7.86>-7.85>-7.84>-7.82 kcal/mol. The poor binding interaction of unsubstituted derivative (C1) showed lowest binding affinity with ΔG of -7.75 kcal/mol.

Table I. Details of protein structures

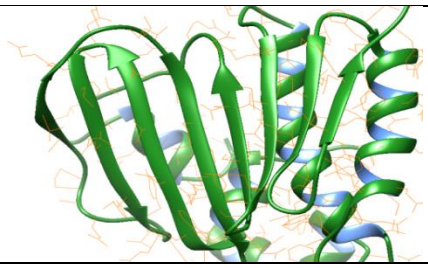
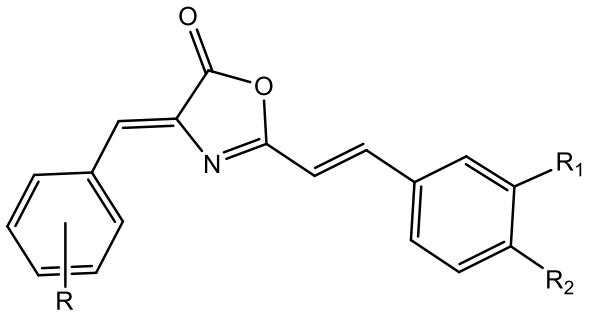
Target enzyme Structural details	DNA Gyrase (1KZN)
	The 3D structure of E. coli 24kDa Domain in Complex with Clorobiocinat the active site (2.3A° resolution)was retrieved from protein data bank (PDB ID: 1KZN).
Organism:	Escherichia coli (strain K12)
Classification:	Isomerase
Total structure weight:	23288.50
Chains:	A
Length:	205

Table.2: Docking scores and details of interactions obtained using SwissDock

S.No	DNA gyrase (PDB ID: IKZN)		Interacting amino acids	Bond distance (AO)
	Energy (ΔG)	Fullfitness		
	Kcal/mol			
CBN (Clorobiocin) In built ligand	-9.67	-1202.57	Arg-136 H-Lig O Arg-136 H-Lig O Arg-136 H-Lig O Asp-73 O-Lig H Asn-46 O-Lig H	2.70 2.15 2.37 1.78 1.97
F1	-8.08	-1302.15	Gly-77 NH-Lig O	2.15
F2	-8.20	-1302.77	Asn-46 H-Lig N	2.70
F3	-8.33	-1309.56	Asn-46 H-Lig N Ala-96 O-Lig H	2.45 1.95
F4	-8.50	-1300.62	Ala-96 O-Lig H	2.03
F5	-8.73	-1286.21	Asp-73 O-Lig H	1.75
F6	-8.98	-1274.50	Asp-73 O-Lig H Arg-136 H-Lig O	1.71 3.77
F7	-8.37	-1293.94	Asn-46 H-Lig N Arg-136 H-Lig O	2.45 2.25
F8	-8.36	-1281.67	Arg-136 H-Lig O Asp-73 O-Lig H	2.06 1.81
C1	-7.75	-1299.95	Arg-136 H-Lig O	2.90
C2	-7.92	-1300.13	Asn-46 H-Lig N	2.42
C3	-7.85	-1313.16	Asn-46 H-Lig N	2.44
C4	-7.84	-1300.54	Arg-136 H-Lig O	2.85
C5	-8.12	-1282.54	Gly-77 NH-Lig O Arg-136 H-Lig O	1.94 3.86
C6	-8.18	-1261.03	Arg-136 H-Lig O	2.73
C7	-7.86	-1302.47	Asn-46 H-Lig N	2.40
C8	-7.82	-1290.41	Arg-136 H-Lig O	1.90



C1-C8 R= H, Cl, OH, 4-OCH_3, 3,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$, 4-Br, 4-NO_2 R_1, R_2= H

F1-F8 R= H, Cl, OH, 4-OCH_3, 3,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$, 4-Br, 4-NO_2 R_1= OCH_3 R_2=OH

Fig 1.Molecules selected for the docking study

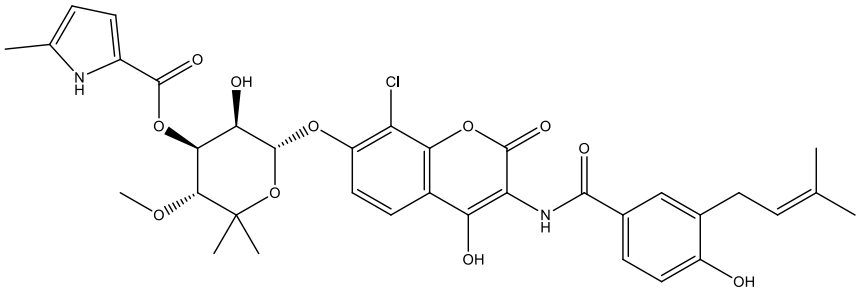


Fig 2.Clorobiocin

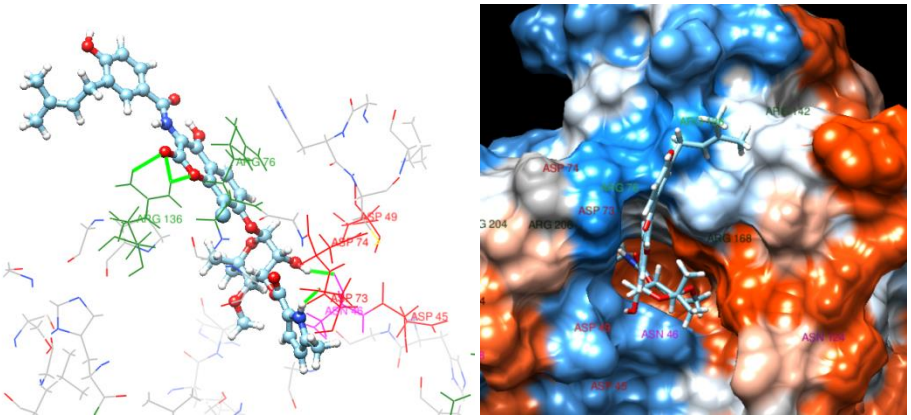


Fig.3 (A) Hypothetical binding mode of Clorobiocin in the active site of DNA Gyrase (B) Hydrophobic representation

Binding mode of 4-substituted benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones

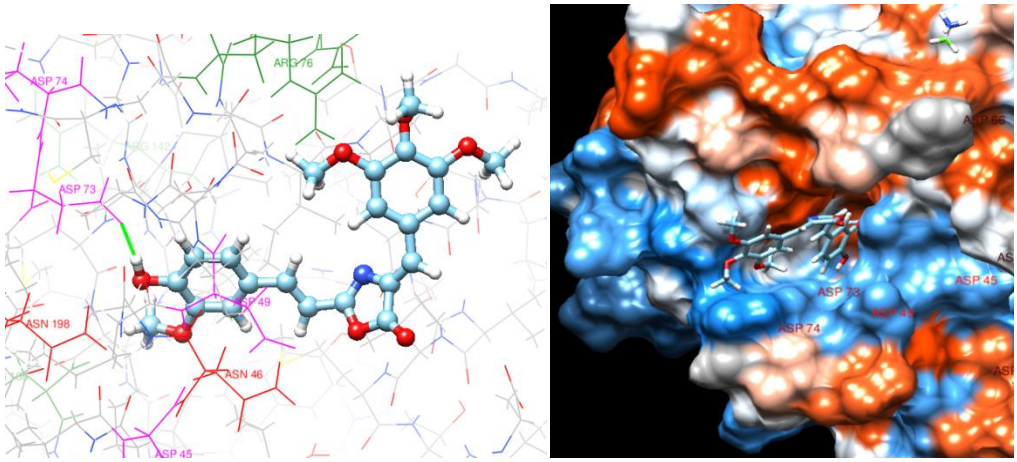


Fig.4 (A) Hypothetical binding mode of F6 in the active site of DNA Gyrase (B) Hydrophobic representation (Asp-73 O-Lig H-1.71; Arg-136 H-Lig O-3.77 Aº)

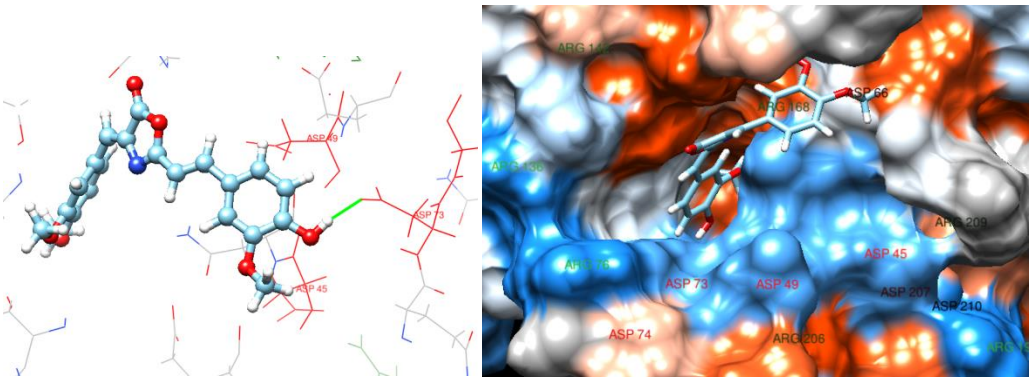


Fig.5 (A) Hypothetical binding mode of F6 in the active site of DNA Gyrase (B) Hydrophobic representation (Asp-73 O-Lig H-1.73 Aº) 4-Substituted benzylidene-2-(styryl) oxazol-5(4H)-ones

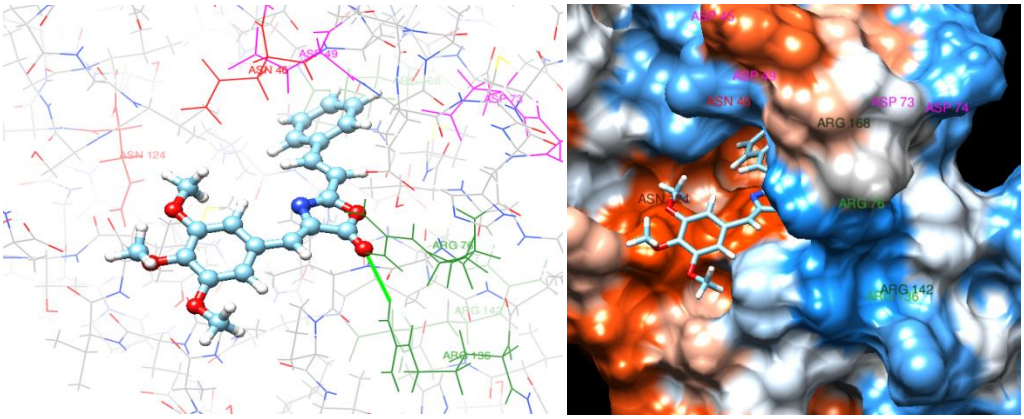


Fig.6 (A) Hypothetical binding mode of C6 in the active site of DNA Gyrase (B) Hydrophobic representation (Arg-136 H-Lig O-2.73 Aº)

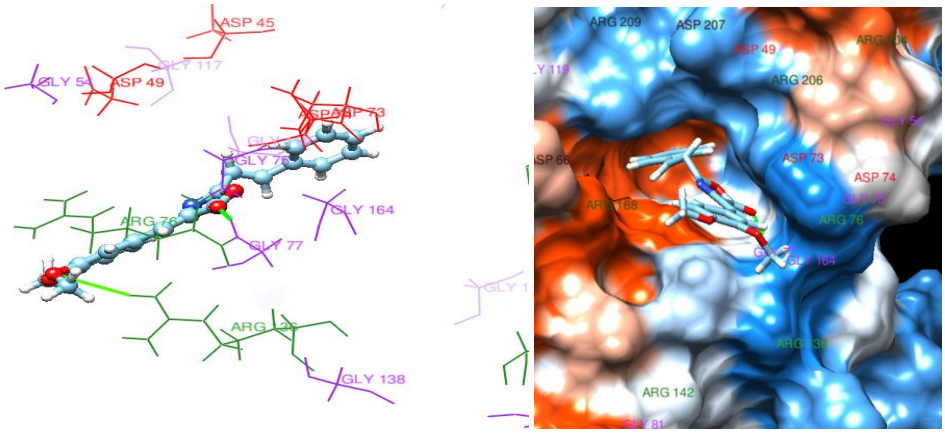


Fig.7 (A) Hypothetical binding mode of F6 in the active site of DNA Gyrase (B) Hydrophobic representation (Arg- 136 H-Lig O-3.86 Aº ;Gly-77 NH-Lig O-1.94 Aº)

Based on the results, it can be suggested that benzylidene ring with electron donating groups is significantly contributing for binding with the DNA gyrase enzyme than compounds with electron withdrawing groups.

Binding mode of 4-substituted benzylidene-2-(styryl) oxazol-5(4H)-ones

Trimethoxy substituted compound (C6) exhibited hydrogen bonding interaction with Arg-136 amino acid residue which is also seen in hydrogen bonding interactions with inbuilt ligand (Arg-136, Asn-46, Asp-73). Most of the compounds showed hydrogen bonding with Arg-136 and Asn-46 amino acid residues.

CONCLUSION

The results of the molecular docking studies suggest that 4-benzylidene-2-(styryl) oxazol-5(4H)-ones and 4- benzylidene-2-(4-hydroxy-3-methoxystyryl) oxazol-5(4H)-ones exhibit good interactions with DNA Gyrase enzyme. Binding modes of the docked compounds revealed their affinity for Arg-136 and Asp-73, two aminoacids residues which are crucial for bacterial resistance. It can be concluded that antibacterial activity exhibited by these oxazolones might be due to their affinity for DNA Gyrase enzyme and proper structural optimization of the title compounds may lead to promising antimicrobial agents.

REFERENCES

1. Franco-Ulloa S, Sala G La, Miscione GP, Vivo M De. Novel Bacterial Topoisomerase Inhibitors Exploit Asp83 and the Intrinsic Flexibility of the DNA Gyrase Binding Site *Int. J. Mol. Sci.* 2018; 19(453); 2-12
2. Rahimi H, Najafi A, Eslami E, Negahdari B, Moghaddam MM. Identification of novel bacterial DNA gyrase inhibitors: An in silico study. *Res. Pharm. Sci*. 2016; 11(3): 250–258.
3. Omar FA, Abel-rasoul M, Sheha MM, Hassan HY, Ibrahiem YM. Synthesis, Antibacterial Activity and Molecular Docking of Substituted Naphthyridines as Potential *DNA Gyrase* Inhibitors. *Chem. Central.* 2018; 3(9): 2604–2612.
4. Durcik M, Tammela P, Barančoková M, Tomasic T, Ilas J, Kikelj D, Zidar

N. Synthesis and Evaluation of N-Phenylpyrrolamides as DNA Gyrase B Inhibitors. *Chem. Med. Chem.* 2018; 13: 186-198.

5. Contreras A, Maxwell A. gyrB mutations which confer coumarin resistance also affect DNA supercoiling and ATP hydrolysis by Escherichia coli DNA gyrase. *Mol. Microbiol.* 1992; 6(12): 1617-24.
6. Munoz R, Maria B, Adela GD. Ser-127-to-Leu Substitution in the DNA Gyrase B Subunit of Streptococcus pneumoniae Is Implicated in Novobiocin Resistance. *J. Bacteriol.* 1995; 177(14): 4166–4170.
7. Thani AB. Antibacterial Resistance via Gyrb24 Targeting. *J. Investig. Genomics.* 2017; 4(1): 00058-00059.
8. Voosala C, Kilaru PS, Dasari UK. A Facile and Efficient Synthesis of 4-Arylidene-2-phenyl-5(4H)-oxazolones and Their Antimicrobial Evaluation against Selected Human and Phytopathogens. *J. Chin. Chem. Soc.* 2016; 63(11): 909–916.
9. Moorkoth S, Srinivasan KK and Bukka D. Evaluation of antimicrobial activity of 4-arylidene-2-phenyl oxazol-5-one derivatives against selected skin microorganisms. *Elixir. Pharm.* 2013; 54: 12315-12318.
10. Jat LR, Mishra R, Pathak D. Synthesis and Anticancer Activity Of 4-Benzylidene-2- Phenyloxazol-5(4h)-One Derivatives Research Article. *Int. J. Pharm. Pharm. Sci.* 2012; 4(1): 378-380.
11. El-Mekabaty A. Erlenmeyer Azlactones: Synthesis, Reactions and Biological Activity. *Int. J. Mod. Org. Chem.* 2013, 2(1): 40-66.
12. Salgin-Goksen U, Gokhan-Kelekci N, Goktas O, Koysal Y, Kilic E, Isik S, Aktay G, Ozalp M. 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones:synthesis analgesic-anti-inflammatory and antimicrobial activities. *Bioorg. Med. Chem.* 2007; 15(17): 5738–5751.
13. Mariappan G, Saha BP, Datta S. Design, synthesis and antidiabetic evaluation of oxazolone derivatives. *J. Chem. Sci.* 2011; 123(3): 335-341.
14. Rodrigues Pereira E, Sancelme M, Voldoire A, Prudhomme M. Synthesis and antimicrobial activities of 3-N-substituted-4,5-bis(3-indolyl)oxazol-2-ones. *Bioorg Med Chem Lett.* 1997; 7(19): 2503-2506.
15. Sadula A, Peddaboina UR, Subhashini NJP. Synthesis and characterization of novel chalcone linked imidazolones as potential antimicrobial and antioxidant agents. *Med. Chem. Res.* 2015; 24(2): 851–859.
16. Sova, Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Min. Rev. Med. Chem.* 2012; 12(8): 749-67.
17. Shi C, Zhang X, Sun Y, Yang M, Song K, Zheng Z, Chen Y, Liu X, Jia Z, Dong R, Cui L, Xia X. Antimicrobial Activity of Ferulic Acid Against Cronobacter sakazakii and Possible Mechanism of Action. *Foodborne Pathog. Dis.* 2016; 13(4): 196-204.
18. Borges A, Ferreira C, Saavedra MJ, Simoes M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb. Drug Resist.* 2013; 19(4): 256-65.

19. Kayahara H, Miao Z, Fujiwara G. Synthesis and biological activities of ferulic acid derivatives. *Anticancer Res.* 1999; 19(5A): 3763-3768.
20. Jeong YC, Jae HM, Keun HP. Isolation and identification of 3-methoxy-4 hydroxybenzoic acid and 3-methoxy-4- hydroxycinnamic acid from hot water extracts of Hovenia dulcis Thumb and confirmation of their antioxidative and antimicrobial activity. *Kor. J. Food Sci. Technol.* 2000; 32(6): 1403-1408.
21. Bayoumi WA, Abdel-Rhman SHM, Shaker ME. Synthesis and Evaluation of New 2-Iminothiazolidin-4-one and Thiazolidin-2,4-dione Derivatives as Antimicrobial and Anti-inflammatory Agents. *Open Chem J.* 2014; 1: 33-38.
22. Lafitte D, Lamour V, Tsvetkov PO, Makarov AA, Klich M, Deprez P, Moras D, Briand C, Gilli R. DNA gyrase interaction with coumarin-based inhibitors: the role of the hydroxybenzoate isopentenyl moiety and the 5'-methyl group of the noviose. *Biochem.* 2002; 41(23): 7217-7223.
23. Grosdidier A, Zoete V, Michielin O. SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucl. Aci. Res.* 2011; 39(2): W270–W277.
24. Rajitha G, Chandi Priya S, Yamini Latha T. Synthesis and biological evaluation of β-amino naphthyl substituted chalcones for anti-inflammatory and antioxidant activities. *J. Chem .Pharm. Res.* 2015; 7(4): 80-84.