Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2016, 5 (5): 1089-1096

(International Peer Reviewed Journal)

Synthesis, Characterization, Antibacterial and Docking Study of Sulfonamide Derivatives

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Accepted on 24th August 2016

ABSTRACT

In this study we synthesized new sulfonamide derivatives by condensing p-toluene sulfonyl chloride with five different amines. All the synthesized sulfonamides were characterized by IR, ^{1}H-NMR, ^{13}C- NMR spectral analysis and were evaluated for their antibacterial activity against two bacterial strains Gram positive Staphylococcus aureus and Gram negative Escherichia coli using Disc diffusion method. Three compounds exhibited significant antibacterial activity. The theoretical binding mode of the target molecules was evaluated by molecular modeling studies, which revealed a new scaffold for enhancing the antibacterial activity of the synthesized compounds.

Keywords: Dehydrosqualene, *Staphylococcus aureus*, *Escheria coli*, Molecular modeling.

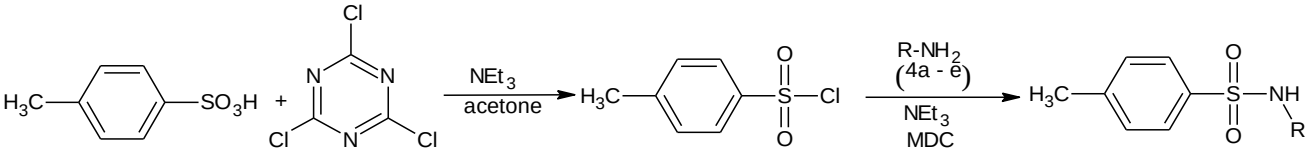
INTRODUCTION

Sulfonamides constitute an important class of antimicrobial agents in the world owing to their low cost, low toxicity and excellent activity against bacterial diseases [1]. Sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase in bacteria and catalyze the conversion of p-amino benzoic acid into an essential nutrient for some bacteria [2]. Sulfonamides (sulfa drugs) were the first drugs largely employed and systematically used as preventive and chemotherapeutic agents against various diseases [3]. Out of 30 drugs containing this moiety are in clinical use, including antihypertensive agent bosentan [4], antibacterial [5], antiprotozoal [6], antifungal [7], anti-inflammatory [8], nonpeptidic vasopressin receptor antagonists [9] and translation initiation inhibitors [10]. Some important sulfonamide derivatives used as carbonic anhydrase inhibitors of commercial importance [11]. They were also effective for the treatment of urinary, intestine and ophthalmic infections, scalds, ulcerative colitis [12], rheumatoid arthritis [13], male erectile dysfunction as the phosphodiesterase-5 inhibitor sildenafil – better known under its commercial name, Viagra [14] and obesity [15]. More recently, sulfonamides are used as an anticancer agent [16], as the antiviral HIV protease inhibitor amprenavir [17] and in Alzheimer's disease [18]. After the introduction of penicillin and other antibiotics, the popularity of sulfonamides decreased. However, they are still considered to be useful in certain therapeutic fields, especially in the case of ophthalmic infection in urinary and gastrointestinal tract. Besides, sulfa drugs are till today among the drugs of first selection

(together with ampicillin and gentamycin) as chemotherapeutic agents in bacterial infections by E. coli in human. The potentially active sulfonamide derivatives deserve more detailed experimental and systematic theoretical studies using updated computer programs and recently available knowledge on structure activity relations.

MATERIALS AND METHODS

Chemicals were purchased from Merck India, Spectrochem and Sigma–Aldrich, solvents and chemicals used were of AR grade. The purity of the compounds was confirmed by thin layer chromatography using precoated thin layer chromatographic plates and solvent systems of Petroleum ether / Ethyl acetate (7:3). Chromatography was performed using Merck silica gel 60 (0.025-0.04 mm), and reaction was monitored by thin-layer chromatography (TLC) on silica gel plates. Visualization was done with UV light (254 nm) or by using iodine chamber. Melting points were recorded using open capillary tubes. Proton NMR spectra were recorded on Bruker 500 MHz instrument with chemical shifts (δ in ppm) reported relative to tetramethylsilane as internal standard.



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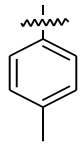
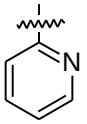
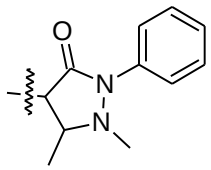
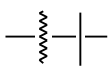
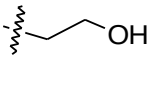
R					

Scheme

Synthesis of 4-methylbenzenesulfonyl chloride (3): To 20mM of p-toluene sulfonic acid was added 20mM of triethylamine in 40 mL of acetone. To this mixture 20mM of cyanuric chloride was added and was heated under reflux for 20 hours. After cooling to room temperature the solution was filtered. Solvent was removed under vacuum, the sulfonyl chloride so obtained was purified by column chromatography.MP 70-71 ^{0}C **(Lit [19]).**

General procedure for the synthesis of Target sulfonamides (5a-e): Amines **(4a-e)** (0.0093M) were taken in methylene dichloride (20 mL) and the reaction mixture was cooled to 0^0 C, to this triethylamine (0.0279 M) was added under stirring at 0^0 C, to this mixture Tosyl chloride (**3**) (0.0111 M) was added and stirring was continued for 2 hour at room temperature, completion of the reaction was monitored by TLC. Reaction mixture was diluted with MDC and quenched with ice cold water. The organic layer was separated, washed with brine solution and dried over anhydrous sodium sulphate and concentrated to obtained crude products **(5a-e)** which were purified by column chromatography using Petroleum ether: Ethyl acetate (7:3) as eluent.

Antibacterial activity: Preliminary antibacterial screening [20] was performed by the agar diffusion method using paper disc. The sterilized (autoclaved at 120°C for 30 min), liquefied muellerhinton agar (40–50 °C) was inoculated (1 mL/100 mL of medium) with the suspension of the microorganism (matched to McFarland Barium sulphate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper discs impregnated with the test compounds (500 mg mL^{-1} in dimethyl sulphoxide) were placed on the solidified medium. The plates were refrigerated for 2 h at 4°C and then incubated at 37°C for 24 h. A

series of glass tubes containing different concentrations of the synthesized compounds (in dimethyl sulphoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism which contains 105 colony forming units per milliliter. One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of microorganism. The tubes were incubated at 37°C for 24 h. The turbidity produced in each tube was recorded by using a UV–visible spectrometer. The minimum inhibitory concentration (MIC /g mL^{-1}) was considered to be the lowest concentration which exhibited the same turbidity as the blank tube. The observed MICs (g mL^{-1}) are presented in table 2.

Molecular docking: The structures of the synthesized compounds were drawn using Chemdraw [21]. Ligand optimization was carried out using macro molecular force field (MMF) followed by energy minimization protocol [22, 23]. Several ligand conformations were generated based on bond energy, CHARM energy, dihedral energy, electrostatic energy, initial potential energy values. The drug likeliness was evaluated using the Lipinski rule of 5 via Lipinski drug filter protocol [24], these studies were performed using Discovery studio 3.5 (Accelrys). Dehydrosqualene synthase of *S. aureus* was considered as a major enzyme for the bacterium to survive inside the host cell [25]. In this study the 3-D crystal structure of dehydrosqualene synthase (PDB ID: 3ACX) complex with inhibitor, BPH-673, was retrieved from the protein data bank to study the binding mode of inhibitors. Prior to protein preparation, the inhibitor, BPH-673 and water molecules were deleted from the protein to obtain clean protein. The structure thus obtained was optimized classically using CHARMm force field implemented in the DS 3.5, minimized with conjugate gradient energy minimization protocol followed by convergence energy minimization (0.001 kcal/mole), that readied the structures for docking and simulations [26]. Active site residues Arg171, Asp48, Val133, Asn168, His18, Tyr129, Tyr248, and Tyr41 were selected for molecular docking studies [27]. For molecular docking studies, a flexible docking approach was employed using the Lead IT [28] software in which C(30) carotenoid dehydrosqualene synthase was considered as receptor protein. The docking results for receptor-ligand complex comprised intermolecular interaction energies, namely, hydrogen bonding and hydrophobic and electrostatic interaction. Receptor-ligand complex with least binding energy was used to infer the best binding compound. The best conformations were selected based on the least docking energy value [29, 30].

RESULTS AND DISCUSSION

Present work describes a convenient and efficient synthetic route to synthesize sulfonamide derivatives, we believe the procedure can be conveniently reproduced. The structures of the compounds **5a-e** were confirmed by its spectral studies. IR spectrum of compound **5a**, shows strong characteristic stretching frequency of $SONH_2$, C-N, C-S at 1334, 1277, 2362 cm^{-1} respectively. The ^{1}HNMR spectrum shows chemical shift at δ 7.95 which corresponds to aromatic protons of toluene nucleus, a doublet at δ 7.76 (J = 8.0 Hz) correspond to same aromatic toluene nucleus. The two doublets at δ 7.35 (J = 8.0 Hz) and (J = 8.0 Hz) corresponds to protons of aniline nucleus. Finally singlet at δ 2.35 is due to -CH_3 protons and NH proton resonates at 5.3ppm. The ^{13}C NMR spectral data showed the aromatic carbon signals at 136.70,134.02, 131.29, 131.3, 129.82, 122.05 and aliphatic carbons at 21.35ppm. Spectral interpretation confirms the assigned structure for **5a** and all the other derivatives **5b-e** were characterized similarly and are in good agreement with their assigned structures.

4-methyl-N-(4-methyl)benzene-1-sulfonamide (5a): Light yellow solid, MP-102^{o}C, IR (KBr): 1334 cm^{-1} (S=O str), 1277 cm^{-1} (>C=N str), 2362 cm^{-1} (>C-S str), 3028 cm^{-1} (Ar-H str), 2921cm^{-1} (-C-H str); ^{1}H NMR (500 MHz, $CDCl_3$, ppm):δ 2.35 (s, 6H, H-CH_3), 7.35 (d, J = 8.0 Hz, 2H, Ar-H), 6.91 (d, J = 8.0 Hz, 2H, Ar-H), 7.76 (d, J = 8.0 Hz, 2H, Ar-H), 7.95 (d, J = 8.0 Hz, 2H, Ar-H), 5.3 (s, 1H,N-H); **^{13}C**-NMR(500 MHz, $CDCl_3$, ppm): δ 136.70,134.02,131.29,131.3, 131.29,129.82, 122.05, 22.05, 21.35.

4-methyl-N-(pyridine-2yl)benzene-1-sulfonamide(5b): Creamy solid, MP-122^{o}C, IR (KBr): 1329 cm^{-1} (S=O str), 1256 cm^{-1} (>C=N str), 2328 cm^{-1} (>C-S str), 3086 cm^{-1} (Ar-H str), 2922 cm^{-1} (-C-H str); ^{1}H

NMR (500 MHz, $CDCl_3$, ppm): δ 2.34 (s, 3H, H-CH_3), 7.74 (d, J = 8.0 Hz, 2H, Ar-H), 7.55(t, J = 8.0 Hz,1H, Ar-H), 7.40 (d, J = 8.0 Hz, 2H, Ar-H) 7.20 (d, J = 8.0 Hz, 2H, Ar-H), 6.81 (d, J = 8.0 Hz, 2H, Ar-H) 6.63 (t, J = 8.0 Hz, 2H, Ar-H) 4.0 (s, 1H, N-H); **^{13}C**-NMR(500 MHz, $CDCl_3$, ppm): δ 146.72 , 139.79, 138.00,137.31,136.23, 129.65,128.30, 119.68, 109.57, 21.53.

N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-4-methylbenzene-1-sulfonamide (5c): White solid, MP-174^{o}C, IR (KBr):1327cm^{-1} (S=O str), 1299 cm^{-1} (>C=N str), 2339 cm^{-1} (>C-S str), 1672 cm^{-1} (>C=O str), 3041 cm^{-1} (Ar-H str), 2823 cm^{-1} (-C-H str); ^{1}H NMR (500 MHz, $CDCl_3$, ppm):δ d(1.12, 3H, CH-CH_3) 2.25 (s, 3H, H-CH_3), 3.1(s, 3H, N-CH_3), 3.25(q, 1H) 3.79 (d,1H, J = 8.0 Hz) 6.90 (t,1H, Ar-H) 7.21 (d, 2H, J = 8.0 Hz, Ar-H), 7.35(d,2H, J = 8.0 Hz, Ar-H)7.70 (d,2H, J = 8.0 Hz, Ar-H) 7.76 (d, 2H, J = 8.0 Hz, Ar-H), 6.21(s, 1H, N-H); **^{13}C**-NMR(500 MHz, $CDCl_3$, ppm): δ 161.81 , 136.31 , 134.49, 135.8 , 129.02, 127.53, 127.21, 125.29, 124.40, 35.08, 29.31, 21.72, 11.39**.**

N-tert-butyl-4-methylbenzene-1-sulfonamide (5d): Light brown solid MP-98^{o}C, IR (KBr): 1327 cm^{-1} (S=O str), 1200 cm^{-1} (>C=N str), 2340 cm^{-1} (>C-S str), 3266 cm^{-1} (Ar-H str), 2971cm^{-1} (-C-H str); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.4 (s, 3H, H-CH_3), 1.24 (s, 9H, H-CH_3), 7.80 (d, J = 8.0 Hz, 2H, Ar-H), 7.41 (d, J = 8.0 Hz, 2H, Ar-H), 5.0 (s, 1H, N-H); **^{13}C**-NMR(500 MHz, $CDCl_3$, ppm): δ 142.77,140.60, 129.46, 127.05, 54.51, 30.12, 21.47.

***N*-(2-hydroxyethyl)-4-methylbenzene-1-sulfonamide (5e):** Yellow liquid, IR (KBr): 1323 cm^{-1} (S=O str), 1215 cm^{-1} (>C=N str), 2335 cm^{-1} (>C-S str), 3273cm^{-1} (Ar-H str), 2935 cm^{-1} (-C-H str); ^{1}H NMR (500 MHz, $CDCl_3$ ppm): δ 2.35 (s, 3H, H-CH_3), 7.65 (d, J = 8.0 Hz, 2H, Ar-H), 7.23 (d, J = 8.0Hz, 2H, Ar-H), 3.5 (t, 2H, H-CH_2), 3.3 (t, 2H, H-CH_2), 6.0 (s, 1H, N-H), 3.1(s, 1H, O-H); **^{13}C**-NMR(500 MHz, $CDCl_3$, ppm): δ 140.60, 136.6, 129.3, 128.8, 60.90, 45.20, 21.36.

Antibacterial activity: In search of new antibacterial agents, all the newly synthesized compounds **(5a-e)** were screened for their in vitro antibacterial activity against *Staphylococcus aureus*, *Escherichia. coli* bacterial strains. The antibacterial activity was measured by minimum inhibitory concentration (MIC) using the Disc diffusion method. During the antibacterial screening, compounds 5b, 5c and 5e displayed significant activity against *Staphylococcus aureus* and *Escherichia coli*. The corresponding MIC values for compounds 5b, 5c and 5e against *Staphylococcus aureus* and *Escherichia coli* were 6.25, 5.77 and 5.33 µg ml^{-1} and values were comparable to the standard drug, ciprofloxacin. The results are tabulated in table 1.

Molecular docking: The molecular docking studies were carried out to understand the binding mode and mechanism of active inhibitors with the crystal structure of the C(30) carotenoid dehydrosqualene synthase (PDB ID: 3ACX). Docking energy data for all the synthesized compounds along with standard ciprofloxacin is included in table 1. Docking results show that ciprofloxacin had less docking energy compared with the new target compounds. The docking energy for all the compounds ranged from –5.89 to –11.49 kcal mol^{-1}. All the compounds could dock in the active site of dehydrosqualene synthase effectively. The binding mode of the most potent compounds 5b, 5c and 5e is shown in fig. 1. All the three compounds were involved in H-bonding with the active site residues Asp48, Tyr41, and His18 with the high docking energies indicating that those are directly involved in inhibiting the dehydrosqualene synthase. The results of docking presented in table 1. The binding modes of these compounds are discussed below.

Binding mode of compound 5b: Figure 1 shows the H-bond interactions with the active site residues of dehydrosqualene synthase, where O9 and N15 groups of compound 5b accepts the hydrogen bond interactions with the His18 (1.83 and 2.7 A°). The NH_2 group of Arg171 and OH group of Tyr248 donates the hydrogen bond with the O10 group of compound 5b (2.02 and 1.75 A°), whereas pi system of the compound 5b accepts the Hydrogen bond interaction with the OH group of Tyr41 (2.99 A°).

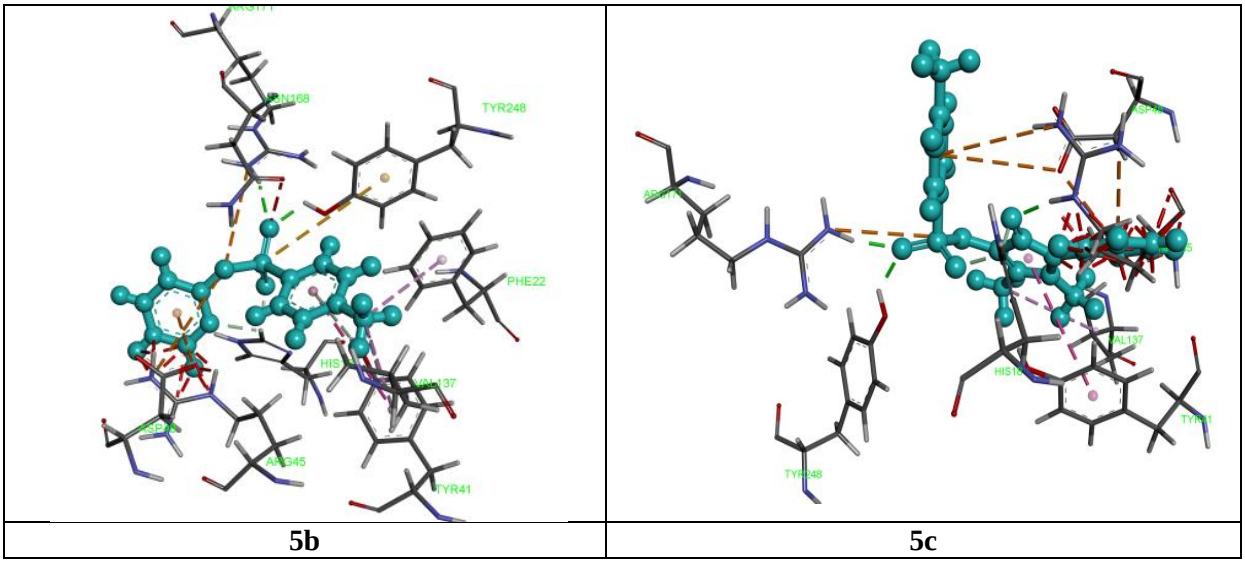
Binding mode of compound 5c: The NH1 group of His18 establishes bivalent H-bond interactions with the O10 and O18 groups of compound 5c. The NH and NH_2 group of Arg45 and Arg171 donates the H-bond with the O18 and O9 group of compound 5c. Another hydrogen bond interaction establishes between OH group of Tyr248 and O9 group of compound 5c.

Binding mode of compound 5e: The O9 group of compound 5e also forms bivalent H-bond interactions with NH_2 group of Arg171 and OH group of Tyr248, whereas OH group of main amino acid residue Tyr41 accepts the H-bond with the OH group of compound 5e.

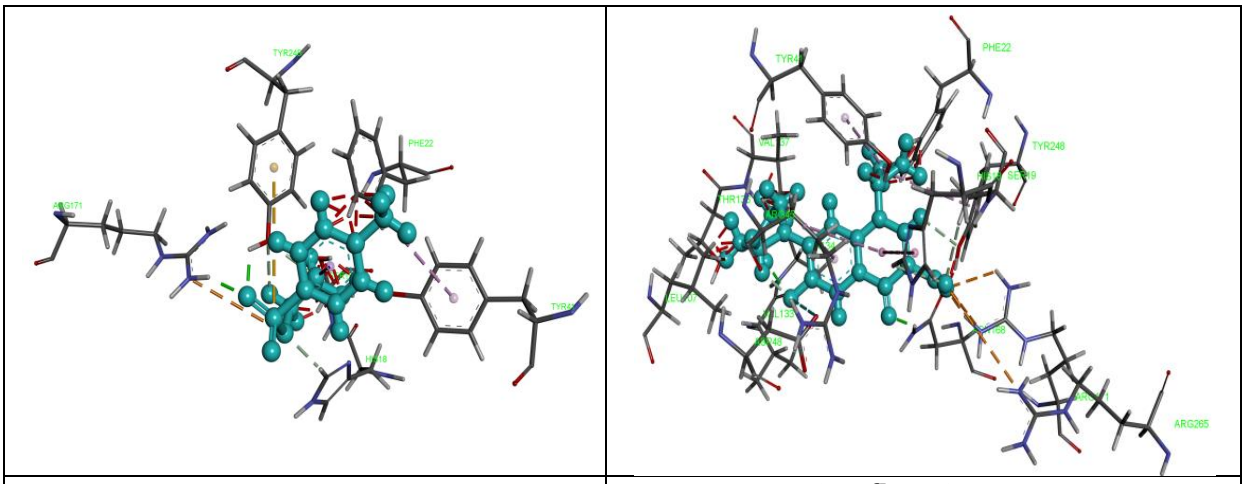
Binding mode of compound STD: The O2 group of ciprofloxacin accepts hydrogen from HD22 group of ASN168 forming monovalent conventional hydrogen bond, COO^- group of val133 forms hydrogen interaction with H41 group of ciprofloxacin, Finally another monovalent hydrogen bond forms between OG1 group of Thr136 and H42 group of standard ciprofloxacin.

Table 1: Docking output of the new compounds

Name	Docking score	interactions	Interacted amino acid residues
5a	-5.893	10	ARG171,ARG45,TYR248,HIS18,ASP48,TYR41,VAL137,ARG265
5b	-11.414	13	ARG45,ARG171,TYR248,HIS18,ASP48,TYR41,VAL137,PHE22
5c	-11.496	12	ARG171,ARG45,TYR248,HIS18,ASP48,TYR41,VAL137
5d	-6.91	11	ARG45,ARG171,ASN168,TYR248,HIS18,SER19,TYR41
5e	-11.476	10	ARG171,TYR248,TYR41,HIS18,TYR248
Ciprofloxacin	-9.21	18	ARG171,ARG265,ASN168,VAL133,THR136,HIS18,SER19
			TYR248,ALA134,ASP48,VAL133,TYR41,ARG45



5b **5c**



5e	**Ciprofloxacin**

Fig 1: Docking binding structures of active molecules (5b, 5c, 5e) and standard ciprofloxacin

APPLICATIONS

The antibacterial screening was carried out for all the compounds, among the tested compounds only 5b, 5c and 5e compounds emerged as most active at concentration level of 6.25 µg, 5.77 µg and 5.33 µg respectively. The standard drug is active at concentration level of **3.12** µg, remaining two compounds 5a and 5d shown poor antibacterial activity. Hence from the result it is concluded that compounds 5b, 5c and 5e are good antibacterial agents compared to standard ciprofloxacin against two strains of bacteria ***Staphylococcus aureus*** and ***Escherichia coli*** as shown in table-1.

Table:2 Antibacterial evaluation of 5a-eMinimum inhibitory concentrations (MIC)/ µg mL^{-1}

Compound	**(MIC)/ µg mL^{-1}**	
	Staphylococcus aureus	***Escherichia coli***
5a	>100	>100
5b	6.25	6.25
5c	5.77	5.77
5d	>100	>100
5e	5.33	5.33
DMF	-	-
Ciprofloxacin	**3.12**	**3.12**
Blank	-	-

Lower MIC values indicate higher antimicrobial activity
Bold values indicate the reference values using standard drugs

CONCLUSIONS

We have synthesized some novel sulfonamide derivatives and evaluated them for their in vitro antibacterial activity. Compounds 5b, 5c and 5e have shown significant antibacterial activity against S. aureus and E. coli (MIC 6.25, 5.77 and 5.33 µg mL^{-1} respectively) relative to ciprofloxacin. Thus, the above compounds can be considered as lead compounds for enhancing their activity and development of more potent antibacterial agents. Furthermore, molecular docking studies revealed the essential groups, which bind with the active site of the dehydrosqualene synthase.

ACKNOWLEDGEMENTS

The authors are thankful to Tumkur University and University College of Science for the laboratory facilities. Vivek Chandramohan, wish to thank the Management, Principal, Director and Head of the department of the Siddaganga Institute of Technology, Tumkur, Karnataka, India. The authors also appreciate KBITS for their support in providing them with the required computational resources for carrying out this work. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

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