



Received on 26 June 2019; received in revised form, 18 January 2020; accepted, 02 April 2020; published 01 May 2020

## SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME COMPOUNDS OF 1,8-NAPHTHYRIDINE-3-CARBOXAMIDE

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### Keywords:

1-ethyl-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide, antibacterial, <sup>1</sup>H NMR, MS

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**ABSTRACT:** Various nitrogenous heterocyclic compounds like 1, 8-naphthyridine-3-carboxamide are synthesized and studied for their wide range of biological activity. Versatile activity of this scaffold will increase interest from innovator on significance of their antibacterial activity and will influence their role in drug discovery. Synthesis and Antibacterial evaluation of ten derivatives of 1-ethyl-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide (SD-1 – SD-10) is discussed in this paper. Synthesis of novel 1, 8-naphthyridine-3-carboxamide derivatives starting from substituted pyridine with ethoxy-methylenemalonate ester by cyclo-condensation reaction affords 1,8-Naphthyridine-3-carboxylate at high temperature and at specific reaction condition. All 1, 8-naphthyridine-3-carboxylate compounds were treated with an excess of substituted aniline with mild reaction conditions. All the compounds obtained were purified by using the recrystallization method. Structure confirmation of synthesized compounds was accomplished on the basis of IR, <sup>1</sup>H NMR, and MS. The synthesized compounds were tested against bacterial strain, *Escherichia coli* (Gram-negative) *Staphylococcus aureus* (Gram-positive). Many of the compounds exhibited significant antibacterial profile.

**INTRODUCTION:** Infectious diseases caused by bacteria, which speak to a critical wellbeing risk all through the world, create problems for healing facilities like hospitals, cause increased mortality, and affect healthcare system<sup>1</sup>. *Staphylococcus epidermidis*, *S. aureus*, *Streptococcus pneumonia*, including *enterococci* like *Enterococcus faecalis*, *Enterococcus faecium* Gram-positive pathogen and Gram-negative include *Enterobacteriaceae* like *Escherichia coli* and *Klebsiella*, *Chlamydomphila pneumonia*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycoplasma pneumonia*, and *Acinetobacter* are the cause of disease<sup>2</sup>.

These bacteria are adding value to infectious diseases. The large mortality rate is associated with the very intense issue of emergence of an extensive spread of resistance to present therapy, and the cause behind it is extended, large, improper use of and also making abuse of antibacterial agent which was reported earlier. So, efforts to discover new antibacterial agent with high potent activity against active pathogens is needed<sup>3</sup>.

Fluoroquinolones are the broad class of synthetic antibacterial agent which was introduced in practice to treat urinary infections which were caused by Gram-negative bacteria in human being. The first synthetic quinolone is nalidixic acid, which was discovered in 1962 as an antimalarial by product and also active against Gram-negative bacterial infections. Recently, 4-quinolone, mostly fluoroquinolone, is common in use to treat bacterial infection caused at upper and lower respiratory, and some epidermal, bone, tissue infections also

	<b>QUICK RESPONSE CODE</b> <b>DOI:</b> 10.13040/IJPSR.0975-8232.11(5).2374-79
	This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a>
DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2374-79">http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2374-79</a>	

including pneumonia infections<sup>4</sup>. Along with this broad antibacterial activity, 4-quinolone shows different biological activities like anti-tubercular<sup>4</sup> anti-cancer, anti-HIV, anti-malarial, anti-hypertensive, etc.<sup>5</sup>

Here, a continuous attempt on structural modifications of 4-Quinolone and available therapy have guided us to discover 1, 8-Naphthyridine-3-carboxamide analog<sup>6</sup>. At N-1, C-5, C-6, C-7 positions of 1,8-naphthyridine substitution like alkyl, aryl or halo as well as nitro, were carried out. Substitution on C-3 help to increase the overall activity of the 1,8-naphthyridine and interpret SAR of 1, 8-Naphthyridine- 3- carboxamide. The formed carboxamide will provide interaction with respective enzyme and may give significant activity<sup>7</sup>.

**MATERIALS AND METHODS:** All chemicals used were procured from commercial sources such as Merck (India), Sigma-Aldrich and Loba Chemie. Veego VMP-D digital melting point apparatus was used to determine the melting point of synthesized compound and was uncorrected. The purity of synthesized compounds was assessed by thin layer chromatography (TLC). Prepared TLC silica plates 4 cm × 1 cm, “Silica Gel G 60, F 254” procured from Merck and were used for observing R<sub>f</sub> values

of all the compounds. TLC plates were visualized under UV visible light. Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded in KBr on “JASCO FTIR-4100” is reported in cm<sup>-1</sup>. Proton Nuclear Magnetic Resonance Spectroscopy (<sup>1</sup>H NMR) were recorded using DMSO solvent by “BRUKER AVANCE II 400 MHz NMR Spectrometer” (NIPER, Mohali, Punjab) trimethyl silane (TMS) as an internal standard and chemical shifts were given in parts per million (ppm).

### Experimental:

**General Chemistry:** Route for the synthesis of 1, 8-Naphthyridine are summarized in the scheme. The reaction of 2-aminopyridine with ethoxymethylene-malonate ester provided Schiff bases. For the cyclization of intermediate put it into refluxing diphenyl ether afforded 1,8-Naphthyridine-3-carboxylates. Formed 1, 8-Naphthyridine-3-carboxylate allow to react with suitable halogenoalkanes in the presence of base sodium hydride using solvent dry DMF and ethyl 1-alkyl-1, 8-naphthyridine-3-carboxylate was formed. Formed ethyl 1-alkyl-1, 8-naphthyridine-3-carboxylate was treated with an excess of substituted aniline by the One-Pot synthesis method to gave the carboxamide. The chemical structure of the afforded compounds was characterized by IR, <sup>1</sup>H NMR, Mass<sup>6,8,9</sup>.

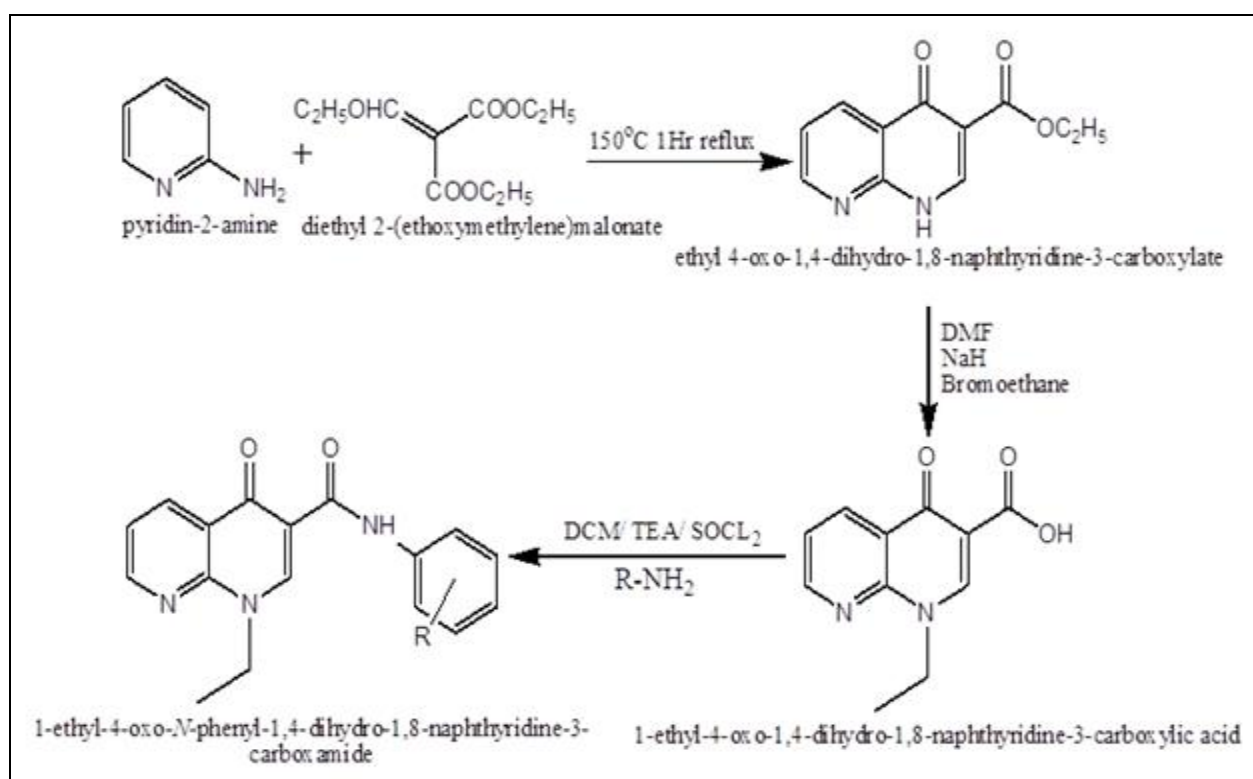


FIG. 1: SCHEME FOR SYNTHESIS

**Scheme of Synthesis:**

**Step 1: General procedure for Synthesis of ethyl 4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxylate:** Take 2-aminopyridine (100 mmol), and ethoxymethylenemalonic ester (110 mmol) in RBF and this mixture was stirred at RT for 10 min; further it was heated at 150 °C for 60 min. The reaction mixture was evaporated in a vacuum to remove residual ethanol and provide condensation products. This product was poured with a thin stream into stewing diphenyl ether (150 mL), and it was refluxed for 20 min. The oily solution formed was allowed to cool to room temperature and add petroleum ether (75 mL). The shiny yellow precipitate formed and collected by filtration and washed with petroleum ether. Washing led to complete removal of diphenyl ether. Compounds had very poor solubility in DMSO, so it was too difficult to characterize their chemical structure by <sup>1</sup>H NMR <sup>6,8</sup>.

**Step 2: General procedure for the synthesis of 1-ethyl-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxylic acid:** 1, 8-naphthyridine-3-carboxylate (5 mmol) and dry DMF (30 mL) take in RBF, and it was stirred at RT for 10 min, and then 60% NaH (15 mmol) and bromoethane (15 mmol) were added. Reaction completion was indicated by TLC. After reaction completion mixture was added into water (75 mL), and was extracted with ethyl acetate. The organic layer was made acidic by HCl and allowed to evaporate further residue formed was crystallized by using acetone. The obtained yellow solid dissolved in water and made basic with sodium bicarbonate, and the aqueous mixture extracted with ethyl acetate. The organic layer was washed with water, then dried over anhydrous sodium sulfate, filtered, and evaporated <sup>8</sup>.

**Step 3: General procedure for the synthesis of 1-ethyl-4-oxo-N-substituted phenyl-1, 4-dihydro-1, 8-naphthyridine-3-carboxamide:** 1-ethyl-4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxylic acid (5.0 mmol) and substituted anilines (5.0 mL) were added in Dichloromethane, Triethylamine and Thionyl Chloride this mixture was subjected to One-Pot synthesis. Reaction completion was checked by TLC monitoring. After completion, evaporate solvent by using a vacuum evaporator. Residual solid is partitioned between water and dichloromethane. The aqueous phase was extracted

with dichloromethane and the combined organic layers were washed with H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, evaporated <sup>9</sup>.

**RESULTS:**

**Synthesis of N-(2-chlorophenyl)-1-ethyl-4-oxo-1, 4-dihydro- 1, 8- naphthyridine- 3- carboxamide:** Starting from step 2 and 2-chloroaniline compound was isolated as yellow solid. 87%, 120 °C - 121 °C R<sub>f</sub>: 0.72 (*n*-Hexane: Ethyl Acetate-4:2 v/v). IR: 3350.71 (N-H stretching); 2924.52 (C-H Aromatic Stretching); 2855.10 (C-H Aliphatic stretching); 1743.33 (C=O Stretching); 1693.19 (C=O Amide Stretching); 1585.20 (C=C Aromatic stretching); 612.288 (C-Cl Stretching). <sup>1</sup>H NMR: 4.32-4.39 (*q*, 2H), 1.13-1.16 (*t*, 3H), 6.73-8.30 (*m*, 7H), 9.15 (*s*, H), 10.12 (*s*, H). MW: 327, ESI-MS: *m/z*, 328.79 [M+1]<sup>+</sup>.

**Synthesis of N-(3-chlorophenyl)-1-ethyl-4-oxo-1, 4- dihydro- 1, 8- naphthyridine- 3-carboxamide:** Starting from step 2 and 3-chloroaniline and compound isolated as yellow solid. 85% 125 °C - 126 °C; R<sub>f</sub>: 0.69 (*n*-Hexane: Ethyl Acetate-3:2 v/v). IR: 3191.61 (N-H stretching); 2898.49 (C-H Aromatic Stretching); 2842.56 (C-H Aliphatic stretching); 1682.59 (C=O Stretching); 1786.72 (C=O Stretching); 1585.20 (C=C Aromatic stretching); 616.14 (C-Cl Stretching). <sup>1</sup>H NMR: 4.37-4.39 (*q*, 2H), 1.15-1.20 (*t*, 3H), 6.75-8.25 (*m*, 7H), 9.12 (*s*, H) 10.08 (*s*, H). MW: 327, ESI-MS: *m/z*, 328.36 [M+1]<sup>+</sup>.

**Synthesis of N-(4-chlorophenyl)-1-ethyl-4-oxo-1, 4- dihydro- 1, 8-naphthyridine- 3- carboxamide:** Starting from step 2 and 4-chloroaniline and compound isolated as yellow solid. 88% 122 °C - 123 °C R<sub>f</sub>: 0.65 (*n*-Hexane: Ethyl Acetate-5:2 v/v). IR: 3181.97 (N-H stretching); 2922.59 (C-H Aromatic Stretching); 2850 (C-H Aliphatic stretching); 1744.30 (C=O Stretching); 1693.19 (C=O Stretching); 1484.92 (C=C Aromatic stretching), 617.109 (C-Cl Stretching). <sup>1</sup>H NMR: 4.28-4.30 (*q*, 2H), 1.20-1.21 (*t*, 3H), 6.42-8.27 (*m*, 7H), 9.02 (*s*, H), 9.72 (*s*, H). MW: 327, ESI-MS: *m/z*, 328.79 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-N-(2-fluorophenyl)-4-oxo-1, 4- dihydro- 1, 8- naphthyridine-3-carboxamide:** Starting from step 2 and 2-fluoroaniline and compound isolated as yellow solid. 80% 122 °C - 121 °C R<sub>f</sub>: 0.59 (*n*-Hexane: Ethyl Acetate-3:2); IR:

3148.22 (N-H stretching); 2925.48 (C-H Aromatic Stretching); 2854.13 (C-H Aliphatic stretching); 1739.48 (C=O Stretching); 1644.02 (C=O Stretching); 1495.53 (C=C Aromatic stretching); 936.306 (C-F Stretching). <sup>1</sup>H NMR: 4.32-4.38 (*q*, 2H), 1.23-1.26 (*t*, 3H), 6.72-8.10 (*m*, 7H), 9.19 (*s*, H), 10.52 (*s*, H). MW: 311, ESI-MS: *m/z*, 318.10 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-N-(4-fluorophenyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and 4-fluoroaniline and compound isolated as yellow solid. 74% 113 °C - 114 °C R<sub>f</sub>: 0.62 (n-Hexane: Ethyl Acetate-3:2; UV). IR: 3247.54 (N-H stretching); 3020.94 (C-H Aromatic Stretching); 2961.16 (C-H Aliphatic stretching); 1692.23 (C=O Stretching); 1657.52 (C=O Stretching); 1432.85 (C=C Aromatic stretching); 916.986 (C-F Stretching). <sup>1</sup>H NMR: 4.37-4.39 (*q*, 2H), 1.84-1.85 (*t*, 3H), 6.87-8.07 (*m*, 7H), 9.10 (*s*, H), 10.08 (*s*, H). MW: 311, ESI-MS: *m/z*, 312.31 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-4-oxo-N-(o-tolyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and o-toluidine and compound isolated as yellow solid. 64% 142°C - 143°C R<sub>f</sub>: 0.64 (n-Hexane: Ethyl Acetate-4:3). IR: 3238.86 (N-H stretching); 2922.59 (C-H Aromatic Stretching); 2857.02 (C-H Aliphatic stretching); 1753.94 (C=O Stretching); 1690 (C=O Stretching); 1520.6 (C=C Aromatic stretching); <sup>1</sup>H NMR: 4.27-4.32 (*q*, 2H), 1.17-1.20 (*t*, 3H), 6.96-8.06 (*m*, 7H), 9.12 (*s*, H), 2.56 (*s*, 3H), 10.03 (*s*, H). MW: 307, ESI-MS: *m/z*, 308.05 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-4-oxo-N-(m-tolyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and m-toluidine and compound isolated as yellow solid. 65% 150°C - 151°C R<sub>f</sub>: 0.72 (n-Hexane: Ethyl Acetate-4:2; UV). IR: 3204.15 (N-H stretching); 2940.91 (C-H Aromatic Stretching); 2883.06 (C-H Aliphatic stretching); 1763.58 (C=O Stretching); 1694.16 (C=O Stretching); 1580.38 (C=C Aromatic stretching). <sup>1</sup>H NMR: 4.13-4.17 (*q*, 2H), 1.14-1.15 (*t*, 3H), 6.99-8.20 (*m*, 7H), 9.22 (*s*, H), 10.09 (*s*, H), 2.19 (*s*, 3H). MW: 307, ESI-MS: *m/z*, 308.44 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-4-oxo-N-(p-tolyl)-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and p-toluidine and compound

isolated as yellow solid. 68% 147 °C - 148 °C R<sub>f</sub>: 0.67 (n-Hexane: Ethyl Acetate-3:2; UV). IR: 3247.54 (N-H stretching); 3016.12 (C-H Aromatic Stretching); 2909.09 (C-H Aliphatic stretching); 1664.27 (C=O Stretching); 1494.56 (C=C Aromatic stretching). <sup>1</sup>H NMR: 4.30-4.34 (*q*, 2H), 1.00-1.01 (*t*, 3H), 6.45-7.47 (*m*, 7H), 9.02 (*s*, H), 10.01 (*s*, H), 2.56 (*s*, 3H). MW: 307, ESI-MS: *m/z*, 308.89 [M+1]<sup>+</sup>.

**Synthesis of 1-ethyl-N-(2-ethylphenyl)-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and 2-ethylaniline and compound isolated as yellow solid. 78% 136 °C - 137 °C. R<sub>f</sub>: 0.56 (n-Hexane: Ethyl Acetate-4:2; UV). IR: 3279.36 (N-H stretching); 2983.34 (C-H Aromatic Stretching); 1753.94 (C=O Stretching); 1680 (C=O Stretching); 1484.92 (C=C Aromatic stretching). <sup>1</sup>H NMR: 4.07-4.13 (*q*, 2H), 1.04-1.17 (*t*, 3H), 6.51-7.93 (*m*, 7H), 9.12 (*s*, H), 10.03 (*s*, H), 2.28-2.32 (*q*, 2H). MW: 321, ESI-MS: *m/z*, 322.09 [M+1]<sup>+</sup>.

**Synthesis of N-(2,5-dimethylphenyl)-1-ethyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide:**

Starting from step 2 and 2,5-dimethylaniline and compound isolated as yellow solid. 75% 124°C - 125 °C R<sub>f</sub>: 0.61 (n-Hexane: Ethyl Acetate-6:4; UV). IR: 3177.15 (N-H stretching); 2921.63 (C-H Aromatic Stretching); 2840.63 (C-H Aliphatic stretching); 1714.41 (C=O Stretching); 1660.41 (C=O Stretching); 1453.10 (C=C Aromatic stretching). <sup>1</sup>H NMR: 4.17-4.21 (*q*, 2H), 1.24-1.25 (*t*, 3H), 6.21-7.91 (*m*, 7H), 9.12 (*s*, H), 10.13 (*s*, H), 2.22 (*s*, 3H), 2.24 (*s*, 3H). MW: 321, ESI-MS: *m/z*, 322.22 [M+1]<sup>+</sup>.

**In-vitro Antibacterial Screening:**

The antibacterial screening of the synthesized compounds was done by well plate method. The synthesized compounds were tested against bacterial strain, *Escherichia coli* (Gram-negative) *Staphylococcus aureus* (Gram-positive). Stock solution of compound was prepared in Dimethyl Sulphoxide (DMSO). Freshly prepared suspension of test organism in 1ml of sterile normal saline solution and was standardized to 10<sup>7</sup> CFU/mL. Agar plate was prepared using nutrient agar (Hi-Media). 100µL suspension of organism was seeded on culture plates. Then in each agar plate 4 cavities/well were prepared using a sterilized cork borer.

100  $\mu$ L of each dilution (50, 75, and 100  $\mu$ g/mL) of compounds were transferred to the wells. Standard drug ciprofloxacin was employed at a concentration of 50  $\mu$ g/mL. Control was maintained for each bacterial strain where pure solvent DMSO was

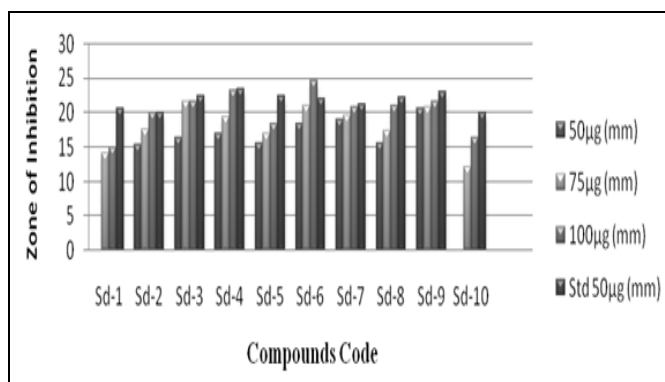
inoculated into the well. The plates were incubated at 37  $^{\circ}$ C, and zones of inhibition were measured at the end of 24 h. Standard drug Ciprofloxacin used as reference<sup>10</sup>.

**TABLE 1: IN-VITRO ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST *ESCHERICHIA COLI***

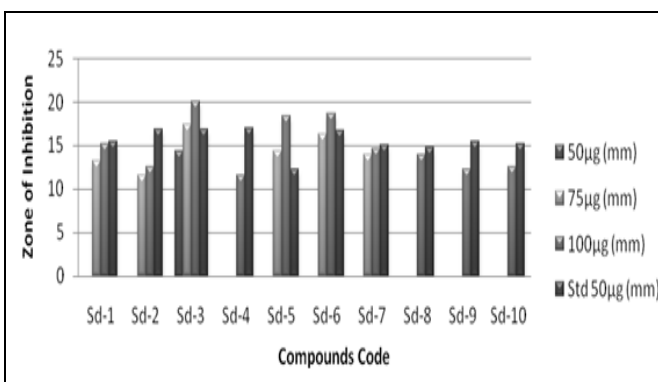
S. no.	Compound code	Zone of Inhibition (mm)			
		50 $\mu$ g	75 $\mu$ g	100 $\mu$ g	Ciprofloxacin std 50 $\mu$ g
1	SD-1	NI	14.2	14.9	20.5
2	SD-2	15.4	17.6	20	20
3	SD-3	16.4	21.7	21.7	22.5
4	SD-4	17	19.4	23.2	23.4
5	SD-5	15.6	17	18.3	22.5
6	SD-6	18.4	21	24.6	22
7	SD-7	18.9	19.6	20.8	21.1
8	SD-8	15.5	17.4	21	22.2
9	SD-9	20.6	20.7	21.6	23
10	SD-10	NI	12.1	16.3	20

**TABLE 2: IN-VITRO ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST *S. AUREUS***

S. no.	Compound code	Zone of Inhibition (mm)			
		50 $\mu$ g	75 $\mu$ g	100 $\mu$ g	Ciprofloxacin std 50 $\mu$ g
1	SD-1	NI	13.2	15.2	15.4
2	SD-2	NI	11.5	12.5	16.9
3	SD-3	14.3	17.4	20	16.9
4	SD-4	NI	NI	11.6	17
5	SD-5	NI	14.3	18.4	12.3
6	SD-6	NI	16.3	18.7	16.7
7	SD-7	NI	13.9	14.6	15.1
8	SD-8	NI	NI	14	14.8
9	SD-9	NI	NI	12.2	15.4
10	SD-10	NI	NI	12.6	15.2



**FIG. 2: ZONE OF INHIBITION OF SYNTHESIZED COMPOUNDS AGAINST *E. COLI***



**FIG. 3: ZONE OF INHIBITION OF SYNTHESIZED COMPOUNDS AGAINST *S. AUREUS***

**DISCUSSION:** The IR spectrum of compounds showed peaks for N-H stretching for amide in between 3000-3300  $\text{cm}^{-1}$ , C=O amide stretching is obtained at 1675-1700  $\text{cm}^{-1}$ , C-Cl, and C-F Stretching obtained between 600-650 and 900-950 respectively.  $^1\text{H}$  NMR spectrum showed not only the absence of -OH proton but also the presence of singlet at 10-10.3 for -NH proton. The mass spectrum showed (M+1) fragmentation base peak

with respect to the molecular weight of each molecule. Hence all compounds were synthesized in an accurate manner. The compounds synthesized were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, by the well-plate method. Ciprofloxacin was used as a standard for antibacterial activity, and synthesized compounds showed moderate activity.

**CONCLUSION:** All the compounds were accurately synthesized characterized and were evaluated for inhibition of gram-positive and gram-negative bacteria. Compounds SD-1, SD-3, SD-5, SD-6 shown significant activity against *S. aureus* (Gram-positive), for *E. coli* (Gram-negative) SD-3, SD-6, SD-7, SD-9 were significantly active at the concentration of 100 µg respectively. From the comparative study of analogs with quinolone in the active site of topoisomerase II, synthetic studies, and preliminary antibacterial evaluation, it can be suggested that further modification or increasing the bulkiness at carbon number three would further improve the selectivity of compounds towards topoisomerase II.

**ACKNOWLEDGEMENT:** The authors are thankful to Poona College of Pharmacy, Pune for providing the necessary laboratory facilities to carry out the study.

**CONFLICTS OF INTEREST:** The author declared that they have no conflicts of interest.

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#### How to cite this article:

Suryawanshi MR and Dupade DD: Synthesis and antibacterial activity of some compounds of 1,8-naphthyridine-3-carboxamide. *Int J Pharm Sci & Res* 2020; 11(5): 2374-79. doi: 10.13040/IJPSR.0975-8232.11(5).2374-79.

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