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STUDY OF ANTIBACTERIAL ACTIVITY OF 7-SUBSTITUTED 1-CYCLOPROPYL-6 -FLUORO-1, 4-DIHYDRO-8-METHOXY-4-OXOQUINOLINE-3-CARBOXAMIDE DERIVATIVES

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ABSTRACT

Here in we report synthesis and antibacterial screening of fourteen novels 7-substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8 methoxy-4-oxoquinoline-3-carboxamide derivatives by substituting fluoro group at C-7 position with heterocyclic amine and substituted heterocyclic amine. All these derivatives were screened for antibacterial against *P. aeruginosa* and *Bacillus subtilis* pathogens and found encouraging result.

KEYWORDS: Antibacterial activity, Quinoline-3-carboxamide, Gatifloxacin, Ciprofloxacin.

INTRODUCTION

The fluoroquinolones are totally synthetic broad spectrum antibacterial agents[1]. Nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8 naphthyridine-3-carboxylic acid) was first drug launched in 1960 as quinolones class of antibiotics drug[2-5]. Initial quinolone drugs such as nalidixic acid[4-6], pipemidic acid, piromidic acid and cinoxacin[4] were useful for urinary tract infections caused by enteric bacteria and to treat bacterial enteritis. Unfortunately, bacteria could develop a rapid resistance to it.The activity of the quinolone drug was further enhanced by doing certain manipulation at N-1, C-5, C-6, C-7, C-8 position of their respective basic molecules.This had led to the synthesis of thousands of compounds and few of these compounds are Norfloxacin, Ciprofloxacin, flumequine, garenoxacin, temafloxacin, sparfloxacine, ofloxacine, levofloxacin, grepafloxacin, clinafloxacin, gatifloxacine, moxifloxacin, enoxacin etc.However in the last few years due to emerging of resistant bacteria has reduced the efficiency of antibiotics due to their excessive use and maltreatment[7-9]. This rapid emergence of drug resistance pathogens has now become a serious public health problem[10-12]. So new classes having effective antimicrobial agents against vancomycin-resistant Staphylococcus aureus (VRSA), methicillin-resistant Staphylococcus aureus (MRSA) and Klebsiella pneumoniae infections are urgently required[13]. In view of these and in continuation of our earlier work on the synthesis of biologically active heterocyclic compounds, we report synthesis of novel 7-substituted-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide derivatives[17-18] with wide spectrum of pharmaceutical application.

MATERIALS AND METHODS

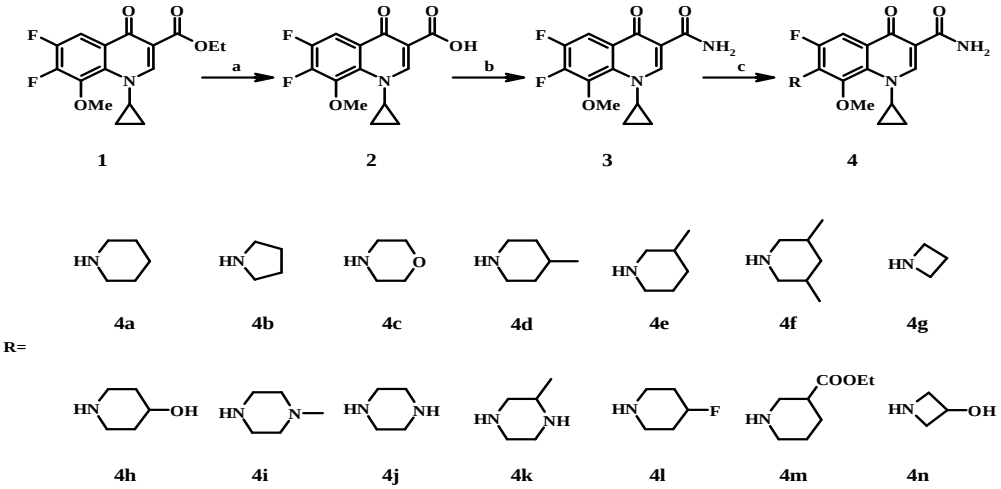
All the raw materials used for synthesis of desired products were obtained from commercial suppliers and was purified as per requirement. The intermediate ethyl 1-cyclopropyl-6,7-difluoro1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate is commercially available and synthesis of this intermediate is also reported in literature. Mass spectra were recorded on 'LCMS-Qp2010s' instrument by direct injection method. Nuclear Magnetic Resonance spectra (^{1}HNMR) were recorded on Bruker advance spectrometer (400 MHz) using DMSO-d_6 or $CDCl_3$ solvent and Tetramethylsilane used as an internal standard. Chemical shift (δ) are reported in parts per million. Reactions were monitored and its purity was checked by Merck pre-coated plate (silica gel 60 F254) Thin Layer Chromatography was visualized with UV light.

Melting points were determined in open capillary tube and are uncorrected.

RESULT AND DISCUSSIONS

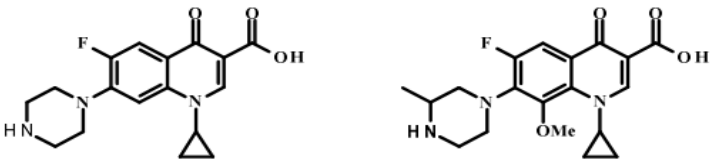
The synthesis of 7-cycloamino substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8-methoxy-4oxoquinoline-3-carboxamide derivatives **4a-n** are presented in Scheme-I.

The synthesis of compound **4a-n** was carried out by hydrolysis of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8methoxy-4-oxoquinoline-3-carboxylate 1 with lithium hydroxide in tetrahydrofuran and water at lower temperature. Reaction of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid **2** with ammonium chloride in presence of coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and1-Hydroxybenzotriazole result in the formation of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide **3**. Compound 4a-4n were synthesized by substituting fluoro group at C-7 position of compound **3** with different cyclo amine and cyclo amine having different hetero atoms or substituted group on it. The structures of the synthesized compounds were confirmed by ^{1}H NMR and mass spectral data. Synthesis of compounds **4a-4n** in **Scheme 1** is reported[17, 18].



(a) LiOH, THF, H_2O, (b) DMF, EDAPC, HOBt, DIPEA, NH_4Cl; (c) RH, DMSO or DMF, Base, 80°C. R= cyclo amine or cyclo amine with different hetero atoms or group.

Scheme. 1: Synthesis of target compound (4a-4n).



Procedure

Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (2)

To the suspension of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (1) (15.4mmol) in THF (50 ml) was added the lithium hydroxide (16.94mmol) solution (25ml water) drop wise at 0°C in 30 minutes. Reaction mass was stirred at room temperature for 4 hour. Solvent was removed under the reduced pressure at 35°C. Cool the reaction mass to 0°C and pH was adjusted (between 5 to 6) by 2 N HCl solution. Reaction mass was filtered and obtained cake was washed with water (3 x 20ml) and dried under reduced pressure at 35°C. Compound (VII) obtained yield 85%; mp 188-190°C; ^{1}H NMR ($CDCl_3$) (400 MHz) δ: 14.39 (s, 1H), 8.875 (s, 1H), 8.075-8.029 (t, 1H, J= 8.4 Hz), 4.151 (s, 3H), 4-145-4.127(m, 1H), 1.303-1.284 (m, 2H), 1.144-1.129 (m, 2H); MS (ESI) m/z 296 (M^{+1}).

Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (3)

To the suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid **(2)** (16.9mmol) in N,N-Dimethylformamide (50mL) were added 1-(3Dimethylaminopropyl)-3-ethylcarbodiimide HCl (25.4mmol) and 1-Hydroxybenzotriazole (18.6 mmol) at 0°C and stirred for 1h. Ammonium chloride (119mmol) and N,N-Diisopropylethylamine (169mmol) were added, After stirring the reaction mixture for 1h at 0°C, allow the mixture to stir at room temperature for 16h, the reaction was quenched to 500mL chilled water, filter and wash the cake with 50mL water. Dry the solid mass under reduced pressure at 40°C. The obtained crude product was further purified and dried. Yield 63%; mp >220 °C; ^{1}H NMR (DMSO-d6, 400 MHz) δ: 8.974 (s, 1H), 8.710 (s, 1H), 7.939 (t, 1H), 7.584 (s, 1H), 4.145-4.111 (m, 1H), 4.067 (s, 3H), 1.178-1.076 (m, 4H), MS (ESI): m/z 295 (M^{+1}).

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4oxoquinoline-3-carboxamide (4a-4k).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3carboxamide **(3)** (1.53mmol) in dimethylsulfoxide (2mL) were heterocyclic amine (3.3mmole) was added. Reaction mass stirred at 80°C.Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4oxoquinoline-3-carboxamide (4l & 4n).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3carboxamide **(3)** (1.53mmol) in N,N-Dimethylformamide (5mL) were heterocyclic amine (1.681 mmole), and N,N-Diisopropylethylamine (7.31mmol) was added. Reaction mass stirred at 80°C. Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.

Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4oxoquinoline-3-carboxamide (4m).: To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3carboxamide **(3)** (1.53mmol) in N,N Dimethylformamide (5ml) were heterocyclic amine (1.688 mmole), and cesium carbonate (Cs_2CO_3) (1.688mmol) was added. Reaction mass was at 80°C.

Concentrate the reaction mass under reduced pressure. Add ice water and stir the reaction mass for 1h. Reaction mass was filtered and dried.

Preparation of compounds (4a-n)

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(piperidin-1-yl)quinoline-3-carboxamide (4a)

A off white colour solid isolated was crystallized from methanol: yield 41%; mp 224-226°C;^{1}H NMR (DMSO-D_6)(400 MHz) δ: 9.104 (s, 1H,), 8.653 (s, 1H), 7.682 (d, 1H, J=12.4 Hz), 7.471 (s,1H), 4.087-4.067 (m, 1H), 3.762 (s, 3H), 3.255 (m,4H), 1.659 (m, 6H), 1.0925 (d, 2H, J= 8 Hz), 0.933 (m, 2H); MS (ESI) m/z 360 (M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(pyrrolidin-1-yl)quinoline-3-carboxamide (4b)

A off white colour solid isolated was crystallized from methanol: yield 75%; mp 220-222°C; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 9.146 (s,1H), 8.594 (s,1H), 7.594 (d,1H, J= 14.4 Hz), 7.409 (s,1H), 4.048-4.022 (m,1H),3.527-3.521 (m, 4H), 3.508 (s, 3H), 1.901 (s,4H), 1.062-1.046 (d, 2H, J= 6.4Hz), 0.889 (m, 2H); MS (ESI) m/z 346(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-morpholino-4-oxoquinoline-3-carboxamide (4c).: A off white colour solid isolated was crystallized from methanol; yield 67%; mp 190-192°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ:9.57 (s, 1H), 8.844 (s, 1H), 7.895 (d, 1H, J=12.8 Hz), 5.664 (s, 1H), 3.959-3.950 (m, 1H), 3.942-3.850 (m,4H), 3.805 (s, 3H), 3.400 (m, 4H), 1.179-1.1444 (m, 2H), 0.957-0.950 (m, 2H); MS (ESI) m/z 362(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(4-methylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (4d).: A off white colour solid isolated was crystallized from methanol; yield 61%; mp 218-220°C;^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.636(s, 1H), 8.829 (s, 1H), 7.848 (d, 1H, J= 12.4Hz), 5.647 (s, 1H), 3.978-3.943 (s, 1H), 3.757 (s, 3H), 3.461-3.196 (m, 2H), 3.166 (t, 2H, J= 12 Hz), 1.746-1.719 (m, 2H), 1.436-1.366 (m, 3H), 1.179-1.128 (m, 2H), 0.995 (d, 3H), 0.961-0.935(m, 2H); MS (ESI) m/z 374(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (4e).: A off white colour solid isolated was crystallized from methanol; yield 46%; mp >230°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ:9.637 (s, 1H), 8.829 (s, 1H), 7.860-7.834(d, 1H, J= 10.4Hz), 5.654 (s, 1H), 3.980-3.945(m, 1H), 3.747 (s, 3H), 3.448-3.385(m, 2H), 3.121-3.090 (m, 1H), 2.809-2.748 (m, 1H), 1.894-1.719 (m, 4H), 1.181-1.105 (m, 3H), 0.979-0.928 (m, 5H), MS (ESI) m/z 374(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3,5-dimethylpiperidin-1-yl)-4-oxoquinoline-3-carboxamide (4f).: A off white colour solid isolated was crystallized from methanol; yield 65 %; mp >230°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.631 (s, 1H), 8.828 (s, 1H), 7.848 (d, 1H, J=12.4 Hz), 5.658(s, 1H), 3.9833.947 (m, 1H), 3.729(s, 3H), 3.400-3.370 (m, 2H), 2.703-2.640 (m, 2H), 1.880-1.831 (m, 2H), 1.169-1.134 (m, 2H), 0.975-0.960 (m, 2H), 0.926- 0.910 (d, 6H), 0.786-0.751 (m, 2H); MS (ESI) m/z 388(M^{+1}).

7-(azetidin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (4g)

A off white colour solid isolated was crystallized from methanol; yield 79 %; mp >230°C;^{1}H NMR ($CDCl_3$) (400 MHz) δ: 9.693 (s,1H), 8.771 (s, 1H), 7.794-7.761 (d, 1H, J=13.2Hz), 5.642 (s, 1H), 4.358-4.313 (m, 4H), 3.897-3.860 (m, 1H), 3.571 (s, 3H), 2.430-2.355 (m, 2H), 1.150-1.099 (m, 2H), 0.985-0.960 (m, 2H), MS (ESI) m/z 332(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-7-(4-hydroxypiperidin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxamide (4h).: A off white colour solid isolated was crystallized from methanol; yield 70 %; mp 224-226°C;^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.611 (1H, s),8.839 (s, 1H), 7.884-7.853 (d, 1H, J=12.4Hz), 5.664 (s, 1H), 3.979-3.914 (m, 2H), 3.775 (s, 3H), 3.568-3.536 (m, 2H), 3.264-3.208 (m, 2H), 2.071-2.030 (m, 2H), 1.751-1.760 (m, 2H),1.170-1.135(m, 2H), 0.98-0.956 (m, 2H); MS (ESI) m/z 376(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxamide (4i).: A off white colour solid isolated was crystallized from methanol; yield 66 %; mp >230°C;^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.598 (s, 1H),8.835 (s, 1H), 7.881-7.850 (d, 1H, J=12.4 Hz), 5.654 (s, 1H), 3.965-3.939 (m, 1H), 3.754 (s, 3H), 3.421 (m, 4H), 2.612-2.579 (m, 4H), 2.383 (s, 3H), 1.160-1.142(m, 2H), 0.954 (m, 2H); MS (ESI) m/z 375(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (4j)
A off white colour solid isolated was crystallized from methanol; yield 42 %; mp 192-194°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.5805 (s, 1H),8.847 (s, 1H), 7.907-7.876(d, 1H, J=12.4Hz), 5.706 (s, 1H), 3.969-3.934 (m, 1H), 3.790 (s, 3H), 3.470-3.459 (m, 2H),3.165-3.141 (m, 2H), 2.409-2.318 (m, 4H), 1.255-0.938 (m, 4H);MS (ESI) m/z 361(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxamide (4k).: A off white colour solid isolated was crystallized from methanol; yield 57 %; mp 188-190°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.600 (s, 1H), 8.847 (s, 1H), 7.903-7.872(d, 1H, J=12.4Hz), 5.740 (s, 1H), 3.965-3.947 (m, 1H), 3.773 (s, 3H), 3.380 (s, 1H), 3.143 (m, 3H), 2.418-2.321 (m, 4H), 1.228-1.213(m, 3H), 1.175-0.896 (m, 4H); MS (ESI) m/z 375(M^{+1}).

1-cyclopropyl-6-fluoro-7-(4-fluoropiperidin-1-yl)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (4l).: yield 21 %; mp 188-190°C; ^{1}H NMR (DMSO-D_6) (400 MHz) δ: 8.984(s, 1H), 7.989 (s, 1H), 7.6847.653 (d, 1H, J= 12.4 Hz), 7.584(s, 1H), 4.972-4.955 (m, 1H), 4.853-4.843(m, 1H), 3.495-3.443 (m, 2H), 3.280-3.213(m, 2H), 3.001 (s, 3H), 2.080-2.001(m, 1H), 1.880-1.688(m, 3H), 1.143-1.051 (m, 2H), 0.943 (m, 2H); MS (ESI) m/z 378(M^{+1}).

Ethyl 1-(3-carbamoyl-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinolin-7-yl)piperidine-3-carboxylate (4m).: yield 20 %; mp 216-218°C; ^{1}H NMR ($CDCl_3$)(400 MHz) δ: 9.600 (s, 1H), 8.839 (s, 1H), 7.8887.857(d, 1H, J=12.4Hz), 5.655 (s, 1H), 4.339-4.323 (m, 2H), 4.175-4.129 (m, 1H), 4.022-3.948 (m, 2H), 3.749 (s, 3H), 3.702-3.637(m, 2H), 3.383-3.364(m, 2H), 3.220-3.194(m, 1H), 2.721 (m, 1H), 2.128 (m, 1H), 1.870-1.718 (m, 3H), 1.214-1.165 (m, 2H), 0.978-0.955 (m, 2H); MS (ESI) m/z 432(M^{+1}).

1-cyclopropyl-6-fluoro-1,4-dihydro-7-(3-hydroxyazetidin-1-yl)-8-methoxy-4-oxoquinoline-3-carboxamide (4n).: A off white colour solid isolated was crystallized from methanol; yield 44 %; mp 216-218°C; (M^{+1} 348),^{1}H NMR (DMSO-D_6) (400 MHz) δ: 9.136(s, 1H), 8.569 (s, 1H), 7.604-7.571 (d, 1H, J= 13.2Hz), 7.404(s, 1H), 5.400 (s, 1H), 4.491-4.473(m, 3H), 3.971(m, 3H), 3.540 (s, 3H), 1.063-1.046 (m, 2H), 0.899 (m, 2H), MS (ESI) m/z 348(M^{+1}).

Antimicrobial susceptibility testing by disc diffusion assay: Various bacterial strains such *as Bacillus subtilis* (NCIM 2250) and *Pseudomonas aeruginosa* (NCIM 2036) were used as test microorganism to evaluate the antimicrobial activity of newly synthesized compounds in **Table-1**. Pure culture of test bacterial strain was picked with a loop, and the growth was transferred into a tube containing 5 ml of a nutrient broth medium, while pure culture of test fungal strain was transferred into a tube containing 5 ml of a MGYP medium. The broth culture was incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This result in a suspension contains 2 x 10^8 CFU/ml of microbial cells.

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The surface of a nutrient agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking several times, rotating the plate approximately 60°C each time to ensure an even distribution of inoculum.

Stock solution [1000 microgram per ml] of each newly synthesize compounds were prepared in dimethylsulfoxide (DMSO). The sterile discs of 6 mm diameter were used in this assay. The disc diffusion assay was carried out by taking concentration 100 microorganism per disc. The discs immersed with compounds were dispensed onto the surface of the inoculated agar plate. Also, Ciprofloxacin (10 microgram/disk, Amphotericin-B (100 units/disk) [Hi-media, disc diameter 6 mm] moistened with DMSO were placed on agar plate as standard. Each disc was pressed down to ensure complete contact with the agar surface. The plates were placed in a refrigerator at to 8°C for 30 minutes after the discs are applied. Then the plates were incubated in incubator at 37°C for 24 hours.

Antibacterial activity of newly synthesized 7-substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide derivatives was evaluated by measuring the zone of growth inhibition against the tested bacteria. The antibacterial activity of the compounds was compared with ciprofloxacin as standard. The results were summarized in **Table 1**.

Table-1: Antibacterial activity of intermediates and compounds 4a-4n & 2-2a.

Compound	Gram negative bacteria *P. aeruginosa*	Gram positive bacteria *Bacillus subtilis*
4a	-	12.11
4b	-	11.23
4c	-	18.23
4d	-	9.56
4e	-	9.65
4f	-	8.11
4g	-	12.45
4h	-	14.23
4i	22.23	22.23
4j	22.36	17.89
4k	20.11	15.66
4l	10.78	20.11
4m	9.88	19.85
4n	8.90	18.23
3	14.22	10.12
2	18.18	14.56
2a	33.30	30.23
5	27.88	20.30

Diameter in mm calculated by Vernier Caliper '-' means no zone of inhibition, NA Not applicable, **2a** (Gatifloxacin) and **5** (Ciprofloxacin).

The screening result of antibacterial activity of compounds **4 a-n**, **3**, **2** & **2a** compound against *Bacillus subtilis* (Gram-positive bacteria) and *P. aeruginosa* (Gram-negative bacteria) is tabulated in **Table-1**.Ciprofloxacin used as a reference standard. Compound **3** show less activity then compound **2** against *Bacillus subtilis and P. aeruginosa* due to replacement carboxylic acid group with carboxamide group at C-3 position. The activity of 4-oxy-3-carboxamide compound **3** was tried to increase by substituting C-7 fluoro group with different cyclo amine or cyclo amine with different hetero atoms or group on it. It was observed that compounds **4 (a-h)** having pyrrolidine, piperidine, piperidine ring with methyl group at different position on it, morpholine or 4-hydroxyl piperidine or azitidine at C-7 position shows nil activity against *P. aeruginosa* (Gram-negative bacteria) but compounds **4 (a-b)** & **4 (d-h)** shows moderate activity against *Bacillus subtilis* (Gram-positive bacteria) except **4c** in which morpholine group is at C-7 position show good activity. Compounds **4i-k** having piperazine or substituted piperazine with methyl group on nitrogen or carbon atom shows good activity against *P. aeruginosa* and *Bacillus subtilis*. Compounds **4l-n** shows moderate activity against *P. aeruginosa* and shows good activity against *Bacillus subtilis*. Based on the screening data in **Table-1** suggest that further modification at C-7 position of compound **3** may lead to a promising antibacterial drug candidate.

Antibacterial studies

Preparation of test compound stocks

20.48 mg of test compound along with standard drug was weighed on analytical balance and dissolved completely in solvent and stored at 2-8°C till further use.

Preparation of inoculum

A loop from agar plate of overnight grown bacterial cultures was taken in media and suspended well by vortex mix and incubated at 35-37°C till turbidity of culture reaches equal or greater than 0.5 McFarland standards by adjusting absorbance between 0.08-0.1 at 625nm wavelength. 0.1mL from above suspension was taken and diluted in 9.9mL of broth.

MIC determination

Stock solutions of test and standard drug was removed from cold condition and brought at room temperature for further dilutions as per below table. Add 1mL of broth dilution to 1mL of the prepared inoculum suspension and incubate at 35-37°C for 16-20 hour.

Stock solution concentration (mg/L)	Volume of stock solution (mL)	Volume broth (mL)	Concentration obtained (mg/mL)
20480	1	9	2048
2048	1	1	1024
2048	1	3	512
2048	1	7	256
512	1	1	128
512	1	3	64
512	1	7	32
256	1	1	16
256	1	3	8
256	1	7	4
128	1	1	2
128	1	3	1
128	1	7	0.5
64	1	1	0.25
64	1	3	0.125
64	1	7	0.0625

Table. 2: Minimum inhibitory concentration (MIC) microgram/mL (Double dilution method).

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
4c	16	>1024	512	>1024
4i	8	8	4	4
4j	16	16	8	8
4k	32	32	8	8
4l	4	8	128	128
4m	8	8	64	128
4n	8	8	64	128
2	64	64	16	16
3	128	128	128	128

Minimum inhibition concentration (MIC) is the lowest concentration of the quinolone that inhibits visible growth of the organism after 18 h at 35°C. Compounds in **Table-2** were tested for in vitro antibacterial activity against (Gram positive bacteria) *Bacillus subtilis & Staphylococcus aureus*, (Gram negative bacteria) *P. aeruginosa & Escherichia coli* and the results are summarized in it. Introduction of amide group at C-3 position in compound **3** was compared with the activity against compound **2** had showed decreased in the activity by two fold against *Bacillus subtilis & Staphylococcus aureus* and by eight fold against *P. aeruginosa & Escherichia coli*. When different groups were introduced at C-7 position of compound **3** and compared the activity against compound **2** & **3** shows alter in the antimicrobial activity. Compound **4i** is highly potent when compared with other compounds in **Table-2** show eight fold increases in the activity against *Bacillus subtilis & Staphylococcus aureus* and four folds increases in the activity against *P. aeruginosa & Escherichia coli*. Compound **4c** shows four fold improvements in activity against *Staphylococcus aureus* but poor activity against *Bacillus subtilis, P. aeruginosa & Escherichia coli*. Compound **4j** show four fold and compound **4k** show two fold improvements in the activity against *Bacillus subtilis & Staphylococcus aureus* and two fold improvement in the activity against *P. aeruginosa & Escherichia coli* respectively. Compound **4l** shows 16 fold and eight fold increase in activity against *Staphylococcus aureus & Bacillus subtilis* respectively but decrease in eight fold activities against *P. aeruginosa & Escherichia coli*. Compound **4n & 4m** show eight fold increases in the activity against *Bacillus subtilis & Staphylococcus aureus* and four fold decrease in the activity against *P. aeruginosa & Escherichia coli*.

CONCLUSION

It is widely believed that carboxylic functional group at C-3[14-16] position of quinolone is very important for binding the DNA gyrase of the bacteria cell. Any modification at C-3 position on quinolone core decreases the antibacterial activity[19-21] with exception of some groups which are converted to carboxylic acid group in vivo. Data in **Table-1** & **Table-2** shows modification of compound **2** to **3** had decrease the antimicrobial activity but by substituting cyclo amine or cyclo amine with different hetero atoms or group at C-7 position improves the antibacterial activity of compounds. By proper substituting fluoro group at C-7 with different group of compound **3** can increase the antimicrobial activity with fewer side effects and could be a drug in future.

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REFERENCES

1. Singh R, Debnath A, Masram DT, Rathore D. Synthesis and Biological Activities of Selected Quinolone-Metal Complexes. Research Journal of Chemical Sciences, 2013; 3(6): 83-94.
2. Schaumann R, Rodloff AC. Activities of Quinolones Against Obligately Anaerobic Bacteria. Anti-Infective Agents in Medicinal Chemistry, 2007; 6: 49-56.
3. Andersson MI, MacGowan AP. Development of the quinolones. Journal of Antimicrobial Chemotherapy, 2003; 51 (Suppl. S1): 1-11.
4. Pramod MS. International Journal of Antimicrobial agent Agents, 1991; 1: 75-96.
5. Lesher GY, Froelich EJ, Gruett MD, Bailey JH. Brundage RP. 1,8-Naphthyridine derivatives. A New Class of Chemotherapeutic Agent. Med. Pharm. Chem, 1962; 5: 1063.
6. Bisacchi GS. Origins of the Quinolone Class of Antibacterials: An Expanded "Discovery Story". J. Med. Chem, 2005; 58: 4874-4882.
7. Harnnett N, Brown S, Krishnan C. Emergence of Quinolone Resistance among Clinical Isolates of Methicillin-Resistant Staphylococcus aureus in Ontario, Canada. Antimicrobial Agents and Chemotheraphy, 1991; 35: 1911-1913.
8. Piddock LJV. Mechanisms of Fluoroquinolone Resistance: An Update 1994-1998. Drugs, 1999; 58: 11-18.
9. Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. International Journal of Antimicrobial Agents, 2000; 16: 5–15.

10. Canton R, Coque TM, Baquero F, Multi-resistant Gram-negative bacilli: from epidemics to endemics. Current opinions in infectious diseases, 2003; 16:315-325.
11. Stevens, Dennis L. Community-acquired Staphylococcus aureus infections: Increasing virulence and emerging methicillin resistance in the new millennium.Current Opinion in Infectious Diseases, 2003; (3): 189-191.
12. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine, 2004; 10 (12): S122-S129.
13. Sunduru N, Gupta L, Chauhan K, Mishra NN, Shukla PK, Chauhan PMS. Synthesis and antibacterial evaluation of novel 8-fluoro Norfloxacin derivatives as potential probes for methicillin and vancomycin-resistant Staphylococcus aureus. European Journal of Medicinal Chemistry, 2011; 46: 1232-1244
14. Sarkozy G. Quinolones: a class of antimicrobial agents. Vet. Med. – Czech, 2001; 46(9–10): 257–274.
15. Sultana N, Arayne MS, Naz A and Mesaik M A. Identification of anti-inflammatory and other biological activities of 3-carboxamide, 3-carbohydrazide and ester derivatives of gatifloxacin. Chemistry Central Journal, 2013; 7: 6
16. Wiles JA. Bradbury BJ. Pucci M J. New quinolone antibiotics: a survey of the literature from 2005 to 2010. Expert Opin. Ther. Patents, 2010: 20: 1295-1319.
17. Munshi ZB, Chaudhari BR. Synthesis and Evaluation of antimicrobial & antifungal activity of novel 1-cyclopropyl-6 fluoro-7-(substituted azole and fused azole)-1, 4-dihydro-8-methoxy-4oxoquinoline-3-carboxamide derivatives. World Journal of Pharmaceutical Research, 2017; 6(1) Issue 1, 2017; 1199-1209.
18. Munshi ZB, Khairnar BJ, Mane DV, Chaudhari BR. Novel Synthesis and antimicrobial activity of 7-substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide derivatives. World Journal of Pharmacy and Pharmaceutical Sciences, 2017; 5(12): 1733-1749.
19. Basuri TS, Modi V, Thakar PM. Quinolones in 2011: An update. Journal of Pharmacy Research, 2011; 4(4):1294-1297.
20. Tillotson GS. Quinolones: structure-activity relationships and future predictions. J.Med. Microbial, 1996; 44: 320-324.
21. PC, Jain A, Jain S. Fluoroquinolone Antibacterials: A Review on Chemistry, Microbiology and Therapeutic Prospects. Acta Poloniae Pharmaceutica - Drug Research, 2009; 66(6): 587-604.