NOVEL SYNTHESIS AND ANTIMICROBIAL 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 of 1-cyclopropyl-6, 7-difluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide. The target compound was synthesized from 1-cyclopropyl-6, 7-difluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid by one pot synthesis using simple reagent and condition. Then different novel derivatives of 7-substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide were synthesized by substituting fluoro group at C-7 position with different cyclo amine or cyclo amine with different hetero atoms. The novel synthesized compounds were characterized then examined for their antibacterial and antifungal activities.

KEYWORDS: Antimicrobial, Quinoline-3-carboxamide, Moxifloxacin, Gatifloxacin, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and 1-Hydroxybenzotriazole.

INTRODUCTION

The fluoroquinolones are totally synthetic broad spectrum antibacterial agents and many of these drugs are used in the treatment of various bacterial infections.[1-2] The first quinolone compound showing antimicrobial activity was serendipitously discovered in the early 1960s as "byproduct" 7-chloro-1,4-dihydro-1-ethyl- quinoline-4-oxy 3-carboxylic acid (**Ib**) during synthesis of antimalerial drug chloroquinoline (**Ia**)[3-5] showing antimicrobial activity against gram-negative bacteria. Its potency and antimicrobial spectrum was very narrow. So it cannot
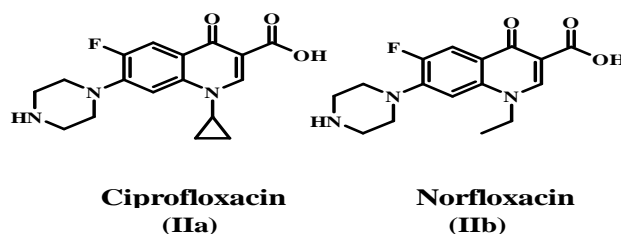
be use for therapy and was considered as lead compound. After doing different changes on this lead compound and studying their microbial activity.

Chloroquine (Ia) → Chloroquine By-Product (Ib) → Nalidixic acid (Ic)
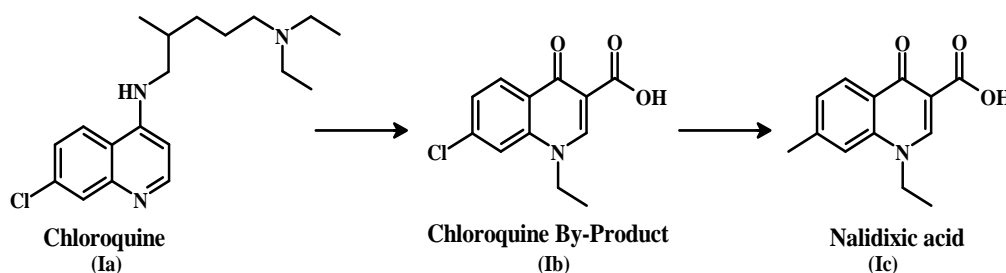
Nalidixic acid (**Ic**) (1-ethyl-1, 4-dihydro-7-methyl-4-oxoquinoline-3-carboxylic acid) launch as drug in 1960s as parent compound of quinolones class of antibiotics.[6] It was discovered by Lescher and colleagues for the treatment of urinary tract infections caused by Escherichia coli and a few other Gram negative pathogens.[7] Its use was limited due to its narrow spectrum of activity, its high protein binding (approximately 90%) and little half life (about 1.5 h). It was active against some Gram negative bacteria. Unfortunately, bacteria could develop a rapid resistance to it.[8, 1] This rapid emergence of drug resistance pathogens like, penicillin-resistant Streptococcus pneumonia, vancomycin-resistant Enterococci, methicillin-resistant Staphylococcus aureus and multi-resistant Salmonellae has now become a serious public health problem so new classes having effective antimicrobial agents against VRSA, MRSA and K. pneumoniae infections are urgently required.[9, 10] There was a significant breakthrough after 20 years in 1980 it was observed that by modifications at C6 and C7 position of lead compound (**IIa**) there was improvement in absorption and activity.

The addition of fluorine molecule at C6 position of quinolone is also known as fluoroquinolones[11] which had increased more than 10-fold in gyrase inhibition and up to 100-fold improvement in MIC.[9] Flumequine (**IVa**) was the first mono fluoroquinolone drug with a fluoro group at position C-6 gave the first indications that modifications of the basic 1,4-dihydro-4-oxoquinoline-3-carboxalic acid core could improve Gram-positive activity.[12]

From 2005-2010 more focus was given on modification at C-7 position of 4-Quinolone because this position strongly influenced the potency, pharmacokinetics and safety profiles of quinolones.[11] Piperazine at position C-7 led to increased spectrum and potency. Ciprofloxacin (**IIa**) and Norfloxacin (**IIb**)[13] [2] are widely used antibiotics in bulk quantities have piperizine at C-7 position.

Ciprofloxacin
(IIa)

Norfloxacin
(IIb)

Pipemidic acid (**III**), Enoxcin (**IV**) and Sarafloxain (**V**) also have piperazine ring at C-7 position. There are data which shows that a piperazine ring may play an important role in inhibiting efflux mechanisms, thereby improving the potency of these drugs.

Pipemidic acid
(III)

Enoxacin
(IV)

Sarafloxacin
(V)
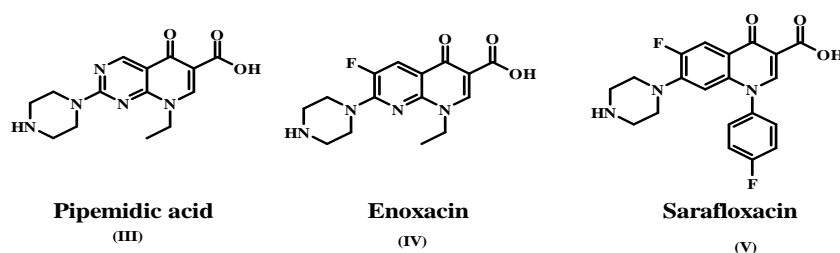
A methoxy group at position C-8 is found in Moxifloxacin (**Va**), Gatifloxacin (**VIa**) and Balofloxacin (**VIIa**) which show good Gram-negative activity and in addition further improved activity against Gram-positive and atypical anaerobic bacteria.[12, 14] They are active against all the primary pathogens that cause typical respiratory disease, e.g. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis.* But Gatifloxacin has been removed from the market because of severe dysglycaemia in some patients.

It has being found that introduction of a cyclopropyl group at N-1 position in ciprofloxacin increased 2-8 fold in potency[9] against Enterobacteriaceae, *Pseudomonas aeruginosa,* and *Staphylo*coccus *aureus* when compared with Norfloxacin[15] and increased in the volume of distribution and reduce the resistance selection.

But Carboxylic acid group at C-3 and Carbonyl group at C-4 position are very important for antimicrobial property of compound and position C-3 and C-4, having a link between the carboxylic acid group and the keto group are generally considered necessary for binding of quinolones to DNA gyrase.[12, 16] Hydrogen atom at C-2 position is very important and very close to this important binding site, and any modification leads to complete lost in antimicrobial activity.

Therefore, we thought to synthesis the fluorinated quinolones with cyclo amine with different hetero atoms at the C-7 position and carboxamide at C-3 position. This present work

describes the synthesis of different novel derivatives of 7-substituted 1-cyclopropyl-6 -fluoro-1, 4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide and their subsequent evaluation for antibacterial and antifungal activities.[19-20]

MATERIALS AND METHODS

All the raw materials used for synthesis are obtained from commercial suppliers and was purified as per requirement. The intermediate ethyl 1-cyclopropyl-6,7-difluoro-1,4-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$_3$ solvents. Tetramethylsilane was used as 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 outline for synthesis of target compounds is given in Scheme-1. Compound (**VII**) 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid was synthesized by hydrolysis of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**VI**) with lithium hydroxide. THF and water was used as solvent. Compound (**VIII**) 1-cyclopropyl-6, 7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide was synthesized by reacting 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid with coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and1-Hydroxybenzotriazole. Reaction carried out in aprotic N,N-Dimethylformamide solvent. Ammonium chloride was used instead of ammonium gas or solution for amidation which is cheaper and safe to handle on large scale. N,N-Diisopropylethylamine used as base. Reaction carried out at low temperature to avoid side reaction. Substitution at C-7 position with different cycloamine was carried out by conventional method. In literature 4-oxoquinoline-3-carboxamide derivatives are synthesized by different methods such that making acylimidazole derivative of 3-carboxylic acid by reacting with 1,1' carbonyldiimidazole in DMF at 100°C to 150°C and then treating its acylimidazole derivative with anhydrous ammonia.[17, 18] Reacting 4-oxoquinoline-3-carboxylate ester with aqueous ammonia and capryl alcohol (Catalytic

amount) was stirred on a steam bath till reaction get completed or with saturated methanolic solution of ammonia in a stainless steel vessel pressure vessel at 150°C for 23 hours[17, 18] or refluxed with hydrazine hydrate for overnight then cool to ambient temperature and this hydrazide is refluxed with Raney nickel in DMF solvent for 36 h.[17] Reacting 4-oxoquinoline-3-carboxylic acid derivatives is reacted with ethyl chloroformate and organic base at 0°C in DMF solvent and then treated with concentrated aqueous ammonia and then stirred for overnight at room temperature.

General procedure for synthesis of VII to IXa- IXn
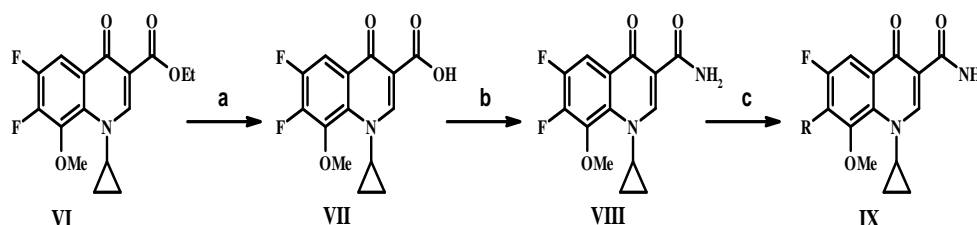
(a) LiOH, THF, H$_2$O, (b) DMF, EDAPC, HOBt, DIPEA, NH$_4$Cl; (c) RH DMSO or DMF, Base, 80°C. R= cyclo amine or cyclo amine with different hetero atoms.

Scheme 1: Synthesis of target compound IX

Procedure; Synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (*VII*)

To the suspension of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**VI**) (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 (*VIII*): To the suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid (*VII*) (16.9mmol) in N,N-Dimethylformamide (50mL) were added 1-(3-Dimethylaminopropyl)-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 obtain 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* 315.10 (M^{+1}).

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

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (*VIII*) (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.
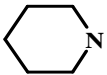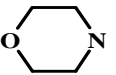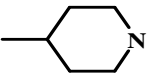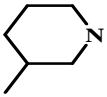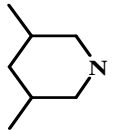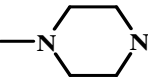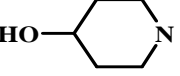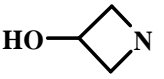
Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (*IXl&IXn*).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (*VIII*) (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.
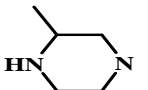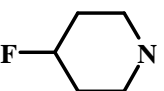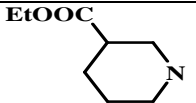
Synthesis of 1-cyclopropyl-6-fluoro-7-(heterocyclic amino)-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (*IXm*).

To a suspension of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (*VIII*) (1.53mmol) in N,N Dimethylformamide (5ml) were heterocyclic amine (1.688 mmole) and cesium carbonate (Cs$_2$CO$_3$) (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.

Table: 1- Physical data of synthesized compounds (IXa- IXn).

No	R1	MF	Mwt	% yield	M.P ($^\circ$C)
IXa		$C_{19}H_{22}FN_3O_3$	359.40	41%	224-226
IXb		$C_{18}H_{20}FN_3O_3$	345.38	75%	220-222
IXc		$C_{18}H_{20}FN_3O_4$	361.38	67%	190-192
IXd		$C_{20}H_{24}FN_3O_3$	373.43	61%	218-220
IXe		$C_{20}H_{24}FN_3O_3$	373.43	46%	>230
IXf		$C_{21}H_{26}FN_3O_3$	387.46	65%	>230
IXg		$C_{19}H_{23}FN_4O_3$	374.42	66%	>230
IXh		$C_{17}H_{18}FN_3O_3$	331	79%	>230
IXi		$C_{19}H_{22}FN_3O_4$	375	70%	224-226
IXj		$C_{18}H_{21}FN_4O_3$	360	42%	192-194
IXk		$C_{19}H_{23}FN_4O_3$	374	57%	188-190
IXl		$C_{19}H_{21}F_2N_3O_3$	377	21%	222-224
IXm		$C_{22}H_{26}FN_3O_5$	431	20%	216-218
IXn		$C_{17}H_{18}FN_3O_4$	347	44%	216-218

Preparation of 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-7-(piperidin-1-yl)quinoline-3-carboxamide (IXa): 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 (IXb): 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 (IXc): 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 (IXd): A off white color 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 (IXe): A off white color 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 (IXf): A off white color 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), 3.983-3.947 (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}).

1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(4-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxamide (IXg): A off white color 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}).

7-(azetidin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide (IXh): 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 (IXi): 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-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxamide (IXj): 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 (IXk): 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 (IXl): yield 21 %; mp 188-190°C; ^{1}H NMR (DMSO-D$_6$) (400 MHz) δ: 8.984(s, 1H), 7.989 (s, 1H), 7.684-7.653 (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 **(IXm)**

yield 20 %; mp 216-218°C; ^{1}H NMR (CDCl$_3$)(400 MHz) δ: 9.600 (s, 1H), 8.839 (s, 1H), 7.888-7.857 (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 (IXn): 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}).

RESULT AND DISCUSSIONS

The outline for synthesis of target compounds is given in Scheme-1. Compound (**VII**) 1-cyclopropyl-6, 7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid was synthesized by hydrolysis of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylate (**VI**) with lithium hydroxide. THF and water was used as solvent. Compound (**VIII**) 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide was synthesized by reacting 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid with coupling reagent 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride and1-Hydroxybenzotriazole.

Reaction carried out in aprotic N,N-Dimethylformamide solvent. Ammonium chloride was used instead of ammonium gas or solution for amidation which is cheaper and safe to handle on large scale. N,N-Diisopropylethylamine used as base. Reaction carried out at low temperature to avoid side reaction. Substitution at C-7 position with different cycloamine was carried out by conventional method. In literature 4-oxoquinoline-3-carboxamide derivatives are synthesized by different methods such that making acylimidazole derivative of 3-carboxylic acid by reacting with 1,l' carbonyldiimidazole in DMF at 100°C to 150°C and then treating its acylimidazole derivative with anhydrous ammonia.[17][18] Reacting 4-oxoquinoline-3-carboxylate ester with aqueous ammonia and capryl alcohol (Catalytic amount) was stirred on a steam bath till reaction get completed or with saturated methanolic solution of ammonia in a stainless steel vessel pressure vessel at 150°C for 23 hours[17] [18] or refluxed with hydrazine hydrate for overnight then cool to ambient temperature and this hydrazide is refluxed with Raney nickel in DMF solvent for 36 h.[17] Reacting 4-oxoquinoline-3-carboxylic acid derivatives is reacted with ethyl chloroformate and organic base at 0°C in DMF solvent and then treated with concentrated aqueous ammonia and then stirred for overnight at room temperature.

Antimicrobial testing (Disc diffusion assay): Various bacterial strains - *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2109) and fungal strain *Candida albicans* (NCIM 3471) were used as test microorganism to evaluate the antimicrobial testing of newly synthesized compounds (Table-1) IX (a-n), IIIV and compound X (a-b).

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 standard (usually to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension contains 2×10^8 CFU/ml of microbial cells.
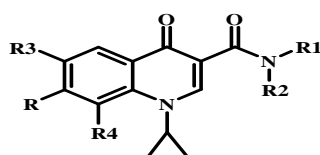
Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The surface of a nutrient agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated

by streaking several times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.
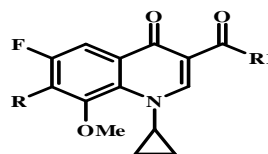
Stock solution [1000 microgram per ml] of each newly synthesized compounds were prepared in dimethylsulfoxide (DMSO). The sterile discs of 6 mm diameter were used in this assay. The disc diffusion assay was carried out by taking concentration 100 microorganism per disc. The discs immersed with compounds were dispensed onto the surface of the inoculated agar plate. Also, Ciprofloxacin (10 microgram/disk, Amphotericin-B (100 units/disk) [Hi-media, Mumbai, 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.

After 24 hours of incubation, each plate was examined. The diameters of the zones of complete inhibition including the diameter of the disc was measured using Vernier caliper, which is held on the back of the inverted petri plate. The results were summarized in Table 1.

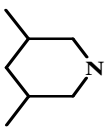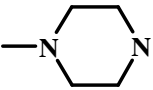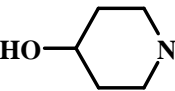**Table: 1: Antibacterial and Antifungal activity data of compounds (IXa - IXn).**
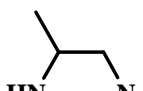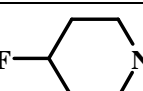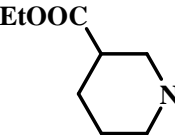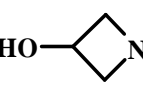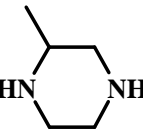
(IX a-n)   (X a-b)

	R	R1	R2	R3	R4	*S.aureus*	*E. coli*	*C.albicans*
IXa	piperidine	H	H	F	OMe	17.43	9.80	-
IXb	pyrrolidine	H	H	F	OMe	15.61	-	-
IXc	morpholine	H	H	F	OMe	19.67	-	-
IXd	methylpiperidine	H	H	F	OMe	11.06	-	-
IXe	methylpiperidine	H	H	F	OMe	10.51	-	-

Code	Structure							
IXf	(2,6-dimethylpiperidin-1-yl)	H	H	F	OMe	9.24	-	-
IXg	(4-methylpiperazin-1-yl)	H	H	F	OMe	23.21	28.69	-
IXh	(azetidin-1-yl)	H	H	F	OMe	14.63	-	-
IXi	(4-hydroxypiperidin-1-yl)	H	H	F	OMe	16.58	-	-
IXj	(piperazin-1-yl)	H	H	F	OMe	19.59	25.11	-
IXk	(3-methylpiperazin-1-yl)	H	H	F	OMe	17.75	21.63	-
IXl	(4-fluoropiperidin-1-yl)	H	H	F	OMe	23.25	12.56	-
IXm	(ethyl piperidine-3-carboxylate) EtOOC	H	H	F	OMe	21.09	9.45	-
IXn	(3-hydroxyazetidin-1-yl) HO	H	H	F	OMe	20.81	11.90	-
VIII	F	H	H	F	OMe	12.88	16.88	-
Xa	F	OH	-	F	OMe	16.23	21.00	-
Xb	(3-methylpiperazine) HN NH	OH	-	F	OMe	33.32	32.10	-
Ciprofloxacin						26.94	32.70	NA
Amphotericin						NA	NA	9.59

Diameter in mm calculated by Vernier Caliper '-' means no zone of inhibition, NA Not applicable Microbial Cultures Used to test antimicrobial Activity, Fungi (Yeast), *Candida albicans,* Gram Positive Bacteria: *Staphylococcus aureus*. *Gram* Negative Bacteria: *Escherichia coli.*

BIOLOGICAL ACTIVITY

Antifungal studies. The antifungal activity was studied against with *Candida albicans* pathogenic fungi. Amphotericin was used as reference for inhibitory activity against fungi.

Compounds (Table-1) IX (**a-n**), **VIII** and compound X (**a-b**) show no activity against *Candida albicans* pathogenic fungi.

Antibacterial studies. All synthesized compounds (Table-1) **IX** (**a-n**), **VIII** and compound **X** (**a-b**) were tested against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Ciprofloxacine used as a reference standard.

It is widely believed that carboxylic acid at C-3 and Carbonyl at C-4 position is necessary for antimicrobial activity. So compound (**Xa**) have slightly better activity as compared to compound **VIII** (Table-1) which have fluoro group at C-7 position and carboxamide at C-3 position against *Staphylococcus aureus* and *Escherichia coli* pathogens. So compound **IX** (**a-n**) (Table-1) were synthesized to increase the activity against both pathogens by substituting different cyclo amine or cyclo amine with different hetero atoms at C-7 position.

Antibacterial screening data (Table-1) shows that all the tested compounds depict moderate to good bacterial inhibition capabilities. As per Structure Activity Relation (SAR) from Table-1 it has been observed that group present at C-7 position play important role in bacterial inhibition capabilities.

Compound **IX**(**a**) showed better activity (piperidine ring at C-7 position) against *S. aureus and E. coli* have better activity against *S. aureus and E. coli* than compound **IX**(**b**) (pyrolidine) and **IX**(**h**) (azitidine) respectively.

When methyl group is introduced on piperidine ring at C-7 position then there is decrease in activity for example compound **IX**(**a**) have better activity against *S. aureus and E. coli* then a compound **IX**(**d**), **IX**(e) and **IX**(**f**) respectively which shows activity against *S. aureus* pathogen and show no activity against *E. coli* pathogen. Activity of methyl piperidene at C-7 position depends upon the position and number of methyl group on piperidine ring if we compare the activity of compound **IX**(**d**), **IX**(**e**) and **IX**(**f**) with compound **IX**(**a**). So when methyl group is introduced at piperidine ring there is decrease in activity against *S. aureus* pathogen and shows no activity against *E. coli* pathogen.

But when fluoro group is present at 4- position of piperidine ring then compound **IX**(**l**) shows better activity than compound **IX**(**n**) and **IX**(**i**) against *S. aureus and E. coli* pathogen but if the hydroxyl group is at 4-position of piperidine ring in compound **IX**(**i**) then there is no activity against *E. coli* pathogen.

When hetero atom is introduced in piperidine ring such as oxygen or nitrogen atom then there is change in the activity of compound. When piperizine group is introduced at C-7 position in compound **IX**(**j**) then it shows good activity against *S. aureus and E. coli* and when morpholine group is introduced at C-7 position in compound **IX**(**c**) then it shows good activity against *S. aureus* pathogen and show no activity against *E. coli* pathogen. This shows that piperizine ring at C-7 position play a very important role in increasing the activity of compound against *S. aureus and E. coli*. But when methyl group is introduced at 4-position of piperazine ring in compound **IX**(**g**) showed very good activity against *Staphylococcus aureus* pathogens and *Escherichia coli* pathogens as compared to all other synthesized molecule in Table -1 but when methyl group was introduced at 3-position of piperizine in compound **IX**(**k**) then there was slight decrease in activity when we compared with compound **IX**(**g**). This show that when methyl group is introduced on nitrogen atom of piperazine ring there is drastic increase in activity against both pathogens and when methyl group is introduced at carbon atom of piperazine ring there is decrease in activity against both pathogens. When ethyl ester group introduces at C-3 position of piperidine ring in compound **IX**(**m**) it shows better activity against *Staphylococcus aureus* pathogens and *Escherichia coli* pathogens.

CONCLUSION

Developed a very efficient, simple and safe method for the synthesis of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxamide. In previous reports ammonium gas (pressure reaction) or solution at higher temperature for amidation. We introduced ammonium chloride for amidation which is cheaper and safe to handle on large scale also.

As per Table-1 Compounds **VII**, **VIII** and **IX** (**a-n**) were synthesized and characterized by ^{1}H NMR and mass. All compounds (Table-1) were screened against *Staphylococcus aureus pathogen* which shows moderate to very good antimicrobial activity. While some compounds shows moderate to very good antimicrobial activity against *Escherichia coli pathogen.* But all compounds show no antifungal activity. Compound **IX**(**g**) had shown very good activity against both pathogens. Above data in Table-1 shows that carboxamide at C-3 position, Carbonyl group at C-4 position with different heterocycles at C-7 position can improve the antimicrobial activity of compounds with less side effects by doing suitable modification on quinolone core at different position.

REFERENCES

1. Sakozy G, Quinolones: a class of antimicrobial agents. Vet. Med-Czech. 2001; 46(9-10): 257.

2. Guillaume A, Jacques G. Pierre, V. Synthesis of mono- and di-substituted 2,4,5-trifluorobenzoic acid synthons, key precursors for biologically active 6-fluoroquinolones. Tetrahedron. 2005; 61: 8394-8404.

3. Schaumann R, Rodloff AC. Activities of Quinolones Against Obligately Anaerobic Bacteria. Anti-Infective Agents in Medicinal Chemistry. 2007; 6: 49-56.

4. Mitscher LA. Bacterial Topoisomerase Inhibitors: Quinolones and Pyridone Antibacterial Agent. *Chem. Rev. 2005;* 105: 559.

5. Bambeke FV. Michot, JM. Eldere JV. Tulkens, PM. Quinolones in 2005: an update. Clinical Microbiology and Infection, 2005; 11: 256-280.

6. 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.

7. Bisacchi GS. Origins of the Quinolone Class of Antibacterials: An Expanded "Discovery Story". J. Med. Chem, 2005; 58: 4874-4882.

8. Emmerson AM, Jones AM. The quinolones: decades of development and use. Journal of Antimicrobial Chemotherapy. 2003; 51(Suppl. S1): 13-20.

9. Andersson MI, MacGowan AP. Development of the quinolones. Journal of Antimicrobial Chemotherapy. 2003; 51(Suppl. S1): 1-11.

10. 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.

11. 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.

12. Appelbaum PC. Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. International Journal of Antimicrobial Agents. 2000; 16: 5-15.

13. Kalkote UR. Sathe VT. Kharul RK. Chavan SP. Ravindranathan T. Quinolones Antibiotics: Study Of Reactivity and Impurity Profile Of Piperazine with Chloro-Fluro-Quinolone Carboxylic Acid In Aqueous Medium. Tetrahedron Letters. 1996; 37: 6785-6786.

14. Sanchez JP, Gogliotti RD. Domagala JM. Gracheck SJ. Huband MD, Sesnie JA, Cohen M.A. Shapiro MA. The Synthesis, Structure-Activity, and Structure-Side Effect Relationships of a Series of 8-Alkoxy and 5-Amino-8-alkoxyquinolone Antibacterial Agent. Med. Chem. 1995; 38: 4478-4487.

15. Sanchez JP, Domagala JM, Hagen SE. Heifetz CL. Hutt MP, Nichols JB, Trehan AK. Quinolone Antibacterial Agent. Synthesis and Structure-Activity Relationships of 8-Substituted Quinoline-3-carboxylic Acid and 1, 8-Naphthyridine-3-carboxylic Acids. Journal of Medicinal Chemistry. 1998; 31: 983-991.

16. Soni K. Fluoroquinolones: Chemistry & Action – A Review. Journal of Pharmaceutical Sciences. 2012; 2: 43-53.

17. Wentland MP, Perni RB, Dorff PH. Brundage RP. Castaldi MJ. Bailey TR. Carabateas PM. BaconER, Young DC. Woods MG, Rosi D. Drozd ML. Kullnig RK, Dutko FJ. 3-Quinolinecarboxamide. A Series of Novel Orally-Active Antiherpetic Agent. J. Med. Chem. 1993; 36: 1580-1596.

18. Wentland MP. Perni RB, Dorff PH. Brundage RP, Castaldi MJ. Bailey TR, Carabateas PM, Bacon ER, Young DC, Woods MG, Rosi D, Drozd ML, Kullnig RK. Dutko FJ. Antiviral Properties of 3-Quinolinecarboxamide: A Series of Novel Non-Nucleoside Antiherpetics Agents. Drug Design and Discovery. 1997; 15: 25-38.

19. Rajput A. P. and Nagarale Deepak V., Der Pharma Chemica, 2016; 8(8): 182-186.

20. Rajput A. P. and Nagarale Deepak V., IJPC, 2016; 06(07): 181-185.