CIBTech Journal of Pharmaceutical Sciences ISSN: 2319–3891 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjps.htm
2014 Vol.3 (4) October-December, pp.32-38/Verma
Research Article

QUINOLONE SYNTHESIS AND EVALUATION –A NOVEL APPROACH

***Verma Vikrant**
Kharvel Subharti College of Pharmacy, Swami Vivekanand Subharti University Meerut
**Author for Correspondence*

ABSTRACT

The importance of the quinolone as a nucleus with medicinal properties is well established, since many decades. Literature survey reveals that the heterocyclic quinolone nucleus has been consistently rewarded as a promising molecule because of its broad spectrum pharmacological activities like anti HIV, antibacterial, antiplatlet, antibiotic, antitumor, FMS Kinase Inhibitor etc. Quinolones are well known for their antibacterial properties. Differently substituted quinolones have been synthesized and evaluated to explore the effect of substitution at different sites on the antibacterial activity. Literature survey reveals that substitution at 6 and 8 positions can lead to improvement in antibacterial activity. The present study was done to explore the effect of different substituent at 6 and 8 positions on the antibacterial activity.

Keywords: *4-Quinolones, Antibacterial Activity*

INTRODUCTION

The importance of the quinolone as a nucleus with medicinal properties is well established, since many decades. Literature survey reveals that the heterocyclic quinolone nucleus has been consistently rewarded as a promising molecule because of its broad spectrum pharmacological activities like antibacterial1, FMS Kinase Inhibitor, antitumor, anti HIV etc. Modifications are done on the quinolone ring at the 6th and 8th position for the antibacterial activity. Quinolones exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating. The majority of quinolones in clinical use belong to the subset fluoroquinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position.

Quinolones comprise a relatively large, growing and most interesting group of antibacterial drugs which have made a major impact on the field of antimicrobial chemotherapy, particularly in the past few decades. This is because they potentially offer many of the attributes of an ideal antibiotic, combining high potency, a broad spectrum of activity, good bioavailability, oral and intravenous formulations, high serum levels, a large volume of distribution indicating concentration in tissues and a potentially low incidence of side-effects.

MATERIAL AND METHODS

Reagents and Solvents

Chemicals used were of LR grade and obtained from Oualigens fine chemicals, Rankem RFCL Ltd. New Delhi, Central Drug House New Delhi and Sigma Aldrich, USA etc. The solvents used throughout the experiment for running TLC were Dicholoromethane: ethanol in the ratio of 98:2.

All the reactions were monitored by thin layer chromatography (tlc) plates coated with silica gel from Qualigens (India). Plates were visualized by iodine vapours. The plates were also visualized in UV light.

Melting points of compounds were measured by capillary fusion technique.

IR spectra was recorded in solid potassium bromide (KBr) disc on Perkin-Elmer RXI FT-IR spectrometer and values are expressed in cm^{-1}.

^{1}H NMR spectroscopy was recorded in Bruker advanced II 400 spectrometer and MASS spectroscopy in ESI-ToF Mass spectrometer.

General Method of Synthesis:-Synthesis of 6-Substituted Quinolones

General Procedure

A mixture of the appropriate aniline (1 mmol) and diethyl ethoxy methylenemalonate (200 µL, 1 mmol) was heated at 120°C for 1 hr. The crystalline solid obtained after cooling at room temperature was dissolved in diphenyl ether and the solution was refluxed for 2hr. After cooling to room temperature, diethyl ether (3mL) was added and the precipitated solid was filtered and recrystallized from DMF.

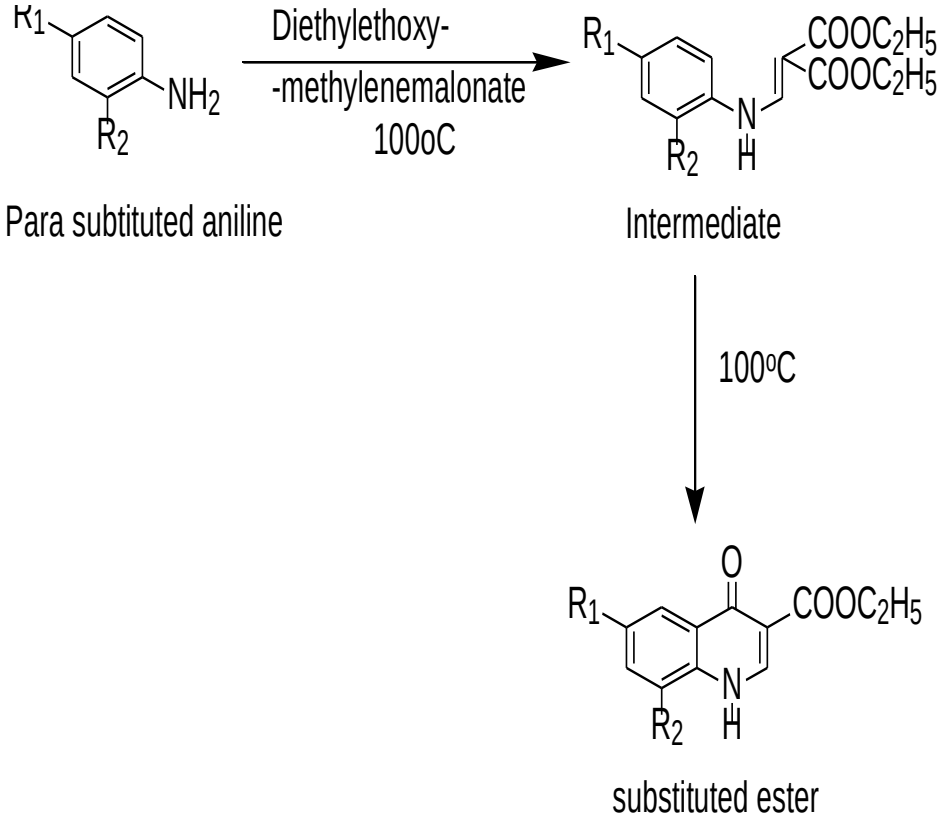
General Method of Synthesis:-Synthesis of 4-Quinolone-3-Carboxylic Acids

General Procedure

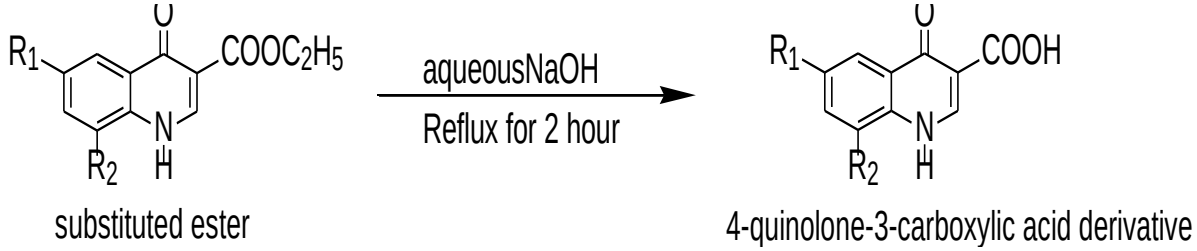
A suspension of the appropriate ester in 10 aq. NaOH (10ml) was refluxed for 2h. After cooling at room temperature, the reaction mixture was acidified using conc. HCl.

The resulting precipitate was filtered and washed with water to give the corresponding 4-quinolone-3-carboxylic acid.

Step 1



Step 2



S. No.	R_1	R_2
1(Q1)	CH_3	CH_3
2(Q2)	CH_3	CN
3(Q3)	$COOCH_3$	CH_3

S.NO	Compound	Percentage yield	Melting point (degree centigrade)	Rf
1.	6,8-Dimethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid	0.80	140-142	0.68
2.	6-Cyano-8-methyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid	0.89	155-159	0.7
3.	8-Methyl-4-oxo-1,4-dihydro-quinoline-3,6-dicarboxylic acid 6-methyl ester	0.98	175-177	0.88

Determination of Minimum Inhibitory Concentrations (MICs) of Antibacterial Agents by Agar Dilution

Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing against which other methods, such as disk diffusion, are calibrated.

MIC methods are widely used in the comparative testing of new agents. In clinical laboratories they are used to establish the susceptibility of organisms that give equivocal results in disk tests, or for tests on organisms where disk tests may be unreliable, and when a more accurate result is required for clinical management.

In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in microplate wells of broth (broth micro dilution) containing dilutions of the antimicrobial agent. The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism known as the MIC.

Although several susceptibility testing media are available in, a clear choice for a reference medium remains to be determined. Mueller-Hinton (MH) agar shows no performance advantages over some other media but is probably the most widely used medium internationally, and there is a USA National Committee for Clinical Laboratory Standards (NCCLS) document which describes procedures for evaluating MH agar (Illig *et al.*, 2008).

The MIC is the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot (Illig *et al.*, 2008).

Antibacterial Study

Antibacterial studies of all the synthesized compounds were conducted against test microorganisms, bacteria (Gram +ve, Gram –ve).

Equipments were cleaned and sterilized by autoclave method at 12 psi for 30 minutes.

Nutrient agar medium was used for the growth of bacteria.

Table: Composition of Nutrient Agar Medium

Composition	Quantity
Peptone	6.0 gm
Yeast extract	3.0 gm
Meat extract	1.5 gm
Agar	15 gm
Distilled water	1 L
PH	6.6-6.8

Test Organisms

1] *Escherichia coli*

2] *Bacillus subtilis*

Standard Drug

Norfloxacin (100µg/mL)

Preparation of Drug Dilutions

The compound were insoluble in water therefore stock solutions of 100mg/ml were prepared in DMSO. Dimethyl sulphoxide [DMSO] used as an inert solvent, which was also used as control.

The stock solution was used for preparation of working solutions with concentrations of 32, 16, 8, 4, 1 µg/ml. stock solution and working solution was prepared in the same way as that of test in concentration of 100mg/ml and 32, 16, 8, 4, 1 µg/ml respectively.

Preparation of Inoculum

- Identified pure cultures were kept as stock strains.
- Fresh culture strains were prepared from stock strains,which were used as inoculum.
- The culture was made to appear in turbidity equivalent to 0.5 Mc Farlands.

Pouring the Plates

Firstly labeled out round Petri plates for each concentration of antibiotics were being tested and prepared the media as per the manufacture instructions (28g in 1000ml distilled water). Cooled the media at water bath at 45^0C and the pH of the medium 7.2 to 7.4 at room temperature. The flask was swirled to mix the contents thoroughly. After that poured into round Petri plates on a level surface to a depth of 3-4 mm(app.19-20ml per plates) and plates were allowed to solidify at room temperature.

Preparation of Control Plates

In addition to preparing antibiotic dilution plates, it was also important to prepare control plates. These plates consist of only the agar-based media with no antibiotic added.

Test and standard drugs were not soluble in water therefore stock solutions were prepared in DMSO.

A control plate with Dmso dilutions was referred as +ve control while without DMSO dilutions was referred as -ve control.

Table 4.1: Antimicrobial activity of quinolin-4-one-3-carboxylic acid derivatives by Agar Dilution method

S.NO.	(MIC-µg/ml Compound Code	*Escherichia coli*	*Bacillus subtilis*
1	Q1	12	16
2	Q2	4	9
3	Q3	18	33
7	Norfloxacin	7	9

Table 4.2: Dilutions of quinolin-4-one-3-carboxylic acid derivatives used in Agar Dilution Susceptibility Tests

S.No.	Antimicrobial concentration (µg/L)	Volume of stock solution in ml.	Volume distilled water in ml.	Antimicrobial concentration obtained (µg/L)	Final concentration (µg/ml) in medium after addition of 19mL of agar
1	1000	0.64	0.36	640	32
2	1000	0.32	0.68	320	16
3	1000	0.16	0.84	160	8
4	100	0.8	0.2	80	4
5	100	0.4	0.6	40	2
6	100	0.2	0.2	20	1

Assay Method

Antimicrobial activity of synthesized compounds was determined using agar dilution method. The apparatus were sterilized. The procedure was carried out to observe strictly sterile conditions

Incubation of Plates

All plates were incubated at 37^0C for 48hrs for bacteria.

Determination of MIC

Effectiveness of a chemotherapeutic agent against a pathogen can be obtained from the minimal inhibitory concentration (MIC).

The MIC is the lowest concentration of a drug that prevents growth of a particular pathogen. Antibiotic plates were read for end points on a dark nonreflecting surface. The MIC was read as the first antibiotic concentration that inhibits the growth of the organism completely.

The minimal inhibitory concentration (MIC-µg/ml) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

The (MIC-µg/ml) of synthesized compound and the standard Norfloxacin is given in table

RESULTS AND DISCUSSION

Results

Quinolin-4-one-3-carboxylic acid derivatives were synthesized and screened for their efficacy as antibacterial agents against various pathogens in-vitro by Agar dilution method. The stock solution(100mg/ml) of Quinolin-4-one-3-carboxylic acid derivatives was prepared in dimethyl sulfoxide and working solution of concentrations 1, 2, 4, 8, 16 and 32 µg/ml in distilled water was prepared. Same dilutions were prepared for the standard drug.

Inhibition of Escherichia Coli

All the synthesized compounds (Q1-Q3) showed inhibition of E.coli at concentration range from 2µg/ml to 16µg/ml.The compound Q2 showed higher activity (less MIC) when compared with standard drug at concentration of 2µg/ml.

Inhibition of Bacillus Subtilis

All compounds exhibited mild to moderate activity against *Bacillus subtilis* at concentration range from 8µg/ml to 32µg/ml. Compound Q2 showed equipotent activity when compared with concentration of standard at 8µg/ml. Compounds Q1 showed inhibition of bacteria at 16 µg/ml while compound Qc showed inhibition of the same at 32 µg/ml.

On the basis of activity it was found that the compound Q2 among the series of synthesized compounds showed good antibacterial activity when compared with the standard drug.

Discussion

The exhaustive literature survey revealed many methods of synthesis of different kinds of quinolin-4-one-3-carboxylic acid derivatives as antibacterial agents.

In the present research work novel quinolin-4-one-3-carboxylic acid derivatives were prepared. All the synthesized compounds showed antibacterial activity against gram+ve and gram –ve species. Novel derivatives were mainly synthesized from the chemical reaction of Para substituted aniline with diethyl ethoxymethylenemalonate in the presence of polyphosphoric acid followed by alkaline hydrolysis.

This study showed the relationship between the antimicrobial activity and certain structural modifications of these new quinolin-4-one-3-carboxylic acid derivatives

The compound Q2 was found to be most effective against *E.coli* and *B.subtilis* in comparison to the standard drug at concentration 2µg/ml, 8µg/ml.

The compounds Qc, Qe were found to be least effective against *E.coli* and *B.subtilis* in comparison to standard drug. Variations in the antimicrobial activity may be distributed to structural changes in synthesized compounds.

REFERENCES

Asahina Y, Hosaka M, Iwase K and Ishizaki T (2005). Synthesis and antibacterial activity of 4-quinolone-3-carboxylic acid derivatives having a trifluoromethyl group as a novel N-1 substituent *Journal of Medicinal Chemistry* **48** 3443.

Batori S, Timari G, Koczka I and Hermecz I (1996). Synthesis and biological evaluation of N-(1-aziridino)-6-fluoro-quinolone-3-carboxylic acids. *Bioorganic & Medicinal Chemistry Letters* **6**(13) 1507.

Beny C, Hadjeri M and Mariotte A (2000). Boumendjel, A convenient method for the synthesis of 3,5,7-trimethoxy-2-phenyl-4-quinolones. *Tetrahedron Letters* **41** 7037-7030.

Chyitzeng C, Longchen Y, Wenchen Y, Fenlo W, Linkao C and Shuanliu Y (2009). Synthesis and antimycobacterial evaluation on arylsulfonyl and arylcarbonyl derivatives of ofoxacin *Journal of the Chinese Chemical Society* **56** 374.

Corelli F, Pasquini S and Mugnaini C (2008). Investigations on the 4-quinolone -3-carboxylic acid motif.1.synthesis and structure activity relationship of a class of human immuno deficiency virus type 1 integrase inhibitors. *Journal of Medicinal Chemistry* **51** 5125.

Foroumadi A, Shafiee A, Emami S and Rajaee M (2005). Synthesis and antibacterial activity of some novel N-(5-benzylthio-1,3,4-thiadiazol-2-yl) and N-(5-benylsulfonyl-1,3,4-thiadiazol-2-yl) piperazinyl quinolone derivatives. *Bioorganic & Medicinal Chemistry Letters* **15** 4488-4492.

Guo HY, Liu JY, Lu Y and Wang YC (2004). The synthesis and activity in vitro of a series of 5-Amino-8-methoxyquinolones *Chinese Chemical Letters* **15**(12) 1397.

He Y, Ellis D, Kuhen KL, Anaclerio B, Wu B, Yin H and Bursulaya B (20069). Design, synthesis and biological evaluations of novel quinolones as HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **16** *4*246-4251.

Illig CR, Wall MJ, Chen J, Meegalla S and Wilson KJ (2008). Synthesis and evaluation of novel 3, 4, 6-substituted 2-quinolones as FMS kinase inhibitors. *Bioorganic & Medicinal Chemistry Letters* **18** 2097-2102.

Jiang J, Liu J and Guo Hui (2007). The synthesis and activity in vitro of a series of 8-difluoromethoxy quinolones. *Chinese Chemical Letters* 1169-1172.

Kappe T, Karem AS and Stadlbauer WJ (1998). Synthesis of benzo-halogenated 4-hydroxy-2(1H) quinolones. *Heterocyclic Chemistry* **25** 5857-862.

Kidwai M, Misra P, Kumar R and Saxena RK (1998). Microwave assisted synthesis and antibacterial activity of new quinolone derivatives. *Monatshefte fur Chemie* **129** 961.

Koyama J, Toyokuni I and Tagahara K (1999). Synthesis of 2-arylquinolone and2-aryl-4-quinolone alkaloids via diels alder reaction of 1,2,3-benzotriazine with enamines. *Chemical and Pharmaceutical Bulletin* **47**(7) 1038.

Kuo CS, Jiauhuang Li, Chiehhsieh M and Mingteng C (1998). Synthesis and antiplatelet activity of phenyl quinolones. *Bioorganic & Medicinal Chemistry Letters* **6** 1657.
Lee J, Lee S and Chang S (1992). New quinolones synthesis of pyrido[3,2-h] quinoline derivatives and their antibaterial activity. *Bioorganic Bull. Korean chem.* **5**(13) 571.
Nagao Y, Ikee Y and Hashimoto K (2007). Nakashima, M.;Synthesis and antibacterial activities af new quinolone derivatives utilizing 1-azabicyclo[1.1.0] butane. *Bioorganic & Medicinal Chemistry Letters* **17** 942-945.
Park MS, Inlee J and Sook Park (2004). Synthesis of new 2,3-dihydro-2-phenyl-4-quinolone derivatives Aza analogs of flavanone M. *Bulletin of the Korean Chemical Society* **25**(8) 1269.
Rajballian S, Forumadi A, Shafiee A and Emami S (2007). Functionalized N-(2-oxyiminoethyl) piperazinyl quinolones as new cytotoxic agents. *Journal of Pharmacy & Pharmaceutical Sciences* **10**(2) 153-158.
Sano T, Toda J, Sakagani M, Goan Y, Simakata M, Horiguchi Y (2000). Synthesis of some methoxy-2-quinolones via pummerer-type cyclization of N-Aryl-N-methyl (phenylsulfinyl) propionamides. *Chemical and Pharmaceutical Bulletin* **48**(12) 1854-1861.
Shafiee A, Foroumadi A (2009). Synthesis and antibacterial activity of N-[2-(2-naphthyl)ethyl]piperazinyl quinolones *Journal of the Iranian Chemical Society* **6**(2) 325.
Souza MLD, Ferreira VF, Lucero BA and Gomes CRB (2006). Synthesis and anti-HSV-1 activity of quinolonic acyclovir analogues. *Bioorganic & Medicinal Chemistry Letters* **16** 1010-1013.
Stadlbauer W and Steinschifter WJ (1994). Synthesis of oxazolo[4,5-c] quinolones by thermolytic degradation of 4-azido-2(1H)-quinolones. *Heterocyclic Chemistry* **31** 1647.
Sun X, Zhang L, Wei C, Piao H and Quan Z (2008). synthesis of a series of 8-alkoxy-5, 6-dihydro-[1, 2, 4] triazino [4, 3-a] quinolin-1-ones with anticonvulsant activity. *European Journal of Medicinal Chemistry* 1265-1270.
Tillotson GS (1996). Quinolones; structure activity relationships and future predictions *Journal of Medical Microbiology* **44** 320.
You QD and Wang H (2008). Design, synthesis and antitumor activity of 3-substituted quinolone derivatives. *Chinese Chemical Letters* **19** 1395.