International Journal of ChemTech Research
ISSN : 0974-4290
Vol.1, No.1, pp 34-45, Jan – March 2009

STUDIES ON SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME SUBSTITUTED FLUOROQUINOLONES'

AKHILES ROY*, S. M. SARDAR#, B.U.SALVE#, D.D.RISHIPATHAK§,

Department of Pharmaceutical chemistry, NDMVPs College of Pharmacy, Gangapur Road,Nashik 422005 (Maharashtra)

***Corres.author: akhilesroy@gmail.com**

 # Presently working at,
Department of Pharmaceutical Chemistry
Sinhgad Institute of Pharmacy,
SR No.45/1,2,3 Off Westerly bypass Mumbai-Pune expressway
Narhe, Pune 411 041 Maharashtra, India

§Presently working at,
Department of Pharmaceutical Chemistry
METs Institute of Pharmacy,
Bhujbal knowledge city, Adgaon, Nashik- 422003

Abstract

In the present study the synthesis of Fluoroquinolone nucleus, 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-ethyl carboxylate has been reported by the microwave-assisted condensation of 3-chloro-4-fluoro aniline and diethyl (ethoxymethylene) malonate (EMME) under solvent free conditions. The microwave-assisted synthesis of Fluoroquinolone nucleus was successfully standardized and optimized making the method easy, more convenient & less time –consuming and eco-friendly requiring less chemicals and reagents and with better yield making the process more economic than other conventional methods. N_1-alkyl / aryl/arylsulphonyl substituted and C_7 piperazinyl derivatives of the title compounds have been synthesized to identify newer fluoroquinolones which have better efficacy, lesser side-effects and well tolerability than

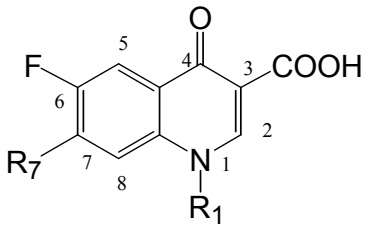
the already available ones. The biological evaluation of the synthesized fluoroquinolone derivatives was carried out using disc method and compounds V_2,VP_2, V_3, VP_{3A}, V_5, VP_5 V_{10},VP_{10} were found to be active against both Gram-positive and Gram-negative bacteria having activity comparable to that of standard drug i.e. Ciprofloxacin.(5 μg/disc) Comparison of activity of the test compounds indicates that benzenesulphonamido moiety at N_1 position and piperazine moiety at C_7 position possibly contributes to activity either due to stronger binding with the receptor or better hydrophilic lipophillic balance of the overall molecule. It is clear from the results that replacement of 7-chloro substituent by piperazine improves the spectrum of activity of fluoroquinolones, showing activity against *Pseudomonas* species as well as Gram-positive and Gram-negative bacteria whereas the 7-chloroquinolones are active against Gram-positive and Gram-negative bacteria.

Key words: Fluoroquinolone, microwave, Gram-positive bacteria, Gram-negative bacteria.

1.Introduction

Fluoroquinolones as a class is now a days one of the frequently prescribed class of antibacterial. Fluoroquinolones have gained stupendous importance during the last two decades because of their potent anti-bacterial activity against wide varieties of gram-positive and gram-negative pathogenic bacteria with minimum toxic side-effects and some what different mechanism of action than other available antibacterial drugs.[1] To date, many fluoroquinolone antibacterial agents have been introduced into clinical use with significant improvement in antibacterial spectrum and activity. A vast array of fluoroquinolones having excellent broad-spectrum activity forms an invaluable part of the present anti-infective armory of the clinicians. A number of these compounds are today's blockbusters of the antibacterial market due to their therapeutic efficacy and tolerable side-effects even, challenging the predominance of well- established β-lactam antibiotics which are becoming more prone to the resistant pathogenic bacteria. The fluoroquinolones are the fastest growing antibacterial class in terms of global revenue, increasingly being used in both the hospital and community sectors to treat a broad range of infection [2]. The boost in fluoroquinolone prescribing was attributable to the introduction and use of newer, broader-spectrum fluoroquinolones with activity against *S. Pneumoniae* (for example, levofloxacin, gatifloxacin, and moxifloxacin). However, increased prescribing has led to the recent emergence of fluoroquinolone-resistant bacteria which has necessitated the search or newer drugs with efficacy against resistant strains and efforts are on worldwide in this direction.[3,4] The present work is a part of these worldwide efforts to develop better Fluoroquinolones than the available ones with respect to activity or toxicity or resistance or all of these..

1.1 Structure-Activity Relationship (SAR): [2, 5, 6]



Position 1: [2 ,5, 6]

Earlier study indicated that substitution at N-1 position is important for anti-bacterial activity. QSAR analysis of a set of N-1 allyl and alkyl derivatives suggested and optimum STERIMOL length of 0.42 nm , corresponding approximately to an ethyl group. STERIMOL is a program that calculates a set of five parameters characterizing size and shape of a substituent. STERIMOL length is defined as length of substituent along the axis of bond between the substituent and the parent molecule. Introduction of a t-butyl group at N-1 produced quinolones with enhanced activity against gram positive bacteria with minor reduction of activity against gram-negative bacteria.

Position - 7: [1, 3,7, 8,]

C-7 substituent is regarded as drug –enzyme interaction domain, it is also concluded that the cell permeability is dominantly controlled. It also affects the interaction with target site. The inhibition of DNA gyrase and cell permeability of quinolones is greatly influenced by the nature of C-7 substituent on the standard structure of 4- quinolone- 3-carboxylic acid C-7 piperazinyl group in addition to C-6 fluorine substituent has anti-bacterial potency for superior to that of earlier classical quinolones against both gram-positive and gram-negative bacteria. The pipeprazine moiety of 7-piperazine quinolones possesses enough structural flexibility to allow product optimization. In general, the substitution of methyl at C-4 position of the piperazinyl group enhances gram-positive anti-bacterial activity with slight decrease in gram-negative activity.

2. Materials and Methods:

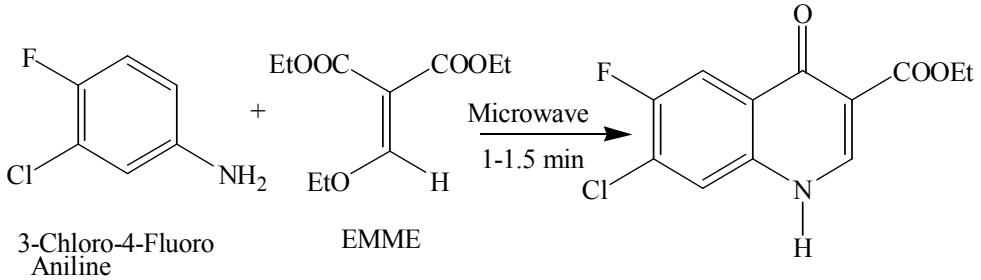
Melting points were taken in open glass capillary using Elico melting point apparatus and are uncorrected. The identification and purity of the synthesized compounds were checked by Thin layer chromatography using silica gel G as adsorbant. The spots were detected by exposure to iodine vapours. Infrared spectra of compounds were recorded on Schimadzu IR 408 spectrophotometer model using Nujol as medium. Proton (1H) NMR spectra of compounds were recorded on BROOT spectrophotometer (800 MHz) using DMSO-d_6 as solvent, at Analytical Center, University of Pune. The elemental analysis (CHN) of compounds was carried out at SAIF IIT-Mumbai. All microwave reactions were carried on Raga's Electromagnetic System with automatic power setting from P-1 to P-10. The reactions were started at power P-5 for initial 30 sec. and after every 30 sec. reaction mixtures were monitored for completion of the reaction with the help of TLC.

2.1 Procedure for Synthesis of 7-Chloro-6-Fluoro-1, 4-dihydro-4-Oxoquinoline-3-Carboxylic acid (4) [4, 9]

Step 1: (Scheme 1)

Equimolar amounts of 3-chloro-4-fluoro aniline **(1)** (1.45 gm, 0.01 mol) (white crystalline solid, m.p. 44 -47 ^{0}C) and diethyl (ethoxymethylene) malonate (EMME) **(2)** (2.16 gm, 0.01mol), (almost colourless liquid, b.p. 279-281 ^{0}C) were taken in a beaker under solvent-free condition, when clear solution was obtained by shaking which was irradiated under microwave for 1-1.5 min at high power (540- 750 watts) by which time the whole reaction mixture was gradually converted into a semisolid mass having white to pale yellow appearance which was washed with acetone to get almost white solid and was recrystalized using N, N- dimethyl formamide (DMF) as solvent.

Scheme 1:



3-Chloro-4-Fluoro Aniline — **1**

EMME — **2**

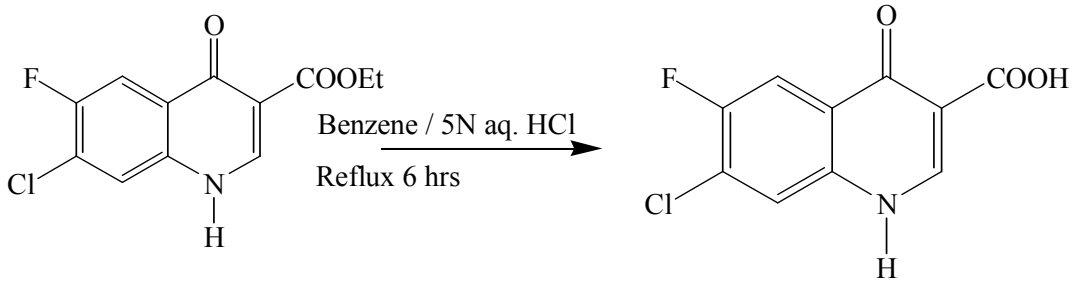
3

Ethyl-7-chloro-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate

Step 2 (Scheme 2)

The product of step-1 (an ethyl ester) **(3)** (2.7 gm , 0.01 mol) was dissolved in 50 ml of benzene and hydrolyzed to corresponding carboxylic acid using 50 ml of 5N aqueous hydrochloric acid . Then the reaction mixture was stirred and heated under reflux for 5-6 hours. The white solid was gradually precipitated at the bottom of aqueous layer. The solid thus obtained was filtered, washed with water till neutral, dried and recrystalized using acetone as solvent to give the product. **(4)**

Scheme 2:



3

4

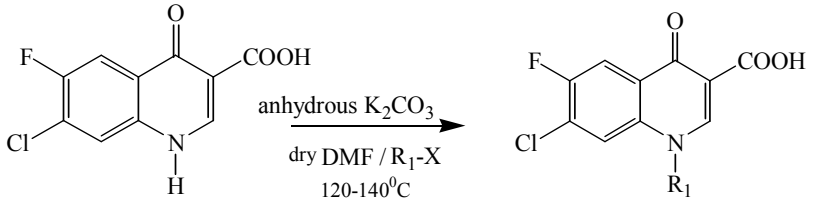
7-chloro-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid

2.2 General Method of Synthesis of n_1-alkyl / aryl/arylSulphonyl -7-Chloro-6-Fluoro-1,4-dihydro-4-Oxoquinoline-3- Carboxylic acid (5) (Scheme 3)[10]

7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3- carboxylic acid **(4)** (2.56 g, 0.01 mol) was added to 10 ml of N, N- dimethyl formamide (DMF), followed by addition of alkyl / aryl/arylsulphonyl halide (0.01 mol). The reaction mixture was heated to dissolve the 3-acid which was partially soluble in cold condition. Then anhydrous potassium carbonate (3.40 g, 0.02mol) was added to the reaction mixture. The whole reaction mixture was heated to 120-140^{0}C and stirred for 5-8 hrs. Then the reaction mixture was poured onto crushed ice or ice cold water, washed with cold water to remove N, N- dimethyl formamide (DMF) and potassium carbonate if any. The solid **(5)** obtained was recrystalized from acetone to give the carboxylic acid.

Scheme 3:

N-1 SUBSTITUTION :-



4 **5**

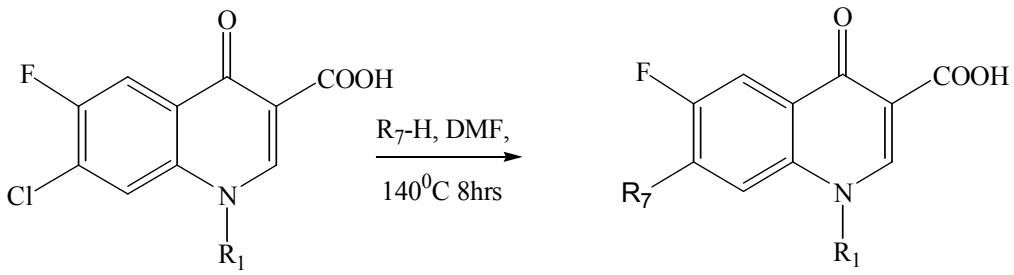
Where, R_1= various alkyl , aryl aryl sulphonyl moieties

2.3 General Procedure for the Synthesis of n_1-alkyl / aryl/arylsulphonyl -7-Piperazinyl -6-Fluoro-1,4-dihydro-4-Oxoquinoline-3- Carboxylic acid (6) (Scheme 4) [10,11]

A mixture of the product i.e., N_1 substituted 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid **(5)** (2.7 mmol) in dry dimethyl sulphoxide and piperazine (18 mmol, 1.6 gm) was heated at 140^{0}C with stirring for 2.5-8 hrs. Cooled to room temperature and then 10 ml of cold water was added to the mixture and acidified to pH 7 with dilute acetic acid. The resulting precipitate **(6)** washed with water, dried and recrystalized from ethanol and DMF.

Scheme 4:

C-7 SUBSTITUTION



5 **6**

2.4 Antimicrobial Screening test [12,13]

The plates were prepared with Mueller Hinton Agar for the use in the Bauer-Kirby method for rapidly growing aerobic organisms. A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum (turbidity so adjusted, as to obtain confluent growth on the Petri plate) and the soaked swab was rotated firmly against the upper inside wall of the tube to express excess fluid. The entire agar surface was streaked of the plate with the swab three times, turning the plate at 60^0 angles between each streaking. The inoculum was allowed to dry for 5-15 minutes with the lid in place. The disc was applied using aseptic technique. The discs were deposited with centers at least 24 mm apart. The inoculated culture medium prepared as above was immediately incubated at 37^0C and was examined after 14-19 hours or later if necessary. The zones showing complete inhibition were measured and the diameters of the zones to nearest millimeter were recorded.

3. Spectral analysis:

1) **N_1 (N'-phenyl carbamoyl methyl) - 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinilone- 3-carboxylic acid. (V_1):**

   I.R. (KBr, cm^{-1}): 3190 (Secondary amide N-H), 1701 (Carboxylic acid C=O), 1600 (Pyridone C=O), 1473 (C-N stretch of quinoline having C=C-N), 1030 (C-F), 754 (C-Cl)

2) **N_1 (p-ethylphenyl sulphonyl) - 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid(V_4):**

   I.R. (KBr, cm^{-1}): 2972 (Ethyl C-H stretch), 1700 (Carboxylic acid C=O), 1600 (Pyridone C=O), 1473 (C-N stretch of quinoline having C=C-N), 1359, (SO_2 symmetric),1155 (SO_2 asymmetric), 1033 (C-F), 734(C-Cl)

3) **N_1 (p-acetamidobenzenesulphonyl) - 7-chloro-6-fluoro-1, 4-dihydro – 4-oxoquinoline-3-carboxylic acid (V_6):**

I.R. (KBr, cm^{-1}): 3185 (Secondary amide N-H), 1700 (Carboxylic acid C=O), 1600 (Pyridone C=O), 1473 (C-N stretch of quinoline having C=C-N), 1359 (SO_2 symmetric) 1172 (SO_2 asymmetric) 1035 (C-F), 734 (C-Cl)

4) **N_1 (3-carboxyphenyl sulphonyl) -7-chloro-6-fluoro-1, 4-dihydro- 4-oxoquinoline-3-carboxylic acid(V_8):**

I.R. (KBr, cm^{-1}): 1700 (Carboxylic acid C=O), 1618 (Pyridone C=O), 1473 (C-N stretch of quinoline having C=C-N), 1379 (Aryl C-O stretch) 1359 (SO_2 symmetric), 1120 (SO_2 asymmetric) 1035 (C-F), 655 (C-Cl)

5) N_1 (2-hydroxyethyl) -7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline- 3-carboxylic acid (V_{10}):

I.R. (KBr, cm^{-1}):1720 (Carboxylic acid C=O), 1612 (Pyridone C=O), 1488 (C-N stretch of quinoline having C=C-N), 1047 (Primary alcohol O-H), 1029 (C-F), 739, (C-Cl)

^{1}H NMR (DMSO- d_6, δ ppm): 1.29 (t, 2H, ethyl C-1), 3.71(t, 2H, ethyl C-2), 4.23 (t, 1H, alcoholic OH), 8.03 (d, 1H, C8 proton), 8.21(d, 1H, C5 proton), 8.49 (s, 1H, C2 proton.) 8.58 (s, 1H, carboxylic-OH)

Elemental analysis ($C_{12}H_9NO_4FCl$), Found (Calcd) %: C, 49.88 (50.42), H, 4.23 (3.75), N, 5.45 (4.92).

6) N_1 (p-tolyl sulphonyl)-7-(N'$_4$-(3-trifluromethylphenyl)-piperazino-6-fluoro- 1, 4-dihydro-4-oxoquinoline -3-carboxylic acid (VP_{3B})

I.R. (KBr, cm^{-1}):2850 (Piperazine C-H), 1710 (Carboxylic acid C=O), 1618 (Pyridone C=O) 1310 (SO_2 symmetric) 1172 (SO_2 asymmetric) 1034 (C-F)

7) N_1 (p-acetamidophenylsulphonyl)-7-piperazino-6-fluoro-1, 4-dihydro- 4-oxoquinoline-3-carboxylic acid (VP_6):

I.R. (KBr, cm^{-1}):3340 (Piperazine N-H), 2853 (Piperazine C-C), 1718 (Carboxylic acid C=O), 1616 (Pyridone C=O), 1484 (C-N stretch of quinoline having C=C-N), 1378 (Aromatic C-N), 1295 (SO_2 symmetric), 1172 (SO_2 asymmetric), 1037 (C-F)

8) N_1 (3-carboxyphenyl)-7-piperazino-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid (VP_8)

I.R. (KBr, cm^{-1}):3422 (Piperazine N-H), 2863 (Piperazine C-H), 1707 (Carboxylic acid C=O) 1618 (Pyridone C=O) 1490 (C-N stretch of quinoline having C=C-N) 1378(SO_2 symmetric), 1126 (SO_2 asymmetric) 1032 (C-F)

Table 1: Physical data of Synthesized r_1 Substituted 3-acid compounds

Structure No.	Substituent R_1	Addendum (R_1-X) X-chloro	Melting point (°C)	Yield (%)	Recrystalizing solvent
V_1.	Acetanilido-	Chloro acetanilide	180-182	65.00	Acetone
V_4.	p-ethylphenyl sulphonyl-	p-ethylphenyl sulphonyl chloride	280-284	81.00	Methanol
V_6.	p-acetamido phenyl sulphonyl-	p-acetamido phenyl sulphonyl-chloride	> 300	77.98	DMF
V_7.	p-acetyl phenyl-	p-chloro acetophenone	294-296	69.89	DMF
V_8.	3-carboxy phenyl sulphonyl-	3-carboxy phenyl sulphonyl chloride	290-292	84.66	Acetone
V_{10}	Hydroxy ethyl-	Ethylene chlorhydrin	205-207	75.70	Methanol
V_{11}.	p-methoxy phenyl sulphonyl-	p-methoxy phenyl sulphonyl-chloride	252-254	70.50	Acetone

Table 2: Physical data of Synthesized r_1 with r_7 Substituted 3-acid compounds

Structure No.	Substituent R_1	Substituent R_7	Melting point (°C)	Yield (%)	Recrystalizing solvent
$VP_{3B.}$	p-tolyl-sulphonyl-	3-trifluromethylphenyl piperazino-	243-245	71.50	Acetone
$VP_{4.}$	p-ethylphenyl sulphonyl-	Piperazino-	268-270	74.76	Acetone-methanol
$VP_{6.}$	p-acetamido phenyl sulphonyl-	Piperazino-	270-272	68.48	DMF
$VP_{8.}$	3-carboxy phenyl sulphonyl-	Piperazino-	278-280	73.00	Acetone
VP_{10}	Hydroxy ethyl-	Piperazino-	222-224	67.98	Methanol

Table 3: Activity of the compounds taken at higher concentration against following Standard strains

Comp. Code	Strength (µg / disc)	Zone of inhibition (in mm)		
		E.coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
V_2	100	33	NA	30
VP_2	100	37	33	33
V_3	100	37	NA	33
VP_{3A}	100	40	34	33
VP_{3B}	100	NSA	NA	NSA
V_5	100	34	NA	30
VP_5	100	37	30	31
V_7	100	NA	NA	NA
V_{10}	100	34	NA	29
VP_{10}	100	39	33	30
Cipro	5	32	30	32

NSA: No significant activity, which signifies that the zone of inhibition is less than 10 mm , NA: No activity

Table 4: Activity of the compounds taken at lower concentration against following Standard strains

Comp. Code	Strength (µg / disc)	Zone of inhibition (in mm)		
		E.coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Staphylococcus aureus ATCC 25923
`V_2	5	26	NA	20
VP_2	5	28	25	22
V_3	5	25	NA	22
VP_{3A}	5	27	24	24
V_5	5	25	NA	22
VP_5	5	28	24	23
V_{10}	5	24	NA	22
VP_{10}	5	26	20	22
Cipro.(V)	5	32	30	32

NA: No activity

Results and discussion

In the present work, the microwave method was used to synthesize ethyl- 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylate from the corresponding 3-chloro-4 fluoroaniline, It was observed that while the yields were almost similar as that using the conventional methods, the time of reaction was very less (only a few minutes) in microwave method compared to the time required for the conventional methods (6-8 hrs). Further, microwave-assisted synthesis of quinolone nucleus could be carried out using the reactants only, without any solvent or any solid support which was an added advantage over the conventional methods requiring solvents.

These observations prompted to optimize the reaction condition with respect to power and time, and after several experiments with various combinations of power and time of the reaction, the method was finally optimized to get very high yield of the product having very good quality which required minimum efforts, solvents and chemicals to purify the products. Physical data of synthesized R_1 substituted 3-acid compounds and R_1 with R_7 substituted 3-acid compounds is reported in **Table 1** and **Table 2** respectively.

All 3- carboxyl fluoroquinolones synthesized were initially tested for their antibacterial activity using standard literature method using doses of 100µgm/disc for test compounds, and the usual dose of 5 µgm/disc for the standard drug, Ciprofloxacin. This was done to identify even the weakly active compounds compared to Ciprofloxacin. The results

(Table 3) show a few compounds to be comparably active as Ciprofloxacin. Then the selected more potent compounds were further screened using the same method as before, but using all test compounds and Ciprofloxacin at doses of 5µgm/disc.

It is interesting to note that compounds (VP_2, VP_{3A}, VP_5, VP_{10},) having piperazine substituent at C_7 position of quinolone ring showed broader spectrum of activity than their analogues (V_2, V_3, V_5, V_{10}) with chloro substituent at C_7 position, and this finding corroborates the similar findings reported in the literature.

The results of secondary screening **(Table 4)** is highly encouraging considering the fact that selected compounds (V_2,VP_2, V_3, VP_{3A}, V_5, VP_5 V_{10},VP_{10}) were found to be comparably active against Gram-positive (*Staphylococcus* spp) and Gram-negative (*Escherichia* spp) pathogenic bacteria at the same dose 5µgm/disc comparable as that of standard drug, Ciprofloxacin. Further, the compounds (VP_2, VP_{3A}, VP_5, VP_{10}) bearing substituent of piperazine at C_7 position of quinolone ring were found to be almost nearly active against *Pseudomonas* spp at the same dose of 5 µgm/disc as the standard drug, Ciprofloxacin.

References

1. Talah A.K., Gadad, Synthesis, antibacterial and antitubercular activities of some7-[4-(5-amino-[1, 3, 4] thiadiazole-2-sulfonyl)-piperazin-1-yl] fluoroquinolonic derivatives. Eur J. Med. Chem, 2006,1-7;
2. Bhanot S.K., Singh M. and Chatterjee N.R., The Chemical and Biological Aspects of Fluoroquinolones: Reality and Dreams, Current Pharmaceutical design**,** 2001, 7, 313-337
3. Foroumadi A., Ghodshi S., Emani S., Najjari S., Samadi N., Ali Faramarzi M., Beikmohammadi L., Shirazi F.H. and Shafie A., Synthesis and antibacterial activity of new fluoroquinolones containing a substituted N-(phenethyl) piperzine moiety. Bioorg. Med. Chem. Lett, 2006, 16, 3499-3503.
4. Shindikar A.V. and Viswanthan C.L., Novel fluoroquinolones: design, synthesis and in vitro activity in mice against *Mybacteririum tuberculosis* H-37 Rv**,** Bioorg. Med. Chem. Lett**,** 2005,15, 1803-6
5. Chu D.T.W. and Fernandes P.B., Structure- Activity Relationships of the Fluoroquinolones, Antimicrobial Agents and Chemotherapy**,** 1989, 33,131- 135

6. Ohta M. and Koga H. Three dimensional Structure Activity Relationship and Receptor Mapping of N_1 - substituents of Quinolone Antibacterial. J. Med. Chem**,** 1991, 34, 131 -139
7 Neito M.J., Alovero F.L., Manzo R.H., and Mazzieri M.R., Benzene sulphonamide analogs of fluoroquinolones antibacterial activity and QSAR studies, Eur. J. Med Chem, 2005, 40, 361-9
8 Neito M.J., Alovero F.L., Manzo R.H., and Mazzieri M.R., A new class of fluoroquinolones: benzenesulphonamide fluoroquinolones (BSFQs) antibacterial activity and SAR studies. Eur. J. Med. Chem, 1999, 34, 209-214
9 Dave C. G. and Joshipura H. M., Microwave assisted Gould - Jacob reaction: Synthesis of 4- quinolones under solvent free conditions, Indian.J.Chem, 2002, 41B, 650 - 652
10 Chen Y.L., Fang K.C., Sheu J.Y., Hsu S.L., and Tzeng C. C., Synthesis and Antibacterial Evaluation of Certain Quinolone Derivatives, J.Med.Chem, 2001, 44, 2374 – 2371
11 D. J.Abraham, Burgers medicinal chemistry and drug discovery, 6^{th} Ed, Wiley Interscience, New Jersy, 2003, 5, 853-855,
12 Bauer A.W., Kirby W.M.M., Sherris J.C., Turck M., Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Path, 1966, 45, 493.
13 Wayne P.A. National Committee for Clinical Laboratory Standards, Performance standards for antibacterial disc susceptibility tests, NCCLS, 2002, 22,1

******