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Synthesis, Characterization and Pharmacological Studies of Some Substituted Fluoroquinolones

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ABSTRACT

Certain N-1 substituted fluoroquinolonic derivatives were synthesized and evaluated for antimicrobial and antioxidant activities. N-1 alkyl/ aryl/ aryl sulphonyl substituted derivatives of the title compounds have been synthesized to identify newer fluoroquinolones which have better efficacy, lesser side effects and well tolerability. The biological evaluation of the synthesized fluoroquinolone derivatives was carried out using agar-well diffusion method and compounds FI-ETH, FII-SUL, FIII-ATY, FIV-BZO, and FV-BZY were found to be active against both Gram-positive and Gram-negative bacteria having activity comparable to that of standard drug i.e. Ofloxacin 10mg/ml. N-1 substituted moiety is mostly active against strain is S. aureus, K. Pneumonia, and E. coli with concentration of 100 - 150µg/ml, and less active against strain is B. subtilis. And secondly, antioxidant activity is that of show the better activity by this four method DPPH Free Radical Scavenging Assay, Hydrogen Peroxide Radical Scavenging Activity, Nitric Oxide Assay, and Reducing Power Assay. All the synthesized compounds show the better antioxidant activity and in fourth one method result is mostly capable reducing power activity of the synthesized compounds.

Key words: Fluoroquinolones, Antibacterials, Antioxidant agent.

INTRODUCTION

The first clinically useful quinolone was naldixic acid, discovered by Lesher and co-workers in 1962, which was generated from chloroquine, an antimalarial agent[1]. It was active against some Gram negative bacteria and had limited usefulness because of its high protein binding (approximately 90%) and little half life (about 1.5 h)[6]. Unfortunately, bacteria could develop a rapid resistance to this agent[7,11]. Flumequine was the first fluoroquinolone which was patented in 1973, after that many fluoroquinolones have been patented and are still used today, including norfloxacin (1978), pefloxacin (1979), enoxacin (1980), fleroxacin (1981), ciprofloxacin (1981) and ofloxacin (1982)[1]. An advantage of these compounds over previous ones is their broad spectrum. A big revolution was made in 1980 ís when an analog of naldixic acid, enoxacin was derived with significantly increased spectrum of activity against Gram negative or Gram positive bacteria[8]. An antioxidant is a molecule capable of inhibiting or preventing the oxidation of other molecules. Antioxidants are substances that may

protect cells from the damaging effects of oxygen radicals, highly reactive chemicals that play a part in atherosclerosis, some forms of cancer and reperfusion injuries (4). Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, (ROS) exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (9). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases[16, 9-10]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases[9]. In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases[12-16].

Methodology

Synthesis of derivative FI-ETH Equimolar amounts of 3-chloro-4-fluoroaniline (i), and diethylethoxymethylene malonate (ii) were condensed at 145^{0}C to get 3-chloro-4-fluoroanilinomethylene malonic diethyl ester (iii), which was then cyclized by heating at 250^{0}C in diphenyl ether to get ethyl 7-chloro-6-fluoro-4-oxo1, 4-dihydroquinoloine-3-carboxylate (iv). Whole reaction mixture change into the semisolid mass having white to pale yellow appearance and washed with acetone to get almost white solid recrystillised using DMF as solvent. Showed in scheme 1 fig. I.

Synthesized derivatives (FII-SUL, FIII-ATY, FIV-BZO, and FV-BZY) are synthesized by this following method. ethyl 7-chloro-6-fluoro-4-oxo1, 4-dihydroquinoloine-3-carboxylate (iv) 0.01 mol was added to 10 ml. of DMF, followed by addition of N-1 position (R) (0.01 mol). The reaction mixture was heated to dissolve the 3-acid which was partially soluble in cold condition, and then anhydrous potassium carbonate (0.02 mol) was added to the reaction mixture. The whole reaction mixture was heated to 120^0 – 140^{0}C and stirred for 5-8 hrs. then, the reaction mixture was poured onto the crushed ice or ice cold water, washed with cold water to remove DMF and potassium carbonate if any, the solid (v) obtained was recrystillised from acetone to get the derivative. Showed in scheme 2 fig. II. All synthesized derivatives checked by TLC technique using adsorbent silica gel G of Merck specialties Pvt. Ltd., Mumbai and each derivatives are isolate & purified by column chromatography using silica gel mesh size 60-120 of Merck specialties Pvt. Ltd., Mumbai.

In-vitro antibacterial activity (5)

Agar- Well Diffusion Method: Petriplates containing 20 ml Muller Hinton medium were seeded with 24 hr culture of bacterial strains. Wells were cut and 20 µl of the given sample (of different concentrations) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Ofloxacin were used as a positive control.

In-vitro antioxidant activity by DPPH Free Radical Scavenging Assay (2, 10)

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the sample Different concentrations (50 – 250 µg/ml) of each sample were added, at an equal volume, to 90% methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid were used as standard controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Hydrogen Peroxide Radical Scavenging Activity (4)

The ability of the sample (FI-ETH, FII-SUL, FIII-ATY, FIV-BZO, and FV-BZY) to scavenge

hydrogen peroxide was determined. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Sample (50 – 250 µg/ml) in distilled water, and 1 ml added to 2.4 ml. of 0.1 M phosphate buffer solution and a hydrogen peroxide solution (600 µl, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged $[H_2O_2]$ = [(Ao - A1)/Ao] × 100 where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample and standard.

Nitric Oxide Assay (13)

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (5 mM), in phosphate-buffered saline, was mixed with different concentrations (50 - 250 µg/ml) of each sample dissolved in methanol and incubated at room temperature for 30 min. After the incubation period, 1.5 ml incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Rutin was used as standard.

Reducing Power Assay (3)

The reducing power assay of sample was determined. Different amounts of each sample (50 -800 µg/ ml) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid used as a standard.

Spectral Analysis

All synthesized derivatives done characterized by ^{1}H NMR, MASS, and FT-IR.

FI-ETH: FT-IR (cm^{-1})

1445 (C-N stretch of quinolines having C=C-N), 1044 (C-F), 751 (C-Cl), 1650 (C=C-C=O), 1733 (C=C-COOR), 1618 (N-H bending).

MS (m/z)

269 (M^+) $C_{12}H_9ClFNO_3$ calculated value: C= 53.53, H= 3.35, Cl= 13.20, F= 7.05, N= 5.2, O= 17.84; 270 (M^+) Found value: C= 53.33, H= 3.33, Cl= 13.14, F= 7.03, N= 5.18, O= 17.77.

$^+$H NMR (dmso d_6 300 MHz) δ ppm= 7.06 (1H, s, J= 7.021), 7.46 (1H, s, J= 7.424), 4.15 (1H, s, J= 4.133), 1.13 (3H, m, J= 1.056), 4.4 (2H, m, J= 4.353), 8.0 (1H, s, J= 7.954).

FII-SUL: FT-IR (cm^{-1})

1450 (C-N stretch of quinolines C=C-N), 1044 (C-F), 637 (C-Cl), 1635 (C=C-C=O), 1733 (C=C-COOR), 3325 (aromatic C-H), 2972 (C-H alkane), 1380 (SO_2 Symmetric), 1274 (SO_2 Asymmetric).

MS (m/z)

423 (M^+), $C_{19}H_{15}ClFNO_5S$ calculated value: C= 53.90, H= 3.54, Cl= 8.39, F= 4.49, N= 3.30, O= 18.91, S= 7.5; 422 (M^+) Found value: C= 53.77, H= 3.53, Cl= 8.37, F= 4.48, N= 3.30, O= 18.86, S= 7.56.

$^+$H NMR (dmso d_6 300 MHz) δ ppm= 7.06 (1H, s, J= 7.021), 7.46 (1H, s, J= 7.424), 1.13 (3H, m, J= 1.056), 4.4 (2H, m, J= 4.353), 8.0 (1H, s, J= 7.954), 7.3 (2H, m, J= 7.414), 7.71 (2H, m, J= 7.734), 2.28 (3H, m, J= 2.281).

FIII-ATY: FT-IR (cm^{-1})

1425 (C-N stretch of quinolines C=C-N), 1044 (C-F), 659 (C-Cl), 1649 (C=C-C=O), 1733 (C=C-COOR), 2973 (C-H alkane).

MS (m/z)

311 (M^+), $C_{14}H_{11}ClFNO_4$ calculated value: C= 54.01, H= 3.5, Cl= 11.41, F= 6.1, N= 4.5, O= 20.5; 311 (M^+) Found value: C= 54.01, H= 3.5, Cl= 11.41, F= 6.1, N= 4.5, O= 20.5.

$^+$H NMR (dmso d_6 300 MHz) δ ppm= 7.83 (1H, m, J= 7.844), 7.54 (1H, m, J= 7.615), 8.75 (1H, s, J= 8.728), 1.13 (3H, m, J= 1.056), 4.4 (2H, m, J= 4.353), 2.27 (3H, s, J= 2.299).

FIV-BZO: FT-IR (cm^{-1})

1466 (C-N stretch of quinolines C=C-N), 1030 (C-F), 618 (C-Cl), 1690 (C=C-C=O), 3099 (aromatic C-H).

MS (m/z)

373 (M^+) $C_{19}H_{13}ClFNO_3$ calculated value: C= 61.12, H= 3.48, Cl= 9.51, F= 5.09, N= 3.75, O= 17.15; 373 (M^+) Found value: C= 61.12, H= 3.48, Cl= 9.51, F= 5.09, N= 3.75, O= 17.15.

$^+$H NMR (dmso d_6 300 MHz) δ ppm= 7.83 (1H, m, J= 7.844), 7.54 (1H, m, J= 7.615), 8.75 (1H, s, J= 8.728), 1.13 (3H, m, J= 1.056), 4.4 (2H, m, J= 4.353), 1.13 (3H, m, J= 1.056), 4.4 (2H, m, J= 4.353), 7.79 (2H, m, J= 7.812), 7.34 (2H, m, J= 7.386), 7.57 (1H, s, J= 7.601).

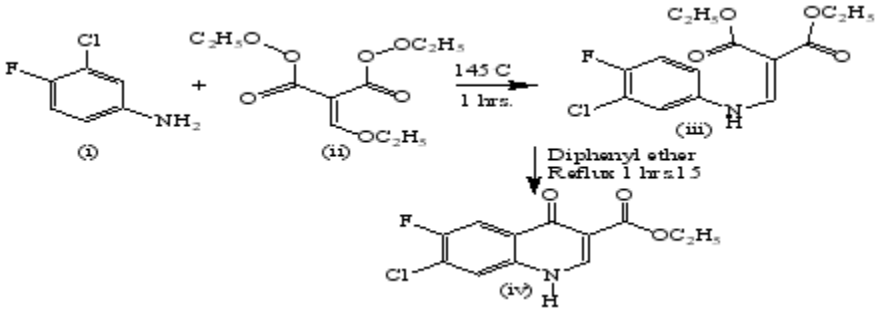
FV-BZY: FT-IR (cm^{-1})

1494 (C-N stretch quinolines having C=C-N), 881 (C-F), 658 (C-Cl), 1655 (C=C-C=O), 3423 (Aromatic C-H), 2969 (C-H alkane).

MS (m/z)

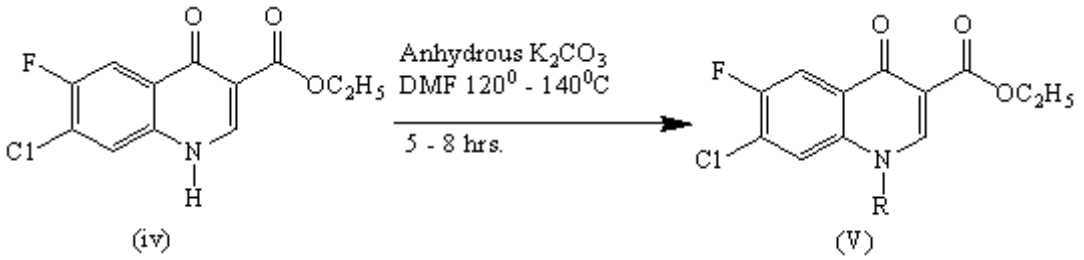
359 (M^+) $C_{19}H_{15}ClFNO_3$ calculated value: C= 63.50, H= 4.17, Cl= 9.88, F= 5.29, N= 3.89, O= 13.37; 359 (M^+) Found value: C= 63.50, H= 4.17, Cl= 9.88, F= 5.29, N= 3.89, O= 13.37.

$^+$H NMR (dmso d_6 300 MHz) δ ppm= 7.23 (1H, s, J= 7.299), 7.97 (1H, m, J= 7.973), 4.26 (2H, m, J= 4.253).



[Where **(i)** = 3-chloro-4-fluoroaniline; **(ii)** = Diethylethoxymethylenemalonte **(iii)** = 3-chloro-4-fluoroanilinomethylene malonic acid diethyl ester **(iv) =** ethyl 7-chloro-6-fluoro-4-oxo1, 4-dihydro-quinoline-3-carboxylate]

Scheme 1:



[Where **(iv) =** ethyl 7-chloro-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylate; **(v)** = ethyl 7-chloro-6-fluoro-4-oxo-1-R, 4-dihydro-quinoline-3-carboxylate; K_2CO_3= Potassium carbonate; DMF= N, N, dimethyl formamide].

Scheme 2:

RESULTS AND DISCUSSION

Chemistry

The preparation of N-1 substituted fluoroquinolone derivative I (ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate) is outlined in scheme 1. 3-chloro-4-fluoroaniline (i) was treated with diethylethoxymethylenemalonate (ii) with 145^{0}C heat for 1 hrs. approximate and then treated with diphenyl ether heated at 250^{0}C approximate and gives its ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (iv) over all yield 80%. The synthetic pathways for preparation of N-1 substitution in (iv) by treated with Anhydrous K_2CO_3, DMF at 120^0- 140^{0}C for 5 - 8 hrs. N-1= R (P-toluenesulphonyl chloride, Acetyl chloride, Benzoyl chloride, Benzyl chloride respectively.) is outlined in scheme 2. The physicochemical parameters are recorded are given in the table 2.

Table 1: Physico-chemical properties of the Derivatives

Derivatives	Substituents R	Melting Point	Yield (%)	R_f	Solvent System
FI-ETH	H	250^0-253^{0}C	80	0.64	Ethyl Acetate:Benzene:Acetone5:2:2
FII-SUL	$C_7H_7ClO_2S$	261^0-266^{0}C	70	0.9	Ethyl Acetate:Benzene:Acetone4:2:2
FIII-ATY	C_2H_3ClO	243^0-248^{0}C	67	0.82	Ethyl Acetate:Benzene:Acetone 5:2.5:2
FIV-BZO	C_7H_5ClO	256^0-260^{0}C	65	0.64	Ethyl Acetate:Chloroform:Acetone 5:2:2
FV-BZY	C_7H_7Cl	257^0-261^{0}C	62	0.48	Ethyl Acetate:Hexane4:1

Table 2: Test Result of Derivatives with Standard Drug Ofloxacin

Organism	Zone of Inhibition (mm) Derivatives with concentration (µg/ml)															Ofloxacin (10mg/ml)
	FI-ETH			FII-SUL			FIII-ATY			FIV-BZO			FV-BZY			
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	
K. Pneumonia	15	21	28	14	22	30	19	24	31	15	19	21	17	19	22	38
B. subtilis	26	34	39	25	35	40	23	36	33	20	23	32	29	32	34	42
S. aureus	22	24	40	20	23	36	29	32	38	22	25	35	23	25	27	41
E. coli	29	30	32	28	29	31	25	30	32	24	29	30	23	26	30	34

Table 3: Antioxidant activity Test Result of Derivatives

Derivatives	DPPH radical scavenging, IC50 (µg/ ml)[a]	H_2O_2 scavenging IC50 (µg/ ml)[b]	NO-scavenging IC50 (µg/ ml)[c]
FI-ETH	81.69	287.71	79.24
FII-SUL	14.98	143.51	61.73
FIII-ATY	68.63	225.76	31.94
FIV-BZO	80.32	245.18	68.34
FV-BZY	92.9	286	83.46667

Where [a] **IC 50** for Ascorbic acid (standard drug) is 32.0 (µg/ml); [b] **IC 50** for Ascorbic acid (standard drug) is 67.36 (µg/ml); [c] **IC 50** for Rutin (standard drug) is 15.07 (µg/ml).

Table 4: Antioxidant activity (Reducing Power Assay) of Derivatives

Concentration (µg/ml)	Ascorbic acid (std. drug) Absorbance	FI-ETH Absorbance	FII-SUL Absorbance	FIII-ATY Absorbance	FIV-BZO Absorbance	FV-BZY Absorbance
50	0.029	0.061	0.092	0.074	0.080	0.084
100	0.036	0.070	0.101	0.083	0.089	0.093
150	0.057	0.098	0.129	0.092	0.106	0.121
200	0.078	0.112	0.140	0.116	0.130	0.145
250	0.105	0.129	0.167	0.133	0.147	0.162

In-vitro antibacterial activity

All synthesized derivatives are active against gram ($+^{ve}$) and ($-^{ve}$) bacterial growth but increasing concentration of the derivatives that is increased inhibitory action on bacterial growth. This inhibition seen on *B. subtilis, S. aureus,* and *E. coli* with concentration of 150 µg/ml. as shows in table 2.

In-vitro antioxidant activity

Antioxidant activity shown is excellent in all synthesized derivatives, and antioxidant activity done by four methods DPPH free radical scavenging assay is capable of hydrogen donating nature and synthesized all derivatives show the hydrogen donating nature, Hydrogen peroxide radical scavenging activity ability to, nitric oxide assay is Outlined in table 3. and reducing power assay is showed the increasing the absorbance of reaction mixture to increase the reducing power, and all synthesized derivatives show the reducing power. outlined in table 4.

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