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DESIGN, SYNTHESIS & EVALUATION OF NOVEL SUBSTITUTED FLUOROQUINOLONE-BENZIMIDAZOLE ANTIBACTERIALS

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ABSTRACT

The current developments of the chemical and biological aspects of fluoroquinolones in a chronological manner touching upon their antibacterial properties based on the structure- activity relationship while pointing out to their mode of action. . Out of more than 8000 analogues of fluoroquinolones synthesized bearing variety of ring systems, a few dozen have been established in the market, and more are in the horizon to be introduced. Bacterial resistance to fluoroquinolones is seriously challenging the clinical applications of this class of antimicrobial agent. Most of the fluoroquinolones currently on the market or under development have only moderate activity against many gram-positive cocci, including staphylococci and streptococci. Fluoroquinolones effectiveness is concentration-dependant. It has been shown that in order for bacteria to be highly susceptible to Fluoroquinolones and prevent resistant mutants, the maximum concentration must be 10 times that of the minimum inhibitory (MIC) concentration when treating against gram-positive bacteria. The antibacterial activity of some substituted Benz imidazole derivatives against Gram negative bacteria Escherichia coli was investigated.

KEYWORDS Fluoroquinolones, Antibacterial, Gram-positive, Resistant mutants, Benzimidazole.

INTRODUCTION

The fluoroquinolones comprise the fastest growing, relatively large, and most interesting group of antibacterial drugs because because of their broad antibacterial spectrum both gram positive and gram negative bacteria and their in-vivo chemotherapeutic efficacy. In multiple species of bacteria, early biochemical evidence indicated that fluoroquinolones damage bacterial DNA and lead to defects in negative supercoiling. This effect was linked to

inhibition of DNA gyrase activity, an enzyme found in all bacteria. In concert with other proteins, gyrase catalyzes changes in the degree of double-stranded DNA supercoiling. In this capacity, it plays a vital role in DNA packing, replication and transcription. The active holoenzyme is a heterotetramer composed of two subunits each of gyrA and gyrB (A2B2). GyrA binds to DNA and mediates strand breakage and rejoining activity, whereas gyrB contains the ATP binding site. GyrA activity involves cleavage of both DNA strands, (mediated by an enzyme-DNA covalent intermediate), passage of DNA through the break and religation of the strand.

Structure-Activity Relationship

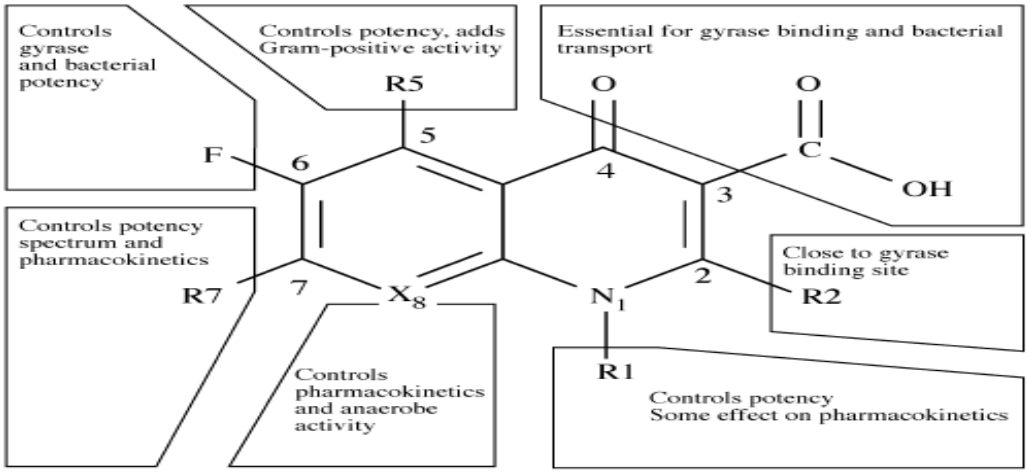


Figure No: 1 Overview Of SAR of Fluoroquinolone

Position 1

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.

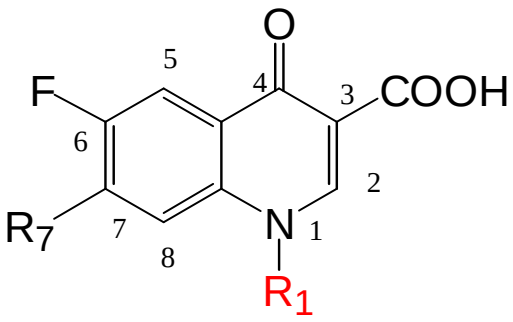
STERIMOL length is defined as length of substituent along the

Axis of bond between the substituent and the parent molecule.

Subsequently, the discovery of potent quinolones with N-1 alkyl and N-1 cyclopropyl substitutions indicated that with respect to an N-1 substituent, in addition to steric bulk, there are other factors such as electronic- π donation and ideal spatial effects that also have a great influence on their biological activities.

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.

In general, cyclopropyl group appears to be optimum for activity. e.g. Ciprofloxacin.

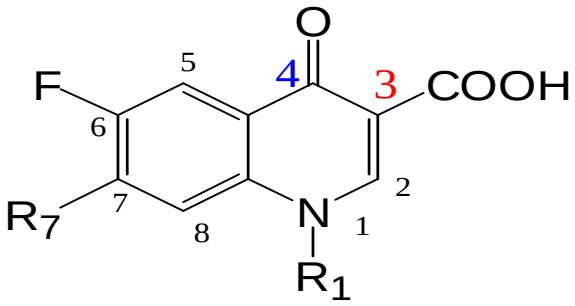


Position - 3

Position 3 and 4, having a link between the carboxylic acid group and the keto group are generally considered necessary for binding of quinolones to DNA gyrase .

Classical studies have produced no active quinolone with a significant modification of C-3 carboxylic acid group, with exception of groups which are

converted in vivo to carboxylic acid group.



Position - 4

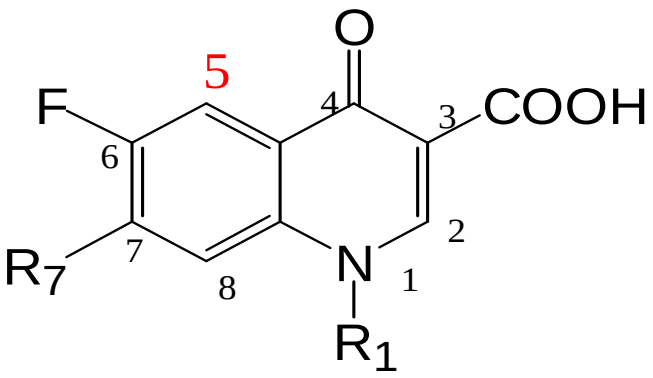
Position - 4 has not been extensively explored and replacement of 4- keto group with other groups has generally produced inactive or weakly active compounds.

Position - 5:

Compounds with small substituents such as nitro, amino, halo,alkyl groups have been synthesized. Among them, C-5 amino group enhances absorption and / or tissue distribution. e.gSparfloxacin .

The incidence of photo toxicity of Sparfloxacin is the lowest of theFluoroquinolones, because of the presence of the 5- amino group,which counteracts the

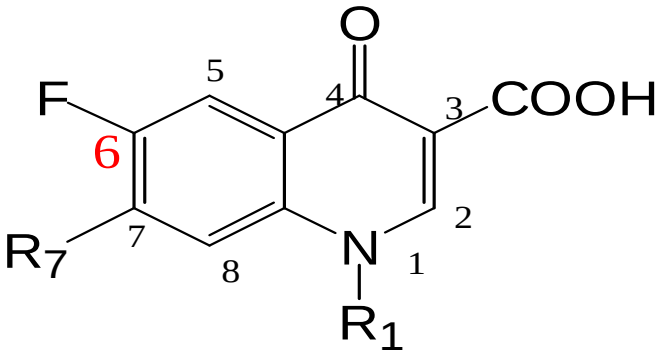
effect of the 8- fluoro substituent .



Position - 6

Of various C-6 substituents, H, Cl , Br , F , CH_3 , S- CH_3, CO CH_3,CN, NO_2 etcthe addition of a fluorine atom resulted in a dramatic increase in anti-bacterial potency .

Fluoro group at C-6 seems to improve both the DNA gyrase complex binding (2 to 17 folds) and cell penetration (1 to 70 folds)of the corresponding derivatives with no substitution at C-



Position - 7

C-7 position has great impact modulating potency, spectrum biopharmaceutics and pharmacokinetics.

C-7 substituent is regarded as drug –enzyme interaction domain, it is also concluded that the cell permeability is dominantly controlled by C-7 substituents. It also affect the interaction with target

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-cacboxylic 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.

In general, quinolones with small or linear C-7 substituents (H , Cl , CH_3 , NH_2 -CH_2-CH_2-NH_2 , NH- CH_3 , NH-NH_2) possess moderate to weak anti-bacterial activities .

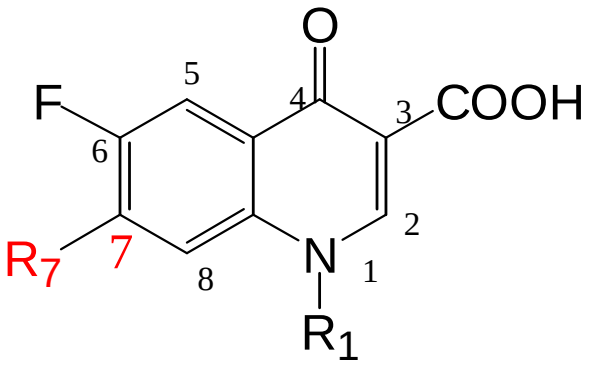
Various substitutions tried at C-7 position are -

- Substituted piperazinyl

-Substituted pyrrolidinyl

-Substituted morpholinyl

The pipeprazine moiety of 7-piperazine quinolones possesses enough structural flexibility to allow product optimization. In general, the substitution of methyl at 4 position of the piperazinylgroup enhances gram-positive anti-bacterial activity with slight decrease in gram-negative activity.



Position – 8

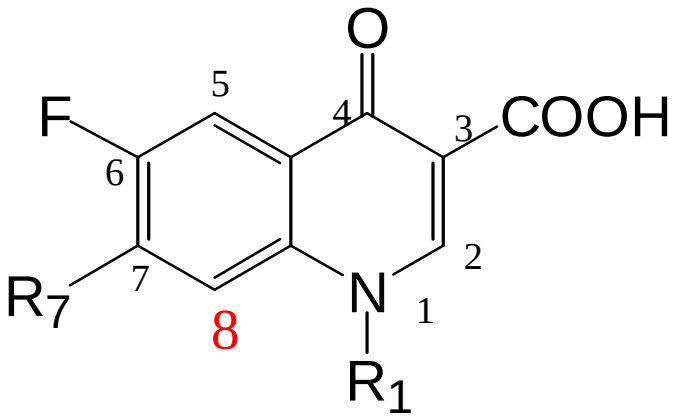
C-8 fluoro or chloro derivatives are more active

in-vivo, owing to better oral absorption.

Oxygen substituent at C-8 position , where substituent is part of ring system has been shown to have better in vivo efficacy .

C-8 methoxy or ethoxy group appears to increase the spectrum of activity.

C-8 methoxy(e.gGatifloxacin) has been shown to contribute significant activity against anaerobes .



MODE OF ACTION

Fluroquinolones antimicrobial agents with highly chemotherapeutic relevance, it is believed that they directly inhibit DNA synthesis and exert their action by inhibiting DNA gyrase and topoisomerase IV, both type second isomerases.

Most fluoroquinolones would have preferential affinity for topoisomerase IV in Gram +ve bacteria such as *Staphylococcus aureus*, but DNA gyrase would be the primary drug target in gram-negative bacteria such as *Escherichia coli.*

In general they act by interfering with the action of bacterial DNA gyrase, which results in the degradation of choromsomal DNA and leads to termination of chromosomal replication and interference with cell division and gene expression.

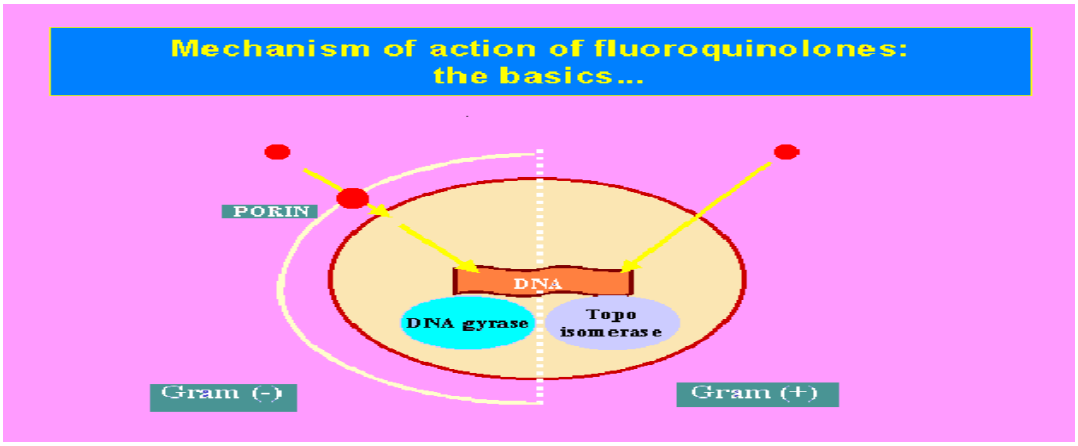


Figure no:2

EXPERIMENTAL WORK

Step 1

General Procedure For Synthesis Of 2-Chloromethyl Benz Imidazole Derivatives:

A) 2 (Chloromethyl) 1*H*benzimidazole[46].

O-phenylenediamine (0.13mol), monochloroacetic acid (0.104 mol) and 25 ml of conc. hydrochloric acid were taken in an RBF and refluxed for 8hrs. Reaction was monitored by TLC. A test portion was dumped in water and basified with ammonia solution. The solid was extracted with ether and TLC of this ether extract was checked for the completion of reaction. After completion of the reaction, the reaction mixture was poured in ice-cold water. It was then basified with conc. ammonia solution. The solid precipitated was filtered immediately and dried.

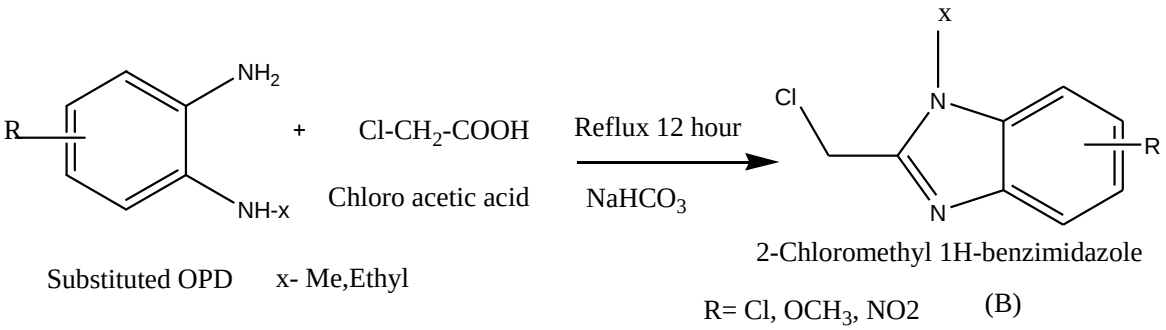


Figure no: 3

Step 2

General Procedure For Synthesis Of N_1-Alkyl / Aryl- Chloro-6-Fluoro-1,4-Dihydro-4-Oxyquinoline-3-Carboxylic Acid

A mixture of 3-chloro-4-fluoroaniline **2a** (0.01 mol) and diethylethoxymethy lenemalonate**2b** (0.01 mol) was heated at 120 -130^{0}C. After 2 h, the resulting EtOH was evaporated off.The crude malonate **2c** was used in the successive reaction without further purification.To crude malonate diphenyl ether (50 mL) was added and refluxed for 2 h. After the solution cooled, the resulting precipitate was filtered off, washed with benzene, and dried. The solid was recrystallized from DMF to give **2d** and characterised by melting point(reported >300, observed >300).

A mixture of **2d** (0.01mol), K_2C0_3 (0.025 mol), alkyl/aryl halide (0.05 mol), and DMF (20 mL) was heated at 80-90 ^{0}C with stirring. After 10 h, the mixture was evaporated to dryness and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with H_20, dried, and evaporated to dryness. The crude product**2e** was used in the successive reaction without further purification. A mixture of crude**2e** (0.0091 mol) and 2 N NaOH (0.05 mol) was refluxed with stirring. After 2 h, the mixture was acidified with AcOH, and the resulting precipitate was filtered off, washed with H_20, and dried. The solid was recrystallized from DMF to yield **2f**and characterised by melting point**.**

A mixture of the product i.e., N_1 substitued 7-chloro-6 –fluoro-1,4-dihydro-4-oxyquinoline-3-carboxylic acid **2f**(2.7 mmol) in dry dimethyl formamide and piperazine (18 mmol) was heated at 140^{o}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

recipitate **2g** washed with water,dried and recrystalized from DMF.And was characterised by H^1NMR,IR and melting point.

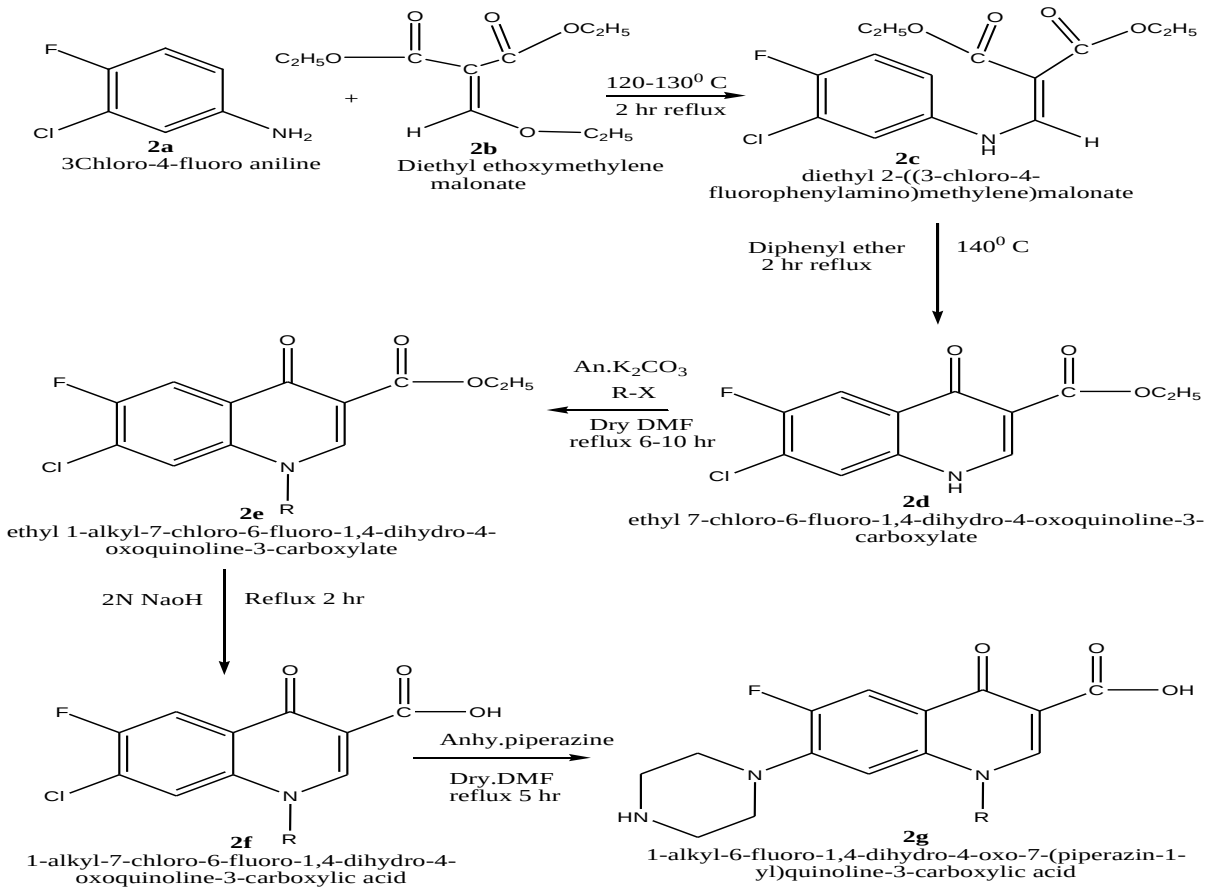


Figure No: 4

Sr.no	R
2g1	-C_2H5
2g2	

Table No: 1

Step 3

General Procedure For Synthesis of Hybrid Molecules : Microwave Assisted Synthesis

To the mixture of Ciprofloxacin/Norfloxacin/Moxifloxacine (3.31g,0.01mol, 255^{0}C) in dried absolute ethanol/ Dimethylformamide (DMF) in sufficient quantityand Triethylamine (TEA) (0.025mol, 3.48ml , 89^{0}C) with stirring , substituted Benz imidazole derivatives(0.01 mol) was added. Then above mixture was refluxed for 15 min in microwave. After refluxing, excess solvent was removed in vacuum or with hot air drier and the residue was obtained. The residue was dissolved in suitable solvent and washed with distilled water. Solvent layer separated and dried with the help of anhydrous sodium sulphate or magnesium sulphate.

Dried solvent layer evaporated and the residue washed with methanol and final compound dried (Yield 85%).

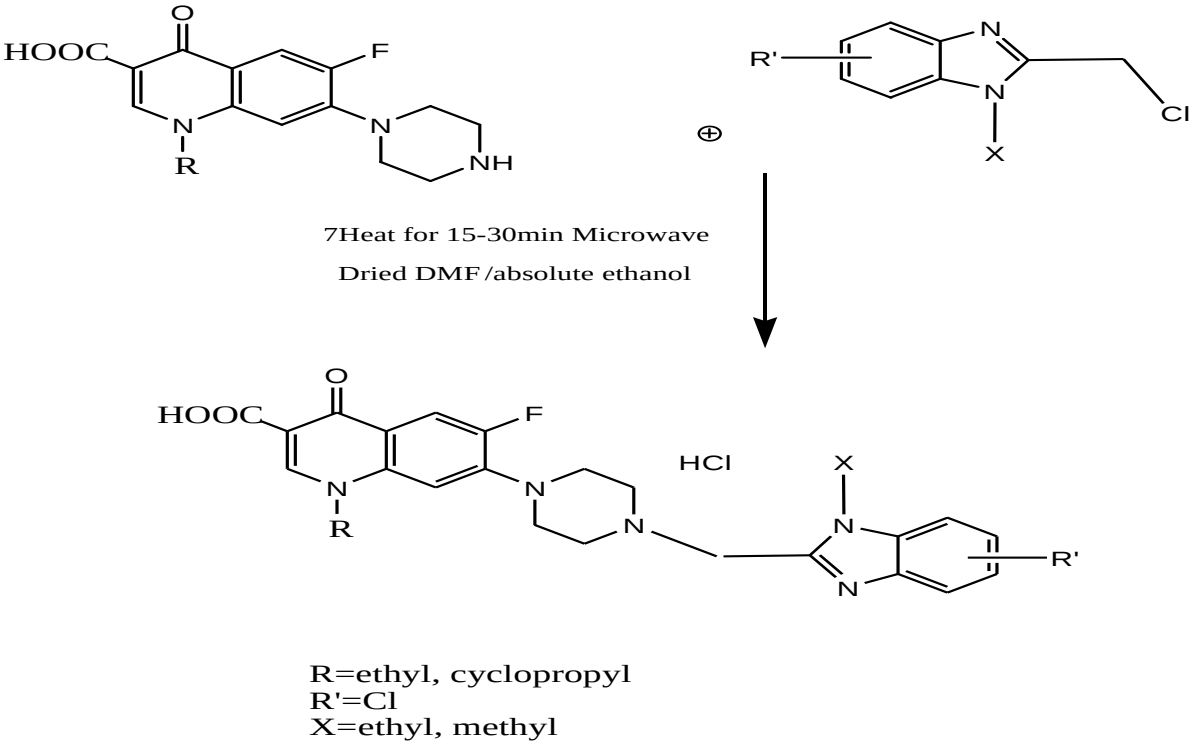


Figure No: 5

SPECTRAL ANALYSIS

Compound Code: 2g1

Structure

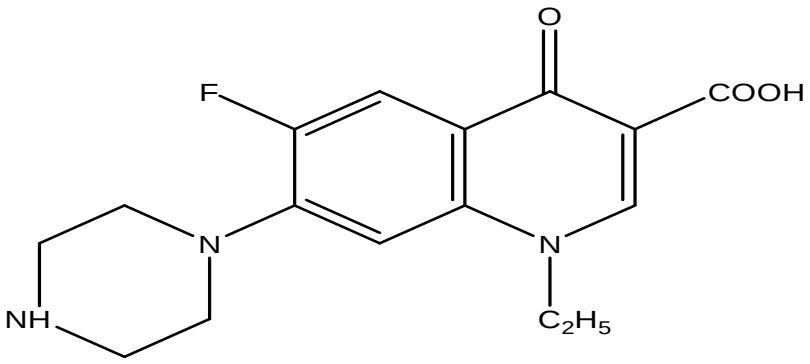


Figure No: 6

Chemical Name: 1-ethyl-6-fluoro-1,4-dihydro-4-oxo -7-(piperazin-1-yl)quinoline-3-carboxylic acid.

Molecular Formula:- $C_{16}H_{18}FN_3O_3$

Molecular Weight :-319.13

IR Graph:-

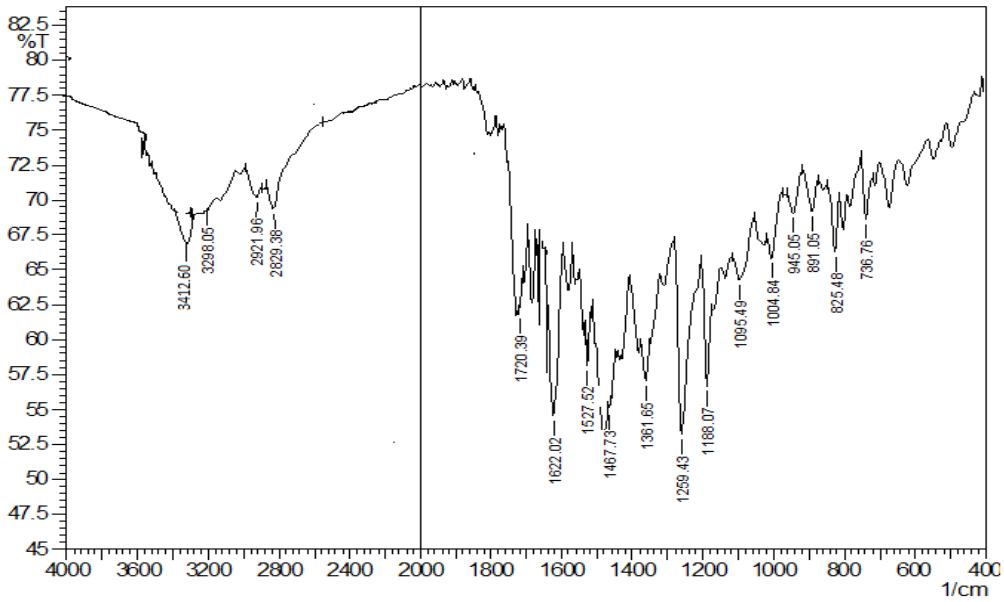


Figure No:7 IR Spectra of Compound Norfloxacin(2g1)

Table No: 2 IR Interpretations of Compound 2g1

Sr.no	Observed Frequency cm^{-1}	Standard Frequency cm^{-1}	Assingement
1	3412.60	2400-3400	O-H Stretch of COOH
2	3298.05	3100-3500	N-H Stretch of secondary amine
3	2921.96	2950-3100	C-H Stretching of aromatic ring.
4	1720.39	1700-1730	C=O Stretching, of –COOH
5	1622.02	1670-1600	Pyridone C=O Stretch
6	1309.58	1350-1280	C-N Stretching of amine
7	1259.43	1320-1210	C-O Streching of acid
8	1188.07	1250-1100	C-F Streching

H^1NMR Spectra of Compound 2g1

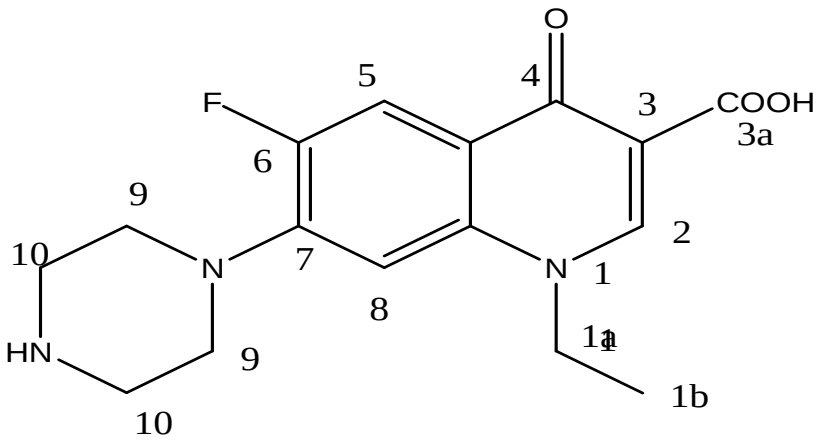


Figure No: 9

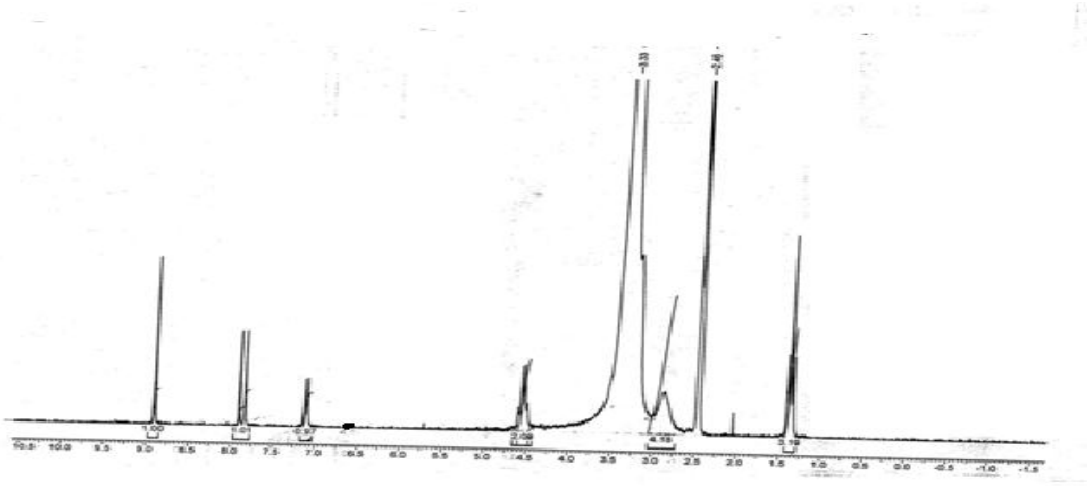


Figure No: 8H[1]NMR of Compound 2g1

Table: 3 Interpretation of H[1]NMR of Compound-2g1

Sr. no	Proton Assignment	Signal	Delta value(ppm)Observed	Delta value(ppm) Standard	Comment
1.	1b (CH_3-)3H	Triplet	1.50-1.57	0.7-1.3	-
2.	1a (-CH_2-)2H	Quartet	4.46-4.57	4.40-4.88	-
3.	2 (Aromatic)1H	Singlet	8.79	6.5-8.0	-
4.	5 (Aromatic)1H	Singlet	7.90-7.92	6.5-8.0	-
5.	8 (Aromatic)1H	Doublet	7.17-7.21	6.5-8.0	-
6.	9 (Piperazine)4H	Multiplet		2.5-3.5	Merge in water of solvent peak
7.	10 (Piperazine) 4H	Multiplet	2.71-2.76	2.5-3.5	
8.	Methanol-d4		2.49	2.50	Solvent
9.	Water in methanol d4		3.33	3.33	Water in solvent

Compound Code: 2g2

Structure

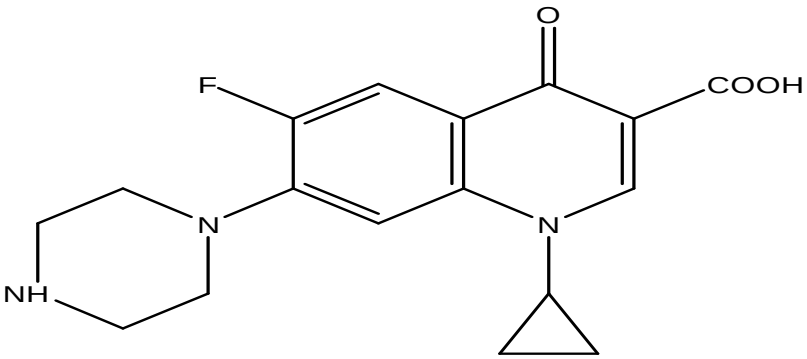


Figure No: 10

Chemical Name: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo -7-(piperazin-1-yl)quinoline-3-carboxylic acid.

Molecular formula:- $C_{17}H_{18}FN_3O_3$

Molecular Weight :-331.34

IR Graph:-

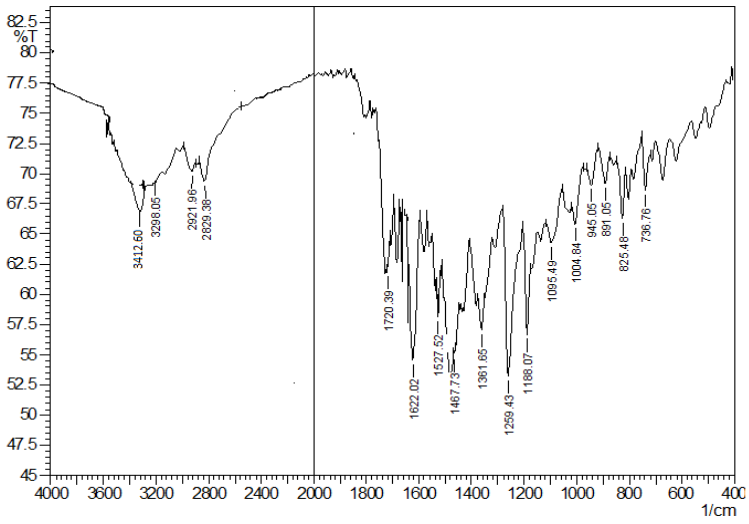


Fig: 11 IR Graphs of Compound 2g2

Table No: 4 IR Interpretations of Compound 2g2

Sr. no	Observed Frequency cm^{-1}	Standard Frequency cm^{-1}	Assingement
1	3412.60	2400-3400	O-H Stretch of COOH
2	3298.05	3100-3500	N-H Stretch of secondary amine
3	2921.96	2950-3100	C-H Stretching of aromatic ring.
4	1720.39	1700-1730	C=O Stretching, of –COOH
5	1622.02	1670-1600	Pyridone C=O Stretch
6	1309.58	1350-1280	C-N Stretching of amine
7	1259.43	1320-1210	C-O Streching of acid
8	1188.07	1250-1100	C-F Streching

H[1]NMR Spectra of

Compound2g2

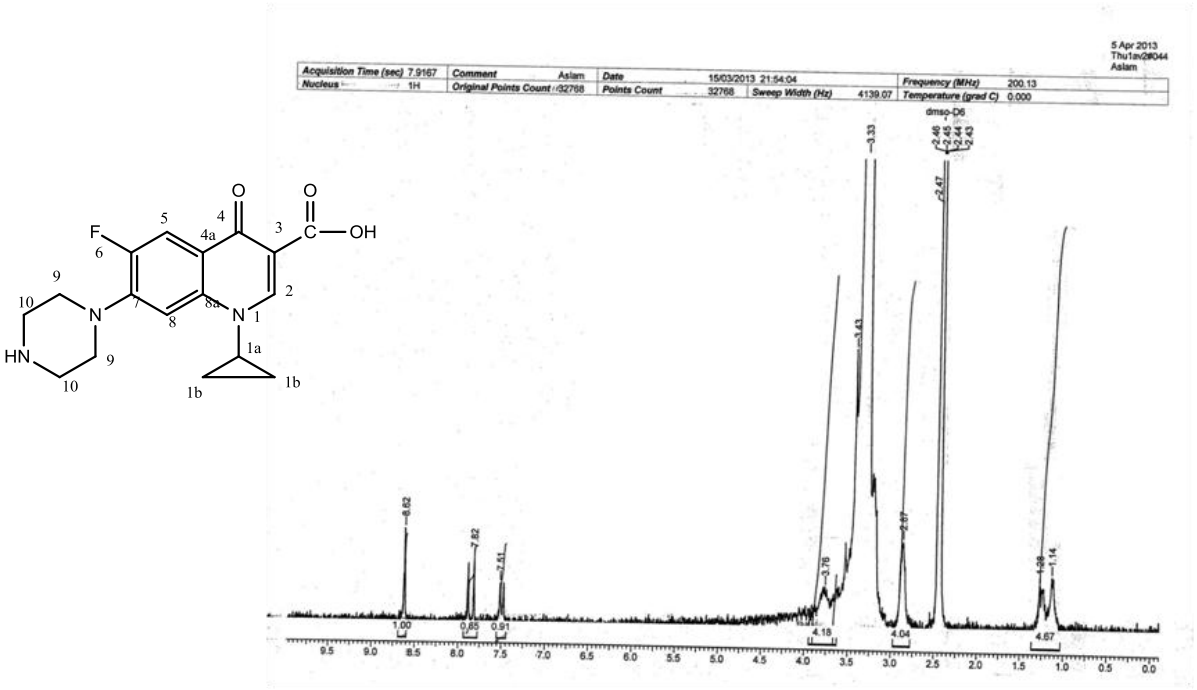


Figure: 12H[1]NMR Spectra of Compound 2g2

Table No: 5 Interpretation of H[1]NMR of Compound 2g2

Sr. no.	Proton Assignment	Signal	Delta value (ppm) Observed	Delta value(ppm) Standarad	Comment
1.	1b (-CH_2-)4H	Multiplet	1.14-1.28	1.2-1.4	
2.	1a (-CH-)1H	Multiplet		3.5-3.8	Might have merge in DMSO-d6 water peak at 3.33
3.	2 (Aromatic)1H	Singlet	8.62	6.5-8.0	-
4.	5 (Aromatic)1H	Doublet	7.82-7.86	6.5-8.0	-
5.	8 (Aromatic)1H	Doublet	7.49-7.51	6.5-8.0	-
6.	9 (Piperazine) 4H	Multiplet	3.72-3.76	2.5-3.5	-
7.	10(Piperazine) 4H	Multiplet	2.80-2.85	2.5-3.5	-
8.	3a (carboxylic) 1H			11.0-12.0	Rarely seen
9.	DMSO-d6		2.45	2.50	Solvent
10.	Water in DMSO-d6		3.33	3.33	Water in solvent

Compound code:- 1A

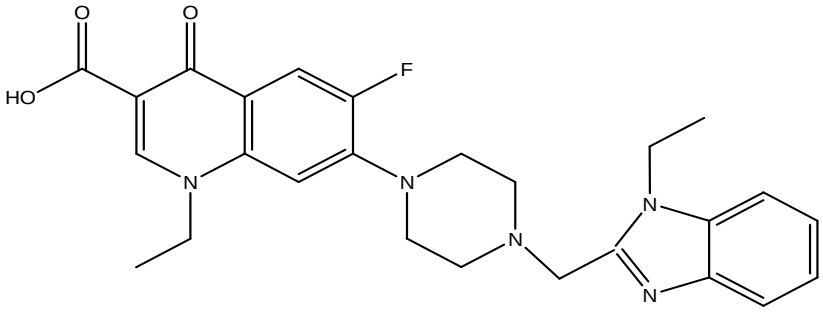


Figure no: 13

IUPAC NAME:- 7-(4-(1-ethyl-benzimidazole)-2-methyl)-piperazinyl-6-fluoro-1-ethyl-4-quinolone-3-carboxylic acid

Melting Point:-190-195^0

Molecular formula:- $^C{}_{26}H_{28}FN_5O_3$

Molecular weight:- 476

Fig.No.14: IR Spectra of 1A

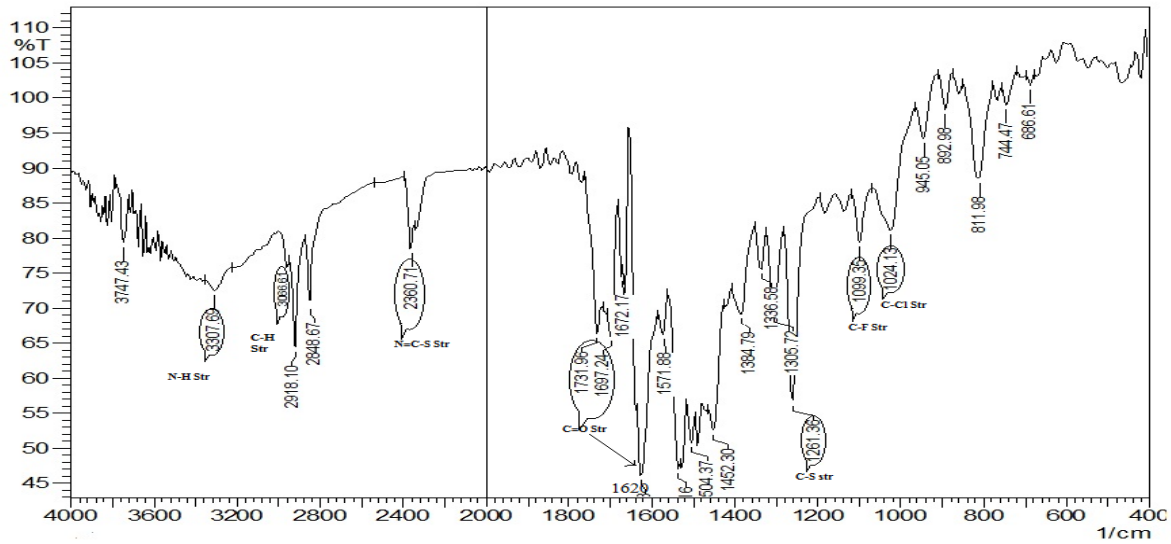


Table No. 6: IR Interpretation of IR spectra of Compound1A

SR. NO.	OBSERVED FREQUENCY cm^{-1}	STANDARD FREQUENCY $cm^{-1(46)}$	ASSIGNMENT
1	3066.61	3150-3050	Aromatic C-H Stretching
2	3380	3500-3100	N-H stretching amine
3	2848.67	2863-2843	$-CH_2-$ Stretching
4	2858.05	2863-2843	$-CH_2-$ Stretching
5	1731.96	1730-1700	C=O Stretching in acid
6	1668.31	1680-1630	Aromatic C=C Stretching
7	1620	1670-1600	C=O Stretching in ketone

8	1672.17	1690-1640	C=N Stretching
9	1099.36	1250-1100	C-F Stretching
10	892.98	900-860	C-H bending(one adjacent hydrogen atoms)
11	811.98	860-800	C-H bending (two adjacent hydrogen atoms)

BIOLOGICAL SCREENING

Antimicrobial Susceptibility Test

All synthesized derivatives of Fluoroquinolone (ciprofloxacin). Final hybrid compounds were screened for their in-vitro antibacterial activity against Gram-negative bacteria P.*aeruginosa ATCC 27853,* gram positive bacteria E.*coli ATCC 25922 and* S.*aureus ATCC 25923.*

Susceptibility Test Procedure[50, 51]:-

1. The plates were prepared with Mueller Hinton Agar for the use in the Bauer-Kirby method for rapidly growing aerobic organisms. For fastidious organisms such as Streptococcus, the agar is supplemented with 5% sterile, defibrinated blood. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Pure cultures were used as inoculum. Three to four similar colonies were selected and transferred into about 5 ml of suitable broth such as Tryptone Soya broth and was incubated at 35^0C for 2-8 hours till light to moderate turbidity developed. If the turbidity in the broth was sufficient, further incubation was not necessary.
3. 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.
4. The disc was applied using aseptic technique. Sample discs* were deposited with centers at least 24 mm apart.
5. The inoculated culture medium prepared as above was immediately incubated at 37^0C and was examined after 14-19 hours or later if necessary.
6. The zones showing complete inhibition were measured and the diameters of the zones to nearest millimeter were recorded. Results are given in Table No. 35.

7. *Note- Sample disc prepared using presterilized disc (6mm diameter) of whatman filter paper no. 1 impregnated with 50µl of 0.2µg/µl sample solution in DMSO by using micropipette.
8. The Standard ciprofloxacin (5µg/disc) and Norfloxacin (10µg/disc) disc were obtained as marketed by M/S. Hi Media Pvt. Ltd., Mumbai (India).

Table No. 7: Zone Size Interpretative Chart:- (Based On Results Obtained Using Mueller Hinton Agar)

S/N	Antimicrobial agent	Disc content (mcg)		Interpretative criteria	
			Sensitive (mm)	Intermediate (mm)	Resistant (mm)
1	Norfloxacin	10	17	13-16	12
2	Ciprofloxacin	5	21	16-20	15

Table No. 8: Quality Control Limits For Antibacterials[50, 51]:-

S/N	Antimicrobial agent	Disc content (mcg)	Diameter of zone of Inhibition (mm)		
			Quality control limits		
			E.coli ATCC25922	*S.aureus* ATCC25923	*P.aeruginosa ATCC27853*
1	Norfloxacin	10	28-35	17-28	22-29
2	Ciprofloxacin	5	30-40	22-30	25-33

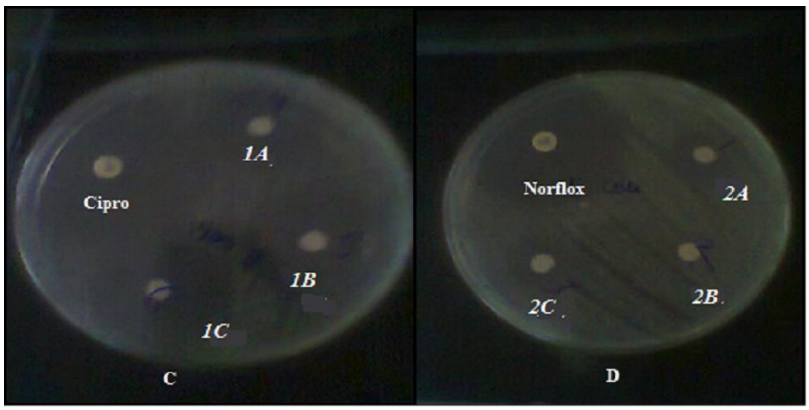


Fig:15 Zone Of Inhibition For E.Coli

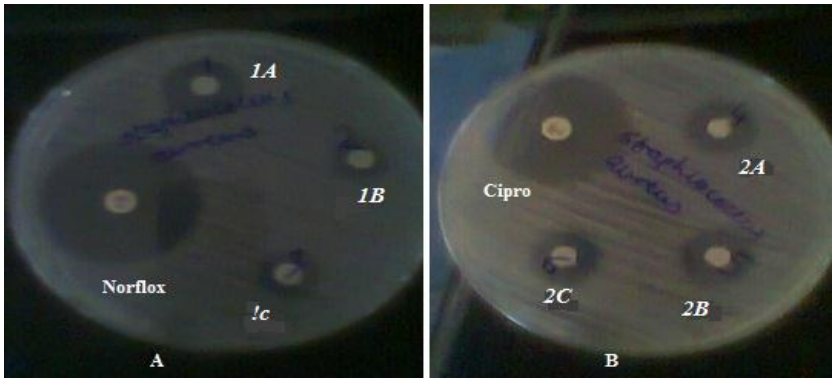


Fig:16 Zone of Inhibition For S. Aureus

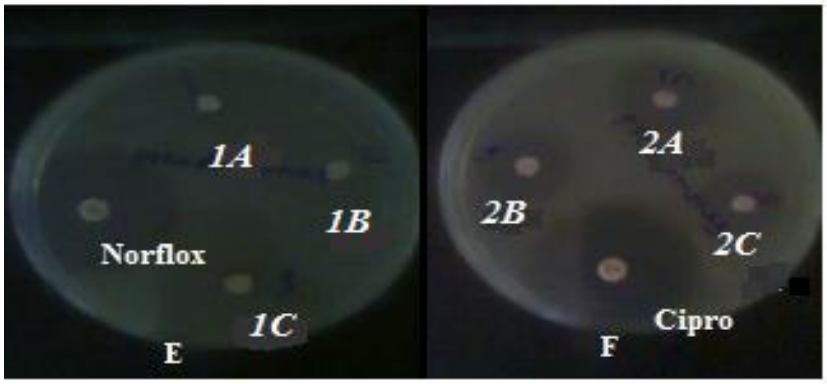


Fig:17 Zone Of Inhibition For P. Aeruginosa

RESULTS AND DISCUSSION

1. Physical Data of Hybrid Compound

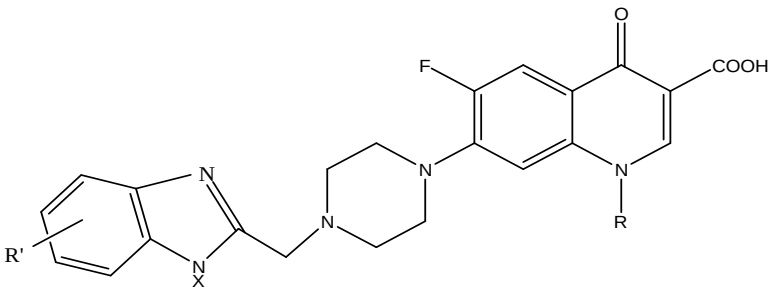


Table: 9 Physical Data of Hybrid Compound

Comp Code	R'-	X-	Mol Formula	Mol Weight	M.P. (^{O}C)	% Yield
1A	H	C_2H_5	$C_{26}H_{28}FN_5O_3$	476	190-195	80-85
1B	H	C_2H_5	$C_{27}H_{25}FN_5O_3$	488	180-183	80-85
1C	H	C_2H_5	$C_{28}H_{27}FN_5O_4$	518	200-203	80-85
2A	Cl	CH_3	$C_{25}H_{26}FN_5O_3Cl$	508.5	220-223	80-85
2B	Cl	CH_3	$C_{26}H_{23}FN_5O_3Cl$	517.5	200-203	80-85
2C	Cl	CH_3	$C_{27}H_{25}FN_5O_4Cl$	536.5	235-242	80-85

All synthesized hybrid compounds were purified by column chromatograpy by using 10:90methanol: dichloromethane as a mobile phase.

2. Spectral Analysis

IR Spectroscopy–

Some synthesized compounds (both final and intermediate) were subjected to IR spectroscopy to confirm their molecular structures by assigning characteristic functional group frequency .The observed value of frequency were compared with standard values required for interpret functional groups.

NMR-

Hybrid compound1AwAS analysed by NMR spectroscopy. Some intermediates were also analysed by NMR.Delta values (in ppm) and total number of proton and carbon in NMR fulfils the criteria of proposed structure.

Mass Spectrometry-

Selected hybrid compound was analysed by mass spectrometry and found to be uncorrected.

3. Biological Evaluation

A. Antibacterial Screening

Table No.10: Report of Antibacterial Susceptibility Test.

S/ N	Compound codes	Disc content (mcg)	Diameter of zone of Inhibition (mm)		
			Quality control limits		
			E.coli	*P. aeruginosa*	*S.aureus*
			ATCC 25922	ATCC 27853	ATCC 25923
1	1A	10	18	No zone*	17
2	1B	10	06	No zone*	13
3	1C	10	17	No zone*	12
4	2A	10	23	18	14
5	2B	10	26	20	15
6	2C	10	28	18	14
7	CIPROFLOXACIN	5	38	31	30
8	NORFLOXACIN	10	36	31	31

*****No zone means zone of inhibition is 0 mm in diameter.

Results are obtained using Mueller Hinton agar

Antimicrobial screening shows that broad spectrum activity of compound 2A, 2B, and 2C. While compound 1A, 1B, 1C are less activity than reference drug, shows no inhibition zone for P. aeruginosa.

CONCLUSION

A series of fluoroquinolone-benzimidazole hybrid was prepared and characterized by NMR, MS, and IR. The synthesized substituted fluoroquinolones were found pure; satisfactory in yield with reproducible results .Thus a series of hybrid molecules has been discovered. Hybrids were subjected to biological screening. Antibacterial activity (MIC) was done and compounds were found to be less and nearly similar active to ciprofloxacin/ norfloxacin.All synthesized derivatives of Fluoroquinolone (ciprofloxacin). Final hybrid compounds were screened for their in-vitro antibacterial activity against Gram-negative bacteria P.*aeruginosa ATCC 27853,* gram positive bacteria E.*coli ATCC 25922 and* S.*aureus ATCC 25923* and found that compounds less active except compound 2A, 2B, 2Care nearly similar to ciprofloxacin/ norfloxacin.

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