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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL MANNICH BASES OF NORFLOXACIN DERIVATIVES

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Abstract: *Introduction of Furoquinolone moiety, in the synthesis and Biological evaluation of novel mannich bases of Norfloxacin derivatives. The synthesized product was characterized by IR &H^{1}NMR and evaluated for antibacterial activity by cup plate method and tube dilution method. The activity of all synthesized compounds has shown good to mild activity against tested microbes. The compound IId has shown good activity for gram positive bacteria, whereas the compound IIe has shown good activity for gram negative bacteria and compounds IIa, IIb and IIc shows moderate activity.*

Key words: *Furoquinolone moiety, IR, H^{1}NMR, Cup plate method, Tube dilution method.*

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INTRODUCTION:

FLUROQUINOLONES

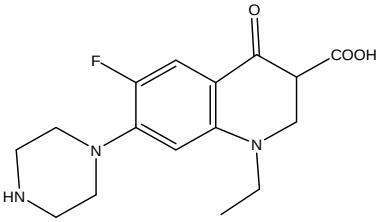
The Quinolones [1] are a family of synthetic broad spectrum antibacterial drugs. Researchers divide the Fluroquinolones Quinolones into generations based on their antibacterial spectrum. The I and II generations are narrow spectrum antibiotics and third and fourth generations of Fluroquinolones are broad spectrum antibiotics.The substitution of fluorine [2] at 6^{th} position is the Fluroquinolones. The Fluroquinolones are 1-substituted 1,4-dihydro 4-oxopyridine 3-carboxylic moiety. These have Nitrogen at 1^{st}positionis essential for anti-bacterial activity. The 3^{rd} position carboxylic group binds to DNA gyrase. The oxo pyridine group is essential and show broad spectrum of activity.TheFluroquinolones have minimal gram positive activity, but they are most active against gram negative bacilli. These are used in the treatment of Staphylococci, Streptococci, Enterococal infections. The antibiotic activity [3]of the Fluroquinolonesresults from their abiity to inhibit its DNA gyrase, an enzyme required for transcription and inhibit only the bacterial enzyme [4]The fluorine substituted Fluroquinolones increases the lipophilicity of the drug to enable it to penetrate in to tissue and cells.

MANNICH REACTION:

Compounds [5] containing at least one hydrogen atom condenses with formaldehyde and primary, secondary amines or ammonia to give a product known as mannich base.The Mannich [6] reaction is one of the most widely utilized chemical transformations for the construction of nitrogen containing compounds, with the increasing occurrence of nitrogen in drugs and natural products, highly asymmetric variants of the mannich [7] reaction are desirable.

NORFLOXACIN:

Norfloxacin [8] is an Organic compound with a quinoline ring fused with piperzineand it is an 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,2,3,4-tetra hydro-quinoline-3-carboxylic acid.



1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,2,3,4-tetrahydro-quinoline-3-carboxylic acid

MECHANISM OF ACTION OF FLUROQUINOLONES:

The Fluroquinolones [9] inhibit the enzyme bacterial DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. In gram negative bacteriathe FQ's inhibits the positive supercoiling of the strands. The DNA gyrase consists of IIA and IIB subunits. The A subunit carries out nicking of DNA, B subunit introduces negativesupercoils and then A subunit introduces negative supercoils and then A subunit reseals the strands. Fluroquinolonesbindto complex DNA gyrase and DNA as a result stabilizes the enzyme and leads to breakage in DNA strandsandfaetal to bacteria.

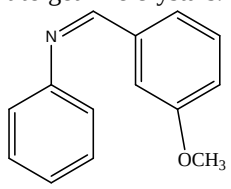
MATERIALS AND METHODS:

All Chemicals were obtained from S.D. Fine chem. Limited Mumbai. All glassware is of Borosilicate grade. Melting Points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G plate. Characterization of synthesized compounds were done by spectral studies. IR spectra were in KBr on a SHIMADZU Spectrophotometer.H^1NMR spectra were recorded on AVANCE 300 MHz Spectophotometer in $CDCl_3$ with TMS as internal standard. The Chemical shift values are in delta (ppm). Physical data, antibacterial activity were recorded in Tables.

RESULTS AND DISCUSSION:

Step I (Synthesis of N-(3-methoxybenzylidene)benzamine:

Equimolar of Aniline and anisaldehyde dissolved in 10ml methanol . A drop of acetic acid was added as a catalyst and reflux it for 1-1.5 hours at 45^oC. A pale yellow coloured product is formed which indicated formation of product. The synthesized product was filtered and dried and recrystalized by using Methanol as a solvent to get fine crystals.



(*Z*)-*N*-(3-methoxybenzylidene)benzenamine

Melting point:75^0c

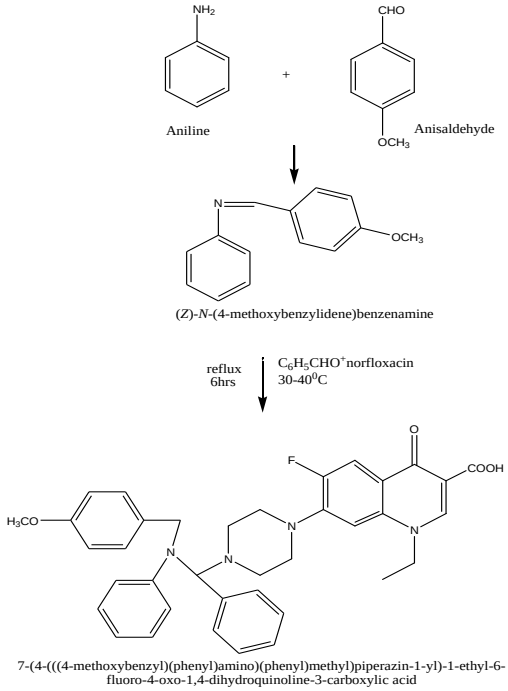
STEP-II: synthesis of 7(4((benzyl(4methoxyphenyl)amino)(phenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

PROCEDURE:

Equimolar quantity of Schiff base,different aldehydes and Norfloxacin were dissolved in 10 ml methanol. Mix well and refluxed at 45^oc for about 6 hrs. The resulted product was concentrated by heating on water bath. Coloured solid precipitate was collected and recrystallised with hot methanol.

PHYSICAL DATA OF THE SYNTHESIZED COMPOUND:

SCHEME-II(synthesis of benzaldehyde-mannich base of Norfloxacin):

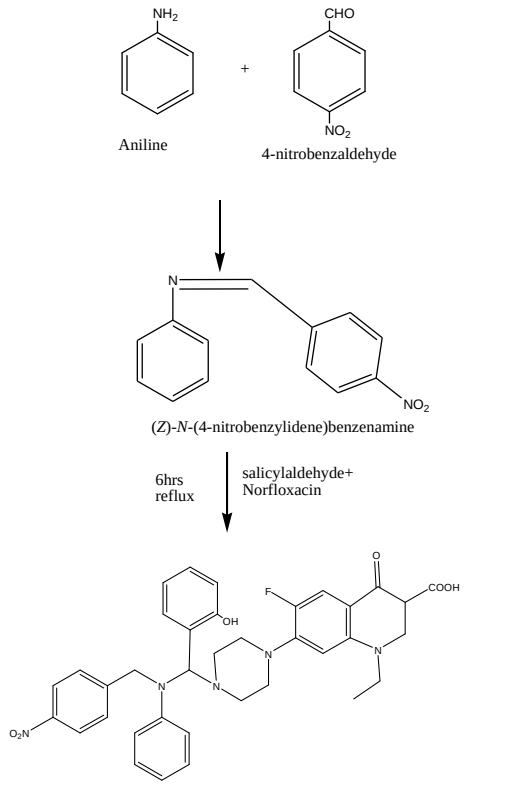


The H^1 NMR Spectral data of the compounds is furnished in Table-1

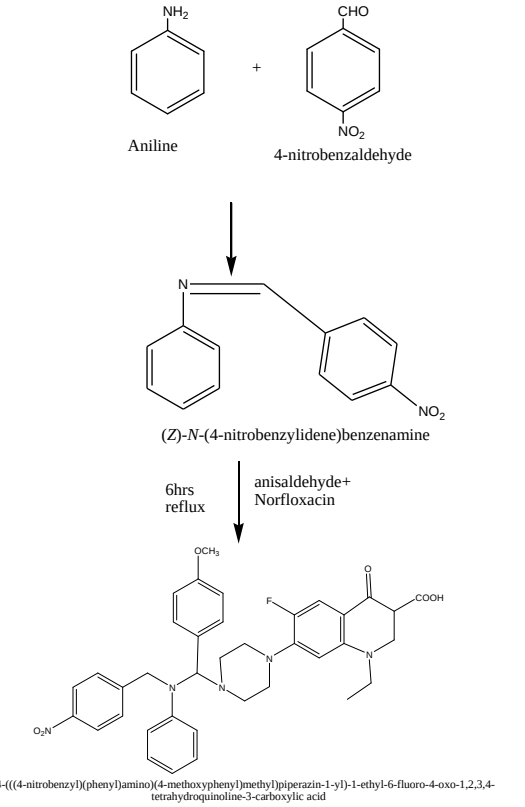
COMPOUNDS	M.P	YIELD	M.F
IIa	105°c	62%	$C_{29}H_{32}FN_4O_4$
IIb	115°c	69%	$C_{37}H_{39}FN_4O_4$
IIc	125°c	78%	$C_{36}H_{36}FN_5O_6$
IId	112°c	81%	$C_{37}H_{36}FN_5O_6$
IIe	118°c	72%	$C_{37}H_{39}FN_4O_5$

Type of protons	Absorbance peak for comp IIa	Absorbance peak for comp IIb
-CH_3	1.415t)	1.419(s)
-CH_2-	2.95(d)	2.805(d)
-COOH	8.964(s)	9.57(s)
Ar-H	7.87-7.945(m)	7.89-7.943(m)
-CH_2-CH_2-H	3.277,3.347(d)	3.257(s)

scheme-III(synthesis of salicylaldehyde-mannich base of Norfloxacin):



scheme-IV(synthesis of anisaldehyde-mannich base of Norfloxacin):



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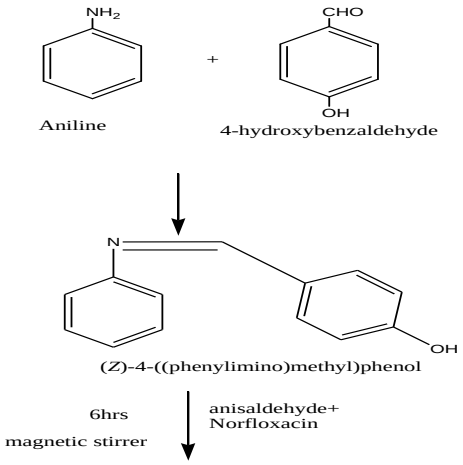
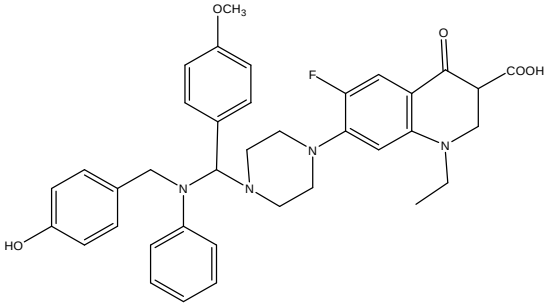
Scheme-V(synthesis of anisaldehyde-mannich base of Norfloxacin):





7-(4-(((4-hydroxybenzyl)(phenyl)amino)(4-methoxyphenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

H[1]NMRSPECTRAL DATA OF SYNTHESIZED COMPOUND-IIa&IIb

The synthesized compounds of the present study is characterized through [1]H NMR Spectra and showed characteristic absorption bands for-CH_2-CH_2, –CH_3, -CH_2-,COOH-H,, Ar-Hgroups

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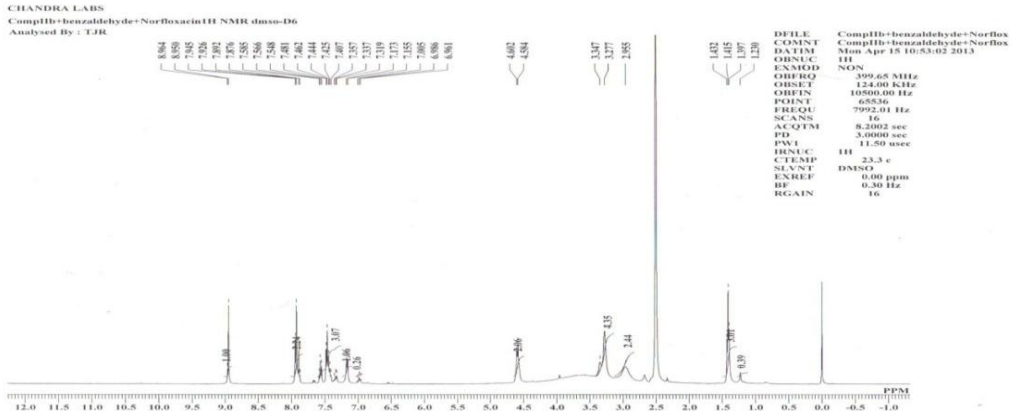


Fig 1: NMR spectral data of compound – IIa

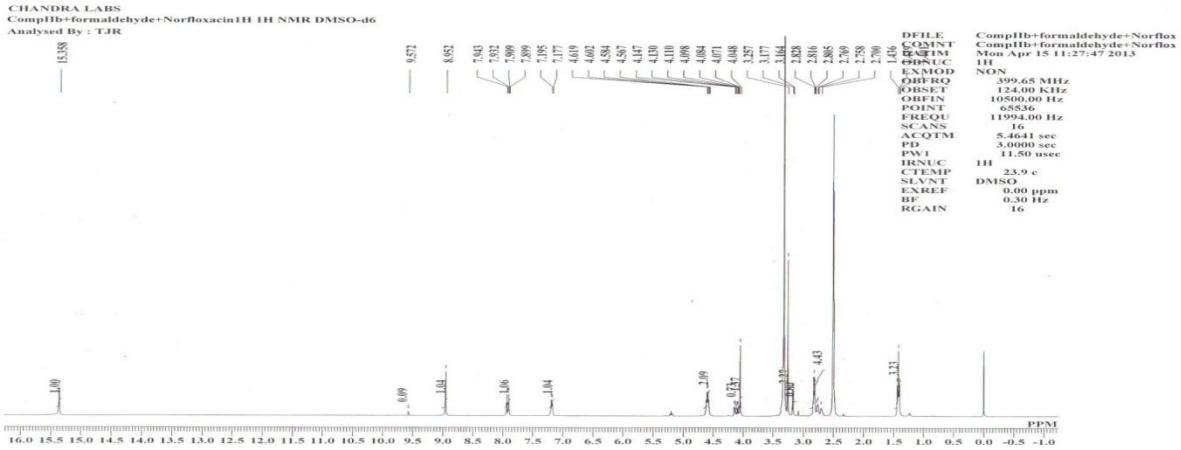


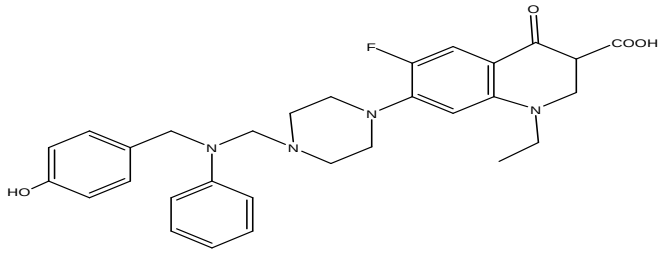
Fig 2: NMR spectral data of compound –Iib

IR SPECTRAL DATA OF SYNTHESIZED COMPOUNDS IIa-IIe

The synthesized compounds of the present study is characterized through IR Spectra and showed expected characteristic absorption bands for –CH_2-CH_3-, C=O,OH-Ar,C-N,COOH,O=C-CH_3, C-H,-C-F,-NO_2 groups.

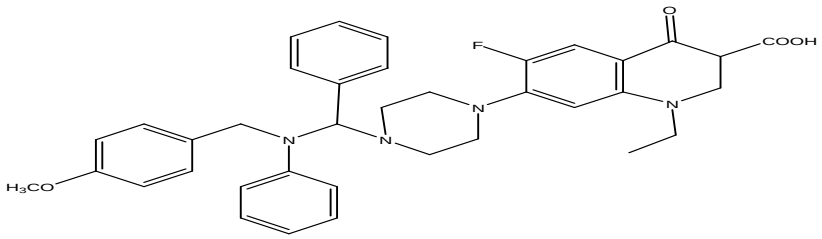
The IR Spectral data of the compounds –IIa-IIe is furnished in Table-2

S. NO	FUNCTIONAL GROUP	COMPOUND-IIa	COMPOUND-IIb	COMPOUND-IIc	COMPOUND-IId	COMPOUND-IIe
1.	$-CH_2$-(1375-1450cm^{-1})	1442.08cm^{-1}	1383.5cm^{-1}	1282.06cm^{-1}	1448.12cm^{-1}	1377.1cm^{-1}
2.	$-CH_2-CH_3$(2840-3000cm^{-1})	2979cm^{-1}	2851.14cm^{-1}	2921.96cm^{-1}	2918.37cm^{-1}	2349.2cm^{-1}
3.	OC-**CH_3**(1245-1030 cm^{-1})	-	1203.04cm^{-1}	-	1157.77cm^{-1}	1191.44cm^{-1}
4.	OH-Ar(3584-3650cm^{-1})	3383.49 cm^{-1}	-	3443.64cm^{-1}	-	3634.2cm^{-1}
5.	C-N(850cm^{-1})	834.7 cm^{-1}	831.46cm^{-1}	849.9cm^{-1}	831.42cm^{-1}	850.2cm^{-1}
6.	C=O(1540-1870 cm^{-1})	1618.54cm^{-1}	1722.02cm^{-1}	1717.80cm^{-1}	1580.8cm^{-1}	1539.7cm^{-1}
7.	COOH(3300-2200cm-1	1735cm-1	3049.32cm-1	3057.97cm^{-1}	2918.37cm^{-1}	3048.8cm^{-1}
8.	C-H_Ar(3100-3000 cm^{-1})	3051.08 cm-1	3049.32cm-1	693.6cm^{-1}	62.9cm^{-1}	850.24cm^{-1}
9.	C-F(1400-730 cm^{-1})	1373.32cm-1	720.4cm-1	1341.2cm^{-1}	1382.86cm^{-1}	1341.72cm^{-1}
10.	$-NO_2$-(1550-1500 cm^{-1})	-	-	1516.82 cm^{-1}	1580.83 cm^{-1}	-



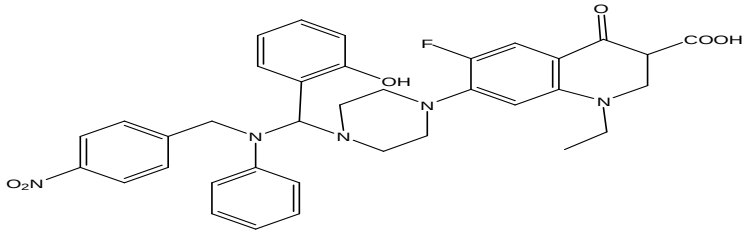
7-(4-(((4-hydroxybenzyl)(phenyl)amino)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

Fig 3: Structure of synthesized compound – IIa



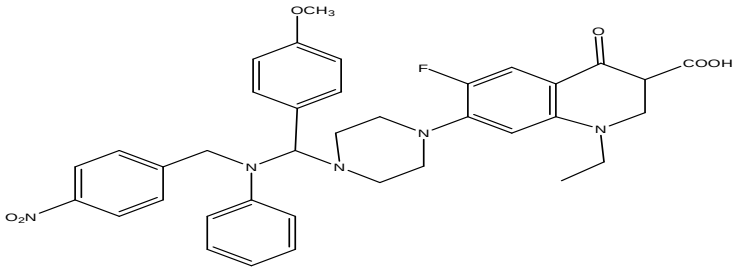
7-(4-(((4-methoxybenzyl)(phenyl)amino)(phenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

Fig 4: Structure of synthesized compound – IIb



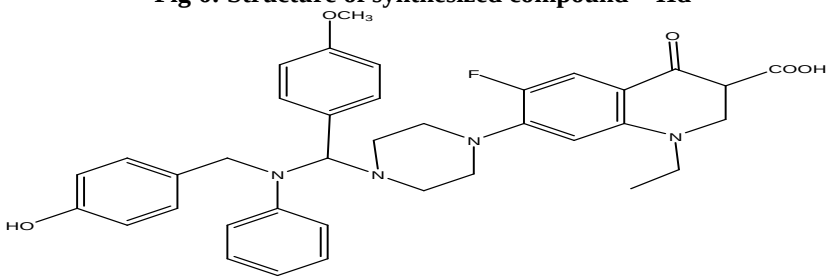
7-(4-(((4-nitrobenzyl)(phenyl)amino)(2-hydroxyphenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

Fig 5: Structure of synthesized compound – IIc



7-(4-(((4-nitrobenzyl)(phenyl)amino)(4-methoxyphenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

Fig 6: Structure of synthesized compound – IId



7-(4-(((4-hydroxybenzyl)(phenyl)amino)(4-methoxyphenyl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid

Fig 7: Structure of synthesized compound – IIe

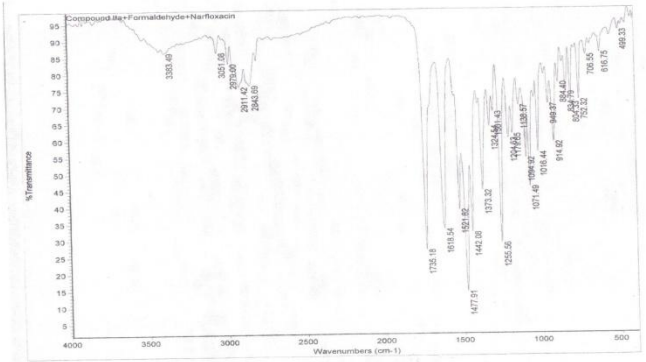


Fig 8: IR spectra of synthesized compound – IIa

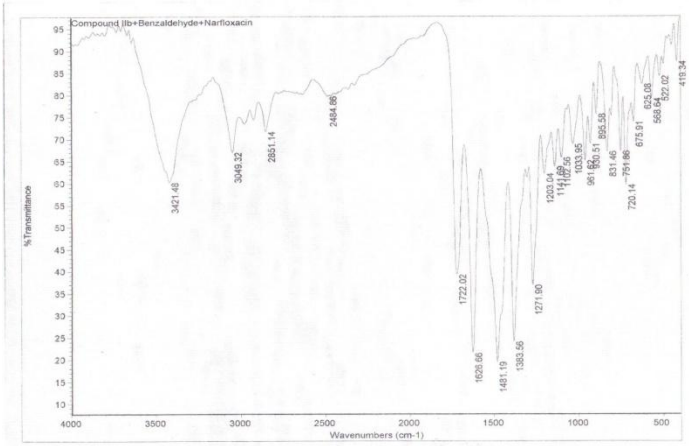


Fig 9: IR spectra of synthesized compound - IIb

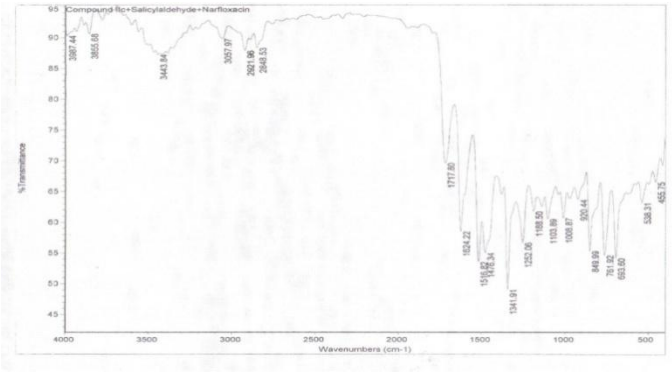


Fig 10: IR spectra of synthesized compound - IIc

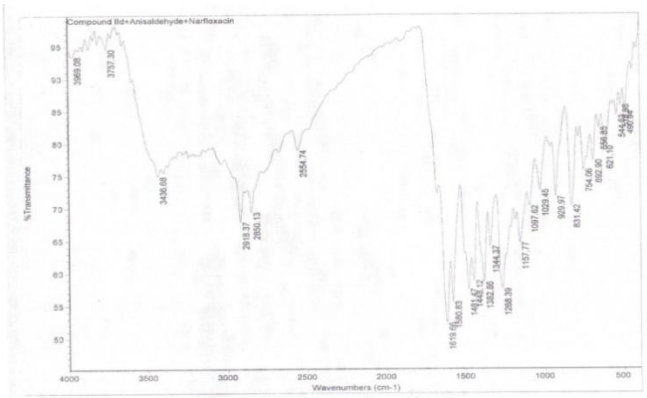


Fig 11: IR spectra of synthesized compound - IId

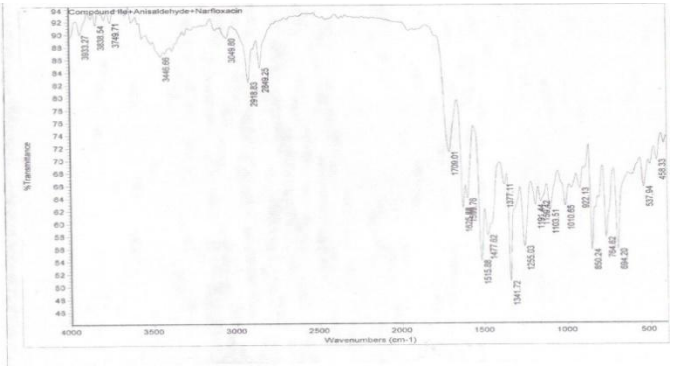


Fig 12: IR spectra of synthesized compound - IIe

ANTIMICROBIAL ACTIVITY:

All the synthesized Norfloxacin derivatives IIa-IIe were characterized and screened for their antimicrobial activities,they were tested against gram positive staphylococcus aureus and gram negative E.coli. The antimicrobial activity of the derivatives was performed by cup plate method at a concentration level of 10µg/ml. Norfloxacin was used as standard drug at a concentration of 10µg/ml.

Table 3 : Antimicrobial activities of synthesized derivatives

Compound	Antimicrobial activity	
	S. aureus	E.coli
IIa	15	10
IIb	19	09
IIc	16	13
IId	22	12
IIe	18	16
Control	--	--
Norfloxacin	20	18

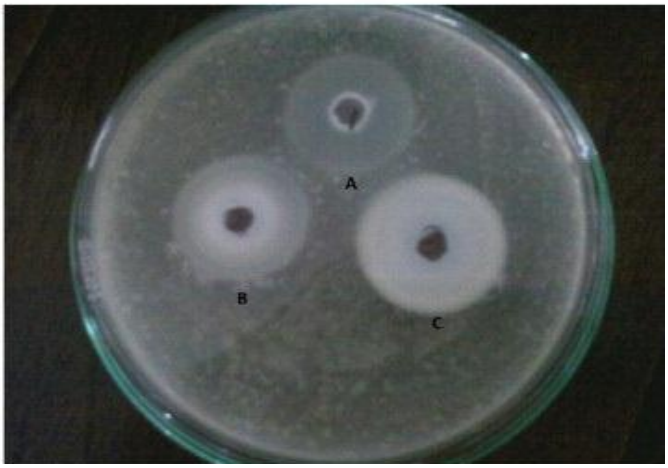


Fig 13: Zone of inhibition of S.aureus.

Note: The zone of inhibition was measured in mm from the one end to another end of inhibition zone at three diagonals and the avg value is recorded.

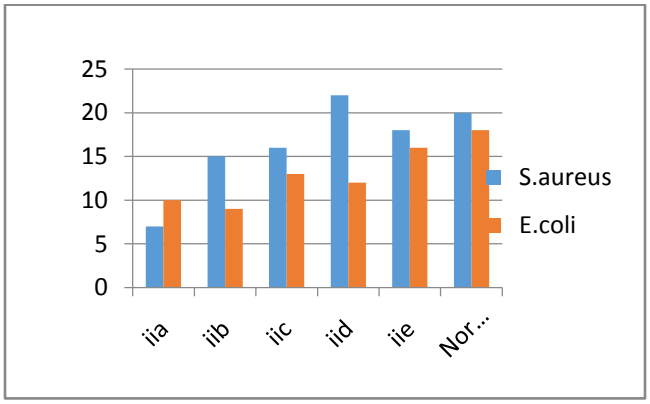


Fig 14: InvitroAntibacterial activity of synthesized compondsIIa-IIe and norfloxacin

The compound IId has good activity for and IIa, IIb, IIc, IIe has shown moderate activity for gram positive bacteria and IIe shown good activity and IIa-IId has showed moderate activity for gram negative bacteria.

CONCLUSION:

Introduction of Furoquinolone moiety, in the synthesis and Biological evaluation of novel mannich bases of Norfloxacin derivatives. The synthesized product was characterized by IR &H^1NMR and evaluated for antibacterial activity by cup plate method and tube dilution method. The activity of all synthesized compounds have shown good to mild activity against tested microbes. The compound IId has shown good activity for gram positive bacteria, whereas the compound IIe has shown good activity for gram negative bacteria and compounds IIa, IIb and IIc shows moderate activity.

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