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Synthesis and Antimicrobial Activity of Ciprofloxacin Schiff and Mannich bases

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Abstract: Ciprofloxacin was incorporated to the new series of Schiff and Mannich bases via Mannich reaction. The new compounds have been evaluated *in vitro* for their antimicrobial activity against various gram positive and gram negative bacteria. A series of these compounds 1 to 6 bearing Mannich base (**5** and **6**) was prepared from Thiadiazole. Schiff bases (**1**), (2), (3) and (4) by Benzothiazole-amine with formaldehyde and secondary/substituted primary amines. Among the synthesized compounds, **Compound 4 [**1-Cyclopropyl-6-fluoro-4-phenylimino-7-piperazine-1-yl-1,4-dihydro-quinoline-3-carboxylic acid**]** showed good activity against *S. aureous, B.subtilis, proteous mirabilis, morganella morganii.* **Compound 5** [1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(3-phenylamino-4H-[1, 2, 4] thiadiazole-5-ylideneamino)-methyl**]**-piperazine-1-yl}-1, 4-dihydro-quinoline-3-carboxylic acid] **& Compound 6 [**7-(4-{[3-(4-Chloro-phenylamino)-4H-[1, 2, 4] thiadiazole-5-ylideneamino]-methyl}-piperazine-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-oxo-1,4-dihydro-quinoline-3-carboxylic acid**]** showed excellent activity against *klabe pneumoniae, S.typhi* & *P.valgaris* . All these synthesized compounds were screened for their antimicrobial activity. Compounds **1, 2, 3, 4, 5 and 6** exhibited promising antibacterial activity. Showed *in vitro* antibacterial & antifungal activity which was comparable to that of activity of parent drug (ciprofloxacin).All the newly synthesized compounds were characterized on the basis of elemental analysis, IR, ^{1}H NMR spectra.
Keywords: Ciprofloxacin, Schiff bases, Mannich bases, Benzothiazole, Antibacterial Activity.

Introduction:

The fluoroquinolones were active against the whole bacterial spectrum responsible for complicated UTI. This has changed during the last few years and many*P.aeruginosa,* enterococcal and staphylococcal strains are intermediately susceptible or resistant to many fluoroquinolones.[4] The fluoroquinolones are a group of antibiotics that have increased in numbers in recent years. Their usefulness has greatly expanded with the introduction of several new quinolones having improved properties. Fluoroquinolones remain the most potentoral antibacterials against pathogens responsible for complicated UTI. Ciprofloxacine is considered to be the standared treatment for patients with complicated UTI and the dosage of 250 mg twice daily has been shown to be efficacious in most circumstances.[5-8] This multicentre, randomized clinical study was designed to compare a once daily regimen with 500 mg to usual twice-daily regimen with 250 mg orally for 7-21(median 8) days in patients with complicated UTI to improve the compliance of the patients.

Literature survey reveals that 2-aminobenzothiazole derivatives were synthesized and screened against Gram + and Gram – bacteria[1.] Schiff and Mannich bases of Ciprofloxacine and its derivatives were synthesized and reported for antibacterial [2], Antifungal [3].

Based on the importance of 2- aminobenzothiazole and their biological activities. The synthetic approach to the title compounds is outlined in SCHEME 1, 2 and 3.

Material and Methods:

M.P. of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using TLC Plates, using chloroform**:** methanol (8:2) solvent system. The developed chromatographic plates were visualized in saturated Iodine chamber .IR Spectra were recorded using KBr on BRUKER Spectrophotometer, NMR spectra in $CDCl_3$ on FT-NMR instrument using TMS as internal standard.

General Procedure for Synthesis of Schiff bases Compounds (1&2):

The Schiff bases was prepared by reaction of equimolar of substituted 2-Aminobenzthiazole (0.45 g) (for compound **1)** and 6-chloro-1, 3-benzothiazole-2-amine (0.55) (for compound **2)** respectively and ciprofloxacin (1.0 g) .Each reactant was dissolved in a minimum amount of ethanol, then mixed together and followed by addition of 5 ml glacial acetic acid .The solution was refluxed for 10 hrs. Then cooled to room temp. and poured in to ice cold water .The solid product was collected through filtration and then were air dried .The product was re-dissolved in ethanol for re-crystallization & then dried to give a product.

General Procedure for Synthesis of Schiff bases Compounds (3&4):

The Schiff bases was prepared by reaction of equimolar of Aniline (for compound **3)** , 4-chloro aniline (for compound **4)** and ciprofloxacin (1.0 g) respectively .Each reactant was dissolved in a minimum amount of ethanol, then mixed together and followed by addition of 5 ml glacial acetic acid .The solution was refluxed for 10 hrs. Then cooled to room temp. and poured in to ice cold water .The solid product was collected through filtration and then were air dried .The product was re-dissolved in ethanol for re-crystallization & then dried to give a product.

General Procedure for Synthesis of Mannich base Compound (5):

Step 1: (5 E)-5-methylimino-N-phenyl -4, 5-dihydro-1, 2, 4-thiadiazole-3-amine were prepared from its phenyl thiourea (2 g) in presence of hydrogen peroxide (10 ml) in slight acidic condition (HCl), the reaction was monitored by its Rf value in the solvent system of methanol & chloroform (9:1) for the completion of the reaction .The products were filtered dried & stored properly.

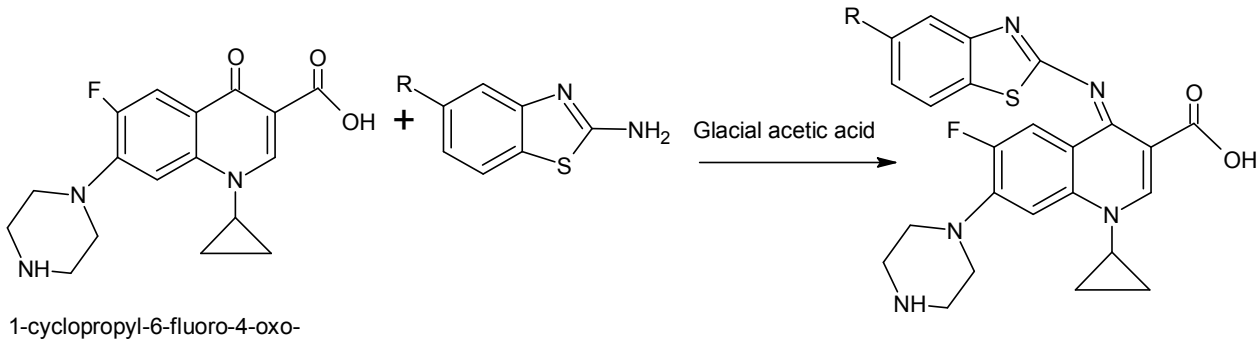
Step 2: The Mannich base of (5E)-5-(methylimino)-N-phenyl-4,5-dihydro-1,2,4-thiadizole-3-amine(1.0 g)were prepared in presence of diethyl amine (4 ml),formaldehyde (1 ml) & methanol (few drops)in ice cold condition by continuous shaking. The resultant products were kept in refrigeration for 48 hrs, after that it was filtered, dried & stored properly.

General Procedure for Synthesis of Mannich base Compound (6):

Step 1: N-(4-chlorophenyl)-5-imino-4, 5-dihydro-1, 2, 4-thiadiazole-3-amine were prepared from its phenyl thiourea (2 g) in presence of hydrogen peroxide (10 ml) in slightly acidic condition (HCl), the reaction was monitored by its Rf value in the solvent system of methanol & chloroform (9:1) for the completion of the reaction .The products were filtered, dried & stored properly.

Step 2: The Mannich base of N-(4-chlorophenyl)-5-imino-4,5-dihydro-1,2,4-thiadizole-3-amine (1.0 g) were prepared in presence of diethyl amine (4 ml),formaldehyde (1 ml) & methanol (few drops) in ice cold condition by continuous shaking. The resultant products were kept in refrigeration for 48 hrs, after that it was filtered, dried & stored properly.

General Reaction for:
Scheme 1:



1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

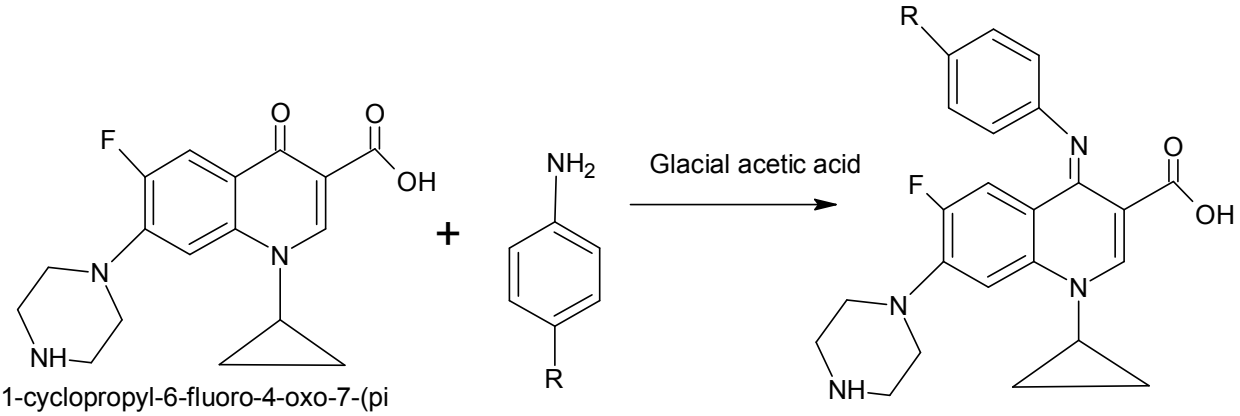
Where R = **H** for **Compound 1**

4-(Benzothiazole-2-ylimino)-1-cyclopropyl-6-fluoro-7-piperazine-1-yl-1, 4-dihydro-quinoline-3-carboxylic acid

R = **Cl** for **Compound 2**

4-(5-Chloro-benzothiazole-2-ylimino)-1-cyclopropyl-6-fluoro-7-piperazine-1-yl-1,4-dihydro-quinoline-3-carboxylic acid

Scheme 2:



1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

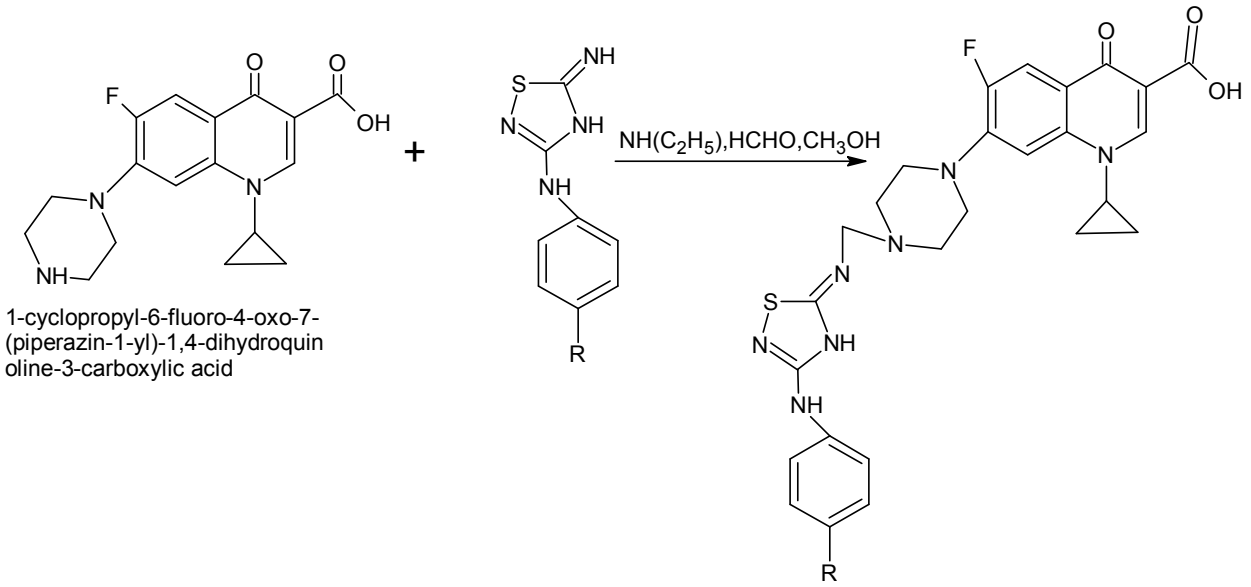
Where R = **H** for **Compound 3**

1-Cyclopropyl-6-fluoro-4-phenylimino-7-piperazine-1-yl-1,4-dihydro-quinoline-3-carboxylic acid

R = **Cl** for **Compound 4**

4-(4-Chloro-phenylimino)-1-Cyclopropyl-6-fluoro-7-piperazine-1-yl-1, 4-dihydro-quinoline-3-carboxylic acid

Scheme 3:



Where R = **H** for **Compound 5**

1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(3-phenylamino-4H-[1, 2, 4] thiadiazole-5-ylideneamino)-methyl]-piperazine-1-yl}-1, 4-dihydro-quinoline-3-carboxylic acid

R = **Cl** for **Compound 6**

7-(4-{[3-(4-Chloro-phenylamino)-4H-[1,2,4] thiadiazole-5-ylideneamino]-methyl}-piperazine-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-oxo-1,4-dihydro-quinoline-3-carboxylic acid

Elemental Analysis :

1-(0.2) Cyclopropane, (0.9) Primary hydrogen, (2-2.4) H-C-COOH acid, (5.2) H-C-F (Fluoride), (7.8) Aromatic.

2-Aromatic (7.6), H-C-F (4.87), H-C-COOH (2.8), H-C-Cl (3.4), H-C-F (4.3)

(1) N-H Str.2750, O-H-str. (broad, H-bonding)

(2) N-H Str.2750, O-H-str. (broad, H-bonding)

(3) O-H (broad, H-bonding) C=C, C-O-H

(4) O-H (broad, H-bonding) C=C, C-O-H

(5) C=O, O-H, N-H, C-H (Str)

(6) C=O, O-H, N-H (Str.), C-H (Str)

Table 1:

PRODUCTS	MOLECULAR FORMULA	MOLECULAR WEIGHT	MELTING POINT	ELEMENTAL ANALYSIS Cal.(Found)(%)	Rf. VALUE
1.	$C_{24}H_{22}FN_5O_2S$	463.53	288-290	C=61.01(60.97) H=5.04 (4.08) N=15.02(14.99)	0.76
2.	$C_{24}H_{21}ClFN_5O_2S$	497.97	168-170	C=58.02(57.98) H=4.05 (4.01) N=14.03(13.97)	0.70
3.	$C_{23}H_{23}FN_4O_2$	406.45	234-236	C=68.05(68.03) H=6.23 (6.19) N=14.54(14.50)	0.59
4.	$C_{23}H_{22}ClFN_4O_2$	440.89	284-286	C=66.01(65.97) H=6.11 (6.07) N=11.36(11.32)	0.70
5.	$C_{26}H_{26}FN_5O_3S$	535.59	308-310	C=58.07(58.03) H=5.02 (4.98) N=18.09(18.05)	0.62
6.	$C_{26}H_{25}ClFN_7O_3S$	570.04	324.326	C=55.03(54.07) H=4.05 (4.01) N=17.05(17.01)	0.66

Note: Mobile Phase :-(Chloroform: Methanol) (9:1)

Spectral Data of Compounds:

Compound 1:

IR (KBr) ν_{max} in cm^{-1}): 1615(N-H), 1100(C-N), 1750(C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O), 3600(O-H), 1178(C-F), 2560(C-S).

^{1}HNMR (CDCl3, ppm): (4H) 3.47-2.74, (1H) 7.4, (NH) 2.0, (5H) 6.4- 7.2, (CH) 7.55-8.12, (CH_2) 1.37, (OH) 11.0, (N-C-CH) 5.8

Compound 2:

IR (KBr) ν_{max} in cm^{-1}): (N-H), 1100(C-N), 1750(C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O), 3600(O-H), 2560(C-S), 725(C-Cl), 1178(C-F)

^{1}HNMR (CDCl3,ppm) : (3H) 8.24-7.56, (NH) 2.0 , (N-CH_2) 1.35, (OH) 11.0, 2(C-CH_2-)-0.41, (C=CH-N)7.8-5.8, (4H) 3.47-2.59, (5H) 6.4-7.45

Compound 3:

IR (KBr) ν_{max} in cm^{-1}): (N-H), 1100(C-N), 1750(C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O), 3600(O-H), 1178(C-F).

^{1}HNMR (CDCl3, ppm): (5H) 6.4-7.47, (OH) 11.0, (1H) 7.4, (N-CH_2)1.35, 2(N-C-CH_2) 0.41, (N-C=CH) 5.77, 2(N-CH_2) 3.47, 2(N-C-CH_2)-2.78, (NH) 2.0

Compound 4:

IR (KBr) ν_{max} in cm^{-1}): (N-H), 1100 (C-N), 1750 (C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O), 3600(O-H), 725(C-Cl), 1178(C-F)

^{1}HNMR (CDCl3, ppm): (4H) 7.2-7.3, (OH) 11.0, (1H) 7.4, (N-CH_2)1.35, 2(N-C-CH_2) 0.41, (N-C=CH) 5.77, 2(N-CH_2) 3.47, 2(N-C-CH_2) 2.78, (NH) 2.0

Compound 5:

IR (KBr) ν_{max} in cm^{-1}): (N-H), 1100(C-N, aromatic), 3400(C-N, aliphatic), 1750(C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O, aromatic), 1725(C=O, ketone) 3600(O-H), 1178(C-F), 2560(C-S).

^{1}HNMR (CDCl3, ppm): (5H) 6.4-7.47, (NH) 2.0, (OH) 11.0, (1H) 7.4, (N-CH_2)1.35, 2(N-C-CH_2) 0.41, (N-C=CH) 5.77, 2(N-CH_2) 3.47, 2(N-C-CH_2) 2.78.

Compound 6:

IR (KBr) ν_{max} in cm^{-1}): (N-H), 1100(C-N, aromatic), 3400(C-N, aliphatic), 1750(C=N), 1550(C=C, aromatic), 3100(C-C, aromatic), 1700(C=O, aromatic), 1725(C=O, ketone) 3600(O-H), 1178(C-F), 2560(C-S),725(C-Cl)

^{1}HNMR (CDCl3,ppm) : (4H) 6.4-7.47, (NH) 2.0, (OH) 11.0, (1H) 7.4, (N-CH_2)1.35, 2(N-C-CH_2)-0.41, (N-C=CH) 5.77, 2(N-CH_2) 3.47, 2(N-C-CH_2) 2.78.

Antimicrobial Activity:

Preparation of Suspension of Bacteria:

2 ml normal saline (0.85% w/v) was taken in test tubes and then plugged with cotton, raped with news paper with help of cello tape. Test tubes were put in autoclave for sterilization for 15 lbs for 20 min. After autoclaving take 1-2 colonies of bacteria from sub cultured bacterial plate with the help of loop. Colonies were dissolved in normal saline with rub on side of test tube with stirring. Turbidity of tubes were marked and add more colonies if needed.

Procedure for Sensitivity Test:

Preparation of Muller Hinton Agar Plates:

3.0 mg Muller Hinton agar media was dissolved properly in 80 mL Distilled water in 250 mL conical flask with stirring (For preparation of four plates).Then mouth of conical flask was plugged with cotton, rapped with news paper with help of cello tape. Conical flask was put in autoclave for sterilization for 15 lbs for 20 min. After autoclaving, warm 20-25 mL media was poured on Petri dish per plate in front of laminar flow. Leave it until media did not solidified in Petri disk. After that plate was put in incubators for drying the water vapour in plate. Now agar plate was ready for use.

Preparation of Compounds Solution:

5 mg compound was dissolved in 1 ml DMSO: PEG=1:10 in test tube with vertex stirring, heated if required (conc. of DMSO was not increased above 1%) Given the code of each tube .Same procedure was adopted for all compounds. Divided the bottom of prepared plate into four quadrants with the help of marker .Given the same code to each quadrant as given to code to test tube containing solution of compound. Swab the one plate from one bacterial suspension with the help of cotton swab, coded the name of bacteria on each plate .Same procedure was adopted for all plates.

With the help of micro pipette ,10-20 µl solution of compound was dropped on same code of quadrant as given on the test tube containing solution of compound .Same procedure was adopted for all compounds in laminar flow . All plates were put in incubator for incubation for 18-24 hrs. After18-24 hrs, view the plate .If the specific compound was sensitive for specific bacteria. Then growth was found in whole plate except where solution of compound was dropped. If the specific compound was not sensitive for specific bacteria. Then growth was found in whole plate including where solution of compound was dropped.

Antibacterial Activity of Synthesized Compounds

Table: 2

Compound code- / Name of bacteria	1	2	3	4	5	6
B.subtilis	++	_	++	++	++	++
S.aurieus	++	++	_	++	++	++
E.coli	_	_	_	++	_	++
S.typhi	_	_	_	_	++	++
Kleb.pneumoniae	_	++	++	++	++	++
Proteus mirabilis	++	_	_	_	_	++
H.pylori	_	_	_	_	_	_
Morganella morganii	++	_	++	++	++	++
P.vulgaris	_	_	_	_	++	++

Concentration: 0.5 microgram/ml

++ =Sensitive (Active), __ = Insensitive (Inactive)

Compounds are Respectively-

Table: 3

Compound 1	$C_{24}H_{22}FN_5O_2S$
Compound 2	$C_{24}H_{21}ClFN_5O_2S$
Compound 3	$C_{23}H_{23}FN_4O_2$
Compound 4	$C_{23}H_{22}Cl\ FN_4O_2$
Compound 5	$C_{26}H_{26}FN_7O_3S$,
Compound 6	$C_{26}H_{25}ClFN_7O_3S$

Zone of Inhibition: (mm) At a Concentration 0.5µg/ml Data of Synthesized Compounds:

Table: 4

Compound code- / **Name of bacteria**	**1**	**2**	**3**	**4**	**5**	**6**
B.subtilis	14	9	16	17	14	13
S.aurieus	13	14	13	18	16	15
E.coli	8	8	11	18	9	17
S.typhi	7	10	10	11	18	16
Kleb.pneumoniae	8	16	18	20	20	15
Proteus mirabilis	15	9	10	10	10	18
H.pylori	6	8	12	11	11	16
Morganella morganii	16	10	19	19	20	18
P.vulgaris	9	9	13	12	18	11
CONTROLE	__	__	__	__	__	__
CIPROFLOXACINE	25	26	25	24	**24**	24

Summary and Conclusion:

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolones drug class. It is a second generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops DNA & protein synthesis. The chemistry & pharmacology of thiadiazole and Benzothiazole have been of great interest because it's various biological activities, so that the biological & pharmacological activity of thiadiazole with Ciprofloxacine and Phenylamine may be taken in to account for synergism. It is well known that the introduction of chlorine atom in organic molecules causes dramatic changes in its biological profile, mainly due to high electro negativity of chlorine, the strong carbon – chlorine bond and increased solubility in lipids. Therefore it was through worthwhile to synthesize better kinds of drugs by incorporating thiadiazole,Benzothiazole and ciprofloxacin. Synthesized compounds were tested against a panel of microorganisms including Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria *S.Typhi, Proteus mirabilis, Hpylori, Morganella morganii, P.vulgaris* using conventional agar- dilution method.

In summary, Sensitivity testing was performed on all compounds. Which showed, some synthetic compounds (1, 2, 3, 4, 5 and 6) were highly sensitive against *B.subtilis, S.aurieus, Kleb.pneumoniae, Morganella morganii* bacteria. Compound 4, 5 and 6 were found to be most highly active .It showed good antimicrobial activities.

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