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SYNTHESIS, CHARACTERIZATION, AND ANTI-BACTERIAL ASSESSMENT OF NEW GATIFLOXACIN ANALOGUES

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ABSTRACT

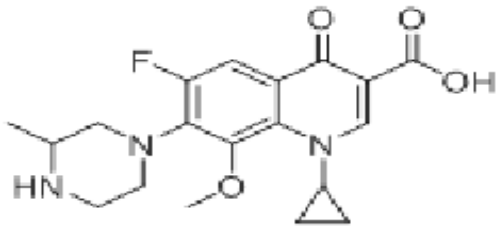
New gatifloxacin analogues were synthesized by coupling with different sulfonamide derivatives (sulfanilamide, and sulfacetamide), using chloroacetyl chloride linker, in order to increase the bulkiness at position 7 of the flouroquinolone by that reduce the efflux of the antibiotic from the bacterial cells and bacterial resistance will reduced consequently. The chemical structures of the synthesized compounds were confirmed and characterized using FT-IR spectroscopy, and elemental microanalysis, in addition to some physicochemical properties as melting points and R_f values. The antibacterial study was showed comparable effect of the prepared compounds with Gatifloxacin. The largest inhibition zone was observed with compound IIa against bacteria Bacillus cereus and the lowest inhibition zone was observed with compound IIa against bacteria Proteus mirabilis.

KEYWORDS: Gatifloxacin, Flouroquinolones, Bacterial resistance, Topoisomerase enzyme.

INTRODUCTION

Gatifloxacin is one of the flouroquinolones synthetic antibacterial compounds, it possess clinical activity against both gram positive and gram negative bacteria, in addition to its effectiveness against anaerobic and mycoplasma infections.[1] Flouroquinolones act through inhibition of DNA synthesis by direct inhibition of topoisomerase II or gyrase enzyme in gram negative bacteria, while in gram positive bacteria, the topoisomerase IV is the primary target for their antibacterial action.[2,3] Flouroquinolones possess broad spectrum activity against many clinically important pathogens which are responsible for variety of infections

including urinary tract infection (UTI), respiratory tract infection (RTI), sexually transmitted disease (STD), and skin infections.[4] New quinolones provide a valid alternative antibacterial therapy, especially in areas where the prevalence of pencillin resistant and macrolide resistant organisms exist.[5] Gatifloxacin (1), and clinafloxacin (2) are related to the fourth generation of flouroquinolones, which show extended activity against both strains of bacteria.[6]



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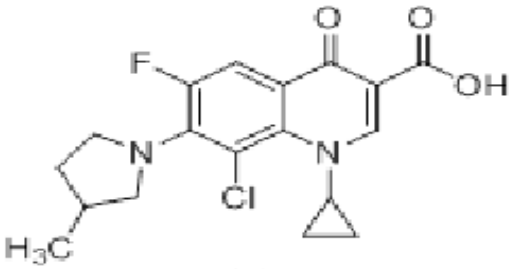

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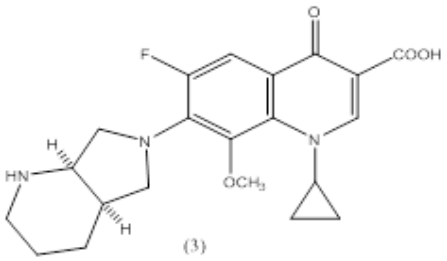
With increasing utilization of fluoroquinolones in both human and veterinary medicines, emerging resistance for these agents is also a growing concern. These findings ponder upon the need for the continued monitoring of quinolone resistance among bacterial pathogens.[7] The mechanism of acquired resistance to the fluoroquinolones is consistent among currently available drugs of this class and is expected to be similar for new and developing agents as well.[8] Resistance to this class of agents occurs primarily *via* two fundamental processes. First, by spontaneous mutations at various locations on the gyrase enzymes, which lower the affinity of the drug at the gyrase DNA complex. Second mechanism that entails resistance to the fluoroquinolones is slow to appear, but when it appears it is mainly due to the efflux mechanism, which pumps the drug back to the cell.[9,10] This is due to the mutation in the genes that code for porins, which are membrane proteins by which quinolones enter Gram negative cells.[11] These mutations raise tolerance limit of antibiotics to four folds and result in either reduced production of outer membrane proteins or stimulated cell efflux system, which lead to active drug expulsion.[9]

It was found that fluoroquinolones with 7 piperazinyl moiety have been reported to possess potent antibacterial activity.[12,13] It could kill most Gram negative bacteria and had greater efficacy on Gram positive bacteria by improving the penetration of the drug through the bacteria cell wall.[14] A recent interesting observation is that increased bulkiness here (R-7) appears to confer protection from the efflux exporter proteins of bacteria, and diminishes the likelihood of bacterial resistance in wild-type bacterial strains. Bulk here also increases anti-anaerobic activity.[15,16] Moxifloxacin (3)[17] and trovafloxacin (4)[18], are the currently available agents with the greatest bulk at this position, and they appear least affected by reserpine-inhibited exporter proteins.[15]



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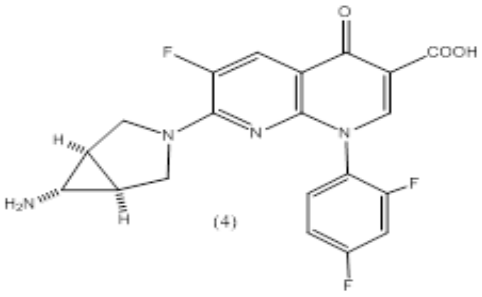

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Depending on this back ground, we synthesized gatifloxacin derivatives by linking of sulfonamide moieties on the secondary amine of piperazine heterocyclic ring to reduce their expulsion from bacterial cells.

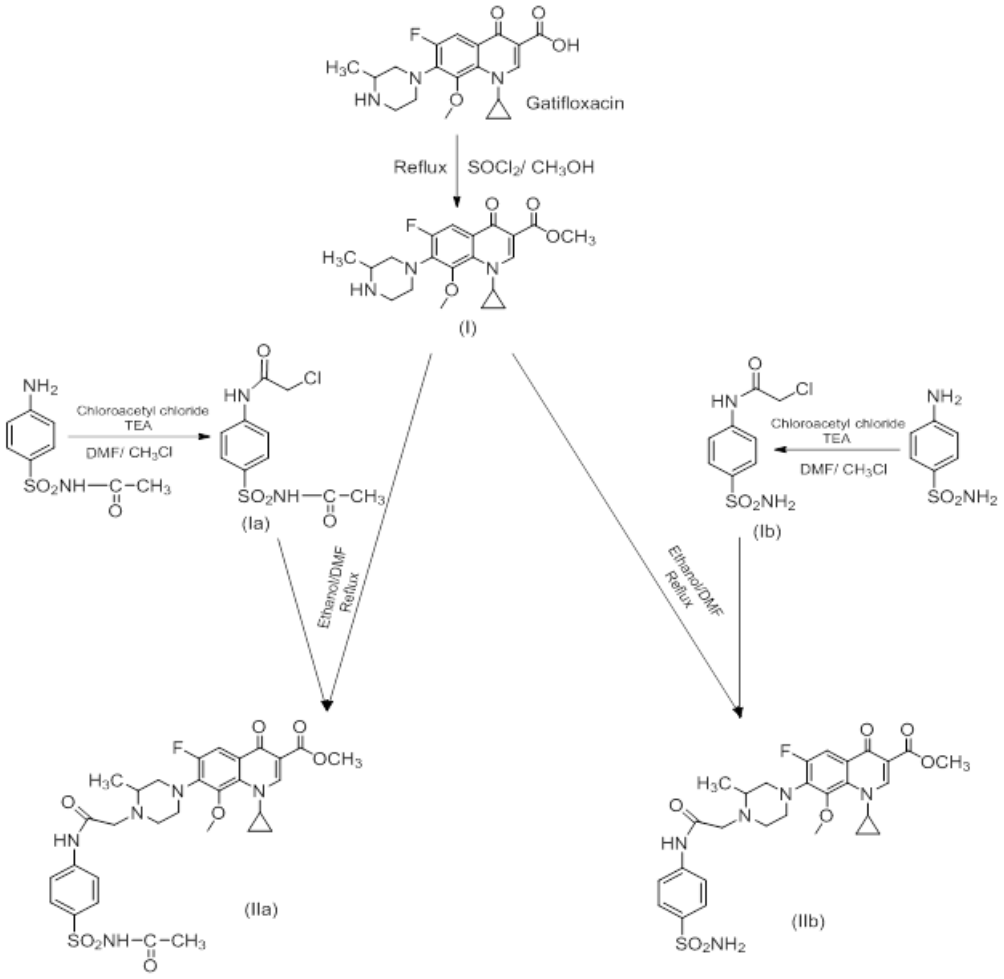
MATERIALS AND METHODS

Experimental

The solvents (anhydrous), and chemicals were of analytical grade and provided from (Merck Germany; Sigma-Aldrich Germany; Fluka Switzerland). Melting points were recorded by capillary tube using thomas hover apparatus (England). Retention factor (R_f) values were measured through using ascending thin layer chromatography (TLC), to ensure the purity and progress of the reaction, using ethyl acetate: ethanol (2:3) as mobile phase[19] Infrared (IR) spectra were recorded at college of pharmacy, kufa university, through using fourier

transform infrared (FT-IR) spectrophotometer, by KBr discs, and elemental microanalysis (CHNO) was recorded using a euro 3000 elemental analyzer (Italy).

Steps of the synthesis of four target compounds and their intermediates were presented in scheme 1. The primary amine of the sulfanilamide and sulfacetamide was reacted with chloroacetyl chloride, which then reacted with the secondary amine of gatifloxacin methyl ester.



Scheme 1: Synthesis of the target compounds (IIa and IIb), and their intermediates.

Chemistry

Synthesis of gatifloxacin methyl ester (I)

Gatifloxacin (2 g, 5.33 mmol), was dissolved in methanol (50 ml), the solution cold to -15^{o} C, then thionyl chloride (0.38 ml, 5.33 mmol) was added drop wise. The reaction mixture kept for 3 hours at 40^{o} C, then undergo reflux for 3 hours, and left overnight at room temperature. Methanol was evaporated, and the residue was re-dissolved in methanol and evaporated. The process was repeated until complete removal of thionyl chloride was achieved. The product

crystalized using ether-ethanol[20] The description and physical parameters were represented in Table 1.

Coupling of sulfonamide derivatives with chloroacetyl chloride

Sulfonamide derivatives (3mmol) were dissolved in DMF:chloroform (20:80) (60ml), and TEA (0.41ml, 3mmol) was added. The mixture was stirred in ice bath and chloroacetyl chloride (0.24ml, 3 mmol in 10ml chloroform) was added drop wise over a period of 1 hour, followed by reflux for 3 hours. Then the solvent was evaporated and the residue was collected and crystallized by using ethanol[21] The description and physical parameters were represented in Table 1.

Coupling of gatifloxacin methyl ester with compounds Ia and Ib

Gatifloxacin methyl ester (I) (2.4 g, 6 mmol), and compounds Ia, Ib (6 mmol) were dissolved in DMF (30 ml), refluxed for 3 hours. Then the solvent was evaporated and the residue was collected and crystallized by using ethanol[21] The description and physical parameters were represented in Table 1.

Spectral Analysis

Synthesis of methyl-1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylate (I)

FT-IR (cm^{-1}): 3,217 (N-H) stretching of secondary amine, 3,010 (C-H) stretching of aromatic, 1,743 (C=O) stretching of ester, 1,724 (C=O) stretching of ketone. CHNO calculated ($C_{20}H_{24}FN_3O_4$): C, 61.68; H, 6.21; N, 10.79; O, 16.43 found: C, 62.05; H, 5.81; N, 10.94; O, 16.0.

N-(4-(N-acetylsulfamoyl)phenyl)-2-chloroacetamide (Ia)

FT-IR (cm^{-1}): 3,292 (N-H) stretching of secondary amine, 1,695 (C=O) stretching of amide, 2,949 and 2,877 (C-H) stretching of alkane, 1,340 and 1,159 (SO_2) stretching, 997 (C-CL) stretching. CHNO calculated ($C_{10}H_{11}ClN_2O_4S$): C, 41.31; H, 3.81; N, 9.64; O, 22.01; S, 11.03 found: C, 41.15; H, 4.01; N, 9.71; O, 22.24; S, 11.12.

2-Chloro-N-(4-sulfamoylphenyl)acetamide (Ib)

FT-IR (cm^{-1}): 3,331 and 3,225 (N-H) stretching of sulfonamide, 1,689 (C=O) stretching of amide, 1,343 and 1,170 (SO_2) stretching, 835 (C-CL) stretching. CHNO calculated ($C_8H_9ClN_2O_3S$): C, 38.64; H, 3.65; N, 11.27; O, 19.30; S, 12.89 found: C, 38.65; H, 3.81; N,

11.24; O, 19.0; S, 12, 76.

Methyl-7-(4-(2-((4-(N-acetylsulfamoyl)phenyl)amino)-2-oxoethyl)-3-methyl piperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (IIa):

FT-IR (cm^{-1}): 3,282 (N-H) stretching of secondary amine, 3,010 (C-H) stretching of aromatic, 1,732 (C=O) stretching of ester, 1,691 (C=O) stretching of amide, 1,317 and 1,161 (SO_2) stretching. CHNO calculated ($C_{30}H_{34}FN_5O_8S$): C, 55.98; H, 5.32; N, 10.88; O, 19.88; S, 4.98 found: C, 56.05; H, 5.41; N, 10.64; O, 19.76; S, 5.14.

Methyl-1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (IIb)

FT-IR (cm^{-1}): 3,329 (N-H) stretching of sulfonamide, 3,219 (N-H) stretching of secondary amine, 3,008 (C-H) stretching of aromatic, 1,734 (C=O) stretching of ester, 1,687 (C=O) stretching of amide, 1,317 and 1,163 (SO_2) stretching. CHNO calculated ($C_{28}H_{32}FN_5O_7S$): C, 55.90; H, 5.36; N, 11.64; O, 18.61; S, 5.33 found: C, 56.14; H, 5.11; N, 11.93; O, 18.75; S, 5.34.

Antibacterial Study

Bacterial Isolates

The bacterial isolates were supplied from Microbiological Laboratory, Department of Biology, Faculty of Science, Kufa University (Gram positive bacteria Bacillus cereus and Gram negative bacteria Proteus mirabilis).The bacteria were activated in nutrient broth and sub cultured three successive times in nutrient agar and stored on nutrient agar slants at 4 °C.

Drug Preparation

An appropriate amount (0.128 g) of prepared compounds was dissolved in 1000 ml sterile distilled water to prepare a solution containing 128 mg/ml.

METHODS

Morphological and Cultural Characteristics

The bacterial growth on blood agar media used to produce single bacterial colony. Bacteria was identified depending on its morphological and cultural characteristics (blood hemolysis, colony shape, size, color, borders, and texture) then it was examined under the microscope with Gram's stain for observation arrangement and reaction bacteria with stain.[22, 23]

Screening for Antibacterial Activity

It was carried out according to well diffusion method[24] The plates of Muller – Hinton agar media was inoculated with bacterial study containing (1.5 × 108) cell with a sterile swabs , then 50 µL of prepared compounds was added to each of wells (8 mm diameter wells cut in the agar gel). Each prepared compound was assayed in triplicate. The inoculated plates were incubated for 24 h at 37ºC in aerobic conditions. The bacterial growth was observed and the inhibition of the bacterial growth was measured in mm.

Determination of Minimum Inhibitory Concentration (MIC) of Prepared Compounds

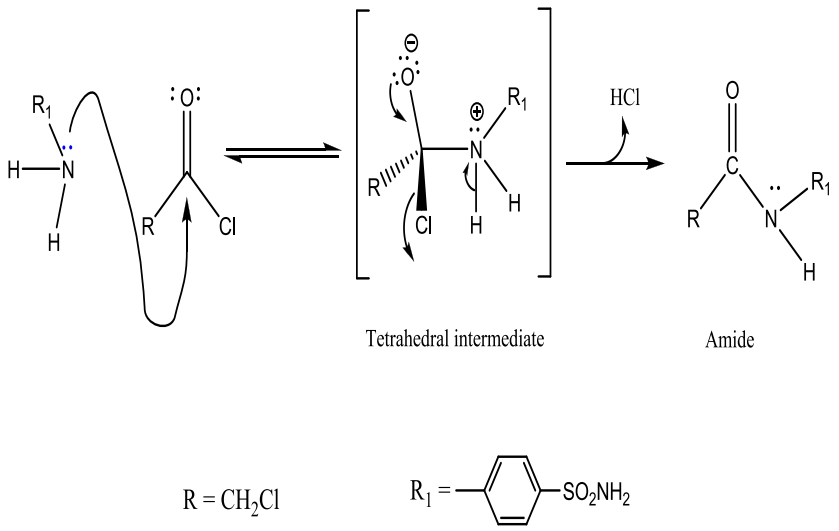
Determination of the minimum inhibitory concentration (MIC) was carried out according to Gupte method[25] The (MIC) to the prepared compound could be find out to bacteria (Bacillus cereus and Proteus mirabilis) with different concentrations of (64, 32, 16, 8, 4, 2 and 1 mg/ml and these different concentrations could be got by placing (1 ml) of the solutions (plants extracts and fractions) containing the concentrations (128, 64, 32 ,16 ,8 ,4 and 2 mg/ml) were represented in varying test tubes, next we added (1 ml) from sterile media (nutrient broth) and followed by inserting a loopful bacterial growth on nutrient broth attenuated to reach the turbidity of 0.5 McFarland. The control tube included pure sterile nutrient broth media was inoculated with bacteria (Bacillus cereus & Proteus mirabilis) merely. All the cultures subsequently incubated for 24 hours next to 37°C. Finally, the bacterial development in the tubes were investigated with turbidity monitor –measuring by eyes.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the final compounds (IIa-IId) and their intermediates was illustrated in scheme 1. Firstly, the carboxyl group of gatifloxacin was protected in the form of methyl ester through reaction with thionyl chloride in methanol to give acyl chloride intermediate which undergo reaction with methanol to give methyl ester of gatifloxacin. The shifting of C=O stretching from 1,722 cm^{-1} to 1,743 cm^{-1}, and disappearance of broad band above 3200 cm^{-1}, indicate the conversion of carboxyl group in gatifloxacin to its methyl ester derivative.[26,27]

N-acytalation of sulfanilamide and sulfacetamide was done by using chloroacetylchloride to get 2-chloro-acetamide derivatives. The conversion of chloroacetyl chloride into amide will occur through nucleophilic acyl substitution reactions which involve tetrahedral intermediate, the mechanism will described in scheme 2.[28]



Scheme 2: Mechanism of synthesis of the intermediate compounds (Ia and Ib).

Nucleophilic substitution occurs selectively at the acyl carbon atom in α-chloroacetyl chloride because of the greater reactivity of nucleophiles toward acid chlorides compared to alkyl chlorides. The reasons for this selectivity are attributed to the differences in the electrophilicity of the two carbon atoms in α-chloroacetyl chloride. Electronically, the carbonyl carbon has two electron-withdrawing groups – the oxygen doubly bonded to it and the (-Cl) bonded to it. On the other hand, the carbon in $–CH_2Cl$ has only one electron-withdrawing group (-Cl). Besides electronics, steric factors also play a role in this selectivity. It is easier for the nucleophile to attack the carbon of the planar carbonyl group in the acid chloride than to attack the tetrahedral carbon in the $–CH_2Cl$ group. The reaction is carried out with triethylamine, which acts as a base to neutralize the hydrogen chloride formed.[29]

The reaction of sulfanilamide is regioselective[30], where the basicity of the amine has a considerable effect on the rate and extent of the reaction, sulfonamides are of low basicity, being fairly strong acids, due to strong electron-withdrawing effect of the $–SO_2$ substitution and stabilization of the resulting anion by resonance. In addition to the sulfonamide group the molecule also possess a free amino group, so, generally the reaction will take place on the free amino group.[31,32]

The IR spectrum of the synthesized compound (Ia and Ib) show disappearance of asymmetric and symmetric stretching vibration bands for primary aromatic NH_2 of the starting compounds (sulfanilamide and sulfacetamide) and appearance of new absorption band at 3132cm-1 for NH stretching vibration of secondary amide, appearance of characteristic bands

at 1689cm-1 for C=O stretching vibration of amide (amide I Band), 1546cm-1 for N-H bending vibration of amide (amide II Band), and at 835cm-1 for C-Cl stretching vibration.

Gatifloxacin methyl ester (compound I) undergo nucleophilic substitution reaction (SN^2) in presence of equimolar of compounds Ia and Ib, when the secondary cyclic amine of piperazine will attack the electrophilic carbon in the above compounds leading to displacement of chlorine atom. The reaction was occurred in presence of equimolar of triethylamine which act as a base to neutralize the hydrogen chloride formed, this reaction lead to formation of compounds IIa and IIb.

The rate of an SN^2 reaction follows second order kinetics, as the rate limiting step depend on the nucleophile concentration, as well as the concentration of the substrate.[33] This mechanism depends on solvent, temperature, and concentration of the nucleophile and that of the leaving group. It is generally favored in primary or secondary alkyl halides with an aprotic solvent as (DMF).[33] In aliphatic heterocyclic compounds, the nitrogen atom is a part of a saturated heterocyclic ring and lone pair of electrons is available for reaction with protons (e.g. piperazine). Compounds of this type are similar in base strength to their open-chain aliphatic counterparts with typical pKa values of 8-9.[34]

Table 1: Physicochemical properties of the synthesized compounds.

Compound	Empirical Formula	Molecular weight	Description	% Yield	Melting point ^oC	R_f values
I	$C_{20}H_{24}FN_3O_4$	389.42	Pale yellow crystals	83	190-192	0.63
Ia	$C_{10}H_{11}ClN_2O_4S$	290.72	Off-white powder	76	215-2-18	0.78
Ib	$C_8H_9ClN_2O_3S$	248.68	Gray powder	80	203-205	0.74
IIa	$C_{30}H_{34}FN_5O_8S$	643.69	Yellow powder	58	228-231 d	0.67
IIb	$C_{28}H_{32}FN_5O_7S$	601.65	Dark brown powder	74	268 d	0.82

Antibacterial study

Morphological and Cultural Characteristics

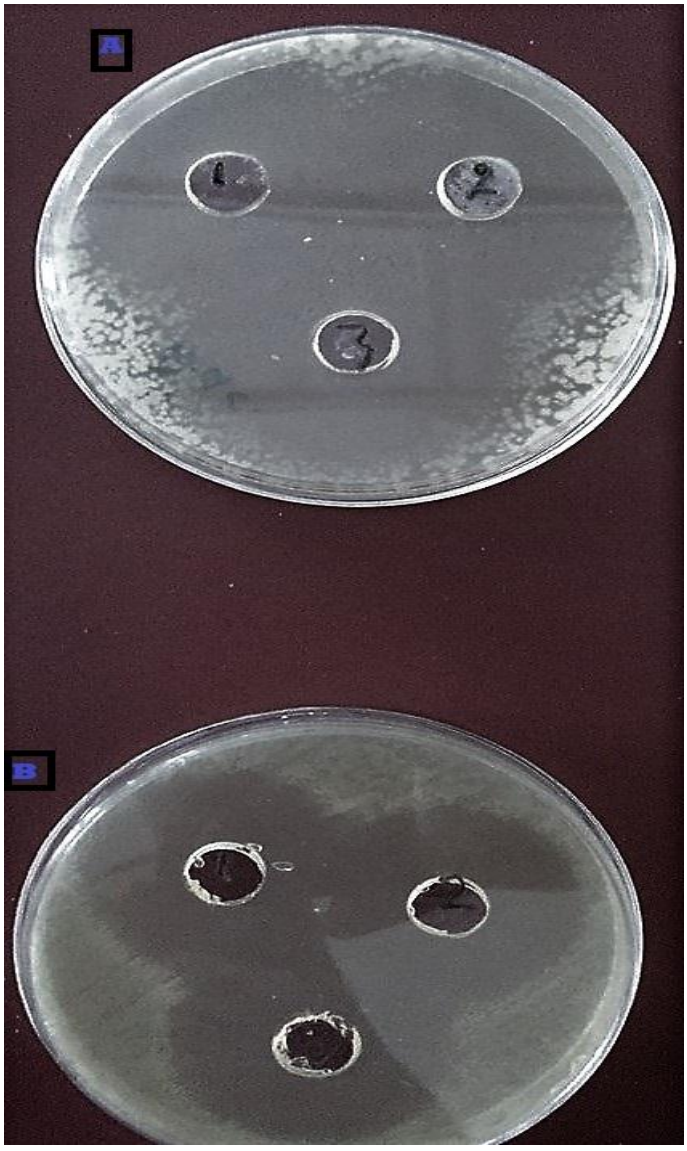
Bacillus cereus is Gram positive rod motile bacteria, colonies shape are circular with a less wavy edge, flat, dull and opaque. The colonies color grey-white and on blood agar media gave β-hemolysis while Proteus mirabilis is Gram negative rod motile bacteria, colonies showed swarming phenomena with diverse fishy odor.

Screening for Antibacterial activity

The results in table 1 & pictures 1 showed that the prepared compounds have antibacterial activity against *Bacillus cereus & Proteus mirabilis* that the topmost inhibition zone diameter (47mm) was observed with compound IIa solution against bacteria *Bacillus cereus* and lowest inhibition zone (37mm) was observed with compound IIa against bacteria *Proteus* mirabilis.

Table 1: The Antibacterial Activity of Gatifloxacin and Compounds I and II.

Compound	Inhibition zone on Bacterial growth (average in mm)	
	Proteus mirabilis	***Bacillus cereus***
Gatifloxacin	38	45
IIa	37	47
IIb	40	42



Picture 1: The Antibacterial Activity of Gatifloxacin, Compound I, and II.

The picture showed the antibacterial activity of Gatifloxacin and the prepared compounds on;

A. Bacillus cereus

B. Proteus mirabilis

The numbers 1, 2 and 3 represented Gatifloxacin, IIa, and IIb, respectively.

Determination of Minimum Inhibitory Concentration (MIC) of Prepared Compounds

The minimum inhibitory concentration (MIC) values of prepared compounds showed that all them had MIC values with 3mg/ml on two selected bacteria (*Bacillus cereus& Proteus mirabilis).*

CONCLUSION

The designed compounds were synthesized successfully and their structures were confirmed, and characterized using elemental microanalysis (CHN), and infrared spectroscopy (FT-IR spectra), their purity was confirmed by their physical data (melting points and R_f values).

The antibacterial study was showed comparable effect of the prepared compounds with Gatifloxacin. The largest inhibition zone was observed with compound IIa against gram positive bacteria and the lowest inhibition zone was observed with compound IIa against gram negative bacteria.

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