Synthesis and characterization

A derivative of levofloxacin and a study of its bacteriostatic activity

Huda ahmed younis a and Raheem Jameel Mahesein b

a,b Department of Pharmaceutical Chemistry, College of Pharmacy, University of .Basrah, ,Basrah, Iraq

***Corresponding Author E-mail: huda.ahmad2014@yahoo.com , phone 009647709011081.**

Abstract:

This study includes two newly synthesized prodrugs of Levofloxacin derivatives mono and dipeptide H2, where was the ~~s~~ynthesized monoPeptide as Levofloxacin-Histadine (L-H), as well as dipeptide H6 as ~~S~~ynthesis of diPeptide (phenylalanine-levofloxacin (GPA-L), using a Levofloxacin substituted. Spectroscopic data were studied for two derivative compounds H2 and H6 and reactivity indices were characterized using techniques FT-IR, ^{1}H-NMR, and ^{13}C-NMR. All the newly produced derivative compounds H2 and H6 have FT-IR spectra that share similarities in certain fingerprint-like bands and other bands. The essential functional group vibration bands. In the DMSO-d6 solvent, the compound H2, and H6 of ^{1}H NMR spectra were studied, Typically, the fitted intensity ratio of the observed compound 1HNMR spectra gives, and the expected signals. The DMSO-d6 solvent was employed to record the 13C NMR spectra of levofloxacin derivatives H2 and H6. portrayed the ^{13}C NMR spectra of generated compounds H1 through H6, comprised of the ^{13}C NMR data. where done Further confirmation of the properties of the developed molecule emerged from ^{13}C-NMR spectra. [1]

The bioactivity was studied for two derivative compounds H2 and H6 against three gram-positive and three gram-negative Bacteria. The mono and dipeptide prodrugs that are derivatives of levofloxacin have appeared to have excellent results against these Bacteria. Antibiotic susceptibility experimental findings revealed that various bacteria had taken different approaches to the tested drugs. most bacteria isolated in gram-positive and gram-negative demonstrated severe sensitivity to the generated

compounds (H2 and H6). The spectral results in the FT-IR analyses as well as the nuclear magnetic resonance studies showed the high validity of the compounds formed (H2 and H6) interaction, as well as the biological results showed a clear positive in killing bacteria of the type of Gram-negative and Gram-Positive.

Keywords: mono and dipeptide prodrug, Levofloxacin-Histadine (L-H) and Glycine phenylalanine- levofloxacin (GPA-L)

1. Introduction

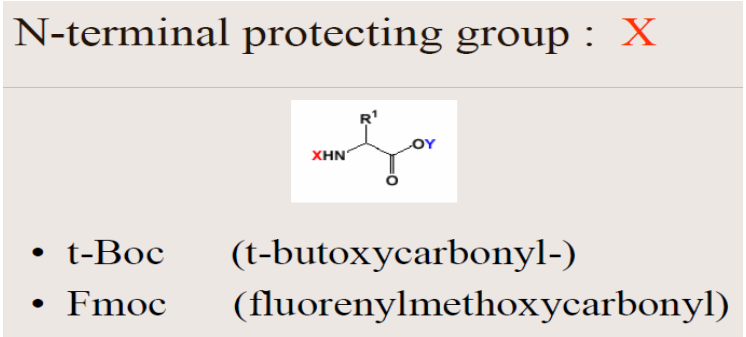
Peptides are named according to the type and arrangement of amino acids, where the name begins with the terminal amino acid that contains a free terminal amine group, which appears on the left side of the peptide, and the syllable (yl) is added at the end of the name of each amino acid except for the last amino acid, which contains a carboxyl group, for example, such as Leu-Gly-Tyr-Cys. The various amino acids in the peptides can be separated by chromatography or electrophoresis and depend on the acid-base behavior [2-4].

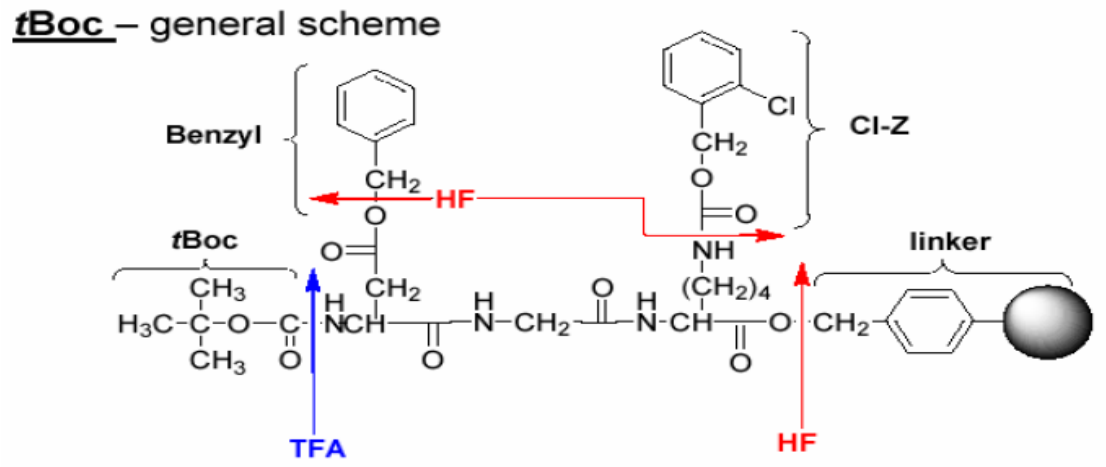
Peptides have two important chemical reactions, in one of these two reactions, Peptides are hydrolyzed by boiling with a strong acid or a strong base to yield their amino acid components freely. The second reaction of the lipids is used to determine the amino acid sequence with its association with 2,4-dinitrofluorobenzene [5].

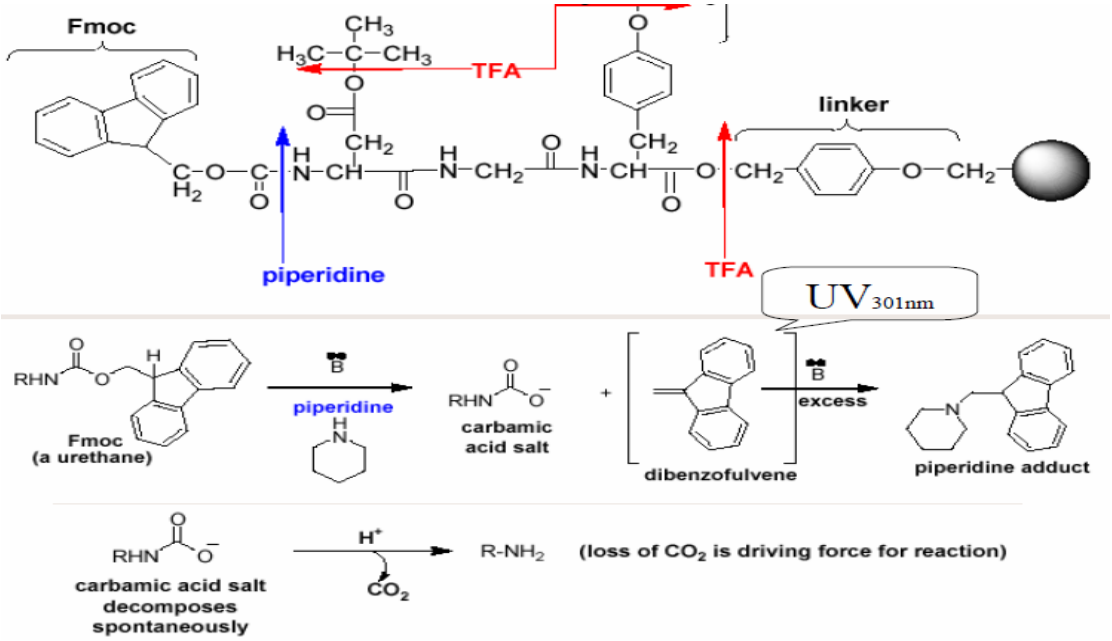
The main part of the synthesis was carried out utilizing this technique (scheme1) [6]. On the Applied Biosystem Model 430A Peptide Synthesizer, these analogs were synthesized. A cartridge containing a Boc-protected amino acid was added sequentially to create synthetic peptides, and 3-(Diethoxy-phosphoryl oxy)-3H-benzo[d]()triazine-4-one (DEPBT) in DMF was added to create activated esters of each amino acid. Nitrogen gas was bubbled through the cartridge to dissolve the amino acids. To facilitate ester formation, N, N-disopropylethylamine was added to the cartridge. This mixture was then poured into the reaction container with the PAM resin's C-terminal residue still attached, vortexed several times, and left to couple to

the resin. The N-terminal Boc protecting group was removed by treatment with trifluoroacetic acid after washing to remove the unreacted chemicals (TFA) [7-10].

Once the critical stages had been completed, the chain was put together after the resin had been rinsed with DMF. After the synthesis, the reaction vessel contained roughly protected peptidyl-PAM resin (usually 30 amino acids). After being treated with trifluoroacetic acid, to remove the last Boc protecting group, the resin has been repeatedly washed using dimethylformamide (DMF) and dichloromethane (DCM) and dried. Anhydrous HF was used to cure the peptidyl-resin (method described later in this section), and the result was often a crude deprotected peptide [11,12].







Scheme(1): Peptide synthesis (Boc and Fmoc methods)

Many peptides' therapeutic effectiveness is further hampered by their missing prolonged half-life in systemic circulation. Polypeptides may exhibit chemical and physical instability in addition to delivery issues, making the formulation work much more difficult [13].

Due to the lack of patient compliance along with all challenges appearing in parenteral delivery, as well as the low oral bioavailability, researchers were prompted to look into different ways to distribute macromolecules. These include rectal, transdermal, pulmonary, ocular, nasal,, and vaginal routes. Using these alternate pathways hasn't been very effective because there aren't any external stimuli to help with absorption. The injection of additional protease inhibitors, absorption enhancers, novel formulation techniques, and irreversible (analogs) or reversible (prodrugs) chemical alterations are only a few methods used to increase the bioavailability of polypeptides. Through chemical derivatization, prodrug techniques temporarily alter a compound's physicochemical characteristics. Such reversible chemical modifications are intended to improve chemical stability, change water solubility, or increase bioavailability [14-17]. At the same time, it maintains the parent drug's natural pharmacological qualities.

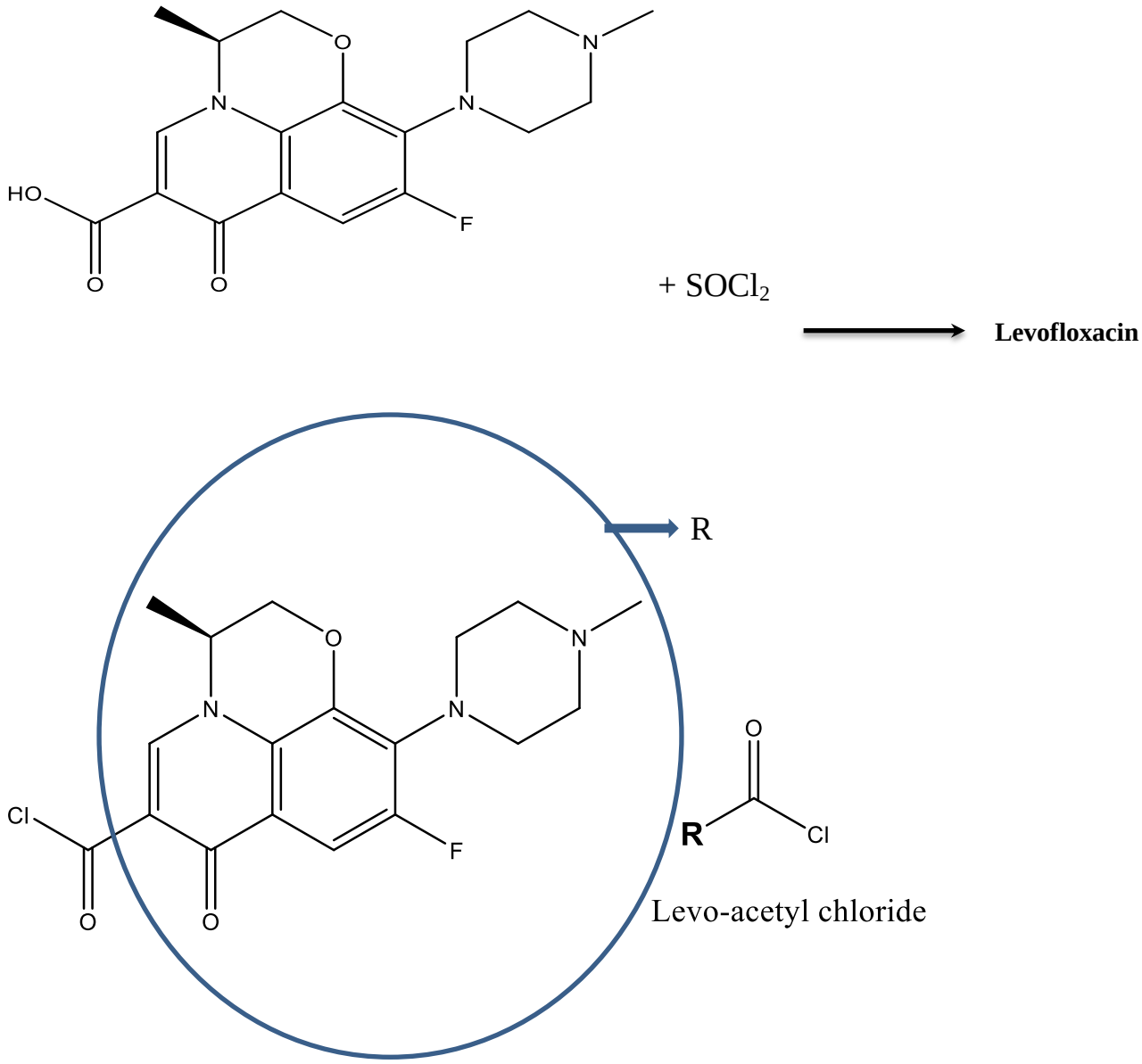
Antimicrobial peptides (AMPs) are crucial elements of the innate immune systems of many species. Various bacteria, fungi, parasites, and viruses can be suppressed by AMPs.This study offers a thorough and systematic introduction to the advancement of research on AMPs. Research on antivirus peptides, specifically anti-coronavirus (COVID-19) peptides, increased in light of the projected global COVID-19 pandemic in 2020. According to their sources, AMPs can be categorized as microorganism antimicrobial peptides, insect antimicrobial peptides, amphibian antimicrobial peptides, and mammalian antimicrobial peptides [18,19].

The Antimicrobial Peptide Structure Classification: Antimicrobial peptides might be separated into 4 classes established on their structural properties: linear-helical peptides, linear extension structures, and both -helix and -sheet peptides**.**

A member of the fluoroquinolone medication class, levofloxacin is a bactericidal antibiotic that prevents bacteria from synthesizing DNA. Levofloxacin encourages DNA strand breaks by preventing DNA-gyrase in sensitive species and supercoiled DNA from relaxing. Compared to ciprofloxacin, the fluoroquinolone levofloxacin has less activity against gram-negative bacteria, most notably Pseudomonas aeruginosa, and more activity against gram-positive bacteria, including Streptococcus pneumonia, which is penicillin-sensitive and resistant. Levofloxacin is effective against various common respiratory pathogens, including Chlamydia pneumonia, Moraxella catarrhalis, Legionella species, and Mycoplasma species. Levofloxacin is recommended above the other fluoroquinolones as second-line antitubercular therapy because it has a stronger in-vitro action against Mycobacterium TB. Fluoroquinolone medication resistance, which chromosome-encoded or plasmid-mediated processes can cause, is becoming a global concern. The body quickly absorbs and evenly distributes levofloxacin. Levofloxacin has a 99% absorption rate. Hence its intravenous and oral formulations are interchangeable. Levofloxacin is mainly cleared through the kidneys (87%) [20-23].

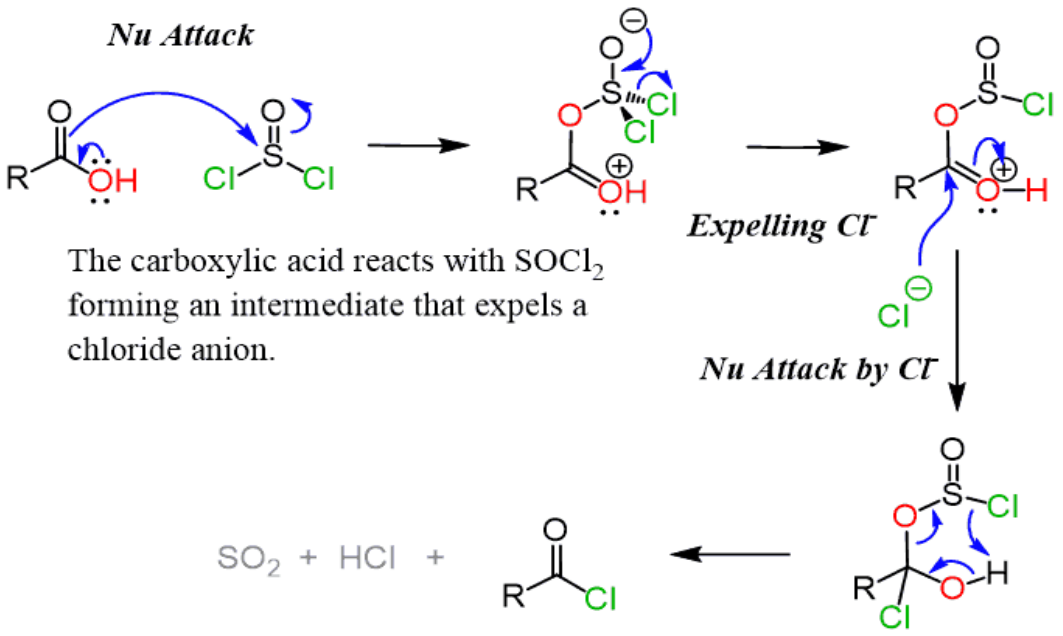
2. Experimental Section

2.1. The LAC (Levofloxacin Acid Chloride) Synthesis



Scheme (2): The LAC (Levofloxacin Acid Chloride) Synthesis

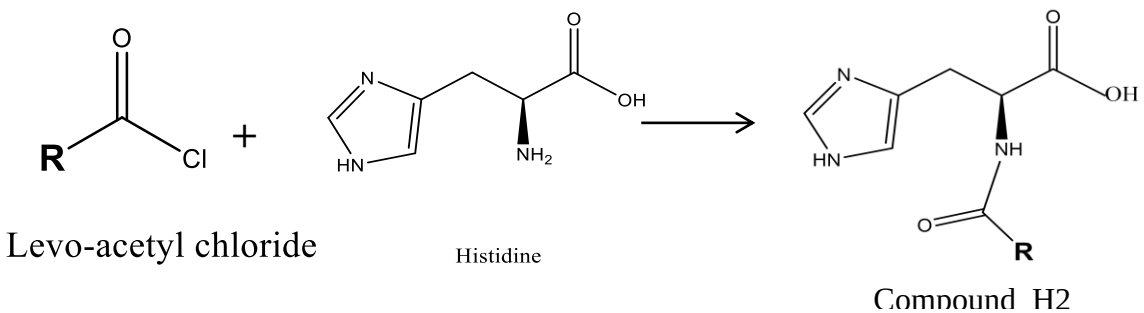
An intermediate expelling a chloride anion is form when levofloxacin carboxylic group is reacted with thionyl chloride. The initial step is forming oxonium, carboxylic oxygen on thionyl sulfur atom that is attacked by the nucleophilic c. It is said as the suggested mechanism [24]. The next step after that is the reaction between chloride ion and the oxonium. The reaction of it is displayed in Scheme (2and 3).



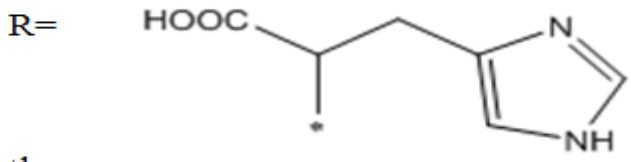
Scheme (3): The LAC forming suggested mechanism

2.1. Synthesisof monoPeptide (levofloxacin acid chloride with Histadine (L-H), H2)

Mixed levofloxacin acid chloride (0.37gm,1mmol) and (0.155gm,1mmol) of histadine in a dry flask containing the two compounds mixed, then 20ml of DMF was added; After 24 hours of continuous stirring, the mixture was processed in a clean petri dish, the solvent evaporated, and the residue cleaned numerous times with 20 mL volumes of Acetone before being harvested. Scheme (4) depicts the Synthesis General Scheme.



where n= number of hydrogen atoms in amino acid group.

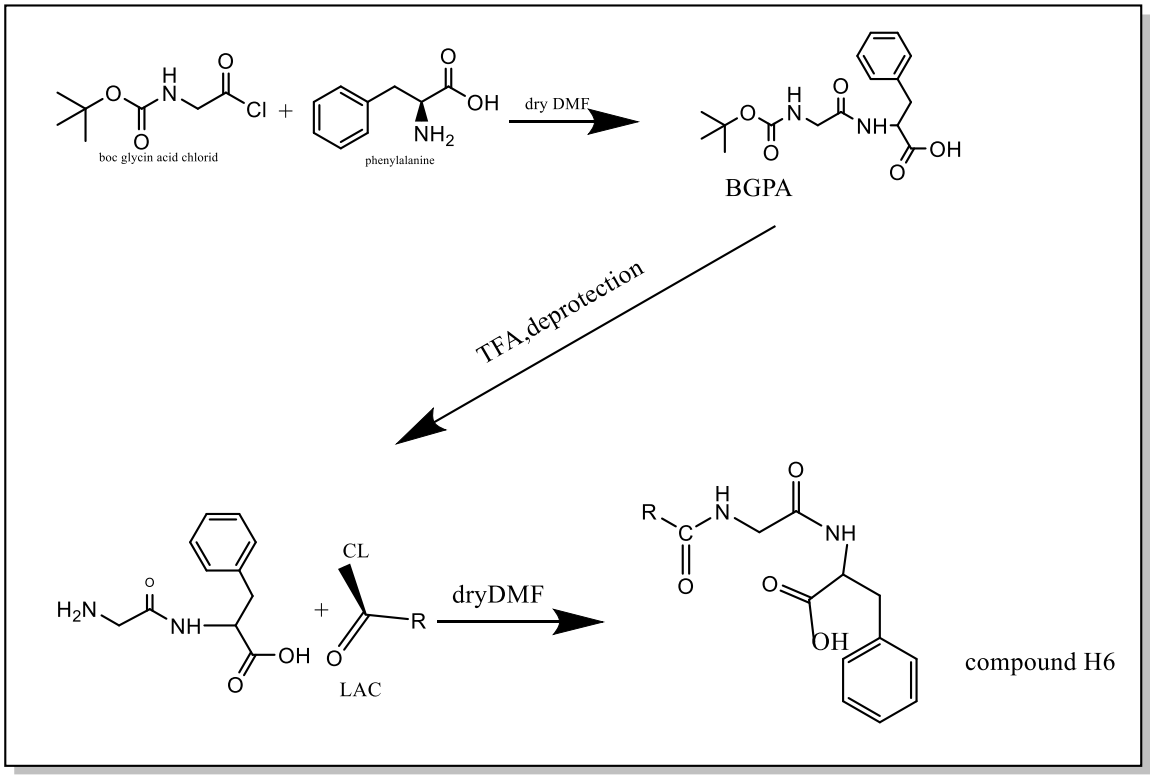


Note: (*) is the position of link

Scheme (4): The Synthesis of Compound H2

2.2. Synthesisof diPeptide (Glycine phenylalanine- levofloxacin (GPA-L) H6)

Mixed Boc-glycine acid chloride (0.194gm,1mmol) and (0.165gm,1mmol) of phenylalanine in a dry flask containing the two compounds were mixed, and then 20ml of dry DMF was put into it. after twenty-four hours of mixing at room temperature, the mixture was diffused in a clean petri dish, The solvent had melted away, and the fraction had been cleaned up lots of times with 20 mL volumes of Acetone then collected. The product was deprotection by adding 10ml of trifluoroacetic acid (TFA) and letting it dry, then taking (0.221gm,1mmol) of finally powder and mixing with (0.37gm,1mmol) of LAC, then 10ml of dry DMF was added, and the mixture let to stirrer for about 20hr, In a hygienic petri dish, the product was separated., let it dry, after that washed the product some time by Acetone then collected. The Synthesis General Scheme is in Scheme (5).



Scheme(5): General scheme for the synthesis of compound H6

2.2.1. Instrumentation.

The infrared spectra were recorded Using potassium bromide disks on a Pye Unicam SP-3-500 infrared spectrophotometer/ SHIMAZU/Japan. ^{1}H-NMR spectra were run at 500 MHz, on a Varian Mercury VX-500/ (INOVA/Switzerland) DMSO-d, the NMR spectrometer using TMS as an internal standard in deuterated dimethylsulphoxide. The microanalytical data were measured in the Central Lab of College of Education for Pure Sciences, Basrah University, Iraq; also All the chemical reactions were monitored, and The melting points were measured [9].

2.2.2. Materials:

Moxalactam or cefoperazone, protects amino acid, amino acids, DCC, thionyl chloride, solvent, and mineral acids.

All materials were supplied from different companies: Schar-Lab/Spain, BIOSYNTH Carbosynth/USA, Riedel-de-Hane/ Germany, Sigma-Aldrich/Germany, Carl Roth/Germany, Santacruz Biotechnology/USA, and GLS SYSTEMS/ India.

2.2.3. Computations:

Computations were performed using Gaussian 16 revision A.03 package and/or Spartan'16 parallel QC program (Wavefunction, Inc., USA). Optimized structures and spectroscopic data were obtained within DFT by employing the widely used wB97X-D/6-31G (d,p) model. Long-range corrected hybrid density functional, the wB97X-D functional [10] , includes empirical damped atom-atom dispersion corrections. wB97X-D is significantly more accurate than the commonly used functional B3LYP. Harmonic vibrational frequencies of the optimized geometries were calculated with the same model in order to verify that they are true minima (with zero imaginary frequencies). Tight SCFconvergence (energy change 1.0e−08 au) and larger integrationgrids are used. (e list of the convergence criteria followed is 5e−9 for RMS density change, 1e−7 for maximum density change, 5e−7 for direct inversion in the iterative subspace)DIES) error convergence, and 1e−5 for orbital gradient convergence. Finally, we used successfully a less-expensive computational model wB97X-D/6-31G (d) without any change in the trends obtained from the basis set 6-31G(d,p).

3. Results and Discussion

The spectrum of infrared [24, 25]

All the newly produced derivative compounds H2 and H6 have FT-IR spectra that share similarities in certain fingerprint-like bands and other bands. The essential functional group vibration bands are presented in Table (1), and the corresponding compound IR spectra are displayed in Figures(1) and (2).

All the predicted bands for normal Levofloxacin, as well as the bonded amino acids glutamine, histadine, hydroxyproline and phenylalanine are present in all FT-IR spectra of levofloxacin derivatives. Levofloxacin derivatives H2 and H6 often have IR spectra that are very similar to those of regular Levofloxacin. It indicates that condensation between levofloxacin and amino acids took place at the chlorine-containing region of typical levofloxacinoyl chloride.

The carbonyl bond of the amide group stretching is attributed to a sturdy band between 1745 and 1685 cm^{-1} in the FT-Infrared spectra of all the newly produced compounds H2 and H6. Those are in harmony with earlier works, and denote the entire condensation of levofloxacin with the study's glutamine, phenylalanine amino acids, hydroxyproline, and histidine. Additionally, the carboxylic groups bond of O-H linked to the amino acid residues is accountable for levofloxacin variants. IR spectra, performing a robust and broad extending band vibration in the range of 3441-3286 cm^{-1}. Furthermore, the N-H bond of a secondary amide can be assigned a medium band in the range of 3258-3124 cm^{-1}.

The levofloxacin derivative compounds H2 and H6 of IR spectra show a negative band at a range of 3041-3024 cm^{-1} because of aromatic straining C-H, as well as powerful bands to moderate ones at the range of 977-619 cm^{-1} because of the bending of C-H bond aromatic. A weak band was observed at the range of 2978-2823 cm^{-1} due to asymmetrical straining of aliphatic C-H bands, whereas a moderate band emerged at the range of 1423-1305 cm^{-1} for the bending of aliphatic C-H bond.

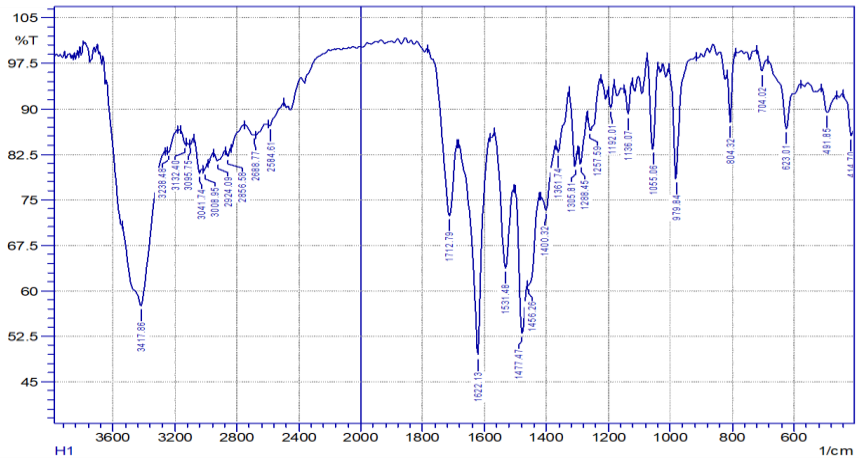
Aromatic (C=C) asymmetrical and symmetrical stretching could be associated with two prominent bands that popped up in the 1544-1455 cm^{-1} and 1479-1431 cm^{-1} ranges, and between.

Further to that, the compounds H2 and H6 of FT-IR spectra illustrate a strong band that is attributed to v(C-N) at the range of 1257-1201 cm^{-1} and assigned to (C-O) at the range of 1138-1124 cm^{-1}.

Table (1): Infrared novel levofloxacin derivatives' selected bands in the cm^{-1} unit

	ν_{aro}(C-H) Bending	ν(C-O)	ν(C-N)	ν_{ali}(C-H) Bending	ν_{as}(C=C) ν_s (C=C)	ν(C=O) amide	ν_{as} (C-H) ν_s (C-H) Aliphatic	ν(C-H) Aromatic	ν(N-H) Amide	ν(O-H)
2	909 s 704 s 673 s	1124 s	1213 s	1361 s	1531 s 1471 s	1712 s	2924 w	3041 w	3023 w	3417
6	835 s 800 s 723 s	1134 s	1201 s	1423 s	1544 w 1471 s	1685 s	2975w	3034 w	-	3431 br

aro=aromatic , ali= aliphatic, as=asymmetrical , s=symmetrical, s= sharp , w=weak , br=broad



Figure(1): The Compound H**2** of FT-Infrared Spectrum

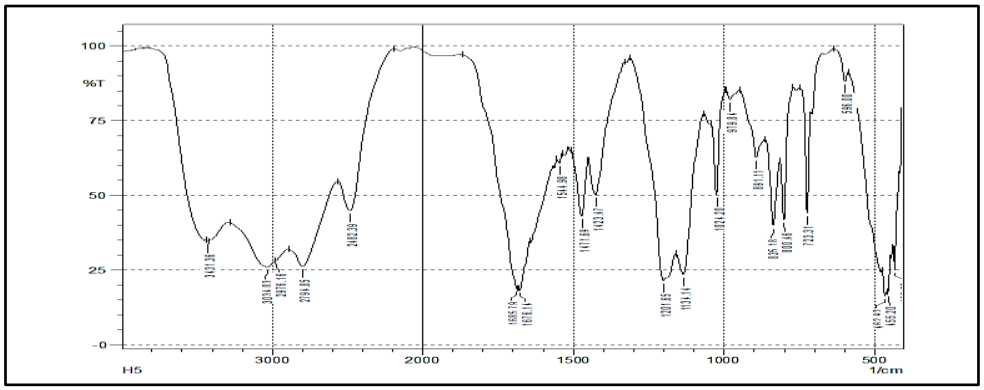
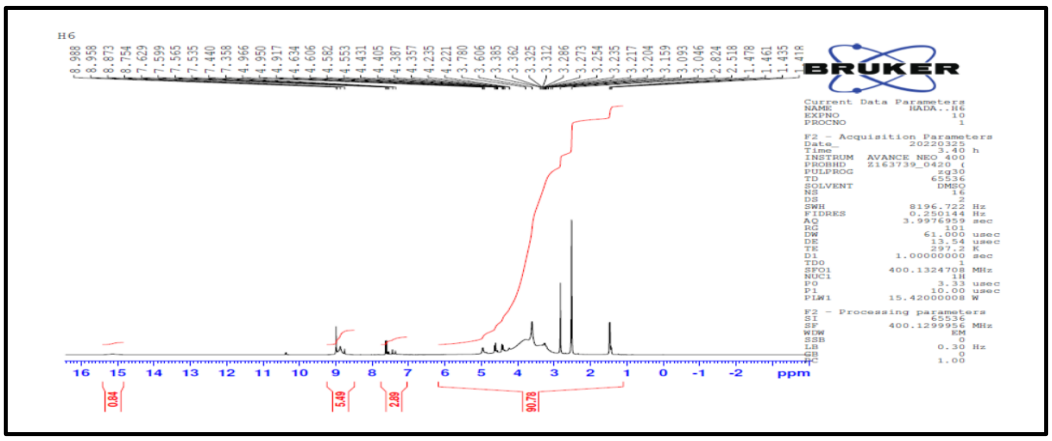


Figure. (2): The Compound H6 of FT-Infrared Spectrum

3.2. The Spectra of ^{1}H–NMR [25]

In DMSO-d6 solvent, the compound H2, and H6 of ^{1}H NMR spectra were restricted. Figures (3) and (4) demonstrate it, accordingly. Typically, the fitted intensity ratio of the observed compound ^{1}H NMR spectra depicts the expected signals. Table provides a breakdown of all levofloxacin derivative H2 and H6 ^{1}H–NMR.



Figure(3): The Spectrum of Compound H2 of the ^{1}H NMR

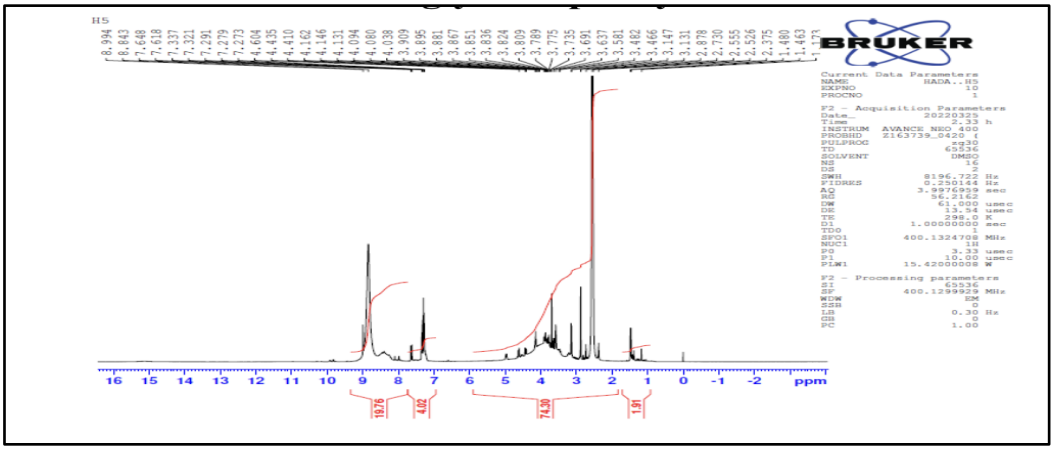


Figure (4): The Compound Spectrum: H6 of the ^{1}H NMR

The ^{1}H NMR spectra of the compounds, shown in Figures above, provided additional proof that Levofloxacin completely condensed with glutamine, histadine, hydroxyproline, and phenylalanine (the amino acids) to form a -CO=NH-R (N-substitution amide) group. Table (2) clearly illustrates a broad singlet sign caused by an amide proton at 7.56 to 15.2 ppm. Additionally, all the compound H2 and H6, ^{1}H–NMR spectra were recorded all the anticipated levofloxacin and amino acid

concentrations that were used in this work. These numbers match the information that was previously reported[27].

The aromatic protons of the phenyl group , the histidine imidazole ring, and the phenyl alanine of the phenyl group can all be attributed to the various signals in the ^{1}H NMR spectra of compound H2 and H6 (shown in Figure (3) and (4), which range in frequency from 7.273 to 8.99 ppm [28] . Further to that, those show a singlet sign that can be attributed to groups of proton methyl (e.g., no.14) centered at about 1.40 ppm. Aliphatic Proton possessed by no.2 and no.3 piperidine cyclic groups can be attributed to two triplet signals between 2.73 and 2.824 ppm, whereas the same cycle group's no.4 and no.5 can be assigned to the other two triplet signs around 3.35 and 3.88 ppm.

The aliphatic cycle proton of CH_2-O, or no.12, produces a di sign ranging from 4.046 to 4.51 ppm. The proton of carboxylic groups in amino acids, on the other hand, could be the source of the singlet sign during strong chemical shifts (weak field) in the 9.2-15.11 ppm range.

Table (2): The ^{1}H NMR data of various organometallic compounds having azomethine groups

Compound	Type of proton	Chemical shift in ppm	Desecription
H2	CH_3	1.461(d) 2.518(S)	(3H), Protons of CH3 group at position 14 (3H), Protons of (N-CH3) group at position 1
	CH_2	2.824 (S) 3.204(m) 3.78(s) 4.357(d)	(2H), Protons of CH2 groups at position 2,3 (2H), Protons of CH2 groups at position 21 (2H), Protons of CH2 groups at position 3,4 (2H), Protons of CH2 groups at position 12
	CH	3.385(br) 4.950(m) 7.352(t) 7.535(d) 7.629(m)	(1H), Protons of CH groups at position 13 (1H), Protons of CH groups at position 19 (1H), Protons of CH groups at position 8 (1H), Protons of CH groups at position 24 (1H), Protons of CH groups at position 23
	H	8.988(S)	(1H), Protons link C groups at position 15
	OH	10.5 (S)	(1H), Protons of COOH group position 20
	NH	8.73(d) 15.2(m)	(1H), Protons of NH group attach to c=o (1H), Protons of NH group in the ring
H6	CH_3	1.482(d) 2.555(s)	(3H), Protons of CH3 group at position 14 (3H), Protons of (N-CH3) group at position 1
	CH_2	2.730(t) 3.585 (t) 3.131(d) 3.842(d) 4.435(d)	(2H), Protons of CH2 groups at position 2,3 (2H), Protons of CH2 groups at position 3,4 (2H), Protons of CH2 groups at position 23 (2H), Protons of CH2 groups at position 19 (2H), Protons of CH2 groups at position 12
	CH	3.482(br) 7.273 7.618(s)	(1H), Protons of CH groups at position 13 (1H), Protons of CH groups at position 25,26,27.28,29 (1H), Protons of CH groups at position 8
	H	8.99(S)	(1H), Protons link C groups at position 15
	OH	10.2	(1H), Protons of COOH group
	NH	8.843-8.994	(1H), Protons OF two NH group

- S is singlet, d is doublet, t is triplet, q is quartet, m is multiplet,br is broad signal

3.3. The Spectra of ^{13}C–NMR

The DMSO-d6 solvent was employed to record the ^{13}C NMR spectra of levofloxacin derivatives H2 and H6. Figures (5) and (6) portrayed the ^{13}C NMR spectra of generated compounds H1 through H6, while Table (3) comprised the 13C NMR data. Further confirmation of the properties of the developed molecule emerged from 13C-NMR spectra [25].

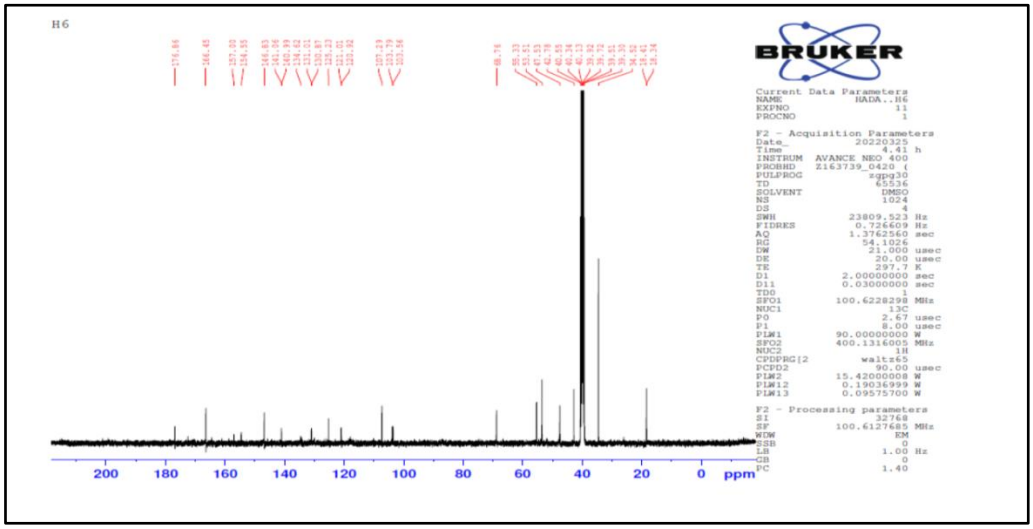


Figure (5): Spectrum of the compound H2 ^{13}C NMR

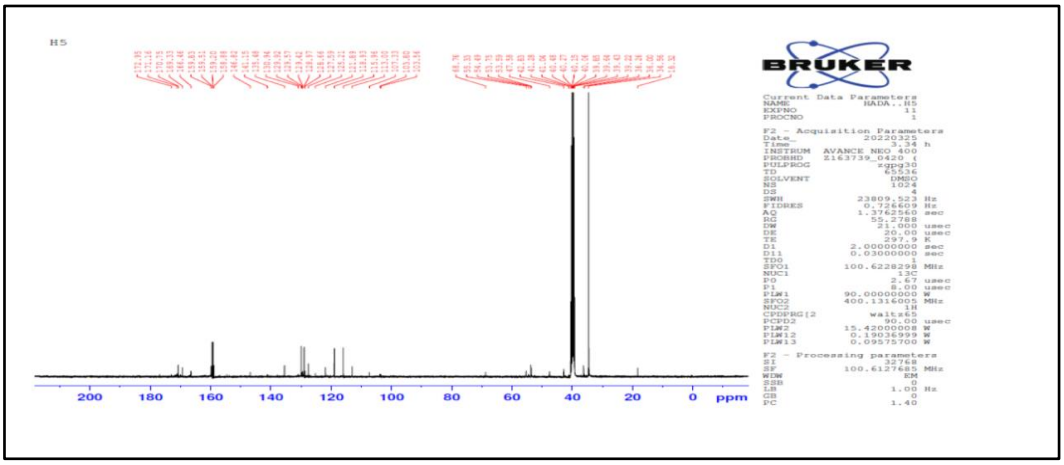


Figure (6): The compound H6 of Spectrum of ^{13}C NMR

Table (3): The ^{13}C-NMR data of all products

Compound	chemical shift (δ) in (ppm)	Description of carbon environment
H2	18.41	Carbon environment at position 14
	34.52	Carbon environment at position 21
	42.78	Carbon environment at position 1
	47.53	Carbon environment at position 2,3
	53.51	Carbon environment at position 4,5
	53,51	Carbon environment at position 19
	55.33	Carbon environment at position 13
	68.76	Carbon environment at position 12
	103.76	Carbon environment at position 8
	103.89	Carbon environment at position 24
	107.29	Carbon environment at position 16
	120.92	Carbon environment at position 9
	121.01	Carbon environment at position 22
	125.23	Carbon environment at position 10
	130.67	Carbon environment at position 6
	134.62	Carbon environment at position 23
	140.99	Carbon environment at position 11
	146.83	Carbon environment at position 15
	154.55	Carbon environment at position 7
	166.45	Carbon environment at position 18
	176.86	Carbon environment at position 20,17
H6	18.32	Carbon environment at position 14
	36.26	Carbon environment at position 23
	39.64	Carbon environment at position 19
	42.28	Carbon environment at position 1
	47.58	Carbon environment at position 2,3
	53.75	Carbon environment at position 4,5,21
	55.33	
	68.76	Carbon environment at position 13
	103.56	Carbon environment at position 12
	107.33	Carbon environment at position 8
	121.69	Carbon environment at position 16
	125.21	Carbon environment at position 9
	127.59-129.57	Carbon environment at position 10
	130.94	Carbon environment at position 25,26,27,28,29
	135.48	Carbon environment at position 6
	141.15	Carbon environment at position 24
	146.82	Carbon environment at position 11
	158.88	Carbon environment at position 15
	166.46	Carbon environment at position 7
	169.33	Carbon environment at position 18
	171.17	Carbon environment at position 20
	172.95	Carbon environment at position 22
		Carbon environment at position 17

4. Bioactivity

Antibiotic susceptibility experimental findings revealed that various bacteria had taken different approaches to the tested drugs. At (250 mg / L) & (400 mg / L), most bacteria isolated in gram-positive and gram-negative demonstrated strict sensitivity to the generated compounds (H2 and H6).

The results of Figures. (7 to10) demonstrate that the monopeptide (H2) antibiotic is more efficacious against streptococcus and Staphylococcus at a concentration of 250 mg/L.

These antibacterial peptides are described as a type of natural microbicide that is especially harmful to bacterial cells while barely toxic to mammalian cells. They function by drawing negatively charged bacterial cells with a reasonably high electrostatic force.

These peptides are categorized according to their content, amino acid sequences, and secondary structures. Despite the absence of an outer membrane or LPS in Gram-positive bacteria, these organisms have a highly anionic structure that makes them a good target for cationic antimicrobial peptides. Cationic antimicrobial peptides are more effective against Staphylococcus strains in which the acid of teichoic has already been transformed, culminating in an improved anionic charge. The antimicrobial peptide is thought to replace the cations customarily dealt with by LPS in Gram-negative bacteria.

Gram-negative bacteria have LPS (Lipopoly Saccharid) structures in their cell walls, with the hydrocarbon chains providing low fluidity to the LPS minor area. In blocking the flow of chemicals like antibiotics, the outer membranes are efficient [29]. The porin channels are frequently used by bacteria with considerable outer membrane components to enter the cell. In general, gram-negative bacteria's Porin channels allow access to hydrophilic substances [30].

Our results demonstrate that (H2) Samples at (100mg/L, and 200mg/L) Concentration levels are less efficient against bacteria of gram-positive and bacteria gram-negative.

On the other hand, at (50 mg/ L), all the samples (H2 and H6) show the lowest inhibition on the various tested bacteria. Low layer-by-layer permeability of the outer membrane to lipophilic Solutions is the cause of this resistance to antibiotics [31]. Drug Synergy is also considered to be a solid clinical notion because it allows for lower drug concentrations than are normally used, resulting in fewer adverse effects while proving the medication's efficacy [32].

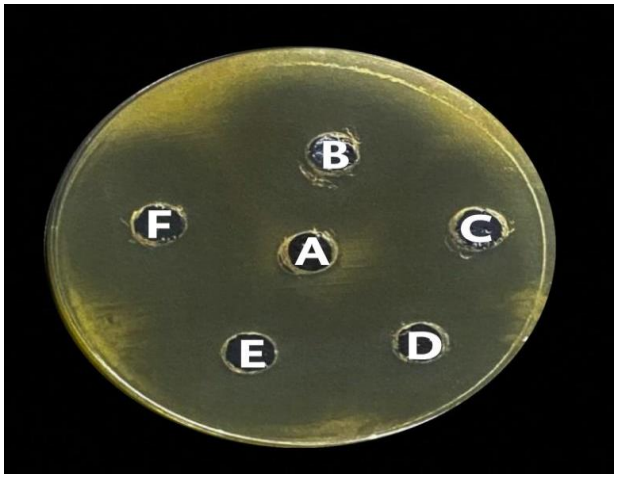
It has been investigated how the dipeptide antibiotic promotes the growth of bacteria. The prepared Samples (H6) that were utilized the most were very efficient against E. coli at 250 and 400 mg/L. (Figures. 11 to 14). Even though pseudomonas exhibited a lesser inhibition of the growth of bacteria in all the doses of an antibiotic (All are shown in figures 11 up to 14).

The two least effective bacterial mechanisms involved in intrinsic resistance have been decreased outer sheath permeability (the LPS (lipopolysaccharide) in gram-negative bacteria was most prominent) and the biological activity of the efflux pump.

Furthermore, the plasmid is an important element of the genetic structure of pseudomonas some of which can confer antibiotic resistance and other compounds of bacterial [33].

The assay for specimen, with one exception, 168 at a dose of 100 mg/L reveals a smaller zone of inhibition than at a concentration of 50 mg/L. To create combinatorial medicines that minimize patient toxicity while maximizing treatment success, the significant synergy and antagonism effects are frequently explored at optimal intermediate medication doses [34].

The level of inhibition present in each sample varies depending on the organism. There might be a diversity of different Zone Sizes preseasons, like the maturity and the quantity of inoculate that is injected into the solid medium, the incubation conditions, the medium composition, and changing receptors on the bacterial cells' surface. Eventually, these peptides are thought to be expansive microbicides [35-37]



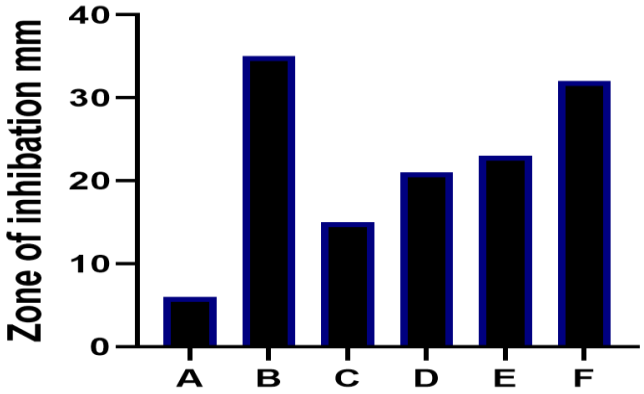
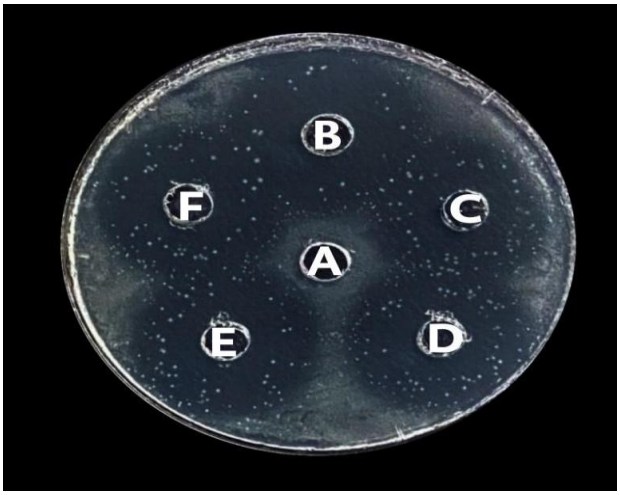


Figure (7): H2 antibacterial activity against the reference strain of Streptococcus A. Levofloxacin 250 micrograms/ml was administered to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.



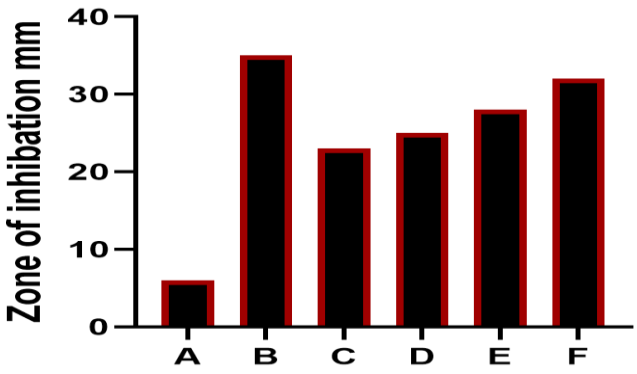
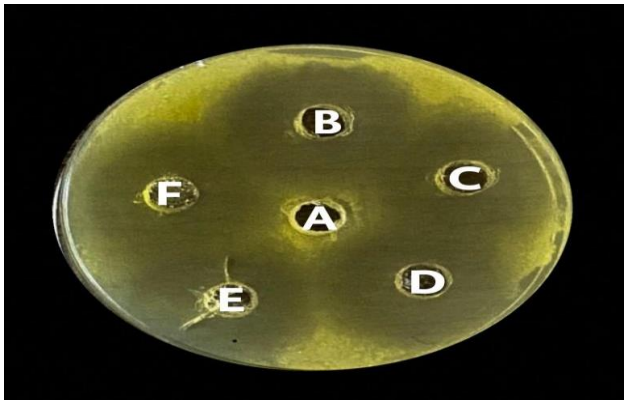


Figure (8): H2 antibacterial action on pseudomonas. A, command. Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.



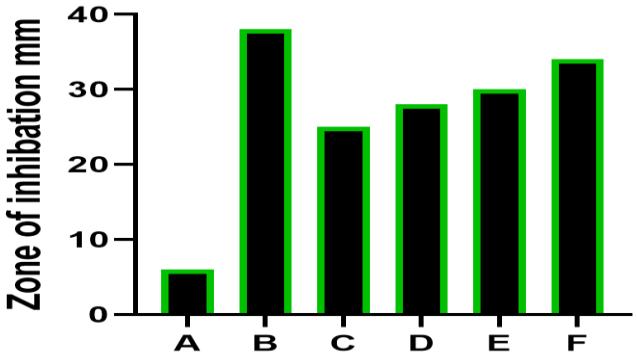


Figure (9): H2 antibacterial effects on Staphylococcus. A, command. Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment.

E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.

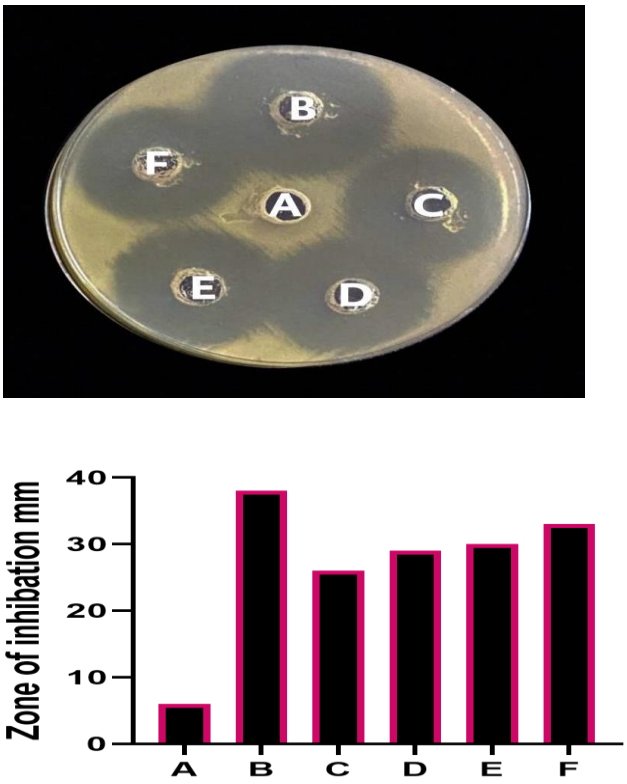


Figure (10): Escherichia coli is susceptible to H2 antibacterial properties. A, command. Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.

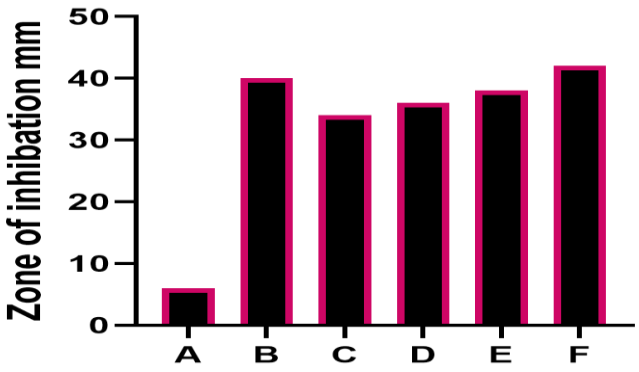


Figure (11): Escherichia coli-targeting (H6) antibacterial action. A, command. Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, the latter are given a 50 microgram/ml treatment. D, also given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.

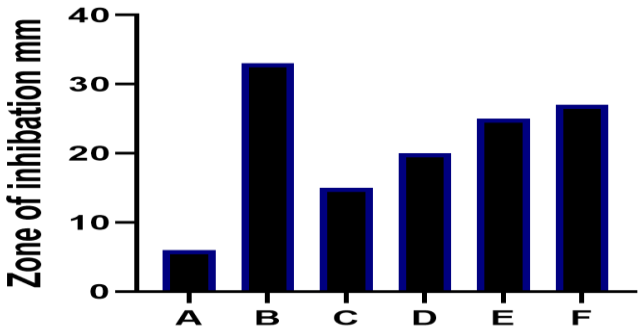
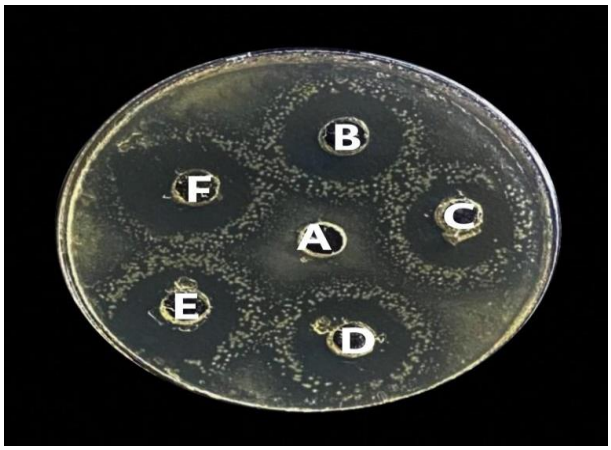


Figure (12): H6 antibacterial activity against the reference strain of Streptococcus A. Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain administered a 100 microgram/ml pretreatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.



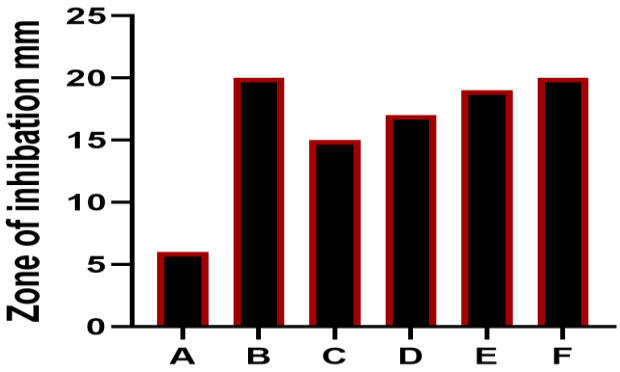
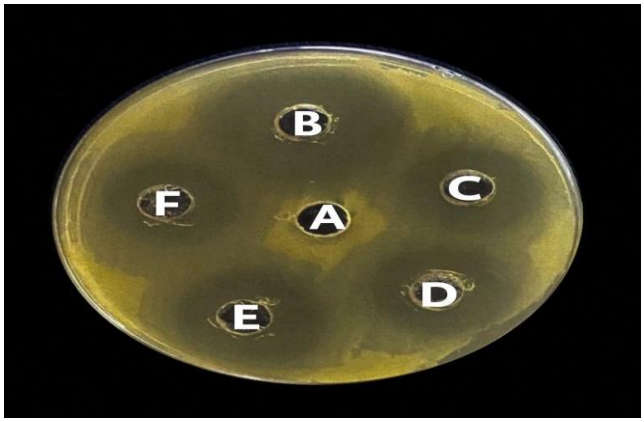


Figure (13): H6 antibacterial action on pseudomonas. A, direction Levofloxacin 250 micrograms/ml was used to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.



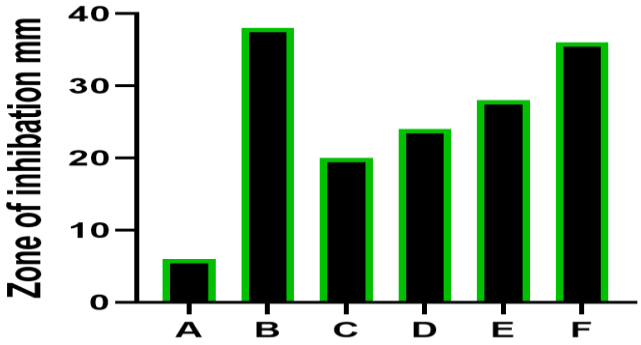


Figure (14): H6 antibacterial ability to tackle Staphylococcus bacteria. A, direct. Levofloxacin 250 micrograms/ml was prescribed to treat the bacterial strain B. C, bacterial strain given a 50 microgram/ml treatment. D, bacterial strain given a 100 microgram/ml treatment. E, a bacterial strain that received 200 g/ml of treatment. F, 400 microgram/ml-treated strain of bacteria.

5. Conclusion:

The studied bioactivity of the two compounds of levofloxacin H2 and H6 derivatives showed excellent results against four types of Gram-positive and Gram-negative bacteria. The spectroscopic data of the two new derivatives of H2 monopeptide and H6 dipeptide were characterized, as well as the interaction indicators were studied using FT-IR, ^{1}H-NMR, and ^{13}C-NMR techniques.

References :

1- Al-Salman H, Ali ET, Jabir M, Sulaiman GM, Al-Jadaan SA.[2020]: 2-Benzhydrylsulfinyl-N-hydroxyacetamide-Na extracted from fig as a novel cytotoxic and apoptosis inducer in SKOV-3 and AMJ-13 cell lines via P53 and caspase-8 pathway. J.European Food Research and Technology. 246, pp. 1591-1608.

2- Wei T, Lu S, Sun J, Xu Z, Yang X, Wang F, Ma Y, Shi Y, Chen X.[2020]: Al. Sanger's reagent sensitized photocleavage of amide bond for constructing photocages and regulation of biological functions. J.Journal of the American Chemical Society. 142 (8), pp. 3806-3813.

3- Zhang H, Chen S.[2022]: Cyclic peptide drugs approved in the last two decades (2001–2021). J.RSC Chemical Biology. 3 (1), pp. 18-31.

4- O Moustafa G, Shalaby A.[2021]: The Importance of Amino Acid and Peptide Chemistry in the Treatment of the Major Diseases: Neuropeptides. J.Egyptian Journal of Chemistry. 64 (8), pp.4469-4486.

5- Ben-Shushan S, Miller Y.[2021]: Neuropeptides: Roles and activities as metal chelators in neurodegenerative diseases. J. The Journal of Physical Chemistry B.125(11), pp.2796-2811.

6- Narambuena CF, Longo GS, Szleifer I.[2015]: Lysozyme adsorption in pH-responsive hydrogel thin-films: the non-trivial role of acid–base equilibrium. J.Soft Matter. 11(33), pp.6669-6679.

7- Baird EE, Dervan PB.[1996]: Solid phase synthesis of polyamides containing imidazole and pyrrole amino acids. J. Journal of the American Chemical Society. 118(26), pp.6141-6146.

8- Brady SF, Hirschmann R, Veber DF.[1977]: Some novel, acid-labile amine protecting groups. J.The Journal of Organic Chemistry. 42 (1), pp.143-146.

9- Schnölzer M, Alewood P, Jones A, Alewood D, Kent SB.[1992]: In situ neutralization in Boc-chemistry solid phase peptide synthesis: Rapid, high yield assembly of difficult sequences. J.International journal of peptide and protein research.40(3-4), pp.180-193.

10- Guy CA, Fields GB.[1997]: [5] Trifluoroacetic acid cleavage and deprotection of resin-bound peptides following synthesis by Fmoc chemistry. J. Methods in enzymology. 289, pp. 67-83.

11- Amblard M, Fehrentz J-A, Martinez J, Subra G.[2006]: Methods and protocols of modern solid phase peptide synthesis. J. Molecular biotechnology. 33, pp. 239-254.

12- Palomo JM.[2014]: Solid-phase peptide synthesis: an overview focused on the preparation of biologically relevant peptides. J. Rsc Advances. 4 (62), 32658-32672.

13- Frokjaer S, Otzen DE.[2005]: Protein drug stability: a formulation challenge. J. Nature reviews drug discovery. 4 (4), pp.298-306.

14- Sinha V, Trehan A.[2003]: Biodegradable microspheres for protein delivery. J. Journal of controlled release. 90(3), pp.261-280.

15- Shantha Kumar T, Soppimath K, Nachaegari S.[2006]: Novel delivery technologies for protein and peptide therapeutics. J. Current pharmaceutical biotechnology. 7 (4), 261-276.

16- Langer R.[1990]: New methods of drug delivery. J. Science. 249(4976), pp. 1527-1533.

17- Oliyai R.[1996]: Prodrugs of peptides and peptidomimetics for improved formulation and delivery. J. Advanced drug delivery reviews. 19(2), pp.275-286.

18- Sun SF.(2004): Physical chemistry of macromolecules: basic principles and issues: John Wiley & Sons.

19- Shoombuatong W, Schaduangrat N, Nantasenamat C.[2018]: Unraveling the bioactivity of anticancer peptides as deduced from machine learning. J. EXCLI journal. 17, pp.734.

20- Rahman AM, Ali R, Jahng Y, Kadi AA.[2012]: A Facile Solvent Free Claisen-Schmidt Reaction: Synthesis of α, α′-bis-(Substituted-benzylidene) cycloalkanones and α, α′-bis-(Substituted-alkylidene) cycloalkanones. J. Molecules. 17(1), pp. 571-583.

21- Lyu Y, Domalaon R, Yang X, Schweizer F.[2019]: Amphiphilic lysine conjugated to tobramycin synergizes legacy antibiotics against wild-type and multidrug-resistant Pseudomonas aeruginosa. J. Peptide Science. 111(1), pp. e23091.

22- Yang X, Ammeter D, Idowu T, Domalaon R, Brizuela M, Okunnu O, et al.[2019]: Amphiphilic nebramine-based hybrids Rescue legacy antibiotics from intrinsic resistance in multidrug-resistant Gram-negative bacilli. J. European journal of medicinal chemistry. 175, pp. 187-200.

23- Ali ET, Al-Salman H, Rasool KH, Jabir MS, Ghimire TR, Shari FH, et al.[2023]: 2-(Benzhydryl sulfinyl)-N-sec-butylacetamide) isolated from fig

augmented trastuzumab-triggered phagocytic killing of cancer cells through interface with Fcγ receptor. J. Natural Product Research. pp. 1-9.

24- Ptaszyńska N, Gucwa K, Olkiewicz K, Heldt M, Serocki M, Stupak A, et al.[2020]: Conjugates of ciprofloxacin and levofloxacin with cell-penetrating peptide exhibit antifungal activity and mammalian cytotoxicity. J. International journal of molecular sciences. 21(13), pp. 4696.

25- Lihumis HS, Al Talebi ZA, Khaleel AK.[2022]: Synthesis and Identification of Some New Heterocyclic Compounds for Levofloxacin Drug Derivatives with Evaluating of Their Biological Efficiency and Antioxidant Activity. J. Journal of Medicinal and Chemical Sciences. 5(4), pp. 596-606.

26- Sarfraz N, Khan I.[2021]: Plasmonic gold nanoparticles (AuNPs): properties, synthesis and their advanced energy, environmental and biomedical applications. J. Chemistry–An Asian Journal. 16(7), pp. 720-742.

27- Medforth CJ.[2022]: NMR Spectroscopy of Porphyrins. J.Fundamentals of Porphyrin Chemistry: A 21st Century Approach. 2, pp. 611-629.

28- Wagh P, Escobar IC.[2019]: Biomimetic and bioinspired membranes for water purification: a critical review and future directions. J. Environmental Progress & Sustainable Energy. 38(3), pp. e13215.

29- Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM.[2020]: Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. J. Advances in Natural Sciences: Nanoscience and Nanotechnology. 11(3), pp. 035010.

30- Ma L, Kohli M, Smith A.[2013]: Nanoparticles for combination drug therapy. J. ACS nano. 7(11), 9518-9525.

31- Al-Bahadily DC, Alkamil RD, Qasim QA, Shari FH, Al-Salman H.[2020]: analyticaL and biological activity study of one of the isolated extracts of truffles ethyl 6-methyl-2-oxo-4-(2-thenyl)-1, 2, 3, 4-tetrahydroperimidine-5-carboxylate (emottc) against three of candida species.pp. 6121-6128.

32- Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM.[2020]: Anticancer activity and toxicity of carbon nanoparticles produced by pulsed

laser ablation of graphite in water. J. Advances in Natural Sciences: Nanoscience and Nanotechnology. 11(3), pp. 035010.

33- Khashan KS, Badr BA, Sulaiman GM, Jabir MS, Hussain SA, editors.[2021]: Antibacterial activity of Zinc Oxide nanostructured materials synthesis by laser ablation method. J. Journal of Physics: Conference Series. 1795(1), pp. 012040.

34- Jihad MA, Noori FT, Jabir MS, Albukhaty S, AlMalki FA, Alyamani AA.[2021]: Polyethylene glycol functionalized graphene oxide nanoparticles loaded with nigella sativa extract: a smart antibacterial therapeutic drug delivery system. J. Molecules. 26(11), pp. 3067.

35- Mohammed MK, Mohammad M, Jabir MS, Ahmed D, editors.[2020]: Functionalization, characterization, and antibacterial activity of single wall and multi wall carbon nanotubes. IOP Conference Series: Materials Science and Engineering. 757(1), pp. 012028.

36- Ali IH, Jabir MS, Al-Shmgani HS, Sulaiman GM, Sadoon AH, editors.[2018]: Pathological and immunological study on infection with Escherichia coli in ale balb/c mice. Journal of Physics: Conference Series.1003, pp. 012009

37- AL-Salman HN, Ali ET, Almukhtar OA, Jabir MS, editors.[2020]: 2-benzhydrylsulfinyl-N-hydroxyacetamide extracted from fig: A good therapeutic agent against Staphylococcus aureus. AIP Conference Proceedings. 2213(1), pp. 020223