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In Silico Study and In Vitro Evaluation of Novel Synthesized Quinolone Derivatives Having Five-Membered Heterocyclic Moieties

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Abstract

Infectious diseases are caused by pathogens, such as viruses, bacteria, fungi, and parasites. Quinolones work by inhibition of bacterial topoisomerase IV and/or gyrase, a group of oxadiazole derivatives were incorporated into C7 piperazine ring of Gatifloxacin, a well-known antibacterial fluoroquinolone, in order to increase bulkiness at C7 leading to reduce bacterial resistance and improve anti-bacterial activity. In the current work , the synthesized compounds V(a-e) were screened for their antibacterial activity against gram negative bacteria: *Klebsiella pneumonia* and *Escherichia coli* and gram positive bacteria *Streptococcus pyougenes* and *Staphylococcus aureus* bacteria, the tested compounds showed an interesting activity against gram positive and gram negative bacteria, these tested compounds give significant antibacterial activity in comparison to Gatifloxacin as a starting compound and DMSO as a control, confirmations and characterization of the chemical structures related to these compounds were performed using ^{1}H-NMR spectroscopy, FT-IR spectroscopy, and some physicochemical properties such as melting points. Docking study of the final synthesized compounds gave evidence about the affinity of these compounds toward topoisomerase IV enzyme, statistical results show the elevated inhibitory zones of the prepared compounds compared with Gatifloxacin, regarding *S. aureus* bacteria the inhibition zone elevated from 18mm in Gatifloxacin to 24 in (Vb and Ve) and 26mm in Vd, also for *K. pneumonia* bacteria the zone of inhibition raised from 18mm in Gatifloxacin to 20mm in Vb and 24mm in Ve.

Keywords: Anti-bacterial; Gatifloxacin; molecular docking; 1,3,4-oxadiazole; topoisomerase IV;

1. Introduction

Invasive pathogens, such as viruses, bacteria, fungus, and parasite infestations, cause infectious illnesses. (1) Antibiotics have been the most commonly used class of medications for destroying infectious illnesses throughout the past century. (2)

Quinolones are one of the largest classes of antibiotics. (3) They work by inhibition of bacterial topoisomerase IV and/or DNA gyrase. (4) Topoisomerase IV is catalyzing the unlinking procedure of post-replication daughter strands. DNA gyrase is regulating bacterial DNA replication and transcription by catalyzing the negative supercoiling of DNA. (5) The action of quinolones of the first generation e.g., nalidixic acid in gram-negative bacteria, whereas activity of gram-negative activity in the second generation e.g. ciprofloxacin in some gram-positive pathogens.

Third generation are more gram-positive than their suppression by first and second generation of DNA gyrase due to their selective inhibition of topoisomerase IV e.g. gatifloxacin (Figure 1). Finally, the fourth generation for example, moxifloxacin display two enzymes, topoisomerase IV and DNA gyrase, such that they can be used to treat quinolone-resistant diseases. (6) Inhibition of both bacterial topoisomerase IV and DNA gyrase, is equally by moxifloxacin, finafloxacin and delafloxacin, in contrast with fluoroquinolones of second generation, trovafloxacin and ciprofloxacin which have an 8-and 19-times higher powers on DNA gyrase, compared with topoisomerase IV. (7)

The position 7 of fluoroquinolone moiety is directly interatomic to DNA gyrase or topoisomerase IV.
In this position, the optimal substitutes were bunches that have a 5- or 6-member heterocycle at least.

Aminopyrrolidines and piperazines are the most frequent of them. (8)

The condition of aminopyrrolidine improves gram positive action, while the effect of piperazine on gram negative microscopic organisms often increases strength.

Furthermore, alkylation (CH_3) of 5membered or 6 membered heterocycles (pyrrolidines and piperazines

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separately) increases grampositive microbiological activity. [9] An interesting perspective afterwards is that increased bulkiness (R-7) here is shown to shield

Aldehyde Compound	Weight (g)
4- bromobenzaldehyde	1.66
4- chlorobenzaldehyde	1.26
4- hydroxybenzaldehyde	1.09
4- nitrobenzaldehyde	1.35
4- methylbenzaldehyde	1.08

microscopic organisms from exporting efflux proteins and to lower the likelihood of resistance to bacteria in wild bacterial strains. In addition, the bulk of anti-anaerobic activity increases here. [10] Medicinal chemistry researchers have executed many work on heterocyclic derivatives due to their various therapeutic applications, range from central nervous system activity to antimicrobial applications. [11] Results of many literatures reveals that 1,3,4-oxadiazole moiety offers a wide spectrum of biological activities including: anti-inflammatory, analgesic, antiviral, antifungal, antibacterial activities. [12]

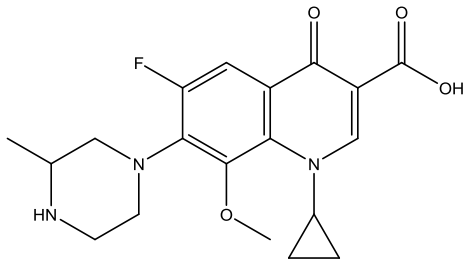


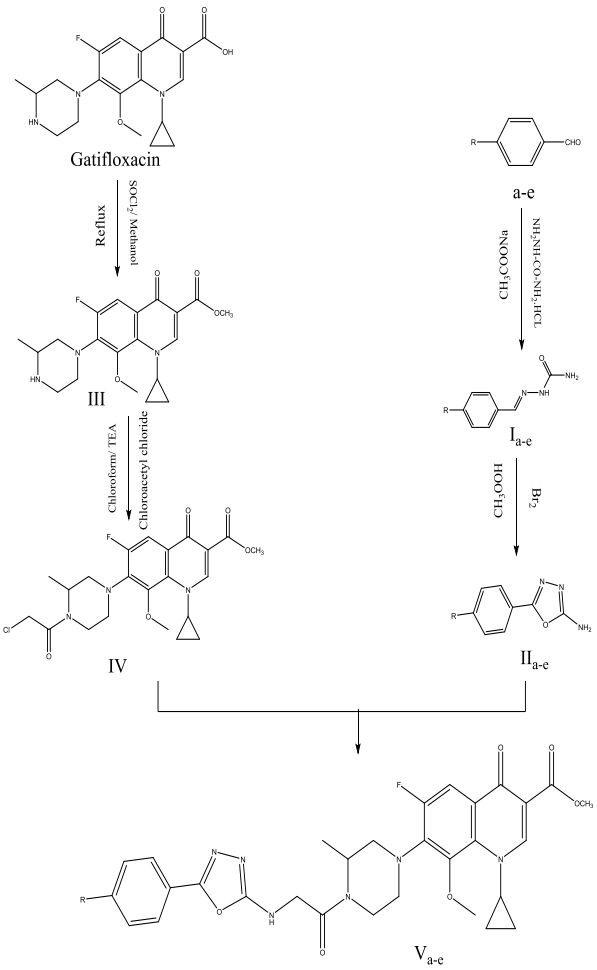
Figure (1): structure of Gatifloxacin

2. Experimental:

All reagents and anhydrous solvents were of analytical grade and were supplied from (England, Germany, China, India, Spain, USA). Melting points were determined by Electro – thermal melting point apparatus capillary tube method. Determination of infrared spectrum by using FTIR- spectrophotometer, were done at the college of pharmacy, Mustansiriyah University, KBr discs was the method for spectrum determination. The ^{1}HNMR spectrum was performed at Tehran University, Iran. Instrument Model: Bruker 500MHz-Avanc using dimethylsulphoxide (DMSO) as a solvent.

Chemical Synthesis

The synthesis of the target compounds (I-Va-e) and their intermediates were achieved following procedures illustrated in scheme (1).



R= Br, Cl, OH, NO_2, CH_3

Scheme (1): Synthesis of the target compounds ($I\text{-}V_{a\text{-}e}$)

Synthesis of Semicarbazone, Compounds (Ia-e) [13]

Semicarbazide Hydrochloride (9 mmol, 1.004 g) and sodium acetate (18 mmol, 1.476 g) was dissolved in 35mL of distilled water and the appropriate aldehydes (that listed in table 1) (9 mmol) were added slowly to the solution. After an hour of stirring the precipitate was filtered, dried and recrystallized from 95% ethanol.

The characterization and physical data are given in table (2).

Table 1: Aldehydes and their weight:

2-(4-bromobenzylidene)hydrazine-1-carboxamide (Ia): FT-IR (KBr, cm^{-1}): 3462 and 3277(N-H primary amide); 3174 (N-H secondary amide); 1705 (C=O amide); 1668 (C=N); 829 (C-Br stretching vibration).

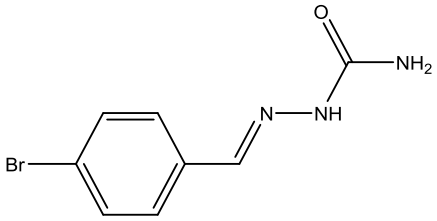


Figure 2: Chemical Structure of Compound Ia

2-(4-chlorobenzylidene)hydrazine-1-carboxamide (Ib): FT-IR (KBr, cm^{-1}): 3464 and 3277(N-H primary amide); 3144 (N-H secondary amide overlap with =C-H of aromatic); 1707 (C=O amide); 1668 (C=N); 705 (C-Cl stretching vibration).

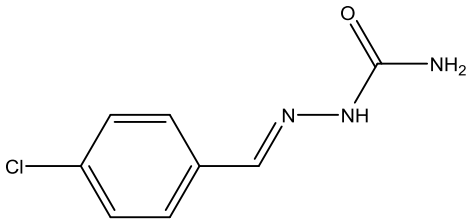


Figure 3: Chemical Structure of Compound Ib

2-(4-hydroxybenzylidene)hydrazine-1-carboxamide (Ic): FT-IR (KBr, cm^{-1}): 3475 and 3338 (N-H primary amide); 3290-2920 (Broad band O-H stretching); 3018 (N-H stretching vibration of secondary amide overlapping with =C-H stretching aromatic); 1687 (C=O amide); 1583 (C=N).

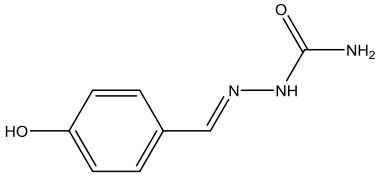


Figure 4: Chemical Structure of Compound Ic

2-(4-nitrobenzylidene)hydrazine-1-carboxamide (Id): FT-IR (KBr, cm^{-1}): 3464 and 3296 (N-H primary amide); 3070 (N-H stretching vibration of secondary amide overlapping with =C-H stretching band); 2954, 2931 and 2829 (C-H alkane); 1695 (C=O amide); 1612 (C=N); 1585 (Asymmetrical stretching of NO_2); 1346 (Symmetrical stretching of NO2).

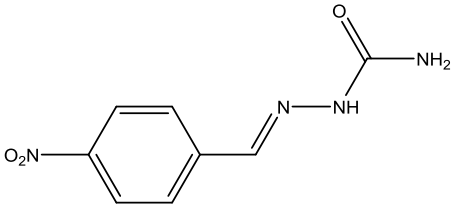


Figure 5: Chemical Structure of Compound Id

2-(4-methylbenzylidene)hydrazine-1-carboxamide (Ie): FT-IR (KBr, cm^{-1}): 3462 and 3286 (N-H primary amide); 3186 (N-H secondary amide); 3066 (C-H stretching of aromatic); 2978 and 2937 (C-H stretching of methyl); 1685 (C=O amide); 1649 (C=N).

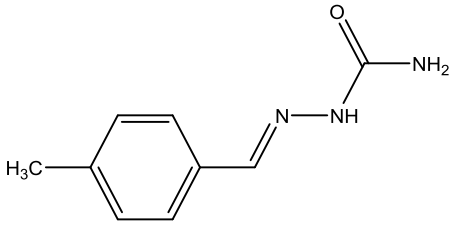


Figure 6: Chemical Structure of Compound Ie

Synthesis of 2- Amino- 5 –Aryl-1, 3, 4-Oxadiazole Derivatives (IIa-e) [14]

Semicarbazone compounds (Ia-e) (6 mmol) and sodium acetate (6 mmol, 0.984 g) were dissolved in 30 mL of glacial acetic acid, and bromine (0.7 mL in 5 mL of glacial acetic acid) was added slowly to it. Solution was stirred for an hour and then transferred on crushed ice. The resulted solid was filtered, then washed with cold water, dried and recrystallized from ethanol (95%).

The characterization and physical data are given in table (2).

5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine (IIa): FT-IR (KBr, cm^{-1}): 3269 and 3105 (N-H primary amine); 1658 (C=N stretching vibration); 1114 (C-O-C stretching vibration); 831 (C-Br stretching vibration).

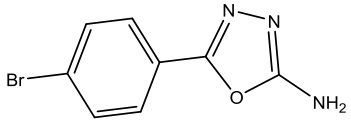


Figure 7: Chemical Structure of Compound IIa

5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (IIb): FT-IR (KBr, cm^{-1}): 3302 and 3105 (N-H primary amine); 1656 (C=N); 1097 (C-O-C stretching vibration); 837 (C-Cl stretching vibration).

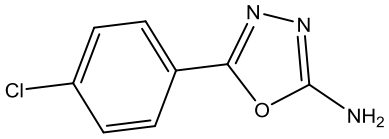


Figure 8: Chemical Structure of Compound IIb

4-(5-amino-1,3,4-oxadiazol-2-yl) phenol (IIc):
FT-IR (KBr, cm^{-1}): 3487 and 3379 (N-H primary amine); 3290-2920 (Broad band O-H stretching); 3018 (C-H aromatic); 1687 (C=N); 1149 (C-O-C stretching vibration).

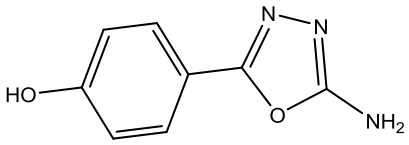


Figure 9: Chemical Structure of Compound IIc

5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine(IIId):
FT-IR (KBr, cm^{-1}): 3456 and 3186 (N-H primary amine); 3086 (C-H aromatic); 1683 (C=N); 1591 and 1338 (Asymmetric and symmetric stretching of NO_2); 1226 (C-N); 1149 and 1195 (C-O-C stretching vibration); 852 (C-N stretching vibration of nitro aromatic compound).

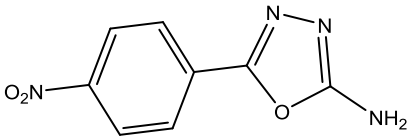


Figure 10: Chemical Structure of Compound IId

5-(p-tolyl)-1,3,4-oxadiazol-2-amine (IIe):
FT-IR (KBr, cm^{-1}): 3294 and 3260 (N-H primary amine); 2937 (CH_3 stretching vibration); 1664 (C=N); 1124 (C-O-C stretching vibration).

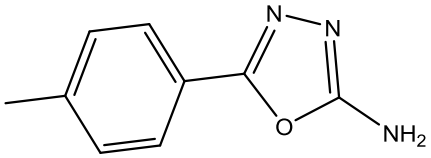


Figure 11: Chemical Structure of Compound IIe

Synthesis of methyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (Compound III): [15]

A mixture of gatifloxacin (1.877g, 5mmol), in absolute methanol (50mL) was cooled down to -15^0 C, then thionyl chloride (1mL) was added drop wise, (the temperature should be kept at -15℃). The reaction mixture was kept at 40 ℃ for three hours, followed by refluxing for 24 hr. (until the HCl gas was ended). Methanol was evaporated, the residue was re-dissolved in methanol and evaporated. The process was repeated several times to ensure complete removal of thionyl chloride, the product was recrystallized by diethyl ether. The characterization and physical data are given in table (2).

FT-IR (KBr, cm^{-1}): 3433 (N-H stretching of piperazinyl moiety); 3078 (C-H stretching of aromatic); 2941 and 2902 (C-H stretching vibration of CH_3 and CH_2); 1734 (C=O ester); 1620 (C=O quinolone); 1359 (C-N stretching vibration); 1215 (C-O stretching vibration of ester); 1045 (C-F).

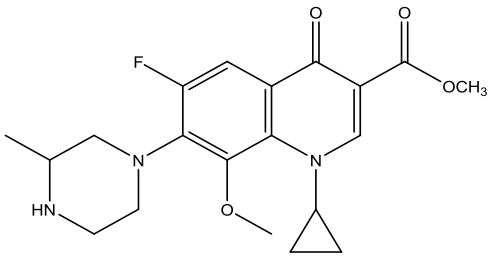


Figure 12: Chemical Structure of Compound III

Synthesis of methyl 7-(4-(2-chloroacetyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (Compound IV): [15]

Compound (III) (1.0g, 2.5 mmol), was dissolved in DMF: Chloroform (1:3) mixture (40mL), then TEA (0.34mL, 2.5 mmol) was added. The reaction mixture was stirred on ice bath, chloroacetylchloride (0.2mL, 2.5 mmol in 10mL Chloroform) was added drop wise with continuous stirring over period of one hour, followed by refluxing of the mixture for seven hours. Then the solvent was evaporated, and the precipitated compound was recrystallized from ethanol. The characterization and physical data are given in table (2).

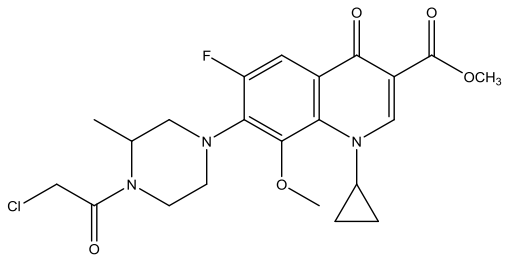


Figure 13: Chemical Structure of Compound IV

FT-IR (KBr, cm^{-1}): 3076 (C-H aromatic); 2939 and 2902 (C-H stretching vibration of CH_3 and CH_2); 1732

(C=O ester); 1653 (C=O amide); 1620 (C=O quinolone); 1278 (C-N stretching vibration); 1053 (C-F); 802 (C-Cl stretching vibration).

Synthesis of Compounds (Va-e) (Coupling Reaction): [16]

A mixture of compounds (IIa-e) (2.15mmol) and compound (IV) (1.0019g, 2.15mmol), were dissolved in DMF (20mL), then TEA (0.3mL, 2.15mmol), was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated; the residue was crystallized from ethanol to give compounds (Va-e). The characterization and physical data are given in table (2).

methyl7-(4-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl) glycyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (Va):

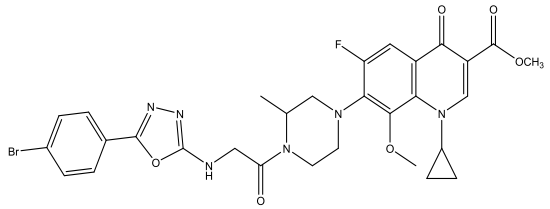


Figure 14: Chemical Structure of Compound Va

FT-IR (KBr, cm^{-1}): 3294 (N-H stretching vibration); 1732 (C=O stretching vibration of ester); 1658 (C=O Stretching vibration of amide coupling with C=O of quinolone); 1610 (C=C stretching vibration of aromatic); 1307 (C-O stretching vibration of ester); 1168 (C-F stretching vibration); 885 (C-Br stretching vibration); ^{1}H-NMR (ppm) (Figure 25): 8.72 (1H, s, Ar-H); 7.72-7.76 (4H, m, Aromatic ring); 7.4 (2H, d, CH of aromatic); 7.32 (1H, t, NH); 4.17 (3H, m-d, CH Cyclopropane CH_2-NH); 3.81 (6H, s, OCH_3); 3.54 (2H, d, CH_2 piperazine); 2.51-3.17 (3H, m, CH_2 and CH piperazine); 1.13-1.32 (4H, d, CH_2Cyclopropan); 1.05 (3H, d, CH_3- piperazine).

methyl7-(4-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl) glycyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (Vb):

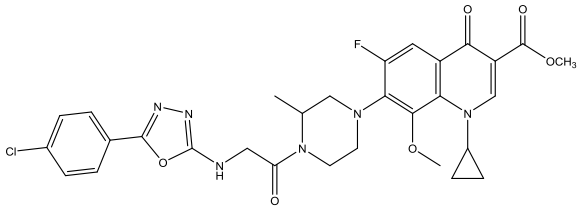


Figure 15: Chemical Structure of Compound Vb

FT-IR (KBr, cm^{-1}): 3296 (N-H stretching vibration); 1732 (C=O stretching vibration of ester); 1656 (C=O Stretching vibration of amide coupling with C=O of quinolone); 1604 (C=C stretching vibration of aromatic); 1307 (C-O stretching vibration of ester); 1166 (C-F stretching vibration); 883 (C-Cl stretching vibration). ^{1}H-NMR (ppm) (Figure 26): 8.72 (1H, s, Ar-H); 7.6-7.78 (4H, m, Aromatic ring); 7.32 (1H, t, NH); 4.18 (3H, m-d, CH Cyclopropane); 3.81 (3H, s-d-s, OCH_3-CH_2-OCH_3-Ar); 3.54-3.56 (2H, d, CH_2 piperazine); 2.51-3.14 (3H, m, CH_2 and CH piperazine); 1.15-1.31 (4H, m, CH_2Cyclopropan); 1.05 (3H, d, CH_3- piperazine).

methyl1-cyclopropyl-6-fluoro-7-(4-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (Vc):

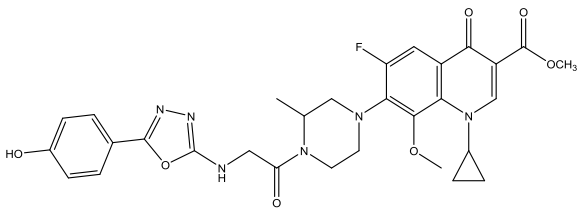


Figure 16: Chemical Structure of Compound Vc

FT-IR (KBr, cm^{-1}): 3286 (N-H stretching vibration); 3194-2943 (O-H stretching); 1734 (C=O stretching vibration of ester); 1653 (C=O Stretching vibration of amide coupling with C=O of quinolone); 1602 (C=C stretching vibration of aromatic); 1311 (C-O stretching vibration of ester); 1155 (C-F stretching vibration). ^{1}H-NMR (ppm) (Figure 27): 10.22(1H, d, OH); 8.72(1H, d, Ar-H); 7.77-7.93 (4H, m, Aromatic ring); 7.69 (1H, t, NH); 4.18 (2H, d, CH Cyclopropane); 3.81 (3H, s, CH_3-O, CH_3OCO); 3.34-3.54 (4H, m ،CH_2 piperazine); 2.51-3.17 (3H, m, CH_2 and CH piperazine); 1.15-1.33 (4H, m, CH_2Cyclopropan); 1.05 (3H, d, CH_3- piperazine).

methyl1-cyclopropyl-6-fluoro-7-(4-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-

methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (Vd):

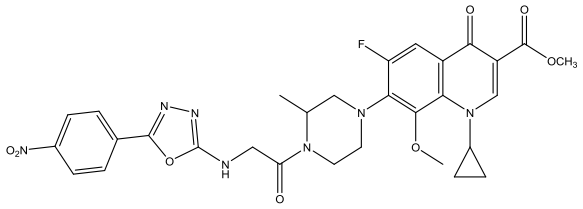


Figure 17: Chemical Structure of Compound Vd

FT-IR (KBr, cm^{-1}): 3454 (N-H stretching vibration); 1732 (C=O stretching vibration of ester); 1654 (C=O Stretching vibration of amide coupling with C=O of quinolone); 1614 (C=C stretching vibration of aromatic); 1309-1276 (C-O stretching vibration of ester overlapping with N-O stretching vibration of NO_2); 1166 (C-F stretching vibration); 883 (C-N stretching vibration of nitro aromatic moiety). ^{1}H-NMR (ppm) (Figure 28): 8.72 (1H, d, Ar-H); 7.94-8.20 (4H, m, Aromatic ring); 7.79 (1H, t, NH); 4.19 (3H, m, CH Cyclopropane); 3.81 (6H, s, CH_3-O); 3.23-3.54 (4H, m ،CH_2 piperazine); 2.51-3.16 (3H, m, CH_2 and CH piperazine); 1.15-1.33 (4H, m, CH_2Cyclopropan); 1.05 (3H, d, CH_3- piperazine).

methyl1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl) glycyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (Ve):

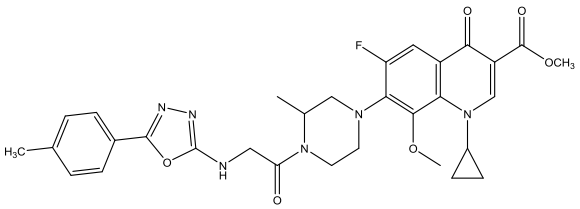


Figure 18: Chemical Structure of Compound Ve

FT-IR (KBr, cm^{-1}): 3271 (N-H stretching vibration); 2906 (C-H of aliphatic stretching vibration); 1730 (C=O stretching vibration of ester); 1660 (C=O Stretching vibration of amide coupling with C=O of quinolone); 1610 (C=C stretching vibration of aromatic); 1311 (C-O stretching vibration of ester); 1170 (C-F stretching vibration). ^{1}H-NMR (ppm) (Figure 29): 8.51 (1H, d, Ar-H); 7.87-7.89 (4H, m, Aromatic ring); 7.56 (1H, t, NH); 4.2 (2H, d, CH Cyclopropane); 3.81 (3H, s, CH_3-O, CH_3OCO); 3.37-3.54 (4H, m ،CH_2 piperazine); 2.51-3.2 (3H, m, CH_2 and CH piperazine); 2.31 (3H, s, CH_3); 1.19-1.4 (4H, m, CH_2Cyclopropan); 1.07 (3H, d, CH_3- piperazine).

Table (2): The characterization and physical data of the synthesized compounds

Compounds and intermediates	Empirical formula	Molecular weight	Description	Yield %	Melting point ℃
Ia	$C_8H_8BrN_3O$	242.08	white powder	94	226-228
Ib	$C_8H_8ClN_3O$	197.62	White powder	91	238-240
Ic	$C_8H_9N_3O_2$	179.18	Beige powder	80	219-220
Id	$C_8H_8N_4O_3$	208.18	Yellow powder	96	203-205
Ie	$C_9H_{11}N_3O$	177.21	white powder	92	220-222
IIa	$C_8H_6BrN_3O$	240.06	Off white powder	70	165-168
IIb	$C_8H_6ClN_3O$	195.61	Off white powder	72	208-210
IIc	$C_8H_7N_3O_2$	177.16	Beige powder	68	205-207
IId	$C_8H_6N_4O_3$	206.16	Yellow powder	93	168-170
IIe	$C_9H_9N_3O$	175.19	White powder	84	251-254
III	$C_{20}H_{24}FN_3O_4$	389.43	Off white powder	92	279-282
IV	$C_{22}H_{25}ClFN_3O_5$	465.91	Yellow powder	92	267-270
Va	$C_{30}H_{30}BrFN_6O_6$	669.51	Off white powder	64	239-241
Vb	$C_{30}H_{30}ClFN_6O_6$	625.05	Off white powder	61	252-255
Vc	$C_{30}H_{31}FN_6O_7$	606.61	Beige powder	58	272-275
Vd	$C_{30}H_{31}FN_6O_7$	606.61	Yellow powder	75	277-279
Ve	$C_{31}H_{33}FN_6O_6$	604.64	White powder	64	244-247

3. Antibacterial study:

In vitro antibacterial effects of the synthesized target compounds were evaluated against both gram negative bacteria, *Klebsiella pneumonia* and *Escherichia coli* and gram positive bacteria *Streptococcus pyougenes* and *Staphylococcus aureus* bacteria at concentrations of (62.5,125, 250 & 500 µg/mL).

Method

In this method, we used Brain Heart Infusion Agar (BHIA). The tested agents were dissolved in dimethyl sulfoxide (DMSO), then 1mL of spore suspension of each type of bacteria was spread evenly on the sterile solid media by using cotton swabs. Wells of 6mm were made in the plates filled with 0.1 mL of each concentration. Then the plates were incubated at 37^{o}C for 24 hours. The zones of inhibition were observed and measured to determine the antibacterial activity of synthesized compounds. [17,18] The antibacterial study was performed at college of Pharmacy, Almustansiriyah University.

4. Docking Study:

Molecular docking approach become a very important tool in the drug design process. By which we can evaluate the interaction occurs between the protein and ligand used in the study.

The docking process involve protein preparation and ligand preparation. Ligands preparation involved protonation of three dimensional structure in MOE 2015.10, partial charge addition, and energy minimization. The protein (2xct) was selected from PDB, it was prepared by removing the solvent molecules (water) and other sites on topoisomerase enzyme II (DNA gyrase) to facilitate the interaction of only ligands and selected receptor, followed by addition of protons that deleted to facilitate the upload and down load of the protein from PDB, then addition of broken bond, and fixation of the potential of the protein molecule. Finally, the active site of the topoisomerase was selected in MOE and determine the amino acids of this site. [19]

5. RESULTS AND DISCUSSION:

5.1 Chemistry:

Reaction between semicarbazide and aromatic aldehyde (Ia-e) considered as one of the most common chemical reactions for the synthesis of hydrazone compound (Schiff base or imine). Schiff bases formation is of reversible style, that depends on acids that catalyse the process that embarked with semicarbazide, primary amine, addition happened by nucleophilic attack for the carbonyl (C=O) group in the aromatic aldehyde, leading to carbinolamine formation by migration of proton from nitrogen (N) to oxygen (O). Hydroxyl group is made a good group to leave by oxygen protonation of carbinolamine with acidic catalysis, and then an iminium ion is produced when hydrogen hydroxide is lost. Finally, the end product was formed by proton loss from nitrogen and regenerating the acid catalyst [20,21].

Compounds (IIa-e) derivatives, 2-amino-5-substituted-1,3,4-oxadiazole, were synthesized in controlled potential electrolysis through the electro oxidation of semicarbazone at a bromine electrode. The oxadiazole ring is the final product of this electrochemical cyclization. Acetic acid was used as a solvent and the electrolyte was bromine. The initial step is deprotonation, which results in the formation of an anion, which then rearranges and evolves into a free radical after one electron oxidation. The free radical then undergoes a second electron oxidation, resulting in the formation of a carbocation. The ring is completed by the development of a carbon-oxygen link. When a proton is lost in the last step, it is possible to make 2-amino -5-substituted-1,3,4-oxadiazole. [22].

The carboxyl group (OH) is routinely protected as an ester, carboxylic acids are converted firstly into acid halide, because this group is regarded as a poor leaving group, so direct nucleophilic acyl substitution of carboxylic acid is challenging in the laboratory. Thus, it is important to improve the acid's reactivity, either by employing a strong acid catalyst to protonate the carboxylic group and make it a better acceptor or by converting the –OH group into a better leaving group. N-acylation of compound (III) was accomplished by utilizing chloroacetylchloride and forming compound (IV). The nucleophilic acyl substitution reactions which involve tetrahedral intermediate will convert chloroacetyl chloride into amide. [23]

The carbon in $–CH_2Cl$, on the other hand, has only one electron-withdrawing group (-Cl). Steric parameters, in addition to electronics, play a part in this selectivity.

Because of the electron-withdrawing effect of the –F substitution and the stabilization of the resultant anion, by resonance, compound III has a low basicity, making it a strong acid, and because the molecule also has a free amino group, the reaction will generally take place on the free amino group. [24] Between the

chloroacetamide derivative (IV) and the produced heterocyclic rings (IIa-e), a nucleophilic substitution reaction (SN_2) was carried out. The electrophilic carbon atom of chloroacetamide (R-CH_2-Cl) will be attacked by the free amino group of compounds (IIa-e). The reaction of an electron pair donor (the nucleophile, Nu) with an electron pair acceptor (the electrophile) is known as nucleophilic substitution. In order for the reaction to take place, an sp3-hybridized electrophile must have a leaving group. [25]

5.2. Pharmacology

Anti-bacterial study

The results of antibacterial sensitivity test for the prepared compounds (Va-e) were screened for their antibacterial activity against gram negative bacteria: *Klebsiella pneumonia* and *Escherichia coli* and gram positive bacteria *Streptococcus pyougenes* and *Staphylococcus aureus* bacteria at concentrations of (62.5,125, 250 and 500 µg/mL), and Figure (19) show the comparison of these activities.

For *S. aureus* bacteria the most powerful compounds acting on this bacterium are Vd and Ve and in comparison with the reference compound (Gatifloxacin), also Vb compound maintain its activity with the decreased concentration.

For *S. pyogenes*, there is some similarity in the activities of Gatifloxacin and compound Ve, but we notice that Ve is more active due to the difference in activity between these two compounds when we decrease concentration also we notice the activities of Vc and Vb which still elevated even when we decreased the concentration.

For *E. coli*, it is clear that Ve compound is the most active one, and some similarity in the activities of Gatifloxacin, Va, Vb and Vc with the priority to Vb and Vc on the Gatifloxacin.

Finally, for *K. pneumonia* the most active one is Vb compound then in the second degree Ve and Vd compounds, all of them are more active than Gatifloxacin.

So as we expect, the antibacterial activities of compound Vc, Vd and Ve are matching the docking scores listed below in table (3) that they exert powerful antibacterial activities than the started compound.

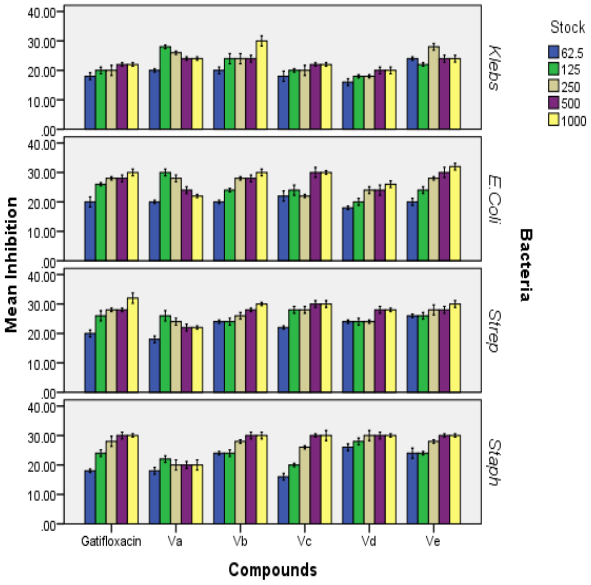


Figure 19: Statistical results for all compounds

5.3. Docking Study:

The docking studies were done by MOE software program (Molecule Operation Environment) also was used for the computational docking analysis. Proto-oncogene topoisomerase II enzyme (PDB ID 2XCT) is the target protein. Gatifloxacin acts as topoisomerase inhibitor alongside with the Va, Vb, Vc, Vd, Ve compounds with different score as shown in table (3). The docking of the chemical structures Gatifloxacin, Va, Vb, Vc, Vd, and Ve as shown in figures (20 to 24).

Table (3): Docking results for compounds (Va-e) and gatifloxacin.

Compound	Docking score
Gatifloxacin	-5.9
Va	-6.16
Vb	-5.87
Vc	-6.4
Vd	-6.4
Ve	-6.22

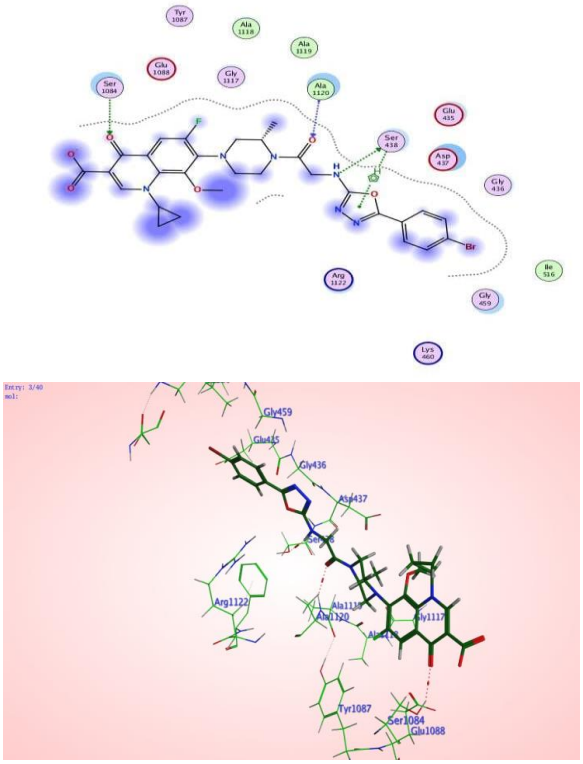


Figure 20: Chemical Structure of Va compound in complex with topoisomerase II enzyme (PDB code 2XCT)

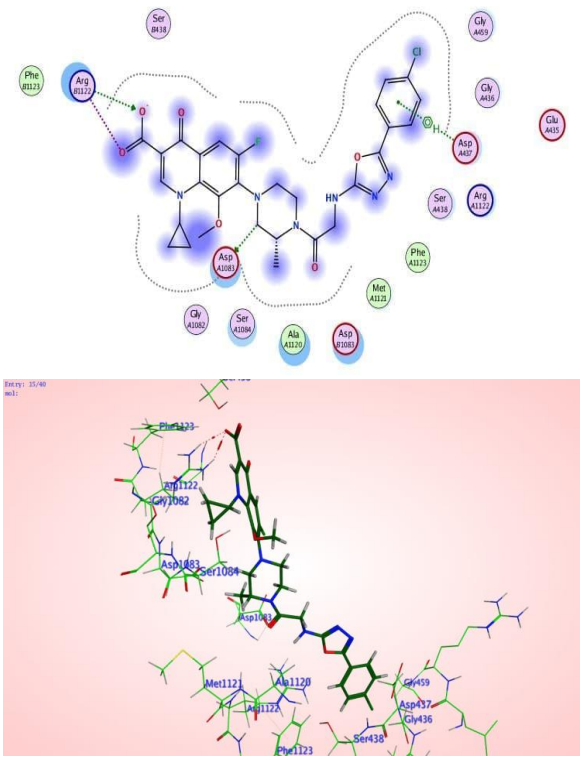
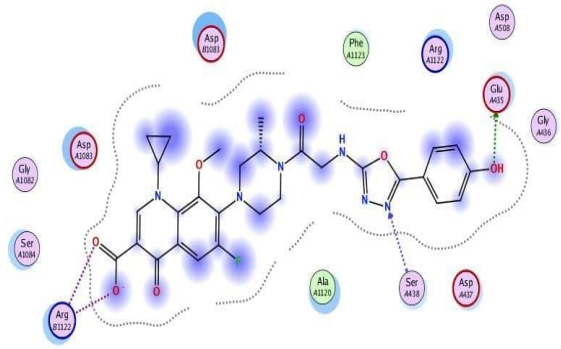


Figure 21: Chemical Structure of Vb compound in complex with topoisomerase II enzyme (PDB code 2XCT)



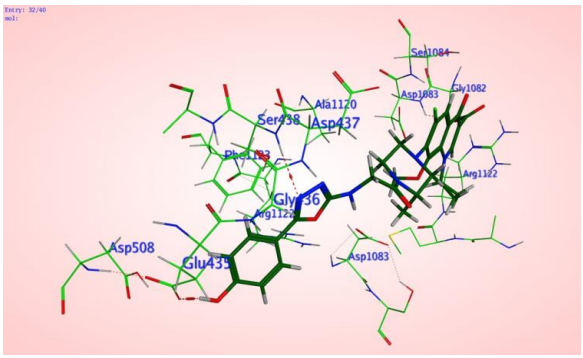
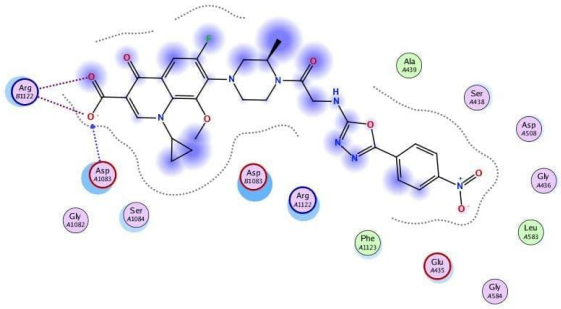


Figure 22: Chemical Structure of Vc compound in complex with topoisomerase II enzyme (PDB code 2XCT)



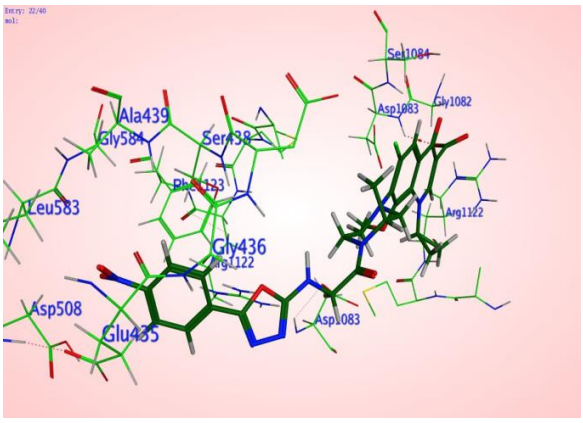


Figure 23: Chemical Structure of Vd compound in complex with topoisomerase II enzyme (PDB code 2XCT)

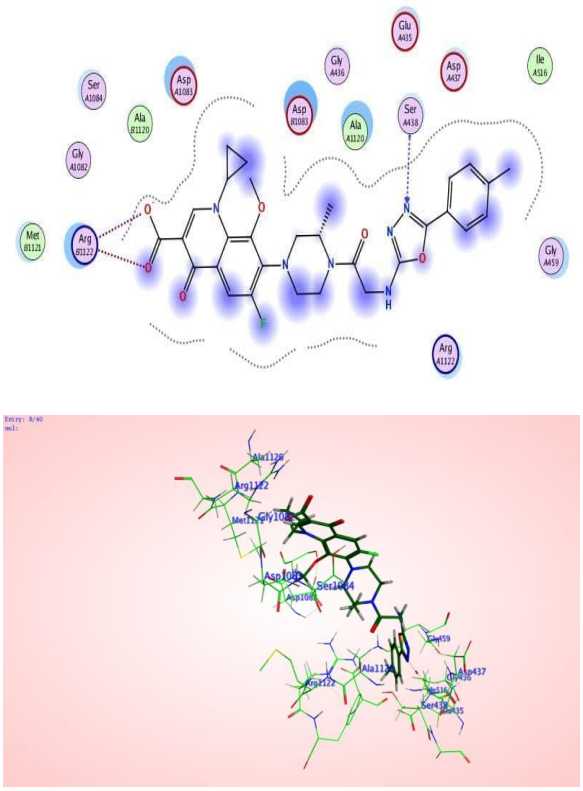


Figure 24: Chemical Structure of Ve compound in complex with topoisomerase II enzyme (PDB code 2XCT)

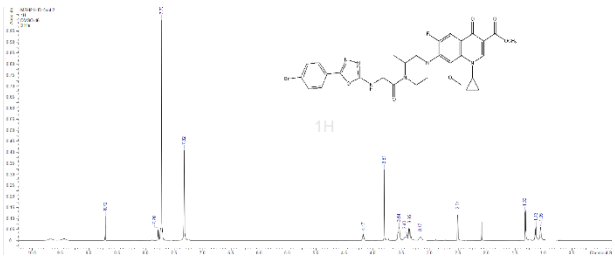


Figure 25: [1]HNMR Data for Compound Va

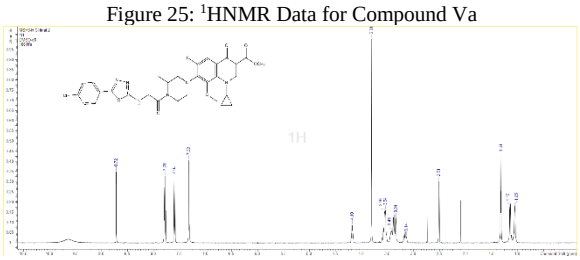


Figure 26: [1]HNMR Data for Compound Vb

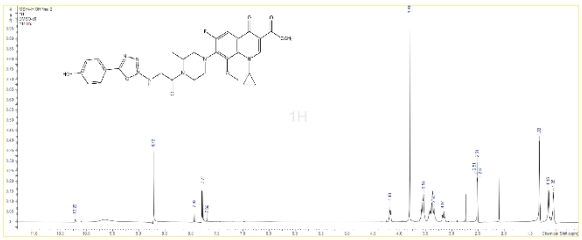


Figure 27: [1]HNMR Data for Compound Vc

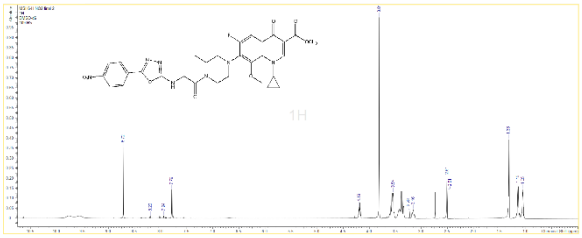


Figure 28: [1]HNMR Data for Compound Vd

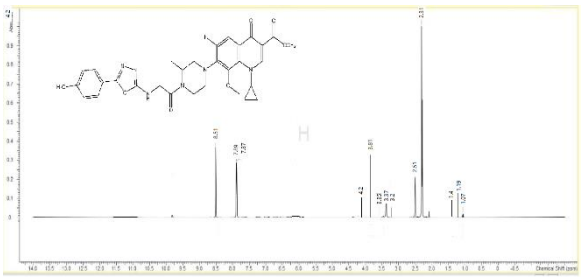


Figure 29: [1]HNMR Data for Compound Ve

6. Conclusions:

The antibacterial study showed that the incorporation of oxadiazole and its derivatives into a secondary amine of piperazine ring in gatifloxacin improves or maintains the antibacterial activity which is compatible with in silico study of the synthesized compounds.

Conflicts of interest

"There are no conflicts to declare".

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