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Design, Synthesis and Pharmacological Evaluation of New Lomefloxacin Derivatives Having Oxadiazole Nucleus

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Abstract

Infectious diseases are accompanied by inflammation as a defense mechanism through the role of arachidonic acid cascade or cyclooxygenase enzyme. Many researches indicated that many types of fluoroquinolone antibiotics have anti-inflammatory effects in addition to their antibacterial activities.

A group of oxadiazole derivatives were incorporated into C7 piperazine ring of lomefloxacin, a well-known antibacterial fluoroquinolone, in order to increase bulkiness at C7 leading to reduce bacterial resistance and improve anti-inflammatory activity.

In the current work, an *in vivo* experiment via visualizing the acute anti-inflammatory activities of the final compounds (VI_{a-f}) were tested encouraging inflammation using the egg-white induced-edema model (EWIE). The doses used were 10mg/kg and 3mg/kg for lomefloxacin and diclofenac, reference substances (RS), respectively. The results for this experiment showed significant ($p<0.05$) reductions in paw edema (PE) for all the compounds comparing that to the control group, propylene glycol (PG). However, compound VI_b produced superior ($p<0.05$) anti-inflammatory activity compared to lomefloxacin.

In vitro antibacterial evaluation using well disc diffusion method (WDDM) showed a comparable effect of the synthesized compound (SC) with lomefloxacin with the largest inhibition zone for compound VI_a against *Staphylococcus epidermis*, and compound VI_c produced superior antibacterial activity against *Klebsiella pneumoniae* when compared with lomefloxacin.

Confirmations and characterization of the chemical structures related to these SCs were performed using ^{1}H-NMR spectroscopy, FT-IR spectroscopy, and some physicochemical properties such as melting point and R_f value.

Docking study of the final synthesized compounds gave evidence about the affinity of these compounds toward topoisomerase IV enzyme.

Keywords: Anti-inflammatory, fluoroquinolone, lomefloxacin, oxadiazole.

INTRODUCTION

The antibiotic resistance (AR) problem is linked to the high and the random use of these compounds; however, reducing the processes of developing new drugs due to economic purposes adds to these factors of encouraging AR. According to many studies, AR bacterial strains are emerged and disseminated via the consumption of these drugs (1).

Fluoroquinolones are considered as one of the most frequent and effective anti-gram negative and positive used compounds in both human and animal medicines. According to studied isolates of *E. coli* from the UK, fluoroquinolones resistance (FR) was increased from 6% in 2001 to 20% in 2006. In Italy, a consistent increase of FR also was noticed in *Klebsiella pneumonia* as 11% to 50% in 2005 and in 2012 respectively (2,3). According to some studies, fluoroquinolones represented by ciprofloxacin (4), alatrofloxacin (5), grepafloxacin (6), gemifloxacin, and moxifloxacin are considered to alter the synthesis and the release of cytokines. This means that compounds act as antibacterial and anti-inflammatory substances at the same time (7,8). It was found that the human GM-CSF and lymphotoxin productions were decreased when ciprofloxacin had been used in a high dose (9).

Some factors affect the properties of fluoroquinolone anti–inflammatory (FA) such as chemical structure and the bulky group substitution at C-7 position that improve the FA such as besifloxacin, a significant inhibitor against cytokines at low concentration (10,11).

Moxifloxacin, a C-7-bulked fluoroquinolone, inhibits the activities of mitogen–activated protein kinase (MAP) and the NF–kB in monocytes and the epithelial cells related to cystic fibrosis (12), increases the efficacy, and decreases the Gram positive resistance. These effects were noticed to increase as bulkiness at C-7 is increased (13).

Depending on this background, lomefloxacin, which is difluorinated broad-spectrum second generation quinolone antibiotic with free secondary amine group in its piperazine moiety at C-7 position became our choice for chemical modification to improve its anti-inflammatory and reduce bacterial resistance by addition of oxadizole ring derivatives that have known anti-inflammatory (14) and antibacterial (15) actions in order to increase bulkiness at C7 of lomefloxacin.

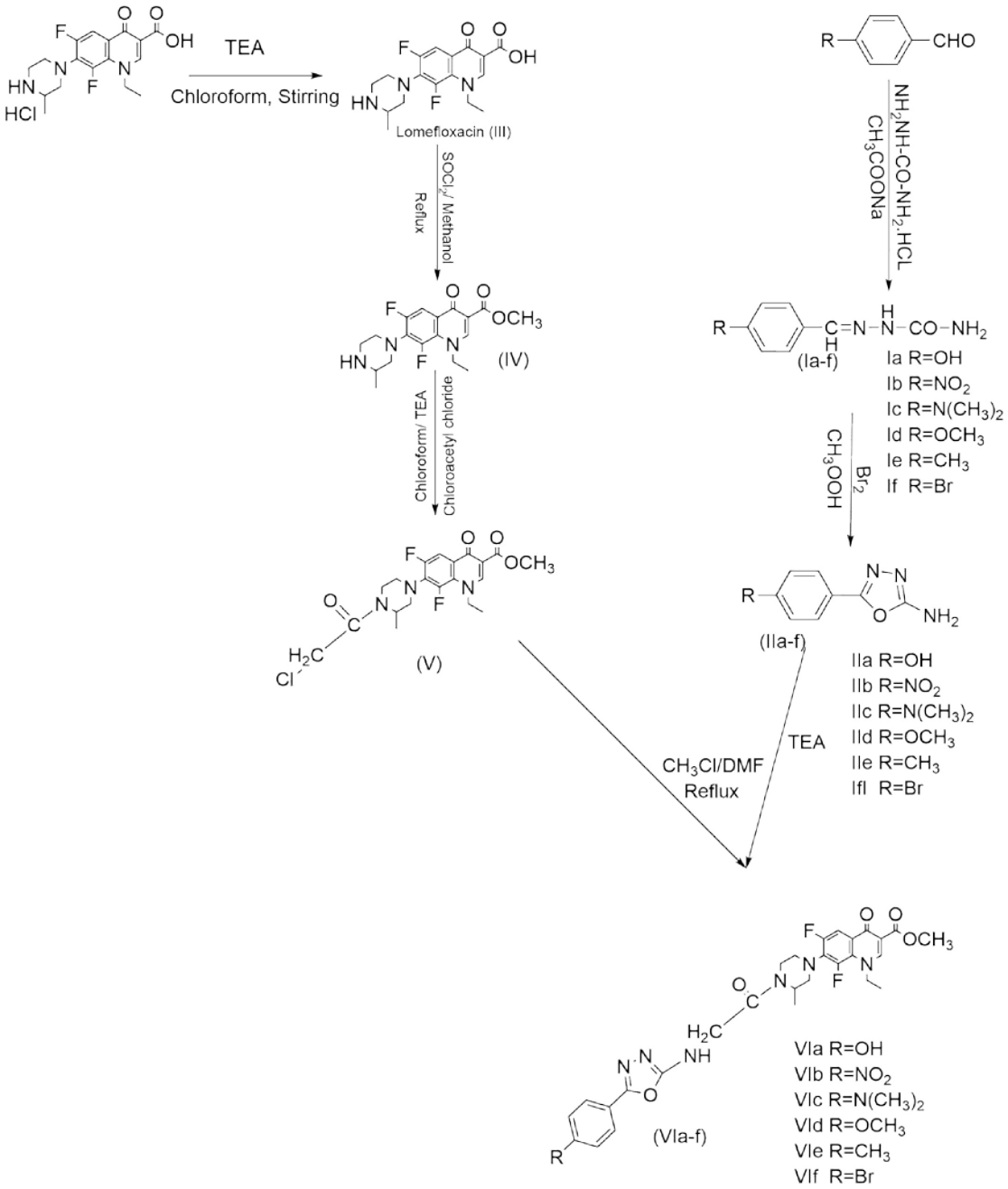
MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

Using chemicals at analytical grades, thin layer chromatography was operated to detect the purity and the melting points of the SCs and other chemical products eluting the system Chloroform: Methanol (6:1) (16) and Acetone: methanol: aq.qmmonia (5:3:2) (17). UV lights were used to reveal the compounds, and FT-IR (shimadzu 8100s spectrometer at the College of Pharmacy, University of Kufa, Iraq) was used to record IR spectra. Bruker 300 MHz-Avanc III (in the University of Mashhad) was used to record ^{1}HNMR spectra.

Typical procedure for the reactions

The SCs ($I-VI_{a-f}$) was synthetized as shown in scheme 1. The aromatic aldehydes react with semicarbazide HCl in the presence of sodium acetate to form semicarbazone, compounds (I_{a-f}) then the cyclization of these compounds was done by the presence of sodium acetate and bromine solution to form compounds (II_{a-f}). The lomefloxacin HCl firstly was converted to the pure lomefloxacin (compound III) by adding trimethylamine in chloroform. The carboxylic acid of lomefloxacin was protected as methyl ester that performed in methanol in the presence of thionyl chloride to form compound (IV) which reacted with chloroacetylchloride to form compound (V) that undergone nucleophilic attack with compounds (II_{a-f}) to form the final target compounds (VI_{a-f}).



Scheme 1: Steps of chemical synthesis of target compounds

Synthesis of Semicarbazone (I_{a-f})

Semicarbazones were synthesized by using different aromatic aldehydes (8.96 mmol), semicarbazide hydrochloride (8.96 mmol, 1g), and sodium acetate (18 mmol, 1.5 g) that were collected in a 100ml conical flask and dissolved in 30-40ml distilled water (DW). After stirring for 30mins, the precipitate was filtered and recrystallized from alcohol (16).

2-(4- hydroxybenzylidene) hydrazine-1- carboxamide (Ia):

FT-IR (cm^{-1}): 3475 and 3261(N-H primary amide); 3400-3000 (O-H); 3022 (N-H secondary amide overlap with =C-H of aromatic); 1695 (C=O amide); 1608 (C=N). Melting point: 222 ^oC . R_f : A=0.84, B=0.95.

2-(4- nitrosibenzylidene) hydrazine-1- carboxamide (Ib):

FT-IR (cm^{-1}): 3450 and 3292(N-H primary amide); 3062 (N-H secondary amide overlap with =C-H of aromatic); 1681 (C=O amide); 1631 (C=N); 1531 and 1348 (Asymmetric and symmetric NO_2); 869 (C-N nitro aromatic moiety). Melting point: 204-206 ^oC . R_f : A=0.88, B=0.84.

2-(4-(dimethylamino) benzylidene) hydrazine-1-carboxamide (Ic):

FT-IR (cm^{-1}): 3464 and 3294(N-H primary amide); 3188 (N-H secondary amide overlap with =C-H of aromatic); 2931,2806 and 2858 (C-H of alkane); 1689 (C=O amide); 1643 (C=N). Melting point: 225 ^oC . R_f : A=0.88, B=0.85.

2-(4- methoxybenzylidene) hydrazine-1- carboxamide (Id):

FT-IR (cm^{-1}): 3454 and 3284(N-H primary amide); 3165 (N-H secondary amide); 2954, 2931 and 2829 (C-H alkane); 1689 (C=O amide); 1645 (C=N); 1251 and 1029 (Asymmetric and symmetric C-O-C). Melting point: 208-210 ^oC. R_f : A=0.92, B=0.97.

2-(4- methylbenzylidene) hydrazine-1- carboxamide (Ie):

FT-IR (cm^{-1}): 3462 and 3280(N-H primary amide); 3190 (N-H secondary amide overlap with =C-H of aromatic); 2989, 2922 and 2856 (C-H stretching of alkane); 1693 (C=O amide); 1666 (C=N). Melting point: 226 ^oC . R_f : A=0.76, B=0.96.

2-(4- bromobenzylidene) hydrazine-1- carboxamide (If):

FT-IR (cm^{-1}): 3464 and 3278(N-H primary amide); 3199(N-H secondary amide);3070 (C-H aromatic); 1670 (C=O amide); 1645 (C=N);690 (C-Br). Melting point: 234-236 ^oC . R_f : A=0.82, B=0.97.

Synthesis of 2- Amino- 5 –Aryl-1, 3, 4-Oxadiazole Derivatives (II_{a-f})

Glacial acetic acid (GAA), in a 100ml-round-bottomed flask supported by a separated funnel to help drop-wise adding bromine (0.7ml in 5ml GAA), at 30-40ml was used to dissolve semicarbazone (5.6mmol) and sodium acetate (5.6mmol, 0.46 g). After 30mins of magnetic stirring, crushed ice was used to pour the solution on. Separation, drying, and recrystallization of the resulted solid were done (18).

4-(5-amino-1,3,4-oxadiazol-2-yl)phenol (IIa):

FT-IR (cm^{-1}): 3473 and 3334 (N-H primary amine); 3473-2613 (O-H); 3026 (C-H aromatic);1693 (C=N);1234 and 1151 (Asymmetric and symmetric C-O-C). Melting point: 181 ^oC . R_f : A=0.82, B=0.90.

5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (IIb):

FT-IR (cm^{-1}): 3471 and 3329 (N-H primary amine); 3097 (C-H aromatic);1712 (C=N);1516 and 1342 (Asymmetric and symmetric NO_2)1151 and 1105 (Asymmetric and symmetric C-O-C). Melting point: 136-138 ^oC . R_f : A=0.48, B=0.86.

5-(4(dimethylamino)phenyl)-1,3,4-oxadiazol-2-amine (IIc):

FT-IR (cm^{-1}): 3475 and 3271 (N-H primary amine); 3018 (C-H aromatic);1687 (C=N);1251 (C-N);1168 and 1130 (Asymmetric and symmetric C-O-C). Melting point: 202-204 ^oC . R_f : A=0.93, B=0.93.

5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (IId):

FT-IR (cm^{-1}): 3444 and 3354 (N-H primary amine); 3132 (C-H aromatic);3074 (C-H of alkane);1660 (C=N);1259 (C-N);1224 and 1180 (Asymmetric and symmetric C-O-C). Melting point:240-242 ^oC . R_f : A=0.90, B=0.96.

5-(p-tolyl)-1,3,4-oxadiazol-2-amine (IIe):

FT-IR (cm^{-1}): 3267 and 3097 (N-H primary amine); 3097 (C-H aromatic);2926 and 2787 (C-H alkane);1656 (C=N);1122 and 1074 (Asymmetric and symmetric C-O-C). Melting point: 268-270 ^oC . R_f : A=0.84, B=0.86.

5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine (IIf):

FT-IR (cm^{-1}): 3292 and 3109 (N-H primary amine); 1683 (C=N); 1076 and 1039 (Asymmetric and symmetric C-O-C); 829 (C-Br). Melting point: 173 ^oC . R_f : A=0.81, B=0.94.

Synthesis of 1- Ethyl -6, 8- Difluoro-4- Oxo-7- (3-Methylpiperazin -1-yl)-1, 4-Dihydroquinoine-3-Carbpxylic Acid (III)

To a suspension of lomefloxacin –HCl (12.9 mmol, 5 g) in dry chloroform (50ml), TEA (12.9 mmol, 1.8 ml) was added drop wise over a period of 10 min. at 0^o C with continues stirring for 2 hours. The reaction mixture was then filtered and dried, and the filtrate was used in the next step without further purification (19,20).

FT-IR (cm^{-1}): 3055-2459 (O-H); 2937 and 2885 (C-H alkane); 1726 (C=O quinolone); 1616 (C=O carboxyl); 1330 (C-N); 1047 (C-F). Melting point: 240 ^oC . R_f : A=0.3, B=0.31.

Synthesis of Methyl -1- Ethyl-6, 8-Difluoro -4- Oxo -7-(3- Methylpiperazin -1-yl)-1, 4-Dihydroquinoline-3-Carboxylate Hydrochloride (IV)

Thionyl chloride (0.21ml, 2.85mmol) was drop-wised added to a suspension of lomefloxacin (1g, 2.85mmol), in absolute methanol (50ml), at a consistent temperature of -15^{0}C. Then, the temperature at 40^{0}C for 3hrs was kept for the reaction mixture. After that, a refluxing step for 30hr was done to be then left at room temperature overnight. Drying and vacuuming were performed to get rid of the solvent with a methanol-based dissolving and evaporating to completely remove residues (21).

FT-IR (cm^{-1}): 3066 (C-H aromatic); 2956 and 2924 (C-H alkane); 1695 (C=O quinolone); 1637 (C=O ester); 1230 (C-O ester); 1002 (C-F). R_f : A=0.35, B=0.2.

Synthesis of methyl 7-(4-(2-chloroacetyl) -3-methylpiperazin -1- yl) -1-ethyl -6, 8-difluoro -4-oxo-1, 4- dihydroquinoline -3- carboxylate (V)

Compound (IV) (1g, 2.76mmol), was dissolved in DMF: Chloroform (1:3) mixture (40ml), then TEA (0.38ml, 2.76mmol) was added. Stirring on ice-dependent bath was performed for the mixture reaction. Moreover, a drop wise addition, accompanied by stirring for 1hr, of chloroacetylchloride (0.22ml, 2.76mmol in 10ml Chloroform) was done. After that, a refluxing step for 30hr was done. Addition of excess-cold H_2O was performed followed by filtering and crystallizing of the precipitated compound to form compound (V) (22).

FT-IR (cm-^{1}): 3057 (C-H aromatic); 2983 and 2941 (C-H alkane); 1722 (C=O quinolone overlap with ester); 1622 (C=O amide); 1253 (C-O ester); 1002 (C-F); 808 (C-Cl). R_f : A=0.34, B=0.49.

Synthesis of Compounds (VI_{a-f})

A mixture of compounds (II_{a-f}) (2.46mmol), and compound (V) (1g, 2.46mmol), were dissolved in DMF (25ml), then TEA (0.34ml, 2.46mmol), was added. Stirring at room temperature for overnight was done for maintaining the mixture reaction. Evaporation, acetone-based trituration, and crystallization were performed to get rid of the solvents forming compounds (VI_{a-f}) (23).

Methyl1-ethyl-6,8-difluoro-7-(4-((5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (VIa)

FT-IR (cm^{-1}): 3421-2980 (O-H overlap with N-H and C-H); 1604 (C=O amide); 1666(C=O quinolone);1637 (C=O ester),1591 (C=N); 866 (C-F). ^{1}H-NMR (ppm) ($C_{28}H_{28}F_2N_6O_6$): 9.6 (1H, s, OH); 8.4 (1H, s, CH of alkene); 7.8-7.5 (3H, m, CH of aromatic), 7.4 (2H, d, CH of aromatic); 6.2 (1H, s , NH); 4.3 (2H, s, CO-CH_2); 3.79 (3H, s, CH of ester); 3.1-2.5 (7H, m, CH_2CH of piperazine overlap with CH_2 of ethyl); 1.3 (3H, t, CH3 of ethyl); 1.1 (3H, d, CH_3 of piperazine). Decomposition point: 240-243 ^{o}C. R_f : A=0.86, B=0.72.

Methyl 1-ethyl-6,8-difluoro-7-(3-methyl-4-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)glycyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (VIb)

FT-IR (cm^{-1}): 3442 (N-H of secondary amide); 1662 (C=O ester); 1606(C=O quinolone overlap with amide);1352-1251 (C-O ester overlap with N-O),1591 (C=N); 881 (C-F). ^{1}H-NMR (ppm) ($C_{28}H_{27}F_2N_7O_7$): 8.4 (1H, s, CH of alkene); 8.4-7.5 (5H, m, CH of aromatic), 6.5 (1H, s , NH); 3.7 (2H, s, CO-CH_2); 3.1 (2H, m, CH_2); 2.4 (3H, s, CH_3 of ester); 2.8-1.3 (7H, m, CH_2CH of piperazine); 1.1 (3H, t, CH3 of piperazine); 0.9 (3H, d, CH_3 of ethyl).Melting point: 260 ^{o}C . R_f : A=0.87, B=0.75.

Methyl 7-(4-((5-(4-(dimethylamino)phenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (VIc)

FT-IR (cm^{-1}): 3425 (N-H of secondary amide); 1662 (C=O ester); 1595(C=O quinolone overlap with amide); 1550 (C=N);1251 and 1230 (C-O ester);1363 (C-N); 866 (C-F). ^{1}H-NMR (ppm) ($C_{30}H_{33}F_2N_7O_5$): 9.6 (1H, s, OH); 8.4 (1H, s, CH of alkene); 7.8-7.5 (3H, m, CH of aromatic), 7.4 (2H, d, CH of aromatic); 6.2 (1H, s , NH); 4.3 (2H, s, CO-CH_2); 3.79 (3H, s, CH of ester); 3.1-2.5 (7H, m, CH_2CH of piperazine overlap with CH_2 of ethyl); 1.3 (3H, t, CH3 of ethyl); 1.1 (3H, d, CH_3 of piperazine).Melting point: 205 ^{o}C . R_f : A=0.48, B=0.77.

Methyl1-ethyl-6,8-difluoro-7-(4-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (VId)

FT-IR (cm^{-1}): 3450 (N-H overlap with C-H); 1651 (C=O ester); 1633(C=O quinolone overlap with amide); 1558 (C=N); 1309 (C-N); 1253 and 1153 (C-O ester); 1016 (C-F). ^{1}H-NMR (ppm) ($C_{29}H_{30}F_2N_6O_6$): 8.5 (1H, s, CH of alkene); 7.9-6.8 (5H, s & d, CH of aromatic); 6 (1H, s , NH); 3.8 (2H, s, CO-CH_2); 3.7 (2H, m, CH_2 of ethyl); 3.4 (3H, s, $CH_3$of methoxy); 2.8 (7H, m, CH_2CH of piperazine); 2.4 (3H, s, CH_3 of ester); 1.2 (3H, t, CH_3 of ethyl); 1.1 (3H, d, CH_3 of piperzine).Melting point: 221 ^{o}C decomposition. R_f : A=0.52, B=0.86.

Methyl 1-ethyl-6,8-difluoro-7-(3-methyl-4-((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)glycyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (VIe)

FT-IR (cm^{-1}): 3442 (N-H secondary amine); 1647 (C=O ester overlap with quinolone); 1558(C=O amide overlap with C=N);1151 and 1114 (C-O ester), 1068 (C-F). ^{1}H-NMR (ppm) ($C_{29}H_{30}F_2N_6O_5$): 8.5 (1H, s, CH of alkene); 7.6 and 7.3 (4H, d, CH of aromatic); 7.2 (1H, s , NH overlap with 1H of aromatic);); 3.8 (2H, m, CH_2 of ethyl); 3.3 (2H, s, CO-CH_2); 3-2.7 (7H, m, CH_2CH of piperazine); 2.5 (3H, s, CH_3 of ester); 2.3 (3H, s, Ar-CH_3); 1.1 (3H, t, CH_3 of ethyl); 0.9 (3H, d, CH_3 of piperzine).Melting point: 225-228 ^{o}C decomposition. R_f : A=0.5, B=0.8.

Methyl 7-(4-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)glycyl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (VIf)

FT-IR (cm^{-1}): 3444 (N-H overlap with C-H); 1691 (C=O ester); 1662 (C=O quinolone overlap with amide); 1629 (C=N); 1153 (C-F); 881 (C-Br). ^{1}H-NMR (ppm) ($C_{28}H_{27}BrF_2N_6O_5$): 8.6 (1H, s , NH); 8.4 (1H, s, CH of alkene); 7.7,7.4 and 7 (5H, d, d, s, CH of aromatic); 3.4 (2H, s, CO-CH_2); 3.2-2.8 (7H, m, CH_2 of ethyl overlap with CH_2CH of piperazine); 2.5 (3H, s, CH_3 of ester); 1 (3H, t, CH_3 of ethyl); 0.9 (3H, d, CH_3 of piperzine).R_f : A=0.36, B=0.84.

Preliminary Pharmacological Studies

Anti-inflammatory Study

The College of Pharmacy, Kufa University approved the protocol for the current study via the ethical committee (ACE file number 3 at 18-02-2018). an *in vivo* experiment via visualizing the acute anti-inflammatory activities of the final compounds (VI_{a-f}) were tested encouraging inflammation using the EWIE. The doses used were 10mg/kg and 3mg/kg for lomefloxacin and diclofenac, a reference drug, respectively. Thickness decreases in paw was considered as a screening basis for the anti-inflammatory effects induced by these compounds (24).

The animal house (AH) at the College of Pharmacy, Kufa University provided the lab animals, albino rats (220±10gm), used for this study and housed in the AH under standardized conditions. Commercial chaw was used for feeding these animals and had free access to water ad libitum. Animals were divided into nine groups (each group consist of 6 rats) as follow:

Group A: The control group served with 6 rats provided with the vehicle, PG at 50% v/v.

Group B: 6 rats exposed to 10mg/kg lomefloxacin, RS, (25), suspended in PG 50% (v/v).

Group C: 6 rats exposed to 3mg/kg diclofenac, RS, (26), suspended in PG 50% (v/v).

Groups (D, E, F, G, H, and I): 6 rats/group exposed to the SC (VI_{a-f}). The doses were used as below. These SCs were suspended in PG 50% v/v.

All the SCs are derivatives of lomefloxacin which is given in a dose of 10mg/kg. According to that, these doses were calculated using the following equation:

$$\frac{\text{Wt.of lomefloxacin}}{\text{M.Wt. of lomefloxacin}} = \frac{\text{Wt.of compound VIa}}{\text{M.Wt. of compound VIa}}$$

Injection of SCs was performed subcutaneously at the planter side of the hind paw at 0.05ml undiluted egg-white in 30min following the IP injection of the substances that belong for each group.

A vernea was used to measure the thickness after drug injections at 0, 30, 60, 120, 180, 240, and 300min time points (TP), table 1.

Statistical Analysis

Mean±SE was used to show and analyze the data using student-*t*-test. For more than 2 comparisons, 2-way-ANOVA test was used. For significant/non-significant results, probability at 0.05 was used.

Table 1: The anti-inflammatory activity of the control via thickness of the paw (mm) at TPs (min)

	0	30	60	120	180	240	300
Control	3.36±0.11	4.40±0.09	5.16±0.07	5.90±0.07	6.01±0.11	5.66±0.06	4.34±0.12
Diclofenac	3.33±0.12	4.37±0.05	4.31±0.06*^{a}	4.28±0.14*^{a}	4.23±0.12*^{a}	3.98±0.08*^{a}	3.48±0.09*^{a}
Lomefloxacin	3.36±0.8	4.42±0.06	4.89±0.07*^{b}	4.86±0.11*^{b}	4.79±0.09*^{b}	4.48±0.09*^{b}	4.01±0.10*^{b}
VIa	3.34±0.09	4.43±0.12	4.40±0.05*^{c}	4.38±0.06*^{c}	4.26±0.04*^{a}	4.10±0.10*^{c}	3.75±0.09*^{c}
VIb	3.32±0.23	4.40±0.16	4.35±0.16*^{a}	4.30±0.07*^{a}	4.11±0.11*^{c}	3.86±0.21*^{d}	3.50±0.18*^{a}
VIc	3.31±0.08	4.51±0.10	4.48±0.05*^{d}	4.41±0.10*^{c}	4.34±0.07*^{d}	4.20±0.04*^{e}	3.65±0.12*^{de}
VId	3.36±0.10	4.49±0.04	4.43±0.09*^{c}	4.38±0.12*^{c}	4.31±0.05*^{d}	4.24±0.08*^{e}	3.71±0.10*^{c}
VIe	3.37±0.07	4.48±0.06	4.41±0.04*^{c}	4.35±0.05*^{c}	4.16±0.12*^{e}	4.01±0.11*^{a}	3.69±0.10*^{d}
VIf	3.32±0.05	4.41±0.10	4.33±0.12*^{a}	4.29±0.04*^{a}	4.20±0.07*^{e}	4.09±0.12*^{c}	3.62±0.04*^{e}

*significantly (p<0.05) differences. Different superscript letters (a, b, c, d, and e) mean significant (p<0.05). differences.

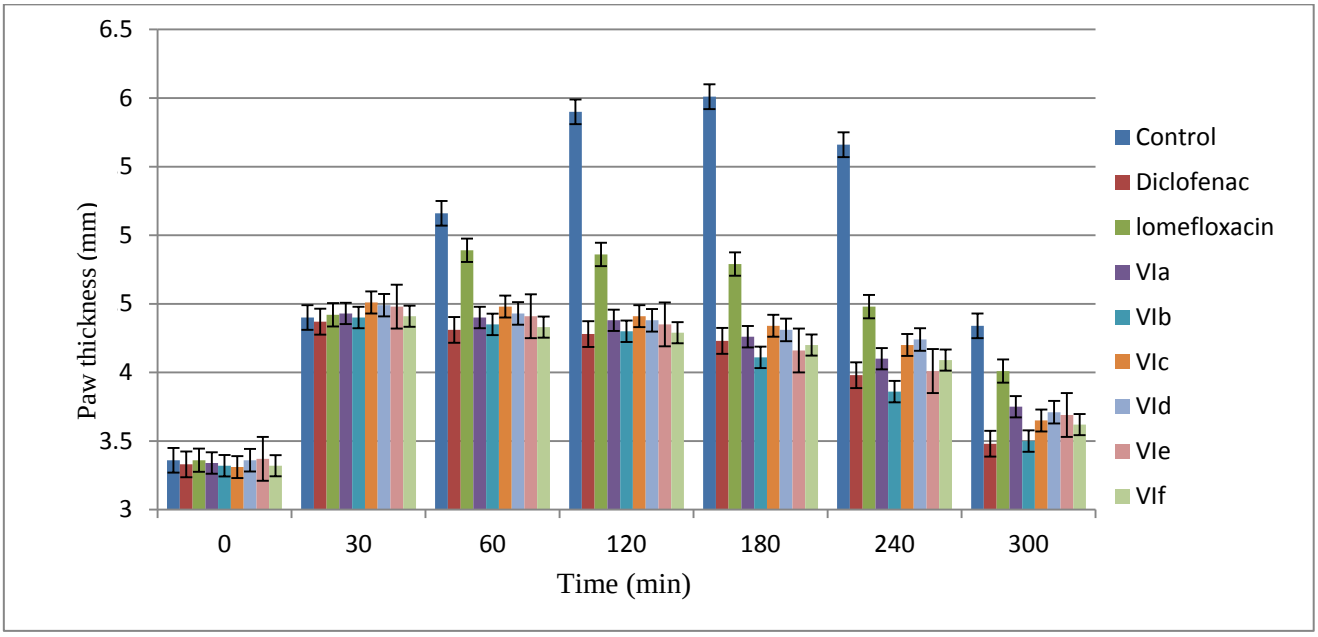


Figure 1: Anti-inflammatory effects of the all compounds on paw thickness.

Anti-bacterial study

The antibacterial evaluation of newly SCs (VI_{a-f}) was done by using WDDM (27). The antimicrobial substance used was applied at 20–100µl using Dimethyl sulfoxide (28) using specific conditions (29).

The lomefloxacin sensitivity disc concentrations is 10mcg (30), so we calculated our derivative strength, table (2, 3, 4,5, 6, and 7) by using their molecular weights in the below equation :

Lomefloxacin strength (10^{-5}g)/ Lomefloxacin M.Wt (351.35g/mol) = Lomefloxacin derivative strength (10 mcg)/Lomefloxacin derivative M.Wt)

Table 2: Lomefloxacin derivative antibacterial sensitivity disc strength

Compounds	M.Wt (g/mol)	Strength (g/ml)
VIa	582.6	16.6 x 10^{-6}
VIb	611.6	17.4 x 10^{-6}
VIc	609.6	17.35 x 10^{-6}
VId	596.6	17 x 10^{-6}
VIe	580.6	16.5 x 10^{-6}
VIf	645.5	18.4 x 10^{-6}

Table 3: The antibacterial activity of lomefloxacin and compounds VI_{a-f}

Compound	Inhibition zone on Bacterial growth (mm)	
	Staphylococcus epidermidis	*Klebsiella pneumoniae*
Lomefloxacin	48	31
VIa	39	30
VIb	35	29
VIc	36	36
VId	37	26
VIe	32	24
VIf	31	25

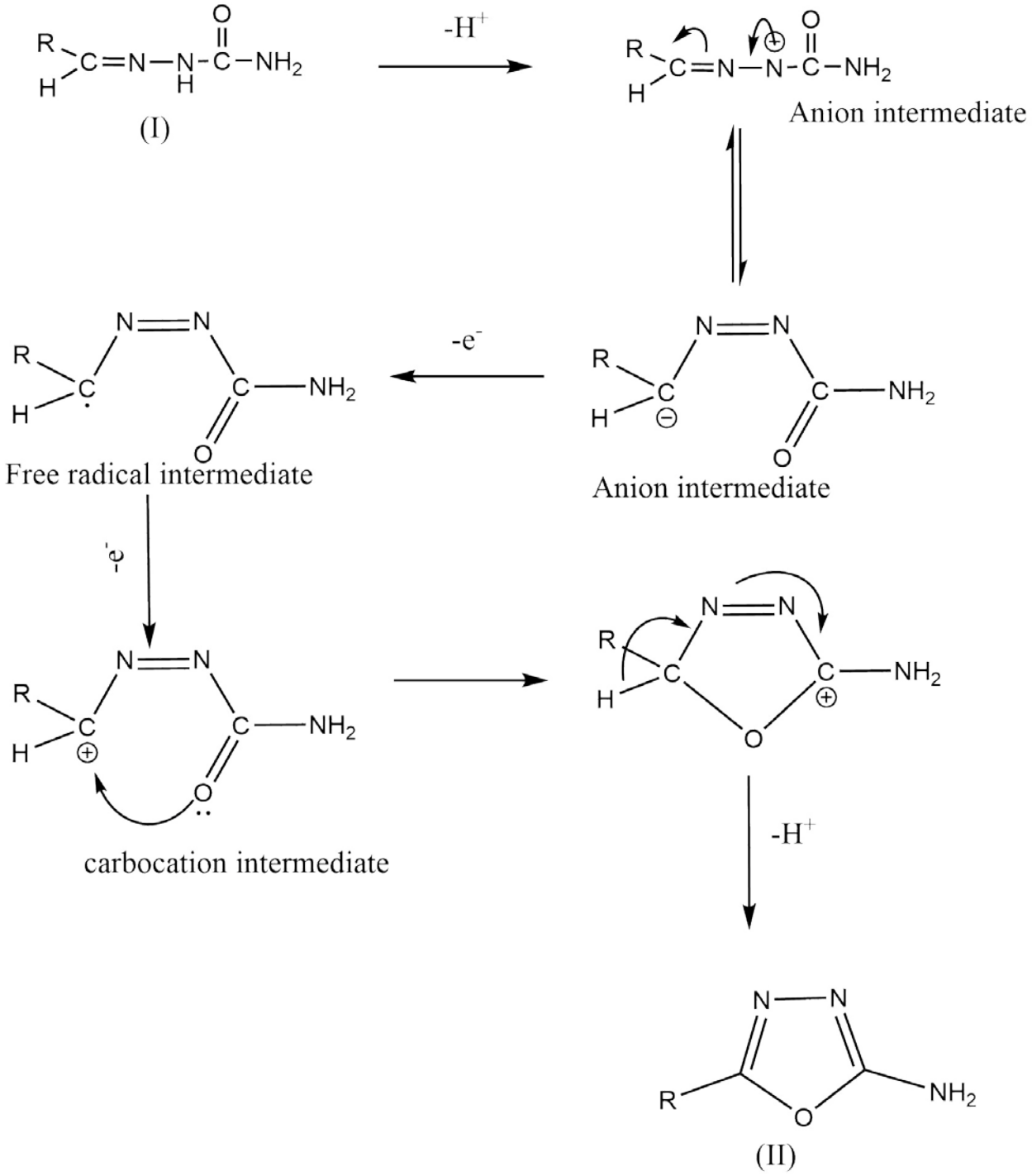
RESULTS AND DISCUSSION

Chemistry

The syntheses of oxadiazole ring derivatives (II_{a-f}) were done firstly by formation of semicarbazone compound through Schiff base reaction of different aromatic aldehyde with semicarbazide in the acidic media that performed through nucleophilic attack of primary amine of semicarbazide on the carbonyl group in aromatic aldehyde and formation of a carbinolamine by proton migration from

nitrogen to oxygen then acid catalyze oxygen protonation to make the hydroxyl as good group to leave (-OH_2), and a hydrogen hydroxide was lost to produce an iminium ion that its proton was lost from nitrogen giving the end product which undergone cyclization in second steps by electrooxidation of semicarbazone at a bromine electrode in controlled potential electrolysis to produce oxadiazole ring as shown in scheme 2.

The lomefloxacin carboxyl group was protected as methyl ester (31) to form compound (IV), and this was done by conversion it to acid halide because of the –OH group is considered as a poor leaving group and direct nucleophilic acyl substitution of carboxylic acid is difficult in the laboratory. Thus, it was needed to promote the reactivity of the acid, either by using 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 (32). The IR spectrum of the synthesized compound showed shifting of the C=O stretching vibration band to higher frequency from 1616 cm^{-1} for lomefloxacin (III) to 1637 cm^{-1} for compound (IV) and disappearing of broad band (from 2459-3055 cm^{-1}) giving an evidence about ester formation.



Scheme 2: Mechanism of synthesis of 2-amino-5-(substituted)-1,3,4-oxadiazole ring (IIa-f)

N-acetylation of compound (IV) was done by using chloroacetylchloride to get compound (V). The conversion of chloroacetyl chloride into amide occurred through nucleophilic acyl substitution reactions that involved tetrahedral intermediate. Selective nucleophilic substitution was performed at the acyl carbon atom in α-chloroacetyl chloride because of the nucleophiles has stronger reactivity toward acid chlorides compared to alkyl chlorides. The selection was done as there were differences in the electrophilic properties of the two carbon atoms in α-chloroacetyl chloride. Electronically for electron-withdrawing (EWD) groups, the carbonyl carbon has the double-bonded oxygen and the -Cl bonded attached to it (33); however, the carbon in $–CH_2Cl$ has only 1 group (-Cl). Steric factors add some potential roles in the selective process. So, nucleophilic attacks of the carbon in the planar carbonyl group in the acid chloride is easier than that in the tetrahedral carbon of the $–CH_2Cl$ group. The reaction was carried out with triethylamine, which acted as a base to neutralize the hydrogen chloride formed (34). In aliphatic heterocyclic compounds as in piperazine, a saturated heterocyclic ring and lone pair of electrons is reacted with protons. For these compounds, the strength for the bases in the compound open-chain aliphatic counterparts is similar with typical pKa values of 8-9 (35,36). The SCs related IR spectrum revealed characteristic bands at 1622 cm^{-1} for C=O stretching vibration of amide (amide I Band) and at 808 cm-1 for C-Cl stretching vibration.

For the synthesis of end products compounds (VI_{a-f}), the nucleophilic substitution reaction (SN^2), was performed between chloroacetamide derivative (V) and the prepared heterocyclic rings (II_{a-f}). The free amino group of compounds II_{a-f} attacked the electrophilic carbon atom of chloroacetamide (R-CH_2-Cl). Nucleophilic substitution is the reaction of an electron pair donor (the nucleophile, Nu) with an electron pair acceptor (the electrophile). An sp3-hybridized electrophile must have a leaving group (LG) in order for the reaction to take place (37).

Pharmacology

Anti-inflammatory study

The tested final compounds were evaluated for their anti-inflammatory activities by comparing them to the control group and the reference groups.

The IP injection results showed significant (p<0.05) reductions in the edematous paw. All SCs VI_{a-f} showed higher anti-inflammatory activities than those in the lomefloxacin. Compound VI_b effect was similar (p>0.05) to that in diclofenac, figure 1.

Anti-bacterial study

Table 3 showed the results of antibacterial sensitivity test for the prepared compounds (VI_{a-f}) and the reference compounds against *Staphylococcus epidermidis* and *Klebsiella pneumonia*. The highest inhibition zone diameter (39mm) was observed with compound VIa solution against *Staphylococcus epidermidis*, and the lowest inhibition zone (24mm) was noticed with compound VIe against bacteria *Klebsiella pneumoniae*. Compound VIc produced better anti-bacterial activity against *Klebsiella Pneumoniae* with inhibition zone at 36 mm, when compared to lomefloxacin.

Docking Study

The docking studies were done by Glide module (Glide version 5.7; Schrodinger, LLC, 2018) set up in Maestro 9 (Maestro 2018-3, Schrodinger, LLC, 2018) as shown in figures 2-8. The positive control (lomefloxacin) showed docking score at -11.3 with topoisomerase IV. Generally, the in silico results were compatible with in vitro results in which all newly synthesized compounds showed antibacterial activity

Table 4: Docking results and the corresponding compounds

Compound	Docking Score
Lomefloxacin	-11.3
VIa	-8.46
VIb	-7.12
VIc	-8.42
VId	-7.40
VIe	-6.63
VIf	-8.62

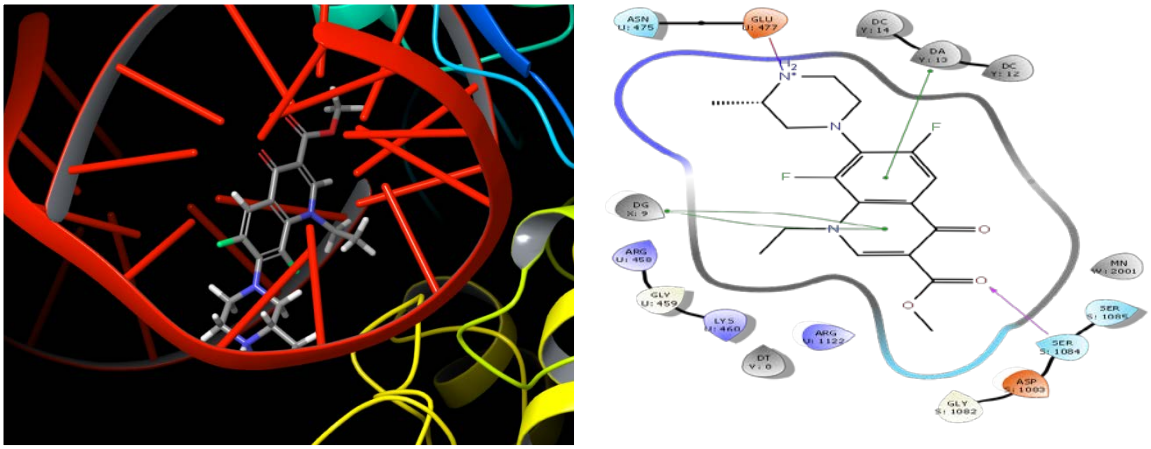


Figure 3: Docking result of lomefloxacin

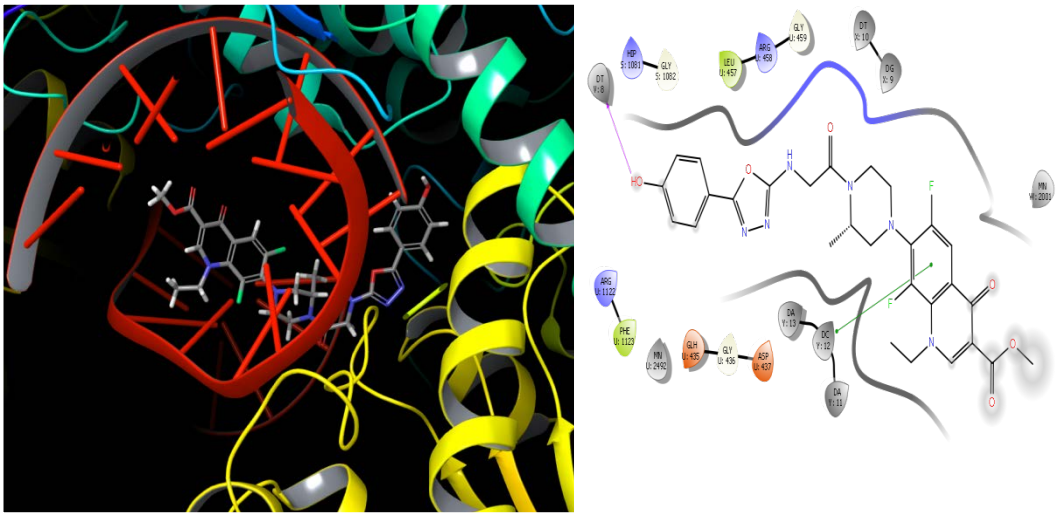


Figure 4: docking result of compound VIa

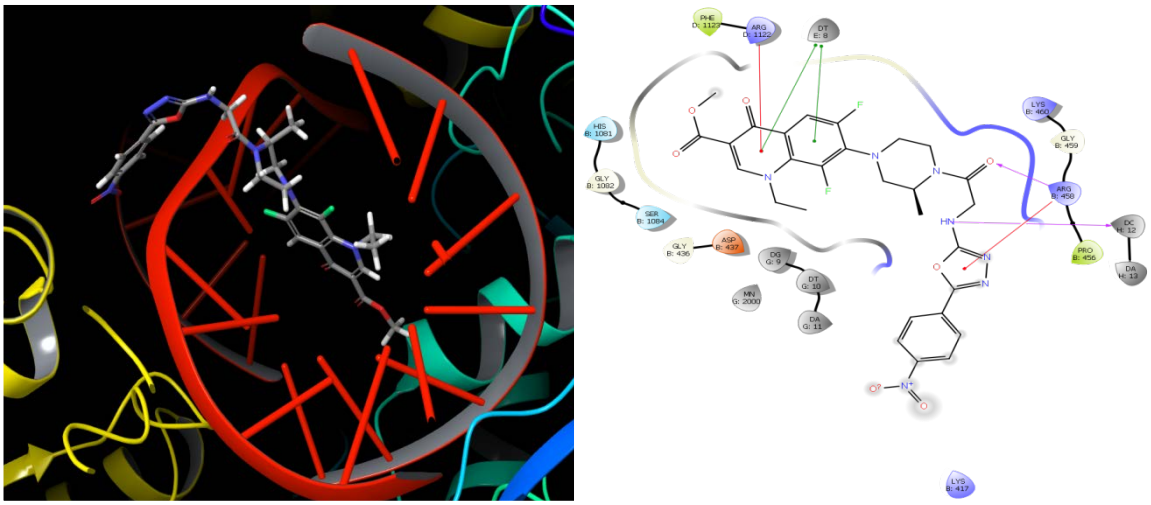


Figure 5: docking result of compound VIb

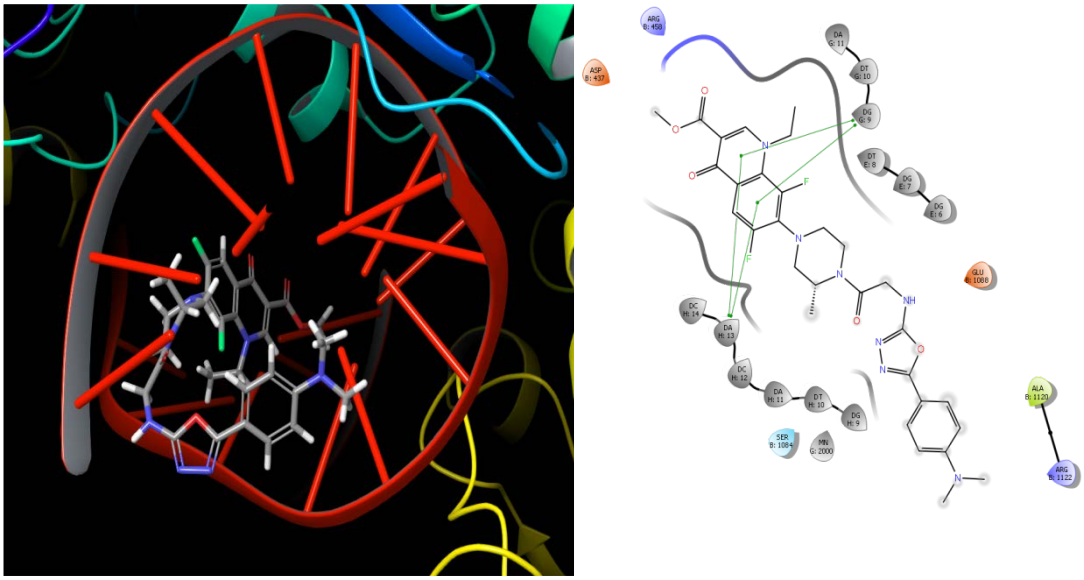


Figure 6: docking result of compound VIc

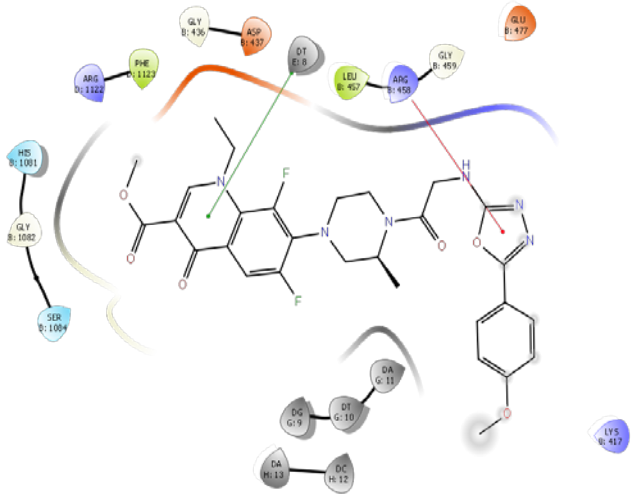


Figure 7: docking result of compound VId

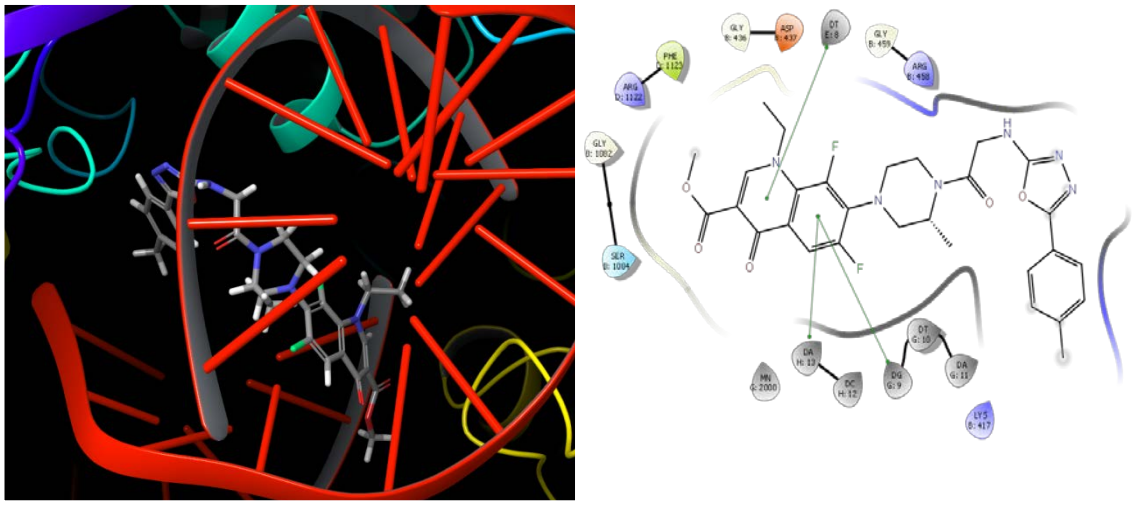


Figure 8: docking result of compound VIe

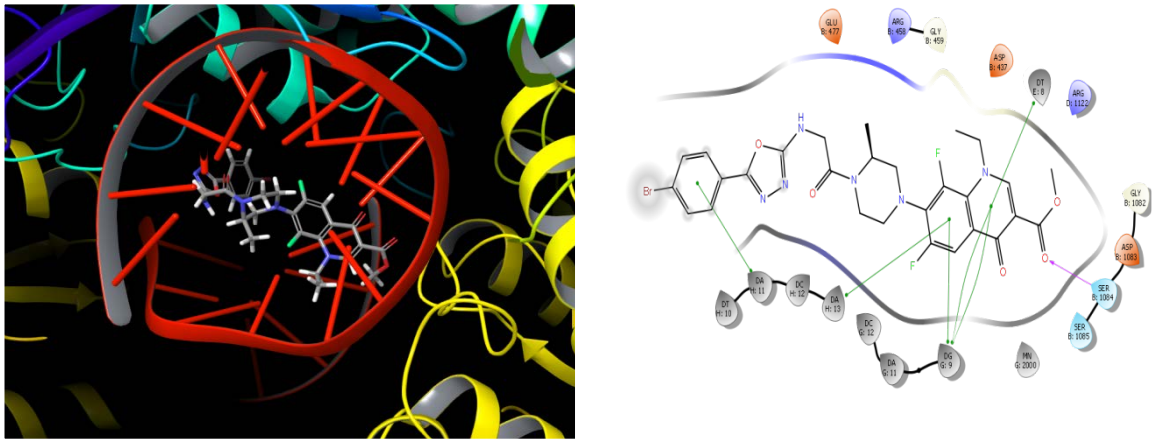


Figure 9: docking result of compound VIf

CONCLUSIONS

In vivo acute anti-inflammatory study proved that the incorporation of oxadiazole pharmacophore into lomefloxacin maintains or enhances its anti-inflammatory activity. In vitro antibacterial study showed that the incorporation of oxadiazole maintains its antibacterial activity, this is compatible with the in silico studies for the topoisomerase IV enzyme.

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