https://doi.org/10.31351/vol34iss1pp165-175

Molecular Docking, Synthesis, and, Biological Activity of New Diclofenac Derivatives Incorporating 1,2,4-Triazole Ring as a Promising Antibacterial Agents

Alyaa N. Doohee[1], Noor H. Naser[1] and Karrar Al-Gburi[*,2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Zahraa University for Women, Holy Karbala, Iraq.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
[*]**Corresponding author**
Received 28/8/2023, Accepted 14/1/2024, Published 29/3/2025

Abstract

NSAIDs, recently have been shown to exert a wide variety of biological activities, such as antibacterial, antifungal, antioxidant, and anticancer activity. The triazole ring has various pharmacological activities of tetrazole ring, anticancer, antidiabetic, antifungal, antioxidant, anticonvulsant, antiviral, and antibacterial properties. Hence, this study aimed to design, synthesize, molecular docking, and assess the antibacterial activity of new diclofenac derivatives incorporating a 1,2,4-triazole ring. Molecular docking studies were carried out using MOE 2015 software using a standard protocol. The new diclofenac derivatives (VIa-d) were synthesized by esterifying diclofenac, reacting the product with hydrated hydrazine and carbon disulfide, and cyclization to form a 1,2,4-triazole ring. Finally, the reaction of the benzoyl chloride derivatives with the primary amino group on the synthesized triazole ring via a nucleophilic substitution reaction. In vitro antibacterial activity of diclofenac, and the synthesized compounds were evaluated using the agar-well diffusion method. Compounds (VIa-d) have shown a higher binding score than the reference ligand (Topotecan) against Human DNA topoisomerase I (PDB: 1K4T), while they were nearly identical to the reference ligand (levofloxacin) against type IV topoisomerase from S. pneumoniae (PDB: 3RAE). All of the compounds inhibited growth of the Staphylococcus aureus and Escherichia coli, with compound VId exhibiting the highest level of activity.

Keywords: Antibacterial activity, Benzoyl chloride derivatives Diclofenac, Molecular Docking, 1,2,4-triazole.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are FDA-approved drugs used widely to treat conditions like fever, inflammation, and pain [(1)]. They are among the most prescribed medication classes, representing nearly 5-10% of all medications prescribed yearly [(2)]. These drugs inhibit cyclooxygenase enzymes thus restricting prostaglandin synthesis, limiting downstream cytokine release, and lowering the inflammatory response [(3)]**.** In addition to these activities (analgesic, pain relief, and anti-inflammatory), NSAIDs, recently have been shown to exert a wide variety of biological activities, such as antibacterial, antifungal, antioxidant, and anticancer activity[(4,5)]**.** Cancer and Bacterial resistance are two of the most serious problems facing public health nowadays. Hence, recent research has focused on discovering new novel compounds that are safe and effective in preventing and treating infection and cancer. Cancer is one of the leading causes of human morbidity and mortality worldwide[(6,7)]. Collectively, there are about 14 million new cases around the world and over 8 million cancer-related deaths per year. Globally, the incidence is not declining and is expected to rise to 22 million new cases per year (a 70% increase) in the next two decades[(8)]. Similarly, bacterial resistance is one of the emerging issues, where, multidrug resistance is caused by pathogens' ability to undergo modifications and mutations that reduce or eliminate antibiotic-target contact[(9)].Diclofenac is a well-tolerated nonsteroidal anti-inflammatory medicine (NSAID) with few documented adverse effects, making it one of the first-choice drugs for the treatment of painful disorders like postoperative pain and inflammatory conditions [(10)]. Additionally, diclofenac has been shown to reduce the growth of a variety of bacteria in the laboratory, including *Escherichia coli* (E. coli), *Staphylococcus aureus* (S. aureus), *Candida albicans*, *Listeria monocytogenes*, *and Mycobacterium tuberculosis*[(11,12)]. The triazole ring is one of the interesting heterocyclic rings. It has

various pharmacological activities of tetrazole ring, anticancer, antidiabetic, antifungal, antioxidant, anticonvulsant, antiviral, and antibacterial properties[13]. Tetrazole ring can mimic various amino acids, and act as bioisosteres of important functional groups, such as amide, ester, and carboxylic acid[14]. Furthermore, it has a synthetic and practical biological value. Therefore,1,2,4-triazole and their fused heterocyclic derivatives have attracted significant attention in chemistry [15]. Hence, this study aims to design, synthesize, molecular docking, and assess the anticancer and antibacterial activity of new diclofenac derivatives incorporating a 1,2,4-triazole ring.

Materials and Methods

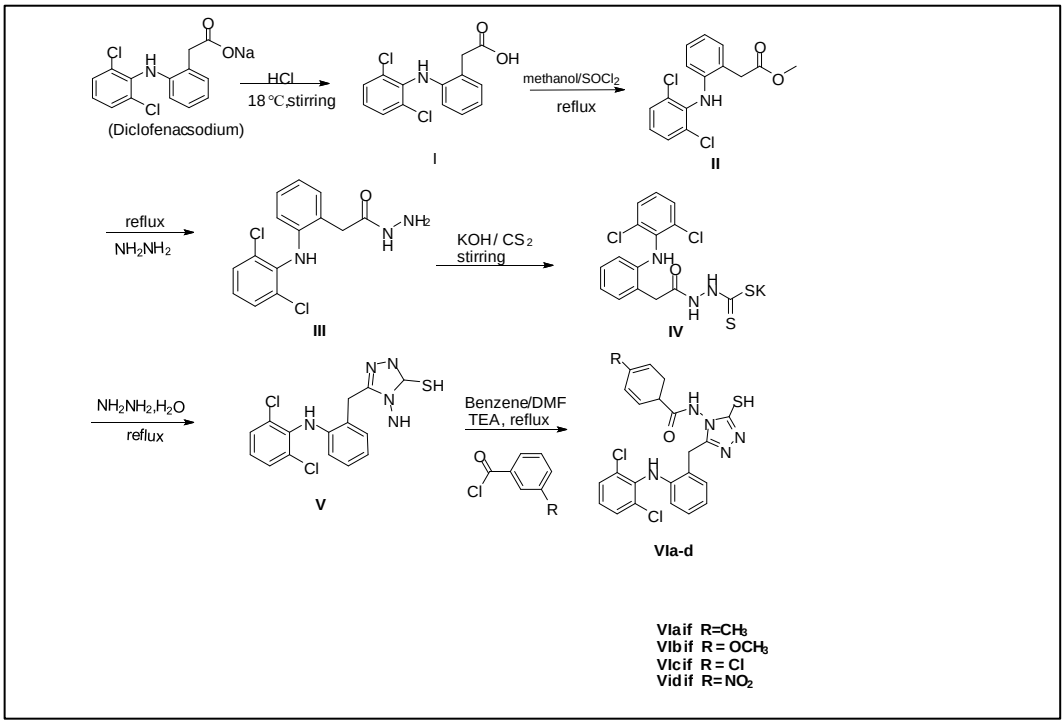
Materials

Chemicals and solvents were purchased from commercial resources, such as Sigma-Aldrich, Merck, and Chem-Lab. Thomas Hover apparatus was used to record melting points. Retention factor values were measured by using TLC to ensure the purity of the synthesized compounds, and the progression of the reaction using a solvent system of n-hexane: ethyl acetate: acetic acid at a ratio of 7:2.5:0.5 [16]. Shimadzu FT-IR spectrophotometer (Japan) was used to record the IR spectra of the synthesized compounds. Proton NMR was recorded using Bruker 500 MHz using DMSO as the solvent. Chemical shifts were shown in ppm and coupling constants (J) were in Hz. Standard abbreviations indicating spin multiplicities are given as follows: s (singlet), d (doublet), t (triplet), q (quartet), br (broad), or m (multiplet).

Chemical Synthesis

General procedures

Compounds **VI a-d** were synthesized as shown in scheme1



Scheme 1. Synthesis of the compounds (VI a- d)

Diclofenac's carboxyl group was first esterified in cold methanol in the presence of thionyl chloride to give compound **(II)**. To get the 1,2,4-triazole-3-thiol heterocyclic ring derivative **(VIa-d)** of diclofenac, the methyl ester **(II)** was allowed to react with hydrazine hydrate to produce hydrazide **(III)**, which was then reacted with carbon disulfide in the presence of potassium hydroxide to produce potassium dithiocarbazinate derivative **(IV)**. Synthesis of target compounds was achieved by reacting the primary amine group in the synthesized ring with different benzoyl chloride derivatives.

Conversion of diclofenac salt to 2-(2-((2,6-dichlorophenyl)amino) phenyl)acetic acid (I) [17].

A minimal volume of a 99% ethanol: THF (3:1) combination was used to dissolve diclofenac sodium (2 g, 6.28 mmol). After cooling to 18 °C with constant stirring for 10 minutes, HCl (2N, 3.1 mL, 6.28 mmol) was added to the mixture, and then the solution was mixed with cold water. The resulting material was filtered and dried to yield compound **I**, which was then used in the next step without further purification. The percent yield, physical data, and Rf values were given in Table (5).

IR (cm^{-1}): 3323 (N-H) of secondary amine, 3200-2500 (O-H) of carboxylic acid, 3072 (C-H) of aromatic, 2985,2887(C-H) of alkane, 1695 (C=O) of carboxylic acid, 1504,1450 (C=C) of aromatic (Skeletal vibration), 1311 (C-O) stretching vibration, 711 (C-Cl) stretching vibration.

Synthesis of methyl 2-(2-((2,6-dichlorophenyl) amino) phenyl) acetate (II)[18][19].

After chilling a solution of compound, **I** (1 g, 3.37 mmol) in 50 milliliters of absolute methanol to -15 ℃, a thionyl chloride solution (0.25 ml, 3.37 mmol) was added drop by drop (the temperature was kept below -10 ℃). After incubation at 40 ℃ for three hours, the mixture was refluxed for 3 hours, and kept at room temperature overnight. The mixture was dried under reduced pressure, then redissolved in methanol and dried. The process was repeated many times to ensure that all thionyl chloride was eliminated. The residue was collected and re-crystallized from methanol-ether to give compound **II**. The percent yield, physical data, and Rf values were given in Table (5). IR (cm^{-1}): 3352 (N-H) of secondary amine, 3086 (C-H) of aromatic, 3030,2951 (C-H) of alkane, (C=O) of ester, 1490,1452 (C=C) of aromatic (Skeletal vibration), 715 (C-Cl) stretching vibration.

Synthesis of 2-(2-((2,6-dichlorophenyl) amino) phenyl) acetohydrazide (III)[20][21].

Diclofenac methyl ester **(I)** (1g, 3.223mmol) and hydrazine hydrate (99%) (2ml, 64.46mmol) were refluxed in ethanol (50 mL) for 30 hours at 100 ℃. The reaction mixture was then combined with ice-cold water. A white solid product was obtained, which was filtered, dried, and recrystallized from ethanol. The percent yield, physical data, and R_f values were given in Table (5). IR (cm^{-1}): 3325 (N-H) of secondary amine overlap with N-H of primary amine, 3072 (C-H) of aromatic, 1637 (C=O) of amide, 1504,1452 (C=C) of aromatic (Skeletal vibration), 715 (C-Cl) stretching vibration.

Synthesis of potassium 2-(2-(2-((2,6-dichlorophenyl) amino) phenyl)acetyl)hydrazine carbodithioate (IV)[22][23].

The hydrazide derivative compound **III** (1g, 3.2mmol) was added slowly to a solution of KOH (0.24g, 4.8mmol) in absolute ethanol (250mL) while stirring, followed by CS_2 (0.36 mL, 6mmol) and kept to react with continuous stirring overnight. After filtering the reaction mixture, the desired precipitate was recovered, washed with (20 mL) ether, and then dried. The salt compound **IV** was isolated and used in the subsequent procedure without additional purification. The percent yield, physical data, and R_f values were given in Table (5). IR (cm^{-1}): 3259,3205 (N-H) of secondary amine and amide, 3039 (C-H) of aromatic, 2978,2943 (C-H) of alkane, 1651 (C=O) of amide, 1508 (C=C) of aromatic (Skeletal vibration), 1261 (C=S) vibration 715 (C-Cl) stretching vibration.

Synthesis of 4-amino-5-(2-((2,6-dichlorophenyl) amino) benzyl)-4H-1,2,4-triazole-3-thiol (V)[18][21].

The potassium salt IV suspension (1g, 2.35mmol) in excess hydrazine hydrate was refluxed until the development of H_2S was ended. After cooling, the reaction mixture was acidified with 10% HCl to produce a cream-colored precipitate. To get crystals, the precipitate was recrystallized from ethanol. The percent yield, physical data, and R_f values were given in Table (5). IR (cm^{-1}): 3425,3400 (N-H) of primary amine, 3323 (N-H) of secondary amine, 3064 (C-H) of aromatic, 3026 (C-H) stretching vibration of alkane, 1635 (C=N) vibration, 1581,1504,1454 C=C of aromatic, 752 (C-Cl) stretching vibration. ^1H NMR ($C_{15}H_{13}Cl_2N_5S$) (500 MHz, DMSO): 9.40 (s,2H, of amine (NH2)), 8.55 (s, 1H, of amine (NH)), 7.5-6.3 (m, 7H, overlap of Ar-H), 4.35 (s,2 H, of CH2), 3.54 (s,1 H, of SH).

Synthesis of the target compounds (VIa-d)[24].

Compound **V** (1g, 2.73mmol) was dissolved in a minimum volume of benzene and dimethyl formamide (DMF) (3:1) mixture then TEA (0.5 mL,) was added slowly and the solution was stirred in an ice bath for 15 min. After that, (2.73 mmol) of different benzoyl chloride derivatives were dissolved in benzene and added to the first mixture with continuous stirring in an ice bath for an hour, and was then refluxed for a specific time. After refluxing, the reaction mixture was filtered and washed with cold water several times to obtain a yellow precipitate. This precipitate was washed with an amount of petroleum ether and dried. The percent yield, physical data, and R_f values are given in Table (5).

N- (3 - (2 - ((2 , 6 – dichlorophenyl) amino) benzyl)-5-mercapto - 4H – 1 , 2 , 4 – triazol – 4 – yl)-4-methylbenzamide (VIa);

IR (cm^{-1}): 3182 (N-H) secondary amine, 3039 (C-H) of aromatic, 1627 (C=O) of amide, 1608 (C=N) stretching vibration, 1570,1492,1454 (C=C) of aromatic, 740 (C-Cl) stretching vibration. ^1H NMR ($C_{23}H_{19}Cl_2N_5OS$) (500 MHz, DMSO): 10.49 (s,1H, of amide (CONH)), 10.41 (s, 1H, of amine (NH)), 8.08-6.34(m, 11H, overlap of Ar-H), 3.9(s,2 H, of CH_2), 3.08 (s,1 H, of SH), 2.39 (s, 3H, of CH_3).

N-(3 -(2 - ((2 , 6 – dichlorophenyl) amino) benzyl)-5-mercapto - 4H -1 , 2 , 4- triazol -4 -yl) -4-methoxybenzamide(VIb);

IR (cm^{-1}): 3217 (N-H) secondary amine, 3062 (C-H) of aromatic, 1662 (C=O) of amide, 1600 (C=N) stretching vibration, 1570,1504,1450 (C=C) of aromatic, 740 (C-Cl) stretching vibration, ^1H NMR ($C_{23}H_{19}Cl_2N_5O_2S$) (500 MHz, DMSO): 10.43 (s,1H, of amide(CONH)), 10.35 (s, 1H, of amine

(NH)), 8.09-6.34 (m, 11H, overlap of Ar-H), 3.84 (s,3 H, of OCH_3), 3.63 (s,1 H, of SH), 3.32 (s, 2H, of CH_2).

4- chloro -N- (3- (2 - ((2 , 6-dichlorophenyl)amino)benzyl) -5- mercapto - 4H -1 ,2 ,4 – triazol – 4 -yl)benzamide (VIc);

IR (cm−1): 3205 (N-H) secondary amine, 3072 (C-H) of aromatic, 1678 (C=O) of amide, 1598 (C=N) stretching vibration, 1558,1498,1465 (C=C) of aromatic, 736 (C-Cl) stretching vibration. ^{1}H NMR ($C_{22}H_{16}C_{l3}N_5OS$) (500 MHz, DMSO): 10.47 (s,1H, of amide (CONH)), 10.44 (s, 1H, of amine (NH)), 8.08-6.34(m, 11H, overlap of Ar-H), 3.04 (s,2 H, of CH_2), 3.08 (s,1 H, of SH).

N-(3-(2-((2,6-dichlorophenyl)amino)benzyl)-5-mercapto-4H-1,2,4-triazol-4-yl)-4-nitrobenzamide (VId);

IR (cm^{-1}): 3217 (N-H) secondary amine, 3016 (C-H) of aromatic, 1654 Asymmetric NO_2, 1593 (C=N) stretching vibration, 1504,1450 (C=C) of aromatic, 1342 symmetric NO_2, 748 (C-Cl) stretching vibration.1HNMR ($C_{22}H_{16}C_{l2}N_6O_3S$) (500 MHz, DMSO): 10.87 (s,1H, of amide(CONH)), 10.65 (s, 1H, of amine (NH)), 8.37-6.33 (m, 11H, overlap of Ar-H), 3.9 (s,2 H, of CH_2), 3.75 (s,1 H, of SH).

Table1. Benzoyl chloride derivatives and their amount

Benzoyl Chloride derivatives	Weight (g)	Molecular weight (g/mol)	Reflux time (h)
4-Methybenzoyl chloride (VIa)	0.4	154.59	6
4-Methoxybenzoyl chloride (VIb)	0.5	170.59	10
4-Chlorobenzoyl chloride (VIc)	0.5	175.01	6
4-Nitrobenzoyl chloride (VId)	0.5	185.56	30

Molecular docking

Molecular docking studies were carried out using MOE 2015 software from chemical computing as per the following steps:

1. Ligand preparation: chemical structures of the designed compounds were drawn using ChemDraw Ultra 20.1.1and the 2D structures were saved in MDL-SD file format. Using MOE 2015, the compounds were prepared using the ligand preparation protocol of protonation, partial charge addition, and energy minimization.

2- Protein preparation: Human DNA topoisomerase I (PDB: 1K4T) and type IV topoisomerase from S. pneumoniae (PDB: 3RAE) were retrieved from the Protein Data Bank (PDB). They were prepared using a standard protocol of water removal, the addition of protons, the addition of broken bonds, and fixation of the potential of the protein molecule. The active site on the topoisomerase enzyme was selected in MOE and amino acids at this site were determined [25,26]. Five distinct protein interactions were allowed for each compound, and the pose with a higher S-Score and proper RMSD value (less than 2) was selected. The binding energy, number of H- H-bonds, and type of amino acids that form the target protein's active site have been shown in Table (3) for 3RAE Protein and Table (4) for 1K4T Protein.

Antibacterial study

The target compounds' in vitro antibacterial properties were assessed against gram-positive and gram-negative bacteria, S. aureus, and E. coli, respectively, using the agar-well diffusion method. This technique utilized Brain Heart Infusion Agar (BHIA). The tested compounds were dissolved in dimethyl sulfoxide, and 1 mL of a spore solution of each bacterium was equally distributed on sterile solid media using cotton swabs. Wells of 6 mm was made in the plates filled with 0.1 mL of each concentration. Then the plates were incubated at 37 ℃ for 24 hours. The zones of inhibition were observed and measured to determine the antibacterial activity of synthesized compounds. Table (2) displays the calculated doses of synthesized compounds:

Table2. Calculated concentrations of diclofenac derivatives.

Compound	Molecular weight (g/mol)	Dose in ppm (µg/mL)
Diclofenac	296.1	29.61
VIa	484.4	48.44
VIb	500.4	50.04
VIc	504.82	50.482
VId	515.37	51.537

Results and Discussion

Molecular Docking and Virtual Screening

The molecular docking approach is quickly becoming an essential tool in the drug design process. It is important in computer-based drug design because it can be used to demonstrate the cooperation between a small molecule and a protein at the nanoscale, allowing the description of the behavior of small particles in a protein active site and explaining key biochemical processes[25]. In this study, the results have shown that all of the ligands studied have a similar position and orientation inside the recognized binding sites of the topoisomerase enzymes, revealing a large space bounded by a membrane-binding domain. The binding free energy results explain that the majority of these compounds have a good binding affinity toward the receptor. Table 3 and Table 4 illustrate the scores of bindings against 1K4T and 3RAE, respectively. In addition, the RMSD values, which represent the average distance between the atoms of the pose and that of the crystallized ligand, of the selected poses are less than 2, which reflects a good fit of the ligands in the same binding site of the crystallized. Docking results of the diclofenac and compounds (**VIa-d**) with the human DNA topoisomerase I (1K4T) enzyme are shown in Table3. The enzyme's active site is made up of Thr C718, PTR C723, Lys C532, Asp C533, Asn C722, DT A10, Arg C364, DA B113, and DC B112. Compounds (**VIa-d**) have shown a higher binding score than the reference ligand (Topotecan), especially compound **VIb**, which had the highest docking scores (-8.0322). The interacting residues and number of interactions are shown in Table (4) and as illustrated in Figure (1-5).

Table 3. Docking Results of Diclofenac and (VIa, VIb, VIc, VId) Compounds against 1K4T Protein

Compound	S-Score	Rmsd	No. of binding sites	Binding amino acids
Topotecan (Reference ligand)	-7.5544	0.8964	6	Lys C532, Asp C533, Arg C364, Da B113. DC B113
Diclofenac (I)	-5.4865	0.7934	7	Lyr B426, DA A114, DA. A113
VIa	-7.7142	1.8906	4	Glu C356, lle C355, Tyr C426, Trp C416
VIb	-8.0322	1.6816	2	DA B113, TyrC426
VIc	-7.9097	1.4551	5	Lys C425, DA B113
VId	-7.8113	1.2121	5	Tyr C426, DT A10, DA B113, lle C355, Glu C356

S-Score (Score of binding), RMSD (Root mean square deviation),

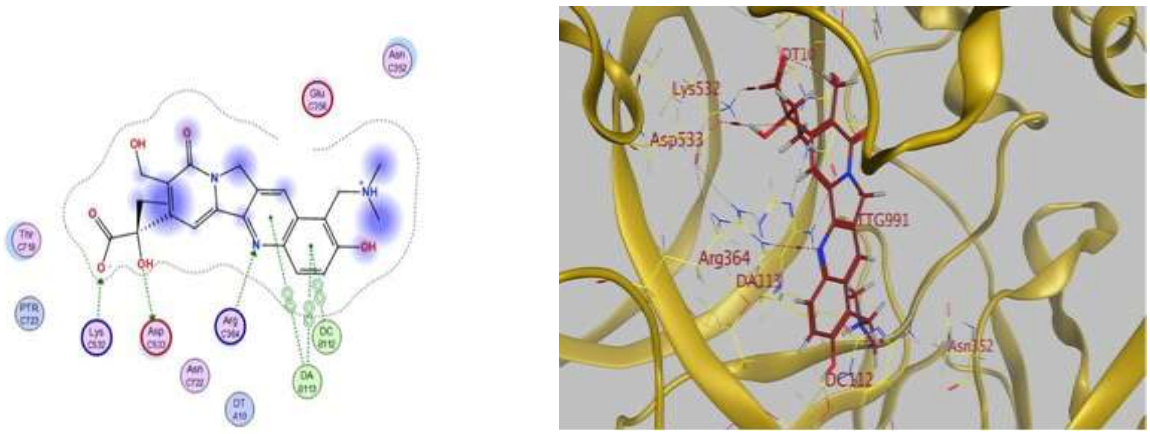


Figure 1. Docking result of reference ligand (Topotecan) with human DNA topoisomerase I enzyme (PDB code:1K4T) (2D and 3D).

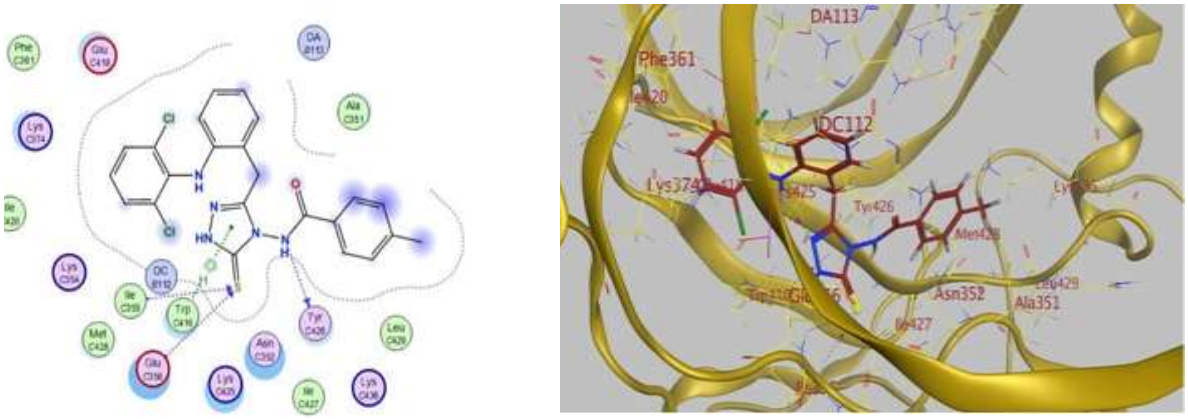


Figure 2.Docking result of compound VIa with human DNA topoisomerase I enzyme (PDB code:1K4T) (2D and 3D).

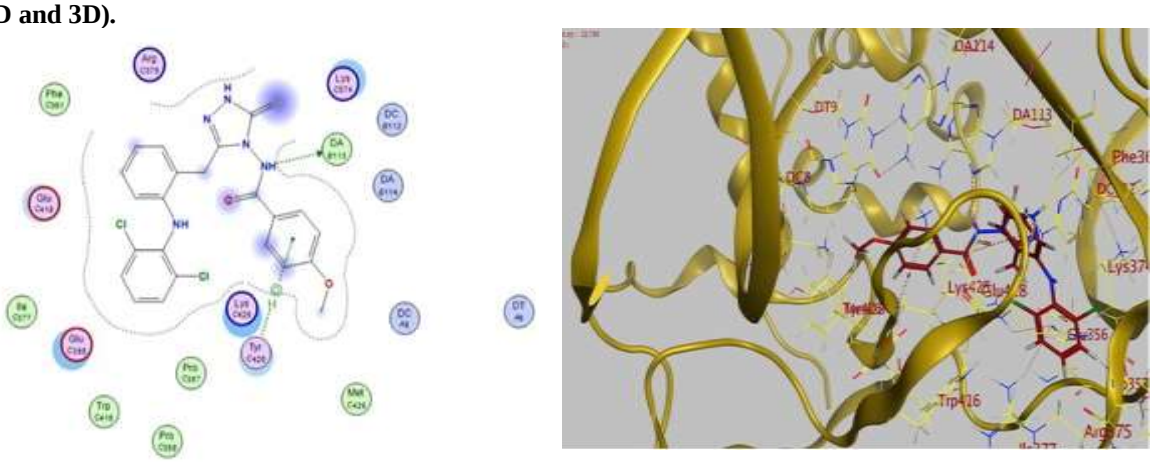


Figure 3. Docking result of compound VIb with human DNA topoisomerase I enzyme (PDB code:1K4T) (2D and 3D).

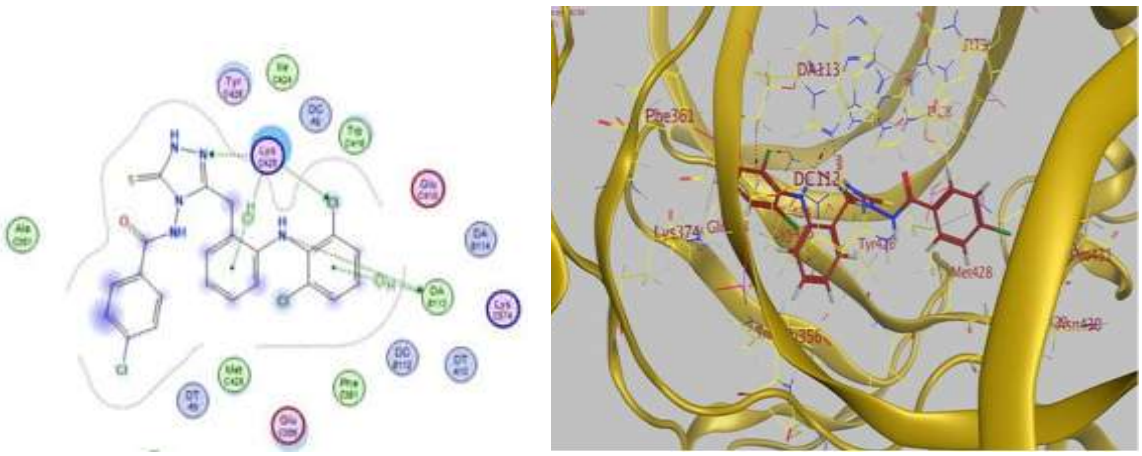


Figure 4. Docking result of compound VIc with human DNA topoisomerase I enzyme (PDB code:1K4T) (2D and 3D)

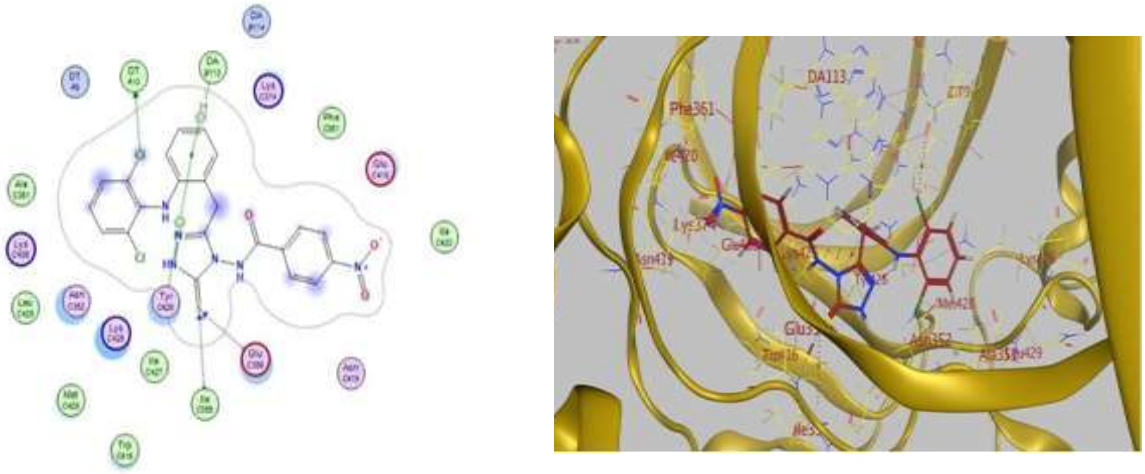


Figure 5. Docking result of compound VId with human DNA topoisomerase I enzyme (PDB code:1K4T) (2D and 3D)

Similarly, docking results of the diclofenac and compounds (**VIa-d**) with type IV topoisomerase from S. pneumoniae (PDB: 3RAE), along with the number of binding and binding amino acids for each compound are shown in Table (3). Ser B79, M G101, DA H5, Glu D475, Gly D457, Arg D456, Asp D510, DG H7, lle D514, DT H5, Glu D433, Glu D474, Ser B80, and Lys D458 make up the enzyme's active site. The docking study revealed that the affinity of design compounds was nearly identical to the reference ligand (Levofloxacin) toward the enzyme as shown in Figures (6-10).

Table 4. Docking Results of Diclofenac and(VIa-d) Compounds with 3RAE Protein

Compound	S-Score	Rmsd	No. of binding sites	Binding amino acids
Levofloxacin (Reference ligand)	-7.4584	1.7733	3	Ser B79, M G101, DA H5
Diclofenac (I)	-5.9379	1.4681	3	MG 101, DA C5
VIa	-7.1055	1.3511	4	Ser A80, DG C7, Arg A28, Lys B458
VIb	-7.1428	1.3880	2	Arg A28
VIc	-7.3903	0.9316	3	Lys 38, Gln 41, Arg 87
VId	-7.0246	1.6092	4	Ser A80, Arg A28, Lys B458

S-Score (Score of binding), Rmsd (Root mean square deviation),

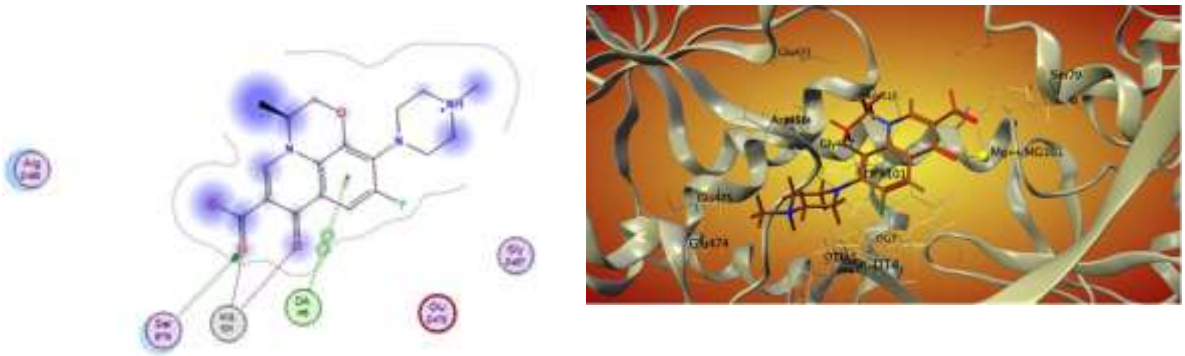


Figure 6. Docking result of reference ligand (Levofloxacin) with topoisomerase IV enzyme (PDB code: 3RAE) (2D and 3D).

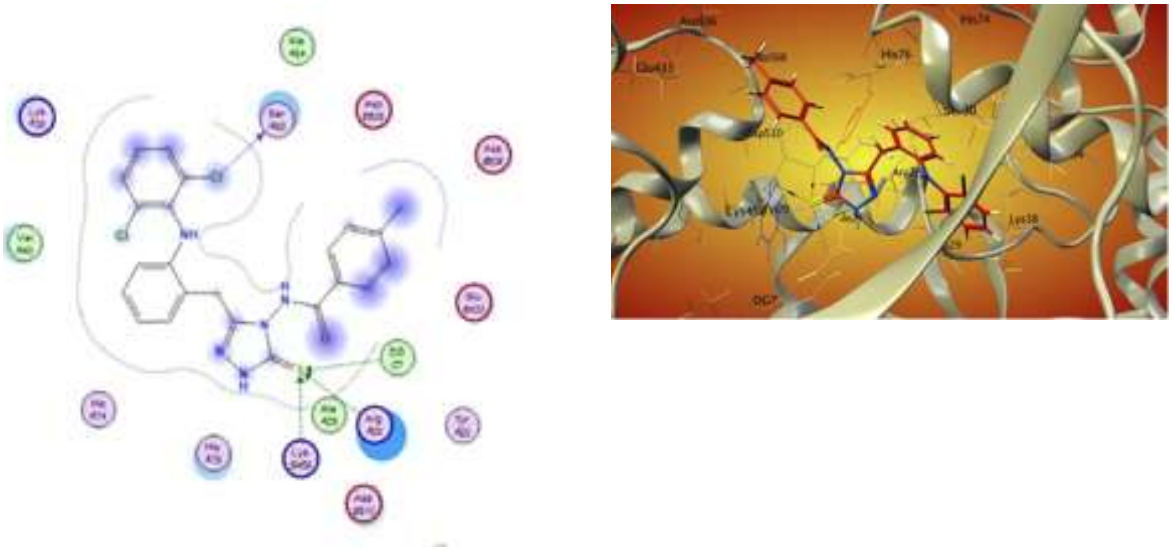


Figure 7. Docking result of compound VIa with topoisomerase IV enzyme (PDB code: 3RAE) (2D and 3D)

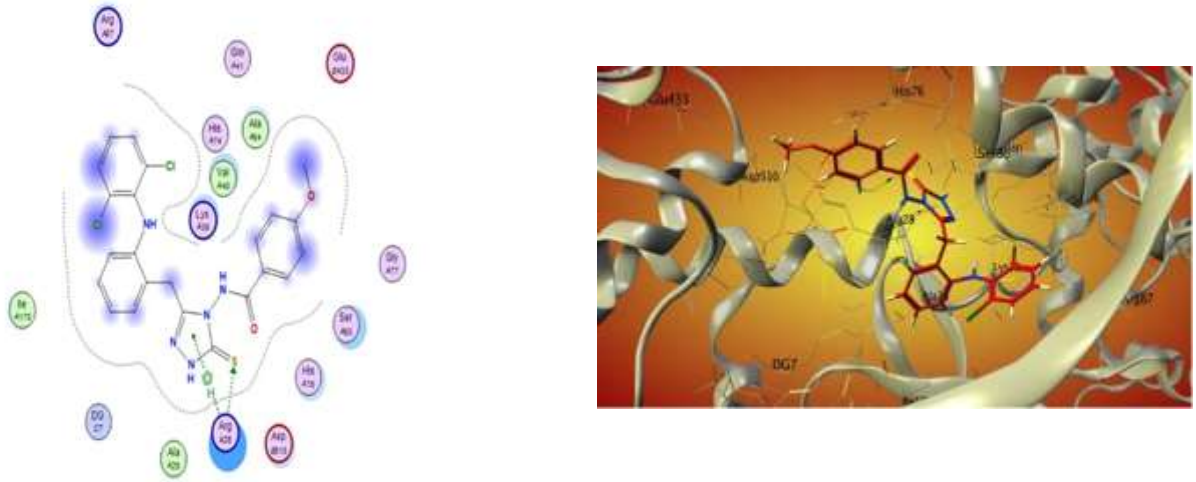


Figure 8. Docking result of compound VIb with topoisomerase IV enzyme (PDB code: 3RAE) (2D and 3D).

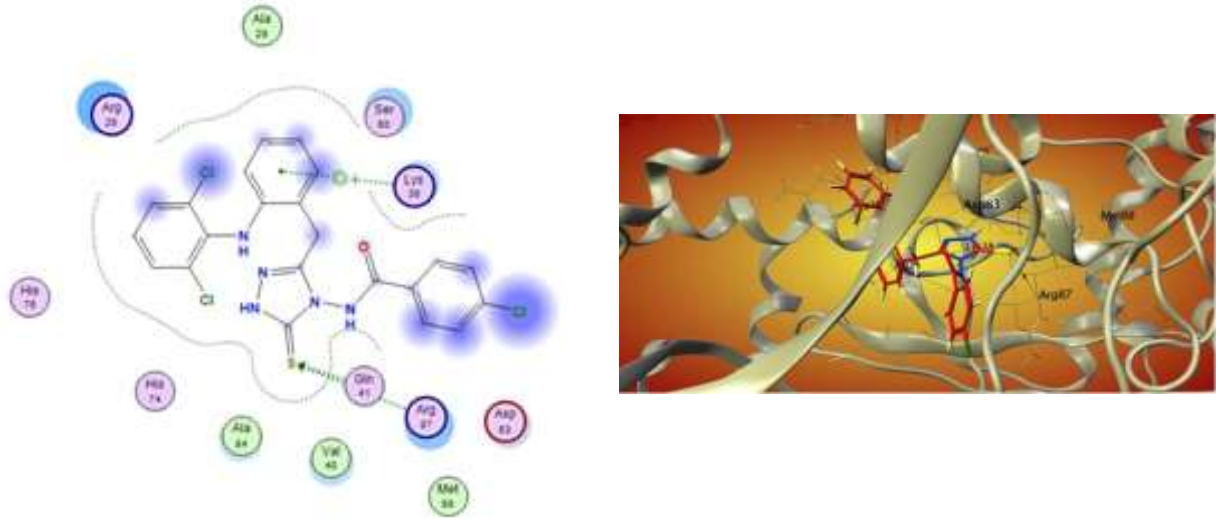


Figure 9. Docking result of compound VIc with topoisomerase IV enzyme (PDB code: 3RAE) (2D and 3D).

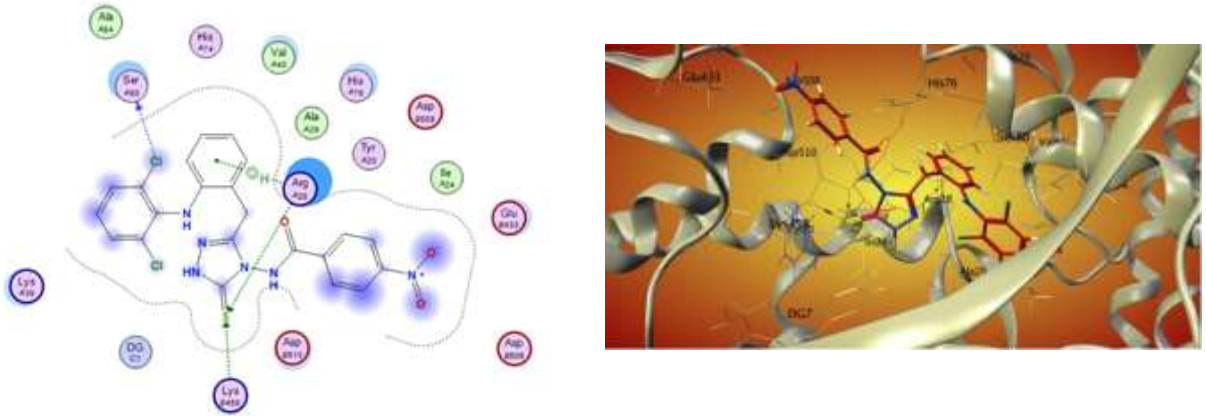


Figure 10. Docking result of compound VId with topoisomerase IV enzyme (PDB code: 3RAE) (2D and 3D).

Chemistry

Scheme 1 depicts the synthetic approach that resulted in the synthesis of the final derivatives of diclofenac **(VIa-VId),** as well as their intermediates. Firstly, methanol and thionyl chloride were used to esterify diclofenac, yielding an acyl chloride intermediate that reacted with an alcohol to form diclofenac methyl ester. The disappearance of broadband of the OH group of carboxylic acid (2500-3200cm^{-1}), and shifting of the C=O stretching vibration of carboxylic acid from 1695 cm^{-1} to 1730 cm^{-1}, the C=O stretching vibration of ester indicate the conversion of the carboxyl group in diclofenac to ester.

The potassium salt intermediate was cyclized with hydrazine hydrate to give the 1,2,4-triazole-3-thiol derivative of diclofenac. This reaction involved several intermediates, the first intermediate being a nucleophile hydrazine attack of the carbonyl moiety with loss of a water molecule, followed by intramolecular cyclization by a neighboring nucleophile amine moiety attacking the carbon of carbon disulfide by nucleophile substitution reaction, the potassium salt was formed with concomitant loss of hydrogen sulfide. The compound **(V)** was released after the potassium salt was acidified with 35% concentrated hydrochloric acid. Final compounds **(VIa-VIb)** were obtained by reacting the free amine in the created heterocyclic ring with various Benzoyl Chloride derivatives. This reaction occurs between the primary amine and acyl carbon through nucleophilic acyl substitution and conversion into an amide. Table 5 shows the melting points and Rf values of the synthesized compounds.

Table5. Characterization and physical data of the intermediates and compounds (VIa-d).

Compounds and intermediates	Empirical formula	Molecular weight (g/mol)	Description	% Yield	Melting point ℃	R_f value
Diclofenac I	$C_{14}H_{11}Cl_2NO_2$	296	White powder	98	160-162	0.85
II	$C_{15}H_{13}Cl_2NO_2$	310	White crystals	92	73-75	0.88
III	$C_{14}H_{13}Cl_2N_3O$	310	White powder	85	120-123	0.32
IV	$C_{15}H_{12}Cl_2KN_3OS_2$	424	brown powder	75	180 d	----
V	$C_{15}H_{13}Cl_2N_5S$	366	Yellow-Brown powder	40	130-133	0.5
VIa	$C_{23}H_{19}Cl_2N_5OS$	484.40	Yellow powder	58	170-173	0.6
VIb	$C_{23}H_{19}Cl_2N_5O_2S$	500.40	Pale Brown powder	62	140-142	0.65
VIc	$C_{22}H_{16}Cl_3N_5OS$	504.82	Yellow powder	45	195-198	0.3
VId	$C_{22}H_{16}Cl_2N_6O_3S$	515.37	Brown powder	68	190-192	0.5

Pharmacology

Antibacterial study

The agar-well diffusion method was used to test the produced compounds for their antibacterial activity in vitro. Antibacterial activity against S. aureus and E. Coli was demonstrated for the synthesized compounds. The compound VId showed the highest antibacterial activity in these bacteria, while the lowest inhibition zone VIa.

Table 6. The antibacterial activity of diclofenac and final compounds (VIa-d)

Compound	Inhibition zone of bacterial growth (mm)	
	S. aureus (Gr+ve)	E. coli (Gr-ve)
Diclofenac	26	16
VIa	23	15
VIb	27	16
VIc	25	14
VId	29	21

Conclusion

The docking results validated the binding of the target compounds **(VIa-d)** to the active site of the human DNA topoisomerase I. Most of the designed compounds have demonstrated a good binding score with the target protein (1K4T) relative to the reference drugs (Topotecan). The strongest S-score compounds were **(VIa and VIc)**. Furthermore, these designed compounds **(VIa-d)** have shown acceptable binding scores to the type IV topoisomerase IV, nearly similar to that of levofloxacin, except compound **VIa**. In vivo, anti-bacterial testing of the synthesized compounds revealed that they are effective against Staphylococcus aureus and Escherichia coli. Compound **VId** exhibited the highest level of activity.

Acknowledgment

We would like to thank members of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Kufa for their assistance during the carrying out of this study.

Conflicts of Interest

"Authors declare no conflict of interest".

Funding

N/A

Ethics Statements

N/A

Author Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Alyaa N. Doohee, N.H. N.; data collection: A. N. D., N.H. N.; analysis and interpretation of results: A. N. D., N.H. N.; draft manuscript preparation; A. N. D., N. H. N., K.Al-G.. All authors reviewed the results and approved the final version of the manuscript.

References

1. Drake TM, Fairfield CJ, Pius R, Knight SR, Norman L, Girvan M, et al. Non-steroidal anti-inflammatory drug use and outcomes of COVID-19 in the ISARIC Clinical Characterisation Protocol UK cohort: a matched, prospective cohort study. Lancet Rheumatol. 2021;3(7):e498–506.
2. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis. 2018;9(1):143–50.
3. Edinoff AN, Houk GM, Patil S, Siddaiah HB, Kaye AJ, Iyengar PS, et al. Adjuvant drugs for peripheral nerve blocks: The role of alpha-2 agonists, dexamethasone, midazolam, and non-steroidal anti-inflammatory drugs. Anesth Pain Med. 2021;11(3).
4. Shneine J. Synthesis, characterization, and antibacterial activity of new schiff's bases with 1, 2, 4-triazole moiety. 2017;(January 2015).
5. Hamadameen BM, Ameen DMH. Synthesis, Characterization, and Antibacterial Activity of Novel Mutual Non-Steroidal Anti-inflammatory Prodrugs. Zanco J Pure Appl Sci. 2019;31(6).
6. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
7. Yadav AR, Mohite SK. Cancer-A silent killer: An overview. Asian Journal of Pharmaceutical Research. 2020;10(3):213.
8. Reynolds BA, Oli MW, Oli MK. Eco-oncology: Applying ecological principles to understand and manage cancer. Ecol Evol. 2020;10(16):8538–53.
9. Bonev BB, Brown NM. Bacterial resistance to antibiotics - from molecules to man. Bacterial Resistance to Antibiotics: From Molecules to Man. 2019. 1–239 p.
10. Derry S, Wiffen PJ, Moore RA. Single dose oral diclofenac for acute postoperative pain in adults. Cochrane Database of Systematic Reviews. 2015;2017(10).
11. Dutta NK, Mazumdar K, Park JH. In vitro synergistic effect of gentamicin with the anti-inflammatory agent diclofenac against Listeria monocytogenes. Lett Appl Microbiol. 2009;48(6):783–5.
12. Zhang S, Qu X, Tang H, Wang Y, Yang H, Yuan W, et al. Diclofenac Resensitizes Methicillin-Resistant Staphylococcus aureus to β-Lactams and Prevents Implant Infections. Advanced Science. 2021;8(13):1–16.
13. Kaur R, Ranjan Dwivedi A, Kumar B, Kumar V. Recent Developments on 1,2,4-Triazole Nucleus in Anticancer Compounds: A Review. Anticancer Agents Med Chem. 2015;16(4):465–89.
14. Mareddy J, Suresh N, Kumar CG, Kapavarapu R, Jayasree A, Pal S. 1,2,3-Triazole-nimesulide hybrid: Their design, synthesis and evaluation as potential anticancer agents. Bioorg Med Chem Lett. 2017;27(3):518–23.
15. Bayrak H, Demirbas A. Synthesis of some new 1 , 2 , 4-triazoles , their Mannich and Schiff bases and evaluation of their antimicrobial activities. 2009;44.

16. Mohammad A, Sharma S, Bhawani SA. Reversed phase thin layer chromatography of five co-administered drugs with surfactant modified solvent systems. Indian Journal of Chemical Technology. 2009;16(4):344–50.
17. S. A. Hasan and A. N. Elias, "Synthesis of new diclofenac derivatives by coupling with chalcone derivatives as possible mutual prodrugs," *Int. J. Pharm. Pharm. Sci.*, vol. 6, no. 1, pp. 239–245, 2014.
18. N. H. Naser, M. F. Mahdi, T. N-A Omar, and A. A. Fadhil, "Synthesis and Preliminary Pharmacological Evaluation of New Analogues of Diclofenac as Potential Anti-inflammatory Agents," *Iraqi J. Pharm. Sci. (P-ISSN 1683 - 3597 , E-ISSN 2521 - 3512)*, vol. 20, no. 1, pp. 25–32, 2017, doi: 10.31351/vol20iss1pp25-32.
19. J. B. Christensen, "A simple method for synthesis of active esters of isonicotinic and picolinic acids," *Molecules*, vol. 6, no. 1, pp. 47–51, 2001, doi: 10.3390/60100047.
20. N. A. Alsaif, M. A. Bhat, M. A. Al-omar, H. M. Al-tuwajiri, A. M. Naglah, and A. Al-dhfyan, "Synthesis of Novel Diclofenac Hydrazones : Molecular Docking , Anti-Inflammatory , Analgesic , and Ulcerogenic Activity," vol. 2020, 2020.
21. V. Sumangala, B. Poojary, N. Chidananda, T. Arulmoli, and S. Shenoy, "Synthesis and biological evaluation of some Schiff bases of 4-amino-5-(4-methylsulfonyl)benzyl-2,4-dihydro-3H-[1,2,4]-triazole-3-thione," *Med. Chem. Res.*, vol. 22, no. 6, pp. 2921–2928, 2013, doi: 10.1007/s00044-012-0294-5.
22. A. Husain, M. a Naseer, and M. Sarafroz, "Drug Synthesis Synthesis and Anticonvulsant Activity of Some Novel Fused," *Acta Pol. Pharm.*, vol. 66, no. 2, pp. 135–140, 2009.
23. B. Sejdiu and D. P. Tieleman, "COX-1 – lipid interactions: arachidonic acid, cholesterol, and phospholipid binding to the membrane binding domain of COX-1," pp. 1–29, 2020.
24. M. Saeedi *et al.*, "Synthesis and biological investigation of some novel sulfonamide and amide derivatives containing coumarin moieties," *Iran. J. Pharm. Res.*, vol. 13, no. 3, pp. 881–892, 2014.
25. Attique SA, Hassan M, Usman M, Atif RM, Mahboob S, Al-Ghanim KA, et al. A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. Int J Environ Res Public Health. 2019;16(6):1–17.
26. Egieyeh S, Egieyeh E, Malan S, Christofells A, Fielding B. Computational drug repurposing strategy predicted peptide-based drugs that can potentially inhibit the interaction of SARS-CoV-2 spike protein with its target (humanACE2). PLoS One. 2021;16(1):1–14.

الارساء الجزيئي والتصنيع والفعالية الحيوية لمشتقات الفولتارين الحاوية على حلقة ١,٢,٤ ترايزول كمضادات بكتيرية جديدة

علياء نوري دوحي[١]، نور هاتف ناصر[١] و كرار الجبوري[٢،*]

[١]فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة الزهراء، كربلاء المقدسة، العراق
[٢]فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة الكوفة، النجف الاشرف، العراق

الخلاصة

تبين من خلال الدراسات السابقة ان مضادات الالتهاب غير الستيرويدية تمتلك عدة فعاليات حيوية مثلاً مضادة ضد البكتيريا والفطريات وكذلك فعالية ضد الخلايا السرطانية. حلقة الترايزول تمتلك فعاليات حيوية عدة منها مضادة ضد البكتيريا والفطريات وكذلك فعالية ضد الخلايا السرطانية. ولذلك كان الهدف من هذه الدراسة هو التصميم والإرساء الجزيئي وفحص الفعالية الحيوية كمضادات بكتيرية لمشتقات الفولتارين الحاوية على حلقة ١,٢,٤ ترايزول. تم عمل الإرساء الجزيئي باستخدام برنامَج MOE بواسطة اتباع الخطوات القياسية. أيضا تم التصنيع المختبري لمشتقات الفولتارين (VIa-d) بأتباع الخطوات التالية: أولا تم عمل استرة الفولتارين ومفاعلة الناتج مع الهيدرازين المائي. بعد ذلك تمت مفاعلة الناتج من التفاعل السابق مع ثاني كبريتيد الكربون. ولتشكيل حلقة ١,٢,٤ ترايزول تم مفاعلة المركب الناتج من التفاعل السابق مع الهيدرازين المائي مرة أخرى. وللحصول على مشتقات الفولتارين الجديدة تم مفاعلة الناتج مع مشتقات مختلفة للبنزويل كلوريد. تقييم النشاط المضاد للبكتيريا في المختبر للفولتارين والمركبات المحضرة باستخدام طريقة انتشار القرص على بكتيريا Staphylococcus aureus and Escherichia coli. أظهرت المركبات (VIa-d) درجة ربط أعلى من (Topotecan) ضد Human DNA topoisomerase I (PDB: 1K4T) بينما كانت مطابقة تقريبًا لمركب (الليفوفلوكساسين) ضد Type IV topoisomerase from S. pneumoniae (PDB: 3RAE). كل المركبات تثبط نمو Staphylococcus aureus and Escherichia coli. مركب VId اظهر اعلى فعالية على البكتريا المذكورة انفا. **الكلمات المفتاحية: الفولتارين، الارساء الجزيئي، حلقة ١,٢,٤ ترايزول، الفعالية البكتيرية , مشتقات البنزويل كلورايد.**