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Design, Synthesis And Molecular Docking Of L-Prolinamide Containing Thiazolidine-4-One Ring System As(ACE Inhibitors)

Mohammed G Mukhlif [1*] , Ayad S Hameed[1]and Firas Shawqi Algburi [2]

[1]University of Tikrit College of Sciences Department of Chemistry

[2]University of Tikrit College of Sciences Department of Biology

Abstract

The present work started with synthesis of five new Schiff bases containing terminal carboxylic acid (A_1-A_5) group through the condensation of para amino benzoic acid and para substituted benzaldehydes , these compounds were subjected to cyclization reaction with thioglycolic acid to from 2,3-disubstituted -1,3thiazolidine-4-one containing carboxylic group(B_1-B_5), these carboxylic acids were converted to corresponding acid chlorides(C_1-C_8) using thionyl chloride, finally L-prolinamides(D_1-D_8) were synthesized by reaction of acid chlorides with L-proline acid, TLC was used to monitor the reactions, and spectroscopic methods such as FT-IR, ^{1}H-NMR, and mass spectrometry were used to describe all structures, Molecular docking was used to investigate the inhibitory action of the produced compounds (D_1-D_8) in the activity of the angiotensin-converting enzyme (ACE), All L-proline amide derivatives showed enzyme inhibitory activity, The derivative D_8 at a concentration of (10^{-2}-10^{-6}M) and docking Score (-7.134) Kcal/mole reveal good inhibition potency in vitro ACE- inhibitory tests using human blood samples., the in vitro results were backed up by good binding results in molecular docking investigations, According to molecular docking , The results were compared to Captopril and Enalapril as a reference drug.

Keywords: 1,3-Thiazolidine-4-one, L-proline, Molecular Docking

Introduction:

Hypertension is a chronic illness that is frequently viewed as a major risk factor for cardiovascular disease. Controlling and preventing this disease has been deemed vital to global health[1].Several studies have revealed stimulation of the renin-angiotensin-aldosterone system has as a contributor to the pathogenesis of hypertension [2]. The renin–angiotensin system (RAS), which regulates blood pressure, is made up of two enzymes: angiotensin-converting enzyme (ACE) and renin [3]. Renin inhibitors, angiotensin II receptor blockers (ARBs), and angiotensin-converting enzyme inhibitors are among the several types of pharmacological drugs now available for the treatment of hypertension (ACEIs) [1]. Synthetic ACE inhibitors including lisinopril, captopril, and enalapril have been explored and used as first-line hypertension medications [4]. Antihypertensive medicines and ACE inhibitors containing proline and proline-like moieties in their structures have shown to be effective, indicating that this moiety is crucial in ACE inhibitors [5]. Proline is the protein's α -pyrrolidine substituted amino acid, L-proline is one of the 20 amino acids that make up the building blocks of proteins in

living beings [6]. Because of their widespread availability and low toxicity, α -amino acids are a popular family of organic molecules in synthetic chemistry [7]. Schiff base organic compounds containing the group of (-CH=N-) group are named after the world Schiff, who discovered in 1864 areformed by the condensation reaction of aldehydes or ketones with primary amines[8]. Heterocyclic compounds are common in nature and have a big influence on human health since structural subunits of these compounds may be found in a lot of natural goods including vitamins, hormones, and medications [9]. Thiazolidine-4-one is a five-membered heterocyclic ring containing N and S heteroatoms and one carbonyl group that is present in the structure of manufactured drugs, It was prepared using a variety of techniques, including the reaction of aldehyde and amine derivativeswith thioglycolic acid. [10]. thiazolidine derivatives are heterocyclic compounds with anticancer, anti-infectious, and antibacterial properties [11]. Molecular docking is a key tool in molecular and structural biology and in drug design and discovery by computer, which is the process of joining two molecules together in a direction and location determined by the geometry and physical properties of the two molecules [12]. Where molecular is a pillar for understanding bio-molecular- pharmacological interactions for the design and discovery of the correct drug as well as studying the mechanistic by placing a molecule (ligand) in the preferred binding site of the specific target regionof the protein-DNA (receptors) mainly to form stable complexes of the potential and more specific activity. It is used to explain how to bind the prepared compounds with the active site of the protein,as the practical application of molecular docking requires the presence of a bank of information to search for the target molecule [13-14].all these facts promote our research group to synthesized eight novel L-proline amide derivatives and their anti-hypertensive activity was evaluated by molecular docking and experimental testing . We hope that the combination of L- proline and other carboxylic acids containing thiazolidine ring system in one frame structure may enhance the anti-hypertensive activity of such compounds.

1. Experimental part

2.1. Chemistry

2.1.1.Material and Methods

All chemicals were purchased and acquired from Sigma-Aldrich and are not purified further. The melting points were determine in an open capillary tube and are unadjusted. Infrared spectra were recorded as FT-IR using a Tensor 27 Bruker Co., Germany spectrometer (4000–600 cm-1). The1HNMR spectra were acquired using DMSO-d6 as a solvent on a Bruker Ultershield 400MHz NMR spectrometer, Co., Germany. The chemical shifts are presented as values in parts per million (ppm). The Agilent Technology MS 5973 equipment was used to analyze mass spectra.

2.1.2. General procedure for Synthesis Schiff's bases(A_1-A_5)

The method of work was adopted in the source [15], with some modifications made where (0.02) mole of benzaldehyde or one of its derivatives and absolute ethanol plus(4drops) of glacial acetic acid was added to a 100 mL two-neck round bottom flask. The mixture was stirred for ten minutes and then (0.02 mole ,3 gm) from(4-amino benzoic acid dissolved in 30 ml of absolute ethanol)was added through a drop-by-drop distillation funnel. The mixture was refluxed for3hr.TLC was used to monitor the reactions and use (Benzene: Methanol) (8:2). The reaction mixture was left to cool, then

stirred for an hour. A precipitate was leached using a Buechner funnel and the product was recrystallized from ethanol. Table (1): physical constants of the prepared Schiff's base.

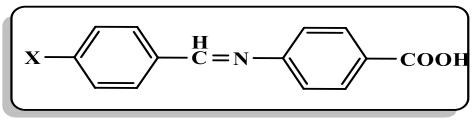


Table (1): The Molecular formula, physical constants and R_f of Schiff's base compounds (A_1-A_5).

Comp. Symb.	- X	M.P. °C	R_f	M.F.	M.Wt.	Yield %	reflux time(hr.)	Colour
A_1	-H	192-194	0.83	$C_{14}H_{11}NO_2$	225.25	73	3	White
A_2	-Cl	263-265	0.73	$C_{14}H_{10}ClNO_2$	259.69	80	3	White
A_3	-Br	287-289	0.76	$C_{14}H_{10}BrNO_2$	304.14	75	3	White
A_4	$-NO_2$	290-292	0.60	$C_{14}H_{10}N_2O_4$	270.24	85	3	Yellow
A_5	$-OCH_3$	289-291	0.62	$C_{15}H_{13}NO_3$	255.27	78	3	White

2.1.3. General procedure for Synthesis 1,3- Thiazolidine-4-one Derivatives(B_1-B_5)

The working method was adopted in the source [16] with some modifications, where 0.02 mole of Schiff base was dissolved in 25 ml of Dioxane and heated with stirring for ten minutes in a two-neck round-bottom flask containing a condenser, anhydrous calcium chloride tube guard, and a distillation funnel. put in a water bath at a temperature of 68 °C. Then 0.02 mol of 2.7 gm of zinc chloride anhydrous dissolved in 10 ml of dioxane as a catalyst was added to 0.02 mol of 2 gm of thioglycolic acid and dissolved in 20 ml of dioxane. Then the mixture was added through the distillation funnel drop by drop, and after the completion of mixing the reaction components, the turbidity of the mixture was observed immediately after the addition. The mixture was refluxed for approximately 18–22 hrs. TLC was used to monitor the reactions and use (Benzene: Methanol) (6:4). After the end of the escalation period, part of the solvent was evaporated, where it was observed that a precipitate was formed. The precipitate was filtered using a Buechner funnel and washed withdistilled water, then left to dry, and then re-washed with chloroform. Physical properties for 1,3- Thiazolidine-4-one Derivatives are given in table 2:

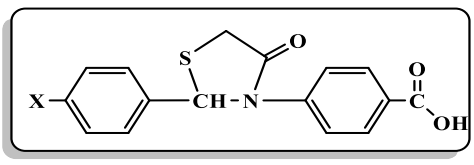


Table (2): The Molecular formula melting point and R_f of compounds (B_1-B_5)

Comp. Symb.	- X	M.P. °C	Rf	M.F.	M.Wt.	Yield%	reflux time(hr)	Colour
B1	-H	245-247	0.70	$C_{16}H_{13}NO_3S$	299.34	70	22	Yellow
B2	-Cl	229-231	0.78	$C_{16}H_{12}ClNO_3S$	333.79	76	20	light Orange

B3	**-Br**	**212-214**	**0.80**	**$C_{16}H_{12}BrNO_3S$**	**378.24**	**72**	**20**	**Copper**
B4	**-NO_2**	**236-238**	**0.75**	**$C_{16}H_{12}N_2O_5S$**	**344.34**	**65**	**22**	**Copper**
B5	**-OCH_3**	**223-225**	**0.83**	**$C_{17}H_{15}NO_4S$**	**329.37**	**80**	**18**	**Yellow**

2.1.4. General procedure for Synthesis Acid Chlorides (C_1-C_8)

The working method was adopted at the source [17] with some modifications in a two-neck round-bottom flask of 100 ml capacity equipped with a condenser and a distillation funnel placed immersed in an oil containing 0.01 mole of carboxylic acid. A drop by drop (0.018 mol/ml) of thionyl chloride $SOCl_2$ was added through the distillation funnel. the mixture refluxed at 75°C for approximately 3–4 hours the excess thionyl chloride was distilled, off and the product was collected. Table (3): physical constants of the prepared acid chlorides.

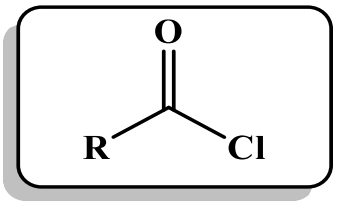
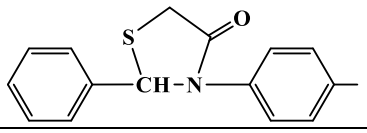
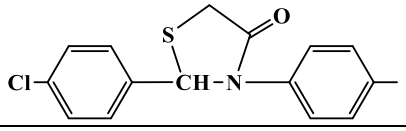
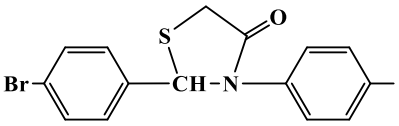
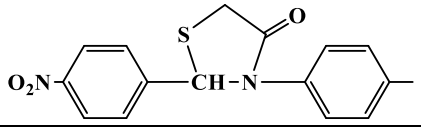
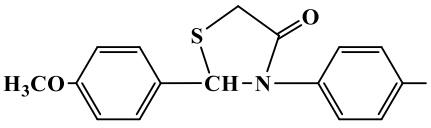
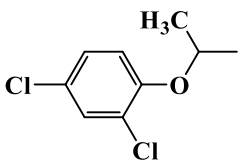
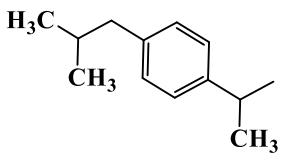
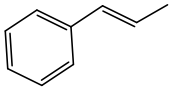


Table (3): The Molecular formula, physical constants of compounds (C_1-C_8).

Comp. Symb.	-R	M.F.	M.Wt.	Yield %	Time (hr.)
C_1		**$C_{16}H_{12}ClNO_2S$**	**317.79**	**88**	**3**
C_2		**$C_{16}H_{11}Cl_2NO_2S$**	**352.23**	**84**	**2.5**
C_3		**$C_{16}H_{11}BrClNO_2S$**	**396.68**	**86**	**3**
C_4		**$C_{16}H_{11}ClN_2O_4S$**	**362.78**	**90**	**2**
C_5		**$C_{17}H_{14}ClNO_3S$**	**347.81**	**92**	**2.5**
C_6		**$C_9H_7Cl_3O_2$**	**251.95**	**88**	**3**

C_7		**$C_{13}H_{17}ClO$**	**224.73**	**90**	**2**
C_8		**C_9H_7ClO**	**166.60**	**93**	**2**

2.1.5. General procedure for Synthesis L-proline amide derivatives (D_1-D_8)

The working method was adopted at the source [18] with some modifications where 0.02 mole, 2.3 gm of L-proline was dissolved in 25 ml of pyridine with stirring for ten minutes in a two-neck round-bottom flask of 100 ml capacity equipped with a condenser and a distillation funnel placed immersed in an ice bath. Then 0.02 mol of carboxylic acid chloride was added through the distillationfunnel drop by drop, and after the completion of mixing the reaction components, turbidity in the mixture was observed immediately after the addition. After the end of the stirring period, acidified water [(Water :HCl) (95: 5)] was added to the reaction mixture, then 100 ml of chloroform was added in the separating funnel, the chloroform layer containing the product was separated , The chloroform layer was washed with a solution (5%) of acidified water, and then with distilled water . The chloroform layer was dried with magnesium sulfate anhydrous. The reaction mixture was left to evaporate where a precipitate was observed. The precipitate was collected and then left to dry. Physical properties for L-proline amide derivatives are given in table 4:

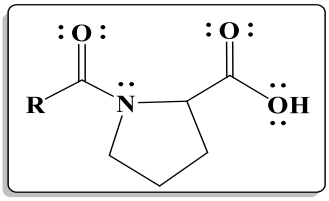
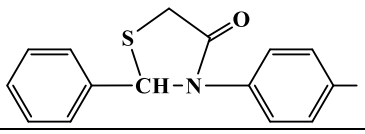
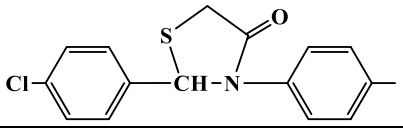
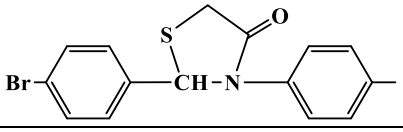
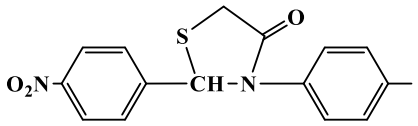
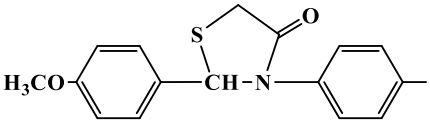
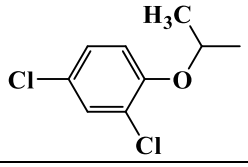
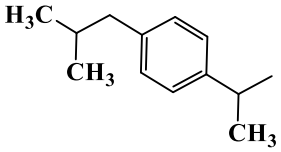
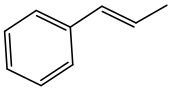


Table (4): The Molecular formula, melting point and R_f of compounds (D_1-D_8)

Comp. Symb.	-R	M.P. °C	R_f	M.F.	M.Wt.	Yield %	Colour
D_1		**115-117**	**0.73**	**$C_{21}H_{20}N_2O_4S$**	**396.46**	**65**	**Light Brown**
D_2		**123-125**	**0.80**	**$C_{21}H_{19}ClN_2O_4S$**	**430.90**	**68**	**Copper**
D_3		**128-130**	**0.78**	**$C_{21}H_{19}BrN_2O_4S$**	**475.36**	**63**	**Brown**

D_4		**131-133**	**0.82**	**$C_{21}H_{19}N_3O_6S$**	**441.46**	**60**	**Brown**
D_5		**118-120**	**0.75**	**$C_{22}H_{22}N_2O_5S$**	**426.49**	**65**	**Copper**
D_6		**65-68**	**0.85**	**$C_{14}H_{15}Cl_2NO_4$**	**332.18**	**70**	**Brown**
D_7		**132-134**	**0.88**	**$C_{18}H_{25}NO_3$**	**303.40**	**65**	**Light yellow**
D_8		**164-167**	**0.85**	**$C_{14}H_{15}NO_3$**	**245.28**	**73**	**Off white**

2.2.Biological activity

2.2.1 Assay for ACE-inhibitory activity in vitro.

An ACE inhibition test was used to evaluate the newly synthesized compound's (D_8) in vitro ACE inhibitory effectiveness. (E0927Hu, Bt-laboratory, Sandwich). An Enzyme-Linked Immunosorbent Assay is included in this kit (ELISA). A human ACE antibody has been pre-coated on the plate. The ACE in the sample is introduced, and it binds to the antibodies that have been coated on the wells. The biotinylated human ACE Antibody is then added to the sample and binds to the ACE. The biotinylated ACE antibody is then bound by Streptavidin-HRP. During a washing phase after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance with the quantity of human ACE present. The process is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm. The experiments were carried for the designed compound (D8), for ACE-inhibitory activity at five different concentrations (10^{-2}-10^{-6}).

2.2.2. Molecular Docking Study.

To learn more about how ACE interacts with the most potent compound, It was hooked to ACE, using Schrödinger Maestro version 12.5.139, MMshare 5.1.139 were utilized to conduct a molecular docking study[19] .The 3D crystal structures of ACE (PDB ID:: 1O86) were acquired from the PDB(protein data bank) (www.rcsb.org). The ligand structures were generated using ChemBioDraw Ultra (v16.0) [20].

3.Results and Discussion

3.1. Synthesis and characterization of Schiff's base (A_1-A_5)

Schiff's bases were synthesized by reaction of benzaldehyde or one of its derivatives and 4-amino benzoic acid, FT IR spectra for compounds (A1-A5) revealed a broad band within the range 2526-3341cm^{-1} which is attributable to the OH group assigned to the carboxylic group , strong absorption

within the range 3066-3073cm^{-1} attributable to the Aromatic (C-H). absorption band within the range 1674-1681cm^{-1} which is attributable to the (C=O) group carboxylic acid. absorption within the range 1622-1636cm^{-1} attributable to the azomethine group (C=N) [21]. In addition to the appearance of stretching absorption of the other groups data are given in table (5).

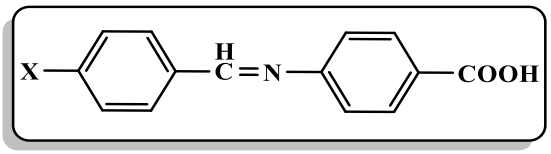


Table (5): IR characteristic absorption of A_1-A_5 cm^{-1}

Comp. Symb.	-X	vO-H	vC-H		vC=O Carboxy.	v C=N	v C=C ring		Others
			Arom.	Aliph.					
A_1	-H	2553-3290	3069	2981	1674	1629	1589	1512	.....
A_2	-Cl	2558-3298	3074	2986	1679	1634	1594	1517	C-Cl at 830
A_3	-Br	2526-3304	3066	2975	1678	1622	1593	1503	C-Br at 705
A_4	-NO_2	2541-3341	3073	2986	1677	1631	1588	1513	C-$NO_{2\ Asym.}$ at 1513 C-$NO_{2\ sym.}$ at 1341
A_5	-OCH_3	2560-3297	3072	2988-2850	1681	1636	1596	1519	ether C-O at 1114δ CH_3 1327

3.2. Synthesis and characterization of 1,3- Thiazolidine-4-one Derivatives(B_1-B_5)

1,3- Thiazolidine-4-one Derivatives were synthesized by reactions of Schiff base and thioglycolic acid using Dioxane as a solvent, FT IR spectra for compounds (B_1-B_5) showed the absence of (vC=N) absorption band for azomethine group. FT-IR spectra for compounds revealed an absorption within the range3058-3130cm^{-1} attributable to the aromatic (C-H), strong absorption band within the range1655-1685cm^{-1} attributable to the(vC=O)carboxylic acid and lactam group [21]. In addition to the appearance of stretching absorption of the other groups data are given in table 6.

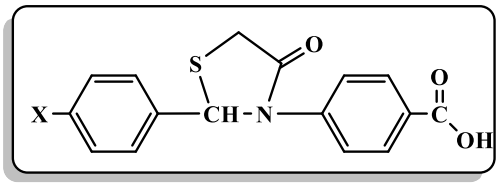
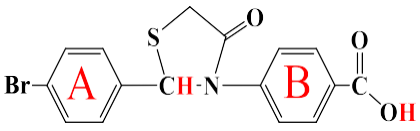
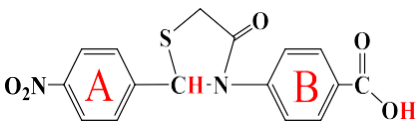


Table (6): IR characteristic absorption of compounds B_1-B_5 cm^{-1}

Comp. Symb.	-X	vO-H	vC-H Arom.	vC-H Asym.	vC-H Sym.	vC=O lactam	vC=O Carboxy.	v C=C ring		v C-S
B_1	-H	2545-3221	3058	2978	2901	1678	1678	1596	1413	692
B_2	-Cl	2551-3221	3130	2979	2902	1685	1685	1590	1487	684
B_3	-Br	2549-3263	3059	2980	2906	1694	1655	1579	1485	683
B_4	$-NO_2$	2542-3259	3068	2973	2985	1683	1683	1594	1515	693
B_5	$-OCH_3$	2554-3268	3064	2926	2911	1699	1660	1584	1490	688

Further identification for compounds (B_3-B_4)was performed using ^{1}H-NMR, spectra of compounds were comprised of a single signal within the range [12.67-12.91(s, 1H)]ppm which ascribed to the carboxylic acid proton, a several different signals within the range [δ 3.99 – 4.04 (s, 2H)] attributed to the protons -CH_2-Heteroring. The^{1}H-NMR data and spectra for compounds B_3-$B_4$are shown in table 7[22].

Table (7): ^{1}H-NMR spectra for compounds B_3-B_4

Comp. Symb.	Structure	Chemical Shift(ppm)	No. of Protons	Type of single	Group
B_3		3.99	2	s	-CH_2-Hetero
		6.52	1	s	-N-CH-Ar.
		7.51-7.53	2	d	Aro. Protons
		7.78-7.80	2	d	
		7.94-7.96	2	d	
		8.62-8.64	2	d	
		12.67	1	s	-OH Carbox.
B_4		4.04	2	s	-CH_2-Hetero
		6.47	1	s	-N-CH-Ar.
		7.56-7.57	2	d	Aro. Protons
		7.78-7.80	2	d	
		8.22-8.24	2	d	
		8.80-8.82	2	d	
		12.91	1	s	-OH Carbox.

3.3. Synthesis and characterization of Acid Chlorides (C_1-C_8)

Acid Chlorides were synthesized by reaction of carboxylic acids derivatives and thionyl chloride ($SOCl_2$), FT IR spectra for compounds (C_1-C_8) showed the absence of OH group stretching vibration bands for carboxylic acids. FT-IR spectra for compounds (C_1-C_8) revealed a sharp band within the range 1778-1785cm^{-1} which is attributable to the (C=O) group acid chlorides, strong absorption within the range 3052-3114cm^{-1} attributable to the Aromatic (C-H) [21]. In addition to theappearance of stretching absorption of the other groups data are given in table (8).

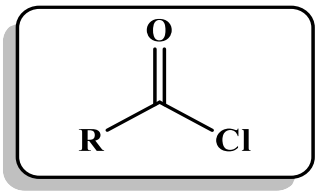
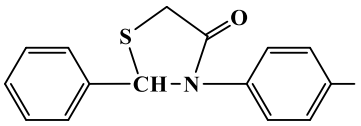
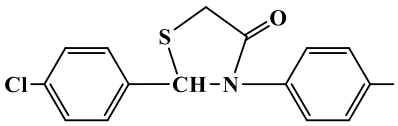
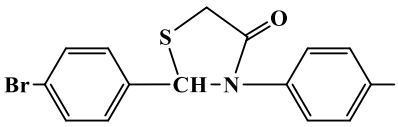
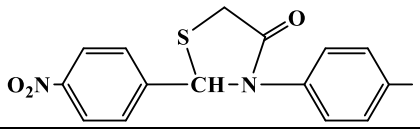
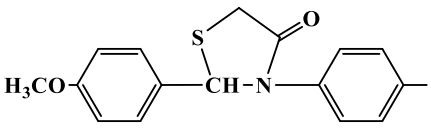
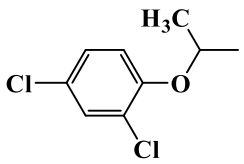
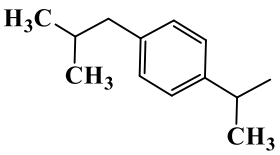
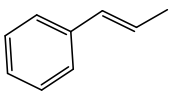


Table (8): IR characteristic absorption of C_1-C_8 cm^{-1}.

Comp. Symb.	-R	νC-H arom.	ν C-H Aliph.		νC=O	ν C=O lactam	νCO-Cl
			Asm.	Sym.			
C_1		3052	2972	2904	1778	1685	690
C_2		3085	2975	2905	1785	1688	705
C_3		3054	2978	2902	1780	1660	692
C_4		3074	2985	2909	1797	1687	695
C_5		3114	2964	2911	1782	1671	712
C_6		3082	2986	2903	1778	—	648
C_7		3021	2956	2870	1781	—	633
C_8		3023	—	—	1780	—	686

3.4. **Synthesis and characterization of L-proline amide derivatives (D_1-D_8)**

L-proline amide derivatives were synthesized by reactions of Acid chlorides compounds and proline acid using pyridine as a solvent, FT IR spectra for compounds (D_1-D_8) showed the absence of NH group stretching vibration bands for proline and (νC=O) group absorption band for acid chloride. FT- IR spectra for compounds revealed an absorption within the range 2974-3094cm^{-1} attributable tothe aromatic (C-H), strong absorption band within the range 1711-1750cm^{-1} attributable to the (νC=O) carboxylic acid group, and absorption within the range 1640-1688 cm^{-1} for the (νC=O) group of amide[23]. In addition to the appearance of stretching absorption of the other groups data are given in table 9 and (Fig.1).

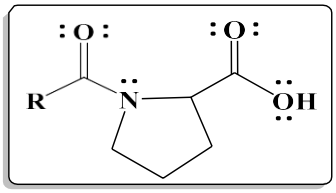
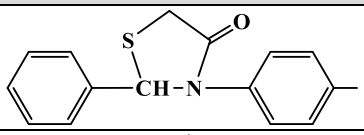
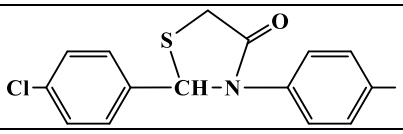
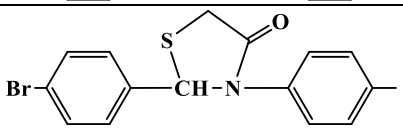
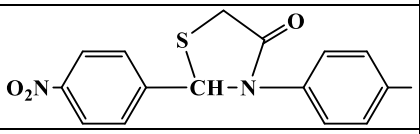
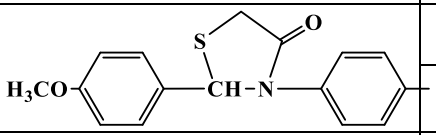
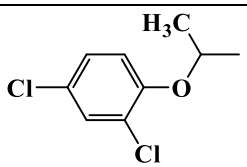
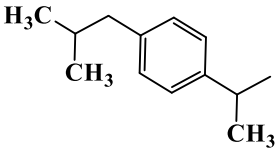
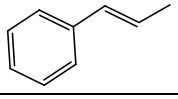


Table (9): IR characteristic absorption of compounds D_1-D_8 cm^{-1}

Comp. Symb.	-R	ν O-H	νC-H arom.	ν C-H Aliph.		νC=O carbox.	νC=O lactam &amid
				Asm.	Sym.		
D_1		**3350** **2578**	**3035**	**2987**	**2906**	**1698**	**1688**
D_2		**3351** **2571**	**3040**	**2989**	**2902**	**1706**	**1697**
D_3		**3320** **2626**	**3043**	**2968**	**2900**	**1722**	**1662**
D_4		**3289** **2443**	**3063**	**2960**	**2894**	**1692**	**1692**
D_5		**3372** **2530**	**3082**	**2984**	**2916**	**1710**	**1695**
D_6		**3383** **2570**	**3098**	**2997**	**2907**	**1754**	**1644**
D_7		**3303** **2521**	**3081**	**2958**	**2916**	**1726**	**1642**
D_8		**3270** **2499**	**3058**	**2958**	**2877**	**1726**	**1643**

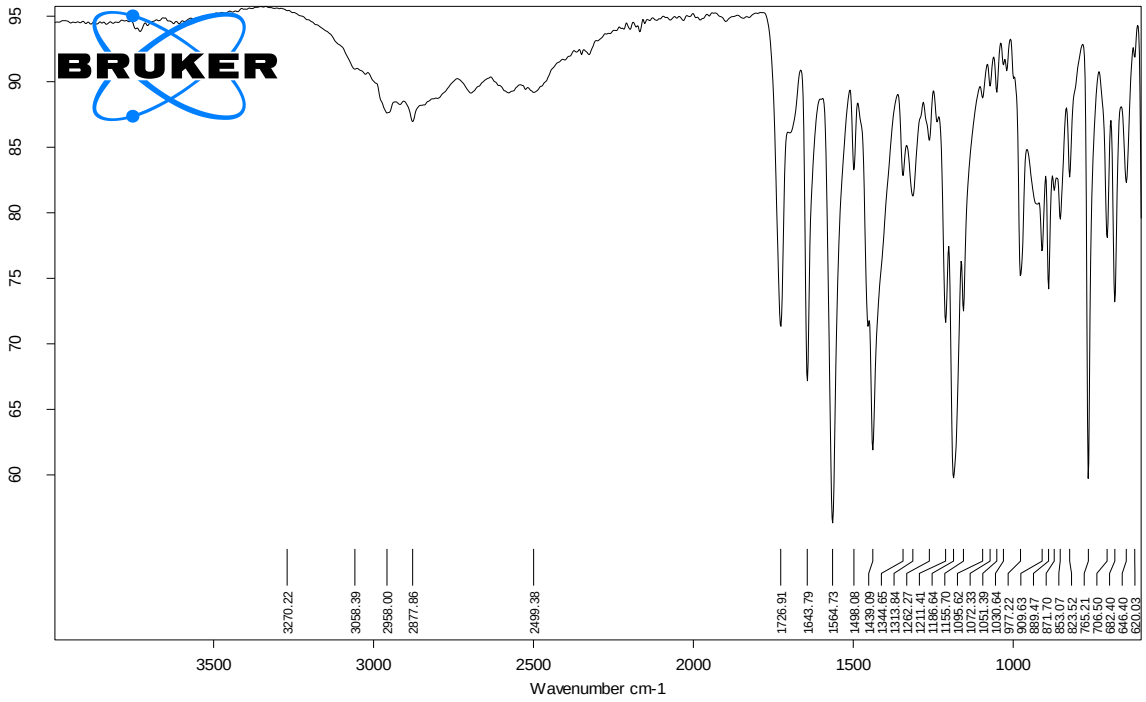
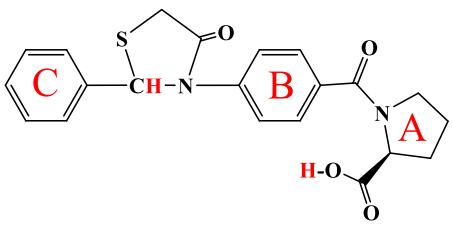
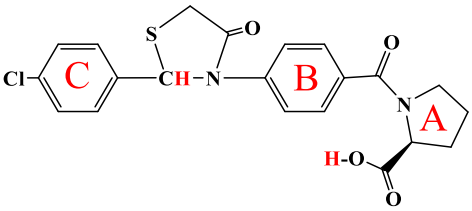
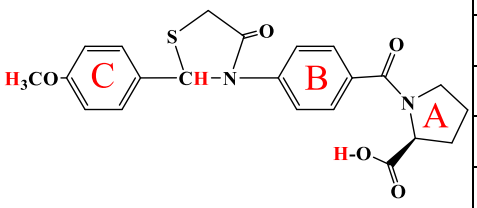


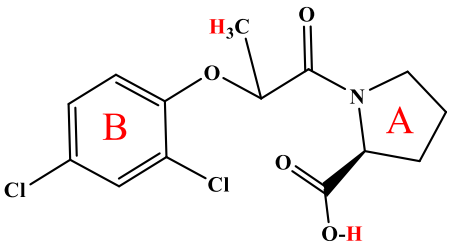
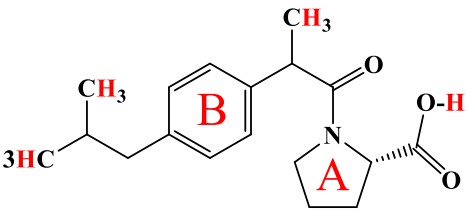
Figure 1: FT-IR spectrum of compound D_8

Further identification for compounds (D_1-D_2,D_5-D_8) was performed using ^{1}H-NMR, spectra of compounds were comprised of a single signal within the range 12.30-12.68(s, 1H) ppm which ascribed to the carboxylic acid proton, a several different signals within the range [δ 1.83 – 4.46 (m, 7H)] attributed to the protons ring five members of proline .The ^{1}H-NMR data and spectra for compounds B_1-$B_5$are shown in table 10 and (Fig.2) as a representative illustration[22].

Table (10): ^{1}H-NMR spectra for compounds D_1-D_8

Comp. Symb.	Structure	Chemical Shift(ppm)	No. of Protons	Type of single	Group
D_1		1.86-1.90	2	m	Aliph. Protons[A]. proline
		2.04-2.08	2	m	
		3.56-3.57	2	t	
		4.44-4.46	1	t	
		4.02	2	s	-C<u>H</u>$_2$- Hetero
		6.50	1	s	-N-C<u>H</u>-Ar.
		7.48-7.51	5	m	Aro. Protons [B,C]
		7.66-7.68	2	d	
		8.02-8.04	2	d	
		12.49	1	s	-O<u>H</u> Carbox.

D2		**1.83-1.87**	**2**	**m**	**Aliph. Protons[A]. proline**
		2.03-2.07	**2**	**m**	
		3.54-3.56	**2**	**t**	
		4.36-4.38	**1**	**t**	
		4.07	**2**	**s**	**-CH2- Hetero**
		6.63	**1**	**s**	**-N-CH-Ar.**
		7.15-7.16	**2**	**d**	**Aro. Protons [B,C]**
		7.37-7.38	**2**	**d**	
		7.59-7.60	**2**	**d**	
		8.22-8.24	**2**	**d**	
		12.68	**1**	**s**	**-OH Carbox.**
D5		**1.85-1.89**	**2**	**m**	**Aliph. Protons[A]. proline**
		2.03-2.07	**2**	**m**	
		3.45-3.47	**2**	**t**	
		4.25-4.27	**1**	**t**	
		3.71	**3**	**s**	**Aro.-O-CH3**
		3.95	**2**	**s**	**-CH2- Hetero**
		6.55	**1**	**s**	**-N-CH-Ar.**
		6.99-7.01	**2**	**d**	**Aro. Protons [B,C]**
		7.63-7.65	**2**	**d**	
		7.73-7.74	**2**	**d**	
		8.03-8.04	**2**	**d**	
		12.46	**1**	**s**	**-OH Carbox.**

D6		**1.42-1.43**	**3**	**d**	**-CH3**
		1.89-1.93	**2**	**m**	**Aliph. Protons[A]. proline**
		2.13-2.16	**2**	**m**	
		3.43-3.45	**2**	**t**	
		4.27-4.29	**1**	**t**	
		5.24-5.28	**1**	**m**	**-CH-**
		6.98-6.99	**1**	**d**	**Aro. Protons[B]**
		7.25-7.27	**1**	**d**	
		7.50	**1**	**s**	
		12.31	**1**	**s**	**-OH Carbox.**
D7		**0.80-0.81**	**6**	**d**	**2(-CH3)**
		1.36-1.37	**3**	**d**	**-CH3**
		1.80-1.87	**1**	**m**	**-CH-**
		2.35-2.37	**2**	**d**	**-CH2-**
		3.67-3.72	**1**	**m**	**-CH-**
		1.92-1.96	**2**	**m**	**Aliph. Protons[A]. proline**
		2.01-2.05	**2**	**m**	
		3.46-3.48	**2**	**t**	
		4.19-4.20	**1**	**t**	

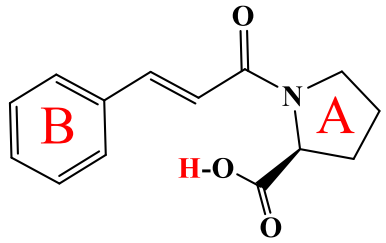
		6.99-7.01	2	d	Aro. Protons[B]
		7.23-7.24	2	d	
		12.30	1	s	-OH Carbox.
D_8		1.85-1.89	2	m	Aliph. Protons[A]. proline
		2.00-2.04	2	m	
		3.48-3.50	2	t	
		4.40-4.42	1	t	
		6.93-6.96	1	d	=CH-
		7.37-7.39	4	m	Aro. Protons[B]&
		7.57-7.58	2	d	Aro.-CH=
		12.49	1	s	-OH Carbox.

Figure 2: ^{1}H NMR spectrum of compound D_8

Compounds were further characterized using mass spectrometry to determine their molecular mass by determining the molecular ion and base Peake. Fig. (3) show the fragmentation pattern of the compounds (D_6-D_8) [24].

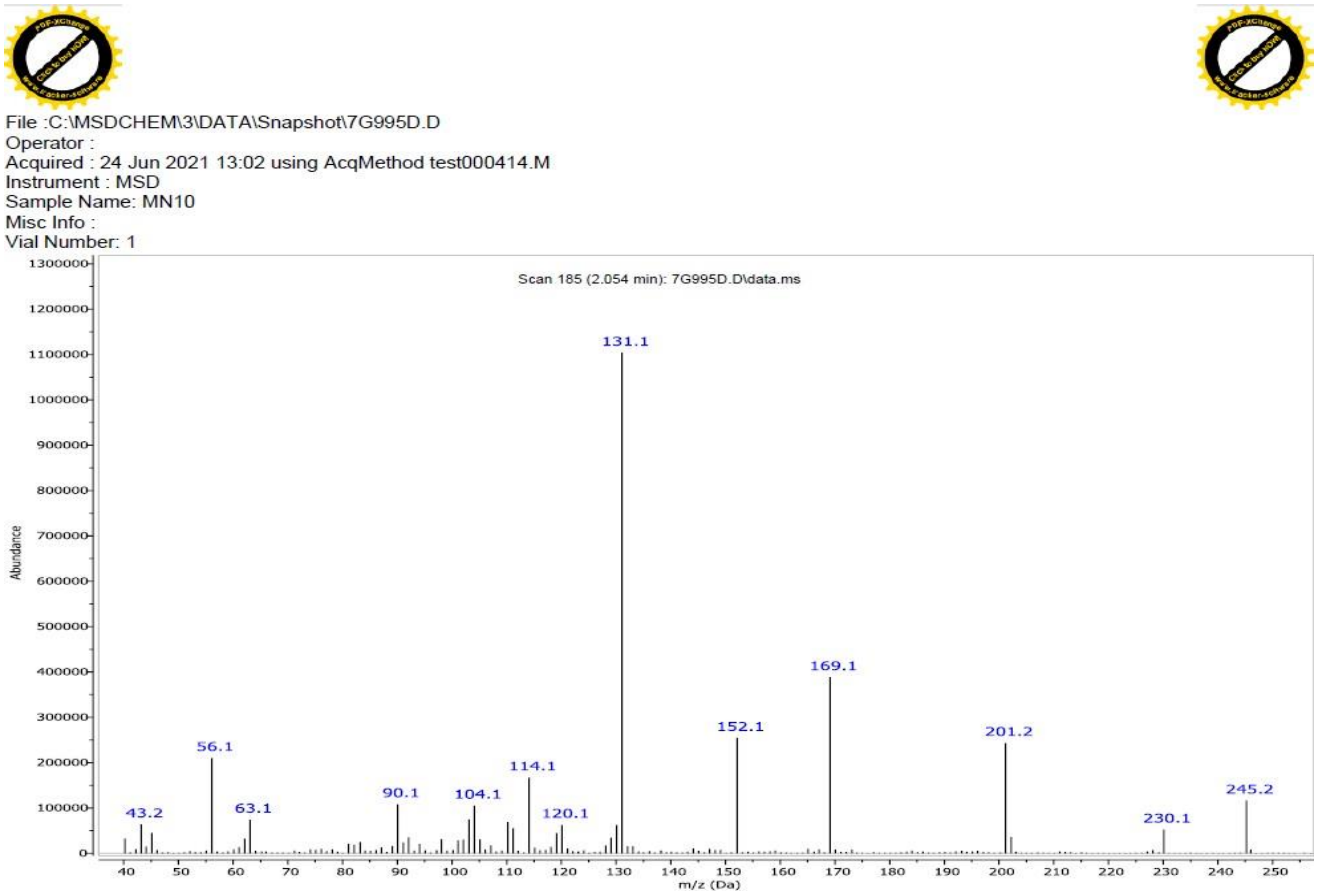
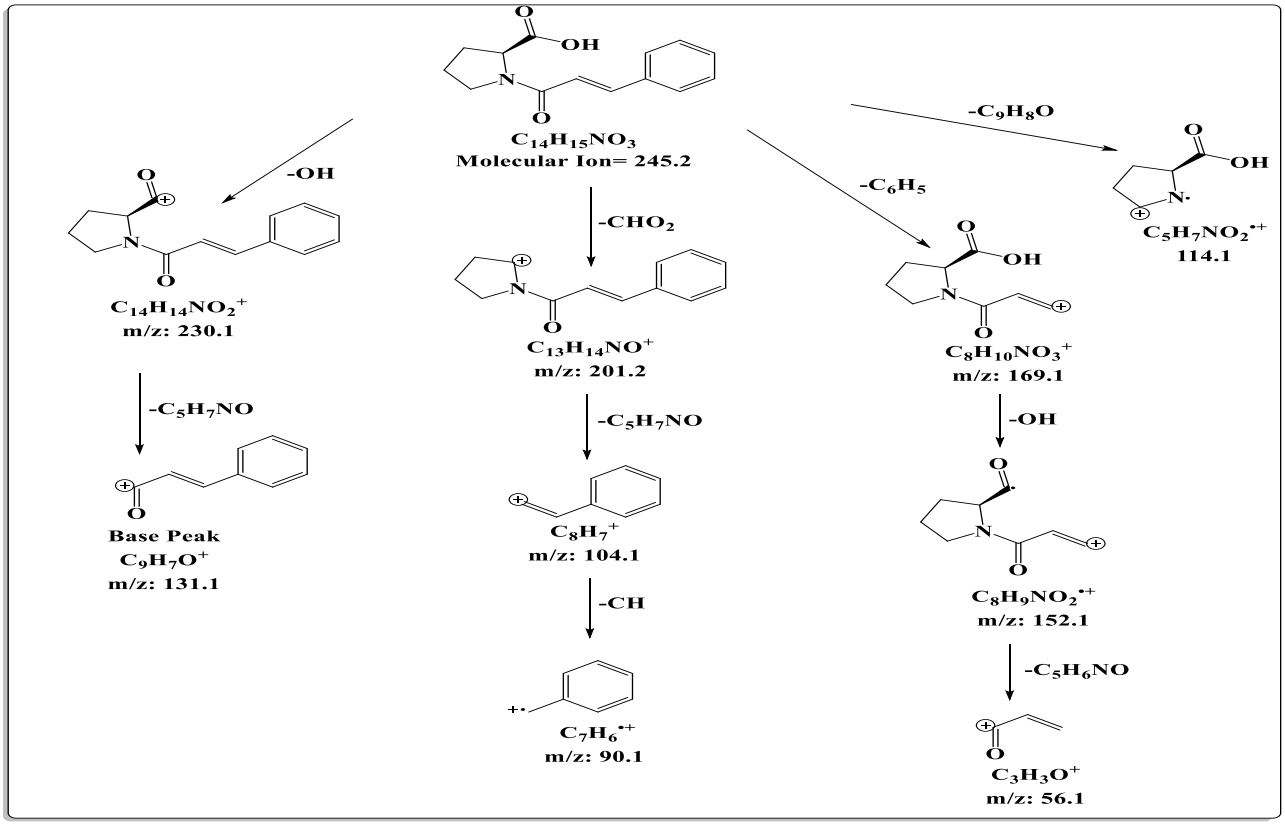


Figure 3: Mass spectrum of compound D_8



Scheme 1: fragmentation pattern of compound D_8

Table (11) shows the m / z values of the M + molecular ion and some of the generated fragments of the prepared compounds.

Table (11): The m / z values of the M + molecular ion and some of compounds D_6-D_8

m/z	Compounds Symb.		
	$M.N_6$	$M.N_9$	$M.N_{10}$
Molecular Ion	$C_{14}H_{15}Cl_2NO_4$	$C_{18}H_{25}NO_3$	$C_{14}H_{15}NO_3$
	332.1	303.1	245.2
Base Peak	$C_6H_5^+$	$C_{13}H_{19}^+$	$C_9H_7O^+$
	77.1	175.0	131.1
Fragments	$C_{14}H_{14}Cl_2NO_3^+$	$C_{18}H_{24}NO_2^+$	$C_{14}H_{14}NO_2^+$
	314.1	288.1	230.1
	$C_{13}H_{17}ClNO_4^+$	$C_{17}H_{24}NO^+$	$C_{13}H_{14}NO^+$
	286.1	260.0	201.2
	$C_{11}H_{11}C_{l2}NO_2^{\bullet+}$	$C_{13}H_{18}NO^{\bullet+}$	$C_8H_{10}NO_3^+$
	259.1	205.1	169.1
	$C_{10}H_9Cl_2O_2^+$	$C_{13}H_{17}O^+$	$C_8H_9NO_2^{\bullet+}$
	231.1	190.1	152.1
	$C_8H_{12}NO_4^+$	$C_{12}H_{17}^+$	$C_5H_7NO_2^{\bullet+}$
	186.1	162.1	114.1
	$C_6H_3Cl_2O^+$	$C_6H_8NO_3^+$	$C_8H_7^+$
	160.1	143.1	104.1
	$C_6H_8NO_3^+$	$C_5H_7NO_2^{\bullet+}$	$C_7H_6^{\bullet+}$
	142.2	114.1	90.1
	$C_5H_7NO_2^+$	$C_7H_7^+$	$C_3H_3O^+$
	113.1	92.1	56.1
	$C_6H_3O^{\bullet+}$	$C_4H_7N^{\bullet+}$	———
	91.1	70.1	———
	C_2H_4O+	$C_3H_7^+$	———
	45.1	44.1	———

The integration of all identification spectroscopic results (FT-IR, 1H-NMRM, Mass), gave a good indication to the structures assigned to these compounds.

3.5. Comparison angiotensin-converting enzyme activity level in serum

The effect of the prepared compound(D_8) on the activity of the angiotensin-converting enzyme was measured; by using ACE Elisa kit, and Table (12) shows the ACE activity level for the prepared compound and the control group.

Table (12): Effect of the prepared compounds (D_8) on the ACE.

Group	Mean± SD	P-Value
control	58.30 ± 9.08	0.0006
$D_8.(10^{-2})M$	13.36 ± 3.42	
control	58.30 ± 9.08	0.0005
$D_8.(10^{-3})M$	18.24 ± 1.63	
control	58.30 ± 9.08	0.0006
$D_8.(10^{-4})M$	16.07 ± 1.78	
control	58.30 ± 9.08	0.0005
$D_8.(10^{-5})M$	17.13 ± 1.52	
control	58.30 ± 9.08	0.0006
$D_8.(10^{-6})M$	15.64 ± 0.893	

Table 12 reveals that the action of the prepared compound (D_8) has resulted in a considerable drop in enzyme activity At concentrations (10^{-3},10^{-4},10^{-5},10^{-6} M), the effectiveness values were (18.24 ± 1.63),(16.07 ± 1.78), (17.13 ± 1.52), and (15.64 ± 0.893) U/L, respectively, compared to the control group (58.30±9.08) U/L. At the level of probability, the produced drug (D_8) with a concentration (10^{-2}M) gave the maximum inhibition (13.36 ± 3.42) U/L compared to the control group (58.30±9.08) U/L. (0.0006) [32]. Table (13) shows the percentages of inhibition with different concentrations of the prepared compound (D_8):

Table (13): The percentages of inhibition with different concentrations of the prepared compound (D_8).

Comp. Symb.	Conce.(M)	Inhibition %
D_8	10^{-2}	77.08
	10^{-3}	68.71
	10^{-4}	72.43
	10^{-5}	70.61
	10^{-6}	73.17

The percentage of ACE inhibition by the different concentrations of the prepared compound D_8 canbe shown in Figure3

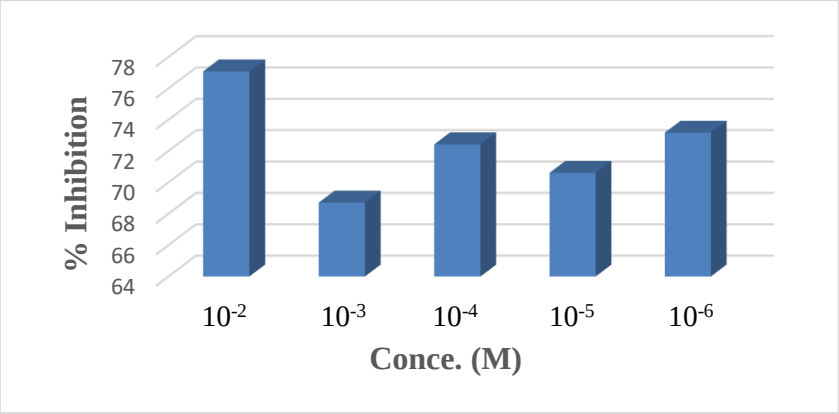


Figure 4: percentage of inhibition with different concentrations of the prepared compound D_8

3.6 Comparison of the concentration effect between the prepared compound

Differences were found between the effect of different concentrations of the prepared compound (D_8) on ACE activity, and Table 11 shows the differences through the mean and standard deviation values.

Table (11): The differences between the different concentrations of the prepared compound (D_8) on the activity of the angiotensin converting enzyme.

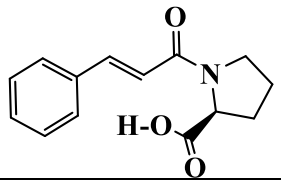
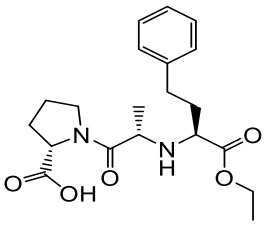
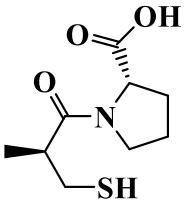
Comp. Symb.	Conce.(M)	Mean± SD
D_8	10^{-2}	13.360 ^{d} ± 3.42
	10^{-3}	18.239 ^{a} ± 1.6
	10^{-4}	16.074 ^{bc} ± 1.777
	10^{-5}	17.130 ^{ab} ±1.518
	10^{-6}	15.641 ^{c} ± 0.893

It can be seen from the table above that there are significant differences in enzyme activity as a result of the effect of different concentrations of the prepared compound (D_8), with a significant difference in enzyme activity with the effect of (D_8) at the concentration (10^{-2}M) and with enzymatic activity (13.360 ± 3.42) U /L compared to concentrations (10^{-3},10^{-4}, 10^{-5},10^{-6} M) and with enzymatic activity (18.239 ± 1.6), (16.074 ± 1.777), (17.130 ±1.518), (15.641 ± 0.893) U /L on Respectively [25].

3.7. Results of molecular docking studies

The molecular docking studies of the organic derivatives of the amino acid (L-proline) gave Information about the value of the docking score of the prepared derivative compared to thecommonly used hypertensive drugs (Enalapril, Captopril). The derivative (D_8) gave a docking score equal to (-7.134Kcal/mole) in comparison with the docking score of hypertensive drugs (Enalapril and Captopril) which equaled (-6.038 Kcal/mole and -5.544) respectively. It is noted that the prepared derivative has a higher docking score than the pressure drugs that were used for comparison, and the reason for this is that the derivative (D_8) has the bonds (Hydrogen Bond, and Salt bridge), While the drugs that were used to compare having the following forces: Enalapril contains three bonds (Hydrogen Bond) and (Coordination bond), As for the drug Captopril, it has (Hydrogen Bond, Coordination bond and Salt bridge)[26], Table 12 shows the information obtained from the molecular docking studies of the prepared derivative (D_8) in comparison with the hypertensive drugs (Enalapril and Captopril).

Table (12): values of the docking score of the derivative (D_8) in comparison with the hypertensive drugs.

Comp. Symb.	Structure	RMSD	Docking Score Kcal/mole
D_8		**0.043**	**-7.134**
Enalapril		**0.048**	**-6.038**
Captopril		**0.044**	**-5.544**

The molecular docking studies of the prepared derivative (D_8) and hypertensive drugs (Enalapril and Captopril) that work as angiotensin converting enzyme inhibitors, which were used to compare its effectiveness with the prepared derivative, revealed different types of bonds with amino acid residues found in the active site of the angiotensin-converting enzyme (ACE). The study found that the (D_8) interacts with amino acid residues through Tow types of bonds: a hydrogen bond with a hydrogen atom at ALA356 in the enzyme's active site, a lone pair of oxygen atoms for the amide carbonyl group in the (D_8) with a distance of 2.04 Å, and it was observed that there is a salt bridge linking the negative charge on the oxygen atom of the carboxyl group of (D_8) with Zn701 ions which are located in the active site of the enzyme and at a distance of 1.98 Å, while the study gave the interactions that occur between the hypertensive drugs and the amino acid residues present in the active site of the enzyme. Enalapril showed the presence of three Hydrogen bonds, the first hydrogen bond linking the lone pair of the oxygen atom of amide carbonyl in the amino acid residue GLU384 and the hydrogen atom bonded to the amide nitrogen atom in (D_8) at a distance of 2.10 Å, the second hydrogen bond between the amino acid residues HIE513 and the lone pair of the oxygen atom of the amide group, with a distance of 2.23 Å, and third hydrogen bond, links the amino acid residue TYR520 and the lone pair belonging to the oxygen atom of the carbonyl carboxyl group, witha distance of 1.87 Å. It also showed the presence of a coordination bond between Zn 701 ions and the lone pair of the oxygen atom of the carbonyl ester group with a distance of 2.00 Å, As for the drug Captopril, it showed the presence of interactions between amino acid residues with (D_8), a one hydrogen bond between amino acid residue ALA356 and the lone pair of the oxygen atom ofcarbonyl amide with a distance of 1.93 Å, the salt bridge between the negative charge on the oxygen atom of the carboxyl group with the Zn 701 ions located in the active site of the enzyme with a distance of 2.15 Å, and also showed the presence of a coordination bond linking the Zn 701 ions with the lone pair of the oxygen atom of the carboxyl group,

at a distance of 2.18 Å[27]. Table 13 shows the number and types of bonds and their binding sites for the organic derivative (D_8) preparedin comparison with the pressure drugs (Enalapril and Captopril).

Table (13): The number and types of bonding sites of the derivative (D_8) in comparison with hypertensive drugs.

Comp. Symb.	Interactions	Distance Å	Bonding	Bonding Types	Binding site of target	Binding site of ligand
D_8	A:ALA356:H7156-:MN11:O4	2.04	Hydrogen bond	Conventional Hydrogen Bond	A:ALA356:H7156	:MN10:O4
	A:Zn701:4690-:MN10:O18	1.98	Salt bridg	Salt Bridge, Attractive Charge	A:Zn701:4690	:MN10:O18
Enalopril (EN)	A:GLU384:OE2-:EN:H9262	2.10	Hydrogen bond	Conventional Hydrogen Bond	A:GLU384:OE2	:EN:H9262
	A:HIE513:HE2-:EN:O9235	2.23	Hydrogen bond	Conventional Hydrogen Bond	A:HIE513:HE2	:EN:O9235
	A:TYR520:HH-:EN:O9237	1.87	Hydrogen bond	Conventional Hydrogen Bond	A:TYR520:HH	:EN:O9237
	A:Zn701:4658-:EN:O9239	2.00	Coordination bond	Metal coordination	A:Zn701:4658	:EN:O9239
Captopril (CA)	A:ALA356:H7124-:EN:O9236	1.93	Hydrogen bond	Conventional Hydrogen Bond	A:ALA356:H7124	:EN:O9236
	A:Zn701:4658-:CA:O9237	2.15	Salt bridg	Salt Bridge, Attractive Charge	A A:Zn701:4658	:CA:O9237
	A:Zn701:4658-:CA:O9238	2.18	Coordination bond	Metal coordination	A A:Zn701:4658	:CA:O9238

And 3D, 2D images were obtained for (D_8) and the hypertensive drugs(Enalapril and Captopril) as shownin the figures below:

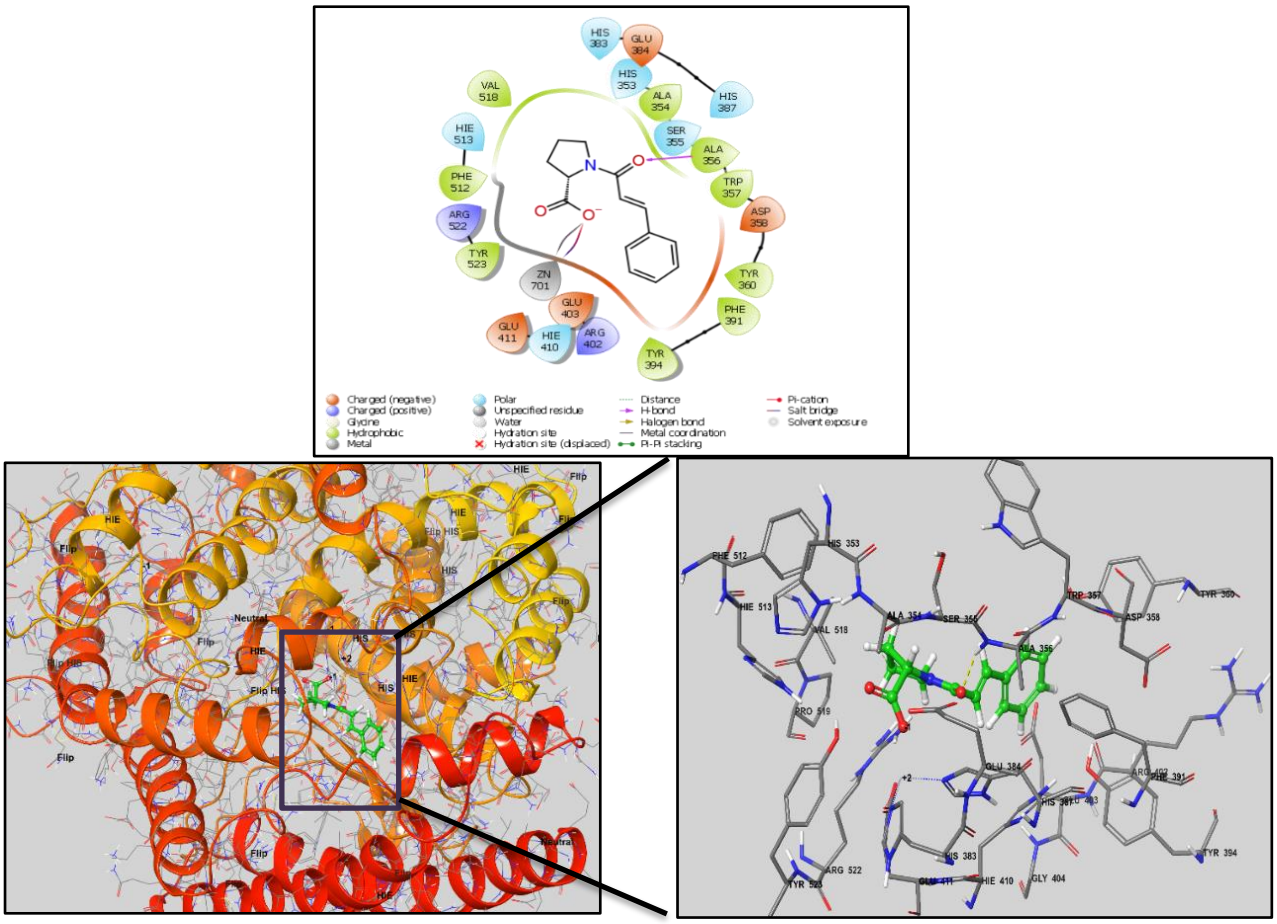


Figure 5: 2D,3D Interaction between D_8 and ACE.

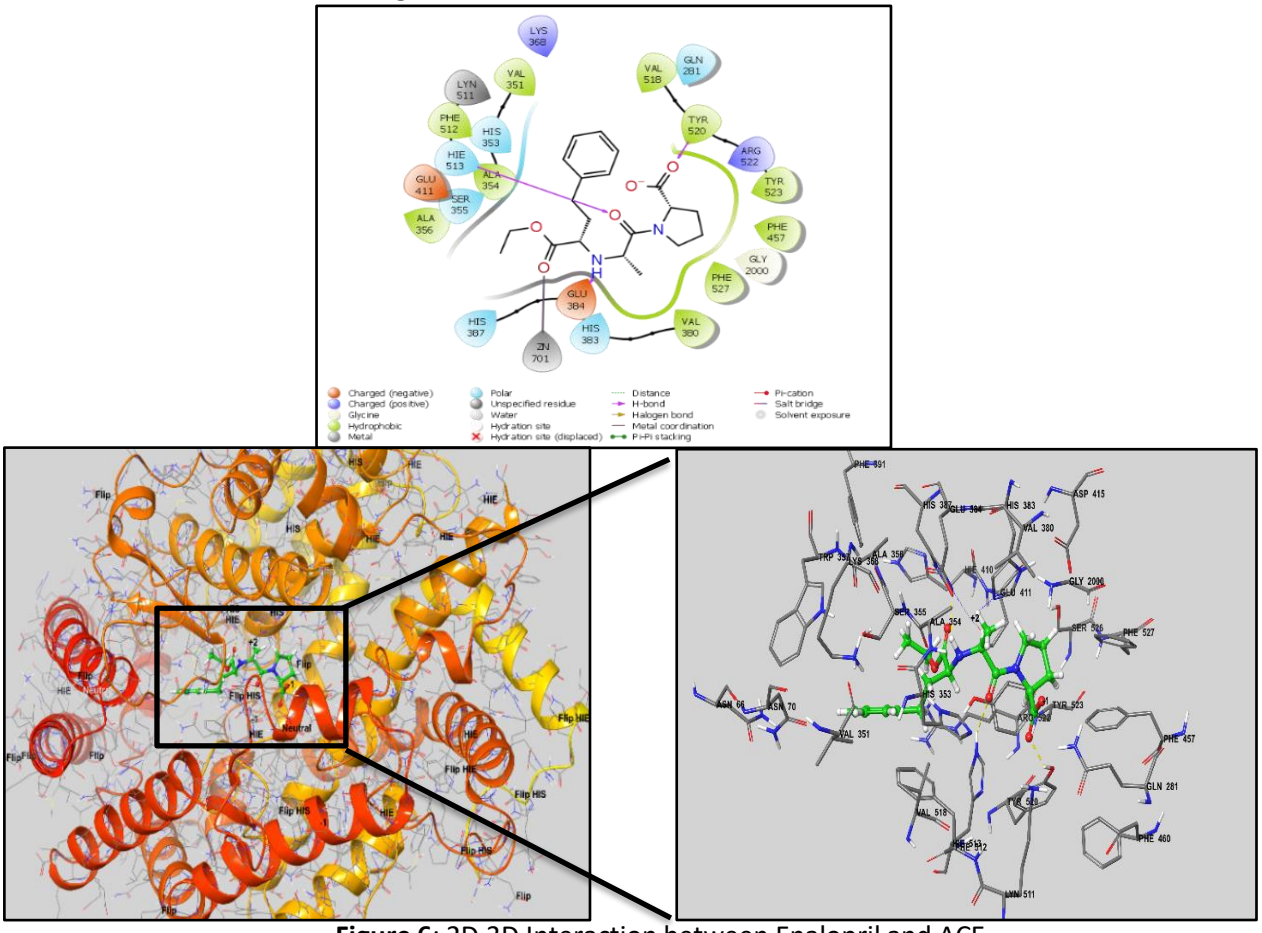


Figure 6: 2D,3D Interaction between Enalopril and ACE.

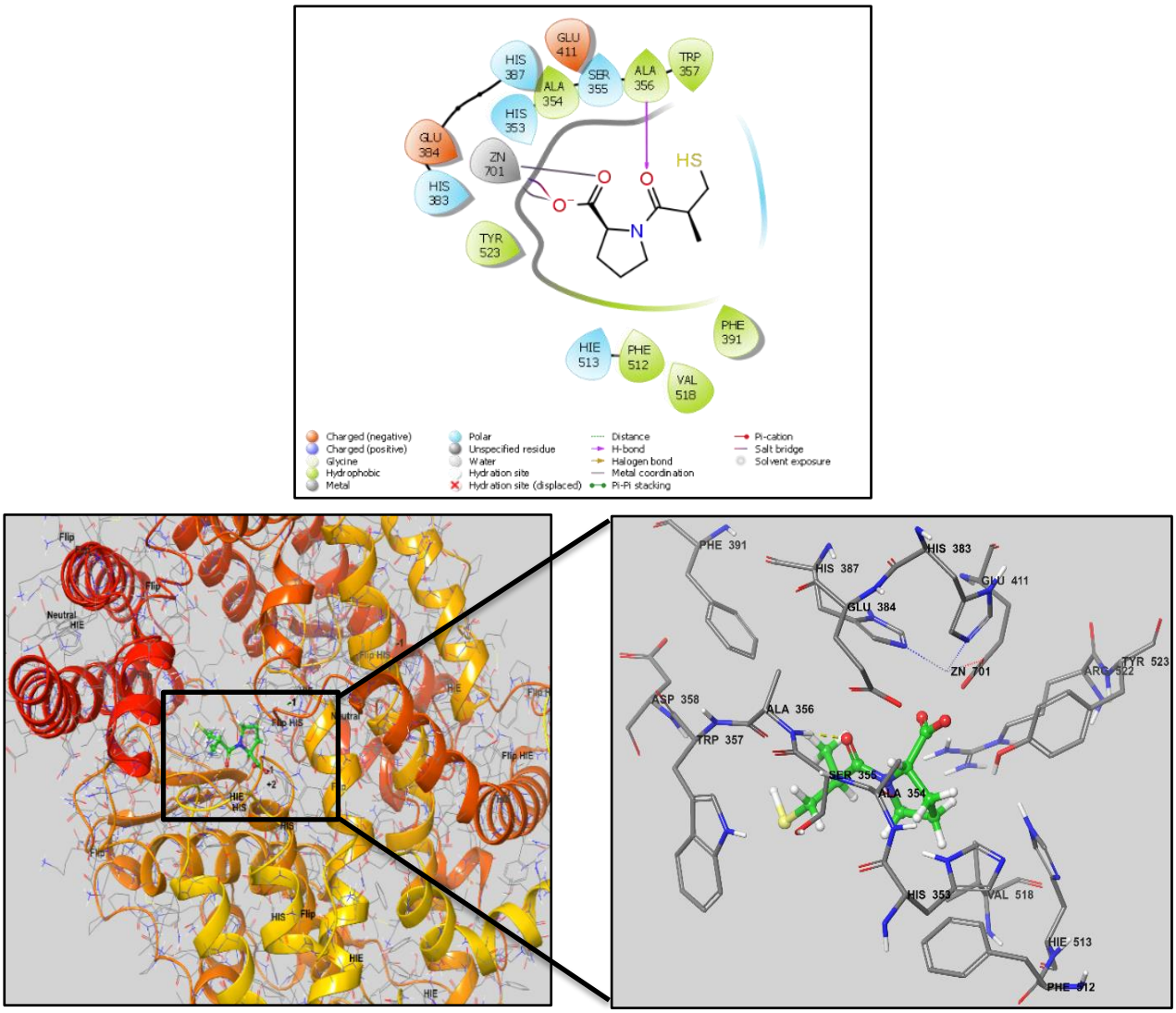


Figure 7: 2D,3D Interaction between Captopril and ACE.

The results of the molecular docking studies of other organic derivatives of an amino acid (L-proline) (D_1-D_4,D_6-D_7) were obtained, and table 12 shows the values of the docking score, The root-mean-square deviation, types of bonds, and the binding sites between the (D_8) and amino acid residues in the active site of the ACE enzyme.

Table (12) : values of the docking score and the number and type of bonds for the prepared derivatives (D_1-D_4,D_6-D_7).

Comp. Symb.	RMSD	Docking Score	Hydrogen Bond	Halogen Bond	Salt bridg	Metal coordination	pi-pi stacking
D_1	0.039	-6.728	ARG522	——	Zn701	Zn701	——
D_2	0.043	-6.882	ALA356 ASP358	——	Zn701	Zn701	——
D_3	0.042	-6.658	ALA356 ASP358	——	Zn701	Zn701	——
D_4	0.039	-6.857	ALA356 ASP358	——	Zn701 ARG124	Zn701	——
D_6	0.041	-6.389	ALA356	ASN66 ASP358	Zn701	Zn701	TRP357
D_7	0.048	-6.553	ALA356	——	Zn701	Zn701 Zn701	TYR523

Also, these interactions that occur between the prepared organic derivatives and the amino acid residues present in the active site of the angiotensin-converting enzyme were clarified by means of 3D and 2D images, as shown in the figures below:

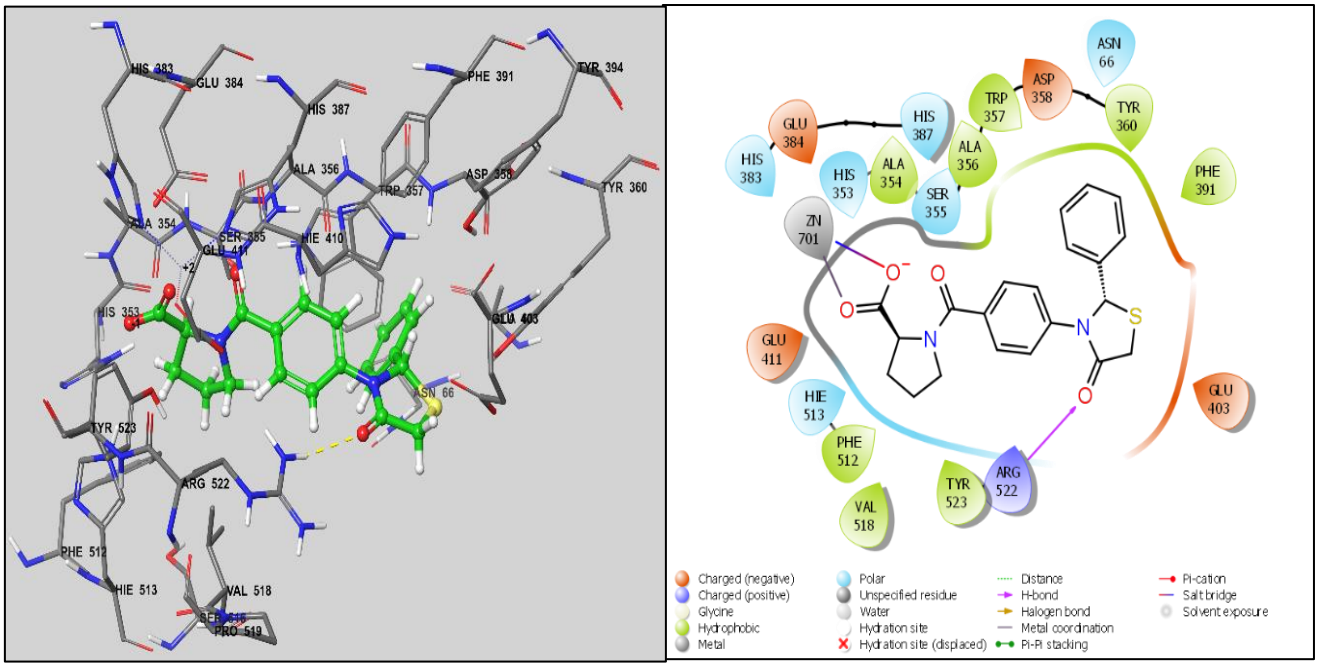


Figure 8: 2D,3D Interaction between $D_1$and ACE.

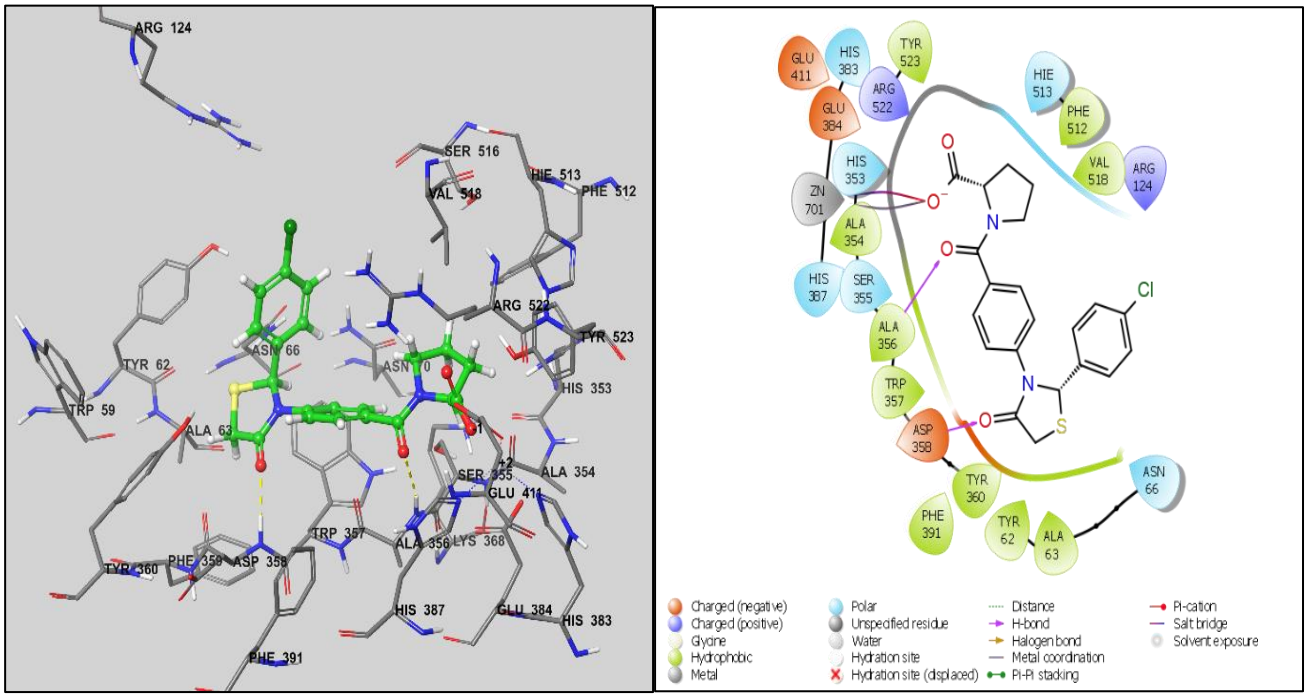


Figure 9: 2D,3D Interaction between $D_2$and ACE.

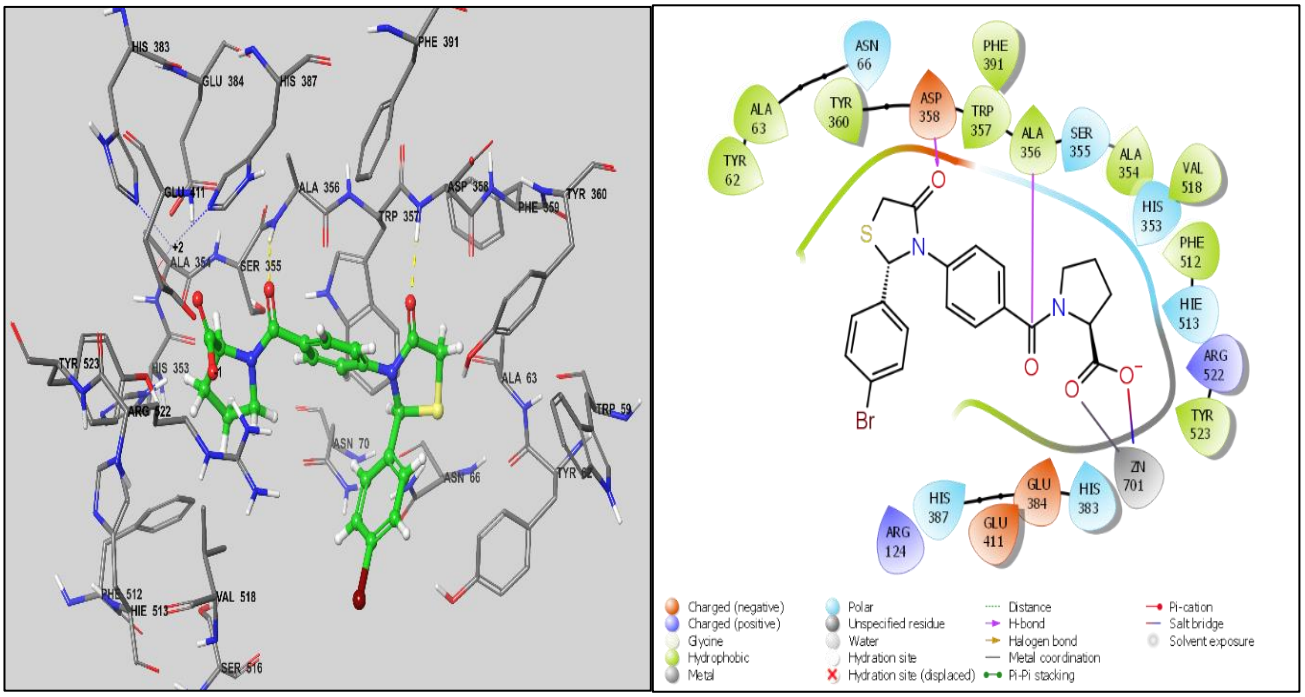


Figure 10: 2D,3D Interaction between $D_3$and ACE.

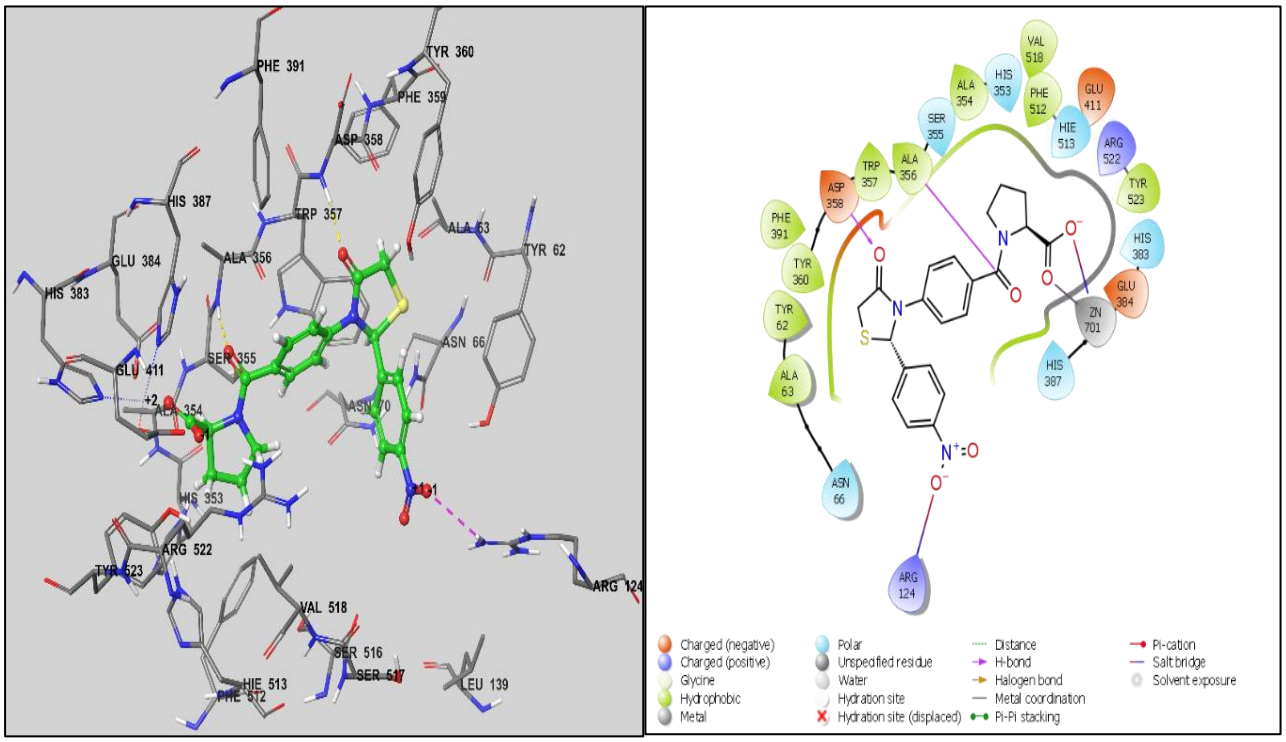


Figure 11: 2D,3D Interaction between $D_4$and ACE.

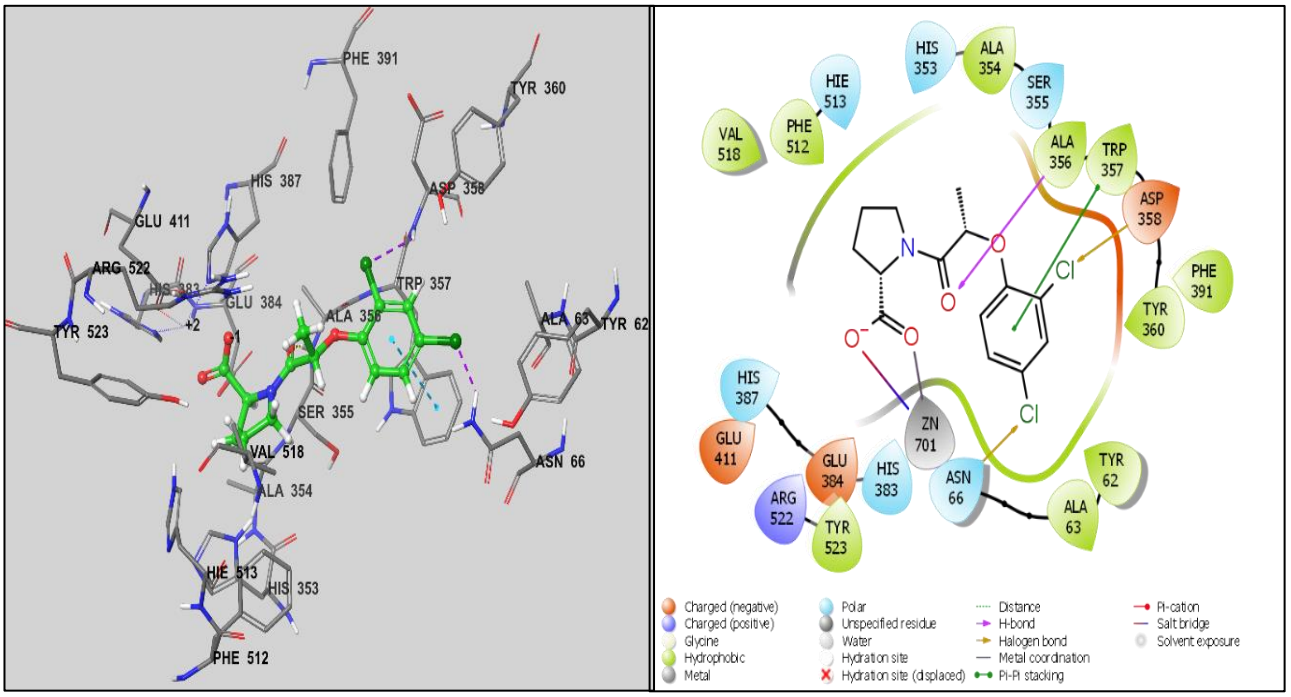


Figure 12: 2D,3D Interaction between $D_6$and ACE.

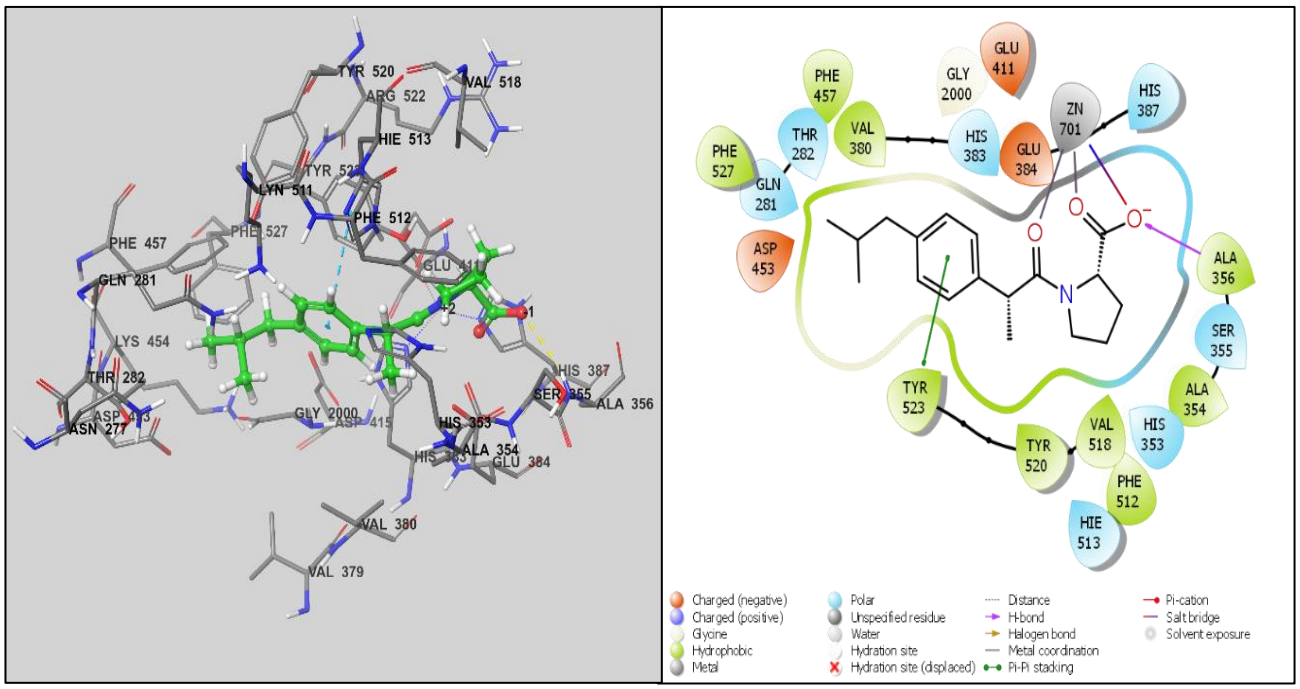


Figure 13: 2D,3D Interaction between $D_7$and ACE

3. Conclusions

In conclusion, we developed an efficient and shortcut method for drug discovery based on both combinatorial chemistry and pharmaceutical design philosophy. We designed eight novel organic derivatives of the amino acid that were produced and tested in vitro as possible ACE inhibitors with fewer side effects. The ACE activity was reduced in vitro by the amino acid chemical derivative D2. The organic derivative is most likely to serve as a prodrug with just a minor anti-ACE effect. Based onthese early and encouraging results, further relevant modifications of the organic derivatives of Proline will be developed. This novel class of drugs, together with its complete antihypertensive activities, will be useful in future medicine development.

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