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Design and Synthesis of Peptide Derivatives Act as DNA Binding Agent and Discovery of Potent Carbonic Anhydrase Inhibitors Using Docking Studies

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NEW peptide series of 2-(2-oxo-2H benzo[h] chromen-4-yl) acetyl derivatives 3-5, 15-20 and their corresponding methyl esters 6-14 were synthesized. Structures of the synthesized products were characterized by correct elemental analysis, IR, Mass and ^{1}H-NMR spectroscopy. DNA binding activities were performed. Some synthesized compounds showed high binding affinity against DNA. Virtual screening using molecular docking studies of the synthesized compounds were performed, the molecular docking results indicate that, most of the synthesized compounds are more suitable inhibitors against carbonic anhydrase isozyme (CA) than reference drug.

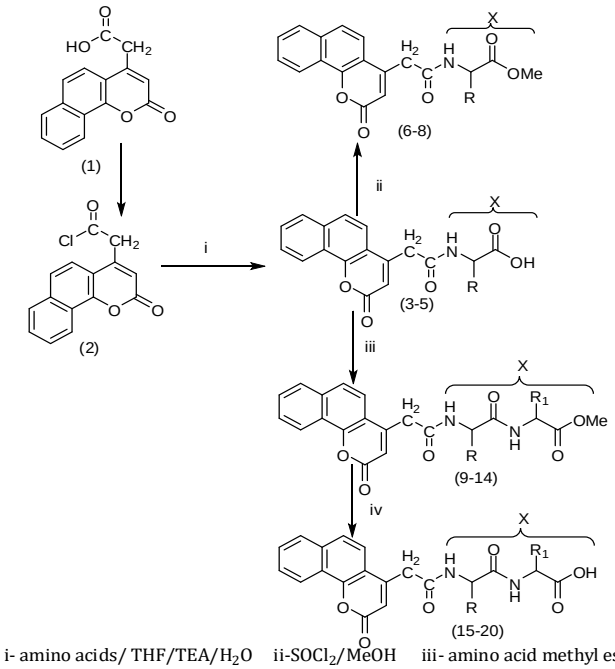
Study of conformational changes of DNA (deoxyribonucleic acid) by ligand binding are a pharmacologic target of many drugs [1-5]. The structural changes of DNA based on the interaction of small molecular weight ligands with DNA have attracted attention in the medicinal design of anticancer and anti-AIDS drugs [6,7]. Some chromen-2-one (coumarine) derivatives have been reported to possess anti-SARS agent[8], antioxidant properties[9], anti-fungal[10], anti-bacterial[11], anti-inflammatory[12], antitumor[13], inhibitors of pancreatic cholesterol[14], estrogen receptor [15] and treatment of Alzheimer diseases[16]. In addition, many drugs consist of amino acid moieties as; AG7088 (which was developed by Pfizer and used for the treatment of rhinovirus, which can cause the common cold), which contain phenylalanine moiety [17] .

There are a variety of mechanisms for the anticancer activity and the most famous mechanism is through the inhibition of carbonic anhydrase isozymes [18-22]. In brief, the CA is a family of metalloenzymes involved in the catalysis of an important physiological reaction: the hydration of CO_2 to bicarbonate and a proton(CO_2+H_2O HCO^{-}_3 $+H^{+}$), where bicarbonate is necessary to synthesis of nucleotides and other cell components such as membrane lipids. The synthesized compounds may decrease supplying of bicarbonate. Based on the foregoing, the present study undertakes synthesis of peptide series and their methyl esters of 2-(2-oxo-2H-benzo[h]-chromen-4-yl)acetyl derivatives, with hope to obtain new candidate(s) as antitumor agents .

Result and Discussion

Chemistry

The formation of dipeptides 3-5 was achieved by the reaction of 2-(2-oxo-2H-benzo[h]chromen-4-yl) acetyl chloride 2 with different type of amino acids (Scheme 1). Also, 3-5 were reacted with thionyl chloride (molar ratio) in methanol to give the corresponding dipeptide methyl esters 6-8 (Scheme 1). Tripeptide methyl esters 9-14 were prepared by the reaction compounds 3-5 with different amino acid methyl esters in presence of tetrahydrofuran and few drops of triethyl amine using carbodiimide technique[18] (Scheme 1). When compounds 14-19 were treated with sodium hydroxide (0.01N) was converted into corresponding tripeptides 15-20 . The results of chemical analyses of the synthesized compounds were summarized in Tables 1-3



R	R_1
3) $CH(CH_3)_2$	
4) $(CH_2)_2SCH_3$	
5) $CH_2C_6H_5$	
6) $CH(CH_3)_2$	
7) $(CH_2)_2SCH_3$	
8) $CH_2C_6H_5$	
9) $CH(CH_3)_2$	$CH(CH_3)_2$
10) $CH(CH_3)_2$	$CH_2C_6H_5$
11) $(CH_2)_2SCH_3$	$CH(CH_3)_2$
12) $(CH_2)_2SCH_3$	$CH_2C_6H_5$
13) $CH_2C_6H_5$	$CH(CH_3)_2$
14) $CH_2C_6H_5$	$CH_2C_6H_5$
15) $CH(CH_3)_2$	$CH(CH_3)_2$
16) $CH(CH_3)_2$	$CH_2C_6H_5$
17) $(CH_2)_2SCH_3$	$CH(CH_3)_2$
18) $(CH_2)_2SCH_3$	$CH_2C_6H_5$
19) $CH_2C_6H_5$	$CH(CH_3)_2$
20) $CH_2C_6H_5$	$CH_2C_6H_5$

i- amino acids/ THF/TEA/H_2O ii-$SOCl_2$/MeOH iii- amino acid methyl esters DCC/THF/ TEA iv- NaOH

Scheme 1

Biological Activity

DNA as an affinity probe for evaluation of biologically active compounds

DNA binding assay

The mechanism of several antitumor compounds and antitumor antibiotics depends on their interaction with DNA. In this work, the antitumor activities of the newly synthesized compounds were determined using DNA binding assay and methyl green. DNA displacement assay[23,24]. In this method, a fixed amount of the ligand is spotted on the RP-18 TLC plates, followed by addition of known amount of

DNA on the same spot. The plate was then developed and the position of unbound DNA was determined by spraying the plates with anisaldehyde reagent. The free DNA was detected as a blue spot (Rf, MeOH–H_2O, 8:2) on RP-18 TLC. It was demonstrated that, when DNA was mixed with compounds known to interact with it, *e.g.* ethidium bromide, the complex was retained at the origin. Compounds with high binding affinity to DNA remained on the base line or migrated for a very short distance, while compounds with poor binding affinity did not cause DNA to be retained at the origin [23].

TABLE 1. Physical data for the compounds (3-20).

Compd.	X	Yield %	M.P. °C	Color	$[\alpha]_D^{20}$	R_F	Molecular Formula (M.Wt.)	Elemental Analysis Calculated/Found		
								C	*H*	*N*
3	L-Val	76	182-84	yellow	+77	0.53	$C_{20}H_{19}NO_5$ (353)	67.98 67.98	5.42 5.40	3.96 3.92
4	L-Met	82	175-77	yellow	+86	0.80	$C_{20}H_{19}NO_5S$ (385)	62.32 62.31	4.97 4.98	3.63 3.61
5	L-Phe	75	191-93	yellow	+119	0.87	$C_{24}H_{19}NO_5$ (401)	71.81 71.80	4.77 4.77	3.49 3.50
6	L-Val -OMe	69	200-202	brown	+80	0.86	$C_{21}H_{21}NO_5$ (367)	68.65 68.63	5.76 5.74	3.81 3.82
7	L-Met -OMe	65	210-12	Red	+98	0.89	$C_{21}H_{21}NO_5S$ (399)	63.14 63.15	5.30 5.30	3.51 3.50
8	L-Phe -OMe	78	193-95	Red	+116	0.60	$C_{25}H_{21}NO_5$ (415)	72.28 72.28	5.10 5.10	3.37 3.35
9	L-Val-L-Val-OMe	86	166-68	Red	+134	0.68	$C_{26}H_{30}N_2O_6$ (466)	66.94 66.91	6.48 6.45	6.00 6.01
10	L-Val-L-Phe-OMe	80	207-209	Red	+128	0.64	$C_{30}H_{30}N_2O_6$ (514)	70.02 70.00	5.88 5.88	5.44 5.46
11	L-Met-L-Val-OMe	75	179-81	Red	+120	0.69	$C_{26}H_{30}N_2O_6S$ (498)	62.63 62.63	6.06 6.03	5.62 5.63
12	L-Met-L-Phe-OMe	78	206-208	Red	+109	0.65	$C_{30}H_{30}N_2O_6S$ (546)	65.92 65.90	5.53 5.52	5.12 5.13
13	L-Phe-L-Val-OMe	80	197-99	Red	+103	0.67	$C_{30}H_{30}N_2O_6$ (514)	70.02 70.00	5.88 5.88	5.44 5.44
14	L-phe-L-Phe-OMe	75	210-12	orange	+85	0.79	$C_{34}H_{30}N_2O_6$ (562)	72.58 72.56	5.37 5.34	4.98 5.00
15	L-Val-L-Val	85	160-62	Red	+98	0.84	$C_{25}H_{28}N_2O_6$ (452)	66.36 66.34	6.19 6.22	6.19 6.20
16	L-Val-L-Phe	70	192-94	Red	+125	0.91	$C_{29}H_{28}N_2O_6$ (500)	69.59 69.60	5.60 5.62	5.60 5.60
17	L-Met-L-Val	78	216-18	Red	+142	0.82	$C_{25}H_{28}N_2O_6S$ (484)	61.97 61.97	5.78 5.80	5.78 5.78
18	L-Met-L-Phe	77	200-202	Red	+162	0.77	$C_{29}H_{28}N_2O_6S$ (532)	65.40 65.39	5.26 5.30	5.26 5.36
19	L-Phe-L-Val	70	209-11	Red	+112	0.94	$C_{29}H_{28}N_2O_6$ (500)	69.59 69.60	5.60 5.64	5.60 5.62
20	L-phe-L-phe	68	187-89	Red	+117	0.81	$C_{33}H_{28}N_2O_6$ (548)	72.25 72.22	5.11 5.14	5.11 5.12

Methyl green-DNA displacement assay

Methyl green reversibly binds polymerized DNA forming a stable complex at neutral pH [24]. The maximum absorption for the DNA-methyl green complex is 642-645 nm. This colorimetric assay was used to measure the displacement of methyl green with DNA by compounds, which have the ability to bind with DNA. The degree of displacement was determined spectrophotometrically by measuring the change in the initial absorbance of the DNA-methyl green solution in the presence of reference compound.

TABLE 2. IR and mass spectra of the synthesized products.

Compd.	IR (ν_{max} cm^{-1}) Selected bands	MS [M^+]
3	3400 (OH); 3331 (NH); 3090 (CH-aromatic), 2969,2931 (CH_3-valine); 1725,1733 (CO).	(353;22%)
4	3380 (OH); 3122 (NH); 3090 (CH-aromatic), 1716,1690 (CO).	(385;43%)
5	3410 (OH);3250 (NH); 3076 (CH-aromatic), 1718,1670 (CO).	(401;56%)
6	3200 (NH), 1720,1700 (CO).	(367;22%)
7	3229 (NH), 1726,1685 (CO).	(399;12%)
8	3233 (NH), 1733,1648 (CO).	(415 ;42%)
9	3300,3125 (NH); 1720-1678 (CO).	(466;82%)
10	3250,3131 (NH); 1725-1680 (CO).	(514;79%)
11	3173,3115 (NH); 1744-1637 (CO).	(498;40%)
12	3173,3115 (NH); 1748-1637 (CO).	(546;47%)
13	3185,3122 (NH); 1750-1699 (CO).	(514;24%)
14	3331,3285 (NH); 1714-1690 (CO)	(562;100%)
15	3342, 3331 (OH,NH); 1718-1690 (CO).	(453 ;84%)
16	3422 cm^{-1}(OH); 3332,3250 (NH) 1750-1702 (CO).	(500;64%)
17	3358,3116 (OH,NH); 1718-1696 (CO).	(484;57%)
18	3382 (OH); 3338,3241 (NH) 1718-1652 (CO).	(532;27%)
19	3414 (OH); 3356,3230 (NH) 1718-1672 (CO).	(500;5%)
20	3416 (OH); 3345,3210 (NH) 1706-1690 (CO).	(548;36%)

TABLE 3. ^{1}H NMR results for the synthesized compounds.

Comp.	^{1}HNMR δ
3	10.33 (s, 1H, OH-COOH, D_2O-exchangeable]), 8.13(d, 1H,NH, D2O-exchangeable]), 7.54-6.89 (m,6H, 2Ar-H), 6.65(s,1H, CH-hetero), 3.78(d, 1H, CH-CH–CH$(CH3)_2$), 3.01(s,2H,CH_2-CO), 2.94 (m, 1H,CH– CH$(CH3)_2$, 1.74(d, 6H,2$(CH_3)_2$).
5	10.63 (s, 1H, OH-carboxylic, D_2O-exchangeable), 8.52(d, 1H,NH, D_2O-exchangeable]), 8.0-7.1 (m,11H,3Ar-H), 6.52(s,1H, CH-hetero), 4.09(t, 1H,CH-CH_2), 2.96 (s, 2H, CH_2- CO), 2.82(d, 2H, CH_2-Ph).
7	8.37(d, 1H,NH, D_2O-exchangeable), 7.92-7.12 (m, 5H,2Ar-H), 6.32(s,1H, CH-hetero), 4.05(t,1H, CH-$(CH_2)_2$), 3.89 (s, 3H, CH_3-OCH_3), 3.01(s,2H, CH_2-CO), 2.69-2.63 (t, 2H, CH_2-SCH_3), 1.84-1.63(m,2H,CH_2-CH_2), 1.32 (s,3H, CH_3).
8	8.54(d, 1H,NH, D_2O-exchangeable), 7.53–6.86 (m, 11H,3Ar-H), 6.46(s,1H, CH-hetero), 4.05(t,1H, CH-$(CH_2)_2$),3.89 (s, 3H, CH_3-OCH_3), 2.95(s,2H, CH_2-CO)and other bands confirmed the structure.
10	8.30(d, 1H,NH, D_2O-exchangeable), 7.73–6.74 (m, 5H,2Ar-H), 6.54(s,1H, CH-hetero), 5.92 (s, 1H,NH, D_2O-exchangeable), 4.05(t,1H, CH-CH_2Ph),3.79 (d,1H, CH-CH$(CH_3)_2$), 3.42 (s, 3H, CH_3-OCH_3), 3.14 (d,2H-CH_2Ph); 2.83 (s,2H, CH_2-CO),2.31-3.12 (m, 1H, CH-CH$(CH_3)_2$), 1.43 (s,6H,2CH_3-$(CH_3)_2$).
11	8.37(d, 1H,NH, D_2O-exchangeable), 7.87–7.00 (m, 6H,2Ar-H), 6.67(s,1H, CH-hetero), 6.02 (s, 1H,NH, D_2O-exchangeable),3.77 (s, 3H, CH_3-OCH_3), 3.63(t,1H, CH-$(CH_2)_2SCH_3$), 3.24(d,1H, CH-CH$(CH_3)_2$), 2.91(s,2H, CH_2-CO), 2.62-2.55 (m, 1H, CH-CH$(CH_3)_2$),2.26-2.16(t,2H,CH_2-SCH_3), 1.91-1.74(m,2H,CH_2-CH_2SCH_3), 1.64(s,3H, CH_3-SCH_3), 1.34 (s,6H,2CH_3-$(CH_3)_2$).
13	8.55(d, 1H,NH, D_2O-exchangeable), 7.58–6.74 (m, 6H,2Ar-H), 6.47(s,1H, CH-hetero), 5.87 (s, 1H,NH, D_2O-exchangeable) 4.28(t,1H, CH-CH_2Ph),3.71 (s, 3H, CH_3-OCH_3), 3.44(t,1H, CH-CH_2Ph), 2.74(s,2H, CH_2-CO), 2.68(d, 1H, CH-Ph) and other bands confirmed the structure.
16	11.04(s,1H,OH-COOH, D_2O-exchangeable), 8.51(d, 1H,NH, D_2O-exchangeable), 7.71–6.98 (m, 6H,2Ar-H), 6.24 (s,1H, CH-hetero), 5.92 (s, 1H,NH, D_2O-exchangeable), 4.05(t,1H, CH-CH_2Ph),3.21(d,1H, CH-CH$(CH_3)_2$), 3.04(d,2H-CH_2Ph); 2.75(s,2H, CH_2-CO),2.31-2.10 (m, 1H, CH-$(CH_3)_2$), 1.43 (s,6H,2CH_3-$(CH_3)_2$).
18	10.94(s,1H,OH-COOH, D_2O-exchangeable), 8.37(s, 1H,NH, D_2O-exchangeable), 7.95–7.11 (m, 6H,2Ar-H), 6.74(s,1H, CH-hetero), 6.11 (s, 1H,NH, D_2O-exchangeable); 3.89 (t,1H, CH-CH_2Ph);3.57 (t,1H, CH-$(CH_2)_2SCH_3$), 3.10(d,2H, CH_2-Ph), 2.78(s,2H, CH_2-CO), 2.17(t,2H, CH_2-SCH_3),1.96-1.90 (m, 2H,CH_2-CH_2SCH_3); 1.51 (s,3H,CH_3-SCH_3).
19	11.03(s,1H,OH-COOH, D_2O-exchangeable), 8.30(d, 1H,NH, D_2O-exchangeable), 7.87–6.94 (m, 6H,2Ar-H), 6.65(s,1H, CH-hetero), 6.32(s, 1H,NH, D_2O-exchangeable); 3.97(t,1H, CH-CH_2Ph),3.49(d,1H, CH-CH$(CH_3)_2$), 3.17 (s, 3H, CH_3-OCH_3), 2.94-2.93(d,2H-CH_2Ph); 2.75(s,2H, CH_2-CO);1.98-1.69 (m, 1H, CH-CH$(CH_3)_2$), 1.57 (s,6H,2CH_3-$(CH_3)_2$).

Results from DNA binding assay (Table 4) revealed that, Compounds 4,7,9,13 and 16 showed the highest affinity for DNA, which was demonstrated by retaining the complex at the origin or by migrating for a very short distances, and by measuring IC_{50} (concentration required for 50% decrease in the initial absorbance of the DNA/methyl green solution). Compounds 6,10,11 and 17 showed moderate activity while compounds 8,18,19 and 20 showed weak activity. The remaining synthesized compounds showed the lowest affinity for DNA, which was demonstrated by migrating for a very long distance at the origin, represented graphically (Fig.1).

TABLE 4. DNA binding activity of the synthesized compounds 3-20 using methyl green DNA displacements assay.

NO.	IC_{50}(μg/ml.)*	IC_{50}(μM.)**	NO.	IC_{50}(μg/ml.)*	IC_{50}(μM.)**
3	23	65.15	**4**	20	51.94
5	ND	ND	**6**	21	55.58
7	19	47.62	**8**	82	197.59
9	18	38.62	**10**	37	71.99
11	41	82.33	**12**	ND	ND
13	12	23.35	**14**	ND	ND
15	ND	ND	**16**	15	30.00
17	39	75.88	**18**	57	107.14
19	68	136	**20**	45	82.11
Dau.	29	53.13			

ND) Not determined (Compounds having IC_{50} value > 100 μ/ml.).

*) IC_{50} Values: Represented IC_{50} obtained from three independent determinations required for 50% decrease in the initial absorbance of DNA-methyl green solution.

**) IC_{50} values: Concentration required for 50% decrease in the initial absorbance of DNA-methyl green solution.

Molecular modeling studies

To predict the anti-tumor behavior of the synthesized derivatives 3-20 on a structural basis, automated docking studies were carried out using Molegro virtual docker [25]. The scoring functions and hydrogen bonds formed with the surrounding amino acids, which are used to predict their binding modes at the active site of the human carbonic anhydrase II (hCA II). The scoring functions were calculated from minimized ligand protein complexes. The protein–ligand complex was constructed based on the X-ray structure (PDB entry 1G54) bounded with its inhibitor FFB-555 [26].

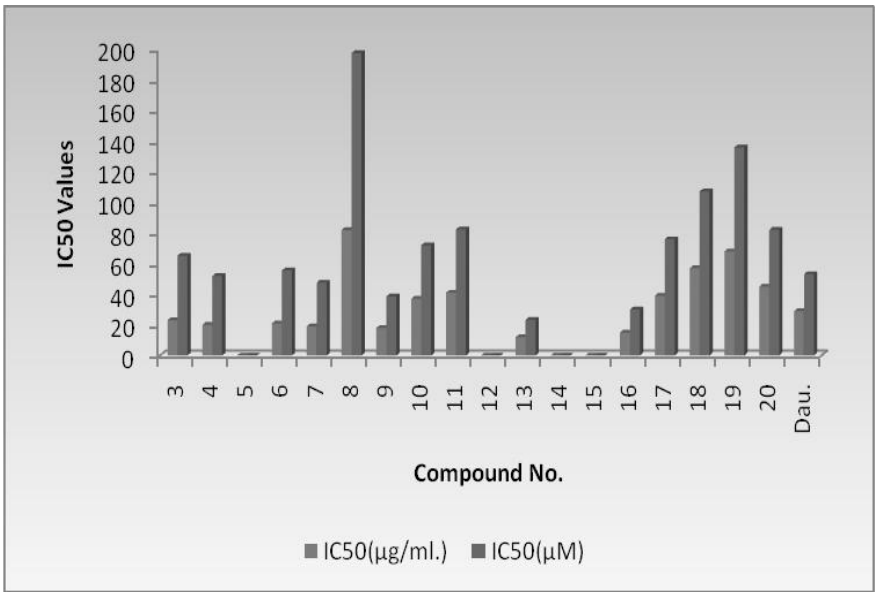


Fig.1. DNA binding activity of compounds (3-20) using methyl green DNA displacements assay as IC_{50} (concentration required for 50% decrease in the initial absorbance of the DNA/methyl green solution).

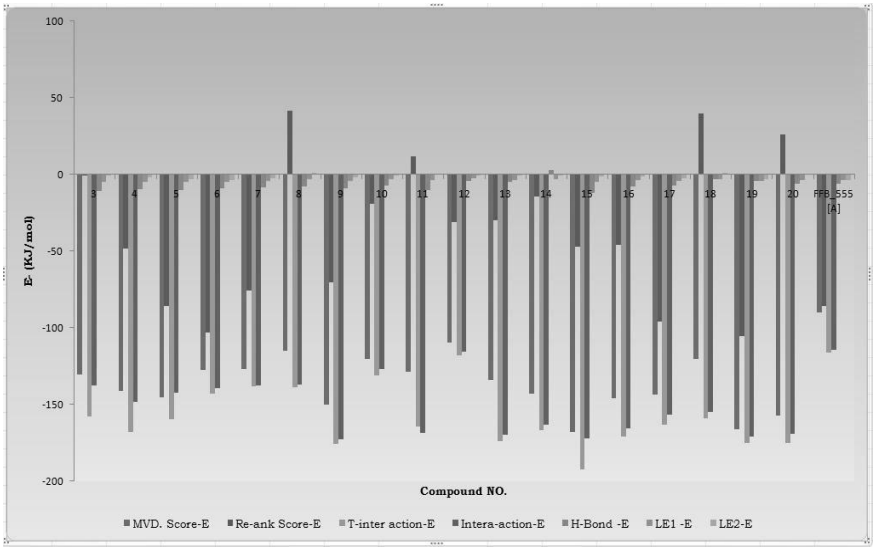


Fig. 2. Showed different scores derived from the MVD docking tools.

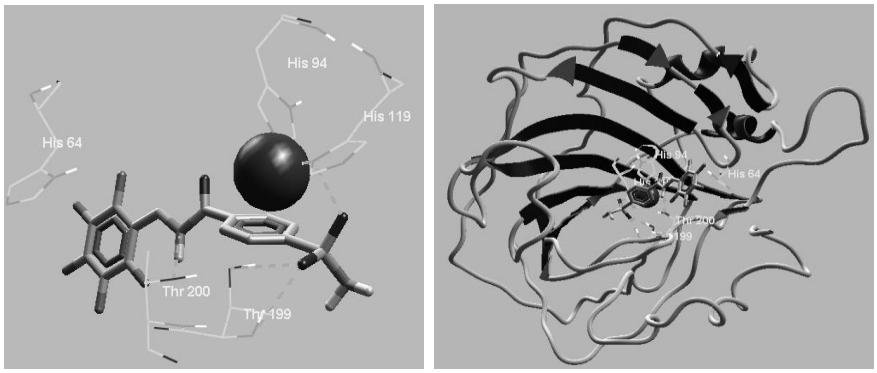
From the comparative docking study of the synthesized compounds with (FFB-555) and observe how the compounds bind to the kinase binding site, based on the knowledge of the structure of similar active sites from analysis of the X-ray crystal structure of human carbonic anhydrase II (hCA II) with (E7070) [20]. We docked the synthesized compounds 3-20 into the empty binding site of (1G54), with its bound inhibitor (FFB-555), Fig. 3 show binding mode of the original ligand into its binding site, while Fig. 4&5 show binding modes of compounds 5 and 19, respectively.

As shown from Tables 5& 6 and Fig. 3-5, the following results can be drawn:

E7070 (the original ligand) reveals MVD score (-90.03) and form three hydrogen bonds: two hydrogen bond with Thr-199 and one hydrogen bond with His-119 (Fig.3).

Compound 5 gave strong binding affinity with MVD score (-145.517) and form 18 bonds with active binding site: two important bonds with Zn metal, two other important bonds with Thr-199, three bonds with (Thr-200 anad His-119), one bond with (Glu-106, His-96 and His-94). (Fig.4).

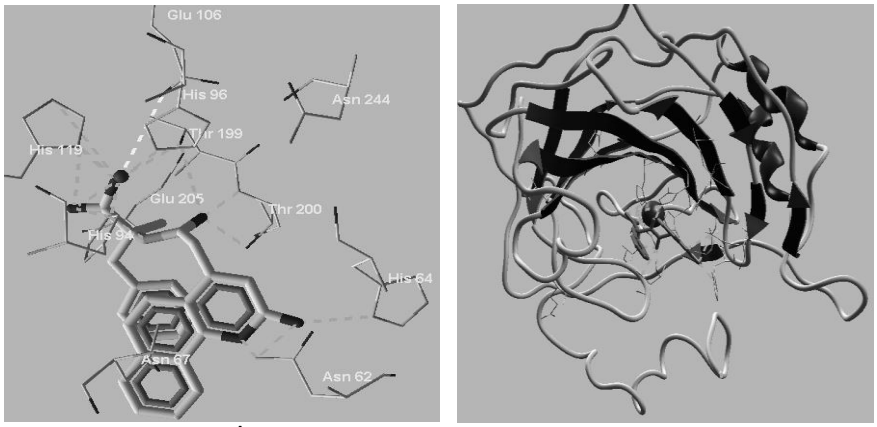
Also, Compound 19 gave strong binding affinity with MVD score (-166.088) and form eighteen bonds with active binding site: two important bonds with Zn metal, three other important bonds with Thr-199, three bonds with (Thr-200 anad His-119), two important bonds with (His-119 , His 94 and Gln-92) and one bond with (Glu-106, His-96, Asn-67, Asn-64 and His-64).



A B

Fig.3. A) Interaction between (FFB) and binding site of CA (1G54, PDB code), which Green dot lines represented hydrogen bonding interaction of ligand (E7070) with binding site. Docked compound (E7070) is represented in stick mode, which atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. Hydrogen atoms of the amino acid residues and ligand were removed to improve clarity.

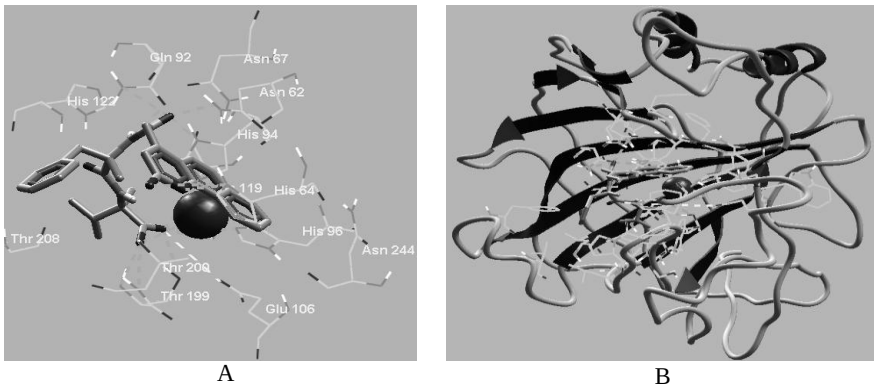
B) A plot of docked ligand (FFB) in active site where the backbone of protein is shown in flat ribbon.



A B

Fig.4. A) Interaction between ligand (5) and binding site of CA 1G54, PDB code), which green dot lines represented hydrogen bonding interaction of ligand (5) with binding site, yellow dot line represented electrostatic interaction of ligand (5) with binding site. Ligand (5) is represented in stick mode, which atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. Hydrogen atoms of the amino acid residues and ligand were removed to improve clarity.

B) A plot of docked ligand (5) in active site where the backbone of protein is shown in flat ribbon.



A B

Fig. 5. A) Interaction between ligand (19) and binding site of CA (1G54, PDB code), which green dot lines represented hydrogen bonding interaction of ligand (19) with binding site. Ligand (19) is represented in stick mode, which atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. Hydrogen atoms of the amino acid residues and ligand were removed to improve clarity.

B) A plot of docked ligand (19) in active site where the backbone of protein is shown in flat ribbon

TABLE 5. Showed different scores derived from the MVD docking tools.

NO.	MVD. Score-E	affinity -E	T-inter action-E	Inter-action-E	H-Bond -E	LE1 -E	LE2 -E
FBB	-90.0384	-85.8086	-115.89	-114.53	-6.09668	-3.60154	-3.43234
3	-130.326	-0.38792	-157.608	-137.279	-10.749	-4.8269	-0.0143674
4	-141.259	-48.099	-167.859	-148.502	-9.81888	-5.04495	-1.71782
5	-145.517	-85.6385	-159.809	-142.487	-10.1736	-4.6941	-2.76253
6	-127.573	-103.273	-142.959	-139.194	-9.00793	-4.55617	-3.68833
7	-126.845	-75.462	-137.947	-137.571	-8.61806	-4.37397	-2.60214
8	-114.609	41.6497	-138.677	-136.846	-7.97827	-2.86522	1.04124
9	-150.096	-69.941	-175.71	-172.752	-9.06891	-4.16934	-1.94281
10	-120.185	-18.9083	-130.676	-126.942	-7.41885	-3.00462	-0.472707
11	-128.714	11.852	-164.444	-168.641	-10.3075	-3.47876	0.320324
12	-109.326	-30.9755	-117.991	-115.643	-4.32672	-2.66649	-0.7555
13	-134.024	-29.6718	-173.946	-169.601	-4.6524	-3.35061	-0.741794
14	-143.097	-14.4901	-166.506	-162.8	2.79054	-3.2522	-0.329321
15	-168.068	-46.8467	-192.27	-172.204	-11.8771	-4.80194	-1.33848
16	-146.016	-45.5448	-170.981	-165.62	-7.8758	-3.744	-1.16782
17	-143.16	-95.9654	-162.882	-156.657	-7.0596	-3.97667	-2.66571
18	-119.972	40.0006	-158.787	-154.502	-3.09716	-2.99929	1.00002
19	-166.088	-105.62	-174.787	-170.656	-4.22561	-4.25866	-2.70821
20	-157.309	26.3942	-174.897	-169.203	-5.7745	-3.65834	0.613818

Where: MVD Score(KJ/mol):Energy score used during docking .
Re-rank Score(Kj/mol):The re-ranking score.
H. Bond Enrgy(Kj/mol) :Hydrogen bonding energy between protein and ligand.
T.interaction(Kj /mol):The total interaction energy between the pose and the target molecule.
Intera-action affinity (Kj /mol): The total interaction energy between the pose and the protein.
LE1(Kj/mol):MolDock Score divided by Heavy Atoms count.
LE2 (Kcal/mol):Rerank Score divided by Heavy Atoms count.

In conclusion, most of the synthesized compounds, showed significant activity, and will be considered as a guide for further future synthetic design . In addition, the docking study of the compounds showed that, the most synthesized compounds act as CA inhibitors more than (E7070). SO, the further modification of these compounds may be promising candidates for clinically useful drug agents.

TABLE 6. Interaction between ligand and amino acid residues derived from MVD docking tools.

Cpd. NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
FBB	4	(ND1)His-119...H	O-24(**O**SO)
		(N)Thr-199...H	O-23(**OS<u>O</u>**)
		(OG1)Thr-199...H	
		(OG1)Thr-200...H	N-7(NH)
3	13	Zn... H	O-24(CO**<u>O</u>**H)
		(N)Thr-200...H	
		(OG1)Thr-200...H	
		(N)Thr-199...H	
		(OG1)Thr-199...H	
		Zn...H	O-23(C**<u>O</u>**OH)
		(OG1)Thr-199...H	
		(ND1)His119...(2)H	
		(OE1)Glu106...E	
		(NE2)His96...H	
		(NE2)His94...H	
		(OG1)Thr-200...H	O-1(Pyrane)
4	12	Zn... H	O-24(CO**<u>O</u>**H)
		(OG1)Thr-199...H	
		(ND1)His119...(2)H	
		(NE2)His96...H	
		(NE2)His94...H	
		Zn... H	O-23(C**<u>O</u>**OH)
		(OG1)Thr-200...H	
		(N)Thr-200...H	
		(OG1)Thr-200...H	
		(OG1)Thr-199...H	
		(NE2)His64...H	O-1(O-Pyrane)
		(ND1)His64...H	
5	18	Zn... H	O-24(CO**<u>O</u>**H)
		(N)Thr-200...H	
		(ND1)His-119...H	

TABLE 6. Contd.

Cpd NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
9		(OG1) Thr-200....H (OG1)Thr-199...H (N)Thr-199...H (OG1) Thr-199....H	O-27(C**O**OCH_3)
		(ND1)His-64...H	O-8(**O**-pyrane)
		(NE2)Gln-92...H	O-7(C**O**CH_2)
		(ND1)His-64...H	O-2(CH_2C**O**-)
10	5	(NE2)Gln-62...H (ND1)His-94...H	O-31(C**O**-pyran)
		(NE2)Gln-62...H	O-28(CO**O**CH_3)
		(NE2)Gln-62...H (ND2)Asn-62...H	O-27(CO**O**CH_3)
11	7	(OG1)Thr-199...H (ND1)His-119...H	O-28(CO**O**CH_3)
		(OG1)Thr-199...H (N)Thr-199...H	O-27(C**O**OCH_3)
		(OG1)Thr-200...H	O-9(O-pyran)
		(ND1)His-94...H	O-8(CONCH-Val)
		(NE2)Gln-62...H	O-2(CH_2C**O**NH)
12	5	(ND2)Asn-67...H (ND1)Asn-62...H	O-38(C**O**-pyran)
		(ND1)Asn-62...H	O-9(O-pyran)
		(OG1) Thr-200....H (N) Thr-200....H	O-2(CH_2C**O**NH)
13	4	(NE2)Gln-62...H	O-38(C**O**-pyran)
		(ND2)Asn-62...H	O-8(CONCH-Val)
		(ND1)His-64....H (ND1)His-94....H	O-3(CH_2C**O**NH)

TABLE 6. Contd.

Cpd NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
		(NE2)His-94...H	
		Zn... H	O-23(C**O**OH)
		(OG1)Thr-199...H	
		(NE2)His-119... H	
		(ND1)His-119...(2)H	
		(OE1)Glu-106...E	
		(NE2)His-96...H	
		(NE2)His-94...H	
		(OG1)Thr-199...H	O-18(C**O**NH)
		(N)Thr-200...H	
		(OG1)Thr-200...H	
6	8	(OH)Tyr-7... H	O-24(CO**O**H)
		(ND2)Asn-67...H	O-18(C**O**NH)
		(ND2)Asn-62...H	
		(OG1)Thr-200...H	O-16(C**O**-pyrane)
		(N)Thr-200...H	
		(OG1)Thr-199...H	
7	3	(NE2)Gln-92...H	O-18(CO**O**CH_3)
		(ND1)His-64...H	O-16(C**O**-pyrane)
		(ND1)His-64...H	O-1(**O**-pyrane)
8	5	(ND2)Asn-62...H	O-18(C**O**NH)
		(ND1)His-64...H	O-7(C**O**-pyrane)
		(ND2)Asn-62...H	
		(ND1)His-94...H	
		(ND2)Asn-62...H	O-2(C**O**-NH)
9	10	(ND1)His-64...H	O-31(C**O**-pyrane)
		(N)Thr-200...H	O-28(C**OO**CH_3)
		(N)Thr-199...H	

TABLE 6. Contd.

Cpd NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
14	4	(NE2)Gln-92....H	O-41(C**O**-pyran)
		(ND2)Asn-62...H	O-8(CH_2C**O**NH-Phe)
		(OG1)Thr-200...H	N-4(CH_2CO**N**H-Phe)
		(OG1)Thr-200...H	O-3(CH_2CO**N**H-Phe)
15	15	(ND2)Asn-62...H	O-33(C**O**-pyran)
		Zn... H	O-28(CO**O**H)
		(OG1)Thr-200...H	
		(N)Thr-200...H	
		(OG1)Thr-199...H	
		(N)Thr-199...H	
		O-28(CO**O**H)	O-27(C**O**OH)
		(OG1)Thr-199...H	
		(OE1)Glu-106...E	
		(NE2)His-96....H	
		(ND1)His-119....(2)H	
		(NE2)His-96....H	
		(ND2)Asn-62...H	O-8(O-pyran)
		(NE2)Gln-92....H	O-2(CH_2C**O**NH)
16	3	(ND1)His-96... (2)H	O-37(C**O**-pyran)
		(NE2)Gln-92... H	O-27(C**O**OH)
17	9	(ND1)His-64... (2)H	O-28(CO**O**H)
		(ND2)Asn-62... H	
		(OG1)Thr-200...H	O-27(C**O**OH)
		(ND1)His-64... (2)H	
		(OG1)Thr-200...H	N-23(**N**H-Val)
		(ND1)His-94... H	O-7(C**O**NH-Val)
		(NE2)Gln-92... H	O-2(C**O**NH-Met)
		(ND1)His-94... H	O-7(C**O**NH-Val)

TABLE 6. Contd.

Cpd NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
18	3	(ND1)His-64...H	O-38(C**O**-pyran)
		(NE2)Gln-92... H	O-27(C**O**OH)
		(OG1)Thr-199...H	O-8(O-pyran)
19	18	(ND)His-64...H	O-37(C**O**-pyran)
		Zn... H	O-28(CO**O**H)
		(N)Thr-200...H	
		(OG1)Thr-199...H	
		(N)Thr-199...H	
		Zn... H	O-27(C**O**OH)
		(OE1)Glu-106...E	
		(OG1)Thr-199...H	
		(NE2)His-96....H	
		(NE2)His-94....H	
		(ND1)His-119....(2)H	
		(ND2)His-119....H	
		(ND2)Asn-64...H	O-8(O-pyran)
		(NE2)Gln-92....H	N-3(CH_2CO**N**H)
		(ND1)His-94....H	O-2(CH_2C**O**NH)
		(NE2)Gln-92....H	(NE2)Gln-92....H
		(NE2)Asn-67....H	(NE2)Asn-67....H
20	9	(OG1)Thr-200...H	O-40(C**O**-pyran)
		(N)Thr-200...H	
		(OG1)Thr-199...H	
		(N)Thr-199...H	
		(ND1)His-64....H	O-7(C**O**NHCOOH)
		(ND2)Asn-62...H	
		(ND1)His-94....H	O-2(CH_2C**O**NH)
		(ND2)Asn-67...H	
		(N)Thr-199...H	O-8(O-pyran)

Expermintal

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Electronic spectra were recorded on a UV-UNICAM 2001 spectrophotometer using 10 mm pass length quartz cells at room temperature. IR spectra were recorded on a Perkin–Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. ^1H NMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. Mass spectra were recorded on a mass spectrometer HP model MS–QPL000EX (Shimadzu) at 70 eV. Elemental analyses (C,H,N) were carried out at the

Microanalytical Center of Cairo University, Giza, Egypt. Biological activities were carried out at the Biogenetic Engineering Center, Molecular Biology Unit, Al-Azhar University, Nasr City, Egypt.

Synthesis of 2-(1-oxo-1H-benzo[h]-chromen-4-yl)acetyl dipeptids (3-5)

General procedures

The desired amino acids (1.5 equiv) were dissolved in water (25 ml) and THF (15 ml) mixture, triethylamine (2 ml) was added, followed by portionwise addition of 2-(1-oxo-1H-benzo- [h]-chromen-4-yl)acetyl chloride (2; 1 equiv) during 30 min. Temperature of the reaction mixture was kept at 10°C during the addition. Stirring was continued for 2 hr at 20°C. Tetrahydrofuran was removed by concentration of the reaction mixture under reduced pressure; water (30 ml) was added and acidified with 2 M HCL to pH 5. The crude products were filtered and recrystallized from Methanol. All the products (3-5) were chromatographically homogeneous by iodine and benzidine development.

Synthesis 2-(1-oxo-1H-benzo[h]-chromen-4-yl)acetyl dipeptide methyl esters (6-8)

General procedures

To a suspension of coupling reaction products (3-5; 1 equiv) in absolute methanol (150 ml) was cooled to -10°C and pure thionyl chlorid (1 equiv) was added dropwise during one hour. The reaction mixture was stirred for an additional 34 hr at room temperature, then kept overnight then the solvent was removed by vacuum distillation. The residual solid material was recrystallized from methanol. All the products (6-8) were chromatographically homogeneous by iodine and benzidine development.

Preparation of 2-(1-oxo-1H-benzo[h]-chromen-4-yl)acetyl tripeptid methyl esters (9-14)

General procedures

To a solution of amino acid methyl ester hydrochloride (1.5 equiv) was dissolved in THF, triethylamine (2 ml) was added, the solution was stirred at 20°C for 30 min and cooled to O°C, dipeptide (3-5;1equiv) in THF (50 ml) and DCC (1 equiv) were added to the above mixture. The reaction mixture was stirred for 6 hr at 0°C and for another 12 hr at room temperature. The crude material is diluted with EtOAc and washed with sat. aq. Na_2CO_3 (×2) and brine (×1), dried over sodium sulfate, evaporated, and purified by methanol to give the desired products. Products 9-14 were to be chromatogramphically homogeneous by iodine and benzidine development.

Preparation of 2-(2-(1-oxo-1H-benzo[h]-chromen-4-yl)acetyl tripeptid methyl esters (15-20)

General procedures

Tripeptide metyl esters (9-14; 0.001mole) were added to a solution of 0.1-N NaOH. The mixture was stirred for 1 hr at (100 °C). The reaction mixture was allowed to cool, then acidified with 1N-HCl to pH=5. The crude products (15-20) were separated, filtered and purified by recrystallization from Methanol. The

products (15-20) were to be chromatogramphically homogeneous by iodine and benzidine development.

Biological Screening

Evaluation of the degree of DNA binding

DNA binding assay on TLC plates

Analyses of the DNA binding affinity of the tested compounds were predeveloped first using methanol-water (8:2). The tested compounds were then applied (5 mg/ml in methanol) at the origin, followed by the spotting of DNA (1 mg/ml in methanol-water mixture (8:2) at the same positions at the origin. Daunomycin was used as a positive control. After complete spotting, the plates were developed with the same solvent system and the positions of DNA were visualized by spraying the plates with anisaldehyde, which produces a blue colour with DNA. The intensity of the colour was proportional to the quantity of DNA added to the plate.

Colorimetric assay for the degree of DNA binding

DNA\methyl green complex (20 mg) was suspended in 100 ml of 0.05M tris-HCl buffer (pH 7.5) containing 7.5 mM $MgSO_4$ and stirred at 37 °C with a magnetic stirrer for 24 hr. The calculated amounts of samples were placed in Eppendorf tubes and (200 µl) of the DNA-methyl green solution was added to each tube. The samples were incubated in dark at room temperature and after 24 hr. the final absorbance of each sample was determined at (642-645) nm. The results were recorded in form of the IC_{50} for each compound, which is the sample concentration required to produce 50% decrease in the initial absorbance of the DNA-methyl green complex. The molar concentration required for 50% decrease in the initial absorbance of the DNA-methyl green complex was calculated and the results are given in Table 4.

Molecular modelling study

Generation of ligand and enzyme structures

Docking study was carried out for the target compounds into (hCAII) using Molegro virtual docker(MVD 2008.2.4.0 Molegro). The crystal structure of the (1G54) complexed with (E7070) was uploaded from protein data bank PDB [25] .

Preparation of small molecule

Molecular modeling of the target compounds were built using ChemDraw Ultra version 8.0.3 and minimized their energy through Chem3D Ultra version 8.0.3/MOPAC, Jop Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1°A and saved as MDL MolFile (*.mol)[27]. Our compounds were introduced into the (1G54) binding site in accordance with the published crystal structures of (E7070) bound to kinase.

Stepwise docking method

Active compounds and hCAII (PDB code 1G54) was uploaded without water molecules. The binding sites of (1G54) were defined close to Zn-Metal ion (volume of approximately 76.8 Å3), The-199, His-119, His-98 and His-96 residues. During docking the grid resolution was set to 0.3 Å, while the binding site radius was set to 9 Å. Scorings generated by MVD. In addition . Docking Score and the following MVD based scores were also calculated for each compound.

(1) MVD score: Scorings generated by MVD.

(2) ReRank:To further increase the docking accuracy, the 10 solutions obtained from the 10 independent docking runs.

(3) T-interaction-E: total interaction energy between ligand and target.

(4) H. Bond: the hydrogen bonding energy between ligand and target.

(5) LE1: ligand efficiency 1, which corresponds to MolDock Scores divided by the number of heavy atoms.

(6) LE2: ligand efficiency 2, which corresponds to the affinity divided by the number of heavy atoms.

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تصميم وتحضير بعض مشتقات البيبتيدات التى تعمل كمرتبطات بالحمض النووى واكتشاف تأثير هذه المركبات على انزيم انهيدرز الكربونيك باستخدام الطرق الميكانيكية

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تحتوي هذه المقالة على وصف لعملية التشييد الكيميائي لمجموعة جديدة مشتقات البيبتيدات لمركب (2-أكسو-2-هيدرو-[هـ]- كرومين-4-يل) كلوريد الأسيتيل ثم تفاعل المركب الاخير مع الأحماض الامينية المختلفة في وجود ثلاثى ايثيل الأمين رباعى الهيدروفيوران وذلك لتحضير مركبات(2-أكسو-2-هيدرو-[هـ]- كرومين-4-يل) اسيتيل أحماض امينية ، وقد تم تفاعل المركبات الأخيرة مع مما يلى من المركبات الأتية:-

أ- كلوريد الثيونيل والكحول الميثيلى لتحضير الاسترات الميثيلية المقابلة.

ب - الاسترات الميثيلية للاحماض الامينية المختلفة وذلك لتحضير مجموعة من الاسترات الميثيلية للبيبتيدات الثنائية بطريقة الكاربودايميد ثم تحويلهم الى البيبتيدات المقابلة وذلك بواسطة هيدروكسيد الصوديوم.

كما تم التأكد من التركيب البنائي لهذه المركبات الجديدة بواسطة التحليل الدقيق للعناصر وطيف الأشعة تحت الحمراء وطيف الرنين النووي المغناطيسي لذرة الهيدروجين- 1، كما تم إنجاز دراسة عن كيفية ارتباط هذه المركبات المشيده مع الحامض النووي. كما تم عمل دراسة لأكتشاف كيفية ارتباط هذه المركبات مع انزيم انهيدرز الكربونيك المسبب للسرطان لوقف نشاطه حيث أعطت هذه المركبات نشاطاً بيولوجيا لوقف نشاط هذا الأنزيم أكثر من الدواء المستخدم.