Synthesis of New Sulfonamide Scaffolds Acting As Anticancer Targeting CAII Protein Based Docking Studies

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Abstract: Recently, sulfonamides have been reported act as anticancer agent through different mechanisms in vitro and/or in vivo, the most important mechanism is through inhibition of the carbonic anhydrase isozymes. The present work aims to synthesis some novel sulfonamide derivatives. The synthesized compounds were characterized by elemental analysis, physical and spectral data (IR, ^{1}HNMR). The synthesized compounds docked into active site target of validated drug of CA. The calculations in-silico were predicted that, the lowest energy of the docked poses of compounds which interact with residues of active site, may be making them possible inhibitors and physiologically active. Some compounds like (**17**) show extensive interactions with the targets, which consider more suitable inhibitor against (CA) than reference drug.
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Keywords: Sulfonamide; Anticancer; carbonic anhydrase CAII; Docking.

1. Introduction:

Sulfonamides important class of drugs, with several types of pharmacological agents possessing antibacterial [1], antithyroid [2], diuretic [3,4],hypoglycaemic [5], and anti-carbonic anhydrase [3-7]. From other studies, aryl/heteroaryl sulfonamides may act as antitumor agents through several mechanisms, most famous suggested mechanism by inhibition carbonic anhydrase isozymes(CA) [8-12]. After widely evaluation, Sulfonamides were found act as carbonic anhydrase (CA) inhibitors [13].

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 ($CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+$). The mechanism of inhibition tumour with Sulfonamide CA inhibitors was suggested by Chegwidden and Spencer [13], sulfonamides may decrease the provision of bicarbonate to synthesis of nucleotides and other cell components such as membrane lipids. Moreover, peptide derivatives possess several biological activities such as anti-tumour effect and DNA binding activity [14,15].

Based on the foregoing, and continuation of reported work [7,14,15], the present study aim to syntheses of bioactive molecule containing sulphanilamide moiety, where were designed like general features of pharmacophore of the sulfonamide CA inhibitors, and investigation inhibition of the synthesized compounds against (CA).

2. Results and discussion:

From analysis feature of the CA active site[11], and pharmacophore acting as carbonic anhydrase inhibitors (Fig. 1), which has been described by Thiry et al. [16].

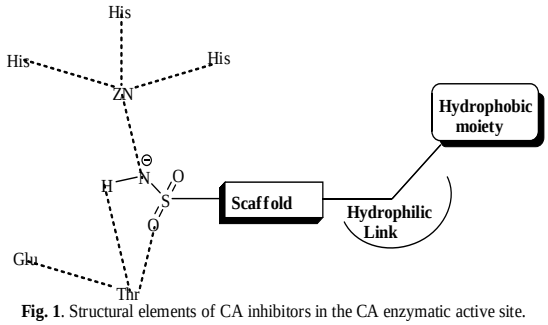


Fig. 1. Structural elements of CA inhibitors in the CA enzymatic active site.

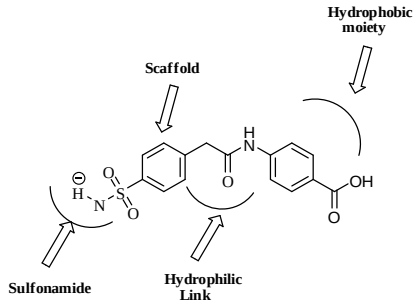
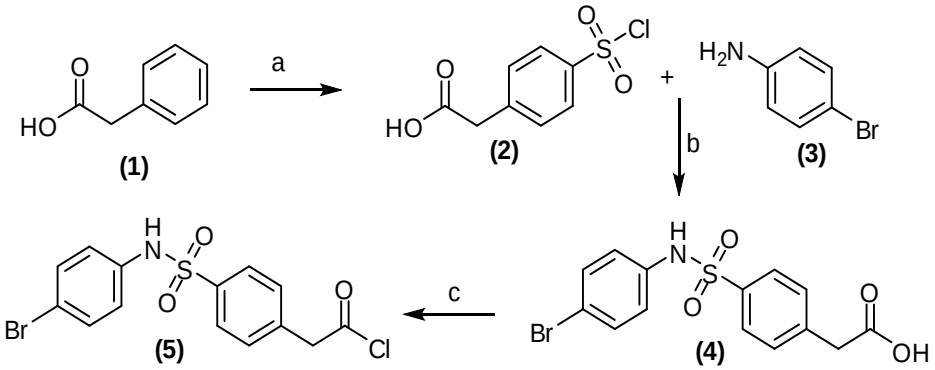


Fig. 2. Representative examples of the synthesized compounds showing compliance to the general feature pharmacophore of sulfonamide compounds acting as carbonic anhydrase inhibitors.

This structural element should be present in the compounds to acting as CA inhibitors: (i) presence of a sulfonamide moiety, which coordinates with the zinc ion of the active site of the CA, and attaching to a scaffold which is usually a benzene ring. (ii) The side chain might posses a hydrophilic link able to interact with the hydrophilic part of the active site, and a hydrophobic moiety which can interact with the hydrophobic part of the CA active site. So that, in this work the synthesized compounds were designed to comply with general character of pharmacophore described earlier [16] (Fig. 2), these compounds were synthesized according to Schemes (1-4).

2.1. Chemistry:

In this paper, aimed to prepare bio molecules contain sulfonamide moiety. The starting intermediate of 2-(4-(N-(4-bromophenyl)sulfamoyl)-phenyl)acetyl chloride (**5**) was carried out according to the three-step-route depicted in (Scheme 1).



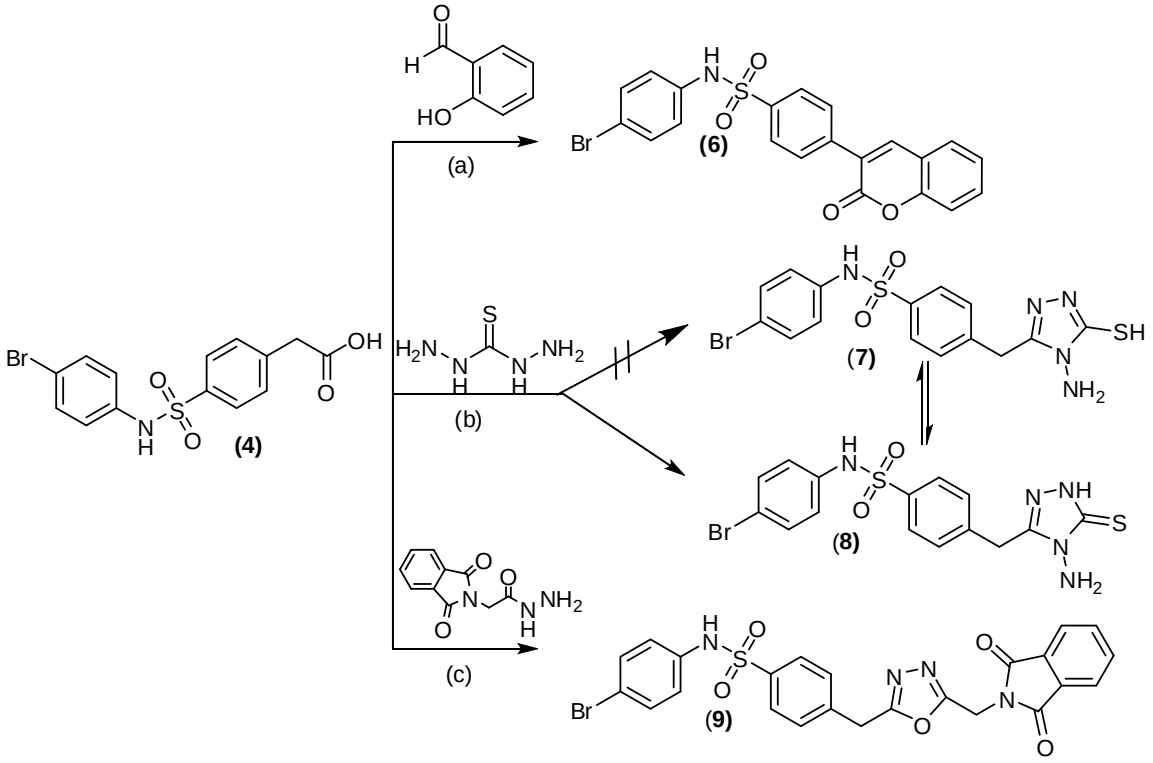
Scheme (1): Reagents and conditions: (a) $ClSO_3H$; (b) i-THF/ TEA; ii- 1N HCl (c) $SOCl_2$, Benzene.

Compound **(2)** was prepared by chlorosulfonation of phenyl acetic acid (**1**) as early report **[17],** which was coupled with p-bromoaniline (**3**) in tetrahydro-furan(THF)-Triethylamin (TEA) media, to afford 2-(4-(N-(4-bromophenyl)sulfamoyl)phenyl)-acetic acid (**4**) in good yield (87%). The IR spectrum of compound **(4)** indicated that presence of a C=O function (1704 cm^{-1}), and its the $^{-1}$HNMR spectrum showed a singlet at (δ_H 10.41 ppm) due to OH protons of carboxylic. The corresponding acid chloride (**5**) was achieved by heating compound **(4)** with thionyl chloride in benzene for 3 hrs (Scheme 1).

Compounds (**6-9**) were synthesized according to the methods described in (**Scheme 2**). Condensation of (**4**) with salicylaldehyde in ethanol with catalytic amount of piperidine gave N-(4-bromophenyl)-4-(2-oxo-2H-chromen-3-yl)benzene-sulfonamide (**6**), the characteristic signal of ^{1}HNMR ^{1}HNMR at (δ_H 7.67ppm) for a methine proton which confirm a cyclization reaction leading to the formation of coumarin moiety. When acid(**4**) was fused with thiocarbohydrazide at 180-195 °C, the 4-((4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl)-N-(4-bromo-phenyl)benzenesulfonamide (**8**) was obtained, while structure of triazol (**7**) was excluded depending on spectral data, the ^{1}HNMR spectrum showed characteristic singlet peak at (δ_H 10.51) ppm due to the NH protons of triazole ring. The cyclocondensation of (**4**) with acid hydrazide in the presence of phosphorus oxychloride afforded N-(4-bromophenyl)-4-((5-((1,3-dioxoiso-indolin-2-yl)met-hyl)-1,3,4-oxadiazol-2-yl)methyl)benzene-sulfonamide **(9)**.

The target compounds (**10-12**) were synthesized according the following procedures as shown in (**Scheme 3**). The compounds (**5**) coupled with p-aminobenzoic acid in THF/TEA/ H_2O media to give 4-(2-(4-(N-(4-bromophenyl)sulfamoyl)phenyl)- acetamido)-benzoic acid (**10**), which esterified to achieved methyl 4-(2-(4-(N-(4-bromophenyl)sulfamoyl) phenyl) acetamido)benzoate (**11**) using $SOCl_2$ in presence of methanol. Compound (**11**) was subjected to hydrozinolysis was afforded corresponding hydrazide (**12**). The structures of the isolated products (**10-12**) were established on the basis of their elemental and spectral analyses.

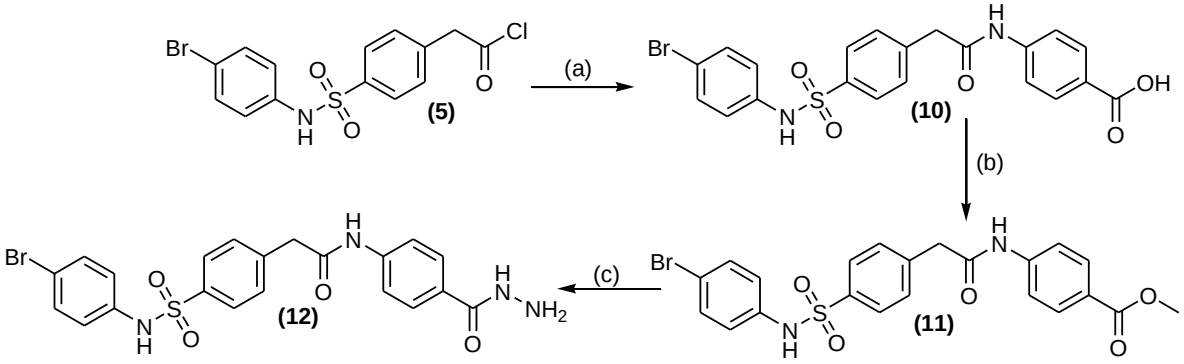
Due to amphoteric character of amino acids in solution ($^+NH_3CH(R)\text{-}COO^-$) [**18]**, the reaction of amino acids as nucleophiles with acid chloride **(5)** might seem hard to conduct, due to decreasing the electron density on nitrogen atom of amino acids. Thus, due to zwitterions the amino acids possess lower nucleophility than amines, and are difficult to react with acid chloride. This reaction facilitate by adding an organic base to improve the reaction rate. The synthetic route for designing the pseudo-peptide containing α-amino acid moiety (**13-18**), was summarized in (**Scheme 4**).



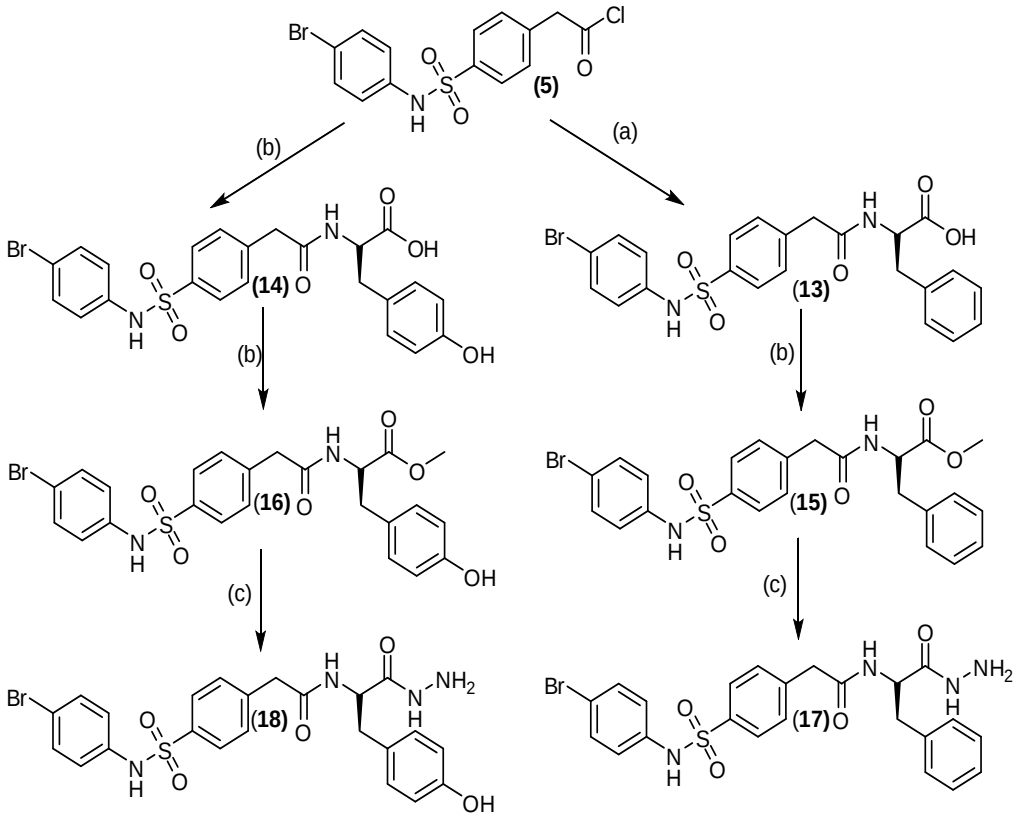
Scheme **(2):** Reagents and conditions:**(a)** EtOH, piperdine, Ref. 3h; (b)180-195 ^{o}C Fusion; (c) $POCl_3$, Ref. 3h. at 80 °C

Synthesis was started, by coupling different chiral α-amino acids (L-Phenylalanine and L-Tyrosine) with compound **(5)** to accomplish chiral free α-amino acid derivatives (**13,14),** which converted into corresponding methyl ester derivatives **(15,16)** under $SOCl_2$-methanol condition. Characteristic ^{1}HNMR of free amino acid derivatives (**13,14**) were displayed peak at (δ_H 10.39,10.41 ppm) for carboxylic (**OH**) proton of new amino acids moiety, which disappeared in corresponding methyl ester derivatives **(15,16)**. The hydrazides derivatives (**17, 18**) were carried out by refluxing compounds **(15,16)** respectively with hydrazine hydrate in absolute ethanol.

In order to established enantiomeric purity of isolated compounds (**13-18**), the specific rotation values were determined, which remained unchanged after repeated crystallization for several times. Also, enantiomeric excess (ee) and diastereoisomeric excess (de) values were determined, these values based on the stereoconfiguration of amino acid of amide part of products (**13,14**), obtained through nucleophilic addition of free amino acids on sulfonamide. Also, from TLC analysis, the optical purity of the resulting compounds was greater than 98%. Thus, as expected, stereochemical configuration at α-carbon atom of the acid was practically unaffected and this synthetic transformation from chiral α-amino acid could be applied to a wide range of compounds without undergoing any significant loss of optical activity.



Scheme **(3):**Reagents and conditions: **(a) i-** pABA., THF/TEA/H_2O, stirr. 3h. at 10 °C, i-1NHCl.; **(b)** $SOCl_2$, MeOH, stirr. 3h., 0-5 °C; (c) NH_2NH_2, EtOH, Ref. 1/$_2$ h. at 120°C



Scheme **(4):**Reagents and conditions: **(a) i-** THF/TEA/H_2O,stirr. 3h. at 10 °C,i- 1N HCl.; **(b)** $SOCl_2$, MeOH, stirr. 3h., 0-5 °C;(c) NH_2NH_2, EtOH, Ref.$1/_2$ h. at 120°C

2.2. Molecular Modeling Studies:

Abbate et all stated that, (**E7070)** acts as a strong CA inhibitor, which considering anti-cancer potent sulfonamide under clinical development for the treatment of several cancer types [**19**].

The X-ray crystal structure of human carbonic anhydrase II (hCA II) with (**E7070**) revealed similar interactions between the inhibitor and the CA active site as those reported by Supuran et al. [**10,20**]. Since, the synthesized sulfonamide derivatives (**4-18**) were designed to complies the general features of sulfonamide pharmacophore act as CA inhibitors. So, to understand the binding mode of protein-ligand interactions, the comparative docking study of our compounds with **(E7070)** were performed, and observe how the compounds bind to the kinase binding site.

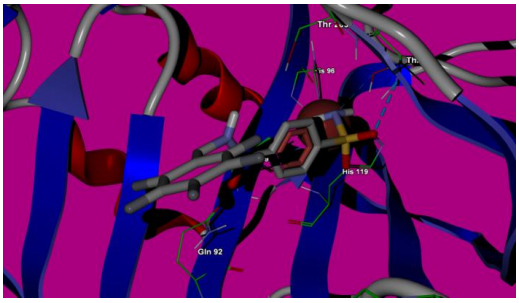
Docking studies were preformed using default parameter, with Molegro virtual docker version 2008 with SYBYL version 7.3. Tripos Inc. [**21**]. The crystal structure of the enzyme (1G54) complexed with **(E7070)** as inhibitor was obtained from protein data bank PDB[**22**]. The MolDock scoring function was applied to evaluate the binding affinities between the (1G54) complexed synthesized inhibitors and **(FBB)** Table(1,2).

2.2.1. Docking reference molecules into active site CA enzyme:

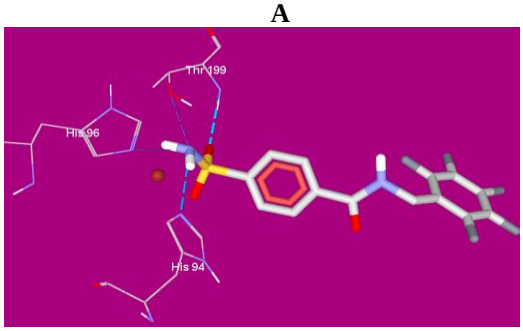
To get a better insight of the 3D-binding orientations of **FBB** into the catalytic site of CA, we performed docking of **FBB** (Figure 3), which is reported to be potent anti-toumer agent experimentally[19], using similar default parameters in the MVD Docking results are graphically in the (Figure 3), while the drug-receptor interactions in terms of number of H bonds, binding energy etc. for each compound are summarized in Table (1,2). Binding mode of the original ligand into its binding site reveals MVD score of -65.49 and form **4** hydrogen bonds: two hydrogen bond with Thr-199, and one hydrogen bond with His-96 and His-94 respectively (Figure 3).

2.2.2. Docking synthesized compounds into active site CA enzyme:

All docked compounds (**FBB** and **4-18**) were represented in stick and the protein represents in ribbon, to clarify suggesting, the preferred binding mode of the synthesized ligands in the active site protein showed in (Figure 4). The results of docking, for instance, no. of H bonds, binding energy etc. for each compound are summarized in (Table 1,2).



A



B

Figure3 A) Interaction between original ligand (**FBB-555**) and binding site of CAII (1G54, PDB code), which blue dot lines represented hydrogen bonding interaction of ligand (**FBB-555**) with binding site. B) A plot of docked ligand (**FBB-555**) in active site where the backbone of protein is shown in flat ribbon. Ligand (**FBB-555**) are represented in stick mode, which atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. A hydrogen atom of the amino acid residues and ligand was removed to improve clarity.

2.2.3. Structures activity relationships:

In order to get a deeper insight into the nature and type of interactions of docked compounds, the complexes between each compound and CAII receptor were visualized, which are depicted in (Figures 4). Since, the H bonds play an important role in the structure and function of biological molecules, the current ligand-receptor interactions were analysed on the basis of H bonding, as described in (Tables 2). In order to reduce the complexity, hydrophobic and π-cation interactions (>6Å) are not shown in the figures.

The compounds (**4-18**) stabilized in the binding pocket of CA by adjusting phenyl ring (sulfonyl pheylacetic) perpendicular with phenyl ring (bromoaniline), except compounds (**8, 11,16**) two phenyl ring parallel together. The compound (**17**) showed highest binding score with active site with comparison to other ligands, which interact with His-94, His-96 and Thr-119, respectively, through their Sulfomyl oxygen atoms (Figure 5). Moreover, which itself stabilized in the binding pocket of CA by adjusting its phenyl ring (sulfonyl pheylacetic) perpendicular to the imidazole rings of His-119 and parallel with His-94 and His 96 amino acid residues through cation-π interactions.

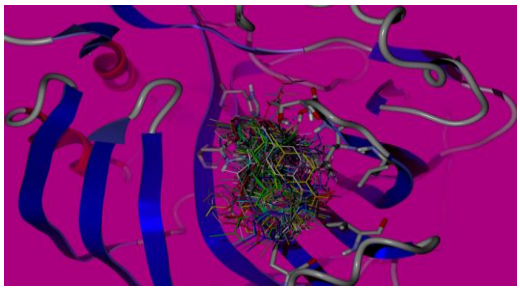
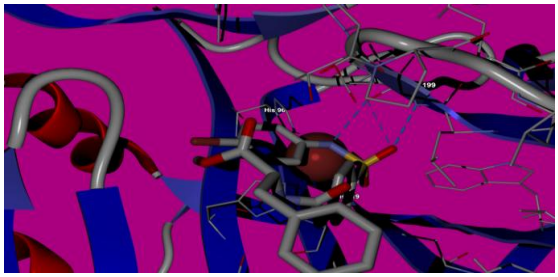
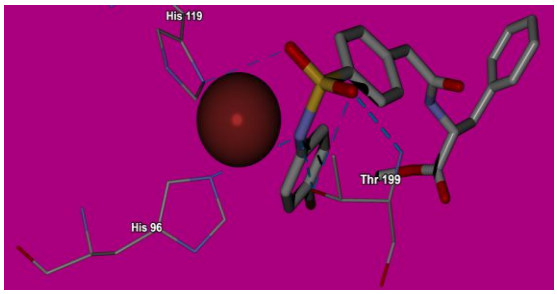


Figure 4. Ribbon representation of CA protein (1G54, PDB code) with all poses (**4-18**) docked into the cavity. All docked ligand are shown in stick form and are coloured according to their atoms name. Hydrogen atoms of the amino acid residues and ligands removed to improve clarity.



A



B

Figure 5 A) Interaction between ligand (**17**) and binding site of CAII (1G54, PDB code), which blue dot lines represented hydrogen bonding interaction of ligand (**17**) with binding site. B) A plot of docked ligand (**17**) in active site where the backbone of protein is shown in flat ribbon. Ligand (**17**) are represented in stick mode, which atoms are colored in dark grey, oxygen in red, nitrogen in blue and sulfur in yellow. A hydrogen atom of the amino acid residues and ligand was removed to improve clarity.

The results obtained clearly reveals that, the amino acid residues close to the reference molecules are

Table 1: Showed different Scores Derived from the MVD Docking Tools.

Ligand	MVD Score	Re.Score	T.INTER	Internal	HBond	LE1	LE3
4	-76.4062	-65.8852	-90.6053	14.1991	-5	-3.63839	-3.13739
6	-85.4753	-63.5909	-108.534	23.0587	-6.56099	-3.05269	-2.2711
8	-88.7253	-17.7448	-98.3169	9.59159	-5.20893	-3.54901	-0.70979
9	-106.457	-81.2188	-112.763	6.30616	-1.15004	-3.04163	-2.32054
11	-72.1198	-59.656	-92.4026	20.2828	-4.4453	-2.40399	-1.98853
12	-73.0243	-45.742	-110.845	37.8204	-4.81999	-2.35562	-1.47555
13	-87.5576	-57.352	-116.289	28.7319	-6.9671	-2.65326	-1.73794
14	-85.2546	-54.5235	-111.033	25.7786	-6.25921	-2.75015	-1.75882
15	-61.8904	-51.3457	-89.757	27.8666	-2.9819	-1.99647	-1.65631
16	-89.2639	-62.3522	-120.436	31.1717	-6.78806	-2.7895	-1.94851
17	-118.913	-89.4697	-126.909	7.99551	-12.4703	-3.60343	-2.7112
18	-92.2735	-43.2216	-120.129	27.8555	-5.61102	-2.71393	-1.27122

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

mostly the same as those observed in the currently synthesized sulfonamide which complexes with protein. However, a significant improvement in the binding energies and binding process interaction observed in case of compounds (**17**) and compared to **E7070** Table (1 and 2). This could probably be due to the presence of phenylanline moiety in the synthesized compounds.

3. Conclusion:

A series of sulfonamide derivatives **(4-18)** were Synthesized. Additionally, the comparative docking experiments was carried out on synthesized compounds **(4-18)** with reference molecules, which indicate that, the synthesized compounds was stabilized in the binding pocket of CAII. Also, perpendicular orientation of phenyl ring (sulfonyl pheylacetic) to the plane of imidazole rings of Histidine residues (His-119), is significant for locking the geometries of compounds in the CAII more than reference drug.

Table (**2**): Interaction between ligand and amino acid residues derived from MVD Docking Tools.

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(**OSO**)
		(OG1)Thr-199...H	
		(OG1)Thr-200...H	N-7(NH)
5	**2**	(OG1)Thr-199...H	OH- Carboxilic
		(N) Thr-199...H	
6	**5**	(NE2)His-96…..H	NH-bromoaniline
		(ND1)His-119…H	S**O**$_2$NH
		(OG1)Thr-199...2H	S**O**$_2$NH and **NH**-Br-aniline

Table (2): Contd.

Cpd.NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
6		(OG1)Thr-200...H	N-7(NH)
8	**3**	(OG1)Thr-199...H	N-Triazol
		(N)Thr-199...H	N-Triazol
		(O)Ser-197...H	N-Triazol
9	**3**	(OG1)Thr-199...H	N-Oxadiazol
		(N)Thr-199...H	N-Oxadiazol
		(OG1)Thr-200...H	N-Phtalyl
10	**3**	(OG1)Thr-199...H	C**O**- Carboxilic
		(N)Thr-199...H	C**O**- Carboxilic
		(ND1)His-119…H	OH- Carboxilic
11	**2**	(OG1)Thr-199...H	C**O**- Carboxilic
		(N)Thr-199...H	C**O**- Carboxilic
12	**2**	(NE)Gln-92...H	C**O**- phenylacetic
		(OG1)Thr-199...H	**N**H-NHNH$_2$
13	**5**	(NE2)His-96...H	SO$_2$**N**H
		(ND1)His-119…H	S**O**$_2$NH
		(OG1)Thr-199...H	SO$_2$**N**H
		(OG1)Thr-199...H	S**O**$_2$NH
		(N)Thr-199...H	S**O**$_2$NH
14	**4**	(ND1)His-119...H	C**O**- phenylacetic
		(O)Ser-197...H	OH-COOH
		(OG1)Thr-199...H	C**O**- phenylacetic
		(N)Thr-199...H	OH-COOH
		(OG1)Thr-199...H	C**O**- phenylacetic
		(N)Thr-199...H	OH-COOH
15	**5**	(NE2)His-96...H	SO$_2$**N**H
		(ND1)His-119…H	S**O**$_2$NH
		(OG1)Thr-199...H	SO$_2$**N**H
		(OG1)Thr-199...H	S**O**$_2$NH
16	**2**	(OG1)Thr-199...H	**O**H-Tyr
		(N)Thr-199...H	
17	**7**	(NE2)His-94.....H	NH-bromoaniline
		(NE2)His-96…..H	
		(ND1)His-119…H	S**O**$_2$NH
		(OG1)Thr-199...H	
		(N)Thr-199...H	

Table (2): Contd.:

Cpd.NO.	NO. of H. Bond	Involved group of amino acid	Involved atom of ligand
		(N)Thr-200...H	
		(O) Pro-201…H	N**H**NH_2
18	**3**	(ND2)Asn-62...H	**O**H-Tyr
		(O)Ser-197...H	**N**H-$NHNH_2$
		(OG1)Thr-199...H	C**O**- $CONHNH_2$

4. EXPERMINTAL:

4.1. Instrumentation and materials:

Melting points were taken on a Griffin melting point apparatus and are uncorrected. Thin layer chromatography (R_f) for analytical purposes was carried out on silica gel and developed with benzene-ethyl acetate (6:1) using iodine-KI (20%) solution as spraying agent. Benzidine, ninhydrin, and hydroxamate tests used for detection reactions. The IR spectra of the compounds were recorded on a Perkin–Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. The ^1H NMR spectra were observed on a Varian Genini-300 MHz spectrometer and chemical shifts (δ) are in ppm. The 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 Centre of Cairo University, Giza, Egypt.

4.2. Synthesise:

4.2.1.2-(4-(N-(4-bromophenyl)sulfamoyl) phenyl)acetic acid(4):

p-Bromoaniline (**3**; 100 mmol) and TEA (1 equiv) were dissolved in THF, 2-(4-(chlorosulfonyl)-phenyl)acetic acid **(2;** 100 mmol) was dissolved in THF was added gradually during 75 min., the temperature of the reaction mixture was kept at (10 °C) until complete addition. The reaction mixture stirred for further 3 h. at room temperature. The solvent was evaporated in vacuo, then mixture acidified with 1N HCl to pH = 5. The crude product (**4**) was filtered and washed with saturated Na_2CO_3, then recrystallized from ethanol and dried to afford the target compound, as grey solid, yield 87%.

mp: 155-57 IR (KBr cm^{-1}) ν: 3260 (NH), 2918 (CH-**ali**), 1704(C=O), 1396 (SO_2); ^1H NMR (500 MHz, DMSO) δ= 10.41 (s, 1H-O**H**), 7.67–6.98 (m, 9H,:[8H,Ar-**H**]+[1H, SO_2N**H**), 3.43(s, 2H-C$\underline{\mathbf{H}}_2$CO); Anal./Calcd. for **$C_{14}H_{12}BrNO_4S$**: (**369**), C (45.52%), H (3.25%), N (3.79%). Found: C (45.42%); H (3.27%); N, (3.78%).

4.2.2. 2-(4-(N-(4-bromophenyl)sulfamoyl)-phenyl)acetyl chloride (5):

To a stirred solution of (**4**; 100 mmol) in benzene (100 ml), was added dropwise with $SOCl_2$ (150 mmol). The reaction mixture was refluxed for 3 h, and the solvent was removed to obtain compound (**5**) as pale yellow oil. Then another 50 ml of benzene was added and evaporated until the excess $SOCl_2$ was completely removed. The crude product was used immediately in the other reactions without further purification.

4.2.3. N-(4-bromophenyl)-4-(2-oxo-2H-chromen -3-yl)benzenesul-fonamide(6):

The solution of salicylaldehyde (100 mmol) in warm absolute ethanol (20 ml), was added methyl 2-(4-(N-(4-bromophenyl)sulfamoyl)phenyl)-acetic acid (**4**; 100 mmol) and three drops of piperidine. The Table (**1**): Showed different Scores Derived from the MVD Docking Tools.

Solution was heated under reflux for 3h. The crystalline product obtained after cooling was separated by filtration and washed three times with absolute ethanol. A pure coumarin Sulfonamide (**6**) was obtained by crystallization with ethanol, as white solid, yield 80%.

Mp: 115-117; IR (KBr cm^{-1}) ν; 3100 (CH aromatic),1668(C=O), 1368 (SO_2); ^1H NMR (500 MHz, DMSO) δ= 7.67-7.01 (m,14H: [1H, CH-methine] + [12H, CH-Ar-**H**]+ [1H, SO_2N**H**]); Anal./Calcd. for **$C_{21}H_{14}BrNO_4S$**: (**455**), C (55.38%), H (3.07%), N (3.07%). Found: C (55.27%); H (3.09%); N, (3.07%).

4.2.4. 4-((4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl)-N-(4-bromophenyl)-benzene sulfonamide (8):

A mixture of the acid (**4;** 100 mmol) and thiocarbodihydrazide (100 mmol) was fused at 180-195 °C for 15 min. Afterwards, the obtained melt was triturated with cold methanol (10 ml) and the solidified product was filtered, washed with methanol and diethyl ether then, crystallized from benzen to afford pure triazole (**8**), yield 69.5% .

Mp:142-44; IR (KBr cm^{-1}) ν; 3508,3406 (NH_2 ,NH), 3082 (CH-**arom**), 1602 (C=N), 1382 (SO_2); ^1H NMR (500 MHz, DMSO) δ = 10.51 (s,1H-N**H**-triazol), 7.67-6.90(m, 9H: [8H, Ar-**H**] + [1H, N**H**SO_2]), 6.00(s, 2H-N$\underline{\mathbf{H}}_2$-triazol), 4.43(s, 2H-C$\underline{\mathbf{H}}_2$CO); Anal./Calcd. for **$C_{15}H_{14}BrN_5O_2S_2$**: (439) C (41.00%), H (3.18%), N (15.95%). Found: C (40.91%); H (3.20%); N (15.90%).

4.2.5. N-(4-bromophenyl)-4-((5-((1,3-dioxoiso-indolin -2-yl)methyl)-1,3,4-oxadiazol-2-yl)methyl)benzene esulfonamid (9):

To an equimolar solution (**4**; 100 mmol) acid and 2-(1,3-dioxoisoindolin-2-yl)acetohydrazide, $POCl_3$ (4 ml) is added and refluxed for 3 h at 80 °C. The excess $POCl_3$ is removed under evaporation and

the residue is poured onto crushed ice. The resulting precipitate was filtered, and washed with saturated sodium bicarbonate solution and then with water, dried and recrystallized from ethanol to get pure oxadiazole(**9**) as yellow solid, in yield 73.2%.
Mp:170-72; IR (KBr cm^{-1}) ν; 3436 (NH), 1636 (C=N), 1134 (C-O-C); ^{1}H NMR (500 MHz, DMSO) δ = 7.67–6.63 (m, 13H:[12H, H-Ar-**H**] + [1H,N**H**SO_2]), 3.61(s, 2H-**C**$\underline{\mathbf{H}}_2$), 2.08 (s, 2H, C$\underline{\mathbf{H}}_2$ Pht.); Anal./Calcd. for $\mathbf{C_{24}H_{17}BrN_4O_5S}$: (**552**), C(52.17%), H(3.07%), N(10.14%). Found: C(52.09%); H(3.10%); N (10.12%).

4.2.6. General procedures for synthesis of free acids (10,13 and 14):

The desired acids (100 mmol) was dissolved in mixture of water (25 ml) and THF (15 ml), triethylamine (2 ml) was added, followed by portionwise addition of acid chloride (**5**; 100 mmol) during 30 min, temperature of the reaction mixture was kept at 10°C during the addition. Stirring was continued for 3 h at 10°C. THF was removed by concentration of the reaction mixture under reduced pressure; water (30 ml) was added and acidified with 1 N HCL to pH =5. The crude products were filtered and recrystallized from appropriate solvent. All the products (**10, 13 and 14**) were chromatographically homogeneous by iodine and benzidine development.

4.2.6.1. 4-(2-(4-(N-(4-bromophenyl)sulfamoyl) phenyl)acetamido)benzoic acid(10):
White crystal from ethanol: yields=71.3%; mp: 147-49 °C; IR (KBr cm^{-1}) ν; 3258 (b, OH,NH), 2926 (CH**ali**), 1702 (C=O), 1326 (SO_2); ^{1}H NMR (500 MHz, DMSO) δ =10.46 (s, 1H, O**H**), 7.66-7.02 (m,14H: [12H-Ar**H**]+ [1H,-N**H**CO], + [1H,N**H**SO_2]), 3.42(s,2H,C$\underline{\mathbf{H}}_2$CO);Anal./Calcd. for$\mathbf{C_{21}H_{17}BrN_2O_5S}$: (**488**), C(51.63%), H(3.48%), N(5.73%). Found: C(51.54%); H(3.50%); N (5.72%).

4.2.6.2. (L)-2-(2-(4-(N-(4-bromophenyl)sulfamoyl)phenyl)acetamido)-3-phenylpropanoic acid (13):
White crystal from ethanol-water: yields= 55%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); $[\alpha]_D^{20}$ = +37(MeOH) mp: 210-12 °C; IR (KBr cm^{-1}) ν; 3256 (b, OH,NH), 2922 (CH-**ali**), 1662 (C=O), 1532(CONH) and 1390(SO_2); ^{1}H NMR (500 MHz, DMSO) δ =10.39 (b, 1H-**OH** carboxylic), 7.68-7.02 (m, 15H: [13H,Ar**H**]+ [1H,N**H**CO] + [1H-N**H**SO_2]), 3.73-3.27 (m, 3H: [2H,C$\underline{\mathbf{H}}_2$CO]+[1H,C**H**-L-Phe]), 2.11-2.04 (d,2H,C$\underline{\mathbf{H}}_2$-L-Phe); Anal./Calcd. for $\mathbf{C_{23}H_{21}BrN_2O_5S}$: (**516**), C(53.48%), H(4.06%), N(5.42%). Found: C(53.93%); H(4.09%); N (5.41%).

4.2.6.3. (L)-2-(2-(4-(N-(4-bromophenyl) sulfamoyl) phenyl)acetamido)-3-(4-hydrox-yphenyl) propanoic acid (14).

White crystal ethanol-water: yields= 63.5%; R_f= 0.55 ($CHCl_3$/MeOH=3/1); $[\alpha]_D^{20}$ = +25 (MeOH); mp: 182-84 °C; IR (KBr cm^{-1}) ν; 3208 (b, OH,NH),3116(CH. **arm**), 2960 (CH **ali**), 1702 (C=O), 1594(CONH) and 1370 (SO_2); ^{1}H NMR (500 MHz, DMSO) δ =10.41 (s,1H-O**H-carboxylic),** 10.40 (s,1H,O**H-L-Tyr**), 7.67-7.02 (m, 14H [12H-Ar**H**]+ [1H,**NHCO**]+ [1H-**NHSO$_2$**]), 3.69-3.43 (m, 3H: [2H,**C**$\underline{\mathbf{H}}_2$CO] +[1H-C**H**-L-Tyr]),2.05-1.81(s,2H-**C**$\underline{\mathbf{H}}_2$-L-Tyr); Anal./ Calcd. for $\mathbf{C_{23}H_{21}BrN_2O_6S}$: (**532**), C(51.87%), H(3.94%), N(5.26%). Found: C(51.79%); H(3.97%); N (5.25%).

4.2.7. General procedures for synthesis acid methyl ester (11, 15 and16):

The acid (**10, 13 and 14**; 100 mmol) in absolute methanol (150 ml) was cooled to 0-5 °C, and pure thionyl chlorid (150 mmol) was added dropwise during one hour. The reaction mixture was stirred for an additional 3 h at room temperature, and kept overnight, and then the solvent was removed by vacuum distillation. The residual solid material was recrystallized from ethanol. All the products **(11,15 and 16)** were chromatographically homogeneous by iodine and benzidine development.

4.2.7.1. Methyl 4-(2-(4-(N-(4-bromophenyl) sulfamoyl)phenylacetamido)benzoate (11):
White crystal: yields= 51.3%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); mp: 215-17 °C; IR (KBr cm^{-1}) ν; 3242 (NH), 2936 (CH**ali**), 1730 (C=O), 1592 (CONH), 1384 (SO_2) cm^{-1} ^{1}H NMR (500 MHz, DMSO) δ = 7.65-6.50 [m, 14H: [12H, Ar-**H**]+ [1H, N**H**CO]+[1H, N**H**SO_2)], 3.77 (s, 3H-OC$\underline{\mathbf{H}}_3$), 3.41 (s, 2H, C$\underline{\mathbf{H}}_2$CO); Anal./Calcd. for $\mathbf{C_{22}H_{19}BrN_2O_5S}$: (**502**), C(52.58%), H(3.78%), N(5.57%). Found: C, (52.49%); H, (3.80%); N, (5.57%).

4.2.7.2. (L)-methyl 2-(2-(4-(N-(4-bromophenyl)-sulfamoyl) phenyl)acetamido)-3-phenylpropa-noate (15):
White crystal : yields= 57.3%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); mp: 134-36 °C; $[\alpha]_D^{20}$ = +36 (MeOH); IR (KBr cm^{-1}) ν; 3266 (NH), 2924 (CH **ali**), 1648(C=O) and 1580(CONH) cm^1; ^{1}H NMR (500 MHz, DMSO) δ =7.76-7.17(m, 15H:[13H-Ar-**H**]+ [1H,**NHCO**]+[1H, **NHSO$_2$**), 3.71 (s, 3H-OC$\underline{\mathbf{H}}_3$), 2.80 (s, 2H,-C$\underline{\mathbf{H}}_2$CO), 2.24-2.10 (t, 1H,**C**$\underline{\mathbf{H}}$-L-Phe), 1.39-1.23(d, 2H,C$\underline{\mathbf{H}}_2$-L-Phe); Anal./Calcd.for $\mathbf{C_{24}H_{23}BrN_2O_5S}$: (**530**), C(54.33%), H(4.33%), N(5.28%). Found: C(54.24%); H(4.36%); N (5.27%).

4.2.7.3. (L)-methyl 2-(2-(4-(N-(4-bromophenyl)-sulfamoyl)phenylacetamido)-3-(4-hydroxyphenyl) propanoate (16).

White crystal: yields= 61%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); mp: 152-54 °C; $[\alpha]_D^{20}$= +25.6 (MeOH); IR (KBr cm^{-1}) ν;3458, 3208 (OH and NH), 1740 (C=O), 1594(CONH) and 1380 (SO_2) cm^1; ^{1}H NMR (500 MHz, DMSO) δ =10.36 (bs, 1H-O**H**-L-Tyr), 7.87-7.02 (m, 14H: [12H- Ar**H**]+ [1H, **NHCO**]+ [1H, **NHSO$_2$]**), 3.62 (s, 3H, OC**H$_3$**), 2.95-1.15 (5H: [2H,C**H$_2$**CO] +[2H,**CH$_2$**-L-Tyr] +[1H,C**H**-L-Tyr]); Anal./Calcd.for **$C_{24}H_{23}BrN_2O_6S$**: (546), C(52.74%), H(4.21%), N(5.12%)found C(52.66%), H(4.23%), N(5.12%).

4.2.8. General procedures for synthesis acid hydrazide (12,17 and 18)

Compound (**11,15 and 16**; 100 mmol) was dissolved in a solution containing ethanol (120 ml) and 80 % hydrazine hydrate (12 ml), the mixture was refluxed for 1/2h., then left overnight at 25ºC. The product was separated, collected by suction filtration, washed with methanol and light petroleum, and recrystallized from ethanol to give compound **(12,17 and18)**

4.2.8.1. 2-(4-(N-(4-bromophenyl)sulfamoyl)phenyl) -N-(4-hydrazinecarbonyl)phenyl)acetamide (12):
white crystal: yields= 58%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); mp: 193-95 °C; IR (KBr cm^{-1}) ν; 3316,3246 (NH_2,NH), 2926 (CH-**ali**), 1632, 1524 (CONH), 1330 (SO_2) cm^{-1} ^{1}H NMR (500 MHz, DMSO) δ =9.21(s, 1H,NH-N**H**NH$_2$), 7.62-7.01 (m, 14H: [12H, Ar-**H**]+ [1H,N**H**CO] + [1H, N**H**SO$_2$]), 4.44(s, 2H,NHN**H$_2$**), 4.29 (s, 2H,C**H$_2$**CO); Anal./Calcd. for **$C_{21}H_{19}BrN_4O_4S$**: (502), C(50.19%), H(3.78%), N(11.15%). Found: C, (50.11%); H, (3.80%); N, (11.13%).

4.2.8.2. (L)-2-(4-(N-(4-bromophenyl)sulfamoyl) phenyl)-N-(1-hydrazinyl-1-oxo-3-phenylpropan-2-yl)acetamide (17):
White crystal: yields= 61.7%; R_f= 0.71 ($CHCl_3$/MeOH=3/1); mp: 170-72 °C; $[\alpha]_D^{20}$ =+ 44 (MtOH); IR (KBr cm^{-1}) ν; 3416,3358 (NH_2,NH), 2932 (CH-**ali**), 1648(C=O) and 1148(SO_2) cm^1; ^{1}H NMR (500 MHz, DMSO) δ =9.26(s, 1H, N**H**NH$_2$), 7.76-7.03 (m, 15H: [13H, Ar**H** + [1H, **NHCO**]+[1H, **NHSO$_2$]**), 4.45(s, 2H,NHN**H$_2$**), 4.34 (s, 2H, C**H$_2$**CO), 3.87-3.84 (m, 3H: [1H-C**H**-L-Phe] + [2H,-C**H$_2$**, L-Phe); Anal./Calcd.for **$C_{23}H_{23}BrN_4O_4S$**: (530) C(52.07%), H(4.33%), N(10.56%). Found: C(51.98%); H(4.36%); N (10.54%).

4.2.8.3. (L)-2-(4-(N-(4-bromophenyl)sulfamoyl) phenyl) -N-(1-hydrazinyl-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)acetamide (18):
White crystal: yields= 88%; R_f= 0.71 ($CHCl_3$/ MeOH=3/1); mp: 162-64 °C; $[\alpha]_D^{20}$= + 38 (EtOH); IR (KBr cm^{-1}) ν; 3394 (b, NH_2,NH), 1650 (C=O), 1632(CONH) and 1158(SO_2) cm^1; ^{1}H NMR (500 MHz, DMSO) δ =10.42 (s, 1H,NH-N**H**NH$_2$) + 10.40 (1H,OH-L-Tyr), 7.75-7.01 (m, 14H: [12H. Ar**H** + [1H, **NHCO**]+ [1H-**NHSO$_2$]**), 4.14(s, 2H, NHN**H$_2$**), 3.87 (s, 2H,C**H$_2$**CO), 2..31-2.15 (t, 1H-CH-L-Tyr), 1.44-1.19 (d,2H, **CH$_2$**-L-Tyr); Anal./Calcd. for **$C_{23}H_{23}BrN_4O_5S$**: (546), C(50.54%), H(4.21%), N(10.25%). Found: C(50.46%); H(4.23%); N (10.23%).

4.3. Molecular Modelling Study:

4.3.1. Generation of Ligand and Enzyme Structures:

Docking study was carried out for the target compounds into (**hCAII**) using Molegro virtual docker with SYBYL version 7.3. Tripos Inc. The crystal structure of the (**1G54**) complexed with (**E7070**) was uploaded from protein data bank PDB**[25].**

4.3.2. 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. Our compounds were introduced into the (**1G54**) binding site accordance the published crystal structures of (**FBB**) bound to kinase.

4.3.3. Stepwise Docking Method:

Molecular docking was carried out using Molegro Virtual Docker (MVD). MVD is based on a differential evolution algorithm; the solution of the algorithm takes into account the sum of the intermolecular interaction energy between the ligand and the protein, and the intramolecular interaction energy of the ligand. The docking energy scoring function is based on a modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included. Full description of the algorithm and its reliability compared to other common docking algorithm can be found in reference **[23]**. The small molecules and the PDB crystal structure atomic coordinates determined by x-ray crystallography of 1G54 were imported, potential binding sites were predicted. The binding cavity was set at X: -6.51, Y: 2.79, Z: 17.44, grid resolution was set to 0.3 Å, while the binding site radius was set to 12 Å. RMSD threshold for multiple cluster poses was set at < 1.00Å. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 10 runs. Scorings Generated by MVD.

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