Molecular Modeling, Synthesis, and preliminary pharmacological evaluation of New Sulfonamide Derivatives as Selective Carbonic Anhydrase XII and IX inhibitors

Samer T. Jasim*, Monther F. Mahdi*
Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

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Corresponding Author email:
Samertj91@uomustansiriyah.edu.iq
Orcid: https://orcid.org/0009-0000-6004-2120

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Abstract:

New benzene sulfonamide compounds 4–10 was modeled at the molecular level to reveal binding opportunities, bond length, angle, and energy scores in the CA II, CAXII, and CAIX active sites. To test their cytotoxic effect against the AMJ-13 Iraqi breast cancer cell line, researchers synthesized the promising compounds from 4-(2-mercapto-4-oxoquinazolin-3(4H)-yl) benzene sulfonamide 3. Derivatives 4–10 have IC50 values between 0.10 and 6.47 M, indicating potent action against the AMJ-13 cell line. The most effective of these compounds were numbers 4, 7, and 10. The highest binding scores in the active site of CAXII and CAIX were seen for the most active drugs, which may explain their inhibitory profile.

Keywords: Quinazoline; Sulfonamide; Carbonic anhydrase IX; XII

النمذجة الجزيئية والتوليف والتقييم الدوائي الأولي لمشتقات السلفوناميد الجديدة كمثبطات انتقائية للأنهيدراز الكربوني الثاني عشر والتاسع

سامر طارق جاسم*، منذر فيصل مهدي*
فرع الكيمياء الصيدلانية / كلية الصيدلة

الخلاصة:

إجراء النمذجة الجزيئية لمجموعة من مركبات بنزين سلفوناميد الجديدة ٤-١٠ داخل الموقع النشط لأنزيمات الكربونيك انهايدريز ٢ و٩و ١٢ لإظهار إمكانيات الارتباط وطول الرابطة والزوايا ودرجات الطاقة. تم تصنيع المركبات الواعدة من مركب البداية، ليتم تقييمها لنشاطها السام للخلايا ضد خط خلايا سرطان الثدي العراقي. أظهرت المركبات ٤-١٠ نشاطا عاليا نحو خط خلية السرطان العراق بقيم من ٠,١٠ – ٦,٤٧ ميكروميتر. كانت المركبات ٤ و٧ و١٠ هي الأقوى في المجموعة. وقد وجد ان المركبات الأكثر نشاطا أظهرت أفضل درجات الربط في الموقع النشط لأنزيمات الكربونيك انهايدريز ١٢ و٩ مما قد يوضح ملف تعريف تثبيطها.

الكلمات المفتاحية: كوينازولين, سلفونمايد, كربونيك انهايدريز ٩ و ١٢

1- Introduction

Carbonic Anhydrases (CA) I, II, III, VII, XIII, and XV are cytoplasmic CAs. Red blood cells, muscle, and the gastrointestinal system are just a few of the many tissues that contain these enzymes. Carbonic anhydrases IV, IX, XII, XIV, and XVII are membrane-bound carbonic anhydrases. These enzymes are located in the kidney, liver, and pancreas, among others, and are linked to the cell membrane. Carbonic anhydrases VA and VB are examples of mitochondrial carbonic anhydrases. The mitochondria house these enzymes, which contribute in controlling the creation of ATP. ATP is the cell's currency, and its

production must be tightly regulated. Carbonic anhydrases VI and XII are secreted carbonic anhydrases. These enzymes help keep the mouth and stomach at a comfortable level of acidity by being released into saliva and digestive juices (1). Drugs belonging to the class of carbonic anhydrase inhibitors are effective against a wide range of diseases and illnesses, including glaucoma, altitude sickness, and epilepsy. These medications help reduce the amount of aqueous humour produced by the eye and the intensity of seizures by suppressing the activity of carbonic anhydrase enzymes (2).

There is mounting proof that carbonic anhydrase enzymes contribute to carcinogenesis and tumour growth. Overexpression of several carbonic anhydrase enzymes has been associated to increased tumour development and metastasis, and their upregulation has been observed in a variety of cancer cell types (3). The breast, lung, kidney, and prostate cancers, among others, are characterised by an overexpression of CA IX. Tumor development and mortality can be encouraged by CAIX because of its role in maintaining a healthy pH level within the tumour microenvironment and in encouraging angiogenesis, the establishment of new blood vessels that bring oxygen and nutrients to the tumour (4). Carbonic anhydrase XII (CAXII) and possibly other carbonic anhydrase enzymes have been shown to be overexpressed in several types of cancer cells, suggesting a role in tumour progression and metastasis (5).

Breast, lung, colorectal, and pancreatic cancers, among others, have been demonstrated to share a poor prognosis with the overexpression of CAIX and CAXII. Increased tumour growth, invasion, and metastasis, as well as resistance to chemotherapy and radiation, have all been associated with overexpression of these enzymes (6,7). Several researchers believe that CAIX and CAXII promote tumour growth by maintaining an acidic pH in the tumour microenvironment. These enzymes create a pH-increasing acidic extracellular environment, which stimulates the action of proteases and other enzymes implicated in tumour invasion and metastasis. The growth of new blood vessels that bring oxygen and nutrients to the tumour is called angiogenesis, and it can be encouraged by an acidic environment (8).

Carbonic anhydrase inhibitors based on sulfonamides show promise as possible anticancer medicines in cancer. These chemicals can limit the action of carbonic anhydrase enzymes, which are critical for tumour development and survival, thus altering the acidic tumour microenvironment (9). Also, preclinical studies have demonstrated that sulfonamide-based carbonic anhydrase inhibitors improve the efficacy of chemotherapy and radiation therapy, suggesting that they may have promise as combination treatments for the treatment of cancer (10,11)

Although sulfonamide-based carbonic anhydrase inhibitors have shown promise in preclinical research and early-phase clinical trials, they still face a number of obstacles before they can be extensively employed in the treatment of cancer (12). Increasing the specificity and selectivity is the main factor to lower the toxicity without sacrificing efficacy (13).

The quinazoline sulfonamides has been discovered to be a specific inhibitor of CA IX and XII enzymes. These chemicals are selective for CA isoforms IX and XII because their quinazoline scaffold interacts with specific residues in the cavity of those enzymes (14).

Overall, quinazoline sulfonamides are interesting prospects for the development of targeted cancer therapeutics due to their structure that allows for specific inhibition of CA IX and XII.

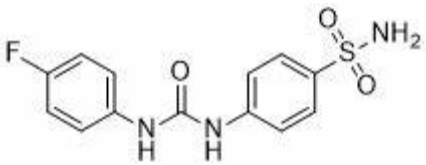


Figure 1: SLC-0111 structure

2. Results and discussion

2.1. Chemistry

Scheme 1 outlined the synthetic procedure for producing the desired chemicals. p-Amino-sulfon-amide and anthranilic acid (18) reacted to provide 4-(2-mercapto-4-oxoquinazolin-3(4H)-yl) benzene sulfonamide 3, an intermediate in the synthesis of 4-(2-mercapto-4-oxoquinazolin-3(4H)-yl) benzene sulfonamide. N(substituted)-2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl) thio) 4-10 (Scheme 1) was obtained by reacting 3 with 2-chloro-N-substituted acetamide in dry acetone and anhydrous K_2CO_3. Bands for NH and CO were found to have been added to IR spectra between 4 and 10 microns. In the ^{1}H-NMR spectra for the range 4-10, the CH2 singlet was at 3.99 ppm, while the NH singlet was at 9.62 ppm. The (C-SH) and (CO) signals were identified in the ^{13}CNMR spectra of 4-10 at 161 and 157 ppm, respectively. The FTIR spectrum of 4 showed C-Cl lines between 838 and 948 cm1, while the 1H-NMR spectrum of 5 showed a singlet at 2.96 ppm due to N-(CH3). The morpholine group in molecule 6 produced a triplet at 3.55 ppm in 1H-NMR.

2.2. Biological Evaluation

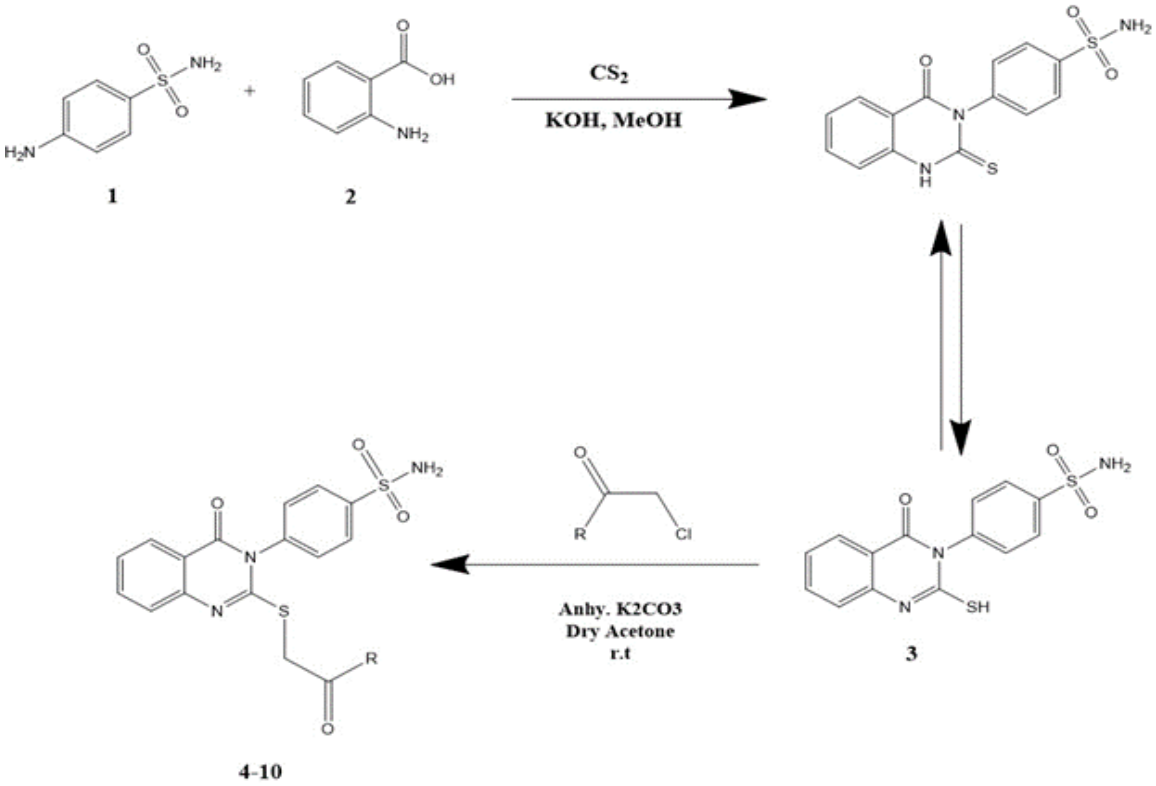
2.2.1. In vitro cytotoxic activity

The MTT assay was used to determine the in vitro cell viability activities of compounds **4-10** against the human Iraqi breast (AMJ-13) cancer cell line. The standard medication was acetazolamide. In comparison to Acetazolamide, Compounds **4–10** in Table 1 (15,16).

Compound 8 had the lowest IC50 value, at 0.10 mM. Derivative 10 of the quinoline was the most potent, followed by derivative 7. Inhibitory action was evaluated for compounds 4–10. Inhibitory activity among the investigated substances, as shown in Table 1. An inhibitory profile was found to be strongest for compound 10, followed by compound 7.

Table 1. Cytotoxic activity against AMJ-13 cell line

Comp.	% of cell death in 24 hr	% of cell death in 48 hr	% of cell death in 72 hr	IC_{50}
3	18.6	32.8	36.6	68.74
4	46.8	50.5	59.4	42.13
5	19.6	39.6	49.0	59.64
6	22.8	26.8	33.9	58.81
7	44.7	49.0	58.9	28.73
8	8.8	25.6	26.7	84.76
9	10.8	40.4	45.1	62.72
10	40.8	60.8	68.2	21.88
Acetazolamide	19.2	20.5	22.9	119.23



Scheme 1. The synthetic pathway for the formation of sulfonamide derivatives.

2.3. Docking study

The docking procedure was optimized using GOLD (Genetic Optimization for Ligand Docking) software at Pharmacy College, Mustansiriyah University. The Protein Data Bank (PDB) IDs 1A42, 5FL4, and 6T5P, respectively, provide access to the 3.0-A resolution crystal structures of carbonic anhydrase II, IX, and XII, respectively. Discovery Studio Visualizer was used to depict the two- and three-dimensional binding modalities of the enzymes and our compounds 4–10.

As can be seen in Table 2, compounds 10, 4, and 5 have the maximum binding ability to CA XII. As seen in Figures 2 and 3, the active site of CA XII is larger than that of CA IX, which may explain why the two enzymes have such different binding capacities.

Table 2. The promising compounds PLP fitness inside the 6T5P (CA XII) active site.

Comp.	PLP fitness	A.A.	Interacting groups	Length
Acetazolamide	55.7143	THR198 HIS117 THR199 HIS91 HIS93	SO_2 SO_2 SO_2 NH_2 NH_2	2.896 2.684 3.024 3.094 3.007
10	80.1479	THR199 HIS119 HIS91 GLN89 ASN64	SO_2 SO_2 NH_2 Oxygen of acetamide Oxygen of acetamide	2.641 2.869 3.095 2.428 2.963
4	78.9912	THR198	SO_2	2.633

		HIS91 THR198 LYS69 ASN64	NH_2 NH_2 Oxygen of acetamide Oxygen of acetamide	2.976 3.021 2.69 2.79
5	76.0641	THR198 THR199 HIS91 HIS117 HIS93	SO_2 SO_2 NH_2 SO_2 NH_2	2.721 2.641 2.810 3.052 2.708

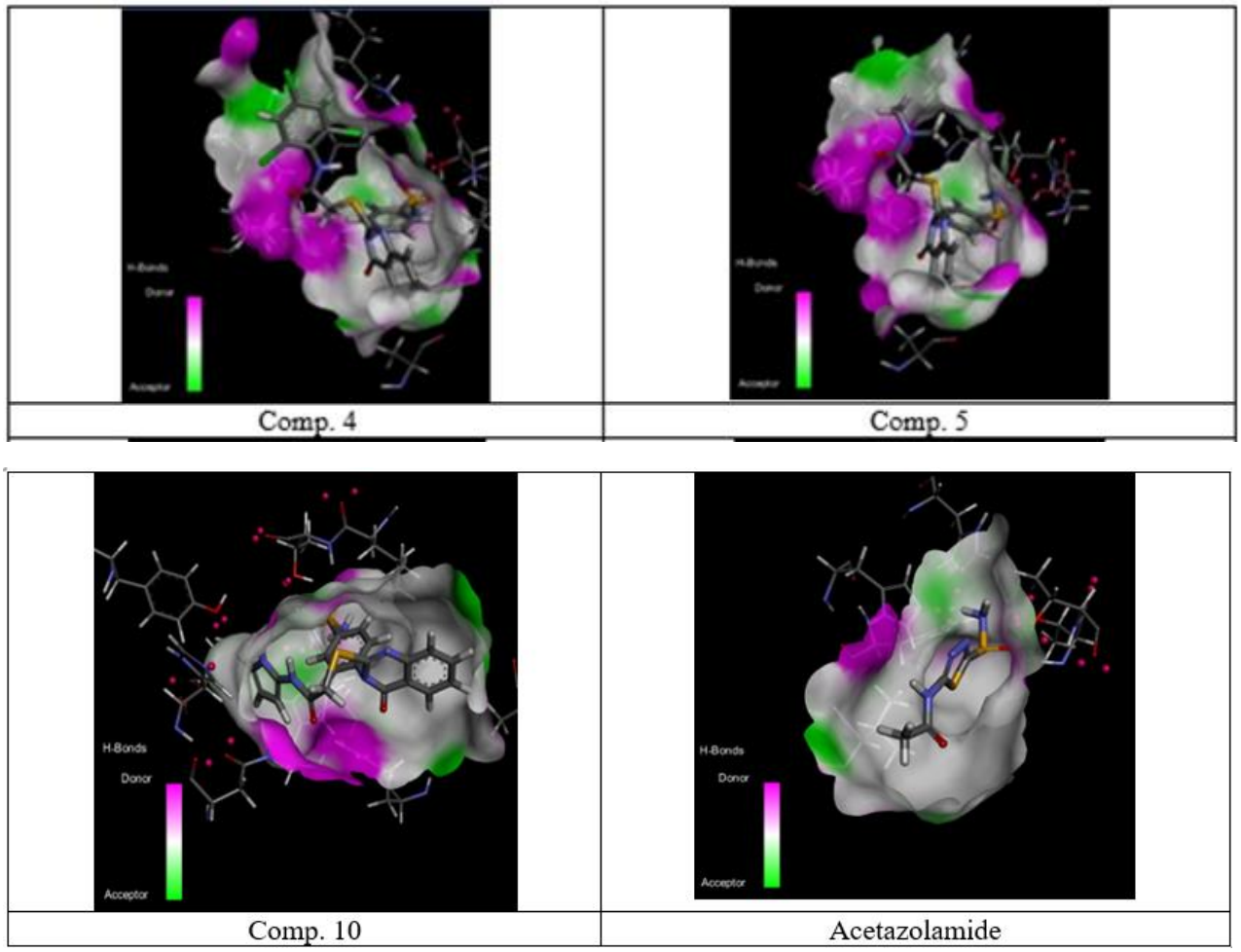


Figure 2. H-Bond interactions of compounds (4, 5,10 and Acetazolamide) in the active site of CA XII (6T5P)

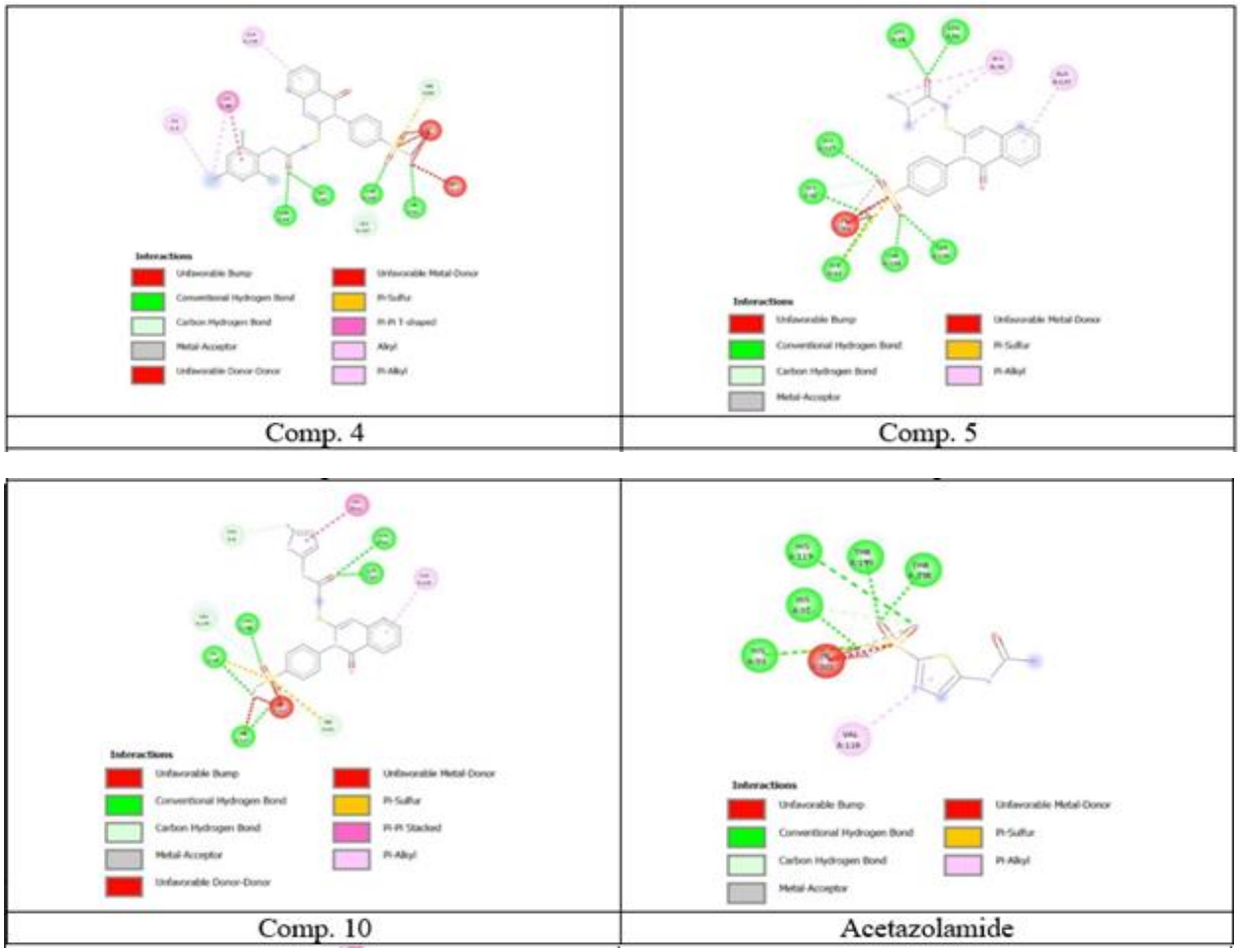


Figure 3. Interactions (2D) of compounds 4-10 and Acetazolamide in the active site of CA XII (6T5P)

Moreover, the compounds have been shown high selectivity to CA IX as shown in **Table 3**. The moderate bulky tails may give the priority for these compounds to be accommodated better in the active site of the CA IX as shown in **Figures 4** and **5**. Interestingly, the high selectivity came together with the high inhibitory percentage in the AMJ-13 cell line study.

Table 3. The promising compounds PLP fitness inside the 5FL4 (CA IX) active site.

Comp.	PLP fitness	A.A.	Interacting groups	Length
Acetazolamide	58.9328	THR199 THR198 HIS117 HIS91 HIS93	SO_2 SO_2 SO_2 NH_2 NH_2	3.051 2.765 2.613 3.042 2.841
4	89.069	THR198 ASN64 HIS91 LYS69	SO_2 Oxygen of thioamide NH_2 Oxygen of thioamide	2.913 2.584 2.991 2.875
6	78.9912	THR200 THR201 HIS94	SO_2 SO_2 NH_2	3.033 2.782 3.036
7	76.0641	HIS93 THR199 THR198 HIS91 HIS117 PRO200	NH_2 SO_2 SO_2 NH_2 NH_2 Nitrogen of acetamide	2.857 2.892 2.616 2.567 2.746 2.89

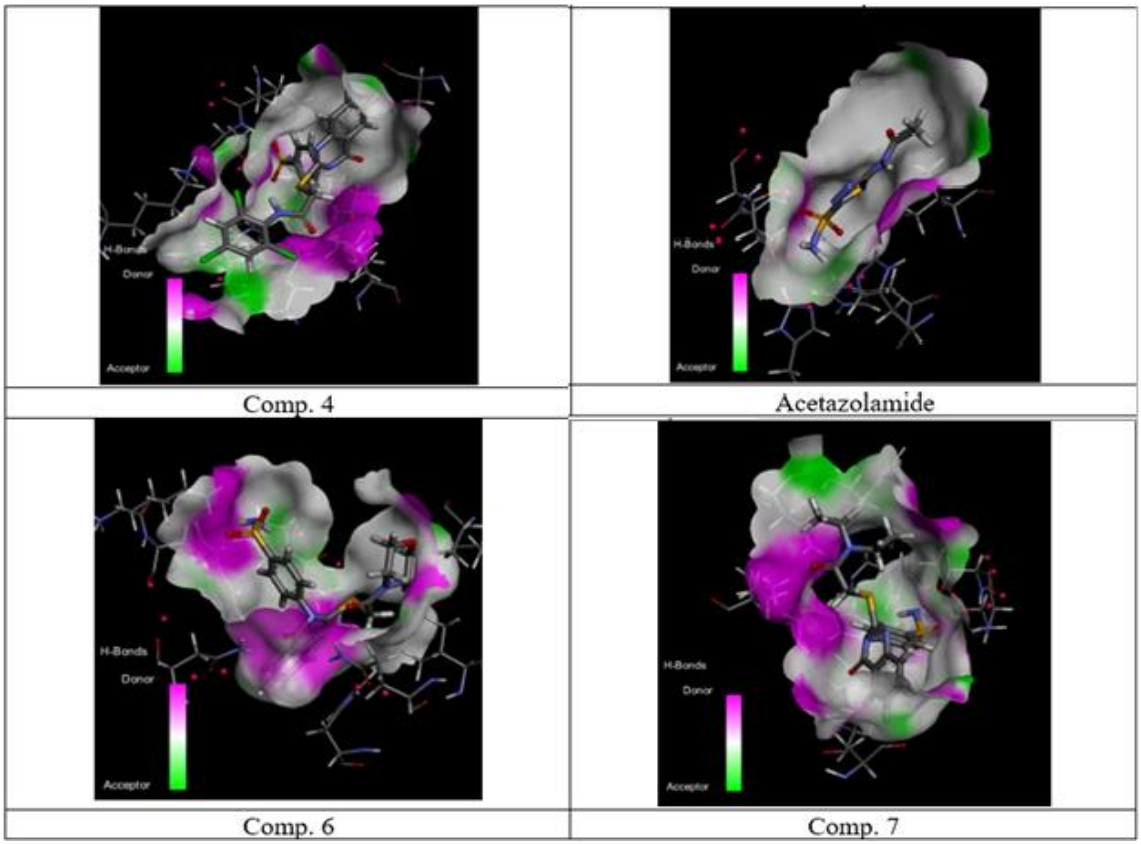


Figure 4. H-Bond interactions of compounds (4, 6, 7, and Acetazolamide) in the active site of CA IX (5FL4)

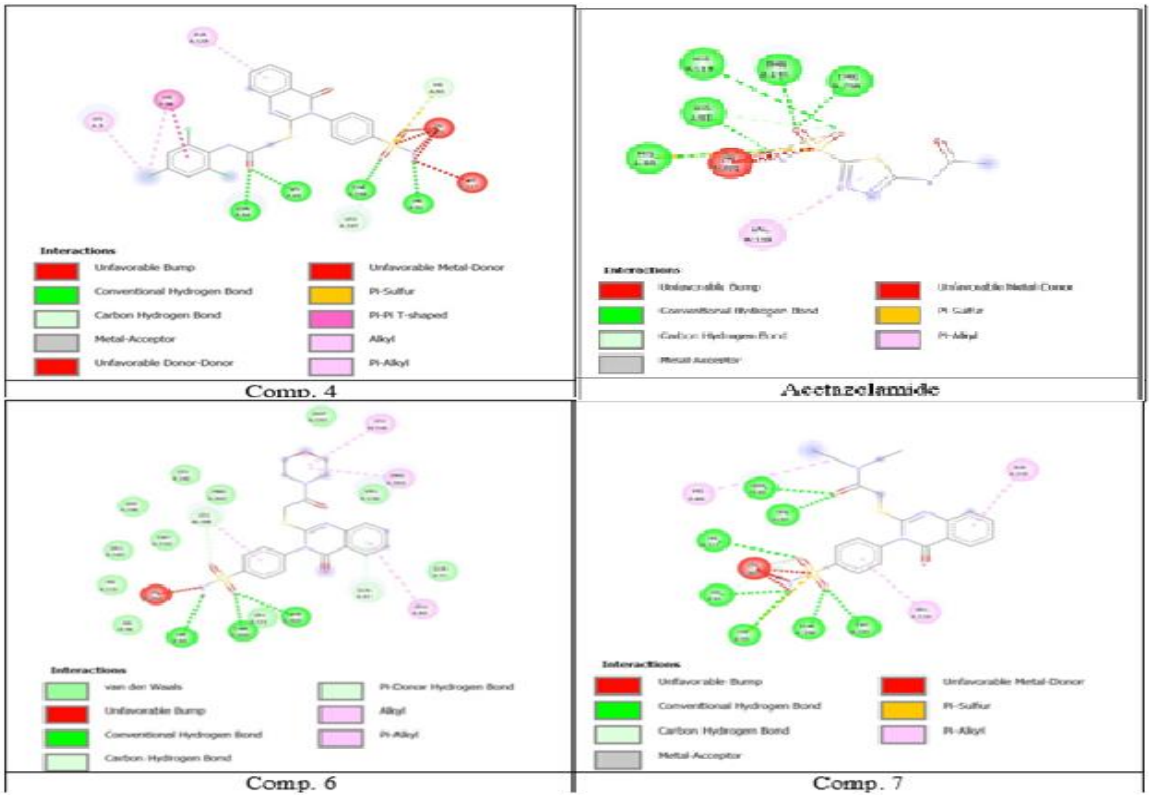


Figure 5. Interactions (2D) of compounds (4, 6, 7, and Acetazolamide) in the active site of CA IX (5FL4)

Compounds 7, 8, and 6 have been showed the lowest probability to bind to CA II as listed in **Table 4**. The active site of the CA II may could not accommodate the bulky tails of compounds 6 and 7 as shown in **figure 6** and **7**.

Table 2. The promising compounds PLP fitness inside the 1A42 (CA II) active site.

Comp.	PLP fitness	A.A.	Interacting groups	Lengt
Acetazolamide	89.617	THR200 HIS119 THR199	N of Thiadiazole ring SO_2 SO_2	2.995 2.995 2.699
7	69.264	GLN92 HIS94	Sulphur of thioamide NH_2	2.565 3.010
8	69.054	His94 HIS 96 THR200 PRO201	NH_2 NH_2 Oxygen of Amide Nitrogen of amide	2.639 2.848 2.648 3.045
6	68.6272	THR200 GLN92 GLN92	SO_2 Sulphur of thioamide Oxygen of thioamide	2.577 2.783 2.649

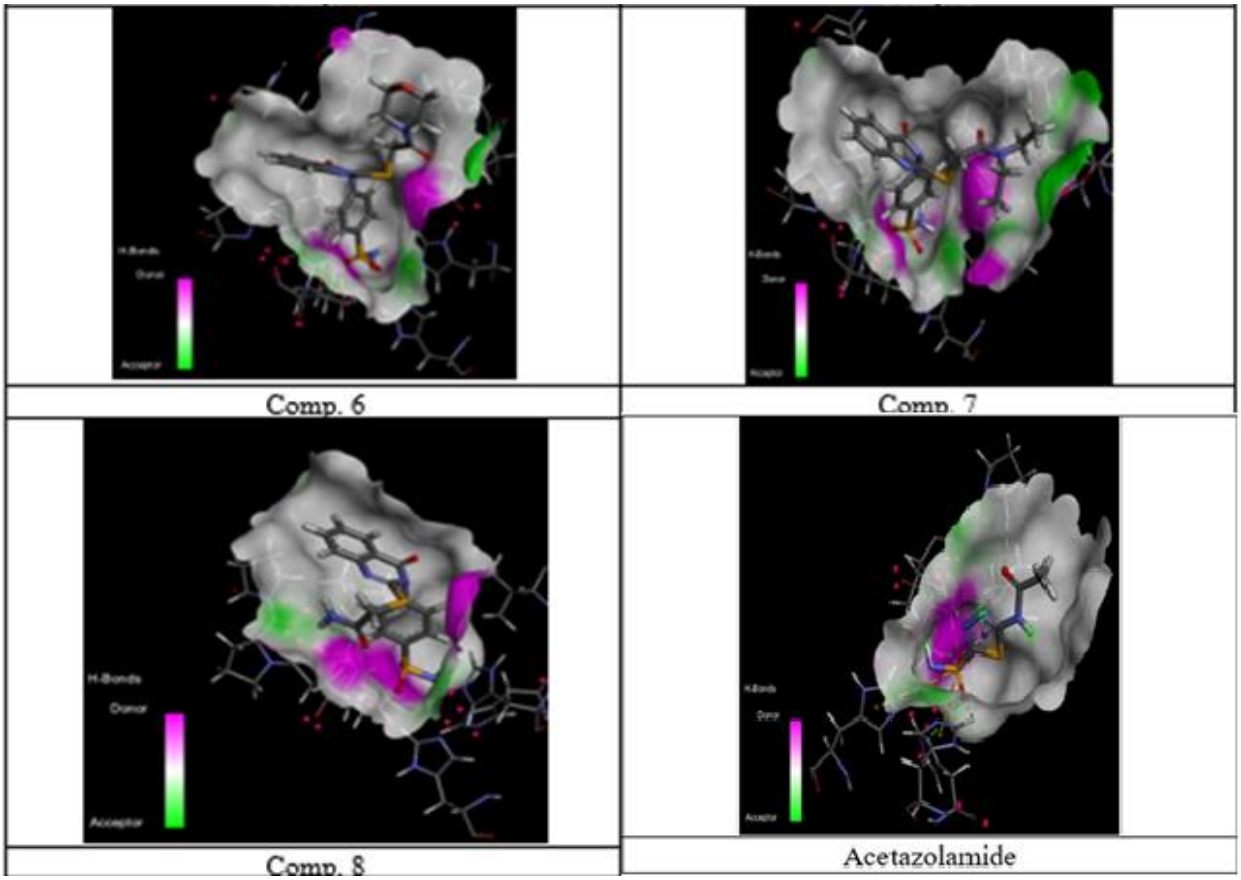


Figure 6. H-Bond interactions of compounds (6, 7, 8, and Acetazolamide) in the active site of CA II (1A42)

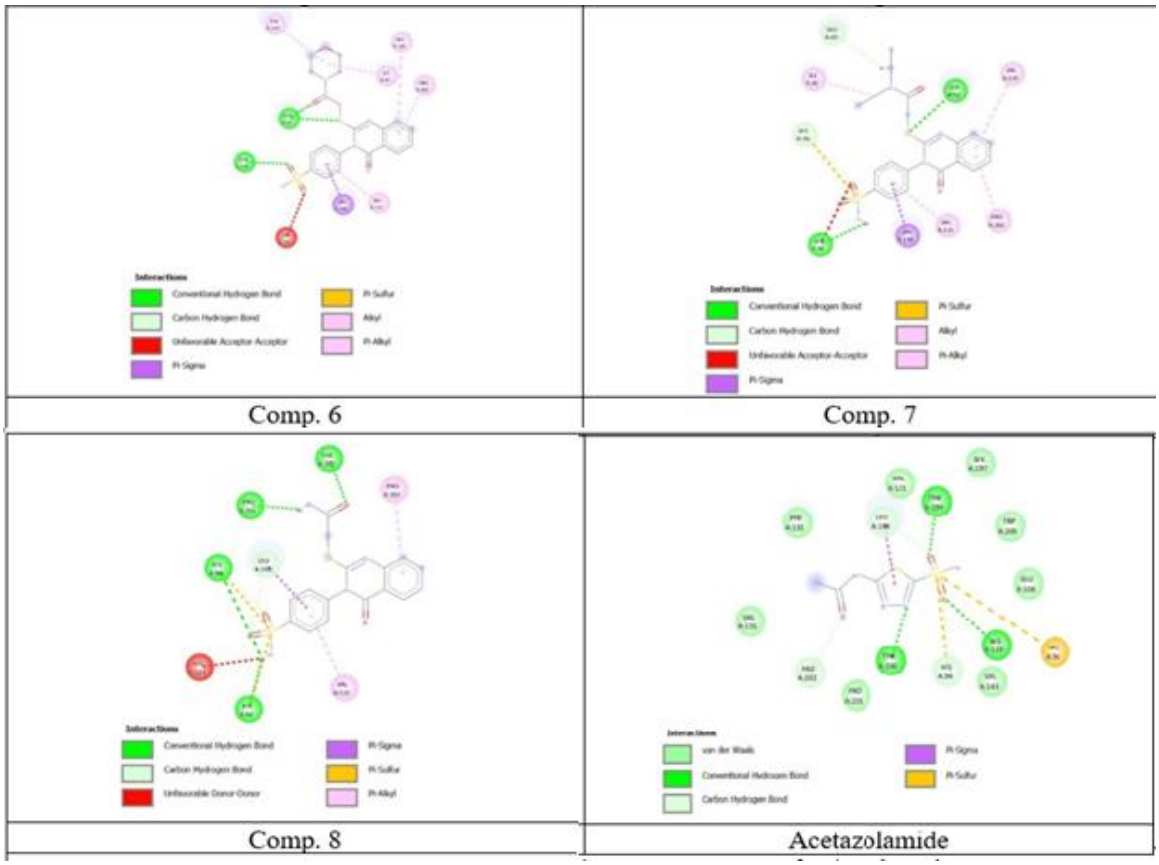


Figure 7. Interactions (2D) of compounds 4-10 and Acetazolamide in the active site of CA II (1A42)

2.4. Physicochemical and pharmacokinetic properties prediction

The target molecule's physicochemical and pharmacokinetic properties were analyzed using the SwissADME system. The compounds' anticipated chemical composition was developed in Sketch (v.12), and then the Swiss ADME software converted the names to SMILE format. All chemicals tested in this study were considered for their potential drug-like effects. The findings are the result of using Lipinski's rule of five. Lipinski's rule of five is a common filter for compounds that have the potential to be used as leads in the design of new medications. A medicine that may be taken orally and follows Lipinski's rule of five will have the following characteristics: a molecular weight of 500, a logarithmic power of 5, a donor of five hydrogen bonds, and an acceptor of ten hydrogen bonds. TPSA was also analyzed because it plays a crucial role in the bioavailability of drugs. Oral bioavailability is expected to be low for medicines with a passively absorbed TPSA larger than 140 Ao. Table (5) shows that all of the compounds we produced entered the systemic circulation, with TPSA values above 100 Ao and bioavailability above 0.55.

Table 5. ADME results of the promising compounds.

Comp No.	No. H-Bond acceptor	No. H-Bond donor	Molar refractivity	TPSA	GI absorption	BBB permeant	Bioavailability Score	Lipinski rule
3	5	1	85.59	142.23	Low	No	0.55	Yes; 0 Violation
4	6	2	138.92	157.83	Low	No	0.55	Yes; 1 Violation MW>500
5	6	1	107.57	149.04	Low	No	0.55	Yes; 0 Violation
6	7	1	120.07	158.27	Low	No	0.55	Yes; 0 Violation
7	6	1	117.19	149.04	Low	No	0.55	Yes; 0 Violation
8	6	2	97.77	171.82	Low	No	0.55	Yes; 0 Violation
9	7	2	121.68	170.72	Low	No	0.55	Yes; 0 Violation
10	7	2	121.52	170.19	Low	No	0.55	Yes; 0 Violation

3. Conclusion

Briefly, we have created a new class of benzene-sulfonamide compounds through synthesis. All of these drugs had markedly higher anticancer activity than the standard treatment, acetazolamide, in a breast (AMJ-13) cancer cell line. The most effective chemicals in this series were 5, 7, and 10. In terms of IC_{50} values, compound 10 was the most active, coming in at 0.10 mM, followed by compounds 7 and 5. In addition, molecular docking analysis confirmed that these active compounds showed a selective fit for the target active sites of the enzymes CA IX, and CA XII, indicating that they likely operate as inhibitors of CA IX and CA XII rather than CA II. The bulkiness of the tail may contribute in deciding the selectivity of the compound to the enzyme, as the compounds with moderately bulky tails showed more selectivity to CA IX, while the compounds with slightly bulky tail have been showed more selectivity to CA XII, and those compounds showed less probability to bind to CA II.

4. Experimental

With an open capillary on a Stuart melting point apparatus, the melting points have been measured (Stuart, UK). Infrared (IR) spectra were obtained from KBr discs by use of a Fourier transform infrared (FT-IR) spectrophotometer (Shimadzu, Japan). An NMR spectrophotometer (Bruker AXS Inc., Switzerland) was used to acquire ^{1}H NMR spectra, with the 1 H frequency set at 500 MHz and the ^{13}C frequency set at 75.65 MHz. In DMSO-d6, chemical shifts are reported as ppm with respect to TMS.

4.1. Chemistry

4.1.1. 4-(2-mercapto-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide 3

A mixture of carbon disulfide (1.8 ml, 0.03 mol) and the *p*-amino sulfonamide (1.67 g, 0.012 mol) has been added dropwise to a refluxed mixture of anthranilic acid (1.8g, 0.01 mol) and potassium hydroxide (2 g, 0.012 mol) in methanol (10 ml). The mixture was refluxed for 3 hr. then the product was filtered, washed with methanol, and dried. The product has been dissolved in KOH solution (10%, 10 ml), filtered, and the conc. HCl has been added dropwaise to the filtrate. The white precipitate obtained was filtered, washed with distilled water, and dried.

Yield 84%; m.p. 251.5 °C. IR: 3288, 3241 (NH_2), 3073 (aromatic CH), 1703 (CO), 1620 (CN), 1324, 1196 (SO_2). ^{1}H NMR: 8.80 (s, 1H, NH), 8.37-7.49 (m, 8H, Ar-H),

7.48-7.25 (s, 2H, NH_2 of sulfonamide). ^{13}C NMR: 161, 157, 144, 139, 136, 134, 127, 125, 122.

4.1.2. 2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-subistituted acetamide (**4-10**)

General Procedure

A mixture of 3 (0.01 mol) and 2-chloro-N-subistituted acetamide derivatives (0.01 mol) in dry acetone (50 mL) and anhydrous K_2CO_3 (0.5g) was stirred at room temp. for 10 h. The mixture has been filtered and the product formed was crystallized from ethanol to give **4-10**.

4.1.2.3. 4-(2-((2-morpholino-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)benzenesulfonamide (**4**). **4:** Yield, 79%; m.p. 262 °C. IR: 3380, 3392 (NH_2), 3012 (aromatic), 2942, 2912, 2896 (aliphatic), 1680, 1650 (2C=O), 1591, 1584, 1562 (3CN), 1386, 1184 (SO_2), 1155, 1127 (2C-O). ^1H NMR: 8.36-7.44 (m, 8H, Ar-H), 7.21 (s, 2H, SO_2NH_2), 4.01 (s, 2H, CH_2), 3.67 (t, 4H, O-CH_2) 3.55 (t, 4H, N-CH_2). ^{13}C NMR: 171, 160, 157, 143, 138, 136, 134, 127, 123, 118, 66, 46.

4.1.2.2. N,N-dimethyl-2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (**5**). **5:** Yield, 77%; m.p. 242.9 °C. IR: 3288, 3241 (NH_2), 3073 (aromatic), 3010 (aliphatic), 1682, 1650 (2CO), 1592, 1584, 1562 (3CN), 1324, 1155 (SO_2). ^1H NMR: 8.26-7.46 (m, 8H, Ar-H), 7.24 (s, 2H, SO_2NH_2), 3.94 (s, 2H, CH_2), 2.96 (s, 6H, $N(CH_3)_2$). ^{13}C NMR: 170, 159, 157, 143, 136, 134, 133, 128, 127, 126, 36, 33.

4.1.2.1. 2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(2,4,6-trichlorophenyl)acetamide (**6**). **6:** Yield, 72%; m.p. 288.5 °C. IR: 3378, 3288, 3241 (NH_2, NH), 3013 (aromatic), 2942 (aliphatic), 1677, 1639 (2CO), 1593 (CN), 1374, 1183 (SO_2), 714 (C-Cl). ^1H NMR: 9.62 (s, 1H, NH amide), 8.33-7.43 (m, 10H, Ar-H), 7.48-7.25 (s, 2H, SO_2NH_2), 3.99 (s, 2H, CH_2). ^{13}C NMR: 167, 160, 157, 143, 139, 136, 134, 133, 131, 129, 128, 127,123, 118, 33.

4.1.2.4. N,N-diethyl-2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (**7**). **7:** Yield, 75%; m.p. 248.8 °C. IR: 3288, 3117 (NH_2), 3104 (aromatic), 3021, 2979 (aliphatic), 1680, 1641 (2C=O), 1593, 1584, 1558 (3CN), 1376, 1183 (SO_2). ^1H NMR: 8.35-7.44 (m, 8H, Ar-H), 7.22 (s, 2H, SO_2NH_2), 4.02 (s, 2H, CH_2), 3.43 (q, 4H, N-CH_2), 1.18 (t, 6H, $2CH_3$). ^{13}C NMR: 170, 160, 157, 143, 138, 136, 134, 127, 123, 118, 41, 33, 13.

4.1.2.5. 2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)acetamide (**8**). **8:** Yield, 81%; m.p. 222.8 °C. IR: 3288, 3240 (NH_2), 3103 (aromatic), 3074, 3010 (aliphatic), 1681, 1641 (2C=O), 1593 (CN), 1324, 1183 (SO_2). ^1H NMR: 8.33-7.10 (m, 8H, Ar-H), 7.03 (s, 2H, SO_2NH_2), 5.83 (s, 2H, NH_2), 3.89 (s, 2H, N-CH_2). ^{13}C NMR: 172, 160, 157, 143, 138, 136, 134, 127, 123, 118, 41, 33, 13.

4.1.2.6. 2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(pyridin-2-yl)acetamide (**9**). **9:** Yield; 87%; m.p. 241 °C. IR: 3288, 3240, 3122 (NH_2, NH), 3067 (aromatic), 2981, 2920 (aliphatic), 1684, 1642 (2C=O), 1604 (CN), 1328, 1173 (SO_2). ^1H NMR: 8.28 (s,1H, NH), 8.27-7.02 (m, 12H, Ar-H), 7.21 (s, 2H, SO_2NH_2), 4.04 (s, 2H, CH_2). ^{13}C NMR: 168, 160, 158, 152, 147, 143, 138, 137, 133, 128, 127, 126, 123, 119, 118, 33.

4.1.2.7. 2-((4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl)thio)-N-(1H-pyrrol-2-yl)acetamide (**10**). **10:** Yield, 78%; m.p. 298.8 °C. IR: 3378, 3288, 3241 (NH_2, NH), 3103 (aromatic), 3012 (aliphatic), 1659, 1642 (2C=O), 1593 (CN), 1324, 1183 (SO_2). ^1H NMR: 9.42 (s,1H, NH pyrrole), 9.40 (s, 1H, NH amide), 8.33-6.21 (m, 11H, Ar-H), 7.21 (s, 2H, SO_2NH_2), 4.06 (s, 2H, CH_2). ^{13}C NMR: 168, 160, 158, 143, 138, 136, 134, 133, 127, 126, 120, 118, 107, 99, 33.

4.2. Biological evaluation

4.2.1. MTT assay

AMJ-13 Iraqi breast cancer has been collected from the country's cancer institute. After incubating the 96-well plate for 24 hours, an MTT test was performed. The cell layer is then washed with 0.25% (w/v) Trypsin and 0.53 mM EDTA. 1% penicillin, streptomycin, and 10% fetal bovine serum have been added to DMEM (Dulbecco's Modified Eagle's Medium) to cultivate the cells. Put in 10% as much reconstituted MTT as there is in the culture medium. Keep warm for 2 hours. After incubation, the formazan crystals formed should be dissolved by adding MTT solubilization solution equal to the volume of the starting culture medium. Calculate the absorbance at a wavelength of 570 nm using spectrophotometry [29]. By utilizing Graph Pad Prism and the Boltzmann sigmoidal concentration-response curve equation, an IC50 value was determined and compared to that of the standard medication acetazolamide. Table 1 displays the findings.

4.3. Molecular docking

GOLD software was used for all molecular modelling analyses. ChemBio3D's MM2 force field was used to perform energy minimizations, and the partial charges were computed mechanically. For this purpose, we used the entry for 6T5P, 1A42, and 5FL4 in the Protein Data Bank as shown in **Tables (2-4)** and **Figures (2-7)**. The energy minimization of the target enzymes was conducted by SPDBV (Swiss Protein Data Bank Viewer) software. The visualization of the complexes was conducted by Discovery Studio software. (i) The enzymes were prepared for docking research by first removing the ligand molecule from the active sites of the enzymes. (ii) The structures were supplemented with hydrogen atoms that had been added in the usual geometric orientation. (iii) The generated model was then applied to active sites prediction of ligand enzymes. (iv) Docking of the target molecules into the enzymes' active sites.

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