ORIGINAL ARTICLE

CONTENTS

Designing and molecular docking analysis of newly acetazolamide derived compounds integrating 4-oxothiazolidine group as possible carbonic anhydrase deactivators

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ABSTRACT

Aim: The study aims to create new acetazolamide products that have a thiazolidin-4-one moiety and test how well they stop the carbonic anhydrase XII enzyme (protein data bank code: (4KP5)) and defeat cancer in silico.

Materials and Methods: We will make four acetazolamide derivatives that have a thiazolidin-4-one group. Acetazolamide will be the starting material. We used the Molecular Operating Environment program 2015.10 to figure out the molecular docking studies. Root Mean Square Deviation (RMSD) and Standard Score (S. score) were used to describe docking how well docked ligands stuck to the target protein carbonic anhydrase XII.

Results: The process of docking showed that the four test ligands (IIIa, IIIb, IIIc, and IIId) bound to the target protein carbonic anhydrase XII more strongly than the reference ligand, which is an acetazolamide.

Conclusions: Of the four chemicals, IIIa had the maximum affinity for binding and a S. score of -7.39. The results propose that the acetazolamide compounds that were designed, especially IIIa, could be carbonic anhydrase inhibitors and anti-cancer drugs that work on the carbonic anhydrase XII enzyme.

KEY WORDS: molecular docking, cancer, acetazolamide derivatives, thiazolidinone moiety, carbonic anhydrase

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INTRODUCTION

Enzymes are very important for many biochemical reactions that happen in living things. Because of how selective and powerful they are at starting certain chemical events, they are very interesting candidates for therapeutic intervention. There has been a rise in the utility of enzymes as promising drug goals in the last few years because they play a role in many diseases, such as cancer, metabolic disorders, and infectious diseases [1-2]. The word "cancer" describes a vast range of disorders that are given rise to the growth of abnormal cells which able to proliferate out of control, infiltrate and damage vital tissue, and destroy it [3]. Cancer is still odd of the most common source of dying around the globe, coming after heart disease [4]. Among the largest problems confronting healthcare agencies is that cancer treatments are failing more often, mostly because tumours are becoming resistant to anticancer drugs [5]. Four new acetazolamide derivatives that have a thiazolidin-4-one moiety are being studied in silico. These derivatives target carbonic anhydrase and are part of new strategies to deal with intrinsic tumour cell heterogeneity, stabilize DNA damage, inactivate drugs, changing targets of drug, and stop cells from dying. These variables all play a big role in drug resistance [6-7]. It seems that tumour protection mechanisms based on the microenvironment have a better chance of being targeted for treatment. The microenvironment is very important in cancer research because it affects how well cancer cells survive and spread [8-9]. The acidity and lack of oxygen in the tumour microenvironment affect how cancer cells work. Acidosis and hypoxia usually make cancer cells more aggressive, move around, and spread, which makes treating tumours much harder. Because of this, therapies that stop these processes are better [10], because of this, there has been a lot of research on the family of carbonic anhydrase enzyme, which affect the pH inside cells [11]. Carbonic anhydrase enzyme (CA) is a group of enzymes which have a metal of zinc ion in the active site and can convert water and carbon dioxide to bicarbonate and a hydrogen ion in a reversible way [12]. There are 15 different types of carbonic anhydrase enzymes in humans. These types have different catalytic activities and locations. CA I, CA III, CA VII, and CA XIII are

examples of cytosolic carbonic anhydrases. CA VA and CA VB are examples of mitochondrial carbonic anhydrases. CA VI is an example of a secretory carbonic anhydrase that can be found in saliva and colostrum. CA IV, CA IX, CA XII, CA XIV, and CA XV are examples of membrane-attached carbonic anhydrases. Three "CA- linked proteins," CA VIII, CA X, and CA XI, are also types that are not active of carbonic anhydrase [13]. There is too much (CA XII) in some solid tumours, but not enough in other healthy tissues. This overexpression helps tumour cells grow, stay alive, and spread in the tumour microenvironment. So, CA XII has been proven as a good objective for cancer examination and management [14]. It changes the acidity, growth, and aggressiveness of tumours, starts the metastatic cascade, and makes tumours less responsive to chemotherapy [15]. When CA XII is overexpressed, it causes cancer to grow. Higher levels of CA XII and CA IX make cancer cells grow faster, start spreading, and make treatments less effective. Hypoxia causes CA XII upregulation in many lines of cells, just like CA IX. However, CA XII expression is found in kidney and breast cells, while CA IX is not. Oestrogen receptors and low oxygen levels also control the expression of CA XII [16]. Targeting CA XII in cancers that show high levels of these markers and stopping their function has been shown to help treat tumours [17]. All catalytic human carbonic anhydrases (hCAs) have a very similar internal binding site that lets zinc ions (Zn2+) bind to them. This is important for carbon dioxide hydration. Because of this, most of the hCA inhibitors found so far have a zinc-attaching part, which is mostly a sulfonamide [18]. Sulfonamides are important because they are used in medicines that have biological activity [19]. Sulfonamides were first widely used as chemotherapeutic drugs and to prevent certain diseases. Since 2005, they have gotten a lot of attention as possible cancer treatments because they can stop carbonic anhydrase [20]. Sulfonamide derivatives can confirm different bindings with the enzyme's unique bipolar structure, based on the groups of substituents on the aromatic moiety, whether they are in the core active site or on the edge [21]. In medicinal chemistry, five-membered heterocyclic chemicals, in specific those with more than one heteroatom, have a wide variety of biological functions [22]. Over time, thiazolidinone has gotten the most attention. It has three distinct atoms: a sulphur atom at stance 1, a nitrogen atom at stance 3, and a carbonyl group at positions 2, 4, or 5 [23]. Researchers are looking into several derivatives of Thiazolidinone with different substituents as possible anticancer agents because thiazolidinone fragments are often used to change lead compounds in anti-tumor drugs [24]. These compounds may have the ability to defeat cancer in a number of methods, like by starting programmed cell death, halting the cell cycle, or making reactive oxygen species. 4-Thiazolidinone-containing chemicals are very good at stopping a number of enzymes, such as tubulin polymerisation, heat shock protein HSP90, carbonic anhydrases, vascular endothelial growth factor receptor 2 (VEGFR2), cell division cycle 25 A (CDC25A), human epidermal growth factor 2 (HER-2), histone deacetyase (HDAC), B-cell leukemia/lymphoma 2 protein (BCl-2), and protein/tyrosine kinases [25-26]. The goal of this study is to make some Inhibitors for Carbonic Anhydrase using derivatives of 4-thiazolidinone. Table (1) shows a list of goal compounds (IIIa to IIId) and the substituents that go with them (R groups). Every R-group stands for a certain chemical part that is linked to the compound's basic structure. This can have a big effect on the physicochemical properties of compound. A molecular docking study was done to see how well the carbonic anhydrase enzyme binds to other molecules. Next, the strongest compounds that have strong enzymatic effects will be chosen for more chemical synthesis.

AIM

The study aims to create new acetazolamide products that have a thiazolidin-4-one moiety and test how well they stop the carbonic anhydrase XII enzyme (protein data bank code: (4KP5)) and defeat cancer in silico.

MATERIALS AND METHODS

SYSTEM AND SOFTWARE CONFIGURATION

The MSI system used in this study had 4.00 GB of random-access memory and Intel Core i3-2330M processors that ran at 2.2 GHz. We set up and installed both ChemDraw 12.0 and MOE 2015.

LIGAND AND RECEPTOR PREPARATION USING MOLECULAR DOCKING TECHNIQUE

The docking process in this work has two parts:

Step 1. Ligand preparation: ChemDraw software (12.0) was utilized to accurately show the molecular structures of the ligand, then, ligand was protonated in 3D, a partial charge was added, energy was lowered, and the findings were restored.

Table 1. Chemicals of Interest, IIIa, IIIb, IIIc, and IIId are target compounds that are OCH_3, Br, OH, and NO_2, respectively

Name	R
IIIa	OCH_3
IIIb	Br
IIIc	OH
IIId	NO_2

Source: compiled by the authors of this study

Table 2. Molecular docking of the test ligands (IIIa-IIId) in comparison to acetazolamide Shows the S score, Rmsd, and main amino acids that are involved in the final ligands' interactions

Name	R group	S score	RMSD	No. of binding sites	binding amino acids
ACZ	****	-5.7	1.5	2	Zn301,Thr198
IIIa	OCH_3	-7.39	1.6	3	Zn301,Thr198,Asn64
IIIb	Br	-7.0	1.9	4	Zn301,Thr198, Asn64,Lys69
IIIc	OH	-6.8	1.6	4	Zn301,Thr198, Asn64,Lys69
IIId	NO_2	-7.0	1.6	4	Zn301,Thr198, Gln89, Trp4

**** - there is no R group

Source: compiled by the authors of this study

Step 2. Protein organization: The crystal framework of Carbonic Anhydrase XII (code: 4kp5) was downloaded to the Molecular Operational Environment (2015) to get the protein ready. The coming steps were taken for preparation of the target protein: only the chains that were involved in the protein action were chosen, small compounds were deleted, water molecules were removed, the active site was built first, the potential of the protein atoms was adjusted, and then hydrogen hide bonds were added. The docking process was done after the MOE had the ligand that had been made earlier from the saved data.

This study is continuation of the article: Ayoub SAZ, Aldabagh NHN, Atiya R (2025) Design and molecular docking study of new acetazolamide derivatives incorporating a 4-Thiazolidinone moiety as potential carbonic anhydrase XII inhibitors. Review of Clinical Pharmacology and Pharmacokinetics – International Edition 39(1): 51-61. https://doi.org/10.61873/RGHB5923.

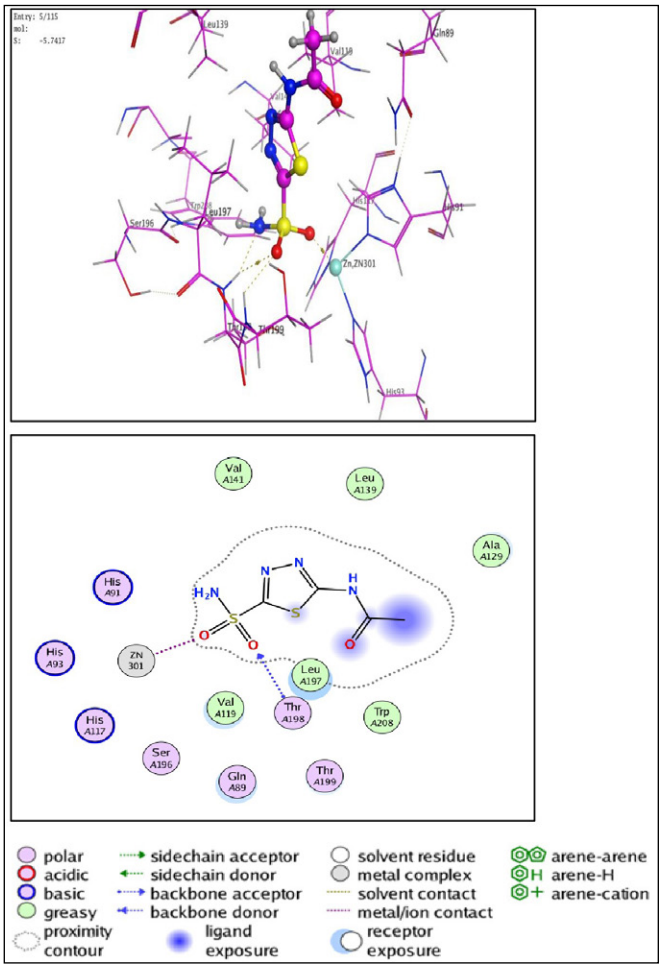

Fig. 1. MOE visualization of reference ligand (acetazolamide) with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

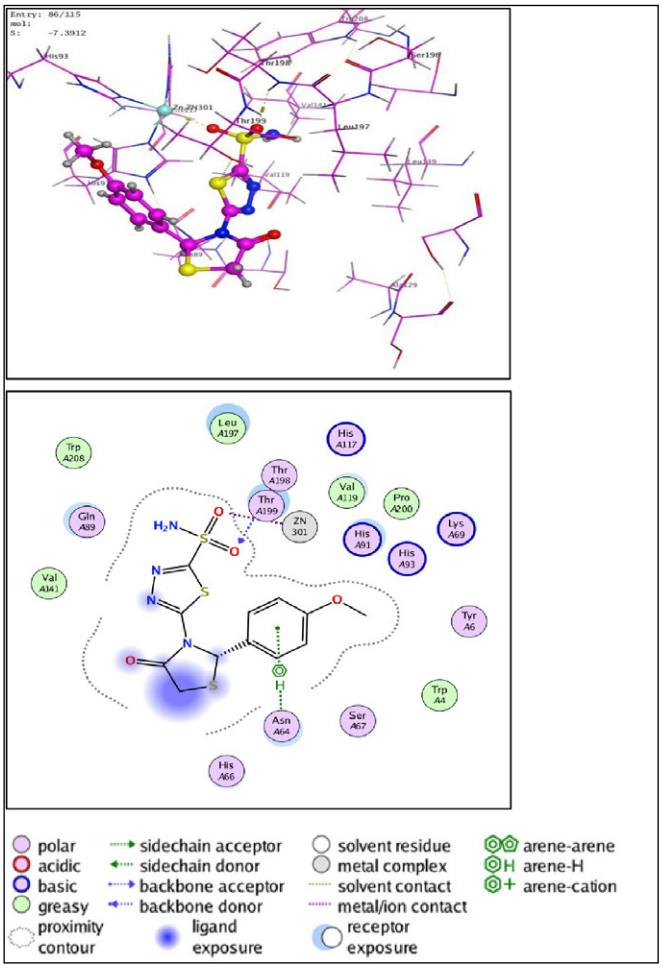

Fig. 2. MOE visualization of compound IIIa with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

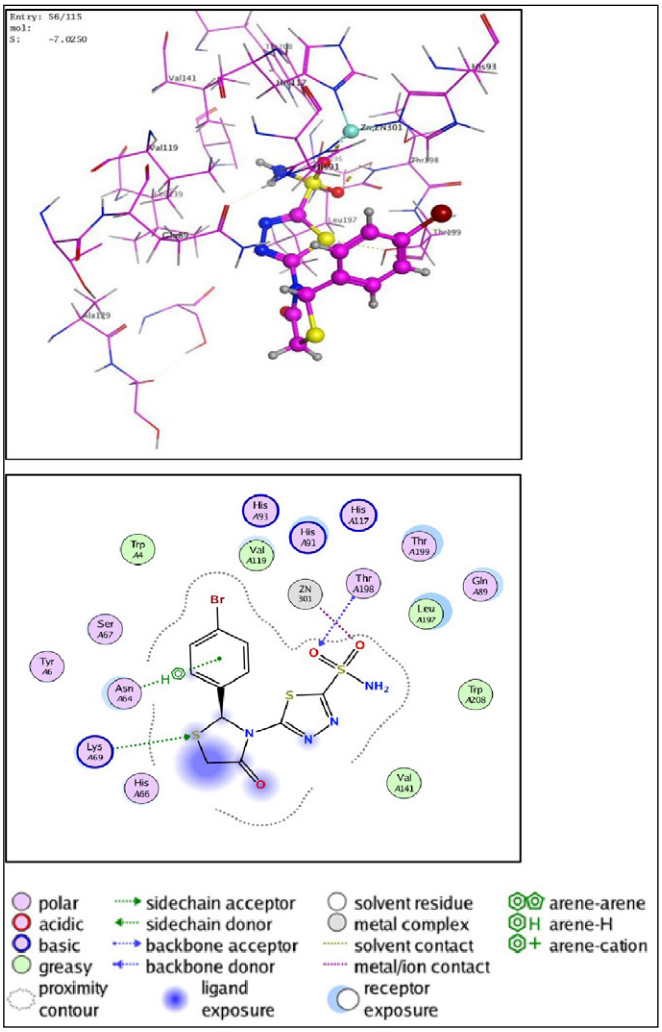

Fig. 3: MOE visualization of compound IIIb with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

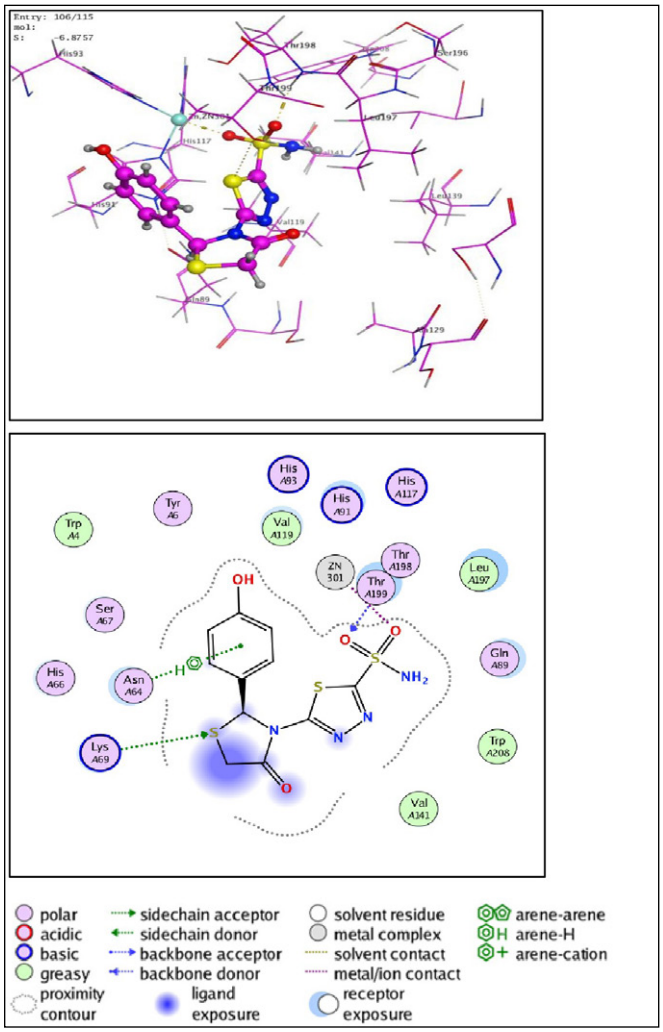

Fig. 4. MOE visualization of compound IIIc with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure

Source: compiled by the authors of this study

RESULTS

Molecular docking shows that some ligands interact strongly with an active site in a target. The MOE was created due to it gives a full graphical picture of where ligands are and how they bind to receptor residues. This makes it easy to see, define, and estimate how proteins bind to ligands. The molecular operating environment makes a unique connection between the suspected compounds and the enzyme CA XII, which is in the alike active site as acetazolamide. It is important to quickly figure out how much the compound stops things from happening and how similar the amino acids are to each other and to active sites. Table (1) shows the interaction parameters for the final compounds IIIa, IIIb, IIIc, and IIId.

These parameters include RMSD and S. score values, as well as the identification of important amino acids that are involved in these interactions. These parameters show how well the designed compounds and the target proteins fit together and how much energy it takes to bind them. The S. score and the root mean deviation (RMSD) show how far apart the target point and the ligand atoms are on average for the anti-cancer compound. This shows how this method works. This means that compounds have the best pose when the S. score is high and the RMSD is low. The S. score for all of the target products (IIIa-IIId) is higher than that of the control (acetazolamide). So, they work better than other carbonic anhydrase inhibitors. In addition, their S. scores are -7.39, -7.02, -6.8, and -7.0, and their RMSD values are 1.6, 1.9, 1.6, and 1.6, as shown in Table (2).

The following figure (1) shows the MOE visualization of Acetazolamide with hCA XII (PDB code: 14KP5)

The ligand-protein interaction as 3D structure is shown in the top image, and the 2D structure is shown in the bottom image. In Figure (1), Acetazolamide only binds to zinc and the amino acid threonine 198 in the active site of hCA XII. Using PDB code 4kp5, Figures (2–5) show how compounds IIIa–IIId dock with hCA XII.

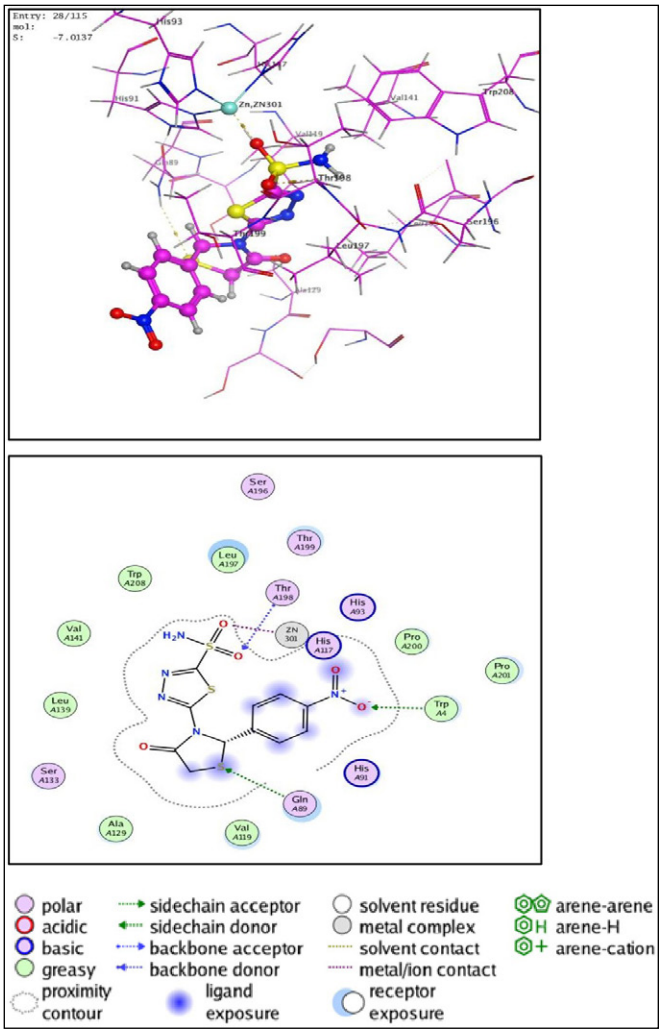

Fig. 5. MOE visualization of compound IIId with carbonic anhydrase XII (PDB code: 4KP5), (above) is the 3D structure (below) is the 2D structure
Source: compiled by the authors of this study

Each figure shows the 3D and 2D structures of the interactions between the ligand and the protein. Every acetazolamide derivative finds zinc and a hydrogen bond with the important amino acid Threonine198. The MOE visualization of compound IIIa with hCA XII is shown in Figure (2). Compound IIIa has the highest S. score of -7.39, which means it has a strong binding affinity. The high S. score could be because of more hydrogen bonding with Asn64, which is important for CAXII's catalytic activity. A methoxy group on this compound may help it fit better inside the receptor pocket. The IIIb binds to important amino acids, especially Lys69 and Asn64, in the active site of carbonic anhydrase XII in ways that are different from the other compounds. Figure (3) shows these interactions in both 3D and 2D forms. In Figure (4), you can see the docking simulation of compound IIIc with hCA XII. It shows that its Thiazolidinone part binds more strongly to the amino acid Lys69, which is vital for substrate interaction at the active site of CAXII. Finally, IIId binds more strongly to the crucial amino acid Trp4 in the active site of CA XII. Figure (5) shows the docking visualization of compound IIId.

DISCUSSION

The active site of human carbonic anhydrase XII has a big conical-figured hole in it that is made up of a hydrophobic part and a hydrophilic part. There is a catalytic cleft inside the hole of this enzyme. Zinc is in this cleft and works as a cofactor. His91, His93, and His117 are three residues of histidine that hold the zinc ion in place and keep its positive charge stable. Threonine 198 is close to the active site and helps keep the enzyme's structure stable. It might also help bind the substrate and keep the transition state stable while catalysis. So, the compounds have to stick to both Thr198 and zinc to stop carbonic anhydrase XII from working. More connecting with other amino acids like Lys67, Gln89, and Asn64 helps the synthesized compounds work better as inhibitors. This is because these amino acids may help the active site cavity stay in the right shape by binding to the substrate and interacting with nearby residues. All of these amino acids are listed in Table (2). Acetazolamide is a very important standard because it is a famous and well-assessed carbonic anhydrase inhibitor. Also, it has a pharmacophore similar to the compounds we looked at.

CONCLUSIONS

The ongoing investigation has shown that four acetazolamide derivatives with thiazolidin-4-one moiety (IIIa-IIId) have both a viable carbonic anhydrase inhibition effect and anti-cancer properties. Most of the compounds had a stronger affinity for the target protein than acetazolamide. The compound that looks the most promising is IIIa, which has an OCH_3 group on the benzene ring and a S. score of -7.39.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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