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Original Research Article

Synthesis and *In Silico* Study of New Acetazolamide Derivatives Incorporating a 1,2,4-Triazole Moiety as Potential Carbonic Anhydrase Inhibitors and Anti-cancer Agents

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ABSTRACT

A significant number of 1,2,4-triazole derivatives are known to possess numerous pharmacological properties. The present study aims to synthesize new acetazolamide derivatives with 1,2,4-triazole moiety and investigate their carbonic anhydrase inhibitory and anticancer activities *in silico*. Three acetazolamide derivatives having a 1,2,4-triazole moiety were synthesized from the reaction between acetazolamide and hydrazine monohydrate as starting materials. The interaction of the synthesized ligands with carbonic anhydrase XII (CAXII) enzyme (PDB code: 4QJW) a putative biomarker for solid tumors was investigated *in silico* via molecular docking simulation. The binding affinities of docked ligands with the target protein CAXII were reported in terms of S score and Root Mean Square Deviation (RMSD), which were calculated using the Molecular Operating Environment (MOE) software 2015 program. The docking results revealed a better binding affinity of the three test ligands (Sa, Sb, and Sc) for the target protein CAXII than the positive control ligand, acetazolamide. Of the three compounds, compound Sc showed the highest binding affinity with an S score of -7.0168. These observations suggest that the newly synthesized acetazolamide derivatives particularly compound Sc hold promise as carbonic anhydrase inhibitors, and anticancer agents targeting the CAXII enzyme.

Keywords: *In silico*, Triazole moiety, Carbonic anhydrase inhibitor, Anticancer.

Introduction

Cancer is the second leading cause of death in the United States of America after cardiovascular diseases, and it is a significant global public health concern.[1,2] The primary cause of cancer death is still metastasis, despite recent therapeutic advancements in the treatment of cancer.[3,4] The primary reason why chemotherapy does not work is that the chemotherapeutic drug do not get to the cancerous cells, hence, the need for increase in the dose. Due to the non-selective nature of the drugs, an increase in dose result in a severe and significant damage to normal cells.[4] Resistance to anticancer drugs can be attributed to many factors, such as conserved but increased drug efflux, genetic mutations and epigenetic modifications, and other cellular and molecular pathways.[5] Due to the limitations, such as drug resistance, high toxicity, adverse effects, and a lack of selectivity of the currently available chemotherapeutic medications, there is the need for the development of novel drug candidates that can address these issues.[6] Carbonic anhydrase (CA) is a type of metalloenzyme that reversibly catalyzes the hydration of carbondioxide(CO_2) to bicarbonate (HCO_3^-). Alpha-CAs are extensively expressed in mammals and may be divided into 16 isoenzymes based on their enzymatic efficiency, subcellular localization, catalytic activity, and sensitivity to several inhibitor classes.[7] There are 12 of the 16 alpha-CA class isoenzymes that are catalytically active in humans.

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These enzymes have a zinc cation in their active site.[8] Different physiological functions and expression patterns are associated with these isoenzymes.[7,8] Several CAs have been linked to the development of neoplasm, and certain cancers have been observed to display extensive expression of CA II and the hypoxia-inducible CA IX and CA XII.[9]

Increased CA IX and CA XII expression increases cancerous cell proliferation, metastasis initiation, and poor treatment response.[10] Like CA IX, hypoxia in several cell lines induces CA XII expression, however, CAXII expression is observed in renal and breast tissue, in contrast to CA IX. Regulation of CAXII expression is influenced by estrogen receptors and hypoxia.[11] Numerous compounds, including sulfonamides, sulfamates, and sulfamides, among others, inhibit these enzymes. In medicine, carbonic anhydrase inhibitors (CAIs) are utilized as diuretics, anti-obesity, anticancer medications, antiglaucoma, and diagnostic aids. Certain CAIs are also used to treat other neurological conditions or as anticonvulsants.[12] The unique chemical properties and diverse biological activity of heterocyclic molecules make them attractive to medicinal chemists in their research and the possibility of using them in treating diseases, including cancer. Heterocyclic molecules are essential since they are the building blocks of many biological chemicals related to living organisms, such as hormones, vitamins, antibiotics, and nucleic acids. Most chemical molecules found in naturally occurring products, physiologically active complexes, and chemicals frequently used in medical chemistry are heterocyclic compounds containing nitrogen atoms.[13] Triazoles have better pharmacological uses among the heterocyclic molecules containing nitrogen heteroatom.[14] Because of its electron-rich characteristics and aromaticity, triazole easily binds to enzymes and receptors and is frequently used in many fields.[15] Structurally, triazoles include two isomers; 1,2,3-triazole and 1,2,4-triazole.[16] By interacting at the receptor's active site as both a donor and an acceptor of hydrogen bonds, the 1,2,4-triazole nucleus functions as a significant pharmacophore and is metabolically stable. The hydrophilicity of the triazole moiety can enhance the ligand's solubility and improve the molecule's pharmacological properties significantly. A significant number of 1,2,4-

triazole derivatives are known to possess anticancer activity.[17] In the light of the above, the present study aim to synthesize novel acetazolamide derivatives, a 1,2,4-triazole ring attached to an aromatic aldehyde, and investigate their potentials as CAIs and anticancer agents *in silico*.

Materials and Methods

Synthesis of acetazolamide derivatives

Compounds Sa – Sc, three acetazolamide derivatives were synthesized according to the scheme depicted in Figure 1.

Compound Sa was synthesized by the reaction of 99.9% hydrazine monohydrate and acetazolamide according to the procedure previously reported by Gümrükçüoğlu *et al*. (2023).[18] Compound Sb was synthesized according to the procedure described by Kapri *et al*. (2020),[19] while compound Sc synthesized according to the procedure described by Jubie *et al*. (2011).[20]

In silico study of compounds Sa – Sc

Computer software and system

An HP computer system with the following specifications was used for the *in silico* study: CPU @ 2.80 GHz,12 GB RAM, 11th Gen Intel(R) and Core (TM) i7-1165G7. ChemDraw Professional Software Pro 20.0 and MOE 2015 were downloaded and installed.

Ligand/receptor preparation and molecular docking

ChemDraw Professional 20.0 was used to precisely generate the ligands molecular structures. The ligands were protonated in a three-dimensional shape, partial charge was added, energy was minimized, and the results were saved. The receptor, which was the crystal structure of artificially produced CA XII (122.06 KD) (PDB code: 4QJW) was downloaded into the Molecular Operational Environment (MOE) via the PDB website. The target protein was prepared according to the following steps:

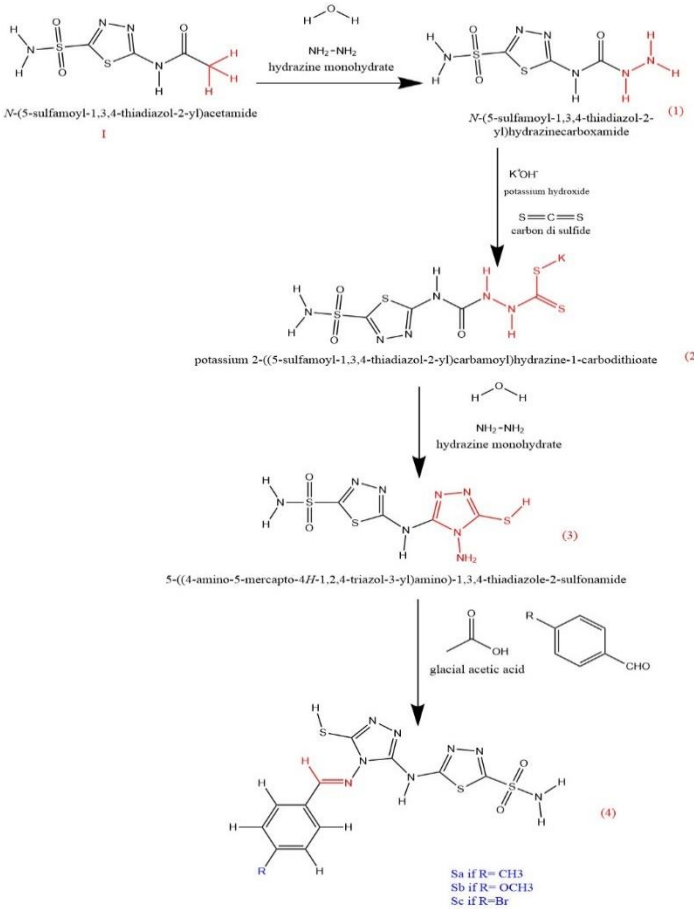

Figure 1: Scheme for the synthesis of compounds Sa – Sc

Extra chains were removed, leaving only the sequences implicated in the protein function. Water molecules were also removed, followed by the addition of H-bonds. Thereafter, the potential of the atoms in the protein structure was fixed and its active site was identified. Then, the previously prepared ligands were added to the MOE using the data that had been saved, to complete the docking process.

Results and Discussion

Molecular docking and virtual screening

Molecular docking simulation technique is used to determine how best a ligand will attach to an active site on a target. Molecular Operating Environment (MOE) was employed in this study because displaying the locations and interactions of ligands with receptor-binding residues offers a compelling visual illustration of the data. MOE facilitates the visualization, characterization, and assessment of protein interactions with ligands. From the MOE data, the test compounds (Sa – Sc) showed selective binding to the CA XII enzyme at the exact principal active site similar to acetazolamide. The inhibitory effects of the synthetic compounds were assessed using S score and root mean square deviation (RMSD) values, as well as the identity of the amino acids that interacted with the ligands at the protein's active site. The S score is a measure of the average distance between the atoms of the ligand that is posed with the protein active site. The amino acid residues of the protein active site to which acetazolamide interacted were Zn301, Thr198, and Thr199, whereas the test compound Sa interacted with the target protein at 5 binding sites, with the residues Thr199, Zn301, GlnA89, Thr198, and LysA69. Compounds Sb, and Sc, both interacted at 6 binding sites, Zn301, Thr198, Thr199, SerA130, SerA133, and LysA69 for compound Sb, and Thr199, Zn301, GlnA89, Thr198, LysA69, and AsnA64 for compound Sc (Table 1). The visualization of the interactions of the positive control ligand (acetazolamide) with the target protein carbonic anhydrase XII (PDB code: 4QJW), as well as those of the test ligands (Sa, Sb, and Sc) with the target protein are shown in Figures 2-5. The highest S score (-7.0168) and the best RMSD (1.2635) were recorded by compound Sc, indicating that the presence of bromine (Br) substituents on the benzene ring enhances the orientation of the ligand in the receptor pocket. The compound Sb having a methoxy (OMe) substituent on the benzene ring formed more hydrogen bonds with numerous amino acid residues of the target protein, giving it more stable orientation and a stronger binding affinity than the other derivatives, with an S score of -6.9471. Compound Sa, a derivative with a methyl substituent gave the lowest S score (-6.8137) among the three derivatives, but it was still better and higher than that of acetazolamide (-5.05608).

Conclusion

The present study has shown the potential carbonic anhydrase inhibitory, and anticancer activities of three acetazolamide derivatives with 1,2,4-triazole moiety (Sa – Sc). Of the three compounds, compound Sc with Br substituents on the benzene ring is thought to have the highest activity with an S score of -7.0168, while compound Sb with a methoxy substituents on the benzene ring, and with S score of -6.9471 has more stable orientation, and a stronger receptor affinity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 1: Molecular docking score of the test ligands (Sa, Sb, and Sc) compared to acetazolamide.

Compound	R group	S. score	RSMD	Number of binding sites	Amino acid residues of the protein active binding sites
Acetazolamide	****	-5.05608	1.8407	3	Zn301, Thr198, Thr199
Sa	CH_3	-6.8137	1.6634	5	Thr199, Zn301, GlnA89, Thr198, LysA69
Sb	OCH_3	-6.9471	1.9199	6	Zn301, Thr198, Thr199, SerA130, SerA133, LysA69
Sc	Br	-7.0168	1.2635	6	Thr199, Zn301, GlnA89, Thr198, LysA69, AsnA64

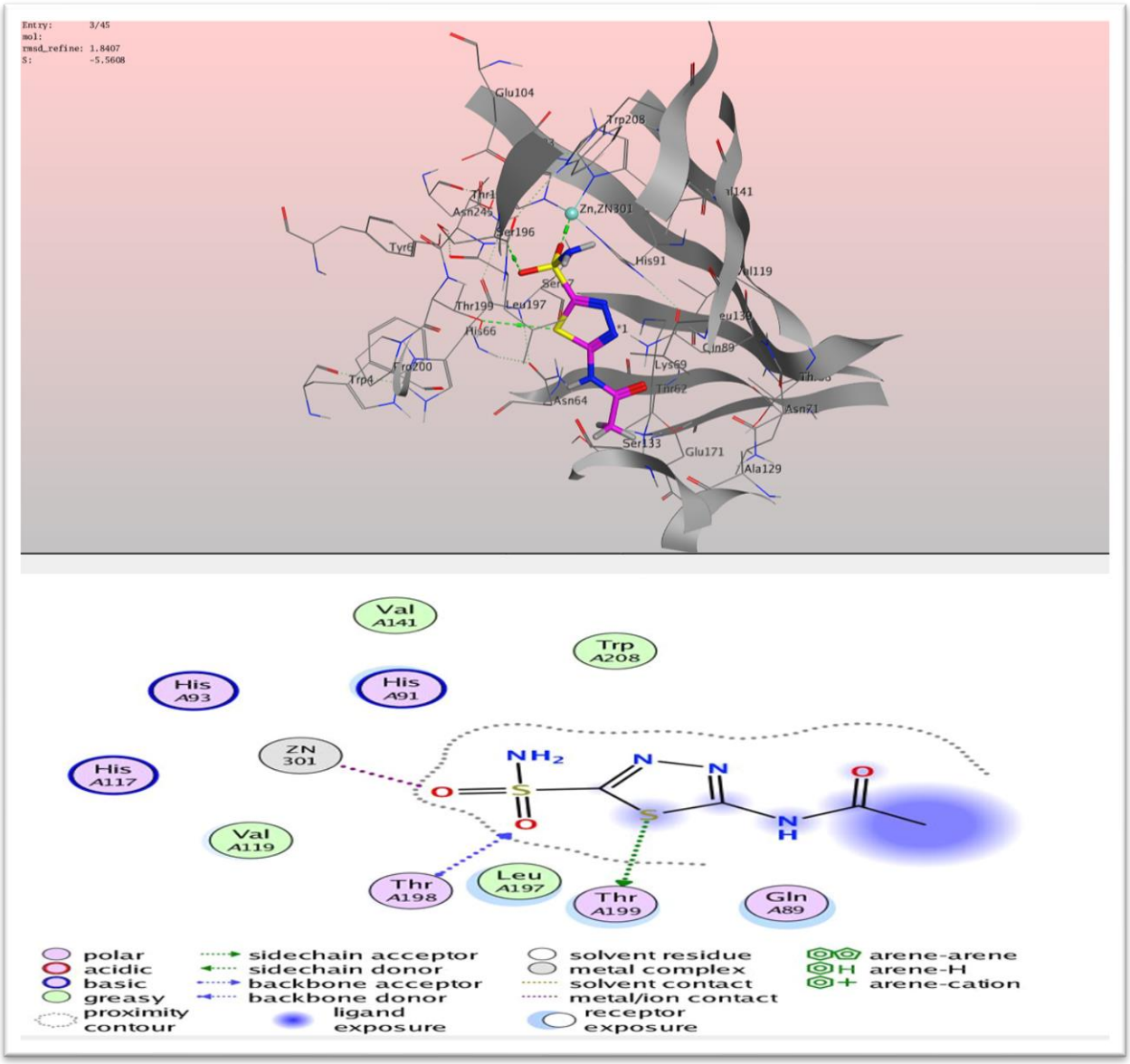

Figure 2: MOE Visualization of acetazolamide (AZM) with carbonic anhydrase XII (PDB code: 4QJW)

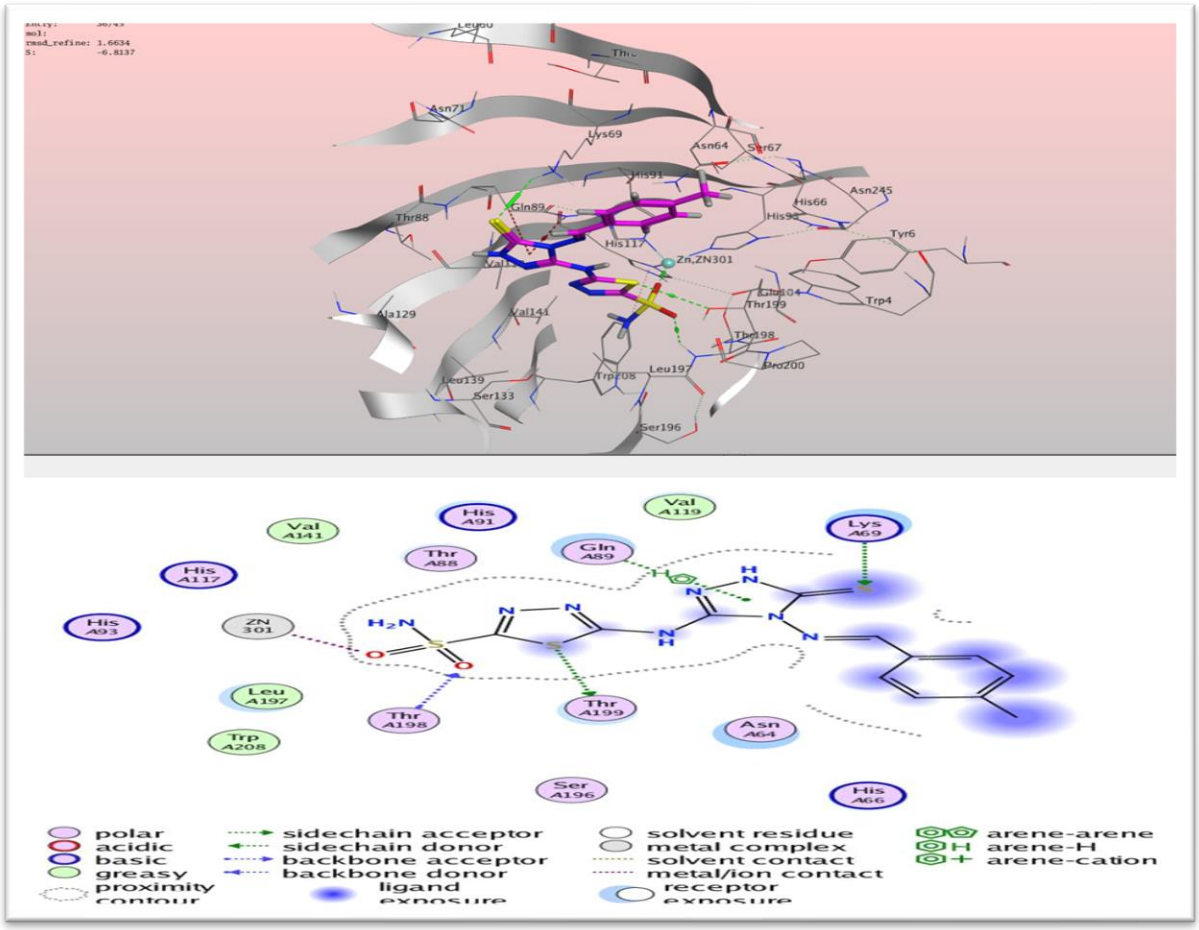

Figure 3: MOE Visualization of Ligand Sa with carbonic anhydrase XII (PDB code: 4QJW)

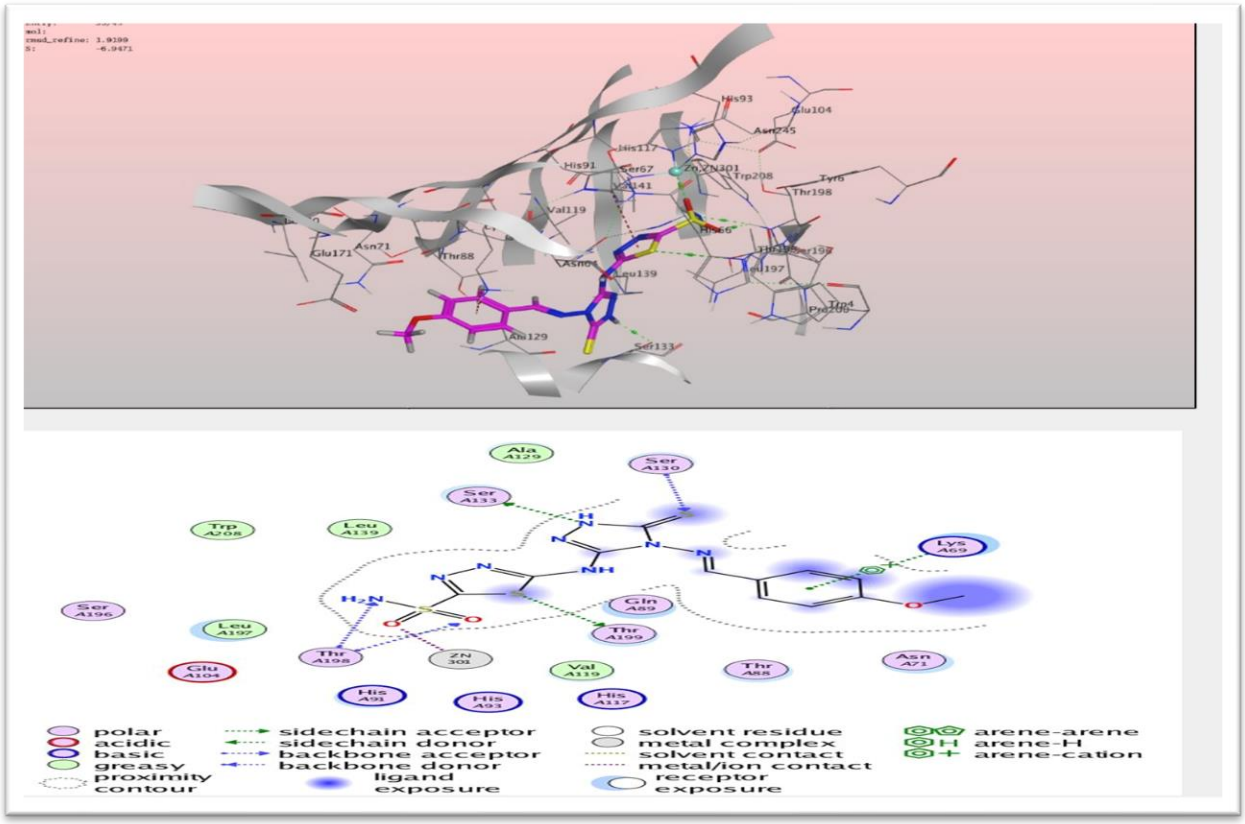

Figure 4: MOE Visualization of Ligand Sb with carbonic anhydrase XII (PDB code: 4QJW)

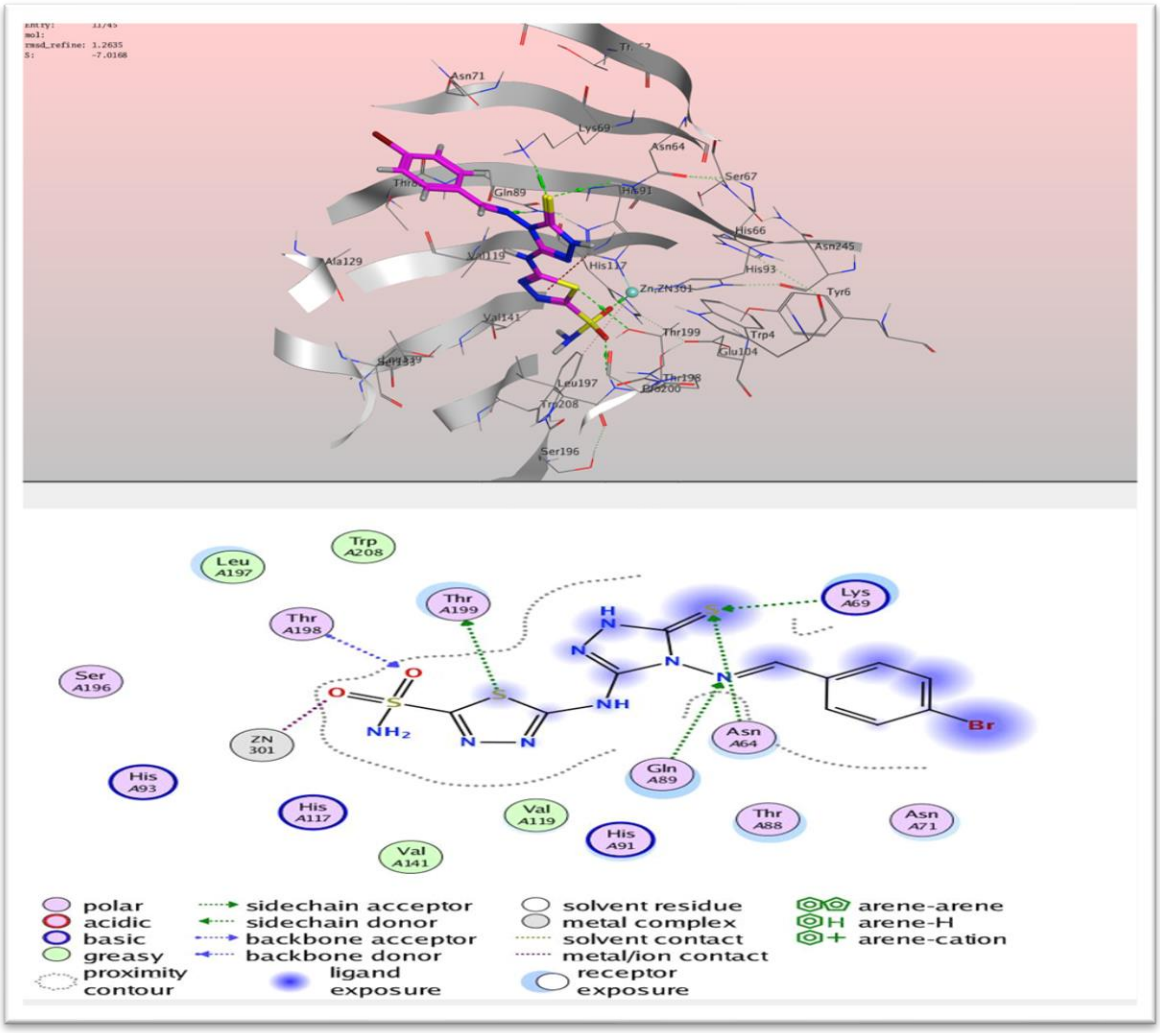

Figure 5: MOE Visualization of Ligand Sc with carbonic anhydrase XII (PDB code: 4QJW)

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