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In Silico Study of New Carbonic Anhydrase Inhibitor Derivatives Bearing 1, 3, 4-Oxadiazole Moiety with Promising Anti-Cancer Activity

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Abstract

Objectives: Molecular docking simulations were performed to assess the theoretical binding affinities of six (6) compounds created where they are derivatives having 1,3,4, oxadiazole moiety, and their target was cancer and Human carbonic anhydrase IX (PDB code: 6U4T). Using ChemDraw Ultra 12.0, the molecular structure was meticulously sketched. Molecular Operating environment software was used to verify the developed compounds by looking at their S. score and Rmsd values. Promising activity was seen with these proteins from the theoretically generated compounds, which exhibited strong binding contacts with the receptor active pocket.

Methods: Chemically by joining together several oxadiazole derivatives, sulfanilamide analogues (IVa-IVd) may be created in the lab. Molecular docking and ligand/receptor priming by MOE software.

Results: Acetazolamide was selected because it had the same pharmacophore as the sulfanilamide group, and cisplatin was used in clinical trials for cancer therapy. IVc and IVa yielded max score and irrevocable relationship compared with acetazolamide and cisplatin.

Conclusion: The MOE docking results validated the potent anticancer activity, the identified compounds showing good binding affinity with target proteins relative to the reference drugs (Acetazolamide and cisplatin). The most effective anticancer compounds were IVc and IVa, which yielded a maximum score with a Rmsd of less than 2, the MOE docking results were able prove this. Compounds IVc and IVa exhibited the greatest cytotoxic impact of the synthetic compounds against MCF7, and all four synthesized compounds showed a superior safety profile than the standards in MCF10a.

Keywords: Cancer, chemotherapy, in silico, oxadiazole moiety, sulfanilamide derivatives, carbonic anhydrase

Introduction

Cancer is a term used to describe a wide range of disorders defined by the growth of aberrant cells with the potential to proliferate uncontrollably and invade and destroy healthy tissue. The incidence of cancer is rising and is now the second-biggest cause of death worldwide.[1-3] Cancer risk mostly depend on demographic factors, including age and sex, with males being more at risk for the disease.[4]

The spread of cancer is medically referred to as metastasis.[5] The tumor mass or whatever ulceration it causes may cause local symptoms.[4] For instance, esophageal cancer can constrict the esophagus, making swallowing difficult or unpleasant, and lung cancer can generate a mass effect that blocks the bronchus, leading to coughing or pneumonia. An initial tumor is often painless, although localized pain may develop as the malignancy progresses.[6] Hypoxia, or low oxygen levels, is a hallmark of the tumor microenvironment in all solid tumors due to the unchecked growth of tumors.[7] Surgical resection, radiation, and chemotherapy are the three mainstays of standard oncology care.[8] It is well-established that tumor cells in humans frequently develop resistance to chemotherapy, and this resistance is especially pronounced in cases of metastatic illness.[9] Because of genotoxicity and resistance to existing anticancer treatments, the search for new small-molecule chemotherapeutic medications that are effective and safe to treat or avoid cancer has accelerated in recent years. Computational chemistry techniques, such as computer-aided drug design (CADD), have the potential to improve the process of finding new chemicals and reduce the expense of synthesis.[4] Humans express 15-class carbonic anhydrase (CA) isoforms, 12 of which are catalytically active and catalyze the reversible hydration of CO_2 to HCO_3. Each of these isoforms has multiple physiological roles and diverse expression patterns.[10,11] Carbonic anhydrase is increased by hypoxia and displays a primarily peri necrotic pattern of tumor cell expression. CA IX and CA XII have been linked to cancer development.[12-14] Sulfonamides have a wide range of biological actions, and members of this pharmacological family are extensively used in clinical settings as antiviral, antibacterial, diuretic, hypoglycemic, antihypertensive drugs, among other things. Recent research has revealed that a range of structurally distinct sulfonamide derivatives have strong anticancer action in vitro and/or in vivo.[15-17] Sulfonamide derivatives share a common aromatic or heterocyclic chemical design in their molecules and depending on the aromatic ring substituent (tail or linker moiety).[18] The heterocyclic ring compound structure has been studied for application in the treatment of a wide range of illnesses, including cancer. Because biological components already present in our bodies, such as DNA, RNA, and vitamins, contain heterocyclic core rings as part of their structure, advances in medicine involving heterocyclic compounds have become increasingly important.[19-21] 1,3,4-oxadiazole is a common nucleus in a wide variety of molecules. Its pharmaceutical efficacy is enhanced by hydrogen bond interactions with biomacromolecules, according to its favorable physical, chemical, and pharmacokinetic features. Various oxadiazole compounds have been shown to have antimicrobial, anti-tuberculous, anti-inflammatory, anti-fungal, anticancer, and anti-diabetic effects.[22] It has been found that

1,3,4-oxadiazole heterocycles are excellent bio isosteres of amides and esters and that their involvement in hydrogen bonding interactions with the receptor significantly increases the biological efficacy,[23] so it is a desirable scaffold for use in drug creation.[24] Depending on this background, novel sulfonamide derivatives bearing a 1,3,4-oxadiazole moiety were designed and synthesized to act as carbonic anhydrase inhibitors.

Methods

Chemical Synthesis

Depending on the previous introduction, novel sulfonamide derivatives bearing a 1,3,4 oxadiazole moiety were designed and synthesized to act as CA inhibitors.

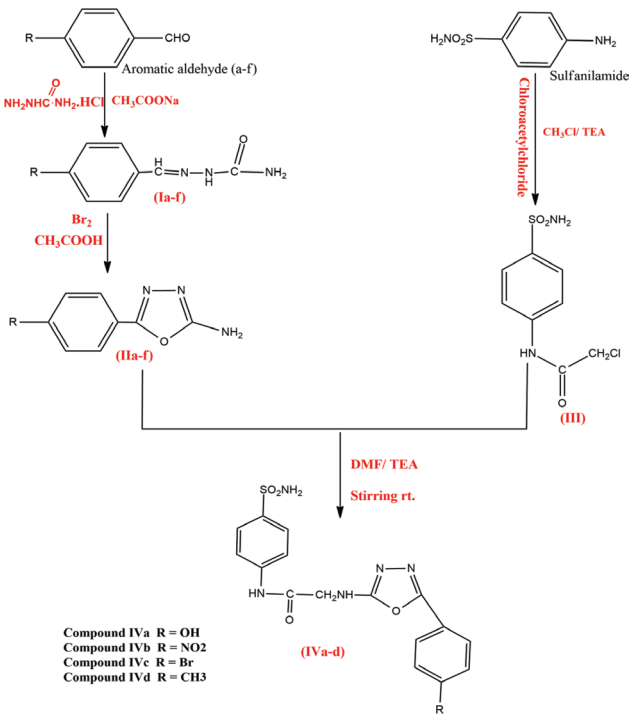
Compounds (IVa-IVd) with sulfanilamide analogues can be synthesized by connecting various oxadiazole derivatives, as depicted in Scheme 1.

Sheehan et al. 2022 described the synthesis of semicarbazone derivatives (Ia-d).[25] Substances (IIa-d) were synthesized according to the method described by Saeed et al. 2019,[26] Chemically, the synthesized substance III is produced according to Mohammed et al. 2015 by reacting sulfanilamide with chloroacetylchloride.[27]

Different final products IVa-d were synthesized by the reaction of compound IIa-d with compound III, according to the procedure by Noor Hatef Aldabagh 2017 [p. 69].[28]

Computer System and Software

The present study was conducted using an MSI system outfitted with 11th Generation Intel Core i7-11800H processors running at 2.30 GHz and 16 GB of random-access memory. MOE 2015 and ChemDraw Ultra 12.0 are both was downloaded and set up.



Scheme 1. **Synthesis of target compounds.**

Ligand/Receptor Preparation and Molecular Docking Protocol

The chemical structures of the ligand molecules were carefully drawn in ChemDraw Professional (12.0) software. Then protonating the ligand in 3D shape, partial charge addition, energy minimization, and finally saving the results.

We use the Protein Data Bank (PDB website (https://www.rcsb.org) to download receptors into the Molecular Operational Environment (MOE). The crystal structure of the extracellular domain of human carbonic anhydrase IX (PDB code: 6U4T) complexed with acetazolamide was revealed Table 1.

The target protein is prepared through the following steps:

The chain sequences that participate in the protein action were only selected; the remaining chains were deleted. The small molecules were deleted. Water molecules were removed also. Adding hydrogen hides bonds; after that, fix the potential of the protein atoms and identify their active site. At the end, the previously prepared ligand is loaded into MOE from saved data, followed by the docking process. A total of 30 poses were used, with each molecule represented by five distinct positions.

Results

The final (IVa-IVd) target compounds were produced by reacting sulfanilamide with aromatic aldehyde derivatives in preliminary stages.

Drug targets, including proteins, lipids, ligands, and nucleic acids, are the focus of molecular docking. By determining an ideal shape for the protein and ligand as well as a relative orientation between the protein and ligand, molecular docking aims to decrease the system's free energy. Research into molecular docking is crucial for identifying disease targets and developing effective pharmaceuticals.[29]

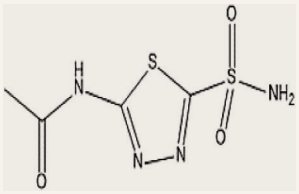
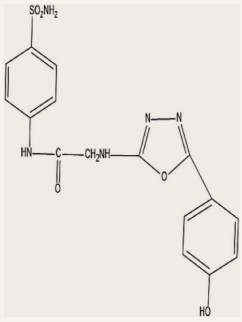
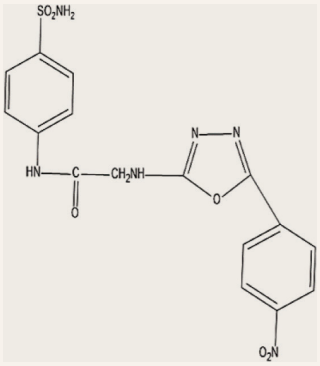
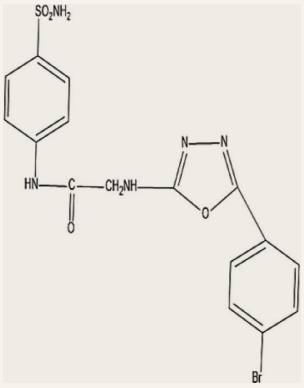
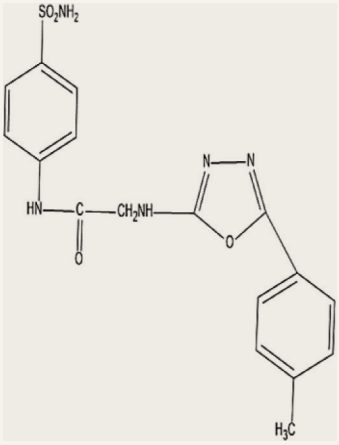
The s. score and rmsd (root mean square deviation) values, which represent the average distance between the atoms of the posed ligand and the ligand for the site of the anti-cancer that was studied, were used to evaluate the inhibitory activities of designed compounds. This information was used to gauge the potency of the compounds.

Acetazolamide interacts with its interaction site, which is composed of Zn301, His94, and Thr199. IVc joined forces with Zn301, Leu198, Thr199 and His4 to create bonds. IVa, in the meantime, bonded with Zn301, Thr199, Leu198, Glu170, and Pro201. The amino acids Zn301, Thr199, Leu198, and Asn62 are all part of the IVd family as well. Zn301, Thr199, and Thr69 are the binding sites of IVb Figures 1–10.

Discussion

This study designed carbonic anhydrase inhibitors bearing 1, 3, and 4-oxadiazole was developed and tested virtually. The Molecular Operating Environment docking results determined the potency of the compounds as anticancer agents toward the carbonic anhydrase IX enzyme, with the majority of the tested compounds showing good binding affinity with target proteins compared to acetazolamide and cisplatin. Compound Sb has the best s. score (–8.37), showing

Table 1. **The binding properties of tested compounds**

Compound structure	Structure	S-Score	Rmsd	No. of binding sites	Binding amino acids
Acetazolamide		–6.8473	1.8074	3	Zn301, His94 and Thr199
Iva		–7.7782	1.3516	5	Zn301, Thr199, Leu198, Glu170, and Pro201
IVb		–7.7141	1.5262	3	Zn301, Thr199 and Thr69
IVc		–7.8577	1.3310	4	Zn301, Leu198, Thr199 and His4
IVd		–7.7143	1.6434	4	Zn301, Leu198, Thr199 and Asn62

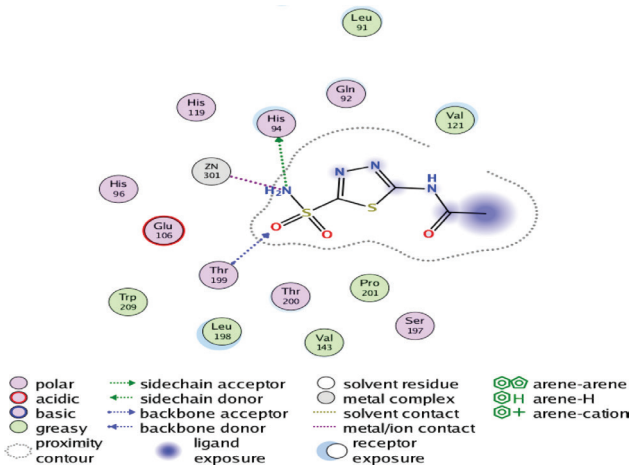


Fig. 1 **Acetazolamide with the human carbonic anhydrase IX (PDB code: 6U4T) (2D).**

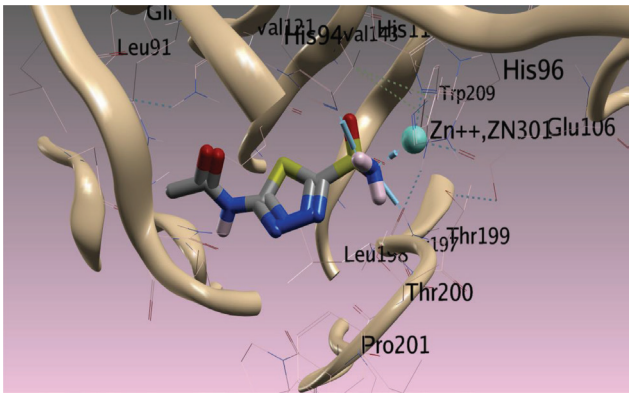


Fig. 2 **Acetazolamide with the human carbonic anhydrase IX (PDB code: 6U4T) (3D).**

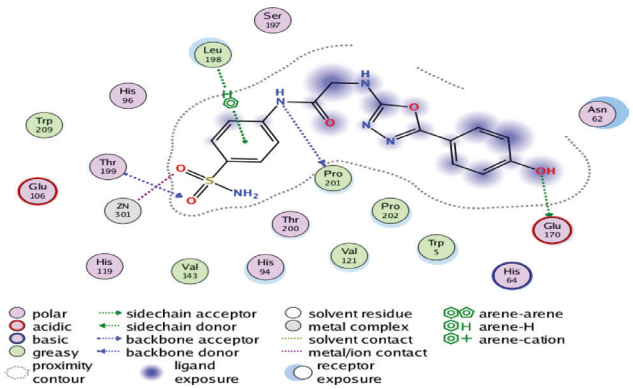


Fig. 3 **IVa with the human carbonic anhydrase IX (PDB code: 6U4T) (2D).**

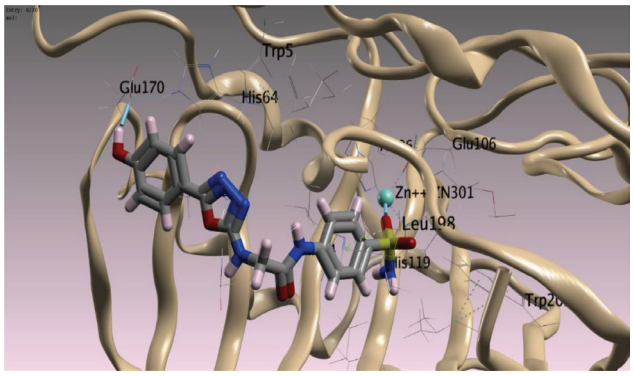


Fig. 4 **IVa with the human carbonic anhydrase IX (PDB code: 6U4T) (3D).**

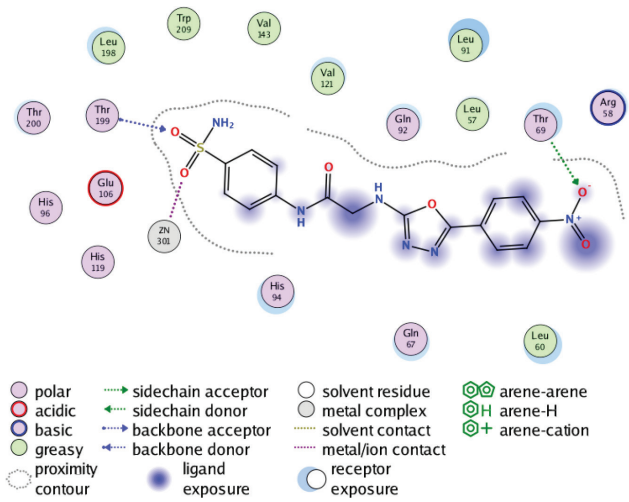


Fig. 5 **IVb with the human carbonic anhydrase IX (PDB code: 6U4T) (2D).**

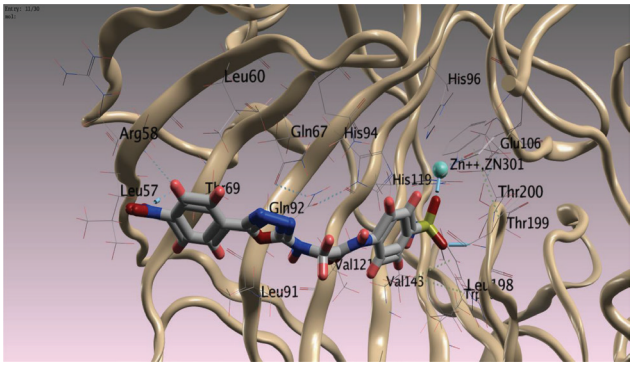


Fig. 6 **IVb with the human carbonic anhydrase IX (PDB code: 6U4T) (3D).**

that Br substitution enhances the orientation of the suggested ligand in the receptor pocket. The Sd derivative with Cl substitution formed more hydrogen bonds with several important amino acid residues in the protein, resulting in a more stable orientation and a tighter binding affinity than other compounds. In compound Sa with a methyl substituent, the S. score is less (–7.46) with good rmsd. In contrast, the inhibitor without substitution shows a high Rmsd (2.2) and a lower s. score (–8.09).

The higher s. score (–7.8577) of compound IVc compared to the reference acetazolamide demonstrates that the Br substitution improves the orientation of the proposed ligand in the receptor pocket. In contrast to the other molecules with an s. score of –7.7782, the IVa derivative with the OH substitution

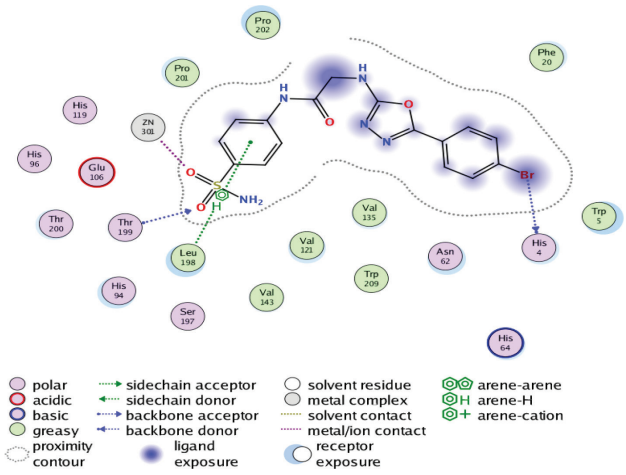


Fig. 7 **IVc with the human carbonic anhydrase IX (PDB code: 6U4T) (2D).**

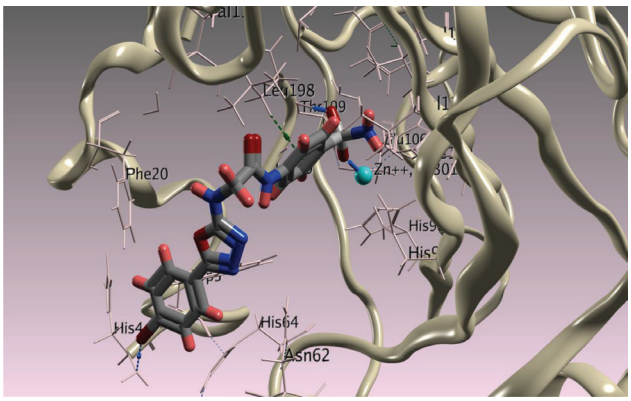


Fig. 8 **IVc with the human carbonic anhydrase IX (PDB code: 6U4T) (3D).**

had a more stable orientation and stronger binding affinity because it had created more hydrogen bonds with numerous key amino acid residues in the protein. The s. score for compound IVd with a methyl substituent fall to –7.7143, while the s. score for compound IVb is –7.7141 with a good rmsd. This demonstrates the significance of benzaldehyde derivatives, which exhibit differences in interaction depending on the groups substituted at position 4 of the benzene ring, and the importance of a substituted oxadiazole ring, which allows for greater flexibility and increases the likelihood of interaction with the receptor.

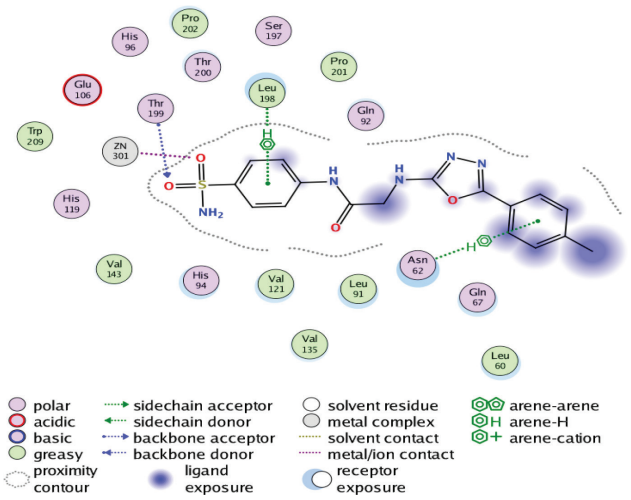


Fig. 9 **IVd with the human carbonic anhydrase IX (PDB code: 6U4T) (2D).**

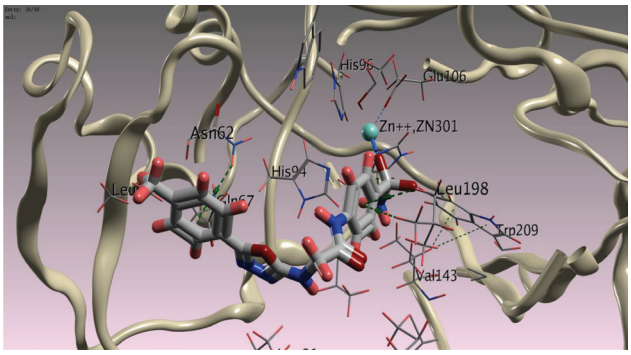


Fig. 10 **IVc with the human carbonic anhydrase IX (PDB code: 6U4T) (3D).**

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Noor H. Naser designed the experiments and performed experiments. Riyam Saad Aljubouri did sampling, performed the experiments, wrote the initial draft of manuscript, and analyzed the data and wrote the manuscript. All authors commented and edited the manuscript.

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