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Comparative In-Silico Evaluation of Common Over-the-Counter Drugs as Potential Binders of Human Carbonic Anhydrase IX Using AutoDock Vina

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ABSTRACT

Carbonic Anhydrase IX (CA-IX) is a hypoxia-induced, tumor-associated zinc metalloenzyme that plays a critical role in regulating pH homeostasis in cancer cells and is considered a promising molecular target in anticancer research [1-3]. Drug repurposing using computational approaches provides a cost-effective strategy for identifying novel interactions between approved drugs and therapeutic targets [4]. In the present study, a comparative molecular docking analysis was performed to evaluate the binding potential of five commonly used over-the-counter (OTC) drugs—paracetamol, ibuprofen, aspirin, caffeine, and cetirizine—against human Carbonic Anhydrase IX using AutoDock Vina [5]. The crystal structure of CA-IX (PDB ID: 3IAI) was prepared by removing co-crystallized ligands while retaining the catalytically essential Zn^{2+} ion. All ligands were docked using a focused grid centered on the Zn^{2+}-containing active site. Docking results revealed that cetirizine exhibited the strongest binding affinity (–7.6 kcal/mol), followed by ibuprofen (–7.2 kcal/mol), paracetamol (–6.6 kcal/mol), aspirin (–6.5 kcal/mol), and caffeine (–5.9 kcal/mol). The study highlights differential binding patterns among OTC drugs and provides preliminary computational evidence supporting their potential interaction with CA-IX, warranting further experimental investigation.

Keywords: Carbonic Anhydrase IX, Drug Repurposing, Molecular Docking, AutoDock Vina, Over-the-Counter Drugs, In-Silico Study

1. INTRODUCTION

1.1 Carbonic Anhydrases and Their Isoforms

Carbonic anhydrases (CAs) are a large family of zinc-dependent metalloenzymes that catalyze the reversible hydration of carbon dioxide into bicarbonate and protons. This fundamental reaction plays a critical role in maintaining acid–base balance, regulating pH homeostasis [1,6] and facilitating carbon dioxide transport in biological systems. Due to their involvement in diverse physiological processes, carbonic anhydrases are widely distributed across various tissues and cellular compartments.

In humans, at least 15 carbonic anhydrase isoforms have been identified, which differ in their tissue distribution, subcellular localization, catalytic activity and physiological function [2,7]. These isoforms are broadly classified into cytosolic (CA I, II, III, VII, XIII), mitochondrial (CA V(A), V(B)), membrane-associated (CA IV, IX, XII, XIV) and secreted forms (CA VI). Among these, CA II is the most abundant and catalytically active isoform, playing a central role in normal physiological functions such as respiration, renal acidification and electrolyte balance [6].

The structural conservation of the catalytic zinc ion across isoforms enables efficient catalysis; however, subtle differences in active-site architecture contribute to isoform-specific functional roles. These structural similarities also pose a major challenge in the development of isoform-selective carbonic anhydrase inhibitors, as many classical inhibitors interact with multiple CA isoforms, leading to undesirable off-target effects [2,3].

1.2 Role of Carbonic Anhydrase in Tumor Progression

Altered cellular metabolism and insufficient oxygen supply are hallmarks of rapidly growing solid tumors, resulting in a hypoxic tumor microenvironment. Under hypoxic conditions, cancer cells undergo metabolic reprogramming characterized by increased glycolysis and excessive production of acidic metabolites [8]. To survive under these conditions, tumor cells rely on efficient pH-regulating mechanisms, among which carbonic anhydrases play a pivotal role.

Carbonic Anhydrase IX (CA-IX) is a transmembrane isoform that is selectively overexpressed in hypoxic tumor tissues and is transcriptionally regulated by hypoxia-inducible factor-1α (HIF-1α) [3,9].CA-IX catalyzes the extracellular hydration of carbon dioxide, leading to the generation of protons and bicarbonate ions. This enzymatic activity contributes to extracellular acidification while maintaining a relatively neutral intracellular pH, thereby creating a microenvironment that favors tumor cell survival, invasion and metastatic potential [8,10].

The acidic extracellular milieu promoted by CA-IX activity enhances tumor aggressiveness, supports resistance to chemotherapy and radiotherapy and facilitates degradation of the extracellular matrix, enabling cancer cell migration. Importantly, CA-IX shows limited expression in most normal tissues, making it an attractive tumor-associated biomarker and therapeutic target [3,9]. Consequently, selective inhibition of CA-IX has gained significant interest as a strategy to disrupt tumor pH regulation while minimizing systemic side effects.

1.3 Rationale for the Present Study

Given the critical role of CA-IX in tumor biology and the challenges associated with isoform-selective inhibition, there is growing interest in exploring alternative strategies such as drug repurposing. Drug repurposing leverages existing drugs with known safety profiles to identify new therapeutic applications, thereby reducing development time and cost. Molecular docking is a widely used in-silico approach that enables rapid screening of potential drug–target interactions and provides insights into ligand binding affinity and orientation within the active site [11,12].

In this context, the present study aims to comparatively evaluate the binding potential of five commonly used over-the-counter (OTC) drugs paracetamol, ibuprofen, aspirin, caffeine and cetirizine against Carbonic Anhydrase IX using AutoDock Vina[13], with the objective of identifying potential interactions that may warrant further experimental investigation.

2. Materials and Methods

2.1 Software and Databases

Molecular docking studies were performed using AutoDock Vina [13]. Protein and ligand preparation was carried out using AutoDockTools (ADT) version 1.5.6. Protein structures were retrieved from the RCSB Protein Data Bank and ligand structures were obtained from the PubChem database [14]. Docking results were visualized and analyzed using molecular visualization tool such as PyMOL.

2.2 Protein Structure Retrieval and Preparation

The three-dimensional crystal structure of human Carbonic Anhydrase IX was retrieved from the RCSB Protein Data Bank (PDB ID: 3IAI), determined by X-ray diffraction at a resolution of 2.2 Å[14]. The protein structure was manually curated to remove co-crystallized ligands and buffer molecules, including acetazolamide (AZM), glycerol (GOL), phosphate (PO4) and Tris buffer (TRS). All water molecules were removed.

The catalytically essential Zn^{2+} ion was retained to preserve the native active-site environment. Polar hydrogens were added, Kollman charges were assigned and AutoDock4 atom types were applied using AutoDockTools. The prepared receptor was saved in PDBQT format for docking.

2.3 Ligand Preparation

The chemical structures of paracetamol, ibuprofen, aspirin, caffeine and cetirizine were retrieved from the PubChem database in SDF format [15]. Each ligand was prepared using AutoDockTools by adding all hydrogens, computing Gasteiger charges, defining rotatable bonds and assigning AutoDock4 atom types. Prepared ligands were saved in PDBQT format.

2.4 Grid Box Definition and Docking Protocol

The docking grid box was centered on the Zn^{2+} ion present in the CA-IX active site to ensure focused active-site docking. Grid box dimensions were set to 22 × 22 × 22 Å. Docking simulations were performed using AutoDock Vina with an exhaustiveness value of 8 and generation of nine binding modes. Binding affinity values (kcal/mol) and RMSD values were used to evaluate docking outcomes [5].

3. Results

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AutoDock Vina generated nine binding conformations for each ligand docked into the active site of Carbonic Anhydrase IX. The best binding pose for each ligand was selected based on the lowest binding energy and RMSD value of 0.0 Å.

Table 1. Comparative molecular docking results of selected over-the-counter drugs with Carbonic Anhydrase IX (CA-IX)

Ligand	Best Binding Affinity (kcal/mol)	Range of Binding Affinities (kcal/mol)	Lowest RMSD (Å)	Interpretation
Cetirizine	−7.6	−7.6 to −6.1	0.0	Strong binding
Ibuprofen	−7.2	−7.2 to −6.4	0.0	Strong binding
Paracetamol	−6.6	−6.6 to −5.5	0.0	Moderate binding
Aspirin	−6.5	−6.5 to −5.4	0.0	Moderate binding
Caffeine	−5.9	−5.9 to −5.1	0.0	Weaker binding

Binding affinity values were obtained using AutoDock Vina. The best docking pose (Mode 1) with an RMSD of 0.0 Å was selected for comparative analysis. Docking simulations were performed using a focused grid box (22 × 22 × 22 Å) centered on the Zn^{2+} ion of Carbonic Anhydrase IX. The affinity range represents the minimum and maximum binding energies observed across all nine docking modes.

The binding affinity order observed was:

Cetirizine > Ibuprofen > Paracetamol > Aspirin > Caffeine

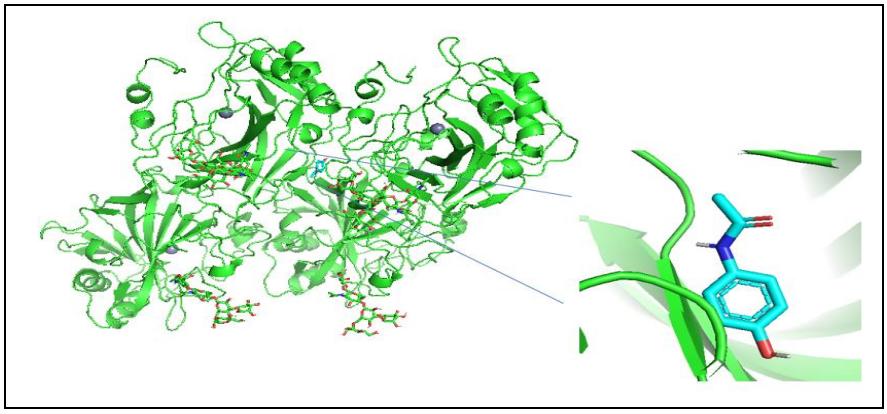

Figure 1: Three-dimensional docking pose of paracetamol in the active site of Carbonic Anhydrase IX (Mode 1).

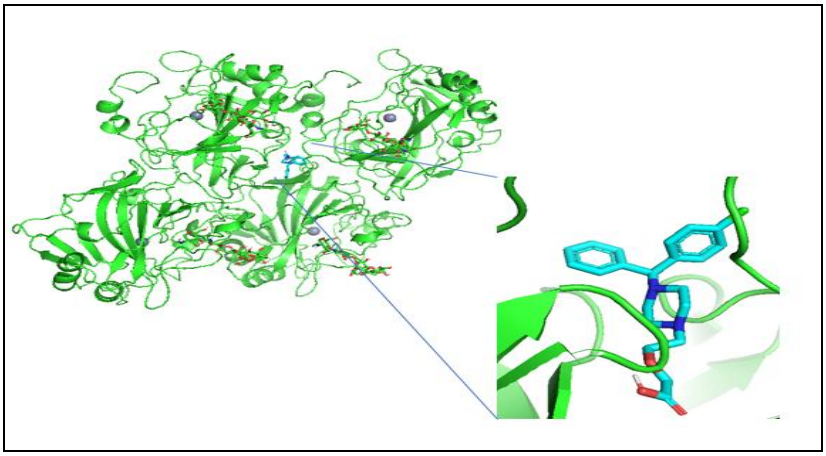

Figure 2: Three-dimensional docking pose of Cetirizine in the active site of Carbonic Anhydrase IX (Mode1).

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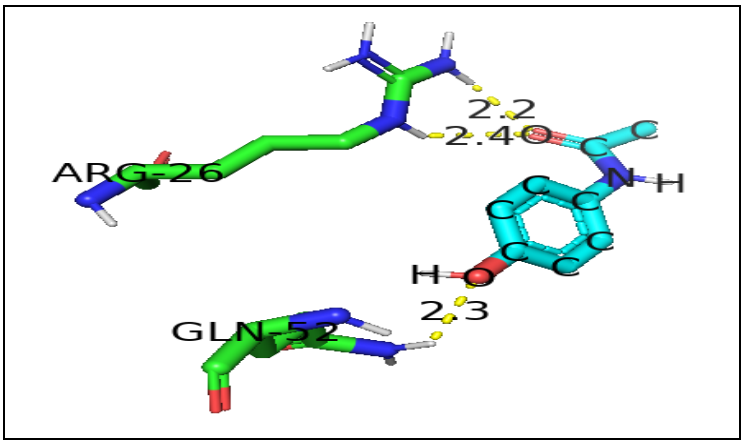

Figure 3: Two-dimensional interaction map showing key residues interacting with paracetamol. (Mode:1)

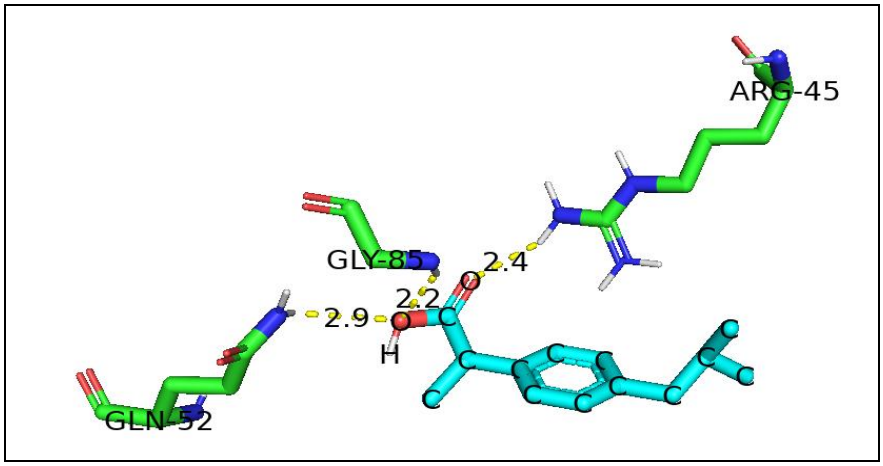

Figure 4: Two-dimensional interaction map showing key residues interacting with Ibuprofen (Mode:1)

4. Discussion

The comparative docking analysis demonstrated that all five OTC drugs exhibited favorable in-silico binding interactions with the active site of Carbonic Anhydrase IX. Cetirizine showed the strongest binding affinity, which may be attributed to its larger molecular framework and the presence of multiple functional groups capable of forming stabilizing interactions within the Zn^{2+}-containing catalytic pocket [2,3]. Ibuprofen also demonstrated strong binding affinity, likely due to enhanced hydrophobic interactions within the active-site cavity.

Paracetamol and aspirin exhibited moderate binding affinities, suggesting stable but comparatively weaker interactions. Caffeine showed the lowest binding affinity among the tested compounds, which may be due to its smaller molecular size and limited functional groups for strong active-site interactions. Although none of these drugs are established CA-IX inhibitors, the observed docking results provide valuable preliminary computational insights into their potential binding capability.

5. Conclusion

The present in-silico study demonstrates that commonly used over-the-counter drugs can interact favorably with human Carbonic Anhydrase IX. Among the evaluated compounds, cetirizine exhibited the strongest binding affinity, followed by ibuprofen, paracetamol, aspirin, and caffeine. These findings highlight differential binding patterns among OTC drugs and support the potential relevance of drug repurposing strategies targeting CA-IX. Further in-vitro and in-vivo studies are required to validate these computational predictions.

6. Acknowledgements

The authors acknowledge the use of freely available computational tools and databases, including RCSB Protein Data Bank, PubChem, AutoDockTools and AutoDock Vina.

7. Conflict of Interest

The authors declare no conflict of interest.

8. REFERENCES

1. Wilson K, Walker J. *Principles and Techniques of Biochemistry and Molecular Biology.* 7th ed. Cambridge University Press; 2010.
2. Lehninger AL, Nelson DL, Cox MM. *Lehninger Principles of Biochemistry.* 7th ed. W.H. Freeman; 2017.
3. Patrick GL. *An Introduction to Medicinal Chemistry.* 6th ed. Oxford University Press; 2017.
4. Katzung BG, Trevor AJ. *Basic and Clinical Pharmacology.* 15th ed. McGraw-Hill Education; 2021.
5. Supuran CT.Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators.*Nat Rev Drug Discov.* 2008;7(2):168–181. doi:10.1038/nrd2467
6. Supuran CT.Carbonic anhydrase inhibitors as emerging drugs for the treatment of cancer.*Expert Opin Investig Drugs.* 2012;21(6):881–898.
7. Pastorekova S, Pastorek J.Cancer-related carbonic anhydrase IX and XII as biomarkers and therapeutic targets.*Biochim Biophys Acta.* 2010;1804(2):326–331.
8. Swietach P, Vaughan-Jones RD, Harris AL.Regulation of tumor pH and the role of carbonic anhydrase IX.*Cancer Metastasis Rev.* 2007;26:299–310.
9. Chiche J, Ilc K, Laferrière J, et al.Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth.*Oncogene.* 2009;28:131–141.
10. McDonald PC, Dedhar S.Carbonic anhydrase IX as a mediator of hypoxia-induced tumor cell migration.*Cell Cycle.* 2014;13(3):356–362.
11. Ashburn TT, Thor KB.Drug repositioning: Identifying and developing new uses for existing drugs.*Nat Rev Drug Discov.* 2004;3:673–683.
12. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery. *Nat Rev Drug Discov.* 2004;3:935–949.
13. Trott O, Olson AJ.AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function.*J Comput Chem.* 2010;31:455–461.doi:10.1002/jcc.21334
14. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res.* 2000;28:235–242.
15. Kim S, Chen J, Cheng T, et al.PubChem in 2021: New data content and improved web interfaces.*Nucleic Acids Res.* 2021;49(D1):D1388–D1395.

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Conflict of Interest Statement:
The authors have no conflicts of interest to declare.