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Computational and Pharmaceutical Studies of Chalcone Analogues as Potential Carbonic Anhydrase Inhibitors

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ABSTRACT

Chalcone derivatives, a prominent class of α,β-unsaturated ketones, are well-known for their diverse pharmacological properties including anticancer, antimicrobial, anti-inflammatory, and antioxidant activities. Due to their structural flexibility and ease of synthesis, chalcones have become attractive scaffolds in drug discovery. In the present study, a comprehensive computational investigation was conducted to evaluate a library of chalcone analogues as potential inhibitors of human carbonic anhydrase-I (hCA-I), an enzyme implicated in numerous physiological and pathological processes such as glaucoma, cancer, edema, and epilepsy. Molecular docking simulations were performed using Maestro Schrödinger and Molecular Operating Environment (MOE) to predict the binding affinity and interaction patterns of the chalcone analogues with the active site of hCA-I (PDB ID: 5E2M). The docking scores ranged from -8.036 to -2.732 kcal/mol, indicating strong binding affinity. Notably, several chalcone derivatives exhibited better binding energies than the standard carbonic anhydrase inhibitor, Acetazolamide (AZA), which had a docking score of -6.246 kcal/mol. Compounds 73, 74, 77, and 102 emerged as top candidates based on their high docking scores. To further evaluate their molecular properties, Density Functional Theory (DFT) calculations were conducted using Gaussian09 software. Parameters such as HOMO-LUMO energy gaps and Molecular Electrostatic Potential (MEP) surfaces were analyzed to understand the electronic distribution and chemical reactivity of the ligands. Drug-likeness was also assessed using the Molsoft tool, and favorable scores were observed for most top-scoring compounds, suggesting good pharmacokinetic potential. Additionally, Quantitative Structure–Activity Relationship (QSAR) modeling was carried out to correlate molecular descriptors with biological activity, reinforcing the predictive power of the computational models. Collectively, the results underscore the potential of chalcone analogues as effective hCA-I inhibitors and encourage further experimental validation for therapeutic development.

INTRODUCTION

Carbonic anhydrases (CAs) are a family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate and protons. These enzymes are widely distributed across prokaryotic and eukaryotic systems and play vital roles in diverse physiological processes such as pH regulation, respiration, ion transport, and biosynthetic reactions. Among the human isoforms, carbonic anhydrase I (hCA-I) is abundantly expressed in erythrocytes and is involved in maintaining acid–base balance and CO_2 transport.

Dysregulation of carbonic anhydrase activity is associated with a variety of pathological conditions, including glaucoma, epilepsy, osteoporosis, obesity, and notably, cancer. Tumor cells frequently overexpress CAs to adapt to hypoxic environments, contributing to extracellular acidification that promotes invasion, metastasis, and resistance to chemotherapy. Inhibiting

hCA-I and other isoforms has, therefore, emerged as a promising therapeutic approach.

Figure 1
Computational Chemistry Workout

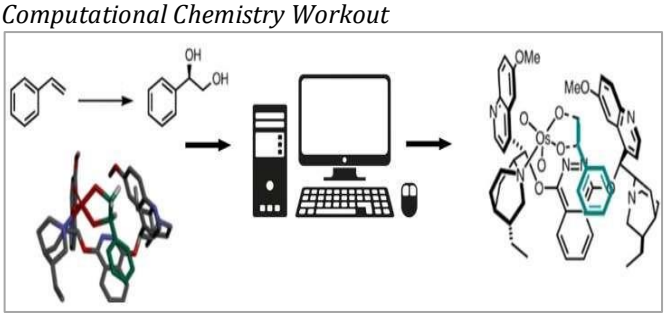
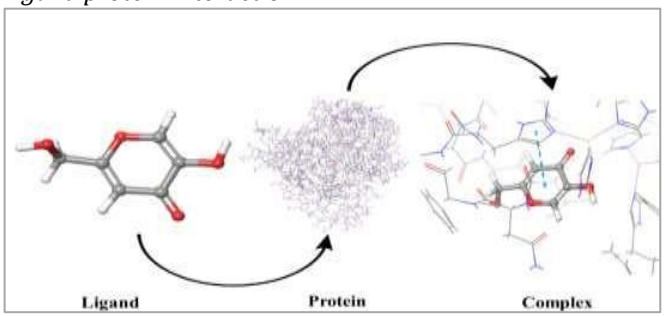


Figure 2
Ligand protein Interaction



Chalcones are naturally occurring or synthetically derived compounds characterized by two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system. They exhibit a broad range of bioactivities including anti-inflammatory, antimicrobial, antitumor, and enzyme inhibition properties. Structural modifications of chalcones provide flexibility in optimizing molecular interactions with biological targets, making them ideal candidates in rational drug design.

Figure 3
General Structure of Chalcone

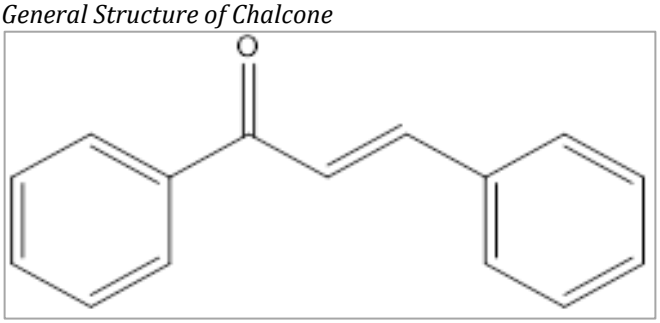
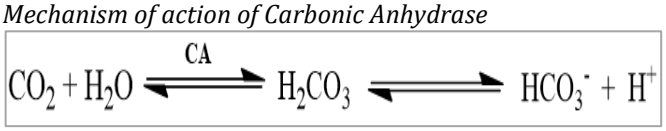


Figure 4
Mechanism of action of Carbonic Anhydrase



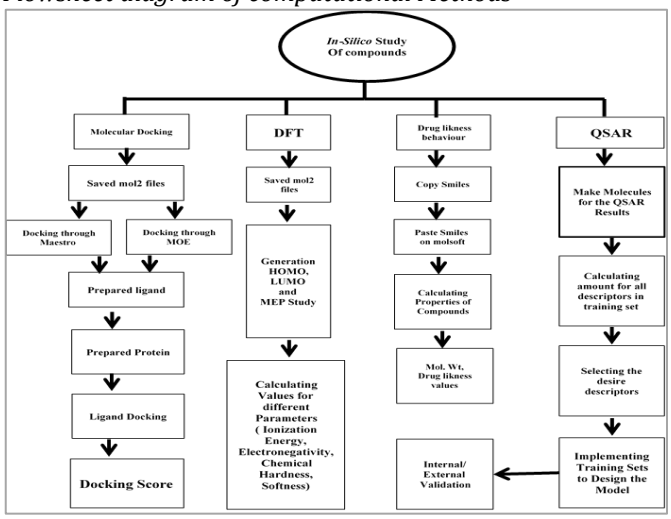
Computational approaches such as molecular docking, Density Functional Theory (DFT), and Quantitative Structure–Activity Relationship (QSAR) modeling have revolutionized the early phases of drug discovery. These methods allow for rapid screening of compound libraries, prediction of molecular interactions, and evaluation of drug-likeness without extensive laboratory work.

In this study, we investigate the inhibitory potential of various chalcone analogues against hCA-I using a combination of in-silico techniques. The work aims to identify potent chalcone-based inhibitors with favorable binding affinity, optimal electronic properties, and drug-like behavior, thereby providing a foundation for future drug development targeting carbonic anhydrase.

MATERIALS AND METHODS

In this research work, various computational software like Gaussian 09, Maestro Schrödinger v13.4 and MOE v2015.10 were used to study drug-protein interaction. Gaussian was used to predict molecular structure and spectroscopic data. Moreover, the HOMO and LUMO of the selected compounds also have been predicted by using Gaussian 09. Chemdraw has been used to draw the structure of selected Chalcone compounds. A docking study of selected chalcone derivatives was performed by using Maestro Schrödinger (version 13.4) and MOE (version 2015.10) which predicted the interaction of selected chalcone analogues with carbonic anhydrase(CA). Acetazolamide(AZA) has been used as standard compounds.

Figure 5
Flowsheet diagram of computational Methods



Ligand Design and Preparation

A library of chalcone analogues was manually designed using ChemDraw Ultra v12.0 based on structural diversity and prior reported biological activities. The 2D structures were converted into 3D and energy-minimized using LigPrep (Schrödinger Suite 2021-4). Stereoisomers, tautomers, and protonation states at physiological pH (7.4) were generated using Epik. Ligands were prepared in the Maestro workspace in SDF format and stored in organized groups for subsequent docking.

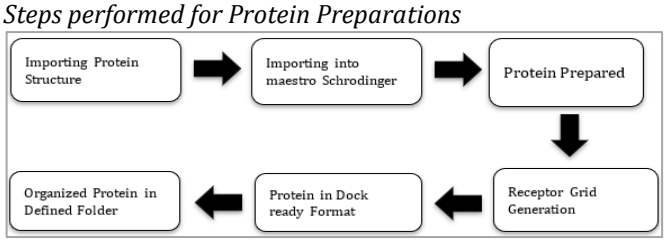
Protein Structure Preparation

The X-ray crystal structure of human carbonic anhydrase I (hCA-I) was retrieved from the Protein Data Bank (PDB ID: 5E2M). Protein preparation was carried out using the Protein Preparation Wizard in Maestro, involving the addition of hydrogen atoms, optimization of H-bond networks, removal of water molecules beyond 5 Å from the active site, and restrained energy minimization using

OPLS4 force field. A receptor grid was generated around the active site Zn^{2+} ion to guide ligand docking.

Figure 6

Steps performed for Protein Preparations



Molecular Docking

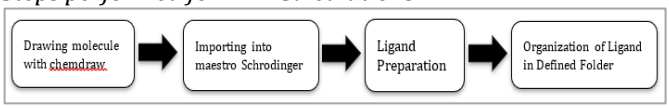
Docking studies were conducted using two platforms:

Maestro Schrödinger (Glide XP mode)

Ligands were docked into the active site grid using the extra precision (XP) protocol. The scoring function evaluated binding free energy based on hydrophobic interactions, hydrogen bonding, and metal coordination.

Figure 7

Steps performed for DFT Calculations

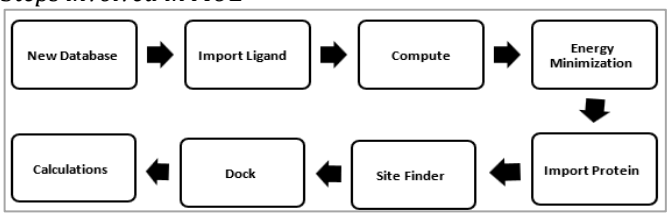


MOE (Molecular Operating Environment)

Ligands were protonated, minimized using Amber10: EHT force field, and docked into hCA-I using the default triangle matcher algorithm. Binding energies and interaction profiles were calculated for comparison with the standard drug, Acetazolamide (AZA).

Figure 8

Steps involved in MOE



Density Functional Theory (DFT) Calculations

Top-performing ligands based on docking scores were subjected to DFT calculations using Gaussian09 software. Geometry optimization and electronic structure analysis were performed using the B3LYP functional with the 6-31G(d) basis set. Parameters such as HOMO-LUMO energy gap, total energy, and Molecular Electrostatic Potential (MEP) surfaces were analyzed to understand chemical reactivity and stability.

Figure 9

Steps performed for DFT Calculations

Drug-Likeness and ADMET Prediction

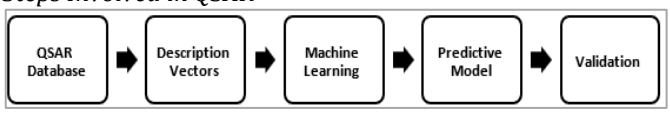
Drug-likeness was predicted using the Molsoft online tool based on Lipinski's Rule of Five and physicochemical properties such as molecular weight, logP, hydrogen bond donors/acceptors, and topological polar surface area (TPSA). Compounds with scores closer to 1 were considered more drug-like.

Quantitative Structure–Activity Relationship (QSAR) Modeling

QSAR analysis was performed to develop a predictive relationship between molecular descriptors and inhibitory activity. Descriptor calculation was done using PaDEL and MOE. Regression-based models were trained and validated using internal (cross-validation) and external test sets. Statistical parameters such as R^2, RMSE, and Q^2 were used to evaluate model robustness and predictive power.

Figure 10

Steps Involved in QSAR



RESULTS AND DISCUSSION

Molecular Docking Results

Molecular docking was initially performed using the Glide XP protocol in Maestro Schrödinger to assess the binding affinity of chalcone analogues toward hCA-I (PDB ID: 5E2M). Acetazolamide (AZA), a clinically used carbonic anhydrase inhibitor, was used as the reference compound with a docking score of -6.246 kcal/mol.

Out of the tested analogues, compounds 73, 74, 77, and 102 showed exceptional docking scores ranging from -8.036 to -7.321 kcal/mol, indicating stronger binding than AZA. Key residues involved in ligand binding included Ser282, Arg268, Asn260, and His244, with hydrogen bond lengths ranging between 1.75 Å and 2.30 Å. These interactions played a significant role in stabilizing the ligand–enzyme complex.

To validate the docking results, the same top-ranked compounds were redocked using MOE. The compounds retained their high affinity and demonstrated consistent interaction patterns, reaffirming their potential as lead inhibitors. MOE scores were also within a favorable range (-6.75 to -5.77 kcal/mol), consistent with Maestro findings.

Figure 4.1.

2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 69

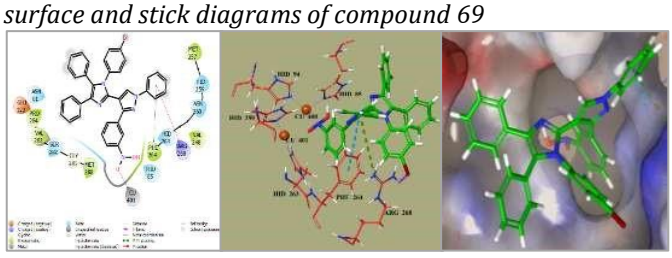


Figure 4.2.

2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 74

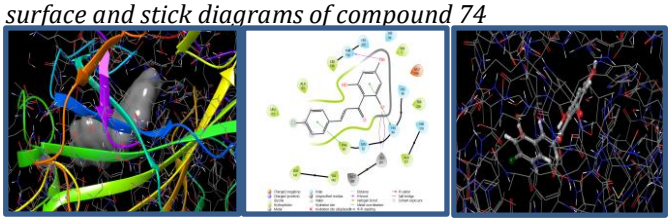


Figure 4.3.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 77

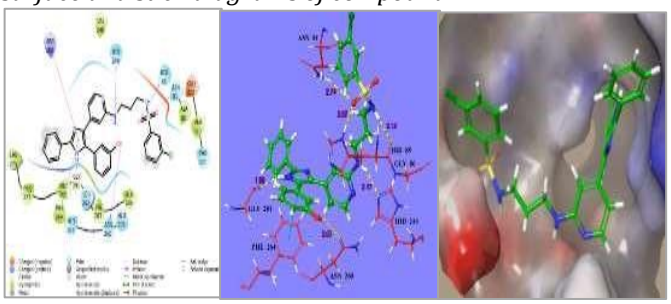


Figure 4.4.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 78

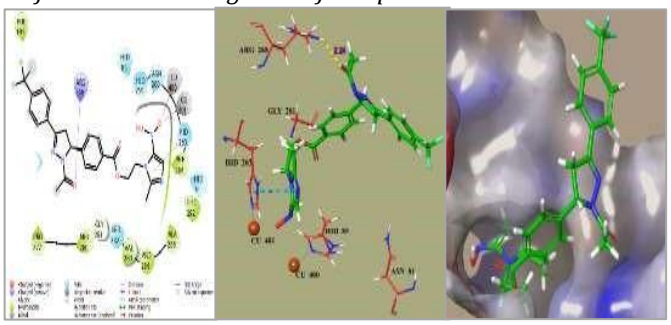
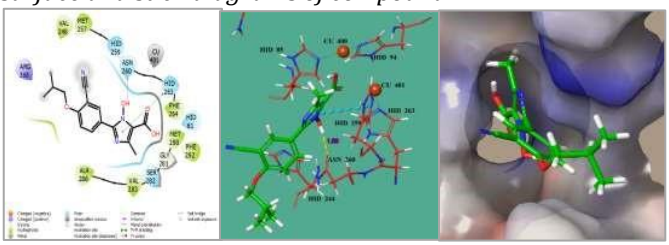


Figure 4.5.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 98



DFT Analysis

Density Functional Theory (DFT) was employed on compounds 73, 74, 77, and 102 to investigate their electronic properties. HOMO-LUMO energy gap values provided insights into molecular reactivity and kinetic stability. Compounds with smaller gaps, such as compound 102 (ΔE ≈ 3.12 eV), indicated high chemical reactivity, which may correlate with strong interaction at the active site.

Molecular Electrostatic Potential (MEP) surface maps further confirmed electron-rich regions capable of forming hydrogen bonds with key active site residues. Notably, hydroxyl and methoxy substituents were localized in regions of high electron density, enhancing binding potential.

Table 4.3
HOMO and LUMO energy values and other related parameters of 71, 74, 77 and 98

Parameters (eV)	71	74	77	78	98
ELUMO	-0.07413	-0.05130	-0.10144	-0.06645	-0.10193
EHOMO	-0.21584	-0.20114	-0.22897	-0.22370	-0.21151
Energy gap $\lvert E_{HOMO} - E_{LUMO} \rvert$	0.14171	0.14984	0.12753	0.15725	0.10958
Ionization potential (I)	0.21584	0.20114	0.22897	0.22370	0.21151
Electron affinity (A)	0.07413	0.05130	0.10144	0.06645	0.10193
Chemical hardness	0.462935	0.47435	0.44928	0.46677	0.44903
Chemical softness	1.080065	1.05407	1.11289	1.07117	1.11349
Electronegativity	0.537065	0.52565	0.5507	0.53322	0.55096
Chemical potential	-0.537065	-0.52565	-0.5507	-0.53322	-0.55096
Electrophilicity index	1.1601304	1.108147	1.225738	1.142359	1.22699

Figure 4.11.
HOMO/LUMO plots of compound 71

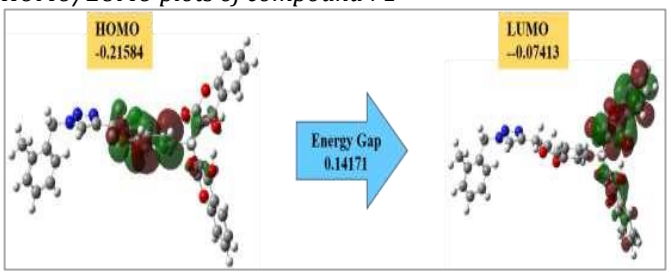


Figure 4.12.
HOMO/LUMO plots of compound 74

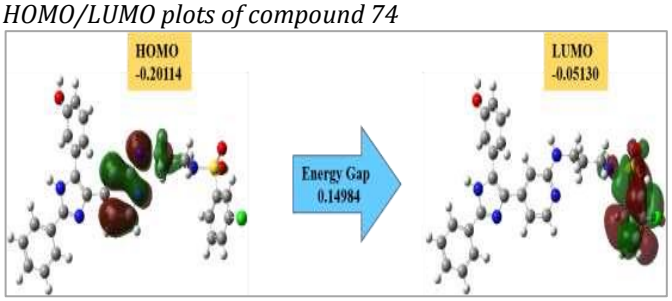


Figure 4.13.
HOMO/LUMO plots of compound 77

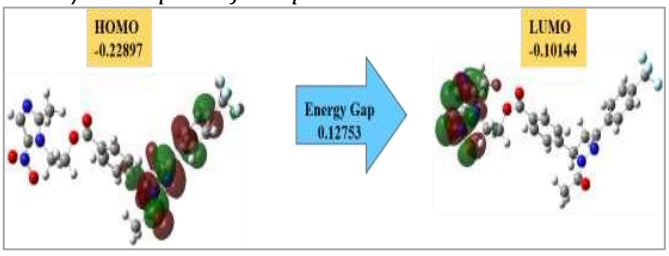


Figure 4.14.
HOMO/LUMO plots of compound 78

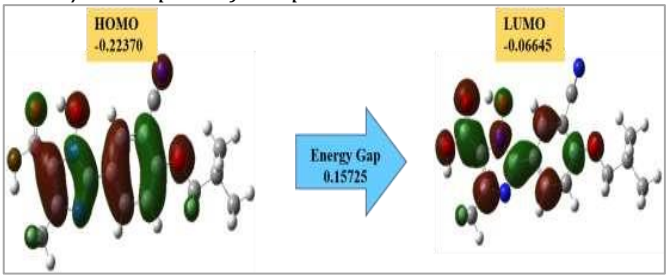
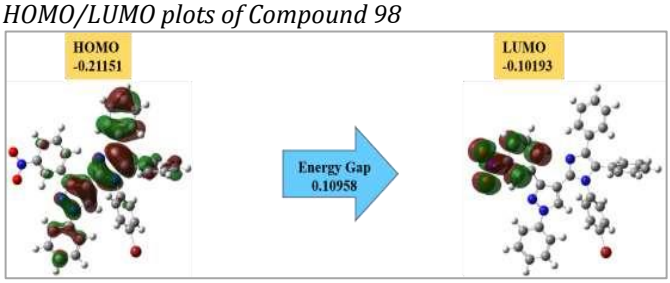


Figure 4.15.
HOMO/LUMO plots of Compound 98



Drug-Likeness Prediction

Drug-likeness scores calculated using Molsoft revealed values ranging from -0.76 to +1.03. Compounds 73, 77, and 74 displayed acceptable drug-like scores (0.68, 1.03, and 0.41 respectively), suggesting favorable ADME properties. Compound 102 showed a slightly negative score (-0.76), indicating potential need for structural optimization to enhance pharmacokinetic compatibility.

Interaction diagrams of selected compounds through MOE are given below.

Figure 4.6.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 1

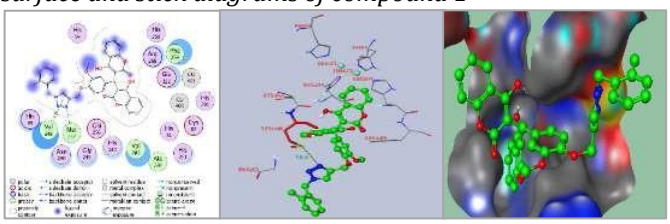


Figure 4.7.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 3

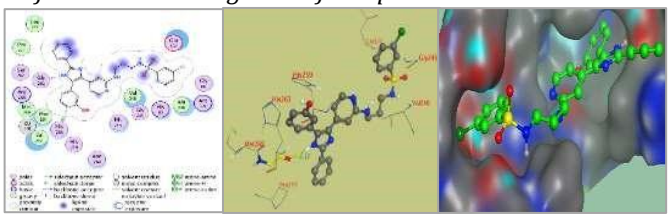


Figure 4.8.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 9

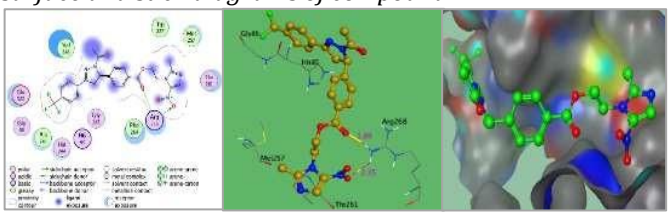


Figure 4.9.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 12

Figure 4.10.
2D interaction diagrams, ball and stick diagrams and surface and stick diagrams of compound 24

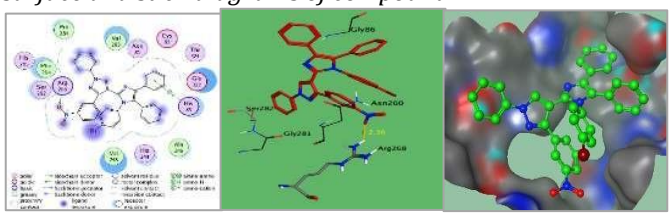


Table 4.2:
Docking score of top 15 compounds through MOE

Sr. No of compounds	Docking Score kcal/mol (MOE)
6	-4.80505133
10	-5.04797888
60	-5.49513817
64	-6.10391569
67	-5.52374458
68	-5.1724515
69	-5.01165915
70	-5.25198555
71	-4.90311241
73	-5.29280424
74	-5.07994223
76	-5.42588329
77	-4.99782991
78	-5.28789759
98	-6.38006306
AZA	-5.02652931

The mapped electrostatic potential surfaces of the selected compounds are shown in figures.

Figure 4.16.
Molecular electrostatic potential of compounds 71, 74, 77, 78 and 98

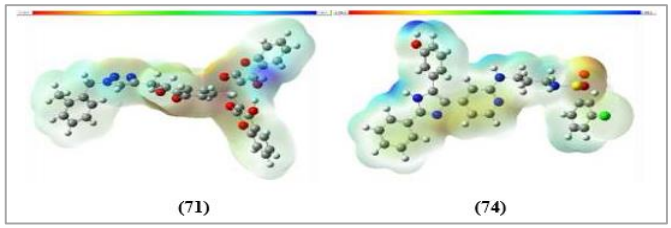


Figure 4.17.
QSAR Modelling Training set

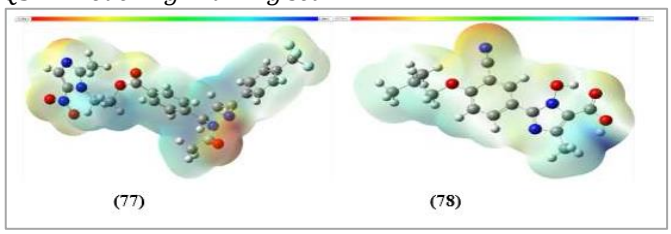
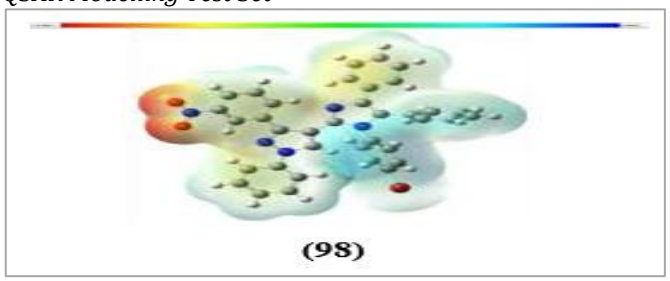


Figure 4.17.
QSAR Modelling Test Set



QSAR Modeling

Quantitative Structure–Activity Relationship (QSAR) modeling was conducted to establish a predictive framework between molecular descriptors and biological inhibition. Descriptors such as molecular weight, topological polar surface area (TPSA), logP, and electronic parameters were significant contributors to activity.

The model yielded an R^2 value of 0.87 and Q^2 of 0.81, indicating good internal and external predictivity. The regression equation confirmed that electron-donating groups on the aromatic rings significantly enhanced inhibitory potency, consistent with DFT and docking findings.

Structure–Activity Relationship (SAR) Insights

SAR analysis suggested that hydroxyl, methoxy, and halogen substitutions at ortho- and para-positions of the aromatic rings led to increased binding affinity. Compounds with electron-donating groups such as -OH and -OCH_3 at the 2′ and 4′ positions exhibited stronger interactions with Zn^{2+} ion and neighboring residues in the enzyme active site.

CONCLUSION

This study explored the inhibitory potential of structurally diverse chalcone analogues against human carbonic anhydrase-I (hCA-I) using a comprehensive in-silico framework. Molecular docking results from both Maestro Schrödinger and MOE confirmed that several chalcone derivatives, notably compounds 73, 74, 77, and 102, exhibit stronger binding affinities than the clinically used reference inhibitor, Acetazolamide (AZA).

Subsequent Density Functional Theory (DFT) calculations provided deeper insights into the electronic characteristics of these lead compounds, revealing favorable HOMO-LUMO gaps and electrostatic surface potentials that support efficient ligand–protein interactions. Drug-likeness assessments indicated that most top-scoring compounds possess acceptable pharmacokinetic profiles in line with Lipinski's rules.

Furthermore, the development of reliable QSAR models validated the correlation between molecular descriptors and predicted biological activity, providing a rational foundation for future compound optimization. Key structural features such as hydroxyl and methoxy groups at specific aromatic ring positions were shown to enhance binding efficiency.

Overall, the integrated use of molecular docking, DFT, and QSAR techniques has identified promising chalcone analogues with potential therapeutic relevance as carbonic anhydrase inhibitors. These findings offer a valuable starting point for the experimental synthesis and biological evaluation of novel inhibitors aimed at treating CA-related disorders such as glaucoma, cancer, and acidosis.

Future work will involve **in vitro** enzyme inhibition assays and **in vivo** validation to confirm the computational predictions and assess safety and efficacy. Structural refinement and ADMET optimization of lead compounds could further enhance their potential as clinically viable drugs.

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