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Molecular Docking Approach on Exploring the Energetics and Binding Stability of Carbonic Anhydrase with Specific Enzyme Inhibiting Drugs in the Presence of Dicyanomethylene-4H-Pyran Dyes: Hydrogen-Bonding Versus Hydrophobic Interactions or Combined Influences?

Sambath Rajesh[a], Babu Chandraja[a], Mani Akshaya[a], Manoharan Yogalaxhsmi[a], Ravichandran Keerthiga[a], Gunasekaran Shoba[b] and Rajendran Kumaran[a*]

[a]P.G and Research Department of Chemistry, Dwaraka Doss Goverdhan Doss Vaishnav College (Autonomous),833, Gokul Bagh, E.V.R. Periyar Road, Arumbakkam, Chennai 600106, Tamil Nadu, India.
[b]Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College (Autonomous),833, Gokul Bagh, E.V.R. Periyar Road, Arumbakkam, Chennai 600106, Tamil Nadu, India.

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KEYWORDS
carbonic anhydrase; DCM; molecular docking: enzyme inhibiting drugs; binding energy

ABSTRACT:
Introduction: Molecular Docking (Mol.Doc) studies were employed to ascertain the energetics and binding stability of zinc-based enzymes, carbonic anhydrase I and II (CA1 and CA2) in the presence of dicyanomethylene (DCM) dye. DCM as the guest, enzyme as the host and non-steroidal anti-inflammatory drug (NSAID) as competing guest molecules.
Objectives: The competitive binding nature of host-guest complexes were ascertained in the presence of NSAID.
Methods: Mol.Doc provides strategic and important information on energetics and binding affinity of host-guest-competing guest system
Results: The binding stability of DCM with CA1 were energetically favoured than CA2. Interestingly, addition of acetazolamide, benzolamide, dorzolamide, ethoxzolamide and methazolamide (competing guest molecules) decreases the binding stability of DCM-CA1 complex. Whereas, dorzolamide and benzolamide enhanced the stability of DCM-CA2 complex. Similarly, the role of DCM on drug-enzyme complex stability provided contrasting results. DCM results in an enhanced stability when docked to drug-CA1 or CA2 complex, which was not observed when drug docked to dye-enzyme complex.
Conclusions: The contribution by number of polar amino acids rather than non-polar amino acids resulted in DCM-CA1 complex and the molecular interaction is equally attributed to hydrogen bonding (HB) and hydrophobic interactions. Conversely, hydrophobic interaction predominates the binding affinity of DCM-CA2 complex.

1. Introduction

Carbonic anhydrase (CA), a naturally ocurring super family of ubiquitous metalloenzyme [1] play a pivotal role in catalyzing the reversible hydration of CO_2 into HCO_3^- and H^+ ions [2]. CA is essential in maintaining the acid-base balance and regulation in red blood cells (RBC) of cow [1]. The stability of CA is attributed largely to its cone-shaped active site, which is primarily composed of central 10-stranded beta-sheet surrounded by alpha helices. The active site of the CA comprises Zinc in tetrahedral geometry with three histidine residues as ligands and water molecule/hydroxide ion coordinating to other sites. The metal in ζ type of CA is Cd^{2+} and remaining CA contains Zn^{2+} ions [3]. Depending on the species, different types of CA isozymes are produced which functions in cytosol (semi-fluid gelatinous matrix in cell) and membranes. In humans, there are 5 cytosolic isozymes (I, II, III, VII, XIII) 5 mitochondrial isozymes (VA, VB) and salivary isozyme (VI) [4]. Cytosolic isozymes of CA impart a catalyzing role in reversible hydration of CO_2 into HCO_3^- and H^+, where CA(I) is found in RBC, thereby contributing to CO_2 transport, CA(II) is efficient isozyme and well-studied isozyme in α-CA family [5], found in kidney, pancreas and brain tissue which contributes in HCO_3^- reabsorption. Other

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CA such as CA(III), which is found in muscle and fat-storing tissues contributes in CO_2 hydration, CA-VII exclusively found in brain maintaining the pH homeostasis, CA-XIII which is less understood in scientific society [6]. Without zinc, the enzyme loses its activity [4], such that the arrangement of Zinc in coordinated histidine residue and water such that it allows the water to convert as OH^- ion which acts as nucleophile that attacks CO_2, which catalyse the formation of HCO_3^-, essential of the activity of CA.

On comparing the activities of CA1 and CA2, CA1 has a turnover frequency of approximately 2.0×10^5 s^{-1} and CA2 has a turnover frequency of about 1.4×10^6 s^{-1} which makes the CA2 as the fastest and unique enzymes [7]. Though, these enzymes play a vital role in metabolism, inhibition of the activities of this enzyme helps in therapeutic applications by regulating the bicarbonate and pH levels in the body, by affecting its ability to catalyze the conversion of CO_2 to HCO_3^- . Inhibitors typically bind to the zinc ion in the active site and inhibit the catalytic activity [8]. It is predominantly achieved by blocking the access to active site by physically obstructing it, thus prevent the substrates from reaching the metal. Since the active site of CA is highly dynamic, it allows itself to adapt to different substrates and reaction conditions such that binding to ligands provides usefull information in the concept of biophysical chemistry.

Inhibition activity of the inhibitor stabilizes the specific conformations which are less favourable for the catalysis process, that leads to reduced enzymatic activity, with stabilized inactive forms of CA such that this specific conformation locks the enzyme for further reaction [9]. Inhibition of CA helps to reduce the effects of glaucoma [7], diuretic effects (Edema and Hypertension), altitude sickness; epilepsy leads to therapeutic implications in ophthalmology, nephrology and neurology such that the role of the above enzyme plays a significant role in the metabolic activity and for survival of healthy life.

Among the various inhibitors, competitive inhibitors which primarily interacts with the zinc ion, [8] by blocking the enzymes activity. Binding of sulfonamides induces conformational changes in the enzyme and stabilizes the inactive forms of enzyme and leads to reduced catalytic activity. Different classes of sulfonamides exhibit different degree of selectivity for specific CA isozymes, based on the arrangement of ligands. Certain sulfonamide inhibits CA2 more effectively while some with CA1, which leads to different therapeutic effects [7]. A study regarding the role of NSAID with CA1 and CA2 as competing ligands in the presence of a fluorophore was carried out.

2. Objectives

The guest molecule employed is an intramolecular charge transfer (ICT) based dye involved in docking studies with several globular proteins in the presence of drugs, flavanoids as competing ligands [10-15]. The energetics and bimolecular interactions have been established by docking 4-(Dicyanomethylene)-2,6-(4-dimethylaminostyryl-4,4' dioic)-4H-Pyran (DCM) family derivatives as the guest molecule with Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), Ovalbumin (OVA), Beta lactoglobin (BLG) in the presence of several drugs has established the binding affinity, binding characteristics, domains, and sites. Moreover, the amino acids involved in the stability of the complexes have been ascertained. Similarly, in the present study, a derivative of DCM family is employed as the guest molecule, docked with CA1 and CA2 in the presence of acetazolamide (AZM), benzolamide (BZM), dorzolamide (DZM), ethoxzolamide (EXM) and methazolamide (MZM), which acts as the competing guest molecule.

The PDB structures of the enzymes are provided in **figures 1a** and **1b** respectively. The structures of DCM dye and the drugs are provided in **figures 2** and **3** respectively. The methodology employed is based on the earlier studies reported in the literature [16-22].

DCM dye with a molecular formula of $C_{19}H_{17}N_3O$ is a synthetic organic dye widely used as fluorescence probe for interaction with simple non-fluorophoric solutes [23-33] and with proteins. DCM has a characteristic structure including a dicyanomethylene group (electron acceptor group) and a dimethylaminostyryl group (electron doctor) is characterized by π-conjugated system. DCM docking predicts the nature of interaction in targeting protein sites to simulate its binding sites and binding affinities [12].

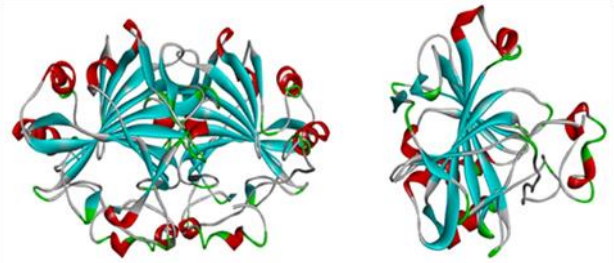

Figure 1: pdb structure [34] of CA I(2FOY) and CA II (2VVB)

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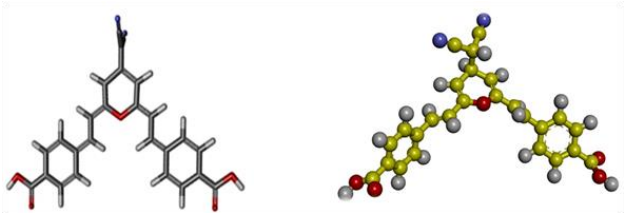

Figure 2: 3d Structure of 4-(Dicyanomethylene)-2, 6-(4-dimethylaminostyryl-4,4' dioic)-4H-Pyran (DCM) dye

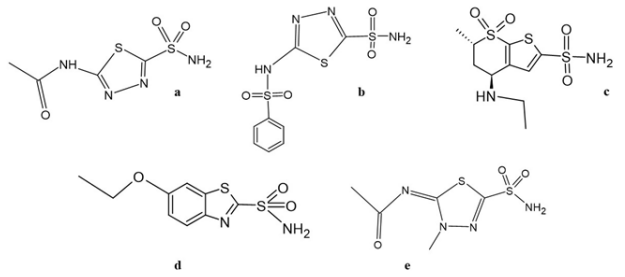

Figure 3: Structure of CA I and CA II enzyme inhibiting drugs. (a) Acetazolamide (AZM), (b) Benzolamide (BZM), (c) Dorzolamide (DZM), (d) Ethoxzolamide (EXM), (e) Methazolamide (MZM)

The interaction and binding affinities were determined based on the bonding between the DCM and targeted proteins. The bonding may be due to hydrogen/hydrophobic interactions. DCM forms a hydrogen bonding (HB) with the specific amino acids in the protein molecules especially with the highly electronegative atom, N or O. These interactions stabilize the binding with the targeted protein active site with DCM dye. From the nature of HB, it is possible to interpret the therapeutic effects of targeted protein within the biological system, higher/stronger the binding, higher the therapeutic effects [16].

Another type of interaction known as hydrophobic interactions which are the interaction between the non-polar molecules in the active site of the protein and DCM. The hydrophobic interaction helps to predict the dissociation of the drug molecule which is beneficial and important for drug efficiency [17]. The combined effects of the HB and hydrophobic interactions between the DCM complex and the targeted proteins results in the overall efficiency of the drug in therapeutic applications. Stronger the interaction between the DCM and target protein leads to increased binding affinity, which results in lesser concentration, is enough for effective treatment with the drug [18]. Hence in docking of CA with the specific enzyme inhibiting drugs, DCM dye is utilized for its binding affinity studies.

3. Methods

The structures of CA1 and CA2 were retrieved systematically, and the docking studies were carried out based on Lipinski rule of five [35,36] and this methodology was applied for AZM, BZM, DZM, MZM and EXM without any modification. Structures were optimised by chem sketch software and were finally saved in PDB format using open Babel converter. The complete docking properties of the guest and competing guest molecule comprising the compound molecular formula, molecular weight and SMILES notations are provided in **table 1**.

By employing SMILES notation properties, the docking procedures were carried out and docking score (Binding energy between host-guest complexes) were ascertained. The results and discussion have been segmented into three divisions and detailed comparison on the outcome of the docking studies has been explained in detail. Ten different conformers of each guest with host molecule were generated through the software auto dock 4.2 and the structure were arranged based on the corresponding energetics ($kcalmol^{-1}$) with the formulation of several possible energy parameters leading to the stability of DCM-CA1 versus DCM-CA2 complex.

Table 1: SMILES notation of CA inhibiting drugs

Ligand	Mol. wt g/mol	Molecular Formula	X Log P	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	Rotatable Bond Count	Topological Polar Surface Area (TSA) in Å²	Heavy atom Count

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AZM	223.3	$C_4H_6N_4O_3S_2$	-0.98	2	7	2	152	15
BZM	320.4	$C_8H_8N_4P_4S_3$	0.46	2	9	4	177	19
DZM	324.4	$C_{10}H_{16}N_2O_4S_3$	-0.74	2	4	3	159	14
EXM	258.3	$C_9H_{10}N_2O_3S_2$	0.36	1	6	3	119	16
MZM	236.3	$C_5H_8N_4O_3S_2$	-0.98	1	6	1	139	14

4. Results

The energetically more stable conformer (CA-1 DCM1) has a binding energy (B.E) of -9.95 kcal/mol and that of the least stable conformer CA-1 DCM10 has B.E of -6.49 kcal/mol. A gradual decrease in the B.E reflects the stability of the various conformers generated, as provided in **figure 4**. The decrease in the extent of B.E is prominent from CA-1 DCM1 to CA-1DCM3, which clearly reveals that all the conformers generated vary in their energetics which in turn provides the nature of bimolecular interaction existing in between CA1 with dye. Interestingly, the numbers of bimolecular interactions in all the conformers are less than 10 except in the case of CA-1DCM4 and CA-1DCM8. Except in the case of the most stable conformer CA-1DCM1, in all other conformer, the number of non-polar amino acids predominates over polar amino acids involved in binding. The representation of the nature of interaction existing between CA1 with DCM dye is provided in **figure 5** and the type of amino acid involved in binding is provided in **figure 6** respectively.

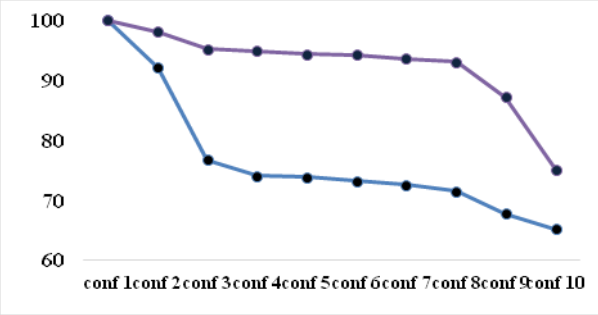

Figure 4: Extent of decrease in the Binding Energy of CA-1 and CA-2 docked with DCM dye. Blue and Yellow lines represents CA1 and CA2 conformers docked with DCM dye.

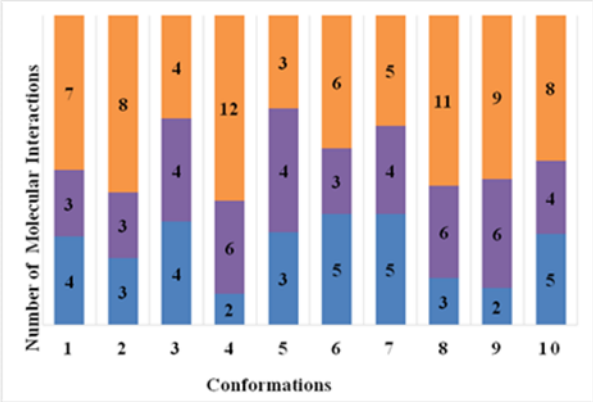

Figure 5: Bimolecular interactions existing between CA-1 and DCM dye. Blue colour represents Hydrogen-Bonding interactions. Violet and Orange colour represents Hydrophobic and van der Waals interactions respectively.

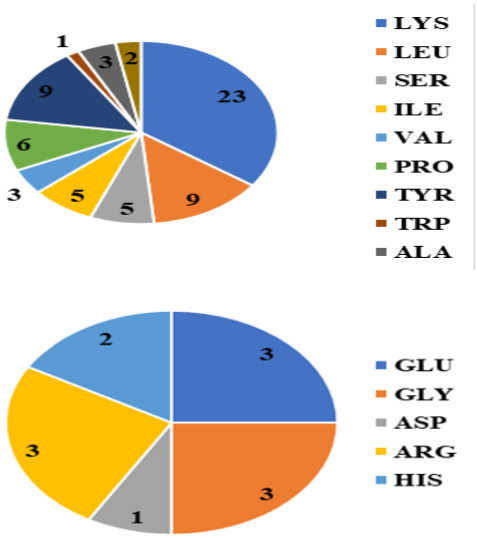

Figure 6: (a) represents the number of polar amino acids of CA-1 involved in bimolecular interaction and

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(b) represents the number of non-polar amino acids of CA-1 involved in bimolecular interaction.

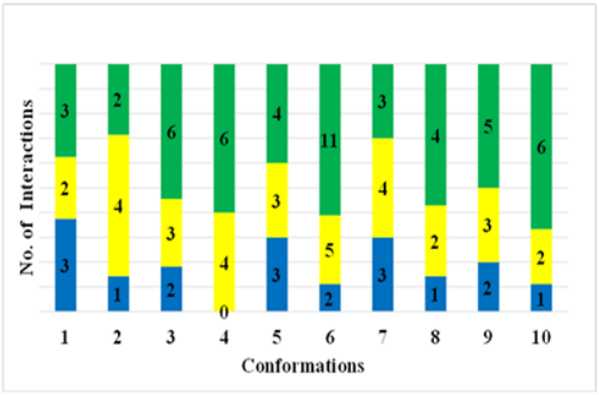

Figure 7: Bimolecular interactions existing between CA-2 and DCM dye. Blue colour represents Hydrogen-Bonding interactions. Yellow and Green colour represents Hydrophobic and van der Waals interactions respectively

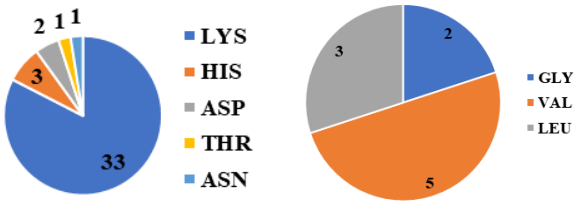

Figure 8: (a) represents the number of polar amino acids of CA-2 involved in bimolecular interaction and (b) represents the number of non-polar amino acids of CA-2 involved in bimolecular interaction.

Interestingly, among the energetically stable conformers (CA1DCM1 and CA1DCM2) HB interaction predominates over hydrophobic interaction. This pattern was also observed in 6th,7th and 10th conformers. The 10th conformer is energetically least stable conformer compared to the 1st conformer. Generation of various conformers of CA1 with DCM dye reveals that HB interactions as well as hydrophobic interaction play a prominent role in binding stability at the host-guest complex.

Further, all the polar amino acids are definitely involved in interactions and among the polar amino acids LYS is most preferred in formation of HB interaction compared to all other amino acids. However, aspartic acid (ASP), arginine (ARG), glutamic acid (GLU), threonine (THR) and serine (SER) are equally involved in binding interaction of DCM dye with CA1. In the case of CA2, the most stable conformer B.E of CA2-DCM1 is less than that of CA1-DCM1. The extent of decrease in the B.E of the most stable with that of least stable conformers in the case of CA2-DCM complex binding was around 25%, whereas in the case of CA1-DCM system, it was found to be 35%. A comparatively lesser decrease in the B.E was visualized in the case of CA2 when docked with dye rather than with CA1-DCM.

Interestingly, the conformers CA2DCM3 to CA2-DCM8 almost possess a similar B.E, which was not resulted in the case of CA1 docked with DCM dye. The variation in the B.E of CA1 docked with DCM with that of CA2 with DCM is provided in figure 4 for better understanding.

The representation of the nature of interaction existing between CA2 with DCM dye is provided in figure 7 and the type of amino acid involved in binding is provided in figure 8 respectively. In CA2-DCM system, the contribution from polar amino acid predominates over non-polar amino acids and total number of molecular interactions in any conformer are less than or equal to 7 except in the case of CA2-DCM6 conformer.

A variation in the number of amino acids involved in any sort of interaction in the case of CA2 with dye is lesser than that of CA 1 docked with DCM. All the conformer possesses HB as well as hydrophobic interaction except the 4th conformer (HB is absent in CA-2DCM4). The bimolecular interactions involved in dye-enzyme complexes are discussed below.

Among the polar amino acids, lysine (LYS) is the only amino acid that is involved in HB interaction. Apart from LYS, ASP and THR are involved in 6th and 8th conformers respectively whereas in CA1 these two amino acids were preferred in binding with the guest molecule. ARG and SER are the only polar amino acids that are not involved in molecular interaction. In the case of non-polar amino acids valine (VAL), tryptophan (TRP) and tyrosine (TYR) are involved predominantly in hydrophobic interaction. Compared to polar amino acids that are not involved in interaction, the non polar amino acids namely alanine (ALA), phenylalanine (PHE), cystine (CYS) and methionine (MET) are not involved in any sort of any bimolecular interaction with the dye molecule. The sulphur containing amino acids as well as the simple amino acids except VAL are not involved in binding compared to other amino acids.

Compared to CA1 interaction versus CA2 towards binding studies with dye, a variation in the amino acids pattern involved in molecular interaction vary based on the conformer which provides an interesting information regarding binding nature and affinity of an enzyme with guest molecule.

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Carbonic Anhydrase inhibiting drugs

Acetazolomide (AZM) belongs to the family of diuretics, chemically referred as N-(5-sulfamoyl-1.3.4-thiadiazol-2yl) acetamide containing an organic sulfonamide functional group. This sulfonamide drug potentially inhibits the enzymatic activity of CA by binding with its active Zn^{2+} ion[19].

Benzolamide (BZM) referred widely as 1,3,4-Thiadiazole-2-sulfonamide,5-((phenylfulfonyl)amino) also belongs to sulfonamide group. Similar to all CA inhibitor it has a potential inhibiting property compared to other inhibitors of same group [37-53].

Ethoxzolamide (EXM) is a sulfonamide drug play a crucial role in maintaining fluid balance and pressure regulation in the body by inhibiting CAI and CAII by reducing aqueous tumor production in eye.

Dorzolamide (DZM), is widely used to treat intraocular pressure conditions like glaucoma by inhibiting the production and working of CA.

Generally, Dorzolamide is effective in inhibiting CA but less potent than acetazolamide. Methazolamide (MZM) inhibits the CA by decreases the bicarbonate reabsorption in the kidneys which helps in properties of diuretics and also helps in treating glaucoma. Based on the above properties, the binding stability and binding energy of all above enzyme inhibiting enzymes, CA1 and CA2 was analyzed and determined using Mol.Doc method by binding it with DCM as the guest molecule. A comparison on the B.E of the dye with that of all the drugs employed with respect to CA1 and CA2 are provided in **figures 9** and **10** respectively.

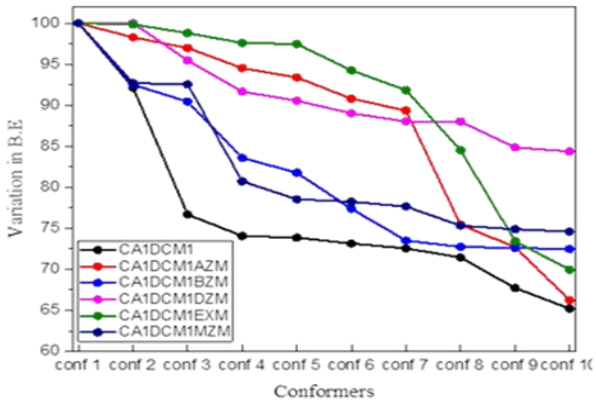

Figure 9: Variation of the Binding Energy of CA-1DCM complex with enzyme inhibiting drugs

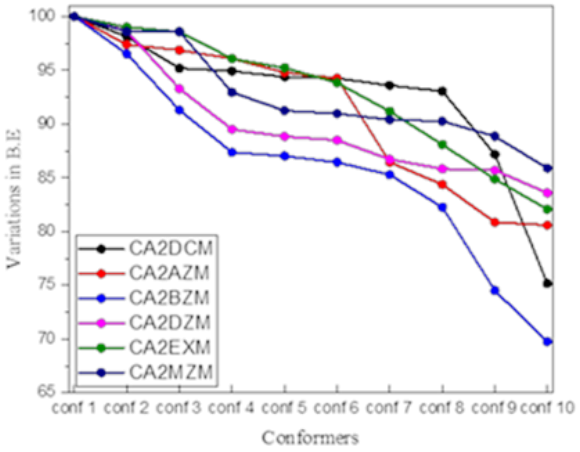

Figure 10: Variation of the Binding Energy of CA-2DCM complex with enzyme inhibiting drugs.

Simultaneous binding of the drug to CA1-DCM system resulted in a gradual decrease in the binding stability of the dye-enzyme complex. The order of decrease in the binding efficiency is MZM > AZM > EXM > BZM > DZM, and in the case of CA1/CA2-DCM dye complex, MZM and AZM increases the binding affinity of dye-enzyme complex, whereas all other drugs decrease the stability of the host-guest complex (**Figure 11** and **12**)

Among the drugs, almost MZM and AZM have the larger efficiency in disrupting the binding affinity of dye-enzyme complex in CA1, whereas these two drugs enhance the binding affinity of CA2 to DCM dye. (**Figure 10**).

Interestingly, when the dye is docked to drug-enzyme complex, the B.E increases significantly such that the presence of DCM dye to the drug-enzyme complex such that the presence of guest-host complex stability decreases in the presence of a competing guest molecule, whereas to that of competing guest-host complex, the addition of guest molecule behavioral pattern is entirely different.

The competitive binding efficiency of dye-enzyme complex, dye-enzyme complex docked with drug, drug-enzyme complex and drug-enzyme complex docked with dye for CA1 and CA2 enzymes provided a large variation in their energetics.

The B.E of CAIDCM1 and that of CA2DCM1 conformer with the drugs employed in the present study is displayed in **figures 11** and **12** respectively.

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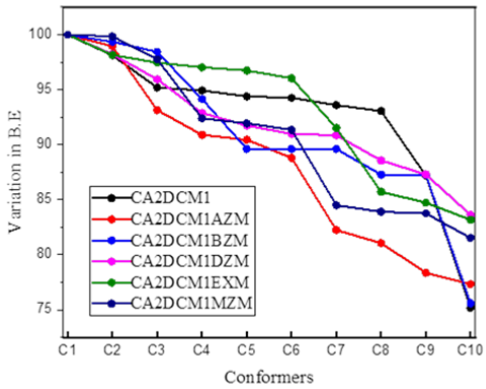

Figure 11: Variation of the Binding Energy of CA-1DCM1 conformer docked with enzyme inhibiting drugs.

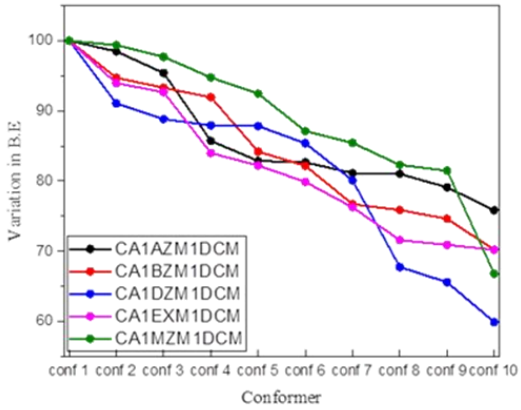

Figure 12: Variation of the Binding Energy of CA-2DCM1 conformer docked with enzyme inhibiting drugs.

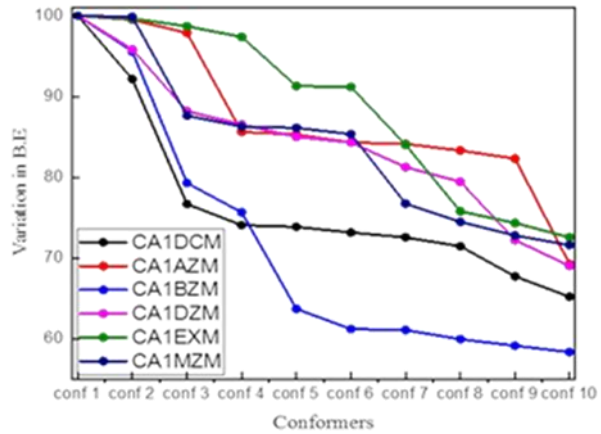

Figure 13: Variation of the Binding Energy of CA-1enzyme inhibiting drug conformer docked with DCM dye.

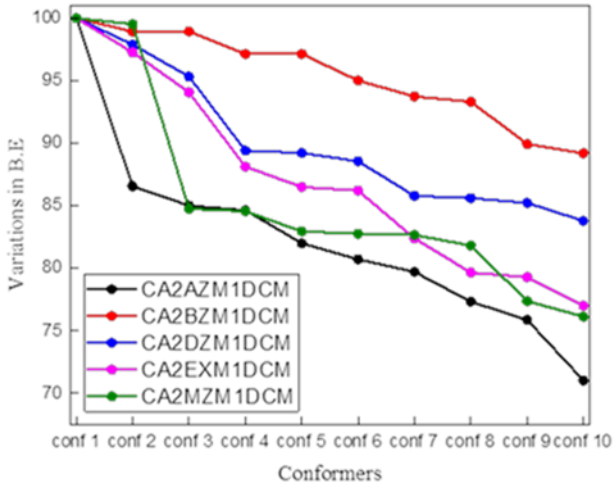

Figure 14: Variation of the Binding Energy of CA-2enzyme inhibiting drug conformer docked with DCM dye

Docking of DCM dye to CA1-drug complex and CA2-drug complex results in enhanced binding stability of drugs bound to enzyme. On the contrary, this pattern of binding stability was not resulted in the case of docking of drugs to CA1-DCM /CA2-DCM complex.

In other words, the simultaneous docking of competing guest molecule to host-guest complex resulted in disruption of the binding nature of CA 1-DCM and CA 2-DCM complexes whereas the role of guest ligands docked to host-competing guest molecule enhance the binding nature which was a significant observation ascertained from molecular docking studies as shown in **figures 13** and **14** respectively.

The dye enhancement on CA1-drug complex as follows: AZM > MZM > DZM > EXM > BZM whereas in the case of CA2-drug complex as follows: EXM > MZM > AZM > DZM > BZM.

It is evident that the binding nature and stability of host-guest- complex depends upon the nature of the drug even though they belong to the same class of inhibiting action.

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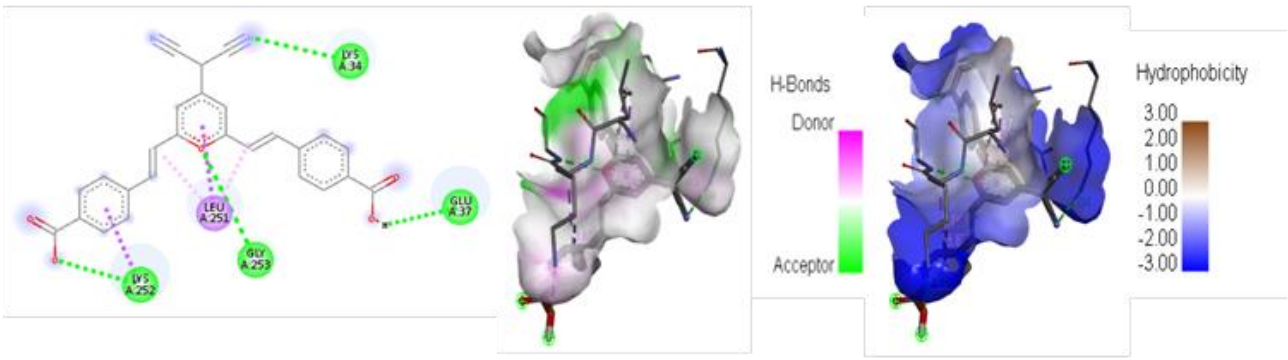

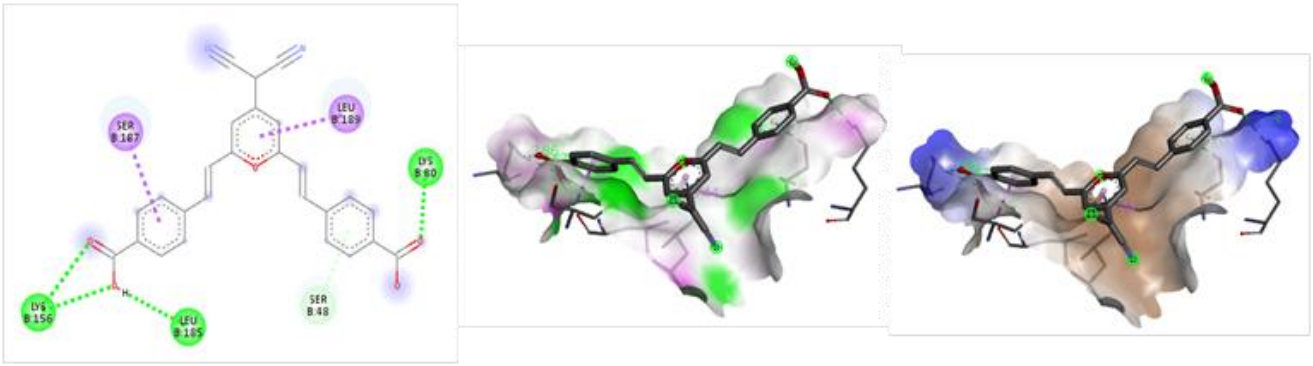

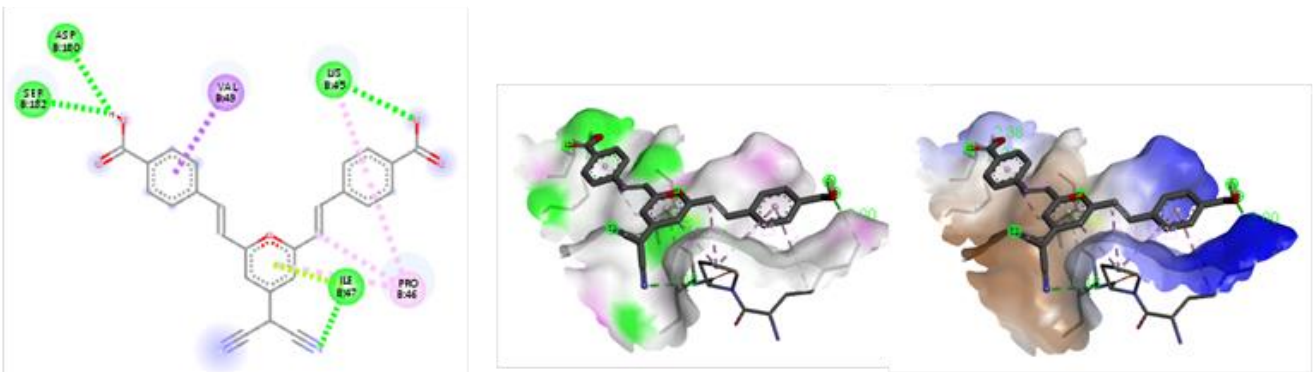

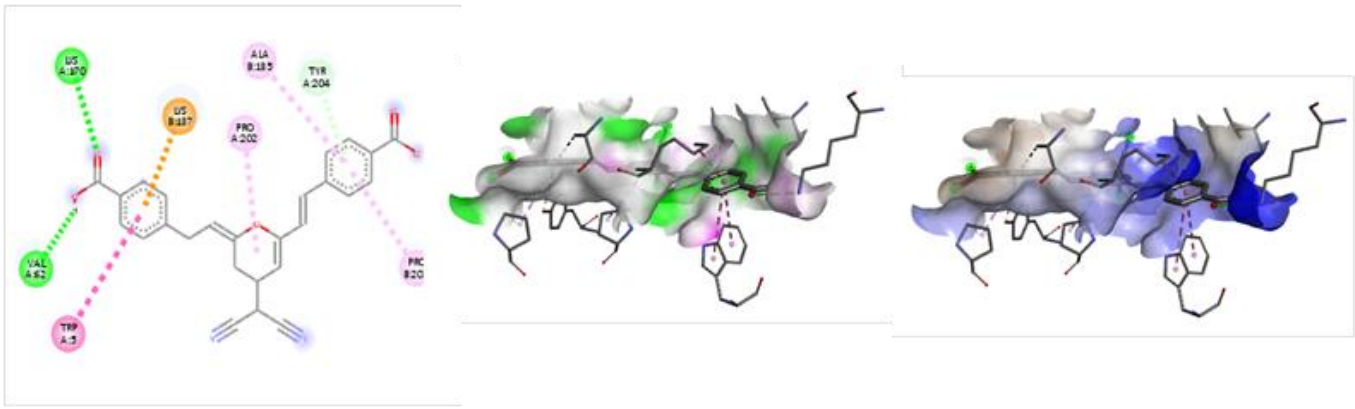

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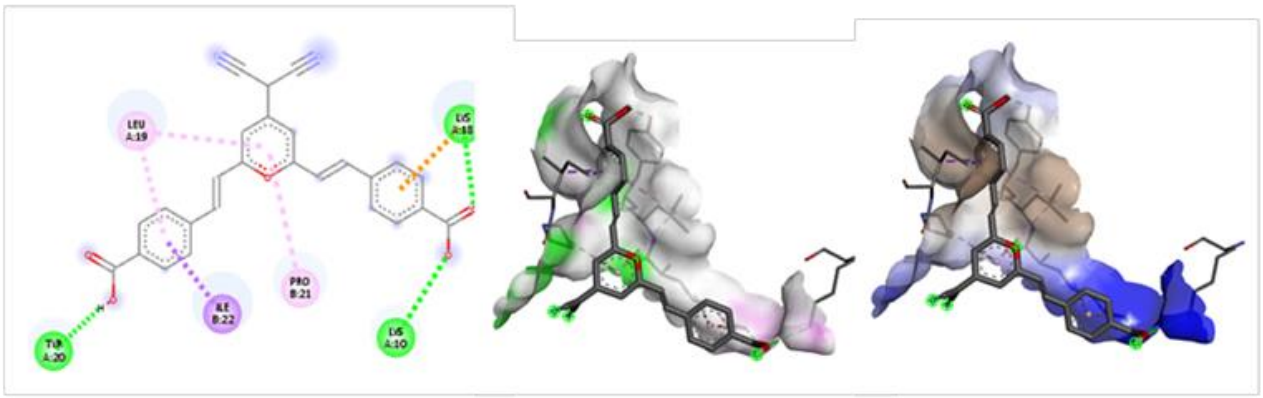

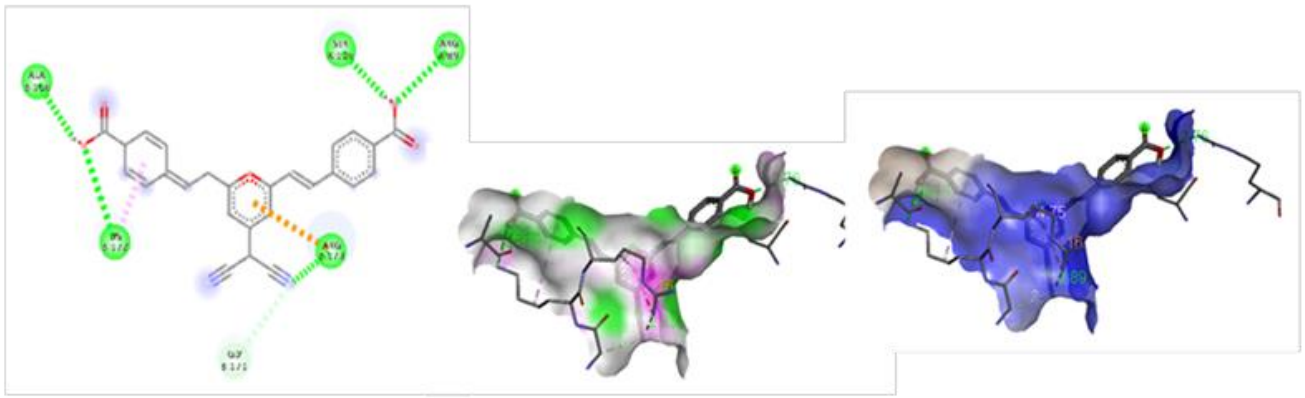

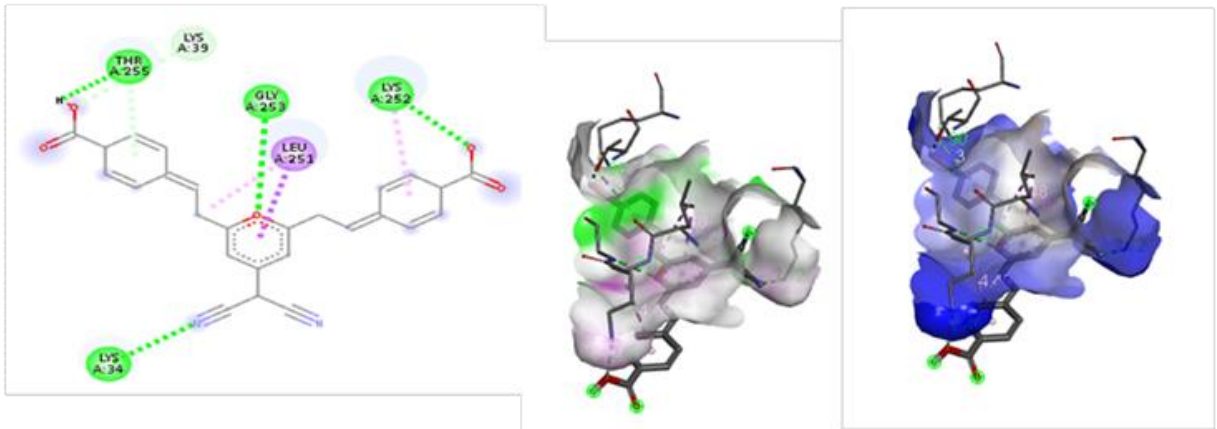

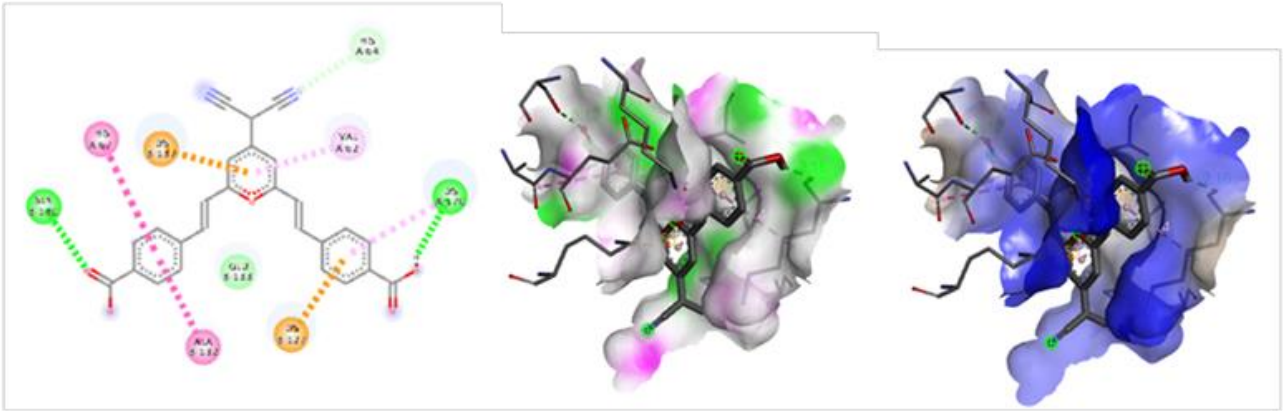

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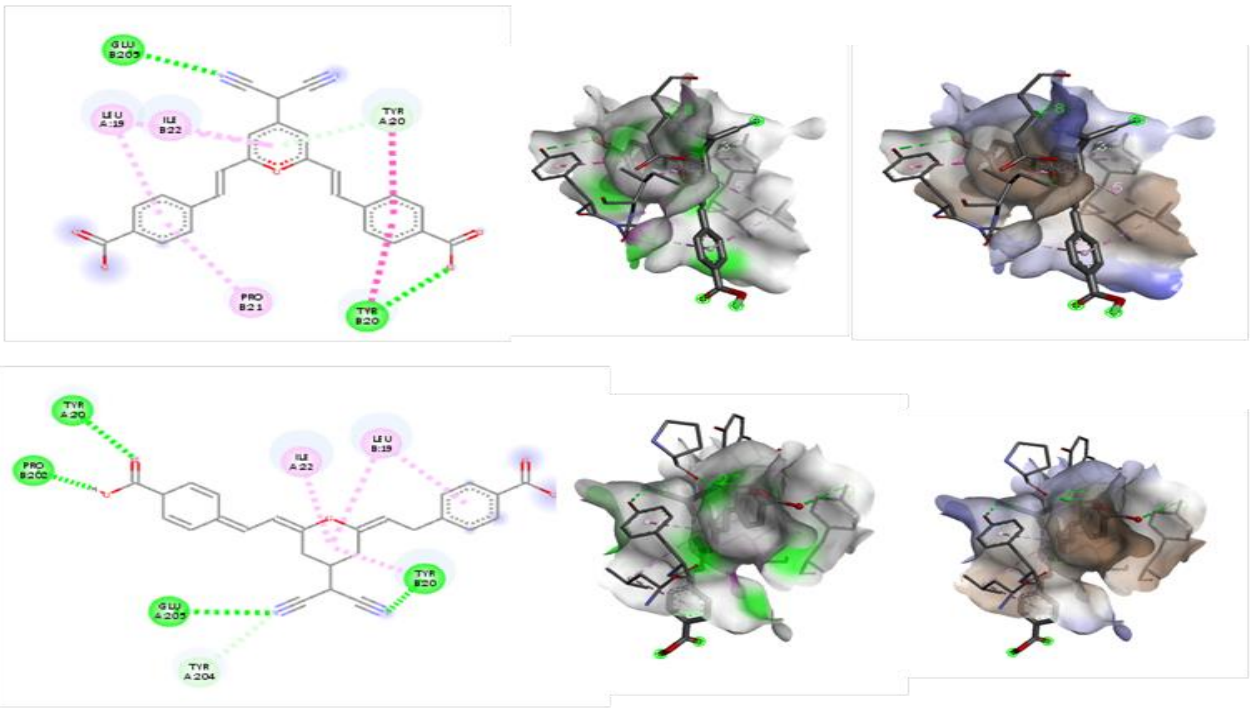
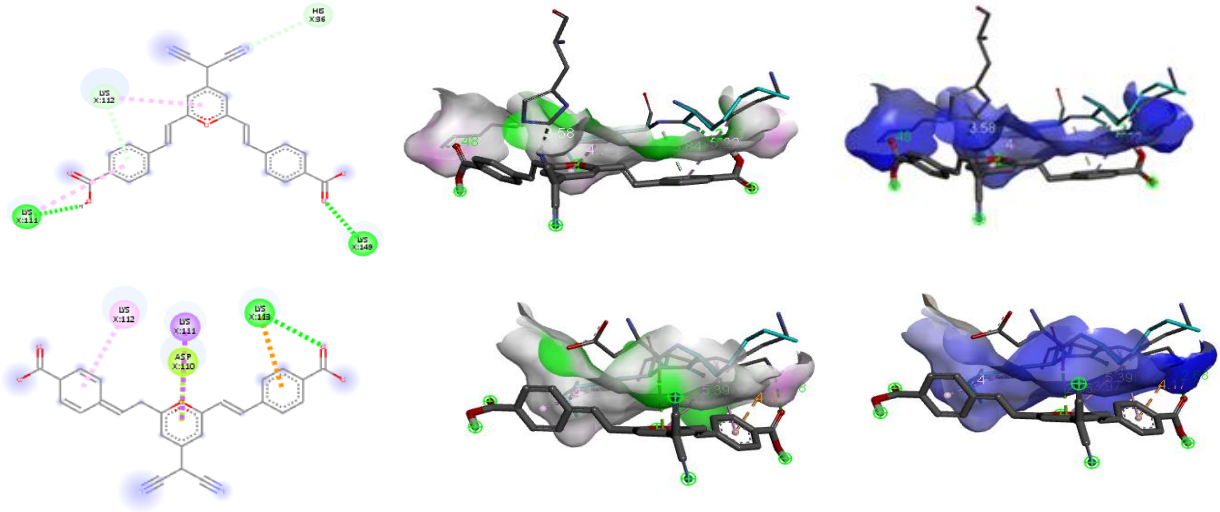


Figure 15: 2D Structures of all the ten conformers of CA-1DCM dye with pictorial representation of the hydrogen-bonding and hydrophobic interaction contour map profile.

The above representation of the conformers clearly reveals the role of the functional groups involved in HB and the amino acid contributing to the stability of the host-guest complex. Interestingly, all the conformers are unique and differ in the pattern of binding distances between the donor and acceptor moieties.



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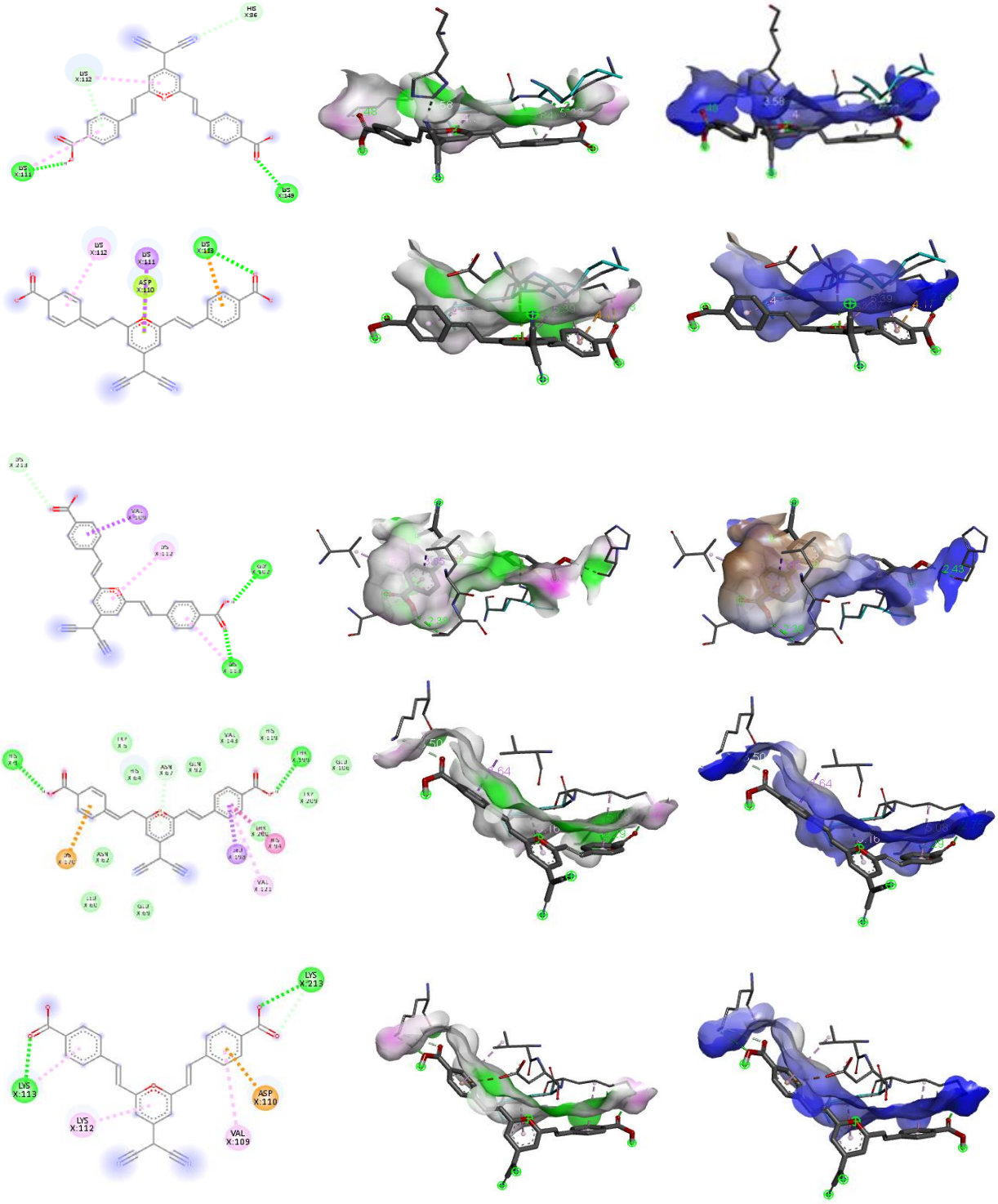

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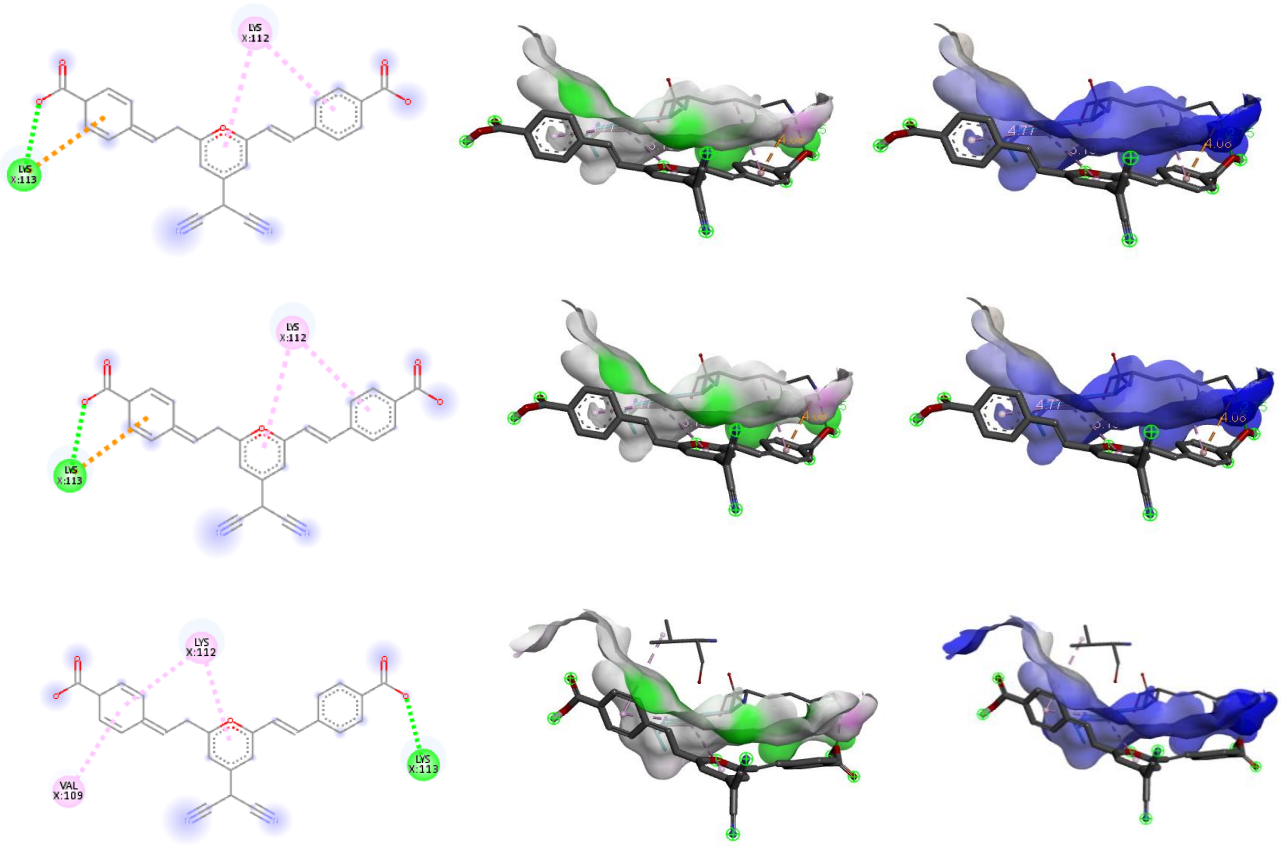

Figure 16: 2D Structures of all the ten conformers of CA-2DCM dye with pictorial representation of the hydrogen-bonding and hydrophobic interaction contour map profile.

5. Conclusion

Molecular docking studies establish authentically in understanding the binding abilities and affinity of various CA inhibiting drugs with Carbonic Anhydrase enzymes. Among the diuretic drugs interactions explored, the binding energy of Acetazolomide has the highest binding affinity with the CA 1, while comparing the binding affinity with CA2, the docking studies illustrates that Ethoxzolamide has the highest affinity towards CA2. Through docking studies, the role of host-guest interactions is largely governed by the competing guest molecules is established, whereas the guest molecule promotes more binding efficiency of the drugs binding with Carbonic Anhydrase

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