RESEARCH ARTICLE

In-Silico Docking Studies of Carbonic Anhydrase Inhibitors in the Management of Neuropathic Pain

Himanshu Singh[1], Reema Sinha[2], Sandeep K. Bansal[2], Rahul Kaushik[3], Krishan K. Verma[3]

Abstract

Background: In the present study, the in-silico docking studies of carbonic anhydrase inhibitors with 4RUX i.e. The crystal Structure of Human carbonic Anhydrase II protein was performed in the management of neuropathic pain.

Materials and Methods: The crystal structure of protein PDB ID: 4RUX was downloaded from RCSB PDB database and the ligand molecules of carbonic anhydrase inhibitors was drew from Marvin Sketch. Docking studies between ligand and protein to predict binding interactions by using AutoDock 4.2 and the receptor ligand complex interaction viewed by using Biovia Drug Discovery studio.

Result: Carbonic anhydrase inhibitors showed binding energy ranging between -5.41 to -8.63. Ganoderic acid A showed better binding energy -8.63 kcal/mol than the standard Acetazolamide -6.22 kcal/mol.

Conclusion: The result clearly indicate that that among carbonic anhydrase inhibitors Ganoderic acid A and Morindone shows better hydrogen bonding and binding affinity towards carbonic anhydrase II (PDB ID: 4RUX). Thus, conclude that among carbonic anhydrase inhibitors Ganoderic acid A (obtained from Ganoderma lucidium) and Morindone (both obtained from Morinda citrifolia (NONI)} provide better pharmacological effect.

Keywords: Carbonic Anhydrase Inhibitors, AutoDock 4.2, 4RUX, Ganoderic Acid A, Acetazolamide.

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Introduction

Pain has arises from the Latin word "Poena" or penalty which means unpleasant sensory and emotional experience associated with actual and potential tissue damage.[1] Pain is the most common reason for people to seek medical attention. Despite this, noniceptive pain is a vital physiological process that signals actual or potential tissue damage. By so doing, it protects the individual from injury and serves the survival of species. By contrast, direct injury to neural tissue can produce nerve or neuropathic pain that last for month or years after any injury has healed.[2]

Neuropathic pain is the group of heterogeneous disease such as diabetes, immune deficiency, malignant diseases, traumatic and ischemic disorders or neuropathic pain with disease or injury of the peripheral or central nervous system.[1]

Neuropathic pain has been defined by the International Association for the Study of Pain (IASP) as "pain caused by a lesion or disease of the somatosensory nervous system.[3] Neuropathic pain is caused from abnormal physiology of central or peripheral nervous system and it is not related to the on going tissue damage or inflammation.[1] It can be caused by traumatic nerve, spinal cord or brain injury (including stroke) or can be associated with diabetic, human immunodeficiency virus/AIDS, and postherpetic neuropathies, or with multiple sclerosis or cancer and/or the toxic effects of chemotherapeutic agents.[2]

Data have indicated that 8% of the general population in UK experience pain of neuropathic origin. In France, 7% of the general population are affected by neuropathic pain.

[1]Noida Institute of Engineering and Technology, Institute of Pharmacy, Greater Noida, India.

[2]Ram-Eesh Institute of Vocational and Technical Education, Gautam Buddh Nagar, Uttar Pradesh, India.

[3]Metro College of Health Sciences and Research, Greater Noida, India.

Corresponding Author Himanshu Singh, Noida Institute of Engineering and Technology, Institute of Pharmacy, Greater Noida, India, Email: rajput.1998himanshusingh@gmail.com

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A study in Canada reported that 17.9% of the general population reported chronic pain with neuropathic symptoms; however, a recent Canadian study has reported lower percentages. A study in the United States revealed that the prevalence rates for neuropathic pain determined by either clinical examination or self reporting were 9.8% and 12.4%, respectively. It is difficult to obtain a true estimate, due to epidemiological studies using different methods assessment and different definitions of neuropathic pain. A recent systematic review of epidermiological neuropathic pain studies across the world by yan Hecke et al. suggests that the prevelance likely lies between 6.9% and 10% in the general population.[3]

Main symptoms of neuropathic pains are sensory abnormalities including:

- *Paresthesias:* numbness or tingling
- *Dysesthesias:* electric shock phenomenon
- *Hyperesthesia:* increased sensitivity to mild painful stimuli
- *Hyperalgesia:* increased sensitivity to normally painful stimuli
- *Hyperpathia:* pain produced by sub threshold stimuli
- *Allodynia:* pain produced by normally non painful of stimuli
- *Pall-Hypoaesthesia:* reduced sensation to vibration
- *Thermal Hypoaesthesia:* reduced sensation to cold or warm stimuli
- *Hypoalgesia:* reduced sensation to painful stimuli
- *Heat and Cold Hyperalgesia:* pain from normally non painful heat and cold stimuli
- *Hypoaesthesia:* reduced sensation to non painful stimuli.[1]

Neuropathic pain is a common condition that results from various aetiologies and can be categorized into either peripheral or central neuropathic pain syndromes. Central neuropathic pain is the result of a central lesion or disease such as stroke, multiple sclerosis or spinal cord injury, whereas peripheral neuropathic pain occurs from dysfunction or damage to peripheral nerves.

Peripheral nerve damage in early life does not simply remove a source input from the somatosensory system, it triggers great change in neural circuitry and leads to long term alterations spinal somatosensory function. However nature of these changes is dependent upon when exactly, in terms of postnatal age, this age nerve damage occurs. A major consequence of nerve damage, in adult man and laboratory animals, is the onset of neuropathic pain, characterized by allodynia and pain hypersensitivity from the partially denervated regions.[7]

Peripheral nerve injury negatively influences spinal gamma aminobutyric acid (GABA)-ergic networks via a reduction in the neuron- specific potassium-chloride {k (+)-Cl (-)} cotransporter (KCC2), which leads to neuropathic allodynia.[6] It has long been recognized that pharmacological manipulation of spinal GABAergic circuits can achieves analgesia. However recently become clear that following peripheral nerve injury (PNI) there are changes in GABAergic function that limit the analgesic effect of spinally applied GABA-A receptor agonists and allosteric modulators and that spinal GABAergic circuits may even promote pathological pain resulting from PNI. The strongest evidence demonstrating that the neuron specific K (+)-Cl (-) cotransporter, is downregulated contributing to a loss of Cl (-) dependent fast inhibitory neurotransmitter and potentially to the generation of GABA-A receptor mediated excitation. While thus has been shown to occur following PNI in outer lamina dorsal horn neurons, and in several other pain models, it is also true that GABA-A agonist and positive allosteric modulators retain anti-allodynic effects and grafting of GABAergic neurons into the spinal cord following alleviates symptoms of neuropathic pain. While brief GABA-A receptor activation leads to Cl (-) – influx dependent hyperpolarization, prolonged receptor engagement leads to a strong HCO_3 (-)-efflux dependent depolarization that has been linked to several neurological disorders. This situation might be exacerbated when KCC2 expression is decreased therefore compromising Cl (-) gradients in GABA responsive neurons. The influence of thus HCO3 (-) dependent depolarization can be mitigated by Carbonic Anhydrase Inhibition.[4] It was shown that spinal inhibition of Carbonic Anhydrase with Acetazolamide reduces neuropathic allodynia in rats and that Acetazolamide and Benzodiazepine have synergistic spinal effects following PNI.[8] This suggest that loss of Cl (-) extrusion capacity impairs the ability of GABA-A receptor engagement to achieve inhibition of spinal network activity. A potential strategy to mitigate this effect, and therefore restore full analgesic efficacy of GABA-A agonists and allosteric modulators, is via inhibition of Carbonic Anhydrase.

Materials and Methods

Retrieval of the structure and Preparation of protein

The three dimensional crystal structure of human carbonic anhydrase ii protein (pdb id 4rux) was downloaded from the RCSB (research collaborator structural bioinformatics) protein data bank.

The downloaded protein structure in their ".pdb" format was cleaned to remove the non-amino acid residues, such as water molecule, ions, ligand are that are in the complex by using SWISS PDB VIEWER and AutoDock Tools.

These has to be done, since, these molecules will interfere with the interaction between the target molecule and protein in AutoDock.

That pdb format file was converted to PDBQT file using AutoDock tools to generate atomic coordinates.

Retrieval of ligand and Preparation of ligand

The chemical structure of ligands was seen from PubChem compound database and ZINC database. And these ligands was drew by MARVIN SKETCH and TRICHOS MOL2 format of this ligand was converted to PDBQT file using AutoDock tools to generate atomic coordinates.

Executing AutoDock

AutoDock software calculates and predicts the interaction between the ligand molecule and protein molecule based on predefined parameters. To be precise, the interactions between the molecules will be calculated at a specified region in the protein. This region was defined, using Grip map option. Ultimately, the software predicts the interaction and binding energy of the ligand molecule and the amino acid present in the GridBox only. Before executing the AutoDock,

the ".pdb" files of the protein and ".mol2" files of ligand was moved into the working folder (ADT folder) where all file beginning files are present.

There are following procedure follow in the analysis of AutoDock:

- Initializing molecules
- Running AutoGrid
- Running AutoDock
- Analyzing interaction energy.

Initializing molecules

Initializing the molecule mainly include addition of hydrogen atoms, compute gasteiger, assign AD4 type atoms in the protein molecule. While for ligand molecule, detect root, torsion tree, choose torsion and detecting the rotatable bonds.

Once the protein molecule is opened it is important to change the view of protein. It will be line view by default which changes to surface view that makes it friendly to set the GridBox.

To initialize macromolecule

Go to *File* >> Read molecule
Select Protein file ("4RUX.pdb" file)
Edit >> Hydrogens>> Add
Edit >> Charges >> Compute gasteiger
Edit >> Hydrogens >> polar only >> ok
Edit >> Atoms >> Assign AD4 type
File >> Save >> write PDBQT >> browse ADT folder and save this file in ADT folder.

To Initialize Ligand Molecule

Go to Ligand >> Input >> open (select mol2 file format in popup window and select the file of ligand)
Ligand >> Torsion tree >> Detect root
Ligand >> Torsion tree >> choose torsions >> done
Ligand >> Output >>Save as PBDQT (save this file in the ADT folder).

Executing AutoGrid

AutoGrid was executed, to define the region in the protein to be analyzed for the interaction with the ligand molecule. The GridBox was set in the AutoDock to cover the identified binding sites.

AutoDock only analyzes the interaction of ligand molecule and the amino acids that are present within the GridBox.

The GridBox options menu shows two fields: the size of the GridBox which was increased or decreased using the no. of points in X/Y/Z dimension and these points was set at 70/70/80 for all ligands and another fields for position of GridBox i.e. Grid spacing can be adjusted in X/Y/Z axis which was taken as 0.375 Angstrom. The current Grid file was saved in the ".gpf" format and running AutoGrid4.

To run AutoGrid

Go to Grid >> Macromolecule >> open (open .pdbqt file from ADT folder)
Grid >> Set map types >>Directly (add any special atoms present in ligand molecule in the prompted box and click on continue).
Grid >> Grid box (for saving grid box coordinates, click on File and choose close saving current).
Grid >> Output >> Save GPF (save this file with protein code .gpf)
Run >> run AutoGrid >> click

- Select Program Pathname: 'AuotGrid4.exe'
- Select Parameter Filename: 'Grid.gpf'
- AutoFill for Log Filename: 'grid.glg'
- Launch

Executing AutoDock:

When the AutoGrid file was successfully completed then the docking file was prepared.

For preparing docking file macromolecule set as rigid file name and the ".PDBQT" file of protein and ligand was selected. Genetic algorithm and docking parameters was taken as default and the output in Lamarckian GA was saved (docking file saved in ".dpf" format) and running AutoDock.

To run AutoDock

Go to *Docking* >> Macromolecule >> set rigid filename

- Select '4RUX.pdbqt' >> open
  Docking >> Ligand >> open
  Docking >> Search Parameters >> Genetic Algorithm
  Docking >> Docking Parameters (accept)
  Docking >> Output >> Lamarckian GA (4.2)
- Save file as 'dock.dpf'
  Run >> Run AutoDock
- Select Program Pathname: 'autodock4.exe"
- Select Parameter Filename: ' dock.dpf'
- AutoFill for Log Filename: 'dock.dlg'
- Launch

Analyzing interaction energy:

After the AutoDock was successfully executed. The result was given in the ten next conformations. These was viewed in the analyzed option in the order of their free energy binding, by choosing the "Play, ranked by energy" option.

To Analyze Interaction Energy

Analyze >> Docking >> open
Select 'dock.dlg' >> open
Analyze >> Macromolecule >> choose
Analyze >> conformation >> play, ranked by energy

- Click on '&' button (open panel to change play options

Set Play Option

- Check 'Build H-bonds'
- Check 'Show Info'
- Build Current Write complex (Save as 'result.pdb' file).[9]

Results and Discussion

In-silico docking study, was carried out to identify the inhibiting potential of carbonic anhydrase inhibitors against human carbonic anhydrase II protein (PDB ID: 4RUX) for the management of neuropathic pain. In this study 17 different carbonic anhydrase inhibitors were selected for the in-silico docking studies. One carbonic anhydrase inhibitor *i.e.* Acetazolamide was taken as positive control for the screening of other inhibitors.

The protein complex of human carbonic anhydrase (4RUX) was downloaded from RCSB PDB and that PDB was cleaned by using SWISS PDB VIEWER and AUTODOCKTOOLS for the removal of non-amino acid residue such as ions, water, ligands, etc. The ligand molecule was drew in the MARVIN SKETCH. The docking studies were performed by using AUTODOCK 4.2. all the 17 carbonic anhydrase inhibitors used in this study were listed in Table 1 these ligands were employed for molecular docking in AUTODOCK 4.2 to predict the interaction between the mentioned ligands and the human carbonic anhydrase II (PDB ID: 4RUX). Analysis of the receptor/ ligand complex models generated after the successful completion of docking was based on the two parameters such as H-bond interaction and binding energy to the active site residues. The interaction images of hydrogen bonding were obtain from analyzing the docking file and it seen in the Biovia Discovery Studio.

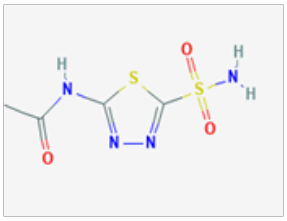
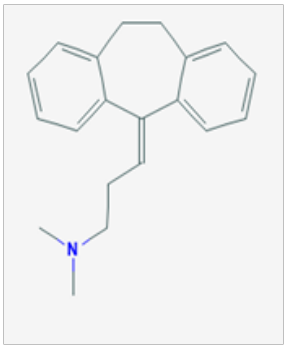
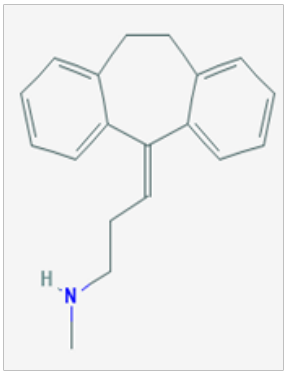
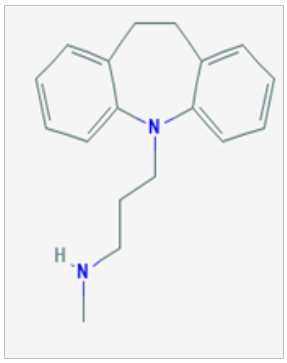
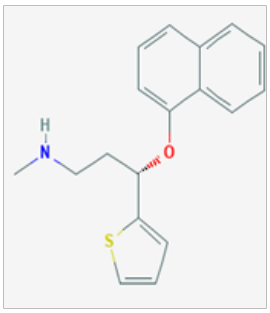
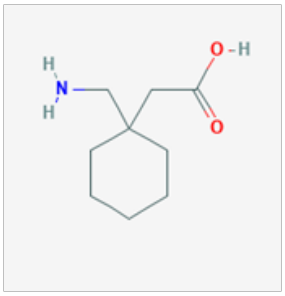
The binding mode of the carbonic anhydrase inhibitors within the active sites of 4RUX has been analyzed. The amino acid residue responsible for binding interaction of Acetazolamide with the enzyme was, HIS A: 119, HIS A: 94, THR A: 199, HIS A: 96 and GLN A: 92.

The potential binding interaction of Ganoderic acid A with 4RUX was found that, HIS A: 119, HIS A: 96, HIS A: 94, THR A: 200, THR A: 199, ASN A: 67 and ASN A: 62.

The potential binding interaction of Morindone with 4RUX was found that, HIS A: 96, HIS A: 119, GLN A: 92, ASN A: 67 and THR A: 199.

These results shows that the effective binding orientations were present in the selected carbonic anhydrase inhibitors

Table 1

Drugs	*Chemical Structure*	*Molecular formula*
Acetazolamide		C4H6N4O3S2
Amitriptyline		C20H23N
Nortriptyline		C19H21N
Desipramine		C18H22N2
Duloxetine		C18H19NOS
Gabapentin		C9H17NO2

(Contd.)

Table 1

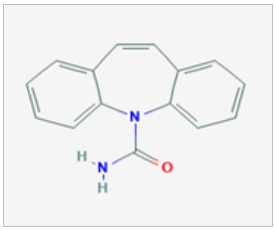
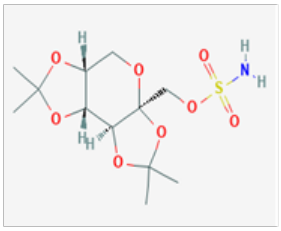
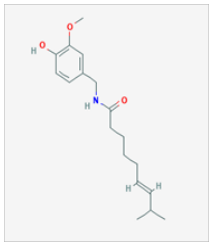
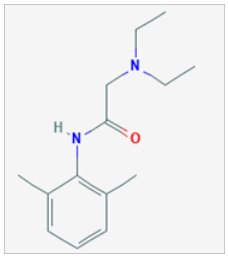
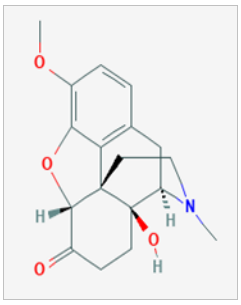
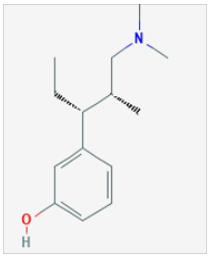
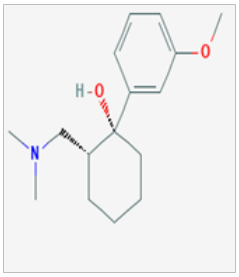
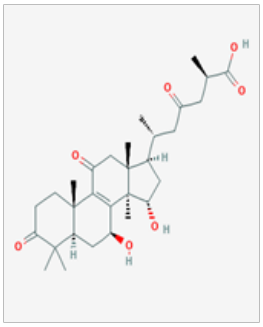
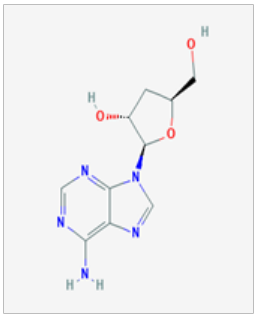
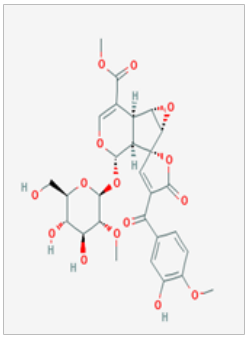
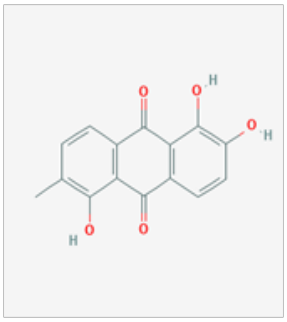
Drugs	*Chemical Structure*	*Molecular formula*
Carbamazepine		C15H12N2O
Topiramate		C12H21NO8S
Capsaicin		C18H27NO3
Lidocaine		C14H22N2O
Oxycodone		C18H21NO4
Tapentadol		C14H23NO

Table 1

Drugs	*Chemical Structure*	*Molecular formula*
Tramadol		C16H25NO2
Ganoderic Acid A		C30H44O7
Cordycepin		C10H13N5O3
Citrifolinoside		C28H30 O15
Morindone		C15H10O5

when compared with the standard Acetazolamide. Interactions images of all inhibitors with 4RUX was given below: Figs 1 to 17.

Binding energy of the individual compound were calculated using the following formula,

Binding Energy = A + B + C + D

Where as,

- A Denotes final Intermolecular energy + Vander Waal energy + hydrogen bonds + desolvation energy+ electrostatic energy (kcal/mol),
- B Denotes final total internal energy (kcal/mol),
- C Denotes torsional free energy (kcal/mol),
- D Denotes unbound system's energy (kcal/mol).

Carbonic anhydrase inhibitors showed binding energy ranging between -5.41 to -8.63. Ganoderic acid A showed better binding energy -8.63 kcal/mol than the standard Acetazolamide –6.22 kcal/mol. All the selected carbonic

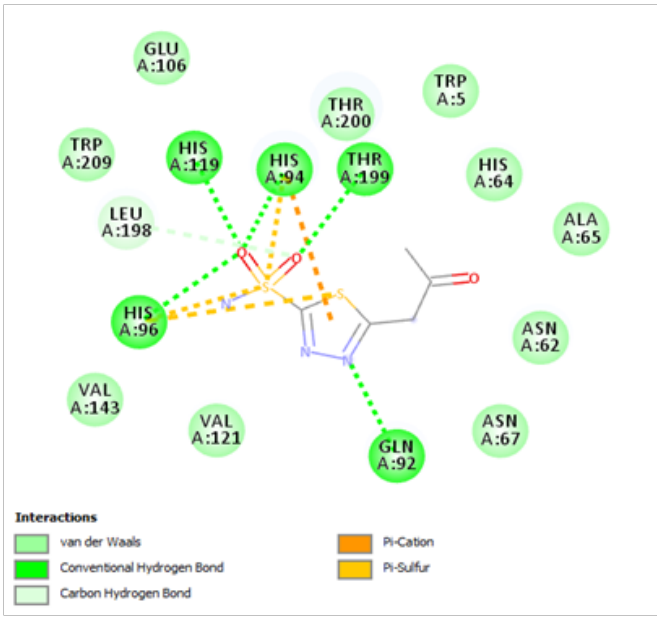


Fig. 1: Interaction image of Acetazolamide (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

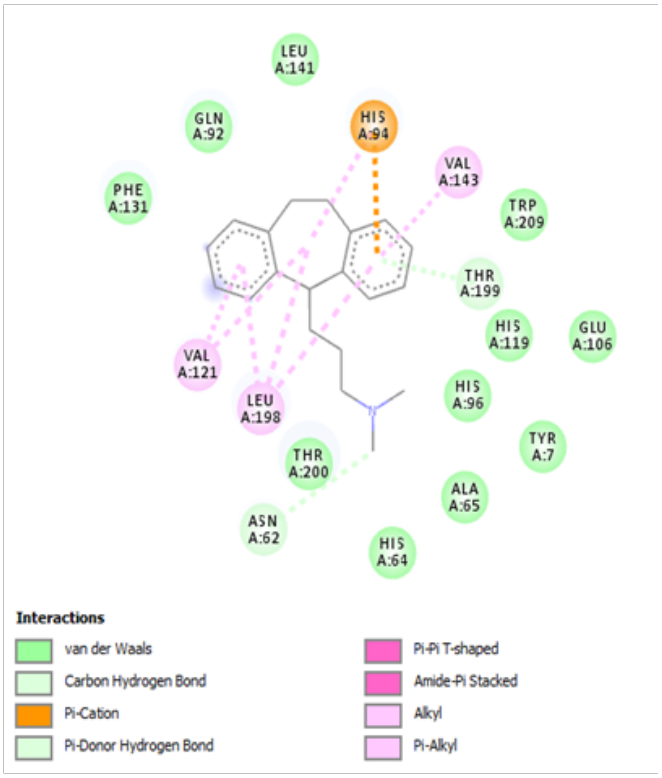


Fig. 2: Interaction image of Amitriptyline (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

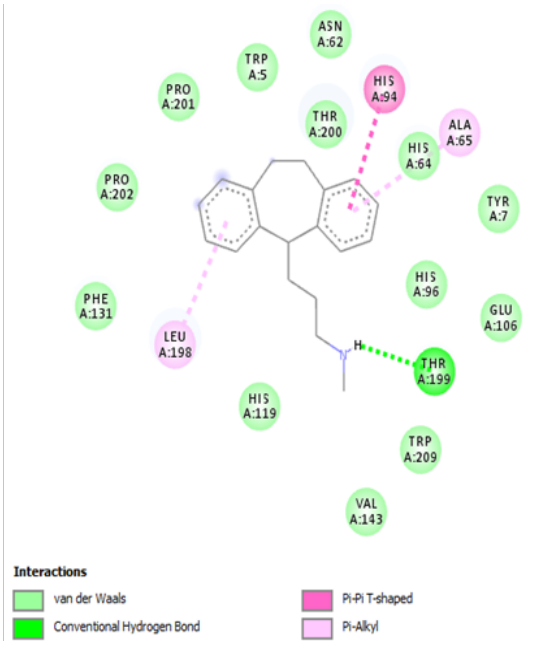


Fig. 3: Interaction image of Nortriptyline (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

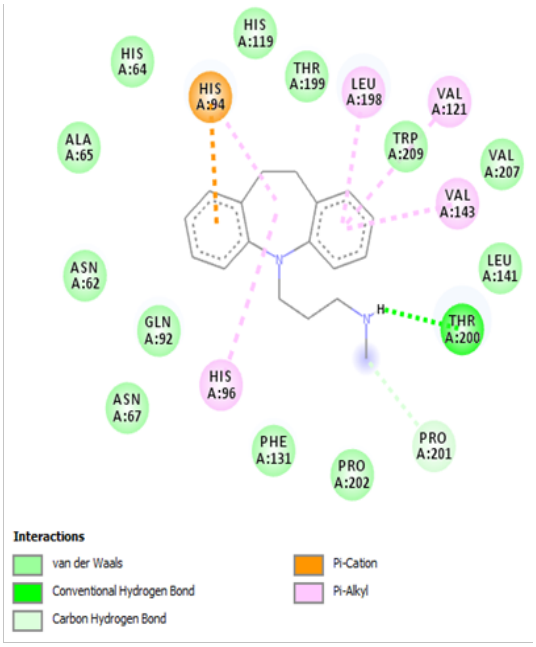


Fig. 4: Interaction image of Desipramine (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

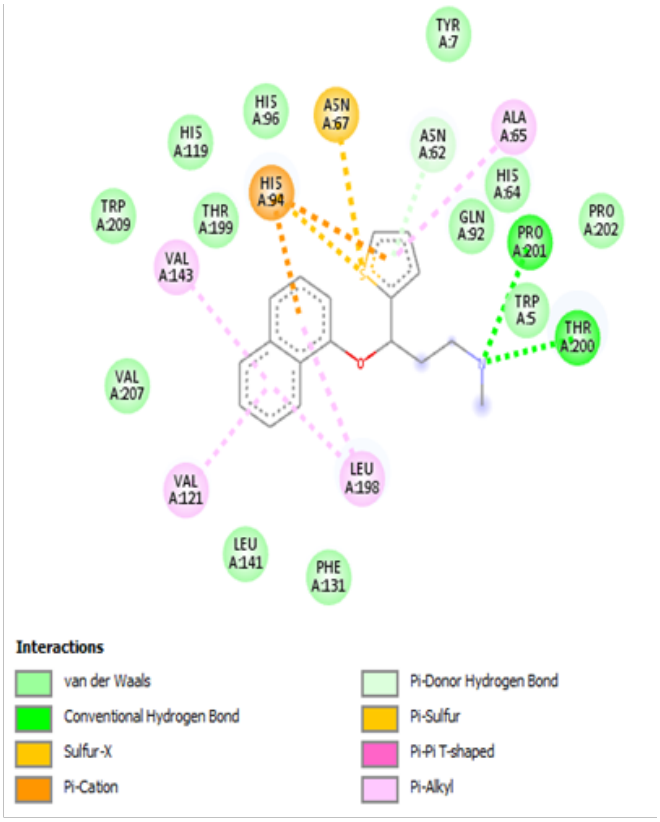


Fig. 5: Interaction image of Duloxetine (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

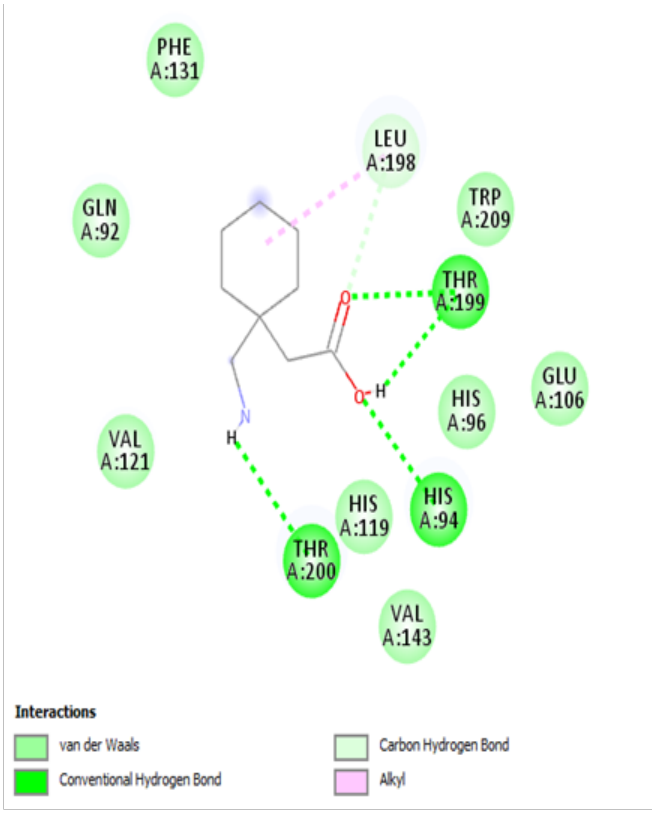


Fig. 6: Interaction image of Gabapentin (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

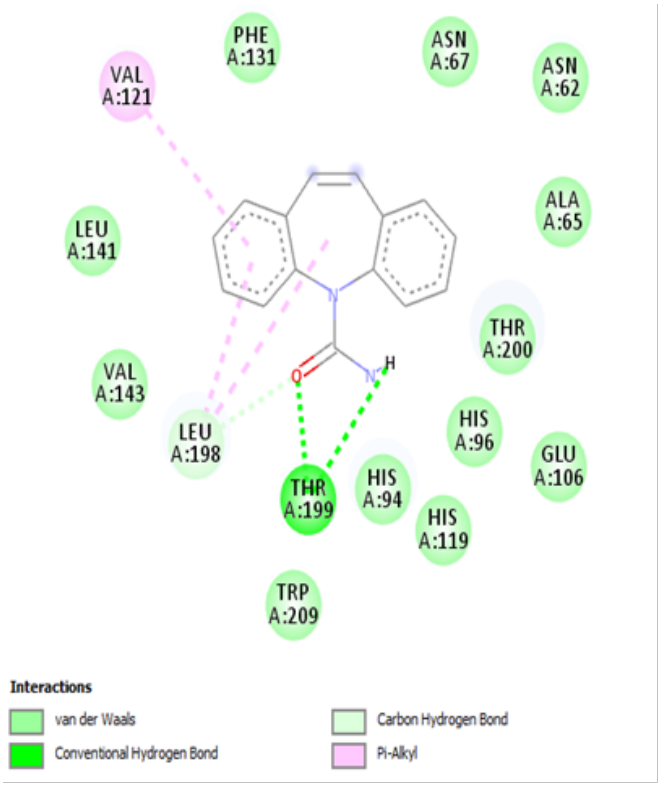


Fig. 7: Interaction image of Carbamazepine (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

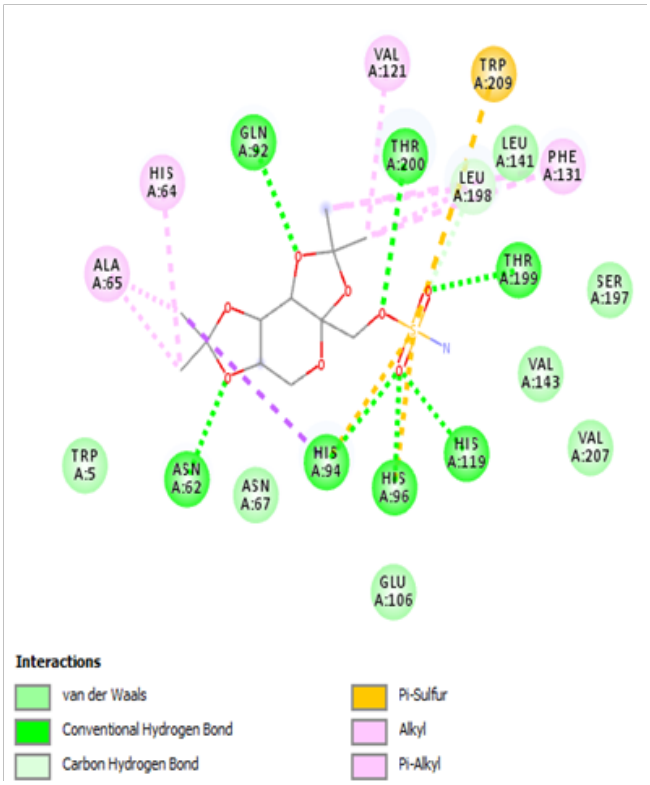


Fig. 8: Interaction image of Topiramate (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

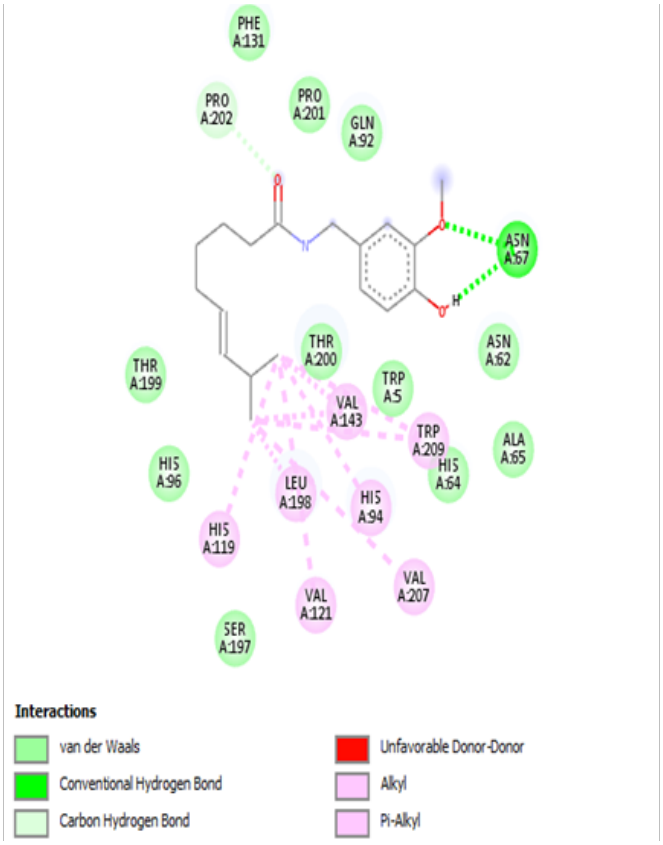


Fig. 9: Interaction image of Capsaicin (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

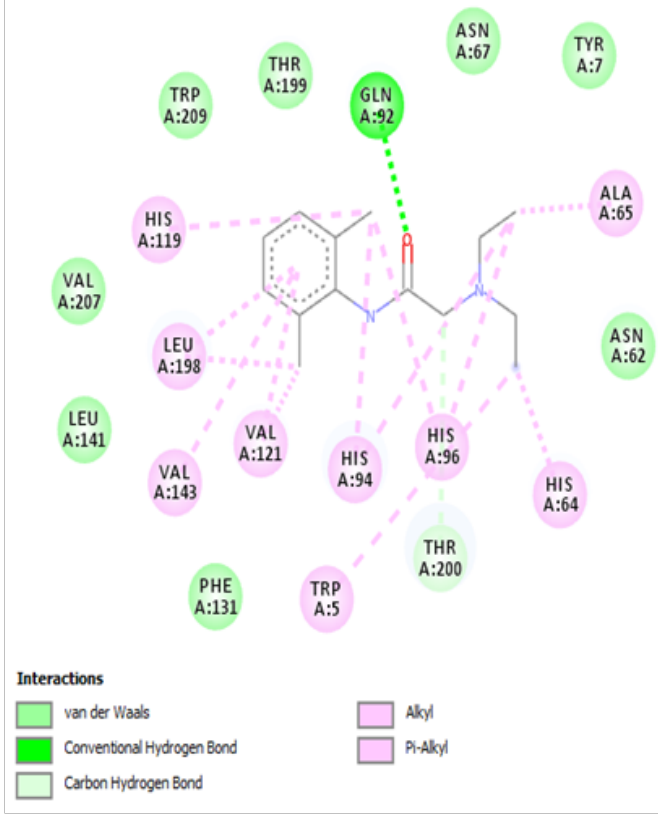


Fig. 10: Interaction image of Lidocaine (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

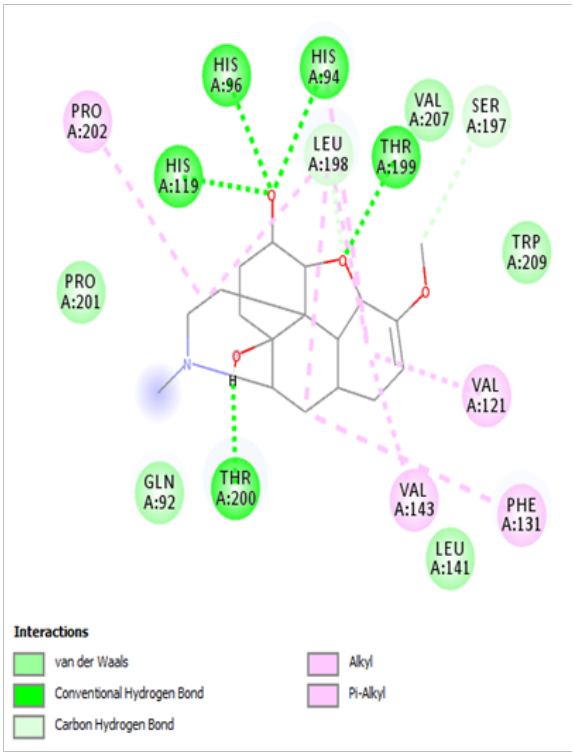


Fig. 11: Interaction image of Oxycodone (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

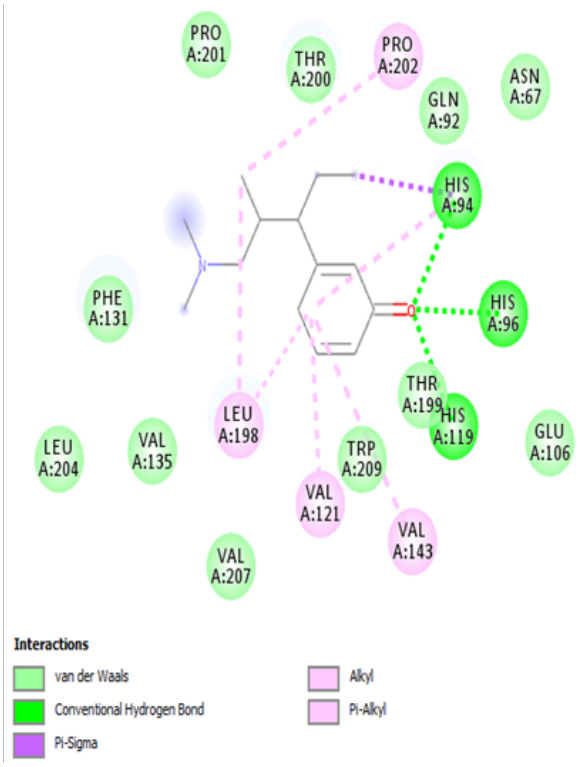


Fig. 12: Interaction image of Tapentadol (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

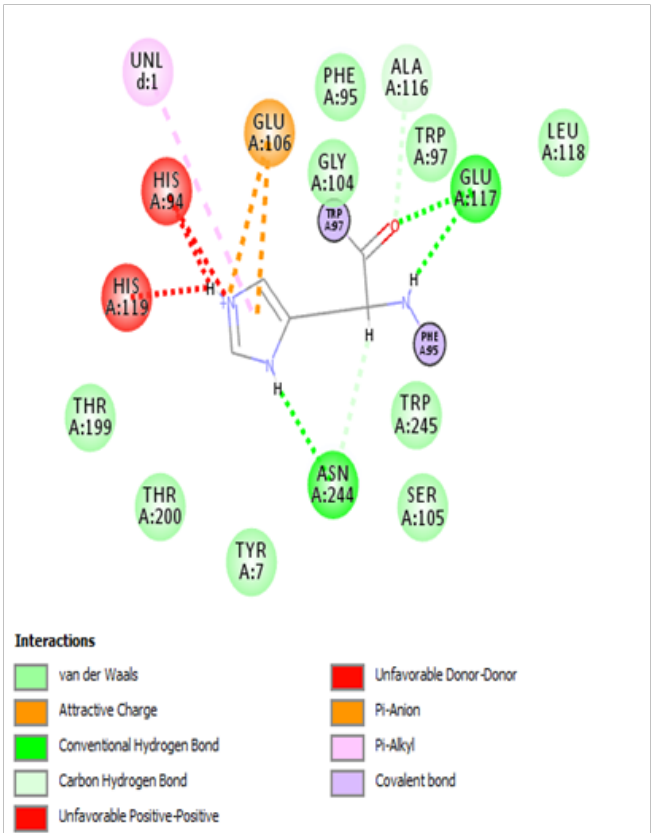


Fig. 13: Interaction image of Tramadol (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

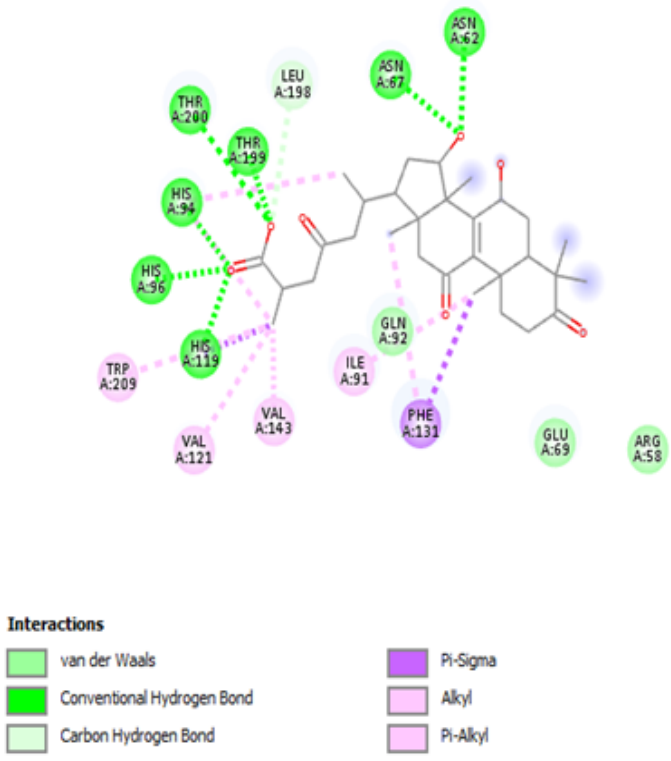


Fig.14: Interaction image of Ganoderic Acid A with Carbonic Anhydrase II (PDB ID: 4RUX)

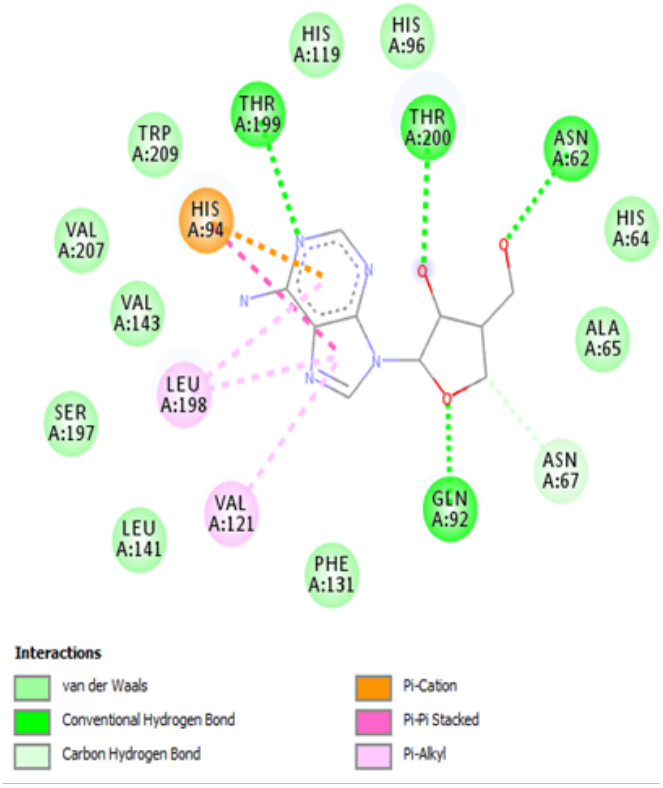


Fig. 15: Interaction image of Cordycepin (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

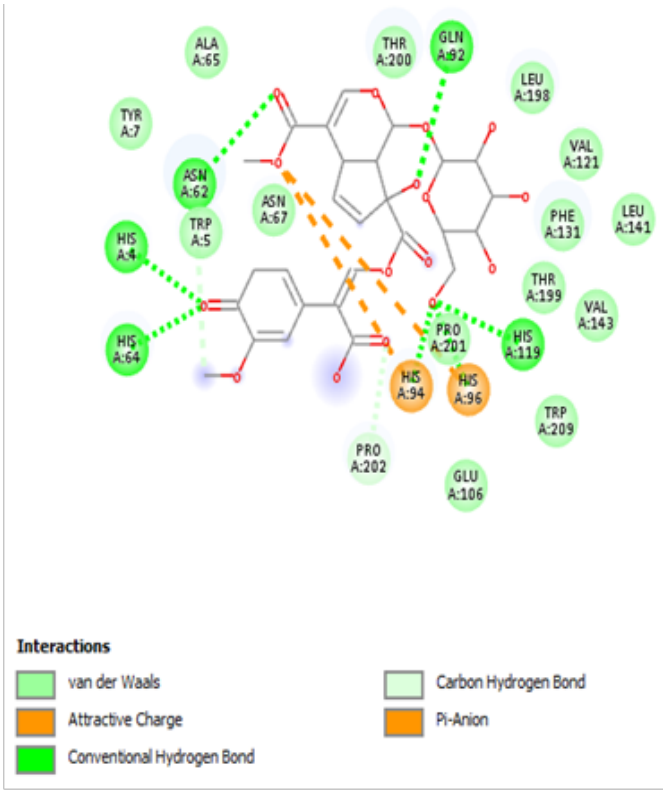


Fig. 16: Interaction image of Citrifolinoside (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX)

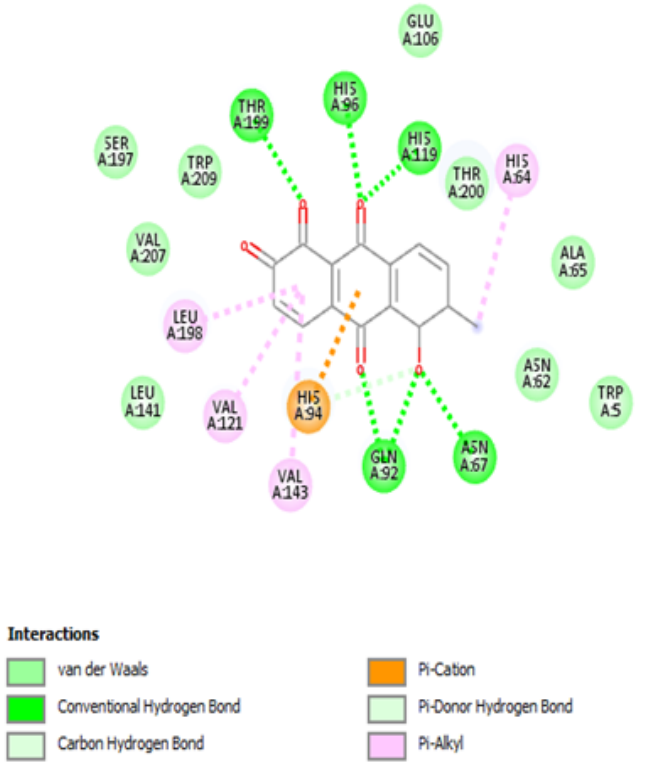


Fig. 17: Interaction image of Morindone (Ligand) with Carbonic Anhydrase II (PDB ID: 4RUX).

anhydrase inhibitor had showed binding energy compared to that of standard. This proves that carbonic anhydrase inhibitors consist of potential carbonic anhydrase II inhibitory binding sites similar to that of standard and are more better than standard.

In addition, one another parameter i.e. hydrogen bonding were also determined. The binding energy and no. of hydrogen bonds of all carbonic anhydrase inhibitors with 4RUX was listed in Table 2. inhibition constant is directly proportional to the binding energy that means Ganoderic acid A showed excellent inhibition constant.

Based on the docking studies, the carbonic anhydrase II inhibitory activity was found to be decreased in the order of Ganoderic acid A, Morindone, Amitriptyline, and Citrifolinoside which posses potential inhibitory binding sites comparision to standard. This may attributed due to the difference in position of functional group or difference in chemical structure.

Conclusion

Docking software serves as better choice in finding drug for neuropathic pain. Nowadays neuropathic pain is common among diabetic patients and aged global populations. The result clearly indicate that that among carbonic anhydrase inhibitors Ganoderic acid A and Morindone shows better hydrogen bonding and binding affinity towards carbonic anhydrase II (PDB ID: 4RUX). Thus, conclude that among carbonic anhydrase inhibitors Ganoderic acid A (obtained from Ganoderma lucidium) and Morindone (both obtained from Morinda citrifolia (NONI)} provide better pharmacological effect.

Table 2

Drugs	*No. of hydrogen bonds*	*Binding energy*
Acetazolamide	06	–6.22
Amitriptyline	0	–8.01
Nortriptyline	01	–7.6
Desipramine	01	–7.63
Duloxetine	02	–5.81
Gabapentin	05	–5.43
Carbamazepine	01	–7.7
Topiramate	06	–7.29
Capsaicin	03	–5.52
Lidocaine	01	–6.54
Oxycodone	03	–7.53
Tapentadol	03	–5.14
Tramadol	02	–6.48
Ganoderic acid A	06	–8.63
Cordycepin	04	–6.5
Citrifolinoside	07	–7.6
Morindone	02	–8.43

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