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HEPATITIS C VIRUS RNA-DEPENDENT RNA POLYMERASE NS5B INHIBITION POTENTIALS OF ANTI-HELMINTHIC DRUG ALBENDAZOLE AND ITS BIOTRANSFORMED METABOLITES: AN *INSILICO* STUDY

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ABSTRACT

Albendazaole, the broad spectrum anti-helminthic benzimidazole derivative and its biotransformed products were studied for Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B inhibition potentials in silico using Sofosbuvir, a potent NS5B inhibitor as reference standard drug. Albendazole and its biotransformed products viz. albendazole sulfoxide (M1), albendazole sulfone (M2) and two unidentified metabolites (M3 and M4) and the standard drug sofosbuvir exhibited binding energies of -6.6, -6.5, -7.2,-7.0,-6.1 and -7.6 Kcalmol^{-1}respectively against Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B (PDB ID: 2GIR).

In the present study, albendazole a broad spectrum anti-helminthic drug showed strong interaction to Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B. Hence, we conclude that albendazole may have an addition impact in treatment of Schistosomiasis and Hepatitis C Virus (HCV) coinfections.

KEY WORDS

Albendazole, albendazole sulfoxide, albendazole sulfone, Hepatitis C Virus RNA-Dependent RNA Polymerase.

1. INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem, with nearly 3% of the world's population persistently infected with the virus (Fan et al., 2007). It is a major cause of liver failure responsible for majority of liver transplants. The RNA-dependent RNA polymerase NS5B in particular has been subject of intense research for developing new drugs in the past decade because of its essential role in viral replication (Wei et al., 2016).

Schistosomiasis is a parasitic disease caused by flukes (trematodes) of the genus *Schistosoma* which remains endemic in about 75 countries worldwide. It continues to be a significant cause of morbidity and mortality (Enain et al., 2015). World Health Organization (WHO) considers schistosomiasis as the second only to malaria in socioeconomic importance worldwide and the third more frequent parasitic disease in public health importance (Elbaz and Esmat, 2013). Hepatic schistosomiasis, or schistosomal hepatopathy, is the most common form of the chronic disease and usually results from heavy *S. mansoni* infection. Albendazole which is a benzimidazole has a fasciolicidal effect on Schistosomiasis (Enain et al., 2015).

Schistosomiasis and Hepatitis C Virus (HCV) coinfection is common in Egypt and other developing countries (Kamal et al., 2001). Co-infections contribute to HIV-related pathogenesis and often increase viral load in HIV-infected people. Patients coinfected with HCV and schistosomiasis exhibit a unique clinical, virological, and histological pattern manifested by viral persistence with high HCV RNA titers, as well as higher necro inflammatory and fibrosis scores in their liver biopsy

samples (Kamal et al., 2001) and a greater mortality rate than those infected with only HCV.

Benzimidazole is a heterocyclic aromatic organic compound with a bicyclic structure consisting of benzene and imidazole rings fusion. The derivatives of benzimidazoles has broad range of biological activity which includes anti-bacterial, anti-fungal, anti-viral anti-tubercular, anti-cancer, anti-tumour, anti-helmintic, anti-hepatitis C virus, anti-allergic, anti-HIV, analgesic, anti-psychotic, anti-depressant, anti-anxiety, anti-hypertensive, anti-ulcer, anti-inflammatory, topoisomeraase inhibitor, thromboxane A2 receptor antagonist and 5HT3 antagonist (Swaraj et al., 2011).

Because of diverse biological activities of benzimidazole derivatives, present investigation is directed to study *in silico* anti-hepatitis C virus activity of albendazole and both its reported and novel metabolites targeting NS5B protein which is a key enzyme in viral replication.

2. MATERIALS AND METHODS:

Molecular docking of anti-helminthic drug albendazole and its biotransformed metabolites viz. albendazole sulfoxide (M1), albendazole sulfone (M2) and two novel metabolites of albendazole (M3 and M4) produced by thermophilic fungus *Rhizomucor pusillus* (Prasad et al., 2011) was performed with NS5B polymerase of Hepatitis C Virus which is RNA dependent RNA polymerase using Auto dock vina software, an interactive molecular graphics programme to understand the protein-ligand interactions (available from http://viba.scripps.edu/).

2.1. Preparation of target protein:

The crystal structures of Hepatitis C Virus RNA-Dependent RNA Polymerase with a resolution of 1.9 A° (PDB ID: 2GIR) with NNI-1 inhibitor (Rao et al., 2011) was obtained from PDB data base (http://www.rcsb.org./pdb).The bound ligand NNI-1 inhibitor was removed from the target protein. All hetero atoms, water molecules were removed, and polar hydrogen atoms were then added, Kollman charges and salvation parameters were assigned by default using Auto dock software.

2.2. Preparation of ligands

The ligands albendazole, albendazole sulfoxide (M1), albendazole sulfone (M2) and other two unidentified metabolites (M3, M4) were drawn using chem Draw software and were converted to 3D PDB format to pdb format from mol format by Accelrys Discovery Studio Visualizer (DS Visualizer). Later Gasteiger charger and hydrogen atoms were added to ligands using Auto dock software.

2.3. Validation of Software

The Autodock Software was validated using the protein NS5B polymerase (PDB ID: 2GIR). The X-ray crystal structure of 2GIR was recovered from protein data bank. The co-crystallized ligand was redocked, reproducing the original interactions of the reference protein-ligand complexes comparing the root-mean square distance of the experimentally determined pose with the docked pose.

3. *In silico* studies: Virtual screening for interaction of albendazole and its 4 metabolites with Hepatitis C Virus RNA-Dependent RNA polymerase by molecular docking

After preparing the ligands as well as receptor Hepatitis C Virus RNA-Dependent RNA Polymerase, both were converted into the pdbqt format using the automated docking tool Auto Dock which were later used for docking. A grid box was prepared to cover the pocket with the main residues of protein binding site by maintaining the grid size of X=40, Y= 40, and Z= 40. An advanced molecular docking program Auto Dock Vina, version 1.1.2 available from http://vina.scripps.edu/download.html was used for docking against the receptors and to estimate the binding affinities (kcal mol^{-1}). The ligands were evaluated *in silico* against Hepatitis C Virus RNA-Dependent RNA Polymerase (PDB ID: 2GIR) enzymes in triplicates and the average of the best conformation was chosen with the lowest docked energy, based on complete docking search (ten runs). The interaction of Hepatitis C Virus RNA-Dependent RNA Polymerase with the ligands, hydrogen bonds, bond lengths and Root Mean Square Difference (RMSD) was analyzed using PyMOL software (http://pymol.sourceforge.net/).

4. RESULTS AND DISCUSSION:

In the present investigation, anti-helmintic drug albendazole and its 4 metabolites viz. albendazole sulfoxide (M1), albendazole sulfone (M2) and two novel metabolites (M3 and M4) produced by biotransformation of albendazole using thermophilic fungal culture *Rhizomucor pusillus* (Prasad et al., 2011) were studied for Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B inhibition potentials in *insilico*so as to use in patients suffering from coinfection of helminthes

and Hepatitis C virus. The structures of all the four ligands were presented in Table 1.

Table.1 showing structures and IUPAC names of albendazole and its metabolites

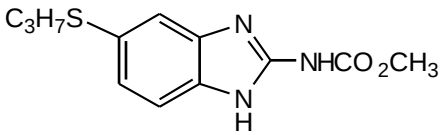
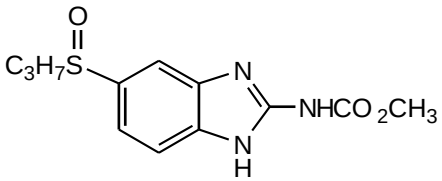
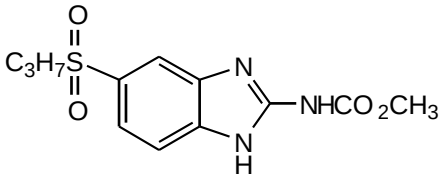
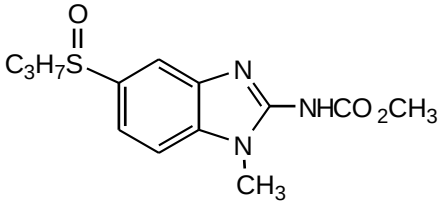
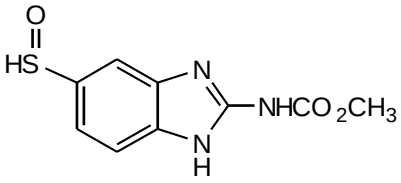
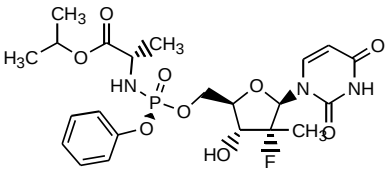
S.No.	Name of the compound	Structure of the compound	IUPAC Name of the compound
1	**Albendazaole (Parent compound)** m/z=265		(5-Propylsulfanyl-1H-benzoimidazol-2-yl)-carbamic acid methyl ester
2.	**Albndazaole sulfoxide (M1)** m/z=282		[5-(Propane-1-sulfinyl)-1H-benzoimidazol-2-yl]-carbamic acid methyl ester
3.	**Albendazole sulfone (M2)** m/z=298		5-(Propane-1-sulfonyl)-1H-benzoimidazol-2-yl]-carbamic acid methyl ester
4.	**Novel metabolite (M3)** m/z=296		[1-Methyl-5-(propane-1-sulfinyl)-1H-benzoimidazol-2-yl]-carbamic acid methyl ester
5.	**Novel metabolite (M4)** m/z=240		Methane; methylN-(5 hydrosulfinyl-1Hbenzimidazol-2-yl) carbamate
6.	**Sofosbuvir (Standard drug)** m/z=529		2-{[5-(2,4-Dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydro-furan-2-ylmethoxy]-phenoxy-phosphorylamino}-propionic acid isopropyl ester

Table.2 showing binding energy, hydrogen bonds, interacting residues of protein and ligands

Name of the Ligand of receptor	Binding Energy Kcal mol^{-1}	Number of hydrogen bonds	Distance (A^0)	Interacting amino acid	Interacting Atom of the ligand
Albendazole	-6.6	3	2.1	Leu-474	HN-1
			2.6	Leu-474	H-10
			2.5	Ser-473	H-10
Metabolite 1	-6.5	3	3.0	Arg-422	NH1-O15
			3.1	Lys-533	NZ-O12
			3.2	Arg-501	NH1-O16
Metabolite 2	-7.2	3	2.6	Leu-474	HN -O
			3.0	Tyr-477	OH-O15
			3.0	Arg-422	NH1-O15
Metabolite 3	-7.0	3	3.1	Arg-422	NH1-O16
			3.0	Tyr-477	OH-O16
			2.6	Leu-474	HN-O
Metabolite 4	-6.1	3	3.3	Leu-536	N-O
	2.2		Phe-472		HN-O
			3.1	Lys-533	NZ-O15
Sofosbuvir	-7.6	4	3.2	Ala-376	O-25
			2.2	Ser-473	OH-17
			2.9	Ser-473	OH-17
			3.4	Tyr-477	O-29

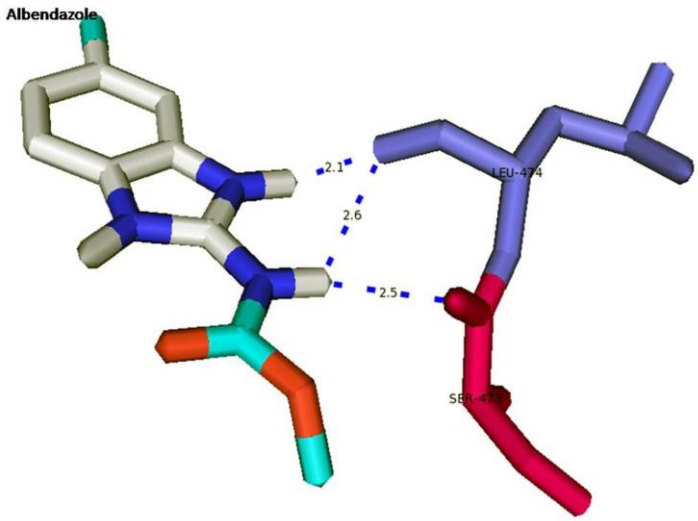


Figure 1. Snapshot of Albendazole and its hydrogen-bond interactions with 2GIR

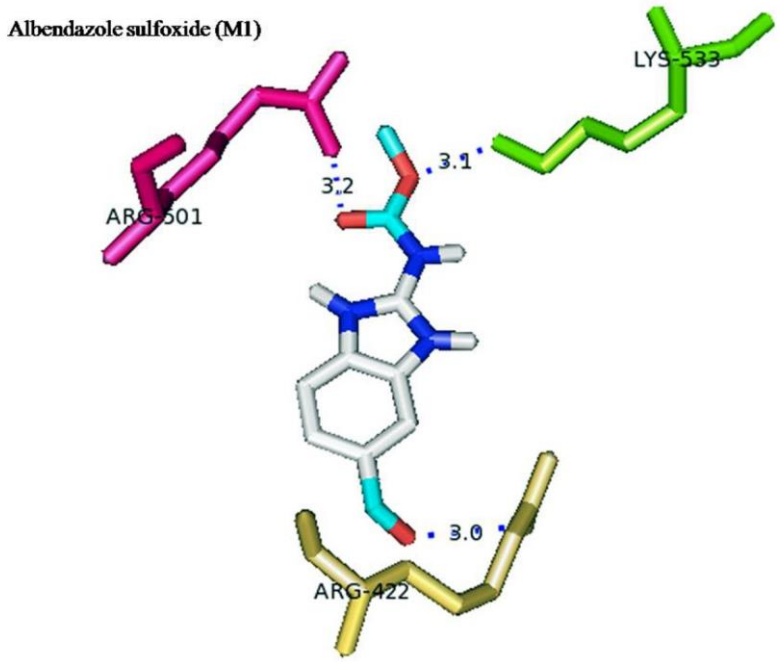


Fig.2 Snapshot of Albendazole sulfoxide (M1) and its hydrogen-bond interactions with 2GIR

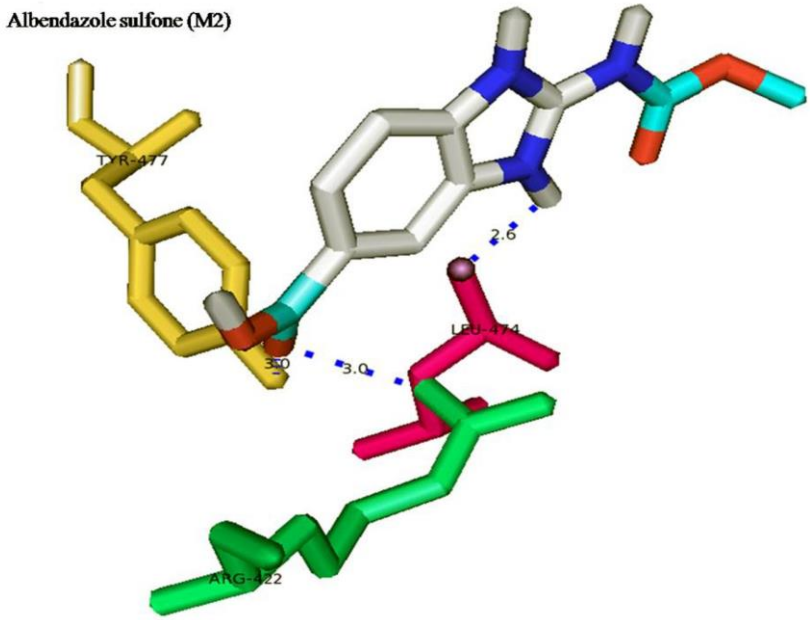


Fig.3 Snapshot of Albendazole sulfone (M2) and its hydrogen-bond interactions with 2GIR

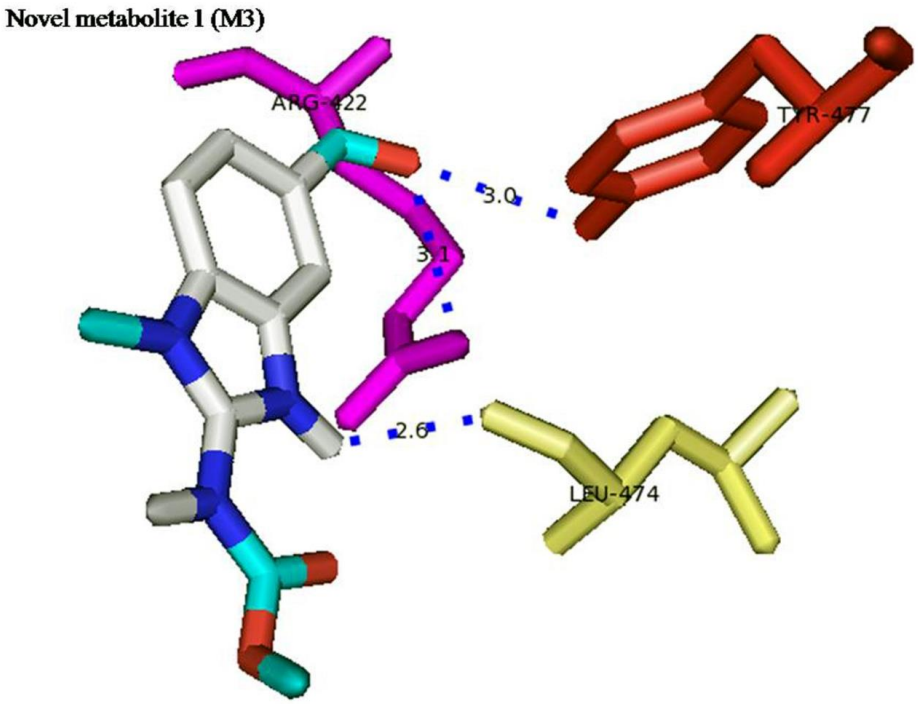


Fig.4 Snapshot of novel metabolite (M3) ofalbendazole and its hydrogen-bond interactions with 2GIR

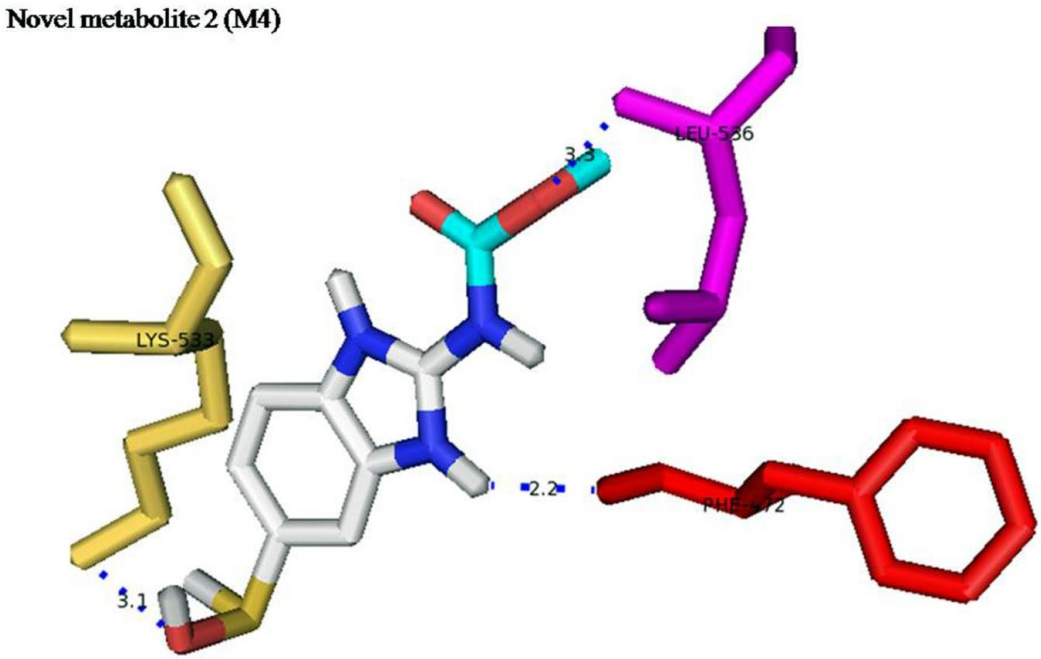


Fig.5 Snapshot of novel metabolite of albendazole (M4) and its hydrogen-bond interactions with 2GIR

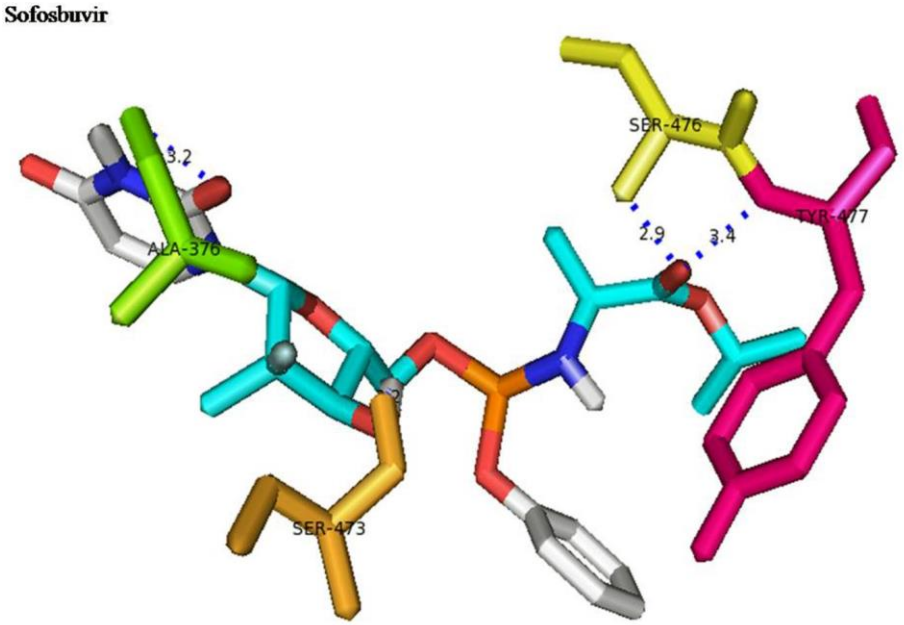


Fig.6 Snapshot of standard drug sofosbuvir and its hydrogen-bond interactions with 2GIR

4.1. *In silico* studies

Molecular docking of test compounds into the active site of Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B was found to be successful based on the formation of complexes of Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B with ligands. The hydrogen bond interactions, binding energy, bond length, RMSD, active site residues and orientation of the docked compound within the active site were visualized. All the test compounds screened showed best fit RMSD value of 0.000, indicating statistically significant interaction. The negative and low value of ΔG indicated a strong and favourable bonding between 2GIR and the ligands in their most favourable conformations. The binding energies for albendazole and its metabolites viz. albendazole sulfoxide (M1), albendazole sulfone (M2) and two novel metabolites of albendazole (M3, M4) and standard drug Sofosbuvir were found to be, -6.6, -6.5, -7.2, -7.0, -6.1 and -7.6 kcal mol-1respectively, indicating a relatively higher interaction of test compounds with Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B. The details of binding energies, the number of hydrogen bonds formed, and catalytic site residues involved in the protein-ligand complex of COX-2 with different ligands is depicted in Table 2 and fig 1-6.

In our earlier studies using the fungal culture *Rhizomucor pusillus,* four metabolites of albendazole were recorded, out of which two were reported earlier (M1, M2) and two were novel metabolites (M3, M4) of albendazole. Our present study is to find the inhibitory potential of these metabolites of albendazole on Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B which is a key protein for novel drug discovery. The *in-silico* studies involved virtual screening for the interaction of albendazole and its metabolites with Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B by molecular docking in comparison with standard reference drug Sofosbuvir.

The NS5B protein is an RNA dependent polymerase and is reminiscent of a human right-hand thumb, palm and fingers sub-domains typical of polymerases (Kukolj et al., 2005). The HCV NS5B polymerase active site is situated in the palm domain. NS5B inhibitors can be classified into nucleoside and non-nucleoside inhibitors (Nis and NNIs, respectively) (Kukolj et al., 2005). Three distinct inhibitor binding sites have been reported, NNI-1, NNI -2, and NNI -3. NNI site I inhibitors target a site on the upper section of the thumb domain, approximately 30Å from the active site at the juncture of the thumb and finger loop, a small section of the fingers domain that extends to interact with the thumb domain (Kukolj

et al., 2005).The important residues in the thumb II region were Ser476 and Tyr477 (Wei et al., 2016).

In the present investigation, the metabolites M2, M3 and the standard drug Sofosbuvir formed hydrogen bonds with amino acid Tyr 477 which is an important residue in the thumb region of NS5B protein and it is also reported that (Wei et al., 2016) inhibitors of crystal structure of NS5B (PDB ID: 2GIR) bound to the thumb II region which clearly states that these metabolites of benzimidazole albendazole derivatives are interacting with thumb II region of NS5B. The binding energy shown by the metabolites M2 and M3 were almost nearer to the standard drug Sofosbuvir which is a potent NS5B inhibitor.

Albendazole, the broad spectrum anti-helmintic benzimidazole derivative and its biotransformed products exhibited strong inhibition potentials of Hepatitis C Virus RNA-Dependent RNA Polymerase NS5B. Hence, it can be concluded that albendazole and its metabolites shows strong impact in treating patients suffering from Schistosomiasis and Hepatitis C Virus (HCV) coinfection.

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