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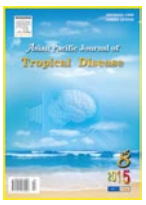

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Prediction of the ligands having the inhibitory activity against the HCV non-structural protein 5B polymerase

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ABSTRACT

Objective: To find similar compounds of rhodanine inhibitors of HCV non-structural protein 5B (NS5B) through exploring the PubChem database.

Methods: We used the data mining of these ligands and we studied molecular docking of these ligands with the enzyme HCV NS5B for knowing inhibitory activity. We used the the Knime software for the data mining and the USCF Chimera and Molecular Operating Environment for study the molecular docking.

Results: As a result, the discovery was two new inhibitors of NS5B HCV, namely CID 211702 and CID 13752.

Conclusions: Two new ligands, CID 211702 and CID 13752, were discovered for the inhibition of the HCV and can be used to invent new medicines against the cancerous diseases.

1. Introduction

Hepatitis C virus (HCV), an enveloped single-stranded positive sense enveloped RNA virus, was discovered in 1989[1]. It is a leading cause of long term liver cirrhosis, resulting in liver transplantation, liver failure and hepatocellular carcinoma[2]. HCV is the primary causative agent for non-A, non-B hepatitis[1]. More than 80% of all infections turns chronic at the risk of developing severe liver disease resulting in hepatocellular carcinoma[3]. Recent advances in development of directly acting antiviral have significantly improved sustained virology response in patients and provided a new hope for cure infected patients[4]. The HCV non-structural protein 5B (NS5B), a 66 kDa RNA dependent RNA polymerase is an important therapeutic target for its important role in replicating the HCV RNA genome.

This target is especially significant from the drug discovery point-of-view since humans lack thier functional equivalent[5]. The combination of crystallographic, biochemical and mutagenesis studies have allowed the identification of at least five non-nucleoside inhibitor binding sites on NS5B enzyme[6].

Compounds bearing the rhodanine scaffold have been previously reported as HCV NS5B inhibitors by us and others[7,8]. Recently, the rhodanine scaffold has been a topic of debate in regards to its potency and selectivity at the step of advanced lead optimization[9]. Importantly, clinical success with the rhodanine analog and epalrestat argues in favor of its safety in humans.

The aim of the present study was to find more similar rhodanine derivatives for development of a new drug with the inhibitory activity against the polymerase enzyme.

2. Materials and methods

Inhibitors similar to rhodanine have been downloaded from the PubChem database. Then data mining of these molecules with the

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Knime software was made and selection was carried out using the rule of Lipinski's rule of five. This is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it being a likely orally active drug in humans. The rule is based on the observation that most orally administered drugs is relatively small and moderately lipophilic molecules[11,12].

The downloading of HCV polymerase was made from the data base-Brookhaven Protein Data Bank (access code 3TYV), the resolution is 1.65 Å. The protein HCV polymerase B was prepared for molecular docking by adding all hydrogen atoms using standard procedures. The water molecules and other heteroatom's were deleted. The binding energy was observed for each ligand protein complex. Selected inhibitors are shown in Figure 1.

3. Results

Tanks data base PubChem (www.pubchem.com) which allows the downloading of structures from a variety of vendors as SDF files was used for screening. The downloaded similar compounds of rhodanine[10] were CID 1273212, CID 83070, CID 1237173, CID 1207359, CID 211702, CID 210591, CID 99598, CID 98686, CID 95741, CID 211718, CID 211716, CID 13752, and they were used for studying the inhibitory activity of HCV NS5B55.

3.1. Data mining of the legends

Figure 2 shows the nodes in the pipeline program KNIME 2.1.1 (http://www.knime.org/). As a first step, we extracted similar compounds of rhodanine from the databases using 3D SMARTS strings. For these compounds we have used the PubChem database[13]. A similarity based search and further screening using Lipinski's rule yielded 12 compounds. We had most of the compounds that had the molecular weight in the range of 250–400, hydrogen bond donor (1 or 2), hydrogen bond acceptor (1–5) and log P (≤ 5.5).

3.2. Molecular docking study

For the score of the obtained complexes, we used USCF Chimera software to gain insight into the molecular mechanism of inhibition. We analyzed the energies of the docked conformation of similar compounds of rhodanine and HCV NS5B (PDB ID: 3TYV) [14] in Table 1.

Table 1

Score of interaction between the similar compound of rhodanine and NS5B.

Legends	G score (kcal/mol)
CID 1273212	-5.8
CID 83070	-5.7
CID 1237173	-6.0
CID 1207359	-5.9
CID 211702	-6.9
CID 210591	-6.0
CID 99598	-5.5
CID 98686	-5.9
CID 95741	-5.9
CID 13752	-6.9
CID 211718	-6.5
CID 211716	-6.3

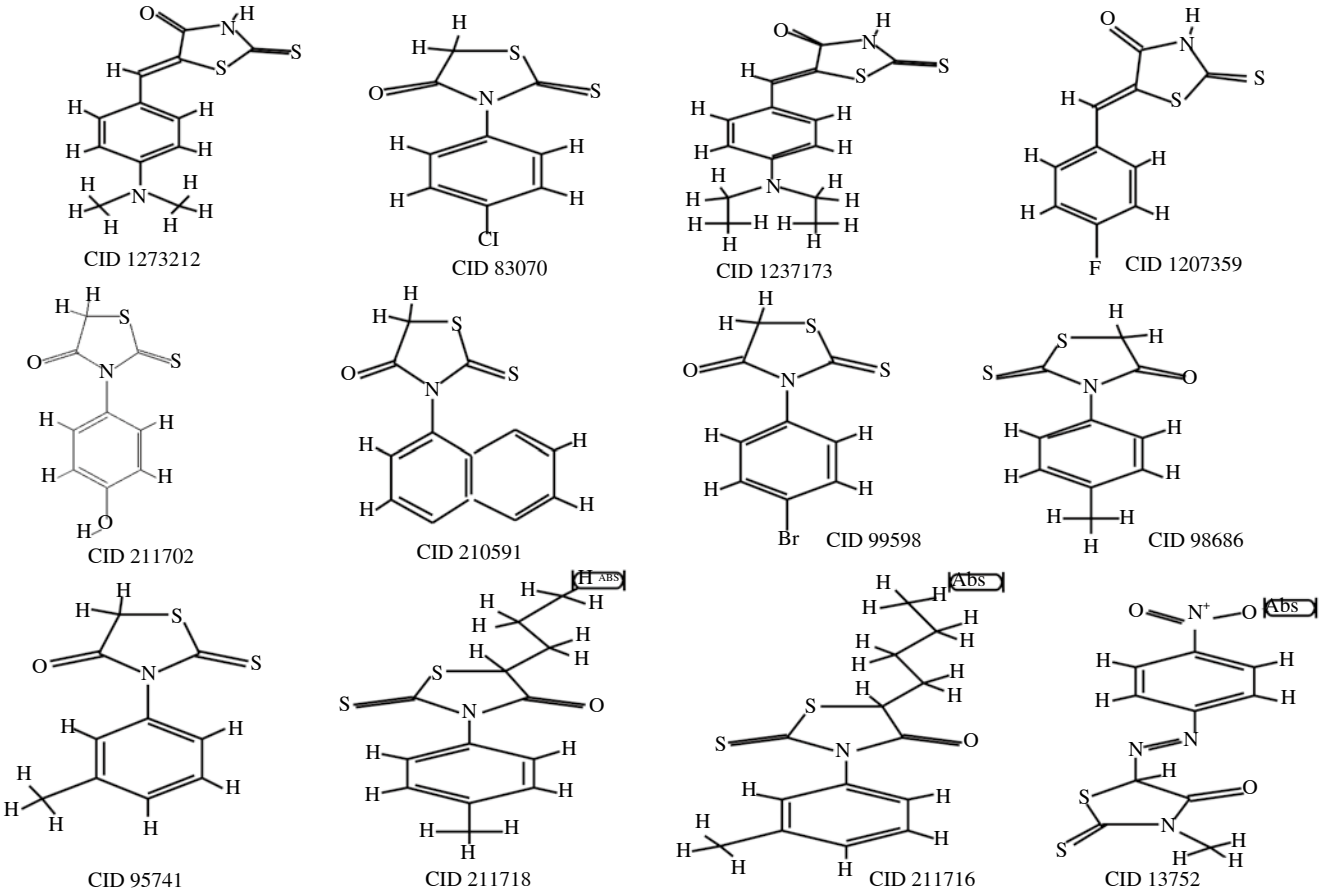

Figure 1. Inhibitors similar to rodhanine.

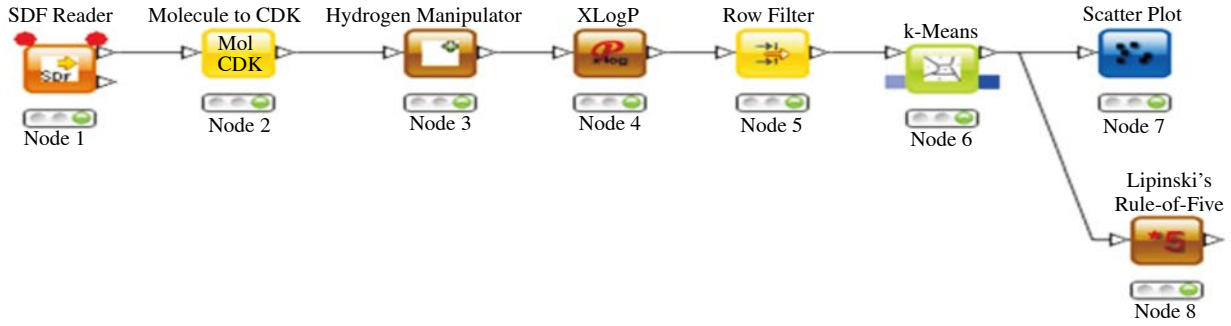

Figure 2. Data mining KNIME for database preparation for the virtual screening.

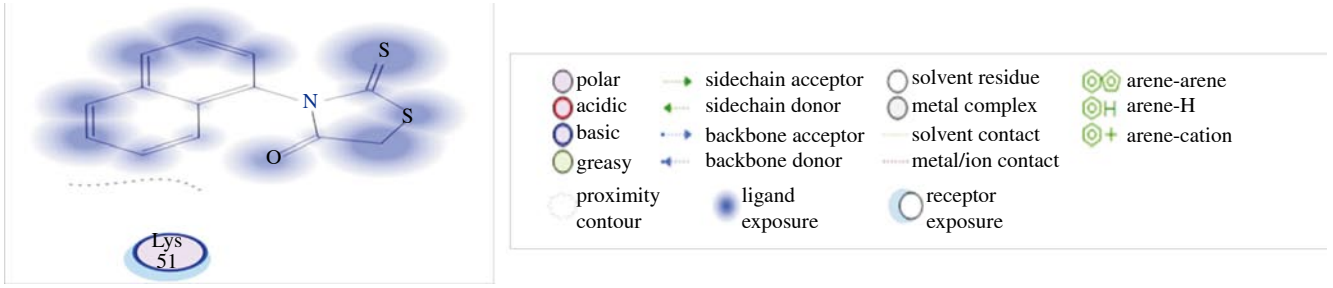

Figure 3. Docking of CID 211702 in the substrate cavity.

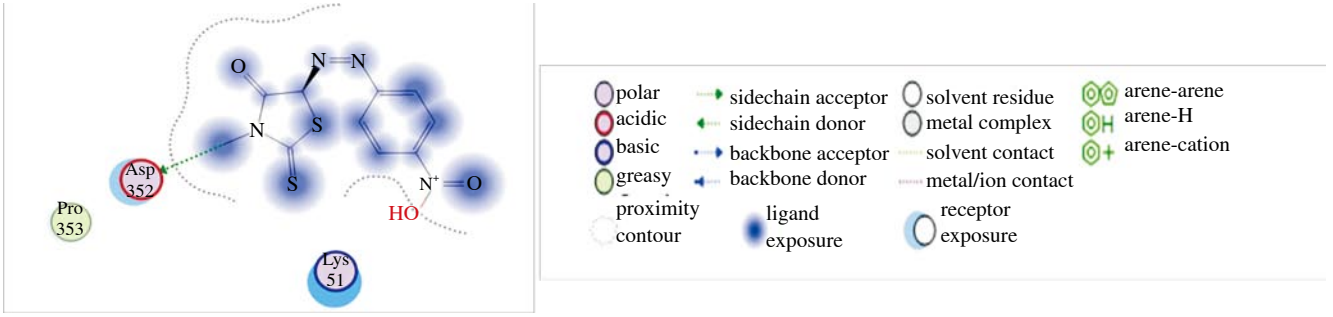

Figure 4. Docking of CID 13752 in the substrate cavity

The scoring function took care of van der Waals term as well as hydrogen bond directionality. The fitness of a candidate solution was evaluated in terms of intermolecular interaction energy between the ligand and the protein, and the intramolecular interaction energy of the ligand. The robustness of the docking process was mitigated by a more stringentre ranking of the top scoring poses.

From the results of Table 2 and Table 3, two ligands CID 211702 and CID 13752 were the best for the enzyme NS5B to complex with. We will study the molecular docking of complexes formed by these ligands and the HCV NS5B (Figures 3 and 4).

To further investigate the interaction modes and selectivity to two legends with the enzyme, molecular modeling was carried out by Molecular Operating Environment software package. 3D structure of the strongest NS5B inhibitors CID 211702 and CID 13752 were built by using the builder interface of Molecular Operating Environment program and were docked into the active site of the protein after energy was minimized. Finally, the geometries of resulting complex were displayed in Figures 5 and 6. In the optimal side chains the high inhibitory activity against NS5B polymerase was showed, indicating that both the open-loop groups have the ability to occupy the active site of NS5B polymerase (Figures 4 and 5, Tables 2 and 3).

Table 2

Distances between the active site amino acids and inhibitor CID 211702.

Compounds	Distance to CID 211702 (Å)
ARGININE 200	2.450
ISOLEUCINE 447	2.708
PROLINE 197	3.436
TYROSINE 448	3.213
SERINE 367	3.419
LEUCINE 384	2.635
METMETHIONINE 414	2.149

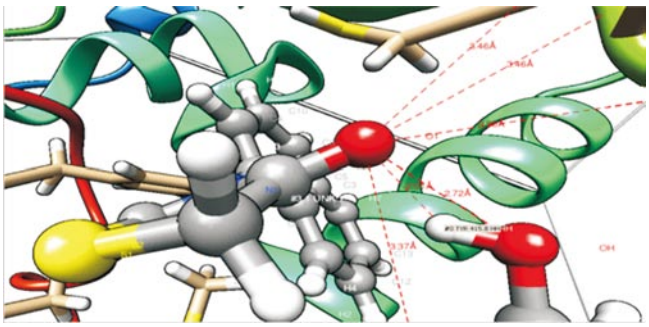

Figure 5. Distances between the active site amino acids and inhibitor CID 211702.

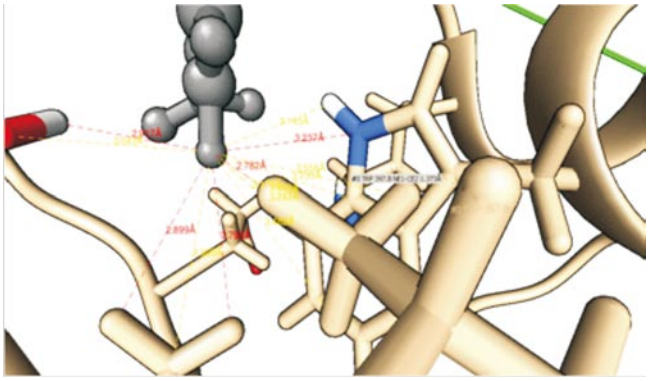

Figure 6. Distances between the active site amino acids and inhibitor CID 13752.

Table 3

Distances between the active site amino acids and inhibitor CID 13752.

Compounds	Distance to CID 13752 (Å)
ARGENINE 394	2.174
PROLINE 404	2.135
TRYPTHOPHANE 97	2.430
SERINE 142	2.205
ALANINE 39	2.793
VALINE 144	2.782
SERINE 142	2.914

4. Discussion

In this work, template-based molecular docking studies were carried out to explore the efficacy of docking technique so as to obtain a better model as compared to that of a simple docking protocol, to facilitate design.

Also more number of hits could be retrieved from the external database (Pub-Chem compound database). The most significant accomplishment of our present work is the identification of a novel scaffold (CID 13752), which could be a precursor to a newer and novel series of inhibitors of NS5B HCV.

In Table 1, the lowest energy corresponding to complex has formed the most stable enzyme-inhibitor. We noticed that the inhibitor CID 13752 and CID 211702 have the lowest energy (-6.9 kcal/mol). They are the most stable complex.

Tables 2 and 3 recorded the distances between the active site of the enzyme and the chosen inhibitors. The measured distances varied between 2.135 Å and 3.436 Å for all studied complexes. The interactions between 2.5 Å and 3.5 Å are considered high and those between 3.1 Å and 3.55 Å are assumed averages. Interactions greater than 3.55 Å are weak or absent[16].

Conflict of interest statement

We declare that we have no conflict of interest.

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