Original Research Article

In-silico ADME and Docking-based Virtual Screening Study aiming at the Sigma-Covalent Inhibition of SARS-CoV-2 RdRp

ABSTRACT

The objectives of the study were to examine the virtual screening of the compounds and sigma-covalent inhibition of SARS-CoV-2 RdRp (*RNA-Dependent RNA-Polymerase),* which is conserved and is an essential enzyme for RNA transcription and replication of this virus. In this study, we collected around 1225 similar compounds of Penciclovir and Acyclovir inhibitors from PubChem and predicted ADME (Adsorption, Distribution, Metabolism and Excretion) molecular descriptors using Swiss-ADME server. Virtually screened 24/1225 compounds based on drug-likeliness five rules (Lipinski, Ghose, Veber, Egan, and Muegge) and lead-likeliness properties. Further 10/24 compounds screened, based on high binding affinity and RMSD<3.5Å against RdRp structure using PyRx docking software. Furthermore, the molecular interactions of 10 compounds studied using Discovery studio software and finally screened five PubChem compounds 57201841, 135408972, 54552823, 135409422 and 467850, based on bioactivity score using Molinsipiration cheminformatics software. All these five compounds showed up anti-SARS CoV-2 activity, though further *in-vitro* studies are required.

Keywords: SARS-CoV-2; RdRp; Penciclovir; Acyclovir; Swiss-ADME.

1. INTRODUCTION

An outbreak of a novel coronavirus named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China in December 2019 [1]. The coronavirus disease 2019 (COVID-19) has been spawned by SARS-CoV-2 and become pandemic and spread around the world [2]. The SARS-CoV-2 belongs to the family of Coronaviridae and has been identified as β-coronavirus and are enclosed in a single-stranded positive-sense RNA virus [3]. The COVID-19 has taken 3.5 million of human lives as of June 2021 globally [1,4]. The rapid transmission of the virus is more over large geographical and demographical area need to design and develop anti- SARS-CoV-2 therapies [5,6]. Typically, several methodologies and strategies were used to discover suitable antiviral medication for various illness types. The present wide variety antiviral medications have been used by conventional testing as one of the most prevalent strategies. Another quick way in antiviral medication development is to check for the previously authorised chemical compounds by means of computer and bioinformatics technologies. Antiviral medicines were analysed in this technique for their effectiveness in inhibiting key enzymes of novel viruses [7]. Several studies have focussed to design and develop the inhibitor molecules against specific receptor enzymes of the SARS-COV-2 [8,10].

The RdRp protein, which is conserved in SARS-COV-2, is known to be RNA-dependent RNA polymerase, and this enzyme is vital for RNA transcription and replication of the virus. The RdRp domain of polymerase is located at the C-terminus and has a conserved Ser-Asp-Asp motif [8]. Inhibition of RdRp enzyme activity by clinical drugs such as Penciclovir and Acyclovir have reported [11]. Clinical reports investigated that these drugs could not cause significant side effects on host over targeting inhibition of RdRp [12]. In addition, Gnidian lamprantha and Betulonal Cassine Xylocarpa natural compounds and derivatives with anti-inflammatory, anti-tumour and anti-virus properties have demonstrated a strong binding affinity to RdRp with promising anti-COVID-19 action although additional research is necessary [13]. The design of computer-based drugs depends on the integrity of the drug receptors used. This test and selection are useful for reducing the discrepancies in dry and wet laboratory studies and the false positives mistake. These RdRp are already targets for pharmacological discovery, as in this investigation. Computational approach helps in saving a huge money that is being spent on the clinical trials [14].

The objectives of the study were to examine the virtual screening of the compounds and sigma-covalent inhibition of SARS-CoV-2 RdRp (*RNA-Dependent RNA-Polymerase),* which is conserved and is an essential for RNA transcription and replication of this virus. The present study was divided into two sections, first section dealt with the virtual screening of chemical compounds based on ADME properties, drug-likeliness Five Rules and lead like friendliness parameters. Subsequent section dealt about the molecular interaction analysis between RdRp protein and the virtual screened compounds followed by bioactivity of the selected compounds.

2. MATERIAL AND METHODS

2.1. Protein and Compounds used in the Present Study

The crystal structure of the SARS-CoV-2 RdRp enzyme also widely known as RNA dependent RNA polymerase (RdRp) [15] downloaded from PDB data bank (the PDB file of 7BW4) which is available at https://www.rcsb.org. The 3D structure was experimentally determined by electron microscopy with a resolution of 3.7 Å, and composed of three chains (A, B and C) and length of 1204 amino acids [16]. Discovery studio software was used to visualize the 3D structure of RdRp protein and removed solvent and any ligand molecules present over 2 chains (A and B) and further used to confirm the exact protein structure for molecular docking using Base by Base approach [17,18]. Recent studies reported the critical residues of active site with enriched aspartates specifically Asp (684,761,762) and Ala685 in RdRp enzyme reaction [19].

Currently the available inhibitors for SARS-CoV-2 RdRp enzyme are Penciclovir and Acyclovir as well reported [20]. Similar 3D conformers of Penciclovir and Acyclovir were collected from PubChem around 1225 compounds in smile canonical file formats for ADME properties screening. Swiss ADME server was used to determine the molecular descriptors of pharmacokinetics, drug-likeness, medicinal chemistry friendliness for collected 1225 compounds [18]. Virtually screened and passed about 58 compounds based on Five Rules such as *Lipinski (MW≤ 500, MLOGP≤ 4.15, N or O≤ 10, and NH or OH≤ 5)*[20], *Ghose (180≤ MW≤ 480, -0.4≤ WLOGP≤ 5.6, 40≤ MR≤ 130, and 20≤ Atoms≤ 70)* [21], *Veber (Rotatable bonds≤ 10 and*

TPSA≤140) [22], *Egan (WLOGP≤ 5.88 and TPSA≤131.6)* [23] *and Muegge (200≤ MW≤ 600, -2≤ XLOGP≤ 5, TPSA≤ 150, Number of rings≤ 7, Number of Carbons >4, Number of Heteroatoms >1, Number of Rotatable Bonds ≤15, H-Bond Acceptors ≤5, and H-Bond Donors≤ 5)* [24]. Further screened about 24 compounds based on lead likeness properties *(250 ≤ MW ≤ 500, XLogP ≤ 3.5 and Number of Rotatable Bonds ≤ 7)* [25] and added the hydrogen bonds to the compounds using Open Babel [26] for molecular docking studies against RdRp protein.

2.2. Molecular Docking of the Compounds against RdRp Structure

Recently several studies used PyRx software particularly for SARS-CoV2 protease inhibition studies using molecular docking [27]. PyRx is a computational drug discovery virtual screening software which can be used to test chemical compounds against possible therapeutic drug targets. PyRx provides a user-friendly docking wizard, making it a powerful tool for computer-aided drug design [28]. The pharmacophores of ligand molecules and receptors, as well as mathematical force field functions, are used to calculate binding affinity [29,30]. All the 24 compounds were loaded along with the RdRp protein and after docking, based on binding affinity and RMSD values the compounds were further screened to 10 for further interactions visualization [27,30].

2.3. Molecular Interaction Analysis

Molecular interactions of side chains of RdRp protein and the compounds were visualized with help of Discovery studio software. The interactions with the side chains, hydrogen bonding, bonding energy with the amino acids were analyzed in this study. Furthermore, binding mode analysis of the compounds and hydrogen bonds networks were investigated using Discovery studio software [31]. The RMSD values of bounded compounds were taken less than 4 Å to establish the structural orientation of the RdRp enzyme due to covalent inhibition [32].

2.4. Bioactivity of the Screened Compounds

A score of bioactivity of chemical compounds has been analysed for many metrics, including 5G ligand (GPCR) and nucleic receptor ligand, ion channel modulation, inhibition of kinases, inhibition of proteases and suppression of enzyme activity using molinspiration cheminformatics software [32]. The bioactivity of chemicals is associated to their pharmacological activity, which pertains to the compounds' potential benefits in living organisms [33]**.** The sum of the activity outputs of components of the compounds used to calculate the bioactivity score of the chemical compounds. The yield of a bioactivity score > 0 means that the compounds are more likely to be active, if the score is lies between -0.5 to 0 means that the compounds are moderate active and if the score < -0.5 means that the compounds are to be considered as inactive [34,35]. The complete process methodology as depicted in the Figure 1.

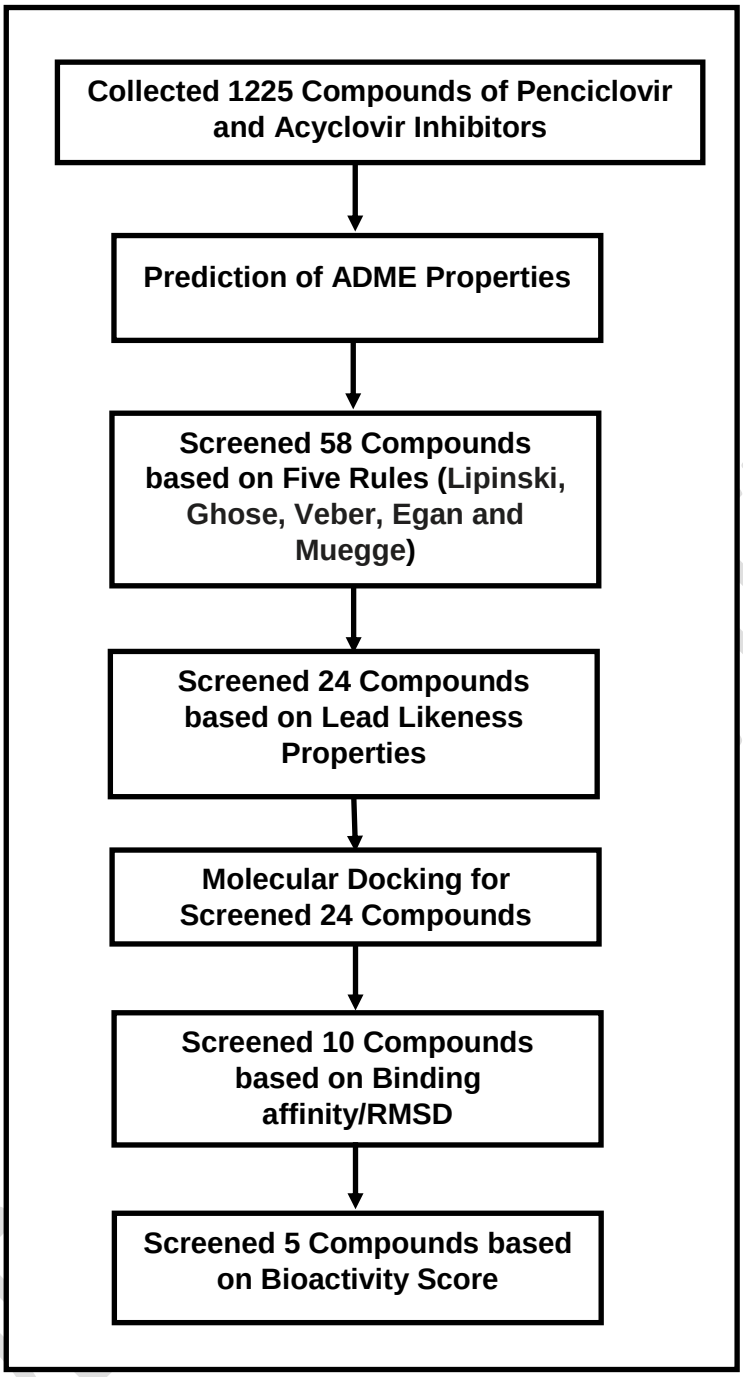


Fig.1. The Methodology used in the Present Study

3. RESULTS AND DISCUSSION

3.1. Virtual Screening of Compounds

In this study, similar 3D conformers of Penciclovir and Acyclovir were collected from PubChem database around 1225 compounds in smile canonical file formats for ADME properties screening. For all 1225 compounds, molecular descriptors of pharmacokinetics, drug-likeness and medicinal chemistry friendliness were determined using Swiss-ADME server. Drug-likeness five rules such as Lipinski, Ghose, Veber, Egan and Muegge applied and virtually screened 58 out of 1225 compounds those passed in all five rules. The bioavailability score (Probability >10% in Rats) of all 58 compounds were 0.55, which indicated that those

compounds were good enduring for gastrointestinal absorption via oral injection. Further applied the lead likeness parameters (250 ≤ MW ≤ 500, XLogP ≤ 3.5 and Number of rotatable bonds ≤ 7) and passed 24 compounds out of 58. Further, hydrogen bonds added for all 24 compounds using OpenBabel for molecular

PubChem ID	**Binding Energy	***Binding Modes	†H-bonds; ††Sn	†††RMSD (A°)

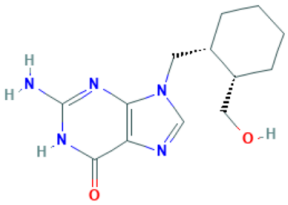
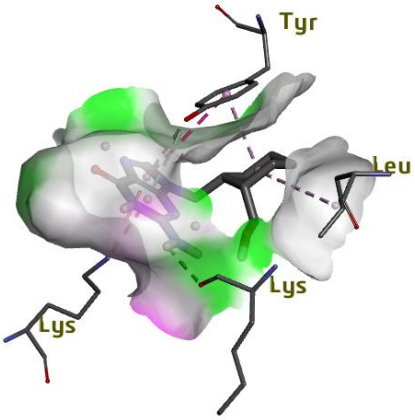
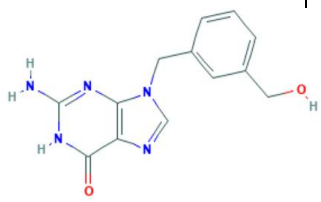
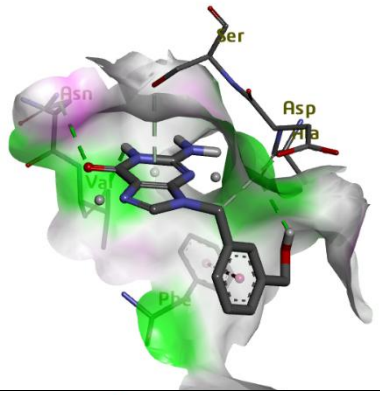
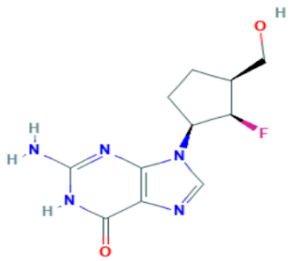
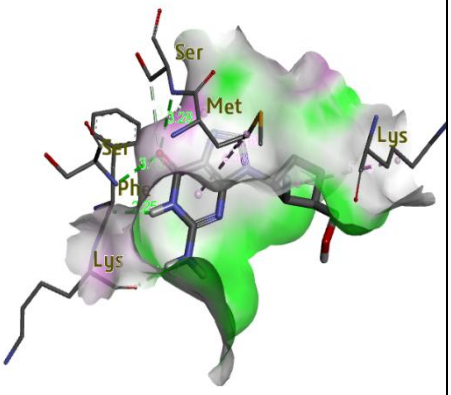
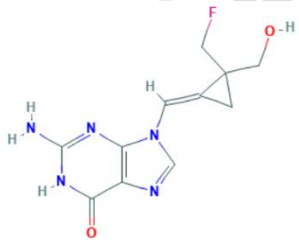
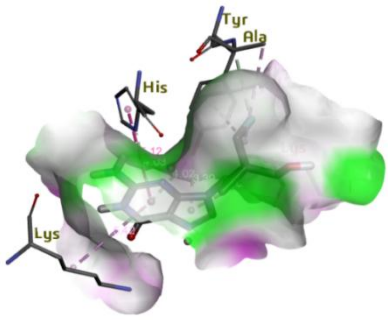
docking studies against RdRp protein.

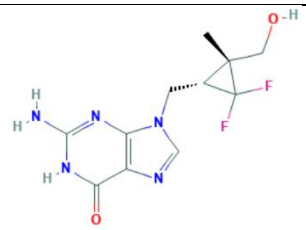
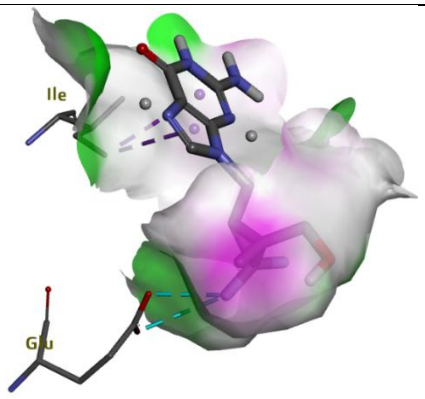
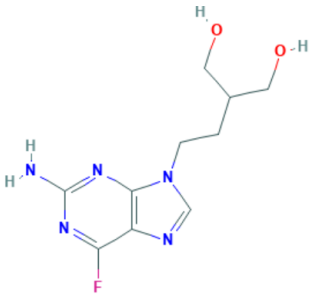
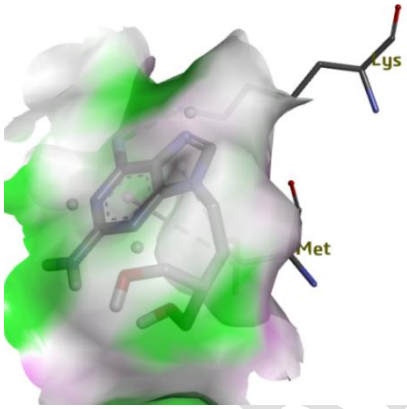
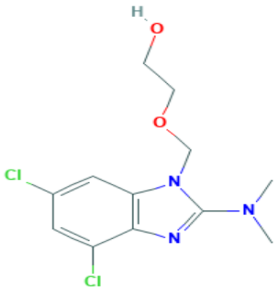
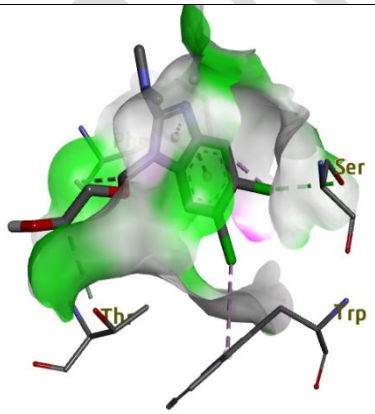
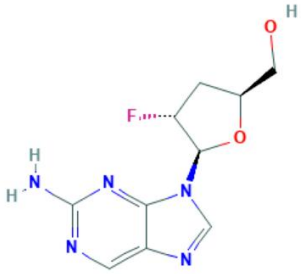
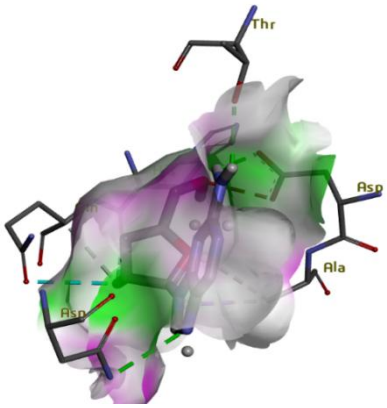
3.2. Molecular Docking Analysis

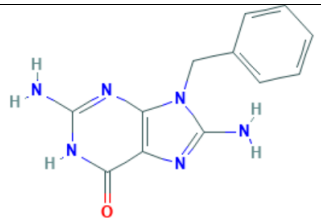
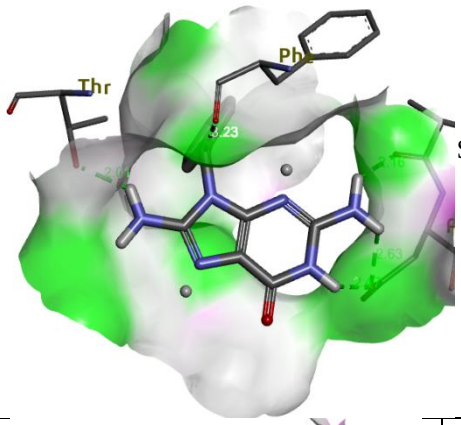
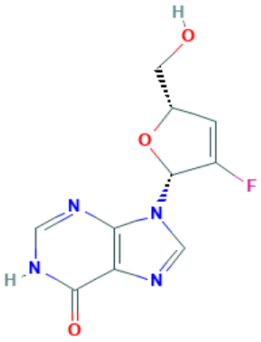
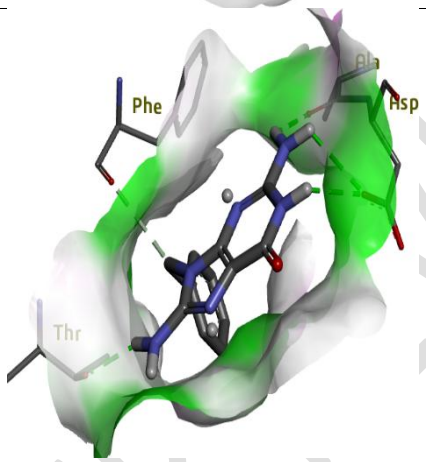
The selected 24 compounds were loaded along with RdRp PDB structure into PyRx docking software with default docking parameters. The docking grid was focused on active site of RdRp structure particularly the critical residues involved in the active site with enriched aspartates specifically Asp (684,761,762) and Ala685 in RdRp enzyme reaction and performed the PyRx docking process. Each compound was produced around 100 different binding modes with various binding energy and RMSD values. However, only 10 compounds were selected based on best binding mode, lowest binding energy and RMSD < 3.5 Å and reported in **Table 1.**

The binding energy of selected 10 compounds with PubChem ID 135409422, 135436426, 135538681, 136036832, 136168880, 54552823, 467850, 57201841, 135407710 and 135408972 were about -6.3, -6.5, -6.4, -6.2, -6.0, -5.8, -5.8, -7.1, -6.1 and -7.1 kcal/mol respectively. The binding energy was used to predict the binding affinity of both receptor and ligand when it is docked, by means that the compounds 57201841 and 135408972 had more binding affinity with target RdRp protein, which indicated that these two compounds were highly stable conformation towards the RdRp protein with lowest binding energy. In the same way, the RMSD values of selected 10 compounds with PubChem ID 135409422, 135436426, 135538681, 136036832, 136168880, 54552823, 467850, 57201841, 135407710 and 135408972 were about 2.05, 2.40, 3.19, 1.96, 2.43, 2.43, 3.40, 2.27, 2.02 and 3.46 Å respectively. All selected compounds have RMSD < 3.5 Å, which indicated that the compounds were very closely interacted within the active site residues of RdRp enzyme.

Table 1. Potential Inhibitor Compounds of Rdrp Protein.

 135409422	-6.3		1,S1	2.05
 135436426	-6.5		3; S1,S2	2.40
 135538681	-6.4		4; S1,S2,S3	3.19
 136036832	-6.2		1,S1	1.96

 136168880	-6		1;S1	2.43
 54552823	-5.8		1;S1	2.43
 467850	-5.8		1,S1,S2	3.40
 57201841	-7.1		3; S1,S2,S3	2.27

 135407710	-6.1		4; S1,S2,S3	2.02
 135408972	-7.1		4; S1,S2,S3	3.46

*****Binding Energy(Kcal/mol):** Binding Energy obtained from PyRx virtual screening tool**,, ***Binding Modes:** The orientation of compound relative to the active site of enzyme**,(**the green color shows the H acceptor and pink shows the H donor **) †H-bonds**: Hydrogen bond between the active site residue to the functional group of the compound **, ††Sn:** Indicate the binding pockets interacting with the compounds**, †††RMSD**: Average root mean square deviation of upper and lower bound values of docking complex in Angstroms (Aº) (all the RMSD values less than 3.5 Aº docking complexes screened).*

3.3. Molecular Interaction Analysis

The Discovery Studio software was used to analyze the interactions including binding residues, bond lengths, binding modes, and hydrogen networks between RdRp structure and the docked compounds. The various binding modes of the docked compounds were 135409422(S1), 135436426(S1,S2), 135538681(S1,S2,S3), 136036832(S1), 136168880(S1), 54552823(S1), 467850(S1,S2), 57201841(S1,S2,S3), 135407710(S1,S2,S3) and 135408972(S1,S2,S3) respectively, which indicated that the orientation of the compounds particularly 135538681, 57201841, 135407710 and 135408972 bound relatively to the active site of enzyme in x,y and z-direction from epic center. However, these four compounds exhibited maximum 3-4 hydrogen bonds than others with critical residues of active site of the RdRp enzyme. It was observed that the number of binding modes of the compounds directly related to the number of hydrogen bonds formation. The number of hydrogen bonds and the various binding modes were reported in **Table 1.** The docked compounds and their molecular interactions with critical residues of active site of RdRp structure can be seen in 3D and 2D forms of **Figure1**.

As shown in **Figure 2** and **Table 2**, the interactions were mostly Conventional hydrogen bonds, Carbon hydrogen bonds, Halogen (Fluorine), Pi-Cation, Pi-donor hydrogen bond, Pi-Sigma, Pi-Alkyl, Pi-Pi-T-shaped and Alkyl bonds with the side chains and active site of the RdRp protein and the docked compound

functional groups. The colored circles represent the critical residues of RdRp and interacting with the functional groups of the compounds.

In case of the compound 57201841, three conventional hydrogen bonds formed between amide functional group to the critical residues Asn496(A), Arg569(A), Thr565(A) and Asp684(A) of active site of RdRp with a bond range 2.04-3.47 A^0 particularly, specific with Aspartate adjoining site. However, a strong covalent Pi-Sigma bond formed with a bond range of 3.85-4.51 A^0 between Ala685(A) to the aromatic aldehyde and amide functional groups of the compound. The compound showed a strong covalent interaction with the critical residues of active site and side chain residues of the RdRp enzyme and it was evidenced that the compound might be act as an efficient inhibitor against the RdRp enzyme. The same conventional hydrogen bond interactions were observed in the cleft site of protein with compound 135408972, with the cleft residues of Ala162(B), Asp163(B), Phe407(A), and Thr409(A) with a bond range 2.01-3.23A^0.

Table 2. Intramolecular Interactions of Two Compounds with Specific Residues of Rdrp Protein.

Compound			RdRp protein	Interaction Type /Bond Distance (A^0)
PubChem CID	Chemical Formula	Functional Group	Residue/Number/Chain	
57201841	$C_{10}H_{12}FN_5O_2$	*NH	Asn496(A)	H-bond/3.32
		*NH	Arg569(A)	H-bond/ 3.47
		*NH	Thr565(A)	H-bond/ 2.43
		*NH	Asp684(A)	H-bond/ 2.04
		***F	His572(A)	H-bond/3.29
		***F	Gln573(A)	H-bond/3.27
		R,**N	Ala685(A)	Pi-Sigma/3.85/4.51
135408972	$C_{12}H_{12}N_6O$	*NH	Ala162(B)	H-bond/2.47
		*NH	Asp163(B)	H-bond/2.63/2.16
		#CH	Phe407(A)	H-bond/3.23
		*NH	Thr409(A)	H-bond/2.01
54552823	$C_{10}H_{14}FN_5O_2$	H	Lys7(C)	H-bond/3.14
		†R,**N	Met3(C)	Pi-Alkyl/4.80/4.86
135409422	$C_{13}H_{19}N_5O_2$	*NH	Arg33(A)	Unfavourable/3.2
		*NH	Lys121(A)	H-bond/2.74
		†R	Leu119(A)	Pi-Alkyl/5.41
		†R	Tyr217(A)	Pi-H-bond/3.89
		†R,**N	Lys50(A)	Pi-Cation/3.38/3.84
467850	$C_{12}H_{15}C_{l2}N_3O_2$	††Cl	Ser26(C)	H-bond/3.26
		††Cl	Trp29(C)	Pi-Alkyl/5.19
		††Cl, †R,**N,C	Phe407(A)	Pi-Pi-Stacked/4.83/4.82/5.81/3.5
		C	Thr409(A)	H-bond/3.67

*†R=Aromatic alkyl, *NH=Amide, **N= Aromatic amide, ***F=Fluorine, ††Cl=Chlorin, #CH=Methyl, H=Hydrogen*

In case of the compound 54552823, a conventional hydrogen bond and non-covalent Pi-Alkyl formed between Lys7(C) and Met3(C) with a bond range 3.14-4.86 A^0. In case of the compound 135409422, the most significant and insignificant two conventional hydrogen bonds formed between amide group to the critical residues Arg33(A) and Lys121(A) of RdRp with a bond range 2.74-3.2 A^0, and non-covalent Pi-Alkyl and Pi-H-bonds formed between Leu119(A) and Tyr217(A) with a bond range 3.89-5.41 A^0. In the case of the compound 467850, two significant conventional hydrogen bonds formed between halogen group (Chlorin) and carbon to the critical residues Ser26(C) and Thr409(A) of RdRp enzyme with a bond range

3.26-3.67 A^0. Although, two non-covalent interactions such as Pi-Alkyl and Pi-Pi-Stacked bonds occurred between

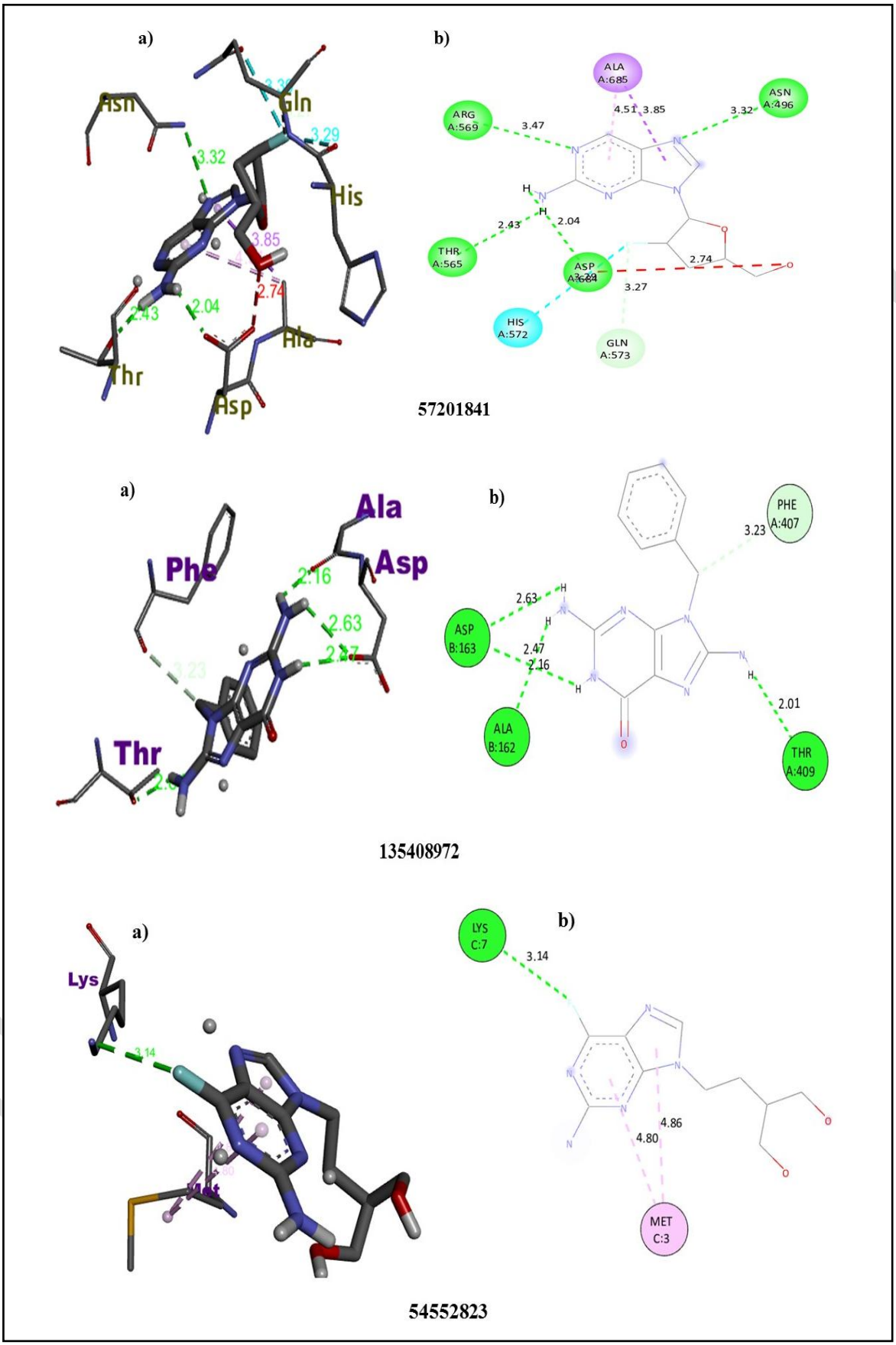


Fig.2. Interactions between RdRp and the Compounds in 3D and 2D (a and b)

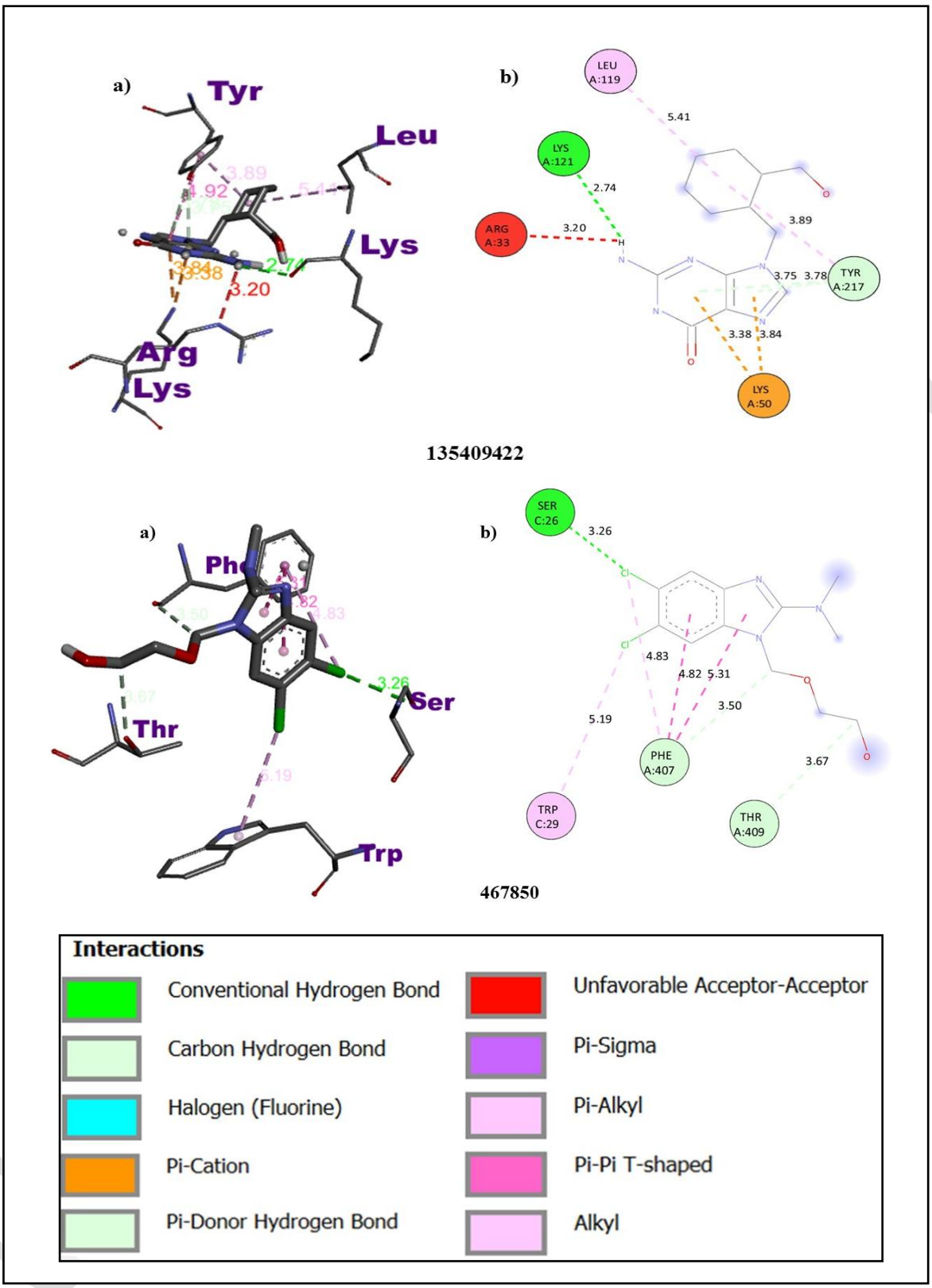


Fig. 2. (Continued.)

aromatic alkyl, halogen (Chlorin) of compounds and the critical residues Trp29(C) and Phe407(A) of to RdRp enzyme with a bond range 3.5-5.81A^0. In this interactions study, we observed that the compounds 57201841 and 135408972 were more efficient inhibitors than others particularly by forming strong covalent interactions with targeted RdRp protein. Summary of intramolecular interactions of the compounds with specific residues of RdRp protein was reported in **Table 2.**

3.4. Bioactivity of the Compounds

Bioactivity score was determined for screened 10 chemical compounds based on *5G protein-coupled receptor (GPCR) ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition* using Molinspiration cheminformatics software. The sum of the activity outputs of components of the compounds used to calculate the bioactivity score of the chemical compounds. The yield of a bioactivity score > 0 means that the compounds are more likely to be active, if the score is lies between -0.5 to 0 means that the compounds are moderate active and if the score < -0.5 means that the compounds are to be considered as inactive. In this study, we observed that 5 compounds 57201841, 135408972, 54552823, 135409422 and 467850 have bioactivity score > 0, in terms of enzyme inhibition by means that these 5 compounds were more active in enzyme inhibition as shown in **Table 3**. The identified these compounds 57201841, 135408972, 135409422 and 467850 showed better bioactivity score than others by means that more active in enzyme inhibition particularly for kinase inhibition and moderate towards in protease inhibition. However, the enzyme inhibition compound 54552823 was more actively used for kinase as well as protease, which revealed that pharmacologically more active, the bioactivity of all these 5 compounds and the favourable effects of chemicals on living organisms. The bioactivity of these five chemicals was beneficial in designing and developing a new functional medicine with improved binding affinity and reduced unwanted effects.

Table 3. Bioactivity Score of the Chemical Compounds According to Molinspiration Cheminformatics software

PubChem ID	**135409422**	**135436426**	**135538681**	**136036832**	**136168880**	**54552823**	**467850**	**57201841**	**135407710**	**135408972**
GPCR ligand	0.5	0.5	0.59	0.57	0.54	0.49	0.07	1	0.4	0.19
Ion channel modulator	-0.02	0.04	0.08	-0.01	0.06	0.38	0.21	0.67	0.11	-0.1
*Kinase inhibitor	0.41	0.47	0.65	0.64	0.45	0.62	0.1	1	0.18	0.19
Nuclear receptor ligand	-0.91	-0.75	-0.69	-0.73	-0.65	-0.4	-0.44	-0.7	-1.03	-1.25
#Protease inhibitor	-0.16	-0.12	0.02	-0.21	0.08	0.07	-0.41	0.2	-0.21	-0.43
†Enzyme inhibitor	0.72	0.84	1.07	1.06	0.97	0.75	0.1	1.46	1.24	0.58

**, #,†Bioactivity Score>0(Active), -0.5 to 0 (Moderate active), <-0.5(Inactive) w.r.t Enzyme Inhibition*

4. CONCLUSION

In this study, virtually screened 24/1225 compounds based on drug-likeliness five rules (Lipinski, Ghose, Veber, Egan, and Muegge) and lead-likeliness properties. Further 10/24 compounds identified with lowest binding energy and RMSD<3.5Å against RdRp structure using molecular docking interaction studies. Furthermore, the interactions (binding mode, binding residues, hydrogen bond networks and bond lengths) of 10 compounds. Furthermore, five PubChem compounds 57201841, 135408972, 54552823, 135409422

and 467850 virtually screened based on drug-likeliness five rules, lead-likeliness properties, high binding affinity against RdRp protein and bioactivity score. Bioactivity score was evaluated for a variety of metrics, including 5G protein-coupled receptor (GPCR) ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition using molinspiration cheminformatics software. Five compounds yielded the bioactivity score>0 means that those were more likely to be active in enzyme inhibition. Additional investigations are required in-vitro. For the modification, the design and development of inherent molecules against viral proteins, the study might serve to analyze compounds in a library of bulk compounds and molecular interactions.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advance knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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