Computational study of novel inhibitory molecule, 1-((4-((2S,3S)-3-amino-2-hydroxy-4-phenylbutyl)piperazine-1-yl)-3-phenylurea, with high potential to competitively block ATP binding to the RNA dependent RNA polymerase of SARS-CoV-2 virus

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\textbf{ABSTRACT}

For coronaviruses, RNA-dependent RNA polymerase (RdRp) is an essential enzyme that catalyses the replication from RNA template and therefore remains an attractive therapeutic target for anti-COVID drug discovery. In the present study, we performed a comprehensive \textit{in silico} screening for 16,776 potential molecules from recently established drug libraries based on two important pharmacophores (3-amino-4-phenylbutan-2-ol and piperazine). Based on initial assessment, 4042 molecules were obtained suitable as drug candidates, which were following Lipinski’s rule. Molecular docking implemented for the analysis of molecular interactions narrowed this number of compounds down to 19. Subsequent to screening filtering criteria and considering the critical parameters \textit{viz.} docking score and MM-GBSA binding free energy, 1-((4-(25,35)-3-amino-2-hydroxy-4-phenylbutyl)piperazine-1-yl)-3-phenylurea (compound 1) was accomplished to score highest in comparison to the remaining 18 shortlisted drug candidates. Notably, compound 1 displayed higher docking score ($-8.069 \text{ kcal/mol}$) and MM-GBSA binding free energy ($-49.56 \text{ kcal/mol}$) than the control drug, remdesivir triphosphate, the active form of remdesivir as well as adenosine triphosphate. Furthermore, a molecular dynamics simulation was carried out (100 ns), which substantiated the candidacy of compound 1 as better inhibitor. Overall, our systematic \textit{in silico} study predicts the potential of compound 1 to exhibit a more favourable specific activity than remdesivir triphosphate. Hence, we suggest compound 1 as a novel potential drug candidate, which should be considered for further exploration and validation of its potential against SARS-CoV-2 in wet lab experimental studies.

\textbf{1. Introduction}

The COVID-19 outbreak caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) initiated in Wuhan, the capital of Hubei province in China in late December 2019 (Liu et al., 2020a; Nittari et al., 2020). Within six-month time the COVID-19 disease has turned into a pandemic and has spread to 215 countries and territories. As of 28th March 2021, more than 126.3 million confirmed cases and over 2.76 million deaths worldwide have been reported due to the COVID-19 disease (World Health Organisation, 2020). SARS-CoV-2 belongs to the family Coronaviridae, sub-family Coronavirus, which includes a large number of pathogenic viruses. Most of these viruses are zoonotic, including the most pathogenic Severe Acute Respiratory Syndrome virus (SARS-CoV), Middle East Respiratory Syndrome virus (MERS-CoV) and the Novel coronavirus (SARS-CoV-2) (Mackenzie & Smith, 2020). SARS-CoV-2 has \textit{~}80\%, \textit{~}50\%, and \textit{~}96\% similarity to the genome of the SARS-CoV, MERS-CoV, and bat coronavirus RaTG13,
respectively (Udugama et al., 2020). Thus, the novel coronavirus was termed SARS-CoV-2 due to 80% genomic similarity shared with earlier SARS-CoV coronavirus, and the disease caused due to SARS-CoV-2 was called coronavirus disease (COVID-19) (Sumit et al., 2020). Owing to the fact that SARS-CoV-2 is spreading quickly across the globe due to its effective human to human transmission, COVID-19 has been declared a pandemic by WHO (El Zowalaty & Jarhult, 2020).

As precautionary measures, most countries around the world are undergoing lockdown for certain periods of time to control further spreading of the disease. Whilst different vaccines across different platforms have been rolled out recently, despite an urgent need, as of now, no specific drug is available against COVID-19. Repurposing of already approved drugs is a strategy, which has been utilized all over the globe to provide a quick medication and relief to the COVID-19 patients (Caly et al., 2020; Choy et al., 2020; Li & De Clercq, 2020; Sanders et al., 2020; Yamamoto et al., 2020). Some of these approved drugs have been found to be partially effective for COVID-19, viz. remdesivir, chloroquine and hydroxychloroquine (Liu et al., 2020b). Notably, the drug remdesivir was found to have higher potential against SARS-CoV-2 in vitro as compared to chloroquine with EC50 values of 0.77 μM and 1.13 μM, respectively. Remdesivir was also found to have higher SI (selectivity index) in comparison to that of chloroquine (>129.87, >88.50, respectively) (Wang et al., 2020). In addition, remdesivir was ranked highest amongst the antiviral drugs for their molecular interaction potential with the RNA-dependent RNA polymerase (RdRp) (Chang et al., 2020). The drug remdesivir was originally developed by Gilead Sciences (USA) for the treatment of Ebola and is known to exhibit antiviral activity against several RNA viruses (Eastman et al., 2020; Tchesnokov et al., 2019). This drug was found to specifically target a viral key component, RdRp (Elfiky, 2020; Gao et al., 2020; Zhang et al., 2020). RdRp, also known as nsp12, catalyses the synthesis of viral RNA and hence plays an important role in the replication process of SARS-CoV-2 virus proliferation (Figure 1) with the assistance of nsp7 and nsp8 as accessory factors (Subissi et al., 2014; Yin et al., 2020).

Because of the crucial role of RdRp in ssRNA replication, this polymerase is an essential enzyme for proliferation of RNA viruses and thus has been targeted for antiviral drug design against hepatitis C virus (HCV) (Shiroti et al., 2002), Zika virus (ZIKV) (Godoy et al., 2017), and coronaviruses (CoVs) (Elfiky, 2020). As reported earlier, the 759 to 761 amino acid stretch is the catalytic site while the amino acids Lys_545, Arg_553 and Arg_555 form the nucleoside triphosphate (NTP) entry channel in RdRp of SARS-CoV-2 (Gao et al., 2020). Hence, the above-mentioned amino acid stretch forming the active site of RdRp pose a potential inhibitory drug target for SARS-CoV-2 (Alexpandi et al., 2020).

Whilst there is an urgent need for a drug molecule with high anti-viral potential, a specific drug to inhibit the replication of the SARS-CoV-2 is yet to be developed. To aid in this endeavour in an efficient and highly specific manner, we initiated virtual screening employing computer aided drug design (CADD) and designed a specific library of novel compounds. This approach led to the origin of a lead molecule, 1-(4-((2S,3S)-3-amino-2-hydroxy-4-phenylbutyl)piperazin-1-yl)-3-phenylurea, with the potential to inhibit RdRp of SARS-CoV-2.

2. Material and methods
2.1. RdRp inhibitor screening and molecular interaction analysis
2.1.1. Screening of potential drug scaffolds against the RdRp
A library of anti-viral drugs consisting of 16,776 scaffolds was prepared by the maestro tool of Schrödinger. The three-dimensional structure of remdesivir TP was taken from the literature (Gordon et al., 2020a, 2020b; Yin et al., 2020). The structures of both, compound 1 and remdesivir TP along with other designed RdRp analogs were prepared by ligprep. All the shortlisted ligands were energy minimized for their 3D molecular structures, optimized for correct Lewis structures, tautomeric and ionization states, ring conformations, chirality parameters, and stereoisomers prior to detailed interaction study with the RdRp active site cavity. The ionization state at pH 7 ± 2 for all the compounds was optimized by Epik. Further, to analyze the inhibitory potential of all the analogs against SARS-CoV-2, the X-ray crystal structure of SARS-CoV-2 RdRp (PDB ID: 6M71) (Gao et al., 2020) was retrieved from the RCSB-PDB database.
2.1.2. Preparation of polymerase structure and active site identification

The retrieved crystal structure of the SARS-CoV-2 RdRp protein was further prepared as receptor by the Maestro Suit of Schrödinger. Missing hydrogen atoms were added, incorrect bond order assignments performed, charge states optimized and orientations of various groups, missing loop and side chains were optimized (Schrödinger Release 2020-1: Protein Preparation Wizard; Epik; Kaur et al., 2017). After removal of steric clashes and strained bonds/angles were removed, overall energy minimization was performed by restrained energy minimization.

The catalytic site residues (Ser_759, Asp_760 and Asp_761) were assigned from the literature (Gao et al., 2020). The grid box x, y, and z coordinates were 113.45, 114.89, and 123.36, respectively. The size of grid box was $30 \times 30 \times 30 \, \text{Å}^3$. The comparative molecular docking study of remdesivir TP, compound 1 and 4 nucleotides (ATP, GTP, UTP, and CTP) was performed by keeping the selected catalytic site residues well within the search grid of the molecular docking tool Glide. The Glide (Grid-Based Ligand Docking with Energetics) algorithm approximates a systematic search of positions, orientations, and conformations of the ligand in the receptor-binding site using a series of hierarchical filters (Friesner et al., 2006; Halgren et al., 2004). The OPLS3e force-field utilized for our studies included all atom types.

2.2. Molecular interaction analysis of designed small molecule library, remdesivir TP and nucleotides within RdRp complex

The molecular interaction study of compound 1 and remdesivir TP as well as all other shortlisted analogs was performed by utilizing the Glide tool of Schrödinger. A site-specific molecular docking of remdesivir TP and other designed analogs (Tables 1 and 2) against RdRp protein of SARS-CoV-2 was performed using the Glide module of Schrödinger suite. The resulting scores viz. docking score and the binding free energy of all the analogs with RdRp were analysed. Out of all shortlisted analogs, compound 1 and remdesivir TP were observed to have maximal docking score. The binding free energy for all the analogs was calculated by prime MM-GBSA. The molecular interaction of compound 1 and remdesivir TP with RdRp was comparatively analysed on the basis of the above-mentioned scores.

The competitive inhibition of ATP binding to the RdRp binding cavity, caused by compound 1 and remdesivir TP was thoroughly compared by analyzing the molecular docking results performed by the Glide tool of Schrodinger. RdRp residues involved in ATP binding were compared with those of compound 1 and remdesivir TP in order to analyze the competitive binding potential of these drug molecules. A cut-off distance of 4.0 Å was used to generate a 2D-ligand protein interaction map.

2.3. MD Analysis of compound 1-RdRp, remdesivir TP-RdRp, and ATP-RdRp complexes

The molecular complexes formed by the molecular docking experiment for compound 1 and remdesivir TP with RdRp were further analyzed for stability by MD simulation studies. MD simulations were performed for 100 ns at 300 K temperature and 1.01325 bar pressure. Added water molecules for compound 1-RdRp, remdesivir TP-RdRp, and ATP-RdRp complexes were 29,312, 29,268, and 30,249, respectively, while 11, 16, and 15 Na$^+$ ions were in the environment to neutralize complexes. The periodic solvation box size for these three was $10 \times 10 \times 10 \, \text{Å}^3$. The columbic cutoff radius was 9 Å. Both, compound 1-RdRp and the remdesivir TP-RdRp complexes were subjected to dynamics study in a virtual environment by utilizing the Desmond module of Schrodinger. The MD simulation was performed for 100 nanoseconds (ns)

| Entry no | Molecules | RMSD (Å) | Docking score | Binding free energy (kcal/mol) |
|----------|-----------|----------|---------------|-------------------------------|
| 1        | Remdesivir TP | 2.54    | -8.199       | -25.26                       |
| 2        | ATP       |         | -8.444       | -23.32                       |
| 2        | Compd. 1  | 2.54    | -8.069       | -49.56                       |

Table 1. Docking score and binding free energy (kcal/mol) for remdesivir TP, ATP and compound 1.
Table 2. SAR analysis.

| Entry No | Compd. | Structure | Docking Score | Binding free energy (kcal/mol) | RMSD (fit on ATP) in Å |
|----------|--------|-----------|---------------|--------------------------------|-----------------------|
| 1        | 2      | 3         | -6.707        | -28.72                         | 4.19                  |
| 2        | 3      | 4         | -6.801        | -37.39                         | 6.98                  |
| 3        | 4      | 5         | -6.836        | -17.17                         | 7.22                  |
| 4        | 5      | 6         | -6.934        | -51.79                         | 6.42                  |
| 5        | 6      | 7         | -6.935        | -40.24                         | 6.42                  |
| 6        | 7      | 8         | -7.058        | -36.32                         | 9.97                  |
| 7        | 8      | 9         | -7.091        | -39.69                         | 2.63                  |

Docking Score: -8.069; and Binding Energy: -49.56 kcal/mol
Table 2. Continued.

| 8 | 9 | Pocket-I | Pocket-II | Compound 1 |
|---|---|----------|-----------|------------|
|   |   |          |           | Docking Score: -8.069; and Binding Energy: -49.56 kcal/mol |
| 9 | 10 | H-     | NH       | -7.144     |
|   |   | N-N    | OH       | -36.94     |
|   |   |         |          | 3.14       |
| 10 | 11 | H-     | NH       | -7.205     |
|   |   | N-N    | OH       | -37.35     |
|   |   |         |          | 8.09       |
| 11 | 12 | H-     | NH       | -7.676     |
|   |   | N-N    | OH       | -30.37     |
|   |   |         |          | 2.41       |
| 12 | 13 | H-     | NH       | -7.726     |
|   |   | N-N    | OH       | -44.16     |
|   |   |         |          | 4.30       |
| 13 | 14 | H-     | NH       | -7.144     |
|   |   | N-N    | OH       | -45.92     |
|   |   |         |          | 4.23       |
Docking Score: -8.069; and Binding Energy: -49.56 kcal/mol
at an optimized temperature of 300 K and 1.01325 bar pressure. In the MD simulation system, the total number of TIP3P water molecules for the compound 1-RdRp and the remdesivir TP-RdRp complexes used were 29,312 and 29,268, respectively, while 11 and 16 Na\(^{+}\) ions (corresponding to a NaCl concentration of 0.15 M, to mimic a physiological ionic concentration) were used respectively to neutralize the MD simulation system. The total energy (kcal/mol) of the system was recorded at an interval of 1.2 ps (Kumar et al., 2020). By performing the MD simulation, we calculated the conformational deviation in \(\text{C}_\alpha\) and the conformational fluctuation in amino acid residues of RdRp in complex with compound 1 and remdesivir TP.

2.4. MD Analysis of compound 1-RdRp, remdesivir TP-RdRp, and ATP-RdRp complexes, molecular binding interfaces

The molecular interface of the complexes of compound 1, remdesivir TP with RdRp were also analysed by MD simulation. The conformational fluctuation in the amino acid residues of binding interfaces of RdRp in complex with compound 1 and remdesivir TP was calculated. The comparative binding interface of the complexes of compound 1, remdesivir TP with RdRp were analysed and particular amino acid residues involved the complex formation identified. Further, the fluctuation in conformational properties of compound 1 and remdesivir TP within their respective complex with RdRp protein were analysed.

2.5. Stereochemical geometry analysis of RdRp in complex with compound 1, remdesivir TP, ATP after MD simulation

Further, the stereochemical geometry for all the amino acid residues of the RdRp protein in complexes with compound 1, remdesivir TP, and ATP were analysed by procheck (Morris et al., 1992). For all three complexes, an acceptable percentage of the amino acid residues were found to be in favoured, additional allowed and generously allowed region. This indicates that the complexes were in stable conformations after MD simulation.

2.6. ADME profile analysis of compound 1 and remdesivir TP

In the process of analog screening for the RdRp, we have also analysed the ADME (Absorption, Distribution, Metabolism, and Excretion) related properties for all the 16,776 structures of the designed anti-viral drug library. For screening of the analogs, we have utilized the gikprop program and non-suitable candidate molecules were filtered out as per the Lipinski rule. Only those analogs having docking scores and binding free energies comparable to remdesivir TP were included for detailed analysis. The analog screening was performed within an acceptable range of parameters which included: predicted octanol/water
partition coefficient (QPlogPo/w) (range: -2.0 to 6.5); logarithm of water solubility (QPlogS) (range: -6.5 to 0.5); number of H-bond donors (HBD) (range: 0.0–6.0); number of H-bond acceptor (HBA) (range: 2–20); number of non-trivial, non-hindered rotational bond (range: 0–15); Predicted human oral absorption (range: >80% is good and <25% is poor) and Lipinski rule (Drug likeness) (mol_MW < 500, QPlogPo/w < 5.0, donorHB ≤ 5, and acceptHB ≤ 10).

Figure 2. Molecular interaction of RdRp nucleotide-binding cavity residues with (A) compound 1; (B) remdesivir TP; and (C) ATP.

Figure 3. Multiple sequence alignment of 452 RdRp sequences retrieved from the NCBI protein database. The alignment is showing the nucleotide binding cavity region of the RdRp protein (YP_009725307.1) (residues range 455 to 814) representing the entire ligand environment of the ATP molecule and the drug molecules, compound 1 and remdesivir TP. Binding residues which form the hydrogen bonds, salt bridge or pi-pi interaction are highlighted by red blocks (compound 1), blue blocks (remdesivir TP) and green blocks (ATP). The full alignment of all 452 RdRp (nsp12 ORF of polyprotein ORF1ab) protein sequences can be retrieved from: https://drive.google.com/file/d/1O7Aara1k16pJbItbIiSjusZ_WcopkQjM/view?usp=sharing.
3. Result and discussion

3.1. RdRp inhibitor screening and molecular interaction analysis

3.1.1. Screening of potential inhibitor scaffolds for RdRp

Remdesivir is a phosphoramidate prodrug of remdesivir triphosphate (remdesivir TP), which is a nucleotide analogue (Siegel et al. 2017) with a complex mechanism of action: the prodrug has the ability to diffuse into cells, where it is metabolised through an enzymatic cascade to remdesivir-monophosphate, a cyano-substituted adenosine monophosphate (AMP) analogue (Eastman et al., 2020). The monophosphate is then readily converted into the active form, remdesivir TP, by cellular nucleoside phosphate kinase. Due to their polarity the ribonucleotide analogues are trapped in the cell where remdesivir TP acts as an inhibitor of viral RdRp, competing with the natural substrate counterpart adenosine triphosphate (ATP) and ultimately leading to premature RNA synthesis termination (Gordon et al., 2020b). Remdesivir TP is known to inhibit replication of RNA-viruses, including that of coronaviruses and SARS-CoV-2 (Sheahan et al. 2017). It competes with its natural counterpart ATP for binding to SARS-CoV-2 RdRp and with a highly similar predicted mode of binding to its active site (Gordon et al.,

![Figure 4. Molecular interactions of the ligands compound 1, remdesivir TP and ATP are shown. The bottom panel of the figure shows residues which are directly interacting with the respective ligand via hydrogen bonds. Hydrogen bonds are drawn as orange dotted lines, ligands are in magenta and the RdRp residues involved in ligand interaction in green. Compound 1 shares two crucial active site residues (Asp_760 and Tyr_619) for RdRp interaction with the ATP molecule, while remdesivir TP shares only one (Tyr_619). Compound 1 forms strong salt bridges with two RdRp residues (Asp_760 and Tyr_619), which also engage in interaction with the ATP molecule, suggesting competitive inhibition of ATP binding to the RdRp binding cavity by compound 1.](image-url)
SARS-CoV-2 RdRp readily incorporates remdesivir TP into the nascent RNA strand (Yin et al., 2020). Ultimately, the incorporation of a single analog into the RNA strand terminates further extension after incorporation of 3 additional upstream nucleotides. The underlying mechanism is not yet fully understood, but emerging evidence suggests that the 1'-cyano group of the analog is hindering translocation of the strand by interaction with RdRp, when at the +3 position (Gordon et al., 2020b; Zhang et al., 2020). Recently, the phase III clinical trial of remdesivir was launched in Wuhan for the treatment of COVID-19 patients. However, the availability of the experimental drug, remdesivir, for treating a very large number of patients in a timely manner is of great concern (Cao et al., 2020). Thus, a library of compounds was designed virtually with a database of 16,776 structures of potential SARS-CoV-2 agonist molecules and searched on the basis of their drug likeness i.e. ADME profile. Out of 16,776 molecules in the database, 4042 molecules were selected for further screening against RdRp protein of SARS-CoV-2 as other structures were eliminated by Lipinski drug likeness filter. Further, compound 1 showed significantly better binding free energy (−49.56 kcal/mol; Table 1, entry 3) as compared to remdesivir TP and ATP (−25.26 and −23.32 kcal/mol, respectively; Table 1, entry 1–2). Hence, compound 1 has the potential to compete with remdesivir TP and ATP binding.

3.1.2. Structure activity relationship (SAR) analysis

Further, a structure activity relationship (SAR) analysis was carried out for the top-ranked molecules of the screened library. The library under study was initially designed on the basis of two important pharmacophores viz. 3-amino-4-phenylbutan-2-ol (blue color; Table 2) and piperazine (black color; Table 2). These two pharmacophores were selected on the basis of their high medicinal importance. The pharmacophore, 3-amino-4-phenylbutan-2-ol, is the main core in many antiviral drugs such as darunavir, ritonavir and saquinavir (Arts & Hazuda, 2012). 3-amino-4-phenylbutan-2-ol was combined with piperazine, a pharmacophore present as main core in a series of FDA approved drugs (Rathi et al., 2016). To study the SAR for the small molecules containing 3-amino-4-phenylbutan-2-ol and piperazine as two important pharmacophores, changes were analyzed at two pockets i.e. Pocket-I (red color; Table 2) and Pocket-II (purple color; Table 2). Initially, the H-atom present in compound 1 was kept at

![Figure 5. Fluctuation in root mean square deviation (RMSD) for (A) compound 1, (B) remdesivir TP, and (C) ATP in complex with RdRp protein (fit on RdRp), respectively, are shown. The root mean square fluctuation (RMSF) for all ligand atoms with respect to the protein for (D) compound 1 and (E) remdesivir TP, and (F) ATP are shown with nitrogen, oxygen and phosphorus drawn in blue, red and brown, respectively.](image-url)
Figure 6. Cα Root Mean Square Fluctuation (RMSF) of all the residues of RdRp in complex with (A) compound 1, (B) remdesivir TP, and (C) ATP. RdRp residues forming H-bonds, hydrophobic interactions, ionic interactions and water bridges with compound 1 are indicated by green bands; red and blue background indicates protein RdRp (receptor) secondary structures, α-helices and β-strands, respectively.
Pocket-I and substitutions were analyzed at Pocket-II for top-ranked compounds. A total of eleven Pocket-II substitutions displayed stronger or comparable free binding energy over remdesivir TP and ATP (compound 2–12; Table 2, entry 1–11). Ten of these analogs were observed to have stronger binding free energy (< \(-25.26\) kcal/mol) as compared to remdesivir TP (Table 1, entry 1), with compound 4 having the lowest binding free energy, through tetrazole substitution (Table 2, entry 3) at Pocket-II. Next, substitutions of top-ranked compounds, which are six analogs (compound 13–19) with lower binding free energy as compared to remdesivir TP (Table 2, entry 12–18), were analyzed at Pocket-I. Altogether, the binding free energy of compound 1 (\(-49.56\) kcal/mol) was comparable to compound 5 and 17 (\(-51.79\) kcal/mol and \(-50.00\) kcal/mol respectively) and significantly higher in the remaining 16 analogues (Table 2). However, none of these eighteen compounds (2–19) exhibits a better docking score than compound 1 (Table 1, entry 3; Table 2, entry 4 and 16). Therefore, compound 1 remains the most favorable candidate compound over the set of substituted analogs as judged on the basis of two factors (docking score and binding free energy) and was selected for further study. Overall, the same criteria predict compound 1 to be favourable over the active form of remdesivir i.e. remdesivir TP as RdRp inhibitor. Thus, compound 1 and remdesivir TP were subjected for detailed comparative studies. The RMSD of docked poses was calculated by imposition of poses with top dock score over lowest dock score. Compound 1 was found to have lowest RMSD except compound 8 and 11 (Table 1, entry 3 and Table 2, entry 7 and 10).

### 3.2. Molecular interaction analysis of compound 1, remdesivir TP, and ATP with RdRp

The molecular interaction analysis of compound 1 and remdesivir TP with SARS-CoV-2 RdRp was performed using the Glide module of Schrödinger suite. The molecular interaction analysis of compound 1 and RdRp revealed that compound 1 has the tendency to form a conformationally fitting...
complex, as indicated in our molecular docking study by hydrogen bond formation, pi-pi interaction, salt bridge interaction, polar interaction, electrostatic interaction (H-bond with: Tyr_619, Asp_623, Asp_760 and Asp_761; pi-pi interaction with Tyr_455; salt-bridge interaction: Asp_760, Asp_761; polar interaction: Ser_814; and electrostatic interaction: Arg_553, Lys_551, Arg_553, Lys_798, Trp_800, and Ser_814) as well as a salt bridge with Lys_551 leading to a stable complex formation within the active site of the RdRp protein. Notably, while remdesivir TP formed multiple hydrogen bonds within the active site of RdRp, it was not observed to engage in any pi-pi interaction within the active site of RdRp, unlike compound 1 (Figure 2A and 2B; Table 3, entry 1 and 3). Additionally, the favourable arrangement of amino groups in compound 1 leads to the formation of salt bridge interactions with adjacent aspartic acid residues (Figure 2A) that contribute significantly to the observed binding energy, which is suggestive of a competitive ligand property of compound 1 for the natural ligand of RdRp, the ATP molecule (Figure 2A and 2C). Furthermore, the hydroxyl group participates in two hydrogen bonds as a proton donor and a proton acceptor group. Hence, compound 1 is predicted to have a higher potential to form a stable complex within the active site of the RdRp protein and thus may provide a consistent inhibitory effect on RdRp polymerase activity, which in turn will lead to the inhibition of SARS-CoV-2 replication.

3.2.1. RdRp binding cavity for compound 1, remdesivir TP and ATP binding

The nucleotide binding cavity of RdRp forms a groove in which the ATP molecule binds, facilitating the polymerase activity of the RdRp protein. ATP binds to the cavity involving the amino acid stretch of RdRp ranging from Lys_545 to Asp_761 (Koulgi et al., 2020). In our molecular docking interaction analysis of ATP with RdRp, a total of 16 amino acid residues from the above-mentioned stretch were directly involved in forming the binding cavity (Figure 2C). The 9 key amino acid residues (Lys_545, Thr_556, Arg_555, Arg_553, Lys_798, Trp_800, and Ser_814) as well as a salt bridge with Lys_551 leading to a stable complex formation within the active site of the RdRp protein. Notably, while remdesivir TP formed multiple hydrogen bonds within the active site of RdRp, it was not observed to engage in any pi-pi interaction within the active site of RdRp, unlike compound 1 (Figure 2A and 2B; Table 3, entry 1 and 3). Additionally, the favourable arrangement of amino groups in compound 1 leads to the formation of salt bridge interactions with adjacent aspartic acid residues (Figure 2A) that contribute significantly to the observed binding energy, which is suggestive of a competitive ligand property of compound 1 for the natural ligand of RdRp, the ATP molecule (Figure 2A and 2C). Furthermore, the hydroxyl group participates in two hydrogen bonds as a proton donor and a proton acceptor group. Hence, compound 1 is predicted to have a higher potential to form a stable complex within the active site of the RdRp protein and thus may provide a consistent inhibitory effect on RdRp polymerase activity, which in turn will lead to the inhibition of SARS-CoV-2 replication.
Amino acid residues (Lys_545 to Asp_761) of the RdRp active site, are involved in interaction with compound 1, remdesivir TP and ATP (Figure 2). Those residues from the respective sequence stretch directly interacting with compound 1, remdesivir TP and ATP are highlighted with red, blue and green blocks respectively in the binding cavity sequence alignment (Figure 3). Amongst all amino acid residues involved in ATP-RdRp binding cavity formation, the residues common amongst ATP and compound 1 in complex with RdRp are Arg_553, Tyr_619, Asp_760 and Asp_761. Out of these common residues, compound 1 forms hydrogen bonds as well as salt bridges with Asp_760 and Asp_761 (Figure 2A and Table 3). Additionally, compound 1 forms a pi-pi interaction with Tyr_455. The high number of common residues between compound 1 and ATP strongly suggests a competitive nature of binding. Further, common RdRp residues for interaction with remdesivir TP and ATP are restricted to Arg_553 and Tyr_619, of which only Arg_553 forms a hydrogen bond with remdesivir TP (Figure 2 and Table 3).

From the binding residues discussed above, compound 1 does not share any H-bonds interactions with specific RdRp residues with remdesivir TP, while compound 1 shares all four (Tyr_619, Asp_623, Asp_760, and Asp_761) interactions with ATP (Figures 2 and 4). This suggests a comparatively weaker tendency of remdesivir TP for competitive binding to the RdRp binding cavity. Hence, here again compound 1 is most likely to compete with the binding of ATP to the RdRp binding cavity more efficiently than the remdesivir TP molecule.

3.3. Molecular dynamic (MD) analysis of compound 1-RdRp, remdesivir TP-RdRp, ATP-RdRp complexes

The binding stability of the drug within the target protein molecule is an important determinant of the effectiveness of the drug molecule towards inhibition of virus replication. Hence, the RdRp complexes with compound 1, remdesivir TP, and ATP were subjected to a molecular stability analysis. To this end, a MD simulation was carried out for these three complexes over 100 ns.

3.3.1. Conformational deviation in Cα of RdRp in complex with compound 1, remdesivir TP and ATP after MD simulation

The root mean square deviation (RMSD) of the Cα of the RdRp protein from the initial to the final conformation

| Entry no | System       | Number (%) of residues in favored region | Number (%) of residues in additional allowed region | Number (%) of residues in additional generously region | Number (%) of residues in the outlier region |
|----------|--------------|------------------------------------------|---------------------------------------------------|---------------------------------------------------------|---------------------------------------------|
| 1        | Compd. 1     | 703 (86.0%)                              | 108 (13.2%)                                       | 4 (0.5%)                                                | 2 (0.2%)                                    |
| 2        | Remdesvir TP | 703 (86.0%)                              | 106 (13.0%)                                       | 5 (0.6%)                                                | 3 (0.4%)                                    |
| 3        | ATP          | 700 (85.7%)                              | 110 (13.5%)                                       | 4 (0.5%)                                                | 3 (0.4%)                                    |
during MD simulation was analysed for all three complexes. The RMSD is very much invariable and within range of ~1.0 Å for a long duration of the simulation experiment, indicating a stable nature of both the compound 1-RdRp and the remdesivir TP-RdRp complexes with ATP-RdRp complex (Figure 5). In case of the compound 1-RdRp complex, the RMSD trajectory of Cα showed an increase from 1.6 to 3.2 Å at the initial 30 ns but remains stable with minimal variation for the following 70 ns. Compound 1 bound to the RdRp protein (fit to RdRp) gave an acceptable variation in RMSD (0.4 Å to 7.2 Å) in between 0 to 32 ns, afterwards (32–100 ns) the RMSD for compound 1 remains stable (Figure 5A) at 4 Å. The trajectories analysis revealed that RMSD fluctuation is mainly caused by the phenyl moiety of 3-amino-4-phenylbutan-2-ol of compound 1. The MD simulation of the remdesivir TP in complex with RdRp was also performed as a positive control (Figure 5B). The MD simulation Cα backbone trajectory analysis revealed an increase in the RMSD profile (1.6–3.2 Å) between 0–21 ns, then the RMSD remains stable till 60 ns, with no further significant variation after 60 ns. The ligand RMSD for remdesivir TP in complex with RdRp (fit to RdRp) remained stable during 2–18 ns, then there is an increase RMSD from 18 to 59 ns, resulting in a 3.5 to 19.7 Å RMSD shift, which is then followed by a RMSD drop to 17.5 Å at the end of the simulation (Figure 5B). The RMSD variation remains stable for significantly long duration of MD simulation for the remdesivir TP in complex with the RdRp protein. Even in case of the ATP-RdRp complex, the Cα backbone is stable at 3.2 Å, while ligand RMSD was observed to be stable between 7.5–9 Å (Figure 5C). Overall, the average RMSDbackbone, RMSD_Cα and RMSD_side-chain for the compound 1-RdRp complex (2.659 Å, 2.256 Å, and 3.484 Å respectively) underwent significantly less deviation than that of the remdesivir TP-RdRp complex (2.623 Å, 2.621 Å, and 3.577 Å, respectively) and the ATP-RdRp complex (3.205 Å, 3.205 Å, and 3.897 Å, respectively).

Further, the root mean square fluctuations (RMSF) for the ligand atoms were also analyzed to understand which of the ligand side chain was most active and flexible with respect to protein interaction during complex formation. The ligand RMSF plot (Figure 5D) for the compound 1-RdRp complex, shows that atoms number 12–18 are highly active during these simulations suggesting their involvement in ligand conformations in accordance with the protein conformational changes. Compound 1 atoms like nitrogen (1, 6, 9, 20, and 22) and oxygen (4 and 21) are majorly hydrogen bond donor/acceptors and are in very close proximity to binding site residues due to H-bond interaction. Further, in comparison to remdesivir TP, compound 1 is highly stable and remains in close proximity of binding cavity. Altogether, the ligand RMSF analysis detected less fluctuation for both, compound 1 (Figure 5D) and ATP (Figure 5F) than for remdesivir TP (Figure 5E).

The stability of all MD simulation systems for the compound 1-RdRp, remdesivir TP-RdRp and ATP-RdRp complex was monitored throughout the entire simulation process. Different parameters including total energy (E), potential energy (E_p), temperature (T), pressure (P), and the total volume (V) were analysed (Figure S1 and Tables S1–S3, supplementary material).

3.3.2. Conformational fluctuation in amino acid residues of binding interfaces of RdRp in complex with compound 1, remdesivir TP and ATP after MD simulation

The detailed analysis of local fluctuation of amino acid residues and binding interfaces of the compound 1-RdRp, remdesivir TP-RdRp, and ATP-RdRp complex were studied by RMSF plot (Figure 6). The most fluctuating amino acid residues in the compound 1-RdRp complex were found within the sequence stretches from Gln_57 to Asn_64 and Arg_105 to Asp_109, and also Glu_919 with RMSF values above 3.0 Å, while the majority of binding site residues showed a RMSF lower than 1.0 Å. Similarly, in the remdesivir TP-RdRp complex, most binding site residues have a RMSF below 1.0 Å, but Asp_62, Lys_103 to Pro_112, Asp_901, Met_902, and Glu_919 were among the most fluctuating residues with RMSF values >4.0 Å. The RMSF of binding site residues (Cα) of the ATP-RdRp complex was within 1.5 Å while most fluctuating residues were Gln_57 to Ile_66, Asp_107, Gly_108, Asp_336, Gly_337, Asn_404, Ala_406, Thr_409, Glu_431, and Gly_432 with a RMSF range 3 to 7.5 Å. The RMSF of backbone Cα of RdRp in complex with compound 1, remdesivir TP and ATP have RMSF values ranging from 0.45 to 5.39 Å, 0.49 to 7.91 Å, and 0.45 to 7.5 Å, respectively. This result suggests a higher stability of RdRp residues in complex with compound 1 over remdesivir TP and ATP.

The interaction fraction analysis detects numerous amino acid residues to be involved in the molecular interaction with the drug molecule through H-bonds, hydrophobic interactions, ionic interactions and water bridges. Compound 1 (Figure 7A and 7B and Figure S4A, supplementary material) maintained interactions with residues Tyr_455, Asp_623, Asp_760, and Asp_761 of RdRp. Remdesivir TP (Figure 7C and 7D and Figure S4B, supplementary material) maintained interactions with residues Lys_551, Arg_553, and Lys_798 of RdRp. ATP (Figure 7E and 7F and Figure S4C, supplementary material) interacts with residues Lys_545, Arg_553, Arg_555, Thr_556, and Arg_624. During MD simulation, compound 1 maintained interaction to catalytic residues while both remdesivir TP and ATP interacted to entry channel residues, which is consistent with our molecular docking interaction results (Figure 2).

3.3.3. The molecular binding of compound 1, remdesivir TP and ATP with RdRp residues during MD simulation

The molecular interaction fraction analysis further suggests that after the MD simulation study of the compound 1-RdRp complex, the molecular interaction between compound 1 and the RdRp protein remains intact for at least 30% of the simulation time including H-bonds, pi-pi hydrophilic interactions and salt bridge (H-bond: Asp_623, and Asp_761; salt bridge: Asp_760; pi-pi interaction: Tyr_455; pi-cation interaction: Lys_621, and Arg_624; and water bridge: Asp_618, and Tyr_619), (refer to Figure 7B for molecular interaction.
interface). Trajectory analysis reveals that nitrogen atoms of the 1-phenyl-3-(piperazin-1-yl) urea moiety of compound 1 are indulged in H-bond formation with Asp_623 while its phenyl group interacted with Lys_621, Arg_624 (via pi-cation) and Tyr_455 (via pi-pi stacking). In comparison to remdesivir TP (Figure 7D) and ATP (Figure 7F), compound 1 is less exposed to water. This difference in the level of water interaction could be responsible for remdesivir TP being less stably bound within the binding cavity of RdRp, while supporting the conformational stability of ATP in the binding cavity. Further, it was observed that some binding site residues such as Trp_617, Asp_618, Tyr_619, Lys_621, Arg_624, Asp_760, and Asp_761 within the compound 1-RdRp complex and Ser_451, Tyr_455, Lys_551, Arg_553, Arg_555, Asp_618, Asp_621, Asp_760, and Lys_798 within the remdesivir TP-RdRp complex, formed more than one specific contact (Figure S4A and S4B, supplementary material; darker shade of orange indicates number of contact types is greater than one). These interactions indicate higher conformational stabilities of compound 1 over remdesivir TP within the binding cavity of RdRp.

3.3.4. MD analysis of compound 1, remdesivir TP and ATP in complex with RdRp

Six properties were analyzed in support of stability of compound 1 over remdesivir TP and ATP in complex with RdRp during the MD simulation study. From the ligand RMSD of compound 1 (0.2–2.2 Å), remdesivir TP (0.4–3.0 Å), and ATP (0.9–3.3 Å) in complex with RdRp (Figure 8), it is evident that the compound 1 RdRp complex exhibits the most stable conformation. The bump in the RMSD deviation of compound 1 at between 25–45 ns can be attributed to fluctuations generated by phenyl group of 3-amino-4-phenylbutan-2-ol of compound 1, which disappears after 40 ns suggesting a stable complex conformation (Figure 8). In contrast, the remdesivir TP RMSD deviation is observed to continuously increase over ~50 ns suggesting continuous conformational change (Figure 8), after which the RMSD stabilizes at ~3.0 Å, which is greater than the highest RMSD deviation value for compound 1 (~2.2 Å). Similarly, the RMSD of ATP stabilized within the initial 10 ns and remained stable during simulation. However, compound 1 found to have lower RMSD than ATP which suggests greater conformational stability of compound 1 over ATP.

Consistently, the radius of gyration (rGYR) is indicating a more constant degree of compactness for compound 1, when complexed within the RdRp binding cavity (Figure 8), for a long duration of the MD simulation, as compared to that of remdesivir TP, while the ATP structure is more compact than compound 1, potentially due to higher intramolecular H-bonding. Notably, there is no intra-molecular hydrogen bonding in compound 1, as there is for both, the ATP and the remdesivir TP molecule and which may influence conformational changes leading to lesser structural and conformational stability of the small molecule ligand. Likewise, the Molecular Surface Area (MolSA), Accessible Surface Area (SASA), and the Polar Surface Area (PSA) for compound 1 and ATP, were rather more stable as compared to that of remdesivir TP in complex with RdRp.

Overall, the limited but acceptable deviation in all the MD simulation parameters for compound 1 in complex with RdRp suggests that compound 1 is stably bound to the RdRp protein. All MD simulation parameters for compound 1 were observed to be favourable to those of remdesivir TP in complex with RdRp. Furthermore, the binding free energies calculated after MD simulation for compound 1 (~50.38 kcal/mol), remdesivir TP (~29.94 kcal/mol), and ATP (~42.13 kcal/mol) are strongly suggesting more stable binding of compound 1 to RdRp in comparison to remdesivir TP and ATP. Hence, compound 1 has the potential to replace ATP during RNA polymerization as competitive inhibitor and, in turn, block SARS-CoV-2 replication more efficiently than remdesivir TP.

Next, the torsional conformation of compound 1, remdesivir TP, and ATP (Figure 9) within the RdRp binding cavity complex during the simulation process was analysed. The intact orientation of every rotatable bond of compound 1, remdesivir TP, and ATP within the complex throughout the MD simulation, again, suggest a more stable complex formation tendency of compound 1 over remdesivir TP and ATP within the active site of RdRp protein (Figure 9).

Overall, the molecular interaction analysis of compound 1 with RdRp suggests a stable complex formation tendency of compound 1 with multiple hydrogen bond, pi-pi interactions and salt bridge formation within RdRp binding cavity. For complex formation tendency within the active site of RdRp compound 1 outperforms remdesivir TP and ATP in multiple parameters, suggesting compound 1 as promising drug candidate for blocking replication of SARS-CoV-2.

Further, to obtain insight into the structural and conformational stability of the compound 1 protein complex, the simulation was extended up to 130 ns (Figure S3, supplementary material). Thermodynamic data for compound 1-RdRp complex was calculated for every 10 ns of the trajectory with an average binding energy of ~58.87 ± 4.43 kcal/mol (Table S4; supporting information) indicating the stability of compound 1 throughout the simulation process. The ligand RMSD was also calculated for every 10 ns of the trajectory frame yielding an average RMSD of 1.62 ± 0.2 Å (Table S4).

3.4. Stereochemical geometry analysis of RdRp in complex with compound 1, remdesivir TP, and ATP after MD simulation

Stereochemical geometry analysis of all three complexes (compound 1-RdRp, remdesivir TP-RdRp, and ATP-RdRp) after MD simulation revealed a comparable number of residues in favoured, additionally allowed and generously allowed region (86.0%,13.2%,0.5%; 86.0%,13.0%,0.6%; 85.7%, 13.5%, 0.5%, respectively); again favourably, all three complexes were observed to have a very small number of residues falling in the outlier region of the Ramachandran plot (0.2%; 0.4%, and 0.4% respectively) (Table 4; Figure S5, supplementary material). In conclusion, all three complexes exhibit a sterically acceptable conformation of the receptor RdRp protein after...
MD simulation, which is indicating all these complexes would be stable under physiological conditions.

3.5. Screening of compound 1, ATP, and remdesivirTP on the PyRx platform

Subsequent ligand analysis with SARS-CoV-2 RdRp of using the PyRx autodock vina software (Dallakyan & Olson, 2015), resulted in binding affinities of compound 1, ATP, and remdesivirTP of −7.4, −7.3, and 7.0 kcal/mol, respectively (Figure S6), which supports our previous findings. Further, the binding free energy was calculated for these docked protein-ligand complexes using MM-GBSA, where compound 1 (−56.32 kcal/mol) and remdesivirTP (−56.06 kcal/mol) exhibited significantly stronger binding free energy as compared to ATP (−31.39 kcal/mol). In conclusion, these results are consistent with our previous findings, where no significant difference was observed in the docking affinity of these three ligands but both remdesivirTP and compound 1 possessed lower binding free energy than ATP.

3.6. ADME profile analysis of compound 1 and remdesivir TP

An extensive ADME profiling for compound 1, remdesivir TP and the prodrug remdesivir was performed using QikProp program. The distributions of the compound molecular weights (MW), lipophilicity (QPlogPo/w), number of hydrogen bond acceptors (HBA) and number of hydrogen bond donors (HBD) were used to assess the ‘drug-likeness’ of a designed small molecule library. Favourably, compound 1 exhibits a less flexible nature with 9 rotational bonds than remdesivir TP (15 rotational bonds) and the remdesivir prodrug (16 rotational bonds). Both, compound 1 and remdesivir have 5 HBD while remdesivir TP has 4 HBD. Compound 1 has approximately half the number of HBA (8.2) compared to remdesivir (16.65) and remdesivir TP (19.65). Both, remdesivir and remdesivir TP are bulky in comparison to compound 1 with molecular weights of 602.583 g/mol 531.205 g/mol and 383.492 g/mol, respectively. The oil/water partition coefficient (QPlogPo/w) for compound 1 (1.444) is higher than that of both remdesivir TP (−1.873) and remdesivir (1.217). The water solubility (QPlogS) for compound 1, remdesivir TP and remdesivir is −2.004, −1.993, and −4.439, respectively, indicating that compound 1 exhibits better oral absorption than remdesivir (: 58.9% and 35.1% oral absorption, respectively).

The oral absorption percentage for remdesivir TP is zero, whereas compound 1 shows 49.0% oral absorption. Compound 1 has molecular weight and H-bond acceptors (2189.1) and remdesivir TP (2189.1) with QPlogPo/w of 0.95 and 0.01, respectively. In this study, we have screened the active form of remdesivir i.e. remdesivir TP, a RdRp inhibitor, and a designed compound library in order to identify inhibitors targeting the RdRp of SARS-CoV-2. We identified compound 1 as top ranked potential inhibitor molecule based on docking score and binding free energy analysis out of all designed analogs and remdesivir TP. The binding site of the RdRp protein carries several key residues functioning in ATP binding and hydrolysis. Out of the key residues for ATP binding, Asp_623, Tyr_619, Asp-760 and Asp_761 were observed to be shared by compound 1 binding to the RdRp active site. This suggests a competitive binding potential of compound 1, which could lead to the inhibition of RdRp protein to bind its natural ligand, the ATP molecule. In case of remdesivir TP, fewer RdRp binding residues (Arg_553 and Tyr_619) are shared with ATP. Further, MD simulation studies indicated a more stable complex formation with RdRp for compound 1 than for remdesivir TP and ATP on the basis of RMSD, which is further corroborated by RMSF data. Ligand RMSD (with respect to ligand), radius of gyration, Molecular Surface Area (MoLSA), Accessible Surface Area (SASA), and the Polar Surface Area (PSA) advocate compound 1 stability in the RdRp binding pocket. The last frame of all three protein-ligand-complex simulations showed robust stereo-chemical geometry of RdRp, with less than 0.5% outlier residues. The thermodynamic data for the compound 1-RdRp complex predicts stability of compound 1 during the 130 ns MD simulation with an average binding free energy of −58.87 ± 4.43 kcal/mol. RdRp docking experiments with these three molecules by PyRx autodock vina software further corroborated compound 1 as promising molecule to target the polymerase protein. Overall, based on extensive in silico studies we propose compound 1 as a novel inhibitor for RdRp, which should be explored and validated further by in vitro and in vivo experimentation.

4. Conclusion

In the present study, we have screened the active form of remdesivir i.e. remdesivir TP, a RdRp inhibitor, and a designed compound library in order to identify inhibitors targeting the RdRp of SARS-CoV-2. We identified compound 1 as top ranked potential inhibitor molecule based on docking score and binding free energy analysis out of all designed analogs and remdesivir TP. The binding site of the RdRp protein carries several key residues functioning in ATP binding and hydrolysis. Out of the key residues for ATP binding, Asp_623, Tyr_619, Asp-760 and Asp_761 were observed to be shared by compound 1 binding to the RdRp active site. This suggests a competitive binding potential of compound 1, which could lead to the inhibition of RdRp protein to bind its natural ligand, the ATP molecule. In case of remdesivir TP, fewer RdRp binding residues (Arg_553 and Tyr_619) are shared with ATP. Further, MD simulation studies indicated a more stable complex formation with RdRp for compound 1 than for remdesivir TP and ATP on the basis of RMSD, which is further corroborated by RMSF data. Ligand RMSD (with respect to ligand), radius of gyration, Molecular Surface Area (MoLSA), Accessible Surface Area (SASA), and the Polar Surface Area (PSA) advocate compound 1 stability in the RdRp binding pocket. The last frame of all three protein-ligand-complex simulations showed robust stereo-chemical geometry of RdRp, with less than 0.5% outlier residues. The thermodynamic data for the compound 1-RdRp complex predicts stability of compound 1 during the 130 ns MD simulation with an average binding free energy of −58.87 ± 4.43 kcal/mol. RdRp docking experiments with these three molecules by PyRx autodock vina software further corroborated compound 1 as promising molecule to target the polymerase protein. Overall, based on extensive in silico studies we propose compound 1 as a novel inhibitor for RdRp, which should be explored and validated further by in vitro and in vivo experimentation.

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Disclosure statement
Authors declare no conflict of interest.
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