Design, synthesis, and molecular dynamics simulation studies of quinoline derivatives as protease inhibitors against SARS-CoV-2

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ABSTRACT
A new series of quinoline derivatives has been designed and synthesized as probable protease inhibitors (PIs) against severe acute respiratory syndrome coronavirus 2. In silico studies using DS v20.1.0.19295 software have shown that these compounds behaved as PIs while interacting at the allosteric site of target Mpro enzyme (6LU7). The designed compounds have shown promising docking results, which revealed that all compounds formed hydrogen bonds with His41, His164, Glu166, Tyr54, Asp187, and showed π-interaction with His41, the highly conserved amino acids in the target protein. Toxicity Prediction by Komputer Assisted Technology results confirmed that the compounds were found to be less toxic than the reference drug. Further, molecular dynamics simulations were performed on compound 5 and remdesivir with protease enzyme. Analysis of conformational stability, residue flexibility, compactness, hydrogen bonding, solvent accessible surface area (SASA), and binding free energy revealed comparable stability of protease:5 complex to the protease:remdesivir complex. The result of hydrogen bonding showed a large number of intermolecular hydrogen bonds formed between protein residues (Glu166 and Gin189) and ligand 5, indicating strong interaction, which validated the docking result. Further, compactness analysis, SASA and interactions like hydrogen-bonding demonstrated inhibitory properties of compound 5 similar to the existing reference drug. Thus, the designed compound 5 might act as a potential inhibitor against the protease enzyme.

HIGHLIGHTS
- Quinoline derivatives have been designed as protease inhibitors against SARS-CoV-2.
- The compounds were docked at the allosteric site of SARS-CoV-2-Mpro enzyme (PDB ID: 6LU7) to study the stability of protein-ligand complex.
- Docking studies indicated the stable ligand-protein complexes for all designed compounds.
- The Toxicity Prediction by Komputer Assisted Technology protocol in DS v20.1.0.19295 software was used to evaluate the toxicity of the designed quinoline derivatives.
- Molecular dynamics studies indicated the formation of stable ligand-Mpro complexes.

KEYWORDS: SARS-CoV-2; PIs; docking; QSTR analysis; molecular dynamics; SASA; RMSD; RMSF; binding free energy

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1. Introduction

The novel coronavirus, leading to coronavirus disease 2019 (COVID-19), has so far spread from China to 219 countries around the world and the fatality rate has hitherto resulted in more deaths (>3%) than severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) combined (Wang et al., 2020; Cheng & Shan, 2020; Zhang & Liu, 2020). Several independent research groups have identified that SARS coronavirus 2 (SARS-CoV-2) belongs to Betacoronavirus, with highly identical genome to bat coronavirus, pointing to bats as the natural host (Ghosh et al., 2020a; Pal et al., 2020). The novel coronavirus (SARS-CoV-2) uses the receptor angiotensin-converting enzyme 2 (ACE2) and mainly spreads through the respiratory tract (Bourgonje et al., 2020). Importantly, increasing evidence showed sustained human-to-human transmission, along with many exported cases across the globe. The clinical symptoms of COVID-19 patients include fever, cough, fatigue and also gastrointestinal infection symptoms (Zhang et al., 2020; Zhao et al., 2020) in a small population of patients. The elderly and people with co-morbidities are more susceptible to infection and serious outcomes, which may be associated with acute respiratory distress syndrome (ARDS) (Chen et al., 2020) and cytokine storm (Coperchini et al., 2020).

Currently, due to the exigency of the finding a suitable therapeutic treatment for COVID-19, several potent antiviral candidates as repurposed under urgent investigation (Ancy et al., 2020) to reduce the time and hassles of approval. Hence, an endeavour of prodigious momentum and magnitude has been set out to develop vaccines against SARS-CoV-2, with successive publication of its genome sequence. Its genome encodes several non-structural and structural proteins, comprised of spike (S), envelop (E), membrane (M) and nucleocapsid (N) proteins (Du et al., 2009; Ghosh et al., 2020b). During viral replication process of SARS-CoV-2 and MERS, the proteolytic cleavage of viral polyproteins into functional subunits is achieved by protease enzymes (Mpro, also known as 3C-like protease) (Bhardwaj et al., 2021; Kumar et al., 2013; Singh et al., 2021). Hence, proteases become a hot spot for drug target against corona viruses without any off-target toxicity (Bhardwaj et al., 2021; Ghosh et al., 2020c; Ghosh et al., 2021; Sharma et al., 2021). Therefore, impeding the Mpro catalytic activity utilising protease inhibitors (PIs) leads to thwarting of viral replication, prompting the amended clinical upshots in case of COVID-19 and associated diseases (Bhardwaj & Purohit, 2020; Ghosh et al., 2020a).

The majority of the candidate vaccines under trial against COVID-19, intend to generate neutralizing antibodies against the viral spike proteins (S) and averting its uptake through human ACE2 receptor, and thus resulting in blockage of infections. The vaccine strategies traditionally employ the whole virus, either attenuated or inactivated, and aspire to procreate a broader, more heterologous polyclonal response against several viral antigens. Subsequently, an emergency use authorization (EUAs) has been issued for several vaccine candidates against SARS-CoV-2 by different national and international drug regulation agencies. SARS-CoV-2 vaccine should match the following parameters to prove to be an ideal vaccine: (i) it should not only be able to provide protection from severe disease but also thwart infection in the vaccinated population, (ii) it should be able to reduce the number of immunocompromised individuals and protect those with history of infection, (iii) it should provide long term memory immune response after a minimal number of immunizations or booster doses, and (iv) it should easily be assessable for worldwide vaccination at the cheapest cost. In this series, six vaccine developers, viz. Pfizer and BioNTech, Curevac, Sanofi-GSK, AstraZeneca and the University of Oxford, Johnson & Johnson and Moderna, have successfully introduced their vaccines, which are being used at present. Several other pharmaceutical giants are still engaged in different levels of clinical trials. In addition to traditional strategies of treatment, several platform strategies, viz. nucleic acid platforms, non-replicating viral vectored platforms, inactivated virus or recombinant subunit vaccines, have also come into existence as unprecedented expeditious vaccine development efforts (Kyriakidis et al., 2021). The human beings are lucky enough to have several vaccines (used under emergency conditions) against SARS-CoV-2 as a great respite. Nevertheless, vaccines, the prophylactic way of thwarting the disease, are not effective enough to guarantee full safety and save the precious lives of human beings, and hence the second line of therapeutic treatment must be there and this necessitates an urgent and undeniable need for safe and effective drug candidates.

The SARS-CoV-2 is a positive-strand virus and hence some broad-spectrum antiviral drugs such as remdesivir, favipiravir, Darunavir, X77 and lopinavir are currently being used for treatment. Recently, 2-deoxy-D-glucose has been approved by the Drug Controller General of India for treatment, however, its utility at the ground level is yet to be proved (Triggle et al., 2021).

Despite all these efforts, a number of aspects of anti-SARS-CoV-2 immunity are still unknown and the world is still facing the issues of morbidity and mortality caused by COVID-19. Thus, along with an ideal vaccine, an effective and safe drug that can abolish the virus from the human body, is privational prerequisite in order to efficiently ebb out the indisposition and mortality, and to establish a vivacity. The N-heterocyclic scaffolds usually perform a vibrant role in the advanced biological activity and thus demonstrate promising therapeutic potential, probably due to charisma of...
nitrogen atoms (Badavath et al., 2020). Quinoline is a hetero-
cyclic aromatic organic compound that contains an electron-
ically rich benzene ring fused with an electronically deficient
pyridine. Quinolines and substituted quinolines obtained
from natural sources and microorganisms show remarkable
biological activities. Many substituted quinoline derivatives
have been synthesized and reported for their biological
activities and are known to possess anti-microbial, anti-viral,
anti-inflammatory, anti-tumor, anti-cancer, anti-dementia,
anti-fungal, hypotensive, anti-HIV, and analgesic properties
(Amer et al., 2018; Cocco et al., 2000; Mukherjee et al., 2001;
Narender et al., 2005). Keeping in view the importance of
quinoline nuclei in medicinal chemistry (Aldahham et al.,
2020) and in the search of new anti-COVID agents, we
have focused on design and development of some novel
quinoline analogues and subjected them to
in silico
studies
against novel coronavirus. The
in silico
studies were per-
formed on a series of quinoline derivatives synthesized by
our research group and out of a library of about one hun-
dred molecules, eight promising molecules were selected for
further studies. Furthermore, molecular dynamics (MD) simu-
lations and other
in silico
pharmacokinetic assessments were
also performed on these molecules. It is worth mentioning
here that the selected quinoline derivatives (1–8) comprised
of amide linkages, similar to well-known Michael acceptor
inhibitors N3, B1 and B2, and thus provided a strong basis to
investigate as plausible anti-COVID candidates (Aldahham et
al., 2020; Gentile et al., 2020; Singh et al., 2021). Design con-
sideration of substituted quinoline derivatives, 1–8, is shown
in Figure 1.

2. Materials and method
2.1. Physicochemical description and bioactivity score
Quinoline derivatives have been designed as possible anti-
viral agents using computational methods, i.e. in silico struc-
ture-based approach. Molinspiration and ChemDraw software
were used to predict the physicochemical descriptors and
pharmaceutically relevant properties of designed
compounds. Assessment of properties was done using a
thumb rule, i.e. Lipinski’s rule of five in order to find out the
dug-like characteristics of compounds and compounds violat-
ing more than one rule were rejected for further studies
(Ghosh et al., 2020a, 2020b, 2020c, 2021; Naaz et al., 2018;
Singh et al., 2021; Srivastava et al., 2018).

2.2. Admet prediction
All in silico pharmacokinetic parameters (viz. aqueous solubil-
ity [log S], skin permeability [log Kp], synthetic accessibility
score, absorption [% ABS], distribution, metabolism, excretion
and toxicity) of newly designed quinoline derivatives were
predicted by using free online software SwissADME and
pkCSM-Biosig (Daina et al., 2017; Ghosh et al., 2020b, 2020c,
2021; Naaz et al., 2018).

2.3. Target prediction
Online available software SwissTargetPrediction was used to
predict the macromolecular targets for designed molecules.
The software is useful to predict off-targets and to evaluate
the possibility of reprocessing therapeutically relevant com-
pounds, based on “similarity principle,” which normally states
that two similar molecules are expected to have similar prop-
ties (Anbazhakan et al., 2019).

2.4. Molecular docking studies and density functional
theory analysis
Selected ligands were optimized and prepared for molecular
simulation by using BIOVIA/Discovery Studio 2020 Client (DS
20.1.0.19295 version) protocol default parameters (Lagos et
al., 2008; Muegge & Martin, 1999; Singh et al., 2016). The 3D
x-ray crystal structure of docking receptor i.e. active site of
main protease enzyme (Mpro, 6LU7: PDB ID, www.rcsb.org),
the catalytic core of SARS-CoV-2 was retrieved as an adduct
from RCSB (www.rcsb.org) protein data bank (Rose et al.,
2013). The ligand was prepared accordingly and docking was

![Figure 1. Design consideration of substituted quinoline derivatives 1–8.](image-url)
performed and the top-ranked key docking pose was selected for further analysis. Overall, the standard measures were employed for preparing, docking, and scoring of ligands with protein (Srivastava et al., 2020). Predicted Ligand binding site for docking simulations is shown in Si-Figure 1.

The structure based density functional theory (DFT) analysis was also performed for the best screened compound using default parameters available in simulation tool panel of BIOVIA/Discovery Studio 2020 Client (DS 20.1.0.19295 version). Analysis of highest occupied molecular orbital and lowest unoccupied molecular orbital was done in solvent free condition with distance dependent dielectric constant parameter (Hoque et al., 2018; Mishra et al., 2021).

2.4.1. Preparation of receptor
Ligand docked with the target protein was extricated and all missing hydrogen atoms were added employing DS v20.1.0.19295. Positions of each atom was optimized by all-atom CHARMM forcefield with Adopted Basis set Newton Raphson (ABNR) minimization algorithm till the root mean square (r.m.s.) gradient for potential energy become $<0.05 \text{kcal mol}^{-1} \text{Å}^{-1}$. Furthermore, target protein was minimized and defined as receptor employing the “Binding Site” 2.4.3 tool in DS 20.1.0.19295. The input site sphere having 5 Å radius on receptor, covered by ligand has been defined as binding site. The minimized receptor obtained this way from target protein was further employed for docking simulations (Srivastava et al., 2020).

2.4.2. Ligand setup
Built-and-edit unit of DS v20.1.0.19295 was employed for creation of 3D structure of each ligand and CHARMM forcefield using ABNR method was applied for minimization of ligands. Conformational selection of ligands was accomplished by MD approach. Moreover, the ligand was heated up to 700 K and then annealed up to 200 K for thirty times. On completion of thirty cycles, the conformation of ligand was obtained and subjected to local energy minimization employing ABNR method. All minimized conformations were then superimposed and the conformation with the lowest energy was selected as the most plausible conformation.

2.4.3. Docking and scoring
The DS v20.1.0.19295 Ligandfit protocol, combined with a shape comparison filter and a Monte Carlo transformational search, was used for docking of ligand with targeted protein. Dreiding forcefield was employed to refine each docked pose by rigid body minimization of ligand with respect to the grid based calculated interaction energy. In docking simulations, the targeted protein receptor conformation was kept rigid. The minimization of docked poses was done employing all-atom CHARMM forcefield and smart minimization method until the r.m.s. gradient for potential energy was $<0.05 \text{kcal mol}^{-1} \text{Å}^{-1}$. While minimization, the binding sites of ligands and protein were concubine flexible employing simulation methods (MD, energy minimization and Monte Carlo simulation). The scoring (Interaction energy, Lig_Internal_Energy, Binding Energy and Dock Score) of ligands are explained in the result and discussion section. All conformations have been refined via LUDI scores. Ligand conformation exhibiting the highest LUDI scores was elected as the best conformation and was taken for further analysis. Negative binding energy of ligands defining the stability of ligand-protein complex, was evaluated by “Calculate Binding Energy protocol” in DS v20.1.0.19295 using the default settings (Srivastava et al., 2018).

2.4.4. Validation of docking protocol
In validation of docking protocols, the native crystallized ligand (remdesivir) and molecules were docked against the active site of Mpro protein receptor. The comparative analysis of docking simulation results alluded that the employed scoring function was appropriate as the root mean square deviation of molecules along with the native crystallized ligand and was under the justifiable limit, i.e., (root-mean square deviation [RMSD]) $<2\text{Å}$. Therefore, the result supported the hypothesis that experimental binding modes could be reproduced with correctness employing pre-mentioned protocol.

2.5. Molecular dynamics simulation
MD simulations were carried out for the target-ligand complexes (protease enzyme-compound 5 and protease enzyme-remdesivir complex) for a period of 100 ns using GROMACS 2019.6 package (GNOningenMAchine for Chemical Simulations) (Pronk et al., 2013; Purohit, 2014; Singh et al., 2021) with CHARMM36 force-field parameters (Huang & Mackerrell, 2013). CgenFF web server was used to generate parameters for ligand molecules (Vanommeslaeghe et al., 2010). A unit cell defined as a cubical box, with a minimal distance of 15 Å from the protein surface to the edges of the box, was generated and solvated using the TIP3P water system. The systems were neutralized by adding appropriate number of sodium counter ions to them. Then the systems were energy minimized using steepest descent methods to remove the bad contacts among the atoms, with 5000 steps and a force convergence of less than 1000 kcal mol$^{-1}$ nm$^{-1}$. Subsequently, the minimized system was subjected to a two-step equilibration; 100 ps of NVT equilibration to maintain the system temperature at 300 K using the V-rescale thermostat (Bussi et al., 2007) and 100 ps of NPT equilibration to maintain a constant pressure of 1 bar for the system, using Parrinello-Rahaman barostat (Parrinello & Rahman, 1981). It also maintained the homogeneous density across the systems. The Linear Constraint Solver algorithm was used for constraining all the bonds (Hess et al., 1997). Further, in order to compute the long-range electrostatics forces, Particle Mesh Ewald (PME) method was employed with a cut off value of 1.0 nm (Darden et al., 1993). The MD simulations were run based on leap-frog algorithm (Van Gunsteren & Berendsen, 1988). A production run of 100 ns without any restraints was carried out for each system on NPT ensemble.
with step size 2 fs. The coordinates were saved every 10 ps during the production run. The resultant MD trajectories were analyzed using XMGRACE 2D plotting tool (Turner, 2005) and VMD 1.9.1 (Schuler et al., 2001).

### 2.6. Binding free energy calculation

The molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA), an efficient and reliable method, was used to investigate residual binding energies in molecular recognition processes (Genheden & Ryde, 2015; Homeyer & Gohlke, 2012; Wang et al., 2018; Sharma et al., 2020; Singh, Bhardwaj, Sharma, et al., 2021). The binding free energy ($\Delta G_{\text{bind}}$) in a solvent medium was calculated as follows:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}})$$  (1)

In Equation (1), $G_{\text{complex}}$ is the total free energy of the protein-ligand complex, $G_{\text{protein}}$ and $G_{\text{ligand}}$ are the total energies of protein and ligand, respectively, alone in a solvent. The free energies for each individual $G_{\text{complex}}$, $G_{\text{protein}}$, and $G_{\text{ligand}}$ were estimated by:

$$G_p = EMM + G_{\text{solv}}$$  (2)

Equation (2), $p$ can be protein, ligand, or complex. $EMM$ is the average molecular mechanics potential energy in vacuum and $G_{\text{solv}}$ is the solvation free energy. The molecular mechanics potential energy was calculated in the vacuum as follows:

$$EMM = E_{\text{bonded}} + E_{\text{non-bonded}}(\text{and} E_{\text{non-bonded}} = E_{\text{vdw}} + E_{\text{elec}})$$  (3)

Here $E_{\text{bonded}}$ is the total bonded interaction energy, like bond, angle, dihedral and improper interactions; $E_{\text{non-bonded}}$ is the total non-bonded interaction energy consisting of both van der Waals ($E_{\text{vdw}}$) and electrostatic ($E_{\text{elec}}$) interactions. $E_{\text{bonded}}$ is taken as zero. The solvation free energy ($G_{\text{solv}}$) is the sum of electrostatic solvation free energy ($G_{\text{polar}}$) and nonpolar solvation free energy ($G_{\text{non-polar}}$), which is determined using the Poisson-Boltzmann (PB) linear equation and the solvent-accessible surface area (SASA), respectively. The binding free energies of the complexes were calculated based on 500 snapshots taken at an equal interval of time during last 10 ns of MD simulation trajectories. The per-residue energy contribution was also computed to understand the contribution of individual amino acids to the total binding energy.

### 2.7. Chemistry

All chemicals were bought from Sigma Aldrich Chemical Company, USA and E. Merck India Ltd, India. All reactions were carried out in oven-dried apparatus and solvents used were dried and distilled. Column chromatography was carried out on silica gel (100–200 mess). Reactions were monitored on TLC, using silica gel 60F254 aluminium plates and visualized under ultraviolet light at 254 nm. Melting points recorded on electro thermal apparatus are uncorrected. NMR spectra were recorded on BRUKER-AV400 spectrometer (Bruker Co., Faellanden, Switzerland) in DMSO-d$_6$ (1H at 400 MHz and 13C at 100 MHz). Chemical shifts (δ) are expressed in parts per million (ppm) and $J$ (coupling constant) values in hertz. Multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and br (broad spectrum). Mass spectra were recorded on Micromass Q-Tof (ESI-HRMS). The elemental analyses were performed on a Perkin-Elmer 240-C equipment.

#### 2.7.1. Synthesis of 8-amino-4-methyl-1H-quinoline-2-one

Aromatic amine (1 mmol) dissolved in ethyl acetocacetate (1 mmol) containing a few drops of H$_2$SO$_4$ (1% mmol) as a catalyst was stirred at 140–150 °C using oil bath. The completion of reaction was monitored on TLC with ethyl acetate/hexane as developing solvent. The newly formed product appeared as a bright blue spot-on TLC when exposed to UV light at 365 nm. The reaction mixture was cooled to room temperature and poured in ice-water mixture and stirred for 10 min. The precipitated product was collected by filtration, washed with water and dried. The final product was purified on silica gel column with ethyl acetate as eluent and recrystallized as white solid using ethanol.

#### 2.7.1.1. 8-Amino-4-methylquinolin-2(1H)-one

Yield 76%, light yellow solid; m.p. 164–168°C; R$_f$ 0.56 (EtOAc:Hexane: 6:4); $^1$H NMR (400 MHz, DMSO-d$_6$) δ ppm: 10.84 (s, 1H, -N-H), 6.85 (d, 2H, $J = 7.98$ Hz, Ar-H), 6.83 (d, 1H, $J = 8.00$ Hz, Ar-H), 6.25 (s, 1H, sp$^2$ -C-H), 4.74 (s, 2H, -N-H), 2.24 (s, 3H, sp$^3$-C-H), $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ ppm: 161.1, 147.4, 141.5, 130.2, 129.2, 122.4, 120.5, 115.3, 113.5, 113.5, 18; HRMS m/z (M + H)$^+$ 274.08; Calcd. For C$_{10}$H$_{11}$N$_2$O: C, 68.95; H, 5.79; N, 16.08; O, 9.18. Found: C, 68.92; H, 5.74; N, 16.04; O, 9.15.

#### 2.7.2. Experimental procedure for synthesis of sulphonamide/benzamide derivatives (1–8)

8-Amino-4-methyl-1H-quinoline-2-one (1 mol) was dissolved in dry DCM and cooled in an ice-bath. A cooled solution of substituted benzenesulfonyl chloride/benzoyl chloride (1.2 mol) and triethylamine (1.2 mol) in dry DCM was added. The reaction mixture was stirred overnight at room temperature. The completion of reaction was monitored on TLC. The reaction mixture was evaporated at room temperature and poured in ice-water mixture and stirred for 10 min. The precipitated product was collected by filtration, washed with water and dried. The product was purified using column chromatography and ethyl acetate/hexane as eluting solvent.

#### 2.7.2.1. N-(4-Methyl-2-oxo-1,2-dihydroquinolin-8-yl)-4-nitrobenzenesulfonamide (1)

Yield 71%, light yellow solid; m.p. 235°C; R$_f$ 0.54 (EtOAc:Hexane: 6:4); $^1$H NMR (400 MHz, DMSO-d$_6$) δ ppm: 10.64 (s, 1H, -N-H), 10.21 (s, 1H,-N-H), 8.10 (d, 2H, $J = 8.00$ Hz, Ar-H), 7.73 (d, 2H, $J = 7.98$ Hz Ar-H), 6.84 (m, 2H, $J = 8$ Hz, Ar-H), 6.75 (d, 1H, $J = 7.98$ Hz, Ar-H), 6.31 (s, 1H, sp$^2$ -C-H), 2.30 (s, 3H, sp$^3$-C-H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ ppm: 161.0, 149.1, 146.5, 145.4, 132.0.
2.7.2.3. 3,4,5-Triisopropyl-N-(4-methyl-2-oxo-1,2-dihydroquinolin-8-yl)thiophene-2-sulfonamide (6).

2.7.2.5. 4-Methyl-N-(4-methyl-2-oxo-1,2-dihydroquinolin-8-yl)benzenesulphonamide (5). Yield 76%, light yellow solid; m.p. 195–198°C; Rf = 0.69 (EtOAc:Hexane: 7:3); 1H NMR (400 MHz, DMSO-d6) ppm: 10.79 (s, 1H, -N-H), 10.07 (s, 1H, -N-H), 7.65 (d, 1H, J = 8.00 Hz, Ar-H), 7.61 (d, 1H, J = 7.94 Hz, Ar-H), 7.15 (d, 2H, J = 7.98 Hz, Ar-H), 6.87 (d, 2H, J = 7.85 Hz, Ar-H), 6.81–6.73 (m, 1H, J = 7.98 Hz, Ar-H), 6.29 (s, 1H, sp3-C-C); 13C NMR (100 MHz, DMSO-d6) ppm: 161.5, 146.2, 137.5, 133.2, 134.2, 130.2, 128.3, 130.7, 128.0, 121.6, 115.3, 113.7, 112.5, 110.6, 108.4, 106.8, 103.3, 101.0, 100.9, 98.3, 72.4, 68.5, 67.5, 66.4, 64.3, 62.4, 56.1, 53.8, 44.7, 42.6, 33.6, 31.8, 29.3, 21.7, 18.5; HRMS m/z (M+H): 382.06, Calcd. For C17H13F3N2O3S: 382.02, Anal. Calcd. For C17H13F3N2O3S: C, 53.40; H, 3.43; F, 14.91; N, 7.33; O, 12.55; S, 8.38. Found: C, 53.38; H, 3.39; F, 14.88; N, 7.29; O, 12.51; S, 8.34.

2.7.2.7. 4-Chloro-N-(4-methyl-2-oxo-1,2-dihydroquinolin-8-yl)benzenesulphonamide (2). Yield 70%, light yellow solid; m.p. 214–219°C; Rf = 0.56 (EtOAc:Hexane: 6:4); 1H NMR (400 MHz, DMSO-d6) ppm: 10.89 (s, 1H, -N-H), 10.09 (s, 1H, -N-H), 7.76 (d, 1H, J= 8.00 Hz, Ar-H), 7.71 (d, 1H, J= 7.80 Hz, Ar-H), 7.57 (d, 2H, J = 7.89 Hz, Ar-H), 6.84 (d, 2H, J = 8.00 Hz, Ar-H), 6.81–6.78 (dd, 1H, J = 7.80 Hz, Ar-H), 6.73 (d, 1H, Ar-H), 6.29 (s, 1H, sp3-C-C); 13C NMR (100 MHz, DMSO-d6) ppm: 161.5, 146.2, 133.6, 130.3, 130.1, 126.5, 121.5, 120.2, 115.5, 115.0, 18.0; HRMS m/z (M+H): 348.03, Calcd. For C17H13ClN2O3S: 347.05, Anal. Calcd. For C17H13ClN2O3S: C, 55.10; H, 3.76; Cl, 10.16; N, 8.03; O, 13.76; S, 9.19. Found: C, 55.06; H, 3.72; Cl, 10.13; N, 8.00; O, 13.72; S, 9.16.

2.7.2.4. N-(4-Methyl-2-oxo-1,2-dihydroquinolin-8-yl)-4-(trifluoromethyl)benzenesulphonamide (4). Yield 76%, light yellow solid; m.p. 222–224°C; Rf = 0.65 (EtOAc:Hexane: 6:4); 1H NMR (400 MHz, DMSO-d6) ppm: 10.75 (s, 1H, -N-H), 9.5 (s, 1H, -N-H), 7.75–7.70 (dd, 2H, J = 8.00 and 2.12 Hz, Ar-H), 6.74–6.78 (dd, 2H, J = 7.98 and 1.83 Hz, Ar-H), 6.92–6.85 (m, 2H, Ar-H), 6.75 (d, 1H, J = 8.00 Hz, Ar-H), 6.38 (s, 1H, Ar-H), 2.39 (s, 3H, sp3-C-H); 13C NMR (100 MHz, DMSO-d6) ppm: 161.4, 147.2, 142.5, 133.4, 134.1, 130.0, 129.1, 128.5, 126.5, 123.5, 122.1, 119.8, 114.8, 115.3, 18.5; HRMS m/z (M+H): 440.60, Calcd. For C26H18F3N2O3S: 440.56, Anal. Calcd. For C26H18F3N2O3S: C, 68.15; H, 7.32, N, 6.36; O, 10.89; S, 7.28. Found: C, 68.12; H, 7.29; N, 6.32; O, 10.85; S, 7.25.
Figure 2. Docking interactions of quinoline derivatives 1–8 and reference drugs with Mpro enzyme receptor.
Figure 2. Continued.
Figure 2. Continued.
Table 2. Drug-likeness properties of quinoline derivatives 1–8.

| Compound | GPCR ligand | Ion channel modulator | Kinase Inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|-----------|-------------|------------------------|------------------|------------------------|-------------------|-----------------|
| 1         | −0.32       | −0.42                  | −0.40            | −0.07                  | −0.30             | −0.17           |
| 2         | −0.20       | −0.42                  | −0.29            | −0.01                  | −0.25             | −0.11           |
| 3         | −0.10       | −0.33                  | −0.21            | 0.05                   | −0.11             | −0.03           |
| 4         | −0.11       | −0.29                  | −0.18            | 0.16                   | −0.12             | −0.06           |
| 5         | −0.21       | −0.44                  | −0.02            | 0.22                   | −0.22             | −0.10           |
| 6         | −0.11       | −0.53                  | −0.18            | −0.00                  | −0.05             | 0.05            |
| 7         | −0.21       | −0.33                  | −0.11            | −0.26                  | −0.42             | −0.11           |
| 8         | −0.35       | −0.40                  | −0.42            | −0.45                  | −0.63             | 0.06            |
| Remdesivir | 0.27       | −0.35                  | 0.20             | −0.48                  | 0.49              | 0.38            |
| Lopinavir  | 0.23       | −0.78                  | 0.55             | −0.66                  | 0.42              | −0.36           |
| Darunavir  | 0.35       | −0.21                  | −0.24            | −0.26                  | 1.15              | 0.31            |
| X77       | 0.23       | 0.09                   | −0.23            | −0.50                  | 0.12              | 0.07            |

GPCR (G-protein coupled receptor-ligand): (−0.62 to −0.39, moderate activity), Ion channel modulator: (0.27 to 0.05, significant activity), Protein kinase inhibitors: (0.40 to 0.18, significant activity), Nuclear receptor: (−0.63 to −0.31, moderately active), Protease activated receptors: (−0.15 to 0.00, moderate activity) and Enzyme inhibitor: (0.61 to −0.48, significant activity).

The bioactivity scores, viz. G-protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor and nuclear receptor ligand were also evaluated to analyse some more drug-like properties. The compounds having bioactivity score more than zero are supposed to be pharmaceutically active, whereas the bioactivity score of compounds between 0.00 and −0.50, shows moderate activity and the score less than −0.50, shows inactivity. On the inspection of results, it was found that almost all compounds 1–8 were found within the permissible range of bioactivity score and thus showed good to moderate drug-like properties. Results are summarized in Table 2.

3.2. In silico ADMET prediction of quinoline derivatives (1–8)

ADMET properties, such as log Kp, log S, synthetic accessibility score and absorption (% ABS), blood brain barrier permeability (log BB), metabolism, excretion and toxicity parameters of all quinoline derivatives (1–8) were calculated by SwissADME online available software and are shown in Tables 3 and 4.

The predicted intestinal absorption was more than 76% for all compounds and thus the compounds qualified the drug-like criteria of ≥70% absorption and hence good intestinal absorption was expected. Water solubility expressed as the decimal logarithms of the molar solubility, i.e. log S (mol/L) indicated the good drug bioavailability as the compounds showed water solubility value <−4 (Ottaviani et al., 2010; Ritchie et al., 2013). The results thus demonstrated that all compounds were in the range of high to moderate solubility. Skin permeability, represented as log Kp (cm/s or cm/h), plays a significant role in transdermal delivery of drugs and the molecule having higher –ve value of log Kp have lesser skin permeability. All molecules showed moderately permeability as value ranged between −2.74 and −2.80 (Potts & Guy, 1992; Singh & Singh, 1993). Compounds 3 and 8 were expected to cross blood brain barrier easily as their log BB values were higher than (−1).

Bioavailability of drug is also influenced by metabolism and cytochrome CYP450 enzymes are the most important class to study this effect. On analysis, most of the
### Table 3. In silico ADMET predication and synthetic accessibility of molecules 1–8.

| Compound | Water solubility (logmol/L) | Intestinal absorption (% Absorbed) | Skin permeability (log Kp) | Blood brain barrier permeability (log BB) | 2D 6 | 3 A 4 Substrate | CYP | Metabolism | Excretion | Oral rat acute toxicity (LD<sub>50</sub>) (log mg/kg/day) | Maximum tolerated dose (human) (log mol/kg) | Synthetic Accessibility |
|----------|----------------------------|------------------------------------|---------------------------|------------------------------------------|------|----------------|-----|-------------|----------|-------------------------------|-------------------------------|------------------------|
| 1        | -3.99                      | 89.82                              | -2.75                     | -0.74                                    | N    | Y              | Y Y | Y Y Y Y N N | N N | 0.41                          | N                             | 3.79                   | 0.00 | 2.88 |
| 2        | -3.55                      | 93.44                              | -2.76                     | -0.44                                    | N    | Y              | Y Y | Y Y Y Y N N | N N | -0.10                         | N                             | 2.49                   | 0.73 | 2.68 |
| 3        | -4.16                      | 93.61                              | -2.74                     | 0.11                                     | N    | Y              | Y Y | Y Y Y Y N N | N N | 0.38                          | N                             | 2.37                   | 0.44 | 3.69 |
| 4        | -4.36                      | 91.81                              | -2.78                     | -0.65                                    | N    | Y              | Y Y | Y Y Y Y N N | N N | 0.12                          | N                             | 2.39                   | 0.12 | 2.77 |
| 5        | -3.45                      | 94.90                              | -2.76                     | -0.26                                    | N    | Y              | Y Y | Y Y Y Y N N | N N | 0.59                          | N                             | 2.50                   | 0.71 | 2.78 |
| 6        | -3.19                      | 77.81                              | -2.64                     | -1.28                                    | N    | Y              | Y Y | Y N Y N N N | N N | -0.07                         | N                             | 2.29                   | 0.30 | 2.97 |
| 7        | -3.38                      | 94.27                              | -2.75                     | -0.01                                    | N    | Y              | Y Y | Y Y Y Y N N | N N | 0.21                          | Y                             | 2.73                   | 0.42 | 2.15 |
| Remdesivir | -3.01                      | 62.56                              | -2.74                     | -1.99                                    | Y    | N              | Y N | N N N N N N | N N | 0.38                          | Y                             | 2.24                   | -0.09 | 2.13 |
| Lopinavir   | -3.11                      | 73.96                              | -2.73                     | -1.44                                    | N    | Y              | N Y | N N N N N N | N N | 0.63                          | Y                             | 2.32                   | 0.81 | 5.67 |
| Darunavir   | -4.59                      | 77.01                              | -2.73                     | -1.10                                    | Y    | Y              | Y Y | Y Y Y Y N N | N N | 0.67                          | N                             | 2.48                   | -0.31 | 5.67 |
| X77        | -2.89                      | 88.55                              | -2.73                     | -1.10                                    | Y    | Y              | Y Y | Y Y Y Y N N | N N | 0.63                          | Y                             | 2.59                   | 0.42 | 3.89 |

**Notes:**
- Y = Yes; N = No.
- Water solubility = < - 4 soluble.
- Intestinal absorption = below 30% indicates poor absorbance.
- Skin permeability = > - 2.5 considered to be permeable.
- Blood brain barrier permeability = < - 1 considered poorly distributed to the brain.
- Total clearance (log CL<sub>tot</sub>) = Lower value indicates high drug half lifetime.
- LD<sub>50</sub> (Lethal Dose) = Lower value predicts minimum toxicity.
- Maximum tolerated dose = > 0.47 predicts lower toxicity.
- Synthetic Accessibility = 1 (very easy) to 10 (very difficult).

#### 3.4. Docking analysis of quinoline derivatives 1–8

Docking is the computational prediction of structures of ligands and proteins. To understand the binding mode of quinoline derivatives, docking was performed using DS (20.10.1925 version) software. Various anti-HIV drugs viz. remdesivir, darunavir, lopinavir, anazapavir, etc. also displayed strong binding affinity toward the active site of Mpro (Beck et al., 2020). Among the compounds screened, results indicated that all compounds had high binding affinity vis-a-vis binding mode of several small molecules within the range of 5.21 Å. The docking results of quinoline derivatives were found to be in the range of 2.83 Å, respectively, and two amino acids His164 and Glu166 at a distance of 3.08 Å. The docking results of quinoline derivatives 1–8 are showed in Table 5. How installation results were calculated based on number of binding affinity. Molecular docking was performed using DS FlexX. The docking results of quinoline derivatives 1–8 with PI were shown in Table 5. Docking results of quinoline derivatives were found to be in the range of 2.83 Å, respectively, and two amino acids His164 and Glu166 at a distance of 3.08 Å. The docking results of quinoline derivatives were found to be in the range of 2.83 Å, respectively, and two amino acids His164 and Glu166 at a distance of 3.08 Å. The docking results of quinoline derivatives 1–8 are showed in Table 5. Docking results of quinoline derivatives were found to be in the range of 2.83 Å, respectively, and two amino acids His164 and Glu166 at a distance of 3.08 Å. The docking results of quinoline derivatives were found to be in the range.
Table 4. Toxicity prediction of quinoline derivatives 1–8 and reference drugs.

| S. No. | AMES Toxicity | Max. tolerated dose (human) | hERG inhibitor | Oral Rat Acute Toxicity (LD50) | Oral Rat Chronic Toxicity (LOAEL) | Hepatotoxicity | Skin Sensation | T. Pyriformis toxicity | Minnow toxicity |
|--------|---------------|-----------------------------|----------------|-------------------------------|----------------------------------|----------------|---------------|---------------------|------------------|
|        | Categorical (Yes/No) | Numeric (log mg/kg/day) | Categorical (Yes/No) | Numeric (mol/kg) | Numeric (log mg/kg_bw/day) | Categorical (Yes/No) | Categorical (Yes/No) | Numeric (log µg/L) | Numeric (log mM) |
| 1      | Yes           | 0.002                       | No             | 3.791                        | 2.021                            | No             | No            | 0.483               | 0.911            |
| 2      | No            | 0.727                       | No             | 2.485                        | 1.326                            | No             | No            | 0.578               | −0.236           |
| 3      | No            | 0.443                       | No             | 2.37                         | 1.244                            | Yes            | No            | 0.300               | −3.575           |
| 4      | No            | 0.117                       | No             | 2.388                        | 1.360                            | No             | No            | 0.760               | 0.938            |
| 5      | No            | 0.708                       | No             | 2.502                        | 1.279                            | No             | No            | 0.579               | −0.019           |
| 6      | No            | 0.297                       | No             | 2.290                        | 1.422                            | No             | No            | 0.394               | 1.505            |
| 7      | No            | 0.415                       | No             | 2.727                        | 1.831                            | No             | No            | 0.627               | 1.130            |
| 8      | Yes           | −0.093                      | No             | 2.337                        | 1.569                            | No             | No            | 0.76                | 0.108            |
|        | Remdesivir    | 0.141                       | No             | 2.459                        | 2.838                            | Yes            | No            | 0.285               | 0.38             |
|        | Lopinavir     | 0.812                       | No             | 2.329                        | 2.412                            | Yes            | No            | 0.285               | −0.302           |
|        | Darunavir     | −0.313                      | No             | 2.487                        | 1.817                            | Yes            | No            | 0.286               | −3.85            |
| X77    | Yes           | 0.421                       | No             | 2.596                        | 0.279                            | Yes            | No            | 0.285               | 1.742            |

LD50 (Lethal Dose) = Lower value predicts minimum toxicity; Maximum tolerated dose = ≤ 0.47 predicts lower toxicity; AMES = Mutagenic or carcinogenic toxicity; LOAEL (Lowest adverse effect level test) = LOAEL ≤ 10 mg per kg per day were labelled as strong chronic toxicity, chemicals with LOAEL > 50 mg per kg per day were labelled as weak chronic toxicity and chemicals with LOAEL ranged from 10 to 50 mg per kg per day were labelled as medium chronic toxicity. T. Pyriformis toxicity = Tetrahymena Pyriformis toxicity, Minnow toxicity = Acute fathead minnow toxicity is basis of hazard and risk assessment for compounds in the aquatic environment. Structure–minnow toxicity relationship as follows: \( \log LC_{50} = -0.94 \log p + 0.94 \log (0.000068 \times p + 1) - 1.25 \) where \( p \) is the n-octanol/water partition coefficient.

Figure 3. DFT analysis of compound 5 and reference drug remdesivir.
Figure 4. RMSD plots of the protease complexes of remdesivir (reference) and compound S (shown in black and red, respectively).

Table 5. Docking interaction of quinoline derivatives 1–8 in ligand—receptor complexes.

| Compound | No. of H-B | Amino acid in H-B | H-B Type | D(Å) | D-A | A-A | No. of π–π/cation B | Amino acid in π–π/cation B | "π–π/π+c=c" monitor |
|----------|------------|-------------------|----------|-------|-----|-----|---------------------|---------------------|-----------------------|
| 1        | 1          | His164            | 1: ND1  | 24    | 2.68 | ND1 | O24                 |                     |                       |
| 2        | 2          | His164            | 2: ND1  | 24    | 2.68 | ND1 | CL23               |                     |                       |
| 3        | 2          | His164            | 3: ND1  | 12    | 3.08 | ND1 | O12                 | 2(π–π) His41        | His41–3              |
| 4        | 3          | His164            | 4: ND1  | 12    | 2.72 | ND1 | O12                 | 2(π–π) His41        | His41–3              |
| 5        | 2          | Glu166            | N: Glu166: O12 | 3.08 | N   | O12 |                      |                     |                       |
| 6        | 3          | Tyr54             | 6: OH-Tyr54: O22 | 2.59 | OH  | O22 |                      |                     |                       |
| 7        | 1          | His41             | 7: NE2  | 15    | 2.72 | NE2 | O15                 |                     |                       |
| 8        | 2          | Tyr54             | 8: OH-Tyr54: O12 | 2.96 | OH  | O12 |                     | His41–3              |
| Ref. 1   | 2          | Glu189            | Ref: N9-Gln189: O16 | 2.90 | N   | O16 |                     |                     |                       |
| Ref. 2   | 1          | Tyr54             | Ref: 2: O22 | 2.73 | OH  | O22 |                     |                     |                       |
| Ref. 3   | 2          | His163            | Ref: N913-4: O | 3.01 | N   | O13 |                     |                     |                       |

H-B = Hydrogen bond, D = Distance (Å), D-A = Donor Atom, A-A = Acceptor Atom, π–π = Pi-Pi bond, π-cation = Pi-cation bond, Ref. 1 = Remdesivir, Ref. 2 = Darunavir, Ref. 3 = X77.

Table 6. Docking scores of quinoline derivatives 1–8 in ligand-receptor docked complexes.

| S. No. | PLP1 | PLP2 | PMF | PMF04 | Lib-DS | Ludi2 | Ludi3 | ΔG | E[C50] |
|--------|------|------|-----|-------|--------|-------|-------|----|--------|
| 1      | 73.30 | 61.87 | 83.14 | 28.98 | 111.82 | 316   | 621   | -4.48 | 6.17 × 10^-7 |
| 2      | 67.43 | 56.93 | 60.08 | -1.76 | 108.14 | 344   | 651   | -4.88 | 3.09 × 10^-7 |
| 3      | 99.60 | 101.11 | 33.09 | -1.11 | 138.32 | 421   | 520   | -5.97 | 6.31 × 10^-6 |
| 4      | 76.46 | 70.82 | 59.87 | 35.39 | 104.02 | 399   | 537   | -5.66 | 4.27 × 10^-6 |
| 5      | 72.76 | 63.04 | 67.44 | 9.03  | 105.92 | 452   | 710   | -6.41 | 7.94 × 10^-8 |
| 6      | 66.64 | 71.22 | 86.22 | 46.35 | 86.70  | 323   | 637   | -4.58 | 4.27 × 10^-7 |
| 7      | 59.12 | 66.91 | 59.10 | -4.92 | 62.41  | 378   | 696   | -5.36 | 1.10 × 10^-7 |
| 8      | 38.51 | 58.25 | 46.95 | -11.35| 75.24  | 359   | 613   | -5.09 | 7.41 × 10^-7 |
| Ref. 1 | 61.42 | 54.78 | 14.43 | -21.95 | 100.33 | 423   | 441   | -6.00 | 3.89 × 10^-7 |
| Ref. 2 | 113.07 | 106.55 | 66.62 | 9.98  | 136.26 | 438   | 447   | -6.21 | 3.38 × 10^-5 |
| Ref. 3 | 119.41 | 108.45 | 83.49 | 28.96 | 93.80  | 550   | 667   | -7.80 | 2.14 × 10^-7 |

PLP = piecewise linear potential, Lib-DS = Dock Score, Ludi2 and Ludi3 = Empirical scoring function, ΔG = binding energy (kcal/mol), E[C50] predicted = 50% effective concentration of compound (µM), Ref. 1 = Remdesivir, Ref. 2 = Darunavir, Ref. 3 = X77.
His41 at a distance from 3.81 to 4.69 Å. Compound 4 having trifluoro methyl group at the para position of the sulfonyl group formed three hydrogen bonds with the amino acid His164 at a distance of 2.72 to 3.01 Å, and two π-π bonds with the amino acid His 41 at a distance of 4.01–5.21 Å.

Compound 5 having methyl group at the para position of the sulphonyl group formed two hydrogen bonds with the amino acids Glu166 and Gln189 at a distance of 2.85–2.90 Å, and Compound 6 bearing thiazole moiety exhibited three hydrogen bonds with Tyr54 at a distance of 2.59 Å, His164 at 3.15 Å and Asp187 at 2.87 Å.

Compound 7 having benzoyl group formed one hydrogen bond with amino acid His41 at a distance of 2.72 Å and one π-π bond with amino acid His41 at a distance of 4.13 Å, and Compound 8 having propargyl group also formed two hydrogen bonds with amino acid Tyr54 at a distance of 2.68 Å to 2.96 Å.

Docking interactions of quinoline derivatives and reference drugs are shown in Figure 2. The binding of all compounds with protease enzyme confirmed their behaviour as PIs.

Analysis of result clearly revealed that all compounds exhibited robust interaction with amino acid residues of catalytic core of target enzyme hence all of them may behave as plausible lead candidates against SARS-CoV-2 and amongst all, compound 5 showed the highest extent of stability and safety profile.

Scoring descriptors, viz. Lib dock score (Lib DS), PLP1, PLP2, PMF, PMF04, DG and EC50 values were calculated for all compounds to analyse the stability of protein-ligand...
complexes. Piecewise linear potential (PLP) functions of a ligand are used to correlate the binding affinity of the ligand to a target receptor protein. The compound having higher PLP scores showed stronger binding with receptor protein. All compounds 1–8 exhibited considerable binding affinity to the receptor protein (Mpro) via PLP 1 (38.51–99.60) and PLP 2 (56.93–101.11) scores.

Lib-Dock score (Lib-DS), another factor generated from docking simulation again supported the interaction of the designed ligands with the Mpro protein in a better way. An extensive study revealed that the Lib-DS values for all molecules (ranging between 62.41 and 138.32) were found to be comparable to the reference drug remdesivir (Lib DS: 100.33), darunavir (136.25) and X77 (93.80). Ludi2 and Ludi3 are empirical scoring functions derived from the Ludi algorithm and used to select the exact conformations of protein-ligand complexes. Free binding energy (ΔG) predicted via Ludi2 for all molecules (ranging between −6.41 and −4.48) was found in close proximity to the remdesivir (−6.00), darunavir (−6.21) and X77 (−7.80). EC50 values, a measure of bearable toxicity and good pharmacological activity, were also evaluated using Ludi3 scoring function ranges between 4.27×10⁻⁶ and 7.94×10⁻⁸, and concluded that the values were found to be comparable and even better in few instances. It can be inferred from the studies that higher ΔG (−ve) values indicated towards stable protein ligand complexes, which, in turn, supported lower EC50 values. Results obtained here, clearly revealed that compounds 1–8 had either exhibited similar mode of binding to the Mpro catalytic site or had an excellent binding affinity with Mpro receptor protein, compared to the references employed in docking analysis. Results are presented in (Table 6).

3.5. Density functional theory analysis

DFT analysis has been done using the discovery studio v20.1.0.19295 software. DFT analyses of compound 5 and the reference drug remdesivir are shown in Figure 3. DFT analysis revealed that the band gap energy between the highest occupied molecular orbital and lowest unoccupied molecular orbital of compound 5 was less than that of remdesivir, which proved that the molecular charge transfer interaction is more feasible and prominent in compound 5 than remdesivir. Hence compound 5 might showed better binding affinity than remdesivir with target enzyme, which was required for good biological activities (Acar et al., 2017; Sulpizi et al., 2002; Tandon et al., 2019).

3.6. Molecular dynamics trajectory analysis

MD simulation presents an appropriate way to study atomic level information about binding of ligands to target molecules (proteins/DNA/RNA) (Hollingsworth & Dror, 2018; Kumari et al., 2021). On the basis of MD trajectories, the RMSD, root-mean square fluctuation (RMSF), radius of gyration (Rg), number of hydrogen bonds and binding free energy of the complexes were computed to analyse and get insight into their structural stabilities, binding modes and binding strengths of the designed molecules.

3.6.1. Conformational stability

The RMSD plots against simulation time, shown in Figure 4, elucidated smaller fluctuations for both reference complex remdesivir (black) and compound 5 (red) after 65 ns of the trajectory indicating attainment of a stable conformation. The average RMSD of the reference complex (0.33 nm) was relatively higher than that for compound 5 complex (0.28 nm). This observation suggested that compound 5 complex was relatively more stable than the reference complex.

3.6.2. Residue flexibility analysis

To evaluate the flexibility of the complexes, fluctuations of each amino acid residue presented by the RMSF plots as a
Figure 8. (a) Hydrogen bond numbers between protease protein and ligands [compound 5 (red) and remdesivir (black)] over MD production run (b) Hydrogen bond numbers between Glu166, Gln189 residues and ligands [compound 5 (red) and remdesivir (black)] over MD production run.

Table 7. Intermolecular interaction energies of the selected protein-ligand complexes.

| Energy components (kJ/mol)                  | Compound 5: protease complex | Remdesivir: protease complex |
|--------------------------------------------|------------------------------|-----------------------------|
| van der Waals energy ($\Delta E_{vdW}$)    | $-121.525$                   | $-161.323$                  |
| Electrostatic energy ($\Delta E_{elec}$)   | $-16.084$                    | $-29.472$                   |
| Polar solvation energy                     | $87.841$                     | $140.105$                   |
| SASA energy                                | $-14.535$                    | $-19.926$                   |
| Binding energy                             | $-64.300$                    | $-70.616$                   |
function of residue number were plotted (Figure 5). High RMSF value (peaks) indicated the presence of loops, turns, terminal ends and loose bonding showing the flexibility of the protein structure while smaller value indicated the presence of secondary structures such as sheets and helices which rendered stability to structure. The graph illustrated that the complex with compound 5 showed high fluctuations than the complex with remdesivir. The catalytic pocket residues showed relatively more fluctuations than other residues (besides the last 100 residues at C terminus) showing a flexible catalytic pocket for compound 5 complex. According to the docking results, the residues Glu 166 and Gln 189 were important and these residues also showed a higher RMSF for compound 5 complex compared to reference complex. The catalytic pocket residues showed relatively more fluctuations than other residues suggesting a flexible catalytic pocket for compound 5 complex. The graph illustrated that the complex with compound 5 showed high fluctuations than the complex with remdesivir. The catalytic pocket residues showed relatively more fluctuations than other residues (besides the last 100 residues at C terminus) showing a flexible catalytic pocket for compound 5 complex. According to the docking results, the residues Glu 166 and Gln 189 were important and these residues also showed a higher RMSF for compound 5 complex compared to reference complex. The catalytic pocket residues showed relatively more fluctuations than other residues (besides the last 100 residues at C terminus) showing a flexible catalytic pocket for compound 5 complex.

3.6.3. Compactness analysis
The radius of gyration (Rg) accounts for the compactness or globular structures of the protein-ligand complexes. Generally, a higher Rg value indicates an expanded or open structural conformation of protein whereas a lower value indicates more compact structure. The plots of Rg versus time (Figure 6) were quite similar for both complexes, having the mean values at 2.24 nm and 2.25 nm, for compound 5 (red) and remdesivir (black), respectively, with protease. Smaller and stable fluctuations in Rg value of compound 5 reiterated the previous finding that the complex was stable and compact resulting in stronger interaction between protease and ligand. The mean Rg values for compound 5 and remdesivir complexes after 65 ns showed the same value (2.23 nm), showing similar compactness in both the structures.

3.6.4. Solvent accessible surface area
SASA accounts for the surface area of a protein-ligand complex which directly interacts with solvent molecules. Higher SASA values indicate an expanded or open structure exposed more to solvent. The SASA values are similar in range for both the complexes (average SASA 151.99 nm² for compound 5 (red) and 150.69 nm² for remdesivir (black). This observation indicates similar solvent interaction and also validates the compactness and residue flexibility analysis results (Figure 7).

3.6.5. Analysis of hydrogen bonding
Hydrogen bond formation between the ligand and target shows the binding stability of a target-inhibitor complex. The simulation results showed a large number of intermolecular hydrogen bonds formed between protein residues and both the ligands (remdesivir and compound 5) indicating a strong interaction (Figure 8a). Figure 8a gives the number of hydrogen bonds formed during the simulations with respect to time. The number of hydrogen bonds for remdesivir complex (average hydrogen bond number 1.65) is higher compared to compound 5 complex (average hydrogen bond number 0.27) as remdesivir is a bulkier molecule (77 atoms) than compound 5 (39 atoms). However, the hydrogen bonds were intact during the entire dynamics for compound 5 complex, indicating stronger binding between compound 5 and protease and leading to a very stable conformation. The docking studies showed that both remdesivir and compound 5 ligands formed two hydrogen bonds with protease residues Glu166 and Gln189. The hydrogen bond analysis of MD simulation also illustrated the bond formation between Glu166, Gln189 residues and both ligands (Figure 8b). The number of hydrogen bonds formed during the simulations is more in case of compound 5 (red) than remdesivir (black). From VMD analysis it was observed that for the last 35 ns, the bond between Glu166 and remdesivir is more stable while for compound 5-protease complex the bond between Gln189 and compound 5 is more stable. This observation illustrates that residue Gln166 is stable.
Figure 10. Snapshots of compound 5: Mpro complex at interval of every 10 ns of MD trajectory.
Figure 11. Snapshots of Remdesivir: Mpro complex at interval of every 10 ns of MD trajectory.
3.6.6. Binding free energy and residue interaction energy

MM/PBSA was widely used for free energy calculation from MD trajectory (Van Aalten et al., 1995; Wang et al., 2018). Binding free energy of both complexes was calculated using MM/PBSA method from 500 snapshots extracted at equal intervals of last 10 ns MD trajectories. The contributions of different interactions were either positive or negative to the binding free energy as summarized in Table 6 for both complexes. The compound 5-protease complex showed lower negative binding energy ($\Delta G = -64.30$ kJ mol$^{-1}$ or $-15.37$ kcal mol$^{-1}$) than remdesivir-protease ($\Delta G = -70.62$ kJ mol$^{-1}$ or $-16.88$ kcal mol$^{-1}$). The binding energy results showed that

in remdesivir-protease complex and residue Gln189 is stable for compound 5-protease complex.

![Scheme 1. Synthesis of compound 8-amino-4-methylquinolin-2(1H)-one. Reagents & condition: (i) $\text{H}_2\text{SO}_4$ (1% mmol), Temp 140–150 °C for 3–4 h.](image1)

![Scheme 2. Synthesis of quinoline derivatives 1–8. Reagents & conditions; A: (i)–(vi) substituted-benzenesulfonyl chloride, Et$_3$N, Dry DCM, Temp 30–36 °C for 8–10 h; B: (vii)–(viii) benzoyl chloride/propargyl bromide, Et$_3$N, Dry DCM, Temp 30–36 °C for 8–10 h.](image2)
the reference complex exhibited more stability than compound 5-protease complex. The difference in the values of binding energies was also reflected in the docking binding energies studies. Decomposition into separate energy terms revealed that the polar solvation energy decreases the binding strength of inhibitors to the protease significantly, and thereby reduced the total binding energy in both complexes due to the positive energy contributions (Table 7). Among the various interactions, van der Waal energy (\(\Delta E_{vdw}\)) showed the most favourable contributions towards the negative binding free energy of both complexes.

In addition to the overall energies of the complex (Table 7), the contributions of individual amino acids to the binding free energy (\(\Delta G_{bind}\)) of both complexes were also computed using the MM/PBSA method and presented in Figure 9. The per-residue interaction energy profiles revealed that compound 5—protease complex differed from reference complex at residue position 49, 61, 165, 167, 168 and 187, indicating that Met49, Met165, Lue167, Pro168 and Asp187 actively participated in interaction to give rise to a stronger binding and stability. There were also energy values that contributed negatively to the stability of compound 5 complex like Arg40, Glu47, Asp48, Tyr54, Arg105 and Glu166. The most unstable residue was Arg40 showing a high positive value 14.19 kJ/mol. The most favourably contributing residue was Met49, having a binding energy of −8.41 kJ/mol, while that for reference complex was also Met49 with −6.32 kJ/mol binding energy.

### 3.6.7. Structural analysis during MD simulations

To analyse the conformational changes in compound 5 and remdesivir in complexed form with Mpro, the structural snapshots at the interval of every 10 ns for the entire 100 ns MD simulation were taken. In case of compound 5: Mpro complex exhibited more fluctuation in first 40 ns and later on due to more compactness in the binding pocket of receptor enzyme (reflected by radius of gyration and SASA analyses) the complex displayed less conformational changes and high stability from 70 ns to 100 ns, as presented in Figure 10.

Similarly, the snapshots were also extracted for Remdesivir: Mpro complex. It was observed that the large size of remdesivir facilitated its additional interactions with main protease atoms/residues as compared to compound 5. Here, remdesivir demonstrated conformational changes only for initial 30 ns and further remains almost constant for entire 100 ns period, also supported by the RMSD assessment. Thus the remdesivir: Mpro complex was also found stable for later 70 ns, as presented in Figure 11.

Overall MD analyses manifested that compound 5-PI complex showed properties comparable to remdesivir–PI complex and both were quite stable complexes.

### 3.7. Chemistry

The synthetic strategy adopted for synthesis of 8-amino-4-methyl-1H-quinoline-2-one from ethyl acetoacetate is shown in Scheme 1. The quinoline derivatives, 1–8, have been synthesized by the reaction of 8-amino-4-methyl-1H-quinoline-2-one with different types of sulphonyl chloride/benzoyl chloride/propargyl bromide as outlined in Scheme 2. Purification and characterization data of these compounds have been provided in supporting information.

### 4. Conclusion

A new series of quinoline derivatives 1–8 have been developed as effective PIs of SARS-CoV-2 using in silico structure-based approach and synthesized via multiple steps. From docking analysis, it was revealed that some of the designed compounds showed high effectiveness and even better results as compared to the reference drugs. Docking results also indicated the good relationship between the predicted EC_{50}, various energy terms and descriptors for all newly reported compounds against SARS-CoV-2. Subsequently, MD simulations positively supported the result of docking analysis by exhibiting comparable results of compound 5 and reference drug remdesivir in terms of radius of gyration, RMSD, RMSF, SASA and binding free energy, that established a robust relation between docking and MD results. In conclusive remark, a comparable stability as well as binding ability of compound 5 and reference drug remdesivir has been found against Mpro of SARS-CoV-2. Therefore, the present study provided a valuable advancement in the development of novel PIs and can be considered as a starting point for lead optimization.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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