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Click triazole as a linker for drug repurposing against SARS-CoV-2: A greener approach in race to find COVID-19 therapeutic

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ABSTRACT

WHO holding the hands of the scientific commune and trying to repurpose the drugs against the SARS-CoV-2. The robust scientific data has illustrated the probable mechanistic path of SARS-CoV-2 entry and action in damaging the cells. Which further has demonstrated Hydroxychloroquine (HCQ; antimalarial drug) as promising drug therapeutic; apart from certain setbacks to be an excellent agent in treating COVID-19. In the present study, we have explored the derivatives of HCQ, conjugated with bioactive agents by the virtue of sustainably modified clicked triazole approach as potential Mpro enzyme inhibitors. In results, we found the chloroquinetrithiazone has strong binding affinity for the Mpro enzyme of SARS CoV-2. We also found the stable binding of CQ-TrOne conjugate with Mpro by MD simulation studies through RMSD, RMSF, and Rg calculations. Moreover, in conjunction with critical reaction coordinate outcomes, binding MMGB/PB energy profile depicted the efficient binding affinity towards Mpro. Also, DFT analyses illustrated the stability of the repurposed drug under study. These significant outcomes have shown high potency of compounds and can be further assessed through \textit{in vitro} and \textit{in vivo} assays to develop the effective drug against COVID-19.

1. Introduction

Since December 2019, a race has begun to bring a conclusion to the chapter entitled “COVID-19 A PANDEMIC”. The year 2020, a mirror couple year, is now 11 months old with an unforgettable & undesirable memory with cases of a novel coronavirus from Wuhua to Worli; pushing the global death toll beyond imagination. SARS CoV-2 has emerged highly infectious with infection of over 25.5 million people worldwide in the large number of countries and caused the lethal results leading to the death. The WHO has shown critical importance on the use of existing FDA approved drugs as an anti-covid agent rather than developing agents from scratch. The biggest question that subsequently became a challenge at the clinical level was ruling out when to use the drug candidate. The idea to start now and move rapidly became a pioneer in coping up with the pandemic constructing SARS-CoV-2 \cite{1,2} as with passing time different strains of Human coronaviruses (HCoVs) have started coming up. 2019n-CoV is a part of the \textit{Betacoronaviruses} genus similar to the Severe Acute Respiratory Syndrome Human coronavirus (SARS-HCoVs) and the Middle-East Respiratory Syndrome Human coronavirus (MERS-HCoVs) attempted to treat by Ebola antiviral Remdesivir, which failed for two major reasons; primarily it works only if given at an early stage and on the other side, the drug is expensive, whereas anti-HIV agent Lopinavir and ritonavir were completely inactive \cite{3}. Chloroquine (CQ) and Hydrochloroquine (HCQ) showed some hope as being a basic unit; it dropped the pH in endosomes restricting the entry of viruses changing the glycosylation of ACE2 receptor and spike protein \cite{4,5}, although the entry mechanism for novel SARS-CoV-2, structured with a Spike, Nucleocapsid, Matrix, and Envelope, and non-structural proteins such as RNA dependent RNA polymerase which is a crucial enzyme in the life cycle of RNA viruses, is still not clear (Fig. 1) (see Fig. 2). (see Scheme 1)

Understanding this, Liu et al. went for \textit{in vitro} studies and came up with important observations: i) The viral RNA copy inhibited by the action of both CQ and HCQ, and ii) the action of CQ and HCQ, blocked the transport of novel coronaviruses from early endosomes (EEs) to endolysosomes (ELs), which is a key step in the release of viral genomes and iii) the cytokines were high in the plasma of infected patients making important observations: i) The viral RNA copy inhibited by the action of both CQ and HCQ, and ii) the action of CQ and HCQ, blocked the transport of novel coronaviruses from early endosomes (EEs) to endolysosomes (ELs), which is a key step in the release of viral genomes and iii) the cytokines were high in the plasma of infected patients making the importance of cytokines with the disease and hydrochloroquine can significantly reduce the production of cytokines as well, making chloroquine and hydrochloroquine an imperative precursor in treatment \cite{6} which is even supported by molecular modelling study from Fantini et al.

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In 2010, the role of Zn$^{2+}$ in inhibiting RNA polymerase in coronavirus and arterivirus was established by in-vitro study [8] and amazingly Chloroquine appeared as an astounding zinc ionophore [9]. However, in the end, HCQ failed at its safe dosage amount; which is around 6–6.5 mg/kg per day and is not up to the mark. Repurposing of ‘existing’ drugs to treat coronavirus is an attractive way of designing drugs from risk-free agents, with promising lower overall improvement costs and petite growth timelines [10].
Table 1
Molecular docking study of Chloroquine derivatives with Mpro of SARS-CoV-2, taking chloroquine (CQ) and Ribavirin (RB) as a reference.

| S. No. | Name               | Structures            | HEX (Rt shape + dars+3) (kJ/mol) | ΔG (kcal/mol) | Swiss Dock Fitness (kcal/mol) |
|-------|--------------------|-----------------------|----------------------------------|---------------|--------------------------------|
| 1     | CQ (Chloroquine)   | ![CQ](image)          | -315.65                          | -7.99         | -1162.43                       |
| 2     | RB (Ribavirin)     | ![RB](image)          | -340.12                          | -6.46         | -1076.71                       |
| 3     | CQ-N3 (Chloroquine-Azide) | ![CQ-N3](image)       | -277.44                          | -8.26         | -1152.74                       |
| 4     | CQ-TrIme (Chloroquinetriimine) | ![CQ-TrIme](image)   | -310.5                           | -9.62         | -1110.68                       |
| 5     | CQ-TrOne (Chloroquinethiazole) | ![CQ-TrOne](image) | -356.58                          | -9.66         | -1219.89                       |
| 6     | CQ-TrAdn (Chloroquinetriadenine) | ![CQ-TrAdn](image) | -315.65                          | -9.60         | -1161.34                       |
| 7     | CQ-TrPyr (Chloroquinetripyridine) | ![CQ-TrPyr](image) | -340.12                          | -9.21         | -1142.83                       |

Scheme 1. Reproposing route for the synthesis of CQ-TrOne.
Sharpless defined Click Chemistry [11] as a stronghold owing to its greener nature (modest reaction conditions, readily and easily available starting materials and reagents, use of no solvent, a benign solvent (such as water), or one that is easily removed, simple product isolation). This powerful toolbox facilitates selective C-Hetero-C bond formation in environment friendly and cost-viable route [12–15], this nonchromatographic high yielding method repurposes drugs by creating a click link between two different bioactive agents. We have made attempt to elucidate the binding affinity of HCQ modified with click ready azide conjugated with existing pharmacophores with a triazole ring. The triazole moiety formed here is not only acting as a linker but it may act as a basic unit (pKa = 9.3), elevating the pH level in eukaryote cells inhibiting the entry of spiked virus as well as the clicked triazole link has also shown medicinal & biological applications [16–18], further the purpose is to explore the potency of click chemistry in drug development for COVID-19 treatment. The novelty of this work included the application of click chemistry and its impact for enhancement of drug binding efficacy and potency to combat the Mpro. Significantly, these results will also be applicable to other strain of virus, since we have employed the widely accepted target model which has high similarity with multiple variants of target Mpro.

Viral RNA replication to yield functional viral protein is a decisive regulatory step and Mpro is the main protease enzyme. Depending on this fact, researchers tried to investigate the action of compounds on this particular protease, inhibiting the replication of the virus. The reported computer-aided assay of HCQ, CQ [19], and Artemisinin analogs [20] and subsequent inhibition of protease enzyme Mpro in terms of binding energy motivated us to use this technique. Molecular dynamics simulation, a tool to analyze the physical movement of atoms and molecules, elucidate the free energy change of the drug upon binding with the target protein through the root mean square deviation (RMSD), the heat-map of decomposing, and other relevant parameters [21,22]. In addition, molecular docking lends a hand in recognizing the mechanistic interaction of the drug with the target receptor [23,24]. Mechanistically, molecular modeling studies are widely employed and proved to be very efficient to understand the background and preliminary efficacy of drugs out of large

Table 2
Pharmacological properties analysis of CQ-TrOne, Chloroquine, Ribavirin, and Favipiravir drugs.

| Drug         | Water solubility (Log mol/L) | Caco-2 permeability (Log Papp in 10-6 cm/s) | Intestinal absorption (human) % Absorbed | Skin Permeability Log Kp | P-glycoprotein substrate (human) (Log L/kg) | Fraction unbound (human) (Fu) | Total Clearance (Log ml/min/kg) |
|--------------|------------------------------|---------------------------------------------|------------------------------------------|--------------------------|---------------------------------------------|-------------------------------|----------------------------------|
| CQ-TrOne    | -4.67                        | 0.692                                       | 77.532                                   | -2.886                   | No                                          | 0.783                         | 0.142                           | 0.978                           |
| Chloroquine | -4.249                       | 1.624                                       | 89.95                                    | -2.679                   | No                                          | 1.332                         | 0.191                           | 1.092                           |
| Ribavirin   | -1.487                       | 0.484                                       | 38.176                                   | -2.746                   | No                                          | 0.102                         | 0.748                           | 0.683                           |
| Favipiravir | -1.556                       | 1.42                                        | 83.471                                   | -2.999                   | No                                          | -0.362                        | 0.696                           | 0.596                           |

Fig. 3. Molecular interaction surface view of drug binding cavity with Mpro enzyme of coronavirus (A) CQ-N3 Mpro enzyme (B) CQ-TrIme Mpro enzyme (C) CQ-TrPyr Mpro enzyme (D) CQ-TrAdn Mpro enzyme (E) CQ-TrOne Mpro enzyme.
datasets [25–27]. To investigate the mode of binding action and binding of drugs with their specific target receptors. Hence, herein details advanced computational assays are employed to study the clicked analogs to acquire the highly competent drug against Mpro of SARS-CoV-2 for further corroboration with investigational lab assay.

2. Material and methods

2.1. SARS-CoV-2 main protease enzyme retrieval and evaluation

To perform the molecular binding analyses of proposed drugs, the main protease enzyme (Mpro) of SARS CoV-2 was used, it has main regulatory role in coronavirus infection, progression, and further regulation. The crystal structure of SARS-CoV-2 main protease was retrieved from the Protein Data Bank (http://www.rcsb.org/) [28] and the structure was energy minimized to avail the confined and stabilized structure. The 3D crystal structure was also assessed for its physicochemical and stereochemical properties using the Ramachandran plot and other physicochemical servers [29,30]. The receptor files were prepared using the Whatif prepdock module for molecular modeling analyses.

2.2. Drug conjugation designing and refinement for molecular modeling analyses

The chloroquine-clicked drug analogs were designed drawn using the Chemdrawn software. Designed conjugates ligands were optimized and energy minimized for molecular docking and molecular dynamics simulations analyses. The drug files (ligands) were assessed and corrected with Ligprep module and further chilarties were optimized.

2.3. Binding analyses of lead conjugates by Molecular docking

To evaluate the binding affinity of proposed drugs with the Mpro enzyme, first molecular docking analyses were conducted. We have employed the HEX 8.0 molecular docking software, which works grounded on a fast Fourier algorithm [29,31]. The shape and dynamics and 3D FFT mode route was opted and further the steps through interface energy with steric shape coordination and the electrostatic potential were investigated. Docking program was set to avail the top 25 drug binding poses with Mpro, which were further analyzed through the drug-protein interaction profilers. Importantly, to strengthen our results we have also used another molecular docking software Swissdock and
molecular contacts, involved non-covalent bonds and binding domains were analyzed using the Ligplot tool.

2.4. Molecular dynamics simulation binding analysis of chloroquine-clicked ligand

The molecular dynamics simulation using the Amber16 program with a build force field (ff14SB and gaff force) and to evaluate the binding mechanism of chloroquine-clicked analogs with the Mpro enzyme. Amber-16 program was used for MD simulation in a set parameter of 1 ns. In explicit water mode, using the steepest descent step method internal approach simulation was initiated \[32\]. The all-atoms MD simulation in explicit solvent water system was run for 1 ns. The binding trajectory was minimized and optimized through the Amber16 force field by employing the 2000 steps steepest descent method, and 3000 steps conjugated gradient method. The dynamics arc was evaluated to read the RMSF, RMSD, and Radius of gyration. Besides, the binding free energy assessment of the complex trajectory was achieved through the MM/PB (GB) SA binding dynamism calculations \[33\].

\[
\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i^m - x_i^t)^2 + (y_i^m - y_i^t)^2 + (z_i^m - z_i^t)^2}
\]

\[
\text{RMSF} = \sqrt{\frac{1}{T} \sum_{i=1}^{T} (x_i - \bar{X})^2}
\]

Where \(N\) denotes the number of atoms, \(x_i^m, y_i^m, z_i^m\) is the Cartesian coordinates of the initial structure, and \(x_i^t, y_i^t, z_i^t\) are the Cartesian coordinates of trajectory at frame \(t\). The number of trajectory frames is denoted by \(T\) and \(\bar{X}\) is the time-averaged position.

\[
\Delta G_{\text{bind}} = \Delta H - T \Delta S
\]

\[
\Delta H = \Delta E_{\text{MM}} + \Delta G_{\text{soln}}
\]

\[
\Delta E_{\text{MM}} = \Delta E_{\text{internal}} + \Delta E_{\text{elec}} + \Delta E_{\text{vdw}}
\]

\[
\Delta G_{\text{soln}} = \Delta G_{\text{GB}} + \Delta G_{\text{SA}}
\]

Where, \(\Delta E_{\text{MM}}\) is denoted as the variation of the MM energy in the gas.
coronavirus main protease (Mpro) was used as a potential target for repurposing therapeutics as it plays essential roles in the induction and life cycle progression of coronavirus. The 3D crystal structure of Mpro has been reported to premeditate through the X-ray diffraction technique in the expression system Escherichia coli. from Bat SARS-like coronavirus. Prior to perform, the molecular modelling studies, the 3D structure of Mpro was examined through the Ramachandran plot along with its 3D structure local quality estimations. The structural sequence plot assessment depicted the > 90.6% residues lie in the favorable region, 9.1% residues lie in the optimal region and only 0.4% residues lie in the outlier region of the Ramachandran plot. Moreover, high structural eminence with superior homology showed the pragmatic in local quality assessment of indigenous structures of Mpro. Also, the Verify3D server affirmed the optimal quality of structure by > 94% of Residues possessing the 3D/1D profiles of native structures. These outcomes gave the refined drug target Mpro for coronavirus for molecular modeling studies.

3.2. Molecular binding docking analyses of chloroquine derivatives with Mpro

The molecular docking assays were employed to study the binding affinity of repurposed drugs. Hex 8.0 module was used, which worked based on a fast Fourier transform by SPF shape density correlations algorithm. Docking assays were conducted at the similar binding sites of molecule N3, a known inhibitor of the Mpro enzyme by analyzing the drug-protein complex. To validate the results, we have compared our target drugs with known Chloroquine and Ribavirin (RB) drugs (chloroquine-conjugates) and monitored through molecular docking. Now, the actual quest was to find whether the conjugates showed a strong binding affinity than the known drug in trials. The favorable outcomes were observed for Chloroquinetrithiazole (CQ-TrOne) with a high binding energy score of −356.58 kJ/mol by Hex 8.0 with shape and conformation docking parameters, among all conjugates and reference known drugs.

Importantly, redocking results by SwissDock analysis of CQ-TrOne supported the obtained data with a high docking score (Table 1). From chloroquine to chloroquine-N3 the docking score dropped by 38.21 kJ/mol defining the role of the substituent in the binding activity. It describes the role of click triazole in enhanced binding affinity towards the Mpro and possible role in COVID-19 treatment. Interestingly, the redocking outcome was apposite and the efficiency of CQ-TrOne was again optimal by molecular binding ΔG energy score of −9.66 kcal/mol, also better than other repurposed candidates (Fig. 3). With these unambiguous results, it can be concluded that CQ-TrOne has a high binding potential with the target Mpro and can be considered as valuable therapeutics for COVID-19 (see Table 2).

3.3. Binding domain analyses of Chloroquinetrithiazole (CQ-TrOne) with Mpro

Elemental information of contact of CQ-TrOne was acquainted with the interaction model of ligands for inflexible binding to target Mpro. The three-dimensional complex structures of Mpro-drug were first analyzed by Chimera modelling application, which depicted the presence of plausible three grooves in SARS-CoV-2 protease, first at 1–98 residues, second at 99–198, and third at 199–306. Primarily CQ-TrOne was founded to bind at binding groove-2 and the coils extended to the other two domains. The conjugate forms two hydrogen bonds at 5A-LYS of bond length 2.42 Å, 131A-ARG- bond length 2.30 Å, and multiple hydrophobic interactions around 137A-LYS-3.99 Å, 286A-LEU-3.04 Å, 286A-LEU-2.77 Å, and one π- cationic interaction: 131A-ARG-4.05 Å. Along with this binding extendee to form the negative protein salt bridge interaction at 290A-GLU-5.03 Å (Fig. 4). These molecular contact analyses revealed the strong binding of lead drug conjugate with catalytic domain-2 and domain-3, results were taken further to study through MD simulation to evaluate the binding statistics.

2.5. Pharmacological analyses of CQ-TrOne and lead compounds

Furthermore, pharmacological assessment of lead drugs, along with known drugs was performed. Pharmacokinetic and pharmacodynamics properties for Chloroquine modified CQ-clicked analogs were studied using SwissADME, pKCSM, ACD/I-Lab, and Molinspiration servers. Many pharmacological parameters including the Lipinski rule of five, drug-likeness, and ADMET (Absorption, metabolism, excretion, and toxicity) were examined.
Fig. 7. Residue wise loading for (A) PC1, (B) PC2, (C) PC3 based on molecular dynamics trajectories from the internal mode. The secondary structures schematics are added to the top and bottom. (D) The dynamical residue cross-correlation map. (E) B-factors atomic fluctuations analysis of each residue of Mpro enzyme of SARS-CoV-2, high fluctuations observed at residues 280–305 amino acids.

Fig. 8. Internal mode PCA agglomerations analysis of conformation by MD (A and B). The trajectory frame depicted from PCA data from red to white to blue in the simulation run and improved by shifting the conformation from red to black color. The trajectory metaphors are alienated into two different agglomerations of the color red and black through the top three PC spaces, (C and D) the superimposed conformation of two agglomerations.
The binding susceptibility of CQ-TrOne to the main protease enzyme was computed through MD simulation analyses to examine the fluctuation breakdown and conformational stabilized binding constancy of the complex. Amber16 program (built with force fields) was engrossed to reconnoiter the CQ-TrOne receptor conformational binding modes through MD simulation run for 1 ns. In the explicit water state, the system molecules were solvated in the octahedron box, and all atoms were allowed to be in a relaxed state. The minimization process was performed utilizing Amber 16, using 2000 steps steepest descent method and 3000 steps conjugated gradient Method. The procured trajectories during the MD simulation run were used for RMSD analysis to gain insight into the structural conformations coverage and the equilibrated stable binding of CQ-TrOne with the target Mpro. The RMSD analysis depicted the range of complex system minimal fluctuation with 0.1–0.5 Å for ligand whereas the receptor showed a fluctuation in a range of 1.2–2.0 Å (Fig. 5A). In addition, the receptor-ligand duo was daged through RMSD vs. density histogram plots, which showed receptor Mpro enzyme and the drug candidate CQ-TrOne were normally distributed around 1.4 and 2.5 Å (Fig. 5E and F). The RMSD values to the MD simulation run suggested the stabilized interaction and ideal for further detailed analysis. Further, to examine every residue dynamics of the target receptor, a RMSF plot was studied. It depicted the affirmative and indigenous fluctuation of the sequence with a peak around 150 residues and in a range from 280 to 305 residues. The fluctuations in the above-stated statement can be reasonably expected due to the proximal binding of the drug candidate with the target protein, it also explains that it is binding in coils and beta sheets of the Mpro enzyme, which has been espied molecular binding exploration (Fig. 5C). With the aim of realizing the compactness and structural activity of macromolecule Mpro, the radii of gyration (Rg) of resulting trajectories were calculated as Rg varies depending upon the folding state of the protein complex. The data indicated fluctuation in an assortment of 21.7–22.2 Å, and this marginal flux showed the stability of the Mpro to CQ-TrOne while binding (Fig. 5B) (see Fig. 6).

As Q of trajectories defines the transition states for all proteins subjected to conformational changes with a free energy barrier, these native contacts were also considered for analysis. The assimilated domino effect describes that the native contacts were preferred by the coarse-grained theoretical models. And, Q value above 96% illustrated the conformation dynamics of the macromolecule system along with the energetics of the bound ligand (Fig. 5D). On the foundation of these substantial outcomes through RMSD, RMSF, Rg, and Q values recommended the stability of the Mpro enzyme–CQ-TrOne complex, that CQ-TrOne can be a
potential drug candidate against SARS-CoV-2.

Principal component and dynamic residues cross-correlation analysis. Principal component analysis (PCA) was executed to get insight into the conformational inconsistencies within the experimental structures and nature of clusters. Conformation frames were divided into two clusters in red and black colors. The rotational calculation of the residues for three different conformations PC1, PC2, and PC3 were obtained through the normal mode molecular dynamics, which signposted that the PCA results were optimal. The conformational analysis revealed that the PC1 cluster has the maximum variability of 31.49% in terms of internal gesticulations of proteins of the Mpro enzyme conformation with the binding of CQ-TrOne, while PC2 and PC3 showed 19.63% & 6.73% the remaining variability of all the atomic motions through the key components respectively (Fig. 7).

We observed that the conformations changed from the black cluster to the red cluster and supplementary recuperated from the first cluster blue to white to red during the molecular dynamics time. Additionally, the two different conformations from the two clusters were allied and superimposed, shown in the plot (Fig. 8). To add on, a dynamical residue cross-correlation map was computed, which designated a high pairwise correlation at binding residues 280–305, close to binding groove-3 of the Mpro enzyme (Fig. 7D).

The atomic fluctuations of the interrelating system were allied with one another; therefore, the correlation was scrutinized by examining the extent of pairwise cross-correlation coefficients. The graph illustrated the correlated residues (>0.8) shown in blue, and anticorrelated residues (<−0.4) shown in red. The light pink and light blue color indicate the high correlated coefficients and anti-correlated coefficients of the pairwise residues of Mpro. To further authenticate the precision of calculations, the normal mode analysis derived atomic fluctuation of Mpro enzyme residues were studied.

The B-factor calculated atomic fluctuations were found confined and stable in correlation to the above results. The obtained plot for b-factor vs residues position of Mpro depicted normalized fluctuation throughout the plot with peaks in the region of 150 and 280–305 residues in the last binding groove when CQ-TrOne was conjugated (Fig. 7E). Effect consequences exhibited the potential binding capacity of CQ-TrOne with the correlating MD calculations.

### 3.4. MM/PB (GB) SA binding energy calculations

To determine the binding energy contribution in the complex trajectory of CQ-TrOne and Mpro of COVID-19, MM/PB (GB) surface area (SA) calculations were performed. In terms of binding energy, the complex was found favorable as the calculated deltaPB and deltaGB were −21.19 kcal/mol and −23.18 kcal/mol respectively. A high van der Waals energy or hydrophobic interaction −58.05 kcal/mol and electrostatic energy −47.50 kcal/mol were obtained from the electrostatic energy distribution in MM energy, which was deliberated as a noteworthy calculation to evaluate binding stability. The polar contribution (GBSOL) and nonpolar (PBSOL) of the solvation of the system were obtained to be −62.43 and −64.43 kcal/mol. Additionally, the total gas-phase energy added a vital contribution to the binding energy with a score of −105.55 kcal/mol of the complex system. Alongside binding energies, H-bond involvements were also evaluated to have information about the dynamic equilibration of. A high number of H-bonding demonstrated the binding stability of CQ-TrOne with the target Mpro enzyme. (Fig. 9A) Besides, to approximate the influence of each residue of the Mpro enzyme in interaction with CQ-TrOne, we executed the per residue-free energy disintegration analysis. The electrostatic free energy decomposition results by

| Drug          | AMES toxicity | Max. tolerated dose (human) (Log mg/kg/day) | hERG I inhibitor | Oral Rat Acute Toxicity (LO50) (mol/kg) | Oral Rat Chronic Toxicity (LOAEL) (Log mg/kg bw/day) | Skin Sensitization | T. Pyriformis Toxicity (Log ug/L) | Minnow toxicity (Log mM) |
|---------------|---------------|---------------------------------------------|------------------|----------------------------------------|---------------------------------------------------|-------------------|---------------------------------|--------------------------|
| CQ-TrOne      | No            | −0.013                                      | No               | 2.626                                  | 1.295                                             | No                | 0.357                           | 0.835                    |
| Chloroquine   | Yes           | −0.167                                      | No               | 2.85                                   | 1.026                                             | No                | 1.558                           | 0.747                    |
| Ribavirin     | No            | 0.878                                       | No               | 1.965                                  | 3.085                                             | No                | 0.285                           | 4.1                      |
| Favipiravir   | No            | 0.999                                       | No               | 2.309                                  | 0.738                                             | No                | 0.187                           | 2.267                    |

Table 3

Toxicity profile of CQ-TrOne, Chloroquine, Ribavirin, and Favipiravir drugs.

![Fig. 10. The Computationally optimized structure of CQ-TrOne on B3LYP/631+G(d,p) and Quantum chemical parameters of CQ-TrOne, CQ, Ribavirin, Favipiravir.](image-url)
MM force field (TELE) exhibited the high energy of the residues of Mpro and contributed significantly to the total binding energy of the Mpro complex with the ligand (Fig. 9C).

Furthermore, hydrophobic interactions, large van der Waals forces (TVDW), and final estimated binding energy (TGBTOT) were also computed in the region of 114–290 residues (coils and beta sheets) of Mpro. This supports the CQ-TrOne backbone binding in binding groove – 2 and 3 of the Mpro enzyme. By the assessed energy (TGBTOT) contribution, the decomposition for the residue was organized and shown by the bar diagram and heat-map for scaling up from large to small (Fig. 9B, D). For binding grooves of the Mpro enzyme, the deliberate energies were centered and disseminated in the row direction red color to blue from –1.5 kcal/mol to 1.5 kcal/mol. These analyses established the prolonged and robust binding of chloroquintrithiazione (CQ-TrOne) with target Mpro of coronavirus and involvement of potential binding energies.

Theoretical analysis of stability and strength of the lead conjugate.

The computation of electronic constraints of a molecule is a modern tool for the elucidation and the extrapolation of its reactivity under a specified condition. The frontier orbitals: HOMO and LUMO described as highest occupied molecular orbital and lowest unoccupied molecular orbital respectively are of myriad prominence in the assessment of the reactivity of a species as they correspondingly delineate its electron acceptance and donation ability. Further, ΔE = ELUMO – EHOMO is a significant factor which processes the kinetic permanence and the intramolecular charge relocation; it has been comprehensively used to enlighten biological activity outcomes. It has been believed that a large energy gap defines the chemical stability of a molecule in a species. Here we computed the HOMO-LUMO gap of CQ-TrOne with DFT/B3LYP/6-31+G(d,p) level of theory to understand quantum chemical parameters associated. The computed result showed a 3.93 eV of HOMO-LUMO gap in compound CQ-TrOne which declares that the molecule is kinetically stable. In biological assessments, several researches have revealed that the placement of LUMO orbitals along with their energy can be influential. Moreover, many other quantum chemical set of parameters can be evaluated to measure the reactivity profile of the molecule. The hardness of a compound is a vital model to assess the reactivity and stability of a species; it outlines the confrontation toward polarization or distortion of the electron density dispersal of a chemical system in an electric field. This notion can be communicated as chemical hardness (η) and softness (σ). The electronegativity (χ) is a degree of a molecule to draw electrons, while the chemical potential (μ) analyses the inclination of an electron to emit from the molecule. The capability of a species to acquire electrons is enumerated by the electrophilicity index. As a result, high values η characterize stable compounds with low reactivity; on the contrary high values for σ, χ, μ, and electrophilicity (ω) signify less stable hence more reactive species. All of the above-mentioned data were calculated from the computationally calculated HOMO-LUMO energies and equated using the mentioned equations (Fig. 10) describes the stability of the molecule CQ-TrOne as possible as other available drugs [34].

3.5. Pharmacological analysis of CQ-TrOne-Mpro

The Pharmacological analysis of CQ-TrOne was conducted to evaluate aptness and sustainability for repurposing. CQ-TrOne with a high molecular weight 516.06 g/mol followed the Lipinski rule of five for high druggability with no violations except bit high molecular weight close to 500 Da (516.6 Da). It was discovered to have high oral absorptions and skin permeability log Kp – 2.886. The primary consideration for optimal drug development and compounds is considered to be high intestine absorption where <30% absorption value is considered to be poorly absorbed. Along with this, CQ-TrOne showed the high plasma concentration by volume of distribution (Vdss) Log L/kg 0.783, with no substrate competence for P-glycoprotein to be directly excreted out. The unbound fraction was calculated to be 0.142 fraction unit. Moreover, the maximum recommended daily dose capability for humans was estimated to be ~0.013 Log mg/kg/day (Table 3). In addition to pharmacodynamics studies, the toxicity profile (side effects) of CQ-TrOne were measured (Table 3). Toxicity profiling depicted, CQ-TrOne is of non-carcinogenic in nature by Ames test. The acute toxicity lethal dosage value (LD50) was calculated to be 2.626 mol/kg, which indicated the concentration that may lead to 50% death of animals in the study. The oral rat chronic toxicity score was studied to 1.295 log mg/kg BW/ day and no side effects with T. Pyriformis and no skin sensitivity to the humans. Also, apart from CQ-TrOne the pharmacological analyses of chloroquine, ribavirin, and favipiravir drugs were performed, shown in the table. Pharmacology parameters calculation and comparative evaluation showed that CQ-TrOne has high druggability, drug-likeness, and potency for repurposing therapeutics inconsideration to chloroquine, ribavirin, and favipiravir drugs.

3.6. Synthetic approach

For repurposing the drugs, we chose non-chromatographic and regioselective click reaction. CQ-based click products are well known in the field of bio for their aforementioned unique properties. We followed Yu et al. reported pathway [35] accompanied by Subhashini et al. [36] and proposed the synthesis of CQ-TrOne. Diabetic drug A and propargyl bromide B were treated in a mild aprotic alkaline medium to generate C with nucleophilic substitution reaction. Then, the prepared CQ-N3 and alkyne linked thiazolidine-2,4-dione C were treated under Cu(II) medium to generate the CQ-clicked repurposed drug.

4. Conclusions

The rapid recognition of effective intrusion against SARS-CoV-2 is a major concern of present times. From the accessible information on their safety profiles, and effectiveness against meticulously related coronaviruses, repurposing existing drugs is a potentially imperative near-term approach to confront COVID-19. Herein this study, we report a potential drug CQ-TrOne as a repurposed therapeutic against the target Mpro of novel coronavirus-2019. Through the molecular docking analysis and molecular dynamics simulation analyses, we report the binding mechanism of CQ-TrOne, with its strong binding affinity in regulatory binding groove- 2 and 3 of the Mpro enzyme. The high efficiency of lead drug and stable binding with Mpro results was well supported with the free binding energy with a high potency of CQ-TrOne in comparison to the drugs including Chloroquine and Ribavirin. The robust binding and stabilized conformational changes of Mpro with the lead drug were in well correlation by RMSD, RMSF, and Rg analyses. Moreover, hydrophobic interactions, H-bonding and free energies calculated through the MM-force field significantly denoted the strong binding capacity of CQ-TrOne. The dynamics residue cross-correlating map quantified the high pairwise correlation in beta-sheets; PCA analysis predicted the nature of cluster conformational variance with a static high number of hydrogen bonds. DFT study unambiguously made a statement on the stability. In addition, the pharmacological and toxicological profiles suggested the potency of the proposed drug and reasoned the importance of CQ-TrOne as SARS-CoV-2 therapeutics.

Author contributions

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Satyaki Chatterjee: Data curation, Software, Formal analysis,
Methodology, Writing - review & editing, Writing - original draft. Neeraj Kumar: Software, Data curation, Methodology, Formal analysis, Writing - review & editing. Hitesh Sehrawat: Data curation, Formal analysis. Nisha Yadav: Data curation, Writing - review & editing, Formal analysis. Vivek Mishra: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Funding acquisition, Supervision, Validation, Visualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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