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SHORT COMMUNICATION

Structure-guided discovery approach identifies potential lead compounds targeting M^{pro} of SARS-CoV-2

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Abstract The ongoing coronavirus disease 19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become fatal for the world with affected population crossing over 25 million in more than 217 countries, consequently declared a global pandemic by the World Health Organization. Unfortunately, neither specific prophylactic or therapeutic drugs nor vaccines are available. To address the unmet medical needs, we explored a strategy identifying new compounds targeting the main protease (M^{pro}) of SARS-CoV-2. Targeting the SARS-CoV-2 M^{pro} crystal structure (PDB ID: 6LU7) a combination of in silico screening, molecular docking, and dynamic approaches, a set of 5000 compounds of the ZINC database were screened. As a result, we identified and ranked the top 20 compounds based on the scores of ligand-interaction, their drug-likeness properties, and their predicted antiviral efficacies. The prominent drug-like and potent inhibitory compounds are 2-[2-(2-aminoacetyl) aminoacetyl] amino-3-(4-hydroxyphenyl)-propanamide (ZINC000004762511), 6′-fluoroaristeromycin (ZINC000001483267) and cyclo (L-histidyl-L-histidyl) (ZINC000005116916) scaffolds. Further in vitro and in vivo validations are required to demonstrate anti-SARS-CoV-2 activities.

Keywords COVID-19 · SARS-CoV-2 · Therapy · Protease · Zinc · Inhibitors

In the late December 2019, hospitals in Wuhan, Hubei, China reported cases of unexplained pneumonia, later named the coronavirus disease 2019 (COVID-19). The responsible causative agent for COVID-19 was identified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. As of now, according to the World Health Organization (WHO) around 25 million laboratory-confirmed cases have been reported worldwide and more than 800,000 of deaths resulting in a fatality rate of 3.2% [2].

European countries and the United States of America have become the epicenters of the SARS-CoV-2 outbreak [3]. In the majority of cases, COVID-19 is either asymptomatic or causing only minor clinical symptoms; however, in patients with medical preconditions, the fatality rates are

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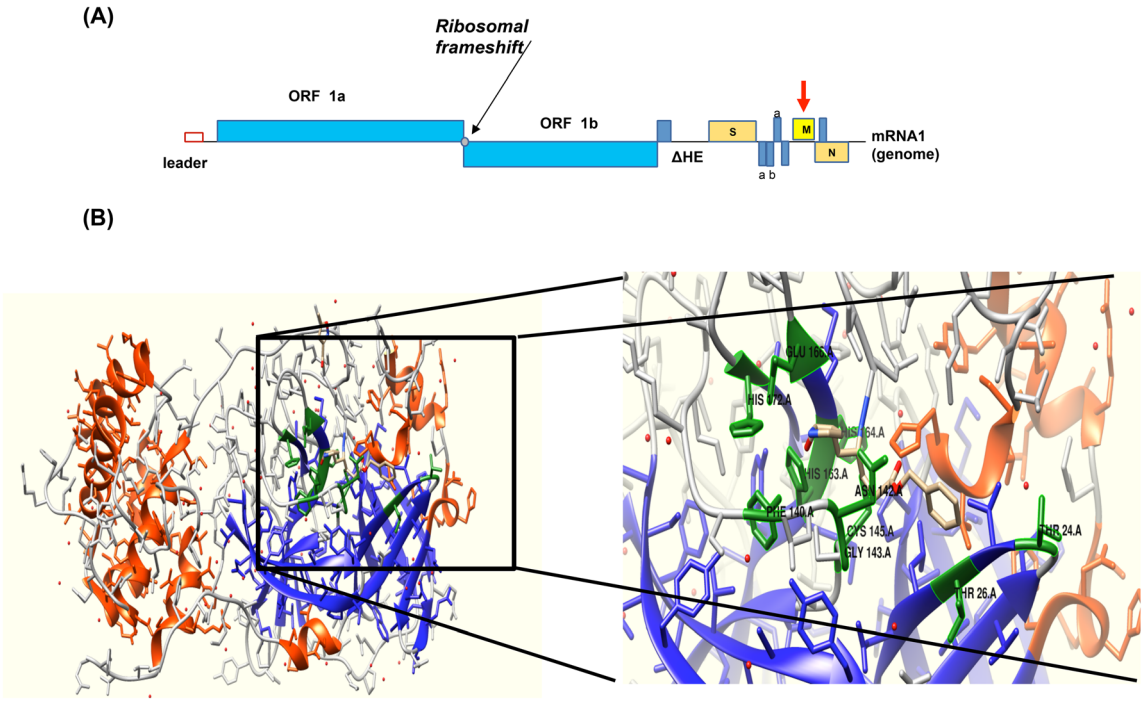

Fig. 1 Structure of SARS-CoV-2 main protease M^{pro}. **a** Schematic presentation of the SARS-CoV-2 genome organization. **b** The 3D of M^{pro} structure. The strand structure is represented in blue and the helix is red. The predicted binding site is represented in green

alarming [4, 5]. As of now, no success is seen in the front of having a specific therapeutic drug or prophylactic vaccine. The current approach to treat COVID-19 relies on the the use of hydroxychloroquine, chloroquine, azithromycin, remdesivir, lopinavir-ritonavir, favipiravir, ribavirin, interferon, and convalescent plasma [6–11]. To quickly address the unmet medical needs, in silico approaches can accelerate drug discovery and development, and can be a complementary method for the classical screening and identification of specific drugs against COVID-19 [12, 13]. Taking the advantage of the main protease (M^{pro}) structure that became available recently [14], we carried out a virtual in silico screening of nearly 5000 ZINC compound database to identify new inhibitors targeting the SARS-CoV-2.

The M^{pro} (PDB ID: 6LU7) structure of SARS-CoV-2 was obtained from protein data bank (PDB) (https://www.rcsb.org/) [14]. The active site prediction was performed using Computed Atlas for Surface Topography of Proteins (CASTp) (https://sts.bioe.uic.edu/castp/index.html?2011).

The ZINC database [15] was used, and a set of 5000 public-available compounds were downloaded in.mol2 format. The predicted active sites from CASTp were then used for molecular simulations, which were performed by using MtiOpenScreen [16], a virtual screening online server-based on AutoDock Vina. The grid box was created based on predicted active site residues using MtiOpenScreen [16] option that allows selecting the docking grid based on specific residues.

To explore more the interaction between our target and top-ranked ligands, we used Ligplot+ [17]. This tool allows analyzing the ligand-receptor interactions by plotting hydrogen bonding and hydrophobic interactions.

Absorption, Distribution, Metabolism, and Excretion (also known as ADME) evaluation has been applied to investigate the pharmacological activity of the compound. The characterization of ADME and Toxicity was performed based on the SWISS ADME server (https://www.swissadme.ch). The rules of Lipinski, Ghose, and Veber applied to predict drug-likeness following molecular weight, LogP, HBD, and number of HPA parameters.

The M^{pro} plays a pivotal role in the replication and transcription of SARS-CoV-2 and thus becomes a apposite drug target [14]. It forms a homodimer of two chains A and B, which are complexed to a native ligand (Fig. 1a). The active site pockets resulted from CASTp has shown that potential site to be localized between THR24, 26, PHE140, ASN142, GLY143, CYS145, HIS163, 164, 172, and GLU166 residues positions in the chain A of the SARS-CoV-2 (Fig. 1b).

The 5000 compounds from the ZINC database were docked using Autodock Vina. Table 1 and supplementary table 1 show the top 20 compounds with binding energies ranging from − 7 to − 6 kcal/mol of chain A active site of M^{pro} (Supplementary figure 1).

The docking simulations showed, by a ranking of binding energies score, that compound 2-[2-(2-

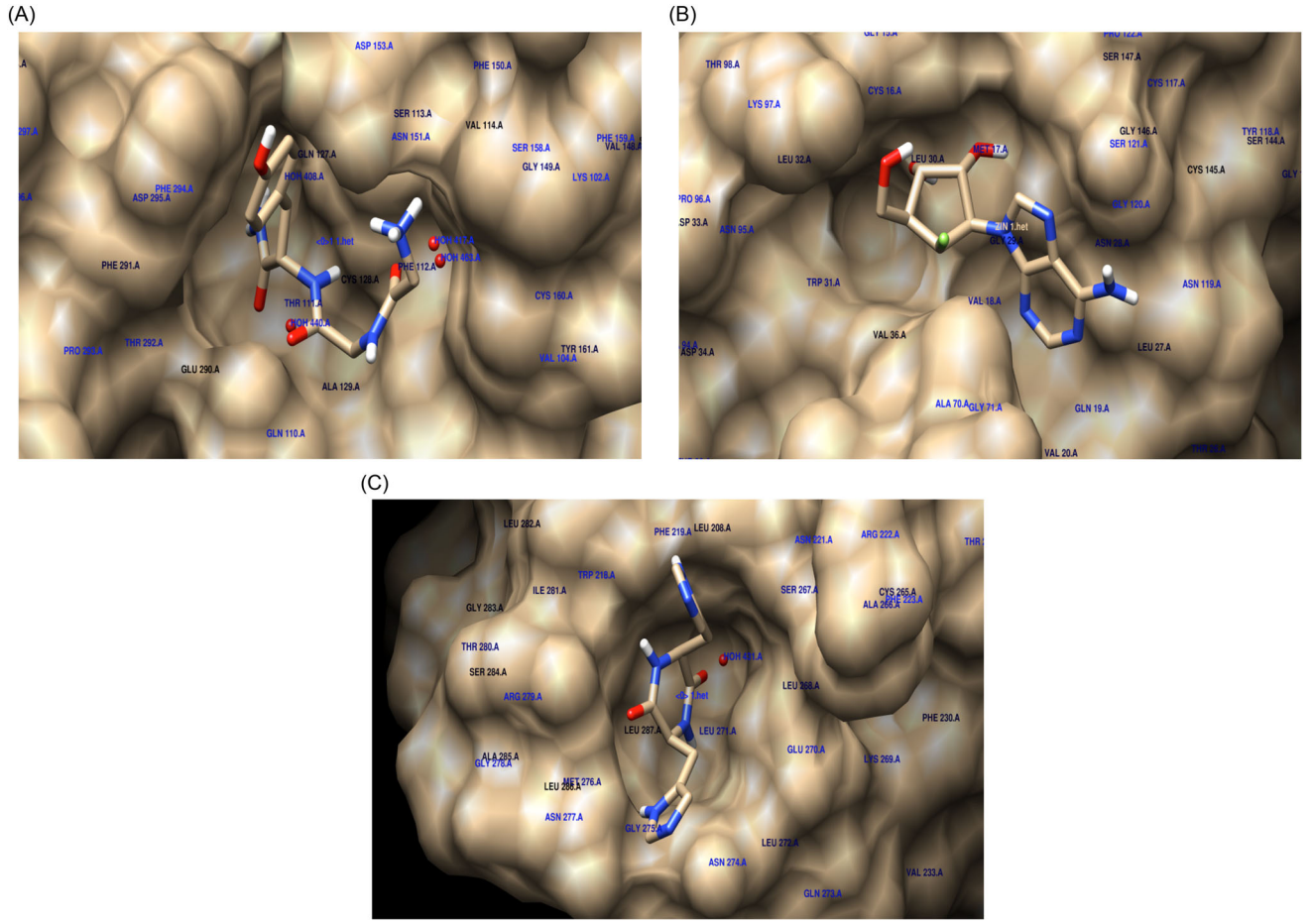

Fig. 2 Docking simulations. The SARS-CoV-2 M^{pro} structure is represented in a surface format with a label of each residue interacting in the zone with the ligand. **a** A complex of the 2-[2-(2-aminoacetyl) aminoacetyl]amino-3-(4-hydroxyphenyl)-propanamide compound with the structure of main protease M^{pro} of the SARS-CoV-2. **b** ZINC000001483267 compound. **c** ZINC000005116916 compound. Amino acids labels are shown in blue and ligand atoms are shown in red, green and blue

aminoacetyl)aminoacetyl]amino-3-(4-hydroxyphenyl)–propanamide (ZINC000004762511) has the best predicted binding energy of −7 kcal/mol. The interaction between the top-ranked hits, according to its docking score and SARS-CoV-2 M^{pro}, has been explored using ligplot+ [16]. The data showed that ZINC000004762511 has Asn151, Thr111, Asp153, Gln110, and Ser158 residues interact with ligand through hydrogen bonding, and Ile106, Thr292, Phe294, and Val104 have a hydrophobic binding (Figs. 2a, 3a). The ZINC000004762511 compound is known as glycylglycyl–L–tyrosinamide, and until to date has not yet been described to have antiviral efficacies.

Moreover, we found that the 6′-fluoroaristeromycin (ZINC000001483267) has a score of − 7 kcal/mol and ligand-interaction show that this compound has Asp289, Glu288, Lys5, Lys137, and Arg131 as hydrogen bonding residues and Thr198, Asp197, Thr199, and Glu290 residues forming hydrophobic binding) (Figs. 2b, 3a). Important to note, it was reported that this compound is an inhibitor of the S-adenosylhomocysteine hydrolase [18].

Furthermore, our data demonstrated that the ZINC000005116916 compound has a lower binding energy of − 6.1 kcal/mol (Figs. 2c, 3c). It interacts through hydrophobic binding with Trp218, Leu220, Asn277, and Arg279 residues. In addition, hydrophobic binding interactions were formed by Leu271, Glu270, Asn274, and Phe219 residues. Furthermore, this compound is known as cyclo(L-histidyl-L-histidyl) and is a part of cyclic peptides that are reported to have a worthy biological function pertinent for the treatment of cardiovascular-related disease, cancer, and infectious diseases [19, 20]. In contrast, regarding other ranked compounds, no available data was published so far.

Based on our computational strategy, the pharmacokinetic properties and drug-likeness of the top 20 ranked scoring molecules that show the potential of M^{pro} inhibitors of the SARS-CoV-2 are shown in supplementary table 1. As per the pharmacokinetic properties, most of the compounds showed low gastrointestinal absorption except six compounds that exhibit higher absorption. These

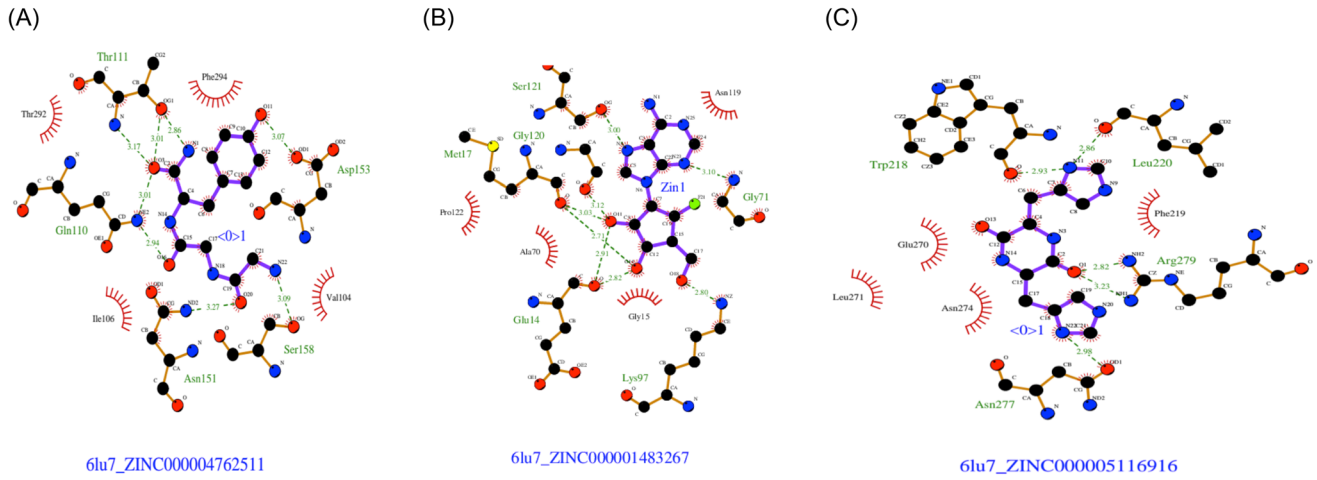

Fig. 3 Main protease M^{pro} of the SARS-CoV-2-ligand interactions with ranked compounds. **a** ZINC000004762511 compound. **b** ZINC000001483267 compound. **c** ZINC000005116916 compound. Residues colored in purple showing hydrogen bonding with residues. Green dash lines represent the hydrogen bond interactions. Green, red, blue, and black are highlighted atoms of ligan

compounds didn't show blood–brain barrier (BBB) penetrability and no inhibition to (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) except one compounds (ZINC000005116916). The drug-likeness forecast following the selected Lipinski, Ghose, and Veber rules and bioavailability scores (Supplementary Table 2), the results of our data recommend that majority of the compounds have no PAIN (pan-assay interference compounds) alerts; in other terms, none of the analyzed hits has reported having false-positive results in high-throughput screenings, and only three compounds have Brenk alerts, a filter determined based on a list of fragments to be putatively toxic [21]. In addition, most of the predicted compounds have shown no Lipinski's violations except five compounds (Supplementary Table 2). The prediction of pharmacokinetics and drug-likeness properties remain a powerful step in in silico drug design due to its capacity to orient and to help scientist to make a decision about which compound to test in vitro according to predicted models.

In the absence of FDA-approved therapies, COVID-19 will continue to raise fatality rates. Several studies were initiated to discover new treatments or to repurpose know drugs quickly [9, 11, 22]. However, these treatments are now under clinical trial investigations [7, 23, 24–26]. We applied a computer-aided drug design to identify new SARS-CoV-2 M^{pro} inhibitors, and identified 20 new prospective inhibitors. Further in vitro and in vivo validations are required to show their efficacy as SARS-CoV-2 main protease inhibitors.

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