

DFT, Molecular Docking, and ADME/Tox Screening Investigations of Market-Available Drugs against SARS-CoV-2

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A series of drugs was investigated to determine structural, electronic and pharmacological properties, as well as the molecular affinity for the main protease of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The drugs were submitted to density functional theory calculations to optimize structures and predict binding preferences. The optimized geometries were used in molecular docking simulations. In the docking study, the receiver was considered rigid and the drugs flexible. The Lamarckian genetic algorithm with global search and Pseudo-Solis and Wets with local search were adopted for docking. Absorption, distribution, metabolism, excretion and toxicological properties were obtained from the Pre-ADMET online server. In this series, the antiviral atazanavir showed the potential to inhibit the main protease of SARS-CoV-2, based on the free binding energy, inhibition constant, binding interactions and its favorable pharmacological properties. Therefore, we recommend carrying out further studies with *in vitro* tests and subsequent clinical tests to analyze its effectiveness in the treatment of SARS-CoV-2.

Keywords: drug repurposing, COVID-19, SARS-CoV-2, molecular docking, density functional theory (DFT)

Introduction

An outbreak of hospitalizations due to a possible pneumonia in December 2019 in the city of Wuhan, China is considered to mark the onset of the current coronavirus pandemic.¹ On February 11, 2020, the World Health

Organization (WHO) officially reported that the disease, known as COVID-19 (coronavirus disease 2019), was caused by a new virus, classified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses.^{2,3} The symptoms of COVID-19 have been reported to include a range of clinical and radiological characteristics, in addition to pneumonia.⁴

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The SARS-CoV-2 is a positive, non-segmented ribonucleic acid (RNA) β -coronavirus (subgenus *sarbecovirus*, subfamily Orthocoronavirinae).⁵ It is one of the seven coronaviruses that affects humans. Four of these, the α -coronaviruses HCoV-229E and HCoV-NL63 as well as the β -coronaviruses HCoV-HKU1 and HCoV-OC43 have low pathogenicity and cause mild respiratory symptoms in humans.^{1,5} The other two β -coronaviruses, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV) cause severe and potentially fatal respiratory infections.⁶

As of March 20, 2021, SARS-CoV-2 infected approximately 122,445,668 people worldwide, resulting in a total of 2,703,547 deaths.⁷ Deeply concerned by the alarming levels of spread and severity, the WHO made the assessment that COVID-19 can be characterized as a pandemic.⁸ Currently, there are some treatment alternatives available for severe cases of SARS-CoV-2, such as the use of remdesivir, which was promoted by the Canadian government agency on July 27, 2020, as well as in other countries.⁹ The response of the research community has been vigorous, with numerous research projects and clinical trials being launched or planned, mainly in the countries most affected by COVID-19.¹⁰

Drug development is a costly and time-consuming process, where most of the new drug candidates are rejected due to the strict safety criteria required by regulatory agencies.^{10,11} Drug repurposing is an effective and quick drug development strategy for finding new inhibitory agents for infectious diseases.¹² The repurposing of market-available drugs to treat new or rare diseases is becoming increasingly attractive because it involves the use of de-risked compounds, with decreased costs and shorter timelines for development.¹³ Both computational data-driven and experimental approaches have been employed for the identification of repurposable drug candidates.¹² Molecular docking is a structure-based computational strategy for predicting the complementarity between a drug ligand and a therapeutic target.¹⁴ Pharmacological analysis of electronic health databases, in combination with other strategies, such as molecular docking, density functional theory (DFT) study, predictions by Pre-ADMET and experimental studies, is also effective for drug repurposing.^{15,16} Using computational screening, it is possible to predict drug's activity for other pathogens, since several drugs have an inhibitory effect and action against various protease pathways present in viruses.¹⁷

Computational strategies based on molecular docking, density functional theory (DFT) and electronic databases have been employed to study and screen the

pharmacological activity of antiviral drugs against key enzymes of SARS-CoV-2.^{1,2,18-21} In these studies, DFT provided fundamental insights based on the frontier orbital energies and spatial distributions as well as optimized geometries.^{2,18} This method has helped scientists to assess the stability of drugs and to compute structural, electronic and thermodynamic properties.²² These properties have been successfully used to better understand the behavior of drugs in biological systems.^{23,24} Frontier molecular orbital energies have been correlated with the calculated and experimentally determined properties of organic molecules screened as drug candidates.²⁵

Molecular docking is widely used to perform virtual screening of organic, inorganic, natural, and synthetic compounds, and to propose and explore structural behavior of a ligand when it binds to a protein target.^{26,27} In the search for new inhibitory agents for the main pathogenic enzymes, molecular docking has been employed to predict the binding affinity of drugs to a receptor and to elucidate the active site.^{28,29}

Pharmacological databases such as Pre-ADMET have been employed to complement drug repurposing studies by elucidating the pharmacological properties of absorption, distribution, metabolism, excretion, and toxicity (ADMET).³⁰ The Pre-ADMET server provides results close to tests performed in laboratories, in addition to simulating Ames tests to obtain toxicological results of mutagenicity and carcinogenicity in mice and rats.³¹

This study aims to screen a series of market-available drug for repurposing to treat the new disease caused by SARS-CoV-2 by using three complementary computational methods. The series includes the anti-influenza and anti-human immunodeficiency virus drugs approved by the Brazilian Health Regulatory Agency (ANVISA), Brazil. Moreover, the main protease inhibitor (M^{pro}) darunavir has been included as a control. The screening is conducted based on structural, electronic, and thermodynamic properties of the drugs under investigation both in vacuum and in the docking site of the main protease of SARS-CoV-2. Moreover, pharmacological database information is collected and used to identify the lead antiviral drug for further testing towards repurposing for the treatment of COVID-19.

Methodology

Computational details

DFT Study

The chemical structures of the anti-human immunodeficiency virus drug atazanavir, antimalarial

chloroquine, and anti-influenza drugs rimantadine, amantadine, zanamivir, oseltamivir and the drug darunavir (for use as a control in the molecular docking studies) were prepared using the Chemcraft software³² in the Cartesian coordinate input file format needed for the subsequent calculations.³³ The geometry of each compound was optimized in vacuum using DFT functional Becke, 3-parameter, Lee-Yang-Parr (B3LYP)³⁴⁻³⁶ and the triple zeta 6-311++G(d,p) basis set^{37,38} as implemented in the Gaussian16 software suite.³⁹ Each molecular geometry obtained was confirmed to be a true minimum in the potential energy surface by vibrational frequency calculations.⁴⁰ The structural and electronic properties obtained from the calculations were analyzed, and the optimized geometries were used as input for the molecular docking simulations and for the development of the ADMET study.

Molecular docking simulation

The main protease (M^{pro} or 3CL^{pro}) of SARS-CoV-2 was obtained from the Protein Data Bank (PDB) database, with the identification code 6Y2E, where this free structure will allow analysis if the Glu288-Asp289-Glu290 region is attractive for the series of drugs in this study. The SARS-CoV-2 M^{pro} was prepared using the CHIMERA v.13.1 software⁴¹ by suppressing water molecules, ions and other residues present in the protein structure. This preparation was conducted to make the molecular affinity tests feasible, as presented in the protocol by Pettersen *et al.*⁴¹ and Araújo *et al.*⁴² The main protease of SARS-CoV-2 is selected as it is responsible for mediating viral replication and transcription, and its inhibition could prevent the development of the pathogen.^{43,44}

Molecular docking was performed using the AutoDock Tools (ADT) version 1.5.6.⁴⁵ The receptor was considered rigid and all ligands were treated as being flexible. Hydrogen was added to the partial charges of Gasteiger and was calculated. Non-polar hydrogen atoms were added to the protease and ligands. Then, a cubic box of 60 × 60 × 60 grid points was created, with a grid resolution of 0.357 Å for the entire target protease (SARS-CoV-2 M^{pro}). The coordinates of the amino acid glutamate-290, X = -13.109, Y = -20.602 and Z = 24.681 were defined as the center of molecular affinity by GASS-WEB,⁴⁶ a web server for identifying enzyme active sites based on genetic algorithms that has an average accuracy of 99%.⁴⁷ The Lamarckian Genetic Algorithm with global search and pseudo-Solis and Wets with local search were adopted for molecular docking. The remaining parameters were adopted from the method of Ramos *et al.*⁴⁸ For the docking of each drug, 100 independent runs were carried out. The molecular affinity

analysis criterion is based on the binding free energy, inhibition constant and hydrogen bonding interactions.⁴⁹

ADME/Tox study

Pharmaceutical properties of ADMET were obtained using the Pre-ADMET⁵⁰ online server, available at no cost for academic purposes.³⁰ Parameters were obtained for the human intestinal absorption potential (HIA), drug absorption capacity (P_{Caco2}) through cell penetration, skin permeability (P_{skin}), potential to cross the blood-brain barrier and reaction with P-glycoprotein. Drug metabolism was analyzed based on the efficiency of inhibition with the subfamilies CYP450. The toxicity parameters were obtained in accordance with the toxicological evaluation in relation to mutagenicity by the Ames test and carcinogenicity in mice and rats.^{31,49}

Results

DFT study

The geometries of the series of antiviral drugs under investigation were optimized using DFT with Gaussian16 software (Figure 1) and confirmed using vibrational frequency analysis.^{51,52} The geometry optimization results were used as input in the molecular docking studies and analyzed to obtain the electronic structure and reactivity parameters.^{53,54}

The spatial distributions of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) for each antiviral drug are presented in Figure 2.⁵⁵ The HOMO and LUMO localizations describe the electron-donating and accepting moieties, respectively.

The DFT calculations provide parameters describing reactivity preferences, such as the HOMO and LUMO energies and the HOMO-LUMO energy gap as well as chemical potential (μ), defined as $\mu = -(E_{\text{LUMO}} + E_{\text{HOMO}})/2$, hardness (η), defined as $\eta = (E_{\text{LUMO}} - E_{\text{HOMO}})/2$, softness (s), defined as $s = 1/\eta$ and global electrophilicity (ω), defined as $\omega = \mu^2/2\eta$.^{56,57} The electrophilicity measures the energy stabilization, defined as the ability of an electrophilic molecule to acquire an additional electron, and the system's resistance to an electron charge exchange with the medium.⁵⁸

Table 1 lists the calculated parameters of the series of antiviral drugs. The HOMO and LUMO energies are related to the propensity of the molecules to donate (ionization potential) and accept electrons (electron affinity), respectively. The HOMO-LUMO energy gap

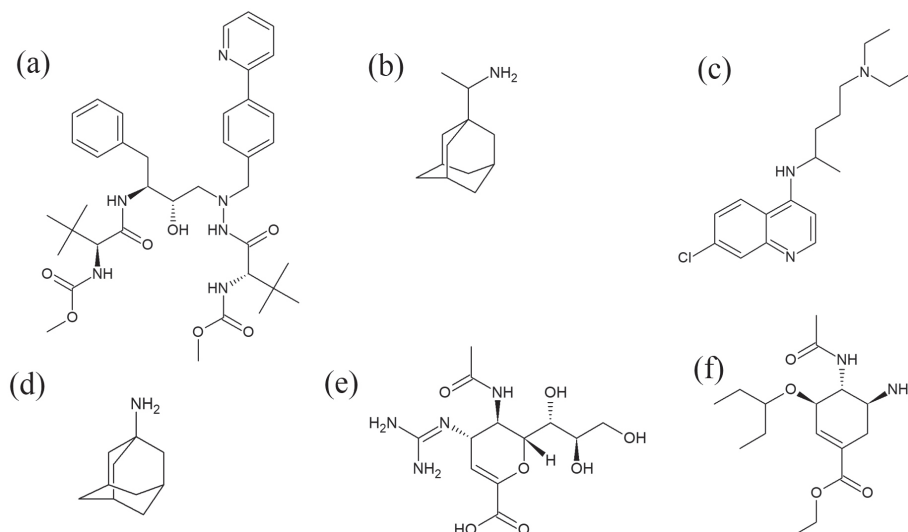


Figure 1. Optimized geometries of (a) atazanavir, (b) rimantadine, (c) chloroquine, (d) amantadine, (e) zanamivir, and (f) oseltamivir, in structural representations prepared with Chemcraft software.³²

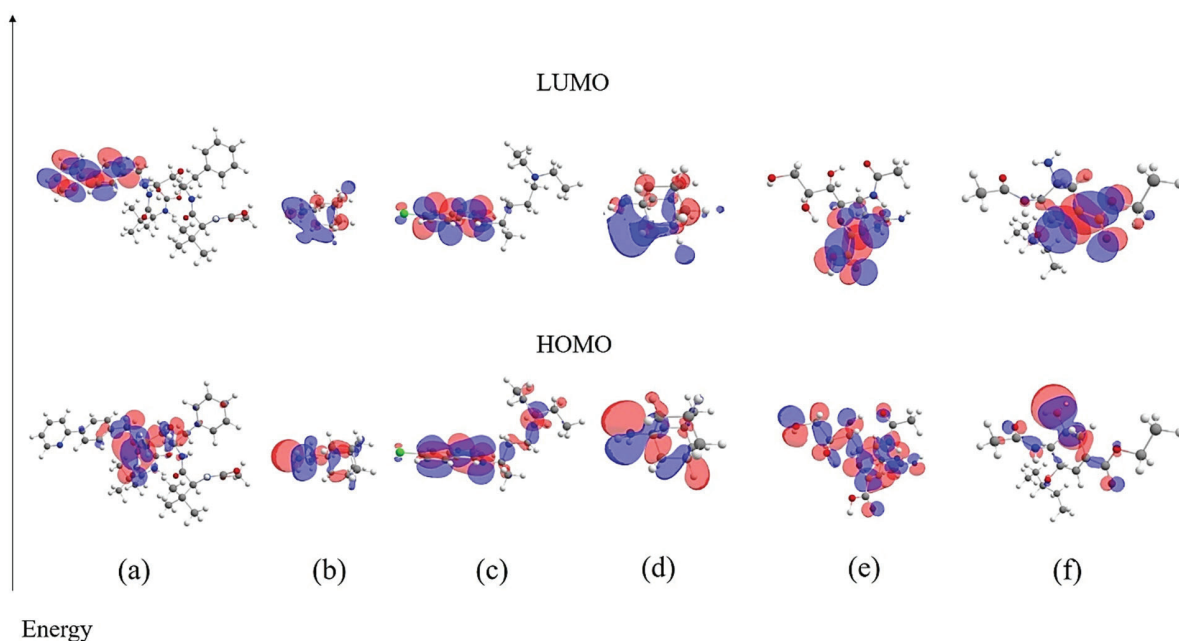


Figure 2. Atazanavir (a), rimantadine (b), chloroquine (c), amantadine (d), zanamivir (e), and oseltamivir (f). The red and blue colors correspond to the positive and negative phases, respectively.

Table 1. Energy values of molecular reactivity descriptors

Property	Atazanavir	Rimantadine	Chloroquine	Amantadine	Zanamivir	Oseltamivir
$E_{\text{HOMO}} / \text{eV}$	-5.92	-6.40	-5.92	-6.65	-6.88	-6.51
$E_{\text{LUMO}} / \text{eV}$	-1.45	-0.45	-1.55	-0.33	-1.91	-1.50
$E_{\text{HOMO-LUMO}} / \text{eV}$	4.47	5.95	4.37	6.32	4.97	5.01
μ / eV	-3.68	-3.43	-3.73	-3.49	-4.40	-4.01
η / eV	2.24	2.98	2.18	3.16	2.49	2.51
s / eV	0.45	0.34	0.46	0.32	0.40	0.40
ω / eV	3.03	1.97	3.19	1.93	3.89	3.20

E_{HOMO} : energy of the highest occupied molecular orbital; E_{LUMO} : energy from the lowest unoccupied molecular orbital; $E_{\text{HOMO-LUMO}}$: energy gap; μ : chemical potential; η : hardness; s : softness; ω : global electrophilicity.

describes the hardness of the molecules, in terms of the Hard and Soft Acids and Bases theory of Pearson^{59,60} for the prediction of chemical reactivity in the context of DFT.^{61,62} The negative values of the chemical potential indicate that the drug compounds are stable. Atazanavir and chloroquine have the highest HOMO energies (ionization potentials) as well as the lowest energy gaps and hardness values. The electrophilicity of zanamavir is the highest, followed by that of oseltamivir, chloroquine, and atazanavir. This order (Table 1) is correlated with the number of hydrogen bond donor sites as expressed by the number of H atoms of hydroxyl and amine groups (Figure 1).

The electrostatic potentials mapped on the electron density surface (Figure 3) visualize the molecular regions with enhanced preference for electrostatic interactions, such as hydrogen bonding, which are prevalent in the binding between drugs and receptors.⁶³⁻⁶⁵ The red and blue regions presented in the electrostatic potential of atazanavir, zanamavir and oseltamivir highlight the regions or functional groups with nucleophilic and electrophilic interaction preferences, respectively.^{58,65} Regions that showed nucleophilic (hydrogen bond acceptor) character were noted at the carbonyl groups and pyridinic N atoms. The

electrophilic H atoms of amine and hydroxyl groups (best seen in Figure 1) are hydrogen bond donors with substantial contributions to docking. Rimantadine and amantadine featured only one hydrogen bond acceptor site (red color) at the amine N atom. Chloroquine had several hydrogen bonding sites with low absolute charges. These results are important for studies of drug-protein interactions.⁶³

Molecular docking simulation

Starting from the optimized geometries of the drugs using DFT and X-ray data of SARS-CoV-2 M^{pro}, molecular docking aims to find the best orientation for binding the drug to the target protease. This approach allows characterization and improved understanding of the bonding interactions at the target binding site.¹⁹ The binding free energies, inhibition constants and hydrogen bonding interaction results obtained from 100 independent docking simulations are presented in Table 2. The binding free energies are in the range from -5.25 to -6.47 kcal mol⁻¹, representative of the docking of other antiviral drugs on the main proteases of coronaviruses.^{2,19,20,66} The standard error of AutoDock in binding free energies is estimated at 0.06 kcal mol⁻¹,

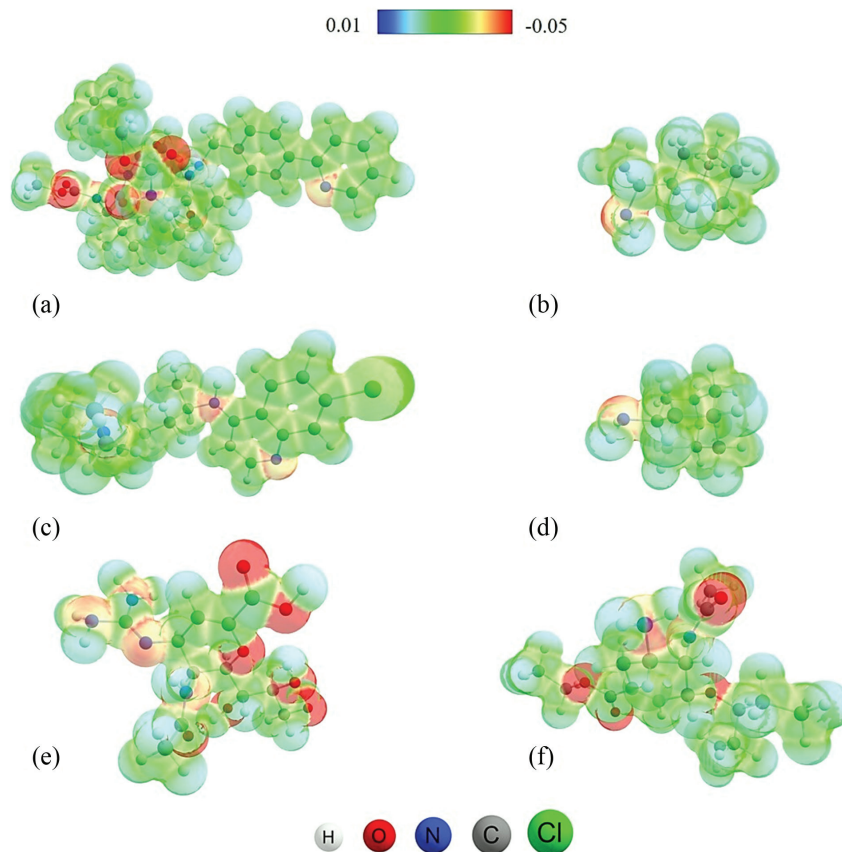


Figure 3. Electrostatic potential mapped on the electron density isosurface of (a) atazanavir, (b) rimantadine, (c) chloroquine, (d) amantadine, (e) zanamavir, and (f) oseltamivir.

Table 2. Molecular affinity parameters. All antiviral drugs interact with Glu288, highlighted in bold

Complex	$\Delta G_{\text{bind}} /$ (kcal mol ⁻¹)	Ki / μM	Number of independent runs	Number of conformations in first cluster	Interacting amino acids through hydrogen bonds
Atazanavir/M ^{pro}	-6.47	18.2	100	1	Lys5, Lys137, Glu290, Glu288
Rimantadine/M ^{pro}	-6.13	31.98	100	61	Glu288
Chloroquine/M ^{pro}	-5.59	79.37	100	61	Glu288
Amantadine/M ^{pro}	-5.58	80.98	100	100	Glu288
Zanamivir/M ^{pro}	-5.3	129.35	100	11	Asp289, Asp197, Glu288 , Lys5, Lys137, Thr199
Oseltamivir/M ^{pro}	-5.25	142.87	100	38	Lys5, Lys137, Glu288 , Asp289
Control					
Darunavir/M ^{pro}	-5.71	65.39	100	3	Glu288 , Leu287, Lys5

Complex: drug and protein; ΔG_{bind} : Gibbs free energy; Ki: inhibition constant; Lys: lysine; Glu: glutamic acid; Asp: aspartic acid; Thr: treonina.

which is within the experimental error.⁶⁷ Thus, obtaining the binding energy (ΔG) will allow to know which drugs express cytotoxic activity *in vitro* against the M^{pro} protein, since the molecular affinity indicates that the protein will bind to the drug, where the pharmacological properties of the drug will act on that target protein.

The results clearly indicate that atazanavir has the highest molecular affinity for the main SARS-CoV-2 protease, with a free binding energy of -6.47 kcal mol⁻¹, followed by rimantadine, chloroquine, amantadine, zanamavir, and oseltamivir. The inhibition constants increase in the same order as the binding free energies. Among the series of antiviral drugs investigated, atazanavir, rimantadine, chloroquine and amantadine are the most promising for binding to the SARS-CoV-2 M^{pro} because of the substantially higher affinity and stronger inhibition of the protease.^{42,68} The antiviral drugs atazanavir, zanamavir, and oseltamivir which contain more hydrogen bond donor and acceptor sites (Figure 3), have fewer conformations in the first cluster and tend to interact with more of the protease amino acids. Atazanavir effectively docks on the protease active center in a single conformation, whereas zanamavir docks in 11 conformations, followed by oseltamivir that docks in 38 conformations and the control darunavir that docks in 3 conformations (Table 2). Each of these four antiviral drugs interacts with several amino acids through hydrogen bonds, including Glu288, whereas the remaining antivirals interact only with Glu288.

The binding free energy calculations conducted using molecular docking are based on force fields that enable the exploration of wide regions of conformational space.⁶⁹ Ligand-protein interactions are defined by a combination of empirical and semi-empirical force field parameters.⁷⁰ It is important to note that the AutoDock calculations consider the desolvation of polar and nonpolar groups using empirical approaches.⁶⁹

Figures 4, 5, 6 and S1 (Supplementary Information (SI) section) present the molecular docking interaction surfaces and detailed representations of the network of hydrogen bonding interactions of each drug with the main protease affinity center. The interactions between atazanavir and the target protease (Figure 4a) include 4 bridging hydrogen bonds to the amino acid residues Lys5 (lysine), Lys137, Glu290 (glutamic) and Glu288. The latter residue can be seen in all hydrogen bonding interactions of the generated complexes, as shown in Figures 4, 5, 6 and S1. Therefore, the amino acid residue Glu288 was identified as a potentially key site to inhibit the SARS-CoV-2 M^{pro} by allowing the antivirals to react and block the active region.^{71,72}

The rimantadine presented the second best result of molecular affinity, with a binding free energy of -6.13 kcal mol⁻¹ and an inhibition constant of 31.98 μM (Table 2). These results highlight the strong docking of the drug on the active site of the protease M^{pro} of SARS-CoV-2,⁷² where the most intense interaction of the complex occurs with the amino acid residue Glu288, which forms a 2.46 Å long hydrogen bridge between the rimantadine O and amino acid N (Figure 4b). In the amino acid residues Arg4 (arginine), Lys5, Leu282 (leucine), Phe291 (phenylalanine), Phe3, Ser284 (serine) and Trp207 (tryptophan) for rimantadine, we observed substantial hydrophobic interactions at the edges of the protease active site, in addition to the hydrogen bonding bridge (Figure 4b).

Chloroquine and amantadine (Figure 5) are comparable in docking effectiveness with the M^{pro} protease of SARS-CoV-2, based on the binding free energy and inhibition constant (Table 2). Chloroquine is docked via a 2.55 Å hydrogen bond with Glu288 and hydrophobic interactions with the amino acid residues Asp289, Glu290, Leu282, Lys5, Leu286, Leu287, Phe3, Phe291, Ser284 and Trp207. The amantadine/M^{pro} complex has a binding free

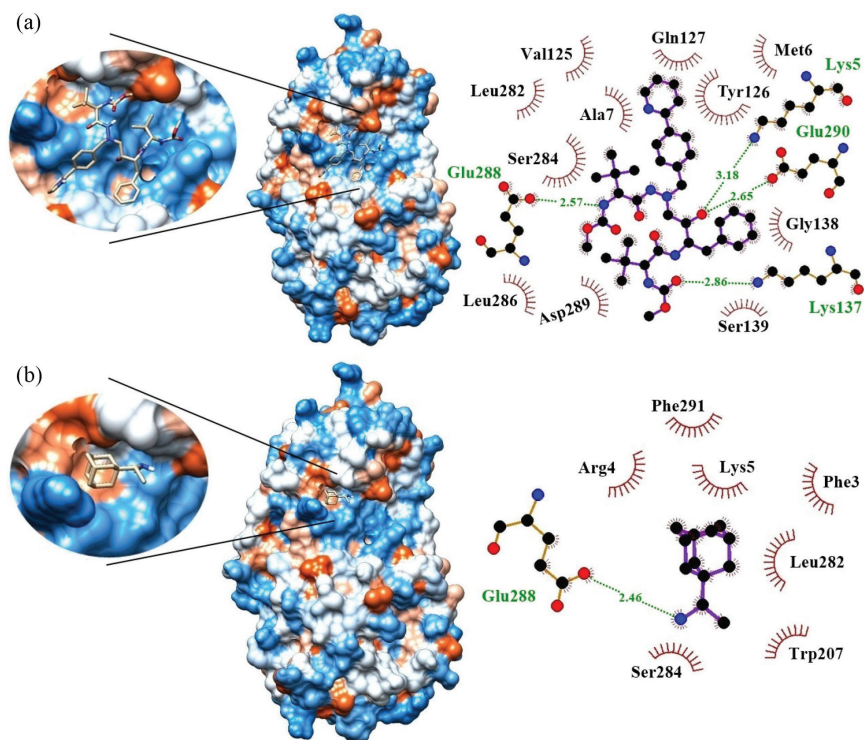


Figure 4. Molecular docking: (a) molecular interaction between atazanavir and protease M^{pro}; (b) molecular interaction between rimantadine and protease M^{pro}. The protease M^{pro} surface color is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively.

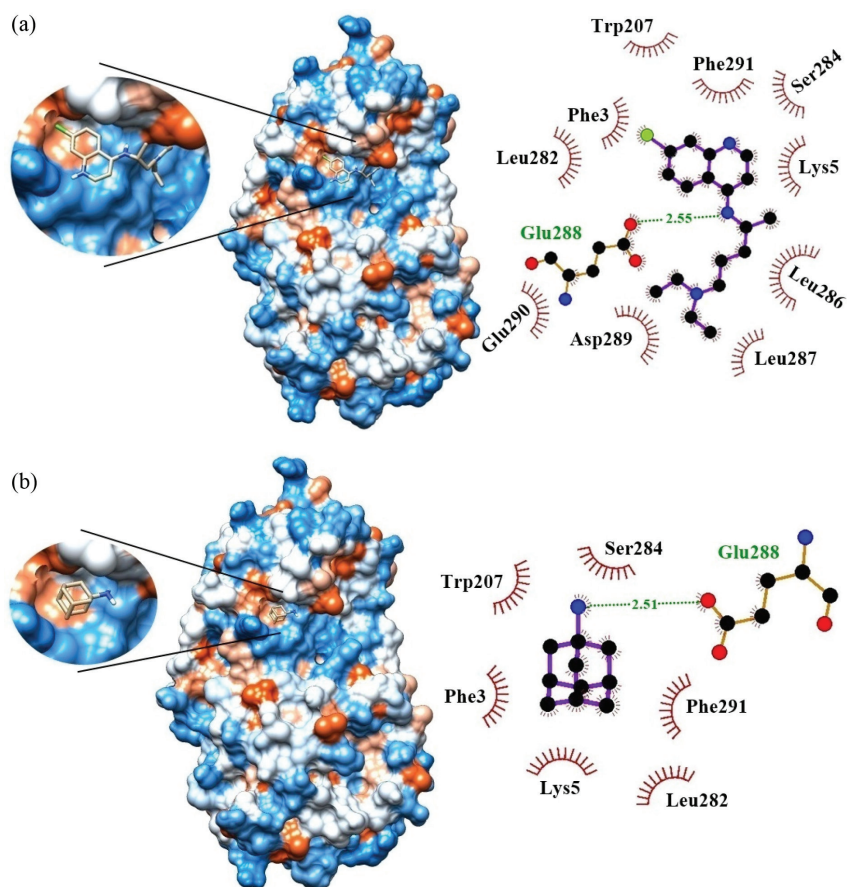


Figure 5. Molecular docking: (a) molecular interaction between chloroquine and protease M^{pro}; (b) molecular interaction between the amantadine and protease M^{pro}. The protease M^{pro} surface color is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively.

energy of $-5.58 \text{ kcal mol}^{-1}$ and an inhibition constant of $80.98 \text{ }\mu\text{M}$ (Table 2). This complex also forms a hydrogen bonding bridge to the amino acid residue Glu288 with a $\text{N}\cdots\text{O}$ distance of 2.51 \AA and hydrophobic interactions with the amino acid residues Lys5, Leu282, Phe291, Phe3, Ser284 and Trp207, which interact at the edges of the SARS-CoV-2 M^{pro} active site (Figure 5).

Zanamivir was also promising when binding SARS-CoV-2 M^{pro} , with a binding free energy of $-5.3 \text{ kcal mol}^{-1}$ and an inhibition constant of $129.35 \text{ }\mu\text{M}$. The docking of zanamivir was stabilized through the formation of six hydrogen bonding bridges with the amino acid residues Asp289, Asp197, Glu288, Lys5, Lys137, and Thr199 (Figure 6). We observed once again that a hydrogen bond is formed at the amino acid residue Glu288, demonstrating that this site is an attractive site in molecular interaction studies for the SARS-CoV-2 M^{pro} .⁷¹ In the molecular interaction between the protease and oseltamivir, there were 4 hydrogen bridges to the amino acid residues Lys5, Lys137, Glu288 and Asp289 (Figure 6), yielding a binding free energy of $-5.25 \text{ kcal mol}^{-1}$ and an inhibition constant of $142.87 \text{ }\mu\text{M}$ (Table 2).

Thus, it is already known that the residue regions at the N terminal (also known as the N finger) interact with two terminal domains of protomers A and B, this interaction

has shown promise for dimerization.⁷² Thus, with these results we show that the Glu288-Asp289-Glu290 region within the extra domain are essential for inhibition of SARS-CoV-2, as also pointed out in studies by He *et al.*⁷² and Vijayakumar *et al.*⁷¹

ADME/Tox study

The results of the absorption (Table 3), distribution (Table 4), metabolism (Table 5) and toxicity parameters (Table 6), predicted by Pre-ADMET were calculated and listed.³⁰ The best results with respect to the human intestinal absorption potential (HIA) were amantadine and rimantadine, both with 100% absorption. The others also have high HIA percentages (85% or higher), with the exception of zanamivir, suggesting that they are well absorbed (Table 3).⁷³

The permeability of Caco-2 cells ($P_{\text{Caco-2}}$) is an important effectiveness evaluation factor for drugs pertaining to transport and absorption. Based on this, it is possible to predict how the drug behaves and moves from the intestine into the patient's bloodstream.^{74,75} The values of $P_{\text{Caco-2}}$ show average permeability for all drugs analyzed, with results from 14.12 to 56.61 nm s^{-1} . We observed that the drugs atazanavir, amantadine and rimantadine have

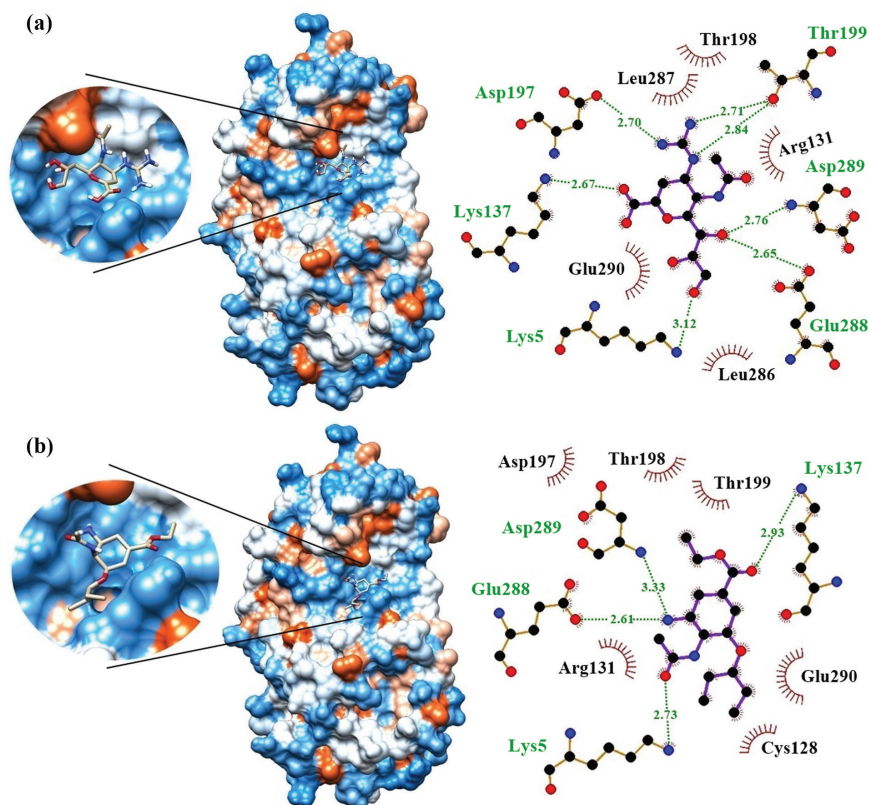


Figure 6. Molecular docking: (a) molecular interaction between zanamivir and protease M^{pro} ; (b) molecular interaction between oseltamivir and protease M^{pro} . The protease surface color is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively.

Table 3. Absorption characteristics of antiviral agents

Drug name	Absorption characteristics		
	HIA / %	P_{Caco-2} / (nm s ⁻¹)	P_{skin}
Amantadine	100.00	21.32	-2.790
Atazanavir	88.54	21.20	-2.108
Chloroquine	98.06	56.61	-2.535
Oseltamivir	87.16	14.12	-3.349
Rimantadine	100.0	21.72	-2.747
Zanamivir	4.065	19.16	-5.137

HIA: human intestinal absorption potential; P_{Caco-2} : permeability of Caco-2 cells; P_{skin} : skin permeability.

similar permeability values, while chloroquine stands out with the best permeability. According to Yazdaniyan *et al.*⁷⁶ permeability values between 4 and 70 nm s⁻¹ are considered average. These below 4 nm s⁻¹ are low and those in the 70-100 nm s⁻¹ range indicate high permeability.⁷⁷ Skin permeability (P_{skin}) is important to determining whether the drug can be applied through transdermal administration.³⁰ All drugs studied showed negative values, indicating that these drugs are not lipophilic, cannot be absorbed by human skin, and must be administered orally.⁷⁸

The distribution characteristics of the drugs were evaluated by binding to plasma protein (PPB), interaction with P-glycoprotein and through the brain-blood participation coefficient (C_{brain}/C_{blood}) (Table 4). The drugs amantadine, atazanavir and chloroquine exhibited high values of 100, 86.56 and 92.53%, respectively, demonstrating a high potential to reach the bloodstream. Oseltamivir, rimantadine and zanamivir are classified as weakly-binding.

The penetration of the blood-brain barrier, reflected in the C_{brain}/C_{blood} values, depends on the polarity of the antiviral compound. The barrier penetration directly affects the drug circulation in the central nervous system, as a rupture of this barrier is the main cause of degenerative diseases.^{79,80} The drugs amantadine, atazanavir, oseltamivir and zanamivir

Table 4. Distribution characteristics of antiviral agents

Drug name	Distribution characteristic		
	PPB / %	C_{brain}/C_{blood}	P-Glycoprotein inhibition
Amantadine	100.0	1.030	non
Atazanavir	86.56	0.285	inhibitor
Chloroquine	92.53	7.734	non
Oseltamivir	37.83	0.123	non
Rimantadine	4.988	2.015	non
Zanamivir	0.000	0.816	non

PPB: plasma proteins binding; C_{brain}/C_{blood} : brain-blood participation coefficient.

have C_{brain}/C_{blood} values of 1.030, 0.285, 0.123 and 0.816, respectively, classified as average absorption, according to Ma *et al.*⁸⁰ Chloroquine and rimantadine, with C_{brain}/C_{blood} values of 7.734 and 2.015, respectively, indicated a greater potential for breaking the blood-brain barrier. Atazanavir was the only drug in this study that inhibited the action of the P-glycoprotein that is responsible for increasing cell resistance in new drugs and transporting various structures for adenosine triphosphate (ATP) binding.^{81,82}

Cytochrome P450 (CYP450) is an important family of monooxygenases that is involved in drug metabolism. Thus, drugs must exhibit a substrate, inducer or inhibitor activity to the enzymes that integrate with CYP450 according to their specificity. When CYP450 inducers are included, a reduction in the effectiveness of the drug can be observed. On the other hand, the consequences of inhibiting CYP450 include increased toxicity of the drug affected by the interaction, or reduced effectiveness, when the drug is a prodrug and depends on the activity of CYP450 enzymes to be activated.^{83,84} Thus, it is expected to obtain inhibiting activity in that CYP2C19, CYP2C9, CYP2D6 and CYP3A4 enzymes and substrates in CYP2D6 and CYP3A4 (Table 5). Amantadine and rimantadine showed negative results for the CYP3A4 substrate. Atazanavir showed negative results

Table 5. Metabolic characteristics of antiviral agents

Drug name	Metabolism					
	CYP2C19 (inhibition)	CYP2C9 (inhibition)	CYP2D6 (inhibition)	CYP2D6 (substrate)	CYP3A4 (inhibition)	CYP3A4 (substrate)
Amantadine	inhibitor	inhibitor	inhibitor	weakly	inhibitor	non
Atazanavir	inhibitor	inhibitor	non	non	inhibitor	substrate
Chloroquine	non	non	inhibitor	substrate	non	substrate
Oseltamivir	non	non	inhibitor	substrate	non	weakly
Rimantadine	inhibitor	inhibitor	inhibitor	substrate	inhibitor	non
Zanamivir	non	non	non	non	non	weakly

CYP: cytochrome.

for the CYP2D6 substrate. Chloroquine and oseltamivir inhibited CYP2D6, with oseltamivir having weak activity on the CYP3A4 substrate. Zanamivir was the least active in the CYP450 family, only showing weak activity towards the CYP3A4 substrate. The results indicated that the drugs amantadine, atazanavir and rimantadine were the most promising candidates based on the effective interactions with the CYP450 enzymes.⁴⁹

In Table 6, the mutagenicity results (Ames test) and the carcinogenic properties in mice and rats are listed. Among the 6 drugs evaluated, amantadine, chloroquine and rimantadine showed mutagenicity based on the Ames tests. The Ames test is accepted by the scientific community as a way of evaluating the mutagenicity of compounds by using different strains of salmonella.⁸⁵ Only amantadine showed carcinogenic activity in mice. For rats, amantadine, chloroquine, and rimantadine showed positive results for carcinogenicity.

Table 6. Toxicological properties of antiviral agents

Drug name	Carcinogenicity		
	Mutagenicity (Ames test)	Mouse	Rat
Amantadine	mutagen	positive	positive
Atazanavir	non-mutagen	negative	negative
Chloroquine	mutagen	negative	positive
Oseltamivir	non-mutagen	negative	negative
Rimantadine	mutagen	negative	positive
Zanamivir	non-mutagen	negative	negative

Table S1 found in the SI section lists the side effects of each drug analyzed in this study.⁸⁶ It can be seen that each drug has a number of serious side effects that must be reported immediately to the doctor and require the immediate suspension of the treatment.

Discussion

The results indicated that all seven drugs dock effectively on the main M^{pro} SARS-CoV-2 protease, based on the negative binding free energies, low inhibition constants and extensive hydrogen bonding and hydrophobic interactions presented in Table 2 and Figures 4, 5, 6 and S1 found in SI section. Among these, the best candidate based on docking was atazanavir, followed by rimantadine, the equally-performing chloroquine and amantadine, and the last on the binding free energy list, zanamivir and oseltamivir. This order is in partial agreement with the E_{HOMO} decrease order (Table 1) of atazanavir comparable to chloroquine, followed by rimantadine, oseltamivir,

amantadine and zanamivir, indicating that the docking strength correlates with the electron donating or ionization potential of the drug. The HOMO and LUMO spatial distributions are localized on the hydrogen bond acceptor and donor moieties, respectively (Figure 2). Moreover, the four drugs with the best docking affinity (lowest binding free energies and inhibition constants) have substantially higher chemical potential, indicative of increased reactivity, than zanamivir and oseltamivir. The hydrogen bond donor and acceptor preferences of the drug molecules highlighted in the electrostatic potential maps (Figure 3) correlate with the hydrogen bonding networks from the docking simulations (Figures 4, 5 and 6).

The binding free energies of atazanavir and rimantadine were close to those calculated for the bis-(3,5,5-trimethylhexyl) phthalate compounds, myristicin, eugenol and 6-shogaol in studies carried out by Tallei *et al.*⁸⁷ for the same target protease (M^{pro}) by molecular docking. Moreover, these two drugs also performed better compared to the main inhibitor (darunavir) of this protease, used as a control in this study, with a binding free energy of -5.71 kcal mol⁻¹ (Table 2). Atazanavir and rimantadine have a high capacity to bind to the main protease, which is responsible for catalyzing the processing of viral polyprotein and, therefore, inhibit viral infection by SAR-CoV-2 in humans.⁸⁷

In addition to having the highest docking affinity with a single conformation, atazanavir, has negative results for carcinogenicity in Ames tests by Pre-ADMET.⁸⁵ Moreover, atazanavir is the only drug in this study to inhibit P-glycoprotein, responsible for preventing the action of new drugs in the body, so its inhibition is essential for the distribution of the drug and transporting various structures for ATP binding.⁸¹ P-Glycoprotein is also located at the villus tip of enterocytes in the gut, thus, drugs with oral administration that inhibit the action of P-glycoprotein cross this physiological barrier effectively for the distribution of its pharmacological properties in the organism.⁸⁸ Knowing that this drug is already available in the pharmaceutical market for the treatment of infections by the human immunodeficiency virus,⁸⁹ human studies are advantageous because of the current need to discover a SARS-CoV-2 inhibitory agent by the drug repositioning method.

These findings demonstrate that both rimantadine and chloroquine are also potentially attractive for studies of the main SARS-CoV-2 protease,⁷¹ having strong hydrogen bonding interactions with the amino acid residue Glu288 and high binding affinities, indicating that both interact strongly with the active site of the key pathogen protein. This molecular affinity indicates that the drugs express

cytotoxic activity against the M^{pro} protein. In a study by Li *et al.*⁹⁰ it was shown that atazanavir and chloroquine has cytotoxic action inhibiting cell viability by 50% (IC₅₀) at a concentration of 7.53 ± 0.31 and 7.16 ± 0.23 μM , respectively. These findings validate the computational prediction by molecular docking, where the drug showed molecular affinity to bind to the M^{pro} protein.

However, chloroquine has highly toxic and its use can lead to complications, for example, in patients with acute or cutaneous porphyria, the use of chloroquine can trigger an acute attack with fever and intense elevations of the aminotransferase, potentially leading to jaundice.⁹¹ Chloroquine also shows positive results for carcinogenicity (Table 6), as in long treatments, it can stimulate cell damage, causing uncontrolled cell division by mitosis, potentially leading to malignant tumors.⁹² In addition, chloroquine may cause hypoglycemia, neuropsychiatric effects, drug interactions and idiosyncratic hypersensitivity reactions, thus further aggravating the treatment outcomes for patients affected by COVID-19, as reported in cases of treatment with chloroquine.⁹³

Conclusions

A series of market-available drugs for the treatment of influenza infections caused by the human immunodeficiency virus and influenza were evaluated for repurposing to inhibit SARS-CoV-2 (M^{pro}) using DFT calculations, molecular docking simulations, and pharmacological database screening. The drugs were found to interact with the protease through hydrogen bonding and hydrophobic interactions, as revealed by docking and DFT studies. The binding affinity was correlated partially with the HOMO energies, indicating that electron donation from the drug to the protein is important. Pharmacological screening identified drugs with high absorption and favorable distribution, metabolic, and toxicological characteristics.

The results identified atazanavir as the drug with the highest docking affinity (-6.47 kcal mol⁻¹) and the strongest inhibition of the main protease, as it formed numerous and strong hydrogen bonds. As well as its molecular affinity stood out in comparison to the main inhibitor of M^{pro} (darunavir) in the Glu288-Asp289-Glu290 region, which obtained a molecular affinity value of -5.71 kcal mol⁻¹. It also has the best pharmacological potential, as evidenced by inhibition of the action of P-glycoprotein, responsible for increasing cell resistance in new drugs and transporting various structures for ATP binding. The toxicological data indicated that atazanavir was the only drug in the series that was negative for both carcinogenicity and mutagenicity. Based on this *in silico* screening, atazanavir was identified

as a good candidate for *in vitro* testing and subsequent clinical testing for the treatment of SARS-CoV-2.

Supplementary Information

Supplementary information (the molecular interaction between darunavir and protease (M^{pro}), along with the color of the protein's surface that is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively, are in Figure S1, and in Table S1 are the common and serious side effects of atazanavir, rimantadine, chloroquine, amantadine, zanamivir and oseltamivir) is available free of charge at <http://jbc.sqb.org.br> as PDF file.

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Author Contributions

Joabe L. Araújo was responsible for conceptualization, data curation, formal analysis, software, investigation, methodology, project administration, supervision, validation, visualization, writing-original draft, writing-review and editing; Lucas A. de Sousa for conceptualization, data curation, formal analysis, supervision, visualization, writing-original draft; Alice de O. Sousa for formal analysis, supervision, writing-original draft; Ruan S. Bastos for conceptualization, formal analysis, supervision, visualization, writing-original draft; Gardênia T. Santos for conceptualization, data curation, supervision, visualization; Mateus R. Lage for conceptualization, data curation,

methodology, supervision, validation, visualization, writing-original draft; Stanislav R. Stoyanov for investigation, methodology, software, supervision, validation, visualization; Ionara N. G. Passos for supervision, visualization, writing-original draft; Ricardo B. de Azevedo for supervision, visualization, writing-original draft; Jefferson A. Rocha for data curation, formal analysis, investigation, supervision, visualization, writing-original draft.

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