Using the Pharmacophoric features of Azithromycin to design potential SARS-CoV-2 inhibitor: A Virtual Screening and Molecular Docking Approach

O. V. Ikpeazu, F. J. Amaku, I. E. Otuokere, and K. K. Igwe

Abstract—The outbreak of novel coronavirus (SARS-CoV-2) found in Wuhan China is rapidly spreading to all nations of the world. Currently, there are no approved drugs for the treatment of the novel coronavirus disease. Meanwhile, repositioning of some antibiotics, antiviral and antimalaria drugs have been employed. In this study, we used azithromycin as a model drug to virtual screen the ZINC database and the molecules obtained were docked against SARS-CoV-2 protein with PDB code: 5r7y. The best five ligands with high affinity for the target protein was compared with the reference molecule (Azithromycin). The docking score for the predicted ligands with high affinity for the target protein include ZINC10635972 (-6.3 kcal/mol), ZINC02651653 (-6.2 kcal/mol), ZINC09728215 (-6.2 kcal/mol), ZINC15003138 (-6.1 kcal/mol), ZINC89836288 (-6.1 kcal/mol) and azithromycin (+28.2 kcal/mol). The lead molecule (ZINC10635972) was observed to interacted with LUE 141, ASN 142, SER 144, SER 46, GLY 166, MET 165, HIS 163, MET 49, HIS 164, PHE 140, which was noticed to fit the class 5 toxicity index. Hence, ZINC10635972 is a promising compound that should further examined as drug candidates before clinical evaluation.

Index Terms—Azithromycin, COVID-19, Pharmacokinetics, Virtual Screening, Molecular Docking

I. INTRODUCTION

The outbreak of novel coronavirus is rapidly spreading with an extremely high degree of virulence. The virus has claimed thousands of lives globally and has impacted negatively to society and the global economy [1]. Meanwhile, the World Health Organization (WHO) has declared that the coronavirus disease is a pandemic.

The novel coronavirus is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [2]. The four phases of transmission are classified into asymptomatic, moderate, extreme, and critical. Symptoms such as fever, dry cough, dyspnea, pneumonia, hypoxemia, encephalopathy, heart failure, and acute kidney injury are associated with SARS-CoV-2. However, the type of symptom observed is a function of the severity of the infection [3]. The novel virus (SARS-CoV-2) is made of structural proteins and non-structural proteins (NSPs). The structural proteins include spike protein (trimeric), membrane protein, envelope protein. These proteins are responsible for the formation of the spherical shape of the virus. Meanwhile, the non-structural proteins (sixteen in number) are responsible for the metabolic and molecular events include transcription and translation [4].

Despite all the effort provided by stakeholders, there is currently no antiviral drug or vaccine available for COVID-19 treatment. Hence, the pandemic is presently managed [5]. It is worth mentioning that repositioning of antiviral, anti-inflammatory, anti-malarial and other pharmacologically active drugs for clinical trials against COVID-19 has been part of the management phase [6]. Azithromycin is an effective antibiotic that has also shown inhibitory activity against viral production and virus-mediated cell death. This antibiotic (azithromycin) was also found to be potent against the treatment of Zika virus.

In this study, we explored the pharmacophoric features of azithromycin for the virtual-screening of the ZINC database for a molecule with inhibitory activity against SARS-CoV-2 via molecular docking approach. The pharmacokinetics and drug-like characteristic of the lead molecule was also validated.

II. MATERIALS AND METHODS

A. Pharmacophore-based Virtual Screening

To initiate effective biological function in a ligand-receptors interaction using the computer-aided approach, it is important to establish robust steric and electronic characteristics of the pharmacophore [7]. To perform the pharmacophore virtual-screening, the chemical structure of Azithromycin was first retrieved from the drug bank online platform in its SMILE format. The UCSF Chimera interface was used to convert the SMILE format of Azithromycin into its 3D model prior to submission to ZINCPharmer with distinct pharmacophoric features (see Table I) [8-10].

The online application “ZINC-pharmer” (http://zincpharmer.csb.pitt.edu/pharmer.html) was employed for pharmacophore-based virtual screening to obtain drug-like compounds having similar pharmacophoric feature with azithromycin on the ZINC database to identify possible 5r7y inhibitors with a robust capacity to stop the spread of COVID-19, the screening was performed [8-10].
B. Receptor and ligand preparation

Molecules were retrieved from the ZINC database in their SDF format and converted to mol2 format for further treatment, the 3D conformation of the ligands was optimized using the MMFF94 force field on Avogadro interface [11]. Prior to the molecular docking step, the optimized 3D structures of the acquired lignans were processed by making use of the dock-prep tools on the UCSF Chimera interface. The complexed crystalized structure of SARS-CoV-2 was retrieved from the Protein Data Bank with ID: 5r7y. The structure COVID-19 was a distinct single chains bounded to ligand (JFM). The preparation of the biological target (5r7y) was performed on the UCSF Chimera interface [9].

C. Molecular docking

All molecular docking was achieved by making use of the AutoDockVina software [8]. We generated grid files to localize the binding positions for specific docking. The grid box that defines the pocket of 5r7y protein was obtained from the AutoDock Vina functionality on UCSF Chimera interface [9]. The grid box size and centre coordinates for the 5r7y were x(9.84876, 10.1046), y (-1.58503, 7.31927) and z (23.884, 9.3706) respectively. The compound with higher binding affinity than the reference inhibitor (chloroquine) is considered for further in silico analysis. Meanwhile, all the hits obtained from the ZINC database were docked to predict their binding conformation and affinity within the pocket of 5r7y was examined.

D. Validation and ADMET analysis

ADME profiling of the lead compound was determined by making use of online SwissADME tool [12, 13]. The significant parameters associated with ADME properties such as Lipinski’s rule of five, the solubility of the drug, pharmacokinetics properties and drug-like characteristics were predicted [14]. Meanwhile, the toxicity of ZINC10635972 was validated on ProTox-II webserver.

E. Quantum chemical calculations

The frontier molecular orbitals energies were estimated and used to calculate the reactivity descriptors such as Electronegativity (χ), hardness (η), softness (S) and electrophilicity index. The energy gap (ΔE) was calculated from the energy difference between LUMO and HOMO energies of the lead molecule [15]. Absolute electronegativity (χ), absolute hardness (η) and electrophilicity index (ω) of ZINC10635972 were also determined. The hardness of a molecule is related to the gap between the HOMO and LUMO orbitals, however, the larger the HOMO-LUMO energy gap the harder the molecule [16, 17]. On the other hand, the global softness of ZINC10635972 is estimated from the inverse of global hardness. Meanwhile, the chemical potential of the lead molecule was also determined. The density functional theory (DFT) calculation was performed on Gaussian 09 equipped with Gaussview 5.0 software package [18]. This was achieved by making use of Becke–Lee Yang–Parr functional (B3LYP) method with 6-311++G (d, p) basis sets [19]. The structure of ZINC10635972 was optimized before theoretical analysis. The ionization potential (IP ≈ - EHOMO) and electron affinity (EA ≈ - ELUMO) were applied to the following equations used to estimate the global reactivity descriptors [20-24].

\[
\text{Electronegativity (}\chi) = \frac{IP + EA}{2}
\]

\[
\text{Hardness (}\eta) = \frac{IP - EA}{2}
\]

\[
\text{Softness (}\delta) = \frac{1}{\eta}
\]

\[
\text{Chemical potential (}\mu) = -\frac{IP - EA}{2}
\]

\[
\text{Electrophilicity index (}\omega) = \frac{\mu^2}{2\eta}
\]

III. RESULTS AND DISCUSSION

Reposition an existing drug for clinical trials against the spread of SARS-CoV-2 has been the only remedy to manage the pandemic. The pharmacophore-base virtual screening of the ZINC database was performed using azithromycin as the model drug via ZINCPharmer. About 50 molecules exhibiting similar pharmacophoric features as azithromycin were retrieved and processed for further use. Fig 1, showed the 3D image of azithromycin and its electronic signals the pharmacophoric. Meanwhile, the coordinate of the pharmacophoric signals was listed in Table I. The small molecules were docked against SARS-CoV-2 protein (5r7y) as displayed in Fig 2.

Fig 1. The 3D image of Azithromycine showing the pharmacophoric signals on the ZINCPharmer online Platform.

Fig 2. The 3d structure of 5r7y.
A. Molecular docking

After successful specific docking of the fifty-one predicted ligands obtained from the virtual screening experiments, the results revealed significant binding of the ligands with the target proteins (5r7y). The docking score values of the azithromycin and the predicted ligands was compared. The top five best ligands with high affinity for the target was reported (see Table II). The docking of azithromycin with 5r7y shows poor interactions in the pocket of the proteins with the affinity of +28.02 kcal/mol. It was also observed to interact with 24 amino acids within the preset pocket and a hydrogen bond formation between HIS 164 and a free hydroxyl group of azithromycin. Meanwhile, the five most favourable predicted ligands was noticed to have -6.3 kcal/mol, -6.2 kcal/mol, -6.2 kcal/mol, -6.1 kcal/mol and -6.1 kcal/mol binding score for ZINC10635972, ZINC02651653, ZINC09728215, ZINC15003138 and ZINC89836288 respectively. Hence, ZINC10635972 was selected as the lead molecule owing to its high-affinity interaction for 5r7y. The ligand interaction of the top five drug-like compounds with the amino acid residues within the pocket of the studied receptor (5r7y) was shown in Figs.3.to 8. Meanwhile, ZINC10635972 was noticed to interacted with eighteen amino acid residues which include LUE 141, ASN 142, SER 144, SER 46, GLY 189, GLU 166, MET 165, HIS163, MET 49, HIS 164, PHE 140, GLY 143, THR 25, CYS 145, HIS 41, CYS 44 and THR 45. The strong affinity of ZINC10635972 for 5r7y could be attributed to the formation of a hydrogen bond between the freely suspended hydroxyl group with GLY143, SER 144 and CYS 145. The free hydroxyl group of ZINC10635972 is quite reactive as it firmly interacted with both hydrophobic and polar amino acids within the pocket of 5r7y protein. The small intermolecular distance between the -OH group and amino acids as well as non-appearance of steric hindrance on the -OH group could also play a role in the enhanced molecular interactions and the high affinity for the target protein.

**TABLE I: THE DIMENSIONS OF THE PHARMACOPHORE FEATURES OF AZITHROMYCIN ON ZINCPHARMER PLATFORM**

<table>
<thead>
<tr>
<th>Pharmacophore Class</th>
<th>X</th>
<th>y</th>
<th>Z</th>
<th>Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>26.50</td>
<td>18.35</td>
<td>26.78</td>
<td>1.00</td>
</tr>
<tr>
<td>Hydrogen acceptor</td>
<td>19.01</td>
<td>18.75</td>
<td>21.34</td>
<td>0.50</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>26.72</td>
<td>23.74</td>
<td>26.03</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**TABLE II: 2D REPRESENTATION, ZINC CODE AND GLIDE SCORE (G-SCORE) VALUE CALCULATED WITH RESPECT TO THE RELATED QUERY, OF THE SHARED BEST 5 HITS.**

<table>
<thead>
<tr>
<th>Code</th>
<th>Score</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>+28.2</td>
<td><img src="https://example.com/azithromycin.png" alt="Image" /></td>
</tr>
</tbody>
</table>

ZINC10635972 -6.3

ZINC02651653 -6.2

ZINC09728215 -6.2

ZINC15003138 -6.1

ZINC89836288 -6.1

Fig. 3. The 3D X-ray crystal structure of 5r7y complex with azithromycin showing also the binding site region and the residues that constitute this binding site region.

Fig. 4. The 3D X-ray crystal structure of 5r7y complex with ZINC10635972 showing also the binding site region and the residues that constitute this binding site region.
B. ADMET assessment of potential SARS-CoV-2 inhibitors

The bioavailability radar of ZINC10635972 revealed the physicochemical space of the molecule necessary to predict its pharmacokinetics and drug-likeness characteristics. As displayed in Fig 9, the physicochemical space of ZINC10635972 is shown in the coloured zone.

The drug-likeness properties of the lead molecule were examine using ADMET predictors (Absorption (A), Distribution (D), Metabolism (M) and Excretion (E)). The analysis gives insight on the possible behaviour of the inside an organism and consequently, impacts the pharmacological activity of the drug. The screening was performed based on a possible violation of fundamental rules such as Lipinski, Ghose, Egan, Verber and Muegge rules using the SWISS-ADME online webserver. However, 0.05 bioavailability score of the lead molecule was estimated, it suggests readily availability of the pharmacophore within the system of an organism. As shown in Table V, the lead molecule was noticed to have 1 violation for Egan and Verber. On the contrary, Lipinski’s, Lipinski, Ghose, and Muegge rules were not violated. Hence, the lead molecule had demonstrated moderate drug-like characteristics. Meanwhile, the lead molecule was noticed to exhibit good solubility (see Table III). As shown in Table IV, ZINC10635972 did not inhibit CYP 1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isoenzyme. However, it was not inhibited by ZINC72170473 (see Table IV). This indicates the elimination of drug-drug interactions, hence, metabolites accumulation within the biosystem is on check. This is own to the fact that the lead molecule, ZINC10635972 did not inhibit liver metabolism by inhibiting CYP1A2, it did not interfere with the metabolism of some therapeutic drugs especially, anti-ulcer, antimalarial, anti-convulsant, anaesthetic and sedative drugs by impeding CYP2C19, it does not stop metabolism of anti-hypersensitive drugs, b blockers, anti-arrhythmic drugs and anti-depressants through the inhibition of CYP2D6, also ZINC10635972 did not stop the metabolism of anti-clotting agents, anti-seizure, management of type-II diabetes, anti-hypertensive, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) by interfering with CYP2D9 and finally did not hinder the oxidation of steroids, fatty acids and xenobiotics as well as for hormone synthesis and breakdown by inhibiting CYP3A4.
The blood-brain barrier (BBB) and passive gastrointestinal absorption (HIA) are pharmacokinetic properties of druglike compounds. The boiled egg model was used to validate the penetration tendency of ZINC10635972 when localized at the interaction site that is barricaded by a permeable membrane. The white and yellow (yolk) region represent the high probability of passive absorption by the gastrointestinal tract and high probability of brain penetration respectively. Fig 9, shows that ZINC10635972 will have high gastrointestinal absorption and easily permeate the blood-brain barrier.

The toxicity prediction indicates that ZINC10635972 is safe and can be given as a drug. As shown in Fig 11, ZINC10635972 was noticed to fall into toxicity class 5 with a similarity index of 51.07% and average accuracy of 67.38%.

Listed in Table IV, the chemical hardness of ZINC10635972 was observed to be >1, this indicates high reactivity of the lead molecule. Hence, ease-interaction with the target molecule.

### IV. Conclusion

In conclusion, molecules retrieve from ZINC database via virtual screening technique were successfully docked against SARS-CoV-2 protein with PDB code 5r7y. The outcome of the docking study showed that ZINC10635972 (-6.3 kcal/mol) had a higher affinity for the target protein than the reference drug (+28.2 kcal/mol). The lead molecule demonstrated good pharmacokinetics properties and drug-like characteristic. Hence, ZINC10635972 is a promising compound worthy of testing for biochemical assays.

### Acknowledgment

Appreciation is extended to the Government of Abia State, Nigeria for her support in this research.

### References


Haider, Z., et al., In Silico discovery of novel inhibitors against main protease (Mpro) of SARS-CoV-2 using pharmacophore and molecular docking based virtual screening from ZINC database. 2020, Preprints.


Dr O.V.Ikepeazu, obtained BSc [Unical] and MSc in Biochemistry at University of Maidaguri, Nigeria in 1984 and 1990 respectively. He obtained his Ph.D in Pharmacological Biochemistry and Toxicology at University of Calabar in Nigeria in 1994. Dr Ikepeazu has lectured in Enugu State University of Science and Technology; Ebonyi State University and Abia State University, Uturu, in Nigeria. He is a member of Nigerian Association of Biochemistry and Molecular Biology.

Dr. F. J. Amaku, obtained BSc pure and applied chemistry from Michael Okpara University of Agriculture, Umudike, Nigeria. MSc in physical chemistry from the University of Badan Oyo State, and Ph.D degree in Chemistry from the University of KwaZulu-Natal Durban, South Africa. He is a member of South African Chemical Institute (SACI) and an Associate Member Royal Society of Chemistry (RSC). He is a lecturer in the department of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

Dr. I. E. Otuokere, is a lecturer in the Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Abia , Nigeria. He obtained his B.Sc degree in Pure and Industrial Chemistry in the year 2000. He bagged his M.Sc degree in Inorganic Chemistry in 2005 and a Ph.D degree in Inorganic Chemistry in 2008. All his degrees are from Nnamdi Azikiwe University, Awka, Nigeria. He is a member of the Chemical Society of Nigeria.