Drug Properties and Drug Ligand-Binding Comparison Analysis on Tenofovir and Zidovudine as a Reverse Transcriptase Inhibitor of HIV-1

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ABSTRACT

Objective: The human immunodeficiency virus (HIV) infection has been a public health concern with no available cure. It is recommended for HIV patients to be supplied with antiretroviral therapy (ART) as their lifelong treatment to help reduce the course of this disease. This paper utilized bioinformatics approaches to examine tenofovir and zidovudine as an inhibitor of reverse transcriptase (RT) enzyme in HIV-1.
Material and methods: The 3D Model of the RT enzyme was generated using Swiss-Model Expasy from the FASTA amino acid sequence obtained from Protein Data Bank (PDB). The enzyme then went through several modifications using PyMOL before inserting them into CASTp: Computed Atlas of Surface Topography of Proteins active site prediction software, as well as PyRx (Python Prescription Virtual Screening Tool) and BIOVIA Discovery Studio 2021 for molecular docking. PreADMET analysis was used to determine the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the two drugs.
Results: The results from molecular docking revealed that tenofovir possessed higher binding affinity towards HIV-1 RT rather than zidovudine. ADMET analysis showed that tenofovir have better Pgp-inhibitor absorption and blood brain barrier (BBB) distribution than zidovudine. Meanwhile, zidovudine possessed higher Fu with carcinogenic properties.
Conclusion: Both drugs exhibited poor at Caco-2 absorption with high passive MDCK permeability, tested positive for human intestinal absorption (HIA), have up to 30% bioavailability, proper plasma protein binding (PPB) and volume distribution (VD), may act as both CYP substrate and inhibitor, have moderate clearance (CL), long half-life ($T_{½}$), and possessed different toxicity and allergic properties.

Keywords: HIV-1, reverse transcriptase (RT), binding affinity, molecular docking, ADMET

INTRODUCTION

The human immunodeficiency virus (HIV) is a virus that targets the immune system of the body, spreading through sexual intercourse, blood transfusions, sharing of needle usage, organ transplantation, open injuries, or horizontal spread from mother to infant during birth or breastfeeding (1-2). HIV replicates on helper T cells by attaching to the CD4 molecule. This results in the annihilation of CD4+ T-cells, causing a steady decline in CD4+ T-cell population and a weakened immune system (3). Without proper antiretroviral treatment, an HIV infection might advance into acquired immunodeficiency syndrome (AIDS). Among other infectious diseases, HIV remains to be one of the most challenging diseases to cure. This is due to the ability of HIV to integrate its genetic material into the host genome. The HIV genome itself consists of two identical single-stranded RNA molecules. Reverse transcriptase (RT) is one of the enzymes encoded by the pol gene in the viral genome, essential for viral replication inside the host cell. Upon cell entry, the single-stranded HIV RNA genome is transcribed into DNA by RT, in which then the RNA strands are also parallelly destroyed enzymatically by RNase. Single-stranded cDNA is converted to double-stranded DNA (proviral DNA) and further incorporated into the host genome utilizing integrase, resulting in HIV infection of the host cells (1,4). The activity of RT is highly error-prone, able to produce one to ten mutations on every mutation cycle, which may encourage HIV mutations that are drug resistant or HIV with a higher infectivity rate (5). Moreover, establishment of a silent or latent infection in the resting CD4+ T cells can also take place, where the infected cells could spread and remain concealed across the

body including the anatomical reservoirs (i.e. brain, genitourinary tract, gastrointestinal tract). Hence, residual viral replication could take place although the virus could not be detected through conventional assays (6). Since no known cure has been found to eradicate HIV infections, antiretroviral therapy (ART) has become a recommended life-long treatment aiming to disrupt viral replication process and eventually delay disease progression. Currently, there are eight ART drugs namely zalcitabine (ddC, Hivid), lamivudine (3TC, Epivir), abacavir (ABC, Ziagen), emtricitabine (FTC, Emtriva), didanosine (ddI, Videx), stavudine (d4T, Zerit), zidovudine (AZT, Retrovir), and Tenofovir disoproxil fumarate (TDF, Viread) have been approved by the FDA (5,7). This study focuses on tenofovir and zidovudine as the ART drugs that target the HIV-1 RT enzyme as a treatment for HIV.

Tenofovir is an ART most often administered as tenofovir disoproxil fumarate. Following its absorption, tenofovir disoproxil fumarate is rapidly converted into tenofovir, which is then metabolized intracellularly into its active anabolite tenofovir diphosphate (8). In in vitro trials, tenofovir displays good inhibitory activity against HIV strains, as well as synergistic or additive activity when it is combined with certain other antiretroviral drugs. Not only that, it shows minimal cytotoxicity and no evidence of reduced mitochondrial DNA synthesis (9).

Zidovudine is an ART with an azido group in place of the 3' hydroxyl group on the sugar moiety. This substitution hinders the formation of phosphodiester links, which are essential to complete nucleic acid chains. After oral intake, it is promptly completely absorbed in the gastrointestinal tract. Zidovudine competitively inhibits HIV's reverse transcriptase enzyme, which is required for the synthesis of the proviral double-stranded DNA from the RNA template, functioning as a chain terminator of DNA synthesis after integrating itself into the nucleotide analogue. The lack of a 3'-OH group in the integrated nucleoside analogue, which hinders the creation of a 5' to 3' phosphodiester linkage also stops viral DNA from growing. This connection is required for DNA chain elongation, stopping it from forming and preventing the growth of viral DNA (10).

In this study, several *in silico* analyses were done to examine the pharmacological properties of tenofovir and zidovudine, as well as their binding affinity with HIV-1 RT enzyme. The binding affinity analysis of both drugs with the RT enzyme was done with PyRx and BIOVIA for molecular docking and visualization. Furthermore, the absorption, distribution, metabolism, excretion, and toxicity of both drugs were also examined utilizing preADMET.

MATERIALS AND METHODS

Molecular Structure Retrieval

The Swiss-Model Expasy (Biozentrum of the University of Basel Klingelbergstrasse, Basel, Switzerland) was first utilized to generate the 3D structure of the HIV reverse transcriptase enzyme using the amino acid sequence (FASTA) obtained from the Protein Data Bank (PDB) database (PDB code: 2ZD1) with default value parameters. The PDB format of the second (02) model generated was utilized for further analysis. Furthermore, the molecular structures of tenofovir and zidovudine were obtained from the Drugbank website, utilizing PDB and SMILES formats for molecular docking and drug properties analysis, respectively.

Drug Properties Analysis

The drug properties analysis was made using the preADMET 2.0 (Yonsei University, Incheon, Republic of Korea) website. All the processes and mechanisms including absorption, distribution, metabolism, excretion, and toxicity of the drugs was extracted using its web server under default parameters. For ADME and toxicity data, their chemical structure was manually drawn using ChemDoodle, provided by preADMET. The structure followed the PubChem database as reference.

RT Enzyme Modification

PyMOL 2.5.2 (DeLano Scientific LLC, South San Francisco, California USA) was used to modify the 3D structure of the previously obtained enzyme, which includes the removal of any solvent or bound molecules, and to minimize the protein by adding hydrogen. The solvent removal was done with the command of “PyMOL> remove solvent”. Any other molecules were clicked and removed from the enzyme. Furthermore, hydrogen was added with the command of “PyMOL> h_add”. The PDB format of the modified enzyme was then utilized for further analysis.

Active Site Prediction

The modified enzyme was uploaded to the CASTp: Computed Atlas of Surface Topography of Proteins 3.0 (University of Minnesota, Minneapolis, USA) (11) website for active site prediction with the default value parameters. Following that, the anticipated results were shown in a three-dimensional model, with the active residues for the binding site highlighted in gray in the Sequence section. Additionally, CASTp also features a viewing list of all pockets that are presently active.

Molecular Docking and Visualization

Molecular docking with PyRx 0.8 (Department of Integrative Structural and Computational Biology, California, USA) was performed to examine the interaction and binding affinity between RT enzymes with tenofovir and zidovudine (12). Structures of the target molecules were required as input files in PDBQT to begin structure-based virtual screening, which include the drug molecules (ligand) and the RT enzyme (macromolecule). The Vina Search Space for both drugs and the RT enzyme were maximized, with center of X: 133.1565, Y: -1.1219, and Z: 71.8217, and dimensions (Å) of X: 108.9952, Y: 77.4336, and Z: 82.7230. The binding affinity result was shown in the form of table. The PyRx docked PDBQT files were then inserted into BIOVIA Discovery Studio Visualizer 2021 21.1.0 (Dassault Systèmes, Vélizy-Villacoublay, France) to view the 3D binding and to assess the specific drug and RT enzyme binding sites. The enzyme was firstly defined as the receptor before visualizing both 2D diagram and 3D ligand interactions.

RESULTS

Preadmet Drug Analysis

The ADMET analysis of both tenofovir and zidovudine was performed using ADMETlab 2.0 by showing each property and predicted value which were used to confirm whether the ADMET results shown are proper or not. The tables below show the results of the properties and predicted value of ADMET analysis of tenofovir and zidovudine.

Table 1. ADMET Analysis Results of Tenofovir using ADMETlab 2.0

Absorption	Distribution	Metabolism	Excretion	Toxicity
Caco-2 Permeability (-5.855)	PPB (88.42%)	CYP1A2 inhibitor (0.954)	CL (7.764)	hERG Blockers (0.04)
MDCK Permeability (8×10^{-6})	VD (0.459)	CYP1A2 substrate (0.239)	T ½ (0.884)	H-HT (0.574)
Pgp-inhibitor (0.815)	BBB (0.553)	CYP2C19 inhibitor (0.948)		DILI (0.931)
Pgp-substrate (0.003)	Fu (2.918%)	CYP2C19 substrate (0.068)		AMES Toxicity (0.32)
HIA (0.005)		CYP2C9 inhibitor (0.877)		Rat Oral Acute Toxicity (0.315)
F20% (0.004)		CYP2C9 substrate (0.548)		FDAMDD (0.871)
F30% (0.008)		CYP2D6 inhibitor (0.042)		Skin sensitization (0.84)
				Carcinogenicity (0.556)
				Eye corrosion (0.009)

				Eye Irritation (0.528)
				Respiratory toxicity (0.915)

Table 2. ADMET Analysis Results of Zidovudine using ADMETlab 2.0

Absorption	Distribution	Metabolism	Excretion	Toxicity
Caco-2 Permeability (-5.795)	PPB (10.495%)	CYP1A2 inhibitor (0.008)	CL (6.172)	hERG blockers (0.003)
MDCK Permeability (8.5×10^{-5})	VD (0.754)	CYP1A2 substrate (0.108	$T_{½}$ (0.951)	H-HT (0.984)
Pgp-inhibitor (0.07)	BBB Penetration (0.862)	CYP2C19 inhibitor (0.814)		DILI (0.975)
Pgp-substrate (0.011)	Fu (76.94%)	CYP2C19 substrate (0.021)		AMES toxicity (0.999)
HIA (0.008)		CYP2C9 inhibitor (0.024)		Rat Oral Acute Toxicity (0.003)
$F_{20\%}$ (0.006)		CYP2C9 substrate (0.799)		FDAMDD (0.019)
$F_{30\%}$ (0.064)		CYP2D6 inhibitor (0.003)		Skin sensitization (0.584)
		CYP2D6 substrate (0.091)		Carcinogenicity (0.942)
		CYP3A4 inhibitor (0.009)		Eye corrosion (0.003)
		CYP3A4 substrate (0.705)		Eye irritation (0.048)
				Respiratory toxicity (0.901)

Table 1 shows the properties and predicted value of ADMET analysis of tenofovir. For the tenofovir absorption, the Caco-2 permeability has a value of -5.855 and MDCK permeability has a value of 8×10^{-6} cm/s. Moreover, the Pgp-inhibitor predicted value is 0.815. Next parameters are Pgp-substrate, HIA, F20%, and F30% which produced results of 0.003, 0.005, 0.004, and 0.008 respectively. Furthermore, the tenofovir distribution shows that the PPB has a value of 88.42%. Volume Distribution (VD) and Blood-Brain Barrier (BBB) have a value of 0.459 and 0.553 consecutively. Lastly, fraction unbound (Fu) has a value of 2.918%

In the tenofovir metabolism, there are CYP1A2 inhibitor, CYP1A2 substrate, CYP2C19 inhibitor, CYP2C19 substrate, CYP2C9 inhibitor, CYP2C9 substrate, and CYP2D6 inhibitor that has a value within the range of 0 to

1. For excretion, there are clearance (CL) and half-life ($T_{½}$) that have values of 7.764 mL/min/kg and 0.884 sequentially. The tests for hERG blockers, H-HT, and AMES toxicity, possessed values of 0.04, 0.574, and 0.32 respectively. Moving on, rat oral acute toxicity, carcinogenicity, eye irritation and corrosion hold values of 0.315, 0.556, 0.528, and 0.009 accordingly. Additionally, the tests for DILI, FDAMDD, skin sensitization and respiratory toxicity all showed high values of 0.931 0.871, 0.84, and 0.915.

Based on Table 2, it can be seen that zidovudine has a Caco-2 permeability value of -5.975. The MDCK permeability is at 8.5 x 10^{-5} cm/s. The result also revealed that zidovudine possessed low Pgp-inhibitor and Pgp-substrate values of 0.07 and 0.011. Lastly, HIA, F20%, and F30% have values of 0.008, 0.006, and 0.064. Furthermore, the distribution of zidovudine is found to have a PPB value at 10.495%, alongside the Volume Distribution (VD) of 0.754 and Blood-Brain Barrier (BBB) which of 0.862. Fraction unbound (Fu) with 76.94%.

The metabolism analysis of zidovudine shows CYPs inhibitor values within the range of 0 to 1. Zidovudine also showed a clearance (CL) value of 6.172 mL/min/kg, supported by a half life ($T_{½}$) of 0.951. Lastly, zidovudine hERG blockers and FDAMDD have low values of 0.003 and 0.019 respectively. In regards to the tests for hERG blockers, H-HT, and AMES toxicity, possessed values of 0.003, 0.984, and 0.999 respectively. Rat oral acute toxicity, carcinogenicity, eye irritation and corrosion hold values of 0.003, 0.942, 0.048, and 0.003 accordingly. Moreover, DILI and FDAMDD have values of 0.975 and 0.019. Lastly, skin sensitization and respiratory toxicity showed values of 0.584 and 0.901.

Active Site Prediction

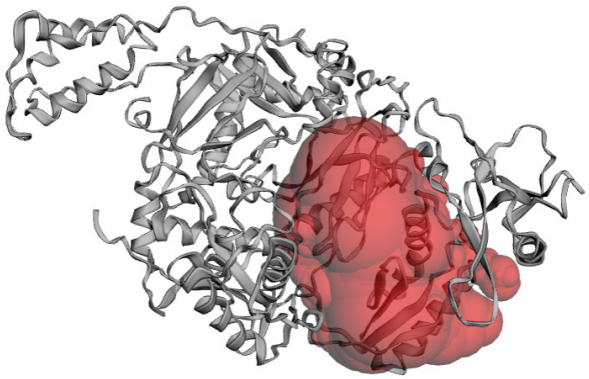

Figure 1. CASTp Active Site Residues Pocket 1

The 3D structure of the RT enzyme was first generated using Swiss Model Expasy using the amino acid sequence obtained from PDB, since the original PDB file was not able to show any result when inputted in the program. Afterwards, the protein was modified using PyMol to remove any solvent, bound molecules, and to minimize the protein through the addition of hydrogen. The active site prediction was then done using CASTp, which showed the predicted active site pockets that were then used to confirm whether the molecular docking drug binding results are located on the predicted active sites. Figure 1 represents the active site (Pocket 1) marked with the color red where the drug binds to the enzyme. Moreover, Table 3 and 4 below depict the amino acid binding sites where tenofovir and zidovudine were successfully bound to (Refer to Figure 2 and Figure 5 for molecular docking results).

Molecular Docking and Visualization

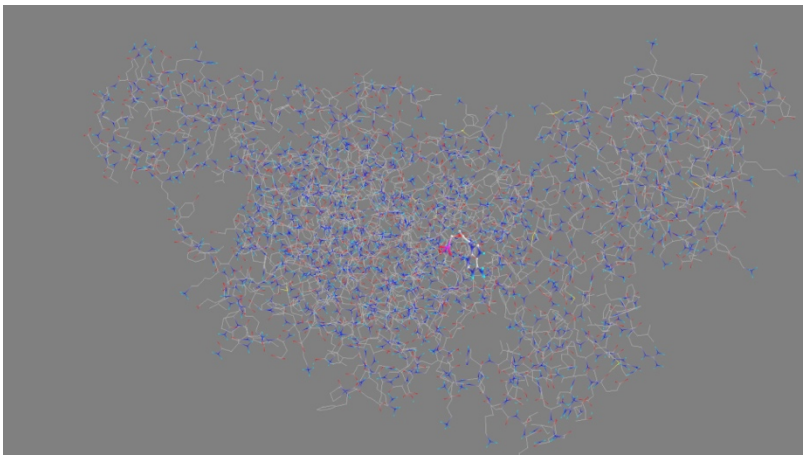

Figure 2. PyRx Visualization Result of RT and Tenofovir

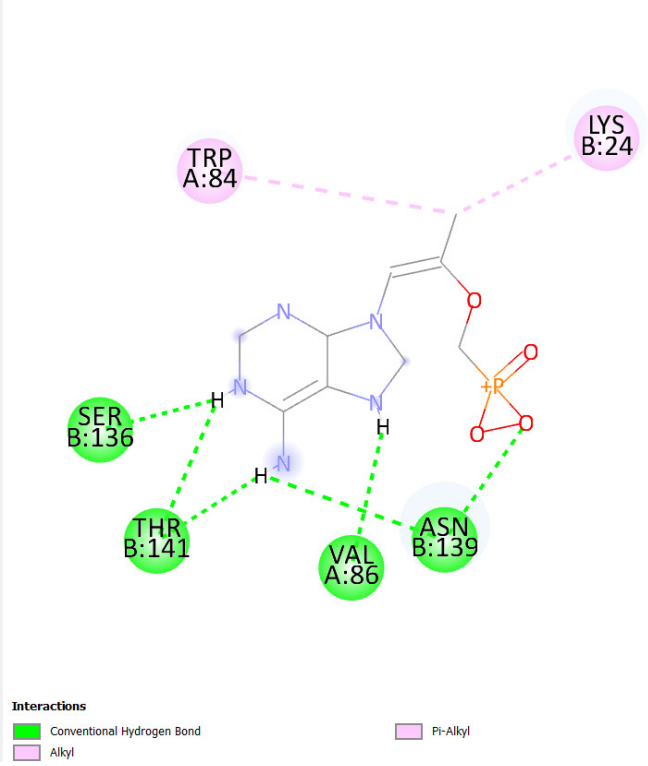

Figure 3. BIOVIA Binding Interactions Result of RT and Tenofovir

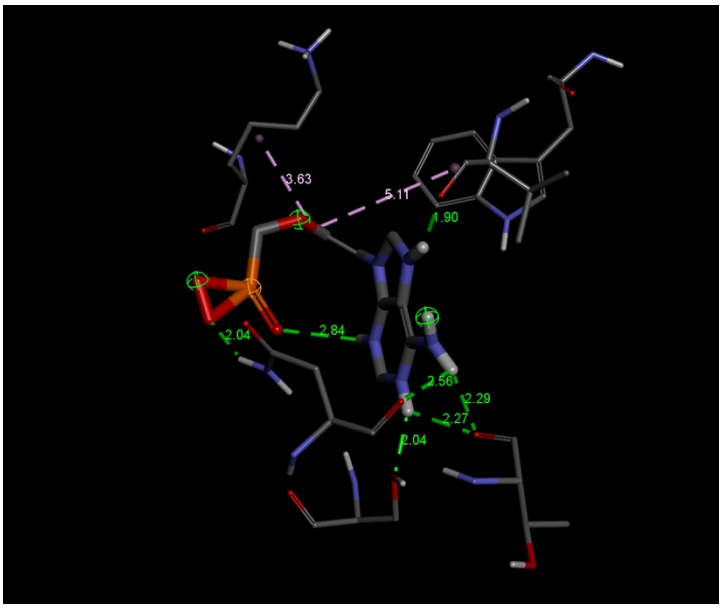

Figure 4. BIOVIA 3D Binding Distance Visualization Result of RT and Tenofovir

After molecular docking of the RT enzyme with tenofovir and zidovudine was conducted using PyRx Virtual Screening Tool and BIOVIA Discovery Studio, both 3D models of the RT enzyme were inserted alongside the drug structure in PyRx. The program displays the binding affinity results measured in kcal/mol

Table 3. RT Active Amino Acid Binding Sites with Tenovofir using CASTp

PocID	Chain	SeqID	AA	Atom
1	A	86	VAL	N
1	A	86	VAL	CA
1	A	86	VAL	C
1	A	86	VAL	O
1	A	84	TRP	C
1	A	84	TRP	O
1	A	84	TRP	CB
1	A	84	TRP	CD1
1	B	24	LYS	CB
1	B	24	LYS	CG
1	B	24	LYS	CD
1	B	136	SER	N
1	B	136	SER	CA
1	B	136	SER	O
1	B	136	SER	CB

1	B	139	ASN	CA
1	B	139	ASN	O
1	B	139	ASN	CB
1	B	139	ASN	ND2
1	B	141	THR	CA
1	B	141	THR	C
1	B	141	THR	O
1	B	141	THR	CB

Table 4. RT Active Amino Acid Binding Sites with Zidovudine using CASTp

PocID	Chain	SeqID	AA	Atom
1	B	24	LYS	CB
1	B	24	LYS	CG
1	B	24	LYS	CD
1	B	139	ASN	CA
1	B	139	ASN	O
1	B	139	ASN	CB
1	B	139	ASN	ND2
1	A	86	VAL	N
1	A	86	VAL	CA
1	A	86	VAL	C
1	A	86	VAL	0
1	A	86	VAL	CB
1	A	86	VAL	CG1
1	A	86	VAL	CG2
1	B	141	THR	N
1	B	141	THR	CA
1	B	141	THR	C
1	B	141	THR	O
1	B	141	THR	CB
1	B	141	THR	OG1
1	B	141	THR	CG2

The highest and lowest binding affinity of RT and tenofovir were found to be -7.2 and -5.8 kcal/mol, respectively. PyRx also provides visualization of the drug binding with the enzyme, which is provided in Figure 2. Moreover, the PDBQT version of the RT enzyme and tenofovir were inserted into BIOVIA for further amino acid binding visualization. There were a total of six amino acids that had binding interactions with tenofovir on pocket one with specific amino acid binding locations. The letter below the amino acid represents the protein chain (A or B) on which it is found, whereas the number denotes the amino acid sequence. Serine 136, Threonine 141, Valine 86, and Asparagine 138 formed a conventional hydrogen bond interaction. The conventional hydrogen bonds differ in distance between each amino acid, starting from the range of 1.90 to 2.84 Å (Figure 4). Moreover, Tryptophan 84 and Lysine 24 were found to form alkyl interaction with the drug, with a longer distance of 3.63 and 5.11 Å (Figure 3 and 4). These findings can further be supported by the active site prediction result from CASTp (Table 3), where Serine 136, Threonine 141, Valine 86, and Asparagine 138, Tryptophan 84 and Lysine 24 are found to be the active binding residues of the protein. Hence, tenofovir is able to bind with the active site of the protein.

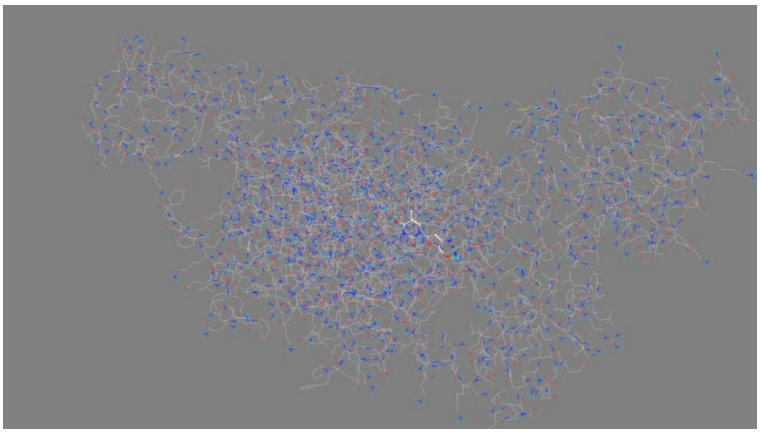

Figure 5. PyRx Visualization Result of RT and Zidovudine

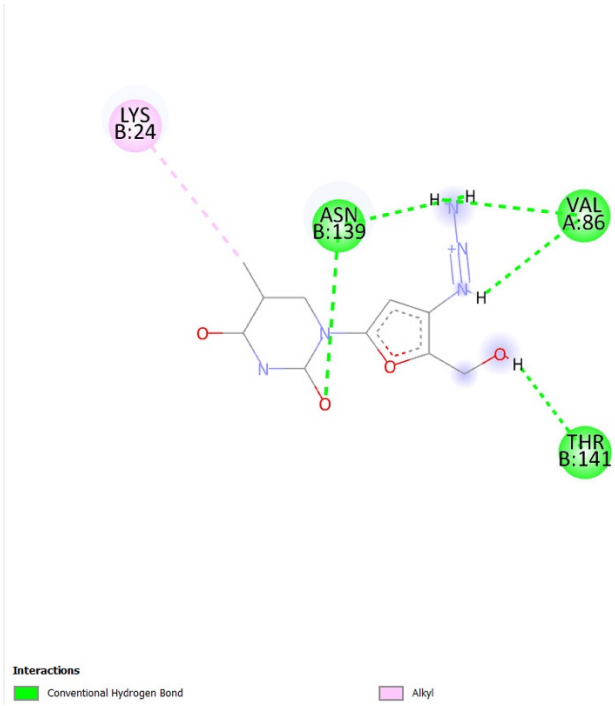

Figure 6. BIOVIA Binding Interactions Result of RT and Zidovudine

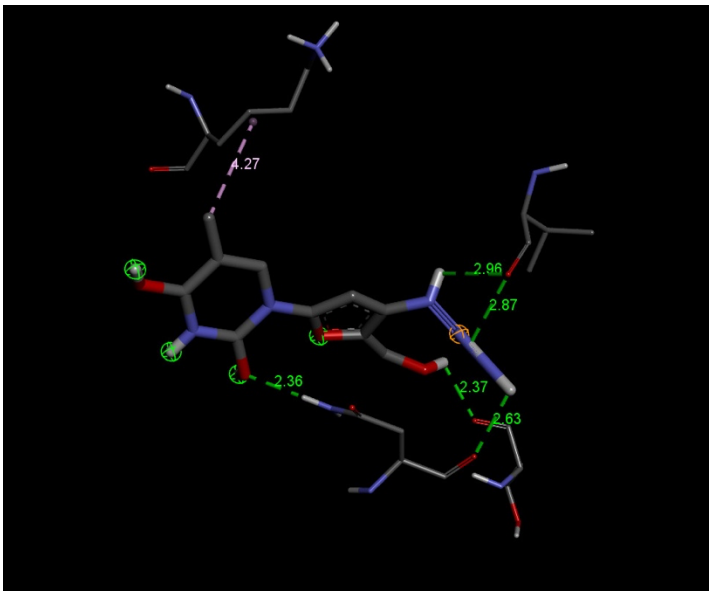

Figure 7. BIOVIA 3D Binding Distance Visualization Result of RT and Zidovudine

Meanwhile, the highest and lowest binding affinity of RT and zidovudine were found to be -7.0 and -5.2 kcal/mol, respectively. In comparison with tenofovir, zidovudine has lower binding affinity with RT. A visualization of zidovudine binding with the enzyme can be found in Figure 5. Similar to tenofovir, the PDBQT version of the RT enzyme and zidovudine were also inserted into BIOVIA for further amino acid binding visualization. Zidovudine also possessed fewer binding interactions with the RT enzyme than tenofovir. There were a total of four amino acids that had binding interactions with zidovudine on pocket one with specific amino acid locations. Similar with tenofovir, the enzyme also shared conventional hydrogen bond and alkyl interactions with zidovudine. Valine 86, Asparagine 139, and Threonine 141 formed a conventional hydrogen bond interaction with varying distance from 2.36 to 2.96 Å, whereas only Lysine 24 formed alkyl interaction with the drug with a distance of 4.27 Å (Figure 6 and 7). Furthermore, these findings can also be supported by the active site prediction result from CASTp (Table 4), where Valine 86, Asparagine 139, Threonine 141, and Lysine 24 were found to be the active binding residues of the protein. Hence, zidovudine is also able to bind with the active site of the protein, but with fewer binding sites than tenofovir.

Table 5. PyRx Binding Affinity Result of RT and Tenofovir

Ligand	Binding affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
RT_tenofovir	-7.2	0	0.0	0.0
RT_tenofovir	-7.2	1	3.678	4.932
RT_tenofovir	-6.6	2	3.527	5.159
RT_tenofovir	-6.4	3	1.635	2.618
RT_tenofovir	-6.3	4	3.747	5.43
RT_tenofovir	-6.0	5	1.563	1.993
RT_tenofovir	-5.9	6	4.165	6.02
RT_tenofovir	-5.9	7	3.497	5.527
RT_tenofovir	-5.8	8	4.74	6.736

Table 6. PyRx Binding Affinity Result of RT and Zidovudine

Ligand	Binding affinity (kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
RT_zidovudine	-7.0	0	0.0	0.0
RT_zidovudine	-6.0	1	2.803	6.772
RT_zidovudine	-5.9	2	2.596	7.458
RT_zidovudine	-5.8	3	2.145	2.66
RT_zidovudine	-5.7	4	2.701	7.08
RT_zidovudine	-5.6	5	2.654	7.016
RT_zidovudine	-5.5	6	2.814	7.403
RT_zidovudine	-5.3	7	10.567	11.82
RT_zidovudine	-5.2	8	2.71	7.41

DISCUSSION

In 2020, the World Health Organization (WHO) estimated that 37.7 million individuals worldwide were living with HIV, with a total of 1,500,000 new cases and 680,000 deaths occurring throughout the same year. Due to the difficulty in curing the disease, the only option left for patients is to take ART as their lifelong-treatment. It was estimated that 73% of the total HIV patients in 2020 were receiving ART. This study specifically examines tenofovir and zidovudine, the two drugs that work by interfering with the activity of RT in HIV-1. As a comparison, tenofovir-based drugs are marketed at a remarkably lower price than zidovudine. Tenofovir itself is a medication which is not only used to treat HIV, but also for other viral infections (i.e. hepatitis B). Meanwhile, zidovudine was only used to treat HIV. Moreover, zidovudine itself is no longer frequently used as the first line of treatment for HIV; instead, it is combined with other ART agents, such as lamivudine (13-15).

Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) are important factors in drug discovery and development. A high-quality drug candidate should not only be effective against the therapeutic target, but should also exhibit adequate ADMET qualities at a therapeutic dose. Thus, many *in silico* models are being created to predict chemical ADMET features (16). To analyze the absorption of both drugs, the parameters used were Caco-2 and MDCK permeability, Pgp-inhibitor and substrate, HIA and $F_{20\%}$ and $F_{30\%}$. The human colon carcinoma cell line, otherwise known as Caco-2, is a human intestinal cell line that can be used to predict the absorption of oral drugs *in vitro*. When these cells are grown on a permeable filter, they are used as an *in vitro* method to assess intestinal permeability and absorption (17). As seen in the results, any value for Caco-2 lower than of -5.15 log cm/s is considered not optimal to be absorbed by the intestines. Tenofovir and zidovudine showed values of -5.855 and -5.795 respectively, indicating that they are lower than the optimal log value of -5.15 and are therefore poorly absorbed by the intestines. Similarly, MDCK, or Madin-Darby canine kidney, is a cell line used to model the renal distal tubule of the kidney (18), and is used to study drug efflux, passive permeability and active transport (19). Drugs that show a value of higher than 20×10^{-6} cm/s in ADMET analyses are considered to have high passive permeability, such as tenofovir and zidovudine with MDCK permeability values of 8×10^{-6} and 8.5×10^{-5} cm/s respectively. Furthermore, the parameters Pgp-inhibitor and Pgp-substrate are also used to assess the body's ability to absorb and distribute uptaken substances and drugs. P-glycoprotein is an efflux membrane transporter, and when inhibited, improves the delivery of drugs. This allows for higher drug bioavailability and better uptake of the drug for the targeted organ (20). Therefore, if the Pgp-inhibitor value is high, it indicates the probability of the drug being a Pgp-inhibitor is high, which may lead to increased drug bioavailability. For instance, tenofovir has a 0.815 probability of being a Pgp-inhibitor compared to zidovudine with only a 0.07 probability of being a Pgp-inhibitor. Since the inhibition of Pgp results in a higher

absorption, this means that tenofovir is absorbed better by the body than zidovudine. Additionally, both tenofovir and zidovudine showed low probabilities of being Pgp-substrates (0.03 and 0.011 accordingly), which may also lead do increased bioavailability. Another parameter is human intestinal absorption (HIA). Both HIA values for tenofovir and zidovudine were found to be lower than 0.3 (0.005 and 0.008), indicating that the drugs are HIA positive and can be absorbed by the intestinal membrane (21). Lastly, F20% $_a$ and F30% represent the 20% and 30% oral bioavailability respectively (22). When the value of F20% is less than 0.02 or 20%, it is considered F20% positive and the drug has a bioavailability of up to 20%. Similar to F30%, when its value is less than 0.03 or 30%, it is considered as F30% positive and the drug also has up to 30% bioavailability (23). Tenofovir and zidovudine have positive F20% values of 0.004 and 0.006 respectively. Moreover, tenofovir and zidovudine are also found to be positive for F30% with values of 0.008 and 0.064, indicating that they have bioavailability in the systemic circulation up to 20% and 30%.

Beside that, to analyze the distribution of both drugs, the parameters used were PPB, VD, BBB, and Fu. The PPB, also known as plasma protein binding, is the binding of a drug to proteins in plasma, and it is one of the most important steps of drug absorption, and consequently has a considerable influence on the pharmacodynamics of a drug. The free concentration of drugs are greatly reduced when they bind to serum proteins, therefore PPB have a direct impact on oral bioavailability (24). Tenofovir and zidovudine were found to have PPB values lower than 90% (88.42% and 10.495%, respectively), indicating that they have proper PPB with a sufficient amount of free-drug concentrations. VD also known as volume distribution is a theoretical term that ties the supplied dose to the actual starting concentration present in the circulation, and it is an important quantity for describing *in vivo* drug distribution (25). A compound with good VD should be within the range of 0.04-20 L/kg. Tenofovir and zidovudine possessed VD values of 0.459 and 0.754 L/kg, which are all within the proper range. Moreover, BBB, also known as blood-brain barrier, are the blood vessels that vascularized the central nervous system (CNS) for drugs that act with a peripheral target. The BBB value for tenofovir was found to be 0.553 cm/s, which is within the medium BBB distribution of 0.3-0.7 cm/s. However, the BBB value for zidovudine was found to be 0.862 cm/s, which are considered to be poor BBB distribution within 0.7-1 cm/s (26). Lastly, fraction unbound (Fu) drugs in plasma will be in an equilibrium condition between being unbound or bound to serum proteins. The Fu of tenofovir has a value of 2.918% which is lower than the optimal value of 5%, indicating the majority of the drug remains bound with proteins in the bloodstream and is less efficient. On the other hand, zidovudine is considered to have a high Fu value of 76.94%.

To analyze the metabolism of both drugs, the parameters used were CYP1A2 inhibitor and substrate, CYP2C19 inhibitor and substrate, CYP2C9 inhibitor and substrate, CYP2D6 inhibitor and substrate. The values of all properties for both tenofovir and zidovudine are within the range of 0 to 1, indicating that the drugs have a probability of being substrate or inhibitor. Moreover, clearance (CL) and half-life (T½) are both important parameters that can be utilized to examine the excretion of both drugs. A drug is considered to have moderate CL if the value is on the range of 5-15 ml/min/kg, and high CL if it is above 15 ml/min/kg. CL values for tenofovir and zidovudine 7.764 and, 6.172 mL/min/kg respectively, which indicate that have moderate clearance. Meanwhile, T½ values for tenofovir and zidovudine were 0.884 and 0.951, which are within the long half-life value of 0.7-1.0.

To analyze toxicity of both drugs, the parameters used were hERG blockers, H-HT, DILI, AMES toxicity, FDAMDD, carcinogenicity, and eye corrosion and irritation, respiratory toxicity, rat oral acute toxicity, and lastly skin sensitization. The human *ether-a-go-go*-related gene (hERG) gene that codes for K^+ channels is crucial for maintaining proper ventricular action potential repolarization in human cardiac myocytes. hERG can be blocked by both cardiac and non-cardiac drugs, making it an important factor to consider when developing drugs (27). When these channels are blocked, they disrupt the cardiac action potential repolarization, which can cause an increase in cardiac arrhythmias (28). A low value of hERG blockers indicates that there is a low probability the drug will block the hERG gene. In the ADMET analysis, the probability that tenofovir and zidovudine would block the hERG gene were 0.04 and 0.003 respectively. These values are considered to be very low (within 0-0.3), especially for zidovudine, indicating that these drugs are not likely to induce toxicity in this aspect and will not affect the hERG gene and cause arrhythmias.

Human hepatotoxicity (H-HT) and drug induced liver injury (DILI) in ADMET analysis are parameters that measure the probability that a drug can induce liver injury that manifests in different forms, such as acute liver failure, hepatitis or cholestasis (29). A value of 0 is considered to be HT-T and DILI negative, while a value of 1 is considered to be HT-T and DILI positive. According to the ADMET analysis, tenofovir has a 0.574 probability of causing human hepatotoxicity, which is lower compared to zidovudine with a probability of 0.984 to be

hepatotoxic, indicating that zidovudine is much more likely to cause toxic reactions in the liver compared to tenofovir. This injury is caused by the inhibition of mitochondrial gamma polymerase, which causes mitochondrial depletion and dysfunction. This in turn will result in microvesicular fat, hepatic failure and lactic acidosis (30). In terms of DILI, tenofovir and zidovudine both have high probability of causing liver injury, with probabilities of 0.931 and 0.975 respectively. Furthermore, AMES toxicity is a test using the bacteria *Salmonella typhimurium* to identify mutagenicity in bacteria (31). Similar to other toxicity parameters, the values shown for AMES test and carcinogenicity in the ADMET analysis indicates the probability of the drug being toxic and carcinogenic. The ADMET analysis shows that tenofovir has a probability of 0.32 of being AMES toxic, compared to zidovudine with an extremely high value of 0.999, indicating that zidovudine is extremely AMES toxic and can cause complications such as DNA mutations that may lead to cancer. Other factors such as FDAMDD, or Food and Drug Administration Maximum (Recommended) Daily Dose, is a measure of the maximum daily dose of a drug. The values shown in the ADMET analysis indicate the probability of the two drugs being positive, which are 0.871 and 0.019 for tenofovir and zidovudine respectively (32). Tenofovir has a much higher probability of being toxic compared to zidovudine, therefore taking more than the maximum recommended daily dose can lead to toxicity when using tenofovir as treatment for HIV.

Lastly, eye corrosion and irritation, and respiratory toxicity are parameters used to measure tissue damage in the eyes and lungs respectively, which can be caused by use of medications. Based on the results of the ADMET analysis, it can be seen that neither tenofovir nor zidovudine are able to cause eye corrosion with values of 0.009 and 0.003, respectively, which are considered to be very low (within 0-0.3). However, when it comes to eye irritation, tenofovir is more likely to cause irritation because of its higher probability of 0.528 compared to zidovudine with only 0.048. Respiratory toxicity for both tenofovir and zidovudine are at a high probability of 0.915 and 0.901 respectively, therefore these drugs must be taken with care when dealing with respiratory issues (32). Acute toxicity is a test that evaluates the effects of a specific dose of a drug administered within a period of 24 hours via a specific route (33). As it can be seen in the ADMET analysis results, tenofovir has a higher probability of being toxic at 0.315, compared to zidovudine with only 0.003. Meanwhile, skin sensitization is the ability of the drug to induce an allergic response to a chemical or to a drug if it comes in contact with the skin (34). The values shown in the ADMET analysis also show the probability that the drug is toxic for consumption, which are 0.84 and 0.584 respectively for tenofovir and zidovudine. This could mean that zidovudine has a higher probability of causing allergic reactions on the skin after being taken or when the skin is exposed to the drug.
Active site prediction is key to understanding molecular interactions of a protein with other molecules such as drugs, and one of the most important factors required to predict binding sites is the protein's physicochemical properties (35). This study utilized CASTp to predict the active site of RT, where it shows the active protein pockets and amino acid sequences. The results from the active site prediction were then utilized for further confirmation whether the molecular docking drug binding results are located on the predicted active sites. Molecular docking itself is a long-established approach of drug development to determine the interaction between a ligand and a three-dimensional structure protein of interest (36). The utilization of drug ligand-binding models is crucial to understand the binding's relative effect (37). With PyRx and BIOVIA, the binding affinity and amino acid binding sites of tenofovir and zidovudine with HIV-1 RT were assessed. The lower the binding affinity (kcal/mol), the better the drug fits into the enzyme binding pocket, and the more stable the ligand-receptor complex obtained (38). The binding affinity itself is greatly influenced by molecular forces such as hydrogen bond and hydrophobic interactions, as well as surrounding solvent (35,39). Hydrogen bonds are not only crucial in making up protein and DNA structure, but they also serve as the key component in drug–receptor interactions. The geometry of hydrogen bonds itself is more variable, and their strength varies depending on the type of donor and acceptor, external environment, and the angle of interaction (40-41).

Through active site prediction and molecular docking, both tenofovir and zidovudine were found to interact with amino acids located on Pocket 1 of the protein. Additionally, tenofovir was found to have a higher affinity for the RT enzyme rather than zidovudine with more amino acid binding sites and molecular bondings, suggesting that tenofovir may bind more firmly with RT and prevent the active sites from binding with viral RNA and initiate the multiplication process. Other than binding affinity itself, drug efficacy is also a point to consider for treatment against HIV. Despite the fact that affinity and efficacy are two separate factors, the binding and activation processes are known to be linked with each other (42). Previous studies have revealed that routine administration of tenofovir was found to have higher performance against HIV infection (43). A retrospective study also found that tenofovir had higher efficacy in comparison to zidovudine (44). In China, HIV treatment using tenofovir is also referred to as a treatment due to its higher effectiveness and activity (45). Hence, more research is still needed to analyze the connection between binding affinity and efficacy of tenofovir and

zidovudine. All in all, this experiment revealed that tenofovir exhibits higher binding affinity, and as found by previous studies, possesses higher efficacy as a treatment against HIV infection.

The application of *in silico* analysis for advancement in drug discovery have several notable limitations. The generated 3D structure of the RT enzyme may contain several errors, which may render the result to be inaccurate (46). In the docking process, some intermolecular interactions such as solvation effect and entropy change predictions are somewhat inaccurate, while some such as halogen bonding and guanine-arginine interactions are rarely considered in scoring function, which have been proven to be significant. Transthyretin-thyroxine complex is one of the examples where molecular docking failed at making the correct prediction of binding mode. It is impossible to predict how many water molecules in the binding pocket will be replaced by potential ligands and how the hydrogen bonding network would be affected by ligand binding. Molecular docking by rigid receptor corresponds to single receptor confirmation, which can lead to false negatives. Lastly, activity spectra against off-target proteins are rarely seen on molecular docking computer screens and only dealt in animal and human trials (47).

CONCLUSION

The results from molecular docking revealed that tenofovir possessed higher binding affinity with more amino acid binding sites towards HIV-1 RT rather than zidovudine. Moreover, ADMET analysis showed that tenofovir have better Pgp-inhibitor absorption and BBB distribution than zidovudine. Meanwhile, zidovudine possessed higher Fu with carcinogenic properties. Both drugs were found to be poor at Caco-2 absorption with high passive MDCK permeability, tested positive for HIA, have up to 30% bioavailability, proper PPB and VD, may act as both CYP substrate and inhibitor, have moderate clearance, long half-life, and exhibited different toxicity and allergic properties.

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