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In silico analysis of cytotoxicity, rate of absorption and molecular docking of natural products against protease, integrase and HIV-1 reverse transcriptase

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Abstract: AIDS is an infectious disease characterized by compromised defense cells, is caused by Human Immunodeficiency Virus - HIV, which affects about 36.7 million people. In its viral multiplication process, HIV requires protease, integrase and reverse transcriptase which are important enzymes in the process. Many therapeutic alternatives in the anti-HIV treatment are in the inhibition of these enzymes, many researches are being directed towards the search of new inhibitors that present better pharmacological profiles. The use of natural products in anti-HIV research has been growing substantially, research groups are betting on these substances in an attempt to offer potent drugs with reduced side effects. The objective of this study was to carry out chemoinformatic studies using cytotoxicity risk prediction tools; prediction of absorption and molecular docking of natural products found in the database of chemical structures (ChEMBL) and literature. The interactions with the targets, the% ABS and cytotoxicological analysis were evaluated. 243 natural products and 18 anti-HIV drugs were analyzed. All molecules had their 3D structures optimized by the methods of mechanical molecules (MM+) and Semi-empirical methods (AM1) (RMS 0.1 kcal /Å.mol in 600 cycles) through HyperChemTM 8.0 software. Structures were imported into the software OSIRIS DataWarrior 4.3.7 for prediction of cytotoxicity risks and absorption rate was calculated based on TPSA. Finally, molecular docking was performed using the software Molegro Virtual Docker 6.0 to calculate the energies of interaction with the protease receptors (PDB ID: 1OHR; reverse transcriptase PDB ID: 1REV and integrase (PDB ID: 3WNH). Of the 243 molecules of natural products with anti-HIV activity, 7 were promising because they did not present cytotoxicity risks (mutagenicity, carcinogenicity, skin irritability and reproductive system effect), better MolDockScore energies for all targets studied simultaneously by varying the interaction energies binder-receptor of -209.47kJ.mol^{-1} at -60.20kJ.mol^{-1} and absorption rate (% ABS) between 34.26% and 90.24%.

Keywords: molecular docking; natural products; HIV; cytotoxicity; rate of absorption.

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1. Introduction

The human immunodeficiency virus (HIV), belonging to the family Retroviridae, genus Lentivirus, which caused the acquired immunodeficiency syndrome (AIDS) that has been a global health problem for more than 28 years[1]. HIV has caused more than 25 million deaths worldwide over the years and according to the World Health Organization (WHO) survey in 2016 there were 36.7 million people infected worldwide[2].

HIV is a ribonucleic acid (RNA) virus that contains a single enzyme, reverse transcriptase, which converts viral RNA into deoxyribonucleic acid (DNA) that can then be integrated into the genome of the host cell it infected. The genus lentivirus, which includes HIV-1 and HIV-2 subspecies, is considered a "slow virus" due to its characteristic of attacking the immune system and presenting a long interweaving between infection and the onset of symptoms[3,4].

In this viral replication process, HIV requires protease, integrase and reverse transcriptase which are important enzymes in the process. Many therapeutic alternatives in the anti-HIV treatment are in the inhibition of these enzymes, many researches are being directed towards the search of new inhibitors that present better pharmacological profiles[5].

A barrier in the treatment of AIDS is the mutation of HIV, which confers resistance against enzyme inhibitors (witch protease, integrase and reverse transcriptase). Because of the high rate of enzyme mutation, the emergence of new resistant strains of HIV has been frequent in recent years. In addition, most of the existing antiretroviral treatments have been shown to be highly cytotoxic to the affected patients[6].

Therefore, one of the strategies used today in modern medical chemistry is the insertion of computational methods for the planning of new drugs[7,8]. Through the *in silico* methods it is possible to perform a virtual screening by analyzing some important aspects of the natural products to obtain drug, such as target anchoring (molecule-receptor system), cytotoxicity and metabolism of the substance (as absorption), and, through pharmacodynamic and pharmacokinetic properties virtually simulated, to select more promising molecules for the treatment of HIV.

Therefore, the search new for antivirals has been focused on compounds that interfere in the viral replication cycle and computational studies have contributed to the prediction of risk of cytotoxicity, prediction of absorption and molecular docking of natural products.

2. Results and Discussion

Initially analyzing the absorption rate of 53 natural products presented lower ABS% than the control drug with lower rate (Ritonavir, 33.22%). This rate refers to the possibility of the bioactive being administered orally, of the 190 that presented% ABS above 33.22%, the risks of cytotoxicity were analyzed, for this study only the bioactive products were considered that did not present expression in the four cytotoxic parameters evaluated, being thus 136 natural products were subjected to molecular anchorage so that their energies interact with the selected targets.

Each molecule presented a total energy of interaction for each target selected in this research, in each molecular target was calculated the median of the energies obtained and were considered the bioactive ones that presented energies lower than the medians calculated, so, it was possible to guarantee that in the end this presented results of the compounds of the best binder-receptor interactions. Thus, crossing all the information obtained in the anchorage, carrying out a multi-target study, 29 natural products presented the best interaction energies with the respective enzymes simultaneously.

Analyzing the interactions with the targets and comparing them with the controls used in this research, the 29 natural products selected after the multi-target analysis showed the best interactions with the reverse transcriptase PDB ID 1REV (-78.86 to -114.16 kJ.mol^{-1}) than the Didanosine, Efavirez, Fosamprenavir, Lamivudine, Nelfinavir, Nevirapine, Tenofovir and Zidovudine. For the protease target PDB ID 1OHR (-44.29 to -104.31 kJ.mol^{-1}) the 29 natural products presented better results than all 19 drugs compared, showing that these natural products have a pharmacological action favored for the inhibition of protease. Finally, the integrin target PDB ID 3WNH (-19.04 to -61.82 kJ.mol^{-1}) showed better interactions than Abacavir, Didanosine, Dolutegravir, Efavirez, Etravirine, Lamivudine, Lopinavir, Nevirapine, Raltegravir and Zidovudine.

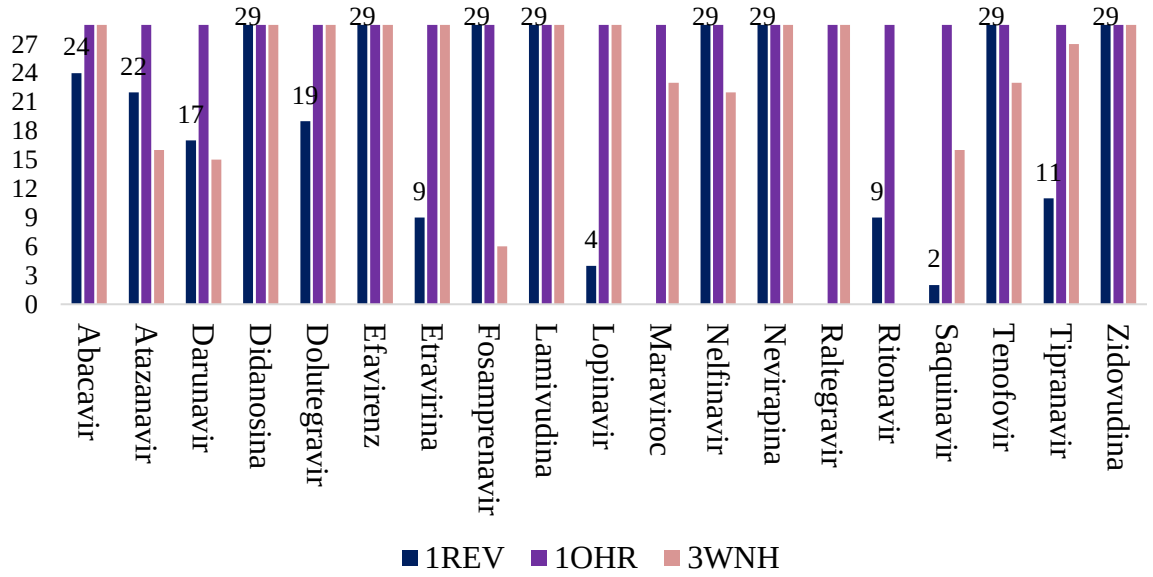

Figure 1. Relationship between the interaction energies of natural products and controls.

3. Materials and Methods

A total of 243 IC_{50} anti-HIV bioactives published in the literature have been selected. These compounds are from different classes of natural products such as flavonoids, terpenes, sisterpenes and others. These compounds were downloaded from the database of natural product structures NPASS (http://bidd2.nus.edu.sg/NPASS). In addition, 19 drugs already used in anti-HIV treatment (protease inhibitors, integrase and reverse transcriptase).

Initially the data of the structures were downloaded from the database in the form of CSV worksheet, from it the information was extracted with the smiles of all the structures contained in the worksheet, these smiles were loaded in the Standardizier 18.16.0 program of ChemAxon to obtain the files (MOL extension) containing each of the 243 natural products, the same was done for the 18 control drugs.

PDB ID 1OHR for protease, PDB ID 1REV for reverse transcriptase and PDB ID 3WNH for integrase were downloaded from the PDB website (https://www.rcsb.org).

In the sequence, the 262 structures were minimized by two optimization methods: molecular (MM +) and semi-empirical (AM1) in HyperChemTM 8.0.6 (RMS 0.1 kcal / Å.mol in 600 cycles).

After the optimization of the forming energies, these molecules were grouped into a single SDF file with MOL extension by the Standardizer program.

In order to predict the risks of cytotoxicity, the OSIRIS DataWarrior 4.7.3 program (http://www.openmolecules.org/datawarrior) was used taking into account four cytotoxic parameters: mutagenicity, carcinogenicity, skin irritability and effect on the reproductive system.

Both previously mentioned targets showed an already crystallographic inhibitor. A template was then made on this molecule and only then molecular docking can be initiated, taking into account the natural products and the molecules of the control drugs.

Finally, the ABS absorption rate was calculated, which expresses the potential oral absorption of the bioactive products, given by the formula[9]:

$$\%ABS = 109 - (0.345xTPSA)^{*}$$

* Where TPSA corresponds to the area of the total topological surface that was given by OSIRIS.

In order to point out the multi-target profile molecules, in silico screening on these molecules, using all the data obtained in the development of this research, molecules with ABS% greater than or equal to the lowest tooth value were taken into account the 19 controls analyzed : Abacavir, Atazanavir, Darunavir, Didanosine, Dolutegravir, Efavirenz, Etravirine, Fosamprenavir, Lamivudine, Lopinavir, Maraviroc, Nelfinavir, Nevirapine, Raltegravir, Ritonavir, Saquinavir, Tenofovir, Tipranavir, Zidovudine

In addition to the absorption rate, we considered the structures that had not present any risk of cytotoxicity, nor molecules whose MolDockScore (total anchorage energy) was higher than the median of the energies obtained for each target, thus considering the compounds that presented the best energies of anchorage.

4. Conclusions

The computational studies applied to medicinal chemistry come as a tool for the screening of bioactives of the best multi-target interactions for HIV-1. The screening proposed by this research, taking into account the absorption rate, toxicity risks and the interactions of Docking Molecular were able to highlight 29 natural products that simultaneously presented better results than the drugs: Didanosine, Efavirez, Lamivudine, Nevirapina and Zidovudina.

For the selected target for the reverse transcriptase (PDB ID 1REV) of the remaining 29 post-screening compounds 15 had the best interaction energies (-138,667 to -179,559 kJ.mol^{-1}), for the protease (PDB ID 1OHR) 15 compounds with energies of -143.450 to -209.476 kJ.mol^{-1}, for the integrase (PDB ID 3WNH) 15 compounds with the best energies (-88,734 to -110,010 kJ.mol^{-1}) were also present.

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Conflicts of Interest

State any potential conflicts of interest here or “The authors declare no conflict of interest”.

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