SHORT COMMUNICATION

Diterpenes isolated from Canistrocarpus cervicornis with virucidal activity against HIV-1: an in silico evaluation

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ABSTRACT

HIV is a public health problem, which makes necessary the development of new drugs. Natural products are known for their anti-HIV potential and a good strategy to suggest its mechanism of action is using in silico tools. Herein, diterpenes 1-3 had the binding mode evaluated in the HIV-1 glycoprotein; and properties ADMET in silico performed. In molecular docking important interactions between the hydrophobic cavity, and 1 and 2 were observed. In the molecular dynamics, 1 remained stable covering the entire hydrophobic cavity and performed hydrogen bond during all simulation. ADMET evaluation showed good properties for the diterpenes. Based on these findings, it was possible to suggest the potential from natural products as entry inhibitor and HIV-1 treatment.

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1. Introduction

According to the World Health Organization, in 2020, 37.7 million people were living with HIV and 680 thousand people died from AIDS-related illnesses (Unaids 2021), demonstrating a public health problem and the need to develop new antiretrovirals. Diterpenes (4R,7R,14S)-4α-7α-diacetoxy-14-hydroxydolast-1(15),8-diene (1), (4R,9S,14S)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (2), and (1S,5R,6R,9R,10R)-9-hydroxy-6,10-dimethyl-2-methylene-10-(4-methyl-3-oxopentyl)-12-oxatricyclo[7.2.1.01,6]dodec-5-yl-acetate (3), isolated from Canistrocarpus cervicornis, were described with activity against HIV-1 strains. Results showed viral multiplication inhibition, low cytotoxicity and high selectivity index. These diterpenes were still evaluated for their ability to alter the replication and infectivity upon direct exposure to viral particles. 1 and 2 showed high activities at a concentration of 25 μM, 99% and 87%, respectively, while 3 showed an inhibition of 19%; suggesting that 1 and 2 could act directly on the virus before of the its penetration into cells (Barros et al. 2016). As the hydrophobic cavity present in HIV-1 glycoproteins is the most exposed site before their conformational changes (Langley et al. 2015), 1 and 2 could bind to it. However, despite of the diterpenes activity, these metabolites usually present low yield in the isolation, hindering more in vitro studies. In this context, the use in silico tools could provide more information about the mechanism of action (Chen and Kirchmair 2020). Thus, this study aimed to build the HIV-1 glycoprotein by comparative modelling to evaluate the diterpenes binding mode into hydrophobic cavity at the glycoprotein using molecular docking and molecular dynamic simulation. In addition, it was evaluated ADMET in silico proprieties.

2. Results and discussion

2.1. Comparative modelling

Four models with the best discrete optimized protein energy score maintained their structures similar to the template, resulting in the low root mean-square deviations (RMSD) value (Table S1). Model 2 was chosen for presenting the best results for 1D-3D scores and Ramachandran plot (Table S1).

2.2. Molecular docking

Hydrophobic cavity at the gp120 is the most exposed site before the glycoprotein conformational changes (Langley et al. 2015) and 1 and 2 could act in it. Binding mode of a series proposed to bind in this site (Curreli et al. 2015) was used to evaluate 1-3. Validation in the hydrophobic cavity showed the best result for GOLD, and ChemPLP function due to preservation of all interactions and RMSD of 1.87 Å, supporting the hypothesis that the experimental binding mode may be reproduced. Diterpenes performed, mainly, Van der Waals interactions (Figure S1). Moreover, 1 performed one hydrogen bond with Asp368 (dOH-O = 3.7 Å), shorter distance than partial agonist (distance 4.5 Å) (Curreli et al. 2015). 2 performed hydrogen bonds with Gly473 (dOH-O of 2.7 Å) and Asp368 (dO-NH of 3.7 Å). As 2 present one more hydroxyl in the
opposite plane to the other groups able to perform hydrogen bond, this could have influenced in the hydrogen bond with Gly473. The epoxide in 3 seems to tension the heptane cycle and induce the preference for a hydrogen bond with Trp427 (dO-NH of 3.2 Å) (Figure S1B).

2.3. Molecular dynamic simulations

To evaluate the interactions of the complexes from docking and the stability of the systems, compared to free glycoprotein, molecular dynamic was performed. Glycoprotein-2 was the first to stabilize, in 5 ns. Next, it was stabilized glycoprotein-1, in 7 ns. Free glycoprotein and complex with 3 stabilized in 13 ns (Figure S2A). 2 was the most stable (Figure S2B). These results showed that 1 and 2 better stability the system. Hydrogen bonds between 1 and hydrophobic cavity occurred throughout the simulation; 2 and 3 had 43.4% and 51.9% hydrogen bonds, respectively, after stabilization (Figure S2C). Hydrogen bond with the Asp368, responsible for the antagonist effect (Curreli et al. 2015), showed a low percentage of binding (Figure S2D). In the representative conformation, 1 performed two hydrogen bonds, with Trp427 (average distance of 3.3 Å) and Gln428 (average distance of 2.0 Å) in 98.1% and 60.1% of simulation after stabilization, respectively. The other residues showed Van der Waals interactions in more than 96% of the simulation after stabilization, with an average distance of 2.9 Å, except with Asp368 (78.5% of simulation after stabilization and average distance of 4.0 Å) (Figure S3A). Asp368, Glu370 and Trp427 are important conserved residues (Andrianov et al. 2019). Visual analysis showed that 1 remained covering the hydrophobic cavity due to the hydrogen bonds; highlighting its potential as entry inhibitor. Hydrogen bond between 2 and Asp368 (average distance of 2.8 Å) occurred in 3.6% of the simulation after stabilization. This hydrogen bond took turns with Gln428 (average distance of 3.0 Å). The stabilization of the 2 was, mainly, by Van der Waals interactions (in more than 90% of the simulation after stabilization and with average distance of 2.8 Å) (Figure S3B). Due to flexibility of the open chain, Asp368 hydrogen bond was formed with 3 in 2.9% of simulation after stabilization, with average distance of 2.5 Å. Hydrogen bonds also occurred with Trp427 (average distance of 2.4 Å) and Gln428 (average distance of 2.5 Å). However, the greatest contribution for stabilization was Van der Waals interaction, in more than 90% of the simulation after stabilization and with average distance of 2.8 Å; except Gly366 (average distance of 4.5 Å) with 50.2% (Figure S3C). The greater instability of 3 could be related the smallest activity.

2.4. In silico ADMET evaluation

Temsavir and diterpenes presented gastrointestinal permeability. Permeability was described for Temsavir (FDA 2020). Diterpenes presented a high capacity to cross the blood-brain barrier. Compounds presented low distribution volume, and they were substrate of the CYP 3A4 and glycoprotein P. Temsavir is substrate for these proteins (FDA 2020). Temsavir and diterpenes showed absence of cardiac toxicity and adverse effects could be present according to the dose. Change in the QT interval for Temsavir
is present in heart problems, over dosage and drug-drug interaction (FDA 2020). Diterpenes showed acute toxicity. Compounds no presented carcinogenicity and mutagenicity. Genotoxicity and reproductive/developmental toxicity were absent in Temsavir and 3. Carcinogenity, genotoxicity and mutagenicity were not observed for Temsavir. Adverse effect on male and female fertility was only observed in rats when the dose was 80 times higher than recommended (FDA 2020). These findings highlight the reliability of in silico results and show good ADMET parameters of the diterpenes.

3. Conclusion

Binding mode of diterpenes in the hydrophobic cavity by molecular docking showed hydrogen bond with Asp368 only with 1 and 2. In molecular dynamic, this interaction showed a low percentage of binding. However, 1 was the only one to perform hydrogen bonds throughout the simulation, remaining stable in the hydrophobic cavity, which could be related to its high virucidal potential. While the open chain instability of 3, it could be related the smallest activity. The ADMET in silico studies showed a good gastrointestinal and BBB permeability; and absence of cardiac toxicity, carcinogenicity and mutagenicity for diterpenes. Based on these findings, it was possible to highlight the potential of natural products for drug design as alternatives to HIV-1 treatment.

Disclosure statement

The authors declare that they have no competing interests.

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