Molecular modeling and synthesis of new targeted HIV latency reversing agents to the lymphatic system

Beatriz Meduqui Rodrigues [1], Juliana Romano Lopes [1], Andressa Francielli Bonjorno [1] e Jean Leandro dos Santos [1]
[1] State University of São Paulo – UNESP, School of Pharmaceutical Science, Araraquara, São Paulo, Brazil, 14800-903

Background

Treatment with antiretroviral therapy (ART), although highly efficient, does not promote the elimination of the HIV virus that remains in a latent state[1], resulting in a viral reservoir in places with limited access to drugs, such as lymph nodes[2]. HIV latency reversing agents have been used in the "kick and kill" strategy, with the objective of reactivating the latent virus and its subsequent elimination[3]. Different compounds have been reported for their ability to reactivate the latent virus, such as histone deacetylase (HDACi)[4] enzyme inhibitors. Studies shows that selective HDAC-3 inhibitors were able to induce the expression of latent HIV virus in cell models[5]. Aiming to delivering these compounds to the lymphatic system, the strategy of this work consists of coupling HDAC-3 inhibitors with fatty acids[6], promoting an increase in its lipophilicity and improved bioavailability.

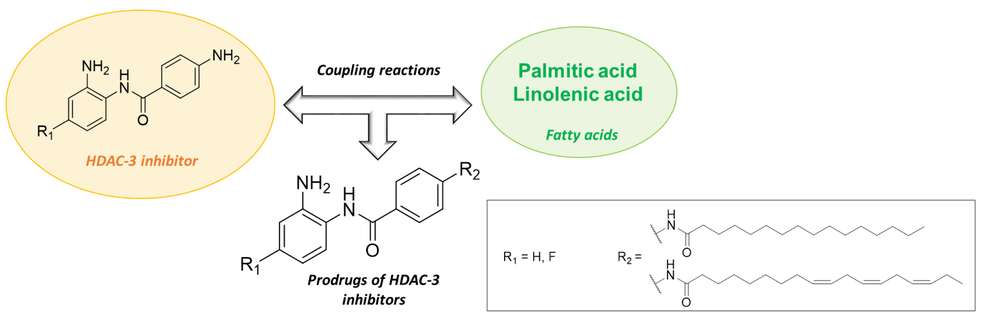
Objectives

Molecular modeling, synthesis and characterization of HDAC-3 selective inhibitor prodrugs.

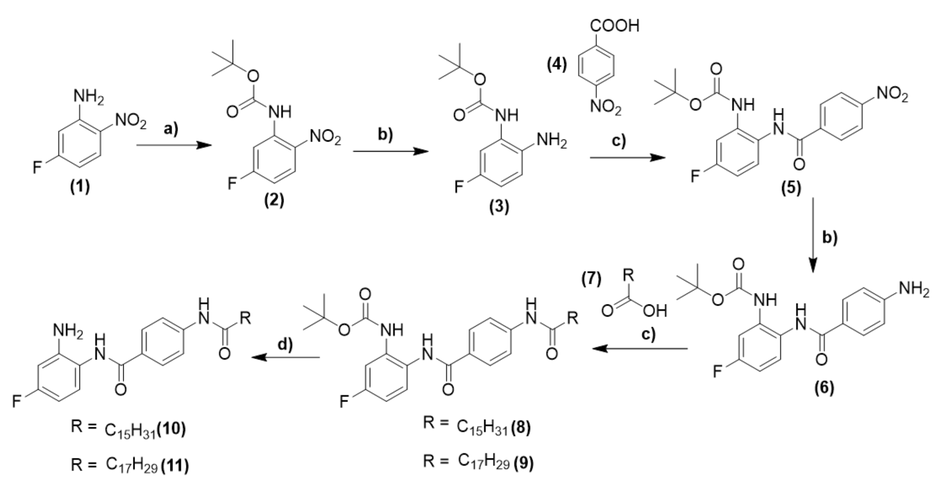
Methods

The designed compounds (Figure 1) were initially evaluated through in silico studies and its theoretical Log P values determined by SwissADME online software. **Molecular docking.** Docking studies were conducted in Maestro (Schrödinger®), with the crystallographic structure of HDAC-3 obtained from the PDB database under code (4A69). The pdb files were imported into the Maestro and prepared using the *Protein Preparation Wizard*. The interaction box ("grid") was defined by the *Receptor Grid Generation*, with dimensions of 10 Å x 10 Å x 10 Å. All ligands were prepared using Ligand Preparation (LigPrep) with the OPLS3 force field and ionization states at pH 7 ± 2. Redocking studies were performed in order to validate the model. **Synthesis.** All compounds were prepared through divergent route, using classic organic reactions, according Scheme 1. **Analytical Methods**. The molecular characterization was performed by ^{1}H and ^{13}C NMR, two-dimensional and infrared (IR). Experimental Log P values were determined by HPLC-UV (C18, mobile phase MeOH:H2O, flow 1mL/min, λ = 210 nm).

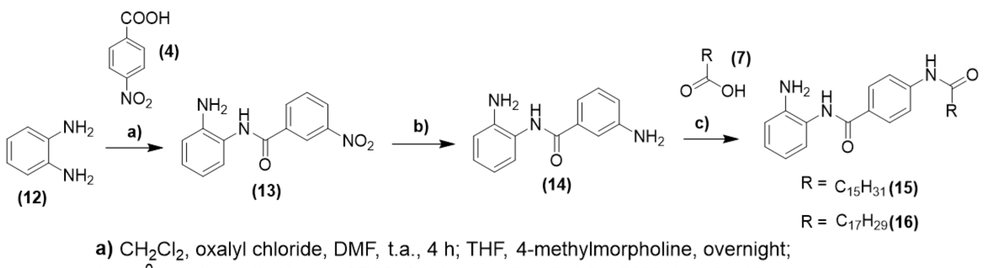
Figure 1. Drug design for HDAC-3 inhibitors prodrugs.



Scheme 1. Synthesis of HDAC-3 prodrugs derivatives.



a) BOC, DMAP, THF, 65 ºC, 24 h; **b)** Fe^{0}, NH_4Cl, H_2O, MeOH 65 ºC, 2-4 h; **c)** HATU, DIPEA, DMF, t.a., 48 h; **d)** TFA, t.a., 3 h.

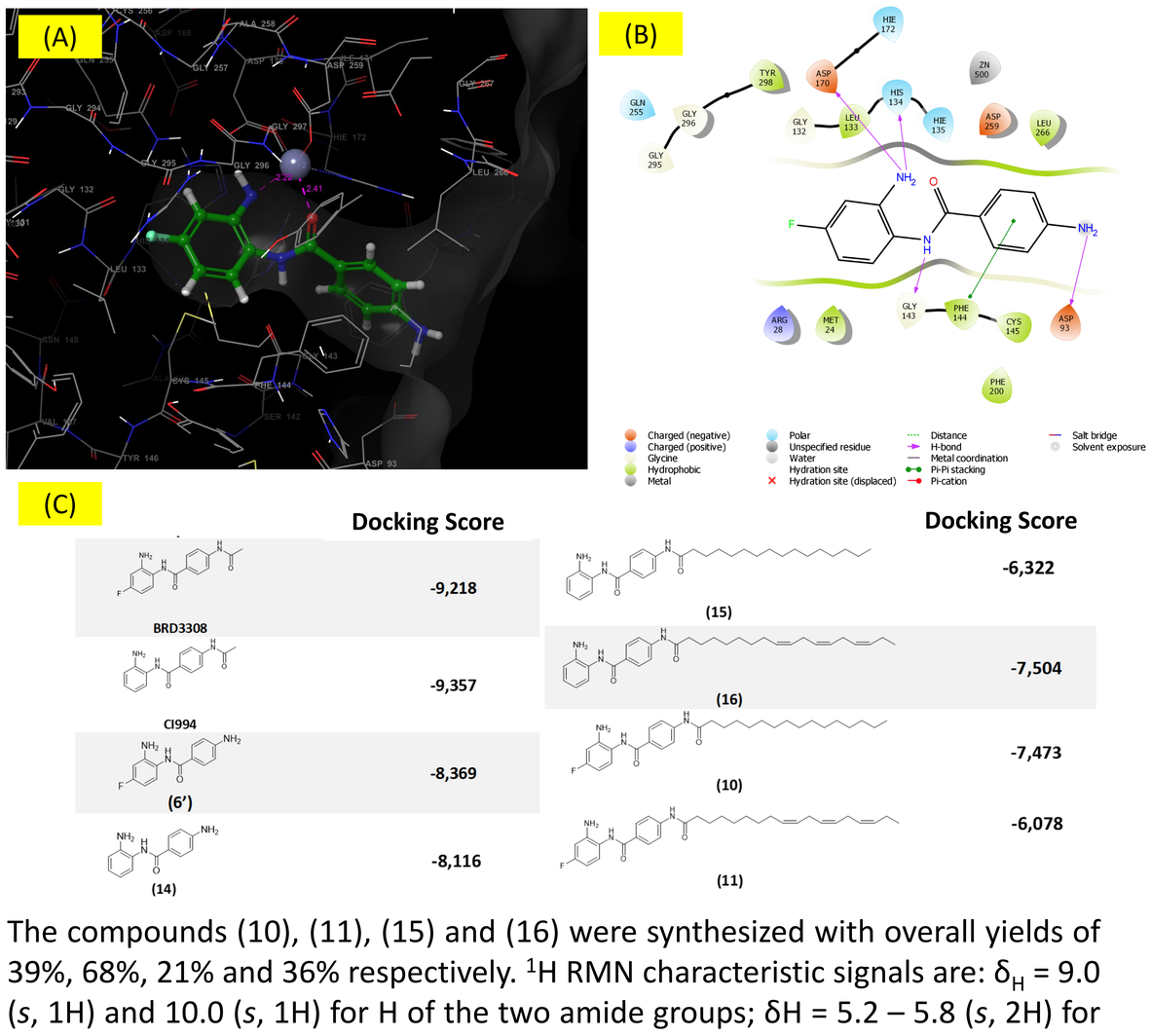


a) CH_2Cl_2, oxalyl chloride, DMF, t.a., 4 h; THF, 4-methylmorpholine, overnight; **b)** Fe^{0}, NH_4Cl, H_2O, MeOH 65 ºC, 2-4 h; **c)** HATU, DIPEA,DMF, t.a., 24-48 h

Results

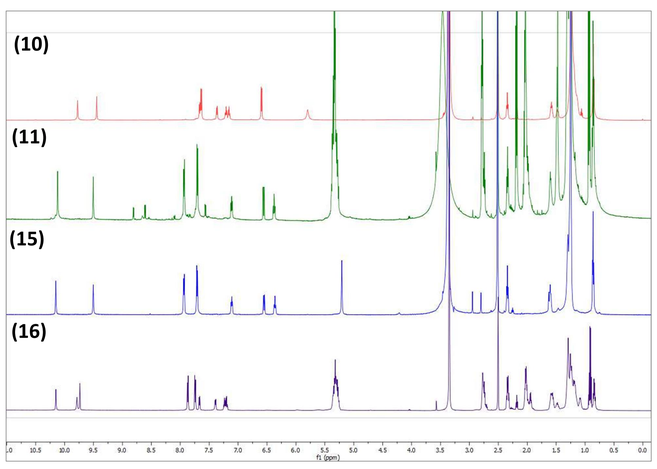
Docking studies showed that the prodrug compounds were able to interact with Zn^{2+} in the active site of HDAC-3. The poses and docking score values were also comparable with BRD3308 and CI994, both HDAC-3 inhibitors.

Figure 2. Molecular docking of the structure (6'). 3D (A) and 2D (B) representation. Determined docking score values (C).



The compounds (10), (11), (15) and (16) were synthesized with overall yields of 39%, 68%, 21% and 36% respectively. ^{1}H RMN characteristic signals are: δ_H = 9.0 (*s*, 1H) and 10.0 (*s*, 1H) for H of the two amide groups; δH = 5.2 – 5.8 (*s*, 2H) for H of the amine group; signals presented in the aromatic region ranging between δ_H = 8.0 – 6.0; and signals from aliphatic hydrogens in the region ranging between δ_H = 3.0 – 1.0 (Figure 2).

Figure 2. Superposition of the ^{1}H spectra of compounds (10), (11), (15) and (16) (DMSO-d6, 600 MHz)..



Conclusions

Four novel prodrugs of HDAC-3 inhibitors were designed and synthesized with yields ranging from 21-68%. The results of the in silico and experimental Log P experiments justify that these compounds can be targeted to the lymphatic system to act as HDAC-3 inhibitors. In the next steps, these compounds will be evaluated in vitro against infected HIV cells in order to characterize its latency-reversing effects.

References

1. Davey, R.T.Jr.; et al. PNAS. 1999, 96 (26), 15109-15114; **2.** Fletcher, C.V.; et al. PNAS. 2014, 111 (6), 2307-2312; **3.** Deeks, S.G.; Overbagh, J.; Phillips, A.; Buchbinder, S. Nature Reviews. 2015, 1, 15035; **4.** Lopes, J.R.; Chiba, D.E.; Dos Santos, J.L. European Journal of Medicinal Chemistry. 2021, 213, 113213. **5.** Barton, K.M.; et al. PLoS One. 2014, 9(8). **6.** Trevaskis, N.L.; Kaminskas, L.M.; Porter, C.J.H. Nature Reviews. 2015, 14, 781-803.

Acknowledgments

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Ref. Process: FAPESP 18/11079-0); This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).