MSc in Biochemistry for Health

Dissertation Project – 2nd Cycle

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TITLE: Development of Novel AZT Derivatives Based on Triazoles

BACKGROUND

The extraordinary development of therapies for HIV treatment over the last decades has extensively reduced the morbidity and mortality associated with the disease. At present, there are over 30 drugs utilized for the treatment of HIV, divided into five main classes, which target different steps in the viral life cycle: 1) viral entry; 2) reverse transcription; 3) integration and 4) viral maturation. However, current therapies have been compromised by the rapid emergence of resistant strains, side effects resulting from extended used of the drugs and poor bioavailability. In addition, the high-cost associated with long-term treatment poses considerable financial pressure for Public Health systems. The development of more cost effective drugs, with increased efficiency and lower side effects is therefore mandatory and will indisputably have a high impact on patient's health and evident financial implications on the costs of the associated treatment.

OBJECTIVES

In this project we will explore the synthesis of novel AZT derivatives via Huisgen 1,3-dipolar cycloaddition (click-chemistry) for the development of novel anti-HIV drugs. The main objective of this project is the development of new generation of antiretrovirals that penetrate in the lymphoid tissues. This project establishes a proof–of-concept for the design and production of cost-effective antiviral agents, by being based on readily available raw materials and employing well established and cost effective chemical methodologies.

PROJECT DESCRIPTION

The emergence of extensively cross-resistant strains of HIV-1, the side effects of long-term use of antiviral drugs and their costs, and the discovery that HIV-1 continues to replicate even in the patients under anti-retroviral therapy, have lead to an urgent need to develop novel anti-HIV drugs with increased efficiency, minimum side effects, higher bioavailability and cost effective production. In this project we will employ 3'-azido-3'-deoxythymidine (AZT, zidovudine) as a scaffold for the development of novel antiviral drugs. AZT is readily available and easily modified molecule, being prone to a wide range of modifications due to the presence of an azide group, easily functionalized via well-established click-chemistry procedures.

This project is organized in 3 main tasks:

- **WP1-** Synthesis of AZT derivatives bearing 1,4 triazoles and 1,5 triazoles
- **WP2-** Synthesis of novel triazolium salts bearing AZT triazole derivatives.
- **WP3-** Test AZT triazole derivatives for anti-retroviral activity against wilds-type HIV-1 and AZT-resistant strains, *in vitro*

Task 1: Synthesis of AZT derivatives via click chemistry using Cu and Ru catalysts

Wang et all showed that the main features which a azole derived AZT must contain to present antiviral activity are a bulky aromatic ring and a 1,5- substitution pattern on the triazole. In this task we will synthesize 1,4 triazoles and 1,5 triazoles via 1,3-Huisgen cycloaddition with different substituents and steric requirements, using both Cu(I) and Ru(II) catalysts, to obtain 1,4 and 1,5 derivatives, respectively.

Task 2: Synthesis of novel triazolium salts bearing AZT triazole derivatives.

Further functionalization of the AZT triazole derivative via quaternization of the triazole nitrogens is possible and has been successfully achieved by Dr. Petronilho group at ITQB. This synthetic tool introduces physiological solubility, due to the existence of a delocalized positive charge at the triazole.

Task 3: Test AZT triazole derivatives for anti-retroviral activity against wilds-type HIV-1 and AZT-resistant strains, *in vitro*

In this task we will determine AZT triazole derivatives ability to block HIV-1 replication in the Jurkat T cell line, in human primary CD4 T cells isolated from peripheral blood and finally, in Tfh isolated from human tonsils. Circulating CD4 T cells and $CXCR5^{+}PD\text{-}1^{+}$Tfh cells will be sorted from peripheral blood and tonsils healthy donors, respectively, by flow cytometry and infected *ex vivo* with HIV-1 NL4.3 or with an AZT-resistant HIV-1 NL4.3 virus that have been supplied to the Soares lab by the HIV-1 AIDS Program. As readout, viral production, will be determined by ELISA and intracellular flow cytometry.

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Thesis										