Fighting multi-drug resistant tuberculosis: paving the way with different approaches

AUTHORS: R. F. de Almeida | J. Marquês, C. Faria, S. Santos, M. S. Santos, Filomena Martins

R&D UNIT: CQE-Ciências

CONTACT: feleitao@fc.ul.pt; rfalmeida@fc.ul.pt

The Disease: Tuberculosis

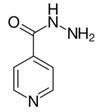
The causative agent: Mycobacterium tuberculosis

10 million new cases in 2018

1.5 million deaths in 2018

(Global TB facts, WHO Global Tuberculosis Report 2019 (refers to 2018 situation)

Pharmacological therapeutic approaches



ISONIAZID (INH)

One of the first-line and most effective drugs to treat tuberculosis

Mutations in the multifunctional catalase-peroxidase enzyme KatG ⇒ Resistance to Isoniazid

3.5 % of new cases and 18 % of previously treated

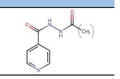
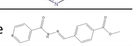
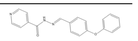
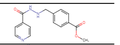
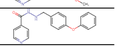
Multidrug-resistant (**MDR**) (isoniazid and rifampicin resistance) or rifampicin-resistant tuberculosis

The development of new effective and low toxicity antitubercular compounds is urgent

TARGTUB
PTDC/MED-QUI/29036/2017

Synthesis and characterization of a series of INH derivatives

THE COMPOUNDS

ID	Name	Structure
INH-C_2	*N'*-acetylisonicotinoylhydrazide	
INH-C_{10}	N'-decanoylisonicotinoylhydrazide	
N33	(*E*)-methyl 4-((2-isonicotinoylhydrazono)methyl)benzoate	
N34	(*E*)-*N'*-(4-phenoxybenzylidene)isonicotinohydrazide	
N33red	methyl 4-((2-isonicotinoylhydrazinyl)methyl)benzoate	
N34red	*N'*-(4-phenoxybenzyl)isonicotinohydrazide	

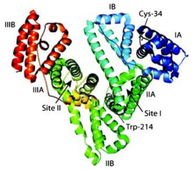
Previous promising results:
N'-decanoylisonicotinoylhydrazide (INH-C_{10}) 6x more potent than isoniazid against the KatG mutated strain, S315T

Evaluating the Therapeutic Potential

Experimental approaches

Ability to bind Human Serum Albumin
Ability to interact with cellular barriers – membranes
Toxicological effects on human cell lines

Human Serum Albumin (HSA)



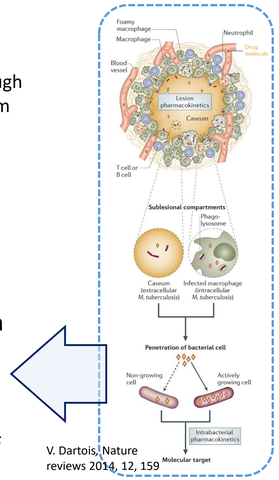
Makes the transport of compounds through the blood stream

Determination of:
- *Dissociation constants (K_d)*
- *Binding site*

Interaction of INH derivatives with systems mimicking:

- plasma membrane of mammals
- cell wall of Mycobacterium tuberculosis

The barriers to be crossed



V. Dartois, Nature reviews 2014, 12, 159

Cell Viability Assays and Fluorescence Spectroscopy

Evaluating toxicological effects

Hep G2 Cells
Immortal human liver cancer cell line

Mtt Assay
assay for assessing cell metabolic activity

RESULT COMPILATION

Compound	K_d (M)	K'_d (M)	Cell Viability IC_{50} (μM)
INH	2.72×10^{-3}	5.38×10^{-3}	> 200
INH-C_2	2.30×10^{-3}	3.50×10^{-3}	n.a.
INH-C_{10}	4.59×10^{-5}	2.16×10^{-4}	>25
N33	1.66×10^{-4}	1.35×10^{-4}	>25
N34	1.26×10^{-4}	2.30×10^{-4}	>200
N33red	5.07×10^{-3}	1.23×10^{-3}	>200
N34red	1.50×10^{-4}	4.69×10^{-4}	48.5

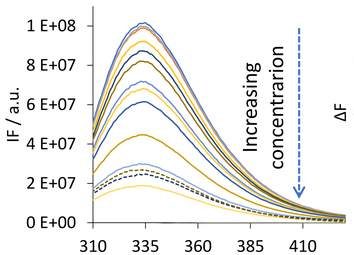
$K'_d - K_d$ in the presence of warfarin

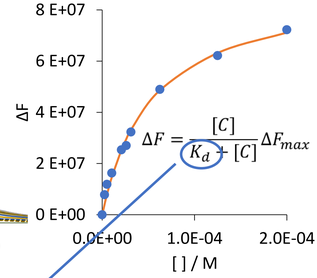
All compounds interact more strongly with HSA than INH, in particular **INH-C_{10}**, with a K_d 2 orders of magnitude stronger

All compounds are less toxic than drugs currently in pharmaceutical use

Viable alternatives to current therapeutics against MDR tuberculosis

Following the interaction Compound-HSA





Dissociation Constant

How strong is the Compound-HSA biding

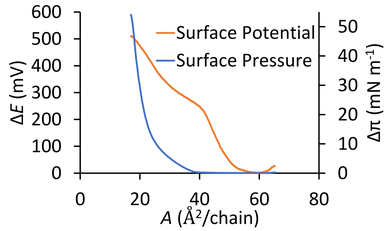
Use of site markers

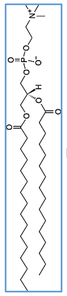
Binding Site

Warfarin
Binds to Sudlow site I

Monolayer compressibility curves

DPPC monolayers





DPPC

Main molecule in pulmonary Surfactant

Next steps
How will the compounds interact with DPPC membranes? Or models of plasma membrane of mammals? Or mycolic acids?

ACKOWLEDGMENTS
PTDC/MED-QUI/29036/2017
UIDB/00100/2020
CEECIND/03247/2018
CEECIND/03414/2018