Modeling the antitubercular activity of isoniazid derivatives: design and synthesis of new lipophilic isonicotinoylhydrazones and hydrazides

Catarina Frazão de Faria[1,2], Tânia Moreira[1], Pedro Lopes[3], Susana Santos[1], Miguel Machuqueiro[1,3], Filomena Martins[1,2]

[1] *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

[2] *Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

[3] *Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*

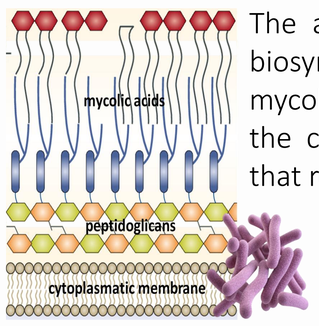
cgfaria@ciencias.ulisboa.pt

Abstract

Multidrug-resistant strains of *Mycobacterium tuberculosis* are currently widespread and proven unresponsive to the two most efficient first line antitubercular drugs: isoniazid (INH) and rifampicin, posing a serious public health threat. In recent years, in the scope of finding novel compounds presenting higher antitubercular activity, several different lipophilic derivatives of INH have been investigated based on QSAR-oriented design. In particular, the work carried out by our team has already identified the *N'*-acyl-C_{10} moiety as an interesting lead, thus prompting the design and synthesis of analogs, such as isonicotinoylhydrazones and hydrazides, herein reported. These new compounds have been purified and characterized by spectroscopic and chromatographic techniques and will undergo further biological evaluation.

INTRODUCTION

Tuberculosis is the leading cause of death worldwide by a single infectious agent, being triggered by the *Mycobacterium tuberculosis* (*Mtb*) bacillus. According to the WHO, it infects almost a quarter of the human population. [1] INH is still the most effective component in all multi-therapeutic regimens, but the increasing resistance of *Mtb* to INH has urged the search for new and effective antitubercular drugs.



The antitubercular activity of INH is due to its ability to inhibit the biosynthesis of mycolic acids, which are an integral component of the mycobacteria cell wall. In the prodrug form, INH undergoes oxidation by the catalase-peroxidase KatG, generating an isonicotinoyl radical (IN$^{\bullet}$) that reacts with NAD^+ to form a covalent adduct as the active metabolite.

Although the exact mechanism of INH resistance is not completely clear, mutations in the *katG* gene, which interfere with the drug activation, are thought to be one of the possible causes.

BACKGROUND

Out of several new INH derivatives investigated, [2] one stood out:

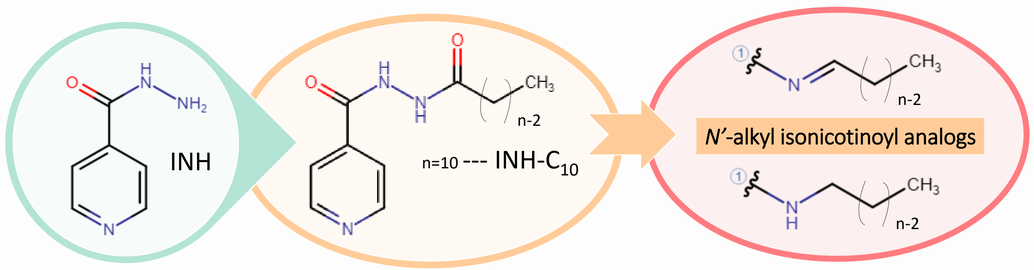
An acylated isonicotinoylhydrazide with a C_{10} aliphatic chain

✓ **More active** against both the wild type strain of *Mtb* and the most common resistant strain, *katG* S315T

Extensive computational and experimental studies departing from this molecule have led to promising findings [3]

GOAL

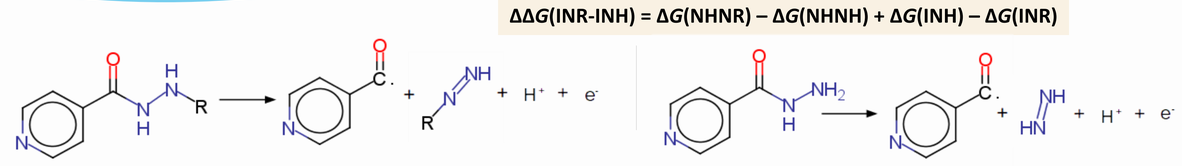
➲ To find INH derivatives that maintain the **high membrane permeability** showcased by INH-C_{10}, but displaying **improved reactivity** to form the isonicotinoyl radical, so far considered to be essential for the mode of action of INH.



CALCULATIONS

❖ **Quantum mechanics: reactivity**

$\Delta\Delta G(\text{INR-INH}) = \Delta G(\text{NHNR}) - \Delta G(\text{NHNH}) + \Delta G(\text{INH}) - \Delta G(\text{INR})$



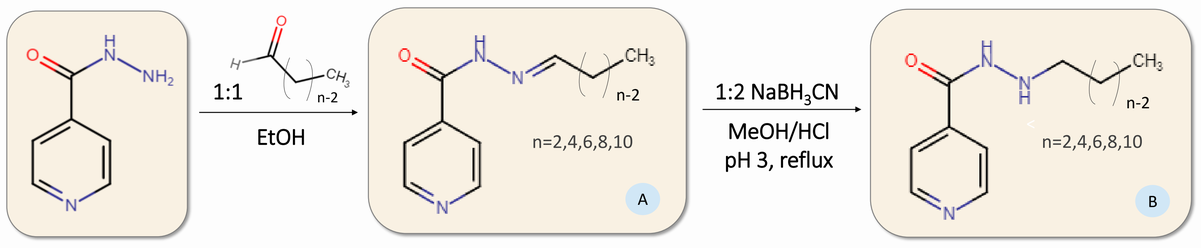
Compound	INH	INH-C_{10}	isonicotinoylhydrazides					isonicotinoylhydrazones				
			n=2	n=4	n=6	n=8	n=10	n=2	n=4	n=6	n=8	n=10
$\Delta\Delta G$ (kcal/mol)	0.0	10.8	-6.8	-9.1	-9.8	-10.4	-10.5	-1.3	-1.3	-1.7	-2.8	-2.4

❖ **Membrane permeability**

Compound	INH	INH-C_{10}	isonicotinoylhydrazides					isonicotinoylhydrazones				
			n=2	n=4	n=6	n=8	n=10	n=2	n=4	n=6	n=8	n=10
Perm. (cm/s)	1.3	27.9	0.6	3.8	5.8	22.0	14.0	-	2.0	3.8	4.2	8.1

SYNTHESIS

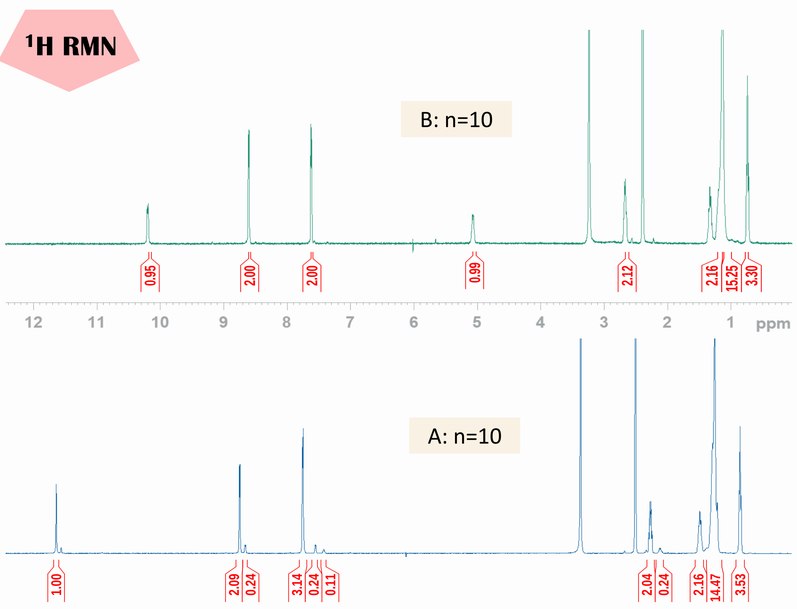
- Isonicotinoylhydrazones were prepared by functionalization of INH at *N'* by **condensation** with the respective aldehyde. The generated Schiff base was recovered as a crystalline solid and recrystallized with ethyl acetate (n=4,6,8,10) or dichloromethane:hexane 2:1 (n=2).
- Isonicotinoylhydrazides were obtained in the form of an oil by **reduction** of the parent hydrazones. After *work-up*,[4] further purification by column chromatography was required to isolate them from a mixture of side-products, e.g. *N'*-di-alkyl analogs.



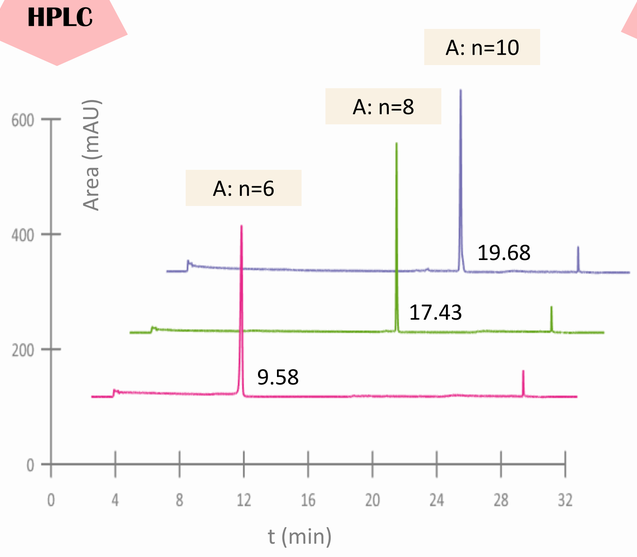
CHARACTERIZATION

A broad **spectroscopic analysis** (IR, $^1H/^{13}C$ NMR, COSY, HMQC, HMBC, NOESY), supported by **HPLC and GC-MS analysis**, was carried out to fully characterize the synthetized compounds and assess their purity.

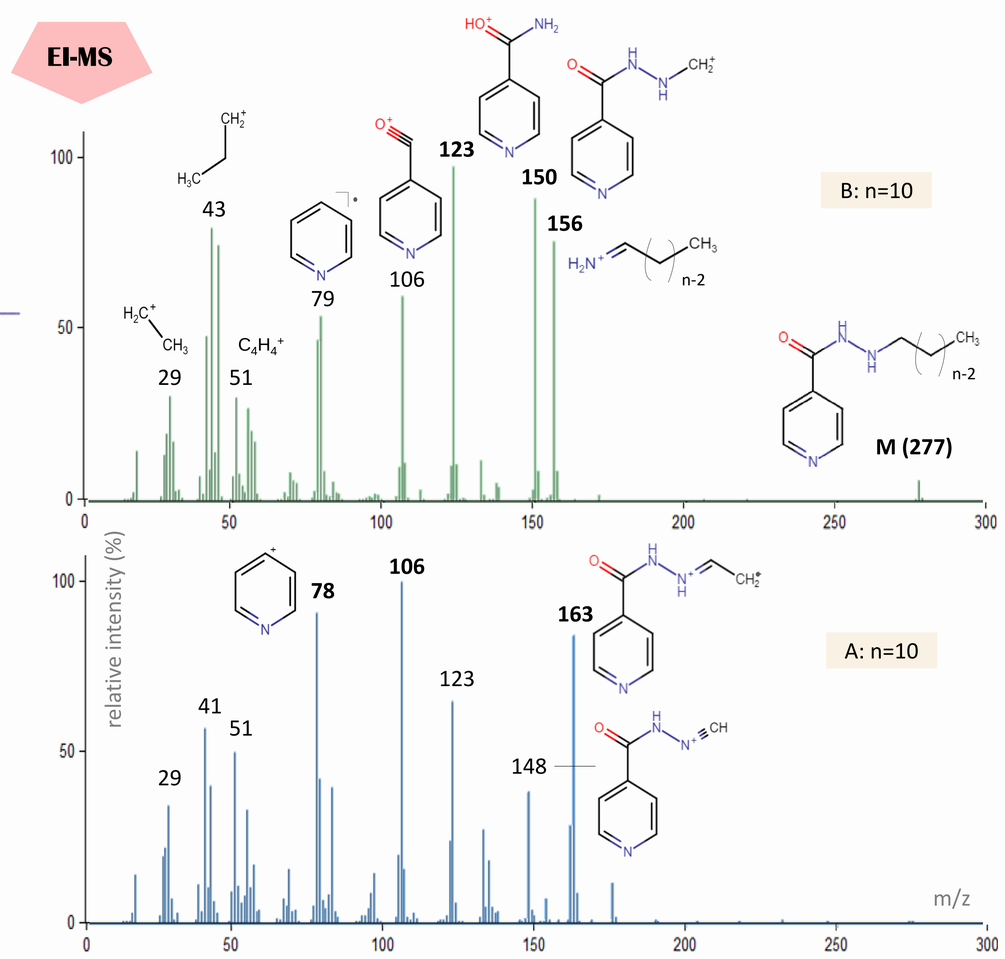
^1H RMN



HPLC



EI-MS



	Compound	INH	INH-C_{10}	isonicotinoylhydrazides			isonicotinoylhydrazones				
				n=6	n=8	n=10	n=2	n=4	n=6	n=8	n=10
HPLC	rt (min)	1.47	19.65	-	-	-	1.68	2.97	9.58	17.43	19.68
GC	rt (min)	-	22.32	16.01	18.49	20.60	11.31	13.43	16.49	18.91	20.93
EI-MS	Base peak	78 | 106	123 | 150	123 | 150			78 |106				
	2nd peak	51	45	100	128	156	122	122	163	163	163
	Parent peak	137	291	221	249	277	163	191	219	247	275

PERSPECTIVES

Biophysical assays
Activity studies
Drug-likeness studies

***N'*-alkyl isonicotinoylhydrazides C_8 and C_{10}** (n=8, 10) were predicted to have the most favorable balance between reactivity and membrane permeability, thus satisfying the goal initially set.

ACKNOWLEDGMENTS

This work was financed by FCT within the framework of project TARGTUB: PTDC/MED-QUI/29036/2017.

REFERENCES

[1] "Global tuberculosis report 2018"; WHO. Retrieved 28-03-2019
[2] F. Martins *et al.*; *Eur. J. Med. Chem.* 81 (2014) 119–138
[3] D. Vila-Viçosa *et al.*; *Mol. Pharmaceutics* 14 (2017) 4597–4605
[4] R. Borsch *et al.*; *J. Am. Chem. Soc.* 93:12 (1971) 2897-2904