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Towards the discovery of new improved antitubercular compounds: rational design and synthesis of novel hydrazones and hydrazides with an isoniazid scaffold

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INTRODUCTION

Tuberculosis, triggered by the *Mycobacterium tuberculosis* (*Mtb*) bacillus, still infects almost a quarter of the human population worldwide.[1] Therapeutic regimens are based on the combination of isoniazid (INH) with milder antibiotics, however **multidrug-resistant strains of *Mtb*** are now widespread and proven **unresponsive to INH**, urging the search for new and effective derivatives with antitubercular activity.

INH's mode of action is grounded on the **formation of the isonicotinoyl radical (IN•)** when oxidized by the catalase-peroxidase KatG. Upon covalent adduction with NAD^+, the generated active metabolite interferes with the mycobacteria cell wall by **inhibiting the biosynthesis of mycolic acids**.

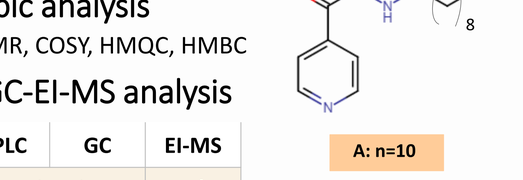
Resistance to INH mainly comes from **mutations in the *katG* gene**, particulary the S315T variant, which hampers a proper drug activation.

AIM

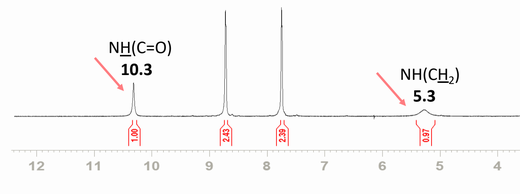
➲ To find **INH derivatives** that maintain the **high membrane permeability** evidenced by INH-C_{10} but displaying **improved reactivity** to form the isonicotinoyl radical.

HIT TO LEAD → LEAD OPTIMIZATION

N'-alkyl isonicotinoylhydrazones and hydrazides



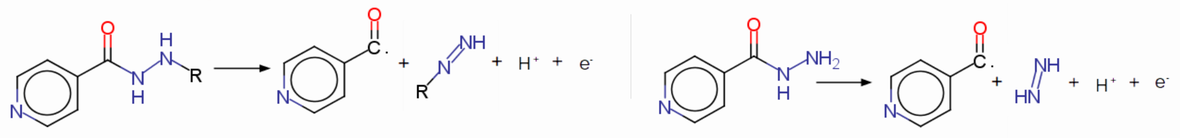
N'-aryl isonicotinoylhydrazides



CALCULATIONS

❖ **Quantum mechanics: reactivity**

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



	INH	INH C_{10}	N33	N34	isonicotinoylhydrazides n=2	n=4	n=6	n=8	n=10	N33 red	N34 red	isonicotinoylhydrazones n=2	n=4	n=6	n=8	n=10
ΔΔG (kcal/mol)	0.0	10.8	-	-	-6.8	-9.1	-9.8	-10.4	-10.5	-8.0	-6.5	-1.3	-1.3	-1.7	-2.8	-2.4

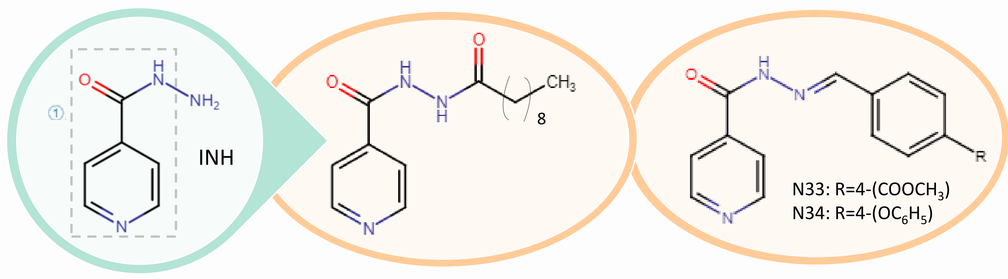
❖ **Membrane permeability**

	INH	INH C_{10}	N33	N34	isonicotinoylhydrazides n=2	n=4	n=6	n=8	n=10	N33 red	N34 red	isonicotinoylhydrazones 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	4.8	15.5	-	2.0	3.8	4.2	8.1

LINE OF RESEARCH

In the scope of a project aiming at developing novel compounds with high antitubercular activity, several different **lipophilic INH derivatives** were investigated by our team based on **QSARS models**. [2]

Highlights **N'-acyl-C_{10} moiety ⇨ INH-C_{10}** **N'-aryl-moiety ⇨ N33 and N34**

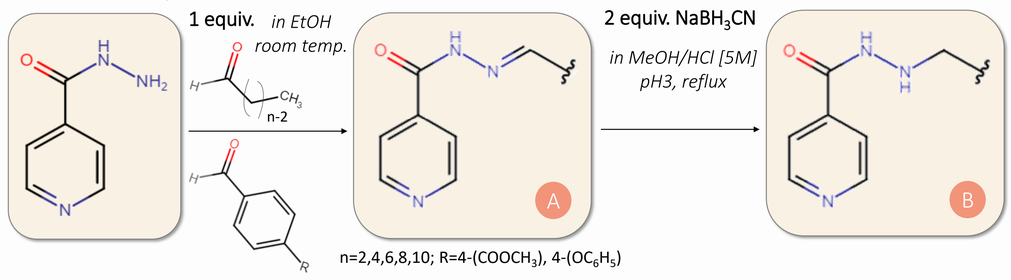


☑ More active than INH against *Mtb katG* **S315T mutant strain**, with MIC values reduced by 2-fold for N33 and N34 and 6-fold for INH-C_{10}.[2]

☑ Other **promising results** regarding INH-C_{10} emerged from extensive **computational and experimental studies**.[3]

SYNTHESIS

INH ▶ Isonicotinoylhydrazones ▶ Isonicotinoylhydrazides



A. **Hydrazones** were recovered as white solids after solvent evaporation and recrystallized with acetone:methanol 2:1 (N33 and N34), dichloromethane:hexane 2:1 (n=2) or ethyl acetate (n=4,6,8,10).

B. **Hydrazides** were obtained in the form of yellow oils after solvent evaporation. Upon *work-up*,[4] a yellowish solid containing a mixture of compounds was generally recovered in the case of the alkyl hydrazides, thus requiring **further purification by column chromatography to isolate the desired products**. With the aryl hydrazides, the recovered solids were whitish, almost pure by TLC, therefore only washed with n-hexane and petroleum ether to remove small impurities.

PURITY ASSESSMENT: 97.6-99.7% [GC-MS]

CHARACTERIZATION

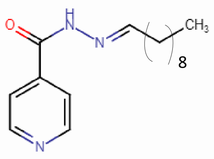
➲ Spectroscopic analysis
FTIR, $^1H/^{13}C$ NMR, COSY, HMQC, HMBC

➲ HPLC and GC-EI-MS analysis

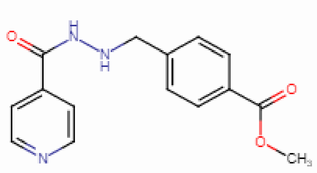
		HPLC	GC	EI-MS
		rt (min)		**m/z**
INH		1.47	10.25	137
INH-C_{10}		19.65	22.32	291
N33		9.17	24.41	283
N34		17.51	28.57	317
isonicotinoylhydrazides	n=6	-	16.01	221
	n=8	-	18.49	249
	n=10	-	20.60	277
	N33red	-	23.31	285
	N34red	-	26.31	319
isonicotinoylhydrazones	n=2	1.68	11.31	163
	n=4	2.97	13.43	191
	n=6	9.58	16.49	219
	n=8	17.43	18.91	247
	n=10	19.68	20.93	275

B: n=10

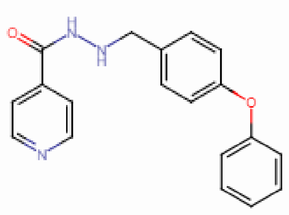
A: n=10



B: N33red

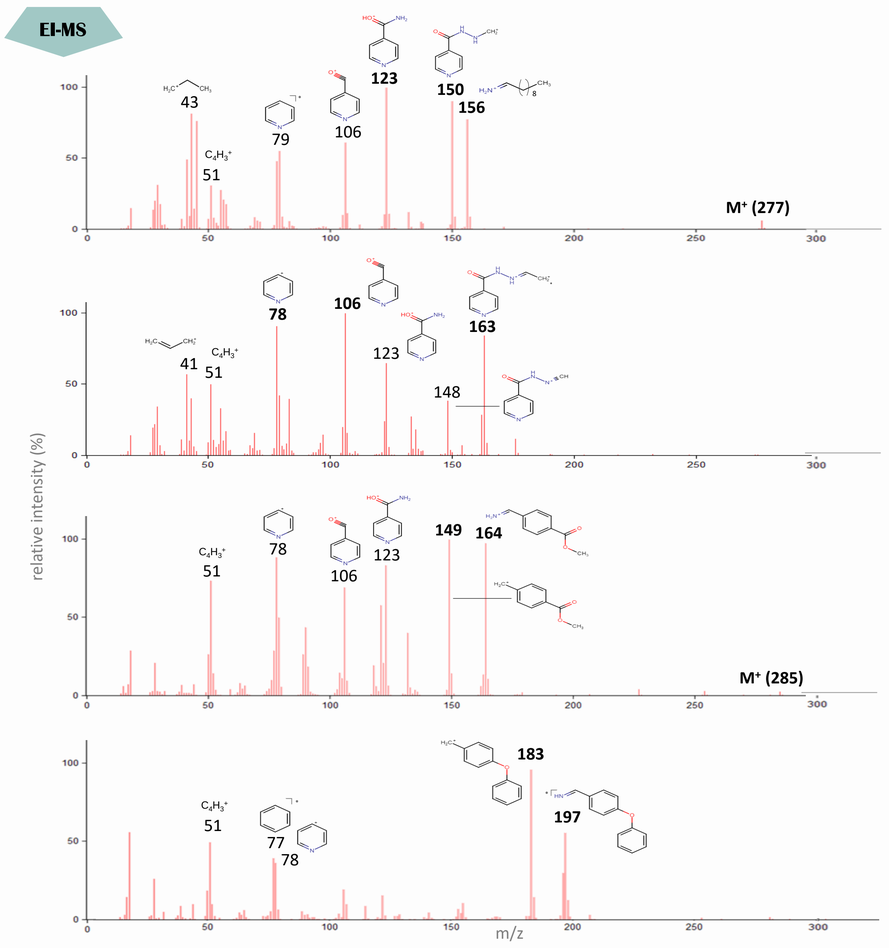


B: N34red



^1H NMR DMSO-d_6

EI-MS



HINTS

➲ *Hydrazides: n=6,8,10; N33red and N34red*

➲ *Hydrazones: n=8,10*

☑ Predicted to have a favorable balance between reactivity and membrane permeability

NEXT

IN VITRO EVALUATION

- *Activity*
- *Cytotoxicity*
- *Biophysical properties*
- *Physicochemical properties*
- *Drug-likeness*

REFERENCES

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ACKNOWLEDGMENTS

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