SYNTHESIS AND EVALUATION OF NOVEL ISONIAZID-BASED COMPOUNDS AS POTENTIAL ANTIMYCOBACTERIAL AGENTS

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Tuberculosis (TB) is infectious disease triggered by mycobacteria, specifically *Mycobacterium tuberculosis* complex (*Mtb.*). One of the major complications of TB therapy is the increasing resistance of mycobacterial strains to conventional drugs. This is very serious problem especially for HIV co-infected patients.[1]

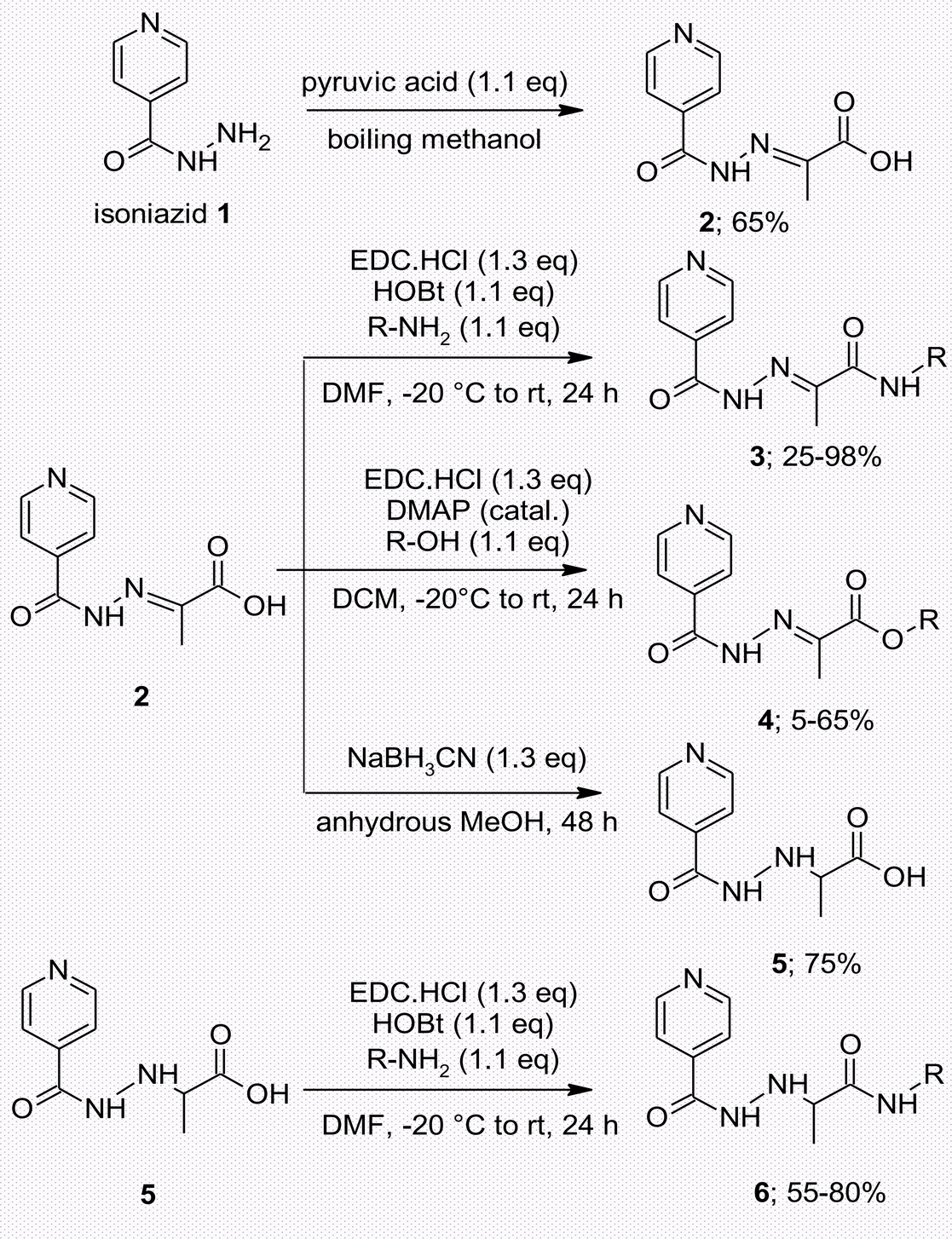

Fig. 1. Examples of structural modifications of isoniazid 1

Isoniazid (INH, 1) is an established, first-line drug against TB with a selectivity for *Mtb*. Its mechanism of action consists primarily in the inhibition of InhA and thus cell wall biosynthesis. However, the development of the resistance has limited its therapeutic potential and that is why many structural modifications of this simple molecule have been synthesized.[1,2]

References
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The prepared derivatives were characterized and their *in vitro* antimycobacterial activity (*Mtb.* $H_{37}Rv$, *M. avium*, two strains of *M. kansasii*) was evaluated and expressed as minimum inhibitory concentrations (MIC). Cytotoxicity and cytostatic properties were also determined (HepG2 and MonoMac6 cell lines).

We have been focused on synthesis and evaluation of novel INH analogues, predominantly its hydrazones with various oxocarboxylic acids (mainly pyruvic acid—the hydrazone 2) that are further modified on free carboxyl group by various amines and phenols to yield amides 3 and esters 4 (*via* EDC-mediated coupling catalysed by 1-hydroxybenzotriazole; HOBt or 4-dimethylaminopyridine; DMAP, respectively). The double bond was reduced in the parent hydrazone 2 and the resulting acid 5 was condensed with various anilines to form 1,2-disubstituted hydrazines 6 (Fig. 1). More than fifty compounds have been prepared within this project (the series includes amides derived from variously substituted anilines, other aromatic amines as well as compounds derived from non-aromatic cyclic and alicyclic amines and also esters of phenols).

R (structure 3)	MIC [μM]			
	Mtb. $H_{37}Rv$		*M. kansasii* 6509/96	
	14 days	21 days	14 days	21 days
4-I-Ph	0.25	0.25	4	4
4-*n*-C_8H_{17}-Ph	0.25	0.25	4	4
4-CF_3O-Ph	≤0.03	≤0.03	2	4
Isoniazid 1	0.5	0.5	16	16

Tab. 1 Antimycobacterial activity of selected aniline-based derivatives

Novel series of INH-based derivatives is easily synthetically available with satisfactory yields and also inexpensive. Structures of prepared molecules were confirmed by spectral methods (NMR, IR, MS). From the pharmacological point of view, these are promising derivatives:

- The best activity against *Mtb.* showed the derivatives of *N*-phenyl-2-[2-(pyridine-4-ylcarbonyl)hydrazinylidene]propanamide substituted by an electron-withdrawing group, an additional aromatic ring or a long alkyl (MIC values of ≤0.03 μM), shown in Tab. 1.
- Derivatives of 4-iodo- and 4-CF_3O-anilines exhibited comparatively high efficacy against atypical mycobacteria (MIC ≥2 μM).
- Importantly, the presented compounds are selective, non-toxic for mammalian cells (HepG2, MonoMac6) and almost all of them are comparable or even significantly superior (17× or more) to parent INH.
- Their activity against drug-resistant mycobacterial strains is also better to parent INH.
- Determination of the mechanism of action is under investigation.

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