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Synthesis and antituberculosis activity of indole–pyridine derived hydrazides, hydrazide–hydrazones, and thiosemicarbazones

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ABSTRACT

We describe the design, synthesis, and in vitro antimycobacterial activity of a series of novel simple hybrid hydrazides and hydrazide–hydrazones combining indole and pyridine nuclei. The compounds are derivatives of 1-acetylindoxyl or substituted indole-3-carboxaldehydes tethered via a hydrazine group by simple C—N or double C=N bonds with 3- and 4-pyridines, 1-oxide 3- and 4-pyridine carbohydrazides. The most active of 15 compounds showed MICs values against an INH-sensitive strain of *Mycobacterium tuberculosis* H37Rv equal to that of INH (0.05–2 μg/mL). Five compounds demonstrated appreciable activity against the INH-resistant *M. tuberculosis* CN-40 clinical isolate (MICs: 2–5 μg/mL), providing justification for further in vivo studies.

Tuberculosis (TB) remains a leading infectious killer in the world. Approximately one-third of the world's population is thought to be infected with the causative agent *Mycobacterium tuberculosis*. Every year about 8 million people develop active, contagious pulmonary TB and more than 1.6 million people die of infection. *M. tuberculosis* rapidly develops genetic resistance to several drugs; thus the anti-tuberculosis chemotherapy regimen recommended by the WHO is based upon a cocktail of four/five first-line drugs, including isoniazid (INH). Nevertheless, multidrug resistant (MDR) TB, extremely drug resistant (XDR) TB and TB-HIV co-infections are rapidly spreading, making the discovery and development of new anti-TB drugs lacking cross-resistance with those currently available an urgent need.[1–5] A current trend for drug design and development is the concept of multi-target 'intramolecular' hybridization.[6] According to this concept, new hybrid drugs should combine different pharmacophores acting at more than one target providing an advantage when treatment is aimed at drug-resistant infectious agents. Hydrazine-derived compounds constitute an important class of potential anti-TB drug candidates, given that the hydrazine-based pharmacophore INH (isonicotinohydrazide) is an invariable anti-tuberculosis pyridine-based medicine.[5,7–9] However, the emergence of INH resistant *M. tuberculosis* strains dictates the necessity of re-designing this old drug in order to create analogs effective against INH resistant strains. To this end, many efforts have been undertaken but without much success.[10] On the other hand, recent studies of INH structural analogs revealed high levels of activity both in vitro and in vivo against sensitive and resistant strains of Mtb.[11–18] Anti-resistant compounds were also found among direct inhibitors of the clinically validated target of INH, the mycobacterial enoyl acyl carrier protein reductase, known as InhA, in the mycolic acid synthetic pathway.[19–21] Noteworthy, INH inhibits this enzyme after preliminary fermentative activation, via an indirect mechanism, whereas some agents active against INH-resistant strains act directly, which may be explained by the concept of allosteric binding of inhibitor to the enzyme target.[22–24] Meanwhile, a number of direct InhA inhibitors possessing antimycobacterial activity were found in a series of indole-containing compounds, being versatile privileged drug-like scaffolds.[21,25–27] Importantly, many known indole-based drugs and bioactive molecules demonstrated an ability to inhibit novel targets, structurally different from those currently known.[28] In addition, indole agents were found recently to affect a number of vital targets in *M. tuberculosis*, in particular as inhibitors of cell wall biosynthesis and bacterial respiration (inhibitors of MmpL3, DprE1, CYP125A1, the *M. tuberculosis* protein tyrosine phosphatase B, indoleamine 2,3-dioxygenase (IDO) enzymes, etc.).[29–38] Based on this background, we designed hybrid structures combining two redox-active heterocyclic scaffolds with previously demonstrated anti-TB activity: hydrophilic electron-deficient pyridines and lipophilic electron-rich indoles tethered through a

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hydrazine pharmacophore. We aimed to synthesize a series of simple hydrazide/hydrazide–hydrazone hybrids from commercially available precursors, anticipating establishment of hybrid molecules with significant antimycobacterial activity due to synergistic effects.

In continuation of our studies of hydrazine-derived antimycobacterials[39] a series of novel 1-acetylindoxyl **1** and substituted indole-3-carboxaldehydes **7**–**12** derived hydrazides and hydrazide–hydrazones (Schiff bases) as well as substituted indole-3-carboxaldehyde derived thiosemicarbazones were synthesized (Schemes 1–4).

Combining indole and pyridine or 1-oxide pyridine nuclei as a single hybrid molecule was performed via a hydrazine group involving a simple C—N or double C=N bond to generate corresponding substituted hydrazides and hydrazide–hydrazones. Recent studies showed that the N-oxidation of a pyridine nucleus mostly resulted in retained or increased minimum inhibitory concentrations (MIC) against *M. tuberculosis*.[40] Certain 1-oxide pyridine derivatives turned out to be active against dormant strains of *M. tuberculosis*.[41] This data provided the stimulus for us to prepare a few *N*-oxidized pyridine hybrids as well. We also paid attention to the data concerning strong antimycobacterial activity of known indole-based Schiff bases and thiosemicarbazones.[42,43] As reported earlier Schiff base $\mathbf{B_1}$ derived from INH and unsubstituted indole-3-carboxaldehyde **7** had a MIC value of 0.0063 μg/mL against *M. tuberculosis*-H37Rv.[44] Moreover, indole-3-carboxaldehyde thiosemicarbazone was reported as an anti-TB agent with in vivo activity in mice.[45] Isatin β-thiocarbohydrazone showed activity against drug resistant mycobacterial strains.[46] Recently, a novel anti-TB thiosemicarbazone drug, perchlozon, was reported by a Russian group.[47] Introduction of COOH or $COOC_2H_5$ groups at the position 2 of the starting indole-3-carboxaldehydes **7**–**12**, that is, in the vicinity of the carbonyl group, allowed attachment of some novel properties to target compounds to enable comparison with unsubstituted **7**.

All compounds synthesized were divided into $\mathbf{A_{1-4}}$, $\mathbf{C_{1-7}}$ hydrazide, $\mathbf{B_{1-9}}$ hydrazide–hydrazone and thiosemicarbazone $\mathbf{D_{1-6}}$ series (Schemes 1–4, Table 1). Series $\mathbf{A_{1-5}}$ was comprised of so-called iproniazid and INH analogs (isonicotinohydrazides), derivatives of pyridine-4-carbohydrazide **3** (INH), pyridine-3-carbohydrazide **4** (nicotinohydrazide), 1-oxide pyridine-4-carbohydrazide **5**; 1-oxide pyridine-3-carbohydrazide **6** bearing an 1-acetyl-3-indolyl unit at the terminal N^2 nitrogen of the hydrazine group. Series $\mathbf{B_{1-9}}$ comprised Schiff bases, derivatives of INH and 1-oxide isonicotinohydrazide **5**, bearing substituted 3-indolyl-methylidene unit at the N^2 nitrogen. Series $\mathbf{C_{1-5,7-9}}$ of iproniazid analogs comprised isonicotinohydrazide **3** and 1-oxide isonicotinohydrazide **5** derivatives bearing substituted/unsubstituted 3-indolyl-methylene unit at the terminal nitrogen of the hydrazine group. The saturated analog $\mathbf{C_6}$ comprised a fully reduced pyridine ring.

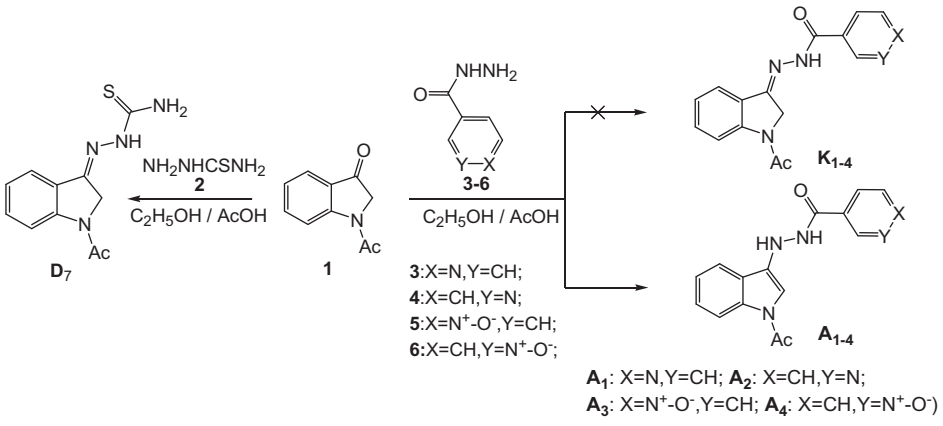
The synthesis of the intermediate and target compounds was performed according to reactions outlined in Schemes 1–4. Acetylindoxyl **1** was generally condensed under reflux with starting hydrazides **3**–**6** to afford target hydrazides $\mathbf{A_{1-4}}$ instead of the corresponding isomeric Knovenagel products $\mathbf{K_{1-4}}$. The NMR ^1H and ^{13}C spectra data indicate that aromatization of $\mathbf{K_{1-4}}$ occurs under reaction conditions due to the known imine–enamine and hydrazone–enhydrazine tautomerism.[48] All reactions were done in ethanol or acetic acid. Diacylated derivative $\mathbf{A_5}$ was obtained by the treatment of $\mathbf{A_1}$ with 4-chlorobenzoylchloride according to a standard method.

The target hydrazide–hydrazones $\mathbf{B_{2-9}}$ were prepared by the reaction of 2-ethoxycarbonyl/2-hydroxycarbonyl-indole-3-carboxaldehydes **7**–**12** with starting hydrazides **3**, **5** under conditions similar to those described above and resulted in the formation of the compound **A**. The hydrazides $\mathbf{C_{1-5,\ 7}}$ were prepared via a two-step approach including the reduction of the starting compounds $\mathbf{B_{1-5,\ 7}}$ by $NaBH_4$ in C_2H_5OH. Hydrazide $\mathbf{C_6}$ with a fully reduced pyridine ring was obtained by the reduction of hydrazone $\mathbf{B_2}$ by hydrogen at 10 atm in ethanol in the presence of 5% Pd/C as catalyst. Diacylated derivatives $\mathbf{C_{8,\ 9}}$ were obtained by the treatment of $\mathbf{C_2}$ with 4-chlorophenylisocyanate and isonicotinoylchloride according standard methods.

Additionally, the condensation of 2-ethoxycarbonyl/hydroxycarbonyl-indole-3-carboxaldehydes **10**, **12**–**16** and ketone **1** with thiosemicarbazide was conducted according to known methods by heating the starting components in ethanol or acetic acid to afford a series of corresponding thiosemicarbazones $\mathbf{D_{1-6}}$. Thiosemicarbazone $\mathbf{D_7}$ was reported by us earlier.[49] The structure and purity of the above indole derivatives were verified by ^1H and ^{13}C NMR spectroscopy, GC–MS and MALDI-TOF mass spectrometry.

Further, a series of **A**–**C** hybrid compounds and thiosemicarbazones **D** were tested for their in vitro antimycobacterial activity against a laboratory strain *M. tuberculosis* H37Rv and against a clinical isolate of INH-resistant *M. tuberculosis* with selective single INH resistance, designated as CN-40. *M. tuberculosis* strain H37Rv (museum strain) was originally obtained from the Institute Pasteur, Paris, France (a kind gift of G. Marchal). The CN-40 strain was isolated from a TB patient at the TB Research Institute of the Russian Academy of Medical Sciences. MICs against *M. tuberculosis* were determined by standard microdilution in microtubes using Dubos medium containing 0.05% Tween 80 (Table 2).

The preliminary studies revealed that thirty of the compounds tested (twenty nine of which were novel) were able to impair



Scheme 1. Synthesis of hydrazides $\mathbf{A_{1-4}}$ and thiosemicarbazone $\mathbf{D_7}$.