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Synthesis, antimycobacterial activity and docking study of 2-aroyl-[1]benzopyrano[4,3-*c*]pyrazol-4(1*H*)-one derivatives and related hydrazide-hydrazones

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ABSTRACT

A new convenient method for preparation of 2-aroyl-[1]benzopyrano[4,3-*c*]pyrazol-4(1*H*)-one derivatives **5b–g** and coumarin containing hydrazide-hydrazone analogues **4a–e** was presented. The antimycobacterial activity against reference strain *Mycobacterium tuberculosis* H37Rv and cytotoxicity against the human embryonic kidney cell line HEK-293 were tested *in vitro*. All compounds demonstrated significant minimum inhibitory concentrations (MIC) ranging 0.28–1.69 μM, which were comparable to those of isoniazid. The cytotoxicity (IC_{50} > 200 μM) to the "normal cell" model HEK-293T exhibited by 2-aroyl-[1]benzopyrano[4,3-*c*]pyrazol-4(1*H*)-one derivatives **5b–e**, was noticeably milder compared to that of their hydrazone analogues **4a–e** (IC_{50} 33–403 μM). Molecular docking studies on compounds **4a–e** and **5b–g** were also carried out to investigate their binding to the 2-*trans*-enoyl-ACP reductase (InhA) enzyme involved in *M. tuberculosis* cell wall biogenesis. The binding model suggested one or more hydrogen bonding and/or arene-H or arene-arene interactions between hydrazones or pyrazole-fused coumarin derivatives and InhA enzyme for all synthesized compounds.

A reemergence of tuberculosis accompanied by an increasing number of drug resistant *Mycobacterium tuberculosis* strains highlights the urgent need of searching and developing of new antitubercular drugs, capable of bypassing the resistance mechanisms. In the last decades, major advances in molecular biology have increased the knowledge of the mechanisms of resistance to the main anti-TB drugs, with the identification of specific gene mutations that are associated with drug resistance[1,2]

Isoniazid (INH), an essential antitubercular agent recommended by the WHO, is a prodrug that penetrates the tubercle bacilli by passive diffusion and is bio-activated by the bacterial anti-oxidant enzyme (KatG).[3,4] It exerts its anti-tubercular activity *via* interference with the synthesis of mycolic acids, which comprise crucial elements of the mycobacterial cell wall**.** Even with the clinical success of isoniazid, severe adverse effects, especially peripheral neuropathy and hepatotoxicity, are associated with INH-based treatment protocols; moreover its usefulness is further limited by the occurrence of resistance.[5] To overcome the resistance,[6] the drug design strategies frequently employ a combination of the INH molecule with other pharmacophores, rendering antitubercular activity. The novel INH hydrazide derivatives appeared to be promising anti-tubercular agents - more effective and less hepatotoxic than isoniazid.[7–16] In the meantime Ellis *at al.*,[17] have described the mechanism of action of the pyridoxal isonicotinoyl hydrazones (PIC) and suggested that hydrazones act as a lipophilic vehicle for the transport of its intact INH moiety into the mammalian cell and the mycobacterium (Fig. 1). The mechanism of antimycobacterial activity of INH[18] and isonicotinoyl hydrazone derivatives passes through formation of electrophilic intermediate species (i.e. a hydrazyl radical or ion) (Fig. 1). The acyl radical being coupled to NADH or NAD + seems to be crucial in yielding adduct responsible for the inhibition of 2-*trans*-enoyl-ACP reductase (InhA), and in restraining the mycobacterial cell wall synthesis. InhA catalyze the final step in the elongation cycle of the bacterial

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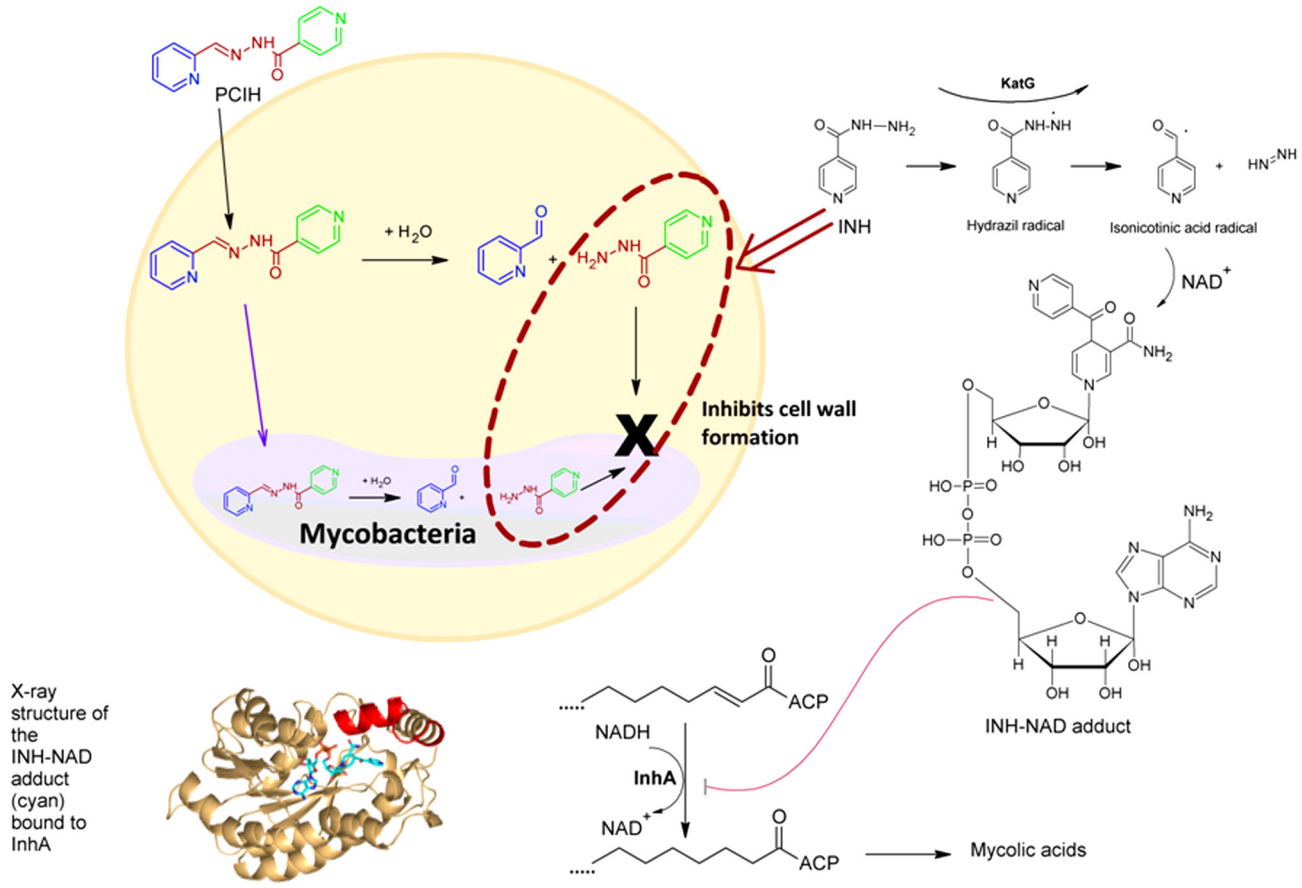

Fig. 1. Schematic representation of the proposed mechanism of action of isoniazid (INH)[18] and hydrazone derivative (2-pyridylcarboxaldehydeisonicotinoylhydrazone – PCIH).[17] X-ray structure of the INH-NAD adduct (cyan) bound to InhA (1zid.pdb).[27] The substrate binding loop is colored red. This portion of the protein (res 196–211) is disordered in the structure of triclosan bound to InhA (2b35.pdb).[24].

fatty acid biosynthesis pathway (type-II fatty acid elongation system- FAS-II) has been recognized as a primary molecular target for developing new anti-TB drugs[19,20] (Fig. 1). There is also evidence that INH can inhibit non-NAD(P) binding proteins such as KasA, the β-ketoacyl-ACP synthase in the FASII pathway.[21] Importantly, since mutations of KatG are the most common mechanisms of resistance to INH,[22,23] inhibitors that bind to the final drug target (s) should be active against the majority of INH-resistant clinicaly isolated strains.[24] Molecular investigation on the genetic basis of the INH-resistance in *M. tuberculosis* strains in Bulgaria targeted two the most frequently reported mutations related to INH resistance, katG 315AGC > ACC and inhA −15C > T[25,26] The global prevalence of the katG S315T substitution in INH-resistant strains highlights the selective advantage conferred by this mutation, which appears to provide the optimal balance between decreased catalase activity and a sufficiently high level of peroxidase activity in KatG. Mutations in the inhA promoter region are thought to increase the InhA protein expression, thereby elevating the drug target levels and producing INH resistance by a drug titration mechanism.

This highlights the crucial need of developing drugs with shorter, simpler regimens as well as with novel mechanisms of action that can be used for treatment of drug resistant forms of the disease. Numerous efforts have been undertaken to develop new INH derivatives and hydrazones[28,29] as anti-TB agents (Saluzide, Ftivazide, Salizide).[30,31] Indeed, the INH derivative LL-3858 is in initial stages of phase II clinical trial for the treatment of TB and may be approved to treat TB in the near future.

Recently we described a series of hydrazide-hydrazones containing coumarin or 2*H*-chromene moieties with potent anti-TB activity and moderate cytotoxicity.[32] Having surveyed comprehensively the literature on pyrazole[33–36] and coumarin[34,37–46] scaffolds as important structural components in the design of anti-tuberculosis agents against *Mycobacterium tuberculosis* we focused on the synthesis of pyrazole-fused coumarin derivatives as less toxic antimycobacterial compounds.

Herein, continuing the research on the biological activity of 2*H*-chromene derivatives, we present the one-pot synthesis, antimycobacterial activity and cytotoxicity of novel 2-aroyl-[1]benzopyrano[4,3-*c*]pyrazol-4(1*H*)-one derivatives **5b–g**. Molecular docking studies were carried out with the InhA enzyme from *M. tuberculosis* in order to explore the structural requirements controlling the observed antimycobacterial activity of **4a–e** and **5b–g**.

The synthesis of hydrazide-hydrazones **4a–e** and *N*-2-substituted coumarin[4,3-*c*]pyrazoles **5b–g** is shown in Scheme 1.

The synthesis of coumarin based hydrazones **4a–e** was achieved by a classical method reported previously.[33] It involves a reaction of 4-chlorocoumarin-3-carbaldehyde **2** with corresponding hydrazides **3a–e** in abs. EtOH. The detailed investigation of the reaction showed that fused 2-aroyl-[1]benzopyrano[4,3-*c*]pyrazol-4(1*H*)-one was formed easily when an electron donating group was present at *p*-position in the benzene ring. The reaction of 4-chlorocoumarin-3-carbaldehyde **2** with **3b–g** in EtOH:CH_2Cl_2 (at a 1:3 ratio) resulted in *N*-2-substituted chromeno[4,3-*c*]pyrazol-4-one analogues **5b–g** (Scheme 1) in nearly quantitative yields (85–92%). Compounds **5b–g** were further purified by column