A class of hydrazones are active against non-replicating *Mycobacterium tuberculosis*

Shilah A. Bonnett, Devon Dennison, Megan Files, Anumita Bajpai, Tanya Parish

TB Discovery Research, Infectious Disease Research Institute, Seattle, WA, United States of America

* tanya.parish@idri.org

Abstract

There is an urgent need for the development of shorter, simpler and more tolerable drugs to treat antibiotic tolerant populations of *Mycobacterium tuberculosis*. We previously identified a series of hydrazones active against *M. tuberculosis*. We selected five representative compounds for further analysis. All compounds were active against non-replicating *M. tuberculosis*, with two compounds demonstrating greater activity under hypoxic conditions than aerobic culture. Compounds had bactericidal activity with MBC/MIC of < 4 and demonstrated an inoculum-dependent effect against aerobically replicating bacteria. Bacterial kill kinetics demonstrated a faster rate of kill against non-replicating bacilli generated by nutrient starvation. Compounds had limited activity against other bacterial species. In conclusion, we have demonstrated that hydrazones have some attractive properties in terms of their anti-tubercular activity.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a global health problem [1]. In 2016, 10.3 million people worldwide became ill with TB and 1.7 million people lost their lives to the disease [1]. While the number of deaths fell ~ 24%, the number of new cases increased slightly to 6.3 million in 2016. Approximately a quarter of the world’s population has latent TB in which patients are asymptomatic and non-infectious. Reactivation of latent infection is observed in 10% of cases representing a large reservoir of infection [2, 3].

During latent infection, *M. tuberculosis* bacilli can persist in the granuloma for years. During this time, the bacteria are in a slow or non-replicating state with low metabolic activity. The metabolic state of the bacilli is influenced by host environmental conditions such as low oxygen and pH, nutrient deprivation, and exposure to RNS and ROS [4], all of which may contribute to antibiotic tolerance. *In vitro*, starvation-induced, non-replicating bacilli are tolerant to isoniazid, rifampicin and metronidazole, but not pyrazinamide, econazole or clotrimazole [5–7]. In contrast, under low oxygen conditions, *M. tuberculosis* enters a non-replicating state that is tolerant to isoniazid, but sensitive to metronidazole [8–13].

The prevalence of latent TB has complicated our ability to eradicate the disease. There is an urgent need for the development of shorter, simpler and more tolerable drug regimens to treat various subpopulations of *M. tuberculosis*. In order to attain a shorter therapy period, new
drugs should be bactericidal and be efficacious against non-replicating and antibiotic tolerant forms of *M. tuberculosis*.

The hydrazone linker (-NH-N = CH-) is a useful synthetic tool enabling the generation of hydrazide-hydrazone derivatives, many of which are pharmacologically-active. Such molecules target wide range of diseases and have anti-microbial, anti-cancer, anti-malarial and anti-inflammatory activities [14–28]. While the mode of action of hydrazones varies depending upon the structural characteristics, several are involved in covalent modification of proteins and/or sequestering of metal ions.

Isoniazid (INH), a first line TB drug is a hydrazine, which is a tight-binding inhibitor of enoyl reductase (InhA) in *M. tuberculosis*; INH is a prodrug which requires activation by the KatG catalase-peroxidase [29–31]. Another anti-tubercular hydrazine, thiacetazone (TAC) covalently modifies hydroxyl-acyl-dehydratases (HadAB and HadBC) following activation by the mono-oxygenase EthA and hydroxymycolate synthase (MmaA4) [32–34]. More recently, 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone, was identified as a selective inhibitor of *M. tuberculosis* methionine aminopeptidase (MetAPs) with activity against replicating and non-replicating bacteria [35]. In addition, quinolone hydrazone derivatives are currently being explored as potential anti-cancer and anti-tubercular drugs [36]. Interestingly, copper (II) and zinc (II) complexes of quinolone hydrazone derivatives have higher anti-tubercular activity than the free hydrazone [36].

We previously identified a series of phenylhydrazones (PHY) in a target-based whole-cell screen [37]. We demonstrated good activity in growth inhibition assays against actively growing wild-type bacteria, as well as improved activity against a strain engineered to under-express the sole signal peptidase, LepB [37]. We conducted a small structure-activity relationship study and identified several modifications which improved potency against *M. tuberculosis*. We selected 3 representative hydrazone compounds (1, 2 and 4) and a hydrazide (3) based on their activity and structure for further characterization in other assay systems that mimic the different environments encountered by *M. tuberculosis* within the host (Fig 1) [37]. The anti-tubercular activity and cytotoxicity for compounds 1–4 have been described elsewhere [37].

**Materials and methods**

**Bacterial culture**

Mycobacteria were cultured in Middlebrook 7H9 medium supplemented with 0.5% w/v Tween 80 and 10% v/v oleic acid, albumin, dextrose, catalase (OADC) supplement (7H9-Tw-OADC) or on Middlebrook 7H10 agar plus 10% v/v OADC. *Escherichia coli* DH5α and *Staphylococcus aureus* RN4220 were grown in LB broth and on LB agar. *Pseudomonas aeruginosa* HER1018 (PAO1) was grown in tryptic soy broth and on tryptic soy agar. *Bacillus subtilis* Marburg was grown in nutrient broth and on nutrient agar. *Saccharomyces cerevisiae* Y187 was grown in YPD broth and on YPD agar supplemented with 0.2% adenine hemisulfate.

**Minimum inhibitory concentration (MIC) determination**

MICs were determined in liquid medium in 96-well, black, clear-bottom plates as described [38]. A 10-point 2-fold serial dilution was run for each compound and bacterial growth was measured by OD<sub>590</sub> after 5 days of incubation at 37˚C. Growth inhibition curves were fitted using the Levenberg–Marquardt algorithm. The IC<sub>90</sub> was defined as the concentration of compound required to inhibit growth by 90%.
Low Oxygen Recovery Assay (LORA)

The Low Oxygen Recovery Assay was carried out as described in 96-well plates [5]. Bacteria (M. tuberculosis strain H37Rv-LUX) were cultured in Dubos medium with supplement (DTA) in the Wayne Model of hypoxia for 18 days to enter hypoxia and used to seed 96-well plates containing compounds. Plates were incubated for 9 days under anaerobic conditions followed by 28h outgrowth under aerobic conditions; as a comparator plates were incubated for 6 days under aerobic conditions. Growth was measured by luminescence. Growth inhibition curves were fitted using the Levenberg–Marquardt algorithm. IC\textsubscript{90} was determined as the minimum concentration required to prevent 90% growth.

Minimum Bactericidal Concentration (MBC)

MBCs were determined as described [39]. Briefly, a late log phase culture (OD\textsubscript{590} 0.6–1.0) was adjusted to an OD of 0.1 in 7H9-Tw-OADC and 50 μL used to inoculate 5 mL of 7H9-Tw-OADC containing compound. Cultures were incubated standing at 37˚C. For starvation, M. tuberculosis H37Rv was resuspended in phosphate buffer saline (PBS) plus 0.05% w/v Tyloxapol and incubated for 2 weeks before the addition of compound. Bacterial viability was determined by plating serial dilutions and enumerating CFUs after four weeks of incubation at 37˚C.

Spectrum

MICs were determined using the serial dilution agar method. Unless otherwise stated, compounds were prepared as an 8-point 2-fold serial dilution in DMSO starting at 100 μM. MIC\textsubscript{99} was defined as the minimum concentration that prevented 99% of growth.

Chemical synthesis

The synthesis of the thiosemicarbazone 5 was according to procedures described previously [37]. Briefly, 1 eq of the acid and 1 eq of the hydrazine were dissolved in anhydrous ethanol and the resulting reaction mixture was refluxed overnight. The acetone was evaporated and...
the crude reaction mixture purified by column chromatography. $^1$H and NMR spectral data were recorded in CDCl$_3$ or Acetone-d$_6$ on a 300 MHz Bruker NMR spectrometer. Column chromatography was carried out on Revelaris flash chromatography system. Reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. HPLC analysis was conducted on an Agilent 1100 series LC system (Agilent ChemStation Rev.A.10.02; Phenomenex-Luna-C18, 4.8 mm × 150 mm, 5 μm, 1.0 mL/min, UV 254nm, room temperature) with MeCN/H$_2$O (0.05% TFA or HCOOH buffer) gradient elution. HPLC-MS was performed on a Gilson 321 HPLC with detection performed by a Gilson 170 DAD and a Finnigan AQA mass spectrometer operating in electrospray ionization mode using a Phenomenex Gemini C18 150x4.6mm column. Yield: (0.011 g, 11%). $^1$H NMR (300 MHz, MeOD): 2.55 (3H, CH$_3$); 7.62–8.51 (6H, m). LCMS–ESI (M+H)$^+$: 245.1.

Results and discussion

The PHY series have good activity against aerobically-cultured, actively growing M. tuberculosis in axenic culture. Our previous work was limited to determining minimum inhibitory concentrations (MICs) under these conditions and demonstrated activity for a range of analogs, with many MICs in the range of 20 μM [37]. However, many compounds that act against actively growing mycobacteria are ineffective against non-replicating or intracellular organisms. Since M. tuberculosis can survive under low oxygen tension, we were interested to determine whether our compounds had activity under this setting, which is relevant to the environment encountered during infection. We were also interested in determining if compounds were bactericidal against replicating and non-replicating bacilli. We selected three representative hydrazones and a hydrazide based on their activity and structure for characterization in other assay systems (Fig 1) [37].

Previously, we reported an MIC for 4 of 3.6 μM against the LepB underexpressing strain and 46 and > 200 μM against wild-type. We repeated the experiment and determined MICs of > 200 μM and 2.3 ± 1.3 μM against the wildtype and LepB underexpressing strain of M. tuberculosis, respectively.

We also examined an additional thiosemicarbazone, 5. Replacing the pyridinyl group in 4 with an isoquinolinyl (5) group had little impact on the MIC against the Lep underexpressing strain (MIC 3.2 ± 2.1 μM) however at least a 40 fold increase in activity against wildtype (MIC 5.3 ± 2.0 μM) was observed.

Hydrazones are active against hypoxically-induced, non-replicating M. tuberculosis

We determined the activity of our compounds using the low-oxygen-recovery assay (LORA) [5, 40]. We determined the IC$_{90}$ (the concentration required to prevent outgrowth by 90%) for bacteria under both aerobic and anaerobic conditions for comparison. Six compounds were tested (Table 1). Five of the compounds were active under aerobic conditions, with IC$_{90}$ < 20 μM. All of the compounds were active under anaerobic conditions, with two compounds, the thiosemicarbazones 4 and 6, showing greater activity under hypoxia (>2-fold difference). Two compounds (3 and 5) were equally active under both conditions. These data demonstrate hydrazine-containing compounds are efficacious against hypoxia-induced non-replicating bacilli.

Hydrazones compounds are bactericidal against replicating M. tuberculosis

We selected three compounds that were active against wild-type M. tuberculosis with an IC$_{90}$ < 100 μM and which were more potent against the LepB underexpressing strain. We first
determined whether compounds had bactericidal or bacteriostatic activity under aerobic conditions (Fig 2). We tested four compounds at varying concentrations. The highest concentration of compound that can be tested in our assay and keep DMSO to 2% is 200 μM. Therefore we selected concentrations to test as follows: for compounds with MIC < 20 μM, we used concentrations as a multiple of the MIC i.e. 10X, 5X, 2.5X, and 1X MIC; for compounds with MICs > 20 μM, we used fixed concentrations of 200, 100, 50, 25 and 20 μM.

All compounds were able to effect a 3 log kill within 21 days. At the highest concentrations compounds 1 and 2 were able to sterilize culture within 7 days, and compound 3 within 14 days. Compounds activity was concentration-dependent, (as defined by CLSI guidelines [41]), since the rate of kill increased with increasing concentration. The MBC, defined as a 3 log kill within 21 days, was determined; compound 1 was 200 μM, compound 2 was 35 μM, compound 3 was 7.8 μM. The compounds were all classified as bactericidal i.e. MBC/MIC of < 4 [41]. For compound 1, the increase in CFUs in the culture treated with 100 μM after day 7 is likely due to the outgrowth of resistant mutants at the lower concentration.

The bactericidal activity of hydrazone compounds is inoculum-dependent

We examined the effect of inoculum size on the efficacy of compound 1 and 2 against M. tuberculosis under aerobic conditions (Fig 3). Cultures were exposed to 10X MIC over a 7 day period. Both compounds behaved in a similar fashion and their effect was inoculum-dependent i.e at high starting inoculum (∼10⁷ CFU/mL), compounds had no impact on bacterial viability. At lower inoculum size, a 2 log reduction in CFU/mL was observed. Complete kill by day 7 was only seen when the inoculum was ≤ 10⁵.

Hydrazones are rapidly bactericidal against starvation-induced, non-replicating M. tuberculosis

One of the complications of LORA, is that it requires a period of outgrowth after exposure to compound under hypoxic conditions when the compound is still present. We used the nutrient starvation model, in which loss of bacterial replication is due to complete starvation in order to determine compound efficacy against non-replicating bacteria. In this model bacilli are starved for 2 weeks before compound exposure, and bacterial viability monitored over 21 days. The dilution step remove any compound carryover during plating. We tested four compounds (Fig 4). Interestingly, all the compounds showed much greater activity i.e. more rapid kill and at lower concentrations, than against replicating bacilli. For all compounds, cultures were sterilized by day 14 even at the lowest concentration tested. We also examined the bactericidal activity of frontline anti-tubercular drugs, isoniazid and rifampicin. While isoniazid lacked bactericidal activity against replicating M. tuberculosis, it exhibited concentration

Table 1. Hydrazone compounds are active under hypoxia. M. tuberculosis was cultured in DTA medium in the Wayne model of hypoxia for 18 days to generate the non-replicating state. Bacteria were inoculated into plates containing compounds and incubated for 9 days under hypoxia (anaerobic) or 6 days in air (aerobic). Growth was measured by luminescence. IC⁹₀ is the concentration required to inhibit growth by 90%. Results are the mean ± standard deviation from a minimum of two experiments.

| Cpd # | Anaerobic IC⁹₀ (μM) | Aerobic IC⁹₀ (μM) |
|-------|---------------------|-------------------|
| 1     | 17 ± 3.0            | > 20              |
| 2     | 22 ± 12             | 6.4 ± 2.4         |
| 3     | 8.3 ± 0.3           | 6.2 ± 4           |
| 4     | 5.5 ± 2.5           | 13 ± 6.3          |
| 5     | 2.5 ± 1.3           | 2.7               |

https://doi.org/10.1371/journal.pone.0198059.t001

A class of hydrazones are active against non-replicating Mycobacterium tuberculosis

PLOS ONE | https://doi.org/10.1371/journal.pone.0198059 October 17, 2018 5 / 11
A class of hydrazones are active against non-replicating *Mycobacterium tuberculosis*. Rifampicin, had no effect against non-replicating bacteria, but was effective against replicating bacteria (S1 Fig).

Fig 2. Kill kinetics against replicating *M. tuberculosis*. Bacterial viability in the presence of compound was determined by counting CFU every 7 days over a 21-day period. Compounds (A) 1, (B) 2, and (C) 3. The dashed line represents the lower limit of detection.

https://doi.org/10.1371/journal.pone.0198059.g002

Fig 3. The effects of inoculum size on compound activity. Aerobic cultures were exposed to compounds at 10X MIC over 7 days. Bacterial viability was determined by counting CFU. (A) DMSO, (B) Compound 1, (C) 2. The starting inoculum was: (●) $10^7$, (■) $10^6$, (▲) $10^5$, (▼) $10^4$, and (♦) $10^3$. The dashed line represents the lower limit of detection.

https://doi.org/10.1371/journal.pone.0198059.g003
Hydrazones are not active against other bacterial species

We were interested to determine the spectrum of activity of a select number of hydrazone compounds. We had previously noted narrow selectivity for *M. tuberculosis* over eukaryotic cells with selectivity indices of <10 [37]. We wanted to determine if this reflected a broad spectrum of activity against all organisms. We measured activity against a range of species on solid medium; for each species we determined the MIC<sub>99</sub>, defined as the minimum concentration required to inhibit growth by 99% (Table 2). Activity against *M. tuberculosis* was lower on

**Table 2. Spectrum of activity for hydrazone compounds.** MIC<sub>99</sub> were measured on solid medium for each species and defined as the concentration required to inhibit growth by 99%.

| IDRI #  | Cpd # | E. coli | M. smegmatis | S. cerevisiae | P. aeruginosa | B. subtilis | S. aureus | M. tuberculosis |
|---------|-------|---------|--------------|--------------|--------------|-------------|-----------|----------------|
| IDR-0107828 | 1     | >100    | >100         | >100         | >100         | >100        | >100      | 3.1            |
| IDR-0111355 | 2     | >100    | >100         | >100         | >100         | >100        | >100      | >100           |
| IDR-0483994 | 3     | >100    | >100         | >100         | >100         | >100        | >100      | 12.5           |
| IDR-0483998 | 4     | >100    | >100         | 50           | >100         | 100         | 100       | >50            |

https://doi.org/10.1371/journal.pone.0198059.t002
solid medium than in liquid, with only 2 of the 4 compounds tested showing appreciable activity. The MIC\textsubscript{99} for compounds 1 and 3 on solid medium was 3.1 and 12.5 μM, respectively, which is 2–6-fold lower than the MIC in liquid culture (S1 Table). Compounds were tested against representative Gram-negative species (Escherichia coli and Pseudomonas aeruginosa), as well as Gram-positive (Bacillus subtilis and Staphylococcus aureus), other mycobacteria (Mycobacterium smegmatis) and another eukaryote, Saccharomyces cerevisiae. Compounds 1, 3 were inactive against all species except M. tuberculosis. Compounds 4 had low activity (MIC\textsubscript{99} = 50 μM) against Sac. cerevisiae and minimal activity against B. subtilis and Staph. aureus. There was no correlation between the activity against M. tuberculosis and other species, since the most active anti-tubercular compounds were inactive against other species.

In conclusion, we have demonstrated that a select number of hydrazone compounds have some attractive properties in terms of their anti-tubercular activity. M. tuberculosis can survive in hostile environments in actively replicating, slow growing or non-replicating states [5, 6, 42, 43]. The presence of slowly replicating and non-replicating persistent forms of M. tuberculosis contributes to latent TB infections and drug tolerance, which ultimately leads to the long treatment therapy [12]. Hypoxia and nutrient deprivation are two conditions M. tuberculosis encounters during infection. The hydrazone compounds were active in two single stress models (hypoxia and starvation) under conditions that promote antibiotic tolerance. In addition, they demonstrated higher rates of kill against non-replicating than replicating bacilli. Our previous work had demonstrated that they are also more potent against a strain of M. tuberculosis with reduced LepB [37]. Since LepB expression is reduced under both nutrient-starved and hypoxic conditions [6, 7], this may account for the increased activity of hydrazone compounds in these conditions.

Supporting information
S1 Fig. Kill kinetics of isoniazid and rifampin against replicating and non-replicating M. tuberculosis.
(DOCX)

S1 Table. In vitro properties of representative hydrazone compounds.
(DOCX)

Acknowledgments
We thank Matthew McNeil and Divya Awasthi for technical assistance.

Author Contributions
Conceptualization: Shilah A. Bonnett, Devon Dennison, Tanya Parish.

Data curation: Shilah A. Bonnett, Tanya Parish.

Formal analysis: Shilah A. Bonnett, Devon Dennison, Megan Files, Anumita Bajpai, Tanya Parish.

Funding acquisition: Tanya Parish.

Investigation: Shilah A. Bonnett, Devon Dennison, Megan Files, Anumita Bajpai.

Methodology: Shilah A. Bonnett, Devon Dennison, Megan Files, Anumita Bajpai, Tanya Parish.

Project administration: Tanya Parish.
Supervision: Shilah A. Bonnett, Tanya Parish.

Validation: Tanya Parish.

Writing – original draft: Shilah A. Bonnett, Tanya Parish.

Writing – review & editing: Shilah A. Bonnett, Tanya Parish.

References
1. Organization WH. Global Tuberculosis Report 2017. World Health Organization, 2017.
2. Smieja MJ, Marchetti CA, Cook DJ, Small FM. Isoniazid for preventing tuberculosis in non-HIV infected persons. The Cochrane database of systematic reviews. 2000;(2):Cd001363, https://doi.org/10.1002/14651858.CD001363 PMID: 10796642
3. Akolo C, Adetifa I, Shepperd S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. The Cochrane database of systematic reviews. 2010;(1):Cd000171, https://doi.org/10.1002/14651858.CD000171.pub3 PMID: 20091503
4. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, et al. Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: Insights into the phagosomal environment. The Journal of Experimental Medicine. 2003; 198(5):693–704, https://doi.org/10.1084/jem.20030846 PMID: 12953091
5. Cho SH, Wart S, Wan B, Hwang CH, Pauli GF, Franzenblau SG. Low-Oxygen-Recovery Assay for high-throughput screening of compounds against nonreplicating Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2007; 51(4):1380–5, https://doi.org/10.1128/AAC.00055-06 PMID: 17210775
6. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling. Molecular Microbiology. 2002; 43(3):717–31, https://doi.org/10.1046/j.1365-2958.2002.02779.x PMID: 11929527
7. Murphy DJ, Brown JR. Identification of gene targets against dormant phase Mycobacterium tuberculosis infections. BMC Infectious Diseases. 2007; 7(1):84, https://doi.org/10.1186/1471-2334-7-84.
8. Wayne LG, Sramek HA. Metronidazole is bactericidal to dormant cells of Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 1994; 38(9):2054–8, https://doi.org/10.1128/AAC.38.9.2054. PMID: 7811018
9. Fattorini L, Piccaro G, Mustazzolino A, Giannoni F. Targeting dormant bacilli to fight tuberculosis. Mediterranean Journal of Hematology and Infectious Diseases. 2013; 5(1):e2013072, https://doi.org/10.4155/mjhid.2013.072 PMID: 24363887
10. Roupie V, Romano M, Zhang L, Korf H, Lin MY, Franken KLMC, et al. Immunogenicity of eight dormancy region-encoded proteins of Mycobacterium tuberculosis in DNA-vaccinated and tuberculosis-infected mice. Infection and Immunity. 2007; 75(2):941–9, https://doi.org/10.1128/IAI.01137-06 PMID: 17140953
11. Wayne LG, Hayes LG. An in vitro model for sequential study of shiftdown of Mycobacterium tuberculosis through two stages of nonreplicating persistence. Infection and Immunity. 1996; 64(6):2062–9. PMID: 8675038
12. Griselda Tudó KL, Denis A Mitchison, Philip D Butcher & Simon J Waddell. Examining the basis of isoniazid tolerance in nonreplicating Mycobacterium tuberculosis using transcriptional profiling. Future Medicinal Chemistry. 2010; 2(8):1371–83, https://doi.org/10.4155/fmc.10.219 PMID: 21426023
13. Wayne LG. Dormancy of Mycobacterium tuberculosis and latency of disease. European journal of clinical microbiology & infectious diseases. 1994; 13(11):908–14.
14. Khan SA, Yusuf M. Synthesis, spectral studies and in vitro antibacterial activity of steroidal thiosemicarbazon and their palladium (Pd (II)) complexes. European journal of medicinal chemistry. 2009; 44 (5):2270–4, https://doi.org/10.1016/j.ejmech.2008.06.006 PMID: 18715679
15. Loncle C, Brunel JM, Vidal N, Dherbomez M, Letoumeux Y. Synthesis and antifungal activity of cholesterol-hydrazide derivatives. European journal of medicinal chemistry. 2004; 39(12):1067–71, https://doi.org/10.1016/j.ejmech.2004.07.005 PMID: 15571888
16. Vicini P, Zani F, Cozzi P, Doychinova I. Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations. European journal of medicinal chemistry. 2002; 37 (7):553–64. PMID: 12126774
17. Bedia KK, Elcin O, Seda U, Fatma K, Nathaly S, Sevim R, et al. Synthesis and characterization of novel hydrazide-hydrazones and the study of their structure-antituberculosis activity. European journal of
18. Küçükgüzeli ŞG, Rollas S, Küçükgüzeli I, Kiraz M. Synthesis and antimycobacterial activity of some coupling products from 4-aminobenzoic acid hydrazones. European journal of medicinal chemistry. 1999; 34(12):1093–100, https://doi.org/10.1016/S0223-5234(99)00129-4.

19. Sriram D, Yogeesswari P, Madhu K. Synthesis and in vitro and in vivo antimycobacterial activity of isonicotinyl hydrazones. Bioorganic & medicinal chemistry letters. 2005; 15(20):4502–5, https://doi.org/10.1016/j.bmcl.2005.07.011.

20. Ventura C, Martins F. Application of quantitative structure-activity relationships to the modeling of antitubercular compounds. 1. The hydrazide family. Journal of medicinal chemistry. 2008; 51(3):612–24, https://doi.org/10.1021/jm701048s PMID: 18176999

21. Imramovs ky A, Polanc S, Vinsova J, Kocevar M, Jampilek J, Reckova Z, et al. A new modification of anti-tubercular active molecules. Bioorganic & medicinal chemistry. 2007; 15(7):2551–9, https://doi.org/10.1016/j.bmc.2007.01.051.

22. Maccari R, Ottana R, Vigorita MG. In vitro advanced antimycobacterial screening of isoniazid-related hydrazides and cyanoboranes: part 14. Bioorganic & medicinal chemistry letters. 2005; 15(10):2509–13, https://doi.org/10.1016/j.bmcl.2005.03.065.

23. Vavrikova E, Polanc S, Kocevar M, Kosmrj J, Horvat K, Bosze S, et al. New series of isoniazid hydrazones linked with electron-donating substituents. European journal of medicinal chemistry. 2011; 46(12):5902–9, https://doi.org/10.1016/j.ejmech.2011.09.054 PMID: 22018878

24. Vergara FM, Lima CH, Henriques M, Candea AL, Lourenco MC, Ferreira Mde L, et al. Synthesis and antimycobacterial activity of N-[(E)-(monosubstituted-benzylidene)]-2-pyrazinecarbohydrazide derivatives. European journal of medicinal chemistry. 2009; 44(12):4954–9, https://doi.org/10.1016/j.ejmech.2009.08.009 PMID: 19765866

25. Todeschini AR, de Miranda ALP, da Silva KCM, Parrini SC, Barreiro EJ. Synthesis and evaluation of anti-tubercular active molecules. Bioorganic & medicinal chemistry. 2007; 41(11):1253–61, https://doi.org/10.1016/j.ejmech.2006.06.009 PMID: 16919372

26. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morighi R, et al. New antitumor imidazo[2,1-b]thiazole guanylhydrazones and analogues. Journal of medicinal chemistry. 2008; 51(4):809–16, https://doi.org/10.1021/jm701246g PMID: 18251494

27. Hu WX, Zhou W, Xia CN, Wen X. Synthesis and anticancer activity of thiosemiacbazones. Bioorganic & medicinal chemistry letters. 2006; 16(8):2213–8, https://doi.org/10.1016/j.bmcl.2006.01.048.

28. Vicini P, Incerti M, Doytchinova IA, La Colla P, Busonera B, Loddo R. Synthesis and antiproliferative activity of benzo[d]isothiazole hydrazones. European journal of medicinal chemistry. 2006; 41(5):624–32, https://doi.org/10.1016/j.ejmech.2006.01.010 PMID: 16540208

29. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature. 1992; 358(6387):591–3, https://doi.org/10.1038/358591a0 PMID: 1501713

30. Musser JM, Kapur V, Williams DL, Kreiswirth BN, van Soolingen D, van Embden JD. Characterization of the catalase-peroxidase gene (katG) and inhA locus in isoniazid-resistant and -susceptible strains of Mycobacterium tuberculosis by automated DNA sequencing: restricted array of mutations associated with drug resistance. The Journal of infectious diseases. 1996; 173(1):196–202. PMID: 8537659

31. Quemard A, Dessen A, Sugantino M, Jacobs WR, Sacchettini JC, Blanchard JS. Binding of catalase-peroxidase-activated isoniazid to wild-type and mutant Mycobacterium tuberculosis enoyl-ACP reductases. Journal of the American Chemical Society. 1996; 118(6):1561–2, https://doi.org/10.1021/ja950996b.

32. Alahari A, Alibaud L, Trivelli X, Gupta R, Lamichhane G, Reynolds RC, et al. Mycolic acid methyltransferase, MmaA4, is necessary for thiacetazone susceptibility in Mycobacterium tuberculosis. Mol Microbiol. 2009; 71(5):1263–77, https://doi.org/10.1111/j.1365-2958.2009.06604.x PMID: 19183278

33. Grzegorzewicz AE, Eynard N, Quemard A, North EJ, Margolis A, Lindenberger JJ, et al. Covalent modification of the Mycobacterium tuberculosis FAS-II dehydratase by Isoxyl and Thiacetazone. ACS infectious diseases. 2015; 1(2):91–7, https://doi.org/10.1021/acsinfecdis.1501713

34. Sacco E, Covarrubias AS, O’Hare HM, Carroll P, Eynard N, Jones TA, et al. The missing piece of the type II fatty acid synthase system from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(37):14628–33, https://doi.org/10.1073/pnas.0704132104 PMID: 17804795

35. John SF, Aniemeke E, Ha NP, Chong CR, Gu P, Zhou J, et al. Characterization of 2-hydroxy-1-naphthaldehyde isonicotinyl hydrazone as a novel inhibitor of methionine aminopeptidases from Mycobacterium tuberculosis. Tuberculosis. 2016; 101:S73–S7, https://doi.org/10.1016/j.tube.2016.09.025.
A class of hydrazones are active against non-replicating *Mycobacterium tuberculosis*.