A Review on Antitubercular Activity of Some 2-isonicotinoylhydrazinecarboxamide, 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine, N’-(E)-heteroaromatic-isonicotino-hydrazide and 1-(7-chloroquinolin-4-yl)-2-(heteroaromatic)methylene hydrazone derivatives

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ABSTRACT
The modification of the isoniazid (INH) structure and with N-substituted 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-amine, 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine derivatives were evaluated in vitro for their anti-tubercular (anti-TB) activity against M. tuberculosis H37Rv, M. avium, M. kansasii and one clinical isolated strain of M. kansasii. 2-Isonicotinoyl-N-(4-octylphenyl) hydrazinecarboxamide displayed an in vitro efficacy comparable to that of INH for M. tuberculosis with MIC of 1–2 μM. Among the halogenated derivatives, the best anti-TB activity was found for 2-isonicotinoyl-N-(2,4,6-trichlorophenyl) hydrazine carboxamide (MIC=4μM). Most of the hydrazinecarboxamides exhibited significant activity against INH-resistant non-tuberculous mycobacteria. Two other series of N’-(E)-heteroaromatic-isonicotino hydrazide derivatives and 1-(7-chloroquinolin-4-yl)-2-[(heteroaromatic)methylene]hydrazine derivatives have been for their in vitro anti-TB activity against M. tuberculosis H37Rv. Several compounds were non-cytotoxic and exhibited significant MIC value (3.12, 2.50, 1.25, or 0.60 μg/mL) compared with ethambutol (3.12 μg/mL) and rifampicin (2.0 μg/mL). These results can be considered an important point for the rational design of new leads for anti-TB compounds.

Keywords: Isoniazid, InhA inhibition, 2-isonicotinoylhydrazine carboxamide, 5-(pyridine-4-yl)-1,3,4-oxadiazol-2-amine, tuberculosis

1. INTRODUCTION
Tuberculosis (TB) is one of the most dangerous infectious diseases known. It is the most important infectious cause of death worldwide. Alarming World Health Organization (WHO) data mainly concerning an increasing number of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) forms of TB, have prompted the development of new, potent, fast-acting anti-TB drugs. Although a new drug, diarylquinoline bedaquiline (TMC-207), has been approved recently, the treatment of MDR-TB and latent forms has still not been satisfactorily resolved. Treatment of the MDR-TB infection requires shortening the total duration of therapy, improving potency against resistant strains and reducing the total expenditure and toxicity. According to the WHO, more than 2 billion people are infected with Mycobacterium tuberculosis and a total of 1.77 million people died from TB in 2007. The lack of new anti-TB drugs, the co-infection with HIV/AIDS, and the advent of resistant strains to the current therapy are the main causes responsible for TB resurgence. Among these problems, the emergence of drug-resistant TB is especially alarming. According to the WHO report, 511,000 cases of MDR-TB, strains resistant to isoniazid (INH) and rifampicin (RIF), occurred in 2007 (4.9% of all cases). Among these cases, 289,000 were new cases and 221,000 were cases that had been previously treated for TB. Another important factor in TB treatment is the advent of XDRTB, which is commonly defined as MDR-TB plus resistance to any fluoroquinolone and to, at least, one of the three injectable second-line anti-TB drugs used in TB treatment (capreomycin, kanamycin, and amikacin). The WHO estimates that 19% of MDR cases are in fact XDR-TB and the cure is possible for up to 50–60% of the people affected.

Isonicotinic acid hydrazide (INH) is a unique first-line anti-TB drug with a high specificity towards M. tuberculosis. Its mechanism of action includes multiple effects on lipids, glycolysis, biomembranes, proteins and nucleic acid synthesis. In fact, INH is a prodrug that has to be activated by a catalase/peroxidase (KatG) inside the mycobacterium to form an isonicotinoyl radical and/or stable oxidative products. KatG couples the isonicotinic acyl with NADH to form an isonicotinic acyl-NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase (InhA), thereby blocking the natural enoyl-AcpM substrate and inhibiting the synthesis of mycolic acid, the main building block of the mycobacterial cell wall.

The discovery of INH is a major milestone in the chemotherapy of TB, but the development of INH-resistance has hampered its wide usage and therapeutic potential. The need to overcome the resistance on INH, which has been found to correlate with five different genes katG, inhA, ahpC, kasA and ndh, led to a huge number of modifications.
A variety of synthetic modifications of INH linked with another active molecule through a methine bridge, by conjugation with an aniline group with electron-withdrawing substituents and via the preparation of a new type of “double” active molecules based on the fluorinated hydrazides of a benzoic acid scaffold as an INH isostere. The modification of the INH structure by introducing a carbonyl group (in place of previously described methine bridge) as the moiety that connects INH to other molecules. The thiocarbonyl linker between the 2-(trifluoromethyl)aniline moiety and INH was shown to contribute to the superior activity of the modified molecule. Furthermore, the conversion of INH to the corresponding 1,3,4-oxadiazoles resulted in compounds with improved lipophilicity and activity; some of these compounds also exhibited significant activity against INH-resistant strains.

1.1 Anti-tubercular Activity
Compounds 1a-v and 2a-d were evaluated for their in vitro anti-TB activity. The MIC is the lowest concentration of the substance at which inhibition of the growth of Mycobacterium species occurs. The in vitro anti-TB activity of the compounds was determined against M. tuberculosis 331/88 (H37Rv; dilution of strain, 10−3), M. avium My 330/88 (resistant to INH, rifampicin, ofloxacin and ethambutol; dilution of strain, 10−5), M. kansasii 235/80 (clinically isolated) (dilution of strain, 10−4) and M. kansasii 6509/96 (dilution of strain, 10−4). The concentrations of the tested compounds were used as follows: 500, 250, 125, 62.5, 32, 16, 8, 4, 2, 1 and 0.5 μM. The same concentrations, over the range of 0.5 to 250 μM, were used for INH. The MIC (in μM) was the lowest concentration at which the complete inhibition of mycobacterium growth occurred.

1.2 Structure of Isonicotinoylhydrazinecarboxamide derivatives (3a-3v)

Compounds

2-Isonicotinoyl-N-(4-methylphenyl) hydrazinecarboxamide (Ia).

2-Isonicotinoyl-N-[4-(propan-2-yl)phenyl]hydrazinecarboxamide (Ib).

2-Isonicotinoyl-N-[4-(tert-butyl)phenyl]hydrazinecarboxamide (Ic).

2-Isonicotinoyl-N-(4-butyl phenyl) hydrazinecarboxamide (Id).

Compounds

N-(4-Bromo-2-fluorophenyl)-2-isonicotinoylhydrazinecarboxamide (II).

N-(2-Bromo-4-fluorophenyl)-2-isonicotinoylhydrazinecarboxamide (Im).

N-(3,4-Dichlorophenyl)-2-isonicotinoyl hydrazinecarboxamide (In).

N-(3-Chloro-4-fluorophenyl)-2-isonicotinoylhydrazinecarboxamide (Io).
2-Isonicotinoyl-N-(4-pentylphenyl) hydrazinecarboxamide (1e).

2-Isonicotinoyl-N-(4-hexylphenyl) hydrazinecarboxamide (1f).

Isonicotinoyl-N-(4-heptylphenyl) hydrazinecarboxamide (1g).

2-Isonicotinoyl-N-(4-octylphenyl) hydrazinecarboxamide (1h).

2-Isonicotinoyl-N-(4-methoxyphenyl) hydrazinecarboxamide (1i).

2-Isonicotinoyl-N-[4-(trifluoromethyl) phenyl] hydrazinecarboxamide (1j).

N-(2,4-Dibromophenyl)-2-isonicotinoyl hydrazinecarboxamide (1k).

N-[4-Chloro-3-(trifluoromethyl)phenyl]-2-isonicotinoylhydrazinecarboxamide (1p).

N-[4-Bromo-2-(trifluoromethyl) phenyl]-2-isonicotinoylhydrazine carboxamide (1q).

N-[4-Fluoro-3-(trifluoromethyl) phenyl]-2-isonicotinoylhydrazine carboxamide (1r).

N-(4-Bromo-3-fluorophenyl)-2-isonicotinoylhydrazinecarboxamide (1s).

N-(3,5-Dichlorophenyl)-2-isonicotinoylhydrazinecarboxamide (1t).

2-Isonicotinoyl-N-(2,4,6-trichloro phenyl) hydrazinecarboxamide (1u).

N-Heptyl-2-isonicotinoylhydrazine carboxamide (1v).
None of the compounds of type 1 exhibited a higher activity than INH (MICs 0.5–1 μM) for Mtb. The best results were observed for the derivative 1h, which reached an almost equal in vitro efficacy as INH, with MICs of 1–2 μM. Among the halogenated molecules, the best activity was found for the 2,4,6-trichloro derivative 1u, which had a MIC of 4 μM, followed by 2,4-dibromo and 3-CF₃,4-bromo substituted molecules 1k and 1q (8 μM). When evaluating particular isomeric substitutions of the N-phenyl ring, we found that among the dichlorinated molecules, the 3,5-dichloro derivative 3t showed a higher activity than the corresponding 3,4- derivative 1n (32/62.5 μM and 125 μM), and for the bromofluoro derivatives, the MICs increased in the order 2-Br,4-F (1m; 16 μM) < 2-F,4-Br (1l; 32 μM) < 3-F,4-Br (1s; 62.5 μM). The comparison of the three molecules with a 3-CF₃,4-halogen substitution led to the following result: the strongest activity against Mtb was with the 3-CF₃,4-Br derivative 1q (8 μM), followed by 3-CF₃,4-Cl 1p and 3-CF₃,4-F 1r (32 μM). When the N-(4-heptylphenyl) derivative 1g and the N-heptyl molecule 1v, which vary by the presence of a phenyl ring, were compared they exhibited identical MICs of 16/32 μM. The influence of the 4-alkyl length on the activity is interesting: the expanding length from methyl 1a to n-butyl 1d increased the activity from 62.5 to 4/8 μM; penty1 1e and hexyl 1f resulted in a milder activity; and heptyl 1g displayed lower MICs comparable to the isopropyl. Finally, the octyl substituted molecule 1h was evaluated and was determined to be the most potent compound in this series. One possible hypothesis that may explain this effect is the similarity of longer alkyl chain with fatty acids, which are structural fragments of biomembranes.

### Table 1: Antitubercular activity of N-substituted 2-isonicotinoylhydrazinecarboxamides 1a-v

<table>
<thead>
<tr>
<th>Compd</th>
<th>M. tuberculosis 331/88</th>
<th>M. avium 330/88</th>
<th>M. kansasii 235/80</th>
<th>M. kansasii 6509/96</th>
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<tr>
<td></td>
<td>MIC [μM]</td>
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<tr>
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<td>14d 16, 21d 32</td>
<td>7d 16, 12d &gt;125</td>
<td>7d 16, 12d 125</td>
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<tr>
<td>1b</td>
<td>16 32</td>
<td>16 32</td>
<td>16 32</td>
<td>8 8 16</td>
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<tr>
<td>1c</td>
<td>8 16</td>
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<td>4 8</td>
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<td>62.5 125</td>
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<td>32 62.5</td>
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<td>1v</td>
<td>16 32</td>
<td>32 62.5</td>
<td>32 125</td>
<td>32 62.5 125</td>
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INH: isoniazid. The best MIC values for each strain are given in bold.

The situation for the atypical mycobacteria was different. Eleven hydrazinecarboxamides showed significantly lower MICs for M. avium than INH: 1a-g, 1i, 1p, 1t and 1v (range, 16 to 125 μM) with the superiority of 1a and 1b (MICs of 16–32 μM) indicating that the best substitution pattern for the phenyl ring is a small 4-alkyl group (1a, 1b) followed by 4-methoxy (3i), and 4-heptyl groups (1g). Identical MIC values were obtained for 1v, the heptyl derivative without a phenyl ring. In general, N-substituted 2-isonicotinoyl hydrazinecarboxamides 1, incorporating either alkyl (1a-g, 1v) or methoxy (1k) groups, exhibited a significantly higher inhibition of M. avium growth than the halogenated derivatives 1j-u (MICs ≥ 125 μM). For both strains of M. kansasii, the alkyl substituted hydrazinecarboxamides 1a-g and 1v showed a higher in vitro potency than the halogenphenyl derivatives 1j-u. Twelve compounds (1a-g, 1n, 1p, 1r, 1t and 1v) inhibited INH-resistant strain 235/80, with MICs ≤ 125 μM, but no molecule produced lower MIC values than INH for clinical strain 6509/96. Only the 4-isopropyl derivative 1b, the most active 2-isonicotinoylhydrazinecarboxamide 1 against all nontuberculous strains in this study (MICs 8–32 μM), displayed a similar in vitro efficacy. The most hydrophilic 4-methoxyphenyl 1i and, surprisingly, the most lipophilic derivative 4-octylphenyl 1h did not exhibit any significant activity. For the strain 6509/96, the presence of two or three chlorine...
atoms (1n, 1t, 1u) or a trifluoromethyl moiety at any position (1j, 1p–r) on the phenyl ring led to better activity among the halogenated compounds [Ventura, et al., 2008].

1.3 Structure of N-Substituted-1,3,4-oxadiazol-2-amines (5a-d)

The 2-isonicotinoyl hydrazine carboxamides 1 act as INH (and then isonicotinic acid) prodrugs that must be activated. None of these compounds showed superiority over INH against drug-susceptible M. tuberculosis. In contrast, a range of these compounds showed notable in vitro activity at micromolar concentrations against the INH-resistant strains of M. avium and M. kansasii. In the case of M. avium, due to the missing catalase/peroxidase enzyme, this observation can be explained on the basis of facilitated liberation of isonicotinoyl radicale from the less stable hydrazide derivative produgs [2]. An examination of the cyclised analogues of the four selected 2-isonicotinoyl hydrazine carboxamides with different substitution patterns (halogens: 1l, 1n and 1s, and methoxy derivative 1i), i.e., N-substituted-5-(pyridine-4-yl)-1,3,4-oxadiazol-2-amines 2a, 2b, 2c and 2d, revealed a complete loss of efficacy (MICs ≥ 125 μM). Having these results in hand, we decided not to further study additional 5-(pyridine-4-yl)-1,3,4-oxadiazol-2-amines 2 as potential anti-TB agents. In fact, the activity values for our 1,3,4-oxadiazol-2-amines are in sharp contrast with the results published [20] where the 2-position of the oxadiazole ring was occupied by alkyls, halogenalkyls and aryls, and the MIC values were within the range of 0.35 to 49.69 μM for Mtb. H37Rv. Additionally, two other 2-substituted 5-(pyridine-4-yl)-1,3,4-oxadiazoles exhibited a remarkable inhibition of M. tuberculosis [20,21].

Table-2: Antitubercular activity of N-substituted 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amines 2a-d

<table>
<thead>
<tr>
<th>Compd</th>
<th>M. tuberculosis 331/88</th>
<th>M. avium 330/88</th>
<th>M. kansasii 235/80</th>
<th>M. kansasii 6509/96</th>
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<tbody>
<tr>
<td></td>
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<td>21 d</td>
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</tr>
<tr>
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Substituted derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazol-2-amines, which avoid any significant activity against Mtb [22]. 5-(Pyridine-4-yl)-1,3,4-oxadiazol-2-amine showed mild anti-TB activity; the replacement of the amino group by mercapto decreased the MIC [23]. The reason for the inactivity of N-(aryl substituted)-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-amines 2 at the observed concentrations when compared to the previously reported derivatives [20,21] with other substituents at position 2, which are based on the hydrocarbon chains or rings, may originate from the changes in the hydrophilic-lipophilic balance due to the presence of an H-N bond as well as possible hydrogen bridge formations, different steric parameters or because these compounds may be metabolised by different ways within the cells.

The biological activity of the INH derivatives to a series of heteroaromatic N’-(E)-Heteroaromatic-Isonicotinohydrazide Derivatives (3a-f and 4a-b) and 7-chloro-4-quinolinylhydrazones (5a-f and 6a-b) evaluated against M. tuberculosis. Recently, anti-TB activity of a series of mono substituted 7-chloro-4-quinolinylhydrazones, which demonstrated relevant MIC between 12.5 and 2.5μg/mL [24]. Hence, this is important to continue the study of the structure-activity relationship (SAR) of this class of compounds. The criteria used to select the five-member heterocyclic nuclei was based on isosteric replacements: (1) substitution of the oxygen atom of the furane ring (a) by sulfur (d) or nitrogen (e) and (2) substitution of –CH= by –N= in the pyrrole ring (e) to give an imidazole ring (f); whereas the six-member heterocyclic (2a-b) was chosen in order to analyze the influence of the introduction of the nitrogen atom in the phenyl ring on the biological activity of this series. Due to the high impact of MDR and XDR in

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TB treatment, there is an urgent need for new drugs to treat this disease efficiently. Isoniazid (INH) derivatives have been found to possess potential anti-TB activities\(^{25,27}\). INH is one of the most powerful synthetic agents against the \(M.\) \textit{tuberculosis} complex and it has an important bactericidal activity against the replicating bacteria. Moreover, INH is a prodrug, which needs a previous \textit{in vivo} activation to exercise its anti-TB activity. The enzyme responsible for this function is called KatG. After INH activation, an isonicotinoyl radical is produced, which reacts with the nicotinamide group of NAD (nicotinamide adenine dinucleotide) to yield the INH-NAD adduct. This adduct mainly inhibits and binds to \textit{trans}-2-enoyl-ACP reductase, encoded by the \textit{InhA} gene, which promotes the elongation phase of the FAS-II (fatty acid synthetase II) system. The inhibition of this enzyme interrupts the mycolic biosynthesis leading to cell lysis\(^{28}\). Due to the significance of this drug for TB treatment, the advent of INH-resistant strains is very alarming. The majority of INH-resistant strains demonstrate deletion or point mutations in the \(M.\) \textit{tuberculosis} \textit{katG} gene, which is responsible for INH activation\(^{29}\). Moreover, it is probable that Mn\(^{3+}\) ions could facilitate the formation of isonicotinic acyl radicals and KatG participates in isoniazid activation by increasing the rate of the conversion of Mn\(^{2+}\) to Mn\(^{3+}\) ions. Due to the ability of hydrazone derivatives in metal chelation\(^{30}\) and generation of metal ion–induced radical intermediates\(^{31,32}\), the potential anti-TB activity of a series of heteroaromatic hydrazones derived from INH (3a-f and 4a-b). Another aim of this article is to compare the biological activity of the INH derivatives to a series of heteroaromatic 7-chloro-4-quinolinylhydrazones (5a-f and 6a-b).

1.4 Structure of \(N’-(E)\)-Heteroaromatic-Isonicotinohydrazide Derivatives (3a-f and 4a-b)

\begin{center}
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<th>Compounds</th>
<th>Compounds</th>
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<tbody>
<tr>
<td>(E)-N’-((5-nitrofuran-2-yl) methylene)isonicotinohydrazide (3a)</td>
<td>(E)-N’-((1H-pyrrol-2-yl) methylene)isonicotinohydrazide (3e)</td>
</tr>
<tr>
<td>(E)-N’-(furan-2-ylmethylene) isonicotinohydrazide (3b)</td>
<td>N’-[(E)-(1H-Imidazol-2-yl) Methylidene]isonicotinohydrazide (3f)</td>
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<tr>
<td>(E)-N’-((5-nitrothiophen-2-yl) methylene)isonicotinohydrazide (3c)</td>
<td>(E)-N’-(pyridin-2-ylmethylene)isonicotinohydrazide (4a)</td>
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<tr>
<td>(E)-N’-(thiophen-2-ylmethylene) isonicotinohydrazide (3d)</td>
<td>(E)-N’-benzylideneisonicotinohydrazide (4b)</td>
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1.5 Structure of 7-Chloro-4-Quinolinylhydrazone Derivatives (5a-f and 6a-b)

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\begin{tabular}{c c}
<table>
<thead>
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<th>Compounds</th>
<th>Compounds</th>
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</table>
| 1-(7-Chloroquinolin-4-yl)-2-[(5-Nitro-Furan-2-yloxy)methylene] (5a) | (E)-4-(2-((1H-pyrrol-2-yl)methylene) hydrazinyl)-7-
\end{tabular}
\end{center}
**1.6 Antitubercular Activity**

The anti-TB activities of the derivatives 3a-f, 4a-b, 5a-f, and 6a-b were assessed against *M. tuberculosis* using the Microplate Alamar Blue Assay (MABA) [33]. When these two different series of compounds were compared, it was observed that among the compounds with five-member heterocyclic nucleus (3a-f vs. 5a-f), the quinoline derivatives are more active than INH derivatives, except in the case of 3e and 5e. The comparison between the six-member compounds (4a-b vs. 6a-b) showed that INH derivatives were more active than quinoline derivatives. These data might indicate that biological activity of quinoline derivatives is more susceptible to bulk effects than INH derivatives. This hypothesis can be more detailed if we compare the five-member heterocyclic nucleus [5d (S), 5b (O), 5e (NH), and 5f (N plus NH)] bounded to quinoline derivatives. It was observed that there is no difference in the biological activity of these compounds (all derivatives showed MIC = 3.12 μg/mL), but with the increase of size ring (six-member compounds, 6a and 6b), the biological activity decreases four times in the case of 6a or completely disappears in the case of 6b. Furthermore, when the compounds are compared into the same series (3a vs. 3b, 5a vs. 5b, and 5c vs. 5d), it was observed that all the nitro derivatives (3a, 5a, and 5c) were more active than the other compounds (3b, 5b, and 5d), suggesting that the nitro group is an important feature to modulation of biological activity in these series. Moreover, when the derivatives 4a and 4b, 6a and 6b are compared, it was observed that the compounds without the nitrogen atom were less active, suggesting that the presence of this atom in the six-member compounds also seems to be important for the biological activity in both series.

**Table-3:** Antitubercular Activities of INH and 7-Chloro-4-quinolinylhydrazone derivatives (3a-f, 4a-b, 5a-f, and 6a-b)

<table>
<thead>
<tr>
<th>Compd</th>
<th>MIC (μg/mL)</th>
<th>Compd</th>
<th>MIC (μg/mL)</th>
<th>Compd</th>
<th>MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>3.12</td>
<td>4a</td>
<td>0.60</td>
<td>5e</td>
<td>3.12</td>
</tr>
<tr>
<td>3b</td>
<td>25</td>
<td>4b</td>
<td>3.12</td>
<td>5f</td>
<td>3.12</td>
</tr>
<tr>
<td>3c</td>
<td>1.25</td>
<td>5a</td>
<td>2.50</td>
<td>6a</td>
<td>12.5</td>
</tr>
<tr>
<td>3d</td>
<td>N.D.c</td>
<td>5b</td>
<td>3.12</td>
<td>6b</td>
<td>Resistant</td>
</tr>
<tr>
<td>3e</td>
<td>1.25</td>
<td>5c</td>
<td>1.25</td>
<td>INH</td>
<td>0.2</td>
</tr>
<tr>
<td>3f</td>
<td>25</td>
<td>5d</td>
<td>3.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**2. DISCUSSION**

Due to the high impact of MDR and XDR in TB treatment, there is an urgent need for new drugs to treat this disease efficiently. In this circumstance, INH derivatives have been found to possess potential anti-TB activities\textsuperscript{25-27}. INH is one of the most powerful agents against the *M. tuberculosis* complex with an important bactericidal activity against the replicating bacteria. Moreover, INH is a prodrug, which needs a previous *in vivo* activation to exercise its anti-TB activity. The enzyme responsible for this function is called KatG. After INH activation, an isonicotinoyl radical is produced, which reacts with the nicotinamide group of NAD (nicotinamide adenine dinucleotide) to yield the INH-
NAD adduct. This adduct mainly inhibits and binds to trans-2-enoyl-ACP reductase, encoded by the \textit{InhA} gene, which promotes the elongation phase of the FAS-II (fatty acid synthetase II) system\textsuperscript{28-30}. The inhibition of this enzyme interrupts the mycolic biosynthesis leading to cell lysis. Due to the significance of this drug for TB treatment, the advent of INH-resistant strains is very alarming. The majority of INH-resistant strains demonstrate deletion or point mutations in the \textit{M. tuberculosis} katG gene, which is responsible for INH activation\textsuperscript{31-36}.

The Isoniazid (INH) derivatives, 2-isonicotinoyl-N-(substituted) hydrazinecarboxamides, None of the compounds exhibited higher activity than INH, but the N-(4-octylphenyl) derivative 1h reached almost the same \textit{in vitro} efficacy with MICs 1-2 μM. Among the halogenated molecules, the best activity was found for the 2,4,6-trichloro derivative 1u, with a MIC of 4 μM. An incorporation of the alkyl substituent on the carboxamide nitrogen of 2-isonicotinoyl hydrazinecarboxamides led to a significantly higher inhibition of the growth of \textit{M. avium} and both \textit{M. kansasii} strains when compared to the halogenated derivatives and INH. Cyclisation of the selected compounds 3 to the corresponding 1,3,4-oxadiazol-2-amines 2 resulted in the loss of anti-TB efficacy\textsuperscript{37}. The INH and 7-chloro-4-hydrazinoquinoline heteroaromatic hydrazone derivatives (3a-f, 4a-b, 5a-f, and 6a-b), among them, four compounds (3f, 5a, 5c, and 6a). These compounds (3a-c, 3e-f, 4a-b, 5a-f, and 6a) were exhibited MIC values between 25 and 0.60 μg/mL. Among these derivatives, 3a-c, 3e, 4a, 5a, 5c-d, and 6a were not cytotoxic to host cells in the effective concentrations to inhibit the growth of \textit{M. tuberculosis}. Furthermore, the compounds 3a, 3c, 3e, 4a, 5a, 5c, and 5d exhibited a significant activity (3.12, 2.50, 1.25, or 0.60 μg/mL) when compared to the ethambutol (MIC=3.12 μg/mL) and rifampicin (2.0 μg/ml), and could be considered a good starting point to find new lead compounds in the fight against TB\textsuperscript{38}. Molecular modelling studies suggested a possible conformation of these novel compounds in the active site of the enzyme InhA.

New drugs for the control of TB are urgently needed, including developments of short-term antibiotic regimens to minimize the emergence of drug resistance and new drugs to treat MDR-TB patients and to eradicate the latent \textit{Mycobacterium species}. Discovery of new synthetic compounds for treatment of TB should have the following major objectives:

1. Developing new drugs from existing lead molecules used to treat other \textit{Mycobacterium species} infections (e.g. Isoniazid derivatives).
2. Modifying an existing drug to improve its anti-TB activity and its pharmacokinetic properties to make it less susceptible to the known mechanism of resistance. This is the strategy adopted in developing new analogues.
3. Discovering new drugs either by random screening or if a specific target is known, by a rational design.
4. Develop faster acting drugs to shorten the duration of treatment.
5. Develop new drugs with different modes of action to resolve the known resistance problem.

3. CONCLUSION
The anti-tubercular pharmacophore moiety of isoniazid has been introduced in a number of various types of molecules to improve their activity against \textit{Mycobacteria} species, as well as their multi-drug resistant tuberculosis. Various types of the most active isoniazid derivatives classified according to their structure are reported and structure-activity relationships are discussed. Therefore this class of compounds could be a good starting point to develop new lead compounds in the treatment of multi-drug resistant tuberculosis.

4. REFERENCES


