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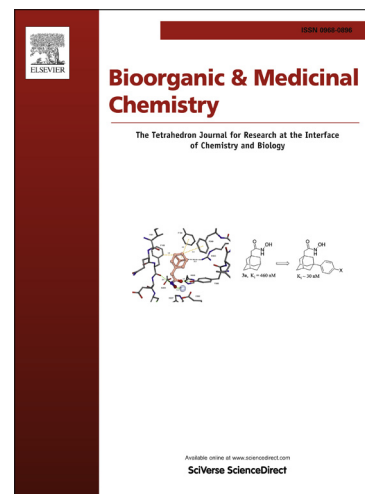
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## Graphical Abstract

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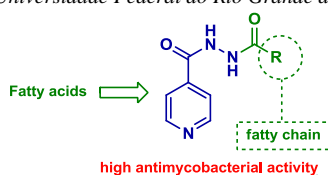
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## Synthesis and antimycobacterial activity of isoniazid derivatives from renewable fatty acids

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### ABSTRACT

This work describes the synthesis of a series of fatty acid hydrazide derivatives of isoniazid (INH). The compounds were tested against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) as well as INH-resistant (ATCC 35822 and 1896 HF) and rifampicin-resistant (ATCC 35338) *M. tuberculosis* strains. The fatty acid derivatives of INH showed high antimycobacterial potency against the studied strains, which is desirable for a pharmaceutical compound, suggesting that the increased lipophilicity of isoniazid plays an important role in its antimycobacterial activity.

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### 1. Introduction

Tuberculosis (TB), a tropical disease of bacterial origin, is more prevalent than ever before and is associated with high levels of morbidity and mortality. Aggravated by the human immunodeficiency virus (HIV), TB was the first reported cause of death among acquired immunodeficiency syndrome (AIDS) patients.<sup>1</sup> The emergence of new TB cases, the increased incidence of multi-drug resistant strains of *Mycobacterium tuberculosis*, the adverse effects of first- and second-line anti-TB drugs, and the increased incidence of TB associated with viral infections have led to renewed research interest in new anti-TB compounds.<sup>2</sup>

In particular, the existence of strains resistant to the two most important anti-TB drugs, isoniazid (INH) and rifampicin (RMP), have serious repercussions for the epidemiology of the disease and for disease control.<sup>3</sup>

INH (1, Scheme 1), an isonicotinic acid-derived hydrazide (pyridine-4-carbohydrazide), is a first-line drug for TB treatment.<sup>4</sup> INH activates the KatG enzyme to form the INH-nicotinamide adenine dinucleotide (NAD) adduct. This adduct inhibits the InhA and enoyl-acyl carrier protein (ACP) reductase enzymes that produce type II fatty acid synthase (FAS II), which synthesizes mycolic acids that lead to cell death.

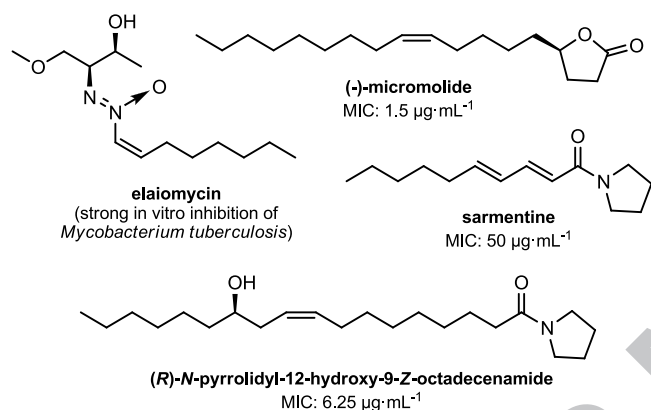
The importance of alkyl chains to antimycobacterial activity has been previously reported. Some compounds with unsaturated long alkyl chains and their minimum inhibitory concentration (MIC) are shown in Figure 1. The various examples include elaiomycin<sup>5</sup> – a hydrazide with a long alkyl chain that is present in natural compound isolated from *Streptomyces* sp. BK190 – micromolide<sup>6,7</sup> – a natural product isolated from the stem bark of *Micromelum hirsutum* (Rutaceae); and sarmentine<sup>8</sup> – pyrrolidine alkaloid isolated from the roots of *Piper sarmentosum*. Another example described the synthesis of new fatty acid amides as well as their antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv, RMP-resistant *M. tuberculosis* (ATCC 35338) and INH-resistant *M. tuberculosis* (ATCC 35822). A compound used this study, (*R*)-*N*-pyrrolidyl-12-hydroxy-9-*Z*-octadecenamide (Figure 1), is a derivative of ricinoleic acid with a pyrrolidine moiety attached at the carbonyl group and it was the most potent among a series of tested compounds.<sup>9</sup>

In a structure–activity relationship study, Ragno *et al.*<sup>10</sup> demonstrated that a high value of log *P* played an important role in antimycobacterial activity because the more lipophilic compounds could have greater antimycobacterial potency.

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High values of log *P* represent an increase in drug permeability through the lipid-rich mycobacterial cell wall. To this end, the hydrophobic synthetic derivative of isoniazid, 1-isonicotinyl-2-nonanoyl hydrazine (INH-C9), promoted increased susceptibility of the bacilli to INH. This improvement seemed to be associated with the presence of the appended fatty acid chain, which may modify the polarity of the drug to make it more likely to permeate the lipid cell wall of the pathogenic agent.<sup>11</sup>

In previous works, we initiated our synthetic program to work with various fatty acid entities including saturated and unsaturated chains derived from vegetable oils, which are common substrates in the chemical industry. Fatty acid derivatives, fatty acid amides,<sup>9,12</sup> fatty *N*-acylamino acids,<sup>13</sup> and fatty *N*-acyl trihalomethylated pyrazoline<sup>14</sup> have important biological and technological activities.



**Figure 1.** Natural and synthetic long alkyl chain-containing compounds with antimycobacterial activity.

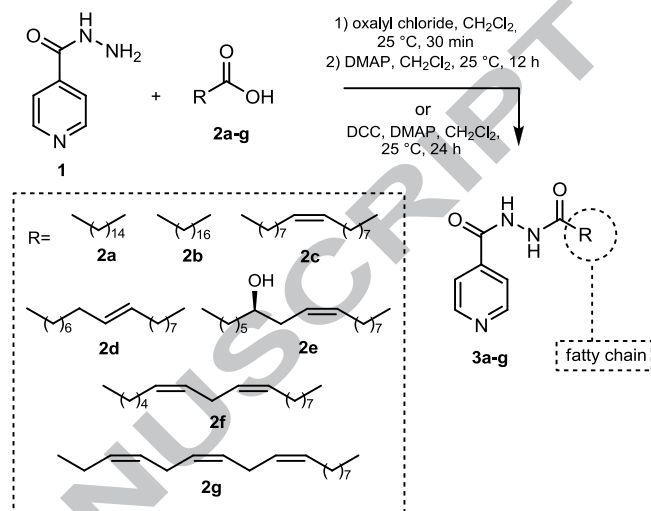
In this study, we describe the design and synthesis of a series of fatty acid hydrazide derivatives of isoniazid constructed by the coupling of isoniazid with fatty acids in different structural arrangements (Scheme 1). These syntheses allow evaluation of the relationship between antimycobacterial activity and the number of carbons, lipophilicity, presence of unsaturated carbons, appended functional groups, and molecular conformation.

## 2. Results and discussion

The first step of this study was the structural design of the INH derivatives, which was based on the natural availability of the fatty acids. The fatty acids were chosen according to several structural requirements to investigate the effect of increasing the number of carbons, increasing the number of unsaturated carbons, changing the conformation of a double bond and inserting a functional group.

INH derivatives **3a-d,f,g** were derived from palmitic (C16:0, **2a**), stearic (C18:0, **2b**), oleic (*cis*-C18:1, **2c**), elaidic (*trans*-C18:1, **2d**), linoleic (*cis,cis*-C18:2, **2f**), and linolenic (*cis,cis,cis*-C18:3, **2g**) acids. As shown in Scheme 1, these compounds were synthesized at room temperature with yields of 60–99% from INH (**1**) and their respective acyl chlorides, which were obtained from the reaction between the appropriate fatty acid and oxalyl chloride. The INH derivative **3e** was synthesized by the reaction

of the ricinoleic acid (*cis*-C18:1,12-OH, **2e**, obtained by castor oil or castor oil biodiesel hydrolysis)<sup>12</sup> with a catalytic amount of (dimethylamino)pyridine (DMAP) and dicyclohexyl carbodiimide (DCC) in CH<sub>2</sub>Cl<sub>2</sub>. All compounds were purified by chromatographic column and identified by FTIR, <sup>1</sup>H and <sup>13</sup>C NMR, and elemental analysis (CHN).



**Scheme 1.** Synthesis of isoniazid derivatives **3a-g**.

The antimycobacterial activity of the compounds was tested and the MIC was determined using the resazurin microtiter assay (REMA).<sup>15</sup> The MIC values were compared with INH (**1**) and RMP as reference compounds. The antimycobacterial activity of the compounds was evaluated using *M. tuberculosis* H37Rv (ATCC 27294), which is susceptible to RMP and INH; RMP-resistant *M. tuberculosis* (RMP<sup>r</sup>, ATCC 35338); INH-resistant *M. tuberculosis* with a mutation at *katG* (INH<sup>r</sup>, ATCC 35822); and INH-resistant *M. tuberculosis* with a mutation at *inhA* (INH<sup>r</sup>, 1896HF). As detailed in Table 1, most of the synthesized compounds displayed MIC values below the 6.25 µg·mL<sup>-1</sup> value postulated by the Global Program for the Discovery of New Anti-Tuberculosis Drugs as an upper threshold for the evaluation of new *M. tuberculosis* therapies.

The Clog *P* values of the compounds **3a-g** were calculated to provide an estimate of their lipophilicity. The results showed that the increased lipophilicity of the isoniazid molecule seems important with regard to antimycobacterial activity.<sup>10</sup> The Clog *P* of the INH derivatives is much higher than INH, indicating that the derivatives are much more capable of permeating the hydrophobic cell wall of mycobacterium.<sup>11</sup>

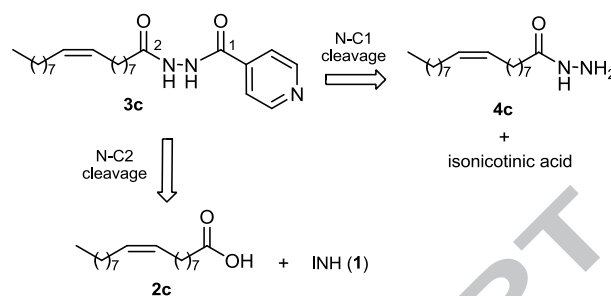
Because both the saturated and unsaturated chain derivatives showed high potency, no clear systematic structure-activity relationship concerning the fatty chain structural arrangement was noted. However, for the isoniazid resistant strain ATCC 35822 only, there was a structure-activity relationship observed where the increase in fatty chain length and the number of unsaturations caused a loss in potency of the tested derivatives. For the other tested strains, the INH derivatives showed diverse behavior.

The evaluation of the monounsaturated compounds with different double bond conformations, **3c** (*cis*-C18:1) and **3d** (*trans*-C18:1), showed that the *cis* double bond appears be

important for antimycobacterial potency, because compound **3c** has the lowest MIC values for most of the strains tested.

Compound **3e** (*cis*-C18:1, 12-OH) was constructed to evaluate the presence of a functional group. This alteration imparts a loss of potency in the INH-resistant strains, which is likely from the polar hydroxyl group causing a lower log *P* value.

To prove whether the potential activity from the tested compounds is related to hybrid fatty acid-INH, the feasible metabolites from the hydrolysis of compound **3c**, oleylhydrazide (**4c**)<sup>14</sup> and **2c** (Scheme 2), were evaluated against all strains *M. tuberculosis*. The results showed that **2c** and **4c** had no antimycobacterial activity against the strains studied (Table 1), strengthening the argument that the potent activity is a result of the fatty acid derivatization of isoniazid.



**Scheme 2.** Products of the hydrolysis of isoniazid **3c**

**Table 1.** In vitro activity, expressed as MIC values ( $\mu\text{g}\cdot\text{mL}^{-1}$ ), of compounds **3a-g** and possible metabolites against *Mycobacterium tuberculosis* strains in comparison to standards (INH and RMP).

Compound	<i>C</i> Log <i>P</i>	MIC ( $\mu\text{g}\cdot\text{mL}^{-1}$ )			
		ATCC 27294 H37Rv	ATCC 35822 INHr <sup>a</sup>	1896-HF INHr <sup>b</sup>	ATCC 35338 RMPr <sup>c</sup>
	6.964	0.015	0.125	0.78	0.003
<b>3a</b> <sup>16</sup>					
	8.022	0.03	0.25	3.12	0.003
<b>3b</b> <sup>16</sup>					
	7.538	0.015	0.25	1.56	0.003
<b>3c</b> <sup>17</sup>					
	7.538	0.03	> 50	6.25	0.0019
<b>3d</b>					
	5.331	0.03	25	12.5	0.015
<b>3e</b>					
	7.054	0.125	2	12.5	0.03
<b>3f</b>					
	6.770	0.06	25	12.5	0.03
<b>3g</b>					
	- <sup>d</sup>	100	> 100	> 100	> 100
<b>2c</b>					
	- <sup>d</sup>	50	50	> 100	50
<b>4c</b> <sup>14</sup>					
<b>INH</b> (1)	-0.668	0.06	2	2	0.015
<b>RMP</b>	- <sup>d</sup>	0.03	0.03	0.125	> 2

<sup>a</sup> INHr, resistant strains with a mutation at the *katG* 315 gene. <sup>b</sup> INHr, resistant strains with a mutation at the *inhA* C(-35)T gene. <sup>c</sup> RMPr, resistant strains with a mutation at the *rpoB* His-526-Tyr gene. <sup>d</sup> not determined.

### 3. Conclusion

In conclusion, the work presented herein demonstrates the synthesis and biological evaluation of fatty acid isoniazides as potent antimycobacterial agents. We are currently evaluating the relationship between antimycobacterial activity and the number of carbons, lipophilicity, presence of unsaturated carbons, and molecular conformation. In general, we can conclude that the insertion of both saturated and unsaturated fatty acid chains into the molecular structure of INH (**1**) produced compounds with significantly improved antimycobacterial properties. We also believe that the increased lipophilicity of isoniazid plays an important role in its antimycobacterial activity. Among the tested derivatives, compound **3a** from palmitic acid could represent a prototype of a new anti-TB drug as it exhibited good MIC values against all studied strains.

### 4. Experimental

#### 4.1. Lipophilicity calculations

The physicochemical parameter,  $C \log P$  (the logarithm of  $n$ -octanol/water partition coefficient  $P$  based on established chemical interactions) was calculated using CS ChemOffice Ultra 12.0 (CambridgeSoft, Cambridge, MA, USA).

#### 4.2. Antimycobacterial assays

The antimycobacterial activity of the INH derivatives was tested and the minimum inhibitory concentration (MIC) was determined using the Resazurin Microtiter Assay (REMA) method. Rifampicin and isoniazid (Sigma Chemical Co.) were used as reference drugs. The bacterial suspensions were prepared in sterile water containing 3 mm glass beads. The suspensions were homogenized by vortex agitation and the turbidity was adjusted to agree with tube one of the McFarland scale ( $3.2 \times 10^6$  colony-forming units/mL). The inoculums were prepared by diluting the bacterial suspension 1:20 in Middlebrook 7H9 medium (4.7 g Middlebrook 7H9 base; Difco, Becton Dickinson). Briefly, the assay is performed in 96-well microplates using 7H9 OADC medium enriched with 10% (v/v) oleic acid-dextrose-albumin-catalase (BBL) and with INH derivatives dissolved in dimethyl sulfoxide (DMSO). The MIC determination was carried out by twofold serial dilutions of the compounds (100.0–3.2  $\mu\text{g/mL}$  range) dispensed into each well of a 96-well microtiter plate. The microplate was incubated at 37 °C for 7 days. After this incubation period, 30  $\mu\text{L}$  of resazurin was added in each well and the plate was incubated for 2 more days at 37 °C. The oxidoreduction of resazurin was used as a color change indicator of when cellular growth was taking place.

#### 4.3. Apparatus and Chemistry

Reagents were purchased from Aldrich Chemical Co. and used without further purification. All organic solvents used for the synthesis were of analytical grade. The solvents were dried and freshly distilled. Column chromatography was performed with Silica Gel 60 A (ACROS Organics, 0.035–0.070 mesh). The reactions were monitored using thin-layer chromatography (TLC) performed with plates containing silica gel (Merck 60GF245) and the spots were visualized using iodine. Yields refer to

chromatographically and spectroscopically homogeneous materials. Melting points were obtained on a Fisatom 430D apparatus and are uncorrected. Infrared spectra were measured using potassium bromide (KBr) pellets or sodium chloride (NaCl) disks on a Shimadzu-IR PRESTIGIE-21 spectrometer. The NMR spectra were recorded on a Varian VNMRs 300 MHz spectrometer ( $^1\text{H}$  at 300 MHz and  $^{13}\text{C}$  at 75.5 MHz, Universidade Federal do Rio Grande do Sul, UFRGS, Brazil) and Bruker DPX 400 spectrometer ( $^1\text{H}$  at 400 MHz and  $^{13}\text{C}$  at 100 MHz, Universidade Federal de Santa Maria, UFSM, Brazil) in deuteriochloroform ( $\text{CDCl}_3$ ) solution. The chemical shift data are reported in units of  $\delta$  (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. The coupling constants ( $J$ ) are reported in Hz and refer to apparent peak multiplicities. CHN elemental analysis was used to ascertain the purity (>95%) of all compounds for which biological data was determined, and was performed on a CHN 2400 elemental analyzer (Universidade de São Paulo, USP, Brazil); all values were within 0.4% of the theoretical values.

#### 4.4. Synthesis

##### 4.4.1. General procedure for the synthesis of INH derivatives **3a-d**, **3f,g**:

Oxalyl chloride (3 mmol) in a  $\text{CH}_2\text{Cl}_2$  solution (3 mL) was slowly added to a round-bottom flask equipped with a magnetic stirring bar containing the appropriate fatty acid **2a-d**, **2f,g** (1 mmol) in a  $\text{CH}_2\text{Cl}_2$  solution (3 mL). The reaction mixture was stirred for 30 min at room temperature and subsequently evaporated under reduced pressure. The fatty acid chloride that was obtained was used directly in the next step without purification to prevent degradation. To a flask containing the appropriate fatty acid chloride,  $\text{CH}_2\text{Cl}_2$  (5 mL), DMAP (1.2 mmol), and INH (**1**, 1.5 mmol) were added. The reaction mixture was stirred for 12 h at room temperature and subsequently extracted with  $\text{CH}_2\text{Cl}_2$ , washed with  $\text{H}_2\text{O}$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. The residual crude product was purified via silica gel column chromatography using a gradient mixture of hexane-ethyl acetate to obtain the pure INH derivatives.

##### 4.4.2. Procedure for the synthesis of INH derivative **3e**:

In a flask containing fatty acid **2e** (1 mmol), isoniazid (**1**, 2 mmol), and 10% DMAP, a solution of DCC (1 mmol) in 3 mL of  $\text{CH}_2\text{Cl}_2$  was added drop-by-drop. The reaction mixture was kept under constant stirring at 25 °C for 24 hours. Then, the precipitated solid was separated by filtration and the solvent was evaporated under reduced pressure. The residual crude product was purified via silica gel column chromatography using a gradient mixture of hexane-ethyl acetate to obtain the pure INH derivative.

##### 4.4.3. *N'*-hexadeca-isonicotinohydrazide (**3a**)

MW 375  $\text{g}\cdot\text{mol}^{-1}$ . White solid. Yield 0.45 g, 90%. Anal. calcd. for  $\text{C}_{22}\text{H}_{37}\text{O}_2\text{N}_3$ : C, 70.36; H, 9.93; N, 11.19; Found C, 69.91; H, 9.95; N, 11.03. Mp 124–126 °C (Lit. 117–119 °C).<sup>16</sup> IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) 1405 (m), 1550 (f), 1598 (F), 2848 (F), 2918 (F), 3030 (m), 3203 (F).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 0.88 (t, 3H,



$J$  6.4 Hz, CH<sub>3</sub>), 1.25 (m, 24H, 12CH<sub>2</sub>), 1.66 (m, 2H, CH<sub>2</sub>β), 2.33 (t, 2H,  $J$  7.3 Hz, CH<sub>2</sub>α), 7.61 (dd, 2H, 2CH-aromatic), 8.67 (dd, 2H, 2CH-aromatic), 9.42 (s, 1H, NH), 10.61 (s, 1H, NH). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ (ppm) 14.1 (CH<sub>3</sub>), 22.7–34.2 (14CH<sub>2</sub>), 120.9 (2CH-aromatic), 138.2 (C-aromatic), 150.5 (2CH-aromatic), 162.6 (CO), 171.7 (CO).

#### 4.4.4. *N'*-octadecaisonicotinohydrazide (3b)

MW 403 g·mol<sup>-1</sup>. White solid. Yield: 0.3 g, 60%. Anal. calcd. for C<sub>24</sub>H<sub>41</sub>O<sub>2</sub>N<sub>3</sub>: C, 70.59; H, 10.24; N, 10.41; found C, 70.59; H, 10.37; N, 10.61. Mp 125–127 °C (Lit. 125–126 °C).<sup>16</sup> IR (KBr): ν (cm<sup>-1</sup>) 1409 (m), 1550 (f), 1597 (F), 2848 (F), 2916 (F), 3014 (m), 3215 (F). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ (ppm) 0.88 (t, 3H,  $J$  6.8 Hz, CH<sub>3</sub>), 1.25 (m, 28H, 14CH<sub>2</sub>), 1.69 (m, 2H, CH<sub>2</sub>β), 2.34 (t, 2H,  $J$  7.78 Hz, CH<sub>2</sub>α), 7.62 (dd, 2H, 2CH-aromatic), 8.7 (dd, 2H, 2CH-aromatic), 9.04 (s, 1H, NH). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ (ppm) 13.9 (CH<sub>3</sub>), 22.5–34 (16 CH<sub>2</sub>), 121.3 (2CH-aromatic), 139.5 (C-aromatic), 150 (2 CH-aromatic), 163.7 (CO), 172 (CO).

#### 4.4.5. (Z)-*N'*-octadeca-9-enoylisonicotinohydrazide (3c)

MW 401 g·mol<sup>-1</sup>. Yellow pasty solid. Yield: 0.44 g, 88%. Anal. calcd. for C<sub>24</sub>H<sub>39</sub>O<sub>2</sub>N<sub>3</sub>: C, 71.78; H, 9.79; N, 10.46; found C, 71.71; H, 9.74; N, 9.88. Mp: 86–88 °C (Lit. 80–81 °C).<sup>17</sup> IR (NaCl, film): ν (cm<sup>-1</sup>) 1463 (m), 1645 (F), 1695 (f), 2854 (f), 2926 (F), 3012 (m), 3221 (F). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 0.86 (t, 3H,  $J$  7.09 Hz, CH<sub>3</sub>), 1.26 (m, 20H, 10CH<sub>2</sub>), 1.64 (m, 2H, CH<sub>2</sub>β), 1.98 (m, 4H,  $J$  6.1 Hz, 2CH<sub>2</sub>-allyl), 2.31 (t, 2H,  $J$  7.4 Hz, CH<sub>2</sub>α), 5.32 (m, 2H,  $J$  4.4 Hz, 2CH-vinyl), 7.61 (d, 2H, 2CH-aromatic), 8.65 (d, 2H, 2CH-aromatic), 9.37 (s, 1H, NH), 10.3 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 14.1 (CH<sub>3</sub>), 22.6–34 (14CH<sub>2</sub>), 121 (2 CH-aromatic), 129.6 (CH-vinyl), 130 (CH-vinyl), 138.4 (C-aromatic), 150.5 (2 CH-aromatic), 162.8 (CO), 171.6 (CO).

#### 4.4.6. (E)-*N'*-octadeca-9-enoylisonicotinohydrazide (3d)

MW 401 g·mol<sup>-1</sup>. White solid. Yield: 0.3 g, 60%. Anal. calcd. for C<sub>24</sub>H<sub>39</sub>O<sub>2</sub>N<sub>3</sub>: C, 71.78; H, 9.79; N, 10.46; found C, 71.90; H, 9.80; N, 9.88. Mp: 100–101 °C. IR (KBr): ν (cm<sup>-1</sup>) 1411 (m), 1550 (f), 1598 (F), 2848 (f), 2920 (F), 3020 (f), 3165 (m). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 0.86 (t, 3H,  $J$  6.1 Hz, CH<sub>3</sub>), 1.26 (m, 20H, 10 CH<sub>2</sub>), 1.65 (m, 2H,  $J$  7.1 Hz, CH<sub>2</sub> β), 1.94 (m, 4H, 2CH<sub>2</sub>-allyl), 2.3 (t, 2H,  $J$  7.5 Hz, CH<sub>2</sub>α), 5.36 (m, 2H, 2CH-vinyl), 7.6 (d, 2H,  $J$  4.8 Hz,  $J$  0.8 Hz, 2CH-aromatic), 8.66 (d, 2H,  $J$  5.3 Hz, 2CH-aromatic), 9.1 (s, 1H, NH), 10.12 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 14 (CH<sub>3</sub>), 22.6–34.2 (14CH<sub>2</sub>), 120.9 (2CH-aromatic), 130.1 (CH-vinyl), 130.5 (CH-vinyl), 138.5 (C-aromatic), 150.6 (2CH-aromatic), 162.6 (CO), 171.2 (CO).

#### 4.4.7. (R,Z)-*N'*-(12-hydroxyoctadec-9-enoyl)isonicotinohydrazide (3e)

MW 417 g·mol<sup>-1</sup>. White solid. Yield: 0.15 g, 60%. Anal. calcd. for C<sub>24</sub>H<sub>39</sub>O<sub>2</sub>N<sub>3</sub>: C, 69.03; H, 9.41; N, 10.06; found C, 61.08; H, 8.42; N, 9.67. Mp: 68–70 °C. IR (KBr): ν (cm<sup>-1</sup>) 1411(m), 1550 (f), 1598 (F), 2848 (f), 2920 (F), 3020 (f), 3165 (m). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ (ppm) 0.90 (m, 3H, CH<sub>3</sub>), 1.28 (m, 16H, 8 CH<sub>2</sub>), 1.52 (m, 2H, CH<sub>2</sub>β), 1.7 (m, 4H, 2CH<sub>2</sub>), 2.02 (m, 2H,

CH<sub>2</sub>), 2.33 (t, 2H,  $J$  7.7 Hz, CH<sub>2</sub>α), 2.48 (t, 1H), 3.9 (m, 1H), 5.47 (m, 2H, CH<sub>2</sub>-vinyl), 7.63 (d, 2H, 2CH-aromatic), 8.69 (s, 2H, 2CH-aromatic), 9.25 (s, 1H, NH), 10.43 (s, 1H, NH). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ (ppm) 14.1 (CH<sub>3</sub>), 22.6–36.8 (13CH<sub>2</sub>), 71.64 (1C), 121.8 (2CH-aromatic), 125.4 (2CH-vinyl), 133 (C-aromatic), 149.2 (2CH-aromatic), 162.5 (CO), 171.9 (CO).

#### 4.4.8. (6Z,9Z)-*N'*-octadeca-6,9-dienoylisonicotinohydrazide (3f)

MW 399 g·mol<sup>-1</sup>. Yellow pasty solid. Yield: 0.45 g, 90%. Anal. calcd. for C<sub>24</sub>H<sub>37</sub>O<sub>2</sub>N<sub>3</sub>: C, 72.14; H, 9.33; N, 10.52; found C, 69.19; H, 9.57; N, 9.32. Mp: 70–72 °C. IR (NaCl, film): ν (cm<sup>-1</sup>) 1465 (m), 1645 (F), 1695 (f), 2854 (f), 2927 (F), 3010 (f), 3217 (m). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ (ppm) 0.81 (t, 3H,  $J$  6.8 Hz, CH<sub>3</sub>), 1.23 (m, 14H, 7CH<sub>2</sub>), 1.58 (m, 2H, CH<sub>2</sub>β), 1.97 (m, 4H, 2CH<sub>2</sub>-allyl), 2.25 (t, 2H, CH<sub>2</sub>α), 2.69 (t, 2H, 2CH-bis-allyl), 5.28 (m, 4H, 2CH-vinyl), 7.56 (dd, 2H, 2CH-aromatic), 8.59 (dd, 2H, 2CH-aromatic), 9.21 (s, 1H, NH), 9.68 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 14 (CH<sub>3</sub>), 22.5–34.1 (12CH<sub>2</sub>), 121.1 (2CH-aromatic), 127.9 (CH-vinyl), 128.1 (CH-vinyl), 129.9 (CH-vinyl), 130.2 (CH-vinyl), 138.6 (C-aromatic), 150.4 (2CH-aromatic), 162.8 (CO), 171.5 (CO).

#### 4.4.9. (9Z,12Z,15Z)-*N'*-octadeca-9,12,15-trienoylisonicotinohydrazide (3g)

MW 397 g·mol<sup>-1</sup>. Yellow pasty solid. Yield: 0.35 g, 71%. Anal. calcd. for C<sub>24</sub>H<sub>35</sub>O<sub>2</sub>N<sub>3</sub>: C, 72.51; H, 8.87; N, 9.78; found C, 69.73; H, 8.73; N, 9.78. IR Mp: 69–71 °C. (NaCl, film): ν (cm<sup>-1</sup>) 1465 (m), 1643 (F), 1695 (f), 2854 (f), 2929 (F), 3014 (F), 3223 (m). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 0.97 (t, 3H,  $J$  7.5 Hz, CH<sub>3</sub>), 1.3 (m, 10H, 5CH<sub>2</sub>), 1.64 (m, 2H, CH<sub>2</sub>β), 2.07 (m, 4H, 2CH<sub>2</sub>-allyl), 2.32 (t, 2H,  $J$  7.4 Hz, CH<sub>2</sub>α), 2.80 (t, 4H,  $J$  5.7 Hz, 2CH<sub>2</sub>-bis-allyl), 5.36 (m, 6H, 3CH<sub>2</sub>-vinyl), 7.62 (dd, 2H, 2CH-aromatic), 9.27 (s, 1H, NH), 10.33 (s, 1H, NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 14.2 (CH<sub>3</sub>), 20.5–34.2 (12CH<sub>2</sub>), 121.1 (2CH-aromatic), 127.8 (CH-vinyl), 128.1 (CH-vinyl), 129.9 (CH-vinyl), 130 (CH-vinyl), 138.4 (C-aromatic), 150.24 (2CH-aromatic), 162.8 (CO), 171.5 (CO).

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### Supplementary Material

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# Synthesis and antimycobacterial activity of isoniazid derivatives from renewable fatty acids

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