Design, Synthesis and Anti-tuberculosis Activity of Hydrazones and N-acylhydrazones Containing Vitamin B₆ and Different Heteroaromatic Nucleus

Thais Cristina Mendonça Nogueira, Lucas dos Santos Cruz, Maria Cristina Lourenço and Marcus Vinicius Nora de Souza

Abstract: Background: The term vitamin B₆ refers to a set of six compounds, pyridoxine, pyridoxal, and pyridoxamine and their phosphorylated forms, among which pyridoxal 5’-phosphate (PLP) is the most important and active form acting as a critical cofactor. These compounds are very useful in medicinal chemistry because of their structure and functionalities and are also used in bioinorganic chemistry as ligands for complexation with metals.

Methods: In this study, a series of hydrazones 1a-g and N-acylhydrazones 2a-f containing vitamin B₆ have been synthesized from commercial pyridoxal hydrochloride and the appropriate aromatic or heteroaromatic hydrazine or N-acylhydrazine. All synthesized compounds have been fully characterized and tested against Mycobacterium tuberculosis.

Results: Among the N-acylhydrazones derivatives 2a-f, 2d (para-pyridine substituted N-acylhydrazone; MIC = 10.90 µM) exhibited the best activity. The ortho-pyridine derivative 2b exhibited intermediate activity (MIC = 87.32 µM), and the meta-pyridine derivative 2c was inactive. In case of the hydrazone series 1a-g, 7-chloroquinoxaline derivative 1f (MIC = 72.72 µM) showed the best result, indicating that the number of nitrogen and chlorine atoms in the radical moiety play an important role in the anti-tuberculosis activity of the quinoxaline derivatives (1f and 1g).

Conclusion: The data reported herein indicates that the isoniazid derivative 2d (MIC = 10.90 µM) exhibited the best activity in the N-acylhydrazine series and; the quinoxaline nucleus derivative 1f (MIC = 72.72 µM) was the most active compound in the hydrazone series.

Keywords: Vitamin B₆, pyridoxal, tuberculosis, drugs, hydrazone, N-acylhydrazone.

1. INTRODUCTION

The term vitamin B₆ refers to a set of six compounds, pyridoxine, pyridoxal, and pyridoxamine and their phosphorylated forms (Fig. 1), among which pyridoxal 5’-phosphate (PLP) is the most important and active form acting as a critical cofactor [1-14]. This compound is involved, for example, in gene expression; lipid, aminoacid, and glucose metabolism and functions; and hemoglobin synthesis [15-25]. Vitamin B₆ is found in a wide variety of foods such as bananas, beef, potatoes, nuts, fish, and enriched cereals [26-34]. Importantly, vitamin B₆ interacts with about thirty-five commercially available drugs, interfering with the metabolism [35-41]. In general, this interaction is based on the reaction between PLP and the drug, which produces a Schiff base that causes inactivation of both compounds [42-45]. These classes of compounds are very useful because in medicinal chemistry of their structure and functionalities and are used in bioinorganic chemistry as ligands for complexation with metals [46, 47]. Considering the importance of vitamin B₆, we aimed to synthesize its derivatives and carry out biological evaluation against tuberculosis (TB). These derivatives were based on pyridoxal, which was coupled with different heteroaromatics containing hydrazine and N-acylhydrazine groups to furnish the respective hydrazones and N-acylhydrazones. The choice for these functional groups was based on their wide spectrum of biological activity against different types of diseases [48-56].

2. EXPERIMENTAL

2.1. General Procedures

Melting points were determined using an MQAPF-302 (MicroQuimica Ltd., Santa Catarina, Brazil) apparatus and reported uncorrected. NMR spectra were recorded on a 400 or 500 MHz Bruker AC spectrometer with tetramethylsilane as the internal standard. Infrared spectra were obtained using a Thermo Nicolet 6700 spectrometer. High-resolution mass spectra were acquired on a Micromass Q-TOF system (Waters, UK). The reaction progress was monitored by thin-layer
chromatography (TLC) on 2.0 cm x 6.0 cm aluminum sheets (silica gel 60, HF-254, Merck) with a thickness of 0.25 mm, under ultraviolet irradiation. For column chromatography, Merck silica gel (70e230 or 230e400 mesh) was used. Solvents and reagents were used without further purification.

2.2. General Procedure for the Synthesis of 1a-g and 2a-f

The desired compounds 1a-g and 2a-f were prepared by reaction between pyridoxal hydrochloride (0.15 g, 0.74 mmol) and the appropriate aromatic or heteroaromatic hydrazine or N-acetylhydrazine (1.1 eq., 0.81 mmol) in ethanol (10.0 mL). The reaction mixture was stirred for 1-48 hours at room temperature. After that, product was purified by washing with cold ethanol (3.0 mL) and cold diethyl ether (3.0 mL), leading to the pure derivatives 1a-g and 2a-f as solid in 42-86% yields.

2.2.1. (E)-5-(hydroxymethyl)-2-methyl-4-((2-phenylhydrazono)methyl)pyridin-3-ol (1a)

Yield: 83%; yellow solid; mp 278°C; IR (KBr pellets) \( \nu_{\text{max}} \) 1548 (C=N), 1281 (C-O) cm\(^{-1}\); \( ^1\)H NMR (400 MHz, TFA-d\(_6\)) \( \delta = 9.04 \) (1H, s, CH), 8.38-8.27 (3H, m, H5' and H6'), 7.57 (1H, d, J = 8.8 Hz, H9'), 7.73 (1H, t, J = 6.7 Hz, H7'), 5.23 (2H, s, H7), 2.84 (3H, s, CH\(_3\)) ppm; \( ^{13}\)C NMR (100 MHz, TFA-d\(_6\)) \( \delta = 155.8, 150.8, 149.7, 148.5, 146.9, 139.1, 138.4, 121.0, 118.2, 115.4, 112.6, 61.3, 16.2 ppm; HRMS \( m/z \): 259.1193 C\(_{12}\)H\(_{14}\)N\(_4\)O\(_2\)\(+\) (calcd. 259.11950).

2.2.2. (E)-5-(hydroxymethyl)-2-methyl-4-((2-(pyridin-2-yl)hydrazono)methyl)pyridin-3-ol (1b)

Yield: 63%; yellow solid; mp 262-264°C; IR (KBr pellets) \( \nu_{\text{max}} \) 1611 (C=N), 1291 (C-O) cm\(^{-1}\); \( ^1\)H NMR (400 MHz, TFA-d\(_6\)) \( \delta = 9.74 \) (1H, s, H6'), 8.78 (1H, s, H9'), 8.65 (1H, s, H10'), 8.36 (1H, s, CH\(_3\)), 8.19 (1H, s, H4), 7.95 (1H, s, H12'), 7.68 (1H, s, H5'), 5.30 (2H, s, H7), 2.95 (3H, s, CH\(_3\)) ppm; \( ^{13}\)C NMR (100 MHz, TFA-d\(_6\)) \( \delta = 155.4, 158.8, 149.7, 148.5, 146.9, 139.1, 138.4, 121.0, 118.2, 115.4, 112.6, 61.3, 16.2 ppm; HRMS \( m/z \): 343.0957 C\(_{17}\)H\(_{15}\)ClN\(_4\)O\(_2\)+1 (calcd. 343.09618).

2.2.3. (E)-5-(hydroxymethyl)-2-methyl-4-((2-(pyrazin-2-yl)hydrazono)methyl)pyridin-3-ol (1c)

Yield: 86%; yellow solid; mp 256-257°C; IR (KBr pellets) \( \nu_{\text{max}} \) 1611 (C=N), 1291 (C-O) cm\(^{-1}\); \( ^1\)H NMR (400 MHz, TFA-d\(_6\)) \( \delta = 8.61 \) (1H, s, CH), 8.43 (1H, d, J = 1.3 Hz, H9'), 8.31 (1H, dd, J = 2.7 and 1.3 Hz, H7'), 8.21 (1H, s, H4), 8.20 (1H, d, J = 2.7 Hz, H6'), 4.82 (2H, s, H7), 2.63 (3H, s, CH\(_3\)) ppm; \( ^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \( \delta = 151.4, 150.4, 142.4, 142.1, 137.0, 136.6, 135.6, 131.8, 129.8, 129.0, 58.7, 14.9 ppm; HRMS \( m/z \): 260.1147 C\(_{12}\)H\(_{13}\)N\(_4\)O\(_2\)+1 (calcd. 260.11475).

2.2.4. (E)-5-(hydroxymethyl)-2-methyl-4-((2-(pyrimidin-2-yl)hydrazono)methyl)pyridin-3-ol (1d)

Yield: 46%; brown solid; mp 193-195°C; IR (KBr pellets) \( \nu_{\text{max}} \) 1526 (C=N), 1283 (C-O) cm\(^{-1}\); \( ^1\)H NMR (500 MHz, TFA-d\(_6\)) \( \delta = 8.65-8.17 \) (3H, m, CH, H6' and H8'), 8.17 (1H, s, H4), 7.09 (1H, t, J = 4.8 Hz, H7'), 4.75 (2H, s, H7), 2.64 (3H, s, CH\(_3\)) ppm; \( ^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \( \delta = 158.9, 158.4, 152.1, 142.3, 136.7, 135.3, 129.1, 127.5, 115.2, 114.1, 57.9, 14.4 ppm; HRMS \( m/z \): 260.1151 C\(_{12}\)H\(_{13}\)N\(_4\)O\(_2\)+1 (calcd. 260.11475).

2.2.5. (E)-4-((2-(6-chloroquinolin-4-yl)hydrazono)methyl)-5-(hydroxymethyl)-2-methylpyridin-3-ol (1e)

Yield: 44%; red solid; mp 268-269°C; IR (KBr pellets) \( \nu_{\text{max}} \) 1581 (C=N), 1281 (C-O) cm\(^{-1}\); \( ^1\)H NMR (400 MHz, TFA-d\(_6\)) \( \delta = 9.74 \) (1H, s, H6'), 8.78 (1H, s, H9'), 8.65 (1H, s, H10'), 8.36 (1H, s, CH\(_3\)), 8.19 (1H, s, H4), 7.95 (1H, s, H12'), 7.68 (1H, s, H5'), 5.30 (2H, s, H7), 2.95 (3H, s, CH\(_3\)) ppm; \( ^{13}\)C NMR (100 MHz, TFA-d\(_6\)) \( \delta = 154.4, 152.2, 152.0, 147.0, 145.3, 143.4, 138.8, 135.5, 134.4, 130.5, 123.2, 123.0, 119.6, 115.9, 113.6, 59.3, 13.3 ppm; HRMS \( m/z \): 343.0957 C\(_{17}\)H\(_{15}\)ClN\(_4\)O\(_2\)+1 (calcd. 343.09618).
2.2.6. (E)-4-((2-(6-chloroquinolin-2-yl)hydrazono)methyl)pyridine-3-ol (1f)

Yield: 49%; yellow solid; mp 203-205°C; IR (KBr pellets) ν max 1669 (C=O), 1540 (C=N), 1251 (C–O) cm⁻¹; 1H NMR (500 MHz, DMSO-d₆): δ = 9.32 (1H, s, H5'), 9.25 (1H, s, H8'), 8.34 (1H, s, CH), 8.28 (1H, s, H4), 8.12 (1H, d, J = 8.8 Hz, H10' or H11'), 8.00 (1H, d, J = 8.3 Hz, H10' or H11'), 5.24 (2H, s, H7), 2.86 (3H, s, CH₃) ppm; 13C NMR (125 MHz, DMSO-d₆): δ = 156.1, 150.6, 149.0, 145.5, 139.5, 139.7, 138.6, 136.8, 132.5, 132.3, 130.1, 128.4, 124.2, 61.3, 16.2 ppm; HRMS m/z: 287.1135 C₁₄H₄₁N₄O₃⁺1 (calcd. 287.11442).

2.2.7. (E)-5-(hydroxymethyl)-4-((2-(quinolin-4-yl)hydrazono)methyl)pyridin-3-ol (1g)

Yield: 71%; yellow solid; mp 203-205°C; IR (KBr pellets) ν max 1686 (C=O), 1533 (C=N), 1275 (C–O) cm⁻¹; 1H NMR (500 MHz, DMSO-d₆): δ = 9.24 (1H, s, H10'), 8.91 (1H, s, H8'), 8.83 (1H, d, J = 1.8 Hz, H7'), 7.88 (1H, s, CH), 8.13 (1H, s, H4), 4.93 (2H, s, H7), 2.67 (3H, s, CH₃) ppm; 13C NMR (125 MHz, DMSO-d₆): δ = 161.6, 152.7, 150.1, 145.5, 144.2, 139.1, 136.4, 130.9, 126.0, 121.9, 58.3, 15.4 ppm; HRMS m/z: 287.1149 C₁₄H₁₄N₅O₃⁺1 (calcd. 287.11442).

2.2.8. (E)-N'-(3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylene)pyridine-2-carbohydrazide (2a)

Yield: 83%; salmon solid; mp 217-219°C; IR (KBr pellets) ν max 1683 (C=O), 1520 (C=N), 1277 (C–O) cm⁻¹; 1H NMR (500 MHz, DMSO-d₆): δ = 13.26 (1H, s, CH), 9.07 (1H, s, H8'), 8.19 (1H, s, H4), 8.05 (2H, d, J = 7.5 Hz, H6' and H10'), 7.68 (1H, t, J = 7.3 Hz, H8'), 7.59 (2H, t, J = 7.6 Hz, H9' and H10'), 5.28 (2H, s, H7), 2.67 (3H, s, CH₃) ppm; 13C NMR (125 MHz, DMSO-d₆): δ = 161.8, 152.8, 145.6, 143.5, 143.0, 136.1, 129.7, 124.5, 121.9, 123.2, 61.3, 16.2 ppm; HRMS m/z: 310.1303 C₁₆H₁₅N₅O₂⁺1 (calcd. 310.13040).

2.2.9. (E)-N'-(3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylene)benzohydrazide (2a)

Yield: 83%; salmon solid; mp 217-219°C; IR (KBr pellets) ν max 1694 (C=O), 1533 (C=N), 1275 (C–O) cm⁻¹; 1H NMR (500 MHz, DMSO-d₆): δ = 13.26 (1H, s, CH), 9.07 (1H, s, H8'), 8.19 (1H, s, H4), 8.05 (2H, d, J = 7.5 Hz, H6' and H10'), 7.68 (1H, t, J = 7.3 Hz, H8'), 7.59 (2H, t, J = 7.6 Hz, H9' and H10'), 5.28 (2H, s, H7), 2.67 (3H, s, CH₃) ppm; 13C NMR (125 MHz, DMSO-d₆): δ = 161.8, 152.8, 145.6, 143.5, 143.0, 136.1, 129.7, 124.5, 121.9, 123.2, 61.3, 16.2 ppm; HRMS m/z: 287.1149 C₁₄H₁₄N₅O₃⁺1 (calcd. 287.11497).

2.2.10. (E)-N'-(3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylene)nicotinohydrazide (2c)

Yield: 79%; pale gray solid; mp 207-208°C; IR (KBr pellets) ν max 1683 (C=O), 1520 (C=N), 1277 (C–O) cm⁻¹; 1H NMR (500 MHz, DMSO-d₆): δ = 13.26 (1H, s, CH), 9.22 (1H, s, CH), 8.78 (1H, d, J = 4.3 Hz, H7'), 8.21-8.18 (2H, m, H4 and H10'), 8.12 (1H, d, J = 7.7 and 1.6 Hz, H9'), 7.75 (1H, ddd, J = 7.5, 4.8 and 1.1Hz, H8'), 4.47 (2H, s, H7), 2.63 (3H, s, CH₃) ppm; 13C NMR (125 MHz, DMSO-d₆): δ = 161.1, 152.8, 148.9, 148.1, 145.4, 143.6, 138.3, 136.7, 130.0, 127.9, 126.1, 123.3, 58.0, 15.0 ppm; HRMS m/z: 287.1137 C₁₄H₁₄N₄O₃⁺1 (calcd. 287.11442).

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization

Commercial pyridoxal hydrochloride (vitamin B₆) and the appropriate heteroaromatic hydrazine or N-acylhydrazine were reacted in ethanol for 1-48 h to give the target hydrazone and N-acylhydrazone derivatives (Scheme 1). Compounds 1a-g and 2a-f were obtained in yields ranging from 42% to 86% (Table 1).

The new compounds 1a-g and 2a-f were identified by detailed spectral analyses, including 1H NMR, 13C NMR, mass spectrometry, and infrared spectroscopy. In general, the 1H NMR spectrum of 1a-g showed signals due to one pyridine proton of the pyridoxal moiety at 8.15-8.40 ppm and the N=CH proton at 8.78-9.81 ppm. In the same manner, compounds 2a-f exhibited signals due to the pyridine proton at 8.13-8.34 ppm, N=CH proton at 8.78-9.81 ppm, and NH proton at 13.26-13.54 ppm.
spectrum, it was possible to observe signals due to the five carbons of the pyridine nucleus of the pyridoxal moiety at 157.5-121.0 ppm for 1a-g and 2a-f; signals due to the carbonyl carbon of 2a-f at 163.2-159.2 ppm; and signals attributed to the C=N hydrazone carbon at 115.2-129.0 ppm for 1a-g and at 143.5-144.7 ppm for 2a-f. Peaks due to the N=CH functional group was also observed in infrared spectra at around 1565 cm\(^{-1}\) for both series of compounds.

### 3.2. Anti-tuberculosis Activity

The activity-structure relationship (SAR) of this class of compounds gives some important information (Fig. 2). Among the N-acylhydrazones 2a-f (Table 2), the best result was obtained with the para-pyridine substituted N-acylhydrazone 2d (isoniazid derivative: 10.90 µM). The ortho-pyridine derivative 2b exhibited intermediate activity at value of 87.32 µM, and the meta-pyridine derivative 2c was...
Fig. (2). Activity-structure relationship (SAR) of the hydrazone and N-acylhydrazone series.

Table 2. The \textit{in vitro} activity of compounds 1a-g and 2a-f against \textit{Mycobacterium tuberculosis} H37Rv strain (ATCC 27294, susceptible to ethambutol).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>MIC (µM)</th>
<th>Mutagenic/Tumorigenic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Ph</td>
<td>&gt;388.67</td>
<td>none / high</td>
</tr>
<tr>
<td>1b</td>
<td>2-Py</td>
<td>387.18</td>
<td>none / none</td>
</tr>
<tr>
<td>1c</td>
<td>2-Pyr</td>
<td>385.71</td>
<td>none / none</td>
</tr>
<tr>
<td>1d</td>
<td>2-Pm</td>
<td>&gt;385.71</td>
<td>none / none</td>
</tr>
<tr>
<td>1e</td>
<td>6-Cl-2-Qu</td>
<td>&gt;291.73</td>
<td>high / none</td>
</tr>
<tr>
<td>1f</td>
<td>6-Cl-Qn</td>
<td>72.72</td>
<td>none / none</td>
</tr>
<tr>
<td>1g</td>
<td>2-Qn</td>
<td>323.29</td>
<td>none / none</td>
</tr>
<tr>
<td>2a</td>
<td>Ph</td>
<td>&gt;350.51</td>
<td>none / none</td>
</tr>
<tr>
<td>2b</td>
<td>2-Py</td>
<td>87.32</td>
<td>none / none</td>
</tr>
<tr>
<td>2c</td>
<td>3-Py</td>
<td>&gt;349.30</td>
<td>none / none</td>
</tr>
<tr>
<td>2d</td>
<td>4-Py</td>
<td>10.90</td>
<td>none / high</td>
</tr>
<tr>
<td>2e</td>
<td>2-Pyr</td>
<td>&gt;348.10</td>
<td>none / none</td>
</tr>
<tr>
<td>2f</td>
<td>2-Pm</td>
<td>&gt;348.10</td>
<td>none / none</td>
</tr>
<tr>
<td>EMB</td>
<td>-</td>
<td>15.3</td>
<td>-</td>
</tr>
<tr>
<td>INH</td>
<td>-</td>
<td>0.46</td>
<td>-</td>
</tr>
</tbody>
</table>

*aCalculated using Data Warrior program. Available online at http://www.openmolecules.org/datawarrior website [Accessed 20 December 2017].
The presence of two nitrogen atoms in the ring, as in the case of pyrimidine 2f and pyrazine 2e, decreased the biological activity of these compounds against the *M. tuberculosis* H37Rv strain. In the case of the hydrazone series 1a-g, the best result was seen for the 7-chloroquinoxaline derivative 1f (72.72 μM), indicating that the number of nitrogen and chlorine atoms in the radical moiety play an important role in the anti-tuberculosis activity of the quinoxaline derivatives (1f and 1g).

**CONCLUSION**

A series of hydrazones and N-acylhydrazones containing vitamin B₆ and different heteroaromatic nuclei were synthesized and evaluated against TB. SAR studies indicated that the best result for N-acylhydrazones was seen for the isoniazid derivative 2d (10.90 μM) and that the quinoxaline derivative 1f (72.72 μM) was the most active compound in the hydrazone series. Due to their structures, this class of compounds can be used as metal ligands in the synthesis of complexes and their potential will be further evaluated.

**CONSENT FOR PUBLICATION**

Not applicable.

**FUNDING**

The authors are grateful to the Brazilian agency CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for fellowships and financial support.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

**ACKNOWLEDGEMENTS**

None.

**REFERENCES**


