

## Synthesis, *in vitro* Antiproliferative and Anti-*Mycobacterium tuberculosis* Activities of Novel $\beta$ -Carboline Derivatives

Flora M. F. Moreira,<sup>a</sup> Julio Croda,<sup>a</sup> Maria H. Sarragiotto,<sup>b</sup> Mary A. Foglio,<sup>c</sup>  
Ana L. T. G. Ruiz,<sup>c</sup> João E. Carvalho<sup>c</sup> and Anelise S. N. Formagio<sup>\*,d</sup>

<sup>a</sup>Faculdade de Ciências da Saúde and <sup>d</sup>Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Rodovia Dourados - Itahum, Km 12, 79804-970 Dourados-MS, Brazil

<sup>b</sup>Departamento de Química, Universidade Estadual de Maringá, Avenida Colombo, 5790, Jardim Universitário, 87020-900 Maringá-PR, Brazil

<sup>c</sup>Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, Universidade Estadual de Campinas, CP 6171, 13083-970 Campinas-SP, Brazil

A series of  $\beta$ -carboline derivatives with amino or guanidinium were synthesized and evaluated *in vitro* against anti-*Mycobacterium tuberculosis* and for antiproliferative activities against nine human cancer cell lines. The compounds 1-(4-hydroxyphenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline (24.9  $\mu\text{g mL}^{-1}$ ) and 1-(4-methoxyphenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline (26.9  $\mu\text{g mL}^{-1}$ ) were the most active against *M. Tuberculosis* (MTB). Compounds 1-(4-hydroxyphenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline and 1-(4-methoxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline, which had the same substituted groups, inhibited the growth of all human tumor cell lines with growth inhibitory activity ( $\text{GI}_{50}$ ) values from 1.37 to 9.20  $\text{mmol L}^{-1}$ . Also in this series, compounds 1-(4-hydroxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline and 1-(3-nitrophenyl)-3-carboxamide(propylamine)  $\beta$ -carboline demonstrated significant activity against NCI/ADR cells. Among compounds with a terminal guanidine group, compounds 1-(4-hydroxyphenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline (27.8  $\mu\text{g mL}^{-1}$ ) and 1-(3-nitrophenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline (37.4  $\mu\text{g mL}^{-1}$ ) demonstrated the greatest activity against MTB. Additionally, compounds 1-(4-methoxyphenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline ( $\text{GI}_{50} = 0.45 \text{ mmol L}^{-1}$ ) effectively inhibited growth and was highly selective against NCI/ADR. The *in silico* study revealed that 1-(4-hydroxyphenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline, 1-(4-methoxyphenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline, 1-(4-hydroxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline, 1-(4-methoxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline and 1-(3-nitrophenyl)-3-carboxamide(propylamine)  $\beta$ -carboline compounds follow the rules established by Lipinski, suggesting that this compound has no problems with oral bioavailability.

**Keywords:** synthesis,  $\beta$ -carboline, *Mycobacterium tuberculosis*, antiproliferative activity

### Introduction

Tuberculosis (TB) exhibits high morbidity and mortality. The long-term treatment regimen can cause patients to be non-compliant in completing the treatment, thus leading to emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) TB strains. Infections caused by MDR-TB and XDR-TB do not respond to first-line drugs that are used to treat TB, and alternative treatment regimens include mostly injected drugs and prolonged treatments.<sup>1,2</sup> Due to the appearance of

resistant strains and given high toxicity of anti-tuberculosis drugs, to the need to develop new drugs that are more effective and less toxic than current drugs, which would reduce time and complexity of treatment, is urgent.<sup>3,4</sup> The discovery of new drugs is also necessary for the treatment of cancer, because most chemotherapeutic agents exhibit severe toxicity and cause many undesirable side effects; additionally, current agents are very expensive, mutagenic, carcinogenic and teratogenic.<sup>5</sup> Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB),<sup>1</sup> and cancer<sup>6</sup> have affected human health for thousands of years and remain a major cause of diseases affecting public health around the world, and the possible interaction of mycobacterial

\*e-mail: aneliseformagio@ufgd.edu.br

pathogens with cancer cells may be influenced by genetic alterations in the tumor cells.

Synthetic  $\beta$ -carboline derivatives exhibit a wide range of pharmacological activities, including antitumor and anti-tuberculosis activities.<sup>7-14</sup> Our research group demonstrated that  $\beta$ -carboline derivatives with various substituents at positions-1, 3 and 9 of the  $\beta$ -carboline skeleton presented significant *in vitro* antitumoral, antiviral, antitrypanosomal and antileishmanial activities.<sup>15-23</sup> Other studies have shown that  $\beta$ -carboline derivatives with a methyl-substituted group at position-1 and a guanidinium group-terminated side chain at C-3 exhibited anti-HIV-1 activity in MT4 cells by hindering the essential interaction of the regulatory protein Tat with trans-activation response region (TAR).<sup>24,25</sup> Some results cited above indicated that  $\beta$ -carboline derivatives containing 4-hydroxyphenyl, 4-methoxyphenyl or 3-nitrophenyl group at C-1 showed potent anticancer activity for some of the human cancer cell lines tested. This led us to study novel  $\beta$ -carboline analogs that might serve as antitumoral and anti-tuberculosis agents as part of our ongoing research program.

Based on the idea that the addition of appropriate substituents at positions-1 and -3 might result in more potent compounds, we synthesized novel 1-substituted-phenyl- $\beta$ -carboline with an amino or guanidinium group-terminated side chain at C-3 and evaluated the *in vitro* anti-tuberculosis, antiproliferative properties and *in silico* study.

## Experimental

### General procedure

<sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury Plus (Palo Alto, EUA) spectrometer operating at 300 and 75.5 MHz, respectively, using deuterated dimethyl sulfoxide (DMSO-*d*<sub>6</sub>), chloroform (CDCl<sub>3</sub>) and methanol (CD<sub>3</sub>OD) as solvent, and tetramethylsilane (TMS) as internal reference. Infrared (IR) spectra were recorded as potassium bromide pellets on a BOMEM spectrometer model MB-100 (Houston, USA). Melting points were determined in a Micro-Química apparatus MQAPF-301 model (Palhoça, Brazil) and are uncorrected. The reactions were monitored by thin layer chromatography (TLC) conducted on Merck TLC plates (Silica Gel 60 F254, Darmstadt, Germany). All reagents were purchased from commercial suppliers.

### Preparation of 1-(substituted phenyl)-3-carboxamide-ethylguanidine- $\beta$ -carboline (**4a-c**)

The 1-(substituted phenyl)-3-carbomethoxy- $\beta$ -carboline were obtained as previously reported.<sup>21</sup> The 1-(substituted-

phenyl)-3-ethylamine-carboxamide  $\beta$ -carboline **2a-c** were obtained by reaction of the methyl esters **1a-c** (2.0 mmol L<sup>-1</sup>) with 1,2-ethylenediamine (6 mmol L<sup>-1</sup>) at room temperature, stirred for 36 h. The formed solids were collected by filtration. The 1-(substituted-phenyl)-3-prophylamine-carboxamide  $\beta$ -carboline **3a-c** were obtained by reaction of the methyl esters **1a-c** (1.7 mmol L<sup>-1</sup>) with 1,3-propanediamine (5 mmol L<sup>-1</sup>) in 25 mL MeOH/CHCl<sub>3</sub> was refluxed for 32 h. The formed solids were collected by filtration.<sup>26</sup>

To a solution of S-methylisothiurea sulfate (0.5 mmol L<sup>-1</sup>) in water (1.5 mL) and 2 mmol L<sup>-1</sup> NaOH (0.5 mL) was added a suspension of  $\beta$ -carboline-3-ethylamine-carboxamide **2a-c** at 0 °C. The mixture was kept under stirring, at room temperature by 1 h and later under reflux for 24 h, was again added a solution of S-methylisothiurea sulfate (0.5 mmol L<sup>-1</sup>) in water (1.5 mL) and 2 mol L<sup>-1</sup> NaOH (0.5 mL) and reflux for 24 h. The formed solids were collected by filtration and washed with cold water for obtained 1-(substituted phenyl)-3-carboxamide-ethylguanidine  $\beta$ -carboline (**4a-c**).<sup>26</sup> The characterization data of the compounds obtained are given bellow that corroborates with correlation spectroscopy (COSY), H<sup>1</sup> detected multiquantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) two-dimensional NMR spectra.

### 1-(4-Hydroxyphenyl)-3-carboxamide(ethylamine) $\beta$ -carboline (**2a**)

Yield 68%; mp 171-174 °C; IR (KBr)  $\nu_{\max}$  / cm<sup>-1</sup> 3090, 1684, 1480, 1460; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.74 (t, 2H, *J* 6.0 Hz, CH<sub>2</sub>-ethyl), 3.07 (t, 2H, *J* 6.0 Hz, CH<sub>2</sub>-ethyl), 7.01 (d, 2H, *J* 8.7 Hz, Phenyl-H); 7.28 (t, 1H, *J* 7.0 Hz, H-6), 7.57 (td, 1H, *J* 7.7, 5.0 Hz, H-7), 7.67 (d, 1H, *J* 8.4 Hz, H-8), 7.99 (d, 2H, *J* 8.7 Hz, Phenyl-H), 8.37 (d, 1H, *J* 7.8 Hz, H-5); 8.73 (s, 1H, H-4); <sup>13</sup>C NMR (75.5 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  41.2, 41.9, 112.1, 112.7, 115.7, 120.3, 121.5, 121.8, 128.5, 129.2, 129.7, 130.0, 134.8, 138.7, 141.4, 141.8, 157.9, 167.1.

### 1-(4-Methoxyphenyl)-3-carboxamide(ethylamine) $\beta$ -carboline (**2b**)

Yield 74%; mp 180-182 °C; IR (KBr)  $\nu_{\max}$  / cm<sup>-1</sup> 3354, 3106, 1686, 1590, 860; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD)  $\delta$  2.84 (t, 2H, *J* 6.0 Hz, CH<sub>2</sub>-ethyl), 3.87 (t, 2H, *J* 6.0 Hz, CH<sub>2</sub>-ethyl); 3.95 (s, 3H, OCH<sub>3</sub>), 7.17 (d, 2H, *J* 8.1 Hz, Phenyl-H), 7.36 (ddd, 1H, *J* 7.8, 7.5, 2.1 Hz, H-6), 7.58 (d, 1H, *J* 7.5 Hz, H-8), 7.61 (ddd, 1H, *J* 7.8, 7.5, 2.1 Hz, H-7), 8.02 (d, 2H, *J* 8.1 Hz, Phenyl-H), 8.24 (d, 1H, *J* 7.8 Hz, H-5), 8.83 (s, 1H, H-4); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD)  $\delta$  37.6, 39.3, 55.4, 113.1, 114.6, 115.1,

121.0, 122.4, 122.8, 130.8, 131.6, 132.3, 132.9, 133.6, 139.5, 141.9, 142.0, 144.3, 162.5.

1-(3-Nitrophenyl)-3-carboxamide(ethylamine)  $\beta$ -carboline (**2c**)

Yield 66%; mp 172-175 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3190, 1636, 1555, 1496, 1420;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$  2.98 (t, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -ethyl), 3.63 (t, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -ethyl), 7.37 (m, 1H, H-6), 7.60 (m, 2H, H-8, Phenyl-H), 7.81 (t, 1H,  $J$  7.8 Hz, H-7), 8.23 (dd, 1H,  $J$  7.8 Hz, H-5), 8.37 (dd, 1H,  $J$  7.0, 2.0 Hz, Phenyl-H), 8.40 (d, 1H,  $J$  7.0 Hz, Phenyl-H), 8.86 (sl, 1H, Phenyl-H), 8.89 (s, 1H, H-4);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$  39.9, 40.7, 112.4, 114.3, 120.8, 121.6, 121.7, 123.4, 123.5, 129.1, 129.9, 131.1, 134.7, 135.0, 138.6, 138.9, 139.4, 141.8, 148.5, 167.1.

1-(4-Hydroxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline (**3a**)

Yield 72%; mp 180-182 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3226, 1640, 1550, 1496, 1440;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.65 (q, 2H,  $J$  6.3 Hz,  $\text{CH}_2$ -propyl), 2.65 (t, 2H,  $J$  6.3 Hz,  $\text{CH}_2$ -propyl), 3.45 (q, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -propyl), 7.00 (d, 2H,  $J$  8.1 Hz, Phenyl-H), 7.29 (t, 1H,  $J$  7.2 Hz, H-6), 7.57 (t, 1H,  $J$  7.2 Hz, H-7), 7.67 (d, 1H,  $J$  8.1 Hz, H-8), 8.02 (d, 2H,  $J$  8.1 Hz, Phenyl-H), 8.36 (d, 1H,  $J$  8.1 Hz, H-5), 8.72 (s, 1H, H-4);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ )  $\delta$  32.8, 36.9, 39.5, 112.0, 112.6, 115.6, 120.1, 121.3, 121.9, 128.3, 128.4, 129.6, 130.1, 133.8, 139.7, 140.9, 141.5, 158.5, 164.9.

1-(4-Methoxyphenyl)-3-carboxamide(propylamine)  $\beta$ -carboline (**3b**)

Yield 60%; mp 188-193 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3200, 1678, 1550, 1520, 1448, 1010;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.64 (qt, 2H,  $J$  6.6 Hz,  $\text{CH}_2$ -propyl), 2.64 (t, 2H,  $J$  6.6 Hz,  $\text{CH}_2$ -propyl), 3.44 (q, 2H,  $J$  6.6 Hz,  $\text{CH}_2$ -propyl), 3.88 (s, 3H,  $\text{OCH}_3$ ), 7.18 (d, 2H,  $J$  8.7 Hz, Phenyl-H), 7.30 (t, 1H,  $J$  7.2 Hz, H-6), 7.58 (t, 1H,  $J$  8.1 Hz, H-7), 7.67 (d, 1H,  $J$  8.1 Hz, H-8), 8.14 (d, 2H,  $J$  8.7 Hz, Phenyl-H), 8.38 (d, 1H,  $J$  7.2 Hz, H-5), 8.75 (s, 1H, H-4);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ )  $\delta$  33.0, 36.9, 37.0, 55.4, 112.3, 112.6, 114.2, 120.1, 121.3, 121.9, 128.4, 129.7, 130.0, 130.1, 133.9, 139.8, 140.4, 141.6, 159.9, 164.5.

1-(3-Nitrophenyl)-3-carboxamide(propylamine)  $\beta$ -carboline (**3c**)

Yield 55%; mp 178-180 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3160, 1686, 1534, 1474, 1440;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.65 (qt, 2H,  $J$  6.5 Hz,  $\text{CH}_2$ -propyl), 2.65 (t, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -propyl), 3.46 (q, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -propyl), 7.33 (t, 1H,  $J$  7.5 Hz, H-6), 7.63 (m, 2H, H-8, Phenyl-H), 7.72

(d, 1H,  $J$  7.8 Hz, H-8), 7.91 (t, 1H,  $J$  7.8 Hz, H-7), 8.33 (dd, 1H,  $J$  8.1, 1.5 Hz, Phenyl-H), 8.45 (d, 1H,  $J$  7.8 Hz, Phenyl-H), 8.64 (d, 1H,  $J$  8.1 Hz, H-5), 8.86 (s, 1H, H-4);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  32.8, 36.8, 37.0, 112.6, 113.7, 120.4, 121.2, 122.2, 123.5, 123.6, 128.9, 130.2, 130.4, 134.7, 135.1, 138.2, 138.9, 139.6, 141.6, 166.5.

1-(4-Hydroxyphenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline (**4a**)

Yield 30%; mp 176-178 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3260, 1670, 1544, 1424, 1460, 1236;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  3.22 (t, 2H,  $J$  6.5 Hz,  $\text{CH}_2$ -ethyl), 3.49 (t, 2H,  $J$  6.5 Hz,  $\text{CH}_2$ -ethyl), 5.50 (s, 2H,  $\text{NH}_2$ ), 6.14 (s, 1H, NH), 6.63 (s, 1H, NH), 7.05 (d, 2H,  $J$  8.4 Hz, Phenyl-H), 7.28 (t, 1H,  $J$  7.5 Hz, H-6), 7.56 (t, 1H,  $J$  7.5 Hz, H-7), 7.67 (d, 1H,  $J$  8.1 Hz, H-8), 8.05 (d, 2H,  $J$  8.4 Hz, Phenyl-H), 8.37 (d, 1H,  $J$  7.8 Hz, H-5), 8.72 (s, 1H, H-4), 8.81 (s, 1H, NH);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ )  $\delta$  37.3, 40.5, 112.4, 113.0, 116.0, 120.5, 121.5, 122.1, 128.6, 128.8, 129.9, 130.5, 134.1, 139.6, 141.3, 141.7, 158.6, 159.6, 163.1.

1-(4-Methoxyphenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline (**4b**)

Yield 45%; mp 190-194 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3320, 1670, 1550, 1520, 1448;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  3.25 (q, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -ethyl), 3.45 (q, 2H,  $J$  6.0 Hz,  $\text{CH}_2$ -ethyl), 3.96 (s, 3H,  $\text{OCH}_3$ ), 5.54 (s, 2H,  $\text{NH}_2$ ), 6.18 (t, 1H,  $J$  7.5 Hz, NH), 7.22 (d, 2H,  $J$  8.7 Hz, Phenyl-H), 7.34 (t, 1H,  $J$  7.0 Hz, H-6), 7.60 (t, 1H,  $J$  7.5 Hz, H-7), 7.70 (d, 1H,  $J$  8.0 Hz, H-8), 8.20 (d, 2H,  $J$  8.7 Hz, Phenyl-H), 8.41 (d, 1H,  $J$  8.1 Hz, H-5), 8.78 (s, 1H, H-4), 8.84 (t, 1H,  $J$  7.5 Hz, NH), 11.81 (s, 1H, NH);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ )  $\delta$  38.6, 40.3, 55.4, 112.3, 112.6, 114.2, 120.8, 121.2, 121.9, 129.7, 129.9, 130.1, 133.8, 138.4, 139.6, 140.5, 141.5, 159.0, 159.9, 165.1.

1-(3-Nitrophenyl)-3-carboxamide(ethyl)guanidine  $\beta$ -carboline (**4c**)

Yield 38%; mp 192-194 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3204, 1668, 1530, 1520, 1448;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  3.22 (m, 2H,  $\text{CH}_2$ -ethyl), 3.42 (q, 2H,  $J$  6.5 Hz,  $\text{CH}_2$ -ethyl), 5.49 (s, 2H,  $\text{NH}_2$ ), 6.15 (t, 1H,  $J$  7.0 Hz, NH), 7.34 (t, 1H,  $J$  7.0 Hz, H-6), 7.60 (d, 1H,  $J$  7.8 Hz, H-8), 7.62 (t, 1H,  $J$  7.8 Hz, Phenyl-H), 7.95 (t, 1H,  $J$  8.0 Hz, H-7), 8.39 (d, 1H,  $J$  7.7 Hz, H-5), 8.45 (d, 1H,  $J$  8.1 Hz, Phenyl-H), 8.65 (d, 1H,  $J$  7.5 Hz, Phenyl-H), 8.86 (s, 1H, Phenyl-H), 8.89 (s, 1H, H-4), 8.90 (s, 1H, NH), 12.02 (s, 1H, NH);  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ )  $\delta$  38.7, 40.5, 112.6, 113.8, 114.2, 120.4, 121.2, 122.3, 123.4, 123.6, 128.9, 130.4, 130.5, 134.4, 135.5, 138.1, 138.9, 140.1, 141.7, 148.3, 159.01, 164.9.

### Anti-*Mycobacterium tuberculosis* activity

*M. tuberculosis* H<sub>37</sub>Rv (ATCC27294) strains were grown in Ogawa-Kudoh (OK) medium for 10 days at 37 °C. For testing, aliquots were removed and cultured in Middlebrook 7H9 broth (Difco, Sparks, USA) supplemented with oleic acid, bovine serum albumin, dextrose and catalase (OADC enrichment BBL/Becton-Dickinson); 0.5% glycerol was added as a carbon source and 0.5% Tween 80 was added to prevent the appearance of lumps. The culture was maintained for 15 days at 37 °C. The bacterial suspensions were prepared and adjusted to the No. 1 of the McFarland scale.

Stock solutions of the test compounds were solubilized in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA) and diluted in Middlebrook 7H9 broth (Difco, Sparks, USA) supplemented with OADC enrichment BBL/Becton Dickinson. Rifampicin and isoniazid were solubilized according to the manufacturer's recommendations (Sigma-Aldrich, St. Louis, USA) and used as positive controls.

Antimycobacterial activity was determined using the resazurin microtiter assay (REMA).<sup>27</sup> Briefly, 100 µL of supplemented Middlebrook 7H9 broth (Difco, Sparks, USA) was dispensed into each well of a sterile flat-bottom 96-well plate; then, serial dilutions of the test compounds (0.98–250 µg mL<sup>-1</sup>) and reference drugs (0.004–1 µg mL<sup>-1</sup>) were prepared. One hundred microliters of bacterial suspension (5 × 10<sup>5</sup> UFC mL<sup>-1</sup>) was then added to each well. Plates were incubated for 7 days at 37 °C, after was added 30 µL of resazurin (Sigma-Aldrich, St. Louis, USA) in sterile water (0.01%) in whole plate, and the samples were incubated for 24 h at 37 °C.

The change in absorbance, at 492 nm wavelength, was measured using a microplate reader TP-Reader (Thermo Plate®, Männedorf, Switzerland). Each compound was analyzed in triplicate on alternate days. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that resulted in 90% inhibition of the growth of *M. tuberculosis*.<sup>28</sup> MIC values were used to classify a compound's activity as follows: inactive, > 150 µg mL<sup>-1</sup>; moderate, between > 10 and < 100 µg mL<sup>-1</sup>; and active, < 10 µg mL<sup>-1</sup>.

### *In vitro* antiproliferative assay

The tested compounds were evaluated *in vitro* against a nine-cell line panel comprising melanoma UACC-62, breast MCF7, lung NCI-460, leukemia K-562, ovarian OVCAR, prostate PCO-3, colon HT29, renal 786-0 and adriamycin drug-resistant ovarian cancer NCI/ADR cells.

The tests were performed using the colorimetric method with sulforhodamine B according to the National Cancer Institute (NCI) standard protocol; doxorubicin was used as a positive control.<sup>29</sup> Assays were performed in a 96-well plate using four serial 10-fold dilutions (0.25–250 µg mL<sup>-1</sup>) for each test compound. The anticancer activity was determined based on concentration-response curves, and three concentration response parameters, growth inhibitory activity (GI<sub>50</sub>), growth inhibition (TGI) and cytotoxic activity (LC<sub>50</sub>) were calculated. The response parameter GI<sub>50</sub> refers to the drug concentration that produces 50% reduction of cell growth when compared to untreated control cells. The TGI and LC<sub>50</sub> parameters refer to the drug concentrations required for total growth inhibition and for 50% cell mortality, respectively. Compounds with GI<sub>50</sub> values < 100 µmol L<sup>-1</sup> were considered active.

### *In silico* study

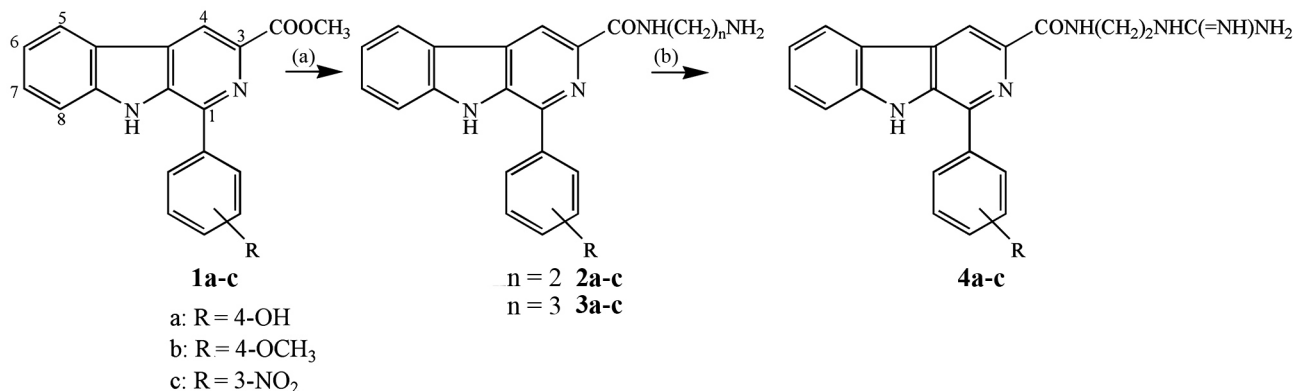
The *in silico* computational study of compounds were performed to determine Lipinski's rules of five<sup>30</sup> (hydrogen bond donors ≤ 5; hydrogen bond acceptors ≤ 10; molecular weight ≤ 500; the Log P is ≤ 5), topological polar surface area (TPSA) and percentage of absorption (%ABS). Calculations were performed using Molinspiration online property calculation toolkit software<sup>31</sup> and OSIRIS property explorer software.<sup>32</sup> The percentage of absorption was estimated using the following equation: %ABS = 10 – [0.345 × TPSA].

## Results and Discussion

### Chemistry

The synthetic pathway for the preparation of 1,3-disubstituted β-carbolines is presented in Scheme 1. The methyl esters in **1a-c** were prepared by a Pictet-Spengler condensation of L-tryptophan with the appropriate aromatic aldehydes in acidic media, subsequent esterification of the resulting carboxylic acids with methanol and sulfuric acid, and oxidation with sulfur in refluxing xylene.<sup>22</sup> Compounds **2a-c** and **3a-c** were obtained by the reaction of β-carboline methyl ester with 1,2-ethylenediamine and 1,3-propanediamine, respectively, and resulted in an amino group-terminated side chain at C-3. Finally, the coupling of β-carboline carboxamide derivatives **2a-c** with S-methylisothiourea yielded compounds **4a-c**, which include a terminal guanidinium group.

The novel compounds **2a-c**, **3a-c** and **4a-c** were characterized using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, as detailed in the Experimental section. The <sup>1</sup>H NMR spectra



**Scheme 1.** Synthetic route for the preparation of the  $\beta$ -carboline derivatives. Reagents and conditions: (a) 1,2-ethylenediamine, at room temperature, 36 h or 1,3-propanediamine, CHCl<sub>3</sub>/MeOH, reflux, 32 h (55-72%); (b) S-methylisothiourea, 2N NaOH, 4 °C to room temperature, reflux, 48 h (30-45%).

of carboxamides **2a-c** and **3a-c** showed additional signals at  $\delta_{\text{H}}$  1.64-3.95 (reflecting the integration of two protons) and at  $\delta_{\text{H}}$  7.01-8.94 (corresponding to aromatic hydrogens). The presence of the ethylamine or propylamine carboxamide in position C-3 group was confirmed by <sup>13</sup>C NMR, which showed signals at  $\delta_{\text{C}}$  32.8-41.9 (CH<sub>2</sub>) and  $\delta_{\text{C}}$  162.5-167.1 (C=O). Derivatives **4a-c** was characterized by the presence of an additional signal at  $\delta_{\text{C}}$  159.01-159.90, corresponding to the guanidinium group.

#### Anti-*Mycobacterium tuberculosis* activity (MTB)

Derivatives **2a-c**, **3a-c** and **4a-c** were evaluated *in vitro* for their antimycobacterial activity against *M. tuberculosis* H37Rv (ATCC 27294) using the REMA method.<sup>27</sup> The MIC values ( $\mu\text{g mL}^{-1}$  and  $\mu\text{mol L}^{-1}$ ) were measured with respect to two standard antitubercular drugs, isoniazid (INH) and rifampicin (RFP), and the screening results are presented in Table 1. Among the nine compounds evaluated against MTB, seven presented moderate activity, with MIC values ranging from 58.3-24.9  $\mu\text{g mL}^{-1}$ ; in particular, compounds **2a** (24.9  $\mu\text{g mL}^{-1}$ ), **2b** (26.9  $\mu\text{g mL}^{-1}$ ), **4a** (27.8  $\mu\text{g mL}^{-1}$ ) and **4c** (37.4  $\mu\text{g mL}^{-1}$ ) presented interesting activity. Compounds **2a** and **2b**, which had *p*-hydroxyphenyl and *p*-methoxyphenyl substituents, respectively, at position-1 and ethylenediamine moieties at C-3 were the most active derivatives in this series. The length of the terminated side chains affected the activities of these compounds. Substituting the guanidinium group led to reduced activity (compare compounds **2a** and **4a**). The effect of the guanidinium group was particularly significant when comparing compounds **2c** (57.9  $\mu\text{g mL}^{-1}$ ) and **4c** (37.4  $\mu\text{g mL}^{-1}$ ). The substituents at positions-1 and 3 strongly affected anti-MTB activities of these compounds.

Earlier studies reported the synthesis and investigations of the antimycobacterial activity, e.g., guanidinium-modified

compounds, which demonstrated potent antitubercular activity against *M. tuberculosis*, aminopyrimidine derivatives exhibit moderate to potent anti-MTB activity, with MIC values ranging from 12.5-3.12  $\mu\text{g mL}^{-1}$ .<sup>33</sup> The introduction of an ethylguanidinium group at the upper rim resulted in high antimycobacterial activities for the unsubstituted, 5,5'-dimethyl-2,2'-bipyridyl and 4,4'-dimethyl-2,2'-bithiazolyl analogs, with MIC values of 1.51 and 2.69  $\mu\text{g mL}^{-1}$ , respectively, values that were similar to those of current commercially available anti-tuberculosis agents.<sup>34</sup>

**Table 1.** Anti-*Mycobacterium tuberculosis* H<sub>37</sub>RV activity of compounds **2a-c**, **3a-c** and **4a-c**

Compound	R	n	MIC <sup>a</sup> / ( $\mu\text{g mL}^{-1}$ )	MIC / ( $\mu\text{mol L}^{-1}$ )
<b>2a</b>	4-OH	2	24.9	75.4
<b>2b</b>	4-OCH <sub>3</sub>	2	26.9	74.6
<b>2c</b>	3-NO <sub>2</sub>	2	57.9	153.5
<b>3a</b>	4-OH	3	> 250	> 500
<b>3b</b>	4-OCH <sub>3</sub>	3	58.3	155.7
<b>3c</b>	3-NO <sub>2</sub>	3	> 250	> 500
<b>4a</b>	4-OH	2	27.8	74.4
<b>4b</b>	4-OCH <sub>3</sub>	2	57.5	142.6
<b>4c</b>	3-NO <sub>2</sub>	2	37.4	88.3
Isoniazid	–	–	0.05	0.3
Rifampicin	–	–	0.01	0.01

<sup>a</sup>MIC = minimum inhibitory concentration (REMA assay), values quoted are the means of results for triplicate samples.

#### Antiproliferative activity

The antiproliferative activities of the synthesized 1,3-disubstituted  $\beta$ -carboline derivatives (**2a-c**, **3a-c** and **4a-c**) were evaluated *in vitro* against nine human tumor cell lines. The results for compounds **2a-c** and **3a-c**,

which were amino group-terminated at C-3, demonstrated that compounds **2a** and **3b**, with *p*-hydroxyphenyl and *p*-methoxyphenyl groups, respectively, at position-1, inhibited growth in all human tumor cell lines with GI<sub>50</sub> values ranging from 1.37-9.20 μmol L<sup>-1</sup>. Also in this series, compounds **3a** (GI<sub>50</sub> = 0.33 μmol L<sup>-1</sup>, TGI = 50.28 μmol L<sup>-1</sup>) and **3c** (GI<sub>50</sub> = 0.71 μmol L<sup>-1</sup>, TGI = 11.08 μmol L<sup>-1</sup>, LC<sub>50</sub> = 26.62 μmol L<sup>-1</sup>) showed significant activity and high selectivity against adriamycin drug-resistant ovarian cancer cells (NCI/ADR) (Tables 2 and 3).

The compounds with terminal guanidinium groups, including compound **4b** (GI<sub>50</sub> = 0.45 μmol L<sup>-1</sup>; TGI = 72.09 μmol L<sup>-1</sup>) effectively inhibited growth and was highly selective against adriamycin drug-resistant ovarian cancer cell lines (NCI/ADR) when compared

with compounds **2a-c**, which are amino group-terminated. However, the guanidinium-terminated compounds did not demonstrate any important interaction that could account for the cell growth inhibition. Substituting the phenyl group with electron-donating substituents at position-1 influenced each series differently.

A previous study evaluated the *in vitro* antitumor activities of several benzenesulfonamide derivatives with various substituted aminoguanidine groups. Compound 1-allyl-2-[4-chlorophenylcarbamoyl]-2-methylthiobenzenesulfonyl-3-(5-nitrofurfurylideneamino) exhibited remarkable activity against 21 human tumor cell lines representing leukemia and melanoma and lung, colon, ovarian, renal, prostate and breast cancers (GI<sub>50</sub> = 0.3-3.0 μmol L<sup>-1</sup>).<sup>35</sup>

**Table 2.** *In vitro* cell growth inhibition (GI<sub>50</sub>) of compounds **2a-c**, **3a-c** and **4a-c** against neoplastic cells

Compound	R	n	GI <sub>50</sub> <sup>a</sup> / (μmol L <sup>-1</sup> )								
			UACC-62 melanoma	MCF7 breast	NCI-460 lung	K-562 leukemia	OVCAR ovarian	PCO-3 prostate	HT29 colon	786-0 renal	NCI/ADR ovarian-resistant
<b>2a</b>	4-OH	2	5.15	9.55	9.64	2.93	9.20	8.69	4.27	9.64	8.18
<b>2b</b>	4-OCH <sub>3</sub>	2	38.75	44.49	44.49	53.07	17.14	*c	> 100	33.11	8.36
<b>2c</b>	3-NO <sub>2</sub>	2	10.34	11.72	88.26	> 100	9.36	*	25.82	21.64	2.42
<b>3a</b>	4-OH	3	25.14	16.19	13.59	13.01	22.75	23.57	23.57	18.71	0.33
<b>3b</b>	4-OCH <sub>3</sub>	3	9.66	6.98	7.35	5.20	7.09	2.39	9.02	4.40	1.37
<b>3c</b>	3-NO <sub>2</sub>	3	43.22	23.94	55.16	18.89	16.76	11.18	27.48	27.48	0.71
<b>4a</b>	4-OH	2	> 100	> 100	- <sup>b</sup>	-	> 100	-	-	3.61	> 100
<b>4b</b>	4-OCH <sub>3</sub>	2	95.93	35.46	27.17	63.41	39.94	15.54	75.64	63.41	0.45
<b>4c</b>	3-NO <sub>2</sub>	2	51.85	99.17	> 100	31.03	87.51	91.82	25.02	31.03	1.71

<sup>a</sup>GI<sub>50</sub> = growth inhibitory activity; <sup>b</sup>not determined; <sup>c</sup>not tested.

**Table 3.** Total growth inhibition (TGI) and lethal concentration (LC<sub>50</sub> - in parentheses) of compounds **2a-c**, **3a-c** and **4a-c** (μmol L<sup>-1</sup>)

Compound	R	n	UACC-62 melanoma	MCF7 breast	NCI-460 lung	K-562 leukemia	OVCAR ovarian	PCO-3 prostate	HT29 colon	786-0 renal	NCI/ADR ovarian-resistant
<b>2a</b>	4-OH	2	15.44 (37.5)	38.27	34.32	23.87	24.75 (51.7)	24.75 (74.42)	73.52	24.75 (64.37)	24.75
<b>2b</b>	4-OCH <sub>3</sub>	2	> 100	> 100	> 100	> 100	> 100	*b	> 100	> 100	26.45
<b>2c</b>	3-NO <sub>2</sub>	2	20.35 (70.93)	22.41 (43.42)	> 100	44.27	19.33 (30.85)	*	> 100	87.41	79.13
<b>3a</b>	4-OH	3	- <sup>a</sup>	42.55	-	36.01	-	> 100	-	-	50.28
<b>3b</b>	4-OCH <sub>3</sub>	3	-	15.38 (32.8)	20.83 (51.25)	15.38 (37.3)	21.47 (46.84)	10.56 (35.2)	21.47 (49.15)	16.71 (46.84)	11.97 (28.94)
<b>3c</b>	3-NO <sub>2</sub>	3	> 100	> 100	-	> 100	> 100	43.09	> 100	> 100	11.08 (26.62)
<b>4a</b>	4-OH	2	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
<b>4b</b>	4-OCH <sub>3</sub>	2	-	> 100	> 100	> 100	> 100	> 100	> 100	> 100	72.09
<b>4c</b>	3-NO <sub>2</sub>	2	90.86	-	-	55.07	-	-	99.28	> 100	> 100

<sup>a</sup>Not determined; <sup>b</sup>not tested.

**Table 4.** Lipinski's rule and percentage of absorption (%ABS), topological polar surface area (TPSA), for compounds **2a-c**, **3a-c** and **4a-c**

Compound	%ABS <sup>a</sup>	TPSA <sup>b</sup> / Å <sup>2</sup>	Lipinski's parameter					
			nHBA <sup>c,h</sup> (nON)	nHBD <sup>d</sup> (nOHNH)	Log P <sup>e,h</sup>	MW <sup>f,h</sup>	n violations <sup>h</sup>	Log S <sup>g,i</sup>
<b>2a</b>	73.11	104.03	6	5	2.02	346.39	0	-4.23
<b>2b</b>	76.91	93.04	6	4	2.56	360.42	0	-4.54
<b>2c</b>	64.28	129.63	8	4	2.43	375.39	0	-4.98
<b>3a</b>	73.11	104.03	6	5	2.29	360.42	0	-4.50
<b>3b</b>	76.91	93.04	6	4	2.83	374.44	0	-4.81
<b>3c</b>	64.28	129.63	8	4	2.71	389.42	0	-5.25
<b>4a</b>	60.74	139.91	8	7	2.08	388.43	1	-3.87
<b>4b</b>	64.53	128.92	8	6	2.61	402.46	1	-4.18
<b>4c</b>	51.90	165.51	10	6	2.49	417.43	1	-4.62

<sup>a</sup>%ABS = 109 - [0.345 × TPSA]; <sup>b</sup>TPSA = topological polar surface area; <sup>c</sup>nHBA [number hydrogen bond acceptor (nON)] ≤ 10; <sup>d</sup>nHBD [number hydrogen bond donors (OHNH)] ≤ 5; <sup>e</sup>Log P (octanol-water partition coefficient) < 5; <sup>f</sup>MW (molecular weight) ≤ 500; <sup>g</sup>Log S (solubility) between -1 and -5; <sup>h</sup>reference 31; <sup>i</sup>reference 32.

### Lipinski's rule of five

The drug-likeness concept helps optimize the pharmacokinetic properties of a compound, such as absorption, distribution, metabolism and excretion (ADME) in the human body.<sup>36</sup> Lipinski's rule of five is a refinement of drug-likeness and is used to predict whether a chemical compound will have pharmacological or biological activity as an orally active drug in humans. This rule was formulated based on the observation that most medication drugs are relatively small and lipophilic molecules.<sup>33</sup> The results of the analysis are shown in Table 4 and indicate that the compounds are in agreement with the values determined by Lipinski, except **4a-c** derivatives, which showed the number of hydrogen bond donors (nHBD) > 5, in violation of the Lipinski rules. The calculated percent absorption (%ABS) of all compounds ranged from 51.90-76.91%, indicating good cell membrane permeability. Another important factor is obtained by the volume analysis and TPSA by the compounds showed lower than 140 Å<sup>2</sup> indicating that these derivatives have good absorption in the intestine, except the compound **4c** (TPSA = 165.51). The compounds **2a-c**, **3a-b** and **4a-c** exhibited good solubility (Log S = -3.87 to -4.98), except the compound **3c** which showed a value of Log S less than -5. Compounds with high solubility are easily metabolized and eliminated from the body, thus resulting in a lower probability of adverse effects and bioaccumulation.

### Conclusions

Thus, our results showed for the first time the synthesis and antitumor and anti-MTB activity of compounds with an amino or guanidinium group-terminated side chain at C-3 of a 1-substituted-phenyl- $\beta$ -carboline nucleus. Compounds

**2a**, **2b** and **4a** were the most active against *M. Tuberculosis* H<sub>37</sub>Rv (ATCC27294). Compound **2a** and **3b** demonstrated promising antiproliferative activity for all cancer cell lines. Eight compounds inhibited the cell growth of adriamycin drug-resistant ovarian (NCI/ADR), showed activity and high selectivity for the **3a**, **3c** and **4b**. Compound **2a** demonstrated promising antiproliferative and anti-MTB activity, in addition to follow as established Lipinski's rule of five, suggesting that this compound has no problems with oral bioavailability, and indicates good permeability a in the plasma membrane of the cell, which may represent a precursor to development of new molecules. Further studies are required to explore the mechanism of action of these compounds in detail.

### Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbc.org.br>, as PDF file.

### Acknowledgments

We are grateful to CNPq, FUNDECT and CAPES for providing financial support and UFGD.

### References

1. World Health Organization (WHO); *Global Tuberculosis Report*, 20<sup>th</sup> ed.; Geneva, 2015, p. 1.
2. Gandhi, N. R.; Nunn, P.; Dheda, K.; Schaaf, H. S.; Zignol, M.; Van Soolingen, D.; Jensen, P.; Bayona, J.; *The Lancet* **2010**, 375, 1830.
3. Ma, Z.; Lienhardt, C.; McIlleron, H.; Nunn, A. J.; Wang, X.; *The Lancet* **2010**, 375, 2100.

4. Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K.; *Nature* **2011**, *469*, 483.
5. Jagetia, G. C.; Venkatesh, P.; Baliga, M. S.; *Bio. Pharm. Bull.* **2005**, *28*, 58.
6. World Health Organization (WHO); *World Cancer Report*, 1<sup>st</sup> ed.; Geneva, 2014, p. 1.
7. Ang, K. K.; Holmes, M. J.; Higa, T.; Hamann, M. T.; Kara, U. A.; *Antimicrob. Agents Chemother.* **2000**, *44*, 1645.
8. Cao, R.; Chen, Q.; Hou, X.; Chen, H.; Guan, H.; Ma, Y.; Peng, W.; Xu, A.; *Bioorg. Med. Chem.* **2004**, *12*, 4613.
9. Cao, R.; Chen, H.; Peng, W.; Ma, Y.; Hou, X.; Guan, H.; Liu, X.; Xu, A.; *Eur. J. Med. Chem.* **2005**, *40*, 991.
10. Cao, R.; Peng, W.; Chen, H.; Hou, X.; Guan, H.; Chen, Q.; Ma, Y.; Xu, A.; *Eur. J. Med. Chem.* **2005**, *40*, 249.
11. Wu, Q.; Cao, R.; Feng, M.; Guan, X.; Ma, C.; Liu, J.; Song, H.; Peng, W.; *Eur. J. Med. Chem.* **2009**, *44*, 533.
12. Wu, J.; Li, C.; Zhao, M.; Wang, W.; Wang, Y.; Peng, S.; *Bioorg. Med. Chem.* **2010**, *18*, 6220.
13. Begum, S.; Hassan, S. I.; Siddiqui, B. S.; *Nat. Prod. Res.* **2004**, *18*, 341.
14. Begum, S.; Ali, S. N.; Siddiqui, B. S.; US 8.420.660, **2013**.
15. Stefanello, T. F.; Panice, M. R.; Ueda-Nakamura, T.; Sarragiotto, M. H.; Auzely-Velty, R.; Nakamura, C. V.; *Antimicrob. Agents Chemother.* **2014**, *58*, 7112.
16. Savariz, F. C.; Foglio, M. A.; Ruiz, A. L.; Costa, W. F.; Silva, M.; Santos, J. C.; Figueiredo, I. M.; Meyer, E.; Carvalho, J. E.; Sarragiotto, M. H.; *Bioorg. Med. Chem.* **2014**, *22*, 6867.
17. Savariz, F. C.; Foglio, M. A.; Carvalho, J. E.; Ruiz, A. L.; Duarte, M. C.; Rosa, M. F.; Meyer, E.; Sarragiotto, M. H.; *Molecules* **2012**, *17*, 6100.
18. Barbosa, V. A.; Formagio, A. S.; Savariz, F. C.; Foglio, M. A.; Spindola, H. M.; Carvalho, J. E.; Meyer, E.; Sarragiotto, M. H.; *Bioorg. Med. Chem.* **2011**, *19*, 6400.
19. Savariz, F. C.; Formagio, A. S. N.; Barbosa, V. A.; Foglio, M. A.; Carvalho, J. E.; Duarte, M. C. T.; Dias Filho, B. P.; Sarragiotto, M. H.; *J. Braz. Chem. Soc.* **2010**, *21*, 288.
20. Tonin, L. T.; Panice, M. R.; Nakamura, C. V.; Rocha, K. J.; Santos, A. O.; Ueda-Nakamura, T.; Costa, W. F.; Sarragiotto, M. H.; *Biomed. Pharmacother.* **2010**, *64*, 386.
21. Formagio, A. S. N.; Santos, P. R.; Zanolli, K.; Ueda-Nakamura, T.; Tonin, L. T. D.; Nakamura, C. V.; Sarragiotto, M. H.; *Eur. J. Med. Chem.* **2009**, *44*, 4695.
22. Formagio, A. S. N.; Tonin, L. T. D.; Foglio, M. A.; Madjarof, C.; Carvalho, J. E.; Costa, W. F.; Cardoso, F. P.; Sarragiotto, M. H.; *Bioorg. Med. Chem.* **2008**, *16*, 9660.
23. Pedroso, R. B.; Tonin, L. T. D.; Ueda-Nakamura, T.; Dias Filho, B. P.; Sarragiotto, M. H.; Nakamura, C. V.; *Ann. Trop. Med. Parasitol.* **2011**, *105*, 549.
24. Yu, X.; Lin, W.; Li, J.; Yang, M.; *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3127.
25. Yu, X.; Lin, W.; Pang, R.; Yang, M.; *Eur. J. Med. Chem.* **2005**, *40*, 831.
26. Ishida, J.; Wang, H.; Bastow, K. F.; Hu, C.; Lee, K.; *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3319.
27. Palomino, J. C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F.; *Antimicrob. Agents Chemother.* **2002**, *46*, 2720.
28. Pavan, F. R.; Poelhsitz, G. V.; Barbosa, M. I.; Leite, S. R.; Batista, A. A.; Ellena, J.; Sato, L. S.; Franzblau, S. G.; Moreno, V.; Gambino, D.; Leite, C. Q.; *Eur. J. Med. Chem.* **2011**, *46*, 5099.
29. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; *J. Natl. Cancer Inst.* **1991**, *83*, 757.
30. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J.; *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
31. <http://www.molinspiration.com> accessed in January 2016.
32. <http://www.organic-chemistry.org/prog/peo> accessed in January 2016.
33. Singh, N.; Pandey, S. K.; Anand, N.; Dwivedi, R.; Singh, S.; Sinha, S. K.; Chaturvedi, V.; Jaiswal, N.; Srivastava, A. K.; Shah, P.; Siddiqui, M. I.; Tripathi, R. P.; *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4404.
34. Mourer, M.; Dibama, M. H.; Constant, P.; Daffé, M.; Regnouf-de-Vains, J. B.; *Bioorg. Med. Chem.* **2012**, *20*, 2035.
35. Brzozowski, Z.; Sączewski, F.; Sławiński, J.; *Eur. J. Med. Chem.* **2007**, *42*, 1218.
36. Vistoli, G.; Pedretti, A.; Testa, B.; *Drug Discovery Today* **2008**, *13*, 285.

Submitted: November 4, 2015

Published online: February 26, 2016

FAPESP has sponsored the publication of this article.