

# Synthesis and Antitubercular Activity of Novel Amino Acid Derivatives

Cristiane F. Da Costa<sup>1,2</sup>, Alessandra C. Pinheiro<sup>1</sup>, Mauro V. De Almeida<sup>2</sup>, Maria C. S. Lourenço<sup>3</sup> and Marcus V. N. De Souza<sup>1,\*</sup>

<sup>1</sup>Instituto de Tecnologia em Fármacos – Farmanguinhos, FioCruz – Fundação Oswaldo Cruz, R. Sizenando Nabuco, 100 Mangueiras, 21041-250 Rio de Janeiro, RJ, Brazil

<sup>2</sup>Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Cidade Universitária, 36036-900 Juiz de Fora, MG, Brazil

<sup>3</sup>Instituto de Pesquisas Clínicas Evandro Chagas, Departamento de Bacteriologia, FioCruz – Fundação Oswaldo Cruz, Av. Brasil, 4365 Mangueiras, Rio de Janeiro, Brazil

\*Corresponding author: Marcus V. N. De Souza, marcos\_souza@far.fiocruz.br

**In this work, 17 new *N*-acylhydrazone derivatives of amino acids have been evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv. The compounds **8b**, **8e**, **8f**, **9a–d**, and **10c** exhibited an important minimum inhibitory concentration activity between 12.5 and 50 µg/mL, which can be compared with that of the tuberculostatic drug *D*-cycloserine (20 µg/mL).**

**Key words:** amino acids, cell wall, hydrazones, tuberculosis

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Tuberculosis (TB) is a serious airborne disease caused by *Mycobacterium tuberculosis*, being considered since 1993 a global health emergency by the World Health Organization (WHO).<sup>a</sup> Control and prevention of TB are major challenge as one-third of the world's population is infected with *Mycobacterium* (1). Some antibiotics employed on tuberculosis treatment affect the metabolism of bacterial cell wall construction, which is a very important cellular component surrounding the cell membrane, providing additional support and protection (2). Basically, it is comprised of three covalently linked substructures: mycolic acids, peptidoglycan, and arabinogalactan, which represent over 60% of cell dry weight (3). The peptidoglycan is formed by extensive chains of polysaccharides and amino acid residues. The *D*-cycloserine, a second-line drug for tuberculosis treatment, binds to the terminal portion of *D*-ala-*D*-ala of a peptide

found in peptidoglycan precursors, interfering in the transpeptidation step (2,4).

In our continuous search of new potent and safe antitubercular agents, we decided to synthesize a new class of *N*-acylhydrazone derivatives of amino acids as attractive *D*-cycloserine analogs. *N*-acylhydrazones are also described with a wide range of pharmacological activities, such as antibacterial agents (5–11).

## Materials and Methods

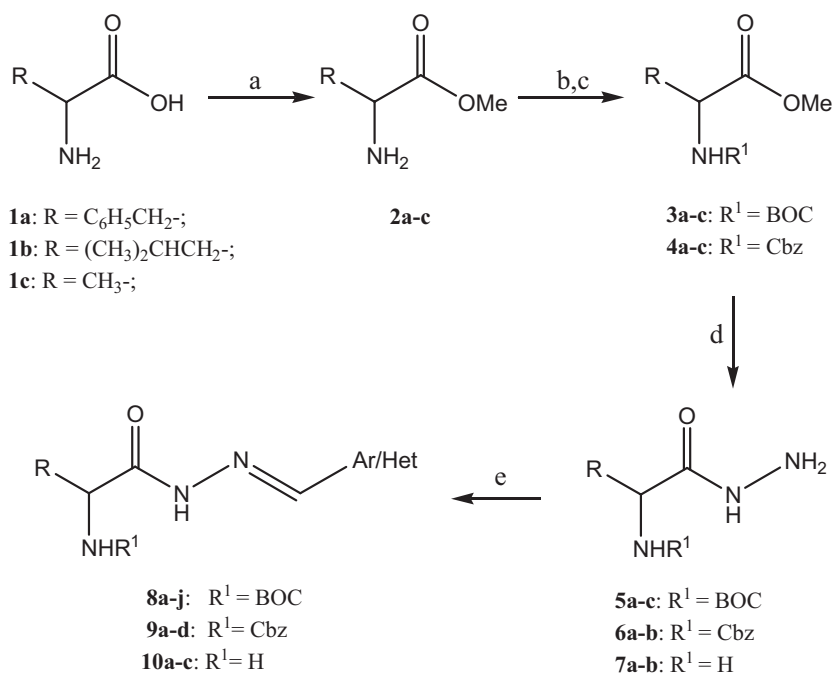
### General procedures

NMR spectra were obtained using a Bruker Avance spectrometer operating at 400 or 500 MHz (<sup>1</sup>H) and 100 or 125 MHz (<sup>13</sup>C), and a Bruker Avance DRX300 spectrometer operating at 300 MHz (<sup>1</sup>H) and 75 MHz (<sup>13</sup>C), in deuterated methanol, chloroform, or dimethylsulfoxide. Chemical shifts are reported in ppm relative to tetramethylsilane. Proton and carbon spectra were typically obtained at room temperature. Thin-layer chromatography was carried out on silica gel plates, using chloroform/methanol mixtures as eluents. For column chromatographic purification, column grade silica gel (0.063–0.200 mm mesh size) was employed. Mass spectra (ESI) were obtained on a ZQ electrospray spectrometer simple quadrupole. Melting points were determined using a Microquímica MQAPF-301 (Microquímica, Santa Catarina, Brazil) digital melting point apparatus.

### Chemistry

The synthetic route used for the preparation of the title compounds is outlined in Scheme 1. The amino acids *L*-phenylalanine **1a**, *L*-leucine **1b**, and *L*-alanine **1c** were employed as starting materials, and *t*-butoxycarbonyl (BOC) and benzyloxycarbonyl (Cbz) protecting groups were included in the core structures of **8a–j** and **9a–d** because of an intrinsic instability observed in non-protected derivatives.

Compounds **8a–j**, **9a–d**, and **10a–c** were prepared from the appropriate amino acids **1a–c**, by esterification, leading to **2a–c**, followed by *N*-protection to furnish **3a–c** or **4a–c**, using (BOC)<sub>2</sub>O or CbzCl, respectively. These compounds were converted into their acyl hydrazone derivatives **5a–c**, **6a–b**, and **7a–b** using 80% aqueous hydrazine hydrate. After washing with cold ethanol the pure products were obtained in 66–85% overall yield. Finally, after condensation reactions of these compounds with substituted aldehydes, the desired derivatives **8a–j**, **9a–d**, and **10a–c** were obtained in 50–84% yield.



**Scheme 1:** Reagents and conditions: (a) SOCl<sub>2</sub>, MeOH, 60 °C, 4 h; (b) (BOC)<sub>2</sub>O, Et<sub>3</sub>N, THF, rt; (c) CbzCl, THF, H<sub>2</sub>O, K<sub>2</sub>CO<sub>3</sub>, 0 °C, 3 h; (d) NH<sub>2</sub>-NH<sub>2</sub>, EtOH, rt, 24 h; (e) Ar/Het-CHO, EtOH, rt, 24 h.

## Experimental

### General procedures for the synthesis of methyl ester derivatives of L-amino acids (2a–c)

To a stirred solution of thionyl chloride (200 mmol) in methanol (100 mL) at 0 °C was added the appropriated L-amino acid (40 mmol). The reaction mixture was stirred for 24 h at room temperature, and the solvent was removed to give **2a–c** in quantitative yield.

**(2S)-1-methoxy-1-oxo-3-phenyl-2-propylammonium hydrochloride 2a:** Yield: 98%, MP: 157–158 °C. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 2.03 (2H, s, NH); 3.19 (1H, dd, *J*<sub>1</sub> = 6.2 Hz, *J*<sub>2</sub> = 14.3 Hz, CHPh); 3.26 (1H, dd, *J*<sub>1</sub> = 6.2 Hz, *J*<sub>2</sub> = 14.3 Hz, CHPh); 3.80 (3H, s, OCH<sub>3</sub>); 4.32 (1H, t, *J* = 6.8 Hz, CHNH<sub>2</sub>); 7.27 (2H, d, *J* = 7.1 Hz, Ph (H2 and H6)); 7.31 (1H, dd, *J*<sub>1</sub> = 4.9 Hz, *J*<sub>2</sub> = 9.6 Hz, H4); 7.37 (2H, t, *J* = 7.1 Hz, Ph (H3 and H5)) ppm NMR-<sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 37.5; 53.7; 55.4; 129.0; 130.2; 130.6; 133.4; 135.5; 170.5 ppm IR (ν, cm<sup>-1</sup>, KBr): 2983, 2841, 2702, 1745. MS/ESI (*m/z*) calcd: 179.0 (M+, free base), found: 179.9 (M+H).

**(2S)-4-methyl-1-methoxy-1-oxo-2-pentylammonium hydrochloride 2b:** Yield: 98%, MP: 134–136 °C. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 1.07 (6H, q, *J* = 7.0 Hz, (CH<sub>3</sub>)<sub>2</sub>); 1.30 (1H, m, CH(CH<sub>2</sub>)<sub>2</sub>); 3.85 (3H, s, OCH<sub>3</sub>); 3.94 (1H, d, *J* = 4.7 Hz, CHNH<sub>2</sub>) ppm NMR-<sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 22.5; 22.7; 25.7; 40.8; 52.6; 53.8; 171.5 ppm IR (ν, cm<sup>-1</sup>, KBr): 2937, 2870, 1737. MS/ESI (*m/z*) calcd: 144.9 (M+, free base), found: 145.9 (M+H).

**(2S)-1-methoxy-1-oxo-2-propylammonium hydrochloride 2c:** Yield: 96%, oil. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 1.54 (3H, d,

*J* = 7.2 Hz, CH<sub>3</sub>); 3.84 (3H, s, OCH<sub>3</sub>); 4.11 (1H, q, *J* = 7.2 Hz, CHNH<sub>2</sub>) ppm NMR-<sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 16.3; 49.9; 53.8; 171.6. IR (ν, cm<sup>-1</sup>, NaCl): 2743, 1726. MS/ESI (*m/z*) calcd: 103.1 (M+, free base), found: 104.2 (M+H).

### General procedures for the synthesis of tert-butoxycarbonylamino derivatives 3a–c

To a reaction mixture containing the appropriate methyl ester derivative **2a–c** (9 mmol) and triethylamine (10.8 mmol) in anhydrous THF (20 mL) at room temperature was added (BOC)<sub>2</sub>O (13.5 mmol). The reaction mixture was stirred for 24 h at room temperature, quenched with water (40 mL), and extracted with ethyl acetate. The combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate 7:3) affording **3a–c** in 65–75% yield.

**Methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanoate 3a:** Yield: 70%, oil. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 1.38 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 2.89 (1H, dd, *J*<sub>1</sub> = 6.2 Hz, *J*<sub>2</sub> = 13.5 Hz, CHPh); 3.09 (1H, dd, *J*<sub>1</sub> = 6.2 Hz, *J*<sub>2</sub> = 13.5 Hz, CHPh); 3.68 (3H, s, OCH<sub>3</sub>); 4.35 (1H, dd, *J*<sub>1</sub> = 5.5 Hz, *J*<sub>2</sub> = 9.0 Hz, CHNH); 5.07 (1H, d, *J* = 7.2 Hz, NH); 7.20 (3H, t, *J* = 7.5 Hz, H2, H4 and H6); 7.27 (2H, t, *J* = 7.5 Hz, H3 and H5) ppm NMR-<sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 29.3; 38.8; 52.7; 56.7; 80.7; 127.3–131.0; 138.5; 157.9; 174.3 ppm IR (ν, cm<sup>-1</sup>, NaCl): 3380, 3028, 2978, 1712, 1496. MS/ESI (*m/z*) calcd: 279.3 (M+), found: 301.9 (M+Na).

**Methyl (2S)-2-[(tert-butoxycarbonyl)amino]-4-methylpentanoate 3b:** Yield: 75%, oil. NMR-<sup>1</sup>H (400 MHz, CDCl<sub>3</sub>) δ:

1.37 (6H, q,  $J_1 = 4.5$  Hz,  $J_2 = 8.0$  Hz,  $(\text{CH}_3)_2$ ); 1.44 (9H, s,  $(\text{CH}_3)_3$ ); 1.50 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ); 1.59 (2H, m,  $\text{CH}_2$ ); 3.79 (3H, s,  $\text{OCH}_3$ ); 4.32 (1H, m,  $\text{CHNH}$ ); 4.89 (1H, d,  $J = 7.2$  Hz, NH) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 22.0; 22.1; 25.0; 28.5; 42.1; 52.2; 52.3; 80.0; 155.6; 174.2 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , NaCl): 3334, 2956, 1728, 1527. MS/ESI ( $m/z$ ) calcd: 245.1 (M+), found: 268.1 (M+Na).

**Methyl (2S)-2-[(tert-butoxycarbonyl)amino]propanoate 3c:** Yield: 65%, oil. NMR- $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 1.37 (3H, t,  $J = 8.0$  Hz,  $\text{CH}_3$ ); 1.43 (9H, s,  $(\text{CH}_3)_3$ ); 3.79 (3H, s,  $\text{OCH}_3$ ); 4.29 (1H, m,  $\text{CHNH}$ ) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 19.2; 28.0; 49.3; 52.5; 80.1; 155.3; 174.1 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , NaCl): 3325, 2943, 1731. MS/ESI ( $m/z$ ) calcd: 203.1 (M+), found: 204.2 (M+H).

### General procedures for the synthesis of benzyloxycarbonylamino derivatives 4a–b

To a reaction mixture containing the appropriate methyl ester derivative **2a–b** (9 mmol), water (50 mL), diethyl ether (40 mL), and sodium bicarbonate (50 mmol) at 0 °C was added dropwise benzyl chloroformate (14 mmol). After 2 h at 0 °C and 1 h at room temperature, the reaction mixture was quenched with pyridine (8 mL), and water was added to solubilize all salts (40 mL). The organic layer was washed with HCl (2.5 N), dried ( $\text{MgSO}_4$ ), filtered, and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate 8:2) affording **4a–b** in 63–75% yield.

**Methyl (2S)-2-[(benzyloxy)carbonylamino]-3-phenylpropanoate 4a:** Yield: 75%, oil. NMR- $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 2.92 (1H, dd,  $J_1 = 9.2$  Hz,  $J_2 = 13.6$  Hz,  $\text{CHPh}$ ); 3.13 (1H, dd,  $J_1 = 9.2$  Hz,  $J_2 = 13.6$  Hz,  $\text{CHPh}$ ); 3.68 (3H, s,  $\text{OCH}_3$ ); 4.44 (1H, dd,  $J_1 = 5.6$  Hz,  $J_2 = 8.8$  Hz,  $\text{CHNH}$ ); 5.06 (2H, s,  $\text{CH}_2\text{O}$ ); 7.18–7.33 (10H, m, Ph) ppm NMR- $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 38.7; 52.8; 57.1; 67.7; 128.0; 128.8; 129.1; 129.6; 130.4; 138.3; 138.4; 158.5; 174.1. IR ( $\nu$ ,  $\text{cm}^{-1}$ , NaCl): 3354, 3030, 2951, 1728, 1518. MS/ESI ( $m/z$ ) calcd: 313.3 (M+), found: 336.0 (M+Na).

**Methyl (2S)-2-[(benzyloxy)carbonylamino]-4-methylpentanoate 4b:** Yield: 63%, oil. NMR- $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.94 (6H, m,  $(\text{CH}_3)_2$ ); 1.53 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ); 1.67 (2H, m,  $\text{CH}_2$ ); 3.74 (3H, s,  $\text{OCH}_3$ ); 4.41 (1H, m,  $\text{CHNH}$ ); 5.12 (2H, s,  $\text{CH}_2\text{O}$ ); 5.17 (1H, d,  $J = 8.0$  Hz,  $\text{CHNH}$ ); 7.36 (5H, s, Ph) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 22.5; 25.4; 42.0; 51.9; 53.2; 67.2; 127.7–129.3; 156.1; 173.8 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , NaCl): 3361, 2958, 2872, 1712, 1516. MS/ESI ( $m/z$ ) calcd: 279.2 (M+), found: 302.2 (M+Na).

### General procedures for the synthesis of N-acyl hydrazine derivatives 5a–c, 6a–b and 7a–b

To a stirred solution of **3a–c** or **4a–c** (10 mmol) in ethanol (20 mL) at room temperature was added  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  (80%, 40 mmol). The reaction mixture was stirred for 24 h at room temperature and concentrated under reduced pressure. The residue was purified by chromatography column (dichloromethane/methanol 9.5:0.5), leading the pure derivative **5a–c**, **6a–b**, or **7a–b** in 59–85% yield.

**Tert-butyl (1S)-1-benzyl-2-hydrazino-2-oxoethylcarbamate 5a:** Yield: 83%, MP: 126.7–127.7 °C. NMR- $^1\text{H}$  (500 MHz,

$\text{CD}_3\text{OD}$ )  $\delta$ : 1.36 (9H, s,  $(\text{CH}_3)_3$ ); 2.83 (1H, dd,  $J_1 = 9.2$  Hz;  $J_2 = 13.5$  Hz;  $\text{CHPh}$ ); 3.04 (1H, dd,  $J_1 = 9.2$  Hz;  $J_2 = 13.5$  Hz,  $\text{CHPh}$ ); 4.25 (1H, t,  $J = 6.5$  Hz;  $\text{CHNH}$ ); 7.18–7.28 (5H, m, Ph) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 29.3; 39.6; 56.3; 80.8; 127.2; 128.5; 128.9; 129.8; 130.2; 131.1; 138.6; 157.6; 173.5 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3327, 3058, 3030, 1689, 1537. MS/ESI ( $m/z$ ) calcd: 279.2 (M+), found: 302.0 (M+Na).

**Tert-butyl (2S)-2-hydrazino-1-isobutyl-2-oxoethylcarbamate 5b:** Yield: 85%, MP: 73.4–75.0 °C. NMR- $^1\text{H}$  (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 1.79 (6H, m,  $(\text{CH}_3)_2$ ); 1.83 (9H, m,  $(\text{CH}_3)_3$ ); 2.18 (2H, m,  $\text{CH}_2$ ); 2.26 (2H, m,  $\text{NH}_2$ ); 2.81 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ); 4.31 (1H, m,  $\text{CHNH}$ ); 8.25 (1H, s,  $\text{OCONH}$ ); 9.70 (1H, s,  $\text{NHN}$ ) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 17.1; 24.9; 28.1; 30.7; 78.7; 153.2 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3354, 3273, 2958, 1681, 1631. MS/ESI ( $m/z$ ) calcd: 245.1 (M+), found: 268.1 (M+Na).

**Tert-butyl (2S)-2-hydrazino-1-methyl-2-oxoethylcarbamate 5c:** Yield: 70%, MP: 138.9–142.6 °C. NMR- $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 1.43 (9H, s,  $(\text{CH}_3)_3$ ); 2.10 (2H, s,  $\text{NH}_2$ ); 4.23 (1H, m,  $\text{CHNH}$ ); 5.37 (1H, d,  $J = 7.0$  Hz,  $\text{CHNH}$ ); 9.49 (1H, s,  $\text{NHN}$ ) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 18.3; 28.0; 47.9; 79.6; 155.6; 175.3 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3329, 3253, 2985, 1676, 1639. MS/ESI ( $m/z$ ) calcd: 203.1 (M+), found: 226.3 (M+Na).

**Benzyl (1S)-1-benzyl-2-hydrazino-2-oxoethylcarbamate 6a:** Yield: 66%, MP: 170.5–171.8 °C. NMR- $^1\text{H}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 2.87 (1H, dd,  $J_1 = 9.2$  Hz,  $J_2 = 13.5$  Hz,  $\text{CHPh}$ ); 3.07 (1H, dd,  $J_1 = 9.2$  Hz,  $J_2 = 13.5$  Hz,  $\text{CHPh}$ ); 4.32 (1H, dd,  $J_1 = 6.0$  Hz,  $J_2 = 8.5$  Hz,  $\text{CHNH}$ ); 4.99 (2H, s,  $\text{CH}_2\text{O}$ ); 5.02 (2H, m,  $\text{CHNH}$ ); 7.19–7.33 (10H, m, Ph) ppm NMR- $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 37.8, 54.9, 65.2, 126.2, 127.4, 127.6, 128.0, 128.2, 129.0, 129.2, 137.0, 138.0, 155.7, 170.7 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3300, 3058, 3030, 1689, 1537. MS/ESI ( $m/z$ ) calcd: 313.1 (M+), found: 336.1 (M+Na).

**Benzyl (2S)-2-hydrazino-1-isobutyl-2-oxoethylcarbamate 6b:** Yield: 71%, MP: 120.9–130.0 °C. NMR- $^1\text{H}$  (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 0.93 (6H, q,  $J_1 = 6.5$  Hz,  $J_2 = 11.5$  Hz,  $(\text{CH}_3)_2$ ); 1.50 (2H, m,  $\text{CH}_2$ ); 1.66 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ); 4.13 (1H, dd,  $J_1 = 6.0$  Hz,  $J_2 = 9.5$  Hz,  $\text{CHNH}$ ); 5.08 (2H, m,  $\text{CH}_2\text{O}$ ); 7.29–7.34 (5H, m, Ph) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 23.0; 26.4; 42.3; 54.4; 67.8; 128.4–130.3; 138.3; 158.5; 174.6 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3305, 3057, 2947, 1683, 1537. MS/ESI ( $m/z$ ) calcd: 279.2 (M+), found: 302.1 (M+Na).

**(2S)-2-amino-3-phenylpropanehydrazide 7a:** Yield: 80%, MP: 91.2–92.4 °C. NMR- $^1\text{H}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 2.80 (1H, dd,  $J_1 = 7.2$  Hz,  $J_2 = 13.2$  Hz,  $\text{CHPh}$ ); 2.97 (1H, m,  $J_1 = 13.2$  Hz,  $J_2 = 11.5$  Hz,  $\text{CHPh}$ ); 3.48 (1H, m,  $\text{CHNH}_2$ ); 7.24 (5H, m, Ph) ppm NMR- $^{13}\text{C}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 42.7; 56.8; 127.9; 129.7; 130.5; 138.9; 175.7 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3348, 3178, 3024, 1670, 1566. MS/ESI ( $m/z$ ) calcd: 179.1 (M+), found: 180.2 (M+H).

**(2S)-2-amino-4-methylpentanehydrazide 7b:** Yield: 60%, oil. NMR- $^1\text{H}$  (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 1.02 (6H, m,  $(\text{CH}_3)_2$ ); 1.76 (2H, m,  $\text{CH}_2$ ); 1.92 (1H, m,  $\text{CH}(\text{CH}_3)_2$ ); 2.10–2.11 (2H, m,  $\text{NHNH}_2$ ); 4.12 (1H, m,  $\text{CHNH}$ ) ppm NMR- $^{13}\text{C}$  (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 23.8; 24.7; 25.7; 42.0; 51.6; 172.4 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , NaCl): 3302, 2964, 1678. MS/ESI ( $m/z$ ) calcd: 131.1 (M+), found: 169.1 (M+Na).

**General procedures for the synthesis of *N*-acyl hydrazone derivatives 8a–j, 9a–d, and 10a–c**

To a stirred solution of **5a–c**, **6a–b**, or **7a–b** (0.5 mmol) in ethanol (10 mL) at room temperature was added the appropriate benzaldehyde (0.52 mmol). The reaction mixture was stirred for 24 h at room temperature and concentrated under reduced pressure. The residue was purified by chromatography column (dichloromethane/methanol 9:1), affording the derivatives **9a–c** or **10a–c** in 25–84% yield.

**Tert-butyl (1*S*)-1-benzyl-2-((2*E*/*Z*)-2-[(5-nitro-2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 8a:** Yield: 70%. MP: 174.7–175.6 °C. NMR-<sup>1</sup>H (400 MHz, DMSO-*d*<sub>6</sub>) δ: 1.32 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 2.79 (1H, m, CHPh); 2.92 (1H, m, CHPh); 4.21 and 4.97 (1H, m, CHNH, (*E*/*Z*)-isomer); 7.30 (5H, m, Ph); 7.20 and 7.53 (1H, m, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.22 and 7.56 (1H, m, H<sub>3</sub>' (*E*/*Z*)-isomer); 8.13 (1H, s, CHNH); 7.96 and 8.16 (1H, s, CH=N (*E*/*Z*)-isomer); 11.77 and 11.94 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (75 MHz, DMSO-*d*<sub>6</sub>) δ: 28.2, 36.2, 37.1, 54.1, 55.3, 78.1, 78.4, 114.7, 115.0, 115.5, 126.4, 128.2, 129.3, 129.5, 131.2, 134.9, 137.7, 138.7, 151.6, 151.7, 151.9, 155.5, 155.7, 169.2, 173.9 ppm IR (ν, cm<sup>-1</sup>, KBr): 3327, 3026, 2983, 1670, 1523, 1489, 1352. MS/ESI (*m/z*) calcd: 402.1 (M+), found: 425.1 (M+Na).

**Tert-butyl (1*S*)-1-benzyl-2-((2*E*/*Z*)-2-[(5-nitro-2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 8b:** Yield: 84%. MP: 180.9–182.8 °C. NMR-<sup>1</sup>H (300 MHz, DMSO-*d*<sub>6</sub>) δ: 1.32 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 2.79 (1H, m, CHPh); 2.92 (1H, m, CHPh); 4.21 and 4.97 (1H, m, CHNH (*E*/*Z*)-isomer); 7.20 and 7.53 (1H, m, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.30 (5H, m, Ph); 7.22 and 7.56 (1H, m, H<sub>3</sub>' (*E*/*Z*)-isomer); 8.12 and 8.13 (1H, s, CHNH); 8.14 and 8.46 (1H, s, CH=N, (*E*/*Z*)-isomer); 11.77 and 11.94 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (75 MHz, DMSO-*d*<sub>6</sub>) δ: 28.1; 36.4; 52.7; 55.2; 78.1; 126.4; 128.1; 129.2; 137.7; 138.1; 140.5; 146.6; 150.5; 155.4; 169.0; 173.5 ppm IR (ν, cm<sup>-1</sup>, KBr): 3336, 2983, 2889, 1676, 1527, 1330. MS/ESI (*m/z*) calcd: 418.1 (M+), found: 457.1 (M+K).

**Tert-butyl (1*S*)-1-isobutyl-2-((2*E*/*Z*)-2-[(5-nitro-2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 8c:** Yield: 54%. MP: 112.4–113.5 °C. NMR-<sup>1</sup>H (500 MHz, CDCl<sub>3</sub>) δ: 0.95 and 1.08 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 1.46 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 1.54 (2H, m, CH<sub>2</sub>); 1.72 and 1.84 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 4.24 and 5.16 (1H, m, CHNH (*E*/*Z*)-isomer); 6.89 (d, *J* = 3.8 Hz) and 7.33 (d, *J* = 3.5 Hz) (1H, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.09 (d, *J* = 3.5 Hz) and 7.36 (d, *J* = 3.5 Hz) (1H, H<sub>3</sub>' (*E*/*Z*)-isomer); 7.83 (1H, m, CHNH); 8.31 and 8.39 (1H, s, CH=N (*E*/*Z*)-isomer); 10.24 and 10.70 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (125 MHz, CDCl<sub>3</sub>) δ: 21.0; 22.8; 24.2; 25.6; 28.1; 49.2; 52.0; 77.8; 129.2; 129.4; 130.4; 130.5; 136.5; 140.3; 146.4; 146.6; 150.3; 150.7; 155.4; 155.5; 169.8; 171.6; 174.5 ppm IR (ν, cm<sup>-1</sup>, KBr): 3215, 2958, 2872, 1693, 1516, 1352. MS/ESI (*m/z*) calcd: 368.2.1 (M+), found: 367.2 (M-H).

**Tert-butyl (1*S*)-1-isobutyl-2-((2*E*/*Z*)-2-[(5-nitro-2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 8d:** Yield: 71%. MP: 103.0–105.4 °C. NMR-<sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) δ: 0.88 and 0.99 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 1.38 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 1.51 (2H, m, CH<sub>2</sub>); 1.63 and 1.73 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 4.01 and 4.84 (1H, m, CHNH (*E*/*Z*)-isomer); 7.01 (m) and 7.51 (d,

*J* = 4.4 Hz) (1H, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.14 (d, *J* = 6.8 Hz) and 7.54 (d, *J* = 4.4 Hz) (1H, H<sub>3</sub>' (*E*/*Z*)-isomer); 8.11 (1H, m, CHNH); 8.17 and 8.51 (1H, s, CH=N (*E*/*Z*)-isomer); 11.66 and 11.89 (H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (125 MHz, DMSO-*d*<sub>6</sub>) δ: 25.6; 28.1; 41.9; 50.2; 80.3; 127.6; 127.9; 137.4; 145.9; 145.4; 152.3; 156.0; 156.5; 170.3 ppm IR (ν, cm<sup>-1</sup>, KBr): 3350, 3134, 2958, 2872, 1687, 1516, 1334. MS/ESI (*m/z*) calcd: 368.2.1 (M+), found: 367.2 (M-H).

**Tert-butyl (1*S*)-1-isobutyl-2-((2*E*/*Z*)-2-[(2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 8e:** Yield: 60%. MP: 140.1–141.5 °C. NMR-<sup>1</sup>H (500 MHz, CDCl<sub>3</sub>) δ: 0.94 and 1.07 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 1.44 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 1.60 and 1.71 (2H, m, CH<sub>2</sub>); 1.80 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub>); 4.25 and 5.43 (1H, m, CHNH (*E*/*Z*)-isomer); 5.22 (1H, m, H<sub>3</sub>'); 6.43 (d, *J* = 3.2 Hz) and 6.72 (d, *J* = 3.6 Hz) (1H, H<sub>4</sub>' (*E*/*Z*)-isomer); 6.47 (m) and 6.76 (d, *J* = 4.0 Hz) (1H, H<sub>2</sub>' (*E*/*Z*)-isomer); 7.46 and 7.48 (1H, s, CHNH (*E*/*Z*)-isomer); 7.78 and 8.13 (1H, s, CH=N (*E*/*Z*)-isomer); 9.95 and 10.53 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 21.6; 23.4; 24.7; 28.4; 41.0; 42.1; 50.0; 52.2; 79.5; 138.7; 144.4; 144.6; 149.3; 149.5; 155.6; 156.3; 169.6; 175.8 ppm IR (ν, cm<sup>-1</sup>, KBr): 3331, 3064, 2966, 2899, 1666. MS/ESI (*m/z*) calcd: 323.2 (M+), found: 322.1 (M-H).

**Tert-butyl (1*S*)-1-isobutyl-2-((2*E*/*Z*)-2-[(2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 8f:** Yield: 69%. MP: 169.5–173.1 °C. NMR-<sup>1</sup>H (400 MHz, DMSO-*d*<sub>6</sub>) δ: 0.88 and 0.98 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 1.38 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 1.46 (2H, m, CH<sub>2</sub>); 1.62 and 1.72 (2H, m, CH(CH<sub>3</sub>)<sub>2</sub> (*E*/*Z*)-isomer); 4.00 and 4.83 (1H, m, CHNH (*E*/*Z*)-isomer); 7.11 (1H, m, H<sub>4</sub>'); 7.40 (d, *J* = 3.0 Hz) and 7.62 (m) (1H, H<sub>3</sub>' (*E*/*Z*)-isomer); 7.44 (d, *J* = 3.2 Hz) and 7.64 (m) (1H, H<sub>2</sub>' (*E*/*Z*)-isomer); 8.15 (1H, s, CHNH); 8.19 and 8.44 (1H, s, CH=N (*E*/*Z*)-isomer); 11.22 and 11.46 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (100 MHz, DMSO-*d*<sub>6</sub>) δ: 23.7; 24.5; 28.2; 49.4; 51.9; 77.8; 129.0; 129.4; 130.8; 138.0; 138.2; 139.0; 139.5; 142.0; 155.4; 155.6; 169.2; 174.1 ppm IR (ν, cm<sup>-1</sup>, KBr): 3367, 2958, 2870, 1689. MS/ESI (*m/z*) calcd: 339.2 (M+), found: 362.1 (M+Na).

**Tert-butyl (1*S*)-1-methyl-2-((2*E*/*Z*)-2-[(5-nitro-2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 8g:** Yield: 54%. MP: 157.5–159.0 °C. NMR-<sup>1</sup>H (500 MHz, CDCl<sub>3</sub>) δ: 1.44 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 1.46 (3H, s, CH<sub>3</sub>); 4.33 and 5.10 (1H, m, CHNH (*E*/*Z*)-isomer); 6.93 (d, *J* = 3.5 Hz) and 7.33 (m) (1H, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.08 (m) and 7.36 (d, *J* = 3.5 Hz) (1H, H<sub>3</sub>' (*E*/*Z*)-isomer); 7.89 and 8.28 (1H, s, CH=N (*E*/*Z*)-isomer); 10.56 and 10.80 (1H, s, NHN (*E*/*Z*)-isomer) ppm NMR-<sup>13</sup>C (125 MHz, CDCl<sub>3</sub>) δ: 16.9; 17.5; 28.2; 46.6; 48.1; 80.3; 113.3; 113.4; 114.2; 132.1; 133.0; 155.8; 156.4; 170.4; 176.2 ppm IR (ν, cm<sup>-1</sup>, KBr): 3327, 3064, 2981, 1670, 1525, 1352. MS/ESI (*m/z*) calcd: 326.1 (M+), found: 325.1 (M-H).

**Tert-butyl (1*S*)-1-methyl-2-((2*E*/*Z*)-2-[(5-nitro-2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 8h:** Yield: 62%. MP: 143.1–145.9 °C. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 1.23 (3H, m, CH<sub>3</sub>); 1.38 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 4.01 and 4.74 (1H, m, CHNH (*E*/*Z*)-isomer); 7.11 (d, *J* = 7.6 Hz) and 7.52 (d, *J* = 4.0 Hz) (1H, H<sub>4</sub>' (*E*/*Z*)-isomer); 7.20 (d, *J* = 7.6 Hz) and 7.54 (d, *J* = 4.0 Hz) (1H, d, H<sub>3</sub>' (*E*/*Z*)-isomer); 8.12 and 8.13 (1H, s, CHNH (*E*/*Z*)-isomer); 8.17 and 8.49 (1H,

s, CH=N (*E/Z*-isomer); 11.74 and 11.88 (1H, s, NHN, (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 16.7; 17.6; 28.2; 46.7; 49.1; 79.2; 129.0; 130.7; 136.5; 140.3; 146.7; 150.4; 150.8; 155.2; 170.1; 174.6 ppm IR (ν, cm<sup>-1</sup>, KBr): 3338, 3103, 2983, 2933, 1678, 1525, 1332. MS/ESI (*m/z*) calcd: 342.1 (M+), found: 343.1 (M+H).

**Tert-butyl (1*S*)-1-methyl-2-((2*E/Z*)-2-[(2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 8i:** Yield: 65%. MP: 158.0–159.6 °C. NMR-<sup>1</sup>H (500 MHz, CD<sub>3</sub>OD) δ: 1.24 (3H, d, *J* = 7.0 Hz, CH<sub>3</sub>); 1.44 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 4.14 and 4.96 (1H, m, CHNH, (*E/Z*-isomer); 7.08 (1H, m, H3'); 7.31 (d, *J* = 3.5 Hz) and 7.49 (d, *J* = 5.0 Hz) (1H, H4' (*E/Z*-isomer); 7.38 (d, *J* = 3.2 Hz) and 7.52 (d, *J* = 5.0 Hz) (1H, H2' (*E/Z*-isomer); 8.12 and 8.35 (1H, s, CH=N (*E/Z*-isomer); 10.88 and 11.30 (1H, s, NHN, (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (125 MHz, CD<sub>3</sub>OD) δ: 18.0; 28.8; 50.8; 80.8; 128.7; 129.5; 130.4; 132.4; 139.6; 140.6; 141.0; 145.4; 157.8; 172.5; 176.6 ppm IR (ν, cm<sup>-1</sup>, KBr): 3332, 3043, 2981, 2889, 1675. MS/ESI (*m/z*) calcd: 281.1 (M+), found: 280.2 (M-H).

**Tert-butyl (1*S*)-1-methyl-2-((2*E/Z*)-2-[(2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 8j:** Yield: 80%. MP: 160.1–161.9 °C. NMR-<sup>1</sup>H (400 MHz, CD<sub>3</sub>OD) δ: 1.36 (3H, d, *J* = 7.2 Hz, CH<sub>3</sub>); 1.44 (9H, s, (CH<sub>3</sub>)<sub>3</sub>); 4.15 and 5.00 (1H, m, CHNH (*E/Z*-isomer); 6.55 (1H, m, H3'); 6.78 (d, *J* = 3.0 Hz) and 7.62 (m) (1H, H4' (*E/Z*-isomer); 6.92 (d, *J* = 3.0 Hz) and 7.64 (m) (1H, H2' (*E/Z*-isomer); 7.82 and 8.06 (1H, s, CH=N (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (100 MHz, CD<sub>3</sub>OD) δ: 18.1; 18.5; 28.5; 48.0; 80.8; 113.3; 136.1; 139.9; 146.0; 146.5; 151.0; 151.3; 157.8; 172.6; 176.9 ppm IR (ν, cm<sup>-1</sup>, KBr): 3331, 3057, 2983, 2875, 1678. MS/ESI (*m/z*) calcd: 297.1 (M+), found: 320.2 (M+Na).

**Benzyl (1*S*)-1-benzyl-2-((2*E/Z*)-2-[(5-nitro-2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 9a:** Yield: 25%. MP: 179.5–181.4 °C. NMR-<sup>1</sup>H (400 MHz, DMSO-*d*<sub>6</sub>) δ: 2.72 (1H, m, CHPh); 3.04 (1H, m, CHPh); 4.33 and 4.90 (1H, m, CHNH (*E/Z*-isomer); 4.90 (2H, s, CH<sub>2</sub>O); 7.23–7.31 (10H, m, 2 Ph); 7.51 (1H, m, H4'); 7.87 (1H, m, H3'); 7.88 (1H, m, CHNH (*E/Z*-isomer); 7.98 and 8.18 (1H, s, CH=N (*E/Z*-isomer); 11.89 and 12.03 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (100 MHz, DMSO-*d*<sub>6</sub>) δ: 36.2, 37.4, 54.5, 55.6, 6.2, 114.5, 115.5, 126.4, 127.5, 128.1, 128.2, 129.2, 131.3, 135.0, 137.0, 138.5, 151.3, 151.6, 156.0, 168.7, 173.4 ppm IR (ν, cm<sup>-1</sup>, KBr): 3306, 3089, 3032, 1693, 1676, 1535, 1352. MS/ESI (*m/z*) calcd: 436.1 (M+), found: 459.1 (M+Na).

**Benzyl (1*S*)-1-benzyl-2-((2*E/Z*)-2-[(5-nitro-2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 9b:** Yield: 77%. MP: 147.6–149.9 °C. NMR-<sup>1</sup>H (400 MHz, DMSO-*d*<sub>6</sub>) δ: 2.83 (1H, m, CHPh); 2.99 (1H, m, CHPh); 4.30 and 5.05 (1H, m, CHNH (*E/Z*-isomer); 4.96 (2H, s, CH<sub>2</sub>O); 7.21–7.35 (10H, m, 2 Ph); 7.53 (d, *J* = 3.2 Hz) and 7.76 (d, *J* = 6.8 Hz) (1H, H4' (*E/Z*-isomer); 7.56 (d, *J* = 3.2 Hz) and 7.85 (d, *J* = 6.4 Hz) (1H, H3' (*E/Z*-isomer); 8.13 (1H, s, CHNH); 8.16 and 8.47 (1H, s, CH=N (*E/Z*-isomer); 11.86 and 12.01 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (100 MHz, DMSO-*d*<sub>6</sub>) δ: 36.5, 37.1, 53.2, 55.6, 65.3, 65.4, 126.5, 127.6, 127.8, 128.1, 128.3, 129.2, 129.4, 129.8, 130.5, 136.9, 137.0, 137.6, 138.0, 140.8, 146.3, 150.6, 150.9, 156.0, 168.8, 173.2. ppm IR (ν, cm<sup>-1</sup>, KBr): 3305, 3062, 2956, 1647, 1531, 1334. MS/ESI (*m/z*) calcd: 452.1 (M+), found: 475.1 (M+Na).

**Benzyl (1*S*)-1-isobutyl-2-((2*E/Z*)-2-[(5-nitro-2-furyl)methylene]hydrazine)-2-oxoethylcarbamate 9c:** Yield: 70%. MP: 89.3–92.6 °C. NMR-<sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) δ: 0.89 and 0.99 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E/Z*-isomer); 1.42 (2H, m, CH(CH<sub>3</sub>)<sub>2</sub>); 1.56 (1H, m, CH<sub>2</sub>); 4.11 and 4.93 (1H, m, H2, (*E/Z*-isomer); 5.03 (2H, m, CH<sub>2</sub>O); 7.14 (d, *J* = 4.0 Hz) and 7.54 (d, *J* = 8.0 Hz) (1H, H4' (*E/Z*-isomer); 7.35 (5H, m, Ph); 7.22 (d, *J* = 4.0 Hz) and 7.68 (d, *J* = 7.8 Hz) (1H, H3' (*E/Z*-isomer); 7.77 (1H, m, CHNH); 7.93 and 8.21 (1H, s, CH=N (*E/Z*-isomer); 11.72 and 11.96 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (500 MHz, DMSO-*d*<sub>6</sub>) δ: 20.8, 21.5, 22.8, 23.2, 24.2, 24.6, 50.0, 52.4, 114.4, 115.2, 127.7, 128.3, 131.1, 135.0, 137.6, 151.7, 156.2, 169.7, 174.6 ppm IR (ν, cm<sup>-1</sup>, KBr): 3209, 3032, 2956, 1691, 1680, 1517, 1352. MS/ESI (*m/z*) calcd: 402.1 (M+), found: 425.1 (M+Na).

**Benzyl (1*S*)-1-isobutyl-2-((2*E/Z*)-2-[(5-nitro-2-thienyl)methylene]hydrazine)-2-oxoethylcarbamate 9d:** Yield: 75%. MP: 90.8–94.0 °C. NMR-<sup>1</sup>H (400 MHz, Acetone-*d*<sub>6</sub>) δ: 0.89 and 0.99 (6H, m, (CH<sub>3</sub>)<sub>2</sub> (*E/Z*-isomer); 1.42 (2H, m, CH<sub>2</sub>); 1.42 and 1.54 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub> (*E/Z*-isomer); 4.12 and 4.92 (1H, m, CHNH (*E/Z*-isomer); 5.04 (2H, m, CH<sub>2</sub>O); 7.36 (5H, m, Ph); 7.52 (d, *J* = 4.4 Hz) and 7.58 (d, *J* = 8.0 Hz) (1H, H4' (*E/Z*-isomer); 7.55 (d, *J* = 4.4 Hz) and 7.70 (d, *J* = 7.8 Hz) (1H, H4' (*E/Z*-isomer); 8.12 (1H, m, CHNH); 8.18 and 8.51 (1H, s, CH=N (*E/Z*-isomer); 11.76 and 11.98 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (100 MHz, Acetone-*d*<sub>6</sub>) δ: 21.1; 23.8; 24.5; 29.1; 49.8; 52.4; 65.4; 127.8; 128.4; 129.5; 130.7; 136.9; 140.7; 146.4; 150.5; 150.9; 156.2; 170.0; 174.4 ppm IR (ν, cm<sup>-1</sup>, KBr): 3331, 3130, 2958, 2870, 1691, 1647, 1516, 1334. MS/ESI (*m/z*) calcd: 418.1 (M+), found: 441.1 (M+Na).

***N'*-[(2*E/Z*)-4-nitrobenzylidene]-(2*S*)-amino-3-phenylpropanehydrazide 10a:** Yield: 41%. NMR-<sup>1</sup>H (400 MHz, CF<sub>3</sub>COOD) δ: 2.89 (2H, s, NH<sub>2</sub>); 2.89 (1H, m, CHPh); 3.07 (1H, m, CHPh); 4.58 and 5.16 (1H, m, CHNH (*E/Z*-isomer); 7.82–7.92 and 8.11–8.55 (9H, m, Ph); 9.43 and 9.53 (1H, s, CH=N (*E/Z*-isomer); 10.77 (1H, s, NHN) ppm NMR- <sup>13</sup>C (100 MHz, CF<sub>3</sub>COOD) δ: 40.2; 56.7; 112.7; 115.5; 121.2; 126.7; 127.0; 133.9; 135.0; 138.2; 142.1; 168.7; 170.6; 183.5 ppm IR (ν, cm<sup>-1</sup>, KBr): 3530, 1597, 1525, 1346. MS/ESI (*m/z*) calcd: 312.1 (M+), found: 335.1 (M+Na).

***N'*-[(2*E/Z*)-4-nitrobenzylidene]-(2*S*)-amino-4-methylpentanehydrazide 10b:** Yield: 39%. MP: 133.5–135.2 °C. NMR-<sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) δ: 0.98 (6H, m, (CH<sub>3</sub>)<sub>2</sub>); 1.63 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub>); 1.81 (2H, m, CH<sub>2</sub>); 2.80 (2H, s, NH<sub>2</sub>); 3.51 and 4.21 (1H, m, CHNH<sub>2</sub> (*E/Z*-isomer), 7.17 (1H, d, *J* = 8.5 Hz, H2 and H6); 7.98 (2H, d, *J* = 8.5 Hz, H3 and H5); 7.54 and 7.82 (1H, s, CH=N (*E/Z*-isomer); 11.75 and 11.82 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (125 MHz, DMSO-*d*<sub>6</sub>) δ: 17.0; 19.5; 31.9; 59.1; 77.7; 123.4; 124.7; 127.2; 128.5; 141.1; 148.7; 174.0 ppm IR (ν, cm<sup>-1</sup>, KBr): 3622, 3342, 2954, 2870, 1695, 1517, 1336. MS/ESI (*m/z*) calcd: 278.1 (M+), found: 277.3 (M-H).

***N'*-[(2*E/Z*)-[(5-nitro-2-furyl)methylene]-(2*S*)-amino-3-methylbutanehydrazide 10c:** Yield: 44%. MP: 125.2–127.4 °C. NMR-<sup>1</sup>H (500 MHz, DMSO-*d*<sub>6</sub>) δ: 0.96 (6H, t, *J* = 7.0 Hz; (CH<sub>3</sub>)<sub>2</sub>); 1.65 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub>); 2.09 (2H, m, CH<sub>2</sub>); 2.55 (2H, s, NH<sub>2</sub>); 3.01 and 3.86 (1H, m, CHNH<sub>2</sub> (*E/Z*-isomer); 6.20 and 6.70 (7.17 (1H, m, H4' (*E/Z*-isomer); 6.31 and 6.97 (1H, m, H3' (*E/Z*-isomer); 7.50 and 7.58 (1H, s, CH=N (*E/Z*-isomer); 9.02 and 9.22 (1H, s, NHN (*E/Z*-isomer) ppm NMR- <sup>13</sup>C (125 MHz, DMSO-*d*<sub>6</sub>) δ: 26.3, 41.9, 70.2, 113.9, 114.1,

115.4, 116.8, 138.0, 139.4, 140.7, 152.7, 153.1, 156.1, 156.8, 173.6 ppm IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3134, 2958, 2872, 1712, 1529, 1352. MS/ESI ( $m/z$ ) calcd: 268, 1.1 (M+), found: 267.2 (M-H).

### Biologic evaluation

The antimycobacterial activities of the compounds **5a–c**, **6a–b**, **7a–b**, **8a–j**, **9a–d**, and **10a–c** have been assessed against *Mycobacterium tuberculosis* ATCC 27294 using the microplate Alamar Blue assay (12). This methodology is nontoxic, uses a thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric methods (13,14). The method is described as follows: 200 mL of sterile deionized water was added to all outer-perimeter wells of 96-sterile-well plates (falcon, 3072; Becton Dickinson, Lincoln Park, NJ, USA) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 mL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA), and successive dilution of the compounds **5a–c**, **6a–b**, **7a–b**, **8a–j**, **9a–d** and **10a–c** was made directly on the plate. The final drug concentrations tested were 0.01–20.0  $\mu\text{g/mL}$ . Plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. 25 mL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake, OH, USA) reagent and 10% Tween-80 was then added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The minimal inhibition concentration (MIC) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

## Results and Discussion

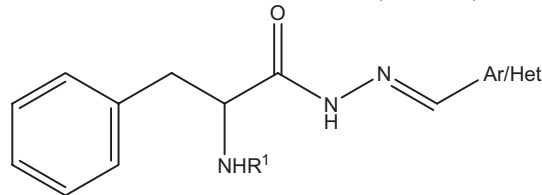
The *in vitro* antitubercular activity of the synthesized compounds is shown in Tables 1–4. The *N*-acyl hydrazine synthesized compound **7a**, an L-phenylalanine derivative, was the only intermediate product active against *M. tuberculosis*, with a MIC of 100  $\mu\text{g/mL}$  (Table 1). The compounds **5a** and **6a** were used as starting materials for the synthesis of **8a–b** and **9a–b**, respectively. No reaction

**Table 1:** The *in vitro* activity of *N*-acyl hydrazines amino acid derivatives **5a–c**, **6a–b**, and **7a–b** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to D-cycloserine)

Compound	R	R <sup>1</sup>	logP <sup>a</sup>	MIC ( $\mu\text{g/mL}$ )
<b>5a</b>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -	BOC	2.380	Resistant
<b>5b</b>	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> CHCH <sub>2</sub> -	BOC	2.229	Resistant
<b>5c</b>	CH <sub>3</sub> -	BOC	0.919	Resistant
<b>6a</b>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -	Cbz	2.790	Resistant
<b>6b</b>	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> CHCH <sub>2</sub> -	Cbz	2.638	Resistant
<b>7a</b>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -	H	-1.363	100
<b>7b</b>	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> CHCH <sub>2</sub> -	H	-1.514	Resistant
<b>Cycloserine</b>	–	–	–	20

<sup>a</sup>Calculated by <http://www.molinspiration.com/cgi-bin/properties>.

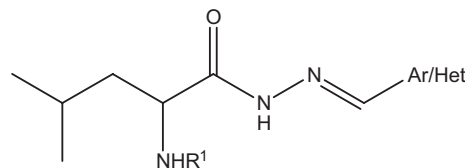
**Table 2:** The *in vitro* activity of *N*-acyl hydrazones L-phenylalanine derivatives **8a–8b**, **9a–b** and **10a** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to cycloserine)



Compound	R <sup>1</sup>	Ar/Het	logP <sup>a</sup>	MIC ( $\mu\text{g/mL}$ )
<b>8a</b>	BOC	5-nitro-2-furyl	5.137	100
<b>8b</b>	BOC	5-nitro-2-thienyl	5.779	50
<b>9a</b>	Cbz	5-nitro-2-thienyl	5.546	12.5
<b>9b</b>	Cbz	5-nitro-2-thienyl	6.188	50
<b>10a</b>	H	<i>p</i> -nitrobenzyl	2.013	Resistant
<b>Cycloserine</b>	–	–	–	20

<sup>a</sup>Calculated by <http://www.molinspiration.com/cgi-bin/properties>.

**Table 3:** The *in vitro* activity of *N*-acyl hydrazones L-leucine derivatives compounds **8c–f**, **9c–d** and **10b–c** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to cycloserine)

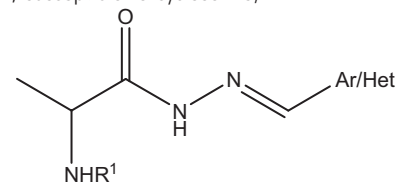


Compound	R <sup>1</sup>	Ar/Het	logP <sup>a</sup>	MIC ( $\mu\text{g/mL}$ )
<b>8c</b>	BOC	2-furyl	4.903	Resistant
<b>8d</b>	BOC	2-thienyl	5.545	Resistant
<b>8e</b>	BOC	5-nitro-2-furyl	4.986	50
<b>8f</b>	BOC	5-nitro-2-thienyl	5.627	50
<b>9c</b>	Cbz	5-nitro-2-furyl	5.395	25
<b>9d</b>	Cbz	5-nitro-2-thienyl	6.037	25
<b>10b</b>	H	<i>p</i> -nitrobenzyl	1.862	Resistant
<b>10c</b>	H	5-nitro-2-furyl	1.242	25
<b>Cycloserine</b>	–	–	–	20

<sup>a</sup>Calculated by <http://www.molinspiration.com/cgi-bin/properties>.

was observed between the unprotected hydrazine **7a** and heteroaromatic aldehydes. However, the coupling reaction between **7a** and *p*-nitrobenzaldehyde was successfully achieved, being the desired compound **10a** inactive against *M. tuberculosis*. Among the **8a** and **8b** *N*-BOC-protected compounds, the derivative **8b**, synthesized from the 5-nitrothiophenecarboxaldehyde, showed a moderate activity, with a MIC of 50  $\mu\text{g/mL}$ . The 5-nitrofuran derivative **9a**, with a MIC of 12.5  $\mu\text{g/mL}$ , exhibited the highest activity among all the compounds synthesized in this study, being more potent when compared with that of the tuberculostatic drug D-cycloserine (20  $\mu\text{g/mL}$ ). These data highlight the importance of 5-nitrofuran groups for the tuberculostatic activity observed (Table 2).

The use of the L-leucine as starting material confirms the importance of the heteroaromatic nuclei containing 5-NO<sub>2</sub> substituents for the biologic activity of the synthesized compounds. Concerning

**Table 4:** The *in vitro* activity of the *N*-acyl hydrazones L-alanine derivatives **8g–8j** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to cycloserine)


Compound	R <sup>1</sup>	Ar/Het	logP <sup>a</sup>	MIC (µg/mL)
<b>8g</b>	BOC	2-furyl	3.593	100
<b>8h</b>	BOC	2-thienyl	4.235	100
<b>8i</b>	BOC	5-nitro-2-furyl	3.676	Resistant
<b>8j</b>	BOC	5-nitro-2-thienyl	4.318	Resistant
<b>Cycloserine</b>	–	–	–	20

<sup>a</sup>Calculated by <http://www.molinspiration.com/cgi-bin/properties>.

compounds containing BOC in their structures, **8e** and **8f** showed activity at concentrations of 50 µg/mL. However, without nitro groups, the compounds **8c** and **8d** proved to be inactive. Then, the nitrated aldehydes were used to obtain the *N*-Cbz-protected analogs **9c** and **9d**, and the unprotected hydrazone **10c**. The three compounds displayed activities of 25 µg/mL. The analogous compound containing the 5-nitro-2-thienyl subunit in their structures was not successfully synthesized, and the *p*-nitrobenzyl derivative **10b** was not active against *M. tuberculosis*, confirming the importance of the heteroaromatic nuclei to the activities observed.

Other amino acids were also used as starting materials, such as L-alanine. However, no relevant activities were detected using the *N*-BOC-protected hydrazones **8g**, **8h**, **8i**, and **8j**, and because of these results, the protected analogs with Cbz and unprotected analogs were not synthesized (Table 4).

## Conclusion

In summary, this work describes the synthesis and biologic evaluation of 17 *N*-acylhydrazone derivatives of amino acids from different amino acids, such as L-phenylalanine, L-leucine, and L-alanine. The protection of these amino acids with Cbz and BOC was necessary for the stability of the derivatives synthesized. The compounds were evaluated against *M. tuberculosis* furnishing promising results, displaying a MIC between 12.5 and 50 µg/mL. Among them, the most promising compound was **9a**, being more potent when compared with that of the tuberculostatic drug D-cycloserine (20 µg/mL).

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## Note

<sup>a</sup>Available at: <http://www.who.int/tb/en/> (accessed 1 February 2011).