Chem Biol Drug Des 2012; **79:** 216–222

Research Letter

doi: 10.1111/j.1747-0285.2011.01269.x

Synthesis and Antitubercular Activity of Novel Amino Acid Derivatives

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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

Received 12 February 2011, revised 16 September 2011 and accepted for publication 29 October 2011.

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).[a] 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

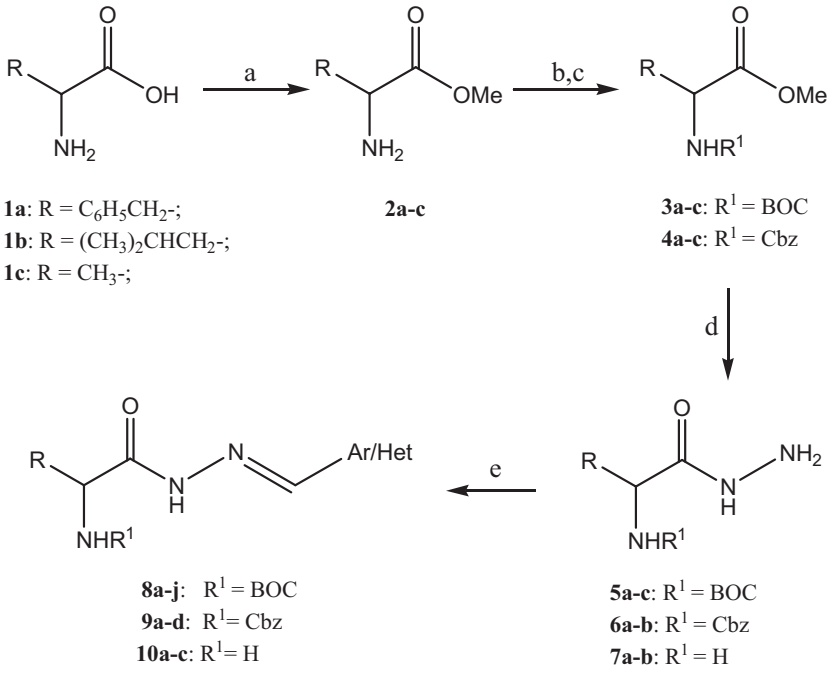
General procedures

NMR spectra were obtained using a Bruker Avance spectrometer operating at 400 or 500 MHz (^{1}H) and 100 or 125 MHz (^{13}C), and a Bruker Avance DRX300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}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 Microquimica MQAPF-301 (Microquimica, 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 $(BOC)_2O$ or CbzCl, respectively. These compounds were converted into their acyl hydrazine 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.



1a: R = $C_6H_5CH_2$-;
1b: R = $(CH_3)_2CHCH_2$-;
1c: R = CH_3-;

2a-c

3a-c: R^1 = BOC
4a-c: R^1 = Cbz

5a-c: R^1 = BOC
6a-b: R^1 = Cbz
7a-b: R^1 = H

8a-j: R^1 = BOC
9a-d: R^1 = Cbz
10a-c: R^1 = H

Scheme 1: Reagents and conditions: (a) $SOCl_2$, MeOH, 60 °C, 4 h; (b) $(BOC)_2O$, Et_3N, THF, rt; (c) CbzCl, THF, H_2O, K_2CO_3, 0 °C, 3 h; (d) NH_2-NH_2, 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.

(2*S*)-1-metoxy-1-oxo-3-phenyl-2-propylamonium hydrochloride 2a: Yield: 98%, MP: 157–158 °C. NMR-^{1}H (500 MHz, CD_3OD) δ: 2.03 (2H, s, NH); 3.19 (1H, dd, J_1 = 6.2 Hz, J_2 = 14.3 Hz, CHPh); 3.26 (1H, dd, J_1 = 6.2 Hz, J_2 = 14.3 Hz, CHPh); 3.80 (3H, s, OCH_3); 4.32 (1H, t, J = 6.8 Hz, $CHNH_2$); 7.27 (2H, d, J = 7.1 Hz, Ph (H2 and H6); 7.31 (1H, dd, J_1 = 4.9 Hz, J_2 = 9.6 Hz, H4); 7.37 (2H, t, J = 7.1 Hz, Ph (H3 and H5) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 37.5; 53.7; 55.4; 129.0; 130.2; 130.6; 133.4; 135.5; 170.5 ppm IR (ν, cm^{-1}, KBr): 2983, 2841, 2702, 1745. MS/ESI (*m/z*) calcd: 179.0 (M+, free base), found: 179.9 (M+H).

(2*S*)-4-methyl-1-metoxy-1-oxo-2-pentylamonium hydrochloride 2b: Yield: 98%, MP: 134–136 °C. NMR-^{1}H (500 MHz, CD_3OD) δ: 1.07 (6H, q, J = 7.0 Hz, $(CH_3)_2$); 1.30 (1H, m, $CH(CH_3)_2$); 3.85 (3H, s, OCH_3); 3.94 (1H, d, J = 4.7 Hz, $CHNH_2$) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 22.5; 22.7; 25.7; 40.8; 52.6; 53.8; 171.5 ppm IR (ν, cm^{-1}, KBr): 2937, 2870, 1737. MS/ESI (*m/z*) calcd: 144.9 (M+, free base), found: 145.9 (M+H).

(2*S*)-1-metoxy-1-oxo-2-propylamonium hydrochloride 2c: Yield: 96%, oil. NMR-^{1}H (500 MHz, CD_3OD) δ: 1.54 (3H, d, J = 7.2 Hz, CH_3); 3.84 (3H, s, OCH_3); 4.11 (1H, q, J = 7.2 Hz, $CHNH_2$) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 16.3; 49.9; 53.8; 171.6. IR (ν, cm^{-1}, 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 $(BOC)_2O$ (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 (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenyl-propanoate 3a: Yield: 70%, oil. NMR-^{1}H (500 MHz, CD_3OD) δ: 1.38 (9H, s, $(CH_3)_3$); 2.89 (1H, dd, J_1 = 6.2 Hz, J_2 = 13.5 Hz, CHPh); 3.09 (1H, dd, J_1 = 6.2 Hz, J_2 = 13.5 Hz, CHPh); 3.68 (3H, s, OCH_3); 4.35 (1H, dd, J_1 = 5.5 Hz, J_2 = 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- ^{13}C (125 MHz, CD_3OD) 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^{-1}, NaCl): 3380, 3028, 2978, 1712, 1496. MS/ESI (*m/z*) calcd: 279.3 (M+), found: 301.9 (M+Na).

Methyl (2*S*)-2-[(tert-butoxycarbonyl)amino]-4-methyl-pentanoate 3b: Yield: 75%, oil. NMR-^{1}H (400 MHz, $CDCl_3$) δ:

1.37 (6H, q, J_1 = 4.5 Hz, J_2 = 8.0 Hz, $(CH_3)_2$); 1.44 (9H, s, $(CH_3)_3$); 1.50 (1H, m, C<u>H</u>$(CH_3)_2$); 1.59 (2H, m, CH_2); 3.79 (3H, s, OCH_3); 4.32 (1H, m, C<u>H</u>NH); 4.89 (1H, d, J = 7.2 Hz, NH) ppm NMR- ^{13}C (125 MHz, $CDCl_3$) δ: 22.0; 22.1; 25.0; 28.5; 42.1; 52.2; 52.3; 80.0; 155.6; 174.2 ppm IR (ν, cm^{-1}, NaCl): 3334, 2956, 1728, 1527. MS/ESI (*m/z*) calcd: 245.1 (M+), found: 268.1 (M+Na).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-propanoate 3c: Yield: 65%, oil. NMR-^{1}H (500 MHz, $CDCl_3$) δ: 1.37 (3H, t, J = 8.0 Hz, CH_3); 1.43 (9H, s, $(CH_3)_3$); 3.79 (3H, s, OCH_3); 4.29 (1H, m, C<u>H</u>NH) ppm NMR- ^{13}C (125 MHz, $CDCl_3$) δ: 19.2; 28.0; 49.3; 525; 80.1; 155.3; 174.1 ppm IR (ν, 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 ($MgSO_4$), filtered, and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate 8:2) affording **4a**–**b** in 63–75% yield.

Methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-phenylpropanoate 4a: Yield: 75%, oil. MR-^{1}H (400 MHz, $CDCl_3$) δ: 2.92 (1H, dd, J_1 = 9.2 Hz, J_2 = 13.6 Hz C<u>H</u>Ph); 3.13 (1H, dd, J_1 = 9.2 Hz, J_2 = 13.6 Hz, C<u>H</u>Ph); 3.68 (3H, s, OCH_3); 4.44 (1H, dd, J_1 = 5.6 Hz, J_2 = 8.8 Hz, C<u>H</u>NH); 5.06 (2H, s, CH_2O); 7.18–7.33 (10H, m, Ph) ppm NMR- ^{13}C (100 MHz, $CDCl_3$) δ: 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 (ν, cm^{-1}, NaCl): 3354, 3030, 2951, 1728, 1518. MS/ESI (*m/z*) calcd: 313.3 (M+), found: 336.0 (M+Na).

Methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-4-methylpentanoate 4b: Yield: 63%, oil. NMR-^{1}H (500 MHz, $CDCl_3$) δ: 0.94 (6H, m, $(CH_3)_2$); 1.53 (1H, m, C<u>H</u>$(CH_3)_2$); 1.67 (2H, m, CH_2); 3.74 (3H, s, OCH_3); 4.41 (1H, m, C<u>H</u>NH); 5.12 (2H, s, CH_2O); 5.17 (1H, d, J = 8.0 Hz, CHN<u>H</u>); 7.36 (5H, s, Ph) ppm NMR- ^{13}C (125 MHz, $CDCl_3$) δ: 22.5; 25.4; 42.0; 51.9; 53.2; 67.2; 127.7–129.3; 156.1; 173.8 ppm IR (ν, 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 $N_2H_4{\cdot}H_2O$ (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 (1*S*)-1-benzyl-2-hydrazino-2-oxoethylcarbamate 5a**: Yield: 83%, MP: 126.7–127.7 °C. NMR-^{1}H (500 MHz, CD_3OD) δ: 1.36 (9H, s, $(CH_3)_3$); 2.83 (1H, dd, J_1 = 9.2 Hz; J_2 = 13.5 Hz; C<u>H</u>Ph); 3.04 (1H, dd, J_1 = 9.2 Hz; J_2 = 13.5 Hz, C<u>H</u>Ph); 4.25 (1H, t, J = 6.5 Hz; C<u>H</u>NH); 7.18–7.28 (5H, m, Ph) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 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 (ν, cm^{-1}, KBr): 3327, 3058, 3030, 1689, 1537. MS/ESI (*m/z*) calcd: 279.2 (M+), found: 302.0 (M+Na).

***Tert*-butyl (2*S*)-2-hydrazino-1-isobutyl-2-oxoethylcarbamate 5b**: Yield: 85%, MP: 73.4–75.0 °C. NMR-^{1}H (300 MHz, DMSO-d_6) δ: 1.79 (6H, m, $(CH_3)_2$); 1.83 (9H, m, $(CH_3)_3$); 2.18 (2H, m, CH_2); 2.26 (2H, m, NH_2); 2.81 (1H, m, C<u>H</u>$(CH_3)_2$); 4.31 (1H, m, C<u>H</u>NH); 8.25 (1H, s, OCONH); 9.70 (1H, s, NHN) ppm NMR- ^{13}C (125 MHz, DMSO-d_6) δ: 17.1; 24.9; 28.1; 30.7; 78.7; 153.2 ppm IR (ν, cm^{-1}, KBr): 3354, 3273, 2958, 1681, 1631. MS/ESI (*m/z*) calcd: 245.1 (M+), found: 268.1 (M+Na).

***Tert*-butyl (2*S*)-2-hydrazino-1-methyl-2-oxoethylcarbamate 5c**: Yield: 70%, MP: 138.9–142.6 °C. NMR-^{1}H (500 MHz, $CDCl_3$) δ: 1.43 (9H, s, $(CH_3)_3$); 2.10 (2H, s, NH_2); 4.23 (1H, m, C<u>H</u>NH); 5.37 (1H, d, J = 7.0 Hz, CHN<u>H</u>); 9.49 (1H, s, NHN) ppm NMR- ^{13}C (125 MHz, $CDCl_3$) δ: 18.3; 28.0; 47.9; 79.6; 155.6; 175.3 ppm IR (ν, cm^{-1}, KBr): 3329, 3253, 2985, 1676, 1639. MS/ESI (*m/z*) calcd: 203.1 (M+), found: 226.3 (M+Na).

Benzyl (1*S*)-1-benzyl-2-hydrazino-2-oxoethylcarbamate 6a: Yield: 66%, MP: 170.5–171.8 °C. NMR-^{1}H (500 MHz, $CDCl_3$) δ: 2.87 (1H, dd, J_1 = 9.2 Hz, J_2 = 13.5 Hz, C<u>H</u>Ph); 3.07 (1H, dd, J_1 = 9.2 Hz, J_2 = 13.5 Hz, C<u>H</u>Ph); 4.32 (1H, dd, J_1 = 6.0 Hz, J_2 = 8.5 Hz, C<u>H</u>NH); 4.99 (2H, s, CH_2O); 5.02 (2H, m, CHN<u>H</u>); 7.19–7.33 (10H, m, Ph) ppm NMR- ^{13}C (100 MHz, $CDCl_3$) δ: 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 (ν, cm^{-1}, KBr): 3300, 3058, 3030, 1689, 1537. MS/ESI (*m/z*) calcd: 313.1 (M+), found: 336.1 (M+Na).

Benzyl (2*S*)-2-hydrazino-1-isobutyl-2-oxoethylcarbamate 6b: Yield: 71%, MP: 120.9–130.0 °C. NMR-^{1}H (500 MHz, CD_3OD) δ: 0.93 (6H, q, J_1 = 6.5 Hz, J_2 = 11.5 Hz, $(CH_3)_2$); 1.50 (2H, m, CH_2); 1.66 (1H, m, C<u>H</u>$(CH_3)_2$); 4.13 (1H, dd, J_1 = 6.0 Hz, J_2 = 9.5 Hz, C<u>H</u>NH); 5.08 (2H, m, CH_2O); 7.29–7.34 (5H, m, Ph) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 23.0; 26.4; 42.3; 54.4; 67.8; 128.4–130.3; 138.3; 158.5; 174.6 ppm IR (ν, cm^{-1}, KBr): 3305, 3057, 2947, 1683, 1537. MS/ESI (*m/z*) calcd: 279.2 (M+), found: 302.1 (M+Na).

(2*S*)-2-amino-3-phenylpropanehydrazide 7a: Yield: 80%, MP: 91.2–92.4 °C. NMR-^{1}H (400 MHz, CD_3OD) δ: 2.80 (1H, dd, J_1 = 7.2 Hz, J_2 = 13.2 Hz, C<u>H</u>Ph); 2.97 (1H, m, J_1 = 13.2 Hz, J_2 = 11.5 Hz C<u>H</u>Ph); 3.48 (1H, m, C<u>H</u>NH_2); 7.24 (5H, m, Ph) ppm NMR- ^{13}C (100 MHz, CD_3OD) δ: 42.7; 56.8; 127.9; 129.7; 130.5; 138.9; 175.7 ppm IR (ν, cm^{-1}, KBr): 3348, 3178, 3024, 1670, 1566. MS/ESI (*m/z*) calcd: 179.1 (M+), found: 180.2 (M+H).

(2*S*)-2-amino-4-methylpentanehydrazide 7b: Yield: 60%, oil. NMR-^{1}H (500 MHz, CD_3OD) δ: 1.02 (6H, m, $(CH_3)_2$); 1.76 (2H, m, CH_2); 1.92 (1H, m, C<u>H</u>$(CH_3)_2$); 2.10–2.11 (2H, m, NHN<u>H</u>$_2$); 4.12 (1H, m, C<u>H</u>NH) ppm NMR- ^{13}C (125 MHz, CD_3OD) δ: 23.8; 24.7; 25.7; 42.0; 51.6; 172.4 ppm IR (ν, 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-^{1}H (400 MHz, DMSO-d_6) δ: 1.32 (9H, s, $(CH_3)_3$); 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, H4′ (*E*/*Z*)-isomer); 7.22 and 7.56 (1H, m, H3′ (*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- ^{13}C (75 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (300 MHz, DMSO-d_6) δ: 1.32 (9H, s, $(CH_3)_3$); 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, H4′ (*E*/*Z*)-isomer); 7.30 (5H, m, Ph); 7.22 and 7.56 (1H, m, H3′ (*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- ^{13}C (75 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (500 MHz, $CDCl_3$) δ: 0.95 and 1.08 (6H, m, $(CH_3)_2$ (*E*/*Z*)-isomer); 1.46 (9H, s, $(CH_3)_3$); 1.54 (2H, m, CH_2); 1.72 and 1.84 (1H, m, $CH(CH_3)_2$ (*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, H4′ (*E*/*Z*)-isomer); 7.09 (d, J = 3.5 Hz) and 7.36 (d, J = 3.5 Hz) (1H, H3′ (*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- ^{13}C (125 MHz, $CDCl_3$) 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^{-1}, 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-^{1}H (500 MHz, DMSO-d_6) δ: 0.88 and 0.99 (6H, m, $(CH_3)_2$ (*E*/*Z*)-isomer); 1.38 (9H, s, $(CH_3)_3$); 1.51 (2H, m, CH_2); 1.63 and 1.73 (1H, m, $CH(CH_3)_2$ (*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, H4′ (*E*/*Z*)-isomer); 7.14 (d, J = 6.8 Hz) and 7.54 (d, J = 4.4 Hz) (1H, H3′ (*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- ^{13}C (125 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (500 MHz, $CDCl_3$) δ: 0.94 and 1.07 (6H, m, $(CH_3)_2$ (*E*/*Z*)-isomer); 1.44 (9H, s, $(CH_3)_3$); 1.60 and 1.71 (2H, m, CH_2); 1.80 (1H, m, $CH(CH_3)_2$; 4.25 and 5.43 (1H, m, CHNH (*E*/*Z*)-isomer); 5.22 (1H, m, H3′); 6.43 (d, J = 3.2 Hz) and 6.72 (d, J = 3.6 Hz) (1H, H4′ (*E*/*Z*)-isomer); 6.47 (m) and 6.76 (d, J = 4.0 Hz) (1H, H2′ (*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- ^{13}C (125 MHz, CD_3OD) 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^{-1}, 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-^{1}H (400 MHz, DMSO-d_6) δ: 0.88 and 0.98 (6H, m, $(CH_3)_2$ (*E*/*Z*)-isomer); 1.38 (9H, s, $(CH_3)_3$); 1.46 (2H, m, CH_2); 1.62 and 1.72 (2H, m, $CH(CH_3)_2$ (*E*/*Z*)-isomer); 4.00 and 4.83 (1H, m, CHNH (*E*/*Z*)-isomer); 7.11 (1H, m, H4′); 7.40 (d, J = 3.0 Hz) and 7.62 (m) (1H, H3′ (*E*/*Z*)-isomer); 7.44 (d, J = 3.2 Hz) and 7.64 (m) (1H, H2′ (*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- ^{13}C (100 MHz, DMSO-d_6) 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; 1556.6; 169.2; 174.1 ppm IR (ν, cm^{-1}, 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-^{1}H (500 MHz, $CDCl_3$) δ: 1.44 (9H, s, $(CH_3)_3$); 1.46 (3H, s, CH_3); 4.33 and 5.10 (1H, m, CHNH (*E*/*Z*)-isomer); 6.93 (d, J = 3.5 Hz) and 7.33 (m) (1H, H4′ (*E*/*Z*)-isomer); 7.08 (m) and 7.36 (d, J = 3.5 Hz) (1H, H3′ (*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- ^{13}C (125 MHz, $CDCl_3$) 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^{-1}, 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-^{1}H (500 MHz, CD_3OD) δ: 1.23 (3H, m, CH_3); 1.38 (9H, s, $(CH_3)_3$); 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, H4′ (*E*/*Z*)-isomer); 7.20 (d, J = 7.6 Hz) and 7.54 (d, J = 4.0 Hz) (1H, d, H3′ (*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- ^{13}C (125 MHz, CD_3OD) δ: 16.7; 17.6; 28.2; 46.7; 49.1; 79.2; 129.0; 130.7; 136.5; 140.3; 146.7;v150.4; 150.8; 155.2; 170.1; 174.6 ppm IR (ν, cm^{-1}, 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-^{1}H (500 MHz, CD_3OD) δ: 1.24 (3H, d, J = 7.0 Hz, CH_3); 1.44 (9H, s, $(CH_3)_3$); 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- ^{13}C (125 MHz, CD_3OD) 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^{-1}, 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-^{1}H (400 MHz, CD_3OD) δ: 1.36 (3H, d, J = 7.2 Hz, CH_3); 1.44 (9H, s, $(CH_3)_3$); 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- ^{13}C (100 MHz, CD_3OD) 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^{-1}, 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-^{1}H (400 MHz, DMSO-d_6) 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_2O); 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- ^{13}C (100 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (400 MHz, DMSO-d_6) 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_2O); 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- ^{13}C (100 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (500 MHz, DMSO-d_6) δ: 0.89 and 0.99 (6H, m, $(CH_3)_2$ (*E/Z*)-isomer); 1.42 (2H, m, CH$(CH_3)_2$); 1.56 (1H, m, CH_2); 4.11 and 4.93 (1H, m, H2, (*E/Z*)-isomer); 5.03 (2H, m, CH_2O); 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- ^{13}C (500 MHz, DMSO-d_6) 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^{-1}, 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-^{1}H (400 MHz, Acetone-d_6) δ: 0.89 and 0.99 (6H, m, $(CH_3)_2$ (*E/Z*)-isomer); 1.42 (2H, m, CH_2); 1.42 and 1.54 (1H, m, CH$(CH_3)_2$ (*E/Z*)-isomer); 4.12 and 4.92 (1H, m, CHNH (*E/Z*)-isomer); 5.04 (2H, m, CH_2O); 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- ^{13}C (100 MHz, Acetone-d_6) 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^{-1}, 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-^{1}H (400 MHz, CF_3COOD) δ: 2.89 (2H, s, NH_2); 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- ^{13}C (100 MHz, CF_3COOD) 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^{-1}, 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-^{1}H (500 MHz, DMSO-d_6) δ: 0.98 (6H, m, $(CH_3)_2$); 1.63 (1H, m, CH$(CH_3)_2$); 1.81 (2H, m, CH_2); 2.80 (2H, s, NH_2); 3.51 and 4.21 (1H, m, $CHNH_2$ (*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- ^{13}C (125 MHz, DMSO-d_6) 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^{-1}, 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)metylene]}-(2*S*)-amino-3-methylbutanehidrazide 10c**: Yield: 44%. MP: 125.2–127.4 °C. NMR-^{1}H (500 MHz, DMSO-d_6) δ: 0.96 (6H, t, J = 7.0 Hz; $(CH_3)_2$); 1.65 (1H, m, $CH(CH_3)_2$); 2.09 (2H, m, CH_2); 2.55 (2H, s, NH_2); 3.01 and 3.86 (1H, m, $CHNH_2$ (*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- ^{13}C (125 MHz, DMSO-d_6) 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 (ν, 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

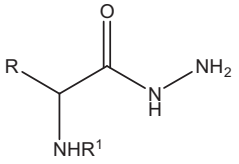
The antimycobacterial activities of the compounds **5a**–**c**, **6a**–**b**, **7a**–**b**, **8a**–**j**, **9a**–**d**, and **10a**–**c** have been assessed against *Mycobacterium tuberculosis* ATTC 27294 using the microplate Alamar Blue assay (12). This methodology is nontoxic, uses a thermally stable 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 μg/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 μg/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 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 μg/mL. The 5-nitrofuran derivative **9a**, with a MIC of 12.5 μg/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 μg/mL). These data highlight the importance of 5-nitrofuranyl 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_2 substituents for the biologic activity of the synthesized compounds. Concerning

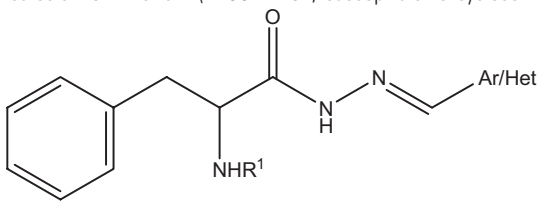
Table 1: The *in vitro* activity of *N*-acyl hydrazines amino acid derivatives **5a–c**, **6a–b**, and **7a–b** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to D-cycloserine)



Compound	R	R^1	logP^a	MIC (μg/mL)
5a	$C_6H_5CH_2$-	BOC	2.380	Resistant
5b	$CH_3(CH_2)_2CHCH_2$-	BOC	2.229	Resistant
5c	CH_3-	BOC	0.919	Resistant
6a	$C_6H_5CH_2$-	Cbz	2.790	Resistant
6b	$CH_3(CH_2)_2CHCH_2$-	Cbz	2.638	Resistant
7a	$C_6H_5CH_2$-	H	−1.363	100
7b	$CH_3(CH_2)_2CHCH_2$-	H	−1.514	Resistant
Cycloserine	–	–	–	20

[a]Calculated by http://www.molinspiration.com/cgi-bin/properties.

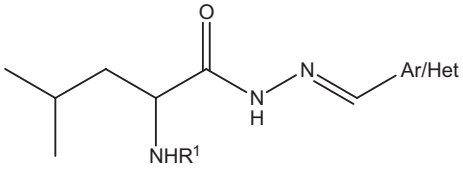
Table 2: The *in vitro* activity of *N*-acyl hydrazones L-phenylalanine derivatives **8a**–**8b**, **9a**–**b** and **10a** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to cycloserine)



Compound	R^1	Ar/Het	logP^a	MIC (μg/mL)
8a	BOC	5-nitro-2-furyl	5.137	100
8b	BOC	5-nitro-2-thienyl	5.779	50
9a	Cbz	5-nitro-2-thienyl	5.546	12.5
9b	Cbz	5-nitro-2-thienyl	6.188	50
10a	H	*p*-nitrobenzyl	2.013	Resistant
Cycloserine	–	–	–	20

[a]Calculated by http://www.molinspiration.com/cgi-bin/properties.

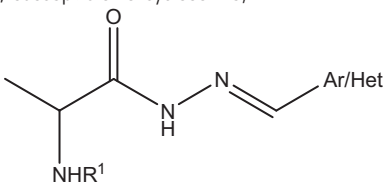
Table 3: The *in vitro* activity of *N*-acyl hydrazones L-leucine derivatives compounds **8c**–**f**, **9c**–**d** and **10b**–**c** against *Mycobacterium tuberculosis* H37Rv strain (ATCC 27294, susceptible to cycloserine)



Compound	R^1	Ar/Het	logP^a	MIC (μg/mL)
8c	BOC	2-furyl	4.903	Resistant
8d	BOC	2-thienyl	5.545	Resistant
8e	BOC	5-nitro-2-furyl	4.986	50
8f	BOC	5-nitro-2-thienyl	5.627	50
9c	Cbz	5-nitro-2-furyl	5.395	25
9d	Cbz	5-nitro-2-thienyl	6.037	25
10b	H	*p*-nitrobenzyl	1.862	Resistant
10c	H	5-nitro-2-furyl	1.242	25
Cycloserine	–	–		20

[a]Calculated by http://www.molinspiration.com/cgi-bin/properties.

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^1	Ar/Het	logP[a]	MIC (μg/mL)
8g	BOC	2-furyl	3.593	100
8h	BOC	2-thienyl	4.235	100
8i	BOC	5-nitro-2-furyl	3.676	Resistant
8j	BOC	5-nitro-2-thienyl	4.318	Resistant
Cycloserine	–	–	–	20

[a]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 then, the most promising compound was **9a**, being more potent when compared with that of the tuberculostatic drug D-cycloserine (20 μg/mL).

Acknowledgments

The authors gratefully acknowledge Farmanguinhos, IPEC, UFJF, CAPES, and CNPq for fellowships.

References

1. Virsdoia V., Shaikh M.S., Manvar A., Desai B., Parecha A., Loriya R., Dholariya K., Patel G., Vorá V., Upadhyay K., Denish K., Shah A., Coutinho E.C. (2010) Screening for *in vitro* antimycobacterial activity and three-dimensional quantitative structure-activity relationship (3D-QSAR) study of 4-(arylamino)coumarin derivatives. Chem Biol Drug Des;76:412–424.
2. Silveira G.P., Nome F., Gesser J.C., Sá M.M. (2006) Estratégias utilizadas no combate a resistência bacteriana. Quim Nova;29:844–855.
3. Souza M.V.N., Ferreira M.L., Pinheiro A.C., Saraiva M.F., Almeida M.V., Valle M.S. (2008) Synthesis and biological aspects of mycolic acids: an important target against *Mycobacterium tuberculosis*. TSWJ;8:720–751.
4. Souza M.V.N., Vasconcelos T.R.A. (2005) Fármacos no combate à tuberculose: passado, presente e futuro. Quim Nova;28:678–682.
5. Rollas S., Küçükgüzel S.G. (2007) Biological activities of hydrazone derivatives. Molecules;1:1910–1939.
6. Dimmock J.R., Vashishtha S.C., Stables J.P. (2000) Anticonvulsant properties of various acetyhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsatured carbonyl compounds. Eur J Med Chem;35:241–248.
7. Çakır B., Dağ Ö., Yıldırım E., Erol K., Şahin M.F. (2001) Synthesis and anticonvulsant activity of some hydrazones of 2-[(3H)-oxobenzoxazolin-3-yl-aceto]hydrazide. J Fac Pharm Gazi;18:99–106.
8. Ergenç N., Günay N.S. (1998) Synthesis and antidepressant evaluation of new 3-phenyl-5-sulfonamidoindole derivatives. Eur J Med Chem;33:143–148.
9. Todeschini A.R., Miranda A.L., Silva C.M., Parrini S.C., Barreiro E.J. (1998) Synthesis and evaluation of analgesic, antiinflammatory and antiplatelet properties of new 2-pyridylarylhydrazone derivatives. Eur J Med Chem;33:189–199.
10. Lima P.C., Lima L.M., Silva K.C., Leda P.H., Miranda A.L.P., Fraga C.A.M., Barreiro E.J. (2000) Synthesis and analgesic activity of novel *N*-acylhydrazones and isosters, derived from natural safrole. Eur J Med Chem;35:187–203.
11. Walcourt A., Loyevsky M., Lovejoy D.B., Gordeuk V.R., Richardson D.R. (2004) Novel arylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine-resistant and -sensitive parasites. Int J Biochem Cell Biol;36:401–407.
12. Franzblau S.G., Witzig R.S., McLaughlin J.C., Torres P., Madico G., Hernandez A., Degnan M.T., Cook M.B., Quenzer V.K., Ferguson R.M., Gilman R.H. (1998) Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the Microplate Alamar blue assay. J Clin Microbiol;36:362–366.
13. Vanitha J.D., Paramasivan C.N. (2004) Evaluation of microplate Alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. Diagn Microbiol Infect Dis;49:179–182.
14. Reis R.S., Neves I. Jr, Lourenço S.L.S., Fonseca L.S., Lourenço M.C.S. (2004) Comparison of flow cytometric and Alamar blue tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid. J Clin Microbiol;42:2247–2248.

Note

[a]Available at: http://www.who.int/tb/en/ (accessed 1 February 2011).