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Triazole-substituted *N*-hydroxyindol-2-carboxylates as inhibitors of isoform 5 of human lactate dehydrogenase (*h*LDH5)

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Electronic Supplementary Information

Experimental Section

Chemistry. Commercially available chemicals were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI, 70 eV, unless otherwise specified) mass spectra were obtained on a Thermo Quest Finningan (TRACE GCQ plus) mass spectrometer. Purity was routinely measured by HPLC on a Waters SunFire RP 18 (3.0 x 150 mm, 5 μm) column (Waters, Milford, MA, www.waters.com) using a Beckmann SystemGold instrument consisting of chromatography 125 Solvent Module and a 166 UV Detector. Mobile phases: 10 mM ammonium acetate in Millipore purified water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5% to 80% of B in 10 minutes and held at 80% for 10 min; flow rate was 0.7 mL/min and injection volume was 30 μL; retention times (HPLC, t_R) are given in minutes. Compound HPLC purity was determined by monitoring at 254 and 300 nm and was found in the range 96-99%, unless otherwise noted. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063–0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. Microwave assisted reaction were run in a CEM or Biotage microwave synthesizer.

1-Azido-2-methyl-3-nitrobenzene (3a).[1]

To a solution of 2-methyl-3-nitroaniline (2.5 g, 16 mmol) in aqueous 4 N HCl (24 mL) cooled to –5 °C was added dropwise a solution of sodium nitrite (1.4 g, 20 mmol) in water (4 mL) and the mixture was stirred at the same temperature for 1 hour. Then, another solution of sodium azide (1.1 g, 17 mmol) in water (4 mL) was added dropwise, keeping the temperature below 0 °C. Stirring was continued for 1 hour at 0 °C and 16 hours at room temperature. The reaction mixture was then

extracted four times with EtOAc. The combined organic phase were washed with a saturated solution of $NaHCO_3$ and brine, then dried and concentrated under vacuum, to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 8:2, R_f = 0.47) to yield pure **3a** (2.4 g, 82% yield) as a yellowish solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H), 7.32-7.41 (m, 2H), 7.55-7.64 (m, 1H).

4-Azido-2-methyl-1-nitrobenzene (3b).

Commercially available 3-methyl-4-nitroaniline (1.8 g, 12 mmol) was submitted to the same procedure described above for the preparation of **3a**.[1] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 9:1, R_f = 0.44) to produce pure **3b** (2.0 g, 93% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.62 (s, 3H), 6.93-7.00 (m, 2H), 8.04-8.08 (m, 1H).

4-Azido-1-methyl-2-nitrobenzene (3c).

Commercially available 4-methyl-3-nitroaniline (4.0 g, 26 mmol) was submitted to the same procedure described above for the preparation of **3a**.[1] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 8:2, R_f = 0.47) to produce pure **3b** (4.2 g, 90% yield) as a light yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.57 (s, 3H), 7.16 (dd, 1H, *J* = 8.2, 2.4 Hz), 7.33 (d, 1H, *J* = 8.2 Hz), 7.64 (d, 1H, *J* = 2.4 Hz).

1-(2-Methyl-3-nitrophenyl)-4-phenyl-1*H*-1,2,3-triazole (5a).[2]

A suspension of **3a** (1.0 g, 5.6 mmol) and ethynylbenzene (**4a**, 1.2 g, 12 mmol) in a 1:1 mixture of *tert*-butanol (12 mL) and water (12 mL) was sequentially treated with a 1M aqueous solution of sodium ascorbate (0.6 mL, 0.6 mmol) and an aqueous solution (0.2 mL) containing 15 mg (0.06 mmol) of copper sulphate pentahydrate. The resulting suspension was heated at 80 °C for 24 hours, then it was cooled to room temperature, diluted with water and repeatedly extracted with EtOAc. The combined organic phase were washed with water, then dried and concentrated under vacuum, to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 7:3;

R_f = 0.24) to yield pure **5a** (1.0 g, 67% yield) as a yellowish solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.36 (s, 3H), 7.36-7.53 (m, 3H), 7.57 (d, 1H, *J* = 8.1 Hz), 7.68 (dd, 1H, *J* = 8.0, 1.6 Hz), 7.88-7.94 (m, 2H), 8.01 (s, 1H), 8.05 (dd, 1H, *J* = 8.1, 1.5 Hz).

Methyl 3-(1-(2-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (5b).

Aryl-azide **3a** (0.11 g, 0.62 mmol) was submitted together with methyl 3-ethynylbenzoate[3] (**4b**, 1.1 eq) to the same procedure described above for the preparation of **5a**,[2] with the following modification: the resulting initial suspension was placed in a sealed vial and irradiated in a microwave synthesizer (150 °C, 250 W) for 15 minutes. The crude product (containing a ~8:2 ratio of the 1,4- vs 1,5-regioisomer) was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.29) to produce pure **5b** (0.15 g, 73% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.37 (s, 3H), 3.96 (s, 3H), 7.56 (t, 1H, *J* = 8.0 Hz), 7.58 (t, 1H, *J* = 7.8 Hz), 7.69 (dd, 1H, *J* = 8.0, 1.6 Hz), 8.05-8.09 (m, 2H), 8.10 (s, 1H), 8.21 (dt, 1H, *J* = 7.7, 1.5 Hz), 8.51 (t, 1H, *J* = 1.7 Hz).

4-Butyl-1-(2-methyl-3-nitrophenyl)-1*H*-1,2,3-triazole (5c).

Aryl-azide **3a** (0.12 g, 0.67 mmol) was submitted together with 1-hexyne (**4c**, 1.6 eq) to the same procedure described above for the preparation of **5a**.[2] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.27) to produce pure **5c** (0.061 g, 35% yield) as a light yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (t, 3H, *J* = 7.3 Hz), 1.44 (sextet, 2H, *J* = 7.3 Hz), 1.75 (quintet, 2H, *J* = 7.4 Hz), 2.29 (s, 3H), 2.83 (t, 2H, *J* = 7.7 Hz), 7.50 (s, 1H), 7.51 (t, 1H, *J* = 8.0 Hz), 7.61 (dd, 1H, *J* = 7.9, 1.5 Hz), 8.02 (dd, 1H, *J* = 8.1, 1.6 Hz).

2-(1-(2-Methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)ethanol (5d).

Aryl-azide **3a** (1.1 g, 6.2 mmol) was submitted together with 1-butyn-1-ol (**4d**, 1.1 eq) to the same procedure described above for the preparation of **5a**.[2] The crude product was purified by flash chromatography (ethyl acetate, R_f = 0.24) to produce pure **5d** (1.5 g, 97% yield) as a light yellow

solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.29 (s, 3H), 3.08 (t, 2H, *J* = 5.8 Hz), 4.04 (t, 2H, *J* = 5.8 Hz), 7.47-7.64 (m, 2H), 7.67 (s, 1H), 8.03 (dd, 1H, *J* = 8.0, 1.0 Hz).

1-(3-Methyl-4-nitrophenyl)-4-phenyl-1*H*-1,2,3-triazole (5e).

Aryl-azide **3b** (1.06 g, 5.93 mmol) was submitted together with ethynylbenzene (**4a**, 1.8 eq) to the same procedure described above for the preparation of **5a**.[2] The crude product (containing a ~7:3 ratio of the 1,4- vs 1,5-regioisomer) was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.34) to produce pure **5e** (0.96 g, 58% yield) as a yellowish solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.75 (s, 3H), 7.40-7.54 (m, 3H), 7.80 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.89-7.95 (m, 3H), 8.22 (d, 1H, *J* = 8.8 Hz), 8.27 (s, 1H).

Methyl 3-(1-(3-methyl-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (5f).

Aryl-azide **3b** (0.39 g, 2.2 mmol) was submitted together with methyl 3-ethynylbenzoate[3] (**4b**, 1.1 eq) to the same procedure described above for the preparation of **5a**,[2] with the following modification: the resulting initial suspension was placed in a sealed vial and irradiated in a microwave synthesizer (150 °C, 200 W) for 15 minutes.The crude product (containing a ~95:5 ratio of the 1,4- vs 1,5-regioisomer) was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.38) to produce pure **5f** (0.68 g, 91% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.76 (s, 3H), 3.96 (s, 3H), 7.57 (t, 1H, *J* = 7.7 Hz), 7.81 (dd, 1H, *J* = 8.9, 2.4 Hz), 7.89 (d, 1H, *J* = 2.4 Hz), 8.06 (dt, 1H, *J* = 8.0, 1.4 Hz), 8.20-8.24 (m, 2H), 8.37 (s, 1H), 8.50 (t, 1H, *J* = 1.4 Hz).

Methyl 3-(1-(4-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (5g).

Aryl-azide **3c** (0.46 g, 2.6 mmol) was submitted together with methyl methyl 3-ethynylbenzoate[3] (**4b**, 1.1 eq) to the same procedure described above for the preparation of **5a**,[2] with the following modification: the resulting initial suspension was placed in a sealed vial and irradiated in a microwave synthesizer (150 °C, 200 W) for 15 minutes.The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.32) to produce pure **5g** (0.77 g, 88% yield) as a

yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.71 (s, 3H), 3.96 (s, 3H), 7.57 (t, 1H, *J* = 7.7 Hz), 7.58 (d, 1H, *J* = 8.2 Hz), 8.02-8.08 (m, 2H), 8.20 (dt, 1H, *J* = 7.8, 1.3 Hz), 8.36 (s, 1H), 8.43 (d, 1H, *J* = 2.4 Hz), 8.50 (t, 1H, *J* = 1.7 Hz).

Methyl 3-(2-nitro-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)-2-oxopropanoate (6a).

Nitrotoluene derivative **5a** (0.800 g, 3.84 mmol) and dimethyl oxalate (2.27 g, 19.2 mmol) were dissolved in anhydrous DMF (7 mL) and the resulting solution was added dropwise under nitrogen to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 4.0 equiv) in DMF (10 mL) at 0 °C.[4] The mixture was stirred at room temperature until consumption of starting material (TLC, 4 h), then it was diluted with 1N HCl and extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 6:4; R_f = 0.26) to yield pure **5a** (1.14 g, 81% yield) as a yellowish solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.90 (s, 3H), 4.35 (s, 2H), 7.35-7.52 (m, 3H), 7.68-7.81 (m, 2H), 7.84-7.90 (m, 2H), 8.04 (s, 1H), 8.32 (dd, 1H, *J* = 7.7, 2.0 Hz).

Methyl 3-(1-(2-(3-methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (6b).

Nitrotoluene derivative **5b** (0.32 g, 0.95 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 6:4; R_f = 0.17) to produce pure **6b** (0.20 g, 50% yield) as an orange solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.90 (s, 3H), 3.95 (s, 3H), 4.37 (s, 2H), 7.56 (t, 1H, *J* = 7.8 Hz), 7.69-7.81 (m, 2H), 8.04-8.16 (m, 2H), 8.12 (s, 1H), 8.32 (dd, 1H, *J* = 7.4, 2.1 Hz), 8.47 (t, 1H, *J* = 1.6 Hz).

Methyl 3-(2-(4-butyl-1*H*-1,2,3-triazol-1-yl)-6-nitrophenyl)-2-oxopropanoate (6c).

Nitrotoluene derivative **5c** (0.25 g, 0.96 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1, R_f = 0.25) to produce pure **6c** (0.18 g, 54% yield) as a yellow oil. ^{1}H NMR

($CDCl_3$) δ (ppm): 0.95 (t, 3H, *J* = 7.2 Hz), 1.22-1.49 (m, 2H), 1.60-1.77 (m, 2H), 2.78 (t, 2H, *J* = 7.7 Hz), 3.92 (s, 3H), 4.29 (s, 2H), 7.53 (s, 1H), 7.63-7.73 (m, 2H), 8.27 (dd, 1H, *J* = 6.7, 3.0 Hz).

Methyl 3-(2-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-6-nitrophenyl)-2-oxopropanoate (6d).

Nitrotoluene derivative **5d** (1.2 g, 4.8 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (ethyl acetate, R_f = 0.20) to produce pure **6d** (1.1 g, 69% yield) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.03 (t, 2H, *J* = 5.9 Hz), 3.92 (s, 3H), 4.00 (t, 2H, *J* = 5.9 Hz), 4.30 (s, 2H), 7.67-7.70 (m, 2H), 7.71 (s, 1H), 8.27 (dd, 1H, *J* = 6.9, 2.8 Hz); signals imputable to the enol form (~ 15%) δ (ppm): 3.85 (s, 3H), 6.51 (s, 1H).

Methyl 3-(2-nitro-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)-2-oxopropanoate (6e).

Nitrotoluene derivative **5e** (0.62 g, 2.2 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 7:3, R_f = 0.21) to produce pure **6e** (0.38 g, 47% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.68 (s, 2H), 7.40-7.53 (m, 3H), 7.89-7.97 (m, 4H), 8.29 (s, 1H), 8.27 (d, 1H, *J* = 8.2 Hz).

Methyl 3-(1-(3-(3-methoxy-2,3-dioxopropyl)-4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (6f).

Nitrotoluene derivative **5f** (0.30 g, 0.89 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 6:4, R_f = 0.25) to produce pure **6f** (0.15 g, 40% yield) as a yellow glass. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 3.99 (s, 3H), 4.69 (s, 2H), 7.58 (t, 1H, *J* = 7.7 Hz), 7.93 (s, 1H), 7.97-8.22 (m, 4H), 8.40 (s, 1H); 8.50 (t, 1H, *J* = 1.4 Hz).

Methyl 3-(1-(4-(3-methoxy-2,3-dioxopropyl)-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)benzoate (6g).

Nitrotoluene derivative **5g** (0.50 g, 1.5 mmol) was submitted to the same procedure described above for the preparation of **6a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1, R_f = 0.28) to produce pure **6g** (0.46 g, 74% yield) as a yellow glass. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.93 (s, 3H), 3.98 (s, 3H), 4.66 (s, 2H), 7.58 (t, 1H, *J* = 7.7 Hz), 8.02-8.10 (m, 2H), 8.16-8.25 (m, 2H), 8.40 (s, 1H), 8.41-8.60 (m, 2H).

Methyl 1-hydroxy-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7a).

Ketoester **6a** (0.50 g, 1.4 mmol) was dissolved in anhydrous DME (1.5 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of $SnCl_2{\cdot}2H_2O$ (3.0 mmol) in DME (1.5 mL) containing activated 4Å molecular sieves.[4] The reaction mixture was stirred under nitrogen at room temperature until consumption of starting material (TLC), then it was diluted with water and extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography over iron-free silica gel[5] (*n*-hexane/ethyl acetate 6:4, R_f = 0.21) to produce pure **7a** (0.29 g, 61% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (s, 3H), 7.36-7.53 (m, 6H), 7.66 (dt, 1H, *J* = 7.9, 1.1 Hz), 7.93-7.98 (m, 2H), 8.29 (s, 1H), 10.50 (bs, 1H). MS *m/z* 335 ($M+H^+$).

Methyl 1-hydroxy-4-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7b).

Ketoester **6b** (0.15 g, 0.35 mmol) was submitted to the same procedure described above for the preparation of **7a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 4:6, R_f = 0.39) to produce pure **7b** (0.052 g, 38% yield) as an off-white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H), 4.02 (s, 3H), 7.39-7.52 (m, 3H), 7.58 (t, 1H, *J* = 7.8 Hz), 7.67 (dt, 1H, *J* = 7.9, 1.0 Hz), 8.06 (dt, 1H, *J* = 7.7, 1.4 Hz) 8.25 (dt, 1H, *J* = 7.9, 1.5 Hz); 8.39 (s, 1H); 8.53 (t, 1H, *J* = 1.6 Hz), 10.55 (bs, 1H). MS *m/z* 392 (M^+).

Methyl 4-(4-butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylate (7c).

Ketoester **6c** (0.175 g, 0.530 mmol) was submitted to the same procedure described above for the preparation of **7a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 6:4, R_f = 0.20) to produce pure **7c** (0.105 g, 63% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.97 (t, 3H, *J* = 7.2 Hz), 1.45 (sextet, 2H, *J* = 7.3 Hz), 1.62-1.82 (m, 2H), 2.83 (t, 2H *J* = 7.7 Hz), 3.99 (s, 3H), 7.29 (dd, 1H, *J* = 7.4, 0.9 Hz), 7.37-7.45 (m, 2H), 7.62 (dt, 1H, *J* = 8.4, 0.9 Hz), 7.81 (s, 1H), 10.64 (bs, 1H). MS *m/z* 315 ($M+H^+$).

Methyl 1-hydroxy-4-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7d).

Ketoester **6d** (0.500 g, 1.57 mmol) was submitted to the same procedure described above for the preparation of **7a**.[4] The crude product was purified by flash chromatography (ethyl acetate/acetone 9:1, R_f = 0.22) to produce pure **7d** (0.228 g, 48% yield) as a white solid. ^{1}H NMR (acetone-d_6) δ (ppm): 3.01 (t, 2H, *J* = 6.0 Hz), 3.90 (t, 2H, *J* = 5.9 Hz), 3.92 (s, 3H), 7.49 (d, 1H, *J* = 0.6 Hz), 7.51-7.58 (m, 2H), 7.62-7.70 (m, 1H), 8.41 (s, 1H), 10.71 (bs, 1H). MS *m/z* 301 (M^+ –H).

Methyl 1-hydroxy-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7e).

Ketoester **6e** (0.32 g, 0.87 mmol) was submitted to the same procedure described above for the preparation of **7a**.[4] The crude product was purified by flash chromatography (*n*-hexane/ethyl acetate 4:6, R_f = 0.34) to produce pure **7e** (0.10 g, 35% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H), 7.25 (d, 1H, *J* = 0.7 Hz), 7.34-7.42 (m, 1H), 7.47-7.55 (m, 2H), 7.70 (d, 1H, *J* = 8.9 Hz), 7.89-7.99 (m, 3H), 8.23 (dd, 1H, *J* = 1.5, 0.6 Hz), 9.30 (s, 1H), 11.74 (bs, 1H).

Methyl 1-hydroxy-5-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7f).

Ketoester **6f** (0.100 g, 0.236 mmol) was submitted to the same procedure described above for the preparation of **7a**.[4] The crude product was purified by flash chromatography (chloroform/methanol 98:2, R_f = 0.15) to produce pure **7f** (0.52 g, 56% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H), 4.03 (s, 3H), 7.14 (s, 1H), 7.56 (t, 1H, *J* = 7.8 Hz), 7.68 (d, 1H, *J* = 8.7 Hz),

7.77 (dd, 1H, *J* = 8.6, 1.8 Hz), 8.02-8.06 (m, 2H), 8.20 (dt, 1H, *J* = 7.8, 1.6 Hz), 8.29 (s, 1H), 8.50 (t, 1H, *J* = 1.6 Hz), 10.76 (bs, 1H).

Methyl 1-hydroxy-6-(4-(3-(methoxycarbonyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylate (7g).

A solution of of ketoester **6g** (0.100 g, 0.236 mmol) in 1 mL of MeOH was treated with 0.21 g of lead powder (325 mesh) and 0.5 mL of HCO_2HNEt_3 (TEAF).[6] The mixture was stirred under reflux at 55 °C for 18 h, cooled to room temperature, and filtered over a pad of Celite. The solvent was removed under reduced pressure and the residue extracted with EtOAc. The organic phase was washed with water, dried and evaporated to afford a crude residue that was purified by column chromatography over iron-free silica gel[5] (chloroform/methanol 98:2, R_f = 0.28) to produce pure **7g** (0.037 g, 40% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.92 (s, 3H), 3.94 (s, 3H), 7.20 (d, 1H, *J* = 0.9 Hz), 7.64 (t, 1H, *J* = 7.8 Hz), 7.81 (dd, 1H, *J* = 8.6, 1.8 Hz), 7.91 (dd, 1H, *J* = 8.7, 0.7 Hz), 7.98-8.04 (m, 1H), 8.13 (dt, 1H, *J* = 1.8, 0.9 Hz), 8.28 (ddd, 1H, *J* = 7.7, 1.8, 1.1 Hz) 8.65 (td, 1H, *J* = 1.6, 0.6 Hz), 9.27 (s, 1H), 10.76 (bs, 1H).

1,3-Bis(1-(4-methyl-3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)propane (9).[2]

A suspension of **3c** (4.0 g, 22.5 mmol) and 1,6-heptadiyne (**8**, 1.0 g, 11 mmol) in a 1:1 mixture of *tert*-butanol (45 mL) and water (45 mL) was sequentially treated with a 1M aqueous solution of sodium ascorbate (2.2 mL, 2.2 mmol) and an aqueous solution (1 mL) containing 56 mg (0.22 mmol) of copper sulphate pentahydrate. The resulting suspension was stirred at room temperature for 48 hours, then it was cooled to room temperature, diluted with water and repeatedly extracted with EtOAc. The combined organic phase were washed with water, then dried and concentrated under vacuum, to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 4:6; R_f = 0.20) to yield pure **9** (3.7 g, 75% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.10 (quintet, 2H, *J* = 7.3 Hz), 2.57 (s, 6H), 2.84 (t, 4H, *J* = 7.4 Hz), 7.73 (d, 2H, *J* = 8.8 Hz), 8.17 (dd, 2H, *J* = 8.2, 2.4 Hz), 8.49 (d, 2H, *J* = 2.4 Hz), 8.77 (s, 2H).

Dimethyl 3,3'-(4,4'-(4,4'-(propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(2-nitro-4,1-phenylene))bis(2-oxopropanoate) (10).

Compound **9** (3.3 g, 7.4 mmol) and dimethyl oxalate (8.7 g, 74 mmol) were dissolved in anhydrous DMF (25 mL) and the resulting solution was added dropwise under nitrogen to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 8.0 equiv) in DMF (75 mL) at 0 °C.[4] The mixture was stirred at room temperature until consumption of starting material (TLC, 16 h), then it was diluted with 1N HCl and extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 2:8; R_f = 0.34) to yield pure **10** (3.1 g, 68% yield) as a yellow solid. Proton NMR in DMSO-d_6 shows a prevalence (~ 60%) of the enol-form, as measured by the integration ratio of the singlet at 6.62 ppm (representing the vinyl proton of the enol form) *vs.* the singlet at 4.67 ppm (corresponding to the methylene protons of the keto-form). ^{1}H NMR (DMSO-d_6) δ (ppm): 2.06-2.19 (m, 2H), 2.81-2.90 (m, 4H), 3.84 (s, 6H), 6.62 (s, 2H), 8.24-8.28 (m, 2H), 8.41 (d, 2H, J = 8.9 Hz), 8.49 (d, 2H, J = 2.0 Hz), 8.80 (s, 2H); signals imputable to the keto-form (~ 40%) δ (ppm): 3.86 (s, 6H), 4.67 (s, 4H), 7.73 (d, 2H, J = 7.9 Hz), 8.62 (d, 2H, J = 2.0 Hz), 8.84 (s, 2H).

Dimethyl 6,6'-(4,4'-(propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(1-hydroxy-1H-indole-2-carboxylate) (11).

An aqueous solution (2.5 mL) containing 4.0 mmol of $H_2PO_2Na•H_2O$ was treated at room temperature with another solution containing compound **10** (0.80 g, 1.3 mmol) in THF (2.5 mL). Palladium 10% on charcoal (13 mg) was then added to the solution and resulting mixture was kept under stirring at room temperature for 12 hours and at 40 °C for 1 hour.[7] Once the disappearance of the precursor is verified by TLC, the reaction mixture is concentrated under vacuum, diluted with water and extracted several times with EtOAc. The combined organic phase were washed with water, then dried and concentrated under vacuum, to afford a crude residue that was purified by column chromatography (*n*-hexane/ethyl acetate 4:6; R_f = 0.20) to yield pure **11** (0.24 g, 34% yield)

as a yellow solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.12 (quintet, 2H, J = 7.0 Hz), 2.84 (t, 4H, J = 7.3 Hz), 3.88 (s, 6H), 7.17 (d, 2H, J = 0.7 Hz), 7.68 (dd, 2H, J = 8.8, 1.8 Hz), 7.85 (d, 2H, J = 8.6 Hz), 7.93 (dd, 2H, J = 1.8, 0.9 Hz), 8.76 (s, 2H), 11.73 (bs, 2H). MS *m/z* 557 ($M+H^+$).

1-Hydroxy-4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (1a).

Methyl ester **7a** (0.260 g, 0.778 mmol) was dissolved in a 1:1 mixture of THF/methanol (8 mL) and treated at room temperature with 2.5 mL of 2N aqueous solution of LiOH. The reaction was monitored by TLC (chloroform/methanol 9:1) and, after consumption of starting material, treated with 1N aqueous HCl and extracted with EtOAc. The organic phase was dried and evaporated to afford pure **1a** (0.244 g, 98% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.35-7.44 (m, 2H), 7.48-7.56 (m, 3H), 7.59-7.65 (m, 2H), 8.00-8.05 (m, 2H), 9.35 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 103.25, 110.51, 112.86, 113.41, 120.82, 124.84, 125.44 (2C), 127.88, 128.21, 128.92 (2C), 129.78, 130.20, 136.99, 146.77, 160.77. MS *m/z* 321 ($M+H^+$). HPLC, t_R 8.6 min.

4-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (1b).

Methyl ester **7b** (0.052 g, 0.13 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1b** (0.022 g, 94% yield) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.41 (s, 1H), 7.48-7.69 (m, 4H), 7.96 (dt, 1H, J = 8.0, 1.4 Hz), 8.27 (dt, 1H, J = 7.6, 1.5 Hz), 8.60 (t, 1H, J = 1.6 Hz), 9.50 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 103.27, 110.53, 112.86, 113.35, 121.33, 124.81, 126.14, 127.88, 128.89, 129.27, 129.60, 129.69, 130.60, 131.51, 136.97, 145.95, 160.73, 167.00. MS *m/z* 365 ($M+H^+$). HPLC, t_R 6.5 min.

4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (1c).

Methyl ester **7c** (0.090 g, 0.29 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1c** (0.077 g, 89% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 0.93 (t, 3H, J = 7.2 Hz), 1.39 (sextet, 2H, J = 7.3 Hz), 1.69 (quintet, 2H, J = 7.5 Hz), 2.75 (t, 2H, J = 7.6 Hz), 7.32 (d, 1H, J = 0.7 Hz), 7.42-7.53 (m, 2H), 7.57 (ddd, 1H, J = 7.1, 2.2, 0.6 Hz),

8.62 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 13.76, 21.77, 24.68, 31.00, 103.41, 110.08, 112.48, 113.30, 121.29, 124.86, 127.68, 129.96, 137.01, 147.53, 160.73. MS *m/z* 301 (M+H$^+$). HPLC, t_R 8.4 min.

1-Hydroxy-4-(4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (1d).

Methyl ester **7d** (0.085 g, 0.28 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1d** (0.078 g, 97% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.90 (t, 2H, *J* = 7.0 Hz), 3.74 (t, 2H, *J* = 6.9 Hz), 7.32 (d, 1H, *J* = 0.7 Hz), 7.46-7.52 (m, 2H), 7.56-7.60 (m, 1H), 8.60 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 29.18, 60.22, 103.39, 110.13, 112.54, 113.34, 121.95, 124.88, 127.70, 129.94, 137.04, 145.05, 160.70. MS (40 eV) *m/z* 287 (M$^+$ – H). HPLC, t_R = 6.6 min (purity = 92%).

1-Hydroxy-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1*H*-indole-2-carboxylic acid (1e).

Methyl ester **7e** (0.028 g, 0.84 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1e** (0.026 g, 95% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.16 (s, 1H), 7.34-7.54 (m, 3H), 7.67 (d, 1H, *J* = 8.8 Hz), 7.88 (dd, 1H, *J* = 8.8, 2.0 Hz), 7.95-7.99 (m, 2H), 8.20 (d, 1H, *J* = 1.8 Hz), 9.29 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.52, 110.70, 113.57, 117.76, 119.73, 120.56, 125.12 (2C), 127.85, 128.45, 128.70 (2C), 129.00, 130.31, 134.68, 146.89, 160.77. MS *m/z* 321 (M +H$^+$). HPLC, t_R 8.8 min.

5-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (1f).

Methyl ester **7f** (0.040 g, 0.11 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1f** (0.039 g, 98% yield) as a yellowish solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.13 (s, 1H), 7.61-7.69 (m, 2H), 7.87-7.70 (m, 2H), 8.18-8.24 (m, 2H), 8.55 (t, 1H, *J* = 1.6 Hz), 9.45 (s, 1H). ^{13}C NMR (DMSO-d_6) δ (ppm): 104.83, 110.73, 113.59, 117.82, 120.22, 120.60, 125.84, 128.58, 129.07, 129.21, 130.31, 130.69, 131.45, 134.91, 146.11, 160.62, 166.80. MS *m/z* 365 (M+H$^+$). HPLC, t_R 6.6 min.

6-(4-(3-Carboxyphenyl)-1*H*-1,2,3-triazol-1-yl)-1-hydroxy-1*H*-indole-2-carboxylic acid (1g).

Methyl ester **7g** (0.025 g, 0.064 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **1g** (0.022 g, 94% yield) as a yellowish solid. ^{1}H NMR (acetone-d_6) δ (ppm): 7.24 (d, 1H, J = 0.9 Hz), 7.66 (t, 1H, J = 7.7 Hz), 7.82 (dd, 1H, J = 8.6, 1.8 Hz), 7.93 (dd, 1H, J = 8.8, 0.6 Hz), 8.05 (dt, 1H, J = 7.8, 1.4 Hz), 8.15 (dt, 1H, J = 2.0, 1.1 Hz), 8.30 (dt, 1H, J = 7.8, 1.6 Hz), 8.69 (t, 1H, J = 1.6 Hz), 9.31 (s, 1H). ^{13}C NMR (acetone-d_6) δ (ppm): 79.14, 101.92, 106.38, 114.61, 120.35, 122.24, 124.79, 127.39, 129.96, 130.56, 132.13, 135.59, 147.81, 161.52, 167.23, 205.60, 206.00. MS m/z 365 ($M+H^+$). HPLC, t_R 6.0 min.

6,6'-(4,4'-(propane-1,3-diyl)bis(1*H*-1,2,3-triazole-4,1-diyl))bis(1-hydroxy-1*H*-indole-2-carboxylic acid) (2).

Methyl ester **11** (0.100 g, 0.180 mmol) was submitted to the same procedure described above for the preparation of **7a**, to produce pure **2** (0.085 g, 89% yield) as a light yellow solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.13 (quintet, 2H, J = 7.4 Hz), 2.85 (t, 4H, J = 7.1 Hz), 7.11 (s, 2H), 7.67 (dd, 2H, J = 8.8, 1.6 Hz), 7.84 (d, 2H, J = 8.6 Hz), 7.93 (bs, 2H), 8.77 (s, 2H). ^{13}C NMR (DMSO-d_6) δ (ppm): 24.64 (2C), 28.45 (1C), 100.52 (2C), 104.87 (2C), 113.17 (2C), 120.49 (4C), 123.68 (2C), 128.39 (2C), 134.00 (2C), 135.53 (2C), 147.64 (2C), 160.80 (2C). MS m/z 546 ($M+NH_4^+$, 5%), 256 ((($M+NH_4^+$)/2 –OH, 40%), 256 ((($M+NH_4^+$)/2 –2OH, 100%). HPLC, t_R = 7.3 min.

Docking calculations. The ligands were built using Maestro 9.0 [*Maestro*, version 9.0;. Schrödinger Inc: Portland, OR, 2009] and were minimized using the conjugate gradient method until a convergence value of 0.05 kcal/(mol•Å) was reached. The minimization was carried out in a water environment model (generalized-Born/surface-area model) using the MMFFs force field and a distance-dependent dielectric constant of 1.0. The human muscle L-LDH M chain was extracted from the minimized average structure of the LDH-compound **1j** complex obtained by us through molecular dynamic simulations.[8] Automated docking was carried out by means of the GOLD

program, version 4.1.1.[9] The region of interest used by GOLD was defined in order to contain the residues within 10 Å from the original position of compound **1j** in the starting LDH structures. The "allow early termination" option was deactivated, the remaining GOLD default parameters were used, and the ligands were submitted to 30 genetic algorithm runs by applying the ChemScore fitness function. The best docked conformation was taken into account. The so obtained complexes were energy minimized using AMBER 11 and the parm99 force field.[10] The complexes were placed in a rectangular parallelepiped water box, an explicit solvent model for water, TIP3P, was used, and the complex was solvated with a 10 Å water cap. Chlorine ions were added as counterions to neutralize the system. Two steps of minimization were then carried out. In the first stage, we kept the complex fixed with a position restraint of 500 kcal/(mol•Å^2) and we solely minimized the positions of the water molecules. In the second stage, we minimized the entire system through 20000 steps of steepest descent followed by conjugate gradient until a convergence of 0.05 kcal/(mol•Å) was attained. All the α carbons of the protein were blocked with a harmonic force constant of 10 kcal/(mol•Å^2).

Enzyme kinetics experiments.

The oxidation of NADH is observed at 340 nm and in the absence of interfering reactions is a direct measure of the reduction of pyruvate to lactate. The apparent Michaelis-Menten constants (K_m) of LDH-A and LDH-B were measured from Lineweaver-Burk plots. The reaction velocity of purified human LDH-A and LDH-B was determined by a decrease in absorbance at 340 nm of NADH and the value of optical density was determined at 30-second intervals for 5 minutes.

***h*LDH5 inhibition assay.**

The effect of the reported compounds on *h*LDH5 enzyme activity was measured using purified human lactate dehydrogenase isoform 5 (*h*LDH5, LDH-A_4, LDH-A Lee Biosolution, Inc.). LDH activity was determined at 37°C by measuring the oxidation of NADH spectrophotometrically at 340 nm as a function of time. The K_m values of *h*LDH5 for NADH and pyruvate were determined in

saturating conditions as described below. In 100 mM sodium phosphate buffer (pH 7.4), 0.005 units of *h*LDH5 were combined with 2 mM sodium pyruvate (pyruvate-saturated) and 12.5 μM to 150 μM NADH, or 200 μM NADH (NADH-saturated) and 25 μM to 1 mM sodium pyruvate, and these conditions were used for all assays. The reaction velocity of *h*LDH5 was determined spectrophotometrically (Lambda 25, PerkinElmer) by a decrease in absorbance at 340nm (ε = 6.2 mM^{-1} cm^{-1}) of NADH. Michaelis-Menten constants for substrates were determined from initial rate measurements at 37 °C by non-linear regression analysis with the GraphPad Prism 3.0. Under these conditions NADH showed a K_m of 20.0 μM and a V_{max} of 62.5 μmol/min/mg, whereas pyruvate showed a K_m of 120 μM and a V_{max} of 62.5 μmol/min/mg. The synthesized compounds were dissolved in stock solutions of DMSO (concentrations of DMSO during the initial rate measurements did not exceed 0.5%). Using 25 μM NADH and 2 mM sodium pyruvate we initially evaluated the percentual inhibition of the compounds: we then carried out kinetic studies only on compounds that showed a ≥50% inhibition (**1a**,**b**,**g** and **2**). Then, we evaluated the apparent K_m' values in the presence of these inhibitors (concentration range = 25-100 μM). From the values of K_m' so obtained, K_i values for each single inhibitor were determined using double-reciprocal plots.

***h*LDH1 inhibition assay.**

The effect of compounds **1a**,**b**,**g** and **2** on *h*LDH1 enzyme activity was measured using purified human lactate dehydrogenase isoform 1 (*h*LDH1, LDH-B_4, LDH-B, ERM® - AD453/IFCC from Sigma) as described before for *h*LDH5, only in pyruvate-saturating conditions. Under these conditions NADH showed a K_m of 27 μM and a V_{max} of 65 μmol/min/mg. The inhibition percentage of these compounds was determined as described above.

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