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# Discovery of *N*-hydroxyindole-based inhibitors of human lactate dehydrogenase isoform A (LDH-A) as starvation agents against cancer cells.

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## 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 ( $\delta$ ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Electron impact (EI, 70 eV) mass spectra were obtained on a Thermo Quest Finningan (TRACE GCQ plus MARCA) mass spectrometer. Purity was routinely measured by HPLC on a Waters SunFire RP 18 (3.0 x 150 mm, 5  $\mu$ m) column (Waters, Milford, MA, [www.waters.com](http://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  $\mu$ L; 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<sub>254</sub>) 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. Precursors **2a-f** are commercially available, whereas **2g**<sup>1</sup> was previously reported.

**5-(4-Methyl-3-nitrophenyl)-1H-tetrazole (2k).** According to Sharpless' synthesis of tetrazoles,<sup>2</sup> benzonitrile **6** (500 mg, 3.08 mmol) was dissolved in water (6 mL) and treated with sodium azide (221 mg, 3.39 mmol) and zinc bromide hydrate (694 mg, 3.08 mmol). The resulting suspension was refluxed for 36 h, then treated with aqueous 3 N HCl (until pH 1) and EtOAc. Stirring was continued until all the white solid was dissolved. The organic phase was separated and the water

phase was extracted again with EtOAc. The combined organic phase was concentrated and the residue was recovered with aqueous 0.25 N NaOH and stirred for 30 min. The suspension was filtered to remove Zn(OH)<sub>2</sub> and the resulting solution was acidified with 3 N HCl to precipitate the tetrazole derivative. The solid was then dissolved in EtOAc and MeOH and the solution was dried and concentrated to give pure **2k** (571 mg, 90 % yield); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 2.61 (s, 3H), 7.76 (d, 1H, *J* = 7.9 Hz), 8.32 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.67 (d, 1H, *J* = 1.6 Hz).

**General procedure for the preparation of ketoesters 3a-g.**<sup>3</sup> Nitrotoluene derivatives **2a-g** (8.5 mmol) and dimethyl oxalate (5.00 g, 42.3 mmol) were dissolved in anhydrous DMF (15 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 (20 mL) at 0 °C. The mixture was stirred at RT until consumption of starting material (TLC, 4-24 h), then it was diluted with 1N HCl or saturated aqueous NH<sub>4</sub>Cl, and extracted with EtOAc. The organic phase was dried and evaporated to afford a crude residue that was purified by column chromatography using the indicated eluant, to give the ketoesters.

**Methyl 3-(2-nitrophenyl)-2-oxopropanoate (3a).**<sup>3</sup> (61% yield from **2a**) *R<sub>f</sub>* = 0.26 (*n*-hexane/EtOAc 7:3); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 3.95 (s, 3H), 4.54 (s, 2H), 7.31-7.68 (m, 3H), 8.18 (dd, 1H, *J* = 8.2, 1.5 Hz); signals imputable to the enol form (~ 30%) δ (ppm): 3.94 (s, 3H), 6.63 (bs, 1H, exchangeable), 6.93 (s, 1H), 7.91 (dd, 1H, *J* = 8.1, 1.4 Hz), 8.23 (dd, 1H, *J* = 8.1, 1.5 Hz).

**Methyl 3-(2-methyl-6-nitrophenyl)-2-oxopropanoate (3b).** (75% yield from **2b**) *R<sub>f</sub>* = 0.33 (*n*-hexane/EtOAc 7:3); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 2.36 (s, 3H), 3.93 (s, 3H), 4.44 (s, 2H), 7.35 (t, 1H, *J* = 7.9 Hz), 7.50 (d, 1H, *J* = 7.3 Hz), 7.86 (d, 1H, *J* = 8.1 Hz); signals imputable to the enol form (~ 10%) δ (ppm): 6.08 (bs, 1H, exchangeable), 6.73 (s, 1H), 7.75 (d, 1H, *J* = 7.7Hz).

**Methyl 3-(2-nitro-6-(trifluoromethyl)phenyl)-2-oxopropanoate (3c).** (87% yield from **2c**) *R<sub>f</sub>* = 0.40 (*n*-hexane/EtOAc 7:3); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 3.96 (s, 3H), 4.69 (s, 2H), 7.66 (t, 1H, *J* =

8.1 Hz), 8.02 (d, 1H,  $J = 7.9$  Hz), 8.26 (d, 1H,  $J = 8.2$  Hz); signals imputable to the enol form (~ 10%)  $\delta$  (ppm): 3.96 (s, 3H), 6.14 (bs, 1H, exchangeable), 6.78 (q, 1H,  $J = 2.2$  Hz), 7.57 (t, 1H,  $J = 8.2$  Hz), 7.93 (d, 1H,  $J = 7.9$  Hz).

**Methyl 3-(2-chloro-6-nitrophenyl)-2-oxopropanoate (3d).** (78% yield from **2d**)  $R_f = 0.24$  (*n*-hexane/EtOAc 8:2);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.94 (s, 3H), 4.70 (s, 2H), 7.43 (t, 1H,  $J = 8.1$  Hz), 7.71 (dd, 1H,  $J = 8.1, 1.1$  Hz), 7.96 (dd, 1H,  $J = 8.1, 1.2$  Hz); signals imputable to the enol form (~ 10%)  $\delta$  (ppm): 3.89 (s, 3H), 6.25 (bs, 1H, exchangeable), 6.72 (s, 1H), 7.62 (d, 1H,  $J = 8.1$  Hz).

**Methyl 3-(2-bromo-6-nitrophenyl)-2-oxopropanoate (3e).**<sup>3</sup> (76% yield from **2e**)  $R_f = 0.56$  ( $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.96 (s, 3H), 4.74 (s, 2H), 7.38 (t, 1H,  $J = 8.1$  Hz), 7.91 (dd, 1H,  $J = 8.1, 1.3$  Hz), 8.02 (dd, 1H,  $J = 8.1, 1.3$  Hz); signals imputable to the enol form (~ 5%)  $\delta$  (ppm): 3.90 (s, 3H), 6.71 (s, 1H), 7.84 (d, 1H,  $J = 8.1$  Hz).

**Methyl 3-(4-bromo-2-nitrophenyl)-2-oxopropanoate (3f).** (78% yield from **2f**)  $R_f = 0.27$  (*n*-hexane/EtOAc 8:2);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.94 (s, 3H), 4.50 (s, 2H), 7.21 (d, 1H,  $J = 8.2$  Hz), 7.77 (dd, 1H,  $J = 8.2, 2.0$  Hz), 8.32 (d, 1H,  $J = 2.0$  Hz); signals imputable to the enol form (~ 20%)  $\delta$  (ppm): 3.91 (s, 3H), 6.72 (bs, 1H, exchangeable), 6.86 (s, 1H), 7.71 (dd, 1H,  $J = 8.6, 2.0$  Hz), 8.04 (d, 1H,  $J = 2.0$  Hz), 8.15 (d, 1H,  $J = 8.6$  Hz).

**Methyl 3-(3-nitrophenyl-2-yl)-2-oxopropanoate (3g).** (76% yield from **2g**)  $R_f = 0.24$  (*n*-hexane/EtOAc 8:2);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.88 (s, 3H), 4.36 (s, 2H), 7.22-7.28 (m, 2H), 7.38-7.45 (m, 3H), 7.51 (t, 1H,  $J = 7.7$  Hz), 7.59 (dd, 1H,  $J = 7.7, 1.8$  Hz), 8.13 (dd, 1H,  $J = 7.9, 1.8$  Hz); signals imputable to the enol form (~ 5%)  $\delta$  (ppm): 3.80 (s, 3H), 5.94 (bs, 1H, exchangeable), 6.52 (s, 1H), 7.86 (dd, 1H,  $J = 8.0, 1.6$  Hz).

**Methyl 3-(2-nitro-4-(tetrazol-5-yl)phenyl)-2-oxopropanoate (3k).** This product was synthesized from **2k** by a slight modification of the general procedure reported above, consisting in the use of 8.0 equivalents of NaH (48% yield from **2k**).  $R_f = 0.14$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1);  $^1\text{H NMR}$

(DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 3.80 (s, 3H), 4.71 (s, 2H), 8.34 (dd, 1H,  $J = 8.5, 1.7$  Hz), 8.43 (d, 1H,  $J = 8.4$  Hz), 8.57 (d, 1H,  $J = 1.6$  Hz); signals imputable to the enol form (~ 40%)  $\delta$  (ppm): 3.76 (s, 3H), 6.67 (s, 1H), 8.65 (dd, 1H,  $J = 8.4, 1.8$  Hz), 8.74 (d, 1H,  $J = 1.8$  Hz).

**General procedure for the reductive cyclization leading to *N*-hydroxyindoles 4a-g.**

Ketoesters **3a-g** (1.0 mmol) were dissolved in anhydrous DME (1.0 mL) and the resulting solution was added dropwise to a cooled (0 °C) solution of SnCl<sub>2</sub>·2H<sub>2</sub>O (2.2 mmol) in DME (1.0 mL) containing activated 4Å molecular sieves. The reaction mixture was stirred under nitrogen at RT 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<sup>4</sup> using the indicated eluant, to give the *N*-hydroxyindoles.

**Methyl 1-hydroxy-1*H*-indole-2-carboxylate (4a).**<sup>5</sup> (63% yield from **3a**)  $R_f = 0.33$  (CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 3.89 (s, 3H), 7.08 (d, 1H,  $J = 0.9$  Hz), 7.13 (ddd, 1H,  $J = 8.1, 7.0, 1.1$  Hz), 7.36 (ddd, 1H,  $J = 8.1, 7.0, 1.1$  Hz), 7.53 (dq, 1H,  $J = 8.4, 0.9$  Hz), 7.66 (dt, 1H,  $J = 8.1, 1.0$  Hz), 10.36 (bs, 1H).

**Methyl 1-hydroxy-4-methyl-1*H*-indole-2-carboxylate (4b).** (56% yield from **3b**)  $R_f = 0.40$  (CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.53 (s, 3H), 3.97 (s, 3H), 6.91 (d, 1H,  $J = 6.8$  Hz), 7.06 (d, 1H,  $J = 0.7$  Hz), 7.25 (dd, 1H,  $J = 8.4, 6.8$  Hz), 7.38 (d, 1H,  $J = 8.4$  Hz), 10.18 (bs, 1H).

**Methyl 1-hydroxy-4-trifluoromethyl-1*H*-indole-2-carboxylate (4c).** (60% yield from **3c**)  $R_f = 0.19$  (*n*-hexane/CH<sub>2</sub>Cl<sub>2</sub> 3:7); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 4.03 (s, 3H), 7.20 (qd, 1H,  $J = 1.7, 1.0$  Hz), 7.40 (t, 1H,  $J = 7.6$  Hz), 7.45 (d, 1H,  $J = 6.6$  Hz), 7.72-7.76 (m, 1H), 10.40 (bs, 1H).

**Methyl 4-chloro-1-hydroxy-1*H*-indole-2-carboxylate (4d).** (72% yield from **3d**)  $R_f = 0.30$  (CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 4.01 (s, 3H), 7.12-7.15 (m, 2H), 7.22-7.30 (m, 1H), 7.45 (d, 1H,  $J = 8.2$  Hz), 10.35 (bs, 1H).

**Methyl 4-bromo-1-hydroxy-1*H*-indole-2-carboxylate (4e).** (58% yield from **3e**)  $R_f = 0.37$  ( $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 4.02 (s, 3H), 7.08 (s, 1H), 7.20 (*pseudo-t*, 1H,  $J = 7.9$  Hz), 7.32 (d, 1H,  $J = 7.3$  Hz), 7.52 (d, 1H,  $J = 8.2$  Hz), 10.33 (bs, 1H).

**Methyl 6-bromo-1-hydroxy-1*H*-indole-2-carboxylate (4f).** (41% yield from **3f**)  $R_f = 0.19$  (*n*-hexane/ethyl acetate 8:2);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.99 (s, 3H), 7.00 (d, 1H,  $J = 0.9$  Hz), 7.21 (dd, 1H,  $J = 8.7, 1.7$  Hz), 7.49 (dd, 1H,  $J = 8.6, 0.7$  Hz), 7.72 (dt, 1H,  $J = 1.5, 0.7$  Hz), 10.22 (bs, 1H).

**Methyl 1-hydroxy-4-phenyl-1*H*-indole-2-carboxylate (4g).** (48% yield from **3g**)  $R_f = 0.28$  (*n*-hexane/ethyl acetate 7:3);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.98 (s, 3H), 7.19 (d, 1H,  $J = 0.9$  Hz), 7.20 (dd, 1H,  $J = 7.0, 1.1$  Hz), 7.39-7.57 (m, 5H), 7.62-7.68 (m, 2H), 10.28 (bs, 1H). MS  $m/z$  267 ( $\text{M}^+$ , 66), 253 ( $\text{M}^+ - \text{CH}_2$ , 70), 190 ( $\text{M}^+ - \text{C}_6\text{H}_5$ , 100).

**Methyl 1-hydroxy-6-tetrazolyl-1*H*-indole-2-carboxylate (4k).** Ketoester **3k** (300 mg, 1.0 mmol) was dissolved in THF (2 mL) and the resulting solution was added to an aqueous solution (2 mL) of sodium hypophosphite monohydrate (0.5 g, 4.7 mmol). Then 15 mg of 10% palladium over charcoal was added and the resulting suspension was stirred at RT overnight. The mixture was concentrated, 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<sup>4</sup> ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1 + 0.1% acetic acid,  $R_f = 0.15$ ) to yield pure **4k** (109 mg, 42% yield);  $^1\text{H NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$  (ppm): 7.11 (d, 1H,  $J = 0.9$  Hz), 7.79 (dd, 1H,  $J = 7.8, 1.5$  Hz), 7.86 (d, 1H,  $J = 7.8$  Hz), 8.17-8.19 (m, 1H).

**Methyl 1-methoxy-6-phenyl-1*H*-indole-2-carboxylate (7).** A solution of **4i** (120 mg, 0.45 mmol) in anhydrous THF was treated with DBU (1.5 mL) and iodomethane (0.8 mL), and the resulting mixture was stirred at RT for 1 hour. An aqueous 1N HCl solution was added and the mixture was extracted with AcOEt. The organic phase was dried and evaporated to afford a crude residue that

was purified by column chromatography (*n*-hexane/ethyl acetate 8:2,  $R_f = 0.48$ ) to give pure **7** (99 mg, 79% yield);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 3.96 (s, 3H), 4.24 (s, 3H), 7.13 (d, 1H,  $J = 0.7$  Hz), 7.37-7.52 (m, 4H), 7.67-7.72 (m, 4H).

**Preparation of final products 1a-g, 1k, 8, and 10h,i. General procedure:** Methyl esters **4a-g**, **4k**, **7**, and **9h,i** (0.5 mmol) were dissolved in a 1:1 mixture of THF/methanol (5 mL) and treated with 1.5 mL of 2N aqueous solution of LiOH. The reaction was monitored by TLC and, after consumption of starting material, treated with 1N aqueous HCl and extracted with EtOAc. The organic phase was dried and evaporated to afford the desired carboxylic acid derivatives.

**1-Hydroxy-1*H*-indole-2-carboxylic acid (1a).**<sup>5</sup> (98% yield from **4a**)  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta$  (ppm): 7.00 (d, 1H,  $J = 0.9$  Hz), 7.08 (ddd, 1H,  $J = 8.1, 6.8, 1.1$  Hz), 7.31 (ddd, 1H,  $J = 8.4, 6.8, 1.1$  Hz), 7.43 (dq, 1H,  $J = 8.4, 1.1$  Hz), 7.63 (dt, 1H,  $J = 8.1, 1.0$  Hz), 11.73 (bs, 1H).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ )  $\delta$  (ppm): 106.17, 110.38, 121.52, 122.96, 123.14, 126.03, 126.38, 136.92, 162.25. MS  $m/z$  177 ( $\text{M}^+$ , 100), 161 159 ( $\text{M}^+ - \text{O}$ , 28), 159 ( $\text{M}^+ - \text{H}_2\text{O}$ , 13), 133 ( $\text{M}^+ - \text{CO}_2$ , 5), 115 ( $\text{M}^+ - \text{H}_2\text{O} - \text{CO}_2$ , 44). HPLC,  $t_R$  7.1 min.

**1-Hydroxy-4-methyl-1*H*-indole-2-carboxylic acid (1b).** (79% yield from **4b**)  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta$  (ppm): 2.48 (s, 3H), 6.88 (d, 1H,  $J = 6.4$  Hz), 7.02 (s, 1H), 7.15-7.27 (m, 2H), 11.37 (bs, 1H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  (ppm): 18.14, 105.06, 108.14, 121.51, 122.83, 126.37, 126.55, 132.72, 137.66, 163.86. MS  $m/z$  191 ( $\text{M}^+$ , 100), 175 ( $\text{M}^+ - \text{O}$ , 6), 146 ( $\text{M}^+ - \text{CO}_2 - \text{H}$ , 5), 129 ( $\text{M}^+ - \text{H}_2\text{O} - \text{CO}_2$ , 19). HPLC,  $t_R$  7.9 min.

**1-Hydroxy-4-trifluoromethyl-1*H*-indole-2-carboxylic acid (1c).** (83% yield from **4c**)  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ )  $\delta$  (ppm): 6.99 (qd, 1H,  $J = 1.7, 0.8$  Hz), 7.43-7.53 (m, 2H), 7.75-7.80 (m, 1H).  $^{13}\text{C}$  NMR ( $\text{acetone-}d_6$ )  $\delta$  (ppm): 103.60, 114.89, 117.91 (q,  $J = 3.0$  Hz), 119.40 (q,  $J = 5.2$  Hz), 123.27 (q,  $J = 34.8$  Hz), 125.16, 125.51 (q,  $J = 260.9$  Hz), 128.31, 136.96, 161.39. MS  $m/z$  245 ( $\text{M}^+$ , 100), 229 ( $\text{M}^+ - \text{O}$ , 9), 183 ( $\text{M}^+ - \text{H}_2\text{O} - \text{CO}_2$ , 33). HPLC,  $t_R$  8.8 min.

**4-Chloro-1-hydroxy-1*H*-indole-2-carboxylic acid (1d).** (99% yield from **4d**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 6.97 (s, 1H), 7.19 (dd, 1H, *J* = 7.3, 0.9 Hz), 7.31 (t, 1H, *J* = 7.8 Hz), 7.44 (d, 1H, *J* = 8.2 Hz). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>) δ (ppm): 103.64, 109.57, 121.14, 123.80, 126.67, 127.22, 127.82, 137.29, 161.65. MS *m/z* 211 (M<sup>+</sup>, 100), 195 (M<sup>+</sup> –O, 10), 149 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub>, 13), 114 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub> –Cl, 34). HPLC, *t*<sub>R</sub> 7.9 min.

**4-Bromo-1-hydroxy-1*H*-indole-2-carboxylic acid (1e).** (97% yield from **4e**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 6.88 (d, 1H, *J* = 0.9 Hz), 7.23 (t, 1H, *J* = 7.8 Hz), 7.35 (dd, 1H, *J* = 7.4, 1.0 Hz), 7.48 (dt, 1H, *J* = 8.1, 1.0 Hz). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>) δ (ppm): 105.06, 110.13, 116.53, 122.72, 124.29, 126.82, 127.38, 136.87, 161.61. MS *m/z* 257 (<sup>81</sup>Br: M<sup>+</sup>, 91), 255 (<sup>79</sup>Br: M<sup>+</sup>, 100), 241 (<sup>81</sup>Br: M<sup>+</sup> –O, 5), 239 (<sup>79</sup>Br: M<sup>+</sup> –O, 8), 114 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub> –Br, 66). HPLC, *t*<sub>R</sub> 8.5 min.

**6-Bromo-1-hydroxy-1*H*-indole-2-carboxylic acid (1f).** (99% yield from **4f**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 7.03 (d, 1H, *J* = 0.7 Hz), 7.22 (dd, 1H, *J* = 8.0, 1.7 Hz), 7.59-7.63 (m, 2H). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>) δ (ppm): 106.35, 113.07, 119.37, 121.32, 124.83, 124.92, 127.42, 137.38, 161.67. MS *m/z* 257 (<sup>81</sup>Br: M<sup>+</sup>, 93), 255 (<sup>79</sup>Br: M<sup>+</sup>, 100), 241 (<sup>81</sup>Br: M<sup>+</sup> –O, 4), 239 (<sup>79</sup>Br: M<sup>+</sup> –O, 7), 114 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub> –Br, 39). HPLC, *t*<sub>R</sub> 8.3 min.

**1-Hydroxy-4-phenyl-1*H*-indole-2-carboxylic acid (1g).** (89% yield from **4g**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 7.03 (d, 1H, *J* = 0.7 Hz), 7.19 (dd, 1H, *J* = 6.6, 1.7 Hz), 7.37–7.57 (m, 5H), 7.64–7.69 (m, 2H). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>) δ (ppm): 105.31, 109.61, 120.50, 121.17, 126.43, 126.73, 128.29, 129.37 (2C), 129.55 (2C), 136.65, 137.43, 140.67, 162.01. MS *m/z* 253 (M<sup>+</sup>, 100), 237 (M<sup>+</sup> –O, 8), 191 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub>, 25), 190 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub> –H, 62), 165 (M<sup>+</sup> –H<sub>2</sub>O –CO<sub>2</sub> –C<sub>2</sub>H<sub>2</sub>, 62). HPLC, *t*<sub>R</sub> 8.9 min.

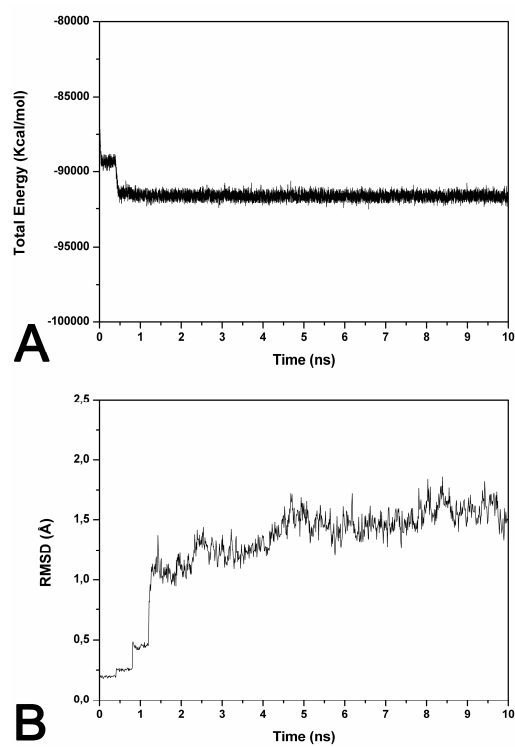
**1-Hydroxy-6-tetrazolyl-1*H*-indole-2-carboxylic acid (1k).** (97% yield from **4k**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 7.11 (d, 1H, *J* = 0.9 Hz), 7.79 (dd, 1H, *J* = 7.8, 1.5 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 8.17-8.19 (m, 1H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 104.63, 108.58, 118.82, 122.66, 123.28, 128.85, 135.40, 160.84. HPLC, *t*<sub>R</sub> 1.4 min.

**1-Methoxy-6-phenyl-1*H*-indole-2-carboxylic acid (8).** (99% yield from **7**)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 4.26 (s, 3H), 7.31 (s, 1H), 7.39-7.53 (m, 4H), 7.67-7.76 (m, 4H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 66.40 (OMe), 107.66, 109.35, 121.19, 121.86, 123.37, 124.59, 127.62 (2C), 128.11, 128.98 (2C), 136.43, 140.16, 141.38, 164.60. MS  $m/z$  267 ( $\text{M}^+$ , 50), 253 ( $\text{M}^+ - \text{CH}_2$ , 30), 237 ( $\text{M}^+ - \text{O} - \text{CH}_2$ , 9), 191 ( $\text{M}^+ - \text{CH}_2 - \text{H}_2\text{O} - \text{CO}_2$ , 40), 190 ( $\text{M}^+ - \text{CH}_2 - \text{H}_2\text{O} - \text{CO}_2 - \text{H}$ , 100), 165 ( $\text{M}^+ - \text{CH}_2 - \text{H}_2\text{O} - \text{CO}_2 - \text{C}_2\text{H}_2$ , 21). HPLC,  $t_{\text{R}} = 8.6$  min (purity = 93%, major impurity accounting for >6% of residual peak area identified as **10i**).

**5-Phenyl-1*H*-indole-2-carboxylic acid (10h).** (99% yield from **9h**)  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  (ppm): 7.27 (d, 1H,  $J = 2.2$  Hz), 7.32-7.35 (m, 1H), 7.42-7.50 (m, 2H), 7.62-7.72 (m, 4H), 7.95-7.97 (m, 1H), 10.93 (bs, 1H).  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  (ppm): 109.12, 113.58, 121.03, 125.29, 127.29, 127.73, 127.76 (2C), 128.93, 129.55 (2C), 134.43, 137.81, 142.75, 162.83. MS  $m/z$  237 ( $\text{M}^+$ , 100), 191 ( $\text{M}^+ - \text{HCOOH}$ , 10), 190 ( $\text{M}^+ - \text{HCOOH} - \text{H}$ , 26), 165 ( $\text{M}^+ - \text{HCOOH} - \text{C}_2\text{H}_2$ , 11). HPLC,  $t_{\text{R}} 8.2$  min.

**6-Phenyl-1*H*-indole-2-carboxylic acid (10i).** (99% yield from **9i**)  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  (ppm): 7.23 (dd, 1H,  $J = 2.2, 0.9$  Hz), 7.34-7.52 (m, 4H), 7.67-7.80 (m, 4H), 10.94 (bs, 1H).  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$  (ppm): 108.70, 111.19, 121.00, 123.38, 126.10, 127.80, 127.89 (2C), 129.62 (2C), 129.65, 138.76, 142.60, 162.60. MS  $m/z$  237 ( $\text{M}^+$ , 38), 191 ( $\text{M}^+ - \text{HCOOH}$ , 40), 190 ( $\text{M}^+ - \text{HCOOH} - \text{H}$ , 100). HPLC,  $t_{\text{R}} = 9.2$  min.

**Figure S1.**



**Figure S1.** Analysis of the MD simulation of the LDH-1j complex. Total energy (kcal/mol) of the system plotted *vs* time (A) and RMSD in angstrom of the  $\alpha$  carbon of the protein from the starting model structure during the simulation (B) are reported.

**Table S1.** Hydrogen Bonds Analysis of the LDH-**1j** interactions during the last 5.5 ns of MD Simulation.<sup>a</sup>

donor	acceptorh	acceptor	distance (Å)	% occupied
WAT@O	LIG@H	LIG@O3	2.6	99
LIG@O1	T248@H1	T248@OG1	2.7	93
LIG@O2	R169@H2	R169@NH2	2.8	88
LIG@O1	R169@H1	R169@NH1	2.8	70
LIG@O3	T248@H2	T248@N	2.9	51
H193@NE2	WAT@H2	WAT@O	2.8	41
H193@NE2	WAT@H1	WAT@O	2.8	37

<sup>a</sup> WAT = water, LIG = ligand **1j**.

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