Supporting Information

Harmaline Analogs as Substrate−Selective Cyclooxygenase−2 Inhibitors

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1. Chemical synthesis, spectroscopic, and chromatographic characterization

6-Methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (1). To a stirred solution of 2-(5-methoxy-1*H*-indol-3-yl)ethan-1-amine (1.9 g, 10 mmol) in CH_2Cl_2 (100 mL) was added triethylamine (1 g, 10 mmol) followed by acetyl chloride (0.7 g, 10 mmol) at 0 ºC. After stirring 5 h at room temperature, toluene (100 mL) followed by $POCl_3$ (19.68 g, 129 mmol, 12 mL) were added dropwise, and the resultant reaction mixture was refluxed for 7 h. The reaction mixture was cooled to room temperature, and the pH was adjusted to 9 by adding a saturated aqueous Na_2CO_3 solution. The product was extracted with CH_2Cl_2 (3x50 mL). After evaporation of the organic layer, the gummy mass was purified by silica gel column chromatography using $CHCl_3$:MeOH:NH_4OH (35:7:1) to give 1 g of pure 6-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (**1**) in 50% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.40 (t, J = 7.5 Hz, 2H), 1.90 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H), 3.83 (s, 3H), 6.92 (dd, J = 9.1, 2.9 Hz, 1H), 7.11 (d, J = 2.9 Hz, 1H), 7.53 (d, J = 9.1 Hz, 1H), 11.63 (br s, 1H). Mass (ESI) $(M+H)^+$ calcd for 215.11; found 215.21. HRMS *m/z* calcd for $[C_{13}H_{14}N_2O + H]^+$ 215.1125; found, 215.1156. HPLC purity 99.9%.

7-Methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (2). To a stirred solution of 2-(6-methoxy-1*H*-indol-3-yl)ethan-1-amine (1.9 g, 10 mmol) in CH_2Cl_2 (100 mL) was added triethylamine (1 g, 10 mmol) followed by acetyl chloride (0.7 g, 10 mmol) at 0 ºC. After stirring 5 h at room temperature, toluene (100 mL) followed by $POCl_3$ (19.68 g, 129 mmol, 12 mL) were added dropwise, and the resultant reaction mixture was refluxed for 7 h. The reaction mixture was cooled to room temperature, and the pH was adjusted to 9 by adding a saturated aqueous Na_2CO_3 solution. The product was extracted with CH_2Cl_2 (3x50 mL). After evaporation of the organic layer, the gummy mass was purified by silica gel column chromatography using $CHCl_3$:MeOH:NH_4OH (35:7:1) to give 1.12 g of pure 7-methoxy-1-

methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole **(2)** in 56% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.39 (t, J = 7.5 Hz, 2H), 1.92 (s, 3H), 2.33 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 6.90-6.95 (m, 2H), 7.53 (d, J = 9.2 Hz, 1H), 11.63 (br s, 1H). HRMS *m/z* calcd for $[C_{13}H_{14}N_2O + H]^+$ 215.1125; found, 215.1163. HPLC purity 99.9%.

9-(4-Chlorobenzyl)-6-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (3). To a cold (0 °C) stirred slurry of NaH (0.3 g, 12.5 mmol) in DMF (50 mL) was added 6-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (1.07 g, 5 mmol). After stirring for 1 h at 0 ºC, 1-(bromomethyl)-4-chlorobenzene (1 g, 5 mmol) was added, and the mixture was stirred 1 h at room temperature. The reaction mixture was then poured into a crushed ice/water bath (100 g) and acidified with 10% aqueous HCl (pH 4). The resultant solid was filtered, washed with cold water, and dried under vacuum. The crude product was purified using silica gel column chromatography (35 : 7 : 1, $CHCl_3$: MeOH : NH_4OH) to give 1 g pure 9-(4-chlorobenzyl)-6-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole **(3)** in 72% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.35 (s, 3H), 2.73 (t, J = 7.5 Hz, 2H) 3.75 (t, J = 7.5 Hz, 2H), 3.83 (s, 3H), 5.81 (s, 2H), 6.72 (dd, J = 9.1, 2.9 Hz, 1H), 7.10 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 2.9 Hz, 1H), 7.95 (d, J = 9.1 Hz, 1H). HRMS *m/z* calcd for $[C_{20}H_{19}ClN_2O + H]^+$ 339.1247; found, 339.1239. HPLC purity 99.9%.

9-(4-Chlorobenzyl)-7-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (4). To a cold (0 °C) stirred slurry of NaH (0.6 g, 25 mmol) in DMF (100 mL) was added 7-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole (2.14 g, 10 mmol). After stirring for 1 h at 0 ºC, 1-(bromomethyl)-4-chlorobenzene (2.05 g, 10 mmol) was added, and the mixture was stirred 1 h at room temperature. The reaction mixture was then poured into a crushed ice/water bath (100 g) and acidified with 10% aqueous HCl (pH 4). The resultant solid was filtered, washed with cold water, and dried under vacuum. The crude product was purified using silica gel column chromatography (35 : 7 : 1, $CHCl_3$: MeOH : NH_4OH) to give 2.1 g

pure 9-(4-chlorobenzyl)-7-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole **(4)** in 64% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.36 (t, J = 7.5 Hz, 2H), 2.22 (t, J = 7.5 Hz, 2H), 3.80 (s, 3H), 6.93 (dd, J = 9.1, 2.9 Hz, 1H), 7.12 (d, J = 2.9 Hz, 1H), 7.34 (d, J = 9.1 Hz, 1H). HRMS *m/z* calcd for $[C_{20}H_{19}ClN_2O + H]^+$ 339.1247; found, 339.1254. HPLC purity 99.9%.

6-Methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylic acid (5). To a stirred solution of 2-(5-methoxy-1*H*-indol-3-yl)ethan-1-amine (1.9 g, 10 mmol) in CH_2Cl_2 (100 mL) was added triethylamine (1 g, 10 mmol) followed by *t*-butyl 2-chloro-2-oxoacetate (1.65 g, 10 mmol) at 0 ºC. After stirring 5 h at room temperature, toluene (100 mL) followed by $POCl_3$ (19.68 g, 129 mmol, 12 mL) were added dropwise, and the resultant reaction mixture was refluxed for 7 h. The reaction mixture was cooled to room temperature, and the pH was adjusted to 9 by adding a saturated aqueous Na_2CO_3 solution. The product was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were evaporated to dryness to give *tert*-butyl 6-methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylate as a gummy mass, to which 2,2,2-trifluoroacetic acid (5 mL) was added. After the reaction mixture was stirred for 1 h at room temperature, it was evaporated to dryness, and the crude product was purified using silica gel column chromatography (35 : 7 : 1, $CHCl_3$: MeOH : NH_4OH) to give 2.2 g of pure 6-methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylic acid (**5**) in 90% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.78 (s, 3H), 6.59 (dd, J = 9.2, 2.4 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 8.6 Hz, 2H), 8.45 (d, J = 9.2 Hz, 1H), 11.53 (br s, 1H), 12.63 (s, 1H). HRMS *m/z* calcd for $[C_{13}H_{12}N_2O_3 - H]^-$ 243.0874; found, 243.0869. HPLC purity 99.9%.

9-(4-Chlorobenzyl)-6-methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylic acid (6). To a cold (0 ºC) stirred slurry of NaH (0.6 g, 25 mmol) in DMF (100 mL) was added 6-

methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylic acid (2.44 g, 10 mmol). After stirring for 1 h at 0 ºC, 1-(bromomethyl)-4-chlorobenzene (2 g, 10 mmol) was added, and the mixture was stirred 1 h at room temperature. The reaction mixture was then poured into a crushed ice/water bath (100 g) and acidified with 10% aqueous HCl (pH 4). The resultant solid was filtered, washed with cold water, and dried under vacuum. The crude product was purified using silica gel column chromatography (35 : 7 : 1, $CHCl_3$: MeOH : NH_4OH) to give 2.3 g pure 9-(4-chlorobenzyl)-6-methoxy-4,9-dihydro-3*H*-pyrido[3,4-*b*]indole-1-carboxylic acid **(6)** in 63% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.43 (t, *J* = 7.5 Hz, 2H) 2.24 (t, *J* = 7.5 Hz, 2H), 3.85 (s, 3H), 5.84 (s, 2H), 6.71 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.54 (d, *J* = 2.9 Hz, 1H), 7.85 (d, *J* = 9.1 Hz, 1H), 12.55 (s, 1H). HRMS *m/z* calcd for $[C_{20}H_{17}ClN_2O_3 - H]^-$ 367.0935; found, 367.0941. HPLC purity 99.9%.

2. LC-MS/MS Assay

We evaluated the substrate-selective COX-2 inhibitory activity of compound **3** in presence of both aa and 2-AG. The assay was performed using a reaction mixture containing 15 nM purified murine COX-2, 30 nM heme, 10 μM AA and 2-AG, 1 μM 5-phenyl-4-pentenyl-1-hydroperoxide (PPHP), and compound **3** or vehicle (DMSO at a final concentration of 5%) in a buffer of 50 mm tris-HCl, pH 8.0, 0.5 mM phenol. following addition of compound **3,** the mixture was incubated for 15-min, and then the reaction was initiated by the addition of both AA and 2-AG together. After 10s, the reaction was quenched by addition of ethyl acetate containing internal standards (PGE_2-d_4 and PGE_2g-d_5) at 0.3 μm. analytes of interest were detected by selected reaction monitoring MS/MS using the following transitions: PGE_2/D_2 *m/z* 370→317; PGE_2-d_4 *m/z* 374→321; PGE_2/d_2-G *m/z* 444→391; PGE_2-G-d_5 *m/z* 449→396. Analyte peak areas were normalized to those of their deuterated internal standards for the quantification of product formation and inhibition.

Table 1s. Statistics of X-ray Data Collection and Structure Refinement

	COX-2•compound **3**
Data Collection	PDB: 6V3R
Wavelength (Å)	0.9792
Resolution range (Å)	102.4 - 2.66 (2.76 - 2.66)
Space group	*C*2
Unit cell a, b, c (Å)	217.2, 124.4, 136.5
α, β, γ (°)	90, 123.8, 90
Total reflections	262,849 (22,097)
Unique reflections	84,726 (7,446)
Multiplicity	3.1 (3.0)
Completeness (%)	98.0 (85.9)
Mean $I/\sigma(I)$	8.43 (1.05)
Wilson B-factor (Å^2)	43.03
R-merge	0.1566 (1.074)
$CC_{1/2}$	0.988 (0.513)
CC*	0.997 (0.823)
Refinement	
R_{work}/R_{free} (%)	22.7/26.7 (36.5/38.9)
Number of atoms	18,844

protein/ligands/water	17,996/532/316
Protein residues	2,208
Root Mean Square bonds/angles (Å²/°)	0.01/0.78
Ramachandran favored /outlier (%)	96/0.18
Average B-factor (Å²)	50
protein/ligands/water	49.9/58.3/40.3

*Number of crystals for both datasets = 1; the values in parentheses are for the highest resolution shell; $R_{merge} = \frac{\sum_{hkl}\sum_i |I_i(hkl) - \overline{I_i(hkl)}|}{\sum_{hkl}\sum_i I_i(hkl)} \times 100\%$; $R = \frac{\sum_{hkl}||F_o| - |F_c||}{\sum_{hkl}|F_o|} \times 100\%$, where F_o and F_c are the observed and calculated structure factors, and R_{free} is the value from the test set (3.0% of all reflections).