Substrate-Selective Inhibition of Cyclooxygenase-2: Development and Evaluation of Achiral Profen Probes

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Supplimental Figures:

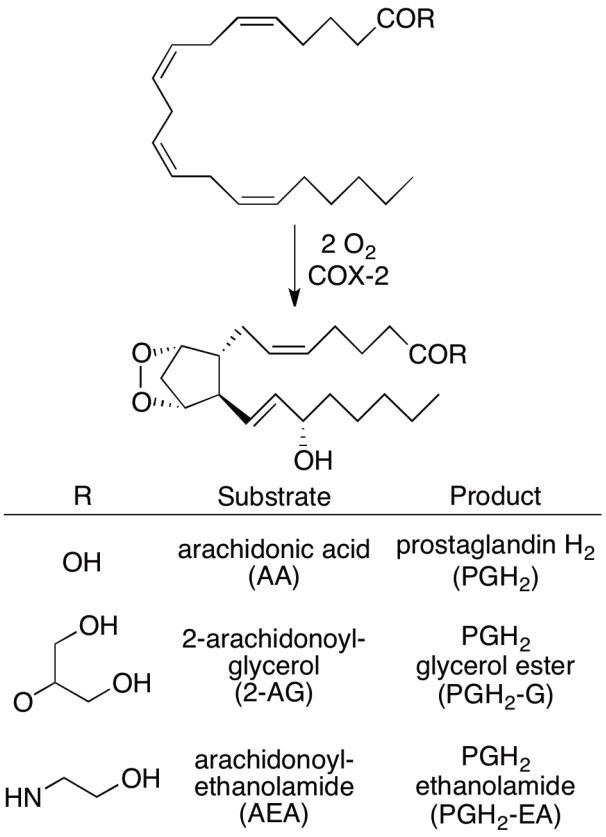

Figure S1. Oxygenation catalyzed by COX-2. Substrates and products are shown underneath.

Table S1. Inhibition of mCOX-2 Dependent Oxygenation of 2-AG and AA by Chiral Profens *In Vitro*[a]

Compound	IC_{50} (μM)[b] 2-AG	% Inhibition[c] AA
7a[d]	0.08	0
7b[d]	3	0
7c[d]	10	0
7d	- (25%)	10
7e	7.3 +/- 0.4 (56%)	20
(*S*)-fenoprofen	0.9 +/- 0.5	30
(*S*)-ketoprofen	0.2 +/- 0.04	55
6d[e]	0.9 +/- 0.2	20
6e[e]	0.3 +/- 0.04	50

[a] IC_{50} values were determined by incubating five concentrations of inhibitor and a solvent control in DMSO with purified murine COX-2 (40 nM) for three min followed by addition of 2-AG or AA (5 μM) at 37 °C for 30 s. [b] Mean ± standard deviation (n = 6); dash (-) indicates < 50% inhibition of 2-AG oxygenation at 10 μM inhibitor. Numbers in parentheses indicate maximum inhibition (when not equal to 100%) at 10 μM inhibitor. [c] % inhibition of AA oxygenation measured at 10 μM inhibitor. [d] Data taken from reference 10. [e] n = 3.

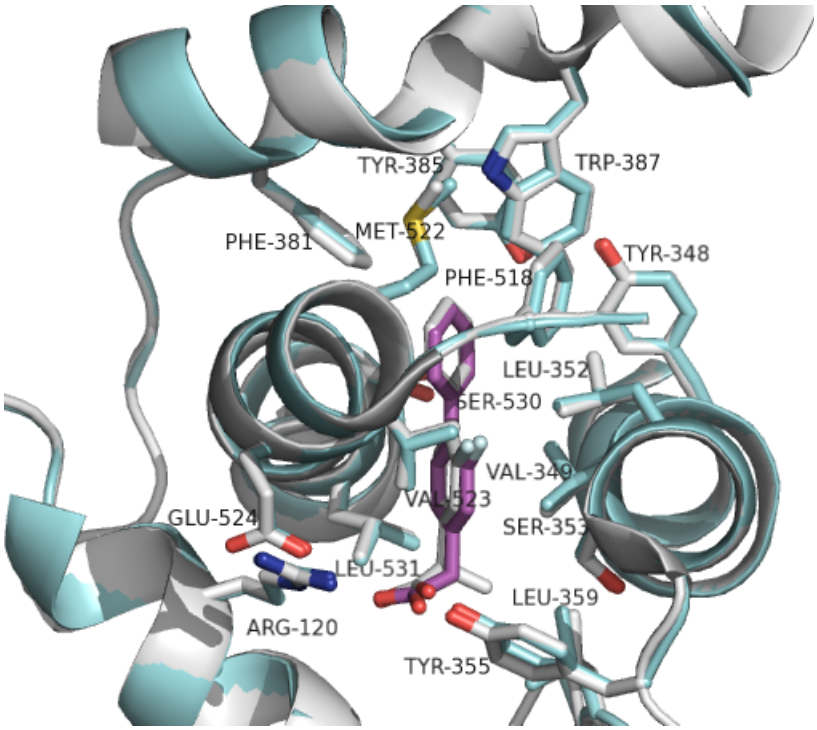

Figure S2. Superimposed active site of the complexes of mCOX-2: desmethylflurbiprofen (PDB id: 4FM5) and mCOX-2:(*R*)-flurbiprofen (PDB id: 3RR3). Desmethylflurbiprofen is illustrated in magenta sticks while corresponding protein is shown in cyan; (*R*)-flurbiprofen is shown in grey with corresponding protein in grey.

Raw material supplies and instrumental:

Silica gel column chromatography was performed using Sorbent silica gel standard grade, porosity 60Å, particle size 32-63 µm (230 x 450 mesh), surface area 500 – 600 m_2/g, bulk density 0.4 g/mL, pH range 6.5 – 7.5, purchased from Sorbent Technologies (Atlanta, GA). Desmethylketoprofen **3e** was purchased from Toronto Research Chemicals. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. ^{1}H and ^{13}C NMR were taken on a Bruker AV-I console operating at 400 MHz. Mass spectrometric analyses were performed on a ThermoElectron Surveyor pump TSQ 7000 instrument in ESI positive or negative ion mode. HPLC was performed with a Waters 2695 Separations Module with detection by a

Waters 2487 Dual λ Absorbance Detector at 260 nm and 285 nm. Light scattering was performed on a Sedex 75.

General synthetic procedures:

General procedure 1: synthesis of benzyl aldehydes 2a-2d[1]: (Methoxymethyl)triphenylphosphonium chloride (4.0 eq) was suspended in dry THF (0.2 M) and cooled to 0 ^{0}C under an argon atmosphere. A solution of potassium *tert*-butoxide in THF (4.0 eq) was added slowly to the suspension and allowed to stir for 45 minutes at 0 ^{0}C. The desired aryl aldehyde (e.g. **1a**) (3.0 mmol, 1.0 eq) was added dropwise and the solution was allowed to stir at room temperature for one hour. The reaction was quenched with saturated NH_4Cl and extracted 3 x with EtOAc. The organic phase was dried with $MgSO_4$, filtered, and concentrated to yield an oil. The oil was then dissolved in a 5:2 THF:5 N HCl solution (0.2 M) and refluxed for one hour. The solution was cooled to room temperature, quenched with saturated $NaHCO_3$ and extracted 3 x with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield an oil that was purified via silica gel column chromatography eluting with 30:1 hexanes:EtOAc.

General procedure 2: synthesis of desmethylprofens 3a-3d: The desired benzyl aldehyde (e.g. **2a)** (2.5 mmol, 1 eq) was dissolved in a 1:1 solution of H_2O:*t*-BuOH (0.1 M). In order, 2,3-dimethyl-2-butene (20 eq), KH_2PO_4 (2.0 eq) and sodium chlorite (2.5 eq) were added to the solution and allowed to stir for 40 minutes at room temperature. The reaction was extracted 3 x with EtOAc and the combined organic layers were washed with a saturated NaCl solution, dried with $MgSO_4$ and concentrated to yield an off-white solid. The crude product was purified via silica gel column chromatography eluting with 9:1 hexanes:EtOAc to give a white solid.

General procedure 3: synthesis of dimethyl profen methyl esters: A racemic profen (e.g. **6a**, 0.5 mmol, 1 eq) was dissolved in MeOH (0.2 M). A drop of concentrated H_2SO_4 was added and the

solution was refluxed for two hours. The reaction was quenched with sat. $NaHCO_3$, extracted 3x with EtOAc, dried with $MgSO_4$ and concentrated to yield the methyl-ester protected racemic profen as an oil. This oil was dissolved in dry THF (0.2 M) and cooled to -78 ^oC under argon. A solution of LDA in THF (1.5 eq) was added slowly and allowed to stir at -78 ^oC for 30 minutes. The solution was warmed to 0 ^oC and HMPA (1.4 eq) was added slowly and allowed to stir at 0 ^oC for 30 minutes. Finally, 1.9 eq. of MeI was added dropwise to the solution and allowed to stir for 30 minutes at 0 ^oC and then thirty minutes at room temperature. The reaction was quenched with NH_4Cl, extracted 3x with EtOAc, dried with $MgSO_4$ and concentrated to yield an orange oil. The crude product was purified via silica gel column chromatography eluting with 9:1 hexanes:EtOAc to give an oil. Note: Compounds **3a-e** can be used as the starting material with this procedure as long as the equivalents of LDA, HMPA, and MeI are doubled from those described above.

General procedure 4: synthesis of cyclopropyl profen methyl esters: A desmethylprofen (e.g. **3a**, 0.5 mmol, 1 eq) was dissolved in MeOH (0.2 M). A drop of concentrated H_2SO_4 was added and the solution was refluxed for two hours. The reaction was quenched with sat. $NaHCO_3$, extracted 3x with EtOAc, dried with $MgSO_4$ and concentrated to yield the methyl-ester protected profen as an oil. This oil was dissolved in dry THF (0.2 M) and cooled to -78 ^oC under argon. A solution of LDA in THF (2.5 eq) was added slowly and allowed to stir at -78 ^oC for 30 minutes. The solution was warmed to 0 ^oC and HMPA (2.0 eq) was added slowly and allowed to stir at 0 ^oC for 30 minutes. Finally, 1.5 eq. of 1,2-dibromoethane was added dropwise to the solution and allowed to stir for 30 minutes at 0 ^oC and then thirty minutes at room temperature. The reaction was quenched with NH_4Cl, extracted 3x with EtOAc, dried with $MgSO_4$ and concentrated to yield an orange oil. The crude product was purified via silica gel column chromatography eluting with 9:1 hexanes:EtOAc to give an oil.

General procedure 5: synthesis of dimethyl (4a-e) and cyclopropyl (5a-e) profens: A methyl-ester protected dimethyl profen *or* a methyl-ester protected cyclopropyl profen (0.2 mmol, 1.0 eq) was dissolved in dry THF (0.2 M). KOTMS (2.0 eq) was added to the reaction flask and refluxed for two hours. The slurry was quenched at room temperature with NH_4Cl, extracted 3 x with EtOAc, dried with $MgSO_4$ and concentrated to yield an off-white solid. The crude product was purified via silica gel column chromatography eluting with 30:1 DCM:MeOH to give a dimethyl profen (e.g. **4a**) *or* a cyclopropyl profen (e.g. **5a**), respectively, as a white solid.

Characterization of compounds:

2-(2-fluoro-[1,1'-biphenyl]-4-yl)acetaldehyde (**2a**): **2a** was prepared via general procedure 1 as a clear oil (56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (t, 1H), 7.60-7.58 (m, 2H), 7.40-7.52 (m, 4H), 7.10-7.08 (m, 2H), 3.77 (d, 2H). MS *m/z* (ESI): calc. for $C_{14}H_{11}FO$ $[M-H]^-$ 213.08, found 213.4.

2-(6-methoxynaphthalen-2-yl)acetaldehyde (**2b**): **2b** was prepared via general procedure 1 as a clear oil (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (t, 1H), 7.72-7.78 (m, 2H), 7.64 (s, 1H), 7.28-7.32 (m, 1H), 7.15-7.20 (m, 2H), 3.95 (s, 3H), 3.83 (d, 2H). MS *m/z* (ESI): calc. for $C_{13}H_{12}O_2$ $[M-H]^-$ 199.08, found 199.4.

2-(4-isobutylphenyl)acetaldehyde (**2c**): **2c** was prepared via general procedure 1 as a clear oil (77% yield). Note: intermediate and product are volatile when heated under reduced pressure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, 1H), 7.16 (d, 2H), 7.10 (d, 2H), 3.65 (d, 2H), 2.47 (d, 2H), 1.85-1.83 (m, 1H), 0.89 (d, 6H). MS *m/z* (ESI): calc. for $C_{12}H_{16}O$ $[M-H]^-$ 175.08, found 175.4.

2-(3-phenoxyphenyl)acetaldehyde (**2d**): **2d** was prepared via general procedure 1 as a clear oil (75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (t, 1H), 7.33-7.31 (m, 3H), 7.13-7.11 (m, 1H), 6.88-7.10-7.08 (m, 4H), 3.66 (d, 2H). MS *m/z* (ESI): calc. for $C_{14}H_{12}O_2$ $[M-H]^-$ 211.07, found 211.27.

2-(2-fluoro-[1,1'-biphenyl]-4-yl)acetic acid (**3a**): **3a** was prepared via general procedure 2 as a white solid (58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, 2H), 7.50-7.35 (m, 4H), 7.20-7.10 (m, 2H), 3.70 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.1, 160.8, 159.2, 135.6, 134.7 (d, J_{C-F} = 150 Hz), 131.0 (d, J_{C-F} = 20 Hz), 129.1, 128.6, 127.8, 125.5 (d, J_{C-F} = 20 Hz), 117.3, 117.1, 40.4. HRMS *m/z* (ESI): calc. for $C_{14}H_{11}FO_2$ $[2M+Na]^-$ 481.1233, found 481.1245.

2-(6-methoxynaphthalen-2-yl)acetic acid (**3b**): **3b** was prepared via general procedure 2 as a white solid (94% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.75-7.65 (m, 3H), 7.38-7.32 (d, 1H), 7.30 (s, 1H), 7.11-7.08 (d, 1H), 3.9 (s, 3H), 3.72 (s, 2H). MS *m/z* (ESI): calc. for $C_{13}H_{12}O_3$ $[M-H]^-$ 215.08, found 215.23.

2-(4-isobutylphenyl)acetic acid (**3c**): **3c** was prepared via general procedure 2 as a white solid (89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, 2H), 7.15 (d, 2H), 3.64 (s, 2H), 2.5 (d, 2H), 1.93-1.82 (m, 1H), 0.94-0.92 (d, 6H). MS *m/z* (ESI): calc. for $C_{12}H_{16}O_2$ $[M-H]^-$ 191.12, found 191.19.

2-(3-phenoxyphenyl)acetic acid (**3d**): **3d** was prepared via general procedure 2 as a white solid (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.23 (m, 3H), 7.12-7.07 (m, 1H), 7.01-6.99 (m, 3H), 6.98-6.88 (m, 2H), 3.62 (s, 3H). MS *m/z* (ESI): calc. for $C_{14}H_{12}O_3$ $[M-H]^-$ 227.08, found 227.07.

Methyl-2-(2-fluoro-[1,1'-biphenyl]-4-yl)-2-methylpropanoate: The methyl-ester protected form of **4a** was prepared via general procedure 3 as an oil (57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.50 (m, 2H), 7.47-7.30 (m, 4H), 7.17-7.10 (m, 2H), 3.70 (s, 3H), 1.61 (s, 6H). MS *m/z* (ESI): calc. for $C_{17}H_{17}FO_2$ $[M+Na]^+$ 295.12, found 295.0.

Methyl-2-(6-methoxynaphthalen-2-yl)-2-methylpropanoate: The methyl-ester protected form of **4b** was prepared via general procedure 3 as an oil (58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.68 (m, 3H), 7.44-7.40 (d, 1H), 7.17-7.11 (m, 2H), 3.92 (s, 3H), 3.66 (s, 3H), 1.67 (s, 6H). MS *m/z* (ESI): calc. for $C_{16}H_{18}O_3$ $[M+Na]^+$ 281.13, found 281.07.

Methyl-2-(4-isobutylphenyl)-2-methylpropanoate: The methyl-ester protected form of **4c** was prepared via general procedure 3 as an oil (85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.20 (d, 2H), 7.10 (d, 2H), 3.68 (s, 3H), 2.47 (d, 2H), 1.90-1.80 (m, 1H), 1.60 (s, 6H), 0.93 (d, 6H). MS *m/z* (ESI): calc. for $C_{15}H_{22}O_2$ $[M+Na]^+$ 257.16, found 257.07.

Methyl-2-methyl-2-(3-phenoxyphenyl)propanoate: The methyl-ester protected form of **4d** was prepared via general procedure 3 as an oil (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.25 (m, 3H), 7.13-6.97 (m, 5H), 6.87-6.82 (m, 1H), 3.70 (s, 3H), 1.58 (s, 6H). MS *m/z* (ESI): calc. for $C_{17}H_{18}O_3$ $[M+Na]^+$ 293.13, found 293.0.

Methyl-2-(3-benzoylphenyl)-2-methylpropanoate: The methyl-ester protected form of **4e** was prepared via general procedure 3 as an oil (64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (m, 3H), 7.68-7.40 (m, 6H), 3.70 (s, 3H), 1.64 (s, 6H). MS *m/z* (ESI): calc. for $C_{18}H_{18}O_3$ $[M+Na]^+$ 305.13, found 305.20.

Methyl-1-(2-fluoro-[1,1'-biphenyl]-4-yl)cyclopropanecarboxylate: The methyl-ester protected form of **5a** was prepared via general procedure 4 as an oil (28% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57-7.49 (m, 2H), 7.45-7.33 (m, 4H), 7.20-7.12 (m, 2H), 3.66 (s, 3H), 1.66-1.62 (dd, 2H), 1.25-1.20 (dd, 2H). MS *m/z* (ESI): calc. for $C_{17}H_{15}FO_2$ $[M+Na]^+$ 293.11, found 293.10.

Methyl-1-(6-methoxynaphthalen-2-yl)cyclopropanecarboxylate: The methyl-ester protected form of **5b** was prepared via general procedure 4 as an oil (22% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70-7.68 (m, 3H), 7.46-7.44 (m, 1H), 7.15-7.12 (m, 2H), 3.92 (s, 3H), 3.62 (s, 3H), 1.67-1.64 (dd, 2H), 1.28-1.24 (dd, 2H). MS *m/z* (ESI): calc. for $C_{16}H_{16}O_3$ $[M+Na]^+$ 279.11, found 279.09.

Methyl-1-(4-isobutylphenyl)cyclopropanecarboxylate: The methyl-ester protected form of **5c** was prepared via general procedure 4 as an oil. Note: the product could not be isolated as a pure compound after column chromatography, but the impurities did not affect the next reaction (41%

crude yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.20 (d, 2H), 7.18-7.13 (d, 2H), 3.68 (s, 3H), 2.44-2.40 (d, 2H), 1.88-1.78 (m, 1H), 1.60-1.55 (dd, 2H), 1.28-1.24 (dd, 2H). MS *m/z* (ESI): calc. for $C_{15}H_{20}O_2$ $[M+Na]^+$ 255.15, found 255.12.

Methyl-1-(3-phenoxyphenyl)cyclopropanecarboxylate: The methyl-ester protected form of **5d** was prepared via general procedure 4 as an oil (17% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.21 (m, 3H), 7.12-7.00 (m, 5H), 6.88-6.84 (m, 1H), 3.63 (s, 3H), 1.59-1.57 (dd, 2H), 1.19-1.16 (dd, 2H). MS *m/z* (ESI): calc. for $C_{17}H_{16}O_3$ $[M+Na]^+$ 291.11, found 291.09.

Methyl-1-(3-benzoylphenyl)cyclopropanecarboxylate: The methyl-ester protected form of **5e** was prepared via general procedure 4 as an oil (18% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.78 (m, 3H), 7.7-7.66 (m, 1H), 7.61-7.54 (m, 2H), 7.52-7.39 (m, 3H), 3.64 (s, 3H), 1.67-1.63 (d, 2H), 1.25-1.21 (dd, 2H). MS *m/z* (ESI): calc. for $C_{17}H_{16}O_3$ $[M+Na]^+$ 303.11, found 303.15.

2-(2-fluoro-[1,1'-biphenyl]-4-yl)-2-methylpropanoic acid (**4a**): **4a** was prepared via general procedure 5 as a white solid (84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.50 (m, 2H), 7.45-7.37-7.35 (m, 4H), 7.17-7.08 (m, 2H), 1.65 (s, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 182.8, 161.9, 158.9, 146.1 (d, J_{C-F} = 150 Hz), 135.6, 131.5 (d, J_{C-F} = 20 Hz), 129.0, 128.5, 127.8, 122.0 (d, J_{C-F} = 20 Hz), 114.3, 113.9, 46.0, 27.0. HRMS *m/z* (ESI): calc. for $C_{16}H_{15}FO_2$ $[2M+Na]^-$ 537.1859, found 537.1871.

2-(6-methoxynaphthalen-2-yl)-2-methylpropanoic acid (**4b**): **4b** was prepared via general procedure 5 as a white solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.71 (m, 3H), 7.51-7.49 (m, 1H), 7.17-7.12 (2H), 3.93 (s, 3H), 1.70 (s, 6H). MS *m/z* (ESI): calc. for $C_{15}H_{16}O_3$ $[M-H]^-$ 243.11, found 243.31.

2-(4-isobutylphenyl)-2-methylpropanoic acid (**4c**): **4c** was prepared via general procedure 5 as a white solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.30 (d, 2H), 7.11-7.07 (d, 2H),

2.58-2.53 (d, 2H), 1.91-1.80 (m, 1H), 1.60 (s, 6H), 0.92-0.89 (d, 6H). MS *m/z* (ESI): calc. for $C_{14}H_{20}O_2$ $[M-H]^-$ 219.15, found 219.13.

2-methyl-2-(3-phenoxyphenyl)propanoic acid (**4d**): **4d** was prepared via general procedure 5 as a white solid (95% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.36-7.24 (m, 3H), 7.13-6.98 (m, 5H), 6.85-6.80 (m, 1H), 1.58 (s, 6H). MS *m/z* (ESI): calc. for $C_{16}H_{16}O_3$ $[M-H]^-$ 255.11, found 255.07.

2-(3-benzoylphenyl)-2-methylpropanoic acid (**4e**): **4e** was prepared via general procedure 5 as a white solid (89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.88-7.76 (m, 3H), 7.68-7.53 (m, 3H), 7.47-7.39 (m, 3H), 1.64 (s, 6H). MS *m/z* (ESI): calc. for $C_{17}H_{16}O_3$ $[M-H]^-$ 267.11, found 267.13.

1-(2-fluoro-[1,1'-biphenyl]-4-yl)cyclopropanecarboxylic acid (**5a**): **5a** was prepared via general procedure 5 as a white solid (77% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.54-7.52 (m, 2H), 7.45-7.36 (m, 4H), 7.23-7.15 (m, 2H), 1.74-1.71 (dd, 2H), 1.34-1.31 (dd, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 181.1, 160.9, 158.9, 140.0 (d, J_{C-F} = 150 Hz), 135.5, 130.7 (d, J_{C-F} = 20 Hz), 129.0, 128.5, 127.7, 126.4 (d, J_{C-F} = 20 Hz), 118.4, 118.2, 28.0, 18.0. HRMS *m/z* (ESI): calc. for $C_{16}H_{13}FO_2$ $[M-H]^-$ 255.0827, found 255.0835.

1-(6-methoxynaphthalen-2-yl)cyclopropanecarboxylic acid (**5b**): **5b** was prepared via general procedure 5 as a white solid (78% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.70-7.68 (m, 3H), 7.49-7.47 (m, 1H), 7.14-7.11 (m, 2H), 3.91 (s, 3H), 1.74-1.71 (dd, 2H), 1.36-1.34 (dd, 2H). MS *m/z* (ESI): calc. for $C_{15}H_{14}O_3$ $[M-H]^-$ 241.09, found 241.2.

1-(4-isobutylphenyl)cyclopropanecarboxylic acid (**5c**): **5c** was prepared via general procedure 5 as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.27-7.24 (d, 2H), 7.07-7.03 (d, 2H), 2.47-2.41 (d, 2H), 1.89-1.80 (m, 1H), 1.66-1.62 (dd, 2H), 1.25-1.21 (dd, 2H), 0.91-0.86 (d, 6H). MS *m/z* (ESI): calc. for $C_{14}H_{18}O_2$ $[M-H]^-$ 217.13, found 217.30.

1-(3-phenoxyphenyl)cyclopropanecarboxylic acid (**5d**): **5d** was prepared via general procedure 5 as a white solid (59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.19 (m, 3H), 7.09-6.96 (m, 5H), 6.87-6.83 (m, 1H), 1.70-1.67 (dd, 2H), 1.3-1.28 (dd, 2H). MS *m/z* (ESI): calc. for $C_{16}H_{14}O_3$ $[M-H]^-$ 253.09, found 253.27.

1-(3-benzoylphenyl)cyclopropanecarboxylic acid (**5e**): **5e** was prepared via general procedure 5 as a white solid (59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83-7.81 (m, 3H), 7.73-7.68 (m, 1H), 7.61-7.56 (m, 2H), 7.49-7.39 (m, 3H), 1.74-1.71 (dd, 2H), 1.33-1.30 (dd, 2H). MS *m/z* (ESI): calc. for $C_{17}H_{14}O_3$ $[M-H]^-$ 265.09, found 265.29.

Separation of compounds 7d, 7e: Racemic profen (e.g. **6d**) was dissolved in a 90:10 solution of hexanes:IPA with 0.1% TFA for a final concentration of 10 mg/mL. An aliquot of 30 μL was injected onto a Chiralcel® AD column using an isocratic gradient of 90:10 hexane:IPA with 0.1% TFA. Compound **7d** eluted at 7.7 minutes and compound **7e** eluted at 14.2 minutes. Fractions were collected manually. The identities of the enantiomers were identified after separation via optical rotation.[2, 3] Enantiomeric excess was determined by re-injecting the separated enantiomers (e.g. **7d**) and integrating each peak (Figure S3). Profens **7d** and **7e** were isolated at

98% ee and 99% ee, respectively.

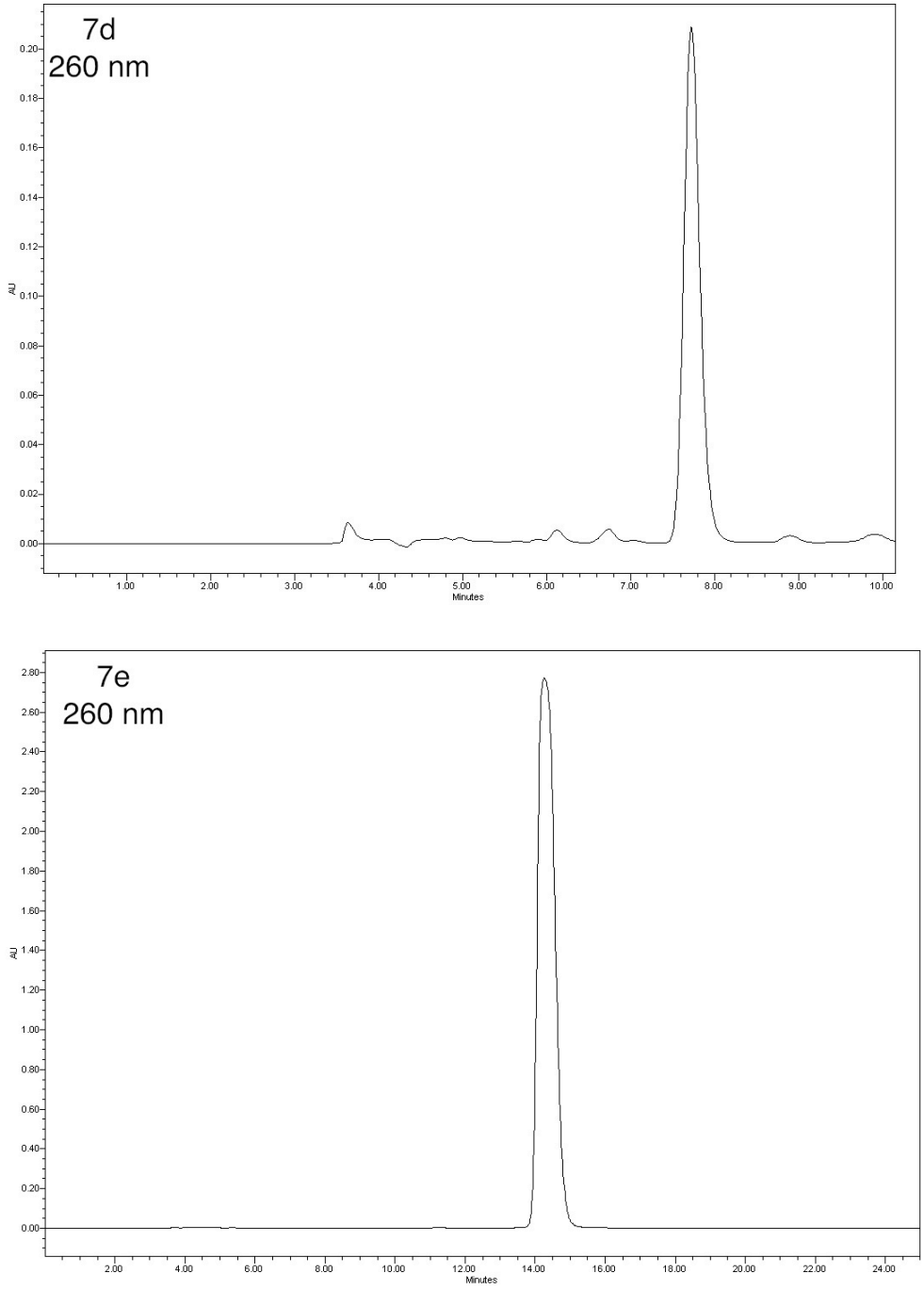

Figure S3. Spectra of **7d** (top) and **7e** (bottom) at 260 nm.

General procedure for *in vitro* assay: Desired concentrations of inhibitor in DMSO or blank (DMSO) were incubated with mCOX-2 (40 nM) for 3 min in 100 mM Tris-HCl with 0.5 mM phenol,

pH 8.0 at 37 °C. After pre-incubation of enzyme and inhibitor, 5 μM of AA or 2-AG was added at 37 °C. Thirty seconds later, the reaction was quenched with 200 μL of ice-cold ethyl acetate containing 0.5 % acetic acid (v/v) and 1 μM PGE_2-d_4 and PGE_2-G-d_5. The solution was then vortexed and the aqueous layer frozen. The organic layer was removed and evaporated to near-dryness under nitrogen. The samples were reconstituted in 1:1 MeOH:H_2O and chromatographed using a Luna C18(2) column (50 × 2 mm, 3 μm) (Phenomenex, Torrance, CA). The elution method was run at a flow rate of 0.4 mL/min and consisted of a linear gradient 20-95% B solvent over five minutes, followed by a one minute linear gradient 95-20% B solvent and a thirty second isocratic gradient at 20% B solvent. Solvent A consisted of 5 mM ammonium acetate pH = 3.5 and solvent B consisted of 6% solvent A in ACN. MS/MS was conducted on a Quantum triple quadrupole mass spectrometer operated in positive ion mode utilizing a selected reaction monitoring method with the following transitions: *m/z* 370→ 317 for PGE_2/D_2, *m/z* 374 → 321 for PGE_2-d_4, *m/z* 444 → 391 for PGE_2/D_2-G and *m/z* 449 → 396 for PGE_2/D_2-G-d_5. Analyte peak areas were normalized to the appropriate internal standard to determine the amount of product formation, and inhibition was determined by normalization to a DMSO control.

General procedure for RAW cells assay: RAW 264.7 macrophages were plated onto 60x15mm collagen coated dishes at 2 million cells per dish in DMEM with GlutaMax® with 10% HI-FBS. The cells were then stimulated overnight with 20 ng/ml of granulocyte-macrophage colony-stimulating factor. The media was replaced in the morning with DMEM with GlutaMax® and the cells were stimulated with 100 ng/ml lipopolysaccharide and 20 units/ml interferon γ. Two hours after stimulation varying doses of inhibitor were added to the cell media and 6 hours after stimulation the media was collected and extracted in acidified ethyl acetate spiked with deuterated internal standards. The organic layer was dried down under a stream of nitrogen gas

and reconstituted in 1:1 water:methanol and analyzed by LC-MS/MS for prostaglandin levels using the same instrumentation, column, solvents and conditions as described for the *in vitro* assay above.

General procedure for *in vivo* mouse assay: The following animal protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee, and all procedures were performed in accordance with national guidelines and regulations. Male C57BL/6 mice were dosed by intraperitoneal (i.p.) injection with 10 mg/kg of compound. For compound **3a** at the designated time points of one hr, two hr, and four hr, mice were euthanized. Separately, mice were dosed with either **3a** or **7a** i.p. with 10 mg/kg at 0, 8, 24, 32, 48 and 56 hr. Blood was collected from the mice at 72 hr. All blood samples were immediately collected into heparinized syringes by cardiac puncture. The 16 hr time points for compounds **7a**, **3a** and (*S)*-flurbiprofen were extracted from murine plasma as follows: 200 μL plasma was diluted 5:1 (*v:v*) with 1% acetic acid (aqueous) then 100 μL acetonitrile and 1.2 mL hexane were added. The plasma samples were mixed well and centrifuged to promote phase separation. The upper, organic layer was removed and dried. The samples were reconstituted in 9:1 isopropyl alcohol:hexane (*v:v*) immediately prior to analysis on an isocratic system where the mobile phase was 98:2 isopropyl alcohol:hexane with 0.1% acetic acid. Chiral separation occurred on a Chiralcel OD column (25 x 0.46 cm) and fluorescence detection was employed (λ_{ex} = 248 nm, λ_{em} = 312 nm).

The one, two and four hr timepoints for **3a** were extracted from murine plasma as follows: 200 μL murine plasma was spiked with flurbiprofen (internal standard) and diluted with 1 mL 1% acetic acid (aqueous) and loaded onto a pre-conditioned OASIS HLB solid phase extraction column. The OASIS HLB cartridge had been conditioned with 3 mL methanol and 2 mL of 1% acetic acid (aq). The loaded cartridge was washed with 3 mL of 1% acetic acid (aq) and 1.5 mL of

1% acetic acid (aq) with 20% methanol. The cartridge was eluted with 2 mL methanol and the eluant was dried. The samples were reconstituted in 100 μL methanol and 50 μL water immediately prior to analysis on a gradient of 30% B to 75% B over 11 min, where A = water and B = 1:1 methanol:acetonitrile (*v:v*), each with 0.1% acetic acid. Separation occurred on a Zorbax C18 column (15 x 0.21 cm) and fluorescence detection was employed (λ_{ex} = 285 nm, λ_{em} = 340 nm).

General procedure for crystallization, X-ray data collection, structure determination and refinement: Crystallization was performed as previously described with modest modification.[4] Purified mCOX-2 (10 mg/mL) was reconstituted with a 2-fold molar excess of Fe^{3+}-protoprophyrin IX. After dialysis against 20 mM sodium phosphate buffer pH 6.7,100 mM NaCl, 0.01% NaN_3, 0.6% (w/v) β-OG, β-OG concentration was adjusted to 1.2% and 10-fold molar excess of chemical **3a** was added prior to setup. Crystallization was performed using hanging drop vapor diffusion method by combining 3.5 μL of the protein solution with 3.5 μL 50 mM EPPS pH 8.0, 80~120 mM $MgCl_2$, 20~25% (v/v) PEG MME-550 equilibrating over reservoir solution containing 0.5 mL of 50 mM EPPS pH 8.0, 100~120 mM $MgCl_2$, 20~25% (v/v) PEG MME-550 at 291 K. Crystals were mounted after about 3 weeks growth and transferred to the stabilization solution [50 mM EEPS, 28% (v/v) PEG MME 550, 100 mM $MgCl_2$] for ~10 s and flash frozen for crystal transportation.

Data sets were collected on an ADSC Quantum-315 CCD using the synchrotron radiation X-ray source at a wavelength of 0.97929 Å with 100 K liquid nitrogen streaming at beamline 24-ID-E of the Advance Photon Source at the Argonne National Laboratory. Diffraction data were collected and processed with HKL 2000.[5] Initial phases were determined by molecular replacement using a search model (PDB 3NT1) with Phaser. Solution with four molecules in the asymmetric unit was

obtained. The models were improved with several rounds of model building in COOT[6] and CCP4 suite.[7] There were no significant structural differences evident among the monomers. Global non-crystallographic symmetry was applied during the refinement. The ligand was built using SMILE and waters were adding from COOT.[6] The final model has R_{work} and R_{free} 0.240 and 0.289, respectively. The potential of phase bias was excluded by simulated annealing using Phenix.[8] The values of the Ramachandran plot for the final refinement of the structure were obtained with PROCHECK.[9] Data collection and refinement statistics are reported in Table S1. The atomic coordinates and structure factor have been deposited in the Protein Data Bank under the access id 4FM5 (http://www.rcsb.org/). The illustrations were prepared using the coordinates of monomer A with Pymol (Schrödinger, LLC).

Table S2. Data collection and refinement statistics of mCOX-2: 3a complex.

	mCOX-2:**3a** complex (PDB: 4FM5**)**
Data collection	
Space group	$P2_12_12$
Cell dimensions	
a, b, c (Å)	181.63, 135.70, 125.15
α, β, γ (°)	90, 90, 90
Resolution (Å)	50.0-2.80 (2.90-2.80)*
Total reflections	303710
Unique reflections	74531
R_{sym}	0.094 (0.498)
Mean I/σ(I)	6.92 (2.03)
Completeness (%)	98.46 (81.56)
Multiplicity	4.1 (4.0)
Wilson B-factor	48.97
Refinement	
Resolution (Å)	49.86-2.81 (2.89-2.81)*
R_{work}/R_{free}	0.240/0.289 (0.335/0.367)
No. atoms	18701
Protein	17896
Ligand	624
Solvent	181
B-factors	45.9

Protein	45.4
Ligand	62.7
Solvent	37.3
RMS (bonds, Å)	0.016
RMS (angles, °)	1.87
Ramachandran Plot	
Preferred	95.91% (2110)
Allowed	3.95% (87)
Outliers	0.14% (3)

The values in parentheses are for the highest resolution shell; R_{sym} = $\frac{\sum_{hkl}\sum_{i}\left|I_i(hkl)-\overline{I_i(hkl)}\right|}{\sum_{hkl}\sum_{i}I_i(hkl)}\times 100\%$; R = $\frac{\sum_{hkl}\left\|F_o\right|-\left|F_c\right\|}{\sum_{hkl}\left|F_o\right|}\times 100\%$, where F_o and F_c are the observed and calculated structure factors, R_{free} = test set 5.0%.
This crystal structure has been deposited in the Protein Data Bank (PDB: 4FM5).

References:

1. Zhao, Y.-M.; Gu, P.; Tu, Y.-Q.; Zhang, H.-J.; Zhang, Q.-W.; Fan, C.-A., One-Pot Synthesis of Aminoenone via Direct Reaction of the Chloroalkyl Enone with NaN3: Rapid Access to Polycyclic Alkaloids. *J. Org. Chem.* **2010,** *75* (15), 5289-5295.
2. Brebion, F.; Delouvrié, B.; Nájera, F.; Fensterbank, L.; Malacria, M.; Vaissermann, J., Highly Diastereoselective Conjugate Addition to Alkylidene Bis(Sulfoxides): Asymmetric Synthesis of (+)-erythro-Roccellic Acid. *Angew. Chem. Int. Ed.* **2003,** *42* (43), 5342-5345.
3. Monti, S.; Manoli, F.; Sortino, S.; Morrone, R.; Nicolosi, G., Binding of a chiral drug to a protein: an investigation of the 2-(3-benzoylphenyl)propionic acid/bovine serum albumin system by circular dichroism and fluorescence. *Phys. Chem. Chem. Phys.* **2005,** *7* (23), 4002-4008.
4. Duggan, K. C.; Hermanson, D. J.; Musee, J.; Prusakiewicz, J. J.; Scheib, J. L.; Carter, B. D.; Banerjee, S.; Oates, J. A.; Marnett, L. J., (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. *Nat. Chem. Biol.* **2011**, *7* (11), 803-809.
5. Otwinowski, Z.; Minor, W., Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods in Enzymology: Macromolecular Crystallography, part A*. C.W. Carter, Jr. & R. M. Sweet, Eds., Academic Press, New York, 1997; Vol. 276, p. 307-326.
6. Emsley, P.; Lohkamp, B., Scott, W.G., Cowtan, K., Features and development of Coot. *Acta Cryst.* **2010**, *D66*, 486-501.
7. Potterton, E.; Briggs, P.; Turkenburg M.; Dodson E. A graphical user interface to the CCP4 program suite. *Acta Cryst.* **2003**, *D59*, 1131-1137.
8. Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P.

H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Cryst.* **2010**, *D66*, 213-221.

9. Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton J. M. PROCHECK: a program to check the stereochemical quality of protein structures, *J. Appl. Cryst.* **1993**, *26*, 283-291.
10. Duggan, K. C.; Hermanson, D. J.; Musee, J.; Prusakiewicz, J. J.; Scheib, J. L.; Carter, B. D.; Banerjee, S.; Oates, J. A.; Marnett, L. J., (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. *Nat. Chem. Biol.* **2011,** *7* (11), 803-809.