Selective Visualization of Cyclooxygenase-2 in Inflammation and Cancer by Targeted Fluorescent Imaging Agents[†]

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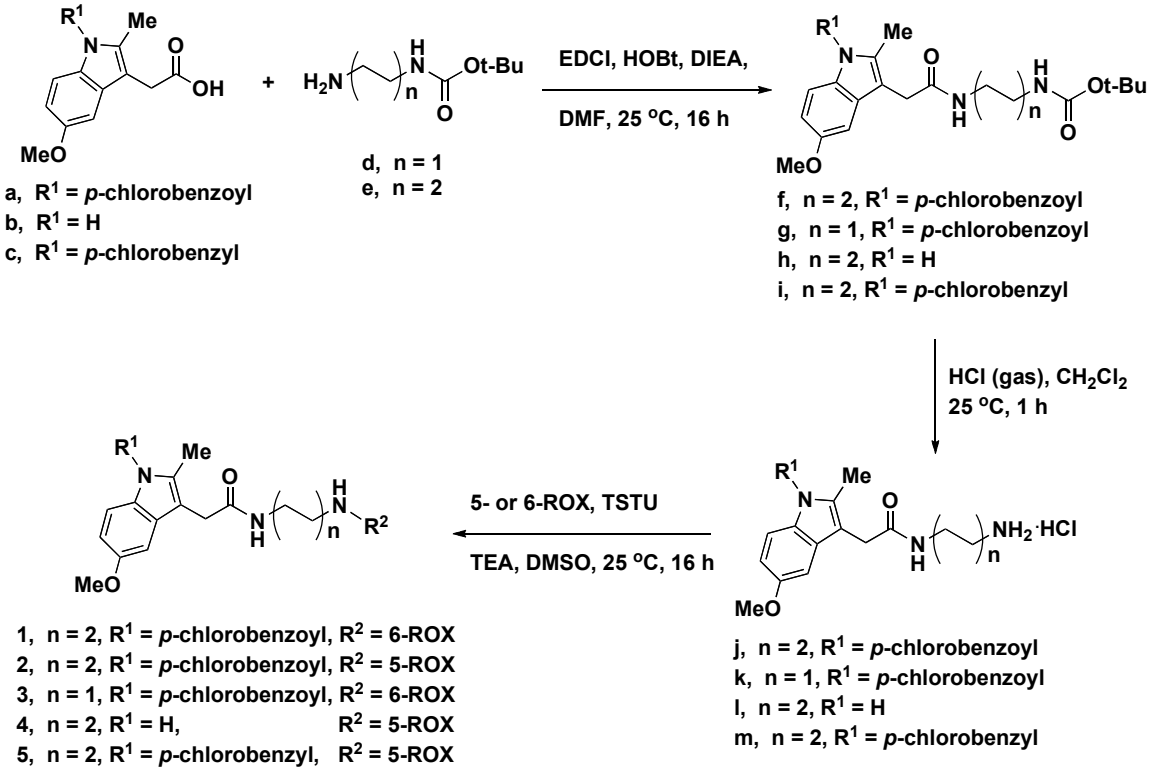
Supplemental Data

Chemistry. 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. HPLC-UV analysis was performed on a Waters 2695 Separation Module in-line with a Waters 2487 Dual Wavelength Absorbance detector. All the reagents and solvents were purchased from the Aldrich Chemical Company (Milwaukee, WI) and were used without further purification. ^{1}H NMR was taken on a Bruker AV-I console operating at 400.13 MHz. ^{1}H COSY experiments were acquired using a 9.4 T Oxford magnet equipped with a Bruker AV-I console operating at 400.13 MHz. Experimental conditions included 2048 x 512 data matrix, 13 ppm sweep width, recycle delay of 1.5 seconds and 4 scans per increment. The data was processed using squared sinebell window function, symmetrized, and displayed in magnitude mode. ^{13}C direct detection, HSQC and HMBC NMR experiments were acquired using an 11.7 T Oxford magnet equipped with a Bruker DRX console operating at 500.13 MHz. Multiplicity-edited HSQC experiments were acquired using a 2048 x 256 data matrix, a J(C-H) value of 145 Hz which resulted in a multiplicity selection delay of 34 ms, a recycle delay of 1.5 seconds and 16 scans per increment along with GARP decoupling on ^{13}C during the acquisition time (150 ms). The data was processed using a p/2 shifted squared sine window function and displayed with CH/CH_3 signals phased positive and CH_2 signals phased negative. J_1(C-H) filtered HMBC experiments were acquired using a 2048 x 256 data matrix, a J(C-H) value of 9 Hz for detection of long range couplings resulting in an evolution delay of 55ms, J_1(C-H) filter delay of 145 Hz (34 ms) for the suppression of one-bond couplings, a recycle delay of 1.5 seconds and 128 scans per increment. The HMBC data was processed using a p/2 shifted squared sine window function and displayed in magnitude mode. Mass spectrometric and LC-MS analyses were performed on a ThermoElectron Surveyor pump and

autosampler operated in-line with a Quantum triple quadrupole instrument in ESI positive or negative ion mode.

Conjugation of indomethacin **(a)** or related carboxyl precursors **(b-c)** with mono BOC-alkyldiamines (**d-e**) was performed using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole hydrate (HOBt), N,N-diisopropylethylamine (DIEA) to afford compounds **f-i**. Treatment of compounds **f-i** with HCl (gas) gave compounds **j-m**. Succinimidyl esters of 5- or 6-carboxy-X-rhodamines (5- or 6-ROX) {Uddin, et al. *Org. Lett.* **2008**, *10,* 4799-4801} were generated *in situ* using *N*,*N*,*N*,*N*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU). These succinnimidyl esters were then reacted with free amines of compounds **j-m** in presence of triethylamine (TEA) to give the target fluorescent conjugates **1-5** (Scheme 1).



Scheme 1. Synthesis of fluorescent conjugates **1-5**.

***t*-Butyl 4-[2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamido]butylcarbamate (f). General Procedure.** To a stirred solution of indomethacin (**a**, 3.57 g, 10 mmol) in DMF (250 mL) was added N-BOC butanediamine (**e**, 5 g, 30 mmol), HOBt (2.02 g, 15 mmol), DIEA (3.88 g, 30 mmol), EDCI (2.10 g, 11 mmol) at 25 ºC. The resultant mixture was stirred for 16 h at 25 ºC. Removal of solvent *in vacuo* afforded a residue, to which 100 mL water was added and extracted with EtOAc (3 X 75 mL). Combined organic layers was dried over Na_2SO_4, concentrated *in vacuo* and crystallized from *n*-hexane as yellow crystals (**f**, 4.6 g, 82%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29 (s, 9H, C(CH_3)$_3$), 1.48-1.56 (m, 4H, CCH_2CH_2C), 2.24 (s, 3H, CH_3), 3.33-2.37 (m, 2H, CCCCH_2), 3.42-3.48 (m, 2H, CH_2CCC), 3.67 (s, 2H, CH_2CO), 3.78 (s, 3H, OCH_3), 6.67 (dd, J = 9.2, 2.5 Hz, 1H, indolyl H-6), 6.72-6.75 (m, 1H, N*H*COO), 6.91 (d, J = 9.2 Hz, 1H, indolyl H-7), 7.20 (d, J = 2.5 Hz, 1H, indolyl H-4), 7.65 (d, J = 8.5 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.72 (d, J = 8.5 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.11-8.15 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{28}H_{34}ClN_3O_5$ $(M+Na)^+$, 550.22; found 550.40.

***t*-Butyl 4-[2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamido]ethylcarbamate (g).** Synthetic procedure for compound **g** was similar to that of described for compound **f**. Yellow solid (74%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, C(CH_3)$_3$), 2.28 (s, 3H, CH_3), 3.39-3.48 (m, 2H, CCH_2), 3.57-3.66 (m, 2H, CH_2C), 3.73 (s, 2H, CH_2CO), 3.76 (s, 3H, OCH_3), 6.69 (dd, J = 9, 2.5 Hz, 1H, indolyl H-6), 6.72-6.77 (m, 1H,

N*H*COO), 6.94 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.19 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.63 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.67 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.12-8.18 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{26}H_{30}ClN_3O_5$ $(M+H)^+$, 500.19; found 500.24.

***t*-Butyl 4-{2-(5-methoxy-2-methyl-1*H*-indol-3-yl)acetamido}butylcarbamate (h).** Synthetic procedure for compound **h** was similar to that of described for compound **f**. Yellow crystals (66%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.40-1.46 (m, 4H, CC*H*$_2$C*H*$_2$C), 2.31 (s, 3H, C*H*$_3$), 3.34-2.38 (m, 2H, CCCC*H*$_2$), 3.40-3.52 (m, 2H, C*H*$_2$CCC), 3.66 (s, 2H, C*H*$_2$CO), 3.75 (s, 3H, OC*H*$_3$), 6.66 (dd, *J* = 9.3, 2.6 Hz, 1H, indolyl H-6), 6.73-6.79 (m, 1H, N*H*COO), 6.89 (d, *J* = 9.3 Hz, 1H, indolyl H-7), 7.25 (d, *J* = 2.6 Hz, 1H, indolyl H-4), 8.09-8.14 (m, 1H, N*H*COCH$_2$), 11.80 (br s, 1H, N*H*). Mass (ESI): calcd for $C_{21}H_{31}N_3O_4$ $(M+H)^+$, 390.23; found 390.40.

***t*-Butyl 4-[2-{1-(4-chlorobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamido]butylcarbamate (i).** Synthetic procedure for compound **i** was similar to that of described for compound **f.** The compound **c** was generated using a literature method {Maguire, A. R. et al. *Bioorg. Med. Chem.* **2001**, *9*, 745-762}. Yellow solid (80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 (s, 9H, C(C*H*$_3$)$_3$), 1.39-1.47 (m, 4H, CC*H*$_2$C*H*$_2$C), 2.33 (s, 3H, C*H*$_3$), 3.35-2.39 (m, 2H, CCCC*H*$_2$), 3.44-3.49 (m, 2H, C*H*$_2$CCC), 3.69 (s, 2H, C*H*$_2$CO), 3.74 (s, 3H, OC*H*$_3$), 5.54 (s, 2H, Ar-C*H*$_2$), 6.69 (dd, *J* = 9.3, 2.4 Hz, 1H, indolyl H-6), 6.74-6.77 (m, 1H, N*H*COO), 6.93 (d, *J* = 9.3 Hz, 1H, indolyl H-7), 7.12 (d, *J* = 2.4 Hz, 1H, indolyl H-4), 7.16 (d, *J* = 8.5 Hz, 2H, *p*-

chlorobenzyl H-2, H-6), 7.46 (d, J = 8.5 Hz, 2H, *p*-chlorobenzyl H-3, H-5), 8.01-8.05 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{28}H_{36}ClN_3O_4$ $(M+H)^+$, 514.24; found 514.32.

***N*-(4-Aminobutyl)-2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide hydrochloride (j). General Procedure.** HCl (gas) was bubbled through a solution of compound **f** (1.0 g) in CH_2Cl_2 (10 mL) for 1 h at 25 ºC. Removal of solvent *in vacuo* afforded a yellow residue, where *n*-hexane was added (20 mL) and stirred for 30 min, then filtered that gave the compound **j** as a brown solid (0.79 g, 98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.45-1.58 (m, 4H, CC*H*$_2$C*H*$_2$C), 2.22 (s, 3H, C*H*$_3$), 2.70-2.79 (m, 2H, C*H*$_2$CCC), 3.01-3.09 (m, 2H, CCCC*H*$_2$), 3.50 (s, 2H, C*H*$_2$CO), 3.71 (s, 3H, OC*H*$_3$), 6.54 (dd, J = 9.1, 2.5 Hz, 1H, indolyl H-6), 6.80 (d, J = 9.1 Hz, 1H, indolyl H-7), 7.14 (d, J = 2.5 Hz, 1H, indolyl H-4), 7.62 (d, J = 8.8 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.66 (d, J = 8.8 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.25 (br s, 3H, N*H*$_3^+$), 8.52-8.59 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{23}H_{26}ClN_3O_3$ $(M+H)^+$, 428.17; found 428.22.

***N*-(4-Aminoethyl)-2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide hydrochloride (k).** Synthetic procedure for compound **k** was similar to that of described for compound **j**. Brown solid (99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (s, 3H, C*H*$_3$), 2.78-2.98 (m, 2H, C*H*$_2$C), 3.34-3.45 (m, 2H, CC*H*$_2$), 3.55 (s, 2H, C*H*$_2$CO), 3.74 (s, 3H, OC*H*$_3$), 6.53 (dd, J = 9, 2.4 Hz, 1H, indolyl H-6), 6.82 (d, J = 9 Hz, 1H, indolyl H-7), 7.12 (d, J = 2.4 Hz, 1H, indolyl H-4), 7.48 (d, J = 8.8 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.65 (d, J = 8.8 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.26 (br s, 3H, N*H*$_3^+$), 8.55-8.62 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{21}H_{22}ClN_3O_3$ $(M+H)^+$, 400.13; found 400.19.

***N*-(4-Aminobutyl)-2-{5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide hydrochloride (l).** Synthetic procedure for compound **l** was similar to that of described for compound **j**. Brown solid (97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42-1.58 (m, 4H, CCH_2CH_2C), 2.25 (s, 3H, CH_3), 2.73-2.80 (m, 2H, CH_2CCC), 3.05-3.14 (m, 2H, CCCCH_2), 3.56 (s, 2H, CH_2CO), 3.75 (s, 3H, OCH_3), 6.57 (dd, J = 9.2, 2.5 Hz, 1H, indolyl H-6), 6.83 (d, J = 9.2 Hz, 1H, indolyl H-7), 7.19 (d, J = 2.5 Hz, 1H, indolyl H-4), 8.32 (br s, 3H, NH_3^+), 8.54-8.60 (m, 1H, N*H*COCH$_2$), 12.30 (br s, 1H, N*H*). Mass (ESI): calcd for $C_{16}H_{23}N_3O_2$ $(M+H)^+$, 290.18; found 290.06.

***N*-(4-Aminobutyl)-2-{1-(4-chlorobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide hydrochloride (m).** Synthetic procedure for compound **m** was similar to that of described for compound **j**. Brown solid (98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38-1.54 (m, 4H, CCH_2CH_2C), 2.24 (s, 3H, CH_3), 2.71-2.80 (m, 2H, CH_2CCC), 3.11-3.20 (m, 2H, CCCCH_2), 3.58 (s, 2H, CH_2CO), 3.77 (s, 3H, OCH_3), 5.66 (s, 2H, Ar-CH_2), 6.55 (dd, J = 9.1, 2.5 Hz, 1H, indolyl H-6), 6.82 (d, J = 9.1 Hz, 1H, indolyl H-7), 7.08 (d, J = 2.5 Hz, 1H, indolyl H-4), 7.26 (d, J = 8.5 Hz, 2H, *p*-chlorobenzyl H-2, H-6), 7.47 (d, J = 8.5 Hz, 2H, *p*-chlorobenzyl H-3, H-5), 8.27 (br s, 3H, NH_3^+), 8.45-8.54 (m, 1H, N*H*COCH$_2$). Mass (ESI): calcd for $C_{23}H_{28}ClN_3O_2$ $(M+H)^+$, 414.19; found 414.26.

***N*-{(6-Carboxy-X-rhodaminyl)but-4-yl}-2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide (1). General Procedure.** To a solution of 6-ROX (5.7 mg) in DMSO (5 mL) was added TSTU (2.5 mg) and TEA (20 µL). The reaction mixture was stirred for 2 h at room temperature. In a separate flask, *N*-(4-aminobutyl)-2-[1-(4-chlorobenzoyl)-5-methoxy-2-

methyl-1*H*-indol-3-yl]acetamide hydrochloride (**j**) (4.6 mg) in DMSO (10 mL) was treated with a drop of TEA. After stirring for 5 min, the succinimidyl ester of 6-ROX was added slowly to the free amine solution through a cannula and stirred for 24 h at 25 ^{o}C. Removal of solvent gave the crude product, which was purified by a silica gel column chromatography to give *N*-[(6-carboxy-X-rhodaminyl)but-4-yl]-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetamide **1** (6 mg, 63%) as a deep blue solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38-1.44 (m, 4H, CCH_2CH_2C), 1.74-1.77 (m, 4H, rhodaminyl two CH_2), 1.92-1.97 (m, 4H, rhodaminyl two CH_2), 2.18 (s, 3H, CH_3), 2.53 (s, 4H, rhodaminyl two CH_2), 2.84-2.87 (m, 4H, rhodaminyl two CH_2), 3.00-3.05 (m, 2H, CH_2CCC), 3.15-3.20 (m, 10H, rhodaminyl four CH_2 and CCCCH_2), 3.42 (s, 2H, CH_2CO), 3.67 (s, 3H, OCH_3), 6.11 (br s, 2H, rhodaminyl two C=C*H*), 6.6435 (dd, *J* = 9, 1.9 Hz, 1H, indolyl H-6), 6.9115 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.069 (d, *J* = 1.9 Hz, 1H, indolyl H-4), 7.58 (s, 1H, carboxyaryl H-7), 7.6145 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.661 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 7.98-8.02 (m, 2H, carboxyaryl H-5 and N*H*COCH$_2$), 8.0855 (d, *J* = 7.5 Hz, 1H, carboxyaryl H-4), 8.62-8.64 (m, 1H, N*H*COAr). Mass (ESI): calcd for $C_{54}H_{54}ClN_5O_7$ $(M+H)^+$, 944.37; found 944.45.

***N*-[(5-Carboxy-X-rhodaminyl)but-4-yl]-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetamide (2)**. Synthetic procedure for compound **2** was similar to that of described for compound **1**, where 5-ROX was conjugated with compound **j**. Deep blue solid (69%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.41-1.60 (m, 4H, CCH_2CH_2C), 1.69-1.81 (m, 4H, rhodaminyl 2 X CH_2, H-6 and H-6'), 1.88-1.99 (m, 4H, rhodaminyl 2 X CH_2, H-2 and H-2'), 2.20 (s, 3H, CH_3), 2.43-2.48 (s, 4H, rhodaminyl 2 X CH_2, H-7 and H-7'), 2.84-2.95 (m, 4H, rhodaminyl 2 X CH_2, H-1 and H-1'), 3.07-3.12 (m, 2H, CH_2CCC), 3.16-3.27 (m, 10H, CCCCH_2, rhodaminyl 4 X CH_2,

H-5 and H-5'; H-3 and H-3'), 3.45 (s, 2H, C*H*$_2$CO), 3.70 (s, 3H, OC*H*$_3$), 6.10 (s, 2H, rhodaminyl 2 X C=C*H*), 6.66 (dd, *J* = 9, 1.9 Hz, 1H, indolyl H-6), 6.94 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.12 (d, *J* = 1.9 Hz, 1H, indolyl H-4), 7.24 (d, *J* = 9.2 Hz, 1H, carboxyaryl H-7), 7.63 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.66 (d, *J* = 8.7 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.05-8.10 (m, 1H, N*H*COCH$_2$), 8.12 (dd, *J* = 9, 1.9 Hz, 1H, carboxyaryl H-6), 8.41 (d, *J* = 1.4 Hz, 1H, carboxyaryl H-4), 8.69-8.80 (m, 1H, N*H*COAr). Mass (ESI): calcd for $C_{56}H_{54}ClN_5O_7$ $(M+H)^+$, 944.37; found 944.48.

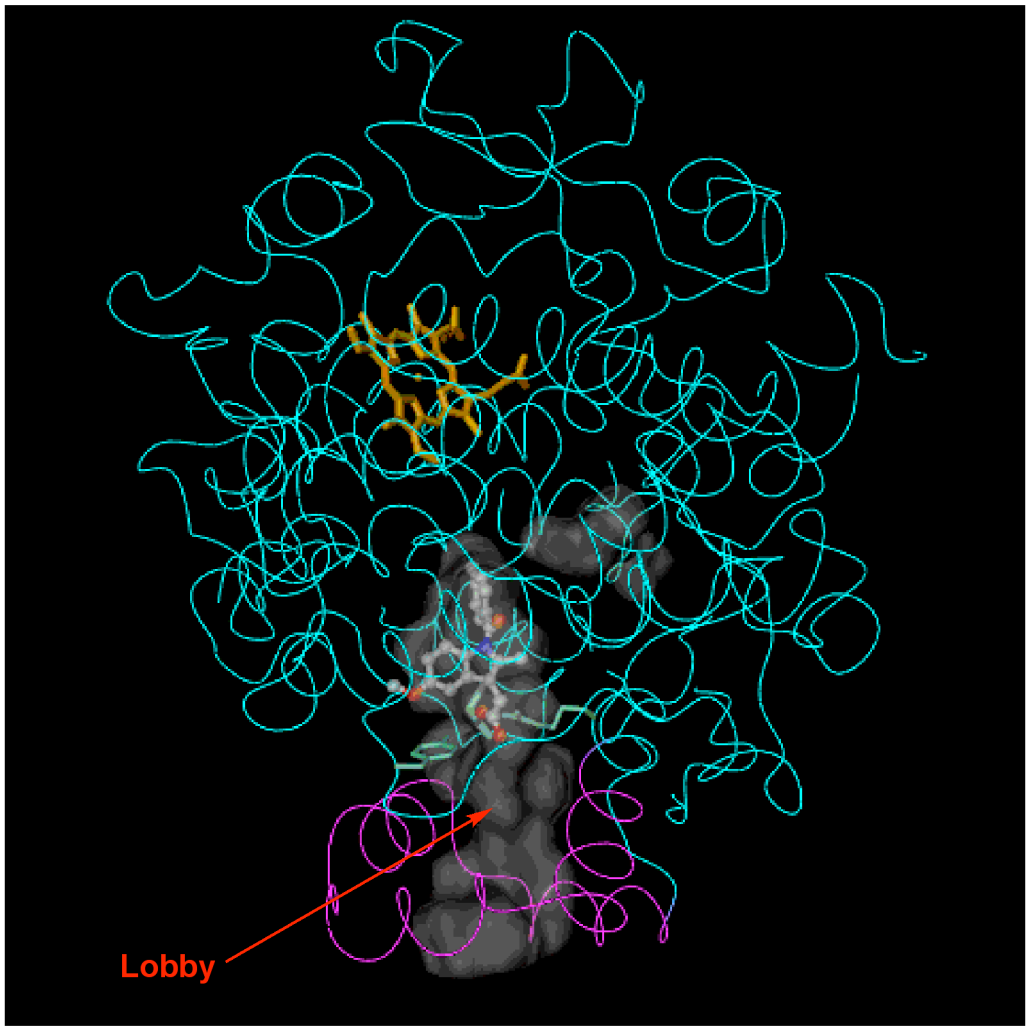
***N*-[(6-carboxy-X-rhodaminyl)eth-2-yl]-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetamide (3).** Synthetic procedure for compound **3** was similar to that of described for compound **1**, where 6-ROX was conjugated with compound **k**. Deep blue solid 67%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.72-1.75 (m, 4H, rhodaminyl two C*H*$_2$), 1.94-2.00 (m, 4H, rhodaminyl two C*H*$_2$), 2.16 (s, 3H, C*H*$_3$), 2.54 (s, 4H, rhodaminyl two C*H*$_2$), 2.85-2.87 (m, 4H, rhodaminyl two C*H*$_2$), 3.01-3.05 (m, 2H, C*H*$_2$C of linker), 3.16-3.22 (m, 10H, rhodaminyl four C*H*$_2$ and linker CC*H*$_2$), 3.43 (s, 2H, C*H*$_2$CO), 3.66 (s, 3H, OC*H*$_3$), 6.12 (br s, 2H, rhodaminyl two C=C*H*), 6.6524 (dd, *J* = 9, 1.9 Hz, 1H, indolyl H-6), 6.9216 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.070 (d, *J* = 1.8 Hz, 1H, indolyl H-4), 7.60 (s, 1H, carboxyaryl H-7), 7.6246 (d, *J* = 8.8 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.671 (d, *J* = 8.8 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 7.97-8.03 (m, 2H, carboxyaryl H-5 and N*H*COCH$_2$), 8.0865 (d, *J* = 7.6 Hz, 1H, carboxyaryl H-4), 8.63-8.65 (m, 1H, N*H*COAr). Mass (ESI): calcd for $C_{56}H_{50}ClN_5O_7$ $(M+H)^+$, 916.34; found 916.59.

***N*-[(5-Carboxy-X-rhodaminyl)but-4-yl]-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]acetamide (4)**. Synthetic procedure for compound **4** was similar to that of described for compound **1**, where

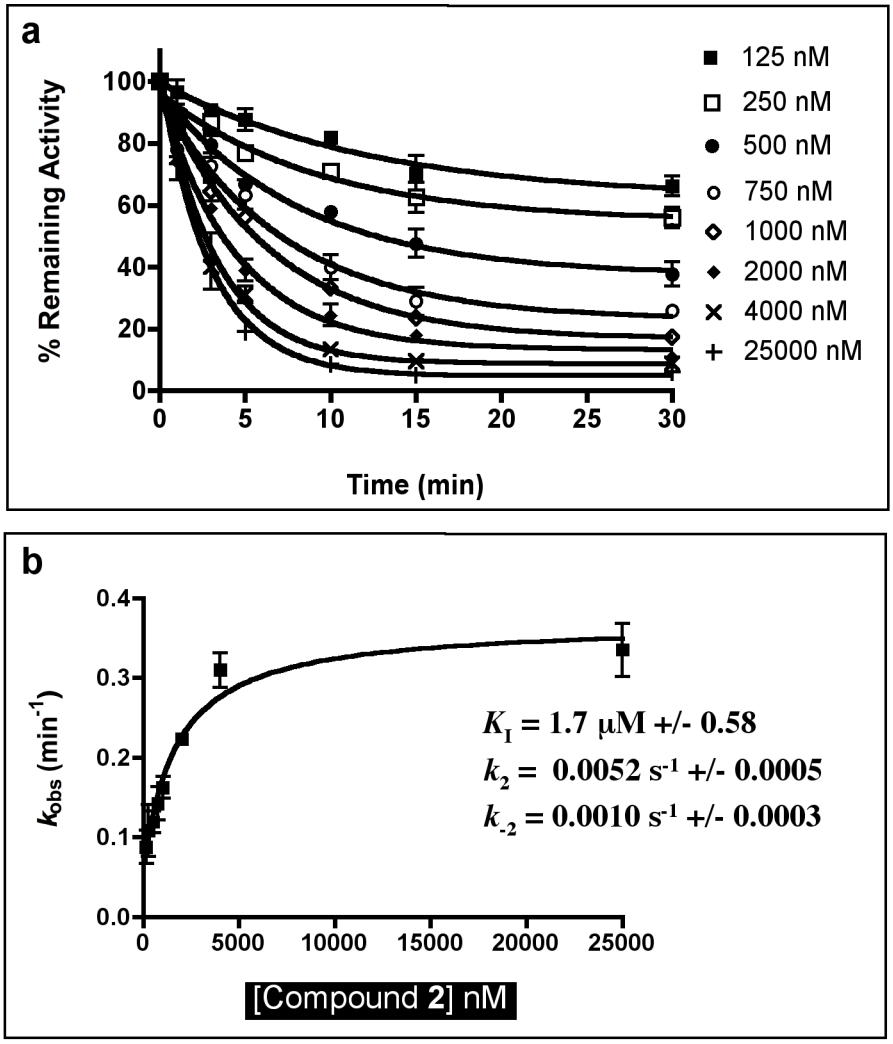
5-ROX was conjugated with compound **l**. Deep blue solid (62%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.38-1.58 (m, 4H, CCH_2CH_2C), 1.71-1.82 (m, 4H, rhodaminyl 2 X CH_2, H-6 and H-6'), 1.85-1.95 (m, 4H, rhodaminyl 2 X CH_2, H-2 and H-2'), 2.23 (s, 3H, CH_3), 2.45-2.50 (s, 4H, rhodaminyl 2 X CH_2, H-7 and H-7'), 2.85-2.94 (m, 4H, rhodaminyl 2 X CH_2, H-1 and H-1'), 3.05-3.14 (m, 2H, CH_2CCC), 3.17-3.24 (m, 10H, CCCCH_2, rhodaminyl 4 X CH_2, H-5 and H-5'; H-3 and H-3'), 3.47 (s, 2H, CH_2CO), 3.76 (s, 3H, OCH_3), 6.11 (s, 2H, rhodaminyl 2 X C=C*H*), 6.68 (dd, *J* = 9, 1.9 Hz, 1H, indolyl H-6), 6.97 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.15 (d, *J* = 1.9 Hz, 1H, indolyl H-4), 7.23 (d, *J* = 9.2 Hz, 1H, carboxyaryl H-7), 8.06-8.11 (m, 1H, N*H*COCH$_2$), 8.14 (dd, *J* = 9, 1.9 Hz, 1H, carboxyaryl H-6), 8.44 (d, *J* = 1.4 Hz, 1H, carboxyaryl H-4), 8.70-8.79 (m, 1H, N*H*COAr), 11.89 (br s, 1H, N*H*). Mass (ESI): calcd for $C_{49}H_{51}N_5O_6$ $(M+H)^+$, 806.38; found 806.51.

***N*-[(5-Carboxy-X-rhodaminyl)but-4-yl]-2-[1-(4-chlorobenzyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetamide (5)**. Synthetic procedure for compound **5** was similar to that of described for compound **1**, where 5-ROX was conjugated with compound **m**. Deep blue solid (69%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.45-1.64 (m, 4H, CCH_2CH_2C), 1.66-1.80 (m, 4H, rhodaminyl 2 X CH_2, H-6 and H-6'), 1.89-1.99 (m, 4H, rhodaminyl 2 X CH_2, H-2 and H-2'), 2.22 (s, 3H, CH_3), 2.41-2.45 (s, 4H, rhodaminyl 2 X CH_2, H-7 and H-7'), 2.85-2.95 (m, 4H, rhodaminyl 2 X CH_2, H-1 and H-1'), 3.11-3.14 (m, 2H, CH_2CCC), 3.16-3.27 (m, 10H, CCCCH_2, rhodaminyl 4 X CH_2, H-5 and H-5'; H-3 and H-3'), 3.55 (s, 2H, CH_2CO), 3.72 (s, 3H, OCH_3), 5.65 (s, 2H, Ar-CH_2), 6.13 (s, 2H, rhodaminyl 2 X C=C*H*), 6.72 (dd, *J* = 9, 1.9 Hz, 1H, indolyl H-6), 6.95 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.14 (d, *J* = 1.9 Hz, 1H, indolyl H-4), 7.25 (d, *J* = 9.2 Hz, 1H, carboxyaryl H-7), 7.25 (d, *J* = 8.5 Hz, 2H, *p*-chlorobenzyl H-2, H-6), 7.48 (d, *J* = 8.5 Hz, 2H, *p*-chlorobenzyl H-

3, H-5), 8.08-8.12 (m, 1H, N*H*COCH$_2$), 8.14 (dd, *J* = 9, 1.9 Hz, 1H, carboxyaryl H-6), 8.42 (d, *J* = 1.4 Hz, 1H, carboxyaryl H-4), 8.72-8.82 (m, 1H, N*H*COAr). Mass (ESI): calcd for $C_{56}H_{56}ClN_5O_6$ $(M+H)^+$, 930.39; found 930.47.



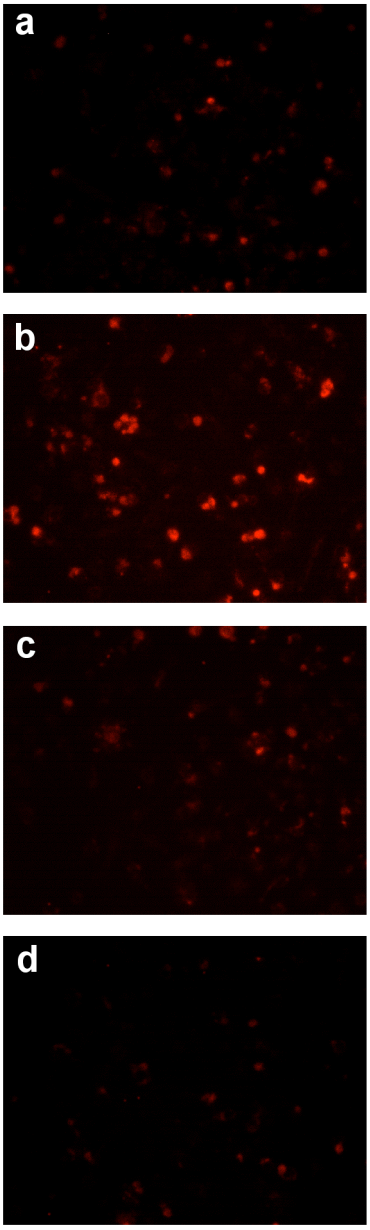
S Figure 1 | COX-2 crystal structure. COX-2 active site is located at the top of a deep channel that runs from the membrane-binding domain (violet) to the catalytic domain (blue). NSAIDs and COXIBs fill the active site immediately adjacent and above the constriction comprised of Arg-120, Tyr-355, and Glu-524 (blue stick structures), which separates the active site from the membrane-binding domain. A molecule of indomethacin is positioned in the active site. The lower portion of the channel, termed the lobby, is right below the constriction and has a large volume, which may accommodate an organic functional group tethered to an NSAID molecule.



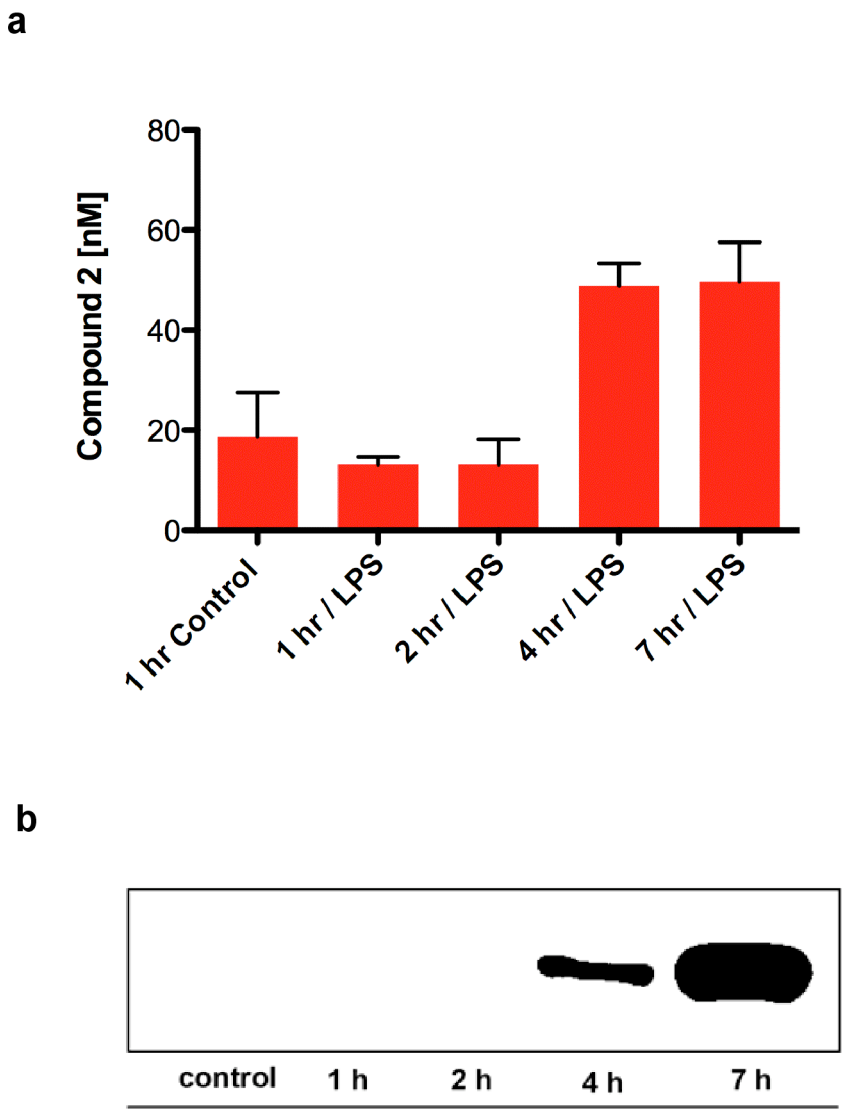
S Figure 2. Kinetics of the time-dependent inhibition of hCOX-2 by compound 2. The purified hCOX-2 enzyme was reconstituted with heme and pre-incubated with the inhibitor at 37 °C for various times (0, 0.5, 10, 15, 20, 25, 30, 45, 60, and 120 min) prior to the addition of the substrate (50 μM). **a,** Time-dependent inhibition of hCOX-2 by compound **2** at various concentrations (125, 250, 500, 750, 1000, 2000, 4000, 25000 nM) and times up to 30 min. **b,** Secondary plot of k_{obs} versus inhibitor concentration used to generate values for K_I, k_2 and k_{-2} using the following equations:

Eq. 1 $E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [EI] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EI^*$ Where: $K_I = (k_{-1}/k_1)$

Eq. 2 $k_{obs} = ((k_2*[I]/(K_I+[I])) + k_{-2}$



S Figure 3. Labeling of COX-2-expressing cells by compounds 1 and 3 (negative control). The experimental protocols are described in Materials and Methods. **a,** Control RAW264.7 cells treated with 200 nM compound **1** for 30 min. **b,** LPS-activated RAW264.7 cells treated with 200 nM compound **1** for 30 min. **c,** LPS-activated RAW264.7 cells pretreated with 10 μM indomethacin for 20 min prior to compound **1** treatment. **d,** LPS-activated RAW264.7 cells treated with 200 nM compound **3** for 30 min.

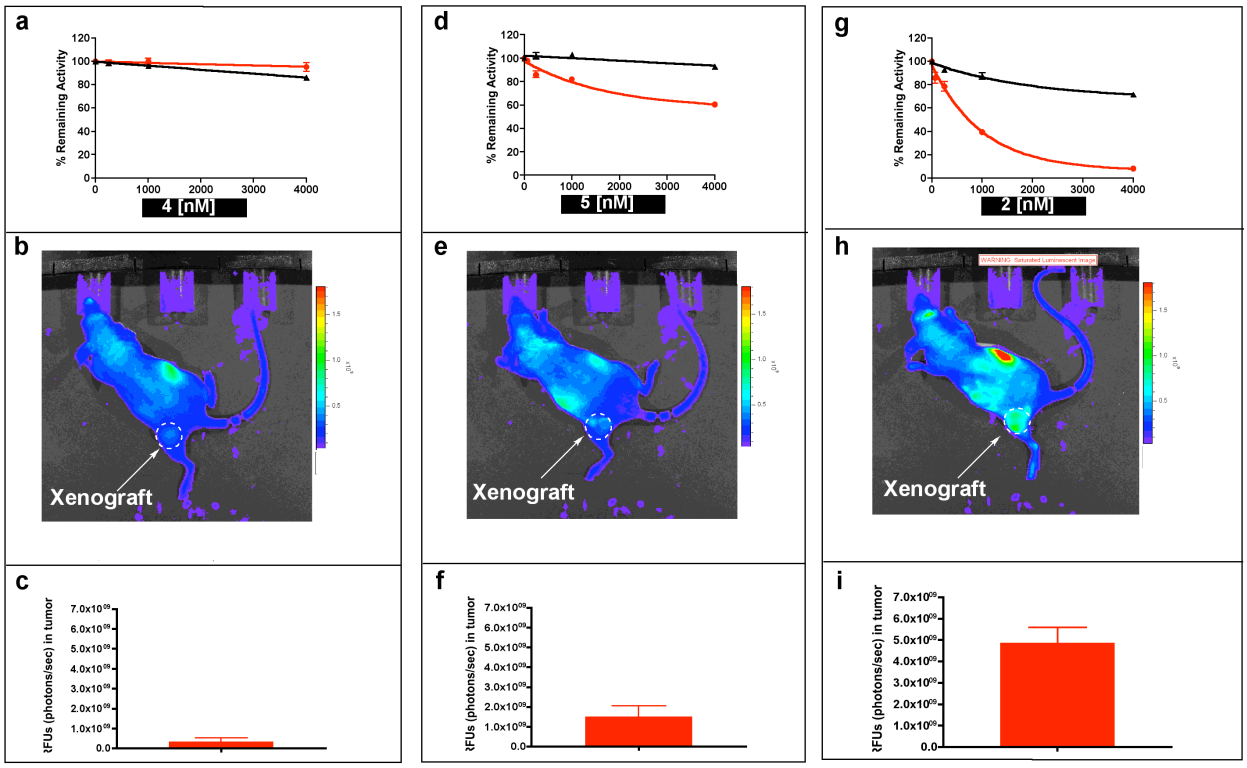


S Figure 4. Correlation of Compound 2 uptake in LPS-activated RAW264.7 cells with COX-2 status. RAW264.7 cells (passage 10) were grown in DMEM, high glucose (Invitrogen, Carlsbad, CA) plus 10 % heat-inactivated FBS (Atlas Biologicals, Ft Collins, CO) in 150 mm plates (Sarstedt, Newton, NC) until 50% confluence, scraped, and resuspended in 50 ml serum-free DMEM at $4x10^6$ cells/ml. Cells (3 ml, $12x10^6$) in a vented 50 ml tube were combined with 3 ml serum-free DMEM +/- LPS and murine IFNγ (200 ng/ml LPS, 10 u/ml, final) and incubated on a rocker platform in the 37°C/5% CO_2 incubator. Compound **2** [200 nM, final] was added to the cell suspension for 30 min followed by centrifugation, one wash with 50 ml PBS, and centrifugation, and by addition of 6 ml growth medium for 25 min for washout of non-specific uptake. After the washout, the cells were centrifuged, washed once with PBS, resuspended in 300 µl PBS, and frozen at -20°C. The time of the final resuspension was counted as the time point of LPS activation of COX-2. For analysis of **2** uptake by the cells, the cells (200 µl) were sonicated for 20 sec x 5 pulses at 35% power, and the proteins were precipitated with acetonitrile. Solutions were centrifuged and analyzed by a previously described HPLC system.

Experimental samples were quantitated against a standard curve. For Westerns, the cells (100 µl) were treated with M-PER (Pierce, Rockford, IL). Protein (5 µg/lane) was loaded on 7.5% SDS/PAGE resolving gels (Bio-Rad) and transferred onto 0.2 µm nitrocellulose (Bio-Rad), probed with murine COX-2 antibody (#160126, Cayman Chemical, Ann Arbor, MI) followed by secondary anti-rabbit HRP (401B, Promega, Madison, WI). SuperSignal West Pico Chemoluminescent Substrate (Thermo Scientific, Rockford, IL) was used per vendor's instructions.

Stoichiometry of compound 2 binding to COX-2 in activated RAW264.7 cells. Calculations are based on an initial 200 µl cell lysate at 7 hr post-LPS treatment. The difference in **2** levels in LPS-induced (9.8 pmol) and uninduced cells (2.65 pmol) = 7.13 pmol uptake due to the presence of COX-2. This represents 4.29×10^{12} molecules. Comparison of COX-2 amounts in the RAW cells to standard amounts of COX-2 by Western blotting indicates the presence of 500,000 COX-2 molecules per cell in maximally LPS-activated RAW cells. Each 200 µl sample contained 9×10^6 cells corresponding to a total of 4.5×10^{12} molecules of COX-2 per sample.

Thus, in a 200 µl sample, 4.29×10^{12} molecules of compound **2** were taken up by 4.5×10^{12} molecules of COX-2 = 0.90 molecule compound **2** per COX-2 molecule.



S Figure 5. ***In vivo*** **labeling of COX-2-expression by compounds 4, 5, or 2 in the 1483 HNSCC xenografts correlates to their COX-2 inhibitory potency.** The inhibitory potency of these compounds against hCOX-2 at 3 μM are as follows: compound **4**, no inhibition; compound **5**, 30% inhibition; compound **2**, 90% inhibition. **a,d,g,** COX purified enzyme inhibition curves of compounds **4**, **5** and **2** respectively. **b,e,h,** Nude mice bearing 1483 HNSCC xenografts on the left flank were dosed (ip) with 2 mg/kg compounds **4**, **5**, and **2** respectively. Fluorescence images were taken in a Xenogen IVIS (DsRed filter, 1 s, f2, 1.5 cm depth) imaging system at 3.5 hrs post-injection. **c,f,i,** Quantified fluorescence of ROIs (1483 HNSCC xenograft) represented as bars (RFUs, photons/sec) that correlated with the COX-2 inhibitory potencies of ligands **4**, **5**, and **2**, respectively.

S Figure 6 – Chromatography of compound 2.

A & B. HPLC with UV detection of compound **2** standard (A) and extracted tumor (B). Detection is at 581 nm. HPLC conditions are given below:

Component A: H_2O with 5 mM octanesulfonic acid (pH ~ 3)
Component B: Acetonitrile with 10% component A
Gradient: 40% B to 90% B in 9.5 min followed by 5.5 min hold at 90% B
Flow: 1.0 mL/min
Column: Supelco C18 25 x 0.46 cm, 5μm, held at 40°C

C & D. HPLC with fluorescent detection of compound **2** standard (C) and extracted tumor (D). Excitation λ = 581 nm and emission λ = 610 nm. HPLC conditions are identical to A & B (above).

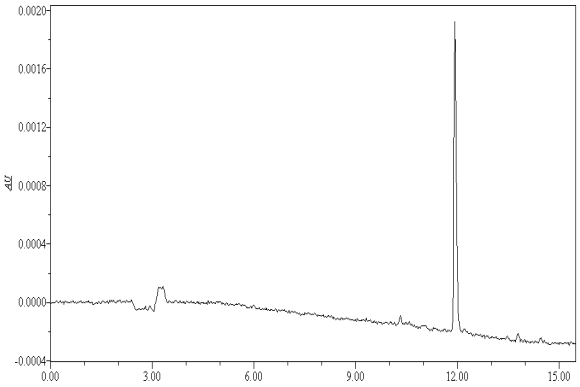
E & F. HPLC with UV detection of compound **2** standard (E) and extracted tumor (F) under an alternative chromatographic regime. Detection is at 581 nm. HPLC conditions are given below:

Component A: H_2O with 0.5% formic acid
Component B: methanol:acetonitrile (1:1, v/v) with 0.5% formic acid
Gradient: 30% B to 90% B in 9.5 min followed by 6.0 min hold at 90% B
Flow: 0.5 mL/min
Column: Phenomenex POLAR-RP 15 x 0.2 cm, 4 μm, held at 40°C

G & H. HPLC with fluorescent detection of compound **2** standard (G) and extracted tumor (H). Excitation λ = 581 nm and emission λ = 610 nm. HPLC conditions are identical to E & F (above).

Notes on Figure 6.
1. Compound **2** elutes at 12.0 min for A-D, 15.3 min for E-H.
2. Minor peaks observed in some chromatograms comprise less than 5% of total peak area.

Figure 6 A (left) & B (right)



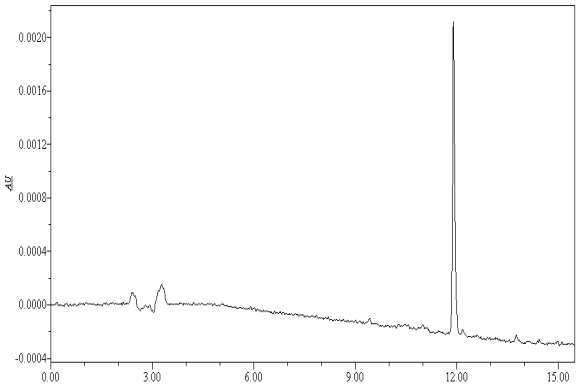
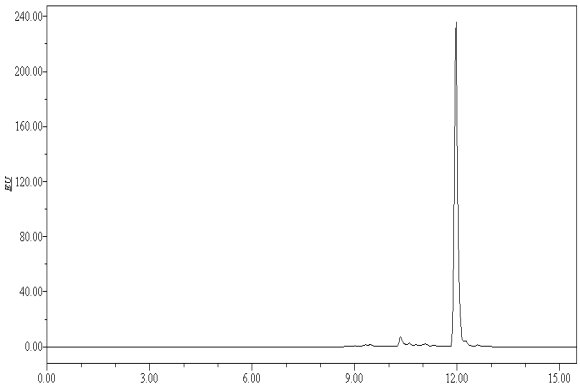


Figure 6 C (left) & D (right)



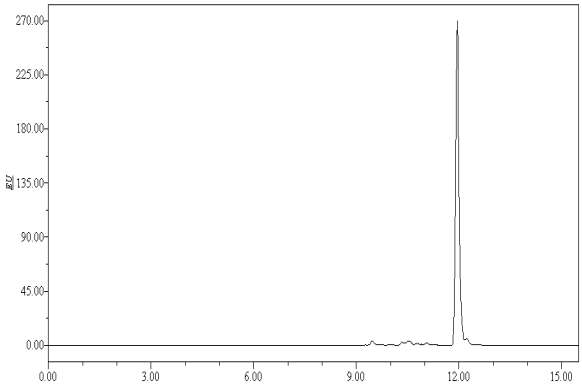
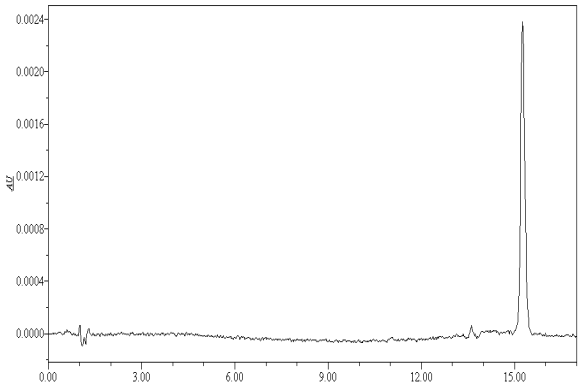


Figure 6 E (left) & F (right)



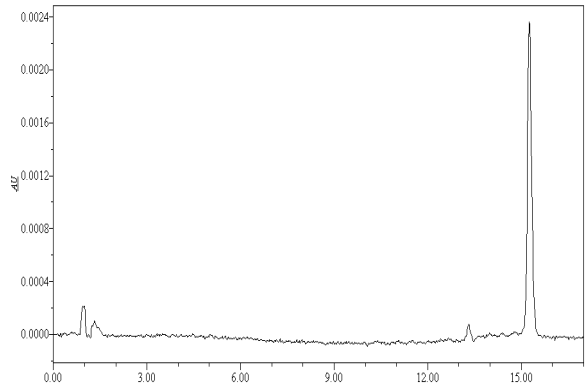


Figure 6 G (left) & H (right)