

Fluorinated Cyclooxygenase-2 Inhibitors as Agents for PET Imaging of Inflammation and Cancer

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Supplementary Information

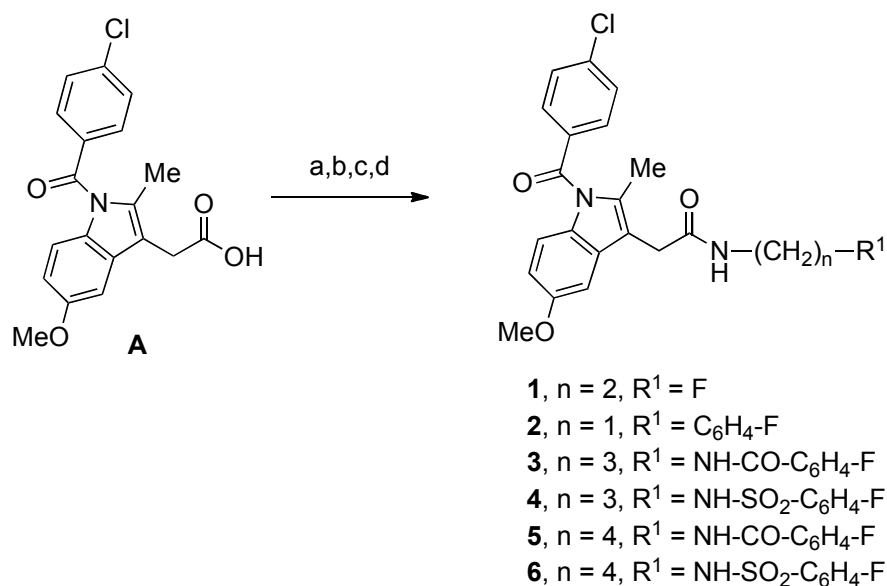
• General

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 m²/g, bulk density 0.4 g/mL, pH range 6.5 – 7.5, purchased from Sorbent Technologies (Atlanta, GA). All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. ¹H NMR was taken on 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 were processed using squared sinebell window function, symmetrized, and displayed in magnitude mode. Multiplicity-edited HSQC experiments were acquired using a 2048 x 256 data matrix, a $J(\text{C-H})$ 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 were 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. $J_1(\text{C-H})$ filtered HMBC experiments were acquired using a 2048 x 256 data matrix, a $J(\text{C-H})$ value of 9 Hz for detection of long range couplings resulting in an evolution delay of 55ms, $J_1(\text{C-H})$ 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 were processed using a $p/2$ shifted squared sine window function and displayed in magnitude mode. Mass spectrometric analyses were performed on a ThermoElectron Surveyor pump TSQ 7000 instrument in ESI positive or negative ion mode. The radiochemical yield was determined by TLC using a radioactivity scanner (Bioscan, Inc., Washington, D.C.).

• Synthesis of Fluorinated Indomethacin Derivatives

Our laboratory has shown that conversion of carboxylic acid-containing NSAIDs (i.e., indomethacin) into neutral amide derivatives is a facile method to generate a structurally diverse series of selective COX-2 inhibitors (1). We used this method to develop fluorinated compounds that bind to COX-2 *in vitro* and *in vivo*. Indomethacin derivatives having a single amide linkage were synthesized by coupling of indomethacin with 4-fluoroethylamine or 4-fluorobenzylamine using ethyl-1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI), 1-hydroxybenzotriazole hydrate (HOBt), and *N,N*-diisopropylethylamine (DIEA) in *N,N*-dimethylformamide (DMF) to afford conjugates **1** and **2**. Alternatively, reaction of indomethacin with mono BOC-protected alkyldiamine in the presence of EDCI followed by treatment with HCl (gas) gave indolylamidoalkylamine hydrochloride salts. Treatment of these salts with triethylamine (TEA) followed by conjugation with either *p*-fluorobenzoylchloride or *p*-fluorobenzenesulfonylchloride afforded fluoro-compounds **3—6**.



Scheme 1s. (a) $H_2N-(CH_2)_2-F$ or $H_2N-CH_2-C_6H_4-F$, EDCI, DIEA, HOBt, DMF, 25 °C, 16 h, (b) $H_2N-(CH_2)_n-NH-BOC$ ($n = 3$ or 4), EDCI, HOBt, DIEA, DMF, 25 °C, 16 h, (c) HCl (gas), CH_2Cl_2 , 0–25 °C 1 h, (d) Cl-CO- C_6H_4-F or Cl-SO₂- C_6H_4-F , TEA, CH_2Cl_2 , 25 °C, 16 h.

***N*-(2-Fluoroethyl)-2-{1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide (1). General procedure.**

To a stirred solution of indomethacin (3.57 g, 10 mmol) in *N,N*-dimethylformamide (DMF) was added 2-fluoroethylamine (0.63 g, 10 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (2.02 g, 15 mmol), *N,N*-diisopropylethylamine (DIEA) (3.88 g, 30 mmol), ethyl-1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (2.10 g, 11 mmol) at 25 °C. The resultant mixture was stirred for 16 h at 25 °C. The solvent was removed, water (100 mL) was added and extracted with EtOAc (3 X 75 mL). The organic layer was collected, washed with water and evaporated *in vacuo*. The crude product was purified using silica gel column chromatography (35 : 7 : 1, $CHCl_3$: MeOH :

NH₄OH) to give compound **1** as yellow solid (3.0 g, 75%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.23 (s, 3H, CH₃), 3.34-3.40 (m, 2H, CH₂), 3.68 (s, 2H, CH₂CO), 3.78 (s, 3H, OCH₃), 4.25-4.31 (m, 2H, CH₂), 6.70 (dd, *J* = 9.0, 2.2 Hz, 1H, indolyl H-6), 6.95 (d, *J* = 9.0 Hz, 1H, indolyl H-7), 7.08 (d, *J* = 2.4 Hz, 1H, indolyl H-4), 7.67 (d, *J* = 8.8 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.83 (d, *J* = 8.8 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 8.49-8.54 (m, 1H, NHCOCH₂). Mass (ESI) *m/z* [M+H]⁺ calcd 403.11; found 403.18.

2-{1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}-*N*-(4-fluorobenzyl)acetamide (2). Yellow solid (77%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.22 (s, 3H, CH₃), 3.65 (s, 2H, CH₂CO), 3.79 (s, 3H, OCH₃), 4.24 (d, *J* = 6 Hz, 2H, CH₂Ar), 6.72 (dd, *J* = 9.0, 2.2 Hz, 1H, indolyl H-6), 6.96 (d, *J* = 9.0 Hz, 1H, indolyl H-7), 7.10 (d, *J* = 2.4 Hz, 1H, indolyl H-4), 7.16 (d, *J* = 8.4 Hz, 2H, 4-fluorobenzyl H-3, H-5), 7.36 (d, *J* = 8.4 Hz, 2H, 4-fluorobenzyl H-2, H-6), 7.68 (d, *J* = 8.8 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.85 (d, *J* = 8.8 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 8.47-8.52 (m, 1H, NHCOCH₂). Mass (ESI) *m/z* [M+H]⁺ calcd 465.13; found 465.20.

***t*-Butyl 4-[2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamido]propylcarbamate. General Procedure.** To a stirred solution of indomethacin (3.57 g, 10 mmol) in DMF was added *N*-BOC propylenediamine (5.0 g, 30 mmol), HOBT (2.02 g, 15 mmol), DIPEA (3.88 g, 30 mmol), EDCI (2.10 g, 11 mmol) at 25 °C. The resultant mixture was stirred for 16 h at 25 °C. Removal of solvent *in vacuo* afforded a residue, to which 100 mL water was added and extracted with EtOAc (3 X 75 mL). The combined organic layers were dried over Na₂SO₄, concentrated *in vacuo* and

the product was crystallized from *n*-hexane as yellow crystals (3.5 g, 64%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.31 (s, 9H, C(CH₃)₃), 2.02-2.07 (m, 2H, CCH₂C) 2.23 (s, 3H, CH₃), 3.39-3.48 (m, 2H, CCH₂), 3.57-3.66 (m, 2H, CH₂C), 3.73 (s, 2H, CH₂CO), 3.76 (s, 3H, OCH₃), 6.69 (dd, *J* = 9, 2.5 Hz, 1H, indolyl H-6), 6.72-6.77 (m, 1H, NHCOO), 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, 4-chlorobenzoyl H-3, H-5), 7.67 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 8.01-8.04 (m, 1H, NHCCH₂). Mass (ESI): *m/z* [M+H]⁺ calcd 514.20; found 514.29.

***t*-Butyl 4-[2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-**

yl}acetamido]butylcarbamate. Yellow solid (60%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.25-1.45 (m, 13H, C(CH₃)₃), 2.21 (s, 3H, CH₃), 2.42-2.48 (m, 4H, CCH₂CH₂C), 2.82-2.88 (m, 2H, CCCCH₂), 3.00-3.05 (m, 2H, CH₂CCC), 3.45 (s, 2H, CH₂CO), 3.75 (s, 3H, OCH₃), 6.68 (dd, *J* = 9, 2.5 Hz, 1H, indolyl H-6), 6.73-6.76 (m, 1H, NHCOO), 6.935 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.185 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.63 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.675 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.98-8.01 (m, 1H, NHCCH₂). Mass (ESI) *m/z* M⁺ calcd 528.22; found 528.32.

***N*-(4-Aminopropyl)-2-{1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-**

yl}acetamide hydrochloride. General Procedure. HCl (gas) was passed through a solution of *t*-butyl 4-[2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamido]propylcarbamate (1.0 g) in CH₂Cl₂ (10 mL) for 2 h at 25 °C. Removal of solvent *in vacuo* afforded a yellow residue, to which *n*-hexane was added (20 mL) and stirred for 30 min to make good slurry, which was filtered to afford the desired *N*-(4-

aminopropyl)-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetamide hydrochloride as brown solid (0.83 g, 99%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.60-1.65 (m, 2H, CCH₂C), 2.25 (s, 3H, CH₃), 2.78-2.98 (m, 2H, CH₂C), 3.34-3.45 (m, 2H, CCH₂), 3.55 (s, 2H, CH₂CO), 3.74 (s, 3H, OCH₃), 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, 4-chlorobenzoyl H-3, H-5), 7.65 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 8.26 (br s, 3H, NH₃⁺), 8.54-8.60 (m, 1H, NHC(=O)CH₂). Mass (ESI) *m/z* (M-Cl)⁺ calcd for 414.15; found 414.22.

***N*-(4-Aminobutyl)-2-{1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}acetamide hydrochloride.** Yellow solid (95%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.45-1.51 (m, 2H, CCH₂CC), 1.54-1.58 (m, 2H, CCCH₂C), 2.22 (s, 3H, CH₃), 2.70-2.78 (m, 2H, CCCCH₂), 3.03-3.07 (m, 2H, CH₂CCC), 3.50 (s, 2H, CH₂CO), 3.75 (s, 3H, OCH₃), 6.68 (dd, *J* = 9, 2.4 Hz, 1H, indolyl H-6), 6.91 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.15 (d, *J* = 2.4 Hz, 1H, indolyl H-4), 7.63 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.68 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.95-8.08 (br s, 3H, NH₃⁺), 8.24-8.27 (m, 1H, NHC(=O)CH₂). Mass (ESI) *m/z* (M-Cl)⁺ calcd 428.17; found 428.28.

2-{1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}-*N*-(3-(4-fluorobenzamido)propyl)acetamide (3). **General Procedure.** To a stirred solution of *N*-(4-aminopropyl)-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetamide hydrochloride (178 mg, 0.37 mmol) in CH₂Cl₂ (4 mL) was added triethylamine (100 mg). The resultant solution was stirred for 5 min at room temperature.

After cooling to 0°C, 4-fluorobenzoyl chloride was added drop-by-drop while maintaining the reaction temperature < 10 °C. The reaction mixture was stirred for 12 h. Removal of solvent *in vacuo* afforded a residue. Water (10 mL) was added and extracted with EtOAc (3 X 10 mL). The combined organic layers were dried over Na₂SO₄. The solvent was removed completely. The residue was purified using silica gel column chromatography (35 : 7 : 1, CHCl₃ : MeOH : NH₄OH) to give compound **3** as yellow solid (161 mg, 72%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.97-2.04 (m, 2H, CCH₂C) 2.23 (s, 3H, CH₃), 3.40-3.49 (m, 2H, CCH₂), 3.58-3.67 (m, 2H, CH₂C), 3.74 (s, 2H, CH₂CO), 3.79 (s, 3H, OCH₃), 6.72 (dd, *J* = 9, 2.5 Hz, 1H, indolyl H-6), 6.95 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.20 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.45 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzoyl H-3, H-5), 7.64 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.66 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.86 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzoyl H-2, H-6), 8.02-8.05 (m, 1H, NHCOCH₂), 8.06-8.09 (m, 1H, NHCOAr). Mass (ESI) *m/z* [M+H]⁺ calcd 536.17; found 536.24.

2-{1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}-*N*-{3-(4-fluorophenylsulfonamido)propyl}acetamide (4). Yellow solid (74%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.99-2.05 (m, 2H, CCH₂C) 2.21 (s, 3H, CH₃), 3.38-3.46 (m, 2H, CCH₂), 3.60-3.67 (m, 2H, CH₂C), 3.75 (s, 2H, CH₂CO), 3.81 (s, 3H, OCH₃), 6.76 (dd, *J* = 9, 2.5 Hz, 1H, indolyl H-6), 6.96 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.21 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.44 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzenesulfonyl H-3, H-5), 7.63 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.66 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.87 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzenesulfonyl H-2, H-6), 8.12-8.15 (m, 1H,

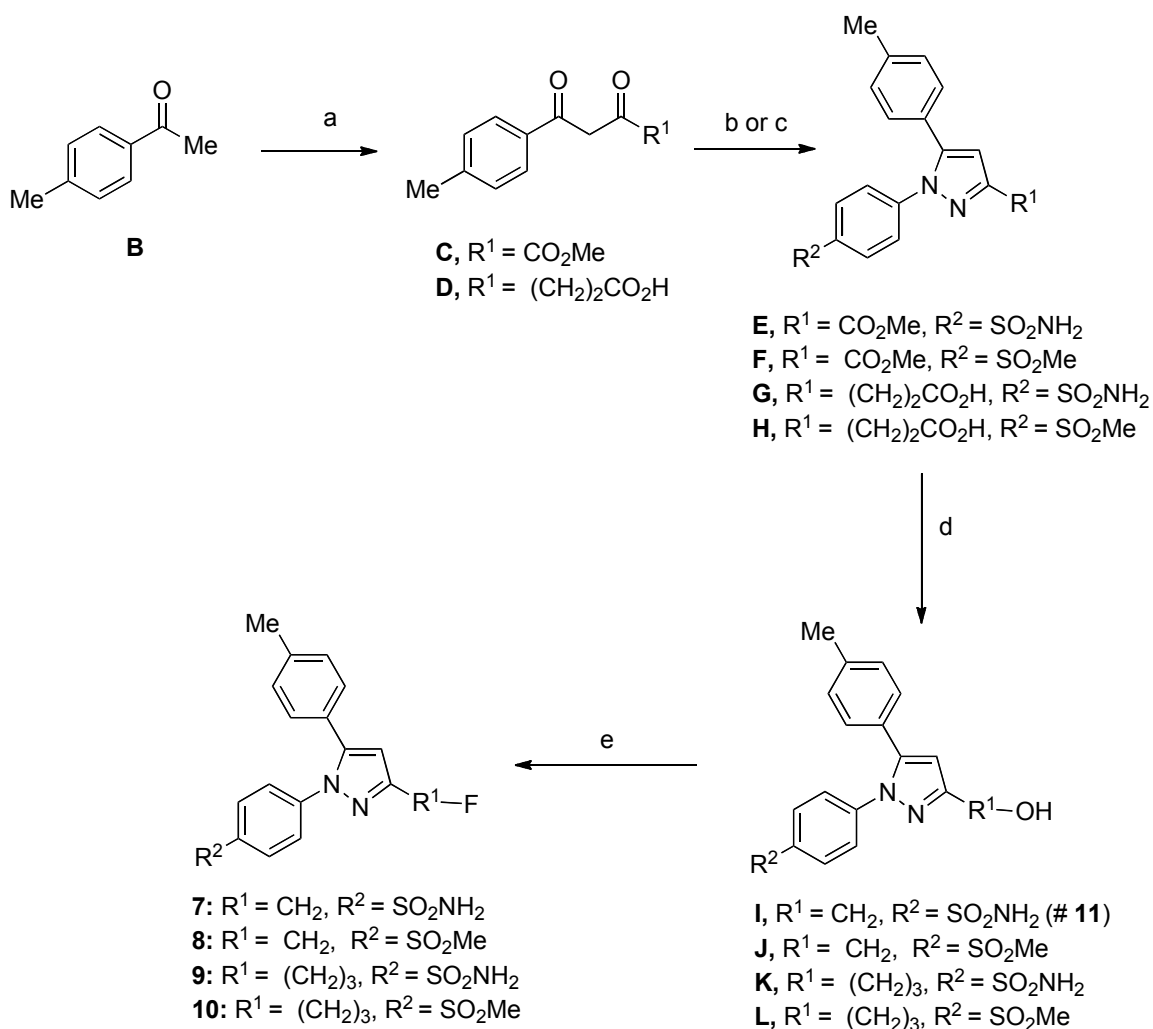
*NHCOCH*₂), 8.26-8.29 (m, 1H, *NHSO*₂Ar). Mass (ESI) *m/z* [M+H]⁺ calcd 572.13; found 572.21.

2-{1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}-*N*-{3-(4-fluorobenzamido)butyl}acetamide (5). Yellow solid (67%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.45-1.54 (m, 4H, CCH₂CH₂C), 2.22 (s, 3H, CH₃), 2.70-2.80 (m, 2H, CH₂CCC), 3.04-3.11 (m, 2H, CCCCH₂), 3.75 (s, 2H, CH₂CO), 3.76 (s, 3H, OCH₃), 6.70 (dd, *J* = 9, 2.4 Hz, 1H, indolyl H-6), 6.93 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.19 (d, *J* = 2.4 Hz, 1H, indolyl H-4), 7.43 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzoyl H-3, H-5), 7.63 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.64 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.87 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzoyl H-2, H-6), 8.04-8.06 (m, 1H, *NHCOCH*₂), 8.07-8.10 (m, 1H, *NHCO*Ar). Mass (ESI) *m/z* [M+H]⁺ calcd 550.18; found 550.28.

2-{1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl}-*N*-{3-(4-fluorophenylsulfonamido)butyl}acetamide (6). Yellow solid (69%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.47-1.56 (m, 4H, CCH₂CH₂C), 2.23 (s, 3H, CH₃), 2.71-2.81 (m, 2H, CH₂CCC), 3.05-3.10 (m, 2H, CCCCH₂), 3.76 (s, 2H, CH₂CO), 3.82 (s, 3H, OCH₃), 6.77 (dd, *J* = 9, 2.5 Hz, 1H, indolyl H-6), 6.97 (d, *J* = 9 Hz, 1H, indolyl H-7), 7.22 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.45 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzenesulfonyl H-3, H-5), 7.64 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-3, H-5), 7.67 (d, *J* = 8.7 Hz, 2H, 4-chlorobenzoyl H-2, H-6), 7.88 (d, *J* = 8.7 Hz, 2H, 4-fluorobenzenesulfonyl H-2, H-6), 8.13-8.16 (m, 1H, *NHCOCH*₂), 8.27-8.30 (m, 1H, *NHSO*₂Ar). Mass (ESI) *m/z* [M+H]⁺ calcd 586.15; found 586.24.

• Synthesis Fluorinated Celecoxib Derivatives

A second set of fluorinated COX-2 ligands was generated based on a fluoromethyl or a SO₂Me pharmacophore substitution on the selective COX-2 inhibitor, celecoxib. An ultrasonication-assisted Claisen condensation of 4-methylacetophenone with dimethyloxalate in the presence of sodium methoxide afforded methyl 2,4-dioxo-4-(*p*-tolyl)butanoate. Alternatively, the reaction of 4-methylacetophenone with succinic anhydride in the presence of lithium diisopropylamide (LDA) yielded 4,6-dioxo-6-(*p*-tolyl)hexanoic acid. The pyrazole intermediates were synthesized by condensation of 2,4-dioxo-4-(*p*-tolyl)butanoate or 4,6-dioxo-6-(*p*-tolyl)hexanoic acid with either *p*-methylsulfonylphenylhydrazine hydrochloride or *p*-sulfonylamidophenylhydrazine hydrochloride in reflux conditions. The 1,5-regioisomers were generated almost exclusively by carrying out the reaction in the presence of the hydrochloride salt of the substituted phenylhydrazine in refluxing methanol (2). When required, the 1,5-diarylpyrazoles were separated from the minor 1,3-diarylpyrazole isomers by flash chromatography. The pyrazole intermediates were reduced to the corresponding alcohols. Diethylaminosulfurtrifluoride (DAST) mediated fluorination of these alcohols gave the desired fluoro-compounds **7**–**10** in good yields.



Scheme 2s. (a) Dimethyloxalate, 25 % NaOMe/MeOH, ultrasound, 45 °C, 16 h, or LDA, succinic anhydride, THF, -78 °C, (b) H₂NSO₂-C₆H₄-NH-NH₂.HCl or MeSO₂-C₆H₄-NH-NH₂.HCl, MeOH, reflux 16 h, (c) H₂NSO₂-C₆H₄-NH-NH₂.HCl or MeSO₂-C₆H₄-NH-NH₂.HCl, TEA, MeOH, Reflux 16 h, (d) LAH, THF, reflux 16 h, (e) DAST, CH₂Cl₂, 25 °C, 3 h.

Methyl 4-(4-methylphenyl)-2,4-dioxobutanoate (C), General Procedure:

To a stirred solution of dimethyl oxalate (4.70 g, 40 mmol) in MeOH (60 mL) was added 4-methylacetophenone (9.04 g, 40 mmol) followed by 25% NaOMe in MeOH (17 mL).

The reaction mixture was ultrasonicated with for 16 h at 45 °C. After cooling to room temperature, the reaction was poured into 1N HCl (125 mL) and cool to 0 °C. The solid formed was filtered, washed with cold water and dried to afford methyl 4-(4-methylphenyl)-2,4-dioxobutanoate as a yellow solid (7.93 g, 65 %). In DMSO this exists in the enol-form exclusively. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.36 (s, 3H, CH₃), 3.66 (s, 3H, CO₂CH₃), 6.76 (s, 1H, =CH), 6.82 (d, *J* = 8.5 Hz, 2H, 4-methylphenyl H-2, H-6), 7.35 (d, *J* = 8.5 Hz, 2H, 4-methylphenyl H-3, H-5), 16.28 [br s, 1H, OH (enol)]. Mass (ESI) *m/z* [M+H]⁺ calcd 221.07; found 221.16.

6-(4-Methylphenyl)-4,6-dioxohexanoic acid (D), General Procedure:

To a stirred solution of *N,N*-diisopropylamine (5.05 g, 50 mmol) in THF (125 mL) was added a 1.6 M solution of *n*-butyl lithium in THF (31.25 mL, 50 mmol) at 0 °C under Argon. The reaction mixture was further cooled to -78 °C and stirred for 10 min at -78 °C followed by 4-methylacetophenone (12.8 g, 50 mmol) in THF (25 mL). The reaction mixture was stirred for 30 min at -78 °C. Then succinic anhydride (2.00 g, 20 mmol) in 50 mL THF was added and stirred for 1 h at -78 °C and 1 h at room temperature. The reaction was poured into 5% HCl (125 mL), then ether (100 mL) was added for extraction. The organic layer was separated and washed with 10% NaOH. The aqueous layer was acidified with 4N HCl, which was extracted with ether (3 X 100 mL). The combined organic layer was dried with Na₂SO₄ and evaporated to dryness and the product **D** was recrystallized from ether as a yellow solid (11.76 g, 68 %). In DMSO this exists in the enol-form exclusively. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.35 (s, 3H, CH₃), 2.64-2.69 (m, 2H, CH₂), 3.12-3.15 (m, 2H, CH₂), 6.75 (s, 1H, =CH), 6.84 (d, *J* = 8.5 Hz,

2H, 4-methylphenyl H-2, H-6), 7.36 (d, $J = 8.7$ Hz, 2H, 4-methylphenyl H-3, H-5), 12.35 [br s, 1H, COOH], 17.14 [br s, 1H, OH (enol)]. Mass (ESI) m/z [M-H]⁻ calcd 233.09; found 233.22.

Methyl 5-(4-methylphenyl)-1-(4-sulfonamidophenyl)-1H-pyrazole-3-carboxylate (E),

General Procedure:

To a stirred solution of 6-(4-methylphenyl)-4,6-dioxohexanoic acid (2.05 g, 6 mmol) in EtOH (60 mL) was added 4-sulfonamidophenylhydrazine hydrochloride (1.5 mL, 6.1 mmol). The reaction mixture was refluxed for 24 h. After cooling to room temperature the solvent was removed in vacuo. The residue was purified using a silica gel column chromatography (7 : 3, Hexane : EtOAc) to give the title compound **E** as a pale yellow amorphous solid (2.37 g, 76 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.34 (s, 3H, CH₃), 3.90 (s, 3H, CO₂CH₃), 7.25 (s, 1H, =CH), 7.36 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.50 (s, 2H, SO₂NH₂), 7.72 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.75 (d, $J = 8.6$ Hz, 2H, 4-sulfonylphenyl H-2, H-6), 7.83 (d, $J = 8.6$ Hz, 2H, 4-sulfonylphenyl H-3, H-5). Mass (ESI) m/z [M+H]⁺ calcd 372.09; found 372.18.

Methyl 5-(4-methylphenyl)-1-(4-methylsulfonylphenyl)-1H-pyrazole-3-carboxylate

(F):

Yellow solid (78 %). $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 2.35 (s, 3H, CH_3), 3.26 (s, 2H, SO_2CH_3), 3.90 (s, 3H, CO_2CH_3), 7.25 (s, 1H, =CH), 7.37 (d, $J = 8.5$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.60 (d, $J = 8.7$ Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.75 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.97 (d, $J = 8.7$ Hz, 2H, 4-methylsulfonylphenyl H-2, H-6). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 371.10; found 371.21.

3-[1-(4-Sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoic acid (G).

A mixture of 6-(4-methylphenyl)-4,6-dioxohexanoic acid (20 mmol), 4-sulfonamidophenylhydrazine hydrochloride (20 mmol), and TEA (20 mmol) in MeOH (150 mL) was stirred at 25 °C for 16 h. The mixture was then concentrated *in vacuo* to a residue, which was partitioned between Et_2O (150 mL) and 5% aq HCl (150 mL). The ether layer was separated, washed with 5% aq HCl (2 X 40 mL), and brine (40 mL), dried (Na_2SO_4 , 1 g), filtered and concentrated to a residue. The crude residue was purified using a silica gel gravity column chromatography (35 : 7 : 1 – CHCl_3 : MeOH : NH_4OH) to give 3-[1-(4-sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoic acid (G) as a yellow solid (83 %). $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 2.37 (s, 3H, CH_3), 2.65 (t, $J = 8.0$ Hz, 2H, CH_2), 2.87 (t, $J = 8.0$ Hz, 2H, CH_2), 6.26 (s, =CH), 7.36 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.53 (s, 2H, SO_2NH_2), 7.70 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.75 (d, $J = 8.9$ Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.85 (d, $J = 8.9$ Hz, 2H, 4-sulfonamidophenyl H-3, H-5), 10.40 (s, 1H, CO_2H). Mass (ESI) $(\text{M}-\text{H})^-$ calcd 384.11; found 384.18.

3-[1-(4-Methylsulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl]propanoic acid (H).

Yellow solid (85 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.32 (s, 3H, CH₃), 2.65 (t, *J* = 8.0 Hz, 2H, CH₂), 2.87 (t, *J* = 8.0 Hz, 2H, CH₂), 3.23 (s, 3H, SO₂CH₃), 6.26 (s, =CH), 7.36 (d, *J* = 8.7 Hz, 2H, 4-methylphenyl H-3, H-5), 7.62 (d, *J* = 8.7 Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.71 (d, *J* = 8.7 Hz, 2H, 4-methylphenyl H-2, H-6), 7.96 (d, *J* = 8.7 Hz, 2H, 4-methylsulfonylphenyl H-2, H-6), 10.40 (s, 1H, CO₂H). Mass (ESI) (M-H)⁻ calcd 383.11; found 383.32.

3-(Hydroxymethyl)-1-(4-sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazole

(I, # 11), General Procedure:

To a stirred solution of compound **E** (1.85 g, 5 mmol) in THF (100 mL) was added lithium aluminium hydride (0.3 g). The reaction mixture was refluxed for 16 h. After cooling to room temperature the reaction was quenched with H₂O (50 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by a silica gel column chromatography (100% EtOAc) to afford the alcohol **I** (# 11) as a white solid (1.2 g, 69 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.33 (s, 3H, CH₃), 3.97 (t, *J* = 8.0 Hz, 1H, OH), 4.85 (d, *J* = 9.0 Hz, 2H, CH₂), 7.24 (s, 1H, =CH), 7.39 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-3, H-5), 7.52 (s, 2H, SO₂NH₂), 7.73 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-2, H-6), 7.76 (d, *J* = 8.6 Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.84 (d, *J* = 8.6 Hz, 2H, 4-sulfonamidophenyl H-3, H-5). Mass (ESI) *m/z* [M+H]⁺ calcd 344.10; found 344.19.

3-(Hydroxymethyl)-1-(4-methylsulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole (J).

White solid (69 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 2.33 (s, 3H, CH_3), 3.26 (s, 2H, SO_2CH_3), 3.98 (t, $J = 8.0$ Hz, 1H, OH), 4.81 (d, $J = 9.0$ Hz, 2H, CH_2), 7.25 (s, 1H, =CH), 7.35 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.65 (d, $J = 8.7$ Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.73 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.95 (d, $J = 8.7$ Hz, 2H, 4-methylsulfonylphenyl H-2, H-6). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 343.10; found 343.03.

3-(3-Hydroxypropyl)-1-(4-sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazole (K).

White solid (76 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.83 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.42 (m, 2H, CH_2), 3.62 (m, 2H, CH_2), 3.74 (m, 1H, OH), 7.24 (s, 1H, =CH), 7.42 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.55 (s, 2H, SO_2NH_2), 7.74 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.73 (d, $J = 8.5$ Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.85 (d, $J = 8.5$ Hz, 2H, 4-sulfonamidophenyl H-3, H-5). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 372.13; found 372.20.

3-(3-Hydroxypropyl)-1-(4-methylsulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole (L).

White solid (70 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.85 (m, 2H, CH_2), 3.25 (s, 2H, SO_2CH_3), 2.30 (s, 3H, CH_3), 2.44 (m, 2H, CH_2), 3.65 (m, 2H, CH_2), 3.75 (m, 1H, OH), 7.25 (s, 1H, =CH), 7.33 (d, $J = 8.7$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.63 (d, $J = 8.6$ Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.75 (d, $J = 8.7$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.92 (d, $J = 8.6$ Hz, 2H, 4-methylsulfonylphenyl H-2, H-6). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 371.14; found 371.05.

3-(Fluoromethyl)-1-(4-sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazole (7),

General Procedure:

To a stirred solution of alcohol **I** (1.72 g, 5 mmol) in CH₂Cl₂ (100 mL) was added diethylaminosulfurtrifluoride (DAST) (0.82 g, 5 mmol). The reaction mixture was stirred for 16 h at room temperature. The reaction was quenched with H₂O (50 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by a silica gel column chromatography (100% EtOAc) to afford the fluoride **I** as a white solid (1.3 g, 70 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.30 (s, 3H, CH₃), 5.45 (d, *J* = 14.0 Hz, 2H, CH₂), 7.22 (s, 1H, =CH), 7.42 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-3, H-5), 7.54 (s, 2H, SO₂NH₂), 7.74 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-2, H-6), 7.77 (d, *J* = 8.6 Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.86 (d, *J* = 8.6 Hz, 2H, 4-sulfonamidophenyl H-3, H-5). Mass (ESI) *m/z* [M+H]⁺ calcd 346.09; found 346.17.

3-(Fluoromethyl)-1-(4-methylsulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole (8).

White solid (74 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.32 (s, 3H, CH₃), 3.27 (s, 2H, SO₂CH₃), 5.41 (d, *J* = 14.0 Hz, 2H, CH₂), 7.23 (s, 1H, =CH), 7.34 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-3, H-5), 7.64 (d, *J* = 8.7 Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.74 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-2, H-6), 7.97 (d, *J* = 8.7 Hz, 2H, 4-methylsulfonylphenyl H-2, H-6). Mass (ESI) *m/z* [M+H]⁺ calcd 345.10; found 345.23.

3-(3-Fluoropropyl)-1-(4-sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazole (9).

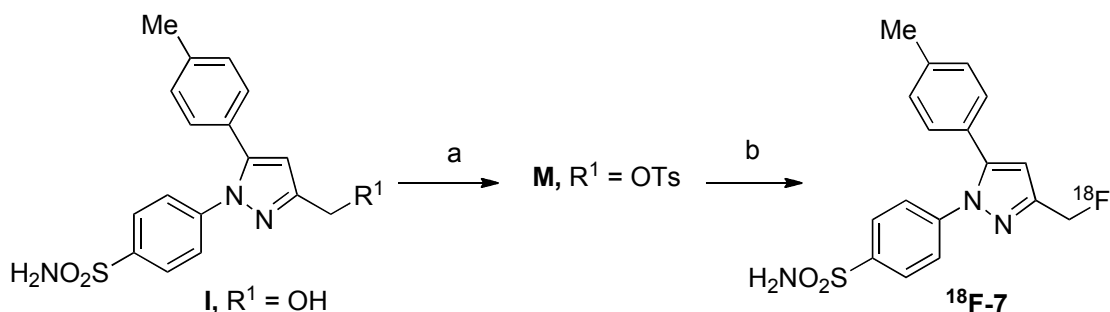
White solid (76 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.83 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.42 (m, 2H, CH_2), 3.82 (m, 2H, CH_2), 7.24 (s, 1H, =CH), 7.42 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.55 (s, 2H, SO_2NH_2), 7.74 (d, $J = 8.8$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.73 (d, $J = 8.5$ Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.85 (d, $J = 8.5$ Hz, 2H, 4-sulfonamidophenyl H-3, H-5). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 374.13; found 372.22.

3-(3-Fluoropropyl)-1-(4-methylsulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole (10).

White solid (89 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.88 (m, 2H, CH_2), 3.28 (s, 2H, SO_2CH_3), 2.34 (s, 3H, CH_3), 2.48 (m, 2H, CH_2), 3.85 (m, 2H, CH_2), 7.25 (s, 1H, =CH), 7.36 (d, $J = 8.7$ Hz, 2H, 4-methylphenyl H-3, H-5), 7.65 (d, $J = 8.6$ Hz, 2H 4-methylsulfonylphenyl H-3, H-5), 7.74 (d, $J = 8.7$ Hz, 2H, 4-methylphenyl H-2, H-6), 7.95 (d, $J = 8.6$ Hz, 2H, 4-methylsulfonylphenyl H-2, H-6). Mass (ESI) m/z $[\text{M}+\text{H}]^+$ calcd 374.13; found 374.25.

• **Radiochemistry.**

The radiochemistry for compound **7** was performed using a fluorodetosylation strategy. The tosylate was generated from the corresponding alcohol using Ts₂O. A microwave-assisted nucleophilic ¹⁸F-fluorodetosylation reaction of the tosylate precursor using carrier-free K¹⁸F/Kryptofix 2.2.2. complex afforded compound [¹⁸F]-**7** in good radiochemical yield (25 %) and high purity (> 99 %)



Scheme 3s. (a) Ts₂O, pyridine, dimethylaminopyridine, CH₂Cl₂, 25 °C, 16 h; (b) K¹⁸F/Kryptofix2.2.2, dimethyl sulfoxide, microwave, 165 °C, 3 min.

{1-(4-Sulfonamidophenyl)-5-(4-methylphenyl)-1H-pyrazol-3-yl}methyl 4-methylbenzenesulfonate (M).

To a stirred solution of alcohol **I** (343 mg, 1 mmol) in CH₂Cl₂ (100 mL) was added pyridine (250 mg), DMAP (35 mg) and Ts₂O (417 mg, 1.3 mmol). The reaction mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo. The residue was purified using a silica gel column chromatography (100% EtOAc) to afford the compound **M** as a white solid (470 mg, 65 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.31 (s, 3H, CH₃), 2.37 (s, 3H, CH₃), 5.14 (s, 2H, CH₂), 7.26 (s, 1H, =CH), 7.44 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-3, H-5), 7.49 (d, *J* = 8.5 Hz, 2H, 4-methylbenzenesulfonate H-3, H-5), 7.55 (s, 2H, SO₂NH₂), 7.75 (d, *J* = 8.8 Hz, 2H, 4-methylphenyl H-2, H-6), 7.79 (d,

$J = 8.5$ Hz, 2H, 4-methylbenzenesulfonate H-2, H-6), 7.82 (d, $J = 8.6$ Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.88 (d, $J = 8.6$ Hz, 2H, 4-sulfonamidophenyl H-3, H-5).
Mass (ESI) m/z $[M+H]^+$ calcd 498.11; found 498.25.

Synthesis of radiotracer ^{18}F -7.

The $[^{18}\text{F}]$ -fluoride anion (27 mCi) was trapped (adsorbed) onto an anion (K_2CO_3 contained) exchange resin (QMA-cartridge) and eluted with a solution of Kryptofix2.2.2 (15 mg) and K_2CO_3 (15 μL , 1 M) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (8 : 2 v/v). The solvent was evaporated using a stream of nitrogen (5-8 psi, 20-30 mL/min) at 85 °C and co-evaporated to dryness with CH_3CN (2 X 200 μL) to remove the residual traces of water. A solution of tosylate precursor (6 mg) in anhydrous DMSO (1 mL) was added to the $\text{K}^{18}\text{F}/\text{Kryptofix2.2.2}$ complex. The reaction mixture was microwaved at 165 °C for three times (1 min each) in a sealed vial. Purification of the crude product was accomplished using HPLC (Varian Microsorb C18, Dynamax 250 X 10, 8 μm , EtOH/ NaH_2PO_4 (10 mM) 50 : 50, flow rate 5 mL/min, R_f 26.7 min). The ^{18}F -7 compound peak was collected and washed with Deionized- H_2O (140 mL). The solvent was evaporated and the radiotracer was formulated with EtOH/Saline (1:9 v/v, 10 mL, 6 mCi, radiochemical yield 25%, radiochemical purity 99%).

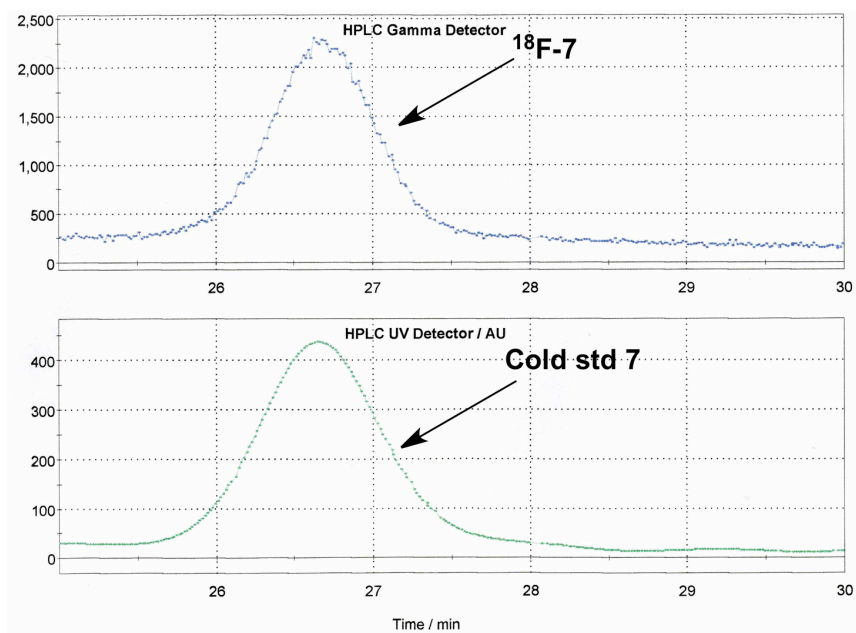


Fig. S1. Typical HPLC chromatogram of ^{18}F -**7** compared with cold standard **7** as shown at R_f 26.7 min. The HPLC was performed using a Varian Microsorb C18, Dynamax 250 X 10, 8 μm column. The HPLC solvent was EtOH/ NaH_2PO_4 (10 mM) 50 : 50, flow rate 5 mL/min (isocratic elution).

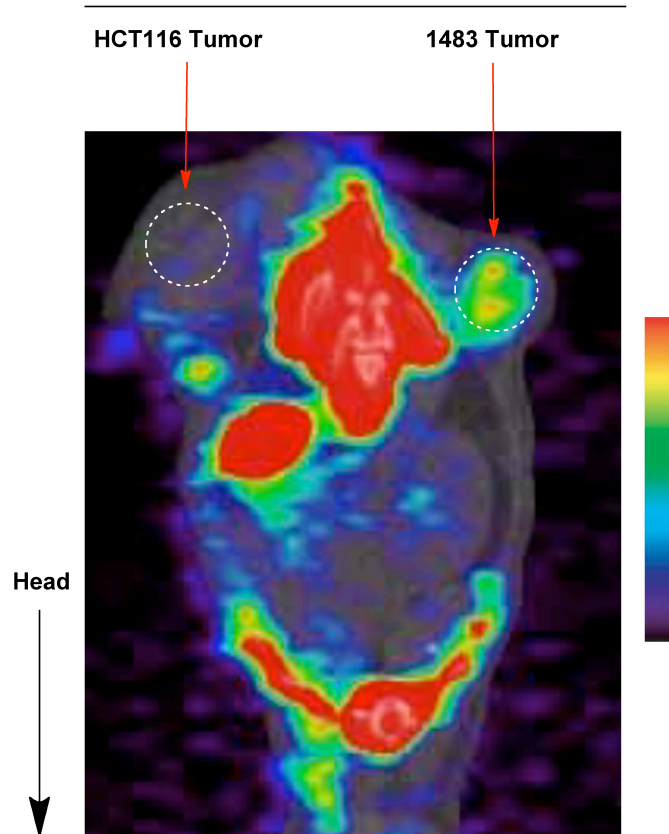


Fig. S2. *In vivo* PET imaging of HCT116 (COX-2-negative) or 1483 HNSCC (COX-2-positive) tumors by ^{18}F -7. HCT116 cells were implanted at the right hip and 1483 cells were implanted in the left hip of the same animal with 3 weeks growth. The HCT116 and 1483 HNSCC tumor bearing female nude mice were dosed by retroorbital injection with compound ^{18}F -7 (100 μL , ~ 350 μCi , r.o.) under anesthesia. At 3 h post-injection, the animals were imaged in the microPET/CT instrument (30 min acquisition). The image depicts a female nude mouse with significant uptake of radiotracer in the COX-2 expressing HNSCC 1483 tumor (dotted circle) compared to minimal uptake in the COX-2 negative HCT116 tumor (dotted circle).

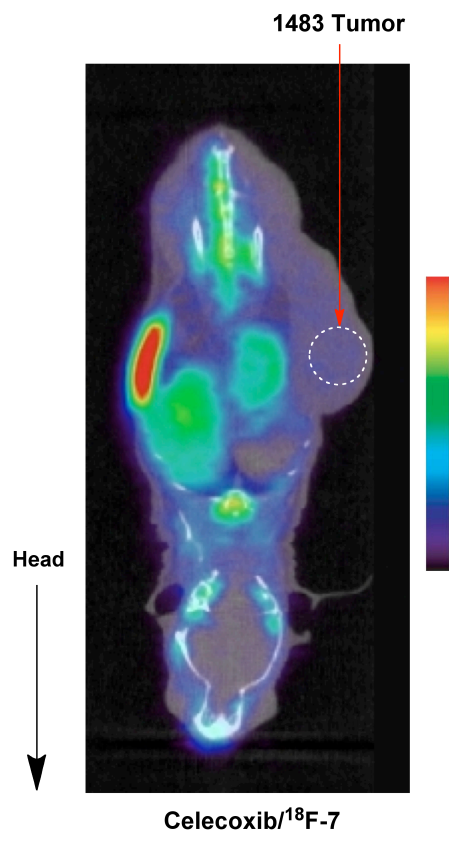


Fig. S3. *In vivo* PET imaging of celecoxib predosed mice with COX-2-expressing human 1483 HNSCC tumors by ¹⁸F-7. Female nude mice bearing 1483 HNSCC xenografts were dosed by injection with celecoxib (20 mg/kg, i.p.) immediately prior to compound ¹⁸F-7 (100 μ L, \sim 350 μ Ci, r.o.) under anesthesia. At 3 h post injection of compounds, the animals were imaged in the microPET/CT instrument (30 min acquisition). The image depicts a nude mouse with minimal ¹⁸F-7 uptake in the 1483 tumor (dotted circle), indicating the efficient blockage of the COX-2 active site to radiotracer in the tumor.

References

1. Kalgutkar AS, Marnett AB, Crews BC, Remmel RP, et al. Ester and Amide Derivatives of the Nonsteroidal Antiinflammatory Drug, Indomethacin, as Selective Cyclooxygenase-2 Inhibitors. *J Med Chem* 2000;43:2860–70.
2. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, et al. Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib). *J Med Chem* 1997;40:1347-75.