SUPPORTING INFORMATION

Targeted Detection of Cyclooxygenase-1 in Ovarian Cancer

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List of Contents

1. General Methods ………………………………………………………………………. page# S3

2. Chemical Synthesis and Characterization ………………………..………………….. page# S4

3. Animal Experiments ……………………………………………….……….…….. page# S7

1. General Methods

ACS grade reagents and anhydrous solvents were used for the synthesis of CMP. All chemicals, including 4-methylacetophenone (compound **1**) were obtained from Sigma-Aldrich, Fisher Scientific, or TCI America. Plastic-backed silica gel micro-thin-layer chromatography plates were employed for qualitative analysis of crude reaction mixtures. Spots were visualized with an ultraviolet illuminator at 254 nm to 365 nm wavelength. Compounds were purified by flash or gravity silica column chromatography. Compound purity was assessed by chromatography on a Waters HPLC system equipped with a photo diode array (PDA) detector and a C-18 column. Eluting solvents were component A (5 mM aqueous octanesulfonic acid (pH ~ 3)) and component B (CH_3CN/component A 9:1) employed as a gradient of 40% - 90% component B. ^1H NMR and ^1H COSY analyses of compounds were carried out on a Bruker AV-I at 600 MHz. The acquired data were processed by a squared sine-bell window symmetrized in magnitude mode. Mass spectrometric analysis employed a quantum triple quadrupole instrument equipped with a ThermoElectron Surveyor pump. Melting points of compounds were determined on a Gallenkamp 7936G capillary melting point apparatus. A T-format Quanta Master QM-9 fluorometer with Felix software was used to evaluate the photophysical properties of CMP.

2. Chemical Synthesis and Characterization

6-(4-Methylphenyl)-4,6-dioxohexanoic acid (2). To a stirred solution of *N,N*-diisopropylamine (55.7 g, 550 mmol) in tetrahydrofuran (125 mL) was added a 1.6 M solution of *n*-butyl lithium in tetrahydrofuran (50 mmol) at 0 ^{o}C under argon. The reaction mixture was further cooled to -78 ^{o}C and stirred for 10 min at -78 ^{o}C followed by addition of compound **1** (12.8 g, 50 mmol) in tetrahydrofuran (25 mL). The reaction mixture was stirred for 30 min at -78 ^{o}C. Then succinic anhydride (2 g, 20 mmol) in 50 mL tetrahydrofuran was added, and the reaction mixture was stirred for 1 h at -78 ^{o}C followed by 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 and then extracted with ether (3 X 100 mL). The combined organic layer was dried with Na_2SO_4 and evaporated to dryness. The product, ***6-(4-***methylphenyl)-4,6-dioxohexanoic acid (**2**) was recrystallized from ether as a yellow solid (15.7 g, 65% yield). In DMSO the compound exists in the enol-form exclusively. ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.35 (s, 3H), 2.64-2.69 (m, 2H), 3.12-3.15 (m, 2H), 6.75 (s, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 2H), 12.35 [br s, 1H], 17.14 [br s, 1H]. HRMS *m/z*: calculated for $[C_{13}H_{14}O_4\text{-}H]^-$ 233.0912; found 233.2232.

3-(1-(4-Methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propanoic acid (3). A mixture of compound **2** (4.69 g, 20 mmol), 4-methoxyphenylhydrazine hydrochloride (3.5 g, 20 mmol), and triethylamine (2 g, 20 mmol) in methanol (150 mL) was stirred at 25 ºC for 16 h. The mixture was then concentrated *in vacuo* to a residue, which was partitioned between ether (150 mL) and 5% aqueous HCl (150 mL). The ether layer was separated, washed with 5% aqueous HCl (2 X 40 mL), and brine (40 mL), dried (Na_2SO_4), filtered and concentrated to a residue. The crude residue

was purified using a silica gel gravity column chromatography (35 : 7 : 1 – $CHCl_3$: CH_3OH : NH_4OH) to give 3-(1-(4-methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propanoic acid (**3**) as a yellow solid (5.4 g, 80 % yield). ^1H NMR (600 MHz, DMSO-d_6) δ 2.26 (s, 3H), 2.62 (t, *J* = 8.0 Hz, 2H), 2.84 (t, *J* = 8.0 Hz), 3.75 (s, 3H), 6.40 (s, 1H), 6.92 (d, *J* = 8.5 Hz, 2H), 7.06 (d, *J* = 8.5 Hz, 2H, 4-methylphenyl H-2, H-6), 7.11 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 10.40 (s, 1H). HRMS *m/z*: calculated for $[C_{20}H_{20}N_2O_3\text{-}H]^-$ 335.1554; found 335.2413.

N-(4-Aminobutyl)-3-(1-(4-methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propenamide hydrochloride (4). To a stirred solution of compound **3** (3.4 g, 10 mmol) in *N,N*-dimethylformamide (250 mL) was added N-BOC butanediamine (5.6 g, 30 mmol), hydroxybenzotriazole (2 g, 15 mmol), *N,N*-diisopropylethylamine (3.9 g, 30 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.1 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, followed by extraction with ethyl acetate (3 X 75 mL). Combined organic layers were dried over Na_2SO_4, concentrated *in vacuo*, and crystallized from *n*-hexane as yellow crystals of *tert*-butyl (4-(3-(1-(4-methoxyphenyl)-5-(*p*-tolyl)-1*H*-pyrazol-3-yl)propanamido)butyl)carbamate. HCl (gas) was bubbled through a solution of *tert*-butyl (4-(3-(1-(4-methoxyphenyl)-5-(*p*-tolyl)-1*H*-pyrazol-3-yl)propanamido)butyl)carbamate in dichloromethane (10 mL) for 1 h at 25 ºC. Removal of solvent *in vacuo* afforded a yellow residue, to which *n*-hexane was added (20 mL). The resultant suspension was stirred for 30 min and then filtered to give N-(4-aminobutyl)-3-(1-(4-methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propenamide hydrochloride (**4**) as a brown solid (4.4g, 99% yield). ^1H NMR (600 MHz, DMSO-d_6) δ 1.42-1.52 (m, 4H), 2.24 (s, 3H), 2.40-2.49 (m, 2H), 2.71-2.74 (m, 2H), 2.80-2.84 (m, 2H), 3.05-3.11 (m, 2H), 3.75 (s, 3H), 6.31 (s, 1H), 6.92 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J*

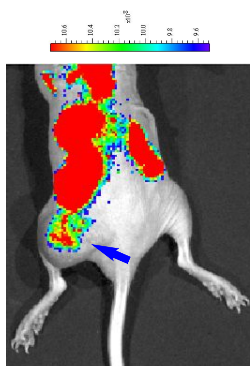
= 8.4 Hz, 2H), 7.89 (br s, 3H), 7.96-7.99 (m, 1H). HRMS *m/z*: calc'd for $[C_{24}H_{30}N_4O_2+H]^+$ calculated 407.2454; found 407.3176.

N-((5-Carboxy-X-rhodaminyl)but-4-yl)-3-(1-(4-methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propenamide (5). To a solution of 5-carboxy-X-rhodamine-acid (5.35 mg, 0.01 mmol) in DMSO (5 mL) was added *N*-succinimidyl)-*N*,*N*,*N′*,*N′*-tetramethyluronium tetrafluoroborate (2.4 mg, 0.008 mmol) and triethylamine (20 μL). The reaction mixture was stirred for 2 h at room temperature. In a separate flask, compound **4** (4.42 mg, 0.01 mmol) in DMSO (10 mL) was treated with a drop of triethylamine. After stirring for 5 min, the succinimidyl ester of 5-carboxy-X-rhodamine was added slowly to the free amine solution through a cannula and the resulting mixture was 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*-((5-carboxy-X-rhodaminyl)but-4-yl)-3-(1-(4-methoxyphenyl)-5-(p-tolyl)-1H-pyrazol-3-yl)propenamide (**5**) as a deep blue solid (5.5 mg, 60% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.40-1.65 (m, 8H), 1.70-1.82 (m, 4H), 1.84-1.95 (m, 4H), 2.23 (s, 3H), 2.39-2.51 (s, 6H), 2.68-2.94 (m, 8H), 3.07-3.12 (m, 4H), 3.15-3.28 (m, 10H), 3.74 (s, 3H), 6.12 (s, 2H), 6.32 (s, 1H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.95-8.00 (m, 1H), 8.16-8.21 (m, 1H). HRMS *m/z*: calc'd for $(C_{57}H_{58}N_6O_6+H)^+$ 923.4476; found 923.5034.

3. Animal Experiments

Establishment of xenografts or allografts in nude mice. Female NU-Fox1nu nude mice (6 to 7 weeks old) were purchased from the Charles River Laboratories. OVCAR-3 and ID8-NGL cells were suspended in cold phosphate-buffered saline containing 30% Matrigel (1×10^6 cells in 100 µL). Cells were implanted individually on the left rear flank of each animal. Tumors were allowed to grow for 2 to 3 weeks to achieve a size of 750 to 1,000 mm^3.

In vivo imaging of tumor xenografts of ovarian cancer. Tumor-bearing female nude mice were injected i.p. with CMP (1 or 2 mg/kg in DMSO) and then imaged under light anesthesia (2% isoflurane) in a Xenogen IVIS 200 instrument (DSRed filter, 1.5-cm depth, and 1 s exposure). Imaging was performed at various time points between 0.25 h and 4.00 h post-dosing of CMP. In some cases, mice were pretreated with an i.p. dose of indomethacin (10 mg/kg) 1 h before CMP (2 mg/kg) to block the COX-1 active site.



ID8-NGL Tumor + CMP
Full body distribution

Figure S1. In vivo optical imaging of mice bearing ID8-NGL tumor on the left flank. The mouse was injected with CMP (i.p., 2 mg/kg in DMSO). Imaging at 3.5 h post-injection revealed tumor uptake due to COX-1 targeting with full body distribution using IVIS 200 imaging system.