Supplementary Information

Chemistry Synthesis

General

All melting points were determined in open capillary tubes using an Optimelt automated melting point system (Stanford Research Systems) and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVIII 400 or AVIII 500 in an appropriate deuterated solvent ($CDCl_3$, CD_3CN or d_6-DMSO). Chemical shifts are reported as parts per million (δ) relative to tetramethylsilane which was used as an internal standard. Coupling constants are given in Hz and coupling patterns are abbreviated as: s (singlet), d (doublet), t (triplet), q (quadruplet), dd (doublet of doublets), dt (doublet of triplets) td (triplet of doublets) and m (multiplet). High resolution ESI mass spectra were acquired on a Bruker Apex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer with an Apollo II ESI/MALDI dual source in positive ion mode. Samples were dissolved in methanol or acetonitrile and injected at 180 μL/h, using a syringe pump. Column chromatography was undertaken on Merck 60 silica gel (40-63 μm).

Chemical Synthesis

4-(2-Bromoacetyl)phenethyl acetate (2). A solution of bromine (4.4 g, 28 mmoles) in ethyl acetate (12 mL) was added over 1 min to a stirred solution of 4-acetylphenethyl acetate **1** (5.2 g, 25 mmoles) in ethyl acetate (70 mL) containing a catalytic amount of aluminium chloride (~50 mg) at room temperature (RT). The resultant solution was protected from light. The bromine solution decolourised immediately giving a yellow solution. After 40 min reaction, HPLC analysis indicated 3.4% of starting material, 76% of product and 14% of the dibromo side-product. After stirring for 2 h the ethyl acetate layer was washed with sodium hydrogen carbonate (10%, 50 mL) and water (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to give a light brown oil (7.0 g). HPLC analysis of the final mixture indicated 2% starting material, 81% of monobrominated product and 11% of the dibrominated side product. Attempts to crystallise the desired product **2** from the mixture using a variety of solvents was unsuccessful. This mixture was used directly in the next step.

4-(6-Chloroimidazo[1,2-*a*]pyridin-2-yl)phenethyl acetate (3). A mixture of the crude 4-(2-bromoacetyl)phenethyl acetate **2** (7.0 g), 2-amino-5-chloropyridine (3.0 g, 24.6 mmoles) and

ethanol (70 mL) was stirred and heated to reflux for 2.5 h. Sodium bicarbonate (1.2 g, 14.4 mmoles) was then added slowly in portions and the solution was left to reflux for an additional 5 h. Another portion of sodium bicarbonate (0.5 g, 6 mmoles) was then added slowly and reflux was continued for another 1.5 h. The reaction was cooled to room temperature and left to stir for 12 h. The precipitate formed was filtered, washed with cold ethanol:water (9:1), water (3 x 20 mL) and dried under reduced pressure to give a pale yellow solid (4.75 g). A small sample was recrystallised from ethanol to give the acetate **3**. ^{1}H NMR (500 MHz, d_6-DMSO): δ 1.99 (s, 3H, $COCH_3$), 2.92 (t, *J* = 6.9 Hz, 2H, $OCH_2C\underline{H_2}$), 4.24 (t, *J* = 6.9 Hz, 2H, $OC\underline{H_2}CH_2$), 7.29 (dd, *J* = 9.6, 2.0 Hz, 1H, ArH), 7.33 (d, *J* = 8.1 Hz, 2H, 2 x ArH), 7.62 (d, *J* = 9.6 Hz, 1H, ArH), 7.89 (d, *J* = 8.1 Hz, 2H, 2 x ArH), 8.34 (s, 1H, ArH), 8.80 (d, *J* = 2.0 Hz, 1H, ArH). ^{13}C NMR (125 MHz, d_6-DMSO): δ 21.6 ($CO\underline{C}H_3$), 35.0 ($OCH_2\underline{C}H_2$), 65.1 ($O\underline{C}H_2CH_2$), 110.5 (ArCH), 118.3 (ArCH), 119.9 (ArC), 125.7 (ArCH), 126.6 (ArCH), 129.8 (ArCH), 130.2 (ArCH), 132.6 (ArC), 138.8 (ArC), 144.2 (ArCH), 146.3 (ArC), 171.2 ($O\underline{C}OCH_3$). HRMS (+ESI): Found *m/z* 315.08991, $(M+H)^+$, $C_{17}H_{16}ClN_2O_2$ calculated 315.08948.

2-(6-chloro-2-(4-(2-hydroxyethyl)phenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N,N*-dimethyl-2-oxoacetamide (4). The acetate **3** (2.4 g, 7.6 mmoles) was added to a stirred solution of oxalyl chloride (3.7 g, 31 mmoles) in dichloromethane (25 mL). After 2 min *N,N*-diisopropylethylamine (1.5 mL, 8.4 mmoles) was added dropwise over 5 min. After stirring for 45 min at RT, the reaction mixture was concentrated under a stream of nitrogen, followed by high vacuum to give a yellow solid. The solid was re-dissolved in a solution of dimethylamine in tetrahydrofuran (2 M, 7.5 mL, 15.2 mmoles) and pyridine (12 mL, 150 mmoles) and left to stand at -20°C for 12 h. The mixture was warmed to room temperature, diluted with ice (100 g) and 2 M hydrochloric acid (50 mL, 100 mmoles) and then extracted with chloroform (3 x 80 mL). The organic extracts were combined and washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a brown oil. The oil was azeotroped with toluene (2 x 20 mL) and concentrated under vacuum. The residue was re-dissolved in methanol (30 mL) and potassium carbonate (2.06 g, 15 mmoles) in water (15 mL) was added. The hydrolysis reaction was stirred at reflux until completion (2-3 h), as indicated by TLC. The hot reaction mixture was cooled to RT, concentrated under a stream of nitrogen and extracted with ethyl acetate (3 x 50 mL). The organic extracts were evaporated to dryness under reduced pressure to give a light brown oil, which solidified after standing at 4°C for 12 h. The product was collected and washed with ice cold methanol:water (1:1) (3 x 3 mL) and dried to give **4** as pale yellow crystals (1.4 g, 53%), m.p. 173.6-173.7°C. ^{1}H NMR (500 MHz, d_6-DMSO): δ 2.35 (s, 3H, NCH_3), 2.77 (s, 3H, NCH_3), 2.80 (t, *J* = 7.0 Hz, 2H, $OCH_2C\underline{H_2}$), 3.65 (td,

J = 7.0, 5.0 Hz, 2H, OC$\underline{H_2}$CH$_2$), 4.68 (t, *J* = 5.0 Hz, 1H, OH), 7.33 (d, *J* = 8.1 Hz, 2H, 2 x ArH), 7.39 (d, *J* = 8.1 Hz, 2H, 2 x ArH), 7.88 (dd, *J* = 9.5, 2.1 Hz, 1H, ArH), 7.97 (dd, *J* = 9.5, 0.6 Hz, 1H, ArH), 9.70 (dd, *J* = 2.1, 0.6 Hz, 1H, ArH). ^{13}C NMR (125 MHz, d$_6$-DMSO): δ 32.9 (NCH$_3$), 36.4 (NCH$_3$), 38.8 (HOCH$_2$$\underline{C}H_2$), 62.0 (HO$\underline{C}H_2CH_2$), 118.1 (ArCH), 118.2 (ArC), 122.6 (ArC), 126.5 (ArCH), 128.2 (ArCH), 129.2 (ArCH), 130.2 (ArC), 131.8 (ArCH), 141.1 (ArC), 145.9 (ArC), 157.2 (ArC), 164.7 (CO$\underline{C}$ON), 181.7 ($\underline{C}$OCON). HRMS (+ESI): Found *m/z* 372.11030, (M+H)$^+$, $C_{19}H_{19}ClN_3O_3$ calculated 372.11095.

2-(6-Chloro-2-(4-(2-fluoroethyl)phenyl)imidazo[1,2-*a*]pyridin-3-yl)-*N,N*-dimethyl-2-oxoacetamide (PBR316). Diisopropylamine (1.0 mL, 6.0 mmoles), perfluoro-1-butanesulfonyl fluoride (0.35 mL, 2.0 mmoles) and triethylamine trifluoride (0.33 mL, 2 mmoles) were added to a stirred mixture of **4** (0.37 g, 1.0 mmoles) in dry acetonitrile (4 mL) and heated to 40^{o}C for 3.5 h. The solvent and volatiles were evaporated under a stream of nitrogen to give a yellow oil. TLC analysis of the crude mixture showed two major compounds. The crude product was purified by flash chromatography using ethyl acetate:heptane (1:1) to give PBR316 (140 mg) as a tan solid, as well as the starting alcohol **4** (72 mg). Recrystallisation from ethyl acetate gave PBR316 as colourless crystals (98 mg, 36%), m.p. 128.6-128.8°C. ^{1}H NMR (500 MHz, d$_6$-DMSO): δ 2.34 (s, 3H, NCH$_3$), 2.77 (s, 3H, NCH$_3$), 3.03 (t, *J* = 6.2 Hz, 1H, FCH$_2$C$\underline{H_A}$H$_B$), 3.05 (dt, *J* = 25.0, 6.2 Hz, 2H, FC$\underline{H_2}$CH$_2$), 4.70 (dt, *J* = 47.0, 6.2 Hz, 2H, FC$\underline{H_2}$CH$_2$), 7.39 (d, *J* = 8.3 Hz, 2H, 2 x ArH), 7.42 (d, *J* = 8.3 Hz, 2H, 2 x ArH), 7.88 (dd, *J* = 9.5, 2.1 Hz, 1H, ArH), 7.98 (dd, *J* = 9.5, 1.0 Hz, 1H, ArH), 9.70 (dd, *J* = 2.1, 1.0 Hz, 1H, ArH).^{13}C NMR (125 MHz, d$_6$-DMSO): 33.73 (NCH$_3$), 36.9 (d, *J* = 20.1 Hz, FCH$_2$$\underline{C}H_2$), 37.3 (NCH$_3$), 84.7 (d, *J* = 165.9 Hz, F$\underline{C}$H$_2$CH$_2$), 119.0 (ArCH), 119.2 (ArC), 123.6 (ArC), 127.4 (ArCH), 129.2 (ArCH), 130.3 (ArCH), 131.7 (ArC), 132.8 (ArCH), 140.0 (ArC), 146.8 (ArC), 158.0 (ArC), 165.6 (CO$\underline{C}$ON), 182.6 ($\underline{C}$OCON). HRMS (+ESI): Found *m/z* 374.10600, (M+H)$^+$, $C_{19}H_{18}ClFN_3O_2$ calculated 374.10716.

4-(6-Chloro-3-(2-(dimethylamino)-2-oxoacetyl)imidazo[1,2-a]pyridin-2-yl)phenethyl 4-methyl benzenesulfonate (5). Method 1: The alcohol **4** (460 mg, 1.24 mmoles) in dry acetonitrile (5 mL) at 40^{o}C was treated with TMHDA (1 mL, 5.0 mmoles), followed by tosyl chloride (0.45 g, 2.5 mmoles). The reaction mixture was stirred at 40^{o}C for 1 h and then at RT overnight. To the mixture was then added dichloromethane (10 mL) and water (10 mL). The organic extract was washed with additional water (2 x 5 mL) and tartaric acid (10%, 5 mL), dried (Na_2SO_4), filtered and then concentrated under reduced pressure to give a yellow oil which crystallised upon standing. Recrystallisation from ethyl acetate and heptane gave the corresponding tosylate **5** as an off-white

powder (254 mg, 39%). Method 2: The tosylation reaction was repeated using the dry grind method. To dry, potassium carbonate (250 mg) was added to the alcohol **4** (200 mg) followed by tosyl chloride (200 mg). The dry mixture was then ground thoroughly and continuously in a mortar for 12 min with a pestle. To the mixture was then added a pellet of potassium hydroxide (~200 mg) and ground for another 5 min to decompose excess tosyl chloride. The damp pasty mixture was then triturated with a mixture of dichloromethane:chloroform (1:1) (6 x 10 mL), decanting into a filtered funnel each time. The paste was then triturated with a further 3 x 10 mL of chloroform and filtered. The filtrate was concentrated to give a dark yellow solid. The solid was then triturated with ethyl acetate with stirring for 20 min and filtered. The filtrate was then again concentrated and the residual solid triturated again with ethyl acetate and the solid filtered to give a second crop of crystals. This process was repeated once more to give a third crop of a yellow solid. The first crop of the yellow solid was recrystallised from ethyl acetate:heptane to give pale yellow prisms of tosylate **5**. The second and third crops, as well as the residue from the above recrystallisation, which also contained some starting material analysed by TLC, (100% ethyl acetate) were purified by flash chromatography on silica gel to give additional tosylate **5** (152 mg, 53%), m.p. 103.6-103.8°C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 2.32 (s, 3H, $ArCH_3$), 2.41 (s, 3H, NCH_3), 2.76 (s, 3H, NCH_3), 2.98 (t, *J* = 6.2 Hz, 2H, $OCH_2C\underline{H}_2$), 4.28 (t, *J* = 6.2 Hz, 2H, $OC\underline{H}_2CH_2$), 7.28 (d, *J* = 8.2 Hz, 2H, 2 x ArH), 7.38 (d, *J* = 8.2 Hz, 2H, 2 x ArH), 7.46 (d, *J* = 8.3 Hz, 2H, 2 x ArH), 7.72 (d, *J* = 8.3 Hz, 2H, 2 x ArH), 7.87 (dd, *J* = 9.4, 2.1 Hz, 1H, ArH), 7.97 (dd, *J* = 9.4, 0.8 Hz, 1H, ArH), 9.96 (dd, *J* = 2.1, 0.8 Hz, 1H, ArH). ^{13}C NMR (100 MHz, d_6-DMSO): δ 21.1 ($Ar\underline{C}H_3$), 32.9 ($OCH_2\underline{C}H_2$), 34.2 (NCH_3), 36.4 (NCH_3), 70.8 ($O\underline{C}H_2CH_2$), 118.1 (ArCH), 118.2 (ArC), 122.7 (ArC), 126.5 (ArCH), 127.5 (ArCH), 128.2 (ArCH), 129.4 (ArCH), 130.2 (ArCH), 130.9 (ArC), 131.9 (ArCH), 132.2 (ArC), 138.4 (ArC), 144.9 (ArC), 145.9 (ArC), 156.9 (ArC), 164.6 (CO$\underline{C}$ON), 181.6 ($\underline{C}$OCON). HRMS (+ESI): Found *m/z* 526.11908, $(M+H)^+$, $C_{26}H_{25}ClN_3O_5S$ calculated 526.11980.

Biodistribution and displacement results of [^{18}F]PBR316 in mice with PC-3M-Luc-C6 tumours measured by γ-counter post PET imaging

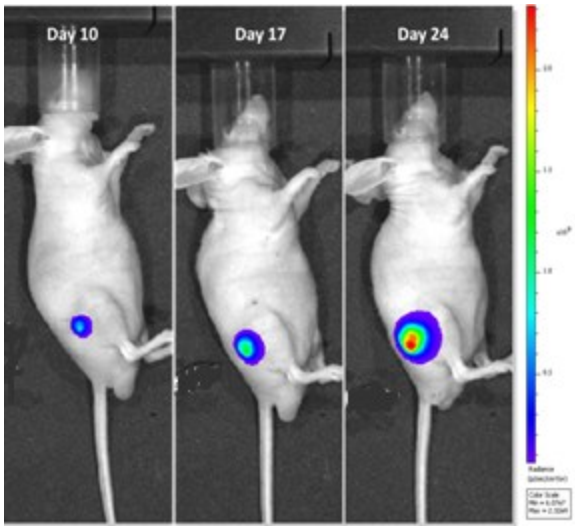

Figure 1. Bioluminescence imaging of PC-3M-Luc-C6 xenograft mice administered with luciferase expressing cells, 10, 17 and 24 days after cell injection.

Biodistribution and displacement results of [^{18}F]PBR316 in mice with PC-3M-Luc-C6 tumours measured by the γ–counter post PET imaging study

Biodistribution of [^{18}F]PBR316 in mice measured by γ-counter

Table 1 summarises the tissue time-course distribution of [^{18}F]PBR316 in mice with PC-3M-Luc-C6 tumours measured in the γ–counter post PET imaging study. The uptake in the kidney, blood and heart decreases throughout the time of measurement. The uptake in the tumour increases from 30 min to 4 h (1.8-3.6 % ID/g).

% ID/g	30 min	1 h	1.5 h	3 h	4 h
Kidney	36.6±1.4	20.4±4.6	23.2±6.1	18.8±2.3	16.7±0.4
Heart	20.8±2.5	8.9±1.2	7.9±1.8	4.1±0.3	4.5±0.9
Blood	0.62±0.01	0.31±0.02	0.32±0.11	0.34±0.08	0.28±0.01
Muscle	1.4±0.2	1.7±0.1	1.1±0.4	1.5±0.4	1.2±0.2
Tumour	1.8±0.4	1.9±0.3	2.4±0.5	3.8±0.1	3.6±0.6

Table 1 Tissue

time-course distribution of [^{18}F]PBR316 in mice with PC-3M-Luc-C6 tumours measured by the γ–counter post PET imaging study. Results are mean ± SD, *n* = 3 percent injected dose per gram (% ID/g).

Displacement [^{18}F]PBR316 in mice measured by γ-counter

The displacement study with PK11195 confirmed the PET results (Fig 2). PK11195 displace the uptake of [^{18}F]PBR316 in the heart and kidney by 77 and 55 % ($p < 0.05$), compared to the uptake of [^{18}F]PBR316 at 30 min p.i. A non-significant difference was observed in the muscle and blood. The uptake of [^{18}F]PBR316 in tumour was significantly increased (83 %; $p < 0.05$), compared

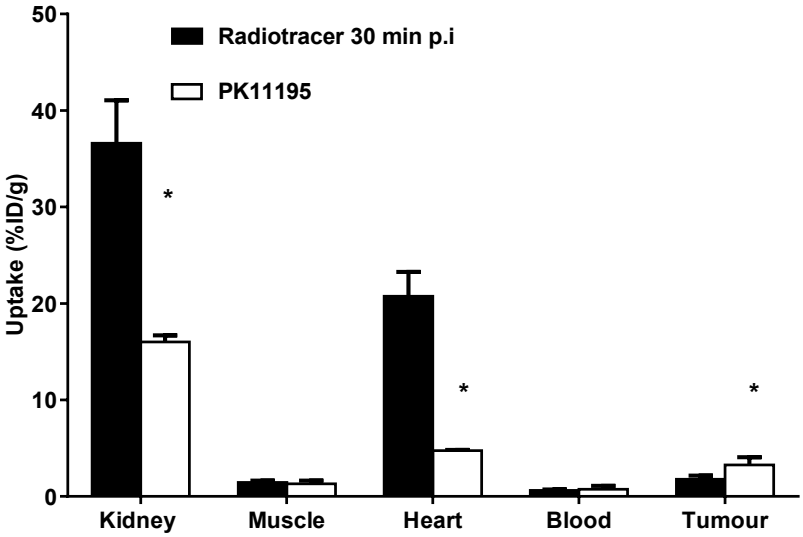

Figure2. Effect of the displacement drug PK11195 (1 mg/kg, injected 30 min after the radiotracer) on [^{18}F]PBR316 uptake in organs and PC-3M-Luc-C6 tumour of mice sacrificed 60 min p.i.; compared with uptake of [^{18}F]PBR316 at 30 min p.i., measured by γ-counter. Results are mean ± SD, unit is % injected dose/g (% ID/g) tissue, (*n* = 3, *$p < 0.05$)