Electronic Supplementary Material

Preclinical Evaluation of a Novel TSPO-PET Ligand 2-(7-Butyl-2-(4-(2-[^{18}F]Fluoroethoxy)phenyl)-5-Methylpyrazolo[1,5-*a*]Pyrimidin-3-yl)-*N*,*N*-Diethylacetamide (^{18}F-VUIIS1018A) to Image Glioma

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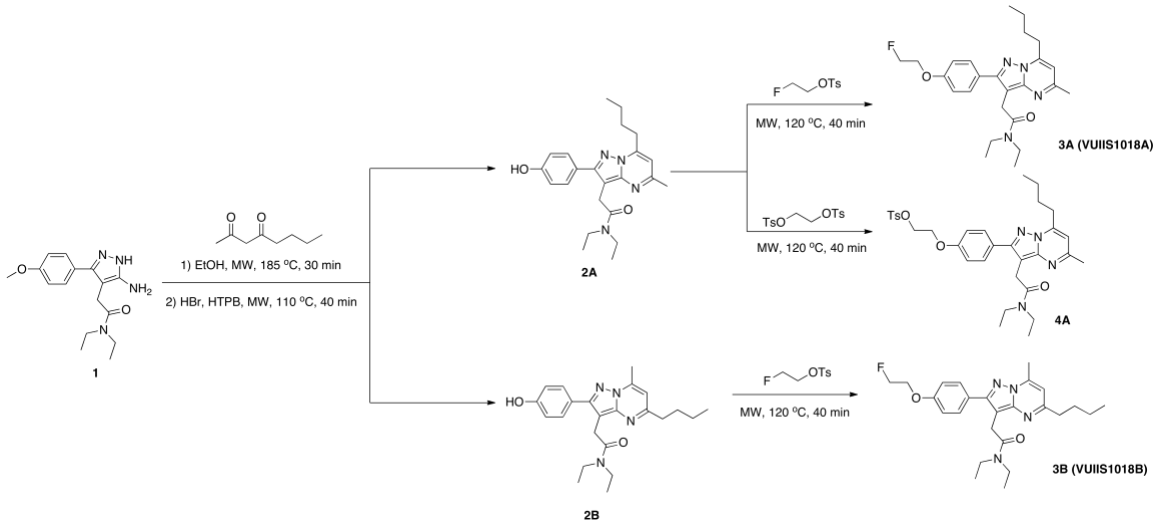

Scheme 1. Synthetic route for **VUIIS1018A** (**3A**), **VUIIS1018B** (**3B**) and precursor (**4A**) for radiosynthesis.

All commercially available reagents were used without further purification. Microwave reactions were carried out with a Biotage Initiator TM Sixty microwave system (Uppsala, Sweden). Reaction residues were purified using a CombiFlash purification system (Teledyne Isco) with silica cartridges. Further reversed-phase HPLC purification was performed with a Gilson preparative separation system (Gilson Inc.; USA). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 600 MHz spectrometer in the Vanderbilt Small Molecule NMR Facility. Chemical shifts are reported in ppm using the residual of chloroform as the internal standard (7.26 ppm for ^{1}H and 77.160 ppm for ^{13}C). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. High-resolution mass spectra were acquired with a Waters 2690 Alliance LC system with Thermo Finnigan TSQ 7000 Triple Quadrupole mass spectrometer equipped with a dual channel ESI-CI source. All compounds used for biological assays were purified

by HPLC and were ≥ 95% purity based on analytical LC/MS monitored at 254 nm.

2. Synthetic and Characterization Data

a. *2-(5-amino-3-(4-methoxyphenyl)-1H-pyrazol-4-yl)-N,N-diethylacetamide (**1**)*

Compound **1** was synthesized and characterized according to previously published methods [1, 2].

b. 2-(7-butyl-2-(4-hydroxyphenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide (**2A**) and 2-(5-butyl-2-(4-hydroxyphenyl)-7-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide (**2B**)

To a solution of **1** (300 mg, 1.0 mmol) in 20 mL EtOH was added 2,4-octanedione (114 mg, 1.0 mmol). The reaction mixture was irradiated with microwaves in a sealed vial at 185 °C for 30 min. The reaction progression was determined by LC/MS. When complete, the reaction mixture was concentrated *in vacuo* and the residue purified with column chromatography to afford 360 mg (89%) of product as white crystals. This product was a mixture of *N*,*N*-diethyl-2-(7-butyl-2-(4-methoxyphenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide and *N*,*N*-diethyl-2-(5-butyl-2-(4-methoxyphenyl)-7-methylpyrazolo[1,5- *a*]pyrimidin-3-yl)acetamide.

To a solution of the mixture of *N*,*N*-diethyl-2-(7-butyl-2-(4-methoxyphenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide and *N*,*N*-diethyl-2-(5-butyl-2-(4-methoxyphenyl)-7-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)acetamide (300 mg, 0.73 mmol) in HBr (aqueous, 7.0 mL) was added a catalytic amount of hexadecyltributylphosphonium

bromide (HTPB, 40 mg, 0.079 mmol). The reaction mixture was sealed then irradiated with microwaves at 110 °C for 40 min and monitored with LC/MS. When complete, the mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with DCM (100 mL × 3). The organic layers were pooled and concentrated *in vacuo*. The residue was then purified with a Gilson preparative purification system, which afforded **2A** (130 mg, 45%) and **2B** (100 mg, 35%) as white crystals.

2A:^{1}H-NMR ($CDCl_3$, 600 MHz) δ 7.65 (d, 2H, *J* = 8.6 Hz), 6.82 (d, 2H, *J* = 8.6 Hz), 6.46 (s, 1H), 3.97 (s, 2H), 3.49 (m, 2H), 3.37 (m, 2H), 3.13 (t, 2H, *J* = 7.8 Hz), 2.54 (s, 3H), 1.83 (m, 2H), 1.48 (m, 2H), 1.13 (t, 3H, *J* = 7.1 Hz), 1.08 (t, 3H, *J* = 7.1 Hz), 0.98 (t, 3H, *J* = 7.3 Hz).^{13}C-NMR ($CDCl_3$, 150 MHz) δ 157.52, 157.07, 155.22, 129.92, 125.17, 115.63, 106.58, 100.03, 42.53, 40.84, 29.61, 28.43, 27.90, 24.60, 22.46, 14.07, 13.85, 12.94. HRMS calcd. $C_{23}H_{30}N_4O_2$ for m/z = 395.2369 $(M + H)^+$, found 395.2430.

2B: ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 7.71 (d, 2H, *J* = 8.4 Hz), 6.84 (d, 2H, *J* = 8.5 Hz), 6.50 (s, 1H), 3.98 (s, 2H), 3.55 (m, 2H), 3.37 (m, 2H), 2.77 (t, 2H, *J* = 7.7 Hz), 2.74(s, 3H), 1.75 (m, 2H), 1.41 (m, 2H), 1.13(t, 3H, *J* = 7.1 Hz), 1.07 (t, 3H, *J* = 7.1 Hz), 0.95(t, 3H, *J* = 7.4 Hz).^{13}C-NMR ($CDCl_3$, 150 MHz) δ 170.86, 161.35, 157.09, 155.51, 147.37, 144.82, 130.01, 125.13, 115.66, 107.55, 100.41, 42.65, 40.91, 37.84, 30.98, 28.44, 22.40, 17.02, 14.17, 13.93, 12.95. HRMS calcd $C_{23}H_{30}N_4O_2$ for m/z = 395.2369 $(M + H)^+$, found 395.2437.

c. 2-(7-butyl-2-(4-(2-fluoroethoxy)phenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide (**3A**)

To a solution of **2A** (130 mg, 0.33 mmol) in 12 mL anhydrous THF was added 100% NaH (27.4 mg, 1.14 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. To the reaction was added 2-fluoroethyl-4-methylbenzenesulfonate (248.8 mg, 1.14 mmol). The reaction mixture was then sealed and irradiated with microwaves at 120 °C for 40 min. The reaction progress was determined by LCMS. When completed, the reaction mixture was quenched then diluted with 1N HCl (100 mL) and extracted with DCM (100 mL × 3). The organic layers were pooled and concentrated *in vacuo.* The residue was then purified with a Gilson preparative purification system, which afforded **3A** (100 mg, 70%) as white crystals. ^{1}H-NMR (CDCl3, 600 MHz) δ 7.84 (d, 2H, *J* = 8.7 Hz), 7.01 (d, 2H, *J* = 8.8 Hz), 6.51 (s, 1H), 4.82 (t, 1H, *J* = 4.1 Hz), 4.74 (t, 1H, *J* = 4.7 Hz), 4.28 (t, 1H, *J* = 4.2 Hz), 4.24 (t, 1H, *J* = 4.3 Hz), 3.94 (s, 2H), 3.55 (m, 2H), 3.40 (m, 2H), 2.79 (t, 2H, *J* = 7.7 Hz), 2.75 (s, 3H), 1.74 (m, 2H), 1.41 (m, 2H), 1.20 (t, 3H, *J* = 7.1 Hz), 1.10 (t, 3H, *J* = 7.1 Hz), 0.95 (t, 3H, *J* = 7.3 Hz). ^{13}C-NMR (CDCl3, 150 MHz) δ 170.22, 161.31, 158.61, 155.02, 130.11, 129.95, 126.93, 114.65, 107.54, 100.96, 82.46, 81.33, 67.18, 67.04, 42.43, 40.67, 37.74, 30.97, 28.13, 22.42, 17.02, 14.38, 13.92, 13.07. HRMS calcd $C_{25}H_{33}FN_4O_2$ for m/z = 441.2588 $(M + H)^+$, found 441.2672.

d. 2-(5-butyl-2-(4-(2-fluoroethoxy)phenyl)-7-methylpyrazolo[1,5-*a*]pyrimidin-3-yl)-*N*,*N*-diethylacetamide (**3B**)

To a solution of **2B** (60 mg, 0.15 mmol) in 12 mL anhydrous THF was added 100% NaH

(11.8 mg, 0.49 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. To the reaction mixture was added 2-fluoroethyl-4-methylbenzenesulfonate (107 mg, 0.49 mmol). The reaction mixture was sealed and irradiated with microwaves at 120 °C for 40 min. The reaction progress was determined with LCMS. When completed, the reaction mixture was slowly quenched then diluted with 1N HCl (50 mL) and extracted with DCM (50 mL × 3). The organic layers were pooled and concentrated *in vacuo.* The residue was then purified with a Gilson preparative purification system, which afforded **3B** (46 mg, 70%) as white crystals. ^{1}H-NMR (CDCl3, 600 MHz) δ 7.79 (d, 2H, *J* = 8.7 Hz), 7.00 (d, 2H, *J* = 8.8 Hz), 6.51 (s, 1H), 4.81 (t, 1H, *J* = 4.1 Hz), 4.73 (t, 1H, *J* = 4.1 Hz), 4.28 (t, 1H, *J* = 4.1 Hz), 4.23 (t, 1H, *J* = 4.3 Hz), 3.94 (s, 2H), 3.50 (m, 2H), 3.40 (m, 2H), 3.14 (t, 2H, *J* = 7.6 Hz), 2.75 (s, 3H), 1.85 (m, 2H), 1.49 (m, 2H), 1.19 (t, 3H, *J* = 7.1 Hz), 1.11 (t, 3H, *J* = 7.1 Hz), 0.99 (t, 3H, *J* = 7.4 Hz). ^{13}C-NMR (CDCl3, 150 MHz) δ 169.88, 158.30, 157.21, 154.31, 148.30, 147.48, 129.78, 129.71, 126.85, 114.37, 106.41, 100.34, 82.22, 81.09, 66.94, 66.81, 42.06, 40.34, 29.34, 27.89, 27.75, 27.66, 24.51, 22.23, 14.06, 13.62, 13.59, 12.82. HRMS calcd $C_{25}H_{33}FN_4O_2$ for m/z = 441.2588 $(M + H)^+$, found 441.2672.

e. 2-(4-(7-butyl-3-(2-(diethylamino)-2-oxoethyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-2-yl)phenoxy)ethyl 4-methylbenzenesulfonate (**4A**)

To a solution of **2A** (140 mg, 0.36 mmol) in 12 mL anhydrous THF was added 100% NaH (27.4 mg, 1.14 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. To the reaction mixture then was added ethane-1,2-diyl bis(4-methylbenzenesulfonate) (422.3 mg, 1.14 mmol). The reaction mixture was then sealed and irradiated with microwaves at

120 °C for 40 min. The reaction progress was determined with LCMS. When completed, the reaction mixture was carefully quenched then diluted with 1N HCl (100 mL) and extracted with DCM (100 mL × 3). The organic layers were pooled and concentrated *in vacuo*. The residue was then purified with a Gilson preparative purification system, which afforded **4A** (180 mg, 84%) as white crystals. ^{1}H-NMR (CDCl3, 600 MHz) δ 7.82 (d, 2H, *J* = 8.3 Hz), 7.75 (d, 2H, *J* = 8.8 Hz), 7.35 (d, 2H, *J* = 8.2 Hz), 6.85(d, 2H, *J*= 8.8 Hz), 6.51 (s, 1H), 4.38 (t, 2H, *J* = 6.8 Hz), 4.18 (t, 2H, *J* = 4.9 Hz), 3.90 (s, 2H), 3.51 (m, 2H), 3.40 (m, 2H), 3.14 (t, 2H, t = 7.74 Hz), 2.54 (s, 3H), 2.45 (s, 3H), 1.85 (m, 2H), 1.49 (m, 2H), 1.19 (t, 3H, *J* = 7.1 Hz), 1.11 (t, 3H, *J* = 7.1 Hz), 0.99 (t, 3H, *J* = 7.1 Hz).^{13}C-NMR (CDCl3, 150 MHz) δ 169.86, 157.86, 157.23, 154.25, 144.71, 132.61, 129.73, 127.75, 126.96, 114.28, 106.44, 100.34, 67.83, 65.17, 42.07, 40.34, 42.07, 40.34, 29.35, 27.87, 27.66, 24.48, 22.23, 21.40, 14.08, 13.62, 12.82. HRMS calcd $C_{32}H_{40}N_4O_5S$ for m/z = 593.2719 $(M + H)^+$, found 593.2794.

Table 1. Parameter estimates for [^{18}F]VUIIS1018A pharmacokinetics in normal brain and tumor tissue using a 2-tissue, 4-parameter model for both normal 60-min dynamic scan. Results = Mean ± SEM.

Region	K_1	k_2	k_3	k_4
Tumor (n = 7)	0.831 ± 0.202	3.115 ± 1.404	0.298 ± 0.084	0.014 ± 0.002
Brain (n = 7)	0.610 ± 0.215	2.320 ± 1.074	0.152 ± 0.058	0.052 ± 0.017

References

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