1. Synthesis and characterization of the radiolabeling precursor for ^{11}C-EKAP and EKAP

1.1 General Information

All reagents and solvents were obtained from commercial sources (Sigma-Aldrich, VWR and Fisher Scientific) and used without further purification unless noted otherwise. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on an Agilent™ 400 MHz or 600 MHz NMR spectrometer. Chemical shifts are reported in parts per million, with the solvent resonance as the internal standard ($CDCl_3$: 7.26 ppm; DMSO-d_6: 2.49 ppm in ^{1}H NMR and $CDCl_3$: 77.0 ppm; DMSO-d_6: 39.7 ppm in ^{13}C NMR). Multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet); coupling constants *J* are given in hertz (Hz). Melting point was determined on an Electrothermal™ Mel-Temp instrument. High resolution mass spectrometry was run on a 9.4T Bruker Qe FT-ICR MS system. Chiral-HPLC was performed with a Varian Prostar system including a Prostar 210 pump and a Prostar 325 UV detector at 254 nm wavelength. All chemicals used were of ≥ 95% purity, based on HPLC, LC-MS, or NMR.

1.2 Synthesis and characterization of the radiolabeling precursor (*(S)*-**11**)

Benzylglycine (**1**) was protected with Boc-anhydride, followed by a coupling amidation with serine methyl ester (**3**) to afford compound **4**. It was treated with $SOCl_2$ and cyclized to produce the piperazinedione (**5**), which was then reduced to piperazine (**6**) using $LiAlH_4$. Coupling of compound **6** and dichlorophenylacetic acid (**7**) led to compound

8, which was oxidized through Swern oxidation to provide aldehyde (**9**). Compound 9 inevitably racemized when triethylamine was used in the oxidation reaction. The racemic aldehyde then underwent a reductive amination with diethylamine, followed by cleavage of the benzyl protecting group to afford racemic compound **11**.

N-Benzyl-N-(tert-butoxycarbonyl)glycine (**2**): To a suspension of *N*-benzylglycine hydrochloride (**1**, 10.17 g, 50.43 mmol) and $(Boc)_2O$ (13.58 g, 60.36 mmol) in deionized (DI) water (150 mL) was added triethylamine (TEA, 14.1 mL, 101.09 mmol). The mixture was stirred at room temperature for 3 h, and quenched with 12 N HCl (3.70 mL, 151.29 mmol) until the pH of the mixture reached 2. The mixture was extracted with EtOAc (100 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford compound **2** as a white solid (11.1 g, 83%) in a ~1:1 mixture of two rotamers, which was used in the next step of synthesis without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz): Rotamer A: δ 1.48 (s, 9H), 3.82 (s, 2H), 4.55 (rotamer B, s, 1H), 4.51 (rotamer A, s, 1H), 7.17-7.38 (m, 5H), 9.30 (br s, 1H). M.P. 103-104 ^{o}C.

Methyl N-benzyl-N-(tert-butoxycarbonyl)glycyl-D-serinate (**4**): To a solution of compound **2** (11.10 g, 41.84 mmol) in anhydrous CH_2Cl_2 (50 mL) was added 1,1'-carbonyldiimidazole (CDI, 6.78 g, 41.84 mmol) at room temperature under argon. The solution was stirred at room temperature for 1 h and then added slowly via a cannula to a pre-cooled (0 ^{o}C) solution of *D*-serine methyl ester hydrochloride (**3**, 6.51 g, 41.84 mmol) and TEA (5.8 mL, 41.84 mmol) in anhydrous CH_2Cl_2 (30 mL). The mixture was stirred at room temperature for 16 h and washed with saturated $NaHCO_3$ solution (80 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was

purified on a silica gel column eluting with 30-60% acetone/hexanes to afford compound **4** as a white solid (6.1 g, 48%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.50 (s, 9H), 3.78 (s, 3H), 3.82-3.94 (m, 2H), 4.45 (br s, 1H), 4.54-4.70 (m, 2H), 7.19-7.39 (m, 5H). M.P. 105-107 ^{o}C.

(R)-1-Benzyl-3-(hydroxymethyl)piperazine-2,5-dione (**5**): To a solution of compound **4** (6.1 g, 16.6 mmol) in anhydrous MeOH (60 mL) under argon was dropwise added $SOCl_2$ (6.1 mL, 83.2 mmol). The mixture was stirred at room temperature for 4 h and concentrated *in vacuo*. The resulting solid was washed with Et_2O (18 mL × 2), re-dissolved in a mixture of MeOH and NH_4OH (1:1, *v/v*, 60 mL) and stirred for 16 h. The mixture was concentrated *in vacuo*, and the crude product was purified on a silica gel column eluting with 10% THF/hexanes to afford compound **5** as a white solid (4.0 g, 80%), ^{1}H-NMR (CD_3OD, 400 MHz): δ 3.69-3.78 (m, 2H), 4.05 (s, 2H), 3.99-4.08 (m, 1H), 4.59 (d, J = 14.89 Hz, 1H), 4.77 (d, J = 14.87 Hz, 1H), 7.21-7.38 (m, 5H). M.P. 183-186 ^{o}C.

(S)-(4-Benzylpiperazin-2-yl)methanol (**6**): To a solution of compound **5** (4.0 g, 17.1 mmol) in anhydrous THF (60 mL) under argon was dropwise added a solution of $LiAlH_4$ (14.2 mL, 2.4 M in THF). The mixture was heated to reflux for 2 h, cooled to room temperature and quenched sequentially with DI water (1.3 mL), NaOH solution (3.9 mL, 2 M), and DI water (1.3 mL). The suspension was filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with CH_2Cl_2/MeOH/NH_4OH (200:10:1) to give compound **6** as yellow oil (2.5 g, 71%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.88 (t, J = 10.28 Hz, 1H), 2.09 (m, 1H), 2.62 (br s, 1H), 2.71

(d, *J* = 10.99 Hz, 2H), 2.85-2.95 (m, 2H), 2.97-3.02 (m, 2H), 3.49 (d, *J* = 5.55 Hz, 2H), 3.52-3.58 (m, 2H), 7.23-7.27 (m, 1H), 7.34 (d, *J* = 3.90 Hz, 2H), 7.30-7.38 (m, 2H).

(S)-1-(4-Benzyl-2-(hydroxymethyl)piperazin-1-yl)-2-(3,4-dichlorophenyl)ethan-1-one (**8**): To a solution of compound **7** (1.95 g, 9.5 mmol) in anhydrous CH_2Cl_2 (20 mL) under argon was added CDI (1.54 g, 9.5 mmol). The solution was stirred at room temperature for 1 h and added slowly via a cannula to a pre-cooled (0 ^oC) solution of compound **6** (1.96 g, 95 mmol) in anhydrous CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 16 h and washed with saturated $NaHCO_3$ solution (30 mL × 3). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 40-70% EtOAc/hexanes to afford compound **8** as a white solid (1.77 g, 37%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.93-2.29 (m, 2H), 2.75-3.10, (m, 2H), 3.33-3.67 (m, 4H), 3.67 (rotamer B, s, 1.2H), 3.72 (rotamer A, s, 0.8H), 3.88 (br s, 1H), 3.75-4.10 (m, 2H), 4.37-4.45 (rotamer A, m, 0.4H), 4.56-4.62 (rotamer B, m, 0.6H), 7.07 (dd, *J* = 2.15, 8.28 Hz, 1H), 7.24-7.42 (m, 7H). M.P. 144-145 ^oC.

4-Benzyl-1-(2-(3,4-dichlorophenyl)acetyl)piperazine-2-carbaldehyde (**9**): To a solution of oxalyl chloride (0.46 mL, 5.39 mmol) in anhydrous CH_2Cl_2 (30 mL) under argon and cooled to -78 ^oC was dropwise added a solution of DMSO (0.64 mL, 8.98 mmol) in anhydrous CH_2Cl_2 (2 mL), followed by a solution of compound **8** (1.77 g, 4.49 mmol) in CH_2Cl_2 (5 mL). TEA (2.5 mL, 17.96 mmol) was added after 30 min, and the mixture was stirred for another 15 min, then slowly warmed to 0 ^oC. The reaction was quenched with DI water and extracted with CH_2Cl_2 (20 mL × 2). The combined organic phase was

dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 30-50% EtOAc/hexanes to afford compound **9** as a yellow solid (0.9 g, 51 %). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.05 (td, J = 3.42, 11.71 Hz, 1H), 2.28 (dd, J = 4.27, 12.04 Hz, 1H), 2.76 (d, J = 11.80 Hz, 1H), 3.0 (d, 1H), 3.33 (td, J = 3.52, 12.47 Hz, 1H), 3.43 (dt, J = 2.01, 11.97 Hz, 1H), 3.45-3.61 (3H, m), 3.76 (s, 2H), 5.11-5.15 (m, 1H), 7.13 (dd, J = 1.83, 8.26 Hz, 1H), 7.20-7.43 (m, 7H), 9.45 (s, 1H). M.P. 144-145 ^{o}C.

1-(4-Benzyl-2-((diethylamino)methyl)piperazin-1-yl)-2-(3,4-dichlorophenyl)ethan-1-one (**10**): To a solution of compound **9** (0.33 g, 0.84 mmol) in anhydrous 1,2-dichloroethane (13 mL) under argon were added 3Å molecular sieves (0.30 g) and diethylamine (DEA, 0.25 g, 2.52 mmol), followed by AcOH (0.14 mL, 2.52 mmol). After stirring for 30 min, $NaBH(OAc)_3$ (0.53 g, 2.52 mmol) was added in three portions and the reaction mixture was kept stirring for 16 h. The reaction was quenched with saturated $NaHCO_3$ solution until the pH reached 8.0, then extracted with CH_2Cl_2 (10 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 20-30% MeOH/CH_2Cl_2 to afford compound **10** as pale yellow oil (0.34 g, 90%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 7.06 Hz, 6H), 1.77-2.02 (m, 2H), 2.40 (q, J = 7.08 Hz, 4H), 2.45-3.00 (m, 5H), 3.30-3.45 (m, 3H), 3.58 (s, 1H), 3.67 (d, J = 2.96 Hz, 1H), 4.34-4.36 (rotamer A, m, 0.5H), 4.53-4.55 (rotamer B, m, 0.5H), 7.01 (dd, J = 8.32, 13.95 Hz, 1H), 7.15-7.25 (m, 5H), 7.27-7.32 (m, 2H).

2-(3,4-Dichlorophenyl)-1-(2-((diethylamino)methyl)piperazin-1-yl)ethan-1-one (**11**): Compound **10** (0.42 g, 0.90 mmol) was dissolved in a mixture of THF and DI water

(1:1, *v/v*, 8 mL) and degassed with argon for 10 min. To this solution were added 12 N HCl (0.8 mL) and 5% Pd/C powder (0.60 g). The resulting suspension was stirred under an atmosphere of hydrogen at room temperature for 3 h. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was diluted with DI water, basified with 2 M Na_2CO_3 solution, and extracted with CH_2Cl_2 (5 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with CH_2Cl_2/MeOH/NH_4OH (200-100:10:1) to afford the racemic compound **11** as colorless oil (0.20 g, 76%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.94 (t, 6H), 2.46 (q, J = 6.91 Hz, 2H), 2.47-3.16 (m, 9H), 3.61 (s, 1H), 3.69 (s, 1H), 3.38-3.72 (m, 1H), 4.36 (rotamer A, m, 0.5H), 4.56 (rotamer B, m, 0.5H), 7.01 (dd, J = 7.96, 17.43 Hz, 1H), 7.27 (s, 1H), 7.31 (d, J = 8.15 Hz, 1H); ^{13}C-NMR: ($CDCl_3$, 151 MHz): δ 169.35, 168.51, 135.99, 135.49, 132.36, 130.89, 130.77, 130.32, 130.28, 128.56, 128.52, 128.45, 128.38, 126.55, 53.32, 52.92, 51.09, 47.69 (2C), 47.49, 47.35 (2C), 47.26, 46.16, 46.14, 45.98, 43.13, 39.89, 39.29, 38.24, 12.01(2C), 11.83 (2C); HRMS: calculated for $C_{17}H_{25}Cl_2N_3O$ ($[M + H]^+$), 358.1447; found, 358.1446.

(R)- and (S)-2-(3,4-Dichlorophenyl)-1-(2-((diethylamino)methyl)piperazin-1-yl)ethan-1-one (*(R)*-**11** *and (S)*-**11**): Resolution of the two enantiomers was conducted on a Chiralcel OD semi-preparative column (10 µm, 10 × 250 mm). Compound **11** (0.20 g, 0.56 mmol) was dissolved in EtOH (2.0 mL) and 50 µL of the solution was injected in each run. Mobile phase: 40/60 ^{i}PrOH/hexanes, flow rate: 4 mL/min, the first compound eluting out from the column was *(S)*-**11**, with retention time of 5.72 min, while the second

compound was *(R)*-**11**, with retention time of 11.55 min. Both enantiomers were isolated in > 95% enantiomeric excess (*ee*).

1.3 Synthesis and characterization of EKAP (**12**) and its fumarate salt (**13**)

Methyl (R)-4-(2-(3,4-dichlorophenyl)acetyl)-3-((diethylamino)methyl)piperazine-1-carboxylate (**12**): To a solution of compound *(S)*-**11** (0.13 g, 0.36 mmol) in anhydrous CH_2Cl_2 (2.5 mL) under argon and cooled to 0 ^oC was added methyl chloroformate (0.14 mL, 1.80 mmol), followed by TEA (0.10 mL, 0.72 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h, then quenched with DI water and extracted with CH_2Cl_2 (2 mL × 2). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column eluting with 2-5% MeOH/CH_2Cl_2 to afford compound **12** as colorless oil (0.12 g, 80%). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.36-7.26 (m, 2H), 7.07-6.97 (m, 1H), 4.64-4.54 (m, 1H), 4.46-4.36 (m, 1H), 4.13 (d, J = 13.38 Hz, 1H), 4.09 (d, J = 13.19 Hz, 1H), 3.74-3.64 (m, 3H), 3.65 (s, 3H), 3.53-3.43 (m, 1H), 2.86 (dd, J = 4.02 13.35 Hz, 1H), 2.78-2.68 (m, 2H), 2.51-2.41 (m, 4H), 0.91 (t, J = 6.94 Hz, 6H).

Methyl (R)- and (S)-4-(2-(3,4-dichlorophenyl)acetyl)-3-((diethylamino)methyl) piperazine-1-carboxylate fumarate (**13**): To a solution of compound **12** (0.12 g, 0.29 mmol) in Et_2O (0.6 mL) was added a solution of fumaric acid (0.035 g, 0.30 mmol) in MeOH (0.07 mL). The white precipitate was collected through filtration and recrystallized in Et_2O to afford compound **13** as a white solid (0.095 g, 61%). ^{1}H-NMR (DMSO-*d6*, 400 MHz): δ 0.89 (t, J = 6.82 Hz, 6H), 2.20-2.35 (m, 1H), 2.38-2.46 (m, 4H overlap with H_2O peak in

DMSO), 2.70-2.79 (m,1H), 2.81-2.97 (m, 2H), 3.58 (s, 3H), 3.67-3.95 (m, 4H), 3.95-4.05 (m, 1H), 4.19 (m, 1H), 4.44 (m, 1H), 6.59 (s, 2H), 7.19 (d, J = 8.33 Hz, 1H), 7.47 (s, 1H), 7.54 (d, J = 8.24 Hz, 1H), 13.02 (s, 2H). ^{13}C-NMR (CD_3OD, 75 MHz): δ 173.08 (2C), 170.57, 157.78, 136.93, 136.02 (2C), 133.22, 132.72, 131.92, 131.54, 130.59, 53.68 (2C), 52.64, 47.00, 44.60, 42.32 (2C), 40.06 (2C), 8.95 (2C). M.P. 137 ºC. $[\alpha]^{22}_D$ = -32.32º (c = 0.5, H_2O). Compound **13** was assigned the *R*-configuration based on the result of negative rotation, in reference to the lead compound GR103545 (*R*-enantiomer), which has an optical rotation of $[\alpha]^{22}_D$ = -25.60º (c = 0.5, H_2O). HRMS: calculated for $C_{19}H_{27}Cl_2N_3O_3 \cdot C_4H_4O_4$ ($[M + H]^+$), 416.1502; found, 416.1504.

1.4 Synthesis and characterization of *(S)*-enantiomer (**14**) and its fumarate salt (**15**)

Methyl (S)-4-(2-(3,4-dichlorophenyl)acetyl)-3-((diethylamino)methyl)piperazine-1-carboxylate (**14**): Compound **14** was prepared in procedures similar to those described above for compound **12** using *(R)*-**11** as the starting material. Yield: 80%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.93 (t, J = 6.91 Hz, 6H), 2.42-2.58 (m, 4H), 2.76 (m, 2H), 2.88 (dd, J = 3.72, 13.37 Hz, 1H), 3.45-3.58 (m, 1H), 3.65 (s, 3H), 3.62-3.75 (m, 3H), 4.11 (d, J = 13.66 Hz, 1H), 4.16 (d, J = 13.45 Hz, 1H), 4.43 (m, 1H), 4.61 (m, 1H), 7.04 (m, 1H), 7.32 (m, 2H).

Methyl (S)-4-(2-(3,4-dichlorophenyl)acetyl)-3-((diethylamino)methyl)piperazine-1-carboxylate fumarate (**15**): Compound **15** was prepared in procedures similar to those described above for compound **13**. Yield: 61%. ^{1}H NMR (MeOD, 400 MHz): δ 1.22 (t, J = 7.21 Hz, 6H), 3.05-3.23 (m, 6H), 3.72 (s, 3H), 3.75-4.09 (m, 8H), 4.89 (m, 1H overlap

with H_2O peak in MeOD), 6.70 (s, 2H), 7.19 (dd, *J* = 1.98, 8.32 Hz, 1H), 7.46 (d, *J* = 1.98 Hz, 1H), 7.48 (d, *J* = 8.29 Hz, 1H). $[\alpha]^{22}_D$ = 32.00° (*c* = 0.05, H_2O).

2. Radiosynthesis of ^{11}C-EKAP

2.1 General information

The semi-preparative HPLC system includes a Shimadzu™ LC-20A pump, a Knauer K200 UV detector, and a Bioscan γ-flow detector. The analytical HPLC system include a Shimadzu™ LC-20A pump, a Shimadzu™ SPD-M20A PDA or SPD-20A UV detector, and a Bioscan γ-flow detector. ^{11}C-Carbon dioxide was produced via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction with the PETtrace cyclotron (GE Medical Systems) using 16.5 MeV proton irradiation of nitrogen gas containing 0.5% oxygen. Waters Classic C18 Sep-Pak cartridges were purchased from Waters Associates.

^{11}C-EKAP was synthesized from the secondary amine precursor (*(S)*-**11**), which was pre-treated with CO_2 to provide the carbamate intermediate in the presence of cesium carbonate (Cs_2CO_3) and tetrabutylammonium triflate (TBAOTf) in DMF, followed by reaction with ^{11}C-MeOTf, in a procedure similar to the radiosynthesis of ^{11}C-GR103545 (*1*).

2.2 Pre-treatment of the precursor (*(S)*-**11**)

To the solution of compound *(S)*-**11** (1 mg) in anhydrous DMF (0.3 mL) were added TBAOTf (2.0 mg) and Cs_2CO_3 (2.0 mg). The mixture was sonicated for 1 min and bubbled

with CO_2 at 25 mL/min for 5 min. This CO_2 pre-treated solution of the precursor in DMF is ready to use.

2.3 Radiosynthesis of ^{11}C-EKAP

The cyclotron produced ^{11}C-CO_2 was reacted with hydrogen at 400 °C under a nickel catalyst to afford ^{11}C-CH_4, which was converted to ^{11}C-CH_3I by a gas phase reaction with iodine (*2*). ^{11}C-CH_3I was swept through the silver triflate column at 190 °C in an FxC module (*3, 4*) and the resulting ^{11}C-CH_3OTf was bubbled into the CO_2 pre-treated solution of the precursor in DMF until activity peaked. The reaction mixture was heated at 45 °C for 5 min, cooled to room temperature, diluted with 1.5 mL of 0.1 M ammonium formate solution and injected onto the semi-preparative HPLC column (Gemini C18, 10 µm, 10 × 250 mm). The column was eluted with a mobile phase of 37% MeCN and 63% 0.1 M ammonium formate solution at a flow rate of 5 mL/min. The radioactivity fraction eluting between 11-12 min was collected, diluted with 50 mL of DI water containing 400 mg of United States Pharmacopeia (USP) grade ascorbic acid, and loaded onto a Waters Classic C18 SepPak cartridge. The SepPak was rinsed with 10 mL of 1 mM HCl and dried, then eluted with 1 mL of USP absolute ethanol (Pharmco-AAPER) followed by a solution of 3 mg of USP ascorbic acid in 3 mL of USP saline (American Regent). The resulting solution was passed through a sterile 0.22 µm membrane filter (13 mm, Millipore MILLEX GV) into a sterile dose vial containing a mixture of 7 mL USP saline, 7 mg of USP ascorbic acid and 200 µL of 4.2% USP $NaHCO_3$ (Abraxis). The inactive *(S)*-enantiomer was synthesized in similar procedures.

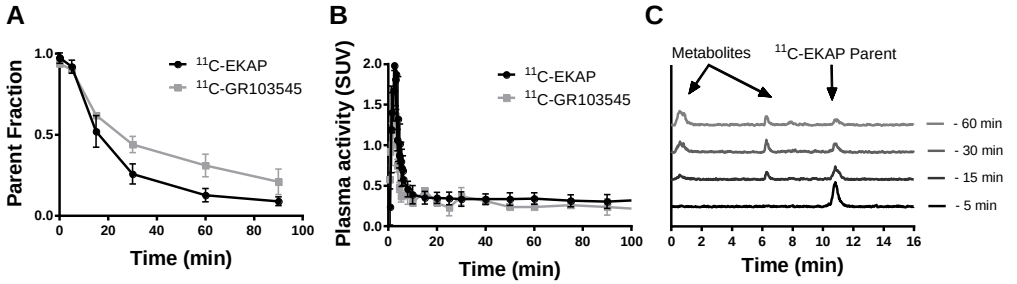
3. Plasma metabolite analysis and input function measurement

3.1 Method

Arterial blood samples were collected at preselected time points and assayed for radioactivity in whole blood and plasma with gamma counters (Wizard 1480/2480, Perkin Elmer, Waltham, MA, USA). Six samples, drawn at 0, 5, 15, 30, 60 and 90 min, were processed and analyzed to measure the radiotracer metabolite profile by HPLC using the column-switching method (5). Whole blood samples in EDTA tubes were centrifuged at 2,930 g at 4 °C for 5 min to separate the plasma. Supernatant plasma was collected and activity in 0.2 mL aliquots was counted on a gamma counter. Plasma samples were then mixed with urea (8 M) to denature plasma proteins, filtered through a 1.0 μm Whatman 13 mm CD/X syringe filter and loaded onto an automatic column-switching HPLC system connecting a capture column (4.6 × 19 mm) self-packed with Phenomenex Strata-X polymeric SPE sorbent and eluting with 1% MeCN in water at 2 mL/min for 4 min. The trapped activity in the capture column was then back flushed and eluted through a Phenomenex Luna C18 phenyl hexyl analytical column (4.6 × 250 mm, 5 μm) with 45% MeCN in 0.1 M ammonium formate (pH = 6.4) at a flow rate of 1.8 mL/min. The eluent fractions were collected with an automated fraction collector (Spectrum Chromatography CF-1). Activity in the whole blood, plasma, filtered plasma-urea mix, filter, and HPLC eluent fractions was all counted with the automatic γ counters. The sample recovery rate, extraction efficiency, and HPLC fraction recovery were monitored. The un-metabolized

parent fraction was determined as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected and fitted with inverted gamma function and corrected for filtration efficiency. The arterial input function (AIF) was then calculated as the product of the total counts in the plasma and the interpolated parent fraction at each time point.

3.2 Metabolite analysis results



SUPPLEMENTAL FIGURE 1. Parent fraction in plasma (A) and metabolite-corrected plasma activity over time (B) for ^{11}C-EKAP and ^{11}C-GR103545, and HPLC chromatograms from metabolite analysis of ^{11}C-EKAP (C).

4. Time activity curve (TAC) analysis and kinetic modeling

4.1 General information

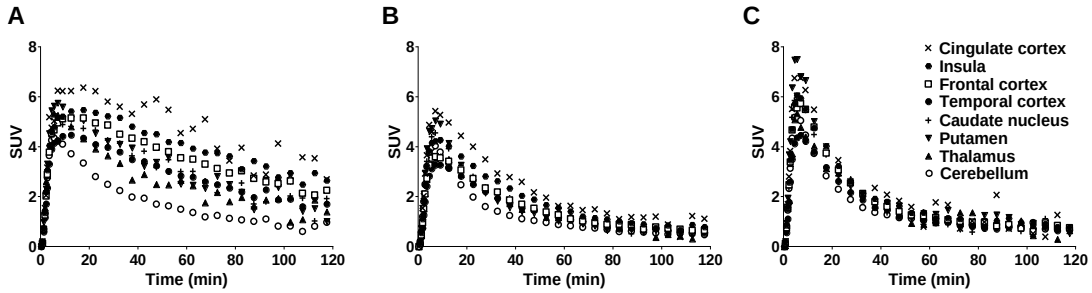
High-resolution magnetic resonance (MR) images were acquired with a Siemens 3T Trio scanner to assist with image co-registration and anatomical localization of regions

of interest (ROIs). The MR image was registered to an atlas and to the PET images, as previously described (*6*).

PET emission data were attenuation-corrected using the transmission scan, and dynamic images were reconstructed using a Fourier rebinning and filtered back projection algorithm. Using the MR images, and the following ROIs were defined: amygdala, brain stem, caudate nucleus, cerebellum, cingulate cortex, frontal cortex, globus pallidus, hippocampus, insula, nucleus accumbens, occipital cortex, pons, putamen, substantia nigra, temporal cortex and thalamus. For each PET scan, radiotracer concentrations over time, i.e. time-activity curves (TACs) were generated for the ROIs. Tissue to metabolite-corrected plasma activity ratio over time was calculated for the cingulate cortex, temporal cortex and cerebellum regions to project the radiotracer equilibration approaching time.

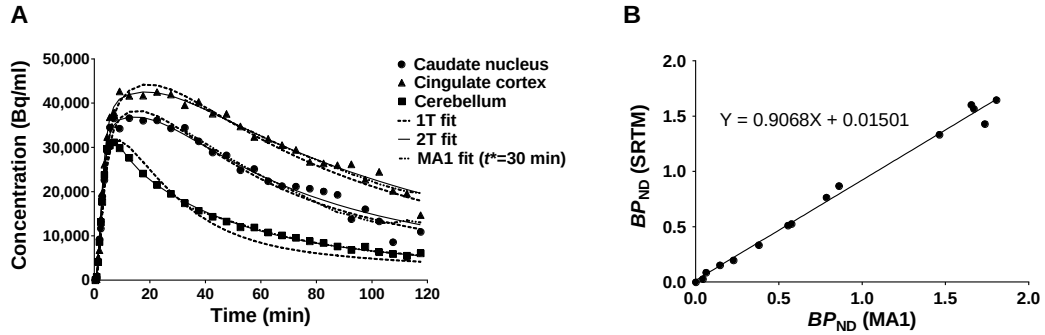
Regional TACs were fitted and analyzed with the one-tissue and two-tissue compartment (1TC, 2TC) models (*7*), as well as the multilinear analysis (MA1) method with a starting time (t^*) of 30 min (*8*). Regional distribution volume (V_T, mL/cm^3) was calculated from kinetic analysis of regional TACs using the metabolite-corrected arterial plasma concentration as the input function (*9*). Akaike information criterion (AIC) (*10*) and visual assessment of fitting curves were used to evaluate the goodness-of-fits.

4.2 Dose-dependent blocking scans



SUPPLEMENTAL FIGURE 2. Regional TACs of ^{11}C-EKAP in a baseline scan (A) and blocking scans with the KOR selective antagonist LY2456302 at a dose of 0.05 mg/kg (B) and 0.3 mg/kg (C), showing dose-dependent blockade of ^{11}C-EKAP binding.

4.3 Goodness-of-fits of kinetic models



SUPPLEMENTAL FIGURE 3. Comparison of curve fitting with the 1TC, 2TC models and MA1 (t* = 30 min) method in selected brain regions (A); and correlation of regional BP_{ND} values derived with the SRTM and MA1 models (B).

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