Supporting Information (SI)

Radiosynthesis of ^{11}C-Levetiracetam: A Potential Marker for PET Imaging of SV2A Expression

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1. General Method and Material

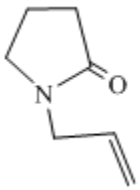
1.1 General Materials.

Levetiracetam standard compound and its racemic mixtures were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA) and from USP (Rockville, MA USA), respectively. Levetiracetam carboxylic acid (Levetiracetam carboxylic acid) was provided by Shanghai Biosundrug Co., Ltd (Shanghai, China). The other reagents and solvents were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA) or Alfa-Aesar (Ward Hill, MA USA), and used without further purification unless otherwise stated. Tetrahydrofuran (THF) was freshly distilled over lithium aluminum hydride prior to use. Solid phase extraction cartridges were obtained from Waters Associates (Milford, MA, USA).

1.2 General Methods

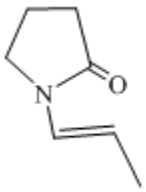
^{1}H NMR spectra were obtained using a Varian Mercury 500-MHz instrument (Wayne State University Lumigen Instrumentation Center), and the chemical shifts were reported in ppm on the δ scale relative to an internal TMS standard. Mass spectrometry (Wayne State University Lumigen Instrumentation Center) were recorded in the + ion mode using electrospray ionization. High pressure liquid chromatography (HPLC) analyses were performed using a Waters HPLC system equipped with a Waters 486 UV detector and a sodium iodide scintillation detector, operated by Waters Empower 2 software. The purification of ^{11}C-LEV was performed on an Astec Chirobiotic T Chiral Column (5 μm, 250 × 10 mm) for semi-preparative HPLC, with the mobile phase 10% ethanol in water at a flow rate of 3.0 mL/min. The effluent from the HPLC column was monitored using a radiodetector. The identification of the final product ^{11}C-LEV was confirmed by analytic HPLC using Astec Chirobiotic T Chiral Column (5 μm, 250 × 4.6 mm) with the same mobile phase at a flow rate of 1.0 mL/min through a UV detector (λ = 210 nm) followed by a gamma radioactivity detector, or Phenomenex Luna C18 reversed phase analytic column (5 μm, 250 × 4.6 mm) with the 6% acetonitrile in 10 mM sodium acetate buffer at a flow rate of 1.0 mL/min through a UV detector (λ = 210 nm) followed by a gamma radioactivity detector. A dose calibrator (Capintec) was used for all radioactivity measurements.

2. Syntheses of precursors and reference compounds



2.1 *N*-allyl-2-pyrrolidone (LEV-ene)

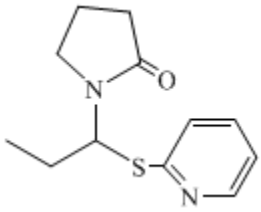
N-allyl-2-pyrrolidone (LEV-ene) was prepared by reaction allyl bromide with 2-pyrrolidone in KHMDS/THF solution at room temperature (RT) according to reported methods with minor modifications.[1] In detail, 2- pyrrolidinone (5.0 mmol) was dissolved in 10 mL anhydrous THF at RT, followed by cooling to -65-78 °C with a dry ice/acetone bath. KHMDS (5.5 mmol, 0.5 M in toluene) was diluted with 11 mL anhydrous THF, and then dropwise added to above 2-pyrrolidinone solution. The resulting reaction mixture was stirred for 1 h and allylbromide (6.0 mmol) in 5.0 mL of anhydrous THF was added dropwise. The addition afforded milk-like solution was stirred for 1 h, and then warmed to RT under nitrogen and kept stirring overnight. The mixture was quenched by a saturated ammonium chloride solution (40 mL) and then extracted with ethyl acetate (30 mL×3). The organic phases were combined, washed successively with 1.0 M HCl (50 mL), and brine (2 x 50 mL) and then dried over sodium sulfate. Flash column chromatography (3-5% methanol/dichloromethane) gave the product as clear yellow oil (0.47 g, 76%). HPLC (C18 semi-preparative column with 20% ethanol in water, 3.0 mL/min, t_R = 12.5 min) analyzed that the purity was more than 98%. The analytical data (NMR, MS) matched those reported in the literature for *N*-allyl-2-pyrrolidone.[2]



2.2 1-Propenyl-pyrrolidin-2-one (Iso-LEV-ene)

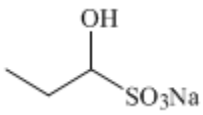
Diisopropylamine (2.2 mmol) was dissolved in dry tetrahydrofuran (5.0 mL) under an argon atmosphere, followed by cooling to -65-78 °C with a dry ice/acetone bath for 10 min. A hexane solution of n-butyllithium (2.2 mmol) was added dropwise to above solution of diisopropylamine. The resulting LDA solution was stirred for 30 min at -65-78 °C. The solution of LEV-ene (1.0 mmol) in tetrahydrofuran (5.0 mL) was added dropwise to the LDA mixture. The resulting

mixture was allowed to stir for 90 min in at -65-78 °C, then rose to 0 °C and continued for additional 3 h reaction. HPLC (the same condition with analysis of LEV-ene) monitored the completion of the reaction. After completion of the reaction, the reaction mixture was treated with methanol (0.2 mL), and then 10 mL ethyl acetate, and quenched by 40 mL saturated ammonium chloride solution. The mixture was then extracted, and water phase was extracted with diethyl ether (20 mL x3) and combined the organic phase, and followed by washing with water (50 mL), brine (50 mL), and dried over sodium sulfate. Flash column chromatography (3-5% methanol/dichloromethane) gave the product as clear yellow oil (0.56 g, 45%). HPLC (t_R= 14.5 min) analyzed that the purity was more than 93%. The analytical data (NMR, MS) matched those reported in the literature for (E)-1-(prop-1-enyl)pyrrolidin-2-one.[2]



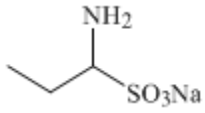
2.3 1-(1-(pyridin-2-ylthio)propyl)pyrrolidin-2-one (LEV-S-Py)

Levetiracetam carboxylic acid (1.3 mmol) was dissolved and stirring in dichloromethane (10 mL) under a nitrogen atmosphere, followed by cooling to 0 °C with an ice/water bath for 10 min. Triethylamine (2.6 mmol) was added to above levetiracetam carboxylic acid solution. After 15 min, isobutyl chloroformate (1.3 mmol) was added dropwise to above solution and stirring for 1 h. N-hydroxypyride-2-thione (1.3 mmol) was dissolved in 4.0 mL dichloromethane, then added dropwise to above colorless solution at water-ice bath, then reacted under the light for 50 min. HPLC (analytical C18 column with 42% acetonitrile/ water, 1.0 mL/min) monitored the completion of the reaction. The reaction solution was concentrated, and purified with silica gel flash column chromatography (3-5% methanol/dichloromethane), yielding the product (0.24 g, 75%). HPLC (t_R = 3.0 min) analyzed that the purity was more than 98%. ^{1}H NMR ($CDCl_3$): δ = 8.40 (s, 1H), 7.50-7.55 (t, 1 H), 7.35-7.40 (t, 1 H), 7.05 (s, 1H), 5.95 (t, 1 H), 3.60 (t, 1 H), 3.30 (t, 1 H), 2.42-2.36 (t, 1 H), 2.24-2.34 (t, 1 H), 1.90-2.02 (t, 2 H), 1.80-1.90 (t, 2 H), 1.0 (t, 3 H) ppm. MS calculated for $C_{12}H_{16}N_2OS$, $[M+H]^+$=237.10; found 237.10.



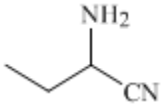
2.4 Sodium 1-hydroxypropane-1-sulfonate, 3

A solution of sodium bisulfite (10 mmol) in water (3.0 mL) was slowly added to the solution of propionaldehyde (10 mmol) in of methanol (20 mL). The mixture was stirred for 60 min at RT, and then the solvent was removed. Additional 5 mL of methanol was added and evaporated to eliminate water. The product (0.82 g, 51%) as white solid was obtained after washed with ether (8.0 mL) and dried. ^{1}H NMR (D_2O): δ = 4.12-4.16 (t, 1H), 1.72-1.84 (t, 1 H), 1.42-1.52 (t, 1 H), 0.84-0.92 (t, 3H) ppm. MS calculated for $C_3H_7NaO_4S$, $[M+Na]^+$=184.99; found 184.90.



2.5 Sodium 1-aminopropane-1-sulfonate, 4

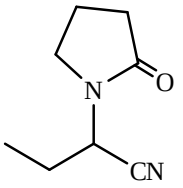
Compound **4** was prepared according to the reported method.[3] Briefly, an aqueous solution of ammonia (30%, 54 µmol) was added to a solution of sodium 1-hydroxypropane-1-sulfonate, **3** (54 µmol) in CD_3OD:D_2O 1:1 (0.8 mL), and the mixture was left at room temperature. After 1 h reaction, the compound **4** was obtained and used without further purification according to the report.[3]



2.6 2-Aminobutanenitrile, 5'

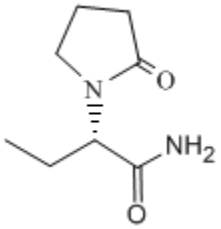
A 50 mL round-bottom flask, which equipped with a stirrer bar, contained magnesium sulfate (11.6 mmol), ammonium chloride (11.6 mmol), and sodium cyanide (22.0 mmol). The mixture was added 93 mmol of 7 M ammonia in methanol, stirred and cooled to 0 °C. After 10 min, the resulting suspension was added propionaldehyde (23.2 mmol). The mixture was stirring for 1 h at 0 °C, then increased to RT and continued to react for 4 h. The solvent was then removed under reduced pressure while maintaining the internal temperature <30 °C until nearly all of the methanol and ammonia were removed. The resulting slurry of inorganic salts and the product was diluted with 15 mL of methyl tertiary-butyl ether **(**MTBE), stirred at RT for 30 min, and filtered. The inorganic wet cake was washed with 5.0 mL of MTBE, and the solvent was removed under reduced pressure, yielding product 2-aminobutanenitrile (1.14g, 62.5%) as colorless liquid. ^{1}H NMR ($CDCl_3$): δ = 3.48-3.51 (d, 1H), 1.56-1.62 (t, 2 H), 0.84-0.92 (t, 3H)

ppm. MS calculated for $C_4H_8N_2$, $[M+H]^+$=85.07; found 85.02. In addition, compound **5'** also could been prepared according to the reported method.[4]



2.7 2-(2-Oxo-1-pyrrolidinyl)butanenitrile, 2'

The solution of 2-aminobutanenitrile (2.5 mmol) in 10.0 mL dichloromethane was added sodium sulfate (3.0 mmol) at RT. The mixture was cooled to 0 ^oC, followed by the addition of potassium hydroxide (3eqs) and tetrabutylammonium bromide (40.5 mg, 0.125 mmol). A solution of 4-chlorobutyryl chloride (3.0 mmol) in dichloromethane (2.0 ml) was added dropwise at 0 ^oC with vigorous stirring, then increased to RT and stirred overnight. The resulting slurry of inorganic salts and the product was filtered. The inorganic wet cake was washed with 5.0 mL of dichloromethane. The solvent was removed under reduced pressure, and then purified with silica gel flash column chromatography (45% ethyl acetate/hexane or 2.5% methanol/dichloromethane), yielding product (277 mg, 72.8%). HPLC (t_R = 8.5 min; condition: analytical C18 column, 15% acetonitrile /10 mm monopotassium phosphate; 1.0 mL/min) analyzed that the purity was more than 98%. The analytical data (NMR, MS) matched those reported in the literature for 2-(2-Oxo-1-pyrrolidinyl)butanenitrile.[5]



2.8 (S)-*α*-ethyl-2-oxo-1-pyrrolidine acetamide (LEV)

2-(2-Oxo-1-pyrrolidinyl)butanenitrile **2'** was hydrolyzed using different method, yielding the LEV (~50%). **Method 1** Acid hydrolysis: Compound **2'** (66 μmol) in dichloromethane solution was added to 95% sulfuric acid (5 eqs, 330 μmol), then heated at 100 ^oC for 10 min, then was added ~30% aqueous ammonia (330 μmol) to adjust pH near 7. The mixture was purged to remove the solvent, analyzed by chiral HPLC (t_R = 9.0 min, matched with standard LEV) showed all compound **2'** was consumed and formed LEV (~50%). **Method 2** Base hydrolysis: Compound **2'** (66 μmol) was dissolved in 0.1 M sodium hydroxide solution (0.4 mL), and

followed by the addition of hydrogen peroxide (200 µL), then heated at 60 ^{o}C for 10 min. Acetic acid (330 µmol) was added to adjust pH near 7.0. The mixture was purged to remove the solvent, analyzed by chiral HPLC (t_R = 9.0 min, matched with standard LEV) showed all compound 2' was consumed and formed LEV(~50%). The analytical data (NMR, MS) matched those reported in the literature for (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide.[5]

3. Radiolabelling procedures

3.1 Production of ^{11}C-HCN.

^{11}C labeled carbon dioxide (^{11}C-CO_2) was produced by the ^{14}N(p,*a*)^{11}C reaction with 11 MeV protons from a CTI RDS-112 cyclotron using a commercial N_2 target containing 1% O_2. ^{11}C-CO_2 was purged from the target in a stream of helium and trapped and cleared of residual target gas oxygen using a Carbosphere™ trap. The ^{11}C-CO_2 was released from the trap upon heating (300 ^{o}C) in a stream of helium (100 mL/min) and passed (together with hydrogen stream, 100 mL/min) through a preheated 450 ^{o}C quartz tube filled with nickel catalyst. The gas mixture containing the ^{11}C-CH_4 was introduced, together with ammonia stream (7-8 mL/min) into a quartz tube containing platinum (preheated to 950 ^{o}C) where ^{11}C-HCN formed by coupling between ^{11}C-CH_4 and ammonia. The gas mixture of ^{11}C-HCN and ammonia from this column is directly delivered into the reactor.

3.2 Radiosynthesis of ^{11}C-amnionitrile 5

Pre-precursor sodium 1-hydroxypropane-1-sulfonate (3.5-4.0 mg) was dissolved in water (300 µL), followed by addition of 10 µL aqueous ammonia in reaction vial. Sodium 1-aminopropane-1 sulfonate was generated in situ as precursor after sealed the vial and heated at 60 ^{o}C for 30 min. The precursor solution was then cooled to 0°C without further purification for labeling. ^{11}C-HCN from its module was bubbled in reaction vial, and the resulting solution was heated at 60 ^{o}C for 3.0 min. Cooled to RT, the reaction mixture passed through C18 plus Sep-Pak cartridge, followed by the dry of the cartridge with N_2, then eluted the cartridge using 1.5 mL of acetonitrile, and dried by sodium sulfate, yielding the product ^{11}C-amnionitrile **5** (85.0 ± 5.0%) for next step reaction.

3.2 Radiosynthesis of compound 2

^{11}C-amnionitrile in acetonitrile (1.5 mL) was added potassium carbonate (4.0-5.0 mg), and then followed by additions of tetrabutylammonium bromide (5.0 µmol) in dichloromethane (50µl) and 4-chlorobutyryl chloride (50 µmol) in dichloromethane (100µl). The mixture was kept at RT

for 5 min with stirring. HPLC analysis showed the form of compound **2** (35.0 ± 5.0%) by comparison with cold standard compound. Without purification, the crude product was used for next step reaction.

After base hydrolysis of compound **2**, ^{11}C-LEV was obtained after HPLC purification with about 1.0 % radiochemical yield.

3.3 Radiosynthesis of ^{11}C-aminobutanamide 6

^{11}C-amnionitrile in acetonitrile (1.5mL) was added hydrogen peroxide (200 µL) and 1.0 M sodium hydroxide solution (100 µL). The mixture was heated at 60°C for 3 min, cooled to RT. HPLC analysis showed the form of compound **6** (78 ± 2.5%) by comparison with cold standard compound.

After purification by HPLC and solution exchange by C18 plus Sep-Pak cartridge, then dried by sodium sulfate, the compound **6** was used to produce ^{11}C-LEV using the same method as produce standard compound of LEV. The radiochemical yield of ^{11}C-LEV was also about 1.0% after HPLC purification.

3.4 Multistep one-pot radiosynthesis, purification and formulation of ^{11}C-LEV

^{11}C-LEV was prepared using a multistep in one-pot method in a fully automated multipurpose PET module developed in house.[6] Briefly, the precursor propionaldehyde (6.0 µl) was dissolved in tetrahydrofuran (250µL), followed by the addition of ammonia in methanol (20 µL) in reaction vial. The mixture was heated at 60°C for 30-60 min, and then cooled to -5~-10°C. ^{11}C-HCN from its module was bubbled in reaction vial, and the resulting solution was kept at 0~10°C for 5 min. This mixture was purged with nitrogen to removed unreacted ammonia, and then added the solution of triethylamine (14 µL) in dichloromethane (100 µl). After 30 seconds, the resulting solution was added the solution of 4-chlorobutyryl chloride (10 µL) in dichloromethane (100 µl), and kept at 0~10°C for 5 min. The solution of 2.0 M sodium tert-butoxide in tetrahydrofuran (100 µL) was added to above reactants, mixed and kept at 0~5°C for 5 min, and then removed the most of solvent by heat at 60°C and the purge of nitrogen flow. The above reactants was added the mixture of hydrogen peroxide (100 µL) in dimethyl sulfoxide (100 µL), heated at 60°C for 3 min, and then cooled to RT and purged, followed by the addition of 1.0 mL 1.0% acetate acid to neutralize the reaction, finally injected to semi-preparative HPLC column for purification, which provided enantiomeric separation. The radioactive fraction containing ^{11}C-LEV was collected. Radiochemical identity is confirmed by the analytical HPLC of sample

^{11}C-LEV. The typical of semi-preparative HPLC and analytical HPLC were shown in **Figure 1**. Usually, starting with ~1000 mCi (37 GBq) of ^{11}C-CO_2, 8.0-12 mCi (296-444 MBq) at end of synthesis (EOS) of ^{11}C-LEV was routinely obtained and ready for further biological evaluation in 50 ± 5.0 min from end of bombardment (including 6 min for collection of the ^{11}C-CO_2 and conversion to ^{11}C-HCN and HPLC purification time) with more than 98% radiochemical and enantiomeric purities. The specific activity was more than 17.0 GBq/μmol at EOS based on HPLC analysis.

3.5 Preliminary evaluation of ^{11}C-LEV for PET imaging of SV2A expression.

We first evaluated the tracer ^{11}C-LEV *in vitro* stability of ^{11}C-LEV, After 90 min of incubation with the formulation solution, > 98% of ^{11}C-LEV remained stable.

PET imaging of SV2A with ^{11}C-LEV in normal mouse will provide valuable information regarding the level and extent of tracer accumulation in the brain to quantify the SV2A expression. The 60 min dynamic microPET/CT imaging was performed for assessment of SV2A expression in mice. PET/CT scans will be performed using a rodent scanner (microPET R4; Siemens Medical Solutions) on normal mice (n = 3). Each mouse will be injected with 500 μCi of tracer through the tail vein under isoflurane anesthesia. A dynamic scan will be performed immediately after the injection for 1 h. After PET imaging, the mouse will be scanned by CT to obtain anatomical image. The images will be reconstructed by an OSEM 2D algorithm, and analyzed using the AMIDE. For each PET/CT scan, regions of interest will be drawn over the brain, normal tissue, and major organs on decay-corrected images. The radioactivity accumulation within the brain and other organs will be obtained from the maximum value within the multiple regions of interest and then converted to percentage injected dose per gram of tissue (%ID/g). A SV2A blockade experiment will be performed by co-injecting the tracer with a saturating dose of LEV to evaluate the imaging specificity of SV2A. The dynamic micro-PET/CT imaging in mice (n = 3), as shown in Figure.2, showed high initial uptake of the tracer in most organs, followed by gradual washout, but brain uptake was accumulated at 60 min. The imaging specificity of SV2A expression was subsequently validated through a blocking experiment with cold LEV.

3.6 Discussion

Although we succeed to produce ^{11}C-LEV that can meet the preclinical study requirements, we realize that the radiochemical yield, specific activity, radiochemical purity and chemical purity

still need to be improved to meet the potential future clinical application. We will optimize the reaction conditions and develop an automated radiosynthesis in order to shorten the synthesis time and increase the radiochemical yield. Regarding to specific activity, we recognize that high specific activity is important for a successful brain imaging agent and that our specific activity is low. The possible reason for the low specific activity might be related to our purification method, which uses a chiral semi-preparative HPLC column that is eluted with 10.0% ethanol in water solution. This condition can clearly separate ^{11}C-LEV from its isomer R-^{11}C-LEV, shown in **Figure 1A**. We analyzed the ^{11}C-LEV sample using an analytical C18 column (6.0% acetonitrile in 10.0 mM sodium acetate buffer) and confirmed that the purified ^{11}C-LEV did not contain the observable cold LEV (as shown in **Figure 1B)**. However, analysis of the sample using an analytical chiral HPLC column showed that it contained trace amounts of dimethyl sulfoxide (DMSO). Unfortunately, a C18 column cannot separate the isomer from ^{11}C-LEV. To be cautious with respect to our reported data, we have calculated the specific activity including the chemical impurity, but we state that the specific activity is more than 17 GBq/μmol at the end of synthesis (EOS). We are also working on the optimization of the purification and analysis condition to increase specific activity, radiochemical purity and chemical purity.

Preliminary evaluations of ^{11}C-LEV demonstrated its potential for specific PET imaging of SV2A expression.

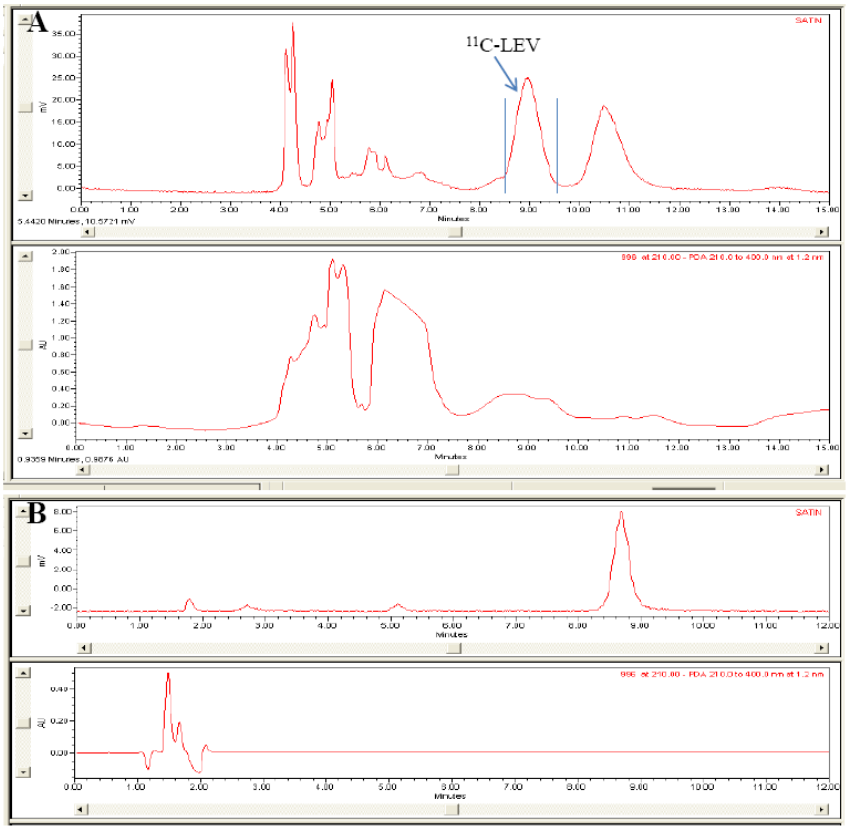


Figure 1. HPLC chromotogram of ^{11}C-LEV. (A) Chiral HPLC separation of crude ^{11}C-LEV (R_t = 9.0 min). (B) Analytical HPLC of pure ^{11}C-LEV (R_t = 8.6 min) at UV absorption (210 nm)

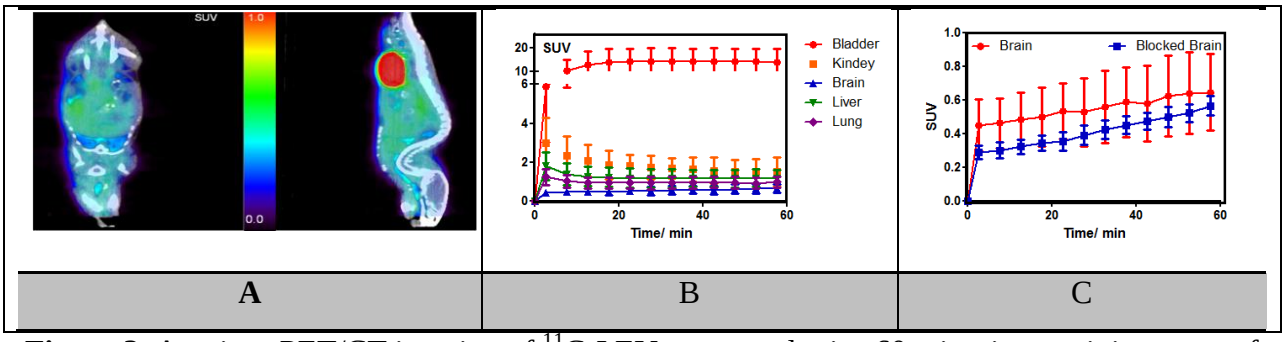


Figure 2. A. micro-PET/CT imaging of ^{11}C-LEV on normal mice 60 min; time-activity curve of ^{11}C-LEV in mice; block study of ^{11}C-LEV in mice brain.

4. Reference

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