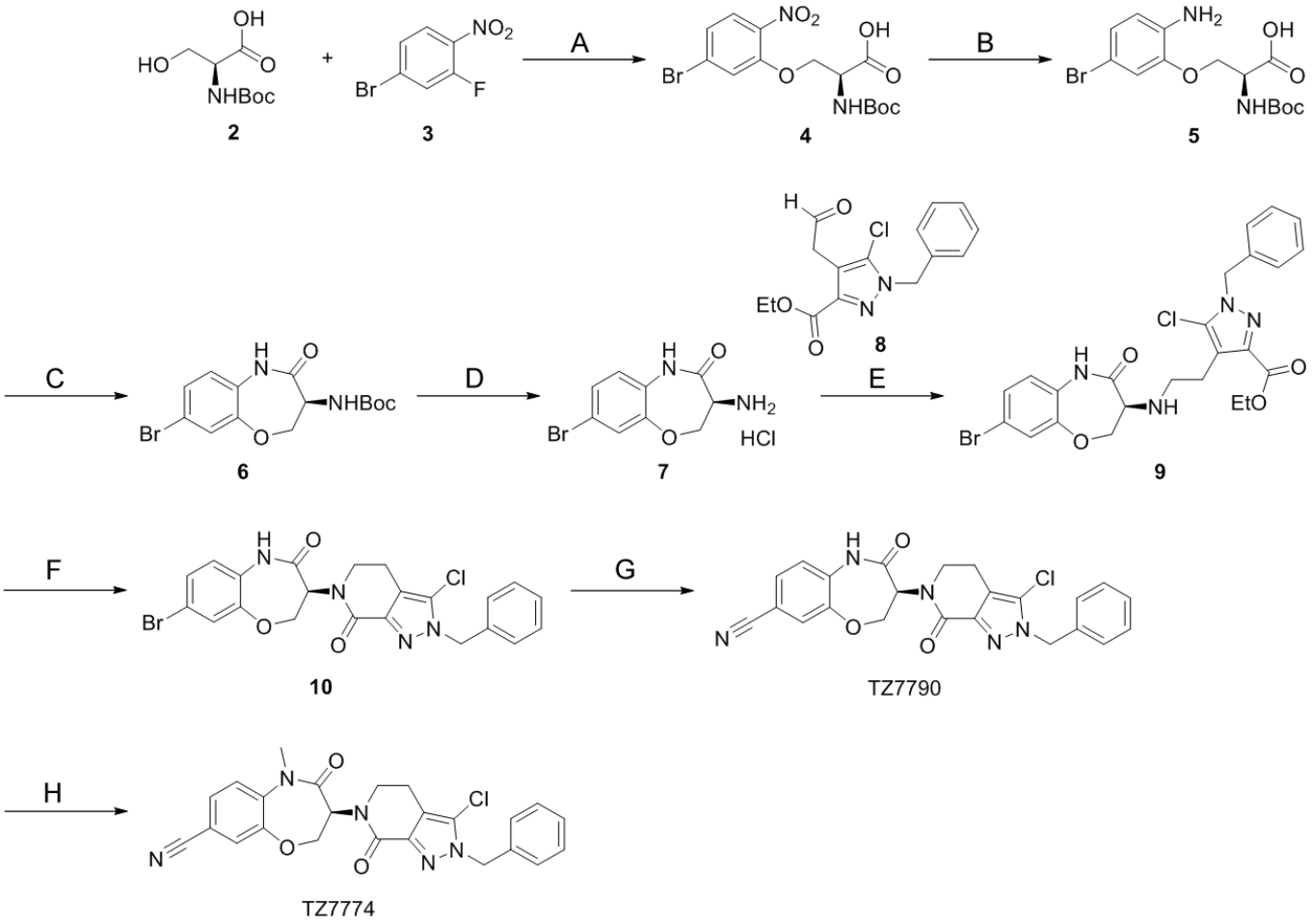
Supplementary Information

Experimental section

Chemicals

All commercially obtained solvents and reagents were used as received without further purification. All reactions were conducted in a nitrogen atmosphere. Normal-phase thin-layer chromatography (TLC) was performed using Silica gel 60 F254 (1.05715.0009, Merck, USA), reagent grade solvent, and UV light (254 nm) or phosphomolybdic acid as visualization reagent. Column chromatography was carried out using silica gel (AP-300S Taiko-shoji) with solvent mixtures as specified. ^{1}H and spectra were acquired for samples dissolved in $CDCl_3$ or DMSO-d_6 using the JEOL JNM-ECA500 spectrometer operating at a frequency of 500 MHz for ^{1}H, with tetramethylsilane (TMS) used as an internal reference. ^{1}H NMR spectra chemical shifts are given as δ values (ppm), with the singlet of tetramethylsilane set at 0 ppm as the reference. Splitting patterns are indicated as follows: s for singlet, d for doublet, t for triplet, dd for doublet of doublets, ddd for doublet of doublet of doublets, td for triplet of doublets, m for multiplet. Electrospray ionization (ESI)-mass spectrometry measurements were conducted using the shimadzu LC-MS2020 spectrometer. The solvents were removed by evaporation under reduced pressure using a rotary evaporator.

Synthesis of intermediate compounds



Scheme S1. Synthesis of standard compound TZ7774 and precursor Dm-NCGG501. Agents and reaction conditions: (A) NaH (60%), DMF, 0 °C to rt; (B) Fe, H_2O, EtOH, 80 °C; (C) HATU, TEA, DMSO, rt; (D) 4 M HCl/EtOAc, 0 °C to rt; (E) 5, 2-picoline boran, 8, HOAc, MeOH, rt; MeOH, rt; (F) $AlMe_3$, toluene, 0 °C to 100 °C; (G) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C, under Ar; (H) Cs_2CO_3, MeI, DMF, 0 °C to rt.

(S)-3-(5-Bromo-2-nitrophenoxy)-2-((tert-butoxycarbonyl)amino)propanoic Acid (4) [1]

Following the method by Huang et al. [1], 4 was obtained, yielding 26 mg (6.4%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.38 (9H, s), 4.33-4.49 (3H, m), 7.12 (^{1}H, *J* = 8.0 Hz, d), 7.35 (^{1}H, dd, *J* = 8.5, *J* = 2.0 Hz), 7.65 (^{1}H, *J* = 2.0 Hz, d), 7.84 (^{1}H, *J* = 9.5 Hz, d); LRMS (ESI-): *m/z* [M+H]$^-$: 403.

(S)-3-(2-Amino-5-bromophenoxy)-2-((tert-butoxycarbonyl)amino)propanoic Acid (5) [1]

Following the method by Yoshikawa et al. [1], 5 was obtained, yielding 19 mg (88%). LRMS (ESI-): *m/z* [M+H]$^-$: 373.

(S)-tert-Butyl (8-Bromo-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-yl)carbamate (6) [1]

Following the method by Y Huang et al. [1], 6 was obtained, yielding 10 mg (67%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.36 (9H, s), 4.24-4.40 (3H, m), 7.00-7.06 (^{1}H, m), 7.14 (^{1}H, *J* = 8.5 Hz, d), 7.29-7.36 (2H, m), 10.02 (^{1}H, s); LRMS (ESI-): *m/z* [M+H]$^-$: 355.

(S)-3-Amino-8-bromo-2,3-dihydrobenzo[b][1,4]oxazepin-4(5H)-one Hydrochloride (7) [1]

Following the method by Huang et al. [1], 7 was obtained, yielding 35 mg (quant). LRMS (ESI+): *m/z* [M+H]$^+$: 257.

(S)-Ethyl 1-Benzyl-5-chloro-4-(2-((7-cyano-5-methyl-4-oxo2,3,4,5-tetrahydrobenzo[b][1,4]oxazepin-3-yl)amino)ethyl)-^{1}H-pyrazole3-carboxylate (9) [1]

Following the method by Huang et al. [1], 9 was obtained, yielding 42 mg (64%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.38 (3H, *J* = 8.5 Hz, t), 2.59-2.67 (^{1}H, m), 2.75-2.96 (3H, m), 3.59 (^{1}H, dd, *J* = 10.5, 6.0 Hz), 4.07-4.15 (^{1}H, m), 4.31-4.44 (3H, m), 5.40 (2H, s), 6.81 (^{1}H, *J* = 8.5 Hz, d), 7.15-7.34 (6H, m), 7.40 (^{1}H, s); LRMS (ESI-): *m/z* [M-H]$^-$: 545.

(S)-3-(2-Benzyl-3-chloro-7-oxo-4,5-dihydro-2H-pyrazolo[3,4-c]pyridin-6(7H)-yl)-8-bromo-5-methyl-2,3-dihydrobenzo[b][1,4]oxazepin-4(5H)-one (10) [1]

Following the method by Huang et al. [1], 10 was obtained, yielding 50 mg (68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.68-2.77 (^{1}H, m), 2.89-2.99 (^{1}H, m), 3.64-3.74 (^{1}H, m), 4.45 (^{1}H, dd, *J* = 11.0, *J* = 5.2 Hz), 4.61 (^{1}H, dd, *J* = 11.4, *J* = 2.4 Hz), 5.36-5.50 (3H, m), 6.79 (^{1}H, d, *J* = 8.8), 7.18 (^{1}H, dd, *J* = 9.0, *J* = 2.2 Hz), 7.27-3.36 (5H, m), 7.60 (^{1}H, s); LRMS (ESI+): *m/z* [M-H]$^+$: 501.

(3S)-3-(2-Benzyl-3-chloro-7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-c]pyridin-6-yl)-4--oxo-2,3,4,5-tetrahydro-1,5-benzoxazepine-8-carbonitrile (TZ7790) [1]

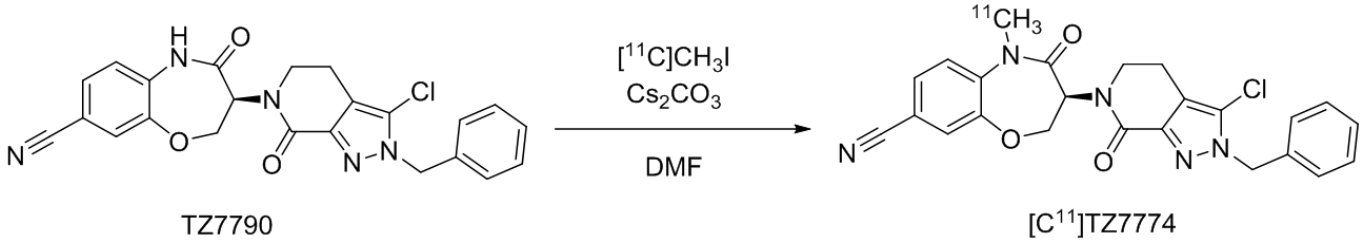
Following the method by Huang et al. [1], TZ7790 was obtained, yielding 11 mg (27%). ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 2.76 (^{1}H, dt, *J* = 10.5, 6.6 Hz), 2.93 (^{1}H, q, *J* = 7.9 Hz), 3.78 (2H, dd, *J* = 7.5, 5.5 Hz), 4.49 (^{1}H, dd, *J* = 12.0, 4.5 Hz), 4.66(^{1}H, dd, *J* = 12.0, 4.0 Hz), 5.19 (^{1}H, br s), 5.40 (2H, s), 6.96 (^{1}H, d, *J* = 8.0 Hz), 7.28-7.36 (6H, m), 7.38 (^{1}H, d, *J* = 2.0 Hz), 7.73 (^{1}H, br s); LRMS (ESI+): *m/z* [M-H]$^+$: 448.

(3S)-3-(2-Benzyl-3-chloro-7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-c]pyridin-6-yl)-5--methyl-4-oxo-2,3,4,5-tetrahydro-1,5-benzoxazepine8-carbonitrile (TZ7774) [2]

Following the method by Yoshikawa et al. [2], TZ7774 was obtained, yielding 8.6 mg (87%). to give TZ7774 (8.6 mg, 87%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 2.69 (^{1}H, dt, *J* = 16.0, 4.8 Hz), 3.05 (^{1}H, ddd, *J* = 15.5, 10.4, 5.2 Hz), 3.40 (3H, s), 3.52-3.60 (^{1}H, m), 4.174.25 (^{1}H, m), 4.45 (^{1}H, dd, *J* = 10.3, 8.3), 4.69 (^{1}H, dd, *J* = 12.0, 10.0 Hz), 5.40 (^{1}H, s) 5.90 (^{1}H, dd, *J* = 11.5, 8.0 Hz), 7.24-7.37 (6H, m), 7.48 (^{1}H, d, *J* = 2.0 Hz), 7.57 (^{1}H, d, *J* = 8.3, 1.8 Hz); LRMS (ESI+): *m/z* [M-H]$^+$: 461.

Radiochemistry

Radio-labeling was conducted according to the previously reported method [3].



Scheme S2. Radiosynthesis of [^{11}C]TZ7774. TZ7790 was methylated with [^{11}C]CH_3I with Cs_2CO_3 in DMF DMF at rt for 10 min.

(3S)-3-(2-Benzyl-3-chloro-7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-c]pyridin-6-yl)-5-[^{11}C]methyl-4-oxo-2,3,4,5-tetrahydro-1,5-benzoxazepine8-carbonitrile ([^{11}C]TZ7774)

(3S)-3-(2-Benzyl-3-chloro-7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo[3,4-c]pyridin-6-yl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzoxazepine-8-carbonitrile (TZ7790) (0.50-2.6 mg, 1.05-5.44 μmol) was dissolved in DMF (300 μL), added potassium carbonate (20 mg, 145 μmol). [^{11}C] methyl iodide was prepared following general methods, transferred to the reaction vial and heated to rt for 10 min. The reaction mixture was purified by HPLC (column: CAPCELL PAK C18, UG120, 10 mmI. D. × 250 mm, UV = 260 nm) eluting with a 55% CH_3CN in 0.1% formic acid at a flow rate of 5 mL/min. The radioactive peak corresponding to TZ7774 was corrected in a flask, evaporated to dryness in a rotary evaporator and dissolved in 3 mL of saline with 7.5 μL of Tween-80 filtered through a 0.22 μm Milex filter. The radiochemical purity, the chemical purity and the molar activity of [^{11}C]TZ7774 were analyzed by HPLC (column: CAPCELL PAK C18, UG120, 4.6 × 150 mm, UV = 260 nm) eluting with a 55% CH_3CN in 0.1% formic acid at a flow rate of 1.0 mL/min. [^{11}C]TZ7774 was reliably obtained 1336.6 ± 492.8 MBq in an 3.4 ± 1.2% decay-corrected radiochemical yield, a molar activity of 137.8 ± 54.3 GBq/μmol, with a chemical purity 82.3 ± 19.4 % and with a radiochemical purity > 99.9 % (n = 9).

References

[1] Huang T, Gu J, Jiang H, Liang Q, Perlmutter JS and Tu Z. Radiosynthesis and characterization of a carbon-11 PET tracer for receptor-interacting protein kinase 1. Nucl Med Biol 2022; 110-111: 18-27.

[2] Yoshikawa M, Saitoh M, Katoh T, Seki T, Bigi SV, Shimizu Y, Ishii T, Okai T, Kuno M, Hattori H, Watanabe E, Saikatendu KS, Zou H, Nakakariya M, Tatamiya T, Nakada Y and Yogo T. Discovery of 7-Oxo-2,4,5,7-tetrahydro-6 H-pyrazolo[3,4- c]pyridine derivatives as potent, orally available, and brain-penetrating receptor interacting protein 1 (RIP1) kinase inhibitors: analysis of structure-kinetic relationships. J Med Chem 2018; 61: 2384-2409.

[3] Sakai T, Ogata A, Ikenuma H, Yamada T, Hattori S, Abe J, Imamura S, Ichise M, Tada M, Kakita A, Koyama H, Suzuki M, Kato T, Ito K and Kimura Y. A novel PET probe to selectively image heat shock protein 90α/β isoforms in the brain. EJNMMI Radiopharm Chem 2024; 9: 19.

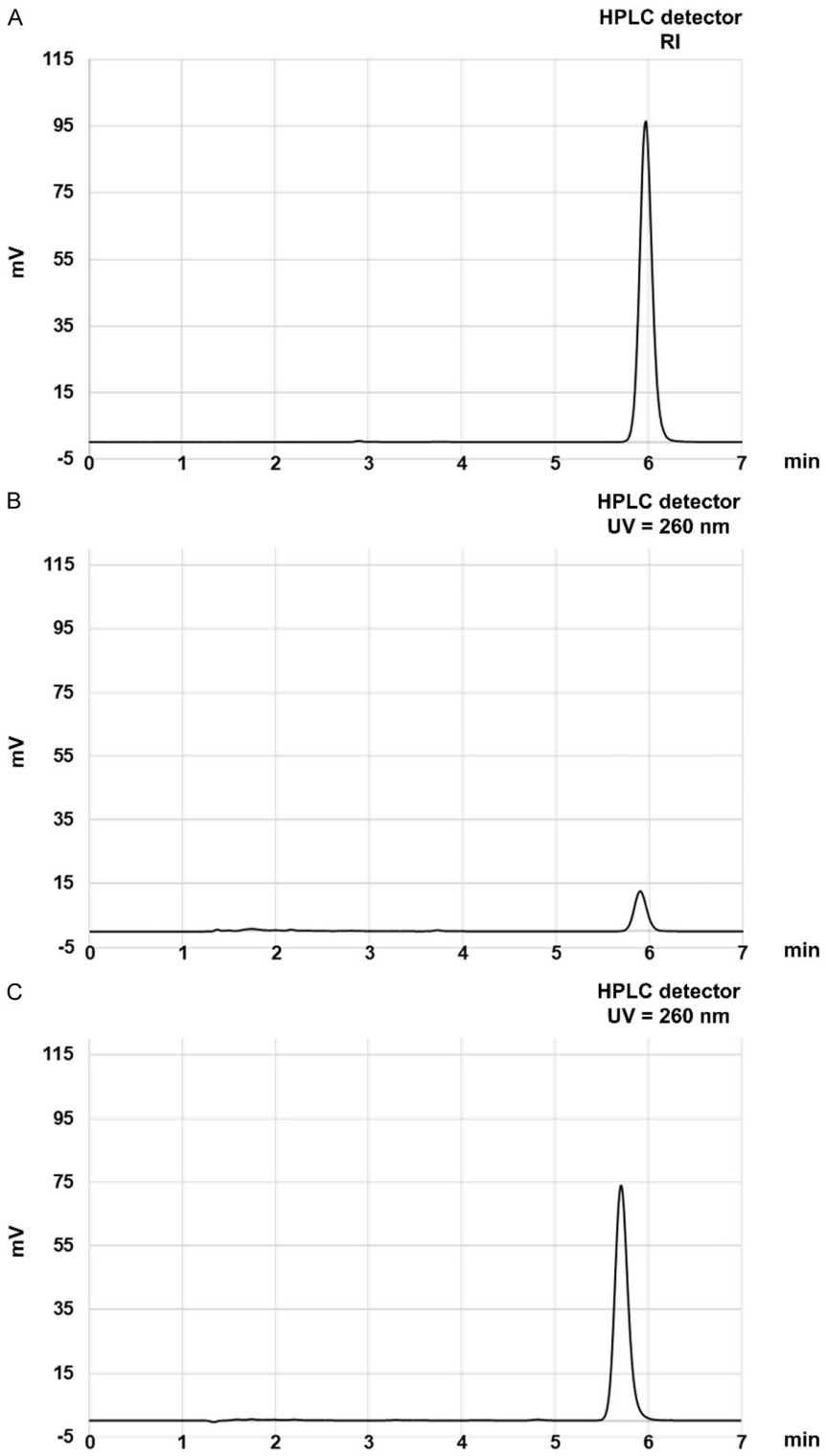


Figure S1. HPLC chromatogram of [^{11}C]TZ7774 and TZ7774. [^{11}C]TZ7774 (A), TZ7774 (B) and [^{11}C]TZ7774 with TZ7774 was analyzed by HPLC (column: CAPCELL PAK C18, 4.6 × 150 mm, UG120) eluting with around 55% CH_3CN in 0.1% formic acid at a flow rate of 1.0 mL/min. HPLC peaks were detected with RI (A, rt, 5.95 min), UV (B, rt 5.84 min) and UV (C, rt 5.72 min).

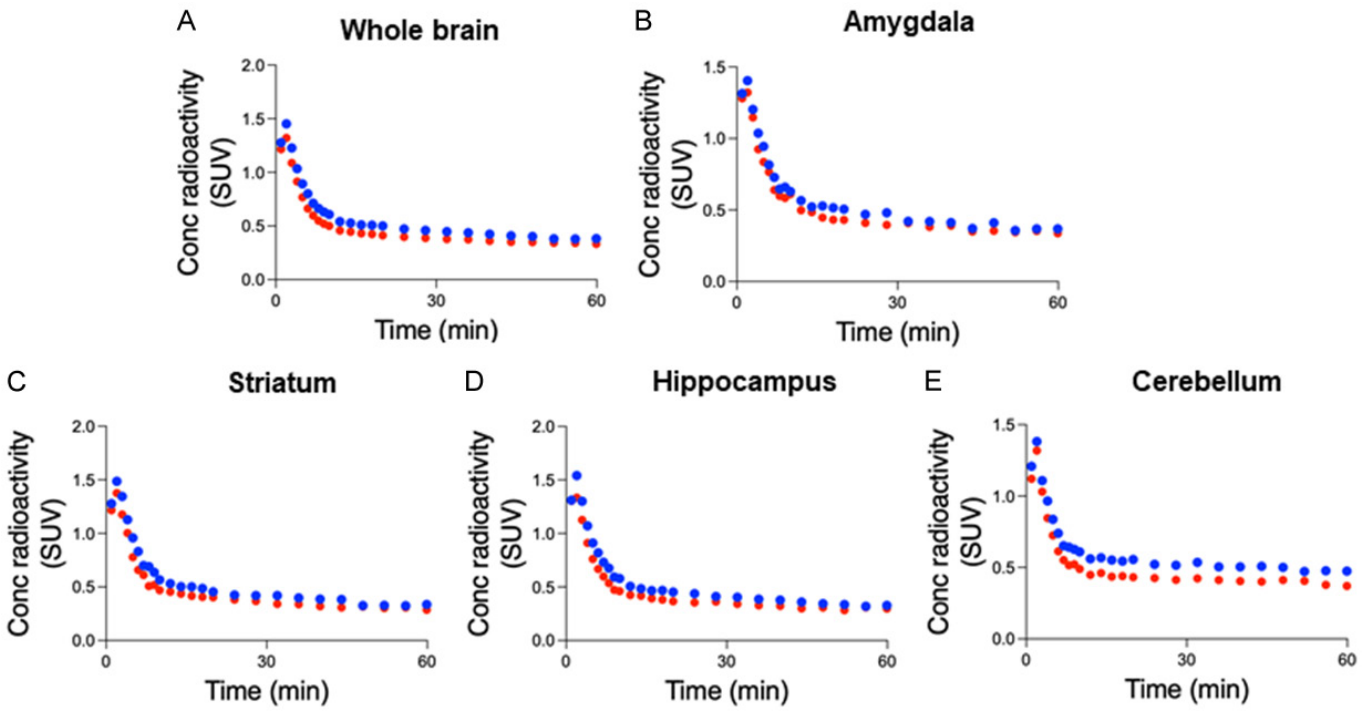


Figure S2. PET imaging study of [11C]TZ7774 in rats includes averaged time-activity curves for different brain regions. The regions analyzed are: the whole brain (A), amygdala (B), striatum (C), hippocampus (D), and cerebellum (E). The data compare the results after injection of [11C]TZ7774 with (blue circles) and without (red circles) the administration of 1 mg/kg of GSK'963 as a blocking agent.

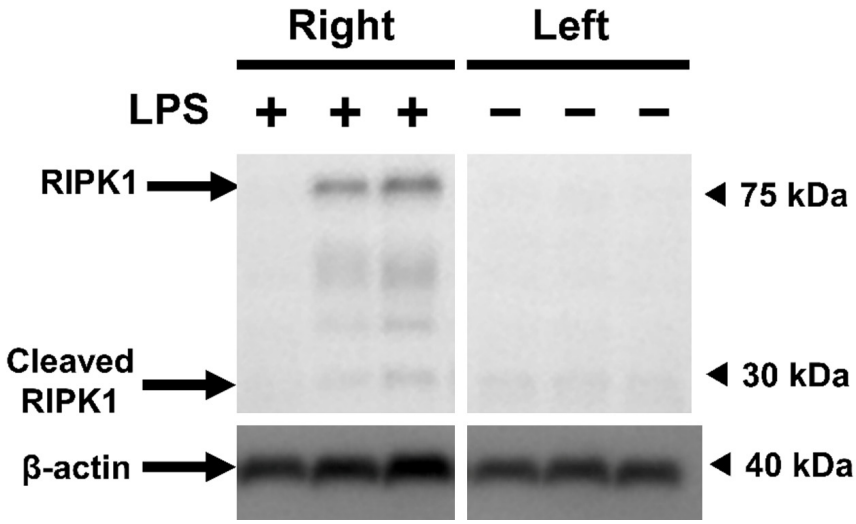


Figure S3. RIPK1 protein expression in homogenates of right and left striatum of rats injected with LPS (25 µg) in the right brain striatum.

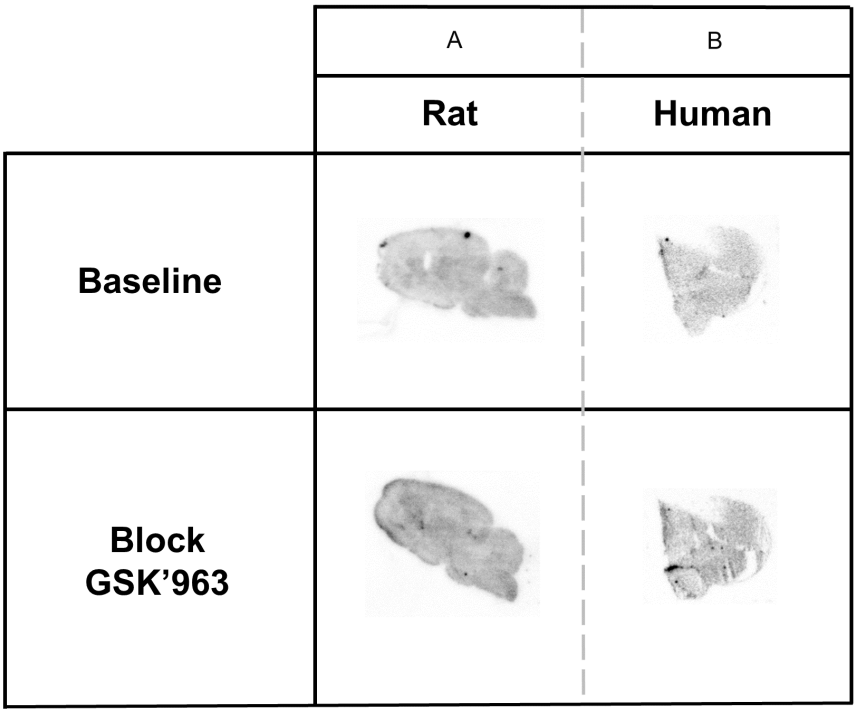


Figure S4. Autoradiography of a sagittal section of a healthy rat (A) and frontal sections of a healthy human (B), with or without the addition of GSK'963 (10 μM) as a blocking agent.