Supporting Information

^{99m}Tc-cyclopentadienyl Tricarbonyl Chelate-labeled Compounds as Selective Sigma-2 Receptor Ligands for Tumor Imaging

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Author Contributions:
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Contents

1. General information and some parts of evaluation of the radiotracers in experimental section

General information

All reagents and chemicals were purchased from commercial source and used without further purification unless otherwise stated. ^{99m}Tc-pertechnetate was eluted from a commercial ^{99}Mo–^{99m}Tc generator obtained from Beijing Atomic High-tech Co. Haloperidol was obtained from Sigma-Aldrich Co. Ltd. (Beijing, China). Siramesine was synthesized according to the method in the literature.[1] Reactions were monitored by thin layer chromatography (TLC) (TLC silica gel 60 F_{254} plates, E. Merck). Flash column chromatography was conducted on silica gel (45–75 μm) from Qingdao Haiyang Chemical Co., Ltd. The mobile phase was reported in the experimental procedure. ^{1}H NMR spectra were recorded on a Bruker Avance III (400 MHz) NMR spectrometer in $CDCl_3$ at room temperature with TMS as an internal standard. Chemical shift (δ) were reported in ppm values downfield from tetramethylsilane and coupling constants (*J*) were reported in Hertz (Hz). Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). ^{13}C NMR spectra were recorded on a Bruker Avance III (100 MHz) NMR spectrometer. Mass spectra were acquired by Quattro micro API ESI/MS (Waters, USA). High-resolution mass spectrometry (HRMS) was performed on a LCT Premier XE ESI-TOF mass spectrometry instrument (Waters, USA). X-ray crystallography data were collected on a Bruker Smart APEX II diffractometer (Bruker Co., Germany). Melting point (Mp) of solid compounds was tested on a WRX-4 micro melting point apparatus (Shanghai Yice instrument Co., LTD, China) and was uncorrected. The purity of rhenium compounds and ferrocene precursors were analyzed by high performance liquid chromatography (HPLC). All final complexes were tested with a purity of > 95%.

HPLC separations and analyses were performed on a Waters 600 system (Waters, corporation, USA) equipped with a Waters 2489 UV–VIS detector, and a Raytest Gabi NaI (Tl) scintillation detector (Raytest, Germany) and on a Shimadzu SCL-20AVP system (Shimadzu Corporation, Japan) equipped with a Bioscan Flow Count 3200 NaI/PMT γ-radiation scintillation detector. Samples were analyzed and separated on an Agela Venusil MP C18 column (250 mm × 4.6 mm, 5 μm) using acetonitrile with 0.1% trifluoroacetic acid (TFA) and water with 0.1% TFA as mobile phase at a flow rate of 1 mL/min.

Male ICR mice (4–5 weeks, 22–25 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. All procedures related to animal experiments were performed in compliance with relevant laws and institutional guidelines. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of Beijing Normal University.

X-ray Crystallography

Single-crystal X-ray diffraction measurements were conducted on a Bruker Smart APEXII CCD crystal diffractometer at 150(2) K using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). An empirical absorption correction was applied using the SADABS program.[2] All structures were solved by direct methods and refined by full-matrix least-squares on F^2 using the SHELXL-97 program package. All of the hydrogen atoms (except solvent H_2O) were geometrically fixed using the riding model.[3]

In vitro radioligand competition studies

σ Receptor Binding Assays

The σ_1 and σ_2 receptor affinities were determined by radioligand competition binding assay which was previously reported.[4,5] Briefly, the σ_1 receptor binding assay was performed using rat brain membrane homogenates and the σ_1 specific radioligand (+)-[^{3}H]pentazocine. For σ_2 receptors, it was conducted by using rat liver membrane homogenates and [^{3}H]DTG in the presence of 10 μM dextrallorphan for selective masking of σ_1 receptor binding. Nonspecific binging was determined with 10 μM haloperidol. K_i values were calculated according to the Cheng−Prusoff equation and represent the mean ± standard deviation (SD) from at least two independent experiments, each performed in triplicate.

VAChT Binding Assays

Affinity of compound **20a** for the vesicular acetylcholine transporter (VAChT) was also determined in radioligand competition binding assays.[6] Radioligand competition binding assays were performed using membrane homogenates obtained from PC12 cells stably transfected with rat VAChT (obtained from Ali Roghani, Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, Lubbock, TX) and (−)-[^{3}H]vesamicol (1 nM working concentration). Assays were performed with compound **20a** in 50 mM Tris−HCl, pH 7.4, by incubation at room temperature for 120 min. Nonspecific binding was determined in the presence of 10 μM of (−)-vesamicol. K_i values were calculated according to the Cheng−Prusoff equation and represent the mean ± standard deviation (SD) from two single experiments, each performed in triplicate.

Other receptor and transporter Binding Assays

To test the selectivity of compound **20a**, the radioligand competition binding assays of dopamine D_{2L} receptors, NMDA receptors, opiate receptors, dopamine transporter (DAT), Norepinephrine transporter (NET) and serotonin (5-hydrotryptamine) transporter (SERT) were performed based on the methods reported in the literature. The detailed protocols are listed as follows.

Dopamine D_{2L} receptors [7,8]

Source:	Human recombinant CHO cells
Vehicle:	1.00% DMSO
Incubation Time/Temp:	2 hours at 25 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 1.4 mM Ascorbic Acid, 0.001% BSA, 150 mM NaCl
$K_{d:}$	0.080 nM
B_{max}:	0.48 pmole/mg Protein
Ligand:	0.16 nM [^{3}H]Spiperone
Non-specific Ligand:	10.0 μM Haloperidol
Specific Binding:	85%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

NMDA receptors[9]

Source:	Wistar Rat cerebral cortex
Vehicle:	1.00% DMSO
Incubation Time/Temp:	45 minutes at 25 °C
Incubation buffer:	10 mM Tris-HCl, pH 7.4
$K_{d:}$	8.40 nM
B_{max}:	0.78 pmole/mg Protein
Ligand:	4.0 nM [^{3}H]TCP

Non-specific Ligand:	1.0 µM Dizocilpine ((+)-MK-801)
Specific Binding:	94%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Opiate receptors, Non-selective[10,11]

Source:	Wistar Rat brain
Vehicle:	1.00% DMSO
Incubation Time/Temp:	40 minutes at 25 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4
$K_{d:}$	1.40 nM
B_{max}:	0.095 pmole/mg Protein
Ligand:	1.0 nM [^{3}H]Naloxone
Non-specific Ligand:	1.0 µM Naloxone
Specific Binding:	85%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Dopamine Transporter (DAT)[12,13]

Source:	Human recombinant CHO-S cells
Vehicle:	1.00% DMSO
Incubation Time/Temp:	3 hours at 4 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 µM Leupeptin, 10 µM PMSF
$K_{d:}$	0.58 nM
B_{max}:	0.047 pmole/mg Protein
Ligand:	0.15 nM [^{125}I]RTI-55
Non-specific Ligand:	10.0 µM Nomifensine
Specific Binding:	90%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Norepinephrine Transporter (NET)[14]

Source:	Human recombinant MDCK cells
Vehicle:	1.00% DMSO
Incubation Time/Temp:	3 hours at 4 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 µM Leupeptin, 10 µM PMSF
$K_{d:}$	0.024 µM
B_{max}:	2.50 pmole/mg Protein
Ligand:	0.20 nM [^{125}I]RTI-55
Non-specific Ligand:	10.0 µM Desipramine
Specific Binding:	75%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Serotonin Transporter (SERT)[15,16]

Source:	Human recombinant HEK-293 cells
Vehicle:	1.00% DMSO
Incubation Time/Temp:	60 minutes at 25 °C
Incubation buffer:	50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl
$K_{d:}$	0.078 nM
B_{max}:	4.40 pmole/mg Protein

Ligand:	0.40 nM [^{3}H]Paroxetine
Non-specific Ligand:	10.0 μM Imipramine
Specific Binding:	95%
Quantitation Method:	Radioligand Binding
Significance Criteria:	≥ 50% of max stimulation or inhibition

Radiochemistry

The general procedure for preparing radioligand [^{99m}Tc]**20b**–**25b** and [^{99m}Tc]**29b**–**30b** were through double ligand transfer (DLT) reaction which was reported previously.[17-19] Briefly, 1.0 mg of ferrocene precursor (**36**–**41** or **43**–**44**) and 3.0 mg of $Mn(CO)_5Br$ were dissolved in 0.6 mL of DMF, 0.5−1.0 mL of ^{99m}Tc-pertechnetate was added successively. The mixture was stirred in a 10 mL of sealed vial and heated to 140 or 150 °C for 60 min. After cooled to room temperature, followed by addition of 3 mL of H_2O, the crude product was extracted with $CHCl_3$ and then passed through a 0.22 μm hydrophobic membrane. The residue was purified by HPLC.

The eluent for purification of [^{99m}Tc]**20b**–**25b** and [^{99m}Tc]**30b** was 40% acetonitrile (with 0.1% TFA) and 60% H_2O (with 0.1% TFA). The identification of the radiotracer was performed by co-injected and co-eluented with the corresponding rhenium compound **20a**–**25a** and **30a** with the same mobile phase (Agela Venusil MP C18 column, 250 × 4.6 mm, 5 μm, 1 mL/min).

The eluent for purification of [^{99m}Tc]**29b** was 35% acetonitrile (with 0.1% TFA) and 65% H_2O (with 0.1% TFA). The identification of the radiotracer was performed by co-injected and co-eluented with rhenium compound **29a** with the same mobile phase (Agela Venusil MP C18 column, 250 × 4.6 mm, 5 μm, 1 mL/min).

Measurement of log *D* values

The log *D* values of [^{99m}Tc]**20b**–**25b** and [^{99m}Tc]**29b**–**30b** were determined by measuring the distribution of the radiotracer between 1-octanol and 0.05 M sodium phosphate buffer at pH 7.4 according to papers.[5,17,18] The two phases were pre-saturated with each other. 1-octanol (3 mL) and sodium phosphate buffer (3 mL) was pipetted into a 15 mL plastic centrifuge tube and the new purified radioactive product in saline was added. The tube was vortexed for 3 min and followed by centrifugation for 5 min (3500 rpm, Anke TDL80-2B, China). About 50 μL of the 1-octanol layer was weighed in a tared tube. The buffer layer was removed and about 500 μL of the buffer layer was weighed in a second tared tube. The activity in both tubes was measured in an automatic γ-counter (Wallac 1470 Wizard, USA). Accurate volumes of each counted phase were determined by weight and known densities. The distribution coefficient was determined by calculating the ratio of cpm/mL of 1-octanol layer to that of buffer layer and expressed as log *D*. Samples from the 1-octanol layer were redistributed until consistent distribution coefficient values were obtained. The measurement was carried out in triplicate and repeated three times.

***In vitro* evaluation of the ^{99m}Tc-labeled complexes in DU145 prostate tumor cells**

DU145 human prostate cells (National Platform of Experimental Cell Resources for Sci-Tech) were routinely grown as monolayer in RPMI-1640 medium (Macgene Biotech Co., Ltd, Beijing, China) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) antibiotics (penicillin 100 U/mL, and streptomycin 100 μg/mL) in an atmosphere containing 5% CO_2 at 37 °C.

DU145 prostate cells were first seeded in 24-well plates with equal number of cells (2×10^5 cells/well) in each well and incubated with 1 mL of RPMI 1640 medium supplemented with 10% FBS overnight at 37 °C to allow a firm adherence. For the cell binding assay, cells in each well were incubated with [^{99m}Tc]**20b**, [^{99m}Tc]**23b**, [^{99m}Tc]**29b** and [^{99m}Tc]**30b** (11–15 kBq) which were dissolved in 1 mL of RPMI 1640 medium (in triplicate) at room temperature. The medium was removed quickly from 15 min to 120 min at an interval of every 15 min and the cells were rinsed twice with cold phosphate buffer and then lysed with 1 mL of NaOH (1 M). The blocking studies of [^{99m}Tc]**20b** and [^{99m}Tc]**23b** were a little different from those of [^{99m}Tc]**29b** and [^{99m}Tc]**30b**. For [^{99m}Tc]**20b** and [^{99m}Tc]**23b**, each kind of inhibitors with gradient concentration (haloperidol: 2 μM, 10 μM, 20 μM; ISO-1: 2 μM, 10 μM, 20 μM, 40 μM) and radiotracer (11 kBq) in 1 mL of RPMI 1640 medium was added and incubated with the cells for 60 min (in triplicate) at room temperature. While for [^{99m}Tc]**29b** and [^{99m}Tc]**30b**, haloperidol with various concentration (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M haloperidol) in 1 mL fresh medium with radio-labeled complexes (15 kBq) were co-incubated with the cells for 60 min (in triplicate) at room temperature. After removing the medium quickly from the wells, cells were rinsed twice with ice-cold phosphate-buffer saline containing 0.2% BSA and then lysed with 1 mL of NaOH (1 M). The cell-binding radioactivity was detected by a gamma counter (Wallac 1470 Wizard, PerkinElmer, USA). The administered [^{99m}Tc]**20b**, [^{99m}Tc]**23b**, [^{99m}Tc]**29b** and [^{99m}Tc]**30b** (11–15 kBq) in each well was used as the administered tracer dose. The cell binding (%) is given by the formulas:

binding (%) = CPM (in the cell suspension) /CPM (administered tracer dose) × 100%.

The %blocking is calculated by [(radioactivity accumulation under blocking condition)–(radioactivity accumulation under control condition)]/(radioactivity accumulation under control condition) × 100%. Significant differences between control and blocking groups were determined by Student's *t* test (independent, two-tailed). The criterion for significance was $p\leq0.05$.

In vitro evaluation of the ^{99m}Tc-labeled complexes in C6 glioma tumor cells

C6 glioma cells (Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China) were routinely grown as monolayer in HAM'S F10 medium (Macgene Biotech Co., Ltd, Beijing, China) supplemented with 15% (v/v) horse serum, 2.5% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) antibiotics (penicillin 100 U/mL, and streptomycin 100 μg/mL) in an atmosphere containing 5% CO_2 at 37°C.

The preparation of C6 glioma cells in 24-well plates with HAM'S F10 medium was similar with the description of DU145 cells. The cell uptake and blocking studies of [^{99m}Tc]**20b**, [^{99m}Tc]**21b,** [^{99m}Tc]**24b** and [^{99m}Tc]**29b** were performed similar with the methods conducted for DU145 prostate tumor cells. For [^{99m}Tc]**20b**, ISO-1 was used as the blocking agent. While for [^{99m}Tc]**21b,** [^{99m}Tc]**24b** and [^{99m}Tc]**29b**, haloperidol was used as the blocking agent.

Biodistribution and blocking studies in ICR male mice

All animal experiments were carried out in compliance with the national laws related to the care and experiments on laboratory animals. For the biodistribution experiment, The purified [^{99m}Tc]**20b**, [^{99m}Tc]**23b**, [^{99m}Tc]**25b** and [^{99m}Tc]**29b** (185–370 kBq in 0.1 mL saline containing 7% ethanol) were injected via tail vein.

Mice were sacrificed by decapitation at 2, 15, 30, 60, 120, and 240 min (n = 5 for each time point). The organs of interest including blood, brain, heart, liver, spleen, kidneys, muscle, and thyroid were removed, weighed and counted in an automatic γ-counter (Wallac 1470 Wizard, USA). The results were expressed in terms of the percentage of injected dose per gram (% ID/g) of blood or organs.

For the blocking studies, ICR male mice were injected with haloperidol (0.1 mL, 1.0 mg/kg) via tail vein 5 min prior to the radiotracer injection. All mice were sacrificed by decapitation at 60 or 120 min postinjection. The blood and organs of interest were isolated and analyzed as described above for the biodistribution study. Significant differences between control and blocking groups were determined by Student's *t* test (independent, two-tailed). The criterion for significance was $p \leq 0.05$.

In vivo biodistribution and blocking studies of [^{99m}Tc]20b in Balb/c nude mice bearing C6 glioma xenografts

Balb/c nude mice, bearing with C6 glioma xenografts, were purchased from Vital River Laboratory Animal Technology Co. Ltd. (China). Each mouse was planted with 1×10^6 glioma cells and the tumor was grown for about 11 days before experiment.

For the biodistribution studies, mice were injected [^{99m}Tc]**20b** (370 kBq, 0.1 mL) via tail vein and sacrificed by decapitation at 120 and 240 min postinjection. Blood, tumor and other organ samples were removed, weighed and counted in an automatic γ-counter (Wallac 1470 Wizard, USA). The results were expressed in terms of the percentage of injected dose per gram (%ID/g) of blood or organs. For the blocking studies, 1.0 mg/kg (0.1 mL) blocking agents (haloperidol, **36** and siramesine) were injected 5 min prior to [^{99m}Tc]**20b** (370 kBq, 0.1 mL). At 240 min, animals were sacrificed and the samples were obtained and disposed as described for the biodistribution studies. Significant differences between control and blocking groups were determined by Student's *t* test (independent, two-tailed). The criterion for significance was $p \leq 0.05$.

Small animal SPECT/CT imaging studies

Small animal SPECT/CT imaging studies were carried out using the NanoScan SPECT/CT tomography (Mediso Ltd, Budapest, HUN). Nude mice bearing C6 glioma xenografts was injected with [^{99m}Tc]**20b** (22 MBq, 0.15 mL) via tail vein. The animal was imaged at 180 min postinjection after anesthetized with inhalation of 2% isoflurane. A total of 24 projections were acquired in a 256 × 256 acquisition matrix with a minimum of 50000 counts per projection. Images were reconstructed using an ordered-subset expectation maximization (OSEM) algorithm. Prior to each SPECT imaging, conebeam CT (180 projections, 1 s/projection, 45 kVp) images were acquired on the NanoScan SPECT/CT system. The SPECT and CT fusion images were obtained using the automatic fusion feature of the InVivoScope software (Mediso Ltd, Budapest, HUN).

In vivo radiometabolic stability of [^{99m}Tc]20b

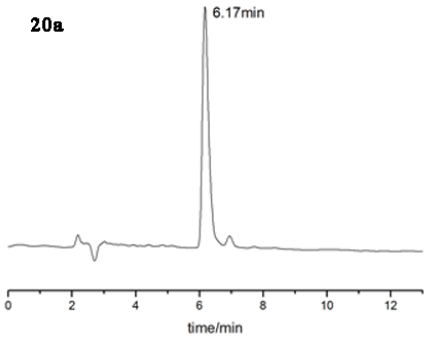
The *in vivo* metabolism of [^{99m}Tc]**20b** was performed as previously reported.[5] Briefly, male ICR mice were sacrificed by decapitation at 15 and 30 min after injection of 0.15 mL of [^{99m}Tc]**20b** (18.5 MBq) saline solution via tail vein. The brain and liver were collected, washed and homogenized with a labGEN 7 homogenizer in 2 mL of cold acetonitrile. After high speed centrifugation, the radiometabolite was

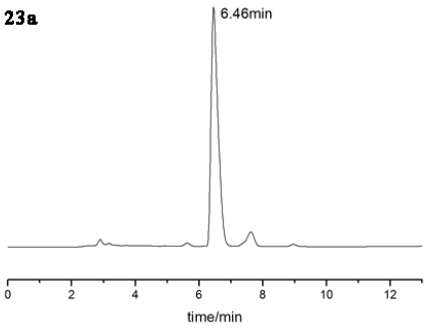
extracted by acetonitrile and filtered by a 0.22-μm organic millipore filter. The filtrates were concentrated and analysed on a radio-HPLC system (Waters 600 system, Agela Venusil MP C18, 250 mm × 4.6 mm, 5 μm) with eluent 40% CH_3CN (0.1% TFA) and 60% H_2O (0.1% TFA) at a flow rate of 1 mL/min.

2. Purity of key target compounds.

UV-wavelength: 254 nm

Compd	Flow rate (mL/min)	Mobile phase (acetonitrile (0.1% TFA)/water (0.1% TFA))	Column (Agela Venusil MP C18, 5 μm)	Retention time (RT, min)	Purity (%)
20a	1	50 : 50	4.6 × 250 mm	6.17	97.40
21a	1	50 : 50	4.6 × 250 mm	6.55	97.34
22a	1	50 : 50	4.6 × 250 mm	6.77	98.41
23a	1	50 : 50	4.6 × 250 mm	6.46	95.06
24a	1	50 : 50	4.6 × 250 mm	6.88	97.17
25a	1	50 : 50	4.6 × 250 mm	8.07	98.51
27a	1	40: 60	4.6 × 250 mm	7.95	99.37
28a	1	40: 60	4.6 × 250 mm	8.05	99.43
29a	1	40: 60	4.6 × 250 mm	8.12	99.52
30a	1	40: 60	4.6 × 250 mm	9.02	98.27
31a	1	40: 60	4.6 × 250 mm	8.80	98.77
32a	1	40: 60	4.6 × 250 mm	9.29	99.05
36	1	45 : 55	4.6 × 250 mm	6.33	97.69
37	1	45 : 55	4.6 × 250 mm	5.94	94.64
38	1	45 : 55	4.6 × 250 mm	7.87	99.25
39	1	45 : 55	4.6 × 250 mm	6.71	99.89
40	1	45 : 55	4.6 × 250 mm	6.34	95.18
41	1	45 : 55	4.6 × 250 mm	8.37	99.05
43	1	40: 60	4.6 × 250 mm	5.87	99.35
44	1	40: 60	4.6 × 250 mm	6.40	98.28
45	1	40: 60	4.6 × 250 mm	6.07	95.89
46	1	40: 60	4.6 × 250 mm	6.30	98.95


20a
6.17min
0
2
4
6
8
10
12
time/min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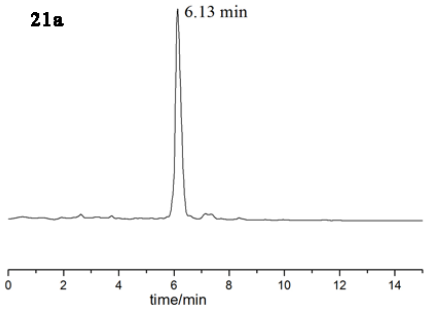
time/min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2
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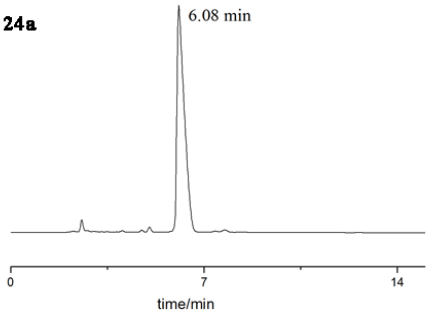
12
14
time/min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7
14
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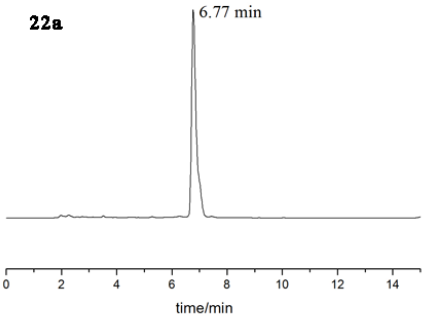
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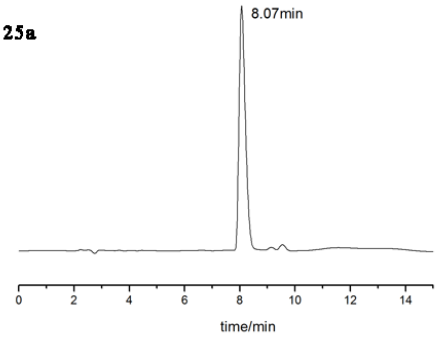
8
10
12
14
time/min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2
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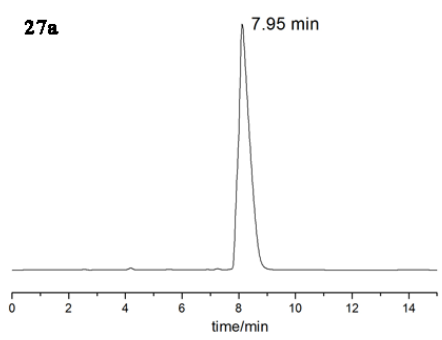
8
10
12
14
time/min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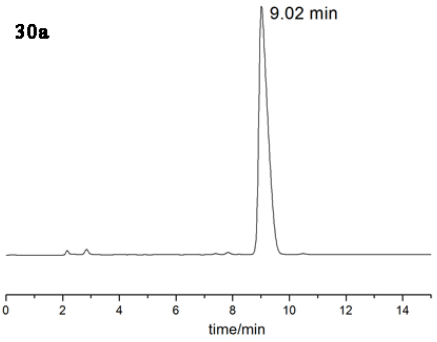
7.95 min
0 2 4 6 8 10 12 14
time/min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9.02 min
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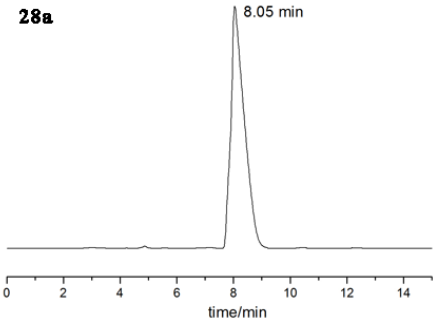
time/min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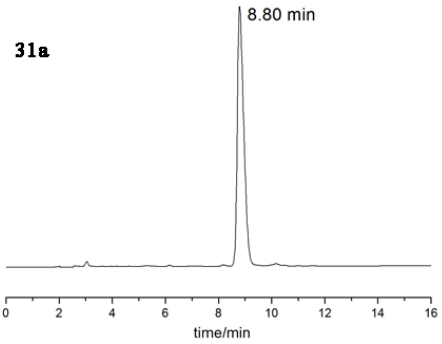
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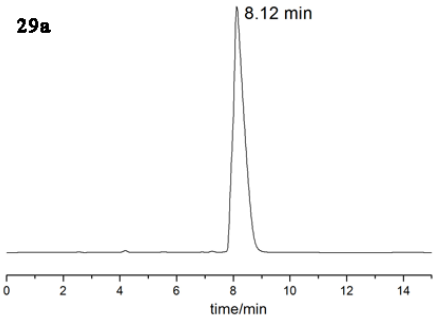
time/min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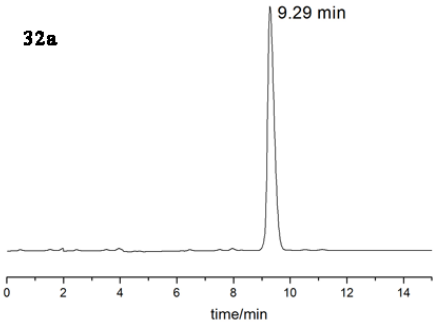
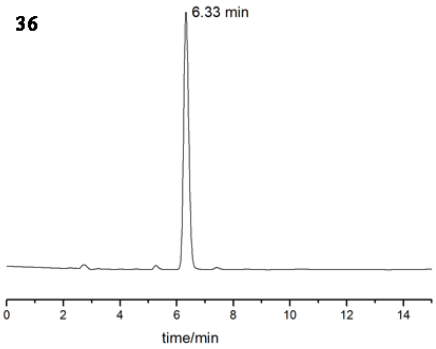
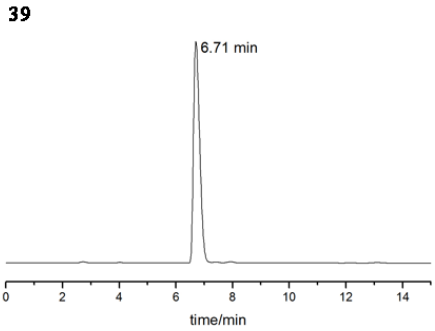
time/min


32a
9.29 min
0 2 4 6 8 10 12 14
time/min

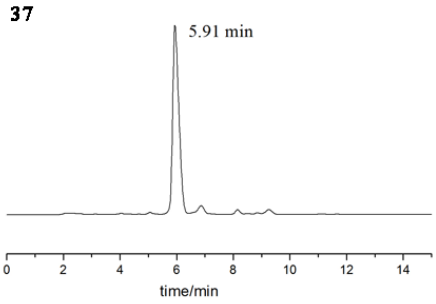
36



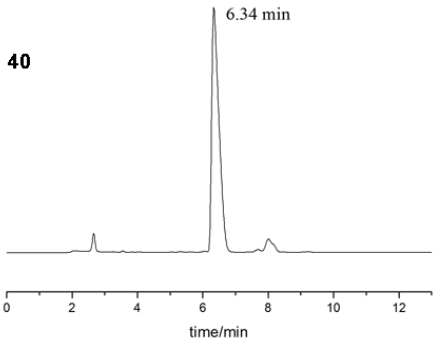
39



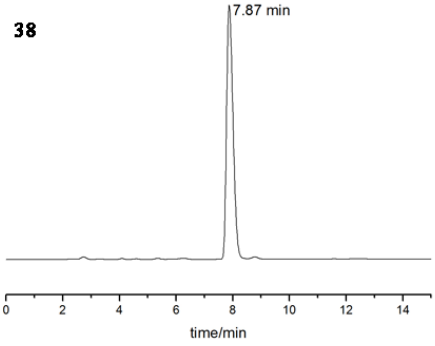
37



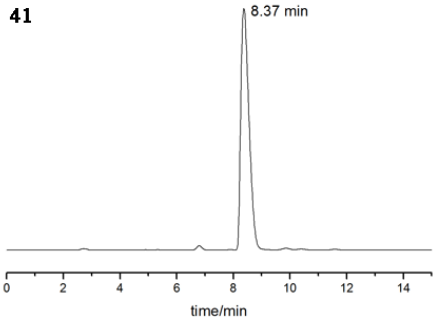
40



38



41



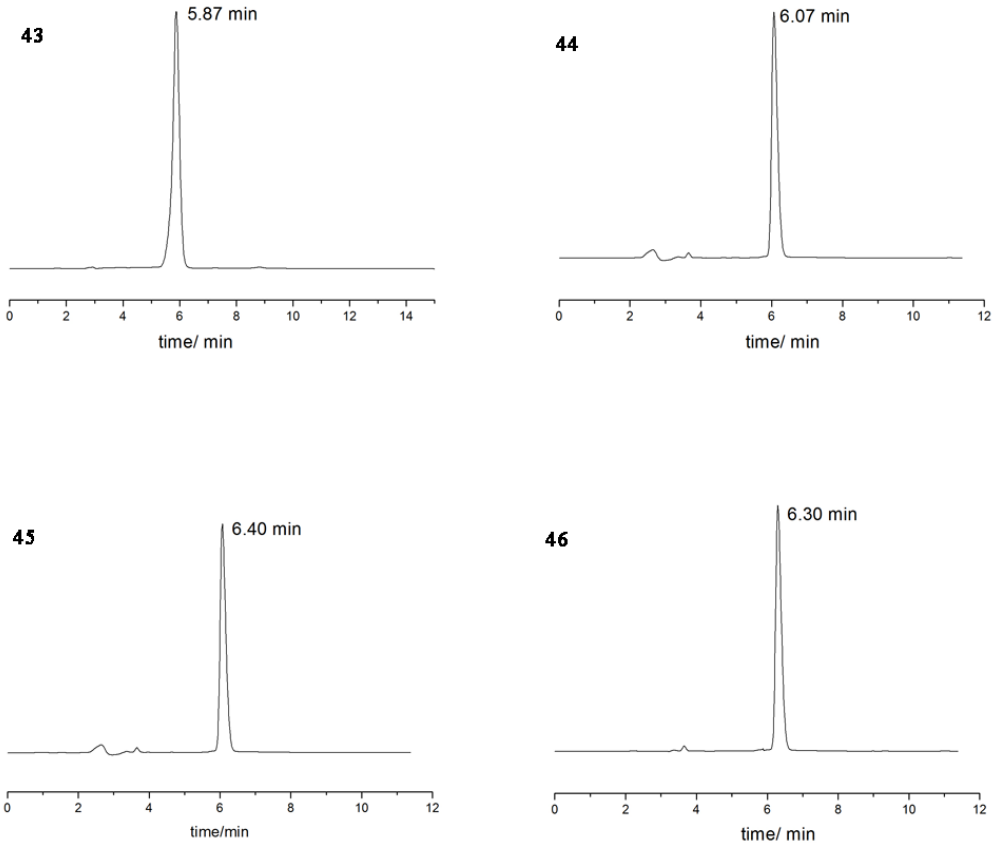
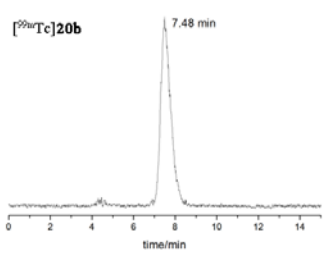
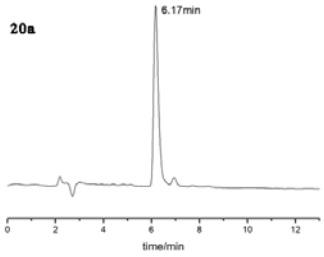
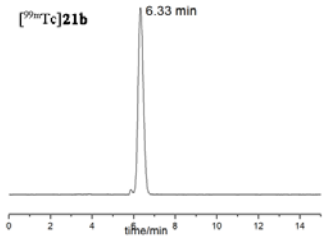


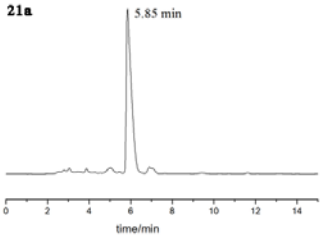
Figure S1. HPLC profiles of **20a–25a**, **27a–32a**, **36–41** and **43–46.**

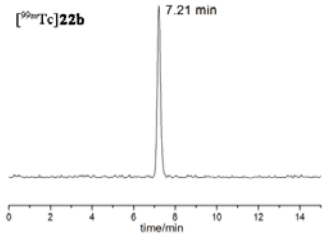
3. HPLC chromatograms of 20a–25a and [^{99m}Tc]20b–25b, 29a–30a and [^{99m}Tc]29b–30b.

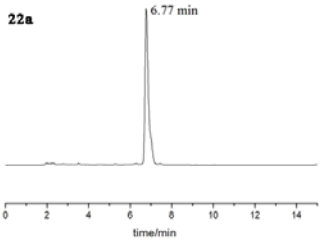

[99mTc]20b
7.48 min
time/min

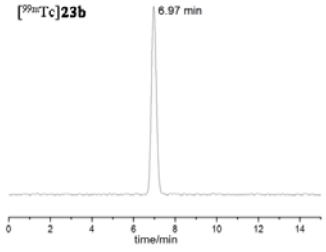

20a
6.17min
time/min

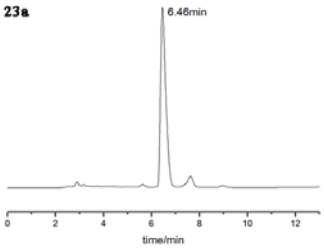

[99mTc]21b
6.33 min
time/min

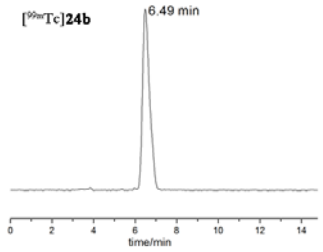

21a
5.85 min
time/min

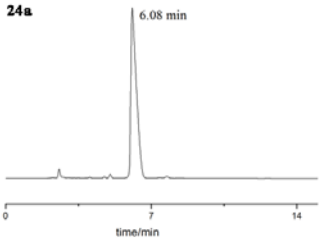

[99mTc]22b
7.21 min
time/min

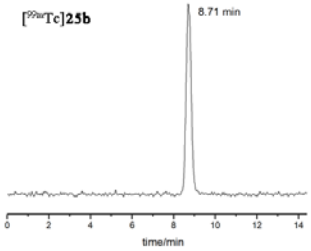

22a
6.77 min
time/min

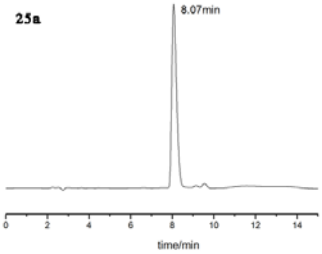

[99mTc]23b
6.97 min
time/min


23a
6.46min
time/min


[99mTc]24b
6.49 min
time/min


24a
6.08 min
time/min


[99mTc]25b
8.71 min
time/min


25a
8.07min
time/min

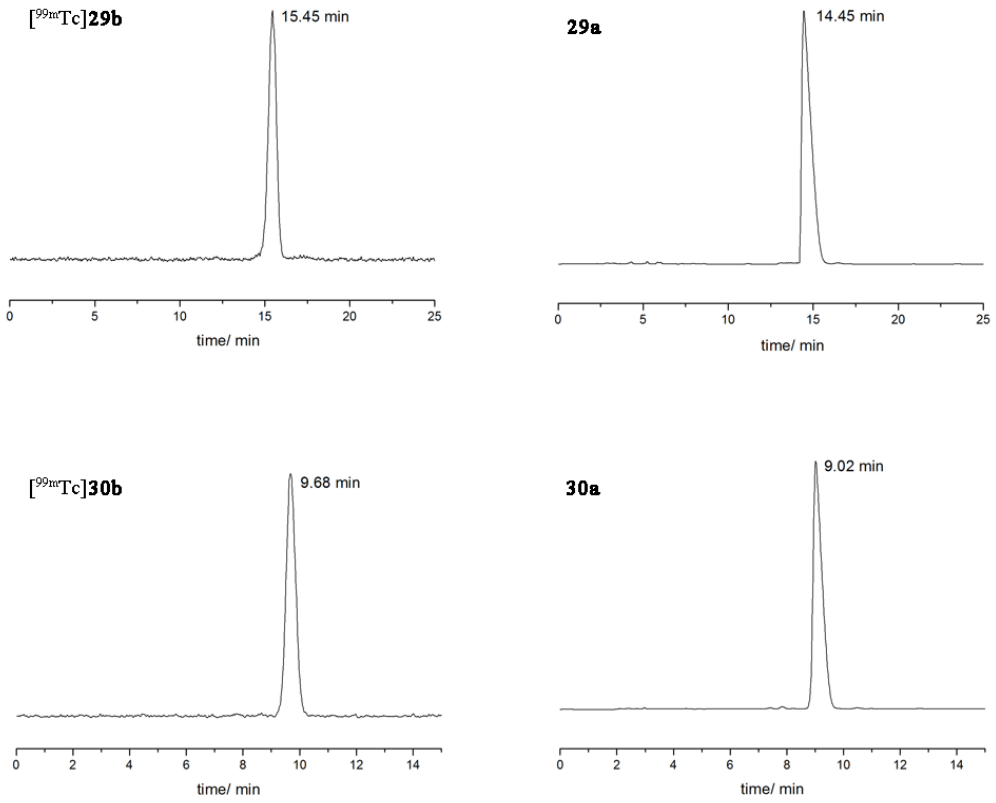


Figure S2. HPLC co-elution profiles of **20a**–**25a** and [^{99m}Tc]**20b**–**25b**, **29a**–**30a** and [^{99m}Tc]**29b**–**30b**. [^{99m}Tc]**20b** t_R (RI) = 7.48 min, **20a** t_R (UV) = 6.17 min (The difference of t_R values between **20a** and [^{99m}Tc]**20b** mainly due to the longer distance between the UV detector and scintillation detector in the HPLC); [^{99m}Tc]**21b** t_R (RI) = 6.33 min, **21a** t_R (UV) = 5.85 min; [^{99m}Tc]**22b** t_R (RI) = 7.21 min, **22a** t_R (UV) = 6.77 min; [^{99m}Tc]**23b** t_R (RI) = 6.97 min, **23a** t_R (UV) = 6.46 min; [^{99m}Tc]**24b** t_R (RI) = 6.49 min, **24a** t_R (UV) = 6.08 min; [^{99m}Tc]**25b** t_R (RI) = 8.71 min, **25a** t_R (UV) = 8.07 min; Conditions: CH_3CN (0.1% TFA)/H_2O (0.1% TFA) = 50/50, v/v, flow rate = 1 mL/min. [^{99m}Tc]**29b** t_R (RI) = 15.45 min, **29a** t_R (UV) = 14.45 min; Conditions: CH_3CN (0.1% TFA)/H_2O (0.1% TFA) = 65/35, v/v, flow rate = 1 mL/min. [^{99m}Tc]**30b** t_R (RI) = 9.68 min, **30a** t_R (UV) = 9.02 min; Conditions: CH_3CN (0.1% TFA)/H_2O (0.1% TFA) = 60/40, v/v, flow rate = 1 mL/min.

4. X-ray crystallographic data for compound 31a

Table S1. Crystal data and structure refinement for compound **31a**

Crystal parameter	Crystal data
Empirical formula	$C_{23}H_{25}N_2O_6Re$
Formula weight	611.65
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic,
space group	P -1

Unit cell dimensions	a = 9.569(2) Å alpha = 89.068(5) deg. b = 9.937(3) Å beta = 68.454(5) deg. c = 12.748(3) Å gamma = 84.701(5) deg.
Volume	1122.4(5) $Å^3$
Z	2
Calculated density	1.810 Mg/m^3
Absorption coefficient	5.455 mm^{-1}
F(000)	600
Crystal size	0.270 x 0.090 x 0.080 mm
	1.718 to 27.531 deg
Limiting indices	-7<=h<=12, -12<=k<=12, -14<=l<=16
Reflections collected / unique	7418 / 5115 [R(int) = 0.0261]
Completeness to theta = 25.242	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.75 and 0.51
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5115 / 0 / 295
Goodness-of-fit on F^2	1.059
Final R indices [I>2 sigma(I)]	R1 = 0.0311, wR2 = 0.0719
Rindices (all data)	R1 = 0.0364, wR2 = 0.0748
Extinction coefficient	n/a
Largest diff. peak and hole	1.557 and -0.876 e. $Å^{-3}$

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **31a**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor

	x	y	z	U(eq)
C(1)	6652(5)	5954(5)	1301(4)	27(1)
C(2)	5368(5)	7328(5)	-32(4)	24(1)
C(3)	3614(5)	5930(5)	1727(4)	25(1)
C(4)	5576(5)	9406(4)	1786(4)	19(1)
C(5)	4052(5)	9521(4)	1829(4)	20(1)
C(6)	3186(5)	8807(4)	2763(4)	19(1)
C(7)	4144(5)	8244(4)	3330(4)	18(1)
C(8)	5630(5)	8621(4)	2711(4)	18(1)
C(9)	3537(5)	7533(5)	4438(4)	20(1)
C(10)	3996(6)	6750(6)	6113(4)	32(1)
C(11)	5337(6)	6367(5)	6469(4)	25(1)
C(12)	6442(6)	7414(6)	6249(4)	31(1)
C(13)	8083(6)	8764(5)	4775(4)	30(1)
C(14)	9021(5)	8838(6)	3520(4)	27(1)
C(15)	9480(5)	10080(5)	3042(4)	25(1)
C(16)	10360(5)	10168(5)	1901(4)	21(1)
C(17)	10589(6)	12510(5)	2027(5)	35(1)
C(18)	8505(6)	6345(5)	4631(4)	33(1)
C(19)	9035(6)	6303(6)	3363(5)	34(1)

C(20)	9445(5)	7687(5)	2870(4)	26(1)
C(21)	10274(5)	7778(5)	1717(4)	22(1)
C(22)	10736(5)	8984(5)	1230(4)	19(1)
C(23)	11903(6)	7923(5)	-569(4)	28(1)
N(1)	4518(4)	7214(4)	4947(3)	21(1)
N(2)	7376(4)	7466(4)	5011(3)	20(1)
O(1)	7685(4)	5199(4)	1182(4)	38(1)
O(2)	5571(4)	7434(4)	-980(3)	33(1)
O(3)	2730(4)	5152(4)	1891(4)	39(1)
O(4)	2201(4)	7343(4)	4843(3)	35(1)
O(5)	10942(4)	11300(3)	1347(3)	24(1)
O(6)	11602(3)	9119(3)	115(3)	21(1)
Re(2)	4969(1)	7283(1)	1548(1)	16(1)

Table S3. Bond lengths [Å] and angles [deg] for **31a**

C(1)-O(1)	1.149(6)
C(1)-Re(2)	1.920(5)
C(2)-O(2)	1.156(6)
C(2)-Re(2)	1.909(5)
C(3)-O(3)	1.158(6)
C(3)-Re(2)	1.907(5)
C(4)-C(8)	1.417(6)
C(4)-C(5)	1.433(6)
C(4)-Re(2)	2.292(4)
C(4)-H(4)	0.9500
C(5)-C(6)	1.402(6)
C(5)-Re(2)	2.297(4)
C(5)-H(5)	0.9500
C(6)-C(7)	1.434(6)
C(6)-Re(2)	2.294(4)
C(6)-H(6)	0.9500
C(7)-C(8)	1.431(6)
C(7)-C(9)	1.507(6)
C(7)-Re(2)	2.304(4)
C(8)-Re(2)	2.296(4)
C(8)-H(8)	0.9500
C(9)-O(4)	1.222(5)
C(9)-N(1)	1.337(6)
C(10)-N(1)	1.465(6)
C(10)-C(11)	1.527(7)
C(10)-H(10A)	0.9900
C(10)-H(10B)	0.9900
C(11)-C(12)	1.502(7)
C(11)-H(11A)	0.9900
C(11)-H(11B)	0.9900
C(12)-N(2)	1.503(6)
C(12)-H(12A)	0.9900

C(12)-H(12B)	0.9900
C(13)-N(2)	1.487(6)
C(13)-C(14)	1.524(7)
C(13)-H(13A)	0.9900
C(13)-H(13B)	0.9900
C(14)-C(20)	1.363(7)
C(14)-C(15)	1.402(7)
C(15)-C(16)	1.394(7)
C(15)-H(15)	0.9500
C(16)-O(5)	1.370(6)
C(16)-C(22)	1.405(6)
C(17)-O(5)	1.433(6)
C(17)-H(17A)	0.9800
C(17)-H(17B)	0.9800
C(17)-H(17C)	0.9800
C(18)-N(2)	1.433(7)
C(18)-C(19)	1.506(8)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
C(19)-C(20)	1.527(7)
C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900
C(20)-C(21)	1.396(7)
C(21)-C(22)	1.375(6)
C(21)-H(21)	0.9500
C(22)-O(6)	1.369(5)
C(23)-O(6)	1.429(6)
C(23)-H(23A)	0.9800
C(23)-H(23B)	0.9800
C(23)-H(23C)	0.9800
N(1)-H(1)	0.96(6)

O(1)-C(1)-Re(2)	176.7(4)
O(2)-C(2)-Re(2)	175.9(4)
O(3)-C(3)-Re(2)	175.9(5)
C(8)-C(4)-C(5)	107.9(4)
C(8)-C(4)-Re(2)	72.2(3)
C(5)-C(4)-Re(2)	72.0(2)
C(8)-C(4)-H(4)	126.1
C(5)-C(4)-H(4)	126.1
Re(2)-C(4)-H(4)	121.5
C(6)-C(5)-C(4)	108.2(4)
C(6)-C(5)-Re(2)	72.1(2)
C(4)-C(5)-Re(2)	71.6(3)
C(6)-C(5)-H(5)	125.9
C(4)-C(5)-H(5)	125.9
Re(2)-C(5)-H(5)	122.1
C(5)-C(6)-C(7)	108.6(4)
C(5)-C(6)-Re(2)	72.4(3)

C(7)-C(6)-Re(2)	72.2(2)
C(5)-C(6)-H(6)	125.7
C(7)-C(6)-H(6)	125.7
Re(2)-C(6)-H(6)	121.4
C(8)-C(7)-C(6)	107.1(4)
C(8)-C(7)-C(9)	130.3(4)
C(6)-C(7)-C(9)	122.2(4)
C(8)-C(7)-Re(2)	71.6(2)
C(6)-C(7)-Re(2)	71.4(2)
C(9)-C(7)-Re(2)	127.1(3)
C(4)-C(8)-C(7)	108.2(4)
C(4)-C(8)-Re(2)	71.9(3)
C(7)-C(8)-Re(2)	72.2(2)
C(4)-C(8)-H(8)	125.9
C(7)-C(8)-H(8)	125.9
Re(2)-C(8)-H(8)	121.7
O(4)-C(9)-N(1)	124.0(4)
O(4)-C(9)-C(7)	119.8(4)
N(1)-C(9)-C(7)	116.0(4)
N(1)-C(10)-C(11)	110.3(4)
N(1)-C(10)-H(10A)	109.6
C(11)-C(10)-H(10A)	109.6
N(1)-C(10)-H(10B)	109.6
C(11)-C(10)-H(10B)	109.6
H(10A)-C(10)-H(10B)	108.1
C(12)-C(11)-C(10)	115.2(4)
C(12)-C(11)-H(11A)	108.5
C(10)-C(11)-H(11A)	108.5
C(12)-C(11)-H(11B)	108.5
C(10)-C(11)-H(11B)	108.5
H(11A)-C(11)-H(11B)	107.5
C(11)-C(12)-N(2)	110.8(4)
C(11)-C(12)-H(12A)	109.5
N(2)-C(12)-H(12A)	109.5
C(11)-C(12)-H(12B)	109.5
N(2)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	108.1
N(2)-C(13)-C(14)	109.7(4)
N(2)-C(13)-H(13A)	109.7
C(14)-C(13)-H(13A)	109.7
N(2)-C(13)-H(13B)	109.7
C(14)-C(13)-H(13B)	109.7
H(13A)-C(13)-H(13B)	108.2
C(20)-C(14)-C(15)	120.1(4)
C(20)-C(14)-C(13)	119.8(5)
C(15)-C(14)-C(13)	120.1(5)
C(16)-C(15)-C(14)	121.1(5)
C(16)-C(15)-H(15)	119.4
C(14)-C(15)-H(15)	119.4

O(5)-C(16)-C(15)	126.8(4)
O(5)-C(16)-C(22)	115.0(4)
C(15)-C(16)-C(22)	118.2(4)
O(5)-C(17)-H(17A)	109.5
O(5)-C(17)-H(17B)	109.5
H(17A)-C(17)-H(17B)	109.5
O(5)-C(17)-H(17C)	109.5
H(17A)-C(17)-H(17C)	109.5
H(17B)-C(17)-H(17C)	109.5
N(2)-C(18)-C(19)	106.7(4)
N(2)-C(18)-H(18A)	110.4
C(19)-C(18)-H(18A)	110.4
N(2)-C(18)-H(18B)	110.4
C(19)-C(18)-H(18B)	110.4
H(18A)-C(18)-H(18B)	108.6
C(18)-C(19)-C(20)	111.3(5)
C(18)-C(19)-H(19A)	109.4
C(20)-C(19)-H(19A)	109.4
C(18)-C(19)-H(19B)	109.4
C(20)-C(19)-H(19B)	109.4
H(19A)-C(19)-H(19B)	108.0
C(14)-C(20)-C(21)	119.0(5)
C(14)-C(20)-C(19)	122.0(5)
C(21)-C(20)-C(19)	119.0(5)
C(22)-C(21)-C(20)	121.9(5)
C(22)-C(21)-H(21)	119.1
C(20)-C(21)-H(21)	119.1
O(6)-C(22)-C(21)	124.2(4)
O(6)-C(22)-C(16)	116.2(4)
C(21)-C(22)-C(16)	119.6(4)
O(6)-C(23)-H(23A)	109.5
O(6)-C(23)-H(23B)	109.5
H(23A)-C(23)-H(23B)	109.5
O(6)-C(23)-H(23C)	109.5
H(23A)-C(23)-H(23C)	109.5
H(23B)-C(23)-H(23C)	109.5
C(9)-N(1)-C(10)	120.6(4)
C(9)-N(1)-H(1)	127(4)
C(10)-N(1)-H(1)	112(4)
C(18)-N(2)-C(13)	110.4(4)
C(18)-N(2)-C(12)	112.6(4)
C(13)-N(2)-C(12)	109.1(4)
C(16)-O(5)-C(17)	115.5(4)
C(22)-O(6)-C(23)	115.5(4)
C(3)-Re(2)-C(2)	90.5(2)
C(3)-Re(2)-C(1)	92.2(2)
C(2)-Re(2)-C(1)	90.7(2)
C(3)-Re(2)-C(4)	154.33(19)
C(2)-Re(2)-C(4)	98.87(18)

C(1)-Re(2)-C(4)	111.43(19)
C(3)-Re(2)-C(6)	94.57(18)
C(2)-Re(2)-C(6)	120.56(17)
C(1)-Re(2)-C(6)	147.92(19)
C(4)-Re(2)-C(6)	60.09(16)
C(3)-Re(2)-C(8)	136.75(18)
C(2)-Re(2)-C(8)	132.10(18)
C(1)-Re(2)-C(8)	94.15(18)
C(4)-Re(2)-C(8)	35.97(15)
C(6)-Re(2)-C(8)	60.31(15)
C(3)-Re(2)-C(5)	119.72(18)
C(2)-Re(2)-C(5)	93.33(18)
C(1)-Re(2)-C(5)	147.76(19)
C(4)-Re(2)-C(5)	36.38(15)
C(6)-Re(2)-C(5)	35.56(15)
C(8)-Re(2)-C(5)	60.19(15)
C(3)-Re(2)-C(7)	102.54(19)
C(2)-Re(2)-C(7)	153.40(18)
C(1)-Re(2)-C(7)	111.61(18)
C(4)-Re(2)-C(7)	60.25(15)
C(6)-Re(2)-C(7)	36.34(15)
C(8)-Re(2)-C(7)	36.26(14)
C(5)-Re(2)-C(7)	60.07(15)

Symmetry transformations used to generate equivalent atoms:

Table S4. Anisotropic displacement parameters (A^2 x 10^3) for **31a**. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
C(1)	24(2)	28(3)	29(3)	0(2)	-11(2)	-2(2)
C(2)	23(2)	17(2)	31(3)	1(2)	-9(2)	2(2)
C(3)	23(2)	19(2)	34(3)	-3(2)	-13(2)	3(2)
C(4)	21(2)	16(2)	18(2)	4(2)	-4(2)	-5(2)
C(5)	24(2)	17(2)	21(2)	3(2)	-10(2)	-3(2)
C(6)	17(2)	19(2)	19(2)	-2(2)	-7(2)	1(2)
C(7)	17(2)	18(2)	20(2)	1(2)	-9(2)	-1(2)
C(8)	14(2)	22(2)	19(2)	2(2)	-6(2)	-4(2)
C(9)	14(2)	27(2)	19(2)	1(2)	-4(2)	-3(2)
C(10)	25(2)	56(4)	21(2)	17(2)	-10(2)	-22(2)
C(11)	29(2)	31(3)	16(2)	7(2)	-7(2)	-6(2)
C(12)	31(3)	39(3)	25(3)	1(2)	-11(2)	-7(2)
C(13)	24(2)	34(3)	32(3)	1(2)	-8(2)	-4(2)
C(14)	11(2)	49(3)	22(2)	10(2)	-7(2)	-4(2)
C(15)	15(2)	40(3)	21(2)	-9(2)	-10(2)	6(2)
C(16)	15(2)	24(2)	28(2)	0(2)	-12(2)	-2(2)
C(17)	32(3)	25(3)	55(4)	-11(2)	-25(3)	2(2)

C(18)	35(3)	31(3)	36(3)	6(2)	-16(2)	-5(2)
C(19)	26(3)	35(3)	39(3)	11(2)	-10(2)	-5(2)
C(20)	15(2)	41(3)	28(3)	13(2)	-14(2)	-8(2)
C(21)	15(2)	26(2)	28(2)	4(2)	-9(2)	-4(2)
C(22)	13(2)	27(2)	17(2)	2(2)	-6(2)	-2(2)
C(23)	28(2)	35(3)	23(2)	-9(2)	-11(2)	-2(2)
N(1)	16(2)	32(2)	15(2)	6(2)	-4(2)	-6(2)
N(2)	19(2)	25(2)	19(2)	7(2)	-9(2)	-6(2)
O(1)	26(2)	33(2)	58(3)	-8(2)	-19(2)	8(2)
O(2)	41(2)	34(2)	20(2)	-2(2)	-8(2)	5(2)
O(3)	27(2)	22(2)	74(3)	5(2)	-23(2)	-9(2)
O(4)	19(2)	61(3)	25(2)	14(2)	-6(2)	-16(2)
O(5)	20(2)	19(2)	35(2)	-1(1)	-12(2)	-2(1)
O(6)	21(2)	23(2)	16(2)	1(1)	-3(1)	-4(1)
Re(2)	15(1)	16(1)	17(1)	3(1)	-6(1)	-2(1)

Table S5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **31a**

	x	y	z	U(eq)
H(4)	6404	9788	1235	23
H(5)	3692	9999	1313	24
H(6)	2138	8712	2984	22
H(8)	6501	8386	2889	22
H(10A)	3418	5956	6177	39
H(10B)	3318	7476	6620	39
H(11A)	4949	6181	7286	30
H(11B)	5881	5520	6067	30
H(12A)	7113	7196	6674	37
H(12B)	5889	8311	6515	37
H(13A)	8736	8829	5217	36
H(13B)	7290	9531	5002	36
H(15)	9186	10875	3504	30
H(17A)	9491	12703	2373	52
H(17B)	11022	13267	1553	52
H(17C)	11011	12388	2619	52
H(18A)	8075	5493	4946	39
H(18B)	9357	6468	4874	39
H(19A)	8228	6005	3133	41
H(19B)	9928	5636	3057	41
H(21)	10527	6983	1257	27
H(23A)	12521	7241	-324	42
H(23B)	12447	8138	-1360	42
H(23C)	10948	7569	-493	42
H(1)	5550(70)	7400(60)	4690(50)	42(17)

5. Binding affinities of compound 20a for the additional receptors and transporters in the CNS

Table S6. Binding affinities of compound 20a for the additional receptors and transporters in the CNS*

Receptors/transporters	% Inhibition	K_i (nM)
Dopamine D_{2L} receptor	7	
NMDA receptor	18	
Opiate receptor (non-selective)	18	
DAT	14	
NET	11	
SERT	17	
VAChT		7026 ± 163

*% inhibition was determined at the concentration of 10 μM.

6. *In vitro* evaluation of [^{99m}Tc]21b and [^{99m}Tc]24b in C6 glioma cells

In vitro cell binding and blocking studies of [^{99m}Tc]**21b** and [^{99m}Tc]**24b** were carried out in C6 glioma cells (Figure S3). [^{99m}Tc]**21b** and [^{99m}Tc]**24b** displayed comparable uptakes with 4.88% and 5.12%, respectively. Compared to [^{99m}Tc]**20b**, treatment with 10^{-6} mol/L of haloperidol couldn't lead to significant reduction of the radiotracer uptake, suggesting high nonspecific binding of [^{99m}Tc]**21b** and [^{99m}Tc]**24b** in C6 glioma cells.

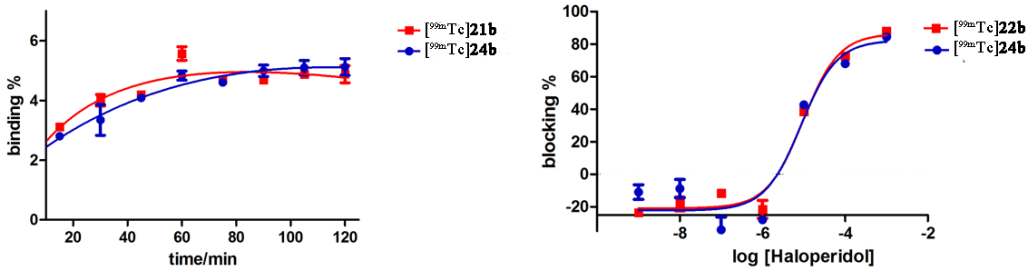


Figure S3. The *in vitro* uptakes of [^{99m}Tc]**21b** and [^{99m}Tc]**24b** in C6 glioma cells.

7. Biodistribution and blocking studies of [^{99m}Tc]25b in male ICR mice

Considering nanomolar affinity of [^{99m}Tc]**25b** for σ_1 receptors, the *in vivo* biodistribution and blocking studies were carried out in male ICR mice. [^{99m}Tc]**23b** showed much lower brain uptake than [^{99m}Tc]**20b** and [^{99m}Tc]**23b**. Moreover, pretreatment with haloperidol didn't lead to significant reduction of accumulation in the organs known to express σ_1 receptors, suggesting high nonspecific binding of [^{99m}Tc]**25b** *in vivo*.

Table S7. Biodistribution of [^{99m}Tc]**25b** in male ICR mice[a]

Organ	2 min	15 min	30 min	60 min	120 min	240 min
Blood	1.73 ± 0.19	0.69 ± 0.09	0.46 ± 0.02	0.38 ± 0.09	0.28 ± 0.03	0.30 ± 0.05

Brain	1.05 ± 0.06	0.95 ± 0.08	0.73 ± 0.10	0.50 ± 0.09	0.32 ± 0.07	0.32 ± 0.04
Heart	11.81 ± 0.83	3.13 ± 0.29	2.10 ± 0.56	1.42 ± 0.18	0.94 ± 0.15	0.95 ± 0.17
Liver	8.47 ± 0.82	19.87 ± 2.56	21.97 ± 3.75	26.49 ± 3.99	26.96 ± 2.37	27.07 ± 1.90
Spleen	4.31 ± 0.63	6.64 ± 1.20	5.86 ± 1.10	3.99 ± 0.54	1.78 ± 0.32	1.04 ± 0.21
Lung	38.74 ± 5.39	10.32 ± 1.79	7.01 ± 2.03	5.59 ± 0.92	4.08 ± 0.63	3.89 ± 0.69
Kidney	17.87 ± 2.31	14.99 ± 1.32	12.18 ± 1.00	11.77 ± 1.20	11.28 ± 1.75	11.25 ± 1.97
Small intestine[b]	3.66 ± 0.41	6.14 ± 1.68	7.63 ± 2.42	9.92 ± 0.60	9.16 ± 0.81	9.85 ± 1.51
Stomach[b]	1.29 ± 0.18	2.04 ± 0.40	1.74 ± 0.44	1.76 ± 0.43	1.06 ± 0.24	0.82 ± 0.12
Muscle	3.60 ± 0.42	2.09 ± 0.25	1.37 ± 0.32	0.94 ± 0.21	0.53 ± 0.06	0.49 ± 0.05
Thyroid[b]	0.13 ± 0.03	0.11 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.05 ± 0.01

[a]Data are expressed as percentage of injected dose per gram, means ± SD, n = 5.
[b]Percentage of injected dose per organ.

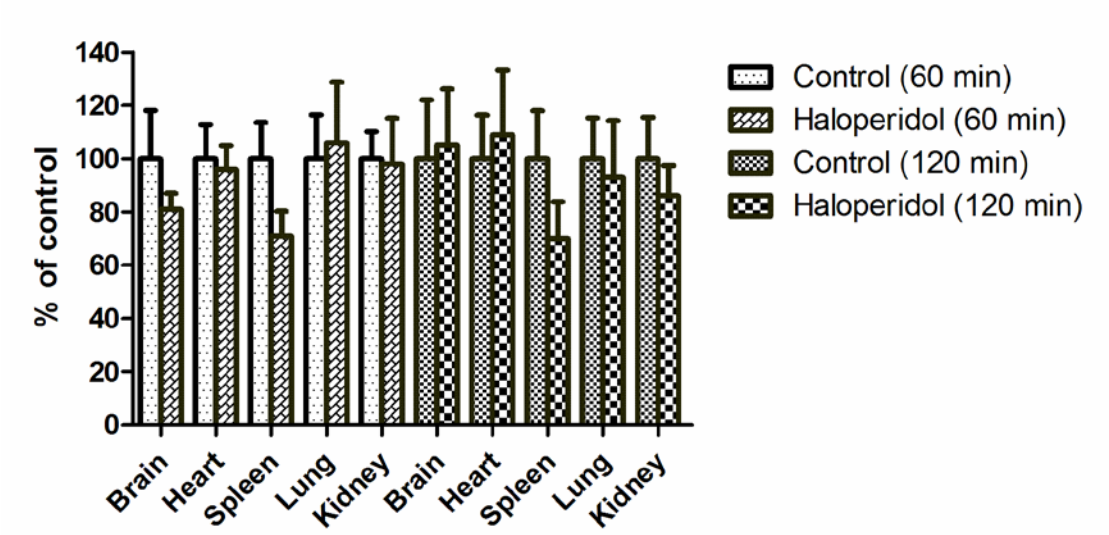


Figure S4. Effects of pretreatment with blocking agents 5 min prior to the injection of [^{99m}Tc]**25b** on the biodistribution in male ICR mice. Student's *t* test (independent, two-tailed) was performed, and $p > 0.05$ (except in the spleen 60 min after intravenous injection).

8. Small animal SPECT/CT imaging of [^{99m}Tc]20b

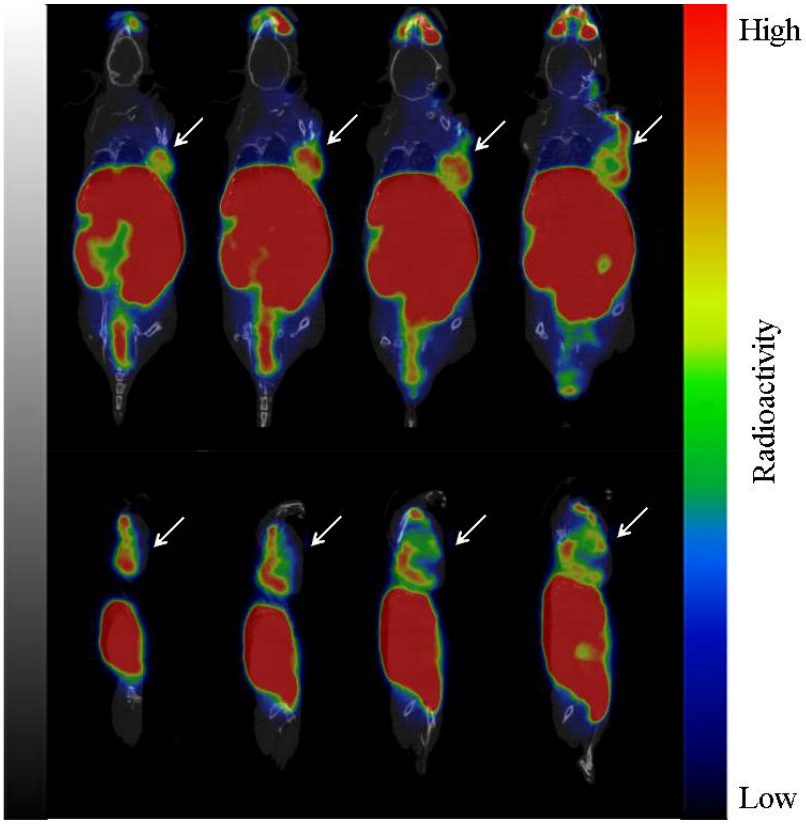


Figure S5. Representative coronal and sagittal plane slices of NanoScan SPECT/CT fusion images of [^{99m}Tc]**20b** (22.2 MBq, 0.15 mL) in male Balb/c nude mouse bearing C6 glioma xenografts after 180 min postinjection. Isoflurane was used for anesthesia.

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