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Synthesis and biodistribution of novel ^{99m}Tc-nitrido methylpiperidine dithioformate derivatives as potential brain imaging agents

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Three dithioformate ligands with methyl substituted on the piperidine rings, potassium 1-(2-methylpiperidine-1-yl)-dithioformate (2-mp), potassium 1-(3-methylpiperidine-1-yl)-dithioformate (3-mp) and potassium 1-(4-methylpiperidine-1-yl)-dithioformate (4-mp) were synthesized. The corresponding ^{99m}Tc-nitrido complexes were prepared in high yield (>95%) through the $[^{99m}TcN]^{2+}_{int}$ intermediate and characterized by thin layer chromatography and high-performance liquid chromatography. All the neutral ^{99m}Tc-nitrido complexes were stable under physiological conditions and lipophilic with log *P* values between 1.40 and 1.58. *In vivo* biodistribution results showed that all the ^{99m}Tc-nitrido complexes displayed high brain uptakes and long retention times. Among them, ^{99m}TcN-4mp, demonstrated the best properties for brain imaging with the brain uptake 3.40±0.24, 3.22±0.31, 2.72±0.28 and 2.22±0.18% ID/g at 5, 30, 60 and 120 min p.i., respectively. Moreover, the influence of stereochemistry of the ^{99m}TcN complexes with methyl substitution on ortho, meta and para positions on piperidine ring on the biodistribution properties were investigated with B3LYP/6-31G*(LANL2DZ for Tc) method using the Gaussian 03 program package.

Keywords: ^{99m}Tc-nitrido; brain imaging agent; methylpiperidine dithioformate derivatives

Introduction

^{99m}Tc is the most widely used radionuclide in diagnostic nuclear medicine due to its almost ideal physical decay properties ($T_{1/2}$ = 6.02 h, E_γ = 140 keV) and the availability through commercial ^{99}Mo/^{99m}Tc generator. At present, ^{99m}Tc-d,l-HMPAO[1–2] and ^{99m}Tc-ECD[3–4] are the major ^{99m}Tc-based imaging agents used in the clinic for the assessment of brain perfusion. Nonetheless, the performance characteristics of them still do not meet the requirements to an ideal brain perfusion imaging agent.[5–7] Search for new ^{99m}Tc-based brain perfusion imaging agents with optimal characteristics remains a subject of interest in the radiopharmaceutical field.

In the past few years, various neutral and lipophilic complexes containing $[^{99m}TcN]^{2+}$core were proposed to design new ^{99m}Tc-based brain imaging agents.[8–11] Compared with the conventional $[^{99m}TcO]^{3+}$ core, the $[^{99m}TcN]^{2+}$core is more stable toward re-oxidation and reduction.[12] In our laboratory, the synthesis and biodistribution of $[^{99m}TcN(RR'NCS_2)_2]$ complexes were investigated.[13–19] It was found that the brain uptakes and brain-to-blood ratios of these complexes were strongly affected by the substitution R and R′ bound to the–NCS_2 moiety. Recently, we reported the biodistribution of two ^{99m}Tc-nitrido complexes containing an *N*-heterocyclic ring in the framework, ^{99m}TcN(PPEDTC)$_2$ (PPEDTC: *N*-2-(1-piperidyl)ethyl dithiocarbamate) and ^{99m}TcN(PREDTC)$_2$ (PREDTC: *N*-2-(1-pyrrolidino)ethyl dithiocarbamate).[20] It was found that the complex ^{99m}TcN(PPEDTC)$_2$, containing the piperidine ring, could cross the blood–brain barrier, and had much higher brain uptake than the ^{99m}TcN(PREDTC)$_2$ complex. However, ^{99m}TcN(PPEDTC)$_2$ showed high blood retention, which resulted in poor brain-to-blood ratios. According to these preliminary results, we decided to further investigate the biological behavior of these ^{99m}Tc-nitrido complexes containing the piperidine ring as potential brain perfusion imaging agents. In this study, three novel ^{99m}Tc-nitrido dithioformate complexes with methyl substitution on different positions of the piperidine rings were prepared and their biological evaluations were investigated. Moreover, the effects of stereochemistry of methyl substitution position on piperidine rings were investigated in order to explain the different biological properties of the above ^{99m}Tc-nitrido complexes.

Results and discussion

Radiochemistry

The ^{99m}Tc-nitrido complexes were prepared by a two-step procedure, as reported in Scheme 1. The SDH kit vial, containing the following lyophilized formulation: 0.05 mg $SnCl_2 2H_2O$,

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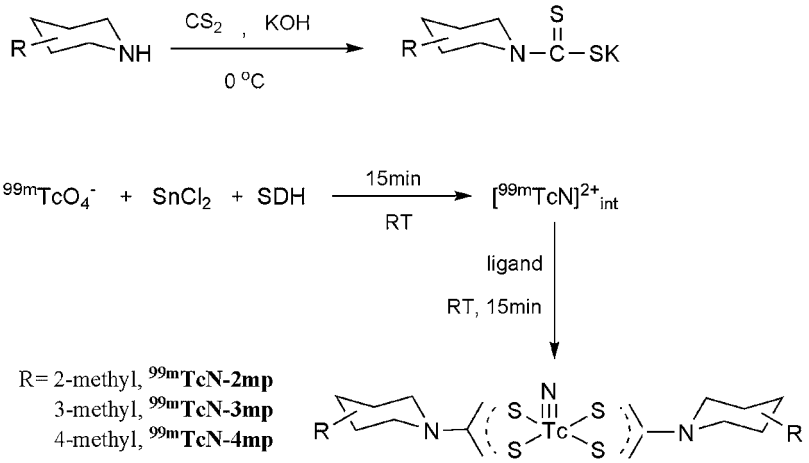

Scheme 1. Synthetic routes of the dithioformate ligands and the corresponding ^{99m}Tc-nitrido complexes.

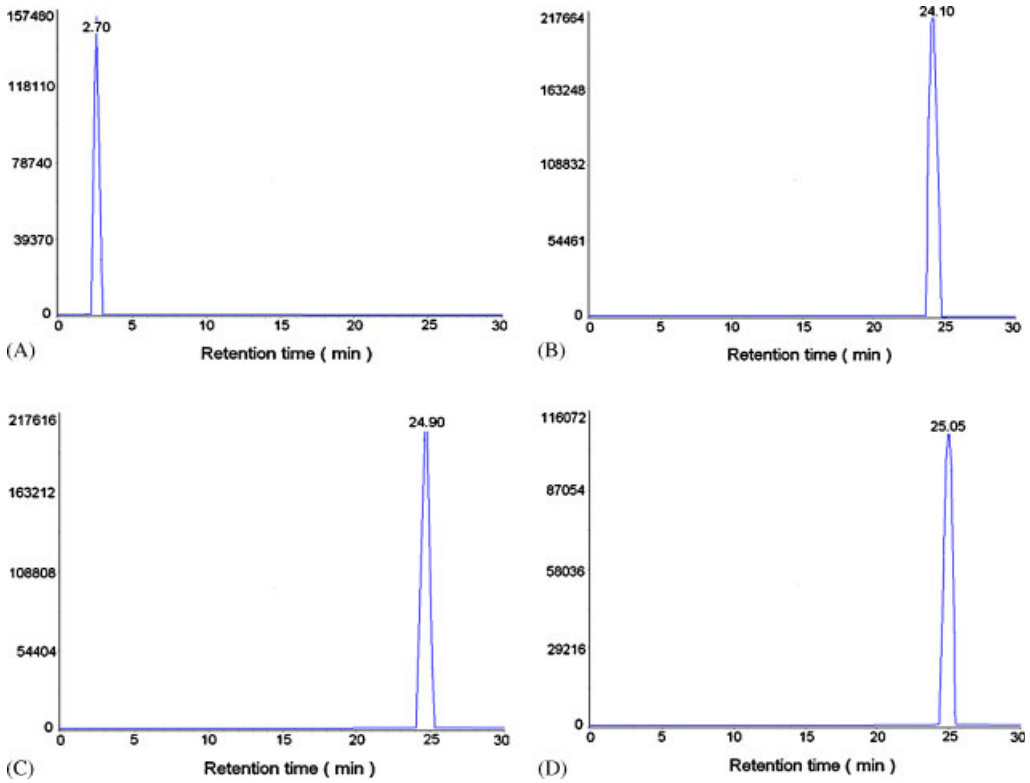

Figure 1. The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate (A) , ^{99m}TcN-2mp (B), ^{99m}TcN-3mp (C) and ^{99m}TcN-4mp (D).

5.0 mg succinic dihydrazide (SDH) and 5.0 mg propylenediamine tetraacetic (PDTA) acid was used for preparation of the $[^{99m}TcN]^{2+}$ intermediate. Quality control of the $[^{99m}TcN]^{2+}$ intermediate was performed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The ^{99m}Tc-nitrido dithioformate complexes were obtained with high yield (>95%) by reaction of the corresponding ligand and $[^{99m}TcN]^{2+}$ intermediate at room temperature for 15 min. The final ^{99m}Tc-nitrido complexes were further purified by HPLC to remove the excess ligands before the injection of ^{99m}Tc-nitrido complexes to mice for *in vivo* studies.

TLC and HPLC analyses were used to evaluate the radiochemical purity (RCP) and the stability of the complexes. TLC was performed on a polyamide film with a mixture of CH_2Cl_2 and CH_3OH ($V/V = 9:1$) as the mobile phase. R_f values for $[^{99m}TcN]^{2+}$ intermediate was 0–0.1 and for the final ^{99m}Tc-nitrido complexes was 0.7–1.0. The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate and final ^{99m}Tc-nitrido complexes are shown in Figure 1(A), (B), (C) and (D), respectively. The retention times of the final complexes ranged from 24.10 to 25.05 min, while that of $[^{99m}TcN]^{2+}$ intermediate was 2.70 min.

The paper electrophoresis results showed these ^{99m}Tc-nitrido complexes were neutral. The log *P* values of **^{99m}TcN-2mp**, **^{99m}TcN-3mp** and **^{99m}TcN-4mp** were 1.49, 1.56 and 1.58 at pH 7.0, respectively, from which one can expect that these complexes may cross the blood–brain barrier (BBB).

The *in vitro* stability of these complexes was evaluated by measuring the RCP at different time points. The results showed that all the complexes were stable over 6 h in the medium of preparation at room temperature. After 4 h of incubation with mouse plasma at 37°C, the RCP of each complex was still over 90%, which suggested that these ^{99m}Tc-nitrido complexes possessed high stability *in vitro*.

Biodistribution studies

Biodistribution studies of these ^{99m}Tc-nitrido complexes were carried out in normal Kunming mice. All the radiolabeled complexes were purified by HPLC before injection to remove excess of cold ligands. The biodistribution data are shown in Table 1. All the complexes displayed high initial brain uptake at 5 min p.i., with the activity in the range of 2.57 ± 0.17–3.55 ± 0.11% ID/g. Significant accumulation of radioactivity was observed in the livers, suggesting that the radiotracers were excreted mainly via the hepatobiliary system due to the lipophilic character of these complexes. These complexes also exhibited high initial myocardial uptake (15.10–11.19% ID/g at 5 min p.i.), but the washout from the hearts was rapid (6.68–6.10% ID/g at 30 min p.i.).

The brain uptake at 5 min p.i. was 2.57 ± 0.17% ID/g for **^{99m}TcN-2mp**, 3.55 ± 0.11% ID/g for **^{99m}TcN-3mp** and 3.40 ± 0.24% ID/g for **^{99m}TcN-4mp**. It correlated well with the order of lipophilicity (log *P*): **^{99m}TcN-2mp** < **^{99m}TcN-3mp** ≈ **^{99m}TcN-4mp**. The 2-methyl substituted derivative, **^{99m}TcN-2mp**, also displayed the highest blood uptake, very slow blood clearance and low brain-to-blood ratio (*B*/Bl < 1) in the series. The 4-methyl substituted derivative, **^{99m}TcN-4mp**, exhibited a greater initial brain uptake and the longest brain retention (3.40 ± 0.24% ID/g at 5 min p.i. and 2.72 ± 0.28 % ID/g at 60 min p.i.). The brain-to-blood ratios rose with time and were 1.11 at 5 min p.i. and 2.42 at 60 min p.i.

Table 2 shows, the comparison of the biodistributions of **^{99m}TcN-4mp**, ^{99m}TcN(PPEDTC)$_2$[20] and ^{99m}Tc-d,l-HMPAO[21] in Kunming mouse. Compared with the previously reported complex ^{99m}TcN(PPEDTC)$_2$, the complex **^{99m}TcN-4mp** showed higher brain uptake and brain-to-blood ratios. Although the complex **^{99m}TcN-4mp** showed a slightly lower brain uptake and retention than ^{99m}Tc-d,l-HMPAO, the low blood uptake and fast blood clearance of **^{99m}TcN-4mp** resulted in more favorable brain-to-blood ratios. These results indicate that **^{99m}TcN-4mp** can be characterized as a candidate for further evaluation as a potential brain imaging agent.

In our previous study, it was found that the *in vivo* biodistrbution patterns of these ^{99m}Tc-nitrido complexes [^{99m}TcN(RR′NCS$_2$)$_2$] are determined by several factors, including the molecular weight, lipophilicity and the nature of the lateral group R and R′ bound to the–NCS$_2$ moiety.[13–20] As this study,

Table 1. Biodistribution of the ^{99m}Tc-nitrido complexes in mice ($n = 3$)

	% Injected dose/g ± SD			
Organ	5 min	30 min	60 min	120 min
^{99m}TcN-2mp				
Brain	2.57 ± 0.17	2.46 ± 0.23	1.61 ± 0.21	0.93 ± 0.04
Heart	12.76 ± 0.64	6.10 ± 1.01	5.17 ± 0.86	3.53 ± 0.26
Liver	35.36 ± 5.31	39.27 ± 2.72	32.93 ± 2.73	30.76 ± 3.07
Lungs	16.07 ± 1.18	11.50 ± 2.92	10.47 ± 1.59	6.36 ± 1.15
Kidney	20.45 ± 2.75	15.85 ± 0.35	12.63 ± 0.43	9.24 ± 0.37
Stomach	1.54 ± 0.13	2.03 ± 0.17	2.58 ± 0.97	2.11 ± 0.23
Blood	9.57 ± 0.56	6.13 ± 5.46	5.46 ± 0.41	3.19 ± 0.33
Brain/blood	0.27	0.40	0.29	0.29
^{99m}TcN-3mp				
Brain	3.55 ± 0.11	3.26 ± 0.30	1.88 ± 0.14	0.98 ± 0.04
Heart	11.19 ± 1.79	6.16 ± 1.26	3.66 ± 0.63	2.46 ± 0.39
Liver	37.78 ± 2.38	41.99 ± 1.57	38.56 ± 5.65	36.06 ± 1.50
Lungs	17.45 ± 1.57	8.98 ± 1.89	6.89 ± 1.17	5.09 ± 0.38
Kidney	18.19 ± 1.50	8.83 ± 1.07	6.35 ± 0.59	5.60 ± 0.88
Stomach	2.31 ± 0.68	1.77 ± 0.33	1.53 ± 0.26	2.22 ± 0.99
Blood	3.24 ± 0.31	2.24 ± 0.13	1.58 ± 0.27	1.37 ± 0.33
Brain/blood	1.09	1.46	1.19	0.72
^{99m}TcN-4mp				
Brain	3.40 ± 0.24	3.22 ± 0.31	2.72 ± 0.28	2.22 ± 0.18
Heart	15.10 ± 0.70	6.68 ± 1.18	6.01 ± 0.82	4.55 ± 0.67
Liver	46.09 ± 2.09	45.52 ± 4.57	35.75 ± 1.46	32.65 ± 5.98
Lungs	15.29 ± 1.43	10.53 ± 1.67	8.20 ± 0.84	4.29 ± 0.44
Kidney	13.87 ± 1.43	8.86 ± 1.44	6.80 ± 1.82	5.70 ± 0.13
Stomach	1.91 ± 0.51	1.96 ± 0.25	2.32 ± 0.30	2.79 ± 0.72
Blood	3.05 ± 0.19	2.53 ± 0.19	1.12 ± 0.11	0.87 ± 0.08
Brain/blood	1.11	1.27	2.42	2.55

Table 2. Biodistributions of ^{99m}TcN-4mp, ^{99m}TcN(PPEDTC)$_2$ and ^{99m}Tc-d,l-HMPAO

	^{99m}TcN-4mp		^{99m}TcN(PPEDTC)$_2$		^{99m}Tc-d,l-HMPAO	
t/min	5	60	5	60	5	60
Brain uptake (% ID/g)	3.40 ± 0.24	2.72 ± 0.28	1.89 ± 0.07	1.02 ± 0.14	4.00 ± 1.46	4.13 ± 1.13
Blood uptake (% ID/g)	3.05 ± 0.19	1.12 ± 0.11	6.53 ± 0.67	5.05 ± 1.24	6.43 ± 1.17	3.23 ± 1.12
Brain/blood	1.11	2.42	0.29	0.20	0.62	1.28

the three complexes have the same molecular weight, and no significant differences in HPLC retention times and log *P* values were observed. The differences in brain uptake and retention must rely on their different stereochemistry. In order to explain the influence of stereochemistry of these ^{99m}Tc-nitrido complexes on the biodistribution properties, the stereochemistry of these complexes were investigated with B3LYP/6-31G* (LANL2DZ for Tc) method using the Gaussian 03 program package. The optimized geometries of **^{99m}TcN-2mp**, **^{99m}TcN-3mp** and **^{99m}TcN-4mp** are showed in Figure 2.

It was reported that the electrostatic desolvation free energy of ^{99m}Tc complexes (ΔE) was the important factor to affect the brain uptake.[22] There was correlation between brain uptake and ΔE. The difference of the brain uptakes may be attributed to the different ΔE generated by the stereochemistry. Therefore, the electrostatic desolvation free energies of ^{99m}Tc-nitrido complexes were calculated with B3LYP/6-31G*(LANL2DZ for Tc) method in water. The calculation results showed that the ΔE (electrostatic dehydration free energies) were 19.9, 19.4 and 19.1 kcal/mol for **^{99m}TcN-2mp**, **^{99m}TcN-4mp** and **^{99m}TcN-3mp**, respectively, from which one can conclude that the complex **^{99m}TcN-3mp** will show the highest brain uptakes, while **^{99m}TcN-2mp** will show the lowest brain uptake. The prediction is in good agreement with the experimental data.

The relative energies of these ^{99m}Tc-nitrido complexes demonstrated that the stability of ^{99m}Tc-nitrido complexes with *m*- and *p*-methyl were comparable, while that with *o*-methyl was much more unstable. It was probably due to the instability of **^{99m}TcN-2mp** resulting in some unfavorable properties, such as high blood uptake and low brain-to-blood ratios. The investigation of the structure–activity relationship of the ^{99m}Tc-nitrido complexes is in progress.

Experimental

General

2-Methylpiperidine (99%), 3-methylpiperidine (98%) and 4-methylpiperidine (98%) were purchased from Alfa Aesar China (Tianjin) Co., Ltd. All other chemicals were of reagent grade and used without further purification. Technetium-99m as sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from commercial ^{99}Mo/^{99m}Tc generator (China Institute of Atom Energy) just before use. The SDH kit was provided by courtesy of Beijing Shihong Pharmaceutical Center. Paper electrophoresis experiments were performed on Whatman 3MM chromatography paper (Whatman, England). Polyamide-6 film (SIJIA Co., China) was used for TLC. Reversed-phase high-pressure liquid chromatography experiments were performed on a SHIMADZU system with SCL-10AVP HPLC pump system (SHIMADZU Corporation, Japan) and liquid scintillation analyzer (Packard BioScience Co., USA), using a C-18 column (4.6 × 250 mm, 5 μm particle size, Alltech Associates, Inc., USA) and methanol:water mixture as the mobile phase at a flow rate of 1 mL/min. IR spectra were obtained with an AVATAR 360 FT-IR spectrometer (Nicolet Co., USA), using KBr pellets. NMR spectra were recorded on a Bruker Avance-500 (500 MHz) spectrometers. ESI-MS spectra were obtained with a LCMS2010 ESI mass spectrometer (SHIMADZU Co., Japan).

Synthesis

*Potassium 1-(2-methylpiperidine-1-yl)-dithioformate (**2-mp**)*

2-Methylpiperidine (0.99 g, 10 mmol) was dissolved in ethanol (10 mL), and 2.0 mL of a 5 mol/L KOH solution (10 mmol) was added. While the solution was stirred in an ice bath at 0°C, 0.7 mL of carbon disulfide was added dropwise. The solution was stirred at room temperature for 3 h. The solvent was removed under vacuum. The solid residue was recrystallized from water/acetone to give potassium 1-(2-methylpiperidine-1-yl)-dithioformate (**2-mp**) as yellow solid (1.18 g, 55%). IR/cm^{-1}: 2931 (C–H), 1466, 1398 (C–N), 951 (C = S). ^{1}H-NMR (500 MHz, D_2O) δ: 1.09–1.10 (m, 3H $CHCH_3$), 1.30–1.64 (m, 6H, $CH(CH_2)_3$), 2.98–3.04 (m, 1H, $CHCH_3$), 5.32–5.81 (m, 2H, NCH_2). ESI-MS: calculated for *m/z* $C_7H_{12}NS_2$ (M^-): 174, found 174.

*Potassium 1-(3-methylpiperidine-1-yl)-dithioformate (**3-mp**)*

It was prepared from 3-methylpiperidine by the same procedure as a yellow solid in 47% yield. IR/cm^{-1}: 2927 (C–H), 1459, 1403 (C–N), 951 (C = S).^{1}H-NMR (500 MHz, D_2O) δ: 1.07–1.15 (m, 3H $CHCH_3$), 1.27–1.78 (m, 6H, $CH(CH_2)_2$ and $N(CHH)_2$), 2.78–3.24 and 5.31–5.80(m, 3H, $CHCH_3$ and $N(CHH)_2$). ESI-MS: calculated for *m/z* $C_7H_{12}NS_2$ (M^-): 174, found 174.

*Potassium 1-(4-methylpiperidine-1-yl)-dithioformate (**4-mp**)*

It was prepared from 4-methylpiperidine as a yellow solid in 54% yield. IR/cm^{-1}: 2952 (C–H), 1458, 1409 (C–N), 954 (C = S).^{1}H-NMR (500 MHz, D_2O) δ: 0.81–0.85 (m, 3H $CHCH_3$), 1.03–1.23 and 1.61–1.79(m, 5H, $CH(CH_2)_2$), 3.02–3.28 and 4.81–5.26(m, 4H, $N(CH_2)_2$). ESI-MS: calculated for *m/z* $C_7H_{12}NS_2$ (M^-): 174, found 174.

Radiochemistry

Preparation of ^{99m}TcN-dithioformate complexes

1 mL of saline containing $^{99m}TcO_4^-$ (370 MBq) was added into an SDH kit containing 0.05 mg of stannous chloride dihydrate, 5.0 mg of SDH, 5.0 mg of PDTA acid, and the mixture was kept at room temperature for 15 min to obtain the $[^{99m}TcN]^{2+}$ intermediate. The $[^{99m}TcN]^{2+}$intermediate was added to a vial containing 1 mg of the synthesized dithioformate ligand. The

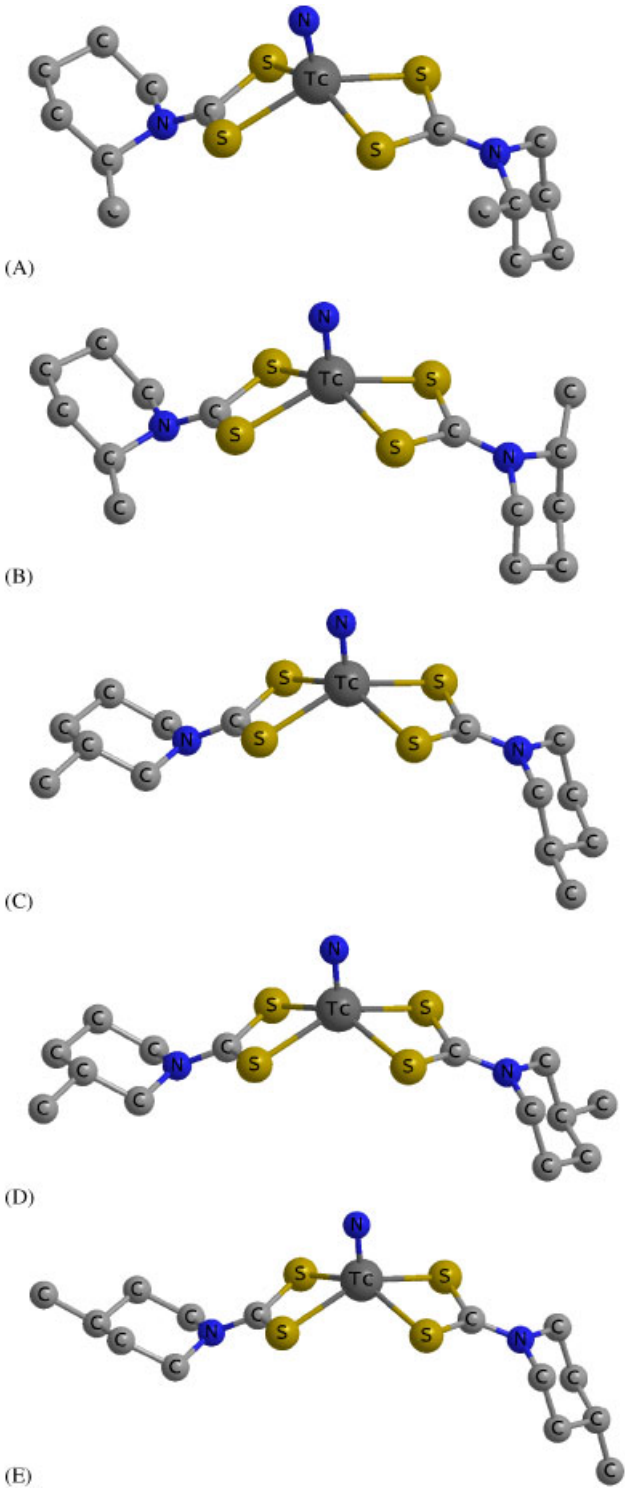

Figure 2. The optimized geometries of ^{99m}TcN-2mp (A: the methyl substitution on the same side; B: the methyl substitution on the opposite side), ^{99m}TcN-3mp (C: the methyl substitution on the same side; D: the methyl substitution on the opposite side) and ^{99m}TcN-4mp (E). And all the hydrogen atoms are hided.

mixture was kept at room temperature for 15 min. The crude product was purified by HPLC using a C-18 column with a mobile phase of water (A): methanol (B) mixture (0–10 min, 70% B, 10–20 min, 70–90% B, 20–30 min, 90–100% B) at a flow rate of 1 mL/min. The collected HPLC fraction of the final complex was evaporated and diluted with ethanol (0.2 mL) and saline (5 mL).

The $[^{99m}TcN]^{2+}$ intermediate and the final complexes were identified by TLC and HPLC.

In vitro *stability*

The *in vitro* stability of these ^{99m}Tc-nitrido complexes was evaluated by monitoring the RCP at different time points. Each ^{99m}Tc-nitrido complex was kept at room temperature for 6 h. The RCP was determined by TLC and HPLC chromatography at 30 min, 1, 2, 4 and 6 h.

To 900 μL of fresh mouse plasma, 100 μL of the ^{99m}Tc complex solution was added and incubated at 37°C. At 30 min, 2 and 4 h, 100 μL aliquots were withdrawn and treated with 200 μL ethanol to precipitate the proteins. Samples were then cooled at 4°C and centrifuged at 3000 rpm. The supernatant was analyzed by TLC and HPLC.

Paper electrophoresis

2 μL of the respective test solution was spotted at the center of the Whatman 3MM chromatography paper strips (10 cm × 1 cm), which were pre-treated with phosphate buffer (0.05 mol/L, pH = 7.4). The analyses were carried out using phosphate buffer (0.05 mol/L, pH = 7.4) at 150 V for 2 h. The developed paper strips were left to dry, and the distribution of radioactivity on the strip was determined.

Octanol/water partition coefficient

The octanol/water partition coefficient was measured by mixing 100 μL of the respective test solution with 1.9 mL of phosphate buffer (0.05 mol/L, pH = 7.0) and 2 mL of octanol in a centrifuge tube. The tube was vortexed for 2 min and then centrifuged at 4000 rpm for 5 min. The counts in 100 μL aliquots of both organic and inorganic layers were determined by use of a NaI well-type γ-counter. The partition coefficient ($\log P$) was calculated using the following equation: P = (activity in octanol − background activity)/(activity in aqueous layer − background activity). The measurement was repeated three times.

Biodistribution studies

The *in vivo* biodistribution studies were performed in normal Kunming mice (18–20 g weight). For the biodistribution studies, solutions of HPLC purified ^{99m}Tc-nitrido complexes were diluted to a radioactivity concentration of approximately 7.4 MBq/mL. A volume of 0.1 mL of the diluted tracer solution containing about 740 kBq of ^{99m}Tc complex was injected into the mice via the tail vein. Then the mice (n = 3) were sacrificed by cervical dislocation at 5, 30, 60 and 120 min post-injection. The organs or tissues of interest were removed, weighted and measured in a well-type NaI (Tl) γ-counter. The percentage of injected dose per gram (% ID/g) for each sample was calculated by comparing its activity with appropriate standard of injected dose (ID), the values were expressed as mean ± SD.

All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experiment.

Calculation

Density functional theory, employing B3LYP/6-31G* (LANL2DZ for Tc) method, has been used to investigate the

stereochemistry of the ^{99m}Tc-nitrido complexes using the Gaussian 03 program package. The geometries of the ^{99m}Tc-nitrido complexes with methyl substitution on *o*-, *m*- and *p*-positions on piperidine ring were fully optimized to the convergences criteria of 3.0×10^{-4}, 4.5×10^{-4}, 1.2×10^{-3}, 1.8×10^{-3} as acceptance thresholds for the gradients of the root mean square (RMS) force, maximum force, RMS displacement and maximum displacement vectors, respectively. The obtained geometries have been characterized with the vibrational analysis at the same calculation level.

The solvent effects of the ^{99m}Tc-nitrido complexes have been performed with the PCM model[23–29] with water ($\varepsilon = 78.39$) as solvent at a temperature of 298 K.

Conclusion

In conclusion, the ^{99m}Tc-nitrido dithioformate complexes with methyl substitution on the piperidine rings can be easily prepared in high yield (>95%) by reacting the corresponding ligand with the $[^{99m}TcN]^{2+}_{int}$ intermediate. All the neutral ^{99m}Tc-nitrido complexes were stable in *vitro*. The log *P* values of the complexes were within the range of 1.0–2.5, and their molecular weight did not exceed 600 Da, thereby satisfying the basic pre-requisites for BBB penetration. *In vivo* biodistribution showed that all the ^{99m}Tc-nitrido complexes had significant initial brain uptake as expected. Despite no great difference in HPLC retention times and log *P* values was observed when methyl position changed, it was found that brain uptake and blood clearance of these complexes were still influenced by their different stereochemistry. The 4-methyl substituted derivative, **^{99m}TcN-4mp**, showed the most promising biological properties as a potential brain imaging agent in the series.

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