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ARTICLE TYPE

Synthesis and biodistribution of novel ^{99m}Tc labeled 4-nitroimidazole dithiocarbamate complexes as potential agents to target tumor hypoxia

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The sodium 3-(4-nitro-1H-imidazolyl)propyl dithiocarbamate (N4IPDTC) was synthesized and radiolabelled with $[^{99m}TcO]^{3+}$, $[^{99m}Tc{\equiv}N]^{2+}$ and $[^{99m}Tc(CO)_3]^{+}$cores to produce ^{99m}TcO-N4IPDTC ,^{99m}TcN-N4IPDTC and $^{99m}Tc(CO)_3$-N4IPDTC, respectively. All of the three complexes were prepared with high radiochemical purity and had good in vitro stability over a period of 6 h. The partition coefficient results showed that ^{99m}TcO-N4IPDTC and ^{99m}TcN-N4IPDTC were lipophilic, while $^{99m}Tc(CO)_3$-N4IPDTC was hydrophilic. The tumor cell experiments and the biodistribution in mice bearing S 180 tumor showed that all of the complexes exhibited good hypoxic selectivity and accumulation in the tumor. Among them, ^{99m}TcO-N4IPDTC had advantages of higher tumor uptake, tumor/blood and tumor/muscle ratios. Planar scintigraphic imaging studies showed there was a visible accumulation in tumor sites, suggesting its potential usefulness as a tumor hypoxia imaging agent.

Introduction

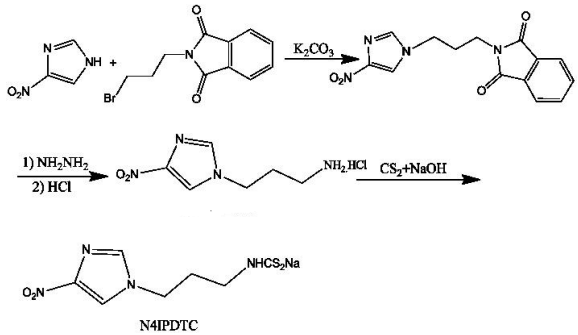
Tumor hypoxia is an important factor in resistance to radiotherapy and chemotherapy. [1] So, the early diagnosis of tumor hypoxia will play a critical role in the tumor treatment planning. Due to the noninvasiveness of single photon emission computed tomography (SPECT) and positron emission tomography (PET) to image tumor hypoxia, many researches are focused on developing targeted radiopharmaceuticals for tumor hypoxia imaging. In the development of hypoxia imaging agents, nitroimidazole derivatives are enzymatically reduced and accumulated in hypoxic regions, therefore labeled nitroimidazole analogues have received great attention. Currently, PET tracer [^{18}F]Fluoromisonidazole ([^{18}F]FMISO) is one of the most clinically studied hypoxia markers.[2-3] However, the short half life, high cost and limited availability of the [^{18}F] isotope are realistic limitations. By comparison, technetium-99m has ideal physical and chemical characteristics, inexpensive cost and in-house availability. Therefore, there has been considerable clinical interest in developing ^{99m}Tc-labeled nitroimidazole derivatives for targeting tumor hypoxia. Recently, several ^{99m}Tc labeled nitroimidazole analogues (including 2-nitroimidazole, 4-nitroimidazole and 5-nitroimidazole) have been reported.[4-13] However, there exist some limitations for these complexes, such as the relative lower tumor uptakes or slow clearance from blood. Thus, ongoing research is in progress to discover an ideal hypoxia imaging agent.

As well-known, the 2-nitroimidazole derivatives have been the most widely studied for their potential for hypoxia imaging, just because 2-nitroimidazole has the more positive single electron reduction potential (SERP) value, which can be efficiently reduced and retained in hypoxia cells. However, SERP is not the only factor which affects the uptake and retention of the imaging agent inside the hypoxia cell. The lipophilicity and the structure of the complex may also play an important role in deciding the overall behavior of the hypoxia imaging agent. Although the SERP of 4-nitroimidazole is less positive compared to 2-nitroimidazole, 4-nitroimidazole is much cheaper than 2-nitroimidazole. Moreover, several 4-nitroimidazole derivates as potential clinical hypoxia imaging agents have been successfully synthesized.[14-17] This fact shows the feasibility of 4-nitroimidazole derivates as potential hypoxia imaging agents. It is known that the $[^{99m}TcO]^{3+}$, $[^{99m}Tc{\equiv}N]^{2+}$ and $[^{99m}Tc(CO)_3]^{+}$cores may exhibit high stabilities and the presence of them in the molecular structures of radiopharmaceuticals may change their biological behaviors. Moreover, $[^{99m}TcO]^{3+}$, $[^{99m}Tc{\equiv}N]^{2+}$ and $[^{99m}Tc(CO)_3]^{+}$cores have been discovered to complex well with ligands containing sulfur atoms, as in dithiocarbamates. [18-20] The above background encouraged us to prepare several ^{99m}Tc labeled 4-nitroimidazole dithiocarbamate complexes by using different ^{99m}Tc cores to find good tumor hypoxia imaging agents. In this study, the synthesis and biological evaluation of novel ^{99m}Tc labeled 4-nitroimidazole dithiocarbamate complexes for tumor hypoxia imaging are reported.

Results and discussion

Chemistry

The 3-(4-nitro-*1H*-imidazolyl)propyl dithiocarbamate (N4IPDTC) was prepared by reacting 3-(4-nitro-*1H*-imidazolyl) propylamine hydrochloride with carbon disulfide in NaOH solutions. The reaction is schematically shown in Scheme 1.

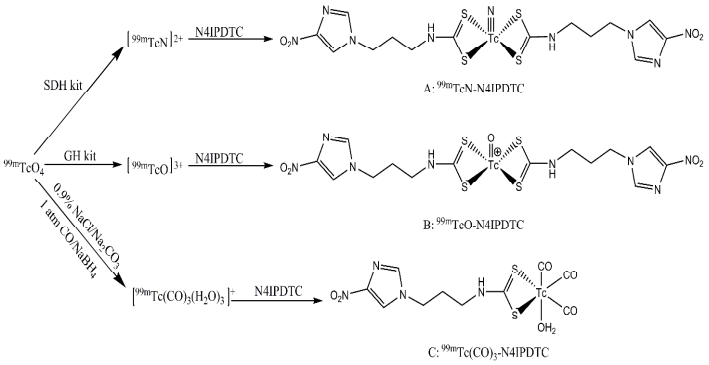


Scheme 1: Synthesis of N4IPDTC

Radiolabeling and quality control

The preparations of ^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC can be carried out by using the following procedures in Scheme 2.

For labeling, ^{99m}TcN-N4IPDTC was prepared by adding N4IPDTC to the $[^{99m}TcN]^{2+}$ intermediate, which was produced by the reaction of $[^{99m}TcO_4]^-$ with succinic dihydrazide (SDH) in the presence of stannous chloride as reducing agent. The $[^{99m}TcN]^{2+}$ core is a proper substrate for the substitution reaction with N4IPDTC to prepare ^{99m}TcN-N4IPDTC with high yield.



Scheme 2: Preparation procedures of ^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC

^{99m}TcO-N4IPDTC was prepared by ligand-exchange reaction with ^{99m}Tc-glucoheptonate (GH). ^{99m}Tc-GH is a suitable substrate for the substitution reaction with N4IPDTC to give the final complex ^{99m}TcO-N4IPDTC. As for preparing ^{99m}Tc(CO)$_3$-N4IPDTC, the H_2O molecule in the fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor is readily substituted by sulfur atoms in the N4IPDTC ligand. The N4IPDTC ligand displaces the two H_2O molecules of the fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor.

The radiochemical purities of the complexes were assessed by HPLC. The retention time of $[^{99m}TcN]^{2+}$, $[^{99m}TcO]^{3+}$ and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was 4.73 min, 4.13 min, and 15.60 min, respectively, while that of ^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC was 18.45 min, 11.74 min and 7.38 min (Figure 1). The mean radiochemical purities of the products were all over 95% immediately after preparation.

In vitro stability study

The complexes were stable over 6 h in the reaction mixture at room temperature. On the other hand, no decomposition of the above complexes occurred over 6 h at 37 °C in mouse serum, suggesting they had good in vitro stability.

Determination of the partition coefficient

The partition coefficient (log P) values of ^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC were 0.60±0.01, 0.27±0.01 and -0.71±0.01, suggesting ^{99m}Tc(CO)$_3$-N4IPDTC was hydrophilic, while ^{99m}TcN-N4IPDTC and ^{99m}TcO-N4IPDTC were lipophilic. Further more, ^{99m}TcN-N4IPDTC was more lipophilic than ^{99m}TcO-N4IPDTC.

In vitro cell uptake

The effect of hypoxic and aerobic conditions on the accumulation of the three ^{99m}Tc complexes in S180 cells as a function of time is illustrated in Figure 2. From Figure 2, it is shown that the uptake of the three ^{99m}Tc complexes in hypoxic cells is constantly more than that in aerobic cells, suggesting all of them exhibit preferential uptake in hypoxic conditions.

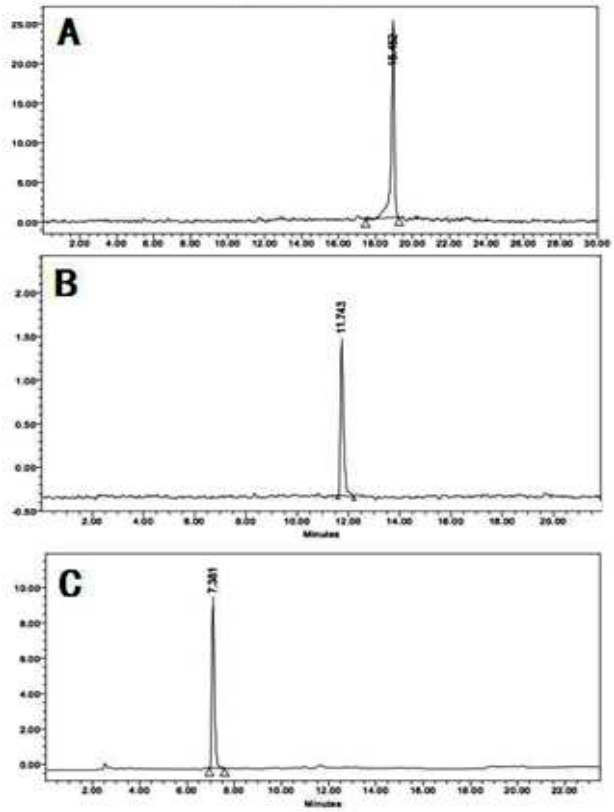


Figure 1 HPLC pattern of ^{99m}TcN-N4IPDTC(A), ^{99m}TcO-N4IPDTC(B) and ^{99m}Tc(CO)$_3$-N4IPDTC(C)

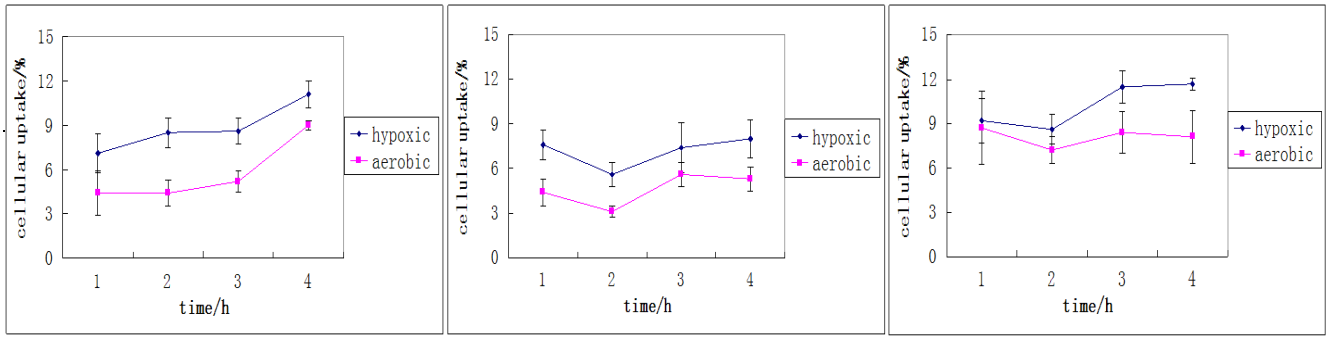


Figure 2 Cellular uptake of ^{99m}TcN-N4IPDTC(A), ^{99m}TcO-N4IPDTC(B) and ^{99m}Tc(CO)$_3$-N4IPDTC(C)

Biodistribution studies

The results of biodistribution of the complexes are shown in Table 1.

As described in Table 1, all of the three complexes have a certain uptake and good retention in tumor. The muscle uptakes are low so the T/N ratios are high. Activity accumulation in the kidney, liver, feces and urine shows that the major route of excretion is renal and hepatobiliary. In the limits of our study, the results demonstrate that different ^{99m}Tc core for preparing the complexes may exhibit significant impact on the tumor uptake, T/B and T/N ratios. By comparison, interestingly, ^{99m}TcN-N4IPDTC (log P: 0.60) and ^{99m}TcO-N4IPDTC (log P: 0.27) show much higher tumor uptakes, along with higher lipophilicity than ^{99m}Tc(CO)$_3$-N4IPDTC(log P: -0.71). Among the three complexes, ^{99m}TcN-N4IPDTC is more lipophilic than the others, thus possibly making the former much higher liver uptakes than the latter. Among them, the tumor uptake, T/N ratio and T/B ratio of ^{99m}TcO-N4IPDTC is the highest, showing the most promising properties for further studies in other animal models.

Although many ^{99m}Tc labelled 4-nitroimidazole derivates have been evaluated as hypoxia imaging agents, their direct comparison is not easy due to both the different kind of the tumors and the heterogeneity in animal models. Because the biodistribution studies of ^{99m}TcO-N4IPDTC and ^{99m}Tc-N4IPA (N4IPA:1-(4-nitroimidazole-1-yl)-propanhydroxyiminoamide) were performed in the same animals bearing S180 tumor,[14] the direct comparison between them are as follows. The tumor uptake of ^{99m}TcO-N4IPDTC is much higher than that of ^{99m}Tc-N4IPA. The tumor uptake of ^{99m}TcO-N4IPDTC (2.63±0.35 %ID/g) is nearly eight times better than that of ^{99m}Tc-N4IPA (0.34±0.06 %ID/g) at 4 h post-injection. With regard to the T/B ratio, there is no great difference between the two complexes. As for the T/N ratio, ^{99m}Tc-N4IPA is superior to ^{99m}TcO-N4IPDTC. The T/N ratio of ^{99m}Tc-N4IPA (8.60) is more than two times better than that of ^{99m}TcO-N4IPDTC (3.87) at 4 h post-injection. To be a good tumor hypoxia imaging, its detectability of tumor depends on both the absolute tumor uptake and tumor to background ratio. From the above point of views, ^{99m}TcO-N4IPDTC sounds more potential usefulness as a tumor hypoxia imaging agent and needs further investigation.

Table 1 Biodistribution of ^{99m}TcN-N4IPDTC(A), ^{99m}TcO-N4IPDTC(B) and ^{99m}Tc(CO)$_3$-N4IPDTC(C) in mice bearing S180 tumor(%ID/g)[a]

Complex	A		B		C	
Time	2 h	4 h	2 h	4 h	2 h	4 h
Heart	1.60 ± 0.24	1.27 ± 0.48	0.93± 0.03	0.88 ± 0.22	0.30 ± 0.03	0.21 ± 0.03
Liver	35.7 ± 2.70	34.2 ± 5.00	6.81±1.28	6.21±0.84	2.20 ± 0.21	1.06 ± 0.20
Lung	5.03 ± 1.38	5.71 ± 0.94	1.77 ± 0.17	1.41 ±0.18	0.64 ± 0.06	0.45 ± 0.08
Kidney	13.1 ± 1.50	12.3 ± 2.40	16.7 ± 3.11	13.2 ± 1.18	3.28 ± 0.42	2.31 ± 0.37
Spleen	5.99 ±0.82	6.48± 0.82	1.17 ± 0.23	1.07 ± 0.13	0.29 ± 0.05	0.25 ± 0.05
Muscle	0.74 ± 0.26	0.59 ± 0.09	0.64 ± 0.17	0.68 ± 0.13	0.24 ± 0.06	0.20 ± 0.04
Tumor	1.11 ± 0.24	1.15 ± 0.24	2.84 ± 0.19	2.63 ± 0.35	0.49 ± 0.10	0.36 ± 0.08
Blood	1.20 ± 0.23	0.71 ± 0.21	1.91 ± 0.18	1.59 ± 0.33	0.62 ± 0.03	0.43 ± 0.07
Feces	25.4 ± 6.38	33.2 ± 8.77	17.9 ± 4.23	11.6 ± 3.17	11.4 ± 3.30	14.3 ± 2.61
Urine	32.8 ±12.4	35.1 ± 10.4	62.1 ±17.1	64.6±9.30	22.3 ± 4.67	20.3 ± 5.17
T/N	1.50	1.95	4.44	3.87	2.04	1.80
T/B	0.93	1.62	1.49	1.65	0.79	0.84

[a] All data are the mean percentage (n=5) of the injected dose of the three complexes per gram of tissue, ± the standard deviation of the mean. T/N=tumor-to-muscle, T/B= tumor-to-blood.

SPECT Imaging Study

The SPECT imaging results showed the tumor uptake was clearly observable (Figure 3), however, the high uptake of ^{99m}TcO-N4IPDTC in the liver and kidneys is the drawback of the complex. The imaging findings were similar to the biodistribution results in mice.

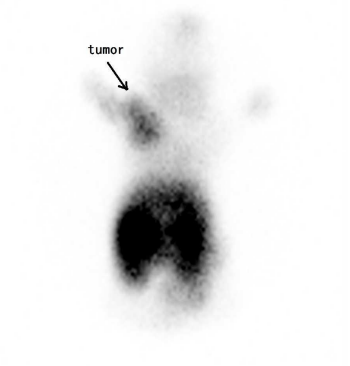


Figure 3 SPECT image of ^{99m}TcO-N4IPDTC in mice bearing S180 tumor

Experimental section

General

4-nitroimidazole was purchased from Alaf Aesa, China. Succinic dihydrazide (SDH) kit, which contains 0.05 mg of stannous chloride dihydrate, 5.0 mg of succinic dihydrazide (SDH) and 5.0 mg of propylenediamine tetraacetic acid (PDTA) and Glucoheptonate (GH) kit containing 0.1 mg of stannous chloride dihydrate, 20.0 mg of GH were obtained from Beijing Shihong Pharmaceutical Center, Beijing Normal University, China. All other chemicals were of reagent grade and were used without further purification. ^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy (CIAE). IR spectrum was obtained with an AVATAR 360 FT-IR spectrometer using KBr pellets. NMR spectrum was recorded on a 400 MHz Bruker Avance spectrophotometer. Elemental analyses were performed on a Vario EL elemental analyzer model. HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5C, 250 4.6 mm), Shimadzu LC-20AT series.

Chemistry

3-(4-nitro-1*H*-imidazolyl) propylamine hydrochloride (compound 1) was prepared as reported previously.[21] The synthetic procedures and the spectral data for N4IPDTC are as follows. 3-(4-nitro-1*H*-imidazolyl) propylamine hydrochloride (0.206 g), carbon disulfide (0.304 g) and NaOH (0.048 g) were dissolved in 25.0 mL water. The mixture was stirred at 3 °C for 2.0 h and continued to react overnight at room temperature. Most of the solvent was removed, and the precipitate was collected by filtration. The crude product was recrystallized from $CH_3OH/CH_3CH_2OCH_2CH_3$ to give yellow crystals (N4IPDTC). Yield 62 %. N4IPDTC was characterized by ^{1}HNMR, ^{13}C-NMR, IR and elemental analysis. ^{1}H NMR δ(D_2O): 8.06 (d, 1H, imi-H), 7.62-7.61 (d, 1H, imi-H), 4.10-4.07 (t, 2H, CH_2), 2.88-2.84 (t, 2H, CH_2), 2.11-2.04 (m, 2H, CH_2), ^{13}C NMR δ(D_2O) : δ211.85 (CS_2), δ161.88 (C), δ149.02 (CH), δ132.84 (CH), δ65.96 (CH_2), δ44.49 (CH_2), δ28.84 (CH_2); IR (KBr)/cm^{-1}: NH:3431.0, NO_2:1535.4, 1379.5, C=S:1046.7; Elemental analysis calculated (%) for $C_7H_9N_4NaO_2S_2$: C, 31.34; N, 20.88; H, 3.38. Found: C, 31.52; N, 20.71; H, 3.35.

Radiolabeling and quality control

The preparations of ^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC were carried out according to our previous reported methods.[18-20] The radiochemical purities of the complexes were assessed by HPLC. The HPLC analysis conditions are as follows. HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5C, 250×4.6 mm), Shimadzu SCL-10A VP series, working at a flow rate of 1.0 mL /min. For ^{99m}TcN-N4IPDTC and ^{99m}TcO-N4IPDTC, water (A) and acetonitrile (B) were used as the mobile phase. For ^{99m}Tc(CO)$_3$-N4IPDTC, water (containing 0.1% TFA) (A) and acetonitrile (containing 0.1% TFA) (B) mixtures were used as the mobile phase. The following gradient elution technique was adopted for the preparation: For ^{99m}TcN-N4IPDTC, 0 min 70 % B, 10 min 70 % B, 15 min 90 % B, 40 min 100 % B; for^{99m}TcO-N4IPDTC, 0 min 50 % B, 20 min 90 % B, 30 min 90 % B, 40 min 100 % B; for ^{99m}Tc(CO)$_3$-N4IPDTC, 0 min 10 % B, 28 min 90 % B, 30 min 100 % B, 40 min 100 % B.

In vitro stability study

^{99m}TcN-N4IPDTC, ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC complexes were incubated in the labeling milieu at room temperature for 6 h, The radiochemical purity was assessed by HPLC. On the other hand, in vitro serum stability studies in mouse serum were also performed using a method reported earlier.[22]

Determination of the partition coefficient (log P)

The partition coefficient (log P) between 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) of the three complexes was measured in order to evaluate their lipophilicity.[18]The measurements were repeated five times and reported as an average of five measurements plus the standard deviation.

In vitro cell uptake

In vitro uptake of the complexes both in hypoxic and aerobic conditions was evaluated by using the previous reported methods.[6] In brief, S180 cells at a concentration of 1.0×10^6 cells/mL were suspended in 20.0 mL DMEM containing 10% (v/v) of fetal bovine serum and incubated at 37.0 °C. The hypoxic and aerobic conditions were conducted following the previous methods. [6] Then, 0.2 mL of the complex (3.7×10^6 Bq/mL) was added to the suspension. 1000 μL aliquots were pipetted at 1.0, 2.0, 3.0 and 4.0 h post-incubation, and were centrifuged at 3000 rpm for 5.0 min. 900 μL supernatant medium was taken for counting (C_{out}) and the left sample containing cells with 100 μL medium was also counted (C_{in}). At each time point, three samples were determined. The cell accumulation, A, was calculated as the following equation: $A = (C_{in} - C_{out}/9) / (C_{in} + C_{out})$

The final results were expressed as mean ± standard deviation.

Biodistribution studies

Animal studies were carried out in compliance with the Regulations on Laboratory Animals of Beijing Municipality and the guidelines of the Ethics Committee of Beijing Normal University. The experiments were approved by the Ethics Committee of Beijing Normal University. 0.1 mL of ^{99m}TcN-N4IPDTC (5.0×10^6 Bq/mL) was injected into the Kunming female mice (18-20 g) bearing S180 tumor via a tail vein. The mice were sacrificed in groups of five at 2 h and 4 h post-injection. The tumor, other organs of interest, blood, urine and feces were collected, weighed and measured for radioactivity. The counting tubes, including a standard equivalent to 1% of the injected dose, were assayed in a well-type NaI(Tl) detector and the results were expressed as the percent uptake of injected dose per gram of tissue (% ID/g). The final results are expressed as mean±standard deviation. The biodistribution studies of ^{99m}TcO-N4IPDTC and ^{99m}Tc(CO)$_3$-N4IPDTC were conducted in the same way.

SPECT imaging studies

0.2 mL of ^{99m}TcO-N4IPDTC (1.85×10^8 Bq/mL) was injected intravenously through trail vein in mice (18~22g) bearing S180 tumor. A dual-head SPECT (Skylight; Philips, Milpitas, CA,

USA), using a low-energy parallel-hole collimator (diameter 3.5mm), was used for SPECT imaging studies. Static images were acquired at 4 h after injection.

Conclusion

In summary, a novel ligand N4IPDTC was successfully synthesized and its ^{99m}Tc-nitrido core, ^{99m}Tc-oxo core and $[^{99m}Tc(CO)_3]^+$ core complexes were prepared with high yields through ligand-exchange reactions. The preliminary studies showed all of them had a certain hypoxic selectivity and tumor uptake. Especially for ^{99m}TcO-N4IPDTC, it is prepared from a kit without the need for purification and shows high tumor uptake, tumor/blood and tumor/muscle ratios, suggesting it would be a promising hypoxia imaging agent.

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Notes and references

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