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Synthesis and biological evaluation of novel ^{99m}Tc labeled ornidazole xanthate complexes as potential hypoxia imaging agents

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Abstract In this study, ornidazole xanthate (ONXT) ligand 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. The tumor cell experiments and the biodistribution in mice bearing S180 tumor showed that all of the complexes had a certain hypoxic selectivity and tumor uptake. Among them, ^{99m}TcO-ONXT exhibited the highest tumor uptake and tumor/muscle ratio. Planar scintigraphic imaging studies showed the tumor detection was observable, suggesting it would be a potential radiotracer to target tumor hypoxia.

Keywords Ornidazole · Xanthate · ^{99m}Tc · Hypoxia · Tumor imaging

Introduction

In the development of hypoxia imaging agent, nitroimidazole derivates are thought to undergo bioreduction reaction in the hypoxic cell and thus can be retained in hypoxic tissue [1, 2]. Therefore, different kinds of positron emission tomography (PET) and single photon emission computed tomography (SPECT) radiotracers containing nitroimidazole pharmacophore have been evaluated as hypoxia imaging agents [3–21]. At present, [^{18}F] Fluoromisonidazole (^{18}F-FMISO), a 2-nitroimidazole derivative, is one of the most widely used PET radiopharmaceuticals for clinical imaging of hypoxia. Compared to ^{18}F, ^{99m}Tc is the most common radionuclide in routine nuclear medicine due to its ideal physical decay properties and the availability through ^{99}Mo/^{99m}Tc generator. Thus, developing ^{99m}Tc-labeled nitroimidazole derivatives to target tumor hypoxia is still one of the major focuses of ongoing research.

Recently, we have reported the synthesis and biodistribution of a novel ^{99m}Tc-DMSAMe (DMSAMe: dimercaptosuccinic acid metronidazole ester) as a potential agent for imaging tumor hypoxia [16]. As reported, ^{99m}Tc-DMSAMe was synthesized through easy procedures and exhibited a certain tumor uptake and a relatively high tumor-to-muscle ratio. However, the tumor-to-blood ratio of ^{99m}Tc-DMSAMe was not satisfactory. Thus, it may be of great interest to probe other better ^{99m}Tc labeled nitroimidazole derivatives for imaging tumor hypoxia. Bearing in mind ornidazole is a commercial 5-nitroimidazole derivative and its molecular structure has a pendant –CH_2OH group, thus making it possible to react with carbon disulfide in NaOH solutions to produce the ornidazole xanthate (ONXT). Based on our previous reported work [22–25], it can be assumed that ONXT can also be used to form stable ^{99m}TcO, ^{99m}TcN and $[^{99m}Tc(CO)_3]$ complexes on the basis of efficient binding of the group to four sulfur atoms. The above background encouraged us to prepare several ^{99m}Tc labeled ornidazole xanthate complexes by using different ^{99m}Tc cores to find good radiotracers for targeting tumor hypoxia. In this study, the synthesis and biological evaluation of novel ^{99m}Tc labeled ornidazole xanthate complexes for imaging tumor hypoxia are reported for the first time.

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Experimental

Materials and methods

Ornidazole was purchased from J&K CHEMICA, 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, 5.0 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 500 MHz Bruker Avance spectrophotometer. Elemental analyses were performed on a Vario EL elemental analyzer model.

Synthesis of ornidazole xanthate (ONXT)

The synthesis of ONXT was carried out according to the literature [22]. In brief, 0.439 g of ornidazole, carbon disulfide (0.456 g) and NaOH (0.120 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 produce ONXT as a yellow solid (0.279 g, 43.9 %).

Radiolabeling and quality control techniques

The preparations of ^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT were carried out according to our previous reported methods [25–27].

For ^{99m}TcO-ONXT, 1 mL of saline containing $[^{99m}TcO_4]^-$ (37.0 MBq) was added to a GH kit containing 0.05 mg of stannous chloride dihydrate, 5.0 mg of glucoheptonate (GH). The mixture was kept at room temperature for 15 min. Then, 1.0 mg of ONXT dissolved in 1.0 mL water was added to the mixture and the resulting solution was heated at 100 °C for 30 min.

For ^{99m}TcN-ONXT, 1 mL of saline containing $[^{99m}TcO_4]^-$ (37 MBq) was added to a SDH kit containing 0.05 mg of stannous chloride dihydrate, 5.0 mg of succinic dihydrazide (SDH), 5.0 mg of propylenediamine tetraacetic acid (PDTA). The mixture was kept at room temperature for 15 min. Successively, 1.0 mg of ONXT dissolved in 1.0 mL water was added and the reaction mixture was heated at 100 °C for 30 min.

For ^{99m}Tc(CO)$_3$-ONXT, potassium sodium tartrate (15.0 mg), Na_2CO_3 (5.0 mg), and $NaBH_4$ (10.0 mg) were added to a 10 mL glass vial. The vial was sealed and flushed with CO for 15 min, followed by the addition of 1.0 mL of saline containing $[^{99m}TcO_4]^-$ (370 MBq). The vial was heated at 80 °C for 30 min. After cooling to room temperature, 0.1 mol/L HCl was added to adjust the pH to approximately 8. Then 1 mL of a water solution containing 1.0 mg of the ONXT ligand was added and the reaction vial was incubated on a boiling water bath for 20 min.

The radiochemical purities of the complexes were assessed by HPLC. The HPLC analysis conditions are as follows. HPLC analysis was carried out with a reverse d-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}TcO-ONXT and ^{99m}TcN-ONXT, water (A) and acetonitrile (B) mixtures were used as the mobile phase. For ^{99m}Tc(CO)$_3$-ONXT, 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}TcO-ONXT, 0 min 50 % B, 20 min 90 % B, 30 min 90 % B, 40 min 100 % B; for ^{99m}TcN-ONXT, 0 min 70 % B, 10 min 70 % B, 15 min 90 % B, 40 min 100 % B; for ^{99m}Tc(CO)$_3$-ONXT, 0 min 10 % B, 28 min 90 % B, 30 min 100 % B, 40 min 100 % B.

In vitro stability study

^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT complexes were incubated in the labeling milieu at room temperature for 6 h. On the other hand, the stability in human serum albumin (HSA) was determined by incubating complexes in the solution of HSA at 37 °C for 6.0 h. The radiochemical purities of the complexes were assessed by HPLC.

In vitro cell uptake

In vitro uptake of the complexes both in hypoxic and aerobic conditions was evaluated by using the previous reported methods [17]. 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 [17]. Then, 0.2 mL (0.74 MBq) of the complex 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 ± SD.

Determination of the partition coefficient

The partition coefficient (log *P*) between 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) of the complex was measured in order to evaluate their lipophilicity. In a centrifuge tube, containing 2 mL of each phase, 0.1 mL of the labeled complex solution was added, and the mixture was shacked on a Vortex mixer for 1 min and then centrifuged at 5000 g for 5 min. Three samples (0.1 mL each) from each layer were counted in a well gamma γ-counter. The partition coefficient, *P*, was calculated as the mean value of counts per minute in octanol divided by that of the buffer. Usually the final partition coefficient value was expressed as log *P*. The log *P* value was reported as an average of three measurements plus the standard deviation.

Biodistribution study

The Kunming male mice (18–20 g, five animals per group) bearing S180 tumor were injected via a tail vein with ^{99m}TcO-ONXT (0.1 mL, 7.4×10^5 Bq). At 2 h and 4 h post-injection, the mice were sacrificed by neck dislocation. The tumor, other organs of interest and blood were collected, weighed and assayed for radioactivity. The results were expressed as the percent uptake of injected dose per gram of tissue (% ID/g). The final results are expressed as mean ± SD. Animal studies were performed in compliance with the national laws related to the ethics during experimentation. The biodistribution studies of ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT were conducted in the same way.

SPECT imaging studies

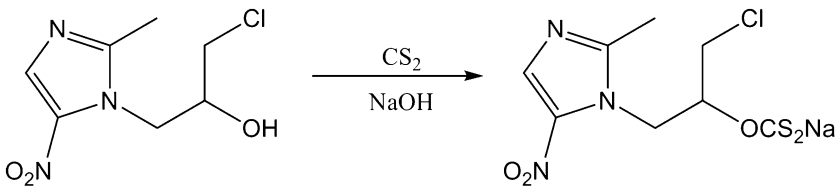
0.2 mL of ^{99m}TcO-ONXT (111 MBq) was injected intravenously through trail vein in mice (18–22 g) bearing S180 tumor. A dual-head SPECT (Skylight; Philips, Milpitas, CA, USA), using a low-energy parallel-hole collimator (diameter 3.5 mm), was used for SPECT imaging studies. Static images were acquired at 4 h after injection.

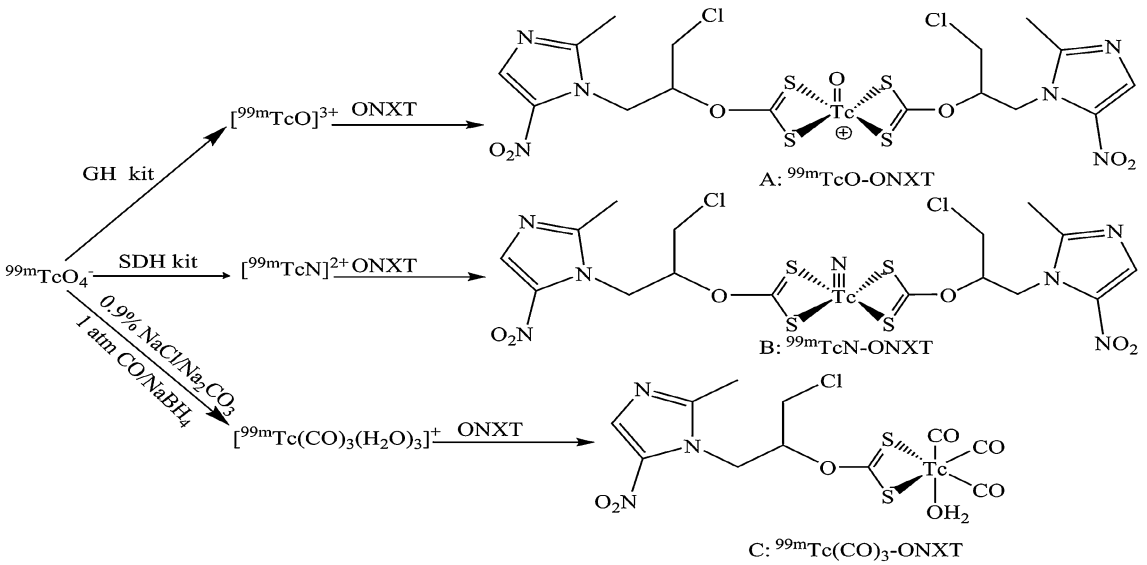
Results and discussion

Synthesis and radiolabeling

ONXT was prepared by reacting ornidazole with carbon disulfide in NaOH solutions. The reaction equation is shown in Scheme 1. It was characterized by IR, ^{1}H NMR,

Scheme 1 Synthesis of ONXT





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

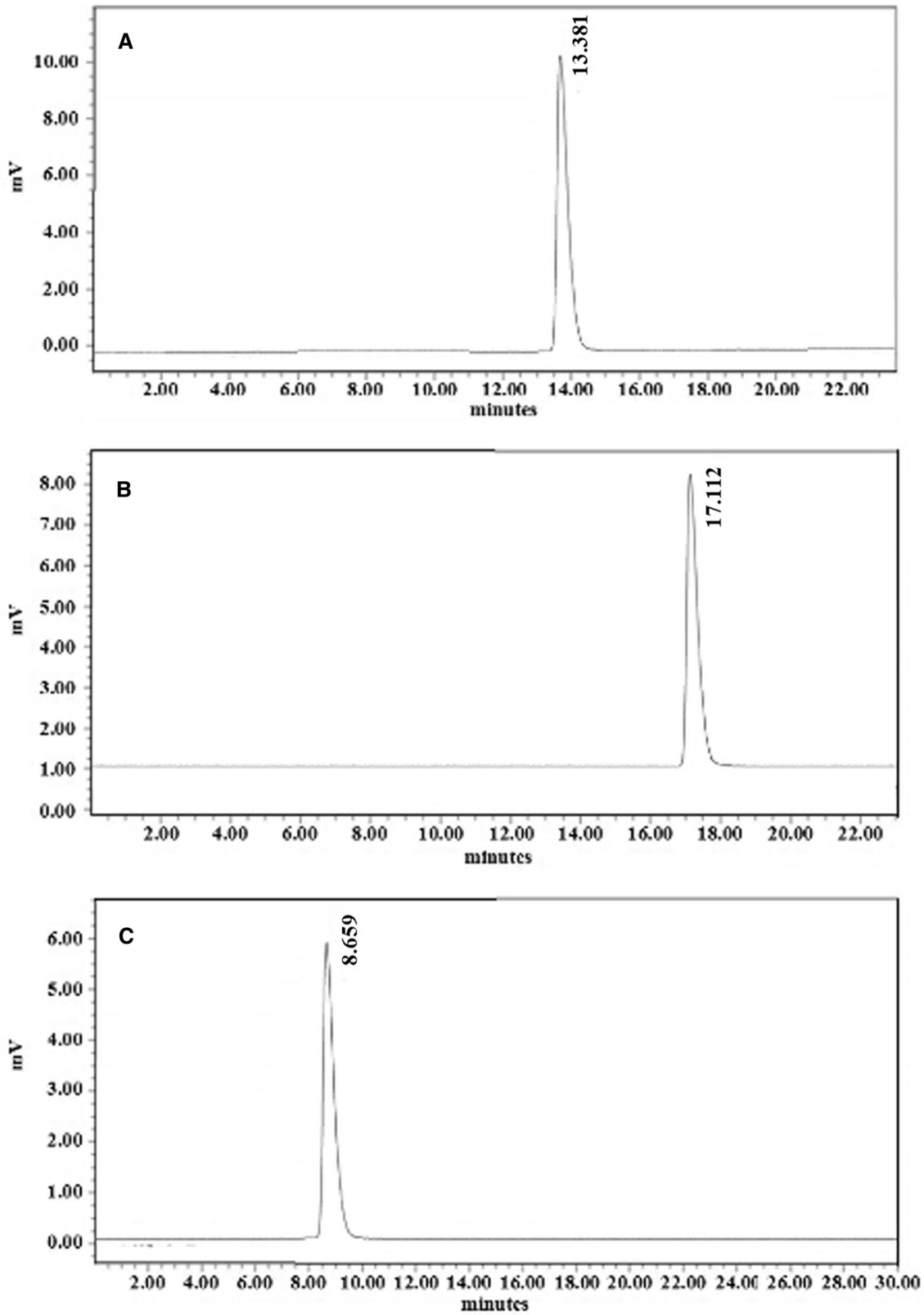


Fig. 1 HPLC patterns of ^{99m}TcO-ONXT (**a**), ^{99m}TcN-ONXT (**b**) and ^{99m}Tc(CO)$_3$-ONXT (**c**)

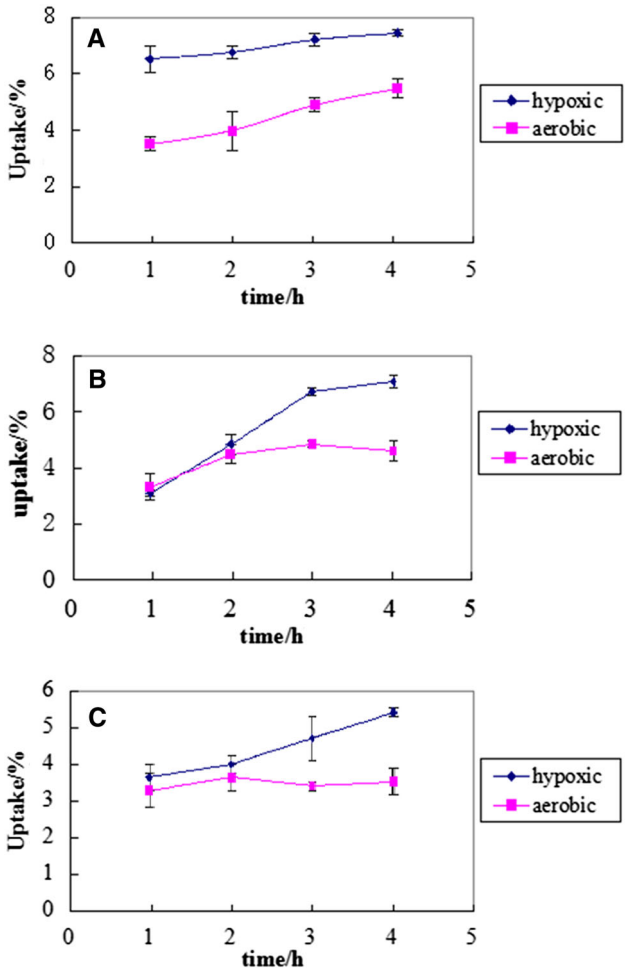


Fig. 2 Cellular uptake of ^{99m}TcO-ONXT (**a**), ^{99m}TcN-ONXT (**b**) and ^{99m}Tc(CO)$_3$-ONXT(**c**)

^{13}C NMR and Elemental analysis. IR(KBr)/cm^{-1}: νC–O:3421.2, νNO$_2$:1636.0,1354.6, νC=S:1083.8. ^{1}H-NMR (D$_2$O): δ7.76(s, 1H, CH), δ4.53–4.50(d, 2H, CH$_2$), δ3.74–3.74 (d, 2H, CH$_2$), δ3.08–3.02 (tt, H, CH), δ2.06 (s, 3H, CH$_3$); ^{13}C-NMR (D$_2$O): δ208.47 (CS$_2$); δ160.36 (C); δ150.80 (C); δ127.85 (CH); δ61.14 (CH$_2$); δ47.78 (CH); δ42.48 (CH$_2$); δ18.29 (CH$_3$). Elemental analysis calculated (%) for C$_8$H$_9$N$_3$NaO$_3$S$_2$Cl: C, 30.24; N, 13.22; H, 2.85. Found: C, 30.07; N, 13.49; H, 3.02.

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

For labeling, ^{99m}TcO-ONXT was prepared by ligand-exchange reaction with ^{99m}Tc-glucoheptonate (GH) [24]. ^{99m}Tc-GH is an ideal substrate for the substitution reaction with ONXT to produce the final complex ^{99m}TcO-ONXT.

^{99m}TcN-ONXT was prepared by adding ONXT 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 ONXT to prepare ^{99m}TcN-ONXT with high yield [23]. As for preparing ^{99m}Tc(CO)$_3$-ONXT, the H$_2$O molecule in the fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor is readily substituted by sulfur atoms in the ONXT ligand [25]. The ONXT ligand displaces the two H$_2$O molecules of the fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor.

The radiochemical purities of the complexes were assessed by HPLC. The retention time of [^{99m}TcO]$^{3+}$, [^{99m}TcN]$^{2+}$ and [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was 4.13, 4.73, and 15.60 min, respectively, while that of ^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT were found to be

Table 1 Biodistribution of ^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT in mice bearing S180 tumor (% ID/g)

Complex	^{99m}TcO-ONXT		^{99m}TcN-ONXT		^{99m}Tc(CO)$_3$-ONXT	
Time	2 h	4 h	2 h	4 h	2 h	4 h
Heart	0.64 ± 0.10	0.62 ± 0.05	0.38 ± 0.03	0.40 ± 0.08	0.77 ± 0.06	0.30 ± 0.02
Liver	8.88 ± 1.47	8.14 ± 0.91	5.00 ± 0.58	5.06 ± 0.39	9.59 ± 0.83	9.39 ± 1.00
Lung	5.11 ± 0.40	5.67 ± 0.71	1.43 ± 0.22	1.23 ± 0.45	2.93 ± 0.27	1.01 ± 0.17
Kidney	11.42 ± 1.36	13.01 ± 1.46	4.07 ± 0.59	3.92 ± 0.47	8.51 ± 0.21	8.24 ± 0.22
Spleen	0.98 ± 0.13	1.45 ± 0.14	0.82 ± 0.17	0.93 ± 0.31	0.46 ± 0.05	0.40 ± 0.03
Muscle	0.54 ± 0.16	0.38 ± 0.03	0.76 ± 0.07	0.72 ± 0.09	0.49 ± 0.07	0.46 ± 0.03
Tumor	2.04 ± 0.09	1.90 ± 0.19	1.09 ± 0.06	1.04 ± 0.21	1.33 ± 0.09	1.05 ± 0.20
Blood	1.27 ± 0.14	1.28 ± 0.16	0.86 ± 0.20	0.46 ± 0.04	0.52 ± 0.04	0.49 ± 0.03
T/N ratio	3.78	5.00	1.43	1.44	2.71	2.28
T/B ratio	1.61	1.48	1.27	2.26	2.56	2.14

All the 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

13.38, 17.11 and 8.66 min (Fig. 1). The mean radiochemical purities of the products were all over 95 % immediately after the 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 HSA, suggesting they had good in vitro stability.

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 Fig. 2. From Fig. 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.

Partition coefficient (log *P*)

The logP values of ^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT were found to be -1.42 ± 0.01, -1.29 ± 0.01 and -0.63 ± 0.01, respectively, indicating all of them were hydrophilic. Moreover, ^{99m}TcO-ONXT and ^{99m}TcN-ONXT were more hydrophilic than ^{99m}Tc(CO)$_3$-ONXT.

Biodistribution study

The results of biodistribution of the complexes in mice bearing S180 tumor are shown in Table 1. Results of biodistribution in mice bearing S180 tumor of recently reported ^{99m}Tc complexes as hypoxia imaging agents are shown in Table 2 for comparison.

As noted from Table 1, ^{99m}TcO-ONXT, ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT have a relatively high tumor uptake and good tumor retention. The blood and muscle uptakes are low so the *T/B* and *T/N* ratios are better. The high concentration in the liver and kidney shows that the major route of excretion is renal and hepatobiliary. By comparison, ^{99m}TcO-ONXT has a lower muscle uptake and higher tumor uptake, so the *T/N* ratio of ^{99m}TcO-ONXT is much higher than that of ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT. However, owing to the higher blood uptake, the *T/B* ratio of ^{99m}TcO-ONXT is lower than that of ^{99m}TcN-ONXT and ^{99m}Tc(CO)$_3$-ONXT at 4 h post-injection. The above facts prove that different ^{99m}Tc core for preparing the complexes may exhibit significant impact on the tumor uptake, *T/B* and *T/N* ratios.

Table 2 Comparison of biodistribution of some reported ^{99m}Tc complexes

Complex	1	2	3	4	5	6	7	8	9	10
Time p.i. (h)	2	2	2	2	2	2	2	2	2	2
Tumor uptake (% ID/g)	2.04 ± 0.09	1.09 ± 0.06	1.33 ± 0.09	1.54 ± 0.26	0.49 ± 0.07	0.29 ± 0.02	0.25 ± 0.06	0.37 ± 0.05	0.40 ± 0.10	0.25 ± 0.02
T/N ratio	3.78	1.43	2.71	2.49	6.66	2.42	3.20	0.63	3.01	1.01
T/B ratio	1.61	1.27	2.56	0.62	1.00	9.67	0.84	0.66	0.35	0.63
Reference	This study	This study	This study	16	18	19	20	21	21	21

Complex 1 ^{99m}TcO-ONXT, *2* ^{99m}TcN-ONXT, *3* ^{99m}Tc(CO)$_3$-ONXT, *4* ^{99m}Tc-DMSAMe, *5* ^{99m}Tc-N2IPA, *6* ^{99m}Tc-HYNIC-MN, *7* [^{99m}Tc(CO)$_3$(MN-TZ-BPA)]$^+$, *8* [^{99m}Tc(CO)$_3$(BPA-PEG$_3$-NIM)]$^+$, *9* [^{99m}Tc(CO)$_3$(AOPA-PEG$_3$-NIM)], *10* [^{99m}Tc(CO)$_3$(IDA-PEG$_3$-NIM)]$^-$

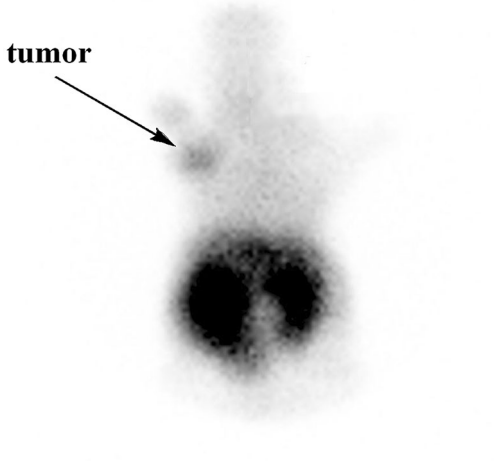


Fig. 3 SPECT image of ^{99m}TcO-ONXT in mice bearing S180 tumor

As seen from Table 2, ^{99m}TcO-ONXT exhibits a much higher tumor uptake when compared to the other nine complexes. With regard to *T/N* ratio, ^{99m}TcO-ONXT is superior to the other complexes except ^{99m}Tc-N2IPA. Moreover, ^{99m}TcO-ONXT also has the higher *T/B* ratio than other complexes except for ^{99m}Tc-HYNIC-MN and ^{99m}Tc(CO)$_3$-ONXT. As a good tumor hypoxia imaging agent, its detectability of tumor depends on both the absolute tumor uptake and tumor to background ratio. From the above point of views, ^{99m}TcO-ONXT exhibits more potential usefulness as a radiotracer for imaging tumor hypoxia.

SPECT imaging studies

The SPECT imaging results showed the tumor uptake was observable (Fig. 3), however, the high uptake of ^{99m}TcO-ONXT in the liver and kidneys is the drawback of the complex. The imaging findings were similar to the biodistribution results in mice. Low uptake in the thyroid is suggestive of in vivo stability of ^{99m}TcO-MNXT. The imaging results of ^{99m}TcO-ONXT exhibits its very promising property for further studies in more extensive preclinical animal models.

Conclusion

In present study, a novel ligand ONXT was synthesized and its ^{99m}Tc-oxo core, ^{99m}Tc-nitrido core and ^{99m}Tc tricarbonyl core complexes were successfully prepared with high yields through ligand-exchange reactions. The preliminary studies showed all of them had a certain hypoxic selectivity and a relatively high tumor uptake and good target to non-target ratios. Especially for ^{99m}TcO-ONXT, 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 potential radiotracer to target tumor hypoxia.

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