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Research Article

Synthesis and biological evaluation of a novel asymmetrical ^{99m}Tc-nitrido complex of metronidazole derivative

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Abstract: The novel dithiocarbamate derivative of metronidazole, potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate (MNIE-DTC), was synthesized as the pharmacophore-containing bifunctional ligand. The corresponding asymmetrical ^{99m}Tc-nitrido complex, expected as a tumor hypoxia marker, had been successfully obtained by the addition of the biphosphine ligand PNP5 (PNP5 = *N*-ethoxethyl-*N*,*N*-bis[2-(bis(3-methoxypropyl)phosphino)ethyl]-amine) and the dithiocarbamate ligand (MNIE-DTC) to the ^{99m}Tc-nitrido precursor solution at 100°C for 15 min. The radiochemical purity of the product was above 95% as measured by thin-layer chromatography and high-performance liquid chromatography. *In vitro* studies showed that the complex possessed good stability under physiological conditions. Its partition coefficient studies indicated that it was a lipophilic complex. The electrophoresis results showed that the complex was cationic. Biological evaluation of the complex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ performed in Kunming mice bearing H22 tumor showed that the complex had a moderate tumor uptake (0.57 ± 0.06 %ID/g at 1 h), and the ratios of tumor/blood and tumor/muscle were 2.46 and 1.31 at 1 h p.i., and reached 4.52 and 2.86 at 4 h p.i., respectively.

Keywords: ^{99m}Tc-nitrido complex; tumor hypoxia; biodistribution; potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate

Introduction

The existence of hypoxic cells in most tumors has long been recognized as a major problem in radiotherapy and is also a potential problem in the chemotherapy of cancer. Nuclear medicine offers a non-invasive method for demonstrating tumor hypoxia. Many radiolabeled nitroimidazole-containing compounds have been developed for tumor hypoxia imaging agent.[1–14] The nitro group undergoes an enzyme-mediated one-electron reduction in viable cells to a radical anion. Under hypoxic condition, the radical anion is further reduced to some products, which are trapped within the cells by binding to cellular components.[15] An ideal hypoxia imaging agent should meet the optimum characteristics, such as ease of preparation, stability, rapid accumulation in tumors, sufficient retention time therein, and rapid clearance from other tissues to provide better contrast between lesion and background.[13] Despite their preferential accumulation in hypoxic tissues, ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21 have not evolved as the marketed radiopharmaceuticals due to their slow plasma clearance and high hepatic uptake.[3,5] The lipophilicity of these compounds appears to play a significant role.[12] If the ^{99m}Tc radiotracer is too lipophilic, it often shows a high protein binding and longer retention in background tissues. If the ^{99m}Tc radiotracer is too hydrophilic, it tends to have poor penetration across the membrane.

Recently, a series of asymmetrical heterocomplexes of the type $[^{99m}TcN(PNP)(XY)]^{+/0}$ reported by Duatti and his coworkers have been used in the development of new radiopharmaceuticals.[16,17] This novel type of mixed-ligand complex was efficiently prepared by reacting the precursor complex $[^{99m}TcN(PNP)]^{2+}$ that was strongly electrophilic with bidentate chelating ligands carrying soft π-donor coordinating atoms such as S, O, and N without the removal of both the

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^{99m}Tc≡N multiple bond and the ancillary PNP ligand. This has led us further to discover a new class of imaging agent, such as myocardial imaging agents ^{99m}TcN-DBODC5 [18,19] and some receptors imaging agents.[20,21] The ether groups of the PNP ligand can modify the lipophilicity of ^{99m}Tc-nitrido heterocomplexes. Hence in this study, in order to develop a new hypoxia-imaging agent, a new dithiocarbamate ligand, potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate (MNIE-DTC), was designed and synthesized as the bidentate ligand to react with $[^{99m}TcN(PNP5)]^{2+}$ to obtain the asymmetrical ^{99m}Tc-nitro complex. *In vivo* biodistribution study of this complex was performed in mice bearing H22 tumor.

Results and discussion

Chemistry

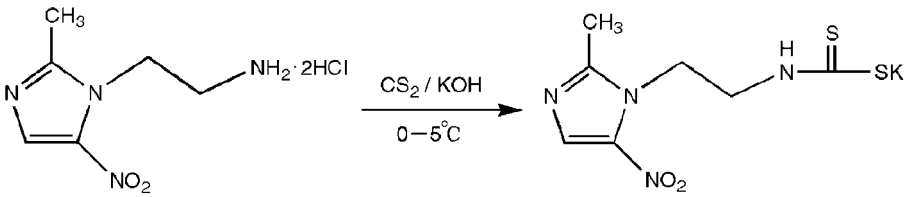
The novel dithiocarbamate derivative with metronidazole was prepared by treatment of the amine with carbon disulfide in basic condition (Scheme 1).

Potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate (MNIE-DTC) was synthesized in a moderate yield of 42% and the product was characterized by IR spectrum, NMR spectrum and Elemental analyses. IR/cm^{-1}: 3175 (–NH), 2962 (–CH_2), 1464, 1499 (C–N), 952 (C═S). ^{1}H NMR (400 MHz, D_2O) δ: 7.92 (s, 1H, CH), 4.46 (t, 2H, CH_2N), 3.90 (t, 2H, CH_2NH), 2.38 (s, 3H, CH_3). ^{13}C NMR (400 MHz, D_2O) δ: 13.062 (CH_3), 45.392 (CH_2), 45.916 (CH_2), 133.076 (imi-C), 138.820 (imi-C), 152.504 (imi-C), 213.385 (CS_2). Anal. (%) Calculated C: 29.55, H: 3.19, N: 19.70; found C: 29.39, H: 3.54, N: 19.40.

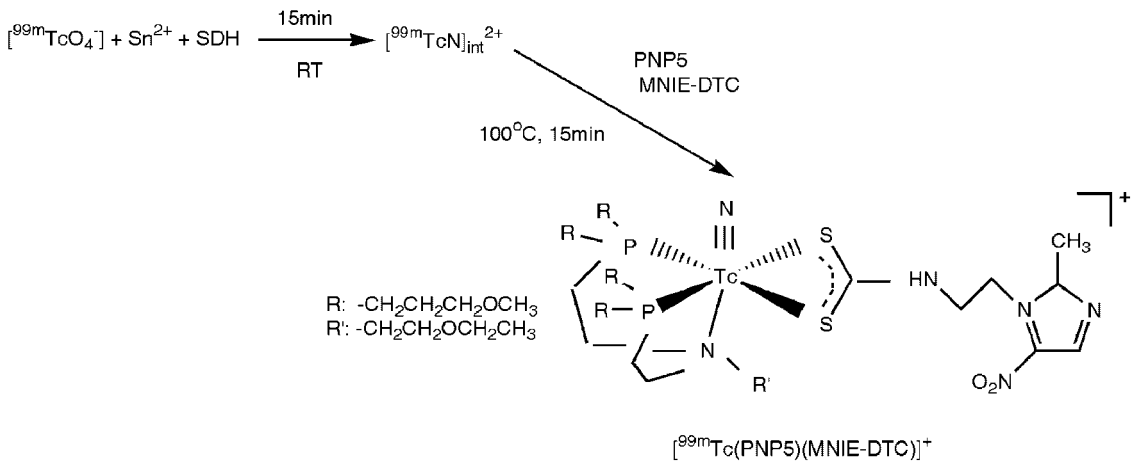
Radiochemistry

The complex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ can be prepared by a two-step procedure, as reported in Scheme 2.

The SDH kit vial, containing the following lyophilized formulation: 0.05 mg $SnCl_2 \cdot 2H_2O$, 5.0 mg succinic dihydrazide (SDH) and 5.0 mg propylenediamine tetra-acetic acid (PDTA) was used for preparing 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 final asymmetrical ^{99m}Tc-nitrido heterocomplex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ was obtained in high yield (>90%) by the addition of PNP5 ligand and MNIE-DTC ligand to the prepared $[^{99m}TcN]^{2+}$ intermediate at 100°C for 15 min. TLC and HPLC analyses were used to evaluate the radiochemical purity (RCP) and the stability of the complex. TLC was performed on a polyamide film with a mixture of acetone and saline (V/V = 1:6) as the



Scheme 1 Synthesis of MNIE-DTC.



Scheme 2 Synthesis of the complex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$.

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Table 1 R_f values for $[^{99m}TcN]^{2+}_{int}$, $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ and other components

Component	$[^{99m}TcO_4]^-$	$^{99m}TcO_2 \cdot nH_2O$	$[^{99m}TcN]^{2+}_{int}$	$^{99m}TcN(PNP5)(MNIE\text{-}DTC)$
R_f	0.1	0.1	0.9–1.0	0.3–0.6

mobile phase. R_f values for $[^{99m}TcN]^{2+}$ intermediate, $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ and other possible components are reported in Table 1.

The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate and $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ are shown in Figure 1. The retention time of the final asymmetrical ^{99m}Tc-nitrido heterocomplex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ was found to be about 21.7 min, while that of $[^{99m}TcN]^{2+}$ intermediate was 2.7 min. The paper electrophoresis pattern of $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ showed that the complex moved to the point of cathode (percentage of radioactivity: 96.20%), suggesting that it is a cationic complex.

The *in vitro* stability of the complex was evaluated by measuring the RCP at different time points. The RCP was still over 95% after 8 h, which suggested that the complex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ possessed a great stability *in vitro*.

The partition coefficient (P) of the complex $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ was 7.37 ± 0.03 at pH 7.0, which is much lower than ^{99m}Tc-BMS181321 (about 40)[3] and ^{99m}Tc-BRU59-21 (about 12).[5] This indicated that the liver uptake for $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ might be modified as expected.

Biodistribution

Biological evaluation of $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$ was performed in Kunming male mice bearing H22 tumor. The result is shown in Table 2. The complex had a moderate tumor uptake (0.57 ± 0.06 %ID/g at 1 h) and good retention (0.43 ± 0.09 %ID/g at 4 h). The complex also showed low uptake in the normal tissues and fast clearance from blood. The ratios of tumor/blood and tumor/muscle rose with time and were 2.46 and 1.31 at 1 h p.i., 4.52 and 2.86 at 4 h p.i., respectively.

Hypoxia is commonly seen at the rapidly growing tumor. In Chen's study,[22] a potential hypoxia imaging agent, ^{99m}Tc-DTPA-metronidazole, showed specific accumulation in tumor tissue in the H22 tumor-bearing mice. It indicated that the H22 tumor might be more hypoxic than the normal tissues in this model. The uptake in tumor and ratio of tumor/muscle can be used as important indicators in evaluating a hypoxia marker. Compared with other ^{99m}Tc-labeled nitroimidazoles, such as ^{99}Tc-BMS181321 and ^{99m}Tc-BRU59-21, the complex $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$ showed

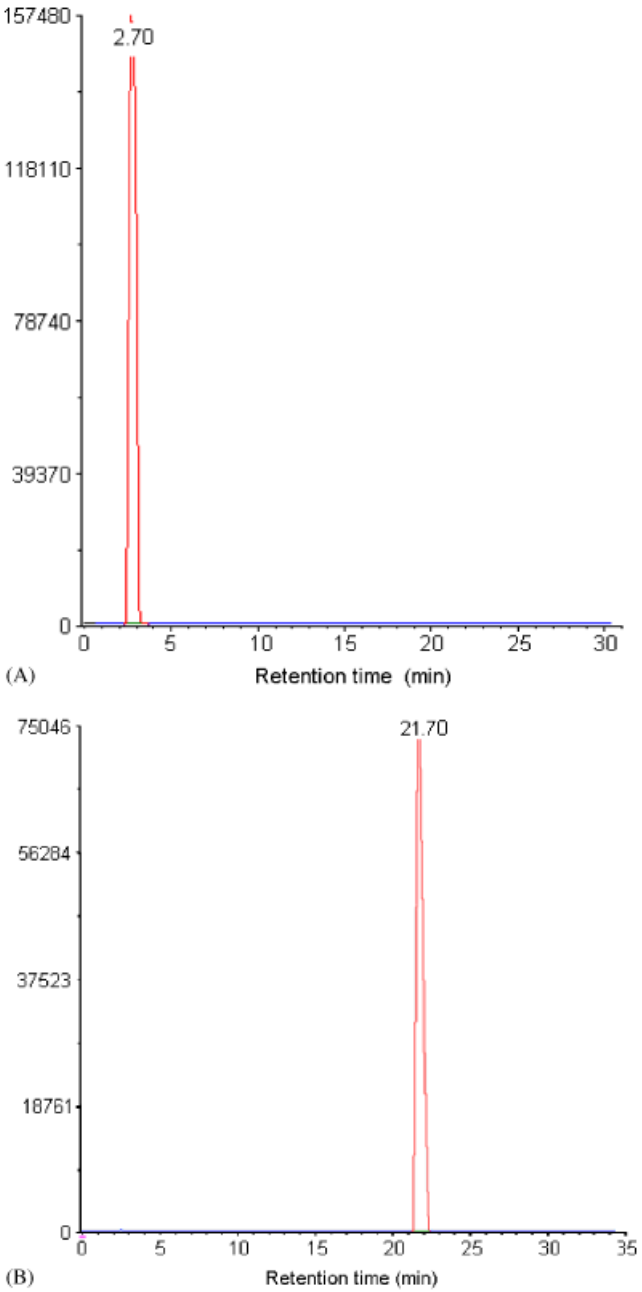

Figure 1 The HPLC chromatograms of $[^{99m}TcN]^{2+}$ intermediate (A) and $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ (B). Figure available in colour online at www.interscience.wiley.com

similar tumor uptake and slightly lower tumor/muscle ratio (Table 3). It was perhaps due to the different tumor models in mice used in the experiments. The

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complex $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$ demonstrated a far lower uptake in liver than ^{99}Tc-BMS181321 and ^{99m}Tc-BRU59-21. For example, the liver uptake was 1.74 ± 0.41 %ID/g for $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$, 8.79 ± 3.05 %ID/g for ^{99m}Tc-BMS181321 and 8.37 ± 0.87 %ID/g for ^{99m}Tc-BRU59-21 at 2 h post-injection.[3,5] As seen from Table 3, the complex $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$ also demonstrated a higher tumor/blood ratio than ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21. It was probably due to the low lipophilicity of this complex resulting in lower liver uptake and fast plasma clearance.

Experimental

Materials

2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethylamine dihydrochloride was purchased from ACROS Organics Company. The SDH kit was donated by Beijing Shihong Pharmaceutical Center. The aminodiphosphine ligand PNP5 was prepared according to the literature methods.[18] $^{99m}TcO_4^-$ was obtained by elution with saline from a ^{99}Mo-^{99m}Tc generator (China Institute of Atom Energy, China). All other chemicals were of reagent grade and were used without further purification. The murine hepatoma cell line H22 was kindly provided by the College of Life Science, Beijing Normal University. IR spectrum was obtained with an AVATAR 360 FT-IR spectrometer using KBr pellets. NMR spectrum was recorded on a Bruker Avance 500 (400 MHz) spectrometer with D_2O as a solvent. Elemental analysis was performed on a Vario EL elemental analyzer model. TLC and HPLC analyses were used to evaluate the RCP of the radiolabeled compounds. The TLC was performed on a polyamide strip and eluted with acetone/saline = 1:6 (V/V). HPLC chromatograms were obtained on a SHIMADZU SCL-10AVP system with a liquid scintillation analyzer (Packard BioScience Co., USA). The ODS-C18 column (5 μm, 250 × 4.6 mm, Alltech Associates, Inc., USA) was eluted at a flow rate of 1 mL/min according to the procedure described in the experimental part.

Table 2 Biodistribution of $[^{99m}TcN(PNP5)(MNIE\text{-}DTC)]^+$ in mice bearing H22 tumor ($n = 5$)

	%ID/g ± SD		
	60 min	120 min	240 min
Tissue			
Heart	0.69 ± 0.12	0.42 ± 0.08	0.23 ± 0.05
Liver	2.93 ± 0.28	1.74 ± 0.41	1.70 ± 0.12
Lung	0.87 ± 0.16	0.64 ± 0.15	0.39 ± 0.04
Blood	0.23 ± 0.02	0.15 ± 0.01	0.09 ± 0.01
Kidney	2.77 ± 0.21	1.36 ± 0.07	0.89 ± 0.08
Muscle	0.43 ± 0.10	0.19 ± 0.09	0.15 ± 0.06
Bone	0.31 ± 0.03	0.18 ± 0.03	0.08 ± 0.02
Spleen	0.42 ± 0.10	0.41 ± 0.16	0.41 ± 0.10
Tumor	0.57 ± 0.06	0.45 ± 0.05	0.43 ± 0.09
Ratios			
Tumor/blood	2.46	3.09	4.52
Tumor/muscle	1.31	2.32	2.86

Synthesis of potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate

Potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate (MNIE-DTC) was prepared by reacting 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethylamine dihydrochloride with an equivalent amount of carbon disulfide in KOH solution. Potassium hydroxide (5.7 mmol) was dissolved in water, the solution cooled in an ice bath and then added to 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethylamine (1.9 mmol) under stirring, followed by carbon disulfide (1.9 mmol). The mixture was stirred for 2 h in an ice bath. The solvent was removed under reduced pressure and the

Table 3 Comparison of the partition coefficient value and biodistribution data between $[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$, ^{99m}Tc-BMS181321 and ^{99m}Tc-BRU59-21

Complex	$[^{99m}TcN(PNP)(MNIE\text{-}DTC)]^+$	^{99m}Tc-BMS181321	^{99m}Tc-BRU59-21
The partition coefficient *P*	7.37	40	12
Time p.i. (h)	2	2	2
Liver (%ID/g)	1.74 ± 0.41	8.79 ± 3.05	8.37 ± 0.87
Tumor (%ID/g)	0.45 ± 0.05	0.55 ± 0.08	0.37 ± 0.14
Tumor/muscle	2.32	2.63	3.84
Tumor/blood	3.09	0.31	0.86
Animal	Kunming mice	C_3H mice	C_3H mice
Tumor model	H22	KTH-C	KTH-C
Reference	Present study	3	5

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residue was filtered off. The crude product was recrystallized from ethanol/water to give yellow crystals of potassium 2-(2-methyl-5-nitro-1*H*-imidazolyl)-ethyl-dithiocarbamate.

Preparation of the heterocomplex (^{99m}TcN(PNP5)(MNIE-DTC))$^+$

The asymmetrical heterocomplex was prepared by a two-step procedure. First, 1 mL saline-containing $^{99m}TcO_4^-$ (about 37 MBq) was added into a SDH kit and kept for 15 min at room temperature to obtain the $[^{99m}TcN]^{2+}$ intermediate. Second, the PNP5 ligand (1.0 mg) and the dithiocarbamate ligand (1.0 mg) were added into the $[^{99m}TcN]^{2+}$ intermediate, and the reaction mixture was sealed and heated at 100°C for 15 min.

The $[^{99m}TcN]^{2+}$ intermediate and final asymmetrical heterocomplex were characterized by TLC and HPLC. In HPLC about 5 μL of the test solution was injected into the column. Water (solvent A) and acetonitrile (solvent B) each containing 0.1% trifluoroacetic acid were used as the mobile phase in the following gradient (0–2 min, 5% B, 2–30 min, 5–100% B, 30–60 min, 100–5% B).

Determination of the partition coefficient for the complex

The partition coefficient of the complex was determined by measuring the activity that partitioned between the *n*-octanol and aqueous phosphate buffer (0.025 mol/L, pH 7.0) under strict equilibrium conditions. Two milliliters of 1-octanol and 2 mL of $[^{99m}$TcN(PNP5)(MNIE-DTC)$]^+$ phosphate buffer were mixed in a centrifuge tube. The mixture was vortexed at room temperature for 5 min and then centrifuged at 5000 r/min for 5 min. The counts in 0.1 mL samples of both organic and inorganic layers were determined by a well-type NaI(Tl) gamma-counter. The measurement was repeated three times. The partition coefficient (P) was calculated by the following equation: P = (cpm in octanol − cpm in background)/(cpm in buffer − cpm in background).

Stability study

The stability of the complex in the reaction mixture was assayed by measuring the RCP through TCL at 1, 2, 4, 6 and 8 h after preparation at room temperature.

Paper electrophoresis

The sample of the complex was spotted on chromatography paper strips (10 cm × 1 cm) which were pre-treated with phosphate buffer (0.025 mol/L, pH = 7.0). The analyses were carried out using phosphate buffer (0.025 mol/L, pH = 7.0) at 150 V for 2 h. Then the strips were left to dry, and the distribution of radioactivity on the strip was determined.

Biodistribution study

In vivo biodistribution study of $[^{99m}$TcN(PNP5)(MNIE-DTC)$]^+$ was carried out in the Kunming mice bearing H22 tumor. *In vivo* growth was initiated by hypodermic injection of approximately 1×10^6 H22 cells into the left front leg of Kunming mice (~20 g body weight, male). Over the course of 10–12 days, tumor grew to a leg diameter of 10–15 mm. The ^{99m}Tc-complex $[^{99m}$TcN(PNP5)(MNIE-DTC)$]^+$ (100 μL, 74 kBq) was injected into the mice via the tail vein. Then the mice ($n = 5$) were sacrificed by cervical dislocation at 60, 120 and 240 min post-injection. The organs or tissues of interest were removed, weighted, and measured in a well-type NaI(Tl) gamma-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 are expressed as mean ± SD. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

Conclusion

The dithiocarbamate derivative of metronidazole (MNIE-DTC) was synthesized. The corresponding asymmetrical ^{99m}Tc-nitrido complex $[^{99m}$TcN(PNP5)(MNIE-DTC)$]^+$ had been successfully prepared in two steps with high yield. Biological evaluation of the complex performed in Kunming mice bearing H22 tumor showed that the complex had a moderate tumor uptake, low liver uptake and high tumor/blood ratio due to the low lipophilicity of this complex. The result suggested that the ether group could decrease the lipophilicity and improve the biological properties of ^{99m}Tc-labeled metronidazole complexes. The ^{99m}Tc-nitrido complex $[^{99m}$TcN(PNP5)(MNIE-DTC)$]^+$ is a very promising candidate for further research as a hypoxia-imaging agent.

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