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Synthesis, radiolabeling and biological evaluation of butene amine oxime containing nitrotriazole as a tumor hypoxia marker

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Abstract ^{99m}Tc-BnAO, as a nonnitroaromatic hypoxia marker, is the subject of intensive research in recent years. In this study, a butene amine oxime–nitrotriazole (BnAO–NT) was synthesized and radiolabeled with ^{99m}Tc in high yield. Cellular uptakes of ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO were tested using murine sarcoma S180 and hepatoma H22 cell lines. The highest hypoxic cellular uptake of ^{99m}Tc-BnAO–NT was 27.11 ± 0.73 and 14.85 ± 0.83 % for the S180 and H22 cell lines, respectively, whereas the normoxic cellular uptake of the complex was about 4–8 % for both cell lines. For ^{99m}Tc-BnAO, the highest hypoxic cellular uptake was 30.79 ± 0.44 and 9.66 ± 1.20 % for the S180 and H22 cell lines, respectively, while the normoxic cellular uptake was about 5 % for both cell lines. Both ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO complexes showed hypoxic/normoxic differentials in the two cell lines, but the results were more significant for the S180 cell line. The in vitro results suggested that S180 may be better than H22 cell line in hypoxic biological evaluation of BnAO complexes. The biodistribution study was tested using a S180 tumor model. The complex ^{99m}Tc-BnAO–NT showed a selective enrichment in tumor tissues: At 4 h, the tumor-to-muscle ratio was 3.79 ± 0.98 and the tumor-to-blood ratio was 2.31 ± 0.34. Compared with the results of ^{99m}Tc-BnAO, the latter was at the same level. In vitro and in vivo studies demonstrated that ^{99m}Tc-BnAO–NT could be a hypoxia-sensitive radiotracer for monitoring hypoxic regions in a sarcoma S180 tumor.

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1 Introduction

When it is beyond the capability of accompanying vasculature to supply oxygen, the growth of tumors can result in hypoxia, which is a common characteristic of many solid tumors [1]. Tumor hypoxia can cause adverse effects in therapeutic oncology, such as resistance to conventional radiotherapy and chemotherapy [2–5]. To improve the therapeutic efficacy, it is important to develop hypoxia markers that can detect hypoxic regions within tumors effectively. As such, there are a lot of hypoxia markers for PET and SPECT imaging [6–9], and they are mainly divided into two classes: compounds with nitroimidazole and compounds without nitroimidazole.

^{99m}Tc-BnAO is a nonnitroaromatic ^{99m}Tc-labeled hypoxia marker, and its hypoxic biological evaluation has been reported [10, 11]. ^{99m}Tc-BnAO is similar to BMS181321 (^{99m}Tc-[PnAO-1-(2-nitroimidazole)]), which also showed a significant hypoxic/normoxic differential. However, the mechanism for why ^{99m}Tc-BnAO was retained in hypoxic cells was unknown. Jia et al. [12] proposed that the mechanism may relate to the interconversion between the penta-coordinated mono-oxo form and hexa-coordinated di-oxo form of the ^{99m}Tc-BnAO complex. Lately, some derivatives of ^{99m}Tc-BnAO have been extensively studied by Hsia et al. [13] and Sun et al. [14], such as HL-91-ET, OH-BnAO, EtO-BnAO and Et-BnAO. The biological results indicated that hypoxia uptake was weakly correlated with the lipophilicity of those radiotracers.

Although the reduction potential of 3-nitro-1,2,4-triazole is more negative than that of 2-nitroimidazole, 3-nitro-1,2,4-triazole has been used as redox center in hypoxia markers [15–18]. In our previous work, ^{99m}Tc-labeled hydroxy-iminoamide complex containing nitrotriazole (NTPA) exhibited a good uptake in hypoxic S180 cells and a high tumor-to-muscle ratio [19]. Recently, two ^{99m}Tc-labeled PnAO complexes containing nitrotriazole were prepared; in vitro and in vivo results showed that both complexes displayed significant hypoxic/normoxic differential [20].

In 2011, Hsia et al. [21] synthesized BnAO–NI, which contained two redox centers, 2-nitroimidazole and ^{99m}Tc-BnAO. It was reported that ^{99m}Tc-BnAO–NI did not achieve a higher specific uptake in hypoxic tissue than ^{99m}Tc-BnAO, although ^{99m}Tc-BnAO–NI was more lipophilic than ^{99m}Tc-BnAO. In our preceding work, we found that ^{99m}Tc-labeled PnAO-NT (^{99m}Tc-[PnAO-1-(3-nitro-1,2,4-triazole)]) had a higher hypoxic cellular uptake than BMS181321 (^{99m}Tc-[PnAO-1-(2-nitroimidazole)]) with the same cell line [20, 22]. Hence, it is interesting and necessary to explore the effect of 3-nitro-1,2,4-triazole on the hypoxia uptake of ^{99m}Tc-BnAO derivatives.

In this work, 3,3,10,10-tetramethyl-1-(3-nitro-1,2,4-triazole)-4,9-diazadodecane-2,11-dionedioxime (BnAO–NT) was synthesized and labeled with ^{99m}Tc in a superior yield (Fig. 1). Some of its physicochemical properties such us stability, protein binding, electrical property and lipophilicity were investigated. The cellular uptakes of ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO were performed using the S180 and H22 cell lines. The biodistribution of ^{99m}Tc-BnAO–NT in mice bearing a S180 tumor was also studied.

2 Experimental section

2.1 General

3-Nitro-1*H*-1,2,4-triazole was acquired from J&K (Beijing, China), and 1,4-diaminobutane and *N,N*-diisopropylethylamine (98 %) were provided by Acros Organics (Geel, Belgium). All other reagents were of analytical grade without further purification. The ^{99}Mo–^{99m}Tc generator was purchased from China Institute of Atom Energy (Beijing). Mass spectra were measured on a Bruker APEX IV FTMS (Faellanden, Switzerland), positive mode, ESI. NMR spectra were obtained on Bruker (400 MHz) spectrometers (Bruker, Faellanden, Switzerland). RP-HPLC analyses were performed on a reversed-phase column (Agilent HC-C18, 4.6 × 150 mm, size 5 μm), a Waters 1525 binary HPLC pumps, and a Waters 2478 UV absorbance dual λ detector (Milford, MA USA). The elution was monitored with a Packard 500 TR flow scintillation radioactivity detector (Meriden, CT, USA). The radioactivity was detected by 2470 WIZARD^2 Automatic Gamma Counter (PerkinElmer, MA, USA). Murine sarcoma S180, hepatocarcinoma H22 cell lines, and male Kunming mice were purchased from Department of Laboratory Animal Science, Peking University. Dulbecco's modified Eagle's medium (DMEM) was from Gibco BRL Life Technologies (Grand Island, NY, USA), and fetal bovine serum was from Hyclone (Logan, UT, USA). The JPSJ-605 dissolved oxygen meter was purchased from REX Instrument Factory of Shanghai Precision & Scientific Instrument Co., LTD (Shanghai, China). The ^{99m}Tc-BnAO kit was purchased from Beijing Xin branch of Star Medical Technology Co., LTD. (Beijing, China).

2.2 Synthesis of BnAO–NT

The synthesis route for the compound BnAO–NT is depicted in Scheme 1, which is similar to that reported in our previous work [20]. 1,3-Diaminopropane was replaced with 1,4-diaminobutane. The compound **2** (3-chloro-3-methyl-2-butanoneoxime) was prepared by the addition of concentrated hydrochloric acid, isoamyl nitrite and 2-methylbut-2-ene. The substitution reaction of compound **2** with an excess of 1,4-diaminobutane produced the compound **3** (*N*-(4-aminobutyl)-3-amino-3-methyl-2-butanone-oxime). 1-Bromo-3-methylbut-2-ene was added to a mixture of K_2CO_3 and 3-nitro-1-*H*-1,2,4-triazole in acetone solution to get the compound **4** (3-methyl-1-(3-nitro-1*H*-1,2,4-triazole-1-yl)-2-butene). Concentrated hydrochloric acid was added to the mixture of compound **4** and isoamyl nitrite to get the compound **5** (3-chloro-3-methyl-1-(3-nitro-1*H*-1,2,4-triazol-1-yl)butan-2-one oxime). BnAO–NT was then prepared by substitution reaction of compounds **3** and **5** in dry acetonitrile. The crude product was purified by column chromatography

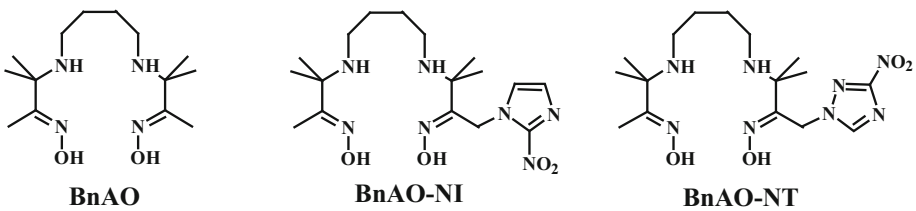
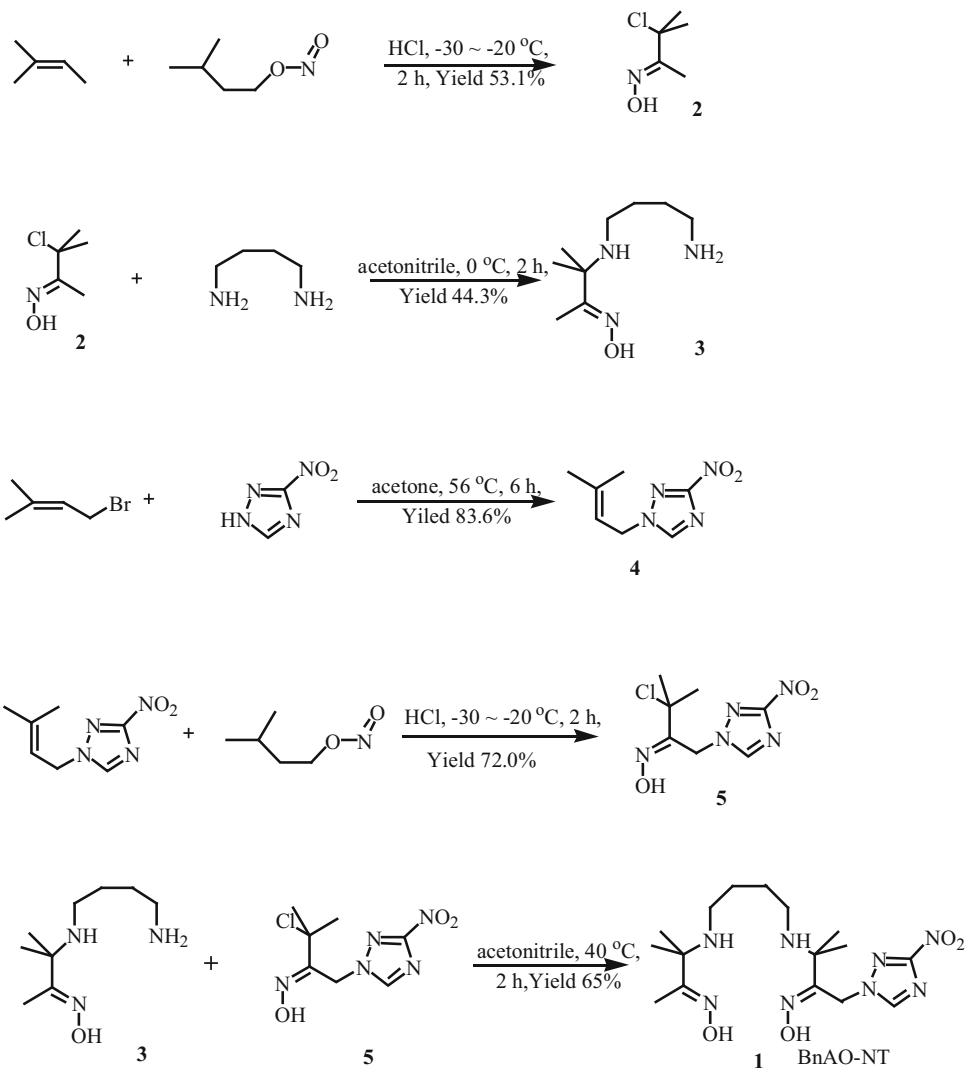


Fig. 1 Chemical structures of BnAO derivatives

Scheme 1 Synthesis of ligand BnAO–NT



(silica gel, CH_2Cl_2:CH_3OH, 4:1) and recrystallized with CH_2Cl_2 to provide a white solid (yield 65 %). HRMS (ESI): mass calculated for BnAO–NT ($C_{16}H_{30}N_8O_4$), 399.24628; *m/z* found, 399.24708 ($M + H^+$). ^1H NMR (DMSO-d_6) δ11.41 (s, 1H, C=N–OH), 11.17 (s, 1H, C=N–OH) 8.84 (s, 1H, triazole-H), 5.20 (s, 2H, NCH_2), 2.71 (t, 2H, CH_2NH), 2.31 (t, 2H, CH_2NH), 1.83 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.63 (s, 3H, N=CCH_3), 1.41 (s, 6H, $C(CH_3)_2$), 1.27 (s, 6H, $C(CH_3)_2$).

2.3 Radiolabeling

$Na^{99m}TcO_4$ was obtained from a ^{99}Mo–^{99m}Tc generator. To get a high labeling yield, the effects of the dosage of stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$), intermediate ligand (sodium tartrate solution) and ligand BnAO–NT were investigated. For comparison, the radiolabeling of ligand BnAO was also performed and analyzed using the same method.

The best radiolabeling method for ^{99m}Tc-BnAO–NT we screened is as follows: $^{99m}TcO_4^-$ (10 μL, 3–5 MBq), the ligand solution (20 μL, 2 mg/mL), phosphate buffer solution (100 μL, pH 7.4, 0.2 mol/L) and sodium tartrate solution (25 μL, 2 mg/mL) were mixed in a 2-mL centrifugal tube. Then the fresh $SnCl_2$ solution (0.5 μL, 2 mg/mL) was added. The mixture was stirred in a water bath at a temperature of 75 °C for 15 min and then filtrated by a 0.22-μm filter. As for ^{99m}Tc-BnAO, $^{99m}TcO_4^-$ (10 μL, 3–5 MBq) was added to ^{99m}Tc-BnAO kit and kept at room temperature for 15 min. ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO were analyzed utilizing the radio high-performance liquid chromatography (radio-HPLC). Solvent systems for HPLC analysis: flow rate 1.0 mL/min; phase $A_{solvent}$, 0.1 M ammonium acetate; phase $B_{solvent}$, CH_3CN. Gradient: 0–10 min 90–30 % $A_{solvent}$; 10–15 min 70–20 % $A_{solvent}$; 15–17 min 20 % $A_{solvent}$; 17–20 min 20–70 % $A_{solvent}$ (Fig. 2).

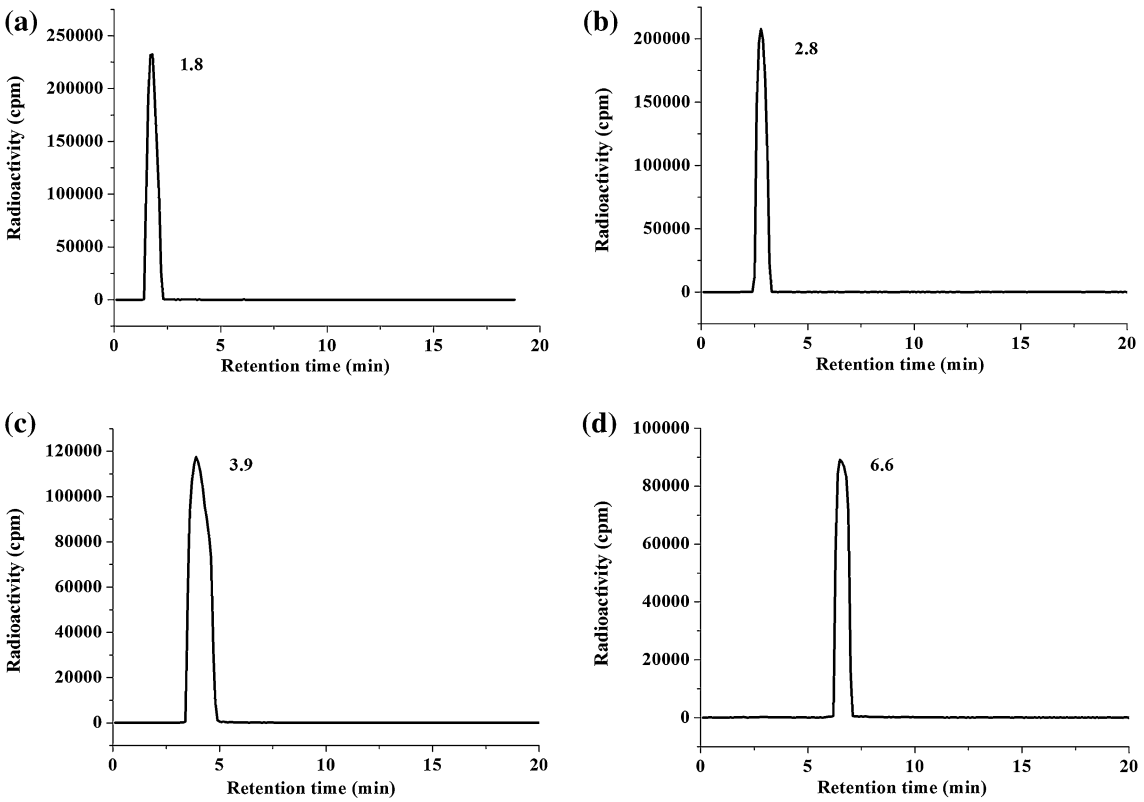


Fig. 2 HPLC chromatograms of ^{99m}Tc-labeled complexes: (**a**) ^{99m}Tc-sodium tartrate, (**b**) $^{99m}TcO_4^-$, (**c**) ^{99m}Tc-BnAO and (**d**) ^{99m}Tc-BnAO–NT

2.4 Stability, protein binding, partition coefficient and electrical property

2.4.1 Stability

In vitro stability was determined in phosphate buffer and mouse serum. The labeled complex ^{99m}Tc-BnAO–NT was dissolved in the phosphate buffer (0.1 M, pH 7.4) and incubated at room temperature after preparation. Certain aliquots of samples were taken at different time points (1, 2, 8 h), and the radiochemical purity was analyzed by HPLC.

The stability of the complex ^{99m}Tc-BnAO–NT in mouse serum was also determined for 8 h with a similar method as reported [23]. The labeled complex (0.1 mL) was dissolved in 1 mL mouse serum and incubated at 37 °C after preparation. Then certain samples were taken at different time points (1, 2, 8 h). Ethanol (200 μL) was added to each sample to precipitate the protein. The samples were centrifuged, filtrated by 0.22-μm filter, and finally, the supernatant was tested by HPLC.

2.4.2 Protein binding

The serum protein binding experiment was investigated along with that of the stability in mouse serum [23]. The radioactivity of the full sample was A_{protein}, and that of the supernatant was B_{protein}. The percentage of protein binding was calculated as $(A_{\text{protein}} - B_{\text{protein}})/A_{\text{protein}}$.

2.4.3 Octanol/water partition coefficient ($P_{o/w}$)

Octanol (1 mL), ^{99m}Tc-labeling solution (0.5 mL) and saline (0.5 mL) were combined in a 5-mL centrifugal tube. The tube was vigorously vortexed for 5 min and centrifuged at 3000 rpm for another 5 min. The radioactivities of a 100 μL solution of each phase were measured. The partition coefficient $P_{o/w}$ was the ratio of radioactivity of the octanol layer to that of the water layer. The process was repeated three times.

2.4.4 Paper electrophoresis

The experiment was taken by the Whatman 3 paper using the method as reported [24]. The labeling complex was put in the middle of the paper, and after the solvent was dried, the paper was placed in the phosphate buffer solution (pH 7.4, 0.05 M) under a voltage of 200 V. One hour later, the paper was taken out, dried, cut into sections of 1 cm and counted for radioactivity. Therefore, the paper electrophoresis map was obtained.

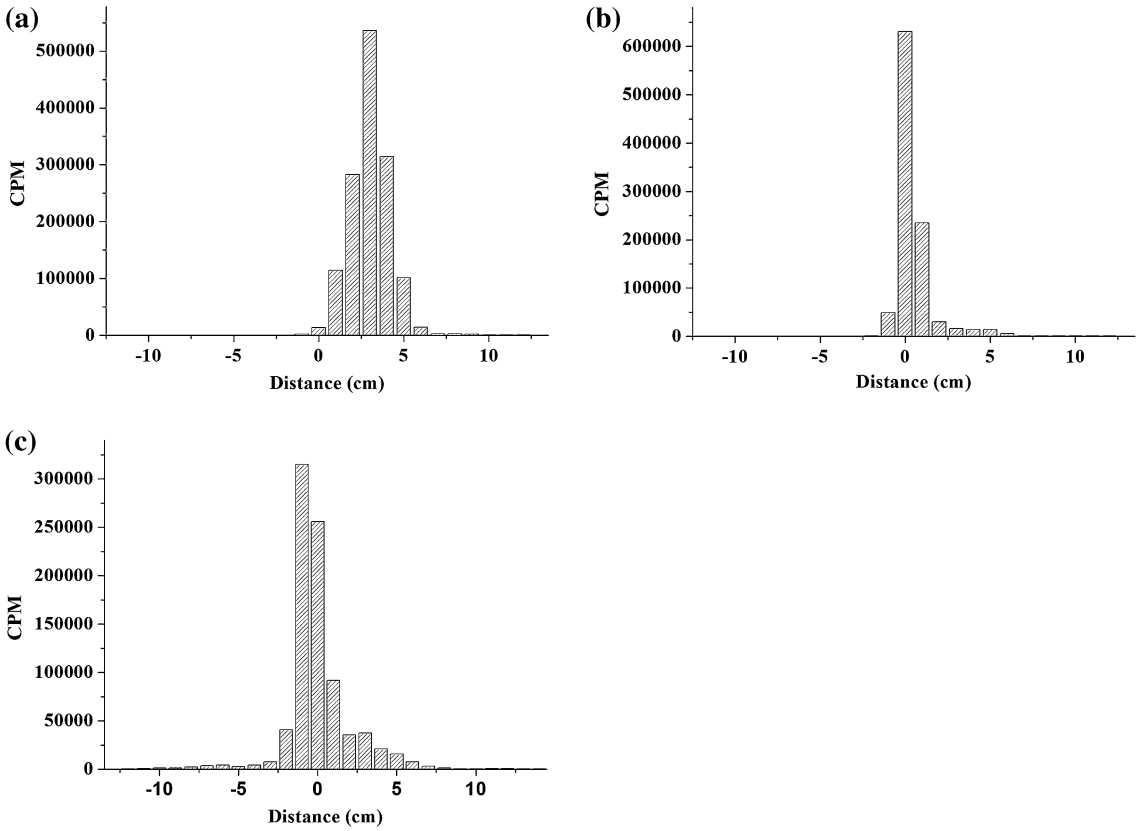


Fig. 3 Paper electrophoresis patterns of the complexes: (**a**) $^{99m}TcO_4^-$, (**b**) ^{99m}Tc-BnAO and (**c**) ^{99m}Tc-BnAO–NT

2.5 In vitro study

In vitro cellular uptake experiments were performed according to the literature methods [20, 22]. The S180 or H22 cells were suspended in fresh DMEM medium with 10 % (v/v) fetal bovine serum at a cell concentration of approximately 10^6 cells/mL. Aliquots of 20 mL were placed into glass vials and then incubated at 37 °C with gentle magnetic stirring under normoxic (95 % air with 5 % carbon dioxide) or hypoxic (95 % nitrogen with 5 % carbon dioxide) conditions. In hypoxic condition, the oxygen concentration was 0.00 mg/L after 60 min equilibration, which was determined by the JPSJ-605 dissolved oxygen meter. The ^{99m}Tc-labeling complex was added to each glass vial at an activity of approximately 0.1 MBq/mL, and the concentration was roughly 1 μg/mL. More than 1 mL of the sample was removed every 30 min. For each sample, five 200-μL aliquots were pipetted and centrifuged at 1500 rpm for 5 min. One hundred and sixty microliters aliquot of supernatant was removed and measured. The radioactivity of the supernatant was marked as A_{cell}, and that of the residue containing cells and medium was regarded as B_{cell}. The cell uptakes were calculated as % uptake $= [(B_{cell} - A_{cell}/4)/(A_{cell} + B_{cell})] \times 100$ %. The cells viability was determined by the trypan blue exclusion assay for 4 h.

2.6 Biodistribution study

All the animal experiments were performed in accordance with the national laws related to the conduct of animal experimentation. Hypodermic injection of approximately 10^6 cells into the left front leg of male Kunming mice was performed to establish the S180 tumor model. Tumors grew to diameters of 10 mm during 8–10 days. The complex ^{99m}Tc-BnAO–NT was administered by tail vein injection (about 0.1 MBq, 0.1 mL, 1.5 μg). Five mice were killed at 0.5, 1, 2, 4 and 8 h after injection. Various organs and tissues (blood, brain, muscle, tumor, heart, stomach, kidney, spleen, intestine, liver and lung) were excised, washed, weighed and counted for radioactivity in a gamma counter. The percent of injected dose per gram (% ID/g) was calculated, and the final results were expressed as mean ± SD (standard deviation).

3 Results and discussion

3.1 Radiolabeling

The labeling yields of the ^{99m}Tc-complexes were tested using the radio high-performance liquid chromatography.

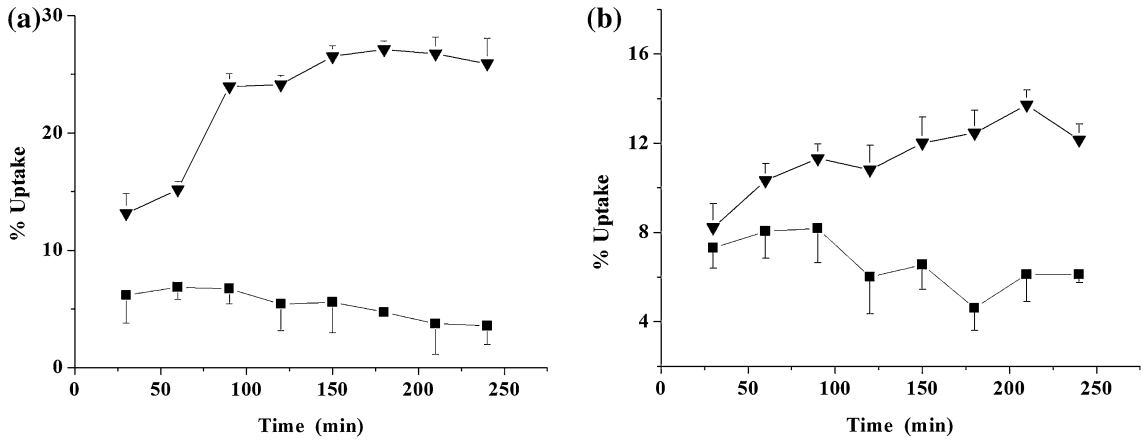


Fig. 4 Uptake of ^{99m}Tc-BnAO–NT in S180 (**a**) and H22 (**b**) cells under hypoxic conditions (*down triangle*), normoxic conditions (*square*)

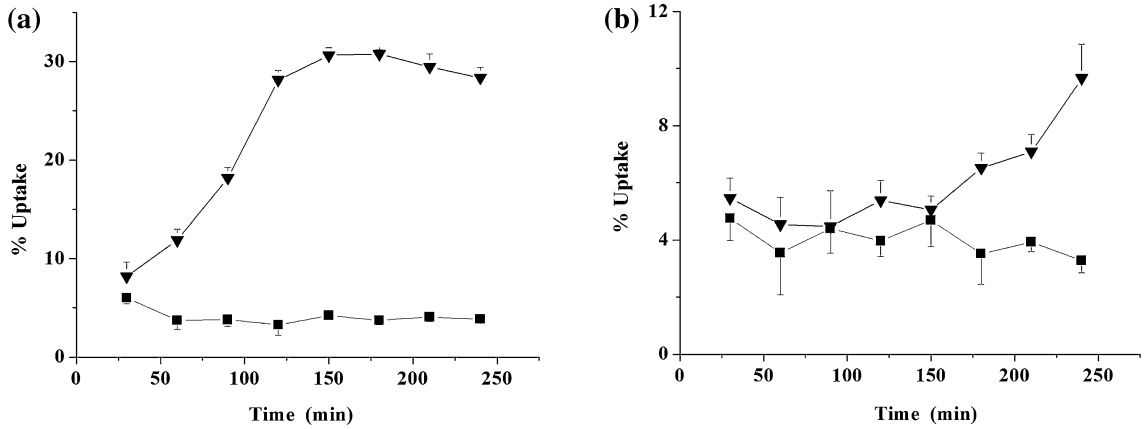


Fig. 5 Uptake of ^{99m}Tc-BnAO in S180 (**a**) and H22 (**b**) cells under hypoxic conditions (*down triangle*), normoxic conditions (*square*)

The retention times of ^{99m}Tc-sodium tartrate and $^{99m}TcO_4^-$ were 1.8 and 2.8 min, respectively, while the radioactivity signal of ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NT showed a distinct peak at 3.9 and 6.6 min, respectively. The labeling yields of ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO were both above 98 %.

3.2 Stability, protein binding, partition coefficient and electrical property

^{99m}Tc-BnAO–NT was observed to be stable in the prepared medium and in mouse serum as their radiochemical purities remained above 95 % for up to 8 h. The protein binding ratios of ^{99m}Tc-BnAO–NT were 30.49, 32.68 and 27.39 % at 1, 2 and 8 h, respectively. The octanol/water partition coefficient of ^{99m}Tc-BnAO–NT was 0.26 ± 0.01, compared with 0.089 ± 0.007 for ^{99m}Tc-BnAO [21], which demonstrated that ^{99m}Tc-BnAO–NT was more lipophilic than ^{99m}Tc-BnAO.

The results of the paper electrophoresis of $^{99m}TcO_4^-$, ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NT are presented in Fig. 3. As $^{99m}TcO_4^-$ was negatively charged, it shifted toward the anode. The paper electrophoresis demonstrated that ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NT were neutral under physiological condition.

3.3 In vitro study

In vitro studies of ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO were performed using the S180 and H22 cell lines. Hepatocarcinoma H22, liver cancer cells, is one of widely used tumor models [25, 26]. Cellular uptakes of ^{99m}Tc-BnAO–NT under normoxic and hypoxic conditions are plotted versus time in Fig. 4.

As for the S180 and H22 cell lines, the significant differences in cellular uptake between normoxic and hypoxic conditions were observed. The maximum hypoxic cellular uptakes of ^{99m}Tc-BnAO–NT under normoxic conditions

Table 1 Biodistribution of ^{99m}Tc-BnAO–NT in mice bearing S180 tumor (% ID/g)

Tissue	0.5 h	1 h	2 h	4 h	8 h
Blood	0.96 ± 0.54	1.00 ± 0.37	0.80 ± 0.17	0.64 ± 0.10	0.52 ± 0.21
Brain	0.13 ± 0.03	0.16 ± 0.05	0.20 ± 0.03	0.22 ± 0.04	0.19 ± 0.02
Muscle	0.47 ± 0.11	0.71 ± 0.28	1.03 ± 0.15	0.42 ± 0.15	0.32 ± 0.08
Tumor	0.97 ± 0.48	1.52 ± 0.57	1.78 ± 0.26	1.35 ± 0.41	0.72 ± 0.19
Heart	0.70 ± 0.20	0.90 ± 0.23	1.21 ± 0.36	1.31 ± 0.28	0.72 ± 0.14
Stomach	0.67 ± 0.32	1.14 ± 0.38	2.00 ± 0.48	1.70 ± 0.50	1.01 ± 0.14
Spleen	2.03 ± 1.13	3.17 ± 1.41	4.85 ± 0.17	1.81 ± 0.42	0.91 ± 0.28
Intestine	1.42 ± 0.55	3.54 ± 0.17	3.23 ± 0.79	2.88 ± 0.65	1.85 ± 0.37
Kidney	0.56 ± 0.22	0.79 ± 0.27	0.99 ± 0.25	0.77 ± 0.30	0.49 ± 0.18
Lung	0.88 ± 0.19	1.31 ± 0.39	1.32 ± 0.22	1.29 ± 0.27	0.57 ± 0.12
Liver	2.91 ± 1.42	7.07 ± 2.02	4.56 ± 0.58	4.15 ± 0.15	2.32 ± 0.92
T/B	1.10 ± 0.46	1.52 ± 0.49	1.96 ± 1.11	2.31 ± 0.34	1.49 ± 0.29
T/M	1.74 ± 0.72	2.22 ± 0.93	1.75 ± 0.37	3.79 ± 0.98	2.29 ± 0.59

were 27.11 ± 0.73 and 14.85 ± 0.83 % for S180 and H22 cells, respectively. The maximum hypoxic uptake in H22 cells was only half of that in S180 cells. In S180 cells, the initial hypoxic cellular uptake was 13.15 ± 1.68 % at 30 min, and then, it reached up to 15.12 ± 0.68, 23.95 ± 1.09 and 27.11 ± 0.73 % at 60, 90 and 180 min. However, normoxic cellular uptake was 6.16 ± 2.38 % at 30 min, and there was little significant increase over 4 h. Under hypoxic condition, the initial cellular uptake in H22 cells was 7.77 ± 1.73 % at 30 min, and then, it reached up to 11.64 ± 0.82, 12.51 ± 1.44 and 14.85 ± 0.83 % at 90, 150 and 210 min. Under normoxic conditions, the cellular uptakes of ^{99m}Tc-BnAO–NT fluctuated with time and there was no fixed trend.

^{99m}Tc-BnAO, the complex containing no nitrotriazole, showed a significant difference in cellular uptake between normoxic and hypoxic uptakes in KHT sarcoma cells [21]. In this work, an in vitro study of ^{99m}Tc-BnAO was also carried out using S180 and H22 cell lines. According to Fig. 5, the uptake of ^{99m}Tc-BnAO steadily increased with time under hypoxic condition, but fluctuated with time at the range about 5 % under normoxic condition. At the time of 30 min, the uptake was only about 8.18 ± 1.47 % in hypoxic cells. Subsequently, there was a significant increase over 4 h. The uptakes were 11.89 ± 1.12, 18.18 ± 1.07 and 28.13 ± 0.97 % at 60, 90 and 120 min. Then, there was a slight increase, and maximum uptake was about 30.79 ± 0.44 % at 180 min. Under normoxic conditions, the uptake was 6.01 ± 0.62 % at 30 min, and there was little significant increase over 4 h. It was shown that ^{99m}Tc-BnAO was enriched in hypoxic S180 cells selectively. However, the result was not satisfactory like that of ^{99m}Tc-BnAO–NT in H22 cells. The difference between normoxic and hypoxic uptakes was not observed in the initial 150 min, and the maximum hypoxic uptake was only 9.66 ± 1.20 % at 240 min.

The results exhibited that ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO selectively accumulated in hypoxic S180 and H22 cells, although the results in hypoxic H22 cells were unsatisfactory. Some studies have explored the cell dependence of hypoxia markers. For example, the uptake and retention of ^{64}Cu-ATSM in MDA468, FaDu and R3327-AT cells was cell line-dependent [27]. Hoigebazar et al. [28] also determined the uptake of ^{68}Ga-labeled DOTA-nitroimidazole derivatives in the Hela, CHO and CT-26 cell lines, and CT-26 showed the greatest radiotracer uptake. However, the cell dependence of hypoxia markers in S180 and H22 cells has rarely been reported. According to Figs. 4 and 5, it could be seen that the cell lines dramatically affected the cellular uptake under hypoxic conditions, but weakly affected the cellular uptake under normoxic conditions. The difference between S180 and H22 cells suggested that the hypoxic cellular uptake of ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO was cell-dependent, and S180 cells may be better than H22 cells in the term of hypoxia evaluation.

For the uptake of ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NT in S180 cells, their maximum hypoxic cells were 30.79 ± 0.44 and 27.11 ± 0.73 %, respectively. Obviously, the nitrotriazole may not play the key role in the cells' uptake of ^{99m}Tc-BnAO–NT. The conclusion was similar to that of ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NI described by Hsia et al. [21]. In their work, the cellular uptake of ^{99m}Tc-BnAO–NI in KHT cells was lower than that of ^{99m}Tc-BnAO.

3.4 Biodistribution study

During in vitro study, the radiotracer uptake was higher for S180 cells than H22 cells, thus the investigation of the biodistribution of ^{99m}Tc-BnAO–NT in Kunming male mice bearing a S180 tumor was carried out. The biodistribution results are listed in Table 1.

The radioactivities of complex ^{99m}Tc-BnAO–NT in the liver, spleen, intestine and kidney were clearly higher than that in other tissues, which was probably related to its lipophilicity, and this result suggested that the complex was cleared away through both the hepatobiliary pathway and urinary pathway. As we could see in Table 1, the tumor uptake increased in the first 2 h and then decreased in the subsequent 4 h: 0.5 h after injection, the tumor uptake was 0.97 ± 0.48 % ID/g, and increased to 1.78 ± 0.26 % ID/g at 2 h, and then decreased to 0.72 ± 0.19 % ID/g at 8 h. The complex may undergo an enrichment and then clearance process. The tumor-to-blood ratio was 2.31 ± 0.34, and tumor-to-muscle ratio was 3.79 ± 0.98 at 4 h. The results showed that ^{99m}Tc-BnAO–NT had high tumor specificity. The results were similar to that of ^{99m}Tc-BnAO [24] using the same S180 tumor. The maximum tumor uptake of ^{99m}Tc-BnAO was 1.60 ± 0.35 % ID/g at 0.5 h. The tumor-to-blood ratio was 2.40 ± 1.04, and the tumor-to-muscle ratio was 3.94 ± 1.25 at 4 h. The tumor specificity of ^{99m}Tc-BnAO–NT did not significantly increase after introducing the redox center 3-nitro-1,2,4-triazole.

4 Conclusion

This study demonstrated that ^{99m}Tc-BnAO–NT and ^{99m}Tc-BnAO could accumulate in the S180 and H22 cell lines under hypoxic conditions, especially in the S180 cells. When combined with 3-nitro-1,2,4-triazole, ^{99m}Tc-BnAO–NT was more lipophilic than ^{99m}Tc-BnAO. However, ^{99m}Tc-BnAO–NT did not exhibit higher cellular uptake in vitro and the biodistribution of ^{99m}Tc-BnAO and ^{99m}Tc-BnAO–NT were similar. Therefore, 3-nitro-1,2,4-triazole moiety did not have a significant influence on the hypoxic uptake of ^{99m}Tc-BnAO–NT. Both in vitro and in vivo studies hinted that ^{99m}Tc-BnAO–NT may be a hypoxia-sensitive radio probe for monitoring hypoxic regions in the sarcoma S180 tumor model.

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