J Radioanal Nucl Chem (2015) 306:477–482
DOI 10.1007/s10967-015-4109-2

Synthesis and evaluation of a ^{99m}Tc-labeled deoxyglucose derivative as a potential agent to target tumor

Yue Wang[1] · Xiaoqing Song[1] · Junbo Zhang[1]

Received: 24 January 2015 / Published online: 2 April 2015

Abstract In this study, ethylene diamine tetraacetic acid-deoxyglucose (EDTADG) ligand was successfully synthesized and then radiolabeled with ^{99m}Tc directly to produce ^{99m}Tc-EDTADG with high radiochemical purity. ^{99m}Tc-EDTADG showed good in vitro stability. The partition coefficient and electrophoresis results showed it was hydrophilic and negatively charged. The biodistribution study in mice bearing S180 tumor showed that ^{99m}Tc-EDTADG had high accumulation in tumor tissue with high tumor-to-muscle and tumor-to-blood ratios. Single photon emission computed tomography imaging clearly visualized the tumor, suggesting it could be considered as a potential agent for tumor imaging.

Keywords D-glucose derivative · ^{99m}Tc · SPECT · Tumor diagnosis

Introduction

^{18}F-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), an analogue of glucose, can enter the cell membrane using the same transporters as glucose. It is phosphorylated by hexokinase to ^{18}F-FDG-6-phosphate. This metabolite does not undergo further metabolism and hence is trapped in the cell. Because of its property of trapping in the cells, [^{18}F]FDG has been wildly used to diagnose cancer as a positron emission tomography (PET) radiopharmaceutical [1–4]. Although [^{18}F]FDG is widely used in PET imaging for diagnosis of some kinds of tumor, the use of [^{18}F]FDG for PET imaging in clinical practice is still limited by factors such as limited availability and high cost. Meanwhile, generator produced isotope, such as ^{99m}Tc, has inexpensive cost and suitable physical and chemical characteristics. Moreover, the availability of a generator and kit chemistry for preparing ^{99m}Tc radiopharmaceuticals may have a great impact on nuclear medicine. Thus, using ^{99m}Tc to label glucose derivatives is one of the major focuses of ongoing research. To date, some ^{99m}Tc labeled glucose derivatives have been synthesized and evaluated as tumor imaging agents [5–15]. Among them, ^{99m}Tc-ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG) exhibits the most promising property for tumor imaging and enters the Phase II clinical trial studies. However, the tumor-to-background ratio of ^{99m}Tc-ECDG is unsatisfactory. Therefore, further investigation to discover novel ^{99m}Tc labeled glucose analogues for tumor imaging is still necessary.

Because nonfunctionalized glucose compounds are generally weak ligands for complexing with ^{99m}Tc, structure modified with D-glucose or D-glucosamine was the reasonable way to develop new ^{99m}Tc labeled glucose derivatives as tumor imaging agents. It is known that ethylene diamine tetraacetic acid (EDTA) is a common and cheap chelator and can be used as a bifunctional chelating agent (BFCA) to form ^{99m}Tc complex on the basis of efficient binding of ^{99m}Tc to free carboxylic groups. The purpose of this study was to covalently couple EDTA bisanhydride with the free amino group of glucosamine to form the ethylene diamine tetraacetic acid-deoxyglucose (EDTADG) ligand and to evaluate the feasibility of ^{99m}Tc-EDTADG as a potential tumor imaging agent.

✉ Junbo Zhang
zhjunbo@bnu.edu.cn

[1] Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China

Experimental

Materials and methods

D-glucosamine hydrochloride was purchased from J&K CHEMICA, 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. ESI-MS spectrum was recorded on a LC–MS Shimadzu 2010 series. Elemental analyses were performed on a Vario EL elemental analyzer model. HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5 μm, 250 × 4.6 mm), Shimadzu LC-20AT series.

Synthesis of EDTADG

215 mg D-glucosamine hydrochloride was dissovled in 3 mL water to a flask, and then 0.15 mL triethylamine was added. 128 mg ethylene diamine tetraacetic acid dianhydride was added into the solution successively and reacted in 50 °C oil bath for 6 h. The mixture was purified by chromatography on G15 Sephadex using water as eluent to obtain EDTADG water solution, and then the solid product (300 mg) was obtained by lyophilization.

The ligand EDTADG product was characterized by FT-IR (KBr), 400 M ^{1}HNMR spectroscopy, ESI-MS and elemental analysis. The solvent of NMR spectrum analysis was D_2O.

Radiolabeling of EDTADG and quality control techniques

25 mg of EDTADG was dissolved in 1 mL of saline in a 10 mL vial, then 50 μL of $SnCl_2{\cdot}2H_2O$/HCl solution (4 mg $SnCl_2{\cdot}2H_2O$/1 mL 1 N HCl) was added into the vial. After adjusting the pH of the above solution to 7–8 with NaOH solution, 1 mL of saline containing $^{99m}TcO_4^-$ (370 MBq) was added. Then the resulting solution was heated at 100 °C for 20 min. The radiochemical purity (RCP) of the product was identified by thin layer chromatography (TLC) by using a polyamide strip as a stationary phase and saline as eluent. High performance liquid chromatography (HPLC) analysis was carried out with a reversed-phase column (Kromasil 100-5 μm, 250 × 4.6 mm) on the Shimadzu LC-20AT at a flow rate of 1.0 mL/min. 10 % acetonitrile in a phosphate-buffered saline (pH 7.8) was used as the mobile phase.

In vitro stability study

The stability of the complex was assayed by measuring the RCP in the reaction mixture for 6 h at room temperature (25 °C). To estimate the serum stability of ^{99m}Tc-EDTADG, 0.1 mL of ^{99m}Tc-EDTADG was incubated in 0.6 mL of mouse serum at 37 °C for 4 h and then the RCP of the complex was analyzed by TLC.

Human serum albumin binding assay

10 μL of ^{99m}Tc-EDTADG (370 KBq) was added in 100 μL human serum albumin (100 mg/mL) in the centrifuge tube. After incubation at 37 °C for 2 h, the serum protein was precipitated by adding 1 mL trichloroacetic acid (250 mg/mL) to the mixture. The supernatant and precipitate were separated by centrifugation at 2000 g for 5 min. Then the radioactivity of each phase was measured separately. This experimental procedure was repeated three times and the percentage of human serum protein binding was determined as the following equation:

$$\text{Serum protein binding } \% = (\text{counts per minute in precipitate})/(\text{counts per minute in precipitate} + \text{counts per minute in supernatant}) \times 100\,\%.$$

Determination of the partition coefficient

The partition coefficient was determined by mixing ^{99m}Tc-EDTADG with an equal volume of 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at $5000\times g$ for 5 min. From each phase 0.1 mL of the aliquot was pipetted and counted in a well γ-counter. Each measurement was repeated three times. The partition coefficient, P, was calculated using the following equation:

$$P = (\text{cpm in octanol} - \text{cpm in background})/(\text{cpm in buffer} - \text{cpm in background})$$

Usually the final partition coefficient value was expressed as log *P*.

Paper electrophoresis

1 μL of ^{99m}Tc-EDTADG was spotted at the center of the Whatman No. 1 chromatography paper strips (15 × 1 cm), which were impregnated with the phosphate buffer (0.025 mol/L pH 7.4). The analyses were carried out using phosphate buffer at 150 V for 2 h. The developed paper strips were dried, and the distribution of radioactivity on the strip was measured.

Cell uptake assay

To evaluate if the uptake of ^{99m}Tc-EDTADG in cells is mediated by means of a D-glucose mechanism, various concentrations of D-glucose (1 and 10 mg) was added to each culture tube that containing 8 mL (2×10^6 cells/mL) murine sarcoma S180 tumor cell suspension. After adding ^{99m}Tc-EDTADG, the mixtures were incubated during 2 h and the cells were centrifuged at $2000 \times g$ for 5 min. The supernatant was removed and the percentage of cell uptake is calculated as residue counts/total counts × 100 %.

Biodistribution study

In vivo growth was initiated by hypodermic injection of approximately 2×10^6 S180 cells into the left front leg of male mice. Over the course of 7 days, tumors grew to diameters of 10–15 mm. 0.1 mL of ^{99m}Tc-EDTADG (7.4×10^5 Bq) was injected into the mice bearing S180 tumor via a tail vein. The mice were sacrificed at 0.5, 2 and 4 h post-injection. The tumor, other organs of interest and blood were collected, weighed and measured for radioactivity. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID/g). All biodistribution studies were performed in compliance with the national laws related to the conduct of animal experimentation.

SPECT image study

A dual-head single photon emission computed tomography (SPECT) (Skylight; Philips, Milpitas, CA, USA), using a low-energy parallel-hole collimator, was used for imaging studies. 0.1 mL of ^{99m}Tc-EDTADG (18.5 MBq) was injected intravenously through tail vein in mice (18–20 g) bearing S180 tumor. At 30 min after injection, the mice were anaesthetized with sodium pentobarbital for SPECT image study. Static images were acquired using 128 × 128 matrix for 20 min. The region of interest (ROI) between tumor tissue and contralateral muscle (background) was used to determine tumor-to-background ratio.

Results and discussion

Synthesis and radiolabeling

EDTADG was prepared by reacting D-glucosamine with ethylenediaminetetraacetic dianhydride in the presence of triethylamine. The reaction equation is shown in Scheme 1. It was characterized by IR, ^{1}H NMR, Elemental analysis, MS. IR (KBr)/cm^{-1}: 3336, 2975, 1384 (N–H); 3336 (O–H); 1633 (C = O); 1041 (C–O–C). ^{1}HNMR (D_2O), δ: 5.09 (d, $J = 3.42$ Hz, αH-1′a), 5.07 (d, $J = 3.42$ Hz, αH-1′b), 4.46 (d, $J = 8.17$ Hz, βH-1′), 3.81–3.52(m, 5H), 3.40 (s, 2H), 3.27–3.09 (m, 3H), 2.96 (s, 1H), 2.62–2.58(m, 1H). Elemental analysis: Anal. Calcd for $C_{22}H_{38}N_4O_{16}\cdot 3H_2O$: C 39.75; H 7.16; N 8.06. Found: C 39.52; H 6.63; N 8.38. MS (ESI): m/z 615.3, $[M + H]^+$.

The preparation of ^{99m}Tc-EDTADG was based on the reaction of $[^{99m}TcO_4]^-$ with EDTADG in the presence of stannous chloride as reducing agent to produce the final product. The RCP of ^{99m}Tc-EDTADG was more than 90 % by TLC. $^{99m}TcO_4^-$ and $^{99m}TcO_2.nH_2O$ stayed at the origin spot while ^{99m}Tc-EDTADG migrated to $R_f = 0.8$–1.0. The RCP of the complex was also checked by HPLC. The retention time of ^{99m}Tc-EDTADG was 2.9 min (Fig. 1), while that of $^{99m}TcO_4^-$ was found to be 5.8 min. A single product with high RCP (>95 %) was obtained.

Stability study

^{99m}Tc-EDTADG was stable over 6 h in the reaction mixture at room temperature. On the other hand, in mouse serum at 37 °C, the RCP of the complex was more than 90 % even up to 4 h after synthesis (Fig. 2), suggesting ^{99m}Tc-EDTADG had good in vitro stability.

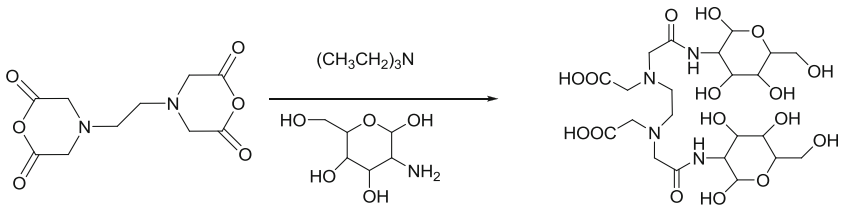
Human serum albumin binding assay

As described in the experiment of human serum albumin binding assay [16, 17], the percentage of human serum protein binding ability for ^{99m}Tc-EDTADG was measured. The result showed that the percentage of human serum protein binding of ^{99m}Tc-EDTADG was 23.51 ± 1.14 %. It is pertinent to note that, when compared with ^{99m}Tc-CPADG (CPADG: 2-[(3-carboxy-1-oxopropyl)amino]-2-deoxy-D-glucose [14]) (19.33 ± 2.86 %), there was no great difference between the two complexes ($P > 0.05$). Moreover, the percentage of human serum protein binding for ^{99m}Tc-EDTADG was much lower than the values of ^{99m}TcN-PRODTC (PRODTC: proline dithiocarbamate, 87.42 ± 0.01 %) [16], ^{99m}TcO-PHEDTC (PHEDTC: phenylalanine dithiocarbamate, 85.68 ± 0.61 %) [17] and ^{99m}TcN-PHEDTC (86.32 ± 0.19 %) [17].

Partition coefficient (log *P*)

The log P value of ^{99m}Tc-EDTADG was found to be −2.54 ± 0.06, indicating it was hydrophilic. Moreover, ^{99m}Tc-EDTADG was more hydrophilic than ^{99m}Tc-CPADG (log P: −2.41), ^{99m}TcN-DGDTC (DGDTC: deoxyglucose dithiocarbamate [11]) (log P: −1.30), $^{99m}Tc(CO)_3$-DGDTC (log P: −0.96) and ^{99m}TcO-DGDTC (log P: −0.73). A higher hydrophilicity of ^{99m}Tc-EDTADG may possibly be related to its structure containing more sugar molecules.

Scheme 1 Synthesis of EDTADG



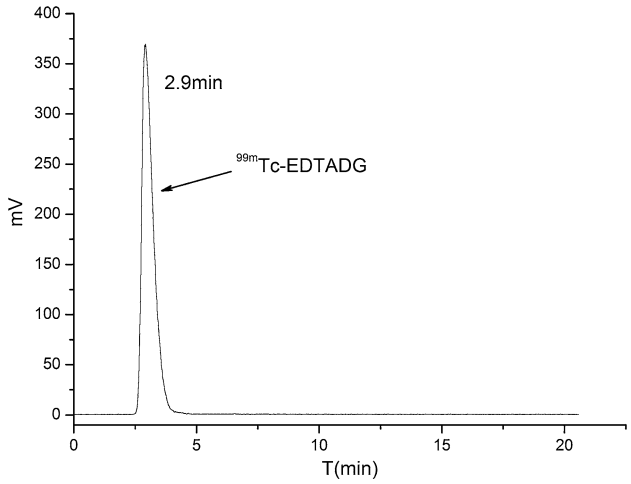


Fig. 1 HPLC pattern of ^{99m}Tc-EDTADG

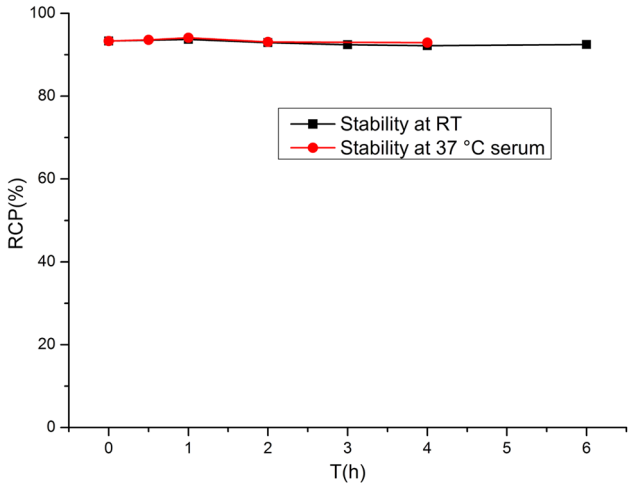


Fig. 2 Stability study of ^{99m}Tc-EDTADG

Paper electrophoresis

Nearly 90 % of radioactivity of ^{99m}Tc-EDTADG was moved to anode, suggesting it was negative charged.

Cell uptake assay

In order to determine whether the uptake of ^{99m}Tc-EDTADG via glucose transport pathyway, we carried out the blocking experiment using D-glucose according to the Ref. [6]. Unfortunately, adding D-glucose at a concentration of 1 and 10 mg per tube, ^{99m}Tc-EDTADG showed similar or slightly higher tumor cell uptake at 1 and 10 mg D-glucose (0.89 ± 0.05 and 0.86 ± 0.08 %) than in glucose-free medium(0.76 ± 0.07 %). These results suggest that, although ^{99m}Tc-EDTADG is a glucose analogue, its uptake mechanism is not transported via the glucose transporters.

Biodistribution study

The result of biodistribution of ^{99m}Tc-EDTADG in mice bearing S180 tumor is shown in Table 1. Results of biodistribution of recently reported ^{99m}Tc labeled glucose analogues as tumor imaging agents are shown in Table 2 for comparison. As described in Table 1, the tumor uptakes of ^{99m}Tc-EDTADG are 2.25 ± 0.28, 1.11 ± 0.14, and 1.02 ± 0.13 % ID/g at 0.5, 2 and 4 h post-injection, respectively. The tumor-to-muscle ratio can maintain a high level (3.46, 2.96, 3.34 at 0.5, 2 and 4 h post-injection, respectively). The tumor-to-blood ratio increases with time

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

Tissue	30 min	2 h	4 h
Heart	0.71 ± 0.09	0.31 ± 0.03	0.28 ± 0.03
Liver	0.75 ± 0.05	0.37 ± 0.06	0.28 ± 0.03
Lung	1.50 ± 0.31	0.42 ± 0.04	0.37 ± 0.05
Kidney	6.34 ± 0.65	2.50 ± 0.23	2.12 ± 0.23
Spleen	0.65 ± 0.14	0.32 ± 0.06	0.27 ± 0.04
Stomach	0.40 ± 0.18	0.24 ± 0.10	0.20 ± 0.12
Bone	1.01 ± 0.21	0.56 ± 0.11	0.53 ± 0.06
Muscle	0.65 ± 0.14	0.37 ± 0.10	0.31 ± 0.06
Intestine	2.03 ± 0.63	0.76 ± 0.25	0.69 ± 0.21
Tumor	2.25 ± 0.28	1.11 ± 0.14	1.02 ± 0.13
Blood	1.56 ± 0.12	0.39 ± 0.03	0.31 ± 0.03
T/N ratio	3.46	2.96	3.34
T/B ratio	1.45	2.84	3.30

All the data are the mean percentage ($n = 5$) of the injected dose of ^{99m}Tc-EDTADG per gram of tissue, ±the standard deviation of the mean

T/*N* tumor to muscle, *T*/*B* tumor to blood

Table 2 Comparison of biodistribution of ^{99m}Tc-EDTADG with some ^{99m}Tc glucose derivatives

Complex	^{99m}Tc-EDTADG	^{99m}Tc-CPADG	^{99m}TcN-DGDTC	^{99m}Tc(CO)$_3$-DGDTC	^{99m}TcO-DGDTC
Animal	Kunming mice	Kunming mice	Kunming mice	Kunming mice	Kunming mice
Tumor type	S180 tumor	S180 tumor	S180 tumor	S180 tumor	S180 tumor
Time p.i. (h)	4	4	4	4	4
Log P	−2.54	−2.41	−1.30	−0.96	−0.73
Tumor uptake (%ID/g)	1.02 ± 0.13	1.01 ± 0.06	1.16 ± 0.57	2.77 ± 0.11	3.53 ± 0.85
Liver uptake (%ID/g)	0.28 ± 0.03	1.01 ± 0.12	2.00 ± 0.34	4.08 ± 0.38	15.29 ± 5.22
Lung uptake (%ID/g)	0.37 ± 0.05	0.46 ± 0.05	1.49 ± 0.14	2.23 ± 0.66	8.03 ± 2.09
Kidney uptake (%ID/g)	2.12 ± 0.23	5.63 ± 0.96	7.71 ± 1.40	5.21 ± 0.51	15.12 ± 3.56
T/N ratio	3.34	5.05	1.68	7.29	4.21
T/B ratio	3.30	1.91	2.32	1.67	0.76
Reference	Present study	[14]	[11]	[13]	[12]

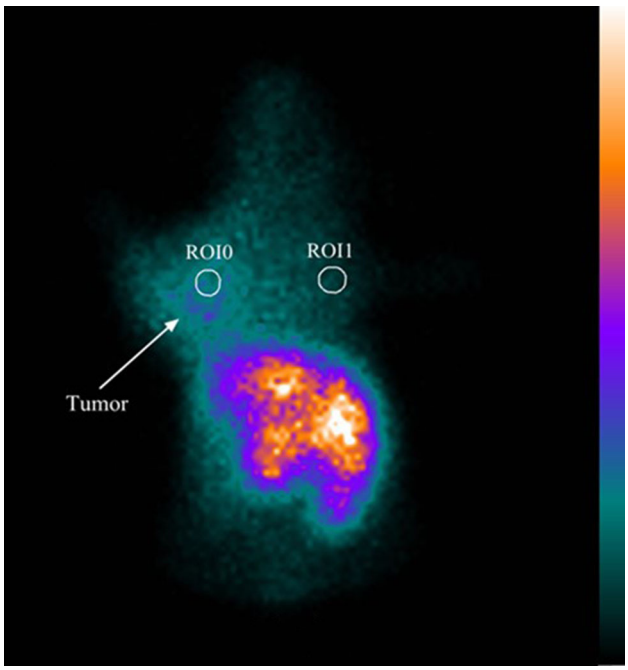


Fig. 3 SPECT image of ^{99m}Tc-EDTADG in S180 tumor-bearing mice

and can reach 3.30 at 4 h post-injection because of the faster clearance of the blood. The kidney uptake is relatively high, suggesting the main routes of excretion is via the urinary tract. Low uptake in the stomach is suggestive of in vivo stability of ^{99m}Tc-EDTADG.

By comparison (Table 2), interestingly, among the five complexes, ^{99m}Tc-EDTADG is more hydrophilic than the others, thus possibly making the former much lower liver uptakes than the latter. Moreover, the lung and kidney uptakes of ^{99m}Tc-EDTADG are also lower so that a better target to non-target ratio may be obtained. Among them, the T/B ratio of ^{99m}Tc-EDTADG is the highest. From the above point of views, ^{99m}Tc-EDTADG shows the very promising properties as a potential tumor imaging agent.

SPECT image study

The SPECT imaging results showed the tumor uptake was visible (Fig. 3). The ROI ratio of ^{99m}Tc-EDTADG uptake for the tumor site versus the corresponding non-tumor region (T/NT ratio) was 2.79, suggesting it would be considered as a potential tumor imaging agent. The imaging findings were in keeping with the biodistribution results in mice. Better scintigraphic image should be acquired by using micro-SPECT on mice imaging in due course.

Conclusions

In summary, EDTADG ligand was successfully synthesized and its ^{99m}Tc complex was achieved in high yield. The high tumor localization, low background uptake, high tumor/blood and tumor/muscle ratios of the complex in mice exhibited promising properties, suggesting the possibility of ^{99m}Tc-EDTADG as a novel agent to target tumor.

Acknowledgments The work was financially supported, in part, by National Natural Science Foundation of China (21171024, 81101069), Beijing Natural Science Foundation (7112035).

References

1. Jaini S, Dadachova E (2012) FDG for therapy of metabolically active tumors. Semin Nucl Med 42:185–189
2. Yang DJ, Kong F-L, Oka T, Bryant JL (2012) Molecular imaging kits for hexosamine biosynthetic pathway in oncology. Curr Med Chem 19:3310–3314

3. Goreti RM, Falconer RA, Santos I (2013) Carbohydrate-based molecules for molecular imaging in nuclear medicine. Eur J Org Chem 2013:1401–1414
4. Sun YY, Chen Y (2009) Cancer drug development using glucose metabolism radiopharmaceuticals. Curr Pharm Des 15:983–987
5. Liu TL, Zhang JB, Wang XB, Yang JG, Tang ZG, Lu J (2014) Radiolabeled glucose derivatives for tumor imaging using SPECT and PET. Curr Med Chem 21:24–34
6. Yang DJ, Kim CG, Schechter NR, Azhdarinia A, Yu DF, Oh CS, Bryant JL, Won JJ, Kim EE, Podoloff DA (2003) Imaging with ^{99m}Tc ECDG targeted at the multifunctional glucose transport system: feasibility study with rodents. Radiology 226:465–473
7. Chen XJ, Li L, Liu F, Liu BL (2006) Synthesis and biological evaluation of technetium-99m-labeled deoxyglucose derivatives as imaging agents for tumor. Bioorg Med Chem Lett 16:5503–5506
8. Branco de Barros AL, Cardoso VN, Mota LdG, Leite EA, de Oliveira MC, Alves RJ (2010) A novel D-glucose derivative radiolabeled with technetium-99m: synthesis, biodistribution studies and scintigraphic images in an experimental model of Ehrlich tumor. Bioorg Med Chem Lett 20:2478–2480
9. Chen Y, Huang ZW, He L, Zheng SL, Li JL, Qin DL (2006) Synthesis and evaluation of a technetium-99m-labeled diethylenetriaminepentaacetate–deoxyglucose complex ([^{99m}Tc]-DTPA-DG) as a potential imaging modality for tumors. Appl Radiat Isot 64:342–347
10. Fernandez S, Crocamo N, Incerti M, Giglio J, Scarone L, Rey A (2012) Preparation and preliminary bioevaluation of a ^{99m}Tc(CO)$_3$-glucose derivative prepared by a click chemistry route. J Label Compd Radiopharm 55:274–280
11. Zhang JB, Ren JL, Lin X, Wang XB (2009) Synthesis and biological evaluation of a novel ^{99m}Tc nitrido radiopharmaceutical with deoxyglucose dithiocarbamate, showing tumor uptake. Bioorg Med Chem Lett 19:2752–2754
12. Lin X, Jin ZH, Ren JL, Pang Y, Zhang WF, Huo JF, Wang XB, Zhang JB, Zhang YY (2012) Synthesis and biodistribution of a new ^{99m}Tc-oxo complex with deoxyglucose dithiocarbamate for tumor imaging. Chem Biol Drug Des 79:239–245
13. Lin X, Chao XY, Zhang JB, Jin ZH, Zhang YY (2014) Preparation and biodistribution of a ^{99m}Tc tricarbonyl complex with deoxyglucose dithiocarbamate as a tumor imaging agent for SPECT. Bioorg Med Chem Lett 24:3964–3967
14. Wang Y, Zhu JJ, Song XQ, Wang XB, Yang JG, Zhang JB (2014) Synthesis and evaluation of ^{99m}Tc-2-[(3-carboxy-1-oxopropyl)amino]-2-deoxy-D-glucose as a potential tumor imaging agent. Bioorg Med Chem Lett 24:3882–3885
15. Liu LQ, Zhao MC, Wang Z, Qin YY, Wang XB (2014) Synthesis and biological evaluation of novel technetium-99m-labeled HYNIC-D-glucose as a potential tumor imaging agent. J Radioanal Nucl Chem 301:731–737
16. Liu M, Lin X, Song XQ, Cui Y, Li PW, Wang XB, Zhang JB (2013) Synthesis and biodistribution of a novel ^{99m}Tc nitrido radiopharmaceutical with proline dithiocarbamate as a potential tumor imaging agent. J Radioanal Nucl Chem 298:1659–1663
17. Zhu JJ, Wang Y, Li ZX, Fang SA, Zhang JB (2014) Synthesis and biological evaluation of novel ^{99m}Tc-oxo and ^{99m}Tc-nitrido complexes with phenylalanine dithiocarbamate for tumor imaging. J Radioanal Nucl Chem 302:211–216