MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Q. Ruan, X. Zhang, X. lin, X. Duan and J. Zhang, *Med. Chem. Commun.*, 2018, DOI: 10.1039/C8MD00146D.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/medchemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

View Article Online
DOI: 10.1039/C8MD00146D

ARTICLE TYPE

Novel ^{99m}Tc labelled complexes with 2-nitroimidazole isocyanide: Design, synthesis and evaluation as potential tumor hypoxia imaging agents

Qing Ruan[a], Xuran Zhang[a], Xiao Lin[a,b], Xiaojiang Duan[a] and Junbo Zhang[a*]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Radiolabelled 2-nitroimidazoles have been used for imaging hypoxia. With the aim to develop novel ^{99m}Tc radiotracers for imaging hypoxia, four novel 2-nitroimidazole isocyanide derivatives (2a, 2b, 2c, 2d) were synthesized and radiolabelling was carried out for preparing their corresponding ^{99m}Tc complexes. These ^{99m}Tc complexes were stable in vitro and could exhibit good hypoxic selectivity. The partition coefficient results indicated they were hydrophilic, and an evaluation of biodistribution in mice bearing S180 tumors indicated that all of the complexes could accumulate in tumor. Among them, ^{99m}Tc-2c exhibited the highest tumor uptake, tumor/blood and tumor/muscle ratios at 2 h post-injection. Further, single photon emission computed tomography (SPECT) imaging studies indicated clear accumulation in tumor, suggesting ^{99m}Tc-2c was a promising candidate for hypoxia imaging.

Introduction

Tumor hypoxia is one of the major causes of resistance to conventional radiotherapy and chemotherapy. Therefore, the evaluation of oxygenation status of solid tumor plays an important role in tumor treatment planning.[1-4] With the development of noninvasive imaging techniques, positron emission tomography (PET) and single photon emission computed tomography (SPECT) with hypoxia targeted radiopharmaceuticals are considered to be reasonable alternatives. In the development of hypoxia imaging agent, nitroimidazole derivatives are enzymatically reduced and accumulated in hypoxic regions, therefore most labelled probes for tumor hypoxia have been based on nitroimidazole analogues. [5, 6] In particular, 2-nitroimidazole derivatives have been found be more suitable for detection of tumor hypoxia, because 2-nitroimidazole has a more positive single electron reduction potential (SERP) value, which means it can be efficiently reduced and retained in hypoxic cells.[7-9] [^{18}F]Fluoromisonidazole ([^{18}F]FMISO), a 2-nitroimidazole radiotracer, is one of the most clinically studied radiotracers for hypoxia imaging.[10] However, to some degree, the limited availability of the [^{18}F] isotope and limited PET scanners restricted its widely clinical use, especially in many developing countries. Because there have been larger number of SPECT scanners in the whole world and progresses being made in hardware and image reconstruction algorithms have optimized the spatial resolution of images of SPECT,[11] to develop effective SPECT tracers for hypoxia imaging still exists great necessity. Since the discovery of ^{99m}Tc in the late 1930s and its introduction into nuclear medicine via the ^{99}Mo/^{99m}Tc generator in the 1950s, ^{99m}Tc has been the most widely used SPECT isotope. Up to date, many ^{99m}Tc labelled 2-nitroimidazole analogues have been reported.[12-15] However, properties of these ^{99m} Tc complexes are not yet ideal and ongoing efforts should be made to develop novel ^{99m}Tc labelled 2-nitroimidazole analogues for tumor hypoxia imaging.

Isocyanide (CN-R) is regarded as a monodentate ligand to strongly coordinate with ^{99m}Tc(I) core and [^{99m}Tc(I)(CO)$_3$]$^+$ core to produce stable ^{99m}Tc complexes([^{99m}Tc(CN-R)$_6$]$^+$ and [^{99m}Tc(CO)$_3$(CN-R)$_3$]$^+$) with high yields.[15-21] With the aim to develop novel ^{99m}Tc labelled 2-nitroimidazole analogues for tumor hypoxia imaging and explore the influence of different length of the alkyl spacer between the 2-nitroimidazole moiety and the ^{99m}Tc core, in this study, by using 2-nitroimidazole as the targeting vector and isonitrile to coordinate with ^{99m}Tc, we synthesized four novel 2-nitroimidazole isocyanide derivatives and labelled it with ^{99m}Tc in an effort to develop novel tumor hypoxia imaging agents.

Results and discussion

Chemistry

Synthesis of four novel 2-nitroimidazole isocyanide derivatives was achieved by reaction of the corresponding amino derivatives of 2-nitroimidazole (1) with different isocyanide-containing active esters in the presence of triethylamine, as shown in Scheme 1. The synthesis methods were very convenient to obtain the desired products. The ligands (**2a, 2b, 2c, 2d**) were characterized by the ^{1}H NMR, ^{13}C NMR, IR and HR-MS, and the results obtained were in agreement with the proposed structures.

Radiolabelling and quality control

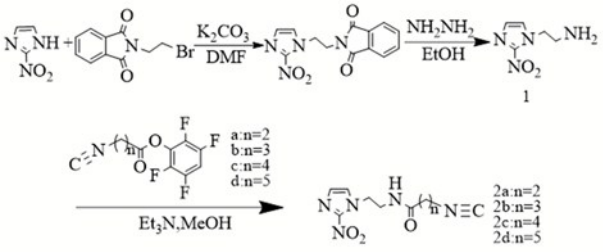
^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d complexes can be prepared by direct labelling method with high radiochemical purities (RCP). The preparation of these complexes does not require heating and it can be easily formed at room temperature, while the preparation of ^{99m}Tc-MIBI (MIBI: 2-methoxy-2-isobutyl isonitrile) needs heating at 100 °C for 15 min. Just like ^{99m}Tc-MIBI, the proposed structure of these complexes would be a monovalent cation with a central technetium core that is surrounded by six same ligands coordinated through the isonitrile carbon [Scheme 2].[19]

The RCP of the complexes were assessed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). By TLC, in methanol, ^{99m}Tc complexes migrated at the front (R_f = 0.8–1.0), while $^{99m}TcO_4^-$, $^{99m}TcO_2 \cdot 2H_2O$ remained at the origin (R_f = 0–0.1). By HPLC, single radioactive peaks were observed with retention times (t_R) of 14.35 min, 14.63 min, 14.88 min and 15.38 min corresponding to ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d, respectively. While the retention times of $^{99m}TcO_4^-$ was 3.33 min. The mean radiochemical purities of the final products were all over 95%, suggesting they can be used for in vitro and in vivo studies without further purification (Fig. 1).

As the structural characterization of ^{99m}Tc complexes is difficult to verify at the no-carrier-added level, corresponding rhenium analogue can be synthesized at macroscopic level.[22] Tc and Re belong to the same group and they have similar chemistry. The structure of ^{99m}Tc complex can be studied by using stable rhenium as a surrogate for ^{99m}Tc. The co-injection HPLC pattern of ^{99m} Tc-2c and Re-2c was showed in Fig. 2. It could be noted that the radioactivity profile of ^{99m} Tc-2c (t_R=14.86 min) nearly matched with the UV-chromatograms (t_R=14.48 min) of Re-2c, suggesting ^{99m} Tc-2c possessed the proposed structure.

In vitro stability study

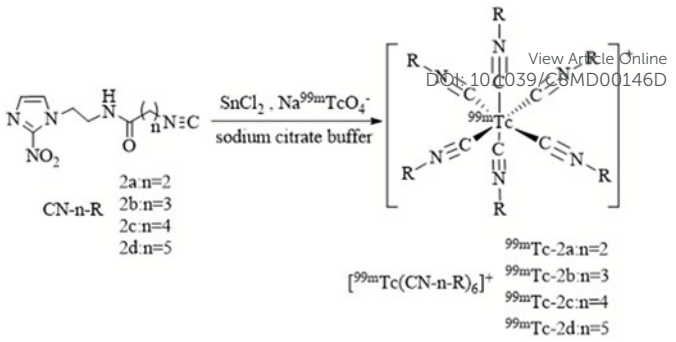
The stability of the ^{99m}Tc complexes was assayed by measuring the radiochemical purity (RCP). The RCP of the complexes was more than 90% and all of the complexes did not show decomposition for 6 h in the labelling milieu at room temperature and in the mouse serum at 37 °C, demonstrating their good in vitro stability.



Scheme 1 Synthesis of **2a, 2b, 2c** and **2d**.

Determination of the partition coefficient

The partition coefficient (log P) values of ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d were −2.65 ± 0.14, −1.95 ± 0.13, −1.27 ± 0.04 and −0.45 ± 0.05, respectively, suggesting they were hydrophilic. Moreover, the log P values of the corresponding ^{99m}Tc



Scheme 2 Preparation procedures for ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d.

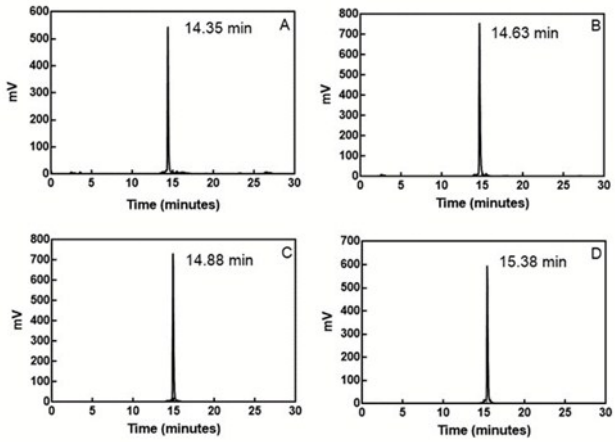


Figure 1 HPLC patterns of ^{99m}Tc-2a (A), ^{99m}Tc-2b (B), ^{99m}Tc-2c (C) and ^{99m}Tc-2d (D).

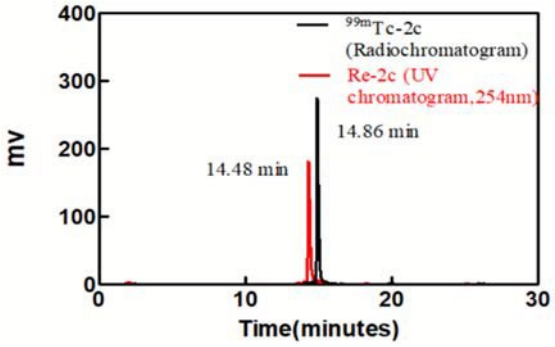


Figure 2 Co-injection analysis of ^{99m}Tc-2c and Re-2c

complexes are in accordance with the structure of the ligands, since incorporation of more CH_2 group increases the lipophilicity.

In vitro cellular uptake

The cellular uptake analysis of ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d was carried out using S180 cell under both hypoxic and aerobic conditions. As presented in Fig. 3, the uptake of the four ^{99m}Tc complexes in hypoxic cells were persistently higher than that in aerobic cells, suggesting they had good hypoxia selectivity. Significant differences between each point in hypoxic and aerobic conditions were determined by Student's t test (independent, two-

tailed). The criterion for significance was $p < 0.05$.

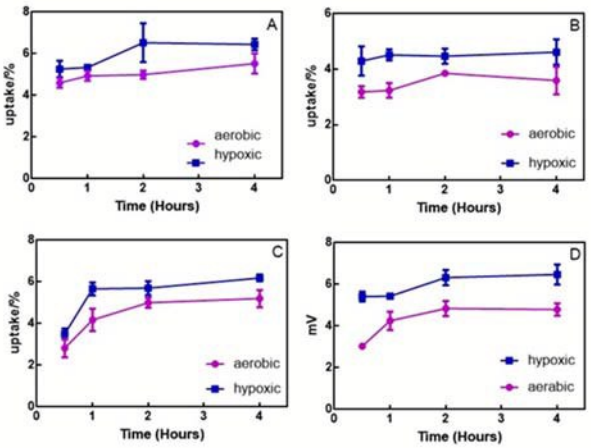


Figure 3 Cellular uptakes of ^{99m}Tc-2a (A), ^{99m}Tc-2b (B), ^{99m}Tc-2c (C) and ^{99m}Tc-2d (D).

Biodistribution studies

Biodistribution studies were performed in kunming mice bearing S180 tumor, which is a representative hypoxia model of solid tumors.[23-25] We conducted biodistribution studies at 0.5, 1 and 2 h after ^{99m}Tc complexes were injected into mice. The results of ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d were shown in Tables 1-4, respectively. They all had a relatively high tumor uptake. Muscle uptakes were low, hence the tumor/muscle ratios were high. The initial accumulations in blood were high, but the clearance of activity from blood is faster than that of tumor. For the four ^{99m}Tc complexes, excretion occurred through both the urinary and hepatobiliary tract. Low uptake in the thyroid and stomach suggested no $^{99m}TcO_4^-$ occured.

Table 1 Biodistribution of ^{99m}Tc-2a in mice bearing S180 tumor(n=5, T/B = tumor-to-blood ratio, T/N = tumor-to-muscle

%ID/g ± s	0.5 h	1 h	2 h
Heart	1.04 ± 0.18	0.32 ± 0.09	0.28 ± 0.06
Liver	4.77 ± 0.55	1.86 ± 0.30	1.52 ± 0.34
Lung	2.26 ± 0.27	0.81 ± 0.04	0.61 ± 0.13
Kidney	8.80 ± 1.15	6.80 ± 2.01	5.28 ± 1.21
Spleen	0.77 ± 0.13	0.33 ± 0.07	0.24 ± 0.04
Stomach	0.33 ± 0.18	0.29 ± 0.17	0.26 ± 0.05
Bone	1.01 ± 0.15	0.49 ± 0.08	0.28 ± 0.07
Muscle	0.64 ± 0.22	0.19 ± 0.02	0.16 ± 0.02
Intestine	1.96 ± 0.53	0.75 ± 0.20	0.58 ± 0.26
Tumor	1.27 ± 0.37	0.83 ± 0.19	0.63 ± 0.09
Blood	2.87 ± 0.66	0.96 ± 0.08	0.70 ± 0.08
Thyroid(%ID)	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.01
T/B	0.44	0.86	0.91
T/N	1.98	4.47	3.86

Table 2 Biodistribution of ^{99m}Tc-2b in mice bearing S180 tumor (n=5, T/B = tumor-to-blood ratio, T/N = tumor-to-muscle)

%ID/g ± s	0.5 h	1 h	2 h
Heart	0.47 ± 0.05	0.34 ± 0.02	0.26 ± 0.03
Liver	4.11 ± 0.49	3.20 ± 0.35	2.83 ± 0.26
Lung	0.99 ± 0.06	0.78 ± 0.02	0.64 ± 0.09
Kidney	7.72 ± 0.60	8.15 ± 0.42	8.22 ± 0.29
Spleen	0.53 ± 0.04	0.47 ± 0.07	0.39 ± 0.05
Stomach	0.10 ± 0.02	0.11 ± 0.04	0.09 ± 0.02
Bone	0.52 ± 0.10	0.47 ± 0.10	0.42 ± 0.12
Muscle	0.21 ± 0.03	0.14 ± 0.03	0.12 ± 0.02
Intestine	1.14 ± 0.49	0.51 ± 0.15	0.36 ± 0.09
Tumor	0.92 ± 0.18	0.63 ± 0.07	0.52 ± 0.08
Blood	1.17 ± 0.08	0.81 ± 0.09	0.53 ± 0.04
Thyroid(%ID)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01
T/B	0.79	0.78	1.00
T/N	4.28	4.57	4.32

View Article Online
DOI: 10.1039/C8MD00146D

Table 3 Biodistribution of ^{99m}Tc-2c in mice bearing S180 tumor (n=5, T/B = tumor-to-blood ratio, T/N = tumor-to-muscle)

%ID/g ± s	0.5 h	1 h	2 h
Heart	0.64 ± 0.07	0.35 ± 0.03	0.29 ± 0.04
Liver	6.77 ± 0.81	4.93 ± 0.37	4.10 ± 0.55
Lung	2.31 ± 0.40	1.29 ± 0.08	1.01 ± 0.16
Kidney	5.70 ± 0.38	5.18 ± 0.39	4.91 ± 0.66
Spleen	1.19 ± 0.16	0.63 ± 0.06	0.69 ± 0.16
Stomach	0.35 ± 0.10	0.24 ± 0.08	0.53 ± 0.20
Bone	1.00 ± 0.04	0.67 ± 0.10	0.60 ± 0.08
Muscle	0.37 ± 0.04	0.21 ± 0.03	0.16 ± 0.04
Intestine	3.64 ± 0.53	1.45 ± 0.40	0.85 ± 0.21
Tumor	1.55 ± 0.19	0.93 ± 0.15	0.83 ± 0.14
Blood	1.31 ± 0.20	0.87 ± 0.01	0.63 ± 0.12
Thyroid(%ID)	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
T/B	1.18	1.06	1.32
T/N	4.15	4.39	5.05

Table 4 Biodistribution of ^{99m}Tc-2d in mice bearing S180 tumor (n=5, T/B = tumor-to-blood ratio, T/N = tumor-to-muscle)

%ID/g ± s	0.5 h	1 h	2 h
Heart	0.70 ± 0.17	0.55 ± 0.13	0.40 ± 0.08
Liver	15.67 ± 0.37	9.49 ± 0.69	6.96 ± 0.61
Lung	3.09 ± 0.92	3.76 ± 1.60	3.73 ± 0.45
Kidney	7.09 ± 1.42	3.36 ± 0.28	2.74 ± 0.41
Spleen	1.42 ± 0.12	0.94 ± 0.15	0.80 ± 0.12
Stomach	0.43 ± 0.02	0.31 ± 0.07	0.40 ± 0.14
Bone	1.06 ± 0.39	0.97 ± 0.24	0.60 ± 0.15
Muscle	0.42 ± 0.13	0.29 ± 0.08	0.22 ± 0.07
Intestine	3.70 ± 0.77	1.30 ± 0.16	1.47 ± 0.33
Tumor	1.30 ± 0.14	1.10 ± 0.23	0.78 ± 0.06
Blood	1.93 ± 0.21	1.47 ± 0.13	1.03 ± 0.13
Thyroid(%ID)	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00
T/B	0.67	0.75	0.75
T/N	3.11	3.77	3.45

The comparison results of biodistribution of the four ^{99m}Tc complexes in mice bearing S180 tumor at 2 h post-injection are shown in Table 5. It was found that with the increase of log P of the complex, the uptake in the liver was increased and kidney uptake was decreased. By comparison, ^{99m}Tc-2c has a lower muscle and blood uptake and higher tumor uptake, so the tumor/blood and tumor/muscle ratios of ^{99m}Tc-2c is much higher than that of other three complexes.

By the analysis of previously reported data, we can find that the tumor uptake of ^{99m}Tc-2c even the tumor/blood and tumor/muscle ratios were higher than those of some reported ^{99m}Tc-labelled 2-nitroimidazole derivatives. In 2017, Vats et al. reported a ^{99m}Tc-'4+1' mixed ligand complex of 2-nitroimidazole with isocyanide for hypoxia imaging. The tumor uptake of ^{99m}Tc-2c (0.93 ± 0.15) was more than two times better than that of ^{99m}Tc(NS_3) (2NimNC) (0.40 ± 0.07) at 1 h post-injection. As for the uptake of non-target organs and tissues, ^{99m}Tc-2c was much better than that of ^{99m}Tc(NS_3) (2NimNC). The liver uptake of ^{99m}Tc-2c (4.93±0.37) was less than half of that of ^{99m}Tc(NS_3) (2NimNC) (12.42±0.42), and the intestine uptake of ^{99m}Tc-2c (1.45 ± 0.40) was merely less than one thirteenth of that of ^{99m}Tc(NS_3) (2NimNC) (20.86 ±0.62) at 1 h post-injection. As for the tumor/blood and tumor/muscle ratios, ^{99m}Tc-2c was superior to ^{99m}Tc(NS_3)(2NimNC) at 1 h post-injection.[15]

Table 5 Comparison of biodistribution of the ^{99m}Tc complexes at 2 h post-injection.

	^{99m}Tc-2a	^{99m}Tc-2b	^{99m}Tc-2c	^{99m}Tc-2d
Log P	-2.65	-1.99	-1.27	-0.44
Liver	1.52 ± 0.34	2.83 ± 0.26	4.10 ± 0.55	6.96 ± 0.61
Kidney	8.42 ± 1.18	8.22 ± 0.29	4.91 ± 0.66	2.74 ± 0.41
Tumor	0.63 ± 0.09	0.52 ± 0.08	0.83 ± 0.14	0.78 ± 0.06
T/B	0.91	1.00	1.32	0.75
T/N	3.86	4.32	5.05	3.45

SPECT/CT imaging studies

As ^{99m}Tc-2c had a higher tumor uptake, tumor/blood and tumor/muscle ratios than the other three complexes, it was selected as a promising candidate for further SPECT/CT imaging studies. The SPECT/CT imaging results of ^{99m}Tc-2c showed the tumor uptake was observable (Fig. 4). However, the appreciable uptake of ^{99m}Tc-2c in the liver and kidneys is the defect of the complex. The value of the region-of-interest (ROI) ratio of the uptake for the tumor site to the corresponding non-tumor region was 5.39±0.67. The imaging data in mice were consistent with the biodistribution results in mice.

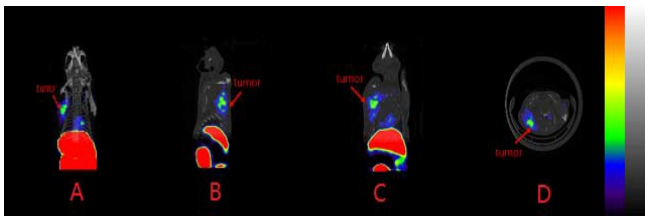


Figure 4 SPECT/CT images of ^{99m}Tc-2c at 2 h post-injection, visual imaging(A), sagittal section(B), coronal section(C) and transverse section(D).

Experimental section

General

All chemicals were purchased from commercial sources and used without further purification. ^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy (CIAE). The murine sarcoma S180 cell line was obtained from Peking University Health Science Center (Beijing, China). 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. High performance liquid chromatography (HPLC) was performed on a Waters 600 binary HPLC pump and a Waters 2487 UV absorbance dual λ detector (Milford, MA USA) with a reversed-phase column (Kromasil C18, 250×4.6 mm).

Chemistry

Compound 1 (2-(2-nitro-1*H*-imidazol-1-yl)ethan-1-amine) was prepared according to literature previously described.[26] The synthetic procedures and the spectral data for the final products (**2a, 2b, 2c** and **2d**) were as follows.

Synthesis of **2a**. Compound 1 156 mg (1 mmol), Compound a (2, 3, 5, 6-tetrafluorophenyl 3-isocyanopropanoate) 247 mg (1 mmol) and triethylamine 0.21 mL (1.5 mmol) were dissolved in 15 mL methanol. The mixture was stirred at room temperature for 6 h. The solvent was removed under a reduced pressure and the residue was purified by chromatography (CH_2Cl_2/CH_3OH) to give **2a** (3-isocyano-*N*-(2-(2-nitro-1*H*-imidazol-1-yl)ethyl) propanamide) as white solid (yield, 64%). IR (KBr)/cm $^{-1}$: 3342.78 (νNH), 1487.18, 1359.87 (νNO_2), 1670.43 (νCO), 2152.65 (νNC); ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.29 (s, 1H), 7.03 (s, 1H), 4.49-4.46 (t, 2H), 3.60-3.58 (t, 2H), 3.53-1.3.51 (t, 2H),2.54-2.51 (t, 2H); ^{13}C NMR (100 MHz, D_2O) δ(ppm): 172.62, 151.97, 144.32, 128.72, 127.27, 48.34, 38.67, 38.11, 34.55; HR-MS (ESI): found 238.0938, calcd 238.0934, $C_9H_{12}N_5O_3$[M+H$^+$].

Synthesis of **2b**. Following the same procedure used in the synthesis of **2a, 2b** was isolated as white solid (yield, 35%). IR (KBr)/cm $^{-1}$: 3254.05 (νNH), 1489.11, 1327.08 (νNO_2), 1633.78 (νCO), 2150.72 (νNC); ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.28 (s, 1H), 7.05 (s, 1H), 4.49-4.46 (t, 2H), 3.57-3.55 (t, 2H), 3.32-3.30 (t, 2H), 2.19-2.16 (m, 2H), 1.71-1.59 (t,2H); ^{13}C NMR (100 MHz, D_2O) δ(ppm): 175.55, 151.33, 143.70, 128.73, 127.72, 49.83, 40.56, 37.95, 32.43, 22.99; HR-MS (ESI): found 252.1098, calcd 252.1091, $C_{10}H_{14}N_5O_3$ [M+H$^+$].

Synthesis of **2c**. Following the same procedure used in the synthesis of **2a, 2c** was isolated as white solid (yield, 53%). IR (KBr)/cm $^{-1}$: 3366.99 (νNH), 1491.04, 1363.73 (νNO_2), 1655.00 (νCO), 2146.86 (νNC); ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.26 (s, 1H), 7.03 (s, 1H), 4.46-4.45 (t, 2H), 3.56-3.54 (t, 2H), 3.33-3.31 (t, 2H), 2.05-2.03 (m, 2H),1.44-1.42 (m, 4H); ^{13}C NMR (100 MHz, D_2O) δ(ppm): 176.87, 150.82, 144.331, 128.56, 127.58, 49.01, 40.86, 37.65, 34.21, 26.89, 22.83; HR-MS (ESI): found 266.1244 calcd 266.1247, $C_{11}H_{16}N_5O_3$ [M+H$^+$].

Synthesis of **2d**. Following the same procedure used in the synthesis of **2a, 2d** was isolated as white solid (yield, 69%). IR (KBr)/cm $^{-1}$: 3342.78 (νNH), 1487.18, 1359.87 (νNO_2), 1670.43 (νCO), 2152.65 (νNC); ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.27(s, 1H), 7.05 (s, 1H), 4.48-4.46 (t, 2H), 3.56-3.54 (t, 2H), 3.35-3.32 (t, 2H), 2.04-2.02 (t, 2H),1.52-1.50 (m, 2H), 1.38-1.31 (m, 2H), 1.20-1.15 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ(ppm): 177.04, 150.33, 144.67, 128.53, 127.39, 49.52, 41.04, 37.92, 35.18, 27.80, 25.07, 24.32; HR-MS (ESI): found 280.1406 calcd 280.1404, $C_{12}H_{18}N_5O_3$ [M+H$^+$].

Radiolabelling and quality control

The preparation procedures for ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d are as follows: 2 mg **2a** (**2b, 2c, 2d**) was dissolved in 0.3mL saline, then 0.2 mL sodium citrate buffer (0.1mol/L, pH=4.0) was add. After 0.03 mL $SnCl_2{\cdot}2H_2O$ (1mg/mL) was added, 0.5mL of saline containing $^{99m}TcO_4^-$ (37–74 MBq) was added right away. The mixture was kept at room temperature for 30 min. The radiochemical purity (RCP) of ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d was checked by TLC and HPLC. The TLC was performed on a polyamide strip and eluted with methanol. For HPLC, water (A) and acetonitrile (B) mixture were adopted as the mobile phase at a flow rate of 1.0 mL/min, and the analytical conditions was described in Table 6.

Table 6 The analytical conditions for HPLC

Time/min	0	5	15	20	22	32	35
A/%	90	90	30	30	10	10	90
B/%	10	10	70	70	90	90	10

Preparation of rhenium analogue

In order to validate the proposed structure of ^{99m}Tc-2c, we synthesized the stable complex Re-2c. The preparation procedures for Re-2c were as follows: 100 mg of 2c was dissolved in 0.3 mL saline, then 1 mL of sodium citrate buffer (0.1 mol/L, pH=4.0) was added. After the addition of 1.5 mg $SnCl_2{\cdot}2H_2O$, 2 mg $KReO_4$ was added right away. The mixture was kept at room temperature for 30 min. The desire product was purified by HPLC under conditions described in Table 6. Then the solvent was removed and brown residue was obtained. The product was analyzed by ^{1}H NMR and HR-MS. ^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.30 (s, 1H), 7.08 (s, 1H), 4.52-4.48 (t, 2H), 3.60-3.57 (t, 2H), 3.39-3.36 (t, 2H), 2.10-2.06 (m, 2H), 1.54-1.47 (m, 4H); HR-MS(ESI): $[(C_{11}H_{15}N_5O_3)_6ReNa]^{2+}$: m/z calcd. for $C_{66}H_{90}N_{30}O_{18}ReNa$ 900.3249. found 900.3245.

***In vitro* stability study**

The stability studies of ^{99m}Tc-2a, ^{99m}Tc-2b, ^{99m}Tc-2c and ^{99m}Tc-2d were performed by checking the RCP in the labelling milieu at room temperature for 6 h. In vitro serum stability studies in mouse serum were also assessed by a method reported earlier.[27] The RCP was checked by TLC and HPLC.

Determination of the partition coefficient (log *P*)

The lipophilicity of the four ^{99m}Tc complexes were evaluated by the partition coefficients (log P) between 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) according to the previously reported method.[28] Briefly, the complex was mixed with an equal volume of the two phase. The mixture was vortexed for 3 min and then centrifuged at 10000 g for 5 min. From each phase, 0.1 mL of aliquot was pipetted and counted in a γ-counter. The partition coefficient, P, was calculated as the mean value of each counts per minute per milliliter of 1-octanol layer divided by that of the buffer. The final partition coefficient value was expressed as log P ± SD.

***In vitro* cellular uptake**

In vitro uptake of the ^{99m}Tc complexes were performed using the murine sarcoma S180 cell lines. The cells were incubated in hypoxic and aerobic conditions and evaluated by the previously reported methods.[29] Briefly, S180 cells were suspended in fresh DMEM growth medium having 10 % (v/v) of fetal bovine serum at a cell concentration of 2×10^6 cells / mL. Aliquots of 20 mL were put into glass vials and incubated at 37 °C under an atmosphere of 95% nitrogen plus 5% carbon dioxide (hypoxic conditions, oxygen concentration <10 ppm) or 95% air plus 5% carbon dioxide (aerobic conditions). After a 30 min equilibration period, 0.2 mL (3.7 MBq) of the ^{99m}Tc labelled complex was mixed to the suspension. 1 mL of cell suspension were pipetted and centrifuged at 3000 g for 5 min at 0.5, 1, 2 and 4.0 h post-incubation. 0.9 mL of supernatant was taken for counting (C_{out}) and the residual sample containing cells with 0.1 mL of supernatant was also counted (C_{in}). The studies were measured five times at each time point. The cell accumulation, A, was calculated as follows: $A = (C_{in} - C_{out}/9) / (C_{in} + C_{out})$. The final results were expressed as the average of the measurements ± the standard deviation.

Biodistribution studies

All biodistribution studies were performed according to the Regulations on Laboratory Animals of Beijing Municipality and all animal procedures were carried out in compliance with the Guidelines for Care and Use of Laboratory Animals of Beijing Normal University and approved by the Animal Ethics Committee of Beijing Normal University.

Experiments were performed using Kunming female mice (18-22 g) bearing S180 tumors.[30, 31] 0.1 mL of ^{99m}Tc complexes (7.4×10^5 Bq mL^{-1}) was injected via a tail vein. The mice were sacrificed in groups of five at 0.5, 1 and 2 h post-injection. The tumor, muscle, blood and other organs of interest were collected, weighed and counted using Gamma Counter. The final results were expressed as the percent uptake of injected dose per gram of tissue ± standard deviation (% ID/g ± SD).

SPECT/CT imaging studies

SPECT/CT images were acquired on a small animal Triumph SPECT/CT system (Trifoil imaging, USA). The SPECT data was reconstructed with HiSPECT software and analyzed with the 40 vivoquant software. The Kunming female mice (18–20 g) bearing S180 tumor were injected via a tail vein with ^{99m}Tc-2c (0.1 mL, 1.11×10^6 Bq). SPECT/CT images were acquired at 2 h after injection. The ROIs for the tumor site and the corresponding non-tumor region were selected and determined. The final results were expressed as mean ± standard deviation(n=3).

Conclusion

In the present study, four novel 2-nitroimidazole isocyanide derivatives (**2a, 2b, 2c, 2d**) were synthesized and their corresponding ^{99m}Tc complexes can be prepared by direct labelling method without the need for heating. These ^{99m}Tc complexes exhibited hydrophilicity and in vitro stability as well as hypoxic selectivity. The preliminary biological evaluation in mice bearing S180 tumor showed that they could accumulate in the tumor. Especially for ^{99m}Tc-2c, the biodistribution and SPECT imaging studies in mice bearing S180 tumor showed it had an obvious tumor uptake and good target/no-target ratios, suggesting it would a potential tracer for hypoxia imaging in tumor.

Conflict of interest

The author(s) confirm that this article content has no conflicts of interest.

Acknowledgments

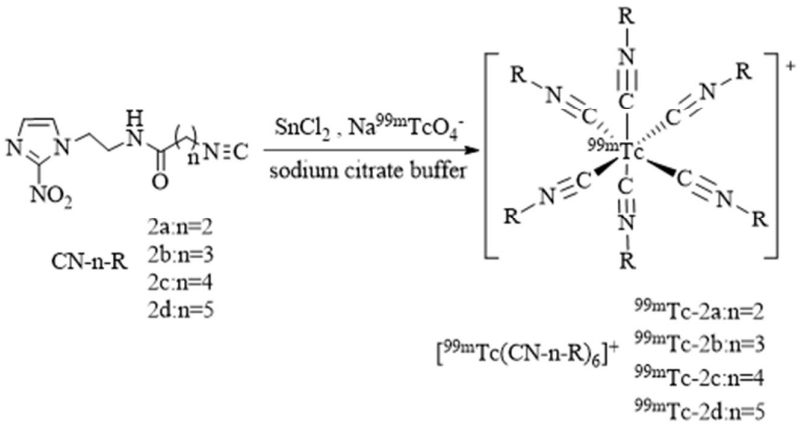
The work was financially supported, in part, by the National Natural Science Foundation of China (21771023), the foundation of Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education.

Notes and references

[a] Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, 100875, P. R. China. Fax: +86 10 6220 5562; Tel: +86 10 6220 8126; E-mail: zhjunbo@bnu.edu.cn.
[b] College of Water Sciences, Beijing Normal University, No19, Xinjiekouwai Street, Beijing 100875, People's Republic of China

1. S. T. Astner, K. Shi, P. Vaupel and M. Molls, *Experimental Oncology*, 2010, **32**, 149.
2. P. Cabral and H. Cerecetto, *Anti-Cancer Agents in Medicinal Chemistry*, 2017, **17**, 318.
3. M. B. Mallia, A. Mathur, H. D. Sarma and S. Banerjee, *Cancer Biotherapy & Radiopharmaceuticals*, 2015, **30**, 79.
4. R. A. Medina, E. Mariotti, D. Pavlovic, K. P. Shaw, T. R. Eykyn, P. J. Blower and R. Southworth, *Journal of Nuclear Medicine*, 2015, **56**, 921.
5. J. H. Tocher, *General Pharmacology*, 1997, **28**, 485.
6. K. A. Krohn, J. M. Link and R. P. Mason, *Journal of Nuclear Medicine*, 2008, **49**, 129S.
7. Y. Joyard, J. V. Le, H. Castel, C. Diouf, L. Bischoff, C. Papamicaël, V. Levacher, P. Vera and P. Bohn, *Bioorganic & Medicinal Chemistry Letters*, 2013, **23**, 3704.
8. M. B. Mallia, C. Kumar, A. Mathur, H. D. Sarma and S. Banerjee, *Nuclear Medicine & Biology*, 2012, **39**, 1236.
9. M. Lei, Y. Wang and T. Chu, *European Journal of Medicinal Chemistry*, 2012, **58**, 50.
10. S. T. Lee and A. M. Scott, *Seminars in Nuclear Medicine*, 2007, **37**, 451.
11. A. Rahmim and H. Zaidi, *Nuclear Medicine Communications*, 2008, **29**, 193.
12. T. Chu, R. Li, S. Hu, X. Liu and X. Wang, *Nuclear Medicine & Biology*, 2004, **31**, 199.
13. M. B. Mallia, S. Subramanian, A. Mathur, H. D. Sarma and S. Banerjee, *Nuclear Medicine & Biology*, 2014, **41**, 600.
14. M. B. Mallia, S. Mittal, H. D. Sarma and S. Banerjee, *Bioorganic & Medicinal Chemistry Letters*, 2016, **26**, 46.
15. K. Vats, M. B. Mallia, A. Mathur, H. D. Sarma and S. Banerjee, *Chemistryselect*, 2017, **2**, 2910.
16. M. J. Abrams, A. Davison, A. G. Jones, C. E. Costello and H. Pang, *Inorganic Chemistry*, 1983, **14**, 2798.
17. X. Chen, Y. Guo, Q. Zhang, G. Hao, H. Jia and B. Liu, *Journal of Organometallic Chemistry*, 2008, **693**, 1822.
18. G. Y. Hao, J. Y. Zang, L. Zhu, Y. Z. Guo and B. L. Liu, *Journal of Labelled Compounds & Radiopharmaceuticals*, 2004, **47**, 513.
19. S. Yang, X. Wang, H. Guo, J. Liu, F. Wang and X. Zhang, *Journal of Radioanalytical & Nuclear Chemistry*, 2008, **278**, 165.
20. J. Giglio, S. Fernández, H. J. Pietzsch, S. Dematteis, M. Moreno, J. P. Pacheco, H. Cerecetto and A. Rey, *Nuclear Medicine & Biology*, 2012, **39**, 679.
21. X. Duan, X. Zhang, Q. Gan, S. a. Fang, Q. Ruan, X. Song and J. Zhang, *MedChemComm*, 2018, DOI: 10.1039/C7MD00635G.
22. S. Seifert, J. U. Künstler, E. Schiller, H. J. Pietzsch, B. Pawelke, R. Bergmann and H. Spies, *Bioconjugate Chemistry*, 2004, **15**, 856.
23. M. Arai，T. KawaChi, A. Setiawan and M. Kobayashi, *ChemMedChem*, 2010, **5**, 1919.
24. H. Huang, H. Zhou, Z. Li,. X. Wang and T. Chu, *Bioorganic & Medicinal Chemistry Letters*, 2012, **22**, 172.
25. Z. Li, X. Lin, J. Zhang, X. Wang, Z. Jin, W. Zhang and Y. Zhang, *Nuclear Medicine & Biology*, 2016, **43**, 165.
26. M. P. Hay, W. R. Wilson, J. W. Moselen, B. D. Palmer and W. A. Denny, *Journal of Medicinal Chemistry*, 1994, **25**, 381.
27. L. Mei, W. Sun and T. Chu, *Journal of Radioanalytical & Nuclear Chemistry*, 2014, **301**, 831.
28. J. Zhang, Q. Yu, J. Huo, P. Yan, S. Yang, Y. He, T. Tang, C. Yang and X. Wang, *Journal of Radioanalytical & Nuclear Chemistry*, 2010, **283**, 481.
29. Y. Zhang, T. Chu, X. Gao, X. Liu, Z. Yang, Z. Guo and X. Wang, *Bioorganic & Medicinal Chemistry Letters*, 2006, **16**, 1831.
30. X. Lin, Z. Jin, J. Ren, Y. Pang, W. Zhang, J. Huo, X. Wang, J. Zhang and Y. Zhang, *Chemical Biology & Drug Design*, 2012, **79**, 239.
31. Z. Li, J. Zhang, Z. Jin, W. Zhang and Y. Zhang, *MedChemCommun*, 2015, **6**, 1143.

View Article Online
DOI: 10.1039/C8MD00146D



^{99m}Tc-2c can be prepared by direct labelling method without the need for heating and would be a promising probe for hypoxia imaging.