RESEARCH ARTICLE

 View Article Online
 View Journal | View Issue

 Cite this: *Med. Chem. Commun.*,
 2018, 9, 705

Novel ^{99m}Tc -labelled complexes with thymidine isocyanide: radiosynthesis and evaluation as potential tumor imaging tracers

 Xiaojiang Duan,^a Xuran Zhang,^{ab} Qianqian Gan,^a Si'an Fang,^a Qing Ruan,^a
 Xiaoqing Song^a and Junbo Zhang *^a

A novel thymidine isocyanide (CN-TdR) functionalized at the N3 position of thymidine was synthesized and then radiolabelled with $^{99m}\text{Tc(I)}$ and $[\text{}^{99m}\text{Tc(II)}(\text{CO})_3]^+$ cores to produce $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ and $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$, respectively. Both of them were prepared with high radiochemical purity and were stable over 6 h in saline at ambient temperature and in serum at 37 °C. The partition coefficient results demonstrated that they were hydrophilic. The cell internalization studies showed that their uptake might be mediated by nucleoside transporters. Biodistribution of these complexes in mice bearing the S180 tumor showed that they accumulated in the tumor with high uptake and cleared rapidly from blood and muscles, producing high tumor/blood and tumor/muscle ratios. Between them, $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ exhibited advantages concerning a higher tumor uptake, tumor/blood ratio and tumor/muscle ratio at 60 min post-injection. Single photon emission computed tomography imaging studies showed that there was a clear accumulation in tumor sites, suggesting that $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ could be a promising candidate for tumor imaging.

 Received 16th December 2017,
 Accepted 27th February 2018

DOI: 10.1039/c7md00635g

rsc.li/medchemcomm

Introduction

Sustained proliferation is one of the hallmarks of cancer, and the rate of cell division is an important prognostic characteristic of malignancy.^{1,2} The ability to assay tumor cell proliferation by non-invasive imaging, such as PET (positron emission tomography) and SPECT (single photon emission computed tomography), plays a vital role in improving the diagnosis, grading, and staging of cancer. Moreover, it can also predict the treatment response after anti-tumor therapy. For many years, researchers have focused on the metabolic process of thymidine, which is the most specific way to measure cell proliferation. Thymidine is the only nucleoside incorporated into DNA, but it is not a constituent of RNA. In fact, two thymidine analogs labelled with positron radionuclides, ^{18}F -FLT (3'-deoxy-3'-[^{18}F]fluorothymidine) and ^{18}F -FMAU (2'-deoxy-2'-[^{18}F]fluoro-5-methyl-1-beta-D-arabinofuranosyl uracil), have been developed and evaluated as potential PET radiopharmaceuticals.^{3–7} It is noteworthy that ^{18}F -FLT has been widely used in the PET imaging of cancer.⁸ However, the limited availability of ^{18}F and PET scanners poses some prac-

tical problems, especially in a lot of developing countries. In contrast, ^{99m}Tc is readily available at a reasonable cost and has ideal physical and chemical characteristics, making it the most widely used radionuclide for SPECT imaging. In addition, the progress in hardware and image reconstruction algorithms has greatly optimized the spatial resolution of SPECT images. Taken together, the attempt to develop ^{99m}Tc -labelled thymidine derivatives as potential tumor imaging agents is worth continuing.

In 2003, R. Schibli *et al.* reported for the first time six $\text{M}(\text{CO})_3$ ($\text{M} = ^{99m}\text{Tc}$, Re) labelled thymidine derivatives at the C5' position, and they tested the affinity of the complexes for hTK-1 (human cytosolic thymidine kinase-1) *in vitro*.⁹ In the following ten years, many ^{99m}Tc -labelled thymidine derivatives have been prepared and evaluated.^{10–20} Unfortunately, none of them had the ideal properties to be used in clinical imaging. In a previous work of our group, a series of $^{99m}\text{Tc}(\text{CO})_3$ -labelled thymidine macrocyclic analogs were synthesized and their biodistributions were systematically evaluated.²¹ However, due to the fact that the macrocyclic coordination moiety was too bulky, the biological characteristics of those complexes were not satisfactory. The preparation of novel ^{99m}Tc -labelled thymidine analogs is still considered to be of great necessity.

Isocyanide (CN-R) is a σ donor and a π acceptor ligand and can act as a monodentate ligand to strongly coordinate with $^{99m}\text{Tc(I)}$ and $[\text{}^{99m}\text{Tc(II)}(\text{CO})_3]^+$ cores, which produces

^a Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, P.R. China.

E-mail: zhjunbo@bnu.edu.cn; Fax: +86 10 6220 5562

^b Department of Isotopes, China Institute of Atomic Energy, P. O. Box 2108, Beijing 102413, P.R. China

stable ^{99m}Tc complexes ($[\text{}^{99m}\text{Tc}(\text{CN-R})_6]^+$ and $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-R})_3]^+$) with high yields.^{22–26} With the aim of developing novel ^{99m}Tc -labelled thymidine analogs for tumor imaging, in this study, by using thymidine as the targeting vector and isocyanide to coordinate with ^{99m}Tc , we prepared a thymidine isocyanide ligand by conjugating an isocyanide-containing active ester with amino-thymidine and labelled it with a $^{99m}\text{Tc}(\text{I})$ core and a $[\text{}^{99m}\text{Tc}(\text{I})(\text{CO})_3]^+$ core in an effort to develop novel tumor imaging agents.

Results and discussion

Chemistry

Amino-thymidine (NH_2 -3-TdR, compound 6) was prepared by multi-step reactions from an initial thymidine. The reaction route of compound 6 is shown in Scheme 1. Thymidine isocyanide (CN-TdR, compound 11) was prepared by reacting compound 6 with compound 10 in the presence of triethylamine. The reaction is schematically shown in Scheme 2. CN-TdR and the intermediates were characterized by ^1H NMR, ^{13}C NMR and MS.

Radiolabelling and quality control

Preparations of the metal complexes (Re and ^{99m}Tc) were carried out using the procedure in Scheme 3. The retention time of $^{99m}\text{TcO}_4^-$ was 3.93 min, while the retention time of $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ (12) was 10.87 min (Fig. 1). A single product with high radiochemical purity (>98%) was obtained.

The retention time of $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was 12.17 min, while that of $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$ was 11.10 min (Fig. 2). The radiochemical purity of the $^{99m}\text{Tc}(\text{CO})_3$ -labelled complex was very high (>98%). To verify the speculative structure of $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$ (13b), we synthesized the stable complex $[\text{Re}(\text{CO})_3(\text{CN-TdR})_3]^+$ (13a). After co-injection of the ^{99m}Tc and Re complexes, their retention times were similar (Fig. 2), suggesting that $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$ did possess the speculative structure.

Scheme 1 Synthesis of NH_2 -3-thymidine (compound 6). Reagents and conditions: (a) TBDMS-Cl, $\text{C}_5\text{H}_5\text{N}$, 1 h, rt; 23 h, 65 °C; 65 °C to rt; H_2O , 15 min, rt; (b) 1,3-dibromopropane, K_2CO_3 , rt, DMF; (c) NaN_3 , DMF, rt; (d) 10% Pd/C, H_2 , MeOH, rt; (e) concentrated HCl, EtOH, rt.

Scheme 2 Synthesis of thymidine isocyanide (CN-TdR, compound 11). Reagents and conditions: (f) formic acid, acetic anhydride, 95 °C, N_2 ; (g) 2,3,5,6-tetrafluorophenol, DMF, DCC; (h) Burgess reagent, DCM; (i) Et_3N , MeOH.

In vitro stability and partition coefficients

In the *in vitro* stability study, the complexes exhibited that they had good *in vitro* stability over a period of 6 h. Respectively, the $\log P$ values of $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ and $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$ were -3.71 ± 0.05 and -1.31 ± 0.05 , indicating that even though both of them were hydrophilic, the former was more hydrophilic than the latter.

In vitro cell experiment

Human thymidine kinase 1 (TK1) is located in the cytosol, and the potential imaging complex has to pass through the cell membrane before it can be phosphorylated. If this transport is not achieved actively *via* nucleoside transporters, then it must occur by passive diffusion.²⁷ In order to investigate the major cell internalization routes of the complexes, the incorporation of complexes 12 ($[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$) and 13b ($[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3]^+$) into HCT116 colorectal carcinoma cells was evaluated. The HCT116 cell line was chosen for its TK1 overexpression and the prominent selectivity of the enzyme for thymidine and thymidine analogs.^{28,29} Another reason was that the HCT116 cells were adherent cells suitable for examining the effects of 5-FU on cellular internalization. Internalization was measured with and without pretreatment

Scheme 3 Preparation procedures of metal complexes and their speculative structures.

Fig. 1 HPLC profiles of $^{99m}\text{TcO}_4^-$ (a) and $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ (b).

Fig. 2 HPLC profiles of $^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3^+$ (a) and $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$ (b) ($M = \text{Re}, ^{99m}\text{Tc}$).

with 5-FU (5-fluorouracil)^{18,29} and excessive thymidine. As a thymidylate synthase inhibitor, 5-FU can increase the uptake of 5-iododeoxyuridine or ^{18}F -FLT *via* inhibiting the endogenous thymidine synthesis, increasing the levels of TK1 and inducing re-distribution of nucleoside transporters.^{29–32} The results of internalization experiments are presented in Fig. 3.

In previous literature studies, researchers proposed that the uptake of N3 functionalized thymidine complexes occurred *via* passive diffusion and endocytosis.^{18,33,34} In the present work, pretreatment with 5-FU and blocking with thymidine had an effect on the internalization of 12 and 13b into cells, proving that their uptake was mediated, at least in part, by nucleoside transporters.

Biodistribution studies

The biodistribution results of $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ and $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$ in mice bearing the S180 tumor are shown in Tables 1 and 2.

As demonstrated in Tables 1 and 2, both $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ and $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$ were accumulated in the tumor. The uptake in the kidney was appreciable, reflecting urinary elimination of these two water-soluble complexes. Their blood and muscle clearance was faster than that of the tumor, therefore, the tumor/blood and tumor/muscle ratios increased with time. Low uptake in the thyroid and stomach suggested that no $^{99m}\text{TcO}_4^-$ was internalized. At 60 min post-

injection, $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ exhibited a higher uptake at the site of the tumor and a higher target/non-target ratio than $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$. The liver uptake of $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ was much lower than that of $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$, and the kidney uptake of $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ was higher than that of $^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3^+$. The findings were in agreement with the log*P* values of these complexes. $^{99m}\text{Tc}(\text{CN-TdR})_6^+$ was

Fig. 3 Internalization into the human colorectal carcinoma HCT116 cell line over a period of 2 h at 37 °C, with and without pretreatment with 5-FU and blocking experiments with 10.0 mg thymidine. The uptake of the control was arbitrarily set to 100%. Statistical significance was determined using a bilateral *t* test of equal variance. Statistical significance is reported for * $p < 0.05$ and ** $p < 0.01$.

Table 1 Biodistribution of [$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$ in mice bearing the S180 tumor (mean $\pm$ SD, $n = 5$, %ID g^{-1})

Tissue	5 min	30 min	60 min
Heart	2.40 $\pm$ 0.31	0.42 $\pm$ 0.09	0.13 $\pm$ 0.02
Liver	1.80 $\pm$ 0.12	0.48 $\pm$ 0.06	0.26 $\pm$ 0.04
Gallbladder ($n = 4$)	—	—	4.76 $\pm$ 1.26
Lung	5.08 $\pm$ 0.64	0.90 $\pm$ 0.17	0.26 $\pm$ 0.03
Kidney	12.39 $\pm$ 2.02	3.21 $\pm$ 0.33	1.78 $\pm$ 0.19
Spleen	1.38 $\pm$ 0.17	0.27 $\pm$ 0.05	0.14 $\pm$ 0.03
Stomach	0.56 $\pm$ 0.08	0.15 $\pm$ 0.06	0.06 $\pm$ 0.02
Bone	2.06 $\pm$ 0.24	0.58 $\pm$ 0.05	0.31 $\pm$ 0.09
Muscle	2.00 $\pm$ 0.39	0.37 $\pm$ 0.14	0.11 $\pm$ 0.03
Intestine	3.11 $\pm$ 1.94	0.52 $\pm$ 0.12	0.20 $\pm$ 0.09
Tumor	3.20 $\pm$ 0.15	1.19 $\pm$ 0.28	0.70 $\pm$ 0.14
Blood	6.61 $\pm$ 0.68	0.89 $\pm$ 0.17	0.18 $\pm$ 0.04
Thyroid (%ID)	0.05 $\pm$ 0.02	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
Tumor/blood	0.48	1.33	3.81
Tumor/muscle	1.60	3.19	6.24

Table 2 Biodistribution of [$^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3$] $^+$ in mice bearing the S180 tumor (mean $\pm$ SD, $n = 5$, %ID g^{-1})

Tissue	5 min	30 min	60 min
Heart	1.29 $\pm$ 0.12	0.21 $\pm$ 0.09	0.13 $\pm$ 0.09
Liver	7.63 $\pm$ 1.98	2.11 $\pm$ 0.76	0.73 $\pm$ 0.17
Gallbladder ($n = 4$)	—	—	33.4 $\pm$ 6.43
Lung	2.45 $\pm$ 0.27	0.56 $\pm$ 0.21	0.27 $\pm$ 0.19
Kidney	5.89 $\pm$ 0.85	1.78 $\pm$ 0.57	0.76 $\pm$ 0.17
Spleen	0.94 $\pm$ 0.08	0.18 $\pm$ 0.06	0.16 $\pm$ 0.08
Stomach	0.51 $\pm$ 0.13	0.33 $\pm$ 0.20	0.79 $\pm$ 0.45
Bone	1.62 $\pm$ 0.13	0.25 $\pm$ 0.11	0.13 $\pm$ 0.05
Muscle	1.11 $\pm$ 0.14	0.15 $\pm$ 0.04	0.10 $\pm$ 0.06
Intestine	13.37 $\pm$ 3.57	2.01 $\pm$ 0.64	1.33 $\pm$ 0.59
Tumor	2.87 $\pm$ 0.80	0.73 $\pm$ 0.22	0.60 $\pm$ 0.24
Blood	2.66 $\pm$ 0.27	0.63 $\pm$ 0.13	0.25 $\pm$ 0.14
Thyroid (%ID)	0.05 $\pm$ 0.01	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
Tumor/blood	1.08	1.16	2.44
Tumor/muscle	2.59	4.96	6.16

more hydrophilic so it exhibited a lower liver uptake and a higher kidney uptake.

Table 3 shows a comparison of the biodistributions of some ^{99m}Tc -labelled thymidine derivatives. As for the tumor uptake, there was no great difference among the four complexes. However, the liver, blood and muscle uptake values of [$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$ were the lowest so it showed the highest tumor-to-muscle, tumor-to-blood and tumor-to-liver ratios. As a promising tumor imaging agent, its detectability in the tu-

mor is related to the absolute tumor uptake and tumor-to-background ratios. Based on the above data, [$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$ is more likely to be developed as a good tracer for tumor imaging than [$^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3$] $^+$.

SPECT/CT imaging studies

Because [$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$ showed a higher tumor uptake and tumor-to-muscle ratio than [$^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3$] $^+$, it was selected as a promising candidate for further SPECT imaging study. As shown in Fig. 4, there was a significant uptake in the tumor site. The kidneys and gallbladder were also visualized. As shown in the time-activity curves of the tumor and muscles (Fig. 5), the uptake of [$^{99m}\text{Tc}(\text{CO})_3(\text{CN-TdR})_3$] $^+$ in the tumor was $5.78 \pm 1.12\%$ ID g^{-1} at 5 min post injection, and the value of the uptake decreased to $0.52 \pm 0.02\%$ ID g^{-1} at 60 min post injection. The uptake value in the muscles declined more rapidly over time, resulting in an increase of the ROI (region of interest) ratio from 1.84 ± 0.16 to 2.73 ± 0.07 . The imaging findings were in accordance with the biodistribution results in mice.

Conclusions

In summary, a novel thymidine isocyanide (CN-TdR) was successfully synthesized and radiolabelled with $^{99m}\text{Tc}(\text{I})$ and [$^{99m}\text{Tc}(\text{I})(\text{CO})_3$] $^+$ cores in high yields. They were hydrophilic and stable *in vitro*. The cell internalization studies showed that they may enter cells *via* nucleoside transporters. The preliminary *in vivo* studies showed that both of the complexes were accumulated in the tumor. In particular, for [$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$, the biodistribution and SPECT imaging studies in mice bearing the S180 tumor showed that it had obvious tumor uptake and high target/no-target ratios. The possibility of the complex as a radiotracer for tumor proliferation imaging needs to be further studied.

Experimental

General

For all reactions, the chemical reagents were purchased from commercial suppliers and were used without any further purification. The $^{99}\text{Mo}/^{99m}\text{Tc}$ generator was obtained from Beijing Atom High-Tech Co. Column chromatography was performed using silica gel from Qingdao Ocean Chemicals (Qingdao, Shandong, China). ^1H and ^{13}C spectra were recorded

Table 3 Comparison of the biodistributions of some ^{99m}Tc -labelled thymidine derivatives

Complex	[$^{99m}\text{Tc}(\text{CN-TdR})_6$] $^+$	^{99m}Tc -TMHEA	^{99m}Tc -TMHHA	$^{99m}\text{Tc}(\text{CO})_3\text{-}12\text{N}3\text{-}5\text{-TdR}$
Time p.i. (min)	60	60	60	120
Tumor (%ID g^{-1})	0.70 $\pm$ 0.14	0.62 $\pm$ 0.12	0.88 $\pm$ 0.22	0.63 $\pm$ 0.14
Muscle (%ID g^{-1})	0.11 $\pm$ 0.03	0.38 $\pm$ 0.06	0.49 $\pm$ 0.20	0.22 $\pm$ 0.05
Blood (%ID g^{-1})	0.18 $\pm$ 0.04	0.48 $\pm$ 0.09	0.83 $\pm$ 0.22	0.67 $\pm$ 0.08
Liver (%ID g^{-1})	0.26 $\pm$ 0.04	4.17 $\pm$ 0.19	5.39 $\pm$ 0.28	11.70 $\pm$ 4.83
Tumor/muscle	6.24	1.85	1.62	2.88
Tumor/blood	3.81	1.27	1.07	0.95
Tumor/liver	2.69	0.15	0.16	0.05
Reference	Present study	20	20	21

Fig. 4 Static SPECT image of $[^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ in mice bearing the S180 tumor at 1 h post injection ($n = 3$).

Fig. 5 Time-activity curves of the tumor and muscles in mice bearing the S180 tumor ($n = 3$).

on a 400 MHz Bruker Avance 500 spectrometer (Bruker, Billerica, MA, USA). Mass spectrometry was conducted using a Shimadzu LC-MS 2010 series instrument (Shimadzu, Kyoto, Japan). The MS spectra were recorded using an AB SCIEX TripleTOF™ 5600 (AB Sciex, Concord, Canada). The radiochemical purity was determined by HPLC performed using a Waters 600 binary HPLC pump and a Waters 2489 UV absorbance dual detector (Waters, Milford, USA) with a reversed-phase C-18 column (Kromasil C18, 4.6 mm × 250 mm). The murine sarcoma S180 cell line was acquired from the Peking University Health Science Center (Beijing, China). The radiochemical purity (RCP) of the radiocomplex was evaluated by HPLC using water (containing 0.1% TFA) (A) and acetonitrile (containing 0.1% TFA) (B) as the mobile phases. The gradient elution technique was adopted: 0 min 10% B, 2 min 10% B, 10 min 90% B, 18 min 90% B, and 25 min 10% B.

Synthesis of the ligand

Compound 2 was synthesized as reported previously,³⁵ and the isocyanide-containing active ester (compound 10) was prepared according to a previous literature report.³⁶

3-(3-Bromopropyl)-1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)-oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (3). Compound 2 (1.0 g, 2.12 mmol) and 1,3-dibromopropane (1.28 g, 3.36 mmol) were added into 10 mL DMF (*N,N*-dimethylformamide) (10 mL). After the addition of K_2CO_3 (600 mg, 4.24 mmol), the reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was poured into ethyl acetate (100 mL) and then washed with water (3×50 mL). The organic phase was dried with MgSO_4 and evaporated under vacuum. The crude product was purified by silica gel column chromatography using PE/EtOAc (petroleum ether/ethyl acetate, v/v = 5 : 1) to give compound 3 as a colorless oil (1.0 g, yield 80%). ^1H NMR (400 MHz, chloroform-*d*) δ 7.45 (s, 1H), 6.35 (dd, $J = 7.8, 5.9$ Hz, 1H), 4.39 (dt, $J = 5.3, 2.4$ Hz, 1H), 4.08 (tdt, $J = 12.9, 9.1, 4.2$ Hz, 2H), 3.93 (q, $J = 2.6$ Hz, 1H), 3.80 (ddd, $J = 40.8, 11.4, 2.6$ Hz, 2H), 3.40 (t, $J = 6.9$ Hz, 2H), 2.31–2.15 (m, 3H), 2.04–1.94 (m, 1H), 1.91 (s, 3H), 0.91 (s, 9H), 0.88 (s, 9H), 0.10 (s, 6H), 0.07 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.03, 150.56, 133.35, 109.59, 87.56, 85.30, 72.07, 62.78, 41.24, 39.97, 30.90, 30.18, 26.69, 25.74, 25.56, 18.16, 17.75, 13.02, -4.83, -5.03, -5.57, -5.64. HRMS (m/z): 591.2279 (calc. 591.2279 [$\text{C}_{25}\text{H}_{47}\text{BrN}_2\text{O}_5\text{Si}_2$] H^+).

3-(3-Azidopropyl)-1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)-oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (4). To a stirred solution of compound 3 (1.0 g, 1.69 mmol) in DMF (10 mL), NaN_3 (1.1 g, 16.9 mmol) was added. The mixture was stirred at room temperature overnight. After completion of the reaction, the mixture was poured into ethyl acetate (100 mL) and then washed with water (3×50 mL). The ethyl acetate was separated, dried with MgSO_4 and evaporated under vacuum. The residue was purified by silica gel column chromatography using PE/EtOAc (v/v = 5 : 1) to give compound 4 as a colorless oil (800 mg, yield 85.5%). ^1H NMR (400 MHz, chloroform-*d*) δ 7.47 (d, $J = 1.1$ Hz, 1H), 6.36 (dd, $J = 7.9, 5.8$ Hz, 1H), 4.40 (dt, $J = 5.5, 2.5$ Hz, 1H), 4.11–3.97 (m, 2H), 3.94 (q, $J = 2.5$ Hz, 1H), 3.81 (ddd, $J = 41.6, 11.4, 2.5$ Hz, 2H), 3.35 (t, $J = 6.9$ Hz, 2H), 2.27 (ddd, $J = 13.1, 5.8, 2.6$ Hz, 1H), 2.06–1.85 (m, 6H), 0.93 (s, 9H), 0.89 (s, 9H), 0.11 (s, 6H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.50, 150.93, 133.69, 110.09, 87.92, 85.62, 72.39, 63.09, 49.46, 41.54, 38.94, 27.33, 26.03, 25.84, 18.49, 18.08, 13.35, -4.54, -4.74, -5.28, -5.36. HRMS (m/z): 554.3190 (calc. 554.3188 [$\text{C}_{25}\text{H}_{47}\text{N}_5\text{O}_5\text{Si}_2$] H^+).

3-(3-Aminopropyl)-1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)-oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (5). A solution of compound 4 (1.0 g, 1.81 mmol) in methanol (20 mL) was hydrogenated using a 10% Pd/C catalyst (100 mg) for 12 h under atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through Celite and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography with DCM/MeOH (dichloromethane/methanol, v/v = 10 : 1) to give

compound **5** as a white solid (667 mg, yield 70%). ^1H NMR (400 MHz, chloroform-*d*) δ 8.47 (s, 2H), 7.51 (s, 1H), 6.35–6.26 (m, 1H), 4.38 (dt, J = 5.7, 2.7 Hz, 1H), 4.10 (t, J = 6.1 Hz, 2H), 3.92 (q, J = 2.5 Hz, 1H), 3.80 (ddd, J = 44.7, 11.4, 2.3 Hz, 2H), 3.00 (t, J = 6.4 Hz, 1H), 2.33–2.15 (m, 3H), 2.02–1.93 (m, 1H), 1.90 (s, 3H), 0.91 (s, 9H), 0.88 (s, 9H), 0.09 (s, 6H), 0.07 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 164.41, 151.08, 134.51, 110.12, 88.03, 85.81, 72.27, 63.03, 41.70, 38.06, 37.04, 26.10, 25.91, 25.47, 18.56, 18.15, 13.38, –4.45, –4.69, –5.20, –5.27. MS (m/z): 528.3428 (calc. 528.3289 $[\text{C}_{25}\text{H}_{49}\text{N}_3\text{O}_5\text{Si}_2]\text{H}^+$).

3-(3-Aminopropyl)-1-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (6). Compound **5** (2.0 g, 3.8 mmol) was dissolved in EtOH (50 mL), and then concentrated HCl (1.0 mL) was added. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, the solvent was removed under vacuum. The residue was redissolved in MeOH (20 mL), and was neutralized with 2 M NaOH before removing the solvent under reduced pressure. The residue was purified by column chromatography with DCM/MeOH (*v/v* = 4:1) to give compound **6** as a white solid (1.08 g, yield 95%). ^1H NMR (400 MHz, deuterium oxide) δ 7.64 (s, 1H), 6.25 (t, J = 6.7 Hz, 1H), 4.47–4.39 (m, 1H), 3.98 (t, J = 6.6 Hz, 3H), 3.84–3.68 (m, 2H), 3.00 (t, J = 7.4 Hz, 2H), 2.40–2.26 (m, 2H), 1.98 (p, J = 6.9 Hz, 2H), 1.87 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 165.49, 151.64, 135.79, 110.57, 86.60, 85.93, 70.44, 61.24, 38.71, 38.39, 37.12, 25.09, 12.37. MS (m/z): 300.1 (calc. 300.2 $[\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_5]\text{H}^+$).

***N*-(3-(3-((2*R*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)propyl)-3-isocyanopropanamide (11).** To a stirred solution of compound **6** (336.0 mg, 1.122 mmol) in MeOH (10 mL), compound **10** (277.4 mg, 1.122 mmol) and trimethylamine (10 μL) were added. The mixture was stirred at room temperature overnight, and then the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography with DCM/MeOH (10:1, *v/v*) to afford compound **11** as a pale yellow oil (210.0 mg, yield 34%). ^1H NMR (400 MHz, methanol-*d*₄) δ 7.83 (d, J = 1.2 Hz, 1H), 6.30 (t, J = 6.7 Hz, 1H), 4.40 (dt, J = 6.8, 3.6 Hz, 1H), 4.02–3.94 (m, 2H), 3.91 (q, J = 3.5 Hz, 1H), 3.84–3.69 (m, 4H), 3.23 (t, J = 6.9 Hz, 2H), 2.63–2.55 (m, 2H), 2.32–2.17 (m, 2H), 1.91 (d, J = 1.2 Hz, 3H), 1.83 (p, J = 6.9 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 170.2, 164.1, 155.3, 151.1, 135.3, 109.4, 87.6, 85.8, 70.8, 61.6, 40.1, 38.8, 37.7, 36.9, 35.3, 27.3, 12.2. MS (m/z): 381.1671 (381.1774 $[\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_6]\text{H}^+$). HRMS (m/z): 381.1768 (calc. 381.1774, $[\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_6]\text{H}^+$).

Preparation of $[\text{Re}(\text{CO})_3(\text{CN-TdR})_3]^+$ (**13a**)

Bromopentacarbonylrhenium(i) ($[\text{Re}(\text{CO})_5\text{Br}]$) (10 mg) was added into water (5 mL) in a pear-shaped flask and then was heated to reflux for 24 h. Successively, 500 μL of the above solution (about 1.0 mg intermediate) was transferred into another 5 mL pear-shaped flask and 250 μL of CN-TDR (**11**) (20.0 mg mL^{-1}) was added, and then the mixture was heated

at 90 °C for 3 h. After completion of the reaction, the mixture solvent was purified by HPLC to obtain compound **13a**. HRMS (m/z): 1411.4498 (calc. 1411.4491 $[\text{C}_{54}\text{H}_{72}\text{N}_{12}\text{O}_{21}\text{Re}]^+$).

Radiolabelling and quality control

The preparations of the metal complexes were carried out using the procedure in Scheme 3. $[\text{Re}(\text{CO})_3(\text{CN-TdR})_3]^+$ (**12**) was prepared by the following procedure: 1.0–2.0 mg CN-TdR was dissolved in 700 μL of saline in a 10 mL vial, and then 200 μL CB (citrate buffer, 0.1 M, pH = 6.6) and 20 μL of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ solution (1 mg $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ /1 mL) were added into the vial. After addition of 100 μL of saline containing $^{99\text{m}}\text{TcO}_4^-$ (37 MBq), the resulting solution was heated at 100 °C for 30 min. The radiochemical purity of the product was determined by HPLC.

$[\text{Re}(\text{CO})_3(\text{CN-TdR})_3]^+$ (**13b**) was prepared by the following procedure: after the $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor was prepared,^{21,37} 1.0 mol L^{-1} HCl was added to adjust the pH to 7–8. Then, 1.0–2.0 mg CN-TdR was added to 0.5 mL of $[\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (37 MBq), and the reaction mixture was heated at 100 °C for 30 min. The radiochemical purity of the product was determined by HPLC.

In vitro stability and partition coefficients

To evaluate the stabilities of the two radiocomplexes, **12** and **13b** were incubated in saline at room temperature for 6 h or in mouse serum at 37 °C for 6 h, respectively. The radiochemical purities of the complexes were measured by HPLC. The proteins of the mouse serum need to be removed before measurement by HPLC.

In a 2 mL centrifuge tube, partition coefficients ($\log P$) between 1-octanol and phosphate buffer (0.025 mol L^{-1} , pH 7.4) of the complexes were measured. With an equal volume of 1-octanol and phosphate buffer, the mixture was vigorously vortexed for 1 min and then centrifuged at 5000 rpm for another 5 min. Three samples (100 μL) from each layer were measured using a gamma counter in triplicate. The partition coefficients were calculated as follows: P = (counts per minute in 1-octanol/counts per minute in phosphate buffer). Usually, $\log P$ was the final expression for the partition coefficient.

In vitro cell experiment

The HCT116 cell line was obtained from China Infrastructure of Cell Line Resource and was maintained as per manufacturer's recommendations. Cells suspended in 4 mL of culture medium were seeded into 6-well plates (~1 000 000 cells per well). The plates were incubated for 48 h in a 5% CO_2 -humidified atmosphere at 37 °C. The medium was removed and washed with 1.0 mL of fresh culture medium. 0.1 mL of medium containing 740 kBq of the radiotracer and 1.0 mL of fresh medium or 1.0 mL of fresh medium containing 10.0 mg thymidine were added into the wells. After incubation for 2 h at 37 °C, the medium was removed and the cells were washed with 2 $\times$ 1.0 mL cold PBS (4 °C, 0.01 M, pH = 7.4).

After detachment by addition of 2×1.0 mL 1 M NaOH, the cells were collected and counted in a gamma counter (Wizard 2480, PerkinElmer). The protein content was measured spectrophotometrically using a Micro BCA protein assay reagent (Macgene, Beijing, China). Each experiment was performed five times. In the FU (5-fluorouracil) pretreatment assay, twenty-four hours after being seeded, the exponentially growing cells were exposed to 200 μ M 5-FU for another 24 h. The results were expressed as the ratio of the percentage of the initial activity to the amount of protein (%ID per mg protein). Taking the cellular uptake in the absence of modulators as the control, the data were finally expressed as (%ID per mg per control) $\times$ 100%.

Biodistribution studies

All animal studies were carried out in compliance with the Regulations on Laboratory Animals of Beijing Municipality. The animal experiments were performed with approval from the Ethics Committee of Beijing Normal University. Approximately 1×10^6 S180 cells were implanted in the left front armpit of male mice by subcutaneous injection. After one week, tumors grew to diameters of 7–10 mm. 0.1 mL of the ^{99m}Tc -labelled radiotracer (37 kBq) was injected into the mice bearing the S180 tumor *via* a tail vein. The mice were sacrificed by neck dislocation at 5 min, 30 min, and 60 min post-injection. The tumors, organs of interest and blood were collected, weighed and measured for radioactive counts. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID g^{-1}).

SPECT/CT imaging studies

A Triumph SPECT/CT scanner (TriFoil Imaging, California, USA) was used for the SPECT/CT imaging studies. 0.1 mL of $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ (18.5 MBq) was injected intravenously through a tail vein into a Kunming mouse bearing the S180 tumor. For static scans, SPECT/CT imaging was performed at 60 min after injection. After being anesthetized with 1.5% isoflurane in air at 500 mL min^{-1} , the mouse was fixed, and sixty minute dynamic acquisitions were initiated at the time of $[\text{}^{99m}\text{Tc}(\text{CN-TdR})_6]^+$ injection.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The work was financially supported, in part, by the National Natural Science Foundation of China (21771023).

References

- 1 D. Hanahan and R. A. Weinberg, *Cell*, 2011, **144**, 646.
- 2 Z. W. F. Kandeel, in *Molecular Imaging Probes for Cancer Research*, ed. X. Chen, World Scientific Publishing, Singapore, 2012, ch. 8, p. 219.
- 3 A. Khalil, K. Ishita, T. Ali and W. Tjarks, *Future Med. Chem.*, 2013, **5**, 677.
- 4 M. Peck, H. A. Pollack, A. Friesen, M. Muzi, S. C. Shoner, E. G. Shankland, J. R. Fink, J. O. Armstrong, J. M. Link and K. A. Krohn, *Q. J. Nucl. Med. Mol. Imaging*, 2015, **59**, 95.
- 5 A. F. Shields, J. R. Grierson, B. M. Dohmen, H.-J. Machulla, J. C. Stayanoff, J. M. Lawhorn-Crews, J. E. Obradovich, O. Muzik and T. J. Mangner, *Nat. Med.*, 1998, **4**, 1334.
- 6 H. Sun, A. Sloan, T. J. Mangner, U. Vaishampayan, O. Muzik, J. M. Collins, K. Douglas and A. F. Shields, *Eur. J. Nucl. Med. Mol. Imaging*, 2005, **32**, 15.
- 7 D. Soloviev, D. Lewis, D. Honess and E. Aboagye, *Eur. J. Cancer*, 2012, **48**, 416.
- 8 C. C. Zhang, Z. M. Yan, W. L. Li, K. Kuszpit, C. L. Painter, Q. Zhang, P. B. Lappin, T. Nichols, M. E. Lira, T. Affolter, N. R. Fahey, C. Cullinane, M. Spilker, K. Zasadny, P. O'Brien, D. Buckman, A. Wong and J. G. Christensen, *Clin. Cancer Res.*, 2012, **18**, 1303.
- 9 R. Schibli, M. Netter, L. Scapozza, M. Birringer, P. Schelling, C. Dumas, J. Schoch and P. A. Schubiger, *J. Organomet. Chem.*, 2003, **668**, 67.
- 10 Y. Zhang, X. Dai, D. F. Kallmes and D. Pan, *Tetrahedron Lett.*, 2004, **45**, 8673.
- 11 M. Schmid, B. Neumaier, A. T. Vogg, K. Wczasek, C. Friesen, F. M. Mottaghy, A. K. Buck and S. N. Reske, *Nucl. Med. Biol.*, 2006, **33**, 359.
- 12 S. Celen, T. de Groot, J. Balzarini, K. Vunckx, C. Terwinghe, P. Vermaelen, L. Van Berckelaer, H. Vanbilloen, J. Nuyts, L. Mortelmans, A. Verbruggen and G. Bormans, *Nucl. Med. Biol.*, 2007, **34**, 283.
- 13 M. Stichelberger, D. Desbouis, V. Spiwok, L. Scapozza, P. A. Schubiger and R. Schibli, *J. Organomet. Chem.*, 2007, **692**, 1255.
- 14 B. Teng, Y. P. Bai, Y. Chang, S. Z. Chen and Z. L. Li, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3440.
- 15 D. Desbouis, H. Struthers, V. Spiwok, T. Kuster and R. Schibli, *J. Med. Chem.*, 2008, **51**, 6689.
- 16 H. Struthers, B. Spingler, T. L. Mindt and R. Schibli, *Chem. – Eur. J.*, 2008, **14**, 6173.
- 17 H. Struthers, A. Hagenbach, U. Abram and R. Schibli, *Inorg. Chem.*, 2009, **48**, 5154.
- 18 H. Struthers, D. Viertl, M. Kosinski, B. Spingler, F. Buchegger and R. Schibli, *Bioconjugate Chem.*, 2010, **21**, 622.
- 19 L. Chun Xiong, W. Zheng Wu, J. Quan Fu, T. Jie, T. Cheng and Z. Jian Kang, *Chin. Chem. Lett.*, 2011, **22**, 1309.
- 20 C. Lu, Q. Jiang, C. Tan, J. Tang and J. Zhang, *Molecules*, 2012, **17**, 8518.
- 21 X. Duan, T. Liu, Y. Zhang and J. Zhang, *Molecules*, 2016, **21**, 510.
- 22 M. J. Abrams, A. Davison, A. G. Jones, C. E. Costello and H. Pang, *Inorg. Chem.*, 1983, **14**, 2798.
- 23 F. E. Hahn, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 650.
- 24 S. Yang, X. Wang, H. Guo, J. Liu, F. Wang and X. Zhang, *J. Radioanal. Nucl. Chem.*, 2008, **278**, 165.
- 25 G. Y. Hao, J. Y. Zang, L. Zhu, Y. Z. Guo and B. L. Liu, *J. Labelled Compd. Radiopharm.*, 2004, **47**, 513.

- 26 X. Chen, Y. Guo, Q. Zhang, G. Hao, H. Jia and B. Liu, *J. Organomet. Chem.*, 2008, **693**, 1822.
- 27 J. Zhang, F. Visser, K. M. King, S. A. Baldwin, J. D. Young and C. E. Cass, *Cancer Metastasis Rev.*, 2007, **26**, 85.
- 28 E. T. McKinley, G. D. Ayers, R. A. Smith, S. A. Saleh, P. Zhao, M. K. Washington, R. J. Coffey and H. C. Manning, *PLoS One*, 2013, **8**, e58938.
- 29 S. J. Lee, S. Y. Kim, H. C. Jin, S. J. Oh, S. R. Jin, S. H. Yong, T. W. Kim and D. H. Moon, *Biochem. Pharmacol.*, 2010, **80**, 1528.
- 30 Y. M. Dupertuis, M. Vazquez, J. P. Mach, N. De Tribolet, C. Pichard, D. O. Slosman and F. Buchegger, *Cancer Res.*, 2001, **61**, 7971.
- 31 M. Perumal, R. G. Pillai, H. Barthel, J. Leyton, J. R. Latigo, M. Forster, F. Mitchell, A. L. Jackman and E. O. Aboagye, *Cancer Res.*, 2006, **66**, 8558.
- 32 J. Pressacco, B. Mitrovski, C. Erlichman and D. W. Hedley, *Cancer Res.*, 1995, **55**, 1505.
- 33 M. D. Bartholoma, A. R. Vortherms, S. Hillier, J. Joyal, J. Babich, R. P. Doyle and J. Zubieta, *Dalton Trans.*, 2011, **40**, 6216.
- 34 Y. Byun, J. H. Yan, A. S. Al-Madhoun, J. Johnsamuel, W. L. Yang, R. F. Barth, S. Eriksson and W. Tjarks, *J. Med. Chem.*, 2005, **48**, 1188.
- 35 D. Kachare, X.-P. Song and P. Herdewijn, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2720.
- 36 Y. Mizuno, T. Uehara, H. Hanaoka, Y. Endo, C. W. Jen and Y. Arano, *J. Med. Chem.*, 2016, **59**, 3331.
- 37 R. Alberto, R. Schibli, A. Egli, A. P. Schubiger, U. Abram and T. A. Kaden, *J. Am. Chem. Soc.*, 1998, **120**, 7987.