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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Preparation and bioevaluation of ^{99m}Tc nitrido radiopharmaceuticals with pyrazolo[1,5-a]pyrimidine as tumor imaging agents

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Abstract The 7-(2-aminoethylamino)-5-methyl-3-cyano-pyrazolo[1,5-a]pyrimidine (AMCPP) was synthesized and conjugated with *N*-mercaptoacetylglycine (MAG), *N*-mercaptoacetylphenylalanine (MAF), and *N*-mercaptoacetylvaline (MAA), respectively. These three compounds were labeled successfully with $[^{99m}TcN]^{2+}$ intermediate in high radiochemical purities. Biodistribution in tumor-bearing mice demonstrated that the three new complexes showed high tumor-to-muscle (T/M) ratios and rapid clearance from the blood, muscle, liver, kidney, and lung. Among them, the ^{99m}TcN-MAG-AMCPP showed the most favorable characteristics. The tumor/blood and tumor/muscle ratios reached 1.50 and 1.15 at 30 min post-injection, 2.20 and 1.83 at 60 min post-injection.

Keywords Pyrazolo[1,5-a]pyrimidine · Tumor imaging · $[^{99m}TcN]^{2+}$ complexes

Introduction

Tumor is among the most common causes of death in the world. Recently, the therapeutic potential of small molecule inhibitors of cyclin-dependent kinases (CDKs) for use as antitumor agents for the treatment of cancer has come under much attention (Lane *et al*., 2001; Sielecki *et al*., 2000). This approach to cancer therapy differs from the traditional use of cytotoxic agents in that the target tissue is the vasculature encompassing the neoplasm, not the tumor cells (Fraley *et al*., 2002; Wu *et al*., 2004; De bondt *et al*., 1993; Richardson *et al*., 2006). A large number of different structural compounds, including indolin-2-ones, 4-anilinopyrazolo[4,3-d]pyrimidines, purines, phthalazines, and quinazolines were reported as antitumor agents used in clinics or in clinical trials (Arora and Scholar, 2005; Meijer and Raymond, 2003; Havlicek *et al*., 2005; Knockaert *et al*., 2002).

Pyrazolo[1,5-a]pyrimidine derivatives are developed as a new class of CDKs inhibitors. These compounds are currently being evaluated as antitumor drugs in a growing number of therapeutic areas. Among them, 3-cyano-5,7-disubstistituted pyrazolo[1,5-a]pyrimidine derivatives showed significant antitumor activities (Berman *et al*., 2000; Lawrie *et al*., 1997; Williamson *et al*., 2005). Gong et al. discovered that the amino group at position 7 of pyrazolo-[1,5-a]pyrimidine core structure enhanced the antitumor activity (Li *et al*., 2006).

Amongst the various chemical forms and preformed precursors of technetium used for radiolabeling, $[^{99m}TcN]^{2+}$ has gained interest owing to the presence of the nitrido N^{3-} ligand as strong π-electron donor that leads to formation of kinetically inert complexes (Liu and Edwards, 1999). The $[^{99m}TcN]^{2+}$ core exhibits a very high chemical stability toward oxidation–reduction reactions and pH variations, and high affinity toward chelating ligands containing sulfur atoms. The presence of the $[^{99m}TcN]^{2+}$ core in the molecular structure of a radiopharmaceutical may dramatically alter its biological behavior.

Benoist et al. reported a novel bifunctional chelating agent (BFCA), MAG-N-OPh, which formed a stable and inert

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^{99m}Tc-complex in high yield under mild conditions (Gal *et al*., 2006). To link to the target molecule, it was synthesized MAG as the precursor of the BFCA according to the previous modified methods (Qi *et al*., 2002), and introduced solubilizing basic ethylenediamine at the 7-position to the 3-cyanopyrazolo[1,5-a]pyrimidine, thus affording a new N_3S type ligand. This report constitutes the first of its kind in using the 3-cyanopyrazolo[1,5-a]pyrimidine in the preparation of ^{99m}TcN complexes as targeted agents for tumor imaging.

Toward developing new better tumor imaging agents, the authors introduced hydrophobic groups such as isopropyl group and benzyl group to MAG, and synthesized ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP. A series of experiment was carried out in order to evaluate the bioactivity of the three complexes.

Materials and methods

^{99}Mo/^{99m}Tc generator was obtained from the China institute of atomic energy (CIAE). All other chemicals were of analytical grade and were used without further purification. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer operating in $CDCl_3$ or DMSO-d^6 at 400 and 100 MHz, respectively. The IR spectra were recorded on a Nicolet-AVATAR 360 FT–IR spectrometer using KBr pellets in the 4000–400 cm^{-1} regions. ESI–MS were performed on Waters LCT Premier XE. HPLC analyses were performed on a Shimadzu SCL-10 AVP equipped with a Packard 500 TR series flow scintillation analyzer. A C-18 reversed-phase Alltima column (5u 150 mm × 4.6 mm) was used for radiochemical purity analysis.

Ligand synthesis

Tritylmercaptoacetic acid (2)

New distilled mercaptoacetic acid **1** (0.7 mL, 10 mmol) was dissolved in the mixture of 6 mL of dichloromethane and 6 mL acetic acid. Trityl chloride (2.79 g, 10 mmol) was added to the solution. $BF_3{\cdot}Et_2O$ (2 mL) was added dropwise as catalyst, and the color of reaction solution turned yellow-green. After the reaction solution had been stirred at room temperature for 60 min, the solvent was removed in vacuo. Water (10 mL) was added to the residue. The white precipitate was collected by filtration and washed with distilled water, acetonitrile and cool ether to obtain the compound **2** (3.18 g, 95%). m.p.: 159–160°C; IR (KBr, cm^{-1}): ν 1705, 1488, 1446, 1412, 1292, 749, 701; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.98 (2H, s), 7.16 (3H, m), 7.23 (6H, m), 7.35 (6H, d, $J = 7.6$ Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 34.3, 67.3, 127.0, 128.1, 129.5, 143.8, 173.9.

Tritylmercaptoacetic acid *N*-hydroxysuccinimido ester (3)

Compound **2** (1.0 g, 3 mmol) and *N*-hydroxysuccinimide (NHS) (0.345 g, 3 mmol) were dissolved in anhydrous THF at −10°C. DCC (0.619 g, 3 mmol) in 5 mL of anhydrous THF was added dropwise. The clear reaction mixture was stirred for 2 h at −5°C and then overnight at room temperature. White precipitate was removed by filtration. Solvent was removed in vacuo to obtain the light yellow crude product and the crude product was recrystallized in ethyl acetate to obtain the compound **3** (1.06 g, 82%) as white crystal. m.p.: 188–189°C; IR (KBr, cm^{-1}): ν 1810, 1782, 1737, 1627, 1445, 1245, 1207, 1064, 742, 699; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.74 (4H, s), 3.11 (2H, s), 7.16 (3H, m), 7.25 (6H, m), 7.35 (6H, d, $J = 7.8$ Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 25.5, 31.4, 68.1, 127.1, 128.2, 129.5, 143.6, 165.1, 168.6.

N-[(Tritylmercapto)acetyl]glycine (Tr-MAG) (4a)

Compound **3** (0.30 g, 0.7 mmol) was dissolved in acetonitrile (15 mL). Glycine (0.105 g, 1.4 mmol) in sodium hydroxide (0.2 M, 1 mL) was added dropwise. The reaction solution was stirred for 2 h at 50–60°C. Distilled water (1 mL) was added to the reaction mixture and the pH was adjusted to 1. The acetonitrile was removed in vacuo and a white solid precipitated. The white residue was recrystallized from ethyl acetate to give compound **4a** (0.22 g, 78%); m.p.: 164–166°C; IR (KBr, cm^{-1}): ν 3434, 3344, 2960, 2926, 1732, 1619, 1527, 1488, 1445, 1247, 1224, 1211, 741, 704; ^{1}H-NMR ($CDCl_3$): δ 3.13 (2H, s), 3.62 (2H, d, $J = 5.1$ Hz), 6.46 (1H, t, $J = 4.9$ Hz), 7.15(3H, m), 7.22 (6H, m), 7.36 (6H, d, $J = 7.6$ Hz); ^{13}C-NMR ($CDCl_3$): δ 35.4, 41.6, 67.9, 127.1, 128.2, 129.4, 143.8, 169.0, 172.2.

N-[(Tritylmercapto)acetyl]phenylalanine (Tr-MAF) (4b)

4b was obtained with the same method of **4a** using phenylalanine instead of glycine (yield: 59%); m.p.: 128–130°C; IR (KBr, cm^{-1}): ν 3448, 3340, 3027, 2928, 1727, 1627, 1528, 1493, 1443, 1223, 1203, 1184, 742, 700; ^{1}H-NMR ($CDCl_3$): δ 3.07 (4H, m), 4.48 (1H, dm, $J = 6.2$ Hz), 6.54 (1H, d, $J = 6.2$ Hz), 7.10 (2H, m), 7.25 (18H, m); ^{13}C-NMR ($CDCl_3$): δ 36.0, 37.0, 53.8, 68.1, 127.2, 127.3, 128.2, 128.7, 129.4, 129.5, 135.5, 143.9, 169.1, 173.6.

N-[(Tritylmercapto)acetyl]valine (Tr-MAA) (4c)

4c was obtained with the same method of **4a** using valine instead of glycine (yield: 65%); m.p.: 146–148°C; IR (KBr,

cm^{-1}): *v* 3434, 3339, 3055, 2960, 2929, 1739, 1720, 1636, 1534, 1489, 1441, 1422, 1381, 1304, 1273, 1213, 1082, 1035, 1000, 885, 757, 738, 700; ^{1}H-NMR ($CDCl_3$): δ 0.88 (6H, d, $J = 6.8$ Hz), 2.10 (1H, m), 3.13 (2H, s), 4.31 (1H, dd, $J = 4.9$, 3.2 Hz), 6.62 (1H, d, $J = 8.2$ Hz), 7.22 (3H, m), 7.28 (6H, m), 7.38 (6H, d, $J = 7.6$ Hz); ^{13}C-NMR ($CDCl_3$): δ 17.9, 18.8, 31.0, 36.2, 57.5, 68.2, 127.2, 128.2, 129.6, 144.0, 168.8, 175.4.

2-(Ethoxymethylene)malononitrile (6)

Malononitrile (9.9 g, 0.150 mol), triethoxymethane (37.4 ml, 0.225 mol) and acetic anhydride (35.4 ml, 0.375 mol) were refluxed for 6 h. Then a little charcoal was added. The solvent was removed from the mixture by distillation. The hot residue was filtered and the filter cake was washed with hot ethanol, the filtrate was placed in a refrigerator overnight. After filtration, the crystalline was washed with ice-cold ethanol and dried to get the product **6** (17.4 g, 88%). m.p.: 66–68°C; IR (KBr, cm^{-1}): *v* 3443, 3031, 2944, 2228, 1611, 1315, 1009, 883.

3-Amino-4-cyanopyrazole (7)

Compound **6** (15 g, 0.123 mol) was carefully added to 85% hydrazine hydrate (12 ml, 0.248 mol) at room temperature. The resulting mixture was refluxed for 1 h. To the resulting solidified mass was added 10 mL of water. The solution stay in a refrigerator overnight and the mushy solution was filtered and the solid was washed with cold water and dried to get the product **7** (10.7 g, 81%). m.p.: 174–176°C; IR (KBr, cm^{-1}): *v* 3416, 3342, 3147, 2955, 2238, 1645, 1570, 1522, 1221, 1034, 716.

5-Methyl-3-cyano-7-hydroxypyrazolo[1,5-a]pyrimidine (8)

A mixture of **7** (9.0 g, 0.083 mol), ethyl acetoacetate (16.25 g, 0.125 mol), and acetic acid (85 ml) was refluxed for 4 h. After cooling to room temperature, the pale white solid was filtered and washed with ethanol, dried with suction, and recrystallized from ethyl acetate to obtain the solid product **8** (13.01 g, 90%). m.p.:>250°C; MS(ESI): M = 174.2 (Found: 175.2).

5-Methyl-3-cyano-7-chloropyrazolo[1,5-a]pyrimidine (9)

Phosphoryl trichloride (0.78 ml, 8.6 mmol) was added dropwise into a solution of **8** (1.0 g, 5.7 mmol) in anhydrous pyridine (0.5 g, 6.3 mmol). The mixture was heated to 85°C slowly, and then stirred at 120°C for 1 h. After it was cooled to 60°C, chloroform (25 ml) was added and stirred for 1 h. Cold water (30 ml) was added to the solution. The insoluble materials was filtered off, the organic layer of the filtrate was washed with cold water to pH 7, concentrated, and recrystallized from ethyl acetate/cyclohexane to give the compound **9** (0.95 g, 87%). m.p.: 213–215°C; MS(ESI): M = 192.0 (Found: 192.9).

7-(2-Aminoethylamino)-5-methyl-3-cyanopyrazolo[1,5-a]pyrimidine (AMCPP) (10)

A mixture of **9** (0.963 g, 5 mmol), ethylenediamine (0.60 g, 10 mmol), and ethanol (30 ml) was heated to reflux for 4 h. Solvent was removed in vacuo to obtain the crude product and the crude product was recrystallized in methanol to obtain the compound **10** (0.86 g, 81%). m.p.: 203–205°C; IR (KBr, cm^{-1}): *v* 3354, 3293, 3112, 2944, 2861, 2221, 1637, 1578, 1459, 1311, 1193, 944; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ 2.51 (3H, s), 2.79 (2H, m), 3.17 (2H, m), 6.44 (1H, s), 8.56 (1H, s); ^{13}C-NMR (DMSO-d^6, 100 MHz): δ 24.5, 40.2, 44.5, 77.8, 88.5, 114.5, 146.1, 147.3, 150.1, 162.7; MS(ESI): M = 216.2 (Found: 217.3).

Conjugation of Tr-MAG and AMCPP (Tr-MAG-AMCPP) (11a)

A mixture of **10** (108 mg, 0.5 mmol), triethylamine (0.105 ml, 0.75 mmol) and anhydrous dichloromethane (4 ml) was stirred at room temperature for 5 min. Then **4a** (196 mg, 0.5 mmol) and HOBt (75 mg, 0.55 mmol) were added. DCC (115 mg, 0.55 mmol) in 2 ml of anhydrous dichloromethane was added dropwise to the solution at 0°C. The mixture was stirred for 30 min at 0°C and then overnight at room temperature. White precipitate was removed by filtration. The organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude was purified by column chromatography (silica gel; petroleum ether/ethyl acetate = 1:5) to afford the desired compound **11a** (56 mg, 19%). mp: 166–168°C; IR (KBr, cm^{-1}): *v* 3384, 3311, 3096, 3048, 2931, 2844, 2223, 1658, 1637, 1585, 1535, 1467, 1443, 1368, 1310, 1245, 1195, 1116, 1038, 745, 702, 673, 622; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.54 (3H, s), 3.18 (2H, s), 3.48 (2H, m), 3.55 (4H, m), 5.98 (1H, s), 6.53 (1H, t, $J = 5.6$ Hz), 6.73 (1H, m), 7.09 (1H, m), 7.23 (3H, m), 7.29 (6H, m), 7.39 (6H, m), 8.03 (1H, s); ^{13}C-NMR (DMSO-d^6, 100 MHz): δ 24.5, 35.9, 40.1, 41.2, 45.7, 65.9, 77.8, 88.3, 114.3, 126.8, 128.0, 129.0, 144.0, 146.1, 147.2, 150.5, 162.7, 167.6, 169.1; MS(ESI): M = 589.7 (Found: 590.6).

Conjugation of Tr-MAF and AMCPP (Tr-MAF-AMCPP) (11b)

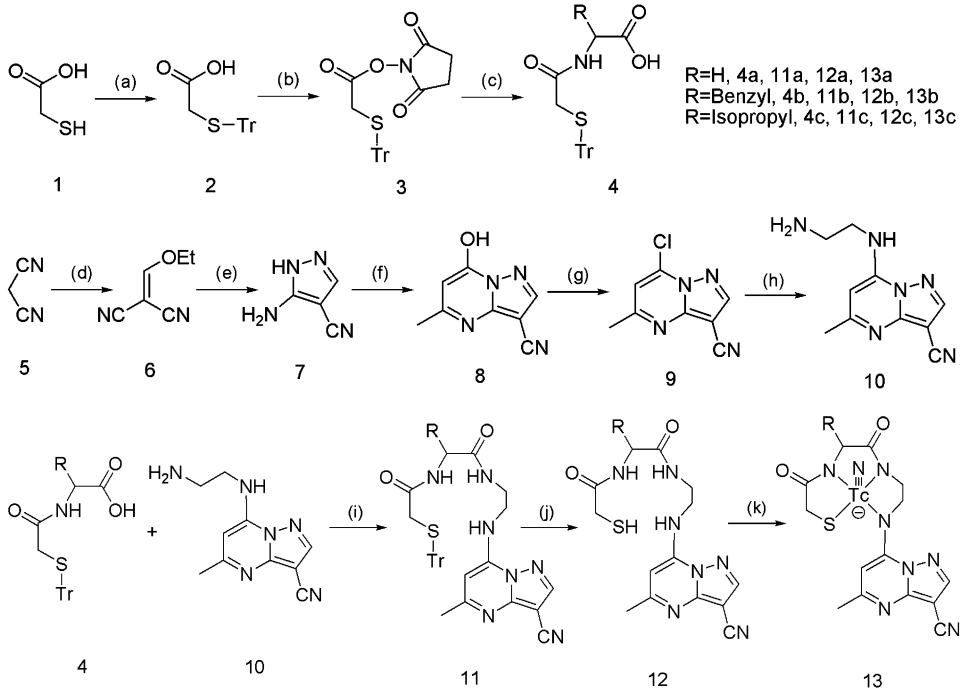
11b was prepared with the same procedure as **11a** synthesis (yield: 23%). m.p.: 208–210°C; IR(KBr, cm^{-1}): ν 3377, 3266, 3084, 3058, 2931, 2844, 2221, 1630, 1583, 1536, 1493, 1444, 1374, 1310, 1245, 1192, 1119, 1026, 744, 700, 674, 623; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.56 (3H, s), 2.89 (2H, m), 3.11 (2H, s), 3.33 (3H, m), 3.51 (1H, m), 4.25 (1H, m), 5.93 (1H, s), 6.15 (1H, t), 6.95 (1H, m), 7.09 (1H, m), 7.12 (5H, m), 7.23 (8H, m), 7.28 (7H, m), 7.98 (1H, s); ^{13}C-NMR (DMSO-d^6, 100 MHz): δ 24.5, 35.9, 37.5, 37.6, 40.1, 41.2, 54.2, 65.7, 77.8, 88.4, 114.3, 126.2, 126.7, 127.9, 128.0, 129.0, 137.5, 144.0, 146.1, 147.1, 150.5, 162.6, 167.1, 171.3; MS(ESI): M = 679.8 (Found: 680.7).

Conjugation of Tr-MAA and AMCPP (Tr-MAA-AMCPP) (11c)

11c was prepared with the same procedure as **11a** synthesis (yield: 19%). m.p.: 202–203°C; IR(KBr, cm^{-1}): ν 3440, 3090, 3048, 2961, 2924, 2223, 1633, 1585, 1536, 1465, 1444, 1368, 1308, 1236, 1193, 1113, 1029, 744, 701; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.85 (6H, dd, J = 7.8, 6.8 Hz), 2.04 (1H, m), 2.57 (3H, s), 3.19 (2H, s), 3.45 (3H, m), 3.60 (1H, m), 3.87 (1H, t), 5.96 (1H, s), 6.24 (1H, m), 6.43 (1H, m), 6.97 (1H, m), 7.24 (9H, m), 7.33 (6H, m), 7.87 (1H, s); ^{13}C-NMR (DMSO-d^6, 100 MHz): δ 17.9, 19.0, 24.5, 30.2, 35.8, 37.5, 41.2, 58.0, 65.9, 77.8, 88.4, 114.4, 126.8, 128.0, 129.0, 144.1, 146.1, 147.1, 150.4, 162.6, 167.4, 171.3; MS(ESI): M = 631.8 (Found: 632.6).

Preparation of ^{99m}TcN-MAG-AMCPP analogs

The tritylated compound **11a** (5 mg) was treated with trifluoroacetic acid under cation trapping conditions (5% triethylsilane) at room temperature. After removing the solvent under a stream of nitrogen, the residue was neutralized with 0.1 M NaOH. The solution was extracted with dichloromethane, and the aqueous phase was under nitrogen protection. 1 ml of saline containing $[^{99m}TcO_4]^-$ (15 MBq) was added to a kit containing 0.05 mg of stannous chloride dehydrate, 5.0 mg of succinic dihydrazide (SDH), 5.0 mg of propylenediamine tetraacetic acid (PDTA). The mixture was kept at room temperature for 15 min. Successively, 1 ml of the MAG-AMCPP solution was then added and the reaction allowed to stand for 15 min at 100°C to give the final complex ^{99m}TcN-MAG-AMCPP (**13a**). ^{99m}TcN-MAF-AMCPP (**13b**) and ^{99m}TcN-MAA-AMCPP



Scheme 1 Synthesis of ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP

(a) TrCl, $BF_3{\cdot}Et_2O$, CH_2Cl_2/CH_3COOH; (b) NHS, DCC, anhydrous THF; (c) CH_3CN. **4a**, glycine; **4b**, phenylalanine; **4c**, valine; (d) triethyl orthoformate, acetic anhydride; (e) 85% hydrazine; (f) ethyl acetoacetate, CH_3COOH; (g) $POCl_3$, anhydrous pyridine, $CHCl_3$; (h) ethylenediamine, EtOH; (i) HOBt, DCC, Et_3N, anhydrous CH_2Cl_2; (j) Et_3SiH, TFA; (k) ^{99m}Tc-SDH.

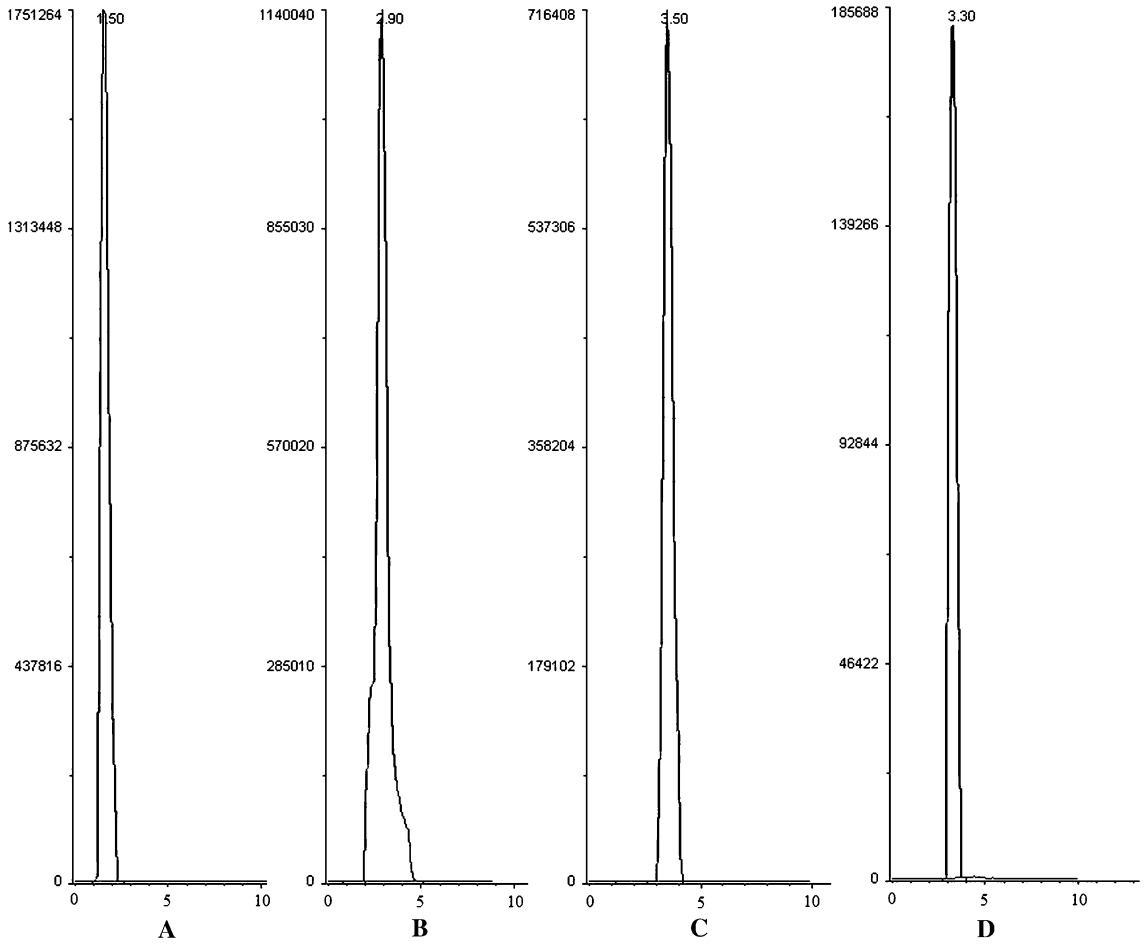


Fig. 1 *Radio*-HPLC chromatograms. **a** $^{99m}TcN^{2+}$. **b** ^{99m}TcN-MAG-AMCPP (**13a**). **c** ^{99m}TcN-MAF-AMCPP (**13b**). **d** ^{99m}TcN-MAA-AMCPP (**13c**)

Table 1 Partition coefficients of ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP

^{99m}TcN-MAG-AMCPP	^{99m}TcN-MAF-AMCPP	^{99m}TcN-MAA-AMCPP
logP -2.71 ± 0.02	1.13 ± 0.03	-1.55 ± 0.03

(**13c**) were prepared using the same method as ^{99m}TcN-MAG-AMCPP (**13a**).

Characterization of ^{99m}TcN-MAG-AMCPP analogs

Formation of the complexes and nature of the species formed was determined by *Radio*-HPLC analysis. Solvent system used for elution was water (solvent A) and acetonitrile (solvent B). The HPLC gradient system for analysis of the product started with 100% A/0% B with a linear gradient to 0% A/100% B from 0 to 30 min. The flow rate was 1.0 ml/min. Five microliters of the sample was used for analysis. Recovery was determined by summing the total counts in all fractions and comparing them to the total injected activity.

Partition coefficient

The partition coefficient was determined by mixing the complex with an equal-volume of 1-octanol and phosphate buffer (0.025 M, pH 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5000 r/min for 5 min. From each phase, 0.1 ml of the aliquot was pipetted and counted in a well-type NaI(Tl) detector. The measurement was repeated three times. Care was taken to avoid cross contamination between the phases. The partition coefficient, P, was calculated using the following equation:

P = (cps in octanol-cps in background)/(cps in buffer-cps in background)

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

Biodistribution

Biodistribution studies were performed in Kunming female mice (weighing 18–20 g) bearing S 180 tumor, which grew

Table 2 Biodistribution of ^{99m}TcN-MAG-AMCPP in mice bearing S 180 tumor (%ID/g)[a]

Tissue	5 min	30 min	60 min	120 min
Heart	0.53 ± 0.04	0.05 ± 0.05	0.06 ± 0.01	0.03 ± 0.01
Liver	8.24 ± 0.68	2.89 ± 0.09	0.16 ± 0.01	0.16 ± 0.02
Spleen	0.24 ± 0.04	0.10 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Lung	0.94 ± 0.13	0.12 ± 0.03	0.10 ± 0.01	0.06 ± 0.01
Kidney	4.09 ± 0.29	0.67 ± 0.01	0.45 ± 0.02	0.61 ± 0.08
Bone	0.34 ± 0.05	0.14 ± 0.01	0.08 ± 0.01	0.04 ± 0.01
Muscle	0.51 ± 0.09	0.13 ± 0.01	0.06 ± 0.01	0.03 ± 0.01
Stomach	0.37 ± 0.02	0.22 ± 0.01	0.08 ± 0.02	0.02 ± 0.01
Large intestine	1.47 ± 0.07	0.17 ± 0.02	0.18 ± 0.02	0.09 ± 0.01
Small intestine	1021 ± 0.13	0.89 ± 0.11	0.75 ± 0.10	0.21 ± 0.022
Blood	1.06 ± 0.14	0.10 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Brain	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Tumor	0.52 ± 0.04	0.15 ± 0.02	0.11 ± 0.02	0.06 ± 0.01
T/M ratio	1.02	1.15	1.83	2.00
T/B ratio	0.49	1.50	2.20	1.50

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

All data are the mean percentage ($n = 3$) of the injected dose of ^{99m}TcN-MAG-AMCPP per gram of tissue, ± the standard deviation of the mean

Table 3 Biodistribution of ^{99m}TcN-MAF-AMCPP in mice bearing S 180 tumor (%ID/g)[b]

Tissue	5 min	30 min	60 min	120 min
Heart	0.05 ± 0.01	0.08 ± 0.03	0.03 ± 0.01	0.04 ± 0.01
Liver	16.93 ± 1.15	4.27 ± 0.44	3.89 ± 0.08	3.05 ± 0.19
Spleen	2.32 ± .022	1.49 ± 0.27	2.29 ± 0.24	0.84 ± 0.17
Lung	1.55 ± 0.21	0.42 ± 0.09	0.20 ± 0.04	0.08 ± 0.01
Kidney	13.12 ± 1.13	2.46 ± 0.38	1.02 ± 0.12	1.16 ± 0.19
Bone	0.51 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.15 ± 0.01
Muscle	0.32 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.08 ± 0.02
Stomach	1.30 ± 0.12	0.32 ± 0.02	0.27 ± 0.03	0.48 ± 0.05
Large intestine	0.64 ± 0.07	0.29 ± 0.01	0.06 ± 0.01	0.08 ± 0.02
Small intestine	0.80 ± 0.08	0.67 ± 0.06	0.59 ± 0.06	0.54 ± 0.06
Blood	1.53 ± 0.17	0.33 ± 0.02	0.21 ± 0.01	0.13 ± 0.01
Brain	0.06 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
Tumor	0.60 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.04 ± 0.01
T/M ratio	1.88	3.50	3.75	0.50
T/B ratio	0.39	0.64	0.71	0.31

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

All data are the mean percentage ($n = 3$) of the injected dose of ^{99m}TcN-MAF-AMCPP per gram of tissue, ± the standard deviation of the mean

Table 4 Biodistribution of ^{99m}TcN-MAA-AMCPP in mice bearing S 180 tumor (%ID/g)[c]

Tissue	5 min	30 min	60 min	120 min
Heart	0.63 ± 0.05	0.31 ± 0.02	0.20 ± 0.01	0.20 ± 0.02
Liver	4.16 ± 0.61	0.75 ± 0.12	0.70 ± 0.10	0.56 ± 0.06
Spleen	0.41 ± 0.05	0.27 ± 0.03	0.25 ± 0.05	0.16 ± 0.01
Lung	1.05 ± 0.10	0.81 ± 0.11	0.43 ± 0.06	0.27 ± 0.03
Kidney	5.21 ± 0.32	1.71 ± 0.19	1.25 ± 0.17	1.09 ± 0.19
Bone	0.45 ± 0.03	0.29 ± 0.03	0.29 ± 0.05	0.22 ± 0.01
Muscle	0.63 ± 0.06	0.15 ± 0.02	0.12 ± 0.01	0.10 ± 0.01
Stomach	2.17 ± 0.26	0.42 ± 0.02	0.36 ± 0.04	0.26 ± 0.03
Large intestine	2.06 ± 0.17	0.20 ± 0.02	0.20 ± 0.03	0.54 ± 0.05
Small intestine	1.09 ± 0.18	0.88 ± 0.15	0.65 ± 0.09	0.23 ± 0.04
Blood	1.46 ± 0.13	1.12 ± 0.12	0.93 ± 0.11	0.60 ± 0.07
Brain	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Tumor	0.61 ± 0.05	0.35 ± 0.04	0.30 ± 0.03	0.24 ± 0.05
T/M ratio	0.97	2.33	2.50	2.40
T/B ratio	0.42	0.31	0.32	0.40

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

All data are the mean percentage ($n = 3$) of the injected dose of ^{99m}TcN-MAA-AMCPP per gram of tissue, ± the standard deviation of the mean

to a leg diameter of 10–15 mm. ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP (0.1 ml, 7.4×10^5 Bq) were injected via a tail vein and the injected radioactivity was measured with a well-type NaI(Tl) detector, respectively. The mice were sacrificed at 5, 30, 60, and 120 min 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 carried out in compliance with the national laws related to the conduct of animal experimentation.

Results and discussion

Chemistry of ^{99m}TcN-MAG-AMCPP analogs

Synthesis of ^{99m}TcN-MAG-AMCPP analogs was performed according to the procedure outlined in Scheme 1. The radiochemical purity of the complexes was routinely checked by HPLC. The HPLC pattern of ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP are shown in Fig. 1. It was observed that the retention time of $[^{99m}TcN]^{2+}_{int}$ was 1.5 min, while that of ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP were found to be 2.9, 3.5, and 3.3 min, respectively. The radiochemical purity of the three products was over 90% immediately after the preparation.

Partition coefficient

The partition coefficients (logP) of ^{99m}TcN-MAG-AMCPP, ^{99m}TcN-MAF-AMCPP, and ^{99m}TcN-MAA-AMCPP are shown in Table 1 and all of them were hydrophilic. ^{99m}TcN-MAG-AMCPP was the highest hydrophilic one and ^{99m}TcN-MAA-AMCPP was more hydrophilic than ^{99m}TcN-MAF-AMCPP.

Biodistribution study

The results of biodistribution are summarized in Tables 2, 3, and 4. There were significant similarities in biodistribution pattern between these three complexes. The accumulation of the three complexes in all of the organs or tissue was highest in the 5 min post-injection and then decreased. Although the uptake in tumor of the three complexes was comparably low, the clearance rate in the tumor was slower than in other organs or tissue, as time lapsed, the activity in the tumor exceeded than that in most other organs or tissue. For ^{99m}TcN-MAG-AMCPP, the tumor/blood and tumor/muscle ratios reached 1.50 and 1.15 at 30 min post-injection, 2.20 and 1.83 at 60 min post-injection. For ^{99m}TcN-MAF-AMCPP, the tumor/blood and tumor/muscle ratios reached 0.64 and 3.50 at 30 min post-injection, 0.71 and 3.75 at 60 min post-injection. For ^{99m}TcN-MAA-AMCPP, the tumor/blood and tumor/muscle ratios reached 0.32 and 2.50 at 60 min post-injection, 0.40 and 2.40 at 120 min post-injection. Early hepatic and renal activity reflected the three complexes were excreted through hepatobiliary as well as renal system. The three complexes were hydrophilic so that they were unable to cross the blood brain barrier, thus making their brain uptake much lower. Among them, ^{99m}TcN-MAG-AMCPP showed the most favorable characteristics with the highest tumor/blood ratios. It is deemed worthwhile to modify the structure of AMCPP suitably to render it less hydrophilic to enhance the tumor uptake of its ^{99m}Tc labeled complex.

Conclusion

In summary, the new 3-cyanopyrazolo[1,5-a]pyrimidine AMCPP was successfully synthesized and conjugated with MAG, MAF, and MAA, respectively. The three compounds were labeled with technetium-99m in high radiochemical purities through a ligand exchange reaction, which could be easily used for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. The three complexes demonstrated high tumor-to-muscle (T/M) ratios and rapid clearance from the blood, muscle, liver, kidney, and lung. Though observations made in biodistribution studies and the clearance from the non-target tissue were encouraging, the tumor uptake of the three complexes was comparably low as in case of other ^{99m}Tc labeled tumor imaging agents reported. Attempts to increase the tumor uptake of the ^{99m}Tc labeled 3-cyanopyrazolo[1,5-a]pyrimidine derivatives by structural modification are underway.

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