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SYNTHESIS, RADIOLABELING AND STABILITY OF NEW NITROPHENOL COMPLEXES OF Technetium-99m AS POSSIBLE HYPOXIA IMAGING RADIOPHARMACEUTICALS

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The synthesis of seven ^{99m}Tc-labeled nitrophenol radiosensitizers (N_2OS chelates) was undertaken for evaluating their *in vitro* biostabilty as possible hypoxia tumor imaging agents. The title compounds (**2** – **7**) were successively synthesized, characterized, and finally radiolabeled (^{99m}Tc-$NaTcO_4$, stannous chloride, pH 10) to obtain the new complexes (**8a** – **8f**) for evaluation. The purity and stability of complexes (in human and rat serum) were evaluated by chromatographic methods (radio-TLC, ITLC, HPLC). The most stable complex (over 6 h) was ^{99m}Tc-labeled 3-[3′-N-(2″-hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-methyl)thiourea (**8e**). Biodistribution studies of **8e** in mammary tumor-bearing rats are in progress.

Many radiolabeled nitroimidazole derivatives have been developed for non-invasive imaging of hypoxic tissues. The most important compounds contain iodine-123 [1, 2], fluorine-18 [3, 4] and bromine-82 [5]. Linder, et al. [6, 7] have also reported preparation of technetium-99m (^{99m}Tc) complexes of nitroimidazole-BATO derivatives. Di Rocco et al. [8] showed that these complexes bind to ischemic tissue of cerebral infarction in rats and in hypoxic myocardium in rabbits. In order to develop agents for non-invasive imaging of hypoxic cells, a nitrophenol ligand, N,N-bis(2-hydroxy-5-nitrobenzyl)-1,3-diamino-2-hydroxypropane (HNBAHP) had previously been synthesized as an approach to tissue hypoxia imaging. This ligand system incorporated the radiosensitizer properties of aromatic nitro-compounds [9, 10] and the stability of technetium-aminophenol complexes [11, 12] into one moiety. Preliminary biodistribution (imaging) data for HNBAHP after intravenous administration demonstrated that most of radioactivity remained in the abdominal area, with negligible radioactivity in the tumor within the first 20 h. Thus, the applicability of these complexes in nuclear medicine was restricted because of a high radioactivity level in the abdominal area and the slow accumulation of radioactivity in the tumor.

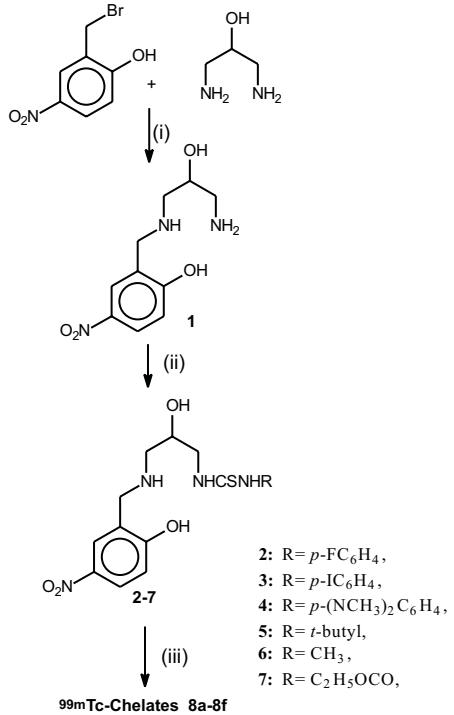
In order to improve the biodistribution and pharmacokinetics of Tc-labeled complexes of HNBAHP, we have synthesized a series of asymmetric nitrophenol-containing ligands (**2** – **7**) were prepared and labeled with technetium-99m complexes. These preparations were studied for biostability in human and rat serum.

Results and discussion

Chemistry.

Preparation of 3-[3′-N-(2″-hydroxy-5″-nitro-benzylamino)-2′-propanol]-1-substituted-thioureas. Sulfur-donor compounds produce suitable and stable complexes in comparison to nitrogen and oxygen. Amine **1** tends to be a hydrophilic compound and the resulting conjugates (**2** – **7**) would have higher lipophilic properties. In this respect, various aryl and alkyl thiourea derivatives were produced and labeled with ^{99m}Tc in order to evaluate their hypoxic cell binding. In the first step, compound **1** was prepared from 1,3-diamino-2-hydroxypropane and 2-hydroxy-5-nitrobenzylbromide. Various N_2SO ligands (**2** – **7**) were synthesized with high yield by condensation of aryl/alkyl isothiocyanates and **1** in order to form possible chelates for hypoxic cell detection (Scheme 1). All organic ligands have been characterized by elemental analysis, ^{1}H and ^{13}C NMR spectroscopy, FAB-MS, and FTIR, and the obtained results were consistent with the anticipated compounds. The synthesized compounds are soluble in MeOH and DMSO.

Radiolabeling of 3-[3′-N-(2″-hydroxy-5″-nitro-benzylamino)-2′-propanol]-1-substituted-thioureas. The radiolabeled technetium complexes (**8a** – **8f**) were prepared with high radiochemical purity and yields: a simple, rapid labeling procedure afforded products that required no further purification. The labeled compounds were stable in aqueous soluti-



Scheme 1. Reagents and conditions for preparation of compounds **1** – **8f**: (i) C_2H_5OH, 0 – 25°C; (ii) RN=C=S, C_2H_5OH, 0 – 25°C; (iii) $SnCl_2$, $[TcO_4]^-$, room temperature.

ons for up to 24 h; no significant amount of other radioactive species was detected by HPLC 24 h after labeling. In the HPLC analysis of each radiolabeled technetium complex, only one radioactive species was detected, with the retention times given in Table 1.

Partition coefficients (log *P*) of complexes. The calculated partition coefficients (log *P*) are presented in Table 2. These log *P* values are within the range (0.9 – 2.5) quoted for lipophilicity suitable for crossing the blood brain barrier.

Stability testing. The stability of the synthesized complexes was checked by radio-TLC (RTLC) for up to 8h after labeling. The complex/colloidal technetium ratio of **8d**, **8e** and **8f** stayed constant, while the others showed decomposition within 3 – 8 h (Table 3).

The biostability remained almost constant within 12 h after labeling only for compound **8e**, even in presence of serum proteins. Due to these results of stability testing for **8e**, the complex was chosen for further biodistribution studies.

To conclude, a series of [^{99m}Tc]-3-[3′-N-(2″-hydroxy-5″-nitro-benzylamino)-2′-propanol]-1-substituted-thiourea complexes have been successfully prepared. The easy synthesis of new N_2OS-^{99m}Tc complexes and their lipophilicity can lead to new hypoxic tumor imaging agents in brain and soft tissues. The radiolabeling method requires no subsequent chromatographic purification of the target compounds. Total labeling and formulation of labeled compounds required about 10 – 15 min. The most stable radiolabeled complex (**8e**) was stable in aqueous solutions (serum and urine) at least for 8 h. Feasibility of production and stability in formulated product as well as in biological media makes compound **8e** an interesting candidate for further tumor imaging studies.

Materials and methods

Chemistry. Chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). 4-Dimethylaminophenyl isothiocyanate (97%) was purchased from Lancaster Caledon Laboratories LDT (Georgetown, Ontario, USA). The ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AM-300 spectrometer using tetramethylsilane as internal standard. All chemicals were recrystallized before use. The IR spectra were recorded in KBr pellets over the range of 4000 – 400 cm^{-1} with a Nicolet DX FTIR spectrophotometer. The FAB mass spectra were recorded with an AEI-MS-12 mass spectrophotometer. Thin layer chromatography (TLC) of non-radioactive products was performed on TLC aluminum sheets, 20 × 20 cm, silica gel 60 F254, (E-Merck, EM Science, Gibbstown, NJ., USA). The analytical HPLC (Shimadzu LC-10AT) used to determine specific activity was fitted with two detector systems, a flow scintillation analyzer (Packard 150 TR), a UV – VIS detector (Shimadzu), and a Si Kromasil 100, (5 μm, 250 × 4.6 mm, M & W) in an Inchrom column. An acetonitrile – water (55 : 45) mixture was used as eluent at a flow rate of 2 ml/min (R_t = 6.4 – 7 min). Melting points were measured with a Thomas Hoover capillary melting point apparatus without corrections. Elemental analyses for C, H and N were performed by Microanalysis Laboratory at the Department of Chemistry, University of Alberta, Canada. $Na^{99m}TcO_4$ from a Mallincrodt Mo/^{99m}Tc generator was purchased from the Edmonton Radiopharmaceutical Center.

1-N-(2′-Hydroxy-5′-nitrobenzylamino)-2-propanol-1,3-diamine (1). A re-crystallized portion of 1,3-diamino-2-propanol (90 mg, 1 mmole) was dissolved in absolute ethanol (20 ml) and cooled in an ice bath. 2-Hydroxy-5-nitrobenzyl bromide (2.6 g, 0.0112 mol) was dissolved in absolute ethanol (5 ml), and added dropwise through a dropping funnel. After addition, the flask was warmed to room temperature and stirred vigorously for 4 h. The mixture was then evaporated *in vacuo* and the residue dispersed in ammonia – methanol solution (30 ml, 2 M) at 0°C followed by overnight stirring. The yellow colored heavy precipitate was then filtered off and washed with pre-cooled methanol (3 × 10 ml). The precipitate was dried under reduced pressure and weighed to give compound **1** (85 mg, 35 %); m.p., 189 – 191°C; ^{1}H NMR in DMSO (δ, ppm): 7.89 (d, 1H, H_6-nitrophenol, J H_6H_4 3.06 Hz), 7.83 (dd, 1H, H_4-nitrophenol, J H_4H_6 = 3.05 Hz, J H_4H_3 9.15 Hz), 6.22 (d, 1H, H_3-nitrophenol, J H_3H_4 9.16 Hz), 5.41 (bs, 6H, CH_2N & OH phenolic & NH_2 & NH), 2.57 – 2.83 (m, 5H, CH_2NH, CHOH); ^{13}C NMR in DMSO (δ, ppm): 44.46, 49.48, 51.49, 67.7, 117.58, 124.52, 125.77, 125.98, 131.03, 175.07; anal. calcd. for $C_{10}H_{15}N_3O_4$ (%): C 49.79, H 6.27, N 17.42; found (%): C 49.4, H 6.35, N 17.1.

3-[3′-N-(2″-Hydroxy-5″-nitro-benzylamino)-2′-propanol]-1-(4′-fluorophenyl)thiourea (2a). Compound **1** (144.6 mg, 0.6 mmol) was dissolved in absolute ethanol (12 ml) and the solution was stirred in a flask cooled at 0°C for 0.5 h. then, 4-fluorophenylisothiocyanate (92.5 mg, 0.6 mmol) dissolved in absolute ethanol (5 ml) was added portion wise during 20 min. After addition, the reaction mixture was warmed to room temperature and stirred for 2 days. The reaction monitoring by TLC using methanol – chloroform (3 : 1, v/v) mixture as the mobile phase demonstrated the formation of two products with R_f values of 0.3 and 0.7. The mixture was evaporated *in vacuo* and the residue was washed with ether (3 × 10 ml) and purified by silica column chromatography using methanol – chloroform (3 : 1, v/v) mixture as the mobile phase. The first eluted fraction gave 36 mg of **2a** (11%).

Monosubstituted **2a**: m.p., 158 – 160°C; ^{1}H NMR in DMSO (δ, ppm): 13.91 (s, 1H, OH-phenolic), 8.13 (d, 1H,

Table 1

Chromatographic Data for ^{99m}Tc Complexes (8a – 8g) in Solution ($^{99m}TcO_4$, R_f = 1.0, R_t = 2.5 min; ^{99m}Tc colloid, R_f = 0.0)

Method	Solvent	Compound					
		8a	**8b**	**8c**	**8d**	**8e**	**8f**
R_f of RTLC	MEK	1.0	0.8	0.9	1.0	0.7	0.8
	Saline	0.3	0.2	0.3	0.1	0.2	0.0
	Acetate buffer (pH 5.6)	0.2	0.1	0.3	0.2	0.1	0.0
	CH_3CN/H_2O (1:1)	0.8	0.6	0.9	0.8	0.9	0.9
R_t of HPLC, min	CH_3CN/H_2O	10.0	9.8	11.0	11.5	10.5	11.0

H_6-nitrophenol, J H_4H_6 3.05 Hz), 7.87 (dd, 1H, H_4-nitrophenol, J H_4H_6 3.06 Hz, J H_4H_3 9.46 Hz), 7.50 (m, 2H, aromatic, CH=C-F), 7.08 – 7.14 (m, 2H, CH=CH-CF), 6.27 (d, 1H, H_3-nitrophenol, J H_3H_4 9.46 Hz), 5.71 (bs, 1H, NH), 4.73 (d, 1H, CH-benzylic, J HH 16.78 Hz), 4.42 (d, 1H, CH-benzylic, J HH 16.78 Hz), 4.39 (m, 1H, CHOH), 3.96 – 4.02 (m, 1H, CHOH-CH_2NHC=S, J HH 6.41 Hz), 3.52 – 3.60 (m, 1H, CHOH-CH_2NHC=S, J HH 6.71 Hz), 2.87 – 2.91 (dd, 1H, CHOHCH_2NHCH_2, J HH 11.90 Hz), 2.69 – 2.77 (m, 1H, CHOHCH_2NHCH_2, J HH 12.56 Hz); IR spectrum in KBr (ν_{max}, cm^{-1}): 3254, 3059, 1595, 1514, 1279, 1085, 829; MS (electron spray, *m/z*): M^+, 395.1; ^{19}F NMR in DMSO (δ, ppm): 46.15; anal. calcd. for $C_{17}H_{19}N_4O_4FS$ (%): C 51.77, H 4.86, N 14.2; found (%): C 51.4, H 5.2, N 13.8.

Disubstituted **2b**: m.p., 123 – 125°C; ^{1}H NMR in DMSO (δ, ppm): 9.65 (bs, 1H, OH-phenolic), 8.06 (dd, 1H, H_4-nitrophenol, J H_4H_3 8.85 Hz, J H_4H_6 1.84 Hz), 7.91 (d, 1H, H_6-nitrophenol, J H_4H_6 1.84 Hz,), 7.68 (bs, 1H, NH), 7.27 – 7.36 (m, 4H, aromatic, CH=C-F), 7.06 – 7.16 (m, 4H, CH=CH-C-F), 6.94 (d, 1H, H_3-nitrophenol, J H_3H_4 8.85 Hz), 5.02 (s, 2H, CH_2-benzylic), 4.18 (m, 1H, CHOH), 4.42 (d, 1H, CH-benzylic, J HH 16.78 Hz), 4.39 (m, 1H, CHOH), 3.96 – 4.02 (m, 1H, CHOH-CH_2NHC=S, J HH 6.41 Hz), 3.52 – 3.60 (m, 1H, CHOH-CH_2NHC=S, J HH 6.71 Hz), 2.87 – 2.91 (dd, 1H, CHOHCH_2NHCH_2, J HH 11.90 Hz), 2.69 – 2.77 (m, 1H, CHOHCH_2NHCH_2, J HH 12.56 Hz). MS (electron spray, *m/z*): [MH^+], 548; [$M^+ + Na^+$], 570; ^{19}F NMR in DMSO δ, ppm): 48.07; ^{13}C NMR in DMSO (δ, ppm): 47.77, 50.36, 54.40, 54.49, 64.70, 68.06, 114.35, 114.64, 114.75, 114.90, 115.04, 115.34, 123.47, 124.30, 125.40, 126.92, 132.06, 135.33, 157.16, 157.16, 157.34, 160.35, 160.53, 180.92, 182.76; anal. calcd. for $C_{24}H_{23}N_5O_4F_2S_2$ (%), C 52.64 H 4.23 N 12.79; found (%): C 52.1 H 3.8 N 12.2.

3-[3′-N-(2″-Hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-iodophenyl)thiourea (3). Compound (3) was prepared according to the procedure used for **2a** and gave only one major product: yield, 157 mg (52%); R_f, 0.4; m.p., 156 – 158°C; ^{1}H NMR in DMSO (δ, ppm): 14.15 (s, 1H, OH-nitrophenol), 8.12 (d, 1H, H_6-nitrophenol, J H_4H_6 3.05 Hz), 7.86 – 7.90 (dd, 1H, H_4-nitrophenol, J H_4H_6 3.05 Hz, J H_4H_3 9.15 Hz), 7.59 (dd, 4H, aromatic, J $H_{2'}H_{3'}$ 8.54 Hz), 6.28 (d, 1H, H_3-nitrophenol, J H_3H_4 9.46 Hz), 5.57 (bs, 1H, NH), 4.73 (d, 1H, CH_2-benzylic, J HH 16.64 Hz), 4.42 (d, 1H, CH_2-benzylic, J HH 16.18 Hz), 4.36 (m, 1H, CHOH), 3.94 – 4.07 (m, 1H, CHOHCH_2NHCS, J H 3.73 z), 3.49 – 3.56 (m, 1H, CHOHCH_2NHCS, J HH 13.74 Hz), 2.87 (dd, 1H, CHOHCH_2NH, J HH 12.2 Hz), 2.71 (dd, 1H, CHOHCH_2NH, J HH 9.16 Hz); ^{13}C NMR in DMSO (δ, ppm): 181.48 (C=S), 177.5 (C-NO_2), 142.39 (C-NHC=S, aromatic), 136.32(C-H, aromatic), 130.00 (C_1-CH_2), 127.17 (C-H, aromatic), 124.78 (C-H, aromatic), 124.20 (C-OH, phenolic), 118.65 (C-H, aromatic), 111.93 (C-H, aromatic), 85.88 (C-I), 67.07 (CH-OH), 53.08 (CH_2NC=S), 52.32 (CH_2NHCH_2), 43.06 (CH_2-benzylic); IR spectrum in KBr (ν_{max}, cm^{-1}): 3274, 2932, 2858, 1588, 1481, 1286, 1098; MS (electron spray, *m/z*): M^+, 503; [M^+ + K+], 543; anal. calcd. for $C_{17}H_{19}N_4O_4IS$ (%): C 40.65, H 3.81, N 11.15; found (%): C 40.1, H 4.1, N 11.5.

3-[3′-N-(2″-Hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-dimethylaminophenyl)thiourea (4). Compound *4* was prepared according to the procedure used for **2b** and gave only one major product: yield, 113 mg (45%); R_f, 0.6; m.p., 201 – 203°C; ^{1}H NMR in DMSO (δ, ppm): 8.46 (s, 1H, OH-nitrophenol), 8.09 (d, 1H, H_6-nitrophenol), 7.86 (q, 1H, H_4-nitrophenol), 7.27 – 6.17(ABq, 4H, C_6H_4N), 6.22 (d, 1H, H_3-nitrophenol), 5.40 (bs, 1H, NH), 4.68 – 4.45 (ABq, 2H, benzylic), 4.35 (m, 1H, CHOH), 3.55 – 4.02 (m, 2H, CH_2NHCS), 2.87 (s, 6H, $N(CH_3)_2$), 2.77 – 2.81(m, 2H, CH_2NH); anal. calcd. for $C_{19}H_{25}N_5O_4S$ (%), C 54.4, H 6.01, N 16.69; found (%) C 54.0, H 6.3%, N 16.1.

3-[3′-N-(2″-Hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-t-butyl)thiourea (5). Compound **5** was prepared according to the procedure used for **2a** and gave only one major product: yield, 96 mg (45%); R_f, 0.75; m.p., 176 – 179°C; ^{1}H NMR in DMSO (δ, ppm): 10.61 (s, 1H, OH-nitrophenol), 7.98 (d, 1H, H_6-nitrophenol, J H_6H_4 3.05 Hz), 7.79 (dd, 1H, H_4-nitrophenol, J H_4H_6 3.05 Hz, J H_4H_3 9.46 Hz), 7.46 (s, 1H, NH), 6.11 (d, 1H, H_3-nitrophenol, J H_4H_3 9.46 Hz), 4.38 (dd, 2H, CH_2-benzylic, J HH 18.59 Hz), 4.13 (m, 1H, CHOH), 3.73 (dd, 2H, CH_2NHCS), 2.62 (mm, 2H, CH_2NHCH_2). 1.50 (s, 9H, $NHC(CH_3)_3$); anal. calcd. for $C_{15}H_{24}N_4O_4S$ (%), C 50.55, H 6.79, N 15.72; found (%): C 50.1, H 6.3, N 15.9.

3-[3′-N-(2″-Hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-methyl)thiourea (6). Compound **6** was prepared according to the procedure used for **2a**. The first eluted fraction was separated: yield, 84 mg (44%); R_f, 0.6; m.p. 201 – 203°C; ^{1}H NMR in DMSO (δ, ppm): 10.78 (s, 1H, OH-nitrophenol), 8.03 (d, 1H, H_6-nitrophenol, J H_4H_6 3.06 Hz), 7.80 (dd, 1H, H_4-nitrophenol, J H_4H_6 3.05 Hz, J H_4H_3 9.15 Hz), 6.16 (d, 1H, H_3-nitrophenol, J H_3H_4 9.4 Hz), 4.50 (d, 1H, CH_2-benzylic, J HH 14.65 Hz), 4.33 (d, 1H, CH_2-benzylic, J HH 14.95 Hz), 4.20 (bs, 1H, OH), 3.99 (dd, 1H, CHOHCH_2NHC=S, J HH 13.89 Hz), 3.58 (dd, 1H, CHOHCH_2NHC=S, J HH 12.9 Hz), 2.87 (s, 3H, NHCH_3), 2.82 (m, 1H, CHOHCH_2NHCH_2, J HH 14.34 Hz), 2.68 (m, 1H, CHOHCH_2NHCH_2, J HH 13.34 Hz); anal. calcd. for $C_{12}H_{18}N_4O_4S$ (%): C 45.85, H 5.77, N 17.82; found (%): C 45.2, H 5.5, N 18.2.

3-[3′-N-(2″-Hydroxy-5″-nitrobenzylamino)-2′-propanol]-1-(4′-ethoxycarbonyl)thiourea (7). Compound **7** was prepared according to the procedure used for **2a** and gave one major disubstituted product eluted: yield, 58 mg (26%); R_f, 0.8; mp: 82 – 85°C (with decomp.); ^{1}H NMR in DMSO (δ, ppm): 10.95 (s, 1H, OH-nitrophenol), 10.18 (s, 1H, NH), 7.97 (d, 1H, H_6-nitrophenol, J H_4H_6 2.75 Hz), 7.85 (dd, 1H, H_4-nitrophenol, J H_4H_6 2.75 Hz, J H_4H_3 9.16 Hz), 6.26 (d, 1H, H_3-nitrophenol, J H_4H_3 9.16 Hz), 5.65 (bs, 1H, NH), 4.75 – 4.38 (m, 2H, benzylic), 4.06 – 4.21 (m, 4H, OCH_2CH_3), 3.87 – 3.23 (m, 6H, CHOH & CH_2NHCS & CH_2NH & OH), 1.24 (m, 6H, OCH_2CH_3); anal. calcd. for $C_{14}H_{20}N_4O_6S$ (%): C 44.15, H 5.41, N 15.04; found (%): C 44.8, H 5.8, N 15.4.

Radiolabeling of compounds 2 – 7 with Technetium-99m. The ligands (0.5 – 1 mg) were added to a sterile, nitrogen-purged vial and then dissolved in 0.2 ml of 0.1 N NaOH solution, followed by the addition of 4.6 ml of saline solution. The pH was then adjusted at about 10 with 1.0 N

Table 2

Partition Coefficients Calculated for ^{99m}Tc Labeled Compounds (*n* = 5)

Compound	8a	8b	8c	8d	8e	8f
log *P*	1.51	1.98	1.87	2.23	1.58	0.97

Table 3.

Complex/Colloidal ^{99m}Tc Ratio for 8a – 8f Complexes in the Presence of Serum Proteins (RTLC, 8 h after labeling, n = 5)

Compound	Time, h					
	1	2	3	4	6	8
8a	0.97 ± 0.04	95 ± 0.03	0.86 ± 0.02	0.64 ± 0.03	0.53 ± 0.04	0.32 ± 0.03
8b	0.98 ± 0.03	0.88 ± 0.05	0.70 ± 0.05	0.62 ± 0.04	0.32 ± 0.03	0.12 ± 0.02
8c	0.96 ± 0.05	0.90 ± 0.06	0.79 ± 0.04	0.68 ± 0.03	0.56 ± 0.05	0.48 ± 0.05
8d	0.95 ± 0.03	0.93 ± 0.03	0.85 ± 0.05	0.79 ± 0.04	0.66 ± 0.05	0.49 ± 0.05
8e	0.99 ± 0.04	0.97 ± 003	0.98 ± 0.04	0.96 ± 0.03	0.98 ± 0.03	0.95 ± 0.04
8f	0.98 ± 0.04	0.85 ± 0.05	0.83 ± 0.06	0.72 ± 0.05	0.29 ± 0.06	0.15 ± 0.04

HCl. This solution was mixed with $Na^{99m}TcO_4$, followed by the addition of 0.2 ml of freshly prepared saturated stannous tartrate solution. The radiochemical yield (> 97% in each case) was determined by HPLC [Waters C-18 reverse phase Radial-Pak cartridge, a gradient system of pH 5.6 NaOAc buffer (A) and THF (B) with flow rate of 2.0 ml/min [1 – 10 min, 100% A to 100% B; 10 – 20 min, 100% B to 100% A; 20 – 25 min, 100% A] and ITLC and paper chromatographic methods [ITLC-SG/MEK, Whatman No.1 (W1) CHR paper/saline and W1 CHR paper/H_2O:CH_3CN (1:1)]. These analyses were usually carried out within 30 min after labeling. The stability of the labeled compounds was also monitored by HPLC up to 12 h. Paper electrophoresis was performed on Whatman No. 1 CHR paper (30 × 2 cm) using 0.05 M acetate buffer (pH 7.0) at 200 V for 1.5 h. Another paper strip spotted with $Na^{99m}TcO_4$ was run simultaneously as the control. The analytical data are listed in Table 1. The pH value of the final solution was adjusted at 7.5 with 0.01 M $NaHCO_3$ solution and the solution was passed through a 0.22 μm membrane filter to free the product from larger colloidal particulates and microorganisms. This solution was used in biological studies.

Determining the partition coefficients (log *P*) for the complexes. The partition coefficients were determined by mixing the complexes (105 – 106 dpm) with 1.0 ml 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 rpm for 5 min. From each phase 0.1 ml of the liquid was pipetted and counted in a well γ-counter. The measurements were repeated three times. Care was taken to avoid the contamination between phases (Table 2.).

Testing the stability of complexes in final product. A sample of complex (0.5 mCi) was kept at room temperature for 8 h while checked by RTLC at various time intervals (2, 4, 6 and 8 h). Micropipet samples (50 μl were taken from the shaking mixture and the ratio of free technetium colloid to complex was checked by radio thin layer chromatography (eluent: 10% NH_4OAc buffer – methanol, 1 : 1). The patterns for technetium colloid and complexes did not change for 8 h.

Testing the stability of complexes *in vitro* in human and mice serum. A mixture of 5 parts of serum and one part radiopharmaceutical (0.2 mCi) was shaken in a 37°C incubator under nitrogen atmosphere. Micropipet samples (50 μl were taken from the shaking mixture every 30 min. The ratio of technetium colloid (R_f = 0) to complexes was checked by RTLC (eluent: pH 5.6 NH_4OAc buffer – methanol, 1 : 1).

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СИНТЕЗ И ИССЛЕДОВАНИЕ СТАБИЛЬНОСТИ НОВЫХ НИТРОФЕНОЛЬНЫХ КОМПЛЕКСОВ ТЕХНЕЦИЯ-99 ДЛЯ РАДИОПРЕПАРАТОВ, ИСПОЛЬЗУЕМЫХ В ДИАГНОСТИКЕ ОПУХОЛЕЙ

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Описан синтез семи нитрофенольных препаратов технеция-99 для диагностики опухолей. Стабильность меченых препаратов в сыворотке крови исследована хроматографическими методами. Наибольшая стабильность (6 ч) наблюдалась для комплекса ^{99m}Tc-3-[3′-N-(2″-гидрокси-5″-нитробензиламино)-2′-пропанол]-1-(4′-метил)тиомочевины.