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Preparation and evaluation of ^{99m}Tc-labeled porphyrin complexes prepared using PNP and HYNIC cores: studying the effects of core selection on pharmacokinetics and tumor uptake in mouse model

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Abstract

Porphyrins are tetrapyrrolic macrocyclic ligands known for their affinity towards neoplastic tissues and once radiolabeled with a suitable diagnostic radioisotope could potentially be used for the imaging of the tumorous lesions. In the present study, an unsymmetrically substituted porphyrin derivative namely 5-(*p*-amino-propyloxyphenyl)-10,15,20-tris(carboxymethyleneoxyphenyl)-porphyrin was synthesized and modified further to enable radiolabeling with ^{99m}Tc using two different ^{99m}Tc-cores viz. ^{99m}Tc-HYNIC (hydrazino nicotinic acid) and ^{99m}Tc(N)PNP2 (PNP2 = Bis-[(2-dimethylphosphino)ethyl]-methoxy-ethylamine) in order to study the effect of employing different ^{99m}Tc-cores on the tumor affinity and pharmacokinetic behavior of the resultant ^{99m}Tc-labeled porphyrin complexes. ^{99m}Tc-porphyrin complexes were characterized by reversed phase HPLC studies and could be prepared with >95% radiochemical purity under the optimized radiolabeling conditions. Both the ^{99m}Tc-complexes were found to be adequately stable in human blood serum till 3 h post-preparation. Bio-distribution studies, carried out in Swiss mice bearing fibrosarcoma tumors, revealed relatively higher tumor uptake for ^{99m}Tc-HYNIC-porphyrin complex (3.95±1.42 and 3.28±0.27 %IA/g) compared to that exhibited by ^{99m}Tc(N)PNP-DTC-porphyrin complex (1.52±0.53 and 1.56±0.10 % IA/g) at 1.5 and 3 h post-administration, although the former complex exhibited comparatively lower lipophilicity in octanol-water system. Higher uptake and longer retention in the blood was observed for ^{99m}Tc-HYNIC-porphyrin complex (6.63±0.75 and 4.36±0.25 % IA/g) compared to that exhibited by ^{99m}Tc(N)PNP-DTC-porphyrin complex (2.41±0.54 and 2.30±0.16 % IA/g) at both 1.5 and 3 h post-administration. However relatively lesser liver uptake was observed for the former complex (19.26±3.48 and 18.45±1.05 % IA/g) than that exhibited by the latter one (39.37±3.88 and 34.15±8.25 % IA/g) at both 1.5 and 3 h post-administration. The

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study indicates that the *in-vivo* behavior exhibited by the ^{99m}Tc-labeled porphyrins not only depend on their lipophilicity/hydrophilicity but also governed by the Tc-cores employed for the radiolabeling.

Keywords: Porphyrin, PNP2, HYNIC, ^{99m}Tc, ^{99m}Tc-cores, Pharmacokinetics

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Introduction

Over the last several years, porphyrin and its derivatives have attracted considerable attention and exhibited great promise for developing agents suitable for tumor diagnosis and targeted tumor therapy.[1-6] The growing interest in the use of porphyrin and its derivatives has been primarily due to their low toxicity, biocompatibility, water solubility, ability to form both thermodynamically and kinetically stable metal complexes, and intrinsic specificity for tumours, both with and without the presence of a coordinate metal ion in the central core.[7] The utility of porphyrin derivatives has already been approved by the US-FDA (Food and Drug Administration of United States of America) for carrying out photodynamic therapy (PDT), a therapeutic modality used for the treatment of certain types of tumors, accessible either superficially or endoscopically.[8-13] While PDT is an established procedure and has made significant inroads and undergone phenomenal expansion and growth, it has its own shares of limitations which include inability to treat or image deep-seated tumors, masking of the tumors due to the internal haemorrhage, low sensitivity, low repeatability due to fluorescence quenching, inability to record photographically the fluorescence observed endoscopically.[8-14] A promising approach to circumvent some of these disadvantages is to incorporate a suitable radionuclide in the porphyrin moiety and using the radiolabeled porphyrin for either tumor diagnosis or targeted tumor therapy.[15,16]

Although a plethora of studies have been conducted for developing suitable radiolabeled porphyrin derivatives, none of these agents has reached the pinnacle of regular clinical exploitation. In this context, studying the effect of structural modifications of radiolabeled porphyrins on the tumor affinity and pharmacokinetic behavior still constitutes an active field of research in radiopharmaceutical sciences.

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The remarkable prospects associated with the use of radiolabeled porphyrin derivatives for tumor imaging led to a considerable amount of fascinating research and development of innovative strategies for radiolabeling a variety of porphyrin derivatives with a range of diagnostic radionuclides, including ^{18}F, ^{68}Ga, ^{99m}Tc, and ^{67}Ga.[17-21] Among the diagnostic radionuclides used for radiolabeling, the possibility of using ^{99m}Tc seemed enticing due to its favorable nuclear decay characteristics, well established polyvalent redox chemistry, ready cost-effective accessibility through $^{99}Mo/^{99m}Tc$ radionuclide generators and widespread clinical utility.[22-24] Additionally, availability of several novel ^{99m}Tc synthons as precursors including $[^{99m}Tc(CO)_3(H_2O)_3]^+$, $[^{99m}Tc(N)]^{2+}$, $[^{99m}Tc(N)PNP]^{2+}$ (Bis-[(2-dimethylphosphino)ethyl]-methoxyethylamine) and ^{99m}Tc-HYNIC (hydrazinonicotinamide) offer the scope to fine tune the chemical properties of the radiolabeled species.[25,26] Each of these complexes, although having the same set of radioisotope and carrier moiety (but having different ^{99m}Tc-cores) has different chemical properties owing to the difference in their size, hydrophilicity/lipophilicity, charge etc. and therefore, expected to have different tumor affinity and pharmacokinetic behavior.[22] Thus ^{99m}Tc-labeling employing different cores not only provide the unique flexibility of maneuvering the chemical properties of the resultant ^{99m}Tc-complexes, but also enable to tune their biological behavior. Towards this, in the present study, an attempt has been made to radiolabel an in-house synthesized porphyrin derivative namely, 5-(*p*-aminopropyleneoxyphenyl)-10,15,20-tris-(*p*-carboethoxymethyleneoxyphenyl)-porphyrin with ^{99m}Tc using two different cores and study the effects of using two different kinds of ^{99m}Tc-cores on the tumor uptake and pharmacokinetic behavior in animal model. Herein, we describe synthesis of the modified porphrin derivatives, ^{99m}Tc-labeling of the porphyrin derivatives using ^{99m}Tc-HYNIC as well as $^{99m}Tc(N)PNP$ core

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and biological behavior of the ^{99m}Tc-labeled porphyrins, formulated using the two different cores, in the small tumor bearing animal model.

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Experimental

Materials and methods

Pyrrole, 4-hydroxybenzaldehyde, ethylbromoacetate, 3-(Boc-amino)propylbromide and trifluoroacetic acid used for the synthesis of the porphyrin derivative were purchased from Aldrich Chemical Company (USA). Propionic acid, nitrobenzene and anhydrous potassium carbonate were obtained from S.D. Fine Chemicals (India). Column chromatography was performed with silica gel (60-120 mesh size) obtained from Merck (India). Analytical thin-layer chromatography (TLC) was performed with silica gel plates (Silica Gel 60 F_{254}), procured from Merck (India). Whatman 3 MM cellulose chromatography paper, procured from Aldrich Chemical Company (USA), was used for the paper chromatography (PC) studies. All other chemicals used were purchased from reputed local manufacturers and were of analytical grade.

Fourier Transform Infra-Red (FT-IR) spectra were recorded in a JASCO FT/IR-420 spectrophotometer (Japan). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were acquired using a 300 MHz Varian VXR 300s spectrometer (USA). Mass spectra were recorded using Varian 410 Prostar Binary LC mass spectrometer (USA) employing electron spray ionization (ESI) technique. All radioactive countings, except the coutings related to the biodistribution studies, were done by using a well-type NaI(Tl) scintillation counter, procured from Electronic Corporation of India Limited (India), setting the baseline at 100 keV and using a window of 100 keV, so as to utilize the 140 keV gamma photon emission of ^{99m}Tc.

The high performance liquid chromatography (HPLC) system (PU 1580) used for the present study was obtained from JASCO (Japan). A C-18 reverse phase HiQSil (250×4 mmol) column was used and the elution profile was monitored by detecting the radioactivity signal

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using a NaI(Tl) detector coupled with the HPLC system. All the solvents used for HPLC analyses were of HPLC grade and degassed as well as filtered prior to use.

All experiments related to human subjects were performed in accordance with the Guidelines of Institutional Medical Ethics Committee (IMEC) of Bhabha Atomic Research Centre (BARC) and these experiments were approved by the IMEC, BARC. Informed consents were obtained from all the human participants involved in this study.

Swiss mice (6-8 weeks age) bearing fibrosarcoma tumors were used as animal models for the biological studies. All the animals used for the present study were bred and reared in the laboratory animal house facility of our Institute following the standard management practice. Radioactive countings associated with the animal studies were performed using a flat-type NaI(Tl) scintillation detector, procured from Electronic Corporation of India Limited (India) employing the same counting set-up mentioned earlier. Animal studies reported in the present article were approved by the Institutional Animal Ethics Committee (IAEC) of BARC and all the animal experiments were carried out in strict compliance with the institutional (IAEC, BARC) guidelines following the relevant national laws (Prevention of Cruelty to Animals Act, 1960) related to the conduct of animal experimentation.

Synthesis of modified porphyrin derivatives

*Synthesis of 4-carboethoxymethyleneoxybenzaldehyde (**i**)*

4-carboethoxymethyleneoxybenzaldehyde was synthesized by following the procedure reported in the literature.[27] The crude product was purified by silica gel column chromatography using $CHCl_3$ as eluting solvent (R_f=0.5) whereby pure 4-carboethoxymethyleneoxybenzaldehyde (**i**) was obtained as a colorless viscous liquid (5.7 g, yield 70%).

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FT-IR (neat, ν cm^{-1}) = 1754 (>C=O). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.24 (t, 3H, J = 7.8 Hz, -$COOCH_2C\mathbf{H}_3$), 4.20 (q, 2H, J = 7.8 Hz, -$COOC\mathbf{H}_2CH_3$), 4.75 (s, 2H, -$OC\mathbf{H}_2$), 6.97 (d, 2H, J = 7.2 Hz, Ar**H**), 7.85 (d, 2H, J = 7.1 Hz, Ar**H**), 9.90 (s, 1H, >C**H**O). ^{13}C-NMR ([D_6]DMSO, δ ppm): 14.02 (CH_2-$\mathbf{C}H_3$), 60.84 (O-$\mathbf{C}H_2$-CH_3), 64.75 (O-$\mathbf{C}H_2$-C=O), 115.06 (*m*-phenyl), 130.16 (*p*-phenyl), 131.72 (*o*-phenyl), 162.48 (*p*-O-phenyl), 168.21 (O=**C**-O), 191.36 (O=**C**H-). ESI-MS (m/z): (calc.) $C_{11}H_{12}O_4$: 208.21; (obs.) [M+H] 209.15

*5,10,15-tris-(p-carboethoxymethyleneoxyphenyl)-20-(p-hydroxyphenyl)porphyrin **(ii)***

Pyrrole (1.50 g, 22.4 mmol) was added to the refluxing mixture of 4-carboethoxymethyleneoxybenzaldehyde (**i**) (3.5 g, 16.8 mmol), 4-hydroxybenzadehyde (0.68 g, 5.6 mmol), propionic acid (25 mL) and nitrobenzene (8 mL) in a drop-wise manner and the refluxing was continued for one hour after the addition of pyrrole was completed.[28] The resultant reaction mixture was allowed to cool overnight at 4 °C. Subsequently nitrobenzene and propionic acid were removed from the reaction mixture by distillation under reduced pressure which gave the crude product. The crude product was purified by silica gel column chromatography using 0.2% methanol in chloroform as eluting solvent whereby 122 mg of pure 5,10,15-tris-(*p*-carboethoxymethyleneoxyphenyl)-20-(*p*-hydroxyphenyl)porphyrin (**ii**) was obtained (yield 4%).

UV-Vis (λ_{max}, nm): 418, 515, 552, 594, 650. FT-IR (KBr, ν cm^{-1}): 3210-3080 (-OH), 1754 (>C=O). ^{1}H-NMR ($CDCl_3$, δ ppm): -2.75 (s, 2H, >N**H**), 1.40 (t, 9H, J = 6.0 Hz, -$COOCH_2C\mathbf{H}_3$), 4.45 (q, 6H, J = 6.0 Hz, -$COOC\mathbf{H}_2CH_3$), 4.90 (s, 6H, -$OC\mathbf{H}_2$-), 7.17-7.31 (m, 8H, Ar**H**), 8.03-8.14 (m, 8H, Ar**H**), 8.85 (8H, s, pyrrole). ESI-MS (m/z): (calc.) $C_{56}H_{48}O_{10}N_4$ 936.34; (obs.) [M+H] 937.25

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*5-(p-Boc-aminopropyleneoxyphenyl)-10,15,20-tris-(p-carboethoxymethyleneoxyphenyl)-porphyrin (**iii**)*

N-Boc-3-bromopropylamine (48 mg, 0.20 mmol) was added to a solution of 5,10,15-tris-(*p*-carboethoxymethyleneoxyphenyl)-20-(*p*-hydroxyphenyl)porphyrin (**ii**) (120 mg, 0.13 mmol), and anhydrous K_2CO_3 (28 mg, 0.20 mmol) in dry acetone and was kept under refluxing conditions for 8 h. The progress of the reaction was monitored by TLC using 0.1% of methanol in chloroform as eluting solvent. After completion of the reaction, solvent was evaporated and the residue was dissolved in chloroform. The chloroform layer was washed with brine and subsequently evaporated in a rotary evaporator. The crude product (**iii**) thus obtained was purified by silica gel column chromatography using 0.05% methanol in chloroform as eluting solvent which resulted formation of 121 mg (yield 86%) of pure (**iii**).

FT-IR (KBr, ν cm^{-1}): 1754 (>C=O). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.39-1.44 (m, 9H, -$COOCH_2C\mathbf{H}_3$), 1.49 (s, 9H, -$C(C\mathbf{H}_3)_3$), 2.12-2.13 (m, 2H, -$OCH_2C\mathbf{H}_2CH_2$-), 2.85 (m, 2H, -$OCH_2CH_2C\mathbf{H}_2$-NH-), 4.38-4.45 (m, 2H, -$OC\mathbf{H}_2CH_2CH_2$-), 4.38-4.45 (m, 6H, -$COOC\mathbf{H}_2CH_3$), 4.90 (s, 6H, -$OC\mathbf{H}_2$-), 7.27-7.53 (m, 8H, Ar**H**), 8.11-8.53 (m, 8H, Ar**H**), 8.84-8.94 (m, 8H, pyrrole). ESI-MS (m/z): (calc.) $C_{64}H_{63}O_{12}N_5$ 1094.2; (obs.) [M] 1094.2

*5-(p-aminopropyleneoxyphenyl)-10,15,20-tris-(p-carboethoxymethyleneoxyphenyl)-porphyrin (**iv**)*

5-(*p*-aminopropyleneoxyphenyl)-10,15,20-tris-(*p*-carboethoxymethyleneoxyphenyl)-porphyrin (**iv**) was prepared by Boc-deprotection of compound (**iii**), which was carried out by stirring a mixture of (**iii**) (120 mg, 0.11 mmol) with trifluoroacetic acid (2 mL) in dry

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dichloromethane (2 mL) for a period of 3 h at room temperature. The reaction was subsequently quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL) solution. The organic layer was separated, dried and concentrated under vacuum whereby 110 mg of the 5-(*p*-aminopropyleneoxyphenyl)-10,15,20-tris-(*p*-carboethoxymethyleneoxyphenyl)-porphyrin (**iv**) (yield 90 %) was obtained.

FT-IR (KBr, ν cm^{-1}): 3310 (-NH_2), 1754 (>C=O), 1650(-NH bending). ^{1}H-NMR ($CDCl_3$, δ ppm): -2.89 (s, 2H, >N**H**), 1.28 (t, 9H, J = 7.4 Hz, -$COOCH_2C\mathbf{H}_3$), 2.10 (m, 2H, -$OCH_2C\mathbf{H}_2CH_2$-), 2.85 (t, 2H, J = 7.8 Hz, -$OCH_2CH_2C\mathbf{H}_2$-), 3.95 (t, 2H, J = 7.4 Hz, -$OC\mathbf{H}_2CH_2CH_2$-), 4.28 (q, 6H, J = 7.4 Hz, -$COOC\mathbf{H}_2CH_3$), 4.80 (s, 6H, -$OC\mathbf{H}_2$-), 7.18-7.30 (m, 8H, Ar**H**), 8.02-8.10 (m, 8H, Ar**H**), 8.90 (m, 8H, pyrrole). ESI-MS (m/z): (calc.) $C_{59}H_{55}O_{10}N_5$ 994.10; (obs.) (M) 993.8.

*5,10,15-tris-(p-carboxymethyleneoxyphenyl)-20-(p-dithiocarbamoylpropyleneoxyphenyl)-porphyrin (**v**)*

5,10,15-tris-(*p*-carboxymethyleneoxyphenyl)-20-(*p*-dithiocarbamoylpropyleneoxyphenyl) -porphyrin (**v**) was prepared following a two-step reaction procedure. In the first step, NaOH (2 N, 5 mL) solution was added to the porphyrin derivative (**iv**) (50 mg, 0.05 mmol) dissolved in tetrahydrofuran (5 mL) and the reaction mixture was stirred for 48 h at room temperature. After completion of the reaction, solvent was evaporated and the crude product, thus obtained, was directly used for the subsequent step. The hydrolyzed product was allowed to react with slight excess of CS_2 under ice cold conditions and subsequently stirred for 24 h at room temperature. The solvent was evaporated under vacuum after the completion of stirring and the crude product thus obtained was purified by semi-preparative HPLC employing 0.1% TFA in water (A) and 0.1 % TFA in acetonitrile (B) as the mobile phase (0-28 min 90% A to 10% A, 28-30 min 10% A,

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30-32 min 10% A to 90% A). Flow rate of the mobile phase was maintained at 2 mL/min. Post-purification ~ 25 mg (yield 50%) of compound (**v**) was obtained.

FT-IR (neat, ν cm^{-1}): 3436 (-COOH), 1754 (>C=O), 1447 (-C=S). ^{1}H-NMR (CD_3OD, δ ppm): 2.12 (m, 2H, -$OCH_2CH_2CH_2$-), 3.00 (t, 2H, J = 6 Hz, -$OCH_2CH_2CH_2$-), 3.80-3.91 (m, 2H, -$OCH_2CH_2CH_2$-NH-), 4.71 (s, 6H, -OCH_2-), 7.33-7.39 (m, 8H, Ar**H**), 8.04-8.10 (m, 8H, Ar**H**), 8.87 (m, 8H, pyrrole). ESI-MS (m/z): (calc.) $C_{54}H_{42}O_{10}N_5S_2$ 985.0; (obs.) [M-S-2H+Na] 973.4

Synthesis of HYNIC-Boc (6-Boc-hydrazinopyridine-3-carboxylic acid)

Synthesis of HYNIC-Boc i.e. 6-Boc-hydrazinopyridine-3-carboxylic acid was achieved by a two-step procedure. In the first step, hydrazine hydrate (8 mL, 80%) was added to 6-chloronicotinic acid (1 g, 6.35 mmol) and the reaction mixture was stirred at 100 °C for 4 h. After the completion of the reaction, the reaction mixture was allowed to attain the room temperature and the solution was concentrated under reduced pressure which resulted formation of a white solid. The solid was subsequently dissolved in water and pH of the solution was adjusted to ~5.5. The precipitate formed were filtered, washed with ethanol and dried in vacuum to give 845 mg of 6-hydrazinopyridine-3-carboxylic acid. In the next step, Boc protection of hydrazine group was carried out by stirring a solution of 6-hydrazinopyridine-3-carboxylic acid (200 mg, 1.31 mmol) with triethylamine (0.365 mL, 2.62 mmol) and di-tert-butyl-dicarbonate (285 mg, 1.31 mmol) in DMF (N,N'-dimethyl formamide) for 16 h at room temperature. The reaction mixture was concentrated under reduced pressure to give a brown solid. The crude product was purified by silica gel column chromatography using ethyl acetate (R_f = 0.2) as the eluting solvent which resulted formation of pure 6-Boc-hydrazinopyridine-3-carboxylic acid (230 mg, 69.5% yield).

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^{1}H-NMR (CD_3OD, δ ppm): 1.42 (s, 9H, -C**H**$_3$), 6.53 (d, 1H, J = 8.7 Hz, *m*-Ar**H**), 7.96 (d, 1H, J = 8.7 Hz, *p*-Ar**H**) 8.57 (s, 1H, *o*-Ar**H**). ESI-MS (m/z): (calc.) $C_{11}H_{15}N_3O_4$ 253.8; (obs.) $[M+Na]^+$ 275.9

*Conjugation of 5-(p-aminopropyleneoxyphenyl)-10,15,20-tris-(p-carboethoxymethylene-oxyphenyl)-porphyrin (**iv**) with 6-Boc-hydrazinopyridine-3-carboxylic acid (**vi**)*

Conjugation of 5-(*p*-aminopropyleneoxyphenyl)-10,15,20-tris-(*p*-carboethoxymethyleneoxyphenyl)-porphyrin (**iv**) with 6-Boc-hydrazinopyridine-3-carboxylic acid was carried out by room temperature stirring of a mixture of compound (**iv**) (40 mg, 0.04 mmol) and 6-Boc-hydrazinopyridine-3-carboxylic acid (11 mg, 0.04) in presence of HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, 0.08 mmol, 30 mg), and diisopropylethylamine (14 µL, 0.08 mmol) in dry dimethylformamide (3 mL) for 12 h. The crude reaction mixture was purified by preparative TLC using 7% MeOH in $CHCl_3$ (R_f = 0.3) as mobile phase which resulted formation of 34.0 mg (yield 70%) of purified (**vi**).

^{1}H-NMR ($CDCl_3$, δ ppm): 1.27 (s, 9H, -C(C**H**$_3$)$_3$), 1.46 (t, 9H, J = 6 Hz, -COOCH$_2$C**H**$_3$), 3.80 (t, 2H, J = 6Hz, -OC**H**$_2$CH$_2$CH$_2$-NH-), 4.44-4.54 (m, 2H, -OCH$_2$CH$_2$C**H**$_2$-NH**)**, 5.00 (s, 6H, -OC**H**$_2$-), 6.76 (s, 1H, ArH), 7.81 (s, 1H, ArH), 7.54-7.58 (m, 8H, Ar**H**), 8.08 (m, 8H, Ar**H**), 8.51-8.56 (m, 8H, pyrrole). ESI-MS (m/z): (calc.) $C_{70}H_{68}O_{13}N_8$ 1229.3; (obs.) [M] 1229.6

*Preparation of hydrolyzed product of compound **vi** (**vii**)*

Compound (**vi**) (30 mg, 0.02 mmol) was hydrolyzed in acidic medium using TFA (trifluoro acetic acid) and dichloromethane (1:1 v/v, 3 mL) by room temperature stirring for 3 h

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to remove the Boc protection of the hydrazine moiety. The reaction mixture was subsequently evaporated using rotary evaporator and was directly used for base hydrolysis which was carried out by room temperature stirring of Boc de-protected product with a solvent mixture of NaOH (2 N) and tetrahydrofuran (1:1 v/v) for 48 h. After the completion of hydrolysis, absolute separation of the layers was observed with hydrolyzed product being present in the aqueous layer. The organic layer was decanted and the aqueous reaction mixture was dried under vacuum, which provided compound 22 mg (yield 90%) of (**vii**).

^{1}H-NMR (D_2O, δ ppm): 3.47-3.66 (m, 2H, -$OCH_2CH_2C\mathbf{H}_2$-NH-), 6.88 (d, J = 10Hz, 2H, ArH), 7.42 (m, 8H, Ar**H**), 7.82 (d, J = 10Hz, 2H, ArH), 7.42 (m, 8H, Ar**H**), 8.86 (m, 8H, Ar**H**), 8.51 (m, 8H, pyrrole). ESI-MS (m/z): (calc.) $C_{59}H_{48}O_{11}N_8$ 1045.0; (obs.) [M] 1044.6

Preparation of $[^{99m}Tc(N)PNP]^{2+}$ core

$[^{99m}Tc(N)PNP]^{2+}$ core was prepared by a two-step process following the procedure reported in the literature.[29,30] The first step involved the preparation of $[^{99m}TcN]^{2+}$ core which was achieved by adding freshly eluted $^{99m}TcO_4^-$ (1 mL, 12 mCi) to succinic dihydrazide (5 mg) dissolved in ethanol (0.25 mL) and incubating the reaction mixture at room temperature for 30 min after the addition of 100 µL of freshly prepared $SnCl_2$ solution (1 mg/mL). $[^{99m}TcN]^{2+}$ intermediate thus prepared was characterized by PC studies using a combination of two different solvent systems viz. normal saline and ethanol:chloroform:toluene:0.5 M ammonium acetate (6:3:3:0.5 v/v). In the second step, freshly prepared $[^{99m}TcN]^{2+}$ core (0.5 mL, ~5 mCi) was added with an ethanolic solution (0.4 mL) of PNP2 (0.2 mg) and the reaction mixture was incubated at room temperature for 15 min. The characterization of $[^{99m}Tc(N)PNP]^{2+}$ core was carried out by RP-HPLC using gradient elution technique employing 0.1% TFA in water (A) and 0.1 % TFA in acetonitrile (B) as the mobile phase

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(0-4 min 95% A, 4-10 min 95% A to 5% A, 10-25 min 5% A to 25 %, 25-30 min 25% A to 95% A, 30-32 min 95% A). Flow rate of the mobile phase was maintained at 1 mL/min.

Radiolabeling of compound (v) using $[^{99m}T(N)PNP]^{2+}$ core

Compound (**v**) was labeled with ^{99m}Tc using $[^{99m}Tc(N)PNP]^{2+}$ core following a procedure which involved the refluxing of compound (**v**) (1 mg, 1 μmol) with freshly synthesized $[^{99m}Tc(N)PNP]^{2+}$ core (~5 mCi, 0.9 mL) in a boiling water bath for 30 min. $[^{99m}Tc(N)PNP]^{2+}$-labeled porphyrin complex was characterized by RP-HPLC using the same gradient system mentioned earlier.

Radiolabeling of compound (vii) with ^{99m}Tc

Radiolabeling of compound (**vii**) with ^{99m}Tc was carried out by following the generalized procedure usually employed for ^{99m}Tc labeling of HYNIC-coupled ligands.[31,32] Stock solutions of $SnCl_2$ (1 mg/mL) and EDDA (ethylenediamine-N,N'-diacetic acid, 50 mg/mL) were prepared. Compound (**vii**) (0.5 mg, 0.478 μmol) was dissolved in PBS (phosphate buffered saline, 0.2 mL) and the resulting solution was transferred to a glass vial containing of tricine (20 mg), EDDA (10 mg) and $SnCl_2$ (100 μg) followed by the addition of freshly eluted $^{99m}TcO_4^-$ (1 mL, 12 mCi). The reaction mixture was subsequently incubated in a boiling water bath for 30 min. Radiolabeling yield of ^{99m}Tc-labeled HYNIC-coupled porphyrin derivative was determined by RP-HPLC using the protocol mentioned earlier. Attempts were also made to purify the radiolabeled reaction mixture in order to improve the percentage radiolabeling yield. Purification was carried out using C-18 Sep-pak® cartridges by employing a standard protocol. The cartridge was pre-conditioned by passing 4 mL of ethanol followed by 2 mL of double distilled water prior to the loading of the

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radiolabeled preparation. Free radiometal was eluted using 600 µL of double distilled water and subsequently radiolabeled porphyrin was eluted out from the column using 1 mL of ethanol. Ethanol present in this purified preparation was removed by gentle warming and the preparation was reconstituted with normal saline before using for biological studies.

Determination of partition coefficient (Log $P_{o/w}$)

The partition coefficients of ^{99m}Tc-labeled porphyrin complexes were determined in octanol-water system by following the protocol mentioned below. An aliquot of the ^{99m}Tc-labeled porphyrin complex (100 µL) was thoroughly mixed with water (900 µL) and octanol (1 mL) using a vortex mixer and subsequently centrifuged at 3000 rpm for 5 min so as to achieve a clear separation between the two layers. Equal aliquots (100 µL) were withdrawn from both the layers and the radioactivity associated with each layer was determined using a well-type NaI(Tl) scintillation detector. Partition coefficients (Log $P_{o/w}$) of ^{99m}Tc-labeled porphyrin complexes were calculated from these data.

***In-vitro* serum binding and stability studies**

In-vitro serum studies were carried out by following the standardized protocol, which is briefly mentioned below. Aliquot of ^{99m}Tc-labeled porphyrin complex (100 µL) was incubated with human serum (400 µL) at 37 °C up to 3 h. For determining the serum binding, serum proteins were precipitated by adding equal volume of acetonitrile to it. The mixture was subsequently centrifuged at 15000 rpm for 4 min and radioactivity associated with the supernatant and pellet were counted separately using a NaI(Tl) detector. Percentage serum binding of ^{99m}Tc-labeled porphyrin complexes was calculated from the counts observed in both

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the fractions. For determining the serum stability, aliquots (100 μL) were withdrawn at regular intervals and the serum proteins were precipitated by adding equal volume of acetonitrile to it. The mixtures were subsequently centrifuged and the supernatants were analyzed by HPLC using the protocol mentioned above.

Biodistribution studies

Biological studies, involving pharmacokinetic evaluation and tumor specificity of the ^{99m}Tc-labeled porphyrin complexes, were carried out by performing biodistribution studies in Swiss mice bearing fibrosarcoma tumors. Solid tumors were developed in Swiss mice by implantation of about 10^6 murine fibrosarcoma cells (~ 100 μL), obtained from the National Center for Cell Sciences (NCCS, India), subcutaneously on the dorsum of each mouse. The tumors were allowed to grow until they become ~10 mm in diameter; subsequent to which, the animals were used for the experiments. Each animal, weighing 20-25 g, was intravenously injected with ~100 μL of the radiolabeled preparation (~100 μCi, 3.7 MBq) through one of the lateral tail veins. Biological distribution of both the radiotracers was studied for two different post-administration time points viz. 1.5 h and 3 h and three animals were used for each time point. The animals were sacrificed immediately after the designated post-administration time points through CO_2 asphyxia. Blood samples of the animals were withdrawn by cardiac puncture immediately after sacrifice. Subsequently, the organs/tissues were excised, washed with saline, dried, weighed in a weighing balance and radioactivity associated with each organ/tissue was measured using a flat-type NaI(Tl) counter. The percent injected activity (%IA) in various organs, tissue and tumor was calculated from the above data and expressed as %IA per gram (%IA/g) of organ/tissue.

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Results

Synthesis of modified porphyrin derivatives

A porphyrin derivative, namely 5-(*p*-aminopropyleneoxyphenyl)-10,15,20-tris-(*p*-carboethoxymethyleneoxyphenyl)-porphyrin (**iv**) was synthesized and further modified [(**v**) and (**vii**)] in order radiolabel the derivatives using two different ^{99m}Tc cores, e.g. PNP and HYNIC following a multi-step reaction procedure (Scheme 1 and 2). Various intermediate compounds and the final products were characterized by standard spectroscopic techniques such as, FT-IR and ^{1}H-NMR spectroscopy as well as by ESI-Mass spectrometry. Synthesis of compound (**ii**) involving the formation of a macrocyclic porphyrin ring was the step having lowest yield owing to the formation of multiple by-products and repeated column chromatographic purification steps required for obtaining the porphyrin derivative (**ii**) in its pure form. Formation of the porphyrin moiety was confirmed by the presence of an intense Soret band (418 nm) followed by four Q-bands (515, 552, 594, 650 nm) observed in the UV-Vis spectrum, which is known to be a characteristic of the porphyrin derivatives.[3] Further confirmatory evidences in favour of the formation of compound (**ii**) were obtained from ESI-MS and ^{1}H-NMR spectroscopy. The presence of NMR signal corresponding to two highly shielded protons in the negative region (at -2.75 ppm) of the ^{1}H-NMR spectrum as well as obtaining the molecular ion peak in the expected region of the mass spectrum provided strong evidence in favour of formation of porphyrin ring structure. Unsymmetrical porphyrin derivative thus synthesized was further derivatized in the peripheral region of the porphyrin ring via an O-alkylation reaction at the phenolic moiety resulting the incorporation of a Boc-NH-propyl linker which was subsequently subjected to Boc-deprotection resulting into the formation of the desired porphyrin derivative (**iv**). This porphyrin derivative (**iv**) was further derivatized following two separate reaction procedures to render it

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suitable for radiolabeling with ^{99m}Tc using two different stabilizing chelate structures viz. PNP and HYNIC. The PNP2 (1-methoxy-2-bis(diphenylphosphino)diethylaminoethane) derivative and HYNIC used in present study were synthesized separately following the reported protocols.[29,33]

Preparation of $[^{99m}TcN]^{2+}$ and $[^{99m}Tc(N)PNP]^{2+}$ core

$[^{99m}TcN]^{2+}$ core was synthesized following a reported procedure [29,30] and characterized by PC using a combination of two sets of solvent systems viz. ethanol:chloroform:toluene:0.5 M ammonium acetate (6:3:3:0.5 v/v) and normal saline. In the former solvent system, $[^{99m}TcN]^{+2}$ intermediate was found to remain near the point of application (R_f = 0-0.2); while in the latter solvent system, it moved towards the solvent front (R_f = 0.7-1). These studies showed that $[^{99m}TcN]^{+2}$ intermediate was obtained with >95% radiochemical purity. Synthesized $[^{99m}TcN]^{+2}$ intermediate was subsequently used for preparation of $[^{99m}Tc(N)PNP]^{2+}$ core which was characterized by RP-HPLC.[30] In HPLC chromatogram free/unlabeled $[^{99m}TcN]^{2+}$ core was found to be eluted at 3.0 min whereas $[^{99m}Tc(N)PNP]^{2+}$ core exhibited retention time of 15.0 and 17.5 min (obtained as a doublet). RP-HPLC studies showed that $[^{99m}Tc(N)PNP]^{2+}$ intermediate could be prepared with >95 % radiochemical purity under the reaction conditions mentioned earlier.

Preparation and characterization of ^{99m}Tc-complexes of (v) and (vii)

Radiolabeling of compound (**v**) with ^{99m}Tc was carried out using $[^{99m}Tc(N)PNP]^{2+}$ core and the radiolabeled product was characterized by RP-HPLC. ^{99m}Tc-labeled porphyrin derivative (**v**) was obtained with a radiochemical purity of >95% as determined by RP-HPLC studies where

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^{99m}Tc-complex of compound (**v**) was eluted with a retention time of ~18 min whereas [^{99m}Tc(N)PNP]$^{2+}$ exhibited retention time of 15.0 and 17.5 min under identical conditions (Fig. 1). On the other hand, compound (**vii**) containing a HYNIC group attached to one of the peripheral positions of the porphyrin macrocyle was radiolabeled with ^{99m}Tc in presence of tricine and EDDA as co-ligands. The radiolabeled complex (^{99m}Tc-labeled **vii**) was characterized by HPLC studies where free $^{99m}TcO_4^-$ eluted from the column at 3.5 min whereas radiolabeled complex exhibited retention time of 15 min (Fig. 2). ^{99m}Tc-labeled (**vii)** was obtained with a radiochemical purity of ~80%, which was further improved to >95% through post-labeling purification using a preconditioned Sep-pak® cartridge.

^{99m}Tc-labeled porphyrin complexes, prepared either HYNIC or PNP-DTC route, were obtained with high specific activities. Specific activity of ^{99m}Tc(N)PNP-DTC-porphyrin complex was calculated to be 5 mCi/mg where as the same for ^{99m}Tc-HYNIC-porphyrin was found to be 14 mCi/mg.

Determination of partition coefficient ($LogP_{o/w}$)

The partition coefficients of ^{99m}Tc-labeled complexes of (**v**) and (**vii**) were determined using octanol-water system and were found to be -0.79±0.03 and -1.86±0.02, respectively. These values indicate the hydrophilic nature of both the complexes with latter being relatively more hydrophilic than the former. This may be due to the presence of more polar groups in ^{99m}Tc-HYNIC complex compared to those present in ^{99m}Tc(N)PNP complex of the porphyrin derivatives.

***In-vitro* serum binding and stability studies**

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In-vitro serum binding and stability studies were carried out for ^{99m}Tc-complexes of (**v**) and (**vii**) revealed that while 84.7% of the former complex was present in the bound state, only 54.7% was found to be bound with serum for the latter one. However, both the radiolabeled complexes showed excellent serum stability as both the complexes retained >95% radiochemical purity when incubated in human blood serum till 3 h post-preparation.

Biodistribution studies

Biodistribution studies, carried out in Swiss mice bearing fibrosarcoma tumor, revealed significant tumor uptake for both the radiolabeled complexes within 1.5 h of administration (1.52±0.53 and 3.95±1.42 %IA per g for ^{99m}Tc(N)PNP-DTC-porphyrin and ^{99m}Tc-HYNIC-porphyrin, respectively) and retention therein till 3 h post-injection (1.56±0.10 and 3.28±0.27 %IA per g for ^{99m}Tc(N)PNP-DTC-porphyrin and ^{99m}Tc-HYNIC-porphyrin, respectively), up to which the studies were continued (Table 1). However, a careful look at the results of the biodistribution studies divulges considerable difference in the tumor uptake as well as distribution pattern of ^{99m}Tc-labeled porphyrins prepared through two different routes. A graphical comparison between blood, liver and tumor uptake values exhibited by both the complexes at 1.5 h post injection has been shown in Fig. 3. It is noteworthy to mention that ^{99m}Tc-porphyrin prepared through HYNIC route showed much higher tumor uptake and better retention of activity in the tumor compared to those exhibited by ^{99m}Tc-porphyrin prepared through PNP route. On the other hand, ^{99m}Tc-porphyrin derivative prepared through PNP route showed much lower blood uptake (2.41±0.54 and 2.30±0.16 %IA at 1.5 h and 3 h post-administration, respectively) than that exhibited by ^{99m}Tc-labeled porphyrin complex prepared through HYNIC route (6.63±0.75 and 4.36±0.25 %IA at 1.5 h and 3 h post-administration,

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respectively). This has resulted in almost similar tumor to blood ratios for both the ^{99m}Tc-labeled complexes [0.62±0.08 and 0.67±0.07% IA/g for ^{99m}Tc(N)PNP-DTC-porphyrin complex and 0.53±0.14 and 0.75±0.02% IA/g for ^{99m}Tc-HYNIC-porphyrin complex at 1.5 and 3 h post-administration, respectively] at both the time points (Table 1). The liver uptake of the ^{99m}Tc(N)PNP-DTC-porphyrin complex (39.37±3.88 and 34.15±8.25 %IA at 1.5 h and 3 h post-administration, respectively) was found to be much higher compared to that of ^{99m}Tc-HYNIC-porphyrin complex (19.26±3.48 and 18.45±1.05 %IA at 1.5 h and 3 h post-administration, respectively) at both the time points and this is in well-correlation with the $LogP_{o/w}$ values exhibited by the complexes. High liver uptake observed with ^{99m}Tc(N)PNP-DTC-porphyrin could be due to the comparatively higher lipophilicity of the complex which again can be attributed to the presence of four phenyl groups on PNP2 backbone. Both the complexes exhibited clearance via hepatobiliary as well as renal pathway, although primary clearance was through the hepatobiliary route, which is an inherent property exhibited by majority of the radiolabeled porphyrins.

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Discussion

Porphyrins and their derivatives generated considerable interest and provided unprecedented opportunities in the development of potential radiopharmaceuticals by virtue of their ability to mimic essential chemicals in the human body, solubility in serum, rapid wash-out and inherent ability to localize preferentially in tumor lesions.[1-6,34] The possibilities to modify the chemical structure of porphyrins in order to alter their lipophilicity/hydrophilicity, which markedly affects the tumor uptake and clearance from the non-target organs, make these macromolecules an attractive target for developing tumor-specific agents having potential for either diagnosis or targeted therapy.[35]

In the quest for developing a radiolabeled porphyrin suitable for diagnostic imaging, maximum attention was focused towards using ^{99m}Tc owing to widespread availability of SPECT (single photon emission computed tomography) instrumentation compared with that for PET (positron emission tomography) in most nuclear medicine facilities, the near ideal imaging characteristics of ^{99m}Tc via SPECT, its rich and diverse redox chemistry, and its ability to form a variety of complexes with different porphyrin derivatives.[22,23] The ability to attain high specific activities for targeting low concentration targets through the radiolabeling procedures employing novel ^{99m}Tc-carbonyl, ^{99m}Tc-nitrido and ^{99m}Tc(III) cores constitute another additional advantage of using ^{99m}Tc for developing radiolabeled porphyrins for diagnostic applications.[22]

As the tumor accumulation and cell membrane penetration properties of porphyrin derivatives are strongly dependent on their structures, selection of an appropriate porphyrin is of prime importance for propitious outcome. In this investigation, we have undertaken the synthesis of an unsymmetrically substituted porphyrin derivative namely 5-(*p*-amino-propyloxyphenyl)-10,15,20-tris(carboxymethyleneoxyphenyl)-porphyrin and modified it further to perform radio

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labeling with ^{99m}Tc using two different ^{99m}Tc-cores viz. ^{99m}Tc-HYNIC and ^{99m}Tc(N)PNP2 with an objective to study the effect of different ^{99m}Tc-cores on the tumor affinity as well as pharmacokinetic behavior of the ^{99m}Tc-labeled porphyrins.

Among the BFCAs (bi-functional chelating agents) that have been studied for radiolabeling with ^{99m}Tc, HYNIC has shown considerable promise.[24] Our interest in using HYNIC as BFCA for the present work has been primarily due to its excellent labeling efficiency and the ability to introduce the hydrophilic character in the resulting complex which consequently can lead to better *in-vivo* pharmacokinetic behavior of the ^{99m}Tc-labeled porphyrin with respect to undesirable uptake in the non-target organs.[22] HYNIC could be linked with porphyrin derivative via an amide bond between the carboxylic group of the former and free amine group present in the latter.

For ^{99m}Tc-labeling of the porphyrin derivative using ^{99m}Tc-nitrido core, the $[^{99m}Tc(N)PNP]^{2+}$ core seems to be an obvious choice, as it offers an unique route for the preparation of asymmetric ^{99m}Tc-complexes owing to the enticing chemical characteristics of the electrophilic nitrido metal fragment $[^{99m}Tc(N)PNP]^{2+}$. The structural framework of $[^{99m}Tc(N)PNP]^{2+}$ moiety contains a pseudo tridentate diphosphine ligand (PNP) coordinated to the ^{99m}TcN group and two labile sites, which complete the pseudo-octahedral environment.[23] The appearance of two peaks in the HPLC chromatogram of $[^{99m}Tc(N)PNP]^{2+}$ moiety is probably due to the presence of weak donor ligands, such as H_2O, Cl^- etc. (available in the reaction mixture) which may occupy these two labile positions [Fig. 1(b)]. The $[^{99m}Tc(N)PNP]^{2+}$ metal fragment is an activated intermediate which selectively reacts with dithiocarbamate derivative of the porphyrin moiety carrying π-donor atoms to form the final complex.[23] The resultant ^{99m}Tc(N)PNP-DTC-porphyrin complex is relatively lipophilic due to introduction of non-polar

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PNP2 moiety compared to the ^{99m}Tc-porphyrin complex formed by using HYNIC as BFCA and this is evident from the corresponding LogP values exhibited by the radiolabeled porphyrin complexes.

Owing to its higher lipophilicity, ^{99m}Tc(N)PNP-DTC-porphyrin complex was expected to exhibit superior tumor uptake; although non-specific accumulation was also expected to be higher. In the same lines, ^{99m}Tc-HYNIC-porphyrin complex was expected to yield relatively lower non-specific accumulation compared to its PNP2 counterpart, along with lower tumor uptake and retention. However, the results of biodistribution studies in tumor bearing small animal model revealed that the expected behavior has not exactly been realized. The probable reason behind the observed results could be the relatively higher blood uptake and longer retention therein exhibited by the ^{99m}Tc-HYNIC-porphyrin complex than that by ^{99m}Tc(N)PNP-DTC-porphyrin complex which might have led to the higher tumor uptake in case of the former complex. These results of the present study indicate that not only the hydrophilicity/lipophilicity of the radiolabeled porphyrin, but the other features such as, charge, size and nature of functional groups present in the coordination sphere could also affect the overall *in-vivo* performance exhibited by the same porphyrin when labeled with same radioisotope using different approaches.

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Conclusions

A porphyrin derivative was derivatized differently in order to radiolabel it with ^{99m}Tc by using two different cores viz. PNP and HYNIC, resulting in the formation of two different ^{99m}Tc complexes of the same porphyrin. Both the ^{99m}Tc-labeled porphyrin derivatives were prepared with >95% radiochemical purity under the optimized radiolabeling conditions and exhibited adequate *in-vitro* stability in human serum. However, it was found that selection of the core has a profound effect on the tumor uptake as well as on the pharmacokinetic behavior of the resultant ^{99m}Tc-complexes. ^{99m}Tc-porphyrin complex prepared through the PNP route, although comparatively more lipophilic, exhibited lesser blood uptake and higher liver accumulation compared to those showed by ^{99m}Tc-porphyrin complex, prepared through the HYNIC route. On the other hand, even though the former complex is more lipophilic in nature, it exhibited significantly inferior tumor uptake and retention, compared to those shown by the latter. This observed phenomenon could be attributed to the higher uptake and relatively longer retention of the ^{99m}Tc-porphyrin complex, prepared through the HYNIC route, in the blood which possibly enhanced the rate of its passive diffusion across the cell membrane in the tumorous tissues leading to the higher tumor accumulation.

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Conflict of interest

Bhabha Atomic Research Centre is a constituent unit of Department of Atomic Energy, Government of India and all research activities of the institute are entirely funded by the Government of India. The authors declare that there are no competing financial interests.

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References

1. J. P. Rovers, M. L. de Jode and M. F. Grahn. *Lasers Surg. Med.,* 2000, **27**, 235-240.

2. M. Khurana, S. Ulrich, A. Kim, Y. Moriyama, G. Netchev, M. K. Akens, H. L. Anderson and B.C. Wilson. *Photochem. Photobiol.,* 2012, **88**, 1531-1538.

3. S. Vedachalam, B. Choi, K. K. Pasunooti, K. M. Ching, K. Lee, H. S. Yoon and X. W. Liu. *Med. Chem. Commun.,* 2011, **2**, 371-377.

4. S. Y. Park, K. T. Oh, Y. T. Oh, N. M. Oh, Y. S. Yound and E. S. Lee. *Chem. Commun.*, 2012, **48**, 2522-2524.

5. Y. Zhang and J. F. Lovell. *Theranostics*, 2012, **2**, 905-915.

6. K. Berg, P. K. Selbo, A. Weyergang, A. Dietze, L. Prasmickaite, A. Bonsted, B. O. Engesaeter, E. Angell-Petersen, T. Warloe, N. Frandsen and A. Hogset. *J. Microsc.,* 2005, **218**, 133-147.

7. M. Imran, M. Ramzan, A. K. Qureshi, M. A. Khan and M. Tariq. *Biosensors,* 2018, **8**, 95.

8. M. R. Hamblin and E. L. Newman. J. *Photochem. Photobiol. B: Biol.,* 1994, **23**, 3-8.

9. J. Moan and K. Berg. *Photochem. Photobiol.,* 1992, **55**, 931-948.

10. T. Gianferrara, A. Bergamo, I. Bratos, B. Milani, C. Spagnul, G. Sava and E. Alessio, *J. Med. Chem.,* 2010, **53**, 4678-4690.

11. M. G. H. Vicente. *Curr. Med. Chem. Anticancer Agents,* 2001, **1**, 175-194.

12. A. Policard. *C. R. Soc. Biol.,* 1924, **91**, 1423-1428.

13. Z. Huang. *Technol. Cancer Res. Treat.,* 2005, **4**, 283-293.

14. H. D. Sarma, T. Das, S. Banerjee, M. Venkatesh, P. B. Vidyasagar and K. P. Mishra. *Cancer Biother. Radiopharm.,* 2010, **25**, 47-54.

View Article Online
DOI: 10.1039/C8MD00559A

15. T. Das, S. Chakraborty, H. D. Sarma and S. Banerjee. Curr. Radiopharm., 2012, **5**, 340-347.

16. M. Bhadwal, T. Das, H. D. Sarma and S. Banerjee. *Mol. Imaging Biol.,* 2015, **17**, 111–118.

17. J. H. Lee, S. Shao, K. T. Cheng, J. F. Lovell and C. H. Paik. *J. Liposome Res.,* 2015, **25** 101-106.

18. S. J. Shetty, S. Murugesan, S. R. Chatterjee, S. Banerjee, T. S. Srivastava, O. P. D. Noronha and A. M. Samuel. *J. Label. Compd. Radiopharm.,* 1996, **37**, 411-418.

19. A. V. C. Simoes, S. M. A. Pinto, M. J. F. Calvete, C. M. F. Gomes, N. C. Ferreira, M. Castelo-Branco, J. Llop, M. M. Pereira and A. J. Abrunhosa. *RSC Adv.,* 2015, **5**, 99540.

20. A. Paknafas, Y. Fazaeli, A. R. Jalilian, A. Ahmadi, S. Feizi, M. Kamalidehghan, A. Rahiminejad and A. Khalaj. *Iran J. Pharm. Res.,* 2013, **12**, 735-744.

21. M. Subbarayan, S. J. Shetty, T. S. Srivastava, O. P. D. Noronha, A. M. Samuel and H. Mukhtar. *Biochem. Biophys. Res. Commun.,* 2001, **281**, 32-36.

22. S. Liu and D. S. Edwards. *Chem. Rev.,* 1999, **99**, 2235-2268.

23. A. Duatti. Nucleus No., 2012, **52**, 41-49.

24. S. Liu and S. Chakraborty. *Dalton Trans.,* 2011, **40**, 6077-6086.

25. K. O. Mang'era, E. Bellande, R. Pasqualini and A. Verbruggen. *J. Pharm. Biomed. Anal.,* 1996, **14**, 1241-1244.

26. M. A. Me´Ndez-Rojas, B. I. Kharisov and A. Y. Tsivadze. *J. Coord. Chem.,* 2006, **59**, 1–63.

27. M. Bhadwal, S. Mittal, T. Das, H. D. Sarma, S. Chakraborty, S. Banerjee and M. R. A. Pillai, *Quart. J. Nucl. Med. Mol. Imaging,* 2014, **58**, 224-233.

View Article Online
DOI: 10.1039/C8MD00559A

28. A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff. *J. Org. Chem*., 1967, **32**, 476.

29. C. Bolzati, A. Boschi, L. Uccelli, F. Tisato, F. Refosco, A. Cagnolini, A. Duatti, S. Prakash, G. Bandoli and A. Vittadini. *J. Am. Chem. Soc.,* 2002, **124**, 11468-11479.

30. K. Vats, M. B. Mallia, A. Mathur, H. D. Sarma and S. Banerjee. *J. Radioanal. Nucl. Chem.,* 2016, **308**, 363-369.

31. Z. Kazemi, M. H. Zahmatkesh, S. M. Abedi and S. J. Hosseinimehr. *Curr. Radiopharm.,* 2017, **10,** 123-130.

32. N. Rahmanian, S. J. Hosseinimehr, A. Khalaj, Z. Noaparast, S. M. Abedi and O. Sabzevari, *Med. Chem. Res.,* 2018, **27**, 890-902.

33. M. J. Abrams, M. Juweid, C. I. TenKate, D. A. Schwartz, M. M. Hauser, F. E. Gaul, A. J. Fuccello, R. H. Rubin, H. W. Strauss and A. J. Fischman. *J. Nucl. Med.,* 1990, **31**, 2022-2028.

34. I. Batinic-Haberle, J. S. Reboucas and I. Spasojevic. *Antioxid. Redox Signal.,* 2010, **13**, 877-918.

35. S. Chakraborty, T. Das, H. D. Sarma and S. Banerjee. *J. Porphyr. Phthalocyanines,* 2012, **16**, 64-71.

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DOI: 10.1039/C8MD00559A

Figure legends

Scheme 1: Synthetic procedure for preparation of porphyrin derivatives **v** and **vii** (a) K_2CO_3, dry acetone, reflux, 8 h (b) propionic acid, nitrobenzene, reflux, 2 h (c) N-Boc-3-bromopropylamine, dry acetone, reflux, 8 h (d) Trifluoroacetic acid: CH_2Cl_2 (1:1), 3 h, R.T. stirring (e) 2 N NaOH:THF (1:1), R.T. stirring, 48 h (f) CS_2, R.T. stirring, 12 h (g) HATU, DIPEA, DMF, R.T. stirring, 12 h (i) Trifluoroacetic acid: CH_2Cl_2 (1:1), 3 h, R.T. stirring (j) 2N NaOH:THF (1:1), R.T. stirring, 48 h

Scheme 2: Probable structures for (a) ^{99m}Tc(N)PNP-DTC-porphyrin and (b) ^{99m}Tc-HYNIC-porphyrin

Fig. 1: HPLC profiles of (a) $^{99m}TcO_4^-$, (b) $[^{99m}Tc(N)PNP]^{2+}$ core and (c) ^{99m}Tc(N)PNP-DTC-porphyrin

Fig. 2: HPLC profile of ^{99m}Tc-HYNIC-porphyrin

Fig. 3: Graphical representation of uptake of ^{99m}Tc(N)PNP-DTC-porphyrin and ^{99m}Tc-HYNIC-porphyrin complexes in blood, liver and tumor at 1.5 h post-injection

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Table 1: Biodistribution pattern of ^{99m}Tc(N)PNP-DTC-porphyrin and ^{99m}Tc-HYNIC-porphyrin complexes in Swiss mice bearing fibrosarcoma bearing (n=3)

Organ	**Injected activity per gram (%IA/g) of organ/tissue**			
	^{99m}Tc(N)PNP-DTC-porphyrin		**^{99m}Tc-HYNIC-porphyrin**	
	1.5 h	**3 h**	**1.5 h**	**3 h**
Blood	2.41±0.54	2.30±0.16	6.63±0.75	4.36±0.25
Lung	3.16±0.87	3.80±1.45	5.54±0.94	3.07±0.76
Heart	0.77±0.22	2.69±0.90	2.94±0.75	2.08±0.28
Stomach	16.74±2.51	5.82±0.73	5.21±1.31	3.92±0.29
GIT	10.23±1.66	15.92±2.05	8.54±0.40	12.00±0.95
Liver	39.37±3.88	34.15±8.25	19.26±3.48	18.45±1.05
Spleen	10.46±1.42	15.21±2.12	5.58±0.65	5.53±0.53
Kidney	6.27±0.97	9.70±1.45	9.53±0.83	9.16±1.00
Muscle	0.92±0.01	0.20±0.01	0.79±0.12	0.37±0.15
Tumor	1.52±0.53	1.56±0.10	3.95±1.42	3.28±0.27
T/B	0.62±0.08	0.67±0.07	0.53±0.14	0.75±0.02
T/M	1.50±0.56	77.66±5.34	3.69±0.79	9.21±1.99

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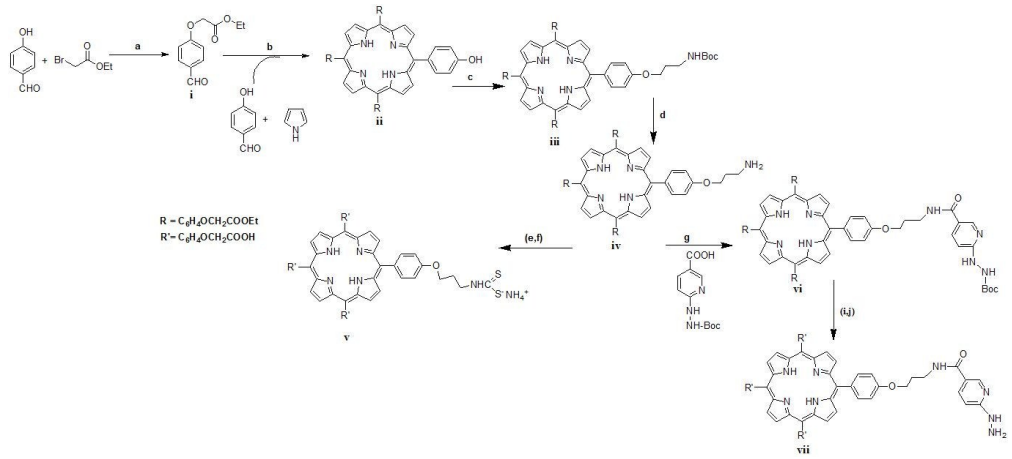

Synthetic procedure for preparation of porphyrin derivatives v and vii (a) K_2CO_3, dry acetone, reflux, 8 h (b) propionic acid, nitrobenzene, reflux, 2 h (c) N-Boc-3-bromopropylamine, dry acetone, reflux, 8 h (d) Trifluoroacetic acid: CH_2Cl_2 (1:1), 3 h, R.T. stirring (e) 2 N NaOH:THF (1:1), R.T. stirring, 48 h (f) CS_2, R.T. stirring, 12 h (g) HATU, DIPEA, DMF, R.T. stirring, 12 h (i) Trifluoroacetic acid: CH_2Cl_2 (1:1), 3 h, R.T. stirring (j) 2N NaOH:THF (1:1), R.T. stirring, 48 h

360x163mm (96 x 96 DPI)

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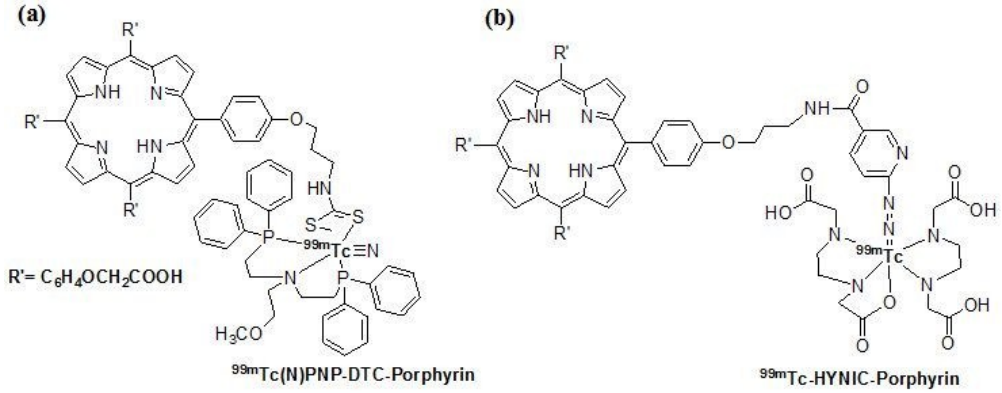

Probable structures for (a) ^{99m}Tc(N)PNP-DTC-porphyrin and (b) ^{99m}Tc-HYNIC-porphyrin

194x76mm (96 x 96 DPI)

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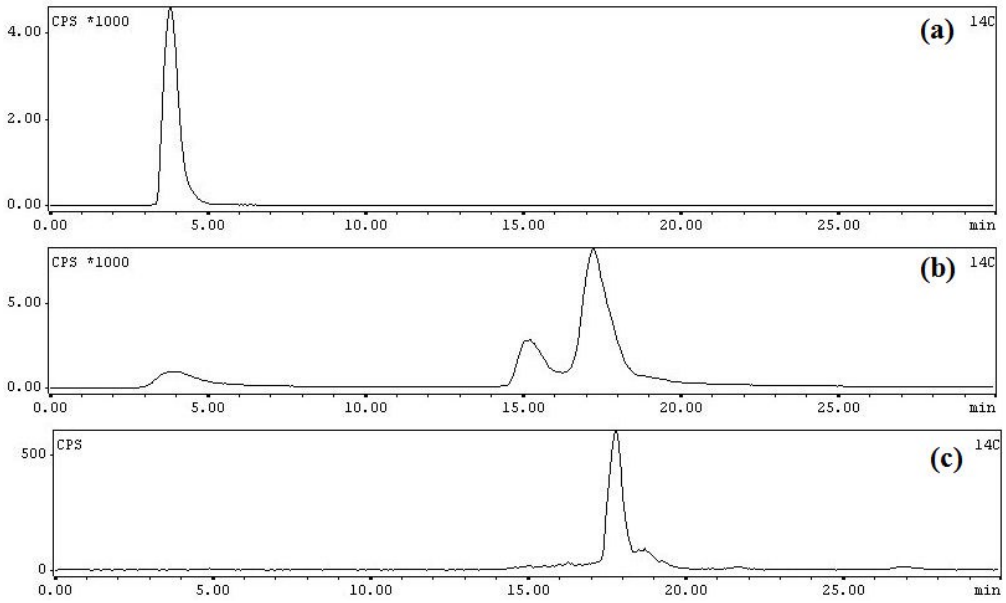

HPLC profiles of (a) $^{99m}TcO_4^-$, (b) $[^{99m}Tc(N)PNP]^{2+}$ core and (c) $^{99m}Tc(N)$PNP-DTC-porphyrin

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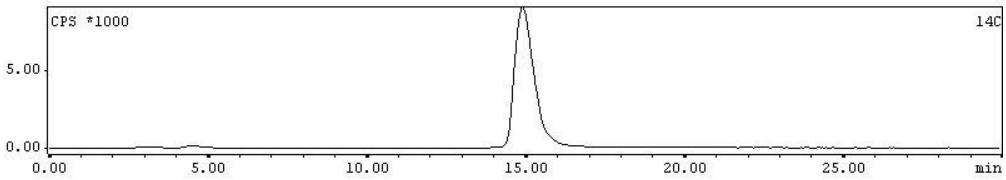

HPLC profile of ^{99m}Tc-HYNIC-porphyrin

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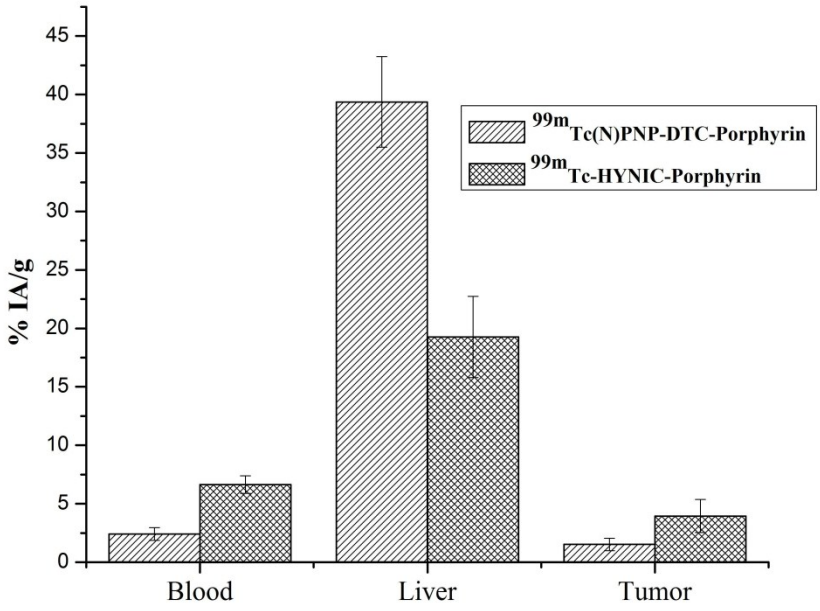

Graphical representation of uptake of ^{99m}Tc(N)PNP-DTC-porphyrin and ^{99m}Tc-HYNIC-porphyrin complexes in blood, liver and tumor at 1.5 h post-injection

872x674mm (96 x 96 DPI)

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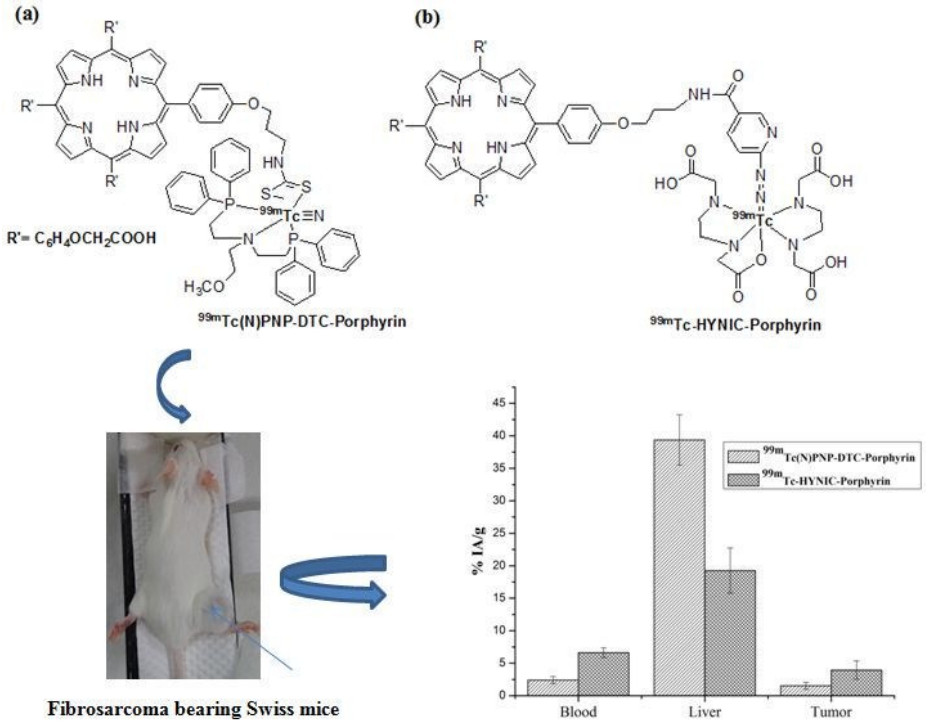
Graphical abstract



Demonstration of effect of using two different ^{99m}Tc-cores for radiolabeling of same ligand: differential *in-vivo* outcome