Full title

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Short title

Imaging Prostate Cancer

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3621

Imaging Prostate Cancer (PCa) with [^{99m}Tc(CO)$_3$]Finasteride Dithiocarbamate

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Abstract

This investigation aimed to modify finasteride (**1**) to finasteride dithiocarbamate (**2**) for subsequent synthesis of the rhenium analogue (**3**) and [^{99m}Tc]tricarbonyl complexes (**4**), to assess its prostate cancer (PCa) targeting potential in a rat model. To validate the identity of (**4**), reference (**3**) has been synthesized using *fac*-[Net$_4$]$_2$[ReBr$_3$(CO)$_3$] precursor and characterized by ^{1}H-NMR, ^{13}C-NMR , ESI-MS and elemental analysis. The analogue (**4**) was synthesized using *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ precursor and its structure was confirmed by comparative HPLC using (**3**) as a reference. Further, the suitability of (**4**) as a PCa imaging agent was investigated *in vitro* and *in vivo.* At room temperature (**4**) had ≥ 99% radiochemical purity and remained ≥84% stable in serum. In pre-clinical studies, biodistribution of (**4**) in histopathologically established rat model showed adequately high *in vivo* uptake in the prostate attracting the possibility of using it for non-invasive imaging of PCa.

Key words: Finasteride dithiocarbamate, tricarbonyl technique, prostate cancer, animal model.

Introduction

Prostate cancer (PCa) is the most common cancer identified in men and remains the third leading cause of mortality [1]. PCa is a complicated disease and its in time screening, diagnosis and staging is of immense clinical importance for specific and appropriate treatment [2,3]. Recent development in diagnostic and treatment techniques of PCa has enhanced the capacity of clinicians and the confidence of patients [4]. However, the role of advanced imaging modalities like ultrasound (US), magnetic resonance imaging (MRI) and computed tomography (CT) based on anatomic and functional imaging has been proven limited, in early diagnosis and staging of PCa [5-9].

However, the nuclear medicine imaging technique has shown promise, as it characterized and targeted PCa on the basis of metabolic changes, which always appear in early stages of the disease [10-16]. The availability of a wide variety of radionuclides both as gamma and beta emitters have further improved the diagnostic and treatment capacities of this technique. Recently, some promising radiopharmaceuticals have been reported for the early diagnosis and treatment of PCa [17-25]. The effectiveness of radiopharmaceuticals has increased confidence of the investigators to look for novel and more specific agents.

Finasteride (**1**) is a synthetic 4-azasteroid drug intended for treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate to improve symptoms, reduce the risk of acute urinary retention, and reduce the risk of the need for surgery including transurethral resection of the prostate. This drug is a specific competitive inhibitor of type II 5α-reductase, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone (DHT). In humans, type I 5α-reductase is predominant in the sebaceous glands of most regions of skin, including scalp, and liver. Type I 5α -reductase is responsible for approximately one-third of circulating DHT. The type II 5α-reductase isozyme is

primarily found in prostate, seminal vesicles, epididymides, and hair follicles as well as liver, and is responsible for two-thirds of circulating DHT. Although FNS is highly selective for type II 5α-reductase over the type I isoenzyme, chronic treatment with this drug may have some effect on type I 5α-reductase [26,27].

The aim of this study was to investigate the characteristics of (**1**) for non-invasive diagnosis of PCa. In this effort, we hereby report the derivatization of (**1**) to (**2**) for subsequent synthesis of analogues (**3**) and (**4**) (**scheme 1**) tricarbonyl complexes. To establish the identity of (**4**), reference (**3**) has been synthesized using *fac*-$[Net_4]_2[ReBr_3(CO)_3]$ precursor and characterized by ^{1}H-NMR, ^{13}C-NMR , ESI-MS and elemental analysis. The analogue (**4**) was synthesized using *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor and its structure was confirmed by comparative HPLC using fully characterized (**3**) as reference standard. Further, the suitability of (**4**) as a PCa imaging agent was investigated *in vitro* and *in vivo.*

Results and Discussion

Derivatization and labeling

In this investigation finasteride (**1**) was dervatized to its dithiocarbamate (**2**) and subsequently labelled with the radiometal [^{99m}Tc] using the tricarbonyl technique, to assess its preclinical imaging potential for targeting PCa in rat. Our attempt to characterize [^{99m}Tc]tricarbonly complex by preparing the [^{99}Tc] analogue complex was unsuccessful. The subsequent employment of the congener cold rhenium ($^{185/187}$Re), which is commonly used as technetium surrogate, gave a crystalline product. The precursors *fac*-$[Net_4]_2[ReBr_3(CO)_3]$ and *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ were used for the synthesis of rhenium tricarbonyl complex (**3**) and radioactive technium-99^m (^{99m}Tc) tri-carbonyl complex (**4**). The (**3**) was characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-MS, HPLC and elemental analysis. The structure of (**4**) was confirmed by comparative HPLC using (**3**) as a reference.

*Synthesis and characterization of [Re(CO)$_3$(**2**)]* (**3**)

Finasteride dithiocarbamate (**2**) was prepared in good yield and fully characterized by NMR, MS, HPLC and elemental analysis. Thereafter (**2**) was reacted with an equimolar amount of the Re tricarbonyl precursor $[Net_4]_2[ReBr_3(CO)_3]$ in methanol to produce *fac*-$[Re(CO)_3(S\text{-}S)]$ complex in which the sulfur of CS_2 coordinates to the *fac*-$[Re(CO)_3]^+$ core through sulfur atoms. HPLC analysis confirmed the synthesis of a single complex.

The ESI-MS of (**3**) in the positive region shows a sole main signal with the characteristic isotopic pattern of ions that contain Re and high resolution mass spectrum (HRMS) evidence that this signal at 737.1715 (*m*/*z*) fully agreeing with the proposed stiochiometery.

The numbering of the carbon atoms is illustrated in scheme 1. ^{1}H and ^{13}C chemical shifts assignments for (**2**) and (**3**) were based on the combined analysis of a series of $^{1}H–^{1}H$ and $^{1}H–^{13}C$ correlation and are given in methodology section.

As can be seen, upon coordination large downfield shifts ranging from 212.6 to 215.3 ppm is noted for C-26 attached to both sulfur atoms of (**2**) and (**3**), due to complexation with Re-metal**.** The corresponding proton on C-26 also experiences an analogous downfield shift upon coordination ranging from 3.20 to 3.91 ppm. ^{1}H and ^{13}C chemical shifts for the rest of the atoms do not show any changes upon coordination, in view of the fact that they are secluded from the Re.

*Synthesis and characterization of [^{99m}Tc(CO)$_3$(**2**)]* (**4**)

The radio-complex (**4**) was synthesized through a ligand substitution reaction using the precursor *fac*-[$^{99m}Tc(H_2O)_3(CO)_3]^+$ and the corresponding ligand (**2**). The mixture was evaluated by HPLC and quantitatively a single complex was observed, which eluted at 18.7 min. The identity of (**4**) was established by comparative HPLC analysis using a well defined Re-complex (**3**), as a reference, which eluted at 18.4 min. The HPLC profile of the (**4**) gave two peaks at retention 3.8 and 18.7 min. The peak observed at 18.7 min of retention represented (**4**) while at 3.8 min the undesirable species.

At room temperature (**4**) behave normally with the maximum radiochemical purity (RCP) of 99.25 ± 0.20% after 30 min after labeling. However, a decrease in the RCP was observed with time and after 4 hr, it dropped to 91.65 ± 0.18%. The overall RCP profile of (**4**) is shown in Fig 1. Further, the participation coefficient (*P*) value calculated for (**4**) was 0.42 ± 0.03, suggesting lipophilicity.

In serum at 37 ˚C the stability of (**4**) up to 16 hr. is shown in Fig. 2. The labeled moiety was found tagged more than 90 % up to 4 hrs. However, after 16 hr post labeling the undesirable species reached to 16.85 %.

The blood kinetic profile of (**4**) showed dual pharmacokinetic compartmental model, wherein a sudden decrease in activity was detected immediately after 15 seconds of administration and subsequent by slow clearance after 25 min. The first disappearance of (**4**) from blood indicating either its rapid uptake by different organs or excretion from the blood. Further, slow disappearance of (**4**) from the body after 25 min is indicative of slow release from the tissues into the systemic pool.

In this study changes in activity of serum prostatic acid phosphatase were assessed using PCa model and normal male rats. The results showed that exposure of rats to different concentrations of testosterone propionate caused a significant increase in serum prostatic acid phosphatase by 76.50 ± 2.82 %. The level of serum prostatic acid phosphatase observed for control was 2.62 ± 0.55 (U/L). Significantly higher activity of serum prostatic acid phosphatase observed for PCa model rats confirmed prostate cancer in model rats.

Biodistribution

Biodistribution data of (**4**) in rat model is shown in Fig 3 (a). Initially, a high uptake was seen in blood which gradually decreased from 10.75 ± 0.55 to 5.6 ± 0.45 % within 6 hrs. A similar profile was observed in all other organs with the exception of the prostate, wherein a gradual uptake was observed. The highest uptake (22.94 ± 1.25 %) in the prostate was observed after 4 hrs p.i. The disappearance of activity from both liver and kidneys indicated the clearance of (**4**) from hepatobiliary and urinary systems. Blocking by an excess of unlabeled (**2**) derivative significantly reduced the uptake to 7.22 ± 0.55% I.D/g after 4 hr (Fig 3 (b)). The prostate to

blood ratio reached 5 and prostate to muscle 20.15 after 4 hr p.i. as shown in Fig 4. No significant variation was observed in the uptake of the other tissues of both groups.

Experimental

Materials

All reagents and solvents used in this study were reagent grade. HPLC solvents (HPLC grade) were filtered through membrane filter (0.22 micrometer, Millipore, Milford, MA) and degassed by helium flux. Finasteride was obtained from Selleckchem 9330 Kirby Drive, STE 200, Houston, TX 77054 USA. A Varian 400 MHz nuclear magnetic resonance (NMR) spectrometer was employed to record ^{1}H and ^{13}C spectra using DMSO. Esquire 3000 plus Ion Trap Mass Spectrometer was used for direct mass determination. Perkin Elmer 2400 automatic elemental analyzer was used for elemental analysis. HPLC analysis was performed on Shimadzu equipped with UV detector operating at 254 nm, flow scintillation analyzer, binary gradient pump and degasser. Water:methanol (W:M) was used as the mobile phase and C-18 column (Brounlee, 150 x 4.6 mm) as the stationary phase, working at a flow rate of 1 mL/min. The elution gradient condition were 100% water in 0-3 min, 60% water in 4-7 min, 45% water in 8-11 min, 25% water in 12-14 min, 100% methanol in 15-20 min and 50% water in 21-25 min. Ludlum well counter and Capintech dose calibrator were used for activity measurement and radiopharmaceutical dose adjustment. The Isolink kit (Mallinckrodt), was used for preparation of *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^{+}$ precursor as per instructions of the manufacturer.

Methods

Synthesis of Finasteride dithiocarbamate **(2)**

Finasteride (**1**) was derivatized to (**2**) using an already reported method [10]. Briefly, 11 mL tetrahydrofuran (THF), 1 mmol of (**1**) and 1.2 gm of sodium hydroxide (NaOH) were gently mixed and incubated for 15 min in freezer at 4 ˚C. One mL carbon disulfide was added to the mixture and incubated at same temperature for 4 hrs. The mixture was processed in an automatic shaker at room temperature for 12 hrs. The reaction mixture was filtered (Whatman™ 8.5cm grade 1) and the filtrate was dried in an oven (Fisher scientific ™ Isotemp; 120 Volt, 60 MHz, 50 to 250 ˚C) at 40 ˚C. The product was re-crystallized from methanol to give (**2**) in good yield (352.7 mg, 71.36 %) and characterized. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.99 (s, 3H; H-18), 1.29 (s, 3H; H-19), 1.32 (s, 9H; H-23-25), 6.95 (d, $J_{1,2}$ = 9.54 Hz 1H; H-1), 6.66 (d, $J_{2,1}$= 9.54 Hz, 1H; H-2), 3.42 (dd, $J_{5,6b}$ =10.24 Hz, $J_{5,6a}$ = 3.12 Hz 1H; H-5), 1.69 (dddd, J_{gem}= 14.04, $J_{6b,5}$ =10.24 Hz, $J_{6b,7a}$ = 3.95 Hz, $J_{6b,7b}$ =1.7 Hz 1H; Hb-6), 1.68 (dddd, J_{gem}=14.04, $J_{6a,7b}$=6.87, $J_{6a,7a}$=3.95, $J_{6a,7b}$=1.7 Hz;1H; Ha-6), 1.53 (dddd, J_{gem} 14.04, $J_{7a,6a}$ =3.95, $J_{7a,6b}$ = 3.95, $J_{7a,8}$ = 1.7 Hz; 1H; Ha-7), 1.28 (dddd, J_{gem} =14.04, $J_{7b,6a}$ = 3.95, $J_{7b,6a}$=1.7, $J_{7b,8}$ =1.7 Hz, 1H; Hb-7), 1.43 (dddd, $J_{8,7a}$ =1.7, $J_{8,7b}$=1.70, $J_{8,9}$ = 13.1, $J_{8,14}$ =1.2 Hz, 1H; H-8), 1.50 (ddd, $J_{9,8}$ =13.1, $J_{9,11b}$ =8.1, $J_{9,11a}$= 3.6 Hz, 1H; H-9), 1.39 (dddd J_{gem}=13.1, $J_{11a,12a}$ =8.1, $J_{11a,12b}$ =9.9, $J_{11a,9}$ =3.6 Hz, 1H; Ha-11), 1.63 (dddd J_{gem} =13.1, $J_{11b,9}$ =8.1, $J_{11b,12a}$ =3.2, $J_{11b,12b}$ =3.2 Hz; 1H; Hb-11), 1.50 (ddd, J_{gem} =13.1, $J_{12a,11a}$ =8.1, $J_{12a,11b}$= 3.2 Hz, 1H;Ha-12), 1.59 (ddd, J_{gem}=13.1, $J_{12b,11a}$ =9.9, $J_{12b,11b}$ =3.2, 1H; Hb-12), 1.85 (ddd, $J_{14,8}$ =1.2, $J_{14,15a}$ = 8.0, $J_{14,15b}$ = 3.9 Hz, 1H; H-14), 1.90 (dddd J_{gem} =13.7, $J_{15a,14}$= 8.0, $J_{15a,16a}$ =6.9, $J_{15a,16b}$ = 3.9 Hz, 1H; Ha-15), 1.88 (dddd, J_{gem} =13.7, $J_{15b,14}$=3.9, $J_{15b,16a}$=6.9,$J_{15b,16b}$= 7.8 Hz, 1H; H-15), 1.65 (dddd, J_{gem}=14.0, $J_{16a,15a}$ =6.9, $J_{16a,15b}$ =6.9, $J_{16a,17}$=8.1, 1H; Ha-16), 1.85 (dddd, J_{gem} =14.0, $J_{16b,17}$ =8.1, $J_{16b,15a}$ =6.7, $J_{16b,15b}$=8.1 Hz, 1H; Hb-16), 2.62 (dd, $J_{17,16}$ =8.1, $J_{17,16}$=3.9 Hz, 1H; H-17), 3.20 (s, 1H; H-

26) and 1.39 (s,-NH). ^{13}C-NMR (400MHz, DMSO): δc (ppm), 171.9 (C-20), 162.9 (C-3), 145.5 (C-1), 120.9 (C-2), 62.8 (C-5), 56.6 (C-14), 50.8 (C-22), 50.1 (C-17), 46.5 (C-9), 43.2 (C-13), 42.4 (C-10), 39.1 (C-12), 212.6 (C-26), 35.9 (C-8), 30.7 (C-6), 28.8 (C-23,24,25), 28.3 (C-7), 27.8 (C-16), 26.2 (C-15), 25.4 (C-19), 21.8 (C-11) and 14.6 (C-18). HPLC t_R= 15.9 min, ESI MS (positive mode, m/z): Calcd for $C_{24}H_{36}N_2Na_2O_2S_2$ 494.202, found 494.198. elemental analysis: anal. Calcd C,58.27 H, 7.34 N, 5.66 % found C, 58.25 H, 7.36 N, 5.63 %

*Synthesis of [Re(CO)$_3$(**2**)]* **(3)**

In 10 mL methanol, 0.1 mmol (77 mg) of $[Net_4]_2[ReBr_3(CO)_3]$ and 0.1 mmol (49.42 mg) of (**2**) were mixed using an automatic shaker at room temperature. The mixture was heated at reflux for 5 hours, the volume reduced by half and the mixture aged in the refrigerator overnight. The precipitated solid was washed with methanol, and the product was re-crystallized from methanol to give (**3**) in good yield (45.94 mg, 62.31 %). Thereafter, (**3**) was characterized using ^{1}H-NMR, ^{13}C-NMR, HPLC, MS and elemental analysis. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.99 (s, 3H; H-18), 1.29 (s, 3H; H-19), 1.32 (s, 9H; H-23-25), 6.95 (d, $J_{1,2}$ = 9.54 Hz 1H; H-1), 6.66 (d, $J_{2,1}$= 9.54 Hz, 1H; H-2), 3.42 (dd, $J_{5,6b}$ =10.24 Hz, $J_{5,6a}$ = 3.12 Hz 1H; H-5), 1.69 (dddd, J_{gem}= 14.04, $J_{6b,5}$ =10.24 Hz, $J_{6b,7a}$ = 3.95 Hz, $J_{6b,7b}$ =1.7Hz 1H; Hb-6), 1.68 (dddd, J_{gem}=14.04, $J_{6a,7b}$=6.87, $J_{6a,7a}$=3.95, $J_{6a,7b}$=1.7 Hz;1H; Ha-6), 1.53 (dddd, J_{gem} 14.04, $J_{7a,6a}$ =3.95, $J_{7a,6b}$ = 3.95, $J_{7a,8}$ = 1.7 Hz; 1H; Ha-7), 1.28 (dddd, J_{gem} =14.04, $J_{7b,6a}$ = 3.95, $J_{7b,6a}$=1.7, $J_{7b,8}$ =1.7 Hz, 1H; Hb-7), 1.43 (dddd, $J_{8,7a}$ =1.7, $J_{8,7b}$=1.70, $J_{8,9}$ = 13.1, $J_{8,14}$ =1.2 Hz, 1H; H-8), 1.50 (ddd, $J_{9,8}$ =13.1, $J_{9,11b}$ =8.1, $J_{9,11a}$= 3.6 Hz, 1H; H-9), 1.39 (dddd J_{gem}=13.1, $J_{11a,12a}$ =8.1, $J_{11a,12b}$ =9.9, $J_{11a,9}$ =3.6 Hz, 1H; Ha-11), 1.63 (dddd J_{gem} =13.1, $J_{11b,9}$ =8.1, $J_{11b,12a}$ =3.2, $J_{11b,12b}$ =3.2 Hz; 1H; Hb-11), 1.50 (ddd, J_{gem} =13.1, $J_{12a,11a}$ =8.1, $J_{12a,11b}$= 3.2 Hz, 1H;Ha-12), 1.59 (ddd, J_{gem}=13.1, $J_{12b,11a}$ =9.9, $J_{12b,11b}$ =3.2, 1H; Hb-12), 1.85 (ddd, $J_{14,8}$ =1.2, $J_{14,15a}$ = 8.0, $J_{14,15b}$ = 3.9 Hz, 1H; H-14), 1.90 (dddd

J_{gem} =13.7, $J_{15a,14}$= 8.0, $J_{15a,16a}$ =6.9, $J_{15a,16b}$ = 3.9 Hz, 1H; Ha-15), 1.88 (dddd, J_{gem} =13.7, $J_{15b,14}$=3.9, $J_{15b,16a}$=6.9,$J_{15b,16b}$= 7.8 Hz, 1H; H-15), 1.65 (dddd, J_{gem}=14.0, $J_{16a,15a}$ =6.9, $J_{16a,15b}$ =6.9, $J_{16a,17}$=8.1, 1H; Ha-16), 1.85 (dddd, J_{gem} =14.0, $J_{16b,17}$ =8.1, $J_{16b,15a}$ =6.7, $J_{16b,15b}$=8.1 Hz, 1H; Hb-16), 2.62 (dd, $J_{17,16}$ =8.1, $J_{17,16}$=3.9 Hz, 1H; H-17), 3.91 (s,1H; H-26) 1.39 (s,-NH) and 2.3 (H_2O). ^{13}C-NMR (400MHz, DMSO): δc (ppm), 171.9 (C-20), 163.6 (C-28), 162.9 (C-3), 162.2 (C-27), 160.5 (C-29), 145.5 (C-1), 120.9 (C-2), 62.8 (C-5), 56.6 (C-14), 50.8 (C-22), 50.1 (C-17), 46.5 (C-9), 43.2 (C-13), 42.4 (C-10), 39.1 (C-12), 215.3 (C-26), 35.9 (C-8), 30.7 (C-6), 28.8 (C-23,24,25), 28.3 (C-7), 27.8 (C-16), 26.2 (C-15), 25.4 (C-19), 21.8 (C-11) and 14.6 (C-18). HPLC t_R=18.2 min, HRMS (ESI) positive mode, m/z): Calcd for $C_{27}H_{38}N_2O_6ReS_2$ 737.1729, found 737.1715. elemental analysis: anal. Calcd C, 44.11 H, 5.20 N, 3.81 % found C, 44.09 H, 5.22 N, 3.82 %

*Synthesis of [^{99m}Tc(CO)$_3$(**2**)]* (**4**)

*[^{99m}Tc(CO)$_3$(**2**)]* (**4**) was synthesized by injecting freshly eluted 20 mCi (2 mL in saline) of sodium pertechnetate to the Isolink kit (consisting of 7.15 mg sodium carbonate, 2.85 mg sodium tetraborate, 2.85 mg sodium borano bicarbonate dehydrate and 8.5 mg sodium tartrate dihydrate). The mixture was incubated at 100 ˚C for 10 min and the pH was adjusted to 8 using 0.1 N HCl, after cooling the mixture. HPLC was used to confirm the synthesis of *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ precursor (t_R=4.5 min). One milliliter of the *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ precursor was added to a separate nitrogen gas filled vial containing (**2**) (100 µL, 10^{-3} M) and incubated at 100 ˚C for 20 min. After cooling the identity of the mixture was tested by HPLC, synthesis of single (**4**) complex with more than 95% yield (HPLC at t_R= 18.4 min) was observed. The identity of (**4**) was ascertained by comparative HPLC with (**3**) as a reference. Further, after labeling the stability of (**4**) was analyzed by HPLC up to 4 h.

Partition coefficient (*P*)

Partition coefficient (*P*) of the (**4**) was measured using the method reported in reference 10. Briefly, at room temperature equimolar amount of (**4**), octanol and phosphate buffer (PB) was vortexed and centrifuged at 2000 rpm/min for 5 min. Thereafter, an aliquot was withdrawn for determination of activity using a well counter interface with scalar count rate monitor with the formula: *P*= CPM in octanol - CPM in background / CPM in buffer - CPM in background.

***In vitro* stability in serum**

In vitro stability of (**4**) in serum at 37 ˚C up to 16 hrs after labeling was determined by TLC. 0.2 mL (1 mCi) (**4**) was incubated with 1.8 mL serum at 37 ˚C up to 16 hrs. During incubation period aliquots were taken and spotted on the TLC strips. After drying the TLC strips were developed in a mobile phase (CH_2Cl_2:CH_3OH (9:1) (v/v)). Thereafter, the TLC strips were divided into two equal parts and analyzed for radioactivity in each half strip separately using a well counter interface with scalar count rate monitor.

Pharmacokinetics

A pharmacokinetics study of the 4 was investigated by injecting i.v. 10 MBq of the (**4**) to the rats. At different time points after i.v. injection blood was taken from the rat and counted for activity using a well counter interface with scalar count rate monitor.

PCa rat model

PCa was induced in rat by the reported method [14]. Briefly, testosterone propionate 50 mg/ kg (body weight) was intraperitoneally (i.p) injected to each for twenty one days. After twenty-four hours 100 mg/kg testosterone propionate in 0.3 mL propylene glycol for 3 days

was i.p. injected to the same animal followed by i.v injection of 50 mg/kg body weight methyl-*N*-nitrosourea. The animal was then placed on 4 mg testosterone propionate i.p. injection alternatively for three months.

Serum prostatic acid phosphatase

Serum prostatic acid phosphatase was measured using the reported method [13]. Briefly, 1.0 mL each of serum (obtained from PCa model and or control rat), *p*-nitrophenyl phosphate and 0.1 N citrate buffer were taken in a clean sterilized test tube. After swirling the mixture was placed in water bath (at 37 ˚C) for 20 min and the reaction was arrested with 0.1 N NaOH. A UV visible spectrophotometer (operated at 415 nm) was used for determination of serum prostatic acid phosphatase.

Histopathology

Hematoxylin/eosin staining was used for verification of PCa in rat tissue. PCa tissues were soaked in 10 % formalin for 12 hrs followed by dehydration using different grades alcohol and finally embedded in paraffin wax. 5 μm slice of the PCa tissue was stained with hematoxylin/eosin stain and transverse section was mounted with dibutyl phthalate xylene and finally examined under microscope for changes in suspected PCa tissues.

Biodistribution in rat

Biodistribution of (**4**) was assessed in two groups of nine male rats. 0.2 MBq of the labeled moiety freshly prepared containing 20 μg (**4**) as an optimal amount to the rat or 200 μg unlabelled (**2**) was additionally i.v. injected in the blocking experiment. All rats were sacrificed in accordance with the approval of the ethics committee and guidelines of NMRL-Vol. I, II at different time points pi. One gram each of muscle, liver, spleen, kidney,

duodenum, colon and blood and prostate were precisely taken using an analytical balance, and activity was estimated using a well counter interface with a scalar count rate monitor.

Conclusion

In this study finasteride was derivatized to its dithiocarbamate for synthesis of novel *fac*-$[Re/^{99m}Tc(CO_3)]^+$ complexes. The Re-complex with finasteride dithiocarbamate was well characterized for validation of the corresponding analogue [^{99m}Tc]tricarbonyl complex. In the design of Re and [^{99m}Tc]tricarbonyl complexes, the active pharmacophore was distanced from the metal core to preserve its competitive inhibition capacity. In pre-clinical studies ^{99m}Tc tri-carbonyl complex has revealed plausibly high *in vivo* uptake in the prostate suggesting the possibility of using it as a non-invasive imaging of PCa.

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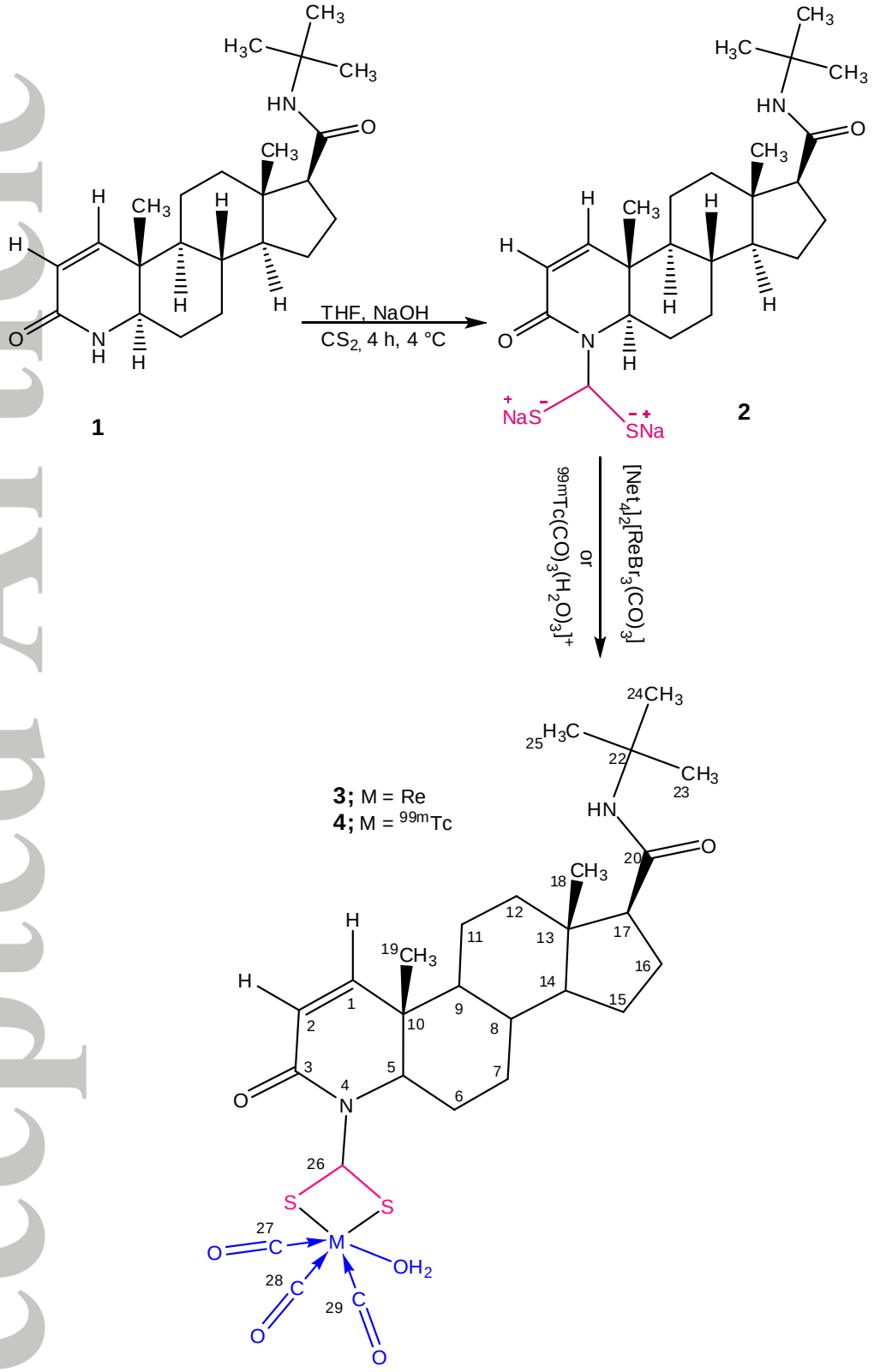


Scheme 1. **Synthetic scheme for ligand 2 and complexes 3-4**

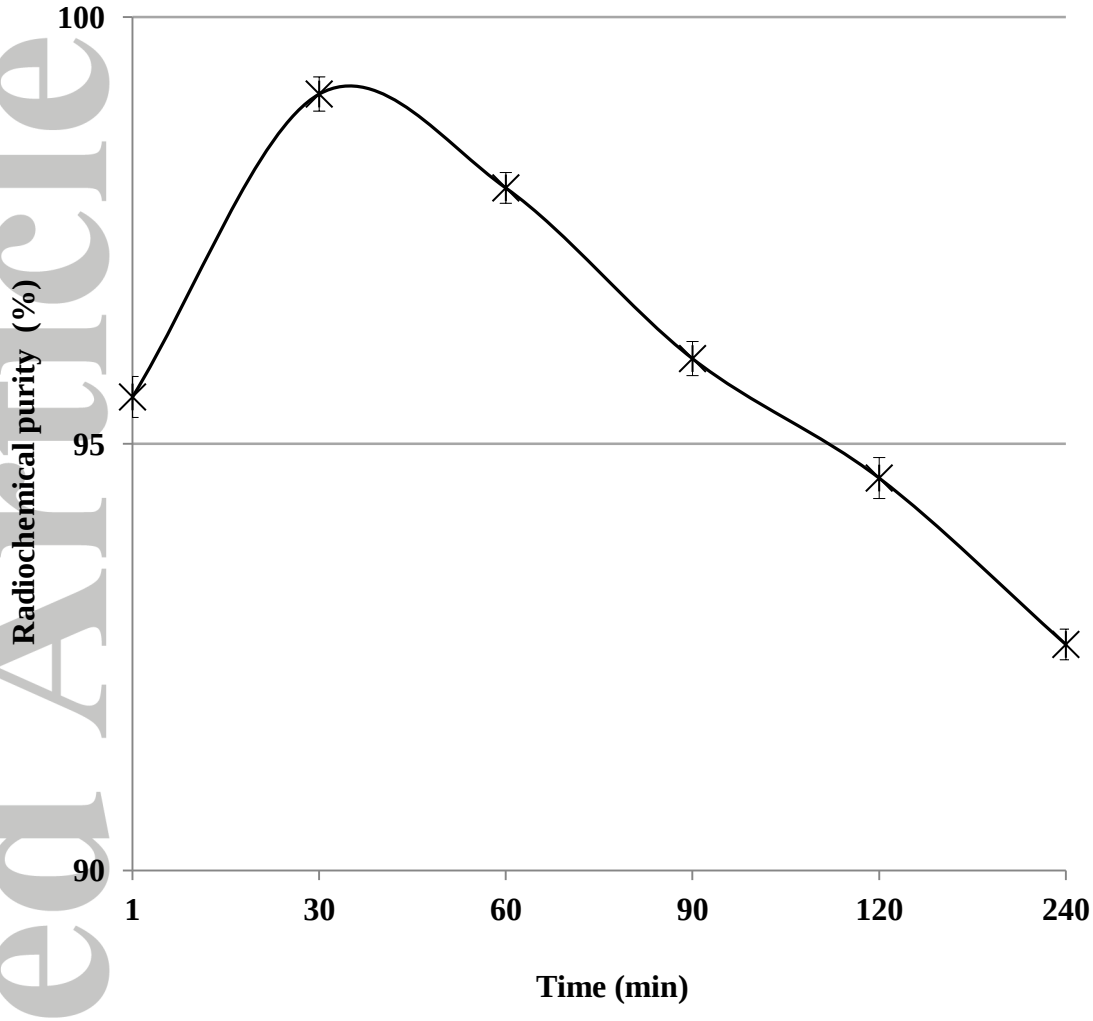


Fig 1. **The overall radiochemical purity profile of 4**

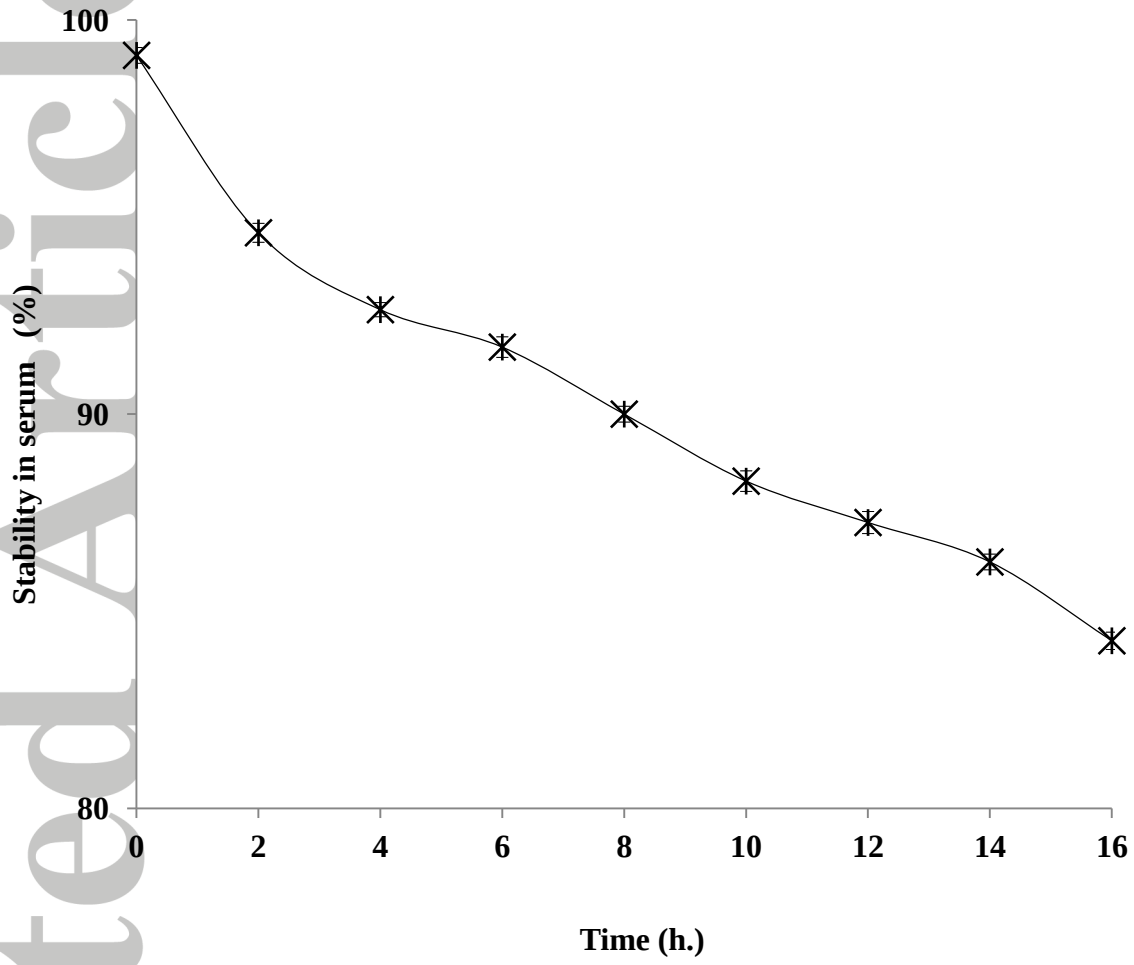
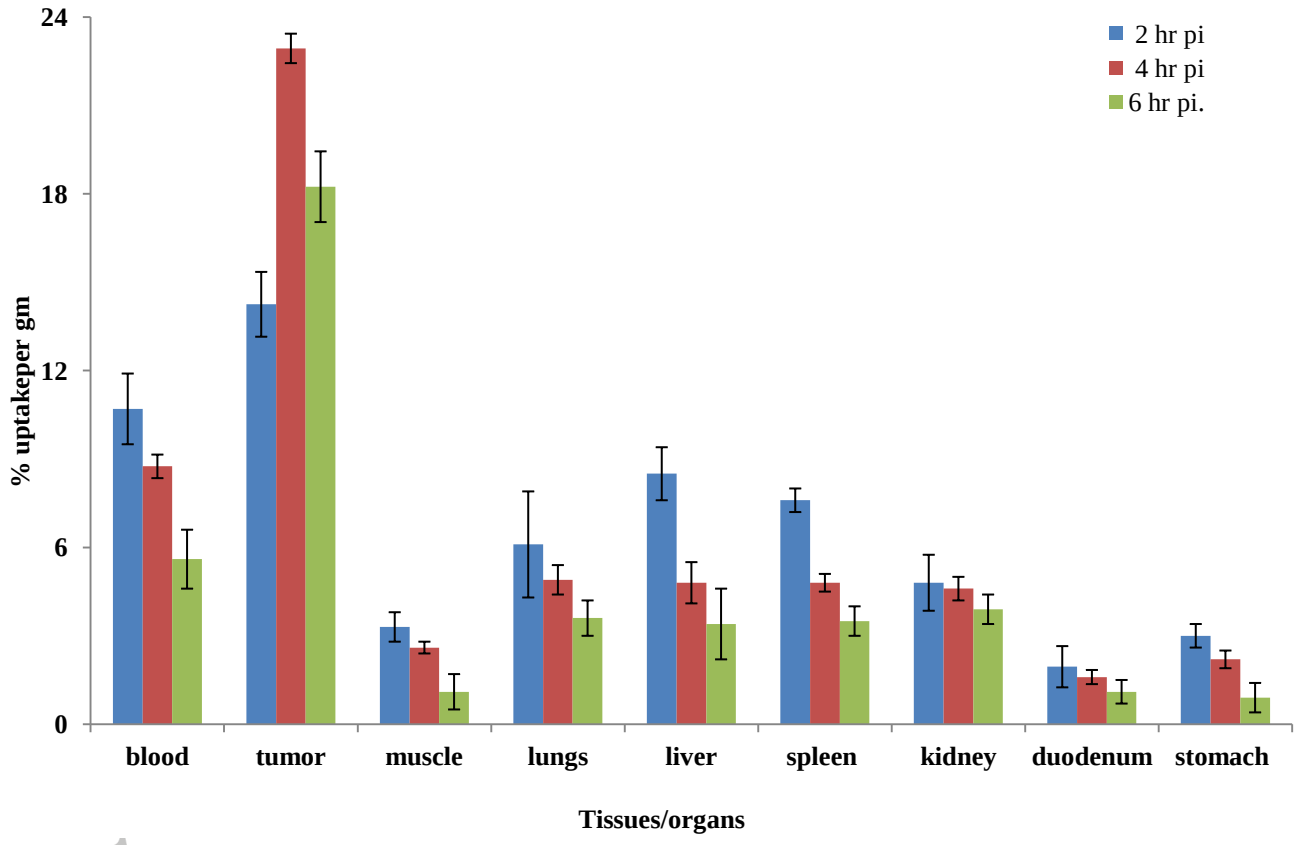
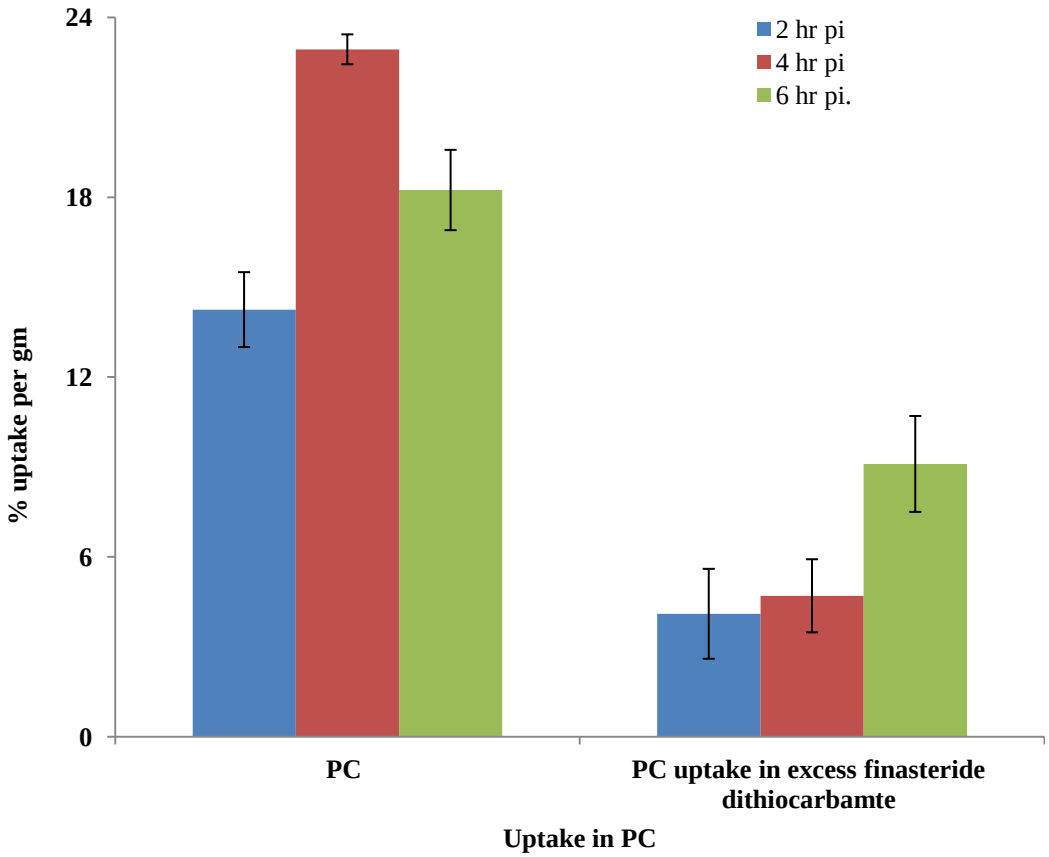


Fig 2. ***In vitro*** **stability of 4 in serum at 37 ˚C**



(a)

Fig 3. (a) Biodistribution of 4 in model rats



(b)

Fig 3. (b) Blocking experiment

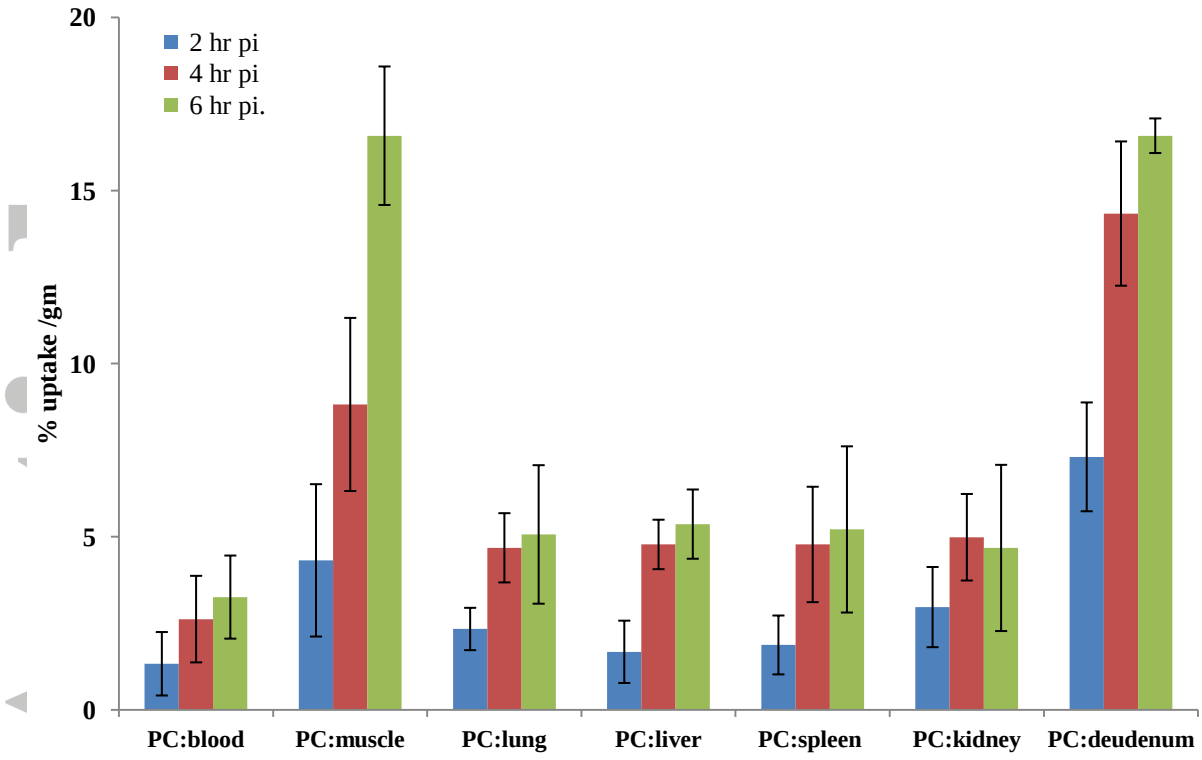


Fig. 4 The prostate to blood and muscle ratio