Original Article

# Target Specific Uptake of a Newly Synthesized Radiolabeled 5α-Reductase Inhibitor "Tc-99m-17-Oxo-17a-Aza-D-Homo-5-Androsten-3β-yl Phenoxyacetate (Tc-99m-17a-Aza Steroid)" in Rat Prostatic Neoplastic Lesions

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## Abstract

**Objective:** Considering the 5α-reductase (5AR) inhibitory activity of the oximes and the importance of the ester group in increasing the anti-androgenic property, we reasoned to synthesize a compound having a lactam group in ring D and an ester group at the 3 β position of the androsterone nucleus. The study aims to radiolabel 17-oxo-17a-aza-D-homo-5-androsten-3β-yl phenoxyacetate (17a-aza steroid) with Tc-99m to evaluate its targeted uptake in experimentally induced prostate carcinogenesis in rats. **Materials and Methods:** The prediction of the optimal interaction and binding affinity of Tc-99m-17-oxo-17a-aza-D-homo-5-androsten-3 β-ylphenoxyacetate (Tc-99m-17a-aza steroid) toward 5AR inhibitor was done using Biopredicta Vlife MDS tool. Tc-99m-17a-aza steroid was developed by direct radiolabeling protocol. The radio-pharmacological characteristics (serum stability, plasma protein-binding ability, and lipophilicity) of the complex were evaluated. Further, the bio-distribution studies of the complex were performed in rats with experimentally induced prostate carcinogenesis. **Results:** The *in-silico* analysis exhibits favorable binding of Tc-99m-17a-aza toward 5AR with D score-130.97. The radiochemical purity of Tc-99m-17a-aza was 96.79%. The radio-complex maintained stability in the rat serum for a period of 6 h (hours). Plasma protein binding and Log *P*o/w value were observed to be 86.23 ± 7.08% and 0.118 ± 0.045, respectively. A significantly enhanced percent-specific uptake was observed in the prostate of rats with induced prostate carcinogenesis. **Conclusion:** The study concludes that Tc-99m-17a-aza exhibits prostate specificity and can be explored further for its potential as a radionuclide imaging probe.

**Keywords:** *17a-aza steroid, 5α-reductase, experimental prostate carcinogenesis, radiolabeling*

## Introduction

Prostate cancer is the second most common cancer found in men after lung cancer and the fifth leading cause of death in men.[1] The screening, diagnosis, and characterization pose a major challenge due to the heterogeneity and complexity of this disease. More than 95% of endogenous androgen, i.e., testosterone (T) is produced by the testis and is taken up from the systemic circulation by the prostatic glandular and stromal cells. Within the prostate, the steroidal 5α-reductase (5AR) enzyme catalyzes the irreversible conversion of testosterone (T) to the corresponding dihydrotestosterone (DHT), which acts in a paracrine fashion to stimulate prostate growth.[2] The higher concentration of intracellular DHT, in addition to its higher affinity for the androgen receptor, support the importance of steroidal 5AR in normal and pathologic prostate physiology disorders including prostate cancer.

Functional imaging modalities with radiopharmaceuticals, namely single photon-emission computed tomography and positron emission tomography provide diagnostic cross-sectional high contrast images that help in localization of the desired radiopharmaceutical in the diseased tissue. In radionuclide imaging, drug development research to develop new more specific radiotracers has increased substantially. Drugs that have high specificity for particular receptors or organs are radiolabeled with available radionuclides to achieve the maximum target to nontarget ratio and help in the

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characterization of disease at the molecular level and also useful for treatment response evaluation. Out of the conveniently used radionuclides in radionuclide imaging, Tc-99m has the ideal characteristics and is cost-effective. It is easily available from a generator in the form of pertechnetate (Tc-99mO4-) and its gamma energy of 140 keV makes it suitable for gamma scintillation imaging.[3,4] An earlier study from our laboratory (Jan *et al.,* 2017*)* has reported the effective radiolabeling of Finasteride, an inhibitor of 5AR, with Tc-99m and described its potential as a radionuclide imaging probe in the detection of prostate cancer.[5] However, better radioimaging needs higher resolution that further requires more specific uptake of the imaging probe. In search for such a compound with more specific uptake than finasteride, our team has synthesized 17oxo 17a D homo 5 androsten 3 β yl ester steroids. This compound has also shown anti-proliferative and 5AR inhibitory property.[6] From this observation, it can be inferred that the location of the aza group in the parent steroid skeleton brings about notable changes in the pharmacological activity. Since, this group of steroids displayed significantly better anti-proliferative activity as compared to reference drug finasteride, in a human prostate cancer cell line (DU-145) and concomitant increase in the serum androgen level of testosterone compared to control; therefore, we reasoned to utilize the potential of 17-Oxo-17a-aza-D-homo-5-androsten-3 β-yl phenoxyacetate (17a-aza steroid) steroid in prostate cancer imaging. The present study has successfully radiolabeled a compound with a general structure containing a lactam moiety in the ring D and an ester moiety at the 3 β position of the androsterone nucleus to make a more potent 5AR inhibitor with Tc-99m. The newly synthesized radio-complex has also been evaluated for its target-specific uptake in the prostate of rats with experimentally induced prostate carcinogenesis.

## Materials and Methods

### Molecular docking analysis of Tc-99m-17a-aza steroid with 5α-reductase receptor

The optimal interaction and binding affinity of Tc-99m-17a-aza steroid toward 5AR was determined by using Biopredicta Vlife MDS tool. The crystallographic structure of 5AR inhibitor (PDB ID: 4AT0) was acquired as PDB files from protein data bank (www.rcsb.org). Final protein in. mol format was considered after the removal of bound water, co-factors and external ligand from the environment. At the same time, protein structure integrity was accessed and checked for its missing residues, followed by the insertion of loop regions using Loop Builder tool. For ligand optimization, mol2 file was processed with ChemDraw Ultra 12.0 software. Energy minimization and optimization of ligands were done by Merck Molecular Force Field, an in built tool of Vlife MDS with default parameters of root mean square gradient of 0.01 kcal/mol and the iteration limit to 10,000. Conformer with least energy was selected for further detailed interaction studies. The docking program was run on BioPredicta module equipped with grip docking to execute molecular docking calculations. Results obtained with best fit in terms of least binding energy (D score) was observed taken forward for the subsequent analysis of binding mode (ligand pose) and their interactions between ligands and receptors.

### Animals

Healthy adult 6–7 week male Sprague Dawley rats of weight 200–250 g were procured from the Central Animal House of the institute. The animals were housed under the hygienic conditions in polypropylene cages in the departmental animal house and were maintained on a standard laboratory pelleted feed. The procedures involving animals were carried in accordance with the standard recommendations for care and use of laboratory animals and the protocols carried were approved by the Institutional Animal Ethics Committee.

### Synthesis and radiolabeling of 17-oxo-17a-aza-D-homo-5-androsten-3β-yl phenoxyacetate (17a-aza steroid)

The steroidal 5AR inhibitor, 17a-aza steroid was synthesized in reference to the scheme adopted as shown by Dhingra *et al*.[6] and characterized by IR and $^{1}$HNMR.

Optimization of reaction constituents such as $SnCl_2.2H_2O$, 17a-aza steroid and Tc-99m$O_4^-$activity, as well as stability of the radio complex with pH and time, was evaluated to obtain the maximum radiochemical yield. Finally, radiolabeling of 17a-aza steroid with Tc-99m was performed by adding 7.4 MBq of Tc-99m$O_4^-$ to a vial containing 20 µg of 17a-aza steroid (1 mg/1 ml in 10% ethanol). For the reduction of Tc-99m$O_4^-$, 40 µg of $SnCl_2.2H_2O$ (1 mg/ml in 0.1N HCl) was added to the mixture and the pH was adjusted to 7 using 0.01N NaOH. The reaction mixture was kept at the room temperature in dark for 10 min (minutes) to complete the reaction.

### Radiochemical purity analysis

Ascending chromatography technique was used to calculate the percentage labeling of 17a-aza steroid with Tc-99m by measuring the free pertechnetate and hydrolyzed fraction in acetone and a mixture of Pyridine: Acetic acid: Water (3:5:1.5 v/v), respectively. In acetone solvent, the free pertechnetate moved to solvent front whereas, both hydrolyzed and $^{99m}$Tc-aza steroid remained at the application point, while in Pyridine: Acetic acid: Water (3:5:1.5 v/v) mixture, the free pertechnetate and $^{99m}$Tc-aza steroid moved to solvent front while hydrolyzed ($^{99m}$Tc-RH) remained at the application point.

Briefly, the strips of (instant thin layer chromatography [ITLC]) were taken and cut into appropriate length and width and a single spot of radio-complex formulation was put on the strip at the point

of origin. The strips were then put in tubes which were formerly filled with solvents, namely acetone and a mixture of Pyridine: Acetic acid: Water (3:5:1.5 v/v) as mobile phases to measure the percentages of free Tc-99m fraction and hydrolyzed Tc-99m fraction, respectively. The strips were then taken out and counts of activity across the strips were measured in a gamma-scintillation probe (ECIL, Hyderabad, India). The percentages of both the free pertechnetate and hydrolyzed fractions were calculated, and the percentage of the radiolabeled compound was evaluated by the formula as below:

**Percentage of bound fraction = (100% free pertechnetate + % hydrolyzed fraction)**

*Paper electrophoresis*

To determine the charge of the radio-complex, paper electrophoresis was carried using a sodium phosphate buffer solution as the mobile phase and Whatman No. 1 paper as the stationary phase. A spot was applied at a marked central point on the strip and the voltage of 300V was applied and the procedure was run for 1 h. The strip was dried and activity across the strip was counted in a well type gamma scintillation camera to check the movement of radio-complex.

### Partition coefficient measurement log *P*o/w

To determine the *in vivo* behavior like movement of radio-complex across cell membrane, lipophilicity (hydrophobicity) was determined by obtaining a parameter called partition coefficient *P*, which is defined as the ratio of the concentrations of a neutral compound in organic ($C_{org}$) and aqueous ($C_{aq}$) phases under the equilibrium conditions.

### Serum stability and plasma protein binding of the radio-complex

Briefly, serum was prepared from the blood samples drawn from rats by puncturing the retro-orbital plexus, followed by incubating 900 µl of serum with 100 µl of the radio-complex under physiological conditions up to 6 h. The serum radio-complex samples were applied on (ITLC-silica gel) strips and developed in 100% acetone to measure the percentage of free activity which if increased is directly linked with degradation of the labeled complex.

Plasma protein binding of the radio-complex was evaluated by incubating 900 µl of rat plasma with 100 µl of the radio-complex at 37°C up to 1 hr in a test tube. Then 1 ml of 10% TCA (trichloroacetic acid) was added, and the tube was centrifuged at 2000 rpm for 5 min. The supernatant was collected, and the pellet was again suspended in 1 ml of 5% TCA and centrifuged. The supernatant was collected and radioactivity was measured in both the precipitate and supernatant fraction in a well-type gamma counter. Protein binding of the complex was expressed as a fraction of radioactivity bound to protein as a percent of total activity.

### Prostate cancer induction

Prostate cancer was induced in rats by subjecting to MNU (*N*-methyl-*N*-nitrosourea) carcinogen and hormone (testosterone propionate [TP]) treatment as described by Jan *et al*.[5] Briefly, intraperitoneal (i.p.) injections of TP (50 mg/kg body weight) were administered to each rat daily for 21 consecutive days. On day 23, rats received daily i.p. injections of 100 mg TP/kg body weight in 0.3 mL propylene glycol for 3 days. On day 27, a single intravenous (i.v.) dose (50 mg/kg body weight) of MNU (dissolved in saline at 10 mg/mL) was administered to all rats through the tail vein. One week after MNU administration, rats received i.p. injection of 4 mg TP/kg body weight alternatively for 120 days.

### Confirmatory studies

*Prostatic acid phosphatase determination*

Prostatic acid phosphatase (PAP) blood levels are correlated well with prostate cancer progression, and therefore, the PAP activity levels were measured in the blood of MNU + TP treated rats by the method of Tenniswood *et al*.[7]

### Histopathology

The histopathological evaluation of prostate cancer in rats was done by hematoxylin/eosin (H/E) staining as described by Jan *et al*.[5] Briefly, an overnight fixation in 10% formalin was done following which prostate tissues were dehydrated using different grades of alcohol and finally embedded in the paraffin wax. The tissues were then sliced into 5 µm sections and placed on glass slides to be stained with H/E stain. Stained transverse sections were permanently mounted with dibutyl phthalate xylene and were observed under a light microscope for preneoplastic/neoplastic changes in MNU-/T-treated tissue sections.

### Blood pharmacokinetics of Tc-99m-17a-aza steroid

Blood pharmacokinetics of the radio-complex was assessed by administering 200 µCi of activity of radiocomplex in rats through a penile vein and the blood was taken out at different time intervals through the ocular vein. The radioactivity was counted in a well-type scintillation counter, and the data were expressed as the counts per ml of blood.

### Bio-distribution studies

The bio-distribution experiment was performed by intravenously administering (via a penile vein) 200 µCi of the radio-complex to the healthy and MNU + TP treated rat. The animals were sacrificed at different time intervals, postinjection, and tissues of interest (heart, lung, liver, kidney, small intestine, large intestine, colon, blood, and other organs) were excised, weighed, and were counted in well scintillation counter. The percentages of injected dose per gram of tissue (ID/g ± SD) were calculated by comparing with standard activity representing the injected dose per animal.

### Statistical analysis

Each experimental parameter was repeated three times, and differences in the data were evaluated with a one-way analysis of variance (ANOVA) test. Results are reported as mean ± standard deviation (SD). The level of significance was set at $P \leq 0.05$.

## Results

### Molecular docking analysis of Tc-99m-17a-aza steroid with 5α-reductase receptor

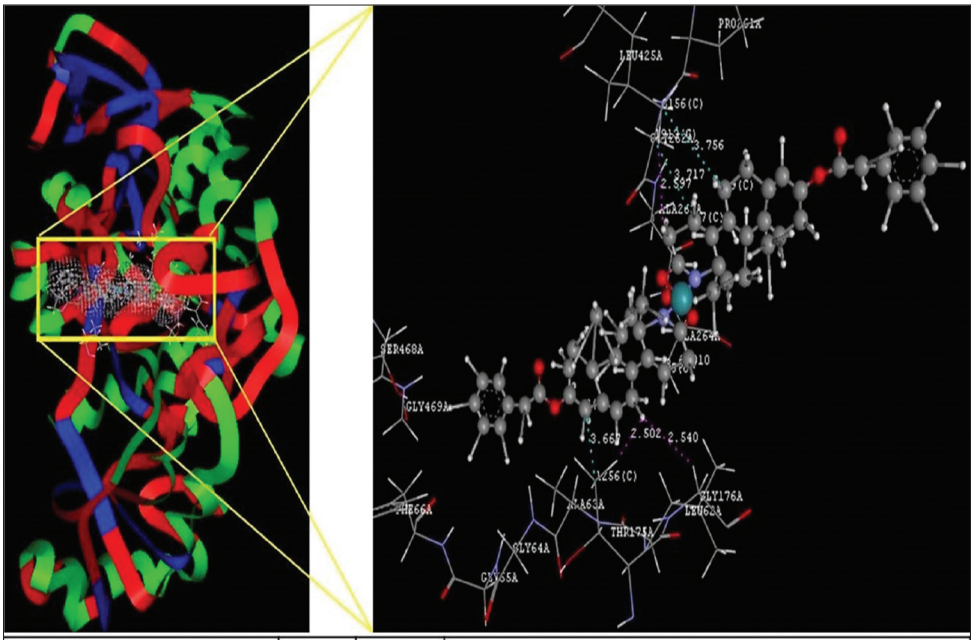
The binding interaction studies of Tc-99m-17a-aza steroid with 5AR receptor (PDB ID: 4AT0) revealed active involvement of various amino acid residues through noncovalent interactions including hydrophobic and van der Waals forces, as mentioned in Figure 1. The docking results also indicated, Tc-99m-17a-aza steroid showed promising binding affinity toward 4AT0 receptor with binding energy (–130.97 kcal/mol) [Figure 1]. Low D score indicates favorable binding energy.

### Synthesis and radiolabeling of 17-oxo-17a-aza-D-homo-5-androsten-3β-yl phenoxyacetate (17a-aza steroid) with Tc-99m

The compound, 17a-aza steroid was synthesized and was characterized by evaluating melting point, IR, and 1H NMR the results are shown in Figure 2. Then the synthesized compound was tagged with Tc-99m by direct labeling protocol and was characterized by ITLC.

### Radiochemical purity and physicochemical characterization

The radio-complex exhibited 96.79% labeling efficiency when subjected to ITLC after 10 min of incubation at the room temperature. The standardization of labeling yield was carried by varying the concentrations of $SnCl_2.2H_2O$, 17a-aza steroid, and activity of Tc-99m$O_4$, as shown in Figure 3. The stability of the radio-complex was observed by varying the pH from pH 2 to pH 10 and the results from the analysis are shown in Figure 4a. The percent radiochemical purity at pH 2 was 92.72% and the radiochemical purity increased to 97.24% at pH 4 and there was no significant variation in percent radiochemical purity from pH 4 to pH 7. After pH 7, the percent radiochemical yield decreased significantly to 86.84% at pH 10. Hence, the pH of the formulation for further experimentation was maintained at 7. Paper electrophoresis depicted that the synthesized radio-complex is neutral in charge as no movement was observed toward the cathode and anode. Log Po/w value of Tc-99m-17a-aza steroid obtained was 0.118 ± 0.045, specifying that the radio-complex is lipophilic in nature.




| Compound | Ligand pose | D score | Forces involved in interaction with active amino acid residues | |
|---|---|---|---|---|
| | | | Hydrophobic interaction | Vander wall forces |
| $^{99m}$Tc-17-oxo-17a-aza-D-homo-5-androsten-3β-ylphenoxyacetate | 2 | -130.97 | Leu425A (3.75Å), Ala264A (3.52Å), Gly262A (3.71Å), Thr175A (3.66Å) | Leu425A (2.59Å), Ala264A (3.01Å), Gly176A (2.54Å), Thr175A (2.50Å) |

**Figure 1: Surface view and three-dimensional ball and stick representation of docked Tc-99m-17-oxo-17a-aza-D-homo-5-androsten-3β-ylphenoxyacetate 4AT0 complex and ligand pose and docking score of ligands with 4AT0 receptor**

The plasma protein binding of the radio-complex was observed to be 86.23 ± 7.08%.

The stability of the radio-complex at the room temperature was evaluated to observe any disintegration and estimate the shelf-life of the radio-complex. The results of the analysis are shown in Figure 4b. From the analysis, the radio-complex was found to be stable at room temperature for a period of 2 h. The percent radiochemical yield was appreciably increased when the incubation time was increased from 0 min (84.94%) to 10 min (96.79%) and decreased to 93.77% after 30 min. The incubation time of 10 min was standardized for the synthesis of radio-complex. Further, the radio-complex was stable in *in vitro* conditions at the room temperature for 6 h in rat serum, as shown in Figure 4c.

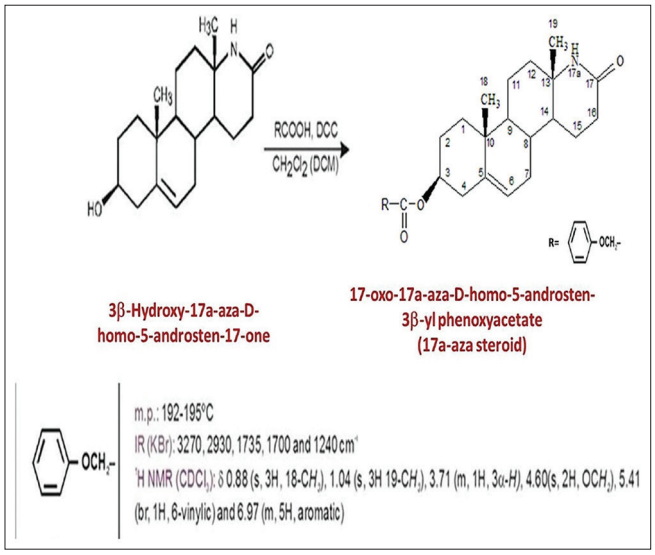



**Figure 2: The structure and characteristics of 17-oxo-17a-aza-D-homo-5-androsten-3 β-yl phenoxyacetate (17a-aza steroid). The D ring in the structure is represented from $C_{13}$ to $N_{17a}$**

## Prostatic acid phosphatase determination

PAP activity levels were significantly amplified in MNU + TP treated rats in comparison with that of healthy rats [Figure 5 I].

## Histological evaluation of prostate cancer

The histological examination of prostate tissue sections of rats treated with carcinogen MNU and hormone TP, revealed preneoplastic changes, described by proliferation in the number of epithelial cells called prostatic intraepithelial neoplasia (PIN) when paralleled with control. Further, high grade PIN (HGPIN) with patterns like tufting and cribiform were detected in the prostate of rats exposed to MNU and TP. Also in some prostate sections treated with MNU + TP, glands were seen to be abundantly filled with epithelial cells as shown in Figure 5 II.

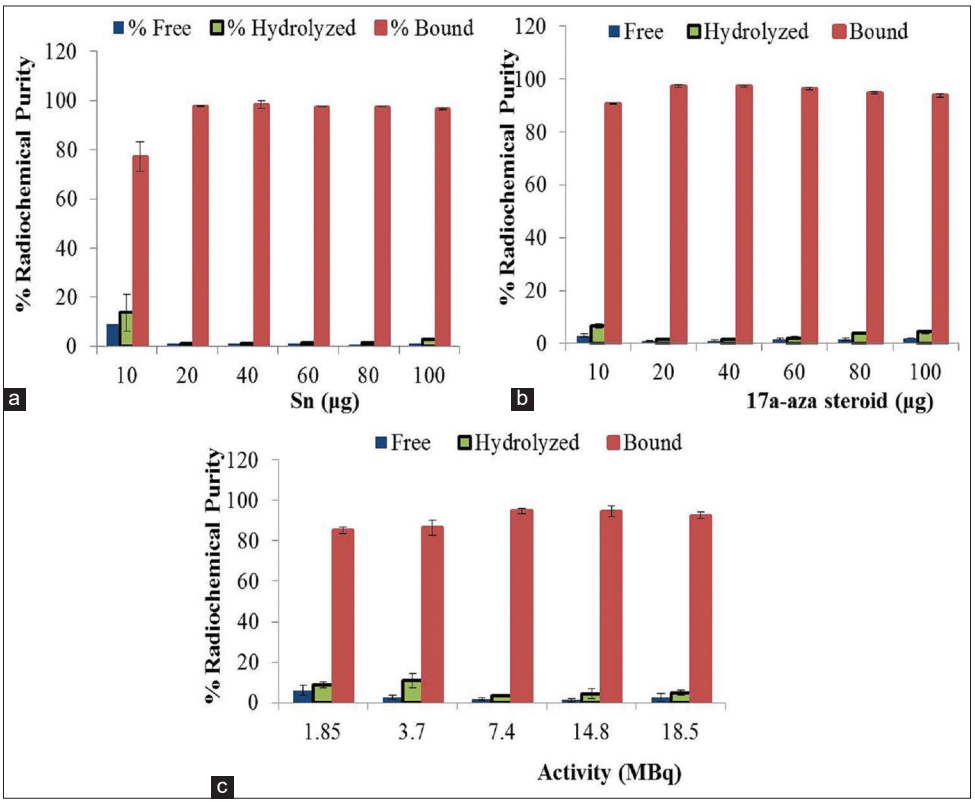



**Figure 3: Percent radiolabeling efficiency of Tc-99m-17a-aza steroid with different amounts of chemical constituents: (a) $SnCl_2.2H_2O$ (10-100 µg) (b) 17a-aza steroid (10-100 µg) and (c) Tc-99m$O_4^-$ activity (1.85MBq-18.5MBq) estimated by the ascending chromatographic technique. Each experiment was repeated three times, and statistical evaluation was done using the one-way ANOVA**

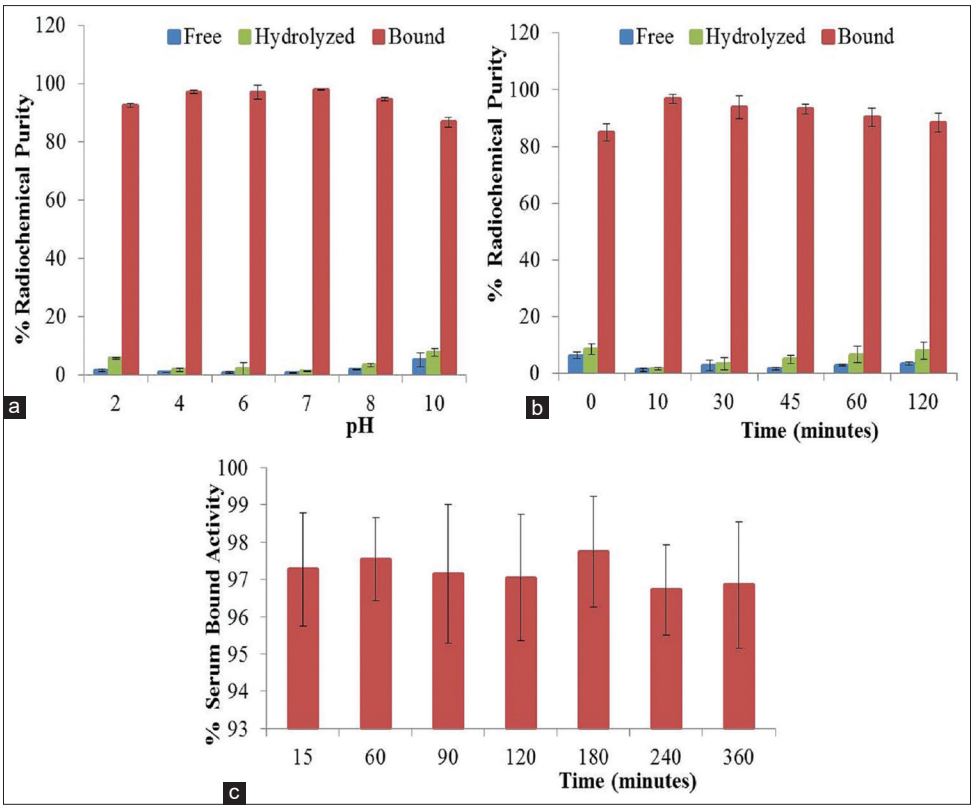



**Figure 4: The graphs represent the percent bound activity of Tc-99m-17a-aza steroid as a function of (a) pH (b) Incubation time at the room temperature and (c) Storage time in serum. Each experiment was repeated three times, and statistical evaluation was done using one-way analysis of variance**

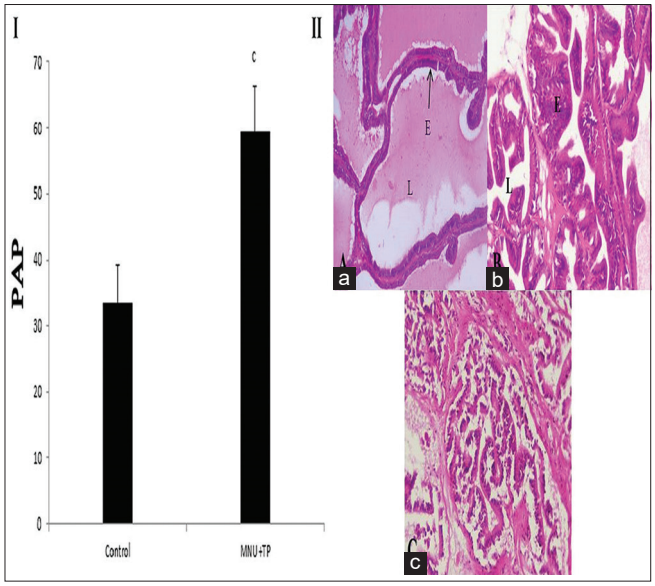



**Figure 5 I: The bars and error bars represent the mean ± SD of PAP values; *n* = 7 per group. [a]*P* ≤ 0.05, [b]*P* ≤ 0.01, [c]*P* ≤ 0.001 by Student's-*t* test when PAP levels in serum of MNU + testosterone propionate rats were compared with PAP levels in serum of normal control rats. (II) Histology of prostate ×20 magnification treated with MNU + testosterone propionate. (a) Normal control group: epithelium was tall columnar. (b and c) MNU + testosterone propionate -treated rats: hyperplastic and dysplastic changes were seen within the glandular epithelium with grade prostatic intraepithelial neoplasia (high grade prostatic intraepithelial neoplasia) as epithelial cells were increased in number when compared to control, (c) Gland filled with epithelial cells abundantly**

## Blood pharmacokinetics of Tc-99m-17a-aza steroid

After the intravenous administration of Tc-99m-17a-aza steroid in rats, two peaks were observed in the blood. The first peak was witnessed after 50 s of i.v. administration of the radio-complex and the second peak was detected after 50 min of i.v. administration. This clearly showed that the radio-complex followed a two-compartmental model of uptake [Figure 6a].

## Bio-distribution

The results of percent specific uptake from the bio-distribution studies in rats of Tc-99m-17a-aza steroid in the control group and in MNU + TP treated group are shown in Tables 1 and 2, respectively. In both groups, the pattern of uptake by various organs was similar. The maximum percent specific uptake of Tc-99m-17a-aza steroid in both groups was observed in kidneys followed by liver, spleen, lungs, and bladder. In healthy rats, there were no significant differences in percent specific uptake values up to 4 h of administration of radio-complex in the prostate, but at 6 h, percent-specific uptake in the prostate was significantly decreased when compared with percent-specific uptake at 2 h. The study observed that the percent-specific uptake of Tc-99m-17a-aza steroid in the prostate of MNU + TP treated rats was increased significantly when compared with the percent-specific uptake in the prostate of normal healthy rats at different time

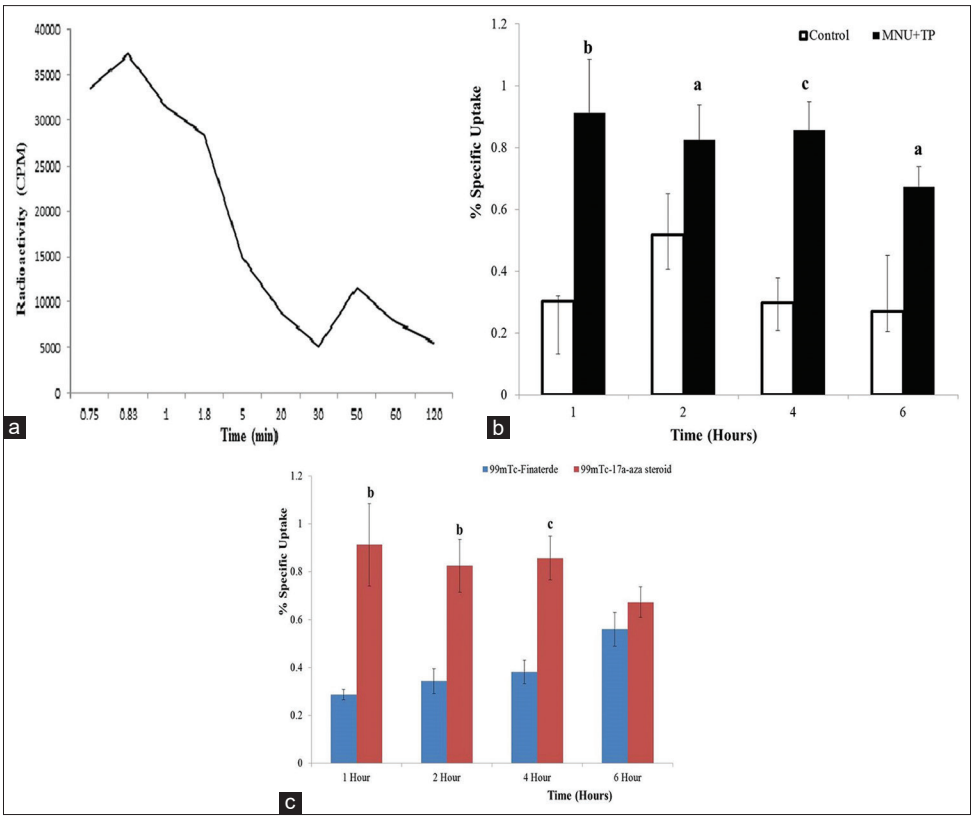



**Figure 6: (a) The graph represents the mean CPM for radioactivity in blood as a function of time (0 − 120 min) (b) Bars and error bars represent the Mean ± SD of percent specific uptake values of Tc-99m-17a-aza steroid in the prostate of normal control versus MNU + testosterone propionate treated rats. *N* = 3 for each observation. [a]*P* ≤ 0.05, [b]*P* ≤ 0.01, [c]*P* ≤ 0.001 following Student's *t*-test when the values of control were compared with values of MNU + testosterone propionate treated rats at different time intervals. (c) Bars and error bars represent the mean ± SD of percent-specific uptake values of the prostate in MNU + testosterone propionate treated rats of Tc-99m-finasteride and Tc-99m-17a aza steroid (*N* = 3). [a]*P* ≤ 0.05, [b]*P* ≤ 0.01, [c]*P* ≤ 0.001 following Student's *t*-test when percent-specific uptake values of Tc-99m-17a-aza steroid of the prostate were compared with percent-specific uptake values of the prostate of Tc-99m-finasteride in MNU + testosterone propionate treated rats**

**Table 1: Bio-distribution data of Tc-99m-17a-aza steroid in normal control rats**

| Organ | First h | Second h | Fourth h | Sixth h |
|---|---|---|---|---|
| Prostate | 0.303±0.017 | 0.518±0.133 | 0.298±0.079 | 0.269±0.182 |
| Bladder | 1.758±0.706 | 1.71±0.550 | 1.121±0.896 | 1.840±0.724 |
| Kidney | 7.476±1.300 | 9.093±0.897 | 13.726±2.948 | 15.403±2.545 |
| Liver | 4.44±0.836 | 5.293±1.504 | 9.423±1.188 | 13.303±1.618 |
| Spleen | 4.002±0.245 | 5.816±0.886 | 8.33±1.334 | 9.04±1.165 |
| Lung | 3.145±0.465 | 7.207±4.919 | 3.354±2.237 | 4.292±1.191 |
| Thyroid | 0.35±0.053 | 0.512±0.106 | 0.422±0.095 | 0.031±0.007 |
| Heart | 0.416±0.005 | 0.547±0.065 | 0.535±0.274 | 0.557±0.061 |
| Brain | 0.217±0.065 | 0.064±0.005 | 0.133±0.027 | 0.096±0.074 |
| Muscle | 0.337±0.003 | 0.462±0.241 | 0.126±0.015 | 0.160±0.06 |
| Bone | 1.77±0.14 | 0.694±0.077 | 0.163±0.011 | 0.59±0.085 |
| Stomach | 0.124±0.065 | 0.101±0.042 | 0.094±0.045 | 0.091±0.013 |
| Small intestine | 0.639±0.277 | 1.276±0.526 | 0.659±0.150 | 0.944±0.074 |
| Large intestine | 0.654±0.294 | 0.691±0.178 | 0.635±0.079 | 0.915±0.186 |

Each value represents the mean±SD ($n$=3) of ID/g±SD of Tc-99m-17a-aza steroid in normal control rats, The statistical significance was considered at the level of $P$<0.05 following the ANOVA test. SD: Standard deviation, ID/g: Injected dose per gram, ANOVA: Analysis of variance

intervals. The detailed comparison of percent-specific uptake of the radio-complex in the prostate between the two groups is illustrated in Figure 6b. From the comparative study, it can be seen that the percent-specific uptakes after 1 h, 2 h, 4 h, and 6 h of administration of radio-complex in the prostate of normal healthy rats observed were 0.303%, 0.518%, 0.298%, and 0.269% which were significantly increased to 0.913% ($P \leq 0.0l$), 0.825% ($P \leq 0.05$), 0.857% ($P \leq 0.001$), and 0.673% ($P \leq 0.05$), respectively, in carcinogen-induced rats.

**Table 2: Tabular representation of bio-distribution data of Tc-99m-17a-aza steroid in rats treated with *N*-methyl-*N*-nitrosourea + testosterone propionate**

| Organ | First h | Second h | Fourth h | Sixth h |
|---|---|---|---|---|
| Prostate | 0.913±0.172 | 0.825±0.110 | 0.857±0.091 | 0.673±0.064 |
| Bladder | 1.465±0.175 | 0.979±0.247 | 0.897±0.456 | 0.871±0.578 |
| Kidney | 6.283±0.746 | 7.37±1.162 | 7.836±1.861 | 8.08±1.784 |
| Liver | 3.48±0.676 | 6.767±0.496 | 8.693±0.784 | 9.673±0.457 |
| Lung | 2.903±0.553 | 2.44±0.216 | 1.263±0.253 | 1.2±0.07 |
| Spleen | 3.873±0.600 | 6.76±0.911 | 4.61±0.026 | 8.77±0.645 |
| Thyroid | 0.356±0.163 | 0.698±0.037 | 0.654±0.099 | 0.684±0.198 |
| Heart | 0.045±0.036 | 0.404±0.217 | 0.231±0.171 | 0.337±0.054 |
| Brain | 0.139±0.124 | 0.257±0.139 | 0.149±0.085 | 0.132±0.048 |
| Muscle | 0.097±0.075 | 0.262±0.023 | 0.330±0.194 | 0.310±0.022 |
| Bone | 0.437±0.237 | 1.381±0.609 | 2.24±1.062 | 2.038±1.163 |
| Stomach | 0.142±0.038 | 0.091±0.029 | 0.112±0.033 | 0.069±0.023 |
| Small intestine | 0.482±0.554 | 0.563±0.037 | 0.400±0.015 | 0.378±0.193 |
| Large intestine | 0.264±0.121 | 0.383±0.034 | 0.878±0.439 | 0.973±0.641 |

Each value represents the mean±SD ($n$=3) of ID/g±SD of Tc-99m-17a-aza steroid in rats treated with MNU + TP, The statistical significance was considered at the level of $P$<0.05 following the ANOVA test. SD: Standard deviation, ID/g: Injected dose per gram, MNU + TP: *N*-methyl-*N*-nitrosourea + testosterone propionate, ANOVA: Analysis of variance

### Comparative uptake of Tc-99m-17a-aza steroid and Tc-99m-finasteride in the prostate of MNU + testosterone propionate treated rats

In comparison to percent-specific uptake values of Tc-99m-finasteride, the percent-specific uptake values of Tc-99m-17a-aza steroid in the prostate of MNU + TP treated rats were significantly increased after 1, 2, and 4 h of administration in animals. The results are shown in Figure 6c. The percent-specific uptake values of Tc-99m-finasteride in the prostate of MNU + TP treated rats observed after 1$^{st}$, 2$^{nd}$, and 4$^{th}$ h postadministration were 0.287%, 0.343%, and 0.381% which were significantly decreased when compared to percent specific uptake values of Tc-99m-17a-aza steroid (0.913%, 0.825%, and 0.857%) with $P \leq 0.01$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

## Discussion

The present study was an attempt to discern the specificity of a newly synthesized drug toward the prostate gland to exploit its potential as a radionuclide probe for the detection of the prostatic lesions. Hence, for the primary analysis molecular docking, a computational approach was adopted for the prediction of binding affinity of Tc-99m-17a-aza steroid toward 5AR. Due to a favorable binding score of –130.97 the radio-synthesis of Tc-99m-17a-aza steroid was carried out using the direct radiolabeling protocol. The radio-complex was developed with >90% labeling efficiency and was stable under physiological conditions for 6 h at the room temperature.

Percentage protein binding of the radiopharmaceutical is important in determining it's *in vivo* reversible and irreversible interactions with proteins, which bears a direct influence on the pattern of bio-distribution, rate of elimination, and the uptake at the target site The protein binding of Tc-99m-17a-aza steroid *in vitro* was found to be 86.23 ± 7.08%. The high plasma binding indicates that the majority of the radio-complex is bound to plasma and a small quantity shall be available for its distribution in tissue. This indicates a long plasma half-life of the radio complex *in-vivo*. Therefore, high plasma binding of the synthesized radiochemical was directly correlated with the delayed uptake in the prostate of rats after i.v administration. Further, the Log-*P* value of the radio-complex deciphers its lipophilic nature, and thus, it can easily cross the cellular membranes in *in vivo* conditions.

For the bio-distribution studies, the animals subjected to MNU + TP treatment were analyzed histopathologically as well as for PAP levels to confirm prostate carcinogenesis. PAP is secreted by the prostate epithelial cells and its increased levels are correlated with the advancement of prostate cancer.[8] The efficacy of PAP as a prostate cancer marker has been reported in previous studies wherein its usefulness in predicting intermediate to high-risk prostate cancer has been highlighted. In our study, the levels of PAP were increased significantly in MNU + TP treated rats in comparison to control rats indicating the successful induction of prostate carcinogenesis in MNU + TP treated rats. Further, histological evaluation in the treated group confirmed neoplastic changes with increased cellular activity (hyperplasia) and high HGPIN.

The blood pharmacokinetics of the radio-complex depicted two peaks. While the first peak reveals its uptake by organs, the second peak depicted its delayed discharge from different organs into systematic circulation. From the bio-distribution pattern of the radio-complex, the highest percent-specific uptake after 1 h of administration

was witnessed in the kidneys trailed by the liver, spleen, lung, and bladder. The uptake in kidneys specifies the excretion of the radio-complex through the renal route. The activity in the liver specifies the metabolism of the compound in the liver. The percent-specific uptake of Tc-99m-17a-aza steroid in the prostate of normal control and MNU + TP treated rats was quite similar, but in comparison to the normal control group, the uptake in the prostate of MNU + TP treated group was significantly increased after different time intervals of administration, as shown in Figure 6b. We also have found higher specific uptake of Tc-99m-17a-aza steroid in prostate of MNU + TP treated rats in comparison to older analog Tc-99m-finasteride.

## Conclusion

In conclusion, the newly synthesized steroid 17a-aza has a better prospect in prostate cancer management. However, the study concludes that it can be explored further for its potential as a radionuclide probe in prostate cancer imaging and treatment response evaluation.

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