J Radioanal Nucl Chem (2011) 288:411–416
DOI 10.1007/s10967-010-0936-3

Radiosynthesis and biodistribution of ^{99m}Tc-tricarbonyl complex of temafloxacin dithiocarbamate: a potential *Streptococci pneumoniae* infection radiotracer

Syed Qaiser Shah · Muhammad Rafiullah Khan

Received: 10 November 2010 / Published online: 4 January 2011

Abstract In the current investigation the complexation of derivatized temafloxacin with ^{99m}Tc using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was assessed. The tricarbonyl complex of the temafloxacin dithiocarbamate (^{99m}Tc(CO)$_3$-TAND) was characterized in terms of radiochemical purity (RCP) yield in saline, in vitro radiochemical stability in serum, in vitro binding with *Streptococci pneumoniae* and biodistribution in male Wister rats (MWR) artificially infected with living and heat killed *Streptococci pneumoniae*. The ^{99m}Tc(CO)$_3$-TAND complex showed 98.10 ± 15% RCP value at 30 min of the reconstitution and remained more than 90% stable up to 120 min in normal saline at room temperature. In serum a stable behavior with the appearance of 15.30% unwanted side product up to 16 h of incubation was observed. A saturated in vitro binding with *Streptococci pneumoniae* was observed. The complex showed almost six times higher uptake in the infected muscle as compared to the inflamed and normal muscles of the MWR infected with living *Streptococci pneumoniae*. Insignificant difference in the uptake of the tracer in the infected, inflamed and normal muscles of the MWR infected with heat killed *Streptococci pneumoniae* was noted. Based on the elevated RCP in saline, in vitro stability in serum at 37 °C, saturated in vitro binding with pathogens and better biodistribution behavior with higher accumulation of the tracer in target organs confirmed the suitability of the ^{99m}Tc(CO)$_3$-TAND complex as promising infection radiotracer.

Keywords Temafloxacin dithiocarbamate (TAND) · *Streptococci pneumoniae* · Infection

Introduction

Technetium-99m (^{99m}Tc) labeled quinolones are nowadays frequently used to identify deep soft tissue infection for accurate in time decision and management [1, 2]. Recently, the reported ^{99m}Tc-labeled bioactive molecules have revealed high radiochemical yield, in vitro permanence in serum, in vitro binding with bacteria, soaring target to non target ratio and precise scintigraphic localization of infectious foci [3–24].

Temafloxacin (TAN) (Fig. 1a) [1-(2,4-Difluorophenyl)-6-fluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic] is another broad-spectrum fluorinated quinolone showing advantageous in vivo and in vitro pharmacokinetics. TAN akin to other quinolones has proven potent against respiratory pathogens including *Staphylococcus aureus, Moraxella catarrhalis, Haemophilus influenzae, Legionella pneumophila* and *Klebsiella pneumoniae*. It is reported that TAN, has revealed almost eight fold in vivo higher potency against *Streptococci pneumoniae* and *Streptococcus pyogenes* [25, 26].

To make use of the higher activity of TAN, to diagnosis of infection caused by *Streptococci pneumoniae*, in the present investigation, TAN was devitalized to temafloxacin dithiocarbamate (TAND) and radiolabeled with technetium-99m (^{99m}Tc) using the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. The ^{99m}Tc- tricarbonyl temafloxacin dithiocarbamate (^{99m}Tc(CO)$_3$-TAND) complex was radiochemically and

S. Q. Shah (✉)
Nuclear Medicine Research Laboratory (NMRL), University of Peshawar, Peshawar, KPK, Pakistan
e-mail: ssqaiser2002@yahoo.com

M. R. Khan
Phytopharmaceutical & Neutraceuticals Research Laboratory (PNRL), University of Peshawar, Peshawar, KPK, Pakistan

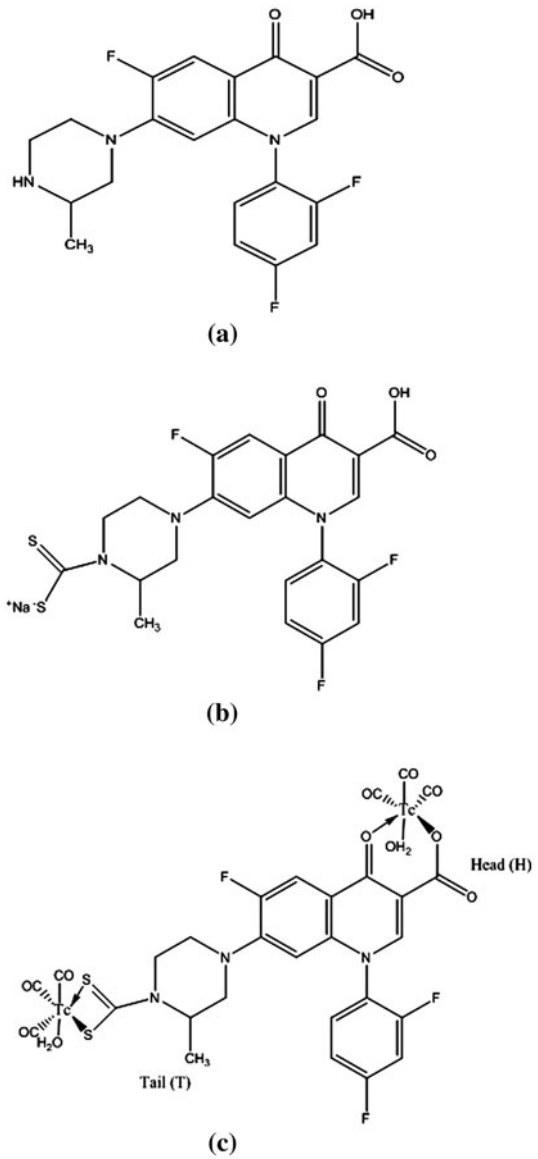

Fig. 1 **a** Chemical structure of the temafloxacin (TAN). **b** Temafloxacin dithiocarbonate (TAND). **c** Proposed structure of the $^{99m}Tc(CO_3)$-TAND complex

biologically characterized in terms of radiochemical stability in normal saline, in vitro permanence in serum, in vitro binding with *Streptococci pneumoniae* and biodistribution in Wister male rats (WMR) artificially infected by *Streptococci pneumoniae*.

Experimental

Materials

Temafloxacin (TAN) (Shanghai Sciencya Biotechnology Co., Ltd. Shanghai, China), TLC (Merck) and all the other chemicals and solvents of analytical grade (Sigma). RP-HPLC (Shimadzu, Japan) well counter and scalar count rate meter (Ludlum, USA) Dose calibrator (Capintech, USA) and Gamma camera GKS-1000 (GEADE Nuclear-medizine system, Germany).

Method

Tricarbonyl radiocomplexation of temafloxacin dithiocarbamate

Temafloxacin (TAN) was derivatized to temafloxacin dithiocarbamate (TAND) using the reported method [22] by mixing equal amount of TAN, tetrahydrofuran (THF) and carbon disulfide (CS_2) in the presence of sodium hydroxide. Then 74 MBq (0.5 mL) of sodium pertechnetate ($Na^{99m}TcO_4^-$) and 0.1 mol/mL HCL solution to the Isolink kit and incubated for 15 min at room temperature. Thereafter, 2 mg of the TAND was added to the Isolink kit and incubated the reaction mixture for 15 min.

Partition coefficient (*P*)

In equal amount the freshly prepared $^{99m}Tc(CO)_3$-TAND complex, octanol and phosphate buffer (PB) was vortexed for 5 min followed by centrifugation at 5000 rpm/min for 10 min. Thereafter, 0.1 mL of the reaction mixture was taken at different intervals and measured in the in well counter interface with scalar count rate meter (WCCRM) using the equation. $P = \text{CPM in octanol} - \text{CPM in background/CPM in buffer} - \text{CPM in background}$.

Radiochemical characterization

The $^{99m}Tc(CO)_3$-TAND complex was radiochemically characterized in saline using the HPLC method reported earlier [22]. Briefly, to the Shimadzu HPLC unit interface with UV detector (working at 254 nm), flow scintillation analyzer, binary pump an online degasser and C-18 (4.6 × 150 mm) column, 5 µL of the $^{99m}Tc(CO)_3$-TAND complex was injected. 1 mL/min elution was allowed for 20 min using water:methanol (W:M) 0–3 min (100:00), 3–7 min (60:40), 7–10 min (55:45), 10–13 (25:75), 13–16 (00:100) and 16–20 (50:50) as the mobile phase. The radio-eluents collected during 15 min of the process were counted for activity using WCCRM.

Stability in serum

The stability of the $^{99m}Tc(CO)_3$-TAND complex in serum was determined by using the thin layer chromatography.

0.2 mL of the ^{99m}Tc(CO)$_3$-TAND complex was incubated at 37 °C with 1.8 mL of serum for 16 h. Aliquots at 0, 2, 4, 6, 8, 10, 12, 14 and 16 h during incubation were taken and spotted on the TLC strips. The strips were then developed for separation of various component of the complex in saline and CH_2Cl_2:CH_3OH (9:1) (v/v). After development the radiostrips were divided into two parts and measured for activity in each part using WCCRM.

In vitro binding with *Streptococci pneumoniae*

In vitro binding capability of the ^{99m}Tc(CO)$_3$-TAND complex was investigated using the reported method [27]. Briefly, 0.1 mL of the sodium phosphate buffer (Na-PB) was mixed with 10 MBq of the ^{99m}Tc(CO)$_3$-TAND complex in clean and pyrogen free test tube. Thereafter, 0.8 mL (50%, v/v) 0.01 M acetic acid containing approximately 1×10^8 colony forming units (CFU) of *Streptococci pneumoniae* was added followed by incubation at 4 °C for 1 h and the pH was adjusted to 5. The reaction mixture was then centrifuged at 2000 rpm for 10 min. After that the supernatant was removed and the bacterial pellets were resuspended in 2 mL Na-BP. The reaction mixture was re-centrifuged at 2000 rpm/min for another 10 min. Next, the bacterial pellets were measured for percent in vitro uptake in WCCRM.

Biodistribution in MWR

The in vivo (percent per gram) accumulation of the ^{99m}Tc(CO)$_3$-TAND complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR artificially infected with living and heat killed *Streptococci pneumoniae* was studied. Sixteen healthy MWR (weight, 160–180 g) were selected and alienated into two groups (A and B) each having eight MWR. All the MWR were intramuscularly injected 0.2 mL sterile turpentine oil into their left thigh. Thereafter, 0.2 mL of the living *Streptococci pneumoniae* was injected I.M. to the left of the MWR of group A and heat killed to the group B. 24 h, after 0.2 mL of the freshly prepared ^{99m}Tc(CO)$_3$-TAND radiotracer was intravenously (I.V.) injected to all the MWR. Next, all the MWR were sacrificed in accordance with the rules of the Nuclear Medicine Research Laboratory (NMRL), University of Peshawar (Part-I and II). Then in vivo (percent per gram) accumulation of the ^{99m}Tc(CO)$_3$-TAND complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR artificially infected with living and heat killed *Streptococci pneumoniae* were calculated using WCCRM.

Results and discussion

Radiochemistry of the ^{99m}Tc(CO)$_3$-TAND complex

Temafloxacin (TAN) a bidentate chelator as shown in Fig. 1a was derivatized to tetradentate temafloxacin dithiocarbamate (TAND) as shown in Fig. 1b for much stronger and stable complexation with ^{99m}Tc using the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. The tetradentate TAND under substitution reaction easily replaced the H_2O from the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor and gave a stable ^{99m}Tc-tricarnoly temafloxacin dithiocarbamate complex as shown in Fig. 1c. The proposed radiochemical arrangement of the ^{99m}Tc(CO)$_3$-TAND complex will have a square planner bipyramidal geometry with ^{99m}Tc(CO)$_3$:TAND ratio of 2:1 [28].

The HPLC radiochromatogram of the ^{99m}Tc(CO)$_3$-TAND complex showed two radiopeaks at 4.1 and 14.4 min of retention as shown in Fig. 2. The radiopeak observed at 14.4 min of retention correspond to the radiochemical yield of the ^{99m}Tc(CO)$_3$-TAND complex.

The ^{99m}Tc(CO)$_3$-TAND complex behave normally and stable in normal saline at room temperature. The radiochemical stability of the ^{99m}Tc(CO)$_3$-TAND complex at 30, 60, 90, 120 and 240 min of reconstitution in given in Fig. 3. The highest radiochemical stability value observed was 98.10 ± 0.15% recorded at 30 min after reconstitution. The value of the radiochemical stability went down from 98.10 ± 0.15% to 90.50 ± 0.18% within 240 min.

Lipophilicity of the ^{99m}Tc(CO)$_3$-TAND complex

The participation coefficient (*P*) value of the ^{99m}Tc(CO)$_3$-TAND complex was 0.44 ± 0.01 signifying lipophilicity.

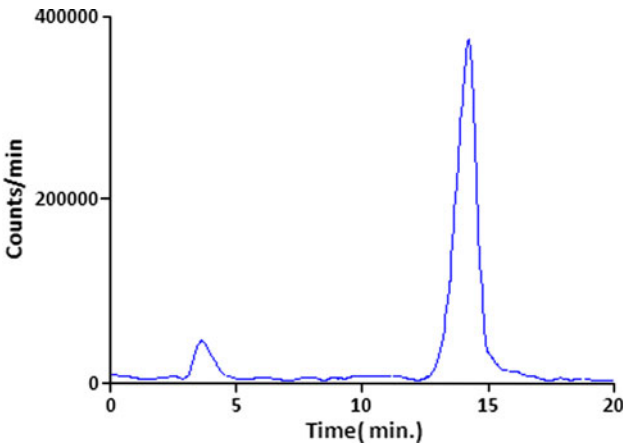

Fig. 2 HPLC ^{99m}Tc(CO)$_3$-Temafloxacin dithiocarbamate chromatogram

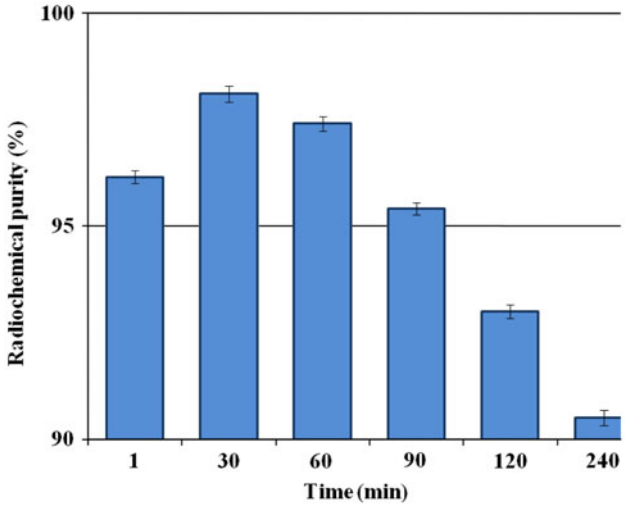

Fig. 3 Stability of the $^{99m}Tc(CO)_3$-TAND complex in normal saline at different intervals

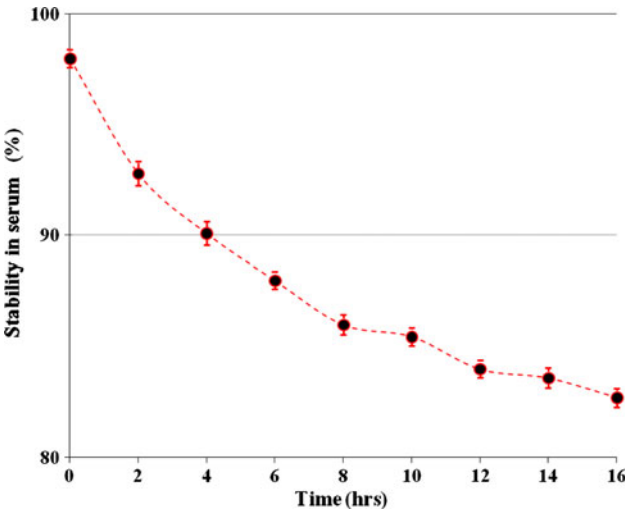

Fig. 4 In-vitro stability of the $^{99m}Tc(CO)_3$-TAND complex in serum at 37 °C up to 16 h

Stability in serum

In vitro stability profile of the $^{99m}Tc(CO)_3$-TAND complex is given in Fig. 4. It was observed that the $^{99m}Tc(CO)_3$-TAND complex was remained more than 90% stable up to 4 h under incubation. The stability value decreased by 15.30% due the formation of undesirable side product within 16 h

In vitro binding with *Streptococci pneumoniae*

Streptococci pneumoniae showed in vitro saturated binding with the $^{99m}Tc(CO)_3$-TAND complex at different intervals

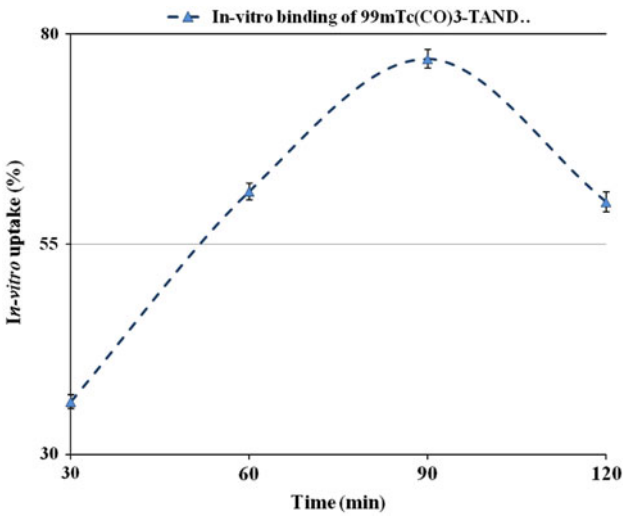

Fig. 5 In-vitro binding of the $^{9m}Tc(CO)_3$-TAND complexes with *Staphylococcus aureus* at 30, 60, 90 and 120 min

with a maximum value of 77.00% at 90 min. The in vitro binding affinity of the *Streptococci pneumoniae* with the $^{99m}Tc(CO)_3$-TAND complex is given in Fig. 5.

Biodistribution in MWR

The in vivo (percent) accumulation of the $^{99m}Tc(CO)_3$-TAND complex in (per gram) blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR of group A and B is given in Table 1. In the beginning of I.V injection of the $^{99m}Tc(CO)_3$-TAND complex to the MWR of group A and B, high level of activity was observed which went down to 19.20 ± 0.14% from 4.00 ± 0.16% with 120 min. Similarly, the level of radioactivity in per gram of the liver, spleen, stomach and intestines was decreased from 14.50 ± 0.18% to 4.50 ± 0.00%, 8.50 ± 0.14% to 4.00 ± 0.14% and 7.80 ± 0.14% to 3.85 ± 0.18% respectively within 120 min of the I.V injection of the complex. However, an opposite behavior was seen in kidneys wherein the activity goes up with time from 8.25 ± 0.18% to 23.10 ± 0.19% within 120 min. Insignificant marginal variation in the uptake values in liver, spleen, stomach, intestines and kidneys. Six times higher accumulation of the radiotracer was seen in the infected muscle than inflamed and normal muscles of the MWR of group A. However, no difference in the percent accumulation of the radiotracer in the infected, inflamed and normal muscles of the MWR of the group B was noted. The uptake ratios of the radiotracer in the infected muscles of the MWR of the group A and B are illustrated Fig. 6. In the urinary system the appearance of the activity of the $^{99m}Tc(CO)_3$-TAND radiotracer and disappearance from the circulatory system confirmed the normal path for excretion from the MWR of group A and B.

Table 1 Percent uptake of the ^{99m}Tc(CO)3-TAND complex in various organs of *Staphylococcus aureus* infected Male Wister rats

Organs/tissues (gm)	Percent in vivo absorption at different intervals (in min)							
	Alive *S. aureus*				Heat killed *S. aureus*			
	30	60	90	120	30	60	90	120
Infected muscle	7.25 ± 0.18	12.25 ± 0.15	15.40 ± 0.14	12.00 ± 0.16	2.50 ± 0.14	3.00 ± 0.16	3.00 ± 0.16	2.80 ± 0.18
Inflamed muscle	3.50 ± 0.14	4.00 ± 0.18	3.00 ± 0.18	3.00 ± 0.15	3.00 ± 0.16	3.50 ± 0.17	3.00 ± 0.20	3.00 ± 0.14
Normal muscle	3.00 ± 0.16	2.50 ± 0.14	2.50 ± 0.17	2.50 ± 0.18	2.50 ± 0.18	2.50 ± 0.18	2.50 ± 0.00	2.50 ± 0.14
Blood	19.20 ± 0.14	11.00 ± 0.18	8.45 ± 0.16	4.00 ± 0.16	19.00 ± 0.18	10.40 ± 0.15	8.00 ± 0.18	3.85 ± 0.00
Liver	14.50 ± 0.18	10.00 ± 0.15	8.10 ± 0.14	4.50 ± 0.00	15.50 ± 0.15	9.45 ± 0.14	8.00 ± 0.18	4.70 ± 0.19
Spleen	8.50 ± 0.14	7.00 ± 0.18	5.85 ± 0.19	4.00 ± 0.14	9.00 ± 0.18	7.25 ± 0.16	6.00 ± 0.18	4.10 ± 0.00
Kidney	8.25 ± 0.18	15.00 ± 0.14	19.45 ± 0.16	23.10 ± 0.19	8.50 ± 0.14	15.20 ± 0.18	20.00 ± 0.15	23.40 ± 0.16
Stomach & intestines	7.80 ± 0.14	6.75 ± 0.18	5.90 ± 0.00	3.85 ± 0.18	8.00 ± 0.15	6.80 ± 0.18	5.75 ± 0.14	3.90 ± 0.17

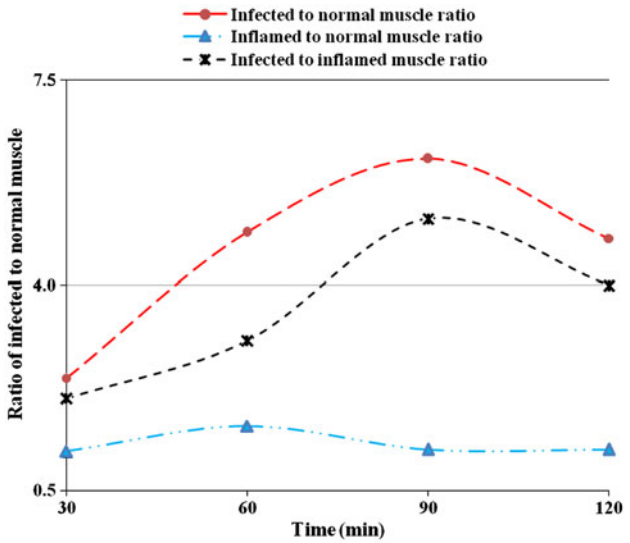

Fig. 6 Infected to normal, inflamed to normal and infected to inflamed muscles uptake ratio of the ^{99m}Tc(CO)$_3$-TAND complex at 30, 60, 90 and 120 min of I.V injection

Conclusion

^{99m}Tc-tricarbonyl complexation of the temafloxacin dithiocarbamate was investigated in terms of RCP yield in saline, in vitro radiochemical stability in serum, in vitro binding with *Streptococci pneumoniae* and biodistribution in male Wister rats (MWR) artificially infected with living and heat killed *Streptococci pneumoniae.* The elevated RCP values in saline, in vitro stability in serum at 37 °C, saturated in vitro binding with *Streptococci pneumoniae* and better biodistribution behavior with higher accumulation of the complex in target organ confirmed the feasibility of the ^{99m}Tc(CO)$_3$-TAND complex as potential in vivo infection radiotracer.

References

1. Gallagher H, Ramsay SC, Barnes J, Maggs J, Cassidy N, Ketheesan N (2006) Neutrophil labeling with [^{99m}Tc]-technetium stannous colloid is complement receptor 3-mediated and increases the neutrophil priming response to lipopolysaccharide. Nucl Med Biol 33:433
2. Basu S, Chryssikos T, Moghadam-Kia S, Zhuang H, Torigian DA, Alvai A (2009) Positron emission tomography as a diagnostic tool in infection: present role and future possibilities. Semin Nucl Med 39:36
3. Chattopadhyay S, Das SS, Chandra S, De K, Mishra M, Sarkar BR, Sinha S, Ganguly S (2010) Synthesis and evaluation of ^{99m}Tc-moxifloxacin, a potential infection specific imaging agent. Appl Radiat Isot 68:314
4. Motaleb MA (2007) Preparation of ^{99m}Tc-cefoperazone complex, a novel agent for detecting sites of infection. J Radioanal Nucl Chem 272:167
5. Motaleb MA (2007) Preparation and biodistribution of ^{99m}Tc-lomefloxacin and ^{99m}Tc-olfloxacin complex. J Radioanal Nucl Chem 272:95
6. Zhang J, Guo H, Zhang S, Lin Y, Wang X (2008) Synthesis and biodistribution of a novel ^{99m}TcN complex of ciprofloxacin dithiocarbamate as a potential agent for infection imaging. Bioorg Med Chem Lett 18:51
7. Roohi S, Mushtaq A, Jehangir M, Ashfaq MS (2006) Synthesis, quality control and biodistribution of ^{99m}Tc-Kanamycin. J Radioanal Nucl Chem 267:561
8. Oh SJ, Ryu J, Shin JW, Yoon EJ, Ha H, Cheon JH, Lee HK (2002) Synthesis of ^{99m}Tc-ciprofloxacin by different methods and its biodistribution. Appl Radiat Isot 57:193
9. EL-Gany EA, EL-Kolaly MT, Amine AM, EL-Sayed AS, Abdel-Gelil F (2005) Synthesis of ^{99m}Tc-pefloxacin: a new targeting agent for infectious foci. J Radioanal Nucl Chem 266:131
10. Motaleb MA (2009) Preparation, quality control and stability of ^{99m}Tc-sparafloxacin complex, a novel agent for detecting sites of infection. J Label Compd Radiopharm 52:415
11. Xia J, Wang Y, Yu J, Li S, Tang L, Zheng M, Liu X, Li G, Cheng D, Liang S, Yin D (2008) Synthesis, in vitro and in vivo behavior of ^{188}Re(I)-tricarbonyl complexes for the future functionalization of biomolecules. J Radioanal Nucl Chem 275:325
12. Zhang J, Wang X, Jin C (2007) Synthesis and biodistribution of the ^{99m}Tc(CO)$_3$-DEDT complex as a potential new radiopharmaceutical for brain imaging. J Radioanal Nucl Chem 272:91

13. Djokic DD, Jankovic DL, Stamenkovic LL, Pirmettis I (2004) Chemical and biological evaluation of ^{99m}Tc $(CO)_3$ and ^{99m}Tc complexes of some IDA derivatives. J Radioanal Nucl Chem 260:471
14. Xia J, Long S, Yu J, Wang Y, Cao Z (2009) Pyridyl derivatives provide new pathways for labeling protein with *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$. J Radioanal Nucl Chem 281:493
15. Zhang JB, Wang XB, Jin C (2006) Synthesis of $^{99m}Tc(CO)_3$-NOET via $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor and comparative biological studies with ^{99m}TcN-NOET. J Radioanal Nucl Chem 269:227
16. Qaiser SS, Khan AU, Khan MR (2010) Synthesis, biodistribution and evaluation of ^{99m}Tc-Sitafloxacin kit: a novel infection imaging agent. J Radioanal Nucl Chem 284:189
17. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis of ^{99m}Tc-nitrifuratonin a novel radiotracer for in vivo imaging of *Escherichia coli* infection. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0697-z
18. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biodistribution of ^{99m}Tc-rifampicin: a novel radiotracer for in vivo infection imaging. Appl Radiat Isot 68:2255
19. Shah SQ, Khan AU, Khan MR (2010) ^{99m}Tc-Novobiocin: a novel radiotracer for infection imaging. Radiochimica acta (in press)
20. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis, biodistribution and scintigraphy of the ^{99m}Tc-Teicoplanin complex in artificially infected animal models. J Label Compd Radiopharm. Online published on 4 November 2010
21. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biological evaluation of ^{99m}TcN-sitafloxacin dithiocarbamate as potential radiotracer for *Staphylococcus aureus* infection. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0833-9
22. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biological evolution of $^{99m}Tc(CO)_3$-sitafloxacin dithiocarbamate complex: a promising Staphylococcus aureus infection radiotracer. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0880-2
23. Shah SQ, Khan MR (2010) Radiocomplexation and biological characterization of the ^{99m}TcN-Trovafloxacin dithiocarbamate: a novel methicillin-resistant *Staphylococcus aureus* infection imaging agent. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0903-z
24. Shah SQ, Khan MR (2010) Radiolabeling of gemifloxacin with technetium-99m and biological evaluation in artificially Streptococcus pneumoniae infected rats. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0916-7
25. Segreti J (1991) In vitro activity of temafloxacin against pathogens causing sexually transmitted diseases. Am J Med 91:S24
26. Jacobs MR (1991) Evaluation of the bactericidal activity of temafloxacin. Am J Med 91:S31
27. Welling MM, Paulusma-Annema A, Batler HS, Pauwels EKJ, Nibbering PH (2000) Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Eur J Nucl Med 27:292
28. Baldas J, Bonnyman J, Poer PM, Williams GA, Mackay MF (1981) Synthesis And Structure of bis(diethyldithiocarbamato)nitridotechnetium(V)—a technetium-nitrogen triple bond. J Chem Soc Dalton Trans 9:1798