

Radio-Synthesis, and In-vivo Skeletal Localization of ^{177}Lu - zoledronic Acid as Novel Bone Seeking Therapeutic Radiopharmaceutical

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Abstract

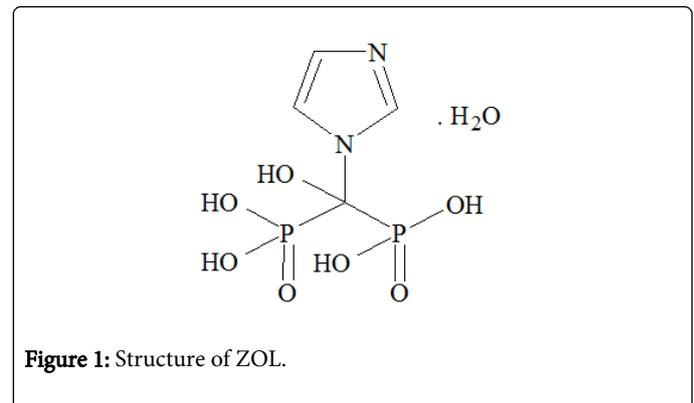
Radiocomplexation of zoledronic acid (ZL), a potent bisphosphonate with Lutetium-177, was investigated. The ^{177}Lu -ZL complex was assessed for its radiochemical purity, in vitro stability and bone uptake by scintigraphic images. A stable ^{177}Lu -ZL complex was observed with $> 99.0 \pm 0.5\%$ radiochemical yield and remained stable for more than 7 hours in vitro by mixing 12mg ZL, 5mCi activity of $^{177}\text{LuCl}_3$ produced by thermal neutron irradiation ($\sim 1.2 \times 10^{14}$ n.cm $^{-2}$ s $^{-1}$) of enriched Lu_2O_3 samples in Pakistan Research Reactor-1 (PARR-1), at pH 7-8. Radiochemical purity was determined by PC, ITLC and HPLC. The biodistribution of ^{177}Lu -ZL in rabbit was studied by SPECT imaging. The ^{177}Lu -ZL showed significant uptake in the bone, which was scintigraphically confirmed after whole body scanning. Due to better physical properties of ^{177}Lu as compared to Sm-153 and acceptable biodistribution of the compound, ^{177}Lu -ZL seemed to be an interesting new candidate for bone pain palliation therapy of skeletal metastases.

Keywords : Zoledronic acid; ^{177}Lu -ZOL; Bone metastases; Bone pain palliation

Introduction

A larger percentage of patients suffering from primary breast, lungs and prostate cancer developed metastasis in bones in the advance stage of their diseases. Bone metastases occur frequently in 70% of patients with breast or prostate cancer and in 40% of patients with lung cancer [1,2], while the bone pain is the most prominent symptom associated with bone metastases. The pain becomes progressively severe as the disease advances. Targeted radiotherapy is considered a standard treatment in the management of bone metastasis using suitable radionuclides linked to bone seeking ligands [3-9]. Targeted radionuclide therapy, involves the specific deposition of ionizing radiation at the skeletal with the minimum radiation induced bone marrow suppression, and found to be an effective treatment for the palliation of pain [10,11]. A great deal of effort has been made in identifying a potential therapeutic radionuclide used for palliation of bone metastasis with more favorable decay characteristic [12-14]. In order to develop effective radiopharmaceuticals for bone pain palliation, it is essential to carefully consider the choice of appropriate radionuclides as well as the carrier moiety with suitable pharmacokinetic properties that could result in good in- vivo localization in bone and concentrate in skeletal lesions, with minimum exposure to red bone marrow.

Bisphosphonate (BPs) ligands are known to form stable chelates with many metals including lanthanides and act as carrier ligands



owing to their high bone affinity and selective localization in skeletal lesions. Ethylenediaminetetramethylene phosphonic acid (EDTMP) is one of the most commonly used bisphosphonate ligands which form stable complexes with different radio metals, particularly with ^{177}Lu (^{177}Lu -EDTMP), has been utilized to palliate metastatic bone pain as a new bone seeking radiopharmaceutical [15]. BPs with an imidazole ring shows higher affinity for bone mineral and zoledronic acid (ZOL) [2-(imidazole-1-yl)-hydroxy-ehtylidene-1,1-bisphosphonic acid], Figure 1 is a bisphosphonate containing imidazole ring and the most potent of the clinically tested compound, could be chosen as another carrier ligand in developing bone pain palliation agent using radio lanthanides. ZOL can reduce the incidence and delay the onset of skeletal complication in patients with breast cancer, multiple myeloma, prostate cancer and other solid tumors. The complex of ZOL and its derivatives with $^{99\text{m}}\text{Tc}$ have been extensively studied as potential bone imaging agents [2,16-18].

^{177}Lu is presently being considered as one of the most promising radionuclide for targeted palliative care in painful bone metastasis owing to its suitable decay characteristics [$T_{1/2}=6.73$ d, E_{β}

(max)=497 keV, E_{γ} =113 keV (6.4%), 208 keV (11%)]. ^{177}Lu decay to stable ^{177}Hf , and its long half-life provides logistic advantage for facilitating supply to places far away from the reactor. The high cross section of the target radionuclide [$^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ =2100barns] allows large scale production using moderate flux reactors. Additionally, its 208-keV γ -emission (11% abundance) allows for imaging of its distribution to facilitate dose calculation [19-21].

Keeping in view the specific advantages of using ^{177}Lu in palliative radiotherapy of bone pain, the aim of this study was to develop new ^{177}Lu -ZOL bone seeking radiopharmaceutical used in the bone pain palliation. The present work describes the preparation of ^{177}Lu -ZOL complex, and imaging studies to evaluate its efficacy as novel bone pain palliation bone seeking radiopharmaceutical.

Experimental

Materials and methods

Enriched $^{176}\text{Lu}_2\text{O}_3$ (purity > 99.99%, ^{176}Lu ~52.10%) used as target for the production of $^{177}\text{LuCl}_3$ was obtained from Trace Science International Canada. ZOL (99.6% pure) used was purchase form Molekula Limited, UK. All other chemicals are of analytical grade and used without further purification. What man 3MM chromatographic paper (UK) and ITLC-SC (Gelman Sciences Institute USA), were used for radio chromatography. Radio-chromatography were performed by thin layer chromatography scanner Bio scan Inc., USA connected with NaI(Tl) detector. High performance liquid chromatography (HPLC) studies were conducted using Hitachi L6200 HPLC system with NaI(Tl) detector integrated with Bio scan Chrom Lite.

The radionuclide purity of $^{177}\text{LuCl}_3$ was determined by γ -ray spectrometer, which consisted of p-type coaxial high purity germanium (HPGe) detector having 60% relative efficiency and 1.95 FWHM at 1,332 KeV γ -ray of ^{60}Co . The detector was connected to Ortech-570 amplifier Trump PCI 8K ADC/MCA card with Gamma Vision-32 ver. 6 software. Full peak energy calibration of the detector was performed using point calibration source ^{152}Eu covering the energy range 59-1, 480KeV. All other activity measurement were made with NaI(Tl) scintillation detector (Capintech, Inc) calibrated with 3.7 MBq ^{137}Cs standard source.

Scintigraphic images of whole body were recorded using dual-head-digital single photon emission computed tomography (SPECT) gamma camera (Siemens Ecam, USA) integrated with Esoft Syngo software at Gujranwala Institute of Nuclear Medicine (GINUM) Gujranwala, Pakistan. The rabbit was placed on a flat hard surface with both hind legs spread out and all legs fixed with surgical tape, then an aliquot of 0.2 ml containing 15 MBq of ^{177}Lu -ZOL was injected intravenously into the marginal ear vein of rabbit. Diazepam (5 mg) was used for anesthazing rabbits before recording the scintigraphic images.

Production of $^{177}\text{LuCl}_3$

$^{177}\text{LuCl}_3$ was produced by irradiation of 1 mg of enriched $^{176}\text{Lu}_2\text{O}_3$ in thermal neutron flux of $\sim 1.2 \times 10^{14}$ $\text{ncm}^{-2}\text{s}^{-1}$ at (PARR-I). First, $^{176}\text{Lu}_2\text{O}_3$ was transfer into quartz ampoule, and sealing of ampoule was done with oxyacetylene. Sealed quartz ampoule containing $^{176}\text{Lu}_2\text{O}_3$ was shifted into aluminum capsule, which was cold welded using carver press at 1400 psi pressure. The target was subjected to irradiation at PARR-I for 12 hr. After cooling of 8 hr, the irradiated target dissolved in 5ml of 1.0 mol L^{-1} HCl with few minutes of heating

at 80 °C in a hot cell. The $^{177}\text{LuCl}_3$ diluted to 10ml with double distilled water for preparation of stock solution. The mixture was filtered through 0.22 μm Millipore filter for sterilization. Total activity produced was measured with dose calibrator.

The radiolabeling of ^{177}Lu -ZOL complex

In order to attain maximum radiochemical purity (%RCP), many factors were investigated such as the amount of ligand (ZOL), amount of $^{177}\text{LuCl}_3$, pH value and the incubation time. The radiolabeling of ZOL with $^{177}\text{LuCl}_3$ was carried out in 10 ml vial.

Effect of amount of the ZOL

A stock solution of ZOL was prepared by dissolving 100 mg of ZOL in double distilled water by adding few drop of 1.0 molL^{-1} sodium hydroxide in order to obtain a concentration of 4 mg mL^{-1} . An aliquot of ZOL solution containing various concentrations (2.0-14 mg), with addition of 2 mg, was placed in a reaction vials respectively, 185MBq of activity of $^{177}\text{LuCl}_3$ was also added and pH of the solution was adjusted to 7-8 with phosphate buffer.

Effect of amount of $^{177}\text{LuCl}_3$

The fixed amount (12 mg) of ZOL was labeled with $^{177}\text{LuCl}_3$, which varied from 18.5 MBq to 222 MBq. The pH of the solution adjusted to 8 with phosphate buffer and %RCP was measured after 30 min. A high RCP (>99%) was obtained when 55MBq $^{177}\text{LuCl}_3$ was used. As the amount of $^{177}\text{LuCl}_3$ was increased, no significant difference was observed.

Effect of pH value

Fixed amount of ZOL (12 mg) and 185MBq activity of $^{177}\text{LuCl}_3$ was added into 10 reaction vials. The pH value were adjusted from 2-12 with phosphate buffer. After incubation time of 30 minute the %RCP were checked using ITLC.

Effect of incubation time

After fully overtaxing the mixture containing 12mg ZOL, 185MBq activity and pH 8 the %RCP was checked for different time starting from 10 minute up to 7 hours.

Quality control of ^{177}Lu -ZOL

Instant thin layer chromatography (ITLC)

Radionuclide purity as well as radiochemical purity was determined by Instant thin layer chromatography (ITLC) and paper chromatography (PC). An aliquot of ^{177}Lu -ZOL were spotted with a syringe at 2 cm from the bottom on 2 \times 14 cm strips. Strips were developed into mobile phase chamber containing ammonium hydroxide:methanol:water (1:2:2) as eluting solvent. The chromatogram were eluted up to 10cm, dried and subjected to 2 π scanner to get actigram which show the radiochemical purity.

High performance liquid chromatography (HPLC)

To verify the complex formation of single species, one of the reaction mixture was analyzed by HPLC using reversed phase C_{18} column waters (3.9 \times 300 mm) attached to NaI(Tl) detector. HPLC was performed using a mixture of water:methanol (2:3) as eluent. Initially, 20 μl of $^{177}\text{LuCl}_3$ at pH 7 [1 ml LuCl_3 =5 mCi] was injected

into the column and elution was monitored by activity profile. Similarly, 20 μl of test solution [1 ml ^{177}Lu -ZL=5mCi] was injected into the column and elution was monitored. Chromatogram was obtained using Bioscan Chrom Lite software attached to Hitachi L6200 HPLC system.

Images study in rabbit:

Dual-headed single photon emission computed tomography (SPECT) gamma camera was used to obtain the images of rabbit. ^{177}Lu -ZOL (~15MBq) was injected intravenously into the rabbit through the ear vein. Next day, before imaging procedure the rabbit was anesthetized. After 30 minute the rabbit was fixed on a board covering with piece of cloth for immobilization during the entire scanning procedure and whole body images were obtained at 24 h, 2.5 d and 7 d post injection using SPECT gamma camera.

Results and Discussion

Production of $^{177}\text{LuCl}_3$:

Irradiation of $^{176}\text{Lu}_2\text{O}_3$ at thermal flux of $\sim 1.2 \times 10^{14} \text{ ncm}^{-2}\text{s}^{-1}$ for 12 hr has resulted $^{177}\text{LuCl}_3$ with specific activity of 11.1 GBq/mg (300 mCi/mg) at 8 hour after the end of bombardment (EOB). Radionuclide purity was checked using HPGe detector using γ -ray spectrum. Analysis of γ -ray spectrum revealed different peaks at 72, 113, 208 and 250 keV, which correspond to photo peaks of ^{177}Lu . The radionuclide purity was found to be >99%.

Radiolabelling of ^{177}Lu -ZOL

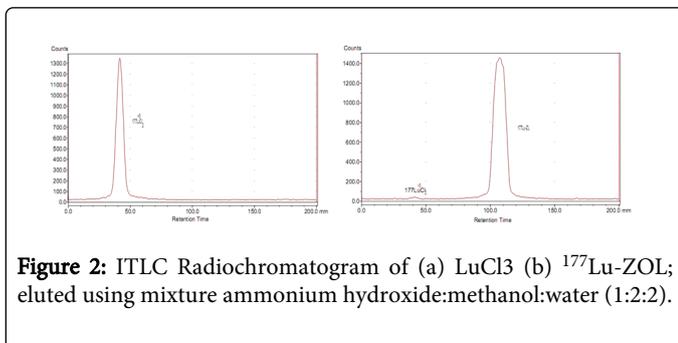


Figure 2: ITLC Radiochromatogram of (a) LuCl_3 (b) ^{177}Lu -ZOL; eluted using mixture ammonium hydroxide:methanol:water (1:2:2).

After drying the developed Whatman 3MM and ITLC strips, it was subjected to 2 π scanner to get actigram which depicted the %RCP of the ^{177}Lu -ZOL. The labeling yield was found to be 99.10 ± 0.40 . ^{177}Lu -ZOL move with solvent front with $R_f=0.7 \pm 0.03$, while free $^{177}\text{LuCl}_3$ remained at the point of spotting ($R_f=0.0-0.1$) shown in the Figure 2a, 2b.

Elution was repeated thrice for calculating %RCP and R_f values. Optimization of radiolabeling condition of ^{177}Lu -ZOL was performed by varying several reaction parameter, such as the amount of ZL, amount of $^{177}\text{LuCl}_3$, pH value and incubation time.

Effect of amount of ligand ZOL

A aliquot of ZL solution at various concentrations, 2, 4, 6, 8, 10, 12 and 14 mg were taken in a reaction vial and 185MBq of activity was added, pH of the solution were adjusted to 7-8. %RCP of these formulations were determined to be 88.01 ± 0.9 , 91.6 ± 0.76 , 94.2 ± 1.0 , 95.1 ± 0.16 , 98.1 ± 0.3 , 99.1 ± 0.5 , 99.1 ± 0.6 respectively. The results

are shown in Figure 3a, which signify that when amount of the ligand ZL was 12 mg or more, the overall labeling yield was >99%.

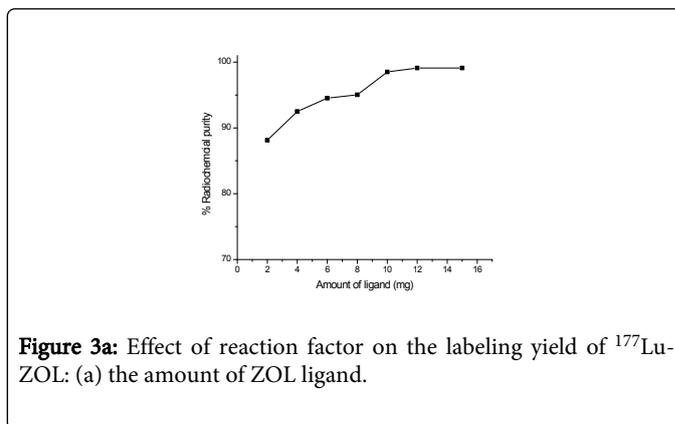


Figure 3a: Effect of reaction factor on the labeling yield of ^{177}Lu -ZOL: (a) the amount of ZOL ligand.

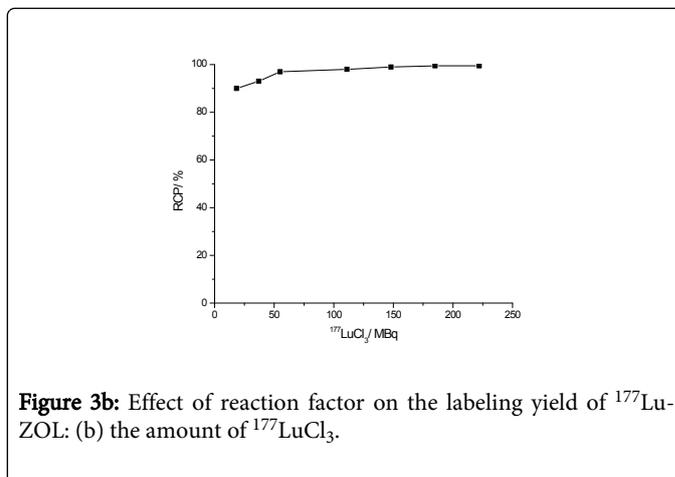


Figure 3b: Effect of reaction factor on the labeling yield of ^{177}Lu -ZOL: (b) the amount of $^{177}\text{LuCl}_3$.

Effect of amount of $^{177}\text{LuCl}_3$:

Reaction vials containing fixed amount of ZOL (250 μl , 12 mg) and various amounts of $^{177}\text{LuCl}_3$, 18.5 MBq, 37 MBq, 55 MBq, 111 MBq, 148 MBq, 185 MBq, 222 MBq, and pH of the solutions were adjusted to 7-8. The %RCP of each reaction vial were checked after 30 min and found to be 89.5 ± 0.5 , 93.0 ± 0.2 , 96.5 ± 0.4 , 98.4 ± 0.9 , 98.9 ± 0.1 , 99.3 ± 0.35 , 99.2 ± 0.8 respectively shown in Figure 3b. The results suggested that activity of 111MBq above has no significant effect on radiolabeling yield of complex.

Effect of the pH value

Fixed amount of ZOL (250 μl , 12 mg) and 185 MBq activity of $^{177}\text{LuCl}_3$ were added into cylindrical vials. The pH values of vials were adjusted 2-12 respectively with Phosphate buffer. The mixture was reacted at room temperature for 30 min. The results shown in Figure 3c indicate that when the pH value is the range of 7-8, an RCP>99% can be obtained.

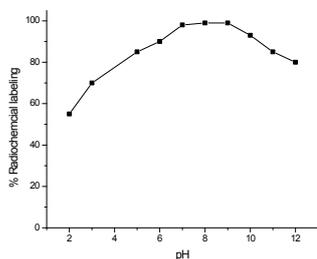


Figure 3c: Effect of reaction factor on the labeling yield of ^{177}Lu -ZOL: (c) The pH value.

Effect of incubation time

One of the reaction mixture at 8 pH value, containing (250 μl , 12 mg) ZOL and 185 MBq of $^{177}\text{LuCl}_3$ activity at room temperature, the %RCP was checked after regular time interval ranging from 10 min to 7 hours. The results shown in Figure 3d, the high RCP >99% was obtained at 30 min after which no significant change was observed.

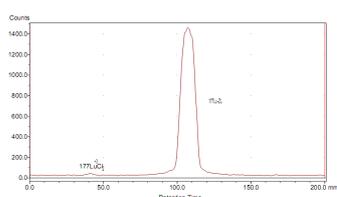


Figure 3d: Effect of reaction factor on the labeling yield of ^{177}Lu -ZOL: (d) The incubation time.

HPLC studies:

The HPLC chromatogram in Figure 4a,4b clearly showed two distinct peaks at different retention time. The first peak belongs to Free $^{177}\text{LuCl}_3$, whereas second peak correspond to ^{177}Lu -ZOL complex, depicting the formation of complex with single species. The results obtained from HPLC are in comparison with ITLC.

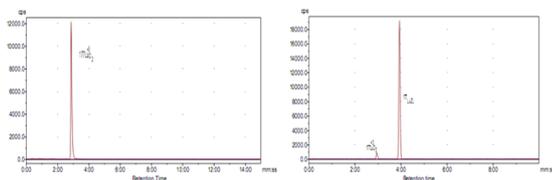


Figure 4: HPLC radiochromatogram of (a) free $^{177}\text{LuCl}_3$ (b) complex ^{177}Lu -ZOL; on reverse phase column using ethanol:water (3:2) as mobile phase.

Imaging study in rabbit:

Whole body images of normal rabbits at 1 d, 2.5 d and 7 d after ^{177}Lu -ZOL administration are presented in Figure 5a,5b,5c. ^{177}Lu in rabbit skeleton was visualized after accumulation of injected labeled zoledronate. The tracer is clearly visible in skeleton at 1 d and 2.5 d post administration with slight activity in upper part of body, whereas at 7 d, due to clearance of activity from other organs, the skeleton image is dominant. Table 1 presents the activity ratio of bone to kidneys and upper part of the body. The bone uptake is quite high, and ratio increases with lapse of time.

Ratio	1 Day	2.5 Day	7 Day
Bone/LK	10.87	10.76	12.29
Bone/RK	12.52	12.76	15.66
Bone/U&B	5.71	10.42	14.72
Bone/Lungs	5.14	8.54	15.07

Table 1: Digital data of ^{177}Lu -ZOL distribution in rabbit. LK= Left kidney, RK = Right Kidney, U&B= Urinary Bladder.

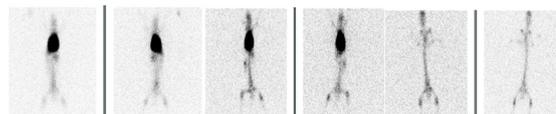


Figure 5: SPECT whole body images (a), (b) and (c) of rabbit recorded at 1 d, 2.5 d, and 7 d, post injection ^{177}Lu -ZOL, respectively showing skeletal uptake of the therapeutic radiopharmaceutical (Activity of ^{177}Lu -ZOL = ~15MBq) (A= anterior and B= posterior).

Currently, for the examination/imaging of bones $^{99\text{m}}\text{Tc}$ phosphate complexes such as $^{99\text{m}}\text{Tc}$ -PyP (pyrophosphate), $^{99\text{m}}\text{Tc}$ -MDP, $^{99\text{m}}\text{Tc}$ -HEDP (etidronat), $^{99\text{m}}\text{Tc}$ -EDTMP (oksabifor) were evaluated, but the biggest interest in the radionuclear diagnosis of the skeleton is given to the bisphosphonate of the latest generation – zoledronic acid (which is successfully used for treatment of bone metastases). Zoledronic acid has maximum affinity to the areas of high metabolism and accelerated resorption in the bone tissue [17]. The $^{99\text{m}}\text{Tc}$ -ZOL is used for finding centers of pathologic changes of different origin and dissemination in the skeleton: primary and metastatical malignant tumors, osteomyelitis, bone-joint tuberculosis, arthritis of different origin and others.

The high affinity of biphosphonates towards bone is based on their ability to become incorporated into the hydroxyapatite crystal by chemisorption on to the surface of bone. $^{99\text{m}}\text{Tc}$ -ZOL and $^{99\text{m}}\text{Tc}$ -MDP were administered intravenously to the rabbits for scintigraphic studies. Between $^{99\text{m}}\text{Tc}$ -ZOL and $^{99\text{m}}\text{Tc}$ -MDP, there were no significant differences in the ratios of femur/back ground (BG) and lumbar vertebrae/BG, whereas epiphysis/BG and the kidney/BG ratios of $^{99\text{m}}\text{Tc}$ -MDP were higher than $^{99\text{m}}\text{Tc}$ -ZOL in the statics imaging studies [2].

Lanthanides (Lu is a member) are usually most stable in solution as trivalent ions (M^{3+}) with the exception of cerium and europium which can exist as quadrivalent and bivalent species. Lanthanides in aqueous

solution bind to water molecule, and due to their large size, their coordination number are usually 7 and 10. Very few six coordinate species are known, while coordination numbers of 8 and 9 are the most common. Lanthanides form stable complexes with nitrogen or oxygen donor chelators. Figure 6 shows the predicted structure of ^{177}Lu -ZOL. In summary, the rabbit SPECT imaging results shows that ^{177}Lu -ZOL possess excellent characteristics for the promising application as a novel bone therapeutic radiopharmaceutical.

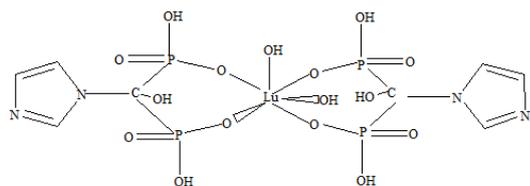


Figure 6: Proposed structure of ^{177}Lu -ZOL.

Conclusion

Optimization of reaction conditions for preparation of ^{177}Lu -ZOL having a radiochemical purity of 99% has been achieved. The complex was found stable up to 7 hours. Scintigraphic studies in rabbits show high uptake of complex in skeleton up to 7 days, the duration of studies. Due to better physical properties of ^{177}Lu compared to ^{153}Sm (FDA, USA approved drug ^{153}Sm -EDTMP) and acceptable biodistribution results of ^{177}Lu -ZOL seemed to be a potential new candidate for clinical trials for bone pain palliation therapy.

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