Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2011, 3(3):140-148
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Cyclam based hybrid compound for imaging and therapy of Bone Metastasis

Tyagi Amit Kumar[1*], Sharma Rajbala[1], Sharma Himanshu[2], Katara Antariksh[3]

Meerut College, CCS University, Meerut, India
Meerut Institute of Engineering and Technology, Meerut, India
Rameesh Institute of Voc. & Tech. Education, Greater Noida, India

---

ABSTRACT

Present study shows the synthesis of 7 however, an alternative route was used in this work, in which a commercially available intermediate, 5 (1,4,7,10-tetraazacyclododecane-1,4,7- tris(t-butyl)acetate: DO3A-t-Bu-ester) was used as starting material instead of cyclam and a higher overall yield (77%) was achieved. A convergent approach was chosen to synthesize the hybrid ligand, in which the derivatives of a bisphosphonate and Cyclam metal chelator (7) were synthesized. The carboxylate acid substituted bisphosphonate, 3-bis-(dibenzyloxyphosphoryl) propanoic acid (3), was synthesized by a literature procedure with an overall yield of 27%. Blood kinetics findings in rabbits are an indication of the excellent in vivo stability of the ^{99m}Tc-AI and non-osseous tissue uptake of ^{99m}Tc-AI is minimal and when assessed with its rapid blood clearance accounts for the low soft tissue activity. Rapid incorporation of phosphonates into calcified tissue leads to their short presence in the circulation. By Receptor binding studies of Scatchard plot analysis revealed affinity of the AI on tumor cell lines (Soas-2) and Scintigraphy observed that the uptake of the conjugate was faster than that of the ALN and MDP alone.

INTRODUCTION

The connective tissue provides an internal support system in all vertebrates. It is also the major source of inorganic ions and actively participates in calcium homeostasis in the body (1). To maintain its normal function, bone, a specified form of connective tissue is continuously being resorbed and rebuilt throughout the skeleton. In healthy individuals, bone resorption and formation are well balanced with the bone mass maintained in a steady state. A number of bone diseases including osteoporosis, Paget's disease, osteopetrosis, bone cancer, etc. occurs due to imbalances in this homoeostasis (2). Over the past decade, our understanding of bone biology has improved dramatically (3, 4). Many molecules have been identified as new therapeutic agents for the treatment of bone disease, out of these most important is bis-phosphonate, which are analogs of inorganic pyrophosphate that are taken up by bone, bind to the bone mineral surface, and inhibit osteoclastic bone resorption (5). Sodium alendronate is a potent inhibitor of bone resorption that has been extensively studied in the treatment of postmenopausal

osteoporosis and in the prevention of postmenopausal bone loss (6–9), which can be utilized for the enhancement of bone mineral density (BMD) of the spine, hip, and total body. (6, 10–13) These changes in bone density are associated with a significant reduction of fracture incidence at both vertebral and non-vertebral sites including the hip (6, 7, 14–16).

Bisphosphonates are used clinically as skeletal markers in the form of ^{99m}Tc derivatives, with nuclear medicine applications. Skeletal localization of phosphonates based bone imaging agents depends on several factors including bone–blood flow, enzyme activity (especially alkaline phosphatase), and active bone mineralization of hydroxyapatite crystals. Radiotracers have reported to be localized in these sites of increased mineralization activity in disease states. Amorphous calcium phosphate present in many bone lesions in high concentrations, which has a greater affinity for bone imaging agents, is also known to play a part in radionuclide accumulation (17–20). The –OH on the phosphonates groups are suggestive of the bone matrix affinity (21). There are other mechanisms of localization which involves calcium fixed to mitochondria rendering site for the deposition of the bone imaging agents are reported in the literature (22-24) Phosphonic acid derivatives and agents more elaborate derivatives are widely used as complexing for calcium, zinc, (25) and/or a number of bivalent metals (26). They are involved in a large number of biochemical processes and disease treatments or diagnosis (scintigraphy in the case of ^{99m}Tc) (27–31) and therapy (^{90}Y, ^{153}Sm, and ^{188}Re) (34-36). Thus, the binding of polyphosphates and bisphosphonates to calcified tissues is the basis for the use of these compounds as skeletal markers in nuclear medicine when linked to ^{99m}Tc. ^{99m}Tc-labeled compound used clinically in nuclear medicine are methylenediphosphonate (MDP), dicarboxypropanediphosphonate (DPD), hydroxyethylidine diphosphonate (HEDP) and ethylenediaminetetramethylene phosphonate (EDTMP) (30).

Bisphosphonates are a group of compounds with a chemical structure similar to that of the natural inorganic pyrophosphate (PPi), an endogenous regulator of bone mineralization, but differing in the central atom where BPs have a methylene carbon rather than an oxygen atom in PPi. This structural feature renders BPs resistant to hydrolysis under acidic conditions or by pyrophosphatases. Varieties of BPs could be obtained by tuning the R2 side chain while leaving the R1 group intact as either –OH or –H (31). It has recently become clear that BPs first bind avidly to the bone mineral surface and are subsequently internalized selectively by osteoclasts, where they inhibit the osteoclastic activity and induce apoptosis. In addition, BPs have been found to inhibit tumor cell adhesion to mineralized bone as well as tumor cell invasion and proliferation (33-34).

The binding of the hydroxyl groups of BPs to Ca^{2+} in hydroxyapitate of bone is responsible for the accumulation of BPs in bone. However, it reduces the coordination sites of 99mTc-MDP in vivo and subsequently 99mTc-MDP decomposes into 99mTcO4 and BP components. Therefore the bone uptake of 99mTc-MDP is mainly dependent on the osteoblastic activity, and the purely osteolytic lesion is poorly detectable (35). DOTA (1, 4, 7, 10-tetraazacyclododecane- N, N0, N00, N000-tetraacetic acid) is a commonly used bifunctional chelator for radioimmuno diagnosis and radioimmuno therapy because itis able to form thermodynamically stable and kinetically inert complexes with many divalent or trivalent metal ions (36–37). To date, few conjugates of DOTA and BPs have been reported but recent research has focused on the improvement of the binding affinity of BP to bone for the applications as delivery vehicles of MRI contrast agents and palliation agents for certain bone diseases (38-39).

MATERIALS AND METHODS

All chemicals used in present study of analytical grade purchased from Sigma, Aldrich and Merck chemical Co. All the solvents were used after distillation. TLC was run on the silica gel coated aluminium sheets (silica gel 60 F_{254}, E Merck, Germany) and visualized in UV light. Melting points were determined by using Thomas Hoover apparatus and are uncorrected. IR spectra were recorded on the FT-IR perking Elmer spectrum BX spectrophotometer. NMR spectra were obtained by using Brucker NMR instrument 400 MHz. The FAB-MS spectra were recorded from JEOL SX 102/DA-6000 spectrometer using m-Nitro benzyl alcohol as matrix. EI-MS spectra were recorded on a JEOL SX102/DA (KV 10 mA) instrument. Elemental analysis was done on elementar analysen system Gmbh variable system. Radio complexation and radio chemical purity was checked by instant strip chromatography (silica gel impregnated paper chromatography) with IILC-SG (Gelman sciences, Ann arbar, MI, USA)

Synthesis of Hybrid Cyclam

Compound 3:- To a suspension of methylene bis (phosphonic dichloride) (5.00 g, 20.0 mmol) in dry toluene (10 mL), a mixture of benzyl alcohol (8.70 mL, 84.0 mmol) and pyridine (6.79 mL, 84.0 mmol) was added drop wise by an addition funnel while the temperature was maintained at 0 ^{0}C. The reaction was allowed to reach room temperature and the mixture was stirred for 16 h. The solids formed during the reaction were removed by filtration and washed twice with toluene. The filtrate was washed twice with 2 M NaOH and then once with water. After the removal of bulk solvent, the residue was submitted to column chromatography on silica gel eluting with 100% EtOAc. Evaporation of appropriate fractions afforded 1 as colourless oil.

Take Compound 1(5.80 g, 10.8 mmol) in THF (50 mL) was slowly added to a suspension of NaH (0.27 g, 11.3 mmol) in THF (100 mL) at 0^{0}C. After the addition was completed, the reaction mixture was allowed to reach 20 ^{0}C and stirred for 30 min. Ethyl bromoacetate (1.91 g, 11.4 mmol) was then added. A white precipitate appeared during the addition. After the mixture was stirred for 48 h, the white solids were removed by filtration and the concentrated residue was submitted to column chromatography on silica gel eluting with EtOAc/Hexane (v/v: 7:3). Evaporation of appropriate fractions gave 2 as colourless oil. A solution of 2 (4.1 g, 6.60 mmol) in methanol (20 mL) was added to 40 mL of a KOH solution (0.46 g, 8.21 mmol) in 50% aq. methanol at 0 ^{0}C. The mixture was stirred at room temperature until 2 disappeared as monitored by TLC. After removal of methanol, the residual was extracted with ethyl ether. The aqueous layer was acidified to pH 2 by adding aqueous $KHSO_4$ (1 M) and then extracted with of $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated in vacuum to give 3 as colourless oil (over all yield 27%): 1H NMR ($CDCl_3$, 500 MHz): d 2.90 (2H, td, J = 6.5, 16.5 Hz), 3.28 (1H, tt, J = 5.5, 24.0 Hz), 4.95–5.05 (8H, m), 7.20–7.35 (20H, m). MS (MALDI-TOF) 617.6 (M+Na+).

Compound 7:- Compound 4 (11.52 g, 19.36 mmol) was dissolved in 100 mL of acetonitrile containing 2 equiv of K_2CO_3. The resulting mixture was stirred at 50^{0}C for 16 h. After removal of the solids, the filtrate was dried under vacuum. The residue was redissolved in $CHCl_3$ and washed with water thoroughly. The evaporation of $CHCl_3$ afforded 5 in quantitative yield. To 50 mL of 5 (5.26 g, 10.2 mmol) in acetonitrile, K_2CO_3 (3.37 g, 24 mmol) was added followed by methyl chloroacetate (1.14 g, 10.5 mmol) in 5 mL of acetonitrile. The resulting mixture was stirred at 55 ^{0}C for 2 days. After removal of the solids and evaporation of the solvent, the residue was redissolved in $CHCl_3$ and washed thoroughly by water. The organic layer was then dried over sodium sulphate. Removal of the solvent under vacuum gave 6 as brown oil, which was used for the next step without purification. A fivefold excess of ethylenediamine was cooled in an ice bath and then mixed with a concentrated solution of 6 in THF. The reaction mixture was

stirred at room temperature for 72 h. After evaporation of THF, the excess amine was distilled off at 50 ^{0}C under high vacuum. Compound 7 was obtained as white foam with an overall yield of 77%, which showed high chemical purity as evidenced by: 1H NMR ($CDCl_3$, 400 MHz) d 1.45 (27 H, s), 2.52–3.33 (30H, m), 8.76 (1H, br); 13C NMR ($CDCl_3$, 100 MHz) d 28.45, 42.33, 43.03, 52.22, 52.71, 53.79, 55.15, 56.50, 57.15, 58.42, 81.10, 81.22, 170.84, 170.87, 172.68; MS (MALDI-TOF) 615.8 (M+H+).

Compound 8:- In a RBF, compound 7 (200 mg, 0.33 mmol), compound 3 (212 mg, 0.36 mmol), DCC (74 mg, 0.36 mmol), and HOBt (48 mg,0.36 mmol) were stirred in 20 mL of dry CH_2Cl_2 while cooling in an ice bath.

The mixture was then allowed to stand at room temperature with constant stirring for 48 h. The precipitate, dicyclohexylurea, was filtered off, and the filtrate was washed sequentially with aqueous $KHCO_3$ solution 3 times and brine 1 time, and concentrated under reduced pressure to give white foam. The residue was purified by column chromatography (silica gel 60–230 mesh) using 100% EtOAc to 10:1 $CHCl_3$/MeOH for elution.

Compound 8 is found as a sticky oil (192 mg, 50%): 1H NMR ($CDCl_3$, 500 MHz) d 1.36 (27H, multiple),1.80–3.45 (28H, br), 3.70 (1H, tt, J = 6.0, 24.0 Hz), 4.97 (8H, m), 7.18 (20H, m),8.71 (1H, br), 8.84 (1H, br); 13C NMR ($CDCl_3$, 100 MHz) d 28.08, 28.17, 31.61 (t,J = 4 Hz), 33.08 (t, J = 135 Hz), 39.54, 46.45, 48.71 (br), 52.23 (br), 55.69, 55.72,55.83, 56.26, 68.17, 68.23, 82.00, 128.14, 128.52, 128.63, 136.58, 169.81 (t,J = 8 Hz), 171.67, 172.55; 31P NMR ($CDCl_3$, 162 MHz): d 25.89 (s); Anal. Calculated for C61H88N6O14P2_6.5H_2O: C 55.99, H 7.78, N 6.42. Found: C 55.80, H 7.62, N 6.68; MS (MALDI-TOF): 1191.6 (M+).

Compound 9:- Compound 8 (40 mg, 0.03 mmol) was dissolved in 3 mL of a mixed solvent of trifluoroacetic acid (TFA) and $CHCl_3$ (v/v: 1:2). The reaction mixture was stirred at room temperature overnight. After removal of bulk solvent under reduced pressure, the residue was dissolved in deionised water and then mixed with 40 mg of Pd/C. The reaction was allowed to proceed in a hydrogenation shaker for 24 h. After removal of the solids, evaporation of water under vacuum gave the target compound (9) as a colourless thick oil in nearly quantitative yield: 1H NMR (D_2O, 400 MHz) d 4.02–2.50 (br); 13C NMR (D_2O,100 MHz): d 31.14, 32.35, 33.93, 35.17, 36.41, 38.96, 39.0, 46.0–52.10 (br), 52.10–54.24 (br), 55.0, 174.10; 31P NMR (D_2O, 162 MHz): d 20.54. MS (MALDI TOF) 701.8 (M+K+).

Chemical Scheme for synthesis noted in Scheme 1 and 2.

Bone Metastasis Evaluation Technique:

DTPA challenge. ^{99m}Tc-AI and ^{99m}Tc-MDP (300 MBq) were incubated at different concentration 25, 50, and 100 mM DTPA, and maintained at 37°C up to 24 h. periodically, samples were removed, spotted on a 10-cm ITLC-SG strip, and developed in 0.9% NaCl. Once the solvent front had reached the end of the strip, it was removed from the solvent and cut at R_f of 0.5. The two portions were assayed for radioactivity, and the amount of intact chelate was determined.

In-Vitro Serum Stability Assay: The fresh human serum was prepared by allowing blood collected from healthy volunteers to clot for 1h at 37°C in a humidified incubator maintained at 5% carbon dioxide, 95% air. Then the sample was centrifuged at 400 rpm and the serum was filtered through 0.22 micron syringe filter into sterile plastic culture tubes. The above freshly prepared technetium radio complex were incubated in fresh human serum at physiological conditions i.e. at 30°C at a concentration of 100 nM/mL and then analyzed by instant thin layer

chromatography (ITLC-SG) at different time intervals to detect any dissociation of complex. Percentage of free pertchnetate at a particular time point that was estimated using Saline and Acetone as mobile phase, represented percentage dissociation of the complex at that particular time point in serum.

Blood kinetic studies: In normal rabbit weighing about 2 to 2.5 kg, 10 MBq of the ^{99m}Tc AI was administered intravenously through the dorsal ear vein of the animal. At different time interval starting from 5 min to 24 h persistence of the activity in terms of percentage administered dose in samples at different time intervals was calculated using gamma counter.

Receptor binding studies. Exponentially growing cells 0.1 x 10^6 cells/PD (Soas-2) were plated at a uniform cell density and incubated overnight. Monolayer culture (Soas-2) of the cell lines were washed twice for 2 minutes with ice cold binding buffer (25 mM HEPES, 10 mM $MgCl_2$ and 1% BSA). The cell line culture were then incubated for 40 min with labelled AI (1 nM–8 nM) in the absence and presence of the 100 folds excess unlabeled AI for estimation of total binding and non-specific binding respectively. Specific binding was obtained by subtracting non-specific binding from total binding. At the end of each experiment, the cells were washed with ice cold binding buffer three times for three minutes. The cells were lysed with 200 µL of Lysis buffer. The cell-associated radioactivity was determined by gamma scintillation counting. Scatchard plot analysis was done using EQUILIBRATE software from graph pad found in picture 1.

Receptor-binding analysis

The binding affinity of the compound to the BMP (Bone Morphogenetic Protein) receptor was examined. Various concentration of examined solution in phosphate-buffered saline (100 µM) and BMP-2 (100 µM) were kept in 96-well microtiter plate (Nunc) overnight at 4 ^{0}C. FGF-2 (100 µM) was also coated to compare the specific binding of compound and BMP-2 to the BMP receptor. Wells were blocked with 1% BSA for 2 h at 37 ^{0}C and then further incubated for 4 h at room temperature. The wells were then incubated overnight at 4 ^{0}C with 1:1000 mouse anti-BMPRIA, anti-BMPRII antibody, and anti-FGFR2, followed by reaction with 1:2000 horseradish peroxidise conjugate rabbit anti-mouse IgG (Sigma) for 45 min at room temperature. The bound antibodies were detected using ABTS (2, 2'-Azinobis-diammonium salt, Sigma). The reaction was stopped after 5 min by the addition of 1% sodium dodecylsulfate (SDS), and the absorbance was measured at 405 nm. The wells without peptide coating were blocked with BSA, and the amount of BMP receptor binding was used as a background control presented in Table 1 and 2.

RESULTS AND DISCUSSION

A convergent approach was chosen to synthesize the hybrid ligand , in which the derivatives of a bisphosphonate and Cyclam metal chelator (7) were synthesized separately as shown in Schemes .The carboxylate acid substituted bisphosphonate, 3-bis-(dibenzyloxyphosphoryl) propanoic acid (3), was synthesized by a literature procedure with an overall yield of 27% . The synthesis of 7 however, an alternative route was used in this work, in which a commercially available intermediate, 5 (1,4,7,10-tetraazacyclododecane-1,4,7- tris(t-butyl)acetate: DO3A-t-Bu-ester) was used as starting material instead of cyclam and a higher overall yield (77%) was achieved. Alkylation of 5 with methyl chloroacetate afforded 6, and then 7 was obtained by reacting 6 with excess ethylene diamine. Subsequent conjugation of 3 with 7 via the standard DCC/HOBt procedure (DCC: dicyclo-hexylcarbodiimide; HOBt: 1-hydroxybenzotriazol) (Scheme 2) afforded an orthogonally protected DO3A-BP (8) in a yield of 50%. Compound 8

was obtained with high chemical purity via the column chromatography eluted with $CHCl_3$/MeOH (10:1). The 1H NMR spectrum of 8 showed two well-separated peaks between 8.6 and 9.0 ppm, which can be ascribed to the amide protons on the linkage between the DOTA and BP moieties. A broad peak between 1.8 and 3.5 ppm was observed for the ethylene protons of the macrocycle and it overlapped with the methylene proton signals on the pendent arms. The product, 9 (DO3A-BP), was obtained in nearly quantitative yield after a two-step deprotection, where the t-butyl and benzyl protecting groups were removed by trifluoroacetic acid and Pd/Ccatalyzed hydrogenation, respectively. Bisphosphonates are typically protected in the form of tetra alkyl bisphosphonate esters and deprotection was conducted by either acid hydrolysis or silylation–

dealkylation, however low yields were commonly seen due to the decomposition under the acidic condition of the hydrolysis. Our choice of using benzyl rather than alkyl as the BP protecting group enabled us to selectively deprotect 8 in quantitative yield by two separate procedures, which is advantageous to the formation of macrocyclic metal complexes; and provide a UV chromophore for the monitoring of the synthetic procedures.

Blood kinetics. The blood activity curves in rabbits (Fig. 1) show that the blood clearance of ^{99m}Tc-AI initially is faster than ^{99m}Tc-MDP due to which there was less activity present in the blood at 2 h post-injection. This accounts for high bone/blood and bone/muscles ratio exhibited by ^{99m}Tc-AI, which is comparable to that of ^{99m}Tc-MDP. These findings in rabbits are an indication of the excellent in vivo stability of the ^{99m}Tc-AI. The images also show that non-osseous tissue uptake of ^{99m}Tc-AI is minimal and when assessed with its rapid blood clearance accounts for the low soft tissue activity. Rapid incorporation of phosphonates into calcified tissue leads to their short presence in the circulation.

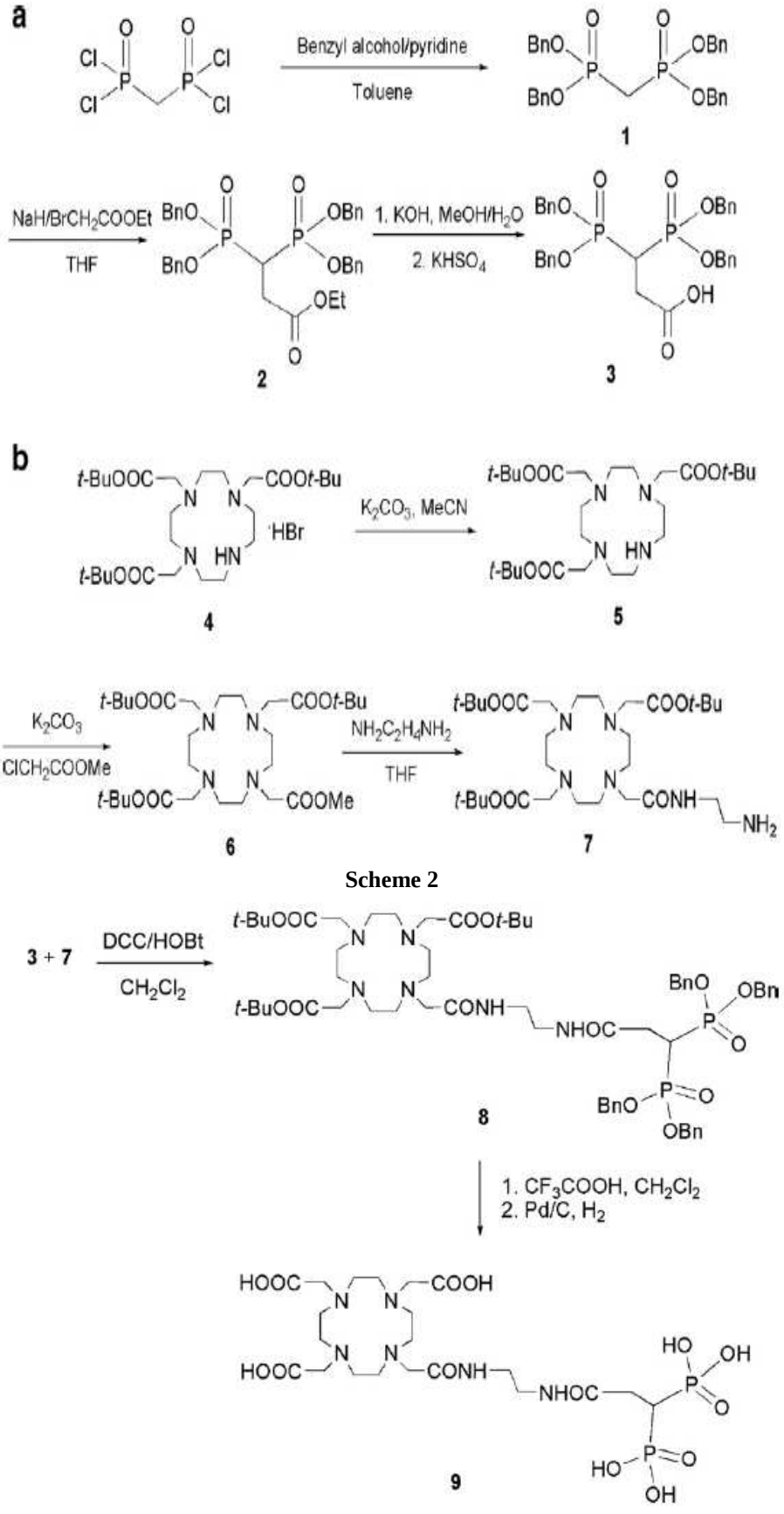
Receptor Binding Studies. Scatchard plot analysis revealed affinity of the AI on tumor cell lines (Soas-2). K_D was found to be 5.5 nM.

Scintigraphy. Localisation of ^{99m}Tc-labeled AI in rabbit over time was determined by gamma camera. Images showed rapid accumulation of radioactivity in bone. Imaging of animals was carried out at different time intervals after administering labelled compound intravenously. With in 30 minutes labelled AI conjugate accumulated in the joints region. After 1.5 h the conjugate was localized in the whole skeleton of rabbit. Accumulation at the head region was very prominent. A comparison study was conducted for the indole derivative (AI) and MDP in normal rabbit of the same age and weight demonstrated comparable quality at 3 h with ^{99m}Tc-MDP and at 1.5 h with ^{99m}Tc-AI post-injection (image not shown). It was observed that the uptake of the conjugate was faster than that of the ALN and MDP alone. Thus it is concluded that the synthesized conjugate was a better/superior bone imaging agent.

Table 1:

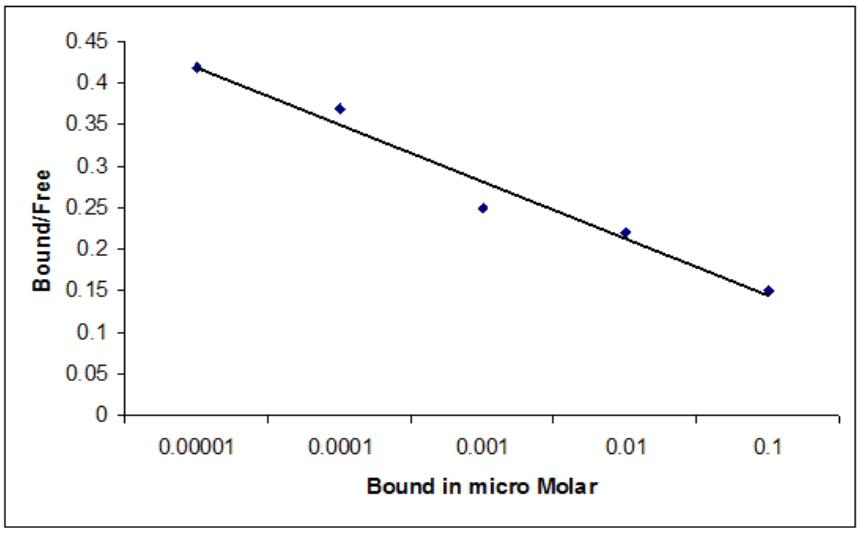
ABS at 405 nm						
----------	Compound (0.1 µM)	Compound (1 µM)	Compound (10 µM)	BMP	FGF	BSA
BMPRIA	0.36	0.39	0.34	0.85	0.28	0.32
BMPRI I	0.42	0.45	0.46	0.96	0.30	0.39
FGFR2	0.27	0.28	0.31	0.25	0.92	0.21

Scheme 1



Scheme 2

Picture 1:



Cell associated ^{99m}Tc radioactivity per 106 cells following incubation of Soas-2 cells with increasing concentrations (0.00001µM – 0.1µM) of 99mTc- AI ; Scatchard Plot of the specific binding data to the ratio of bound to free (B/F) for Soas-2

Table 2: Binding with Soar -2-Cells

Bound in micromolar	Bound/Free
0.00001	0.36
0.0001	0.34
0.001	0.29
0.01	0.24
0.1	0.23
1	0.21

Acknowledgement
The authors are thankful to the V. C. of Meerut University, Meerut and other institutions for providing research facilities to carry out the work.

REFERENCES

[1] Marks SC. Jr., and Odgren, P. R., *Academic Press*, **2002**, 2nd ed. pp 3-15,
[2] Odgren PR, Martin TJ., *Science*. **2000**,289, pp 1508-1514.
[3] Ducy P, Zhang R, Geoffroy V, Ridall, AL. Karsenty G., *Cell*, **1997**, 89, pp 747-754.
[4] Takahashi, N., Udagawa, N., Takami, M. and Suda, T., *Academic Press*, **2002**, 2, pp 109-126.
[5] Fleisch H., *Endocr Rev*, **1998**,19, pp 80–100.
[6] Liberman UA, Weiss SR, Bro¨ll J, Minne H, Quan NH. Bell NH, Rodrigez-Portales J., *N Engl J Med*, **1995**, 333, pp 1437–43.
[7] Black DM, Cummings SR, Karpf DB. Cauley JA. Thompson DE. Nevitt MC. Bauer DC., *Lancet*, **1996**, 348, pp 1535–41.
[8] McClung M, Clemmesen B, Daifotis A, Gilchrist NL, Eisman J, Weinstein RS, El Hajj Fuleihan G, Reda C, Yates AJ, Ravn P., *Ann Intern Med* , **1998**, 28, pp 523–561.
[9] Hosking D, Chilvers CED, Christiansen C, Ravn P, Wasnich R, Ross P, McClung M., *N Engl J Med*., **1998**, 338, pp 485–92.

[10] Adami S, Passeri M, Orolani S, Broggini M, Carratelli L, Caruso I, Gandolini G. *Bone*, **1995**, 17, pp 383–90.
[11]Chesnut CH, McClung MR, Ensrud KE, Bell NH, Genant HK, Harris ST, Singer FR., *Am J Med*, **1995**, 99, pp 144–52.
[12] Devogelaer JP, Broll H, Correa-Rotter R, Coming DC, Nagant De Deuxchaisnes C, Geusens P., *Bone*, **1996**, 18, pp 141–50.
[13] Tucci JR, Tonino RP, Emkey RD, Peverly CA, Kher U, Santora AC., *Am J Med*., **1996**, pp 101, 488–1.
[14] Karpf DB, Shapiro DR, Seeman E, Ensrud KE, Johnston CC, Adami S, Harris ST., *JAMA*, **1997**, 277, pp 1159–64.
[15] Cummings SR, Black DM, Thompson DE, Applegate WB, Barre H, Connor E, Musliner TA., *JAMA*, **1998**, 280, pp 2077–82.
[16] Bergstrom JD, Bostedor RG, Masarachia PJ, Rewszka AA, Rodan GA., *Arch Biochem Biophys*, **2000**, 373, pp 231- 41.
[17] Bisaz, S.; Jung, A.; Fleisch, H. Clin., *Sci. Mol. Med*., **1978**, 54, pp 265.
[18] Jung A, Bisaz S, Fleisch H., *Calcif Tissue Res*, **1973**, 11, pp 269.
[19] Richards AGJ., *Nucl. Med*, **1974**, 15, pp 1057-60.
[20] Fitton A, McTavish D. Pamidronate., *Drugs*, **1991**, 41, pp 289.
[21] Francis MD, Ferguson DL, Tofe AJ, Bevan JA, Michaels SEJ., *Nucl. Med* **1980**, 21, pp 1185.
[22] Mu¨hlbauer RC. Bijvoet, O. L. M., Fleisch, H. A., Canfield, R. E., Russell, R. G. G., Eds., *Elsevier: Amsterdam*, **1995**, pp 171–187.
[23] Jones AG, Francis MD, Davis MA., *Semin. Nucl. Med*, **1976**, 6, pp 3-18.
[24] Rodan GA, Fleisch HJ., *Clin. Invest*, **1996**, 97, pp 2692.
[25] Dufau, C.; Benramdane, M.; Leroux, Y.; El Manouni, D.; Neuman, A.; Prange, T.; Sylvestre, J. P.; Gillier, H., *Relat. Elem*., **1995**, 107, pp 145.
[26] Kubicek, V.; Vojtı´sek, P.; Rudovsky, J.; Hermann, P.; Lukes, I., *Dalton Trans*., **2003**, 20, pp 3927.
[27] Davis M, Jones AG., *Semin. Nucl. Med*, **1976**, 6, pp 19-31.
[28] Arteaga de Murphy, C.; Melendez-Alafort, L.; Montoya- Molina, C. E.; Sepulveda-Mendez, *J. Nucl. Med. Biol.*, **1997**, 24, pp 27–33.
[29] . Volkert, W. A.; Edwards, B.; Simon, J.; Wilson, D. A.; McKenzie, E. H.; Oberle, P.; Holmes, R. A., *Int. J. Radiat. Appl. Instrum*. B **1986**, 13, pp 31.
[30] Pauwels EK, Blom J, Camps JA, Hermans J, Rijke AM., *Eur. J. Nucl. Med* **1983**, 8, pp 118.
[31] . Hosain, F., Spencer, R. P., Couthon, H. M., Sturtz, G. L., *J. Nucl. Med.* **1996**, 37, pp 105.
[32] Clezardin, P.; Fournier, P.; Boissier, S.; Peyruchaud, O., *Curr. Med. Chem.* **2003**, 10, pp 173.
[33] Stresing, V.; Daubine, F.; Benzaid, I.; Monkkonen, H.; Clezardin, P., *Cancer Lett*., **2007**, 257, pp 16.
[34] Schwartz, Z.; Shani, J.; Soskolne, W. A.; Touma, H.; Amir, D.; Sela, J., *J. Nucl. Med.*, **1993**, 34, pp 104.
[35] De Leon-Rodriguez, L. M.; Kovacs, Z., *Bioconjug. Chem*., **2008**, 19, pp 391.
[36] . Forster, G. J.; Santos, E. B.; Smith-Jones, P. M.; Zanzonico, P.; Larson, S. M., *J. Nucl. Med.* **2006**, 47, pp 140.
[37] Li, M.; Meares, C. F.; Salako, Q.; Kukis, D. L.; Zhong, G. R.; Miers, L.; DeNardo, S. *J. Cancer Res*., **1995**, 55, pp 5726s.
[38] Kubicek, V.; Rudovsky, J.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.;Kolar, Z. I.; Wolterbeek, H. T.; Peters, J. A.; Lukes, I. J., *Am. Chem. Soc.*, **2005**, 127, pp 164-77.
[39] Vitha, T.; Kubicek, V.;Hermann, P.; Elst, L. V.;Muller, R. N.; Kolar, Z. I.;Wolterbeek,H. T.; Breeman,W. A.; Lukes, I.; Peters, J., *A. J. Med. Chem*, **2008**, 51, pp 677.