

Note

Synthesis of novel bone-targeted agents for treatment of osteoporosis

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Novel bone-targeted agents have been designed and synthesized by the combination of raloxifene and bisphosphonates. The anti-osteoporosis effect has been evaluated by bone mineral density (BMD) obtained from ovariectomized (OVX) mice *in vivo*. The results indicate that the compounds **8** and **9** not only prevent ovariectomy induced loss of bone but also enhance BMD to 7.09 and 12.59% compared to sham-operated control, respectively.

Keywords: Bone, osteoporosis, bisphosphonates, Raloxifene

IPC: Int.Cl.⁷ C 07 D

Osteoporosis is a common and significant health problem, which is characterized by a deterioration of the skeleton leading to a higher incidence of bone fractures. In USA, 1.5 million people per year suffer debilitating and painful osteoporotic fracture¹. Estrogen deficiency in postmenopausal women was considered to be the main factor leading to bone loss; therefore, estrogen replacement therapy was first used in the prevention and treatment of osteoporosis. But its side effects in other tissues, such as vaginal bleeding and increased risk of breast and endometrial cancer, preclude widespread acceptance²⁻⁴. Researchers have searched for some new methods to treat this disease such as selective estrogen receptor modulators (SERMs) and bisphosphonates (BPs), etc. Raloxifene is the best known member of SERMs family and has been approved by the US FDA for the prevention of osteoporosis. The most common adverse effects are hot flushes and leg cramps, and the drug is also associated with an increased risk of

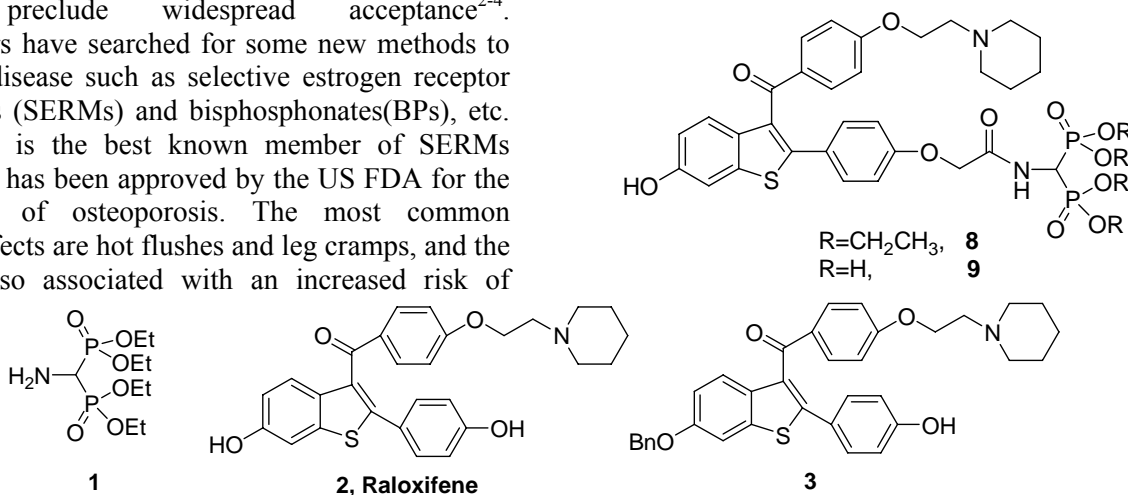
thromboembolic events. BPs are another type of therapeutic agents for the treatment of osteoporosis, which can inhibit bone resorption. On the other hand, BPs are also reported for their use as drug carrier because of their high affinity for bone tissues⁵.

Therefore, it is desirable to selectively deliver raloxifene to the affected part of bone, thereby reducing the possible adverse effects. We report herein the synthesis and the anti-osteoporosis effect of the novel conjugates of raloxifene functionalized with aminomethylenebisphosphonate **8** and **9**.

Chemistry

The aminomethylenebisphosphonate **1** was prepared by a modification of the literature procedure⁶. Raloxifene was mono-protected by treating with benzyl bromide and NaH⁷. The O-alkylation of protective raloxifene **3** with ethyl bromoacetate and sodium hydride offered ethyl 6-protective raloxifene-4'-oxy-methylenecarboxylated **4**. Hydrolysis of **4** with 1 mol/L of aqueous LiOH gave acid **5**.

After conversion of **5** to acid chloride **6** by treating with oxalyl chloride in the presence of the catalytic amount of N, N-dimethyl formamide (DMF), the conjugate **7** was obtained by coupling of **6** with aminomethylenebisphosphonate **1** in 44% yield. Hydrogenolysis of **7** with Pd/C (10%) and H₂ in ethanol gave the compound **8** in 91% yield. The targeted compound **9** was prepared by hydrolysis of the compound **8** with trimethylsilyl bromide (TMSBr) in 75% yield (**Scheme 1**).



Bioassay *in vivo*

The anti-osteoporosis effects of compounds **8** and **9** on ovariectomized mice were examined. Twenty-five female 10-week-old Kunming mice, weighing 30.0 ± 2.1 g, were maintained in a 12h light-dark cycle at $20 \pm 1^\circ\text{C}$ with *ad libitum* access to food and water. Bilateral ovariectomies were performed except on sham-operated controls. Mice were grouped into treatment units of $n = 5$ to include: (i) sham-operated controls (Sham), (ii) ovariectomized controls (OVX), (iii) ovariectomized treated with alendronate (OVX+Alen), (iv) ovariectomized treated with raloxifene (OVX+Ralo), the compound **8** (OVX+**8**) and **9** (OVX+**9**), respectively. Sham and OVX mice were injected with distilled water and other OVX mice were treated with the tested compounds at a dose of $4 \mu\text{mol L}^{-1}/\text{kg}$ i.p. once a day for 4 weeks. After the treatment, uterine weights were tested and BMD were measured by peripheral quantitative computed tomography (pQCT) at the proximal tibia. The results are shown in **Figure 1** and **Table I**.

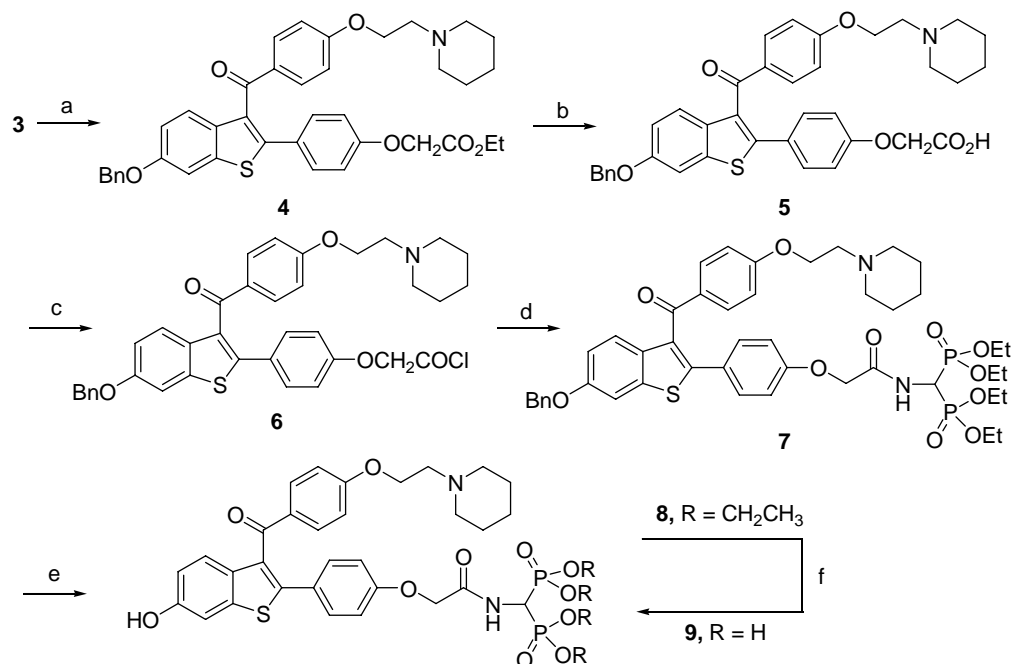
Results and Discussion

The data in **Table I** indicates that the compounds **8** and **9** show marginal effect on increasing the uterine weights compared to raloxifene. The result in

Figure 1 indicate that a significant 22% reduction in BMD was observed for OVX controls compared to Sham, and compounds **8** and **9** not only prevent this loss of bone but also enhance BMD to 7.09 and 12.59% compared to Sham, respectively. The anti-osteoporosis effect of **9** is more potent than **8**. The reason may be that the acid **9** has higher affinity for bone tissues than the ester **8**. Comparison of conjugate **9** to raloxifene showed that the conjugate **9** not only improved curative effect, but also increased the selectivity, reducing the effect on uterus. The pharmacokinetics of conjugate **9** is still in a way. The fact that the conjugates of raloxifene and bisphosphonates exhibit a good effect on inhibiting the reduction of BMD suggests it is a useful way to provide bone-targeted SERMs for treatment of osteoporosis.

Experimental Section

Melting points were determined on Fisher-Johns melting point apparatus and are uncorrected. The ^1H NMR spectra were taken on a Mercury-400(400Mz) with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Microanalyses were performed on Element VE instruments.



a: $\text{BrCH}_2\text{COOEt}$, NaH , THF, r.t., 3hr; b: 1N LiOH , THF, $40\sim 50^\circ\text{C}$, 8hr; c: $(\text{COCl})_2$, DMF, 0°C , r.t., 3hr; d: **1**, pyridine, CH_2Cl_2 , 5hr; e: Pd(C) , H_2 , ethanol, r.t., 24hr; f: TMSBr , CH_2Cl_2 , r.t., 48hr.

Scheme I

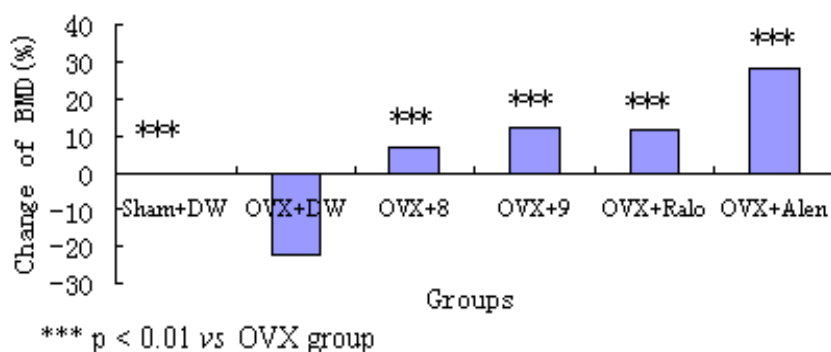


Figure 1 — Effect of the compounds on BMD of tibia in OVX mouse model

Table 1 — Uterine weights of the mice

Group	Weight of the uteri	P value	
		vs sham	vs OVX
Sham group	135.6±59.1		>0.05
OVX group	28.6±13.4	<0.01	
OVX+Alen	22.8±1.8	<0.01	>0.05
OVX+Ralo	43.4±4.3	<0.01	>0.05
OVX+8	36.0±5.3	<0.01	>0.05
OVX+9	31.7±5.6	<0.01	>0.05

P > 0.05, no significant correlation

Raloxifene-Bn-bisphosphonate ester conjugate 7.

Sodium hydride (15 mg, 0.35 mmole) was added to a solution of **3** (112.6 mg, 0.2 mmole) in dry THF (5 mL). The mixture was stirred at room temperature for 30 min, and ethyl 2-bromoacetate (0.026 mL, 0.23 mmole) was added slowly. The reaction mixture was stirred continuously for 2hr, poured into ice water, and extracted with ethyl acetate (30 mL). The organic layer was dried over MgSO₄. The solvent was removed and the crude product was purified by column chromatography on silica gel using 30% methanol/CH₂Cl₂ as eluent to give **4** (71 mg, 54%). ¹H NMR (400 MHz, CDCl₃): δ 1.27(3H, t, J=7.1Hz), 1.50(6H, br), 2.54(4H, br), 2.79(2H, t, J=6.0Hz), 4.12(2H, t, J=6.0Hz), 4.25(2H, q, J=7.1Hz), 4.55(2H, s), 5.14(2H, s), 6.67(4H, dd, J=2, 3Hz), 7.04(1H, d, J=2.4Hz), 7.32-7.54(9H, m), 7.75(2H, d, J=2.2Hz).

To a solution of **4** (126 mg, 0.194 mmole) in THF (30 mL) was added 5mL 1N LiOH aqueous. The mixture was stirred at 40-50°C for 8hr, and concentrated under reduced pressure. To the residue was added 6N HCl to bring the pH to 5, and extracted with chloroform. The organic layer was dried over Na₂SO₄. The solvent was removed to afford the

yellow foam **5** (109 mg, 90.8%). m.p.78°C. ¹H NMR (600MHz, DMSO-*d*₆): 1.56(6H, br), 2.54(4H, br), 2.79(2H, t, J=6.0Hz), 4.12(2H, br), 4.55(2H, s), 5.14(2H, s), 6.67(4H, dd, J=2.3, 8.8Hz), 7.04(1H, d, J=2.3Hz), 7.32-7.54(9H, m), 7.75(2H, d, J=2.2Hz).

To a solution of **5** (62 mg, 0.1 mmole) in dry CH₂Cl₂ was added one drop of DMF, and then oxalyl chloride (0.014 mL, 0.18 mmole) at 0°C. The reaction mixture was stirred for 1hr and allowed to reach the room temperature. The mixture was concentrated to give **6** and then dissolved in 6 mL CH₂Cl₂. To this mixture was added **1** (41.2 mg, 0.14 mmole) and pyridine (0.6 mL) at room temperature. The mixture was stirred for 5hr, and washed with water. The organic layer was dried over Na₂SO₄. Flash chromatography (CH₂Cl₂/CH₃OH; 20/1) gave **7** as pale yellow solid (40 mg, 44.2%). ¹H NMR (400MHz, CDCl₃): δ 1.25-1.44(18H, br), 1.61(4H, m), 2.53(4H, br), 2.79(2H, t, J=5.6Hz), 4.10-4.23(10H, m), 4.65(2H, s), 4.99(2H, s), 5.06(1H, td, J=10.2, 21.5Hz), 6.77(2H, d, J=9.0Hz), 6.84(2H, q, J=2.0, 6.9Hz), 7.00(1H, dd, J=2.3, 9.0Hz), 7.08(1H, d, J=2.0Hz), 7.31-7.39(7H, m), 7.57(1H, d, J=9.0Hz), 7.74(2H, q, J=1.8, 7.0Hz); MS(ESI): m/z 905.3 (M⁺-1), 907.3(M⁺+1).

Raloxifene-bisphosphonate ester conjugate 8.

A solution of **7** (40 mg, 0.044 mmole) and 10% Pd(C) (30 mg) in ethanol (5 mL) was stirred under a balloon of hydrogen for 36 hr at room temperature. The Pd(C) was removed by filtration, the residue washed with methanol (5 mL), and the combined organic solution concentrated *in vacuo*. Flash chromatography (CH₂Cl₂/CH₃OH; 20/1) gave **8** as pale yellow solid (33 mg, 91.7%). ¹H NMR(600MHz, CDCl₃): δ 1.25-1.45(18H, br), 2.71(4H, m), 2.94(2H, br), 4.11-4.25(10H, m), 4.49(2H, s), 5.03(1H, td, J=10.2, 21.5Hz), 6.64(4H, t, J=9.1Hz), 6.99(1H, dd, J=2.4, 8.8Hz), 7.09(2H, d, J=9.0Hz), 7.28(1H, d, J=2.4Hz),

7.63(2H, d, $J=8.8\text{Hz}$), 7.71(1H, d, $J=8.8\text{Hz}$); MS (ESI): m/z 815.3(M^+-1), 817.2(M^++1).

Raloxifene-bisphosphonate acid conjugate 9. Trimethylsilyl bromide (0.1 mL, 0.76 mmole) was added slowly to a solution of **8** (51 mg, 0.062 mmole) in dry dichloromethane (5 mL). The mixture was stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the residue was stirred with methanol (2 mL) for 30 min. The mixture was filtered to afford the product as cream-coloured powder (44 mg, 90%). m.p. 249°C . ^1H NMR(400MHz, D_2O): δ 1.23 (6H, br), 2.71(4H, br), 2.94(2H, br), 3.97(1H, t, $J=18.7\text{Hz}$), 4.14(2H, br), 4.48(2H, s), 6.10(2H, d, $J=8.4\text{Hz}$), 6.25(2H, d, $J=8.0\text{Hz}$), 6.75(2H, d, $J=8.4\text{Hz}$), 6.86(1H, dd, $J=2.2$,

8.8Hz), 7.07(1H, d, $J=8.8\text{Hz}$), 7.21(2H, d, $J=8.0\text{Hz}$), 7.34(1H, d, $J=2.2\text{Hz}$); ^{31}P NMR: δ -11.29; Anal. Calcd for $\text{C}_{31}\text{H}_{34}\text{N}_2\text{O}_{11}\text{P}_2\text{S}\cdot 1.0\text{H}_2\text{O}$: C, 51.52; H, 5.02; N, 3.88. Found: C, 51.24; H, 5.02; N, 3.66 %.

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