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Novel Bone-targeted Agents for Treatment of Osteoporosis

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Abstract: The novel bone-targeted agents were designed and synthesized by the combination of raloxifene and bisphosphonates. The anti-osteoporosis effect was evaluated by bone mineral density (BMD) obtained from OVX mice in *vivo*. The results indicated that the compound **8**, **9** not only prevented ovariectomy induced loss of bone but also enhanced BMD to 0.87% and 19.67% compared to Sham, respectively.

Keywords: Osteoporosis, bone-targeted, raloxifene, bisphosphonate.

Osteoporosis is a common and significant health problem, which affects most of elderly female and many male population. This disease is characterized by a deterioration of the skeleton leading to a high incidence of bone fractures. In USA, 1.5 million people per year suffer debilitating and painful osteoporotic fracture[1]. Estrogen deficiency in postmenopausal women was considered to be the main factor leading 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 acceptance[2-4]. Researchers have searched for some new methods to treat this disease such as selective estrogen receptor modulators (SERMs) which are compounds with selective estrogen agonist activity in bone but with estrogen antagonist activity or no activity in reproductive tissues, bisphosphonates(BPs), *etc*. Raloxifene is the best known member of SERMs family and has been approved by the 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 a drug carrier because of its high affinity to bone tissues[5].

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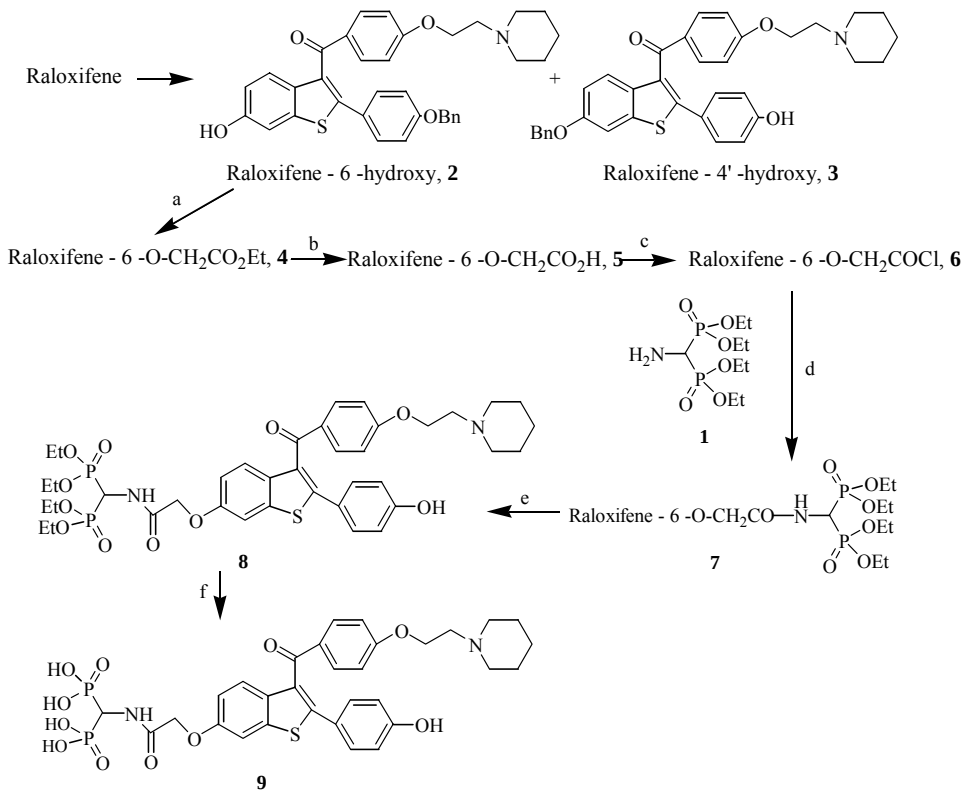
Scheme 1



(a) $BrCH_2COOEt$, NaH, THF, r.t. 6 h, (b) 1 mol/L LiOH, THF, 40~50℃,12 h, (c) $(COCl)_2$, DMF, 0 ℃-r.t., 3 h, (d) pyridine,CH_2Cl_2, 5 h, (e) Pd/C, H_2, methanol, r.t., 24 h, (f) TMSBr, CH_2Cl_2, r.t., 48 h.

Therefore, it is desirable to selectively deliver raloxifene to the affected part of bone, thereby reducing the possible adverse reactions. Herein, we would like to report the synthesis and the anti-osteoporosis effect of the novel conjugates of raloxifene functionalized with aminomethylenebisphosphonate.

The aminomethylenebisphosphonate **1** was prepared by modification of the literature procedure[6] in a total yield of 55%. Raloxifene was mono-protected by treating with benzyl bromide and NaH to afford a mixture of chromatographically separable benzyl ethers **2**, **3** in 20% yield, respectively[7].

The *O*-alkylation of protective raloxifene **2** with ethyl bromoacetate and sodium hydride offered ethyl 4-protective raloxifene-6-oxymethylenecarboxylated **4** in 70%. Hydrolysis of **4** with 1 mol/L of aqueous LiOH gave acid **5** in 95% yield. 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 89% yield. Hydrogenolysis of **7** with Pd/C (10%) and H_2 in methanol gave the compound **8** in 70% yield. The

targeted compound **9** was prepared by hydrolysis of the compound **8** with trimethylsilyl bromide (TMSBr) in 75% yield (**Scheme 1**).

Results and Discussion

The anti-osteoporosis effects of compounds **8** and **9** on ovariectomized mice were tested. The results are shown in **Table 1**.

The data in **Table 1** indicated the compounds **8** and **9** had marginal effect on increasing the uterine weights; A significant 22% reduction in BMD was observed for OVX controls compared to Sham**,** and **8**, **9** not only prevented this loss of bone but also enhanced BMD to 0.87% and 19.67% compared to Sham, respectively. And, the anti-osteoporosis effect of **9** is more potent than **8**. The reason may be that the acid **9** has higher affinity to bone tissues than the ester **8**. Comparison of conjugate **9** to raloxifene alone and the pharmacokinetics of conjugate **9** are still in a way. The fact that the conjugates of raloxifene and bisphosphonates have exhibited a good effect on inhibiting the reduction of BMD suggests it is a useful way to provide bone-targeted SERMs for treatment of osteoporosis.

Table 1 Effects of the compounds on BMD of tibia and uterine weights of the mice in OVX mouse model

Group	Weight of the uteri	Change of BMD	P Value *vs* sham(Change of BMD)	P Value *vs* OVX(Weight of the uteri)
Sham group	135.6±59.1			>0.05
OVX group	28.6±13.4	- 22.48%	<0.01	
OVX+**Alen**	22.8±1.8	28.31%	<0.01	>0.05
OVX+**8**	30.4±3.3	0.87%	<0.01	>0.05
OVX+**9**	34.2±4.0	19.67%	<0.01	>0.05

References and Notes

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8. Compound **9:** ^{1}H NMR(400MHz, D_2O, δ ppm): 1.07(br, 2H), 1.18(br, 4H), 2.03(br, 4H), 2.27(br, 2H), 3.58(br, 2H), 3.97(t, 1H, J=18.7), 4.48(s, 2H), 6.10(d, 2H, J=8.4), 6.25(d, 2H, J=8.0), 6.75(d, 2H, J=8.4), 6.86(dd, 1H, J=2.2, J=8.8), 7.07(d, 1H, J=8.8), 7.21(d, 2H, J=8.0), 7.34(d, 1H, J=2.2). ^{31}P NMR δ_{ppm} -11.29. Anal. Calcd. for $C_{31}H_{34}N_2O_{11}P_2S\cdot1.5H_2O$: C, 50.84; H, 5.06; N, 3.83 Found: C, 50.81; H, 5.02; N, 3.68

Bioassay *in vivo*

Twentyfive female 10-week-old Kunming mice, weighting 30.0±2.1g, were maintained in a 12 h light-dark cycle at 20 ± 1^{o}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: (1) sham-operated controls (Sham), (2) ovariectomized controls (OVX), (3) ovariectomized treated with alendronate(OVX+Alen), the compound **8** (OVX+8) and **9** (OVX+9), respectively. Sham and OVX mice were injected distilled water and other OVX mice were treated with the tested compounds at a dose of 4 μmol/L.kg^{-1} once a day for 4 weeks. After the treatment, uterine weights were tested and BMD were measured by pQCT(Stratec, XCT research SA) at the proximal tibia.

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