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RESEARCH PAPER

Synthesis and *in vivo* bioactivity of lipophilic alendronate derivatives against osteoporosis

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Abstract

New lipophilic alendronate amidated derivatives ***1a–1d*** anchored alkyl chains (C_nH_{2n+1}, $n = 12, 14, 16, 18$) had been obtained through the reaction of alendronate with carboxylic acid under anhydrous condition. The physicochemical parameters, such as the solubility and partition coefficient $P_{o/w}$ in *n*-octanol/water, were determined through calculation by performing reversion phase high-performance liquid chromatography (RP-HPLC). The results showed that the derivatives had improved lipophilicity compared with alendronate. The *in vivo* bioactivities of the derivatives were investigated using the hindlimb unloading growing rats' model. The results showed that the derivatives had *in vivo* bioactivity against hindlimb unloading growing rats' bone loss, which indicated that the lipophilic derivative would be a promising new potent bisphosphates for treatment of the osteoporosis.

Keywords: Alendronate, derivatives, lipophilicity, *in vivo* bioactivity, osteoporosis

Introduction

Osteoporosis is a common chronic disease of the human skeleton and is associated with high morbidity, mortality, and loss of independence (Im et al., 2004; Adachi et al., 2005; Coxon et al., 2006; Dunford et al., 2006; Choi et al., 2008; Berry et al., 2010). Nitrogen-containing bisphosphonates (NBPs) have been shown to inhibit osteoclast-mediated bone resorption, to significantly increase bone mineral density (BMD) of the hip and spine, and have been extensively used for the treatment of osteoporotic patients all over the world (Rogers, 2003; Feldstein et al., 2009; Hwang et al., 2010; Ito et al., 2010). These oral NBPs include once-a-week alendronate (marketed in the USA since 2000), once-a-week risedronate (since 2002), and once-a-month ibandronate (since 2005) (Widler et al., 2002; Abelson et al., 2010).

However, recent research showed that the major disadvantage of the clinically utilized NBPs is their poor oral absorption from the gastrointestinal (GI) tract, typically <1% is absorbed. (Ezra & Golomb, 2000; Leu et al., 2006; Iafisco et al., 2008). In addition, the potential for an increased risk of the GI adverse events has been noted with alendronate sodium, especially when taken incorrectly (Li et al., 2005; Kanis et al., 2008; Grima et al., 2010). Many patients with fragility fracture do not undergo osteoporosis management and are at high risk for subsequent fractures (Blouin et al., 2008; Hagino et al., 2009). Moreover, recent clinical applications have disclosed an unexpected side effect, osteonecrosis of the jaw.

Several factors contribute to NBPs potency and efficacy as inhibitors of bone resorption, such as the ability of binding affinities to hydroxyapatite (HAP) (Gittens et al., 2005; McLeod et al., 2006; Nancollas et al., 2006; Wright et al., 2006; Mukherjee et al., 2008), the treatment with the farnesyl diphosphate synthase (FDPS) (Ebetino et al., 2005; Dunford et al., 2006; Shull et al., 2006; Maalouf et al., 2007), which was the intracellular target of BPs. However, research results showed that the properties of high affinity to HAP and target to FDPS could not improve NBPs absorption and the poor absorption of BPs was due to its hydrophilic character, which could be enhanced either by changing the permeability properties of BPs or by altering the physicochemical properties of its permeability (Ezra & Golomb, 2000).

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Many research works had been done to increase the effect of NBPS to osteoporosis. One strategy for improving oral absorption of these types of molecules is to develop more lipophilic derivatives. Raiman et al. (2001, 2003) found the clodronate esters could increase the clodronate absorption and further suggested that at least three phosphate hydroxyl groups need to be substituted until the permeation route is changed from paracellular to transcellular. Monteil et al. (2005) formed various 1-hydroxy-1,1-bisphosphonate partial esters, which obtained from (alkyl or phenyl) bis(trimethylsilyl) phosphite and aromatic or aliphatic acid chlorides, followed by methanolysis. They suggested that these ester derivatives had anti-angiogenic and antitumor effects in breast carcinoma models and would have potent biological applications. Troutman et al. (2004) synthesized three tripivaloyloxymethyl esters of isoprenoid methylenediphosphonate with significantly increased lipophilicity and might act as important farnesyl diphosphate prodrugs. The above research results showed that enhancing the lipophilicity of BPs would increase its permeability through cell membrane and its absorption (Vachal et al., 2006). However, reports concerning the design of prodrug of alendronate with improving lipophilicity have not been published. In fact, there still exists the need for design of bioreversible alendronate prodrugs.

The aim of the present article was to increase the lipophilicity of alendronate through changing its molecular structure by introducing long alkyl into its chain and form its lipophilic derivatives. The structure and physicochemical property of the derivatives were investigated in detail in this article. In addition, the *in vivo* bioactivities of synthesized derivatives against osteoporosis were tested using hindlimb unloading growing rats' model.

Materials and methods

Materials

Alendronate sodium [(4-amino-1-hydroxybutylidene) bisphosphonic acid sodium salt, $C_4H_{13}NNaO_7P_2$, $M_w = 272.09$ Da] was supported by Shenyang Dongrui Technology (Shenyang, China). Fatty acids, such as lauric acid ($C_{11}H_{23}$–COOH), tetradecylic acid ($C_{13}H_{25}$–COOH), hexadecouldoic acid ($C_{15}H_{29}$–COOH), and octadecanoic acid ($C_{17}H_{33}$–COOH), were all supported by Tianjin Chemical Agent Factory (Tianjin, China). *N*,*N*′-Dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinamide (NHS) were supported by GL Biochem Ltd. (Shanghai, China). The solvents, such as *N*,*N*-dimethylformamide (DMF), acetoacetate, anhydrous methanol, and dichloromethane, were supported by Xi'an Chemical Agent Company (Xi'an, China). DMF was freshly distilled in the metallic sodium reflux under nitrogen until the water content must low 0.01%. Other solvents were used without further treatment. Reactions were protected from atmospheric moisture by $CaCl_2$ drying tubes. All reaction mixtures and column eluents were monitored by thin-layer chromatography (TLC) using commercial glass backed TLC plates (Merck Kieselgel 60 F_{254}). The plates were observed under UV light at 254 and 365 nm. The ELC developer triketohydrindene hydrate was obtained from Xi'an Chemical Agent Company (Xi'an, China) and used without further treatment.

The melting point of compounds was determined by the WRS-1B Digital Melting Point Apparatus, Shanghai Shenguang Instrument Co. Ltd. (Shanghai, China). The sample is heated at a rate of 2°C/min until it melts. The melting point was obtained automatically.

FTIR spectra were recorded on a WQF-310 FTIR spectrometer (The Second Optical Instruments Plant of Beijing). Optical grade potassium bromide (KBr; Beijing Beijing Fine Chemical Corporation, Yixing, China) was used as a background material. ^{1}H- and ^{13}C-NMR spectra were performed with Super Conducting Fourier Digital NMR Spectrometer, AVANCE (Bruker, Switzerland), 300 MHz instrument using tetramethylsilane $(CH_3)_4Si$ (TMS) as internal standard and DMSO-d_6 at 20°C. All chemical shifts were reported as δ values (in ppm). Mass spectra with ionization energy maintained at 70 eV usage were taken on AXIMA-CFR™ plus MALDI-TOF Mass Spectrometer, KRATOS GROUP PLC (Shimadzu, UK).

The solvents for analysis of physicochemical property of derivatives, such as ethanol, propylene glycol (PG), isopropyl alcohol (IPA), oleyl alcohol (OA), acetonitrile (AN), ethyl acetate (EA), and isopropyl myristate (IPM), were used. Sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), and sodium hydroxide (NaOH) were used for prepared phosphate-buffered solution (PBS). The PBS (100 mM) was prepared by dissolving 1.482 g sodium dihydrogen phosphate (NaH_2PO_4, 358.14 g/mol) and 14.499 g disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$, 156.01 g/mol) in 500 mL distilled water and the pH of the solution was adjusted to 7.4 using NaOH (1 M). AN and methanol used were of HPLC grade and purchased from Sigma Corp. Other reagents were of analytical grade.

General procedure for the synthesis of 4-alkyl-amide-1-hydroxybutylidene (*1a*, BP-C_{12}) bisphosphonates

Chemicals such as 32.52 mg (1 mmol) alendronate sodium, 13.81 mg (1.2 mmol) NHS, 24.76 mg (1.2 mmol) DCC, and 20.03 mg (1 mmol) lauric acid were mixed together in anhydrous DMF and stirred at 0°C for 5 h and at room temperature overnight (Figure 1). The reaction was carried out under anhydrous condition and at pH values of 7.0–9.0 (Gu et al., 2010). The reaction was monitored by TLC (MeOH/$CHCl_3$, 90:10, Rf 0.34), used triketohydrindene hydrate as the developer and the terminal point of the reaction was defined when there was no red point appeared. The reaction mixture was allowed to stir, a by-product DCU was filtered, and the filtrate was evaporated under reduced pressure. The product after deionized water washing for several times and 23.56 mg

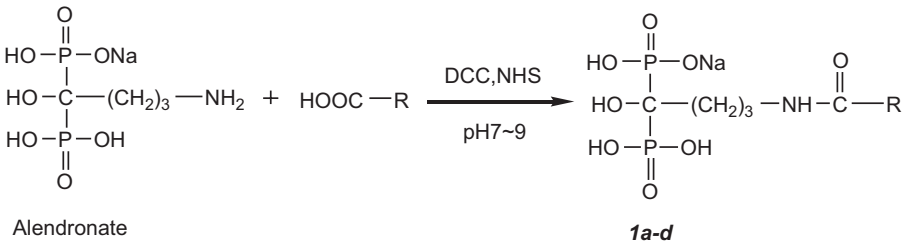


Figure 1. The chemical synthesis of lipophilic derivatives ***1a–1d***. (R = $-C_{11}H_{23}$, $-C_{13}H_{25}$, $-C_{15}H_{31}$, $-C_{17}H_{33}$).

1a was obtained. The chemical structure data of ***1a*** was as follows:

(4-Lauramide-1-hydroxybutylidene) bisphosphonate (***1a***, BP-C_{12}) as a white powder (31.1 mg, yield 90%): R_f = 0.34 ($CHCl_3$/MeOH, 9:1), m.p.: 197–200°C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 2.0 (s, 4H, OH), 8.0 (s, 1H, CONH), 0.94 (s, 3H,CH_3), 1.32(d, 20H, CH_2), 3.2(d, 2H, CCH_2), 2.2 (d, 2H, $COCH_2$), 1.5(s, 2H, CH_2NH) ppm; ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 10.3 (CH_2NH), 14.1(CH_3), 29.5(CH_2), 37.7 ($CO\underline{C}H_2$),41.0($C\underline{C}H_2$), 74.3 ($HO\underline{C}CH_2$), 173.4 (CONH) ppm; ^{31}P NMR: δ +27.7 ppm. IR (KBr) υ = 3398, 2925, 2854, 1630 cm^{-1}; AXIMA-CFR plus MALDI-TOF MS (70 eV) *m/z*: 430 (M–Na^+), 326 (M–CH_3–8 CH_2), 298 (M–CH_3–10 CH_2). Anal. calcd. for $C_{15}H_{34}Na_2NO_8P_2$: C 38.79, H 7.33, O 27.59, found: C 39.26, H 7.21, O 27.48.

Other three derivatives *(4-myristamide-1-hydroxybutylidene) bisphosphonate* (***1b***, BP-C_{14}), *(4-palmitoylamide-1-hydroxybutylidene) bisphosphonate* (***1c***, BP-C_{16}), and *(4-stearylamide-1-hydroxybutylidene) bisphosphonate* (***1d***, BP-C_{18}) were obtained through the same reaction processing but different fat acids, such as tetradecylic acid, hexadecouldoic acid, and octadecanoic acid, respectively. For the derivatives have the same chemical structure and the difference between them was just the length of alkyl chains introduced, so we did not show the chemical structure data of ***1b–1d***. The high-performance liquid chromatography (HPLC) of the derivatives were presented in our pervious paper (Lu et al. 2010).

General processing for solubility determination

An excess amount of derivative was added to the various pure solvents and PBS (pH 7.4). The solutions were shaked at 37°C for >48 h. The solutions were then centrifuged at 7500 rpm for 5 min, and the supernatant was assayed by HPLC (Water ALLIANCE 2695) after appropriate dilution. The Agilent CLC-ODS C_{18} (4.6 mm × 250 mm, 5 μm, Agilent Technologies Inc., Santa Clara, CA) column was used from ambient temperature to 50°C. The mobile phase was an aqueous solution mixture of MeOH (HPLC grade) and H_2O (distilled) at pH 7.2, which contained 5 mmol/L ammonium dihydrogen phosphate, 2 mmol/L dodecoulde tributyl bromination, and 1.5 mmol/L disodium ethylenediaminetetraacetic acid. The sample concentration was 1.0 mg/mL with the mobile phase liquid as the solvent. The flow rate was 1.0 mL/min and 20 μL of samples were injected. The HPLC trace was monitored with a differential refractive index detector (Water 2414).

General processing for partition coefficient determination

The derivative solution (50 μg/mL) was prepared with hydrophilic phase saturated with lipophilic phase. The derivative concentrations present in lipophilic and liquid phases were measured simultaneously. One milliliter of this solution was then transferred to 10 mL centrifuge tube containing 1 mL of lipophilic phase saturated with hydrophilic phase. The tube was vortexed for 30 min and centrifuged at 3000 *g* for 5 min, and the derivative concentrations present in both phases were determined by HPLC. The partition coefficient $P_{o/w}$ of derivatives could be calculated by the following equation:

$$P_{o/w} = \frac{[C]_{n-\text{octanol}}}{[C]_{\text{water}}}$$

Where $[C]_{n\text{-octanol}}$ was the concentration of derivatives in the lipophilic phase, and the $[C]_{\text{water}}$ was the corresponding derivative concentration in the liquid. Units for both concentrations were in mol/L.

Animals and experimental groups

Twenty-four virgin male Sprague-Dawley rats, 3-months old and weighing approximately 213 ± 5 g, were obtained from the Fourth Military Medical University (Xi'an, China). The 24 rats were divided into four groups as follows: group (1) (control, the rats were freely act and feed), group (2) (suspended control, the rats were suspended by the tail with the head down ~30°), group (3) (the rats were suspended by the tail with the head down ~30°, with intragastric administration of *alendronate* at the dosage of 1 mg/kg/day), and group (4) (the rats were suspended by the tail with the head down ~30°, with intragastric administration of alendronate derivative ***1a*** at the dosage of 1 mg/kg/day). Animals were housed in a temperature-controlled room (23 ± 1°C) with 12-h light–dark cycles. The experiment lasted for 28 days and at the termination, the rats were killed by exsanguination under ether anesthesia and the right femur of each animal was harvested until mechanical testing. The blood sample was obtained from arteria carotis of each rat under ether anesthesia and the serum was obtained through centrifugating (3000 rpm/min, 10 min) the blood. The blood

was stored under −70°C at refrigerator and was tested within 3 days.

BMD and body weight measurement

The BMD (mg/cm^2) was measured by PRODIGY Direct Digital DEXA (dual-energy X-ray absorptiometry) Bone Densitometry (GE Prodigy, Model 8743-BX/1L, Lunar, Madison, WI). Body weight of rat was measured by electronic balance (MP6001, quantity sensitive: 0.01 g, China) at 6, 11, 16, 21, and 28 days.

Serum alkaline phosphatase and calcium measurement

The serum alkaline phosphatase (ALP), calcium (Ca), and phosphorus (P) were detected using fully automatic biochemical meter (7080, HITACHI, Japan).

Biomechanical testing

Femurs were harvested, cleaned of superficial tissues, and frozen −20°C until tested. The biomechanical testing of the right femur was measured using the texture analyzer (QTS-25, UK). Three-point Flexure Bond Test was used and the measurement conditions were as follows: sample span: 20 mm, plunger speed; 12 mm/min, and load range: 50.0 kg. The mechanical properties of maximum load (ML, N), maximum deformation (MD, %), elastic load (EL, N), and elastic deformation (ED, %) were recorded.

Statistical analyses

Data were analyzed using the SPSS statistical package (V.10; Chicago, IL) by Tukey's test and one-way analysis of variance (ANOVA). Statistically significant differences ($P < 0.05$) were found between or within the experimental groups by ANOVA, individual differences were assessed by post-hoc analyses (Tukey's test). Data are expressed as mean ± standard deviation (SD) in the text and tables, and as mean ± standard error of the mean (SEM) in the figures.

Results and discussion

Properties of the derivatives

Derivatives ***1a–1d*** had higher $P_{o/w}$ value than that of alendronate (Table 1). That was they had the improved solubility in *n*-octanol organic phase, which indicated the improved lipophilicity of the derivatives. Moreover, the longer the alkyl chain introduced, the higher the partition coefficient was in *n*-octyl alcohol/water and as a result the more lipophilic the derivatives. In fact, we did not mean that the higher the $P_{o/w}$, the higher the bioavailability of drugs. Here we just showed that the higher the $P_{o/w}$ value, the more lipophilic the derivatives. So we did not introduce the larger alkyl chain (C_nH_{2n+1}, $n > 18$) into alendronate molecule.

Alendronate had the little solubility in water and its solubility in PBS was 13.5 ± 2.8 μg/mL (Table 2). Compared with alendronate, the derivative had the relative low solubility in PBS, which was about 0.01 ± 0.003 and 0.007 ± 0.0005 μg/mL for ***1a*** and ***1b***, respectively. But the derivatives with longer alkyl chains introduced, such as ***1c*** and ***1d***, almost had no measurable solubility values in PBS. This result indicated that the derivatives had the improved lipophilicity and had a very low aqueous solubility. This could be due to the lower polarity than that of alendronate for the organic alkyl chain introduced.

We also selected several different solvents, such as PG, ethanol, AN, IPA, EA, OA, and IPM, as organic solvents to investigate derivatives' solubility. There were differences in polarity among these solvents with a rank order of PG > ethanol > AN > IPA > EA > OA > IPM. All the derivatives had the increasing solubility with the solvents' polarity decreasing, and had the best solubility in EA. After that the solubility decreased with the polarity decreasing and almost had no measurable value in IPM for ***1a*** and ***1b***. The solubility of derivatives in different organic solvents was different with different alkyl chain introduced.

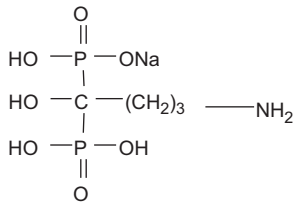
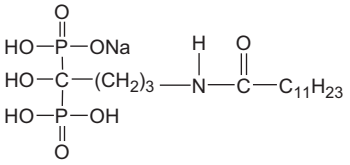
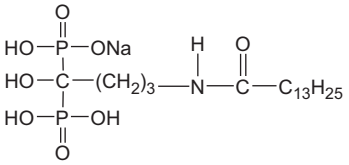
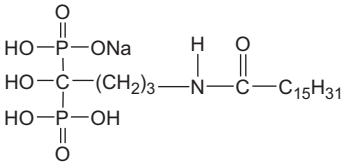
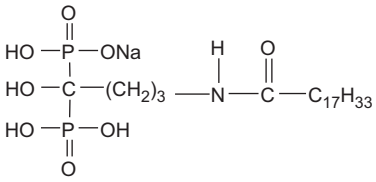
In vivo bioactivity against bone loss

Compared with the data in group (1), the rats in suspended groups (2), (3), and (4) all had the decreasing body weights and had statistical significant differences (P = 0.001, 0.011, 0.01) vs. control group (1) (Figure 2, left). Among the three suspended groups, the rats in groups (3) and (4) had some slight increasing body weight than that of rats in group (2), but there were no statistical significant differences between them (P = 0.019, 0.025). This result indicated that intragastric administration of alendronate and its derivative might have some beneficial effects on improvement of the rats' body weights.

The BMD value at any point in time was a combined function of one's peak bone mass and the amount of bone loss since skeletal maturation. A low BMD value was likely due to progressive bone loss. The BMD values of the rats in the suspended control group (2) had the significant decreasing (P = 0.005), comparing with that of the control group (1), which indicated the bone loss of the suspended rats (Figure 2, right). Compared with group (2), the BMD values of rats in groups (3) and (4) all had the significant increasing value (P = 0.003, 0), which indicated that the intragastric administration of *alendronate* and its derivative ***1a*** could increase the BMD of the suspended rats, which will combine with the increasing bone mass.

The value of ALP and P content in group (2) decreased (P = 0.002, 0.007), although the serum Ca content increased (P = 0.009), which was almost the same symptom as that of osteoporosis comparing with that of group (1) (Figure 3). The ALP value of rats in group (3) with alendronate given had the significant increase (P = 0.004), comparing with that of group (2). The value of P content in serum was almost having no change vs. group (2). However, the Ca content in serum

Table 1. Chemical structure of alendronate and its derivatives ***1a–1d***.

Compounds	Chemical structures	$P_{o/w}$ ($n=3$)
Alendronate (BP-C_0)	 (4-Amino-1-hydroxybutylidene) bisphosphonate	0.012 ± 0.0006
1a (BP-C_{12})	 (4-lauramide-1-hydroxybutylidene) bisphosphonate	0.027 ± 0.0006
1b (BP-C_{14})	 (4-myristamide-1-hydroxybutylidene) bisphosphonate	0.045 ± 0.0004
1c (BP-C_{16})	 (4-myristamide-1-hydroxybutylidene) bisphosphonate	0.053 ± 0.0005
1d (BP-C_{18})	 (4-stearylamide-1-hydroxybutylidene) bisphosphonate	0.062 ± 0.0003

Note: Considering the relationship between $P_{o/w}$ and the bioavailability of drugs is not that the $P_{o/w}$ is larger, the bioavailability is higher. So, the derivatives (BP-C_n) with $n > 18$ were not obtained in our experiment.

was decreased ($P = 0.002$). The value of ALP and P contents in serum all increased in group (4) vs. group (2) ($P = 0.027$, 0.026), and the Ca content in serum decreased ($P = 0.023$). The value of ALP, P, and Ca contents had no statistical significant differences between the groups (3) and (4).

The activity and osteogenesis of the osteoblastic were related with the content of ALP in serum, the more activity of the osteoblast, the more the ALP content. The contents of P and Ca in serum were the parameter which reflected the condition of bone metabolism. The low ALP values of the suspended groups (2) suggested that the suspending in rats' tail make its hindlimb unloading and cause the symptom of osteoporosis or bone loss, which showed the lower content of ALP and P, whereas the higher content Ca in serum. However, the intragastric administration of *alendronate* and its derivative ***1a*** change the above symptom and made the condition become better, that was the increasing ALP and P content while the decreasing Ca content in serum. We could know that the alendreonate and its derivative could improve the activity of osteoblast and decrease the loss of calcium, and as a result enhance the bone mineralization.

The biomechanical data of the rats' right femur were listed in Table 3. Compared with the suspended control group (2), groups (3) and (4) all had the increasing value of ML, EL, ED, σ_b, σ_s, ε_b, U, EJ, K, and E, which indicated that the intragastric administration of *alendronate* and its derivative ***1a*** improved the biomechanical properties of the rats' right femur, increased the bone elasticity, and increased the bioactivity against osteoporosis.

Table 2. Equilibrium solubility of derivatives in different solvents ($n=3$).

	Solubility (μg/mL)				
		Derivatives			
Solvents	Alendronate	***1a***	***1b***	***1c***	***1d***
Phosphate buffer (PBS)	13.5 ± 2.8	0.01 ± 0.003	0.007 ± 0.0005	—*	—*
Propylene glycol (PG)	2.6 ± 0.4	0.04 ± 0.005	0.02 ± 0.006	—*	—*
Ethanol	0.004 ± 0.0005	0.1 ± 0.07	0.06 ± 0.008	0.003 ± 0.0002	—*
Acetonitrile (AN)	—*	0.15 ± 0.02	0.2 ± 0.05	0.6 ± 0.01	0.1 ± 0.03
Isopropyl alcohol (IPA)	—*	3.4 ± 1.3	3.9 ± 1.8	2.7 ± 0.7	2.3 ± 0.2
Ethyl acetate (EA)	—*	2.3 ± 0.2	3.1 ± 0.1	3.3 ± 2.5	5.7 ± 0.7
Oleyl alcohol OA)	—*	0.3 ± 0.07	0.6 ± 0.03	1.3 ± 0.08	1.7 ± 0.3
Isopropyl myristate (IPM)	—*	—*	—*	0.009 ± 0.0005	0.01 ± 0.006

*No measurable values.

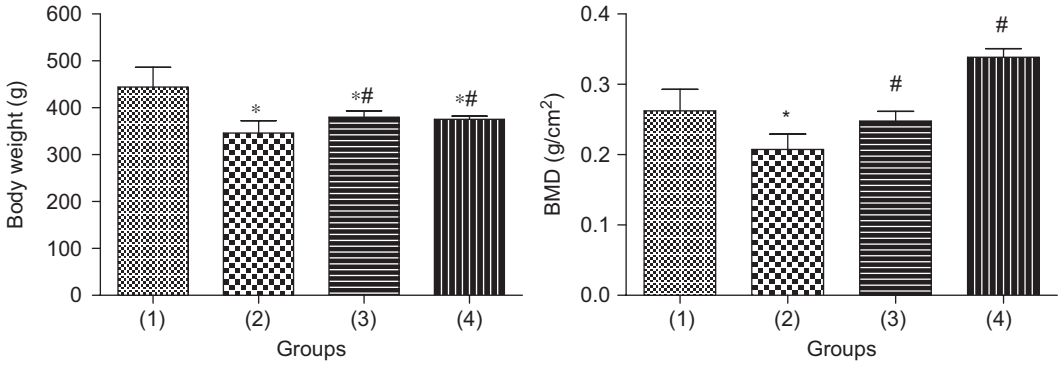


Figure 2. The body weight and bone mineral density (BMD) of rats in different groups. (*$P<0.05$ vs. control group (1); #$P<0.05$ vs. suspend control group (2). Group (1): control, the rats were freely act and feed; Group (2): suspended control, the rats were suspended by the tail with the head down ~30°); Group (3): the rats were suspended by the tail with the head down ~30°, with intragastric administration of *alendronate* at the dosage of 1 mg/kg/day; Group (4): the rats were suspended by the tail with the head down ~30°, with intragastric administration of alendronate derivative ***1a*** at the dosage of 1 mg/kg/day.

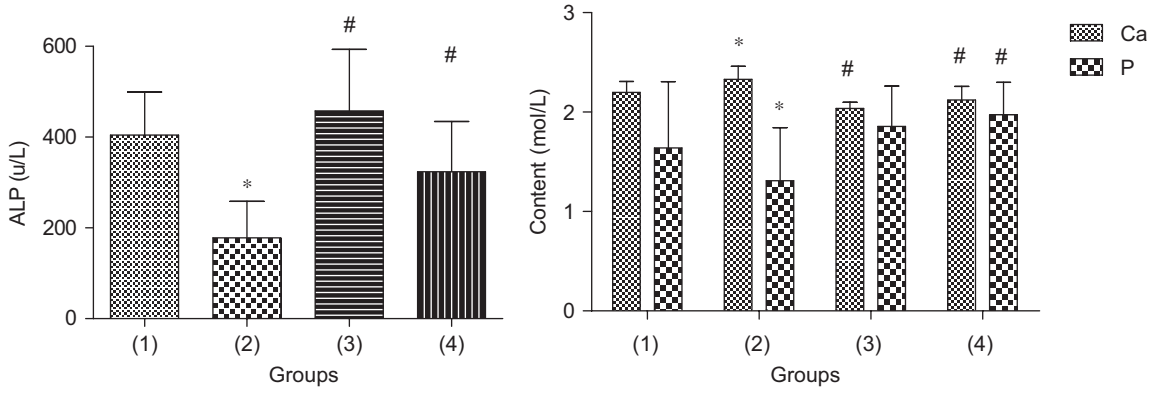


Figure 3. Alkaline phosphatase (ALP), P, and Ca contents of the rats in all groups. (*$P<0.05$ vs. control group (1); #$P<0.05$ vs. suspend control group (2). Group (1): control, the rats were freely act and feed; Group (2): suspended control, the rats were suspended by the tail with the head down ~30°); Group (3): the rats were suspended by the tail with the head down ~30°, with intragastric administration of *alendronate* at the dosage of 1 mg/kg/day; Group (4): the rats were suspended by the tail with the head down ~30°, with intragastric administration of alendronate derivative ***1a*** at the dosage of 1 mg/kg/day.

Conclusion

New compounds, such as (4-alkyl-amide-1-hydroxybutylidene) bisphosphonates, had been prepared. Compare with alendronate, the derivatives had a very low aqueous solubility and had little or even no measurable solubility values in PBS. While it had different solubility in organic solvents with different polarities. The partition coefficient $P_{o/w}$ of derivatives in *n*-octanol/water mixture was calculated and the results showed that the derivatives had the high $P_{o/w}$ data than that of alendronate and the longer the alkyl chain was introduced, the higher the partition coefficient was. All of the above results showed through long alkyl chain

Table 3. Biomechanical data of rats' right femur ($n = 6$, mean ± SEM).

Group	(1)	(2)	(3)	(4)
ML (N)	166.23 ± 19.98	124.73* ± 7.12	147.69# ± 17.00	137.19# ± 2.80
MD (mm)	1.17 ± 0.31	0.93 ± 0.19	0.77 ± 0.05	0.76 ± 0.06
EL (N)	69.465 ± 22.516	36.330* ± 11.824	81.30# ± 23.13	54.55# ± 12.10
ED (mm)	0.51 ± 0.27	0.22* ± 0.07	0.35# ± 0.09	0.31# ± 0.03
σ_b (MPa)	194.16 ± 7.84	156.81* ± 23.30	182.16# ± 9.37	185.61# ± 12.17
σ_s (MPa)	59.32 ± 25.14	31.68* ± 6.58	86.41# ± 26.10	65.35# ± 18.61
ε_b (%)	0.067 ± 0.016	0.035* ± 0.007	0.048# ± 0.011	0.05# ± 0.01
U (N × mm)	17.90 ± 2.39	4.17* ± 2.40	14.96# ± 7.73	8.67# ± 2.59
EJ ($\times 10^3$, N·mm^2)	29.21 ± 3.87	28.53 ± 5.85	38.83# ± 5.09	28.87 ± 4.94
K ($\times 10^{-3}$, m/N)	7.50 ± 1.48	5.87* ± 0.73	7.33# ± 0.61	6.16# ± 0.52
E ($\times 10^3$, MPa)	4.44 ± 0.51	3.74* ± 0.32	4.23# ± 0.36	4.04# ± 0.64

Abbreviations: ML: maximum load; MD: maximum deflection; EL: elastic load; ED: elastic deflection; σ_b: maximum bending stress; σ_s: elastic bending stress; ε_b: maximum strain; U: bending energy; EJ: bending rigid coefficient; K: toughness coefficient; E: elastic modulus. Group (1): control, the rats were freely act and feed; group (2): suspended control, the rats were suspended by the tail with the head down ~30°; group (3): the rats were suspended by the tail with the head down ~30°, with intragastric administration of *alendronate* at the dosage of 1 mg/kg/day; group (4): the rats were suspended by the tail with the head down ~30°, with intragastric administration of alendronate derivative ***1a*** at the dosage of 1 mg/kg/day.
*$P < 0.05$ vs. control group (1); #$P < 0.05$ vs. suspend control group (2).

introduced into its R_2 chain, the derivatives had the increasing lipophilicity.

The animal experiment results showed that the derivatives could have as good *in vivo* bioactivity against hindlimb unloading growing rats' bone loss as that of alendronate did in improving the suspended rats' bone properties, such as the increasing of ALP and BMD. This synthesized lipophilic derivative would be a promising new potent bisphosphates for treatment of the osteoporosis.

Declaration of interest

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