

## Supporting Information

**Development of three-component conjugates: To get nano-globes with porous surface, high *in vivo* anti-osteoporosis activity and minimal side effects**

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### Contents

**The synthesis and structural data of 4a-c**

## Synthesis of conjugates 4a-c

### 17 $\beta$ -amino-11 $\alpha$ -hydroxyandrost-1,4-diene-3-one (1)

To a solution of 5.0 g (16.6 mmol) of 11 $\alpha$ -hydroxyandrost-1,4-dien-3,17-dione in 50 mL of anhydrous methanol, 12.8 g (16.6 mmol) of anhydrous ammonium acetate was added. After the salt dissolved completely, 1.2 g (18.0 mmol) of NaBH<sub>3</sub>CN was added partly. The mixture was stirred at room temperature for 24 h and TLC (ethyl acetate: petrol ether, 2:1) indicated the disappearance of 11 $\alpha$ -hydroxyandrost-1,4-dien-3,17-dione. After removal of methanol under reduced pressure, the residue was added into 20 mL of water. Potassium carbonate was added to adjust pH to 10, and the mixture was extracted with 100 mL of chloroform. The organic phase was dried over anhydrous sodium sulphate. After filtration and evaporation under reduced pressure the residue was recrystallized with methanol to yield the title compound 4.51 g (90 %) as a yellow powder. Mp 154-158 °C. ESI-MS (m/e): 302 [M + H]<sup>+</sup>. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = 28.2 (c = 0.01, CH<sub>3</sub>OH). IR (KBr): 1658, 3372, 3289, 3380 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  = 7.16 (d, *J* = 10.2 Hz, 1H), 6.25 (dd, *J* = 10.2 Hz, *J* = 1.8 Hz, 1H), 6.05 (s, 1H), 3.72 (w, 1H), 2.75 (m, 1H), 2.76 (t, *J* = 3.0 Hz), 2.53 (w, 2H), 2.00 - 2.20 (m, 9H), 1.56 (m, 1H), 1.40 (s, 3H), 1.33 (m, 2H), 0.70 (s, 3H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  = 186.2, 166.1, 154.2, 132.4, 124.0, 119.9, 66.7, 60.0 (C<sub>11</sub>), 58.1, 47.8, 43.0, 40.0, 38.8, 33.9, 33.3, 31.1, 30.4, 26.6, 13.8.

### 17 $\beta$ -(3-Carboxypropionylamino)-androst-1,4-diene-3-one (2)

To a solution of 560 mg (2.0 mmol) of **1** in 10 mL of anhydrous THF, 220 mg (2.0 mmol) of succinic anhydride was added, and the reaction mixture was stirred to form a clean solution. After addition of 25 mg (0.2 mmol) of 4-dimethylamino-pyridine (DMAP)

the reaction mixture was stirred at room temperature for 24 h and TLC (ethyl acetate: petroleum ether, 1:1) indicated complete disappearance of **1**. The reaction mixture was evaporated under reduced pressure, and the residue was extracted with ethyl acetate (3 × 50 mL). Then the combined ethyl acetate phase was successively washed with 5% KHSO<sub>4</sub> (3 × 50 mL) and saturated aqueous NaCl (3 × 50 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure to give 770 mg (96%) of the title compound as a yellowish powder. Mp 123 - 124 °C,  $[\alpha]_D^{20} = 29.0$  (c = 0.10, MeOH), ESI-MS 400 [M - H]<sup>-</sup>.

**17β-[Aminocarbonylethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Phe-OBzl]-androst-1,4-diene-3-one (3a)**

At 0 °C to the solution of 200 mg (0.50 mmol) of 17β-(3-carboxylpropionyl- amono)-androst-1,4-diene-3-one (**2**) and 75 mg (0.55 mmol) of 1-hydroxybenzo triazole (HOBT) in 20 mL of anhydrous tetrahydrofuran (THF), 113 mg (0.55 mmol) of dicyclohexylcarbodiimide (DCC) was added. The solution was stirred at 0 °C for 30 min. Then a solution 410 mg (0.46 mmol) of HCl·Arg(Tos)-Gly-Asp(OBzl)-Phe- OBzl, which was prepared by following a known procedure,<sup>24</sup> in 30 mL of anhydrous THF was added. At 0 °C the reaction mixture was adjusted to pH 8.5 with N-methylmorpholine (NMM), then stirred at room temperature for 8 h, and TLC (chloroform/methanol, 5:1) indicated the disappearance of **2**. The precipitates of dicyclohexylurea (DCU) were removed by filtration. The filtrate was evaporated under reduced pressure, and the residue was dissolved in 50 ml of ethyl acetate. The solution was washed successively with a 5% aqueous solution of sodium bicarbonate, a 5% aqueous solution of citric acid and a saturated aqueous solution of sodium chloride. The organic phase was dried over

anhydrous sodium sulfate. After filtration and evaporation under reduced pressure the obtained residue was purified on silica gel column (chloroform/ether, 30:1) to provide 510 mg (94%) of the title compound as yellowish powder. Mp 107 - 108 °C,  $[\alpha]_D^{20} = -12.0$  ( $c = 0.10$ , MeOH), ESI-MS 1198  $[M + H]^+$ .

**17 $\beta$ -[Aminoethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Val-OBzl]-androst-1,4- diene-3-one (3b)**

According to the procedure of the preparation of **3a** from 200 mg (0.50 mmol) of **2** and 380 mg (0.46 mmol) of HCl·Arg(Tos)-Gly-Asp(OBzl)-Val-OBzl, which was prepared by following a known procedure, to provide 460 mg (86%) of the title compound as yellowish powder. Mp 105 - 106 °C,  $[\alpha]_D^{20} = -4.0$  ( $c = 0.13$ , MeOH), ESI-MS 1150  $[M + H]^+$ .

**17 $\beta$ -[Aminoethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Ser-OBzl]-androst-1,4- diene-3-one (3c)**

According to the procedure of the preparation of **3a** from 180 mg (0.47 mmol) of **2** and 400 mg (0.5 mmol) of HCl·Arg(Tos)-Gly-Asp(OBzl)-Ser-OBzl, which was prepared by following a known procedure, to provide 500 mg (86%) of the title compound as yellowish powder. Mp 104 - 105 °C,  $[\alpha]_D^{20} = -12.0$  ( $c = 0.11$ , MeOH), ESI-MS 1138  $[M + H]^+$ .

**Synthesis of 17 $\beta$ -(aminocarbionylethylcarbonyl-Arg-Gly-Asp-Phe)-and- rost-1,4- diene-3-one (4a)**

At 0 °C a mixture of 400 mg (0.33 mmol) of 17 $\beta$ -[amino-carbonylethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Phe-OBzl]-androst-1,4-diene-3-one (**3a**), 1.7 mL of dime-thyl sulfide, 1.7 mL of phenyl methyl ether and 8.5 mL of CF<sub>3</sub>CO<sub>2</sub>H/CF<sub>3</sub>SO<sub>3</sub>H (4:1) was

stirred for 1 h. After removing  $\text{CF}_3\text{CO}_2\text{H}/\text{CF}_3\text{SO}_3\text{H}$  the residue was triturated with ether and then purified on Sephadex-G10 column (water: acetic acid, 100: 5). The fraction was lyophilized and 220 mg (76%) of **4a** was obtained as yellowish powder. Mp 114 - 116 °C,  $[\alpha]_{\text{D}}^{20} = -19.0$  (c = 0.1, MeOH), ESI-MS 877  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 500 MHz)  $\delta = 10.90$  (b, 2H), 8.13 (s, 1H), 8.00 (s, 1H), 7.96 (s, 1H), 7.90 (s, 1H), 7.80 (s, 1H), 7.05 - 7.25(m, 5H), 6.35 (dd,  $J = 10.0$  Hz,  $J = 3.2$  Hz, 1H), 6.30 (d,  $J = 3.2$  Hz, 1H), 6.10 (d,  $J = 3.2$  Hz, 1H), 4.86 (m, 1H), 4.55 (m, 1H), 4.23 (m, 1H), 4.20 (b, 1H), 4.10 (s, 2H), 3.58 (t,  $J = 10.0$  Hz, 1H), 3.16 (m, 1H), 3.05 (m, 1H), 2.75 (m, 1H), 2.65 (m, 2H), 2.46 (t,  $J = 12.0$  Hz, 4H), 2.42 (b, 1H), 1.45 - 2.30 (m, 23H), 1.46 (s, 3H), 1.16 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 125 MHz)  $\delta = 187.2, 180.4, 178.0, 177.4, 175.2, 171.3, 163.5, 157.8, 144.2, 141.2, 139.4, 136.3, 132.6, 129.8, 128.2, 127.6, 123.5, 64.4, 60.1, 59.7, 55.9, 51.5, 46.2, 45.1, 43.1, 42.8, 40.1, 38.5, 37.3, 35.8, 31.1, 30.2, 30.0, 29.0, 28.9, 27.1, 25.8, 24.9, 18.4, 17.2, 15.7$ . Anal. Calcd. for  $\text{C}_{44}\text{H}_{60}\text{N}_8\text{O}_{11}$ : C, 60.26; H, 6.90; N, 12.78. Found: C, 60.45; H, 7.16; N, 12.56.

### **Synthesis of 17 $\beta$ -(aminocarbonylethylcarbonyl-Arg-Gly-Asp-Val)-androst-1,4-diene-3-one (4b)**

Using the procedure of preparing **4a** from 400 mg (0.34 mmol) of 17 $\beta$ -[aminocarbonylethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Val-OBzl]-androst-1,4-diene-3-one (**3b**) 230 mg (81%) of **4b** was obtained as yellowish powder. Mp 114 - 115 °C,  $[\alpha]_{\text{D}}^{20} = -10.0$  (c = 0.11, MeOH), ESI-MS 829  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 500 MHz)  $\delta = 10.90$  (b, 2H), 8.13 (s, 1H), 8.00 (s, 1H), 7.96 (s, 1H), 7.90 (s, 1H), 7.80 (s, 1H), 6.35 (dd,  $J = 11.0$  Hz,  $J = 3.2$  Hz, 1H), 6.29 (d,  $J = 3.2$  Hz, 1H), 6.09 (d,  $J = 3.0$  Hz, 1H), 4.86 (m, 1H), 4.55 (m, 1H), 4.53 (m, 1H), 4.20 (b, 1H), 4.10 (s, 2H), 3.58 (t,  $J = 10.0$  Hz, 1H),

3.16 (m, 1H), 2.78 (d,  $J = 11.0$  Hz, 2H), 2.75 (m, 1H), 2.65 (m, 2H), 2.46 (t,  $J = 12.0$  Hz, 4H), 2.33 (b, 1H), 1.45 - 2.30 (m, 21H), 1.46 (s, 3H), 1.16 (s, 3H), 1.01 (d,  $J = 11.0$  Hz, 6H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta = 187.3, 180.2, 178.4, 177.2, 175.2, 171.3, 163.0, 157.6, 144.2, 139.7, 136.1, 132.6, 129.1, 123.5, 64.3, 60.2, 60.0, 55.4, 51.2, 46.2, 45.1, 43.1, 42.9, 40.1, 38.8, 37.2, 35.8, 31.1, 30.2, 30.0, 29.6, 28.9, 27.1, 25.8, 24.9, 18.4, 17.2, 16.5, 15.7$ . Anal. Calcd. for  $\text{C}_{40}\text{H}_{60}\text{N}_8\text{O}_{11}$ : C, 57.96; H, 7.30; N, 13.52. Found: C, 58.17; H, 7.46; N, 13.29.

### **Synthesis of 17 $\beta$ -(aminocarbonylethylcarbonyl-Arg-Gly-Asp-Ser)-androst-1,4-diene-3-one (4c)**

Using the procedure of preparing **4a** from 400 mg (0.34 mmol) of 17 $\beta$ -[aminocarbonylethylcarbonyl-Arg(Tos)-Gly-Asp(OBzl)-Ser-OBzl]-androst-1,4-diene-3-one (**3c**) 230 mg (82%) of **4c** was obtained as yellowish powder. Mp 111 - 112 °C,  $[\alpha]_{\text{D}}^{20} = -18.0$  ( $c = 0.12$ , MeOH), ESI-MS 817  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (DMSO- $d_6$ , 500 MHz)  $\delta = 11.00$  (b, 2H), 8.12 (s, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 7.80 (s, 1H), 6.35 (dd,  $J = 10.0$  Hz,  $J = 3.2$  Hz, 1H), 6.30 (d,  $J = 3.2$  Hz, 1H), 6.10 (d,  $J = 3.2$  Hz, 1H), 4.86 (m, 1H), 4.55 (m, 1H), 4.53 (m, 1H), 4.20 (b, 1H), 4.10 (s, 2H), 4.02 (d,  $J = 12.0$  Hz, 2H), 3.58 (t,  $J = 10.0$  Hz, 1H), 3.16 (m, 1H), 2.78 (d,  $J = 11.0$  Hz, 2H), 2.65 (m, 2H), 2.46 (t,  $J = 12.0$  Hz, 4H), 2.30 (b, 1H), 1.45 - 2.30 (m, 20H), 1.46 (s, 3H), 1.16 (s, 3H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125 MHz)  $\delta = 187.2, 180.0, 178.2, 175.2, 175.0, 170.2, 164.0, 144.2, 139.2, 136.6, 132.6, 128.2, 123.5, 63.9, 60.4, 59.8, 56.7, 55.0, 50.5, 46.7, 44.3, 43.2, 42.1, 40.0, 38.5, 37.6, 35.2, 31.6, 30.3, 30.0, 29.2, 28.9, 25.8, 24.9, 18.4, 17.2, 15.7$ . Anal. Calcd. for  $\text{C}_{38}\text{H}_{56}\text{N}_8\text{O}_{12}$ : C, 55.87; H, 6.91; N, 13.72. Found: C, 56.09; H, 7.07; N, 13.50.

### **Stereoview of intramolecular hydrogen bond defined three-component conjugates**

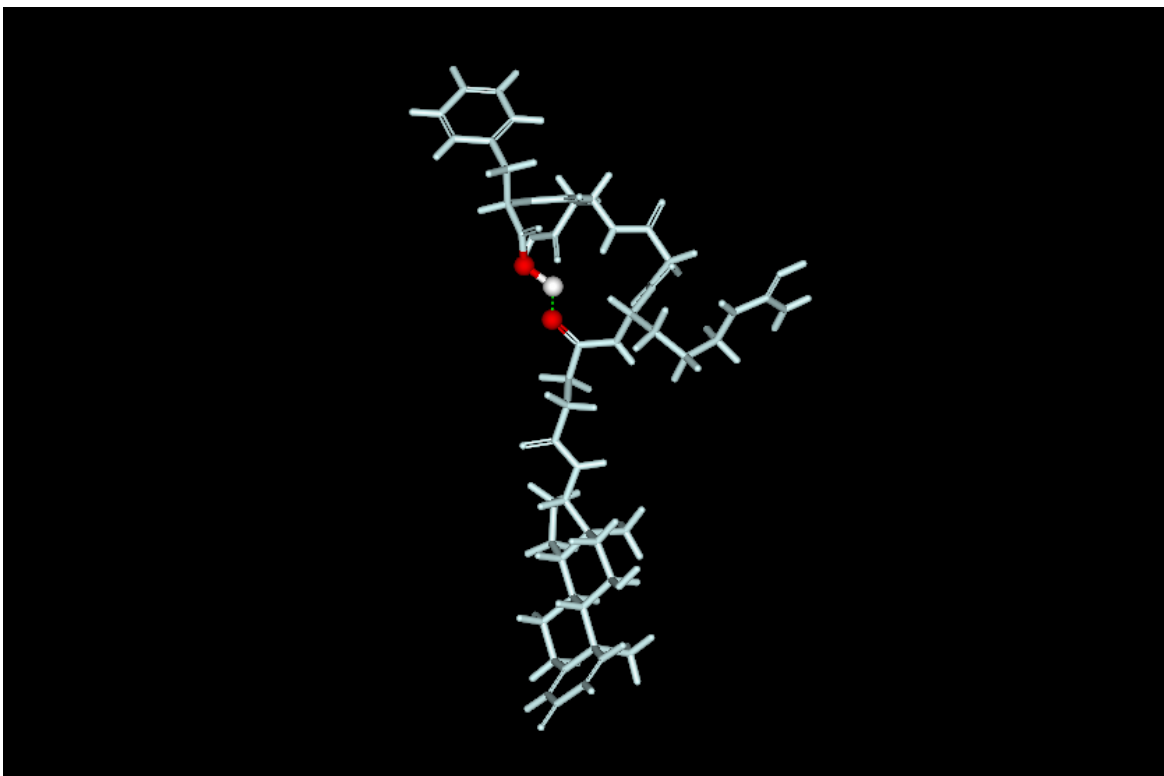


Figure 1 Stereoview of 17 $\beta$ -(aminoethylcarbonyl-Arg-Gly-Asp-Phe)-androst-1,4-diene-3-one.

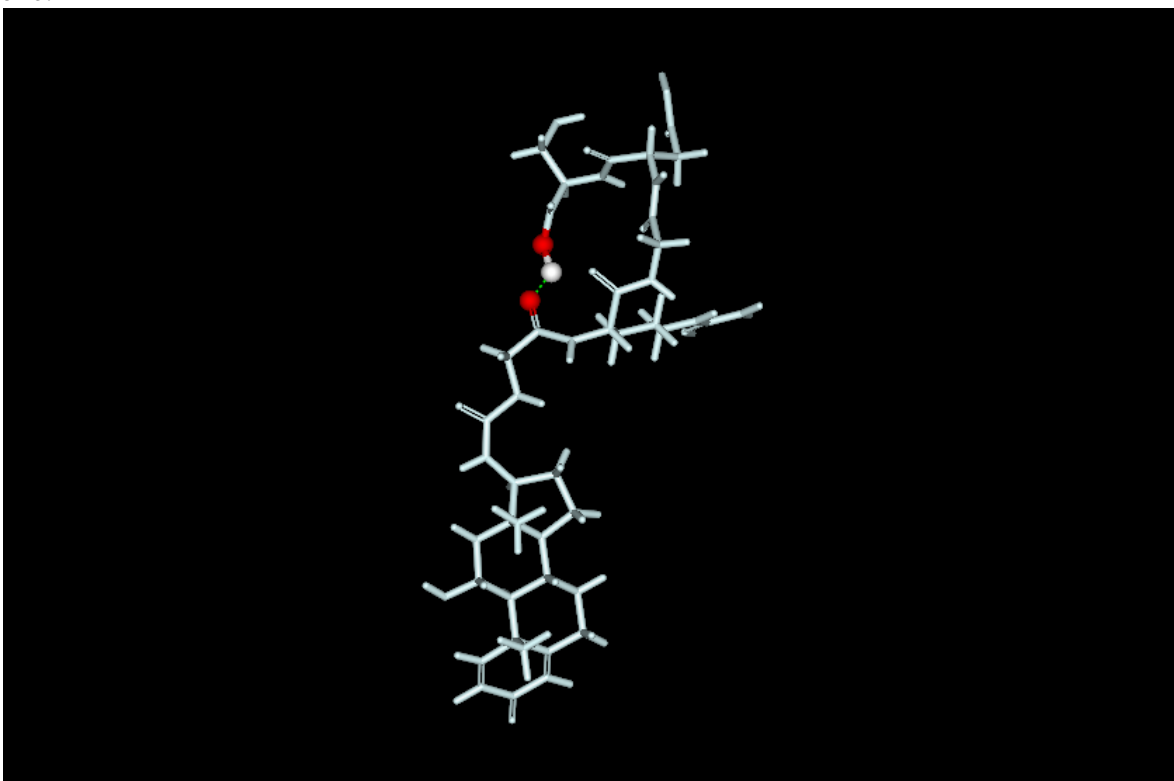


Figure 2 Stereoview of 17 $\beta$ -(aminoethylcarbonyl-Arg-Gly-Asp-Ser)-androst-1,4-diene-3-one.

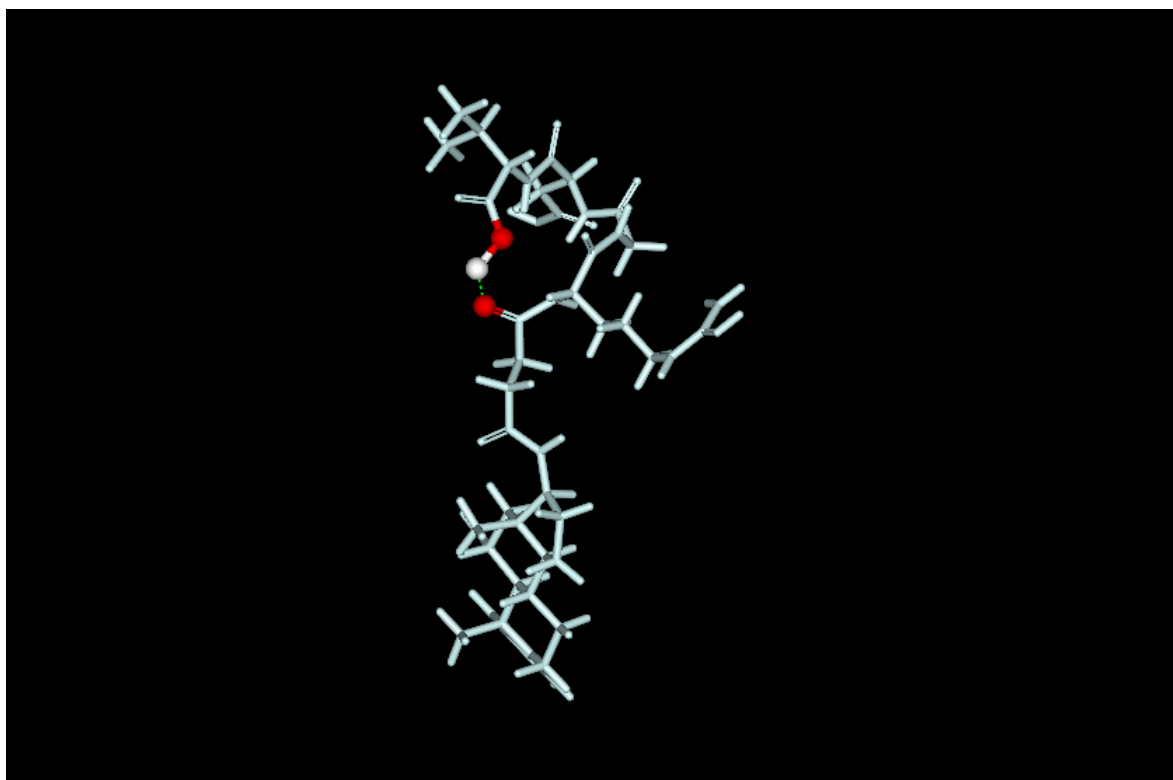


Figure 3 Stereoview of 17 $\beta$ -(aminoethylcarbonyl-Arg-Gly-Asp-Val)-androst-1,4-diene-3-one.

### Food and water for mice

Here we induced the mice to develop osteoporosis by intramuscularly injecting of 6.3 mg/kg of prednisone twice a week for 4 weeks. In order to help the mice developing osteoporosis the mice in model group (prednisone alone) and **4a-c** treating groups were given special food of low calcium and phosphorus (0.1 % of calcium and 0.4 % of phosphorus) and distilled water (**References:** 1. A. Gomes, S. Haldar, B. Giri, R. Mishra, A. Saha, S. Dasgupta, A. Gomes, Experimental osteoporosis induced in female albino rats and its antagonism by Indian black scorpion (*Heterometrus bengalensis* C.L. Koch) venom. *Toxicon* **2009**, 53, 60-68. 2.A.A.S. Islam, L.R.H. Yoshikawa, Y. Shiratsuchi, M. Ohishi, Healing of fractures in osteoporotic rat mandible shown by the expression of bone morphogenetic protein-2 and tumour necrosis factor- $\alpha$ . *British Journal of Oral and*



Maxillofacial Surgery **2005**, 43 383-391). On the other hand, in order to show the bone mineral density, calcium content and phosphorous content of health mice the mice in blank control were give normal food of standard calcium and phosphorus (1.76 % of calcium and 1.01 % of phosphorus) and running water. Since the present paper aimed at the utility of **4a-c** for preventing the glucocorticoids treated patients to develop secondary osteoporosis, the model group (prednisone alone) served also the positive control. Therefore the comparison of the bone mineral density, calcium content and phosphorous content of the mice in the model group and in **4a-c** treated groups should accurately explain the efficacy of **4a-c**.