Chapter 4

Modification of Sex Hormones with RGD-Peptide: A Strategy of Improving HRT and Other Secondary Osteoporosis Therapy

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Additional information is available at the end of the chapter

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1. Introduction

In the time of transition from premenopausal state to postmenopausal state the capacity of ovary producing sex hormones including estrogens, progesterone and testosterone cuts down [1]. Due to the menopause the level of serum oestrogen dramatically decreases, which increases the production of bone-resorbing cytokines and osteoblasts and then increases the number and activity of osteoclast, thereby increasing the bone loss [2]. Hormonal replacement therapy (HRT) is able to prevent bone loss for sex hormones-deficient menopausal women and consequently is of clinical importance for the treatment of osteoporosis. [1-3] In Europe and USA the osteoporosis prevention of 25-50% of the post-menopausal women rely on HRT [2,5, 6]. In past years, however, the large international studies, such as the randomized Woman Health Initiative, the observational Million Women Study and the Women's International Study of long Duration, discussed both of the adverse and beneficial effects of post-menpausal HRT [7]. In respect of the adverse effects, the discussion was focused on HRT induced risk of breast cancer [8-11], venous thromboembolism [12], stroke and myocardial infarction [13], as well as coronary heart diseases [14]. To limit these adverse effects a series of regimens of HRT, such as continuous combination of oestrogen and progestogen or continuous oestrogen and interruptted progestogen [15], and with dehydroepiandrosterone as a new strategic tool [16], were developed. In general these regimens confer no positive result, and thus new strategies are still needed.

Osteoporosis relates to both the decrease of the formation of osteoblast-modulated bone and the increase of the resorption osteoclast-modulated bone. Estrogen directly up-modulates the activity and the proliferation of osteoblasts, and/or regulats the gene expression in osteoblasts

and osteoclasts [17-20]. Bone resorption is regulated by the adhesion of osteoclasts to the surface of the bone, which is mediated by the receptor $\alpha_v\beta_3$ integrin and its recognition to RGD (Arg-Gly-Asp) containing protein of osteoclasts [21]. These suggest that the activity and proliferation of osteoblasts and the adhesiveness of osteoclasts can be simultaneously up-regulated with estrogen and down-regulated with RGD peptide, respectively. On the other hand, it was explored that the covalent modifications of hydrocortisone and estrone with kyotorphin (a dipeptide, Tyr-Arg) may increase the analgesic activities of hydrocortisone and estrone [22], as well as the covalent modifications of hydrocortisone and prednisolone with urotoxins (Gly-Asp-Gly, His-Gly-Gly, His-Gly-Lys and His-Gly-Lys-$NHNH_2$) may increase the immunosuppressive activities of hydrocortisone and prednisolone [23]. Similarly, the anti-osteoporosis activities of estrone and estradiol were enhanced by growth hormone releasing peptides (GHRPs: Tyr-Gly-Gly-Phe-Met-NH_2, Tyr-Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Leu-NH_2, Tyr-Gly-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Gly-NH_2 and Tyr-Gly-Gly-Phe-Gly) [24-26]. In this context a strategy to enhance anti-osteoporosis potency and reduce adverse effects of HRT was practiced by covalent modifications of sex hormone with RGD-peptides.

2. Covalent modifications of estrogen with RGD-peptides and ip treated ovariectomy mice

Estrogens including estrone, estradiol, estriol, conjugated estrogen and tibolone have been widely used in HRT. Upon the promotion of the enzyme both estrone and estradiol can be converted to ertriol. Conjugated estrogen is an oral estrogen isolated from the urine of gravid horse and contains estrone monosodium sulfate (50.0% - 63.0%), equilin monosodium sulfate (22.5% - 32.5%), a few of 17α-estradiol monosodium sulfate and equilenin monosodium sulfate. Tibolone is an analog of norethynodrel. Of these estrogens, estrone and estradiol are the common parents and estradiol is the major agents of HRT. Thus estradiol and estrone were covalently modified by RGD-peptides (**1-9**, Figure 1) and evaluated with ip treated ovariectomy mice [27].

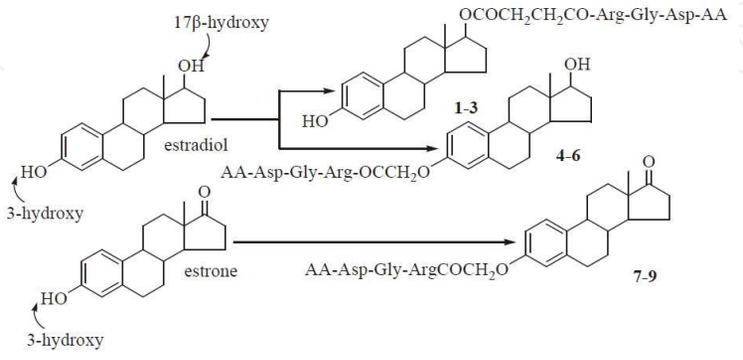

Figure 1. Structures of conjugates of estradiol-RGD-tetrapeptides. In **1**, **4,7** AA = Ser, in **2**, **5, 8** AA = Val, in **3**, **6, 9** AA = Phe.

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2.1. Covalent modification of estradiol with RGD-tetrapeptides decreasing bone turnover

Using succinyl group as the linker the covalent modifications of the 17β-hydroxy of estradiol with RGD-tetrapeptides provided conjugates **1-3**, and using carbonylmethyl group as the linker the covalent modifications of the 3-hydroxy of estradiol or estrone with RGD-tetrapeptides provided conjugates **4**-**9** (Figure 1). The changes of the levels of the serum calcium and serum alkaline phosphatase (ALP) of the mice receiving ip injection of **1-6** for 4 weeks are shown in Figure 2. After the treatments of conjugates **1-6** the levels of serum calcium and serum ALP of the treated mice are significantly lower than that of ovariotomy and estradiol treated mice. This means that the ip injection efficacy of conjugates **1-6** in decreasing the serum calcium and serum ALP is significantly higher than that of estradiol. Due to serum ALP been the biomarker of bone turnover low serum ALP means conjugates **1-6** benefits the inhibition of bone turnover.

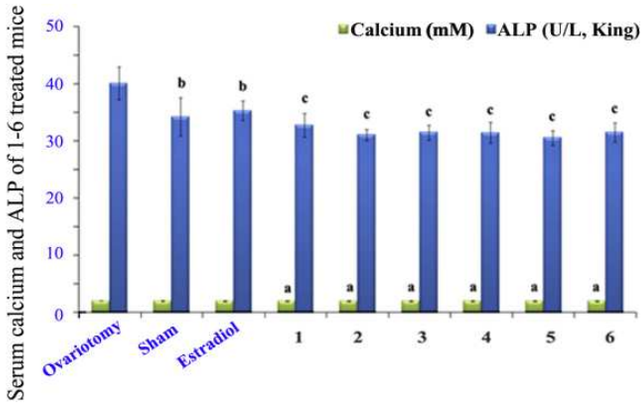

Figure 2. Serum calcium and serum ALP of **1-6** treated mice. Dose = 110.3 μmol/kg, n=12, a) Compared to ovariotomy P<0.05; b) Compared to ovariotomy P<0.01; c) Compared to ovariotomy and estradiol P<0.01. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

2.2. Covalent modification of estradiol with RGD-tetrapeptides inhibiting bone loss

The effects of ip injection of **1-6** for 4 weeks on the bone loss of the mice are shown in Figure 3. The level of bone loss is represented with the weight of dry femur and the weight of femur ash. The data indicate that the weight of dry femur and the weight of femur ash of **1-6** treated mice are significantly higher than those of ovariotomy and estradiol treated mice. This means that ip injection efficacy of **1-6** in inhibiting the bone loss is significantly higher than that of estradiol, and the covalent modification of estradiol with RGD-tetrapeptides benefits the inhibition of bone loss.

2.3. Covalent modification of estrone with RGD-tetrapeptides inhibiting bone turnover

Using carbonylmethyl group as the linker the covalent modifications of the 3-hydroxy of estrone with RGD-tetrapeptides provided conjugates **7**-**9** (Figure 4). The effects of ip injection

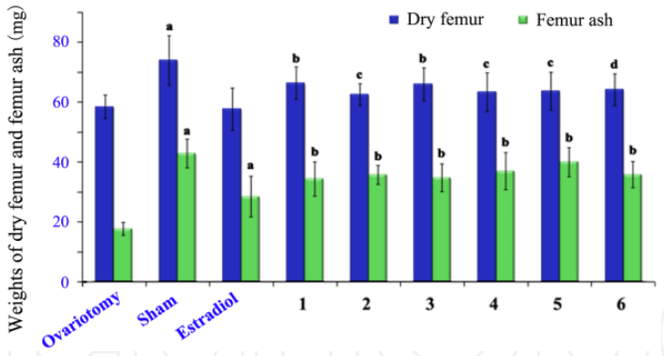

Figure 3. Weight of dry femur and femur ash of **1-6** treated mice. Dose =110.3 μmol/kg, n=12; a) Compared to ovariotomy P<0.01; b) Compared to ovariotomy and estradiol P<0.01; c) Compared to ovariotomy and estradiol P<0.05; d) Compared to ovariotomy P<0.01, to estradiol P<0.05. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

of **7-9** for 4 weeks on serum calcium and serum ALP of the mice are shown in Figure 4. The serum calcium and serum ALP of **7-9** treated mice are significantly lower than that of ovariotomy and estrone treated mice. This means that the ip injection efficacy of conjugates **7-9** in decreasing the serum calcium and serum ALP is significantly higher than that of estrone. Due to serum ALP reflecting the level of bone turnover and low serum ALP corresponding with low bone turnover, **7-9** benefits the inhibition of bone turnover.

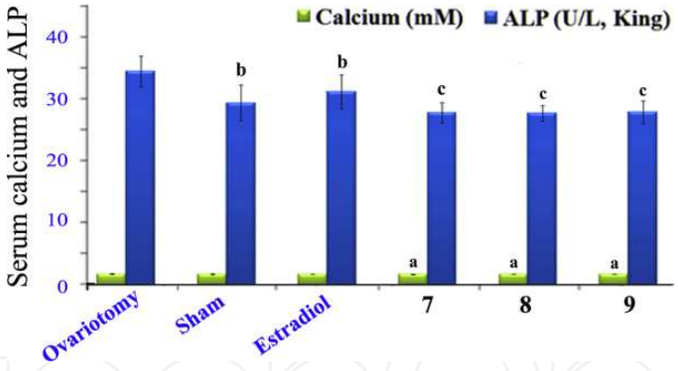

Figure 4. Serum calcium and ALP of **7-9** treated mice. Dose = 110.3 μmol/kg, n=12, a) Compared to ovariotomy P<0.05; b) Compared to ovariotomy P<0.01; c) Compared to ovariotomy and estrone P<0.01. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

2.4. Covalent modification of estrone with RGD-tetrapeptides preventing bone loss

The effect of ip injection of **7-9** for 4 weeks on the bone loss of the mice is shown in Figure 5. The weight of dry femur and the weight of femur ash of **7-9** treated mice are significantly higher than that of ovariotomy and estrone treated mice. Due to the weight of dry femur and the weight of femur ash reflecting the level of bone loss of osteoporosis mice this comparison means that ip injection efficacy of **7-9** in inhibiting bone loss is significantly higher than that of estrone and the covalent modification enhances the inhibition of estrone in bone loss.

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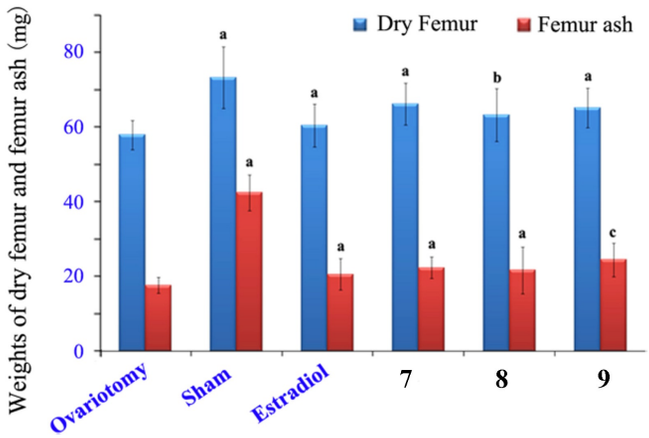

Figure 5. Weight of dry femur and femur ash of conjugates **7-9** treated mice. Dose =110.3 μmol/kg, n=12; a) Compared to ovariotomy P<0.01; b) Compared to ovariotomy P<0.05; c) Compared to ovariotomy P<0.01, to estrone P<0.05. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

2.5. Covalent modification of estrogen with RGD-tetrapeptides inducing no endometrial cell hyperplasia

The effects of ip injection of **1-9** for 4 weeks on endometrial cell hyperplasia of the mice were also observed. The weight of the uteri of ovariotomy, estradiol and estrone treated mice is significantly higher than that of **1-9** treated mice. Due to the weight of the uteri reflecting the level of endometrial cell hyperplasia of treated mice this comparison means that ip injection efficacy of **1-9** in inducing endometrial cell hyperplasia is significantly lower than that of estradiol and estrone and the covalent modification induces no observable endometrial cell hyperplasia.

2.6. Summary of covalent modification of estrogen with RGD-tetrapeptides

With RGD-tetrapeptides modifying one hydroxyl group of estradiol and estrone resulted in 9 conjugates. On ovariotomy mouse model and at 110.3 μmol/kg of ip dose their anti-osteoporosis activities were significantly higher than that of estradiol and estrone themselves. In contrast to estradiol and estrone themselves, the anti-osteoporosis therapy of these conjugates induced no endometrial cell hyperplasia. It is commonly accepted that osteoporosis relates to both the decrease in bone formation modulated by osteoblasts and the increase in bone resorption modulated by osteoclasts. In HRT, estradiol and estrone are used to treat the decrease in skeletal muscle and bone by the direct modulation of osteoblastic activity and proliferation or by the regulation of gene expression in osteoblasts and osteoclasts. Bone resorption is regulated by the binding of osteoclasts to the bone surface and, therefore, depends upon osteoclast adhesiveness. This bone adhesion process is mediated by RGD-tetrapeptides binding integrin receptor on cell surface. This action of RGD-tetrapeptides should be responsible for both the increased anti-osteoporosis activity and the decreased endometrial cell hyperplasia of the conjugates. Due to ovariotomy mouse model simulates the bone loss

condition of postmenopausal women these RGD-tetrapeptides modified estradiol and estrone should be promising candidates for HRT use.

3. Covalent modification of estrogen with RGD-octapeptides and orally treated ovariectomy mice

It was explored that the modification of RGD-tetrapeptides with oligopeptides usually increased their bioactivities [28, 29], suggesting the modification of RGD-tetrapeptides with RGD-tetrapeptides may result in increase of the activity of down-regulating proliferation of osteoblasts and the adhesiveness of osteoclasts. In this context estradiol and estrone were modified with RGD-octapeptides (**10-21**, Figure 6) to evaluate the oral activity on ovariectomy mice [30, 31].

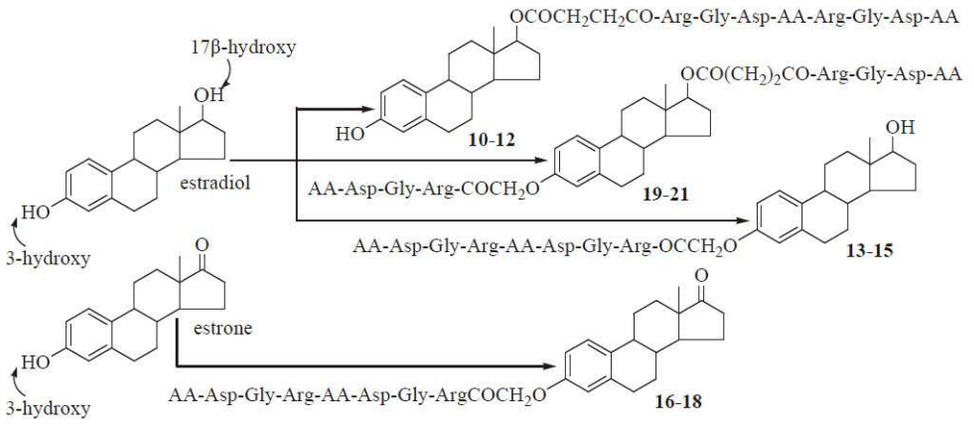

Figure 6. Structures of conjugates of RGD-octapeptides and estradiol. In **10**, **13, 16, 19** AA = Ser, in **11**, **14**, **17, 20** AA = Val, in **12**, **15, 18, 21** AA = Phe.

3.1. Covalent modification of estradiol with RGD-octapeptides inhibiting bone turnover

Using succinyl group as the linker the 17β-hydroxy of estradiol was modified with RGD-octapeptides and provided **10-12**, using carbonylmethyl group as the linker the 3-hydroxy of estradiol was modified with RGD-octapeptides and provided **13-15** (Figure 6). The effect of oral administration of **10-15** for 4 weeks on serum calcium and serum ALP of the mice are shown in Figure 7. The data indicate that the serum calcium and serum ALP of **10-15** treated mice are significantly lower than that of ovariotomy and estradiol treated mice. This means that the frequency of bone turnover of **10-15** orally treated mice is significantly lower than that of estradiol treated mice, the efficacy of oral **10-15** in inhibiting bone turnover is significantly higher than that of estradiol.

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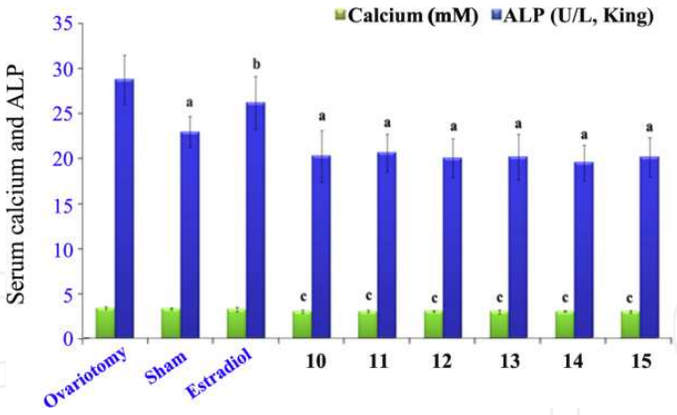

Figure 7. Serum calcium and ALP of **10-15** treated mice. Dose = 110.3 nmol/kg, n=12, a) Compared to ovariotomy and estradiol P<0.01; b) Compared to ovariotomy P<0.01; c) Compared to ovariotomy P<0.01, to estradiol P<0.05. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

3.2. Covalent modification of estradiol with RGD-octapeptides preventing bone loss

The effect of orally administration of **10-15** for 4 weeks on the bone loss of the treated mice is shown in Figure 8, of which the activity is represented with dry femur weight and femur ash weight. The data indicate that both the weights of dry femur and femur ash of **10-15** treated mice are significantly higher than that of ovariotomy and estradiol treated mice. This means that when orally dosed **10-15** effectively inhibit the mice to lose femur and their efficacy is significantly higher than that of estradiol, and the covalent modification of estradiol benefits the inhibition of bone loss.

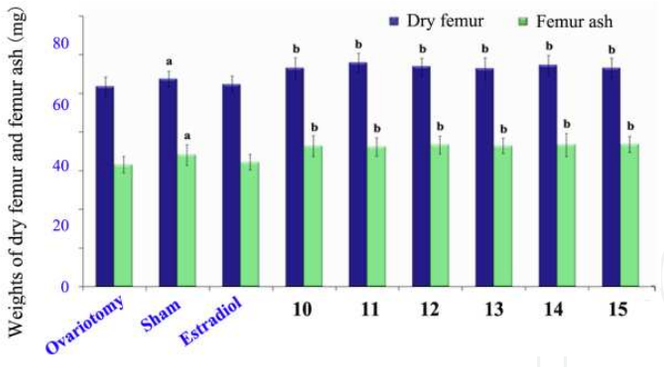

Figure 8. Weight of dry femur and femur ash of conjugates **10-15** treated mice. Dose =110.3 nmol/kg, n=12; a) Compared to ovariotomy P<0.05; b) Compared to ovariotomy and estradiol P<0.01. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

3.3. Covalent modification of estrone with RGD-octapeptides inhibiting bone turnover

Using carbonylmethyl group as the linker the 3-hydroxy of estrone was modified with RGD-octapeptides and provided **16-18** (Figure 6). The effects of oral administration of **16-18** for 4 weeks on serum calcium and serum ALP of the mice are shown in Figure 9. The data indicate

that the serum calcium and serum ALP of **16-18** treated mice are significantly lower than that of ovariotomy and estrone treated mice. This means that the frequency of bone turnover of **16-18** orally treated mice is significantly lower than that of estrone treated mice, the efficacy of oral **16-18** in inhibiting bone turnover is significantly higher than that of estrone.

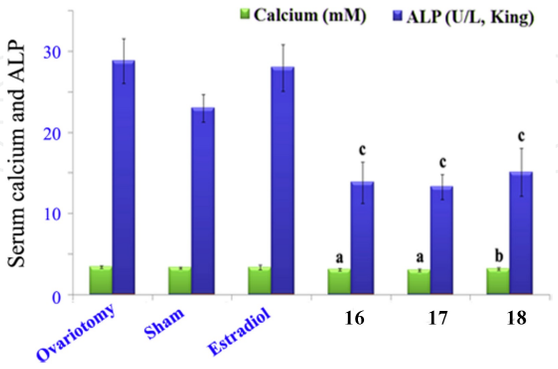

Figure 9. Serum calcium and serum ALP of **16-18** treated mice. Dose = 110.3 nmol/kg, n=12, a) Compared to ovariotomy P<0.01, to estrone P<0.05; b) Compared to ovariotomy P<0.05; c) Compared to ovariotomy and estrone P<0.01. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

3.4. Covalent modification of estrone with RGD-octapeptides preventing bone loss

The effect of orally administration of **16-18** for 4 weeks on the bone loss of the treated mice is shown in Figure 10, their activities are represented with dry femur weight and femur ash weight. The data indicate that both the weights of dry femur and femur ash of **16-18** treated mice are significantly higher than that of ovariotomy and estradiol treated mice. This means that upon oral administration **16-18** effectively inhibit the mice losing femur, their efficacies are significantly higher than that of estrone, and the covalent modification of estrone prevents the bone loss.

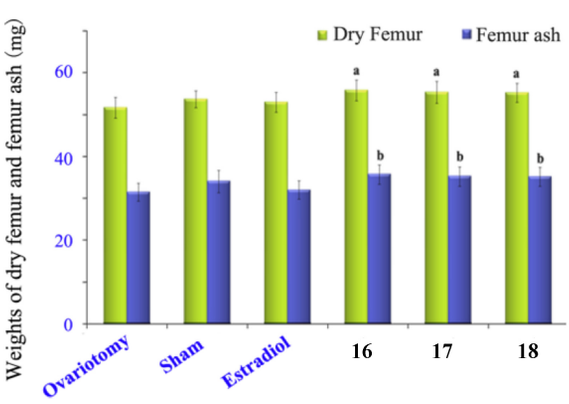

Figure 10. Weight of dry femur and femur ash of conjugates **16-18** treated mice. Dose =110.3 nmol/kg, n=12; a) Compared to ovariotomy and estrone P<0.01; b) Compared to ovariotomy P<0.01, to estrone P<0.05. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

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3.5. Covalent modification of estradiol with two RGD-octapeptides inhibiting bone turnover

Using succinyl group as the linker of the 17β-hydroxy and using carbonylmethyl group as the linker of the 3-hydroxy estradiol was simultaneously modified with RGD-tetrapeptides and provided **19-21** (Figure 6). The effects of oral administration of **19-21** for 4 weeks on serum calcium and serum ALP of the mice are shown in Figure 11. The data indicate that the serum calcium and serum ALP of **19-21** treated mice are significantly lower than that of ovariotomy and estradiol treated mice. This means that the frequency of bone turnover of **19-21** orally treated mice is significantly lower than that of estradiol treated mice, the efficacy of oral **19-21** in inhibiting bone turnover is significantly higher than that of estradiol.

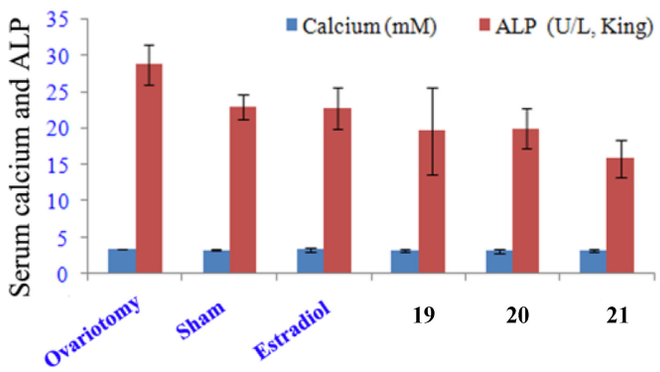

Figure 11. Serum calcium and serum ALP of **19-21** treated mice. Dose = 110.3 nmol/kg, n=12. The statistical analysis of the data was carried out by use of an ANOVA test and $p<0.05$ was considered significant. For serum calcium a) Compared to ovariotomy $P<0.05$; For serum ALP b) compared to ovariotomy and estradiol $P<0.01$.

3.6. Covalent modification of estradiol with two RGD-octapeptides preventing bone loss

The effect of orally administration of **19-21** for 4 weeks on the bone loss of the treated mice is shown in Figure 12, their activities are represented with dry femur weight and femur ash weight. The data indicate that both the weights of dry femur and femur ash of **19-21** treated mice are significantly higher than that of ovariotomy and estradiol treated mice. This means that upon oral administration **19-21** effectively inhibit the mice losing femur, their efficacies are significantly higher than that of estradiol, and the covalent modification of estrone prevents the bone loss.

3.7. Covalent modification of estradiol with RGD-octapeptides inducing no endometrial cell hyperplasia

The effect of orally administration of **10-21** for 4 weeks on the endometrial cell hyperplasia of the mice was observed, of which the inhibition is represented with uteri weight. The data indicate that the weight of the uteri of **10-21** treated mice is significantly lower than that of ovariotomy and estradiol treated mice. This means that, in contrast to estradiol and estrone, oral administration of **10-21** induces no observable endometrial cell hyperplasia, and the covalent modification of estradiol and estrone with RGD-octapeptides limits the dose-related adverse effects of estradiol.

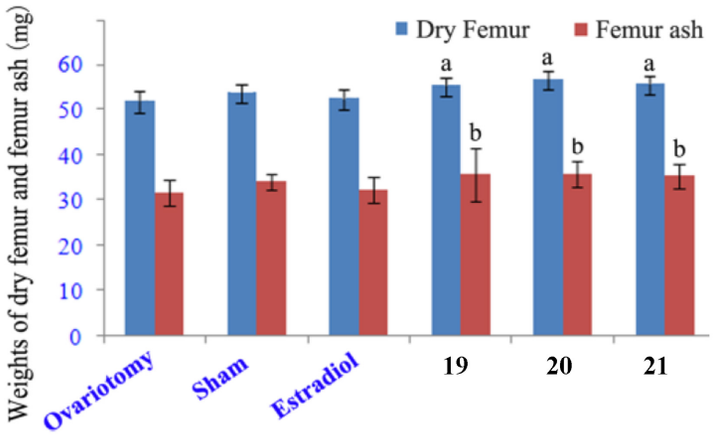

Figure 12. Weight of dry femur and femur ash of conjugates **19-21** treated mice. Dose =110.3 nmol/kg, n=12, weights of dry femurs and femur ashes are represented as X±SD mg; a) Compared to ovariotomy, estradiol P<0.01; b) Compared to ovariotomy P<0.01, to estradiol P<0.05.

3.8. Covalent modification of estradiol with RGD-octapeptides having no thrombosis risk

The effect of orally administration of **10-21** for 4 weeks on thrombosis risk of the mice was observed, of which the risk is represented with tail bleeding time. The data indicate that the tail bleeding time of **10-21** treated mice is significantly longer than that of ovariotomy, estradiol and estrone treated mice. This means that, in contrast to estradiol and estrone, oral administration of **10-21** induces no observable thrombosis risk, and the covalent modification of estradiol and estrone with RGD-octapeptides limits the dose-related adverse effects of estradiol.

3.9. Summary of covalent modification of estrogen with RGD-octapeptides

With RGD-octapeptides modifying one hydroxyl group of estradiol and estrone or with RGD-tetrapeptides simultaneously modifying two hydroxyl groups of estradiol resulted in 12 conjugates. On ovariotomy mouse model and at 110.3 nmol/kg of oral dose their anti-osteoporosis activities were significantly higher than that of estradiol and estrone themselves. In contrast to estradiol and estrone themselves, the anti-osteoporosis therapy of these conjugates induced no endometrial cell hyperplasia and thrombosis risk. Comparing to RGD-tetrapeptide modified estradiol and estrone the effective dose of RGD-octapeptide modified estradiol and estrone is 1000 folds lower. This means that the anti-osteoporosis efficacy of RGD-octapeptide modified estradiol and estrone is 1000 folds higher than that of RGD-tetrapeptide modified estradiol and estrone. Reasonably, this dramatically enhanced efficacy could attitude to the introduction of RGD-octapeptides. Furthermore, due to ovariotomy mouse model simulates the bone loss condition of postmenopausal women and high activity these RGD-octapeptides modified estradiol and estrone should be preferentially promising candidates for HRT use.

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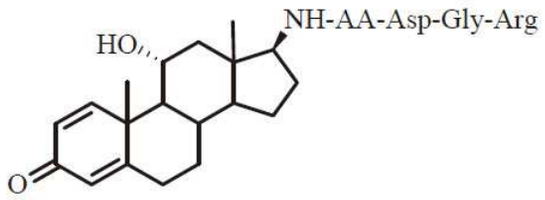
4. Direct covalent modification of androgen with RGD-tetrapeptides

In the improvements of the efficacy of HRT, the anti-osteoporosis efficacy of androgen is found to be higher than that of estrogen, inducing no endometrial cell hyperplasia and having no thrombosis risk. Particularly in the research of androgen, 17β-amino-11α-hydroxyandrost-1,4-diene-3-one is disclosed as a new androgen. Comparing to estrone and estrogen 17β-amino-11α-hydroxyandrost-1,4-diene-3-one has higher anti-osteoporosis activity and raises no endometrial cell hyperplasia and thrombosis risk. Thus 17β-amino-11α-hydroxyandrost-1,4-diene-3-one is selected as the androgen and directly and covalently modified with RGD-tetrapeptides (**22-24**, Figure 13) [32].



22-24

Figure 13. Structures of conjugates of androgen and RGD-tetrapeptides. In **22** AA = Ser, in **23** AA = Val, in **24** AA = Phe.

4.1. Direct covalent modification of androgen with RGD-tetrapeptides inhibiting bone turnover

The direct covalent modification of the 17β-amino of 17β-amino-11α-hydroxyandrost-1,4-diene-3-one (androgen) with RGD-tetrapeptides provided **22-24** (Figure 13). The effect of oral administration of **22-24** plus intramuscular prednisone for 4 weeks on serum calcium and serum ALP of the mice is shown in Figure 14. The data indicate that the serum calcium and serum ALP of oral administration of **22-24** plus intramuscular prednisone treated mice are significantly lower than that of prednisone alone and oral administration of estradiol plus intramuscular prednisone treated mice. This means that the frequency of bone turnover of **22-24** orally treated mice is significantly lower than that of androgen treated mice, the efficacy of oral **22-24** in inhibiting bone turnover is significantly higher than that of estradiol.

4.2. Direct covalent modification of androgen with RGD-tetrapeptides preventing bone loss

The effect of oral administration of **22-24** plus intramuscular prednisone for 4 weeks on the bone loss of the treated mice is shown in Figure 15, their activities are represented with dry femur weight and femur ash weight. The data indicate that both the weights of dry femur and femur ash of oral adminis-tration of **22-24** plus intramuscular prednisone treated mice are significantly higher than that of intramuscular prednisone alone and oral administration of estradiol plus intramuscular prednisone treated mice. This means that upon oral administra-

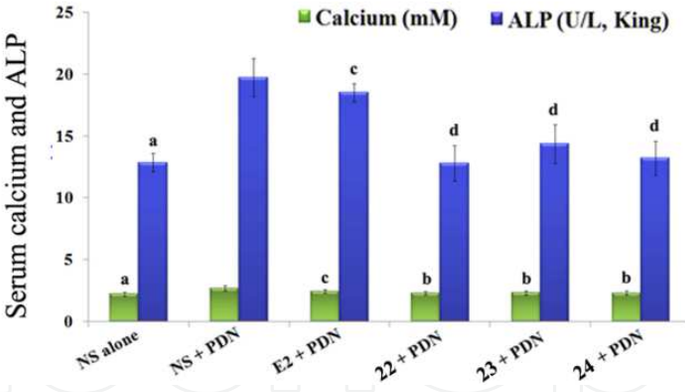


Figure 14. Serum calcium and ALP of **22-24** treated mice. ip Dose of prednisone (PDN): 6.3 mg/kg, twice a week; oral dose of **22-24**: 110 nmol/kg, once a day; oral dose of estradiol (E2): 110 nmol/kg, once a day; n = 12. a) Compared to NS + PND and E2 + PND $p < 0.01$; b) Compared to NS + PND $p < 0.01$, to E2 + PND $p < 0.05$; c) Compared to NS + PND $p < 0.05$; d) Compared to NS + PND and E2 + PND $p < 0.01$. The statistical analysis of the data was carried out by use of an ANOVA test and $p<0.05$ was considered significant.

tion **22-24** effectively inhibit the mice losing femur, their efficacies are significantly higher than that of estradiol, and the direct covalent modification of androgen prevents the bone loss.

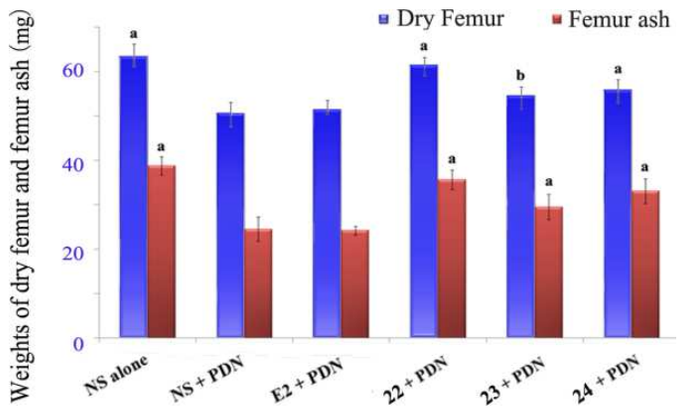


Figure 15. Weight of dry femur and femur ash of conjugates **22-24** treated mice. ip Dose of prednisone (PDN): 6.3 mg/kg, twice a week; oral dose of **22-24**: 110 nmol/kg, once a day; oral dose of estradiol (E2): 110 nmol/kg, once a day; n = 12. a) Compared to NS + PND and E2 + PND $p < 0.01$; b) Compared to NS + PND and E2 + PND $p < 0.05$. The statistical analysis of the data was carried out by use of an ANOVA test and $p<0.05$ was considered significant.

4.3. Direct covalent modification of androgen with RGD-tetrapeptides increasing total vBMD

CT measured 3D bone geometry and the size-independent vBMD, as well as pQCT quantitatively measured 3D bone geometry and size-independent vBMD were used to represent the anti-osteoporosis efficacy of **22-24** and are shown in Figure 16. The data indicates that the total vBMD of the femurs of NS plus intramuscular prednisone treated mice is significantly lower than that of the femurs of NS alone treated mice. This means that intramuscular administration of prednisone effectively induces the mice to decrease the total vBMD. The total vBMDs of the

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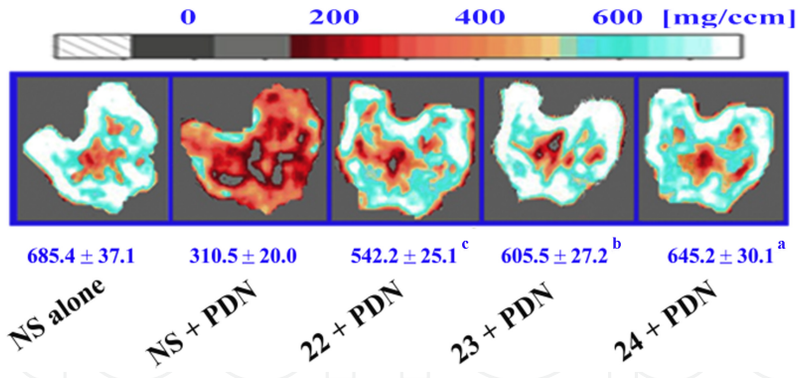

Figure 16. Total vBMD and images of pQCT scanning at a distance from the proximal femur growth palate corresponding to < 6 % of the total length of the femur of **22-24** treated mice. ip Dose of prednisone (PDN): 6.3 mg/kg, twice a week; oral dose of **22-24**: 110 nmol/kg, once a day; n = 12. a) Compared to NS alone , NS + PND, 23 + PDN and 22 + PDN p< 0.01; b) Compared to NS alone , NS + PND and 22 + PDN c) Compared to NS alone and NS + PND p< 0.01. The statistical analysis of the data was carried out by use of an ANOVA test and p<0.05 was considered significant.

femurs of oral administration of **22-24** plus intramuscular prednisone treated mice are significantly higher than that of the femurs of NS plus intramuscular prednisone treated mice. This means that upon oral administration **22-24** effectively prevent intramuscular administration of prednisone treated mice decreasing total vBMD.

4.4. Direct covalent modification of androgen with RGD-tetrapeptides increasing trabecular vBMD

Figure 17 indicates that the trabecular vBMD of the femurs of NS plus intramuscular administration of prednisone treated mice is significantly lower than that of the femurs of NS alone treated mice. This means that prednisone effectively induces the mice to decrease trabecular vBMD. The trabecular vBMDs of the femurs of oral administration of **22-24** plus intramuscular administration of prednisone treated mice are significantly higher than those of the femurs of NS plus intramuscular administration of prednisone treated mice. This means that upon oral administration **22-24** effectively prevent intra-muscular administration of prednisone treated mice decreasing trabecular vBMD.

4.5. Direct covalent modification of androgen with RGD-tetrapeptides inducing no endomtrial cell hyperplasia

The effect of oral administration of **22-24** plus intramuscular administration of prednisone for 4 weeks on the endometrial cell hyperplasia of the mice was observed, and their inhibition activities are represented with uteri weight. The data indicate that the weight of the uteri of oral administration of **22-24** plus intramuscular administration of prednisone treated mice is significantly lower than that of intramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that, in contrast to oral administration of estradiol, oral administration of **19-21** induces no observable endometrial cell hyperplasia, and the direct covalent modification of androgen with RGD-tetrapeptides induces no dose-related adverse effects of estradiol.

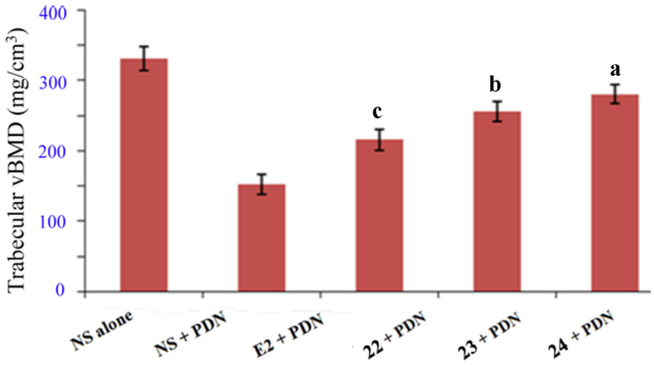

Figure 17. Trabecular vBMD of the femurs of the treated mice at a distance from the proximal femur growth palate corresponding with < 6 % of the total length of the femurs of **22-24** treated mice. a) Trabecular vBMD is represented with mean ± SD mg/cm^3, n = 12, PDN = prednisone, dose = 110 nmol/kg. b) Compared to NS + PDN, **22 +** PDN and **23 +** PDN p< 0.01; c) Compared to NS + PDN and **22 +** PDN p< 0.01; d) Compared to NS + PDN p< 0.01.

4.6. Direct covalent modification of androgen with RGD-tetrapeptides having no thrombosis risk

The effect of oral administration of **22-24** plus intramuscular administration of prednisone for 4 weeks on thrombosis risk of the mice was observed, and the risk is represented with tail bleeding time. The data indicate that the tail bleeding time of oral administration of **22-24** plus intramuscular administration of prednisone treated mice is significantly longer than that of ntramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that, in contrast to oral administration of estradiol, oral administration of **22-24** induces no observable thrombosis risk, and the direct covalent modification of androgen with RGD-tetrapeptides induces no dose-related adverse effects of estradiol.

4.7. Summary of direct covalent modification of androgen with RGD-tetrapeptides

RGD-octapeptides directly modifying the 17β-amino group of 17β-amino-11α-hydroxyandrost-1,4-diene-3-one was performed by amidation and resulted in 3 conjugates. On prednisone treated mouse model and at 110 nmol/kg of oral dose their anti-osteoporosis activities were significantly higher than that of estradiol. In contrast to estradiol, the anti-osteoporosis therapy of these conjugates induced no endometrial cell hyperplasia and thrombosis risk. Comparing to RGD-tetrapeptide modified estradiol the effective dose of RGD-octapeptide modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one is 1000 folds lower. This means that the anti-osteoporosis efficacy of RGD-octapeptide modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one is 1000 folds higher than that of RGD-tetrapeptide modified estradiol. Reasonably, this dramatically enhanced efficacy could attitude to the introduction of 17β-amino-11α-hydroxyandrost-1,4-diene-3-one. In addition to premenopausal women and in older men, secondary osteoporosis is common in the patients treated with glucocorticoids and in prostate cancer patients receiving androgen deprivation therapy (ADT) in particular. Glucocorticoids are ubiquitously prescribed in the fields of rheumatol-

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ogy, respirology, neurology, hematology, dermatology, gastroenterology, and transplant medicine. Chronic exposure to pharmacological doses of glucocorticoids causes multiple deleterious effects on osteopenia, osteoporosis and bone fracture. Prostate cancer is one of the most common diseases in the older men. After the surgery or radiation therapy the male patients with localized or metastatic prostate cancer are generally given ADT. Though male patients on ADT usually have good prognosis, osteoporosis is a very common consequence of this therapy and up to 20% of the patients will fracture within 5 years. To prevent osteoporotic fracture in the female patients treated with glucocorticoids and the male patients receiving ADT novel effective agents are needed. The ability of these RGD-octapeptides modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one to prevent prednisone treated mouse developing osteoporosis suggests that these conjugates should be promising candidates of secondary osteoporosis therapies.

5. Indirect covalent modification of androgen with RGD-tetrapeptides

For androgen a parallel covalent modification with the direct covalent modification is an indirect strategy. In brief, between the 17β-amino group of 17β-amino-11α-hydroxyandrost-1,4-diene-3-one and RGD-tetrapeptides as a linker, succinyl group, is inserted to provide RGD-tetrapeptides indirectly modified androgen (Figure 18) [33].

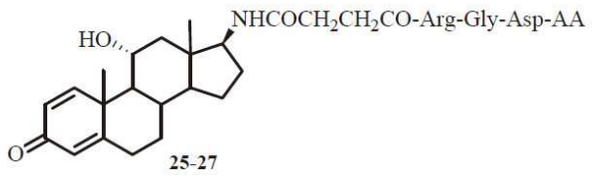

Figure 18. Structures of conjugates of androgen, succinyl group and RGD-tetrapeptides. In **25** AA = Ser, in **26** AA = Val, in **27** AA = Phe.

5.1. Indirect covalent modification of androgen with RGD-tetrapeptides inhibiting bone turnover

The effect of oral administration of **25-27** plus intramuscular administration of prednisone for 4 weeks on serum calcium and serum ALP of the mice is shown in Figure 19. The data indicate that the serum calcium and serum ALP of oral administration of **25-27** plus intramuscular administration of prednisone treated mice are significantly lower than those of intramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that the frequency of bone turnover of oral administration of **25-27** treated mice is significantly lower than that of oral administration of estradiol treated mice, the efficacy of oral administration of **25-27** in inhibiting bone turnover is significantly higher than that of oral administration of estradiol.

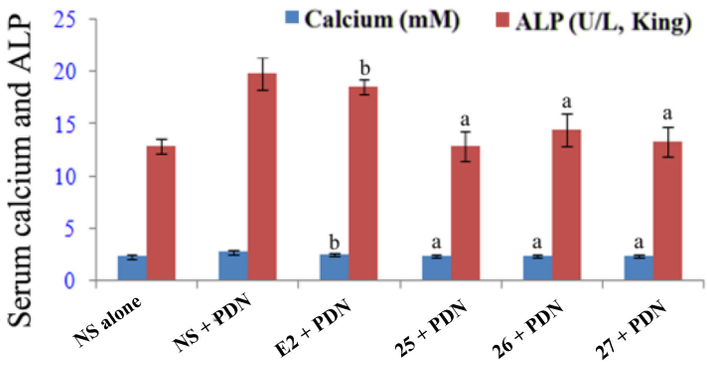

Figure 19. Serum calcium and ALP of **25-27** treated mice. ip Dose of prednisone (PDN): 6.3 mg/kg, twice a week; oral dose of **25-27**: 110 nmol/kg, once a day; oral dose of estradiol (E2): 110 nmol/kg, once a day; n = 12. **For serum Ca^{+2}:** a) Compared to NS + PND $p < 0.01$, to E2 + PND $p < 0.05$; b) Compared to NS + PND $p < 0.05$. **For serum ALP:** a) Compared to NS + PND and E2 + PND $p < 0.01$; b) Compared to NS + PND $p < 0.05$.

5.2. Indirect covalent modification of androgen with RGD-tetrapeptides preventing bone loss

The effect of oral administration of **25-27** plus intramuscular administration of prednisone for 4 weeks on the bone loss of the treated mice is shown in Figure 20, their activities are represented with dry femur weight and femur ash weight. The data indicate that both the weights of dry femur and femur ash of oral administration of **25-27** plus intramuscular administration of prednisone treated mice are significantly higher than those of intramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that upon oral administration **25-27** effectively inhibit the mice to losing femur, their efficacies are significantly higher than that of oral administration of estradiol, and the direct covalent modification of androgen prevents the bone loss.

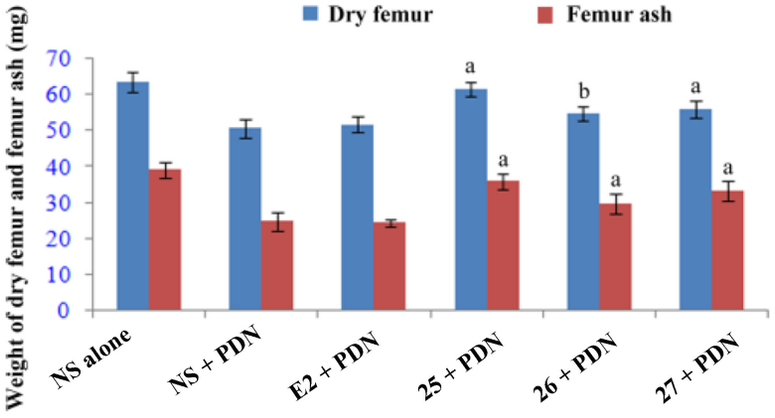

Figure 20. Weight of dry femur and femur ash of conjugates **25-27** treated mice. ip Dose of prednisone (PDN): 6.3 mg/kg, twice a week; oral dose of **25-27**: 110 nmol/kg, once a day; oral dose of estradiol (E2): 110 nmol/kg, once a day; n = 12. **For dry femur:** a) Compared to NS + PND and E2 + PND $p < 0.05$; b) Compared to NS + PND and E2 + PND $p < 0.05$. **For femur ash:** a) Compared to NS + PND and E2 + PND $p < 0.01$.

5.3. Indirect covalent modification of androgen with RGD-tetrapeptides increasing total vBMD

CT measured 3D bone geometry and the size-independent vBMD, as well as pQCT quantitatively measured 3D bone geometry and size-independent vBMD were used to represent the anti-osteoporosis efficacy of **25-27** and are shown in Figure 21. The data indicates that the total vBMD of the femurs of NS plus intramuscular administration of prednisone treated mice is significantly lower than that of the femurs of NS alone treated mice. This means that intramuscular administration of prednisone effectively induces the mice to decrease the total vBMD. The total vBMDs of the femurs of oral administration of **25-27** plus intramuscular administration of prednisone treated mice are significantly higher than that of the femurs of NS plus intramuscular administration of prednisone treated mice. This means that upon oral administration **25-27** effectively inhibit intramuscular administration of prednisone treated mice decreasing total vBMD.

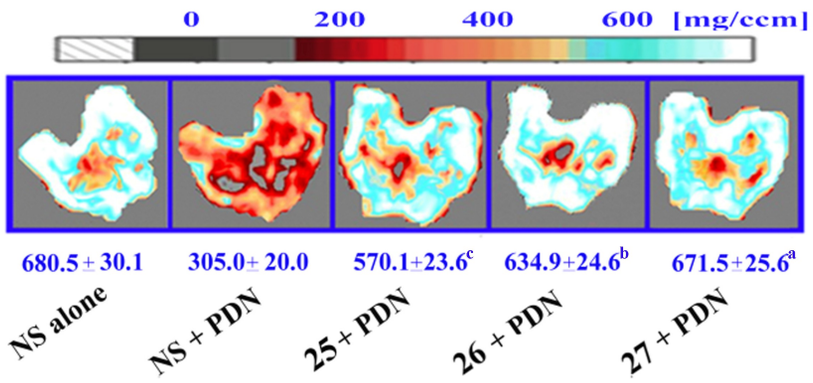

Figure 21. Total vBMD and images of pQCT scanning at a distance from the proximal femur growth palate corresponding to < 6 % of the total length of the femur of **25-27** treated mice.

5.4. Indirect covalent modification of androgen with RGD-tetrapeptides increasing trabecular vBMD

Figure 22 indicates that the trabecular vBMD of the femurs of NS plus intramuscular administration of prednisone treated mice is significantly lower than that of the femurs of NS alone treated mice. This means that prednisone effectively induces the mice to decrease trabecular vBMD. The trabecular vBMDs of the femurs of oral administration of **25-27** plus intramuscular administration of prednisone treated mice are significantly higher than that of the femurs of NS plus intramuscular administration of prednisone treated mice. This means that upon oral administration **25-27** effectively inhibit intramuscular administration of prednisone treated mice decreasing trabecular vBMD.

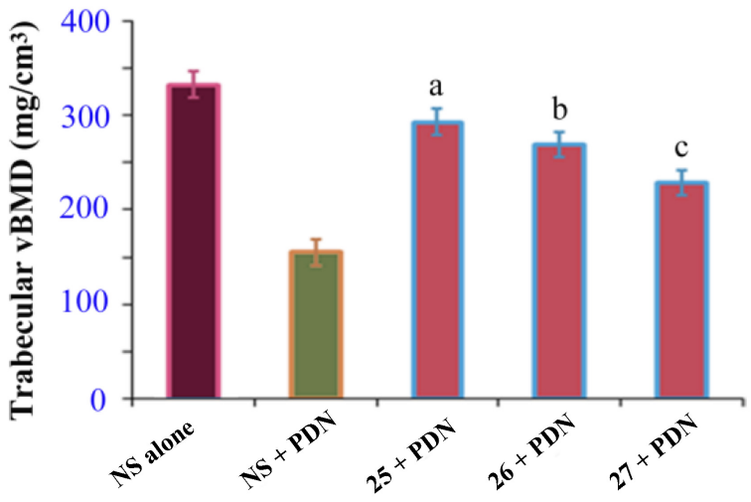

Figure 22. Trabecular vBMD in the femurs of treated mice at a distance from the proximal femur. Growth plate corresponds with < 6% of the total length of the femur of the treated mice. Trabecular vBMD is represented with mean ± SD mg/cm^3, n = 12, PDN = prednisone, dose of **25-27** = 110 nmol/kg. a) Compared to NS + PDN, **25 +** PDN and **26 +** PDN $p < 0.01$; b) Compared to NS + PDN and **27 +** PDN $p < 0.01$; c) Compared to NS + PDN $p < 0.01$.

5.5. Indirect covalent modification of androgen with RGD-tetrapeptides inducing no endomtrial cell hyperplasia

The effect of oral administration of **25-27** plus intramuscular administration of prednisone for 4 weeks on the endometrial cell hyperplasia of the mice was observed, and their inhibition activities are represented with uteri weight. The data indicate that the weight of the uteri of oral administration of **25-27** plus intramuscular administration of prednisone treated mice is significantly lower than that of intramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that, in contrast to oral administration of estradiol, upon oral administration **25-27** induces no observable endometrial cell hyperplasia, and the direct covalent modification of androgen with RGD-octapeptides induces no dose-related adverse effects of estradiol.

5.6. Indirect covalent modification of androgen with RGD-tetrapeptides having no thrombosis risk

The effect of oral administration of **25-27** plus intramuscular administration of prednisone for 4 weeks on thrombosis risk of the mice was observed, and the risk is represented with tail bleeding time. The data indicate that the tail bleeding time of oral administration of **25-27** plus intramuscular administration of prednisone treated mice is significantly longer than that of ntramuscular administration of prednisone alone and oral administration of estradiol plus intramuscular administration of prednisone treated mice. This means that, in contrast to oral administration of estradiol, upon oral administration **25-27** induces no observable thrombosis risk, and the indirect covalent modification of androgen with RGD-octapeptides induces no dose-related adverse effects of estradiol.

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5.7. Summary of indirect covalent modification of androgen with RGD-tetrapeptides

RGD-tetrapeptides indirectly modifying the 17β-amino group of 17β-amino-11α-hydroxyandrost-1,4-diene-3-one was performed by inserting a succinyl functional group and resulted in 3 conjugates. On prednisone treated mouse model and at 110 nmol/kg of oral dose their anti-osteoporosis activities were significantly higher than that of estradiol. In contrast to estradiol, the anti-osteoporosis therapy of these conjugates induced no endometrial cell hyperplasia and thrombosis risk. In respect to inhibiting the prednisone treated mice to lose total vBMD, trabecular vBMD, femur ash weight, femur Ca^{2+} and bone mineral content 110 nmol/kg of RGD-tetrapeptides indirectly modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one was more effective than 110 nmol/kg of RGD-tetrapeptides directly modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one, and this increased efficacy could be attributed to the insertion of a succinyl group. Similarly, the ability of these RGD-octapeptides indirectly modified 17β-amino-11α-hydroxyandrost-1,4-diene-3-one to prevent prednisone treated mouse developing osteoporosis and high activity suggests that these conjugates should be preferentially promising candidates for secondary osteoporosis therapies.

6. Nano-structures of RGD-peptides modified estrogen and androgen

Self-organization or self-assembly practically leads to the formation of various ordered nanostructures in solution, at bulk state, and on a solid surface [34,35]. Numerous self-assembling substances, such as highly fluorinated amphiphilic molecules[36], amphiphilic triblock copolymers with polyrotaxane as a central block [37], amphiphilic dodecyl ester derivatives from aromatic amino acids [38], dendritic molecules [39], the shape anisotropy of non-spherical colloidal building blocks [40], alkylated polycyclic aromatic hydrocarbons [41], porphyrins, graphenes and fullerenes [42], were designed. Of the self-assembling molecules, peptides have been considered a set of particular substance [43-51]. In respect of the self-assembly the formation of nano-structure is an inherent property of organic compounds. In this context, the nano-structures of **10**-**15** and **22-27** in aqueous are given below to explore the relationships between the nano-structure and the concentration or pH, as well as to correlate the nano-feature with the pharmacological activity.

6.1. Nano-aggregators from modification of 17β-hydroxy of estradiol with RGD-octapeptides

As explained by Figure 6, using succinyl group and RGD-octapeptides modifying the 17β-hydroxy of estradiol provides **10-12**. Figure 23 demonstrates that in water **10** forms stick like nano-aggregators of 161.1 nm in diameter and 222.2-888.9 nm in length, **11** forms maize like nano-aggregator of 388.9 nm in length, **12** forms solid pipe like nano-aggregator of 3.6 nm in diameter and 263.9 nm in length.

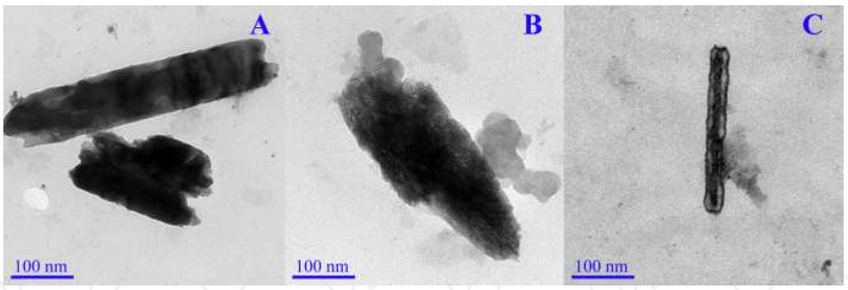

Figure 23. TEM images of **10** - **12** formed nano-aggregators. A) Stick like nano-aggregators of **10**; B) Maize like nano-aggregators of **11**; C) Solid pipe like nano-aggregator of 12.

6.2. Nano-aggregators from modification of 3-hydroxy of estradiol with RGD-octapeptides

As seen in Figure 6, carbonylmethyl and RGD-octapeptides modifying the 3-hydroxy of estradiol provides **13-15**. Figure 24 demonstrates that in water **13** forms porous nano-aggregators of 133.3-430.6 nm in length, **14** forms maize like nano-aggregator of 111.1-600.0 nm in length, **15** forms nano-globes of 66.7-237.5 nm in diameter.

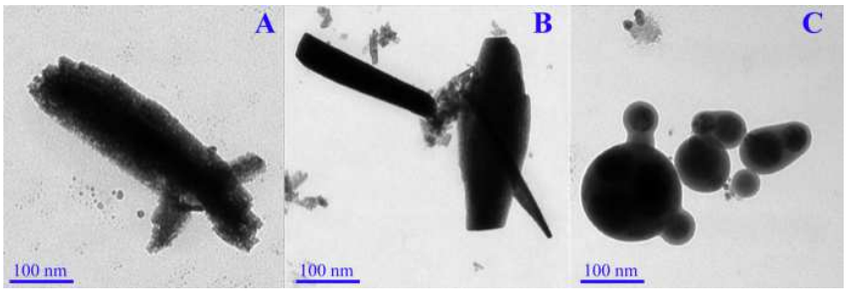

Figure 24. TEM images of **13** - **15** formed nano-aggregators. A) Porous nano-aggregators of **13**; B) Porous nano-aggregators of **14**; C) Nano-globes of **15**.

6.3. TEM image of nano-globes of androgen having RGD-tetrapeptides modified 17β-hydroxy

The nano-structures of **22-24** were explained with TEM nano-images and are shown with Figures 25-27. Figure 26 indicates that in 1.1 mM aqueous solution **22** forms numerous smaller globes aggregated nano-globe of 400 nm in diameter, dispersing globes of 55 - 200 nm in diameter, and dispersing globes of 18 - 146 nm in diameter. Figure 41 indicates that in 1.1 mM aqueous solution **23** forms nano-globe of 312.5 nm in diameter having small globes, blocks and awls on surface, nano-globes of 21.9 - 82.9 nm in diameter and nano-globes of 22.9 - 194.3 nm in diameter. Figure 27 indicates that in 1.1 mM aqueous solution **24** forms nano-globe of 183 nm in diameter having a number of nano-particles on surface, hemisphere of 275 nm in diameter having some smaller globes on incomplete surface and nano-globes of 48 - 188 nm in diameter.

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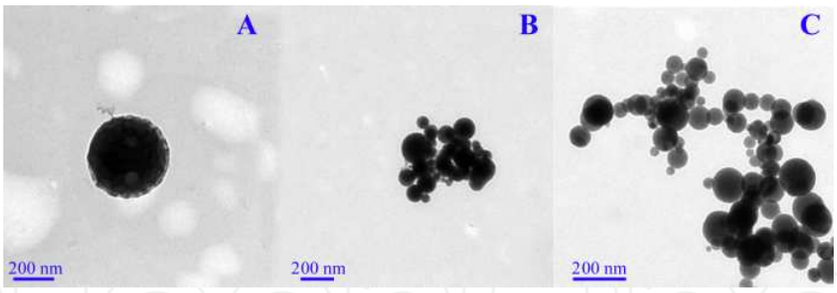

Figure 25. TEM images of 1.1 mM of **22** in ultrapure water. A) Numerous smaller globes aggregated nano-globe of 400 nm in diameter; B) Dispersing globes of 55 - 200 nm in diameter; C) Dispersing globes of 18 - 146 nm in diameter.

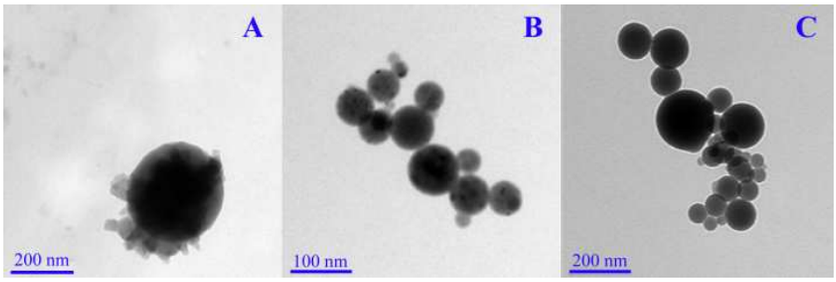

Figure 26. TEM images of 1.1 mM of **23** in ultrapure water. A) Nano-globe of 312.5 nm in diameter having small globes, blocks and awls on surface; B) Nano-globes of 21.9 - 82.9 nm in diameter; C) Nano-globes of 22.9 - 194.3 nm in diameter.

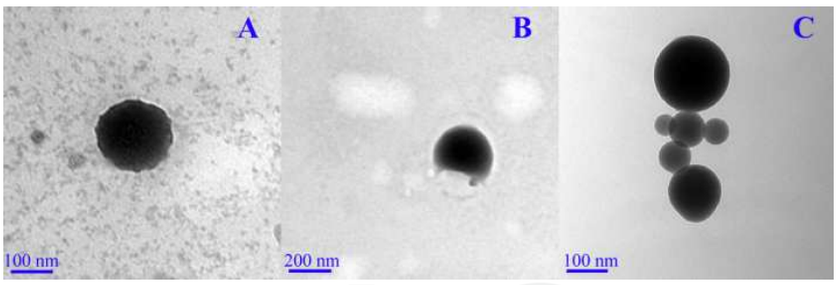

Figure 27. TEM images of 1.1 mM of **24** in ultrapure water. A) Nano-globe of 183 nm in diameter having a number of nano-particles on surface; B) A hemisphere of 275 nm in diameter having some smaller globes on incomplete surface; C) Nano-globes of 48 - 188 nm in diameter.

6.4. SEM image of nano-globes of androgen having RGD-tetrapeptides modified 17β-hydroxy

The nano-structures of **22-24** were explained with SEM nano-images and are shown with Figures 28-30. Figure 29 indicates that in solid state **22** exists as globes of 3.3 - 14.2 μm in diameter. Figure 44 indicates that in solid state **23** exists as eggs of 9.6 × 11.5 μm to 19.5 × 27.0 μm in diameter, of which surfaces have small eggs, and one egg remains its tail been incomplete. Figure 30 indicates that in solid state **24** exists as beads of 9.2 × 10.0 μm to 21.4 × 22.8 μm in diameter, and beads remain been incomplete.

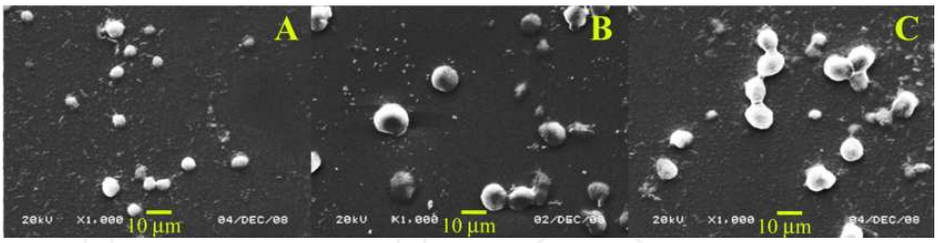

Figure 28. SEM images of **22** in solid state. A) Globes of 3.3 - 6.6 µm in diameter; B) Globes of 3.3 - 14.2 µm in diameter; C) Globes of 3.3 - 11.7 µm in diameter.

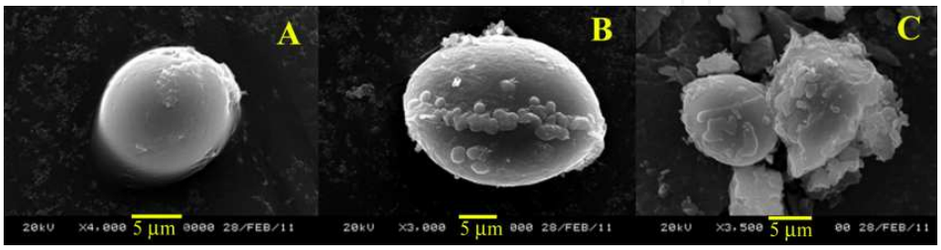

Figure 29. SEM images of **23** in solid state. A) Egg of 12.9 × 14.4 µm in diameter; B) Egg of 19.5 × 27.0 µm in diameter; C) Egg of 9.6 × 11.5 µm in diameter and egg remains its tail been incomplete.

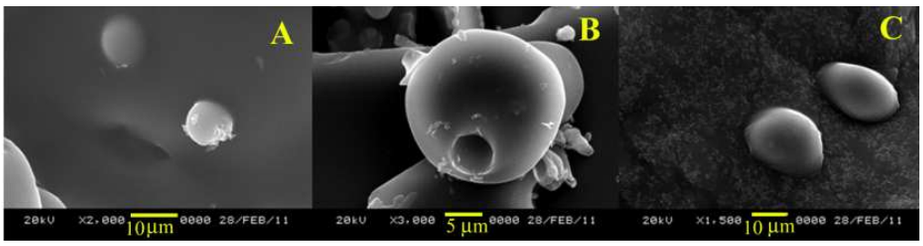

Figure 30. SEM images of **24** in solid state. A) Bead of 9.2 × 10.0 µm in diameters, and bead remains been incomplete; B) Hollow bead of 21.4 × 22.8 µm in diameters; C) Bead of 12.6 × 21.1 µm in diameter, and bead of 15.3 × 22.1 µm in diameter.

6.5. TEM image of nano-globes of androgen having RGD-tetrapeptides and succinyl modified 17β-hydroxy

The TEM images (Figures 31-33) demonstrate that in water **25-27** consistently form nano-globes with porous surface. The comparison of the nano-globes of **22-24** having no porous surface and the nano-globes of **25-27** having porous surface gave us an impression that the insertion of succinyl was a key to form the nano-globes with porous surface, 17β-ethyl-carbonylaminoandrost-1,4-diene-3-one was responsible for forming nano-globe, and RGD-tetrapeptide was responsible for characterizing the surface feature and size of the nano-globes, in particular. For instance, RGDS causes **25** to form dispersing nano-globes of 8 - 150 nm in diameter and having porous surfaces, RGDV causes **26** to form dispersing nano-globes of 29 - 150 nm in diameter and having porous surfaces, and RGDF causes **27** to form dispersing nano-globes of 76 - 343 nm in diameter and having porous surfaces.

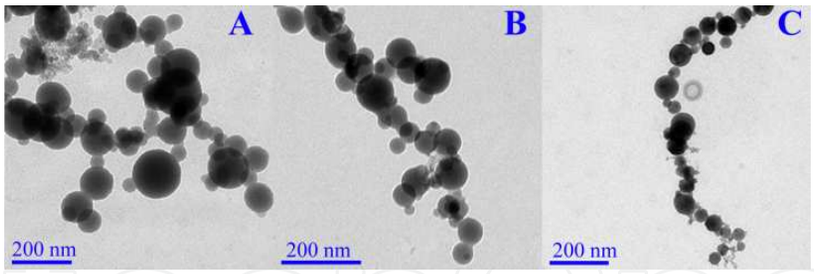

Figure 31. TEM images of 1.1 μM of **25** in ultrapure water. A) Dispersing globes of 8 - 150 nm in diameter; B) Dispersing globes of 17 - 94 nm in diameter; C) Dispersing globes of 27 - 82 nm in diameters.

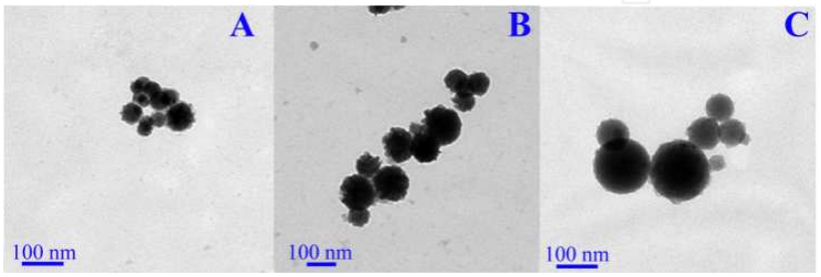

Figure 32. TEM images of 1.1 μM of **26** in ultrapure water. A)Dispersing globes of 29 - 69 nm in diameter; B) Dispersing globes of 70 - 120 nm in diameter; C) Dispersing globes of 67 - 150 nm in diameter.

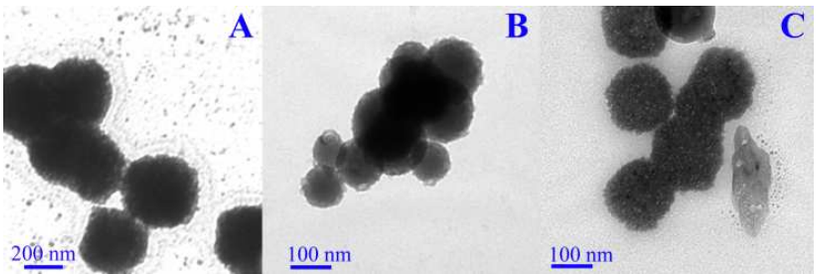

Figure 33. TEM images of 1.1 μM of **27** in ultrapure water. A) Dispersing globes of 320 - 343 nm in diameter; B) Dispersing globes of 76 - 139 nm in diameter; C) Dispersing globes of 120 - 171 nm in diameter.

6.6. SEM image of nano-globes of androgen having RGD-tetrapeptides and succinyl modified 17β-hydroxy

The SEM image (Figures 34-36) demonstrates that in solid state **25-27** exist as nano-globes of 15 nm - 6.4 μm in diameter, nano-pine seeds of 286 nm - 2.7 μm in length, nano-eggs of 1.3 - 12.9 μm in length, nano-pinecones of 5.0 - 5.6 μm in length, nano-gear of 10 μm in diameter, nano-calabash of 4 μm in length, and uncompleted nano-calabash of 11.3 μm in length. The coexistence of nano-globe having nano-egg, nano-pine seed having nano-pinecone, and uncompleted nano-calabash having nano-calabash implies that the nano-egg, nano-pinecone and nano-calabash are built by nano-globes, nano-pine seeds and uncompleted nano-calabash. The correlation of the molecular constitutions and the nano-structures gave us an impression

that for **25** - **27** 17β-ethylcarbonyl-amino-androst-1,4-diene-3-one was responsible for forming a globe-like body, and RGD-tetrapeptide was responsible for characterizing globe-like body.

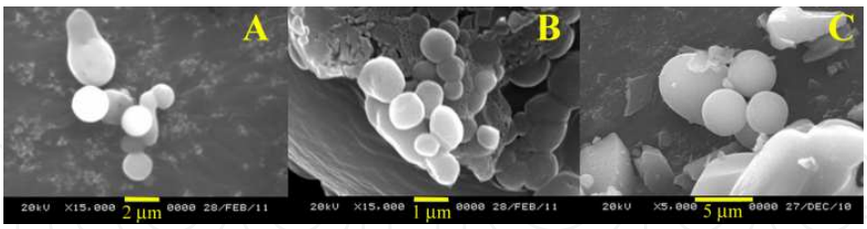

Figure 34. SEM images of **25** in solid state. A) Nano-globes of 15 nm - 2 μm in diameter and nano-calabash of 4 μm in length; B) Nano-globes of 600 nm - 1.3 μm in diameter and nano-egg of 1.3 μm in length; C) Nano-globes of 3.0 - 5.0 μm in diameter and uncompleted nano-calabash of 11.3 μm in length.

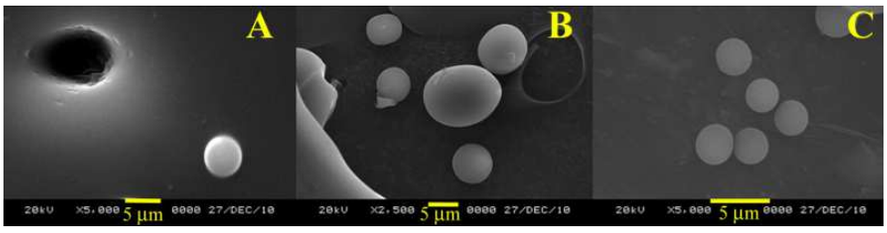

Figure 35. SEM images of **26** in solid state. A) Globe of 2.9 μm in diameter; B) Globes of 5.0 - 6.4 μm in diameter and eggs of 5.7 - 12.9 μm in length; C) Globes of 2.8 - 3.5 μm in diameter.

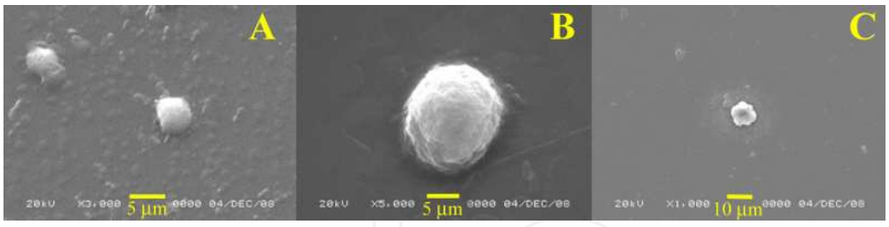

Figure 36. SEM images of **27** in solid state. A) Pine seeds of 7.1 - 9.4 μm in length; B) Globe of 8.1 μm in diameter; C) Gear of 10 μm in diameter.

6.7. Summary of the nano-structures of RGD-peptides modified sex hormones

In water RGD-peptides modified sex hormones generally formed diverse nano-species via self-assembly. Due to all non-covalent bond interactions could be involved into the self-assembly the size and the feature of the nano-species of RGD-peptides modified sex hormones clearly depend on the concentration of their aqueous solution. Similarly, due to all non-covalent bond interactions could be involved into the self-assembly the size and the feature of the nano-species usually depend on the chemical structures of the sex hormones and the sequence of the RGD-peptides. In addition, the RGD-peptides modified sex hormones possessed various anti-osteoporosis activities. Thus the feature and the size of their nano-species could be

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correlated with their anti-osteoporosis activities. Therefore by selecting the concentration and by modifying the chemical structure we are able to optionally get the desirable nano-structure and consequently to optionally get desirable anti-osteoporosis activity.

7. Conclusions

Secondary osteoporosis is common in premenopausal women with osteoporosis and in older men, and is a major problem in clinical practice. More than one third of women with postmenopausal osteoporosis have identifiable secondary causes that contribute to bone loss. The secondary causes of osteoporosis in older men account for 50% - 80% of the cases of bone loss leading to fracture. Besides, secondary osteoporosis is common in the patients treated with glucocorticoids and in prostate cancer patients receiving ADT in particular. Glucocorticoids are ubiquitously prescribed in the fields of rheumatology, respirology, neurology, hematology, dermatology, gastroenterology, and transplant medicine. Chronic exposure to pharmacological doses of glucocorticoids causes multiple deleterious effects on osteopenia, osteoporosis and bone fracture. Prostate cancer is one of the most common diseases in the older men. After the surgery or radiation therapy the male patients with localized or metastatic prostate cancer are generally given ADT. Though male patients on ADT usually have good prognosis, osteoporosis is a very common consequence of this therapy and up to 20% of the patients will fracture within 5 years. To prevent osteoporotic fracture in premenopausal women with osteoporosis, the female patients treated with glucocorticoids and the male patients receiving ADT RGD-peptides modified sex hormones were provided. On ovariotomy and prednisone induced osteoporosis mice either ip injection or orally dosed the modified hormones were able to enhance the efficacy and minimize the adverse effects. By forming nano-species their therapy could be further improved.

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