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Syntheses of (±)-Shinflavanone and Its Structural Analogues as Potent Inhibitors of Bone Resorption Pits Formation

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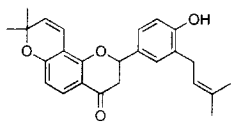
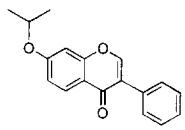
Abstract: The first total syntheses of (±)-shinflavanone and its structural analogues were achieved. (±)-Shinflavanone, appears to be a strong inhibitor of bone resorption pits formation by osteoclast-like cell induced by 1α, 25-dihydroxy vitamine D_3 (IC_{50} = 0.70 μ g/mL)

Glycyrrhiza glabra L. (Fabaceae) is the origin of the crude drug licorice that has been used widely in Europe and its vicinity since ancient times. *G. glabra* (known in commerce as Russian licorice and Spanish licorice) is an economically valuable plant as a source of glycyrrhizin (medicine and sweetening agent) and of a flavonoid rich fraction, containing glabridin and glabrene, which is used in cosmetics as a depigmentary agent.[1,2] Some bioactive flavonoids, such as glabrol[3] and shinflavanone[4] have been isolated from commercial licorice and their structures were elucidated on the basis of spectroscopic evidence. In recent years the number of reports referring to biological activity of licorice constituents has dramatically increased, and either flavonoids or isoflavonoids were identified as the active principles.[5,6] Furthermore the structure of these flavonoids, including shinflavanone, have some similarity as compared to ipriflavone which is known to have some anti-osteoporosis activity.[7] Therefore, our research interest in this field also has been focused in the syntheses of the natural products and evaluation of their inhibition ability of osteoclast cell activity to generate potent osteoporosis therapy agents.



Shinflavanone



Ipriflavone

In this report, we reveal the first chemical syntheses of (±)-shinflavanone and its analogues and their inhibitory activity of resorption pits formation by osteoclast-like cell (OCL) induced by 1α, 25-dihydroxy vitamine D_3.

The synthetic route of (±)-shinflavanone (**10**) began with isoprenylation of 2, 4-dihydroxyacetophenone (**1**) with 2-methyl-but-3-en-2-ol and boron trifluoride-etherate in dioxane. In the above reaction, a mixture of

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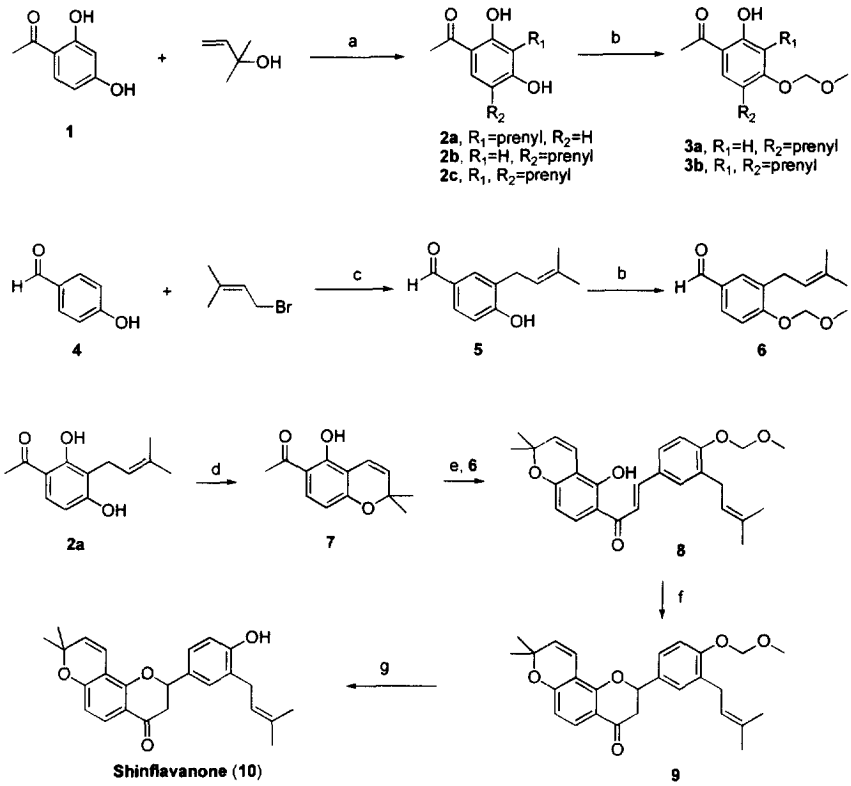
three products was obtained as was already reported.[8] The major component was identified as 5-C-prenylresacetophenone (**2b**), the next major product was 3-C-prenylresacetophenone (**2a**), and the third

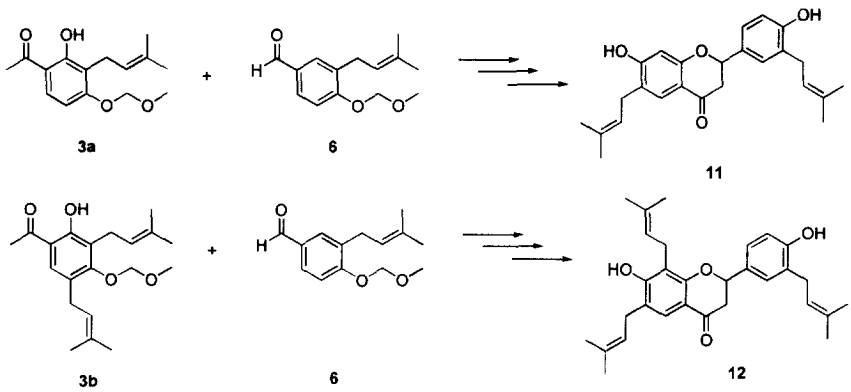
SCHEME 1



(a) $BF_3{\cdot}OEt_2$, dioxane; (b) methoxymethyl chloride, K_2CO_3, acetone; (c) 10% KOH; (d) DDQ, benzene; (e) 50% KOH, EtOH; (f) Na_2CO_3, EtOH; (g) 3N-HCl, MeOH

product obtained in the lowest yield was found to be 3,5-di-C-prenylresacetophenone (**2c**). The resulting 3-C-prenylresacetophenone (**2a**) was cyclized with DDQ in benzene to afford 6-acetyl-5-hydroxy-2, 2-dimethylchromene (**7**) in nearly quantitative yield. The other products **2b** and **2c** were treated with methoxymethyl chloride and K_2CO_3 to obtain protected compounds **3a** and **3b** in good yields. 4-Methoxymethyloxy-3-(3-methyl-2-butenyl)benzaldehyde (**6**), counterpart compound for condensation, was prepared from isoprenylation of *p*-hydroxybenzaldehyde with 3-methyl-2-butenyl bromide in alkaline solution in 22% yield followed by the protection using methoxymethyl chloride and K_2CO_3 in 95% yield. 6-Acetyl-5-hydroxy-2, 2-dimethylchromene (**7**) was condensed with 4-methoxymethyloxy-3-(3-methyl-2-butenyl)benzaldehyde (**6**) in the presence of concentrated alcoholic alkali to afford the corresponding chalcone **8** in 35% yield. Subsequent cyclization with dilute alkali yielded flavanone **9** in 52% yield.

SCHEME 2



Finally, demethoxymethylation of **9** was achieved in methanolic hydrochloric acid for 10 min at reflux to provide (±)-shinflavanone (**10**), 4'-hydroxy-3'-(3", 3"-dimethylallyl)-6"', 6"'-dimethylpyrano[2"', 3"':7, 8]flavanone, in 34% yield. The final step also produced some of the by-product (18% yield), 1,1-dimethylbenzopyran, which was formed by the cyclization of the prenyl group with the neighboring hydroxyl group in the condition used. The synthesized compound **10**[8a] was spectroscopically identical with the corresponding naturally occurring shinflavanone. Compound **11**[8b] and **12**,[8c] new analogues of (±)-shinflavanone (**10**), were synthesized using analogous procedure with comparable yields.

Table 1. Effects of Bone Resorption Inhibitors on Pits Formation and Their Cytotoxicity on Osteoclastic Cells. [a]

Compound No.	HS #	Numbers of Pits (inhibition %)				IC_{50}s of Pits Formation(μ g/mL)	Cytotoxicity CC_{50}, μ g/mL
		0.37 μ g/mL	1.11 μ g/mL	3.33 μ g/mL	10.00 μ g/mL		
10	HS-1311	74 (48.3)	68 (54.1)	54 (62.2)	32 (78.1)	0.70	16.8
11	HS-1289	112 (21.7)	87 (41.2)	84 (41.3)	23 (84.2)	5.7	18.2
12	HS-1317	150 (<5)	120 (18.9)	108 (24.4)	80 (45.2)	10.9	10.4
Control		143	148	143	146		
Herbimycin A		54 (37.8)	75 (50.7)	cytotoxic	cytotoxic	1.10	1.72

[a] All the values are stated as the mean of at least three determinations.

Compounds **10**, **11**, and **12** were assayed for their ability to inhibit the resorption pits formation by OCL induced by 1α, 25-dihydroxy vitamine D_3 (Table 1).[10-12] The synthesized natural product **10**, (±)-shinflavanone, appears to be a strong inhibitor (IC_{50} = 0.70 μ g/mL) of bone resorption pits formation by osteoclast-like cell induced by 1α, 25-dihydroxy vitamine D_3 as shown in table 1 (ipriflavone shows only 31% inhibition at 5 μ g/mL). Compound **11** and **12**, regioisomers of glabrol, showed decreased activity. Compounds **10** showed lower cytotoxicity (CC_{50} = 16.8 μ g/mL) as compared to herbimycin A (CC_{50} = 1.72 μ g/mL).[13] There has been no report about the osteoporosis related activity with shinflavanone (**10**). These results imply that shinflavanone could be a good candidates for osteoporosis therapy. In conclusion, we firstly synthesized (±)-shinflavanone and its structure analogues and found that (±)-shinflavanone is a potent inhibitor of bone

resorption pits formation by OCL induced by 1α, 25-dihydroxy vitamine D_3.

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9. (a) **10** [synthetic (±)-shinflavanone]: a yellowish solid; mp = 94-96℃; *Rf* 0.25(SiO_2, 75% EtOAc-Hexane); ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.45(s, 3H), 1.47(s, 3H)1.79(s, 6H), 2.80(dd, 1H, *J*=3.2, 16.9Hz), 3.02(dd, 1H, *J*=12.8, 16.9Hz), 3.39(d, 2H, *J*=7.0Hz), 5.38(dd, 1H, *J*=3,2, 12,8Hz), 5.38(t, 1H, *J*=7.0Hz)5.57(d, 1H, *J*=10.0Hz), 6.49(d, 1H, *J*=8.6Hz), 6.64(d, 1H, *J*=10.0Hz), 6.85(d, 1H, *J*=8.2Hz), 7.20(s, 1H), 7.24(d, 1H, *J*=8.2Hz), 7.74(d, 1H, *J*=8.6Hz), ^{13}C-NMR (75.5MHz, $CDCl_3$) δ 17.8, 25.7, 28.0, 28.3, 29.6, 44.0, 77.2, 79.6, 109.4, 111.2, 114.7, 115.9, 116.0, 121.4, 125.5, 127.4, 128.0, 128.1, 128.8, 130.9, 135.2, 154.9, 158.0, 159.8, 191.6, HRMS (EI), *m/z* 390.1825 (calculated for $C_{25}H_{26}O_4$ 390.1831). (b) **11** : a yellowish solid; mp = 58-59℃; *Rf* 0.25(SiO_2, 75% EtOAc-Hexane); ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.76(d, 12H, *J*=2.4Hz), 2.76(dd, 1H, *J*=3.2, 3.0Hz), 3.04(dd, 1H, *J*=13.2, 13.2Hz), 3.34(dd, 2H, *J*=7.4, 7.2Hz), 5.29-5.37(m, 3H), 5.67(s, 1H), 6.45(s, 1H), 6.77(s, 1H), 6.84(d, 1H, *J*=8.8Hz), 7.18(s, 1H), 7.20(d, 1H, *J*=3.6Hz), 7.69(s, 1H), ^{13}C-NMR (75.5MHz, $CDCl_3$) δ 14.1, 17.8, 21.0, 25.8, 28.6, 29.6, 44.1, 60.5, 79.7, 103.5, 114.4, 115.8, 121.3, 121.4, 122.4, 125.6, 127.4, 128.2, 128.3, 130.7, 134.9, 154.8, 162.1, 162.3, 191.8, HRMS (EI), *m/z* 392.1995 (calculated for $C_{25}H_{28}O_4$ 392.1988). (c) **12** a yellowish viscous liquid; *Rf* 0.25(SiO_2, 75% EtOAc-Hexane); ^{1}H-NMR (300MHz, $CDCl_3$) δ 1.64(s, 3Hz), 1.76(d, 15H, *J*=8.8Hz), 2.82(dd, 1H, *J*=3.2, 3.0Hz), 3.01(dd, 1H, *J*=13.2, 13.0Hz), 3.35(d, 6H, *J*=7.0Hz), 5.23-5.30(m, 4H), 6.12(s, 1H), 6.84(d, 1H, *J*=8.8Hz), 7.19(s, 1H), 7.61(s, 1H), HRMS (EI), *m/z* 460.2604(calculated for $C_{30}H_{36}O_4$ 460.2615).
10. **Resorption Pit Assay and Quantitation of Pits** : Drops of the osteoclast like multinucleated cell population[11,12] were added on bone slices placed in a 96-well culture dish with and without samples. After incubation for 48 h, bone slices were placed for 30 min in 1M NaOH and cleaned by ultrasonication to remove adherent cells. The bone slices were then stained with Mayer's hematoxylin solution(hematoxylin, 1g/L; $NaIO_3$, 0.2g/L; $AlNH_4(SO)_4 12H_2O$, 50g/L; CH_3COOH, 7.5g/L; pH 2.8) for 50 sec, washed with distilled water, cleaned by ultrasonication, and finally air dried. Resorption pits visualized by Mayer's hematoxylin staining were identified by light microscopy with a X5 objective lens. Using an image analysis software(Image-pro plus, Media Cybernetics, MD, USA), the numbers of pits were counted.
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