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Osteogenic constituents from *Pterospermum acerifolium* Willd. flowers

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ABSTRACT

Phytochemical investigation of ethanol extracts of the *Pterospermum acerifolium* flowers led to the isolation and identification of two new flavones, 4′-(2-methoxy-4-(1,2,3-trihydroxypropyl) phenoxy luteolin (**1**) and 5,7,3′-trihydroxy-6-*O*-β-D-glucopyranosyl flavone (**2**), and one new lactone, 3,5-dihydroxyfuran-2(5H)-one (**3**) along with 14 known compounds (**4**–**17**). The structure of compounds **1**–**17** was established based on MS, 1D and 2D NMR, spectroscopic analysis. Eight of these compounds (**1**–**6**, **8** and **9**) were assessed for osteogenic activity by using primary cultures of rat osteoblast. The compounds **1**, **3** and **4** significantly stimulated osteoblast differentiation and mineralization as evident from a marked increase in expression of alkaline phosphatase and alizarin red-S staining of osteoblasts.

Pterospermum acerifolium Willd. belongs to the family Sterculiaceae, is widely distributed in Indian sub-Himalayan tract, outer Himalayan valley and also cultivated in Pakistan and North America. This plant is commonly known as Kanak Champa (in Hindi).[1] The alcoholic and aqueous extracts from the fruits of this plant exhibited diverse medicinal properties, including anti-hyperglycemic, analgesic, antipyretic, anti-inflammatory activities.[2] The bark of the plant has been known for the treatment of diabetes.[3] Previous phytochemical investigation of this plant showed the presence of epi-catechin, isolariciresinol-3-*O*-β-D-xyloside eriodictyol, 5,7,4′-trihydroxy-5′-methoxyisoflavone-3′-*O*-β-D-glucoside, apigenin-6-*C*-β-D-glucoside,[4] kaempferol-3-*O*-galactoside and glycosides of luteolin and quercetin.[5] The constituents of flower were 24-β-ethylcholest-5-en-3β-*O*-α-cellobioside, 3,7-diethyl-7-methyl-1:5-pentacosanolide, *n*-hexacosane-1,26-diol dilignocerate, friedelan-3α-ol and its β-isomer, β-amyrin, β-sitosterol, *n*-triacontanol, *n*-hexacosane-1,26-diol, myristic, palmitic, stearic, arachidic, behenic, lignoceric, oleic, linoleic, linolenic acids,[6] kaempferol, 4′ methoxy-kaempferol and kaempferide-7-*O*-β-D-glucopyranoside[7] while taraxerol, friedelin, 1-friedelen-3-one and β-sitosterol-3-*O*-β-D-glucoside were known from the leaves of *P. acerifolium*.[3] The trunk bark of *P. acerifolium* contained cystine, glycine, alanine, tyrosine, leucine amino acids[8] and acid-polysaccharides.[9] The known constituents of fruits are protocatechuate, vanillic acid and protocatechuic acid.[2]

After the menopause, decreased bone formation relative to resorption results in negative bone balance at the tissue level, leading to osteoporosis. At the cellular levels, defective osteoblast function primarily contributes to negative bone balance, indicating that osteoporosis is more of a disease of lack of bone formation than increased resorption. Thus, anabolic/osteogenic therapy, or stimulating the function of osteoblasts, is the preferred pharmacological intervention for osteoporosis.[10] In our continued search for osteogenic compounds from Indian medicinal plants,[11,12] we embarked on isolation of bioactive compounds from the flower of *P. acerifolium*. The ethanol extract of the flowers of *P. acerifolium* was successively fractionated with *n*-hexane, chloroform, *n*-butanol and water. The chloroform and *n*-butanol soluble fractions were subjected to repeated column chromatography[13] over flash silica gel, resin, sephadex LH-20 and reverse phase C18 silica gel. The chloroform fraction afforded four compounds (**1** and **15**–**17**) in which compound (**1**) named pterospermin A (Fig. 1) was found to be new and the column chromatography of *n*-butanol fraction led to isolation of two new compounds named pterospermin B (**2**) and pterospermin C (**3**) along with 11 known compounds (**4**–**14**). All known compounds (**4**–**17**) were identified by comparing their spectroscopic data with those previously reported in literature. The known compounds (Fig. 1) were identified as trans-tiliroside (**4**),[14] linalool-3-rutinoside (**5**),[15] (6*R*,9*S*)-3-Oxo-α-ionol-β-D-glucopyranoside (**6**),[16] luteolin (**7**),[17] luteolin-7-*O*-β-glucoside (**8**),[18] luteolin-7-β-*O*-neohesperidoside (**9**),[19] apigenin (**10**),[20] 3′-methoxy-apigenin (**15**),[21] apigenin-7-β-*O*-neohesperidoside (**12**),[22] vitexin (**13**),[23] (3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methyl-dihydro-furan-2-one (**14**),[24] *p*-hydroxy cinnamic acid (**11**),[25] vanillic acid (**16**)[26] and 1-undecene (**17**).[27] Compounds **4**–**6** and

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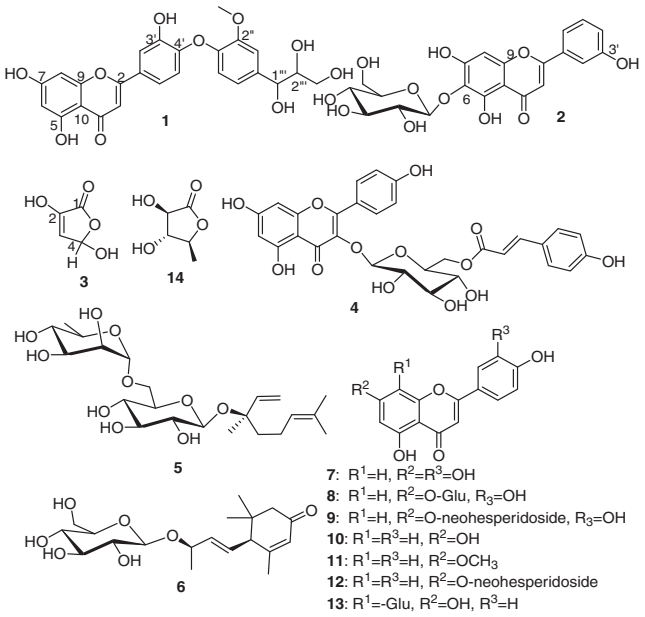

Figure 1. Structural formula of isolated compounds (**1**–**14**).

9–**15** were isolated first time from this plant. In the present communication we describe the isolation and structure elucidation of three new compounds and evaluation of osteogenic activity of eight compounds (**1**–**6**, **8** and **9**).

The compound **1** was obtained as a yellow amorphous solid with $[\alpha]_D^{30.5}$ −50.7 (*c* 0.065, DMSO). The HREIMS shows $[M+1-H_2O]^+$ ion peak at *m/z* 465.1146 and the negative mode ESI-MS of compound **1** shows the peaks at *m/z* 481 for $[M-H]^-$ corresponding to the molecular formula $C_{25}H_{22}O_{10}$. The IR spectrum indicated the presence of hydroxyl (3632 cm^{-1}), a chelated hydroxyl (3550 cm^{-1}), α, β unsaturated carbonyl (1666 cm^{-1}) and C=C, conjugated with carbonyl (1594 cm^{-1}). The UV absorption at 254, 268 and 346 nm in methanol was indicative of flavone structure.[28] The ^1H NMR spectrum (Table 1), showed proton signals at δ 6.81 (1H, s, δ_C 104.2) and 12.86 (br s) correspond to H-3 and chelated hydroxy proton at C-5, respectively. Other meta coupled proton signals at δ 6.19 (1H, d, *J* = 1.2 Hz, δ_C 99.1) and δ 6.50 (1H, d, *J* = 1.2 Hz, δ_C 94.3) correspond to the H-6 and H-8 protons of ring A of flavonoid unit. The protons of ring B showed an ABX system in which the signals at δ 7.62 (1H, s, δ_C 115.0), δ 7.05 (1H, d, *J* = 8.5 Hz, δ_C 117.7) and δ 7.57 (1H, d, *J* = 8.5 Hz, δ_C 120.1) were related to the H-2′, H-5′ and H-6′ protons, respectively. The presence of an another ABX system was also indicated by the signals at δ 7.01 (1H, s, δ_C 112.0), δ 6.87 (1H, d, *J* = 7.9 Hz, δ_C 120.8) and δ 6.80 (1H, d, *J* = 7.9 Hz, δ_C 115.5) correspond to the H-3″, H-5″ and H-6″ protons and showed the presence of an additional trisubstituted aromatic ring D attached to the flavonoid moiety. This system was evidence with analysis of the ^1H–^1H COSY and HMBC spectrum. Moreover, four proton signals at δ 4.98 (1H, d, *J* = 7.0 Hz, δ_C 76.6), δ 4.24 (1H, br m, δ_C 78.2), δ 3.63 (1H, m, δ_C 60.2) and 3.38 (1H, m, δ_C 60.2) were sequenced as H-1‴, H-2‴ H-3‴a and H-3‴b showed the presence of –CHOH–CHOH–CH_2OH moiety confirmed by ^1H–^1H COSY correlations between corresponding protons. The HMBC correlation between proton δ 4.98 with carbons δ_C 127.2 (C-4′), 112.0 (C-3″), 120.8 (C-5″) and protons δ 7.01 and 6.87 with δ_C 76.6 (C-1‴) indicated that 1,2,3-trihydroxypropyl moiety was attached on ring D at 4″ position. The chemical shift values related to 1‴ was not support the ether linkage between 1‴ and 4′ as previously reported flavonolignans.[29,30] A three-proton methoxyl singlet was observed at δ 3.77 (3H, s, δ_C 55.9) assigned to aromatic methoxyl group at C-2″ by HMBC

Table 1
^1H and ^{13}C data of compound **1** in DMSO-d_6

Position	δ_H (*J* in Hz)	δ_C
1	–	–
2	–	163.2
3	6.81, s	104.2
4	–	182.0
5	–	161.6
6	6.50, d (1.2)	99.1
7	–	164.4
8	6.19, d (1.2)	94.3
9	–	157.5
10	–	104.2
1′	–	123.8
2′	7.62, s	115.0
3′	–	143.8
4′	–	147.3
5′	7.05, d (8.5)	117.7
6′	7.57, d (8.5)	120.1
1″	–	147.3
2″	–	147.8
3″	7.01, s	112.0
4″	–	127.2
5″	6.87, d (7.9)	120.8
6″	6.80, d (7.9)	115.5
OCH_3	3.77, s	55.9
1‴	4.98, d (7.0)	76.6
2‴	4.24 br m	78.2
3‴a	3.63 br s	60.2
3‴b	3.38 br m	–

spectrum. The UV spectrum of compound **1** shows a bathochromic shift of 21 nm (λ_{max} 254–275 nm) in the presence of NaOAc suggesting the free hydroxyl group at 7-position hence the ring D would be attached with flavonoid moiety either at 3′ or 4′ position. The correlation between H-5′ and 2″-OCH_3 observed in the NOESY spectrum verified a 4′-O-1″ ether linkage.[31] The ^{13}C NMR spectrum of compound **1** showed 25 carbon resonances. The DEPT NMR experiment permitted differentiation of 25 carbons into 11 methine, one methylene, one methyl and 12 quaternary carbons, of which 15 carbons were attributed to a flavone nucleus, six carbons to ring D, three of 1,2,3-trihydroxypropyl moiety, and one of methyl carbon. On the basis of above analysis of spectroscopic data, suggested the presence of luteolin[18] moiety (**7**), isolated from the same plant and 2-methoxy-4-(1′,2′,3′-trihydroxypropyl) phenyl was attached to the luteolin nucleus at 4′ position by ether linkage. Accordingly, the structure of compound **1** was established as 4′-(2-methoxy-4-(1,2,3-trihydroxypropyl) phenoxy luteolin, a new natural product.

Compound **2**, a yellow amorphous solid, with $[\alpha]_D^{31.5}$ +53.0 (*c* 0.075, Pyridine), was assigned a molecular formula of $C_{21}H_{20}O_{11}$ by FABMS, which exhibited $[M+H]^+$ ion peak at *m/z* 449. The IR spectrum showed the presence of chelated hydroxyl (3461 cm^{-1}) and α, β unsaturated carbonyl functional group (1659 cm^{-1}). The UV absorption at 222 and 325 nm was indicative of flavone structure.[28] The analysis of the ^1H and ^{13}C NMR spectrum of compound **2** revealed the presence of an isolated proton signal at δ 6.82 (1H, s, δ_C 100.0). The appearance of the cross peaks of proton δ 6.82 with carbons δ_C 127.6 (C-6), 159.4 (C-7), 158.5 (C-9), 103.4 (C-10) in HMBC spectrum confirm the proton as H-8 of ring A of flavonoid unit. The ring B showed three ortho coupled protons and one meta coupled proton in which the signals at δ 8.58 (1H, dd, *J* = 1.9, 1.7 Hz, δ_C 115.2), δ 7.43 (1H, m, δ_C 120.7), δ 8.51 (1H, dd, *J* = 8.3, 7.5 Hz, δ_C 130.2) and δ 7.90 (1H, m, δ_C 117.4) were related to the H-2′, H-4′, H-5′ and H-6′, respectively. The complete assignment of this system was achieved by considering ^1H–^1H COSY as well as HMBC spectrum in which H-2′ correlates with δ_C 165.4 (C-2), 120.7 (C-4′), 117.4 (C-6′), H-4′ with 117.4 (C-6′) and H-6′ with 165.4 (C-2), 115.2 (C-2′), 120.7 (C-4′). The proton signals at δ 7.0

Table 2
^{1}H and ^{13}C data of compound **2** in pyridine-d_5

Position	δ_H (J in Hz)	δ_C
1	–	–
2	–	165.4
3	7.0, s	103.4
4	–	183.2
5	–	158.5
6	–	127.6
7	–	159.4
8	6.82, s	100.0
9	–	158.5
10	–	105.1
1′	–	123.1
2′	8.58, dd, 1.9, 1.7	115.2
3′	–	163.2
4′	7.43, m,	120.7
5′	8.51, dd, 8.3, 7.5	130.2
6′	7.90, m	117.4
1″	5.34, d, 7.1	109.5
2″	4.34, m	76.1
3″	4.34, m	78.4
4″	4.52, m	70.5
5″	4.05, m	78.9
6″a	4.71, dd, 11.6, 5.2	61.3
6″b	4.56, dd, 11.6, 2.5	–

(1H, s, δ_C 103.4) and δ 13.61 (br s) correspond to the H-3 and chelated hydroxy, respectively. An aliphatic proton signal at δ 5.34 (d, J = 7.1 Hz, δ_C 109.5) arising from anomeric proton of sugar moiety and other sugar protons comes at δ 4.71 (1H, dd, J = 11.6, 5.2 Hz, δ_C 61.3), δ 4.56 (1H, dd, J = 11.6, 2.5 Hz, δ_C 61.3), δ 4.52 (1H, m, δ_C 70.5), δ 4.34 (2H, m, δ_C 78.4, 76.1), δ 4.05 (1H, m, δ_C 78.9). Acid hydrolysis of compound **2** on TLC yielded glucose as determined by TLC comparison with standard. The coupling constant of the signal resulting the anomeric proton of the glucopyranoside indicated the glucosidal linkage to have β-configuration. The location of the glycosyl residue at C-6 was confirmed by HMBC correlation between anomeric proton (δ 5.34) and C-6 (δ_C 127.6). The above data (Table 2), established the aglycone as 5,6,7,3′-tetrahydroxyflavone[32] and showed glucose moiety attached to 6 position. Thus compound **2** was identified as 5,7,3′-trihydroxy-6-*O*-β-D-glucopyranosylflavone, a new compound.

Compound **3**, a greenish powder with $[\alpha]_D^{30.6}$ −53.1 (*c* 0.065 DMSO), was assigned a molecular formula of $C_4H_4O_4$ by HREIMS mass spectrum which exhibit $[M+H]^+$ ion peak at *m/z* 117.1149. ^{1}H NMR spectra of compound showed two proton signals at δ 5.44 (1H, d, J = 7.3 Hz, δ_C 100.6) and 7.37 (1H, d, J = 7.3 Hz, δ_C 142.6) which were coupled with each other in COSY spectra. The ^{13}C spectra had four carbon signals. The signal at δ_C 165.9 correspond the carbonyl carbon of α, β unsaturated lactone and the peak at δ_C 151.8 and δ_C 142.6 indicative of α and β unsaturated carbon atoms of lactone, respectively. The downfield shift of α carbon was due to attachment of hydroxyl group. The carbon signal at δ_C 100.6 indicate the presence of hydroxyl group attached to C-4, so the structure of compound **3** was established as 3,5-dihydroxyfuran-2(5H)-one.

Compounds **1**–**6**, **8** and **9** were tested for osteogenic activity using primary cultures of rat calvarial osteoblasts and bone marrow cells (BMC) following previously described protocol,[33] wherein stimulation of alkaline phosphatase (ALP) production indicated increased osteoblast differentiation.[11,34,35] ALP was measured by using *p*-nitrophenylphosphate (PNPP) as a substrate as described before[33] and data were normalized using total protein concentration determined by Bradford assay. Bone morphogenetic protein-2 (BMP-2) was used as a positive control for osteoblast differentiation.

Effect of compounds on the viability of osteoblasts was assessed by MTT assay.[34] Ex vivo mineralization was performed by culturing BMC for 21 days in α-MEM medium containing 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid in presence or absence of compounds. Cells were then stained with alizarin red-S and dye was extracted to quantify the extent of osteoblast mineralization as described before.[11,33,35] Transcript levels of osteogenic genes including BMP-2, collagen type 1 (Col1) and runt-related transcription factor 2 (runx2) were determined in calvarial osteoblasts by qPCR using an optimized protocol.[34]

Primer pairs used were: BMP-2 – 5′CGGACTGCGGTCTCCTAA3′ (sense), 5′GGGGAAGCAGCAACACTAGA3′ (antisense); runx2– 5′CC ACAGAGCTATTAAAGTGACAGTG3′ (sense), 5′AACAAACTAGGT TTA GAGTCATCAAGC3′ (antisense); Col1 – 5′CATGTTCAGC TTG TGG AC-CT3′ (sense), 5′CGAGCTGACTTCAGGGATGT3′ (antisense); GAPDH (house-keeping gene) – 5′CAGCAAGGAT ACTGAGA GCAAGAG3′ (sense), 5′GGA TGGAATTGTGAG GGAGATG3′ (antisense). Data are expressed as mean ± SEM. The data obtained in experiments with multiple treatments were subjected to one-way ANOVA test of significance using PRISM 5.0 software.

As shown in Figure 2, BMP-2 significantly stimulated osteoblast ALP production compared to control cells (receiving vehicle). Variation in ALP production observed with control and BMP-2 treated cells can be attributed to batches of osteoblasts harvested from separate sets of animals. Figure 2 further showed that **1**, **3** and **4** significantly increased osteoblast ALP activity compared to control. Maximum increase in ALP activity was observed at 10 and 100 pM, and at these concentrations, the increase in ALP activity was comparable to BMP-2. Interestingly, **1**, **3** and **4** failed to stimulate

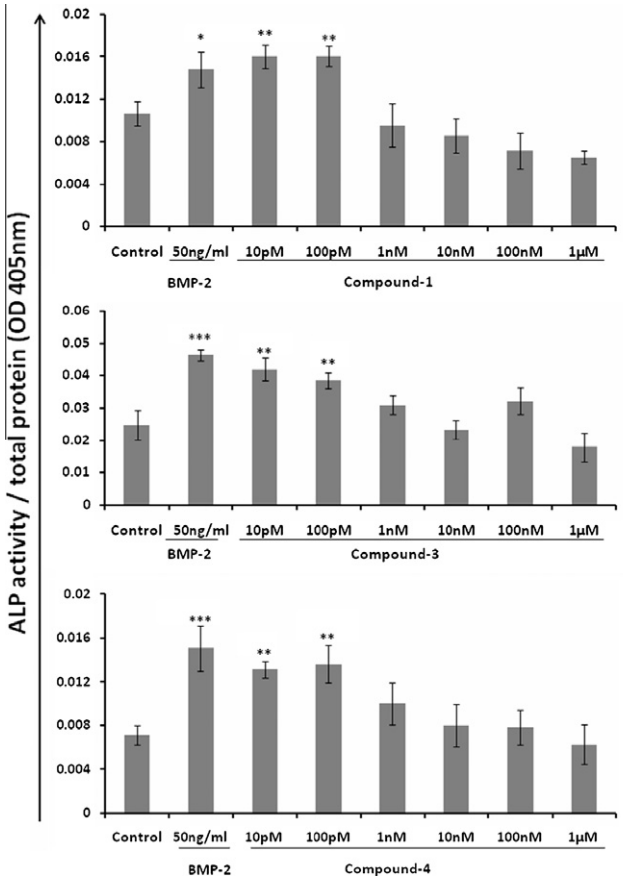

Figure 2. Effect of **1**, **3** and **4** on osteoblast differentiation. Rat calvarial osteoblasts (2×10^3 cells) were seeded in 96-well plate and treated with increasing concentrations of **1** or **3** or **4** for 48 h. BMP-2 was used as a positive control. ALP activity was quantified spectrophotometrically at 405 nm. Data are presented after normalization with total protein. Values are obtained from three independent experiments in the replicate of six/treatment point and expressed as mean ± SEM; $^{*}P$ <0.05 and $^{**}P$ <0.01 compared with control.

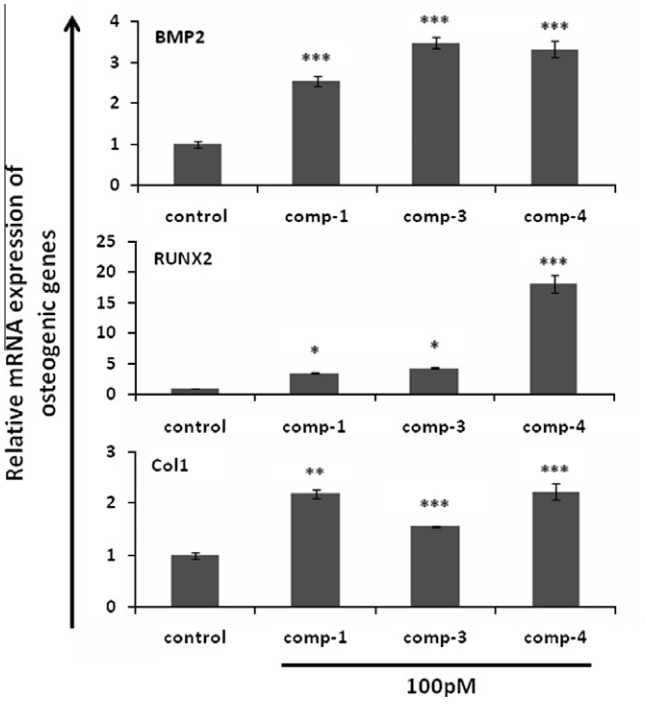

Figure 3. Effect of **1**, **3** and **4** on mRNA levels of osteogenic genes. Rat calvarial osteoblasts were treated with 100 pM **1**, **3** or **4** for 48 h. qPCR for BMP-2, runx2 and col1 mRNAs was performed. At 100 pM **1**, **3** and **4** increased the mRNA levels when compared to control. Values are obtained from four independent experiments in triplicate/treatment point and expressed as mean ± SEM; $^{**}P$ <0.01 and $^{***}P$ <0.001 compared with control.

osteoblast ALP activity at concentrations higher than 100 pM. Loss of stimulatory effect of **1**, **3** and **4** on osteoblasts at higher concentrations[36] may be due to the unfavorable balance between estrogen receptors and peroxisome proliferator-activated and determine the biological effects of receptors, which are reciprocally activated by phytoestrogens and determine the biological effects of phytoestrogens on bone.[37] Furthermore, phytoestrogens at higher concentrations may result in a much greater induction of cytochrome P450-dependent enzymes[38], which in turn could rapidly metabolize these compounds leading to abolition of effect. Since all three compounds at 100 pM maximally stimulated osteoblast ALP activity, this concentration was used in the remaining experiments.

Compounds **2**, **5**, **6**, **8** and **9** had no effect on osteoblast ALP activity (data not shown) while compounds **1**, 3 and **4** showed a significant increase in ALP activity at 10 and 100 pM. At higher concentrations, **1**, **3** and **4** however, failed to increase ALP activity. None of these three compounds at all concentrations affect osteoblast viability assessed by MTT. These data indicate that **1**, **3** and **4** increase osteoblast differentiation and are not associated with osteoblast proliferation. For additional confirmation of the differentiation promoting effects of **1**, **3** and **4** on osteoblasts, we next studied their effects on the expression of osteogenic genes. qPCR data show that **1**, **3** and **4** increased mRNA levels of BMP-2, runx2 and Col1 in osteoblasts to varying extents (Fig. 3).

Calvarial osteoblasts represent osteoblasts from membranous bones that do not exhibit osteoporotic bone loss. Therefore, we next studied the effect of **1**, **3** and **4** on the mineralization of BMC from femur of 21 days old rats.[38] As shown in Figure 4, **1**, **3** and **4** significantly increased mineralization of BMC, suggesting significant osteogenic effect exerted by these compounds due to likely enhancement of bone marrow osteoprogenitors. It is noteworthy that luteolin and kaempferol have been shown to stimulate in vitro osteoblast differentiation at micromolar concentrations.[33]

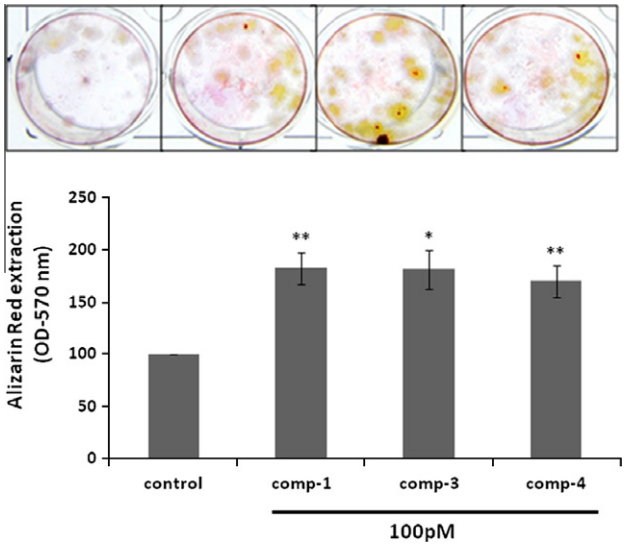

Figure 4. Effect of **1**, **3** and **4** on mineralization of BMC. BMCs (2×10^5 cells) were seeded in 12-well plates and incubated with 100 pM **1**, **3** or **4** for 21 days. At the end of incubation, cells were fixed and stained with alizarin red-S (upper panel – representative photomicrograph). Stain was extracted and OD measured colorimetrically. Values are obtained from four independent experiments in triplicate/treatment point and expressed as mean ± SEM; $^{**}P$ <0.01 and $^{***}P$ <0.001 compared with control.

Our data show that **1** (a novel luteolin analog) and **4** (a kaempferol analog) stimulated osteoblast differentiation at a picomolar range, suggesting their more potent osteogenic effect over luteolin and kaempferol, respectively. Further structure–activity relationship reveal that the presence of a 2-methoxy-4-(1,2,3-trihydroxypropyl) phenoxy substitution at 4′ position of luteolin (compound **1**) made the compound more osteogenic while the glucoside or neohesperidoside substitution on 7-position of luteolin (compounds **8** and **9**), losses the activity. Taken together, our data suggest that **1**, **3** and **4** by stimulating osteoblast differentiation holds bone anabolic potential.

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References and notes

1. DellaGreca, M.; Monaco, P.; Pinto, G.; Pollio, A.; Previtera, L.; Kirtikar, K. R.; Basu, B. D., 2nd ed. In *Indian Medicinal Plants*; Lalit Mohan Basu: Allahabad, 1933; Vol. I, p 374.
2. Selim, Mohamed A.; El-Askary, Hesham I.; Sanad, Ola A.; Ahmed, Mohamed N.; Sleem, Amany A. *Bull. Fac. Pharm., Cairo Univ.* **2006**, *44*, 119.
3. Mamun, M. I. R.; Nahar, Nilufar; Mosihuzzaman, M.; Andersson, Rolf *J. Bangladesh Chem. Soc.* **2002**, *15*, 91.
4. Mamun, M. I. R.; Nahar, Nilufar; Mosihuzzaman, M.; Andersson, Rolf *Dhaka Univ. J. Sci.* **2003**, *51*, 209.
5. Gunasegaran, R.; Subramanian, S. Sankara *Indian J. Pharm. Sci.* **1979**, *41*, 72.
6. Rizvi, S. A. I.; Sultana, Tajwar *Phytochemistry* **1972**, *11*, 856.
7. Varshney, Suresh C.; Rizvi, S. A. I.; Gupta, P. C. *Planta Med.* **1972**, *21*, 358.
8. Tandon, S. P.; Tiwari, K. P.; Saxena, V. K. *Proc. Indian Natl. Sci. Acad. A* **1970**, *40*, 217.
9. Bishnoi, Purnima; Gupta, Purna C. *J. Chem. Soc.* **1979**, *7*, 1680.
10. Trivedi, R.; Mithal, A.; Chattopadhyay, N. *Curr. Mol. Med.* **2010**, *10*, 14.
11. Rawat, P.; Kumar, M.; Sharan, K.; Chattopadhyay, N.; Maurya, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4684.
12. Maurya, R.; Yadav, D. K.; Singh, G.; Bhargavan, B.; Murthy, P. S. N.; Sahai, M.; Sing, M. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 610.

13. Flowers of *P. acerifolium* Willd. (family Sterculiaceae) were collected from Lucknow, India in March 2008 and authenticated by Dr. A.K. Mangal, Central Council for Research in Ayurveda and Siddha (CDRI plant code No. 4740). The air-dried, powdered flowers of *P. acerifolium* (6 kg) was extracted with EtOH (×5) for 48 h at room temperature. The solvent was evaporated under reduced pressure to obtain an extract (1 kg), which was fractionated successively with *n*-hexane, $CHCl_3$, *n*-BuOH/water. The chloroform fraction (25 g) was subjected to silica gel column chromatography (230–400 mesh, 250 g), with the gradient of hexane–EtOAc (99:1, 3:1, 1:1, pure EtOH) as eluent and four fractions (F1–F4) were collected according to TLC analysis. On repeated flash chromatography of fractions F1–F3 afforded compound **17** (140 mg) from *n*-hexane–EtOAc (9:1), compound **16** (100 mg) from *n*-hexane–EtOAc (4:1), compound **15** (50 mg) from methanol–chloroform (1:19) and compound **1** (15 mg) from *n*-hexane–EtOAc (1:8). The *n*-BuOH fraction (200 g) was purified over silica gel column chromatography (230–400 mesh, 1200 g), with the gradient of $CHCl_3$–MeOH (99:1, 9:1, 4:1, 3:1, 1:1 and pure MeOH) as eluent and six fractions (F5–F10) were collected according to TLC analysis. Fraction F6 was purified on silica gel column chromatography eluted with $CHCl_3$–MeOH (19:1) to afforded compound **3** (10 mg), compound **7** (45 mg), compound **10** (40 mg), compound **11** (80 mg) and compound **14** (20 mg). Purification of F7 fraction on silica gel followed by C18 column chromatography eluted with MeOH–H_2O (3:7) afforded compound **2** (70 mg), compound **4** (500 mg), compound **8** (30 mg) and compound **13** (50 mg). Fraction F8 afforded compound **5** (50 mg), compound **6** (70 mg), compound **9** (10 mg) and compound **12** (30 mg) by CC over Sephadex-LH25 eluted with MeOH–H_2O (3:7), followed by C18 and normal silica gel CC eluted with MeOH–H_2O (3:7) and MeOH–$CHCl_3$ (3:17), respectively.
14. Budzianowaski, J.; Skrzypczak, L. *Phytochemistry* **1994**, *38*, 997.
15. Voirin, S.; Baumes, R.; Bayonove, C. *Carbohydr. Res.* **1990**, *39*, 207.
16. Wang, M.; Shao, Y.; Huang, T. C.; Wei, G. J.; Ho, C. T. J. *Agric. Food Chem.* **1998**, *46*, 2509.
17. Markham, K. R.; Chari, V. M. *The Flavonoids: Advances in Research*; Chapman and Hall: London, 1982. pp 19–134.
18. Markham, K. R.; Geiger, H. *The Flavonoids: Advances in Research Since 1986*; Chapman and Hall: London, 1982. pp 19–134.
19. Li, Y. L.; Li, J.; Wang, N. L.; Yao, X. S. *Molecules* **2008**, *13*, 1931.
20. Shen, C.; Chang, Y.; Ho, L. *Photochemistry* **1993**, *34*, 1976.
21. Park, Y.; Moon, B. H.; Yang, H.; Lee, Y.; Lee, Y.; Lee, E.; Lim, Y. *Magn. Reson. Chem.* **2007**, *45*, 1072.
22. Tang, T.; Chen, K.; Cosentino, M.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 455.
23. Peng, X.; Zheng, Z.; Cheng, K. W.; Shan, F.; Ren, G. X.; Chen, F.; Wang, M. *Food Chem.* **2008**, *106*, 475.
24. Ley, S. V.; Dixon, D. J.; Guy, R. T.; Palomero, M. A.; Polara, A.; Rodriguez, F.; Sheppard, T. D. *Org. Biomol. Chem.* **2004**, *2*, 3618.
25. Temussi, F. *Bull. Environ. Contam. Toxicol.* **2001**, *67*, 352.
26. Lee, C. K.; Lee, P. H.; Kuo, Y. H. *J. Chin. Chem. Soc.* **2001**, *48*, 1053.
27. Liard, A.; Marek, I. J. *Org. Chem.* **2000**, *65*, 7218.
28. Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systemic Identification of Flavonoids*; Springer: New York, 1970.
29. Huang, H.; Liu, Y.; Meng, Q.; Wei, S.; Cui, H.; Zhang, C. *Biochem. Syst. Ecol.* **2010**, *38*, 656.
30. Bouaziz, M.; Veich, N. C.; Grayer, R. J.; Simmonds, M. S. J.; Damak, M. *Phytochemistry* **2002**, *60*, 515.
31. Chang, C. L.; Wang, G. J.; Zhang, L. J.; Tsai, W. J.; Wu, Y. C.; Kuo, Y. H. *Phytochemistry* **2010**, *71*, 271.
32. Gao, H.; Kawabata, J. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1859.
33. Siddiqui, J. A.; Swarnkar, G.; Sharan, K.; Chakravarti, B.; Sharma, G.; Rawat, P.; Kumar, M.; Khan, F. M.; Pierroz, D.; Maurya, R.; Chattopadhyay, N. *Mol. Cell. Endocrinol.* **2010**, *323*, 256.
34. Trivedi, R.; Kumar, S.; Kumar, A.; Siddiqui, J. A.; Swarnkar, G.; Gupta, V.; Kendurker, A.; Dwivedi, A. K.; Romero, J. R.; Chattopadhyay, N. *Mol. Cell. Endocrinol.* **2008**, *289*, 85.
35. Sharan, K.; Swarnkar, G.; Siddiqui, J. A.; Kumar, A.; Rawat, P.; Kumar, M.; Nagar, G. K.; Manickavasagam, L.; Singh, S. P.; Mishra, G.; Wahajuddin; Jain, G. K.; Maurya, R.; Chattopadhyay, N. *Menopause* **2010**, *17*, 577.
36. Dang, Z. C.; Lowik, C. *Trends Endocrinol. Metab.* **2005**, *16*, 207.
37. Mense, S. M.; Chhabra, J.; Bhat, H. K. *J. Steroid Biochem. Mol. Biol.* **2008**, *110*, 157.
38. Siddiqui, J. A.; Sharan, K.; Swarnkar, G.; Rawat, P.; Kumar, M.; Manickavasagam, L.; Maurya, R.; Pierroz, D.; Chattopadhyay, N. *Menopause* **2011**, *18*, 198.