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Preliminary communication

Synthesis and evaluation of new coumarin–pyridine hybrids with promising anti-osteoporotic activities☆

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ABSTRACT

Anti-osteoporotic effects of the newly synthesized coumarin–pyridine hybrids were evaluated in primary cultures of rat calvarial osteoblasts in vitro. Compounds **6a**, **i**, **j** and **k** were potent in stimulating osteoblast differentiation and mineralization as assessed by the alkaline phosphatase production and alizarin red-S staining assay, respectively. These compounds were also found to be nontoxic in osteoblast cells as compared to the control group in an MTT assay. Furthermore, the effect of these compounds on the transcript levels of osteogenic genes revealed that the compound **6j** robustly enhanced mineralization of the osteogenic genes in calvarial osteoblasts. In this context, compound **6j** was selected as a potential lead for further structural optimization in the development of new anti-osteoporotic agents.

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1. Introduction

Osteoporosis is a progressive skeletal disorder, due to the unequal coupling between osteoclast mediated bone resorption and osteoblast mediated bone formation [1]. It is a wide spread disease in the elderly population and effects up to 50% of females [2]. It is characterized by decrease in bone mineral density and consequent increase in bone fragility, which leads to a high risk of fractures [3–5]. The main risk factors for osteoporosis are ageing (decline in the supply of osteoblasts), estrogen deficiency (excessive bone resorption), calcium deficiency, Vitamin D deficiency and fractures [6]. This has been widely recognized as a 'silent disease' and in the event of complicated fracture it becomes clinically apparent [7]. The FDA-approved medical options include bisphosphonates (alendronate, ibandronate, risedronate and zoledronic acid), calcitonin, estrogen hormone therapy, parathyroid hormone (teriparatide) and estrogen agonist/antagonist (raloxifene) [8]. However, with the long-term usage of these drugs most of the patients experience undesirable side effects, such as osteonecrosis of the jaw (ONJ), unusual thigh bone fractures and oesophageal cancer, etc [9]. In 2012, The European Medicines Agency (EMA) has withdrawn intranasal calcitonin due to risk of hypercalcaemia followed by cancer [10]. Therefore, there is a constant need for a new class of bone anabolic agents for the treatment of osteoporosis.

Coumarins are an important class of plant secondary metabolites which possess a diverse range of biological activities [11]. It is also reported that coumarins posses bone anabolic effects [12]. Inspired from the medicinal chemistry hybridisation approach, we recently identified compound **1** ethyl 4-(8-*sec*-butyl-3-(methoxycarbonyl)-2-oxo-2*H*-chromen-6-yl)-2-methyl-5-oxo-1,4,5,6, 7,8 hexahydroquinoline-3-carboxylate (at 100 pM) as a hit compound, that showed approximately 65–70% increased mineralization compared to control untreated cells (Fig. 1) [13]. Further, compound **1** did not exhibit any toxicity after oral administration. A hit compound may have a simpler pharmacophore moiety that exists within its structure, which through modification could exhibit improved activity and be more useful as a scaffold for further analogue design [14]. The exquisite potency and structural novelty encouraged us to further explore the chemical space for the development of new class of bone anabolic agents.

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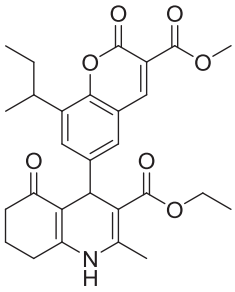


Fig. 1. Hit compound **1**.

Interestingly, recent report suggests that the pyridine ring containing compounds may modulate anti-osteoporotic activity [15]. Furthermore, Saito et al. reported substituted thienopyridine derivatives as bone anabolic agents [16]. This prompted us to synthesize new coumarin containing pyridine hybrids as potential anti-osteoporotic agents (Fig. 2) [17].

2. Chemistry

The synthesis of target and intermediate compounds is outlined in Scheme 1. The Duff reaction on ortho-substituted phenols (**2a**–**d**) in the presence of hexamethylenetetraamine (HMTA) and TFA at 120 °C gave aromatic dicarbaldehydes (**3a**–**d**). These dicarbaldehyde intermediates were then engaged in the Knoevenagel-type reaction with different active methylene compounds resulted in the formation of coumarinic compounds (**4a**–**f**) [13]. Further, these coumarinic aldehyde compounds were subjected to Hantzsch dihydropyridine synthesis via a multi-component reaction, involving coumarinic aldehydes, active methylene compounds and ammonium acetate (nitrogen donor) in the presence of acetic acid glacial. For symmetrical Hantzsch dihydropyrimidines (**5a**–**c**), two equivalent of ethyl/methyl acetoacetate were used while for unsymmetrical Hantzsch polyhydroquinolines (**5d**–**m**), uniequivalent of ethyl/methyl acetoacetate and different 1,3-cyclohexadiones were employed. Finally, aromatisation with the 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of tetrahydrofuran (THF) as a solvent at room temperature gives the desired coumarin–pyridine hybrids (**6a**–**m**) [18]. All the synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR, IR spectroscopy and ESI-MS.

3. Pharmacology

3.1. Culture of calvarial osteoblasts

Mice calvarial osteoblast cultures were performed using 25–30 calvariae from 1 to 2 day old mice pups (both sexes). Calvarias were surgically isolated from skull; sutures were segregated and adherent tissue material cleaned by gentle scrapping. Isolated calveriae were pooled and kept for repeated digestion (15 min/digestion) with 0.1% dispase and 0.1% collagenase-1 was used to release cell. Supernatant of first digestion was discarded, cells from the next three digestions were pooled and cultured in α modified essential medium (α-MEM) containing 10% FCS and 1% penicillin/streptomycin (complete growth medium). For experiment 70–80% confluent mice calvarial osteoblast were used [13,19].

3.2. Alkaline phosphatase assay

At 70–80% confluent mice calvarial osteoblast was used for trypsinization and 2×10^3 cells were seeded onto 96-well plates for alkaline phosphatase (ALP) measurement. Cells were treated with or without compound (**6a**, **i**, **j** and **k**) or vehicle for 48 h in α-MEM supplemented with 10% FCS, 10 mM β-glycerophosphate, 50 μg/mL ascorbic acid, and 1% penicillin/streptomycin (osteoblast differentiation medium). At the end of incubation period, cells were washed with phosphate-buffered saline (PBS) and kept in −80 °C for 30 min and then at 37 °C for 30 min. ALP activity was measured using *p*-nitrophenylphosphate (PNPP) as substrate and absorbance was read at 405 nm [19].

3.3. Mineralized nodule formation assay

For mineralization mice calvarial osteoblast were seeded onto 12-well plates (10,000 cells/well) in osteoblast differentiation medium. Mice calvarial osteoblasts were cultured with or without compound 18 for 21 days and after every 48 h media were changed. At the end of the experiment, cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 15 min. The fixed cells were stained with alizarin red-S (40 mM, pH 4.5) for 30 min followed by washing with water. Stained cells were first photographed under light microscope, and extraction of alizarin stain was carried out by using 10% (v/v) acetic acid with shaking at room temperature for 30 min. Cells were scrapped out from wells, centrifuged (20,000 × *g* for 15 min), and supernatant was collected. To the supernatant, 10%

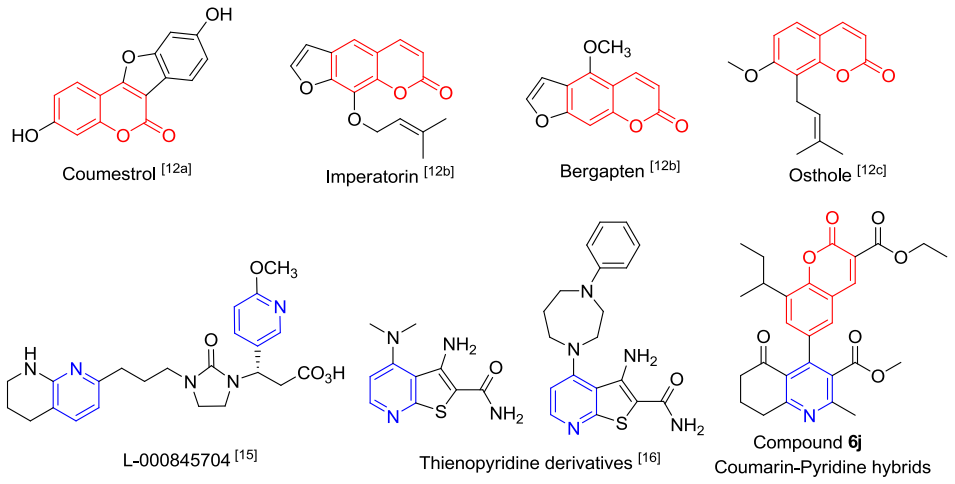
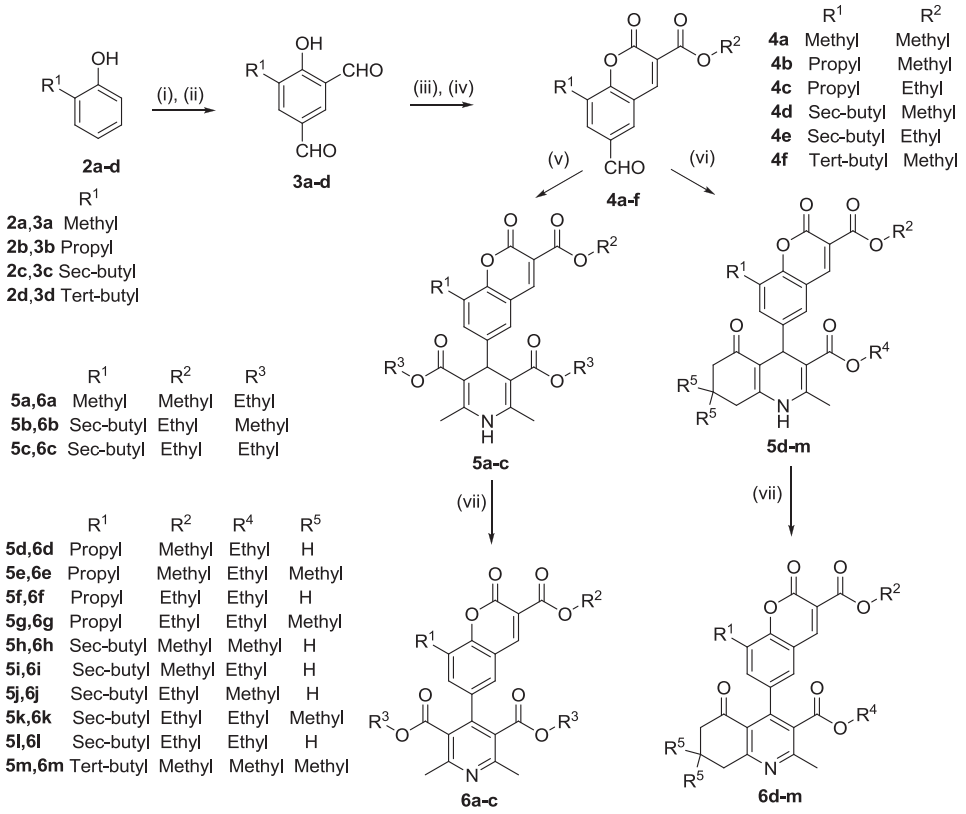


Fig. 2. - Designing of coumarin–pyridine hybrids based on coumarin and pyridine scaffolds showing anti-osteoporotic effects.



Scheme 1. Synthesis of coumarin–pyridine hybrids. Reagents and conditions: (i) HMTA, TFA, 120 °C, 4 h. (ii) aq H_2SO_4, 100 °C, 2 h. (iii) diethyl/methyl malonate, EtOH, piperidine, reflux, 30 min (iv) Glacial AcOH, rt. (v) ethyl/methyl acetoacetate, NH_4OAc, AcOH, EtOH, reflux, 5 h. (vi) ethyl/methyl acetoacetate, different 1,3-cyclohexadiones, NH_4OAc, AcOH, EtOH, reflux, 5 h. (vii) DDQ, THF, rt, 1 h.

(v/v) ammonium hydroxide was added to bring the pH of the solution to 4.5 for colour formation. Absorbance of the solution was read at 405 nm [19,20].

3.4. Cytotoxicity assay

Mice calvarial osteoblast was used to test toxicity of synthesized compounds (**6a**, **i**, **j** and **k**) 2×10^3 cells. Cells were seeded onto 96-well plates and treated with or without compounds (**6a**, **i**, **j** and **k**) at various concentrations (100 pM–1 μM) for 48 h. After incubation, the cells were washed with PBS and were treated with MTT solution (5 μg/10 mL in α-MEM devoid of Phenol Red) for 4 h. Formazon crystals formed were dissolved in DMSO and OD was taken at 540 nm.

4. Results and discussion

4.1. Preliminary screening of coumarin–pyridine hybrids using osteoblast differentiation assay

The synthesized compounds (**6a**–**m**) were screened for alkaline phosphatase (ALP) activity in primary mice calvarial osteoblasts, which is an important marker of osteoblast differentiation. Cells were treated with hybrid compounds to evaluate ALP activity at concentrations ranging from 1 pM to 1 μM and optical density was determined at 405 nm. Among all compounds screened, compound **6a** at 1 μM and 10 nM, compound **6i** at 10 nM, compound **6j** at 1 μM, 10 nM and 100 pM and compound **6k** at 1 pM and 100 pM were found to significantly increase ALP activity over control (Fig. 3). Though most compounds exhibited ALP activity at much lower concentrations (nM concentrations), the compounds **6j** and **6k** showed significant increase of ALP activity at concentration as low as 100 pM.

4.2. Mineralization ability of active compounds *6a, i, j* and *k* in calvarial osteoblast cells

After ALP activity, the most active compounds **6a**, **i**, **j** and **k**, were further evaluated for their osteogenic activity by mineralisation assay. At the end of differentiation process of calvarial osteoblast cells, with or without treatment with the compounds at active concentration for 21 days were fixed and stained with alizarin red-S stain. In this process calcium has been deposited for the newly formed mineralized nodules. Interestingly compounds **6a**, **i**, **j** and **k** showed significant increase of mineralizing ability as compared to the control not treated cells. From the extraction of alizarin red-S stained cultures, quantification of mineralization is determined by the measurement of optical density. Quantification data suggests that compound **6a** at 1 μM ($P < 0.001$ vs corresponding control group) concentration, show 2 fold increase in mineralization, compound **6i** at 10 nM concentration, ($P < 0.001$, vs corresponding control group) exhibited 3 fold increase in mineralization, compound **6j** at 100 pM ($P < 0.001$ vs corresponding control group) concentration showed approximately 3.5 fold increased mineralization, while **6k** at 1 pM concentration showed 2 fold increase in mineralization ($P < 0.001$, as compared to control) (Fig. 4).

4.3. Active compounds *6a, i, j* and *k* increase osteoblastogenesis related marker gene expressions

In order to further validate and identify the most potent osteogenic agent, we further examined the effect of compounds on the

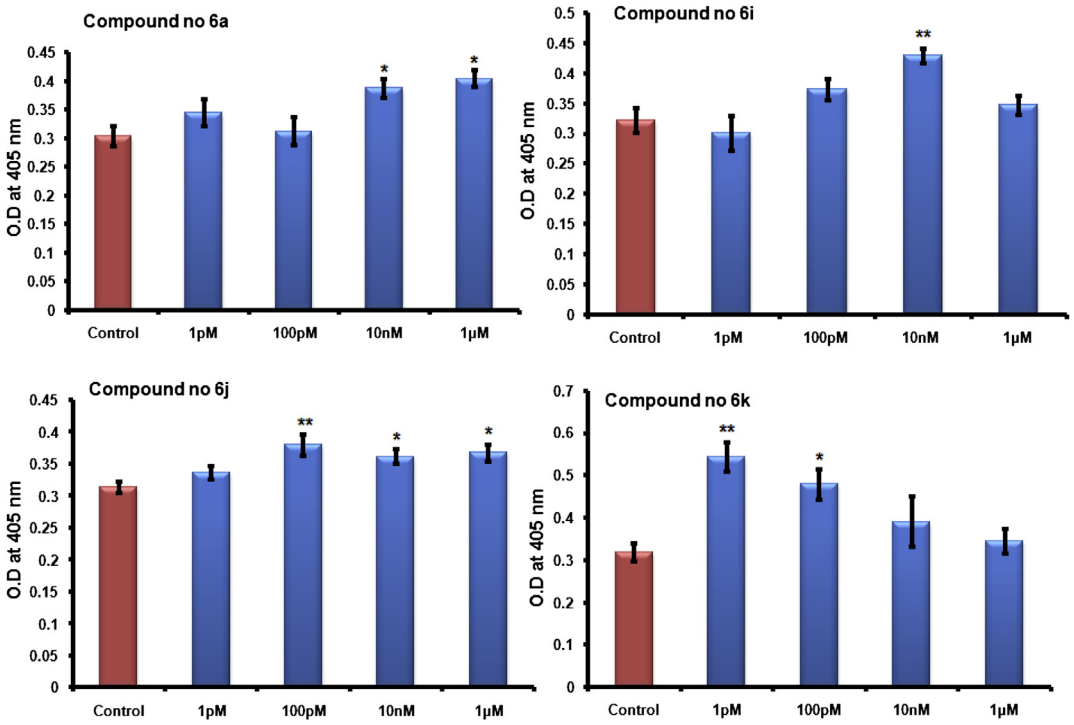


Fig. 3. Activity of the compounds **6a**, **i**, **j** and **k** as assessed by alkaline phosphatase (ALP) activity in calvarial osteoblast cells (for details see text). At the end of the experiment, ALP activity was measured colorimetrically as described before. Data shows mean ± S.D. of three independent experiments $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.01$ as compared with untreated cells taken as control.

expression of osteogenic genes (Runx-2, BMP-2 and COL-1) in calvarial osteoblasts by quantitative PCR (qPCR) [21,22]. In this study glyceraldehydes 3-phosphate dehydrogenase (GAPDH) a house keeping gene was used as the internal control. Runx-2, a bone-specific transcription factor and BMP-2 are key regulators of osteoblast differentiation [21,23]. Whereas, COL-1 is well known to be involved in the matrix maturation and mineralization process [24].

The transcript levels of Runx-2, BMP-2 and COL-1 were assessed by qPCR after treatment with compounds and results were expressed as fold change over untreated cells. Our data shows that the expression of Runx-2 gene was increased by both **6i** (~5.2 fold) and **6j** (~5.0 fold). In case of BMP-2, mRNA expression was increased by **6i** (~4.8 fold), **6a** (~2.5 fold) and **6j** (~2.4 fold). In case of COL 1, mRNA expression was increased by only **6j** (~3.1 fold) (Fig. 5).

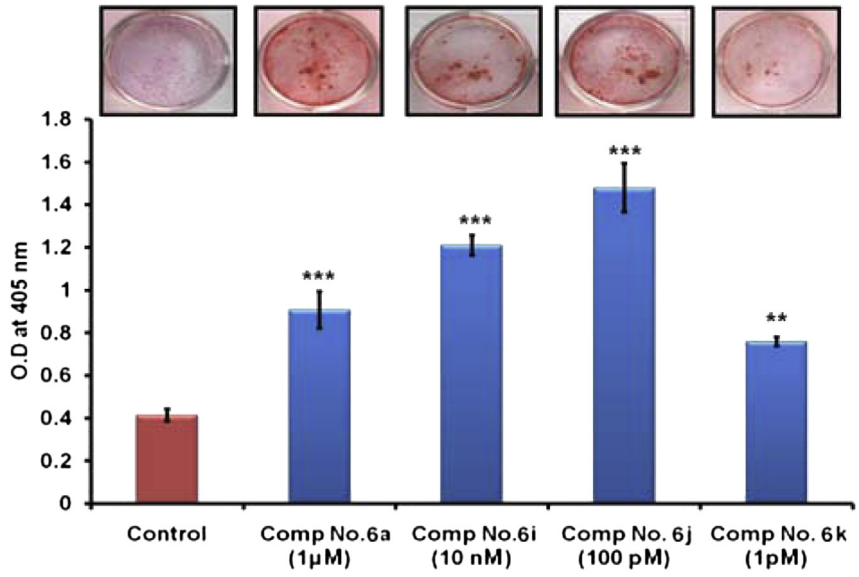


Fig. 4. - Mineralizing ability of compounds **6a**, **i**, **j** and **k** as compared to the control not treated cells in calvarial osteoblasts cells. Calvarial osteoblasts were grown in osteoblast differentiation medium as described before (see text for details). At the end of the experiments, cells were stained with alizarin red-S. Photomicrographs show increased formation of mineralized nodules by compound treatment compared to the vehicle treated cells. Bar diagram in the lower panel show quantification of mineralization by extraction of alizarin red-S dye. Data represents mean ± S.E. of three independent experiments ($^{*}p < 0.05$, $^{**}p < 0.001$, and $^{***}p < 0.001$ as compared to control).

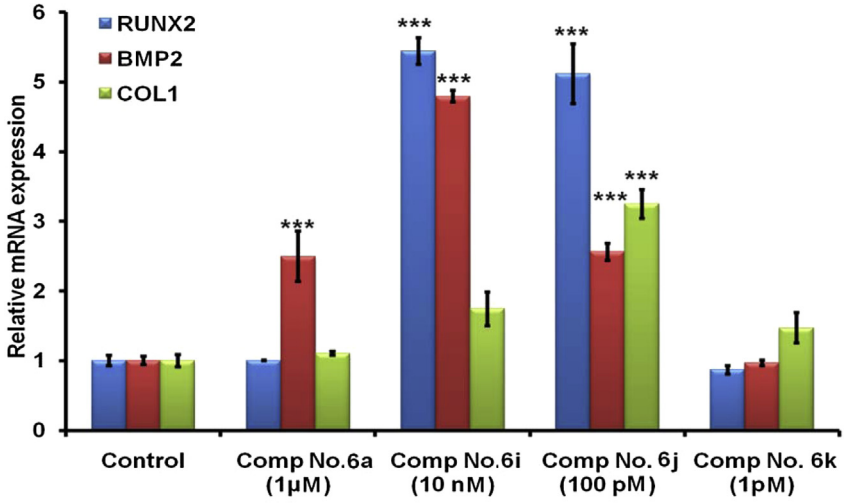


Fig. 5. - Effect of compounds **6a**, **i**, **j** and **k** on the expression of osteogenic genes (Runx-2, BMP-2 and CoL-1) in calvarial osteoblasts as compared to control by qPC.

Thus, on the basis of qPCR data the compound **6j** was the most active that caused marked increase in expression of the osteogenic genes (Runx-2, BMP-2 and COL-1). Furthermore, the active compounds **6a**, **i**, **j** and **k**, were found to be non-toxic, when assessed using MTT assay in osteoblast cells as compared to the control group at doses ranging from 1 pM to 1 μM (Fig. 6).

In terms of structure activity relationship (SAR), in general most unsymmetrical coumarin–pyridine hybrids (**6d**–**m**), exhibited significant activity (**6j**, **i** and **k**), than the symmetrical counterparts (**6a**–**c**), which underscores the importance of the unsubstituted cylohexanone moiety in the lower part of the molecule. While, on the upper part of the hybrid the presence of *sec*-butyl (R^1) side chain (**6j**, **i** and **k**) seems to enhance the activity, with the exception of **6h** and **l**. Most interestingly, this study has revealed that hydrogen bonding modification of the nitrogen ring does not effect the activity, as both coumarin–dihydropyridine hybrids (hydrogen bond donors) (Hit compound –Fig. 1) and coumarin–pyridine hybrids (hydrogen bond acceptors) retains the activity, suggesting that the binding sites for both are very similar.

5. Conclusion

A series of coumarin–pyridine hybrids were synthesized and evaluated for their anti-osteoporotic activity in calvarial osteoblast

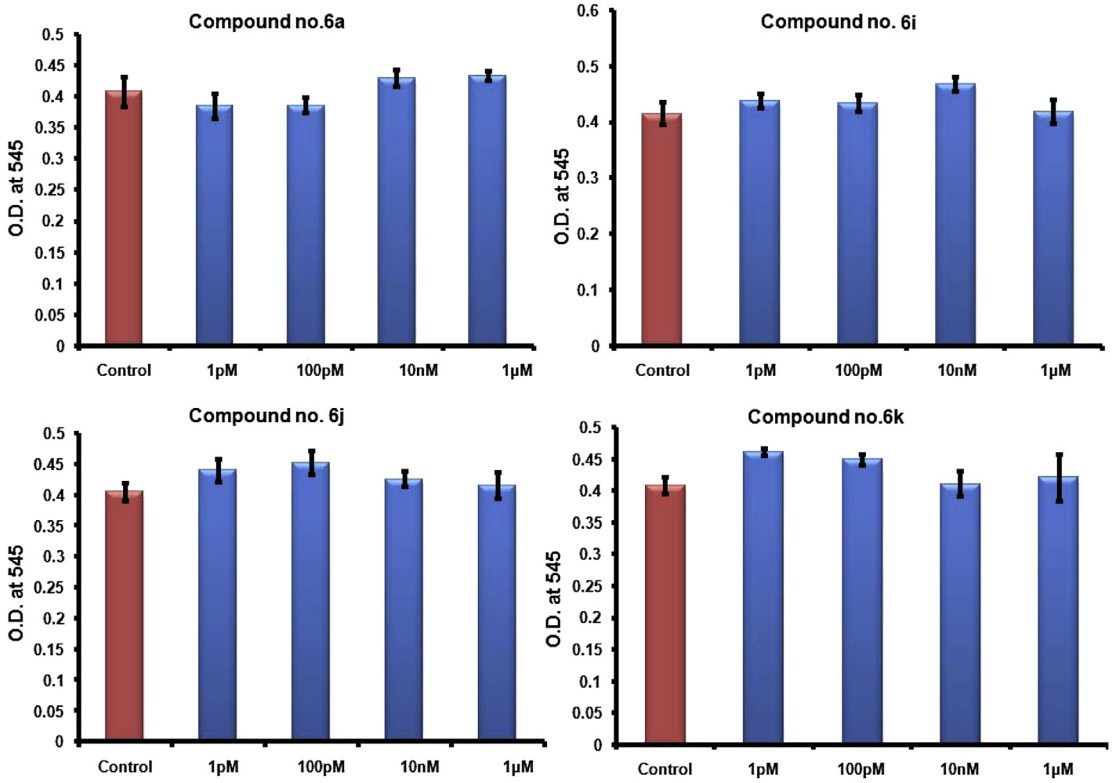


Fig. 6. - Bioactive compounds **6a**, **i**, **j** and **k** were assessed for cell viability and toxicity. Cells were cultured in differentiation medium and treated with various concentrations of the compound ranging from 100 pM to 1 μM for 48 h and cell viability assessed by MTT assay. The percent viable cells were calculated as compared to untreated cells taken as control.

cells. Out of all compounds screened, compounds **6a**, **i**, **j** and **k** significantly increased the ALP activity and potentially increased the mineralization in calvarial osteoblast cells by alizarin red-S staining. Furthermore, the compound **6j** increased the expression of osteogenic genes (RUNX2, BMP-2 and COL-1). Taken together, this study has identified compound **6j** as a new class of promising lead among the series of coumarin–pyridine hybrids. Further detailed studies (in vivo and pharmacokinetic) on **6j** are planned so as to advance this molecule in preclinical trials.

6. Experimental

6.1. General information

All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (60–120 and 100–200 mesh). All reactions were monitored by TLC; silica gel plates with fluorescence F254 were used. Melting points were uncorrected. The ^{1}H NMR, and ^{13}C NMR spectra were determined on 200, 300, 400 MHz and 50, 75, 100 MHz, respectively, using $CDCl_3$ and DMSO-d_6 as solvents and TMS as internal standard. All chemical shifts were given in ppm. IR spectra were recorded on in the range of 500–4000 cm^{-1} and multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet).

6.2. General synthetic procedure for preparation of 5-alkyl-4-hydroxy-benzene-1,3-dicarbaldehyde (**3a**–**f**)

2-alkyl phenol (1.0 equiv.) and hexamethylenetetramine (1.2 equiv.) were dissolved in TFA and the solution was heated at 120 °C for 3 h. After cooling to room temperature 10% aq.H_2SO_4 (25 mL) was added and again the temperature maintained (at 90–100 °C) for two more hours. The solution was basified with $NaHCO_3$ to pH 8 and extracted 3-fold with 50 mL of $CHCl_3$. The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product was purified on a silica gel column (100–200 mesh) using hexane–ethylacetate (12:88, v/v) as eluent to afford compounds **3a**–**f** in good yields.

6.2.1. 5-Methyl-4-hydroxy-benzene-1,3-dicarbaldehyde (**3a**)

White solid; yield: 60%; mp: 125–127 °C; IR (neat):3262, 2865, 1703, 1626, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 11.82 (s, 1H), 9.97 (s, 1H), 9.90 (s, 1H), 7.97 (d, 1H, J = 1.8 Hz), 7.93 (brs, 1H), 2.33 (s, 3H); ESI-MS: m/z: 164 $(M + H)^+$.

6.2.2. 5-Sec-butyl-4-hydroxy-benzene-1,3-dicarbaldehyde (**3c**)

Oily, yield 55%, IR (neat): 3267, 2862, 1709, 1622, 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 11.99 (s, 1H), 10.05 (s, 1H), 9.97 (s, 1H), 8.09 (brs, 1H), 8.01 (brs, 1H), 3.27–3.10 (m, 1H), 1.74–1.57 (m, 2H), 1.26 (d, 3H, J = 7.0 Hz), 0.86 (t, 3H, J = 7.3 Hz); ESI-MS: m/z: 207 $(M + H)^+$.

6.2.3. 5-Tert-butyl-4-hydroxyisophthalaldehyde (**3d**)

Oily, yield 60%, IR (neat): 3262, 2865, 1703, 1626, 1013 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 12.39 (s, 1H), 9.99 (s, 1H), 9.93 (s, 1H), 8.07 (brs, 1H), 7.99 (brs, 1H), 1.46 (s, 9H); ESI-MS: m/z: 207 $(M + H)^+$.

6.3. General synthetic procedure for preparation of methyl-8-sec-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (**4d**)

A solution of *5-secbutyl-4-hydroxy-benzene-1,3-dicarbaldehyde* (0.5 g, 2.42 mmol), dimethyl malonate (0.32 g, 2.42 mmol) in methanol (25 mL) was treated with piperidine (0.2 mL) and refluxed. Most of the excess solvent was evaporated under reduced pressure, and the residue was neutralized with acetic acid. To this residue water (25 mL) was added and extracted 3-fold with 20 mL of $CHCl_3$. The combined organic layers were dried on Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product thus obtained was purified over column chromatography to furnish (0.44 g, 64% yield) of compound **4d**.

Compounds **4a**–**f** was synthesized by following the above method.

6.3.1. Methyl-8-sec-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (**4d**)

White solid, yield: 64%; mp 122–123 °C; IR (KBr): 3062, 1749, 1609, 1576, 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.04 (s, 1H), 8.62 (s, 1H), 8.05 (brs, 1H), 7.98 (brs, 1H), 3.98 (s, 3H), 3.51–3.40 (m, 1H), 1.79–1.69 (m, 2H), 1.32 (d, 3H, J = 7 Hz), 0.88 (t, 3H, J = 7.4 Hz); ^{13}C NMR: ($CDCl_3$, 75 MHz) δ: 190.2, 163.4, 156.6, 155.9, 149.0, 137.5, 133.0, 131.9, 129.9, 118.9, 118.1, 53.2, 33.5, 29.7, 20.5, 12.0; ESI-MS: m/z: 289 $(M + H)^+$.

6.3.2. Ethyl-8-sec-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (**4e**)

White solid, yield: 65%; mp 89–90 °C; IR (KBr): 3042, 1747, 1601, 1589, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.05 (s, 1H), 8.60 (s, 1H), 8.05 (brs, 1H), 8.01 (brs, 1H) 4.44 (q, 2H, J = 7.1 Hz), 3.51–3.40 (m, 1H), 1.78–1.69 (m, 2H), 1.43 (t, 3H, J = 7.1 Hz), 1.33 (d, 3H, J = 7 Hz), 0.88 (t, 3H, J = 7.4 Hz); ^{13}C NMR: ($CDCl_3$, 75 MHz) δ: 190.2, 162.7, 156.5, 155.8, 148.5, 137.3, 132.9, 131.7, 129.9, 119.2, 118.0, 62.2, 33.4, 29.6, 20.4, 14.2, 12.0; ESI-MS: m/z: 303 $(M + H)^+$.

6.3.3. Methyl-8-tert-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate (**4f**)

White solid, yield: 68%; mp 92–93 °C; IR (KBr): 3048, 1741, 1611, 1584, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.05 (s, 1H), 8.62 (s, 1H), 8.16 (brs, 1H), 8.02 (brs, 1H), 3.98 (s, 3H), 1.55 (brs, 9H); ^{13}C NMR: ($CDCl_3$, 75 MHz) δ: 190.2, 163.3, 157.7, 155.3, 149.3, 139.7, 132.5, 131.7, 130.7, 118.6, 118.4, 53.1, 35.4, 29.6; ESI-MS: m/z: 289 $(M + H)^+$.

6.4. General synthetic procedure for preparation of dimethyl4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5b**)

To a solution of *ethyl 8-sec-butyl-6-formyl-2-oxo-2H-chromene-3-carboxylate* (1.0 equiv.) (**4e**) in ethanol, methyl acetoacetate (2.0 equiv.), ammonium acetate (3.0 equiv.) and 3 mL acetic acid was added. The reaction mixture was continued under reflux condition for 8 h. After completion of reaction (monitor by TLC), the reaction mixture was cooled to room temperature. The solid thus obtained was filtered and chromatographed over EtOAc furnished compound **5b** as white solid.

The compounds (**5a**–**c**) were prepared in a manner similar to the procedure described above.

6.4.1. Dimethyl-4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5b**)

White solid, yield: 81%; mp 247–248 °C; IR (KBr): 3004, 1680, 1596, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.48 (s, 1H), 7.51 (d, 1H, J = 1.7 Hz), 7.29 (d, 1H, J = 2.1 Hz), 6.09 (s, 1H), 5.04 (s, 1H), 4. 40 (q, 2H, J = 7.1 Hz), 3.64 (brs, 6H), 3.36 (q, 1H, J = 6.8 Hz), 2.38 (s, 6H), 1.67 (t, 2H, J = 7.5 Hz), 1.40 (t, 3H, J = 7.2 Hz), 1.25 (d, 3H, J = 6.9 Hz), 0.83 (t, 3H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 167.7, 163.3, 157.3, 151.4, 149.9, 144.8, 134.4, 132.7, 125.9, 117.7, 116.9, 103.4, 61.7, 50.9, 39.3, 33.0, 29.5, 20.6, 19.4, 14.2, 11.7; ESI-MS: (m/z): 498 $(M + H)^+$.

*6.4.2. Diethyl-4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**5c**)*

White solid, yield: 83%; mp 240–241 °C; IR (KBr): 3011, 1677, 1591, 1033 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.48 (s, 1H), 7.52 (d, 1H, $J = 1.8$ Hz), 7.31 (d, 1H, $J = 2.0$ Hz), 6.09 (s, 1H), 5.04 (s, 1H), 4.40 (q, 2H, $J = 7.1$ Hz), 4.13–4.06 (m, 4H), 3.34 (q, 1H, $J = 7.0$ Hz), 2.38 (brs, 6H), 1.69–1.64 (m, 2H), 1.40 (t, 3H, $J = 7.1$ Hz), 1.26–1.20 (m, 9H), 0.83 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 167.4, 163.3, 157.3, 155.7, 151.4, 149.9, 144.9, 144.5, 134.4, 132.8, 126.2, 117.6, 116.9, 103.6, 103.5, 61.7, 61.5, 59.8, 39.3, 33.2, 29.6, 22.9, 20.5, 19.4, 14.3, 14.2, 13.8, 11.9; ESI-MS: (m/z): 526 $(M + H)^+$.

*6.5. General synthetic procedure for preparation of (±)ethyl-4-(8-sec-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5i**)*

To a solution of methyl-8-*sec*-butyl-6-formyl-2-oxo-2*H*-chromene-3-carboxylate (1.0 mmol) (**4d**) in ethanol, ethyl acetoacetate (1.0 mmol), 1,3-cyclohexadione (1.0 mmol), ammonium acetate (3.0 mmol) and 3 mL acetic acid was added. The reaction mixture was continued under reflux condition for 7–8 h. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature. The solid thus obtained was filtered and chromatographed over EtOAc:Hexane, provided compound (**5i**) in good yield.

Compounds **5d**–**m** was synthesized in a similar pathway.

*6.5.1. (±)Ethyl 4-(3-(ethoxycarbonyl)-2-oxo-8-propyl-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo 1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5g**)*

White solid, yield: 75%; mp 238–239 °C; IR (KBr): 3002, 1685, 1593, 1042 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 9.15 (s, 1H), 8.73 (s, 1H), 7.47 (s, 1H), 7.41 (s, 1H), 4.91 (s, 1H), 4.28 (q, 2H, $J = 6.6$ Hz), 3.96 (q, 2H, $J = 6.8$ Hz), 2.76–2.58 (m, 2H), 2.41–1.96 (m, 7H), 1.61–1.51 (m, 2H), 1.30 (t, 3H, $J = 7.0$ Hz), 1.12 (t, 3H, $J = 7.0$ Hz), 1.01 (s, 3H), 0.89 (t, 3H, $J = 7.1$ Hz), 0.84 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): 195.6, 167.2, 163.3, 157.6, 151.8, 150.0, 148.8, 144.5, 144.1, 135.7, 130.1, 126.6, 117.3, 116.9, 111.5, 105.2, 61.8, 60.0, 50.8, 40.9, 36.4, 32.7, 31.3, 29.5, 27.1, 22.9, 19.4, 14.3, 13.9; ESI-MS: (m/z): 522 $(M + H)^+$.

*6.5.2. (±)Methyl-4-(8-sec-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5h**)*

White solid, yield: 85%; mp 240–241 °C; IR (KBr): 3006, 1694, 1594, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.55 (s, 1H), 7.59 (s, 1H), 7.34 (d, 1H, $J = 1.7$ Hz), 7.24 (s, 1H), 5.15 (d, 1H, $J = 3.7$ Hz), 3.92 (s, 3H), 3.60 (s, 3H), 3.35–3.26 (m, 1H), 2.52 (brs, 2H), 2.39–2.34 (m, 5H), 2.03–1.88 (m, 2H), 1.66 (d, 2H, $J = 8.3$ Hz), 1.25 (d, 3H, $J = 6.8$ Hz), 0.85–0.79 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 195.9, 167.7, 163.7, 157.6, 151.4, 150.9, 144.9, 134.7, 133.4, 126.1, 117.5, 116.1, 112.5, 104.8, 52.7, 51.0, 37.0, 36.2, 33.4, 29.4, 27.1, 21.1, 20.4, 19.1, 11.8; ESI-MS: (m/z): 480 $(M + H)^+$.

*6.5.3. (±)Ethyl-4-(8-sec-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5i**)*

White solid, yield: 85%; mp 263–264 °C; IR (KBr): 3013, 1679, 1599, 1016 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.55 (s, 1H), 7.60 (d, 1H, $J = 1.8$ Hz), 7.34 (d, 1H, $J = 2.1$ Hz), 7.04 (s, 1H), 5.15 (s, 1H), 4.09–4.01 (m, 2H), 3.93 (s, 3H), 3.35–3.27 (m, 1H), 2.50 (d, 2H, $J = 5.1$ Hz), 2.41 (s, 3H), 2.35–2.32 (m, 2H), 2.05–1.88 (m, 2H), 1.68–1.63 (m, 2H), 1.26–1.16 (m, 6H), 0.86–0.79 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 195.9, 167.2, 163.8, 157.6, 151.4, 150.8, 144.6, 134.6, 133.5, 126.3, 117.4, 116.2, 112.6, 105.1, 59.9, 52.7, 36.9, 36.3, 33.4, 29.5, 27.2, 21.1, 20.5, 19.1, 14.3, 11.8; ESI-MS: (m/z): 494 $(M + H)^+$.

*6.5.4. (±)Methyl-4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5j**)*

White solid, yield: 86%; mp 229–230 °C; IR (KBr): 3005, 1680, 1597, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.52 (s, 1H), 7.58 (s, 1H), 7.34 (d, 1H, $J = 1.7$ Hz), 7.21 (s, 1H), 5.15 (d, 1H, $J = 5.3$ Hz), 4.40 (q, 2H, $J = 7.1$ Hz), 3.60 (s, 3H), 3.30 (p, 1H, $J = 6.6$ Hz), 2.52 (brs, 2H), 2.40 (s, 3H), 2.34 (s, 2H), 2.04–1.95 (m, 2H), 1.71–1.63 (m, 2H), 1.40 (t, 3H $J = 7.1$ Hz), 1.23 (t, 3H $J = 6.9$ Hz), 0.84–0.77 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): 195.8, 167.7, 163.1, 157.6, 151.3, 150.3, 144.9, 134.6, 133.2, 126.1, 117.5, 116.5, 112.5, 104.7, 61.7, 51.0, 37.0, 36.1, 33.4, 29.4, 27.1, 21.1, 20.4, 19.1, 14.2, 11.8; ESI-MS: (m/z): 494 $(M + H)^+$.

*6.5.5. (±)Ethyl-4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5k**)*

White solid, yield: 88%; mp 225–226 °C; IR (KBr): 3011, 1686, 1590, 1045 cm^{-1}; 1H NMR ($CDCl_3$, 300 MHz) δ: 8.50 (s, 1H), 7.56 (d, 1H, $J = 1.8$ Hz), 7.37 (d, 1H, $J = 1.8$ Hz), 6.75 (s, 1H), 5.13 (s, 1H), 4. 39 (q, 2H, $J = 7.1$ Hz), 4.08–4.04 (m, 2H), 3.30 (p, 1H, $J = 6.6$ Hz), 2.45 (s, 4H), 2.39–2.10 (m, 3H), 1.67–1.60 (m, 2H), 1.40 (t, 3H, $J = 7.1$ Hz), 1.25–1.15 (m, 6H), 1.07 (s, 3H), 0.89–0.77 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):195.6, 167.2, 163.1, 157.6, 151.3, 150.2, 149.3, 144.8, 134.7, 133.2, 126.2, 117.3, 116.6, 111.3, 105.1, 61.7, 59.9, 50.7, 40.7, 36.4, 33.4, 32.6, 29.7, 26.9, 20.5, 19.2, 14.3, 11.9; ESI-MS: (m/z): 536 $(M + H)^+$.

*6.5.6. (±)Ethyl-4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (**5l**)*

White solid, yield: 89%; mp 215–216 °C; IR (KBr): 3003, 1681, 1597, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.52 (s, 1H), 7.59 (d, 1H, $J = 1.4$ Hz), 7.35 (d, 1H, $J = 1.9$ Hz), 7.27 (s, 1H), 5.15 (s, 1H), 4.41–4.35 (m, 2H), 4.08–4.02 (m, 2H), 3.34–3.25 (m, 1H), 2.51 (s, 2H), 2.41 (s, 3H), 2.34–2.31 (m, 3H), 2.03–1.97 (m, 2H), 1.70–1.61 (m, 2H), 1.39 (t, 3H, $J = 7.1$ Hz), 1.25 (t, 3H, $J = 6.8$ Hz), 1.22–1.14 (m, 3H), 0.85–0.79 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz):195.8, 167.2, 163.1, 157.6, 151.2, 150.2, 144.6, 134.5, 133.2, 126.2, 117.4, 116.5, 112.6, 104.9, 61.7, 59.8, 37.1, 36.3, 33.4, 29.5, 27.2, 21.1, 20.5, 19.1, 14.3, 11.9; ESI-MS: (m/z): 508 $(M + H)^+$.

*6.6. General synthetic procedure for preparation of diethyl 4-(3-(methoxycarbonyl)-8-methyl-2-oxo-2H-chromen-6-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (**6a**)*

To a solution of diethyl 4-(3-(methoxycarbonyl)-8-methyl-2-oxo-2*H*-chromen-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1.0 mmol) (**5a**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of tetrahydrofuran (THF) as a solvent at room temperature to gave compound **6a**. The reaction mixture was continued under room temperature for 1 h. After completion of reaction (monitored by TLC), the reaction mixture solvent was evaporated under reduced pressure, and chromatographed over EtOAc:Hexane, provided pure compound (**6a**) in good yield.

Compounds **6a**–**m** were synthesized in similar pathway.

*6.6.1. Diethyl4-(3-(methoxycarbonyl)-8-methyl-2-oxo-2H-chromen-6-yl)-2,6-dimethyl pyridine-3,5-dicarboxylate (**6a**)*

Oily; yield: 70%; IR (KBr): 3019, 1692, 1215, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.83 (s, 1H), 7.61 (s, 1H), 7.42 (s, 1H), 4.02 (q, 4H, $J = 7.0$ Hz), 3.89 (s, 3H), 2.52 (brs, 6H), 2.39 (s, 3H), 0.91–0.86 (m, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz): 166.6, 163.0, 155.6, 155.0,

152.8, 148.6, 143.7, 134.7, 131.8, 127.1, 126.4, 125.3, 118.0, 117.3, 61.3, 52.5, 22.5, 14.7, 13.4; ESI-MS: (m/z): 468 $(M + H)^+$

*6.6.2. Dimethyl 4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,6-dimethy lpyridine-3,5-dicarboxylate (**6b**)*

White solid, yield: 73%; mp 96–97 °C; IR (KBr): 3015, 1685, 1200, 760 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.80 (s, 1H), 7.61 (s, 1H), 7.35 (s, 1H), 4.31 (q, 2H, $J = 7.0$ Hz), 3.30–3.23 (m, 1H), 2.52 (brs, 6H), 1.66–1.59 (m, 2H), 1.31 (t, 3H, $J = 7.5$ Hz), 1.22 (d, 3H, $J = 6.8$ Hz), 0.78 (t, 3H, $J = 7.5$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): 167.2, 162.5, 155.7, 155.3, 151.9, 148.5, 144.1, 134.2, 132.1, 131.0, 126.6, 126.3, 118.3, 117.6, 61.2, 52.4, 32.6, 29.0, 22.6, 20.2, 14.0, 11.6; ESI-MS: (m/z): 496 $(M + H)^+$.

*6.6.3. Diethyl 4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,6-dimethyl pyridine-3,5-dicarboxylate (**6c**)*

Oily, yield: 66%; IR (KBr): 3021, 1690, 1208, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.81 (s, 1H), 7.63 (s, 1H), 7.36 (s, 1H), 4.31 (q, 2H, $J = 7.0$ Hz), 3.97 (q, 4H, $J = 6.8$ Hz), 3.29–3.22 (m, 1H), 2.53 (brs, 6H), 1.65–1.61 (m, 2H), 1.31 (t, 3H, $J = 7.1$ Hz), 1.22 (d, 3H, $J = 7.0$ Hz), 0.87–0.77 (m, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz): 166.6, 162.4, 155.6, 155.1, 148.4, 144.0, 134.2, 132.2, 131.2, 126.4, 117.5, 61.2, 32.8, 28.9, 22.5, 20.1, 14.0, 13.4, 11.7; ESI-MS: (m/z): 524 $(M + H)^+$.

*6.6.4. Ethyl 4-(3-(methoxycarbonyl)-2-oxo-8-propyl-2H-chromen-6-yl)-2-methyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6d**)*

White solid, yield: 72%; mp 123–124 °C; IR (KBr): 3013, 1683, 1219, 775 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.76 (s, 1H), 7.50 (s, 1H), 7.36 (s, 1H), 3.94–3.84 (m, 5H), 3.14 (*t*, 2H, $J = 5.4$ Hz), 2.83–2.69 (m, 2H), 2.57–2.51 (m, 5H),2.11–2.07 (m, 2H), 1.66–1.59 (m, 2H), 0.91 (t, 3H, $J = 7.1$ Hz), 0.82 (t, 3H, $J = 7.2$ Hz); ESI-MS: (m/z): 478 $(M + H)^+$.

*6.6.5. Ethyl 4-(3-(methoxycarbonyl)-2-oxo-8-propyl-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6e**)*

White solid, yield: 75%; mp 129–130 °C; IR (KBr): 3011, 1676, 1210, 769 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.77 (s, 1H), 7.51 (s, 1H), 7.35 (s, 1H), 3.94–3.84 (m, 5H), 3.08 (s, 2H), 2.84–2.66 (m, 2H), 2.52 (brs, 5H), 1.66–1.59 (m, 2H), 1.06 (s, 3H), 1.04 (s, 3H), 0.91 (t, 3H, $J = 7.1$ Hz), 0.83 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): 196.7, 166.5, 164.8, 163.0, 157.1, 155.8, 151.9, 148.9, 145.8, 134.7, 133.7, 129.4, 128.5, 126.5, 123.5, 117.3, 116.9, 61.1, 52.4, 32.9, 30.2, 22.7, 22.2, 20.7, 13.3; ESI-MS: (m/z): 506 $(M + H)^+$.

*6.6.6. Ethyl 4-(3-(ethoxycarbonyl)-2-oxo-8-propyl-2H-chromen-6-yl)-2-methyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6f**)*

Oily, yield: 75%; IR (KBr): 3018, 1690, 1220, 773 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.73 (s, 1H), 7.50 (s, 1H), 7.35 (s, 1H), 4.30 (q, 2H, $J = 6.7$ Hz), 3.92 (q, 2H, $J = 5.0$ Hz), 3.14 (s, 2H), 2.78–2.51 (m, 7H), 2.09 (brs, 2H), 1.66–1.56 (m, 2H), 1.31 (t, 3H, $J = 7.1$ Hz), 0.91 (t, 3H, $J = 7.2$ Hz), 0.82 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): 196.7, 166.6, 164.8, 162.5, 157.1, 155.9, 151.9, 148.6, 145.8, 134.6, 133.7, 129.4, 128.5, 126.6, 123.5, 117.6, 117.0, 61.2, 61.2, 32.9, 30.3, 22.7, 22.2, 20.7, 13.4; ESI-MS: (m/z): 492 $(M + H)^+$.

*6.6.7. Ethyl 4-(3-(ethoxycarbonyl)-2-oxo-8-propyl-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6g**)*

Oily, yield: 76%; IR (KBr): 3023, 1675, 1211, 778 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.74 (s, 1H), 7.51 (s, 1H), 7.35 (s, 1H), 4.30 (q, 2H, $J = 7.3$ Hz), 3.92 (q, 2H, $J = 6.5$ Hz), 3.08 (s, 2H), 2.84–2.65 (m, 2H), 2.52 (brs, 5H), 1.69–1.56 (m, 2H), 1.31 (t, 3H, $J = 7.0$ Hz), 1.06 (s, 3H), 1.04 (s, 3H), 0.91 (t, 3H, $J = 7.3$ Hz), 0.83 (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): 196.7, 166.5, 163.3, 162.5, 157.6, 155.9, 148.6, 145.6, 134.5, 133.5, 129.3, 128.5, 126.6, 122.4, 117.6, 117.0, 61.2, 61.2, 52.7, 46.5, 32.0, 30.2, 27.7, 27.5, 22.8, 22.2, 14.0, 13.4; ESI-MS: (m/z): 520 $(M + H)^+$.

*6.6.8. Methyl 4-(8-sec-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo 5,6,7,8-tetrahydroquinoline-3-carboxylate (**6h**)*

White solid, yield: 72%; mp 130–131 °C; IR (KBr): 3017, 1680, 1205, 771 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.74 (s, 1H), 7.48 (s, 1H), 7.36 (s, 1H), 3.84 (s, 3H), 3.43 (s, 3H), 3.34–3.22 (m, 1H), 3.14 (t, 2H, $J = 5.8$ Hz), 2.59–2.50 (m, 5H), 2.12–2.06 (m, 2H), 1.66–1.61 (m, 2H), 1.22 (d, 3H, $J = 6.9$ Hz), 0.79 (t, 3H, $J = 6.5$ Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): 196.6, 167.1, 164.8, 163.0, 157.1, 155.8, 151.4, 149.1, 146.1, 133.8, 133.2, 131.8, 129.3, 126.3, 123.6, 117.2, 116.9, 52.4, 52.3, 32.8, 28.7, 22.8, 20.7, 20.3, 11.6; ESI-MS: (m/z): 478 $(M + H)^+$.

*6.6.9. Ethyl 4-(8-sec-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6i**)*

Oily, yield: 70%; IR (KBr): 3025, 1680, 1223, 765 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.77 (s, 1H), 7.50 (s, 1H), 7.38 (s, 1H), 3.93–3.84 (m, 5H), 3.27–3.20 (m, 1H), 3.14 (t, 2H, $J = 6.2$ Hz), 2.57 (s, 2H), 2.51 (brs, 3H), 2.10–2.06 (m, 2H), 1.65–1.59 (m, 2H), 1.23 (brs, 3H), 0.84–0.77 (m, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz): 196.7, 166.6, 164.8, 163.1, 157.1, 155.8, 151.8, 149.1, 146.0, 133.2, 132.2, 129.3, 126.5, 126.4, 123.6, 117.2, 117.0, 61.2, 52.4, 32.9, 28.9, 28.7, 22.7, 20.7, 20.3, 13.4, 11.6; ESI-MS: (m/z): 492 $(M + H)^+$.

*6.6.10. Methyl 4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6j**)*

White solid, yield: 75%; mp 158–159 °C; IR (KBr): 3010, 1678, 1225, 774 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.71 (s, 1H), 7.48 (s, 1H), 7.35 (s, 1H), 4.30 (q, 2H, $J = 7.1$ Hz), 3.43 (s, 3H), 3.34–3.24 (m, 1H), 3.14 (t, 2H, $J = 5.4$ Hz), 2.59–2.50 (m, 5H), 2.12–2.07 (m, 2H), 1.65–1.60 (m, 2H), 1.31 (t, 3H, $J = 6.8$ Hz), 1.22 (d, 3H, $J = 7.2$ Hz), 0.79 (t, 3H, $J = 6.8$ Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): 196.6, 167.1, 164.8, 162.5, 157.1, 155.8, 151.4, 148.7, 146.1, 133.8, 133.1, 131.7, 129.3, 126.4, 123.6, 123.5, 117.6, 117.0, 61.1, 52.2, 39.2, 32.8, 29.0, 28.7, 22.8, 20.7, 14.0, 11.6; ESI-MS: (m/z): 492 $(M + H)^+$.

*6.6.11. Ethyl 4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6k**)*

Oily, yield: 65%; IR (KBr): 3024, 1695, 1216, 758 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.74 (s, 1H), 7.50 (s, 1H), 7.35 (s, 1H), 4.30 (q, 2H, $J = 6.8$ Hz), 3.91 (q, 2H, $J = 7.4$ Hz), 3.34–3.24 (m, 1H), 3.08 (s, 1H), 2.52 (brs, 5H), 1.63–1.62 (m, 2H), 1.30 (t, 3H, $J = 7.0$ Hz), 1.21 (d, 3H, $J = 6.5$ Hz), 1.06 (s, 3H), 1.03 (s, 3H), 0.83–0.79 (m, 6H); ESI-MS: (m/z): 534 $(M + H)^+$.

*6.6.12. Ethyl 4-(8-sec-butyl-3-(ethoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2-methyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6l**)*

White solid, yield: 69%; mp 95–96 °C; IR (KBr): 3028, 1676, 1209, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.73 (s, 1H), 7.49 (s, 1H), 7.37 (s, 1H), 4.30 (q, 2H, $J = 7.0$ Hz), 3.89 (q, 2H, $J = 7.5$ Hz), 3.28–3.20 (m, 1H), 3.30 (t, 2H, $J = 5.5$ Hz), 2.56–2.51 (m, 5H), 2.12–2.06 (m, 2H), 1.65–1.60 (m, 2H), 1.31 (t, 3H, $J = 7.1$ Hz), 1.21 (d, 3H, $J = 5.4$ Hz), 0.85–0.77 (m, 6H); ^{13}C NMR (DMSO-d_6, 50 MHz): 196.7, 166.6, 164.7, 162.5, 157.1, 155.8, 151.5, 148.8, 146.0, 133.9, 133.1, 132.0, 129.3, 126.4, 123.6, 117.5, 116.9, 61.2, 32.9, 29.0, 28.7, 22.7, 20.7, 20.1, 14.0, 13.4, 11.7, 11.5; ESI-MS: (m/z): 506 $(M + H)^+$.

*6.6.13. Methyl 4-(8-tert-butyl-3-(methoxycarbonyl)-2-oxo-2H-chromen-6-yl)-2,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate (**6m**)*

White solid, yield: 68%; mp 145–146 °C; IR (KBr): 3014, 1683, 1218, 772 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 8.76 (s, 1H), 7.58 (s, 1H), 7.37 (s, 1H), 3.84 (s, 3H), 3.46 (s, 3H), 3.08 (s, 2H), 2.50 (brs, 5H), 1.44 (brs, 9H), 1.07 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): 196.7, 167.2, 163.4, 162.9, 157.6, 155.3, 152.4, 149.5, 145.8, 135.7, 133.3, 130.9, 126.3, 127.7, 122.4, 117.6, 116.8, 52.7, 52.3, 46.5, 38.9, 34.4, 32.0, 29.4, 27.6, 22.8; ESI-MS: (m/z): 506 $(M + H)^+$.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.060.

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