< Supporting Information >

Discovery of a Novel Benzopyranyl Compound as a Potent

* In Vitro and In Vivo Osteogenic Agent

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### Table of Contents

I. General Information about Synthesis ........................................ S3

II. Full Characterization of $6b$ as a Novel Osteogenic Agent ............. S4

III. Synthetic Procedure and Compound Characterizations .................. S7

IV. General Information about Biological Procedure .......................... S15

V. Supporting Figure ............................................................... S17

VI. NMR Data ................................................................. S19

VII. Reference ................................................................... S31
I. General Information about Synthesis

$^1$H and $^{13}$C NMR spectra were recorded on a Varian Inova-500 [Varian Assoc., Palo Alto, USA], and chemical shifts were measured in ppm relative to internal tetramethylsilane (TMS) standard or specific solvent signal. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); bs (broad singlet), etc. Coupling constants were reported in Hz. Routine mass analyses were performed on LC/MS system equipped with a reverse phase column (C-18, 50 × 2.1 mm, 5 μm) and photodiode array detector using electron spray ionization (ESI). The HRMS analyses were conducted at the Mass Spectrometry Laboratory of Seoul National University by direct injection on JEOL JMS AX505WA spectrometer using electron impact (EI) method. The purity was determined used by Shimadzu SCL-10AVP HPLC [Japan] instrument with Shimadzu Shim pack VP-ODS (C18, 150 × 4.6 mm) column.

All reagents in this synthetic procedure were purchase from Sigma-Aldrich [MO, USA] and TCI [Japan]. The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60 F$_{254}$ 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with anisaldehyde staining solution followed by heating. Silica gel 60 (0.040–0.063 mm) used in flash column chromatography was purchased from Merck [Germany]. All reactions were conducted in oven-dried glassware under dry argon atmosphere, unless otherwise specified. CH$_2$Cl$_2$ was distilled from CaH$_2$ immediately prior to use. Other solvents and organic reagents were purchased from commercial venders and used without further purification unless otherwise mentioned.
II. Full Characterization of 6b as a Novel Osteogenic Agent

2,2-Dimethyl-4-(3-nitrophenyl)-2H-chromen-7-ol (Compound 6b)

Yellow solid (83% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 8.22–8.20 (m, 2H), 7.67 (d, $J = 8.0$ Hz, 1H), 7.56 (t, $J = 8.0$ Hz, 1H), 6.75 (d, $J = 8.5$ Hz, 1H), 6.45 (d, $J = 2.5$ Hz, 1H), 6.35 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.56 (s, 1H), 5.42 (bs, 1H), 1.50 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 157.3, 155.0, 148.6, 140.6, 135.0, 133.2, 129.5, 128.1, 126.3, 123.8, 122.8, 115.1, 108.2, 104.7, 76.5, 27.6; HRMS (EI+) m/z calculated for C$_{17}$H$_{15}$NO$_4$ [M]$^+$: 297.1001; Found: 297.1003.

HPLC Spectrum

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**MASS Data**

**LRMS**

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Base: m/z 242.799508  
TIC: 2323019  
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Time Run: 17.51.47  
R.T.: 1.04.26

**HRMS**

File: 5795  
Sample: 4b  
Instrument: JEOL JMS600  
Inlet: Direct Probe  
Scan: 78-103  
Base: m/z 282.169958  
TIC: 1699276  
Date Run: 03-16-2010  
Time Run: 16.38.43  
R.T.: 1.02.45

Selected Isotopes: $C_{48}H_{146}O_{10}N_{10}$

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$^1$H and $^{13}$C NMR Data
III. Synthetic Procedure and Compound Characterizations

General procedure for the cyclization of hydroxyacetophenone

A solution of hydroxyacetophenone (1.0 equiv.) in EtOH was treated with pyrrolidine (3.0 equiv.) and then ketone (cyclopentanone 3.0 equiv., acetone 10.0 equiv., or ethyl levulinate 3.0 equiv.) and then heated at reflux for about 48 h. After the completion of reaction monitored by TLC, the reaction mixture was concentrated in vacuo. Evaporated residue was redissolved in ethyl acetate and washed several times with 1N HCl solution. After washing with brine, the combined organic layer was dried over anhydrous MgSO₄, filtrated, and evaporated in vacuo. Purification by silica-gel flash column chromatography gave the desired compound 2a~2d.

To a solution of purified compound 2 (1.0 equiv.) and imidazole (1.5 equiv.) dissolved in CH₂Cl₂, tert-butyldimethylsilyl chloride (TBDMSCl, 1.2 equiv.) was added and then stirred for about 3 h at room temperature. After the reaction completion monitored by TLC, the reaction mixture was diluted with ethylacetate and then washed with brine. The separated organic layer was dried over anhydrous MgSO₄, filtrated and evaporated in vacuo. The resulting mixture was purified with silica-gel flash column chromatography to provide desired product 3a~3d.

Compound 2a

Colorless crystal (69% from 2',4'-dihydroxyacetophenone); this compound was previously reported.¹
**Compound 2b**

![Compound 2b](image)

Colorless crystal (64% from 2',3'-dihydroxy-4'-methoxyacetophenone); this compound was previously reported.¹

**Compound 2c**

![Compound 2c](image)

Colorless crystal (58% from 2',4'-dihydroxyacetophenone); this compound was previously reported.¹

**Compound 2d**

![Compound 2d](image)

Colorless crystal (68% from 2',4'-dihydroxyacetophenone); 

\[ ^1H \text{-NMR (500 MHz, CDCl}_3\]): \delta 8.74 (bs, 1H), 7.77 (d, \text{ } J = 8.5 \text{ Hz, } 1H), 6.55 (dd, \text{ } J = 8.5 \text{ and } 2.0 \text{ Hz, } 1H), 6.39 (d, \text{ } J = 2.0 \text{ Hz, } 1H), 4.15 (q, \text{ } J = 7.0 \text{ Hz, } 2H), 2.79–2.60 (AB q, \text{ } J_{AB} = 16.5 \text{ Hz, } 2H), 2.53–2.49 (m, 2H), 2.21–2.15 (m, 1H), 2.05–1.99 (m, 1H), 1.40 (s, 3H), 1.26 (t, \text{ } J = 7.0 \text{ Hz, } 3H); ^{13}C \text{-NMR (125 MHz, CDCl}_3\): \delta 192.2, 173.9, 165.0, 162.2, 129.1, 113.8, 110.7, 103.8, 80.5, 61.2, 47.0, 34.6, 29.1, 23.8, 14.4.]

**General procedure for the formation of vinyl triflate**

A solution of compound 3 (1.0 equiv.) and 2,6-di-tert-butyl-4-methylpyridine (DTBMP, 1.3 equiv.) in anhydrous CH₂Cl₂ under N₂ atmosphere at 0 °C was treated with triflic anhydride
(Tf₂O, 1.2 equiv.). The reaction was stirred for 10 min at the same temperature. The reaction mixture was filtered to remove any solid and the filtrate was concentrated in vacuo. The residue was redissolved in ethyl acetate and washed with sat. NaHCO₃ solution and brine. The combined organic layer was dried over anhydrous MgSO₄, filtrated, and evaporated in vacuo. Purification by silica-gel flash column chromatography gave the desired compound 4a~4d.

**General procedure of Suzuki coupling for compound 6**

Compound 4 (1.0 equiv.), arylboronic acid derivative (1.1 equiv.), Pd(PPh₃)₄ (5 mol%) and Na₂CO₃ (3.0 equiv.) were suspended in solvent mixture of EtOH:toluene:H₂O (1:1:0.5) and the reaction mixture was stirred at 70 °C for about 3 h. After reaction completion monitored by TLC, the resulting mixture was diluted with ethyl acetate and then washed with brine. The organic layer was dried over anhydrous MgSO₄, filtrated, and evaporated in vacuo. Purification by silica-gel flash column chromatography gave the desired compound 5a~5m.

To a solution of purified compound 5 (1.0 equiv.) dissolved in tetrahydrofuran (THF), tetrabutylammonium fluoride (1M solution in THF, 1.5 equiv.) was added at 0 °C and then stirred for about 30 min at the same temperature. After the reaction completion monitored by TLC, the reaction mixture was diluted with ethylacetate and then washed with brine. The separated organic layer was dried over anhydrous MgSO₄, filtrated and evaporated in vacuo. Purification by silica-gel flash column chromatography gave the desired compound 6a~6m.
**Compound 6a**

Yellow solid (87% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 8.25 (dd, $J = 7.0$ and 2.0 Hz, 2H), 7.51 (dd, $J = 7.0$ and 2.0 Hz, 2H), 6.77 (d, $J = 8.5$ Hz, 1H), 6.44 (d, $J = 2.5$ Hz, 1H), 6.34 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.56 (s, 1H), 5.16 (s, 1H), 1.50 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 157.3, 155.0, 147.5, 145.8, 133.5, 129.7, 128.4, 126.4, 123.9, 115.1, 108.1, 104.7, 76.5, 27.6; LRMS (ESI) m/z calculated for C$_{17}$H$_{16}$NO$_4$ [M+H]$^+$: 298.11; Found: 298.08.

**Compound 6b**

Yellow solid (83% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 8.22–8.20 (m, 2H), 7.67 (d, $J = 8.0$ Hz, 1H), 7.56 (t, $J = 8.0$ Hz, 1H), 6.75 (d, $J = 8.5$ Hz, 1H), 6.45 (d, $J = 2.5$ Hz, 1H), 6.35 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.56 (s, 1H), 5.42 (bs, 1H), 1.50 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 157.3, 155.0, 148.6, 140.6, 135.0, 133.2, 129.5, 128.1, 126.3, 123.8, 122.8, 115.1, 108.2, 104.7, 76.5, 27.6; HRMS (EI+) m/z calculated for C$_{17}$H$_{15}$NO$_4$ [M]$^+$: 297.1001; Found: 297.1003.

**Compound 6c**

Yellow solid (77% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.95 (d, $J = 7.5$ Hz, 1H), 7.60 (t, $J = 7.5$ Hz, 1H), 7.49 (t, $J = 7.5$ Hz, 1H), 7.35 (d, $J = 7.5$ Hz, 1H), 6.44 (d, $J = 8.5$ Hz, 1H), 6.40 (d, $J = 2.0$ Hz, 1H), 6.25 (dd, $J = 8.5$ and 2.0 Hz, 1H), 5.35 (s, 1H), 1.48 (s, 3H), 1.45 (s, 3H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 157.6, 154.3, 149.7, 133.5, 133.0, 132.2, 131.5, 128.9, 126.1, 125.8, 124.3, 115.1, 108.3, 104.4, 76.5, 28.1, 26.8; LRMS (ESI) m/z calculated for C$_{17}$H$_{16}$NO$_4$ [M+H]$^+$: 298.11; Found: 298.19.
Compound 6d

Yellow solid (81% from compound 4b); \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.23–8.20 (m, 2H), 7.69 (d, \(J = 7.5\) Hz, 1H), 7.57 (t, \(J = 7.5\) Hz, 1H), 6.45–6.40 (m, 2H), 5.62 (s, 1H), 5.58 (bs, 1H), 3.90 (s, 3H), 1.55 (s, 6H); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)): \(\delta\) 148.5, 148.3, 140.7, 140.4, 135.0, 134.7, 133.4, 129.4, 128.4, 123.8, 122.8, 115.9, 103.8, 77.0, 56.3, 27.6; LRMS (ESI) \(m/z\) calculated for C\(_{18}\)H\(_{18}\)NO\(_5\) [M+H]\(^+\): 328.12; Found: 327.99.

Compound 6e

Yellow solid (79% from compound 4c); \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.22–8.19 (m, 2H), 7.68 (d, \(J = 8.0\) Hz, 1H), 7.55 (t, \(J = 8.0\) Hz, 1H), 6.75 (d, \(J = 8.5\) Hz, 1H), 6.42 (d, \(J = 2.5\) Hz, 1H), 6.33 (dd, \(J = 8.5\) and 2.5 Hz, 1H), 5.62 (s, 1H), 5.31 (bs, 1H), 2.23–2.19 (m, 2H), 1.96–1.90 (m, 2H), 1.77–1.64 (m, 4H); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)): \(\delta\) 157.2, 155.0, 148.6, 140.6, 135.0, 133.9, 129.5, 127.3, 126.1, 123.8, 122.8, 115.9, 108.1, 104.8, 87.4, 39.0, 23.7; LRMS (ESI) \(m/z\) calculated for C\(_{19}\)H\(_{18}\)NO\(_4\) [M+H]\(^+\): 324.12; Found: 324.14.

Compound 6f

Yellow solid (83% from compound 4d); \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.22–8.20 (m, 2H), 7.67 (d, \(J = 7.5\) Hz, 1H), 7.56 (t, \(J = 7.5\) Hz, 1H), 6.72 (d, \(J = 8.5\) Hz, 1H), 6.44 (d, \(J = 2.5\) Hz, 1H), 6.36 (dd, \(J = 8.5\) and 2.5 Hz, 1H), 5.45 (s, 1H), 4.12 (q, \(J = 7.0\) Hz, 2H), 2.60–2.47 (m, 2H), 2.16–2.08 (m, 2H), 1.46 (s, 3H), 1.22 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)): \(\delta\) 174.2,
158.5, 154.7, 148.6, 148.5, 140.4, 135.0, 134.4, 129.5, 126.2, 125.7, 123.7, 122.8, 113.9, 108.4, 104.5, 78.0, 60.9, 35.9, 29.6, 26.0, 14.3; LRMS (ESI) m/z calculated for C_{21}H_{22}NO_6 [M+H]^+: 384.14; Found: 384.22.

**Compound 6g**

White solid (81% from compound 4a); ^1^H-NMR (500 MHz, CDCl₃): δ 7.25 (t, J = 8.5 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 6.76–6.74 (m, 1H), 6.71–6.70 (m, 2H), 6.38 (d, J = 2.5 Hz, 1H), 6.23 (dd, J = 8.0 and 2.5 Hz, 1H), 5.47 (s, 1H), 2.95 (s, 6H), 1.47 (s, 6H); ^1^C-NMR (125 MHz, CDCl₃): δ 156.8, 155.0, 150.8, 139.7, 135.4, 129.2, 127.1, 126.1, 117.8, 116.3, 113.4, 112.5, 107.7, 104.2, 76.5, 41.1, 27.9; LRMS (ESI) m/z calculated for C_{19}H_{22}NO_2 [M+H]^+: 296.17; Found: 296.34.

**Compound 6h**

White solid (64% from compound 4a); ^1^H-NMR (500 MHz, MeOD): δ 7.11 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 6.69 (s, 1H), 6.63 (d, J = 7.5 Hz, 1H), 6.29 (d, J = 2.5 Hz, 1H), 6.26 (dd, J = 8.5 and 2.5 Hz, 1H), 5.41 (s, 1H), 1.41 (s, 6H); ^1^C-NMR (125 MHz, MeOD): δ 159.8, 156.1, 148.8, 141.2, 136.7, 130.1, 127.8, 126.3, 119.9, 117.0, 116.2, 116.0, 108.7, 104.9, 77.1, 28.0; LRMS (ESI) m/z calculated for C_{17}H_{18}NO_2 [M+H]^+: 268.13; Found: 267.98.

**Compound 6i**

White solid (83% from compound 4a); ^1^H-NMR (500 MHz, CDCl₃): δ 7.24 (t, J = 8.0 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.83 (dd, J = 8.5 and 2.5 Hz, 1H), 6.77–6.76 (m, 1H), 6.40 (d, J = 2.5 Hz, 1H),
6.29 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.49 (bs, 2H), 5.47 (s, 1H), 1.46 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 156.8, 155.6, 154.8, 140.5, 134.4, 129.8, 126.9, 126.5, 121.4, 116.0, 115.8, 114.9, 107.9, 104.4, 76.6, 27.7; LRMS (ESI) $m/z$ calculated for C$_{17}$H$_{17}$O$_3$ [M+H]$^+$: 269.12; Found: 269.13.

**Compound 6j**

White solid (77% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.95–7.93 (m, 2H), 7.54 (d, $J = 7.5$ Hz, 1H), 7.48 (t, $J = 7.5$ Hz, 1H), 6.77 (d, $J = 8.0$ Hz, 1H), 6.45 (d, $J = 2.5$ Hz, 1H), 6.33 (dd, $J = 8.0$ and 2.5 Hz, 1H), 5.81 (bs, 1H), 5.51 (s, 1H), 2.64 (s, 3H), 1.49 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 199.0, 157.3, 155.0, 139.5, 137.4, 134.1, 133.8, 128.8, 127.9, 127.2, 126.5, 115.6, 108.0, 104.5, 76.5, 27.7, 27.0; LRMS (ESI) $m/z$ calculated for C$_{19}$H$_{19}$O$_3$ [M+H]$^+$: 295.13; Found: 295.11.

**Compound 6k**

White solid (85% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.34 (t, $J = 1.5$ Hz, 1H), 7.22 (d, $J = 1.5$ Hz, 2H), 6.80 (d, $J = 8.5$ Hz, 1H), 6.41 (d, $J = 2.5$ Hz, 1H), 6.34 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.49 (s, 1H), 5.17 (bs, 1H), 1.47 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 157.2, 154.9, 141.9, 135.1, 132.9, 127.9, 127.8, 127.4, 126.4, 115.1, 108.1, 104.6, 76.4, 27.6; LRMS (ESI) $m/z$ calculated for C$_{17}$H$_{15}$ClO$_2$ [M+H]$^+$: 321.04; Found: 321.10.
Compound 6l

White solid (82% from compound 4a); $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 7.22 (d, $J = 8.5$ Hz, 2H), 6.91 (d, $J = 8.5$ Hz, 1H), 6.77 (d, $J = 8.5$ Hz, 2H), 6.39 (d, $J = 2.5$ Hz, 1H), 6.22 (dd, $J = 8.5$ and 2.5 Hz, 1H), 5.41 (s, 1H), 2.98 (s, 6H), 1.45 (s, 6H); $^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 156.7, 155.1, 150.3, 134.4, 129.7, 126.9, 125.2, 116.5, 112.8, 107.6, 104.3, 76.5, 41.0, 27.9; LRMS (ESI) $m/z$ calculated for C$_{19}$H$_{22}$NO$_2$ [M+H]$^+$: 296.17; Found: 296.07.

Compound 6m

White solid (70% from compound 4a); $^1$H-NMR (500 MHz, MeOD): $\delta$ 7.05 (d, $J = 8.5$ Hz, 2H), 6.83 (d, $J = 8.5$ Hz, 1H), 6.73 (d, $J = 8.5$ Hz, 2H), 6.28–6.25 (m, 2H), 5.36 (s, 1H), 1.40 (s, 6H); $^{13}$C-NMR (125 MHz, MeOD): $\delta$ 159.7, 156.3, 148.5, 136.3, 130.6, 129.9, 127.8, 125.4, 116.6, 116.4, 108.7, 104.9, 77.1, 28.0; LRMS (ESI) $m/z$ calculated for C$_{17}$H$_{18}$NO$_2$ [M+H]$^+$: 268.13; Found: 268.27.
IV. General Information about Biological Procedure

**ALP assay and staining**

To assess ALP activities, C3H10T1/2 cells were seeded into 24-well plate at $3 \times 10^4$ cells/well. After 24 hrs, each small molecule (0.25, 0.5, 1, 2 and 4 µM) or vehicle was treated and Wnt-3a (100 ng/ml) was used as a control. After incubation for 72 hrs, cell lysates were prepared and ALP activities were measured via the previously described manners. All data were expressed as means ± SDs of triplicate wells. ALP staining was carried out using an alkaline phosphatase kit according to the manufacturer's instructions (Promega, Southampton, U.K.). Briefly, after 5 min fixation with 10% formalin, the osteogenic culture was incubated in a mixture of nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate $p$-toluidine salt within 1 h. The resulting blue, insoluble, and granular dye deposit indicated sites of alkaline phosphatase activity.

**Establishment of TOPflash transfected C3H10T1/2 cells**

To generate the high-throughput screening system, $3 \times 10^5$ C3H10T1/2 cells were plated onto a 60-mm culture dish. After 24 h incubation at 37°C, TOPflash plasmid was transfected by using Lipofectamine Plus system (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. To set up for the stable cell line selection, standard media was replaced by media containing 1,000 µg/ml G418 after 48 h. Stably transfected TOPflash-C3H10T1/2 cells were established 3 weeks later, based upon this selection process. $1 \times 10^4$ TOPflash-C3H10T1/2 cells were plated and incubated for 24 h followed by treatment of small molecules (2 µM) or Wnt-3a (100 ng/mL) for 48 h before cell harvest. The dual luciferase assay was performed using the Promega Luciferase assay system and activity was
measured using a luminometer (Lumat LB 9507, Berthold, Germany). All data were expressed as means ± SDs of triplicate wells.

**Animal experiments**

Female C57BL/6 10-week old mice were purchased from Japan SLC Lab (Hamamatsu, Japan). All animal experiments were performed under approval from the Institutional Animal Care and Use Committee of Seoul National University and complied with the National Research Council’s “Guidelines for the Care and Use of Laboratory Animals” (revised 1996). 12-Week-old female C57BL/6 mice were divided into five groups, as described above. On study day 0, bilateral OVX or Sham-op was performed by the standard method for “OVX” or “Sham-op” groups, respectively. OVX mice were permitted to lose bone mass for 4 weeks, in order to establish osteopenia, before the initiation of treatments. Compound 6b, at a dose of 5 or 10 mg/kg/d, was administered orally, using an oral zonde needle, from study day 28 to day 56 (for 4 weeks, 5 days per week). The compound was prepared in a 40% PEG400 / 5% DMSO vehicle (Sigma-Aldrich, MO). For OVX + estradiol group, 60 mg/kg of 17β-estradiol was weekly injected intramuscularly. BMD (g/cm²) and body composition was measured, using a Lunar PIXImus densitometer (software version 2.0; GE Lunar, Madison, WI), on weeks 0, 4, and 8, for the remaining mice. For three-dimensional measurement with Micro-CT, femurs were obtained 4 weeks after treatment, fixed overnight in 70% ethanol and analyzed using a Micro-CT scanner and associated analysis software (model 1076, Skyscan, Antwerp, Belgium) at 9-μm voxel size.
V. Supporting Figure

**Fig. S1** Representative compounds without osteogenic activity based on our initial library screening.

**Fig. S2** Alizarin red staining of cell calcification treated with 6b (4 μM) in osteogenic medium (OM) containing L-ascorbic acid and β-glycerophosphate.
Fig. S3  6b does not affect the cell viability in various cell lines monitored by mitochondrial activity using WST-1 assay at the varied concentration for 24 h incubation.
VI. NMR Data

6a
6c
6d
6e
6g
6h
6i
$6k$
61
VII. Reference