Bioorganic & Medicinal Chemistry Letters 27 (2017) 2401–2406

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel 5,6-dehydrokawain analogs as osteogenic inducers and their action mechanisms

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ARTICLE INFO

Article history:
Received 6 February 2017
Revised 3 April 2017
Accepted 5 April 2017
Available online 6 April 2017

Keywords:
5,6-Dehydrokawain
Kavalactone
MC3T3-E1
Mineralization
Osteoblast

ABSTRACT

An imbalance between bone resorption by osteoclasts and bone formation by osteoblasts can cause bone loss and bone-related disease. In a previous search for natural products that increase osteogenic activity, we found that 5,6-dehydrokawain (**1**) from *Alpinia zerumbet* promotes osteoblastogenesis. In this study, we synthesized and evaluated series of 5,6-dehydrokawain analogs. Our structure-activity relationships revealed that alkylation of *para* or *meta* position of aromatic ring of **1** promote osteogenic activity. Among the potential analogs we synthesized, (*E*)-6-(4-Ethylstyryl)-4-methoxy-2*H*-pyran-2-one (**14**) and (*E*)-6-(4-Butylstyryl)-4-methoxy-2*H*-pyran-2-one (**21**) both significantly up-regulated Runx2 and Osterix mRNA expression at 10 μM. These osteogenic activities could be mediated by bone morphogenetic protein (BMP) and activation of p38 MAPK signaling pathways. Compounds **14** and **21** also inhibited RANKL-induced osteoclast differentiation of RAW264 cells. These results indicated that novel 5,6-dehydrokawain analogs not only increase osteogenic activity but also inhibit osteoclast differentiation, and could be potential lead compounds for the development of anti-osteoporosis agents.

Osteoporosis, characterized by systemic dysfunction in bone mass and strength that leads to fragility fractures, is a growing medical and socioeconomic issue. Osteoblastic bone formation and osteoclastic bone resorption are tightly coordinated during the remodeling cycle and loss of bone mass results from an imbalance of these fundamental processes.[1,2] Recent therapies for osteoporosis have mainly focused on the suppression of bone resorption by osteoclasts.[2] For example, bisphosphonates and selective estrogen receptor modulators have been used clinically to prevent bone loss.[1,2] However, by the time they initiate such therapies, many osteoporotic patients have already lost substantial amounts of bone. Therefore, anabolic treatments that enhance osteogenesis are needed.[3] In this regard, the ability of some chemical components in food and medicinal plants to promote osteogenesis has been studied.[4–7] In particular, the ability of isoflavones such as daidzein and genistein, which abundant in soybeans, to prevent bone loss has been extensively investigated using various experimental models and in clinical studies.[8–10] Recently, the synthesis and osteogenic activity of daidzein analogs was reported.[11,12] These analogs are expected to be lead compounds for osteoporosis treatments.

As a part of our exploration of natural products that enhance osteogenic activities, we identified the kavalactone 5,6-dehydrokawain (**1**) from *Alpinia zerumbet*,[13] and showed that it has osteogenic activity (Fig. 1).[14] To develop 5,6-dehydrokawain analogs that have more potent osteogenic activity than the natural parent products, and to reveal structure–activity relationships,

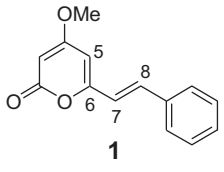

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Fig. 1. Structure of 5,6-dehydrokawain (**1**).

Abbreviations: ALP, alkaline phosphatase; αMEM, alpha minimum essential medium; BMP, bone morphogenetic protein; DMSO, dimethyl sulfoxide; HRMS, high-resolution mass spectrometry; MAPK, mitogen-activated protein kinase; ODM, osteoblast differentiation medium; RANKL, receptor activator of nuclear factor-κB ligand; TRAP, tartrate-resistant acid phosphatase.

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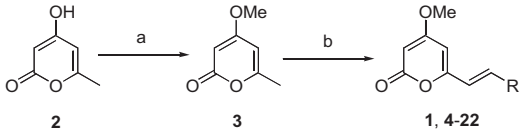
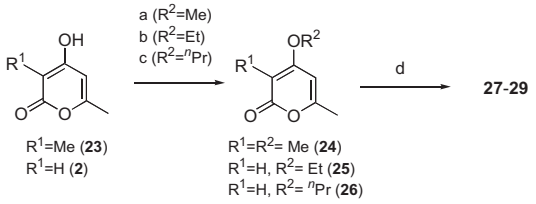
http://dx.doi.org/10.1016/j.bmcl.2017.04.016
0960-894X/

here we synthesized a series of chemically modified 5,6-dehydrokawain analogs and evaluated their osteogenic activity using the mouse osteoblast cell line MC3T3-E1. For those compounds that had potent activities, we investigated the molecular mechanisms underlying the osteogenic activity and the effects against osteoclastogenesis using RANKL-induced RAW264 cells.



Scheme 1. Synthesis of 5,6-dehydrokawain and its analogs. Reagents and conditions: (a) Me_2SO_4, K_2CO_3, DMSO, rt; (b) RCHO, $Mg(OMe)_2$, MeOH, 60 °C.



Scheme 2. Synthesis of 5,6-dehydrokawain analogs **27**–**29**. Reagents and conditions: (a) dimethyl sulfate, K_2CO_3, DMSO, rt; (b) diethyl sulfate, K_2CO_3, DMSO, rt; (c) dipropyl sulfate, K_2CO_3, DMSO, rt; (d) benzaldehyde, $Mg(OMe)_2$, MeOH, 60 °C.

The synthesis of 5,6-dehydrokawain analogs was performed using an aldol reaction (Scheme 1).[15,16] Commercially available 4-hydroxy-6-methylpyrone was methylated by dimethyl sulfate, and condensed with a variety of aldehydes by magnesium methoxide in anhydrous methanol. Only 3-ethylbenzaldehyde was synthesized as previously described.[17] Other aldehydes were obtained from commercial suppliers and used without further purification. For condensation, the mixtures were heated at reflux for 3–6 h and then cooled. The solvent was removed *in vacuo* and the crude product was purified by preparative HPLC.

To prepare pyrones **27**–**29**, commercially available pyrones were reacted with each alkylating reagent and then condensed with benzaldehyde (Scheme 2). The detailed method, synthetic yields, melting points, ^1H and ^{13}C NMR, HRMS data, purity data are described in Supplementary data.

To evaluate the effects of these compounds on osteoblast differentiation, MC3T3-E1 cells were cultured with the synthesized derivatives in osteoblast differentiation medium (ODM) containing ascorbic acid and β-glycerophosphate which were essential for the expression of osteoblast phenotype.[18] After 4 and 10 days, alkaline phosphatase activity and osteoblastic mineralization, respectively, were assessed. Alkaline phosphatase activity is an early-stage osteogenesis marker, whereas mineralization characterizes late-stage osteogenesis.[19]

We first synthesized compounds **1** and **4**–**14** as a small-scale library for modification of the phenyl ring in 5,6-dehydrokawain (**1**) and evaluated osteogenic activities in an initial screen (Fig. 2A). Synthetic **1** (60 μM) enhanced ALP activity by approximately 1.25-

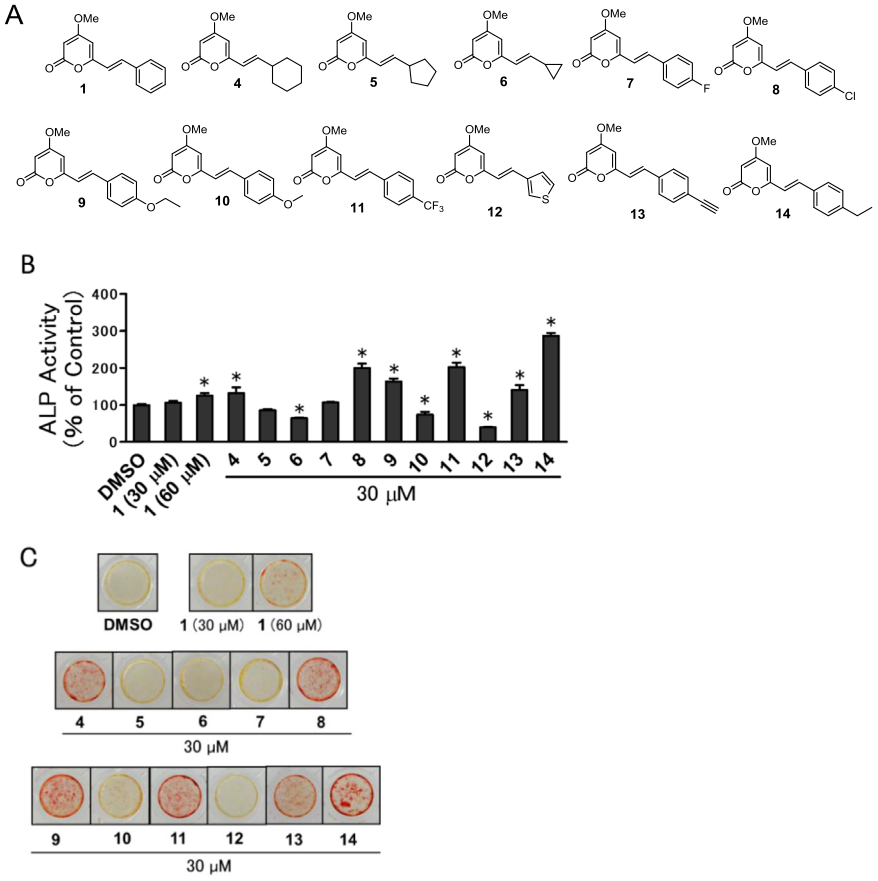


Fig. 2. Structures of synthesized compounds **1** and **4**–**14** (A), and their effects on ALP activity (B) and mineralization (C) in MC3T3-E1 cells. The cells were cultured with or without test compounds in an osteoblast differentiation medium containing ascorbic acid (50 μg/mL) and β-glycerophosphate (10 mM) for 4 days (ALP activity) and 10 days (Mineralization). Mineralization was visualized by Alizarin Red S staining and photographed. The data represent means ± SD of triplicate analysis. The photographs show representative views of triplicate analyses. $^*P < 0.05$ versus the vehicle control by Dunnett's test.