Bioorganic Chemistry 78 (2018) 94–102

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Synthesis of some novel orcinol based coumarin triazole hybrids with capabilities to inhibit RANKL-induced osteoclastogenesis through NF-κB signaling pathway

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ARTICLE INFO

Article history:
Received 18 September 2017
Revised 6 February 2018
Accepted 6 March 2018
Available online 7 March 2018

Keywords:
Orcinol
Coumarin traizoles
RANKL
Osteoclastogenesis
TRAP-staining assay

ABSTRACT

A total of twenty-two novel coumarin triazole hybrids (**4a**-**4k** and **6a**-**6k**) were synthesized from orcinol in good to excellent yields of 70–94%. The structures of all the synthesized compounds were elucidated by spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, and HRMS. The anti-inflammatory potential of synthesized compounds was investigated against the proinflammatory cytokine, TNF-α on U937 cell line and compounds **4d**, **4j**, and **6j** were found to exhibit promising anti-inflammatory activity. These three compounds were further screened against TNF-α on LPS-stimulated RAW 264.7 cells, which confirm their anti-inflammatory potential. Furthermore, the above said active compounds were tested for their inhibitory effect on RANKL-induced osteoclastogenesis in RAW 264.7 cells by using tartrate resistant acid phosphatase (TRAP) staining assay at 10 μM. Molecular mechanism studies demonstrated that compound **4d** exhibited dose dependent inhibition of RANKL-induced osteoclastogenesis by suppression of the NF-kB pathway. Thus, compound **4d** is a promising candidate for further optimization to develop as a potent anti-osteoporotic agent.

1. Introduction

Bone is an active and dynamic tissue, provides support to the skeletal system, protects the internal organs and acts as a reservoir of hematopoiesis. Bone remodeling is regulated by the osteoclasts and osteoblasts [1]. Especially, osteoclasts are bone specific multinucleated cells generated from hematopoietic monocyte precursor cells which help in resorption of the bone. On the other hand, the osteoblasts are derived from a mesenchymal stem cell that differentiates into a pre-osteoblastic stromal cell and then into an osteoblasts which helps in the formation of bone. Enhanced bone degradation by osteoclasts result in the development of bonelytic diseases such as rheumatoid arthritis, periodontitis, Paget's disease and malignant bone diseases [2–4]. The receptor activator of nuclear factor-kappa B ligand (RANKL) is a member of the tumor necrosis factor (TNF) cytokine super family and plays an essential role in bone resorption. RANKL regulates the development, maintenance and activation of osteoclasts. RANKL specifically binds to its receptor (RANK), which results in differentiation of osteoclasts leading to bone resorption [5,6].

In view of this, present treatment to bonelytic diseases mainly focuses on the inhibition of osteoclastogenesis. Bisphosphonates are antiresorptive drugs, which are synthetic analogues of pyrophosphates, most commonly used medication for the treatment of osteoporosis and they act by inhibiting osteoclast activity [7]. In addition to this, bisphosphonate have specific serious adverse effects such as renal toxicity and jaw necrosis [8]. Estrogen replacement therapy also suppresses osteoporosis, but it suffers from drawbacks like increased risk of breast cancer and stroke [9]. Therefore, there is huge demand for the development of better and alternate strategies for treatment of osteoporosis.

Literature review reveals that most of the drugs are produced from natural products or their chemically modified structures. Hence, they play a vital role in discovering new drugs. In search of new class of antiresorptive drug also we found that the plenty of biologically active natural products have been used for their

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https://doi.org/10.1016/j.bioorg.2018.03.005
0045-2068/

discovery [10–12]. In particular, coumarins have been found to be an attractive class of oxygenated heterocyclic systems, which exhibit a wide range of biological activities including anti-coagulants [13,14], anti-HIV agents [15], anti-oxidants [16] and anti-inflammatory [17–19] activities. More significantly they have been shown to possess anti-osteoporotic activity [20–22]. Naturally occurring coumarins esculetin, aesculin, osthole, and bergapten were reported to inhibit the osteoclastogenesis and stimulating osteoblast formation (Fig. 1) [23–25]. Keeping these aspects in mind, initially we have synthesized coumarin from orcinol (3,5-dihydroxytoluene), as this is a ubiquitous building block in lichen secondary metabolites and also found to accumulate in a number of lichen species [26–29]. In addition, 1,2,3-triazole is known to be an important pharmacophore in biological systems and its substituted analogues have diverse applications in the field of medicinal chemistry [30–34].

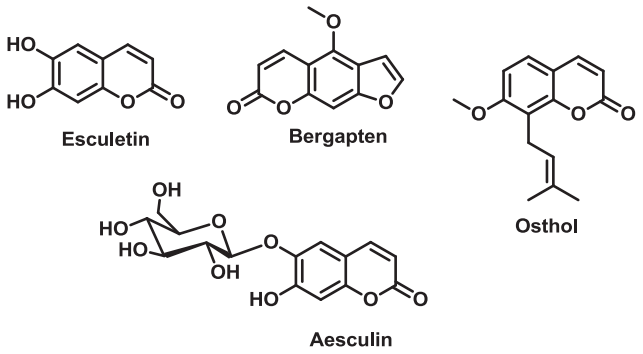


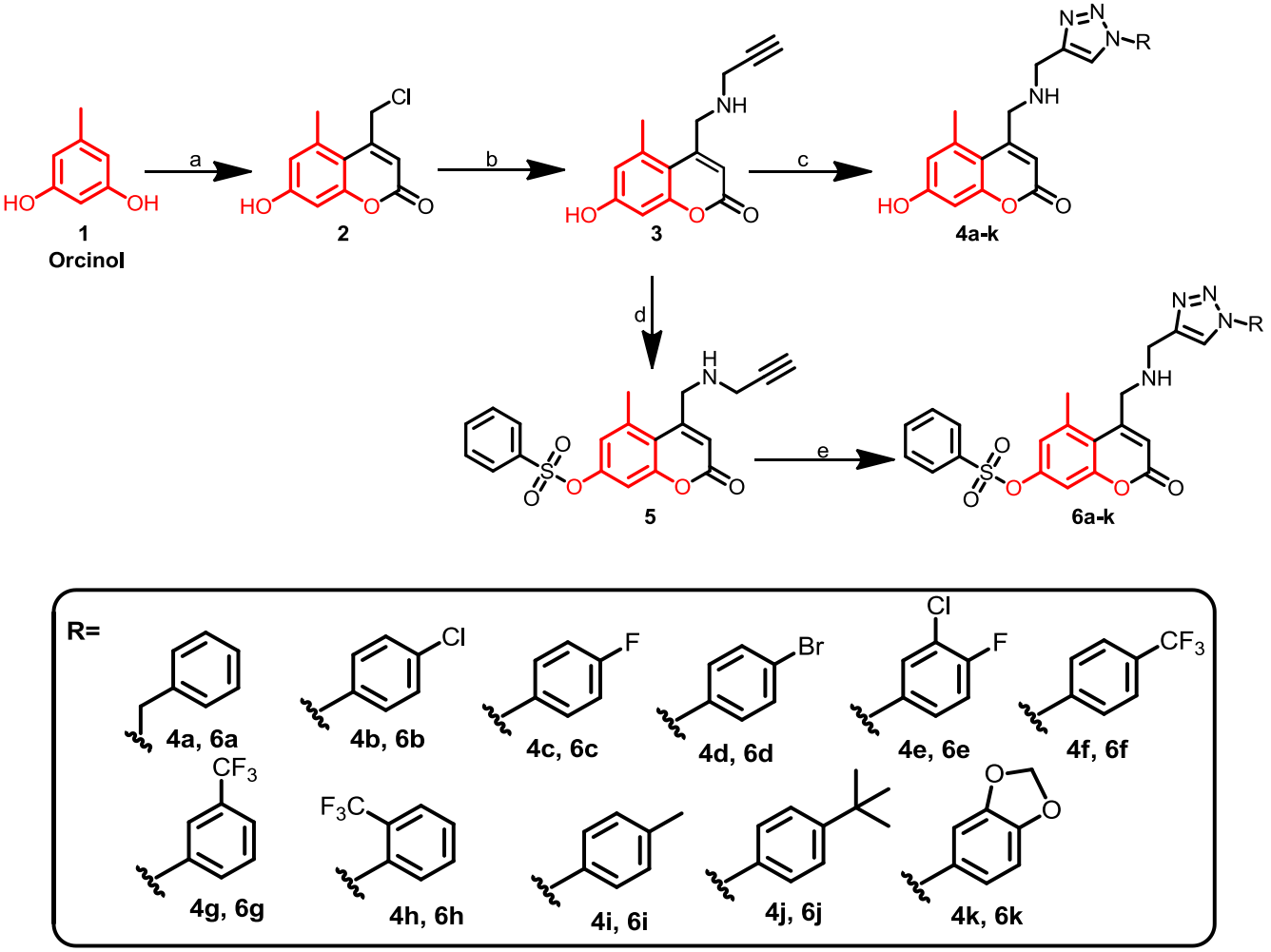
Fig. 1. Naturally occurring coumarins as anti osteoporotic agents.

In view of significant biological activities attributed to coumarin and triazole moieties, we have targeted our research in combining these two ligands through a dialkyl amino chain to synthesise some new chemical entities with enhanced therapeutic activities. Herein, we report the synthesis and anti-inflammatory activity of 7-hydroxy coumarin triazoles (**4a**-**k**) and 7-benzene sulfonate coumarin triazole hybrids (**6a**-**l**). The active compounds (**4d**, **4j**, and **6j**) were further investigated on RANKL-induced osteoclast in RAW 264.7 cells at 10 μM.

2. Results and discussion

2.1. Chemistry

The synthesis of novel structurally diverse 7-hydroxy and 7-benzene sulfonate coumarin 1, 2, 3-triazole hybrids were carried out by employing the protocols as given in Scheme 1. In the first step, we performed the Pechmann cyclization of orcinol **1** with 4-chloroethylacetoacetate in H_2SO_4 at room temperature for 3 h obtained the compound **2** (90% yield). Compound **2** was further reacted with propagyl amine in presence of



Scheme 1. Synthesis of compounds **4a-4k** and **6a-6k**. Reagents and conditions: (a) ethyl 4-chloro acetoacetate, H_2SO_4, rt, 3 h; (b) propagyl amine, DIEA, ethanol, 60 °C, 5 h; (c), (e) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF-H_2O (1:1), rt; (d) benzene sulphonyl chloride, K_2CO_3, acetone, rt, 1 h.