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Synthesis and evaluation of antibacterial and anti-inflammatory properties of naturally occurring coumarins

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ABSTRACT

Coumarins are a group of heterocyclic compounds naturally present in a large variety of plant families. Nevertheless, oxyprenylated coumarins have been only recently seen as valuable and promising biologically active phytochemicals. In this study, we synthesized three naturally occurring *O*-prenylcoumarins (**1**), (**2**), and (**3**), and evaluated their antibacterial and anti-inflammatory properties in view of their therapeutic potential against periodontal disease. The three *O*-prenylcoumarins were synthesized using well-known schemes leading to the chromen-2-one nucleus. The periodontal pathogen *Porphyromonas gingivalis* was found to be highly susceptible to all three *O*-prenylcoumarins with minimal inhibitory concentration values in the range of 12.5–25 mg/ml; the non-prenylated forms of the coumarins did not show any activity. The antibacterial activity of (**1**), (**2**), and (**3**) appeared to result from its ability to permeate the cell membrane. Using the U937-3xkB-LUC human monocytic cell line, compounds (**2**) and (**3**) dose-dependently inhibited lipopolysaccharide-induced NF-kB activation, while (**1**) did not. The non-prenylated forms of the coumarins were either inactive or much less potent. In conclusion, *O*-prenylcoumarins (**2**) and (**3**) by exhibiting a dual mode of action including antibacterial and anti-inflammatory activities may represent promising targeted therapeutic agents for localized treatment of periodontal diseases.

1. Introduction

Periodontal diseases are common chronic inflammatory disorders in adults. More specifically, approximately 5–15% of the population is affected by severe forms of the disease (Pihlstrom et al., 2005; Burt, 2005). If left untreated, periodontal diseases may result in tooth loss and systemic complications, such as diabetes, cardiovascular diseases and preterm low birth weight babies (Pizzo et al., 2010). Specific Gram-negative anaerobic bacteria, including *Porphyromonas gingivalis*, that colonize the periodontal pocket are the primary etiologic factor of periodontal diseases (Holt and Ebersole, 2005; Berezow and Darveau, 2011). However, the continuous and excessive host inflammatory response to these pathogens that results in the secretion of cytokines and matrix metalloproteinases modulates periodontal tissue destruction (Liu et al., 2010). Given this complex etiopathogenesis, the use of therapeutic drugs with dual antibacterial and anti-inflammatory properties represents a valuable adjunctive therapy to control this disease.

Coumarins are a group of heterocyclic compounds naturally present in a large variety of plant families. Numerous biological activities, including antimicrobial, anti-inflammatory, anti-cancer and antioxidant properties, have been demonstrated in coumarins and their derivatives (Borges et al., 2005). Since the discovery of the first coumarin more than 200 years ago (Vogel, 1820), a huge number of coumarins and analogues have been either isolated or synthesized. Until the last two decades, less attention has been dedicated to oxyprenylated coumarins. However, they have been recently re-considered as valuable and promising biologically active phytochemicals. In this study, we synthesized three coumarin derivatives (**1**), (**2**), and (**3**) (Fig. 1) and evaluated their antibacterial and anti-inflammatory properties in view of their therapeutic potential against periodontal disease. 4-Isopentenyloxy-5-methylcoumarin (**1**) has been first isolated in 1973 from *Gerbera crocea* Kuntze and *Gerbera serrata* Druce (sin. *G. asplenifolia*) (Fam. Asteraceae) (Bohlmann et al., 1973), 6-isopentenyloxy-7-methoxycoumarin (**2**) has been obtained in 1979 from

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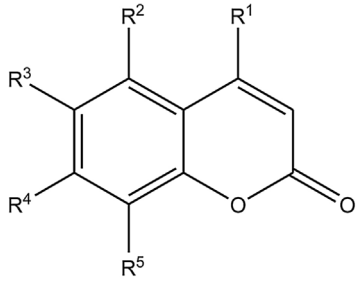


(**1**) R^1 = isopentenyloxy, R^2 = Me, R^3 = R^4 = R^5 = H

(**2**) R^1 = R^2 = H, R^3 = isopentenyloxy, R^4 = OMe, R^5 = H

(**3**) R^1 = R^2 = R^3 = H, R^4 = OMe, R^5 = isopentenyloxy

Fig. 1. Structure of coumarins (**1**), (**2**), and (**3**).

Haplophyllum pedicellatum Bunge ex Boiss (Fam. Rutaceae) (Abyshev and Gashimov, 1979), and 8-isopentenyloxy-7-methoxycoumarin (**3**) has been extracted in '80s from *Artemisia carvifolia* Besser (Barua et al., 1980), *Artemisia laciniata* Willd., *Artemisia armeniaca* Lam., *Artemisia tanacetifolia* Georgi (Szabo et al., 1985), *Melampodium divaricatum* DC. (Fam. Asteraceae) (Borges-del-Castillo et al., 1984), *Coleonema calycinum* (Steud.) I. Williams (Gray et al., 1986), *Flindersia australis* (Fam. Rutaceae) (Reisch and Podpetschnig, 1987), F. Muell., and *Cyperus incompletus* Boeckeler (Fam. Cyperaceae) (Dini et al., 1993).

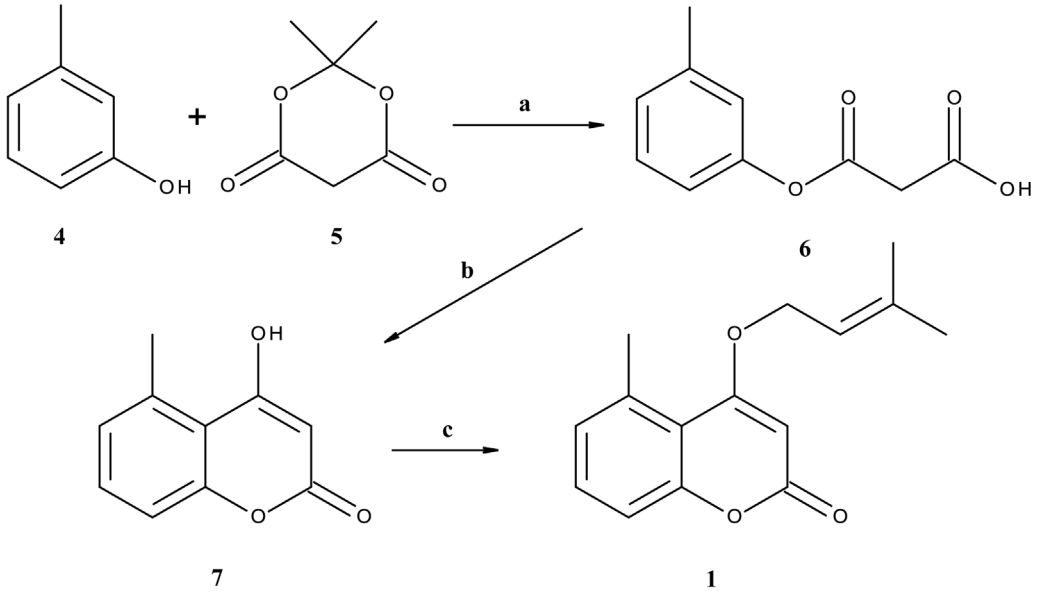
2. Results and discussion

The three *O*-prenylcoumarins (Fig. 1) have been synthesized using well-known schemes leading to the chromen-2-one nucleus. In particular, compound (**1**) has been synthesized starting from commercially available m-cresol (**4**) and Meldrum's acid (**5**) that were made to react under solvent-free conditions for 24 h at 120 °C, yielding the monoester (**6**), that in turn was cyclised in the presence of a catalytic amount of conc. H_2SO_4 at 120 °C for 5 h to provide the coumarin (**7**). The synthesis of (**1**) was then finalized by alkylation of the OH group with 3,3-dimethylallyl bromide in acetone at 80 °C for 1 h in the presence of K_2CO_3 as the base (Scheme 1). Compound (**2**) has been obtained by a two-step procedure from commercially available 2-methoxyhydroquinone (**8**) and 3,3-diethoxyethyl propionate (**9**) that were let to react in the presence of H_3PO_4 85% for 2 h at 100 °C followed by crystallization to yield pure 6-hydroxy-7-methoxycoumarin (**10**). This latter was then prenylated by the usual way (Scheme 2). Compound (**3**) has been synthesized from commercially available 3-methoxycatechol (**11**) that has been submitted to a Pechmann reaction with propiolic acid (**12**) in the presence of catalytic amounts of conc. H_2SO_4 at 120 °C. The so obtained 8-hydroxy-7-methoxycoumarin (**13**) was then prenylated in the usual way as described above (Scheme 3).

The three synthesized *O*-prenylcoumarins were first tested for their antibacterial activities against two strains of *P. gingivalis*, a major causative pathogen of chronic periodontitis (Holt and Ebersole, 2005; Berezow and Darveau, 2011). This Gram negative anaerobic bacterium was found to be highly susceptible to all three *O*-prenylcoumarins with MIC and MBC values in the range of 12.5–25 μg/ml (Table 1). Doxycycline used as reference molecule showed a MIC of 0.78 μg/ml and a MBC of 12.5 μg/ml. Compounds (**7**), (**10**), and (**13**), the non-prenylated forms of (**1**), (**2**), and (**3**) respectively, did not show any antibacterial activity against *P. gingivalis* at concentrations up to 200 μg/ml (data not shown). To determine whether the antibacterial activity of compounds (**1**), (**2**), and (**3**) was specific to *P. gingivalis*, we also tested their effect on two additional oral bacterial species *Fusobacterium* nucleatum and *Streptococcus mutans*. As reported in Table 1, all three *O*-prenylcoumarins were poorly active with MIC and MBC values >200 μg/ml in most cases.

To investigate the antibacterial mode of action of the three *O*-prenylcoumarins on *P. gingivalis*, we evaluated their ability to permeate the bacterial cell membrane using the SYTOX® Green dye, which penetrates damaged cell envelope and react with DNA. As reported in Table 2, after the addition of (**1**), (**2**), (**3**) or ethanol used as positive control, an increase in fluorescence was observed following a 60 min exposure, indicating permeabilization of the cell membrane of *P. gingivalis* (ATCC 33277). The effect of the three *O*-prenylcoumarins was more pronounced when they were used at a concentration corresponding to 4-fold the MIC value.

Therapeutic approaches that inhibit inflammatory mediator production by immune cells represent an interesting strategy for controlling inflammatory diseases such as periodontal diseases (Souza et al., 2012). The transcription factor NF-kB is activated by a



Scheme 1. Reagents and conditions: (a) 120 °C, 24 h; (b) H_2SO_4 conc. (cat.), 120 °C, 5 h; (c) 3,3-dimethylallyl bromide (1 eq.), acetone, K_2CO_3 (2 eq.), 80 °C, 1 h.