Phytochemistry Letters 13 (2015) 399–405

Contents lists available at ScienceDirect

Phytochemistry Letters

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

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

Jabrane Azelmat[a], Serena Fiorito[b], Vito Alessandro Taddeo[b], Salvatore Genovese[b], Francesco Epifano[b], Daniel Grenier[a,*]

[a] Oral Ecology Research Group, Faculty of Dentistry, Université Laval, Quebec City, Quebec, Canada
[b] Department of Pharmacy, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 3 August 2015
Accepted 4 August 2015
Available online xxx

Keywords:
Antibacterial
Anti-inflammatory
Coumarin
Periodontal disease

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.

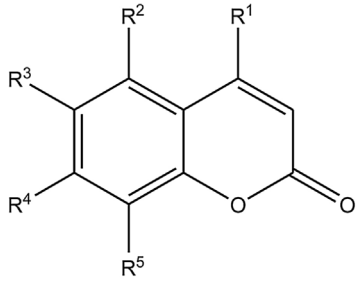
1. Introduction

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

* Corresponding author at: Oral Ecology Research Group, Faculty of Dentistry, Universite Laval, 2420 Rue de la Terrasse, Quebec City, Quebec G1V 0A6, Canada. Fax: +1 418 656 2861.
E-mail address: Daniel.Grenier@greb.ulaval.ca (D. Grenier).

http://dx.doi.org/10.1016/j.phytol.2015.08.008
1874-3900/



(**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).

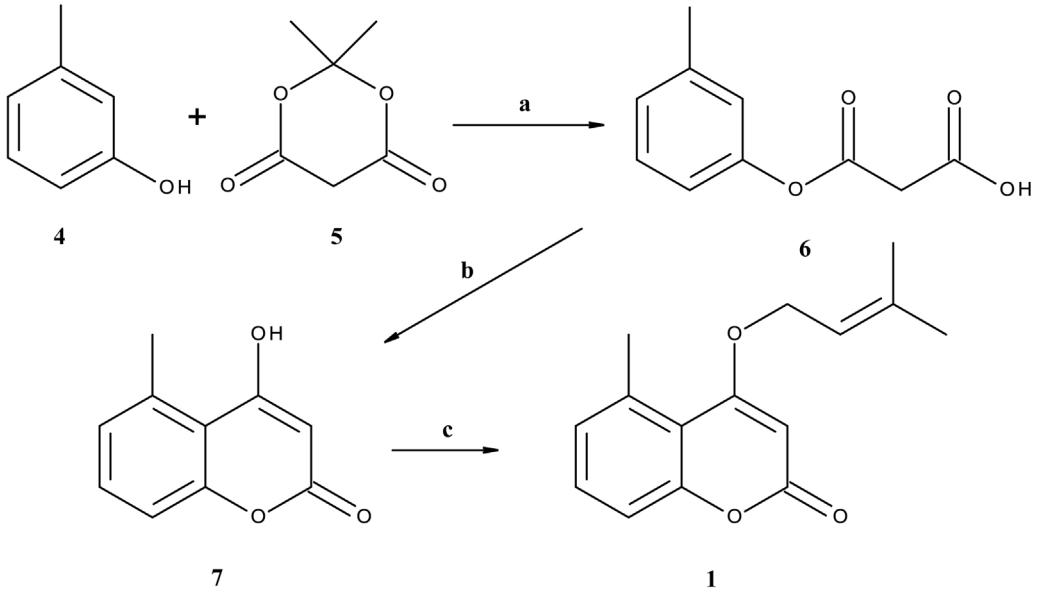
2. Results and discussion

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).

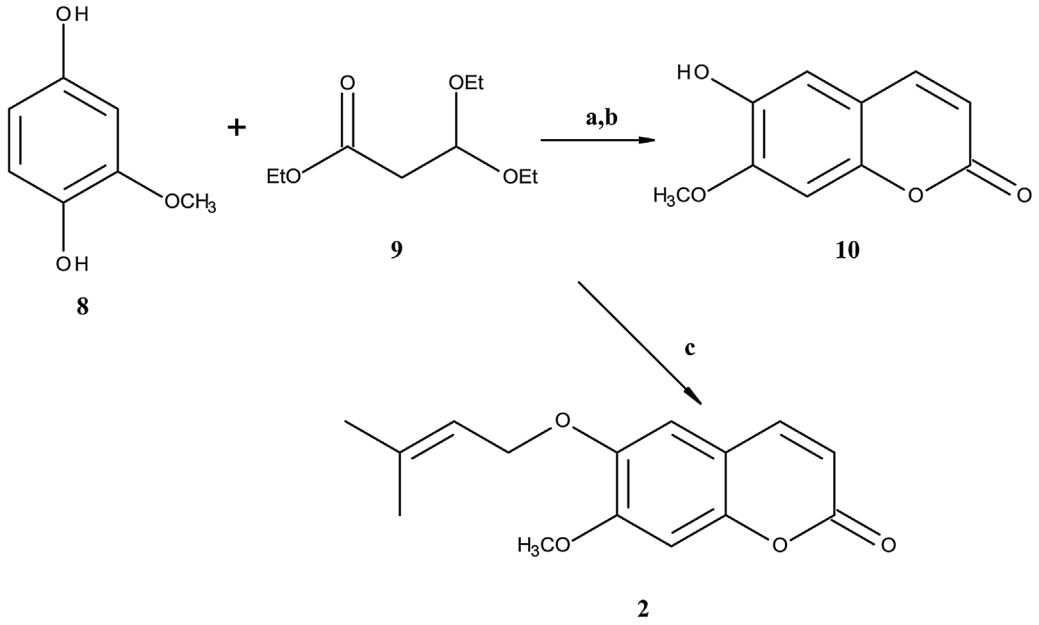
The three synthesized *O*-prenylcoumarins were first tested for their antibacterial activities against two strains of *P. gingivalis*, a major causative pathogen of chronic periodontitis (Holt and Ebersole, 2005; Berezow and Darveau, 2011). This Gram negative anaerobic bacterium was found to be highly 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.

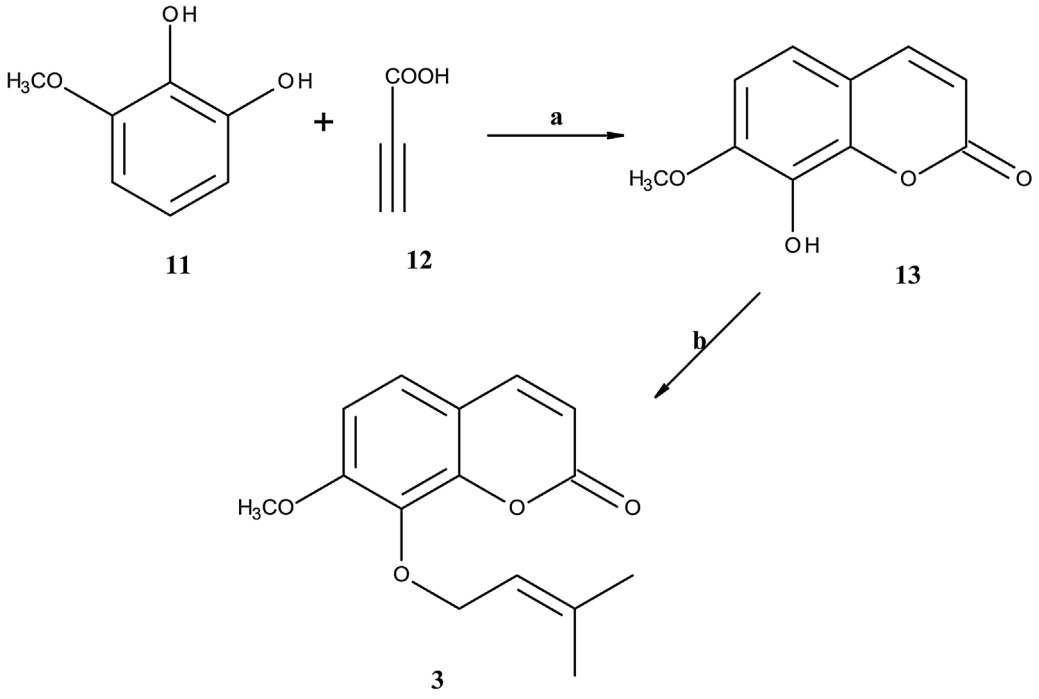
Therapeutic approaches that inhibit inflammatory mediator production by immune cells represent an interesting strategy for controlling inflammatory diseases such as periodontal diseases (Souza et al., 2012). The transcription factor NF-kB is activated by a



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.



Scheme 2. Reagents and conditions: (a) H_3PO_4, 100 °C, 2 h; (b) crystallization (c) 3,3-dimethylallyl bromide (1 eq.), acetone, K_2CO_3 (2 eq.), 80 °C, 1 h.



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

wide variety of stimuli including bacterial pathogens and has many target genes comprising genes encoding cytokines, adhesion molecules, and matrix metalloproteinases (Kumar et al., 2004). NF-kB is thus a central player in inflammatory diseases such as periodontitis and inhibition of its activation should be considered as a promising therapeutic strategy (Gupta et al., 2010). In the present study, we used the U937-3xkB-LUC cell line to evaluate the ability of compounds (**1**), (**2**), or (**3**) to inhibit activation of the NF-kB signaling pathway induced by LPS. To exclude the possibility that cell toxicity might have been responsible for any decrease in NF-kB activation, the cytotoxic effect of (**1**), (**2**), and (**3**) on U937-3xkB-LUC cell line was evaluated using an MTT test. No detectable cytotoxic effect was observed after a 6 h treatment of the cells with the compounds at concentrations up to 50 μg/ml (data not shown).

Fig. 2 shows that *Aggregatibacter actinomycetemcomitans* LPS induced activation of NF-kB signaling pathway, while the *O*-prenylcoumarins had no effect. Compounds (**2**) and (**3**) dose-dependently inhibited LPS-induced NF-kB activation, while (**1**) did not. More specifically, at a concentration of 12.5 μg/ml, compounds (**2**) and (**3**) decreased LPS-induced NF-kB activation by 94.7% and 48.9%, respectively. Given that the transcription factor NF-kB controls the expression of a large array of genes involved in inflammation (Kumar et al., 2004), compounds (**2**) and (**3**) by inhibiting NF-kB activation show potential to reduce the host

Table 1
Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of compounds (**1**), (**2**), and (**3**).

Strain	(1)		(2)		(3)		Reference[a]	
	MIC[b]	MBC[c]	MIC	MBC	MIC	MBC	MIC	MBC
P. gingivalis ATCC 33277	25	25	12.5	25	25	25	0.78	12.5
P. gingivalis W83	25	25	12.5	12.5	12.5	12.5	0.78	12.5
F. nucleatum ATCC 25586	>200	>200	>200	>200	100	100	0.78	50
S. mutans ATCC 35668	>200	>200	>200	>200	>200	>200	0.05	1.56

[a] Doxycycline was used as reference antibiotic for *P. gingivalis* and *F. nucleatum* while penicillin G was used for *S. mutans*.
[b] MIC in μg/ml.
[c] MBC in μg/ml.

Table 2
Effect of compounds (**1**), (**2**), and (**3**) on permeabilization of *P. gingivalis* ATCC 33277 cells, as determined using the SYTOX® Green Dye. Compounds were used at the MIC as well as four-fold the MIC value. Ethanol was used as positive control.

Conditions[a]	Relative fluorescence units (x10^3)	
	0 min	60 min
Positive control	254 ± 3	369 ± 3
(1) MIC	0	22 ± 3
(1) four-fold MIC	6 ± 4	236 ± 13
(2) (MIC)	0	33 ± 5
(2) four-fold MIC	0	122 ± 7
(3) MIC	0	89 ± 17
(3) four-fold MIC	0	371 ± 7

[a] Background values of fluorescence were substracted.

inflammatory response associated with periodontal diseases. Compounds (**7**) and (**10**), the non-prenylated forms of (**1**) and (**2**) respectively, did not attenuate the LPS-induced NF-kB activation while compound (**13**) was much less active than (**3**) (Fig. 3).

From the data reported in this study in regard to the comparison of the antibacterial and anti-inflammatory activities of prenylated coumarins and parent unprenylated compounds, it appears that the linkage of a terpenyl side chain to the benzochromene core markedly improves the pharmacological effects. The addition of either a geranyloxy or a 3,3-dimethylallyloxy moiety not only increases the lipophylicity of each molecule, thus allowing it to better permeate the cell membrane, but it may allow the compound to trigger a specific target in the endocellular environment leading to the herein reported activities. Prenylation of secondary metabolites having a phenylpropanoid, polyketide, or alkaloid cores is a common biosynthetic reaction occurring in nature, mainly in bacteria, fungi, and plants (Kuzuyama et al., 2005). In most cases, the addition of an isoprenoid side chain to an oxygen or to a nitrogen atom leads to a pharmacologically more efficient compound than the parent non-prenylated one. One of the clearest examples of this phenomenon was described by Kretzschmar et al. (2010), who reported that prenylated genistein and naringenin were able to exert a higher estrogenic effect than their respective parent compounds. Such a phenomenon have been also observed with coumarins and other structurally related phenylpropanoids and polyketides (Bruyere et al., 2011; Genovese et al., 2015).

While periodontal disease is initiated by a specific group of Gram negative bacteria, tissue and bone destruction is mainly modulated by an uncontrolled inflammatory response (Berezow and Darveau, 2011; Liu et al., 2010). *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.

3. Experimental

3.1. Chemistry

All starting materials were obtained from Sigma–Aldrich Chemical Co. (Milano, Italy), and all solvents were analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.32 MHz). $CDCl_3$ was used as the solvent and tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm). Reactions were routinely monitored by thin layer chromatography (TLC) using Merck silica gel F_{254} plates. Melting points were measured on a Büchi melting point apparatus and are uncorrected. Gas chromatography (GC) analysis was performed on a Hewlett-Packard gas chromatograph, model 5890, equipped with a flame ionisation detector (FID) and coupled to an electronic integrator. The chromatograph was fitted with a methyl silicone column (12.5 m × 0.25 mm, 0.25 mm film thickness). GC analytical conditions were as follows: carrier gas, He (purity > 99.8%); flow rate, 0.9 ml/min; and injector and detector temperatures, 280 °C and 250 °C, respectively. The oven temperature was programmed from 50 to 270 °C at a rate of 10 °C/min. Quantitative data were obtained by electronic integration of FID area data without the use of response factor correction. GC/MS analysis was performed using a Hewlett-Packard 6890 chromatograph combined with HP ChemStation Software and equipped with an HP 5973 mass selective detector. Operation conditions were as follows: carrier gas, He (purity > 99.8%); ionisation voltage, 70 eV; scanning speed, 1 s over 40–300 amu range; and ion source temperature, 180 °C. The column and conditions of use were the same as reported above. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyser.

3.1.1. Compound **6** (3-(3-methylphenoxy)-3-oxopropanoic acid)

A solvent-free mixture of Meldrum's acid (**5**) (5.0 mmol) and m-cresol (**4**) (7.0 mmol) was made to react at 120 °C for 24 h. After warming, the reaction mixture was added to a 10% solution of $NaHCO_3$ (20 ml) and extracted with EtOAc (3 × 20 ml). The organic phases were discarded, while the aqueous solution was then acidified to pH 1 with 10% HCl and then extracted with CH_2Cl_2 (3 × 20 ml). The collected organic phases were dried and evaporated under vacuum. The desired product was obtained as waxy white solid (m.p. 51–53 °C) in 88% yield. All analytical data were identical to those already reported for the same compound (Matsui, 1957).

3.1.2. Compound **7** (4-hydroxy-5-methyl-2H-chromen-2-one)

The monoester (**6**) (3.0 mmol) was added of 3 drops of conc. H_2SO_4 and the resulting mixture was made to react at 120 °C for 5 h. After warming, the latter was diluted with H_2O (20 ml) and extracted with EtOAc (3 × 20 ml). The collected organic phases were dried and evaporated under vacuum. The desired product was obtained as a white solid (m.p. 230–232 °C) in 71% yield. All analytical data were identical to those already reported for the same compound (Padwal et al., 2011).

3.1.3. Compound **1** (5-methyl-4-[(3-methylbut-2-enyl) oxy]-2H-chromen-2-one)

The same general procedure for the prenylation of phenylpropanoid nuclei as already reported was followed (Curini et al., 2003). The desired product was obtained as a yellowish solid (m.p. 123–125 °C) in 92% yield. All analytical data were identical to those already reported for the same compound (Bohlmann et al., 1973).

3.1.4. Compound **10** (6-hydroxy-7-methoxy-2H-chromen-2-one)

A solution of 2-methoxyhydroquinone (**8**) and 3,3-diethoxyethylpropionate (**9**) in conc. H_3PO_4 was well stirred for 2 h at 100 °C.

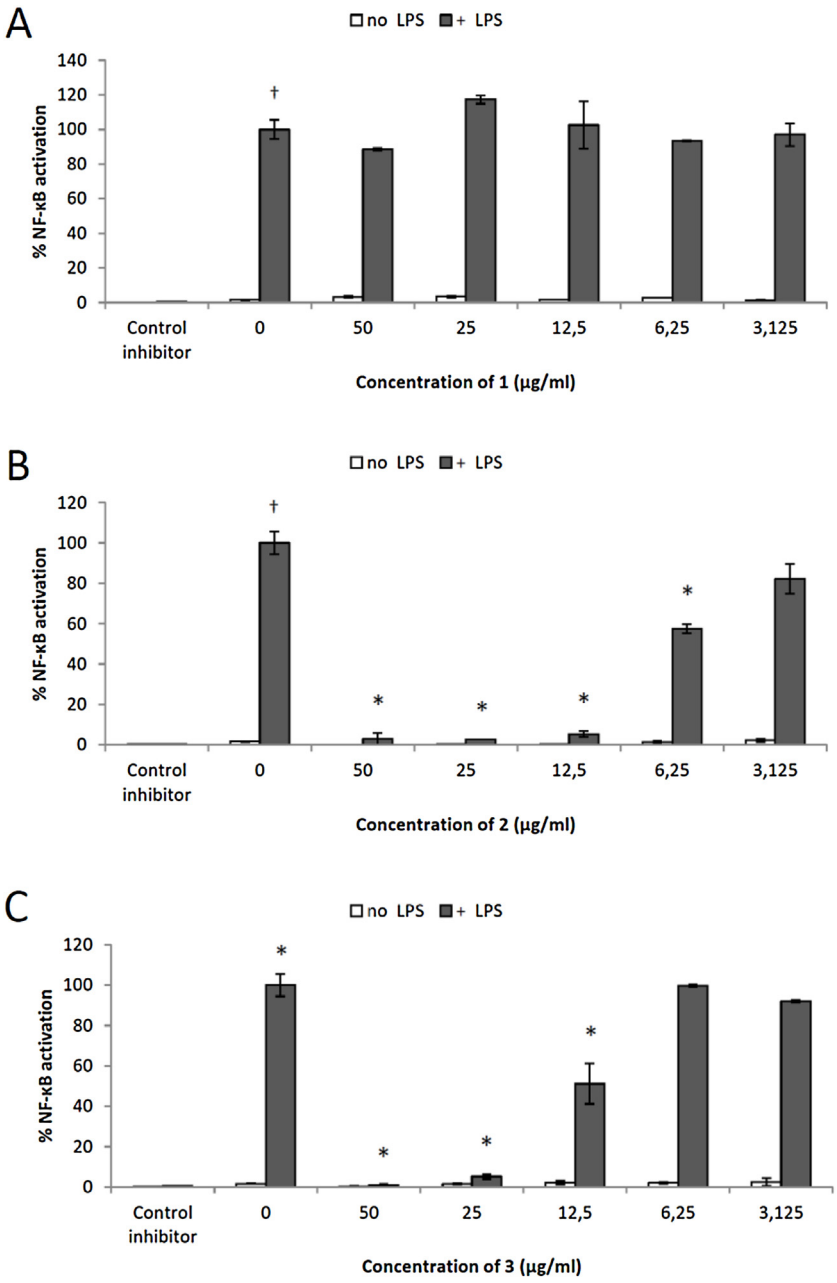


Fig. 2. Effect of compounds (**1**), (**2**), or (**3**) on *A. actinomycetemcomitans* LPS-induced NF-kB activation using the U937-3xkB-LUC cell line model. . . . , significant increase compared to non-stimulated cells. *, significant decrease compared to non-treated cells.

The resulting mixture was then poured into icy water and the resulting yellow solid formed filtered under vacuum. The desired product was obtained (m.p. 230–232 °C) in 62% yield. Analytical data were identical to those obtained for a commercial sample used for comparison.

*3.1.5. Compound **2** (6-[(3-methylbut-2-enyl)oxy]-7-methoxy-2H-chromen-2-one)*

The same general procedure for the prenylation of phenylpropanoid nuclei as already reported was followed (Curini et al., 2003). The desired product was obtained as a brownish solid (m.p. 165–168 °C) in 89% yield. All analytical data were identical to those already reported for the same compound (Abyshev and Gashimov, 1979).

*3.1.6. Compound **13** (8-hydroxy-7-methoxy-2H-chromen-2-one)*

The same general procedure for the Pechmann reaction as already reported was followed (Curini et al., 2003). The desired product was obtained as a brownish solid (m.p. 169–171 °C) in 38% yield. Analytical data were identical to those obtained for a commercial sample used for comparison.

*3.1.7. Compound **3** (7-methoxy-8-[(3-methylbut-2-enyl) oxy]-2H-chromen-2-one)*

The same general procedure for the prenylation of phenylpropanoid nuclei as already reported was followed (Curini et al., 2003). The desired product was obtained as a yellowish solid (m.p. 211–214 °C) in 78% yield. All analytical data were identical to those already reported for the same compound (Abyshev and Gashimov, 1979).

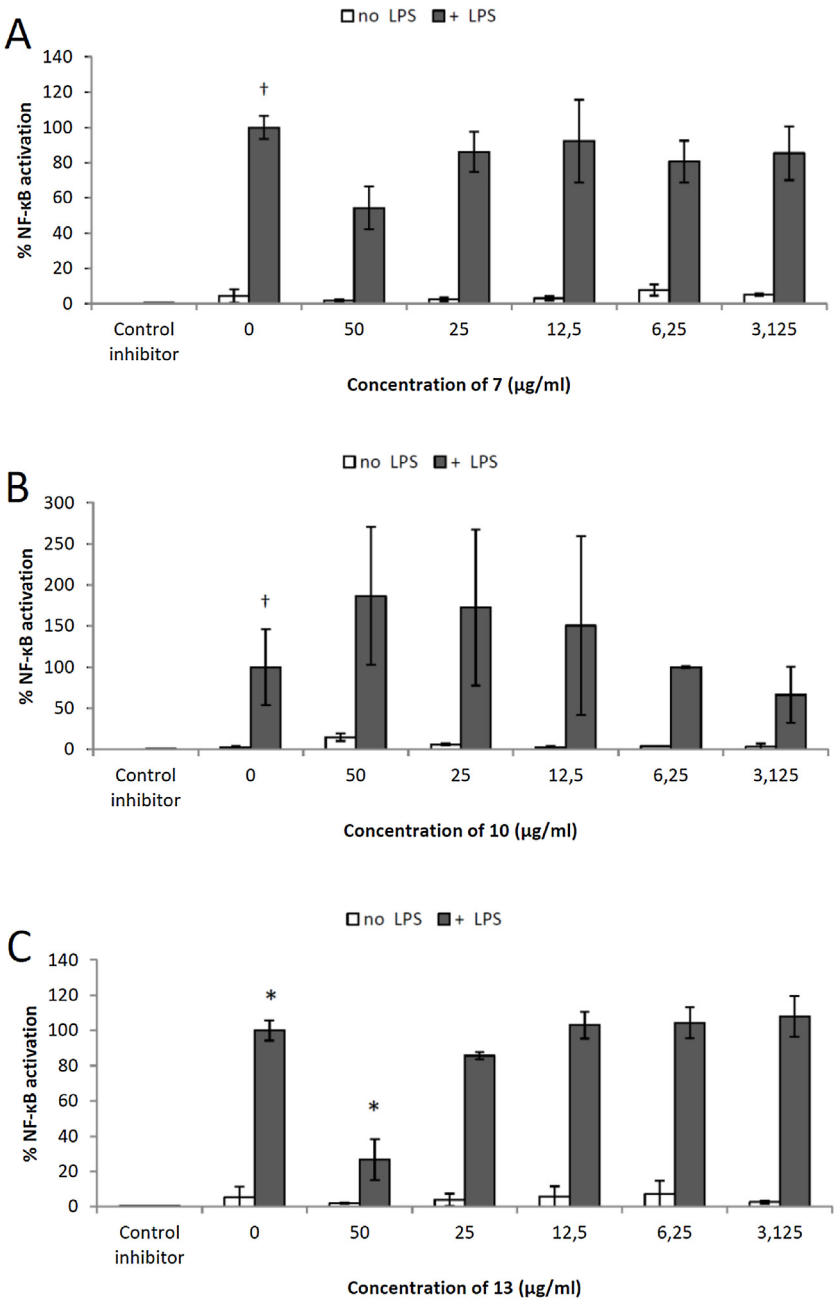


Fig. 3. Effect of compounds (**7**), (**10**), or (**13**) on *A. actinomycetemcomitans* LPS-induced NF-kB activation using the U937-3xkB-LUC cell line model. . . . , significant increase compared to non-stimulated cells. *, significant decrease compared to non-treated cells.

3.2. Stock solutions of coumarins

Stock solutions of the tested compounds were prepared in dimethyl sulfoxide at 5 mg/ml and kept at 4 °C protected from light.

3.3. Bacteria and growth conditions

P. gingivalis (ATCC 33277 and W83), *F. nucleatum* (ATCC 25586), and *S. mutans* (ATCC 35668) were used in this study. Bacteria were routinely grown in Todd Hewitt broth (THB; BD-Canada, Mississauga, ON, Canada) supplemented with 0.001% hemin and 0.0001% vitamin K, and cultures were incubated at 37 °C in an anaerobic chamber (N_2:H_2:CO_2/80:10:10).

3.4. Broth microdilution assay for determination of minimal inhibitory concentrations and minimal bactericidal concentrations

Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined using a broth microdilution assay. Overnight bacterial cultures were diluted in fresh broth medium to obtain an optical density at 660 nm (OD_{660}) of 0.2. Equal volumes (100 µl) of bacteria and two-fold serial dilutions (from 400 µg/ml) of compounds (**1**), (**2**), or (**3**) in culture medium were mixed into the wells of a flat-bottomed 96-well microplate. Control wells with no bacteria or with solvent alone were also prepared. Doxycycline and penicillin G were used as reference compounds. After an incubation of 24 h at 37 °C, bacterial growth was recorded visually. The MIC values (µg/ml) of the tested compounds were determined as the lowest concentration at which

no growth occurred. To determine the MBC values (μg/ml), aliquots (5 μl) of each well showing no visible growth were spread on blood-supplemented THB agar plates, which were incubated for 3 days at 37 °C. The MBC values were determined as the lowest concentration at which no colony formation occurred. The MIC and MBC values were determined in three independent experiments to ensure reproducibility.

3.5. Membrane permeabilization assay

The ability of compounds (**1**), (**2**), and (**3**) to permeate *P. gingivalis* cells was evaluated using the SYTOX Green dye (Life Technologies Inc., Burlington, ON, Canada), which binds to nucleic acid of bacteria once the bacterial membrane is compromised. Briefly, 1.25 mM of SYTOX Green dye was added to *P. gingivalis* cells suspended in 10 mM HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) pH 7.0 to an OD_{660} of 0.4. Aliquots of 100 μl were added to wells of a 96-well black microplate prior to adding compound (**1**), (**2**), or (**3**) at concentrations corresponding to the MIC value and to 4-fold the MIC value. The incubation was carried out in a microplate reader (Synergy 2; BioTek Instruments, Winooski, VT, USA) at 37 °C for 60 min, prior to record the fluorescence resulting from the binding of the dye to bacterial DNA, following excitation at 485 nm and emission at 528 nm. Ethanol 70% was used as positive controls. Three assays were performed and the means ± standard deviations were calculated.

3.6. Determination of NF-kB activation

The human monoblastic leukemia cell line U937-3xkB-LUC was kindly provided by Dr. Rune Blomhoff (University of Oslo, Norway). This cell line consists in the U937 cell line stably transfected with a construct containing 3 NF-kB binding sites from the Ig κ light chain promoter coupled with the gene encoding firefly luciferase (3x-kB-*luc*) (Carlsen et al., 2002). Cells were routinely grown at 37 °C in a 5% CO_2 atmosphere in RPMI-1640 medium (Life Technologies Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 μg/ml penicillin-streptomycin and 75 μg/ml hygromycin B. One hundred microliters of the cell suspension were seeded on a black bottom, black walls 96-well microplate. Ten microliters of the tested compounds (final concentrations of 50, 25, 12.5, 6.25, and 3.125 μg/ml) were then added. Moreover, an assay was performed using a commercial inhibitor (BAY-11-7082; EMD Millipore, Billerica, MA, USA; 25 mM) of the NF-kB signaling pathway. Following a 30 min incubation, *A. actinomycetemcomitans* lipopolysaccharide (LPS; 1 μg/ml) was added to induce activation of the NF-kB signaling pathway, and the plate was further incubated at 37 °C (5% CO_2) for 6 h. NF-kB activation was then monitored using the Bright-Glo Luciferase Assay System (Promega, Madison, WI, USA) by adding 100 μl of luciferase substrate to the wells at room temperature. Luminescence was recorded using the luminometer option of a Synergy 2 microplate reader (BioTek Instruments) within 3 min after substrate addition. Moreover, following a treatment of U937-3xkB-LUC with the tested compounds for 6 h, an MTT (3-[4,5-diethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay was performed according to the manufacturer's protocol (Roche Diagnostics, Mannheim, Germany) to determine the effect of the compound on the cell viability. All assays were performed in triplicate and the means ± standard deviations were calculated.

Acknowledgements

We are grateful to R. Blomhoff and H. Carlsen (University of Oslo, Norway) for providing the U937-3xkB-LUC cell line. This study was supported by the Laboratoire de Contrôle Microbiologique de l'Université Laval. The authors report no conflicts of interest related to this study.

References

Abyshev, A.Z., Gashimov, N.F., 1979. Khimiya Prirod. Soed. 6, 846–848.
Barua, N.C., Sharma, R.P., Madhusudanan, K.P., Thyagaraan, G., Herz, W., 1980. Phytochemistry 19, 2217–2218.
Berezow, A.B., Darveau, R.P., 2011. Periodontology 2000 (55), 36–47.
Bohlmann, F., Zdero, C., Franke, H., 1973. Chem. Ber. 106, 382–387.
Borges, F., Roleira, F., Milhazes, N., Santana, L., Uriarte, E., 2005. Curr. Med. Chem. 12, 887–916.
Borges-del-Castillo, J., Martinez-Martin, A.I., Rodriguez-Luis, F., Rodriguez-Ubis, J.C., Vazquez-Bueno, P., 1984. Phytochemistry 23, 859–861.
Bruyere, C., Genovese, S., Lallemand, B., Ionescu-Motatu, A., Curini, M., Kiss, R., Epifano, F., 2011. Bioorg. Med. Chem. 21, 4173–4178.
Burt, B., 2005. J. Periodontol. 76, 1406–1419.
Carlsen, H., Moskaug, J.Ø., Fromm, S.H., Blomhoff, R., 2002. J. Immunol. 168, 1441–1446.
Curini, M., Epifano, F., Maltese, F., Marcotullio, M.C., Prieto Gonzale, S., Rodriguez, J. C., 2003. Aust. J. Chem. 56, 59–60.
Dini, A., Ramundo, E., Saturnino, P., Scimone, A., Stagno d'Alcontre, I., 1993. Biochem. System Ecol. 21, 305–307.
Genovese, S., Fiorito, S., Epifano, F., Taddeo, V.A., 2015. Curr. Med. Chem. 22 (1) doi: http://dx.doi.org/10.2174/0929867322666150716114758.
Gray, A.I., Meegan, C.J., O'Callaghan, N.B., 1986. Phytochemistry 26, 257–260.
Gupta, S.C., Sundaram, C., Reuter, S., Aggarwal, B.B., 2010. Biochim. Biophys. Acta 1799, 775–778.
Holt, S.C., Ebersole, J.L., 2005. Periodontology 2000 (38), 72–122.
Kretzschmar, G., Zierau, O., Wober, J., Tischer, S., Metz, P., Vollmer, G., 2010. J. Steroid Biochem. Mol. Biol. 118, 1–6.
Kumar, A., Takada, Y., Boriek, A.M., Aggarwal, B.B., 2004. J. Mol. Med. 82, 434–448.
Kuzuyama, T., Noel, J.P., Richard, S.B., 2005. Nature 435, 983–987.
Liu, Y.C., Lerner, U.H., Teng, Y.T., 2010. Periodontology 2000 (52), 163–206.
Matsui, K., 1957. Nippon Kagaku Zasshi 78, 517–520.
Padwal, J., Lewis, W., Moody, C.J., 2011. Org. Biomol. Chem. 9, 3484–3493.
Pihlstrom, B.L., Michalowicz, B.S., Johnson, N.W., 2005. Lancet 366, 1809–1820.
Pizzo, G., Guiglia, R., Russo, L.L., Campisi, G., 2010. Eur. J. Internal Med. 21, 496–502.
Reisch, J., Podpetschnig, E., 1987. Pharmazie 42, 754–756.
Souza, J.A., Rossa, C., Garlet, G.P., Nogueira, A.V., Cirelli, J.A., 2012. J. Appl. Oral Sci. 20, 128–138.
Szabo, G., Greger, H., Hofer, O., 1985. Phytochemistry 24, 537–541.
Vogel, A., 1820. Gilbert's Am. Phys. 64, 161.