SYNTHESIS OF 6-*TERT*-OCTYL AND 6,8-DI*TERT*-BUTYL COUMARINS, TWO COUMARINS OF BIOLOGICAL INTEREST

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ABSTRACT

In this study, the synthesis of new coumarins with aliphatic chains is discussed. The incorporation of the 6-*tert*-octyl and 6,8-di*tert*-butyl chains into a coumarin structure from alkylphenols, allows obtaining hydrophobic coumarins with good yields. These coumarins can be potential modulators of TRPV1 receptors. Synthesis and spectroscopic data of these new coumarins are analyzed.

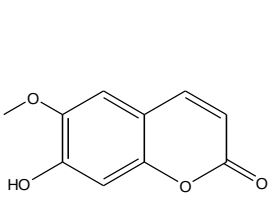
Keywords: Coumarins, Alkylcoumarins, TRPV1.

INTRODUCTION

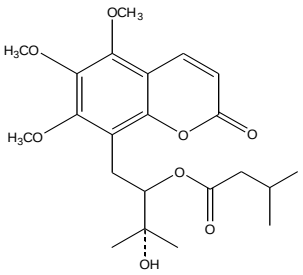
The past ten years have seen the emergence of specific small molecule antagonists targeting receptors on sensory neurons that detect painful stimuli.[1] Among these new therapeutic targets, TRPV1 has attracted the most attention.[2] TRPV1 (formerly known as the vanilloid receptor VR1) is probably still best recognized as the receptor for capsaicin (Figure 1, I), which is responsible for the piquancy of hot chili peppers.[3] The search of new modulating ligands of TRPV1 receptors, agonists or antagonists, constitutes a strategy in the development of new drugs for the pain treatment.[2]

Coumarins constitute a group of natural compounds, widely distributed in the plant kingdom.[4] Some coumarin derivatives are also known to act beneficially on human health due to their therapeutic effects such as inhibitory activities against various tumor cells, mycobacteria, antioxidant, antihyperglycemic, antifungal, and anti-asthmatic, which have been extensively studied in the medical and pharmaceutical fields for the treatment of human diseases.[5]

Previous studies have demonstrated that some coumarins exert an antinociceptive action. Scopoletin (Figure 1, I) isolated from the *Polygala sabulosa* plant has shown a high *nociceptive activity* in mice, that have been induced visceral pain.[6] Muralatin R (Figure 1, II) was found to be capable of activating the transient receptor potential vanilloid 1 (TRPV1) channel through desensitization mechanism.[7]



Scopoletin (I)



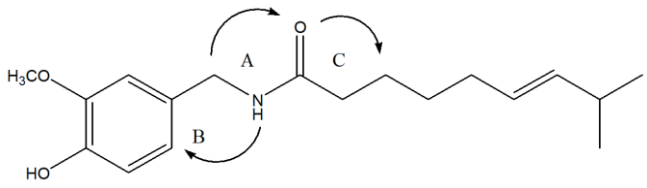
Muralatin R (II)

Figure 1. Chemical structures modulating the TRPV1 receptor.

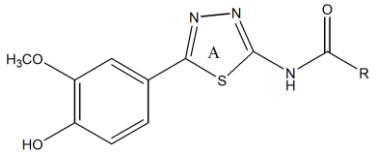
Compounds analogous to Capsaicin have been designed and synthesized by several research groups for studies with the TRPV1 receptor.[8] Our working group has designed and synthesized structures analogous to capsaicin, which incorporate an increasing the conformational restriction on the amide bond. (Figure 2, III, region: A) with the incorporation of the heterocyclic, such as the azoles[9] or a chalcone[10] (Figure 2; IV and V respectively) and the action of these molecules in transfected mouse cells have been studied to evidence the efficiency of the chemical structure on the TRPV1 receptor. [9, 10]

In this work the synthesis of new coumarins is reported, which incorporate both greater conformational restriction in B region of Capsaicin (Figure 2, VI) and hydrophobic units in the coumarin structure.

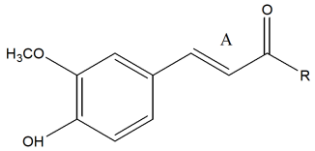
The incorporation of hydrophobic chains in the coumarin structure allows to observe the conformational effect of both chains in the modulation with the TRPV1 receptor. The amide function will be linked by derivatization of the methyl ester group at R3 position, by direct substitution with primary amines or through an acid chloride and subsequent substitution. In addition, the dipole moment has been increased and new hydrogen receptors region have been incorporated on the molecule.



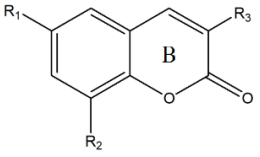
Capsaicin (III)



Tiadiazol (IV)



Chalcone (V)



Alkyl coumarins (VI)

Figure 2. Compounds with conformational restrictions.

EXPERIMENTAL

All solvents and reagents were purchased from Merck and Aldrich Companies. Experiments ^{1}H-NMR and ^{13}C-NMR were carried out in a Bruker Ascend T 400 MHz multicore equipment, at room temperature with deuterated solvents of $CDCl_3$ and DMSO. The chemical displacements are informed in delta units in parts per million (δ ppm), relative to the internal TMS standard. Fusion points were determined in a hot plate capillary fusion equipment. IR spectra were recorded on a Nicolet Nexus 470 FTIR instrument.

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Synthesis of 2-hydroxi-3,5-*ditert*-butilbenzaldehyde.

Formylation of di*tert*-butylphenol with hexamethylenetetramine in acid medium were implemented according to Duff formylation method.[11]

A mixture of 10.42 g (5.02 x 10^{-2} mol) of 2,4-di*tert*-butylphenol and 14.02 g (100 mmol) of hexamethylenetetramine in 50 ml of glacial acetic acid was stirred at 118°C for 3 h. Then, reaction mixture was cooled to room temperature, and a solution of 12.4 ml HCl 5 M is added slowly. The resulting solution was again heated to the same temperature (118°C) for half an hour. After cooling, the mixture was extracted with 100 ml of hexane (2x 50 mL).

The organic phase (*n*-hexane) was washed with 20 ml of water and 10 ml of a saturated dissolution of sodium chloride. Then, this organic phase was filtered by a thick silica gel column chromatography, which was successively washed with hexane (4x50 ml). The combined organic phase was concentrated in a rotary evaporator to afford 8.25 g of 2-hydroxy-3,5-di*ter*butylbenzaldehyde (yield: 73.2%): Pf: 42 °C; R_f = 0.85 (20%, ethylacetate/n-hexane); FTIR (KBr, ν cm^{-1}): 2958.5; 2872.1; 2743.0; 1650.4 (CO), 1470.0; 1375.3; 1260.7; 1212.6; 1169.4; 744.4; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.65 (1H, s, -OH), 9.87 (1H, s,-CHO), 7.60 ($1H_6$, d, 4.0 Hz), 7.35 ($1H_4$, d, 4.0 Hz), 1.44 (9H, s, t-Bu), 1.33 (9H, s, t-Bu); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 197.37, 159.10, 141.63, 137.60, 131.91, 127.85, 120.00, 35.02, 34.25, 31.32, 29.27.

Synthesis of 2-hydroxy-5-*tert*-octylbenzaldehyde.

Formylation of *tert*-octylphenol was carried out according to the Levin´s formylation method.[12]

Solid magnesium wires (2.0 g, 80 mmol) are added to a mixture of methanol (37.3 ml) and toluene (16.0 ml), then magnesium methoxide drops are added. The mixture is stirred at reflux temperature until the disappearance of solid magnesium. Then, 4-*tert*-octylphenol (26.8 g, 0.13 mol) is added to the reaction flask and is kept under stirring for an additional hour. To eliminate the excess of methanol, a methanol/toluene azeotropic mixture is used. For this, 33.0 ml of toluene are added, keeping the temperature to 50°C. A solution of paraformaldehyde (12.0 g, 0.4 mol) in toluene (20 ml) are slowly added, with continuous distillation of the azeotrope for 2 hours.

Then, the mixture is cooled to room temperature and sulfuric acid (20%, 80 ml) is slowly added. The reaction mixture is continuously stirred at 50°C until all the solid formed is dissolved. The final product is extracted with toluene (2x40 ml), washed with 10% sulfuric acid (2x 15 ml) and water (15 ml) is added. The organic phase is dried over anhydrous magnesium sulfate, filtered and the solvent is removed under vacuum. The mixture of products is purified by a rapid method of silica gel chromatography column 60 G (2% ethyl acetate in n-hexane), being obtained a white solid (yield: 19.0 g, 63%): Pf = 38°C. R_f = 0.86 (20% ethyl acetate/n-hexane); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.90 (1H, s,-OH), 9.91 (1H, s, -CHO), 7.58 ($1H_4$, dd, J= 12 Hz, J=4 Hz), 7.50 ($1H_6$, d, J= 4 Hz), 6.94 ($1H_3$, d, J=8 Hz), 1.75 (2H, s, $-CH_2$-), 1.39 (6H, s, $-CH_3$), 0.75 (9H, s, t-Bu); ^{13}C-NMR (100MHz, $CDCl_3$): δ 196.86, 159.43, 141.80, 135.48, 130.51, 119.98, 116.98, 56.65, 37.96, 32.95, 31.84, 31.47.

Synthesis 6-*tert*-octyl-2-oxo-2H-chromene-3-methylcarboxylate

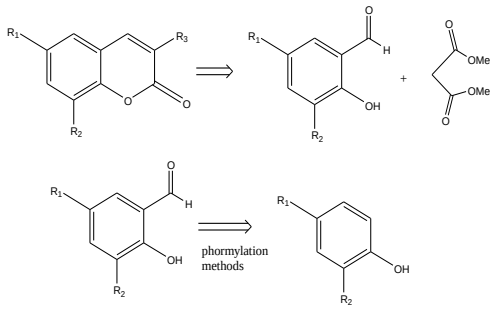
Procedure: 1 mmol (225 mg) of 2-hydroxy-5-tertoctyl benzaldehyde and 1.1 mmol of methyl malonate (0.13 mL) are added to a round-bottomed flask containing absolute alcohol (25 mL), and piperidine drops as catalyst. The temperature is raised to reflux and kept under stirring for 3 h. The progress of the reaction was followed visually by thin layer silica gel chromatography, with fluorescence indicator and UV lamp. The workup is performed by adding the reaction mixture at room temperature to a separatory funnel containing 20 mL water and extracting with ethyl acetate (2x20 mL), and the organic phases are concentrated. The reaction products are separated on a chromatographic column using Silicagel G and as eluent a solution of increasing concentration of ethyl acetate in n-hexane. After purification 210 mg of the compound is obtained (yield 75%): Pf = 41-43 ºC, R_f = 0.48(20% Ethyl Acetate/n-hexane); ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.55 ($1H_4$, s), 7.65 ($1H_5$, dd, J= 8.0 Hz, J=4.0 Hz), 7.53 ($1H_7$, d, J=4.0 Hz), 7.23 ($1H_8$, d, J=8.0 Hz), 3.89 (3H, s), 1.72 (2H, s), 1.35 (6H, s), 0.66 (9H, s). ^{13}C-NMR (DMSO-d6, ppm): 163.65, 156.90, 153.12, 149.66, 147.21, 133.06, 126.45, 117.36, 117.15, 115.91, 56.59, 52.65, 38.37, 32.26, 31.74, 31.39.

Synthesis 6,8-di*tert*-butyl-2-oxo-2H-chromene-3-methylcarboxylate.

Procedure: 3.15 mmol (585 mg) of 2-hydroxy-3,5-diterbutyl benzaldehyde and 3.77 mmol of methyl malonate (0.498 mg, 0.4 mL) are added to a round-bottomed flask containing absolute alcohol (25 mL), and piperidine drops as catalyst. The temperature is raised to reflux and kept under stirring for 3 h. The progress of the reaction was followed visually by thin layer silica gel chromatography, with fluorescence indicator and UV lamp. The workup is performed by adding the reaction mixture at room temperature to a separatory funnel containing 20 mL water and extracting with ethyl acetate (2x20 mL), and the organic phases are concentrated. The reaction products are separated on a chromatographic column using Silicagel G and as eluent a solution of increasing concentration of ethyl acetate in n-hexane. After purification 210 mg of the compound is obtained (yield 64%): R_f = 0.57 (20% Ethylacetate/*n*-hexane); IR (KBr, ν cm^{-1}) 2964.5, 1760.2, 1742.8, 1621.3, 1581.5, 1469.8, 1342.1, 1246.4, 1206.0, 1150.4;^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.58 ($1H_4$, s), 7.72 ($1H_5$, d, J=0 Hz), 7.43 ($1H_7$, d, J=0 Hz), 3.97 (3H, s, OCH_3), 1.53 (9H, s, t-Bu), 1.38 (9H, s, t-Bu); ^{13}C-NMR (100 MHZ, $CDCl_3$, ppm): δ 164.05, 156.63, 152.15, 150.54, 147.23, 137.49, 129.92, 124.01, 117.85, 116.64, 57.65, 35.16, 34.71, 31.29, 29.78.

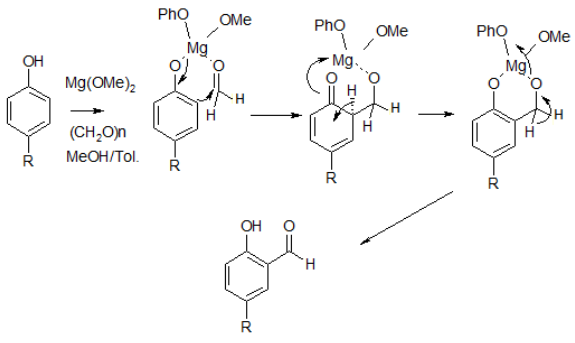
RESULTS AND DISCUSSION

The synthesis of hydrophobic coumarins was performed according to the retrosynthetic methodology shown in Scheme I. The corresponding alkyl aldehydes were obtained by the Levin and Duff synthesis methodology.



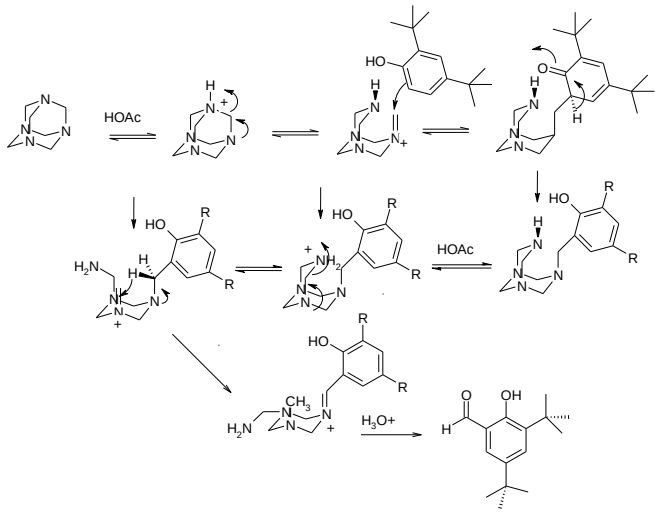
Scheme I. Retrosynthesis of coumarins.

The Levin formylation method uses a magnesium salt to obtain an ortho formyl group.[11] The salt is formed by dissolving magnesium in the phenol. According to the proposed mechanism (Scheme II), the magnesium salt formed (bisphenoxide) subsequently reacts with formaldehyde dissolved in toluene. Methanol is extracted in a toluene/methanol azeotropic mixture. The product is obtained by an acid *work-up* to generate the corresponding alkyl salicylaldehyde by approximately 65%.



Scheme II. Proposed mechanism for the Levin formylation.

The Levin methodology applied in the formylation of 2,4-diterbutyl phenol gives a low yield, probably because of increasing the steric factors around the hydroxyl group in the formation of the magnesium salt, so the synthesis route was modified by applying the Duff formylation methodology. This synthetic way[11] considers obtaining aldehydes from a phenol (or alkylphenol) (Scheme III), by reacting 2,4-di*tert*-butylphenol with hexamethylentetraamine in acetic acid medium. The reaction in these conditions provides aldehyde with a yield of 75%.



Scheme III. Proposed mechanism for Duff formylation.

The synthesis of coumarins were carried out with good yields, by the reaction of both alkylsalicylaldehydes with dimethyl malonate in ethanol at reflux temperature, in the presence of piperidine as catalyst. The IR spectrum confirms the presence of the C3-substituted coumarin structure by the intense vibrational frequencies typical of the conjugated carbonyl of the pyran ring and the methyl ester at 1761.0 and 1743.0 cm^{-1}, respectively. The coumarin 6-*tert*-octyl-2-oxo-2H-chromene-3-methylcarboxylate (chemical structure, Figure 3) presents a chemical displacement for the hydrogen H_4 at δ 8.85 ppm, and the chemical displacement for the hydrogens of the aromatic ring at δ (ppm) 7.65 (H_7, dd, J=8.0 Hz, J=4.0 Hz); 7.53 ($1H_5$, d, J=4.0 Hz) and 7.23 ($1H_8$, d, J=8.0 Hz). The methyl group of the ester is displaced to δ 3.89 ppm (3H, s). The presence of the aliphatic side chain is confirmed by the high-field signals of δ (ppm) 1.72 (2H, s), δ(ppm) 1.35 (6H, s) and δ (ppm) 0.66 (9H, s) and the signals at δ(ppm) 52.65, 38.37, 32.26, 31.74, 31.39 and the grouping of the conjugated carbonyl of the pyrone ring of δ(ppm) 163.65, 153.12, 149.66 at the ^{13}C-NMR spectrum.

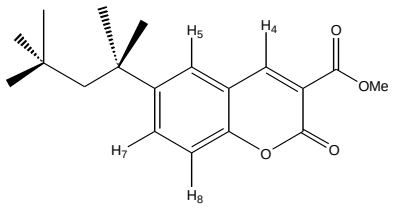


Figure 3. Structure of 6-*tert*-octyl-2-oxo-2H-chromene-3-methylcarboxylate.

The coumarin 6,8-di*tert*-butyl-2-oxo-2H-chromene-3-methylcarboxylate (Figure 4) presents in the FTIR the signals of both carbonyls at 1.742,7 and 1.760,2 cm^{-1} (carbonyl ester and lactone, respectively). ^{1}H-NMR spectrum shows the H_4 hydrogen signal of the lactone ring at δ (ppm) 8.58 ($1H_4$, s) and the aromatic ring at δ(ppm) 7.72 ($1H_5$, d, J=1.4 Hz) and δ(ppm) 7.50 ($1H_7$, d, J=1.4 Hz). At δ(ppm) 3.97 (3H, s, OCH_3) the methyl ester and both *tert*-butyls groups are at δ (ppm) 1.53 (9H, s, t-Bu) and δ(ppm) 1.38 (9H, s, t-Bu), respectively. The ^{13}C-NMR spectroscopy shows the signals of both *tert*-butyl groups at δ (ppm) 35.16, 34.71, 31.29, 29.78 and of the pyrone ring carbons (C2, C3, and C4) at δ (ppm) 164.05, 150.54, and 152.15, respectively.

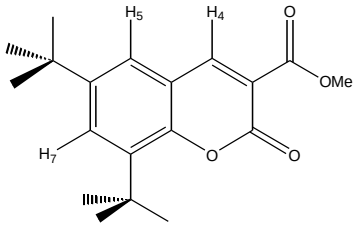


Figure 4. Structure of 6,8-ditert-butyl-2-oxo-2H-chromene-3-methylcarboxylate.

CONCLUSIONS

An attractive synthesis route has been designed in order to prepare hydrophobic coumarins using alkylphenols. The intermediate aldehydes and the coumarins were obtained with good yields and high purity under smooth reaction conditions. 6-alkyl and 6,8-dialkyl coumarins such as their corresponding aldehydes were completely characterized by spectroscopic techniques, allowing to identify the characteristic signals. Thanks to this route, the access to a wide set of alkyl coumarins is very important to study their conformational restrictions and evaluate their biologic properties. In addition, it is important to underline that their biological activities are under study and their data will be subsequently informed.

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