Synthesis of Novel Kavain-like Derivatives and Evaluation of their Cytotoxic Activity

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Reações de acoplamento do tipo Heck, Sonogashira-Hagihara, Suzuki-Miyaura e reação de aldolisação catalizadas por metal foram utilizadas para a obtenção de três séries de δ-valerolactonas substituídas em posições 3, 4, 5 e 6 do anel lactônico. As 26 δ-valerolactonas sintetizadas foram testadas contra três linhagens celulares e cinco delas exibiram uma moderada atividade citotóxica.

Palladium-catalyzed cross coupling reactions (Sonogashira-Hagihara, Suzuki-Miyaura, and Heck) coupling and nickel hydride-mediated tandem isomerization aldolisation have been used for the synthesis of three series of δ-valerolactones substituted in positions 3, 4, 5 and 6 of the lactone ring. The 26 kavain-like derivatives were tested against three cell lines and five of them exhibited a weak cytotoxic activity.

Keywords: δ-valerolactones, kavain analogues, Heck, Sonogashira-Hagihara, Suzuki-Miyaura, tandem isomerisation-aldolization, cytotoxicity

Introduction

The extract of roots and stems of a plant called kava, Piper methysticum Forster, Piperaceae, is used in the traditional medicine of the Pacific cultures (Polynesia, Melanesia, Micronesia) for the treatment of several disorders.1,2 The main substances responsible for the therapeutic actions of kava extract are constituted by the styrylpyrone skeleton and they are named kavalactones or kavapyrones. The kavalactones possesses a molecular framework constituted by two rings - the aromatic moiety (benzene nucleus) and the valerolactone ring (4-O-Methyl) - linked by a spacer containing the ethylene or ethane subunit (Figure 1). The literature reports 18 isolated and characterized kavalactones but the six most important for the pharmacological effects are Kavain 1, Methysticin 2, Desmethoxyyangonin 3, Dihydrokavain 4, Dihydromethysticin 5 and Yangonin 6 (Figure 1).

Despite the fact that pharmacological activities found for the kava extracts have been known for a long time, the investigations with the isolated kavalactones remained poorly studied.1,3 The styril lactones class has already been investigated as cytotoxic and antitumor agents.4 Altholactone, a furano-pyrone first isolated in 1977 and recently identified as the major compound from the stem bark of Goniothalamus arvensis, is known to

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\begin{array}{c|c|c|c|c}
\text{Kavalactones} & R^1 & R^2 & C5-C6 & C7-C8 \\
1 & Kavain & H & H & = \\
2 & Methysticin & OCH_3O & = & = \\
3 & Desmethoxyyangonin & OCH_3O & = & = \\
4 & Dihydrokavain & H & H & = \\
5 & Dihydromethysticin & OCH_3O & = & = \\
6 & Yangonin & OCH_3 & H & = \\
\end{array}
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Figure 1. General structure of Kavalactones and the most representative derivatives isolated from P. methysticum.
possess significant cytotoxicity against several tumor cell lines (Figure 2). More recently, a simple styryl lactone goniothalamin, structurally closer of the kavalactones, also exhibited significant effect on various cancer cell lines (MCF-7, P-388, WEHI164, HG-27). Because of the biological interest focused on this class of compounds, there is a tremendous potential for medicinal chemists to study structure-activity relationships of kavain derivatives in order to find a chemotherapeutic agent for the treatment of cancer.

The array of transition-metal-catalyzed cross-coupling reactions can easily be considered nowadays as the unique tool in the field of organic synthesis. The carbon-carbon bond formation reactions are among the most important processes in chemistry because they represent key steps in the synthesis of the more complex molecules from simple building blocks. Among them, the metal-catalyzed coupling reaction between phenyl, vinyl halides with allylic alcohols has become an important method to prepare building blocks for the synthesis of both natural products and analogs of large interest in medicinal chemistry. Consequently, the development of many fields of the organometallic chemistry has given support for the synthesis of new hits and lead compounds. Its scope and relevance for other areas of the chemistry, as well as the deep understanding of its numerous facets is very impressive. This is mostly due to the very large diversity of the catalysis available to provide efficient methods for the synthesis of small biologically active molecules with excellent yields.

The δ-valerolactones are an important class of organic compounds due to their high occurrence in nature, and also because they are versatile building blocks in the synthesis of a wide range of natural and synthetic products. In a previous work, we described the one-pot synthesis of some δ-valerolactones by the reaction of ethyl acetate with several aldehydes in the presence of LDA. These compounds were tested in vivo and the first results revealed a potential analgesic effect. More recently, two approaches using Heck and Suzuki-Miyaura reactions for the synthesis of yangonin, demethoxyyangonin, kavain-rac and derivatives were published by our research group. Continuing these investigations, in the present work we report the synthesis of three sets of novel kavain-like derivatives (Figure 3) through different cross-coupling approaches and their in vitro cytotoxicity evaluation against three cell lines.

In order to generate diversity, we have designed the structures bearing substituents in all positions of the lactone nucleus. These methods allow us to synthesize three sets of novel valerolactones substituted at C-3, C-4, C-5 and C-6 positions, as shown in Figure 3. For this proposal, we have focused our attention, specifically, on four procedures of cross-coupling reactions: Suzuki-Miyaura, Sonogashira, Heck-Mizoroki and Nickel hydride-mediated tandem isomerization-aldolization reaction.

**Experimental**

**Chemical analysis**

Solvents were distilled prior to use, following standard procedures, and reactions were performed under nitrogen or argon atmosphere. Silica gel 60 F254 plates were used to monitor synthetic transformation, visualization being done under UV light, 2% KMnO4 or anisaldehyde sulphuric 2% solutions. Chromatographic purifications were carried out using 70-230 mesh silica gel. Melting points were determined on a System Kofer type WME apparatus and were uncorrected. Infrared (FT-IR) spectra were recorded with a Perkin Elmer 1600 spectrometer. Nuclear magnetic resonance spectra (1H NMR and 13C NMR) spectrometer at 270 MHz and 75.5 MHz, respectively. Chemicals shifts (δ values) are given in parts per million downfield from tetramethylsilane as the internal standard. Mass spectral analyses were performed at the Centre Régional de Mesures Physiques de l’Ouest (CRMPO) in Rennes (France).

**General procedure aldolisation/lactonization reaction of 8a-8g**

To a cold (0 °C) solution of diisopropylamine (0.77 mL, 5.54 mmol) in THF (12 mL) under argon was
added n-buthyllitium (2.22 mL, 5.54 mmol, 2.5 mol L⁻¹ in hexanes) and the reaction mixture stirred 45 min at 0 °C. Ethyl acetooacetate (0.3 mL, 2.31 mmol) was added dropwise (20 min) at 0 °C and then the corresponding aldehyde (2.54 mmol) was slowly added and the reaction mixture stirred 20 min at 0 °C. After addition of cold water (75 mL), the mixture was left to stand at room temperature for 1-3 h. The crude mixture was extracted with Et₂O (3 x 20 mL) and the aqueous layer acidified with hydrochloric acid (pH 1) under ice bath. The solid thus obtained was filtered off, washed with cold water and Et₂O.

The compounds 8a, 8b and 8c have already being described by some of authors in reference 9.

4-hydroxy-6-(4-methoxyphenyl)-5,6-dihydropyran-2-one (8d): 355 mg (70%) isolated yield yellow solid, mp 136-138 °C. ¹H NMR (DMSO-d₆, 270 MHz) δ 2.49 (m, 2H), 3.75 (s, 3H), 5.03 (s, 1H), 5.36 (d, 1H, J 8.7 Hz), 6.94 (d, 2H J 8.3 Hz), 7.30 (d, 2H J 8.3 Hz); ¹³C NMR (DMSO-d₆, 67.5 MHz) δ 34.0 (CH₃), 55.2 (OCH₃), 76.0 (CH), 91.0 (CH), 113.8 (2 CH), 128.0 (2CH), 131.0 (C), 159.0 (C), 167.0 (C), 173.0 (C). FT-IR (KBr) ν_max/cm⁻¹: 1712, 1727 and 1289. HMRS or HRMS (EI) Found: 220.0743. Calc. for C₁₂H₁₀O₂: 220.0736.

(E)-4-methoxy-6-styryl-5,6-dihydropyran-2-one (9a): 128 mg (80%) isolated yield as a pale white solid, mp 141-143 °C. ¹H NMR (CDCl₃, 270 MHz): δ 2.52 (m, 2H), 3.67 (s, 3H), 4.93-5.01 (m, 1H), 5.11 (s, 1H), 6.16 (dd, 1H, J 16.0, 6.3 Hz), 6.64 (d, 1H, J 16.0 Hz), 7.15-7.32 (m, 5H); ¹³C NMR (CDCl₃, 67.5 MHz): δ 33.0 (CH₃), 56.0 (OCH₃), 76.0 (CH), 90.5 (CH), 125.5 (CH), 126.7 (2 x CH), 128.3 (CH), 128.7 (2 x CH), 133.4 (CH), 135.7 (C), 166.7 (C), 172.3 (C). FT-IR (KBr) ν_max/cm⁻¹: 1713, 1625. HRMS (EI) Found: 230.0935. Calc. for [M⁺⁺] C₁₄H₁₄O₃: 230.0943.

4-(4-hydroxy-6-oxo-3,6-dihydropyran-2-yl)benzonitrile (8e): 377 mg (76%) isolated yield yellow solid, mp 115-120 ºC. ¹H NMR (DMSO-d₆, 270 MHz) δ 2.63-2.85 (m, 2H), 5.08 (s, 1H), 5.54 (dd, 1H J 11.0, 8.7 Hz), 7.65 (d, 2H J 8.0 Hz), 7.88 (d, 2H J 8.0 Hz); ¹³C NMR (DMSO-d₆, 75 MHz) δ 33.9 (CH₃), 75.3 (CH), 90.8 (CH), 111.0 (C), 118.6 (CN), 127.2 (2CH), 132.5 (2CH), 144.6 (C), 166.3 (C), 172.7 (C). FT-IR (KBr) ν_max/cm⁻¹: 1700, 1720, 1289. HMRS (EI) Found: 171.0669. Calc. for C₉H₇N₂O₂: 171.0684.

4-(3-bromophenyl)-4-hydroxy-5,6-dihydropyran-2-one (8f): 236 mg (40%) isolated yield yellow solid, mp 165-167 °C. ¹H NMR (DMSO-d₆, 270 MHz) δ 2.74-2.85 (m, 2H), 5.04 (s, 1H), 5.44 (d, 1H J 9.0 Hz), 7.40 (d, 2H J 8.0 Hz), 7.60 (d, 2H J 8.0 Hz); ¹³C NMR (DMSO-d₆, 67.5 MHz): δ 34.0 (CH₃), 75.4 (CH), 90.7 (CH), 121.4 (C), 128.6 (2CH), 131.4 (2 CH), 138.5 (C), 166.5 (C), 172.7 (C). FT-IR (KBr) ν_max/cm⁻¹: 1648, 1589. HRMS (EI) Found: 267.9739. Calc. for C₁₁H₇O₂³Br: 267.9735.

6-(4-fluorophenyl)-4-methoxy-5,6-dihydropyran-2-one (9c): 106 mg (66%) isolated yield white solid, mp 191-193 °C. ¹H NMR (CDCl₃, 270 MHz): δ 2.50-2.82 (m, 2H), 3.75 (s, 3H), 5.21 (s, 1H), 5.40 (dd, 1H J 12.0, 3.6 Hz), 7.02-7.08 (m, 2H), 7.34-7.39 (m, 2H); ¹³C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 56.0 (OCH₃), 76.5 (CH), 90.5 (CH), 115.5 (2CH), 127.7 (2CH), 137 (C), 161.0 (CF J 246.8 Hz), 166.6 (C), 172.4 (C). FT-IR (KBr) ν_max/cm⁻¹: 1711, 1618. HRMS (EI) Found: 222.0695. Calc. for C₁₀H₇F₂O₂: 222.0692.

Dried aceton (3 mL) was added to the lactones 8a-8g (150 mg, 0.750 mmol). Powdered anhydrous K₂CO₃ (210 mg, 1.50 mmol) and (CH₃)₂SO (150 μL, 1.50 mmol) were added and the suspension was stirred overnight at room temperature. The reaction was diluted with EtOAc (26 mL) and was washed with 0.5 mol L⁻¹ HCl (26 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL) and the combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The product was purified by Flash chromatography (solvent = to 50% EtOAc in cyclohexane).

General procedure methylation reaction of 9a-9g

The compounds 8a, 8b and 8c have already being described by some of authors in reference 9.

4-(4-hydroxy-6-oxo-3,6-dihydropyran-2-yl)benzonitrile (9d): 96 mg (60%) isolated yield white solid,
mp 169-171 °C. 1H NMR (CDCl₃, 270 MHz): δ 2.57-2.80 (m, 2H), 3.77 (s, 3H), 5.24 (s, 1H), 5.45 (dd, 1H, J 11.5, 4.4 Hz), 7.0 (d, 2H, J 8.2 Hz), 7.70 (d, 2H J 8.2 Hz); 13C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 56.0 (OCH₃), 76.5 (CH), 90.5 (CH), 112.5 (C), 118.3 (CN), 126.5 (2CH), 132.6 (2CH), 143.3 (C), 160.0 (C), 172.0 (C). FT-IR (KBr) $\nu_{max}$/cm$^{-1}$: 2229, 1704, 1618. HRMS (EI) Found: 229.0740. Calc for C₁₃H₁₁NO₂: 229.0739.

4-methoxy-6-(4-methoxyphenyl)-5,6-dihydropyran-2-one (9e): 120 mg (75%) isolated yield white solid, mp 103-105 °C. 1H NMR (CDCl₃, 270 MHz): δ 2.51-2.89 (m, 2H), 3.78 (s, 3H), 5.24 (s, 1H), 5.36 (dd, 1H, J 12.0, 3.9 Hz), 6.90 (d, 2H, J 8.7 Hz), 7.30 (d, 2H J 8.7 Hz); 13C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 55.0 (OCH₃), 76.0 (CH), 90.6 (CH), 114.0 (2CH), 125.6 (2CH), 130.0 (C), 159.8 (C), 167.0 (C), 172.0 (C). FT-IR (KBr) $\nu_{max}$/cm$^{-1}$: 1716, 1626. HRMS (EI) Found: 234.0902. Calc. for C₁₃H₁₃O₂: 234.0892.

6-(4-bromophenyl)-4-methoxy-5,6-dihydropyran-2-one (9f): 154 mg (74%) isolated yield white solid, mp 161-163 °C. 1H NMR (CDCl₃, 270 MHz): δ 2.54-2.83 (m, 2H), 3.79 (s, 3H), 5.24 (s, 1H), 5.45 (dd, 1H, J 10.8, 5.4 Hz), 7.30 (d, 2H, J 8.1 Hz), 7.54 (d, 2H, J 8.1 Hz); 13C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 56.0 (OCH₃), 76.0 (CH), 90.7 (CH), 122.6 (CH), 127.7 (2CH), 132.0 (2CH), 137.4 (C), 167.0 (C), 172.0 (C). FT-IR (KBr) $\nu_{max}$/cm$^{-1}$: 1702, 1620. HRMS (EI) Found: 281.9883. Calc. for C₁₃H₁₃BrO₂: 281.9892.

Sonogashira reaction of 4-methoxy-6-(4-(2-fluorophenyl)phenyl)-5,6-dihydropyran-2-one (11)

The toluene (1 mL) was added 6-(4-iodophenyl)-4-methoxy-5,6-dihydropyran-2-one (50 mg, 0.150 mmol), followed by K₂PO₄ (64 mg, 0.270 mmol), S-Phos (1.3 mg, 0.003 mmol), 2-fluorophenyloboronic acid (31.5 mg, 0.225 mmol) and Pd(OAc)₂ (4 mg, 0.0015 mmol). The mixture was stirring at 80 °C for 24 h. The reaction was filtered through Celite® and the filtrate was dried and concentrated in vacuo. The product was purified by column chromatography (EtOAc/cyclohexane, 1:1) to afford 42 mg (72%) isolated yield white solid, mp 157-159 °C. 1H NMR (CDCl₃, 270 MHz): δ 2.60-2.90 (m, 2H), 3.80 (s, 3H), 5.26 (s, 1H), 5.48 (dd, 1H, J 11.9, 4.1 Hz), 7.12-7.60 (m, 8H); 13C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 56.2 (OCH₃), 76.9 (CH), 90.7 (CH), 116.0 (CH), 124.3 (C), 124.4 (CH), 126.0 (2CH), 128.0 (CH), 129.0 (2CH), 130.6 (CH), 136.2 (C), 137.6 (C), 160.9 (CF J 247 Hz), 166.7 (C), 172.5 (C). FT-IR (KBr) $\nu_{max}$/cm$^{-1}$: 1707, 1621. HRMS (EI): Found: 288.1003. Calc. for C₁₆H₁₁O₂F: 298.1005.

Sonogashira reaction of 4-methoxy-6-(4-(2-phenylethynyl)phenyl)-5,6-dihydropyran-2-one (12)

The DMF (1 mL) was added 6-(4-iodophenyl)-4-methoxy-5,6-dihydropyran-2-one (39 mg, 0.1168 mmol), followed by PdCl₂(PPh₃)₂ (0.84 mg, 0.0012 mmol), Cul (0.23 mg, 0.0012 mmol), 1-ethylnylbenzene (20 µL, 0.175 mmol) and iPr₂NEt (30 µL, 0.175 mmol). The mixture was stirring 60 h at room temperature. The reaction was filtered through Celite® and the filtrate was diluted with water and ethyl acetate. The organic layer was stripped off, then water. The organic layer was separated, dried and concentrated in vacuo. The product was purified by column chromatography (EtOAc/cyclohexane, 1:1). To afford

Heck reaction of (E)-methyl 3-(4-(4-methoxy-6-oxo-3,6-dihydro-2H-pyran-2-yl)phenyl)acrylate (10)

The DMF (1 mL) was added 6-(4-iodophenyl)-4-methoxy-5,6-dihydropyran-2-one (30 mg, 0.090 mmol), Pd(PPh₃)₃ (1.3 mg, 0.009 mmol). Methyl acrylate (20 µL, 0.180 mmol) and Et₃N (25 µL, 0.180) were then added, and the mixture was stirring at 80 °C for 16 h. The reaction was filtered through Celite® and the filtrate was diluted with
32 mg (90%) isolated yield white solid, mp 185-187 °C.

1H NMR (CDCl₃, 270 MHz): δ 2.56-2.85 (m, 2H), 3.78 (s, 3H), 5.25 (s, 1H), 5.40 (dd, 1H, J 4.4, 5.4 Hz), 7.34-7.57 (m, 9H); 13C NMR (CDCl₃, 67.5 MHz): δ 35.0 (CH₃), 56.2 (OCH₃), 76.7 (CH), 88.7 (C), 90.0 (C), 90.6 (CH), 123.0 (C), 123.6 (C), 126.0 (2CH), 128.3 (4CH), 131.6 (CH), 132.0 (2CH), 138.2 (C), 166.5 (C), 172.4 (C). FT-IR (KBr) ν_max/cm⁻¹: 1711, 1619, 835, 755, 692. HRMS (EI): Found: 304.1110. Calc. for C₁₇H₁₄O₃: 304.1099.

General procedure for the aldolization/lactonization reaction of 3-oxo-5-phenylpentanoate (15): 992 mg (65%) isolated yield pale yellow oil. 1H NMR (CDCl₃, 270 MHz): δ 1.24 (t, 3H, J 7.1 Hz), 2.80-3.00 (m, 4H), 3.40 (s, 2H), 4.16 (q, 2H, J 7.1 Hz), 7.15-7.35 (m, 5H); 13C NMR (CDCl₃, 67.5 MHz): δ 14.0 (CH₃), 29.3 (CH₂), 44.3 (CH₂), 49.3 (CH), 61.3 (CH), 126.0 (CH), 127.6 (2CH), 128.4 (2CH), 140.4 (C), 167.0 (C), 201.7 (C). FT-IR (KBr) ν_max/cm⁻¹: 699, 754, 1719, 1739. HRMS (EI): Found: 220.1100. Calc. for C₁₆H₁₈O₃: 220.1099.

Ethyl 5-(4-methoxyphenyl)-3-oxopentanoate (16): 1.13 g (65%) isolated yield pale yellow oil. 1H NMR (CDCl₃, 270 MHz): δ 1.18 (s, 3H), 1.20 (s, 3H), 1.28 (t, 3H, J 7.2 Hz), 2.82 (s, OH), 4.11-4.22 (m, 3H), 5.27 (dd, 2H, J 17.1, 10.5 Hz), 5.89 (dd, 1H, J 17.1, 10.5, 6.6 Hz); 13C NMR (CDCl₃, 67.5 MHz): δ 14.0 (CH₃), 19.8 (CH), 22.3 (CH₂), 46.5 (C), 60.6 (CH₃), 77.8 (CH), 117.4 (CH₂), 136.2 (CH), 177.2 (C). FT-IR (KBr) ν_max/cm⁻¹: 1719, 3497. HRMS (EI): Found: 250.1208. Calc. for C₁₇H₁₆O₃: 250.1205.

General procedure aldolisation/lactonization reaction of 17, 17b, 17b', 17d and 17h

To a cold (~78 °C) solution of Diisopropylamine (0.8 mL, 5.68 mmol) in THF (13 mL) under argon was added n-buthyllithium (2.3 mL, 5.68 mmol 2.5 mol L⁻¹ in hexane) and HMPA (0.4 mL, 2.27 mmol) the reaction mixture was stirred for 15 min. The ester (2.27 mmol) was added dropwise (30 min) at ~78 °C and then the corresponding aldehyde (2.72 mmol) was slowly added and the reaction mixture stirred 30 min at ~78 °C. After addition of NaOH 1 mol L⁻¹ (100 mL), the mixture was left to stand at room temperature for 1 h 30 min - 3 h. The crude mixture was extracted with Et₂O (3 x 20 mL) and the aqueous layer was purified with hydrochloric acid (pH 1) under ice bath. The oil obtained was purified by column chromatography (CHCl₃:AcOEt, 8:2).

Trans-5-(4-methoxybenzyl)-6-phenyl-dihydro-3H-pyran-2,4-dione (17): 211 mg (30%) isolated yield white solid, mp 100-115 °C. 1H NMR (CDCl₃, 270 MHz): δ 2.57-2.64 (dd, 2H, J 13.9, 4.9), 2.92-3.08 (m, 1H), 3.66...
(s, 2H), 3.67 (s, 3H), 5.25 (d, 1H, J 8.2 Hz), 6.75 (dd, 4H, J 37.8, 10.8), 7.15-7.30 (m, 5H); 1H NMR (CDCl$_3$, 75.5 MHz): δ 3.18 (CH$_3$), 47.4 (CH$_3$), 53.5 (OCH$_3$), 79.6 (CH$_2$), 114.0 (4CH), 127.3 (C), 128.7 (4CH), 130.0 (C), 135.6 (C), 158.5 (C), 166.6 (C), 201.2 (C). White solid, mp 110-115 $^\circ$C. FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 1582, 1666, 1721, 1754. HRMS (EI): Found: 310.1191. Calc. for C$_{15}$H$_{18}$O$_4$: 310.1205.

Cis-5-benzyl-6-phenyl-dihydro-3H-pyran-2,4-dione (17b): 89 mg (14%) isolated yield white solid, mp 96-100 $^\circ$C. 1H NMR (CDCl$_3$, 270 MHz): δ 2.63-2.98 (m, 2H), 3.19-3.22 (m, 1H), 3.46 (syst AB, 2H, J 20.4 Hz), 5.72 (d, 1H, J 3.8 Hz), 6.92-7.40 (m, 10H); 13C NMR (CDCl$_3$, 67.5 MHz): δ 30.5 (CH$_2$), 45.5 (CH$_2$), 54.4 (CH$_3$), 78.7 (CH$_2$), 126.2 (2CH), 128.7 (4CH), 129.7 (4CH), 134.4 (C), 137.0 (C), 167.0 (C), 201.2 (C). FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 1591, 1664, 1721, 1744. HRMS (EI): Found: 280.1085. Calc. for C$_{12}$H$_{10}$O$_2$: 280.1099.

Trans-5-benzyl-6-phenyl-dihydro-3H-pyran-2,4-dione (17b): 127 mg (20%) isolated yield white solid, mp 96-100 $^\circ$C. 1H NMR (CDCl$_3$, 270 MHz): δ 2.77-3.12 (m, 2H), 3.17-3.24 (m, 1H), 3.45 (s, 2H), 5.35 (d, 1H, J 8.1 Hz), 6.99-7.44 (m, 10H); 13C NMR (CDCl$_3$, 67.5 MHz): δ 32.8 (CH$_2$), 47.4 (CH$_3$), 53.5 (CH$_2$), 79.6 (CH$_2$), 126.2 (2CH), 128.7 (4CH), 129.7 (4CH), 136.6 (C), 138.2 (C), 166.6 (C), 201.9 (C). FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 1591, 1664, 1721, 1744. HRMS (EI): Found: 280.1085. Calc. for C$_{12}$H$_{10}$O$_2$: 280.1099.

Trans-5-benzyl-6-(4-methoxyphenyl)-dihydro-3H-pyran-2,4-dione (17d): 225 mg (30%) isolated yield white solid, mp 102-116 $^\circ$C. 1H NMR (CDCl$_3$, 270 MHz): δ 2.63-3.09 (m, 2H), 3.20-3.26 (m, 1H), 3.43 (syst AB, 2H, J 20.4 Hz), 3.67 (s, 3H), 5.25 (d, 1H, J 8.2 Hz), 7.23-7.76 (m, 9H); 13C NMR (CDCl$_3$, 67.5 MHz): δ 30.6 (CH$_2$), 45.6 (CH$_3$), 54.4 (OCH$_3$), 55.5 (CH), 78.7 (CH$_2$), 114.0 (4CH), 127.3 (C), 129.0 (4CH), 130.0 (C), 137.2 (C), 160.0 (C), 167.2 (C), 201.5 (C). FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 1601, 1688. HRMS (EI): Found: 310.1191. Calc. for C$_{15}$H$_{18}$O$_4$: 310.1205.

General procedure for alldolization of an allylic alcohol with an aldehyde by means of a nickel complex as catalyst (20b-20d and 20f)

A 1 mol L$^{-1}$ solution of LiBHEt$_3$ in THF (3 mL) (54 µL, 0.0567 mmol) was added to a solution of [NiCl$_2$(dppe)] (30 mg, 0.0567 mmol) at room temperature under nitrogen. The reaction mixture was stirred for 5 min before to be transferred into a flask containing anhydrous MgBr$_2$ (10 mg, 0.0567 mmol). This reaction mixture was stirred 5 min at room temperature and was cooled to –50 $^\circ$C. Then, the aldehyde (2.08 mmol) and allylic alcohol (1.89 mmol) were added successively. The reaction mixture was raised to room temperature and the reaction was monitored by TLC until the disappearance of the allylic alcohol. The reaction mixture was quenched with a saturated solution of NH$_4$Cl (15 mL) and the aqueous phase was extracted with Et$_2$O (3 × 50 mL). The organic phase was dried and concentrated under reduced pressure. After purification by column chromatography on silica gel (pentane:ether, 8:2) the desired aldot products were isolated.

(4S,5S)-ethyl 5-hydroxy-2,2,4-trimethyl-3-oxo-phenylpentaqoate (20b): 489 mg (93%) isolated yield colourless oil. 1H NMR (CDCl$_3$, 270 MHz): δ 1.10 (d, 3H, J 7.2 Hz), 1.30 (t, 3H, J 7.1 Hz), 1.35 (s, 3H), 1.40 (s, 3H), 3.15 (m, 1H), 4.25 (q, 2H, J 13.9, 7.1 Hz), 5.05 (d, 1H, J 3.0 Hz), 7.20-7.10 (m, 5H); 13C NMR (CDCl$_3$, 67.5 MHz): δ 11.5 (CH$_3$), 14.1 (CH$_2$), 21.7 (2CH$_3$), 48.4 (CH$_3$), 56.5 (C), 61.6 (CH$_2$), 115.1 (CH), 127.6 (4CH), 137.0 (C), 173.1 (C), 214.1 (C). FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 701, 1702, 1731, 3517. HRMS (EI): Found: 272.1080. Calc. for C$_{10}$H$_8$O$_2$ [M-PhCHOH]$^+$: 272.1099.

(4S,5S)-ethyl 5-(4-fluorophenyl)-5-hydroxy-2,2,4-trimethyl-3-oxopentaqoate (20c): 530 mg (95%) isolated yield colourless oil. 1H NMR (CDCl$_3$, 270 MHz): δ 1.10 (d, 3H, J 6.9 Hz), 1.30 (t, 3H, J 7.2 Hz), 1.35 (s, 3H), 1.40 (s, 3H), 3.15 (m, 1H), 4.25 (q, 2H, J 13.9, 7.2 Hz), 5.05 (d, 1H, J 3.0 Hz), 7.05 (m, 2H), 7.35 (m, 2H); 13C NMR (CDCl$_3$, 67.5 MHz): δ 11.5 (CH$_3$), 14.1 (CH$_2$), 21.7 (2CH$_3$), 48.4 (CH$_3$), 56.2 (C), 61.6 (CH$_2$), 115.1 (2CH), 127.6 (2CH), 137.0 (C), 162 (CF, J 241.6 Hz), 173.1 (C), 214.1 (C). FT-IR (KBr) $\nu_{\text{max}}$ /cm$^{-1}$: 834, 850, 1700, 1731, 3515. HRMS (EI): Found: 296.1408. Calc. for C$_{16}$H$_{14}$F: 296.1424.

(4S,5S)-ethyl 5-hydroxy-5-(4-methoxyphenyl)-2,2,4-trimethyl-3-oxopentaqoate (20d): 465 mg (80%) isolated yield colourless oil. 1H NMR (CDCl$_3$, 270 MHz): δ 1.05 (d, 3H, J 6.9 Hz), 1.30 (t, 3H, J 7.2 Hz), 1.35 (s, 3H), 1.40 (s, 3H), 3.05 (m, 1H), 3.80 (s, 3H), 4.20 (q, 2H, J 13.9, 7.2 Hz).
Hz), 5.05 (d, 1H, J 3.4 Hz), 6.85 (d, 2H, J 6.7 Hz), 7.25 (d, 2H, J 6.7 Hz); 13C NMR (CDCl3, 170 MHz): δ 11.9 (CH3), 14.1 (CH3), 21.7 (2CH3), 48.4 (CH3), 56.4 (C), 6.6 (CH3), 73.2 (CH), 113.6 (2CH), 127.1 (2CH), 133.5 (C), 158.9 (C), 173.2 (C), 214.1 (C). FT-IR (KBr) νmax/cm−1: 830, 1702, 1308. HRMS (EI): Found: 308.1628. Calc. for C15H12O5: 308.1624.

(4S,5S)-ethyl 5-(4-bromophenyl)-5-hydroxy-2,4,3-trimethyl-3-oxopentanoate (20f): 629 mg (90%) isolated yield colourless oil. 1H NMR (CDCl3, 270 MHz): δ 0.98 (d, 3H, J 5.4 Hz), 1.25 (t, 3H, J 7.8 Hz), 1.28 (s, 3H), 1.32 (s, 3H), 3.00 (m, 1H), 3.48 (s, OH), 4.18 (q, 2H, J 13.9, 7.8 Hz), 4.92 (d, 1H, J 2.8 Hz), 7.15 (d, 1H, J 8.1 Hz), 7.43 (d, 2H, J 8.1 Hz). 13C NMR (CDCl3, 67.5 MHz): δ 11.3 (CH3), 14.0 (CH3), 21.7 (2CH3), 48.3 (CH3), 56.4 (C), 61.6 (CH2D), 72.8 (CH), 121.2 (C), 127.7 (2CH), 133.4 (2CH), 140.4 (C), 173.1 (C), 214.1 (C). FT-IR (KBr) νmax/cm−1: 1702, 1731, 3511. HRMS (EI): Found: 356.0623. Calc. for C16H21O4: 356.0623.

General procedure for the preparation of lactones (21b-21d and 21f)

The aldehyde product (2.88 mmol) was dissolved in toluene (20 mL) and p-toluenesulfonic acid monohydrate (70 mg, 0.288 mmol) added. The solution was stirred 8 h at 80 °C. The mixture washed with saturated aqueous NaHCO3 solution (10 mL) and brine (10 mL). The organic phase was dried and evaporated. The oily residue was purified by column chromatography on silica gel using (pentane:ether, 6:4) to give the lactones as an inseparable cis/trans mixture.

3,3,5-trimethyl-6-phenyl-dihydro-3H-pyran-2,4-dione (21b): 401 mg (70%) cis/trans (25:75) isolated yield white solid, mp 98-104 °C. 1H NMR (CDCl3, 270 MHz): δ 1.00 (d, 3H, J 7.4 Hz), 1.55 (s, 3H), 1.63 (s, 3H), 3.02-3.07 (m, 1H), 5.16 (d(trans), 1H, J 11.2 Hz), 5.82 (d(cis), 1H, J 3.3 Hz), 7.30-7.50 (m, 5H). 13C NMR (CDCl3, 67.5 MHz): δ 10.5 (CH3), 23.0 (CH3), 25.5 (CH3), 47.6 (CH), 60.8 (C), 81.1 (CH), 125.7 (CH2), 128.8 (OCF, 135.3 (C), 174.2 (C), 209.7 (C). FT-IR (KBr) νmax/cm−1: 701, 727, 1744, 1713. HRMS (EI): Found: 232.1090. Calc. for C14H12O3Br: 232.1099.

6-(4-fluorophenyl)-3,3,5-trimethyl-dihydro-3H-pyran-2,4-dione (21c): 504 mg (60%) cis/trans (20:80) isolated yield white solid, mp 90-94 °C. 1H NMR (CDCl3, 270 MHz): δ 0.90 (d, 3H, J 7.3 Hz), 1.43 (s, 3H), 1.49 (s, 3H), 2.86-2.95 (m, 1H), 5.05 (d(trans), 1H, J 11.3 Hz), 5.70 (d(cis), 1H, J 3.1 Hz), 7.00-7.10 (m, 2H), 7.25-7.33 (m, 2H); 13C NMR (CDCl3, 67.5 MHz): δ 9.2 (CH3), 21.0 (CH3), 25.4 (CH3), 47.0 (CH), 61.6 (C), 80.3 (CH), 115.8 (2CH), 127.6 (2CH), 131.1 (C), 162.5 (CF, J 247.8 Hz), 174 (C), 209.5 (C). FT-IR (KBr) νmax/cm−1: 833, 848, 1714, 1745. HRMS (EI): Found: 250.0999. Calc. for C14H14O3F: 250.1005.

6-(4-methoxyphenyl)-3,3,5-trimethyl-dihydro-3H-pyran-2,4-dione (21d): 452 mg (60%) cis/trans (13:87) isolated yield white solid, mp 100-106 °C. 1H NMR (CDCl3, 270 MHz): δ 0.97 (d, 3H, J 6.8 Hz), 1.53 (s, 3H), 1.55 (s, 3H), 2.84-2.93 (m, 1H), 3.85 (s, 3H), 5.10 (d(trans), 1H, J 11.2 Hz), 5.75 (d(cis), 1H, J 3.1 Hz), 6.90-7.00 (m, 2H), 7.22-7.43 (m, 2H); 13C NMR (CDCl3, 67.5 MHz): δ 11.0 (CH3), 22.4 (CH2), 22.7 (CH3), 48.0 (CH), 55.7 (OCF), 61.6 (C), 81.2 (CH), 114.3 (2CH), 128.9 (2CH), 132.0 (C), 160.7 (C), 174.5 (C), 208.4 (C). FT-IR (KBr) νmax/cm−1: 817, 842, 1711, 1740. HRMS (EI): Found: 262.1201. Calc. for C15H15O3Br: 262.1205.

6-(4-bromophenyl)-3,3,5-trimethyl-dihydro-3H-pyran-2,4-dione (21f): 580 mg (65%) cis/trans (13:87) isolated yield white solid, mp 98-106 °C. 1H NMR (CDCl3, 270 MHz): δ 0.94 (d, 3H, J 6.7 Hz), 1.50 (s, 3H), 1.52 (s, 3H), 2.74-2.80 (m, 1H), 5.09 (d(cis), 1H, J 11.3 Hz), 5.75 (d(trans), 1H, J 3.1 Hz), 7.27 (d, 2H, J 8.5 Hz), 7.53 (d, 2H, J 8.5 Hz); 13C NMR (CDCl3, 67.5 MHz): δ 10.4 (CH3, 23.4 (CH3), 22.5 (CH3), 47.5 (CH), 51.7 (C), 80.3 (CH3), 127.3 (C), 128.7 (2CH), 132.1 (2CH), 135.5 (C), 173.7 (C), 207.2 (C). FT-IR (KBr) νmax/cm−1: 816, 859, 1712, 1739. HRMS (EI): Found: 312.0169. Calc. for C14H14BrO3: 312.0184.

Biological

Cell culture and treatments

In vitro studies were performed using two tumor cell lines, a human skin melanoma A375 human cells (ATCC CRL-1619) and a murine cancer cell line B16-F1 (melanoma; ATCC CRL-6323). Cytotoxicity was also evaluated on a non-tumorigenic human keratinocyte cell line HaCaT (ATCC, Rockville, Md., USA; human keratinocytes). A375M and B16 cell lines were grown in RPMI 1640 medium containing 10% FCS, 500 U mL−1 penicillin, 500 μg mL−1 streptomycin. HaCaT cells were maintained as previously described for B16 and A375M except the use of Dulbecco’s modified Eagle’s medium (DMEM) as culture medium.

After 24 h incubation at 37 °C under respectively 5% carbon dioxide for HaCaT and 10% for B16 and A375M cells to allow attachment, the cells were treated with
different concentrations of the compounds (100, 50, 25, 12.5, 6.25, 3.13 μmol L⁻¹) and incubated for 48 h for B16 and 72 h for human cell lines under the same conditions. Stock solutions of compounds were prepared in DMSO (50 mmol L⁻¹) so as the amount of this solvent was adjusted to give a final concentration lower than 0.1%. Control cultures received DMSO alone.

**MTT bioassay**

MTT assay was performed as previously described. Briefly, the cells were set up 3000, 1500, 2000 cells per well of a 96 well for respectively A375M, HaCaT and B16 cells. They were incubated at 37 °C in a humidified 5% or 10% CO₂ air mixture and treated with cisplatine, used as positive control and with the tested compounds (10% CO₂) and incubated for 48 h with (CH₃)₂SO, K₂CO₃, acetone; r.t.; 16 h.

Following three different established protocols, the para-position of the benzene nucleus of iodo lactone 9g was modified aiming to introduce diversity. We selected the organometallic cross coupling reactions Heck (route i), Suzuki (route ii) and Sonogashira (route iii) to furnish compounds 10, 11 and 12 as outlined in Scheme 2. Following three different established protocols, the para-position of the benzene nucleus of this series was modified aiming to introduce diversity at lactones. For this purpose, the iodo lactone 9g as the starting material, we selected the organometallic cross coupling reactions Heck (route i), Suzuki (route ii) and Sonogashira (route iii) to furnish compounds 10, 11 and 12 as outlined in Scheme 2.

**Results and Discussion**

**Chemistry**

Among several works which deal with the synthesis of different δ-lactones, the aldol reactions are frequently used to prepare these heterocycles. Because of that and due to our previous experience in performing this reaction, we selected synthetic route presented in Scheme 1 approach 1 to synthesize the first set of lactones (8a-8g and 9a-9g, Scheme 1). The synthesis was carried out by an aldolisation-lactonization process using ethyl acetacetate in reaction with several aromatic aldehydes, in the presence of LDA, as published before. The nature of the substituent can affect the reactivity of the aromatic aldehyde, as well as the bioactivity of final compounds. So, we have chosen some substituents that could, possibly, activate or deactivate the ring (Table 1) to produce the lactones 8a-8g in moderate to good yields (33-90%). After isolation and purification, these polar intermediary δ-oxo-lactones (8a-8g) were methylated using a classical procedure with (CH₃)₂SO in acetone, leading to the formation of targeted enol ether 9a-9g with good yields (60-80%).

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>8 (yield %)</th>
<th>9 (yield %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-CH=CH-Ph</td>
<td>8a (90)</td>
<td>9a (80)</td>
</tr>
<tr>
<td>2</td>
<td>-Ph</td>
<td>8b (86)</td>
<td>9b (76)</td>
</tr>
<tr>
<td>3</td>
<td>p-F-C₆H₄</td>
<td>8c (53)</td>
<td>9c (66)</td>
</tr>
<tr>
<td>4</td>
<td>p-OCH₃C₆H₄</td>
<td>8d (70)</td>
<td>9d (75)</td>
</tr>
<tr>
<td>5</td>
<td>p-CN-C₆H₄</td>
<td>8e (76)</td>
<td>9e (60)</td>
</tr>
<tr>
<td>6</td>
<td>p-Br-C₆H₄</td>
<td>8f (40)</td>
<td>9f (74)</td>
</tr>
<tr>
<td>7</td>
<td>p-I-C₆H₄</td>
<td>8g (33)</td>
<td>9g (73)</td>
</tr>
</tbody>
</table>

**Scheme 1.** Reagents and conditions: (i) LDA, R.CO, THF; 0 °C to room temperature; 1-3 h. (ii) (CH₃)₂SO, K₂CO₃, acetone; r.t.; 16 h.

**Scheme 2.** Reagents and conditions: (i) H₂C=CHCO₂CH₃, Et₃N, Pd(PPh₃)₄, DMF; 80 °C; 16 h; (ii) (OH)₂BO₂-F-C₆H₄=Pd(OAc)₂, S-Phos, Toluene; 70 °C; 24 h; (iii) H₂C=CHPh, iPr₂NEt, PdCl₂(PPh₃)₂, Cul, DMF; r.t.; 60 h.
Initial experiments, carried out under Heck conditions with bromopyrone 9f in opposition to methyl acrylate, have failed. If time was prolonged, temperatures were increased and/or mineral bases like CH₃COONa were used, more side products resulting from decomposition of bromopyrone were observed by ¹H NMR. The iso-o-lactone derivative 9g was briefly evaluated (entry 7, Table 1) and Heck reaction occurred in DMF at 80 °C with methyl acrylate, Pd(PPh₃)₄ and Et₃N, affording compound 10 in good yield (Scheme 2). Some optimization was required to carry out Suzuki reaction with 9g and (OH)₂B-α-F-C₆H₄. Test experiments have shown that the reaction was best performed using Pd(PPh₃)₄ and 2-(2,6-dimethoxyphenyl)-dicyclohexyl-phosphine (S-Phos) as ligand in toluene at 70 °C, affording compound 11 in good yield (Scheme 2, route b).

There are several protocols published in the literature showing the use of Sonogashira reaction. We have selected to react 9g with phenylacetylene in the presence of PdCl₂(PPh₃)₄ and CuI in DMF at room temperature and the desired product 12 was obtained in a very good yield (90%).

With successful introduction of substituents at the para-position of benzene at C-5 of the lactone ring, a different approach was considered in order to elaborate the modifications at positions 5 and 6. As illustrated in Scheme 3, a strategy for the synthesis could involve the aldol condensation of the ethyl acetate 13 with acrolein to afford allylic alcohol 14 with excellent yield. The next step was the formation of β-ketoesters 15 and 16 through Heck reactions by the treatment with two different iodobenzenes (C₆H₄-I and p-OMeC₆H₄-I, respectively). In some cases, the isomerisation is considered undesirable but this one with allylic alcohol 14 proved to be an elegant shortcut to carbonyl compounds, which led to the formation of new bond carbon-carbon in good yield. Then, the transformation of 15 and 16 into 4-oxo-lactones 17, 17b, 17b', 17d and 17h was accomplished by aldolisation/cyclisation reaction under basic conditions with different aldehydes, but in relatively low yields (Table 2). The low yields obtained for 15 and 16 (Table 2) suggested that the presence of the group CH₃CH₂Ph had a strong negative impact on the formation and/or the reactivity of the dianion. Compounds 17, 17b, 17b', 17d and 17h were obtained as a diastereoisomers mixture of cis and trans isomers (40:60 ratio, respectively, as determined by ¹H NMR in the crude mixture). However, only in the case of 17b and 17b' in which the separation of cis and trans isomers could have been carried out.

The last series in which is a substitution in all positions of the oxo-lactone ring was prepared in good yield by the reaction of allylic alcohol 19 with different aldehydes (Scheme 4). The key step was the aldol reaction through a nickel-mediated tandem reaction as discussed bellow. The synthesis started with the aldolisation between ethyl isobutyrate 18 and acrolein to furnish the allylic intermediate 19 with excellent yield. With 19 in hand, the proclivity of this allylic alcohol for aldolisation in the presence on nickel hydrides was probed. The nickel hydrides can be attractive catalysts, since they can be prepared from nickel dichloride precursors bearing a large variety of ligands, including chiral non racemic derivates. In fact, it has been demonstrated that [NiHCl-(dppe)]/ MgBr₂ combination is a very active catalytic system for the regioselective tandem isomerization-aldolisation reaction of allylic alcohols with aldehydes. Therefore, we utilized this source of nickel hydrides for the successful synthesis of the intermediates 20b-20d and 20f. The conversion of these intermediates into δ-valerolactones 21b-21d and 21f was easily performed by heating in the presence of p-TSA in toluene at 80 °C as a mixture of trans and cis stereoisomers (Table 3). The configurational assignments of these diastereoisomers were based on ¹H NMR chemical shifts and coupling constants of the protons at the lactone ring junction (see experimental).

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R¹</th>
<th>Yield %</th>
<th>mp° (C)</th>
</tr>
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<tr>
<td>1</td>
<td>H</td>
<td>Ph</td>
<td>cis</td>
<td>17b</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>Ph</td>
<td>trans</td>
<td>17b'(20)</td>
</tr>
<tr>
<td>3</td>
<td>OCH₃</td>
<td>Ph</td>
<td>trans</td>
<td>17d (30)</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>OCH₃CH₂</td>
<td>trans</td>
<td>17d (30)</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>CH(CH₃)₂</td>
<td>trans</td>
<td>17h (31)</td>
</tr>
</tbody>
</table>

The products mp (°C) for decomposition.
The cytotoxicity essay is a good first step, a rapid and cost-effective tool to help choose an optimal candidate lead compound. In this work, we have evaluated the potential cytoxicity of valerolactones 8a-g, 9a-g, 10, 11, 12, 17b, 17b', 17d, 17h, 21b-21d and 21f against two human melanone cell lines: A375-M and B16 and against the human normal keratinocytes HaCaT. The results are tabulated and compared to standard drug cisplatin data.

According to the results, the valerolactones did not affect the cell viability for most of the compounds even at 100 µmol L$^{-1}$ (see experimental). Among the 26 valerolactones tested on the three cell lines, 5 of them exhibited an IC$_{50}$ < 100 mmol L$^{-1}$ (Table 4) but the most active compound (8g) was ten-fold less active than the positive control cisplatin on HaCaT and A375M cell lines. These five compounds, 8g, 8f, 17d, 12 and 9g, were more active on human keratinocytes (HaCaT) than on the other cell lines in which confer a more specificity against this cell line.

### Table 3. δ-lactones 15n-q series 3

<table>
<thead>
<tr>
<th>Entry</th>
<th>R$^4$</th>
<th>Ratio cis:trans</th>
<th>21 (yield %)</th>
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<tr>
<td>1</td>
<td>-C$_6$H$_5$</td>
<td>25:75</td>
<td>21b (70)</td>
</tr>
<tr>
<td>2</td>
<td>p-F-C$_6$H$_4$</td>
<td>20:80</td>
<td>21c (60)</td>
</tr>
<tr>
<td>3</td>
<td>p-OCH$_3$-C$_6$H$_4$</td>
<td>13:87</td>
<td>21d (60)</td>
</tr>
<tr>
<td>4</td>
<td>p-Br-C$_6$H$_4$</td>
<td>13:87</td>
<td>21f (65)</td>
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### Table 4. Cell growth inhibition of the most active compounds on the three cell lines HaCaT, A375M and B16. The MTT assay was used to evaluate the cytotoxicity of the compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ on HaCaT (µmol L$^{-1}$)</th>
<th>IC$_{50}$ on A375M (µmol L$^{-1}$)</th>
<th>IC$_{50}$ on B16 (µmol L$^{-1}$)</th>
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<tbody>
<tr>
<td>8f</td>
<td>60 ± 10</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>8g</td>
<td>29 ± 6</td>
<td>65 ± 10</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>9g</td>
<td>&gt; 100</td>
<td>90 ± 8</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>17d</td>
<td>68 ± 11</td>
<td>102 ± 10</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>12</td>
<td>88 ± 12</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3 ± 1.5</td>
<td>8.5 ± 4.9</td>
<td>14 ± 3</td>
</tr>
</tbody>
</table>

The IC$_{50}$ value, relative to untreated control, represents the concentration that inhibited cell viability by 50%.
data provide a rank order of activity for those compounds: (8g > 8f > 17d > 12 > 9g), with the valerolactone 8g having a greater overall activity especially for HaCaT cells and could be a potential candidate as a lead compound according to NCI criteria.17

Due to the low number of active compounds we were not able to discuss the structure-activity relationship of series, but some remarks could be suggested for series 1. The structures with iodine (8g, 9g), bromine (8f) or 2-phenylethynyl on para- position of the 6-phenyl ring (structure 12) exhibited a higher activity. Moreover, comparing 8f, 8g, 9f and 9g, it can be observed that the substitution at carbon 4 with an hydroxyl enhanced the cytotoxic effect and, especially on keratinocytes compared to the corresponding 4-methoxy-substituted derivatives (8f > 9f and 8g > 9g).

Conclusions

In conclusion, we have demonstrated that transition metal catalyzed reactions can be very useful for the synthesis of the three series of δ-valerolactones designed for these studies. Among the 26 compounds tested on the three cell lines, most of them exhibited a weak cytotoxic activity and 8g was the most active. In such cases, further investigation is required to determine the anticancer potentiality of the valerolactones synthesized. Long-term objectives have been studied in order to establish structure-activity relationships (SAR) for such lactones, mainly regarding cytotoxicity and analgesic properties. Corresponding studies will be reported in due course.

Acknowledgments

This work was supported by CAPES/COFECUB project 418/03 and MCT/CNPq Universal/2003 and for grants to V.L.E.L.. The authors thank Centre Régional de Mesures Physiques de L’Ouest (CRMPO) in Rennes (France) for performing the HRMS analyses.

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Received: December 11, 2008
Web Release Date: September 19, 2009