Article

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 catalisadas 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 kavaïn-like derivatives were tested against three cell lines and five of them exhibited a weak cytotoxic activity.

Keywords: δ-valerolactones, kavaïn analogues, Heck, Sonogashira-Hagihara, Suzuki-Miyaura, tandem isomerization-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.[1-3] The main substances responsible for the therapeutic actions of kava extract are constituted by the styrilpyrone 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
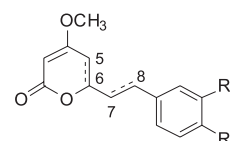
	Kavalactones	R^1	R^2	C5-C6	C7-C8
1	Kavain	H	H	–	=
2	Methysticin	OCH₂O		–	=
3	Desmethoxyyangonin	OCH₂O		=	=
4	Dihydrokavain	H	H	–	–
5	Dihydromethysticin	OCH₂O		–	–
6	Yangonin	OCH_3	H	=	=

Figure 1. General structure of Kavalactones and the most representative derivatives isolated from *P. methisticum*.

time, the investigations with the isolated kavalactones remained poorly studied.[1,3] The styryl 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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possess significant cytotoxicity against several tumor cell lines (Figure 2).[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, WEH1164, HG-27).[3] 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 kavaïn derivatives in order to find a chemotherapeutic agent for the treatment of cancer.[4]
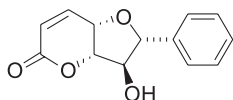

Figure 2. Altholactone – (*Goniothalamus* sp.).

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.[5] 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.[6] 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.[7] In a previous work, we described the one-pot synthesis of some δ-valerolactones by the reaction of ethyl acetoacetate with several aldehydes in the presence of LDA. These compounds were tested *in vivo* and the first results revealed a potential analgesic effect.[8] 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.[9] 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.
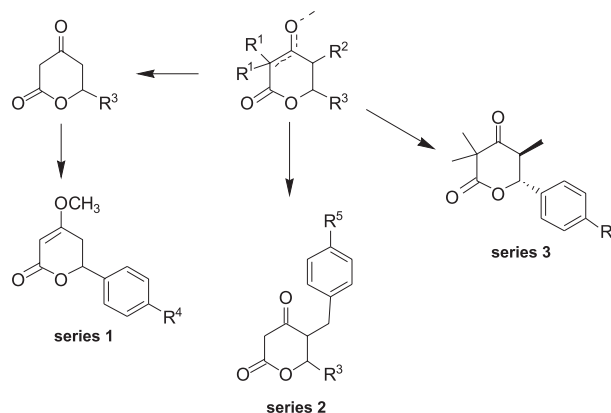

Figure 3. General structure of the three series.

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,[10,11] Sonogashira,[6] Heck-Mizoroki[12] and Nickel hydride-mediated tandem isomerization-aldolization reaction.[5]

Experimental

Chemical analysis

Solvents were distilled prior to use, following standard procedures, and reactions were performed under nitrogen or argon atmosphere. Silica gel 60 F_{254} plates were used to monitor synthetic transformation, visualization being done under UV light, 2% $KMnO_4$ or anisaldehyde sulphuric 2% solutions. Chromatographic purifications were carried out using 70-230 mesh silica gel. Melting points were determined on a System Kofler 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 acetoacetate (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 Et$_2$O (3 × 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 Et$_2$O.

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. ^{1}H NMR (DMSO-d$_6$, 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); ^{13}C NMR (DMSO-d$_6$, 67.5 MHz): δ 34.0 (CH$_2$), 55.2 (OCH$_3$), 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 ou HRMS (EI) Found: 220.0743. Calc. for C$_{12}$H$_{12}$O$_4$: 220.0736.

4-(4-hydroxy-6-oxo-3,6-dihydro-2H-pyran-2-yl) benzonitrile (8e): 377 mg (76%) isolated yield yellow solid, mp 115-120 °C. ^{1}H NMR (DMSO-d$_6$, 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); ^{13}C NMR (DMSO-d$_6$, 75 MHz) δ 33.9 (CH$_2$), 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 C$_{11}$H$_9$NO$_3$: 171.0684.

6-(4-bromophenyl)-4-hydroxy-5,6-dihydropyran-2-one (8f): 236 mg (40%) isolated yield yellow solid, mp 165-167 °C. ^{1}H NMR (DMSO-d$_6$, 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); ^{13}C NMR (DMSO-d$_6$, 67.5 MHz): δ 34.0 (CH$_2$), 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. HMRS (EI) Found: 267.9739. Calc for C$_{11}$H$_9$O$_3$^{79}Br: 267.9735.

4-hydroxy-6-(4-iodophenyl)-5,6-dihydropyran-2-one (8g): 240 mg (33%) isolated yield yellow solid, mp 153-155 °C. ^{1}H NMR (DMSO-d$_6$, 270 MHz): δ 2.49-2.72 (m, 2H), 4.93 (s, 1H), 5.31 (d, 1H *J* 8.1 Hz), 7.15 (d, 2H *J* 5.4 Hz), 7.66 (d, 2H *J* 8.1 Hz); ^{13}C NMR (DMSO-d$_6$, 67.5

MHz):δ 34.0 (CH$_2$), 75.4 (CH), 90.7 (CH), 94.4 (C), 128.6 (2CH), 137.2 (2CH), 138.9 (C), 166.4 (C), 172.6 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1678, 1649. HMRS (EI) Found: 315.9586. Calc. for C$_{11}$H$_9$O$_3$I: 315.9597.

*General procedure methylation reaction of **9a-9g***

Dried acetone (3 mL) was added to the lactones **8a-8g** (150 mg, 0.750 mmol). Powdered anhydrous K$_2$CO$_3$ (210 mg, 1.50 mmol) and (CH$_3$Me)$_2$SO$_4$ (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 Na$_2$SO$_4$ and concentrated *in vacuo*. The product was purified by Flash chromatography (solvent = 0 to 50% EtOAc in cyclohexane).

(E)-4-methoxy-6-styryl-5,6-dihydropyran-2-one (9a): 128 mg (80% yield) isolated as a pale white solid, mp 141-143 °C. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl3, 67.5 MHz): δ 33.0 (CH$_2$), 56.0 (OCH$_3$), 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$^{+•}$] C$_{14}$H$_{14}$O$_3$: 230.0943.

4-methoxy-6-phenyl-5,6-dihydropyran-2-one (9b): 122 mg (76%) isolated yield white solid, mp 143-145 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 2.53-2.86 (m, 2H), 3.76 (s, 3H), 5.22 (s, 1H), 5.40 (dd, 1H *J* 12.0, 3.6 Hz), 7.15-7.40 (m, 5H); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.0 (OCH$_3$), 76.5 (CH), 90.6 (CH), 126 (3CH), 128,6 (2CH), 138.2 (C), 166.8 (C), 172.5 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1714, 1621. HRMS (EI) Found: 204.0764. Calc. for C$_{12}$H$_{12}$O$_3$: 204.0786.

6-(4-fluorophenyl)-4-methoxy-5,6-dihydropyran-2-one (9c): 106 mg (66%) isolated yield white solid, mp 191-193 °C. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.0 (OCH$_3$), 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 C$_{12}$H$_{11}$O$_3$F: 222.0692.

4-(4-methoxy-6-oxo-3,6-dihydro-2H-pyran-2-yl) benzonitrile (9d): 96 mg (60%) isolated yield white solid,

mp 169-171 °C. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.0 (OCH$_3$), 76.5 (CH), 90.5 (CH), 112.5 (C), 118.3 (CN), 126.5 (2CH), 132.6 (2CH), 143.3 (C), 166.0 (C), 172.0 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 2229, 1704, 1618. HRMS (EI) Found: 229.0740. Calc for C$_{13}$H$_{11}$NO$_3$: 229.0739.

4-methoxy-6-(4-methoxyphenyl)-5,6-dihydropyran-2-one (9e): 120 mg (75%) isolated yield white solid, mp 103-105 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 2.51-2.89 (m, 2H), 3.78 (s, 3H), 3.81 (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); ^{13}C NMR (CDCl3, 67.5 MHz): δ 35.0 (CH$_2$), 55.0 (OCH$_3$), 56.0 (OCH$_3$), 77.0 (CH), 90.6 (CH), 114.0 (2CH), 126.5 (2CH), 130.0 (C), 159.8 (C), 167.0 (C), 172.0 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1716, 1626. HRMS (EI) Found: 234.0902. Calc. for C$_{13}$H$_{14}$O$_4$: 234.0892.

6-(4-bromophenyl)-4-methoxy-5,6-dihydropyran-2-one (9f): 154 mg (74%) isolated yield white solid, mp 161-163 °C. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl3, 67.5 MHz): δ 35.0 (CH$_2$), 56.0 (OCH$_3$), 76.0 (CH), 90.7 (CH), 122.6 (C), 127.7 (2CH), 132.0 (2CH), 137.4 (C), 167.0 (C), 172.0 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1702, 1620. HRMS (EI) Found: 281.9883. Calc. for C$_{12}$H$_{11}$O$_3$^{79}Br: 281.9892.

6-(4-iodophenyl)-4-methoxy-5,6-dihydropyran-2-one (9g): 114 mg (73%) isolated yield yellow solid, mp 121-123 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 2.54-2.82 (m, 2H), 3.78 (s, 3H), 5.24 (s, 1H), 5.40 (dd, 1H, *J* 10.8, 5.4 Hz), 7.30 (d, 2H, *J* 8.1 Hz), 7.54 (d, 2H, *J* 8.1 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.0 (OCH$_3$), 76.4 (CH), 90.6 (CH), 94.0 (C), 127.8 (2CH), 137.8 (2CH), 138.0 (C), 166.4 (C), 172.3 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1704, 1620. HRMS (EI): Found: 329.9749. Calc. for C$_{12}$H$_{11}$O$_3$I: 329.9753.

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(PPh$_3$)$_4$ (1.3 mg, 0.009 mmol). Methyl acrylate (20 μL, 0,180 mmol) and Et$_3$N (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

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 26 mg (78%) isolated yield of a yellow solid, mp 157-159 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 2.57-2.81 (m, 2H), 3.79 (s, 3H), 3.81 (s, 3H), 5.26 (s, 1H), 5.45 (dd, 1H, *J* 10.8, 5.4 Hz), 6.60 (d, 1H, *J* 16.2 Hz), 7.45 (d, 2H, *J* 8.1 Hz), 7.55 (d, 2H, *J* 16.2 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 51.7 (OCH$_3$), 56.2 (OCH$_3$), 76.4 (CH), 90.6 (CH), 118.6 (CH), 126.4 (2CH), 128.3 (2CH), 134.7 (C), 140.3 (C), 144.0 (CH), 166.5 (C), 167.2 (C), 172.3 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1704, 1620. HRMS (EI): Found: 288.0991. Calc. for C$_{16}$H$_{16}$O$_5$: 288.0998.

Suzuki reaction of 4-methoxy-6-(4-(2-fluorphenyl)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 K$_3$PO$_4$ (64 mg, 0.270 mmol), S-Phos (1.3 mg, 0.003 mmol), 2-fluorophenylboronic 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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.2 (OCH$_3$), 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), 160.09 (CF *J* 247 Hz), 166.7 (C), 172.5 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1707, 1621. HRMS (EI): Found: 288.1003. Calc for C$_{18}$H$_{15}$O$_3$F: 298.1005.

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

The DMF (1 mL) was added to 6-(4-iodophenyl)-4-methoxy-5,6-dihydropyran-2-one (39 mg, 0.1168 mmol), followed by PdCl$_2$(PPh$_3$)$_2$ (0.84 mg, 0.0012 mmol), CuI (0.23 mg, 0.0012 mmol), 1-ethynylbenzene (20 μL, 0.175 mmol) and iPr$_2$NEt (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

32 mg (90%) isolated yield white solid, mp 185-187 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 2.56-2.85 (m, 2H), 3.78 (s, 3H), 5.25 (s, 1H), 5.40 (dd, 1H, *J* 10.8, 5.4 Hz), 7.34-7.57 (m, 9H); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 35.0 (CH$_2$), 56.2 (OCH$_3$), 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 C$_{20}$H$_{16}$O$_3$: 304.1099.

*General procedure for aldolization reaction (**14** and **19**)*

To a stirred solution of THF (70 mL) and diisopropylamine (4.35 mL, 30.96 mmol), at −78 °C was added *n*-BuLi (12.4 mL, 30.96 mmol, 2.5 mol L^{-1} in hexane). The reaction was stirred at −78 °C for 15 min followed by of dropwise addition of the ester (25.8 mmol). Upon completion of addition of ester, the reaction was stirred 50 min. Distilled acrolein (1.54 mL, 27 mmol) in THF (10 mL) was added via cannula, and reaction stirred at −78 °C for 10 min. The reaction was quenched by the rapid injection of a saturated NH$_4$Cl solution (10 mL) immediately poured into a separatory funnel containing diethyl ether (50 mL), and washed with brine (2 x 20 mL). The organic layer was dried, filtered, and evaporated in vacuo to give a yellow oil which was purified by column chromatography (CH$_2$Cl$_2$:EtOAc, 9:1).

*Ethyl 3-hydroxypent-4-enoate (**14**):* 3.24 g (95%) isolated yield pale yellow oil. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 1.28 (t, 3H, *J* 7.1 Hz), 2.51-2.57 (m, 2H), 3.29 (s, OH), 4.16 (q, 2H, *J* 7.1 Hz), 4.51-4.58 (m, 1H), 5.15 (dd, 2H, *J* 16.9, 10.5 Hz), 5.89 (ddd, 1H, *J* 16.9, 10.5, 5.6 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 14.0 (CH$_2$), 41.2 (CH$_2$), 60.6 (CH$_3$), 68.8 (CH), 115.1 (CH$_2$), 138.8 (CH), 172.0 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}:: 1715, 3434. HRMS (EI): Found: 98.0368. Calc for C$_5$H$_6$O$_2$ [M-EtOH]$^+$: 98.0368.

*Ethyl 3-hydroxy-2,2-dimethylpent-4-enoate (**19**):* 3.97 g (97%) isolated yield pale yellow oil. ^{1}H NMR (CDCl$_3$, 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 (ddd, 1H, *J* 17.1, 10.5, 6.6 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 14.0 (CH$_2$), 19.8 (CH$_3$), 22.3 (CH$_3$), 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: 116.0841. Calc for C$_6$H$_{12}$O$_2$ [M-C$_3$H$_3$O]$^+$: 116.0837.

*General procedure for the Heck reaction (**15** and **16**)*

To the DMF (15 mL) were added ethyl 3-hydroxypent-4-enoate (1 g, 6.94 mmol), NaHCO$_3$ (1.16 g, 13.88 mmol),

NBu$_4$Br (1.16 g, 13.88 mmol), 1-iodobenzene and Pd(OAc)$_2$ (25 mg, 0.0094 mmol). The mixture was stirring at 60 °C for 16 h. The reaction was filtered through Celite® and the filtrate was diluted with water and Et$_2$O. The organic layer was separated, dried and concentrated *in vacuo*. The product was purified by column chromatography (CH$_2$Cl$_2$ 100%).

*Ethyl 3-oxo-5-phenylpentanoate (**15**):* 992 mg (65%) isolated yield pale yellow oil. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 14.0 (CH$_3$), 29.3 (CH$_2$), 44.3 (CH$_2$), 49.3 (CH$_2$), 61.3 (CH$_2$), 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 C$_{13}$H$_{16}$O$_3$: 220.1099.

*Ethyl 5-(4-methoxyphenyl)-3-oxopentanoate (**16**):* 1.13 g (65%) isolated yield pale yellow oil. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 1.24 (t, 3H, *J* 7.5 Hz), 2.82-3.04 (m, 4H), 3.40 (s, 2H), 3.76 (s, 3H), 4.16 (q, 2H, *J* 7.5 Hz), 6.80 (d, 2H, *J* 8.1 Hz), 7.07 (d, 2H, *J* 8.1 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 14.1 (CH$_3$), 28.5 (CH$_2$), 44.6 (CH$_2$), 49.0 (CH$_2$), 55.3(OCH$_3$), 61.3 (CH$_2$), 114.1 (2CH), 130.8 (2CH), 132.5 (CH), 157.9 (C), 167.0 (C), 202.0 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 699, 754, 1719, 1739. HRMS (EI): Found: 250.1208. Calc for C$_{14}$H$_{18}$O$_4$: 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*-buthyllitium (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 Et$_2$O (3 × 20 mL) and the aqueous layer acidified with hydrochloric acid (pH 1) under ice bath. The oil obtained was purified by column chromatography (CHCl$_2$: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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 75.5 MHz): δ 31.8 (CH$_2$), 47.4 (CH$_2$), 53.5 (OCH$_3$), 79.6 (CH), 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 °C. FT-IR (KBr) ν$_{max}$/cm^{-1}: 1582, 1666, 1721, 1754. HRMS (EI): Found: 310.1191. Calc. for C$_{19}$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 °C. ^{1}H 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 30.5 (CH$_2$), 45.5 (CH$_2$), 54.4 (CH), 78.7 (CH), 126.2 (2CH), 128.7 (4CH), 129.7 (4CH), 134.4 (C), 137.0 (C), 167.0 (C), 201.2 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1591, 1664, 1721, 1744. HRMS (EI): Found: 280.1085. Calc. for C$_{18}$H$_{16}$O$_3$: 280.1099.

Trans-5-benzyl-6-phenyl-dihydro-3H-pyran-2,4-dione (17b'): 127 mg (20%) isolated yield white solid, mp 96-100 °C. ^{1}H 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 32.8 (CH$_2$), 47.4 (CH$_2$), 53.5 (CH), 79.6 (CH), 126.2 (2CH), 128.7 (4CH), 129.7 (4CH), 136.6 (C), 138.2 (C), 166.6 (C), 201.9 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1591, 1664, 1721, 1744. HRMS (EI): Found: 280.1085. Calc. for C$_{18}$H$_{16}$O$_3$: 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 °C. ^{1}H 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), 7.23-7.26 (m, 9H); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 30.6 (CH$_2$), 45.6 (CH$_2$), 54.4 (OCH$_3$), 55.5 (CH), 78.7 (CH), 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) ν$_{max}$/cm^{-1}: 1601, 1688. HRMS (EI): Found: 310.1191. Calc. for C$_{19}$H$_{18}$O$_4$: 310.1205.

Trans-5-benzyl-6-isopropyl-dihydro-3H-pyran-2,4-dione (17h): 173 mg (31%) isolated yield white solid, mp 131-133 °C. ^{1}H NMR (CDCl$_3$, 270 MHz): δ 0.94 (d, 3H, *J* 6.7 Hz), 1.00 (d, 3H, *J* 6.7 Hz), 2.04 (m, 1H), 2.72-2.87 (m, 1H), 2.80-3.09 (m, 2H), 3.20 (syst AB, 2H, *J* 19.0 Hz), 4.12 (dd, 1H, *J* 8.0, 4.2 Hz), 7.08-7.27 (m, 5H); ^{13}C NMR (CDCl3, 67.5 MHz): δ 15.2 (CH$_3$), 19.6 (CH$_3$), 29.6 (CH), 33.0 (CH$_2$), 46.8 (CH$_2$), 50.5 (CH), 82.3 (CH), 127.2 (CH), 129.0 (4CH), 136.5 (C), 167.0 (C), 201.8 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1598, 1672. HRMS (EI): Found: 246.1250. Calc. for C$_{15}$H$_{18}$O$_3$): 246.1256.

General procedure for aldolization 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 °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 aldol products were isolated.

(4S,5S)-ethyl 5-hydroxy-2,2,4-trimethyl-3-oxo-5-phenylpentanoate (20b): 489 mg (93%) isolated yield colourless oil. ^{1}H 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 11.5 (CH$_3$), 14.1 (CH$_3$), 21.7 (2CH$_3$), 48.4 (CH), 56.5 (C), 61.6 (CH2), 115.1 (CH), 127.6 (4CH), 137.0 (C), 173.1 (C), 214.1 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}:: 701, 1702, 1731, 3517. HRMS (EI): Found: 172.1080. Calc. for C$_9$H$_{16}$O$_3$ [M-PhCHOH]$^+$: 172.1099.

(4S,5S)-ethyl 5-(4-fluorophenyl)-5-hydroxy-2,2,4-trimethyl-3-oxopentanoate (20c): 530 mg (95%) isolated yield colourless oil. ^{1}H 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 11.5 (CH$_3$), 14.1 (CH$_3$), 21.7 (2CH$_3$), 48.4 (CH), 56.2 (C), 61.6 (CH2), 115.1 (2CH), 127.6 (2CH), 137.0 (C), 162 (CF, *J* 241.6 Hz), 173.1 (C), 214.1 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 834, 850, 1700, 1731, 3515. HRMS (EI): Found: 296.1408. Calc. for C$_{16}$H$_{21}$O$_4$F: 296.1424.

(4S,5S)-ethyl 5-hydroxy-5-(4-methoxyphenyl)-2,2,4-trimethyl-3-oxopentanoate (20d): 465 mg (80%) isolated yield colourless oil. ^{1}H 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), 5.05 (d, 1H, *J* 3.4 Hz), 6.85 (d, 2H, *J* 6.7 Hz), 7.25 (d, 2H, *J* 6.7 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 11.9 (CH$_3$), 14.1 (CH$_3$), 21.7 (2CH$_3$), 48.4 (CH), (OCH$_3$), 56.4 (C), 61.6 (CH$_2$), 73.2 (CH), 113.6 (2CH), 127.1 (2CH), 133.5 (C), 158.9 (C), 173.2 (C), 214.1 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 830, 1702, 1730, 3508. HRMS (EI): Found: 308.1628. Calc. for C$_{17}$H$_{24}$O$_5$: 308.1624.

(4S,5S)-ethyl 5-(4-bromophenyl)-5-hydroxy-2,2,4-trimethyl-3-oxopentanoate (20f): 629 mg (90%) isolated yield colourless oil. ^{1}H NMR (CDCl$_3$, 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, 2H, *J* 8.1 Hz), 7.43 (d, 2H, *J* 8.1 Hz); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 11.3 (CH$_3$), 14.0 (CH$_3$), 21.7 (2CH$_3$), 48.3 (CH), 56.4 (C), 61.6 (CH$_2$), 72.8 (CH), 121.2 (C), 127.7 (2CH), 133.4 (2CH), 140.4 (C), 173.1 (C), 214.1 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 1702, 1731, 3511. HRMS (EI): Found: 356.0623. Calc. for C$_{16}$H$_{21}$O$_4$^{79}Br): 356.0623.

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

The aldol 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 NaHCO$_3$ 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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 10.5 (CH$_3$), 23.0 (CH$_3$), 25.5 (CH$_3$), 47.6 (CH), 60.8 (C), 81.1 (CH), 125.7 (CH), 128.8 (4CH), 135.3 (C), 174.2 (C), 209.7 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 701, 727, 1744, 1713. HRMS (EI): Found: 232.1090. Calc. for C$_{14}$H$_{16}$O$_3$: 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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 9.2 (CH$_3$), 21.0 (CH$_3$), 25.4 (CH$_3$), 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) ν$_{max}$/cm^{-1}: 833, 848, 1714, 1745. HRMS (EI): Found: 250.0999. Calc. for C$_{14}$H$_{15}$O$_3$F: 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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 Mhz): δ 11.0 (CH$_3$), 22.4 (CH$_3$), 22.7 (CH$_3$), 48.0 (CH), 55.7 (OCH$_3$), 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) ν$_{max}$/cm^{-1}: 817, 842, 1711, 1740. HRMS (EI): Found: 262.1201. Calc. for C$_{15}$H$_{18}$O$_4$: 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. ^{1}H NMR (CDCl$_3$, 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); ^{13}C NMR (CDCl$_3$, 67.5 MHz): δ 10.4 (CH$_3$), 23.4 (CH$_3$), 22.5 (CH$_3$), 47.5 (CH), 51.7 (C), 80.3 (CH), 127,3 (C), 128.7 (2CH), 132.1 (2CH), 135.5 (C), 173.7 (C), 207.2 (C). FT-IR (KBr) ν$_{max}$/cm^{-1}: 816, 859, 1712, 1739. HRMS (EI): Found: 312.0169. Calc. for C$_{14}$H$_{15}$O$_3$^{79}Br: 312.0184.

Biological

Cell culture and treatments

In vitro studies were performed using two tumor cell lines 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.[13,14] 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% CO$_2$ air mixture and treated with cisplatine, used as positive control and with the tested compounds (**8a-8g**, **9a-9g**, **10**, **11**, **12**, **17b**, **17b'**, **17d**, **17h**, **21b-21d** and **21f**) at different concentrations for 48 h or 72 h. Four hours before the end treatment time, 10µL of MTT were added to each microwell and plates were replaced at 37 °C. After 4 h, the supernatant was removed and crystals of formazan were dissolved in 150 µL of DMSO. The optical density of each well sample was measured with a microplate spectrophotometer reader at 540 nm. Each experiment was repeated at least three times and three different wells were used for each concentration to allow the determination of the IC$_{50}$ (cytotoxicity for which the absorbance is reduced to 50%).

## Results and Discussion

Chemistry

Among several reports which deal with the synthesis of different δ-lactones, the aldol reactions are frequently used to prepare these heterocycles.[9] 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 acetoacetate in reaction with several aromatic aldehydes, in the presence of LDA, as published before.[9] 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[15] with (CH$_3$)$_2$SO$_4$ in acetone, leading to the formation of targeted enol ether **9a-9g** with good yields (60-80%).

Table 1. δ-lactones **9a-9g** series 1

Entry	R^3	8 (yield %)	9 (yield %)
1	-CH=CH-Ph	**8a** (90)	**9a** (80)
2	-Ph	**8b** (86)	**9b** (76)
3	*p*-F-C$_6$H$_4$	**8c** (53)	**9c** (66)
4	*p*-OCH$_3$-C$_6$H$_4$	**8d** (70)	**9d** (75)
5	*p*-CN-C$_6$H$_4$	**8e** (76)	**9e** (60)
6	p-Br-C6H4	**8f** (40)	**9f** (74)
7	p-I-C6H4	**8g** (33)	**9g** (73)
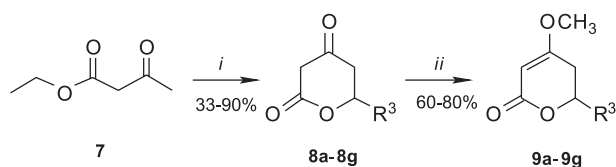
Scheme 1. Reagents and conditions: (*i*) LDA, R$_3$CHO, THF; 0 °C to room temperature; 1-3 h. (*ii*) (CH$_3$)$_2$SO$_4$, K$_2$CO$_3$, acetone; r.t.; 16 h.

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, using 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.
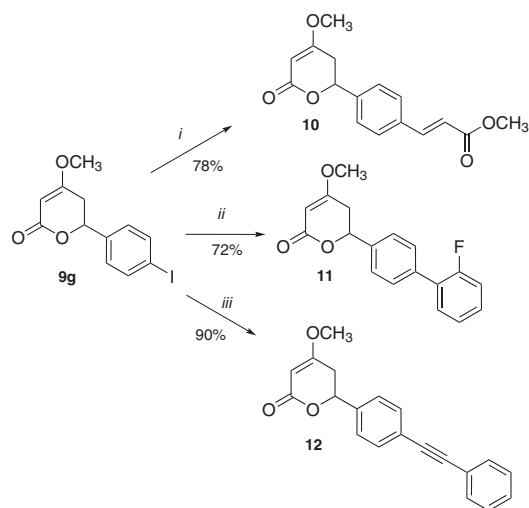
Scheme 2. Reagents and conditions: (*i*) H$_2$C=CHCO$_2$CH$_3$, Et$_3$N, Pd(PPh$_3$)$_4$, DMF; 80 °C; 16 h; (*ii*) (OH)$_2$B-*o*-F-C$_6$H$_4$, Pd(OAc)$_2$, S-Phos, Toluene; 70 °C; 24 h; (*iii*) HC≡C-Ph, iPr$_2$NEt, PdCl$_2$(PPh$_3$)$_2$, CuI, DMF; r.t.; 60 h.

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 CH_3COONa were used, more side products resulting from decomposition of bromopyrone were observed by ^1H NMR. The iodo-δ-lactone derivative **9g** was briefly evaluated (entry 7, Table 1) and Heck reaction occurred in DMF at 80 °C with methyl acrylate , $Pd(PPh_3)_4$ and Et_3N, affording compound **10** in good yield (Scheme 2). Some optimization was required to carry out Suzuki reaction with **9g** and $(OH)_2B$-*o*-F-C_6H_4. Test experiments have shown that the reaction was best performed using $Pd(PPh_3)_4$ and 2-(2,6-dimethoxybiphenyl)-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 $PdCl_2(PPh_3)_4$ 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 (C_6H_5-I and *p*-$OMeC_6H_4$-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,[12,16] 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 CH_2CH_2Ph 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
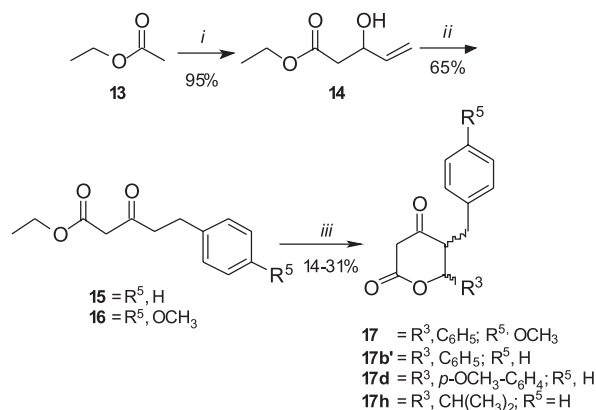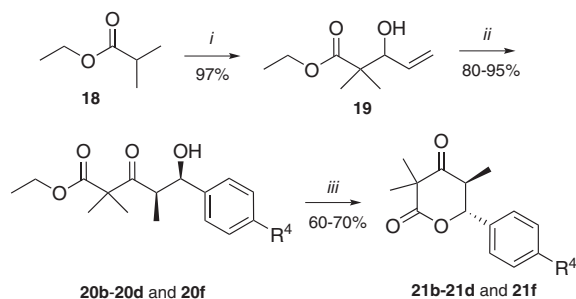

Scheme 3. Reagents and conditions: (*i*) $H_2C=CHCHO$, $C_6H_{15}N$, *n*-BuLi; THF −78 °C; 65 min; (*ii*) *p*-R^5-C_6H_4-I, $NaHCO_3$, NBu_4Br, $Pd(OAc)_2$; DMF 60 °C; 16 h; (*iii*) R^3CHO, $C_6H_{15}N$, *n*-BuLi, HMPA; THF −78 °C; 45 min; H_3O^+; 1h 30 - 3 h r.t.

Table 2. 4-oxo-lactones substituted series 2

Entry	R^5	R^3	Configuration	Yield %	mp[a] (°C)
1	H	Ph	*cis*	**17b** (14)	96-100
2	H	Ph	*trans*	**17b'** (20)	96-100
3	OCH_3	Ph	*trans*	**17** (30)	100-115
4	H	$OCH_3C_6H_4$	*trans*	**17d** (30)	102-116
5	H	$CH(CH_3)_2$	*trans*	**17h** (31)	131-133

[a]The products mp (°C) for decomposition.

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[16] that the [NiHCl-(dppe)]/$MgBr_2$ 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).

Scheme 4. Reagents and conditions: (*i*) $H_2C=CHCHO$, $C_6H_{15}N$, *n*-BuLi; THF −78 °C; 65 min; (*ii*) *p*-R^4-C_6H_4CHO, $NiCl_2$, $MgBr_2$, $LiBH(Et)_3$; THF −50 °C to r.t.; 5 min; (*iii*) *p*-TSA; toluene 80 °C; 8 h.

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

Entry	R^4	Ratio *cis:trans*	21 (yield %)
1	-C_6H_5	25:75	**21b** (70)
2	*p*-F-C_6H_4	20:80	**21c** (60)
3	*p*-OCH_3-C_6H_4	13:87	**21d** (60)
4	*p*-Br-C_6H_4	13:87	**21f** (65)

Biology

The cytotoxicity essay is a good first step, a rapid and cost-effective tool to help choose an optimal candidate a 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 cisplatine 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. The

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

Compounds	IC_{50} on HaCaT (µmol L^{-1})	IC_{50} on A375M (µmol L^{-1})	IC_{50} on B16 (µmol L^{-1})
8f	60 ± 10	> 100	> 100
8g	29 ± 6	65 ± 10	> 100
9g	> 100	90 ± 8	> 100
17d	68 ± 11	102 ± 10	> 100
12	88 ± 12	> 100	> 100
Cisplatin	3 ± 1.5	8.5 ± 4.9	14 ± 3

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

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