Artigo

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF HALOGENATED AROMATIC *BIS*-γ-LACTONES ANALOGOUS TO AVENACIOLIDE

Pedro A. Castelo-Branco*
Departamento de Pesquisa e Pós-Graduação, Instituto Federal Fluminense, Campus Campos-Centro, R. Dr. Siqueira, 273, 28030-130 Campos dos Goytacazes – RJ, Brasil
Mayura M. M. Rubinger, Leandro de C. Alves, Natalia A. Liberto, Thayane C. M. Nepel e Mariana Catrinck
Departamento de Química, Universidade Federal de Viçosa, Av. PH Rolfs, s/n, 36571-000 Viçosa – MG, Brasil
Silvana Guilardi, Hudson A. Silvério e Wilson P. F. Neto
Instituto de Química, Universidade Federal de Uberlândia, Av. João Naves de Ávila, 2121, 38400-902 Uberlândia – MG, Brasil
Laércio Zambolim
Departamento de Fitopatologia, Universidade Federal de Viçosa, Av. PH Rolfs, s/n, 36571-000 Viçosa – MG, Brasil
Dorila Piló-Veloso
Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte – MG, Brasil

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Here we describe the total syntheses and characterization by elemental analyses, infrared and NMR spectroscopy of three new compounds analogous to avenaciolide, a *bis*-γ-lactone isolated from *Aspergillus avenaceus* that possesses antifungal activity, where the octyl group of the natural product was replaced by aromatic groups containing chlorine and fluorine atoms. The effects of the avenaciolide, the novel compounds and their synthetic precursors on mycelia development and conidia germination of *Colletotrichum gloeosporioides* and *Fusarium solani* were evaluated *in vitro*. The title compounds were almost as active as avenaciolide. The absolute structures of the chlorinated analogs were determined by X-ray diffraction analysis.

Keywords: avenaciolide analogues; absolute structural determination; antifungal activity.

INTRODUCTION

Fungal plant pathogens can cause serious yield losses to several plant species interfering directly in food production, especially in the subtropical and tropical regions. Some of the major phytopathogens affecting food production also cause infections in humans, and important economic losses can occur both from agricultural losses and medical care costs. Although many fungicides are commercially available, the effectiveness of the antifungal agents changes due to the emergence of fungal resistance.[1] Research is needed to develop novel antifungals to increase the variety of chemicals available for field applications to control plant diseases. Successful development of such compounds could prove eventually useful to prevent infectious and toxin-producing fungi in both the agricultural and medical fields.

Many antifungal metabolites presenting a wide range of biological activities have been isolated from microorganisms.[2] Avenaciolide (Figure 1) is a naturally occurring antifungal *bis*-γ-lactone isolated from *Aspergillus avenaceus*.[3] Its structure was confirmed by total synthesis and crystallographic studies.[4,5] Avenaciolide has also antibacterial action,[3] inhibits the transport of glutamate in rat liver mitochondria and interferes on the ability of ADP to stimulate the rate of glutamate oxidation.[6] Due to all these important biological activities combined to an interesting bicyclic structure, several synthetic approaches to avenaciolide have been published.[7]

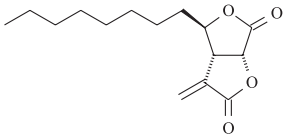
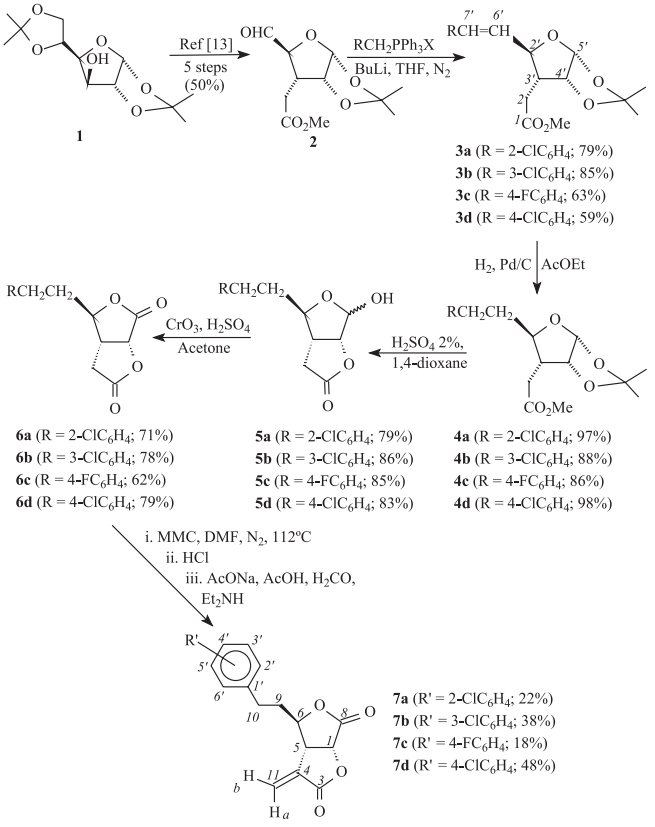


Figure 1. *Chemical structure of avenaciolide (***7e***)*

We have previously described the preparation of some avenaciolide analogs[8,9] which were active against *Colletotrichum gloeosporioides* (Penz.), an important fungal plant pathogen species distributed worldwide. Among these analogs, the (1*R*,5*R*,6*R*)-6-[2-(4-chlorophenyl)ethyl]-4-methylidene-2,7-dioxabicyclo[3.3.0] octan-3,8-dione (Scheme 1, **7d**) was 25% less active than avenaciolide.[9] The molecular structure of **7d** was determined and showed remarkable differences in the torsion angles and bond lengths within the α-methylene-γ-lactone ring when compared to avenaciolide.[9] In order to further investigate the importance of the lateral chain for the structure and the biological activity of these *bis*-γ-lactones we decided to prepare other halogenated aromatic analogues. Here we describe the synthesis and characterization by elemental analyses, infrared and NMR spectroscopies of three new avenaciolide analogues containing aromatic groups with chlorine (*ortho* and *meta*) and fluorine (*para*) substituents **7a**, **7b** and **7c**, respectively (Scheme 1).

The structures of the chlorinated analogs **7a** and **7b** were further investigated by X-ray diffraction and compared to **7d** (Scheme 1) and to avenaciolide **7e** (Figure 1). The compounds **7a-7e** were tested against *C. gloeosporioides* and *Fusarium solani* (Mart.). *Colletotrichum* is one of the most serious plant pathogenic fungal genera, and causes the disease known as anthracnose. *C. gloeosporioides* affects many crops of agricultural importance, resulting in serious yield losses including cereals, legumes and fruits.[10] *F. solani* is considered the major plant pathogen of its genus and cause wilt and root rot in a number of crops including cotton, peas, ornamentals, fruits and cucurbits.[11] This fungus causes sudden death syndrome in soybeans and green beans, leading to root rot, crown and leaf necrosis,

*e-mail: pedro@iff.edu.br



Scheme 1. *Synthesis of the avenaciolide analogs* **7a-7d**

pod abortion, and vascular discoloration of roots and stems.[11] Both fungi can cause keratitis in humans, especially in immunocompromised patients.[12]

RESULTS AND DISCUSSION

Syntheses of *bis*-γ-lactones

The synthetic approach is shown in the Scheme 1. The use of a chiral pool strategy, from readily available enantiopure diacetone-D--glucose (**1**), was suitable for our purposes as it enabled us to prepare the enantiomeric pure avenaciolide analogs **7a**-**7c**, with 2-Cl, 3-Cl, and 4-F substituents, respectively. In order to compare their biological activities, the 4-Cl analog (**7d**) and avenaciolide (**7e**) were also prepared, as described in the literature.[4,9] These compounds and all the new synthetic intermediates (compounds **3a-c** to **6a-c**, Scheme 1) were characterized by elemental analyses, infrared and NMR spectroscopy. The analyses of the NMR spectra were supported by DEPT, COSY, HMBC and HSQC experiments. The data obtained for compounds **3d-e** to **7d-e** were identical to those reported in the literature.[4,9]

The aldehyde intermediate **2** was prepared from diacetone-D--glucose (**1**) as described in the literature.[13] The aromatic side chains were introduced by the Wittig reaction between the aldehyde **2** and the phosphonium ylides prepared *in situ* from the phosphonium salts and butyllithium, yielding mixtures of *Z/E* isomers **3a**-**3c**. The Scheme 1 displays the numeration of C-atoms used for the NMR signals attributions. The signals of the olefinic C-6' and C-7' of the major *Z* isomers were observed at *ca.* 129 and 135 ppm, respectively, in the ^{13}C NMR spectra of the new compounds **3a**-**3c**. Along with these signals, other less intense ones were indicative of the presence of the *E* isomers as minor components (*E*:*Z* = 1:4 to 1:7). The coupling constant (*J*) of *ca.* 11 Hz for the olefinic hydrogens H-6' and H-7' observed in the ^{1}H NMR spectra of these compounds confirmed the *Z* configuration for the main component. The observation of the expected signals in the aromatic range (7.0-7.4 ppm) confirmed the presence of the aromatic groups in the structures of **3a**-**3c**. It was not necessary to separate the *Z/E* isomers, as the mixture was hydrogenated to yield the compounds **4a**-**4c**. Their ^{13}C NMR spectra showed three methylene signals (DEPT) in the range of 29 to 35 ppm, attributed to C-2 and to the two newly hydrogenated carbons C-6' and C-7'. These two signals were correlated (HMQC contour maps) to the ^{1}H NMR multiplets at 1.6-1.9 ppm and 2.6-3.3 ppm (H-6' and H-7', respectively).

The first lactone ring was closed by the reaction of the esters **4a**-**4c** with sulphuric acid (2%) in 1,4-dioxane under reflux (Scheme 1). The infrared spectra of the new compounds **5a**-**5c** showed the expected broad band at *ca.* 3415 cm^{-1} due to the $\nu_{O\text{-}H}$, and a shift of the $\nu_{C=O}$ band to higher wave numbers when compared to the precursor esters. Their ^{1}H NMR spectra showed that **5a**-**5c** were mixtures of isomers with the hydroxyl at 8-C in α and β positions, in the proportion of 1:2 to 1:3 (α:β). Two broad signals were observed for the OH groups at *ca.* 3.6 and 3.3 ppm for the α and β epimers, respectively. The identity of the major product was established by the comparison of the H-8 signals (5.5 to 5.6 ppm), which appeared as singlets for the epimers with the hydroxyl group in β position, and as doublets ($J_{8,1}$ *ca.* 4 Hz) for the minor α isomers. In the spectrum of **5c** these signals were superposed. The correlated C-8 signals were observed at 101 and 96 ppm (less intense), respectively, in the ^{13}C NMR spectra of the mixtures **5a**-**5c**. Two C=O signals were observed in the ^{13}C NMR spectra, the most intense ones at 176 ppm (β isomers) and the less intense at 177 ppm (α isomers).

The *Jones* oxidation of the compounds **5a**-**5c** yielded the *bis*--γ-lactones **6a**-**6c**. The infrared spectrum of **6a** showed a broad band at 1782 cm^{-1} while in the spectra of **6b** and **6c**, two distinct $\nu_{C=O}$ absorptions could be observed (at 1784-1785 and 1790-1803 cm^{-1}). The simplification of the H-1 signal multiplicity in the ^{1}H NMR spectra of these compounds (doublets at 5 ppm, with $J_{1,5}$ *ca.* 8 Hz) and the observation of the two C=O signals in their ^{13}C NMR spectra (8-C at *ca.*170 ppm and 3-C at *ca.* 174 ppm) confirmed the oxidation at C-8.

The reaction of **6a**-**6c** with methylmethoxymagnesium carbonate (MMC, Scheme 1), followed by the addition of HCl, yielded the carboxylic acid intermediates, which were not isolated. To the crude products (yellowish oils) was added a mixture of sodium acetate, acetic acid, formalin, distilled water and ethylamine, yielding the new avenaciolide analogs **7a-7c**. The infrared spectra of the new compounds **7a**-**7c** showed the expected band due to the extra C=C stretching vibration (at *ca.* 1665 cm^{-1}) and a broad band in the C=O region of the γ-lactones, centered at ca. 1780 cm^{-1}. No changes were observed in the C-8 signals in the ^{13}C NMR spectra of compounds **7a**-**7c**, when compared to the spectra of the parent *bis*-lactones. On the other hand, the introduction of the methylidene group α to C-3 caused the expected shift on this C=O signal from *ca.* 174 to 168 ppm. The expected change in the chemical shifts of C-4 signals from *ca.* 33 ppm in the ^{13}C NMR spectra of the parent *bis*-γ-lactones to *ca.* 134 ppm confirmed the formation of compounds **7a**-**7c**. The vinylic H-11a and H-11b of the analogs **7a**-**7c** originated two doublets ($J_{11a,11b}$ = 2 Hz) at *ca.* 6.4 and 5.9 ppm in their ^{1}H NMR spectra. The correlated C-signals were observed at *ca.* 126.5 ppm in the ^{13}C NMR spectra of **7a**-**7c**. The remaining signals were in very good agreement with the proposed structures. In the ^{13}C NMR spectra of the series of fluorinated compounds **3c**-**7c**, the expected coupling constants between the fluorine and the aromatic carbon atoms were observed ($^4J_{1'\text{-}C,F}$ *ca.* 3 Hz, $^3J_{2'\text{-}C,F}$ *ca.* 8 Hz, $^2J_{3'\text{-}C,F}$ *ca.* 21 Hz, and $^1J_{4'\text{-}C,F}$ *ca.* 243 Hz).

Crystal structure determinations

The absolute structures of compounds **7a** and **7b** were determined through X-ray diffraction, in agreement with Flack and Bernardinelli.[14] ORTEP-3[15] representations are shown in Figure 2. The bond distances and angles are within normally expected ranges.

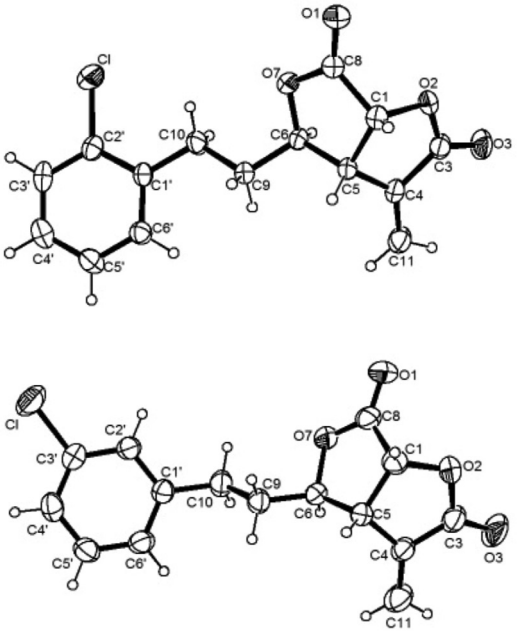


Figure 2. *ORTEP-3 drawings of molecules* **7a** *(top) and* **7b** *(bottom), with the atom numbering schemes. The displacement ellipsoids are drawn at the 30% probability levels*

As a result of the different substituents and of the interactions present in the structures of **7a**, **7b** and **7d**,[9] when compared with avenaciolide **7e**,[5] some torsion angles have important differences, as shown in Table 1.

Table 1. Some torsion angles measured for **7a** and **7b** in comparison with **7d** and **7e**

Torsion angles	7e [8]	7d [14]	7a	7b
O1-C8-O7-C6	175.89	172.3(3)	173.5(2)	178.1(3)
O7-C6-C5-C4	-130.0	-96.6(3)	-130.5(2)	-108.4(2)
O3-C3-C4-C5	169.8	172.4(4)	-173.6(3)	175.4(3)
O3-C3-C4-C11	11.28	-10.3(1)	9.8(5)	5.3(5)
C3-C4-C5-C6	89.2	127.3(3)	94.8(2)	109.9(2)
O7-C6-C9-C10	64.6	64.1(3)	55.6(3)	63.9(3)
C6-C9-C10-C1'	------	171.5(3)	-176.4(2)	177.4(2)

For both compounds the chlorine atom is in the same plane defined by the aromatic ring [0.043(4) Å and 0.007(4) Å for compounds **7a** and **7b** respectively]. Comparing the compounds **7a**, **7b** and **7d**[9] it is observed that the position of the chlorine atom does not significantly change the lengths and angles of the chlorophenyl substituent. On the other hand compounds **7a**-**7d** show different conformations with respect to the *bis*-γ-lactone skeleton. In the compound **7a** the two rings are in twist conformation and in the compound **7b** they are in planar conformation. In the analogue **7d**, the lactone ring containing the chlorophenyl substituent has a twist conformation[9] and in the avenaciolide (**7e**) this ring has an envelope conformation.[5] The other ring of the *bis*-γ-lactone has an envelope conformation in compound **7d** and a twist conformation in **7e**.[5,9]

Compounds **7a** and **7b** show the same type of intramolecular interaction C-H…O involving the oxygen atom O-7 and the carbon atom C-10 with C…O distances of 2.926(3) and 2.928(4) Å respectively. In compound **7a** there is another intramolecular interaction, C-10-H-10a…Cl, with a C…Cl distance of 3.060(3) Å. This interaction occurs because the chlorine atom is in *ortho* position and affects the orientation of the aromatic group.

The crystal packing of **7a** has two intermolecular interactions (C-6-H-6…π and C-9-H-9b...O-1), where the acceptor π is a centroid generated by the aromatic ring[16] with donor-acceptor distances of 3.675(2) and 3.341(3) Å, respectively.

The crystal packing of compound **7b** shows four intermolecular interactions, one of the type C-H...O involving the oxygen atom O-7 and the carbon atom C-1 with C...O distance of 3.316 (4) Å, and three of the type C-X...π (C-3'-Cl...π1, C-8-O-1...π2 e C-3-O-3...π3) with donor-acceptor distances of 3.796(1), 3.787(3) and 3.785(4) Å, respectively. In this case the centroids acceptors π1, π2 and π3 correspond to the rings (O-2-C-1-C-5-C-4-C-3), (O-7-C-6-C-5-C-1-C-8) and (C-1'-C-2'-C-3'-C-4'-C-5'-C-6'), respectively.

Antifungal screening

Avenaciolide (**7e**) and the analogues **7a**-**7d** were tested against the plant pathogenic fungi *C. gloeosporioides* and *F. solani*. The test methodology was chosen in order to allow the use of very low amounts of substances and to provide a fast way to evaluate the antifungal potential of the compounds. Paper discs (6 mm) were dipped into the solutions of the compounds at 1000 and 3000 ppm in dichloromethane. The discs were removed from the solutions and after evaporation of the solvent, they were placed in the center of Petri dishes containing *C. gloeosporioides* or *F. solani* conidia mixed with the BDA media. The negative control discs were prepared in the same way, with solvent only and no activity was observed.

Table 2 shows the inhibition hales caused by compounds **7a**-**7e** after 48 h of incubation at 25 °C. These results showed that the effects of these *bis*-lactones are greater against *C. gloesporioides* than against *F. solani*.

Table 2. Antifungal activity (average inhibition hales in mm and standard deviations) of compounds **7a**-**7e** on *Colletotrichum gloeosporioides* and *Fusarium solani*, after 48 h of incubation at 25 °C (samples prepared from 1000 and 3000 ppm solutions)

Compound	C. gloeosporioides		F. solani	
	1000 ppm	3000 ppm	1000 ppm	3000 ppm
7a	12.1 ± 0.6	18.0± 0.9	8.9 ± 0.2	11.3 ± 0.3
7b	11.4 ± 0.4	19.9 ± 0.1	8.3 ± 0.2	10.4 ± 0.6
7c	12.1 ± 0.8	15.0 ± 0.6	7.7 ± 0.3	8.8 ± 0.3
7d	11.3 ± 0.8	19.8 ± 0.3	7.5 ± 0.5	10.1 ± 0.6
7e	20.2 ± 0.5	21.9 ± 0.2	10.0 ± 0.4	11.3 ± 0.3

It has been shown that other α-methylene-γ-lactones react rapidly with enzymes to form stable adducts, what explains at least in part their biological activity.[17] We had previously observed that the exocyclic double bond conjugated to the lactone carbonyl was necessary for the activity of similar avenaciolide analogues, including **7d**.[8,9] To confirm this hypothesis, compounds **6a**-**6c** were tested for their capacity to inhibit the growth of *C. gloeosporioides*, proving to be inactive, showing the same aspect of the negative control.

The small differences on the aromatic groups of compounds **7a**-**7d** did not cause great differences in their antifungal activities in the *in vitro* test employed (Table 2). In fact, the results for **7b** and **7d** (*meta*- and *para*-Cl) could not be differentiated with respect to *C. gloeosporioides* using the *Scott-Knott* test at 5% of probability.[18] Similarly the inhibition of **7b** and **7d** could not be differentiated at 3000 ppm with respect to *F. solani*, showing a small difference (*ca.* 10%) at the 1000 ppm dose, in favour of **7b**. All the chlorinated compounds were more active than the fluorinated analogue at 3000 ppm with respect to both fungi (14-24%).

Avenaciolide (**7e**) was more active against *C. gloeosporioides* than all the aromatic compounds, indicating that the nature of the side chain is of some importance for their antifungal activity. The difference in the activity decreases with the dose (*ca.* 42% at 1000 ppm to 17% at 3000 ppm). These alterations were less expressive with respect to *F. solani* (Table 2).

EXPERIMENTAL

General

Diacetone-D-glucose (**1**) and the benzyl halides (2-chlorobenzyl bromide, 3-chlorobenzyl bromide and 4-fluorobenzyl chloride) were purchased from Aldrich. Aldehyde **2** was prepared from **1** as described in the literature.[13] Solvents were distilled before use and dried according to standard procedures. Melting points are uncorrected and were obtained on a MQAPF-301 apparatus (Microquimica, Brazil). Optical rotations were obtained with a Bellingham+Stanley Model D polarimeter and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Microanalyses were performed in a Perkin Elmer 2400 elemental analyzer. IR was performed in a Perkin Elmer Paragon 1000 spectrometer (4000-400 cm^{-1}) with samples on KBr pellets (when solids) or deposited as thin films on NaCl plates (when oils). NMR spectra were recorded in deuterochloroform ($CDCl_3$) using a Bruker DRX 400 Avance or a Bruker DPX Avance 200 spectrometers. Chemical shifts δ are given in ppm rel. to TMS as internal standard, and coupling constants *J*, in Hz. The attributions of signals in NMR spectra of the new compounds (**3** to **7**) were supported by 2D experiments (COSY, HSQC and HMBC contour maps).

Preparation of 3a-3c

The appropriate aryl halide [2-chlorobenzyl bromide or 3-chlorobenzyl bromide (2.30 g, 11 mmol), or 4-fluorobenzyl chloride (2.10 g, 11 mmol)] was added to a stirring solution of triphenylphosphine (3.00 g, 11 mmol) in dry benzene (5 mL) at room temperature and under nitrogen atmosphere. The mixture was stirred under reflux for 6 h. The product was filtered, washed with diethyl ether and dried under reduced pressure yielding the corresponding phosphonium salt as a white solid [2-chlorobenzyltriphenylphosphonium bromide (**a**, 4.66 g, 89%), 3-chlorobenzyltriphenylphosphonium bromide (**b**, 4.35 g, 83%) and 4-fluorobenzyltriphenylphosphonium chloride (**c**, 3.99 g, 80%)].

Butyllithium (2.5 mol L^{-1} in hexane, 3.4 mL) was added to a stirring solution of the Wittig salt (**a** and **b**: 4.07 g, 8.7 mmol; or **c**: 3.93 g, 8.7 mmol) in dry tetrahydrofuran (THF; 30 mL) under nitrogen atmosphere. The mixture was stirred for 10 min previous to the addition of a solution of aldehyde **2** [1.80 g (7.4 mmol)] in dry THF (5 mL). After 18 h stirring at room temperature, the mixture was concentrated under reduced pressure, water (25 mL) was added and extractions were performed with diethyl ether (5 x 35 mL). The organic phase was dried over $MgSO_4$, concentrated and submitted to column chromatography on silica gel using 3:1 (*v/v*) hexane/ethyl acetate as eluants, yielding the mixtures of isomers **3a** (2.05 g, 79%), **3b** (2.21 g, 85%), or **3c** (1.56 g, 63%).

*Methyl (2'R,3'R,4'R,5'R)-2-[(Z)-2'-(2-chlorophenyl)vinyl-4',5'-isopropylidenedioxytetra-hydrofuran-3'-yl]acetate and methyl (2'R,3'R,4'R,5'R)-2-[(E)-2'-(2-chlorophenyl)vinyl-4',5'-isopropylidenedioxytetrahydrofuran-3'-yl]acetate (**3a**)*

White crystals; m.p. 85.4-87.5 ºC; IR ν_{max}(KBr)/cm^{-1} 3078, 3056, 3016, 2985, 2962, 2931, 2854, 1731, 1688, 1595, 1552, 1439, 1384, 1373, 1327, 1252, 1212, 1167, 1078, 1136, 1026, 984, 964, 909, 872, 776, 762 and 740; ^{1}H NMR ($CDCl_3$, 400 MHz) – *Z* isomer: δ 1.29 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 2.17-2.31 (m, 1H, H-3'), 2.23 (dd, $J_{2a,2b}$ 17.4, $J_{2a,3'}$ 3.9, 1H, H-2a), 2.46 (dd, $J_{2b,2a}$ 17.4, $J_{2b,3'}$ 11.4, 1H, H-2b), 3.68 (s, 3H, OCH_3), 4.43 (t, $J_{2',3'}$ and $J_{2',6'}$ 9.6, 1H, H-2'), 4.77 (t, $J_{4',5'}$ and $J_{4',3'}$ 3.7, 1H, H-4'), 5.69 (dd, $J_{6',7'}$ 11.3, $J_{6',2'}$ 9.6, 1H, H-6'), 5.88 (d, $J_{5',4'}$ 3.7, 1H, H-5'), 6.89 (d, $J_{7',6'}$ 11.3, 1H, H-7'), 7.20-7.28 (m, 2H, H-3'' and H-5''), 7.33-7.42 (m, 1H, H-4'') and 7.48-7.52 (m, 1H, H-6''); *E* isomer: δ 1.35 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 2.17-2.31 (m, 1H, H-3'), 2.38-2.48 (m, 1H, H-2a), 2.72 (dd, $J_{2b,2a}$ 16.8, $J_{2b,3'}$ 9.9, 1H, H-2b), 3.65 (s, 3H, OCH_3), 4.36 (dd, $J_{2',3'}$ 10.1, $J_{2',6'}$ 7.9, 1H, H-2'), 4.83 (t, $J_{4',5'}$ and $J_{4',3'}$ 3.8, 1H, H-4'), 5.91 (d, $J_{5',4'}$ 3.8, 1H, H-5'), 6.06 (dd, $J_{6',7'}$ 15.8, $J_{6',2'}$ 7.9, 1H, H-6'), 7.03 (d, $J_{7',6'}$ 15.8, 1H, H-7'), 7.20-7.28 (m, 2H, H-3'' and H-5''), 7.33-7.42 (m, 1H, H-4'') and 7.48-7.52 (m, 1H, H-6''); ^{13}C NMR ($CDCl_3$, 100 MHz) – *Z* isomer: δ 26.5 ($2CH_3$), 28.9 (C-2), 46.3 (C-3'), 51.8 (OCH_3), 76.2 (C-2'), 80.8 (C-4'), 105.0 (C-5'), 111.8 ($C(CH_3)_2$), 126.5 (C-5''), 129.2 (C-6' and C-3''), 129.4 (C-4''), 130.6 (C-6''), 133.7 (C-2''), 133.9 (C-7'), 134.4 (C-1'') and 172.5 (C-1); *E* isomer: δ 26.3 ($2CH_3$), 29.0 (C-2), 45.9 (C-3'), 51.8 (OCH_3), 80.7 (C-4'), 81.8 (C-2'), 105.0 (C-5'), 111.8 ($C(CH_3)_2$), 129.1 (C-6' and C-5''), 130.4 (C-7' and C-6''), 133.3 (C-2'') and 134.3 (C-1''). Calculated for $C_{18}H_{21}ClO_5$: C, 61.3; H, 6.0; Found: C, 61.4; H, 6.1.

*Methyl (2'R,3'R,4'R,5'R)-2-[(Z)-2'-(3-chlorophenyl)vinyl-4',5'-isopropylidenedioxytetra-hydrofuran-3'-yl]acetate and methyl (2'R,3'R,4'R,5'R)-2-[(E)-2'-(3-chlorophenyl)vinyl-4',5'-isopropylidenedioxytetrahydrofuran-3'-yl]acetate (**3b**)*

White crystals; m.p. 55.3-58.4 ºC; IR ν_{max}(KBr)/cm^{-1} 3076, 3017, 2990, 2960, 1734, 1683, 1593, 1565, 1475, 1437, 1385, 1331, 1209, 1171, 1074, 1022, 873, 797 and 687; ^{1}H NMR ($CDCl_3$, 400 MHz) – *Z* isomer: δ 1.34 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.22-2.31 (m, 2H, H-2a and H-3'), 2.56 (dd, $J_{2b,2a}$ 17.7, $J_{2b,3'}$ 11.0, 1H, H-2b), 3.69 (s, 3H, OCH_3), 4.59 (t, $J_{2',3'}$ and $J_{2',6'}$ 9.6, 1H, H-2'), 4.81 (t, $J_{4',5'}$ and $J_{4',3'}$ 3.7, 1H, H-4'), 5.65 (dd, $J_{6',7'}$ 11.4, $J_{6',2'}$ 9.6, 1H, H-6'), 5.92 (d, $J_{5',4'}$ 3.7, 1H, H-5'), 6.76 (d, $J_{7',6'}$ 11.4, 1H, H-7'), 7.25-7.29 (m, 3H, H-4'', H-5'' and H-6'') and 7.41 (s, 1H, H-2''); *E* isomer: δ 1.36 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 2.22-2.31 (m, 1H, H-3'), 2.38 (dd, $J_{2a,2b}$ 16.8, $J_{2a,3'}$ 4.6, 1H, H-2a), 2.71 (dd, $J_{2b,2a}$ 16.8, $J_{2b,3'}$ 10.0, 1H, H-2b), 3.66 (s, 3H, OCH_3), 4.85 (t, $J_{4',5'}$ and $J_{4',3'}$ 3.9, 1H, H-4'), 6.11 (dd, $J_{6',7'}$ 15.6, $J_{6',2'}$ 7.8, 1H, H-6'), 6.61 (d, $J_{7',6'}$ 14.6, 1H, H-7'), 7.25-7.29 (m, 3H, H-4'', H-5'' and H-6'') and 7.41 (s, 1H, H-2''); ^{13}C NMR ($CDCl_3$, 100 MHz, major product *Z*) δ 26.5 ($2CH_3$), 29.0 (C-2), 46.5 (C-3'), 51.8 (OCH_3), 75.7 (C-2'), 80.8 (C-4'), 104.9 (C-5'), 111.9 ($C(CH_3)_2$), 127.0 (C-6''), 127.8 (C-6'), 128.7 (C-2''), 128.8 (C-4''), 129.5 (C-5''), 134.3 (C-3''), 135.5 (C-7'), 137.6 (C-1'') and 172.4 (C-1). Calculated for $C_{18}H_{21}ClO_5$: C, 61.3; H, 6.0. Found: C, 60.85; H, 5.9.

*Methyl (2'R,3'R,4'R,5'R)-2-[(Z)-2'-(4-fluorophenyl)vinyl-4',5'-isopropylidenedioxytetra-hydrofuran-3'-yl]acetate and methyl (2'R,3'R,4'R,5'R)-2-[(E)-2'-(4-fluorophenyl)vinyl-4',5'-isopropylidenedioxytetrahydrofuran-3'-yl}acetate (**3c**)*

White crystals; m.p. 101.8-104.7 ºC; IR ν_{max}(KBr)/cm^{-1} 3070, 2989, 2942, 1735, 1601, 1509, 1436, 1385, 1374, 1329, 1209, 1171, 1024 and 845; ^{1}H NMR ($CDCl_3$, 200 MHz) – *Z* isomer: δ 1.32 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.18-2.32 (m, 2H, H-2a and H-3'), 2.54 (dd, $J_{2b,2a}$ 17.5, $J_{2b,3'}$ 11.1, 1H, H-2b), 3.68 (s, 3H, OCH_3), 4.54 (t, $J_{2',3'}$ and

$J_{2',6'}$ 9.5, 1H, H-2'), 4.80 (t, $J_{4',5'}$ and $J_{4',3'}$ 3.8, 1H, H-4'), 5.57 (dd, $J_{6',7'}$ 11.3, $J_{6',2'}$ 9.5, 1H, H-6'), 5.90 (d, $J_{5',4'}$ 3.8, 1H, H-5'), 6.77 (d, $J_{7',6'}$ 11.3, 1H, H-7'), 6.98-7.09 (m, 2H, H-3'' and H-5'') and 7.31-7.40 (s, 2H, H-2'' and H-6''); *E* isomer: δ 3.64 (s, 3H, OCH_3), 5.98 (dd, $J_{6',7'}$ 16.0, $J_{6',2'}$ 9.5, 1H, H-6') and 6.61 (d, $J_{7',6'}$ 16.0, 1H, H-7'); ^{13}C NMR ($CDCl_3$, 50 MHz, major product *Z*) δ 26.5 ($2CH_3$), 29.1 (C-2), 46.5 (C-3'), 51.8 (OCH_3), 75.8 (C-2'), 80.8 (C-4'), 105.0 (C-5'), 111.7 ($C(CH_3)_2$), 115.3 (d, $^2J_{C,F}$ 21.3, C-3'' and C-5''), 127.5 (C-6'), 130.6 (d, $^3J_{C,F}$ 8.0, C-2'' and C-6''), 132.0 (d, $^4J_{C,F}$ 3.4, C-1''), 134.7 (C-7'), 162.3 (d, $^1J_{C,F}$ 246.0, C-4'') and 172.4 (C-1). Calculated for $C_{18}H_{21}FO_5$: C, 64.3; H, 6.3. Found: C, 63.8; H, 6.4.

Preparation of 4a-4c

To a solution of **3a** (1.44 g, 4.1 mmol), **3b** (2.15 g; 6.1 mmol), or **3c** (1.40 g; 4.2 mmol) in ethyl acetate (200 mL for **3a** and **3c**, and 250 mL for **3b**) were added Pd/C 10% (60 mg for **3a** and **3c**, and 258 mg for **3b**). The suspension was shaken under hydrogen atmosphere for 20 h at room temperature and atmospheric pressure. The mixture was filtered and the solvent was removed under reduced pressure yielding the esters **4a** (1.41 g, 97%), **4b** (1.90 g, 88%) and **4c** (1.22 g, 86%), respectively.

*Methyl (2'R,3'R,4'R,5'R)-2-[2'-(2-chlorophenyl)ethyl-4',5'-isopropylidenedioxytetrahydro-furan-3'-yl]acetate (**4a**)*

Colourless oil; $[\alpha]_D^{27}$ +241.7 (*c* 1.20, CH_2Cl_2); IR ν_{max}(film)/cm^{-1} 3068, 2987, 2951, 2868, 1738, 1600, 1572, 1475, 1437, 1381, 1373, 1215, 1167, 1016, 875 and 755; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.64-1.77 (m, 1H, H-6'a), 1.81-1.96 (m, 1H, H-6'b), 2.05-2.17 (m, 1H, H-3'), 2.30 (dd, $J_{2a,2b}$ 16.9, $J_{2a,3'}$ 4.2, 1H, H-2a), 2.64 (dd, $J_{2b,2a}$ 16.9, $J_{2b,3'}$ 10.2, 1H, H-2b), 2.76-2.84 (m, 1H, H-7'a), 2.95-3.03 (m, 1H, H-7'b), 3.69 (s, 3H, OCH_3), 3.74-3.81 (m, 1H, H-2'), 4.77 (t, $J_{4',5'}$ and $J_{4',3'}$ 4.0, 1H, H-4'), 5.85 (d, $J_{5',4'}$ 4.0, 1H, H-5'), 7.13-7.17 (m, 2H, H-3'' and H-5''), 7.23-7.26 (m, 1H, H-6'') and 7.31-7.34 (m, 1H, H-4''); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.4 (CH_3), 26.5 (CH_3), 29.5 (C-2), 30.2 (C-7'), 32.3 (C-6'), 44.7 (C-3'), 51.8 (OCH_3), 79.5 (C-2'), 81.1 (C-4'), 104.7 (C-5'), 111.4 ($C(CH_3)_2$), 126.8 (C-5''), 127.5 (C-3''), 129.5 (C-4''), 130.7 (C-6''), 133.9 (C-2''), 139.3 (C-1'') and 172.6 (C-1). Calculated for $C_{18}H_{23}ClO_5$: C, 60.9; H, 6.5. Found: C, 61.0; H, 6.7.

*Methyl (2'R,3'R,4'R,5'R)-2-[2'-(3-chlorophenyl)ethyl-4',5'-isopropylidenedioxytetrahydro-furan-3'-yl]acetate (**4b**)*

White crystals; m.p. 55.6-58.1 ºC; $[\alpha]_D^{26}$ +62.3 (*c* 0.70, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 2986, 2952, 2862, 1737, 1598, 1560, 1435, 1373, 1334, 1210, 1139, 1041, 1018, 896, 786 and 700; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.64-1.76 (m, 1H, H-6'a), 1.77-1.90 (m, 1H, H-6'b), 2.04-2.17 (m, 1H, H-3'), 2.28 (dd, $J_{2a,2b}$ 17.0, $J_{2a,3'}$ 4.5, 1H, H-2a), 2.59-2.72 (m, 1H, H-7'a), 2.64 (dd, $J_{2b,2a}$ 17.0, $J_{2b,3'}$ 7.2, 1H, H-2b), 2.81-2.90 (m, 1H, H-7'b), 3.69 (s, 3H, OCH_3), 3.72-3.81 (m, 1H, H-2'), 4.76 (t, $J_{4',5'}$ and $J_{4',3'}$ 4.0, 1H, H-4'), 5.84 (d, $J_{5',4'}$ 4.0, 1H, H-5') and 7.05-7.26 (m, 4H, H-2'', H-4'', H-5'' and H-6''); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.9 (CH_3), 27.0 (CH_3), 29.9 (C-2), 32.4 (C-7'), 34.6 (C-6'), 45.2 (C-3'), 52.3 (OCH_3), 79.5 (C-2'), 81.4 (C-4'), 104.8 (C-5'), 111.6 ($C(CH_3)_2$), 126.2 (C-6''), 126.8 (C-4''), 128.7 (C-2''), 134.2 (C-3''), 143.9 (C-1'') and 172.5 (C-1). Calculated for $C_{18}H_{23}ClO_5$: C, 61.2; H, 6.5. Found: C, 61.0; H, 6.5.

*Methyl (2'R,3'R,4'R,5'R)-2-[2'-(4-fluorophenyl)ethyl-4',5'-isopropylidenedioxytetrahydro-furan-3'-yl]acetate (**4c**)*

White crystals; m.p. 79.5-81.5 ºC; $[\alpha]_D^{23}$ +211.4 (*c* 1.23, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 3038, 2984, 2942, 2884, 1735, 1598, 1509, 1439, 1386, 1373, 1220, 1011, 879 and 831; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.32 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.61-1.89 (m, 2H, H-6'a and H-6'b), 2.01-2.16 (m, 1H, H-3'), 2.27 (dd, $J_{2a,2b}$ 16.9, $J_{2a,3'}$ 4.2, 1H, H-2a), 2.63 (dd, $J_{2b,2a}$ 16.9, $J_{2b,3'}$ 10.0, 1H, H-2b), 2.74-2.92 (m, 1H, H-7'a and H-7'b), 3.69 (s, 3H, OCH_3), 3.69-3.81 (m, 1H, H-2'), 4.76 (t, $J_{4',5'}$ and $J_{4',3'}$ 4.0, 1H, H-4'), 5.84 (d, $J_{5',4'}$ 4.0, 1H, H-5'), 6.91-6.99 (m, 2H, H-3'' and H-5'') and 7.11-7.18 (m, 2H, H-2'' and H-6''); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.4 (CH_3), 26.5 (CH_3), 29.4 (C-2), 31.4 (C-7'), 34.5 (C-6'), 44.8 (C-3'), 51.8 (OCH_3), 79.2 (C-2'), 81.1 (C-4'), 104.6 (C-5'), 111.4 ($C(CH_3)_2$), 115.1 (d, $^2J_{C,F}$ 21.0, C-3'' and C-5''), 129.8 (d, $^3J_{C,F}$ 7.7, C-2'' and C-6''), 137.4 (d, $^4J_{C,F}$ 3.2, C-1''), 161.3 (d, $^1J_{C,F}$ 241.8, C-4'') and 172.6 (C-1). Calculated for $C_{18}H_{23}FO_5$: C, 63.9; H, 6.85. Found: C, 64.1; H, 6.9.

Preparation of 5a-5c

To a stirring solution of the esters **4a** (1.30 g, 3.7 mmol), **4b** (1.80 g, 5.1 mmol) or **4c** (1.12 g, 3.3 mmol) in 1,4-dioxane (60 mL for **4a**, 80 mL for **4b** and 50 mL for **4c**) was added an aqueous solution of H_2SO_4 2% (*v/v*) (25 mL for **4a**, 35 mL for **4b** and 23 mL for **4c**). The mixture was stirred under reflux for 3 h. The product was extracted with diethyl ether (350 mL). The organic phase was washed with distilled water (45 mL) and with saturated $NaHCO_3$ aqueous solution (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography with silica gel using 1:1 (*v/v*) hexane/ethyl acetate as eluants, yielding the mixtures of epimers **5a** (0.86 g, 79%), **5b** (1.23 g, 86%), and **5c** (0.75 g, 85%), respectively.

*(1R,5R,6R,8R)-6-[2-(2-chlorophenyl)ethyl)-8-hydroxy-2,7-dioxabicyclo[3.3.0]octan-3-one and (1R,5R,6R,8S)-6-[2-(2-chlorophenyl)ethyl]-8-hydroxy-2,7-dioxabicyclo[3.3.0]octan-3-one (**5a**)*

Colourless oil; IR ν_{max}(film)/cm^{-1} 3413, 3062, 2937, 2864, 1781, 1571, 1475, 1167, 1079, 1050, 971 and 755; ^1H NMR ($CDCl_3$, 400 MHz) - β epimer: δ 1.90-2.00 (m, 1H, H-9a), 2.03-2.16 (m, 1H, H-9b), 2.44 (dd, $J_{4a,4b}$ 18.0, $J_{4a,5}$ 2.0, 1H, H-4a), 2.71-2.84 (m, 2H, H-4b and H-10a), 2.87-2.98 (m, 2H, H-5 and H-10b), 3.35 (br, s, 1H, OH), 3.97 (dt, $J_{6,5}$ 8.2, $J_{6,9a}$ and $J_{6,9b}$ 5.0, 1H, H-6), 4.87-4.92 (m, 1H, H-1), 5.57 (s, 1H, H-8), 7.13-7.24 (m, 3H, H-3', H-5' and H-6') and 7.26-7.35 (m, 1H, H-4'); α epimer: δ 1.90-2.00 (m, 1H, H-9a), 2.40 (dd, $J_{4a,4b}$ 18.0, $J_{4a,5}$ 1.8, 1H, H-4a), 2.44-2.54 (m, 1H, H-9b), 2.64-2.78 (m, 1H, H-5), 2.71-2.84 (m, 2H, H-4b and H-10a), 2.87-2.98 (m, 1H, H-10b), 3.55 (br, s, 1H, OH), 4.02-4.06 (m, 1H, H-6), 4.87-4.92 (m, 1H, H-1), 5.56 (d, $J_{8,1}$ 3.6, 1H, H-8), 7.13-7.24 (m, 3H, H-3', H-5' and H-6') and 7.26-7.35 (m, 1H, H-4'); ^{13}C NMR ($CDCl_3$, 100 MHz) - β epimer: δ 30.2 (C-10), 34.0 (C-4), 37.5 (C-9), 42.6 (C-5), 87.4 (C-6), 88.3 (C-1), 101.1 (C-8), 127.0 (C-5'), 127.7 (C-3'), 129.6 (C-4'), 130.5 (C-6'), 133.8 (C-2'), 138.7 (C-1') and 175.8 (C-3); α epimer: δ 29.8 (C-10), 33.4 (C-4), 34.6 (C-9), 42.0 (C-5), 82.2 (C-1), 82.7 (C-6), 95.7 (C-8), 127.0 (C-5'), 127.8 (C-3'), 129.6 (C-4'), 130.5 (C-6'), 133.8 (C-2'), 138.6 (C-1') and 176.7 (C-3). Calculated for $C_{14}H_{15}ClO_4$: C, 59.5; H, 5.35. Found: C, 59.2; H, 5.1.

*(1R,5R,6R,8R)-6-[2-(3-chlorophenyl)ethyl)-8-hydroxy-2,7-dioxabicyclo[3.3.0]octan-3-one and (1R,5R,6R,8S)-6-[2-(3-chlorophenyl)ethyl]-8-hydroxy-2,7-dioxabicyclo[3.3.0]octan-3-one (**5b**)*

White crystals; m.p. 80.7-83.7 ºC; IR ν_{max}(KBr)/cm^{-1} 3429, 3027, 2923, 2854, 1755, 1595, 1572, 1474, 1447, 1414, 1181, 1060, 1047, 973, 907, 775 and 615; ^1H NMR ($CDCl_3$, 400 MHz) - β epimer: δ 1.89-1.98 (m, 1H, H-9a), 2.06-2.15 (m, 1H, H-9b), 2.44 (dd, $J_{4a,4b}$ 18.0, $J_{4a,5}$ 1.8, 1H, H-4a), 2.63-2.72 (m, 2H, H-4b and H-10a), 2.75-2.85 (m, 1H, H-10b), 2.82 (dd, $J_{4b,4a}$ 18.0, $J_{4b,5}$ 9.1, 1H, H-4b), 2.91-2.96 (m, 1H, H-5), 3.33 (br, s, 1H, OH), 3.95 (dt, $J_{6,5}$ 8.7, $J_{6,9a}$ and $J_{6,9b}$ 5.0,

1H, H-6), 4.91 (d, $J_{1,5}$ 6.3, 1H, H-1), 5.59 (s, 1H, 8-H), 7.06-7.09 (m, 1H, H-4') and 7.18-7.26 (m, 3H, H-2', H-5' and H-6'); α epimer: δ 1.89-1.98 (m, 2H, H-9a and H-9b), 2.42 (dd, $J_{4a,4b}$ 18.0, $J_{4a,5}$ 1.8, 1H, H-4a), 2.63-2.72 (m, 1H, H-10a), 2.75-2.85 (m, 2H, H-5 and H-10b), 3.59 (br, s, 1H, OH), 3.97-4.04 (m, 1H, H-6), 4.89-4.93 (m, 1H, H-1), 5.56 (d, $J_{8,1}$ 4.0, 1H, H-8), 7.06-7.09 (m, 1H, H-4'), 7.18-7.26 (m, 2H, H-2' and H-6') and 7.29-7.33 (m, 1H, H-5'); ^{13}C NMR ($CDCl_3$, 100 MHz) - β epimer: δ 31.9 (C-10), 33.9 (C-4), 39.0 (C-9), 42.6 (C-5), 87.1 (C-6), 88.2 (C-1), 101.0 (C-8), 126.3 (C-6'), 126.5 (C-4'), 128.5 (C-2'), 129.8 (C-5'), 134.2 (C-3'), 143.1 (C-1') and 175.7 (C-3); α epimer: δ 31.6 (C-10), 33.2 (C-4), 36.2 (C-9), 42.0 (C-5), 82.1 (C-1), 82.4 (C-6), 95.6 (C-8), 126.4 (C-6'), 126.6 (C-4'), 128.4 (C-2'), 129.8 (C-5'), 134.2 (C-3'), 142.9 (C-1') and 176.6 (C-3). Calculated for $C_{14}H_{15}ClO_4$: C, 59.5; H, 5.35. Found: C, 59.6; H, 5.5.

*(1R,5R,6R,8R)-6-[2-(4-fluorophenyl)ethyl)-8-hydroxy-2,7-dio-xabicyclo[3.3.0]octan-3-one and (1R,5R,6R,8S)-6-[2-(4-fluorophenyl) ethyl]-8-hydroxy-2,7-dioxabicyclo[3.3.0]octan-3-one (**5c**)*

White solid; m.p. 84.5-87.0 ºC; IR ν_{max}(film)/cm^{-1} 3412, 2937, 2870, 1781, 1601, 1509, 1471, 1293, 1220, 1159, 1079, 1047 and 835; ^{1}H NMR ($CDCl_3$, 200 MHz) - β epimer: δ 1.75-2.00 (m, 1H, H-9a), 2.03-2.14 (m, 1H, H-9b), 2.37-2.49 (m, 1H, H-4a), 2.57-2.82 (m, 3H, H-4b, H-10a and H-10b), 2.86-2.98 (m, 1H, H-5), 3.18 (br, s, 1H, OH), 3.93 (dt, $J_{6,5}$ 8.4, $J_{6,9a}$ and $J_{6,9b}$ 5.0, 1H, H-6), 4.89 (d, $J_{1,5}$ 6.1, 1H, H-1), 5.57 (s, 1H, H-8), 6.91-7.03 (m, 2H, H-3' and H-5') and 7.10-7.17 (m, 2H, H-2' and H-6'); α epimer: δ 1.75-2.00 (m, 1H, H-9a), 2.37-2.49 (m, 1H, H-4a), 2.57-2.82 (m, 5H, H-4b, H-5, H-9b, H-10a and H-10b), 3.50 (br, s, 1H, OH), 3.88-4.04 (m, 1H, H-6), 4.88-4.94 (m, 1H, H-1), 5.50-5.60 (m, 1H, H-8), 6.91-7.03 (m, 2H, H-3' and H-5') and 7.10-7.17 (m, 2H, H-2' and H-6') ^{13}C NMR ($CDCl_3$, 50 MHz) - β epimer: δ 31.5 (C-10), 33.9 (C-4), 39.4 (C-9), 42.6 (C-5), 87.1 (C-6), 88.3 (C-1), 101.0 (C-8), 115.3 (d, $^2J_{C,F}$ 21.0, C-3' and C-5'), 129.7 (d, $^3J_{C,F}$ 7.8, C-2' and C-6'), 136.6 (d, $^4J_{C,F}$ 3.5, C-1'), 161.4 (d, $^1J_{C,F}$ 242.4, C-4'), 175.7 (C-3); α epimer: δ 31.2 (C-10), 33.3 (C-4), 36.6 (C-9), 42.1 (C-5), 82.2 (C-1), 82.4 (C-6), 95.7 (C-8) and 176.5 (C-3). Calculated for $C_{14}H_{15}FO_4$: C, 63.15; H, 5.7. Found: C, 63.0; H, 5.7.

Preparation of 6a-6c

A solution of 2.67 g of CrO_3 in concentrated H_2SO_4 (2.3 mL) was added to 4.0 mL of distilled water. The solution volume was then completed with distilled water up to 10.0 mL. A portion this solution (Jones reagent; 1.9 mL for **5a**, 1.1 mL for **5b** and 1.7 mL for **5c**) was added to a stirring solution of the compounds **5a** (0.75 g, 2.7 mmol), **5b** (0.45 g, 1.6 mmol) or **5c** (0.63 g, 2.4 mmol) in acetone (30 mL). After 5 min at room temperature, a second portion of the Jones reagent (1.9, 1.6 and 1.7 mL, respectively) was added, and the mixture was stirred for further 15 min, previous to the addition of methanol (30 mL). Distilled water (35 mL) was added and the product was extracted with diethyl ether (4 x 40 mL). The organic phase was washed with $NaHCO_3$ saturated solution (40 mL), dried over Na_2SO_4 and concentrated under reduced pressure to yield the *bis*-γ-lactones **6a** (0.53 g, 71%), **6b** (0.35 g, 78%) and **6c** (0.39 g, 62%), respectively.

*(1R,5R,6R)-6-[2-(2-chlorophenyl)ethyl]-2,7-dioxabicyclo-[3.3.0] octan-3,8-dione (**6a**)*

Colourless oil; $[\alpha]_D^{27}$ +119.3 (*c* 1.09, CH_2Cl_2); IR ν_{max}(film)/cm^{-1} 2937, 1782, 1572, 1475, 1445, 1245, 1217, 1145, 1075, 1058, 1005, 932 and 755; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96-2.11 (m, 2H, H-9a and H-9b), 2.53 (dd, $J_{4a,4b}$ 18.2, $J_{4a,5}$ 4.2, 1H, H-4a), 2.70-3.03 (m, 2H, H-10a and H-10b), 2.93 (dd, $J_{4b,4a}$ 18.2, $J_{4b,5}$ 9.4, 1H, H-4b), 3.05-3.13 (m, 1H, H-5), 4.28-4.40 (m, 1H, H-6), 5.06 (d, $J_{1,5}$ 7.9, 1H, H-1) and 7.17-7.36 (m, 4H, H-3', H-4', H-5' and H-6'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.3 (C-10), 32.7 (C-4), 35.3 (C-9), 40.0 (C-5), 76.8 (C-1), 83.9 (C-6), 127.2 (C-5'), 128.2 (C-3'), 129.8 (C-4'), 130.7 (C-6'), 133.8 (C-2'), 137.4 (C-1'), 169.7 (C-8) and 173.8 (C-3). Calculated for $C_{14}H_{13}ClO_4$: C, 59.9; H, 4.7. Found: C, 60.2; H, 4.9.

*(1R,5R,6R)-6-[2-(3-chlorophenyl)ethyl]-2,7-dioxabicyclo-[3.3.0] octan-3,8-dione (**6b**)*

White crystals; m.p. 99.5-101.4 ºC; $[\alpha]_D^{26}$ +16.5 (*c* 1.70, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 3026, 2923, 2864, 1790, 1784, 1598, 1573, 1477, 1363, 1245, 1218, 1078, 1059, 934 and 787; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.02-2.11 (m, 2H, H-9a and H-9b), 2.53 (dd, $J_{4a,4b}$ 18.3, $J_{4a,5}$ 4.2, 1H, H-4a), 2.74 (dt, $J_{10a,10b}$ 14.1, $J_{10a,9a}$ and $J_{10a,9b}$ 8.2, 1H, H-10a), 2.84-2.94 (m, 1H, H-10b), 2.93 (dd, $J_{4b,4a}$ 18.3, $J_{4b,5}$ 7.7, 1H, H-4b), 3.04-3.11 (m, 1H, H-5), 4.30-4.35 (m, 1H, H-6), 5.05 (d, $J_{1,5}$ 8.0, 1H, H-1), 7.08-7.11 (m, 1H, H-6') and 7.20-7.29 (m, 3H, H-2', H-4' and H-5'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.0 (C-10), 32.6 (C-4), 37.0 (C-9), 40.2 (C-5), 76.7 (C-1), 86.5 (C-6), 126.6 (C-6'), 126.8 (C-4'), 128.5 (C-2'), 130.0 (C-5'), 134.5 (C-3'), 141.6 (C-1'), 169.7 (C-8) and 173.4 (C-3). Calculated for $C_{14}H_{13}ClO_4$: C, 59.9; H, 4.7. Found: C, 60.1; H, 4.8.

*(1R,5R,6R)-6-[2-(4-fluorophenyl)ethyl]-2,7-dioxabicyclo-[3.3.0] octan-3,8-dione (**6c**)*

White crystals; m.p. 92.8-94.7 ºC; $[\alpha]_D^{23}$ +117.6 (*c* 1.19, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 3006, 2952, 2924, 2850, 1803, 1785, 1601, 1510, 1366, 1237, 1219, 1157, 1079, 973 and 821; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.96-2.07 (m, 2H, H-9a and H-9b), 2.50 (dd, $J_{4a,4b}$ 17.9, $J_{4a,5}$ 3.8, 1H, H-4a), 2.64-2.92 (m, 2H, H-10a and H-10b), 2.91 (dd, $J_{4b,4a}$ 17.9, $J_{4b,5}$ 9.4, 1H, H-4b), 2.98-3.12 (m, 1H, H-5), 4.26-4.35 (m, 1H, H-6), 5.03 (d, $J_{1,5}$ 7.7, 1H, H-1), 6.96-7.06 (m, 2H, H-3' and H-5') and 7.12-7.19 (m, 2H, H-2' and H-6'); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 29.7 (C-10), 32.7 (C-4), 37.4 (C-9), 40.2 (C-5), 76.8 (C-1), 83.6 (C-6), 115.6 (d, $^2J_{C,F}$ 21.2, C-3' and C-5'), 129.8 (d, $^3J_{C,F}$ 7.8, C-2' and C-6'), 135.3 (d, $^4J_{C,F}$ 3.3, C-1'), 161.6 (d, $^1J_{C,F}$ 243.3, C-4'), 169.8 (C-8) and 173.6 (C-3). Calculated for $C_{14}H_{13}FO_4$: C, 63.6; H, 5.0. Found: C, 63.4; H, 5.1.

Preparation of 7a-7c

A solution of methylmethoxymagnesium carbonate [MMC, 2.0 mol L^{-1} in dimethylformamide (DMF); 6.0, 6.7 and 6.0 mL, respectively] was added to the *bis*-γ-lactones **7a** (0.32 g, 1.1 mmol), **7b** (0.35 g, 1.2 mmol), or **7c** (0.30 g, 1.1 mmol) under nitrogen atmosphere. The mixture was stirred at 112 ºC for 5 h and then was poured over an ice-cold mixture of 6 mol L^{-1} HCl and diethyl ether (5+1 by volume, 21 mL) and stirred in order to dissolve the precipitate formed. The phases were separated and extractions with diethyl ether were performed (2 x 10 mL). The combined organic phases were washed with sodium chloride saturated solution (15 mL), dried over magnesium sulphate and concentrated under reduced pressure. To the yellow oil thus obtained it was added a mixture previously prepared of sodium acetate (106, 125 and 114 mg for **7a**, **7b** and **7c**, respectively), acetic acid (4.5 mL), formalin (3.3 mL) and diethylamine (1.2 mL). The reaction mixture was vigorously shaken for one minute and then heated on a stream bath for 5 min, cooled and poured into water (45 mL) and ether (30 mL). The organic phase was washed with water (15 mL), saturated $NaHCO_3$ aqueous solution (15 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The white solid obtained was purified by column chromatography on silica gel with 1:1 (*v/v*) hexane/ethyl acetate as eluants, yielding compounds **7a** (0.071 g, 22%), **7b** (0.13 g, 38%) and **7c** (0.055 g, 18%).

*(1R,5R,6R)-6-[2-(2-chlorophenyl)ethyl]-4-methylidene-2,7-dioxabicyclo[3.3.0]octan-3,8-dione (**7a**)*

White solid; m.p. 107.0-109.6 ºC; $[\alpha]_D^{27}$ +58.3 (*c* 1.03, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 3064, 2927, 2864, 1779, 1665, 1570, 1475, 1444, 1360, 1298, 1221, 1105, 1067, 1051, 962 and 756; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.99-2.25 (m, 2H, H-9a and H-9b), 2.77-3.07 (m, 2H, H-10a and H-10b), 3.58-3.69 (m, 1H, H-5), 4.34-4.48 (m, 1H, H-6), 5.10 (d, $J_{1,5}$ 8.5, 1H, H-1), 5.88 (d, $J_{11a,11b}$ 2.1, 1H, H-11a), 6.42 (d, $J_{11b,11a}$ 2.1, 1H, H-11b) and 7.14-7.39 (m, 4H, H-3', H-4', H-5' and H-6'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.4 (C-10), 35.8 (C-9), 44.2 (C-5), 74.4 (C-1), 84.4 (C-6), 126.7 (C-11), 127.4 (C-5'), 128.4 (C-3'), 129.9 (C-4'), 130.7 (C-6'), 133.9 (C-2'), 134.4 (C-4), 137.4 (C-1'), 167.7 (C-3) and 170.0 (C-8). Calculated for $C_{15}H_{13}ClO_4$: C, 61.55; H, 4.5. Found: C, 61.5; H, 4.6.

*(1R,5R,6R)-6-[2-(3-chlorophenyl)ethyl]-4-methylidene-2,7-dioxabicyclo[3.3.0]octan-3,8-dione (**7b**)*

Yellowish solid; m.p. 114.0-117.4 ºC; $[\alpha]_D^{26}$ +50.6 (*c* 1.60, CH_2Cl_2); IR ν_{max}(KBr)/cm^{-1} 3020, 2958, 2917, 2849, 1781, 1662, 1598, 1573, 1479, 1297, 1229, 1102, 1070, 963 and 796; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.03-2.16 (m, 2H, H-9a and H-9b), 2.72-2.91 (m, 2H, H-10a and H-10b), 3.57-3.61 (m, 1H, H-5), 4.38-4.42 (m, 1H, H-6), 5.08 (d, $J_{1,5}$ 8.5, 1H, H-1), 5.85 (d, $J_{11a,11b}$ 2.2, 1H, H-11a), 6.44 (d, $J_{11b,11a}$ 2.2, 1H, H-11b), 7.08-7.10 (m, 1H, H-6') and 7.20-7.27 (m, 3H, H-2', H-4' and H-5'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.0 (C-10), 37.6 (C-9), 44.2 (C-5), 74.1 (C-1), 84.0 (C-6), 126.5 (C-11), 126.6 (C-6'), 126.9 (C-4'), 128.5 (C-2'), 130.1 (C-5'), 134.2 (C-4), 134.5 (C-3'), 141.7 (C-1'), 167.7 (C-3) and 169.7 (C-8). Calculated for $C_{15}H_{13}ClO_4$: C, 61.55; H, 4.5. Found: C, 61.5; H, 4.8.

*(1R,5R,6R)-6-[2-(4-fluorophenyl)ethyl]-4-methylidene-2,7-dioxabicyclo[3.3.0]octan-3,8-dione (**7c**)*

Colourless oil; $[\alpha]_D^{23}$ +13.9 (*c* 0.72, CH_2Cl_2IR ν_{max}(film)/cm^{-1} 2927, 2864, 1777, 1665, 1601, 1509, 1454, 1296, 1266, 1218, 1158, 1101, 1066, 1048 and 825; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.03-2.12 (m, 2H, H-9a and H-9b), 2.73-2.91 (m, 2H, H-10a and H-10b), 3.54-3.59 (m, 1H, H-5), 4.40 (dt, $J_{6,5}$ 6.7, $J_{6,9a}$ and $J_{6,9b}$ 4.0, 1H, H-6), 5.07 (d, $J_{1,5}$ 8.4, 1H, H-1), 5.83 (d, $J_{11a,11b}$ 2.1, 1H, H-11a), 6.46 (d, $J_{11b,11a}$ 2.1, 1H, H-11b) and 6.99-7.03 (m, 2H, H-3' and H-5') and 7.15-7.18 (m, 2H, H-2' and H-6'); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 30.6 (C-10), 38.0 (C-9), 44.3 (C-5), 74.1 (C-1), 83.8 (C-6), 115.7 (d, $^2J_{C,F}$ 21.1, C-3' and C-5'), 126.5 (11-C) 129.8 (d, $^3J_{C,F}$ 7.8, C-2' and C-6'), 134.2 (C-4), 135.1 (d, $^4J_{C,F}$ 3.2, C-1'), 161.6 (d, $^1J_{C,F}$ 243.4, C-4'), 167.3 (C-3) and 169.5 (C-8); Calculated for $C_{15}H_{13}FO_4$: C, 65.2; H, 4.7. Found: C, 64.9; H, 4.8.

X-ray crystal structure determination of compounds 7a and 7b

Single crystals of compounds **7a** and **7b** were used for data collection on an Enraf-Nonius Kappa CCD diffractometer using graphite monochromatic Mo Kα radiation (λ = 0.71073 Å). Data collections were made using the Collect program.[19] The final unit cell parameters were based on all reflections. Integration and scaling of the reflections, correction for Lorentz and polarization effects were performed with the HKL Denzo-Scalepack system of programs.[20] Numerical absorption corrections were carried out using the Sortav program.[21]

The structures were solved by direct methods using Shelxs-97.[22] The models were refined by full-matrix least squares on F2 using Shelxl-97.[23] All the hydrogen atoms were stereochemically positioned and refined with the riding model.[23] Anisotropic displacement parameters were used for all non-H atoms. The absolute configurations of both compounds were established by anomalous dispersion effects. Experimental details are summarized in Table 1S, supplementary material. The program Ortep-3[19] was used for graphic representation and the program Wingx[24] to prepare materials for publication. Supplementary crystallographic data can be obtained from the Cambridge Crystallographic Data Centre.[25]

Antifungic assay

Three sterilized Blank paper disks (6 mm) were dipped into the solutions of the *bis*-γ-lactones **6a**-**6c** and **7a**-**7e** (1000 and 3000 mg L^{-1}) in CH_2Cl_2. After 5 min, the disks were removed and allowed to dry in a desiccator, at reduced pressure. The negative check treatment was prepared with solvent only. Each disk was placed in the center of a Petri dish containing *C. gloeosporioides* or *F. solani* conidia (3,4 x 10^5 conidia/mL), the antibiotic streptomicin (50 mg/100 mL) and potato dextrose agar medium (DIFCO). The distances from the center of the disks to the edge of the inhibition zone, observed with the aid of a stereoscopic microscope (Ken-a-vision), were measured after 48 h at 25 ºC. Compounds **6a**-**6c** were inactive. The Petri dishes containing the *bis*-γ-lactones **7a**-**7e** showed a transparent hale with the average diameters listed in Table 2.

CONCLUSIONS

Here we have described the synthesis and characterization of three new avenaciolide analogs, **7a**, **7b** and **7c**, with halogenated aromatic groups in the side chain of the bicyclic *bis*-γ-lactones. The results of the X-ray studies for compounds **7a** and **7b**, compared to the published data for **7d**[9] showed that the position of the chlorine atom in the aromatic ring affects the conformation of the rings of the *bis*-γ-lactone skeleton, the orientation of the aromatic group and the interactions present in the crystal packing. The halogenated compounds **7a**-**7d** and avenaciolide (**7e**) were active against *C. gloeosporioides* and *F. solani* at a very low dose, showing potential applications as agrochemicals. Avenaciolide was more potent, indicating the importance of the nature of the side chain for the activity. These compounds are more effective against *C. gloeosporioides* than *F. solani*. To further investigate the biological activities of this class of compounds and to evaluate their applicability as agrochemicals, other analogues are being prepared and tests of different methodologies, including *in vivo* experiments, will be carried out.

SUPPLEMENTARY MATERIAL

The Table 1S is available free of charge at the http://quimicanova.sbq.org.br, as PDF file.

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25. CCDC 755524 (for **7a**) and 755525 (for **7b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).