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Enantioselective syntheses of (*R*)- and (*S*)-argentilactone and their cytotoxic activities against cancer cell lines

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Abstract—Concise total syntheses of (*R*)- and (*S*)-argentilactone have been developed via enantioselective catalytic allylation (ECA) and ring-closing metathesis pathways (four steps, 39% overall yield and 82–84% ee) from 2-octynal and their in vitro activity against cancer cells is described.

1. Introduction

Natural, synthetic or semi-synthetic compounds may be used to block, reverse, or prevent the development of invasive cancers.[1,2] 6-Substituted derivatives of 5,6-dihydro-α-pyrones such as pironetin (**1**)[3–5] have been found to inhibit the cell cycle progression in the M phase, to exhibit cytotoxic activity such as callystatin A (**2**)[6,7] and spicigerolide (**3**),[8] to induce the apoptotic process such as goniothalamin (**4**)[9–11] or to be an anticancer agent such as fostriecin (**5**) (Fig. 1).[12–15]

Argentilactone (**6**) (Fig. 1) is a natural compound that displays a 5,6-dihydro-2*H*-pyran-2-one moiety and bears the (*R*)-configuration in its natural form. It was isolated from *Aristolochia argentina*

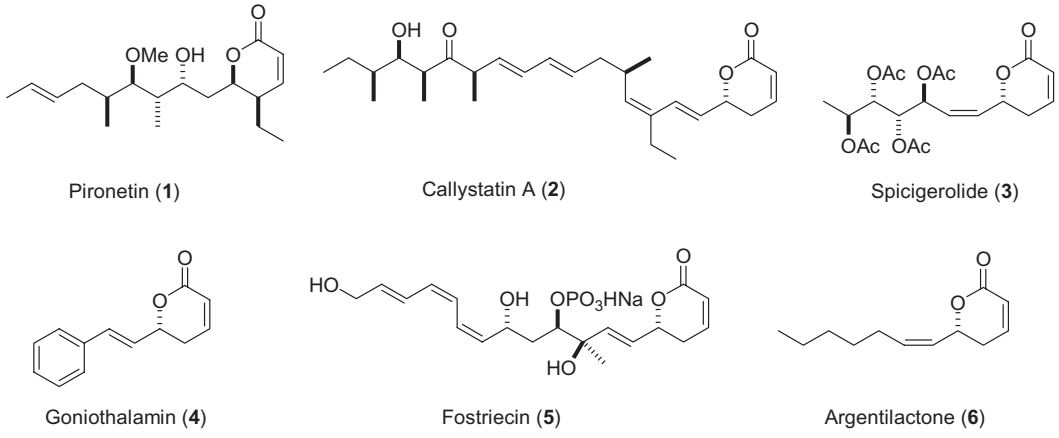

Figure 1. Some examples of 6-substituted natural 5,6-dihydro-pyrones.

Keywords: Argentilactone; Asymmetric synthesis; Cytotoxic activity; Cancer cell.
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(Aristolochiaceae),[16] *Chorisia crispflora* (Bombaceae)[17] and *Annona haematantha* (Annonaceae).[18] This natural pyranone was shown to have in vitro antiprotozoa activity against *Plasmodium falciparum* (ED_{50} = 0.5 μM),[19] *Leishmania panamensis* (ED_{50} = 51.5 μM),[19], and *Leishmania amazonensis* (ED_{50} = 51.5 μM),[18] as well as cytotoxic activity against leukemia cells (P-388, IC_{50} = 21.4 μM).[17]

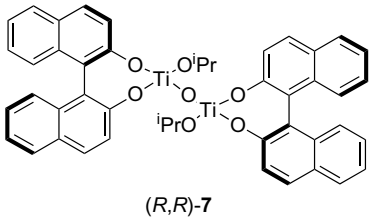
A wide variety of biological functions that emerges through molecular recognition requires strict matching of chirality. Enzymes and receptors sites in biological systems have the ability to distinguish between two enantiomers compounds through differential binding thus eliciting different biological response. The most dramatic example reported is thalidomide, where one enantiomer is a useful drug for prevention of morning sickness, whereas its antipode is highly teratogenic.[20] Inspired by the cytotoxic activity against P-388 leukemia cells reported for natural (*R*)-argentilactone [(*R*)-(**6**)], we decided to extend the evaluation of the cytotoxic activity of (*R*)- and (*S*)-argentilactone [(*R*)- and (*S*)-(**6**)] to other cancer cell lines. In order to successfully implement the study proposed, it was mandatory to provide a short and efficient access to chiral nonracemic argentilactone (**6**). In spite of the biological activities exhibited by (*R*)-argentilactone [(*R*)-(**6**)], the biological profile of its enantiomer, (*S*)-argentilactone [(*S*)-**6**], still remains unknown, particularly, regarding its antiproliferative activity.

Several syntheses of (*R*)-argentilactone [(*R*)-(**6**)][21–27] and its nonnatural enantiomer are reported.[28,29] Usually, the (*R*)-stereogenic center has been introduced from chiral starting material[21–24,26–28] or from asymmetric reduction using enzymes.[29] A noteworthy exception is the asymmetric allylboration of aldehydes with *B*-allyldiisopinocampheylborane developed by Brown and co-workers.[30–32] While high yield and enantiomeric excess are generally achieved, the use of a stoichiometric amount of the chiral allylborane is not the more efficient solution for the transfer of chirality. Catalytic asymmetric protocols are certainly the more attractive alternative to chiral nonracemic 6-substituted 5,6-dihydro-2*H*-pyran-2-ones.[33,34] Enantioselective catalytic allylation (ECA) is one of the fundamental powerful C–C bond-forming reactions that has attracted considerable attention in asymmetric synthesis.[35] This work provides a full account of our previously reported total synthesis of (*R*)-argentilactone [(*R*)-**6**], in four steps and 39% overall yield and 82–84% ee, featuring enantioselective catalytic allylation of 2-octynal (**8**)[36] and ring-closing metathesis as the key steps.[37] Moreover, we present for the first time the in vitro biological activity of (*S*)-argentilactone [(*S*)-(**6**)] against cancer cells and compare these results with those exhibited by its enantiomer, (*R*)-argentilactone [(*R*)-(**6**)].

2. Results and discussion

2.1. Chemistry

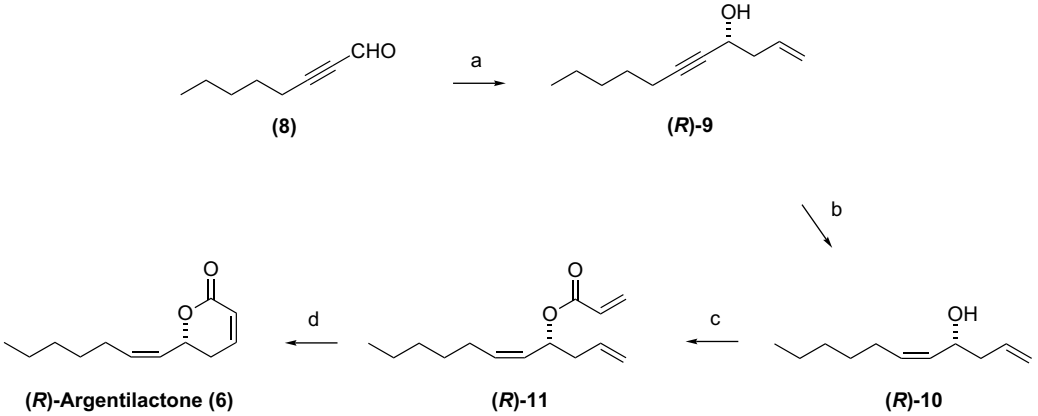
Maruoka and co-workers have developed a new class of highly reactive and selective titanium complexes for the allylation of aldehydes[38–40] featuring the μ-oxo bis(binaphthoxy)(isopropoxy)-titanium complex [(*R*, *R*)-**7**] (Fig. 2), which displays excellent enantioselectivity and yield for the addition of allyltributyltin to aldehydes.[40] The efficiency of this catalyst is proposed to be due to its simultaneous coordination and double activation ability.



(*R*,*R*)-**7**

Figure 2. μ-Oxo bis(binaphthoxy)(isopropoxy)titanium complex (*R*,*R*)-**7** developed by Maruoka and co-workers.[40]

Our approach (Scheme 1) to (*R*)-argentilactone [(*R*)-(**6**)] centered on the enantioselective addition of allyltributyl-



Scheme 1. (a) (*R*)-BINOL (10 mol%), $Ti(OiPr)_4$ (15 mol%), $TiCl_4$ (5 mol%), Ag_2O (10 mol%), allyltributyltin (1.1 equiv), CH_2Cl_2, −20 °C, 24 h (89%; 84% ee); (b) quinoline (2.0 equiv), Lindlar catalyst (10 mol%), EtOAc–1-octene (1:1) (74%); (c) acryloyl chloride (1.8 equiv), Et_3N (3.6 equiv), CH_2Cl_2, 0 °C (86%); (d) Grubbs' catalyst [$(PCy_3)_2Cl_2Ru{=}CHPh$] (10 mol%), CH_2Cl_2 (70%).

tin to 2-octynal (**8**) in CH_2Cl_2, which under the influence of in situ generated chiral (*R*,*R*)-**7** complex afforded **9** in 89% yield and 84% ee (see Section 4 for determination of enantiomeric excess). Despite the recent developments in the catalytic asymmetric allylation promoted by chiral Lewis acids, no successful example with propargylic aldehydes had been reported previously.[35] The conversion of propargylic alcohol **9** to (*Z*)-allylic alcohol **10** was accomplished by treatment of **9** with Lindlar catalyst and quinoline, in the presence of 1-octene as a cosolvent (1:1 v/v of EtOAc–1-octene), and H_2 (1 atm) for 30 min at room temperature, in 74% yield.[41] The absence of 1-octene in this step furnished a complex mixture of byproducts, containing mostly the saturated alcohol formed from reduction of allylic alcohol **10**. Acylation of **10** with acryloyl chloride provided ester **11** in 86% yield after purification by chromatography on silica gel. Finally, ring-closing metathesis of acrylate **11** took place with good yield under the catalysis of Grubb's ruthenium complex $PhCH{=}RuCl_2(PCy_3)_2$[42–44] and did not require the addition of $Ti(OiPr)_4$, as in other instances,[45,46] to afford (*R*)-argentilactone [(*R*)-(**6**)] in 70% yield. In summary, (*R*)-argentilactone [(*R*)-(**6**)] was efficiently obtained in 39% overall yield and 84% ee from 2-octynal providing sufficient amounts for biological evaluation. The same approach afforded (*S*)-argentilactone [(*S*)-**6**] when (*S*,*S*)-**7** was used as catalyst in the catalytic asymmetric allylation step.

2.2. Biological activities

Antiproliferative activity of (*R*)- and (*S*)-argentilactone [(*R*)- and (*S*)-(**6**)] was evaluated in the following human cancer cell lines: MCF-7 (breast), NCI-ADR (breast expressing the multidrug resistance phenotype), NCI 460 (lung, nonsmall cells), UACC62 (melanoma), 786-0 (kidney), OVCAR03 (ovarian), PCO 3 (prostate), and HT-29 (colon), which were grown in vitro (cell lines were kindly provided by Frederick Cancer Research & Development Center—National Cancer Institute—Frederick, MA, USA). Chemotherapic doxorubicin (DOX) was used as positive control.

Both enantiomers of argentilactone (**6**) displayed antiproliferative activity against the cancer cells tested in a concentration-dependent way. At 0.25 μg/mL, only (*S*)-(**6**) inhibited the growing of the cells tested according to the following increasing order: UACC62 (41%), MCF-7 (51%), NCI 460 (60%), HT-29 (63%), PCO 3 (68%), NCI-ADR (78%), and OVCAR03 (85%) (Fig. 3). On the other hand, at this concentration (*S*)-(**6**) showed to be cytotoxic against 786–0 cell line reducing in 34% the initial cell number after 48 h of treatment. In addition to this, (*S*)-(**6**) at 0.25 μg/mL was more efficient than doxorubicin (DOX) in inhibiting the growing of all cell lines studied (data not shown).

It is noteworthy that, although both enantiomers [(*R*)- and (*S*)-(**6**)] display roughly the same IC_{50} (μM) values for MCF-7, NCI 460, and PCO 3, (*R*)-(**6**) was considerably more potent toward NCI-ADR and (*S*)-(**6**) for UACC62 cancer cells (Table 1). Besides this, (*R*)-(**6**) showed to be four times more powerful than reference drug DOX on NCI-ADR cell line. In fact, both enantiomers of argentilactone (**6**) represent a promising carbon–carbon backbone for the development of drugs to be used as chemotherapics.

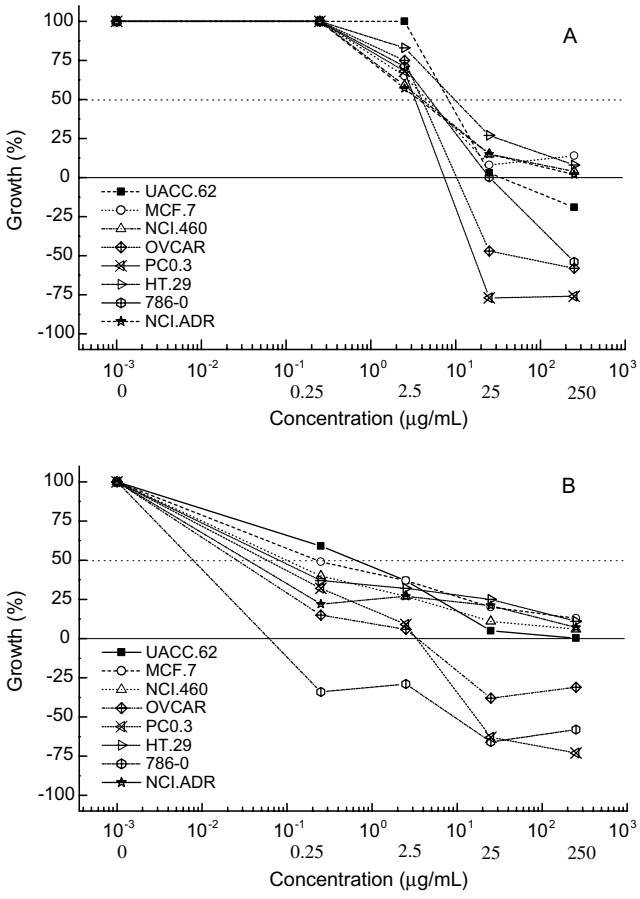


Figure 3. Percent growth of cancer cells for 48 h with different concentrations of (*R*)- or (*S*)-argentilactone. **A**, (*R*)-argentilactone; **B**, (*S*)-argentilactone. Positive values in relation to *y* axis correspond to cytostatic activity while the others refer to cytotoxic activity of compounds analyzed. Data were obtained from a representative experiment carried out in triplicate.

Table 1. Comparison of antiproliferative activity of (*R*)- and (*S*)-argentilactone against cancer cell lines[a]

Cell line	IC_{50} (μM)		
	(*R*)-(6)	(*S*)-(6)	Doxorubicin[b]
MCF-7	14.7	13.9	3.3
NCI-ADR	11.0	>100	48.7
NCI 460	14.3	17.9	1.8
UACC62	55.0	17.8	9.8
786–0	73.6	48.5	>100
OVCAR03	33.8	25.3	11.7
PCO 3	29.0	30.4	18.6
HT-29	42.3	>100	5.3

[a] IC_{50} values (concentration that elicits 50% inhibition) were determinated from nonlinear regression analysis by GraphPad Prism software ($r^2 > 0.9$).

[b] Doxorubicin (DOX) was employed as positive control.

3. Conclusion

The approach presented here for the synthesis of both enantiomers of argentilactone (**6**) represents a short route to these pyranones amenable to provide these compounds in sufficient amounts for biological assays. Both enantiomers displayed antiproliferative activity against the cancer cell lines tested. For MCF-7 (breast), NCI 460 (lung, nonsmall cells), and PCO 3 (prostate) cell lines, similar potency was observed for (*R*)- and (*S*)-(**6**). The natural form of argentilactone [(*R*)-(**6**)] proved to be more potent for breast cancer cells that express multidrug resistance phenotype (NCI-ADR), whereas melanoma cells (UACC62) were more sensitive to (*S*)-(**6**).

The versatility of our synthetic route allows its application to the preparation of both enantiomers of argentilactone analogues to be screened against cancer cell lines in order to improve their antiproliferative activity.

4. Experimental section

4.1. Chemistry

4.1.1. General procedures. Reagents and solvents are commercial grade and were used as supplied, except for dichloromethane and triethylamine that were distilled from calcium hydride. Chromatographic separations were performed using 70–230 mesh silica gel. Thin layer chromatography was carried out on Macherey–Nagel precoated silica plates (0.25 mm layer thickness). IR spectra were obtained on Nicolet Impact 410 FT (film or KBr). ^{1}H NMR and ^{13}C NMR data were recorded on a Varian Gemini 2000 (7.0 T) or Varian Inova (11.7 T) spectrometer. Chemical shifts are reported in δ [ppm relative to $(CH_3)_4Si$] for ^{1}H and $CDCl_3$ for ^{13}C NMR. For ^{1}H NMR, the chemical shifts were followed by multiplicity (s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; q, quartet; m, multiplet) and coupling constant *J* reported in hertz (Hz). High resolution mass spectra (HRMS) were measured on a VG Autospec-Micromass spectrometer. Chiral GC analyses were performed with capillary column CP-Chirasil-DEX CB fused silica WCOT (25 m × 0.25 mm × 0.25 μm) on Agilent 6890 series GC system. Optical rotations were measured at 25 °C with Perkin-Elmer 241 instrument.

4.1.2. Preparation of (*R*)- and (*S*)-1-undec-5-yn-4-ol (9). To a stirred solution of $TiCl_4$ (13 μL, 0.12 mmol) in CH_2Cl_2 (2.4 mL) was added dried Ti(O*i*Pr)$_4$ (110 μL, 0.36 mmol) at 0 °C under argon. The solution was allowed to warm to room temperature. After 1 h, silver(I) oxide (56 mg, 0.24 mmol), recently prepared,[47] was added at room temperature, and the whole mixture was stirred 5 h under exclusion of direct light. The mixture was diluted with CH_2Cl_2 (4 mL) and treated with (*R*)-BINOL (138 mg, 0.48 mmol) at room temperature for 2 h to furnish chiral bis-Ti(IV) oxide (*R*,*R*)-**7**. After cooling this mixture to −15 °C, it was treated sequentially with 2-octynal (300 mg, 2.41 mmol) and allyltributyltin (1.37 g, 4.16 mmol). The whole mixture was allowed to warm to 0 °C and stirred for 24 h. The reaction mixture was quenched with saturated $NaHCO_3$ and extracted with ether. The organic extracts were dried over $MgSO_4$. Evaporation of solvents and purification of this residue by column chromatography on silica (hexane–ethyl acetate, 9:1) furnished (*R*)-1-undec-5-yn-4-ol (**9**) in 89% yield (357 mg). The enantiomeric purity of both enantiomers of **9** was determined to be 82–84% ee by chiral GC analysis (column: CP-Chiralsil-DEX CB fused silica WCOT, 25 m × 0.25 mm × 0.25 μm; conditions—initial temperature/time: 60 °C/1 min, rate: 0.5 °C/min, final temperature/time: 180 °C/70 min, H_2 as the carrier gas and FID detector) and comparing with racemic standard. IR (film): 3356, 3078, 2956, 2933, 2860, 2227, 1641, 1462, 1433, 1379, 1331, 1144, 1036, 995, 914 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.96–5.82 (m, 1 H), 5.21–5.15 (m, 2 H), 4.44–4.37 (m, 1 H), 2.45 (t, 2 H, J = 6.6 Hz), 2.21 (dt, 2 H, J = 7.1 e 1.8 Hz), 1.96 (d, 1 H, J = 5.9 Hz), 1.53–1.30 (m, 6 H), 0.92–0.88 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 133.2, 118.5, 85.9, 80.5, 61.8, 42.6, 31.0, 28.4, 22.2, 18.7, 14.0. HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 166.13576; found: 166.13148. For (*R*)-**9** $[\alpha]_D^{25}$ + 30.0 (*c* 2.0, $CHCl_3$). The same procedure except for the use of (*S*)-BINOL instead of (*R*)-BINOL, afforded (S)-(**9**). For (*S*)-**9** $[\alpha]_D^{25}$ − 30.0 (*c* 2.0, $CHCl_3$).

4.1.3. Preparation of (4*R*,5*Z*)- and (4*S*,5*Z*)-1, 5-undecadien-4-ol (10). To a stirred solution of (*R*)-1-undec-5-yn-4-ol (**9**) (150 mg, 0.89 mmol) in a (1:1, v/v) mixture of EtOAc and 1-octene at room temperature and 1 atm H_2 was added quinoline (210 μL, 1,78 mmol) and Lindlar catalyst (191 mg, 0.09 mmol). The mixture was stirred for 30 min and after TLC indicated only a trace amount of starting material, the mixture was filtered through a pad of Celite using EtOAc as eluent, and then dried over $MgSO_4$. Evaporation of solvents and purification of this residue by column chromatography on silica (hexane–ethyl acetate, 9:1) furnished (4*R*, 5*Z*)-1,5-undecadien-4-ol (**10**) in 74% yield (113 mg). IR (film): 3350, 3076, 3006, 2956, 2925, 2856, 1641, 1466, 1030, 912, 748 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.89–5.75 (m, 1 H), 5.55–5.36 (m, 2 H), 5.18–5.11 (m, 2 H), 4.49 (q, 1 H, J = 6.8 Hz), 2.32–2.27 (m, 2 H); 2.15–2.02 (m, 2 H), 1.66–1.61 (m, 1 H), 1.42–1.29 (m, 6 H), 0.90 (t, 1 H, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.1, 132.5, 131.4, 117.9, 66.8, 42.2, 31.5, 29.4, 27.8, 22.6, 14.1. HRMS (EI) *m*/*z* calculated for $M^{+\cdot}$ = 168.15141; found: 168.15560. For (*R*)-**10** $[\alpha]_D^{25}$ + 25.9 (*c* 1.5, $CHCl_3$). The same procedure except for the use of (*S*)-1-undec-5-yn-4-ol (**9**) instead of (*R*)-1-undec-5-yn-4-ol (**9**), afforded (S)-(**10**). For (*S*)-**10** $[\alpha]_D^{25}$ − 26.0 (*c* 1.5, $CHCl_3$).

4.1.4. Preparation of (4*R*,5*Z*)- and (4*S*,5*Z*)-1,5-undecadien-4-yl propenoate (11). To (4*R*, 5*Z*)-1, 5-undecadien-4-ol (**10**) (110 mg, 0.65 mmol) dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C, were added acryloyl chloride (950 μL, 1.17 mmol) and Et_3N (330 μL, 2.34 mmol). The mixture was warmed to room temperature and stirred for 2 h. The resulting mixture was filtered through a short pad of Celite to remove solid $Et_3N{\cdot}HCl$, poured into water, and the product was extracted with CH_2Cl_2.

Evaporation of solvents and purification of this residue by column chromatography on silica (hexane–ethyl acetate, 9:1) furnished (4*R*, 5*Z*)-1,5-undecadien-4-yl propenoate (**11**) in 86% yield (125 mg). IR (film): 3080, 3014, 2958, 2927, 2858, 1726, 1637, 1404, 1267, 1190, 1043, 984, 808 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.39 (dd, 1 H, $J = 17.2$, 1.5 Hz), 6.10 (dd, 1 H, $J = 17.2$, 10.2 Hz), 5.80 (dd, 1 H, $J = 10.2$, 1.5 Hz), 5.80–5.54 (m, 2 H), 5.40–5.33 (m, 1 H), 5.14–5.06 (m, 2 H), 2.50–2.31 (m, 2 H), 2.19–2.09 (m, 2 H), 1.39–1.26 (m, 6 H), 0.93–0.87 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.1, 134.5, 133.0, 130.2, 128.7, 127.0, 117.7, 69.7, 39.4, 31.5, 29.2, 27.9, 22.6, 14.1. HRMS (EI) *m/z* calculated for $M^{+\cdot} = 222.16198$; found: 222.16354. For (*R*)-**11**, $[\alpha]_D^{25} - 32.7$ (*c* 1.0, $CHCl_3$). The same procedure except for the use of (4S,5*Z*)-1,5-undecadien-4-ol (**10**) instead of (4*R*, 5*Z*)-1,5-undecadien-4-ol (**10**), afforded (4*S*, 5*Z*)-1,5-undecadien-4-yl propenoate (**11**). For (4*S*, 5*Z*)-**11** $[\alpha]_D^{25} + 33.0$ (*c* 1.0, $CHCl_3$).

4.1.5. Preparation of (*R*)- and (*S*)-argentilactone (6). To a stirred solution of bis(tricyclohexylphosphine)benzylidine ruthenium(IV) dichloride (Grubb's catalyst, 22 mg, 10 mol%) in CH_2Cl_2 (7 mL) at 55–60 °C was added (4*R*, 5*Z*)-1,5-undecadien-4-yl propenoate (**11**) (60 mg, 0.27 mmol) dissolved in CH_2Cl_2 (27 mL).The resulting mixture was heated for 4 h. After this period, the mixture was cooled at room temperature and evaporated under reduced pressure, and purification of this residue by column chromatography on silica (hexane–ethyl acetate, 9:2) furnished (*R*)-argentilactone in 70% yield (37 mg). IR (film): 3020, 2956, 2927, 2858, 1722, 1466, 1381, 1244, 1149, 1055, 1022, 816 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.90 (ddd, 1 H, $J = 9.7$, 5.5, 3.1 Hz), 6.05 (ddd, 1 H, $J = 9.7$, 2.2, 1.4 Hz), 5.71–5.52 (m, 2 H), 5.22 (ddd, 1 H, $J = 10.2$, 8.4, 4.9 Hz), 2.49–2.30 (m, 2 H), 2.17–2.03 (m, 2 H), 1.46–1.26 (m, 6 H), 0.90 (t, 3 H, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 144.6, 135.5, 126.3, 121.5, 73.9, 31.5, 29.9, 29.1, 27.8, 22.5, 14.1. HRMS (EI) *m/z* calculated for $M^{+\cdot} = 194.13068$; found: 194.12733. For natural form $[\alpha]_D^{25} - 22.0$ (*c* 2.2, EtOH) {lit. $[\alpha]_D^{25} - 21$ (*c* 2.2, EtOH)}. The same procedure above afforded (*S*)-argentilactone (S)-(**6**) from (4S,5*Z*)-1,5-undecadien-4-yl propenoate (**11**). For the nonnatural argentilactone [(*S*)-**6**]: $[\alpha]_D^{25} + 22.0$ (*c* 2.2, EtOH).

4.2. Biological activities

4.2.1. Biological assay. Since it is known that different cell lines display different sensitivities toward a cytotoxic compound, the use of more than one cell line is, therefore, considered necessary in the detection of cytotoxic compounds. Bearing this in mind, cell lines of different histological origin were used in the present study. Human tumor cell lines UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, nonsmall cells), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786–0 (renal), and NCI-ADR (breast expressing fenotipe multiple drugs resistance) were kindly provided by National Cancer Institute (NCI). Stock cultures were grown in a medium containing 5 mL of RPMI 1640 (GIBCO BRL, Life Technologies) and supplemented with 5% of fetal bovine serum. Gentamicine (50 μg/mL) was added to the experimental cultures. Cells in 96 well plates (100 μL cells/well) were exposed to various concentrations of samples in DMSO (0.25, 2.5, 25, and 250 μg/mL) at 37 °C, 5% of CO_2 in air for 48 h. The final concentration of DMSO did not affect the cell viability. Then, a 50% of trichloroacetic acid solution was added and after incubation for 30 min at 4 °C, washing, and drying, the cell proliferation was determined by spectrophotometric quantification (540 nm) of the cellular protein content using sulforhodamine B assay described by Skehan et al.[48]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.07.044. This consists of spectra of compounds **6**, **9**, **10**, and **11**.

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