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Synthesis, characterisation and cytotoxicity of chloro derivatives of prenylnaphthohydroquinone

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Abstract—From the Diels–Alder adduct between α-myrcene and 2-chloro-1,4-benzoquinone, a family of chloro derivatives of prenylnaphthohydroquinone have been synthesised and evaluated for their cytotoxicity against 14 neoplastic cell lines.

1. Introduction

Mono and diterpenylquinone/hydroquinone derivatives, prepared from the Diels–Alder cycloaddition adduct between α-myrcene or related myrceocommunic acid as dienes and *p*-benzoquinone or 1,4-naphthoquinone as dienophiles, are bioactive compounds against P-388 murine leukaemia, A-549 human lung carcinoma, HT-29 and H-460 human colon carcinoma, MEL-28 human malignant melanoma, MCF-7 mammary gland carcinoma and SF-268 brain carcinoma neoplastic cells lines. These families of compounds have been prepared mostly by chemical modification on the monoterpenic or diterpenic core using reactions like aromatisation, epoxidation, degradative cleavage, reduction, oxidation or acetylation.[1–5]

Regarding the bioactivity of the myrceocommunic acid family,[3] the IC_{50} values found ranged between 0.1 and 10 μM for naphthoquinone derivatives, 2–21 μM for anthraquinone derivatives and most of the *p*-benzoquinone derivatives are more bioactives than avarol and avarone, natural sesquiterpenylquinone/hydroquinone considered as prototypes in these studies.[6,7]

The IC_{50} or GI_{50} values observed for prenylhydroquinone derivatives obtained from the diacetate **1** (Fig. 1) were in the range <0.3 to >30 μM and SAR studies revealed that the cytotoxicity is enhanced when the terpenic part of **1** is aromatised or when the side chain is saturated (IC_{50} = 0.3 μM, P-388; GI_{50} = 1.6 μM, MCF-7);[4,8] this enhancement is higher when both structural characteristics are present (GI_{50} = < 0.3 μM, MCF-7).[8] Improvement of the cytotoxicity is also observed when the side chain is functionalised with an acetate or a methyl ester group (IC_{50} = 0.3 μM, P-388)[4,8] and several compounds of these families are also more potent than avarol and avarone.

To analyse the effect of substitution on the hydroquinonic part of **1**, a new family of prenylacetylhydroquinone derivatives was prepared from the Diels–Alder condensation product between α-myrcene and 2-acetyl-1,4-benzoquinone and this series was evaluated for their cytotoxicity against A-549 and H-116 human colon carcinoma. The IC_{50} values found were in the range 1.3 to

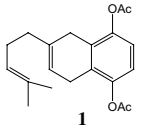

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Figure 1. Structure of the prenylhydroquinone diacetate **1**.

Keywords: Prenylhydroquinones; Chloronaphthohydroquinones; Cytotoxicity; Antineoplastic.
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>14.6 μM, being the aromatised derivative bearing a methyl ester group on the terpenic side chain the most potent of the series.[9] In general, an acetyl group on the hydroquinonic part decreases the antineoplastic potency of **1** and most of the compounds tested were about twice less potent against A-549 cell line than those unsubstituted previously reported.

Chlorine is a recurrent substituent in families of compounds with antitumour activities. 2-Chloro-3-(substituted phenoxy)-1,4-naphthoquinones and related 5,8-dihydroxy-1,4-naphthoquinones,[10] 6-chloro-7-arylamino-5,8-isoquinolinediones,[11] 6-chloro-1,1-dioxo-1,4,2-benzodithiazine derivatives,[12] 2-amino-8-chloro-5, 5-dioxo [1,2,4]triazolo[2,3-*b*][1,4,2]benzodithiazine derivatives,[13] 4-chloro-2-mercapto-5-methyl-benzenesulfonamide derivatives[14] and substituted *E*-3-(2-chloro-3-indolylmethylene)-1,3-dihydroindol-2-ones,[15] among others, have been screened against a wide variety of human tumour cell lines. In some cases, the presence of chlorine improves the cytotoxicity,[16] while in others it is necessary to maintain the chlorinated part of the structure in order to generate new cytotoxic derivatives.[15]

According to these antecedents and following the line of research, related to diacetate **1** we have prepared a new family of prenylhydroquinones from the diacetate **2** (Fig. 2) containing a chlorine substituent on the hydroquinone part, in order to analyse the effect of this substituent on the cytotoxic–antineoplastic properties of these derivatives. All the new compounds have been tested against ovarian, prostate, mammalian, leukaemia, pancreas, colon, cervix and melanoma neoplastic cell lines.

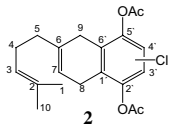
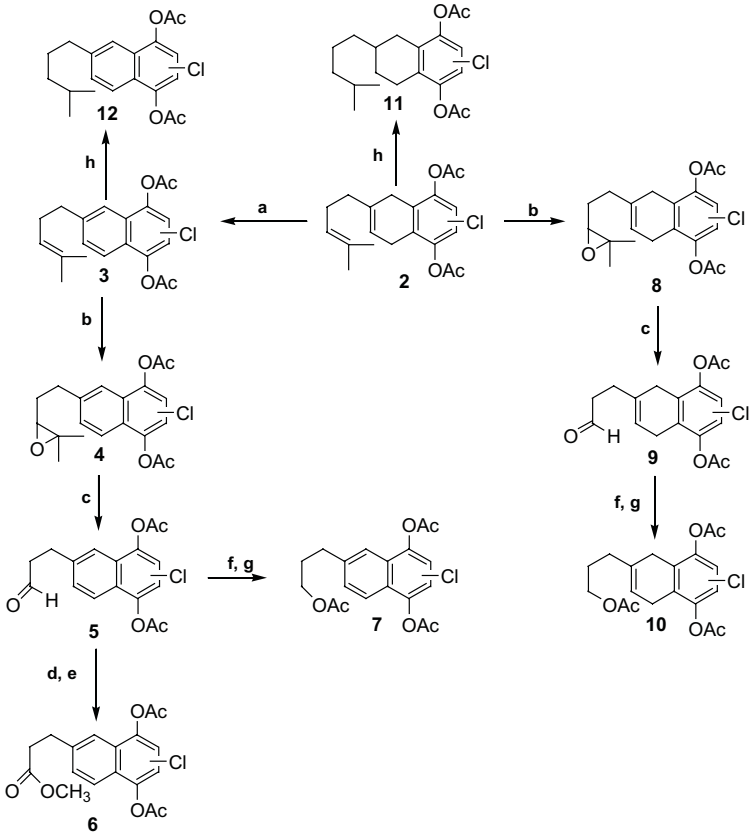


Figure 2. Structure of the starting prenylchlorohydroquinone diacetate **2**.

2. Results and discussion

2.1. Chemistry

The starting prenylchlorohydroquinonic diacetate **2** was obtained as a 1:1 regioisomeric mixture from the Diels–Alder condensation of commercial 2-chloro-1,4-benzoquinone and myrcene, followed by acetylation (with acetic anhydride in pyridine). The presence of both regioisomers was deduced from the complexity of the ^{1}H and ^{13}C NMR spectra, where almost all individual signals for protons and carbons of compound **2**–**12**



Scheme 1. The synthesis of chloro derivatives of prenylnaphthohydroquinone. Reagents and conditions: (a) DDQ, refluxing benzene, 1 h; (b) MCPBA, $NaHCO_3$, CH_2Cl_2, 45 min; (c) H_5IO_6, THF, H_2O, rt, 1 h; (d) $NaClO_2$, NaH_2PO_4, H_2O, *t*-BuOH, 2-methyl-2-butene, rt, 72 h; (e) CH_3I, aqueos NaOH, TEBAC, $CHCl_3$, overnight or CH_2N_2, Et_2O, rt, 30 min; (f) $LiAlH_4$, Et_2O, rt, overnight; (g) Ac_2O, Py, rt, 24 h; (h) H_2, Pd/C, AcOEt, rt, 24 h.

synthesised are double. As a proof, in the ^{1}H spectra the acetate groups of the unsubstituted compound **1** display two singlets at 2.30 and 2.33 ppm,[2] meanwhile four singlets are observed at 2.31, 2.33, 2.39 and 2.40 ppm in the chloro substituted compound **2**.

Chemical modifications of this regioisomeric mixture by reactions such as catalytic hydrogenation with H_2/Pd/C, aromatisation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), epoxidation with *m*-chloroperbenzoic acid (MCPBA), reduction with lithium aluminium hydride (LAH), oxidative cleavage of epoxides with periodic acid (H_5IO_6), acetylation with acetic anhydride/pyridine and methylation of carboxylic group with diazomethane or with methyl iodide under phase transfer catalysis (PTC), afforded the expected chloro derivatives **3**–**12** (Scheme 1). The principal ^{1}H and ^{13}C NMR information according to carbon numbering given in Figure 2, the IR absorptions and other physical data are reported in the experimental part.

2.2. Bioactivity

Compounds **2**–**12** were preliminary evaluated in vitro for their bioactivity against Jurkat 77 cell line and then against prostate DU-45, ovarian IGROV and IGROV-ET, mammalian SK-BR3, melanoma MEL-28, non-small cell lung A-549, leukaemia K-562, pancreas PANC-1, colon HT-29, LOVO and LOVO-DOX, cervix HELA and HELA-APL human neoplastic cell lines. The results are shown in Table 1 and several general observations can be made.

(a) The chloro derivatives of prenylhydroquinone reported are cytotoxic compounds against all the cell lines assayed.
(b) The GI_{50} values are ranged between 0.54 and 7.81 μM, meaning that the chemical transformations achieved on the terpenic part did not modify significantly the bioactivity of compounds **2**–**12** in comparison with those values observed in analogue unsubstituted derivatives of **1** against MCF-7, H-460 and SF-268 antineoplastic cell lines (GI_{50} <0.3 to >30.0 M).[8]
(c) The highest antineoplastic potencies are observed in the aromatised epoxide **4** (GI_{50} 0.537 μM, mammalian SKBR3), in the aromatised triacetate **7** (GI_{50} 0.546 μM, leukaemia K562) and in the saturated aromatised diacetate **12** (GI_{50} 0.604 μM, mammalian SKBR3).
(d) In general, the saturated aromatised diacetate **12** shows great activity against all the cell lines assayed (GI_{50} 0.60–1.89 μM).
(e) A slightly degree of selectivity is observed with the epoxide **4** (mammalian SKBR3), the triacetate **7** (leukaemia K562) and the aromatised saturated diacetate **12** (mammalian SKBR3 and pancreas PANC1).
(f) In general, compounds **3**–**7** and **12**, which are aromatic in the cyclic terpenyl moiety are twice more potent than the unsaturated analogue compounds **2** and **8**–**10**, confirming the importance of the aromatisation on the bioactivity of prenylhydroquinonic derivatives.[1–4,8,9]

3. Experimental

All the new compounds evaluated were obtained as colourless to slightly yellow viscous oils and characterised by IR on a Perkin Elmer FT IR 1600 spectrophotometer, as a film over sodium chloride discs. NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C in deuterochloroform with TMS as internal reference, on a Bruker AVANCE 400 Digital spectrophotometer (Laboratorio Regional de RMN, Universidad Técnica Federico Santa María, Fundación Andes, Convenio C-13672). Chemical shift values are expressed in parts per million followed by multiplicity and coupling constant *J* in hertz. Elemental analysis of carbon and hydrogen was obtained with a Perkin Elmer 2400 Series II CHN Elemental Analyser. Column chromatographic separations were performed on Silicagel 60, 230–400 mesh ASTM.

3.1. Chemistry

3.1.1. Unsaturated diacetate 2. Synthesised by Diels–Alder condensation of 2-chloro-1,4-benzoquinone with myrcene and $BF_3{\cdot}Et_2O$ as catalyst (47%); oil; IR cm^{-1}: 3093 (aromatic CH), 2986–2926 (aliphatic CH), 1766 (ester C=O). ^{1}H NMR ($CDCl_3$) δ: 1.61 (s, 3H, H-1), 1.70 (s, 3H, H-10), 2.12 (m, 4H, H-4, H-5), 2.31, 2.33 (s, 3H, OAc) 2.39, 2.40 (s, 3H, OAc), 3.07–3.19 (m,

Table 1. Cytotoxicity of chloro derivatives of prenylnaphthohydroquinone **2**–**12** (GI_{50} μM)

Compd	DU 145	IGROV	IGROV ET	SKBR 3	MEL 28	A 549	K 562	PANC 1	HT 29	LOVO	LOVO DOX	HELA	HELA APL	JURK 77[a]
2	3.94	2.30	3.33	1.47	1.83	3.86	2.78	2.84	5.10	3.58	3.64	2.09	3.11	1.21
3	2.04	1.23	1.44	1.12	1.22	2.34	1.25	1.49	3.44	2.51	1.57	1.58	1.51	0.14
4	2.89	1.39	2.08	0.54	1.08	1.49	1.16	0.81	4.54	3.08	1.97	1.68	1.98	0.33
5	4.45	3.47	3.14	1.41	1.45	3.79	2.18	2.37	5.85	3.97	3.76	3.92	5.39	0.55
6	1.74	1.50	1.23	7.54	1.00	1.53	1.04	1.43	3.65	1.62	1.57	1.38	1.38	0.43
7	2.02	1.53	1.12	0.93	1.00	1.53	0.55	1.05	3.72	1.71	1.58	1.25	1.67	0.40
8	4.20	3.85	3.01	1.45	2.93	4.36	1.53	1.54	2.18	3.70	3.27	3.62	3.62	1.06
9	7.81	5.32	3.36	3.41	4.66	7.07	3.59	3.15	6.80	7.99	6.32	4.45	7.13	0.82
10	3.18	1.87	1.74	1.48	1.56	2.02	1.02	1.61	4.59	2.28	1.83	1.36	1.66	1.49
11	2.83	2.53	3.38	1.55	2.78	2.40	2.32	1.65	5.67	3.03	3.43	2.40	2.21	0.57
12	1.22	1.13	1.20	0.60	1.06	1.30	1.15	0.76	1.89	1.48	1.13	1.13	1.19	0.59

[a] IC_{50} μM.

4H, H-8, H-9), 5.11–5.13 (m, 1H, H-3), 5.54 (br s, 1H, H-7), 7.06 (s, 1H, H-3′, H-4′). ^{13}C NMR ($CDCl_3$) δ: 17.8 (C-1), 20.3, 20.4 (OAc), 20.7, 20.8 (OAc), 25.7 (C-10), 25.0, 25.6, 26.0, 27.4, 28.0, 37.0 (C-4, C-5, C-8, C-9), 116.5, 116.6, 120.8, 120.9, 123.8 (C-3, C-7, C-3′,C-4′), 124.4, 127.3, 127.6, 130.4, 130.8 131.9, 133.5, 133.6, 142.7, 146.1 (C-2, C-6, C-1′, C-2′, C-3′,C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.7, 168.8 ($OCOCH_3$). Anal. Found (%): C 66.49; H 6.56. Calcd for $C_{20}H_{23}ClO_4$ (%): C 66.20; H 6.39.

3.1.2. Aromatised diacetate 3. Synthesised by DDQ aromatisation of **2** in refluxing benzene (63%); oil; IR cm^{-1}: 3093–3045 (aromatic CH), 2986–2855 (aliphatic CH), 1775 (ester C=O). ^{1}H NMR ($CDCl_3$) δ: 1.56 (s, 3H H-1), 1.70 (s, 3H, H-10), 2.35 (m, 2H, H-4), 2.44, 2.46 (s, 3H, OAc), 2.50, 2.51 (s, 3H, OAc), 2.81 (t, J = 7.7, 2H, H-5), 5.18 (m, 1H, H-3), 7.30, 7.33 (s, 1H, H-3′, H-4′), 7.45 (dd, J = 8.2, 1.7, 1H, 2 × H-7), 7.61, 7.56 (d, J = 1.7, 2 × H-9), 7.74, 7.78 (d, J = 8.2, 2 × H-8). ^{13}C NMR ($CDCl_3$) δ: 17.7 (C-1), 20.4, 20.5 (OAc), 20.9, 21.0 (OAc), 25.6 (C-10), 29.1, 29.6 (C-4), 36.3, 36.4 (C-5), 118.5, 119.6, 120.1, 121.4, 121.7, 123.7, 128.7, 129.6 (C-7, C-8, C-9, C-3′,C-4′), 122.6, 123.2, 125.0, 126.6, 128.6 140.6, 140.9, 141.6, 142.6, 144.0, 144.4 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.0, 168.2 ($OCOCH_3$), 168.9, 169.0 ($OCOCH_3$). Anal. Found (%): C 66.27; H 6.13. Calcd for $C_{20}H_{21}ClO_4$ (%): C 66.57; H 5.87.

3.1.3. Aromatised epoxidiacetate 4. Synthesised by MCPBA epoxidation of **3** in dichloromethane and $NaHCO_3$ (76%); oil; IR cm^{-1}: 3080 (aromatic C–H), 2950–2870 (aliphatic C–H), 1763 (ester C=O). ^{1}H NMR ($CDCl_3$) δ: 1.17 (s, 3H, H-1), 1.28 (s, 3H, H-10), 1.91 (q, J = 6.2, 2H, H-4), 2.45, 2.47 (s, 3H, OAc), 2.50, 2.52 (s, 3H, OAc), 2.79 (t, J = 6.2, 2H, H-5), 2.96 (m, 1H, H-3), 7.31, 7.34 (s, 1H, H-3′, H-4′), 7.43 (dd, J = 8.2, 1.8, 1H, 2 × H-7), 7.60, 7.64 (d, J = 1.8, 1H, 2 × H-9), 7.75, 7.79 (d, J = 8.2, 1H, 2 × H-8). ^{13}C ($CDCl_3$) δ: 18.7 (C-1), 20.5, 20.5 (OAc), 21.0, 21.1 (OAc), 24.8 (C-10), 30.6 (C-4), 33.1, 33.2 (C-5), 58.8 (C-2), 63.7 (C-3), 118.8, 119.7, 120.1, 120.4, 121.7, 122.0, 128.5, 129.4 (C-7, C-8, C-9, C-3′,C-4′), 122.2, 123.1, 125.0, 126.5, 127.1, 128.6, 140.5, 140.6, 140.9, 141.6, 144.2, 144.6 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.9, 169.0 ($OCOCH_3$). Anal. Found (%): C 64.01; H 6.02. Calcd for $C_{20}H_{21}ClO_5$ (%): C 63.74; H 5.62.

3.1.4. Aromatised aldehyde diacetate 5. Synthesised by degradative H_5IO_6 oxidation of **4** in aqueous THF (56%); oil; IR cm^{-1}: 3075 (aromatic C–H), 2960–2870 (aliphatic C–H), 2735 (aldehyde C–H), 1768 (ester C=O), 1720 (aldehyde C=O). ^{1}H NMR ($CDCl_3$) δ: 2.44, 2.46 (s, 3H, OAc), 2.48, 2.51 (s, 3H, OAc), 2.83 (t, J = 7.7, 2H, H-5), 3.09 (dt, J = 7.7, 1.2, 2H, H-4), 7.30, 7.33 (s, 1H, H-3′, H-4′), 7.38, 7.41 (dd, J = 8.6, 1.7, 1H, 2 × H-7), 7.57, 7.62 (d, J = 1.7, 1H, 2 × H-9), 7.73, 7.77 (d, J = 8.6, 1H, 2 × H-8), 9.81 (t, J = 1.2, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ: 20.9, 21.0 (OAc), 21.3, 21.4 (OAc), 28.6, 28.7 (C-4), 45.4 (C-5), 119.4, 120.1, 120.5, 120.9, 122.3, 122.7, 128.6, 129.5 (C-7, C-8, C-9, C-3′, C-4′), 122.8, 123.6, 125.5, 126.9, 127.5, 129.0, 140.1, 140.9, 141.1, 141.2, 144.6, 144.9 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.5, 168.6 ($OCOCH_3$), 169.3, 169.4 ($OCOCH_3$), 201.2, 201.3 (C-3). Anal. Found (%): C 70.12; H 4.95. Calcd for $C_{17}H_{15}ClO_5$ (%): C 70.00; H 4.52.

3.1.5. Aromatised methyl ester diacetate 6. Synthesised by $NaClO_2$ oxidation of the aldehyde **5** in H_2O, *t*-BuOH, NaH_2PO_4 and 2-methyl-2-butene, followed by phase transfer methylation with methyl iodide, aqueous NaOH, $CHCl_3$ and TEBAC (64%); oil; IR cm^{-1}: 3061 (aromatic C–H), 2967–2880 (aliphatic C–H), 1769 (ester C=O), 1742 (methyl ester C=O). ^{1}H NMR ($CDCl_3$) δ: 2.45, 2.47 (s, 3H, OAc), 2.49, 2.51 (s, 3H, OAc), 2.69 (t, J = 7.7, 2H, H-5), 3.11 (t, J = 7.7, 2H, H-4), 3.66 (s, 3H, OMe), 7.30, 7.32 (s, 1H, H-3′, H-4′), 7.39, 7.42 (dd, J = 8.7, 1.7, 1H, 2 × H-7), 7.59, 7.63 (d, J = 1.7, 1H, 2 × H-9), 7.73, 7.78 (d, J = 8.7, 1H, 2 × H-8). ^{13}C NMR ($CDCl_3$) δ: 20.9, 21.0 (OAc), 21.3, 21.4 (OAc), 31.5, 31.6 (C-4), 35.9 (C-5), 52.1 (OMe) 119.3, 120.1, 120.5, 120.8, 122.2, 122.6, 128.6, 129.5 (C-7, C-8, C-9, C-3′, C-4′), 122.7, 123.5, 125.5, 126.9, 127.5, 128.9, 140.1, 141.0, 141.1, 144.6, 144.9 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.5, 169.6 ($OCOCH_3$), 169.3, 169.4 ($OCOCH_3$), 173.4 (C-3). Anal. Found (%): C 59.06; H 5.35. Calcd for $C_{18}H_{17}ClO_6$ (%): C 59.27; H 5.00.

3.1.6. Aromatised triacetate 7. Synthesised by $LiAlH_4$ reduction of the aldehyde **5** in anhydrous diethyl ether, followed by acetylation with acetic anhydride and pyridine (78%); oil; IR cm^{-1}: 3095 (aromatic CH), 2954–2855 (aliphatic CH), 1766 (acetate C=O), 1735 (acetate C=O). ^{1}H NMR ($CDCl_3$) δ: 1.94 (m. 2H, H-4), 1.97, 1.98 (s, 3H, OAc), 2.36, 2.39 (s, 3H, OAc), 2.41, 2.43 (s, 3H, OAc), 2.76 (t, J = 6.8, 2H, H-5), 4.04 (t, J = 6.5, 2H, H-3), 7.22, 7.25 (s, 1H, H-3′, H-4′), 7.35, 7.39 (dd, J = 8.5, 1.6, 1H, 2 × H-7), 7.49, 7.53 (d, 1H, 2 × H-9), 7.66, 7.70 (d, J = 8.5, 1H, 2 × H-8). ^{13}C NMR ($CDCl_3$) δ: 19.4, 19.5 (OAc), 19.8 19.9 (OAc), 20.0 (OAc), 28.9, 29.0, (C-4), 31.5, 31.6 (C-5), 62.6 (C-3), 117.7, 118.6, 119.0, 119.3, 120.7, 121.0, 127.3, 128.3 (C-7, C-8, C-9, C-3′, C-4′), 121.1, 122.0, 124.0, 125.5, 139.4, 139.5, 139.8, 140.4, 143.2, 143.5 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 167.1, 167.2 ($OCOCH_3$), 167.8, 167.9 ($OCOCH_3$), 170.1 ($OCOCH_3$). Anal. Found (%): C 60.52; H 5.18. Calcd for $C_{19}H_{19}ClO_6$ (%): C 60.24; H 5.06.

3.1.7. Unsaturated epoxide diacetate 8. Synthesised by MCPBA epoxidation of the diacetate **2** in dichloromethane and $NaHCO_3$ (76%); oil; IR cm^{-1}: 3065 (aromatic C–H), 2983–2866 (aliphatic C–H), 1763 (ester C=O). ^{1}H NMR ($CDCl_3$) δ: 1.26 (s, 3H, H-1), 1.30 (s, 3H, H-10), 1.68 (m, 2H, H-4), 2.22 (m, 2H, H-5), 2.29, 2.32 (s, 3H, OAc), 2.35, 2.37 (s, 3H, OAc), 2.76 (t, J = 6.3, 1H, H-3), 3.14 (m, 4H, H-8, H-9), 5.57 (br s, 1H, H-7), 7.06 (s, 1H, H-3′, H-4′). ^{13}C NMR ($CDCl_3$) δ: 18.8 (C-1), 20.3, 20.4, 20.7, 20.8 (OAc), 24.8 (C-10), 25.0, 25.6, 26.9, 27.4, 28.0, 33.7 (C-4, C-5, C-8, C-9), 58.4 (C-2), 63.7 (C-3). 117.2, 117.3, 121.0 (C-7, C-3′, C-4′), 124.5, 127.1, 127.3, 130.2, 132.8, 132.9, 142.7, 146.1

(C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.7, 168.8 ($OCOCH_3$). Anal. Found (%): C 66.70; H 6.36. Calcd for $C_{20}H_{23}ClO_5$ (%): C 66.41; H 6.09.

3.1.8. Unsaturated aldehyde diacetate 9. Synthesised by degradative H_5IO_6 oxidation of the epoxide **8** in aqueous THF (56%); oil; IR cm^{-1}: 3083 (aromatic C–H), 2926–2825 (aliphatic C–H), 2731 (aldehyde C–H), 1772 (ester C=O), 1725 (aldehyde C=O). ^1H NMR ($CDCl_3$) δ: 2.29, 2.32 (s, 3H, OAc), 2.35, 2.38 (s, 3H, OAc), 2.61 (t, $J = 6,7$, 2H, H-5), 3.14 (m, 4H, H-8, H-9), 5.54 (br s, 1H, H-7), 7.06 (s, 1H, H-3′, H-4′), 9.78 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ: 20.3, 20.4 (OAc), 20.7, 20.8 (OAc), 25.0, 25.6, 27.5, 28.1, 28.8, 30.3 (C-4, C-8, C-9), 41.5 (C-5), 117.5, 117.6, 121.0 (C-7, C-3′, C-4′), 124.6, 126.7, 127.0, 130.0, 131.1, 131.9, 132.7, 142.7, 146.1 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.8, 168.9 ($OCOCH_3$), 201.7 (C-3). Anal. Found (%): C 60.79; H 5.38. Calcd for $C_{17}H_{17}ClO_5$ (%): C 60.63; H 5.09.

3.1.9. Unsaturated triacetate 10. Synthesised by $LiAlH_4$ reduction of the aldehyde **9** in anhydrous diethyl ether, followed by acetylation with acetic anhydride and pyridine (77%); oil; IR cm^{-1}: 3072 (aromatic C–H), 2954–2850 (aliphatic C–H), 1765 (ester C=O), 1742 (methyl ester C=O). ^1H NMR ($CDCl_3$) δ: 1.79 (m, 2H, H-4), 2.04 (s, 3H, OAc), 2.13 (t, $J = 7.6$, 2H, H-5), 2.30, 2.33 (s, 3H, OAc), 2.35, 2.38 (s, 3H, OAc), 3.11 (m, 4H, H-8, H-9), 4.07, 4.09 (t, $J = 7.1$, 2H, H-3), 5.55 (br s, 1H, H-7), 7.06 (s, 1H, H-3′, H-4′). ^{13}C NMR ($CDCl_3$) δ: 20.3, 20.4 (OAc), 20.7, 20.8 (OAc), 21.0 (OAc), 25.0, 25.6, 26.3, 27.3, 27.7, 33.2 (C-4, C-5, C-8, C-9), 64.0 (C-3), 117.2, 117.3, 121.0 (C-7, C-3′, C-4′), 124.5, 127.1, 127.3, 130.2, 130.4, 132.5, 132.7, 142.7, 146.1 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.8, 168.9 ($OCOCH_3$), 171.1 ($OCOCH_3$). Anal. Found (%): C 60.29; H 5.73. Calcd for $C_{19}H_{21}ClO_6$ (%): C 59.92; H 5.56.

3.1.10. Saturated diacetate 11. Synthesised by catalytic hydrogenation of the unsaturated diacetate **2** with H_2/Pd/C in ethyl acetate (63%); oil; IR cm^{-1}: 3073 (aromatic CH), 2986–2926 (aliphatic CH), 1769 (ester C=O). ^1H NMR ($CDCl_3$) δ: 0.91 (d, $J = 6.6$, 6H, H-1, H-10), 1.14–2.29 (m, 10H, H-2, H-3, H-4, H-5, H-6, H-7), 2.29, 2.31 (s, 3H, OAc), 2.35, 2.37 (s, 3H, OAc), 2.44–2.94 (m, 4H, H-8, H-9), 7.01 (s, 1H, H-3′, H-4′). ^{13}C NMR ($CDCl_3$) δ: 22.6 (C-1, C-10), 20.3, 20.4 (OAc), 20.6, 20.7 (OAc), 23.4, 24.1, 27.8, 27.9, 30.0, 30.7, 32.6, 32.7, 36.3, 39.1 (H-2, H-3, H-4, H-5, H-6, H-7, H-8), 120.4, 120.5 (C-3′, C-4′), 123.9, 124.0, 129.9, 133.0, 143.1, 143.2, 146.5, 146.6 (C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.8, 168.9 ($OCOCH_3$). Anal. Found (%): C 65.78; H 7.69. Calcd for $C_{20}H_{27}ClO_4$ (%): C 65.47; H 7.42.

3.1.11. Saturated aromatised diacetate 12. Synthesised by catalytic hydrogenation of the aromatised diacetate **3** with H_2/Pd/C in ethyl acetate (70%); oil; IR cm^{-1}: 3069–3055 (aromatic C–H), 2950–2855 (aliphatic C–H) 1769 (ester C=O); ^1H NMR ($CDCl_3$) δ: 0.90 (d, $J = 6.6$, 6H, H-1, H-10), 1.29 (m, 2H, H-4), 1.63 (m, 3H, H-2 and H-3), 2.44, 2.47 (s, 3H, OAc), 2.50, 2.52, (s, 3H, OAc), 2.76 (t, $J = 7.5$, 2H, H-5), 7.30, 7.34 (s, 1H, H-3′, H-4′), 7.40, 7.44 (dd, $J = 6.8$, 1.8, 1H, $2 \times$ H-7), 7.59, 7.60 (d, $J = 1.7$, H-9), 7.74, 7.78 (d, $J = 6.8$, $2 \times$ H-8) ^{13}C NMR ($CDCl_3$) δ: 20.5, 20.6 (OAc), 20.9, 21.0 (OAc), 22.6 (C-1, C-10), 27.9, 29.0, 36.4, 36.5, 38.6 (C-2, C-3, C-4, C-5), 118.5, 119.4, 119.8, 120.1 (C-7, C-8, C-9, C-3′, C-4′) 122.6, 124.7, 126.6, 126.9, 128.6, 129.6, 140.5, 140.9, 142.1, 143.1, 144.2, 144.6 (C-6, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′), 168.1, 168.2 ($OCOCH_3$), 168.9, 169.0 ($OCOCH_3$). Anal. Found (%): C 66.38; H 6.56. Calcd for $C_{20}H_{23}ClO_4$ (%): C 66.20; H 6.39.

3.2. Bioactivity

Cells were seeded into 16-mm well (multidishes, NUNC 42001) at concentrations of 2×10^4 cells/wells, in 1-mL aliquots of MEM10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After 3 days at 37 °C, under a 10% CO_2, 98% humidity atmosphere, cells were stained with crystal violet, observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls. All calculations represent the average of duplicated wells.

For Jurkat-77, cells were seeded into 25-mm well (multidishes, NUNC 150318) at concentrations of 13×10^4 cells/mL, in RPMI 1604-SBF10% medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After 4 days at 37 °C, under a 5% CO_2, 90% humidity atmosphere, cells were stained with Blue Tripan, observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls. All calculations represent the average of triplicated wells.

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References and notes

1. Gordaliza, M.; Miguel del Corral, J. M.; Castro, M. A.; Mahiques, M. M.; García-Grávalos, M. D.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1859.
2. Miguel del Corral, J. M.; Gordaliza, M.; Castro, M. A.; Mahiques, M. M.; García-Grávalos, M. D.; San Feliciano, A. *Bioorg. Med. Chem.* **1998**, *6*, 31.

3. Miguel del Corral, J. M.; Gordaliza, M.; Castro, M. A.; Mahiques, M. M.; Chamorro, P.; Molinari, A.; García-Grávalos, M. D.; Broughton, H. B.; San Feliciano, A. *J. Med. Chem.* **2001**, *44*, 1257.
4. Molinari, A.; Oliva, A.; Aguilera, N.; Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; García Grávalos, M. D.; San Feliciano, A. *Bioorg. Med. Chem.* **2000**, *8*, 1027.
5. Molinari, A.; Oliva, A.; Aguilera, N.; Miguel del Corral, J. M.; Gordaliza, M.; Castro, M. A.; García Grávalos, M. A.; San Feliciano, A. *Bol. Soc. Chil. Quim.* **2001**, *46*, 33.
6. Müller, W. G. E.; Maidhof, A.; Zahn, R. K.; Schröder, H. C. M.; Gasic, M. J.; Heidemann, D.; Bernd, A.; Kurelec, B.; Eich, E.; Siebert, G. *Cancer Res.* **1985**, *45*, 4822.
7. Sarin, P. S.; Sun, D.; Thornton, A.; Müller, W. G. E. *J. Natl. Cancer Inst.* **1978**, *78*, 663.
8. Molinari, A.; Oliva, A.; Reinoso, P.; Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; Gupta, M. P.; Solis, P.; San Feliciano, A. *Eur. J. Med. Chem.* **2002**, *37*, 177.
9. Molinari, A.; Oliva, A.; Araya, C.; Miguel del Corral, J. M.; Gordaliza, M.; Castro, M. A.; García Grávalos, M. D.; San Feliciano, A. *Il Fármaco* **2004**, *59*, 651.
10. Chang, H. X.; Chou, T. C.; Savaraj, N.; Liu, L. F.; Yu, C.; Cheng, C. C. *J. Med. Chem.* **1999**, *42*, 405.
11. Ryu, C. K.; Lee, I. K.; Jung, S. H.; Lee, C. O. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1075.
12. Brzozowski, Z.; Sacedilczewski, F.; Gdaniec, M. *Bioorg. Med. Chem.* **2003**, *11*, 3673.
13. Pomarnacka, E.; Gdaniec, M. *Bioorg. Med. Chem.* **2003**, *11*, 1259.
14. Slawinski, J. *Eur. J. Med. Chem.* **2004**, *39*, 179.
15. Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garaliene, V.; Farruggia, G.; Masotti, L. *Bioorg. Med. Chem.* **2004**, *12*, 1121.
16. Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thomson, S. C. *J. Med. Chem.* **1987**, *30*, 1998.