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New cytotoxic-antineoplastic prenyl-1,2-naphthohydroquinone derivatives

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Abstract—Several new prenylnaphthohydroquinone derivatives have been prepared through the Diels–Alder condensation between α-myrcene and 1,2-benzoquinone and evaluated for their cytotoxic activity against A-549, HT-29 and MB-231 cultured cell lines. All of them have shown GI_{50} values in the μM level.
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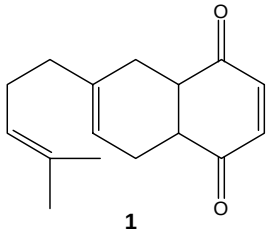
1. Introduction

Prenyl-1,4-naphthohydroquinone derivatives are terpenyl hydroquinones with interesting antitumoural properties against a wide variety of cultured cell lines. They can be synthesised through acetylation and further chemical transformations of the Diels–Alder adduct **1** (Fig. 1), prepared from α-myrcene and 1,4-benzoquinone.[1–4]

In general, all prenyl-1,4-naphthohydroquinone derivatives of **1** are bioactive 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 cell lines (IC_{50} or $GI_{50} < 0.3$- > 30 μM). According to SAR studies, cytotoxicity is enhanced when the carbocyclic part of **1** is aromatised, when the side chain is saturated or when the side chain is functionalized with an acetate or a methoxycarbonyl group. In these cases, the IC_{50} values are below the μM level (0.3 μM, P-388).[3] In addition, the fully aromatised compound with a saturated side chain shows a GI_{50} lower than 0.3 μM (MCF-7).[4] Substituents at the C-2 position of the naphthohydroquinonic core also influence the antitumoural activity of this type of compounds. Introduction of an acetyl group at this position resulted in a decrease of cytotoxicity and only the acetyl derivative bearing a methyl ester group on the terpenic side chain displayed an IC_{50} value in the range of 1 μM against an A-549 cultured cell line.[5] Furthermore, halogens and nitrogen-containing functional groups at 2/3 positions of the naphthohydroquinone ring influence both cytotoxic potency and selectivity against DU-145 prostate carcinoma, SK-BR3 breast adenocarcinoma, MEL-28, A-549, K-562 myelogenous leukaemia, PANC-1 pancreatic epithelioid carcinoma HT-29, LoVo-Dox colon adenocarcinoma and HeLa cervix epithelioid carcinoma neoplastic cell lines.[6,7]



1

Figure 1. Structure of the Diels–Alder product of myrcene and 1,4-benzoquinone.

The antitumoural activity of natural or synthetic 1, 2-naphthoquinone-containing compounds has been studied to a much lesser extent and prenyl-1,2-naph-

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thohydroquinone derivatives of myrcene are not known. Puupehedione, puupehenone, chloropuupehenone, 15-cyanopuupehenone, smenorthoquinone and smenopondiol are examples of natural sesquiterpenyl-1,2-quinone or 1,2-hydroquinone derivatives isolated from marine sponges, which are bioactive against P-388, A-549, HT-29, L1210, KB and LoVo neoplastic cultured cells.[8–14] Synthetic 4,5-diamino-substituted-1,2-benzoquinone, 1,2-pyrano-naphthoquinones, 1,2-furanaphthoquinones and *N*-(3,4-dimethyl-5-isoxazolyl)-4-amino-1,2-naphthoquinone compounds, among others, have been screened against L 1210, HeLa, KB, $HepG_2$ and L-6 cultured cells.[15–17]

Taking into account these antecedents, in this paper we wish to report the preparation of a family of prenyl-1,2-naphthohydroquinone derivatives **2**–**10** (Fig. 2) through chemical modifications of the Diels–Alder product from α-myrcene and 1,2-benzoquinone. The cytotoxic-antineoplastic properties of a regioisomeric mixture of these new compounds have been evaluated against cultured cells of A-549 human lung carcinoma, HT-29 human colon carcinoma and MB-231 breast adenocarcinoma.

2. Results and discussion

2.1. Chemistry

The starting prenyl-1,2-hydroquinonic diacetate **2** was obtained as a 4:1 regioisomeric mixture from the Diels–Alder condensation product of 1,2-benzoquinone with myrcene, followed by acetylation with acetic anhydride in pyridine. The presence of both regioisomers and the ratio was deduced from the ^1H NMR spectra signal of the carbocyclic olefinic proton 6/7, which are differentiated at 5.59 (**2a**) and 5.55 ppm (**2b**), respectively (Fig. 2), and also from the complexity of the ^{13}C NMR spectra of compounds **2**–**10**, where most of the signals for carbons are duplicated. For example, in the ^{13}C spectra the acetate groups of the prenyl-1,4-naphthohydroquinone derivatives display two signals at 168–169 ppm [1–3]; meanwhile, four signals are observed in the 1,2-derivatives **2**–**10**. The regioselectivity and the proposition of **2a** as the major regioisomer can be supported on the heterolytic character of the BF_3-catalysed Diels–Alder reaction and in the higher stability of the electron-deficient centre in the proposed transition model[18,19] (Fig. 3).

The regioisomeric mixture of **2** was chemically modified by catalytic hydrogenation with H_2/Pd/C in EtOAc, aromatisation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), epoxidation with *m*-chloroperbenzoic acid (MCPBA), oxidative cleavage of epoxides with periodic acid (H_5IO_6), chemoselective reduction with sodium borohydride ($NaBH_4$), acetylation with acetic anhydride/pyridine, oxidation of aldehyde with sodium chlorite ($NaClO_2$) and methylation of the carboxylic group with diazomethane, affording the compounds **3**–**10** (Fig. 2). The ^1H and ^{13}C NMR data according to carbon numbering of compound **2** in Figure 4, the IR absorption and other physical information are given in the experimental part.

2.2. Bioactivity

A panel of three human tumour cell lines was used to evaluate in vitro the cytotoxic potential of the compounds **2**–**10**: A-549 lung carcinoma, HT-29 colon carcinoma and MB-231 breast adenocarcinoma. The results obtained are shown in Table 1 and the following general observations can be made.

(a) As it has been observed with 1,4-hydroquinonic compounds,[1–3] the prenyl-1,2-naphthohydroquinone derivatives **2**–**10** reported here are also cytotoxic compounds against the three cell lines assayed.
(b) The GI_{50} (μM) values ranged between 5.51 and >25.0 (A-549), 4.84 and >25.0 (HT-29), and 5.15 and >25.0 (MB-231).

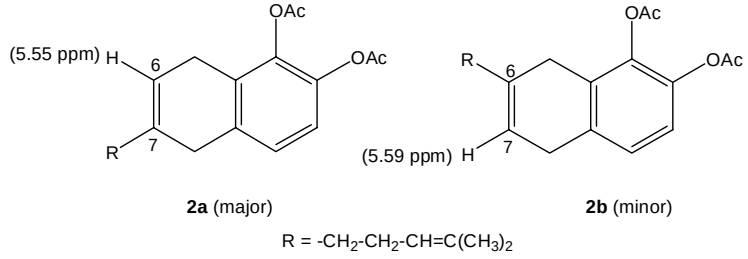

Figure 2. Regioisomers of the Diels–Alder condensation product between myrcene and 1,2-benzoquinone.

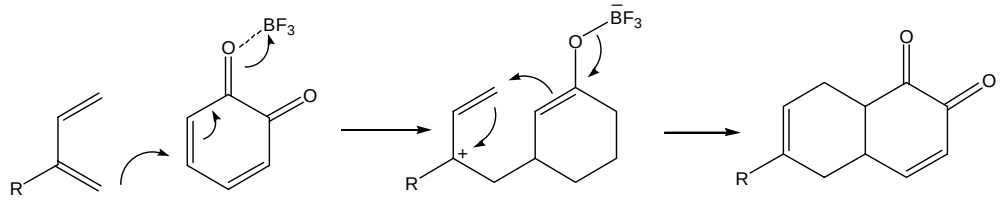

Figure 3. Proposed formation of the major regioisomer **2a**.

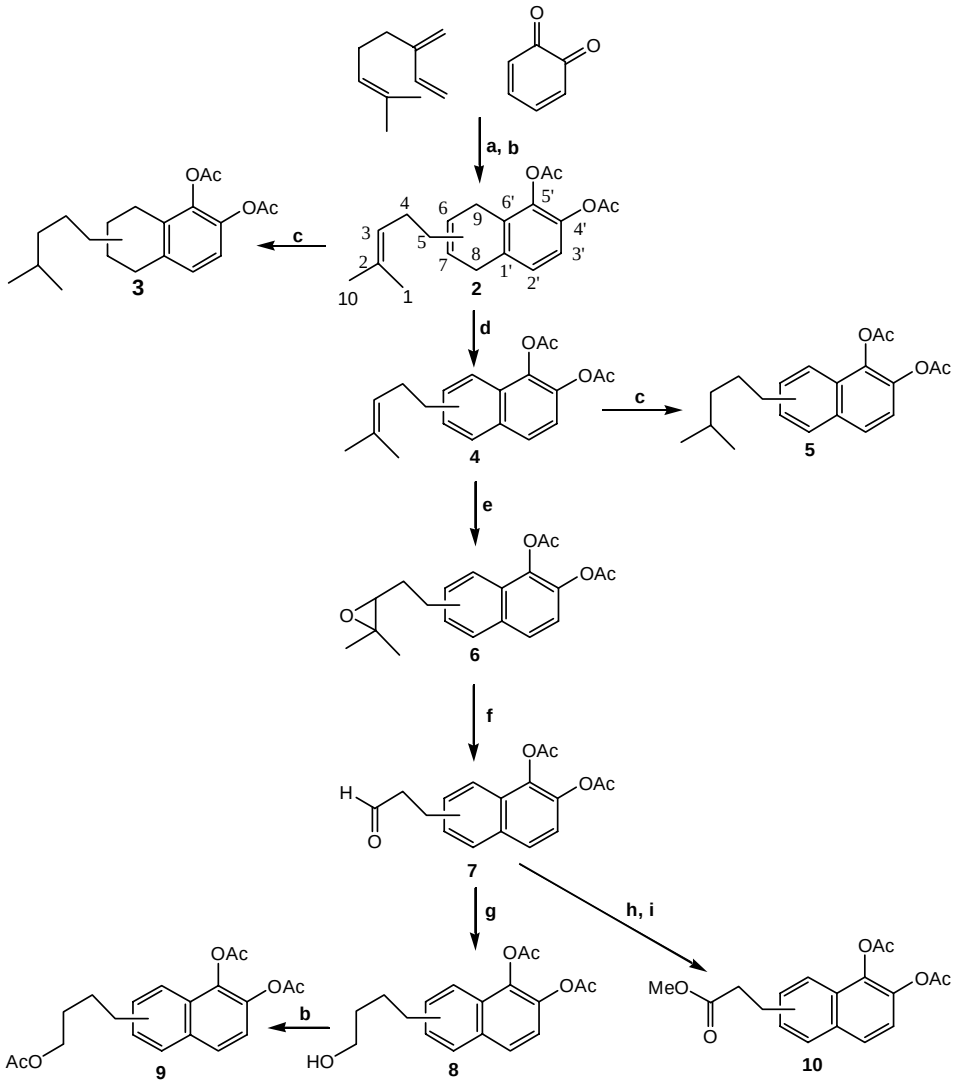

Figure 4. Prenyl-1,2-naphthohydroquinone derivatives **2**–**10** prepared. Reagents and conditions: (a) BF_3·ether, CH_2Cl_2, 60 h; (b) Ac_2O, Py, rt, 24 h; (c) H_2/Pd, AcOEt, rt, 24 h; (d) DDQ, refluxing benzene, 1 h; (e) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 45 min; (f) H_5IO_6, THF, H_2O, rt, 1 h; (g) $NaBH_4$, MeOH, rt, 15 s; (h) $NaClO_2$, NaH_2PO_4, H_2O, *t*-BuOH, 2-methyl-2-butene, rt, 72 h; (i) CH_2N_2, Et_2O, rt, 30 min.

Table 1. Cytotoxicity of prenyl-1,2-naphthohydroquinone derivatives **2**–**10** (GI_{50}, μM)

Compound	A-549	HT-29	MB-231
2	>25.0	>25.0	>25.0
3	24.7	>25.0	12.2
4	5.51	11.6	6.74
5	12.5	13.1	12.2
6	9.93	10.5	10.2
7	10.3	9.99	10.3
8	10.6	10.6	5.95
9	9.58	9.87	9.87
10	5.75	4.84	5.15

(c) According to the GI_{50} values found, the fully aromatised compounds **4**–**10** are more cytotoxic than the unsaturated and saturated derivatives **2** and **3**. These results confirm that aromatisation of the ring fused to the hydroquinone core improves cytotoxicity of both the 1,4 derivatives previously reported,[1–5] as well as of the new 1,2-naphthohydroquinone compounds reported here.

(d) The GI_{50} values of compound **10** against the three lines assayed show that the presence of a methoxycarbonyl substituent on the terpenic moiety is also important for the enhancement of bioactivity in both prenyl-1,2 and 1,4-naphthohydroquinones.[5,20]

3. Experimental

All NMR spectra were recorded in the Centro de Resonancia Magnética Nuclear V Región, located at the Universidad Técnica Federico Santa María, Valparaíso, Chile, on a Avance 400 Digital NMR Bruker spectrometer operating at 400.132 MHz for ^1H and 100.623 MHz for ^{13}C in deuterochloroform with internal TMS as reference. Chemical shifts are expressed in ppm, followed by multiplicity and coupling constant (*J*) in Hertz. IR spectra were recorded on a Perkin-Elmer FT IR 1600 spectrophotometer, as a film over sodium chloride discs. Elemental analysis of carbon and hydrogen was obtained with a Perkin-Elmer 2400 Serie II CHN

Elemental Analyser within ±0.4% of the theoretical values. All the compounds are viscous oils and have been purified by column chromatography on Silicagel 60, 230–400 mesh ASTM, using mixtures of *n*-hexane/ethyl acetate with variable proportions as eluent.

3.1. Chemistry

3.1.1. 1,2-Benzoquinone. Synthesised in situ, according to a described procedure,[21] by the oxidation of an aqueous solution of catechol with an aqueous solution of sodium periodate (1:2 molar ratio), followed by extraction with CH_2Cl_2.

3.1.2. Unsaturated diacetate 2. Synthesised by Diels–Alder condensation of 1,2-benzoquinone with myrcene in CH_2Cl_2 and $BF_3{\cdot}Et_2O$ as catalyst, at room temperature during 60 h. The product was purified by CC with hexane/ethyl acetate 7:3 as eluent (47%); oil; IR cm^{-1}: 3072 (aromatic, olefinic CH) , 2954–2860 (aliphatic CH), 1766 (C=O ester). ^1H NMR ($CDCl_3$) δ: 1.61 (s, 3H, H-1), 1.69 (s, 3H, H-10), 2.06-2.16 (m, 4H, H-4, H-5), 2.26, 2.30, 2.32 (s, 6H, OAc), 3.09–3.40 (m, 4H, H-8, H-9), 5.12 (m, 1H, H-3), 5.55, 5.59 (br s, 1H, H-6, H-7), 6.97–7.05 (m, 2H, H-2′, H-3′). ^{13}C NMR ($CDCl_3$) δ: 17.7 (C-1), 20.4, 20.5, 20.7, 20.8 (OAc), 25.7 (C-10), 25.2, 26.1, 27.7, 29.9, 37.0, 37.1 (CH_2: C-4, C-5, C-8, C-9), 116.8, 118.1, 120.4, 120.5, 123.9, 124.0, 126.1, 126.2 (CH: C-3, C-6, C-7, C-2′, C-3′), 131.8, 133.8,134.0, 135.2, 139.8, 140.1, (C: C-2, C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.1, 168.2, 168.6, 168.7 (OAc). Anal. Calcd for $C_{20}H_{24}O_4$ (%): C, 73.15; H, 7.37. Found (%): C, 73.50; H, 8.10.

3.1.3. Fully saturated diacetate 3. Synthesised by catalytic hydrogenation of the unsaturated diacetate **2** with H_2/Pd/C in ethyl acetate at room temperature during 24 h. The product was purified by CC with hexane/ethyl acetate 3:1 as eluent (63%); oil; IR cm^{-1}: 3073, 3030 (aromatic, olefinic CH) , 2942, 2919 (aliphatic CH), 1773 (C=O ester). ^1H NMR ($CDCl_3$) δ: 0.88 (d, 6H, $J = 6.6$, H-1, H-10), 2.26, 2.30, 2.31 (s, 6H, OAc), 2.47–2.82 (m, 4H, H-8, H-9), 6.91–7.01 (m, 2H, H-2′, H-3′). ^{13}C NMR ($CDCl_3$): δ: 20.3, 20.4, 20.7, 20.8 (OAc), 22.6 (C-1, C-10), 27.9, 33.5, 23.5, 24.6, 28.4, 28.7, 29.0, 30.2, 35.9, 36.6, 39.2 (CH_2: C-3, C-4, C-5, C-6, C-7, C-8, C-9), 27.9, 28.0, 33.2, 33.5 (CH: C-2, C-6; C-7), 119.9, 120.0, 126.7, 127.0 (CH: C-2′, C-3′), 131.1, 136.4, 139.9, 140.2 (C: C-1′, C-4′, C-5′, C-6′), 168.1,168.2,168.6, 168.7 (OAc). Anal. Calcd for $C_{20}H_{28}O_4$ (%): C, 72.26; H, 8.49. Found (%): C, 72.48; H, 8.83.

3.1.4. Unsaturated aromatised diacetate 4. Synthesised by DDQ aromatisation of **2** in refluxing benzene during 1 h. The product was purified by CC with hexane/ethyl acetate 3:1 as eluent (63%); oil; IR cm^{-1}: 3070 (aromatic, olefinic CH) , 2943–2820 (aliphatic CH), 1775(C=O ester). ^1H NMR ($CDCl_3$) δ: 1.56 (s, 3H H-1), 1.68 (s, 3H, H-10), 2.32, 2.45, 2.46 (s, 6H, OAc), 2.35–2.39 (m, 2H, H-4), 2.79 (t, 2H, $J = 7.7$, H-5), 5.17 (m, 1H, H-3), 7.24–7.38 (m, 2H, H-2′, H-3′), 7.63–7.75 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 18.0 (C-1)), 20.4, 20.5, 20.9, 21.0 (OAc), 26.0 (C-10), 30.0, 30.1 36.4, 36.8 (CH_2: C-4, C-5), 119.9, 121.0, 121.4, 121.9, 123.7, 123.8, 126.5, 126.7, 126.9, 128.2, 128.3, 128.9 (CH: C-3, C-6, C-7, C-8, C-9, C-2′, C-3′), 131,2, 132.8, 132.9, 137.3, 138.7, 139.5, 140.7, 141.7 (C: C-2, C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.4, 168.5, 168.9, 169.0 (OAc). Anal. Calcd for $C_{20}H_{22}O_4$ (%): C, 73.60; H, 6.79. Found (%): C, 73.28; H, 6.33.

3.1.5. Saturated aromatised diacetate 5. Synthesised by catalytic hydrogenation of the aromatised diacetate **4** with H_2/Pd/C in ethyl acetate during 24 h. The product was purified by CC with hexane/ethyl acetate 3:1 as eluent (48%); oil; IR cm^{-1}: 3072, 3025 (aromatic, olefinic C–H) , 2942–2919 (aliphatic C-H), 1772 (C=O ester); ^1H NMR ($CDCl_3$) δ: 0.88 (d , 6H, $J = 6.6$, H-1, H-10), 1.62 (m,1H, H-2), 1.22–1.26, 1.67–1.69 (m, 4H, H-3, H-4), 2.32, 2.44, 2.46 (s, 6H, OAc), 2.73 (t, 2H, $J = 7.2$, H-5), 7.24–7.39 (m,2H, H-2′, H-3′) ,7.61–7.65 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 20.7, 20.8, 21.1, 21.2(OAc),, 22.9, 28.2 (C-1, C-10), 29.4, 29.5, 36.4, 36.9, 38.8, 38.9 (CH_2: C-3, C-4, C-5) 31.2 (C-2), 119.8, 120.9, 121.4, 121.9, 126.5, 126.7, 128.1, 128.2, 128.9 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 131.2, 133.0, 136.9, 137.3, 138.8, 139.5, 141.3, 142.3 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.4, 168.5, 168.8, 168.9 (OAc). Anal. Calcd for $C_{20}H_{24}O_4$ (%): C, 73.15; H, 7.37. Found (%): C, 73.28; H, 6.83.

3.1.6. Aromatised epoxidiacetate 6. Synthesised by MCPBA epoxidation of unsaturated aromatised diacetate **4** in dichloromethane and $NaHCO_3$ at room temperature during 45 min. The product was purified by CC with hexane/ethyl acetate 2:1 as eluent (76%); oil; IR cm^{-1}: 3072 (aromatic, olefinic C–H), 2966, 2931, 2861 (aliphatic C–H), 1772 (C=O ester). ^1H NMR ($CDCl_3$) δ: 1.18 (s, 3H, H-1), 1.29 (s, 3H, H-10), 1.91–1.97 (m, 2H, H-4), 2.35, 2.47, 2.48 (s, 6H, OAc), 2.81 (t, 1H, $J = 6.1$, H-3), 2.91-3.03 (m, 2H, H-5), 7.28–7.43 (m, 2H, H-2′, H-3′), 7.69–7.80 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 18.6, 18,7 (C-1), 20.4, 20.5, 20.7, 20.8 (OAc), 24.6, 24.7 (C-10), 30.5, 30.6, 32.7, 33.1 (CH_2: C-4, C-5), 58.6, 58.7 (C-2), 63.6, 63.7 (C-3), 119.7, 121.0, 121.3, 121.8, 126.1, 126.4, 126.6, 127.6, 128.1, 128.4, 130.0 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 131.0, 132.5, 136.9, 138.7, 139.4, 140.4 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.1, 168.2, 168.5, 168.6 (OAc). Anal. Calcd for $C_{20}H_{22}O_5$ (%): C, 70.16; H, 6.48. Found (%): C, 69.80; H, 6.02.

3.1.7. Aromatised aldehyde diacetate 7. Synthesised by degradative H_5IO_6 oxidation of the aromatised epoxidiacetate **6** in aqueous THF at room temperature during 1 h. The product was purified by CC with hexane/ethyl acetate 1:2 as eluent (74%); oil; IR cm^{-1}: 3072, (aromatic, C–H), 2966, 2931 (aliphatic C–H), 2791 (aldehyde C–H), 1766 (C=O ester), 1719 (C=O aldehyde). ^1H NMR ($CDCl_3$) δ: 2.32, 2.44, 2.47 (s, 6H, OAc), 2.83 (t, 2H, $J = 7.6$, H-4), 3.10 (t, 2H, $J = 7.6$, H-5), 7.27–7.38 (m, 2H, H-2′, H-3′), 7.60–7.79 (m, 3H, H-7, H-8, H-9), 9.82, 9.83 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ: 20.5, 20.6, 20.8, 20.9 (OAc), 28.0, 28.4 (C-5), 45.0, 45.2 (C-4), 119.8, 121.2, 121.6, 122.0, 126.2, 126.4, 126.6,

127.3, 128.2, 128.5 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 131.1, 132.5, 137.0, 138.5, 138.9, 139.5 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.2, 168.3, 168.5, 168.6 (OAc), 201.2, 201.3 (C-3). Anal. Calcd for $C_{17}H_{16}O_5$ (%): C, 67.99; H, 5.37. Found (%): C, 68.32; H, 4.95.

3.1.8. Aromatised hydroxyl diacetate 8. Synthesised by $NaBH_4$ reduction of the aromatised aldehyde diacetate **7** in methanol, at room temperature during 15 s. The product was purified by CC with hexane/ethyl acetate 2:1 as eluent (53%); oil; IR cm^{-1}: 3433 (OH), 2976, 2921 (aliphatic CH), 1769 (C=O ester). ^1H NMR ($CDCl_3$) δ: 1.95 (m, 2H, H-4), 2.36, 2.47, 2.49 (s, 6H, OAc), 2.86 (t, 2H, $J = 6.5$, H-5), 3.68 (t, 2H, $J = 6.5$, H-3), 7.27–7.42 (m, 2H, H-2′, H-3′), 7.66–7.78 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 19.0, 19.1, 19.5, 19.6 (OAc), 30.4, 30.9,32.4, 32.5 (CH_2: C-4, C-5), 60.4, 60.5 (C-3), 118.1, 119.3, 119.7, 120.2, 124.6, 124.9, 125.1, 126.2, 126.6, 127.0 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 127.7, 128.2, 131.0, 132.0 136.9, 138.6, 140.0, 141.0 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.3, 168.4, 168.6, 168.7 (OAc). Anal. Calcd for $C_{17}H_{18}O_5$ (%): C, 67.54; H, 6.00. Found (%): C, 68.01; H, 6.36.

3.1.9. Aromatised triacetate 9. Synthesised by acetylation of the aromatised hydroxyl diacetate **8** with acetic anhydride and pyridine in anhydrous diethyl ether at room temperature during 24 h. The product was purified by CC with hexane/ethyl acetate 2:1 as eluent (78%); oil; IR cm^{-1}: 3072 (aromatic CH), 2942, 2861(aliphatic CH), 1766 (C=O aromatic acetate), 1732 (C=O aliphatic acetate). ^1H NMR ($CDCl_3$) δ: 1.98–2.03 (m, 2H, H-4), 2.05 (s, 3H, OAc aliphatic), 2.33, 2.44, 2.47 (s, 6H, OAc aromatic), 2.83 (t, 2H, $J = 7.0$, H-5), 4.12 (t, 2H, $J = 6.5$, H-3), 7.26–7.38 (m, 2H, H-2′, H-3′), 7.64–7.77 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 20.4, 20.5, 20.7, 20.8 (OAc aromatic), 21.1 (OAc aliphatic), 30.0, 30.1, 32.2, 32.6 (CH_2: C-4, C-5), 63.7, 63.8 (C-3), 119.7, 121.0, 121.4, 121.8, 126.2, 126.4, 126.6, 127.6, 128.2, 128.3 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 131.0, 132.6, 136.6, 137.0, 138.8, 139.3, 139.4,140.3 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.1, 168.2, 168.5, 168.6 (OAc aromatic), 171.1 (OAc aliphatic). Anal. Calcd for $C_{19}H_{20}O_6$ (%): C, 66.27; H, 5.85. Found (%): C, 66.52; H, 5.48.

3.1.10. Aromatised methyl ester diacetate 10. Synthesised by $NaClO_2$ oxidation of the aromatised aldehyde diacetate **7** in H_2O, *t*-BuOH, NaH_2PO_4 and 2-methyl-2-butene at room temperature during 72 h. The acid was methylated with diazometane in anhydrous diethyl ether at room temperature during 30 min. The product was purified by CC with hexane/ethyl acetate 1:1 as eluent (64%); oil; IR cm^{-1}: 3061 (aromatic C–H), 2967, 2880 (aliphatic C–H), 1769 (C=O ester), 1738 (C=O methyl ester). ^1H NMR ($CDCl_3$) δ: 2.36, 2.47, 2.50 (s, 3H OAc), 2.72 (t, 2H, $J = 7.6$, H-4), 3.11 (t, 2H $J = 7.6$, H-5), 3.68, 3.69 (s, 3H, OMe), 7.29–7.42 (m, 2H, H-2′, H-3′), 7.63–7.80 (m, 3H, H-7, H-8, H-9). ^{13}C NMR ($CDCl_3$) δ: 20.4, 20.5, 20.8, 20.9 (OAc), 30.9, 31.3, 35.4, 35.5 (CH2: C-4, C-5), 51.7 (OMe), 119.7, 121,121.5, 121.8, 126.3, 126.4, 126.7, 127.4, 128.2, 128.3 (CH: C-6, C-7, C-8, C-9, C-2′, C-3′), 131.1, 132.6, 136.7, 136.9, 138.6, 138.8, 139.4, 139.5 (C: C-6, C-7, C-1′, C-4′, C-5′, C-6′), 168.2, 168.3,168.6, 168.7 (OAc), 173.1 (C-3). Anal. Calcd for $C_{18}H_{18}O_6$ (%): C, 65.45; H, 5.49. Found (%): C, 65.93; H, 5.35.

3.2. Bioactivity

A colourimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[22,23] Cells were seeded in 96-well microtitre plates, at 5×10^3 cells/well in aliquots of 195 μL RPMI medium and were allowed to attach to the plate surface by growing in a drug-free medium for 18 h. Afterwards, samples were added in aliquots of 5 μL (dissolved in DMSO/H_2O, 3:7). After 72 h exposure, the antitumour effect was measured by the SRB method: cells were fixed by adding 50 mL cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates were washed with deionised water and dried; 100 μL of SRB solutions (0,4% wt/vol in 1% acetic acid) was added to each microtitre well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried and bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses were generated automatically by LIMS implementation. Using control OD values (C), test OD values (T) and time zero OD values (T_0), the drug concentration that causes 50% growth inhibition (GI_{50} value) was calculated from the equation: $100 \times [(T - T_0)/C - T_0] = 50$.

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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*, 1859–1864.
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*, 31–41.
3. 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*, 1027–1032.
4. Molinari, A.; Oliva, A.; Reinoso, P.; Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; Gupta, M. P.; Solís, P.; San Feliciano, A. *Eur. J. Med. Chem.* **2002**, *37*, 177–182.
5. Molinari, A.; Oliva, A.; Miguel del Corral, J. M.; Castro, M. A.; Araya, C.; García-Grávalos, M. D.; San Feliciano, A. *Il Fármaco* **2004**, *59*, 651–656.
6. Molinari, A.; Oliva, A.; Ojeda, C.; Escobar, J.; Miguel del Corral, J. M.; Castro, M. A.; Cuevas, C.; San Feliciano, A. *Bioorg. Med. Chem.* **2005**, *13*, 3841–3846.

7. Miguel del Corral, J. M.; Castro, M. A.; Gordaliza, M.; Gordaliza, M.; Martin, L.; Gualberto, S. A.; Gamito, A. M.; Cuevas, C.; San Feliciano, A. *Bioorg. Med. Chem.* **2005**, *13*, 631–644.
8. Ravi, B. N.; Perzanowsky, H. P.; Ross, R. A.; Erdmai, T. R.; Scheuer, P. J.; Finer, J.; Clardy, P. *J. Pure Appl. Chem.* **1979**, *51*, 1893–1900.
9. Amade, P.; Chevolot, L.; Perzanowsky, H. P.; Scheuer, P. J. *Helv. Chim. Acta* **1983**, *66*, 1672–1675.
10. Kondracki, M. L. *Tetrahedron* **1989**, *45*, 1995–2004.
11. Hamann, M. T.; Scheuer, P. J. *Tetrahedron Lett.* **1991**, *32*, 5671–5672.
12. Hamann, M. T.; Scheuer, P. J. *J. Org. Chem.* **1993**, *58*, 6565–6569.
13. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Chahboun, R.; Cortés, M.; Armstrong, V. *Tetrahedron* **1999**, *55*, 15181–15208.
14. Castro, M. E.; Gonzalez-Iriarte, M.; Barrero, A. F.; Salvador-Tormo, N.; Muñoz-Chápuli, R.; Medina, M. A.; Quezada, A. R. *Int. J. Cancer* **2004**, *110*, 31–38.
15. Huang, Z. D.; Chen, Y. N.; Menon, K.; Teicher, B. *J. Med. Chem.* **1993**, *36*, 1797–1801.
16. Kongkathip, N.; Kongkathip, B.; Siripong, P.; Sangma, C.; Luangkamin, S.; Niyomdecha, M.; Pattanapa, S.; Piyaviriyagul, S.; Kongsaeree, P. *Bioorg. Med. Chem.* **2003**, *11*, 3179–3191.
17. Sperandeo, N. R.; Briñón, M. C.; Brun, R. *Il Farmaco* **2004**, *59*, 431–435.
18. Inukai, T.; Kojima, T. *J. Org. Chem.* **1967**, *32*, 869–871.
19. Inukai, T.; Kojima, T. *J. Org. Chem.* **1966**, *31*, 1121–1123.
20. 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*, 1257–1267.
21. Adler, E.; Falkehag, I.; Smith, B. *Acta Chem. Scand.* **1962**, *16*, 529–540.
22. Skenan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D. J.; Waren, T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
23. Faircloth, G. T.; Stewart, D.; Clement, J. J. *J. Tissue Culture Methods* **1998**, *11*, 201–205.