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Original article

Superior anticancer activity of halogenated chalcones and flavonols over the natural flavonol quercetin

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ABSTRACT

A series of chalcone and flavonol derivatives were synthesized in good yield by an eco-friendly approach. A pharmacological evaluation was performed with the human colorectal carcinoma cell line HCT116 and revealed that the anticancer activity of flavonols was higher when compared with that of the respective chalcone precursors. The antiproliferative activity of halogenated derivatives increases as the substituent in the 3- or 4-positon of the B-ring goes from F to Cl and to Br. In addition, halogens in position 3 enhance anticancer activity in chalcones whereas for flavonol derivatives the best performance was registered for the 4-substituted derivatives. Flow cytometry analysis showed that compounds **3p** and **4o** induced cell cycle arrest and apoptosis as demonstrated by increased S, G2/M and sub-G1 phases. These data were corroborated by western blot and fluorescence microscopy analysis. In summary, halogenated chalcones and flavonols were successfully prepared and presented high anticancer activity as shown by their cell growth and cell cycle inhibitory potential against HCT116 cells, superior to that of quercetin, used as a positive control.

1. Introduction

Cancer is one of the leading causes of death in the contemporary western society [1]. Colorectal cancer is the most frequently diagnosed type of cancer in Europe and the second most common cause of cancer death [2]. The search for new molecules with the capacity to inhibit tumour initiation as well as tumour progression is an important contribution to overcome this problem [3,4]. Natural products have been an effective source of bioactive molecules, where the flavonoid scaffold is a central group. This moiety is widely present in fruits, vegetables, tea and certain spices, and has been associated with a range of health benefits due, in part, to its radical quenching properties [5].

Chalcones can be envisaged as open-chain flavonoids, where the two aromatic rings are linked by a conjugated three-carbon system, with a carbonyl and an alkene group. They are often used as precursors in the synthesis of flavonoid derivatives [6,7].

Extensive research on the biological activity of chalcones included anti-inflammatory, antibacterial [8], antileishmanial, antimalarial, antifungal, antituberculosis, antioxidant, antiviral and anticancer activity, a subject that has been reviewed over the years [9]. The chemopreventive potential of some chalcone derivatives is often emphasized [10,11] and includes a number of natural products incorporating this core structure (Fig. 1) [12–14].

Among the bioactive natural products of the flavonol family (Fig. 1), quercetin, a major dietary flavonoid [15–17], has been widely studied, considering the potent antioxidant activity of this compound [18]. A recent report demonstrated that flavonols, in particular those which are less methoxylated, present greater antiproliferative activity than chalcones [19]. At least part of this activity was attributed to the induction of an apoptotic response through caspase activation, an effect which appears to be restricted to cancer cells [20]. The anticancer potential of natural flavonols [21–23] including colon cancer [24,25], is well known, mainly due to the ability of these compounds to interact with several molecular targets important for cancer progression and response to chemotherapy. This comprises the ability to decrease the levels of oncogenes (like K-ras, H-ras and c-myc), to increase the levels of the p53

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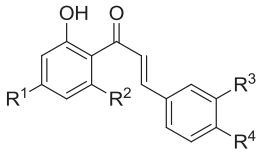
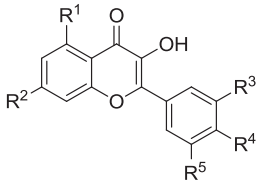


Isoliquiritigenin, R^1=R^4=OH, R^2=R^3=H
Butein, R^1=R^3=R^4=OH, R^2=H,
Flavokavain A, R^1=R^2=R^4=OCH_3, R^3=H
Flavokavain B, R^1=R^2=OCH_3, R^3=R^4=H



Quercetin, R^1=R^2=R^3=R^4=OH, R^5=H
Galangin, R^1=R^2=OH, R^3=R^4=R^5=H
Fisetin, R^1=R^5=H, R^2=R^3=R^4=OH
Kaempferide, R^1=R^2=OH,R^3=R^5=H, R^4=OCH_3
Rhamnazin, R^1=R^4=OH, R^2=R^3=OCH_3, R^5=H
Myrcetin, R^1=R^2=R^3=R^4=R^5=OH

Fig. 1. Naturally occurring chalcone and flavonol derivatives recognized as bioactive molecules.

tumour suppressor and cell cycle control proteins such as p21 and p27, as well as to inhibit tyrosine and serine–threonine kinases (like MAPKs, PI3K and CDKs), important mediators in pathways of cell survival and growth [26–31].

Inspired by the important biological activity of flavonoids, a series of chalcones and flavonols were synthesized using an eco-friendly approach and a panel of 33 compounds was tested in an anticancer screening with HCT116 colon cancer cells.

2. Results and discussion

2.1. Chemistry

The 2-hydroxychalcones **3a**–**r** were synthesized from the reaction of 2-hydroxyacetophenone **2** with the appropriate aromatic aldehyde **1**, using typical procedures for the aldol condensation. The reaction was carried out in aqueous base (3 M NaOH), at room temperature or at 40 °C for chalcone **3i**. The solid product usually precipitated from the reaction media and was used without further purification (Scheme 1). Chalcones **3a**–**h**, **3j** and **3l**–**r** were converted to the corresponding flavonols **4a**–**o**, using the Flynn–Algar–Oyamada method for epoxidation [32,33] and subsequent intramolecular cyclization of the open-chain structure. The reaction was performed with hydrogen peroxide in aqueous base (4 M NaOH) [34], an eco-friendly approach previously used for the synthesis of analogous structures.

2.2. Biological assays

The chalcones **3** and flavonols **4** were tested at 15 μM in a first round of anticancer screening in order to select the most active compounds. HCT116 cells were incubated for 48 h with each of the test compounds and the final number of viable cells was estimated by the MTT assay. The results for the chalcone (Table 1) and flavonol derivatives (Table 2) indicated that most of the compounds decreased cell viability below that of quercetin. This natural flavonol was tested at 30 μM, twice the concentration of the synthetic compounds and the number of viable cells decreased to 60% as compared to control cells.

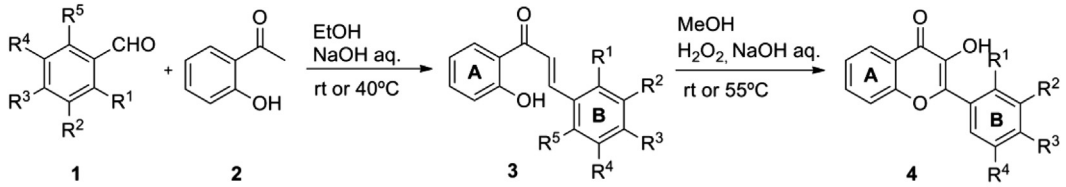
In a recent work by Wiese et al. [35] a selection of methoxylated or chlorinated chalcones, in the B-ring, were tested as inhibitors of breast cancer resistance protein (BCRP/ABCG2), using two different cell lines – MCF-7 MX and MDCK BCRP. The authors clearly observed that compounds with 3,4-dimethoxy substituents were the most active. A different work by Bu et al. [36] reported the synthesis of biaryl-based chalcones with hydroxyl/methoxyl groups or halogen atoms in the B-ring. Compounds with the 4-methoxy and 3,4-dimethoxy substituents exhibited potent inhibitory activities towards CNE2 cell growth, unlike the halogenated analogues, but the substituents in the bi-aryl system also played an important role for anticancer activity.

In the present work and contrarily to these previous reports, molecules with multiple methoxyl substituents in the B-ring (**3i**–**j** and **4g**–**i**) did not show an improved activity in relation to the analogues with one methoxyl substituent (**3e**–**f** and **4d**–**f**) and therefore they were not used in the following experiments. When the potential anticancer activity of chalcones and flavonols with the same substituents were compared (Table 1 *vs* Table 2, respectively) the cyclic structure showed lower IC_{50} values, with the exception of compounds **3f** and **4f**. In general, molecules with halogen substituents in the B-ring showed improved activity relative to the hydroxylated, methylated or methoxylated analogues. Consequently, only halogenated chalcones **3** and flavonols **4** proceeded to further studies where the IC_{50} concentration was calculated from the dose–response curves. The results from the dose–response experiment of the different halogenated compounds with the IC_{50} values and the anticancer activity at 10 μM were summarized in Table 3. These data confirm that flavonols displayed a higher activity as compared with the corresponding chalcones. Chalcones and flavonols presented different kinetics in the dose–response curves, as can be seen in Fig. 2 for compounds **3l** and **4j** shown as an example. Contrarily to flavonols, chalcones are non-active until higher concentrations are reached then, the number of viable cells decreases abruptly with increasing concentration of the compound. Chalcones have higher slope values in the dose–response curves when compared with the corresponding cyclic product (Fig. 2).

This structure–activity analysis also revealed an enhancement in the inhibition of cell growth as the B-ring substituent goes from F to Cl and to Br, either in the 3- or in the 4-position (Table 3). The size of the halogen atom increases in that order, but also the polarizability, a measure of the dynamic response of the electron cloud of the atom to the effect of a nearby ion or dipole. Chalcones with a 4′-halogen substituent (**3m**, **3o** and **3q**) were the only exception as the cell viability remained basically unchanged at 10 μM.

Likewise, the results evidenced that chalcones with the halogen substituent at the 3-position of the aromatic B-ring are more active than those substituted at the 4-position. The opposite happens for brominated flavonols, where the substituent at the 4-position enhances the anticancer activity. This difference in activity related with the halogen substitution pattern of the B-ring may be associated with a more flexible structure of the chalcone, where a distinct spatial arrangement of the molecule could lead to an improved interaction with the active site of the receptor.

A comparison between the anticancer activity of flavonol **4o** at 10 μM (cell viability of 33%) and quercetin at 30 μM (cell viability of



Substituted chalcones, 3a-r

Comp	R^1	R^2	R^3	R^4	R^5
3a	H	H	H	H	H
3b	H	CH_3	H	H	H
3c	H	H	CH_3	H	H
3d	OCH_3	H	H	H	H
3e	H	OCH_3	H	H	H
3f	H	H	OCH_3	H	H
3g	OCH_3	H	H	OCH_3	H
3h	H	OCH_3	OCH_3	H	H
3i	OCH_3	H	OCH_3	H	OCH_3
3j	H	OCH_3	OCH_3	OCH_3	H
3k	OH	OCH_3	H	H	H
3l	H	F	H	H	H
3m	H	H	F	H	H
3n	H	Cl	H	H	H
3o	H	H	Cl	H	H
3p	H	Br	H	H	H
3q	H	H	Br	H	H
3r	H	H	CF_3	H	H

Substituted flavonols, 4a-o

Comp	R^1	R^2	R^3	R^4
4a	H	H	H	H
4b	H	CH_3	H	H
4c	H	H	CH_3	H
4d	OCH_3	H	H	H
4e	H	OCH_3	H	H
4f	H	H	OCH_3	H
4g	OCH_3	H	H	OCH_3
4h	H	OCH_3	OCH_3	H
4i	H	OCH_3	OCH_3	OCH_3
4j	H	F	H	H
4k	H	H	F	H
4l	H	Cl	H	H
4m	H	H	Cl	H
4n	H	Br	H	H
4o	H	H	Br	H

Scheme 1. Syntheses of chalcones **3a**–**r** and flavonols **4a**–**o**.

60%), showed that this newly synthesized molecule is around 6 times more potent than the natural flavonol. The polyhydroxylated structure of quercetin results in a higher hydrophilic character and lower cell bioavailability and this may contribute to the lower biological effect of quercetin compared with the halogenated analogues.

Considering the superior anticancer potential of these halogenated compounds and their ability to inhibit cell growth, the most potent chalcone **3p** and flavonol **4o** were selected to evaluate their capacity to induce cell cycle arrest and apoptosis. The halogenated chalcone **3r**, with low anticancer activity, was used for comparison. The morphological changes in cells after treatment with these compounds evidenced a different outcome between chalcones and flavonols. Flavonols, whose cell viability stabilizes at 30–40% for higher concentrations, seem to be more potent at inhibiting cell growth (even at lower concentrations). In contrast, at higher concentrations, chalcones are better at inducing cell death (as shown by the appearance of floating cells – see Fig. 3), resulting in lower values of cell viability as compared with that induced by flavonols (see Fig. 2).

Table 1
Potential anticancer activity of chalcones **3a**–**c**, **3e**–**f**, **3i**–**r** against HCT116 cells by the MTT assay.

Comp	Cell viability (%) at 15 μM[a]
3a	91.7 ± 4.4^{NS}
3b	85.9 ± 3.5^{NS}
3e	89.6 ± 2.8^{NS}
3i	91.1 ± 3.4^{NS}
3k	$84.7 \pm 3.5^{*}$
3l	$45.3 \pm 4.0^{***}$
3n	$37.5 \pm 2.9^{***}$
3p	$29.6 \pm 3.8^{***}$
3c	89.1 ± 3.9^{NS}
3f	89.1 ± 1.1^{NS}
3j	$83.7 \pm 3.0^{*}$
3r	$85.1 \pm 2.1^{*}$
3m	$74.9 \pm 4.2^{**}$
3o	$69.1 \pm 7.3^{*}$
3q	$68.4 \pm 6.8^{**}$

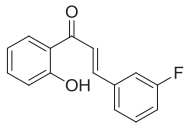
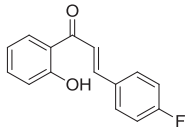
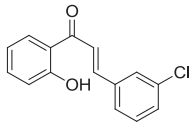
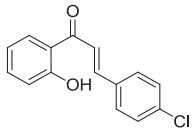
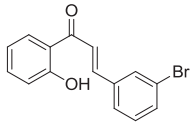
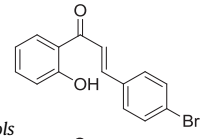
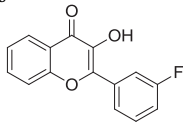
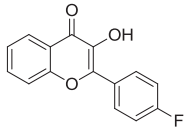
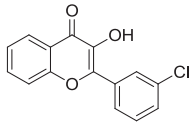
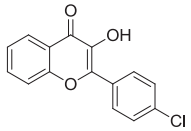
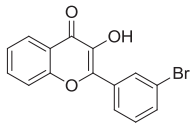
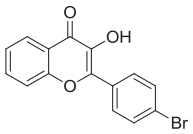
^{NS} not significant ($P > 0.05$), $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, when compared with negative control (100 ± 4.7; cells incubated without compound, 0.5% DMSO only). Quercetin at 30 μM was used as positive control; number of viable cells was $59.7 \pm 4.5^{***}$.
[a] Values are mean ± SEM of 4 independent experiments.

Table 2
Potential anticancer activity of flavonols **4a**–**o** against HCT116 cells by the MTT assay.

Comp	Cell viability (%) at 15 μM[a]
4a	$64.6 \pm 1.5^{***}$
4b	$38.7 \pm 0.7^{***}$
4e	$39.5 \pm 7.3^{***}$
4d	89.1 ± 4.7^{NS}
4h	78.1 ± 8.8^{NS}
4j	$45.9 \pm 4.1^{***}$
4l	$32.9 \pm 1.9^{***}$
4n	$29.2 \pm 3^{***}$
4c	$59.3 \pm 6.3^{**}$
4f	96.0 ± 1.8^{NS}
4g	$76.6 \pm 4.4^{*}$
4i	$70.0 \pm 5.1^{**}$
4k	$49.2 \pm 1.6^{***}$
4m	$38.0 \pm 1.5^{***}$
4o	$29.2 \pm 2.3^{***}$

^{NS} not significant ($P > 0.05$), $^{*}P \leq 0.05$, $^{**}P \leq 0.001$, when compared with negative control (100 ± 4.9; cells incubated without compound, 0.5% DMSO only).
[a] Values are mean ± SEM of 4 independent experiments.

Table 3
Evaluation of the anticancer activity of halogenated chalcones **3** and flavonols **4** against the colorectal carcinoma cell line HCT116 by the MTT assay.

Comp	Cell viability (%) at 10 μM[a]	IC_{50} (μM)
Chalcones		
3l 	90.2 ± 6.2^{NS}	14.5
3m 	85.2 ± 4.5^{NS}	20.5
3n 	75.8 ± 7.1*	13.2
3o 	84.8 ± 4.8^{NS}	20.9
3p 	48.4 ± 6.7**	10.9
3q 	85.5 ± 6.4^{NS}	19.1
Flavonols		
4j 	60.9 ± 3.8**	13.0
4k 	61.1 ± 3.8**	14.4
4l 	43.8 ± 4.4**	8.1
4m 	40.8 ± 0.6**	6.8
4n 	37.6 ± 2.7**	7.5
4o 	32.8 ± 1.7**	4.6

^{NS} not significant ($P > 0.05$), $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, when compared with negative control (100 ± 4.7; cells incubated without compound, 0.5% DMSO only). Quercetin at 30 μM was used as positive control; number of viable cells was 59.7 ± 4.5***.

[a] Values are mean ± SEM of 4 independent experiments.

Cell cycle analysis by flow cytometry for both compounds **3p** and **4o** (Fig. 4) showed that they induced cell cycle arrest at S and G2/M phases. As expected, compound **3r** displayed a much lower activity. Contrarily to chalcone **3p**, flavonol **4o** already attained maximal inhibition of cell cycle at its IC_{50} concentration, which may indicate a higher specificity of the cyclic derivative to induce cell cycle arrest. Analysis by western blot confirmed the higher ability of flavonol **4o** to inhibit cell cycle, in view of a higher induction of the levels of the tumour suppressor p53 and the cell cycle inhibitor p27 (Fig. 5). Cell cycle analysis also revealed that both **3p** and **4o** induced an increase in cells at sub-G1 phase (Fig. 4), typical of apoptosis. This increase was higher for **3p** which agrees with the morphological observations and emphasizes that chalcones **3** have a higher potential to induce cell death than flavonols **4** at concentrations higher than their IC_{50}. To further demonstrate the ability of these compounds to induce cell death by apoptosis, the presence of nuclear condensation was assessed by fluorescence microscopy. This assay showed an increase, in a concentration-dependent manner, for both **3p** and **4o** (Fig. 6). Western blot evidenced that the chalcone and the flavonol also induced the cleavage of poly(ADP-ribose) polymerase 1 (PARP-1) as well as a decrease of total PARP-1 levels (Fig. 5), a marker of induction of apoptosis. Considering the higher levels of these parameters in the tested chalcone, these results corroborate the higher ability of compound **3p** to induce apoptosis as compared with **4o**.

3. Conclusions

A selection of chalcone derivatives was prepared, oxidized by H_2O_2 in aqueous base to generate the corresponding flavonols which were isolated in good yield. Both chalcones and their cyclic

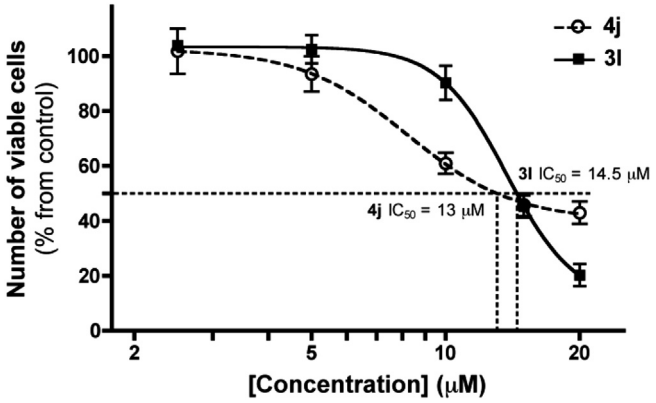


Fig. 2. Dose–response effects of compounds **3l** and **4j** in the number of viable cells after 48 h of incubation. The IC_{50} concentration of tested compounds is also represented.

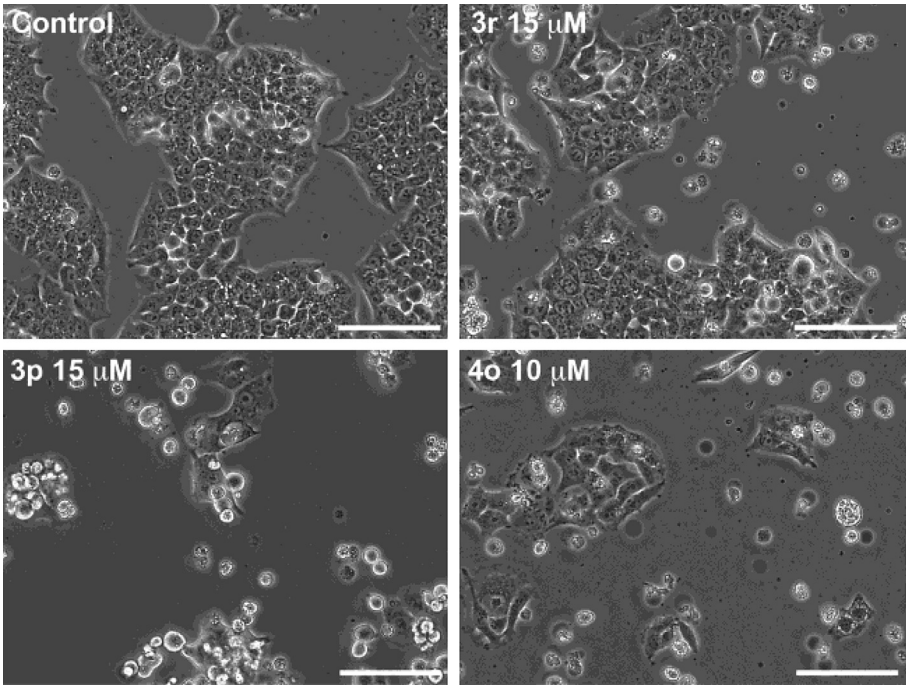


Fig. 3. Effect of compounds **3r**, **3p** and **4o** on cell morphology after 48 h of incubation with HCT116 cells. Bar indicates 100 μm.

derivatives were evaluated for their anticancer activity in human colorectal carcinoma HCT116 cells.

A first round of anticancer screening showed enhanced activity for chalcones **3** and flavonols **4** with halogen substituents in the B-ring when compared with methoxyl/methyl group analogues. In general, the effects observed are related to those identified for the natural flavonoid quercetin, with the advantage of enhanced activity at a much lower concentration range, for the halogenated compounds.

The halogenated compounds were selected for further studies and displayed an enhanced antiproliferative activity as the substituent of the B-ring changed from F to Cl and to Br. The 3-substituted derivatives in chalcones and the 4-substituted products in flavonols exhibited higher anticancer activity. Flow cytometry analysis of the most active chalcone **3p** and flavonol **4o** revealed an induction of cell cycle arrest at S and G2/M phases as well as an increase of sub-G1 cells, typical of apoptosis. These facts were confirmed by western blot and nuclear condensation and indicated that although flavonol **4o** demonstrated a higher cytostatic (growth inhibitory) activity, chalcone **3p** revealed a superior cytocidal (death inducer) capacity by apoptosis.

Compound **4o** proved to be around six times more potent when compared with quercetin. Therefore, this compound family deserves to be explored in further studies to investigate its biological potential and to understand in more detail their anticancer molecular mechanisms of action.

4. Experimental section

4.1. Chemistry

Solvents and chemicals were of analytical grade and were purchased from commercial sources and used without further purification.

All compounds were fully characterized by spectroscopic data and compared with the data available in the literature. The NMR spectra were recorded at room temperature, on a Varian Unity Plus (^{1}H: 300 MHz and ^{13}C: 75 MHz) or on a Bruker Avance 3400 (^{1}H: 400 MHz and ^{13}C: 100 MHz) and deuterated DMSO was used as solvent. The chemical shifts were expressed in parts per million (ppm scale) and the coupling constants, *J*, were reported in Hertz (Hz). The peak patterns were indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet and br, broad. The reactions were monitored by Thin Layer Chromatography (TLC) using silica gel 60 F_{254} plates (Merck). The melting points were determined on a Stuart SMP3 melting point apparatus. Elemental analyses were performed on a LECO CHNS-932 instrument.

4.1.1. General procedure for the synthesis of (2E)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-ones **3**

An aqueous solution of NaOH (3 M, 2–10 mL) was added to a solution of 2-hydroxyacetophenone **2** (1.22–4.88 mmol) and aromatic aldehyde **1** (1.2 equiv.), in EtOH (1–12 mL). The reaction was stirred at room temperature or at 40 °C, for compound **3i**, until the starting material was totally consumed, as evidenced by TLC. The reaction mixture was cooled in an ice-water bath and acidified to pH 2 with concentrated HCl (37%). The solid formed was filtered and washed with distilled water.

*4.1.1.1. (2E)-1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (**3a**).* Isolated as a yellow solid in 77.4% yield. Mp 87–88 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00 (dd, J = 8.4, 1.5 Hz, 1H), 7.02 (td, J = 8.4, 1.2 Hz, 1H), 7.46–7.48 (m, 3H), 7.56 (td, J = 8.4, 1.5 Hz, 1H), 7.83 (d, J = 15.3 Hz, 1H), 7.89–7.92 (m, 2H), 8.03 (d, J = 15.3 Hz, 1H), 8.24 (dd, J = 8.4, 1.5 Hz, 1H), 12.49 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 117.7, 119.2, 120.8, 121.8, 129.0 (2C), 129.2 (2C), 130.9, 131.0, 134.4, 136.3, 144.7, 161.8, 193.6 ppm. Anal. Calcd. for $C_{15}H_{12}O_2$ (224.08): C, 80.34; H, 5.39, found: C, 80.48; H, 5.52.

*4.1.1.2. (2E)-1-(2-Hydroxyphenyl)-3-(3-methylphenyl)prop-2-en-1-one (**3b**).* Isolated as a yellow solid in 55.2% yield. Mp 62–64 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.99 (t, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.56 (t, J = 8.1 Hz, 1H), 7.67 (d, J = 7.5 Hz, 1H), 7.75 (s, 1H), 7.79 (d, J = 15.9 Hz, 1H), 8.10 (d, J = 15.9 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H),

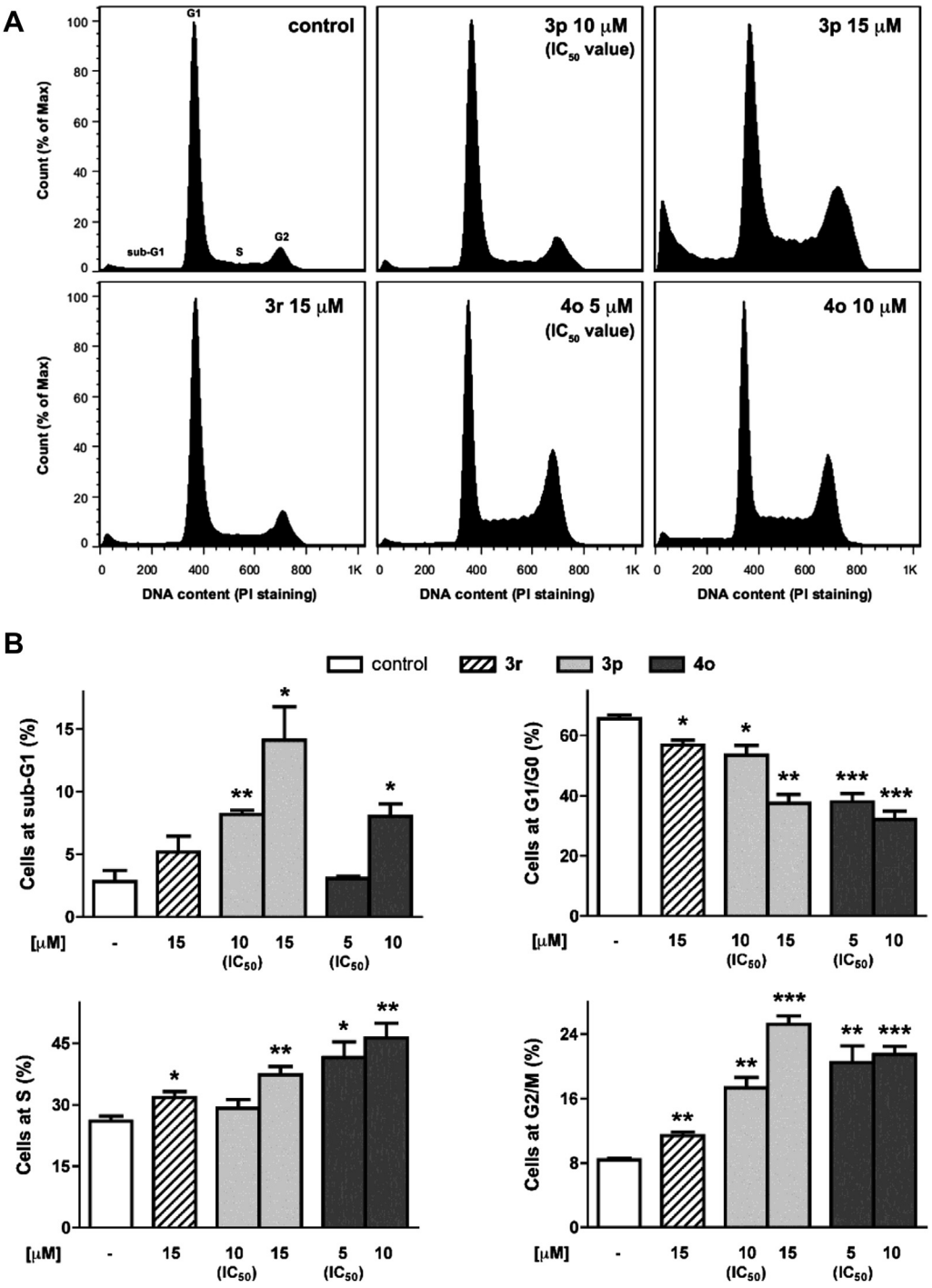


Fig. 4. Effect of compounds **3p**, **3r** and **4o** for 48 h in cell cycle progression of HCT116 cells, assessed by flow cytometry. (A) Distribution of single cells through the phases of cell cycle of a representative experiment. (B) Percentage of cells in the different phases of cell cycle of 3 independent experiments. Values are mean ± S.E.M. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, when compared with control (cells incubated without compound, 0.5% DMSO only).

12.50 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.8, 117.8, 119.0, 120.9, 121.8, 123.7, 126.6, 128.8, 129.4, 130.9, 131.7, 136.2, 138.2, 144.8, 161.9, 193.5 ppm. Anal. Calcd. for $C_{16}H_{14}O_2.0.1H_2O$ (239.90): C, 80.04; H, 5.96, found: C, 79.95; H, 5.99.

*4.1.1.3. (2E)-1-(2-Hydroxyphenyl)-3-(4-methylphenyl)prop-2-en-1-one (**3c**).* Isolated as a yellow solid in 86.1% (lit. [37] 74%) yield. Mp 95–97 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H), 6.98 (t, J = 8.4 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 7.8 Hz, 2H), 7.54 (td, J = 8.4, 1.5 Hz, 1H), 7.80 (d, J = 7.8 Hz, 2H), 7.81 (d, J = 15.6 Hz, 1H), 7.99 (d, J = 15.6 Hz, 1H), 8.24 (dd, J = 8.4, 1.5 Hz, 1H), 12.57 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.1, 117.7, 118.0, 119.1, 120.6, 120.7, 129.2, 129.6, 130.8, 136.3, 141.2, 144.9, 161.9, 193.6 ppm.

*4.1.1.4. (2E)-1-(2-Hydroxyphenyl)-3-(2-methoxyphenyl)prop-2-en-1-one (**3d**).* Isolated as a yellow solid in 79.5% (lit. [38] 46%) yield. Mp 110–112 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.90 (s, 3H), 6.99 (dd, J = 8.4, 1.2 Hz, 1H), 7.04 (t, J = 8.4 Hz, 1H), 7.05 (t, J = 8.1 Hz, 1H),

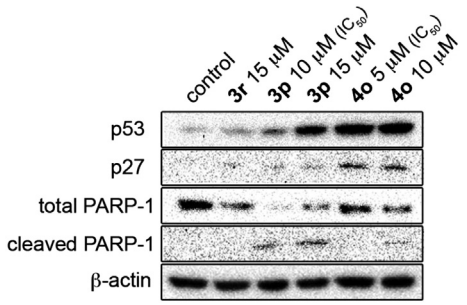


Fig. 5. Effect of compounds **3p**, **3r** and **4o** for 48 h on the expression of p53, p27 and PARP in HCT116 cells, as assessed by western blot. Shown are representative images of two independent experiments. β-actin was used as loading control.

7.12 (d, J = 8.1 Hz, 1H), 7.47 (dt, J = 8.1, 1.5 Hz, 1H), 7.55 (td, J = 8.4, 1.2 Hz, 1H), 7.98 (d, J = 15.6 Hz, 1H), 7.99 (dd, J = 8.1, 1.5 Hz, 1H), 8.15 (d, J = 15.6 Hz, 1H), 8.19 (dd, J = 8.4, 1.2 Hz, 1H), 12.50 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.8, 111.9, 117.8, 119.2, 120.8, 120.8, 121.4, 122.7, 128.8, 130.7, 132.8, 136.2, 139.2, 158.4, 161.8, 193.7 ppm.

*4.1.1.5. (2E)-1-(2-Hydroxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (**3e**).* Isolated as a yellow solid in 91.7% yield. Mp 93–96 °C.

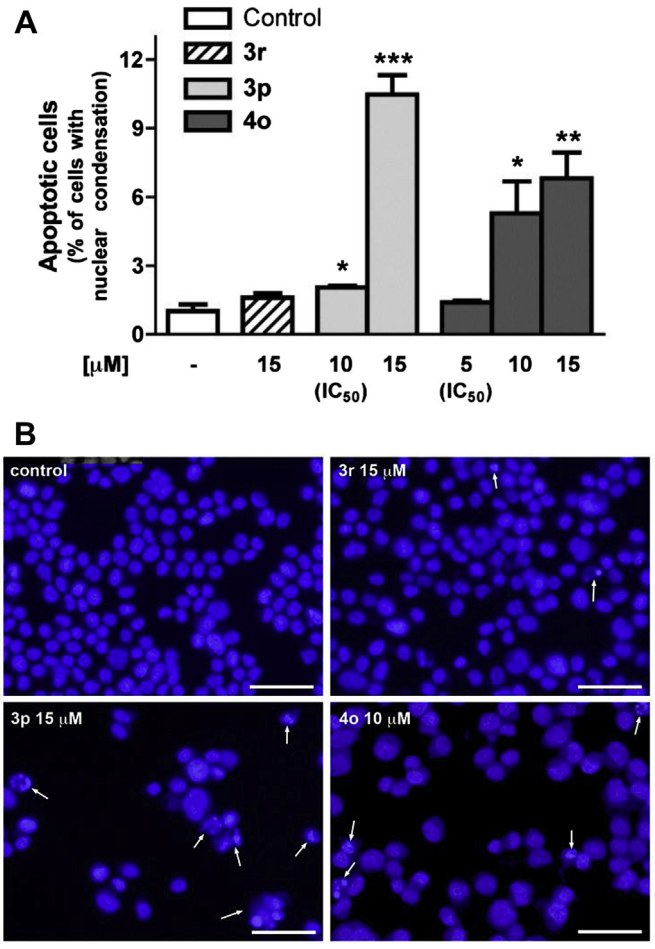


Fig. 6. Effect of compounds **3p**, **3r** and **4o** for 48 h on apoptosis in HCT116 cells, as assessed by nuclear condensation assay. (A) Results given as mean ± SEM of at least 3 independent experiments. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, when compared to control. (B) Representative images where nuclear condensation is shown with an arrow. Bar indicates 50 μm.

^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00 (dd, J = 8.7, 1.8 Hz, 1H), 7.01 (t, J = 8.7 Hz, 1H), 7.06 (d, J = 0.9 Hz, 1H), 7.38 (t, J = 8.1 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.57 (td, J = 8.7, 1.8 Hz, 1H), 7.81 (d, J = 15.6 Hz, 1H), 8.04 (d, J = 15.6 Hz, 1H), 8.27 (dd, J = 8.7, 1.8 Hz, 1H), 12.50 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.3, 113.6, 117.7, 119.2, 120.7, 122.0 (2C), 130.0, 131.0, 135.8, 136.4, 144.8, 159.7, 161.9, 193.7 ppm. Anal. Calcd. for $C_{16}H_{14}O_3 \cdot 0.1H_2O$ (255.90): C, 75.04; H, 5.59, found: C, 75.20; H, 5.67.

*4.1.1.6. (2E)-1-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3f**).* Isolated as a yellow solid in 57.6% (lit. [37] 65%) yield. Mp 94–96 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H), 6.99 (t, J = 8.7 Hz, 1H), 7.00 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 9.0 Hz, 2H), 7.55 (td, J = 8.7, 1.8 Hz, 1H), 7.89 (dd, J = 9.0, 2.4 Hz, 2H), 7.92 (d, J = 15.6 Hz, 1H), 7.82 (d, J = 15.6 Hz, 1H), 8.26 (dd, J = 8.7, 1.8 Hz, 1H), 12.71 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.4, 114.5 (2C), 117.7, 118.9 (2C), 119.1, 120.6, 127.1, 130.8, 131.3, 136.2, 145.1, 161.7, 162.0, 193.6 ppm.

*4.1.1.7. (2E)-3-(2,5-Dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3g**).* Isolated as a yellow solid 74.7% (lit. [38] 38%) yield. Mp 101–103 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H), 3.84 (s, 3H), 6.98 (d, J = 7.8 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 7.04 (d, J = 7.5 Hz, 1H), 7.06 (d, J = 1.5 Hz, 1H), 7.57 (dt, J = 7.8, 1.5 Hz, 1H), 7.58 (dd, J = 7.5, 1.5 Hz, 1H), 8.00 (d, J = 15.6 Hz, 1H), 8.14 (d, J = 15.6 Hz, 1H), 8.25 (dd, J = 7.8, 1.5 Hz, 1H), 12.58 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6, 55.8, 117.7, 112.7, 113.1, 118.8, 119.1, 120.7, 121.5, 123.2, 130.8, 136.3, 139.1, 152.9, 153.3, 163.0, 194.7 ppm.

*4.1.1.8. (2E)-3-(3,4-Dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3h**).* Isolated as a yellow solid 46.7% (lit. [38] 50%) yield. Mp 112–114 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H), 3.86 (s, 3H), 6.99 (dd, J = 8.1, 1.5 Hz, 1H), 7.02 (t, J = 8.1 Hz, 1H), 7.03 (d, J = 8.7 Hz, 1H), 7.42 (dd, J = 8.7, 1.8 Hz, 1H), 7.55 (dd, J = 8.1, 1.5 Hz, 1H), 7.58 (d, J = 1.8 Hz, 1H), 7.81 (d, J = 15.6 Hz, 1H), 7.93 (d, J = 15.6 Hz, 1H), 8.29 (dd, J = 8.1, 1.5 Hz, 1H), 12.79 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6, 55.8, 110.9, 111.5, 117.8, 118.8, 119.0, 120.5, 124.7, 127.2, 130.8, 136.2, 145.7, 149.1, 151.7, 162.2, 193.7 ppm.

*4.1.1.9. (2E)-1-(2-Hydroxyphenyl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**3i**).* Isolated as a yellow solid in 58.0% yield. Mp 132–134 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.86 (s, 3H), 3.93 (s, 6H), 6.32 (s, 2H), 6.96 (d, J = 8.7 Hz, 1H), 6.99 (t, J = 8.7 Hz, 1H), 7.52 (td, J = 8.7, 0.9 Hz, 1H), 7.94 (d, J = 8.7 Hz, 1H), 7.97 (d, J = 15.3 Hz, 1H), 8.24 (d, J = 15.3 Hz, 1H), 12.89 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6, 56.2 (2C), 91.1 (2C), 105.0, 117.8, 118.8, 119.1, 120.7, 129.8, 135.8, 136.4, 161.8 (2C), 162.1, 164.0, 194.3 ppm. Anal. Calcd. for $C_{18}H_{18}O_5.0.1H_2O$ (315.92): C, 69.39; H, 5.80, found: C, 69.17; H, 5.94.

*4.1.1.10. (2E)-1-(2-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3j**).* Isolated as a yellow solid in 67.9% (lit. [37] 55%) yield. Mp 156–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.71 (s, 3H), 3.86 (s, 6H), 6.98–7.03 (m, 2H), 7.26 (s, 2H), 7.56 (td, J = 8.4, 1.5 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H), 7.98 (d, J = 15.6 Hz, 1H), 8.29 (dd, J = 8.4, 1.5 Hz, 1H), 12.63 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.2 (2C), 60.2, 106.9 (2C), 117.7, 119.1, 120.7, 120.9, 130.0, 130.9, 136.3, 140.1, 145.4, 153.1, 161.9, 193.7 ppm.

*4.1.1.11. (2E)-3-(2-Hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (**3k**).* Isolated as a yellow solid in 3.5% yield. Mp 200 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H), 6.98 (t, J = 8.1 Hz, 1H), 7.05 (dd, J = 8.1, 1.5 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.53 (td, J = 8.1, 1.5 Hz, 1H), 7.91 (d, J = 15.6 Hz, 1H), 8.14 (dd, J = 8.1,

1.5 Hz, 1H), 8.16 (d, J = 15.6 Hz, 1H), 9.54 (br s, 1H), 12.52 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.00, 113.99, 117.71, 119.08, 119.15, 119.93, 120.83, 121.09, 121.45, 130.57, 135.96, 139.90, 146.89, 148.06, 161.22, 193.68 ppm. Anal. Calcd. for $C_{16}H_{14}O_4.0.1H_2O$ (271.89): C, 70.63; H, 5.26, found: C, 70.44; H, 5.20.

*4.1.1.12. (2E)-3-(3-Fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3l**).* Isolated as a yellow solid in 99.2% yield. Mp 109–110 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98 (dd, J = 8.1, 1.8 Hz, 1H), 7.01 (t, J = 8.1 Hz, 1H), 7.29 (td, J = 6.0, 1.8 Hz, 1H), 7.49 (dt, J = 8.4, 1.8 Hz, 1H), 7.57 (dt, J = 8.1, 1.8 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 15.6 Hz, 1H), 7.86 (dd, J = 8.4, 1.8 Hz, 1H), 8.03 (d, J = 15.6 Hz, 1H), 8.26 (dd, J = 8.1, 1.8 Hz, 1H), 12.44 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 114.9 (d, J = 21.8 Hz), 117.6 (d, J = 21.2 Hz), 117.7, 119.2, 120.7, 123.3, 125.8 (d, J = 2.2 Hz), 130.9 (d, J = 8.2 Hz), 131.0, 136.5, 137.0 (d, J = 8.0 Hz), 143.2 (d, J = 3.8 Hz), 161.9, 162.5 (d, J = 242.0 Hz), 193.6 ppm. Anal. Calcd. for $C_{15}H_{11}FO_2.H_2O$ (260.08): C, 69.22; H, 5.03, found: C, 69.06; H, 5.15.

*4.1.1.13. (2E)-3-(4-Fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3m**).* Isolated as a yellow solid in 91.0% (lit. [37] 44%) yield. Mp 120–122 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.99 (dd, J = 8.4, 1.6 Hz, 1H), 7.00 (t, J = 8.4 Hz, 1H), 7.30 (dt, J = 8.8, 2.0 Hz, 2H), 7.57 (td, J = 8.4, 1.6 Hz, 1H), 7.83 (d, J = 15.6 Hz, 1H), 7.99 (dt, J = 8.8, 2.0 Hz, 2H), 8.00 (d, J = 15.6 Hz, 1H), 8.24 (dd, J = 8.4, 1.6 Hz, 1H), 12.50 (br s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.0 (d, J = 22.0 Hz, 2C), 117.7, 118.5, 120.7, 121.6 (d, J = 2.0 Hz), 130.9, 131.1 (d, J = 3.0 Hz), 131.6 (d, J = 9.0 Hz, 2C), 136.3, 143.5, 161.8, 163.6 (d, J = 248.0 Hz), 193.5 ppm.

*4.1.1.14. (2E)-3-(3-Chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3n**).* Isolated as a yellow solid in 84.6% yield. Mp 105–107 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00 (dd, J = 8.4, 1.8 Hz, 1H), 7.01 (t, J = 8.4 Hz, 1H), 7.58 (td, J = 8.4, 1.8 Hz, 1H), 7.49 (t, J = 7.2 Hz, 1H), 7.50 (dd, J = 7.2, 2.1 Hz, 1H), 7.79 (d, J = 15.6 Hz, 1H), 7.84 (dd, J = 7.2, 2.1 Hz, 1H), 8.09 (d, J = 2.1 Hz, 1H), 8.11 (d, J = 15.6 Hz, 1H), 8.24 (dd, J = 8.4, 1.8 Hz, 1H), 12.44 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 111.8, 119.2, 120.7, 123.4, 128.2 (2C), 130.5, 130.8, 131.1, 133.9, 136.6, 136.7, 143.0, 161.9, 193.6 ppm. Anal. Calcd. for $C_{15}H_{11}ClO_2.0.1H_2O$ (259.85): C, 69.16; H, 4.33, found: C, 69.24; H, 4.52.

*4.1.1.15. (2E)-3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3o**).* Isolated as a yellow solid in 75.3% (lit. [37] 85%) yield. Mp 143–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.99 (dd, J = 8.1, 1.8 Hz, 1H), 7.01 (t, J = 8.1 Hz, 1H), 7.53 (d, J = 6.6 Hz, 2H), 7.57 (dt, J = 8.1, 1.8 Hz, 1H), 7.81 (d, J = 15.6 Hz, 1H), 7.94 (dd, J = 6.6, 1.8 Hz, 2H), 8.05 (d, J = 15.6 Hz, 1H), 8.24 (dd, J = 8.1, 1.8 Hz, 1H), 12.44 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 117.7, 119.2, 120.8, 122.6, 129.0 (2C), 130.8 (2C), 130.9, 133.3, 135.5, 136.4, 143.3, 161.8, 193.5 ppm.

*4.1.1.16. (2E)-3-(3-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3p**).* Isolated as a yellow solid in 60.8% (lit. [37] 66%) yield. Mp 108–110 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.99 (dd, J = 6.9, 0.9 Hz, 1H), 7.01 (t, J = 6.9 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.57 (dt, J = 6.9, 0.9 Hz, 1H), 7.64 (dd, J = 7.8, 0.9 Hz, 1H), 7.85 (d, J = 15.6 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 15.6 Hz, 1H), 8.23 (s, 1H), 8.29 (d, J = 6.9 Hz, 1H), 12.45 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 111.7, 119.2, 120.7, 122.4, 123.3, 128.6, 131.0 (2C), 131.1, 133.4, 136.6, 136.9, 143.0, 161.9, 193.5 ppm.

*4.1.1.17. (2E)-3-(4-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**3q**).* Isolated as a yellow solid in 80.5% (lit. [37] 58%) yield. Mp 150–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98 (d, J = 8.4 Hz, 1H), 7.00 (td, J = 8.4, 1.5 Hz, 1H), 7.56 (td, J = 8.4, 1.5 Hz, 1H), 7.67 (dd, J = 6.9, 1.8 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 7.87 (dd, J = 6.9, 1.8 Hz, 2H), 8.06 (d, J = 15.6 Hz, 1H), 8.24 (dd, J = 8.4, 1.5 Hz, 1H), 12.44 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 117.7, 119.2, 120.7, 122.6, 124.4, 130.9, 131.0 (2C), 131.9 (2C), 133.7, 136.4, 143.4, 161.8, 193.5 ppm.

*4.1.1.18. (2E)-1-(2-Hydroxyphenyl)-3-[4-(trifluoromethyl)phenyl] prop-2-en-1-one (**3r**).* Isolated as a yellow solid in 80.5% (lit. [39] 46%) yield. Mp 100–102 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00 (d, J = 8.4 Hz, 1H), 7.02 (t, J = 8.4 Hz, 1H), 7.57 (td, J = 8.4, 1.5 Hz, 1H), 7.82 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 16.0 Hz, 1H), 8.11 (d, J = 8.7 Hz, 2H), 8.15 (d, J = 16.0 Hz, 1H), 8.23 (dd, J = 8.4, 1.5 Hz, 1H), 12.29 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 117.7, 119.3, 120.9, 124.8, 125.7 (q, J = 3.8 Hz, 2C), 129.6 (2C), 130.2 (q, J = 31.7 Hz), 131.0, 136.5, 138.5 (q, J = 1.1 Hz), 142.4, 161.7, 193.4 ppm.[1]

*4.1.2. General procedure for the synthesis of 3-hydroxy-2-phenyl-4H-chromen-4-ones **4***

An aqueous solution of H_2O_2 (30%) (151–430 μL) and 4 M NaOH (120–300 μL) was added to a suspension of chalcone **3** (0.44–1.10 mmol) in MeOH (0.2–2 mL). The resulting mixture was stirred at room temperature (compounds **4a**–**d**, **4f**–**i)** or at 55 °C (compounds **4e**, **4j**–**k**), until the starting material was totally consumed as evidenced by TLC. The reaction mixture was cooled in an ice bath and distilled water (2–4 mL) was added. Concentrated HCl (37%) was added until pH 2 and the precipitate was filtered and washed with distilled water.

*4.1.2.1. 3-Hydroxy-2-phenyl-4H-chromen-4-one (**4a**).* Isolated as a white solid in 53.2% yield. Mp 169–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (t, J = 8.7 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.56 (t, J = 7.5 Hz, 2H), 7.74 (d, J = 8.4 Hz, 1H), 7.79 (td, J = 8.7, 1.2 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 9.61 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.4, 121.3, 124.6, 124.8, 127.7 (2C), 128.5 (2C), 129.9, 131.3, 133.7, 139.1, 145.2, 154.6, 173.0 ppm. Anal. Calcd. for $C_{15}H_{10}O_3$ (238.06): C, 75.62; H, 4.23, found: C, 75.54; H, 4.41.

*4.1.2.2. 3-Hydroxy-2-(3-methylphenyl)-4H-chromen-4-one (**4b**).* Isolated as a beige solid in 37.6% yield. Mp 143–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 3H), 7.31 (d, J = 7.5 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 7.47 (td, J = 8.7, 1.5 Hz, 1H), 7.75 (t, J = 8.7 Hz, 1H), 7.81 (dd, J = 8.7, 1.5 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 8.02 (s, 1H), 8.11 (dd, J = 8.7, 1.5 Hz, 1H), 9.56 (s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.2, 118.4, 121.3, 124.6, 124.8, 125.0, 128.0, 128.4, 130.6, 131.3, 133.7, 137.7, 139.0, 145.3, 154.6, 173.0 ppm. Anal. Calcd. for $C_{16}H_{12}O_3$ (252.08): C, 76.18; H, 4.79, found: C, 76.34; H, 4.56.

*4.1.2.3. 3-Hydroxy-2-(4-methylphenyl)-4H-chromen-4-one (**4c**).* Isolated as a white solid in 60.9% (lit. [34] 65%) yield. Mp 192–194 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.37 (s, 3H), 7.36 (d, J = 8.1 Hz, 2H), 7.47 (td, J = 8.1, 1.2 Hz, 1H), 7.77 (dd, J = 8.1, 1.2 Hz, 1H), 7.80 (dd, J = 8.1, 1.2 Hz, 1H), 8.09 (dd, J = 8.1, 1.2 Hz, 1H), 8.12 (d, J = 8.1 Hz, 2H) 9.53 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.0, 118.4, 121.3, 124.5, 124.8, 127.6 (2C), 128.5, 129.1 (2C), 133.6, 138.8, 139.8, 145.4, 154.5, 172.8 ppm.

*4.1.2.4. 3-Hydroxy-2-(2-methoxyphenyl)-4H-chromen-4-one (**4d**).* Isolated as a pale yellow solid in 74.8% (lit. [38] 74%) yield. Mp 211–213 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.79 (s, 3H), 7.08 (t, J = 8.1 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.46 (dd, J = 8.4, 1.5 Hz, 1H), 7.48 (t, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.4, 1.5 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.75 (dt, J = 8.4, 1.5 Hz, 1H), 8.13 (dd, J = 8.4, 1.5 Hz, 1H), 8.93 (s,

[1] The chemical shift of the carbon atom attached to the fluorine atoms was not visible in the spectrum.

1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.8, 112.0, 118.4, 119.9, 120.2, 122.00, 124.5, 124.9, 131.1, 131.8, 133.9, 139.1, 147.1, 155.0, 157.1, 172.7 ppm.

*4.1.2.5. 3-Hydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one (**4e**).* Isolated as a beige solid in 39.2% yield. Mp 133–135 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.09 (dd, J = 8.4, 2.7 Hz, 1H), 7.46 (td, J = 8.4, 1.2 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 7.75–7.82 (m, 4H), 8.12 (dd, J = 8.4, 1.2 Hz, 1H), 9.65 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 113.4, 115.2, 118.5, 120.0, 121.2, 124.6, 124.8, 129.6, 132.5, 133.8, 139.2, 144.8, 154.5, 159.2, 173.0 ppm. Anal. Calcd. for $C_{16}H_{12}O_4$ (284.10): C, 71.82; H, 5.67, found: C, 71.94; H, 5.49.

*4.1.2.6. 3-Hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**4f**).* Isolated as an off-white solid in 74.6% yield. Mp 234–236 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.12 (dd, J = 7.0, 2.0 Hz, 2H), 7.45 (td, J = 8.0, 1.6 Hz, 1H), 7.75 (dd, J = 6.4, 1.6 Hz, 1H), 7.77 (dd, J = 6.4, 1.6 Hz, 1H), 8.09 (dd, J = 8.0, 1.6 Hz, 1H), 8.20 (d, J = 7.0 Hz, 2H), 9.45 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.4, 114.0, 118.3, 121.3, 123.6, 124.5, 124.7, 129.4, 133.8, 138.2, 145.6, 154.4, 160.4, 172.6 ppm. Anal. Calcd. for $C_{16}H_{12}O_4.0.1H_2O$ (269.87): C, 71.16; H, 4.55, found: C, 71.31; H, 4.32.

*4.1.2.7. 2-(2,5-Dimethoxyphenyl)-3-hydroxy-4H-chromen-4-one (**4g**).* Isolated as a pale yellow solid in 67.9% (lit. [38] 45%) yield. Mp 183–185 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (s, 3H), 3.74 (s, 3H), 7.08 (d, J = 7.8 Hz, 1H), 7.09 (d, J = 2.7 Hz, 1H), 7.10 (dd, J = 7.8, 2.7 Hz, 1H), 7.46 (dt, J = 7.8, 1.2 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.76 (dt, J = 7.8, 1.2 Hz, 1H), 8.13 (dd, J = 7.8, 1.2 Hz, 1H), 8.94 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6, 56.3, 113.3, 116.4, 116.7, 118.4, 120.5, 122.0, 124.5, 124.9, 133.5, 139.1, 146.8, 155.0, 172.8 ppm.

*4.1.2.8. 2-(3,4-Dimethoxyphenyl)-3-hydroxy-4H-chromen-4-one (**4h**).* Isolated as a pale yellow solid in 68.8% (lit. [38] 70%) yield. Mp 200–202 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.84 (s, 6H), 7.13 (d, J = 8.7 Hz, 1H), 7.45 (m, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.79 (dd, J = 8.1, 2.1 Hz, 1H), 7.81 (d, J = 1.8 Hz, 1H), 7.86 (dd, J = 8.7, 1.8 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 9.46 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.6 (2C), 111.0, 111.5, 118.4, 121.3, 121.5, 123.6, 124.5, 124.7, 133.6, 138.3, 145.5, 148.4, 150.3, 154.4, 172.6 ppm.

*4.1.2.9. 3-Hydroxy-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-One (**4i**).* Isolated as a pale yellow solid in 39.0% (lit. [37] 38%) yield. Mp 179–181 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 6H), 3.75 (s, 3H), 7.46 (m, 1H), 7.55 (s, 2H), 7.81 (d, J = 3.3 Hz, 2H), 8.10 (d, J = 8.1 Hz, 1H), 9.59 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 50.1 (2C), 60.2, 105.6 (2C), 118.5, 121.2, 124.6, 124.7, 126.6, 133.6, 138.8, 139.1, 145.0, 152.7, 154.4, 172.8 ppm.

*4.1.2.10. 2-(3-Fluorophenyl)-3-hydroxy-4H-chromen-4-one (**4j**).* Isolated as a pale yellow solid in 48.9% yield. Mp 170–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34 (dt, J = 6.8, 2.1 Hz, 1H), 7.46 (td, J = 8.4, 1.6 Hz, 1H), 7.60 (dt, J = 6.8, 2.1 Hz, 1H), 7.77 (dd, J = 8.4, 1.6 Hz, 1H), 7.81 (dd, J = 8.4, 1.6 Hz, 1H), 8.02 (dt, J = 6.8, 2.1 Hz, 1H), 8.07 (dt, J = 6.8, 2.1 Hz, 1H), 8.10 (dd, J = 8.4, 1.6 Hz, 1H), 9.89 (br s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.1 (d, J = 24.0 Hz), 116.6 (d, J = 20.0 Hz), 118.5, 121.2, 123.6 (d, J = 3.0 Hz), 124.6, 124.8, 130.6 (d, J = 9.0 Hz), 133.5 (d, J = 9.0 Hz), 133.9, 139.6, 143.8 (d, J = 3.0 Hz), 154.5, 162.0 (d, J = 242.0 Hz), 173.1 ppm. Anal. Calcd. for $C_{15}H_9FO_3.0.8H_2O$ (270.46): C, 66.57; H, 3.95, found: C, 66.71; H, 3.87.

*4.1.2.11. 2-(4-Fluorophenyl)-3-hydroxy-4H-chromen-4-one (**4k**).* Isolated as a white solid in 72.8% yield. Mp 148–150 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.40 (t, J = 6.6 Hz, 2H), 7.46 (td, J = 8.4, 1.2 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.78 (dt, J = 8.4, 1.2 Hz, 1H), 8.10 (dd, J = 8.4, 1.2 Hz, 1H), 8.27 (dt, J = 6.6, 2.1 Hz, 2H), 9.69 (br s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.4, 115.6 (d, J = 21.8 Hz, 2C), 121.3, 124.6, 124.8, 127.8 (d, J = 3.2 Hz), 130.2 (d, J = 8.6 Hz, 2C), 133.8, 138.9, 144.4, 154.5, 162.6 (d, J = 247.4 Hz), 173.0 ppm. Anal. Calcd. for $C_{15}H_9FO_3.H_2O$ (274.06): C, 65.89; H, 4.04, found: C, 66.03; H, 4.12.

*4.1.2.12. 2-(3-Chlorophenyl)-3-hydroxy-4H-chromen-4-one (**4l**).* Isolated as a white solid in 42.1% (lit. [34] 29%) yield. Mp 151–153 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (td, J = 7.5, 2.1 Hz, 1H), 7.55 (d, J = 6.9 Hz, 1H), 7.58 (t, J = 6.9 Hz, 1H), 7.93 (s, 2H), 8.10 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 6.9 Hz, 1H), 8.26 (s, 1H), 9.92 (s, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 118.5, 121.2, 124.7, 124.8, 126.1, 127.1, 129.5, 130.5, 133.3 (2C), 133.9, 139.7, 143.4, 154.6, 173.1 ppm.

*4.1.2.13. 2-(4-Chlorophenyl)-3-hydroxy-4H-chromen-4-one (**4m**).* Isolated as a white solid in 73.5% (lit. [34] 65%) yield. Mp 200–202 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (td, J = 8.1, 1.2 Hz, 1H), 7.63 (d, J = 9.0 Hz, 2H), 7.75 (d, J = 8.1 Hz, 1H), 7.82 (dt, J = 8.1, 1.2 Hz, 1H), 8.10 (d, J = 8.1, 1.2 Hz, 1H), 8.27 (d, J = 9.0 Hz, 2H), 9.85 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.5, 121.3, 124.7, 124.8, 128.7 (2C), 129.4 (2C), 130.2, 133.9, 134.5, 139.4, 144.0, 154.5, 173.0 ppm.

*4.1.2.14. 2-(3-Bromophenyl)-3-hydroxy-4H-chromen-4-one (**4n**).* Isolated as a white solid in 53.5% yield. Mp 162–164 °C. ^{1}H NMR (100 MHz, DMSO-d_6): δ 7.47 (m, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 3.0 Hz, 2H), 8.11 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 7.8 Hz, 1H), 9.92 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.6, 121.3, 121.8, 124.7, 124.8, 126.4, 129.9, 130.7, 132.5, 133.6, 134.0, 139.6, 143.3, 154.6, 173.1 ppm. Anal. Calcd. for $C_{15}H_9BrO_3$ (315.97): C, 56.81; H, 2.86, found: C, 56.59; H, 2.92.

*4.1.2.15. 2-(4-Bromophenyl)-3-hydroxy-4H-chromen-4-one (**4o**).* Isolated as an off-white solid in 68.2% (lit. [40] 76%) yield. Mp 197–199 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46 (t, J = 8.1 Hz, 1H), 7.76 (d, J = 8.1 Hz, 4H), 8.10 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 8.1 Hz, 2H), 9.84 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 118.4, 121.3, 123.3, 124.6, 124.8, 129.5, 130.5 (2C), 131.6 (2C), 133.9, 139.4, 144.0, 154.5, 173.0 ppm.

4.2. Biological assays

4.2.1. Chemicals and cell culture

The 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), RPMI-1640, antibiotic/antimycotic solution, sodium pyruvate, sodium bicarbonate, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), propidium iodide (PI) and Ribonuclease A (RNase A) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was bought from Biochrom AG (Berlin, Germany). All other reagents were of analytical grade. HCT116 cells (human colorectal carcinoma cell line) were kindly provided by Prof. Raquel Seruca from IPATIMUP, Porto. The cell line was maintained in culture with RPMI 1640 medium containing 6% FBS, 1% antibiotic–antimycotic solution, 0.1 mM sodium pyruvate and 10 mM HEPES under an atmosphere of 5% CO_2 and 95% air at 37 °C.

Stock solutions of quercetin and synthesized chalcones and flavonols were made in dimethyl sulfoxide (DMSO) and kept in aliquots at −20 °C. Compounds were then added to the culture medium just before use in order that the concentration of DMSO was not higher than 0.5% (v/v); controls received DMSO only.

4.2.2. MTT reduction assay

The MTT reduction assay was performed to study the potential of test compounds to decrease the number viable cells, as

previously described [41]. Briefly, HCT116 cells were plated in 24-multiwell culture plates at 70 000 cells per mL (500 μL/well) two days before incubation with test compounds. Cells were then treated with different concentrations of test compounds in fresh complete medium for 48 h. One hour before the end of the incubation period MTT (final concentration 0.5 mg/ml) was added to each well. Then, the medium was removed, and the formazan crystals formed by the cell's capacity to reduce MTT were dissolved with a 50:50 (v/v) DMSO:ethanol solution, and absorbance measured at 570 nm (with background subtraction at 690 nm). The results were expressed as percentage relative to the control (cells without any test compound). The concentration of test compound that decreased the number of viable cells to 50% (IC_{50}) relative to control was calculated using the range of concentrations used (2.5–20 μM) for each compound.

4.2.3. Cell cycle analysis

HCT116 cells were plated in 6-multiwell culture plates at 70 000 cells per mL (2 mL/well) two days before incubation with test compounds. After an incubation period with test compounds of 48 h, both floating and attached cells were harvested by tripsinization, washed with PBS, and fixed with ethanol 70% at 4 °C for 15 min. After rinsing fixed cells with PBS, they were incubated with staining solution (50 μg/ml PI and 20 μg/ml RNase A in PBS) at 37 °C for 15 min. Cell cycle progression was analysed by flow cytometry using a Coulter Epics XL Flow Cytometer (Beckman Coulter Inc., Miami, FL, USA) counting at least 40 000 single cells per sample. Phases of cell cycle were fitted using the mathematical Watson Pragmatic model with the FlowJo Analysis Software (Tree Star, Inc., Ashland, OR, USA).

4.2.4. Nuclear condensation

HCT116 cells were plated in 12-multiwell culture plates at 70 000 cells per mL (1 mL/well) two days before incubation with test compounds. After an incubation period with test compounds of 48 h, both floating and attached cells were harvested by tripsinization, washed with PBS, and fixed with 4% (w/v) paraformaldehyde in PBS for 15 min at room temperature. After rinsing with PBS, cells were attached into a polylysine-treated slide using a Shandon Cytospin 4 (Thermo Scientific, Waltham, MA, USA) and nuclei stained with Hoechst. The percentage of apoptotic cells was calculated from the ratio between cells presenting nuclear condensation and the total number of cells (nuclei stained with Hoechst), from a count higher than 500 cells per slide under a fluorescence microscope. Results are presented as mean ± SEM of at least three independent experiments.

4.2.5. Western blot

HCT116 cells were plated in 6-multiwell culture plates at 70 000 cells per mL (2 mL/well) two days before incubation with test compounds. After an incubation period with test compounds of 48 h, both floating and attached cells were harvested by tripsinization, washed with PBS, and lysed for 15 min at 4 °C with ice cold RIPA buffer (1% NP-40 in 150 mM NaCl, 50 mM Tris–HCl (pH 7.5), 2 mM EDTA) containing 1 mM PMSF, phosphatase inhibitors (20 mM NaF, 20 mM $Na_2V_3O_4$) and protease inhibitor cocktail (Roche, Mannheim, Germany). Protein concentration was quantified using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA) and BSA used as protein standard. For western blot, 20 μg of protein was resolved in SDS-polyacrylamide gel and then electroblotted to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Membranes were blocked in TPBS (PBS with 0.05% Tween-20) containing 5% (w/v) nonfat dry milk, washed in TPBS and incubated with primary antibody overnight. Then, after washing, membranes were incubated with secondary antibody conjugated with horseradish peroxidase and immunoreactive bands were detected using the Immobilon solutions (Millipore) under a chemiluminescence detection system, the ChemiDoc XRS (Bio-Rad Laboratories). Band area intensity was quantified using the Quantity One software from Bio-Rad. The antibodies against p53 (DO-1), p27 (C-19) and PARP-1 (F-2) were purchased to Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibody against β-actin (Sigma–Aldrich) was used as loading control.

4.2.6. Statistical analysis

Values are presented as mean ± S.E.M. of at least 3 independent experiments. Statistical analyses were performed by the Student's *t*-test using the GraphPad Prism 4.0 software (San Diego, CA, USA), and *P*-values ≤ 0.05 were considered statistically significant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.04.064. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] A. Jemal, F. Bray, M. Center, J. Ferlay, E. Ward, D. Forman, CA Cancer J. Clin. 61 (2011) 69–90.
[2] H. Brenner, A. Bouvier, R. Foschi, M. Hackl, I. Larsen, V. Lemmens, L. Mangone, S. Francisci, Int. J. Cancer 131 (2012) 1649–1658.
[3] L. Song, J. Kosmeder II, S. Lee, C. Gerhauser, D. Lantvit, R. Moon, R. Moriarty, J. Pezzuto, Cancer Res. 59 (1999) 578–585.
[4] R. De Vincenzo, C. Ferlini, M. Distefano, C. Gaggini, A. Riva, E. Bombardelli, P. Morazzoni, P. Valenti, F. Belluti, F. Ranelletti, S. Mancuso, G. Scambia, Cancer Chemother. Pharmacol. 46 (2000) 305–312.
[5] S. Kuntz, U. Wenzel, H. Daniel, Eur. J. Nutr. 38 (1999) 133–142.
[6] C. Dyrager, M. Wickstrom, M. Fridén-Saxin, A. Fridberg, K. Dahlén, E. Wallen, J. Gullbo, M. Grothli, K. Luthman, Bioorg. Med. Chem. 19 (2011) 2659–2665.
[7] F. Chimenti, R. Fioravanti, A. Bolasco, P. Chimenti, D. Secci, A. Rossi, M. Yáñez, F. Orallo, F. Ortuso, S. Alcaro, J. Med. Chem. 52 (2009) 2818–2824.
[8] Y. Rao, S. Fang, Y. Tzeng, Bioorg. Med. Chem. 12 (2004) 2679–2686.
[9] Z. Nowakowska, Eur. J. Med. Chem. 42 (2007) 135–137 (and references therein).
[10] S. Won, C. Liu, L. Tsao, J. Weng, H. Ko, J. Wang, C. Lin, Eur. J. Med. Chem. 40 (2005) 103–112.
[11] A. Ullah, F. Ansari, I. Haq, S. Nazir, B. Mirza, Chem. Biodivers 4 (2007) 203–214.
[12] T. Takahashi, N. Takasuka, M. Iigo, M. Baba, H. Nishino, H. Tsuda, T. Okuyama, Cancer Sci. 95 (2004) 448–453.
[13] X. Zi, A. Simoneau, Cancer Res. 65 (2005) 3479–3486.
[14] E. Lee, D. Song, J. Lee, C. Pan, B. Um, S. Jung, Biol. Pharm. Bull. 31 (2008) 1626–1630.
[15] F. Chimenti, R. Fioravanti, A. Bolasco, P. Chimenti, D. Secci, A. Rossi, M. Yáñez, F. Orallo, F. Ortuso, S. Alcaro, R. Cirilli, R. Ferretti, M. Sanna, Bioorg. Med. Chem. 18 (2010) 1273–1279.
[16] S. Caldwell, H. Petersson, L. Farrugia, W. Mullen, A. Crozier, R. Hartley, Tetrahedron 62 (2006) 7257–7265.
[17] R. Václavíková, E. Kondrová, M. Ehrlichová, A. Boumendjel, J. Kovár, P. Stopka, P. Soucek, I. Gut, Bioorg. Med. Chem. 16 (2008) 2034–2042.
[18] H. Kim, S. Kim, B. Kim, S. Lee, Y. Park, B. Park, S. Kim, J. Kim, C. Choi, J. Kim, S. Cho, J. Jung, K. Roh, K. Kang, J. Jung, J. Agric. Food Chem. 58 (2010) 8643–8650.
[19] J. Daskiewicz, F. Depeint, L. Viornery, C. Bayet, G. Comte-Sarrazin, G. Comte, J.M. Gee, I.T. Johnson, K. Ndjoko, K. Hostettmann, D. Barron, J. Med. Chem. 48 (2005) 2790–2804.
[20] U. Wenzel, S. Kuntz, M. Brendel, H. Daniel, Cancer Res. 60 (2000) 3823–3831.

[21] Y. Rao, S. Fang, Y. Tzeng, Bioorg. Med. Chem. 13 (2005) 6850–6855.
[22] L. Howells, R. Britton, M. Mazzoletti, et al., Cancer Prev. Res. 3 (2010) 929–939.
[23] A. Murakami, H. Ashida, J. Terao, Cancer Lett. 269 (2008) 315–325.
[24] J. Hyun, S. Shin, K. So, Y. Lee, Y. Lim, Bioorg. Med. Chem. Lett. 22 (2012) 2664–2669.
[25] M. Cabrera, M. Simoens, G. Falchi, M. Lavaggi, O. Piro, E. Castellano, A. Vidal, A. Azqueta, A. Monge, A. Ceráin, G. Sagrera, G. Seoane, H. Cerecetto, M. González, Bioorg. Med. Chem. 15 (2007) 3356–3367.
[26] J. Spencer, G. Chowrimootoo, R. Choudhury, E. Debnam, S. Srai, C. Rice-Evans, FEBS. Lett. 458 (1999) 224–230.
[27] M. Russo, R. Palumbo, I. Tedesco, G. Mazzarella, P. Russo, G. Iacomino, G. Russo, FEBS Lett. 462 (1999) 322–328.
[28] C. Xavier, C. Lima, A. Preto, R. Seruca, M. Fernandes-Ferreira, C. Pereira-Wilson, Cancer Lett. 281 (2009) 162–170.
[29] C. Xavier, C. Lima, M. Rohde, C. Pereira-Wilson, Cancer Chemother. Pharmacol. 68 (2011) 1449–1457.
[30] J. Araújo, P. Gonçalves, F. Martel, Nutr. Res. 31 (2011) 77–87.
[31] M. Russo, C. Spagnuolo, I. Tedesco, S. Bilotto, G. Russo, Biochem. Pharmacol. 83 (2012) 6–15.
[32] M. Bennett, A. Burke, W. O'Sullivan, Tetrahedron 52 (1996) 7163–7178.
[33] A. Fougerousse, E. Gonzalez, R. Brouillard, J. Org. Chem. 65 (2000) 583–586.
[34] C. Qin, X. Chen, R. Hughes, S. Williams, O. Woodman, J. Med. Chem. 51 (2008) 1874–1884.
[35] K. Kuvale, V. Pape, M. Wiese, Bioorg. Med. Chem. 20 (2012) 346–353.
[36] Y. Zuo, Y. Yu, S. Wang, W. Shao, B. Zhou, L. Lin, Z. Luo, R. Huang, J. Du, X. Bu, Eur. J. Med. Chem. 50 (2012) 393–404.
[37] T. Tran, H. Park, H. Kim, G. Ecker, K. Thai, Bioorg. Med. Chem. Lett. 19 (2009) 1650–1653.
[38] Z. Li, G. Ngojeh, P. DeWitt, Z. Zheng, M. Chen, B. Lainhart, V. Li, P. Felpo, Tetrahedron Lett. 49 (2008) 7243–7245.
[39] M. Liu, P. Wilairat, S. Croft, A. Tan, M. Go, Bioorg. Med. Chem. 11 (2003) 2729–2738.
[40] S. Gunduz, A. Goren, T. Ozturk, Org. Lett. 14 (2012) 1576–1579.
[41] C. Lima, C. Pereira-Wilson, S. Rattan, Mol. Nutr. Food Res. 55 (2011) 430–442.