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Synthetic chalcones, flavanones, and flavones as antitumoral agents: Biological evaluation and structure–activity relationships

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Abstract—A series of synthetic chalcones, flavanones, and flavones has been synthesized and evaluated for antitumor activity against the human kidney carcinoma cells TK-10, human mammary adenocarcinoma cells MCF-7 (estrogen receptor-positive), and human colon adenocarcinoma cells HT-29. The most active series is the chalcone ones with the best results against TK-10 and HT-29 cells. Fourteen out of 53 analyzed compounds resulted very active against at least two of the studied tumoral cells. Alkaline single cell gel electrophoresis, comet assay, was performed as a study of the chromosomal aberrations promoted by the compounds on normal cells. Four active and two inactive chalcones were studied in the comet assay against normal human kidney cells (HK-2). A structure–activity relationship analysis of these compounds was performed and for 4- and 3,4-disubstituted derivatives a quantitative correlation was obtained in the case of anti-HT-29 activity.

1. Introduction

Flavonoids are an extensive group of compounds occurring in plants.[1,2] They are prominent plant secondary metabolites that have been found in dietary components, including fruits, vegetables, olive oil, tea, and red wine.[2] Besides their physiological role in plants, they have shown to possess anxiolytic,[3] anti-inflammatory, antiviral,[4] antiprotozoal,[5] and anticarcinogenic activities.[6] With reference to the antitumor or related activities, it was described for flavonoids antimitotic activity[6f] and/or inhibition of some enzymes[7] like cyclin-dependent kinase, several protein-tyrosine kinases, aromatase, topoisomerase, or protein kinase C.

It has been observed that even a high intake of plant-based dietary flavonoids is safe and not associated with any adverse health effect. In addition, the interaction of dietary flavonoids with the gut has numerous implications for human health and flavonoids in the diet may act as chemopreventive agents against the development of cancer.[8] Apart from their cancer chemopreventive efficacy, such flavonoids could be developed as an alternative medicine to get the beneficial effects in combination treatment by reducing the dose and associated systemic toxicity of chemotherapeutic agents for similar efficacy.[9,10]

As part of our continuous search for potential anticancer drug candidates,[11] and based on the diverse biological activities of flavonoid derivatives, in the present study we have synthesized a series of unnatural flavonoids and examined their in vitro cytotoxic activity on human tumor cell lines, namely kidney carcinoma cells TK-10, human mammary adenocarcinoma cells MCF-7 (estrogen receptor-positive), and human colon adenocarcinoma cells HT-29. In addition, to obtain some preliminary insights into the effect on the normal cells' chromosomal aberrations promoted by these compounds, comet assay was

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performed.[12] A structure–activity relationship analysis was also done in order to understand the structural requirements for optimum activity.

2. Methods and results

2.1. Synthesis

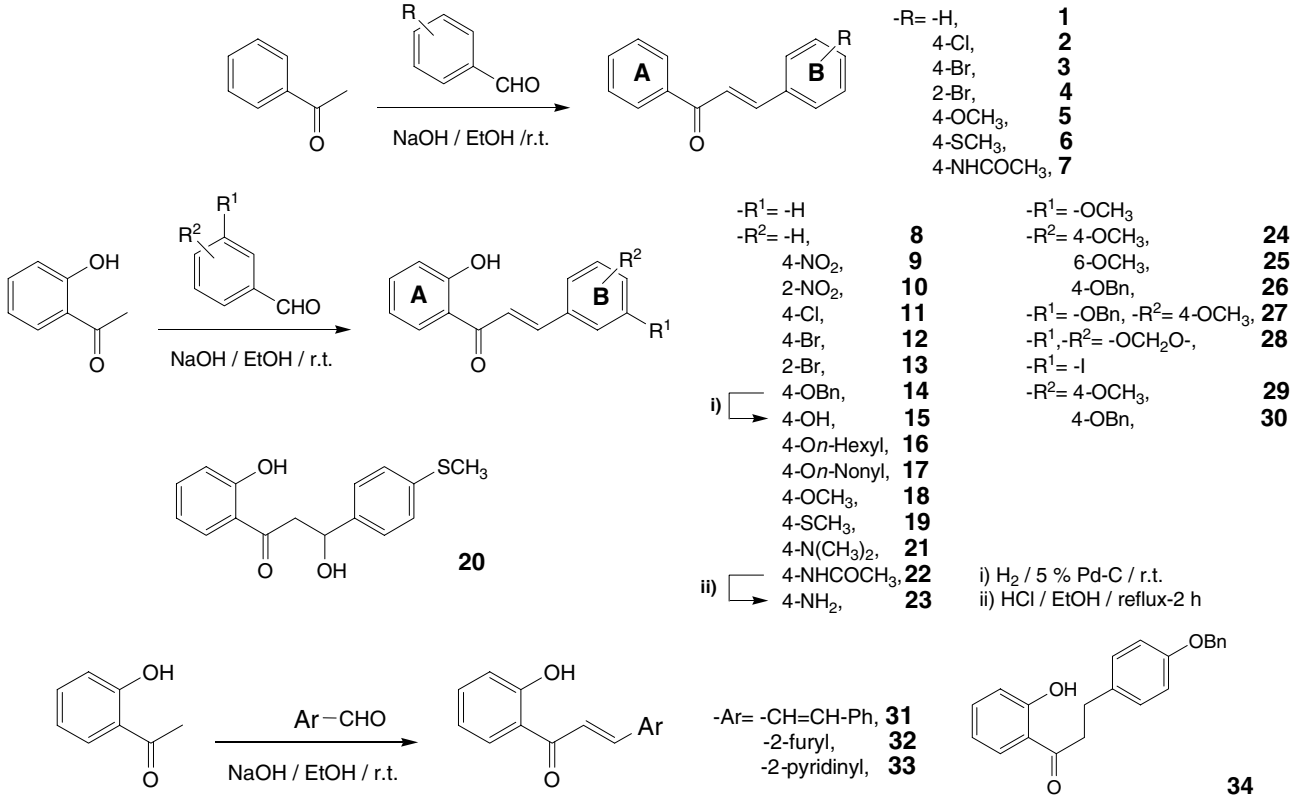
The synthesis of chalcone derivatives substituted in the ring B (Scheme 1) **1**–**5**, **8**–**13**, **18**, **21**, **24**, **25**, and **28** was carried out according to procedures previously described.[10,13] Briefly, we used the aldolic condensation with NaOH as base. In this process, acetophenone or hydroxyacetophenone was reacted with the corresponding aldehyde. In order to modify the lipophilic behavior and volume of chalcones, derivatives **6**, **7**, **14**–**17**, **19**, **22**, **23**, **26**, **27**, **29**, and **30** were prepared using the same methodology. During the synthesis of derivative **19**, aldol **20** was isolated as a secondary product. Further, compounds **31**–**33** were prepared as ring B modified derivatives. Derivative **34** was isolated during derivative **15** synthesis and was used to study the influence of the conjugated enone, Michael system, in the activity. Also, chalcone derivatives with special substitutions in the ring A were developed using adequately substituted hydroxyacetophenone (Scheme 2). Ring B substituted flavanones and flavones were prepared using traditional methodologies (Scheme 3) from the corresponding hydroxychalcones.[6,14] All new compounds were characterized by NMR (^{1}H-, ^{13}C-, COSY, and HETCOR experiments), IR, and MS.

The purity was established by TLC and microanalysis. The stereochemistry around olefinic carbon–carbon bond was established using the corresponding ^{1}H NMR coupling constant. Single crystals of derivative **44** adequate for structural X-ray diffraction studies[15] were obtained by slow evaporation from an Et_2O solution. Figure 1 shows the ORTEP molecular drawing of derivative **44**.

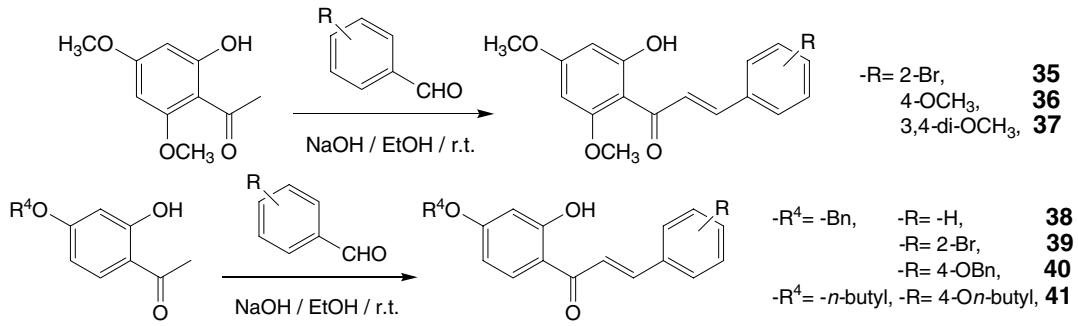
2.2. Biological characterization

2.2.1. Cytotoxicity studies on tumoral cells. The chalcones, flavanones, flavones, and related obtained products were tested at 100 μM doses in aerobic conditions against TK-10 (human kidney carcinoma) (NCI), MCF-7 (human mammary adenocarcinoma) (ATCC HTB-38), and HT-29 (human colon adenocarcinoma) (ATCC HTB-38) tumor lines, according to previously described procedures.[16] Survival percentage (SP) was determined and the results are shown in Figure 2 and collected in Table 1. The IC_{50} concentration (50% inhibitory concentration) was assessed for the most interesting derivatives (Table 2).

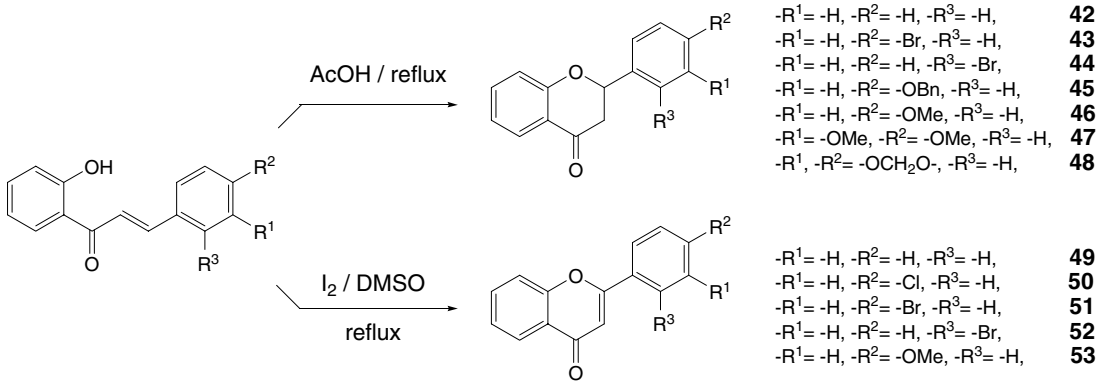
2.2.2. Comet assay. The effect of these compounds against DNA of HK-2 cells (Human kidney cells) was evaluated through the alkaline single cell gel electrophoresis assay. The active derivatives **5**, **8**, **18**, and **33**, and the less active derivatives **12** and **28** were submitted to the comet assay at 20 μM, except for compound **33** at a concentration near to the corresponding $IC_{50,HK-2}$ (see Table 2). Comets were scored visually, and one hundred nucleoids per slide were classified according to the intensity of fluorescence in the



Scheme 1. General procedures used to prepare the desired chalcone derivatives substituted in ring B.



Scheme 2. General procedures used to prepare the desired chalcone derivatives modified in ring A.



Scheme 3. General procedures used to prepare the desired flavanone and flavone derivatives substituted in ring B.

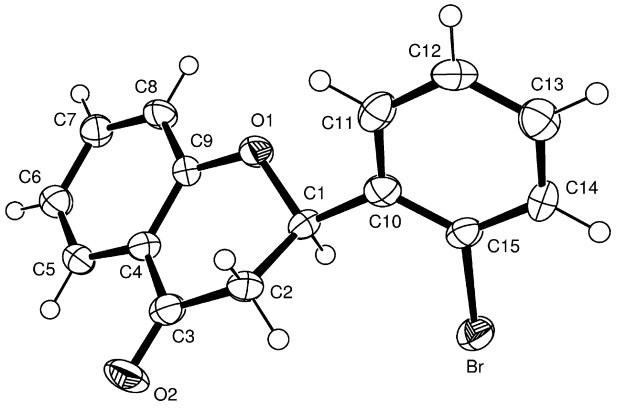


Figure 1. Molecular plot of derivative **44**, showing the labeling of the non-H atoms and their displacement ellipsoids at the 50% probability level.

comet tail.[17] Images were given a value of 0–4 (from undamaged to maximally damaged). DNA damage therefore extends from 0 to 400 arbitrary units and covers a range of strand break frequencies (estimated using X-ray calibration) of 0–3 breaks per 109 Da. The total score was calculated by the following equation: (percentage of cells in class 0×0) + (percentage of cells in class 1×1) + (percentage of cells in class 2×2) + (percentage of cells in class 3×3) + (percentage of cells in class 4×4). Results are shown in Table 3.

2.3. Structure–activity relationships

First, 41 ring-B substituted derivatives were used in the structure–activity relationship studies, namely **1**–**16**, **18**, **19**, **21**–**30**, and **42**–**53**. The different cell-survival percentages (SP) at 100 μM were used as the biological activity. $\log_{10}$ (SP) was used as the dependent variable in the linearization procedure. In the equations n represents the number of data points, r^2 is the correlation coefficient, s is the standard deviation of the regression equation, and F value is related to the F-statistic analysis (Fischer test). As the independent variables were employed the previously tabulated substituent constants related to the electronic, the lipophilic, and the steric properties (Hammett constants σ_m, σ_p, lipophilic constant π, and molar refractivity MR).[18] When the ring B is disubstituted, the sum of the individual parameters was used for lipophilicity ($\sum \pi$) and steric descriptors ($\sum$MR). Some indicator variables were used in order to indicate the presence of OH in ring A (I_{OH} take values 1 if it is present or 0 otherwise), the presence of a flavanone or a flavone (I_C take values 1 if a cycle is present or 0 otherwise), and presence of *ortho* substituent in ring B (I_O take values 1 if it is present or 0 otherwise). The capability of the ring B substituent to participate in a hydrogen bond was taken into account including indicator variables I_{HBD} and I_{HBA} that take values 1 if the group acts donating or accepting hydrogen bond or 0 otherwise.

One-variable and multivariable regressions between the different activities and the physicochemical descriptors were studied. No significative statistical correlation between anti-TK-10 and MCF-7 activities and the studied descriptors was obtained. However, a modest correlation was obtained between anti-HT-29 activities and

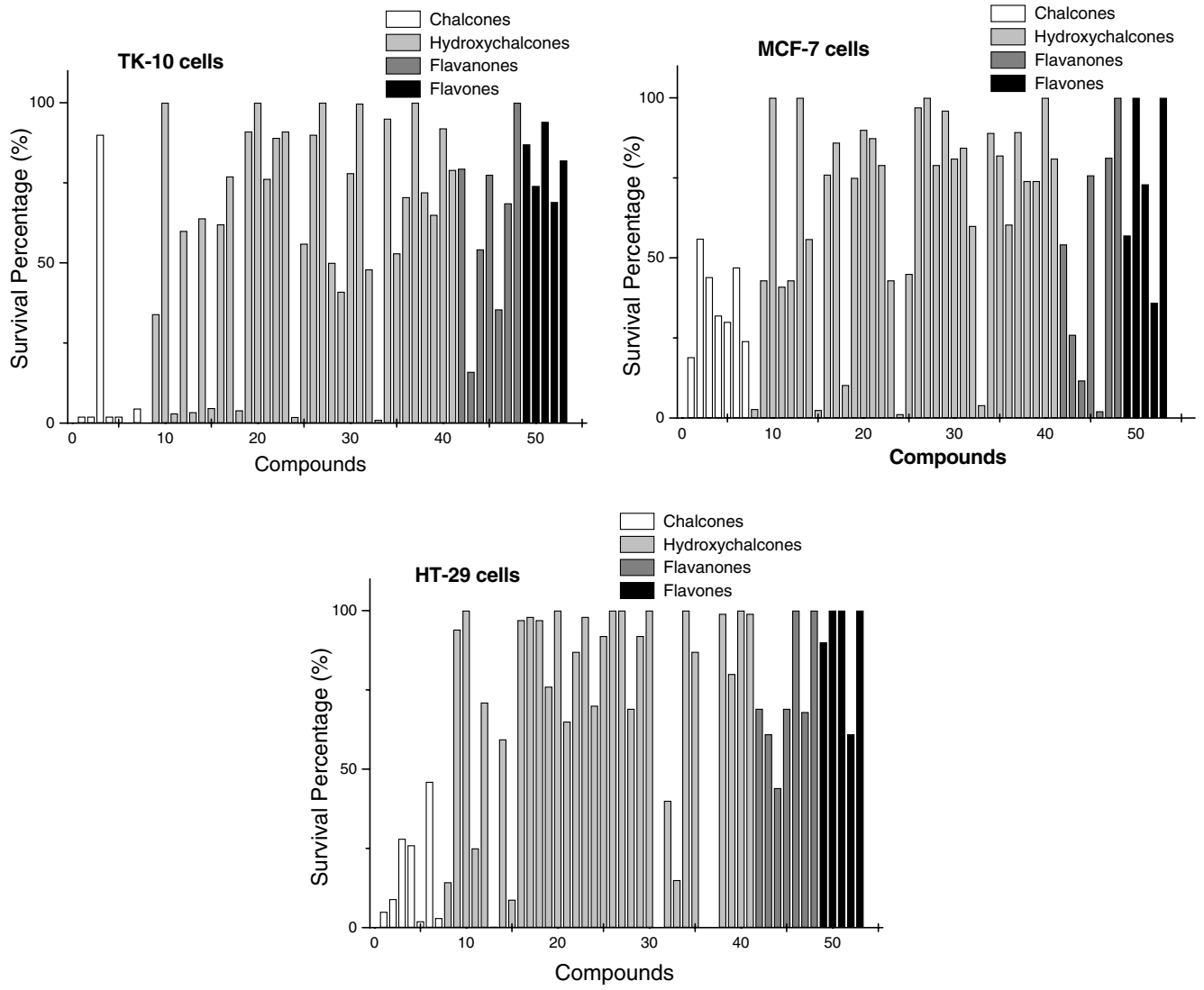


Figure 2. Survival percentages in the different cell-lines of the different series of flavonoids.

some of the studied descriptors (Eq. 1). This modest result could be improved, in the statistical parameters, when *ortho*-ring B substituted derivatives were excluded from the analysis. So, Equation 2 was obtained when derivatives **1**–**3**, **5**–**12**, **14**–**16**, **18**–**19**, **21**–**24**, **26**–**30**, **42**, **43**, **45**–**51**, and **53** were studied. Besides, the correlation matrix for the used physicochemical descriptors was performed and cross-correlations between the descriptors were not obtained. Thus, these parameters are orthogonal, allowing its safely use in the multilinear regression relationship.[19] Clearly, a lack of anti-HT-29 activity, increase of survival percentage, is evidenced when the chalcones are hydroxyl-substituted, in the cases of flavanones and flavones, and with the increment of compounds' volumes. Further, the substituents' capabilities to act as hydrogen bond donor increase the activity of the compounds. Also, lipophilicity (as $\sum \pi$) instead of steric descriptor ($\sum$MR) was included in the study however the statistical quality resulted worse than the one obtained in Equation 1 or 2. Descriptors $\sum \pi$ and $\sum$MR were not studied together because they are not orthogonals.

$$\begin{aligned}\log_{10}(\mathrm{SP}_{\text{HT-29}}) &= 0.85(\pm0.20) - 0.37(\pm0.19)I_{\mathrm{O}} \\ &+ 0.66(\pm0.18)I_{\mathrm{OH}} + 0.88(\pm0.19)I_{\mathrm{C}} \\ &+ 0.32(\pm0.20)(\textstyle\sum \mathrm{MR}) - 0.05(\pm0.04) \\ &\times (\textstyle\sum \mathrm{MR})^2 - 0.26(\pm0.22)I_{\mathrm{HBD}} \\ &n = 41, r = 0.708, r_{\mathrm{adj}} = 0.640, \\ &s = 0.402, F = 5.52, p = 0.0005 \end{aligned} \quad (1)$$

$$\begin{aligned}\log_{10}(\mathrm{SP}_{\text{HT-29}}) &= 0.77(\pm0.16) + 0.83(\pm0.15)I_{\mathrm{OH}} \\ &+ 0.97(\pm0.16)I_{\mathrm{C}} + 0.27(\pm0.15) \\ &\times (\textstyle\sum \mathrm{MR}) - 0.05(\pm0.03) \\ &\times (\textstyle\sum \mathrm{MR})^2 - 0.27(\pm0.17)\mathrm{I}_{\mathrm{HBD}} \\ &n = 34, r = 0.820, r_{\mathrm{adj}} = 0.783, \\ &s = 0.298, F = 11.48, p < 0.0001 \end{aligned} \quad (2)$$

Table 1. Tumor cell cytotoxicity of flavonoids

Compound	Survival percentage[a]		
	TK-10	MCF-7	HT-29
1	2	19	5
2	2	56	9
3	90	44	28
4	2	32	26
5	2	30	2
6	0	47	46
7	5	24	3
8	0	3	14
9	34	43	94
10	100	100	100
11	3	41	25
12	60	43	71
13	3	100	0
14	64	56	59
15	5	3	9
16	62	76	97
17	77	86	98
18	4	10	97
19	91	75	76
20	100	90	100
21	76	87	65
22	89	79	87
23	91	43	98
24	2	1	70
25	56	45	92
26	90	97	100
27	100	100	100
28	50	79	69
29	41	96	92
30	78	81	100
31	100	84	—[b]
32	48	60	40
33	1	4	15
34	95	89	100
35	53	82	87
36	71	60	—
37	100	89	—
38	72	74	99
39	65	74	80
40	92	100	100
41	79	81	99
42	79	54	69
43	16	26	61
44	54	12	44
45	78	76	69
46	36	2	100
47	69	81	68
48	100	100	100
49	87	57	90
50	74	100	100
51	94	73	100
52	69	36	61
53	82	100	100

[a] The results are the means of two different experiments with a SD less than 10% in all cases.
[b] —, not studied.

Table 2. IC_{50} for the most interesting chalcones

Compound	IC_{50}[a,b] (μM)			
	TK-10	MCF-7	HT-29	HK-2
1	42.0	63.0	70.0	30.5
5	53.0	90.0	54.0	23.2
8	79.0	78.0	85.0	24.4
11	—[c]	—	45.0	—
18	67.0	—	—	23.7
24	90.0	95.0	—	—
33	12.2	6.7	13.8	7.9
12	—	—	—	33.8
28	—	—	—	11.6
51	—	—	—	48.0

[a] IC_{50}, concentration that produces 50% inhibitory effect.
[b] The results are means of two different experiments with a SD less than 10% in all cases.
[c] —, not studied.

Table 3. DNA strand breakage (arbitrary units) promoted by chalcones against HK-2 cells

Compound	Number of comets in each category[a,b]					Total score[c]
	0	**1**	**2**	**3**	**4**	
5	37	56	3	2	2	75
8	53	42	2	2	1	56
12	58	38	1	3	0	49
18	53	42	2	2	1	56
28	47	50	0	2	1	60
33	13	64	13	6	4	124
C (−)[d]	47	50	1	1	1	59
C (+)[e]	12	68	19	1	0	107

[a] Compounds were dosed at 20 μM.
[b] The results are means of two different experiments with a SD less than 10% in all cases.
[c] According to the calculus the maximum score is 400.
[d] C (−), control negative, DMSO.
[e] C (+), control positive, HK-2 cells were treated with 50 μM hydrogen peroxide during 15 minutes on ice.

3. Discussion

Fifty-three flavonoids and related compounds were evaluated for their cytotoxic activities against three different human tumoral cells, TK-10, MCF-7, and HT-29. In general, no cytotoxic selectivity between the different cellular lines was observed. Fourteen analyzed compounds were very cytotoxic at least against two of the studied cells (derivatives **1**, **2**, **4**–**8**, **11**, **13**, **15**, **18**, **24**, **33**, and **46**) and clear structure–activity dependence was observed. Thus, chalcones resulted more cytotoxic than flavanones and flavones (compare activities of chalcones **5** and hydroxychalcone **18** to flavanone **46** and flavone **53**). Flavanones' activities could be the result of metabolic transformation into the corresponding hydroxychalcones however in some case could be the result of the own flavanone activity (compare activities of chalcone **12** and flavanone **43**). In general, in the same-substituted series, activity decreases in the order chalcones > flavanones > flavones (i.e., compare activities of derivatives **8**, **42**, and **49**). The most active derivative, namely **33** (see Table 2), belongs to the hydroxychalcone series.

Other structural requirements for the activity could be mentioned. The conjugated enone system results relevant for the activity, that is, aldol **20** is inactive, whereas its parent compounds, **6** and **19**, are more active and

reduced ketone **34** is less active than enone **14**. An extra conjugation does not improve the activity as it can be observed with derivative **31** activity (compare with activity of compound **8**). Further, ring A substitutions produce a lack of activities as can be seen in the activity of compounds **35** and **39** when compared with the parent compounds' activities (derivatives **4** and **13**).

On the other hand, the quantitative structure–activity relationship studies demonstrate the same kind of results mentioned above (see Eqs. 1 and 2). A valid statistical correlation was only obtained for cytotoxicity against HT-29 cells (Eq. 2). In this equation was evidenced the relevance of the chalcone system and the volume and hydrogen bond donor ability of the 4-substituent for anti-HT-29 activity. The relevance of volume could be evidenced for example in chalcones **11** and **12** where the change of chlorine by bromine produces a lack of anti-HT-29 activity or in the case of chalcones **14** and **30** where the inclusion of a 3-iodo substituent produces a clear decrease of activity. Further the relevance of hydrogen bond donor ability could be noted observing the activities of chalcones **15** and **14** where the 4-OH-substituted derivative is the most active of both.

Additionally, the effect of selected compounds against non-tumoral cells, HK-2, was studied. The compounds do not produce relevant DNA strand breakage (see Table 3) being derivative **33** the most harmful one. However, the concentration where **33** shows some comet total score near to positive control is close to three time superior than the corresponding $IC_{50,HK\text{-}2}$ in normal cells (see Table 2).

Currently, there are many approaches that assess a compound's druglikeness partially based on topological descriptors, fingerprints of Molecular Druglikeness structure keys or other properties as *c*log *P* and molecular weights.[20] In the Osiris Property Explorer[21] the occurrence frequency of each fragment is determined within the collection of traded drugs and within the supposedly non-drug-like collection of Fluka compounds. In that case, positive values (0.1–10) point out that the molecule contains predominantly the better fragments, which are frequently present in commercial drugs. In this work, we used the Osiris Properties Explorer tool for calculating the Fragment Based Druglikeness of the most interesting studied compounds, **1**, **2**, **4**–**8**, **11**–**13**, **15**, **18**, **24**, **28**, **33**, **44**, **46**, **51**, and **53**. This tool allows us to predict the undesired effects, like mutagenic, tumorigenic, irritant capacity or reproductive effects (Table 4). In our case, this tool predicts as mutagenic all the studied chalcones, **1**–**7**, while the hydroxylchalcones, except **11** and **15**, flavanones, and flavones are predicted as lacking toxicity risks. Interestingly synthetic derivative **33** (the most cytotoxic studied agent) presents a good druglikeness value and one of the highest drug scores which combines druglikeness, *c*log *P*, log *S*, molecular weight, and toxicity risks in one value and that may be used to judge the compound's overall potential to qualify for a drug.[22] These toxic predictions are in agreement to comet assays performed in this work (see Table 3).

Table 4. Druglikeness properties of some selected derivatives according to Osiris Property Explorer tool[21]

Compound	Toxicity risks[a]				Druglikeness	Drug score
	M[b]	T[c]	I[d]	R[e]		
1	(−)	(+)	(+)	(+)	−2.88	0.25
2	(−)	(+)	(+)	(+)	1.49	0.36
4	(−)	(+)	(+)	(+)	−2.97	0.20
5	(−)	(+)	(+)	(+)	0.83	0.40
6	(−)	(+)	(+)	(+)	0.84	0.34
7	(−)	(−)	(+)	(+)	2.08	0.26
8	(+)	(+)	(+)	(+)	−2.65	0.44
11	(±)	(+)	(+)	(+)	1.58	0.53
12	(+)	(+)	(+)	(+)	−1.38	0.41
13	(+)	(+)	(+)	(+)	−2.85	0.36
15	(±)	(+)	(+)	(−)	0.82	0.35
18	(+)	(+)	(+)	(+)	0.94	0.71
24	(+)	(+)	(+)	(+)	3.18	0.80
28	(+)	(+)	(+)	(+)	1.19	0.66
33	(+)	(+)	(+)	(+)	0.82	0.77
44	(+)	(+)	(+)	(+)	−2.48	0.37
46	(+)	(+)	(+)	(+)	−0.19	0.59
51	(+)	(+)	(+)	(+)	−2.05	0.36
53	(+)	(+)	(+)	(+)	−0.61	0.53

[a] Ranked according to, (+) no bad effect, (±)medium bad effect, (−) bad effect.
[b] M, mutagenic effect.
[c] T, tumorigenic effect.
[d] I, irritant effect.
[e] R, reproductive effect.

These results suggested that the structural features of **33** might be useful in designing new cytotoxic agents.

4. Conclusions

In conclusion, we have developed a wide series of unnatural flavonoids in order to evaluate its antitumoral effects. The results presented above indicate that the in vitro activity of these new compounds against some tumoral cells and its potential use as chemopreventive agents provide supporting evidence for further in vivo studies as anticancer drugs.

5. Experimental

5.1. Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification. Physicochemical and spectroscopical data of derivatives **1**, **8**–**10**, **12**, **14**, **15**, **18**, **21**, **24**–**29**, **32**, **36**–**38**,**40**, **42**, **44**–**49**, **51**, and **53** are in full agreement with those reported.[6a,10,13a,14a,23] All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with sodium sulfate before concentration in vacuo. Melting points were determined using a Leitz Microscope Heating Stage Model 350 apparatus or a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus

pentoxide at 3–4 mmHg, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyser. Infrared spectra were recorded on a Perkin-Elmer 1310 apparatus, using potassium bromide tablets; the frequencies are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Bruker DRX-400 (at 400 MHz) instrument, with tetramethylsilane as the internal reference and in the indicated solvent; the chemical shifts are reported in ppm. *J* values are given in Hz. The ^1H NMR and ^{13}C NMR signals reported were obtained at 303 K. Mass spectra were recorded on a Shimadzu GC-MS QP 1100 EX instrument, using electronic impact at 70 eV.

5.2. General procedure for the synthesis of chalcone derivatives 1–14, 16–19, 21, 22, 24–33, and 35–41

A mixture of the corresponding acetophenone (1 equiv) and the corresponding aldehyde (1 equiv) in anhydrous EtOH (70 mL/23 mmol of acetophenone) was stirred at room temperature during 5 min. Then, NaOH (3 equiv) was added. The reaction mixture was stirred at room temperature until aldehyde consumption. After that, HCl (10%) was added until neutrality. In the cases of the chalcones precipitated, they were filtered and crystallized from MeOH otherwise indicated. In the other cases, the product was purified using column chromatography.

5.2.1. (*E*)-3-(4-Chlorophenyl)-1-phenyl-2-propen-1-one (2). White-yellow solid, mp 114.4–117.1 °C (found: C, 74.3; H, 4.3. $C_{15}H_{11}ClO$ requires C, 74.2; H, 4.6); ^1H NMR ($CDCl_3$) δ: 7.41 (dd, $J_1 = 8.5$, $J_2 = 1.7$, 2H), 7.48–7.64 (m, 6H), 7.77 (d, $J = 15.7$, 1H), 8.03 (dd, $J_1 = 7.9$, $J_2 = 1.7$, 2H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 123.0, 128.9, 129.1, 129.6, 130.0, 133.3, 133.8, 136.8, 138.5, 143.7, 190.6; MS *m*/*z* (abundance, %): 244 ($M^{+\cdot}$+2, 22), 242 ($M^{+\cdot}$, 68), 207 (77), 179 (64), 130 (25), 103 (7), 77 (100); IR *v* 1659, 1402, 822, 776.

5.2.2. (*E*)-3-(4-Bromophenyl)-1-phenyl-2-propen-1-one (3). Pale yellow solid, mp 126.1–128.5 °C (found: C, 62.4; H, 4.1. $C_{15}H_{11}BrO$ requires C, 62.7; H, 3.9); ^1H NMR ($CDCl_3$) δ: 7.49–7.63 (m, 8H), 7.76 (d, $J = 15.7$, 1H), 8.03 (dd, $J_1 = 8.2$, $J_2 = 1.0$, 2H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 123.0, 125.2, 128.9, 129.1, 130.2, 132.6, 133.3, 134.3, 138.5, 143.7, 190.6.

5.2.3. (*E*)-3-(2-Bromophenyl)-1-phenyl-2-propen-1-one (4). Oil (found: C, 62.8; H, 3.7. $C_{15}H_{11}BrO$ requires C, 62.7; H, 3.9); ^1H NMR ($CDCl_3$) δ: 7.28 (dt, $J_1 = 7.9$, $J_2 = 1.6$, 1H), 7.39 (t, $J = 7.4$, 1H), 7.42 (d, $J = 15.7$, 1H), 7.50–7.60 (m, 3H), 7.67 (dd, $J_1 = 8.0$, $J_2 = 1.2$, 1H), 7.76 (dd, $J_1 = 7.8$, $J_2 = 1.6$, 1H), 8.04 (d, $J = 7.9$, 2H), 8.15 (d, $J = 15.7$, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 119.0, 121.0, 127.7, 128.5, 129.3, 130.0, 130.2, 131.6, 134.6, 136.0, 137.5, 145.2, 190.0.

5.2.4. (*E*)-3-(4-Methoxyphenyl)-1-phenyl-2-propen-1-one (5). Pale yellow solid, mp 74.3–77.3 °C (found: C, 80.6; H, 6.0. $C_{16}H_{14}O_2$ requires C, 80.7; H, 5.9); ^1H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 6.96 (d, $J = 8.6$, 2H), 7.43 (d, $J = 15.6$ 1H), 7.51 (dt, $J_1 = 8.1$, $J_2 = 1.0$, 2H), 7.58 (dd, $J_1 = 8.2$, $J_2 = 1.1$, 1H), 7.62 (d, $J = 8.7$, 2H), 7.81 (d, $J = 15.6$, 1H), 8.03 (dd, $J_1 = 8.3$, $J_2 = 1.2$, 2H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 55.8, 114.9, 120.3, 128.1, 128.8, 129.0, 130.6, 132.9, 139.0, 145.1, 162.1, 191.0; MS *m*/*z* (abundance, %): 238 ($M^{+\cdot}$, 67), 207 (17), 179 (7), 130 (3), 103 (8), 77 (100); IR *v* 1713, 1659, 1217, 827, 781.

5.2.5. (*E*)-3-(4-Methylthiophenyl)-1-phenyl-2-propen-1-one (6). White-yellow solid, mp 78.6–80.4 °C (found: C, 75.5; H, 5.3. $C_{16}H_{14}OS$ requires C, 75.6; H, 5.6); ^1H NMR ($CDCl_3$) δ: 2.54 (s, 3H), 7.29 (m, 2H), 7.50 (d, $J = 15.5$, 1H), 7.52 (m, 2H), 7.59 (m, 3H), 7.79 (d, $J = 15.7$, 1H), 8.02 (dd, $J_1 = 8.4$, $J_2 = 1.4$, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 15.6, 121.5, 126.5, 128.9, 129.0, 129.2, 131.9, 133.1, 138.8, 142.8, 144.7, 190.9; MS *m*/*z* (abundance, %): 254 ($M^{+\cdot}$, 100), 207 (60), 179 (20), 105 (25), 77 (57).

5.2.6. (*E*)-*N*-[4-(3-Oxo-3-phenyl-1-propenyl)phenyl] acetamide (7). Yellow solid, mp 177.2–180.1 °C (found: C, 76.9; H, 5.8; N, 5.2. $C_{17}H_{15}NO_2$ requires C, 77.0; H, 5.7; N, 5.3); ^1H NMR (DMSO-d_6) δ: 2.07 (s, 3H), 7.60 (m, 2H), 7.67 (m, 4H), 7.81 (m, 3H), 8.12 (dd, $J_1 = 8.3$, $J_2 = 1.1$, 1H), 10.15 (br s, 1H); ^{13}C NMR (HMQC-HMBC) (DMSO-d_6) δ: 25.0, 119.7, 121.1, 129.3, 129.6, 130.1, 130.7, 133.8, 138.7, 142.5, 144.7, 169.5, 190.0.

5.2.7. (*E*)-3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (11). Yellow solid, mp 146.1–149.4 °C (found: C, 69.3; H, 4.1. $C_{15}H_{11}ClO_2$ requires C, 69.6; H, 4.3); ^1H NMR ($CDCl_3$) δ: 6.97 (dt, $J_1 = 8.1$, $J_2 = 0.9$, 1H), 7.05 (d, $J = 8.4$, 1H), 7.43 (d, $J = 8.5$, 2H), 7.53 (dt, $J_1 = 8.5$, $J_2 = 1.4$, 1H), 7.61 (d, $J = 8.4$ 2H), 7.64 (d, $J = 15.4$, 1H), 7.89 (d, $J = 15.5$, 1H), 7.93 (dd, $J_1 = 8.0$, $J_2 = 1.3$, 1H), 12.74 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 119.1, 119.3, 120.4, 121.1, 129.7, 130.0, 130.2, 133.5, 136.9, 137.3, 144.3, 164.0, 193.9; MS *m*/*z* (abundance, %): 260 ($M^{+\cdot}$+2, 18), 258 ($M^{+\cdot}$, 55), 223 (11), 195 (5), 147 (100), 121 (74), 103 (12); IR *v* 3390, 1644, 1485, 818, 754.

5.2.8. (*E*)-3-(2-Bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (13). Orange-yellow solid, mp 89.5–92.9 °C (found: C, 59.1; H, 3.4. $C_{15}H_{11}BrO_2$ requires C, 59.4; H, 3.7); ^1H NMR ($CDCl_3$) δ: 6.94 (ddd, $J_1 = 7.9$, $J_2 = 7.3$, $J_3 = 1.1$, 1H), 7.03 (dd, $J_1 = 8.4$, $J_2 = 1.0$, 1H), 7.27 (ddd, $J_1 = 7.8$, $J_2 = 7.6$, $J_3 = 1.7$, 1H), 7.38 (t, $J = 7.4$, 1H), 7.50 (ddd, $J_1 = 8.3$, $J_2 = 7.3$, $J_3 = 1.6$, 1H), 7.58 (d, $J = 15.5$, 1H), 7.65 (dd, $J_1 = 8.0$, $J_2 = 8.0$, 1H), 7.74 (dd, $J_1 = 7.8$, $J_2 = 1.6$, 1H), 7.90 (dd, $J_1 = 8.0$, $J_2 = 1.5$, 1H), 8.25 (d, $J = 15.5$, 1H), 12.68 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 119.1, 119.3, 120.3, 123.5, 126.5, 128.1, 128.4, 130.1, 132.0, 134.1, 135.2, 137.0, 144.1, 164.1, 193.9; MS *m*/*z* (abundance, %): 304 ($M^{+\cdot}$+2, 9), 302 ($M^{+\cdot}$, 9), 223 (100), 195 (3), 147 (40), 121 (28), 102 (28), 75 (20); IR *v* 3400, 1644, 1584, 1487, 752.

5.2.9. (*E*)-3-(4-Hexyloxyphenyl)-1-(2-hydroxy phenyl)-2-propen-1-one (16). Yellow solid, mp 88.0–90.0 °C (found: C, 78.0; H, 7.8. $C_{21}H_{24}O_3$ requires C, 77.7; H, 7.5); ^1H NMR ($CDCl_3$) δ: 0.89–0.93 (m, 3H), 1.33–1.36 (m, 4H), 1.78–1.81 (m, 2H), 4.00 (t, $J = 6.6$, 2H), 6.87–6.96 (m, 3H), 7.01 (dd, $J_1 = 8.4$, $J_2 = 0.9$, 1H), 7.47 (ddd, $J_1 = 8.6$, $J_2 = 7.0$, $J_3 = 1.5$, 1H), 7.52

(d, $J = 15.4$, 1H), 7.55–7.62 (m, 2H), 7.89 (d, $J = 15.4$, 1H), 7.91 (dd, $J_1 = 8.0$, $J_2 = 1.5$, 1H), 12.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.0, 22.6, 25.7, 29.1, 31.6, 68.3, 115.1, 117.5, 118.6, 118.7, 120.2, 127.2, 129.5, 130.6, 136.1, 145.5, 161.7, 163.6, 193.7.

5.2.10. (*E*)-1-(2-Hydroxyphenyl)-3-(4-nonyloxyphenyl)-2-propen-1-one (17). Pale yellow solid, mp 75.0–77.0 °C (found: C, 78.9; H, 8.5. $C_{24}H_{30}O_3$ requires C, 78.7; H, 8.3); ^{1}H NMR ($CDCl_3$) δ: 0.89 (t, $J = 6.7$, 3H), 1.22–1.40 (m, 10H), 1.42 –1.48 (m, 2H), 1.76–1.83 (m, 2H), 4.00 (t, $J = 6.6$, 2H), 6.90–6.94 (m, 3H), 7.01 (d, $J = 8.4$, 1H), 7.47 (ddd, $J_1 = 8.5$, $J_2 = 7.1$, $J_3 = 1.4$, 1H), 7.52 (d, $J = 15.4$, 1H), 7.55–7.64 (m, 2H), 7.87–7.91 (m, 2H), 12.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 14.1, 22.7, 26.0, 29.2, 29.3, 29.4, 29.5, 31.9, 68.3, 115.1, 117.5, 118.6, 118.7, 120.2, 127.2, 129.5, 130.6, 136.1, 145.5, 161.7, 163.6, 193.7.

5.2.11. (*E*)-1-(2-Hydroxyphenyl)-3-(4-methylthio phenyl)-2-propen-1-one (19). Yellow solid, mp 75.6–77.0 °C (found: C, 70.9; H, 5.0; S, 11.5. $C_{16}H_{14}O_2S$ requires C, 71.1; H, 5.2; S, 11.9); ^{1}H NMR ($CDCl_3$) δ: 2.55 (s, 3H), 6.96 (t, $J = 8.1$, 1H), 7.05 (d, $J = 8.4$ 1H), 7.30 (d, $J = 8.6$, 1H), 7.52 (m, 4H), 7.64 (d, $J = 15.6$, 1H), 7.91 (d, $J = 15.5$, 1H), 7.95 (d, $J = 1.4$, 1H), 12.85 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 15.5, 119.1, 119.2, 119.4, 120.5, 126.4, 129.4, 130.0, 131.5, 136.7, 143.5, 145.3, 164.0, 194.0. After filtration of derivative **19**, the solvent was evaporated in vacuo and the residue was purified by chromatographic column (SiO_2, petroleum ether:ethyl acetate (0–40%)) isolating derivative **20**.

5.2.12. 3-Hydroxy-1-(2-hydroxyphenyl)-3-(4-methylthiophenyl)-1-propanone (20). Pale yellow solid, mp 119.5–121.5 °C (found: C, 66.5; H, 5.4; S, 10.8. $C_{16}H_{16}O_3S$ requires C, 66.6; H, 5.6; S, 11.1); ^{13}H NMR ($CDCl_3$) δ: 2.51 (s, 3H), 3.18 (s, 1H), 3.36 (dd, $J_1 = 17.5$, $J_2 = 3.2$, 1H), 3.46 (dd, $J_1 = 17.5$, $J_2 = 8.9$, 1H), 5.35 (dd, $J_1 = 8.8$, $J_2 = 2.4$, 1H), 6.92 (m, 1H), 7.00 (d, $J = 8.4$, 1H), 7.29 (d, $J = 8.3$, 2H), 7.31 (d, $J = 8.3$, 2H), 7.44 (m, 1H), 7.71 (dd, $J = 8.0$, $J = 1.5$, 1H), 12.04 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 16.4, 30.1, 47.4, 69.9, 119.0, 119.5, 126.7, 127.3, 129.4, 130.4, 137.3, 138.5, 140.1, 205.7.

5.2.13. (*E*)-*N*-[4-[3-(2-Hydroxyphenyl)-3-oxo-1-propenyl]-phenyl]acetamide (22). Orange-yellow solid, mp 202.2–205.3 °C (from EtOH) (found: C, 72.6; H, 5.1; N, 4.8. $C_{17}H_{15}NO_2$ requires C, 72.6; H, 5.4; N, 5.0); ^{1}H NMR ($CDCl_3$) δ: 3.28 (s, 3H), 7.00 (t, $J = 7.6$, 2H), 7.56 (t, $J = 7.9$, 1H), 7.69 (d, $J = 8.5$, 2H), 7.78 (d, $J = 15.4$, 2H), 7.85 (d, $J = 8.5$, 2H), 7.92 (d, $J = 15.4$, 1H), 8.23 (d, $J = 7.6$, 1H), 10.18 (s, 1H), 12.62 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 24.0, 118.6, 119.7, 120.0, 120.5, 121.6, 129.9, 131.1, 131.6, 137.0, 142.8, 145.6, 162.8, 169.6, 194.4; MS *m*/*z* (abundance, %): 281 ($M^{+\cdot}$, 20), 238 (9), 223 (5), 161 (9), 148 (10), 121 (14), 102 (2), 77 (4).

5.2.14. (*E*)-3-(4-Aminophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (23). Orange solid, mp 153.0–156.0 °C (found: C, 75.0; H, 5.2; N, 5.6. $C_{15}H_{13}NO_2$ requires C, 75.3; H, 5.5; N, 5.9); ^{1}H NMR ($CDCl_3$) δ: 2.55 (s, 2H), 6.96 (t, $J = 7.2$, 1H), 7.05 (d, $J = 8.3$, 1H), 7.29 (d, $J = 8.5$, 2H), 7.52 (d, $J = 8.5$, 1H), 7.60 (d, $J = 8.6$, 2H), 7.63 (d, $J = 15.7$, 1H), 7.88–7.95 (m, 2H), 12.85 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 119.0, 119.2, 119.4, 121.0, 125.0, 126.4, 129.4, 130.0, 131.0, 136.7, 145.4, 164.0, 194.0.

5.2.15. (*E*)-3-(4-Benzyloxy-3-iodophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (30). Oil (found: C, 57.6; H, 3.5. $C_{22}H_{17}IO_3$ requires C, 57.9; H, 3.8); ^{1}H NMR ($CDCl_3$) δ: 5.10 (s, 2H), 6.90 (d, $J = 8.5$, 1H), 6.97 (ddd, $J = 8.2$, $J = 7.4$, $J = 1.2$, 1H), 7.04 (dd, $J = 8.4$, $J = 1.1$, 1H), 7.34–7.59 (m, 7H), 7.61 (d, $J = 8.4$, 1H), 7.81 (d, $J = 15.4$, 1H), 7.93 (dd, $J = 8.1$, $J = 1.4$, 1H), 8.18 (d, $J = 1.8$, 1H), 12.82 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 71.1, 87.1, 112.0, 117.5, 118.6, 118.7, 120.2, 126.3, 126.9, 128.2, 128.7, 129.5, 131.5, 135.4, 136.1, 141.1, 145.5, 161.8, 163.6, 193.7.

5.2.16. (2*E*,4*E*)-1-(2-Hydroxyphenyl)-5-phenyl-2,4-pentadien-1-one (31). Pale-yellow solid, mp 150.0–152.0 °C (from EtOH) (found: C, 81.3; H, 5.3. $C_{17}H_{14}O_2$ requires C, 81.6; H, 5.6); ^{1}H NMR ($CDCl_3$) δ: 6.70 (d, $J = 15.0$, 1H), 6.80–7.15 (m, 4H), 7.20–7.50 (m, 6H), 7.70 (dd, $J = 15.1$, $J = 15.2$, 1H), 7.99 (d, $J = 8.0$, 1H), 12.90 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 111.0–137.0 (12 carbons), 144.5, 161.8, 189.7.

5.2.17. (*E*)-1-(2-Hydroxyphenyl)-3-(2-pyridinyl)-2-propen-1-one (33). Yellow solid, mp 95.8–97.9 °C (found: C, 74.4; H, 4.6; N, 6.0. $C_{14}H_{11}NO_2$ requires C, 74.7; H, 4.9; N, 6.2); ^{1}H NMR ($CDCl_3$) δ: 6.97 (dt, $J_1 = 8.0$, $J_2 = 0.9$, 1H), 7.01 (d, $J = 8.3$, 1H), 7.34 (m, 1H), 7.53 (m, 2H), 7.78 (dt, $J_1 = 7.7$, $J_2 = 1.7$, 1H), 7.88 (d, $J = 15.0$, 1H), 8.06 (dd, $J_1 = 8.0$, $J_2 = 1.3$, 1H), 8.28 (d, $J = 15.0$, 1H), 8.73 (d, $J = 3.9$, 1H), 12.74 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 118.9, 119.3, 120.5, 124.6, 125.1, 126.2, 130.7, 137.0, 137.3, 143.6, 150.7, 153.3, 164.1, 194.6; MS *m*/*z* (abundance, %): 225 ($M^{+\cdot}$, 83), 196 (100), 147 (78), 120 (6), 105 (5), 92 (13), 78 (28); IR *v* 3395, 3050, 1641, 1578, 1338, 1313, 981, 762.

5.2.18. 1-(2-Hydroxyphenyl)-3-(4-benzyloxyphenyl)-1-propanone (34). Oil (found: C, 79.6; H, 5.9. $C_{22}H_{20}O_3$ requires C, 79.5; H, 6.1); ^{1}H NMR ($CDCl_3$) δ: 2.42 (t, $J = 7.8$, 2H), 2.82 (t, $J = 7.8$, 2H), 5.23 (s, 2H), 6.87–6.96 (m, 3H), 7.01 (dd, $J_1 = 8.4$, $J_2 = 0.9$, 1H), 7.47 (ddd, $J_1 = 8.5$, $J_2 = 7.1$, $J_3 = 1.6$, 1H), 7.53–7.64 (m, 7H), 7.97 (dd, $J_1 = 7.9$, $J_2 = 1.5$, 1H), 12.73 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 31.4, 42.0, 70.2, 115.0, 118.6, 118.7, 120.2, 127.1, 127.5, 128.3, 128.7, 129.5, 130.6, 135.9, 136.0, 161.7, 163.6, 193.7.

5.2.19. 3-(2-Bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl)-2-propen-1-one (35). Yellow solid, mp 146.0–147.0 °C (found: C, 55.9; H, 3.9. $C_{17}H_{15}BrO_4$ requires C, 56.2; H, 4.2); ^{1}H NMR ($CDCl_3$) δ: 3.90 (s, 3H), 3.84 (s, 3H), 5.96 (d, $J = 2.4$, 1H), 6.11 (d, $J = 2.4$, 1H), 7.22 (ddd, $J_1 = 7.8$, $J_2 = 7.5$, $J_3 = 1.6$, 1H), 7.34 (d, $J = 7.4$, 1H), 7.62 (dd, $J_1 = 8.0$, $J_2 = 1.1$, 1H), 7.67 (dd, $J_1 = 7.8$, $J_2 = 1.6$, 1H), 7.81 (d, $J = 15.5$, 1H), 8.10 (d, $J = 15.5$, 1H), 14.17 (s, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 55.6, 55.9, 91.3, 93.9, 106.4, 125.8, 127.6, 127.9, 130.2, 130.8, 133.5, 135.7, 140.4, 162.5, 166.4,

168.5, 192.2; MS *m/z* (abundance, %): 364 ($M^{+\cdot}$+2, 33), 362 ($M^{+\cdot}$, 33), 347 (5), 283 (70), 207 (100), 102 (15), 75 (6).

5.2.20. (*E*)-1-(4-Benzyloxy-2-hydroxyphenyl)-3-(2-bromophenyl)-2-propen-1-one (39). Oil (found: C, 64.7; H, 4.4. $C_{22}H_{17}BrO_3$ requires C, 64.6; H, 4.2); ^{1}H NMR ($CDCl_3$) δ: 5.10 (s, 2H), 6.55–6.57 (m, 2H), 7.22–7.45 (m, 7H), 7.57 (d, J = 15.5, 1H), 7.65 (dd, J_1 = 8.1, J_2 = 1.2, 1H), 7.78 (dd, J_1 = 7.8, J_2 = 1.6, 1H), 7.83 (d, J = 9.7, 1H), 8.15 (d, J = 15.5, 1H), 13.27 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 70.7, 102.1, 108.2, 114.1, 120.3, 126.1, 127.7, 127.3, 128.0, 128.3, 128.9, 131.2, 131.9, 133.1, 134.4, 135.6, 144.3, 165.2, 165.6, 192.1.

5.2.21. (*E*)-1-(4-Butoxy-2-hydroxyphenyl)-3-(4-butoxyphenyl)-2-propen-1-one (41). Yellow solid, mp 82.0–84.0 °C (found: C, 74.8; H, 7.6. $C_{23}H_{28}O_4$ requires C, 75.0; H, 7.7); ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, J = 7.4, 3H), 0.99 (t, J = 7.4, 3H), 1.46–1.54 (m, 4H), 1.75–1.82 (m, 4H), 4.01 (t, J = 6.4, 4H), 6.44–6.48 (m, 2H), 6.88–6.96 (m, 2H), 7.44 (d, J = 15.4, 1H), 7.55–7.63 (m, 2H), 7.81 (d, J = 8.6, 1H), 7.85 (d, J = 15.4, 1H), 13.52 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 13.7, 13.8, 19.2, (2 carbons), 31.0, 31.2, 67.9, 68.1, 101.5, 108.0, 114.0, 115.0, 117.8, 127.4, 131.1, 131.3, 144.2, 161.4, 165.7, 166.6, 191.8.

5.3. General procedure for the synthesis of flavanone derivatives (42–48)

A solution of the corresponding 2′-hydroxychalcone (1 equiv) (**8**, **12**–**14**, **18**, **24** or **28**) in AcOH glacial (25.0 mL/mmol of 2′-hydroxychalcone) was heated at reflux during 72 h. Then the mixture was poured into water and extracted with EtOAc (3× 25.0 mL). The organic layer was washed with brine until neutrality and dried with $MgSO_4$ anhydrous. The solvent was evaporated in vacuo and the residue was purified by chromatographic column (SiO_2, petroleum ether:methylene dichloride (0–30%)).

5.3.1. 2-(4-Bromophenyl)-2,3-dihydrochromen-4-one (43). Yellow solid, mp 115.8–119.0 °C (found: C, 59.5; H, 3.6. $C_{15}H_{11}BrO_2$ requires C, 59.4; H, 3.7); ^{1}H NMR ($CDCl_3$) δ: 2.90 (dd, J_1 = 16.8, J_2 = 3.1, 1H), 3.05 (dd, J_1 = 16.8, J_2 = 13.0, 1H), 5.47 (dd, J_1 = 13.0, J_2 = 3.0, 1H), 7.07 (m, 2H), 7.38 (d, J = 8.4, 2H), 7.54 (m, 1H), 7.58 (d, J = 8.5 , 2H), 7.95 (dd, J_1 = 7.8, J_2 = 1.6, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 45.0, 79.2, 118.5, 121.3, 122.2, 123.1, 127.5, 128.2, 132.4, 136.7, 138.2, 161.7, 191.8.

5.4. General procedure for the synthesis of flavone derivatives 49–53

To a solution of the corresponding 2′-hydroxychalcone (1 equiv) (**8**, **11**–**13**, or **18**) in DMSO (10.0 mL/mmol of 2′-hydroxychalcone), I_2 (0.01 equiv) was added. The mixture was heated at reflux during 1 h. Then the mixture was poured into water and extracted with EtOAc (3× 25.0 mL). The organic layer was washed with brine until neutrality and dried with $MgSO_4$ anhydrous. The solvent was evaporated in vacuo and the residue was purified by chromatographic column (SiO_2, petroleum ether:methylene dichloride (0–30%)).

5.4.1. 2-(4-Chlorophenyl)-4*H*-chromen-4-one (50). White solid, mp 172.0–175.4 °C (found: C, 69.9; H, 3.3. $C_{15}H_9ClO_2$ requires C, 70.2; H, 3.5); ^{1}H NMR ($CDCl_3$) δ: 6.81 (s, 1H), 7.45 (t, J = 7.2, 1H), 7.52 (d, J = 8.6, 2H), 7.58 (d, J = 8.4, 1H), 7.73 (dd, J_1 = 7.0, J_2 = 1.6, 1H), 7.89 (d, J = 8.6, 2H), 8.24 (dd, J_1 = 7.9, J_2 = 1.3, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 108.1, 118.4, 124.3, 125.8, 126.2, 128.0, 129.8, 130.7, 134.3, 138.3, 156.6, 162.0, 178.7.

5.4.2. 2-(2-Bromophenyl)-4*H*-chromen-4-one (52). Beige solid, mp 119.5–122.5 °C (found: C, 59.9; H, 2.9. $C_{15}H_9BrO_2$ requires C, 59.8; H, 3.0); ^{1}H NMR ($CDCl_3$) δ: 6.90 (s, 1H), 7.40–7.70 (m, 7H), 8.25 (dd, J_1 = 7.9, J_2 = 1.4, 1H); ^{13}C NMR (HMQC-HMBC) ($CDCl_3$) δ: 108.0, 118.0–138.0 (11 carbons), 157.0, 164.0, 179.0.

5.5. X-ray studies

The crystal and molecular structure of compound **44**, $C_{15}H_{11}BrO_2$, has been determined at 120 K by X-ray diffraction methods. The substance crystallizes in the monoclinic $P2_1/a$ space group with a = 7.976(1), b = 21.126(1), c = 8.107(1) Å, β = 116.54(1)°, and Z = 4. The structure was solved from 1597 reflections with $I > 2\sigma(I)$ and refined to an agreement R1-factor of 0.0508.

Diffraction data and structure solution and refinement. Crystal data, data collection procedure, structure determination method, and refinement results for the compound are summarized in Table 1 (Supplementary material). The hydrogen atoms were positioned stereochemically and refined with the riding model.

Crystallographic structural results and discussion. Atomic fractional coordinates and equivalent isotropic displacement parameters are given in Table 2 (Supplementary material). Intra-molecular bond distances and angles are in Table 3 (Supplementary material). The structure of compound **44** has been deposited in the Cambridge Crystallographic Data Centre, reference number CCDC-631417.

5.6. Biology

5.6.1. Cytotoxicity studies on tumoral cells. *Cells:* an adequate number of cells (TK-10, MCF-7 or HT-29) was maintained in 225 μL of RPMI medium, supplemented with L-glutamine (1%), penicillin/streptomycin (1%), non-essential amino acids (1%), and 10% (v/v) fetal bovine serum (FBS). The cultures were maintained at 37 °C and 5% CO_2 for 48 h. The absorbance at 540 nm before the treatment was determined.

Treatment: Compound solutions were prepared just before dosing. Stock solutions, 1 mM, were prepared in 10% DMSO (Aldrich) and 25 μL (final concentration 100 μM) was added to each well. No effect on cell growth was observed by the presence of DMSO in the culture media. The cells were exposed for 24 h at 37 °C in 5% CO_2 atmosphere.

Sulforhodamine assay: After exposure to the compound, the medium was eliminated and the cells were washed

with PBS. The cells were fixed with 50 μL TCA (50%) and 200 μL of culture medium (without FBS) for 1 h at 4 °C. Then the cells were washed with purified water and treated with Sulforhodamine B (0.4% wt/vol in 1% acetic acid) for 10 min at room temperature. Then plates were washed with 1% acetic acid and dried overnight. Finally, 100 μL of Tris buffer (pH 10.0) was added and absorbance at 540 nm was determined.

Data calculations: The assays were done in duplicate and at least two repetitions per experiment were performed, the absorbances were the means of the two experiments. At the end of the experiment three kinds of measurements were available. Control absorbance (C) that is of cells treated with the solvent; absorbance at time 0 that is before treatment (T_0); and absorbance after the treatment (T). The cell survival percentage (SP) was calculated for all of the compounds as $(T/C) \times 100$. The standard errors were not greater than 10% for most calculated parameters. To determine IC_{50} values, cell growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. The IC_{50} values were determined as the compound concentration required to reduce by half the absorbance of that of the control (without compound).

5.6.2. Comet assay. *Culture of HK-2 cells:* HK-2 cells were routinely cultured in DMEM supplemented with 10% FBS (100 g/mL) and 1% of penicillin/streptomycin in a humidified atmosphere of 5% CO_2 in air at 37 °C.

Sample preparation: Product concentrations were prepared from a stock solution prepared in DMSO. Then, they were diluted in serum-free medium to obtain a final concentration of 20 μM.

Single cell gel electrophoresis (the comet assay): Monolayer HK-2 cells in exponential growth were trypsinized and cell cultures were prepared in a 6-well plate: 2.5×10^6 cells/mL in 2 mL DMEM containing 10% FBS and 1% of penicillin/streptomycin. The cells were exposed to the flavonoid (20 μM in DMSO) for 6 h at 37 °C in 5% CO_2 of air. Control cultures received the equivalent concentration of DMSO to a maximum of 1% of the culture medium. The cultures were washed twice, recovered using trypsin/EDTA, and spun at 200*g* at 4 °C for 3 min. The pellet (approximately 4.5×10^4 cells) was resuspended in LMP agarose for comet analysis. Cells embedded in agarose were lysed with high salt and detergent leaving the DNA as a distinct 'nucleoid'. DNA was allowed to unwind under alkaline conditions. Breaks in the DNA molecule disrupt its complex supercoiling allowing free DNA loops to migrate toward the anode during electrophoresis. DNA damage to the cells can be thus visualized as 'comets'. Cells were suspended in LMP agarose [80 mL of a 1% (w/v) solution in PBS] at 37 °C and pipetted onto a frosted glass microscope slide precoated with a similar solution and amount of buffer (40 mM Hepes–KOH, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/ml BSA, pH 8, 4 °C), blotted dry and incubated either with enzyme or buffer (50 mL) for 45 min (endonuclease III) or 30 min (Fapyglycosylase) at 37 °C. The slides were held in an electrophoresis tank (260 mm wide) containing buffer (1 mM Na_2EDTA, 0.3 M NaOH, pH 12.7, at 4 °C) for 40 min before electrophoresis at 25 V for 30 min. The slides were neutralized by washing 3 times for 5 min each in buffer (0.4 M Tris–HCL, pH 7.5, 4 °C) and stained with DAPI (20 mL of a 5 mg/mL stock solution). DAPI-stained nuclei were evaluated with a Nikon Eclipse TE 300 fluorescence microscope. A total of 100 comets on each comet slide were scored visually and classified as belonging to one of the five classes according to the tail intensity. Each treatment was carried out in duplicate per experiment and each experiment repeated at least twice. DNA damage therefore extends from 0 to 400 arbitrary units. To check the performance of the comet assay, a positive control was included in all the experiments: HK-2 cells were treated with 50 μM hydrogen peroxide during 15 min on ice.

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Supplementary data

Crystallographic data for derivative **44**. Five tables listings of atomic anisotropic displacement parameters (Table S4) and hydrogen atoms' positions and isotropic displacement parameters (Table S5).

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.03.031.

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15. (a) In derivative **44** the phenyl C–C distances in the range from 1.371(6) to 1.410(6) Å correspond to formally resonant C–C bonds. Single C1–C2 and C2–C3 bond lengths in the fused chromane cycle are 1.519(6) and 1.504(6) Å, respectively. π-delocalization at the C3–C4 bond leads to a slightly shorter C–C distance of 1.480(6) Å. Similarly, π-delocalization at the O1-C9 bond makes the O1–C9 length slightly shorter than the O1–C1 distance (1.367(5) and 1.452(5) Å, respectively). The phenyl rings are tilted to each other subtending a dihedral angle of 57.0(2)° (atomic coordinates, bond distances and angles, crystal data, data collection procedure, structure determination method, and refinement results are given as Supplementary information); (b) Enraf-Nonius (1997-2000). COLLECT. Nonius BV, Delft, The Netherlands; (c) Otwinowski, Z.; Minor, W.. In *Methods in Enzymology*; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic Press: New York, 1997; Vol. 276, pp 307–326; (d) PLATON, A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands, A.L. Spek, 1998; (e) Sheldrick, G. M. *SHELXS-97. Program for Crystal Structure Resolution*; University of Göttingen: Göttingen, Germany, 1997; (f) Sheldrick, G. M. *SHELXL-97. Program for Crystal Structures Analysis*; University of Göttingen: Göttingen, Germany, 1997; (g) C.K. Johnson, ORTEP-II.A Fortran Thermal-Ellipsoid Plot Program. Report ORNL-5138, Oak Ridge National Laboratory, Tennessee, USA, 1976.
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