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ORIGINAL CONTRIBUTION

In vitro activity of dietary flavonol congeners against human cancer cell lines

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Abstract
Background Flavonoids have physiological activity and a variety of pharmacological properties, including anticancer activity in vitro, but structure–anticancer activity relationships are unclear.
Aim The objectives of this work were to investigate the activity of dietary flavonol congeners against cell lines derived from human solid tumours and to examine whether the in vitro activity was associated with specific structural feature(s) of the molecules.
Methods Antiproliferative activity of the flavonol congeners was investigated against eight different human cancer cell lines representing different types of human solid tumour, using the sulforhodamine B (SRB) assay in accordance with the instructions published by the NCI. Cell cycle perturbations caused by the congeners were monitored by flow-cytometric analysis of DNA stained with propidium iodide.
Results Most of the flavonols examined had weak antiproliferative and cytotoxic activity. Of all the flavonol congeners tested peracetylated tiliroside found to be the most powerful, with significant antiproliferative and cytotoxic activity. Most flavonols induced similar cell cycle perturbations, whereas induction of apoptosis was significant only for cells treated with peracetylated tiliroside.
Conclusions These findings indicated that the –OH groups of aromatic ring B were not linked to the cytotoxic and antiproliferative activity of the tested flavonols whereas peracetylation of the glycosides resulted in moderate improvement. In contrast, acetylation of tiliroside esterified with coumaric acid at position 5 of the sugar moiety greatly improved the activity of this congener. Overall, the results of this study suggest a critical role of sugar moiety substituents in the anticancer activity of the flavonols.

Keywords Flavonols · Congeners · Tiliroside · Cytotoxicity · Cell cycle

This work is part of C. Tsimplouli's Thesis.

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Introduction

Flavonoids are a group of more than 4,000 polyphenolic compounds that occur ubiquitously in most plants and have been shown to have physiological, pharmaceutical, antioxidant, antiviral, and anticancer activity [1]. They commonly have a generic structure of a chroman nucleus (a benzo ring A, and a heterocyclic ring C) with an aromatic ring B linked at the 2-position. According to the saturation level of the central pyran ring, flavonoids are classified into six main categories: flavones, flavanols, isoflavones,

flavonols, flavanones, and anthocyanines [1, 2]. Flavonoids have been reported to have chemoprotective and cytotoxic activity whereas they have been shown to interfere with cellular proliferation and cell death pathways that lead to tumour development [3]. For these reasons their beneficial effects are unambiguous and much research has been conducted on their putative use as chemoprotective and chemotherapeutic agents [2, 4].

Flavonols have the 3-hydroxyflavone (3-hydroxy-2-phenylchromen-4-one) backbone and their diversity stems from the different position of the phenolic –OH groups. In Western populations, the estimated daily intake is in the range 20–50 mg per day [5]. Amongst them, the most common dietary flavonol quercetin has entered in clinical trials although existing data on the effect of this flavonol seem to be quite controversial [6, 7]. Kaempferol glycosides have also been reported to have in vitro cytotoxic activity, interfering with the cell cycle of human leukemic cell lines and the DNA synthesis pathway, and inducing apoptosis in human leukemic cell lines [8–10]. It has recently been demonstrated that kaempferol and its glycosides may inhibit p90 ribosomal s6 kinases (RSKs), serine/threonine kinases [11]. These are involved in signal transduction and are activated by the MAPK/ERK pathway, suggesting that these compounds and RSK may be important in the development of new targeted cancer chemotherapeutics [12–14].

However, the link between the anticancer activity and structural features of flavonoids is still fragmentary, because experimental results seem quite controversial or the concentrations tested for activity do not fit the acceptance threshold for significant cytotoxic activity [http://dtp.nci.nih.gov/announcements/chg_to_screen.htm] [2, 4]. Hence, in this work our objective was to study the antiproliferative/cytotoxic activity of major flavonols, differing in the number of –OH groups on A and B rings, their glycosides, and their peracetylated derivatives. These were tested against human cancer cell lines in an effort to identify more potent cytotoxic flavonols along with an attempt to search for any structure requirements related to the effectiveness of activity. We therefore tested the ability of 16 flavonols to affect the growth of, and induce cytotoxicity in, eight human solid tumour cell lines and cause cell cycle perturbations.

Materials and methods

Materials

Trichloroacetic acid (TCA), sulforhodamine B (SRB), and Trizma base were purchased from Sigma–Aldrich (St Louis, MO, USA), acetic acid from Fluka (St Gallen, Switzerland), propidium iodide (PI) from Roche (Basel, Switzerland), and all other chemicals from Sigma–Aldrich. All cell culture reagents were purchased from Euroclone Life Sciences Division (Milano, Italy). All flavonol congeners were purchased from Extrasynthese (Lyon, France). Acetylation of the congeners was accomplished according to a published method [15].

Cells

DMS114, H460, MCF7, MB435, DU145, SF268, HT29, and HCT116 cell lines were obtained from the National Cancer Institute, NIH (Bethesda, MD, USA), and were adapted to propagate in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum, 2 mM L-glutamine, and antibiotics. The cultures were grown at 37 °C in a humidified incubator with 5% CO_2-atmosphere.

In vitro cytotoxic activity of the compounds

In vitro cytotoxic activity of all congeners was determined by use of the SRB assay [15]. Cell viability was assessed at the beginning of each experiment by the trypan blue dye exclusion method, and was always greater than 97%. For the SRB assay, cells were seeded into 96-well plates in 100 μL medium at a density of 5,000 to 15,000 cells per well, depending on the cell line, and subsequently, the plates were incubated under standard conditions for 24 h to enable the cells to resume exponential growth before addition of the compounds. In order to measure the starting cell population, cells in one plate were fixed in situ with TCA 50% (*w*/*v*) followed by SRB staining as described elsewhere [10]. To determine cytotoxic activity, all 16 congeners were dissolved in DMSO and then directly added to the cultures at twofold dilutions (from 100 to 6 μM) and incubation was continued for an additional period of 48 h. The final concentration of DMSO in each cell culture was no higher than 0.1%. Cell cultures containing 0.1% DMSO alone served as controls. At this concentration DMSO was found to be inert in all the cell lines tested. The assay was terminated by addition of cold TCA 50% (*w*/*v*) followed by SRB staining and the absorbance was measured at 530 nm, in an EL-311 BioTek microelisa reader (BioTek, Winooski, VT, USA) to determine GI_{50}, TGI, and LC_{50} [16].

Flow cytometric analysis of cell cycle

SF268 cells were used to examine cell-cycle perturbations induced by all the congeners and their acetylated products. Cells were treated with two concentrations of each bioproduct tested, 10 and 30 μM, followed by incubation for 48 h. SF268 cells to which no drug was added were also incubated for 48 h and served as a negative control. After the desired incubation time, aliquots were removed, harvested by centrifugation, suspended and washed in cold

PBS, and finally fixed in ice-cold 70% ethanol. PI was then added at a final concentration of 5 μg/ml and flow-cytometric analysis was performed on an FC500 flow cytometer (Coulter). At least 10,000 events for each sample were counted. Histograms that were generated were subsequently analysed by use of WinMDI software (WinMDI ver 2.8, Scripps Research Institute, USA) [15].

Statistics

Statistical analysis was performed using the unpaired Student *t*-test.

Log*P* evaluation

Log*P* value was investigated for every congener by use of ChemDraw Ultra Version 10.0 supplied by CambridgeSoft (www.cambridgesoft.com).

Results

Cytotoxic and cell antiproliferative activity

Figure 1 shows the structures of all the flavonol congeners tested. The results from measurement of the cytotoxic and cytostatic activity of the flavonols and peracetylated derivatives tested are presented in Table 1 and Fig. 2a; those for the flavonol glycoside and peracetylated derivatives, are presented in Table 2 and Fig. 2b. Only marginal differences were observed for cells treated with the various flavonols. All had moderate growth-inhibiting activity, because GI_{50} was determined to be higher than 10 μM for all the cell lines tested, but none had remarkable cytotoxic activity, as assessed by the corresponding LC_{50} values. Of all the flavonols tested, quercetin and fisetin were slightly more active than the others; their activity was only moderate, however. Modification by peracetylation slightly improved the antiproliferative activity of kaempferol and of myricetin but not their cytotoxic activity. In contrast, acetylation of quercetin and fisetin did not affect the overall activity of these congeners compared with that of the parent compounds. The cell line SF268 was the most sensitive among the eight cell lines used in this study. Glycosylation aggravated the growth-inhibiting activity of both kaempferol and quercetin (Table 2). However, peracetylation significantly enhanced the activity of all the flavonol glycosides. Finally, peracetylated tiliroside (kaempferol-3-*O*-*β*-D-(6″-*E*-*p*-coumaroyl) glycopyranoside) was the most active flavonol congener of all 16 compounds tested. Peracetylated tiliroside had a GI_{50} in the low μM range against all cancer cell lines with a mean GI_{50} value of

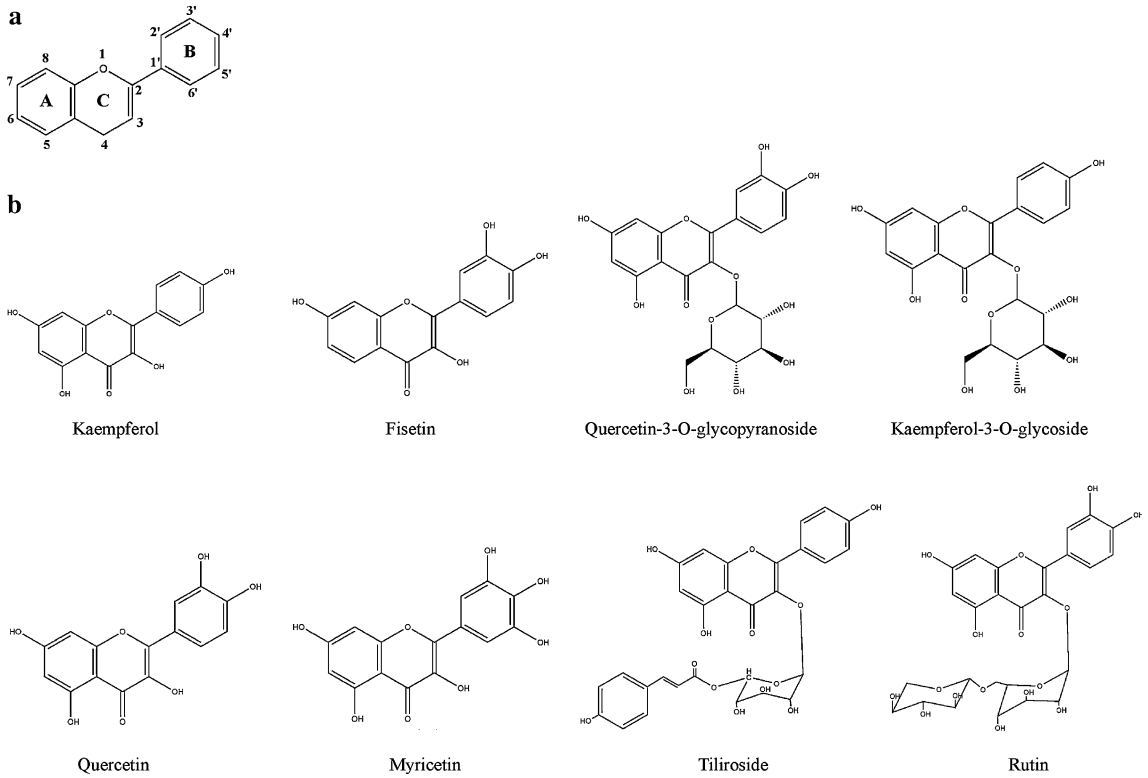


Fig. 1 **a** Flavonols and flavonols glycosides tested for in vitro activity against human cancer cell lines. **b** Basic chemical structure of the flavonoids

Table 1 Cytotoxic activity of flavonols and their peracetylated derivatives

		MCF7[1]	MB435[1]	HT29[2]	HCT116[2]	DMS114[3]	DU145[4]	SF268[5]	NCI-H460[6]	Mean[a]
Kaempferol	GI_{50}	43.8	34.3	26.0	28.9	30.1	33.0	31.3	22.4	31.2
	TGI	92.6	78.7	83.9	70.1	62.9	99.9	90.5	62.9	80.2
	LC_{50}	>100	>100	>100	>100	99.8	>100	>100	>100	>100
Peracetylated kaempferol	GI_{50}	23.9	21.1	18.4	25.0	17.1	27.1	14.5	19.0	20.8
	TGI	53.9	62.8	62.2	72.3	40.4	74.5	51.7	55.4	59.2
	LC_{50}	>100	>100	>100	>100	70.9	>100	>100	>100	>100
Quercetin	GI_{50}	15.4	NT[b]	NT	34.3	NT	NT	16.0	21.0	21.7
	TGI	40.5	NT	NT	97.5	NT	NT	>100	>100	69.0
	LC_{50}	68.2	NT	NT	>100	NT	NT	>100	>100	68.2
Peracetylated quercetin	GI_{50}	22.8	NT	NT	43.8	NT	NT	17.5	34.4	29.6
	TGI	76.9	NT	NT	>100	NT	NT	94.7	>100	85.8
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Fisetin	GI_{50}	21.6	NT	NT	22.8	NT	NT	20.2	15.9	20.1
	TGI	90.1	NT	NT	55.4	NT	NT	71.2	>100	72.2
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Peracetylated fisetin	GI_{50}	20.1	NT	NT	22.3	NT	NT	33.1	37.7	28.3
	TGI	40.4	NT	NT	>100	NT	NT	48.0	93.8	60.7
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Myricetin	GI_{50}	35.5	NT	NT	33.1	NT	NT	31.4	63.7	40.9
	TGI	>100	NT	NT	73.2	NT	NT	79.9	>100	>100
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Peracetylated myricetin	GI_{50}	37.7	NT	NT	21.9	NT	NT	20.3	28.9	27.2
	TGI	>100	NT	NT	>100	NT	NT	77.0	>100	>100
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100

The in vitro anticancer activity of the flavonols and their peracetylated derivatives was determined by use of the SRB assay as instructed by the NCI. All flavonols and their derivatives were tested against a diverse panel of solid tumours. The growth-inhibiting activity (GI_{50}), the cytostatic activity (TGI), and the cytotoxic activity (LC_{50}), were evaluated for each cell line and each compound tested. Results are means from three experiments with a CV $\leq$10%. All values are in micromoles per litre (μM)

[a] Mean values of GI_{50}, TGI, and LC_{50} were calculated for each tested compound

[b] Not tested

[1] Breast cancer

[2] Colorectal cancer

[3] Small-cell lung cancer

[4] Prostate cancer

[5] CNS (glioma) cancer

[6] Non-small-cell lung cancer

6.1 μM (Table 2). In terms of growth inhibition, SF268 and H460 were the cell lines most sensitive to peracetylated tiliroside (Table 2). Peracetylated tiliroside also had the greatest cytotoxic activity among all the flavonols tested; the mean LC_{50} was calculated to be approximately 30 μM.

Apoptosis and cell cycle perturbations

The effect of flavonols, flavonol glycosides, and their derivatives on the cell cycle of SF268 CNS was also studied, because this cell line was found to be the most sensitive to this activity for most of the compounds tested (Tables 1, 2). The concentrations used for treating cells, were selected on the basis of the results obtained from the SRB assay.

Flow-cytometric analysis of the untreated cells revealed the presence of a population of aneuploid cells, approximately 25% of the total cell population (Fig. 3). The diploid cells were distributed in the $G_{0/1}$ phase (52.7%), in the S phase (35.7%), and in $G_{2/M}$ phase (11.6%). For cells treated with 0.1% DMSO (control cell cultures) distribution among the cell-cycle phases was no different from that of the untreated cells (data not shown). Of the four

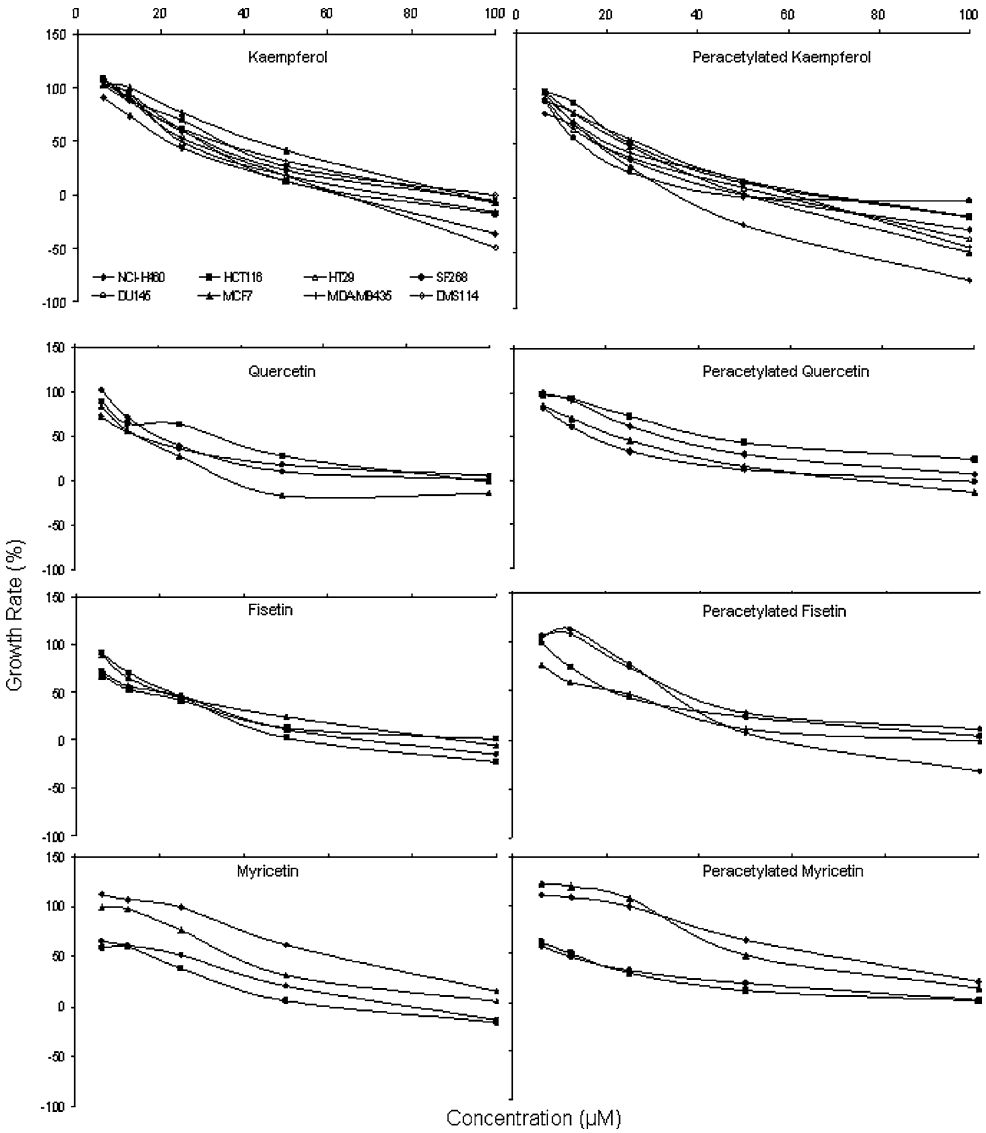
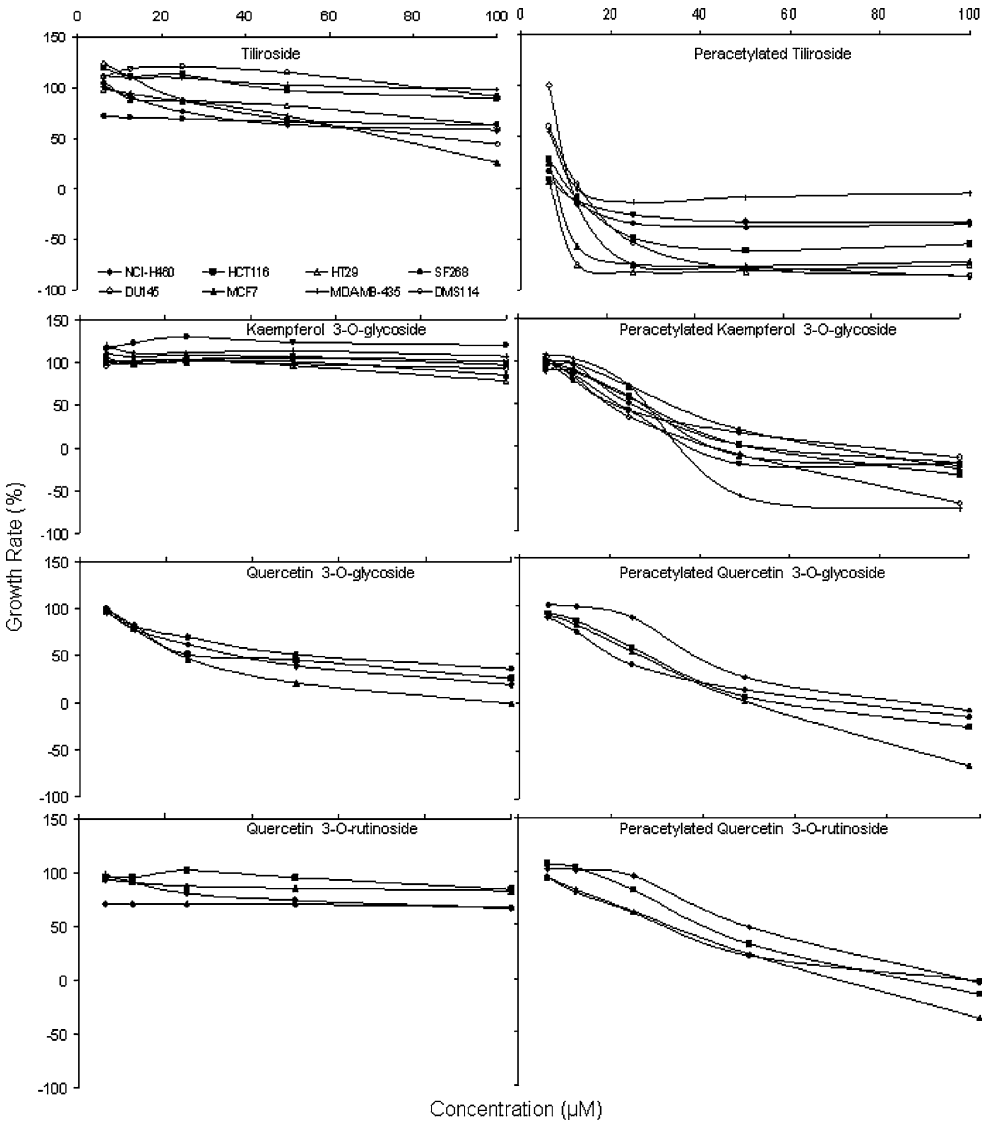


Fig. 2 Growth curves of cells treated with the different flavonols. Cells were exposed to different concentrations of the agents for 48 h and the growth rates were calculated by use of the SRB method (Materials and methods). **a** flavonols and their peracetylated derivatives **b** flavonol glycosides and their peracetylated derivatives. *Points* represent means of three independent experiments, each one run in triplicate. Concentration is in micromoles per litre (μM). CV ≤18%

flavonols tested, fisetin had the highest cytotoxic activity. Treatment of SF268 cell line with 30 μM of the compound for 48 h resulted in the appearance of a sub $G_{0/1}$ phase, apparently consistent with apoptotic cells [17], which was calculated to be 16.1% of the total population, whereas the peracetylated derivative had no significant apoptotic activity at any of the concentrations tested (Fig. 3a). Further, treatment of the cells with 30 μM fisetin, kaempferol, or quercetin for 48 h resulted in a decrease in the $G_{0/1}$ phase and a concurrent increase of the S phase (Fig. 3a), a pattern similar for all three flavonols. The effect on the $G_{2/M}$ phase was unclear and statistically insignificant, because of the presence of the aneuploid cells. Myricetin was inactive at both concentrations under the experimental conditions of this study.

Interestingly, all the flavonol glycosides were completely inactive regarding cytotoxic effect at the concentrations tested for the incubation period indicated (Fig. 3b). However, unlike the other peracetylated flavonols and flavonol glycosides, the activity of peracetylated tiliroside was exceptionally high, because it had significant apoptotic efficacy at both concentrations tested. The apoptotic cell population, designated sub-$G_{0/1}$, increased from 17.9 to 33.4% when the cells were treated with 10 and 30 μM, respectively, of this compound for 48 h. Hence, this compound was the most active among all 16 flavonols tested in this study. Besides the noteworthy effect on cell death, treatment of the same cells with peracetylated tiliroside resulted in significant cell cycle perturbations. A significant reduction of the $G_{0/1}$ phase was observed, with a concomitant increase of the S phase in cells treated with either 10 or 30 μM of the agent, and a statistically significant increase of the $G_{2/M}$ phase was also prominent for cells treated with 30 μM (Fig. 3b). These results suggest that the sub-$G_{0/1}$ cell population originates from cells either in the $G_{0/1}$ phase or at the

Fig. 2 continued



$G_{0/1}$/S boundary; a delay in the S and $G_{2/M}$ phases may also occur.

Discussion

Dietary flavonoids are present in a wide variety of fruit and vegetables and have a wide range of biochemical and pharmacological activity [1]. Many studies have shown that flavonoids can be potent growth inhibitors of a variety of cancer cell lines [3] and result in significant reduction of tumour development in animal models [2]; other studies have tried to propose structural requirements for diverse activity, including cytotoxicity [4, 7, 18]. Our attention was directed toward dietary flavonols, whose structures differ in the number and position of hydroxyl substituents and the existence of a glucose moiety. Some of the flavonol derivatives used in this study have also been reported by others to have improved activity compared with the parent glycosides [8–10, 19] whereas others interfere with important signalling pathways, for example the MAPK pathway [12, 13, 20]. Our objective here was not to address any aspect of the mechanisms of action, because most of these compounds are obviously pleotropic, but rather to study directly

1. the antiproliferative activity of dietary flavonol congeners against cell lines derived from human solid tumours, evaluating them thus as putative chemotherapeutic agents; and
2. whether this in vitro activity, and thus their chemotherapeutic potency, was associated with specific structural feature(s) of the molecules.

In total, 16 flavonols and their glycosides were studied for their ability to kill or delay the proliferation of different human cancer cell lines and to interfere with their cell

Table 2 Cytotoxic activity of flavonol glycosides and their peracetylated derivatives

		MCF7	MB435	HT29	HCT116	DMS114	DU145	SF268	NCI-H460	Mean[a]
Kaempferol-3-*O*-glucoside	GI_{50}	>100	>100	>100	>100	>100	>100	>100	>100	>100
	TGI	>100	>100	>100	>100	>100	>100	>100	>100	>100
	LC_{50}	>100	>100	>100	>100	>100	>100	>100	>100	>100
Peracetylated kaempferol-3-*O*-glucoside	GI_{50}	35.1	29.7	33.6	28.1	30.1	33.2	22.7	25.2	29.7
	TGI	70.9	43.4	65.9	51.9	52.4	79.1	41.9	53.8	57.4
	LC_{50}	>100	73.0	>100	>100	86.5	>100	61.8	>100	>100
Tiliroside	GI_{50}	74.4	>100	68.4	>100	73.5	>100	>100	>100	72.1
	TGI	>100	>100	>100	>100	>100	>100	>100	>100	>100
	LC_{50}	>100	>100	>100	>100	>100	>100	>100	>100	>100
Peracetylated tiliroside	GI_{50}	4.3	6.9	10.4	4.1	9.0	7.4	3.7	3.5	6.1
	TGI	8.1	12.4	16.7	10.9	11.7	13.2	8.8	9.7	11.5
	LC_{50}	12.0	61.4	24.2	27.9	19.7	24.3	35.4	34.7	29.9
Quercetin 3-*O*-glucoside	GI_{50}	23.7	NT[b]	NT	37.9	NT	NT	53.5	38.3	38.4
	TGI	98.2	NT	NT	>100	NT	NT	>100	>100	>100
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Peracetylated quercetin 3-*O*-glucoside	GI_{50}	26.3	NT	NT	28.5	NT	NT	21.0	40.4	29.1
	TGI	50.8	NT	NT	60.0	NT	NT	73.2	88.2	68.1
	LC_{50}	88.3	NT	NT	>100	NT	NT	>100	>100	>100
Rutin (quercetin-3-rutinoside)	GI_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
	TGI	>100	NT	NT	>100	NT	NT	>100	>100	>100
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100
Peracetylated rutin	GI_{50}	32.8	NT	NT	41.1	NT	NT	32.1	47.7	38.4
	TGI	68.6	NT	NT	83.9	NT	NT	93.8	95.9	85.6
	LC_{50}	>100	NT	NT	>100	NT	NT	>100	>100	>100

Flavonol glycosides and their peracetylated derivatives were examined using the same methodology as for the aglycones (Table 1). Results are means from three experiments with a CV ≤15%. All values are in micromoles per litre (μM)

[a] Mean values of GI_{50}, TGI, and LC_{50} were calculated for each tested compound

[b] Not tested

cycle. Flavonol, coumaric acid and their acetylated derivatives were also studied but found to be totally inactive and thus not included (data not shown).

The procedure used by the NCI for its developmental therapeutics program (DTP) (http://dtp.nci.nih.gov/) was selected as appropriate to study the cytotoxic activity of the examined flavonols and derivatives. Cell cycle perturbations caused by the different flavonols were also examined, using a glioma cancer cell line, SF268, which seemed to be the most sensitive to most of the compounds tested. Two different concentrations were tested, chosen according to the mean GI_{50} value of most of the compounds against this cell line, and which, interestingly enough, were close to the plasma concentrations reported by other researchers, 10–50 μmol/L [21]. All flavonols were tested for activity under the same experimental conditions.

Considering 10 μM as the threshold for acceptance of significant antiproliferative activity (http://dtp.nci.nih.gov/announcements/chg_to_screen.html), the aglycones studied herein found to have moderate activity at high micromolar concentrations (Table 1). Their cytotoxic activity was almost negligible, even at concentrations close to or much higher from 100 μM (Table 1). Quercetin, which has been extensively studied [13, 14] had antiproliferative activity above 15 μM against the MCF7 breast cancer cell line, whereas the mean GI_{50} was approximately 22 μM. Fisetin, another well studied flavonol, was found to have a GI_{50} value of approximately 16 μM against the H460 a non-small-cell lung cancer cell line (Table 1). Acetylation did not improve the efficacy of any of the aglycones. The overall differences in activity should be regarded as minor, suggesting that dissimilarities in their structures only marginally affect the antiproliferative and cytotoxic activity of these flavonols, a conclusion which is in agreement with previous reports [4].

With the exception of quercetin-3-*O*-glucoside, which had moderate antiproliferative activity (mean GI_{50} = 38.4 μM, Table 2) the glycosides tested herein were inactive against all cell lines, having neither cytotoxic nor antiproliferative activity. In conclusion, although

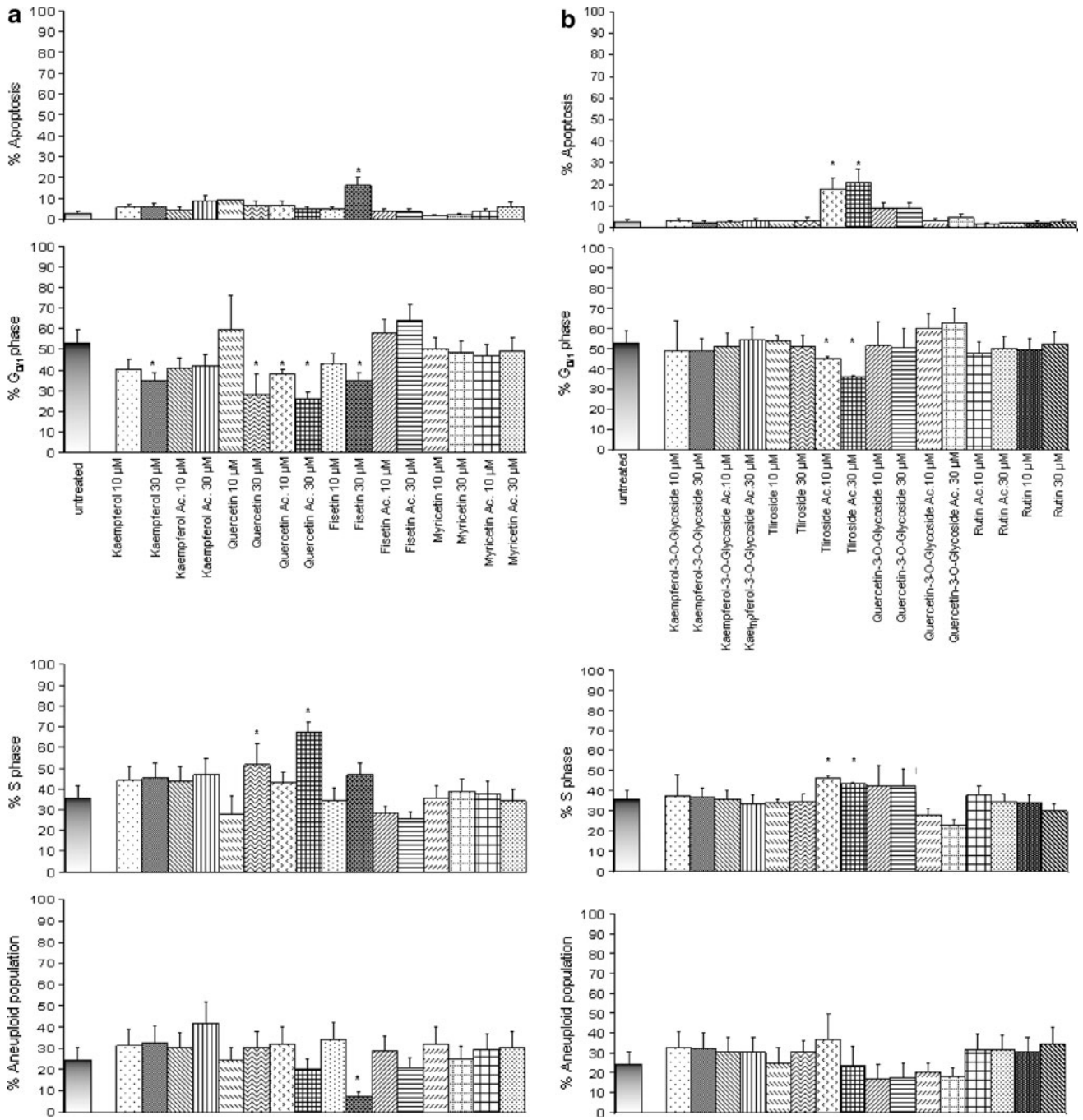


Fig. 3 Flow cytometry histograms of SF268 cells exposed to flavonols and their derivatives. Cell cycle phase distribution of untreated cells or cells exposed to either 10 or 30 µM (micromoles per litre) of flavonols and their derivatives. **a** Aglycones (flavonols) and **b** flavonol glycosides. *Each point* represents the mean and SD from three independent experiments. Acetylated peracetylated compounds: *30*, 30 µM; *10*, 10 µM; $*p < 0.05$. DMSO-treated cells behaved no differently from untreated cells (not shown)

peracetylation improved the antiproliferative activity of the tested flavonol glycosides, the resulting activity for most can be regarded as moderate only. The increase in cytotoxicity could be attributed to the increased lipophilicity of the peracetylated derivatives, which has been reported to favour entry of flavonoids into the cell [2]. Indeed, when the log*P* values were calculated it was found that peracetylation did not affect the log*P* of the aglycones although it increased the log*P* of kaempferol 3-*O*-glucoside by a factor of 6 (from −1 to −0.16) and of quercetin-3-*O*-glucoside by a factor of 12 (from −1.39 to −0.11). Because this was not of benefit to the activity of the latter compound, in accordance with previous reports, increased lipophilicity can only be partially related to potency [13].

Glycosides are the main structural forms of flavonols present in the human diet and thus the main source of aglycones. Glycosides can be absorbed by cells using the intestinal glucose transporter pathway [22–24]. Although the HCT116 and HT29 cells used in this study are of colorectal origin, SGLT1 expression and its effect on the activity of glycosides and their derivatives was not addressed herein. Consequently, it would be of further interest to examine this transporter, because such a study could provide a direct link between the transporter and, consequently, the intestinal glucose transporter pathway, and the beneficial effects of specific nutrients, for example flavonol glycosides.

The most prominent compound in this study among the 16 flavonols tested was peracetylated tiliroside, which was significantly more active than all other compounds tested. This semi-synthetic glycoside (derivative of tiliroside, a 3-*O*-kaempferol glycoside ester with coumaric acid) had significant antiproliferative activity in the low μM range (mean $GI_{50} = 6.1$ μM, Table 2) and cytotoxic activity in the mid to low μM range. Furthermore, its significantly different cytotoxic activity against the individual cell lines, as the LC_{50} value, ranged from 12 μM for the MCF7 breast cancer cell line to 64 μM for the MB435 melanoma cell line (Table 2), strongly suggesting that although the compound is not cytotoxic in general its activity is specific.

The following results:

1. acetylation of the aglycones did not result in increased activity compared with the parent flavonols;
2. peracetylation of the kaempferol glycoside resulted in moderate activity; and
3. tiliroside was totally inactive

strongly suggest that acetylation of the sugar moiety is of importance for the increased activity of these flavonols and that the substantial increase in the activity of tiliroside is because of acetylation of the OH group carried by the coumaric acid. Previous studies on tiliroside and platanoside have also reported that acetylation might be of benefit for kaempferol glycosides [7–10]. Furthermore, much attention has lately been focussed on acetylated kaempferol glycosides, because an acetylated kaempferol glycoside, known as SL0101, has been reported to interfere with the PKC pathway by blocking the function of p90 s6 ribosomal kinase (RSK) and thus has promising in vitro activity against breast and prostate cancer cell lines [12, 20]. Interestingly, the activity of this kaempferol glycoside has been reported to be dependent on acetylation of the OH groups on its rhamnose moiety [13].

Further to the cytotoxicity results, study of the cell cycle perturbations caused by these flavonols against SF268 revealed that peracetylated tiliroside was again the most active, because treatment of the cells with 10 μM of the compound resulted in a substantial increase in the sub $G_{0/1}$ population, which is considered to consist of apoptotic cells, a decrease in the $G_{0/1}$ population, and an increase at the S phase. Reduction of the $G_{0/1}$ phase suggests that the apoptotic population originates from the $G_{0/1}$ phase but this must be further and more extensively studied.

Of the other flavonols examined, fisetin resulted in a substantial increase of the sub-$G_{0/1}$ population and cell cycle perturbations similar to those caused by peracetylated tiliroside when cells were treated with 30 μM of the compound. Treatment with quercetin and kaempferol resulted in apoptosis of negligible statistical significance but, nevertheless, the same pattern of cell cycle perturbations as peracetylated tiliroside and fisetin, i.e. a decrease of the $G_{0/1}$ phase and an increase of the S at the high (30 μM) concentration.

Conclusions

The study reported herein was undertaken to directly evaluate the in vitro antitumour activity of significant dietary flavonols and their derivatives and to associate specific structural feature(s) of these molecules with this activity. The findings of this study imply that:

1. with the exception of acetylated tiliroside the different flavonols have only moderate antiproliferative potency against cells from human solid tumours under the experimental conditions tested, on the basis of the threshold given by the NCI; and
2. the different structural patterns resulted in only minor differences in their in vitro anticancer activity.

However, the results in this report suggest the immense importance of acetylation of hydroxyl groups (–OH) present in the glucose moiety. Although esterification of the flavonol kaempferol with a glucose moiety acidified with a coumaroyl group, substantially reduces its anticancer activity, peracetylation of this derivative resulted in a substantial increase of cytotoxic and cytostatic activity. This is undoubtedly related to the presence of the coumaroyl group, exclusive for this compound, and its acetylation, with the substantial amplification of its anticancer activity giving rise to a highly active compound.

Finally, taking into consideration this study and the immense amount of research on acetylated flavonol glycosides and their cytotoxic activity against novel targets, it would be of great interest to further test the effect of acetylation on other esterified flavonoid glycosides containing either more and/or different alkyl groups, or even different phenolic acids, and to further investigate the possibility that these may be a class of agents with novel, promising anticancer activity.

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