Synthesis, Characterisation and Antitumour Activity of Some Quercetin Analogues

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Quercetin is one of the most abundant naturally occurring flavonoid and is associated with a wide range of biological activities, such as antioxidant, antiinflammatory and anticancer activities. However, there are multiple problems associated with the bioavailability of quercetin, thereby restricting its use. Taking this into consideration, the structure of quercetin was modified by removal of multiple hydroxyl groups and introduction of substituents such as Cl, OCH_3 and N $(CH_3)_2$ on the p-position of the B-ring. The effect of structural modification on the anticancer activity was studied using four different cell lines, including MCF-7, HepG2, HCT-15 and PC-3. Compound 1a has shown an activity better than quercetin in HepG2 cell lines, whereas 1c and 1e showed significant growth inhibition of the HCT-15 cell lines.

Key words: Anticancer activity, quercetin, structural modifications

Flavonoids are polyphenolic compounds possessing a diphenylpropane skeleton. They are divided into various subgroups depending upon the number and the position of the substituents. Quercetin is one of the most abundant flavonoids present in fruits and the vegetables[1]. Structurally, it is associated with a wide range of biological activities, such as antioxidant, antiinflammatory and anticancer activities[2-5]. It is present in the form of glycosides. The polyphenolic glycosides are too polar to penetrate the intestinal membranes and hence are not very easily absorbed. Release of the aglycones by the action of the microfloral enzymes is needed for the compounds to become absorbable. Although aglycones are permeable, the bioavailability is low due to poor water solubility and a greater extent of conjugation[6,7].

Glucuronidation, sulphation and methylation of the multiple phenolic groups and identification of up to 23 different conjugates of quercetin have been reported[8]. The plasma protein binding of quercetin is as high as 99.1%[9]. Multiple hydroxyl (OH) groups, particularly when present at 3' and 4' positions in the molecule, make it susceptible to chemical and microbial degradation in the colon[10]. Important functionalities for the oxygen scavenging action of flavonoids include either the catechol function or the 2,3-double bond along with the 3-OH group[11,12]. In studies of the binding of quercetin with the model membranes, it was observed that the protective action of the flavonoids at the membrane is due to lipophilic as well as the H-bonding interactions of flavonoids[13,14]. Considering the multiple OH groups present in the structure of quercetin and their effect on the metabolism and bioavailability, we decided to modify the structure by reducing the number of OH groups with a view to reduce the degradation. Our previous results with B-ring substituted flavones devoid of hydroxyl groups were encouraging[15]. In view of the importance of the 2,3-double bond, it was decided to retain it. To explore the effect of hydroxy groups at 3 and 7 positions, we decided to synthesise the flavonols.

Compounds 1a-1e were synthesised by Claisen-Schmidt condensation involving the formation of chalcone intermediates, followed by their cyclization to yield flavonols. Briefly, equimolar quantities of 4-substituted benzaldehyde and 2-hydroxyacetophenone/2,4-dihydroxyacetophenone were stirred at room temperature in the presence of potassium hydroxide until complete consumption of aldehyde. The chalcone thus obtained was cyclized in the presence of sodium hydroxide and hydrogen peroxide to give corresponding flavonol (Scheme 1).

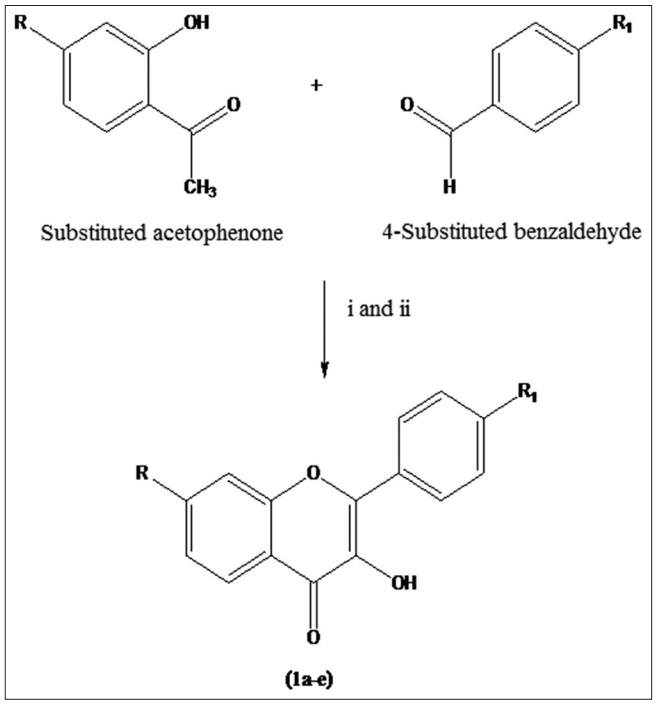
Adopting the above synthetic scheme, five different analogues of quercetin (1a-1e) were synthesised. Spectral analysis of the synthesised compounds is summarised below. Physicochemical properties of the synthesised compounds are listed in Table 1.

Quercetin; Fourier transform infrared spectroscopy (FTIR) (KBr, cm^{-1}): 2715-2897 (aromatic C-H), 1664 (C = O), 3282-3404 (O-H), 1014-1132 (C-O-C); Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) (dimethyl sulfoxide [DMSO]-d_6, δ ppm): 6.149-7.641 (m, 5H, aromatic), 7.641 (d, 1H, 2'-H), 7.501 (dd, 1H, 6'-H), 6.857 (d, 1H, 5'-H), 6.370 (d, 1H, 8-H), 6.149 (d, 1H, 6-H), 9.288-12.462 (s, 5H, OH groups); Carbon-13 nuclear magnetic resonance (^{13}C NMR) (DMSO-d6 at 323K, δ ppm): 176.4, 164.4, 161.2, 157.0, 148.2 147.4, 145.5,136.2, 122.5, 116.1, 115.6, 115.7, 98.7, 98.7, 93.8.

Compound 1a; melting point: 197-198º, FTIR (KBr, cm^{-1}): 2812-2875 (aromatic C-H), 1614 (C=O), 3284 (O-H), 1091-1107 (C-O-C); ^{1}H NMR (DMSO-d_6, δ ppm): 9.63(s), 8.26(s), 8.24(s),

TABLE 1: PHYSICOCHEMICAL DATA

Comp. No.	R	R1	Molecular formula	% yield	M.P. (°)	R_f value	Elemental analysis % calculated (% found)	
							C	H
Quercetin	-	-	$C_{15}H_{10}O_7$		314-316		59.609 (59.587)	3.334 (3.341)
1a	H	Cl	$C_{15}H_9O_3Cl$	69	197-198	0.71	66.150 (66.157)	3.322 (3.181)
1b	H	OCH_3	$C_{16}H_{12}O_4$	67	196-198	0.63	71.635 (71.631)	4.477 (4.413)
1c	H	$N(CH_3)_2$	$C_{17}H_{15}O_3N_1$	61	190-194	0.72	72.583 (72.431)	5.374 (5.391)
1d	OH	OCH_3	$C_{16}H_{12}O_5$	66	192-196	0.69	67.605 (67.571)	4.225 (4.181)
1e	OH	$N(CH_3)_2$	$C_{17}H_{15}O_4N_1$	69	198-200	0.70	68.677 (68.703)	5.085 (5.113)



Scheme 1: Synthesis of 2-(4-substituted phenyl)-3-hydroxy -chromen-4-one and 2-(4-substituted phenyl)-3,7-dihydroxy-chromen-4-one (1a-e).
R = H or OH, R1 = Cl, OCH_3 or N $(CH_3)_2$; (i) EtOH, 1,4-dioxane, 40% w/v KOH; (ii) 1, 4-dioxane, EtOH, 5.4% w/v NaOH, 35% H_2O_2.

8.12(d), 7.81(t), 7.74(d), 7.64(s), 7.62(s), 7.49(t); ^{13}C NMR (DMSO-d6 at 323K, δ ppm): 173.4, 155.0, 144.5, 139.6, 134.9, 134.1, 130.6, 130.6, 129.7, 129.7, 129.0, 125.2, 125.0, 121.7, 118.8.

Compound 1b; FTIR (KBr, cm^{-1}): 2814-2892 (aromatic C-H), 1612 (C=O), 3204 (O-H), 1087-1111 (C-O-C); ^{1}H NMR (DMSO-d_6, δ ppm): 9.26(s), 8.21(s), 8.19(s), 8.12(d), 7.79(t), 7.74(d), 7.47(t), 7.15(s), 7.13(s), 3.87(s); ^{13}C NMR (DMSO-d6 at 323K, δ ppm): 173.2, 160.9, 154.9, 146.1, 138.5, 133.9, 129.8, 125.1, 125.1, 124.9, 121.8, 118.6, 114.5, 114.5, 56.0.

Compound 1c; FTIR (KBr, cm^{-1}): 2810-2902 (aromatic C-H), 1653 (C=O), 3290 (O-H), 1092-1123 (C-O-C), 1232 (C-N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.976 (s, 6H, -N$(CH_3)_2$), 6.945-8.219 (m, 8H, aromatic), 8.821 (s, 1H, -OH).

Compound 1d; FTIR (KBr, cm^{-1}): 2813-2890 (aromatic C-H), 1610 (C=O), 3211 (O-H), 1084-1111 (C-O-C); ^{1}H NMR (DMSO-d_6, δ ppm): 3.806 (s, 3H, -OCH_3), 6.992-7.876 (m, 7H, aromatic), 8.299 (s, 1H, 3-OH), 12.595 (s, 1H, 7-OH).

Compound 1e; FTIR (KBr, cm^{-1}): 2810-2875 (aromatic C-H), 1626 (C=O), 3186 (O-H), 1081-1111 (C-O-C), 1228 (C-N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.745 (s, 6H, -N$(CH_3)_2$), 6.348-7.489 (m, 7H, aromatic), 8.670 (s, 1H, 3-OH), 12.895 (s, 1H, 7-OH).

The cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% foetal bovine serum and 2 mM L-glutamine. For the screening experiment, cells were inoculated into 96 well microtiter plates in 100 µl at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, one 96 well-plate containing 5×10^3 cells/well was fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population at the time of drug addiction (time zero [Tz]). Experimental drugs were initially solubilized in DMSO at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400 and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e., 10, 20, 40, 80 µg/ml[16].

After the addition, plates were incubated at standard conditions for 48 h and the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°. The supernatant was discarded; plates were washed 5 times with tap water and air dried. Sulforhodamine B solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells and plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing 5 times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Per cent growth was calculated on a plate-by-plate basis for test wells relative to control wells and expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells multiplied by 100[17].

Using the six absorbance measurements (Tz, control growth [C] and test growth in the presence of drug at the four concentration levels [Ti]), the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero and [(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz. (Ti-Tz) negative. The results of the anticancer activity of these compounds are shown in Fig. 1.

It was observed that the presence of an additional OH group at C-7 position of the flavonol increased the time required for the chalcone formation as well as the cyclization. The yields of the 7-OH compounds were low as compared to compounds devoid of 7-OH substitution. The formation of the substituted flavonol was confirmed by NMR spectroscopy, where the compounds showed the peak of the enolic OH at 8.25 and 7/8 protons in the aromatic region at 6.96-7.69. The phenolic OH when present showed an exchangeable peak at 12.6 confirming its presence. The elemental analysis was found within satisfactory limits.

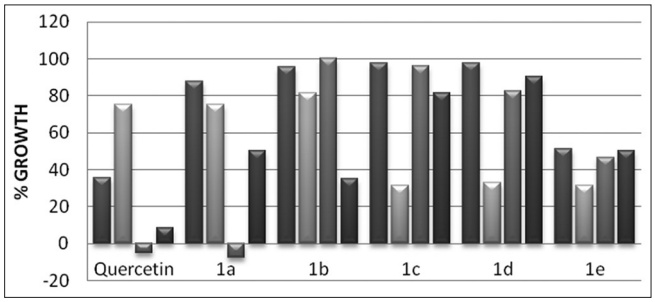


Fig. 1: Effect of synthesized flavonols on cell growth of different human cancer cell lines.
Effect of synthesized flavonols at a concentration of 80 µg/ml on cell growth of different human cancer cell lines. All values are expressed as mean±SD of at least three independent experiments.
PC-3 HCT-15 HEPG2 MCF-7.

The anticancer testing was carried out on four different cell lines, viz. MCF-7, PC-3, HepG2 and HCT-15, which represent the breast cancer, prostate cancer, hepatic cancer and colon cancer cell lines respectively. Breast, prostate and colon cancers represent the cancers of different tissue origin[18] and the corresponding cell lines were therefore selected. The additional choice of HepG2 cell line was based on the literature report that quercetin shows a significant effect on the growth of HepG2 cells[19].

Compound 1a, which is devoid of majority of the OH groups present in quercetin, shows a significant growth inhibitory effect on the HepG2 cell lines. Replacement of the 4'-Cl of 1a by either 4'-OCH_3 (1b) or 4'-$N(CH_3)_2$ (1c) led to loss of activity. However, more substituents need to be explored before commenting on the structural requirements at 4'-position, which affect the anticancer activity. We have previously reported that 1a and 1b, both are devoid of antioxidant activity when compared to quercetin[20]. It may be concluded that although the presence of multiple OH groups is a requirement for the antioxidant activity of flavonoids, the same may not be true for the anticancer activity of these compounds.

Compound 1a probably acts through a pathway, which does not involve a reduction of oxidative stress. Structural similarity of the molecule 1a to quercetin prompted us to conclude that the growth inhibitory pathway may involve alterations in either p53 or cyclin-dependent kinase or caspase and/or Phosphatidylinositide-3 (PI-3) activity[21-24]. Compound 1b displayed growth inhibitory activity against MCF-7 cell lines, although lower than quercetin. Quercetin is reported[25] to inhibit type II estrogen binding sites (ER II) where estradiol acts as an agonist. Although structural details of

binding of quercetin to ER-II is not reported, it probably takes place through H-bonding involving optimally placed phenolic-OH groups as in the case of diethylstilbestrol. Compound 1b may get involved in such H-bonding through 4'-OCH_3 group; it lacks the other OH group in its structure. Taking this into consideration, compound 1d and 1e were synthesised, which contain an additional 7-OH group apart from H-bonding partner at 4'-position. These compounds however did not show any growth inhibitory activity against MCF-7 cell lines. A surprising result was higher growth inhibitory effect of compounds 1c and 1e on the HCT-15 cell lines compared with quercetin. Further work needs to be carried out to ascertain the exact role of 7-OH group and its effect on the inhibition of HCT-15 cell lines.

In conclusion, it can be said that out of the five quercetin analogues devoid of multiple phenolic OH groups, one has shown a promising activity against HepG2 cell lines and two compounds have shown an activity better than quercetin in inhibiting the growth of HCT-15 cell lines. Further studies on these compounds may be useful for understanding the structure-activity relationship of anticancer flavonoids.

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