Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds

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Abstract PURPOSE. To evaluate the inhibition of CYP3A4 activity in human liver microsomes by flavonoids, furanocoumarins and related compounds and investigate possibly more important and potential inhibitors of CYP3A4 in grapefruit juice. **METHODS**. The effects of various flavonoids and furanocoumarin derivatives on CYP3A4 activity in two human liver microsomal samples was determined using quinine as a substrate. All flavonoids and furanocoumarin derivatives were dissolved in DMSO. In all cases, inhibition activities were compared with activities in control incubations containing 0.2% (v/v) DMSO. RESULTS. The results showed that the inhibition of quinine 3-hydroxylation (CYP3A4 activity) by bergapten (67%), and quercetin (55%) was greater than naringenin (39%) and naringin (6%), at the same inhibitor concentration of 100 μM. The results also demonstrated that the furan ring in the furanocoumarins enhanced the inhibitory effect on CYP3A4 activity. Flavonoids with more phenolic hydroxyl (-OH) groups produced stronger inhibition than those with less hydroxyl groups. Of all the chemicals studied, bergapten (5-methoxypsoralen) with the lowest IC_{50} value (19-36 μM) was the most potent CYP3A4 inhibitor. **CONCLUSIONS**. These results suggest that more than one component present in grapefruit juice may contribute to the inhibitory effect on CYP3A4. Bergapten appears to be a potent inhibitor of CYP3A4, and may therefore be primarily responsible for the effect of grapefruit juice on CYP3A4 activity.

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INTRODUCTION

Flavonoids are polyphenolic compounds with antioxidant properties and are widely distributed in foods of plant origin such as vegetables, fruit, tea and wine (1). When administered orally, natural flavonoids such as flavone, tangeretin, and nobiletin can activate rat and human benzo(a)pyrene hydroxylase and some other cytochromes P450 (CYP) activities (2). Interaction between grapefruit juice and clinically used drugs has been reported in recent years. These drugs include cyclosporine, midazolam, triazolam, and calcium antagonists such as felodipine, nifedipine, nitrendipine and nisoldipine (3 and references therein). All of these drugs are substrates for CYP3A4 and undergo extensive metabolism by intestinal CYP3A4 (4-6). CYP3A is the largest subfamily of CYP enzymes expressed in the human liver and gastrointestinal tract (7,8) and is involved in the metabolism of many clinically used drugs and other chemicals. Although the inhibitory effects of grapefruit juice on human CYP3A4 are well documented, its effect on other CYP isoforms remains unclear. Previous *in vivo* studies showed that elimination half-lives of caffeine and coumarin were prolonged by co-ingestion with grapefruit juice. These reactions are considered to be mediated mainly by CYP1A2 and CYP2A6, respectively (9-11).

Interaction between drugs and grapefruit juice results in an increase in the oral bioavailability of drugs. For instance, the consumption of grapefruit juice led to a marked reduction of CYP3A4 activity in the small intestine and was associated with a five times increase in felodipine C_{max} and tripled mean area under the curve (AUC) [3 and references therein]. However, the active components responsible for *in vivo* inhibition of CYP3A4 activity have yet to be fully determined. The predominant flavonoid in grapefruit juice is naringin and is likely to be one of the components in grapefruit

that affects drug metabolism. *In vitro* studies reveal that flavonoids can inhibit microsomal oxidation of felodipine as well as nifedipine (12,13). However, naringin appears to be a weak inhibitor of microsomal felodipine oxidation and its aglycone, naringenin, may be a much more potent inhibitor (12). An *in vivo* study has demonstrated that the increase in felodipine AUC by naringin solution was much less than that observed with grapefruit juice, indicating that other factors were important (14). Further investigation using a naringin capsule formulation resulted in no change in mean or individual nisoldipine pharmacokinetics compared with water (15). A similar phenomenon occurs with quercetin, which is an inhibitor of CYP3A4. No *in vivo* inhibition of CYP3A4 mediated metabolism of nifedipine is produced after ingestion of a high dose of quercetin (16).

Recent studies have shown that in addition to flavonoids, other compounds in grapefruit juice might be involved in the drug interaction. A furanocoumarin, 6',7'-dihydroxybergamottin was currently found to be a potent inhibitor of CYP3A4 in liver microsomes (17). Several components of grapefruit juice were isolated from extracts with ethyl acetate and found to be inhibitors of human liver microsomal CYP3A4. These compounds were identified as furanocoumarin derivatives (18). Later, analysis of ethyl acetate extracts from grapefruit juice revealed the presence of several furanocoumarins of which bergamottin, the parent compound of 6',7'-dihydroxybergamottin, is the major one and was found to be a mechanism-based inactivator of CYP3A4 in human liver microsomes (19). Other components of grapefruit juice may also contribute to the interaction with CYP3A4. The other non-flavonoid components found in grapefruit juice such as limonin and obacunone, a triterpene-derived product, also reduced microsomal testosterone 6β-hydroxylation in human liver (18).

Thus the present study was conducted to examine the effect of flavonoids and furanocoumarin compounds found in grapefruit, on the activity of human liver CYP3A4. Even though 6',7'-dihydroxybergamottin is considered to be an important inhibitor, it is not commercially available and was not included in the current study. This investigation also evaluated the effect of coumarin derivatives because we intended to determine whether coumarins have a different effect from the furanocoumarin derivatives. Quinine was selected as a probe for CYP3A4 as it has been demonstrated to be primarily metabolised by human CYP3A4 (20).

METHODS AND MATERIALS

Chemicals and Reagents

All chemicals and reagents were of analytical grade and distilled water was MilliQ® filtered. Sodium dodecyl sulphate, HPLC-grade acetonitrile and methanol were obtained from BDH Chemicals (Poole, UK). Dimethylsulphoxide (DMSO), tetrabutylammonium bromide, NADPH, bovine serum albumin, flavonoids and coumarin derivatives including, rutin, morin, myricetin, hesperidin, hesperetin, neohesperidin, kaempferol, naringenin, naringin, quercetin, rhoifolin, apigenin, scopoletin, coumarin, 7-ethoxycoumarin, umbelliferone, bergapten, methoxalen and psoralen were purchased from Sigma Chemical Co (St. Louis, MO, USA). Fisetin, galangin, chrysin, and flavone were obtained from Aldrich (Milwaukee, WI, USA). Prunin was purchased from Roth (Karlsrule, Germany). Narirutin was purchased from Indofine (Belle Mead, NJ, USA). Quinine hydrochloride dihydrate was purchased from Merck-Schuchardt (Hohenbrunn, Germany). 3-Hydroxyquinine was a gift from Dr. P Winstanley, University of Liverpool, UK.

Preparation of Human Liver Microsomes

Two human livers used were from Caucasian male donors aged 50 years who had met traumatic deaths. They were neither taking medication nor had significant past medical history. The use of human livers was approved by the Southern Regional Health Authority (Otago) Ethics Committee, Dunedin, New Zealand. Human liver microsomes were prepared by a standard differential ultracentrifugation as previously described (20). The microsomal protein concentration was determined using a BCA reagent as described by the manufacturer (Sigma Chemical Co., St. Louis, MO, USA) and bovine serum albumin was used as a standard.

Inhibition Experiments

The effects of various flavonoids and furanocoumarin derivatives on quinine metabolism were determined in microsomes from two human livers (HL1 and HL2). It has been proposed that grapefruit juice inhibits drug metabolism by selective downregulation of the intesti-

nal CYP3A4 and has no effect on hepatic CYP3A4 (5). The use of liver microsomes instead of intestinal microsomes is still valid as it has been shown that the human CYP3A4 forms expressed in liver and intestine are identical (21). Hence, observations made using hepatic microsomes should be readily applicable to intestinal metabolism. Quinine was used as a substrate at a concentration of 100 μM, approximating the apparent K_m value for quinine 3-hydroxylation determined previously in these livers. The potential inhibitors were studied at three concentrations (10, 100, and 200 μM) chosen to be selective for the respective CYP isoforms on the basis of published K_i or K_m values (13, 22). All inhibitors were dissolved in DMSO. In all cases, inhibited activities were compared with activities in control incubations (a final volume of 0.25 ml) containing 0.2% (v/v) DMSO as appropriate.

IC_{50} values (i.e. inhibitor concentration which produces 50% inhibition) were estimated using the non-linear regression modelling programme, Minim® (courtesy of Dr. R Purves, Department of Pharmacology, University of Otago, Dunedin, New Zealand) by fitting the following equation (23), where [I] was a concentration of inhibitor:

$$i\,(\text{fractional inhibition}) = \frac{\text{inhibited rate}}{\text{control rate}} = \frac{[I]}{[I] + IC_{50}}$$

Human microsomes (1 mg/ml) were incubated with quinine (100 μM) in the presence and absence of inhibitors with 1 mM NADPH in phosphate buffer (0.067 M, pH 7.4) at a final volume of 0.25 ml. Each incubation was performed in duplicate. The reaction was initiated by the addition of NADPH. Incubations were performed at 37°C in a shaking water bath for 15 min. The reaction was terminated by the addition of cold methanol solution (0.5 mL). The samples were vortexed briefly and centrifuged at 2500 g for 10 min. The resultant supernatant (30 μL) was injected onto the HPLC column.

HPLC Assay for 3-Hydroxyquinine

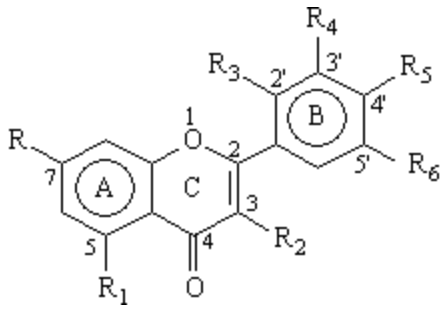
The major metabolite of quinine, 3-hydroxyquinine (3-OHQ) was assayed by a reversed-phase HPLC method (24). The detection limit of the method was 0.1 μM (0.034 μg/ml). The inter- and intra-assay coefficient of variation was less than 7% over the concentration range of 0.1 to 30 μM.

RESULTS

The effects of 10 flavones found in grapefruit, on quinine 3-hydroxylase (i.e. CYP3A4) activity in human liver microsomes were evaluated (Table 1).

These compounds exhibit similar structures with different hydroxyl (OH) substitutions. The results show that two flavone glycosides, rutin and rhoifolin did not inhibit the metabolism of quinine at low and medium concentrations of the inhibitors (10 and 100 μM). Only a moderate inhibition was observed with a 200 μM concentration of these two glycosides. The other 8 flavones produced considerable inhibition in CYP3A4 activity, which was dependent on their concentration and chemical structures. Myricetin was the most potent inhibitor among these flavones whereas apigenin appeared to be a weak inhibitor. As the number of hydroxyl substitutions increased, the inhibition of CYP3A4 activity tended to be progressively enhanced. At a concentration of 100 μM, flavone (without any hydroxyl group) had little effect, and apigenin (with 3 hydroxyl groups) caused 30-37% inhibition. In contrast, morin and quercetin (with 5 hydroxyl groups) produced 34-41% and 55-65% inhibition respectively, and myricetin (with 6 hydroxyl groups) had 78-94% inhibition (Table 1). The inhibitory potency appeared to be in order from flavone < apigenin < kaempferol < chrysin < morin < fisetin < quercetin < myricetin. The hydroxyl groups at R_4 position on the B ring could enhance the inhibitory potency of flavonoids. For example, both fisetin and kaempferol have four hydroxyl groups but the extent of CYP3A4 inhibition was different. At the concentration of 100 μM, fisetin (fourth hydroxyl group at R_4 on the B ring) caused 71-73% inhibition whereas kaempferol (fourth hydroxyl group at R_1 on the A ring) caused only 30-41% inhibition (Table 1). A similar phenomenon was observed with quercetin (fifth hydroxyl group at R_4 on the B ring) which caused 55-65% inhibition at concentration of 100 μM while morin, with the fifth hydroxyl group at R_3 position on the B ring, produced only 34-41% inhibition.

Table 1: Structures of flavones investigated and their inhibition of quinine 3-hydroxylation (CYP3A4 activity) in two human liver microsomes, HL1 and HL2. The inhibitors were studied at three concentrations, 10, 100 and 200 μM. Each value represents the average of two separate determinations that differed by < 10%.

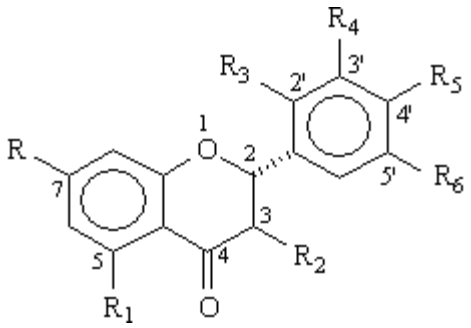


								% Inhibition of quinine 3-hydroxylation		
	R	R_1	R_2	R_3	R_4	R_5	R_6	10μM	100μM	200μM
Flavone	H	H	H	H	H	H	H			
HL1								-14.1	13.3	40.6
HL2								-6.2	22.3	45
Chrysin	OH	OH	H	H	H	H	H			
HL1								19.5	63.3	51.6
HL2								43.1	56.4	56.4
Apigenin	OH	OH	H	H	H	OH	H			
HL1								10.9	29.7	27.1
HL2								18.5	37.4	38
Fisetin	OH	H	OH	H	OH	OH	H			
HL1								14.1	71.1	70.3
HL2								18.5	72.5	74.4
Kaempferol	OH	OH	OH	H	H	OH	H			
HL1								2.3	41.4	41.4
HL2								18.5	29.9	43.1
Morin	OH	OH	OH	OH	H	OH	H			
HL1								4.7	34.4	51.6
HL2								15.6	41.2	60.2
Quercetin	OH	OH	OH	H	OH	OH	H			
HL1								21.1	54.7	61.7
HL2								28	64.9	63
Myricetin	OH	OH	OH	H	OH	OH	OH			
HL1								64.1	78.1	75.8
HL2								82.5	93.6	90.3
Rutin	OH	OH	-O-rutinose	H	OH	OH	H			
HL1								0.8	-10.9	-5.5
HL2								-1.4	9	26.1
Rhoifolin	R-G-[a]	OH	H	H	H	OH	H			
HL1								-0.8	6.3	18.0
HL2								1.4	10.9	26.1

[a] *rhamnose-glucose-, L-rhamnose is linked α 1 → 2 to D-glucose*

The effects of the flavanone compounds found in grapefruit, on the 3-hydroxylation of quinine (CYP3A4 activity) are summarised in Table 2.

Table 2: Structures of flavanones used and their inhibition of quinine 3-hydroxylation (CYP3A4 activity) in two human liver microsomes, HL1 and HL2. The inhibitors were studied at three concentrations, 10, 100 and 200 μM. Each value represents the average of two separate determinations that differed by < 10%



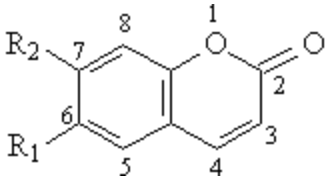
								% Inhibition of quinine 3-hydroxylation		
	R	R_1	R_2	R_3	R_4	R_5	R_6	10μM	100μM	200μM
Galangin	OH	OH	OH	H	H	H	H			
HL1								21.1	50	51.6
HL2								28.9	54.5	49.8
Hesperetin	OH	OH	H	H	OH	OCH_3	H			
HL1								-8.6	28.9	51.6
HL2								9	35.5	58
Naringenin	OH	OH	H	H	H	OH	H			
HL1								8.6	39.1	64.1
HL2								-5.7	34.6	46.9
Naringin	R-G-[a]	OH	H	H	H	OH	H			
HL1								-5.5	6.3	14.1
HL2								23.3	48.9	46.9
Neohesperidin	R-G-[a]	OH	H	H	OH	OCH_3	H			
HL1								7.8	17.2	38.3
HL2								-2.4	8.5	30.3
Hesperidin	R-G-[b]	OH	H	H	OH	OCH_3	H			
HL1								-4.7	6.3	0
HL2								-4.3	20.4	24.2
Narirutin	R-G-[b]	OH	H	H	H	OH	H			
HL1								1.2	-6.3	3.9
HL2								-7.6	-1.4	2.4
Prunin	Glucose-	OH	H	H	H	OH	H			
HL1								14.8	20.3	21.9
HL2								18.5	26.1	14.7

[a] *rhamnose-glucose-, L-rhamnose is linked α 1 → 2 to D-glucose*
[b] *rhamnose-glucose-, L-rhamnose is linked α 1 → 6 to D-glucose*

At low concentration (10 μM) of the flavanones, there was no inhibitory effect, except for galangin, naringin, and prunin. When the concentrations of the flavanones were increased to 100 μM and 200 μM, all compounds (except narirutin) inhibited the activity of CYP3A4. Two flavanone glycosides naringin and neohesperidin produced less inhibitory effect than their corresponding flavanones naringenin and hesperetin. At 200 μM concentration, hesperetin, and naringenin caused a similar extent of inhibition ranging from 47 to 64% (Table 2). Neohesperidin and naringin were weak inhibitors of CYP3A4 as only 14 to 38% inhibition was observed (Table 2). Hydroxyl substitution at R_2 position clearly enhanced the inhibitory effect. For example, at low and medium concentrations (10 and 100 μM) galangin, with a hydroxyl group at R_2 position, showed greater inhibition than hesperidin and naringenin.

Table 3 shows inhibitory effects of four different coumarin derivatives on the activity of CYP3A4.

Table 3: Structures of coumarin derivatives and their inhibition of quinine 3-hydroxylation (CYP3A4 activity) in two human liver microsomes, HL1 and HL2. The inhibitors were studied at three concentrations, 10, 100 and 200 μM. Each value represents the average of two separate determinations that differed by < 10%.



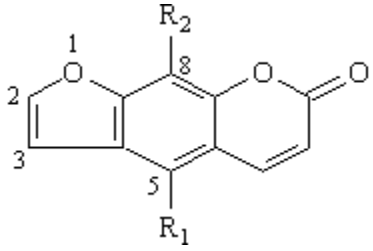
			% Inhibition of quinine 3-hydroxylation		
	R_1	R_2	10μM	100μM	200μM
Coumarin	H	H			
HL1			-7.0	-15.6	-20.3
HL2			7.1	-15.6	-13.7
7-Ethoxycoumarin	H	CH_3CH_2O-			
HL1			5.5	0.8	3.9
HL2			3.3	-6.2	-3.3
Umbelliferone	H	OH			
HL1			7.8	10.9	22.7
HL2			8.1	20.4	41.2
Scopoletin	CH_3O-	OH			
HL1			-3.1	28.9	35.9
HL2			23.2	52.6	50.7

Coumarin and 7-ethoxycoumarin had no effect on the CYP3A4 activity, at the three concentrations studied whereas, umbelliferone and scopoletin produced moderate inhibition of CYP3A4 activity at their medium and high concentrations (100 μM and 200 μM). It was noted that hydroxyl substitution increased the inhibitory effect, as indicated by comparison of coumarin and umbelliferone (Table 3). Further study with furanocoumarin derivatives has shown that they produced a greater degree of inhibition than those observed with coumarin derivatives (Table 4).

Bergapten, methoxalen and psoralen were found to be moderate to potent inhibitors of CYP3A4. Their inhibitory effects were increased with increasing concentrations of the furanocoumarins.

Based on the data presented in Tables 1 to 4, the IC_{50} values were estimated for individual compounds and these are summarized in Table 5.

Table 4: Structures of furanocoumarin derivatives used and their inhibition of 3-hydroxylation of quinine (CYP3A4 activity) in two human liver microsomes, HL1 and HL2. The inhibitors were studied at three concentrations, 10, 100 and 200 μM. Each value represents the average of two separate determinations that differed by < 10%.



			% Inhibition of quinine 3-hydroxylation		
	R_1	R_2	10μM	100μM	200μM
Bergapten	CH_3O	H			
HL1			33.6	67.2	64.8
HL2			43.1	67.8	71.6
Methoxalen	H	CH_3O			
HL1			26.6	71.1	78.1
HL2			9.0	82.7	92.9
Psoralen	H	H			
HL1			1.6	43.8	71.1
HL2			3.8	9.5	42.2

Among the compounds studied, bergapten and methoxalen were the most potent inhibitors of CYP3A4, with IC_{50} values of 19-36 μM and 35-39 μM, respectively (Table 5). Flavonoids including quercetin, fisetin, chrysin, naringenin, and galangin were found to be moderate CYP3A4 inhibitors with IC_{50} ranging from 41 to 136 μM. Naringin and hesperidin were poor inhibitors of CYP3A4 with IC_{50} values of 1349 and 601 μM, respectively.

DISCUSSION

The present study has demonstrated a dose-dependent inhibitory effect of some flavones, flavonones, coumarin and furanocoumarin derivatives on the activity of CYP3A4 in two human liver microsomes. The extent of inhibition on CYP3A4 produced by grapefruit flavonoids and furanocoumarins, e.g. kaempferol, quercetin, naringenin and bergapten, is similar to that observed with grapefruit juice. At a level of 2.5% grapefruit juice with human liver microsomes produced 40-70% inhibition of CYP3A4 activity (25).

Table 5: Derived IC_{50} values for the inhibition of 3-hydroxyquinine formation by flavonoids and coumarin derivatives

Inhibitors	IC50 (μM)	
	Human Liver 1	Human Liver 2
Flavanone (glycosides):		
Neohesperidin	280	291
Hesperidin	n.d.	601
Naringin	1349	n.d.
Narirutin	n.d.	n.d.
Prunin	n.d.	n.d.
Flavanone (aglycones):		
Galangin	136	117
Hesperetin	175	163
Naringenin	139	188
Falvone (glycosides):		
Rhoifolin	n.d.	398
Rutin	n.d.	354
Flavone (aglycones):		
Flavone	224	214
Chrysin	86	70
Fisetin	53	44
Kaempferol	223	540
Morin	188	135
Quercetin	82	41
Myricetin	n.d.	n.d.
Apigenin	n.d.	n.d.
Furanocoumarin derivatives:		
Bergapten	36	19
Methoxalen	35	39
Psoralen	116	257
Coumarin derivatives:		
Scopoletin	307	145
Umbelliferone	543	272
Coumarin	n.d.	n.d.
7-Ethoxycoumarin	n.d.	n.d.

n.d.= not determined, as the data could not be fitted by non-linear regression analysis or these compounds caused no inhibitory effect

There was no inhibition of CYP3A4 observed with glycosides of flavones (rutin and rhoifolin). Rutin and rhoifolin are the rutinose and rhamnose-glucose derivatives of quercetin and apigenin, respectively (Table 1). They are much more water soluble than their corresponding flavones (26). It is possible that the high polarity of rutin and rhoifolin may have interfered with their interaction with the CYP enzyme. This also suggests that the hydroxyl substitution on ring A is an important functional group for the inhibitory effect on CYP3A4. Similar observations were seen with glycosides of flavanones (neohesperidin, hesperidin, narirutin and prunin, Table 2).

Saturation of the C2-C3 double bond in flavanones (Table 2) caused a decrease in inhibitory potency on CYP3A4 activity compared with flavones. Flavones have been described as fairly planar molecules whereas flavanones are bulkier molecules. Thus the conformation of the molecule is probably important (27).

Previous studies have shown that the numbers and positions of hydroxyl groups on the A and B rings of flavonoids are important for the effects on enzyme activities (12,26). It has been proposed that the 7-hydroxyl group preferentially interacts with Fe(III) of CYP (28). Consistent with this, our study has shown that compounds without a free hydroxyl substituent on ring A at the R position had no inhibitory effect, while those with a hydroxyl group were good inhibitors (Table 1). The lower inhibitory effect of naringin, with IC_{50} value 10-fold greater than that of naringenin, could be due to the absence of a free hydroxyl group at the R position on ring A (29). It is known that the 7-hydroxyl group of flavonoids is the first to dissociate and is thus the most likely site of attack by the peroxy radical (30). Similar differences were observed between hesperidin and hesperetin. The IC_{50} value of hesperidin was 3.7-fold higher than that of hesperetin (Table 5). Interestingly, this inhibitory effect mimicked that observed with the inhibition of 11β-hydroxysteroid dehydrogenase in guinea pig kidney microsomes (31). The IC_{50} value of naringin (about 2400 μM) was approximately 8-fold higher than that of naringenin (about 340 μM) (24). Lee et al. (32) also reported that the IC_{50} value of naringin was much greater than naringenin.

The results also show that the inhibitory effect of flavones is related to their molecular structures. Flavones having more hydroxyl substitutions showed stronger inhibition than those with fewer hydroxyl groups. The order of potency is myricetin > quercetin/morin > kaempferol > apigenin > flavone (with 6, 5, 4, 3, 0 hydroxyl groups respectively, Table 1). This was confirmed by the existence of a positive significant correla-

tion ($r = 0.89$, $p < 0.0005$) between number of hydroxyl groups and extent of CYP3A4 inhibition of these six compounds at a concentration of 200 μM. This finding is in agreement with a previous study showing that the ability of flavonoid compounds either to inhibit or stimulate benzo(a)pyrene hydroxylation in human liver microsomes was related to their possession or lack of hydroxyl group respectively (33). The order of inhibitory potency for fisetin (with 4 hydroxyl groups) and chrysin (with 2 hydroxyl groups) were not line in with the mentioned flavones. Fisetin had greater effect than kaempferol which has the same number of hydroxyl groups (Table 1). Chrysin produced more inhibitory effect than apigenin (with 3 hydroxyl groups), even though it has less hydroxyl groups. When the data from fisetin and chrysin were included in the correlation analysis with those six flavone compounds, there was no significant correlation ($r = 0.36$, $p > 0.1$) between number of hydroxyl groups and extent of CYP3A4 inhibition. This suggests that number of hydroxyl groups and position of hydroxyl substitutions are both important. Quercetin has also been reported to be a more effective inhibitor of CYP3A4-catalysed oxidation of felodipine and nifedipine than naringenin and kaempferol. Their order of potency is quercetin > kaempferol > naringenin (with 5, 4, 3 hydroxyl groups respectively) (13).

Furanocoumarin derivatives produced a greater inhibition effect than coumarins indicating that the furan ring increased the potency of inhibition of CYP3A4. Bergapten (5-methoxypsoralen), a furanocoumarin, is present in grapefruit peel oil (34) and some plant foods such as parsley, parsnips and celery (35). In our study, bergapten appears to be the most potent inhibitor of CYP3A4 with IC_{50} value of approximately 25 μM (19 and 36 μM). The IC_{50} value of bergapten was similar to the IC_{50} of bergamottin (22 μM) but 10-fold higher than that of 6',7'-dihydroxybergamottin (2 μM) [25]. Bergapten was approximately 6-fold more potent than naringenin and 50-fold more potent than naringin. This suggests that bergapten may be one of the important components in grapefruit juice which is responsible for the interactions between drugs and grapefruit juice. Several other furanocoumarins have previously been reported to cause mechanism-based inactivation of CYP, e.g., corandrin (36) and methoxalen (8-methoxypsoralen) [37, 38]. 6',7'-Dihydroxybergamottin and its parent compound, bergamottin, found in grapefruit juice have also been shown to cause mechanism-based inactivation of CYP3A4 (17,19). Bergapten was also shown to produce mechanism-based inhibition of CYP3A4 (39). All these compounds contain a furan ring, which has been suggested to be the group responsible for the inactivation of CYP1A based on studies of a series of naturally occurring coumarins (40). Several furans are known to undergo metabolic activation to form reactive epoxide intermediates (41,42). It has been reported that the active metabolites of 8-methoxypsoralen bind covalently to microsomal proteins and inactivate CYP (43). This may imply that diets such as grapefruit juice contribute to the alteration of activity of liver and intestinal CYP3A isoforms through both direct inactivation and inhibition (18).

Grapefruit juice contains many flavonoids and furanocoumarin derivatives that may alter the metabolism of drugs by CYP. Of the flavonoids, naringin is the predominant constituent in grapefruit juice, and presents in concentrations up to 1200 mg/L (2000 μM) [3,44]. Naringenin has been detected in grapefruit juice at a concentration of 240 mg/L (880 μM) [44-46]. Grapefruit juice contains a lower concentration of narirutin with up to 120 mg/L (205 μM) [45]. Quercetin has been found in fresh grapefruit juice at a concentration of 5-6 mg/L or 20 μM (22). Very little hesperidin (up to 16 mg/L or 26 μM), neohesperidin (up to 10 mg/L or 16 μM), and hesperetin (up to 3 mg/L or 10 μM) have been detected in grapefruit juice (45). The concentration of the furanocoumarin, 6',7'-dihydroxybergamottin, in grapefruit juice has been found to average 30 μM or 12 mg/L (17, 25). Bergapten has been detected in grapefruit juice at concentrations up to 7 mg/L (30 μM) [44]. It is extremely difficult to estimate the concentrations of these constituents in vivo as very little is known about disposition of flavonoids in humans (46). A previous study has demonstrated that the grapefruit flavonoids naringin and hesperidin are absorbed from the human gastrointestinal tract after oral administration of pure compounds, but their oral bioavailability was very low, i.e. < 20% (46). The contribution of each component varies according to its inhibitory potency and natural abundance. Thus, the *in vivo* effect of grapefruit juice is obviously complicated.

Naringin was originally thought to be a possible active component. However, as mentioned earlier, when an aqueous solution of naringin was given to volunteers,

it produced little or no interaction with felodipine (14). A recent study concluded that naringin by itself at concentrations found in grapefruit juice does not appear to be capable of producing a clinical drug interaction such as that seen with grapefruit juice (47). The furanocoumarins, bergamottin, 6',7'-dihydroxybergamottin, and related dimers (25,47,48) seem to be more important active ingredients involved in the interaction, although other unidentified substances may be also involved in this clinical interaction. Being a potent inhibitor of CYP3A4, bergapten is likely to be an additional active ingredient in grapefruit juice-drug interactions.

In summary, the present study has demonstrated that besides the flavonoids, other compounds found in grapefruit including furanocoumarins can produce strong inhibition of CYP3A4. The grapefruit juice-drug interactions could involve CYP3A4 inhibition by more than one component present in grapefruit juice. Bergapten was found to be a very potent inhibitor of CYP3A4. Therefore, it may be an important furanocoumarin responsible for the grapefruit juice drug interactions. Concentrations of bergapten (up to 30 μM) detected in grapefruit and commercial grapefruit juice products (44) appear sufficient to cause significant inhibition of CYP3A4 in this study. However, it should be noted that inhibition of CYP3A4 activity *in vitro* does not necessarily imply drug interaction *in vivo*. Further studies will be needed to determine if this furanocoumarin bergapten can influence the CYP enzyme ***in vivo***.

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