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ORIGINAL ARTICLE

# In vitro modulatory effects of flavonoids on human cytochrome P450 2C8 (CYP2C8)

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**Abstract** The inhibitory effects of five flavonoids with distinct chemical classes (flavones [luteolin], flavonols [quercetin and quercitrin], and flavanones [hesperetin and hespiridin]) on cDNA-expressed CYP2C8 were investigated. CYP2C8 was co-expressed with NADPH-cytochrome P450 reductase in *Escherichia coli* and used to characterise potency and mechanism of these flavonoids on the isoform. Tolbutamide 4-methylhydroxylase, a high-performance liquid chromatography-based assay, was selected as marker activity for CYP2C8. Our results indicated that the flavonoids inhibited CYP2C8 with different potency. The order of inhibitory activities was quercetin > luteolin > hesperetin > hesperidin > quercitrin. All of these compounds however exhibited mechanism-based inhibition. A number of structural factors were found to be important for inhibition; these include the molecular shape (volume to surface ratio), the number of hydroxyl groups as well as glycosylation of the hydroxyl group. Quercetin was the most potent inhibitor among the flavonoids examined in this study, and our data suggest that it should be examined for potential pharmacokinetic drug interactions pertaining to CYP2C8 substrates in vivo.

**Keywords** Cytochrome P450 · CYP2C8 · Flavonoids · In vitro inhibition

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## Introduction

Flavonoids represent a group of phytochemicals which are widely found in edible plants. Plant flavonoids have been of interest to scientists for decades, owing to their observed biological effects such as free-radical scavenging, modulation of enzymatic activity, and inhibition of cellular proliferation, as well as their potential utility as antibiotic, anti-allergic, anti-diarrheal, anti-ulcer, and anti-inflammatory agents (Havsteen 2002).

Flavonoids are also well known to interact with enzymes involved in drug metabolism. Among the many drug-metabolising enzyme systems, cytochrome P450 is considered to be important as CYP–flavonoid interactions have been shown to give rise to important pharmacological and toxicological implications. Flavonoids can induce the expression of several CYPs and modulate their metabolic activity, thus enhancing metabolism of certain drugs. On the other hand, inhibition of CYPs involved in carcinogen activation and generation of scavenging reactive species can be beneficial properties of various flavonoids. Modulatory effects of flavonoids have been investigated in CYP1A1 (Zhai et al. 1998), CYP1A2 (Lee et al. 1998), CYP1B1 (Doostdar et al. 2000), CYP3A4 (Chan et al. 1998) and more recently, CYP2C9 and CYP2A6 (Si et al. 2009; Tiong et al. 2010). The flavonoid modulatory potency and the structural features important for their effects on another important CYP2C isoform, CYP2C8, have not been

investigated. The role of the CYP2C8 has recently garnered considerable interest in the fields of drug metabolism and pharmacogenetics. The isoform is now known to play a major role in the metabolism of several therapeutically important drugs, including chemotherapeutic agents (i.e. paclitaxel and all-trans-retinoic acid), anti-malarials (i.e. amodiaquine), anti-diabetic agents (i.e. rosiglitazone and repaglinide), antiarrhythmics (i.e. amiodarone), and HMG-CoA reductase inhibitors (i.e. cerivastatin and fluvastatin) (Totah and Rettie 2005). It also converts endogenous substances, such as arachidonic acid, to biologically active epoxide metabolites (Totah and Rettie 2005). Relevant to this area of study is the use of quercetin, a flavonoid compound, as the in vitro CYP2C8 inhibitor probe in many drug metabolism and pharmacokinetic studies in recent years (Rahman et al. 1994; Li et al. 2002; Gao et al. 2010). Although quercetin exhibited potent inhibitory effect on CYP2C8 in these studies, its relative potency in comparison to other structurally related flavonoid compounds have not been explored. In view of this and the increasing role played by CYP2C8 in drug metabolism, characterisation of the interaction mechanism of flavonoids with this isoform remains an area of research interest. In this study, CYP2C8 was co-expressed with NADPH-cytochrome P450 reductase (OxR) in *Escherichia coli*, and the expressed protein was examined for modulatory effects by five naturally occurring flavonoids with different structural classes. The potency and selectivity of CYP2C8 inhibition, as well as the inhibition mechanism of these compounds were investigated.

## Materials and methods

### Chemicals and reagents

Acetonitrile, phosphoric acid, hydrochloride acid and all other HPLC grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA). Tris base and isopropyl β-D-1-thiogalactopyranoside were acquired from Promega (Madison, WI, USA). Terrific broth media was purchased from Invitrogen Corporation (Carlsbad, CA, USA). Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate dehydrogenase, glucose-6-phosphate (G-6-P), dimethyl sulfoxide (DMSO), magnesium chloride, tolbutamide, 4-hydroxytolbutamide and phenytoin were purchased from Sigma-Aldrich (St. Louis, MI, USA). The five flavonoids used in this study, quercetin dehydrate (purity ≥ 98%), hesperidin (purity ≥ 97%), hesperetin (purity ≥ 95%), luteolin (purity ≥ 98%) and quercitrin (purity ≥78%), were also purchased from Sigma-Aldrich. The *E. coli* bacterial stocks harbouring plasmids, pCW-CYP2C8 and pACYC-OxR, used for the expression of CYP2C8 and OxR, were constructed and prepared in our laboratory previously (Singh et al. 2008).

### Preparation of CYP2C8 monooxygenase systems

Co-expression of CYP2C8 and OxR in *E. coli* DH5α cells and subsequent membrane isolation were carried out as described previously (Singh et al. 2008). The membrane fragments of *E. coli* were stored at −80°C in a 1:1 mixture of pH 7.6 TES (100 mmol/L Tris : 0.5 mmol/L EDTA : 500 mmol/L sucrose buffer) and ice-cold distilled water before utilised in the enzyme assay. Routinely, the CYP2C8 spectral content obtained from culturing experiments was 0.37± 0.05 nmol $mg^{-1}$ ($n$=3).

### Tolbutamide 4-methylhydroxylase assay

Tolbutamide 4-methylhydroxylase assay was carried out essentially according to the published protocol (Miners et al. 1988). A standard 0.5 mL incubation mixture contained 0.4 mg DH5α membrane protein, NADPH generating system (1 mmol/L NADP, 10 mmol/L G-6-P, 2 IU G-6-P dehydrogenase and 5 mmol/L $MgCl_2$) and incubated at 37°C for 120 min. All reactions were started with the addition of NADPH-generating system. Reactions were then stopped by placing the samples in ice followed by addition of 1.0 mL of 0.1 mol/L phosphoric acid. The sample was then extracted with 8 mL of hexane: chloroform: iso-amyl alcohol (1,000: 250: 1 *v*/*v*) to remove excess tolbutamide. After centrifuging the mixture for 5 min at 2,000 rpm, the tube containing the mixture was stored in −80°C freezer for 10 min before the organic layer of the mixture was discarded. Fourteen microlitres of phenytoin (1 g/L), the internal standard, and 8 mL of diethyl ether were later added to the sample and extracted by vortexing for a few minutes. The sample was then centrifuged for 5 min at 2,000 rpm before being stored in the −80°C freezer for 15 min. The resulting organic layer was decanted into a conical tube and then evaporated to dryness using $N_2$ gas. The residue was reconstituted in 60 μL of mobile phase, of which 20 μL was injected onto the HPLC system. The chromatography was performed using an Inertsil® C18 column (15 cm×4.6 mm inner diameter, 4 micron particle size; GL Sciences, USA) and eluted with acetate buffer (10 mmol/L, pH 4.3) : acetonitrile (78:22) at flow rate of 2.0 mL/min. The column was connected to a Perkin Elmer HPLC Series 200 system comprising a pump and a UV detector. Absorbance was monitored at 230 nm. Under these conditions, the retention times for hydroxytolbutamide and phenytoin were 16.2 and 30.6 min, respectively. Standard curves for hydroxytolbutamide were constructed in the range of 4.5–25.0 μmol/L. Unknown concentrations of hydroxytolbutamide were determined by comparison of hydroxytolbutamide:phenytoin peak height ratios with those of the standard curve. Assay validation has been carried out previously in our laboratory. Overall assay within-day precision was determined by

measuring hydroxytolbutamide formation in separate incubations of the same batch of the expressed enzyme. Coefficients of variation for hydroxytolbutamide formation were 7.1%, 5.0% and 3.5% at substrate concentration of 0.1, 0.25 and 1.0 mmol/L, respectively. The corresponding inter-day precision values were 8.9%, 6.3% and 5.7%, respectively. The mean recovery of hydroxytolbutamide, calculated by comparing the peak height for extracted compound with that of an equal amount injected directly into the chromatograph, was 87.0±3.8% for six samples in the concentration range 4.5–25.0 μmol/L.

### Enzyme inhibition experiments

In order to determine the modulatory effects of the flavonoids on CYP2C8, the enzyme assay was carried out in the presence and absence of flavonoids. All flavonoids dissolved well in DMSO and stock solutions were subsequently prepared in this solvent. Individual flavonoid compounds were added to incubations and the final concentration of DMSO did not exceed 0.5% *v*/*v*. Control incubations contained an equivalent volume of DMSO, although it was shown that DMSO had a negligible effect on CYP2C8-catalysed reaction at the concentration (0.5% *v*/*v*) employed. Preliminary experiments were carried out first to determine kinetic parameters Michaelis–Menten constant ($K_m$) and maximum velocity ($V_{max}$) for CYP2C8. All inhibition experiments were subsequently conducted by incubating a single tolbutamide concentration around the determined $K_m$ with a range of flavonoid concentrations to determine $IC_{50}$ values (concentration of inhibitor causing 50% inhibition of enzyme activity). Concentrations used in inhibition experiments for each flavonoid were 20–200 μmol/L for luteolin, 10–200 μmol/L for quercetin, 10–500 μmol/L for quercitrin, and 50–400 μmol/L for hesperetin and hesperidin. To assess mechanism-based activities of the flavonoids, the enzyme assay was conducted with a period of pre-incubation. For this, the reaction mixtures containing 0.1 mol/L phosphate buffer (pH 7.4), NADPH-generating system, expressed CYP2C8 and flavonoids were prepared on ice. After the reaction mixture was pre-incubated at 37°C for 15 min, tolbutamide was added to the mixture to initiate the reaction. The metabolism of the substrate was subsequently analysed as described above. The $IC_{50}$ values with or without pre-incubation were determined for each of the flavonoids studied. In addition, the NADPH-dependency of inhibition was also examined by incubating expressed CYP2C8 and the flavonoid in phosphate buffer for 15 min before addition of tolbutamide and NADPH-generating system to initiate the assay as described above. The velocity rates were subsequently determined and compared to control incubations where NADPH generating system was added at the beginning of the pre-incubation.

### Kinetic analysis

Enzyme kinetic data were analysed by nonlinear least squares regression analysis software EZ-Fit™ (Perrella Scientific, USA) and the kinetic parameters, $K_m$ and $V_{max}$, were determined over the substrate concentration range studied. The $IC_{50}$ values for flavonoid inhibition were interpolated mathematically by SigmaPlot® software (version 2004, Systat Software Inc, USA). This was accomplished by fitting the function $V=100\times IC_{50} / (IC_{50}+C)$ ($V$=activity expressed as percentage of control and $C$=concentration of flavonoid) using the method of Marquardt in the nonlinear regression program of SigmaPlot® software.

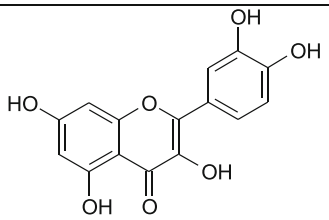
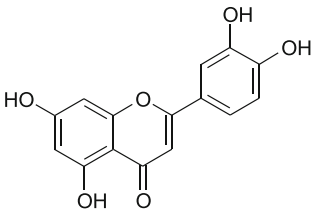
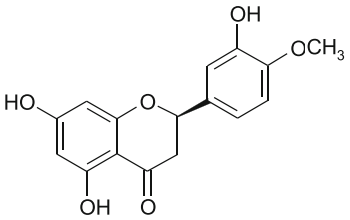
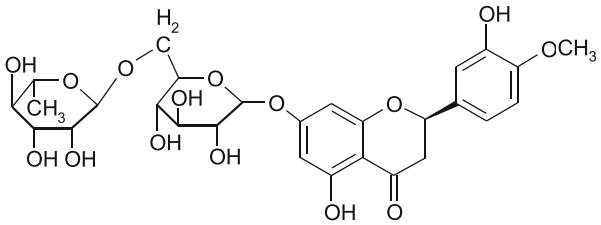
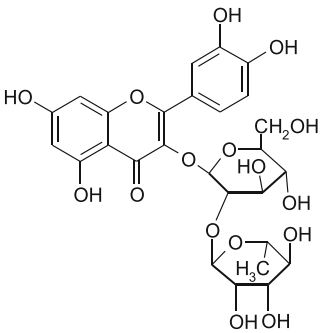
## Results

Kinetic parameters were determined for tolbutamide 4-methylhydroxylation by incubating expressed CYP2C8 at substrate concentration ranging from 200 to 2,000 μmol/L. Hydroxytolbutamide formation by the expressed CYP2C8 was found to best fit the single Michaelis–Menten kinetic, with apparent $K_m$ and $V_{max}$ values of 1.16±0.13 mmol/L and 10.2±1.8 pmol $min^{-1}$ mg $protein^{-1}$ (or 27.6±4.9 pmol $min^{-1}$ nmol $CYP^{-1}$; $n$=3), respectively. These data were in the similar range as reported for CYP2C8 in the literature (Relling et al. 1990; Veronese et al. 1993). Based on these calculated data, subsequent inhibition study with flavonoids was conducted at tolbutamide concentration of 1.0 mmol/L which corresponded to the apparent $K_m$ value calculated above.

Five flavonoid compounds, luteolin, quercetin, quercitrin, hesperetin and hespiridin, were chosen for the study based on their structural classes (see Table 1). All these compounds, dissolved in DMSO, were used separately in the inhibition experiments. Inhibitory effect was examined at 5–7 inhibitor concentrations at a fixed tolbutamide concentration described above. Quercetin, luteolin, hesperetin and hesperidin exhibited inhibitory effects on CYP2C8 activity, giving $IC_{50}$ values of 46.0, 82.0, 168.4 and 274.7 μmol/L, respectively (third column in Table 1). In contrast to the above flavonoids, quercitrin did not show any appreciable inhibition over the range of concentration investigated (10–500 μmol/L). Thus, quercitrin is the least potent inhibitor among the five compounds examined and its $IC_{50}$ value was designated as >500 μmol/L.

To further explore the inhibition mechanism of the four flavonoids showing inhibitory effect (i.e. quercetin, luteolin, hesperetin and hesperidin), kinetic analyses of CYP2C8-catalysed tolbutamide hydroxylase were performed with or without a period of pre-incubation with the flavonoids at selected concentrations. As shown in Fig. 1, pre-incubation of the four flavonoids at their respective selected concentrations

**Table 1** Inhibitory effects of flavonoids on CYP2C8 activity

| *Flavonoid structure* | *Flavonoid name* | *$IC_{50}$ (μmol/L ) without pre-incubation* [a] | *$IC_{50}$ (μmol/L) with pre-incubation*[a] |
|---|---|---|---|
|  | Quercetin (3,5,7,3',4' – Pentahydroxy-flavone) | 46.0±2.2 | 26.7± 2.1 |
|  | Luteolin (5,7,3',4' – Tetrahydroxy-flavone) | 82.0±3.8 | 52.2±4.6 |
|  | Hesperetin (5,7,3' - Trihydroxy-4'-methoxyflavanone) | 168.4±6.1 | 127.6±4.8 |
|  | Hesperidin (Hesperitin 7- O - rutinoside) | 274.7±15.3 | 209.7±13.4 |
|  | Quercitrin (Quercetin 3 - O - rhamnoglucoside) | >500 | n.d.[b] |

*n.d.* Not determined

[a] Data are presented as mean ± SD from triplicate determinations

resulted in greater degree of inhibition for CYP2C8-catalysed tolbutamide hydroxylation, as reflected by the lower velocity rates observed (white-coloured bars vs dark grey bars in Fig. 1).

NADPH-dependency of the inhibition was also examined by pre-incubating the flavonoids with CYP2C8 in the absence of NADPH generating system during the 15-min pre-incubation

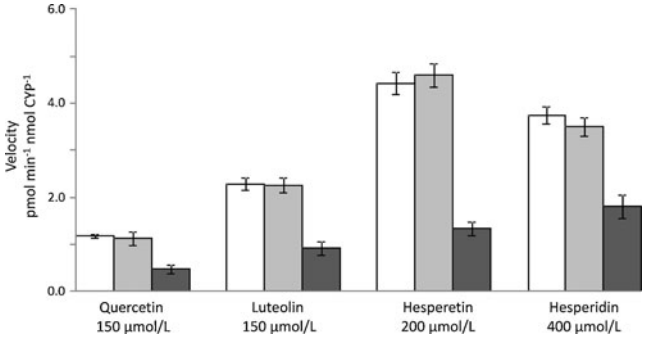



**Fig. 1** Velocity rates for tolbutamide hydroxylation in incubations of tolbutamide (1.0 mmol/L) without (*filled bars with white colour*) or with (*bars with light and dark grey*) pre-incubation with the flavonoid compounds at the indicated concentrations. Pre-incubations were carried out in the absent (*light grey*) or present (*dark grey*) of NADPH generating system during the pre-incubation period. Data are shown as mean ± SD from triplicate determinations

period. As shown in Fig. 1, this manoeuvre resulted in no difference in velocity rates as compared to control incubations without pre-incubation (white-coloured bars vs light grey bars in Fig. 1). Based on the calculated velocities, 15-min pre-incubations (with the flavonoids and in the presence of NADPH generating system) have increased inhibitory potency towards CYP2C8 to 2.5 times for both quercetin and luteolin, 3.3 times for hesperetin, and 2.1 times for hesperidin, respectively. In view of the substantial reduction in the observed CYP2C8 activity, $IC_{50}$ values were re-determined for the four flavonoids with the 15-min pre-incubation treatment at various inhibitor concentrations. Consistent with the enhanced inhibitory effect observed with a single concentration (Fig. 1), quercetin, luteolin, hesperetin and hesperidin exhibited lower $IC_{50}$ values of 26.7, 52.2, 127.6 and 209.7 µmol/L respectively (fourth column in Table 1). A representative $IC_{50}$ plot for hesperetin showing the curves with and without pre-incubation is illustrated in Fig. 2. Quercitrin was not examined for the pre-incubation effect as it did not show appreciable inhibition. Considered collectively, the above data have provided evidence for the mechanism-based inhibition exerted by the investigated flavonoids on CYP2C8.

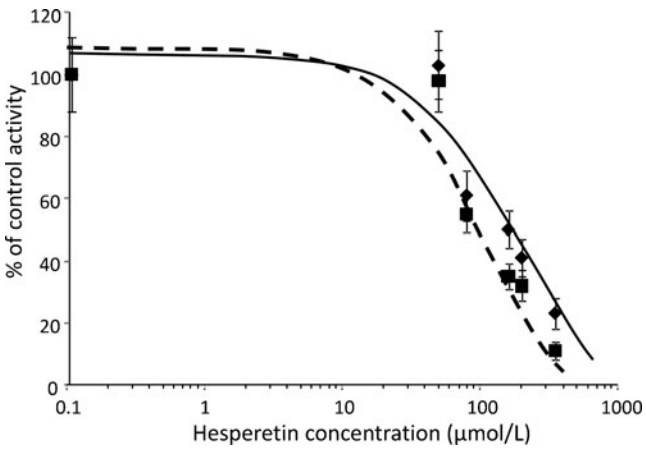



**Fig. 2** Effect of different concentrations of hesperetin on the tolbutamide hydroxylase activity catalysed by CYP2C8 in the absence (*solid line*) and the presence (*dashed line*) of pre-incubation. Data are shown as mean ± SD from triplicate determinations

## Discussion

Tolbutamide hydroxylase assay was used as the activity marker for CYP2C8 as tolbutamide and 4-hydroxytolbutamide were commercially available. This assay is a well established HPLC method commonly used for CYP2C subfamily members. Assay validation has been performed and the protocol has been found to be highly reliable and reproducible (Miners et al. 1988; Baldwin et al. 1999). Tolbutamide hydroxylase activity reported in the present investigation (routinely 2.18–4.13 pmol $min^{-1}$ $mg^{-1}$ or 5.89–11.16 pmol $min^{-1}$ nmol $CYP^{-1}$ at 1.0 mmol/L tolbutamide) was consistent with activity levels reported in the literature which range from 0.44 to 2.48 pmol $min^{-1}$ $mg^{-1}$ (Relling et al. 1990; Veronese et al. 1993). $K_m$ value reported here was also close to that reported by other studies (Veronese et al. 1993). Taken together, data from kinetic characterization indicated that CYP2C8 and OxR have been well expressed in DH5α cells and the two proteins interacted optimally to generate a catalytically active system for kinetic and inhibition studies.

To elucidate the structural features of flavonoids that are responsible for modulating CYP2C8 activity, we examined flavonoid derivatives that have distinct and different structural features. Our results showed that flavonoids affected in vitro CYP2C8-catalysed reaction differently. These differences were related to the chemical structure of the flavonoids. The five compounds investigated belong to three different structural classes. Luteolin, a flavone, differs from flavonol (quercetin) in terms of absence of a hydroxyl group at C3 position. Quercitrin, another flavonol, is the glycosylated form of quercetin. Hesperetin and hesperidin, both flavanone, differ from the others as they are buckled molecules, having the exocyclic phenyl ring B that lies nearly perpendicular to the rest of the molecule. This conformation is due to the absence of C2-C3 double bond in the ring C, unlike flavone and flavonol which are fairly planar molecules. The presence of a C2-C3 double bond restricts the orientation of phenyl ring B, hence the small volume to surface (V/S) ratio in both flavone and flavonol. As with quercitrin, hesperidin is the glycosylated form of hesperetin, having a rutinoside at C7 of the hesperetin.

Based on the $IC_{50}$ values, the order of inhibitory potency among the flavonoids examined was: quercetin > luteolin > hesperetin > hesperidin > quercitrin. Examination of the structure–activity relationship of these compounds seems to suggest the importance of certain structural factors in CYP2C8 inhibition. Planar molecules, quercetin and luteolin, having smaller

(V/S) ratio inhibited more potently than buckled molecules, hesperetin and hesperidin. This may indicate that CYP2C8 binds preferentially planar molecules than buckled molecules which have high (V/S) ratio. This result is in agreement to a study involving CYP1A2 where it was shown that planar molecules possessed higher inhibitory activity (Lee et al. 1998). Another factor that controlled CYP2C8 inhibition was the number of free hydroxyl groups attached to flavonoid nucleus. Among the flavonoids examined, quercetin, with five hydroxyl groups, showed higher inhibitory activity than luteolin which carries four hydroxyl groups. Luteolin, in turn, exhibited two-fold greater potency than hesperetin that carries only three free hydroxyl groups. This result is again in agreement with that of Lee et al. (1998), which showed increasing inhibitory potency toward CYP1A2-mediated caffeine N3-demethylation with an increasing degree of hydroxylation in flavonoids from both flavone and flavonol classes. Several other earlier studies have also demonstrated that the numbers and positions of hydroxyl groups on the A and B rings of flavonoids were important in determining their effects on enzyme activities (Lasker et al. 1984; Guengerich and Kim 1990; Chae et al. 1991). It has been suggested, based on the finding from these studies that free hydroxyl groups, in particular, the C5 and C7 hydroxyl groups, preferentially interact with $Fe^{3+}$ of the CYP active site due to their steric availability and adequate acidity (Li et al. 1994). The results of the present investigation also indicated that glycosylation of the free hydroxyl groups decreased the ability to inhibit CYP2C8. This effect was clearly observed when comparing the inhibitory effect of quercetin with its glycoside, quercitrin as well as that of hesperetin and hesperidin. Rhamnoglucoside, attached to C3 of quercitrin, essentially abolished the potent inhibitory effect seen in quercetin, even though quercetin was the most potent inhibitor among the five flavonoid compounds investigated. Similarly, hesperidin, with a rutinoside attached to its C7, exhibited a 1.6-fold increase in the $IC_{50}$ value when compared to hesperetin. This seems to indicate that the large polar substituent at position C3 or C7 of the flavonoid nucleus diminishes or reduces the affinity of the compounds toward CYP2C8. It is likely that the free hydroxyl groups at these positions are essential for high affinity binding of the flavonoids to CYP2C8. The finding from this study is also consistent with results obtained in other studies (Lee et al. 1998) which showed decrease in inhibitory effect of glycosylated flavonoids when comparison to their respective aglycones was made.

Further investigation on the mechanism of inhibition revealed that the four flavonoids examined, quercetin, luteolin, hesperetin and hesperidin, exhibited mechanism-based inhibition on CYP2C8. The inhibitory effects of these inhibitors increased considerably after pre-incubation with NADPH. Furthermore, control incubations without NADPH did not result in reduced activity, implying NADPH-dependency of the inhibition (Fig. 1). Subsequent incubations at various flavonoid concentrations revealed substantially lower $IC_{50}$ values for quercetin, luteolin, hesperetin and hesperidin (Table 1). Mechanism-based inhibition involves metabolism of drugs or other chemicals to products that, in turn, inactivate CYP enzymes. Mechanisms involved may take several forms, including direct interaction with the haem of CYP leading to formation of haem-protein adduct, as well as formation of metabolite-intermediate (MI) complex that binds tightly to CYP haem (VandenBrink and Isoherranen 2010). In the case of flavonoids, it is well known that CYP enzymes are involved in their metabolism leading to hydroxylated and *O*-demethylated metabolites (Hollman and Katan 1997). It is probable that some reactive metabolites of flavonoids were formed by CYP2C8 in this study, leading to mechanism-based inactivation. This is also consistent with the fact that polyhydroxylated flavonoids were shown to bind to CYP proteins as $Fe^{3+}$ ligands. The free hydroxyl groups, in particular, those attached to C5 and C7, have been suggested to form direct ligand binding to the $Fe^{3+}$ atom at the CYP active site (Beyeler et al. 1988). It is possible that these interactions may lead to formation of MI complex and/or haem-protein adduct, hence inactivation of CYP2C8 seen in the present investigation.

Quercetin was the most potent inhibitor among the flavonoids investigated in the present project. A number of studies have reported the use of quercetin as an inhibitor probe for CYP2C8 in in vitro studies (Rahman et al. 1994; Ono et al. 1996; Li et al. 2002; Gao et al. 2010). Specificity of quercetin toward CYP2C8 was, however, found to be concentration dependent. When used at 100 μmol/L, quercetin inhibited CYP isoform selective substrate probes non-selectively, showing inhibition of 30–100% of all CYPs tested (including CYPs 3A4, 3A5, 2E1, 2D6, 2C8, 2C9, 2B6, 2A6 and 1A2) with isoforms CYPs 2A6, 2B6, 2C8 and 2C9 inhibited to a greater extent (Ono et al. 1996). Another study (Masimirembwa et al. 1999) has indicated that quercetin, when used at a lower concentration (10 μmol/L), exhibited significant selectivity towards paclitaxel 6α-hydroxylase activity mediated by CYP2C8. At this concentration, CYP2C8 was inhibited by more than 80% while other isoforms (CYPs 1A1, 1A2, 2C9, 2D6, 3A4 and 3A5) were inhibited to a lesser degree (about 20%). This showed that quercetin only works well as CYP2C8 isoform-selective inhibitory probe in a narrow concentration range. When used in high concentration, the selectivity seems to be lost, thus it is important to carefully select the inhibitor concentration when any inhibitory probe is to be used in in vitro inhibition study. Although the inhibitory effect of quercetin across various CYP isoforms have been studied, the comparative study of inhibitory effects of flavonoid compounds which are structurally closely related to quercetin on CYP2C8 activity has not been reported. The present project specifically looks into this particular aspect, and the results showed that quercetin was the most potent inhibitor among the flavonoids examined. As discussed

above, molecular shape, position and number of hydroxyl groups as well as glycosylation have been found to be the factors governing the differential inhibitory effect observed.

Studies have shown that humans ingest, on average, 0.78–1.06 g of flavonoid daily (Gosnay et al. 2002; Woods et al. 2003). Inhibition of CYP activities, including CYP2C8 examined here, could be considered relevant in drug–food interaction. Foods contain a great variety of flavonoids, and the overall metabolism and bioavailability of these compounds in man remain an area of research interest. Recent studies have shown that, following ingestion of diets rich in flavonoid, maximum concentration for total flavonoids (both conjugate forms and aglycones) achieved may vary from 3.1 to 22.5 μmol/L (Clifford 2004). However, it is considered unlikely that plasma flavonoid concentration will routinely exceed 10 μmol/L in total after normal dietary intake. Hence, when this is considered with the $IC_{50}$ values of various flavonoids obtained in this study (26.7 μmol/L or larger), the likely in vivo inhibitory effect on CYP2C8-mediated tolbutamide hydroxylation would only be at most marginal as the determined $IC_{50}$s were higher than the typical plasma level attained in our body. Nevertheless, this notion of marginal inhibition may not hold true for other CYP2C8 substrates such as amodiaquine and paclitaxel. Data from other studies have demonstrated lower $IC_{50}$ or $K_i$ values of quercetin and other flavonoids towards CYP2C8 substrates. As discussed above, quercetin inhibited CYP2C8-catalysed amodiaquine N-deethylation potently with a $K_i$ of 2.0 μmol/L (Li et al. 2002). This level is well below the typical flavonoid level attained from the normal diets (about 10 μmol/L) implying potential in vivo interaction between quercetin and amodiaquine via CYP2C8 inhibition. Furthermore, the study by Masimirembwa and coworker which demonstrated potent inhibition of paclitaxel 6α-hydroxylation by 10 μmol/L quercetin (see above) may also have in vivo implication for paclitaxel–quercetin interaction (Masimirembwa et al. 1999). This is also consistent with two other studies which showed low $K_i$ and $IC_{50}$ values for quercetin against CYP2C8-catalysed paclitaxel hydroxylation with values of 1.3 μmol/L and 5.4 μmol/L, respectively (Rahman et al. 1994; Gao et al. 2010). On the basis of the discussion above, it is thus apparent that the potential for clinical interaction between flavonoid and CYP2C8 would depend on the substrate taken as well as the plasma flavonoid attained in individual patients affected. Further studies are needed to obtain additional evidence that quercetin and other flavonoids examined in the present study can be effective in vivo inhibitors of CYP2C8.

In conclusion, the works carried out in this study have confirmed the differential modulatory effects of some naturally occurring flavonoids on CYP2C8, and that certain structural factors have been found to be important to exert inhibitory effect to the isoform. Quercetin was the most potent inhibitor among the flavonoids examined and further work is warranted to study the in vivo interactions between CYP2C8 substrates and this flavonoid.

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**Conflicts of interest** The authors declare that they have no conflict of interest.

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