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Cytochrome P450 inhibitory action of Echinacea preparations differs widely and co-varies with alkylamide content

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Abstract

Echinacea preparations are one of the best selling herbal medicinal products with a well established therapeutic use in the prophylaxis of upper respiratory tract infections. Their consumption is increasing, but information about their ability to inhibit cytochrome P450 enzymes (CYP) is fragmentary. The picture is further complicated by a lack of phytochemical characterization of previously tested preparations. Due to its well characterized immunomodulatory activity, the standardized Swiss registered *Echinacea purpurea* (L.) Moench Echinaforce extract was selected for detailed study. With the single baculovirus-expressed CYP isoforms 1A2, 2C19, 2D9 and 3A4, inhibitory actions were measured by monitoring fluorescent metabolites derived from enzyme substrates (supersome assay). The Echinaforce extract induced mild inhibition of all these isoforms, with CYP 3A4 being the most, and CYP 2D6 the least sensitive enzyme. To assess whether CYP inhibition might be a general feature of Echinacea preparations, an additional nine commercially available preparations were screened using CYP 3A4. All tested preparations were able to inhibit CYP 3A4, but inhibitory potencies (expressed as median inhibitory concentration, IC50) varied by a factor of 150. The alkylamides are thought to be responsible for the immunomodulatory activity of Echinacea, and so the concentration of 2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**) and total alkylamide content were determined in all preparations, and the latter was found to be associated with their CYP 3A4 inhibitory potency. The chemically pure alkylamides dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**) and dodeca-2*E*,4*E*-dieonoic acid isobutylamide (**2**) showed inhibitory activity on CYP 2C19, 2D6 and 3A4. However, unlike the Echinaforce extract, the alkylamides did not induce CYP 1A2 inhibition. Thus, other, as yet unidentified constituents also contribute to the overall weak inhibitory effects seen with Echinacea preparations in-vitro.

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Introduction

Interactions between drugs often derive from differential induction or inhibition of drug metabolizing enzymes such as cytochrome P450 (CYP). The five CYP isoforms 1A2, 2C9, 2C19, 2D6 and 3A4 are thought to be responsible for the metabolic conversion of the majority of currently used prescription drugs (Guengerich 1997; Wrighton 1993), and for this reason, the screening for drug–drug interactions has focused on these isoforms. For newly developed drugs, regulatory agencies now require that interactions involving CYP isoforms be assessed before licensing, and heightened awareness of the possibility that herbal medicinal products might also interfere with drug metabolising enzymes has led to proposals to extend such assessments to herbal medicines (BfArM 2004). A well documented case in point is St John's wort (*Hypericum perforatum*), which can affect the plasma concentrations of a number of conventional medicines, including digoxin, indinavir, irinotecan and ciclosporin, by inducing or inhibiting various CYP isoforms (Izzo 2005).

Echinacea preparations are one of the best selling herbal medicinal products with a well established therapeutic use in the prophylaxis of upper respiratory tract infections (Barnes et al 2005). Their consumption is increasing, but published information about possible interactions with other medicines is scarce. Of the nine Echinacea species, only three (*E. pallida* (Nutt.) Nutt., *E. purpurea* (L.) Moench and *E. angustifolia* (D.C.) Hell.) are commonly used in herbal medicinal preparations (Baum et al 2006). Echinacea preparations are well known

for their immunomodulatory activity, and it is this property which is exploited therapeutically. Several constituents including polysaccharides, alkylamides, and caffeic acid derivatives are thought to be involved in these effects (Barnes et al 2005).

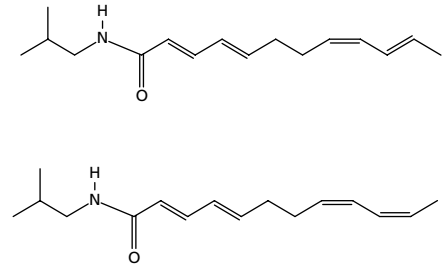
Information about the ability of Echinacea preparations to inhibit CYP enzymes is fragmentary, and the picture is further complicated by a lack of characterization of the extracts used for analysis. For example, Budzinski et al (2000) demonstrated inhibitory effects of Echinacea extracts on purified CYP 3A4 preparations by using enzyme substrates that produced fluorescing metabolites. However, details about the procedures used for the preparation of Echinacea extracts were not provided.

Yale & Glurich (2005) assessed inhibitory effects of extracts of Echinacare (Phytopharmica, Green Bay, WI) capsules (*E. purpurea*—aerial portion only) on baculovirus-expressed CYP isoforms (so-called supersomes (Crespi et al 1997)). The extract inhibited CYP 3A4 and CYP 2C9, but little effect was seen with CYP 2D6. Studies with volunteers have yielded conflicting results about the inhibitory activity of Echinacea preparations. Gorski et al (2004) administered drug probes specific for certain CYP isoforms together with *E. purpurea* root tablets (400 mg, *E. purpurea* root extract lot no: 47266-05, Nature's Bounty, Inc., Bohemia, NY) four times daily, to 12 healthy non-smokers for eight days. There was selective inhibition of CYP 3A and some inhibition on CYP 1A2, but effects on CYP 2D6 or CYP 2C9 were not apparent. In contrast, Gurley et al (2004) failed to observe inhibitory effects on CYP 1A2, CYP 2D6, CYP 3A4 or CYP 2E1 with *E. purpurea* tablets (Wild Oats Markets, Inc, Boulder, CO) given to 12 healthy volunteers. It is likely that variation in the composition of the Echinacea preparations used in those studies was one factor contributing to the differences in their outcomes, but conclusive information is missing.

Even less is known about the nature of Echinacea constituents that might lead to CYP inhibition. In a study with chemically pure alkylamides, Matthias et al (2005) observed that some alkylamides could prevent the CYP-mediated conversion of others in human liver microsomes, suggesting that those agents had CYP inhibiting potential. However, that idea has not been tested directly.

In this study we have aimed to fill some of the gaps in this knowledge. To get a better understanding of the possible range of CYP-inhibiting potencies of Echinacea extracts, we have tested a large selection of commercially available Echinacea extracts. The immunomodulatory activity of Echinacea is well characterized (Brinkborn et al 1999; Gertsch et al 2004), and so the standardized Swiss registered *Echinacea purpurea* (L.) Moench fresh plant tincture Echinaforce extract formed the focus of our studies. To test the idea that possible inhibitory effects of Echinacea extracts might be due to the action of alkylamides, we characterized all tested Echinacea preparations in terms of their alkylamide content and determined median CYP inhibitory concentrations (IC50). These potency estimates were then compared with IC50 values of the chemically pure alkylamide components, dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**, Figure 1) and dodeca-2*E*,4*E*-dieonoic acid isobutylamide (**2**, Figure 1) isolated from Echinacea extracts (Gertsch et al 2004).

Dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**) (MW = 247)



Dodeca-2*E*,4*E*-dienoic acid isobutylamide (**2**) (MW = 251)

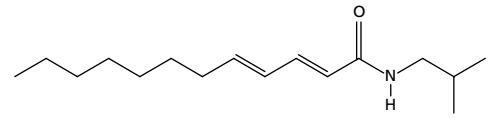


Figure 1 Structures of alkylamides assessed for inhibitory potency against CYP P450 enzymes.

Materials and Methods

Extracts

Two different batches of Echinaforce extract (015232 and 018451) were kindly provided by Bioforce UK. The other Echinacea extracts used in this study are listed in Table 1.

The alkylamides dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**) and dodeca-2*E*,4*E*-dieonoic acid isobutylamide (**2**) were isolated as described by Gertsch et al (2004). The alkylamides were 95% pure as checked by ^{1}H NMR.

Chemicals

Glucose-6-phosphate disodium hydrate salt, glucose-6-phosphate dehydrogenase, β-nicotinamide adenine dinucleotide phosphate, magnesium chloride hexahydrate, sodium citrate tribasic hydrate, ketoconazole, tranylcypromine, furafylline, quinidine hydrochloride monohydrate and trizma base were purchased from Sigma-Aldrich (Poole, Dorset, UK). Potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate 3-hydrate were obtained from VWR International Ltd. Organic solvents were of HPLC grade and were obtained from Sigma-Aldrich (Poole, Dorset, UK). The CYP substrates and metabolite standards 7-benzyl-4-(trifluoromethyl)-coumarin (BFC), 7-hydroxy-4-(trifluoromethyl)-coumarin (HFC), 3-[2-(*N, N*-diethyl-*N*-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC) and 3-[2-(*N, N*-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin hydrochloride (AHMC) were from Gentest (Woburn, UK). 3-Cyano-7-ethoxycoumarin (CEC) and 3-cyano-7-hydoxycoumarin (CHC) were obtained from Molecular Probes (Eugene, OR).

Table 1 Echinacea extracts assessed with their corresponding IC50 values and alkylamide content

Extract	Extract name/ manufacturer and batch no.	Purchased	Ethanol (%)	Echinacea species (tincture of fresh plant/fresh pressed juice)	IC50 values ($\mu g mL^{-1}$) (upper and lower C.L.)*	Total alkylamide content ($\mu g mL^{-1}$)	Alkylamide 1 content ($\mu g mL^{-1}$)	% alkylamide 1 of total alkylamide content	Dry mass ($\mu g mL^{-1}$)
A	Madaus F0500227	Pharmacy Germany	22	*E. purpurea* (juice)	1812 (1343–2447)	1	*trace	trace	43 560
B	Ratiopharm C09664	Pharmacy Germany	22	*E. purpurea* (juice)	1104 (858.7–1419)	3.4	1.7	50	48 520
C	Schoenenberg W-1045	Pharmacy Germany	0	*E. purpurea* (juice)	880.2 (717.9–1079)	1.3	0.2	16	71 704
D	Hervert purpforte 040209	Pharmacy Germany	22	*E. purpurea* (juice)	824.7 (387.6–1755)	1.8	trace	trace	32 240
E	Viridian 1881	Health Food Shop UK	22	*E. purpurea* (tincture)	83.7 (66.1–106.0)	55.9	33.9	61	27 501
F	Salus U0O114/4/3	Pharmacy Germany	50	*E. pallida radix* (juice)	66.0 (61.9–70.6)	19.1	0.3	2	70 639
G	Echinaforce 018451	Bioforce Switzerland	65	*E. purpurea* herb and root (tincture)	27.8 (25.5–30.3)	67.7	33.9	50	15 385
H	Echinaforce 015232	Bioforce Switzerland	65	*E. purpurea* herb and root (tincture)	22.2 (21.4–23.0)	54.2	28.4	52	17 195
I	Holland & Barrett 18827	Health Food Shop UK	65	*E. purpurea* (tincture)	17.0 (14.1–20.5)	98.9	29.3	30	13 005
J	HealthAid B11434	Health Food Shop UK	45	*E. angustifolia* (tincture)	16.7 (15.7–17.8)	130.2	91.8	70	33 771
K	Echinagold 221601-2	Health Food Shop Denmark	50	*E. purpurea* (tincture)	12.7 (11.2–14.5)	1384.1	535.5	39	87 154

*C.L. = 95% confidence limits (*trace < 0.05 $\mu g\ mL^{-1}$).

Analytical data

The concentration of total dry mass (g/100 g m/m) of each extract was assessed using the standard method described in the German 'Homöopathisches Arzneibuch' (HAB 2005). Each extract (3 g) was dried over 2 h at a temperature of 105°C. Using density calculations for each extract, the concentration per mL of extract was calculated.

The analytical determination of the alkylamides was performed according to Bioforces' validated 'Prüfvorschrift PV0988.d07', which used an RP18 column with a gradient of acetonitrile and water with phosphoric acid. The alkylamides were determined against an external working standard at 260 nm.

Supersome assay

A modified in-vitro fluorometric microtitre plate assay was utilised based upon the method described by Crespi et al (1997) and Stresser (2000). The substrates BFC, CEC, and AMMC were used to assess the activity of the CYP isoforms CYP 3A4, CYP 1A2 and CYP 2C19 and CYP 2D6, respectively. Stock solutions of the substrates, 20 mM CEC, 10 mM AMMC and 50 mM BFC were prepared in acetonitrile under reduced lighting conditions and sonicated to ensure homogeneity.

Assays were conducted in black, flat bottomed microtitre 96-well plates (655096) with lids obtained from Greiner (Bio-One Ltd, Gloucestershire, UK). The following human CYP supersomes were purchased from Gentest (Woburn): CYP 3A4 (with cytochrome b5), CYP 1A2 (with cytochrome b5), CYP 2C19 and CYP 2D6*1.

To assess the performance of the assay, the potency of reference inhibitors of each CYP isoform was measured. Solutions of the inhibitors furafylline (5 mM), ketoconazole (0.25 mM), quinidine (0.025 mM) and tranylcypromine (25 mM) were made in acetonitrile. The assay was carried out according to Stresser (2000) and to the manufacturer's instructions (www.gentest.com), with minor modifications. The amount of enzyme used was optimized so that the final amounts of enzyme per well were as follows: CYP 1A2 0.5 pmol, CYP 2C19 1.0 pmol, CYP 2D6 1.5 pmol and CYP 3A4 1.0 pmol. The plates were read in FLUOstar Optima (BMG, Labtech Ltd) for the production of the fluorescent metabolites at the following excitation and emission wavelengths, respectively: CHC 405–410 nm, 460 nm; AHMC 405–410 nm, 460 nm; and HFC 405–410 nm, 530 nm.

Stock solutions of each alkylamide (4 mM) were prepared in ethanol and stored at −20°C before use. The final maximum concentration of alkylamide was 200 μM with a corresponding ethanol concentration of 5%.

Blanks, positive controls, and corrections for solvent effects and intrinsic/quenching fluorescent effects

The activity of CYP isoforms can be inhibited by solvents, including ethanol (Busby et al 1999). To distinguish possible inhibitory actions of the tested Echinacea extracts from unspecific inhibition due to the inevitable presence of ethanol in these extracts, we investigated which concentrations of ethanol could be tolerated by the enzymes. Inhibition by ethanol was low at the concentration ranges tested for the individual Echinacea extracts (maximum ethanol concentration 2%).

The degree of CYP inhibition by test agents was computed as the ratio between the fluorescence measured in the presence of test substance and the fluorescence observed after the enzyme was allowed to act on its substrate in the absence of any inhibitors (positive control, P). To correct for small solvent effects, these positive controls were run with ethanol concentrations equivalent to those present in the reactions with the test substances. The positive control values were corrected for background fluorescence introduced by the enzymes and their substrates (blanks, b). Similarly, the fluorescence readings obtained in the presence of Echinacea extracts were corrected for fluorescence introduced by the extracts. This was achieved by subtracting the readings recorded for extract tests (T) with those recorded for co-factor solutions with extracts but with enzyme and substrate added after addition of the STOP solution (extract blanks, e) (eqn 1):

$$\%\ \text{Enzyme activity} = \left[\frac{(T - \bar{e})}{(\bar{P} - \bar{b})}\right] \times 100$$

None of the Echinacea extracts led to quenching of the fluorescing products of the enzyme reactions. This was ascertained by comparing the fluorescence yielded by a complete reaction with the readings obtained from reactions where extracts were added after termination with STOP solution.

Regression analysis

Three independent experiments run in duplicate were carried out for Echinaforce (Batch 015232) and the alkylamides effects on all four CYP isoforms. Two independent experiments were conducted to record the effects of the other extracts on CYP 3A4. The latter enzyme was chosen due to its abundance in the liver and large involvement in drug interactions.

To estimate median inhibitory concentrations of extracts and alkylamides, non-linear regression analysis was carried out by using the four parameter Hill model (eqn 2):

$$\%\ \text{Enzyme activity} = \text{Min} + (\text{Max} - \text{Min}) / (1 + 10\text{^}((\text{LogEC50} - x) * p)) \quad (2)$$

where Min and Max are the minimal and maximal observed effects, respectively; x is the concentration of test agent; EC50 is the concentration yielding half-maximal response (i.e. IC50); and p is the slope parameter. Due to the correction for readings, the minimal values were equal to 0% and the maximal values were equal to 100%. IC50 values and 95% confidence belts of the regression model were calculated using Graph Pad Prism v.4.03 (Inc, USA).

Results

The accurate functioning of the supersome assay was assessed by analysing the potency of reference inhibitors of

specific CYP isoforms. Median inhibitory concentrations (IC50) were estimated using non-linear regression analysis (four parameter Hill model). The IC50 value of the 1A2 inhibitor furafylline was 1.68 μM, for trancylcypromine (2C19) it was 9.45 μM, for quinidine (2D6) it was 0.0077 μM and for ketoconazole (3A4) it was 0.12 μM, all in good agreement with literature data (Gentest; Yamamoto et al 2002; Zou et al 2004).

At concentrations below 10 μg mL^{-1}, ethanolic Echinaforce extracts did not show significant inhibitory activity on any of the CYP isoforms, 1A2, 2C19, 2D6 or 3A4. The enzymes became sensitive to inhibition in the concentration range between 10 and 500 μg mL^{-1}. For potency comparisons, median inhibitory concentrations (IC50) were estimated by non-linear regression modelling (Hill model). Between-experiment variation was small, and data variation changed little with increases in extract concentration, as illustrated by the response data for CYP 2D6 in Figure 2. All other isoforms yielded data of similar quality (Table 2). Although CYP 3A4 was the most sensitive isoform, and CYP 2D6 most resistant to inhibition by Echinaforce extracts, the differences were not very pronounced. The IC50 values differed by only a factor of three.

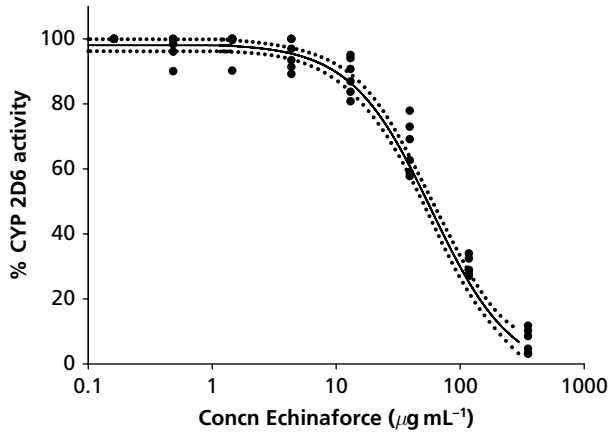


Figure 2 Concentration–response relationship for Echinaforce (batch no. 015232-extract H) on CYP 2D6. Data points are duplicates from three independent experiments with the corresponding regression model (black solid sigmoidal line) and 95% upper and lower confidence limits (dotted lines).

A survey of the inhibitory activity of other commercially available Echinacea extracts on CYP 3A4 isoform was conducted. Although all extracts were able to inhibit CYP 3A4, we observed surprisingly large potency differences (Figure 3 and Table 1). The IC50 value of the most potent extract K (12.71 μg mL^{-1}) was 150 times lower than that of the least effective extract A (1812 μg mL^{-1}).

There were also considerable variations in the alkylamide content of these Echinacea extracts. Extract K contained approximately 1400-fold alkylamide levels than extract A (1384 μg mL^{-1} vs 1 μg mL^{-1}) (Table 1). Higher alkylamide content was associated with greater CYP 3A4 inhibitory activity (with a correlation coefficient of $r^2 = 0.89$), but dry mass content did not correlate with the IC50 values of the extracts.

The association between alkylamide content and CYP 3A4 inhibitory potency prompted us to assess directly whether alkylamides known to be present in Echinacea extracts were able to interfere with CYP isoforms. Studies with the chemically pure alkylamides **1** and **2** (Figure 1) revealed that CYP 2C19, 2D6 and 3A4 could all be inhibited. Generally, **1** showed more pronounced inhibitory action than **2**. The potency differences were particularly marked with CYP 3A4, where over twice the concentration of **2** was required to produce 50% enzyme inhibition (Table 2, Figure 4). The inhibition of CYP 2C19 by **1** was slightly stronger than that by **2** (IC50 of 76.6 vs 93.0 μM). CYP 2D6 was inhibited by both alkylamides, with approximately twice as much **2** required than **1** to cause 50% loss in enzyme activity (40.2 vs 27.4 μM). However, both alkylamides lacked any inhibitory effects on CYP 1A2. The alkylamides were weaker inhibitors of CYP isoforms than their respective reference inhibitors (Table 2).

Discussion

We have shown that all Echinacea extracts studied had the potential to inhibit CYP 3A4. However, it is noteworthy that the inhibitory potency was overall relatively weak and varied considerably between extracts. Our data showed that inhibition of CYP 3A4 was not limited to preparations from *E. purpurea*, but was also seen with extracts derived from *E. pallida*

Table 2 Median inhibitory concentrations (IC50) and the upper and lower 95% confidence limits (depicted in brackets) for Echinaforce, dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetranoic acid isobutylamide (**1**) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (**2**) against the CYP isoforms (CYP 1A2, CYP 2C19, CYP 2D6 and CYP 3A4)

Test substance	CYP isoforms			
	CYP 1A2	CYP 2C19	CYP 2D6	CYP 3A4
Echinaforce Batch 015232 (μg mL^{-1})	30.21 (27.11–33.66)	60.87 (36.66–101.0)	69.40 (57.25–84.14)	22.18 (21.40–23.00)
Alkylamide **1** (μg mL^{-1})	No inhibition	18.9 (14.9–24.0)	6.8 (5.2–8.8)	1.9 (1.7–2.1)
(μM)		76.6 (60.2–97.4)	27.4 (21.1–35.5)	7.7 (7.0–8.5)
Alkylamide **2** (μg mL^{-1})	No inhibition	23.4 (17.4–31.4)	10.1 (7.2–14.2)	5.2 (4.1–6.5)
(μM)		93.0 (69.2–125.10)	40.2 (28.7–56.4)	20.6 (16.4–26.0)
Reference inhibitors	Furafylline	Tranylcypromine	Quinidine	Ketoconazole
(μM)	1.68 (1.36–2.08)	9.45 (8.05–11.10)	0.0077 (0.0069–0.0086)	0.12 (0.10–0.14)

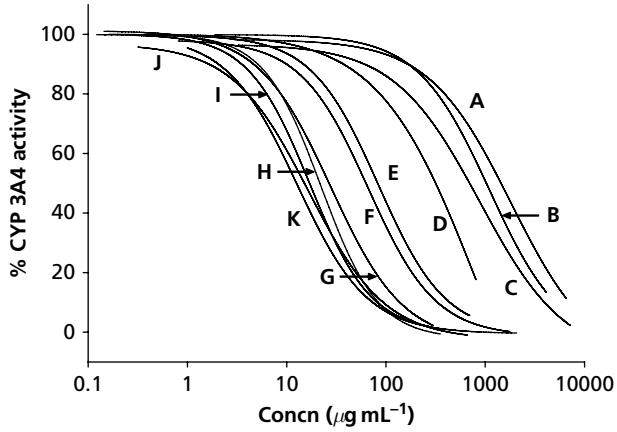


Figure 3 Regression models for the effects of all 10 extracts on CYP 3A4 based on duplicates from two independent experiments for all extracts except extract H where three independent experiments were conducted. For details about the extracts refer to Table 1.

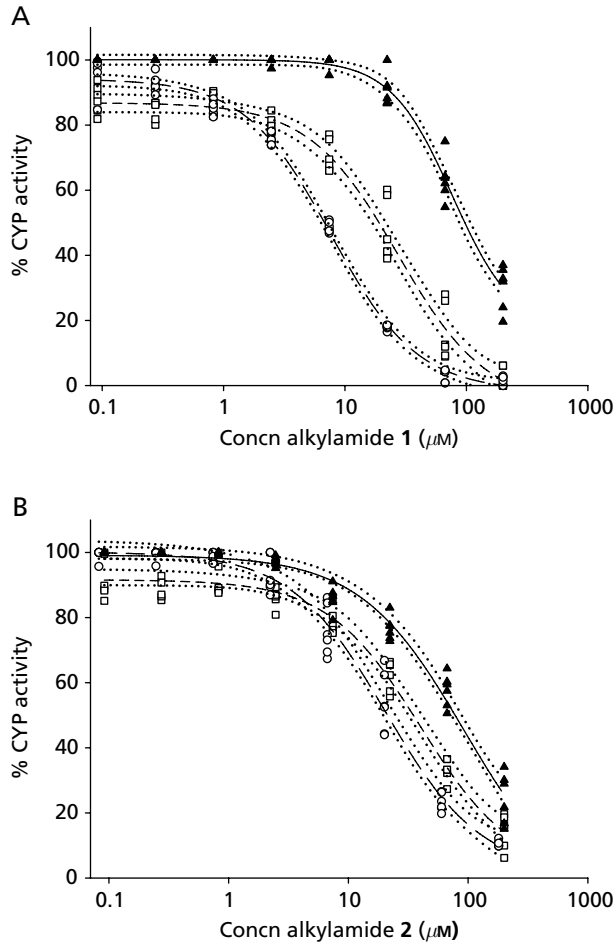


Figure 4 Regression models for the effect of (A) dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetranoic acid isobutylamide (**1**) and (B) dodeca-2*E*,4*E*-dienoic acid isobutylamide (**2**) on CYP 2D6 (open squares, short dashed line), CYP 3A4 (open circles, long dashed line) and CYP 2C19 (closed triangles, solid line). No inhibitory effect was seen with CYP 1A2. Data points are duplicates from three independent experiments. Upper and lower 95% confidence limits of the regression response are shown as dotted lines.

radix and *E. angustifolia*. The Echinaforce extract was tested more extensively and revealed weak in-vitro inhibitory activity also on CYP 1A2, CYP 2C19 and CYP 2D6. It appeared likely that the other preparations were able to inhibit these isoforms, although this awaits experimental confirmation.

Our results agreed with the observations made by Yale & Glurich (2005) with the CYP 3A4 isoform, of which they reported a mild inhibitory activity (IC50 value: 75% full extract concentration) using the same substrate. However, this was not the case for CYP 2D6 for which they reported no effect. It is conceivable that this lack of activity was due to the relative insensitivity of this isoform to Echinacea that became apparent in our studies. The study by Yale & Glurich (2005) assessed a 70% methanol extract of *E. purpurea* that was prepared in house from one Echinacare capsule (Phytopharmica, Green Bay, WI). They employed an assay set up similar to the one used by us, but without a control for intrinsic fluorescence or assessment of quenching effects. The authors acknowledged that this contributed to interferences in the assay.

For the first time, we have demonstrated that alkylamides found in Echinacea species contributed to the inhibitory activity on CYP isoforms 2C19, 2D6 and 3A4. The isomeric alkylamide **1** showed more potent inhibitory activity on CYP isoforms. It is well known that **1** is one of the main components among the numerous alkylamides present in Echinacea species (Gertsch et al 2004). With the exception of the extracts A, C, D and F, **1** was by far the most prevalent of all the alkylamides, representing 30–70% of the total alkylamide content present in the extracts analysed here (Table 1). The good correlation between the alkylamide content of the extracts and their potency in inhibiting CYP 3A4 has provided evidence that alkylamides, at least in part, contributed to the overall inhibitory actions of Echinacea preparations. The CYP inhibition seen with the extracts could not solely be attributed to alkylamides. While the Echinaforce extract inhibited CYP 1A2, this effect was not observed with **1** and **2** in the concentration range where both these alkylamides showed activity with the other CYP isoforms. Furthermore, alkylamides are unstable in aqueous preparations (Gertsch et al 2004) and are better preserved in ethanolic solutions, but the ethanol content of the preparations had little influence on the inhibitory potency of the extracts. For example, extract C did not contain any ethanol, but induced some, albeit weak, inhibition on CYP 3A4. We concluded that other, as yet unidentified constituents also contributed to the overall inhibitory effects seen with Echinacea preparations in-vitro.

Taken together, we have shown that the supersome assay system based on probes that give rise to fluorescent reaction products was useful for the effective and rapid assessment of inhibitory activity of herbal medicinal products on CYP enzymes. Therefore it could be applied to meet the large-scale screening requirements of regulatory authorities.

Although in-vitro screening assays such as the one used in our studies can reveal the potential of Echinacea herbal medicinal products to lead to adverse drug interactions involving CYP inhibition, this cannot indicate that CYP inhibition

will necessarily occur at the doses of Echinacea extracts normally encountered in a clinical situation. An interesting bioavailability study in ten volunteers (Woelkart et al 2006) is available that might provide some guidance in answering this question. A single maximum daily dose of Echinaforce drops (4 mL) or Echinaforce tablets (12 tablets) was administered to volunteers. With a single dose of Echinaforce drops equivalent to 0.07 mg **1**, a maximum serum level of 0.4 ng mL^{-1} (± 0.31 ng mL^{-1}) of **1** was measured after 30 min. Hypothetically, making the worst-case assumption that the same level was reached inside hepatocytes, and ignoring potential losses through distribution, uptake etc., the lowest IC50 recorded for **1** in our study (1.96 μg mL^{-1}) was 4900-fold higher than the anticipated concentration inside hepatocytes. Even taking into account that other, unknown ingredients are likely to be at play, it appears highly unlikely that inhibitory concentrations of **1** will be reached inside the liver. This conclusion needs to be tempered by considering the possibility that the serum levels may be higher after prolonged use of Echinaforce, and that hepatocytes may be more sensitive to CYP inhibition than the purified enzyme preparations used in this study. However, there are currently no data available that would support these possibilities.

The in-vivo findings by Gorski et al (2004) of selective inhibition of CYP 3A4 and 1A2 in volunteers given *E. purpurea* root tablets deserve serious consideration. Interestingly, inhibition of CYP 2D6 was not observed by Gorski et al (2004). Once again this lack of activity may have been due to the relative insensitivity of this isoform that became apparent in our studies. However, in view of the general lack of CYP inhibition in the volunteer study by Gurley et al (2004) the evidence remains somewhat contradictory and it is hard to draw definitive conclusions about the ability of Echinacea preparations to interfere with CYP in "real life". Considering the large variations in inhibitory potency we observed with commercial preparations, and the absence of phytochemical–analytical characterizations of the extracts used in the above human studies, it is essential that future studies, especially with volunteers, are conducted with phytochemically–analytically characterized preparations. Furthermore, conclusions drawn using one specific extract cannot necessarily be generalized to other ones derived from the same botanical drug.

References

Barnes, J., Anderson, L. A., Gibbons, S., Phillipson, J. D. (2005) *Echinacea angustifolia* (D.C.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.)Moench: a review of their chemistry, pharmacology and clinical properties. *J. Pharm. Pharmacol.* **57**: 929–954

Baum, R. B., Binns, S. E., Arnason, J. T. (2006) Integrating recent knowledge about the genus *Echinacea*: morphology, molecular systematics, phytochemistry. *HerbalGram* **72**: 32–46

BfArM (2004) http://www.bfarm.de/cln_042/nn_424630/DE/Arzneimittel/besTherap/amPflanz/ampflanz-node.html_(Last accessed 20.08.2006)

Brinkeborn, R. M., Shah, D. V., Degenring, F. H. (1999) Echinaforce and other Echinacea fresh plant preparations in the treatment of the common cold. A randomized, placebo controlled double-blind clinical trial. *Phytomedicine* **6**: 1–6

Budzinski, J. W., Foster, B. C., Vandenhoek, S., Arnason, J. T. (2000) An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. *Phytomedicine* **7**: 273–282

Busby, W. F. Jr, Ackermann, J. M., Crespi, C. L. (1999) Effect of methanol, ethanol, dimethyl sulfoxide, and acetonitrile on in vitro activities of cDNA-expressed human cytochromes P450. *Drug Metab. Dispos.* **27**: 246–249

Crespi, C. L., Miller, P. V., Penman, B. W. (1997) Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450. *Anal. Biochem.* **248**: 188–190

Gertsch, J., Schoop, R., Kuenzle, U., Suter, A. (2004) Echinacea alkylamides modulate TNF-alpha gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. *FEBS Lett.* **577**: 563–569

Gorski, J. C., Huang, S. M., Pinto, A., Hamman, M. A., Hilligoss, J. K., Zaheer, N. A., Desai, M., Miller, M., Hall, S. D. (2004) The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity in vivo. *Clin. Pharmacol. Ther.* **75**: 89–100

Guengerich, F. P. (1997) Role of cytochrome P450 enzymes in drug-drug interactions. *Adv. Pharmacol.* **43**: 7–35

Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Carrier, J., Khan, I. A., Edwards, D. J., Shah, A. (2004) In vivo assessment of botanical supplementation on human cytochrome P450 phenotypes: *Citrus aurantium, Echinacea purpurea*, milk thistle and saw palmetto. *Clin. Pharmacol. Ther.* **76**: 428–440

Homöopathisches Arzneibuch (HAB) (2005) Bestimmung de Troekenrülks. Deutscher Apotheker Verlag Stuttgart and Govi-Verlag Pharmazeutischer Verlag GmbH. H.2.2.6 p. 4

Izzo, A. A. (2005) Herb-drug interactions: an overview of the clinical evidence. *Fundam. Clin. Pharmacol.* **19**: 1–16

Matthias, A., Gillam, E. M., Penman, K. G., Matovic, N. J., Bone, K. M., De Voss, J. J., Lehmann, R. P. (2005) Cytochrome P450 enzyme-mediated degradation of Echinacea alkylamides in human liver microsomes. *Chem. Biol. Interact.* **155**: 62–70

Stresser, D. M. (2004) High-throughput screening of human cytochrome P450 inhibitors using fluorometric substrates: Methodology for 25 enzyme substrate pairs. In: Yan, Z. and Caldwell, G. W. (eds) *Optimization in drug discovery: in vitro methods* in Kang, Y. J., (series eds) *Methods in pharmacology and toxicology*. Humana Press, Totowa, NJ, USA, pp 215–230

Woelkart, K., Marth, E., Suter, A., Schoop, R., Raggam, R. B., Koidl, C., Kleinhappl, B., Bauer, R. (2006) Bioavailability and pharmacokinetics of *Echinacea purpurea* preparations and their interactions with the immune system. *Int. J. Clin. Pharmacol. Ther.* **44**: 401–408

Wrighton, S. A., Vandenbranden, M., Stevens, J. C., Shipley, L. A., Ring, B. J., Rettie, A. E., Cashman, J. R. (1993) In vitro methods for assessing human hepatic drug metabolism: their use in drug development. *Drug Metab. Rev.* **25**: 453–484

Yale, S. H., Glurich, I. (2005) Analysis of the inhibitory potential of *Gingko biloba, Echinacea purpurea*, and *Serenoa repens* on the metabolic activity of cytochrome P450 3A4, 2D6, and 2C9. *J. Altern. Complement. Med.* **11**: 433–439

Yamamoto, T., Suzuki, A., Kohno, Y. (2002) Application of microtiter plate assay to evaluate inhibitory effects of various compounds on nine cytochrome P450 isoforms and to estimate their inhibition patterns. *Drug Metab. Pharmacokinet.* **17**: 437–448

Zou, L., Henderson, G. L., Harkey, M. R., Sakai, Y., Li, A. (2004) Effects of kava (Kava-kava, 'Awa, Yaquona, *Piper methysticum*) on c-DNA-expressed cytochrome P450 enzymes and human cryopreserved hepatocytes. *Phytomedicine* **11**: 285–294