Inhibition of Human Cytochrome P450 Enzymes by Constituents of St. John’s Wort, an Herbal Preparation Used in the Treatment of Depression

R. SCOTT OBACH

Drug Metabolism Department, Candidate Synthesis, Enhancement, and Evaluation, Central Research Division, Pfizer, Inc., Groton, Connecticut

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ABSTRACT

Commercially available St. John’s wort (Hypericum perforatum) extracts, preparations that are used in the treatment of depression, were examined for the potential to inhibit human cytochrome P450 (CYP) enzyme activities, specifically CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Crude extracts demonstrated inhibition of each of these five enzymes, with CYP2D6, CYP2C9, and CYP3A4 being more sensitive than CYP1A2 and CYP2C19. Extracts were fractionated by HPLC, and each of the fractions was tested for inhibition of these five CYPs to identify individual constituents with inhibitory activity. Several fractions were shown to possess inhibitory activity, including the fractions containing hyperforin (the putative active antidepressant constituent), I3,II8-biapigenin, and hypericin. Hyperforin and I3,II8-biapigenin were isolated from the extract, and inhibition constants for the five CYP activities were measured. In addition, three other constituents, hypericin, quercetin, and chlorogenic acid, were tested for inhibitory activity toward the CYP enzymes. The flavonoid compound I3,II8-biapigenin was shown to be a potent, competitive inhibitor of CYP3A4, CYP2C9, and CYP1A2 activities with Ki values of 0.038, 0.32, and 0.95 μM, respectively. Hyperforin was a potent noncompetitive inhibitor of CYP2D6 activity (Ki = 1.5 μM) and competitive inhibitor of CYP2C9 and CYP3A4 activities (Ki = 1.8 and 0.48 μM, respectively). Hypericin also demonstrated potent inhibition of several CYP activities. These in vitro data indicate that St. John’s wort preparations contain constituents that can potently inhibit the activities of major human drug-metabolizing enzymes and suggest that these preparations should be examined for potential pharmacokinetic drug interactions in vivo.

St. John’s wort (Hypericum perforatum) is a flowering plant indigenous to Europe and North America, of which extracts are retaining a growing popularity in the treatment of depression (Josey and Tackett, 1999). Preparations are available over-the-counter, and as such this agent can be self-prescribed without the recommendation or advice of the physician. In addition, the fact that St. John’s wort is a natural product rather than a chemically synthesized drug can give the impression to the general populace that this agent would be without untoward effects such as drug-drug interactions. Furthermore, herbal products are not subject to the scrutiny of the approval process applied to new drug applications by the U.S. Food and Drug Administration. Thus, herbal agents such as St. John’s wort are not required to undergo careful and scientifically rigorous examinations of clinical efficacy and safety that are required of conventional pharmaceutical products.

Because St. John’s wort is a plant extract, it contains a complex mixture of phytochemicals (Nahrstedt and Butterweck, 1997). Despite the practice of normalizing extracts to a fixed content of the known constituent hypericin (Fig. 1) by measurement of the long-wavelength absorbance of this compound and its analog, pseudohypericin, preparations can vary in composition among different manufacturers and lots. Major constituents of St. John’s wort extracts include several classes of compounds exemplified by flavonoids, flavonol glycosides, biflavones, naphthodianthrones, acylphloroglucinols, and phenylpropanes (Nahrstedt and Butterweck, 1997; Erdelmeier, 1998). Recent investigation has been aimed toward the identification of bioactive constituents that may be wholly or partially responsible for possible therapeutic effect. Hyperforin (Fig. 1), an acylphloroglucinol, has been identified as the possible active constituent in depression (Biber et al., 1998; Laakmann et al., 1998; Singer et al., 1999).

The cytochrome P450 enzymes (CYP) represent a large family of proteins involved in the metabolism of drugs and other xenobiotics, as well as some endogenous substrates (Guengerich, 1995). Drug interactions can frequently arise when drugs are coadministered and one drug inhibits the metabolic clearance of the second drug by inhibition of a specific CYP enzyme (Lin and Lu, 1998). For example, coadministration of the azole antifungal ketoconazole, a potent

ABBREVIATIONS: CYP, cytochrome P450; MS, mass spectrometry.
inhibitor of CYP3A4, can cause marked elevations in systemic exposure of compounds metabolically cleared by this enzyme family. Such an interaction has resulted in the observations of severe adverse drug interactions, including some interactions resulting in death (Honig et al., 1993). Inhibition of CYP enzymes can also be effected by natural products. A notable example of this is the inhibition of CYP3A by grapefruit juice, which can result in elevations of systemic exposure to CYP3A-cleared compounds (Bailey et al., 1998). Thus, it is possible that constituents in herbal preparations could also possess capabilities to inhibit drug-metabolizing enzymes. Although new drug candidates are now routinely examined for the potential to cause drug interactions via inhibition of drug-metabolizing enzymes, herbal preparations are not subject to such examination. Thus, the potential exists that herbal preparations, such as St. John’s wort, could cause drug interactions with concomitantly administered medications.

The objective of these experiments was to determine the potential for St. John’s wort constituents to inhibit human CYP enzymes most commonly involved in the metabolic clearance of drugs: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Extracts of St. John’s wort were fractionated by HPLC to initially identify constituents that inhibited CYP enzyme activities, followed by a full characterization of inhibition kinetics of those constituents that demonstrated inhibition.

### Experimental Procedures

**Materials.** St. John’s wort preparations were obtained from a local pharmacy. Three brands were examined with regard to constituent profile on HPLC: Centrum Herbals (300-mg capsules; Whitehall-Robins Healthcare, Madison, NJ), Quanterra (300-mg capsules; Whitehall-Robins Healthcare, Madison, NJ), and Nature’s Remedy (300-mg capsules; Warner-Lambert Co., Morris Plains, NJ), and Nature’s Resource (150-mg capsules; Nature’s Resource Products, Mission Hills, CA). Recombinant heterologously expressed human CYP enzymes were generated in-house using a baculovirus/Sf9 system (Christopherson et al., 1995). S-Mephenytoin, 4’-hydroxymephenytoin, sulfaphenazole, and furaphylline were obtained from Gentest Corp. (Woburn, MA). Diclofenac, prednisone, metoprolol, ketoprofen, acetaminophen, phenacetin, 6’-hydroxytestosterone, quercetin, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Testosterone was obtained from Steraloids Inc. (Wilton, NH). Bufuralol, 1’-hydroxybufuralol, 4’-hydroxydiclofenac, and 5-(4-hydroxyphenyl)hydantoin were synthesized in-house. [3H2]Acetaminophen was prepared as described (Chan and Pang, 1982). Hypericin was obtained from BIOMOL (Plymouth Meeting, PA). trans-Chlorogenic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI). Solvents and other reagents were obtained from common commercial sources.

**HPLC-Mass Spectrometry (MS) Instrumentation.** HPLC-MS instrumentation used in all experiments consisted of a Hewlett-Packard 1100 quaternary pump with membrane degasser (Hewlett-Packard, Palo Alto, CA), a CTC PAL autoinjector (Leap Technologies, Carrboro, NC), a Spectromonitor 4000 variable-wavelength UV-VIS detector (LDC Analytical, Riviera Beach, FL), and a PE Sciex API 100 mass spectrometer (PE-Sciex, Thornhill, Ontario, Canada). The mass spectrometer possessed a turbo ion spray interface.

**Isolation of Hyperforin.** Hyperforin was isolated from St. John’s wort by the following procedure, adapted from that previously described (Erdelmeier, 1998). Twenty 300-mg St. John’s wort capsules were opened, and the contents were stirred with methyl t-butyl ether (100 ml) under an N2 atmosphere. The mixture was vacuum-filtered through no. 2 filter paper, and the filtrate was evaporated under N2 to yield 1.2 g of residue. The residue was dissolved in 3 ml of methanol, of which 0.5 ml was subjected to preparative HPLC. The column was a Keystone Scientific C18 column (10 × 250 mm) equilibrated in 90% methanol, 10% water at a flow rate of 4 ml/min. The eluent was collected into 1.0-min fractions. Each fraction was flowed into a PE Sciex API100 mass spectrometer operated in the full scan mode with 0.3% formic acid/40% CH3CN at a flow rate of 0.5 ml/min. Fractions containing mass spectral peaks indicative of hyperforin (m/z 537, 277; Brolis et al., 1998) were checked for purity on HPLC-MS using the gradient system described below. Fractions that contained only hyperforin were pooled, and the solvent was removed overnight to yield 4.0 mg of hyperforin as a solid, pale-yellow material. Due to known instability in organic solvent such as hexane (Erdelmeier, 1998; Orth et al., 1999a), stock solutions of hyperforin were prepared in methanol and stored under N2 at 4°C.

**Isolation of I3,I8-Biapigenin.** I3,I8-Biapigenin was isolated using a procedure modified from the literature (Berghofer and Holzl, 1987). The contents of 24 300-mg St. John’s wort capsules were stirred with 300 ml of water and 300 ml of ethyl acetate for 30 min. The layers were separated, and the ethyl acetate layer was washed with water (200 ml). The solvent was evaporated under N2 to yield 6 g of residue. The residue was dissolved in methanol (30 ml) and applied to a Sephadex LH20 column (3 × 30 cm), followed by elution with methanol and collection of 15-ml fractions. Fractions were tested by flow injection on HPLC-MS as earlier; those containing I3,I8-biapigenin (m/z 539) were pooled, the solvent was evaporated, and the resulting material was applied to a second Sephadex LH20 column. Fractions containing I3,I8-biapigenin were evaporated under N2 to yield 69 mg of crude product. The material was applied to preparative HPLC containing a Keystone Scientific C18 column (10 × 250 mm) equilibrated in 0.3% formic acid/CH3CN/CH3OH (67:23:10) at a flow rate of 4 ml/min. Fractions (1-min) were collected, and those containing purified I3,I8-biapigenin were pooled and evaporated under N2. The remaining precipitated material in aqueous solvent was extracted into ethyl acetate, and the solvent was evaporated under N2 to yield purified I3,I8-biapigenin (2.1 mg) as a bright-yellow solid.

**Testing St. John’s Wort Crude Extract for Inhibition of CYP Activities.** The contents of St. John’s wort capsules were combined, and 300 mg was added to a 16 × 125-mm glass test tube containing methanol (3 ml). The mixture was slowly mixed with inversion for 30 min. The mixture was spun in a centrifuge (1000g) for 5 min to remove particulate matter. The supernatant (referred to as a 100 mg Eq/ml solution) was used to make methanolic solutions that were tested directly for inhibition of CYP activities as described below.

**Fig. 1.** Structures of St. John’s wort constituents.
Fractionation of St. John’s Wort Extract. To the contents of a 300-mg St. John’s wort capsule in a 16 × 125-mm glass test tube was added methanol (3 ml), followed by slow mixing with inversion for 30 min. The mixture was subjected to centrifugation (2000g) for 10 min, and the supernatant was removed for HPLC fractionation.

The HPLC system used was adapted from a previously described procedure (Brolis et al., 1998). The HPLC system consisted of a Waters Symmetry C18 column (4.6 × 150 mm) equilibrated in 0.3% formic acid (aqueous component) at a flow rate of 0.8 ml/min. The injection volume was 20 μl. A gradient program was applied as follows: 0 to 10 min, gradient from 100 to 85% aqueous/15% CH3CN; 10 to 30 min, gradient to 70% aqueous/20% CH3CN/10% CH3OH; 30 to 40 min, gradient to 10% aqueous/75% CH3CN/15% CH3OH; 40 to 55 min, gradient to 0% aqueous/85% CH3CN/15% CH3OH; and 55 to 60 min, isotropic at 0% aqueous/85% CH3CN/15% CH3OH. The eluent was monitored at λ = 280 nm, and fractions were collected each minute. An aliquot of each fraction (125 μl; 25 μl for CYP2D6) was transferred to a 16 × 100 sylated test tube, and the solvent was evaporated under N2. Cytochrome P450 activities were tested as described later by adding 0.2 ml of incubation mixture to each of the evaporated fraction tubes.

CYP1A2 Phenacetin O-Deethylase Assay. Phenacetin (50 μM) was incubated with rCYP1A2 microsomes (0.2 mg/ml; 11.7 pmol of CYP/ml), 3.3 mM MgCl2, and 1.3 mM NADPH in a total volume of 0.2 ml of 100 mM KH2PO4, pH 7.5, in the presence and absence of inhibitors. The reactions were commenced with the addition of NADPH, and incubations were conducted in a shaking water bath at 37°C for 30 min. Reactions were terminated by the addition of 20 μl of methanol containing [3H]acetaminophen as internal standard (30 μM) and placed on ice. A portion of the terminated reaction mixture (175 μl) was transferred to a Millipore Multiscreen-HA 0.45-μm mixed cellulose ester 96-well membrane vacuum filtration module. The resulting filtrate was analyzed by HPLC-MS. The system contained a Phenomenex Luna C18 column (2.0 × 50 mm) equilibrated in 10 mM NH4OAc containing 5% CH3CN and 0.9% isopropanol at a flow rate of 0.5 ml/min. The filtered incubation mixtures were injected (30 μl), and the eluent was monitored by selected ion monitoring of m/z 150 (acetylamino) and m/z 153 (trideuterated acetylaminophen internal standard). The eluent flow was diverted to waste for the 1st min to reduce introduction of phosphate buffer into the mass spectrometer. The analyte and internal standard eluted at 1.35 min. Quantification was done from a standard curve of acetylamino with a linear dynamic range from 0.1 to 10 μM.

CYP2C9 Diclofenac 4′-Hydroxylase Assay. Diclofenac (10 μM) was incubated with rCYP2C9 microsomes (0.15 mg/ml; 3.5 pmol of CYP/ml), 3.3 mM MgCl2, and 1.3 mM NADPH in a total volume of 0.2 ml of 100 mM KH2PO4, pH 7.5, in the presence and absence of inhibitors. The reactions were commenced with the addition of NADPH, and incubations were conducted in a shaking water bath at 37°C for 10 min. Reactions were terminated by the addition of 20 μl of methanol containing ketoprofen as internal standard (50 μM) and placed on ice. An aliquot of the terminated reaction mixture (175 μl) was filtered as described earlier, and the resulting filtrate was analyzed by HPLC-MS. The system contained a Phenomenex Luna C18 column (2.0 × 50 mm) equilibrated in 10 mM NH4OAc containing 5% CH3CN and 0.9% isopropanol at a flow rate of 0.5 ml/min. The filtered incubation mixtures were injected (30 μl), and the eluent was monitored by selected ion monitoring of m/z 150 (acetylamino) and m/z 153 (trideuterated acetylaminophen internal standard). The eluent flow was diverted to waste for the 1st min to reduce introduction of phosphate buffer into the mass spectrometer. The analyte and internal standard eluted at 1.35 min. Quantification was done from a standard curve of ketoprofen with a linear dynamic range from 0.1 to 10 μM.

CYP2D6 Bufuralol 1′-Hydroxylase Assay. Bufuralol (10 μM) was incubated with rCYP2D6 microsomes (8.8 μg/ml; 1.62 pmol of CYP/ml), 3.3 mM MgCl2, and 1.3 mM NADPH in a total volume of 0.2 ml of 100 mM KH2PO4, pH 7.5, in the presence and absence of inhibitors. The reactions were commenced with the addition of NADPH, and incubations were conducted in a shaking water bath at 37°C for 10 min. Reactions were terminated by the addition of 20 μl of methanol containing metoprolol as internal standard (2.5 μM) and placed on ice. An aliquot of the terminated reaction mixture (175 μl) was filtered as described earlier, and the resulting filtrate was analyzed by HPLC-MS. The system contained a Phenomenex Luna C18 column (2.0 × 50 mm) equilibrated in 20 mM H2OAc (adjusted to pH 4 with NH4OH) containing 14% CH3CN at a flow rate of 0.5 ml/min. The filtered incubation mixtures were injected (30 μl), and the eluent was monitored by selected ion monitoring of m/z 278 (1′-hydroxybufuralol) and m/z 268 (metoprolol internal standard) with the mass spectrometer operated in the positive ion mode. The eluent flow was diverted to waste for the first 1.3 min. The analyte and internal standard eluted at 1.8 and 2.3 min, respectively. Quantification was done from a standard curve of 1′-hydroxybufuralol with a linear dynamic range from 0.03 to 2 μM.

CYP3A4 Testosterone 6β-Hydroxylase Assay. Testosterone (50 μM) was incubated with rCYP3A4 microsomes (0.86 mg/ml; 72 pmol of CYP/ml), 3.3 mM MgCl2, and 1.3 mM NADPH in a total volume of 0.2 ml of 100 mM KH2PO4, pH 7.5, in the presence and absence of inhibitors. The reactions were commenced with the addition of NADPH, and incubations were conducted in a shaking water bath at 37°C for 10 min. Reactions were terminated by the addition of 20 μl of methanol containing prednisone as internal standard (30 μM) and placed on ice. An aliquot of the terminated reaction mixture (175 μl) was filtered as described earlier, and the resulting filtrate was analyzed by HPLC-MS. Chromatography was done using a Phenomenex Luna C18 column (2.0 × 50 mm) equilibrated in 20 mM H2OAc (adjusted to pH 4 with NH4OH) containing 23% CH3CN at a flow rate of 0.5 ml/min. The filtered incubation mixtures were injected (10 μl), and the eluent was monitored by selected ion monitoring of m/z 305 (6β-hydroxytestosterone) and m/z 359 (prednisone internal standard) with the mass spectrometer operated in the positive ion mode. The eluent flow was diverted to waste for the first 1.2 min. The analyte and internal standard eluted at 1.8 and 2.8 min, respectively. Quantification was done from a standard curve of 6β-hydroxytestosterone with a linear dynamic range from 0.1 to 10 μM.

Data Analysis. IC50 values were determined by fitting the data in Deltagraph (version 4.5; SPSS Inc., Chicago, IL). The data listed represent the average values from two determinations. K values were determined by first assessing the mode of inhibition (i.e., com-
petitive versus noncompetitive) by examination of Dixon and Line-
weaver-Burke plots. The substrate saturation data were then fit
using nonlinear regression in Deltagraph, using the equations:
\[
v = \frac{V_{\text{max}} \cdot [S]}{[S] + K_M + \frac{1}{I}}
\]
or
\[
v = \frac{V_{\text{max}} \cdot [S]}{([S] + K_M) \cdot (1 + \frac{1}{I})}
\]
for competitive and noncompetitive inhibition, respectively.

**Results**

**Inhibition Profiles of HPLC Fractionated St. John’s Wort Extracts.** Three different sources of St. John’s wort extracts were examined with regard to HPLC profile to determine the variability that could be expected from different sources of material. HPLC-UV profiles are presented in Fig. 2, and in-line mass spectrometric detection was used to aid in the identification of constituents by comparison with previously published reports (Brolis et al., 1998; Erdelmeier, 1998). The largest of the UV-absorbing constituents eluted between 18 and 22 min and included several quercetin glycosides such as rutin, hyperoside, and quercitrin. Chlorogenic acid eluted at about 12 min. Quercetin and the flavonoid dimer I3,I8-biapigenin eluted at 34 and 36 min, respectively. Hyperforin and adhyperforin eluted after 50 min, with the former as the major UV peak observed eluting after 50 min. The three different preparations possessed similar profiles with respect to many of the constituents, but the relative abundances of the constituents within a given preparation showed some differences. Two closely eluting peaks, the identity of which are unknown, were observed at 47 to 49 min. The mass spectral data suggested that these could be analogs of hyperforin possessing the addition of a single oxygen atom, possibly oxidative degradants of hyperforin such as orthoforin (Orth et al., 1999b).

The same HPLC system was used to generate fractions that were tested with regard to their ability to inhibit CYP enzyme activities. Elution profiles for the inhibition for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are shown in Fig. 3. The positive controls furaphylline (CYP1A2), sulfaphenazole (CYP2C9), ticlopidine (CYP2C19), quinidine (CYP2D6), and ketoconazole (CYP3A4) were used to confirm inhibitory activity. Fractions eluting in the 35- to 38-min region (quercetin and I3,I8-biapigenin) demonstrated inhibition of each of the CYPs except CYP2C19. The fraction eluting at 51 min, where hyperforin eluted, showed inhibition of CYP2D6, CYP3A4, and CYP2C9. Also, some fractions eluting in the 40- to 48-min region demonstrated inhibition, but little 280-nm absorbing material eluted in this region. When examined, a pure standard of hypericin eluted in this region of the chromatogram, suggesting the possibility that this constituent could be an inhibitor of CYP3A4, -2D6, and -2C9. This possibility was examined (see later). In addition, the materials eluting at 47 to 49 min demonstrated inhibition of CYP3A4, -2D6, -2C9, and -2C19; however these observations were not further pursued. Potent inhibition of CYP2D6 was observed for materials eluting in the 12- to 15-min region, but the identity or identities of these inhibitors remain unknown.

**Fig. 2.** HPLC-UV chromatogram for three commercial St. John’s wort extracts. Methanolic extracts were injected onto HPLC-UV-MS as described in Experimental Procedures. A, Centrum Herbals. B, Nature’s Resource. C, Quanterra. Peaks identified are as follows: 1, chlorogenic acid; 2, quercetin glycosides; 3, quercetin; 4, I3,I8-biapigenin; 5, hypericin; 6, hyperforin analogs; 7, hyperforin; and 8, adhyperforin.
Fig. 3. CYP inhibition profiles of St. John's wort extracts fractionated on HPLC. HPLC fractions were tested for inhibition of CYP activities as described in Experimental Procedures: CYP3A4 testosterone 6β-hydroxylase, CYP2D6 bufuralol 1'-hydroxylase, CYP2C19 S-mephenytoin 4'-hydroxylase, CYP2C9 diclofenac 4'-hydroxylase, and CYP1A2 phenacetin O-deethylase. Substrate concentrations used were as follows: phenacetin (50 μM), diclofenac (10 μM), S-mephenytoin (50 μM), bufuralol (10 μM), and testosterone (50 μM).
Hyperforin and hypericin demonstrated 50% inhibition at concentrations below 10 μM for CYP2D6, CYP3A4, and CYP2C9 activities, whereas quercetin only inhibited CYP1A2 with a potency of less than 10 μM. Chlorogenic acid did not show inhibition for any of the five CYP enzymes examined.

Inhibition constants were measured and mode of inhibition was determined for those compounds exhibiting IC₅₀ values of less than 10 μM. A summary of the data is given in Table 2, and examples of Lineweaver-Burke plots demonstrating the data graphically for interpretation of inhibition mode are given in Fig. 5. Most of the inhibitors demonstrated competitive inhibition with the exception of the inhibition of CYP2D6 by hyperforin and the inhibition of CYP1A2 by quercetin, which both showed noncompetitive inhibition. Again, the most potent inhibition observed was for the effect of I3,II8-biapigenin on CYP3A4 (Kᵢ = 0.038 μM).

### Discussion

The inhibition of CYP enzymes can result in clinical drug interactions whereby the systemic exposure to one drug that is cleared primarily via CYP-mediated biotransformation is elevated when coadministered with a second drug that inhibits this activity. During the past decade, it has become increasingly facile to be able to conduct in vitro experiments with human CYP enzymes or human tissue preparations to measure the inhibition of CYP activities. Such data can be used to predict whether the potential exists for a drug interaction in vivo. Although this is commonly done for drugs, addressing the possibility for drug interactions in vitro for a mixture of compounds, such as herbal preparations or foodstuffs, poses unique challenges. For example, the flavonoid compound naringenin was first believed to be the component in grapefruit juice responsible for the inhibition of CYP3A (Bailey et al., 1998). However, subsequent investigation showed that 6',7'-dihydroxybergamottin, another grapefruit juice constituent, likely is responsible for the inhibition of CYP3A4 by grapefruit juice (Edwards et al., 1999). This example is illustrative of the complexities that can be encountered in attempting to identify CYP inhibitors in a complex mixture.

To address whether any CYP inhibitors are present in St. John’s wort preparations, an approach was taken whereby methanolic extracts of commercially available preparations were fractionated by HPLC, and each fraction was tested for the potential to inhibit human CYP enzymes. This approach...

### Table 1

Inhibition of human CYP enzymes by St. John’s wort constituents

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>CYP1A2 O-Deethylase</th>
<th>CYP2C9 4-Hydroxylase</th>
<th>CYP2C19 S-Mephenytoin 4-Hydroxylase</th>
<th>CYP2D6 Bufuralol 1′-Hydroxylase</th>
<th>CYP3A4 Testosterone 6β-Hydroxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperforin</td>
<td>&gt;100</td>
<td>4.4</td>
<td>31</td>
<td>1.6</td>
<td>2.3</td>
</tr>
<tr>
<td>I3,II8-Biapigenin</td>
<td>3.7</td>
<td>4.0</td>
<td>28</td>
<td>5.7</td>
<td>0.082</td>
</tr>
<tr>
<td>Quercetin</td>
<td>7.5</td>
<td>47</td>
<td>&gt;100</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N.D.</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Hypericin</td>
<td>&gt;100</td>
<td>3.4</td>
<td>37</td>
<td>8.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>

*At concentrations ≥30 μM, chlorogenic acid caused an HPLC-MS interference in the 4-hydroxymephenytoin assay. Hence, the IC₅₀ could not be determined for this compound on CYP2C19 activity (N.D.).
allowed for the identification of individual constituents that demonstrated inhibitory activity, which could then be further examined by testing the pure substances as inhibitors. Using this approach, compounds identified for further testing included quercetin, hypericin, and chlorogenic acid, which are commercially available compounds, as well as I3,II8-biapigenin and hyperforin, which required isolation from the commercially available herbal preparation. Some other fractions showed inhibitory properties, but these were not further pursued at this time. Also, it appeared that the quercetin glycosides, which comprise the major UV peaks eluting at ~20 min, do not appreciably inhibit any of the CYP enzymes examined.

Examination of a crude mixture for a comparison of inhibitory potency toward five CYP activities (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) yielded the result that CYP2D6 was most sensitive. Thus, it could be suggested that substrates of this enzyme would be most likely to be subject to drug interactions with St. John’s wort. However, many other factors are necessary to consider when making this assessment, including the comparative disposition of the individual constituents responsible for inhibition as well as the locations of the affected CYP (intestine, liver, etc.). It is not known whether hepatic or intestinal intracellular concentrations of St. John’s wort constituents achieve values proximate to the in vitro IC50 values. Without this information, it is difficult to quantitatively predict from these in vitro data whether St. John’s wort, at recommended doses, would be more or less prone to cause drug interactions via inhibition of metabolism than conventional antidepressant therapies (e.g., serotonin-selective reuptake inhibitors). Furthermore, if any St. John’s wort constituent inhibits CYP activity in an irreversible manner (i.e., as a suicide substrate), more profound drug interactions are likely.

On closer examination, it was found that several of the individual St. John’s wort constituents examined were capable of inhibiting CYP activities. The biflavone I3,II8-biapigenin demonstrated the greatest potency toward CYP3A4 and CYP2C9, whereas the most potent inhibition of CYP2D6 was exhibited by hyperforin. The effects of flavonoid compounds on CYP activity are precedented (Lee et al., 1998; Zhai et al., 1998). However, the potent inhibition of CYP2D6 by hyperforin was unexpected in consideration of published pharmacophore models of inhibition of this enzyme that place importance on the presence of a basic nitrogen in the structure of potent inhibitors (Strobl et al., 1993; deGroot et al., 1999).

### Table 2

<table>
<thead>
<tr>
<th>St. John’s Wort Constituent</th>
<th>CYP Enzyme</th>
<th>$K_i$</th>
<th>Mode of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperforin</td>
<td>CYP2C9</td>
<td>1.8 ± 0.9</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>1.5 ± 0.9</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td></td>
<td>CYP3A4</td>
<td>0.49 ± 0.24</td>
<td>Competitive</td>
</tr>
<tr>
<td>I3,II8-Biapigenin</td>
<td>CYP1A2</td>
<td>0.95 ± 0.22</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP2C9</td>
<td>0.32 ± 0.14</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>2.3 ± 1.8</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP3A4</td>
<td>0.038 ± 0.006</td>
<td>Competitive</td>
</tr>
<tr>
<td>Hypericin</td>
<td>CYP2C9</td>
<td>1.4 ± 1.1</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>2.6 ± 0.9</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CYP3A4</td>
<td>4.2 ± 2.2</td>
<td>Competitive</td>
</tr>
<tr>
<td>Quercetin</td>
<td>CYP1A2</td>
<td>3.3 ± 0.6</td>
<td>Noncompetitive</td>
</tr>
</tbody>
</table>

*Values are mean ± S.D.

Fig. 5. Lineweaver-Burke plots of inhibition of CYP activities by selected St. John’s wort constituents. A, inhibition of CYP3A4-catalyzed testosterone 6β-hydroxylase by I3,II8-biapigenin (0–0.3 μM). B, inhibition of CYP2C9-catalyzed diclofenac 4’-hydroxylase by I3,II8-biapigenin (0–10 μM). C, inhibition of CYP2D6-catalyzed bufuralol 1’-hydroxylase by hyperforin (0–3 μM). D, inhibition of CYP3A4-catalyzed testosterone 6β-hydroxylase by hyperforin (0–3 μM). Each point represents the average of duplicate determinations.
Furthermore, additional CYP2D6 inhibitory activity was observed in HPLC fractions eluting between 13 and 15 min (Fig. 3), but the constituent compounds responsible for this observation are not known at this time. Thus, it is likely that other St. John’s wort constituents, in addition to the five compounds examined in this report, can inhibit CYP2D6.

It remains to be determined whether the coadministration of St. John’s wort and other medications could result in clinically relevant drug interactions via inhibition of CYP activities. A recent report has demonstrated an interaction between St. John’s wort and digoxin (Johne et al., 1999). In this report, it was shown that multiple daily oral doses of 900 mg/day St. John’s wort extract resulted in a reduction in oral digoxin systemic exposure. Because this interaction appeared to require multiple dosing of St. John’s wort, and because digoxin exposure after oral administration is highly dependent on P-glycoprotein-catalyzed efflux, the authors hypothesized that St. John’s wort administration may have resulted in an induction in P-glycoprotein expression. For inhibition of CYP enzymes, it would be difficult to determine which of the constituents shown to be CYP inhibitors could be responsible for a drug interaction when coadministering St. John’s wort preparations. The potential for in vivo inhibition lies not only with the inhibitory potency ($K_i$) but also with the overall dispositional properties of the inhibitor (i.e., extent of absorption from the gastrointestinal tract, extent of plasma protein binding, uptake into the liver, rate of clearance, etc.). Also, in the case of a complex mixture of compounds, the relative abundance of each compound in the preparation would also have an impact as to the identity or identities of the constituent most responsible for a drug-drug interaction. That is, the most potent inhibitor may be present in the preparation at a much lower quantity than a less potent inhibitor. Thus, although I3, I18-biapigenin is the most potent inhibitor of CYP3A4 tested, it is present in St. John’s wort at levels of 0.1 to 0.5% (Nahrstedt and Butterweck, 1997), whereas hyperforin, which was approximately 13-fold less potent, can be present at levels of up to 5% (Nahrstedt and Butterweck, 1997). Therefore, these in vitro inhibition data should be used to guide the design of relevant clinical experiments for the selection of drugs that should be examined for a potential pharmacokinetic interaction with St. John’s wort. A recent report demonstrated no statistically significant effect on either alprazolam or dextromethorphan disposition in humans when administered after 4 days of St. John’s wort treatment (900 mg/day) (Markowitz et al., 2000). These findings would appear to contradict the in vitro CYP inhibition data. However, further investigation is warranted, as the effect on alprazolam and dextromethorphan was examined only after multiple dosing and only a single source of St. John’s wort preparation was used in the study. Also, as these investigators have pointed out, alprazolam is a low hepatic extraction drug, so effects on CYP3A4 metabolism may be more difficult to discern for this compound compared with a high hepatic or intestinal extraction compound.

In conclusion, several constituents of St. John’s wort, an herbal preparation used in the treatment of depression, have been identified that possess high potency in the inhibition of CYP enzymes involved in the metabolism of drugs. These data merit further investigation of the potential for St. John’s wort to cause drug interactions of clinical relevance. Until further clinical drug interaction experiments are conducted, the coadministration of drugs, especially those primarily cleared via CYP3A4, CYP2C9, and CYP2D6-catalyzed metabolism, with St. John’s wort preparations should be done with caution.

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References

Send reprint requests to: Dr. R. Scott Ohbach, Drug Metabolism Department, Candidate Synthesis, Enhancement, and Evaluation, Central Research Division, Pfizer, Inc., Groton, CT 06340. E-mail: ohbach@pfizer.com

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