INTERACTIONS OF OLOMOUCINE II WITH HUMAN LIVER
MICROSOMAL CYTOCHROMES P450

Michal Siller, Pavel Anzenbacher, Eva Anzenbacherova, Karel Dolezal, Igor Popa and Miroslav Strnad

Department of Pharmacology, Faculty of Medicine and Dentistry, Palacky University, Czech Republic (M.S., P.A.), Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Czech Republic (E.A.), Laboratory of Growth Regulators, Palacky University & Institute of Experimental Botany ASCR, Olomouc, Czech Republic (K.D., I.P., M.S.)
Running title: Olomoucine II metabolism

Correspondence author: Michal Šiller, MSc.

Palacký University at Olomouc, Faculty of Medicine and Dentistry
Department of Pharmacology
Hněvotínská 3, 775 15 Olomouc, Czech Republic
Telephone: +420 58 563 2568
e-mail: Michal.Siller@seznam.cz

Number of text pages: 21
Number of Tables: 0
Number of Figures: 7
Number of References: 25

Word counts:
- Abstract 152
- Introduction 289
- Discussion 549

Abbreviations
CYP, cytochrome P450; \( K_M \), Michaelis-Menten constant; \( V_{max} \), maximum reaction velocity; HPLC, high liquid performance chromatography; LC/MS, liquid chromatography coupled with mass spectrometry
ABSTRACT

Olomoucine II is a cyclin-dependent kinase inhibitor (CDK) and a potential antineoplastic agent since it can arrest animal cell cycles. The presented study examines its interactions with human liver microsomal cytochrome P450 (CYP) enzymes. Spectroscopic and HPLC (high performance liquid chromatography) methods were used to estimate the degree of olomoucine II-mediated inhibition of enzymatic activities of nine drug-metabolising CYPs in vitro. In addition, mass spectrometry coupled with HPLC was used to identify an olomoucine II metabolite (2,5-dihydroxyroscovitine) formed in the reaction mixtures, and CYP3A4 was found to be responsible for the hydroxylation of the N⁶-benzyl ring at position 5 leading to this compound. Olomoucine II significantly inhibited the enzymatic activities of CYP1A2, CYP2C9 and (to a lesser degree) CYP3A4. The results indicate that use of olomoucine II as a drug could affect the activities of CYP3A4, CYP1A2, and CYP2C9 in vivo. Hence, the clinical relevance of these interactions should be carefully evaluated.
INTRODUCTION

Microsomal cytochrome P450 (CYP) enzymes in the liver contribute to the first steps of the metabolism of diverse xenobiotics, including various drugs. However, some drugs may have undesirable effects, such as the inhibition or induction of these enzymes. Hence, interactions between CYPs and drugs can have profound effects, including (reportedly) drug toxicity (e.g. terfenadine, mibefradil) (Wilkinson, 1996; Welker et al., 1998) and, in several cases, sub-therapeutic concentrations of drugs in target tissues (Bachmann et al., 2004). The principal human liver CYPs involved in drug biotransformation are CYP3A4, CYP2D6, CYP2C9, CYP2E1 and CYP1A2, although CYP2C19, CYP2A6, and CYP2B6 may also contribute significantly to the metabolism of some drugs or other xenobiotics (Anzenbacher and Anzenbacherová, 2001; Guengerich, 2005). The potential effects of drugs on specific CYP enzyme activities are generally examined using *in vitro* bacterial systems, each containing single forms of CYP, and/or liver microsomal fractions (Phillips and Shephard, 1996).

Olomoucine II is a C2,N9-substituted 6-(2-hydroxybenzylamino)purine (orthotopolin) inhibitor of cyclin-dependent kinases (CDKs). Such compounds have attracted considerable attention since they can inhibit CDK9, thereby blocking the cell division cycle of animal cells. Thus, there is a possibility that they could be used as antineoplastic drugs (Kryštof et al., 2002), although little is currently known about the potential interactions between olomoucine II and human liver microsomal CYPs. Hence, in the study presented here the potential for the CDK inhibitor olomoucine II to influence the activities of the principal drug-metabolising CYPs of human liver microsomes (CYP3A4, CYP2C9, CYP2D6, CYP2E1, CYP1A2, CYP2A6, CYP2B6 and CYP2C19) was
thoroughly evaluated. In addition, the potential ability of human liver microsomal CYPs to catalyse the formation of olomoucine II metabolites was studied, and a metabolite generated in \textit{in vitro} reaction mixtures (2,5-dihydroxyroscovitine) was identified by tandem mass spectrometry.

\textbf{MATERIALS AND METHODS}

\textbf{Materials}

\textit{Chemicals}

Olomoucine II (2-\{6-[(2-hydroxybenzyl)amino]-9-isopropyl-9H-purine-2-yl\}amino)butan-1-ol; CAS 500735-47-7) was obtained from Sigma Aldrich (Prague, Czech Republic), and two potential metabolites, 2,4-dihydroxy derivate of roscovitine, (2-\{6-[(2,4-dihydroxybenzyl)amino]-9-isopropyl-9H-purine-2-yl\}amino)butan-1-ol and 2,5-dihydroxy derivate of roscovitine, (2-\{6-[(2,5-dihydroxybenzyl)amino]-9-isopropyl-9H-purine-2-yl\}amino)butan-1-ol were synthesised as detailed in the following section (for structures see Figure 1). Diclofenac, 4’-hydroxydiclofenac, bufuralol, 1’-hydroxybufuralol and 6β-hydroxytestosterone were supplied by Ultrafine Chemicals (Salford, UK). 7-Ethoxy-4-(trifluoromethyl)coumarin was purchased from Fluka (Buchs, Switzerland). (S)-(+)\-N-3-benzynirvanol was supplied by BD Gentest (Woburn, MA, USA). All other chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

\textit{Preparation of 2,4-dihydroxyroscovitine and 2,5-dihydroxyroscovitine derivatives}
The precursors (2,6-dichloro-9-isopropylpurine, 2,4-dihydroxybenzylamine hydroiodide and 2,5-dihydroxybenzylamine hydrochloride) required for synthesising the possible olomoucine II metabolites were prepared as previously described (Otyepka et al., 2000; Doležal et al., 2006; Doležal et al., 2007). The appropriate dihydroxybenzylamine isomers (1 mmol) for the metabolites were then added individually, together with triethylamine (6 mmol), to a suspension of 2,6-dichloro-9-isopropylpurine (1 mmol) in ethylacetate. Each reaction mixture was stirred at 78 °C for 5 hours, and then cooled to room temperature. Next, the precipitate was filtered off, washed with cold water (2x10 ml) and diethylether (3x10 ml), and then dried at 60 °C to constant weight. The resultant 2-chloro-6(dihydroxybenzyl)amino-9-isopropylpurines were further substituted with (R/S)-2-aminobutan-1-ol (160°C, 3h). The products, 2,4-dihydroxyroscovitine and 2,5-dihydroxyroscovitine, were purified by extraction in ethylacetate and crystallization from ethanol. Product identities were confirmed by: melting point determination on a Boetius stage, thin layer chromatography (TLC) using WF254 silica gel 60 plates (Merck, Darmstadt, Germany), elemental analysis (C, H, and N) using a EA1108 CHN analyser (Fisons Instruments, San Carlos, CA, USA), and NMR spectroscopy using a Bruker Avance AV 300 spectrometer.

Microsomes and recombinant enzymes

Pooled human liver microsomes (cryopreserved), prepared and sourced in accordance with the regulations of the local ethics committee, were supplied by Advancell (Barcelona, Spain; http://www.advancell.net). The final microsomal mixture reportedly consisted of microsomes obtained from five males and five females,
with a protein content of 38.4 mg.ml\(^{-1}\). Details of the mixture’s CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2E1 and CYP3A4 enzymatic activities can be accessed from the Advancell website (http://www.advancell.net, batch reference no. 102091201). 

*Escherichia coli* bacterial membrane structures (bactosomes) containing recombinant human CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2E1, CYP2D6, CYP2C19 and CYP3A4) coexpressed with human NADPH-cytochrome P450 reductase were purchased from Cypex (Dundee, UK).

**Methods**

*Determination of P450 activities*

The following activities of the selected human liver microsomal CYPs were assayed according to well-established protocols: CYP3A, testosterone 6β-hydroxylation (Guengerich et al., 1986); CYP2E1, p-nitrophenol hydroxylation (Tassaneeyakul et al., 1993); CYP2C9, diclofenac 4-hydroxylation (Crespi et al., 1998a); CYP1A2, 7-ethoxyresorufin *O*-deethylation (Chang and Waxman, 1998); CYP2D6, bufuralol 1´-hydroxylation (Crespi et al., 1998b); CYP2C19, S-mephenytoin 4´-hydroxylation (http://www.cypex.co.uk/2c19info.htm); CYP2A6, coumarin 7-hydroxylation (Waxman and Chang, 1998) and CYP2B6, 7-ethoxy-4-(trifluoromethyl)coumarin *O*-deethylation (Donato et al., 2004). The CYP1A2, CYP2A6 and CYP2B6 activities were calculated from spectral data acquired with a TECAN GENios absorbance-fluorescence-luminescence reader (Tecan Austria, Vienna, Austria), while the activities of CYP2C9, CYP2D6, CYP2C19, CYP2E1, and CYP3A4 were determined by monitoring amounts (in nmol) of metabolites formed (4´-hydroxydiclofenac for CYP2C9, 1´-
hydroxybufuralol for CYP2D6, 4’-hydroxymephytoin for CYP2C19, p-nitrocatechol for CYP2E1 and 6β-hydroxytestosterone for CYP3A4) by reverse phase chromatography HPLC using a Shimadzu (Kyoto, Japan) Class VP system equipped with a LiChroCART® 250-4 LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany) and UV or fluorescence detection, according to the cited literature.

**Inhibition of cytochrome P450 enzymes in microsomal fractions by olomoucine II**

Preliminary experiments were performed using microsomal preparations in the absence of olomoucine II to determine the Michaelis constant (K_M) and limiting velocity (V_max) of each CYP investigated, and to determine suitable substrate concentrations for the inhibition experiments. Generally, inhibition assays were then routinely performed with substrate concentrations corresponding to the respective enzymes’ K_M values and six concentrations of olomoucine II (10, 50, 100, 200, 400 µM; obtained by diluting a 25 mM stock solution of olomoucine II in 100% dimethylsulfoxide as appropriate) plus olomoucine II-free controls. In addition, since some organic solvents can inhibit CYP activities, even in relatively low concentrations (Chauret et al., 1998; Busby et al., 1999) the organic solvent concentrations in the final reaction mixtures were generally less than 1% (v/v) and organic solvent controls without the tested compounds were included in all cases, under otherwise identical experimental conditions. Experimental conditions in inhibition experiments were the same as for determination of individual P450 activities, preincubation of reaction mixtures with olomoucine II as potential CYP inhibitor for 30 min at 37°C was kept in all samples. The amount of human liver microsomes (expressed as amount of P450 in pmol and concentration of human liver microsomal protein in mg/ml in the reaction vessel) in the reaction mixture was (with
DMD #25502

dilutions in accordance with protocols for determination of CYP activities) following: 35 pmol P450 and 0.500 mg protein/ml for CYP1A2, 34 pmol P450 and 0.500 mg protein/ml for CYP2A6, 34 pmol P450 and 0.400 mg protein/ml for CYP2B6, 35 pmol P450 and 0.250 mg protein/ml for CYP2C9, 50 pmol P450 and 0.375 mg protein/ml for CYP2C19, 67.3 pmol P450 and 0.500 mg protein/ml for CYP2D6, 160 pmol P450 and 0.250 mg protein/ml for CYP2E1 and 100 pmol P450 with 0.297 mg protein/ml for CYP3A4.

The inhibitory effect of olomoucine II on each of the examined CYP activities was then evaluated by plotting its concentration against the activities, expressed as means (in nmol product/min/nmol P450) obtained from two parallel assays at each substrate concentration for which there was a between-duplicate difference lower than 15%. In each case where significant inhibition of a CYP was detected the inhibition constant ($K_i$) was determined by constructing Dixon plots, using the scientific graphing software Sigma Plot 8.0.2 (SPSS, Chicago, IL, USA) and data obtained from assays with three substrate concentrations (corresponding to $1/2K_m$, $K_m$ and $2K_m$). The degree of CYP inhibition was then evaluated using Microsoft Excel.

Metabolite formation and its inhibition by carbon monoxide

The effects of carbon monoxide on rates of formation of the olomoucine II metabolite (2,5-dihydroxyros covitine) identified in analyses described below catalysed by the CYPs in the microsomal preparations were also assessed, as follows. Portions of the preparations containing 250 pmol of CYPs were incubated in 1 ml of a 50 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system (0.5 mM NADP, 3.7 mM isocitric acid, and 0.5 unit/ml isocitric acid dehydrogenase) and 5 mM
MgCl₂. Carbon monoxide (Linde Technoplyn, Prague, CZ) was applied by gentle bubbling through the reaction mixture for 1 min after a 10 min pre-incubation period at 37°C. Diluted olomoucine II solutions were then added to the reaction mixtures to a final concentration of 100 μM, and the samples were incubated in a shaking water bath for 30 min at 37°C in sealed air-tight test tubes. Reactions were stopped by adding 20 μl of 70% HClO₄ (to precipitate proteins), the samples were vigorously stirred and centrifuged (5000 rpm for 6 min), and then 200 μl of each supernatant was transferred into an autosampler vial. The amounts of the metabolite formed were then determined by HPLC using a Shimadzu (Kyoto, Japan) Class VP system, equipped with a LiChroCART® 250-4 LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany), and 29% ACN (v/v) in 7.35 mM potassium phosphate buffer (pH 2.2) as the mobile phase.

**Inhibition of microsomal olomoucine II metabolism by specific inhibitors of individual CYP enzymes**

In another series of assays we examined the effects on olomoucine II metabolite formation of the following specific inhibitors of CYP1A2, CYP2C9, CYP3A4, CYP2E1, CYP2D6, CYP2A6, CYP2B6 and CYP2C19 activity: furafylline (22.5 μM), sulfaphenazole (3 μM), ketoconazole (2 μM), diethyldithiocarbamate (75 μM), quinidine (0.3 μM), 8-methoxypsoralen (1 μM), 7-pentoxyresorufin (2 μM) and S-benzylnirvanol (1 μM), respectively (Baldwin et al., 1995; Newton et al., 1995; Dierks et al., 2001; Goldstein et al., 1994, Suzuki et al., 2002). In these experiments each of the inhibitors was pre-incubated with the CYP-containing reaction mixture (see section Metabolite formation and its inhibition by carbon monoxide) prior to adding olomoucine II to a final concentration of 25 μM.
Identification of CYPs involved in metabolite formation in bactosomes

Bactosomes, each containing a single recombinant CYP, were used in an attempt to identify CYPs that participate in the metabolism of olomoucine II, following protocols recommended by the supplier (Cypex). Incubations were performed in 0.1 M Tris-HCl buffer (pH 7.4) containing 5 pmol of the relevant CYP (together with P450 reductase), 5 mM MgCl₂, an NADPH-generating system (0.5 mM NADP, 3.7 mM isocitric acid and 0.5 unit/ml isocitric acid dehydrogenase) and 50 μM olomoucine II. The reactions were terminated by adding 20 μl of 70% HClO₄. Samples were then centrifuged at 5000 rpm for 6 min prior to transferring 200 μl of the resulting supernatants to autosampler vials and analysing them as described above (§ Metabolite formation and its inhibition by carbon monoxide).

Identification of the olomoucine II metabolite by mass spectrometry

To assess the possibility that interactions between olomoucine II and enzymes in the human liver microsomal preparations may result in the production of metabolites, olomoucine II was incubated with microsomal preparations (in triplicate) in 1 ml of Tris-HCl buffer (pH 7.4) containing an NADPH-generating system (0.5 mM NADP, 3.7 mM isocitric acid and 0.5 unit/ml isocitric acid dehydrogenase), 5 mM MgCl₂ and 250 pmol of microsomal CYPs. After 10 min pre-incubation at 37°C, olomoucine II was added to the reaction mixtures to a final concentration of 50 μM. The samples were then incubated for 30 min at 37°C prior to stopping the reactions through addition of 20 μl 70% HClO₄.
After centrifugation at 5000 rpm for 6 min, 200 μl of each supernatant was used to isolate putative olomoucine II metabolites by HPLC, using methods described above (§2.2.3). The fractions containing putative olomoucine II metabolites were evaporated to half their initial volumes under a nitrogen stream, combined, and injected into an ion trap LCQ Fleet (Thermo Fisher Scientific, Waltham, MA, USA) mass spectrometer equipped with an electrospray interface, operated in (+)ESI-MS/MS mode with previously optimized analytical parameters (5 kV spray voltage, 300ºC capillary temperature, sheath gas flow rate 60 arbitrary units, auxiliary gas flow rate 15 arbitrary units, and sweep gas flow rate 8 arbitrary units). A putative olomoucine II metabolite was detected and identified as 2,5-dihydroxyroscovitine. Ion fragments of the compound formed in the (+)ESI-MS/MS experiments correlated well with a molecular structure and theoretical fragmentation mechanisms obtained using Mass Frontier 5.0 software (HighChem).

RESULTS

Olomoucine II inhibition of specific CYP enzyme activities in human liver microsomes

Olomoucine II had no apparent effects on the measured CYP2A6 and CYP2E1 activities, and only weakly interacted with CYP2B6, CYP2C8, CYP2C19 and CYP2D6. These interactions are unlikely to be clinically relevant, since the enzymatic activities were not significantly influenced at physiologically relevant concentrations of the potential inhibitor (≤50 μM; Fig. 2). However, olomoucine II clearly inhibited the enzymatic activities of CYP1A2, CYP2C9, and CYP3A4 at such concentrations (Fig. 2).
DMD #25502

The inhibition of these three drug-metabolising CYPs by olomoucine II is shown in more detail in Figure 3.

In the presence of the highest concentration of olomoucine II (400 µM), the enzymatic activities of CYP1A2, CYP2C9 and CYP3A4 decreased to 24, 26 and 35% of their respective control values, as shown by the plots in Figure 3. The IC₅₀ values for these CYP were calculated and were following: IC₅₀ of 17.0 ± 1.9 µM for CYP1A2, IC₅₀ of 39.1 ± 3.4 µM for CYP2C9 and IC₅₀ of 18.0 ± 3.7 µM for CYP3A4. The degrees to which the enzymatic activities of CYP1A2 and CYP2C9 (and possibly CYP3A4) were inhibited indicated that olomoucine II and these CYPs interacted quite specifically. Hence, Dixon plots were used to reveal the mechanisms behind these enzyme inhibitions, and to determine the respective Kᵢ values.

The Dixon plot for CYP1A2 indicates that it is subject to inhibition by olomoucine II via a mixed mechanism, with a Kᵢ of 50 µM (Fig. 4). In contrast, the Dixon plot for CYP2C9 (Fig. 5), together with the Lineweaver-Burk plot (not shown), indicate that olomoucine II noncompetitively inhibits it, with an estimated Kᵢ of 75 µM. The results also indicated that olomoucine II noncompetitively inhibits CYP3A4 (data not shown).

**Metabolite identification by MS analysis**

To assess the possibility that interactions between olomoucine II and CYP enzymes resulted in the formation of metabolites human liver microsomal preparations were incubated with the compound. A putative metabolite in the resulting mixtures was then detected by HPLC with UV detection (λ = 289 nm). The presence of an olomoucine II-like substance in the samples was further verified by drying and combining the supernatants, fractionating them by HPLC, collecting and semi-drying the corresponding
UV fractions, and finally injecting them into an ion trap mass spectrometer with an electrospray interface. (+)ESI-MS-chromatograms of the potential metabolite revealed a single peak with a retention time of 6.3 min in channel m/z 387, corresponding to a quasi-molecular ion of a dihydroxyroscovitine molecule. The putative olomoucine II metabolite was unequivocally identified as 2,5-dihydroxyroscovitine by comparing its chromatographic retention and mass spectra with those obtained using synthetic standards (2,4-dihydroxyroscovitine and 2,5-dihydroxyroscovitine). The mass spectrum of authentic 2,5-dihydroxyroscovitine, obtained from (+)ESI-MS-chromatograms at m/z 387, and the fragmentation patterns of the metabolite's quasimolecular ion, are shown in Figure 6.

Influence of CO and specific CYP inhibitors on the formation of the olomoucine II metabolite

A carbon monoxide inhibition experiment corroborated the role of CYPs in the metabolism of olomoucine II, since the amount of 2,5-dihydroxyroscovitine formed was reduced by 85 % after carbon monoxide addition (results not shown). In addition, the effects of specific CYP inhibitors on formation of the metabolite were examined to identify the particular CYPs involved in olomoucine II metabolism. The only potent inhibitor of olomoucine II 5-hydroxylation appeared to be ketoconazole, a specific inhibitor of CYP3A4, which resulted in reductions in the amount of 2,5-dihydroxyroscovitine formed to ca. 1 % of control levels (results not shown).

Incubation of olomoucine II with recombinant CYP enzymes
The experiments described in the previous section indicated that CYP3A4 plays a major role in olomoucine II metabolism. Incubation of olomoucine II with \textit{E. coli} bacterial membrane fractions containing recombinant CYPs (1A2, 2A6, 2B6, 2D6, 2E1, 2C9, 2C19 and 3A4) confirmed the participation of CYP3A4 in olomoucine II 5-hydroxylation (Fig. 7).

DISCUSSION

The inhibition experiments showed that olomoucine II influenced the enzymatic activities of at least three CYPs: CYP1A2, CYP2C9 and CYP3A4 (Figures 2 and 3). Standard Dixon plots, used to examine the character of enzyme inhibition, indicated that CYP1A2 enzymatic activity was subjected to a mixed inhibition mechanism, while olomoucine II noncompetitively inhibited CYP2C9 and CYP3A4. In each case, the IC$_{50}$ values were estimated to be in the range of tens of micromolar.

The results also revealed the formation of an olomoucine II-like metabolite in the liver microsomal fraction. Analysis of this metabolite by HPLC-(+)-ESI-MS/MS showed that it possessed an extra hydroxyl group on the N$^6$-benzyl substituent of the parent compound. Comparative analysis of the potential olomoucine II metabolite with synthetic 2,5-dihydroxy- and 2,4-dihydroxy-roscovitine derivative standards confirmed the putative olomoucine II metabolite's 5-hydroxylation of the aromatic ring. Next, we were interested in identifying the particular CYP(s) that participated in the metabolite's formation. Initially, the role of CYP enzymes in the reaction was confirmed by using carbon monoxide to inhibit metabolite formation. Carbon monoxide is known to be a universal CYP inhibitor since it binds strongly to the heme iron, forming a complex that cannot mediate the catalytic reaction due to its inability to bind molecular oxygen (Cooper et al., 1977). The results demonstrated that the level of the hydroxylated metabolite was reduced after treating
the human liver microsomes with carbon dioxide, indicating that CYPs do indeed participate in the biotransformation of olomoucine II.

We then used inhibitors of specific CYPs to identify the CYPs responsible for the formation of the olomoucine II metabolite. Of these, ketoconazole, a specific CYP3A4 inhibitor, was found to inhibit the hydroxylation of olomoucine II most strongly. In addition, the CYP3A4 enzyme was able to efficiently catalyse the 5-hydroxylation of the olomoucine II aromatic ring when olomoucine II was incubated with bactosomes expressing a single CYP. In contrast, there was a lack of, or little, metabolite formation when bactosomes expressing other important drug-metabolising CYPs were used (Figure 7).

Nevertheless, the results concerning the metabolism of olomoucine II and its inhibition of CYP activities do not exclude the possibility that olomoucine could significantly interact with CYP3A4 (which was largely responsible for the metabolite's formation), or CYP1A2 and/or CYP2C9, which were also inhibited by olomoucine II in the microsomal fraction.

The results of a similar study with roscovitine (seliciclib), a 2-hydroxyroscovitine with structural similarities to olomoucine II (see Figure 1), were published during the preparation of this manuscript (McClue and Stuart, 2008). Interestingly, this compound was shown to inhibit the activity of CYP3A4 and CYP2C9, albeit with IC₅₀ values in the micromolar range whereas the results presented in this paper show IC₅₀ values in the tens of micromolar range, i.e. an order of magnitude higher. This difference could be due to the presence of a hydroxy group on the benzene ring of olomoucine II, apparently resulting in its higher polarity. However, a higher IC₅₀ value could be advantageous for olomoucine II, since plasma levels of these compounds are likely to be in the lower (micromolar) concentration ranges (McClue and Stuart, 2008). On the other hand, the results presented
here, and those published by McClue and Stuart (2008), indicate that the potential for drug interactions involving these prospective antineoplastic compounds cannot be excluded, and should therefore be thoroughly tested.
REFERENCES


Footnotes

Acknowledgments

We are grateful for support from the MSM6198959216 and 301/08/1649 projects, and from an internal grant (ref. 91110221) provided by Palacky University.

Reprint requests to: Michal Šiller, MSc.

Palacky University at Olomouc, Faculty of Medicine and Dentistry
Department of Pharmacology
Hvědotínská 3, 775 15 Olomouc, Czech Republic
Telephone: +420 58 563 2568
E-mail: Michal.Siller@seznam.cz
Legends to Figures

Figure 1

Structures of olomoucine II (1a) and two possible metabolites: 2,4-dihydroxyroscovitine (1b) and 2,5- dihydroxyroscovitine (1c).

Figure 2

Inhibition of selected CYP activities in assays with 0, 10, 50, 100, 150, 200 and 400 μM of olomoucine II (white and black indicate the lowest and highest concentrations, respectively, and increasing intensities of gray denote increasing concentrations).

Figure 3

Olomoucine II inhibition of CYP1A2, CYP2C9 and CYP3A4 enzymatic activities.

Figure 4

Dixon plot of olomoucine II inhibition of microsomal CYP1A2 enzymatic activity.

Figure 5

Dixon plot of olomoucine II inhibition of microsomal CYP2C9 enzymatic activity.

Figure 6

Mass spectra of the olomoucine II metabolite (2,5-dihydroxyroscovitine) quasimolecular ion (a) and its fragmentation ions (b & c).
Figure 7

Percentage contributions of each of the examined CYPs to formation of 2,5-dihydroxyrosocvitine from olomoucine II.
Figure 1

(a) 

(b) 

(c)
Figure 2

![Graph showing CYP enzyme activity for different isoforms.](chart.png)
Figure 3
Figure 4
Figure 5

![Graph showing the relationship between 1/(activity CYP1)/(nmol product/min/nmolP450)) and olomoucine II concentration in µM.](image-url)
Figure 6

(a) 

(b) 

(c)
Figure 7

![Graph showing the percent of metabolite formed for different groups. The graph has a bar for each group with 3A4 having the highest percentage at 80%.](image-url)