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Inhibition of human drug metabolizing cytochrome P450 enzymes by plant isoquinoline alkaloids

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ABSTRACT

The human cytochrome P450 (CYP) enzymes play a major role in the metabolism of endobiotics and numerous xenobiotics including drugs. Therefore it is the standard procedure to test new drug candidates for interactions with CYP enzymes during the preclinical development phase. The purpose of this study was to determine *in vitro* CYP inhibition potencies of a set of isoquinoline alkaloids to gain insight into interactions of novel chemical structures with CYP enzymes. These alkaloids ($n = 36$) consist of compounds isolated from the *Papaveraceae* family ($n = 20$), synthetic analogs ($n = 15$), and one commercial compound. Their inhibitory activity was determined towards all principal human drug metabolizing CYP enzymes: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4. All alkaloids were assayed *in vitro* in a 96-well plate format using pro-fluorescent probe substrates and recombinant human CYP enzymes. Many of these alkaloids inhibited the CYP3A4 form, with 30/36 alkaloids inhibiting CYP3A4 with at least moderate potency ($IC_{50} < 10\ \mu M$) and 15/36 inhibiting CYP3A4 potently ($IC_{50} < 1\ \mu M$). Among them corydine, parfumine and 8-methyl-2,3,10,11-tetraethoxyberbine were potent and selective inhibitors for CYP3A4. CYP2D6 was inhibited with at least moderate potency by 26/34 alkaloids. CYP2C19 was inhibited by 15/36 alkaloids at least moderate potently, whereas CYP1A2, CYP2B6, CYP2C8, and CYP2C9 were inhibited to a lesser degree. CYP2A6 was not significantly inhibited by any of the alkaloids. The results provide initial structure–activity information about the interaction of isoquinoline alkaloids with major human xenobiotic-metabolizing CYP enzymes, and illustrate potential novel structures as CYP form-selective inhibitors.

Introduction

Plant alkaloids, one of the largest groups of natural products, represent a highly diverse group of chemical entities. Plants are estimated to produce approximately 12,000 different alkaloids with a wide range of pharmacological properties. Alkaloids can be classified according to their basic heterocyclic ring system. Benzylisoquinoline alkaloids are a group of nitrogen-containing plant secondary metabolites of which approximately 2500 members have been identified. Many of these compounds possess potent pharmacological effects. For example, the well known plant alkaloids include the narcotic analgesics, morphine and codeine, apomorphine (a derivative of morphine) used in Parkinson's disease, the muscle relaxant papaverine, and the antimicrobial agents sanguinarine and berberine. Also several potent anti-cancer drugs have been developed from plant compounds (Stevigny et al. 2005; Ziegler and Facchini 2008; Ziegler et al. 2009). In addition, several Stephania and Corydalis species are used in Traditional Chinese Medicine (TCM) because of their alkaloid content (Mo et al. 2007).

The cytochrome P450 (CYP) enzymes constitute a superfamily of heme-containing mono-oxygenases that catalyse the oxidative metabolism of a wide variety of xenobiotics, including drugs, plant-derived or fungal-derived secondary metabolites consumed with food, and a large number of environmental pollutants, industrial compounds, herbicides, and pesticides. The human CYP forms that metabolize xenobiotics belong to the families CYP1, CYP2 and CYP3. Individual CYP enzymes in these families have broad and overlapping substrate specificities, and are responsible for the metabolism of approximately 70–80% of all currently used drugs (Nebert and Russell 2002; Guengerich et al. 2005; Ingelman-Sundberg 2005). CYP enzymes also play a key role in oxidative reactions in plant secondary metabolism (Ziegler and Facchini 2008), and thus plants have remarkably high numbers of *CYP* genes (Nielsen and Møller 2005).

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Table 1
Experimental conditions in human recombinant CYP enzyme inhibition assays.

CYP	Substrate (μM)	Fluorescent metabolite	Incubation time (min)	Enzyme (pmol)	Excitation/emission (nm)
1A2	7-Ethoxyresorufin (1)	Resorufin	20	0.5	570/615
2A6	Coumarin (10)	7-Hydroxycoumarin	10	0.3	355/460
2B6	EFC (2.5)	HFC	30	0.75	405/535
2C8	DBF (0.5)	Fluorescein	30	1.5	485/535
2C9	MFC (75)	HFC	45	1.5	405/535
2C19	DBF (0.5)	Fluorescein	30	1.5	485/535
2D6	MAMC (20)	HAMC	60	2.0	405/460
3A4	MFC (50)	HFC	30	1.0	405/535

Abbreviations: EFC, 7-ethoxy-4-(trifluoromethyl)coumarin; DBF, dibenzylfluorescein; MFC, 7-methoxy-4-(trifluoromethyl)coumarin; BFC, 7-benzyloxy-4-(trifluoromethyl)coumarin; MAMC, 7-methoxy-4-(aminomethyl)coumarin; HFC, 7-hydroxy-4-(trifluoromethyl)coumarin; HAMC, 7-hydroxy-4-(aminomethyl)coumarin.

To a large extent metabolism determines the pharmacokinetic behaviour of a drug, i.e., the intensity and the duration of action. Modulation of CYP activity via inhibition or induction by drugs and other xenobiotics often is the source of drug interactions. Drug interactions can evoke severe adverse effects, they have resulted in early termination of drug development, refusal to obtain approval, severe prescribing restrictions, and even withdrawal of drugs from the market. The most common mechanism of drug interactions is inhibition of these CYP enzymes (Kalgutkar et al. 2007; Pelkonen et al. 2008). On the other hand, specific CYP inhibitors that do not interact with other targets and have a clear pharmacodynamic profile are potential co-therapeutics, especially in the field of antivirals. The so-called pharmacokinetic boosters inhibit the CYPs metabolizing antiviral drugs, e.g. the HIV-1 protease inhibitor lopinavir, is used to raise the effective concentration of the antiviral drug in the body. The advantages of this approach range from possible reductions in drug load to improvements of patient compliance by achieving longer dosing intervals (Dickinson et al. 2010).

In early drug development, experiments are routinely carried out to determine which CYP enzymes catalyse the metabolism of lead compounds. In addition, the potential of lead compounds to inhibit CYPs can be evaluated with *in vitro* methods. Often, but not always, a compound that inhibits a specific CYP form is also a substrate for that same form. These experiments employ various sources of CYP enzymes, e.g. human liver or cDNA-expressed human enzymes, and probe substrates and inhibitors (Bjornsson et al. 2003; van de Waterbeemd and Gifford 2003; Pelkonen and Raunio 2005).

There is still plenty of scope to enlarge the known chemical space of CYP inhibitors and substrates. The purpose of this study was (1) to screen a series of novel plant-derived isoquinoline alkaloids for their abilities to interact with the most important human liver xenobiotic-metabolizing CYP enzymes, and (2) to search for novel CYP form-selective inhibitory compounds. The inhibition potency of eight protopine, six aporphine, one spirobenzylisoquinoline and one phthalideisoquinoline, and 20 protoberberine alkaloids was determined against recombinant CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 enzymes.

Materials and methods

Chemicals and reagents

The origin of the tested alkaloids is the following (numbering according to Table 2). Alkaloid **1** was isolated by W. H. Perkin in 1918 from *Bocconia cordata* Willd. Alkaloid **2** was isolated by J. Slavik in 1966 from *Hunnemannia fumariifolia* Sweet. Alkaloid **3** was isolated by W. H. Perkin in 1926 from *Papaver somniferum* L. Alkaloid **4** was isolated by J. Slavik from *Chelidonium majus* L. Alkaloid **27** was isolated by J. Slavik from *Meconopsis cambrica* (L.) Vig. Alkaloids **5**, **6**, **8**–**14** and **19**–**26** were from a historical collection gathered by Johannes Gadamer and co-workers at the beginning of the 20th century. Alkaloid **8** thereof is the semisynthetic derivative of **9**. Alkaloids **5**, **6**, **9**–**12**, **14** and **19**–**26** were isolated from *Corydalis cava* L. and alkaloid **13** from *Fumaria vaillantii Loisel*. Alkaloids **15**–**18** are semisynthetic and alkaloids **28**–**36** synthetic (Meyer et al. unpublished). Alkaloid **7** was purchased from Sigma Aldrich (St. Louis, MO, USA). The purity of the alkaloids was higher than 95% as determined by gas chromatography–mass spectrometry and combustion analysis. All structures were confirmed by ^{1}H NMR and mass spectrometry.

cDNA-expressed human wild-type CYPs (Supersomes™) were purchased from BD Biosciences Discovery Labware (Bedford, MA, USA). Substrates and metabolite standards were purchased from BD Biosciences Discovery Labware (Bedford, MA) and Sigma Chemical Company (St. Louis, MO). These were 7-ethoxyresorufin and resorufin for CYP1A2; coumarin and 7-hydroxycoumarin for CYP2A6; 7-ethoxy-4-(trifluoromethyl)coumarin (EFC) and 7-hydroxy-4-(trifluoromethyl)coumarin (HFC) for CYP2B6; dibenzylfluorescein (DBF) and fluorescein for CYP2C8 and CYP2C19; 7-methoxy-4-(trifluoromethyl)coumarin (MFC) and HFC for CYP2C9 and CYP3A4; and 7-methoxy-4-(aminomethyl)coumarin (MAMC) and 7-hydroxy-4-(aminomethyl)coumarin (HAMC) for CYP2D6. All other chemicals used were from Sigma–Aldrich (St. Louis, MO, USA) and were of the highest purity available.

CYP inhibition assays

Incubations were conducted in a 150 μl volume in 96-well microtiter plates based on the general principles originally published by Crespi et al. (1997), using cDNA-expressed recombinant CYP enzymes. Each test compound was screened using four concentrations (mainly 1:10 ratio) ranging from 0.01 to 1000 μM in a duplicate layout. The incubation mixtures contained 100 mM Tris/HCl buffer (pH 7.4), 0.75–2.0 pmol of CYP enzyme, the probe substrate at the concentration corresponding to its measured apparent K_m, and the nicotine adenine dinucleotide phosphate hydrogen (NADPH)-regenerating system (consisting of 1.13 mM NADP, 12.5 mM isocitric acid, 56.33 mM KCl, 187.5 mM Tris/HCl, pH 7.4, 12.5 mM $MgCl_2$, 0.0125 mM $MnCl_2$, 0.075 U ml^{-1} isocitrate dehydrogenase), except 50 mM Tris/HCl buffer (pH 7.4), 0.3 mM NADPH, and 5 mM $MgCl_2$ in a 100 μl incubation volume in the case of CYP2A6. Due to their high lipophilicity the test compounds were dissolved in acetonitrile (ACN) or dimethyl sulfoxide (DMSO) and then further diluted with water. Consequently, the final solvent concentrations in the incubations did not exceed 2%. Only the hydrochloride salts were directly soluble in water. Controls were treated similarly but without the presence of inhibitors. The reactions were initiated by adding 50 μl of the NADPH-regenerating system, except for 25 μl of 1.2 mM NADPH in the case of CYP2A6, after a 10-min preincubation at 37 °C. After incubation (10–60 min), the reactions were terminated by addition of the stop solution (Table 1). In addition, in the case of CYP2A6, immediately before the measurement, 140 μl of 1.6 M glycine–NaOH buffer (pH 10.4) was