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Design and synthesis of selective, high-affinity inhibitors of human cytochrome P450 2J2

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Abstract

The active site topology, substrate specificity, and biological roles of the human cytochrome P450 CYP2J2, which is mainly expressed in the cardiovascular system, are poorly known even though recent data suggest that it could be a novel biomarker and potential target for therapy of human cancer. This paper reports a first series of high-affinity, selective CYP2J2 inhibitors that are related to terfenadine, with K_i values as low as 160 nM, that should be useful tools to determine the biological roles of CYP2J2.

Keywords

Cardiovascular system; Terfenadine; Ebastine; Hydroxylation; Suicide substrates; Monooxygenases

In the human genome, 57 genes have been found to code for cytochromes P450 (CYPs) that are involved in the oxidative metabolism of endogenous compounds and xenobiotics.¹ The main CYPs implicated in drug metabolism, such as CYP3A4, CYP2C9 or CYP2D6, and those responsible for the biosynthesis of steroid hormones have been extensively studied,¹ and several X-ray structures of human CYPs have been recently published.² Much less is known about recently discovered human CYPs such as CYP2J2.^{1,3} This cytochrome seems to be primarily expressed in heart³; it has also been found in kidney, liver, lung,⁴ and the gastrointestinal tract.⁵ Its biological role is presently unclear, even though it has been found to catalyze the oxidation of a few drugs such as ebastine in the intestine.^{6,7} Moreover, recombinant CYP2J2 catalyzes the epoxidation of arachidonic acid to four *cis*-epoxyeicosatrienoic acids (EETs), with regio- and stereo-selectivities that match those of the EETs isolated from heart tissue.³ Some EET-derived metabolites play important roles in regulation of vascular tone⁸ and in a host of processes related to cancer cell behavior, angiogenesis, and tumor pathogenesis.⁹ Very recent data suggest that CYP2J2 promotes the neoplastic phenotype of carcinoma cells and may represent a novel biomarker and potential target for therapy of human cancers.¹⁰ However, little data are presently available on the active site topology and substrate specificity of CYP2J2.¹ This communication reports the design and synthesis of a first series of high-affinity inhibitors of human CYP2J2. These inhibitors should be useful tools to determine the biological roles of this cytochrome.

Hydroxylation of the drug ebastine by recombinant CYP2J2⁷ expressed in baculovirus-infected Sf9 insect cells³ was used as an assay to find CYP2J2 inhibitors. During a first

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screening for such inhibitors, compound **1**, derived from the drug terfenadine by oxidation of its benzylic alcohol function, was found to inhibit CYP2J2¹¹ with an IC₅₀ value of 0.7 ± 0.1 μ M. This value was much lower than the IC₅₀ found for terfenadine itself (8.1 ± 0.4 μ M) (Table 1), that was previously described as an inhibitor of CYP2J2.¹² Then, compound **1**, called terfenadone in the following, was used as a starting point for the design of high-affinity inhibitors of CYP2J2. Ebastine,⁷ terfenadine,¹³ and compound **1** are all hydroxylated by CYP2J2 at a site that is weakly reactive from a chemical standpoint, a CH₃ of the *t*-butyl group (Scheme 1). This regioselectivity in favor of the least reactive part of these substrates implies their strict positioning in the CYP2J2 active site in order to maintain the *t*-butyl group in close proximity of the heme iron for transfer of an oxygen atom from O₂. Therefore, a series of compounds derived from **1** by replacement of its *t*-butyl group with various R groups of different size and polarity was synthesized (Scheme 2). This included compounds bearing functions previously known to lead to suicide inactivation of cytochromes P450 after in situ oxidation.¹⁴ This is the case for the terminal double bond of compound **5**, since terminal alkenes act as mechanism-based inhibitors of cytochromes P450 after N-alkylation of the heme by an intermediate derived from P450-catalyzed oxidation of the substrate double bond.¹⁴ The choice of the CHF₂ and benzodioxole functions of compounds **12** and **13** was also made on the basis of literature data on suicide substrates of cytochrome P450.¹⁴ In situ hydroxylation of the C–H bond of CHF₂ groups leads to an electrophilic intermediate able to acylate the P450 protein, whereas inactivation of cytochrome P450 by benzodioxole derivatives is due to the formation of an iron–carbene bond after oxidation of the benzodioxole CH₂ group.¹⁴ The structure of compounds **5**, **12**, and **13** has been chosen in order that CYP2J2-catalyzed oxidations occur at the site leading to inactivating metabolites, assuming that hydroxylation of **5**, **12**, and **13** should occur on the homobenzylic position as the hydroxylation of compound **1** and terfenadine.

The general synthetic route used for the preparation of terfenadone derivatives (Scheme 2) with R = (CH₂)_{*n*}CH₃ (with *n* = 0–3), (CH₂)_{*n*}OH and (CH₂)_{*n*}OCOCH₃ (with *n* = 2 or 3), Br, CH₂CHF₂, OCH₂O–, and OCH₃ involved an acylation of the benzenic starting compound with 4-chlorobutanoic acid chloride in the presence of FeCl₃, AlCl₃ or SnCl₄ and reaction of the corresponding product with α,α -diphenyl-4-piperidinomethanol. In the case of **7** and **8**, the starting compounds were the acetates of 2-phenylethanol and 3-phenylpropanol, respectively. Deprotection of the alcohol function was done as the last step of the synthesis. Compound **5** was obtained from reaction of **11** with allyltributyltin, in the presence of tetrakis (triphenylphosphine)palladium(0).

The structures of all the terfenadone derivatives listed in Table 1 were completely established from their ¹H NMR and mass spectra; ¹H NMR spectroscopy analysis in the presence of an internal standard showed that all these compounds were more than 95% pure.

Table 1 compares the IC₅₀ values measured for the inhibition of ebastine hydroxylation catalyzed by recombinant CYP2J2. It shows that most of the synthesized terfenadone derivatives are good CYP2J2 inhibitors with IC₅₀ values at the low μ M range. Compounds **4** and **5** had the highest affinity with an IC₅₀ value of 0.4 μ M. In fact, increasing the chain length from R = methyl to R = propyl results in a gradual decrease of the IC₅₀ value, whereas a further increase of the chain length (R = butyl) leads to a loss of affinity. Introduction of a polar function in the R substituent generally leads to a decrease in the affinity of the inhibitors. Compounds such as **10** and **13** in which oxygen atoms have been introduced at benzylic positions exhibit IC₅₀ values one order of magnitude greater than those observed for compounds bearing an alkyl chain (R = Et or Pr, **3** or **4** for instance). Compounds such as **7**, **8**, **9**, and **12** in which an OH, OAc or F substituent have been introduced in the R-chain farther from the phenyl ring exhibit intermediate IC₅₀ values, around 2 μ M. Thus, the best inhibitors (in terms of IC₅₀ value) were compounds **4** and **5**. Preliminary experiments showed that compound **4** is a competitive

inhibitor of CYP2J2-catalyzed hydroxylation of ebastine with a K_i of 160 ± 30 nM and also a competitive substrate of CYP2J2. Compound **5** seems to be a time-dependent inhibitor, as expected for a compound bearing a terminal double bond.¹⁴ Interestingly, compounds **12** and **13** involving a CHF₂ and benzodioxole function, respectively, also led to time-dependent inhibitory effects that suggest a mechanism-based type of inhibition.

Table 2 compares the inhibitory effects of the best inhibitors found for CYP2J2, compounds **4** and **5**, toward the other main human cytochromes P450 that are present in the cardiovascular system, CYP2C8, CYP2C9, CYP2B6, and CYP3A4.²¹ The data clearly show that compounds **4** and **5** are selective inhibitors of CYP2J2, as they are nearly inactive toward CYP2C8 and their IC₅₀ values for CYP2C9, CYP2B6, and CYP3A4 are 1–3 orders of magnitude higher than those observed for CYP2J2.

In conclusion, the aforementioned results have led to the first selective, high-affinity inhibitors of CYP2J2, compounds **4** and **5**, that exhibit IC₅₀ values around 400 nM. Compound **4** is a competitive inhibitor characterized by a K_i of 160 nM, a value that is remarkably low for a human cytochrome P450 inhibitor.¹⁴ Additional studies are underway to determine the type of inhibition exhibited by compounds **5**, **12**, and **13**, and to use these new inhibitors as tools to study the biological roles of CYP2J2 in vitro and in vivo. In light of the recent findings that CYP2J2 promotes the neoplastic phenotype of carcinoma cells, these compounds are also currently being investigated as potential anti-cancer therapeutics.

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15. Ebastine (0.5 μ M) and inhibitor were incubated at 37 °C, for either 2 or 4 min, in the presence of microsomes from baculovirus-infected insect cells (1 nM CYP2J2) in 0.1 M phosphate buffer, pH 7.4, containing 0.1 mM EDTA and a NADPH-generating system, for a total volume of 200 μ L. The reaction was stopped within a few seconds by treatment with 100 μ L of a cold $\text{CH}_3\text{CN}/\text{CH}_3\text{COOH}$ (10:1) mixture and vortexing of the incubate. Proteins were precipitated by centrifugation for 5 min at 10,000 rpm, and the supernatant aliquots were analyzed by HPLC, after injection on a Hypersil C18 column (Thermo, Les Ulis, France). The mobile phase was delivered at a rate of 1 mL/min with a gradient from A (0.1 M acetate, pH 4.6) to B ($\text{CH}_3\text{CN}/\text{CH}_3\text{OH}/\text{H}_2\text{O}$, 7:2:1) (30% up to 100% B in 15 min). The column effluent was monitored at 254 nm.
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Scheme 1.

Formula and site of CYP2J2-dependent hydroxylation of ebastine and terfenadine.

Scheme 2.

General synthetic route used for the preparation of terfenadone derivatives (for the nature of R, see Table 1).

Table 1

Inhibitory effects of terfenadone derivatives toward recombinant CYP2J2

Inhibitor	-R	IC ₅₀ ^a (μM)
Terfenadine		8.1 ± 0.4
Terfenadone, 1	-C(CH ₃) ₃	0.7 ± 0.1
2	-CH ₃	0.7 ± 0.1
3	-CH ₂ -CH ₃	0.6 ± 0.1
4	-CH ₂ -CH ₂ -CH ₃	0.4 ± 0.1
5	-CH ₂ -CH=CH ₂	0.4 ± 0.2
6	-(CH ₂) ₃ CH ₃	0.7 ± 0.2
7	-CH ₂ -CH ₂ -OH	1.3 ± 0.8
8	-CH ₂ -CH ₂ -CH ₂ -OH	1.9 ± 0.3
9	-CH ₂ -CH ₂ -CH ₂ -OAc	2.5 ± 0.5
10	-O-CH ₃	7.6 ± 0.6
11	-Br	4.2 ± 0.6
12	-CH ₂ -CHF ₃	2.2 ± 0.9
13		6.7 ± 2.3

^aCompound concentration leading to 50% inhibition of the hydroxylation of ebastine by CYP2J2 expressed in baculovirus-infected Sf9 insect cells.³ Conditions as described in Ref. 15. Under these conditions, the K_M of ebastine hydroxylation was 0.5 μM. Values are means ± SD from three to four experiments.

^bAr = -C₆H₅-R.

Table 2

Comparison of the inhibitory effects of terfenadone derivatives toward vascular cytochromes P450

Compound	IC ₅₀ (μM) ^a				
	CYP2J2	CYP2B6	CYP2C8	CYP2C9	CYP3A4
4	0.4 ± 0.1	28 ± 1	>100	26 ± 3	7.9 ± 0.5
5	0.4 ± 0.2	21 ± 1	>100	21 ± 1	5.5 ± 1.0

^aCompound concentration leading to 50% inhibition of CYP2B6-catalyzed 7-benzoyloxyresorufin O-deethylation,¹⁶ CYP2C8-catalyzed 6- α -hydroxylation of taxol,¹⁷ CYP2C9-dependent 4'-hydroxylation of diclofenac,¹⁸ and CYP3A4-catalyzed 6- β -hydroxylation of testosterone,¹⁹ respectively. Microsomes from W(R)fur yeast strain expressing each of these cytochromes P450²⁰ were incubated with the corresponding substrate at a concentration equal to the K_m value of the studied reactions (0.5, 5, 10, and 20 μ M, respectively) and a NADPH-generating system. Incubations and analyses of the reaction mixture were performed as described previously.^{16–19} Values are means $\pm$ SD from three to four experiments.