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Imidazopyridines as selective CYP3A4 inhibitors

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ABSTRACT

Cytochrome P450s are the major family of enzymes responsible for the oxidative metabolism of pharmaceuticals and xenobiotics. CYP3A4 and CYP3A5 have been shown to have overlapping substrate and inhibitor profiles and their inhibition has been demonstrated to be involved in numerous pharmacokinetic drug–drug interactions. Here we report the first highly selective CYP3A4 inhibitor optimized from an initial lead with ≈30-fold selectivity over CYP3A5 to yield a series of compounds with greater than 1000-fold selectivity.

Prescription pharmaceuticals are the most common treatment option for physicians. A recent study found 10% of individuals age 75–85 were taking drug combinations that were likely to have drug–drug interactions and that 37% of these patients were concurrently taking 5 or more prescription medications.[1] Because of their central role in drug metabolism, inhibition of cytochrome P450s (CYPs) can significantly alter drug levels in patients taking medications that are metabolized by the inhibited enzyme, occasionally inducing toxicities similar to drug-overdose. During the drug-discovery process, in vitro assays are utilized to determine the risk of a compound to alter the concentrations of concomitantly administered drugs or having its own concentration altered. While great progress has been made in this area, there is a major deficiency with respect to CYP3A4 and CYP3A5. These two enzymes have overlapping substrate and inhibitor profiles and are arguably the most important cytochrome P450 involved in the metabolism of pharmaceuticals based on their overall abundance and the percentage of drugs they metabolize. Suitable chemical tools are not available to differentiate these two enzymes (only pan-substrates and pan-inhibitors are available) and activity is typically expressed as a summation of the activities of the two enzymes.

Associated toxicities due to CYP3A4 related drug–drug interactions have led to clinical failure and withdrawal from market of previously approved pharmaceuticals. Mibefradil (Posicor®), a potent inhibitor of CYP3A4, was withdrawn from the market after numerous reports of serious drug–drug interactions. Terfenadine (Seldane®) and Cisapride (Propulsid®) were withdrawn after patients taking the recommended dose along with CYP3A4 inhibitors such as ketoconazole developed heart arrhythmia leading to heart attack or death.[2]

To date, no substrates have been identified that are metabolized exclusively by only one of the enzymes. However, using recombinantly expressed enzymes, several substrates have been shown to have higher catalytic efficiency for either CYP3A4 or CYP3A5.[3] Similarly, no highly selective inhibitors have been identified, but many do show two to fivefold preference for one of the two enzymes.[4] Many of these have been associated with time-dependent inactivation where CYP3A4 appears to be more susceptible to time-dependent inactivation than does CYP3A5.[5]

This has been shown to have clinical importance because of the polymorphic expression of CYP3A5. CYP3A5*1 leads to the expression of active, full length CYP3A5, but the CYP3A5*3 (22893A→G) allele in intron 3 leads to a frame shift resulting in the majority of the CYP3A5 mRNA coding for inactive protein and loss of CYP3A5 expression.[6] The frequency of having at least one functional CYP3A5*1 allele has varied with different reports but averages approximately 10–30% in the Caucasian population and raises to 50–70% in African Americans.[6] This has been shown to have clinical implications with the immunosuppressant tacrolimus. Plasma concentrations of tacrolimus are vitally important and are regularly monitored with dose adjustment to achieve the desired drug level. Patients that expressed active CYP3A5 (*1/*1 or *1/*3) required approximately twice the dose as patients that did not express CYP3A5 to maintain appropriate tacrolimus concentration.[7] Additionally, clinical drug interactions between the pan-CYP3A4/

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5 substrate midazolam and the mildly CYP3A4 selective (fivefold vs 3A5) inhibitor fluconazole were larger for individuals with the *3/*3 CYP3A5 genotype.

Access to highly selective inhibitors of CYP3A4 will allow researchers to isolate CYP3A5 activity in biologically relevant samples to better assess the potential for deleterious pharmacokinetic drug–drug interactions and make higher quality predictions of pharmacokinetic properties including the effect of CYP3A5*1, or *3 genotype.

We began a program to identify potent and selective CYP3A4 inhibitors mining data from 6000 in-house compounds that had been previously tested in a single point CYP3A4/5 inhibition assay using pooled (150-donor) human liver microsomes. The CYP3A5 content was assumed to be approximately 15% of the total CYP3A4/5 so compounds that previously demonstrated between 80% and 90% inhibition at 10 μM were selected for further evaluation using recombinant CYP3A4 and CYP3A5. After retesting at 10 μM, full IC_{50}s were determined for compounds that appeared to have a level of CYP3A4 versus CYP3A5 selectivity.[8] This led us to identify two imidazopyridines as promising leads (Fig. 1). These compounds had modest selectivity with **1** having a CYP3A4 IC_{50} = 280 nM with 11-fold selectivity over CYP3A5 and **2** having a CYP3A4 IC_{50} = 270 nM with 29-fold selectivity.

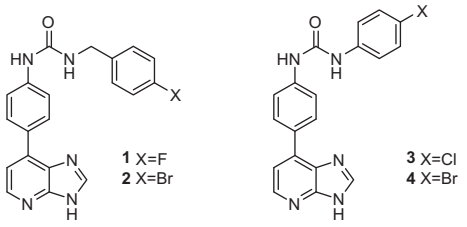
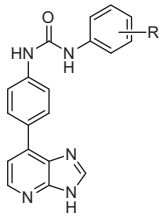


Figure 1. Structure of imidazopyridines.

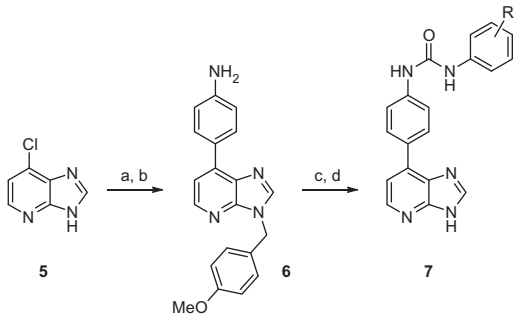
Table 1
P450 inhibition of substituted phenyl urea analogs



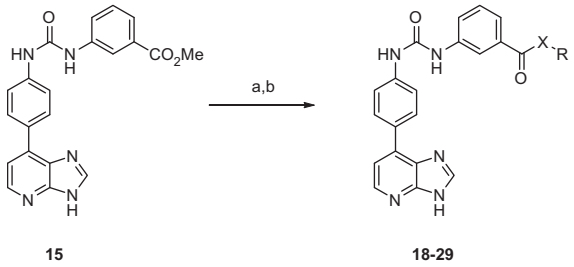
Compd	R	Enzyme IC_{50}[a,b] (μM)		Fold-selective
		CYP3A4	CYP3A5	
3	4-Cl	0.31	34%	>190
4	4-Br	0.21	60	290
8	2-Cl	0.60	6.1	10
9	3-F	0.71	11.4	16
10	3-Cl	0.48	52	110
11	3-Br	0.092	11.4	124
12	H	1.2	44	37
13	3-Me	1.5	34%	>40
14	3-NO_2	0.061	31.5	518
15	3-CO_2Me	0.082	8%	>700
16	4-OMe	0.20	39%	>300
17	4-Ph	0.026	27%[c]	>80[c]

[a] Values are mean of two or more experiments using midazolam hydroxylation as a measure or CYP3A4/5 activity.
[b] Values with a % designation are percent inhibition at the highest concentration tested (60 μM). For selectivity purposes 60 μM is used and a greater than designation is given.
[c] Solubility prevented evaluation of CYP3A5 inhibition above 2 μM.

An initial set of analogs meant to explore what portions of the molecule were amenable to modification resulted in the discovery that the corresponding 4-substituted phenyl analogs **3** and **4** showed similar IC_{50}s for CYP3A4 inhibition but had reduced

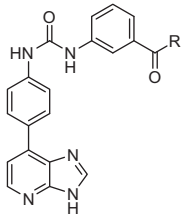


Scheme 1. (a) PMB-Cl, K_2CO_3, DMAc; (b) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, $Pd(PPh_3)_4$, K_2CO_3, THF, 80 °C; (c) TFA; (d) RCNO, toluene.



Scheme 2. (a) LiOH, methanol, THF, rt, 2 h, 90%; (b) (i) N(H)nR, HATU, *N*-methylmorpholine, DMF, rt, 14 h, (*n* = 1–2).

Table 2
Phenyl amide and ester derivatives



18-29

Compd	R	Enzyme IC_{50}[a,b] (μM)		Fold-selective
		CYP3A4	CYP3A5	
15	OMe	0.082	8%	>700
18	OEt	0.044	27%	>1300
19	OH	39%	33%	None
20	NHMe	0.098	45%	>600
21	NHEt	0.055	20.3	368
22	NHiPr	0.090	41%	>600
23	NMe_2	0.11	60	544
24	NEt_2	0.047	8.6	183
25	$NiPr_2$	0.060	1.6	26
26	NHphenyl	0.053	18%	>1100
27	NHcyclopentyl	0.070	6.9	98
28	Piperidine	0.079	5.5	70
29	Morpholine	0.29	60	208

[a] Values are means of two or more experiments.
[b] Values with a % designation are percent inhibition at the highest concentration tested (60 μM). For selectivity purposes 60 μM is used and a greater than designation is given.

CYP3A5 inhibition leading to increased selectivity (Table 1). Given the improved CYP3A4/CYP3A5 selectivity in the phenyl urea series, structure–activity relationship studies (SAR) were initiated on this scaffold. Compounds were easily synthesized as described in Scheme 1. The chloro imidazopyridine (**5**) was first protected with a *p*-methoxybenzyl group. This gave a separable mixture of protected regioisomers (only one shown for clarity), but both protected imidazopyridines underwent smooth Suzuki coupling to yield intermediate **6**. TFA deprotection and exposure to a variety of phenyl isocyanates afforded final products as solids.

Table 1 summarizes the effects of substitution of the terminal phenyl ring upon inhibition of CYP3A4 and CYP3A5 activity using recombinant enzymes.[6] Substitution at all positions of the phenyl ring led to potent CYP3A4 inhibitors with varying degrees of selectivity against CYP3A5. 2- and 3-substituted analogs (**8**–**11**) showed reduced selectivity whereas larger 3-substituted analogs (**14**,**15**) showed improvement in selectivity for CYP3A4. 4-Substituted analogs (**16**,**17**) also showed good levels of selectivity, however for **17**, precipitation at higher concentrations was observed. Given the good potency and nice selectivity profile of ester **15**, this compound was further analoged as shown in Scheme 2.

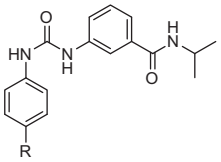
Esters **15** and **18** exhibited strong selectivity and potency for CYP3A4 versus CYP3A5 but when tested in incubations containing 1 mg/ml human liver microsomes or human hepatic S9 the compounds had short half lives of approximately 8 min with or without the addition of NADPH indicating probable hydrolysis by hepatic esterases making **15** and **18** poor in vitro probes. Hence, amides were investigated as more stable surrogates. The parent ester was easily saponified, and a variety of amines were coupled to form the amides (Table 2).

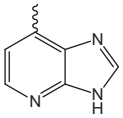
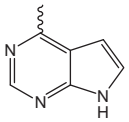
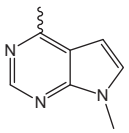
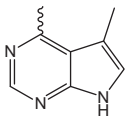
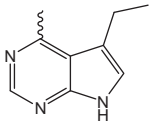
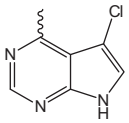
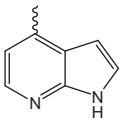
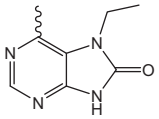
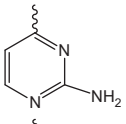
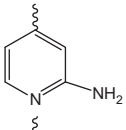
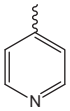
Several of the analogs were highly selective for the inhibition of CYP3A4 versus CYP3A5. A wide range of substituents were acceptable; however, larger substituents particularly with the disubstituted amides exhibited increased CYP3A5 inhibition, thus decreasing the fold-selectivity.

In order to evaluate the necessity of the imidazopyridine group, a variety of compounds were generated using compound **22** as a template (Table 3). Significant loss of CYP3A4 inhibition was observed for all of the tested derivatives.

Of the compounds detailed, compound **17** was the most potent CYP3A4 inhibitor identified; however, compound **17** suffered from

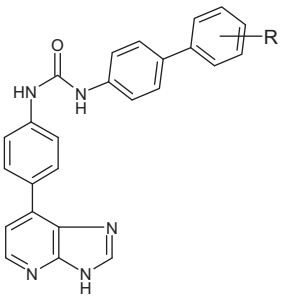
Table 3
Imidazopyridine derivatives of compound **22**



Compd	R	Enzyme IC_{50}[a,b] (μM)		Fold-selective
		CYP3A4	CYP3A5	
22		0.090	41%	>600
33		0.56	34	61
34		2.9	25%	>21
36		22	60	3
37		4.6	8.1	2
38		14	44	3
39		36%	21%	None
40		26	47%	>2
41		28%	7%	None
42		33%	8%	None
43		0.13	0.98	8

[a] Values are means of two or more experiments.
[b] Values with a % designation are percent inhibition at the highest concentration tested (60 μM). For selectivity purposes 60 μM is used and a greater than designation is given.

Table 4
Analogs of compound **17** to retain potency and increase solubility



Compd	R	Enzyme IC_{50}[a] (μM)		Fold-selective
		CYP3A4	CYP3A5	
44	4-Cyano	0.011	33	3000
45	4-Hydroxy	0.030	21	700
46	4-Amino	0.20[b]	26%	>300
47	3-Methoxy	0.048	6.8	143
48	Naphthyl[c]	1.7	13	8
49	Pyridin-3-yl[c]	0.22	30%	>270
50	Pyridin-4-yl[c]	0.36	29%	>160

[a] Values are means of two or more experiments.
[b] CYP3A4 inhibition curve was not sigmoidal.
[c] Phenyl was replaced with naphthylene and pyridine.

poor solubility. A series of substituted 4-phenyl analogs were prepared using similar strategies as in Scheme 1 to try to retain potency but increase solubility (Table 4).

The reported series of imidazopyridines are highly potent inhibitors of CYP3A4 and show the highest degree of specificity between CYP3A4 and CYP3A5 ever presented in the literature. Of particular interest are compounds **14**, **22**, 44 and **45** which had high selectivity and were not rapidly depleted in HLM incubations. Since many drugs are metabolized by CYP3A4/5 these molecules represent the first chemical tools to help understand the relative roles of CYP3A4 and CYP3A5 to enable quantitative prediction of the impact on pharmacokinetics and drug–drug interactions. Assessment of the inhibitors for in vitro metabolism studies is currently underway.

Acknowledgments

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