Prevalence of Scaffolds in Human Cytochrome P450 Inhibitors Identified Using the LOPAC^{1280} Library of Pharmacologically Active Compounds

Richard Kho, Mark R. Hansen, and Hugo O. Villar
Altoris, Inc., San Diego, California, USA

Introduction

The cytochromes P450 (CYP) are oxidative enzymes involved in multiple biochemical pathways, including drug metabolism and clearance of toxicants from the body [1]. Certain drugs or chemicals can inhibit CYP enzyme function, which alters their ability to metabolize drugs. The resulting toxic effects are referred to as drug-drug interactions and are a major concern for drug development. Therefore, CYP inhibition is being considered more thoroughly at earlier stages in drug discovery [2]. Computational methods to study and predict CYP inhibition have garnered significant interest, and methods like pharmacophore analysis and multivariate statistical modeling are widely used [3-5]. As an alternative approach, we present a chemical subgraph method to describe the scaffolds prevalent in compounds that inhibit CYP enzymes. The goal is to organize knowledge about CYP inhibition around chemical substructures, so that simple guidelines can be provided to researchers in their daily efforts to design better drug-like molecules.

Methods

There are two main components to studying the correlations between chemical substructures and CYP inhibition: 1) selecting a representative chemical library for screening in CYP inhibition assays and 2) rapidly identifying meaningful substructures in the chemical library to correlate structural data to assay results. The first component was achieved by screening the LOPAC^{1280} Library of Pharmacologically Active Compounds (Sigma-RBI) in fluorometric CYP inhibition assays (Gentest). LOPAC^{1280} was chosen because it contains a diverse collection of drugs and drug-like molecules, has accompanying Structures Data (SD) electronic files, and is delivered in convenient, assay-ready form. Assays were conducted in 40 µL final volume in 384-well black non-binding polystyrene microtiter plates (Corning #3654). CYP substrates were dissolved in acetonitrile and LOPAC compounds were dried in the 384-well assay plates from 10% DMSO stock. The dried compounds were reconstituted in 20 µL of appropriate NAD(P)(H) reagent for a given CYP isoform, followed by a 30-minute incubation of each microtiter plate at 37 °C. Enzymatic reactions were initiated by addition of appropriate enzyme/substrate reagent. Fluorescence measurements were made using a PerkinElmer VictorV spectrofluorometer (Boston, MA). LOPAC library compounds were assayed in triplicate at a final concentration of 10 µM, and 50% inhibition at this concentration was used as the cutoff for inhibition versus noninhibition.

The substructure parsing component was carried out using SARvision™ software from ChemApps. SARvision is a simple desktop application for chemistry work. It identifies chemical motifs present in large datasets and organizes available biological and biochemical data, such as the results from our CYP inhibition assays, according to the scaffolds it identifies. It facilitates R-table generation and data reporting, and the user can quickly navigate chemical information and create tables for export into applications like Microsoft Word and Excel. A demo version of the software can be downloaded at http://www.chemapps.com/products.html. The correlation between chemical scaffold and CYP inhibition is made using the odds ratio (OR) statistic for binary data, which compares the odds of one outcome (inhibition) to the odds of the other outcome (no inhibition). The OR is defined as the odds of inhibition by population ***a*** divided by the odds of inhibition by population ***b***:

$$OR = \frac{a_{pos} / a_{neg}}{b_{pos} / b_{neg}}$$

An OR of unity implies equal odds of inhibition by ***a*** occurring as by ***b***. In other words, the odds ratio for *a* given scaffold indicates how likely it is to be found in inhibitors of CYP compared to the rest of the scaffolds. Therefore, an odds ratio of less than one (OR < 1) indicates a scaffold that is less likely to be found in CYP inhibitors. To make statistically meaningful inferences, the 95% confidence interval for the OR was also calculated. If the confidence interval includes 1.0, then that scaffold is equally likely to display CYP inhibition, as compared to a random compound in the dataset. For the 15 most prevalent scaffolds, pairwise comparisons were made by calculating odds ratios for two scaffold groups at a time, allowing for direct comparison of two scaffolds to determine whether one should be preferred over another.

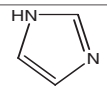
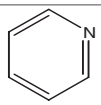
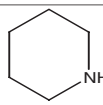
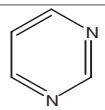
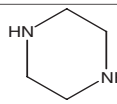
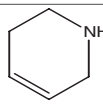
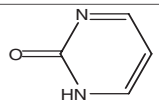
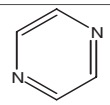
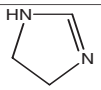
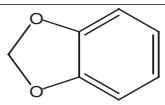
Results and Discussion

Identification of scaffolds

There were 45 chemically-distinct scaffolds in the LOPAC library, as identified by SARvision software (only scaffold groups of 3 or more members were considered). The scaffolds in LOPAC are similar to fragments or heterocycles found in drug or drug-like databases, including CMC and MDDR [6,7].

For the 15 most prevalent scaffolds in LOPAC, the following data are presented in Table 1: 1) structure and name of the scaffold, 2) number of compounds containing the scaffold and percentage of library, 3) number of compounds that

Table 1. Inhibition data and odds ratios for the 15 most prevalent scaffolds in LOPAC[1280]

 **Imidazole** N = 133 (10.4%)[a] 5, 2.18 (0.81, 5.89)[b] 9, 2.12 (1.01, 4.46)[c] 9, 2.22 (1.05, 4.70)[d] 7, 0.44 (0.20, 0.95)[e] 13, 1.78 (0.96, 3.31)[f]	 **Pyridine** N = 105 (8.20%) 8, 5.50 (2.30, 13.0) 4, 1.05 (0.37, 2.99) 7, 2.13 (0.93, 4.88) 12, 1.07 (0.57, 1.99) 13, 2.38 (1.27, 4.45)	 **Piperidine** N = 102 (7.97%) 0, 0 (n/a) 4, 1.09 (0.38, 3.09) 2, 0.53 (0.13, 2.23) 46, 8.62 (5.60, 13.3) 9, 1.55 (0.75, 3.19)	 **Pyrrole** N = 89 (6.95%) 9, 8.05 (3.48, 18.6) 8, 2.92 (1.33, 6.42) 8, 3.06 (1.39, 6.74) 21, 2.74 (1.63, 4.60) 14, 3.23 (1.74, 5.98)	 **Pyrimidine** N = 80 (6.25%) 1, 0.62 (0.08, 4.64) 1, 0.32 (0.04, 2.37) 1, 0.34 (0.05, 2.47) 2, 0.20 (0.05, 0.84) 7, 1.52 (0.68, 3.42)
 **Oxolane** N = 66 (5.16%) 0, 0 (n/a) 0, 0 (n/a) 0, 0 (n/a) 1, 0.12 (0.02, 0.89) 3, 0.73 (0.22, 2.37)	 **Piperazine** N = 61 (4.77%) 3, 2.80 (0.82, 9.59) 0, 0 (n/a) 0, 0 (n/a) 15, 2.84 (1.55, 5.21) 11, 3.73 (1.87, 7.46)	 **Pyridine*** N = 43 (3.36%) 0, 0 (n/a) 3, 2.05 (0.61, 6.88) 2, 1.36 (0.32, 5.81) 11, 2.96 (1.46, 5.99) 6, 2.62 (1.07, 6.39)	 **Oxo-pyrimidine** N = 38 (2.97%) 1, 1.37 (0.18, 10.4) 0, 0 (n/a) 1, 0.74 (0.10, 5.52) 0, 0 (n/a) 0, 0 (n/a)	 **Pyrrolidine** N = 28 (2.19%) 0, 0 (n/a) 2, 2.09 (0.48, 9.05) 2, 2.17 (0.50, 9.44) 2, 0.63 (0.15, 2.70) 4, 2.66 (0.90, 7.84)
 **Pyrazine** N = 23 (1.80%) 2, 5.09 (1.13, 22.9) 0, 0 (n/a) 0, 0 (n/a) 0, 0 (n/a) 0, 0 (n/a)	 **Imidazoline** N = 18 (1.41%) 0, 0 (n/a) 0, 0 (n/a) 0, 0 (n/a) 6, 4.25 (1.57, 11.5) 0, 0 (n/a)	 **Pyrazole** N = 16 (1.25%) 1, 3.44 (0.44, 27.1) 2, 3.91 (0.86, 17.7) 0, 0 (n/a) 1, 0.55 (0.07, 4.20) 0, 0 (n/a)	 **Benzodioxolane** N = 15 (1.17%) 0, 0 (n/a) 2, 4.21 (0.93, 19.2) 4, 10.9 (3.32, 35.4) 0, 0 (n/a) 6, 11.0 (3.82, 31.7)	 **Thiophene** N = 15 (1.17%) 2, 8.28 (1.77, 38.7) 0, 0 (n/a) 0, 0 (n/a) 1, 0.59 (0.08, 4.52) 2, 2.42 (0.54, 10.9)

a Total number of compounds in LOPAC that contain the scaffold; percent of dataset.
b Number of compounds with this scaffold that inhibit CYP 1A2; the odds ratio of the scaffold; 95% confidence interval.
c Analogous data for CYP 2C9.
d CYP 2C19
e CYP 2D6
f CYP 3A4
** Pyridine is represented multiple times due to scaffolds with different bond orders.*
Color gradients provided for visualization purposes: The cells represent the odds ratios for CYP 1A2, 2C9, 2C19, 2D6 and 3A4, respectively. GREEN: OR confidence interval < 1.0; YELLOW: confidence interval includes 1.0; RED: OR confidence interval > 1.0. ***An odds ratio of less than one (OR < 1) indicates a scaffold that is less likely to be found in CYP inhibitors.***

inhibit CYP 1A2, the odds ratio for that scaffold, and the 95% confidence interval, 4) analogous data for CYP 2C9, 5) CYP 2C19, 6) CYP 2D6, 7) CYP 3A4, and 8) color gradient representation of the odds ratio, with yellow representing a confidence interval that contains OR = 1.0, green, a confidence interval below 1.0, and red, a confidence interval above 1.0. There were a total of 289 compounds in LOPAC that inhibited at least one CYP isoform.

CYP 1A2 is the least frequently inhibited isoform in the set

Only 26 compounds inhibited 1A2, and the scaffolds most prevalent in 1A2 inhibitors were thiophene (OR = 8.28), pyrrole (8.05), pyridine (5.50), and pyrazine (5.09). Thiophene and pyrazine containing compounds are known to inhibit CYP enzymes, and have even been investigated as anti-cancer agents via inhibition of CYPs [8].

CYP 2C9 is redundant with 2C19 in inhibition screens

CYP 2C9 and 2C19 share strong sequence homology, and the former is often used in assays in lieu of the latter. This is supported by the current inhibition data and similarities in scaffolds that are relevant to both isoforms. There were 50 inhibitors of 2C9, and 48 inhibitors of 2C19 in the LOPAC library. Of the top 15 scaffolds, both isoforms are equally likely to be inhibited by pyrrole and imidazole scaffolds.

CYP 2D6 is the most frequently inhibited isoform

There were 175 inhibitors of CYP 2D6 in the LOPAC library. Piperidine had the highest OR (8.62), followed by imidazoline (4.25), pyridine (2.96), piperazine (2.84), and pyrrole (2.74). With the exception of the pyrrole, all of these scaffolds are characterized by having an electron lone pair on the ring nitrogen atom not delocalized over the ring, sug-

Table 2. **Pair-wise comparison of scaffolds for their likelihood of CYP inhibition**

	Thiophene	Benzodioxolane	Pyrazole	Imidazoline	Pyrazine	Pyrrolidine	Oxo-pyrimidine	Tetrahydropyridine	Piperazine	Oxolane	Pyrimidine	Pyrrole	Piperidine	Pyridine	Imidazole
Imidazole	≈↑↑≈≈	↑≈←↑←	≈≈↑≈↑	↑↑↑←↑	≈↑↑↑↑	↑≈≈≈≈	≈↑≈↑↑	↑≈≈←≈	≈↑↑←≈	↑↑↑≈≈	≈≈≈≈≈	≈≈≈←≈	↑≈≈←≈	≈≈≈≈≈	≈≈≈≈≈
Pyridine	≈↑↑≈≈	↑≈←↑←	≈≈↑≈↑	↑↑↑←↑	≈↑↑↑↑	↑≈≈≈≈	≈↑≈↑↑	↑≈≈←≈	≈↑↑←≈	↑↑↑↑≈	≈≈≈≈≈	≈≈≈←≈	↑≈≈←≈	≈≈≈≈≈	
Piperidine	←↑↑↑≈	≈≈←↑←	←≈↑↑↑	≈↑↑≈≈	←↑↑↑↑	≈≈≈↑≈	←↑≈↑↑	≈≈≈↑≈	←↑↑≈≈	≈↑↑↑≈	←≈≈↑≈	←≈←↑≈	≈≈≈≈≈		
Pyrrole	≈↑↑≈≈	↑≈≈↑←	≈≈↑≈↑	↑↑↑≈↑	≈↑↑↑↑	↑≈≈≈≈	≈↑≈↑↑	↑≈≈≈≈	≈↑↑≈≈	↑↑↑↑↑	↑≈≈↑≈	≈≈≈≈≈			
Pyrimidine	←↑↑≈≈	↑←←↑←	≈≈↑≈↑	↑↑↑←↑	≈↑↑↑↑	↑≈≈≈≈	≈↑≈↑↑	↑≈≈←≈	≈↑↑←≈	↑↑↑≈≈	≈≈≈≈≈				
Oxolane	←≈≈≈≈	≈←←↑←	←←≈≈↑	≈≈≈←↑	←≈≈↑↑	≈←←≈≈	←≈←↑↑	≈←←←≈	←≈≈←←	≈≈≈≈≈					
Piperazine	≈≈≈≈≈	↑←←↑≈	≈←≈≈↑	↑≈≈≈↑	≈≈≈↑↑	↑←←≈≈	≈≈←↑↑	↑←←≈≈	≈≈≈≈≈						
Tetrahydropyridine	←↑↑≈≈	≈≈←↑←	←≈↑≈↑	≈↑↑≈↑	←↑↑↑↑	≈≈≈≈≈	←↑≈↑↑	≈≈≈≈≈							
Oxo-pyrimidine	≈≈↑←←	↑←←≈←	≈←↑←≈	↑≈↑←≈	≈≈↑≈≈	↑←≈←←	≈≈≈≈≈								
Pyrrolidine	←↑↑≈≈	≈≈≈↑≈	←≈↑≈↑	≈↑↑←↑	←↑↑↑↑	≈≈≈≈≈									
Pyrazine	≈≈≈←←	↑←←≈←	≈←≈←≈	↑≈≈←≈	≈≈≈≈≈										
Imidazoline	←≈≈≈←	≈←←↑←	←←≈≈≈	≈≈≈≈≈											
Pyrazole	≈↑≈≈←	↑≈←↑←	≈≈≈≈≈												
Benzodioxolane	←↑↑←≈	≈≈≈≈≈													
Thiophene	≈≈≈≈≈														

*A graphic representation is presented for odds ratios (OR) comparing inhibition of CYP isoforms 1A2, 2C9, 2C19, 2D6, and 3A4, respectively, between scaffolds. The '≈' sign indicates no greater likelihood of CYP inhibition by one scaffold over another, a left arrow '←' indicates the scaffold on the left (row header) is preferred (less likely to inhibit CYP), and an up arrow '↑' indicates the scaffold on top (column header) is preferred. For example, if a comparison is to be made between the imidazole (row 1) and imidazoline (col. 12) scaffolds, the '↑↑↑←↑' representation would indicate that imidazoline is preferred (i.e. it is less likely to inhibit CYP) for isoforms 1A2, 2C9 and 2C19 (first three ↑ arrows). Imidazole would be preferred over imidazoline for CYP 2D6 (fourth arrow ←), and finally, imidazoline would be preferred for 3A4. *Pyridine is represented multiple times due to scaffolds with different bond orders.*

gesting a mechanistic inhibition via coordination to the heme iron moiety in the P450.

CYP 3A4 is also commonly inhibited

3A4 is the most abundant hepatic CYP isoform. 88 of the LOPAC compounds inhibited 3A4, with benzodioxolane (OR = 11.0), piperizine (3.73), pyrrole (3.23), and pyridine (2.62) most likely to inhibit this isoform.

Pair-wise comparison of scaffolds

Table 2 shows a matrix comparing the OR values of the top 15 scaffolds. This table allows direct comparison of one scaffold versus another for likelihood of CYP inhibition. Oxolane, oxo-pyrimidine, pyrazine and imidazoline are less likely to cause inhibition over other scaffolds. Many scaffolds do not have a particular advantage over another from a CYP inhibition point of view, i.e. scaffolds that have OR confidence intervals containing 1.0, represented as = in Table 2.

Summary

By obtaining reliable, in-house P450 inhibition data on a large library of drug and drug-like compounds (the LOPAC[1280] library), and correlating inhibition data to molecular scaffolds using SARvision software, it was possible to identify and rank order scaffolds found in CYP inhibitors. Such an approach provides correlations between structural features and odds of CYP inhibition. When faced with optimizing a series of compounds against CYP inhibition, these data could be applied to make appropriate substitutions using scaffolds that are less likely to cause CYP inhibition. This is, of course, just one of many factors that need to be considered, including potency, chemical tractability, and additional ADME parameters. The comparison of scaffolds, nonetheless, should provide another bit of information useful for drug design and optimization.

References

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About the Authors

Richard Kho received his Ph.D. in Toxicology from UC Riverside, and has worked in the biotechnology industry as a computational scientist. He joined Altoris, Inc., in 2005 as Research Scientist in Drug Discovery.

Mark R. Hansen received his Ph.D. in Medicinal Chemistry from the University of Texas, Austin. His postdoctoral studies at the University of Colorado were on macromolecular NMR. He has since worked in the biotechnology industry applying structure-based design and computational techniques for drug discovery. He led the development of SARvision software and currently serves as CTO of Altoris, Inc.

Hugo O. Villar received his Ph.D. in Physical Chemistry from Universidad Nacional de La Plata, Argentina. He began his career as a computational chemist at IBM, and later, in not-for-profit organizations including the Molecular Research Institute. Recently, he served in senior management positions at companies including Telik and Triad Therapeutics, where he led projects aimed to the development and application of new techniques for drug discovery. He is currently the CSO of Altoris, Inc.

Ordering Information

Cat. No.	Description	Unit
LO1280 (U.S.)	LOPAC 1280	1 kit
LO3300 (Intl.)	LOPAC 1280	1 kit
UC18	(+/–) Geosmin	5 mg 10 mg
UC70	(±)-d5-Geosmin	5 mg 10 mg
UC126	(±)-4′-Hydroxymephenytoin	5 mg 10 mg
UC148	6-Hydroxychlorzoxazone	5 mg 10 mg
UC160	Hydroxytolbutamide	5 mg 10 mg
UC168	(±)-Bufuralol	5 mg 10 mg
UC169	(±)-Hydroxybufuralol	5 mg 10 mg
UC175	(S)-(+)-Mephenytoin	5 mg 10 mg
UC205	Dextrorphan	5 mg 10 mg
UC213	(R)-(+)-Warfarin	5 mg 10 mg
UC214	(S)-(-)-Warfarin	5 mg 10 mg
UC263	7-Hydroxycoumarin glucuronide	5 mg 10 mg
UC430	1′-Hydroxymidazolam	5 mg 10 mg
UC431	4-Hydroxymidazolam	5 mg 10 mg
UC432	AAMU	5 mg 10 mg
UC455	3-Cyano-7-ethoxycoumarin	5 mg 10 mg
M9194	MDR1 SB-MDR1-*Sf*9-ATPase	500 µl
M9319	rat Mdr1b SB-ratMdr1b-*Sf*9-ATPase	500 µl
M9069	MRP2 SB-MRP2-*Sf*9-VT	500 µl
M9694	rat Mrp2 SB-ratMrp2-*Sf*9-ATPase	500 µl
M9569	MXR SB-MXR-M-VT	500 µl
M9444	MXR (Wild Type) SB-MXR-*Sf*9-VT	500 µl
B2436	BSEP SB-BSEP-*Sf*9-VT	500 µl
M9819	Control defMRP	500 µl
C3992	Control SB-M-CTRL	500 µl
M9944	Control defMXR	500 µl

***For more information or to use the LOPAC Navigator, visit our Web site:* sigma-aldrich.com/LOPAC**