Comparing StarDrop vs. MetaSite Prediction of Sites of Metabolism by CYP3A4, CYP2C9 and CYP2D6: Application of *In Silico* Tools in Drug Discovery Metabolite Identification Studies

V. Sashi Gopaul, Young Shin; Hoa Le, Cyrus Khojasteh and Cornelis Hop
Department of Drug Metabolism and Pharmacokinetics, Genentech, Inc.,1 DNA Way, South San Francisco, CA, USA

INTRODUCTION

Metabolite identification studies play an important role in determining the sites of metabolic liabilities of newly synthesized compounds (NSC) in drug discovery. However, their incompatibility to a high throughput environment is often a challenge. Therefore, the use of *in silico* tools that can predict the sites of metabolism of an NSC and ultimately assist in efforts to improve clearance (CL) is envisaged to enhance the drug design process. In general it is believed that most metabolic liabilities of NSCs are usually P450-mediated reactions. In this study we compare the utility of MetaSite and StarDrop, two predictive softwares available for this purpose. MetaSite is a predictive software for the identification of regioselectivity of metabolism by major P450 isoforms. StarDrop is a data mining software that includes an *in silico* modeling feature to predict the regioselectivity and site of metabolism by CYP3A4, CYP2D6 and CYP2C9 only. Neither software can predict non-P450 catalyzed metabolism nor the rates of metabolism.

OBJECTIVES

Our objectives were: (A) To evaluate the accuracy of MetaSite and StarDrop to predict the site of oxidation by CYP3A4, CYP2D6 and CYP2C9. We only used these enzymes since StarDrop only has these enzymes available. Altogether, 12 substrates[1] of CYP3A4, 9 substrates of CYP2C9 and CYP2D6 were analyzed by each software and the results were compared. (B) To test the accuracy of MetaSite and StarDrop predictions of in-house NSCs metabolized by both P450 and non-P450 enzymes by comparing their predictions with experimental observations. We evaluate the utility of both softwares in drug discovery metabolite identification studies.

Scoring for Objective A:

To measure the degree of prediction by each software, we assigned 3 points if the first major metabolite reported is predicted correctly, 2 points for the second choice and one point for the 3rd choice. No points were given for the 4th choice and beyond. The total points assigned for each enzyme experimentally were compared as a percentage of the total points assigned theoretically for a first choice prediction for all substrates for each enzyme. Assumption: The compounds were not part of the training sets.

Only general trends are described for Objective B.

Objective A

Table 1. Probe Comparisons for CYP3A4

	MetaSite			StarDrop		
	1st	2nd	3rd	1st	2nd	3rd
Testosterone	3			3		
Progesterone	3			3		
Ethynylestradiol	3			3		
Midazolam		2		3		
Phenacetin	3				2	
Nifedipine			1			
Verapamil		2			2	
Aflatoxin			1		2	
Erythromycin		2		3		
Etoposide	3			3		
Tamoxifen		2		3		
Amitriptyline	3			3		
Total Score =	31			30		no match

Reference:

1. David Lewis, *Curr. Med. Chem.*, 2003, **10**, 1955-1972

Table 2. Probe Comparisons for CYP2D6

	MetaSite			StarDrop		
	1st	2nd	3rd	1st	2nd	3rd
Bufuralol	3			3		
Propranolol					2	
Metoprolol	3			3		
Debrisoquine			1	3		
Imipramine				3		
MPTP	3					1
Detromethorphan	3			3		
Codeine	3			3		
Desipramine	3			3		
Total Score =	19			25		no match

Table 3. Probe Comparisons for CYP2C9

	MetaSite			StarDrop		
	1st	2nd	3rd	1st	2nd	3rd
Naproxen	3			3		
Diclofenac	3			3		
Tolbutamide		2		3		
Tielinic Acid						
Mefenamic Acid	3			3		
Flurbiprofen	3			3		
Piroxicam				3		
Lornoxicam				3		
Warfarin		2		3		
Total Score =	16			24		no match

Figure 1. Analysis of Naproxen, a CYP2C9 Substrate

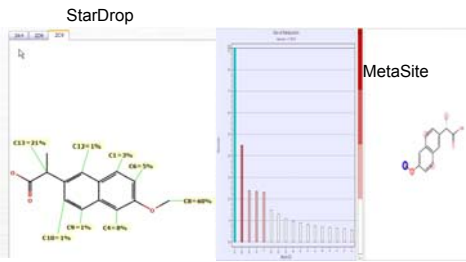


Figure 2. Analysis of Testosterone, a CYP3A4 Substrate

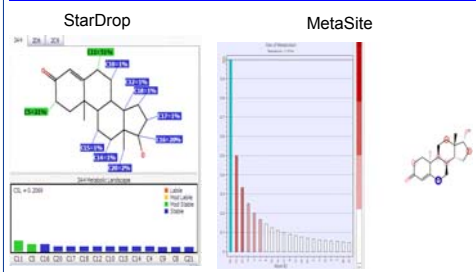
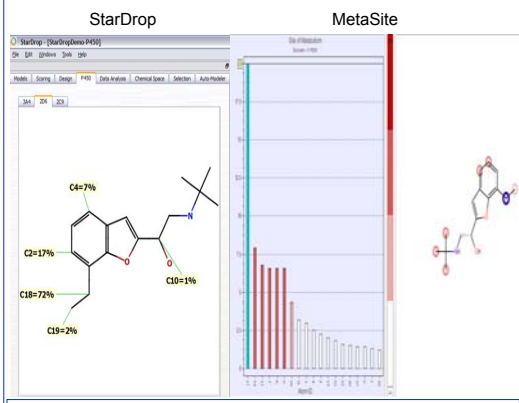


Table 4. Overall Summary of Scores

	CYP3A4	CYP2C9	CYP2D6
MetaSite	31	16	19
StarDrop	30	24	25

Figure 3. Analysis of Bufuralol, a CYP2D6 Substrate



Conclusion of Objective A: Based on the summary of scores, (Tables 1,4), MetaSite and StarDrop are very similar for predicting the correct site of metabolism for CYP3A4. StarDrop appears to be better for predicting CYP2C9 and CYP2D6 metabolism when specific substrates were compared (Tables 2, 3, & 4).

Objective B

To compare the accuracy of both software to predict the metabolic liabilities of 10 in-house NSC with actual experimental observations with a final goal of improving CL.

- The metabolism of 10 NSCs which potentially fit in the enzymatic pocket of protein kinases as shown in Figure 4 and act as their inhibitors were examined by StarDrop & MetaSite
- The results were compared to experimental observations
- General trends and observations are summarized below.

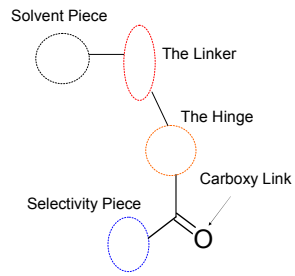


Figure 4. General Structure of 10 NSC

General Trends & Observations

- Non-P450 metabolism such as that surrounding the carbonyl moiety was evidently not predicted by either software
- Neither software could differentiate between isomeric structures. In general, both softwares could predict the observed P450 metabolic sites but not necessarily in a reliable order
- Both software predicted sites of oxidation reasonably well.
- StarDrop was better at predicting appropriate sites of N-dealkylation than MetaSite
- N-dealkylation of substituted heterocyclic rings were not always properly identified (i.e. the α-carbon being oxidized was not always the observed one)
- When oxidation was observed to occur in a particular region containing a phenyl or aryl group, StarDrop identifies the more likely site of oxidation in a ring better than MetaSite
- Based on the observation of these 10 compounds, in general StarDrop appears to have a slight edge at the identification of P450 based metabolic liabilities
- In a series of compounds with mixed P450 and non-P450 soft spots, both softwares can be helpful to localize specific site of P450 reactions identified in a general location during metabolite identifications studies and in turn assist in the modification of soft spots to improve of CL during the drug design process
- In conclusion, MetaSite & StarDrop are very useful tools that can supplement and aid the identification of major metabolites of an unknown xenobiotic

Genentech
IN BUSINESS FOR LIFE