In silico Identification of Metabolic Soft Spots: Case Study Using ACD/ADME Suite Software

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INTRODUCTION

Metabolic stability, determined in liver microsomes, is one of the primary assays used in early drug discovery. A key factor limiting compound half-life is the cytochrome P450 mediated metabolism. High clearance by these enzymes implies a higher and more frequent dosing as well as poses a risk for individual variations in exposure. Experimental identification of metabolic soft spots during lead optimization is a time and resource consuming task as it requires separation of individual metabolites and elucidation of their structure. Here we present a case study illustrating how this workflow can be facilitated by *in silico* regioselectivity prediction tools. Presented examples demonstrate the performance of the ACD/ADME Suite software in identification of their most likely metabolites, thus providing an insight on the structural modifications needed to achieve optimal metabolic stability.

REGIOSELECTIVITY PREDICTION SOFTWARE

The P450 Regioselectivity module is available as a part of ACD/ADME Suite software (www.acdlabs.com/pc_admet).

It predicts the possibility for each atom in the compound of interest to be a metabolism site in human liver microsomes. The results are reported as a calculated score of metabolism, supplemented by the Reliability Index (*RI*) – a quantitative measure of prediction quality. Atoms are color-coded according to score values to easily visualize potentially active sites. Red indicates possible site of metabolism (score >0.6); green – confident prediction of the non-metabolized atom (score <0.4); grey – inconclusive predictions (score between 0.4 and 0.6).

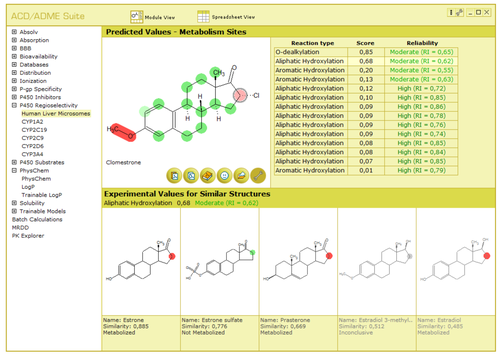


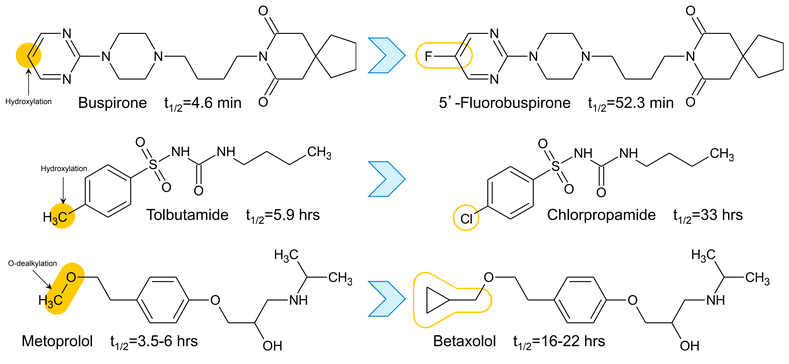
FIGURE 1. ACD/ADME Suite 5.0 Cytochrome P450 Regioselectivity module interface.

PRINCIPLES OF METABOLIC STABILITY OPTIMIZATION

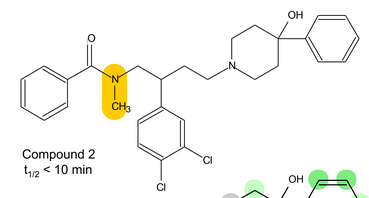
Before switching to the discussion on the potential benefits arising from the use of *in silico* metabolism site predictions, let us review the most popular strategies employed in the quest for improved metabolic stability:

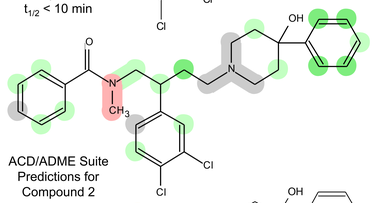
- Blocking site of metabolism
- Cyclization
- Remove labile site
- Reduce lipophilicity
- Replace unstable groups

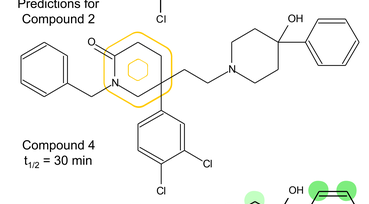
A few classical examples to demonstrate that simple changes in a compound structure may dramatically impact metabolic stability as presented below.



CASE STUDY 1: NEUROKININ-2 ANTAGONISTS


Compound 2
$t_{1/2}$ < 10 min

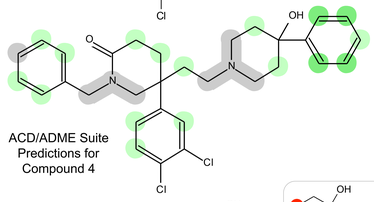

ACD/ADME Suite Predictions for Compound 2


Compound 4
$t_{1/2}$ = 30 min

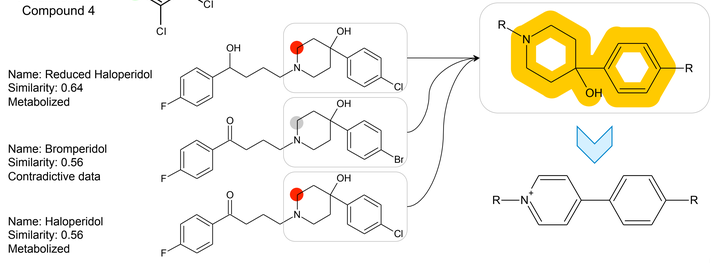
The first case study follows a very detailed metabolic stability optimization study that focuses on the design of novel Neurokinin-2 (NK-2) antagonists [1]. Compound 2 was chosen as lead compound, but it was highly unstable in human liver microsomes, having $t_{1/2}$ < 10 min. The purpose of the original study was to improve *in vitro* metabolic stability to $t_{1/2}$ > 120 min. On the other hand our aim here is to analyze the ability of the P450 Regioselectivity module of the ACD/ADME Suite to reproduce the experimental results therein.

The main reaction that was observed experimentally for the Compound 2 is N-dealkylation. As can be seen on the left, this reaction is also clearly predicted using ACD/ADME Suite.

Cyclization was chosen as a method to improve metabolic stability, resulting in Compound 4, which has a prolonged half-life of 30 min.

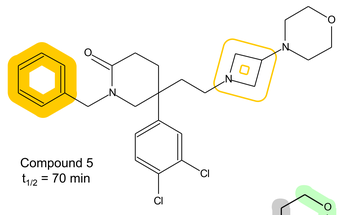

ACD/ADME Suite Predictions for Compound 4

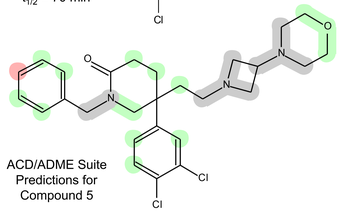
No reliable prediction was obtained for compound 4 using ACD/ADME Suite. However, a solution can be found analyzing most similar metabolism sites of the highest rank prediction in piperidine ring. The analysis of literature data for these compounds reveals the problematic moiety in Compound 4 an its typical reaction – aromatization:

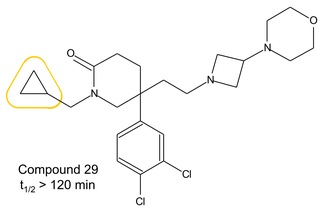


Unsurprisingly, the optimization of metabolic stability was continued by changing this ring system. Azetidine ring was chosen by the authors of experimental study after trying several analogues, because Compound 5 possessed the same potency of interaction with target but at the same time had a noticeably superior metabolic stability ($t_{1/2}$ = 70 min)

However, Compound 5 is still found to be metabolized in N-benzyl ring which is again in agreement with ACD/ADME Suite predictions. Further optimization of metabolic stability resulted in changing the benzyl substituent to an aliphatic ring.

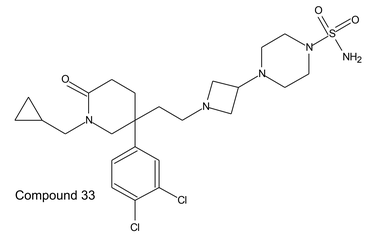

Compound 5
$t_{1/2}$ = 70 min


ACD/ADME Suite Predictions for Compound 5


Compound 29
$t_{1/2}$ > 120 min

Following several further potency optimization steps Compound 33 (UK-224,671) was progressed into clinical development.

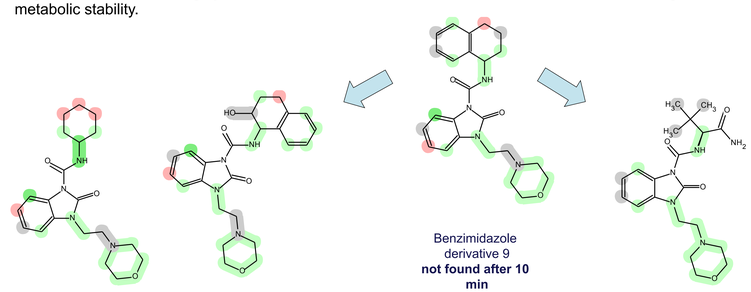
Compound 29 was chosen as the least lipophilic from a series of N-cycloalkylmethyl substituted compounds. It had the desired $t_{1/2}$ > 120 min and good potency.


Compound 33

CASE STUDY 2: RATIONAL OPTIMIZATION OF CB2 ANTAGONISTS

In the case of selective cannabinoid receptor 2 antagonists [2], Benzimidazole derivative 9 was selected as a lead compound, but it was extremely unstable in human liver microsomes. No compound was left after 10 minutes incubation. The major oxidation site was experimentally determined to be located in tetrahydro-naphthalene ring, as it is predicted by ACD/ADME Suite. Therefore, structural changes were introduced into this ring to address metabolic liability. Unfortunately, after introducing the suggested modifications metabolic stability did not improve.

Predicting cytochrome P450 metabolism sites of these compounds using ACD/ADME Suite provides insight on the possible reason of this failure. The suggested cyclic analogues contain metabolism sites in the same place (Derivatives15 and 19). After synthesizing several other analogues, compound 24 having an aliphatic fragment instead of the ring system was selected as the best of the series in terms of CB2 binding and metabolic stability.


Benzimidazole derivative 15
$t_{1/2}$ = 4 min

Benzimidazole derivative 19
$t_{1/2}$ = 2 min

Benzimidazole derivative 9
not found after 10 min

Benzimidazole derivative 24
$t_{1/2}$ =35 min

CONCLUSIONS

Using the ACD/ADME Suite Cytochrome P450 Regioselectivity predictions allows early identification of metabolic soft spots. In these two case studies we have shown how these estimations can guide the optimization of metabolic stability.

In the case of NK-2 antagonists, the predictions of metabolism sites could replace the experimental identification of metabolites. In the second example with CB2 antagonists, using ACD/ADME Suite could have saved some synthesis efforts. As one can see from these examples, metabolic stability optimization using *in silico* predictions before synthesis suggests a rational way to ensure that the structural changes eliminate the most significant metabolic sites.

REFERENCES

[1] MacKenzie et al. *J Med Chem.* **2002**, 45, 5365.
[2] Omura et al. *Bioorg Med Chem Lett.* **2008**, 18, 3310.

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