

Impact of deuteration of a pyrimidine aldehyde oxidase substrate on metabolite formation

Paige Orwig¹, Christina Chan¹, Scott Martin^{1*}, Tom Hunt², Andy Lister², William McCoull², Clare Thomson² and Nicola Colclough¹

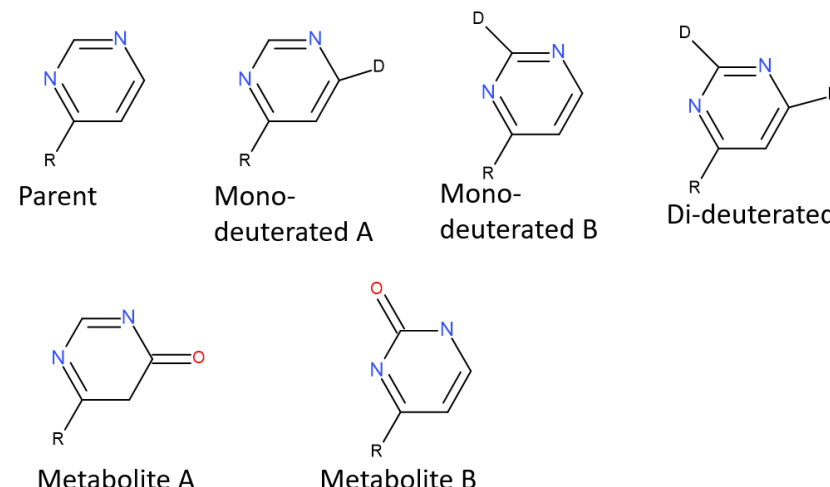
¹DMPK, Oncology R&D, AstraZeneca, Cambridge, UK, ²Advanced Chemistry Oncology R&D, AstraZeneca, Cambridge, UK

Introduction

Aldehyde oxidase (AO) is a molybdenum containing enzyme that is present predominantly in the cytosol of the liver, which catalyses the oxidations of azaheterocycles and aldehydes. The absence or minimal AO activity in preclinical species coupled with the significant underprediction of human in-vivo clearance using in-vitro systems mean AO substrates present a significant risk of sub-optimal exposure in the clinic and potential drug failure.

A range of deuterated analogues of a pyrimidine AO substrate have been synthesised to assess the potential for reduction/elimination of AO metabolism (Figure 1). Together with the parent compound these analogues were incubated in human liver cytosol (HLC) +/- AO specific inhibitor (Raloxifene) and rate and extent of AO metabolite formation assessed.

Figure 1 Pyrimidine AO substrate, deuterated analogues and AO metabolites



Methods

- Parent compound and deuterated versions (mono-deuterated A & B and di-deuterated) of the parent compound were incubated at 5, 10 and 20μM, in duplicate
- In-vitro intrinsic clearance (CL_{int}) experiments in HLC +/- Raloxifene performed using Hamilton Microlab STAR.
- Aliquots taken at 0, 10, 30, 60, 90 and 120 minutes and quenched 1:4 in ice cold acetonitrile containing internal standard
- Samples left on ice for ten minutes and then centrifuged at 3,500 rpm for 10 minutes at 4 °C
- Supernatant transferred and diluted 1:7 in ultrapure water. Samples stored at 4 °C until analysis
- Ultra-high performance liquid chromatography (UHPLC) coupled with high resolution mass spectrometry (HRMS) and ultra-violet spectroscopy (UV) was utilized for quantification of metabolite formation by UV and qualification by HRMS

Results

Figure 2 Formation of AO metabolites A and B

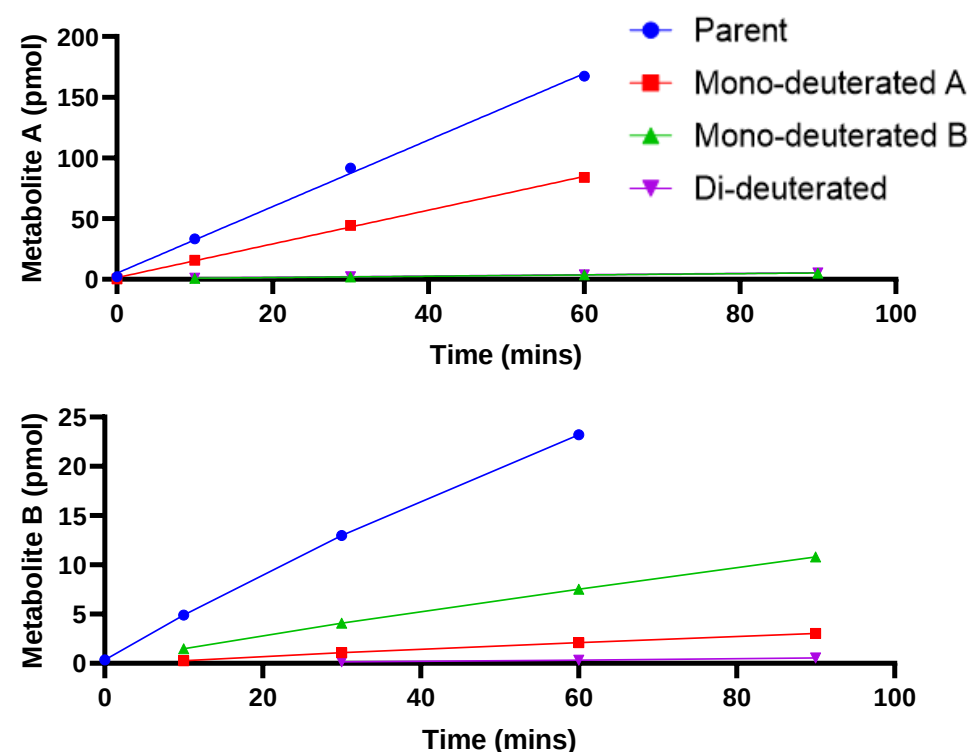


Figure 3 Parent compound and deuterated analogues percent of total metabolites

Compound	Metabolite A (% of metabolism)	Metabolite B (% of metabolism)	Total AO (% of metabolism)
Parent	24	2	26
Mono-deuterated A	12	2	14
Mono-deuterated B	26	<1	26
Di-deuterated	11	<1	11

Key findings

1. Metabolite A proved to be the dominant AO metabolite
2. Deuteration on carbon four (Mono-deuterated A) resulted in a 50% reduction in AO liability
3. Deuteration on carbon two (Mono-deuterated B) did not significantly reduce AO liability
4. Deuteration on carbons two and four resulted in a 60% reduction in AO liability

Conclusion

- AO metabolism is predominantly at the 4 position of the pyrimidine (metabolite A).
- Deuterating the parent pyrimidine only resulted in a small reduction (2-3 fold) in AO liability.
- Deuteration does not provide a significant reduction in AO liability for the pyrimidine AO substrate. It is therefore essential to identify AO substrates early in drug discovery to allow projects to design away from this metabolic route

References

- [1] Manevski, N. et al. Journal of Medicinal Chemistry. 2019;62:10955-10994
- [2] Zientek, M. et al. Drug Metabolism and Disposition. 2010; 38:1322-1327
- [3] Barr, J.T. et al. Drug Metabolism and Disposition. 2014;42:695-699

*Current address Domainex, Cambridge, UK