Computer aided drug design study of alcohol dehydrogenase C from *Mycobacterium tuberculosis*

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Introduction

Mycobacterium tuberculosis

Tuberculosis is one of the oldest, yet still active infectious, diseases caused by the Mycobacterium tuberculosis pathogen. Several antibiotics have been designed to fight the disease, but the increase of bacterial multidrug resistance has made the current treatments much less efficient. The particularity of the mycobacterium is its special cell wall structure which provides a very strong and impermeable barrier to protect the organism against its surrounding, including drugs. The final layer contains long fatty acids which can reach up to 80 carbons in total chain length, the so called mycolic acids.

ADHc function

A protein was identified to be of importance for cell-wall membrane synthesis[1]. It was sequenced and classified as a zinc-containing, long-chain alcohol dehydrogenase oxidizing alcohol to aldehydes *(Fig. 1)* and named ADHc. To confirm the impact of ADHc expression in mycobacteria, the gene was overexpressed in *Mycobacterium bovis BCG* and in *Mycobacterium smegmatis*. It resulted that both liquid cultures had a more hydrophobic cell-wall when ADHc was overexpressed. The amount of lipids was increased by 25% for *M. bovis* and by 60% for *M. smegmatis*.

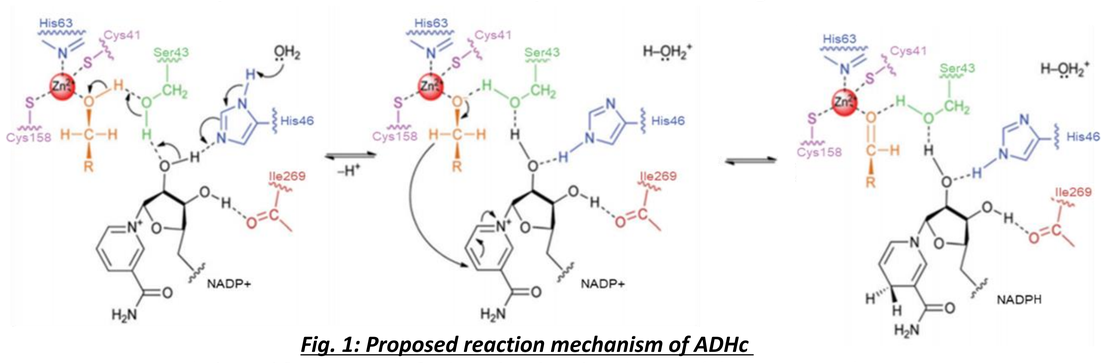


Fig. 1: Proposed reaction mechanism of ADHc
(adapted from Organic & Biomolecular Chemistry, 11(7), 1127–1134.)

ADHc substrates

De Bruyn et al. [2] reported that M. bovis ADHc has in fact a strong preference for aromatic and aliphatic aldehydes and that alcohols are less efficient substrates (*Table 1*). These results are in line with the role of a previously found NADP-dependent aldehyde reducing enzyme necessary for wax synthesis[3]. Interestingly, the most efficient substrate is octanal, a molecule containing a relatively long aliphatic chain, resembling a fatty acid precursor.

SUBSTRATE	K_M (µM)
OCTANAL	9 ± 3
BENZALDEHYDE	11 ± 3
3-METHOXYBENZALDEHYDE	15 ± 3
4-HYDROXYBENZALDEHYDE	17 ± 3
CINNAMALDEHYDE	100 ± 28
CONIFERALDEHYDE	145 ± 40
BUTYRALDEHYDE	200 ± 25
CROTONALDEHYDE	1300 ± 250
CINNAMYL ALCOHOL	7000 ± 400
BUTANOL	300 000 ± 18 400

Table 1: Experimental affinity of ADHc substrates [2]

These data make ADHc an interesting target in ***Mycobacterium tuberculosis*** **disease**.
The aim of this study was to use **computer-aided** structure-based drug design **methods** to identify possible inhibitors of **ADHc**

Method and Results

Model generation and assessment

No experimental 3D structure of the human ADHc has been determined so far. We thus built a structural model of ADHc by comparative modelling. Comparative modelling predicts the 3D structure of a protein sequence (target) using known information from one or more homologous partners (template). The prediction process consists in four steps:

- Template selection, which identifies sequence similarity between the target and at least one known template structure
- Alignment of the target sequence and the templates

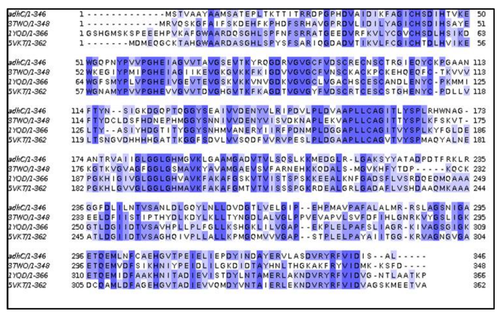


Fig. 2: Alignment of ADHc and three homologs sequences

- Building the models based on the alignment and on the structures of the chosen templates

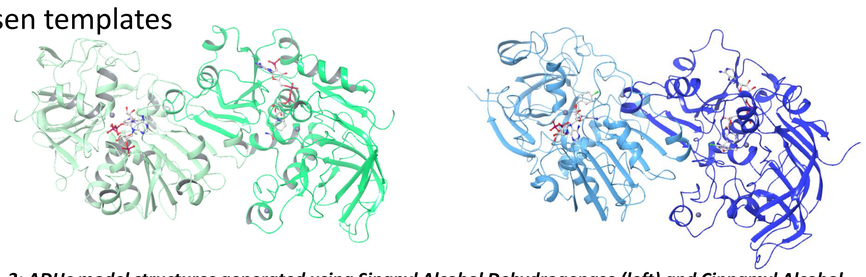


Fig. 3: ADHc model structures generated using Sinapyl Alcohol Dehydrogenase (left) and Cinnamyl Alcohol Dehydrogenase (right) as template

- Model evaluation by docking known ADHc substrates. It revealed poses resembling experimental structures

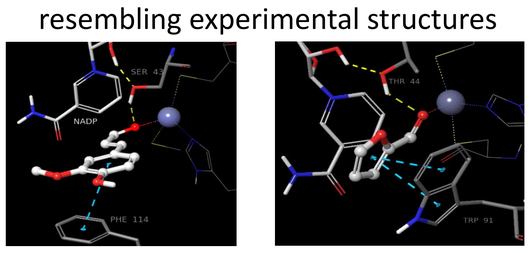


Fig. 4: Docking pose of an ADHc substrate (left) compared to an experimental position (right, PDB:3S2F)

SUBSTRATE	DOCKING SCORE (kcal/mol)	K_M (µM)
3-METHOXYBENZALDEHYDE	-8,284	15 ± 3
BENZALDEHYDE	-7,969	11 ± 3
4-HYDROXYBENZALDEHYDE	-7,935	17 ± 3
CINNAMALDEHYDE	-6,976	100 ± 28
CONIFERALDEHYDE	-6,927	145 ± 40
CINNAMYL ALCOHOL	-6,918	7000 ± 400
BUTYRALDEHYDE	-6,567	200 ± 25
CROTONALDEHYDE	-5,059	1300 ± 250
OCTANAL	-4,982	9 ± 3
BUTANOL	-4,607	300 000 ± 18 400

Table 2: Docking score of ADHc substrates

Structure-based screening strategies

As the experimental binding affinity and pose of a few known substrates of ADHc are in line with the affinity scores of the docked compounds in our 3D model of ADHc, we performed a structure-based drug design using different strategies. We first started with a virtual screening of a large set of compounds coming from subsets of the ZINC database[4] . We then docked a series of smaller sets of compounds selected based on known inhibitors of alcohol dehydrogenases and other metalloenzymes.

Docking results

Diverse ZINC compounds

The best scored compounds possess two aromatic rings featuring π-π interaction with a nearby Phe *(Fig. 5).* The docking results point to the possible contribution of an aromatic moiety to the affinity score as observed for the known substrates. The other top scored molecules also contain at least one aromatic cycle which however are not always engaged in a π-π interaction.

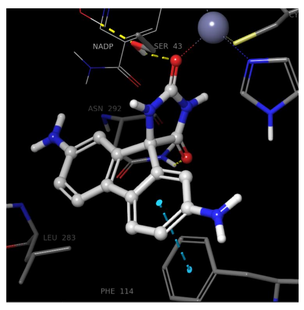


Fig. 5: Docking pose of a ZINC commercial compound in the ADHc model structure

Formamides

Formamides are known inhibitors of alcohol dehydrogenases. We docked all the formamides available from ZINC and found poses very similar to the experimental structures of other alcohol dehydrogenases in complex with formamides *(Fig. 6).*

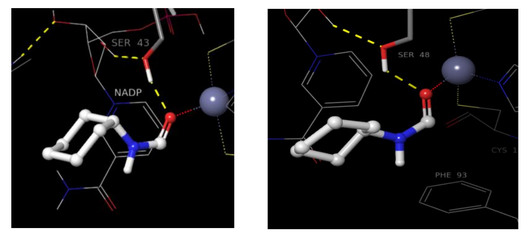


Fig. 6: Comparison of an experimental pose (PDB:1LDY) and a docking pose of cyclohexylformamide

Thiols **Hydroxamates**

Thiols and hydroxamates are known classes of inhibitors of other zinc containing enzymes. The docking revealed that the binding site would be too sterically hindered to allow hydroxamates and thiols to bind to ADHc. Indeed, there is a catalytic serine very close to the zinc ion which could impede the binding of the two oxygens of the hydroxamates or the bulky sulphur atom of the thiols. *(Figs. 7,8)*

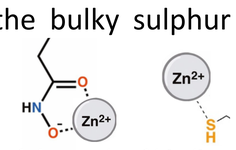


Fig. 7: Representation of the mode of binding of thiols and hydroxamates

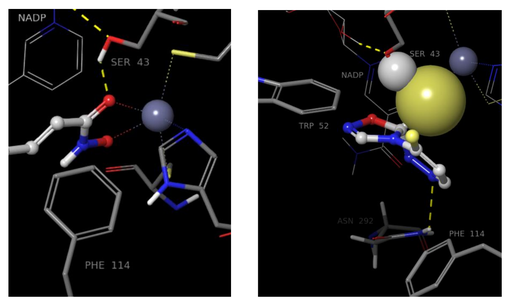
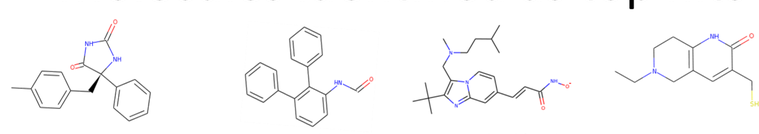


Fig. 8: Docking poses of hydroxamates and thiols and illustration of the steric clash due to the sulfur atom

Conclusions

- We proposed a **first** approach of drug-design targeting **ADHc** against Tuberculosis
- The ADHc protein structure was modelled and **validated** to some extent
- We identified several chemically **diverse** compounds as inhibitor candidates *(Fig. 9)*

Molecules identified as top hits



-14,150 kcal/mol	-12,057 kcal/mol	-11,421 kcal/mol	-7,595 kcal/mol
Diverse ZINC compounds	Formamides	Hydroxamates	Thiols

Fig. 9: Best scored molecules per class of compound and their docking score

1. Stélandre, M., Bosseloir, Y., De Bruyn, J., Maes, P., & Content, J. (1992). *Gene*, *121*(1), 79–86.
2. Wilkin, J. M., De Bruyn, J. (1999). *European Journal of Biochemistry*, *262*(2), 299–307.
3. Wang, L., Takayama, K., Goldman, D.S. & Schnocs. H.K. (1972) *Biochim. Biophys. Acta*, 260 41-48.
4. Sterling, T., & Irwin, J. J. (2015). *Journal of Chemical Information and Modeling*, *55*(11), 2324–2337