Assessing the molecular factors involved in the inhibition of Choline Trimethylamine-Lyase

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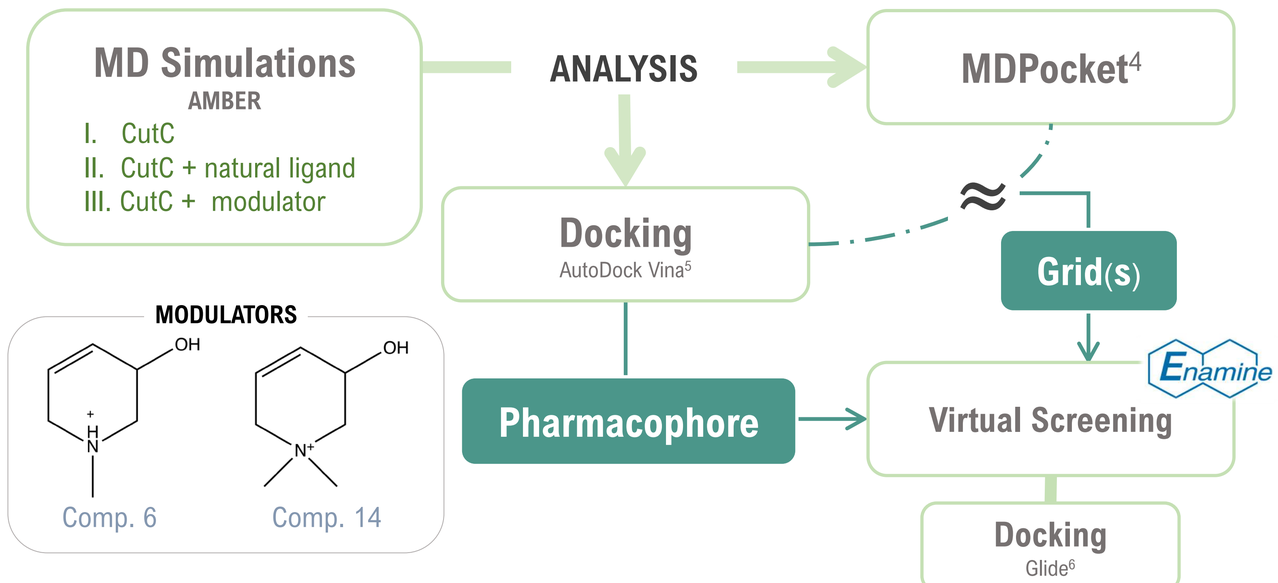
INTRODUCTION

Choline Trimethylamine-Lyase (CutC) is a glycyl radical enzyme present in the human gut microbiota with the ability to cleave the C-N bond of choline, producing trimethylamine (TMA). This metabolite is oxidised into trimethylamine-N-oxide in the liver and is associated with risk of CVDs.[1]

The crystal structures available of CutC pertain to two microorganisms: *D. alaskensis* and *K. pneumoniae*, but the structural differences observed between both systems have led to the focus on the structures of the latter.[2] Different modulators of the activity of CutC have been proposed in the literature[3] but the reaction mechanism of these remain unclear. To assess their stability, selectivity and modulation effects, QM calculations and extensive MD simulations have been performed in absence and presence of some potential inhibitors. Following these results, the binding site and possible tunnels connecting the outer space with the active site have been studied to understand the behaviour of these modulators and use this knowledge to perform Virtual Screening.

METHODS

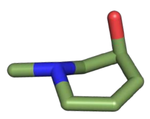


RESULTS

I. MDPocket and docking of Comp. 6 & Comp. 14

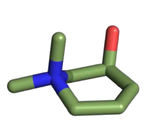
Using MDPocket, 4 regions were determined, and their corresponding grid was defined: ***Binding site***, a ***frontal tunnel***, a tunnel going from the active site towards the exterior of the enzyme, a potential area in which the activating enzyme (CutD) might approach CutC (***CutD tunnel***) and a possible exit tunnel in the back of the protein (***back tunnel***).

Comp. 6 (S)



ΔG_{GAS} = -5.3 kcal/mol
ΔG_{MST} = -10.0 kcal/mol
ΔG_{SMD} = -10.2 kcal/mol

Comp. 14 (S)



ΔG_{GAS} = -3.5 kcal/mol
ΔG_{MST} = -6.7 kcal/mol
ΔG_{SMD} = -7.4 kcal/mol

The most stable conformation was calculated for each modulator and docking was performed for 8 representative structures to see the potential interactions of the modulators with the enzyme. In 6 out of 8 structures, a continuous set of poses is seen throughout the CutD tunnel. MDPocket and docking results served for a refined definition of a grid containing the binding site and the CutD tunnel with the idea of finding a compound that can potentially block this pathway.

The pharmacophore of the region of interest (active site + CutD tunnel) was determined based on the potential residues involved in the reaction mechanism and the most favourable poses of the compounds.

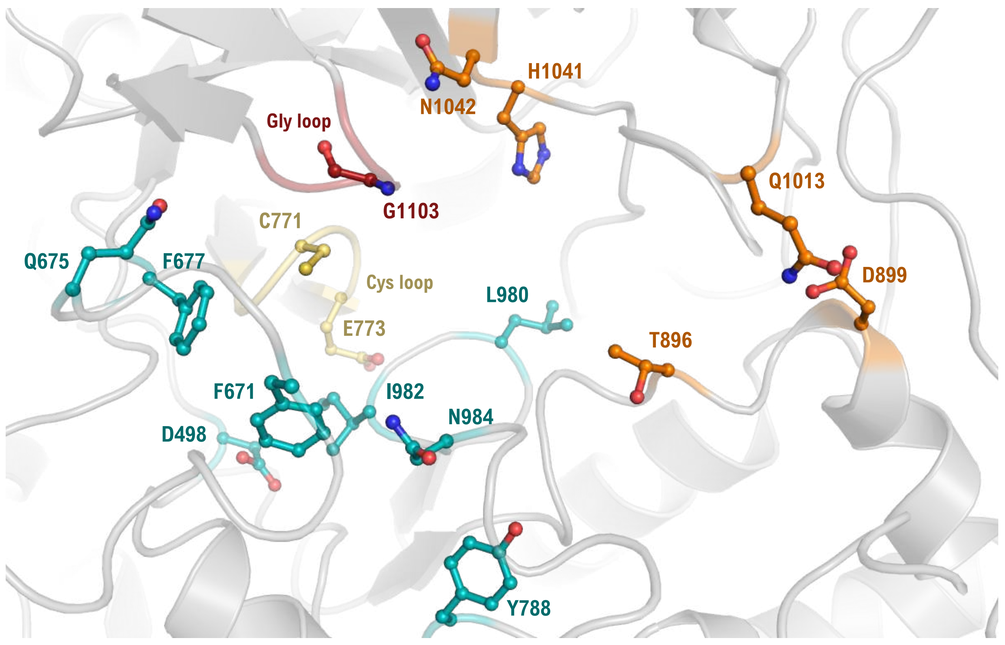


Fig 2. Pharmacophore of the region of interest for Virtual Screening, including the key residues involved in reaction mechanism at the active site (cyan), CutD tunnel (orange) and the Cys (yellow) and Gly (red) loops involved in the cleavage of choline.

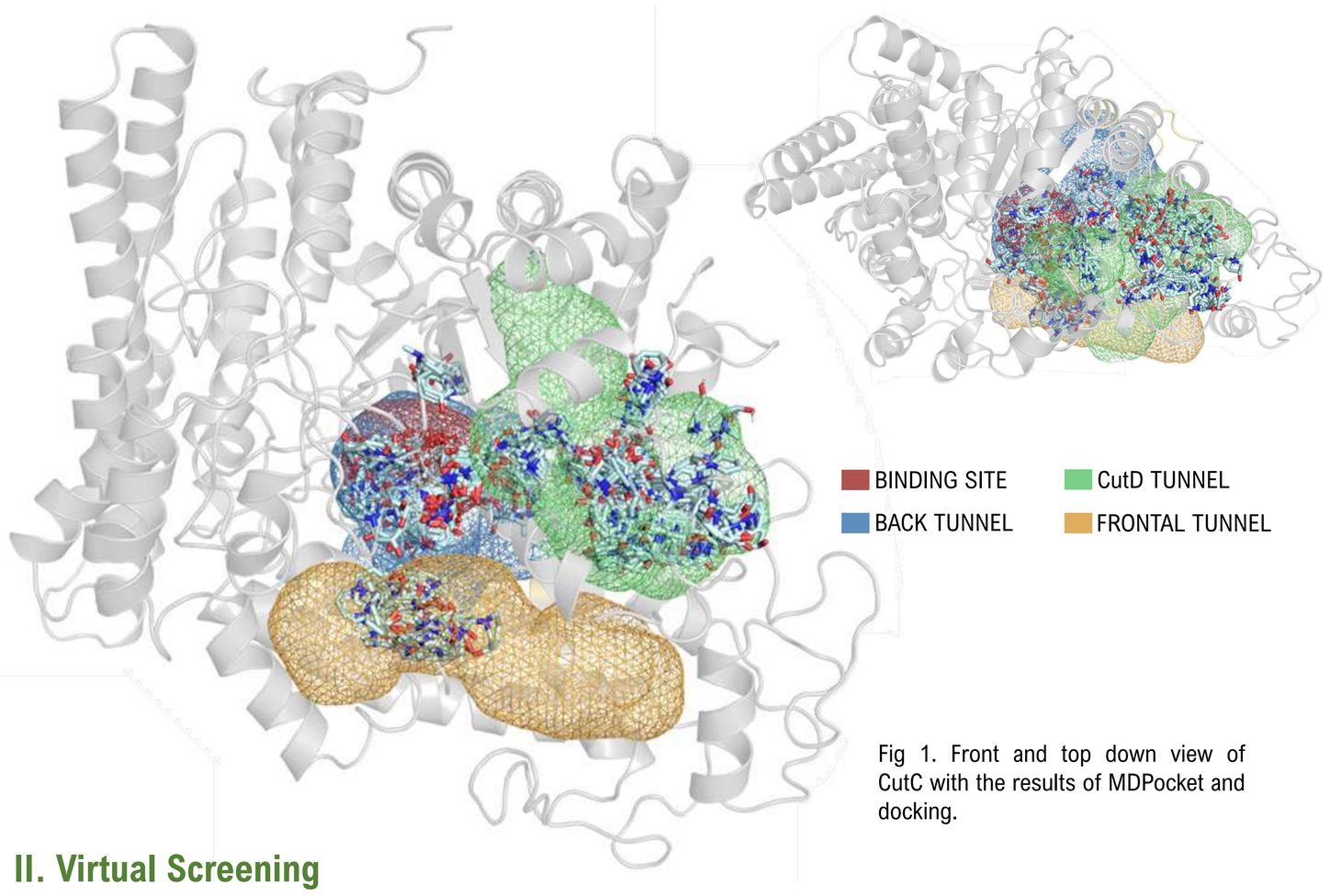
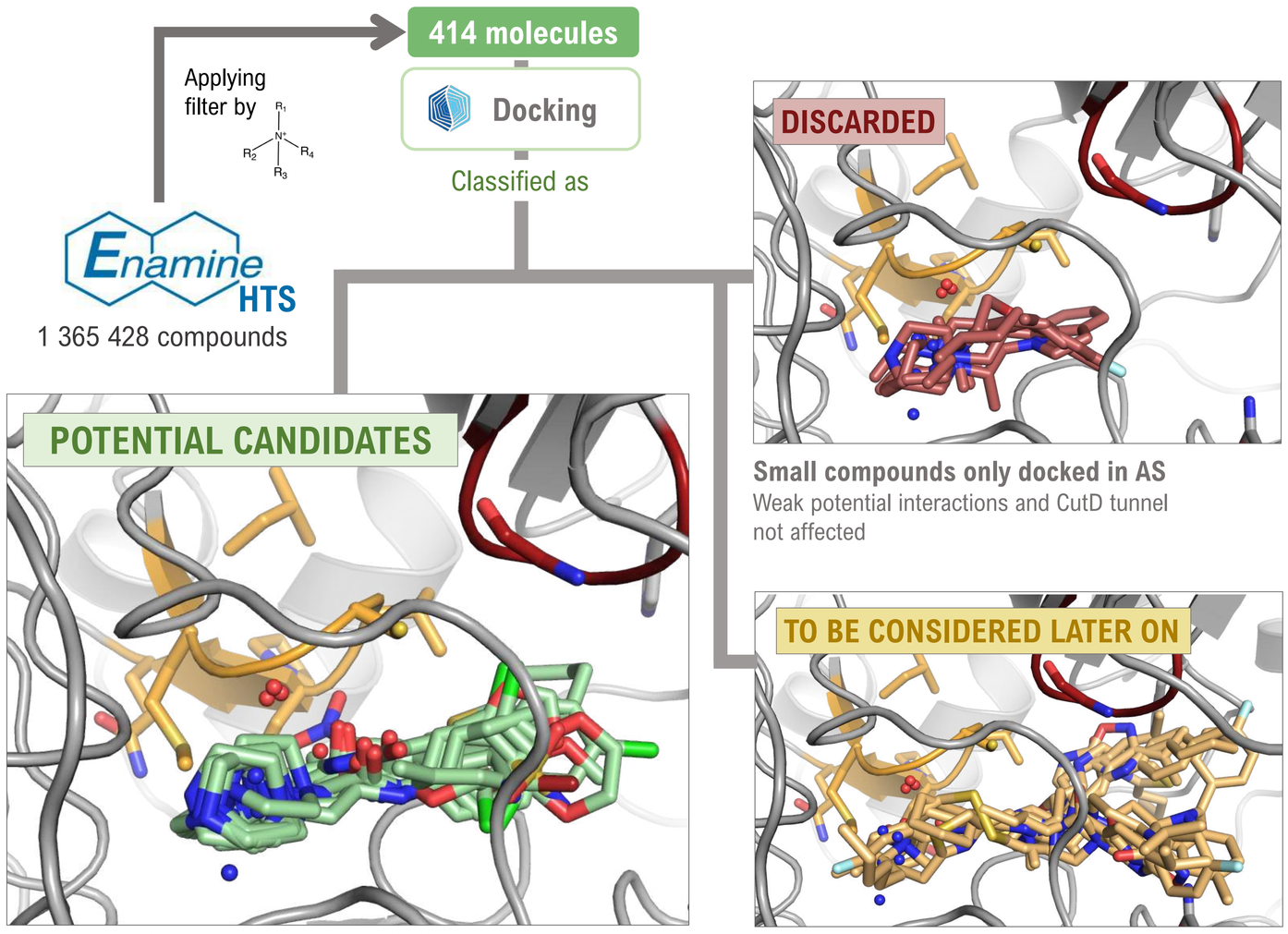


Fig 1. Front and top down view of CutC with the results of MDPocket and docking.

II. Virtual Screening

The Enamine HTS library was used, filtering by compounds containing a quaternary amine, as this feature is key in the interaction between choline and the enzyme, and it is also present in all the modulators proposed in the literature.

As the number of compounds retrieved from the first filter was <1000, docking was performed with all the molecules.



FUTURE PERSPECTIVES

i. Apply positional restraints that are based on the pharmacophore obtained previously and the proposed mechanism of action of CutC.

ii. Redock the most promising compounds to assess the chemical variability that could potentially affect the binding through CutD tunnel and thus, the reactivity of the active site.

iii. Computational fragment-based lead discovery, using the most promising candidate fragments by means of combinatorial methods.

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