

Identification of Novel ID3 Potential Ligands through a Computer-Aided Drug Design approach

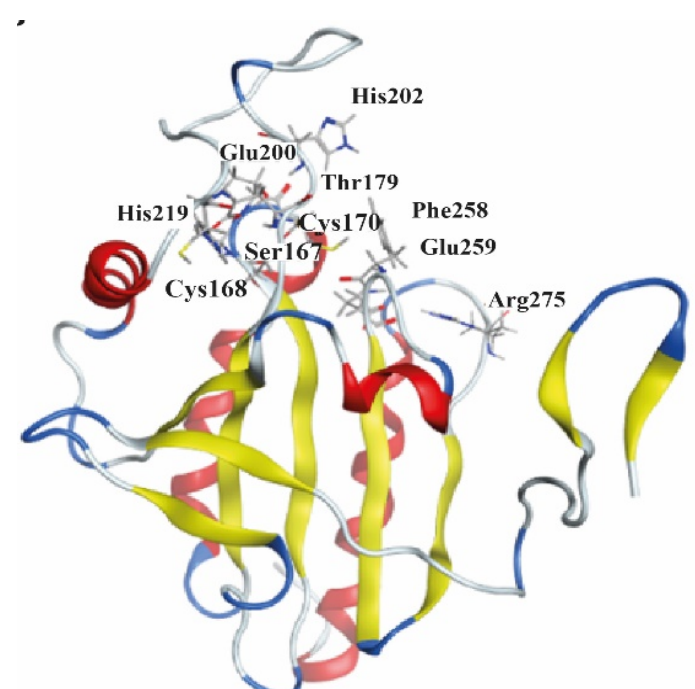
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Iodothyronine Deiodinases (ID) are a family of selenoenzymes involved in the metabolism of thyroid hormones. The three isoforms, iodothyronine deiodinase 1 (ID1), iodothyronine deiodinase 2 (ID2) and iodothyronine deiodinase 3 (ID3) are essential for the conversion of thyroid hormones (TH) to active and inactive forms [1][2]. Each isoform has a different tissue distribution and catalyses deiodination. The ID3 isoform, expressed mainly in brain, pancreas, placenta and skin, was recently discovered to be involved in oncogenic processes (3). The present study aims to find selective inhibitors of iodothyronine deiodinase 3 (ID3) using Structure-Based drug design and Ligand-Based drug design. The experimental work was based on a Computed-Aided Design (CADD) approach. We hence developed a pharmacophore model which will be used to identify novel ligands able to bind ID3.

Fig.1 3D structure of ID3 (PDBID:4TR4). Residues in the catalytic site are labelled and shown as sticks.

COMPUTATIONAL APPROACH

Workflow

Protein Structure Preparation



Ligand Set Preparation

206
50

Molecular docking study

50
13

Dynophores

2 HITS

Ligand Library Preparation

The three-dimensional structure of ID3 was obtained from the Protein Data Bank (PDB). The ID3 inhibitors and substrates were collected from the scientific literature. A Library of ID3 ligands was prepared by filtering the collected compounds based on shared features and affinity values.

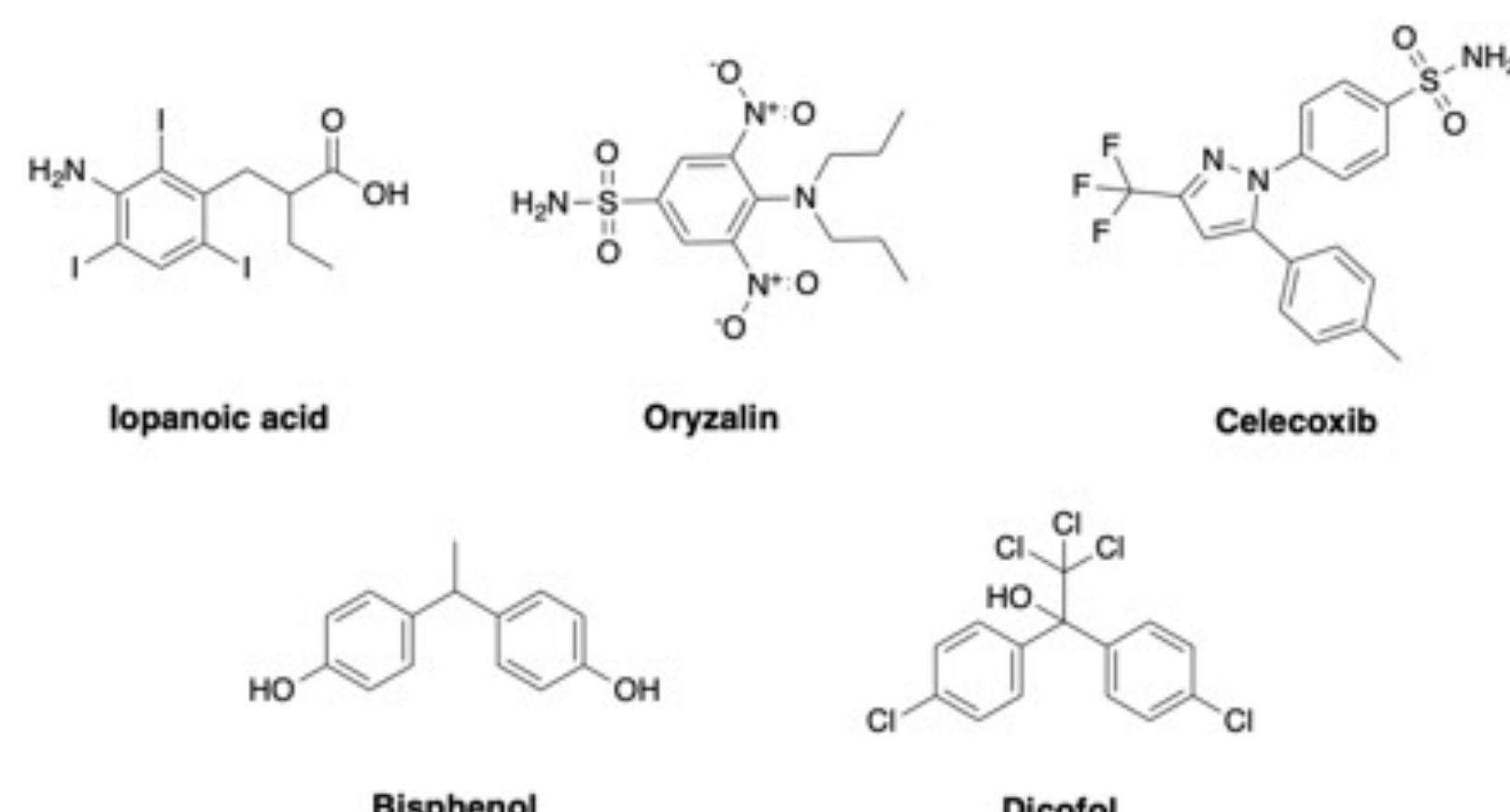


Fig.2 Five of the 50 compounds selected for docking.

Molecular Docking

Molecular Docking of the ligand dataset was performed using GOLD (5). 13 selected enzyme-ligand complexes were subjected to 100 ns MD simulations using Desmond package (6). The catalytic binding pocket of ID3 consists of Phe²⁵⁸, His²⁰², Pro¹⁷¹, Pro¹⁷², Asp²⁷⁸, Cys¹⁷⁰, Ala²⁰¹, Arg²⁷⁵, Gly²⁷⁶ and Glu²⁵⁹. Binding pose analysis was particularly focused on ligand interactions with Cys¹⁷⁰, Glu²⁵⁹, and Arg²⁷⁵.

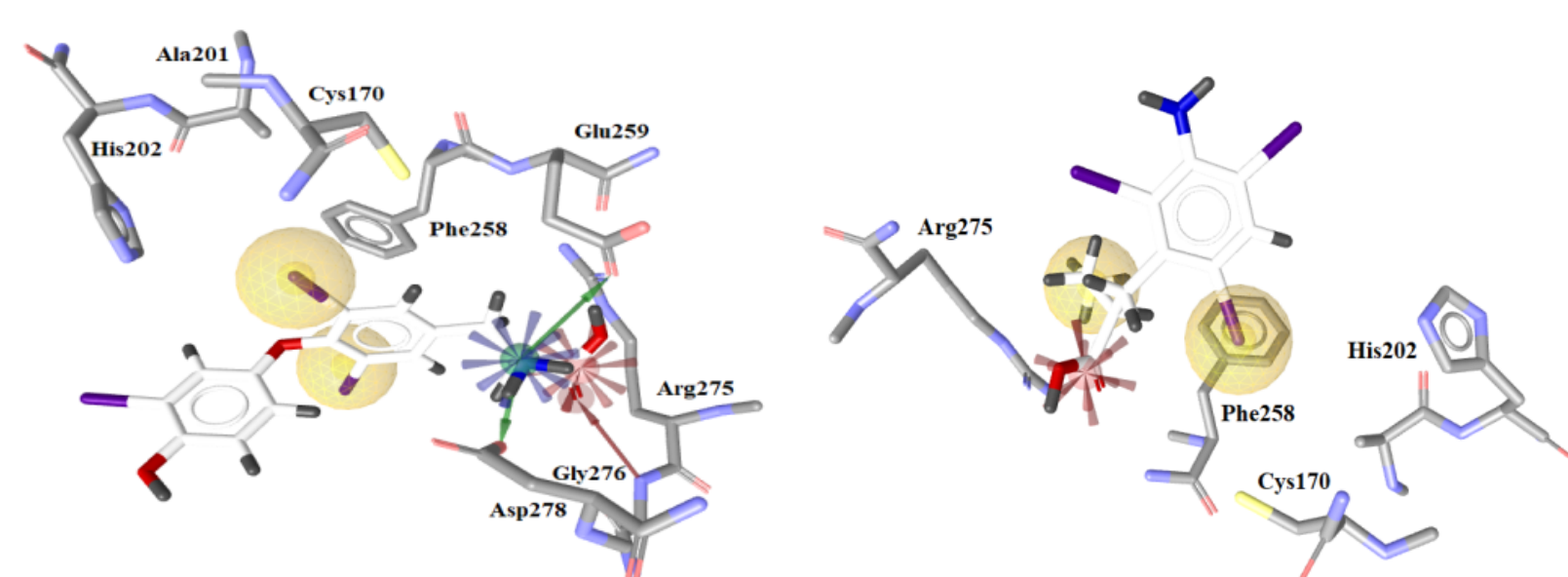


Fig. 5 T3 within ID3 binding site. T3 and the protein residues are depicted as white and gray sticks, respectively. Hydrophobic interactions, Hydrogen Bond donors, Hydrogen Bond Acceptors, Positive Ionizable Areas and Negative Ionizable Areas are shown as yellow spheres and red shown as yellow spheres, green arrows, red arrows, blue and red spherical stars respectively.

Fig. 6 Iopanoic acid within ID3 binding site. Iopanoic acid and the protein residues are respectively. Hydrophobic interactions, Hydrogen Bond donors, Hydrogen Bond Acceptors, Positive Ionizable Areas and Negative Ionizable Areas are shown as yellow spheres and red shown as yellow spheres, green arrows, red arrows, blue and red spherical stars respectively.

Binding Site Analysis and PyRod

Pocket Detection

MD Simulations

Pyrod

dMIFs

Combining data from Moe Pocket Finder and Fpocket calculator, the most plausible 5 binding pockets were determined. Molecular Dynamics (MD) simulations were performed in order to predict protein and ligand behavior. By tracing water molecules in the MD simulations, Dynamic Molecular Interaction Fields (dMIFs) related to hydrogen bond (HB) features (Fig.3), hydrophobic (HI) (Fig.4), ionizable and aromatic interactions, were generated using PyRod (4).

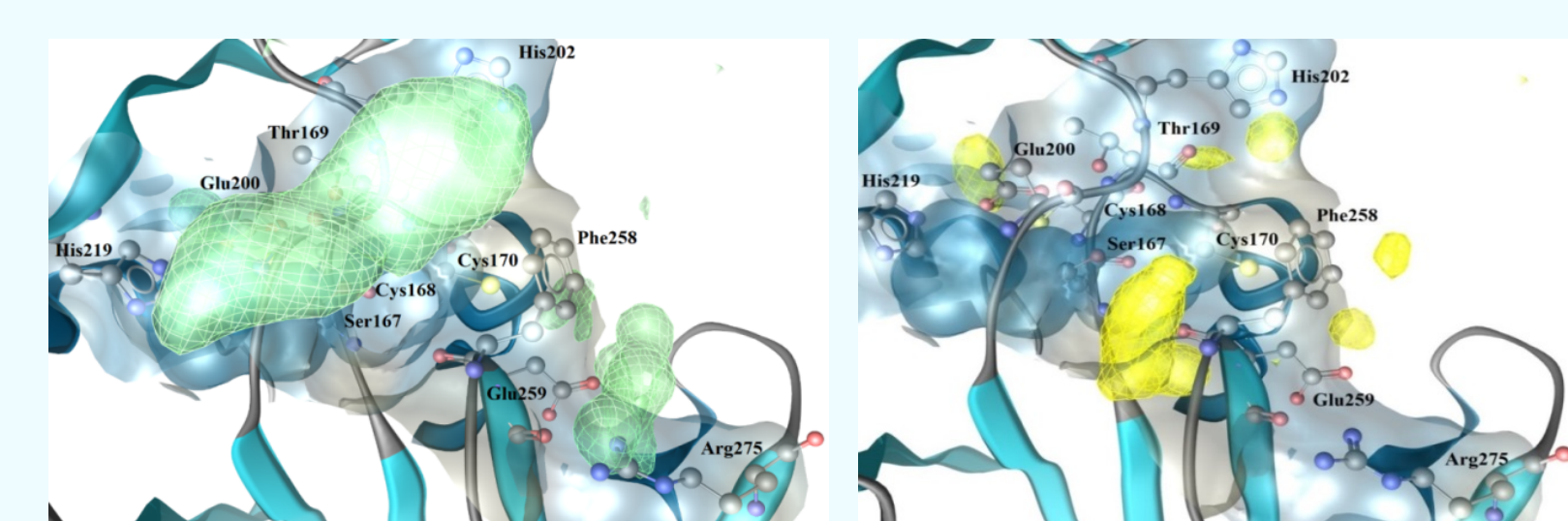


Fig.3 One of the identified binding pockets. The generated dMIFs for HB donor (HBD) is shown as green clouds.

Fig.4 Generated dMIFs for HI shown as yellow clouds.

Dynamic pharmacophores: dynophores

ID3 complexes with Iopanoic acid, Oryzalin, T3, Dicolfol, Fipronil, Celecoxib, Bisphenol, Morine hydrate, Prodiamin and Pirimicarb, were used to perform the MD simulations. Using *DynophoreApp* (7), a LigandScout framework, we created dynophores for each MD trajectory, getting a dynamic view of the ligand-protein interactions. Our results showed that Iopanoic acid and Oryzalin were the only compounds maintaining stable interactions throughout the simulation time.

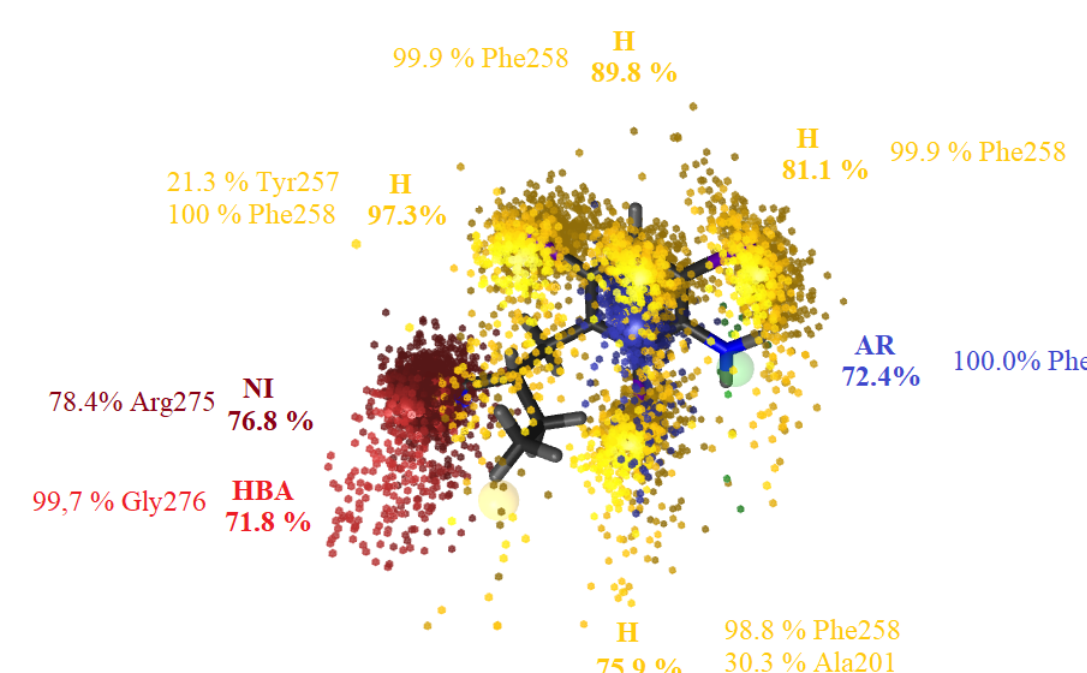


Fig. 7 Iopanoic acid dynophore interactions clouds. Frequencies of occurrence are reported. Aromatic interactions, hydrophobic interactions, HBA and HBD are represented as blue, yellow, red and green cloud points.

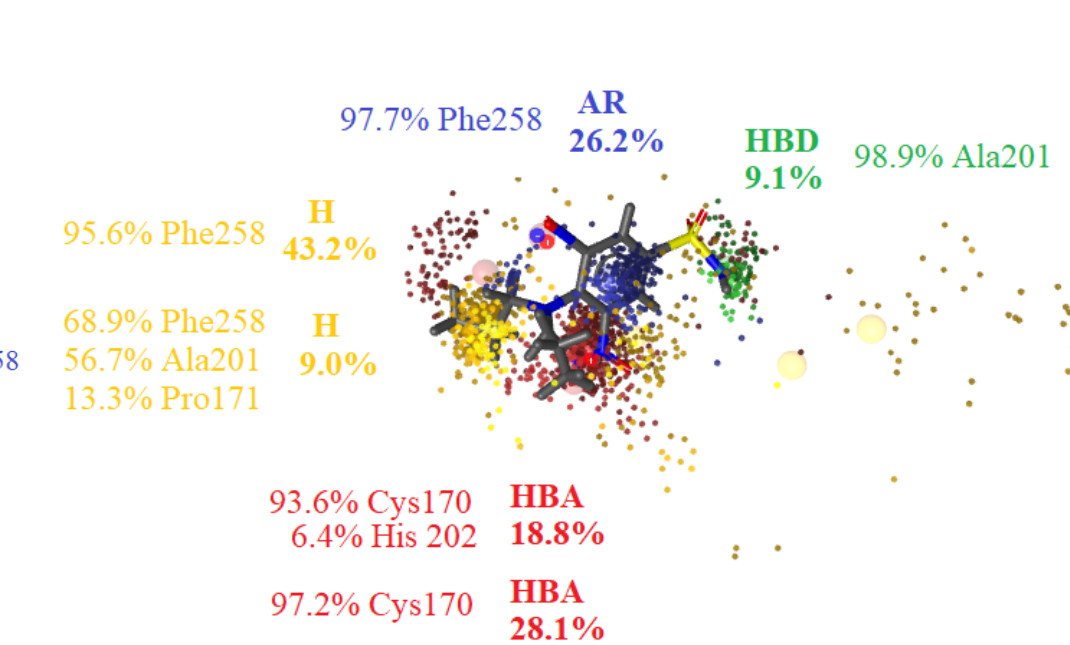


Fig. 8 Oryzalin dynophore interactions clouds. Frequencies of occurrence are reported. Aromatic interactions, hydrophobic interactions, HBA and HBD are represented as blue, yellow, red and green cloud points.

Results

Our results showed Iopanoic acid and Oryzalin exhibiting the most stable interactions with protein through time. In the MD trajectory, the carboxylic group of Iopanoic acid is involved in negative ionizable interactions with Arg²⁷⁵, whereas the Iodine atom and methyl group are involved in hydrophobic interactions with both Ala²⁰¹ and Phe²⁵⁸. NI interactions with Arg²⁷⁵, as well as HI involving Phe²⁵⁸, were confirmed from the generated dynamic pharmacophore. Oryzalin molecular docking results showed IA and HI with Phe²⁵⁸, HBA interactions with Cys¹⁷⁰ and HBD interactions with Asp²⁷⁸. Oryzalin dynophore confirmed HI with Phe²⁵⁸ and HBA interactions with Cys¹⁷⁰. Additionally, PyRod showed interactions that were not obvious from the dynophore and docking studies. HBD interactions were observed mainly with Glu²⁵⁹, His²⁰², Arg²⁷⁵ and Glu²⁰⁰. The HI with Phe²⁵⁸ were confirmed as observed for Iopanoic acid and Oryzalin in both docking and dynophore studies. PyRod also detected HI with Cys¹⁶⁸, Glu²⁰⁰ and His²⁰². NI interactions were found with Arg²⁷⁵ as predicted in Iopanoic acid docking model.

Conclusion

Reported ID3 inhibitors were developed without any previous knowledge of how they bound to ID3. The main purpose of this work was to identify the residues involved in protein-ligand interactions. A CADD approach was hence applied. Our analyses identified Phe²⁵⁸ as crucially involved in the binding event, exhibiting both HI and AR interactions. An important role in ligand binding is likely also played by Arg²⁷⁵, which was identified as involved in NI interactions in both docking and dynophore studies on Iopanoic acid. Additionally, Cys¹⁷⁰ was involved in HBA interactions in both docking and dynophore Oryzalin studies. Our results drew a clear picture of which features are most likely essential to build a solid pharmacophore model suitable for virtual screening.

References

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