

## Pharmacophore-based and shape-based virtual screening of PHZD inhibitors.

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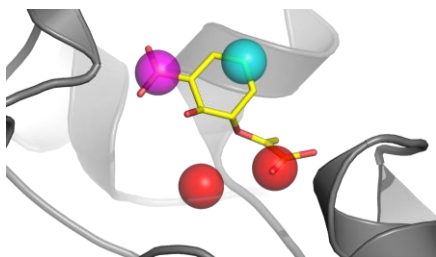
### Introduction

Although traditional drug development approaches lead to drugs that improved life expectancy and revolutionized medicine, they seem to be powerless against the upsurge of bacterial resistance. In fact, the reduction in drug efficiency is an expected effect of the evolutionary pressure posed by antibiotics.<sup>1</sup> In order to overcome this dilemma, bacteria virulence factors have been target for drug development.<sup>2</sup>

Pyocyanin (PYO) is a virulence factor produced by *P. aeruginosa* that reduces ciliary movement and mucus secretion thus impairing the immune response towards infection.<sup>3</sup> Among the enzymes responsible for PYO production PHZD catalyzes the conversion of aminodeoxyisochorismate to trans-2,3-dihydro-3-hydroxyanthranilic, an early intermediate in PYO biosynthesis. Although the kinetic behavior and structural data for this enzyme have long been known, no inhibitors have been reported so far.<sup>4</sup>

### Results and Discussion

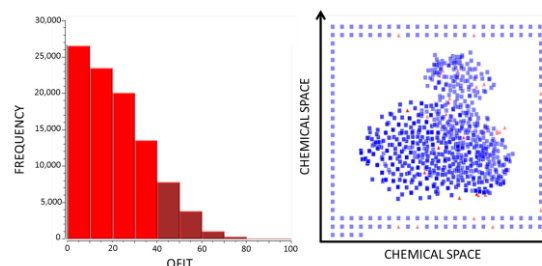
Comparison of HOLO-PHZD crystal structure (PDB code: 1NF8) and APO-PHZD (PDB code: 1NF9) reveals that one water molecule might be displaced due to LYS122 movement and ligand binding. This information was considered to build a UNITY-3D pharmacophore query (Figure 1) that was employed to virtually screen ZINC lead-like molecules. 96317 hits were identified by this approach, as measured by their QFIT values.



**Figure 1.** Pharmacophore query built from the visual analysis of 1NF8 and 1NF9 crystal structures

Considering that this metric has poor correlation to activity, an additional criteria was used to further select putative PHZD inhibitors: Molecules with QFIT > 40 (12711) had their chemical similarity assessed by UNITY\_fingerprints (Figure 2) and representative subset (colored red in chemical space map) was selected for the next step. Although these molecules fulfill the pharmacophore requirements for

PHZD binding, many of them might clash to residues within the active site, once no excluded-volume was employed until this point.



**Figure 2.** Criteria employed to select putative PHZD inhibitors: A) QFIT values; B) Chemical similarity.

In order to overcome this drawback, SURFLEX-DOCK software was employed to build a representation of the active-site (protomol, Threshold= 0.5, Bloat= 1.0), whose volume and chemical similarity towards selected hits was calculated with SURFLEX-SIM software (pre and pos minimization parameters were selected). This strategy takes a few minutes, whereas docking would take several hours. As might be expected, several molecules show poor scoring (<4.5) (Table 1), due to the lack of fit towards the protomol.

**Table 1.** Chemical similarity of putative PHZD inhibitors towards the active-site of the macromolecular target, represented by its protomol.

Mol-ID	SURFLEX-SIM SCORE	Mol-ID	SURFLEX-SIM SCORE
5297	7.81	9740	4.07
5356	7.72	7933	4.04
6923	7.69	3841	4.00

### Conclusion

The integrated use of ligand-based and structure-based strategies afforded the selection of a subset of molecules that fulfills the pharmacophore requirements and fit within PHZD active-site.

### Acknowledgements

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