Structure- Based Druggability assessment of pyocyanin biosynthesis pathway enzymes

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Palavras Chave: *P. aeruginosa*, pyocyanin, druggability

Abstract

In silico tools were employed to select phzF as the most promising target related to the production of pyocyanin, a *P. aeruginosa* virulence factor.

Introduction

Among the manifold reasons for the high attrition rates in drug discovery is the selection of non-druggable targets.[1] Pyocyanin (PYO) biosynthesis inhibition is considered a promising strategy to modulate the virulence of *Pseudomonas aeruginosa*.[2] However there is no experimental data to support the duggability[3] of the enzymes responsible for PYO production (Figure 1). Therefore, as part of an ongoing project to identify lead compounds that are useful to fight resistant *P. aeruginosa*, we employed structure-based approaches to identify and map the binding sites of putative macromolecular targets that would be subjected to virtual screening.

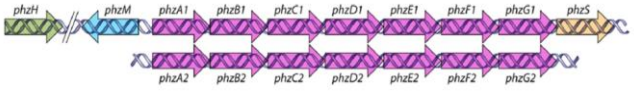

Figure 1: Pyocyanin biosynthetic pathway in *P. aeruginosa*.

Results and Discussion

Among the 10 enzymes involved in PYO biosynthesis, 5 have their crystal structure available. Therefore, we employed MODBASE server to build homology-based models for the 5 remaining enzymes. The low similarity between PhzH / PhzC and their templates (26% and 41% respectively) had a negative impact over the stereochemical quality of the models (> 2.5% disfavored residues in the Ramachandran plot) or the chemical environment into which the residues were placed (less than 70% aminoacids with verify 3D score > 0.2). As consequence, these structures were excluded from further analysis (Figure 2). The remaining structures had their binding sites/ pockets predicted by METAPOCKET and DOGSITE. Although these servers agree on the prediction of residues lining the main binding site of each structure, only DOGSITE takes into account energy descriptors to classify the pockets are druggable or not. According to this server, all structures have druggable binding sites (DOGSITE score > 0.80). In order to prioritize the most promising sites, POCKDRUG server[1] was employed to re-rank them according to their geometry (vol), hydrophobicity (hyd.) and aromaticity (Arom) (Table 1).

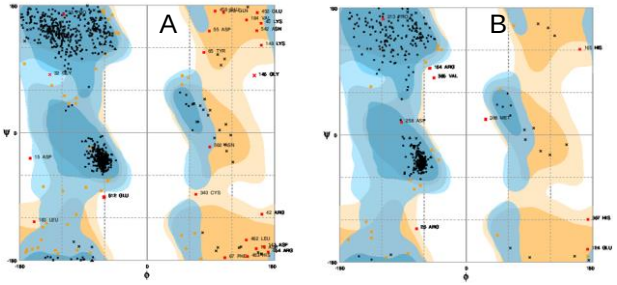

Figure 2. Stereochemical quality of phzH (A) and phzC (B) homology models.

Table 1. Druggable features of selected PYO biosynthesis enzymes.

ID	Vol. (K A^3)	Hyd (Kite)	Arom.	Druggability Score
phzA	5.48	-0.56	0.27	0.80
phzB	18.63	-0.07	0.29	0.98
phzD	2.23	-0.42	0.25	0.77
phzE	10.23	0.13	0.09	0.94
phzF	**4.19**	**0.13**	**0.13**	**0.92**
phzG	4.36	-0.44	0.08	0.66
phzM	7.94	0.49	0.22	0.98
phzS	6.33	-0.21	0.08	0.80

These results point out that phzB, phzM, phzE e phzF are the most promising, but phzM is active only in the presence of phzS (probable transient complex formation). As a consequence, we consider it unsuitable for virtual screening approaches. Among the remaining targets only phzB and phzF are available for *in vitro* screening.

Conclusion

On one side, the *in silico* approaches employed in this work points out that several enzymes involved in PYO production are poor targets for drug development. On the other side, phzF fulfills all requirements to be considered as a druggable target and is available in our lab. Hence, it was selected for pharmacophore based virtual screening.

Acknowledgments

CNPq, FAPESB, CAPES

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