Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Novel 13-Berberine derivatives compounds as Efflux pump inhibitors against MexY variants of *Pseudomonas aeruginosa*

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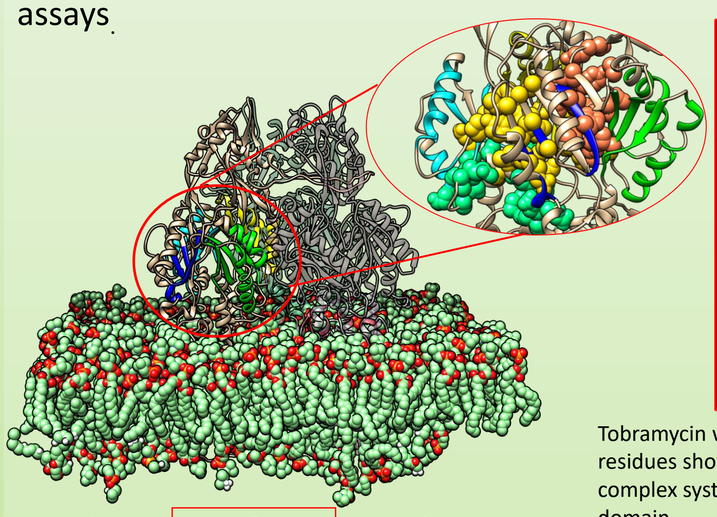
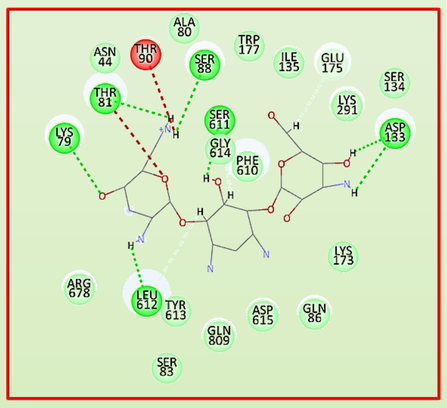
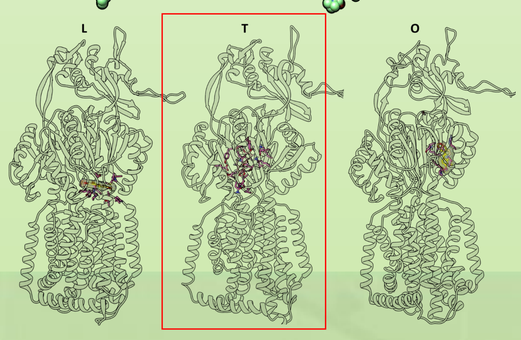
For this project, we started with Berberine, a natural alkaloid that is capable of tightly binding to the MexY protein of the EP (Efflux Pump) system MexXY-OprM in *Pseudomonas aeruginosa*. The mechanism of action of Berberine was elucidated using computational techniques and *in vitro* microbiological assays.

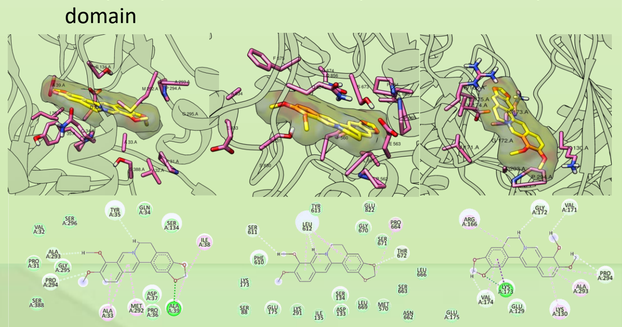




Tobramycin within the deep binding pocket interacts with residues shown. On the left there's the homotrimeric complex system with the main pockets inside the porter domain



The three conformations: loose (L), tight(T) and open(O) were used for the blind docking and molecular dynamics simulation, to assess the Berberine repositioning and its binding affinity. Molecular interaction on the Tight and free gibbs binding energy calculated are depicted in the table below

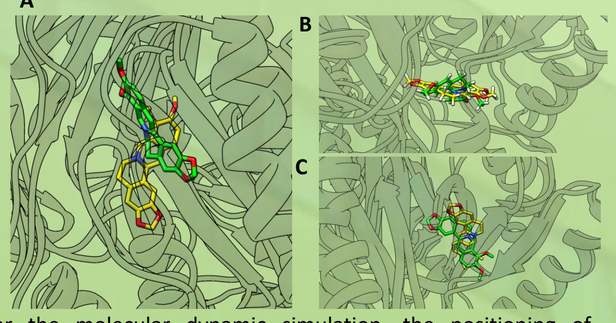


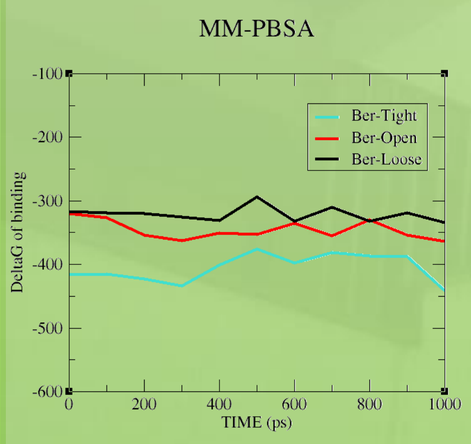
Molecular docking: autodock 2.4.6 with AMBER forcefield based scoring function. GA algorithm 100 runs

Molecular dynamics: GROMAC202.6, CHARMM36m, 100ns production run; PBC conditions; POPC bilayer system; TIP3P water model; NaCl 0,15M

MM/PBSA last ns of MD simulation after stabilization

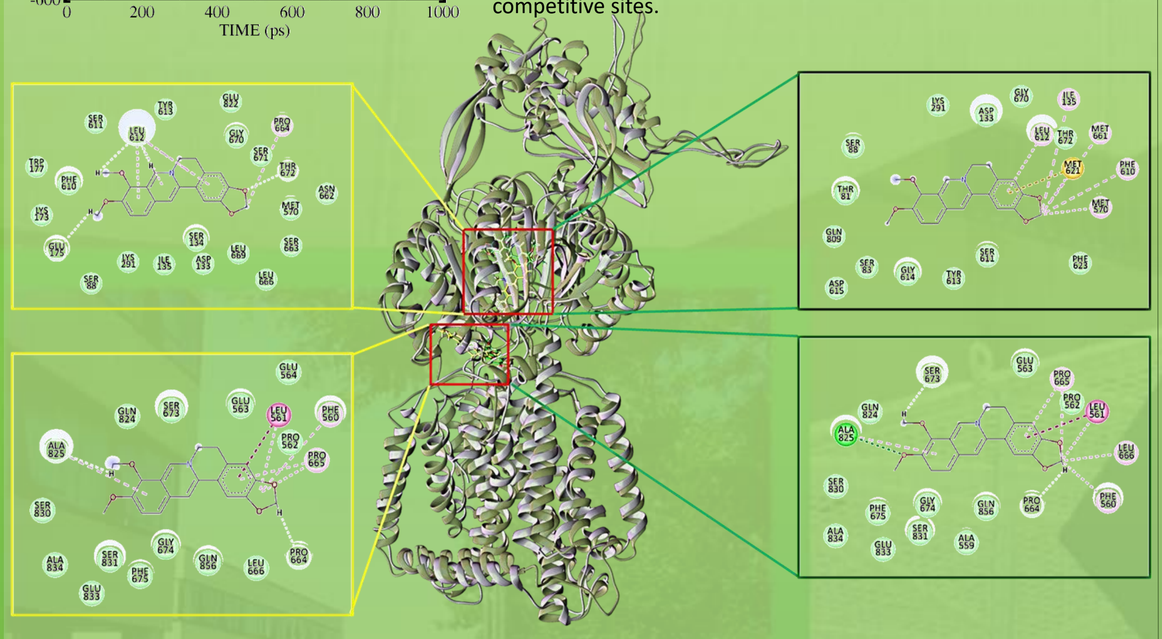
	Loose	Tight DBP	Tight Accessory	Open
Berb	-7,70kcal/mol	-6,97 Kcal/mol	-8,66kcal/mol	-6,32kcal/mol
Tob.	-9,62kcal/mol	-5,96kcal/mol	/	-5.54kcal/mol





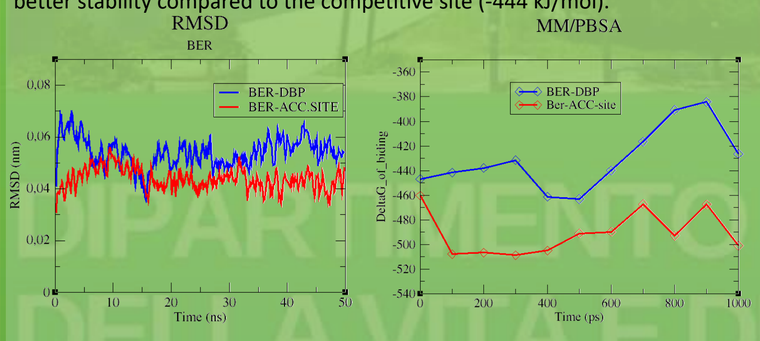
After the molecular dynamic simulation, the positioning of Berberine in each pocket within all the monomer states was compared. The free Gibbs binding energy was calculated

Results: **:** Berberine demonstrates the ability to tightly bind to the T monomer in a competitive manner, similar to Tobramycin. This was confirmed through the calculation of free binding energy using MM/PBSA. Additionally, from the docking analysis, another site known as the non-competitive site exhibited the highest binding affinity energy (-8.66 kcal/mol) compared to other pockets. For this reason, another molecular dynamics simulation was conducted to compare the positioning of Berberine in the homotrimeric tight system within both the competitive and non-competitive sites.



After the MD simulation, it can be observed that Berberine within the non-competitive pocket is more stabilized by hydrogen bond and π-alkyl interactions, whereas within the deep binding pocket it is only stabilized in its benzodioxazolic moiety

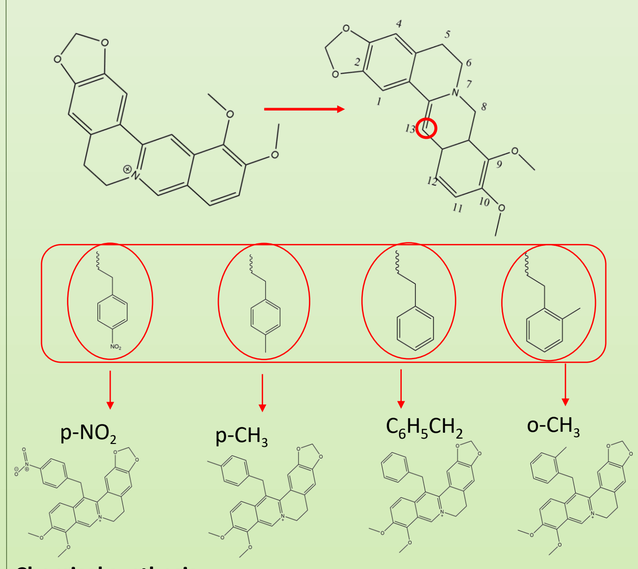
The MM/PBSA calculations also indicate that Berberine within the non-competitive site (-501 kJ/mol) exhibits better stability compared to the competitive site (-444 kJ/mol).



1.Conclusion

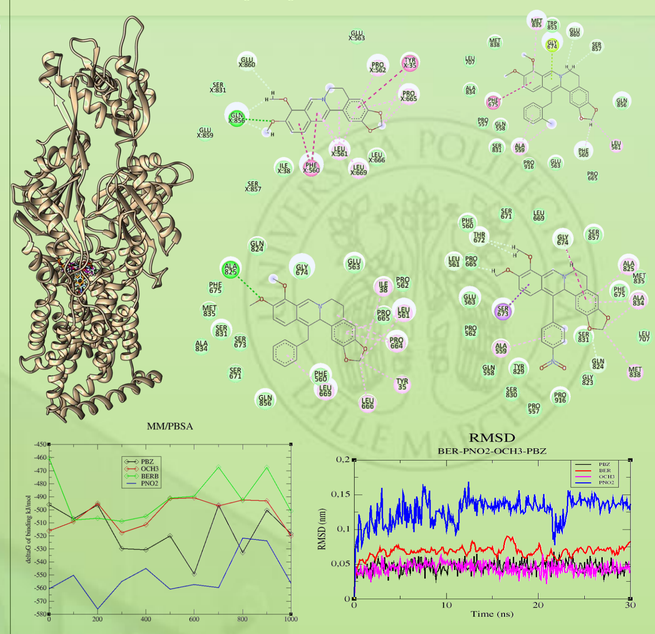
We have hypothesized a non-competitive mechanism within the non-competitive site in the Tight specific conformation of the MexY protein in Pseudomonas aeruginosa. This mechanism is believed to block the rotational function of MexXY-OprM and prevent substrate expulsion.

To increase the activity and binding specificity of the EPI (Efflux Pump Inhibitor), we synthesized berberine derivatives functionalized at the C-13 position. The binding modes of these derivatives were investigated using computational techniques.

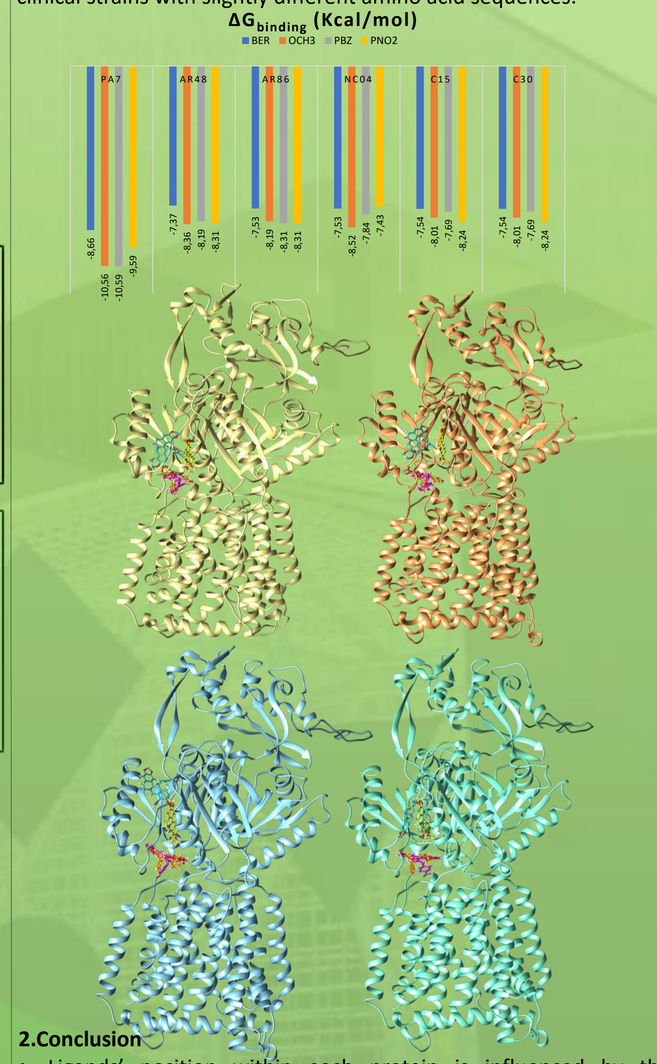


Chemical synthesis:

Berberine reduction to Dihydroberberine (1equiv), with KI (2equiv) in CH_3CN 40mL solution added to benzylbromide substituent. Solution refluxed under stirring for 4h, the extract has been purified with $CHCl_3/CH_3OH$ (50:1) as eluent.



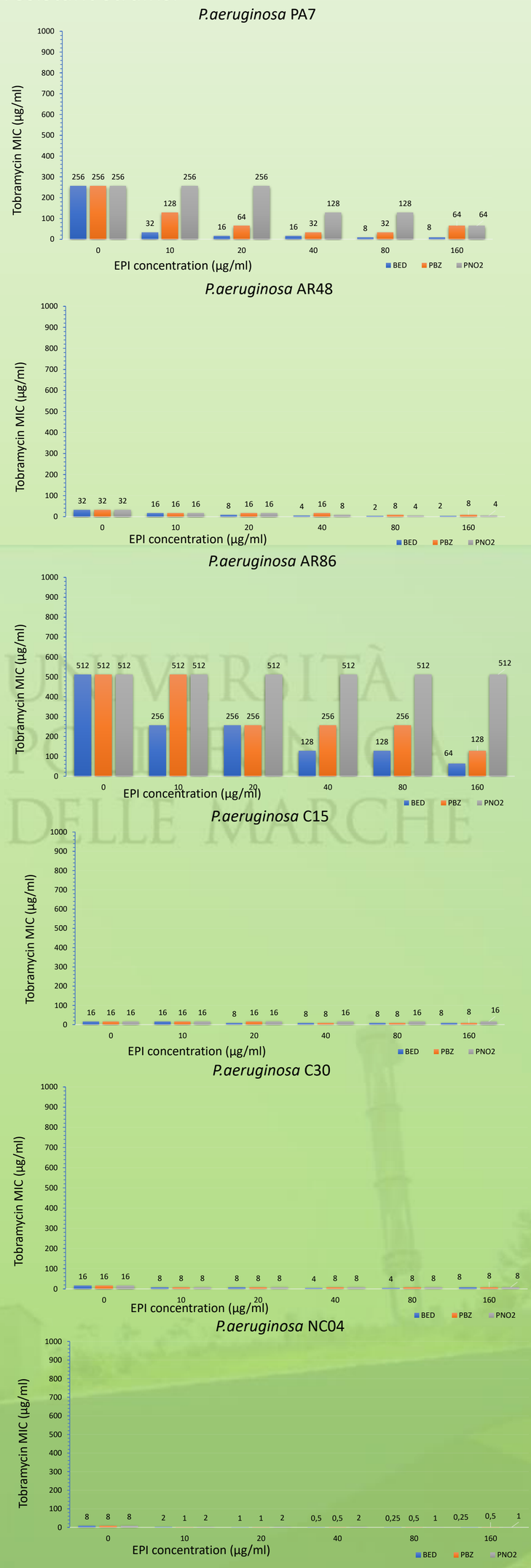
Docking and Molecular Dynamics simulations were conducted within MexY of the highly resistant strain PA7, and the results were analyzed. Docking analysis of all these compounds was also performed in other forms of MexY, particularly in proteins from clinical strains with slightly different amino acid sequences.



2.Conclusion

- Ligands' position within each protein is influenced by the chemical functionalizations and the specific protein type.
- Chemical substituents enhance the binding affinity within the protein pockets.
- Among the compounds analyzed through molecular docking and dynamics, the 13-(3-methylbenzyl)-berberine and 13-benzyl-berberine exhibited the highest scores compared to Berberine. Additionally, the 13-(4-nitrobenzyl)-berberine demonstrated the best binding energy according to the calculated free Gibbs binding energy after the molecular dynamic simulation.

By *in vitro* checkerboard assays, we assessed their inhibitory activity when used in synergy with the aminoglycoside Tobramycin, mostly extruded by this EP in MDR *P. aeruginosa* resistant strains.



Checkerboard assays: were performed using 2-fold dilutions of tobramycin from 0 to 520 µg/ml and berberine derivatives from 10 to 160 µg/ml, preliminarily proven to be devoid of antibacterial activity.

3.Conclusion

The 13(2-methylbenzyl)berberine has its best activity compared to the 13-benzylberberine, both against resistant and susceptible cells. Regarding the 13(4-nitrobenzyl)berberine, there's a reduced or no activity in all strains, probably due to the increasing polarity in the functionalizing group.

- Are ongoing studies for the possibility of the encapsualtion of this berberine derivative within liposomal systems, for increasing it cell membrane permeability.
- Are ongoing *in silico* studies about the membrane permeability of Berberine and its derivative 13-(2-methylbenzyl)derivative and in vitro lyposomal systems encapsulation using different lipidic formulations.

Bibliografia:

1)M.F.Moradali et al.(2017), *Fcimb*, Vol7,39. **2)**D.Dey et al.(2020), *Aac.asm*, Vol64,8 **3)**M.AlMatar et al.(2020), *Pharmacol Rep*. 73(1):1-16. **4)**A.Waterhouse et al.(2018), *Nucleic Acids Res*, 46:W296-W303.**5)**G.M.Morris et al.(2009), *J Comput Chem*. 30(16):2785-2791. **7)**E.Laudadio et al.(2019), *Journal of Natural Products*, 82(7), pp. 1935–1944 **8)**G.Giorgini et al.(2021), *J.Nat.Prod*. 84, 993-1001. **9)**M.J.Abraham(2015), *SoftwareX*, Vol1-2,pp.91