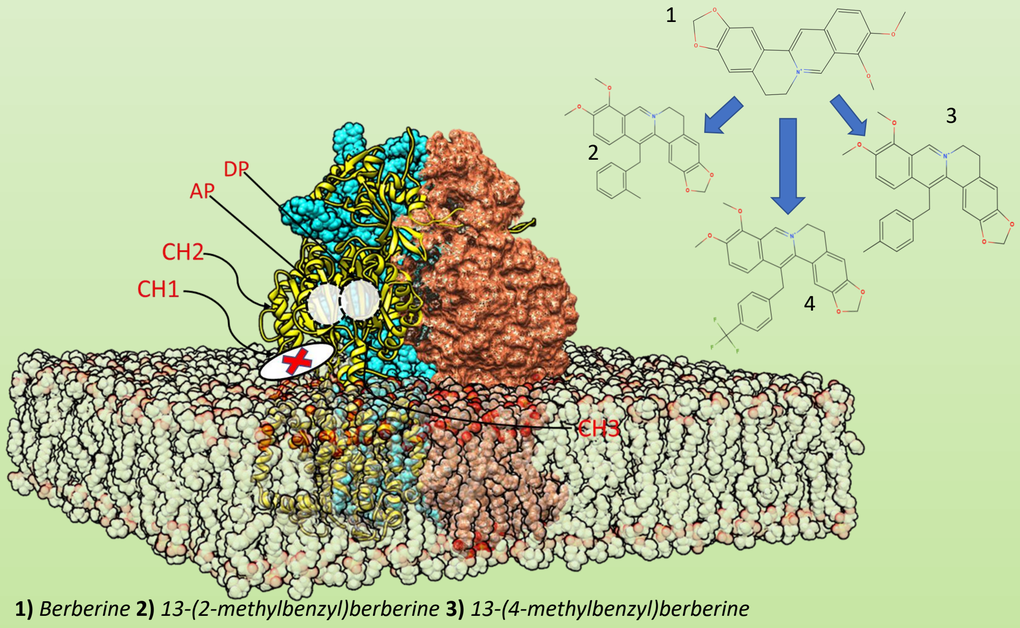
Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Novel 13-Berberine derivatives as EPIs for MexXY system in *Pseudomonas aeruginosa* strains

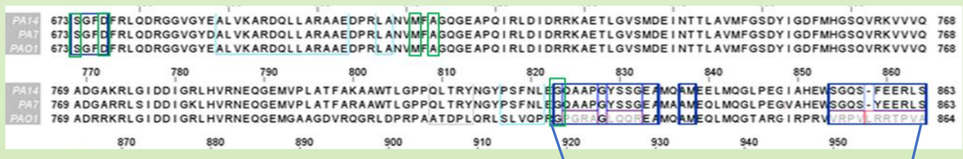
Giorgia Giorgini

Molecular Modeling Laboratory Prof.ssa Galeazzi Roberta



1) *Berberine* **2)** *13-(2-methylbenzyl)berberine* **3)** *13-(4-methylbenzyl)berberine*

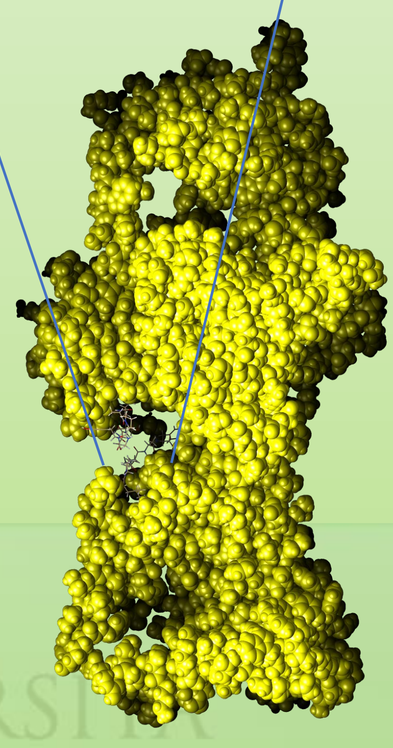
4) *13-(4-trifluoromethylbenzyl)berberine*

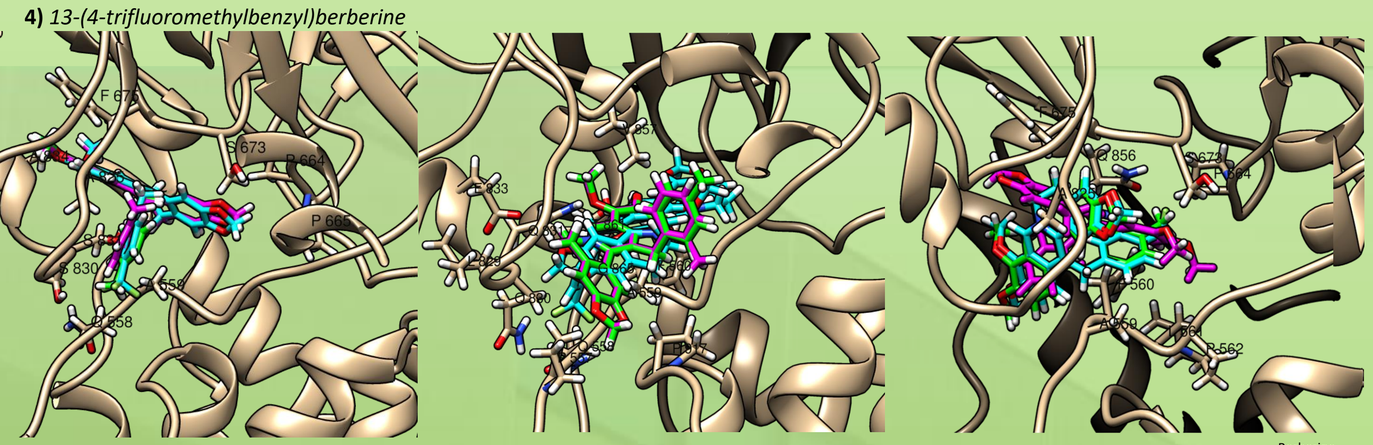


Background:

Pseudomonas aeruginosa is an opportunistic pathogen responsable of lifethreatening infections among immunocompromised patients and it is the principal cause of respiratory infections in cystic fibrosis (CF) patients[1]. MexXY-OprM is the main RND (resistance nodulation division family) efflux pump system due to of the aminoglicoside extrusion (i.e. Tobramycine)[2].

AIMS: A possible strategy is hindering the efflux pump mechanism through efflux pump inhibitors (EPIs) in a combined antibiotic/EPIs therapy.[3] In this study, an *in silico/in vitro* evaluation of the stability and efficacy of three synthetic 13-Berberine derivatives in complex with MexY polymorphic proteins has been carried out, considering three different *P. aeruginosa* strains (PAO1,PA7,PA14)





MexY-PA7-EPIs Molecular Docking MexY-PAO1-EPIs Molecular Docking MexY-PA14-EPIs Molecular Docking

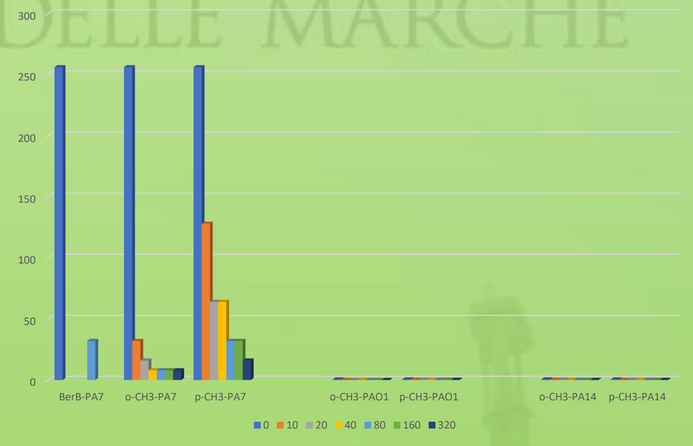
Violet= 13-2-methylbenzylberberine green= 13-4-methylbenzylberberine cyan= 13-4-trifluoromethylbenzylberberine

Molecular docking:
Autodock 4.2.6 Lamarckian Genetic Algorithm. Grid box 50x50x50(Å^3)

Molecular dynamics:
homotrimeric system in POPC bilayer, TIP3P water model used for solvation, NaCl 0.15M physiological condition. Equilibration steps using NVT and NPT ensambles, production phase in NPT using T-coupling Noose-Hoover and Pressure coupling Berendsen barostat (NPT ensamble) T=300K, 1bar. CHARMM36 force field. LINCS alghoritm for constraints.

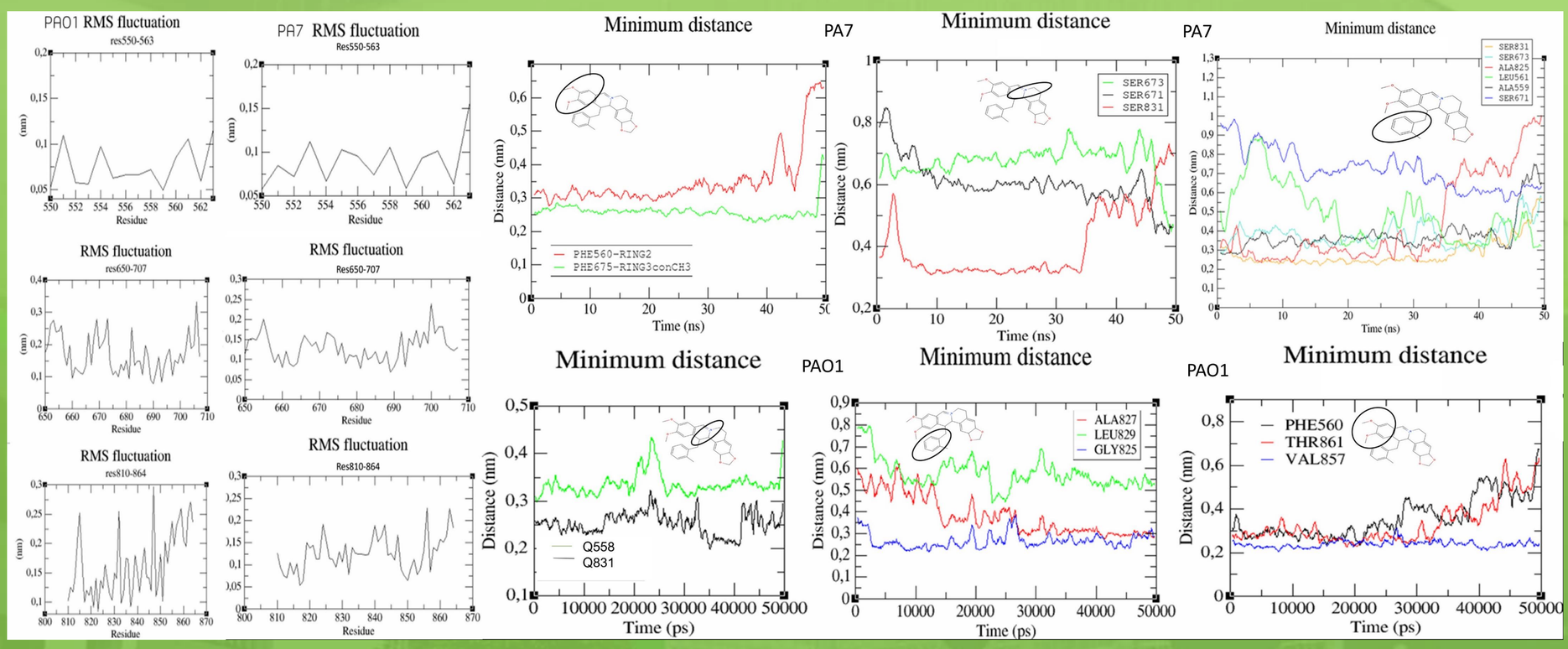
Free Gibbs energy of binding kJ/mol

MIC+EPI µg/mL



First computational step

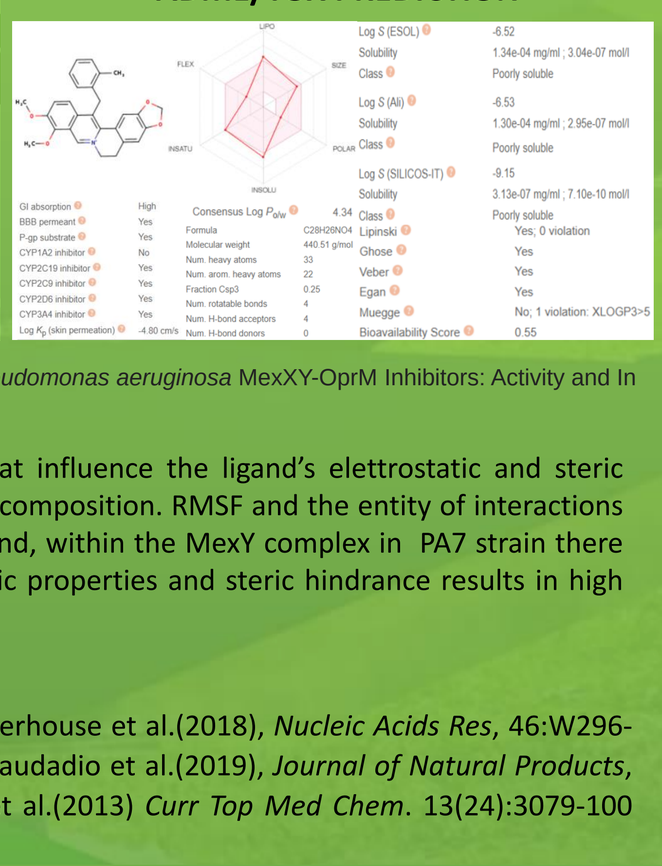
- ✓ Sequences multi-alignment highlighting mutations.
- ✓ Comparison of different ligands poses within inhibition site pocket.
- ✓ Analysis of molecular interactions.
- ✓ Molecular dynamic simulations of macromolecular systems, assessing the ligands' stability within pockets



Second step: *in silico/in vitro* analysis

- ✓ *In vitro* microbiological testing
- ✓ ΔG calculations with MM/PBSA method
- ✓ Analysis of RMSF for pocket's residues
- ✓ Analysis of interacting residues along the MD trajectories.
- ✓ Comparison of MexY PA7 and PAO1 complexes with 13-(2-CH_3benzyl)berberine

ADME/TOX PREDICTION



Giorgini, G.; Mangiaterra, G.; Cedraro, N.; Laudadio, E.; Sabbatini, G.; Cantarini, M.; Minnelli, C.; Mobbili, G.; Frangipani, E.; Biavasco, F.; Galeazzi, R. Berberine Derivatives as *Pseudomonas aeruginosa* MexXY-OprM Inhibitors: Activity and In Silico Insights. *Molecules* **2021**, *26*, 6644. https://doi.org/10.3390/molecules2621664

Conclusion: PA7 MexY protein presents a huge number of residues mutations within the inhibition site with respect to the PAO1 one that influence the ligand's elettrostatic and steric complementariety guiding interactions; thus is useful to consider such variations for choosing the best substituent in function of the aminoacidic composition. RMSF and the entity of interactions during the simulation time evidence both quantitatively and qualitatively the best stability of the orto-methyl-benzyl derivative. For this compound, within the MexY complex in PA7 strain there are efficacious interactions more than in PAO1, evidencing the influence of aminoacidic pocket. The substituent according to its stereoelectronic properties and steric hindrance results in high stability *in silico* and besides confirms the best synergic activity with Tobramycin in the microbiological vitro testing.

References:

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)**K.Katoh et al.(2019), *Briefings in Bioinformatics*, Vol20,4,1160-1166. **6)**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.Mangiaterra et al.(2021), *J.Nat.Prod*. 84, 993-1001. **9)**M.J.Abraham(2015), *SoftwareX*, Vol1-2,pp.91 **10)**P.Ruggerone et al.(2013) *Curr Top Med Chem*. 13(24):3079-100 **11)**I.Alav et al.(2021), *Chem.Rev*. Vol.121,pp.5479-5596