Synthèse de nouveaux antibiotiques potentiels identifiés par criblage virtuel ciblant la protéine FtsZ du divisome bactérien

Synthesis of new potential antibiotics identified by structure based virtual screening targeting FtsZ proteins from the bacterial divisome

Laurent Soulère, Yves Queneau, Oana Dumitrecu, Gérard Lina

Contact: Laurent Soulère (Laurent.soulere@insa-lyon.fr)

Antibiotics are facing an existential crisis less than a century since their introduction. The bacteria-fighting drugs are becoming less effective as a result of their overuse in both humans and animals. At the same time, R&D on new antibiotics has slowed to a crawl, putting the world at risk of entering a dangerous era in which routine infections are untreatable. That is the reason why, there is an incentive to seek and develop new effective agents with improved activity against resistant pathogens.[1]

Like their human homologue tubulin, FtsZ proteins play a major role in bacterial cell division. Subsequently to FtsZ polymerization, the contractile Z ring allows bacterial proliferation. Due to this central role, these proteins have recently been recognized as valuable targets to fight against multi-resistant bacterial infections.[2]

Considering this context, the aim of the work is to synthesize new potential antibiotics identified by structure based virtual screening directed to the PC190723 allosteric binding site of FtsZ proteins (Figure 1).[3]

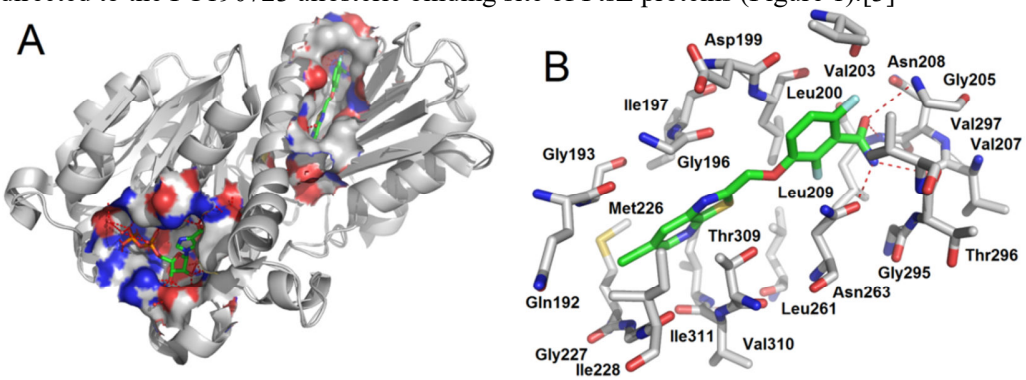

Figure 1: **A**: the 3D-structure of FtsZ from Staphylococcus aureus showing nucleotide (left) and PC190723 (right) binding sites. **B**: The PC190723 binding site with all amino acids interacting with PC190723. (pdb code 4DXD)

The research program for this project consists in the choice of virtual chemical libraries with high structural diversity such as ZINC, Asinex compounds databases... These compounds databases will be then used for docking-based virtual screening targeting the allosteric PC190723 binding site. PC190723 is a potent anti-staphylococcal compound which binds the FtsZ protein acting as polymer stabilizer agent rendering impossible bacterial cell to divide.[4] Since the discovery of this compound, X-Ray structure of the complex between the FtsZ protein of *S. aureus* and PC190723 has been resolved.[5] This structure allows the docking-based virtual screening targeting either the nucleotide binding site[6-8] or the allosteric PC190723 binding site. The latter has been poorly reported in the literature and related studies are deprived of biological evaluations.[3, 9] Subsequently to docking-based virtual screening,[10, 11] Visual inspection of docking results of compounds with high binding potentialities based on scoring function[12, 13] and relative ranking will be performed to select compounds for further biological evaluations. Selected compounds will be either purchased or synthesized to be tested by estimating their MIC. According to biological evaluation, for the promising compounds, structural refinement will be performed using organic synthesis to improve their activity in terms of MIC. Compounds with the best MIC values will then be tested for their ability to interact with FtsZ in particular by Surface Plasmon Resonance (SPR), Isothermal titration calorimetry and by Monitoring FtsZ assembly.

To summarize the research program contains the following milestones:

1) Selections of virtual chemical libraries with structural diversity (Zinc, Asinex…)
2) Docking based virtual screening of the selected databases using the X-ray structure of Ftsz Proteins.
3) Compounds selection following visual inspection of docking results of compounds with high binding potentialities based on scoring function and relative ranking.

4) Synthesis of the compounds selected from the virtual screening.
5) Biological evaluation on bacteria and multi-resistant bacteria (MIC evaluation).
6) Structural refinement using organic synthesis to improve biological activity (MIC evaluation).
7) Estimation for the most promising compounds of their interaction with FtsZ in particular by Surface Plasmon Resonance (SPR), Isothermal titration calorimetry and by Monitoring FtsZ assembly.

Several families of compounds will be identified, synthesized and tested. For example, a screening using a chemical library with high structural diversity, a platinum gold asinex subset library of 12055 compounds, has provided candidates with potential FtsZ inhibition activity. This study, described by L. Soulère and co-workers [3] led to several compounds including a biaryl derivative (Figure 2). It should be noted that biaryl derivatives has been mentioned in the literature as FtsZ inhibitors.[14]

Compounds in this family will be synthesized using Buchwald-Hartwig cross coupling reactions between halogeno-biphenyl and diverse anilines to further study their biological activities regarding MIC value and its effect on FtsZ assembly.[15]

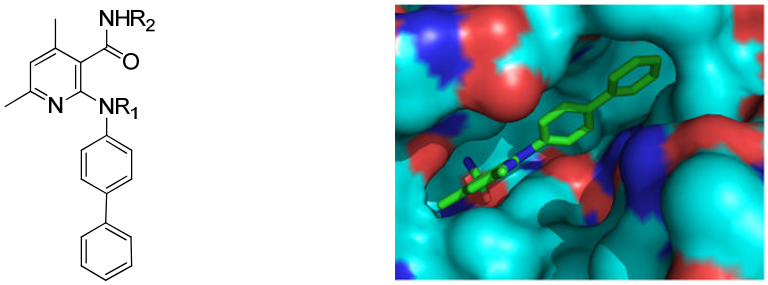

Figure 2: A biaryl derivative as potential FtsZ inhibitors targeting the PC190723 binding site. [3]

All compounds will be tested in collaboration with the CIRI, INSERM U1111, Staphylococcal pathogenesis team headed by Gérard Lina. Compounds with antibacterial activity will be further tested for their affinity to FtsZ in collaboration with the ASPE team (ICBMS). Further synthesis of fluorinated analogs of active compounds will be performed in collaboration with the SURCOOF team (ICBMS).

References

[1] J.S. Bradley, R. Guidos, S. Baragona, J.G. Bartlett, E. Rubinstein, G.G. Zhanel, M.D. Tino, D.L. Pompliano, F. Tally, P. Tipirneni, G.S. Tillotson, J.H. Powers, Anti-infective research and development--problems, challenges, and solutions, Lancet Infect. Dis. 7 (2007) 68-78.
[2] K. Haranahalli, S. Tong, I. Ojima, Recent advances in the discovery and development of antibacterial agents targeting the cell-division protein FtsZ, Bioorg. Med. Chem. 24 (2016) 6354-6369.
[3] L. Soulère, C.O. Soulage, Exploring docking methods for virtual screening: application to the identification of neuraminidase and Ftsz potential inhibitors, Molecular Simulation 43 (2017) 656-663.
[4] D.J. Haydon, N.R. Stokes, R. Ure, G. Galbraith, J.M. Bennett, D.R. Brown, P.J. Baker, V.V. Barynin, D.W. Rice, S.E. Sedelnikova, J.R. Heal, J.M. Sheridan, S.T. Aiwale, P.K. Chauhan, A. Srivastava, A. Taneja, I. Collins, J. Errington, L.G. Czaplewski, An inhibitor of FtsZ with potent and selective anti-staphylococcal activity, Science 321 (2008) 1673-1675.
[5] C.M. Tan, A.G. Therien, J. Lu, S.H. Lee, A. Caron, C.J. Gill, C. Lebeau-Jacob, L. Benton-Perdomo, J.M. Monteiro, P.M. Pereira, N.L. Elsen, J. Wu, K. Deschamps, M. Petcu, S. Wong, E. Daigneault, S. Kramer, L. Liang, E. Maxwell, D. Claveau, J. Vaillancourt, K. Skorey, J. Tam, H. Wang, T.C. Meredith, S. Sillaots, L. Wang-Jarantow, Y. Ramtohul, E. Langlois, F. Landry, J.C. Reid, G. Parthasarathy, S. Sharma, A. Baryshnikova, K.J. Lumb, M.G. Pinho, S.M. Soisson, T. Roemer, Restoring methicillin-resistant Staphylococcus aureus susceptibility to beta-lactam antibiotics, Sci. Transl. Med. 4 (2012) 126ra135.
[6] S. Mikuni, K. Kodama, A. Sasaki, N. Kohira, H. Maki, M. Munetomo, K. Maenaka, M. Kinjo, Screening for FtsZ Dimerization Inhibitors Using Fluorescence Cross-Correlation Spectroscopy and Surface Resonance Plasmon Analysis, PLoS One 10 (2015) e0130933.
[7] F.Y. Chan, N. Sun, M.A. Neves, P.C. Lam, W.H. Chung, L.K. Wong, H.Y. Chow, D.L. Ma, P.H. Chan, Y.C. Leung, T.H. Chan, R. Abagyan, K.Y. Wong, Identification of a new class of FtsZ inhibitors by structure-based design and in vitro screening, J. Chem. Inf. Model. 53 (2013) 2131-2140.
[8] L.B. Ruiz-Avila, S. Huecas, M. Artola, A. Vergonos, E. Ramirez-Aportela, E. Cercenado, I. Barasoain, H. Vazquez-Villa, M. Martin-Fontecha, P. Chacon, M.L. Lopez-Rodriguez, J.M. Andreu, Synthetic inhibitors of bacterial cell division targeting the GTP-binding site of FtsZ, ACS Chem. Biol. 8 (2013) 2072-2083.
[9] P. Vijayalakshmi, J. Nisha, M. Rajalakshmi, Virtual screening of potential inhibitor against FtsZ protein from Staphylococcus aureus, Interdiscip. Sci. 6 (2014) 331-339.
[10] Ulrich Rester, Dock around the Clock - Current Status of Small Molecule Docking and Scoring, QSAR & Comb. Sci. 25 (2006) 605-615.
[11] R. Dias, W.F. de Azevedo, Jr., Molecular docking algorithms, Curr. Drug Targets 9 (2008) 1040-1047.
[12] D.B. Kitchen, H. Decornez, J.R. Furr, J. Bajorath, Docking and scoring in virtual screening for drug discovery: methods and applications, Nat. Rev. Drug. Discov. 3 (2004) 935-949.
[13] R. Wang, L. Lai, S. Wang, Further development and validation of empirical scoring functions for structure-based binding affinity prediction, J. Comput. Aided Mol. Des. 16 (2002) 11-26.
[14] M. Kaul, A.K. Parhi, Y. Zhang, E.J. LaVoie, S. Tuske, E. Arnold, J.E. Kerrigan, D.S. Pilch, A bactericidal guanidinomethyl biaryl that alters the dynamics of bacterial FtsZ polymerization, J. Med. Chem. 55 (2012) 10160-10176.
[15] V. Straniero, C. Zanotto, L. Straniero, A. Casiraghi, S. Duga, A. Radaelli, C. De Giuli Morghen, E. Valoti, 2,6-Difluorobenzamide Inhibitors of Bacterial Cell Division Protein FtsZ: Design, Synthesis, and Structure-Activity Relationships, ChemMedChem 12 (2017) 1303-1318.