Small boron-containing compounds as modulator of the bacterial SOS response via inhibition of the transcriptional repressor LexA.

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Abstract

Aims: In *Escherichia coli* more than 60 genes involved in SOS response network activation are under control of the transcriptional repressor LexA which can be considered as an efficacious target to counteract the bacterial mechanisms of adaptation to antimicrobials. The inhibition of LexA self-cleavage can be exploited as modulator of the bacterial SOS response to increase the life of currently available chemotherapeutic agents by increasing bacterial drug susceptibility and by reducing the emergence of antimicrobial resistance. We investigated the inhibition of LexA autoproteolysis by small boron-containing compounds.

Method and Material: LexA gene was cloned into the overexpression vector pet28b(+) and the protein was purify by His-tagged protein method. Several boronic acid derivatives were tested following the inhibition of LexA protein self-cleavage trough the variation of the first order rate constant by Liquid Chromatography-Mass Spectrometry (LC-MS). *In silico* analysis was performed to predict the binding orientation of inhibitors in the active site upon covalent binding to the catalytic serine 119. The ability of boronic acids to interfere with the SOS response induced activation, was confirmed by filamentation assay.

Findings: Small phenylboronic acid derivatives efficaciously inhibit LexA self-cleavage, specifically, 3-amino- and 3-hydroxy-phenylboronic acid, in about 1:40 LexA:inhibitor ratio.

Molecular modelling analysis showed the formation of an acyl-enzyme intermediate thought interaction with Ser-119. In order to describe the inhibition potency in autoproteolytic enzyme, a new equation was disclosed. The inhibition of bacterial filamentation as results of disturbance of septum formation, firmly supports the interference exerted by the compounds with SOS response.

Conclusion & Significance: Results demonstrate that small boron-containing compounds efficaciously inhibit LexA autoproteolysis disturbing SOS activation. They represent the starting point for a hit-to-lead optimization process toward more effective LexA inhibitors with the aim to prolong the life of new and old antibiotics whose efficacy is threatening by the emergence of resistance.

Image

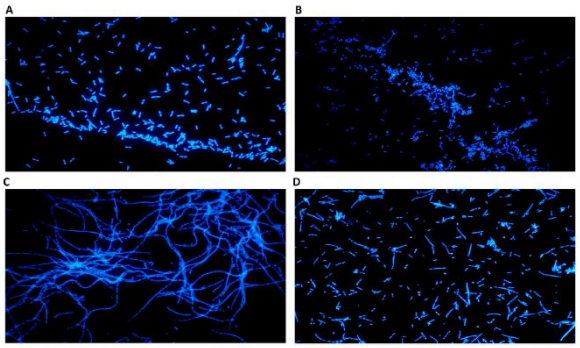


Fig. 6. Fluorescence microscopy of *E. coli* with DAPI. (A) Control; (B) 128 μg/mL compound 3; (C) 0.0039 μg/mL levofloxacin; (D) 0.0039 μg/mL levofloxacin + 128 μg/mL compound 3.

Recent Publications

1. P. Bellio, F. Brisdelli, M. Perilli, A. Sabatini, C. Bottoni, B. Segatore, D. Setacci,G. Amicosante, G. Celenza, Curcumin inhibits the SOS response induced by levofloxacin *in Escherichia coli*, Phytomedicine 21 (2014) 430–434.
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3. G. Celenza, M. Vicario, P. Bellio, P. Linciano, M. Perilli, A. Oliver, J. Blazquez, L. Cendron, D. Tondi, Phenylboronic acid derivatives as validated leads active in clinical strains overexpressing KPC-2: a step against bacterial resistance, ChemMedChem 13 (2018) 713–724.
4. F. Spyrakis, P. Bellio, A. Quotadamo, P. Linciano, P. Benedetti, G. D'Arrigo, M. Baroni, L. Cendron, G. Celenza, D. Tondi, First virtual screening and experimental validation of inhibitors targeting GES-5 carbapenemase, J. Comput. Aided Mol. Des. 33 (2019) 295–305.
5. L. Cendron, A. Quotadamo, L. Maso, P. Bellio, M. Montanari, G. Celenza, A. Venturelli, M.P. Costi, D. Tondi, X-ray crystallography deciphers the activity of broad-spectrum boronic acid β-lactamase inhibitors, ACS Med. Chem. Lett. 10 (2019)650–655.



Biography

Pr. Giuseppe Celenza is Associate Professor in Clinical Biochemistry at the University of l'Aquila (Italy). His research is mainly focused on the study of the biochemical mechanisms of resistance to antimicrobials in pathogenic bacteria, specifically, antibiotic modifying enzymes such as serine and metallo beta-lactamases. In the last years he pointed his attention to the bacterial SOS response mediated by the LexA-RecA system as target to withstand antimicrobial resistance by hindering the bacterial mechanisms of adaptation to antimicrobials and how the modulation of the SOS response activation can be achieved by inhibition of the transcriptional repressor LexA and the bacterial recombinase RecA. of evaluation: measurement, description and judgment. It allows for value-pluralism. This approach is responsive to all stakeholders and has a different way of focusing.

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Notes/Comments: