Biocidal Cationic Macromolecules Irrespective of Bacterial Resistance: Our Best Achievements

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Our Ongoing Studies

Initial Considerations

Difficult-to-treat bacterial infections involving resistant human and plant pathogens, severely afflict hospitals and concern the agri-food sector. Bacteria of the genus Pseudomonas can quickly become resistant to antibiotics and spread such resistance to other bacteria. Species such as P. aeruginosa, P. putida and P. fluorescens, which normally produce antibiotics or help reduce some forms of pollution, can trigger serious nosocomial infections in humans. P. fragi is a major cause of dairy and meat spoilage, while P. syringae can infect a wide range of economically important plant species, including tobacco, kiwi and tomato. Therefore, new strategies and antibacterial agents capable of stopping these bacteria, regardless of their resistance to antibiotics, are urgently needed to limit serious human infections, food waste, plantation extermination and economic losses.

The Promising Candidate Molecule for Our Project

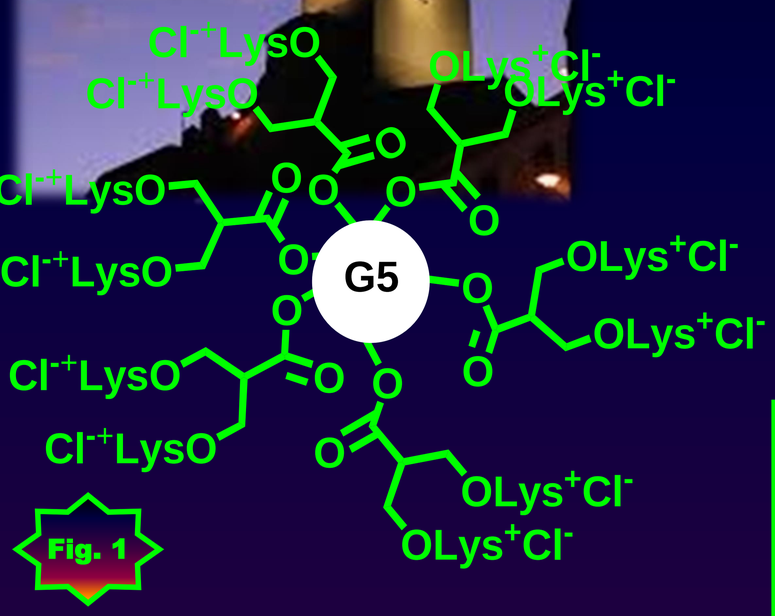

Fig. 1

Lys = lysine; *G5* = generations number

We recently published the auto-biodegradability and rapid bactericidal activity of a fifth-generation dendrimer (G5-PDK) (Fig. 1) against A. baumannii, A. johnsonii, A. junii, A. pittii and A. ursingii (MIC = 3.2-12.7 μM) [1]. Being active against different species of the genus Acinetobacter, we have evaluated the antibacterial effects of G5-PDK also against representatives of other non-fermenting Gram-negative species, such as Pseudomonads. Interestingly, while G5-PDK was practically inactive against the P. aeruginosa isolate tested in these first investigations (MIC => 6.4 μM), it showed very low MICs against other Pseudomonad species belonging to the Fluorescens group (MIC = 0.8 μM).

Fig.1 shows the simplified structure of G5-PDK, which is a cationic dendrimer consisting of two uncharged fifth-generation polyester-based umbrellas, attached to a propanediol (PD) core decorated with 64 lysine hydrochloride salts, which possess a total of 128 cationic groups.

To pursue our aim, in the present study G5-PDK was tested on several species of the genus Pseudomonas. MICs for G5-PDK were obtained by testing a total of 48 strains, including 32 strains of P. aeruginosa, 5 of P. fluorescens, 5 of P. putida, 3 of P. straminea and 1 representative of P. oleovorans, P. fragi and P. syringae species. The MICs observed for G5-PDK against all isolates tested were reported in Table 1, which also collects the selectivity indices (SIs) of G5-PDK for each strain, determined using the LD_{50} of G5-PDK obtained from experiments of cytotoxicity performed on human keratinocytes (Ha-CaT) (Fig 3). Time-kill experiments were reported in Fig. 4.

Table 4. MIC values of G5-PDK obtained on clinical and environmental isolates of the genus Pseudomonas from experiments conducted in triplicate and selectivity indexes (SI) of G5-PDK for each strain.

Strains	MIC μM (μg/mL)	Selectivity Index
P. aeruginosa 1* [1]	>6.4 (>128)	<13
P. aeruginosa 2# [1]	6.4 (128)	13
P. aeruginosa 4# [1]	6.4 (128)	13
P. aeruginosa 7* [1]	>6.4 (>128)	<13
P. aeruginosa 9# [1]	6.4 (128)	13
P. aeruginosa 10* [1]	>6.4 (>128)	<13
P. aeruginosa 11* [1]	>6.4 (>128)	<13
P. aeruginosa 12# [1]	6.4 (128)	13
P. aeruginosa 13* [1]	>6.4 (>128)	<13
P. aeruginosa 14* [1]	>6.4 (>128)	<13
P. aeruginosa 16* [1]	>6.4 (>128)	<13
P. aeruginosa 17* [1]	>6.4 (>128)	<13
P. aeruginosa 18* [1]	>6.4 (>128)	<13
P. aeruginosa 19* [1]	>6.4 (>128)	<13
P. aeruginosa 20* [1]	>6.4 (>128)	<13
P. aeruginosa 244§ [1]	1.6 (32)	51
P. aeruginosa 247* [1]	>6.4 (>128)	<13
P. aeruginosa 248* [1]	>6.4 (>128)	<13
P. aeruginosa 256# [1]	6.4 (128)	13
P. aeruginosa 259* [1]	>6.4 (>128)	<13
P. aeruginosa 402* [1]	>6.4 (>128)	<13
P. aeruginosa 403* [1]	>6.4 (>128)	<13
P. aeruginosa 405* [1]	>6.4 (>128)	<13
P. aeruginosa 426* [1]	>6.4 (>128)	<13
P. aeruginosa 427* [1]	>6.4 (>128)	<13
P. aeruginosa 428* [1]	>6.4 (>128)	<13
P. aeruginosa 432§ [1]	1.6 (32)	51
P. aeruginosa 433* [1]	>6.4 (>128)	<13
P. aeruginosa 434* [1]	>6.4 (>128)	<13
P. aeruginosa 435* [1]	>6.4 (>128)	<13
P. aeruginosa 436* [1]	>6.4 (>128)	<13
P. aeruginosa ATCC* [1]	>6.4 (>128)	<13
P. fluorescens A8# [2]	0.8 (16)	101
P. fluorescens SMM8# [2]	1.6 (32)	51
P. fluorescens SMI1# [2]	0.8 (16)	101
P. fluorescens SMI2# [2]	0.8 (16)	101
P. fluorescens SMI6# [2]	1.6 (32)	51
P. fragi G2# [2]	1.6 (32)	51
P. oleovorans# [1]	0.4 (8)	202
P. putida 262* [1]	3.2 (64)	25
P. putida 407* [1]	3.2 (64)	25
P. putida 409* [1]	3.2 (64)	25
P. putida 410* [1]	6.4 (128)	13
P. putida SMA1# [2]	0.8 (16)	101
P. straminea A5# [2]	1.6 (32)	51
P. straminea A7# [2]	1.6 (32)	51
P. straminea A13# [2]	1.6 (32)	51
P. syringae# [2]	0.2 (4)	404

[1] clinical isolates; [2] environmental isolates; # not pigmented colonies (bacteria not producing pigments); * yellowish-green, green-blue, blue strains (pyoverdine and/or pyocyanin-producers); § brown strains (pyomelanin-producers).

1. Alfei, S.; Caviglia, D.; Piatti, G.; Zuccari, G.; Schito, A.M. Bactericidal Activity of a Self-Biodegradable Lysine-Containing Dendrimer against Clinical Isolates of *Acinetobacter* Genus. *Int. J. Mol. Sci.* 2021, *22*, 7274. https://doi.org/10.3390/ijms22147274.

Strong Correlation Between the Antibacterial Effects of G5-PDK and the Production of Bacterial Pigments

G5-PDK showed MIC > 6.4 μM on 78.1% of the P. aeruginosa strains reported in Table 1 which produced yellowish, yellow-green, green-blue or blue pigmented colonies (* strains in Table 1, Fig. 2), because they are producers of pyoverdine and/or pyocyanin (Fig. 2). Non-pigmented P. aeruginosa isolates (strains 2, 4, 9, 12, Table 1, Fig. 2) showed lower MICs = 6.4 μM. Finally, much lower MICs (1.6 μM) were observed on brown pigmented P. aeruginosa strains 244 (Table 1, Fig. 2) and 432 (Table 1), which produce pyomelanin (Fig. 2) .

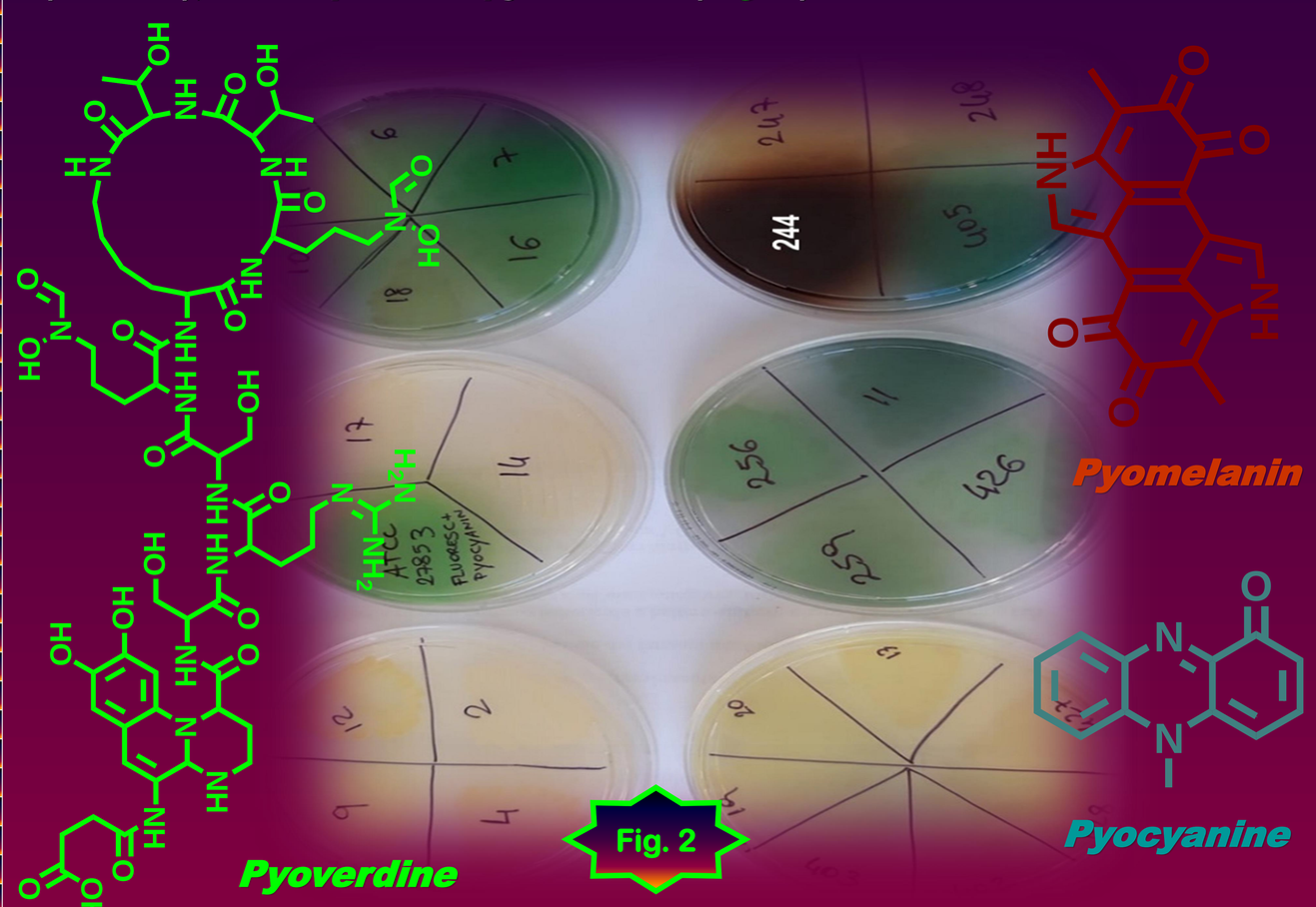

Fig. 2

Pyocyanin and pyoverdine are cationic molecules whose secretion can repel other cationic molecules, such as G5-PDK, thus justifying the low activity of G5-PDK towards the yellow to blue-green pigmented P. aeruginosa isolates of this study. On the contrary, pyomelanin is a negatively charged pigment which, once secreted, creates an anionic environment, favoring the adsorption of cationic antibacterial agents, such as G5-PDK. This may explain the high susceptibility of the pyomelanin producing strains in this study (strains 244 and 432) to G5PDK. Regarding the non-pigmented strains of the P. aeruginosa species (strains 2,4,9 and 12), the interaction of G5-PDK with the bacterial surface was neither hindered nor promoted and MIC = 6.4 μM were observed. Very low MICs (0,2,0.4,1,6 μM) were observed on P. straminea, P. fragi, P. oleovorans and P. syringae because they were unable to produce pigments. With respect to P. fluorescence and P. putida producing pyoverdine only in iron deficiency, higher MICs have been observed for clinical isolates producing pyroverdine to steal iron from the human host.

Time-kill experiments

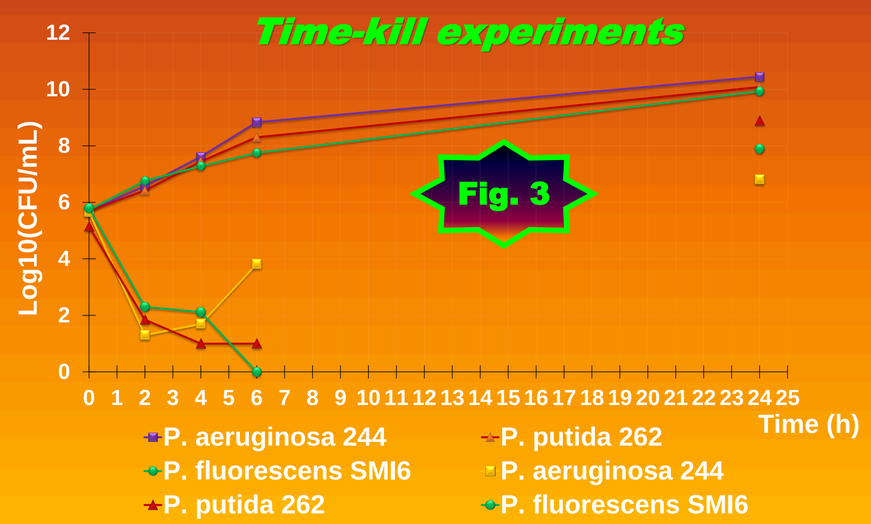

Fig. 3

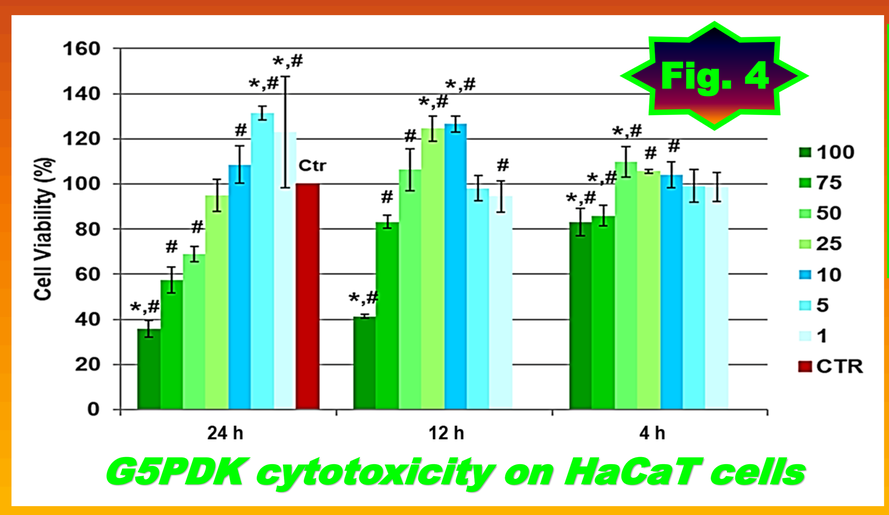

Fig. 4

G5PDK cytotoxicity on HaCaT cells

Conclusions

Overall, G5-PDK due to its low cytotoxicity and high SI, could represent a promising new antibacterial agent to limit serious human infections. Furthermore, it could also find applications to limit food waste and the destruction of economically significant plantations such as tobacco, kiwi and tomato.

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