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Quaternization of fluoroquinolones – novel permanently ionized antibacterials active against biofilms

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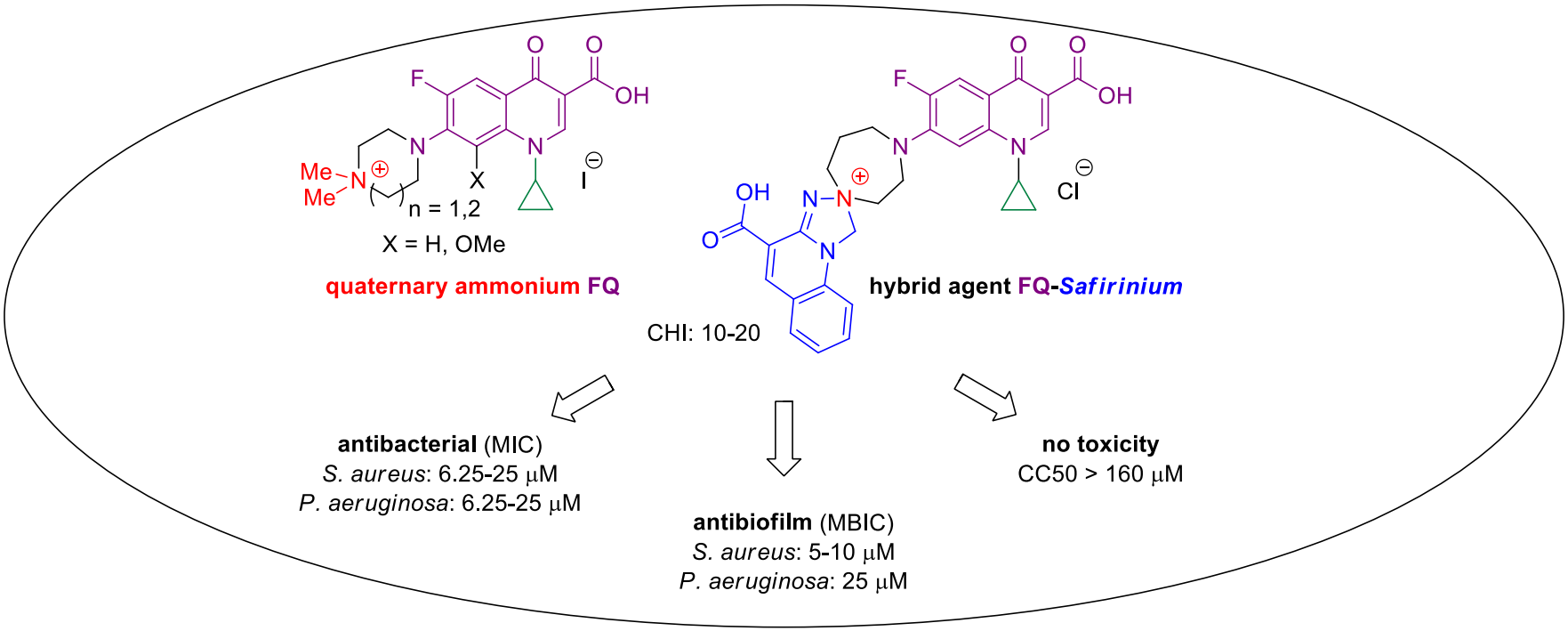
Graphical Abstract



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Abstract:

Increasing antimicrobial resistance poses a critical problem to public health. Many strategies are engaged to combat this problem; one of them is the synthesis of hybrid drugs consisting of diverse bioactive parts merged into one molecule. The risk that bacteria will mutate and evolve defense mechanisms is lower since the various pharmacophoric parts act against different molecular targets.

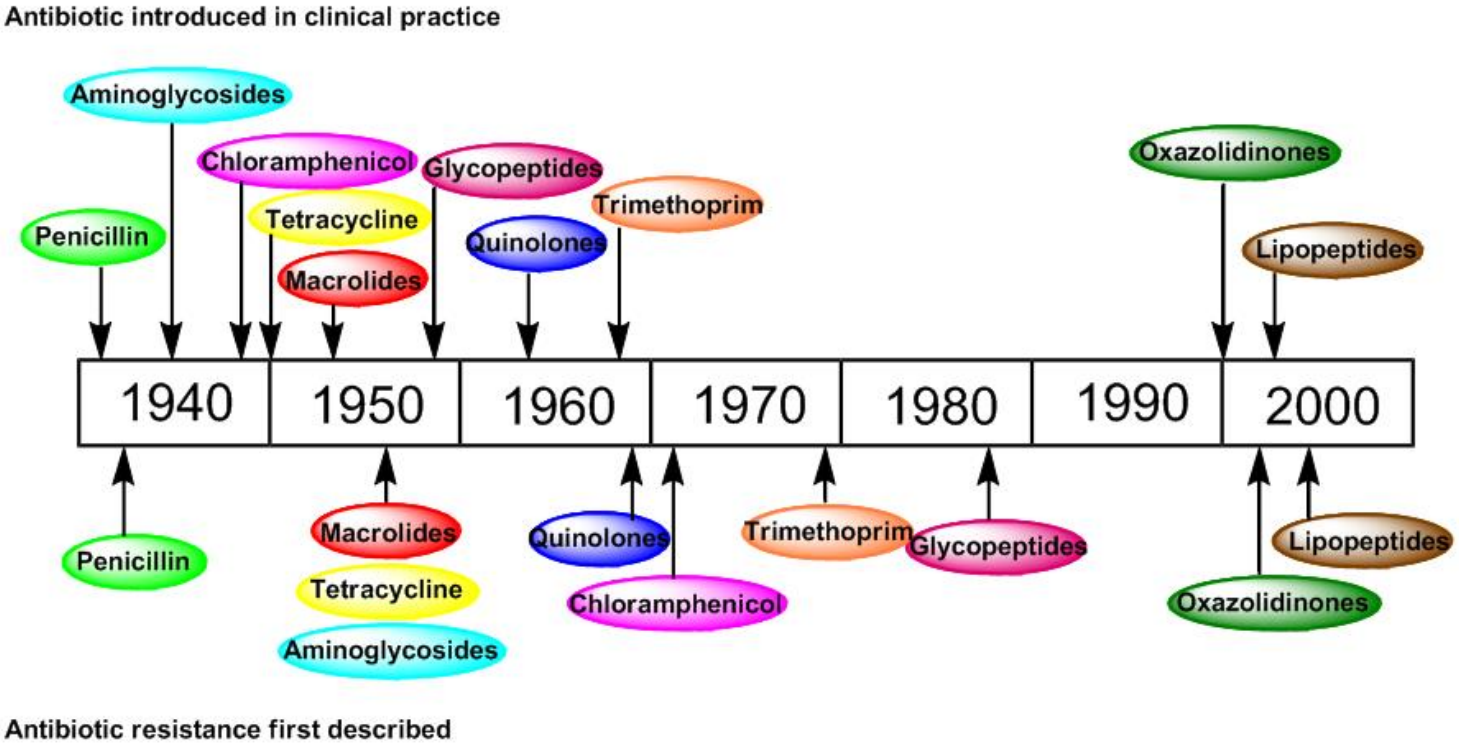
We developed a new class of fluoroquinolone-quaternary ammonium conjugates to obtain inhibitors of bacterial topoisomerases with membrane destabilization activity. Docking studies revealed that compounds can interact with topoisomerases in the fluoroquinolone-binding mode. Hybrids were screened against Gram-positive and negative bacteria, including pathogens from the ESKAPE group and antibiotic-resistant strains, in planktonic and biofilm forms. The most effective were moderately lipophilic (CHI 10-20) cyclopropyl-substituted molecules. Moreover, novel compounds exhibit negligible cytotoxicity.

Keywords: Antibacterial activity; Drug synthesis; Molecular docking; Quinolone, QAC

Introduction

Antimicrobial resistance is one of the most threatening problems of global concern.

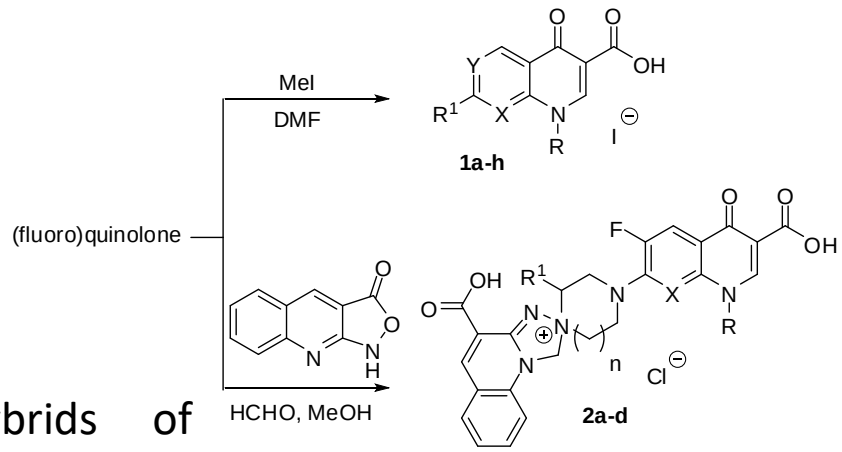
The hardest to combat are multidrug-resistant ESKAPE pathogens due to their ability to form antibiotic-tolerant biofilms.



These facts highlight the need for a renewed research effort by both the academia and pharmaceutical industry in the fight against antibiotic resistance.

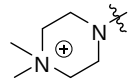
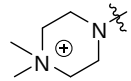
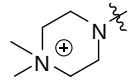
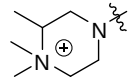
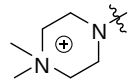
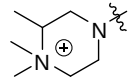
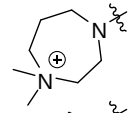
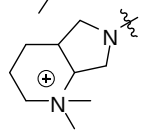
Results and discussion

In the study, a series of novel dimethyl quaternary ammonium fluoroquinolone (FQ) derivatives **1a-h** was designed, synthesized and evaluated in terms of their antibacterial, cytotoxic, and physicochemical properties.



Compound	X	R	R^1	n
2a	N	Et	H	1
2b	CF	Et	Me	1
2c	CH		H	1
2d	CH		H	2

Quaternary hybrids of *Safirinium* dyes and FQs **2a-c** that were synthesized previously were added to the present research for comparison.

Compound	Y	X	R	R^1
1a	N	N	Et	
1b	CF	N	Et	
1c	CF	CH	Et	
1d	CF	CF	Et	
1e	CF	CH		
1f	CF	COMe		
1g	CF	CH		
1h	CF	COMe		

Results and discussion

The tested Gram-positive and Gram-negative pathogenic strains, *i.e. Staphylococcus aureus* and *Pseudomonas aeruginosa*, were sensitive to the majority of the obtained compounds. Derivatives **1e** and **1g**, N^+-dimethyl analogues of ciprofloxacin, displayed broad-spectrum activity with minimum inhibitory concentrations (MIC) of 6.25 µM both selected towards bacteria, while ciprofloxacin hybrid compound **2d** showed slightly weaker effectiveness (MIC of 25 µM).

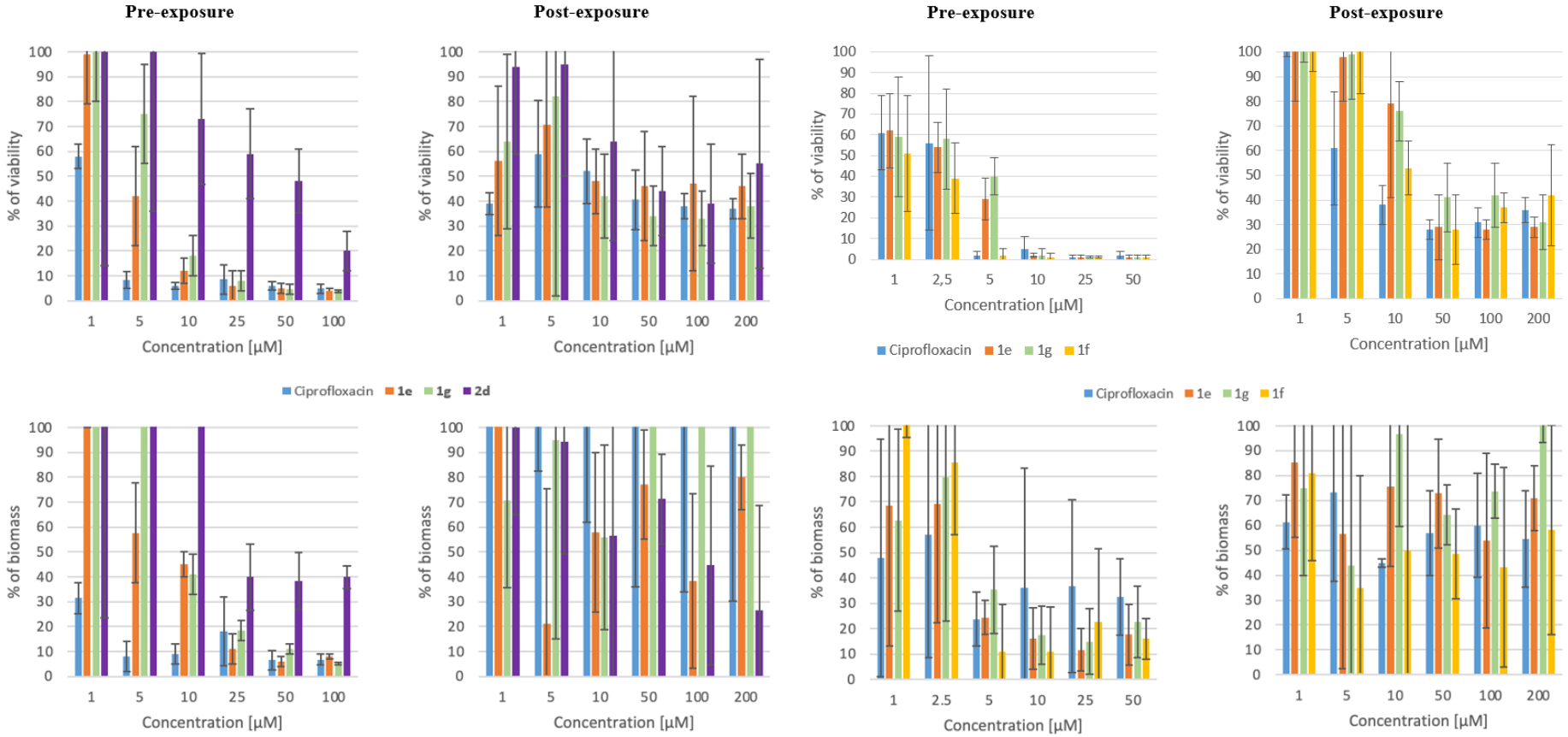
N^+-dimethyl quaternary gatifloxacin derivative **1f** was selective against Gram-positive strain (MIC of 6.25 µM).

Compound / MIC [µM]	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213
1b	>50	50
1c	>50	25
1d	75	25
1e	6.25	6.25
1f	>50	6.25
1g	6.25	6.25
1h	>50	50
2d	25	25
Ciprofloxacin hydrochloride	2.72	1.36

Results and discussion

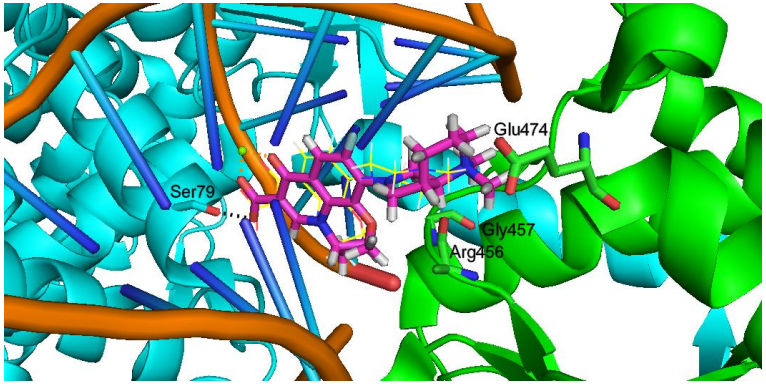
Moreover, the bioactive compounds **1e-g** and **2d** were found to be potent against biofilm in pre- and post-exposure tests with minimum biofilm inhibitory concentrations of 25 µM (**1e**,**g**) against *P. aeruginosa* and 5 (**1f**) or 10 µM (**1e**,**g**) towards *S. aureus*.

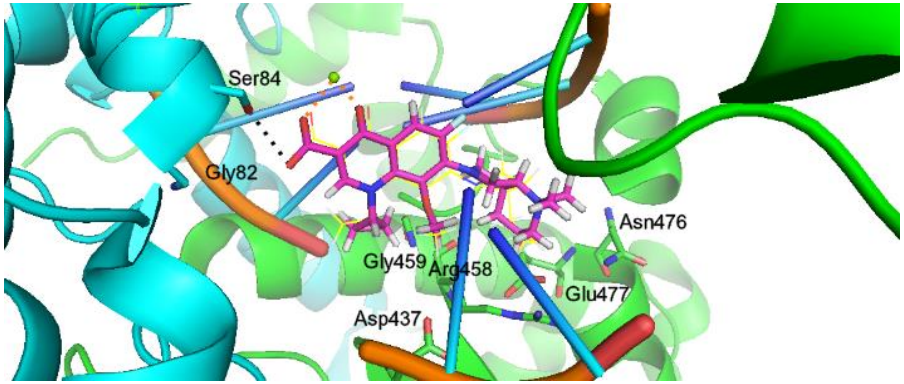
P. aeruginosa ***S. aureus***



Results and discussion

Furthermore, the molecular docking studies indicated that all the synthesized compounds interact in the FQ-binding mode at the active sites of bacterial type II topoisomerases and are able to inhibit the enzymes.





Selectivity of the active molecules towards bacterial cells was confirmed in cytotoxicity experiments that involved noncancerous cell lines (BALB/3T3 murine embryonic fibroblasts).

Compound / IC_{50} [μM]	BALB/3T3
1e	>200
1f	>200
1g	>200
2d	159.92 ± 9.23
Ciprofloxacin hydrochloride	>200

Conclusions

Analysis of the structure-activity relationships (SAR) suggests that the lipophilic cyclopropyl group present within the N1 position of the FQ scaffold was essential for preserving pronounced antibacterial action, whereas quaternization of the terminal nitrogen atom at aliphatic diamine in the N7 position was not detrimental to bioactivity. The above remark was further supported by data obtained with the use of immobilized artificial membrane chromatography (IAM-HPLC), since phospholipophilicity indices (CHI) of the highest active derivatives were in the range of 11.7-18.23. The value determined for ciprofloxacin, the reference drug, was marginally upper or comparable (19.7).

This outcome is principally compelling, as the classic equilibrium-driven interconversion among uncharged and ionized species is absent in the case of studied quaternary FQs. It proves the potential for novel antibacterials development and provides insights into the SAR of antibacterial agents derived from FQs. The described FQ-based compounds might be a promising starting point for the rational design of well-tolerated and highly potent antibiotics.

Acknowledgments

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BEKKER NAWA PROGRAMME

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