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Antibacterial and anti-biofilm activity of quinazolinone derived Schiff base and its Cu(II) complex

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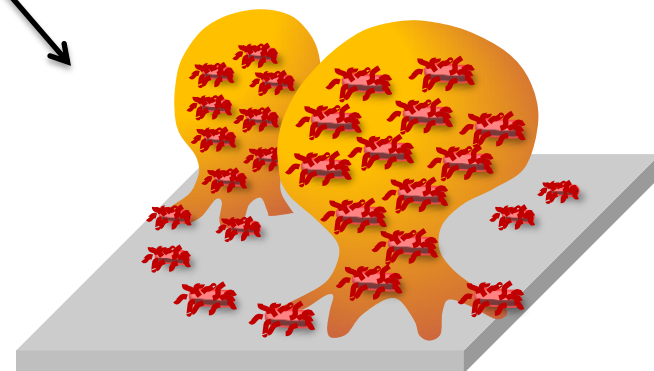
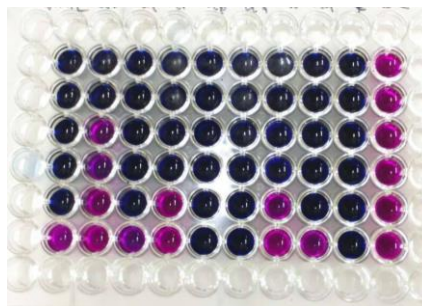
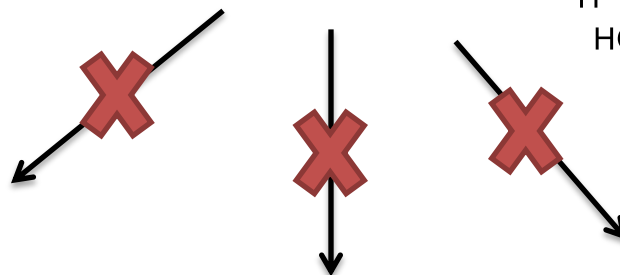
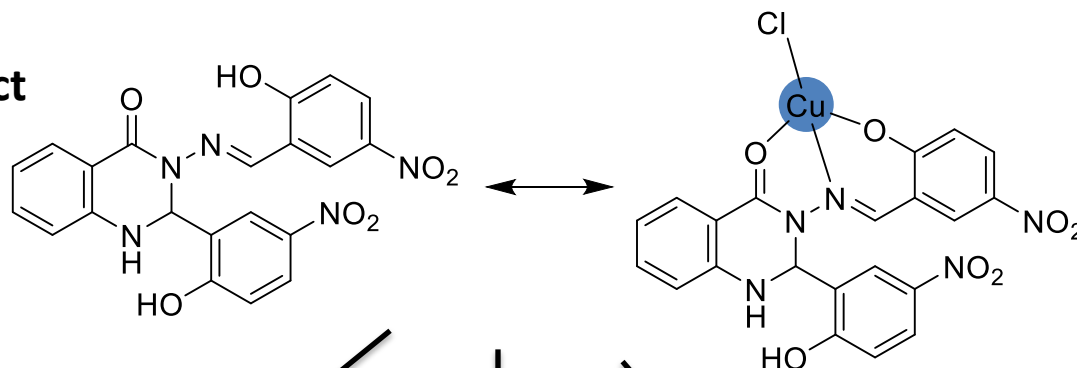
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Antibacterial and anti-biofilm activity of quinazolinone derived Schiff base and its Cu(II) complex

Graphical Abstract



Introduction

- Resistant bacteria are still an important global problem, which causes at least 700 000 deaths per year.
- Treating multi-resistant infections is not only more complicated, but also more expensive.
- Development of new active and safe antibacterial agents is necessary to overcome the risk of losing therapeutic perspectives for treating serious life-threatening infections.
- Schiff bases are a group of compounds with wide spectrum of activity such as antibacterial, anticancer or antioxidant. Due to their structure they are able to serve as ligands in metal complexes, which usually have higher activity than ligands alone.
- The aim of this work was to evaluate antibacterial and anti-biofilm activity of quinazolinone-based Schiff base: 3-[(2-hydroxy-5-nitrobenzylidene)-amino]-2-(2-hydroxy-5-nitrophenyl)-2,3-dihydroquinazolin-4(1*H*)-one and its Cu(II) complex.



Methods

- Minimum inhibitory concentrations (MICs) were tested by broth dilution method in microtitration plates. After incubation, the lowest concentration which inhibited growing od bacteria was evaluated visually. MICs against *M. tuberculosis* were examined using resazurin
- Following incubation, sub-cultivation of aliquots onto agar plates was used to assess **minimum bactericidal concentration (MBCs)**
- **Method of time-kill curves** was used to study dynamics of antibacterial activity against *S. aureus* ATCC 29213.
- Formed biofilm cultivated for 48 hours in tryptic soy broth with 2% of glucose was treated by different concentration of tested compound for 24 hours. **Minimum biofilm eradication concentration (MBEC₉₀)** was counted as the lowest concentration of the compound which increased viability of the cells by 90% compared to the growth control. Viability was measured by MTT method.



Results and discussion

Antibacterial activity of tested compounds

Table 1: Minimum inhibitory concentration (MIC [$\mu\text{g}/\text{mL}$]) / minimum bactericidal concentration (MBC [$\mu\text{g}/\text{mL}$]) against selected bacterial isolates.

Strain/isolate	Ligand	Cu(II) complex
<i>S. aureus</i> ATCC 29213	32/128	16/ >256
MRSA 63718	64 /128	64/64
MRSA SA 630	16/16	8/8
MRSA SA 3202	64/128	16/16
<i>E. faecalis</i> ATCC 29212	256/256	128/>256
<i>M. tuberculosis</i> H37Ra	64	32
<i>M. smegmatis</i> ATCC 700084	128/128	64/64
<i>M. kansasii</i> DSM 44162	128/128	128/128
<i>M. marinum</i> CAMP 5644	64	64



Results and discussion

Antibacterial activity of tested compounds

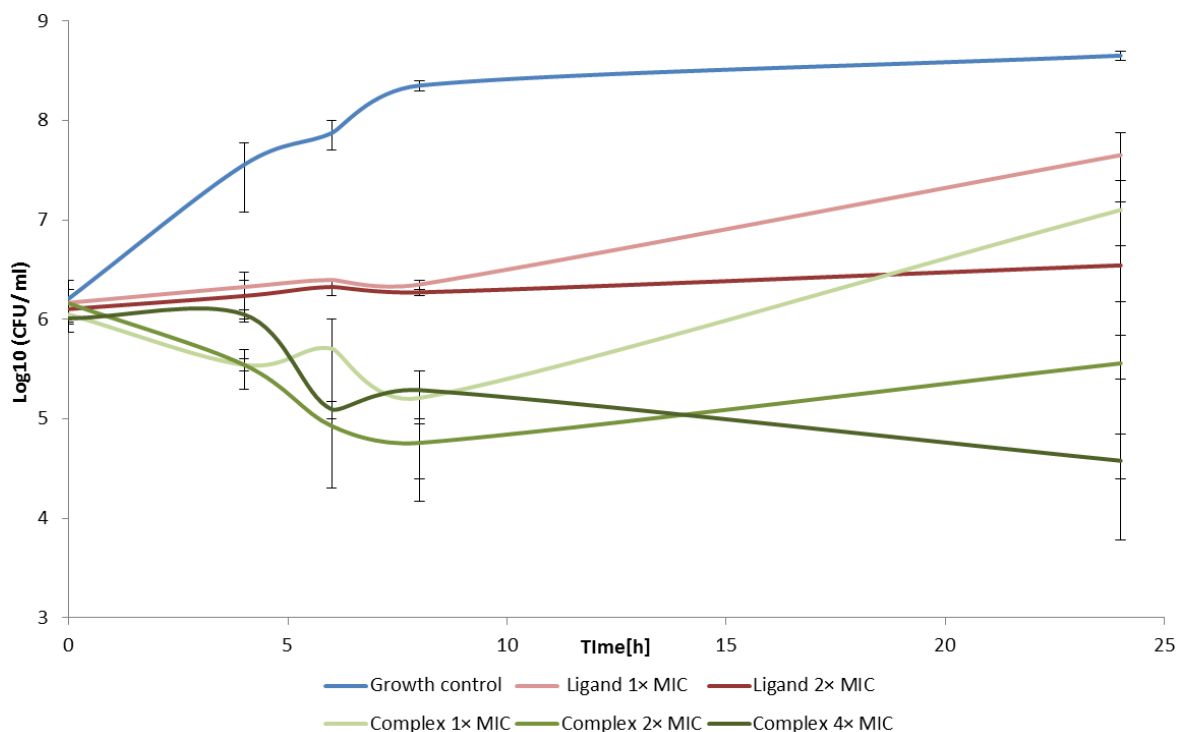
- The compounds showed good antibacterial activity against staphylococci including methicillin resistant isolates.
- Antimycobacterial activity was higher in the case of slowly growing mycobacteria as *M. marinum* and *M. tuberculosis*. Differences among the strains could be caused by differences in their cell wall components.
- The activity of Cu(II) complex was slightly higher than the activity of the ligand.
- *Enterococcus* was less sensitive than staphylococci, which can be caused its higher intrinsic resistance to antibacterial agents in general.



Results and discussion

Dynamics of antibacterial activity

Graph 1: Dynamics of antibacterial activity of both tested compounds against *S. aureus* ATCC 29213.



Results and discussion

Dynamics of antibacterial activity

- All of the compounds demonstrated only bacteriostatic activity, because the decrease of CFU/mL in all times and concentrations was $< 3\log$ compared to the time 0.
- Results were analysed using two-way ANOVA following Tukey test . At $P = 0.05$, the only statistically significant difference in activity was observed between ligand in concentration 1 MIC /24 hours and complex 2–4 MIC/24 hours.
- Thus overall, the difference in bactericidal activity among different concentration of ligands and complexes in different times is not statistically significant.

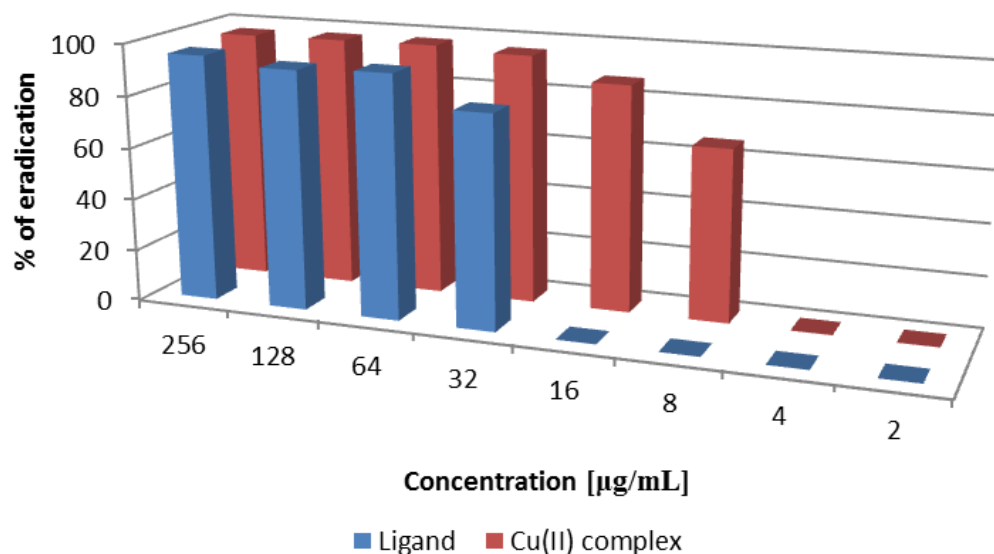


Results and discussion

Anti-biofilm activity against *S. aureus* ATCC 29213

Table 2: Comparison of MICs [$\mu\text{g/mL}$], MBCs [$\mu\text{g/mL}$] and MBECs₉₀ [$\mu\text{g/mL}$] of tested compounds against *S. aureus* ATCC 29213.

Comp.	MIC	MBC	MBEC ₉₀
Ligand	32	128	64
Cu (II) complex	16	>256	32



Graph 2: Eradication activity of tested compounds against formed staphylococcal biofilm.



Results and discussion

Anti-biofilm activity against *S. aureus* ATCC 29213

- The compounds showed very good activity against pre-formed staphylococcal biofilm, when the MBEC₉₀ was only 2-fold higher than MIC against planktonic cells.
- In concentration equal to MICs the anti-biofilm activity of Cu(II) complex was only slightly higher than activity of the ligand (87.3 ± 4% vs. 81.7 ± 13.7%).
- In the concentration equal to ½ MIC, the Cu(II) complex reduced viability of the film by 66.4 ± 10.6%, but the ligand did not have any effect.
- Cu(II) complex is an interesting compound for deeper research in the field of anti-biofilm active agents.



Conclusions

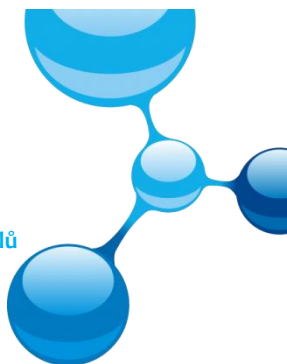
- Quinazolinone –based Schiff base and its Cu(II) complex were tested against a spectrum of bacterial pathogens, as well as against staphylococcal biofilm.
- The compounds showed good activity against staphylococci, which was defined as bacteriostatic using time-kill method.
- Anti-biofilm activity of these compounds is very promising, because the concentration needed to eradicate 90% of matured staphylococcal biofilm was only 2-fold higher than MICs against planktonic cells.
- The compounds are very perspective antibacterial agents and their features should be analysed deeper in following studies.



Acknowledgments

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