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Research article

Critical view on antimicrobial, antibiofilm and cytotoxic activities of quinazolin-4(3*H*)-one derived schiff bases and their Cu (II) complexes

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ABSTRACT

A series of nine 2,3-disubstituted-quinazolin-4(3*H*)-one derived Schiff bases and their three Cu(II) complexes was prepared and tested for their antimicrobial activities against reference strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 and resistant clinical isolates of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecalis* (VRE). All the substances were tested *in vitro* against *Mycobacterium tuberculosis* H_{37}Ra ATCC 25177, *M. kansasii* DSM 44162 and *M. smegmatis* ATCC 700084. While anti-enterococcal and anti-mycobacterial activities were insignificant, 3-[(*E*)-(2-hydroxy-5-nitrobenzylidene)amino]-2-(2-hydroxy-5-nitrophenyl)-2,3-dihydroquinazolin-4(1*H*)-one (**SB3**) and its Cu(II) complex (**SB3–Cu**) demonstrated bacteriostatic antistaphylococcal activity. In addition, both compounds, as well as the other two prepared complexes, showed antibiofilm activity, which resulted in a reduction of biofilm formation and eradication of mature *S. aureus* biofilm by 80% even at concentrations lower than the values of their minimum inhibitory concentrations. In addition, the compounds were tested for their cytotoxic effect on the human monocytic leukemia cell line THP-1. The antileukemic efficiency was improved by the preparation of Cu(II) complexes from the corresponding non-chelated Schiff base ligands.

1. Introduction

Quinazolines and quinazolinones, belonging to the large family of azanaphthalenes, can be considered privileged scaffolds [1], as

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they are characterized by a wide range of biological effects, such as anti(myco)bacterial, antifungal, antiprotozoal, antiviral, anti-cancer, anti-inflammatory or antihypertensive activities [2–5]. In addition, the quinazoline scaffold can be found in many alkaloids isolated from plants that have been used for centuries for a wide range of ailments in traditional folk medicine [6,7]. Based on the position (and number) of the oxo moiety, quinazolinone derivatives can be divided into three types: i) quinazolin-2(1*H*)-ones, ii) quinazolin-4(3*H*)-ones and iii) quinazoline-2,4(1*H*,3*H*)-diones. Whereas, the second group usually receives the most attention [8,9]. Quinazolinone heteroatoms can be functionalized, the simplest nitrogen substitution being the preparation of Schiff bases. Similar to the quinazolinone pharmacophore, Schiff bases are widely used chemical compounds with technical uses (catalysts, dyes), but at the same time they show a number of biological activities, including antibacterial, antifungal, anti-inflammatory or antipyretic [10–14].

The authors have long been engaged in the study of various types of heterocycles [3,15–19], primarily as anti-infective and anti-cancer agents with the aim of obtaining substances with dual (anti-cancer + antibacterial) activity [20–26], because such therapeutics are particularly advantageous in the treatment of oncological patients who have drug-suppressed immunity and for whom even banal infections and opportunistic pathogens are a threat. So impressed with the global interest in quinazolin-4(3*H*)-ones as antibacterial compounds [4,27–31], the previously described anticancer 2,3-disubstituted-quinazolin-4(3*H*)-one derived Schiff bases [32,33] were screened for their antimicrobial activity, and their cytotoxic potential was tested against the human monocytic leukemia cell line THP-1 and thus the spectrum of cytotoxic activities published earlier [32,33] was extended to another line. In addition to the previously described eight derivatives, the studied set was expanded by another compound and three Cu(II) complexes, so a total of twelve compounds were investigated. This study was composed as a proof-of-concept to reveal, at least partially, the structure-activity relationship of tested compounds and the impact on biological activities in the case of their usage as ligands in complexes.

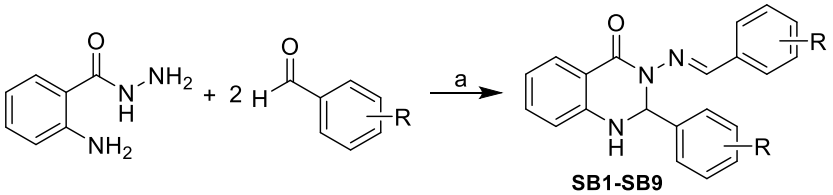
2. Results and discussion

2.1. Chemistry

The series of nine 2,3-disubstituted-quinazolin-4(3*H*)-one derived Schiff bases **SB1–SB9** was prepared by microwave-assisted phosphomolybdic acid (PMoA)-catalysed cyclocondensation reaction reported previously [32,33] and the procedure is depicted in Scheme 1. Mononuclear Cu(II) complexes were prepared by the reaction of copper chloride with equimolar amount of corresponding quinazolin-4(3*H*)-one Schiff base ligand **SB3**, **SB8** and **SB9**. In these Cu(II) complexes Schiff bases act as tridentate ligands through *O*, *N*,*O*-donor centers (Scheme 2). Compounds were obtained in good yields as air stable solids and were characterized by various spectroscopic methods [32]. In addition, the compounds were evaluated for their hydrolipophilic properties (Table 1); lipophilicities as logarithms of ligand capacity factors (log *k*) were experimentally determined by RP-HPLC and as Clog *P* were predicted by ChemBioDraw for all compounds.

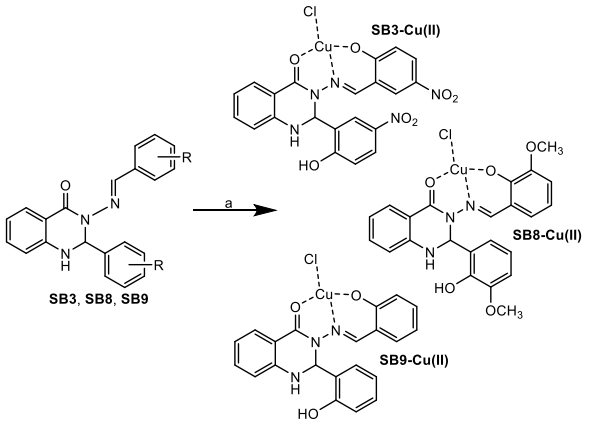
2.2. Interpretation of structural analysis of *SB3–Cu(II)*, *SB8–Cu(II)* and *SB9–Cu(II)* complexes

The UV/Vis spectra indicated significant differences between the ligands and the corresponding copper complexes. In the case of



	R^1	R^2	R^3	R^4	R^5
SB1	H	H	NO_2	H	H
SB2	H	NO_2	Cl	H	H
SB3	OH	H	H	NO_2	H
SB4	H	OCH_3	OH	NO_2	H
SB5	H	OCH_3	OH	OCH_3	H
SB6	H	OCH_3	OH	H	H
SB7	H	OH	OH	H	H
SB8	OH	OCH_3	H	H	H
SB9	OH	H	H	H	H

Scheme 1. Synthesis and structure of quinazolinone-based Schiff base ligands **SB1–SB9**. Reagents and conditions: (a) phosphomolybdic acid on silica gel ($PMoA/SiO_2$), EtOH, MW (200 W, 15 min) [32,33].



Scheme 2. Synthesis and proposed structure of Cu(II) complexes prepared from quinazolinone-based ligands **SB3**, **SB8** and **SB9**. Reagents and conditions: (a) $CuCl_2.2H_2O$, MeOH, 80 °C, 4 h [35].

Table 1
Experimentally determined (log *k*) and predicted (Clog *P*) lipophilicities values of investigated compounds, and *in vitro* antistaphylococcal activities (MIC [μg/ml]; for active agents, MICs also expressed in μM) compared to ampicillin (AMP), ciprofloxacin (CPX), and *in vitro* cell viability (IC_{50} [μM] ± SD, n = 6) on human monocytic leukemia cell line (THP-1) compared to cisplatin (CPT), cytotoxicity against non-cancer human renal proximal tubule epithelial TH-1 cells [33].

Comp.	log *k*	Clog *P*[a]	MIC [μg/ml] ([μM])				IC_{50} [μM]	
			SA	**MRSA1**	**MRSA2**	**MRSA3**	**THP-1**	**TH-1** [33]
SB1	0.7035	3.3548	>256	>256	>256	>256	>50	896
SB2	1.1392	4.4808	>256	>256	>256	>256	>50	>1000
SB3	0.6022	2.9630	32 (71.2)	64 (141)	16 (35.6)	64 (141)	12.7 ± 1.2	432
SB4	0.4470	2.9369	>256	>256	>256	>256	>50	>1000
SB5	−0.6045	1.7993	>256	>256	>256	>256	>50	374
SB6	−0.5500	2.2332	>256	>256	>256	>256	>50	>1000
SB7	−0.6600	1.3408	64 (164)	64 (164)	32 (81.8)	64 (164)	9.5 ± 1.1	178
SB8	0.1689	2.1832	>256	>256	>256	>256	>50	>1000
SB9	0.3153	2.4848	>256	>256	>256	>256	9.8 ± 1.3	–
SB3–Cu	–	2.9424	16 (29.2)	16 (29.2)	8 (14.6)	64 (116)	0.3 ± 1.2	–
SB8–Cu	–	2.8684	64 (123)	16 (30.8)	16 (30.8)	32 (61.7)	0.2 ± 1.4	–
SB9–Cu	–	3.1002	32 (69.8)	8 (17.4)	8 (17.4)	32 (69.8)	0.3 ± 1.4	–
AMP	–	–	2 (5.7)	>16 (>45.8)	>16 (>45.8)	16 (45.8)	–	–
CPX	–	–	1 (3)	8 (24.1)	64 (192)	8 (24.1)	–	–
CPT	–	–	–	–	–	–	16.1 ± 1.3	–

[a] ChemBioDrawUltra 13.0 (CambridgeSoft, PerkinElmer Inc., MA, USA); SA = *Staphylococcus aureus* ATCC 29213; MRSA1–3 = clinical isolates of methicillin-resistant *S. aureus* SA 3202, SA 630 (National Institute of Public Health, Prague, Czech Republic), and 63718 (Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Czech Republic).

ligand **SB3** (see Suppl. Materials, Fig. S1), the appearance of a broad absorption band was observed in the region from approximately 270 to 350 nm, corresponding to π-π transitions caused by the conjugation of phenyl rings with C═C bond and non-bond pairs of *N*–*N*═CH coupling. The presence of an auxochromic NO_2 group in the para position relative to the OH group on the phenyl ring caused a pronounced bathochromic shift of the absorption band to the 430 nm region, which was, moreover, partially enhanced by the presence of the OH group. The absorption in this region can be attributed to *n*-π transitions, which are typical for chromophores and auxochromic groups having heteroatoms linked via multiple bonds [34].

Chelation of the copper atom with **SB3** ligand led to changes in the spectrum as seen in Fig. S1. The coordination of copper with the OH group on the aromatic ring led to a weakening of the auxochromic effect of this group, which was reflected by a shift of the absorption band to a shorter wavelength region with a maximum at 383 nm. Moreover, the conjugation of copper atom with the heteroatoms of the chromophore also partially enhanced this effect. The presence of *d*-*d* transitions was observed as a broad maximum at 689 nm [35]. The second ring (substitution in the 3 position on quinazolinone ring) is not conjugated to the rest of the molecule, so its effect on the changes in the absorption bands is negligible.

In the case of ligands **SB8** and **SB9** (Fig. S2, Fig. S3), the spectra show a slightly different character compared to **SB3**. Ligand **SB8**, containing the OCH_3 group, shows two near maxima in the region of approximately 300–310 nm, which can be attributed to π-π transitions, similar to the case of ligand **SB3**. The second maximum at 368 nm corresponds to *n*-π transitions, similar to that of **SB3**, but here the effect is only due to the presence of the auxochromic OH group, as a result of which the bathochromic shift is significantly smaller. Moreover, the OCH_3 group appears to attenuate the auxochromic effect of the OH group, as shown by the absence of an

absorption band in the region around 332 nm, which is seen in the case of the **SB9**, which does not possess the OCH_3 group in its structure. The presence of the copper atom showed similar trends for both complexes **SB8–Cu** and **SB9–Cu** (Fig. S2, Fig. S3). The broad absorption bands at 418 and 398 nm, respectively is caused by the change in the nature of the *N*–*N*═C chromophore, due to the coordination of the central copper ion via non-covalent interactions with free electron pairs on the OH group on the phenyl ring and the *O*═C–*N*–*N*═C(H) linkage. Since in the case of the complex **SB8–Cu** an OCH_3 group is also present, which does not participate in the coordination, its effect causes a slight shift of the maximum to longer wavelengths compared to **SB9–Cu** due to the auxochromic effect. The band corresponding to the *d-d* transitions was observed at 670 and 676 nm for **SB8–Cu** and **SB9–Cu** complexes [35]. The result of the coordination effect was also reflected in the color change of the complex compounds compared to the non-chelated ligand **SB8** and **SB9** solutions.

The complex formation in IR spectra was inferred from the shifts of the characteristic infrared absorption bands of free ligands. Intense absorption band in the IR spectrum of the **SB3** ligand characteristic for azomethine (CH═*N*) stretching vibration at 1588 cm^{-1} was shifted to lower wavenumber in **SB3–Cu** complex (1575 cm^{-1}), that indicates involvement of azomethine nitrogen in coordination with the copper ion. The same downfield shifts were observed for complexes **SB8–Cu** and **SB9–Cu** (**SB8**/**SB8–Cu** 1608/1582 cm^{-1}; **SB9**/**SB9–Cu** 1609/1598 cm^{-1}). The strong band at 1649 cm^{-1} is assigned to the (C═*O*) stretching vibration of the quinazolinone ring in ligand **SB3**. It is comparable with the data of the similar structures reported previously [32]. In the IR spectra of the Cu complexes absorption frequencies of carbonyl group were shifted downfield indicating the coordination of the carbonyl oxygen with copper ion (**SB3**/**SB3–Cu** 1649/1603 cm^{-1}; **SB8**/**SB8–Cu** 1608/1582 cm^{-1}; **SB9**/**SB9–Cu** 1609/1598 cm^{-1}) [35].

In contrast to the studied ligands (**SB3**, **SB8, SB9**), Cu(II) complexes are difficult to characterize by NMR spectroscopy because these spectra are not very informative. NMR spectra of Cu(II) complexes are heavily affected by the fact that Cu(II) is d^9 metal ion with one unpaired electron and thus Cu(II) is paramagnetic (and also has quadrupole moment). Consequently, chemical shifts and relaxation properties are greatly influenced leading to significant dispersion of chemical shifts. The resonances have very wide half-widths and have been even undetectable in several cases. The ^{1}H NMR spectrum of the Cu(II) complex **SB3–Cu** indicated that the line widths of the proton signals increased, shifted and also new signals appeared. The most visible perturbation was detected for the OH'' (upfield shift change, with respect to the uncomplex compound, was more than 2.5 ppm and marked line broadening) and =CH (downfield shift nearly 2 ppm) signals indicating a different influence of the coordination of the *O*-metal centre to the azomethine nitrogen for different group in the vicinity of Cu(II). However, due to paramagnetic character of Cu(II) complexes and the quadrupolar moment of Cu(II) most of signals in high-resolution NMR were not detectable. Similar effects were observed for the signals corresponding to the carbonyl carbon (Δδ more than 2 ppm), indicating coordination through the carbonyl oxygen, the azomethine nitrogen and the phenolic oxygen [35].

Similar data were observed for complexes **SB8–Cu** and **SB9–Cu**. In the case of compound **SB8**, the signals for Cu(II) complex were even less detectable, but the ^{1}H signals in **SB8–Cu** were also shifted considerably in the complex with respect to the "non-complexed" state, e.g. OH'' (upfield shift up to ~2 ppm) and =CH (downfield also ~ 2 ppm) thus, within a similar range as seen in **SB3–Cu**. The downfield shift of about 3 ppm was also detected for the carbonyl carbon. The same trends were also observed for complex **SB9–Cu**. The elemental analysis data indicated that all copper complexes had a (1:1) metal-ligand stoichiometry (Fig. S4).

2.3. Biological activity

All the tested compounds were evaluated against susceptible collection strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 as well as against resistant clinical isolates of human and veterinary origin; namely on methicillin-resistant *S. aureus* (MRSA) SA 3202, SA 630, 63718 (with the *mecA* gene [36]) and vancomycin-resistant *E. faecalis* isolates (VRE) 342B, 368 and 725B (with the *vanA* gene [37]). Moreover, all the substances were evaluated against fast-growing *Mycobacterium smegmatis* ATCC 700084 and slow-growing *Mycobacterium kansasii* DSM 44162 and *Mycobacterium tuberculosis* H_{37}Ra ATCC 25177. Biological effects were reported as the minimum inhibitory concentrations (MICs). Thus, all these strains/isolates were carefully selected to cover the spectrum of mycobacteria or Gram-positive bacteria long recognized by the WHO as being at risk for resistance and for which new drugs should be developed [38].

Human monocytic leukemia cell line THP-1 was used for cytotoxicity assay, and its values were reported as IC_{50} (Table 1). These data are complemented by the cytotoxicity against non-cancer human renal proximal tubule epithelial TH-1 cells published by Hricoviniova et al. [33]. Due to the remarkable photoactivity of the mentioned compounds that was studied by Hricovini et al. [39,40], these derivatives were also evaluated for their ability to inhibit photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts, i.e., to inhibit electron transport in photosystem (PS) II at the Q_B site of plastoquinone (acceptor side of PS II). As this is an additional test unrelated to anti-invasive activity, the results are reported in the Suppl. Materials. The screening of new compounds on THP-1 cells, as well as PET inhibiting activity, is historically verified by our group in the biological research of all prepared compounds [26,41–52].

2.4. Antistaphylococcal activity

Of the tested Schiff bases (ligands), only compound **SB3** (2-OH-5-NO_2) demonstrated antistaphylococcal potential. Ligand **SB7** (3,4-OH) showed also some antistaphylococcal activity. On the other hand, the Cu(II) complexes expressed approx. Comparable antistaphylococcal activity regardless of the used ligand. At the same time, their cytotoxic efficiency was significantly increased, see IC_{50} values on leukemia THP-1 cells in Table 1. The anti-invasive (i.e., dual) activity of the prepared complexes is higher than the corresponding ligands, which can be explained on the basis of Overton's concept and Tweedy's chelation theory [53]. Thus, chelation

leads to an enhance in the lipophilicity of the compounds, which facilitates their penetration into the cell.

Returning to the activity of the ligands themselves, in general halogenation enhances any biological activity [41,43,51,52,54–56]. However, due to the overall inactivity of **SB2** (3-NO_2-4-Cl) with the highest lipophilicity and, conversely, the activity of **SB3** and the partial activity of **SB7**, it is possible to hypothesize the need for specific substituents in the exact positions of the phenyl rings, namely the keyness of the NO_2 group in the *meta* position, which must be supplemented by another substituent in the *ortho* position (e.g., OH) able to form strong hydrogen bonds with a number of enzymes necessary for the survival of the bacterial cell. Only *para*-NO_2 monosubstitution, or a combination of *meta*-NO_2 and *para*-Cl groups, or, conversely, only *ortho*-OH alone does not show suitable electron-withdrawing properties for sufficient expected interactions. This observed necessity of electron-withdrawing substituents for antibacterial activity is in direct contrast to the anticancer activity on human renal carcinoma Caki-1 and human hepatocellular carcinoma HepG2 cells, where the highest activity was found for the electron-donating groups in compounds **SB5–SB8** [32,33].

On the other hand, the mechanism of anticancer activity of **SB3** was investigated in detail by Zahedifard et al. [57] and it was found that **SB3** induces apoptosis of breast cancer MCF-7 cells through the creation of reactive oxygen species (ROS), leading to disruption of the mitochondrial membrane potential and leakage of cytochrome *c* into the cytosol.

A similar above-mentioned effect of **SB3** was now also demonstrated in *S. aureus*, when respiration inhibition was tested using the standard MTT test (the name is derived from reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) with the most effective compounds. The MTT test may be applied to evaluated cell growth by measuring respiration. A cell respiratory activity (corresponding to cell viability) of less than 70% when exposed to the MIC found for the tested agent is a positive result of the test. Such negligible oxidative metabolism caused by respiratory disturbance inhibits cell growth [58,59]. The lowest multiples of MIC values that achieved more than 70% inhibition of *S. aureus* ATCC 29213 viability [%] are shown in Table 2. Importantly, all tested substances had <70% decrease in viability at values below their MIC values (e.g., **SB3** inhibited respiratory chain by ca. 91% at a value of 0.12 × MIC). These values suggest that the investigated derivatives may act via respiratory chain inhibition, or significantly influence it, compared to, e.g., ampicillin or ciprofloxacin.

2.5. Dynamics of antibacterial activity

Antistaphylococcal activities (expressed as MICs) are followed by determination of minimum bactericidal concentrations (MBCs) based on time-kill curves (dynamics tests), which were measured by the methodology of the Clinical and Laboratory Standards Institute (CLSI) [60]. For a compound to be considered bactericidal, an MBC $\leq$4 × MIC requirement must be met [36,47,48,60,61].

Determination of MBC values is only meaningful for effective compounds, therefore the dynamics of antistaphylococcal efficacy against *S. aureus* ATCC 29213 was evaluated only for derivatives **SB3** at 1 × MIC and 2 × MIC and **SB3–Cu** at all concentrations; other compounds had high MIC values. The influence of the concentration and incubation time of individual substances on the growth of bacteria is shown graphically in Fig. 1. Both compounds acted only bacteriostatically at all times and concentrations. Results were evaluated by two-way ANOVA and Tukey test. A statistically significant difference in activity ($P < 0.05$) was noted only between the activity of the ligand at a concentration of 1 × MIC in 24 h and of the complex at concentrations of 2 × MIC and 4 × MIC in 24 h. Fig. 1 shows that the activity of the ligand itself decreased over time, which was probably caused by the selection of resistant mutants. In conclusion, it can be stated that neither the concentration nor the presence of the complex has a statistically significant effect on the bactericidal activity.

2.6. Antibiofilm activity

Antibiofilm activity against *S. aureus* was evaluated only for compounds that demonstrated activity against planktonic cells, i.e., **SB3** and all Cu(II) complexes. All the compounds inhibited biofilm formation by at least 80% at a concentration 0.5 × MIC, **SB3** even at a value 0.25 × MIC (Fig. 2). This value is the only antibacterial result lower than the IC_{50}.

Compounds **SB3** and **SB3–Cu** eradicated 80% of *S. aureus* biofilm at a concentration 1 × MIC; for the rest compounds it was 2 × MIC (Fig. 3). When the activities of **SB3** and its Cu(II) complex are compared (Fig. 4) at a concentration 1 × MIC, the eradication activity of **SB3–Cu** is only slightly higher than **SB3** (87.3 ± 4% vs. 81.7 ± 13.7%). At a concentration 0.5 × MIC, **SB3–Cu** reduced biofilm viability by 66.4 ± 10.6%, whereas the **SB3** alone had no effect. The higher eradication efficacy of the complex could be explained by its significantly higher cytotoxicity.

Table 2
Lowest MIC values with at least 70% inhibition of *S. aureus* ATCC 29213 respiratory activity; ampicillin (AMP), ciprofloxacin (CPX).

Comp.	Conc.	*S. aureus* Respiration Inhibition [%]
SB3	0.12 × MIC	90.8
SB7	0.5 × MIC	90.1
SB3–Cu	0.5 × MIC	90.2
SB8–Cu	0.25 × MIC	96.2
SB9–Cu	0.5 × MIC	92.9
AMP	16 × MIC	81.9
CPX	32 × MIC	96.1

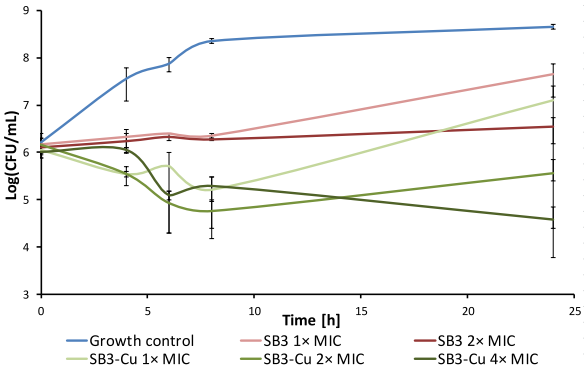


Fig. 1. Dynamics (time-kill curves) of antibacterial activity of compounds **SB3** and **SB3–Cu** on *S. aureus* ATCC 29213.

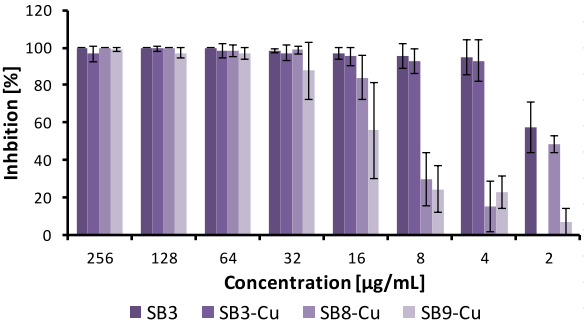


Fig. 2. Inhibition of *S. aureus* ATCC 29213 biofilm growth by **SB3**, **SB3–Cu**, **SB8–Cu** and **SB9–Cu**.

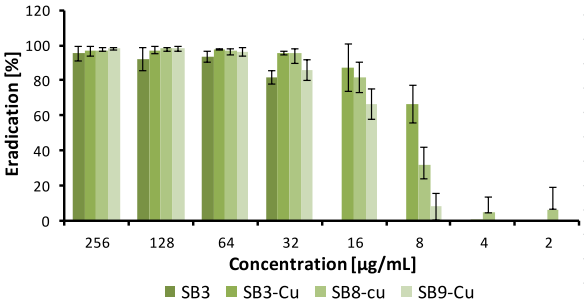


Fig. 3. Eradication of *S. aureus* ATCC 29213 biofilm by **SB3**, **SB3–Cu**, **SB8–Cu** and **SB9–Cu**.

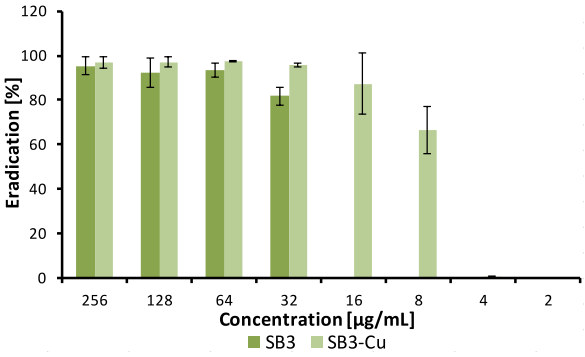


Fig. 4. Comparison of the eradication activity of **SB3** and **SB3–Cu** on mature *S. aureus* ATCC 29213 biofilm.

2.7. Antimycobacterial activity

Antimycobacterial activities of antibacterially active Schiff bases and their Cu(II) complexes were determined against slow-growing strains (*M. tuberculosis*, *M. kansasii*) and fast-growing strain (*M. smegmatis*) of mycobacteria and is summarized in Table 3. As with above-tested Gram-positive bacteria (*S. aureus, E. faecalis*), the Cu(II) complexes demonstrated higher activity against mycobacteria as well. The most active compound **SB8–Cu** was effective against *M. tuberculosis* at a concentration of 16 μg/ml (30.9 μM). In contrast, complex **SB3–Cu** with a nitro-substituted ligand, which showed the highest antistaphylococcal activity, was about 4-fold less active. Thus, substitution with a nitro moiety brings an advantage in terms of antistaphylococcal activity, but does not affect antimycobacterial activity. As with antibacterial activity, antimycobacterial activity also correlates with cytotoxicity. The MIC value of the most effective **SB8–Cu** against *M. tuberculosis* is about 150-fold higher than its cytotoxicity against THP-1 cells (MIC = 30.9 μM vs. IC_{50} = 0.2 μM). The most active ligand, **SB3**, had an MIC against *M. tuberculosis* about 11-fold higher than its IC_{50}. As with Gram-positive bacteria, copper complexation resulted in a significantly weaker increase in the antibacterial effect compared to the cytotoxic effect.

2.8. Anti-enterococcal activity

All the prepared compounds were completely inactive against collection strain of *E. faecalis* ATCC 29212 and to all the VRE isolates specified above [37], see Table 4. This fact confirms the high resistance of enterococci to disinfectants [62,63]. Genomic studies have demonstrated the uniqueness of enterococci, which allows them to overcome exposure to antibacterial chemotherapeutics [64,65].

3. Material and methods

A description of the synthesis of individual compounds, their characterization and a description of the used individual biological tests is given in detail in Supplementary Materials. However, methods described in previous papers [32,57,66–69] were used to prepare the discussed quinazolin-4-one Schiff bases, and recently described methods were used to determine antimicrobial effectivity/cytotoxicity [19,25,26,47,48,51,70,71] of all the investigated derivatives as well as for PET inhibitory activity in spinach chloroplasts [44,46,47,72,73].

4. Conclusion

In summary, the antibacterial, antimycobacterial and antibiofilm activities of nine 2,3-disubstituted-quinazolin-4(3*H*)-one derived Schiff bases **SB1**–**SB9** and three copper complexes were investigated. Activity against staphylococci was generally higher for the Cu(II) complexes than for the non-chelating ligands; the highest activity was observed for the nitro-substituted ligand **SB3** and its complex **SB3–Cu**. The higher activity of all the discussed Cu(II) complexes could be explained by their higher lipophilicity and the better penetration into the cells, and at the same time by their own cytotoxic action, which is probably caused by an increase in the production of ROS. Complexation, however, simultaneously leads to a significant increase in cytotoxicity against eukaryotic cells. In addition, all complexes and **SB3** ligand showed significant antibiofilm activity, which resulted in a reduction of biofilm formation and eradication of mature *S. aureus* biofilm by 80% even at concentrations lower than the MIC value.

The presented data indicate that **SB3–Cu** complex possesses cytotoxic (potentially anti-cancer) and antistaphylococcal/antibiofilm activity. In addition, the preliminary screening of the anticancer activity of these Cu(II) complexes have provided interesting data, and therefore current efforts are focused on investigations using several human cancer cell lines. Test of cytotoxicity on non-cancer cells is also necessary to define therapeutic space and determine safety profile. This step is crucial for further pre-clinical and clinical trials.

Ethical statement

Not applicable.

Table 3
Antimycobacterial activities (MIC [μg/ml]; for active agents, MICs also expressed in μM) of selected 2,3-disubstituted-quinazolin-4(3*H*)-one derived Schiff bases and their Cu(II) complexes compared to isoniazid (INH) and ciprofloxacin (CPX).

Comp.	MIC [μg/ml] ([μM])		
	M. tuberculosis H37Ra ATCC 25177	*M. kansasii* DSM 44162	*M. smegmatis* ATCC 700084
SB3	64 (142)	128	128
SB3–Cu	32 (58.4)	128	64 (116)
SB8	>128	>256	>256
SB8–Cu	16 (30.9)	256	64 (123)
SB9	>128	256	256
SB9–Cu	32 (61.7)	128	64 (123)
INH	8 (58)	4 (29.1)	16 (117)
CPX	16 (48.3)	1 (3.0)	0.125 (0.4)

Table 4
In vitro anti-enterococcal activities (MIC [μg/ml]) compared to ampicillin (AMP), ciprofloxacin (CPX).

Comp.	MIC [μg/ml]			
	E. faecalis ATCC 29213	VRE 342B	VRE 368	VRE 725 B
SB1	>256	>256	>256	>256
SB2	>256	>256	>256	>256
SB3	256	>256	>256	>256
SB4	128	256	256	256
SB5	>256	>256	>256	>256
SB6	>256	>256	>256	>256
SB7	>256	>256	>256	>256
SB8	>256	>256	>256	>256
SB9	>256	>256	>256	>256
SB3–Cu	256	256	256	256
SB8–Cu	>256	>256	>256	>256
SB9–Cu	256	256	256	256
AMP	1	4	4	4
CPX	1	1	1	64

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Data availability statement

The authors declare the all data is included in article/supplementary material and no additional data available.

Statements and declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Dominika Pindjakova: Methodology, Investigation. **Sarka Mascaretti:** Methodology, Investigation. **Jana Hricoviniova:** Methodology, Investigation. **Jan Hosek:** Methodology, Investigation, Data curation. **Jana Gregorova:** Methodology, Investigation. **Jiri Kos:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Alois Cizek:** Validation, Supervision, Data curation, Conceptualization. **Zuzana Hricoviniova:** Writing – review & editing, Validation, Data curation, Conceptualization. **Josef Jampilek:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29051.

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