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Insights into Antimicrobial Potential of Styrylquinoline Derivatives

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Abstract

A total of 64 ring-substituted 2-styrylquinolines were designed and tested against a battery of microbial pathogens: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, clinical isolates of methicillin-resistant *S. aureus* (MRSA), *Mycobacteium tuberculosis* H37Ra/ATCC 25177, *Mycobacterium smegmatis* ATCC 700084, *Mycobacterium kansasii* DSM 44162, *Candida albicans* CCM 8361, *Candida krusei* CCM 8271 and *Candida parapsilosis* CCM 8260. In addition, all compounds were investigated for their anticancer potential on colon cancer cell lines HCT 116 p53+/+ and HCT 116 p53-/- as well as the effect against normal human dermal fibroblasts (NHDF). Twenty derivatives showed remarkable antimicrobial activity comparable to or better than clinically used drugs (ampicillin, ciprofloxacin, rifampicin, amphotericin B). The most effective compounds were substituted on styryl with 2,6-Cl, 2-NO_2, 2,4-NO_2, 4-CN, 2-OAc-5-Cl and 2-OH-5-NO_2. Chlorination of the quinoline at the 5- and 7-position and the free hydroxylic group at the 8-position of the quinoline resulted in increased and broadened efficacy. All the antimicrobial active derivatives did not show significant effect on normal human dermal fibroblasts, except for 2,6-Cl-styryl derivatives. The antimycobacterial active compounds were able to inhibit cellular respiration in all tested mycobacterial species. (*E*)-5,7-dichloro-2-[2-(2-nitrophenyl)vinyl]-quinolin-8-ol (**D4**) and (*E*)-5,7-dichloro-2-[2-(2,4-dinitrophenyl)vinyl]-quinolin-8-ol (**D10**) were the most antimicrobial effective agents simultaneously with high anticancer activity and negligible cytotoxicity against normal human cells. Thus, promising active agents with dual, anti-infectious, and anticancer effects, were identified.

1. Introduction

In the first decade of the 21st century, the "antibiotic era" gradually changed into the "post-antibiotic era". Infectious pathogens, which have been under control since the 1950s thanks to the strong onset of various antibacterial, antituberculosis and antifungal drugs, are increasingly getting out of control and becoming resistant to the used drugs, and the current state of development of new anti-infective drugs is extremely worrying [1–3]. It is undeniable that the increasing microbial load and the development of antimicrobial resistance (AMR) pose a major threat to human health worldwide. The number of deaths associated/caused by AMR is gradually increasing. The main pathogens for resistance-related deaths are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Another high number of deaths is due to *M. tuberculosis* (resistant, multi-resistant, extensively resistant and totally resistant strains). Increasingly frequent infections with non-tuberculous (atypical) strains of mycobacteria causing severe infections of the lungs, bones, digestive system, skin and other soft organs are related to the overall reduced immunity of the human population [4–7]. In addition to the increased risk of patient death, AMR also results in longer hospital stays and increased healthcare costs [4,8,9].

It is understandable that this condition is extremely undesirable in the absence of appropriate treatment and drugs. Currently, various approaches try to overcome the lack of effective drugs. In addition to the application of various synergistic combinations, drug repurposing or nanoparticle (technological)

improvements, designing new entities with a new/innovative mechanism of action or with a multi-target effect is considered the most advantageous [10–19]. This last interesting approach is aimed at the search/design of molecules with multiple mechanisms of action and is used mainly for anti-infective and anticancer drugs, when it is necessary to overcome the resistance [20]. Interestingly, therapeutics active across the species with a so-called dual effect, i.e. with antimicrobial and anticancer or antimicrobial and antiparasitic, etc., can be also found [20–30]. The search for compounds with dual activity is crucial for developing innovative therapeutics that can address multidrug resistance and complex diseases. Such compounds are identified through various methods, including high-throughput screening of natural products and synthetic libraries, computational drug repurposing, and structure-activity relationship (SAR) studies to optimize their efficacy and selectivity. This approach aligns with the concept of privileged structures, which are molecular frameworks frequently found in bioactive compounds and capable of interacting with multiple biological targets. By focusing on such structural motifs, researchers can enhance the likelihood of discovering dual-acting agents through rational drug design, fragment-based screening, and scaffold-hopping strategies.

Azanaphthalenes, specifically quinolines and especially 8-hydroxyquinolines (8-HQ), are a source of such multi-target agents. It is therefore not surprising that 8-HQs have a wide range of biological properties and can therefore be considered as privileged structures of multi-target agents [31–34]. The simple 8-HQ scaffold has unique physicochemical properties and affords the possibility of a large number of modifications (via targeted or diversity-directed synthesis) [34–39]. On the other hand, all these compounds show a mechanism of action that is complex and difficult to determine (e.g. [34–36,38,39]): they are able to chelate various metals [40–44] and affect various enzymatic systems [34,37,39,45–49]. Despite this, quinoline-based compounds form an important scaffold in medicinal chemistry [32,34,35,38], mainly used for the design of anti-infective [35,39,46–52] and anticancer agents [41,44,45,53–58]. It is important to mention that halogenated derivatives of 8-HQ have been approved for their antiseptic use many years ago [59–63].

This contribution is a continuation of the work of our team and deals with the evaluation of antimicrobial (antibacterial, antimycobacterial, antifungal) activity of synthesized ring-substituted 2-styrylquinoline derivatives [64–69]. The discussed compounds were also tested for their anticancer effect [53,54], and therefore suitable molecules with only anticancer or anti-infection potential, or with a dual (anticancer + antimicrobial) effect, can be selected from the biologically investigated compounds in this way.

2. Materials and Methods

2.1. Compounds

All styrylquinoline derivatives investigated in this study were synthesized according to previously reported procedures [53]. Detailed synthetic protocols, purification methods, and physicochemical characterization data for all new compounds are provided in the Supplementary Material.

2.2. Biological Studies

2.2.1. Test Microorganisms

Standard reference bacterial strains *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 2921, *Mycobacteium tuberculosis* H37Ra/ATCC 25177, *Mycobacteium smegmatis* ATCC 700084, and *Mycobacteium kansasii* DSM 44162 were obtained from American Type Culture Collection (ATCC). Clinical isolates of methicillin-resistant *S. aureus* SA 3202, SA 630 [70] were obtained from a collection of the National Institute of Public Health, Prague, Czech Republic, and 63718 [70] from a collection of the Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Czech Republic. *Candida albicans* CCM 8261, *Candida krusei* CCM 8271, and *Candida parapsilosis* CCM 8260 were obtained from a collection of the Czech Collection of Microorganisms, Brno, Czech Republic [71].

2.2.2. Determination of Bacterial Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) were determined using a broth microdilution method in accordance with CLSI guidelines [72], with minor modifications described previously [73]. Stock solutions of the compounds were prepared in DMSO (10 µg/mL) and further diluted in appropriate media: Cation Adjusted Mueller–Hinton broth (CaMH, Oxoid) for staphylococci, brain heart infusion (BHI, Oxoid) for enterococci, and Middlebrook 7H9 (Oxoid) for mycobacteria, to obtain final concentrations ranging from 256 to 0.125 µg/mL. Microplates were inoculated with the tested microorganisms. The final inoculum density was 1.5×10^6 CFU/mL for *M. tuberculosis* and 10^5 CFU/mL for the remaining bacteria. Ampicillin, ciprofloxacin, and rifampicin (Sigma) were used as reference drugs. Drug-free and sterility controls were included. Plates were incubated under appropriate conditions (24 h at 37°C for staphylococci and enterococci, 3 days for *M. smegmatis*, and 14 days for *M. tuberculosis* and *M. kansasii*). MIC values were defined as the lowest concentration completely inhibiting visible growth. For *M. tuberculosis*, MIC determination was performed using the Alamar Blue assay (Oxoid). Following incubation, Alamar Blue reagent (10% v/v) was added, and plates were further incubated for 24 h. The MIC was defined as the lowest concentration preventing the color change from blue (resazurin) to pink (resorufin). All experiments were performed in triplicate.

2.2.3. MTT Assay

The MTT assay was performed as previously described [74–77], with minor modifications. Compounds were dissolved and diluted in appropriate media (CaMH for staphylococci and Middlebrook 7H9 for mycobacteria) to final concentrations ranging from 256 to 0.0625 µg/mL. Bacterial suspensions were prepared at standard densities and added to microplate wells containing the tested compounds. Control wells without compounds were included, and ciprofloxacin (Sigma) served as a positive control. Samples were prepared in duplicate. After incubation under appropriate conditions (24 h for

staphylococci, 3 days for *M. smegmatis*, and 14 days for *M. tuberculosis* and *M. kansasii*), MTT reagent was added to each well (10% v/v), followed by further incubation to allow formazan formation. The reaction was stopped by addition of sodium dodecyl sulfate solution, and absorbance was measured at 570 nm. Cell viability was expressed as a percentage relative to untreated controls using the ratio of absorbance values. A reduction in viability below 70% was considered indicative of cytotoxic activity. The results are presented in Tables 2 and 3.

2.2.4. Determination of Minimum Fungistatic Activity

The minimum fungistatic inhibitory concentrations were evaluated by the microdilution broth method according to the CLSI [78] with some modifications as described previously [73]. The compounds were dissolved in DMSO (Sigma) to get concentration 10 µg/mL and diluted in RPMI-1640 medium (Sigma) to concentrations of 256–1 µg/mL. To prepare fungal inoculum in RPMI-1640 broth, the microorganisms were grown on Sabourad dextrose agar (Sigma) at 37°C for 24 h (*C. albicans*, C. *krusei*) and for 48 h (*C. parapsilosis*). The plate was then inoculated with a pipette, the final concentration of fungal cells was 5×10^2 CFU/mL in each well. The plate was incubated in 37°C for 24 h or 48 h. After incubation the minimal fungistatic concentration was evaluated as a minimal concentration of tested compounds, which visibly inhibited the fungal growth.

2.2.5. Cell Viability Assay

The human colorectal carcinoma cell line HCT 116 with wild type of p53 ($p53^{+/+}$) were obtained from the ATCC. HCT 116 with deletion of TP53 gene ($p53^{-/-}$) was provided from Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice, Poland. The normal human dermal fibroblast cell line (NHDF) was purchased from PromoCell (Heidelberg, Germany). A recently described procedure [53] was used. The results are shown in Table 1.

3. Results and Discussion

3.1. Chemistry

The investigated styrylquinoline derivatives were synthesized using commercially available quinaldines I–III as starting materials. The synthetic route involved an initial condensation step with appropriate aromatic aldehydes (Scheme 1), performed in a boiling mixture of acetic anhydride and 80% acetic acid (3:1, v/v). Under these conditions, all hydroxyl groups present in the substrates underwent acylation, requiring a subsequent deprotection step to regenerate the corresponding hydroxyl functionalities.

Selective removal of acetyl groups was achieved using either pyridine/water or potassium carbonate in methanol (Scheme 2). Treatment with pyridine/water resulted in preferential deacylation of the hydroxyl group substituted with the quinoline moiety, while the acetyl group on the phenyl ring remained intact. In contrast, the use of potassium carbonate in methanol at room temperature enabled complete

deprotection of all hydroxyl groups. These approaches allowed for controlled and selective deacylation of styrylquinoline derivatives depending on the reaction conditions.

Sixty-four compounds were prepared in this way, of which 59 have been previously reported, see [53,54]. Derivatives **D8**, **D11**, **D12**, **D13** and **E2** are novel, have not been described in the literature, and their characteristics are listed in the Supplementary Material. All the discussed compounds were classified into 5 groups according to their structure: **A1**–**15** (2-[(*E*)-2-arylethenyl]quinolin-8-yl acetates), **B1**–**7** (2-[(*E*)-2-arylethenyl]quinolin-8-ols), **C1**–**23** (5,7-dichloro-2-[(*E*)-2-arylethenyl]quinolin-8-yl acetates), **D1**–**15** (5,7-dichloro-2-[(*E*)-2-arylethenyl]quinolin-8-ols), **E1**–**4** (2-[(*E*)-2-arylethenyl]quinolin-4-ols). Their structures and individual substitutions are presented in Table 1.

Since lipophilicity is a key parameter affecting the fate of all bioactive agents [79,80] and influencing their activity [81], lipophilicity values expressed as log *P* were estimated for all compounds, see Table 1. Log *P* values were calculated using ACD/Percepta ver. 2012 (Advanced Chemistry Development. Inc., Toronto, ON, Canada, 2012).

Compounds of series A exhibited log *P* values in the range from 3.51 to 5.22, their deacetylated derivatives of series B had log *P* value in the range 3.35–5.04. 5,7-Dichlorinated derivatives of series C had log *P* ranging from 5.10 to 7.11; while the range of log *P* values of series D was 5.33–7.04. Compounds of series E had log *P* values in the range from 3.73 to 5.53. Thus it can be concluded that all the investigated derivatives are rather lipophilic substances. In general, (*E*)-2-[2-(2-acetoxyphenyl)vinyl]quinolin-8-ol (**B2**) is the least lipophilic (log *P* = 3.35), while (*E*)-5,7-dichloro-2-[2-(2-acetoxy-3,5-dichlorophenyl)vinyl]quinolin-8-yl acetate (**C20**) is the most lipophilic (log *P* = 7.11) of all the discussed compounds. It can be assumed that lipophilicities increase in the series B < A < D < C. But this only applies to halogen-type substitutions on styryl. If NO_2 or CN is used as substituents, a change in order can be traced and the prediction software assigns the C-series compounds higher lipophilicity than the D-series compounds (**C1**–**6** < **D1**–**6**) probably due to intramolecular interactions. The compounds of group E appear to have log *P* values between those of groups A and D.

In addition, the electronic σ parameters illustrating the effect of substituents of the phenyl ring of styryl tail were predicted. These σ parameters are in a wide range from 0.01 (2-OEt), i.e., rather with the properties of donating electrons to the system, up to ~ 1.6 (2,6-F-3-Cl, 2,4-NO_2), i.e., with a very strong electron-withdrawing effect. Also, these σ parameters were predicted using ACD/Percepta ver. 2012.

3.2. Biology

As mentioned above, most of the compounds have been previously described when their anticancer potential was investigated [53,54]. By comparing the IC_{50} values obtained against colon cancer cell lines HCT 116 $p53^{+/+}$ and HCT 116 $p53^{-/-}$ versus normal human dermal fibroblasts (NHDF), their possible therapeutic potential can be seen. Five new compounds were also tested on these human cell lines and all IC_{50} values are shown in Table 1. Considering the previous positive antimicrobial results of selected

styrylquinoline derivatives [47,64–68,82], all the discussed derivatives were subjected to extensive in vitro screening against Gram-positive bacteria, mycobacteria, and candida.

First, universally susceptible collection strains of *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were selected. Some of the compounds were effective against *S. aureus*, so testing continued on staphylococcal strains with an epidemiologically significant type of resistance; clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) of human and veterinary origin (SA 3202, SA 630, 63718) carrying the *mecA* gene were selected [70]. Unfortunately, none of the compounds showed activity against facultatively anaerobic *E. faecalis*, so other similar types of bacteria or resistant isolates of *E. faecalis* were not tested. All the compounds were also in vitro tested against *Mycobacteium tuberculosis* H37Ra/ATCC 25177 and additionally against *Mycobacterium smegmatis* ATCC 700084 and *Mycobacterium kansasii* DSM 44162 as representatives of non-tuberculous mycobacteria causing increasingly frequent and difficult-to-treat mycobacterial infections, especially in immunocompromised patients [7]. *M. tuberculosis* and *M. kansasii* are slow-growing mycobacteria, while *M. smegmatis* is a fast-growing strain. In addition, all the compounds were *in vitro* tested against three representatives of yeasts: *Candida albicans* CCM 8361, *Candida krusei* CCM 8271, *Candida parapsilosis* CCM 8260. Activities are expressed as the minimum inhibitory concentrations (MICs); see Table 1.

Looking at the results from Table 1 and comparing the MIC values of the discussed compounds with their antiproliferative values against cancer cells, it can be stated that the compounds showed greater anticancer activity for which they were primarily designed. Of the 64 compounds, only 20 derivatives showed remarkable antimicrobial activity.

From series A, compounds **A2** (R = 3-OMe, log *P* = 4.02, σ = 0.66) and **A7** (R = 2-Cl, log *P* = 4.46, σ = 1.05) were selective against mycobacteria, and derivative **A9** (R = 2-NO_2, log *P* = 3.78, σ = 1.12) selective against *Candida* sp. On the other hand, agent **A13** (R = 2,6-Cl, log *P* = 5.20, σ = 1.33) was effective in the entire spectrum of tested microorganisms (except *E. faecalis*). All compounds showed insignificant effect against normal human fibroblasts (NHDF). From series B, compound **B4** (R = 2-NO_2, log *P* = 3.64, σ = 1.12) was selective against *Candida* sp. (similar to series A) and derivative **B5** (R = 3,5-OMe, log *P* = 3.93, σ = 0.93) showed selective antistaphylococcal activity. Similar to series A, agent **B6** (R = 2,6-Cl, log *P* = 5.04, σ = 1.33) was effective across the tested spectrum. All compounds also showed negligible activity against NHDF.

By comparing the antimicrobial activities and physicochemical parameters of compounds from series A and B, it can be concluded that lipophilicity does not play a fundamental role in the activity, electronic parameters partially influence the activity (a higher value of σ is advantageous), but the nature and position of the substituents on the phenyl ring of styryl are most significant for the activity (compare the effects of **A12**, **A13**, **A14**). It can be assumed that the hydroxyl group of the quinoline ring is advantageous for activity against staphylococci, compared the activity of **A11**/**B5** (R = 3,5-OMe), as described e.g., [64,68,83]. On the other hand, deacetylation of the C_8-hydroxylic group did not affect the

activity when styryl was substituted with 2-NO_2 or 2,6-Cl (comparable activity of acetylated/deacetylated compounds **A9**/**B4**, **A13**/**B6**).

Within series C, compounds **C1** (R = 2-Cl, log *P* 6.17, σ = 1.05) and **C5** (R = 4-CN, log *P* 5.39, σ = 1.05) were selective against mycobacteria, while derivative **C3** (R = 2-CN, log *P* 6.17, σ = 1.04) showed selective antistaphylococcal activity. On the other hand, compound **C6** (R = 2-NO_2, log *P* = 5.27, σ = 1.12), probably due to chlorination of the quinoline ring nucleus, extended the activity from anticandidal to antistaphylococcal activity as well (as described e.g., by [84,85]. Traditionally, agent **C11** (R = 2,6-Cl, log *P* = 7.10, σ = 1.33) was the most potent derivative against the entire spectrum of microorganisms. As a result of the chlorination of 8-acetoxyquinoline (8-AcQ) (and thus higher lipophilicity), there was also an increase in activity compared to derivative **A13**. The increase in antimicrobial activity associated with higher lipophilicity (chlorination of 8-AcQ) is also associated with higher antiproliferative activity not only against HCT 116 cancer cell lines but also against NHDF cells (**C11** had an IC_{50} = 9.55 μM on NHDF).

Group D showed the highest amount of active substances. In general, halogenation of 8-HQ, i.e. higher lipophilicity leads to higher potency and subsequent deacetylation (release) of the quinoline hydroxyl group further increases activity. Importantly, there was no undesirable increase in antiproliferative activity against NHDF as in the case of the acetylated series C, although the most successful 2,6-Cl styryl substitution (compound **D8**) showing the highest activity against the tested microorganisms showed comparable adverse effects against NHDF (IC_{50} = 6.22 μM) along with **C11**; other derivatives showed an insignificant effect on NHDF cells. As in the previous case with series C, compounds **D1** (R = 2-CN, log *P* = 5.65, σ = 1.04) demonstrated selective antistaphylococcal activity and **D3** (R = 4-CN, log *P* = 5.71, σ = 1.05) selective antimycobacterial activity. In the case of derivative **D4** (R = 2-NO_2, log *P* = 5.45, σ = 1.12), in comparison with the series A, B and C, not only was the activity strengthened, but also its expansion from the original anticandidal to antistaphylococcal and finally to antimycobacterial efficacy. After hydrolysis of the acyl group in the 8-position of the quinoline ring, derivative **D10** (R = 2,4-NO2, log P = 5.33, σ = 1.66) was activated, which was completely inactive in the series A and C. Along with the above-mentioned changes on the quinoline core, new derivatives were activated: **D11** (R = 2-OH-5-Cl, log *P* = 6.25, σ = 1.04) acquired anticandidal and antimycobacterial activity, **D13** (R = 2-OH-5-NO_2, log *P* = 5.68, σ = 1.28) acquired antistaphylococcal and antimycobacterial and especially **D12** (R = 2-OAc-5-Cl, log *P* = 6.18, σ = 0.18) obtained an effect against the entire spectrum of microorganisms.

The last and smallest series E, where the hydroxylic group from position 8 of the quinoline was moved to position 4, were completely inactive.

5,7-Dichloro-8-hydroxyquinoline derivatives (series D) exhibit the broadest spectrum of biological activity among the tested compounds. The most potent agents are those bearing a 2,6-dichloro substitution in the styryl tail (compounds **C11** and **D8**). However, these derivatives also demonstrate relatively high toxicity toward normal human NHDF cells. Consequently, the optimal balance between antimicrobial efficacy and safety appears to be achieved by derivatives **D4** (featuring a 2-NO_2 group) and **D10** (bearing 2,4-NO_2 groups). In addition to their antimicrobial properties, both **D4** and **D10** show excellent antitumor

activity, with IC_{50} values ranging from 0.54 to 0.76 μM (Fig. 1). It is also worth noting that despite the very low MIC values achieved by some standard drugs (e.g. CPX, RIF), the tested compounds exhibit unique properties – not only strong activity against SA strains, but also an extended spectrum of activity including MRSA strains, mycobacterial pathogens (MT, MS, MK) and Candida fungi. Differences in activity against individual microorganisms may result from interactions specific to the structure of cell walls and mechanisms of penetration through the lipid barrier, which opens new perspectives in the design of drugs with a broad spectrum of action.

Since the compounds exhibit activity against aerobic staphylococci but not against facultatively anaerobic enterococci [86–89], suggested that their mechanism of action may be associated with interference in respiratory processes [90]. To verify this hypothesis, an MTT assay was performed for the most active derivatives. This assay enables indirect evaluation of bacterial viability through measurement of metabolic activity. A decrease in respiratory activity below 70% of the control level after exposure to MIC concentrations was considered indicative of a positive effect. Such reduction in oxidative metabolism reflects impaired cellular function and suggests involvement of respiration-related mechanisms [74,77].

The lowest MIC multiples required to achieve > 70% reduction in viability of *S. aureus* ATCC 29213 and MRSA isolate SA 3202 are presented in Table 2. Compounds **A13/B6/C11/D8** (R = 2,6-Cl) and **D12** (R = 2-OAc-5-Cl) did not show any significant inhibition in the MTT assay, even at the highest tested concentration (256 μg/mL). In contrast, compounds **D1** (R = 2-CN), **D10** (R = 2,4-NO2), and **D13** (R = 2-OH-5-NO2) exhibited only limited inhibition at elevated MIC values. These results indicate that, despite pronounced antibacterial activity against staphylococci, the tested styrylquinoline derivatives do not primarily affect cellular respiration and may act via alternative mechanisms. Notably, comparable activity was observed against both methicillin-susceptible *S. aureus* and MRSA strains, suggesting that the presence of the mecA gene, responsible for methicillin resistance [70,91], does not influence the efficacy of these compounds. This supports the hypothesis of a distinct mode of action specific to *Staphylococcus* spp.

Table 2
Lowest MIC values with at least 70% inhibition of respiratory activity of staphylococcal strains.

No.	SA resp. inh. (Conc.)	MRSA1 resp. inh. (Conc.)
D1	96.6% (8× MIC)	92.6% (2× MIC)
D10	96.6% (4× MIC)	84.5% (8× MIC)
D13	80.6% (32× MIC)	83.1% (32× MIC)
CPX	92.5% (32× MIC)	96.5% (32× MIC)

SA = *Staphyloccoccus aureus* ATCC 29213; MRSA1 = clinical isolate of methicillin-resistant *S. aureus* SA 3202; CPX = ciprofloxacin

Quinoline derivatives are known to exhibit antimycobacterial activity, which is often associated with inhibition of mycobacterial ATP synthase and disruption of cellular respiration [48,92,93]. Therefore, in addition to MIC determination, an MTT assay was performed for the most active compounds. Similarly to the previous analysis, a reduction in respiratory activity of mycobacterial cells below 70% of the control after exposure to MIC concentrations was considered indicative of a positive result [74–76]. The lowest MIC multiples required to achieve more than 70% inhibition of mycobacterial viability are summarized in Table 3. Compound **A7** (R = 2-Cl) showed inhibition of respiration at 1× MIC across all tested mycobacterial species. In contrast, disubstituted derivatives **A13/B6/C11/D8** (R = 2,6-Cl) did not exhibit any detectable MTT inhibition, even at the highest tested concentration (256 µg/mL), similarly to the observations for staphylococci. On the other hand, the remaining highly active compounds (**C5**, **D1**, **D10**, **D13**) demonstrated pronounced inhibition of the mycobacterial respiratory chain not only at MIC values but also at sub-MIC concentrations, in some cases as low as one-sixteenth of the MIC (e.g., **C5/D1**, R = 4-CN, against *M. smegmatis*). These findings suggest that derivatives bearing 2,6-dichloro substitution may act via a mechanism distinct from other active styrylquinoline derivatives.

Table 3
Lowest MIC values with at least 70% inhibition of respiratory activity of mycobacterial strains.

No.	MT resp. inh. (Conc.)	MS resp. inh. (Conc.)	MK resp. inh. (Conc.)
A7	75.3% (1× MIC)	91.0% (1× MIC	87.5% (0.5× MIC
C5	89.5% (0.5× MIC)	90.6% (0.063× MIC)	90.9% (0.5× MIC)
D1	92.8% (0.5× MIC)	92.3% (0.063× MIC)	91.4% (0.5× MIC)
D10	94.4% (1× MIC)	86.8% (1× MIC)	92.3% (0.5× MIC)
D13	87.5% (1× MIC)	88.0% (1× MIC)	87.7% (1× MIC)
CPX	95.2% (32× MIC)	94.7% (64× MIC)	95.0% (64× MIC)

MT = *Mycobacteium tuberculosis* H37Ra/ATCC 25177; MS = *Mycobacteium smegmatis* ATCC 700084, MK = *Mycobacteium kansasii* DSM 44162; CPX = ciprofloxacin

This extensive antimicrobial screening succeeded in finding new multi-target *agents* with very good activity against a wide spectrum of microorganisms and a good safety profile. Either these are substances with a selective effect against mycobacteria (**A2**, **A7**, **C1**), staphylococci (**B5**, **C3**, **D1**) or candida (**A9**, **B4**) or, on the contrary, they are compounds with a broad spectrum of antimicrobial effect (**A13**, **B6**, **D4**, **D10**, **D11**, **D12**, **D13**). Most of these compounds simultaneously demonstrated dual activity against colon cancer cell lines.

4. Conclusion

Several compounds from a series of sixty-four ring-substituted 2-styrylquinolines tested against Gram-positive bacteria, slow- and fast-growing mycobacteria, and *Candida* sp. expressed activity comparable

to or higher than clinically used drugs. Antimicrobial active compounds have also shown activity against colon cancer cell lines. Chlorination of the quinoline nucleus and the unsubstituted phenolic moiety at position 8 of quinoline positively affects antimicrobial activity. The crucial factor influencing the activity is not the lipophilicity itself or the electron-withdrawing properties of the styryl tail, but the type of substituent and, especially, the position of the substituents on the styryl. Positions $C_{2,6}$' substituted with chlorine, C_2'/$C_{2,4}$' substituted with a nitro group are preferred. It has been shown that the compounds do not affect cellular respiration in staphylococci, on the other hand, they significantly inhibit cellular respiration in mycobacteria. Thus, this primary screening revealed promising multi-target agents with dual (antimicrobial+ anticancer) activity and insignificantly affecting non-cancerous human cells.

Declarations

Declarations of interest

The authors declare no competing financial or non-financial interests.

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Not required.

Use of AI tools

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Author Contribution

Conceptualization WC, JJ and RM; methodology WC, AMW, AC and JJ; investigation WC, AMW, AC and JJ; original draft preparation WC and JJ; review and editing WC, JJ and RM; project administration and funding acquisition RM.

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Data Availability

Data are available from the corresponding author upon reasonable request.

References

1. Hansson, K. & Brenthel, A. Imagining a post-antibiotic era: a cultural analysis of crisis and antibiotic resistance. *Med Humanit* **48**, 381-388, doi:10.1136/medhum-2022-012409 (2022).
2. Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R. & Martins, C. H. G. Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era. *Plants (Basel)* **12**, doi:10.3390/plants12173077 (2023).
3. Rabi, M. The global (health) governance of antimicrobial resistance. *Globalizations* **21**, 1518-1538, doi:10.1080/14747731.2024.2375455 (2024).
4. *Antimicrobial resistance*, <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> (2024).
5. Antimicrobial Resistance, C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629-655, doi:10.1016/S0140-6736(21)02724-0 (2022).
6. (WHO, Geneva, Switzerland, 2023).
7. Mirabal;, A. B., Nguyen;, A. & Ferrer, G. *Lung Nontuberculous Mycobacterial Infections.*, (StatPearls, 2024).
8. Dadgostar, P. Antimicrobial Resistance: Implications and Costs. *Infect Drug Resist* **12**, 3903-3910, doi:10.2147/IDR.S234610 (2019).
9. Poudel, A. N. *et al.* The economic burden of antibiotic resistance: A systematic review and meta-analysis. *PLoS One* **18**, e0285170, doi:10.1371/journal.pone.0285170 (2023).
10. Jampilek, J. Design and Discovery of New Antibacterial Agents: Advances, Perspectives, Challenges. *Curr Med Chem* **25**, 4972-5006, doi:10.2174/0929867324666170918122633 (2018).
11. Jampilek, J. Drug repurposing to overcome microbial resistance. *Drug Discov Today* **27**, 2028-2041, doi:10.1016/j.drudis.2022.05.006 (2022).
12. Paul, D. *et al.* Artificial intelligence in drug discovery and development. *Drug Discov Today* **26**, 80-93, doi:10.1016/j.drudis.2020.10.010 (2021).
13. Visan, A. I. & Negut, I. Integrating Artificial Intelligence for Drug Discovery in the Context of Revolutionizing Drug Delivery. *Life (Basel)* **14**, doi:10.3390/life14020233 (2024).
14. Rudrapal, M., Paudel, K. R. & Pangeni, R. Editorial: Drug repurposing and polypharmacology: A synergistic approach in multi-target based drug discovery. *Front Pharmacol* **13**, 1101007, doi:10.3389/fphar.2022.1101007 (2022).
15. Weth, F. R. *et al.* Unlocking hidden potential: advancements, approaches, and obstacles in repurposing drugs for cancer therapy. *Br J Cancer* **130**, 703-715, doi:10.1038/s41416-023-02502-9 (2024).

16. Mamun, M. M., Sorinolu, A. J., Munir, M. & Vejerano, E. P. Nanoantibiotics: Functions and Properties at the Nanoscale to Combat Antibiotic Resistance. *Front Chem* **9**, 687660, doi:10.3389/fchem.2021.687660 (2021).
17. Modi, S. *et al.* Nanostructured Antibiotics and Their Emerging Medicinal Applications: An Overview of Nanoantibiotics. *Antibiotics* **11**, 708 (2022).
18. Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A. & Kutlu, O. Therapeutic Nanoparticles and Their Targeted Delivery Applications. *Molecules* **25**, doi:10.3390/molecules25092193 (2020).
19. Li, M. *et al.* Recent advances in nanoantibiotics against multidrug-resistant bacteria. *Nanoscale Adv* **5**, 6278-6317, doi:10.1039/d3na00530e (2023).
20. Chifiriuc, M. C. *et al.* Common themes in antimicrobial and anticancer drug resistance. *Front Microbiol* **13**, 960693, doi:10.3389/fmicb.2022.960693 (2022).
21. Talevi, A. Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. *Front Pharmacol* **6**, 205, doi:10.3389/fphar.2015.00205 (2015).
22. Kabir, A. & Muth, A. Polypharmacology: The science of multi-targeting molecules. *Pharmacol Res* **176**, 106055, doi:10.1016/j.phrs.2021.106055 (2022).
23. Loscher, W. Single-Target Versus Multi-Target Drugs Versus Combinations of Drugs With Multiple Targets: Preclinical and Clinical Evidence for the Treatment or Prevention of Epilepsy. *Front Pharmacol* **12**, 730257, doi:10.3389/fphar.2021.730257 (2021).
24. Makhoba, X. H., Viegas, C., Jr., Mosa, R. A., Viegas, F. P. D. & Pooe, O. J. Potential Impact of the Multi-Target Drug Approach in the Treatment of Some Complex Diseases. *Drug Des Devel Ther* **14**, 3235-3249, doi:10.2147/DDDT.S257494 (2020).
25. Oselusi, S. O., Fadaka, A. O., Wyckoff, G. J. & Egieyeh, S. A. Computational Target-Based Screening of Anti-MRSA Natural Products Reveals Potential Multitarget Mechanisms of Action through Peptidoglycan Synthesis Proteins. *ACS Omega* **7**, 37896-37906, doi:10.1021/acsomega.2c05061 (2022).
26. Doostmohammadi, A., Jooya, H., Ghorbanian, K., Gohari, S. & Dadashpour, M. Potentials and future perspectives of multi-target drugs in cancer treatment: the next generation anti-cancer agents. *Cell Commun Signal* **22**, 228, doi:10.1186/s12964-024-01607-9 (2024).
27. Tran, D. N. *et al.* Dual anticancer and antibacterial activity of fluorescent naphthoimidazolium salts. *RSC Adv* **13**, 36430-36438, doi:10.1039/d3ra06555c (2023).
28. Parchebafi, A. *et al.* The dual interaction of antimicrobial peptides on bacteria and cancer cells; mechanism of action and therapeutic strategies of nanostructures. *Microb Cell Fact* **21**, 118, doi:10.1186/s12934-022-01848-8 (2022).
29. Dong, Z. *et al.* Anticancer Mechanisms and Potential Anticancer Applications of Antimicrobial Peptides and Their Nano Agents. *Int J Nanomedicine* **19**, 1017-1039, doi:10.2147/IJN.S445333 (2024).

30. Tolos , A. M. *et al.* Anticancer Potential of Antimicrobial Peptides: Focus on Buforins. *Polymers* **16**, 728 (2024).
31. Polanski, J., Kurczyk, A., Bak, A. & Musiol, R. Privileged structures - dream or reality: preferential organization of azanaphthalene scaffold. *Curr Med Chem* **19**, 1921-1945, doi:10.2174/092986712800167356 (2012).
32. Solomon, V. R. & Lee, H. Quinoline as a privileged scaffold in cancer drug discovery. *Current medicinal chemistry* **18**, 1488-1508, doi:10.2174/092986711795328382 (2011).
33. Song, Y. n., Xu, H., Chen, W., Zhan, P. & Liu, X. 8-Hydroxyquinoline: a privileged structure with a broad-ranging pharmacological potential. *MedChemComm* **6**, 61-74, doi:10.1039/C4MD00284A (2015).
34. Gupta, R., Luxami, V. & Paul, K. Insights of 8-hydroxyquinolines: A novel target in medicinal chemistry. *Bioorg Chem* **108**, 104633, doi:10.1016/j.bioorg.2021.104633 (2021).
35. Musiol, R., Mrozek-Wilczkiewicz, A. & Polanski, J. Synergy against fungal pathogens: working together is better than working alone. *Curr Med Chem* **21**, 870-893, doi:10.2174/09298673216613121809484 8 (2014).
36. Musiol, R., Serda, M., Hensel-Bielowka, S. & Polanski, J. Quinoline-based antifungals. *Curr Med Chem* **17**, 1960-1973, doi:10.2174/092986710791163966 (2010).
37. Saadeh, H. A., Sweidan, K. A. & Mubarak, M. S. Recent Advances in the Synthesis and Biological Activity of 8-Hydroxyquinolines. *Molecules* **25**, 4321 (2020).
38. Matada, B. S., Pattanashettar, R. & Yernale, N. G. A comprehensive review on the biological interest of quinoline and its derivatives. *Bioorganic & Medicinal Chemistry* **32**, 115973, doi:https://doi.org/10.1016/j.bmc.2020.115973 (2021).
39. Dorababu, A. Recent update on antibacterial and antifungal activity of quinoline scaffolds. *Archiv der Pharmazie* **354**, 2000232 (2021).
40. Prachayasittikul, V., Prachayasittikul, S., Ruchirawat, S. & Prachayasittikul, V. 8-Hydroxyquinolines: a review of their metal chelating properties and medicinal applications. *Drug Des Devel Ther* **7**, 1157-1178, doi:10.2147/dddt.S49763 (2013).
41. Mrozek-Wilczkiewicz, A. *et al.* Investigating the anti-proliferative activity of styrylazanaphthalenes and azanaphthalenediones. *Bioorg Med Chem* **18**, 2664-2671, doi:10.1016/j.bmc.2010.02.025 (2010).
42. Abeydeera, N. *et al.* Harnessing the Dual Antimicrobial Mechanism of Action with Fe(8-Hydroxyquinoline)(3) to Develop a Topical Ointment for Mupirocin-Resistant MRSA Infections. *Antibiotics (Basel)* **12**, doi:10.3390/antibiotics12050886 (2023).
43. Yin, X.-D. *et al.* Design, Synthesis, and Antifungal Evaluation of 8-Hydroxyquinoline Metal Complexes against Phytopathogenic Fungi. *Journal of Agricultural and Food Chemistry* **68**, 11096-11104, doi:10.1021/acs.jafc.0c01322 (2020).
44. Pivarcsik, T. *et al.* Metal Complexes of a 5-Nitro-8-Hydroxyquinoline-Proline Hybrid with Enhanced Water Solubility Targeting Multidrug Resistant Cancer Cells. *Int J Mol Sci* **24**, doi:10.3390/ijms24010593 (2022).

45. Pape, V. F. S. *et al.* Structure-Activity Relationships of 8-Hydroxyquinoline-Derived Mannich Bases with Tertiary Amines Targeting Multidrug-Resistant Cancer. *J Med Chem* **65**, 7729-7745, doi:10.1021/acs.jmedchem.2c00076 (2022).
46. Conan, P. *et al.* Identification of 8-Hydroxyquinoline Derivatives That Decrease Cystathionine Beta Synthase (CBS) Activity. *Int J Mol Sci* **23**, doi:10.3390/ijms23126769 (2022).
47. Szczepaniak, J., Cieslik, W., Romanowicz, A., Musiol, R. & Krasowska, A. Blocking and dislocation of Candida albicans Cdr1p transporter by styrylquinolines. *Int J Antimicrob Agents* **50**, 171-176, doi:10.1016/j.ijantimicag.2017.01.044 (2017).
48. Kos, J. *et al.* Ring-substituted 8-hydroxyquinoline-2-carboxanilides as potential antimycobacterial agents. *Bioorg Med Chem* **23**, 4188-4196, doi:10.1016/j.bmc.2015.06.047 (2015).
49. Kushkevych, I., Vítězová, M., Kos, J., Kollár, P. & Jampílek, J. Effect of selected 8-hydroxyquinoline-2-carboxanilides on viability and sulfate metabolism of Desulfovibrio piger. *Journal of Applied Biomedicine* **16**, 241-246, doi:https://doi.org/10.1016/j.jab.2018.01.004 (2018).
50. Joaquim, A. R. *et al.* Novel Antimicrobial 8-Hydroxyquinoline-Based Agents: Current Development, Structure-Activity Relationships, and Perspectives. *J Med Chem* **64**, 16349-16379, doi:10.1021/acs.jmedchem.1c01318 (2021).
51. Kos, J. *et al.* 8-Hydroxyquinoline-2-carboxanilides as antiviral agents against avian influenza virus. *ChemistrySelect* **4**, 4582–4587, doi:10.1002/slct.201900873 (2019).
52. Kumar, N. *et al.* Quinoline derivatives volunteering against antimicrobial resistance: rational approaches, design strategies, structure activity relationship and mechanistic insights. *Mol Divers* **27**, 1905-1934, doi:10.1007/s11030-022-10537-y (2023).
53. Mrozek-Wilczkiewicz, A. *et al.* The synthesis and anticancer activity of 2-styrylquinoline derivatives. A p53 independent mechanism of action. *Eur J Med Chem* **177**, 338-349, doi:10.1016/j.ejmech.2019.05.061 (2019).
54. Mrozek-Wilczkiewicz, A. *et al.* Design, Synthesis and In Vitro Activity of Anticancer Styrylquinolines. The p53 Independent Mechanism of Action. *PLoS One* **10**, e0142678, doi:10.1371/journal.pone.0142678 (2015).
55. Kuczak, M., Cieślik, W., Musioł, R. & Mrozek-Wilczkiewicz, A. 4-Furanylvinylquinoline derivative as a new scaffold for the design of oxidative stress initiator and glucose transporter inhibitor drugs. *Scientific Reports* **14**, 28454, doi:10.1038/s41598-024-79698-0 (2024).
56. Bissani, C., Gasparin, D. & Pilger, A. 8-Hydroxyquinoline, derivatives and metal-complexes: A review of antileukemia activities. *ChemistrySelect* **8**, doi:10.1002/slct.202204219 (2023).
57. Czaplinska, B. *et al.* Comprehensive exploration of the optical and biological properties of new quinoline based cellular probes. *Dyes and Pigments* **144**, 119-132, doi:https://doi.org/10.1016/j.dyepig.2017.05.029 (2017).
58. Gao, F., Zhang, X., Wang, T. & Xiao, J. Quinolone hybrids and their anti-cancer activities: An overview. *European Journal of Medicinal Chemistry* **165**, 59-79, doi:https://doi.org/10.1016/j.ejmech.2019.01.017 (2019).

59. *Cloxyguin*, <https://go.drugbank.com/drugs/DB15933> (
60. *Clioquinol*, <https://go.drugbank.com/drugs/DB04815> (
61. *Oxyquinoline*, <https://go.drugbank.com/drugs/DB11145> (
62. *Chlorquinaldol*, <https://go.drugbank.com/drugs/DB13306> (
63. *Dequalinium*, <https://go.drugbank.com/drugs/DB04209> (
64. Cieslik, W. *et al.* Contribution to investigation of antimicrobial activity of styrylquinolines. *Bioorg Med Chem* **20**, 6960-6968, doi:10.1016/j.bmc.2012.10.027 (2012).
65. Cieslik, W. *et al.* Investigation of the antimycobacterial activity of 8-hydroxyquinolines. *Med Chem* **11**, 771-779, doi:10.2174/1573406410666150807111703 (2015).
66. Jampilek, J. *et al.* Investigating biological activity spectrum for novel styrylquinazoline analogues. *Molecules* **14**, 4246-4265, doi:10.3390/molecules14104246 (2009).
67. Musiol, R. *et al.* Antifungal properties of new series of quinoline derivatives. *Bioorg Med Chem* **14**, 3592-3598, doi:10.1016/j.bmc.2006.01.016 (2006).
68. Musiol, R. *et al.* Investigating the activity spectrum for ring-substituted 8-hydroxyquinolines. *Molecules* **15**, 288-304, doi:10.3390/molecules15010288 (2010).
69. Serda, M. *et al.* Investigation of the biological properties of (hetero)aromatic thiosemicarbazones. *Molecules* **17**, 13483-13502, doi:10.3390/molecules171113483 (2012).
70. Zadrazilova, I. *et al.* In vitro bactericidal activity of 4- and 5-chloro-2-hydroxy-N-[1-oxo-1-(phenylamino)alkan-2-yl]benzamides against MRSA. *Biomed Res Int* **2015**, 349534, doi:10.1155/2015/349534 (2015).
71. Czech Collection of Microorganisms, Brno, Czech Republic.
72. *National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 11th ed.; M07*. (NCCLS, 2018).
73. R. Schwalbe, L. Steele-Moore & Goodwin, A. C. *Antimicrobial Susceptibility Testing Protocols*. (CRC Press, 2007).
74. *Measuring Cell Viability/Cytotoxicity.*, <www.dojindo.eu.com/Protocol/Dojindo-Cell-Proliferation-Protocol.pdf> (
75. Abate, G., Mshana, R. N. & Miorner, H. Evaluation of a colorimetric assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) for rapid detection of rifampicin resistance in Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* **2**, 1011-1016 (1998).
76. Bueno, J. *Antitubercular In Vitro Drug Discovery: Tools for Begin the Search, Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance*. (InTech, 2011).
77. Grela, E., Kozlowska, J. & Grabowiecka, A. Current methodology of MTT assay in bacteria - A review. *Acta Histochem* **120**, 303-311, doi:10.1016/j.acthis.2018.03.007 (2018).
78. Alexander, B. D. *et al.* Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: M27-A2. (Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2017).

79. Pliska, V., Testa, B. & van der Waterbeemd, H. *Lipophilicity in Drug Action and Toxicology.*, (Wiley-VCH: Weinheim, Germany, 1996).
80. Culen, M., Rezacova, A., Jampilek, J. & Dohnal, J. Designing a dynamic dissolution method: a review of instrumental options and corresponding physiology of stomach and small intestine. *J Pharm Sci* **102**, 2995-3017, doi:10.1002/jps.23494 (2013).
81. Kerns, E. H. & Di, L. *Drug-Like Properties: Concepts. Structure Design and Methods: From ADME to Toxicity Optimization.*, (Academic Press: San Diego, CA, USA, 2008).
82. Cieslik, W., Szczepaniak, J., Krasowska, A. & Musiol, R. Antifungal Styryloquinolines as Candida albicans Efflux Pump Inhibitors: Styryloquinolines are ABC Transporter Inhibitors. *Molecules* **25**, doi:10.3390/molecules25020345 (2020).
83. Zieba, A. *et al.* Design, Synthesis, and Anticancer and Antibacterial Activities of Quinoline-5-Sulfonamides. *Molecules* **29**, 4044 (2024).
84. Dolezal, M. *et al.* Synthesis, antimycobacterial, antifungal and photosynthesis-inhibiting activity of chlorinated N-phenylpyrazine-2-carboxamides. *Molecules* **15**, 8567-8581, doi:10.3390/molecules15128567 (2010).
85. Strharsky, T. *et al.* Study of Biological Activities and ADMET-Related Properties of Novel Chlorinated N-arylcinnamamides. *Int J Mol Sci* **23**, doi:10.3390/ijms23063159 (2022).
86. Portela, C. A., Smart, K. F., Tumanov, S., Cook, G. M. & Villas-Bôas, S. G. Global metabolic response of Enterococcus faecalis to oxygen. *J Bacteriol* **196**, 2012-2022, doi:10.1128/jb.01354-13 (2014).
87. Gilmore, M. S., Clewell, D. B., Ike, Y. & Shankar, N. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection.*, (Massachusetts Eye and Ear Infirmary, 2014).
88. Ramos, S., Silva, V., Dapkevicius, M. L. E., Igrejas, G. & Poeta, P. Enterococci, from Harmless Bacteria to a Pathogen. *Microorganisms* **8**, doi:10.3390/microorganisms8081118 (2020).
89. Gilmore, M. S. *et al.* Genes Contributing to the Unique Biology and Intrinsic Antibiotic Resistance of Enterococcus faecalis. *mBio* **11**, doi:10.1128/mBio.02962-20 (2020).
90. Fraunfelter, V. M. *et al.* Quinoline Compounds Targeting the c-Ring of ATP Synthase Inhibit Drug-Resistant Pseudomonas aeruginosa. *ACS Infect Dis* **9**, 2448-2456, doi:10.1021/acsinfecdis.3c00317 (2023).
91. Nubel, U. *et al.* A timescale for evolution, population expansion, and spatial spread of an emerging clone of methicillin-resistant Staphylococcus aureus. *PLoS Pathog* **6**, e1000855, doi:10.1371/journal.ppat.1000855 (2010).
92. *Bedaquiline*, <go.drugbank.com/drugs/DB08903> (
93. Mapari, M., Bhole, R. P., Khedekar, P. B. & Chikhale, R. V. Challenges in targeting mycobacterial ATP synthase: The known and beyond. *Journal of Molecular Structure* **1247**, 131331, doi:https://doi.org/10.1016/j.molstruc.2021.131331 (2022).

Schemes

Schemes 1 and 2 are available in the Supplementary Files section.

Table 1

Table 1 is available in the Supplementary Files section.

Figures

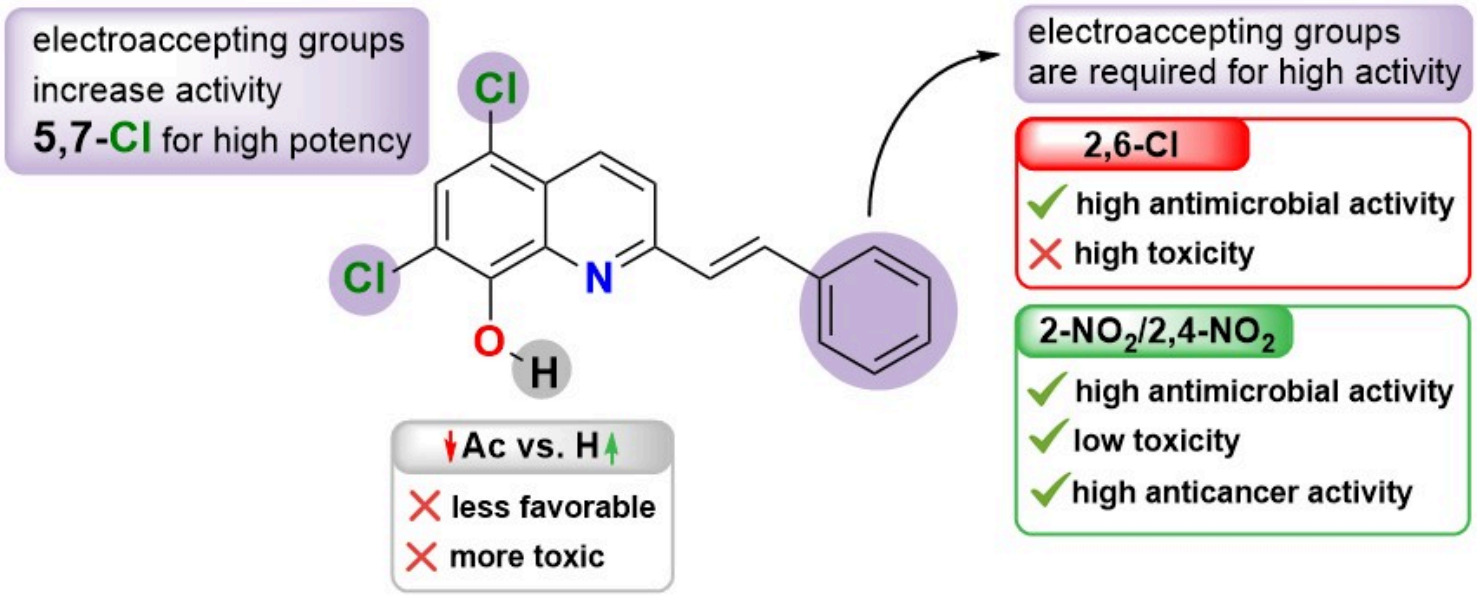


Figure 1

Summary of structure–activity relationships of 2-styrylquinolines.

Supplementary Files

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- SupplementaryMaterial.docx
- scheme1.jpg
- scheme2.jpg
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