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RESEARCH

Quinolinequinone-based sulfonamide bioisosteres as promising antimicrobial agents

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Abstract
In the search for novel antimicrobial agents, a series of aminated quinolinequinone derivatives (AQQ1-13) were synthesized and evaluated for their in vitro antibacterial and antifungal activities. These compounds were constructed *via* sulfonamide linkers incorporating commercially available *p*-aminobenzenesulfonamides bearing diverse heteroaromatic or acyl substituents. The antibacterial activity was assessed against four gram-negative and three gram-positive bacterial strains, while antifungal activity was evaluated against three fungal species using the CLSI-approved broth microdilution method. Several compounds demonstrated moderate antimicrobial activity when compared with standard reference drugs. Notably, three AQQs (AQQ7, AQQ9, and AQQ11) exhibited notable antibacterial activity against *Enterococcus faecalis*, with a minimum inhibitory concentration (MIC) value of 78.12 µg/mL, showing lower MIC values than amikacin under the tested conditions (MIC = 128 µg/mL) under the same conditions. Time-kill studies revealed bactericidal effects at 1× and 4× MIC levels, while combination studies demonstrated synergistic interactions with levofloxacin, particularly for AQQ11. Furthermore, AQQ9 and AQQ11 exhibited antibiofilm activity in combination therapy, including inhibition of biofilm formation and partial disruption of preformed biofilms. In silico ADMET and molecular docking analyses identified AQQ9 as a compound of interest, showing favorable drug-likeness properties and favourable binding interactions toward *E. faecalis* DNA gyrase. Overall, these findings highlight quinolinequinone-based sulfonamide bioisosteres as potential scaffolds for further development of combination therapies targeting gram-positive and biofilm-associated infections.

Keywords Antimicrobial activity · Quinolinequinone hybrids · Sulfonamide bioisosteres · Biofilm inhibition · DNA gyrase inhibition · Molecular docking

Introduction

Infections caused by bacteria have emerged as the most prevalent cause of mortality, raising serious worldwide public health concerns (Premalatha et al. 2024). Antibiotic-resistant strains have emerged quickly as a result of the extensive and frequently improper use of antibiotics, despite the fact that they have been essential in the fight against these illnesses (Naghavi et al. 2024). As an outcome of the extensive development and spreading of antibiotic resistance mechanisms,

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the therapeutic effectiveness of most antibiotics has gradually decreased (Ramu et al. 2024). The first antibacterial agents to show selective toxicity and to be used in clinical settings as early as 1935 were the sulfonamide family of antibiotics by Gerhard Domagk (Domagk 1947). However, they were also among the first to be weakened by the development of antimicrobial resistance (Sköld 2000; Eliopoulos and Huovinen 2001). Since the discovery of the sulfonamide family of antibiotics, sulfonamide (-SO_2NH-) or benzenesulfonamide (Ph-SO_2NH-) moiety has been essential in medicinal chemistry (Fig. 1). This moiety is vital to a large class of physiologically active molecules and continues to be an important pharmacophore in a wide range of pharmacological applications, such as antimicrobial (Ammar et al. 2020; Ragab et al. 2022), antidiabetic (Khamees Thabet et al. 2024), anticancer (Gul et al. 2018), and anti-Alzheimer activity (Gök et al. 2021). The therapeutic and physiological significance of sulfonamide or benzenesulfonamide moiety remains to be a crucial area of research in pharmaceutical and medicinal design and development due to their extensive use and shown effectiveness (Zhao et al. 2019). In recent years, addressing antimicrobial resistance has required a more integrated and multidisciplinary approach. For example, biomarker-based clinical scoring systems incorporating parameters such as lymphocyte count, international normalized ratio, and procalcitonin have improved the assessment of infection severity and risk stratification, emphasizing the urgent need for more effective therapeutic agents (Liu et al. 2022). From a molecular design perspective, structural optimization strategies, or acyl group salt cocrystal formation, have been shown to enhance the physicochemical and biological properties of antifungal agents, particularly in improving solubility, permeability, and activity (Wang et al. 2025). In parallel, biofilm-targeted approaches have gained increasing attention, as biofilm formation represents a major contributor to antimicrobial resistance; recent studies demonstrate that ROS-mediated and stimulus-responsive systems can effectively disrupt biofilm structures and enhance drug efficacy (Wang et al. 2025). Furthermore, combination therapy has emerged as a powerful strategy to combat multidrug-resistant pathogens, with accumulating evidence supporting its role in improving treatment outcomes (Yang et al. 2025) and overcoming resistance mechanisms (Yang et al. 2025). In addition, advances in computational and data-driven methodologies, particularly machine learning-guided reactivity prediction, have enabled more efficient identification of suitable molecular scaffolds and accelerated rational drug design processes (Dou et al. 2024). Taken together, these developments highlight the necessity of designing structurally optimized and multifunctional molecules capable of overcoming resistance, targeting biofilms, and improving therapeutic performance.

The sulfonamide or benzenesulfonamide moiety has been extensively hybridised with a variety of heterocyclic moieties, including as five-membered (thiadiazole and oxazole) or six-membered rings (pyridine, pyrimidine, and diazine), in an attempt to develop a diverse array of sulfonamide-based antibacterial agents (Culletta et al. 2023; Ghomashi et al. 2023). By encouraging certain noncovalent interactions with microbial biological targets, these aromatic heterocycles are known to increase antibacterial activity (Gulçin and Taslimi, 2018). Most important examples include

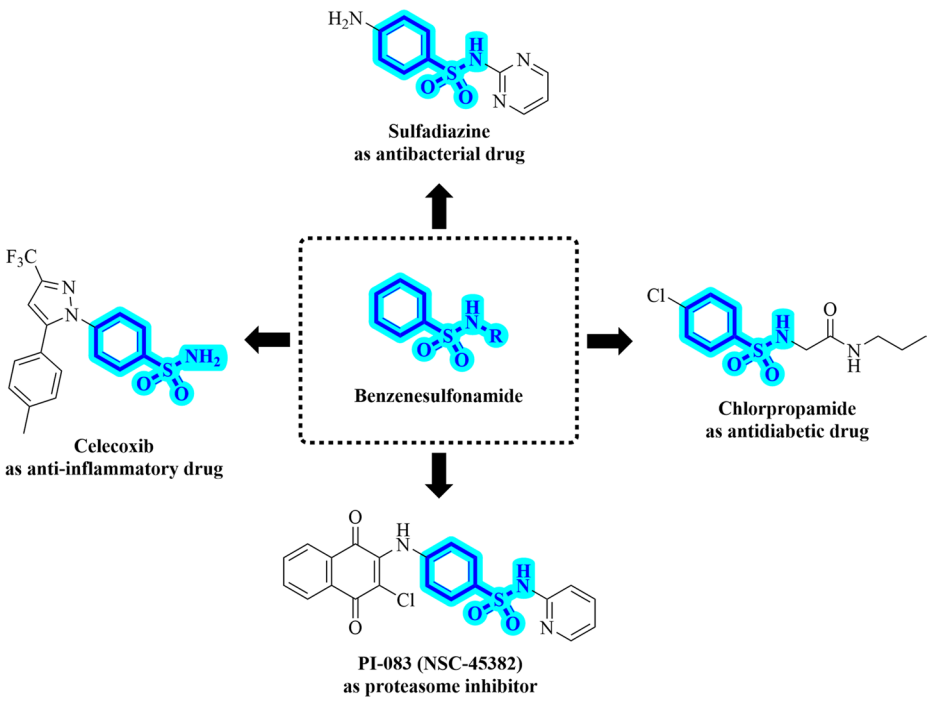


Fig. 1 Structurally diverse sulfonamide-based drugs with different therapeutic applications

sulfabenzamide, sulfamethoxazole, sulfamethizole, sulfadiazine, sulfamethoxypyridazine, sulfamoxole, and sulfathiazole known as sulfa drugs. A wide range of sulfa drugs including a *p*-aminosulfonamide or benzenesulfonamide moiety which is substituted by a heteroaromatic group containing simple substituent(s), such as methyl or methoxy group(s), are currently utilized in clinical practice for the effective treatment of bacterial infections (Fig. 2) (Brown and Wright 2016).

A wide range of pharmacophoric scaffolds have been extensively explored in drug discovery, including 2-azidoethane-1-sulfonyl fluoride (Zhang et al. 2019), coumarin derivatives (Qin et al. 2020b), chalcone derivatives (Xu et al. 2019), indole-based compounds (Qin et al. 2020a), pyrazole-based analogues (Verma et al. 2021), triazine derivatives (Liu et al. 2020), and sulfonyl- or sulfonamide-containing heterocyclic structures (Kumar Verma et al. 2020). These diverse frameworks have demonstrated significant biological activities and have contributed to the development of novel therapeutic agents. In the past several years, 1,4-quinone moiety has emerged as "a lead structure" in the field of drug discovery (Wellington 2015; de Carvalho et al. 2021; Devi et al. 2022; Mone et al. 2023; Pacheco et al. 2023). Several studies have described the synthesis of sulfonamides hybridized with 1,4-quinone moiety as a novel class of proteasome inhibitors. Lawrence et al. reported the synthesis and identification of these proteasome inhibitors containing some common structural features namely a *para*-amino benzenesulfonamide ring attached to various heteroaromatic groups. In this study, PI-083 (NSC-45382), identified as a proteasome inhibitor selective for cancer cell lines over non-transformed cell lines by National Cancer Institute of USA (Kazi et al. 2009), was used in the design of targeted molecules (Lawrence et al. 2010). They identified both the sulfonamide moiety and the chlorine atom as the key elements of the pharmacophore. Xu et al. introduced a new series of PI-083 analogues and investigated the effect of replacing a carbonyl group instead of sulfonyl group (Xu et al. 2012). This work intends to address the critical issue of identifying new compounds with strong antimicrobial activity and low toxicity, building on our earlier results and the ongoing efforts of our research group to uncover novel antibacterial and/or antifungal agents. Herein, we report the synthesis of a new hybrid nucleus containing both sulfonamides and 1,4-quinone moieties to obtain more effective antimicrobial drugs. Their antibacterial and antifungal properties were studied in vitro, and the action mechanism of benzenesulfonamide-containing heterocyclic 1,4-quinone was suggested. Finally, the molecular docking simulation was performed.

Results and discussion

Chemistry

In our previous studies, the developed selective aminoquinones displayed notable in vitro activity to inhibit the growth of gram-positive bacteria and also fungi (Mataraci-Kara et al. 2022a, b, c; ldırım et al. 2022; Yildirim et al. 2022; Sharma et al. 2023; Chen et al. 2024). Both the quinoline-quinone and the aromatic amine moiety were identified as

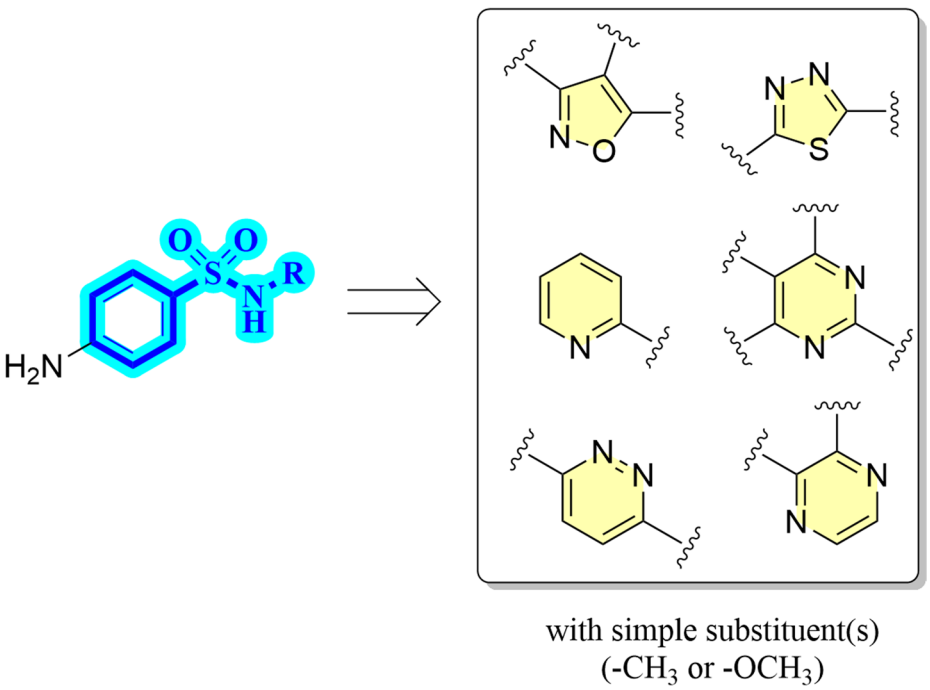
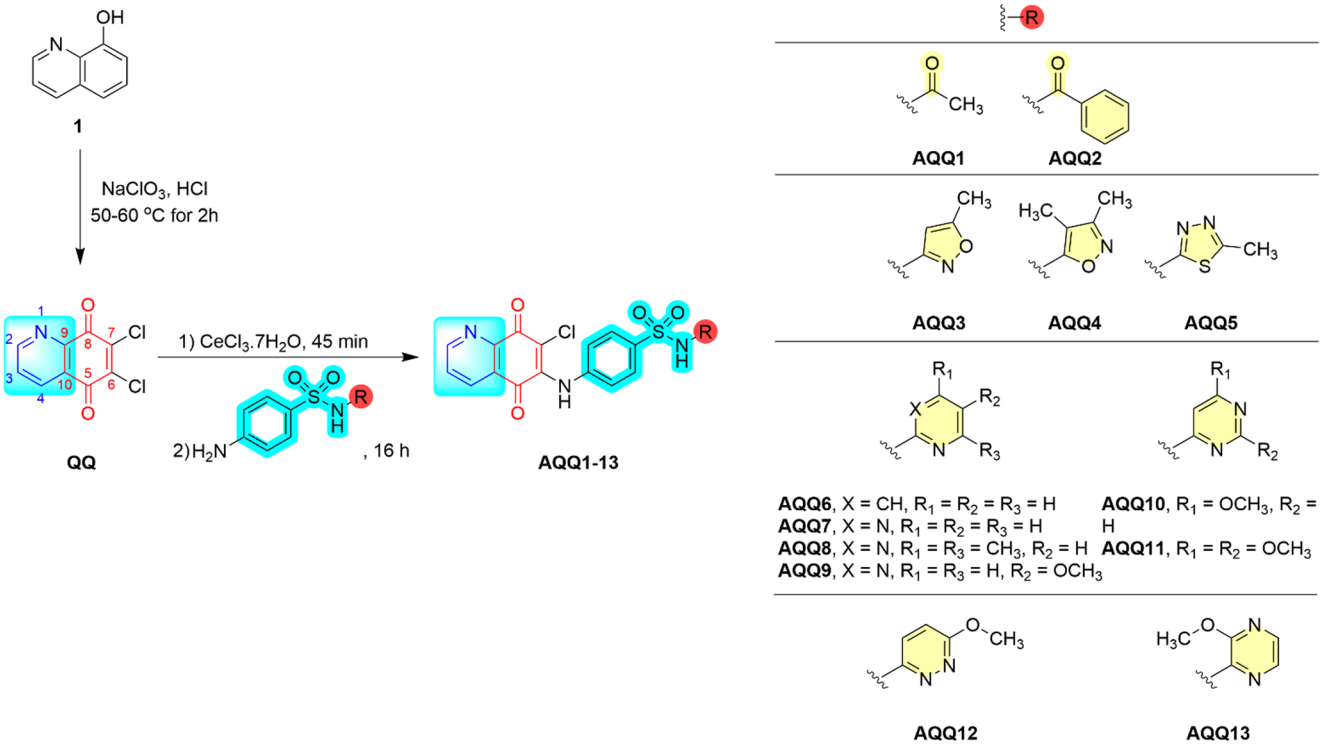


Fig. 2 Examples of known sulfonamide antibiotics known as sulfa drugs

important pharmacophores that contributed to the reported inhibitory action upon analysis of the in vitro data. Based on these findings, this scaffold was selected as a structural template to create a new series of molecules. As part of our ongoing research to identify new antibacterial and/or antifungal lead structures, we investigated the isosteric replacement of substituents on the aromatic amine moiety with a sulfonamide group. As shown in Fig. 2, this alteration was further developed by adding either acyl group or conformationally restricted heterocycles, including isoxazole, thiadiazole, pyridine, pyrimidine, pyrazine, and pyridazine or acyl group (acetyl and benzamide) with different substituent(s), to the sulfonamide moiety. Synthesis of the designed molecules is described in Scheme 1. Briefly, the library of the target aminoquinones (AQQ1-13) was synthesized from commercially available *p*-aminobenzenesulfonamides containing different heteroaromatic or acyl group building blocks and quinolinequinone (QQ) using the protocol employed in our recent publications. In this effort, the quinolinequinone (QQ) was obtained from commercially available 8-hydroxyquinoline (1) as outlined in Scheme 1 *via* a well-established synthetic route (Shaikh et al. 1986). Compound **1** was treated with sodium chlorate ($NaClO_3$) in concentrated hydrochloric acid to afford a chlorinated quinolinequinone (QQ) derivative as a key intermediate. The product distribution of this transformation is highly sensitive to reaction parameters, including the polarity of the reaction medium and the molar ratios of the substrate and reagents (Yoon et al. 2000; Defant et al. 2006; Kadela et al. 2016). Since the precursor (QQ) has two unsymmetrical chlorine atoms which are not electronically equivalent, nucleophilic substitution of chlorine atom located at the C-6 and/or C-7 positions by amines occurs preferentially at these positions. The substitution reaction depends on the nature of the reaction medium and the reagents employed (Kadela-Tomanek et al. 2017; Santoso et al. 2019). Literature reports show that the addition of Lewis acid, such as $CeCl_3.7H_2O$ or $NiCl_2.6H_2O$, can promote preferential nucleophilic substitution at the C-6 position of chlorinated quinolinequinones (QQ). This regioselectivity is attributed to a reduction in electron density at C-6, facilitated by chelation of the Lewis acid, particularly Ce(III), between the pyridine nitrogen and the carbonyl oxygen at the C-8 position (Yoon et al. 2000; Lee et al. 2004). Furthermore, the use of polar solvents such as water or ethanol has been shown to enhance this C-6 selectivity (Yoon et al. 2000). Lewis acid was employed to promote substitution at the C-6 position, which was followed by amination to produce the final products (AQQ1-13) as outlined in Scheme 1. A total of 13 AQQs were synthesized to explore their antimicrobial potential. The R group of the core structure was modified with different heteroaromatic or acyl groups (Fig. 3). All the newly obtained aminated quinolinequinones were fully characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and MS spectral techniques.



Scheme 1 Synthetic pathway of the target molecules (AQQ1-13)

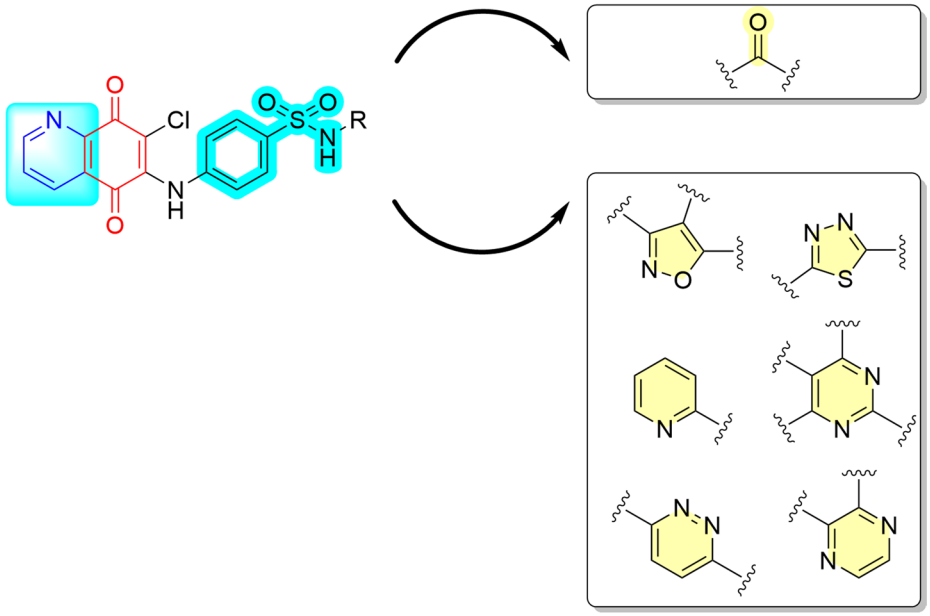


Fig. 3 The core structure of the target molecules

Antimicrobial activity

Determination of minimum inhibitory concentrations (MIC)

To elucidate the significance of the aromatic amine moiety containing a sulfonamide group in conjunction with heteroaromatic or an acyl groups, the in vitro antibacterial and antifungal activities of the target molecules (AQQ1-13) were systematically evaluated as shown in Tables 1 and 2. The antimicrobial activity of aminated QQs (AQQ1-13) showed varying activity across the tested strains. Most AQQs displayed limited biological potency against gram-negative bacteria, with MIC values generally above 2500 µg/mL, except for AQQ3, AQQ5, AQQ7, AQQ8, AQQ10, AQQ11, and AQQ13, which showed weak activity against *E. coli* and *P. mirabilis*. On the other hand, three AQQs (AQQ7, AQQ9, and AQQ11) showed comparatively better activity against gram-positive bacteria, especially *E.*

Table 1 Minimum inhibitory concentration (MIC, µg/mL) values of the aminated QQs (**AQQ1-13**) against selected gram-positive and gram-negative bacteria strains.[a]

ID	Bacteria						
	Gram-negative Bacteria (MIC, µg/mL)				Gram-positive Bacteria (MIC, µg/mL)		
	P. aeruginosa	E. coli	K. pneumoniae	*P.* mirabilis	E. faecalis	S. epidermidis	S. aureus
AQQ1	-	-	-	1250	625	1250	156.25
AQQ2	-	-	-	-	-	-	-
AQQ3	-	625	-	625	312.50	-	-
AQQ4	-	-	-	-	-	-	-
AQQ5	-	312.50	-	-	312.50	-	625
AQQ6	-	-	-	-	-	-	-
AQQ7	-	625	-	625	**78.12**	1250	156.25
AQQ8	-	625	-	1250	-	-	-
AQQ9	-	-	-	1250	**78.12**	625	625
AQQ10	-	625	625	625	312.50	625	-
AQQ11	-	625	-	1250	**78.12**	625	312.50
AQQ12	-	-	-	-	625	-	-
AQQ13	-	625	-	625	625	-	-
Ceftazidime	2.44	ND	ND	ND	ND	ND	ND
Cefuroxime-Na	ND	4.88	4.88	2.44	ND	ND	1.22
Cefuroxime	ND	ND	ND	ND	ND	9.76	ND
Amikacin	ND	ND	ND	ND	128	ND	ND

[a] "-" means > 2500 µg/mL activity.

ND means not determined.

Table 2 Minimum inhibitory concentration (MIC, µg/mL) values of the aminated QQs (AQQ1-13) against selected fungal strains.[a]

ID	Fungi (MIC, µg/mL)		
	C. albicans	C. parapsilosis	C. tropicalis
AQQ1	-	312.50	625
AQQ2	312.50	625	-
AQQ3	-	312.50	312.50
AQQ4	312.50	625	625
AQQ5	312.50	312.50	312.50
AQQ6	-	-	-
AQQ7	-	-	625
AQQ8	312.50	312.50	312.50
AQQ9	-	-	625
AQQ10	312.50	-	-
AQQ11	-	312.50	312.50
AQQ12	312.50	312.50	312.50
AQQ13	156.25	312.50	312.50
Clotrimazole	4.88	ND	ND
Amphotericin B	ND	0.50	1.00

[a] "-" means > 2500 µg/mL activity.

ND means not determined.

faecalis (MIC = 78.12 µg/mL) with 1.5-fold better inhibitory potency compared to the standard reference amikacin. Five AQQs (AQQ1, AQQ7, and AQQ9-11) showed poor antibacterial activity against *S. epidermidis*, while five AQQs (AQQ1, AQQ5, AQQ7, AQQ9, and AQQ11) showed weak antibacterial activity against *S. aureus*. Furthermore, variable antifungal activity was observed, with several AQQs exhibiting MIC values ranging from 156.25 to 625 µg/mL against *Candida* species shown in Table 2. With relatively consistent activity against gram-positive bacterial strains, three AQQs (AQQ7, AQQ9, and AQQ11) showed relatively consistent activity.

A structure-activity relationship (SAR) study for the aminated QQs (AQQ1-13) provided insights into the relationship between structural modifications and antimicrobial activity. Among the tested compounds, three AQQs (AQQ7, AQQ9, and AQQ11) exhibited the highest antibacterial activity against *E. faecalis* (MIC = 78.12 µg/mL). Comparison with AQQ6 suggests that the presence of an additional nitrogen atom may contribute to enhanced antibacterial activity against *E. faecalis*. In addition, two AQQs (AQQ9 and AQQ11) showed relatively higher activity, indicating that methoxy substituents may positively influence biological activity. Dimethyl-substituted AQQs containing either five-membered (AQQ3-5) or six-membered (AQQ8) heterocycles showed a slight increase in antifungal activity. In contrast, the introduction of a methyl group on the heterocyclic ring was associated with reduced antibacterial activity, while a slight increase in antifungal activity was observed, suggesting that steric effects may differentially influence antibacterial and antifungal profiles. Overall,

Fig. 4 The analysis of the time-kill curve. (**A**) AQQ9 and AQQ11 alone or in combination with levofloxacin for *E. faecalis* ATCC 29212 at concentrations of 1 × MIC; (**B**) AQQ9 and AQQ11 alone or in combination with levofloxacin for *E. faecalis* ATCC 29212 at concentrations of 4 × MIC. The horizontal axis illustrates the duration of the killing process, whereas the vertical axis shows the logarithmic survival rates of the bacteria that were tested. *(EF: E. faecalis; LVX: levofloxacin; MIC: minimum inhibitory concentration)*. (***P = 0,0005)

electron-donating groups (such as -OCH_3) appeared to be more favourable than bulkier alkyl groups (such as -CH_3), which may be attributed to a better balance between electronic and steric factors. Although the AQQs were generally less active than the reference antibiotics, their selective activity, particularly against *E. faecalis* and fungal strains, may support further optimisation.

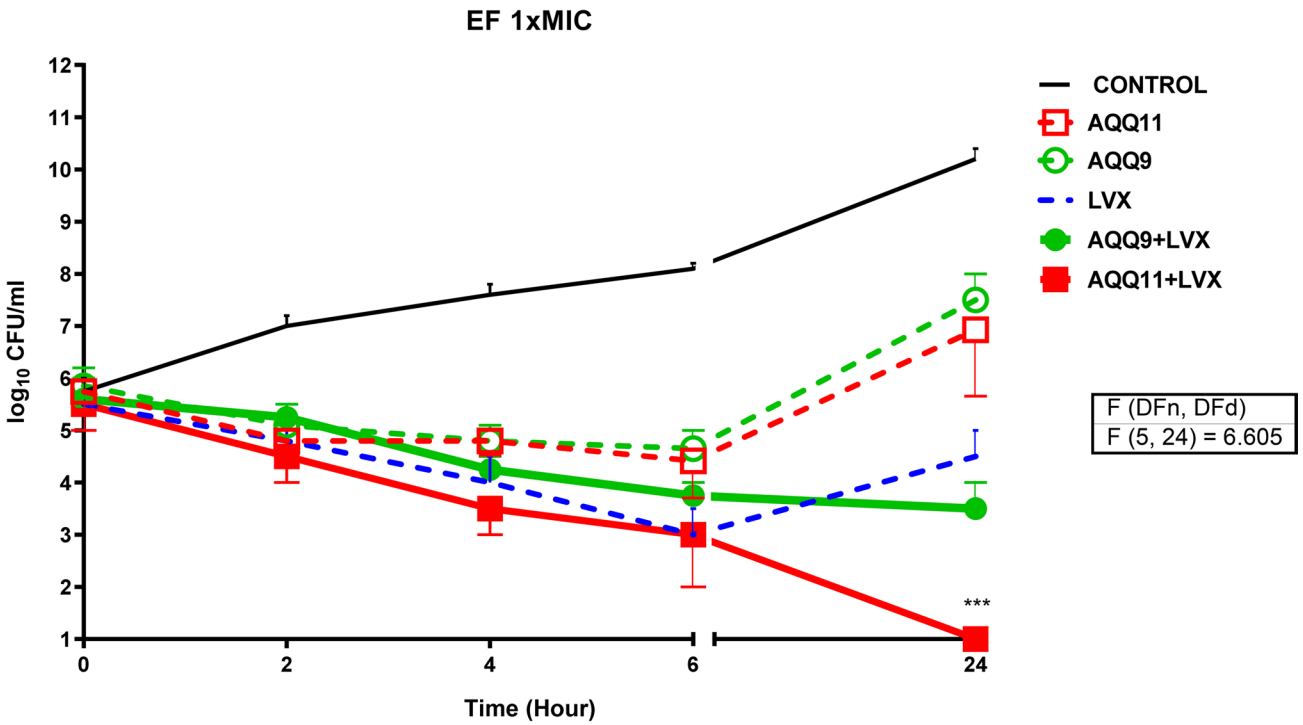
Determination of time-kill curve kinetic study

Time-kill curve (TKC) studies were conducted on *E. faecalis ATCC 29212*, as shown in Fig. 4. Two compounds were selected (AQQ9 and AQQ11) based on their antibacterial activity against *E. faecalis* based on the MIC results. TKC results indicated that neither the tested molecules nor the antibiotic exhibited bactericidal activity against the tested strain at both 1 × and 4 × MIC within 24 h (Fig. 4). On the other hand, potential synergistic effects were observed when combining AQQ11 with levofloxacin at both 1 × and 4 × MIC. Moreover, combining AQQ9 with levofloxacin suggested additive effects against the tested *E. faecalis* strain within 24 h. No antagonism was detected in any combination. Their significant MIC values and ability to combine with levofloxacin, an antibiotic commonly used in the treatment of resistant infections, suggest that these compounds may warrant further investigation.

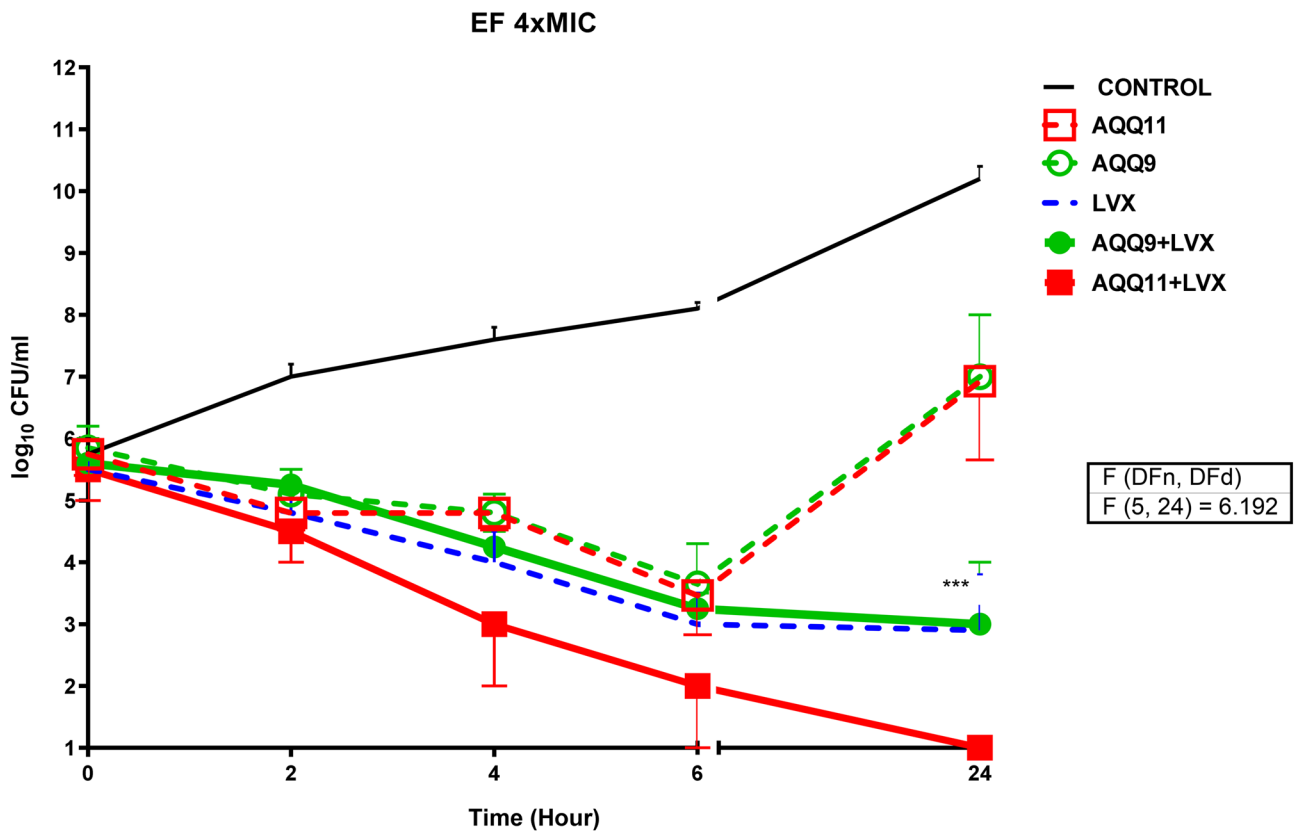
Evaluation of the in vitro antibiofilm activity

Microorganisms can attach to surfaces and form biofilms. Microbial cells inside biofilms have antibiotic resistance that is 10- to 1000-fold higher than that of planktonic counterparts. The significant antibiotic resistance seen in these bacterial populations complicates the effective treatment of biofilm-associated illnesses. Conventional antibiotic therapy fails to completely eradicate bacterial cells ensconced in biofilms, posing challenges for effective treatment. Various approaches and innovative antibiofilm agents are being investigated to address antimicrobial resistance in bacterial biofilm populations (Microbial Biofilms 1995; Donlan 2002; Mohammed et al. 2018; Sharma et al. 2019; Kara et al. 2020).

Because it showed potential synergistic activity in the time-kill curve test, AQQ11 was selected to evaluate its antibiofilm activity. For this purpose, AQQ11 was tested at


EF 1xMIC
log10 CFU/ml
Time (Hour)
CONTROL
AQQ11
AQQ9
LVX
AQQ9+LVX
AQQ11+LVX
F (DFn, DFd)
F (5, 24) = 6.605
***

A


EF 4xMIC
log10 CFU/ml
Time (Hour)
CONTROL
AQQ11
AQQ9
LVX
AQQ9+LVX
AQQ11+LVX
F (DFn, DFd)
F (5, 24) = 6.192
***

B

a sub-MIC level (1/10 × MIC) alone or in combination with LVX, and inhibited biofilm formation when tested alone. The tested molecule and antibiotic showed the highest activity within 6 h compared to previous hours. Cell attachment levels was lower than 50% compared to the control within 6 h when AQQ11 was tested alone or in combination with LVX. Furthermore, our evaluation of the combination effect indicated that it demonstrated increased activity compared to the individual conditions.

Biofilm formation assays showed that of biofilm inhibition were about the same for all tested concentrations of AQQ11. However, enhanced activity was observed in combination of AQQ11 with LVX at 1 × MIC. These results showed that the tested combination may enhance the inhibition of biofilm formation Fig. 5.

In silico ADMET studies

Physico-chemical properties and drug-likeness were predicted through SwissADME server (http://www.swissadme.ch/) for compounds AQQ7, AQQ9, and AQQ11. All the three were found to be compliant with Lipinski's rule-of-five (Ro5) (Lipinski et al. 2001; Lipinski 2004). Abbott bioavailability score was 0.55 due to low aqueous solubility and low GI absorption (Martin 2005). They were also found to have no blood brain barrier (BBB) permeation. CYP2C19, 2C9, and 3A4 inhibition was predicted for all the three and may possibly influence the metabolic profile of drugs metabolized by these enzymes. They were also not a substrate to p-glycoprotein (efflux-pump); hence one can expect that they may evade development of resistance through efflux-pump. Two PAINS (Baell and Holloway 2010) alerts were observed for these compounds due to the presence of halo-enone and quinone portion is one of the concerns.

Molecular docking

SwissTargetPrediction server (http://www.swisstargetprediction.ch/) predicted 100 target proteins in human for the compound AQQ7, AQQ9, and AQQ11. Unfortunately, no orthologous protein was identified for those hundred in *Enterococcus faecalis* through STRING server (https://string-db.org/). Hence, based on the structural features, quinoline-quinone(Onodera et al. 2002) and sulphonamide (Foss et al. 2011), we considered DNA gyrase and dihydrofolate reductase (DHRF) as probable targets. DHRF was then eliminated as amino-group carried bulkier non-hydrolysable quinoline-quinone ring that may hinder the binding of compounds with DHRF. An experimental 3-dimensional X-ray crystal structure of *E. faecalis* (PDB: 4GGL) (Tari et al. 2013) was then selected from protein data bank (https://www.rcsb.org) for molecular docking simulation using AutoDock4 (Table 3). Molecular docking protocol was validated through redocking with the co-crystallized ligand. Top scoring conformer from largest cluster displayed similar orientation as that of co-crystallized ligand with an RMSD value of 1.84 Å. The validated protocol was then adopted for docking AQQ7, AQQ9, and AQQ11. In this case, we picked top scoring conformer from the largest cluster and Ligand Efficiency (LE) was calculated to pick the best-performing compound amongst the three. AQQ9 with estimated Ki value in the sub micromolar concentration exhibited the best LE that was equivalent to CJC (co-crystallized ligand) and the complex was then analysed for molecular level interaction.

Compound AQQ9 was well accommodated in the active site of DNA gyrase of *Enterococcus faecalis* (PDB:4GGL), displaying an extended network of H-bonding and hydrophobic interactions with polar and non-polar amino acids (Fig. 6). Sulphonamide sidechain occupied the space in which the pyrrolopyrimidine ring of cocrystallized ligand was accommodated, called "lipophilic interior pocket" (Adenine binding site). This has kept the quinoline-quinone segment in the pocket where amino-pyrrolidine side chain of cocrystallized ligand was accommodated, called "lipophilic floor". Quinoline-quinone carbonyl oxygen at C5-position engaged with H-bonding interaction with sidechain hydroxyl group of SER122. Sulphonamide functional group established three H-bonding interactions, two of which were with sidechain polar functional groups of ASP75 and THR167, while the third one was with backbone amide NH of GLY79. The fifth H-bonding interaction was found between methoxy oxygen of 2-aminopyrimidine ring and sidechain amide-NH of ASN48 at the mouth of "interior pocket" leading to "lipophilic floor". Hydrophobic interactions were found between (i) Quinoline-quinone ring carbons and VAL96 and (ii) sidechain phenyl ring and ILE80 and ASN48. In case of AQQ7 and AQQ11, the orientation of the molecule inside the pocket reversed and displayed similar interaction pattern. Nitrogen-containing ring of quinoline-quinone has been found to establish hydrophobic interaction with the "lipophilic interior pocket", while the C8-carbonyl oxygen of quinone ring established a H-bonding interaction with side chain oxygen of SER122. Quinone ring thus displayed hydrophobic interaction with "lipophilic floor residues," VAL96 and ILE80. This has put the sulphonamide side chain in "polar anionic pocket" lined by ASP51, GLU52, and ARG78. Oxygen of sulphonamide functional group established H-bonding interaction with side chain guanido-group of ARG78. Additional H-bonding interaction also found between the pyrimidine ring and side chain carbonyl oxygen of ASP51. While all three compounds exhibited favourable interactions, AQQ9 showed

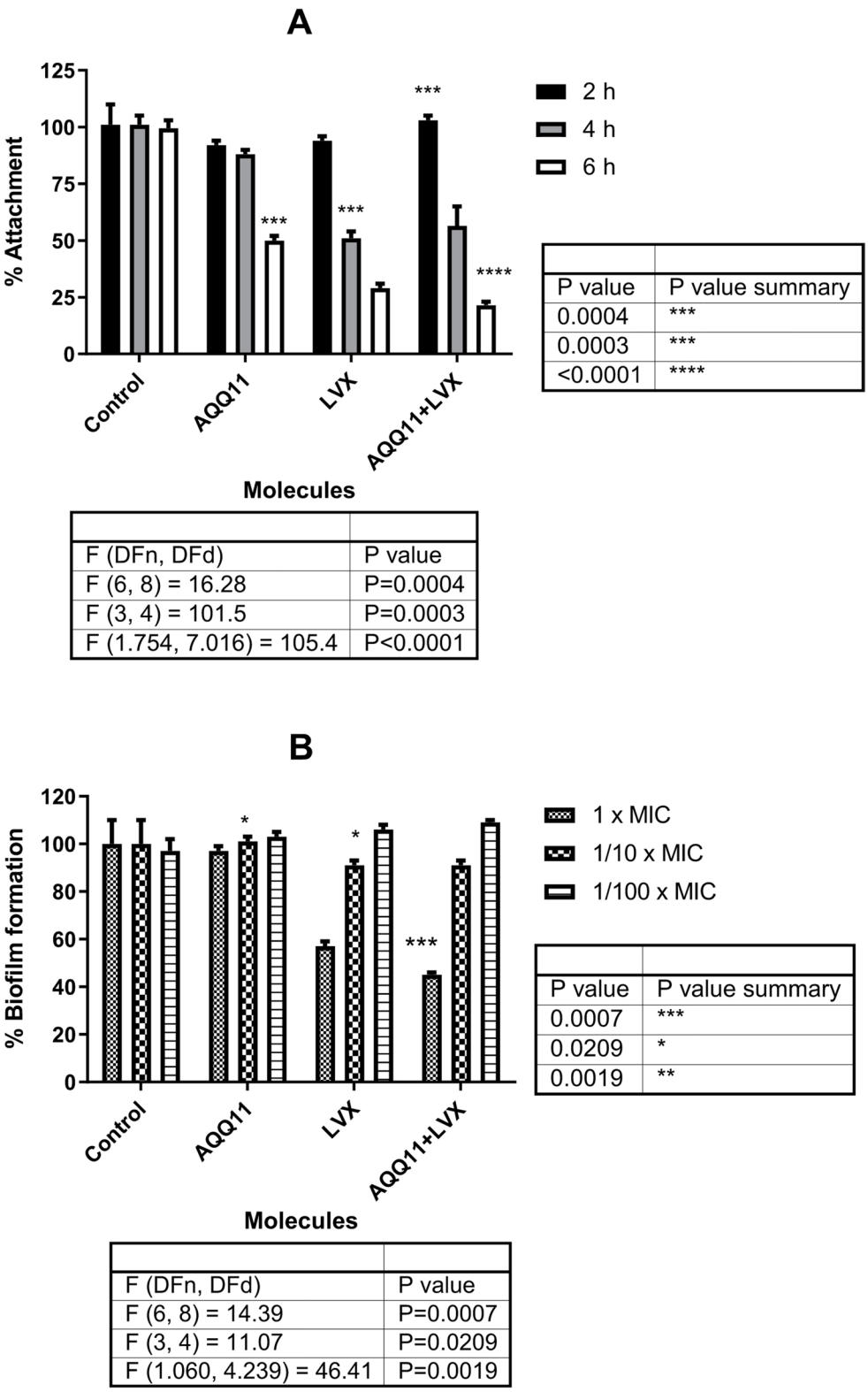


Fig. 5 Inhibition of *E. faecalis*. (**A**) surface attachment to the wells contained 1/10x MIC of molecules and an inoculum of 1×10^6 CFU/200 µL, incubated for 2, 4, or 6 h at 37 °C; (**B**) biofilm formation in each well contained 1 ×, 1/10 ×, or 1/100 × MIC of molecules and an inoculum of 5×10^6 CFU/200 µL, incubated for 24 h at 37 °C. Control bars indicate microorganisms without molecules accepted as 100%. Six wells were used for the tested molecules. Each experiment is representative of three independent tests. All differences between the control and molecules treated biofilms were statistically significant ($p < 0.0001$)

additional interactions with conserved ASN48 at the mouth of the lipophilic interior pocket. This critical interaction was found in CJC either direct or mediated through ordered water molecules in the cavity.

Conclusions

Due to the limitations of the current antibacterial and/or antifungal drugs, the development of new drugs or lead

Table 3 Molecular docking simulation report of AQQ7, AQQ9, and AQQ11

Code	Estimated Free Energy of Binding (Kcal/mole)	Estimated Ki value (μM)	No. of Heavy atoms	Ligand Efficiency* (Kcal/mole)
AQQ7	−7.93	1.53	30	−0.264
AQQ9	−9.89	0.056	32	−0.309
AQQ11	−6.81	10.14	34	−0.200
CJC	−9.78	0.068	30	−0.326

*Estimated Free Energy of Binding/No. of Heavy Atoms, CJC is co-crystallized ligand

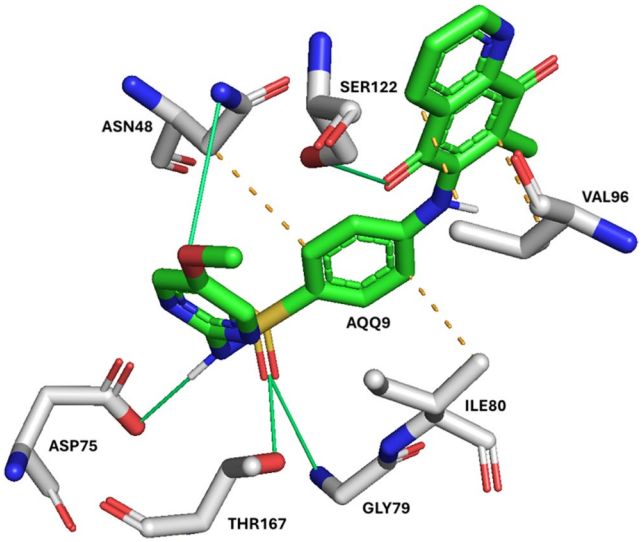


Fig. 6 Interaction of AQQ9 with DNA gyrase of *Enterococcus faecalis* (PDB:4GGL). AQQ9 colored by elements with green carbon, amino acid residues colored by element type with gray carbon, H-bonds represented as green lines and hydrophobic interactions represented as orange dashed-lines

structures for the treatment of infectious diseases is still urgently needed. In this study, a series of aminated quinolinequinones (AQQ1-13) was synthesized and evaluated for antimicrobial activity synthesized from commercially available hydroxyquinoline and aromatic amines containing sulfonamide group with heterocyclic substituent (isoxazole, thiadiazole, pyridine, pyrimidine, pyrazine, and pyridazine) or acyl group (acetyl or benzamide). The freshly designed molecules were confirmed by using spectral techniques, and their biological evaluation was first screened by determining their MIC values against gram-positive and gram-negative bacteria in addition to fungi. The biological evaluation indicated that several compounds exhibited moderate activity, particularly against gram-positive bacteria. Among them, AQQ7, AQQ9, and AQQ11 showed the higher antibacterial activity against *E. faecalis* (MIC = 78.12 μg/mL), with slightly lower MIC values than amikacin under the tested conditions, while most compounds were less active against gram-negative bacteria and fungi. Taking into account the moderate activity of the identified AQQs towards gram-positive bacteria, we were interested in two AQQ analogues (AQQ9 and AQQ11) to further investigate their antimicrobial potential, specifically focusing on their antibiofilm and time-kill kinetics. Although no significant bactericidal activity was observed, combination studies with levofloxacin demonstrated synergistic or additive effects, and enhanced antibiofilm activity was observed under combination conditions. Biofilm formation is known to contribute significantly to persistent and resistant infections. The antibiofilm activity of the compound was evaluated in *E. faecalis*, and increased inhibition was observed in combination with LVX; suggesting improved antibiofilm effects in this combination. In addition, molecular docking studies suggested that AQQ9 exhibited the best ligand efficiency and binding interactions compared to AQQ7 and AQQ11. The fact that just three compounds from the library selectively acting against *Enterococcus faecalis*, clearly ruled out activity due to "dirty" PAINS. Despite limited solubility, its stable H-bonding and hydrophobic contacts with the putative target DNA gyrase suggest that it may serve as a lead scaffold for further studies. Overall, these findings suggest that aminated quinolinequinones may serve as a basis for further investigation as antimicrobial scaffolds, particularly in combination strategies.

Experimental section

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III HD spectrometer with deuterated solvent. Chemical shifts were reported in δ values (ppm) with tetramethylsilane (TMS) as the internal reference, and J values were given in hertz (Hz). The infrared (IR) spectra were recorded on an FTIR spectrometer, using the single reflection diamond ATR module. Mass spectra were obtained on a BRUKER Microflex LT with a MALDI (Matrix Assisted Laser Desorption Ionization)-TOF technique by adding a matrix. Melting points (mp) were determined on a Büchi B-540 melting point apparatus and were uncorrected. TLC was performed on silica gel using aluminum-based DC-plates (Silica gel 60 F_{254}, Merck). Spots were visualized by irradiation with UV light (254–365 nm). Column chromatography was performed using silica gel 60 (63–200 μm particle-sized) with an appropriate solvent system as eluent.

Procedure for the synthesis of the 6,7-dichloroquinoline-5,8-quinone (QQ)

6,7-Dichloroquinoline-5,8-quinone (QQ) was prepared according to the literature from 8-hydroxyquinoline and sodium chlorate in concentrated HCl in 29% yield. Mp 219–220 ºC (Lit. 221–223 ºC) (Shaikh et al. 1986). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ (ppm): 9.40 (d, J=4.39 Hz, 1H, $CH_{aromatic}$), 8.44–8.48 (m, 1H, $CH_{aromatic}$), 7.67–7.72 (m, 1H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *CDCl*$_3$) δ (ppm): 174.6, 173.3 (>C=O), 154.4, 145.8, 143.4, 142.1, 134.6, 127.3, 127.2 ($C_{aromatic}$ and C_q). MS (+ESI) *m/z* (%): 248 (100, $[M+Na\text{-}2\ H]^+$), 250 (93, $[M+Na]^+$); Anal. Calcd. for $C_9H_3Cl_2NO_2$ (228.03).

General procedure for the synthesis of aminated quinolinequinones (AQQ1-13)

A suspension of the QQ (0.150 g, 0.658 mmol) and $CeCl_3.7H_2O$ (0.270 g, 0.734 mmol, 1.1 equiv) in ethanol was stirred at room temperature for 1 h. Then, a suspension of the corresponding amines (0.734 mmol, 1.1 equiv) in ethanol was added to that solution and refluxed for 3–6 h until consumption of the QQ. The reaction mixture was cooled to room temperature. After evaporation of the solvent, the residue was dissolved with $CHCl_3$ (50 mL), and the solution was washed sequentially with water (3 × 30 mL). The organic layer was dried over $CaCl_2$, filtered, and concentrated under reduced pressure, and the residue was purified by means of column chromatography on silica gel to give the corresponding AQQ.

N-((4-((7-chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino) phenyl)sulfonyl)acetamide (AQQ1)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfacetamide (0.103 g, 0.482 mmol) to the general procedure for the synthesis of the AQQ1, the title compound (AQQ1) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 18%. FTIR (ATR) υ (cm^{-1}): 3168, 3141 (NH), 3093, 3000 ($CH_{aromatic}$), 1715, 1677 (>C=O), 1649, 1603, 1562, 1520, 1448, 1405, 1367, 1344, 1322, 1298, 1263, 1239, 1223, 1186 (SO_2), 1158, 1091, 1006. ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ (ppm): 11.99 (br s, 1H, $NHSO_2$), 9.64 (s, 1H, NH), 9.02 (d, J=4.5 Hz, 1H, $CH_{aromatic}$), 8.41 (d, J=7.8 Hz, 1H, $CH_{aromatic}$), 7.83 (dd, J=7.9, 4.8 Hz, 1H, $CH_{aromatic}$), 7.80 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 7.26 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 1.94 (s, 3 H, CH_3). ^{13}C NMR (125 MHz, *DMSO-d*$_6$) δ (ppm): 180.15, 176.14 (>C=O), 169.18, 154.85, 147.98, 144.72, 142.66, 135.00, 133.26, 128.49, 128.38, 128.06, 122.06, 121.60 ($C_{aromatic}$ and C_q), 23.71 (CH_3). MS (MALDI TOF) *m/z*: 408 $[M+3\ H]^+$; Anal. Calcd. for $C_{17}H_{12}ClN_3O_5S$ (405.02).

N-((4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6yl)amino) phenyl)sulfonyl)benzamide (AQQ2)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfabenzamide (0.200 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ2, the title compound (AQQ2) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 92%, mp 211.4–212.8 °C. FTIR (ATR) υ (cm^{-1}): 3600, 3520 (NH), 3059 ($CH_{aromatic}$), 2948 ($CH_{aliphatic}$), 1678, 1634 (>C=O), 1601, 1584, 1575, 1518, 1498, 1456, 1438, 1407, 1348, 1328, 1296, 1259, 1223, 1193 (SO_2), 1164, 1084, 1064, 1025. ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ (ppm): 12.46 (br s, 1H, $NHSO_2$), 9.66 (s, 1H, NH), 9.01 (dd, J=4.7, 1.5 Hz, 1H, $CH_{aromatic}$), 8.41 (dd, J=7.9, 1.6 Hz, 1H, $CH_{aromatic}$), 7.92 (d, J=8.7 Hz, 2 H, $CH_{aromatic}$), 7.88 (d, J=7.5 Hz, 2 H, $CH_{aromatic}$), 7.82 (dd, J=7.8, 4.7 Hz, 1H, $CH_{aromatic}$), 7.63 (t, J=7.4 Hz, 1H, $CH_{aromatic}$), 7.50 (t, J=7.8 Hz, 2 H, $CH_{aromatic}$), 7.30 (d, J=8.7 Hz, 2 H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *DMSO-d*$_6$) δ (ppm): 180.13, 176.13 (>C=O), 165.90, 154.85, 147.96, 144.77, 142.65, 135.01, 133.71, 133.34, 132.06, 129.09, 128.86, 128.65, 128.47, 128.05, 122.09, 121.62 ($C_{aromatic}$ and C_q). MS (MALDI TOF) *m/z*: 469 $[M+2\ H]^+$; Anal. Calcd. for $C_{22}H_{14}ClN_3O_5S$ (467.03).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (AQQ3)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfamethoxazole (0.183 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ3, the title compound (AQQ3) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 68%, mp 230.8–232.3 °C. FTIR (ATR) υ (cm^{-1}): 3189 (NH), 3096, 3007 ($CH_{aromatic}$), 2915, 2863, 2815 ($CH_{aliphatic}$), 1682, 1652 (>C=O), 1621, 1605, 1561, 1522, 1475, 1409, 1374, 1346, 1299, 1266, 1217, 1160 (SO_2), 1091, 1042, 1008. ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ (ppm): 11.32 (s, 1H, $NHSO_2$), 9.60 (br s, 1H, NH), 9.01 (s, 1H, $CH_{aromatic}$), 8.40 (s, 1H, $CH_{aromatic}$), 7.82 (s, 1H, $CH_{aromatic}$), 7.75 (d, J=6.6 Hz, 2 H, $CH_{aromatic}$), 7.25 (d, J=6.5 Hz, 2 H, $CH_{aromatic}$), 6.16 (s, 1H, $CH_{aromatic}$), 2.31 (s, 3 H, CH_3). ^{13}C NMR (125 MHz, *DMSO-d*$_6$) δ (ppm): 180.11, 176.07 (>C=O), 170.73, 158.04, 154.82, 147.95, 144.36, 142.63, 134.96, 133.49, 128.43, 128.01, 127.52, 122.46, 121.29, 95.89 ($C_{aromatic}$ and C_q), 12.53 (CH_3). MS (MALDI TOF) *m/z*: 445 $[M+H]^+$; Anal. Calcd. for $C_{19}H_{13}ClN_4O_5S$ (444.03); HRMS(+ESI)

m/z calcd for $C_{19}H_{14}ClN_4O_5S$ $[M+H]^+$: 445.0373; found: 445.0356; HRMS(-ESI) m/z calcd for $C_{19}H_{12}ClN_4O_5S$ $[M-H]^+$: 443.0217; found: 443.0213.

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide (AQQ4)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfisoxazole (0.193 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ4, the title compound (AQQ4) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 50%, mp 219.8–220.2 °C. FTIR (ATR) υ (cm^{-1}): 3225 (NH), 3063, 3000 ($CH_{aromatic}$), 2926 ($CH_{aliphatic}$), 1679, 1661 (>C=O), 1584, 1575, 1560, 1518, 1464, 1396, 1339, 1300, 1261, 1221, 1189 (SO_2), 1153, 1138, 1117, 1088, 1025. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 10.92 (br s, 1H, $NHSO_2$), 9.65 (s, 1H, NH), 9.01 (s, 1H, $CH_{aromatic}$), 8.41 (s, 1H, $CH_{aromatic}$), 7.82 (s, 1H, $CH_{aromatic}$), 7.66 (s, 2 H, $CH_{aromatic}$), 7.26 (s, 2 H, $CH_{aromatic}$), 2.09 (d, J=2.5 Hz, 3 H, CH_3), 1.63 (d, J=2.5 Hz, 3 H, CH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.18, 176.10 (>C=O), 161.85, 156.01, 154.83, 147.97, 144.31, 142.65, 134.95, 134.05, 128.39, 128.00, 127.38, 122.67, 120.93, 105.67 ($C_{aromatic}$ and C_q), 10.81, 6.31 (CH_3). MS (MALDI TOF) *m/z*: 459 $[M+H]^+$; Anal. Calcd. for $C_{20}H_{15}ClN_4O_5S$ (458.05).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide (AQQ5)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfamethizole (0.196 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ5, the title compound (AQQ5) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 97%, mp>300 °C. FTIR (ATR) υ (cm^{-1}): 3328 (NH), 3078, 3007 ($CH_{aromatic}$), 2874, 2737 ($CH_{aliphatic}$), 1675, 1664 (>C=O), 1592, 1567, 1535, 1503, 1439, 1407, 1297, 1256, 1219, 1189 (SO_2), 1138, 1085, 1023. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 9.57 (s, 1H, NH), 9.00 (s, 1H, $CH_{aromatic}$), 8.40 (d, J=7.8 Hz, 1H, $CH_{aromatic}$), 7.86–7.78 (m, 1H, $CH_{aromatic}$), 7.73–7.65 (m, 2 H, $CH_{aromatic}$), 7.22 (d, J=8.5 Hz, 2 H, $CH_{aromatic}$), 2.47 (s, 3 H, CH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.18, 176.04 (>C=O), 168.16, 154.94, 147.97, 143.38, 142.72, 136.53, 134.94, 128.37, 127.95, 126.38, 122.71, 120.21, 113.01 ($C_{aromatic}$ and C_q), 16.54 (CH_3). MS (MALDI TOF) *m/z*: 463 $[M+2\,H]^+$; Anal. Calcd. for $C_{18}H_{12}ClN_5O_4S_2$ (461.00).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(pyridin-2-yl)benzenesulfonamide (AQQ6)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfapyridine (0.180 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ6, the title compound (AQQ6) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 33%, mp 250.7–252.4 °C. FTIR (ATR) υ (cm^{-1}): 3340, 3219 (NH), 3059 ($CH_{aromatic}$), 2815 ($CH_{aliphatic}$), 1677, 1660 (>C=O), 1629, 1561, 1524, 1461, 1381, 1357, 1298, 1272, 1219, 1184 (SO_2), 1167, 1139, 1086, 1003. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.62 (br s, 1H, $NHSO_2$), 9.57 (br s, 1H, NH), 9.04–8.97 (m, 1H, $CH_{aromatic}$), 8.39 (d, J=7.8 Hz, 1H, $CH_{aromatic}$), 8.02 (s, 1H, $CH_{aromatic}$), 7.86–7.75 (m, 3 H, $CH_{aromatic}$), 7.72 (t, J=7.8 Hz, 1H, $CH_{aromatic}$), 7.23–7.11 (m, 3 H, $CH_{aromatic}$), 6.88 (s, 1H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 179.94, 176.10 (>C=O), 154.81, 153.78, 147.67, 143.04, 142.90, 141.40, 135.16, 128.12, 127.23, 122.85, 119.36, 114.56, 114.36 ($C_{aromatic}$ and C_q). MS (MALDI TOF) *m/z*: 442 $[M+2\,H]^+$; Anal. Calcd. for $C_{20}H_{13}ClN_4O_4S$ (440.03); HRMS(+ESI) m/z calcd for $C_{20}H_{14}ClN_4O_4S$ $[M+H]^+$: 441.0424; found: 441.0410; HRMS(-ESI) m/z calcd for $C_{20}H_{12}ClN_4O_4S$ $[M-H]^+$: 439.0268; found: 439.0236.

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(pyrimidin-2-yl)benzenesulfonamide (AQQ7)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfadiazine (0.121 g, 0.482 mmol) to the general procedure for the synthesis of the AQQ7, the title compound (AQQ7) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 11%. FTIR (ATR) υ (cm^{-1}): 3281 (NH), 3081, 3037, 3007 ($CH_{aromatic}$), 2919, 2863, 2807, 2722 ($CH_{aliphatic}$), 1684, 1664 (>C=O), 1605, 1579, 1562, 1490, 1439, 1407, 1337, 1300, 1258, 1215, 1165 (SO_2), 1139, 1095, 1060, 1023. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.69 (s, 1H, $NHSO_2$), 9.58 (s, 1H, NH), 9.01 (d, J=4.6 Hz, 1H, $CH_{aromatic}$), 8.51 (d, J=4.8 Hz, 2 H, $CH_{aromatic}$), 8.40 (d, J=7.8 Hz, 1H, $CH_{aromatic}$), 7.89 (d, J=8.7 Hz, 2 H, $CH_{aromatic}$), 7.82 (dd, J=7.8, 4.7 Hz, 1H, $CH_{aromatic}$), 7.23 (d, J=8.7 Hz, 2 H, $CH_{aromatic}$), 7.06 (t, J=4.8 Hz, 1H, $CH_{aromatic}$). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.18, 176.10 (>C=O), 158.79, 157.42, 154.85, 148.00, 143.91, 142.73, 134.97, 130.28, 128.41, 128.36, 128.01, 122.25, 120.73, 112.58 ($C_{aromatic}$ and C_q). MS (MALDI TOF) *m/z*: 443 $[M+2\,H]^+$; Anal. Calcd. for $C_{19}H_{12}ClN_5O_4S$ (441.03).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (AQQ8)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfamethazine (0.201 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ8, the title compound (AQQ8) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 43%, mp > 200 °C. FTIR (ATR) υ (cm^{-1}): 3274 (NH), 3093, 3067 ($CH_{aromatic}$), 2919, 2789 ($CH_{aliphatic}$), 1677, 1658 (>C=O), 1588, 1556, 1485, 1431, 1374, 1354, 1343, 1317, 1269, 1187 (SO_2), 1149, 1104, 1078. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.66 (br s, 1H, $NHSO_2$), 9.58 (br s, 1H, NH), 9.01 (dd, J=4.7, 1.5 Hz, 1H, $CH_{aromatic}$), 8.39 (dd, J=7.8, 1.5 Hz, 1H, $CH_{aromatic}$), 7.90 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 7.82 (dd, J=7.8, 4.7 Hz, 1H, $CH_{aromatic}$), 7.22 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 6.77 (s, 1H, $CH_{aromatic}$), 2.26 (s, 6 H, CH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.24, 176.07 (>C=O), 156.68, 154.86, 148.02, 143.42, 142.77, 134.94, 128.77, 128.35, 127.97, 122.26, 122.11, 120.14 ($C_{aromatic}$ and C_q), 23.33 (CH_3). MS (MALDI TOF) *m/z*: 469 $[M]^+$; Anal. Calcd. for $C_{21}H_{16}ClN_5O_4S$ (469.06).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(5-methoxypyrimidin-2-yl)benzenesulfonamide (AQQ9)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfameter (0.135 g, 0.482 mmol) to the general procedure for the synthesis of the AQQ9, the title compound (AQQ9) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 7%. FTIR (ATR) υ (cm^{-1}): 3360, 3315 (NH), 2920, 2848 ($CH_{aliphatic}$), 1677, 1656 (>C=O), 1562, 1461, 1423, 1333, 1296, 1273, 1152 (SO_2), 1090, 1012. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.39 (s, 1H, $NHSO_2$), 9.57 (s, 1H, NH), 9.01 (d, J=4.7 Hz, 1H, $CH_{aromatic}$), 8.40 (d, J=7.8 Hz, 1H, $CH_{aromatic}$), 8.30 (s, 2 H, $CH_{aromatic}$), 7.86 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 7.82 (dd, J=7.8, 4.7 Hz, 1H, $CH_{aromatic}$), 7.22 (d, J=8.6 Hz, 2 H, $CH_{aromatic}$), 3.80 (s, 3 H, OCH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 184.95, 180.83 (>C=O), 159.61, 156.26, 154.86, 152.76, 149.86, 148.58, 147.50, 139.72, 139.58, 133.16, 132.94, 132.75, 127.01, 125.43 ($C_{aromatic}$ and C_q), 61.51 (OCH_3). MS (MALDI TOF) *m/z*: 473 $[M+2\ H]^+$; Anal. Calcd. for $C_{20}H_{14}ClN_5O_5S$ (471.04).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(6-methoxypyrimidin-4-yl)benzenesulfonamide (AQQ10)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfamonomethoxine (0.203 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ10, the title compound (AQQ10) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 97%, mp 250.3–252.0 °C. FTIR (ATR) υ (cm^{-1}): 3186 (NH), 3085, 3026 ($CH_{aromatic}$), 2870, 2719 ($CH_{aliphatic}$), 1679, 1632 (>C=O), 1597, 1579, 1560, 1514, 1486, 1427, 1392, 1343, 1320, 1287, 1216, 1149 (SO_2), 1132, 1087, 1027. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.78 (s, 1H, $NHSO_2$), 9.60 (s, 1H, NH), 9.01 (s, 1H, $CH_{aromatic}$), 8.42–8.39 (m, 2 H, $CH_{aromatic}$), 7.81 (s, 3 H, $CH_{aromatic}$), 7.23 (s, 2 H, $CH_{aromatic}$), 6.35 (s, 1H, $CH_{aromatic}$), 3.85 (s, 3 H, OCH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.11, 176.06 (>C=O), 170.38, 158.95, 154.82, 147.94, 144.00, 142.68, 134.95, 129.63, 128.39, 128.00, 127.67, 122.51, 120.89, 112.98, 91.35 ($C_{aromatic}$ and C_q), 54.68 (OCH_3). MS (MALDI TOF) *m/z*: 473 $[M+2\ H]^+$; Anal. Calcd. for $C_{20}H_{14}ClN_5O_5S$ (471.04).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (AQQ11)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfadimethoxine (0.150 g, 0.482 mmol) to the general procedure for the synthesis of the AQQ11, the title compound (AQQ11) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 9%. FTIR (ATR) υ (cm^{-1}): 3259 (NH), 3100 ($CH_{aromatic}$), 2952 ($CH_{aliphatic}$), 1681, 1562 (>C=O), 1509, 1477, 1444, 1409, 1383, 1357, 1320, 1296, 1263, 1205, 1146 (SO_2), 1088, 1062. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 11.49 (s, 1H, $NHSO_2$), 9.61 (s, 1H, NH), 9.01 (dd, J=4.7, 1.6 Hz, 1H, $CH_{aromatic}$), 8.40 (dd, J=7.8, 1.6 Hz, 1H, $CH_{aromatic}$), 7.86–7.80 (m, 3 H, $CH_{aromatic}$), 7.25 (d, J=8.7 Hz, 2 H, $CH_{aromatic}$), 5.96 (s, 1H, $CH_{aromatic}$), 3.81 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.17, 176.11 (>C=O), 172.15, 160.44, 154.85, 147.98, 144.26, 142.70, 134.97, 128.44, 128.01, 122.41, 121.08, 85.08 ($C_{aromatic}$ and C_q), 55.01, 54.29 (OCH_3). MS (MALDI TOF) *m/z*: 503 $[M+2\ H]^+$; Anal. Calcd. for $C_{21}H_{16}ClN_5O_6S$ (501.05).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(6-methoxypyridazin-3-yl)benzenesulfonamide (AQQ12)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfamethoxypyridazine (0.203 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ12, the title compound (AQQ12) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as an orange solid. Yield: 95%, mp 256.3–257.3 °C. FTIR (ATR) υ (cm^{-1}): 3272,

3141 (NH), 3063 ($CH_{aromatic}$), 2941 ($CH_{aliphatic}$), 1672, 1658 (>C=O), 1567, 1525, 1500, 1483, 1459, 1409, 1371, 1298, 1244, 1217, 1139 (SO_2), 1084, 1018. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 9.56 (s, 1H, NH), 9.00 (s, 1H, $CH_{aromatic}$), 8.39 (d, *J*=7.6 Hz, 1H, $CH_{aromatic}$), 7.85–7.77 (m, 1H, $CH_{aromatic}$), 7.75 (d, J=7.4 Hz, 3 H, $CH_{aromatic}$), 7.38 (br s, 1H, NH), 7.21 (d, *J*=7.8 Hz, 3 H, $CH_{aromatic}$), 3.84 (s, 3 H, OCH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.18, 176.02 (>C=O), 154.83, 147.99, 142.79, 134.94, 128.34, 127.95, 126.70, 122.75, 112.96 ($C_{aromatic}$ and C_q), 54.98 (OCH_3). MS (MALDI TOF) *m/z*: 473 $[M+2\ H]^+$; Anal. Calcd. for $C_{20}H_{14}ClN_5O_5S$ (471.04).

4-((7-Chloro-5,8-dioxo-5,8-dihydroquinolin-6-yl)amino)-N-(3-methoxypyrazin-2-yl)benzenesulfonamide (AQQ13)

By subjecting the mixture (QQ and $CeCl_3.7H_2O$) and sulfalene (0.203 g, 0.724 mmol) to the general procedure for the synthesis of the AQQ13, the title compound (AQQ13) was purified by column chromatography on silica gel using hexane/ethyl acetate (5:2, v/v) as eluent to afford the title compound as a red solid. Yield: 81%, mp 237.7–238.0 °C. FTIR (ATR) υ (cm^{-1}): 3384, 3241 (NH), 3093 ($CH_{aromatic}$), 2989, 2956 ($CH_{aliphatic}$), 1680, 1607 (>C=O), 1579, 1560, 1518, 1467, 1434, 1402, 1372, 1350, 1311, 1295, 1263, 1215, 1180 (SO_2), 1156, 1136, 1088, 1001. ^{1}H NMR (500 MHz, *DMSO-d_6*) δ (ppm): 10.81 (s, 1H, $NHSO_2$), 9.59 (s, 1H, NH), 9.01 (s, 1H, $CH_{aromatic}$), 8.39 (d, *J*=6.3 Hz, 1H, $CH_{aromatic}$), 7.89 (d, *J*=7.4 Hz, 2 H, $CH_{aromatic}$), 7.85–7.66 (m, 3 H, $CH_{aromatic}$), 7.23 (d, *J*=8.3 Hz, 2 H, $CH_{aromatic}$), 3.91 (s, 3 H, OCH_3). ^{13}C NMR (125 MHz, *DMSO-d_6*) δ (ppm): 180.14, 176.06 (>C=O), 154.83, 147.96, 143.86, 142.72, 138.48, 134.95, 134.64, 133.62, 128.38, 128.21, 127.99, 122.24, 120.69 ($C_{aromatic}$ and C_q), 54.19 (OCH_3). MS (MALDI TOF) *m/z*: 473 $[M+2\ H]^+$; Anal. Calcd. for $C_{20}H_{14}ClN_5O_5S$ (471.04).

Biological studies

Determination of biological activity

The minimum inhibitory concentrations (MICs) of the substances were assessed utilizing the broth microdilution method sanctioned by the Clinical and Laboratory Standards Institute (CLSI) (1997; 2020). Three gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 4352, and *Proteus mirabilis* ATCC 14153), four gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 29212), and three fungi (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750) were cultured according to CLSI protocols (1997; 2020). Mueller-Hinton broth for bacteria and RPMI-1640 medium for the yeast strain were used as the test media. Stock solutions of the substances under analysis were prepared in DMSO. Serial two-fold dilutions from 1250 to 0.06 µg/mL were conducted in media. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 CFU/mL for bacteria and 0.5×10^3 to 2.5×10^3 CFU/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller–Hinton broth were incubated at 35 °C for 18–20 h while the trays containing RPMI-1640 medium were incubated at 35 °C for 46–50 h.

The minimum inhibitory concentration (MIC) was established as the lowest concentration of the tested extracts that exhibited complete inhibition of visible growth. As a control, antimicrobial effects of the solvents were investigated against test microorganisms. The results were evaluated according to the values of the controls. Ceftazidime, cefuroxime, cefuroxime-Na and amikacin for bacteria; clotrimazole and amphotericin B for yeast were used as reference antimicrobials at the assays. The MIC values of the reference antimicrobials were within the accuracy range in CLSI throughout the study (1997; 2020). Experiments were conducted in triplicate.

Determination of time-kill curves

The bactericidal and synergistic activity of selected molecules, which was chosen based on its MIC value, AQQ9 and AQQ11 alone or in combination with conventional antibiotic, levofloxacin, was assessed using the time-kill curve (TKC) method at one and four times the MIC against *E. faecalis* ATCC 29212 strain. Molecule-free controls were included for the tested strain. The test tubes containing MHB with and without (growth control) molecule in a final volume of 10 mL were incubated in a 37 °C calibrated shaking water bath, and viable counts were determined at 0, 2, 4, 6, and 24 h intervals after inoculation by subculturing 0.1 mL serial dilutions onto TSA plates. All tests were performed in triplicate. The lower limit of detection for the time-kill assay was 1 $\log_{10}$ CFU/mL.

The bactericidal activity was defined as a ≥ 3 $\log_{10}$ CFU/mL reduction from the initial inoculum. The impact of combining the data was analysed in relation to its more potent component. Synergy and antagonism were described as a 2 $\log_{10}$ reduction or increase in the viable count of the combination after 24 h relative to the more active of the two drugs when used alone, respectively.

Determination of the antibiofilm activity

Biofilm attachment and inhibition of biofilm formation assays were performed as previously described with some modifications. For biofilm attachment, an overnight culture of *E. faecalis* ATCC 29212 was diluted 1/50 to obtain 1×10^6 – 1×10^7 CFU/200 mL in TSB supplemented with 1% glucose. Then the strains were added to each well of 96-well tissue culture microtiter plates with 1/10 × MIC of tested molecules or antibiotics alone or in combination. The plates were allowed to incubate for 2, 4, and 6 h at 37 °C. The positive control was the studied strain in the medium alone. After incubation, each well was washed with PBS solution three times, and OD was measured at 595 nm (Mataraci-Kara et al. 2021).

For inhibition of the biofilm formation, the tested strain was incubated in its medium and molecules at 1 × and 1/10 × in addition to 1/100 × MIC at 37 °C for 24 h in microtiter plates. Six wells were used for each molecule. The positive control was the tested strain in its medium without molecules. After incubation, each well was washed with PBS solution thrice, and OD was measured at 595 nm (Mataraci-Kara et al. 2021).

Statistical analysis

All experiments were performed in three independent assays. Two-way ANOVA Tukey's multiple comparison test was used to compare differences between control and molecules alone or combination with levofloxacin. The p-value was calculated for each graph as statistically significant.

Molecular docking and in silico ADMET studies

Experimental X-ray crystallographic structure of *Enterococcus faecalis* DNA gyrase (PDB: 4GGL) was downloaded from protein data bank (https://www.rcsb.org). The structure was prepared for molecular docking using Dock Prep utility in Chimera (Pettersen et al. 2004; Yang et al. 2012) and subsequently using MGLTools-1.5.7 (Morris et al. 2009). Structures of AQQ7, AQQ9, and AQQ11 were prepared through SwissParm server (http://swissparam.ch/) and subsequently through MGLTools-1.5.7. For docking the grid box of dimension 40 × 40 × 40 was centred around co-crystallized ligand CJC and map types were generated through autogrid4 for atom types in AQQ7, AQQ9, and AQQ11. Subsequently docking was performed with 300 GA runs with other parameters kept at default value using autodock4. Top scoring conformer from the largest cluster was then used to prepare the complexes and the interactions were studied through PLIP server (Salentin et al. 2015; Adasme et al. 2021).

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Data availability No datasets were generated or analysed during the current study.

Declarations

Clinical trial number Not applicable.

Competing interests The authors declare no competing interests.

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