Review

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Future Medicinal Chemistry

Novel sulfonamide derivatives as a tool to combat methicillin-resistant *Staphylococcus aureus*

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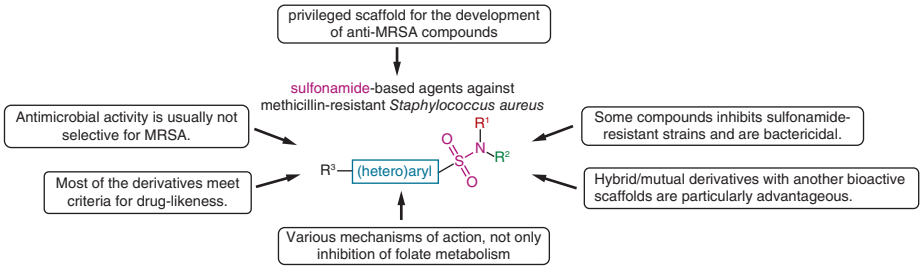
Increasing resistance in *Staphylococcus aureus* has created a critical need for new drugs, especially those effective against methicillin-resistant strains (methicillin-resistant *Staphylococcus aureus* [MRSA]). Sulfonamides are a privileged scaffold for the development of novel antistaphylococcal agents. This review covers recent advances in sulfonamides active against MRSA. Based on the substitution patterns of sulfonamide moieties, its derivatives can be tuned for desired properties and biological activity. Contrary to the traditional view, not only *N*-monosubstituted 4-aminobenzenesulfonamides are effective. Novel sulfonamides have various mechanisms of action, not only `classical´ inhibition of the folate biosynthetic pathway. Some of them can overcome resistance to classical sulfa drugs and cotrimoxazole, are bactericidal and active *in vivo*. Hybrid compounds with distinct bioactive scaffolds are particularly advantageous.

Graphical abstract:



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In 2017, the WHO published a list of priority pathogens to support the research and development of new antibiotics targeting these pathogens and to address the growing global resistance to antibiotics. The pathogens were divided into three groups based on their urgency. The second high-priority group included methicillin- and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA, respectively). MRSA is a frequent cause of hospital-acquired infections (HA-MRSAs), and it is also reported as a causative agent of community-acquired MRSA (CA-MRSA) infections, even in individuals without any risk factors. Infections caused by MRSA increase mortality, length of hospital stay and costs compared with infections caused by methicillin-susceptible strains of *Staphylococcus aureus* (MSSA). MRSA was classified as a pathogen with high mortality, transmissibility, community burden and a very high healthcare burden [1].

MRSA, first described in 1961, caused an infection that reached epidemic proportions. It is the leading cause of Gram-positive bacterial infections worldwide and its incidence is increasing. MRSA can cause diseases ranging from superficial skin and soft tissue infections, endocarditis, osteomyelitis, necrotizing pneumonia, fasciitis and myositis to central nervous system infections. Resistance to methicillin and almost all other β-lactams is caused

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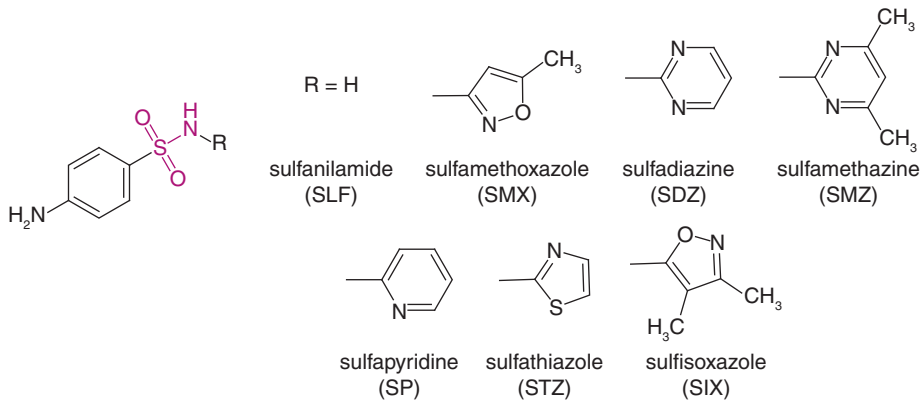

Figure 1. Examples of 'classical' sulfonamides.

by the presence of the *mecA* gene, which is translated into PBP2a, which has a low affinity for these drugs. Unfortunately, the therapeutic options for oral therapy of these strains are limited as they are often multidrug resistant. Linezolid, clindamycin, rifampicin, fluoroquinolones (FQs), fusidic acid and some tetracyclines are drugs with proven activity. In addition to the development of acquired resistance, they have many drawbacks, including toxicity. Thus, trimethoprim–sulfamethoxazole (also called cotrimoxazole) is among the drugs of choice for the oral therapy of CA-MRSA. It is inexpensive, well tolerated, bactericidal and has sufficient tissue penetration. However, clinical and experimental reports show a mixture of successes and failures with this drug, although *in vitro*, cotrimoxazole appears to have the strongest bactericidal activity against MRSA compared with linezolid, rifampicin, clindamycin, vancomycin and minocycline [2–4].

The discovery of novel antimicrobial agents, not only against MRSA, relies on several approaches. One of these is the modification of old and approved antibiotics to circumvent, among other things, the resistance mechanisms that have evolved and developed against them. This has been successful, for example, for newer tetracyclines, cephalosporines and glycopeptides [5].

Sulfonamides (Figure 1) are the oldest class of synthetic antimicrobial drugs. They have been in clinical use since the 1930s and have proven efficacy against many pathogens and clinical infections. 'Classical' sulfonamide drugs, that is, 4-aminobenzenesulfonamides with a free primary amino group and at most one substituent on the sulfonamide nitrogen, inhibit dihydropteroate synthase (DHPS), which catalyzes the condensation of *p*-aminobenzoic acid (PABA) with dihydropterin pyrophosphate to form 7,8-dihydropteroate. Sulfonamides compete with its natural substrate, PABA, thereby blocking folate biosynthesis and subsequently leading to defective thymidine biosynthesis. They also lead to the formation of pterin-sulfa dead-end metabolic products. The anionic species formed by the deprotonation of sulfonamide nitrogen is a bioactive compound occupying the same pocket in the enzyme as ionized PABA, but its limited lipid solubility prevents the crossing of biological barriers, favoring a unionized form of these drugs. Sulfonamides alone are bacteriostatic against *S. aureus*. The development of acquired resistance involves PABA overproduction, the release of thymidine, the upregulation of enzymes and most common alterations in DHPS [2,6–9]. The addition of trimethoprim, a dihydrofolate reductase inhibitor, to sulfonamide, mainly in a fixed combination, provides blockage of the second step in the folate biosynthetic pathway, the so-called sequential blockade. This orally bioavailable combintion turns antimicrobial action into bactericidal and reduces the emergence of resistance. However, resistance can develop, and variable rates of resistance have been reported worldwide [2,7–9].

In addition, other mechanisms of action (MoAs) of sulfonamide derivatives have been proposed and confirmed, here not only for classical ones. The interference of sulfonamides, including clinically used drugs, with peptidoglycan formation by inhibiting certain enzymes involved in its biosynthesis (MurB, MurD, MurE) has also been reported in various pathogens, including *S. aureus*. Peptidoglycan is an essential component of the cell wall, and its disruption leads to bacterial death [10]. Sulfonamides have been also shown to be inhibitors of serine/threonine kinase (Stk1/PknB), leading to the increased susceptibility of MRSA to sublethal concentrations of β-lactams, thereby reversing acquired resistance [11]. Mafenide, a higher homologue of classical sulfonamides with an inserted methylene linker between the benzene ring and the primary amino group, does not inhibit DHPS, but it has

been found to be an inhibitor of dihydrofolate synthase, nucleic bases and nucleotides biosynthesis [12]. It may also indirectly disrupt the transport system for folic acid [13].

Another proposed and studied MoA was the inhibition of carbonic anhydrases (CAs). These metalloenzymes are involved in pH maintenance and CO_2 and bicarbonate-dependent biosynthetic processes because CAs catalyze the interconversion between these two small molecules. Various sulfonamides have been described as their potent inhibitors in active *in vitro* as well as in whole-cell assays [14]. β-CA, originally reported to originate from *S. aureus* (SauBCA), was inhibited by various sulfa drugs in the nanomolar-to-micromolar range. The best inhibitor was H_2-antihistamine famotidine [15]. However, the presence of CAs in *S. aureus* was questioned and the enzyme was then attributed to *Mammaliicoccus* (formerly *Staphylococcus*) *sciuri* [16]. There is, however, a surprising link here – *M. sciuri* carries virulence and drug-resistant genes that can be transferred to *S. aureus*. Suppressing CAs from *M. sciuri* by sulfonamide-based inhibitors (e.g., antiglaucoma drugs including acetazolamide, diuretics and antiseizure medications, as well as antimicrobials mafenide and classical 4-aminobenzenesulfonamides) may result in halting the spread of antibiotic-resistant gene transfers [17].

Sulfonamides are also capable of inhibiting other enzymes, proteins and biomacromolecules including human ones, thus contributing to off-target side effects and interactions [18,19,20]. In addition to the traditional use of 4-aminobenzenesulfonamides as antibacterial and antiprotozoal drugs, it has been found that they can boost the activity of antifungals and even revert resistance to azoles [21].

From a medicinal chemistry perspective, sulfonamide derivatives have exhibited a range of pharmacological activities widely utilized in drug design, and the sulfonamide group is considered a versatile pharmacophore [22,23]. The synthesis and investigation of novel sulfonamides are among the most current research topics. Approved sulfa drugs have several limitations, and novel antibacterial sulfonamides should have key advantages, including the inhibition of drug-resistant pathogens (no cross-resistance), low toxicity and reduced side effects [24].

This review covers recent advances (2018–2022) in the field of sulfonamides potent against MRSA.

Sulfonamides active against methicillin-resistant *Staphylococcus aureus*

Modification of 4-aminobenzenesulfonamides: the primary amino group

Schiff bases

The preparation of Schiff bases from the primary amino group of sulfonamides represents a frequent and synthetically easy approach, supported by the well-known antimicrobial activity of such derivatives. It also allows the combination of two bioactive scaffolds [25,26].

Krátký *et al.* designed and prepared mutual compounds by combing two antimicrobial scaffolds and substituted salicylaldehydes and three classical sulfonamides (sulfamethoxazole, SMX; sulfathiazole, STZ; sulfamethazine, SMZ) to prepare imines [23] (Figure 2; **1**). The best activity was associated with 3,5-dihalogenated salicylaldehydes, favoring heavier halogens; SMZ followed by STZ was found to be the most preferred sulfonamide. They were active against Gram-positive cocci including MRSA and, importantly, also cotrimoxazole-resistant clinical isolates (*Staphylococcus epidermidis* and *Staphylococcus hominis*) at minimum inhibitory concentrations (MICs) from 3.91 μM. Thus, no cross-resistance to antibiotics was detected. Based on MIC and minimum bactericidal concentration (MBC) values, these derivatives are bactericidal, unlike the parent sulfonamides. The SMZ derivative 4-[(3,5-dichloro-2-hydroxybenzylidene)amino]-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide prevents biofilm formation in MRSA. These compounds did not cause haemolysis and some of them avoided strong cytotoxicity (HepG2) [23]. Analogous derivatives obtained from sulfadiazine (SDZ) have previously been reported (Figure 2; **1**) [27]. Here too, iodinated 3,5-dihalogenosalicylidene compounds were the most potent (MICs from 7.81 μM). In terms of selectivity, 2,5-dihydroxybenzaldehyde-based imines showed the optimal profile. These derivatives also exhibited antimycobacterial and antifungal properties.

Analogous 4-[(2-hydroxy-3,5-diiodobenzylidene)amino]-*N*-(5-methylisoxazole-3-yl)benzenesulfonamide inhibited MRSA (MIC 9.5 μg/ml; Figure 2; **1**, R^1 = 3,5-I_2) [28]. In this group of derivatives, activity is not necessarily related to the presence of halogens, as 4-[(5-*tert*-butyl)-2-hydroxybenzylidene]amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide was also active (Figure 2; **1**, derivative of SMZ, R^1 = 5-*t*Bu) [29].

An identical approach was used for the nonclassical sulfonamide drug, mafenide, which has a distinct MoA and no cross-resistance. The range of aldehydes was also expanded to include ketones, and furfural- and thiophen-2-carbaldehyde-based carbonyl compounds. These Schiff bases (Figure 2; **2** and **3**) exhibited potent broad-spectrum activity against Gram-negative and Gram-positive pathogens, including multidrug-resistant isolates (MRSA, VRSA,

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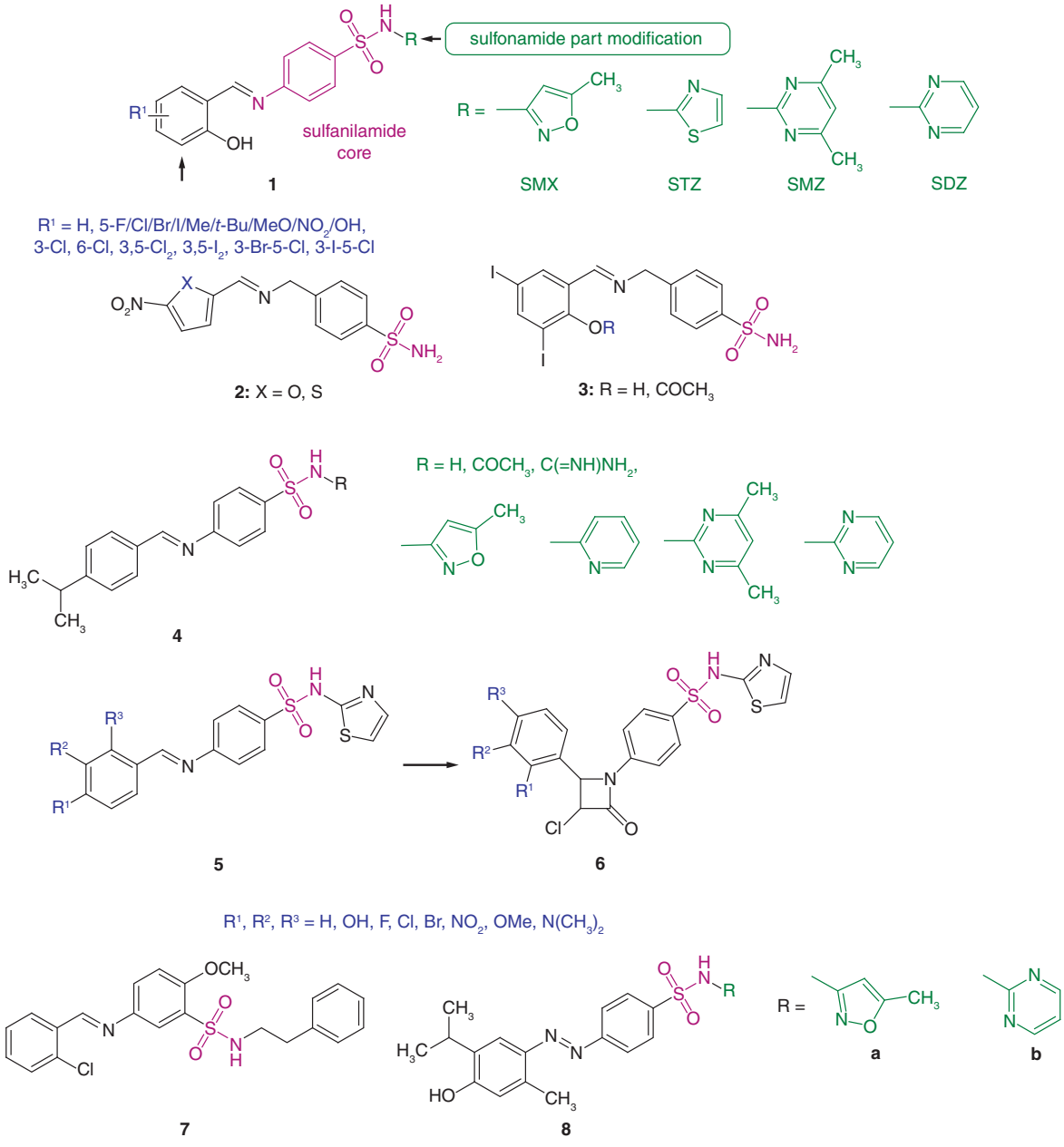

Figure 2. Schiff bases and diazo compounds derived from sulfonamides.
SMX: Sulfamethoxazole; SMZ: Sulfamethazine; SDZ: Sulfadiazine; STZ: Sulfathiazole.

vancomycin-resistant enterococci [VRE], *S. epidermidis*), mycobacteria and fungi (MIC ≥3.9 μM). The MIC for staphylococci started at 7.81 μM. Interestingly, the iodinated salicylic and thiophene derivatives were bactericidal, whereas 5-nitrofurfurylidene exhibited a rather bacteriostatic effect. Time–kill interactions with MRSA and VRSA were also studied [12].

Other successful examples of Schiff bases were bases derived from terpene *p*-cuminal and sulfanilamide (SLF), sulfapyridine (SP), SDZ, SMX, sulfacetamide and sulfaguanidine (Figure 2; **4**), and additionally with sulfanilic acid and a related drug, sulfone dapsone. Among other bacterial and fungal pathogens, all were active against MRSA with an MIC and MBC of 6.25–50 and 12.5–100 μg/ml, respectively. The most active imines were those obtained from SLF, SMX and sulfacetamide, while dapsone derivatives showed the lowest efficacy. Some compounds were equally active for MRSA and MSSA, but a few of them were more effective on MSSA (for SP 8×). Molecular docking indicated DHPS inhibition [30].

PBP2a was proposed as a target of β-lactam-STZ hybrids (Figure 2; **6**) that were prepared from imines (Figure 2; **5**). Some of the imines and lactams inhibited MSSA (MIC of 0.0625–4 μg/ml) without strong cytotoxic (IC_{50} >100 μM for HeLa) and haemolytic properties. Molecular docking indicated their ability to bind to PBP2a from MRSA. However, no results of whole-cell assays for MRSA were reported. The most active analogues were substituted with either 2-OH and/or a methoxy group [31].

Also, imines of nonclassical sulfonamides inhibited MRSA. Ten *N*-substituted 5-[(2-chlorobenzylidene)amino]-2-methoxybenzenesulfonamides were investigated against bacteria, including MSSA and MRSA. Only the *N*-phenethyl derivative (Figure 2; **7**) exhibited an MIC lower than 1000 μg/ml, but it was still relatively high (312–625 μg/ml). Its replacement by cyclohexyl, 3-pyridyl or phenyls resulted in either negligible or no activity [32].

A summary of MIC values for each group of derivatives is provided in Supplementary Table 1.

Diazo compounds

Utilizing computational predictions, SMX conjugated with thymol linked via a diazo bond (Figure 2; **8a**) was synthesized as the most prospective phytochemical–sulfonamide compound for targeting DHPS *in silico*. Its MIC against multidrug-resistant *S. aureus* clinical isolates was 20 μg/ml, while MBC exceeded 40 μg/ml, indicating a bacteriostatic action along with low *in vivo* toxicity for rats [33].

In a follow-up study [34], thymol was conjugated with other sulfa drugs (sulfacetamide, SDZ, sulfaguanidine, SMX, SLF, sulfanilic acid and SP) in an identical manner. They were evaluated against MRSA and VRE isolates with MIC and MBC values of 20–80 and 40–160 μg/ml, respectively. The highest activity was associated with SMX, SDZ and, surprisingly, sulfanilic acid. The most potent SDZ derivative (Figure 2; **8b**) was nontoxic for human umbilical cord blood lymphocytes. The computational study suggested targeting DHPS as well, but there was no experimental confirmation.

These studies as well as the aforementioned work [30] are successful examples of sulfonamide conjugates with naturally occurring phytochemicals [35].

N-mono- & N,N-disubstitution

Low anti-MRSA activity was demonstrated by STZ tethered to phthalazines (Figure 3; **9**). Among the four derivatives, the 4-phenyl analogue inhibited MRSA with an MIC and MBC of 940 and 1880 μg/ml, respectively, as well as no antibiofilm activity. Other bacterial and especially fungal pathogens were slightly more sensitive [36].

SLF-based aminophosphonates (Figure 3; **10**) were predominately active against *Escherichia coli*, but some of them inhibited MSSA and MRSA with an MIC from 16 μg/ml and low hemolytic potency [37]. In a subsequent study, sulfonamide (SLF, STZ, SMX, SDZ, SP and sulfisoxazole [SIX])-based aminophosphonates with the naturally occurring antimicrobial phenol emodin (Figure 3; **11**) were reported, thus forming mutual antibacterial agents [38]. Derivatives of SIX, SP and isopropyl esters are the most efficient molecules, with superiority of diisopropyl [(4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)({4-[*N*-(4,5-dimethylisoxazol-3-yl)sulfamoyl]phenyl}amino)methyl]phosphonate (MIC: 0.25 μg/ml for MRSA). MRSA strains were predominantly more susceptible than MSSA (eight times for the title compound) and this modification enhanced the anti-MRSA activity of the parent SIX and emodin by 32 and 64 times, respectively; however, this was not a general rule. In addition, the conjugates exhibited broad-spectrum antibacterial activity.

Wang *et al.* merged 2-aminothiazole and oxime, which are often present in β-lactams (cephalosporins, monobactams), plus various sulfonamide scaffolds (SLF, sulfacetamide, SDZ, SMZ, SMX, SIX, sulfamono/dimethoxine etc.) into a single molecular entity (Figure 3; **12**) to target especially drug-resistant Gram-negative infections [39]. In addition to acylation of the primary amine, methylene group(s) may have been inserted between this moiety and benzene ring. All compounds also showed activity against MRSA and MSSA strains (MIC of 1–64 μg/ml and 8–128 μg/ml, respectively). Optimal anti-MRSA activity was found for SMZ and SDZ derivatives, most when n = 0 and R = 4,6-dimethylpyrimidine-2-yl, followed by an *N*-allyl analogue. They also showed low hemolytic potency. The insertion of an alkylene group was detrimental, but these derivatives were still active. These compounds exhibited broad-spectrum activity and unfortunately, in terms of MRSA, an advanced investigation was performed with Gram-negative *Acinetobacter baumannii*.

Based on the molecular hybridization approach, 19 quinazolinone-benzenesulfonamide derivatives (Figure 3; **13**) were synthesized and screened against various bacteria, including MRSA and yeast. The most potent compound was *N*-(2-methyl-4-nitrophenyl)-2-[(4-oxo-3-(4-sulfamoylphenyl)-3,4-dihydroquinazolin-2-yl]thio)acetamide (Figure 3; **13**, R = 2-CH_3-4-NO_2). Its MIC was 5 μg/ml for MRSA (0.62 μg/ml for MSSA),

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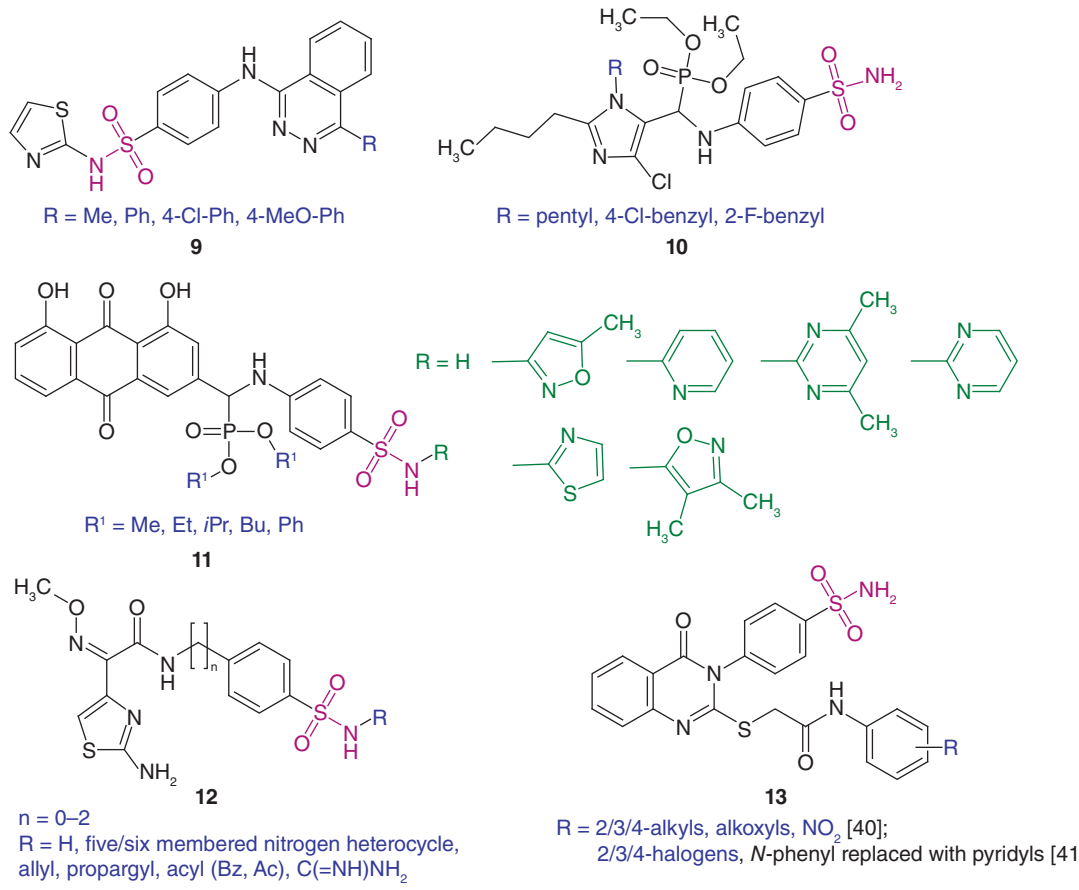

Figure 3. Novel *N*-mono- and *N,N*-disubstituted 4-aminobenzenesulfonamides.

which is identical to the 4-ethoxy analogue, followed by 4-methoxy and 3-methyl derivatives (7.81 μg/ml), and MBC of 9.01 μg/ml, thus indicating bactericidal action. However, unlike with MSSA, some compounds were inactive and had higher MIC values, suggesting partial resistance. The most active compound was conjugated with copper oxide nanoparticles (CuONPs) and incorporated into two nanoformulations; chitosan nanoparticles (CNP) and CuONPs incorporated into chitosan. Conjugates with CuONP exhibited lower anti-MRSA activity, but both chitosan nanoformulations, especially with CuONPs–CNP, were more active with MIC values of 2.5 and 1.25 μg/ml and MBC values of 4.5 and 2.25 μg/ml, respectively. Their activity towards DNA gyrase from *S. aureus* was assayed (IC_{50} 10.57–27.32 μM) and molecular docking revealed that sulfonamide binds in the same manner as that of the co-crystallized ligand ciprofloxacin (CIP). A cytotoxicity evaluation of the compound and its nanoconjugates (Vero cells) found them to be relatively safe (IC_{50} ≥543.37 μg/ml) [40].

The authors then expanded the series by adding halogens as substituents and replacing the phenyl group with isosteric pyridyls (Figure 3; **13**) [41]. Of the 14 sulfonamides, nine were active against MRSA, with MICs ranging from 10 μM in favor of the 4-Br derivative, followed by the 2-F and 5-chloro-2-pyridyl analogues (60 μM). Furthermore, they were investigated against a panel of bacteria and fungi with variable efficacy. The most active quinazolines were then conjugated to zinc oxide nanoparticles (ZnONPs). Here, the best inhibition of MRSA was found with 2-F/4-Br derivatives-ZnONPs (6 μM). Cytotoxicity was investigated in HepG2, breast cancer (MCF-7) and normal kidney epithelial (Vero) cell lines. The compounds and especially their ZnONPs were toxic to cancer cells (IC_{50} from 1.93 μM) and to Vero to a lesser extent (≥219.62 μM); nanoformulations were universally more toxic. Sulfonamides also demonstrated intracellular killing, immunomodulatory potential consisting of increased spleen and thymus weights, and activation of $CD4^+$ and $CD8^+$ T lymphocytes.

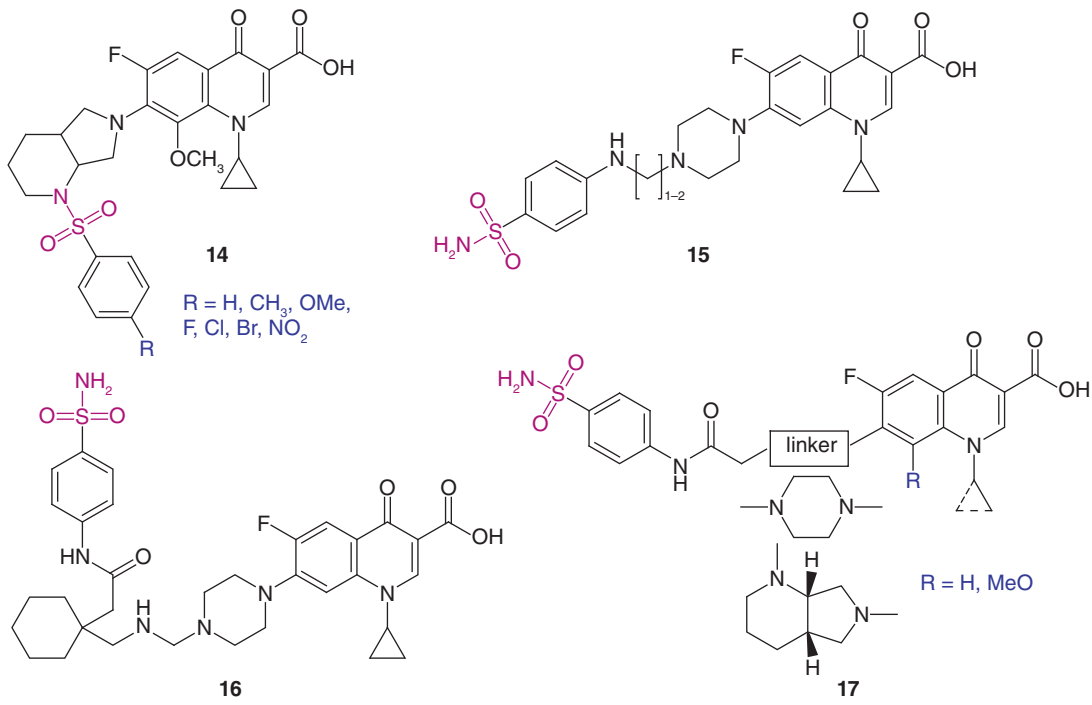

Figure 4. Fluoroquinolone–sulfonamide conjugates.

Fluoroquinolone conjugates

Highly potent fourth-generation broad-spectrum FQ moxifloxacin was converted into sulfonamides through its secondary amino group (Figure 4; **14**). Seven compounds together with their carboxamide and methyleneamine analogues were evaluated against a panel of bacteria, mycobacteria and yeasts. Of the sulfonamides, the 4-Br derivative showed the greatest activity against both MSSA and MRSA in terms of inhibition zone (26 and 22 mm, respectively), but subsequent MIC determination showed quite comparable activity for all compounds (<2.59 μg/ml) and preferential activity for Gram-positive organisms. Unfortunately, due to the concentration range used, it was not possible to distinguish particular derivatives, any activity towards MRSA and MSSA, or make a direct comparison to parent moxifloxacin, carboxamide and alkylamine isosteres. *In silico* exploration indicated the inhibition of staphylococcal DNA gyrase, as did FQ. Neither cytotoxicity, nor a DNA gyrase supercoiling assay, was performed for sulfonamides [42].

Dual CIP–SLF derivatives (Figure 4; **15**) were also identified as potent anti-MRSA hits. The presence of a quinolinone moiety was essential for high activity, as SLF conjugated with piperazine alone was ineffective. The length of the linker connecting SLF and piperazine had only a minimal effect on activity. The methylene derivative (n = 1) was more active against MSSA (0.9 vs 3.7 μM), while the ethylene linker was superior against MRSA (3.7 vs 7.7 μM). This activity was accompanied by low MIC values, and this was also true against other Gram-positive and -negative collections and clinically isolated strains. Importantly, both derivatives were better against MRSA than analogous PABA derivatives. The gabapentin–SLF–CIP triple hybrid (Figure 4; **16**) showed an MIC for MSSA and MRSA of 0.74–1.5 μM, thus being the most potent anti-MRSA compound in this series. Notably, all these Mannich bases were superior to the parent antimicrobials. Based on the relationship with FQ and docking studies with DNA gyrase from *S. aureus*, this enzyme was proposed as their target with a moxifloxacin-like binding mode [43].

Another linker, an amide bond, was used to combine three FQs (norfloxacin, CIP, moxifloxacin) with SLF as one of the investigates scaffolds. These conjugates (Figure 4; **17**) were evaluated for their antibacterial and antimycobacterial activity. Of these, the CIP-based one showed negligible toxicity in the RAW 264.7 cell line (18% at 25 μg/ml), which was even less than the parent CIP. Antimicrobial activity against various bacteria and fungi was evaluated. Antifungal activity was low, but most of the compounds showed broad-spectrum antibacterial activity. The CIP derivative was the most potent against both MSSA and MRSA with identical MIC values (<1.16 μg/ml,

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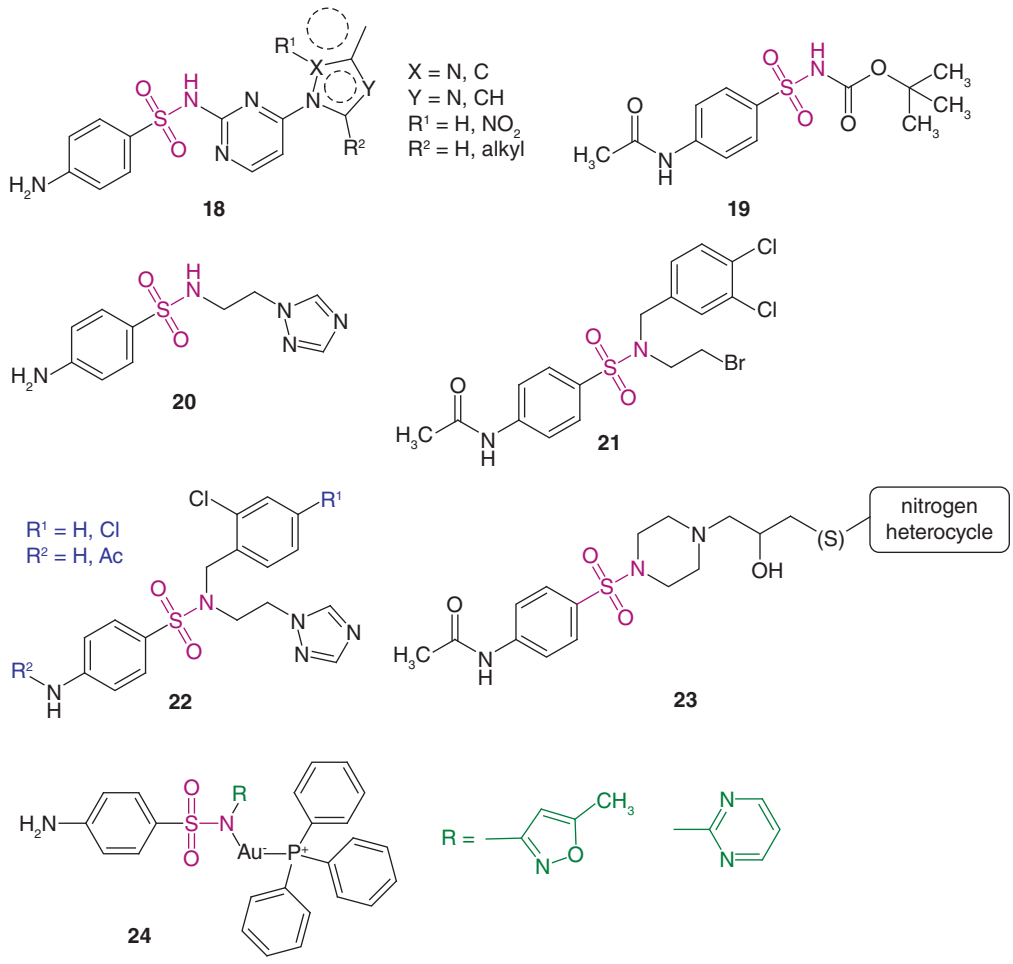

Figure 5. New 4-aminobenzenesulfonamides, compounds with the *N,N*-disubstituted sulfonamide group and sulfonamide complexes.

comparable to the parent drug), followed by the moxifloxacin and norfloxacin derivatives with a higher MIC than their FQ precursors (2.44–4.88 and 9.67–19.34 μg/ml, respectively). The CIP analogue was generally the most active within this series. The derivatives were active inhibitors in the DNA supercoiling assay (for CIP analogue IC_{50} = 7.41 μM). MoA involving binding to a DNA gyrase of a mycobacterial and staphylococcal origin were studied *in silico*. Molecular modelling revealed that the compounds adopted a binding mode similar to the parent FQ. Sulfamoyl moiety provided advantageously additional interactions with basic amino acids residues. The derivative also showed the best calculated binding energy to the enzyme of *S. aureus* origin [44].

Other modifications of 4-aminobenzenesulfonamides: new classical sulfonamides, sulfonamide *N,N*-disubstitution & others

SDZ hybrids with substituted imidazoles, 1,2,4-triazoles, indoles and benzimidazoles (Figure 5; **18**) were prepared and investigated against a panel of human pathogens with an MIC of 1–512 μg/ml. MICs against one MRSA and three *S. aureus* strains started at 2 μg/ml. Interestingly, MRSA was predominantly more susceptible than MSSA (up to 64 times). The presence of indole was crucial for the best anti-*S. aureus* action, while other heterocycles resulted in lower activity. The 2-propylimidazolyl derivative resulted in the strongest inhibition of *S. aureus* among those structures without any *ortho*-condensed substituents (MIC: 8–64 μg/ml). This activity was accompanied by the inhibition of Gram-negative species and antifungal properties at similar effective concentrations. Sulfonamides were nontoxic at 64 μg/ml for lung cancer (A549) and human lung epithelium cell (BEAS-2B) lines. The docking study suggested a MoA involving the inhibition of DNA gyrase. However, advanced microbiological assays were performed with *Enterococcus faecalis* and their extrapolation to *S. aureus* would be inappropriate [45].

Triazoles are a well-established scaffold in medicinal chemistry. Their introduction has been demonstrated to be beneficial for improving interactions with biological targets and increasing water solubility. Specifically, they are known pharmacophores for antifungal and also anti-Gram-positive activities (e.g., triazole antifungals). Accordingly, novel sulfonamides-derived 1,2,4-triazoles and their synthetic precursors were evaluated *in vitro* against a wide range of Gram-positive and negative bacteria and fungi. Focusing on MRSA, the vast majority showed negligible activity (MIC >500 μM) and five derivatives were more potent (including *N*-acetylated molecules; Figure 5; **19**–**22**), but still with only mild inhibition (**19**: MIC 180 μM; **20**: 148 μM; **21**: 270 μM; **22**: $R^1 = R^2 = H$: 160 μM; $R^1 = H$, $R^2 = Ac$: 290 μM; $R^1 = Cl$, $R^2 = Ac$: 270 μM). Interestingly, the presence of triazole is not required for efficacy against MRSA and deacetylation of the primary amino group is advantageous. A preliminary MoA suggested binding to DNA, microsomal heme and also the production of a small amount of reactive oxygen species (ROS). The molecule **22** ($R^1 = R^2 = H$) was highly cytotoxic for human breast cancer cell line MCF-7 (6.33 μg/ml) [46].

Hu *et al.* combined four scaffolds (4-aminobenzenesulfonamide, piperazine, hydroxyethyl and azoles; Figure 5; **23**) to target drug-resistant pathogens [47]. Although their focus was on *E. coli*, the piperazines showed mild activity against MRSA (≥302 μM) and MSSA, which was comparable in most cases, but for some compounds, the MSSA strains were significantly more susceptible. Synergy with norfloxacin against *S. aureus* was identified.

Metal complexes

A different tool to revert acquired resistance to sulfonamides, boost their activity against susceptible pathogens or extend the spectrum of activity is their complexation. Metal coordination can improve the biological activity of both ligands and metal ions, ideally combining two antimicrobial pharmacophores acting via a distinctive MoA. Transition metal ions, which easily change their oxidation state, can nonspecifically disrupt biomolecules by either redox processes or by generating reactive species. In fact, it could be a single-molecule combination therapy. This modification can also modulate lipophilicity and other physicochemical properties important for drug action and the permeation of biological barriers. Sulfonamides are frequently used ligands due to their structural features offering mono- or more commonly polydentate binding and several different coordination modes. Developing complexes with old drugs has been established and considered a viable approach to revisit them. On the other hand, toxicological aspects should not be ignored [6,48]. However, the vast majority of such complexes have been evaluated only against MSSA.

Mizdal *et al.* reported on five sulfonamide complexes with gold: SDZ–Au–PPh_3; SDZ–Ph_2P–Au–Au–PPh_2; SMX–Au–PPh_3; SMX–Au–Au–PPh_2; sulfamethoxazolate–Au–PPh_3 [24]. All these complexes inhibited one MRSA and five clinically isolated *S. aureus* strains with MIC values of 1–8 μg/ml, whereas SMX produced a significantly lower activity (64–512 μg/ml). In general, complexes with triphenylphosphine and only one gold(I) atom were more efficacious (Figure 5; **24**). A time–kill assay indicated bactericidal action after 6 h of treatment by three PPh_3 complexes at MIC. A checkerboard assay identified synergy with trimethoprim, CIP and rifampicin. Moreover, the compounds interfered with MRSA biofilm formation at concentrations corresponding to their MIC, even at subinhibitory concentrations. Importantly, gold complexes did not promote toxicity on *Caenorhabditis elegans*.

Sulfonamides not derived from a 4-aminobenzenesulfonamide scaffold

A total of 19 4,5-dimethoxy-*N*,*N*-dimethyl-2-[(6-substituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)methyl]benzenesulfonamides (Figure 6; **25**) inhibited MRSA strains with an MIC and MBC of 10–80 and 20–150 μg/ml, respectively, favoring derivatives with 3-methyl- or 2-chloro-4-nitrophenyl substitution and disfavoring the 3-methoxybenzylidene group. They were also active against MSSA and other Gram-positive, -negative and fungal species at similar concentrations. A cytotoxicity assessment using two cell lines (MCF7/S0.5, HK-2) indicated that the compounds were safe at antimicrobial concentrations [49].

Three sulfonamides were also included in the larger series of 2-(4-methylsulfonylphenyl)indoles (Figure 6; **26**). The fluorinated (R = F) derivative caused 96% inhibition of MRSA strain growth at a concentration of 32 μg/ml with MIC = 2 μg/ml, and it was similarly active against other bacteria. Cytotoxicity was determined against a human embryonic kidney cell line and via hemolysis. This compound was cytotoxic (IC_{50} = 2.987 μg/ml) but not hemolytic. The remaining two analogues showed only a negligible inhibition of MRSA. In addition, all were dual or selective inhibitors of COX-2 and exhibited anti-inflammatory activity [50].

Employing computational strategies, interactions of sulfonamides based on Ala–Ala and Pro–Ala dipeptides with PBP2a were studied as potential inhibitors of this protein. Based on *in silico* results, two Pro–Ala dipeptides (Figure 6; **27**) were evaluated against four clinical MRSA isolates with an experimentally confirmed presence of

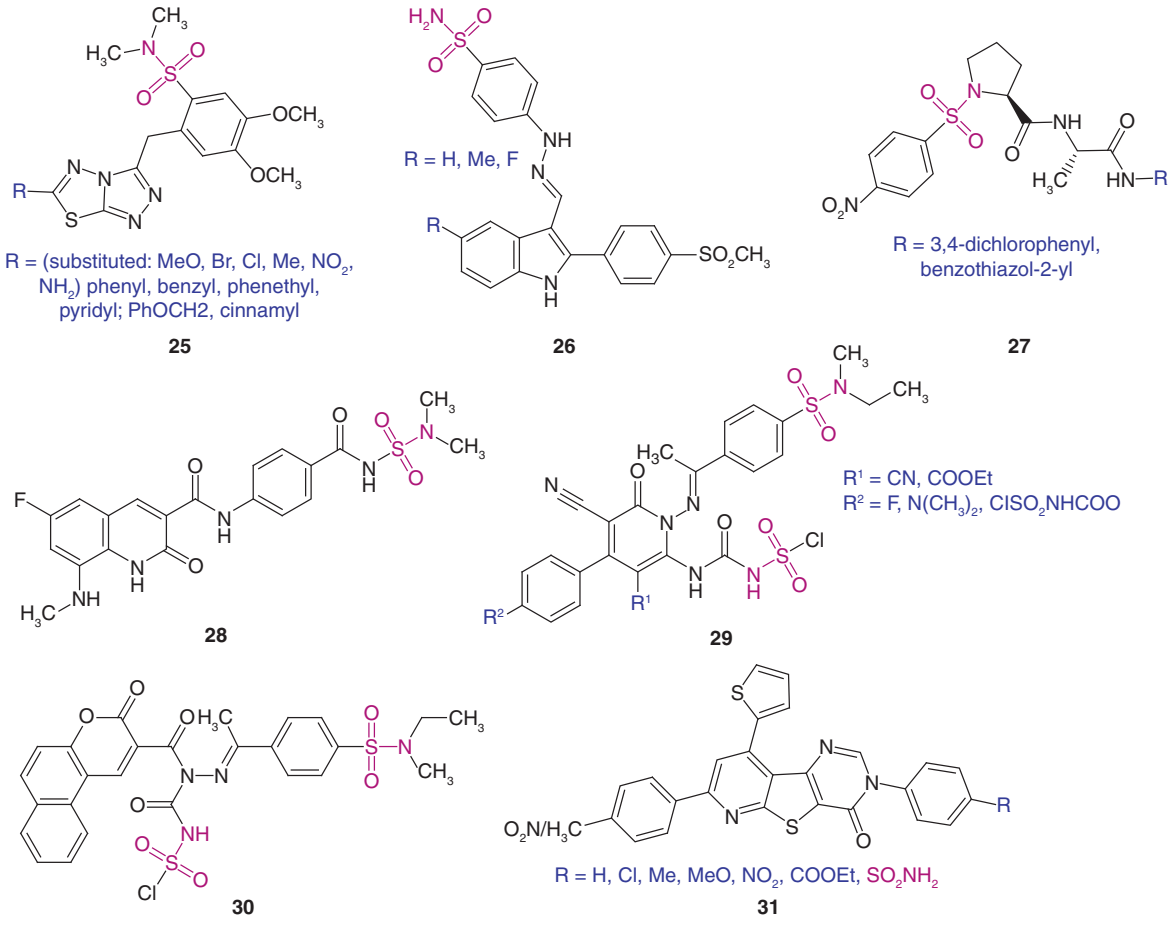

Figure 6. Sulfonamides effective against methicillin-resistant *Staphylococcus aureus* that are not 4-aminobenzenesulfonamide derivatives (part I).

mecA. The inhibitors showed moderate inhibition of two MRSA strains at 10 μg/ml in terms of inhibition zone. Slightly increased activity was observed in the presence of oxacillin, suggesting a possible synergy. This may consist of restoring susceptibility to oxacillin by interaction with PBP2a [51].

The sulfonamide–FQ hybrid (Figure 6; **28**) inhibited MRSA with an MIC of 1 μg/ml for both CA- and HA-MRSA (comparable to vancomycin), along with showing even better activity against enterococci, including VRE, other Gram-positive cocci and Gram-negative species. This compound had only a negligible effect on HepG2 cells and hERG (IC_{50} >50 and >30 μM, respectively), but showed some affinity to human topoisomerase IIα (IC_{50} = 20.9 μM). The MoA could be inhibition of the gyrase B subunit [52].

A series of unusual chlorosulfonyl derivatives (Figure 6; **29** and **30**) were investigated as potential anticancer (Caco-2, A549, HEP-2) and antimicrobial agents (Gram-positive and -negative bacteria, fungi, mycobacteria). The highest inhibition zone for both MSSA and a clinical isolate of MRSA was observed when R^1 = COOEt and/or R^2 was a chlorosulfonylamino group (Figure 6; **29**), among ureas especially benzocoumarine **30** (Figure 6). Most of the remaining compounds were active only against MSSA. Inhibition of Gram-negative and fungal species was lower. Molecular docking demonstrated that some compounds were able to inhibit dihydrofolate reductase, although the study was performed with a human enzyme [53].

The sulfonamide group was also investigated within arene-linked fused pyrimidinone-thieno[2,3-*b*]pyridine hybrids as potential antibacterials (Figure 6; **31**). Sulfonamides showed the best efficacy against MSSA with an MIC of 1.7–1.8 μM. All remaining substituents resulted in lower activity; the nitro and ethoxycarbonyl groups were better than the others. Two sulfonamides also inhibited five collection Gram-positive and negative strains and two MRSA strains with similar MIC values (1.7–3.6 μM). These compounds exhibited anti-inflammatory properties through COX-2 inhibition with a submicromolar IC_{50}. However, this activity was partially overshadowed by

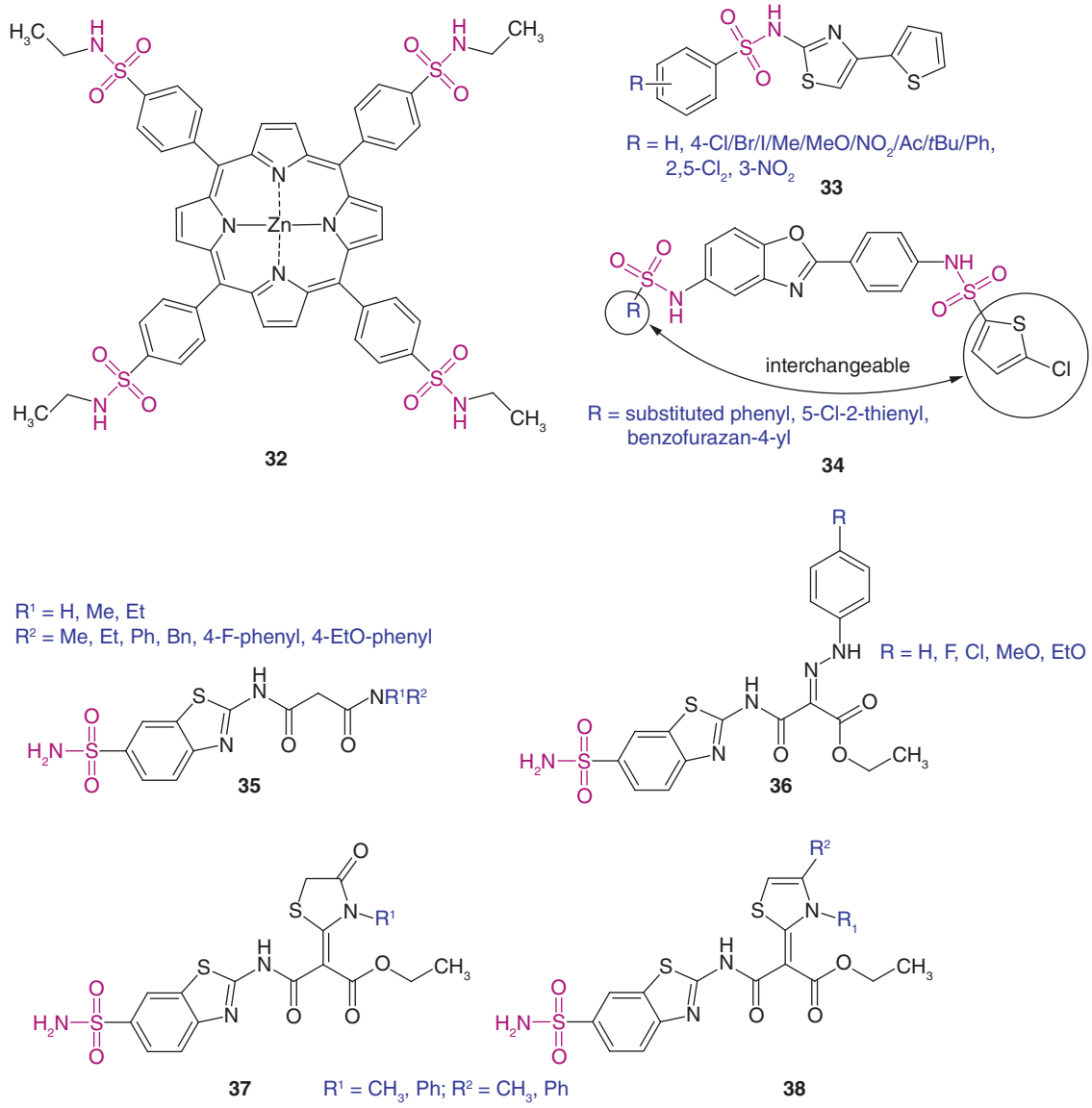

Figure 7. Sulfonamides effective against methicillin-resistant *Staphylococcus aureus* that are not 4-aminobenzenesulfonamide derivatives (part II).

their nonselective cytotoxicity evaluated using four cell lines (hepatocellular carcinoma HepG2, breast epithelial MCF-10A, breast carcinoma MCF-7 and colon cancer Caco-2 cell lines) with IC_{50} values ranging from 12.5 to 14.6 μM. Here, sulfonamides were slightly less toxic than the remaining derivatives tested. For all assays, both 4-nitrophenyl and *p*-tolyl derivatives exhibited identical activity [54].

Photodynamic therapy (PDT) represents an alternative method to antibiotic chemotherapy. It is based on the generation of ROS upon light irradiation of a photosensitizer in the presence of molecular oxygen. For targeting Gram-positive bacteria, negatively charged molecules can be effective and also selective. The presence of a sulfonamide group promoted intracellular accumulation [55]. Sulfonamides can be also utilized in the PDT of drug-resistant infections by them being introduced into photosensitizing scaffolds and acting via multiple MoA [56].

Phthalocyanines are non-naturally occurring synthetic molecules resembling natural porphyrins proposed for PDT. Phthalocyanines bearing four or eight sulfonamide units were effective in the inactivation of MSSA, but not MRSA [57]. However, based on MoA, there is no expectation of decreased activity for this resistant pathogen.

In another study, porphyrins were conjugated with sulfonamides. 5,10,15,20-Tetrakis[4-(*N*-ethylsulfamoyl)phenyl]porphyrin free base, its zinc(II) complex (Figure 7; **32**) and a hydrolysis product

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with free sulfonic groups were evaluated towards MRSA. They were efficient at reducing MRSA viability in a concentration-dependent manner and with bactericidal action. Comparing the photodynamic efficiency of each, sulfonic acid proved to be the most effective, followed by the zinc complex and then by the free sulfonamide ligand. The generation of ROS, such as $^1O_2$, was confirmed experimentally and postulated as MoA during light irradiation. Photoinactivation activity was enhanced in the presence of potassium iodide [56].

Inspired by STZ, 12 substituted *N*-[4-(thiophen-2-yl)thiazol-2-yl]benzenesulfonamides (Figure 7; **33**) were prepared. The original amino group was replaced by different groups and thiophene was introduced on the thiazole ring. These derivatives were selective against various *S. aureus* strains, including one MRSA and one clinical isolate with MIC values of 0.25–200 and 1–100 μg/ml for MSSA and MRSA, respectively. Introducing a bulky lipophilic substituent in the *para*-position of the phenyl ring (R) led to an increase in activity, specifically iodine followed by phenyl and *tert*-butyl (an MIC for MRSA of 1, 2.5 and 6.25 μg/ml, respectively). All derivatives were superior to SDZ and STZ (MIC ≥200 μg/ml). Compounds demonstrating favorable selectivity indexes, SI (determined in a human fibroblast cell line MRC-5; again, favoring the iodinated derivative), were further evaluated for MoA, but disruption of the membrane potential and DNA interactions were not responsible for the antibacterial effect. Interactions with DHPS of *S. aureus* origin were then investigated using molecular docking, molecular dynamics simulations and dynamic 3D pharmacophores. These results suggested binding them in a PABA active site of the enzyme [58].

Bis–sulfonamide–benzoxazole hybrids (Figure 7; **34**) derived from known GroEL/ES chaperonin inhibitors were identified as active compounds against resistant Gram-positive cocci, but not Gram-negative species. They inhibited the proliferation of MRSA and VRE with EC_{50} values as low as 0.53 μM along with showing moderate toxicity for human nontumor cell lines (kidney HEK 293 and THLE-3 liver cells; IC_{50} ≥11 μM). Some selectivity indexes were acceptable (i.e., above 10), favoring 3,4-dichlorophenyl derivative (SI = 46). 5-Chlorothiophen-2-yl is essential for high activity, preferentially bound to an aniline amino group; the second substituent should be phenyl substituted by halogen(s), CF_3 and/or NO_2. The investigation suggested an innovative MoA consisting of binding to unknown sites outside the ATP pocket. Attempts to generate resistant MRSA mutants resulted in a rapid development of resistance to one compound out of the two investigated; however, after subsequent culturing in the absence of the inhibitors, the bacteria regained sensitivity, suggesting a reversible nature of resistance, maybe based on efflux pumps [59].

Novel 2-aminobenzothiazole-6-sulfonamides (Figure 7; **35–38**) were designed as dual inhibitors of DNA gyrase/topoisomerase IV. They were evaluated against seven strains (Gram-positive, Gram-negative, one fungal), including MSSA and MRSA (also resistant to CIP). Focusing on MRSA, the activity was as follows for particular scaffolds: malondiamides **35** (MIC 8–>250 μg/ml, MBC 16–>250 μg/ml), hydrazineylidenes **36** (MIC 62.5–>250 μg/ml, MBC 125–>250 μg/ml), thiazolidinones **37** (MIC 4–62.5 μg/ml, MBC 8–125 μg/ml) and thiazolines **38** (MIC 31.25–250 μg/ml, MBC 62.5–>250 μg/ml). *N*-(4-fluorophenyl)amide (**35**, R^1 = H, R^2 = 4-F-phenyl) and *N*-methyl thiazolidinone (**37**, R^1 = Me) exhibited the lowest MIC and MBC values for MRSA (4–8 and 8–16 μg/ml, respectively). Generally, the halogenation of **35** and **36** as well as the methylation of thiazoline/thiazolidinones **37** and **38** improved their activity; contrarily, bulkiness and a high level of hydrophobicity were not recommended. In some cases, these sulfonamides were stronger inhibitors of MRSA than MSSA. In addition, almost all compounds exhibited broad-spectrum antimicrobial properties as well as acceptable safety profiles (cytotoxicity for lung tissue fibroblasts WI38; IC_{50} 145.5 and 120.7 μM). These two most potent compounds inhibited the proposed enzymes (but of an *E. coli* origin) at low micromolar levels. The interactions were investigated by molecular docking [60].

Sulfonamide–oxadiazole hybrids

Screening the chemical library of seven sulfonamide–1,3,4-oxadiazole hybrids revealed significant antistaphylococcal activity against MSSA and clinical MRSA isolates with an MIC of 2–32 μg/ml for five hits. The high level of activity was related to the presence of 3,5-dimethylpiperidine bound via a sulfonamide linkage. Changing this moiety (methyl(s) removal, opening the ring, replacement by morpholine or furan-2-ylmethyl) was disadvantageous. Moreover, the best molecule (Figure 8; **39**) was also active against other resistant Gram-positive pathogens, including VRSA and VRE. The action was only bacteriostatic, which was confirmed by MIC/MBC determination as well as a time–kill analysis. Activity against Gram-negative strains was negligible, probably due to being a substrate for efflux pumps. Resistance-generation experiments failed to induce rapid resistance in MRSA. This compound was

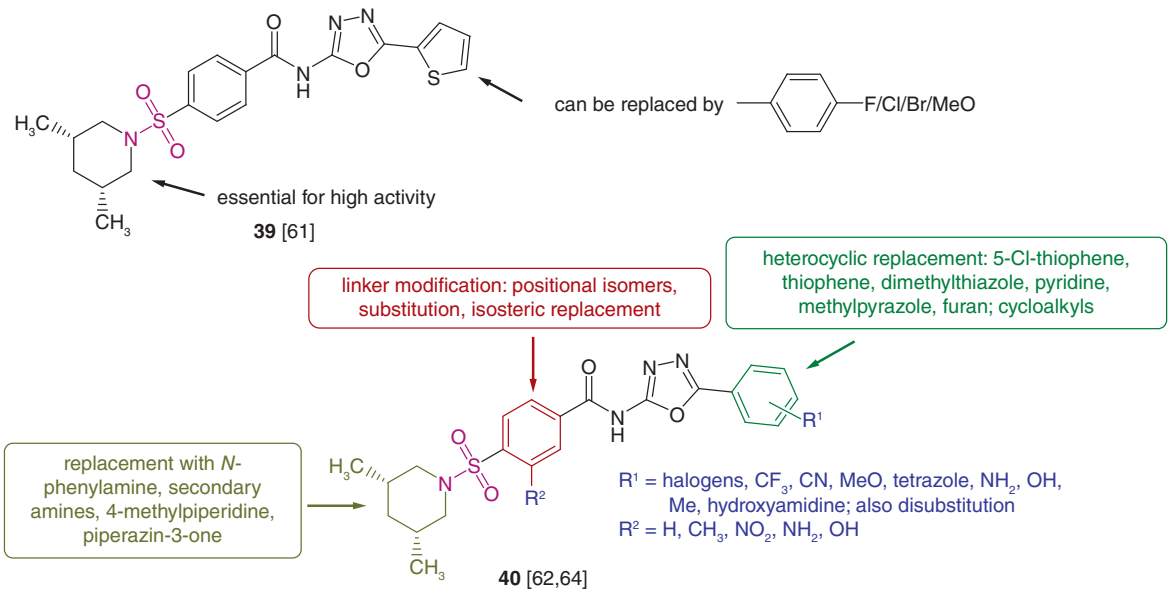

Figure 8. Sulfonamide–oxadiazole–benzamide antimicrobials.

also nontoxic to mammalian cells (macrophages, Caco-2). In a murine model of a skin wound infection, it showed an equipotency to fusidic acid in reducing the MRSA burden after 5 days of treatment [61].

Subsequently, systematic modification of this hit was initiated. Modifications covered the sulfonamide groups (various cyclic and aliphatic amines, heterocyclic amines) and position 5 of oxadiazole (heterocycles, substituted phenyls). The most efficient change was the replacement of 2-thienyl with 4-chlorophenyl (MIC: 1 μg/ml). Three other analogues had an MIC of 4 μg/ml (4-Br/F/MeO-phenyl; Figure 8; **39**), while the remaining ones were less effective. The presence of oxadiazole–carboxamide liner was also essential [61].

A follow-up study thoroughly explored the chemical space of the derivatives: 4-[(3,5-dimethylpiperidin-1-yl)sulfonyl]-*N*-(phenyl-1,3,4-oxadiazol-2-yl)benzamide scaffold (Figure 8; **40**) was modified as follows: substitution of the phenyl moiety, its replacement with heterocycles, replacement of piperidine with cyclic and acyclic amines, and modification of the *p*-phenylene linker connecting the sulfonamide and carboxamide moieties. Focusing on phenyl, for the inhibition of MSSA and MRSA, the presence (**40**, R^1) of 3-Cl/F and especially 3,5-Cl_2 and 4-CF_3 groups were superior to others (MIC: 0.5 and 0.25 μg/ml, respectively), while hydrophilic substituents led to low activity. Heterocyclic replacement identified 5-chloro-2-thienyl as the most potent (MIC: 2 μg/ml). Piperidine can be changed to *N*-methyl-*N*-phenylsulfonamide (the lowest MIC: 0.25 μg/ml), closely followed by *N*-isopropyl-*N*-phenylsulfonamide and 4-methylpiperidine (0.5 μg/ml). An aromatic linker was advantageously methylated (R^2 = Me; 0.25 μg/ml) but replacing benzene with thiophene or pyridine led only to a small decrease in activity [62].

Based on its activity profile, the MoA of 4-{[(3*S*,5*R*)-3,5-dimethylpiperidin-1-yl]sulfonyl}-3-methyl-*N*-[5-(4-(trifluoromethyl)phenyl]-1,3,4-oxadiazol-2-yl)benzamide (Figure 8; **40**, R^1 = CF_3, R^2 = Me) was investigated in depth. The generation of resistant mutants was unsuccessful; then, based on a structural analogy with the previously reported inhibitor of lipoteichoic acid (LTA) biosynthesis, this action was investigated and confirmed for *S. aureus*. The degree of LTA synthesis inhibition corresponded to the MIC values [62]. Interestingly, replacement of the sulfonamide moiety with different substituents turned the MoA into different ones [63].

Another trifluoromethyl analogue (Figure 8; **40**, R^1 = CF_3, R^2 = H) also inhibited other Gram-positive multidrug-resistant bacteria, such as VRSA, VRE and β-lactam-resistant *Streptococcus pneumoniae* at concentrations similar to those of MRSA and MSSA (0.2–2 μM). A cytotoxicity assessment (human keratinocyte cells HaCaT) showed no apparent toxicity at 64 μg/ml but were toxic at 128 μg/ml. MoA was investigated using global proteomics to identify proteins altered after sulfonamide treatment. This resulted in effects on several bacterial processes (nucleic acids synthesis, nucleotide metabolism, translation, cell wall, amino acid and carbohydrate biosynthesis, iron acquisition and diminishing virulence). Cytidine diphosphate-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase (PgsA) was identified as the most important target. PgsA is an essential protein involved in phospholipid synthe-

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sis, but the inhibitor also affects phosphoglucomutase (PgcA) synthesizing glucose-1-phosphate from glucose-6-phosphate (the first step in LTA biosynthesis) and interferes with the formation of Glc_2-diacylglycerol. Based on additional experiments, the molecule inhibits LTA biosynthesis in two distinct manners as the main MoA. First, it directly binds to PgcA, thereby inhibiting Glc_2–DAG biosynthesis. Second, it downregulates PgsA expression, causing an effect on phosphatidyl glycerol. This multiple MoA explains why attempts to generate resistant clones failed (no changes in MIC over 65 passages). This feature is positive in preventing acquired resistance. Moreover, the sulfonamide reduced the MRSA load in a skin infection in a murine model comparable to clindamycin and mupirocin and decreased levels of proinflammatory cytokines (e.g., TNF-α, IL-1β) in MRSA-infected wounds [64].

This study also covered numerous me-too derivatives involving sulfonamide substitution (primary amine, cyclic, bicyclic and acyclic secondary amines), phenyl ring replacement by heterocycles (Figure 8), substitution of this (Figure 8, R^2), a linker between benzene and oxadiazole (reverse amide, *N*-methylation), and the replacement of the 4-CF_3-phenyl moiety by cycloalkyls; in sum, 20 compounds. However, none of them showed lower MIC values than the title 4-CF_3 derivative. The greatest activity was associated with the thiophen-2,4-diyl linker, the hydroxy group (R^2 = OH) and 4-methylpiperidine (MIC 1 μg/ml, i.e., ∼2 μM) [64].

Two highly efficient compounds from this group (Figure 8; **40**, R^1 = CF_3, R^2 = H or CH_3) were subjected to an antibiofilm evaluation. They inhibited MRSA and VRE biofilms with minimum biofilm inhibitory concentrations (0.0625–0.5 μg/ml) below the compounds' MIC. Thus, the mode of biofilm inhibition was not entirely due to killing bacteria. In addition, these sulfonamides showed synergistic activity when combined with the nonsulfonamide wall teichoic acid inhibitors targocil and tunicamycin. Moreover, a strong synergy was found with the latter compound in inhibiting biofilm formation. On the other hand, the oxadiazoles did not eradicate the established biofilms (EC >256 μg/ml) [65].

Conclusion

Despite significant advances in the development of sulfonamide-derived compounds against MRSA, there are still some obstacles and gaps. First, many compounds have only been evaluated *in vitro* against MSSA without any data for MRSA and need to be re-evaluated for this purpose. Second, different authors have used various methods to determine activity (the disc diffusion method, broth methods, only antiproliferative assays), which complicates making a direct comparison of potential drugs in development. For compounds with validated anti-MRSA activity, the MoA is often unknown and therefore it is not possible ascertain that it is different from the parent 4-aminobenzenesulfonamides. In some reports, the proposed MoA is based only on *in silico* studies and has not been confirmed experimentally. Most reports lack a direct comparison of the activity of novel sulfonamide derivatives with parent or classical sulfa drugs; thus, it is not possible to conclude whether these modifications are advantageous. A direct comparison of activity against sulfonamide (cotrimoxazole)-resistant strains would also be useful for this purpose but is mostly missing. Only a few studies have reported *in vivo* results.

But still, sulfonamides are a promising and potent group with potential to combat MRSA.

Future perspective

Although the classical antimicrobial 4-aminobenzenesulfonamides have been widely regarded as old and practically abandoned drugs, there has been a recent return to them as antimicrobials. *S. aureus* including MRSA is one of the pathogens for which they are being studied and becoming more popular. This particular bioactivity will be further exploited and investigated. The spectrum of sulfonamide derivatives investigated will be broader than just the aforementioned 4-aminobenzenesulfonamide derivatives. New analogues with novel MoAs beyond folate biosynthesis will be targeted and studied. The sulfonamide group is easily synthetically available, and this scaffold can be modified with three substituents, thus providing variability in physicochemical and biological properties. In particular, hybrid/mutual derivatives with different antimicrobial scaffolds will be designed to tackle antibiotic resistance and achieve low MIC/MBC values, excellent selectivity and optimal pharmacokinetics. Novel structure–activity relationships will be described to provide more convenient sulfonamides highly effective against MRSA.

Executive summary

Introduction

- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a highly problematic pathogen causing a wide range of community and hospital-associated infections.
- Sulfonamides are a privileged scaffold for the development of compounds active against MRSA.

Sulfonamides active against methicillin-resistant *Staphylococcus aureus*: mechanisms of action & their role

- Their mechanism of action is multifaceted and contrary to the traditional view, inhibition of not only dihydropteroate synthase but also other molecular targets (DNA gyrase, lipoteichoic acid biosynthesis and so on) have been proposed and identified.
- Some compounds are also able to inhibit even sulfonamide-resistant strains and are bactericidal in contrast to the 'classical' 4-aminobenzenesulfonamides.
- Antimicrobial activity is usually not selective for methicillin-sensitive *Staphylococcus aureus* (MSSA) and MRSA, but they are often active against other Gram-positive pathogens, less frequently against Gram-negative pathogens and mycobacteria, and some of them also inhibit human pathogenic fungi. For some of the reported compounds, selectivity for prokaryotic cells (lower selectivity indexes) is a major concern.

Sulfonamides active against methicillin-resistant *Staphylococcus aureus*: structure–activity relationships

- For some compounds, the presence of a sulfonamide moiety is crucial for the mechanism of action and its modification, even disubstitution, has led to a complete abolition of activity (e.g., sulfanilamide derivatives and their complexes).
- Other compounds also require a sulfonamide group, but the possibilities of substitution are more variable and not as stringent.
- The last group consists of derivatives, where the sulfonamide moiety was only one of the possible substituents studied, for example, by the concept of (bio)isostery, and although it often improves biological properties, it can be replaced by other groups.
- The presence of any sulfonamide moiety is not explicitly translated into potent anti-MRSA activity. Some sulfonamides were also completely inactive (hence not included in this review).
- Hybrid/mutual derivatives with another bioactive/antimicrobial scaffolds (e.g., salicylic, fluoroquinolone and oxadiazole, naturally occurring terpenes) are particularly advantageous in terms of high potency.
- Most of the reported derivatives meet the criteria for drug-likeness.

Research gaps

- Many antistaphylococcal compounds have only been evaluated *in vitro* against MSSA without any data for MRSA.
- Various methods have been used to determine activity, which complicates making a direct comparison of potential drugs. Most reports lack a direct comparison of the activity with established 4-aminosulfonamides.
- For many sulfonamides, their mechanism of action is often unknown or suggested only based on *in silico* studies.
- Only a few studies have reported *in vivo* results.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.4155/fmc-2023-0116

Author contributions

M Krátký is responsible for conceptualization, investigation and writing – original draft preparation.

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Competing interests disclosure

The author has no competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

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