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Design and synthesis of benzenesulfonanilides active against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*

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Abstract—Vancomycin is mainly used as an antibacterial agent of last resort, but recently vancomycin-resistant bacterial strains have been emerging. Although new antimicrobials have been developed in order to overcome drug-resistant bacteria, many are structurally complex β-lactams or quinolones. In this study, we aimed to create new anti-drug-resistance antibacterials which can be synthesized in a few steps from inexpensive starting materials. Since sulfa drugs function as *p*-aminobenzoic acid mimics and inhibit dihydropteroate synthase (DHPS) in the folate pathway, we hypothesized that sulfa derivatives would act as folate metabolite-mimics and inhibit bacterial folate metabolism. Screening of our sulfonanilide libraries, including benzenesulfonanilide-type cyclooxygenase-1-selective inhibitors, led us to discover benzenesulfonanilides with potent anti-methicillin-resistant *Staphylococcus aureus* (MRSA)/vancomycin-resistant *Enterococcus* (VRE) activity, that is, *N*-3,5-bis(trifluoromethyl)phenyl-3,5-dichlorobenzenesulfonanilide (**16b**) [MIC = 0.5 μg/mL (MRSA), 1.0 μg/mL (VRE)], and 3,5-bis(trifluoromethyl)-*N*-(3,5-dichlorophenyl)benzenesulfonanilide (**16c**) [MIC = 0.5 μg/mL (MRSA), 1.0 μg/mL (VRE)]. These compounds are more active than vancomycin [MIC = 2.0 μg/mL (MRSA), 125 μg/mL (VRE)], but do not possess an amino group, which is essential for DHPS inhibition by sulfa drugs. These results suggested that the mechanism of antibacterial action of compounds **16b** and **16c** is different from that of sulfa drugs. We also confirmed the activity of these compounds against clinical isolates of Gram-positive bacteria.

1. Introduction

Infectious diseases are a major cause of death,[1] especially in developing countries.[2,3] These diseases are killing about 15 million people every year, which correspond to about one third of the numbers of deaths.[1] Nosocomial infectious diseases, such as methicillin-resistant *Staphylococcus aureus* (MRSA),[4,5] present a particular threat to postsurgical or elderly patients. MRSA is thought to be one of the resident microbiota, which is harmless to healthy people, but intractable in immunosuppressed patients, and has been implicated in postoperative wound infection, bone infection (osteomyelitis), infectious endocarditis (IE), and organ abscesses. In the United States, the number of MRSA-infected patients in 2005 was estimated to be 94,000, and the number of deaths was estimated to be 18,000, exceeding the number of deaths due to AIDS.[6] In England and Wales, mortality from MRSA rose from 50 in 1993 to 1600 in 2005.[7] In the 1940s, infection with *S. aureus* was cured with penicillin. Thereafter, penicillin-resistant *S. aureus* emerged and spread around the world as an increase in the use of penicillin. Methicillin (**1**) (Fig. 1) was developed in the 1960s to treat penicillin-resistant *S. aureus* and came into wide use in Europe and the United States, but resistance quickly developed.[8]

β-Lactam antibiotics, including penicillin and methicillin (**1**), act by inhibiting transpeptidases (also known as penicillin binding protein; PBP), which play a role in peptidoglycan synthesis for construction of the bacterial cell-wall. MRSA is characterized by production of PBP2′, an enzyme to which β-lactams do not bind.[9] Vancomycin (**2**, VCM) (Fig. 1), a glycopeptide-type antibiotic that is effective in the treatment of Gram-positive

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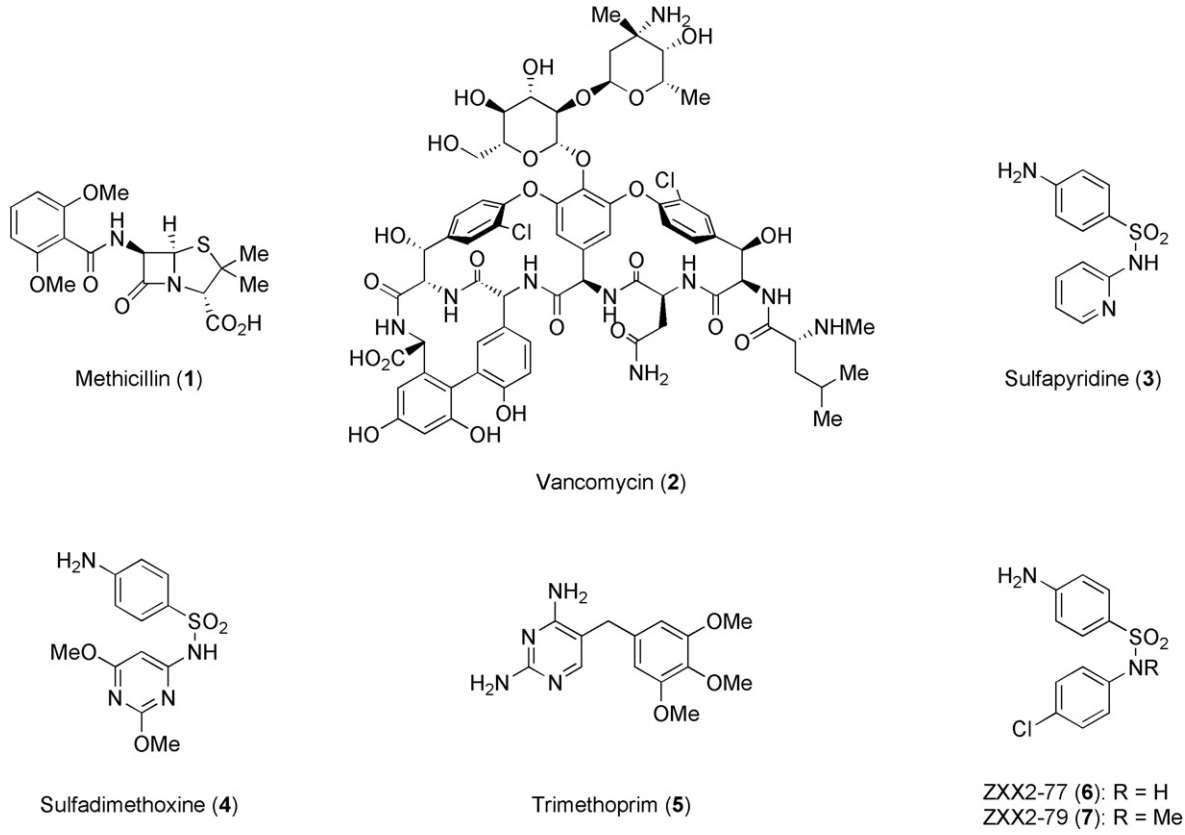


Figure 1. Chemical structures of antibacterial agents **1**–**5**, and our COX-1 selective inhibitors **6** and **7**.

bacterial infections, was developed for the treatment of MRSA[10–12]; it is also useful in patients who are allergic to β-lactam antibiotics.[12] However, vancomycin-resistant *Enterococcus* (VRE) has emerged.[4,13] Consequently, many new antibacterials have been developed to treat drug-resistant bacteria. However, most are structurally complex β-lactam or quinolone antimicrobials which are not easy or cheap to synthesize.[14] Thus, we aimed to create new agents with simple structures that would be easily synthesizable from inexpensive starting materials, and that would exhibit antimicrobial activity through a different mechanism from that of currently used drugs.

For this purpose, we focused on sulfonamides, often called simply sulfa drugs [e.g., sulfapyridine (**3**), sulfadimethoxine (**4**) (Fig. 1)], which possess a simple chemical structure with a 4-aminobenzenesulfonamide skeleton. They have been developed early by G. Domagk, but have been hardly used owing to the emergence of sulfa-resistant strains.[15] It is well known that sulfa drugs act as dihydropteroate synthase (DHPS) inhibitiors in the synthetic pathway of folate, thereby causing DNA and RNA synthesis inhibition,[16,17] because they act as analogues of *p*-aminobenzoic acid (PABA), a substrate of DHPS (Fig. 2). Mammals, including humans, cannot biosynthesize folate, which is an essential nutrient, to lack an enzyme necessary for folate biosynthesis. They take in folate as vitamin from eatables and drinkables. The difference in folate requirement between bacteria and mammals results in selective toxicity. In addition, trimethoprim (**5**), which is effective against sulfa-resistant strains, inhibits dihydrofolate reductase (DHFR) (Fig. 2),[18] so that DHFR inhibitors are also of interest as candidate antimicrobials.

We hypothesized that sulfa derivatives would act as folate metabolite-mimics and inhibit folate metabolism. Previously, we developed benzenesulfonanilide-type cyclooxygenase-1 (COX-1)-selective inhibitors **6** and **7** (Fig. 1), which resemble sulfa derivatives.[19] Thus, we examined the antibacterial activities of these compounds and their derivatives against MRSA and vancomycin-resistant *Enterococcus* (VRE), and found that benzenesulfonanilides **16b** and **16c** have potent anti-MRSA/VRE activity. These compounds can be synthesized in a single step from commercially available reagents. Here we describe the design and synthesis of these compounds, and the results of a structure–activity relationship study. We also confirmed the activity of these compounds against clinical isolates of Gram-positive bacteria.

2. Results and discussion

Compounds **8**–**11** were obtained previously during our search for COX-1-selective inhibitors (Table 1).[19] Derivatives of them were prepared according to Schemes 1–3, mostly in a single step. N-alkylation of sulfonamide was carried out by reaction of compounds **16b** and **16c** with

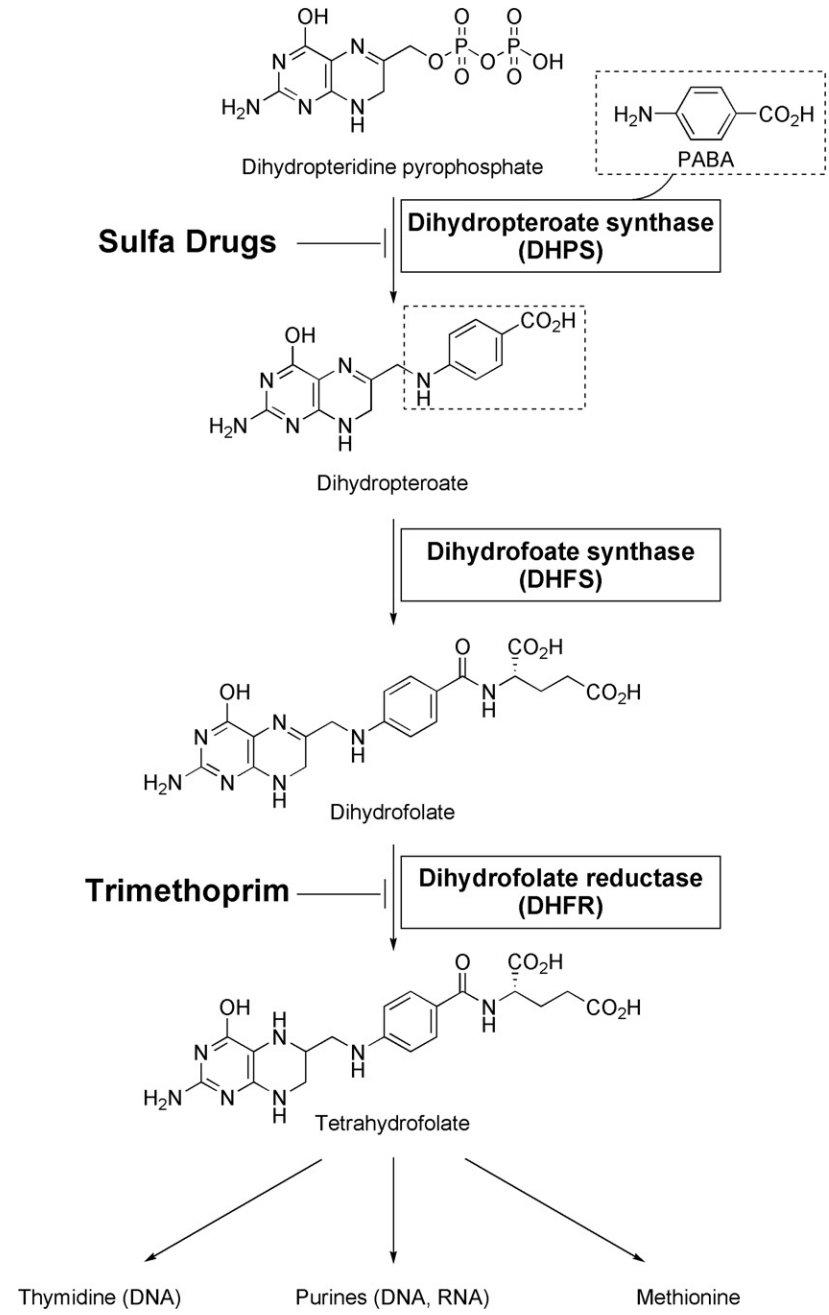


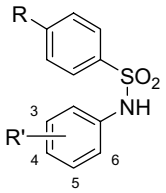
Figure 2. Schematic illustration of relationships between the folate biosynthetic pathway and antimicrobial agents.

methyl iodide after treatment with NaH in anhydrous DMF. As shown in Scheme 2, compound **21a**, in which the anilino moiety is replaced with a pyridine ring, was prepared by coupling reaction with 2-chloro-5-(trifluoromethyl)pyridine, 3,5-dichlorobenzenesulfonamide (**20**), and potassium carbonate using copper as a catalyst in anhydrous DMF.

At the first screening, anti-MRSA activities of compounds **8**–**11** were assessed by the disc diffusion method. Briefly, to a paper disc on agar medium containing bacterial test strain was added 10 μL of a solution of test compound in DMSO at 20 mM, and the plate was incubated at 37 °C for 20 h. The size of the inhibitory zone, within which the test strain did not grow, was measured and categorized as follows: minus (no inhibitory), plus/minus (0–1 mm), plus 1 (1–15 mm), plus 2 (15–20 mm), and plus 3 (>20 mm).

Compound **9h**, whose anilino ring possesses bis-trifluoromethyl groups at meta positions, showed moderate anti-MRSA activity, but most compounds were inactive (Table 1). Further structure development of compound **9h** led to the series **12**, within which most compounds showed anti-MRSA activity (Table 2). Interestingly, it was found that the anti-MRSA activity tended to increase with increasing electron-withdrawing character (Table 2).

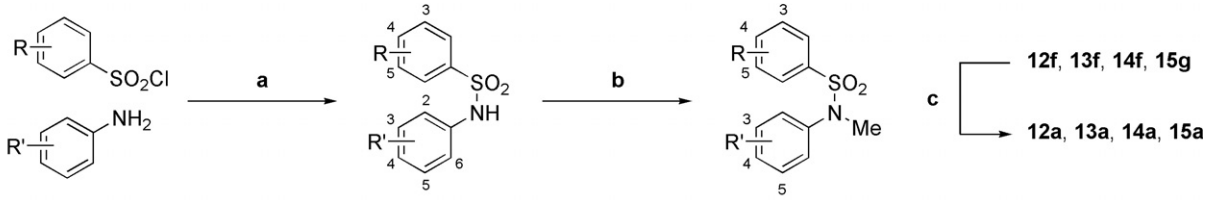
Table 1. Anti-MRSA activities of compounds **8**–**11** which were prepared during our search for cyclooxygenase-1 inhibitors[a]



Compound	R′	Inhibitory zone at 200 μmol			
		8 (R = CO_2H)	**9** (R = NO_2)	**10** (R = NH_2)	**11** (R = NHMs)
a	H	n.t.[b]	—	—	—
b	2-Me	—	n.t.	—	—
c	3-Me	n.t.	+1	—	—
d	4-Me	—	—	—	—
e	3,5-diMe	—	—	—	—
f	4-OMe	—	—	—	—
g	4-CF_3	n.t.	—	—	—
h	3,5-bisCF_3	n.t.	+3	+1	±
i	4-Cl	n.t.	n.t.	—	±
j	4-I	—	n.t.	—	n.t.

[a] Anti-MRSA activities were determined by the disc diffusion method as described in Section 4.
[b] n.t.: not tested.

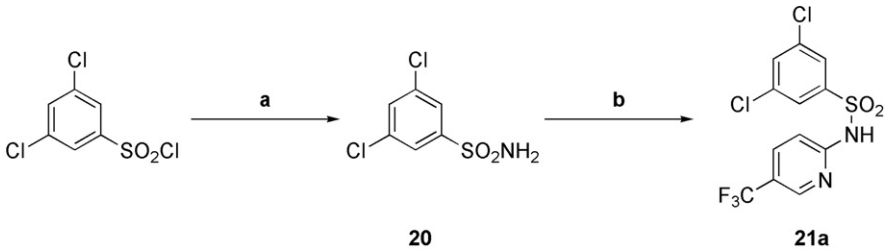
Next, the minimum inhibitory concentration (MIC) of each compound bearing a 3,5-bis-trifluoromethylphenyl group against MRSA was determined by means of the microdilution susceptibility test. Again, anti-MRSA



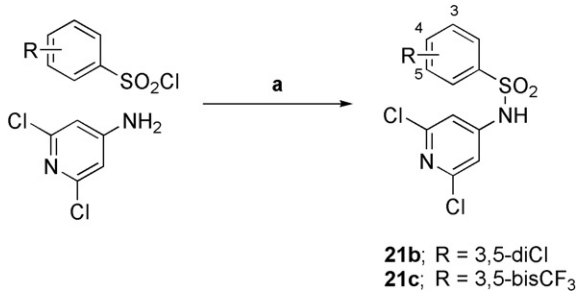
Compound	R	R'
12a	4-NH_2	3,5-bisCF_3
12b	4-OMe	3,5-bisCF_3
12c	4-Me	3,5-bisCF_3
12d	4-Cl	3,5-bisCF_3
12e	4-CF_3	3,5-bisCF_3
12f	4-NO_2	3,5-bisCF_3
13a	3,5-bisCF_3	4-NH_2
13b	3,5-bisCF_3	4-OMe
13c	3,5-bisCF_3	4-Me
13d	3,5-bisCF_3	4-Cl
13e	3,5-bisCF_3	4-CF_3
13f	3,5-bisCF_3	4-NO_2
14a	4-NH_2	3,5-diCl
14b	4-OMe	3,5-diCl
14c	4-Me	3,5-diCl
14d	4-Cl	3,5-diCl
14e	4-CF_3	3,5-diCl
14f	4-NO_2	3,5-diCl
15a	3,5-diCl	4-NH_2
15b	3,5-diCl	4-OMe
15c	3,5-diCl	4-Me
15d	3,5-diCl	4-Cl
15e	3,5-diCl	3-CF_3
15f	3,5-diCl	4-CF_3
15g	3,5-diCl	4-NO_2

Compound	R	R'
16a	3-Cl	3,5-bisCF_3
16b	3,5-diCl	3,5-bisCF_3
16c	3,5-bisCF_3	3,5-diCl
16d	3,5-bisCF_3	2,4-diCl
16e	3,5-bisCF_3	2,6-diCl
17a	4-F	3,5-bisCF_3
17b	3,5-bisCF_3	2,3-diF
17c	3,5-bisCF_3	2,4-diF
17d	3,5-bisCF_3	2,5-diF
17e	3,5-bisCF_3	2,6-diF
17f	3,5-bisCF_3	3,4-diF
17g	3,5-bisCF_3	3,5-diF
17h	3,5-diCl	3,5-diF
19a	3,5-diCl	3,5-diCl, 4-OH
19b	3,5-bisCF_3	3,5-diCl, 4-OH

Scheme 1. Reagents and yields: (a) pyridine, 11–99%; (b) H_2, Pd/C, EtOH or Sn, HCl, THF, 39–99%; (c) MeI, NaH, DMF, 33–58%.



Scheme 2. Reagents and yields: (a) NH_3 aq, 88%; (b) 2-chloro-5-(trifluoromethyl)pyridine, K_2CO_3, Cu, DMF, 32%.



Scheme 3. Reagent and yield: (a) pyridine, 22–65%.

activity tended to increase with increasing electron-withdrawing character. Compounds **13** with the sulfonamide group reversed in the structure also showed a similar tendency. Among these compounds, **12d** possessing a chloro substituent at the para position of the benzenesulfonyl group exhibited potent anti-MRSA activity (Table 3). We then examined compounds **14** and **15**, which possess chlorine substituents in place of the bis-trifluoromethyl groups of **12** and **13** (Table 3). Their anti-MRSA activities were similar to those of **12** and **13**, and also tended to increase with increasing electron-withdrawing character.

The anti-MRSA activities of **16a**, a regioisomer of **12d**, and **16b**, a 3,5-dichlorobenzenesulfonanilide derivative of **12d**, were evaluated (Table 3). Surprisingly, **16b** exhibited more potent anti-MRSA activity (MIC = 0.5 μg/mL) than vancomycin (**2**) (MIC = 2.0 μg/mL). Then, the anti-MRSA activity of **16c**, in which the the sulfonamide group is reversed compared with the structure of **16b**, as well as **16d** and **16e**, which are regioisomers of **16c**, was evaluated (Table 3). Although compounds **16d** and **16e**, which possess chloro substituents at ortho and para, or bis-ortho positions on anilino moieties, showed poor anti-MRSA activity, **16c** showed potent activity (MIC = 0.5 μg/mL).

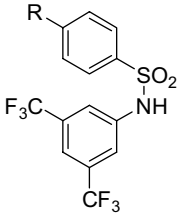
Since compounds **16b** and **16c** showed more potent anti-MRSA activity than vancomycin (**2**), their anti-vancomycin-resistant *Enterococcus* (anti-VRE) activity was evaluated. Compounds **16b** and **16c** both showed potent anti-VRE activity (MIC = 1.0 μg/mL). Next, compound **17** bearing di-fluorine substituents were evaluated (Table 3). As in the cases of **16d** and **16e**, di-fluoro substitution at ortho and para, or bis-ortho positions on the anilino moiety negated the anti-MRSA and anti-VRE activities. Considering that the most potent compound in Table 3 is **17g**, and that **16b** and **16c** show potent anti-MRSA/VRE activities, introduction of either halogens or trifluoromethyl groups at the meta positions seems optimal. Compounds **18a** and **18b**, *N*-methylated derivatives of **16b** and **16c**, showed no activity (Table 3), indicating that these compounds require NH-sulfonamide structure for anti-MRSA/VRE activity. Further structural development by the introduction of a hydroxyl group at the para position of the anilino moiety of **19b** resulted in less potent anti-MRSA/VRE activity than that of **16c** (Table 3). Replacement of phenyl rings with nitrogenous heterocycles was unfavorable (Table 4). The most potent compounds in this study were **16b** and **16c**. In summary, as shown in Figure 3, benzenesulfonanilide-type anti-MRSA/VRE compounds require an NH-sulfonamide moiety with halogen or trifluoromethyl substituents at the meta positions of both phenyl rings.

We next evaluated the antibacterial activities of compounds **16b** and **16c** against strains isolated from patients with urinary tract infection at Okayama University hospital. These compounds were effective against staphylococci and enterococci, while sulfa drugs were ineffective (Table 5). These compounds do not possess an amino group on the benzenesulfonyl moiety, though an amino group is essential for DHPS inhibition by sulfa drugs (Fig. 2). These results suggested that the mechanism of the antibacterial action of **16b** and **16c** is different from that of sulfa drugs. In an acute toxicity study, compounds **16c** and **17g** were administered at the dose of 100 mg kg^{-1} ip to mice, and no toxic symptoms were observed up to 14 days. Although these compounds were developed on the basis of our hypothesis that they would be folate metabolism inhibitors, further studies will be needed to confirm this and to establish the action mechanism in detail.

3. Conclusion

We discovered the potent benzenesulfonanilide-type anti-MRSA/VRE compounds **16b** and **16c**, which are available by one-step synthesis from commercially available reagents, based on our hypothesis that they would be folate metabolism inhibitors. Structure–activity relationship study revealed that a NH-sulfonamide moiety with halogen atoms or trifluoromethyl groups at the meta positions of both phenyl rings is required for potent anti-MRSA/VRE activity. These compounds do not possess an amino group on the benzenesulfonyl moiety, indicating that their mechanism of antibacterial action is different from that of sulfa drugs.

Table 2. Anti-MRSA activity of compounds **12** bearing trifluoromethyl groups[a]



Compound	R	Inhibitory zone at 200 μmol
a	NH_2	+1
b	OMe	+1
c	Me	—
d	Cl	+2
e	CF_3	+3
f	NO_2	+3

[a] Anti-MRSA activity was determined by the disc diffusion method as described in Section 4.

4. Experimental

4.1. General methods

Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on JASCO FT/IR350 (KBr). ^{1}H NMR spectra were recorded on a VarianVXR-300 (300 MHz) or VarianVXR-500 (500 MHz) spectrometer. Proton chemical shifts were referenced to the TMS internal standard. Elemental analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer and results were within ±0.4% of the theoretical. FAB-MS was carried out with a VG70-SE. TLC analysis was carried out on Merck Kieselgel 60F_{254}.

4.2. General procedure for synthesis of substituted benzenesulfonanilides from anilines (GP-A)

To a solution of aniline (0.5 mmol) in pyridine (1 mL) was added benzenesulfonyl chloride (0.5 mmol) at 0 °C. The mixture was stirred at room temperature overnight, then acidified with 2 N HCl solution, and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography or recrystallized using an appropriate solvent to give a substituted benzenesulfonanilide.

4.3. General procedure for reduction of nitro-substituted benzenesulfonanilides

4.3.1. GP-B-1. Nitro-substituted benzenesulfonanilide (2.5 mmol) was dissolved in EtOH (10 mL) and hydrogenated (1 bar H_2) over 10% palladium on charcoal. The mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography or recrystallized from an appropriate solvent to give an amino-substituted benzenesulfonanilide.

4.3.2. GP-B-2. To a solution of substituted nitrobenzenesulfonanilide (1.0 mmol) in THF (4 mL) were added tin powder (3.5 mmol) and concd HCl, then the mixture was refluxed for 30 min. After the reaction was finished, the mixture was basified with 2 N NaOH solution. The mixture was filtered through Celite and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography or recrystallized using an appropriate solvent to give corresponding substituted aminobenzenesulfonanilide.

4.4. General procedure for synthesis of *N*-methylated benzenesulfonanilides (GP-C)

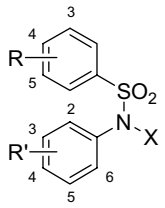
To a solution of sodium hydride (2.0 mmol) (60% in mineral oil, washed with *n*-hexane) in DMF (3 mL) was added a solution of substituted benzenesulfonanilide (1.0 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 15 min, then methyl iodide (1.2 mmol) was added, and stirring was continued at room temperature overnight. To the reaction mixture was added water, and the organics were extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography or recrystallized using an appropriate solvent to give the corresponding *N*-methylated benzenesulfonanilide.

4.4.1. 4-Amino-*N*-3,5-bis(trifluoromethyl)phenylbenzenesulfonanilide (12a). According to the general procedure (GP-B-1), **12a** was obtained in 84% yield as colorless solid after column chromatography (eluent: MeOH/$CHCl_3$ = 1:100). Mp 171.5–172.5 °C; IR (KBr) cm^{-1}: 3508, 3410 (NH_2), 3245 (NH), 1376, 1189 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.80 (s, 1H), 7.66 (s, 1H), 7.60 (s, 2H), 7.43 (d, 2H, J = 8.7 Hz), 6.55 (d, 2H, J = 8.7 Hz), 6.12 (s, 2H); FAB-MS *m*/*z*: 384 $[M]^+$, 385 $[M+H]^+$. Anal. Calcd for $C_{14}H_{11}F_6N_2O_2S$: C, 43.76; H, 2.62; N, 7.29. Found: C, 43.83; H, 2.83; N, 7.22.

4.4.2. *N*-3,5-Bis(trifluoromethyl)phenyl-4-methoxybenzenesulfonanilide (12b). According to the general procedure (GP-A), **12b** was obtained in 75% yield as yellow needles after recrystallization from CH_2Cl_2/*c*-hexane. Mp 100.5–101.5 °C; IR (KBr) cm^{-1}: 3223 (NH), 1378, 1129 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.09 (s, 1H), 7.75 (d, 2H, J = 8.2 Hz), 7.74 (s, 1H), 7.64 (s, 2H), 7.11 (d, 2H, J = 8.2 Hz), 3.80 (s, 3H); FAB-MS *m*/*z*: 399 $[M]^+$, 400 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}F_6NO_3S$: C, 45.12; H, 2.78; N, 3.51. Found: C, 45.05; H, 2.90; N, 3.65.

4.4.3. *N*-3,5-Bis(trifluoromethyl)phenyl-4-methylbenzenesulfonanilide (12c). According to the general procedure (GP-A), **12c** was obtained in 99% yield as white powder after recrystallization from EtOAc/*n*-hexane. Mp 148.0–150.0 °C; IR (KBr) cm^{-1}: 3249 (NH), 1379, 1136 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.72 (d, 2H, J = 8.2 Hz),

Table 3. Anti-MRSA/VRE activities of compounds **12**–**19** evaluated with the microdilution susceptibility test[a]



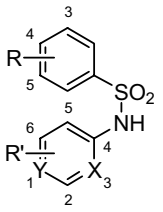
Compound	R	R′	X	MIC (μg/mL)	
				MRSA[b]	VRE[c]
Vancomycin (**2**)	—	—	—	2.0	125
12a	4-NH_2	3,5-bisCF_3	H	125	62.5
12b	4-OMe	3,5-bisCF_3	H	62.5	62.5
12c	4-Me	3,5-bisCF_3	H	62.5	62.5
12d	4-Cl	3,5-bisCF_3	H	7.8	7.8
12e	4-CF_3	3,5-bisCF_3	H	15.6	7.8
12f	4-NO_2	3,5-bisCF_3	H	31.3	3.9
13a	3,5-bisCF_3	4-NH_2	H	>250	>250
13b	3,5-bisCF_3	4-OMe	H	>250	125
13c	3,5-bisCF_3	4-Me	H	>250	>250
13d	3,5-bisCF_3	4-Cl	H	31.3	62.5
13e	3,5-bisCF_3	4-CF_3	H	7.8	3.9
13f	3,5-bisCF_3	4-NO_2	H	7.8	7.8
14a	4-NH_2	3,5-diCl	H	250	31.3
14b	4-OMe	3,5-diCl	H	250	125
14c	4-Me	3,5-diCl	H	125	62.5
14d	4-Cl	3,5-diCl	H	31.3	62.5
14e	4-CF_3	3,5-diCl	H	15.6	7.8
14f	4-NO_2	3,5-diCl	H	>250	62.5
15a	3,5-diCl	4-NH_2	H	>250	>250
15b	3,5-diCl	4-OMe	H	125	125
15c	3,5-diCl	4-Me	H	>250	62.5
15d	3,5-diCl	4-Cl	H	>250	125
15e	3,5-diCl	3-CF_3	H	125	62.5
15f	3,5-diCl	4-CF_3	H	31.3	31.3
15g	3,5-diCl	4-NO_2	H	7.8	15.6
16a	3-Cl	3,5-bisCF_3	H	31.3	15.6
16b	3,5-diCl	3,5-bisCF_3	H	0.5	1.0
16c	3,5-bisCF_3	3,5-diCl	H	0.5	1.0
16d	3,5-bisCF_3	2,4-diCl	H	>250	>250
16e	3,5-bisCF_3	2,6-diCl	H	>250	>250
17a	4-F	3,5-bisCF_3	H	15.6	62.5
17b	3,5-bisCF_3	2,3-diF	H	15.6	62.5
17c	3,5-bisCF_3	2,4-diF	H	>250	>250
17d	3,5-bisCF_3	2,5-diF	H	15.6	62.5
17e	3,5-bisCF_3	2,6-diF	H	>250	>250
17f	3,5-bisCF_3	3,4-diF	H	3.9	7.8
17g	3,5-bisCF_3	3,5-diF	H	2.0	7.8
17h	3,5-diCl	3,5-diF	H	31.3	7.8
18a	3,5-diCl	3,5-bisCF_3	Me	>250	>250
18b	3,5-bisCF_3	3,5-diCl	Me	>250	>250
19a	3,5-diCl	3,5-diCl, 4-OH	H	31.3	31.3
19b	3,5-bisCF_3	3,5-diCl, 4-OH	H	125	62.5

[a] The microdilution susceptibility test was performed as described in Section 4.
[b] Anti-MRSA activity was examined against methicillin-resistant *Staphylococcus aureus* OM584.
[c] Anti-VRE activity was examined against *Enterococcus faecium* FN-1.

7.57 (s, 1H), 7.52 (s, 2H), 7.29 (d, 2H, J = 8.2 Hz), 7.03 (s, 1H), 2.41 (s, 3H); FAB-MS m/z: 383 $[M]^+$, 384 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}F_6NO_3S$: C, 47.00; H, 2.89; N, 3.65. Found: C, 47.09; H, 3.01; N, 3.60.

4.4.4. *N*-3,5-Bis(trifluoromethyl)phenyl-4-chlorobenzenesulfonanilide (12d). According to the general procedure (GP-A), **12d** was obtained in 48% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp

Table 4. Anti-MRSA/VRE activities of compounds **21** evaluated with the microdilution susceptibility test[a]



Compound	R	R′	X	Y	MIC (μg/mL)	
					MRSA[b]	VRE[c]
Vancomycin (**2**)	—	—	—	—	2.0	125
a	3,5-diCl	CH	N	C–CF_3	>250	>250
b	3,5-diCl	2,6-diCl	CH	N	15.6	7.8
c	3,5-bisCF_3	2,6-diCl	CH	N	15.6	7.8

[a] The microdilution susceptibility test was performed as described in Section 4.
[b] Anti-MRSA activity was examined against methicillin-resistant *Staphylococcus aureus* OM584.
[c] Anti-VRE activity was examined against *Enterococcus faecium* FN-1.

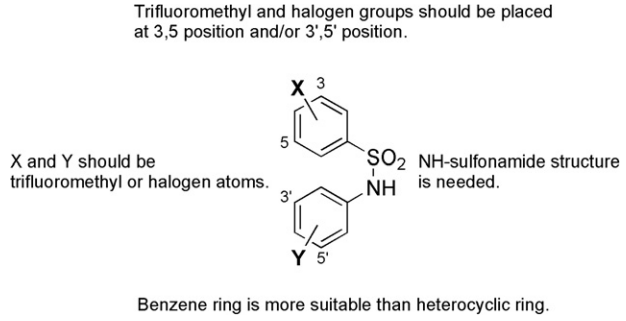


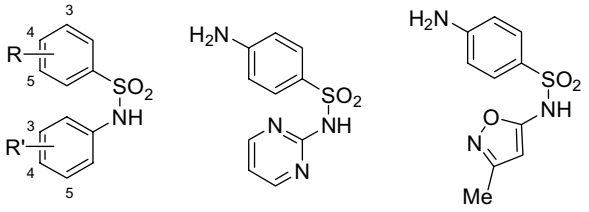
Figure 3. Important structural factors for antibacterial activity of benzenesulfonanilide derivatives.

115.0–117.0 °C; IR (KBr) cm^{-1}: 3265 (NH), 1345, 1157 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.29 (br s, 1H), 7.82 (dd, 2H, J = 6.6, 2.1 Hz), 7.79 (s, 1H), 7.68 (dd, 2H, J = 6.6, 2.1 Hz), 7.66 (s, 2H); FAB-MS *m/z*: 403 $[M]^+$, 404 $[M+H]^+$, 405 $[M+2]^+$, 406 $[M+2+H]^+$. Anal. Calcd for $C_{14}H_8ClF_6NO_2S$: C, 41.65; H, 2.00; N, 3.47. Found: C, 42.01; H, 2.27; N, 3.31.

4.4.5. *N*-3,5-Bis(trifluoromethyl)phenyl-4-trifluoromethylbenzenesulfonanilide (12e). According to the general procedure (GP-A), 12e was obtained in 99% yield as white solid after recrystallization from EtOAc/*n*-hexane. Mp 122.0–124.0 °C; IR (KBr) cm^{-1}: 3269 (NH), 1325, 1161 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.95 (d, 2H, J = 8.3 Hz), 7.78 (d, 2H, J = 8.3 Hz), 7.65 (s, 1H), 7.55 (s, 2H), 7.05 (s, 1H); FAB-MS *m/z*: 437 $[M]^+$, 438 $[M+H]^+$. Anal. Calcd for $C_{15}H_8F_9NO_2S$: C, 41.2; H, 1.84; N, 3.20. Found: C, 41.06; H, 1.94; N, 3.40.

4.4.6. *N*-3,5-Bis(trifluoromethyl)phenyl-4-nitrobenzenesulfonanilide (12f). According to the general procedure (GP-A), **12f** was obtained in 76% yield as colorless solid

Table 5. Antibacterial activities of our compounds and sulfa drugs on clinical isolates[a]



14e, **15f-g**, **16b-c** Sulfadiazine (**22**) Sulfamethoxazole (**23**)

Compound	R	R′	Staphylococci (13)[b]	Enterococci (12)	*Pseudomonas aeruginosa* (14)	*E. coli* (11)
14e	4-CF_3	3,5-diCl	13[c]	12	1	0
15f	3,5-diCl	4-CF_3	13	9	0	0
15g	3,5-diCl	4-NO_2	13	1	0	0
16b	3,5-diCl	3,5-bisCF_3	13	10	5	2
16c	3,5-bisCF_3	3,5-diCl	13	12	2	1
22	—	—	0	0	1	5
23	—	—	0	0	2	7

[a] The assay was performed by the disc diffusion method as described in Section 4.
[b] The number of strains.
[c] The number of strains against which the compounds showed confirmed antibacterial activity.

after column chromatography (eluent: MeOH/$CHCl_3$ = 1:100). Mp 143.5–144.0 °C; IR (KBr) cm^{-1}: 3242 (NH), 1348, 1170 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.53 (s, 1H), 8.40 (d, 2H, J = 9.0 Hz), 8.06 (d, 2H, J = 9.0 Hz), 7.82 (br s, 1H), 7.68 (br s, 2H); FAB-MS m/z: 414 $[M]^+$, 415 $[M+H]^+$. Anal. Calcd for $C_{14}H_8F_6N_2O_4S$: C, 40.59; H, 1.95; N, 6.76. Found: C, 40.56; H, 2.13; N, 6.65.

4.4.7. *N*-4-Aminophenyl-3,5-bis(trifluoromethyl)benzenesulfonanilide (13a). According to the general procedure (GP-B-2), **13a** was obtained in 39% yield as pale yellow solid after recrystallization from EtOAc/*n*-hexane. Mp 181.5–182.5 °C; IR (KBr) cm^{-1}: 3471, 3357 (NH_2), 3239 (NH), 1362, 1171 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 9.79 (s, 1H), 8.47 (s, 1H), 8.12 (br s, 2H), 6.63 (dd, 2H, J = 8.6, 2.0 Hz), 6.41 (dd, 2H, J = 8.6, 2.0 Hz), 5.09 (s, 1H); FAB-MS m/z: 384 $[M]^+$, 385 $[M+H]^+$. Anal. Calcd for $C_{14}H_{10}F_6N_2O_2S$: C, 43.76; H, 2.62; N, 7.29. Found: C, 43.68; H, 2.72; N, 7.25.

4.4.8. 3,5-Bis(trifluoromethyl)-*N*-4-methoxyphenylbenzenesulfonanilide (13b). According to the general procedure (GP-A), **13b** was was obtained in 89% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 87.0–90.4 °C; IR (KBr) cm^{-1}: 3286 (NH), 1331, 1139 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.08 (s, 2H), 8.03 (s, 1H), 6.97 (d, 2H, J = 6.8, 2.3 Hz), 6.83 (d, 2H, J = 6.8, 2.3 Hz), 6.68 (br s, 1H), 3.78 (s, 3H); FAB-MS m/z: 399 $[M]^+$, 400 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}F_6NO_3S$: C, 45.12; H, 2.78; N, 3.51. Found: C, 44.98; H, 2.91; N, 3.42.

4.4.9. 3,5-Bis(trifluoromethyl)-*N*-4-methylphenylbenzenesulfonanilide (13c). According to the general procedure (GP-A), **13c** was obtained in 67% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 109.0–112.0 °C; IR (KBr) cm^{-1}: 3237 (NH), 1362, 1148 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.09 (s, 2H), 8.02 (s, 1H), 7.11 (d, 2H, J = 8.3 Hz), 6.93 (d, 2H, J = 8.3 Hz), 6.44 (s, 1H), 2.38 (s, 3H); FAB-MS m/z: 383 $[M]^+$, 384 $[M+H]^+$. Anal. Calcd for $C_{15}H_{11}F_6NO_2S$: C, 47.00; H, 2.89; N, 3.65. Found: C, 47.00; H, 2.97; N, 3.57.

4.4.10. 3,5-Bis(trifluoromethyl)-*N*-4-chlorophenylbenzenesulfonanilide (13d). According to the general procedure (GP-A), **13d** was was obtained in 78% yield as white powder after recrystallization from EtOAc/*n*-hexane. Mp 148.0–151.0 °C; IR (KBr) cm^{-1}: 3226 (NH), 1282, 1140 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.15 (s, 2H), 8.06 (s, 1H), 7.29 (d, 2H, J = 6.8, 2.0 Hz), 7.03 (d, 2H, J = 6.8, 2.0 Hz), 6.63 (s, 1H); FAB-MS m/z: 403 $[M]^+$. Anal. Calcd for $C_{14}H_8ClF_6NO_2S$: C, 41.65; H, 2.00; N, 3.47. Found: C, 41.86; H, 2.20; N, 3.29.

4.4.11. 3,5-Bis(trifluoromethyl)-*N*-4-trifluoromethylphenylbenzenesulfonanilide (13e). According to the general procedure (GP-A), 13e was obtained in 40% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 142.0–143.0 °C; IR (KBr) cm^{-1}: 3283 (NH), 1325, 1124 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.21 (s, 2H), 8.07 (s, 1H), 7.58 (d, 2H, J = 8.7 Hz), 7.22 (d, 2H, J = 8.7 Hz), 7.09 (br s, 1H); FAB-MS m/z: 437 $[M]^+$. Anal. Calcd for $C_{15}H_8F_9NO_2S$: C, 41.20; H, 1.84; N, 3.20. Found: C, 40.92; H, 1.95; N, 3.34.

4.4.12. 3,5-Bis(trifluoromethyl)-*N*-4-nitrophenylbenzenesulfonanilide (13f). According to the general procedure (GP-A), **13f** was obtained in 53% yield as yellow needles after recrystallization from EtOAc/*n*-hexane. Mp 183.0–185.0 °C; IR (KBr) cm^{-1}: 3332 (NH), 1366, 1183 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.55 (br s, 1H), 8.51 (s, 1H), 8.41 (s, 2H), 8.16 (dd, 2H, J = 7.1, 2.3 Hz), 7.36 (dd, 2H, J = 7.1, 2.3 Hz); FAB-MS m/z: 414 $[M]^+$, 415 $[M+H]^+$. Anal. Calcd for $C_{14}H_8F_6N_2O_4S$: C, 40.59; H, 1.95; N, 6.76. Found: C, 40.80; H, 2.25; N, 6.70.

4.4.13. 4-Amino-*N*-3,5-dichlorophenylbenzenesulfonanilide (14a). According to the general procedure (GP-B-1), **14a** was obtained in 99% yield as white needles after recrystallization from CH_2Cl_2/*n*-hexane. Mp 120.0–122.0 °C; IR (KBr) cm^{-1}: 3395 (NH), 1316, 1160 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.46 (br s, 1H), 7.42 (dd, 2H, J = 6.9, 1.8 Hz), 7.17 (t, 1H, J = 1.8 Hz), 7.03 (d, 2H, J = 1.8 Hz), 6.57 (dd, 2H, J = 6.9, 1.8 Hz), 6.10 (s, 2H); FAB-MS m/z: 316 $[M]^+$, 317 $[M+H]^+$, 318 $[M+2]^+$, 319 $[M+2+H]^+$. Anal. Calcd for $C_{12}H_{10}Cl_2N_2O_2S$: C, 45.44; H, 3.18; N, 8.83. Found: C, 45.72; H, 3.14; N, 9.16.

4.4.14. *N*-3,5-Dichlorophenyl-4-methoxybenzenesulfonanilide (14b). According to the general procedure (GP-A), **14b** was obtained in 94% yield as white needles after recrystallization from MeOH. Mp 128.0–130.0 °C; IR (KBr) cm^{-1}: 3315 (NH), 1332, 1162 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.71 (br s, 1H), 7.73 (d, 2H, J = 8.9 Hz), 7.22 (s, 1H), 7.10 (d, 2H, J = 8.9 Hz), 7.07 (s, 2H), 3.81 (s, 3H); FAB-MS m/z: 331 $[M]^+$, 332 $[M+H]^+$, 333 $[M+2]^+$, 334 $[M+2+H]^+$, 335 $[M+4]^+$, 336 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_{11}Cl_2NO_3S$: C, 47.00; H, 3.34; N, 4.22. Found: C, 47.31; H, 3.55; N, 4.21.

4.4.15. *N*-3,5-Dichlorophenyl-4-methylbenzenesulfonanilide (14c). According to the general procedure (GP-A), **14c** was obtained in 90% yield as white columnar crystals after recrystallization from EtOAc/*n*-hexane. Mp 146.0–147.0 °C; IR (KBr) cm^{-1}: 3232 (NH), 1327, 1166 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.83 (br s, 1H), 7.70 (d, 2H, J = 8.5 Hz), 7.41 (d, 2H, J = 8.5 Hz), 7.23 (t, 1H, J = 2.0 Hz), 7.08 (d, 2H, J = 2.0 Hz), 2.36 (s, 3H); FAB-MS m/z: 315 $[M]^+$, 316 $[M+H]^+$, 317 $[M+2]^+$, 318 $[M+2+H]^+$, 319 $[M+4]^+$, 320 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_{11}Cl_2NO_2S$: C, 49.38; H, 3.51; N, 4.43. Found: C, 49.14; H, 3.43; N, 4.13.

4.4.16. 4-Choro-*N*-3,5-dichlorophenylbenzenesulfonanilide (14d). According to the general procedure (GP-A), **14d** was obtained in 92% yield as white needles after recrystallization from MeOH/CH_2Cl_2. Mp 130.0–132.0 °C; IR (KBr) cm^{-1}: 3224 (NH), 1335, 1169 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.95 (br s, 1H), 7.81 (dd, 2H, J = 6.7, 2.0 Hz), 7.68 (dd, 2H, J = 6.7, 2.0 Hz), 7.27 (t, 1H, J = 1.8 Hz), 7.09 (d, 2H,

J = 2.0 Hz); FAB-MS m/z: 335 $[M]^+$, 336 $[M+H]^+$, 337 $[M+2]^+$, 338 $[M+2+H]^+$, 339 $[M+4]^+$, 340 $[M+4+H]^+$. Anal. Calcd for $C_{12}H_8Cl_3NO_2S$: C, 42.82; H, 2.40; N, 4.16. Found: C, 43.07; H, 2.64; N, 4.12.

4.4.17. *N*-3,5-Dichlorophenyl-4-trifluoromethylbenzenesulfonanilide (14e). According to the general procedure (GP-A), **14e** was obtained in 60% yield as white needles after recrystallization from $CHCl_3$/*c*-hexane. Mp 118.0–119.0 °C; IR (KBr) cm^{-1}: 3255 (NH), 1326, 1132 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.11 (s, 1H), 8.02 (s, 4H), 7.29 (t, 1H, J = 1.9 Hz), 7.11 (d, 2H, J = 1.9 Hz); FAB-MS m/z: 369 $[M]^+$, 370 $[M+H]^+$, 371 $[M+2]^+$, 372 $[M+2+H]^+$. Anal. Calcd for $C_{13}H_8Cl_2F_3NO_2S$: C, 42.18; H, 2.18; N, 3.78. Found: C, 42.43; H, 1.88; N, 3.44.

4.4.18. *N*-3,5-Dichlorophenyl-4-nitrobenzenesulfonanilide (14f). According to the general procedure (GP-A), **14f** was obtained in 99% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 186.0–188.0 °C; IR (KBr) cm^{-1}: 3265 (NH), 1350, 1176 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.19 (br s, 1H), 8.41 (dd, 2H, J = 7.0, 2.0 Hz), 8.06 (dd, 2H, J = 7.0, 2.0 Hz), 7.31 (t, 1H, J = 2.0 Hz), 7.11 (d, 2H, J = 2.0 Hz); FAB-MS m/z: 346 $[M]^+$, 347 $[M+H]^+$, 348 $[M+2]^+$, 349 $[M+2+H]^+$, 350 $[M+4]^+$, 351 $[M+4+H]^+$. Anal. Calcd for $C_{12}H_8Cl_2N_2O_4S$: C, 41.51; H, 2.32; N, 8.07. Found: C, 41.33; H, 2.22; N, 8.16.

4.4.19. *N*-4-Aminophenyl-3,5-dichlorobenzenesulfonanilide (15a). According to the general procedure (GP-B-2), **15a** was obtained in 76% yield as white needles after recrystallization from $CHCl_3$/*n*-hexane. Mp 175.0–176.0 °C; IR (KBr) cm^{-1}: 3396, 3333 (NH_2), 3063 (NH), 1335, 1166 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 9.69 (s, 1H), 7.93 (t, 1H, J = 1.9 Hz), 7.59 (d, 2H, J = 1.9 Hz), 6.66 (dd, 2H, J = 8.8, 2.2 Hz), 6.42 (dd, 2H, J = 8.8, 2.2 Hz), 5.06 (s, 2H); FAB-MS m/z: 316 $[M]^+$, 317 $[M+H]^+$, 318 $[M+2]^+$, 319 $[M+2+H]^+$, 320 $[M+4]^+$, 321 $[M+4+H]^+$. Anal. Calcd for $C_{12}H_{10}Cl_2N_2O_2S$: C, 45.44; H, 3.18; N, 8.83. Found: C, 45.32; H, 2.88; N, 8.45.

4.4.20. 3,5-Dichloro-*N*-4-methoxyphenylbenzenesulfonanilide (15b). According to the general procedure (GP-A), **15b** was obtained in 90% yield as pale red plates after recrystallization from MeOH. Mp 88.0–90.0 °C; IR (KBr) cm^{-1}: 3294 (NH), 1340, 1173 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.12 (br s, 1H), 7.94 (t, 1H, J = 2.0 Hz), 7.62 (d, 2H, J = 2.0 Hz), 6.97 (d, 2H, J = 9.2 Hz), 6.85 (d, 2H, J = 9.2 Hz), 3.69 (s, 3H); FAB-MS m/z: 331 $[M]^+$, 332 $[M+H]^+$, 333 $[M+2]^+$, 334 $[M+2+H]^+$, 335 $[M+4]^+$, 336 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_{11}Cl_2NO_3S$: C, 47.00; H, 3.34; N, 4.22. Found: C, 46.91; H, 3.16; N, 4.02.

4.4.21. 3,5-Dichloro-*N*-4-methylphenylbenzenesulfonanilide (15c). According to the general procedure (GP-A), **15c** was obtained in 99% yield as white needles after recrystallization from MeOH. Mp 123.0–125.0 °C; IR (KBr) cm^{-1}: 3210 (NH), 1340, 1163 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.29 (br s, 1H), 7.93 (td, 1H, J = 2.0 Hz), 7.66 (d, 2H, J = 2.0 Hz), 7.09 (d, 2H, J = 8.5 Hz), 6.97 (d, 2H, J = 8.5 Hz), 2.21 (s, 3H); FAB-MS m/z: 315 $[M]^+$, 316 $[M+H]^+$, 317 $[M+2]^+$, 318 $[M+2+H]^+$, 319 $[M+4]^+$, 320 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_{11}Cl_2NO_2S$: C, 49.38; H, 3.51; N, 4.43. Found: C, 49.52; H, 3.73; N, 4.24.

4.4.22. *N*-4-Chlorophenyl-3,5-dichlorobenzenesulfonanilide (15d). According to the general procedure (GP-A), **15d** was obtained in 65% yield as pale red solid after recrystallization from $CHCl_3$/*c*-hexane. Mp 145.5–146.0 °C; IR (KBr) cm^{-1}: 3275 (NH), 1339, 1170 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.63 (s, 1H), 7.97 (t, 1H, J = 1.9 Hz), 7.71 (d, 2H, J = 1.9 Hz), 7.35 (dd, 2H, J = 8.9, 2.1 Hz), 7.12 (dd, 2H, J = 8.9, 2.1 Hz); FAB-MS m/z: 335 $[M]^+$, 336 $[M+H]^+$, 337 $[M+2]^+$, 338 $[M+2+H]^+$, 339 $[M+4]^+$, 340 $[M+4+H]^+$, 341 $[M+6]^+$. Anal. Calcd for $C_{12}H_8Cl_3NO_2S$: C, 42.82; H, 2.40; N, 4.16. Found: C, 42.79; H, 2.22; N, 4.06.

4.4.23. 3,5-Dichloro-*N*-3-trifluoromethylphenylbenzenesulfonanilide (15e). According to the general procedure (GP-A), **15e** was obtained in 64% yield as white solid after recrystallization from CH_2Cl_2/*n*-hexane. Mp 139.5–141.5 °C; IR (KBr) cm^{-1}: 3283 (NH), 1325, 1170 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.89 (br s, 1H), 7.97 (t, 1H, J = 2.0 Hz), 7.72 (d, 2H, J = 2.0 Hz), 7.47 (m, 4H); FAB-MS m/z: 369 $[M]^+$, 370 $[M+H]^+$, 371 $[M+2]^+$, 372 $[M+2+H]^+$. Anal. Calcd for $C_{13}H_8Cl_2F_3NO_2S$: C, 42.18; H, 2.18; N, 3.78. Found: C, 42.35; H, 2.27; N, 3.72.

4.4.24. 3,5-Dichloro-*N*-4-trifluoromethylphenylbenzenesulfonanilide (15f). According to the general procedure (GP-A), **15f** was obtained in 65% yield as white solid after recrystallization from $CHCl_3$/*c*-hexane. Mp 145.5–146.0 °C; IR (KBr) cm^{-1}: 3282 (NH), 1324, 1144 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.06 (s, 1H), 7.98 (t, 1H, J = 1.8 Hz), 7.79 (d, 2H, J = 1.8 Hz), 7.65 (d, 2H, J = 8.3 Hz), 7.31 (d, 2H, J = 8.3 Hz); FAB-MS m/z: 369 $[M]^+$, 370 $[M+H]^+$, 371 $[M+2]^+$, 372 $[M+2+H]^+$, 373 $[M+4]^+$, 374 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_8Cl_2F_3NO_2S$: C, 42.18; H, 2.18; N, 3.78. Found: C, 42.35; H, 2.05; N, 3.59.

4.4.25. 3,5-Dichloro-*N*-4-nitrophenylbenzenesulfonanilide (15g). According to the general procedure (GP-A), **15g** was obtained in 69% yield as yellow plates after recrystallization from EtOAc/*n*-hexane. Mp 190.0–192.0 °C; IR (KBr) cm^{-1}: 3298 (NH), 1335, 1174 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.45 (s, 1H), 8.17 (dd, 2H, J = 9.3, 2.2 Hz), 8.00 (t, 1H, J = 2.0 Hz), 7.85 (d, 2H, J = 2.0 Hz), 7.35 (dd, 2H, J = 9.3, 2.2 Hz); FAB-MS m/z: 346 $[M]^+$, 347 $[M+H]^+$, 348 $[M+2]^+$, 349 $[M+2+H]^+$, 350 $[M+4]^+$, 351 $[M+4+H]^+$. Anal. Calcd for $C_{12}H_8Cl_2N_2O_4S$: C, 41.51; H, 2.32; N, 8.07. Found: C, 41.65; H, 2.23; N, 7.85.

4.4.26. *N*-3,5-Bis(trifluoromethyl)phenyl-3-chlorobenzenesulfonanilide (16a). According to the general procedure (GP-A), **16a** was obtained in 63% yield as white granules after recrystallization from EtOAc/*n*-hexane. Mp 135.0–137.0 °C; IR (KBr) cm^{-1}: 3271 (NH), 1341, 1132 (SO_2);

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.61 (br s, 1H), 8.18–8.15 (m, 1H), 7.72 (s, 1H), 7.70–7.66 (m, 2H), 7.62 (s, 2H), 7.61–7.57 (m, 1H); FAB-MS *m/z*: 403 $[M]^+$, 404 $[M+H]^+$, 405 $[M+2]^+$, 406 $[M+2+H]^+$. Anal. Calcd for $C_{14}H_8ClF_6NO_2S$: C, 41.65; H, 2.00; N, 3.47. Found: C, 41.95; H, 2.31; N, 3.47.

4.4.27. *N*-3,5-Bis(trifluoromethyl)phenyl-3,5-dichlorobenzenesulfonanilide (16b). According to the general procedure (GP-A), **16b** was obtained in 64% yield as white powders by flash column chromatography (eluent: EtOAc/*n*-hexane = 1:8). Mp 110.0–111.0 °C; IR (KBr) cm^{-1}: 3258 (NH), 1345, 1163 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.15 (s, 1H), 8.02 (t, 1H, J = 1.8 Hz), 7.86 (s, 1H), 7.78 (d, 2H, J = 1.8 Hz), 7.67 (s, 2H); FAB-MS *m/z*: 437 $[M]^+$, 438 $[M+H]^+$ 439 $[M+2]^+$, 440 $[M+2+H]^+$, 441 $[M+4]^+$, 442 $[M+4+H]^+$. Anal. Calcd for $C_{14}H_7Cl_2F_6NO_2S$: C, 38.38; H, 1.61; N, 3.20. Found: C, 38.36; H, 1.77; N, 3.08.

4.4.28. 3,5-Bis(trifluoromethyl)-*N*-3,5-dichlorophenylbenzenesulfonanilide (16c). According to the general procedure (GP-A), **16c** was obtained in 79% yield as white solid after recrystallization from $CHCl_3$/*n*-hexane. Mp 128.0–128.5 °C; IR (KBr) cm^{-1}: 3261 (NH), 1361, 1173 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.09 (s, 1H), 8.13 (s, 1H), 8.32 (br s, 2H), 7.36 (t, 1H, J = 1.8 Hz), 7.11 (d, 2H, J = 1.8 Hz); FAB-MS *m/z*: 437 $[M]^+$, 438 $[M+H]^+$, 439 $[M+2]^+$, 440 $[M+2+H]^+$, 441 $[M+4]^+$, 442 $[M+4+H]^+$. Anal. Calcd for $C_{14}H_7Cl_2F_6NO_2S$: C, 38.38; H, 1.61; N, 3.20. Found: C, 38.47; H, 1.84; N, 3.05.

4.4.29. 3,5-Bis(trifluoromethyl)-*N*-2,4-dichlorophenylbenzenesulfonanilide (16d). According to the general procedure (GP-A), **16d** was obtained in 47% yield as white granules after recrystallization from EtOAc/*n*-hexane. Mp 110.0–112.0 °C; IR (KBr) cm^{-1}: 3259 (NH), 1281, 1177 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.70 (br s, 1H), 8.56 (s, 1H), 8.23 (s, 2H), 7.63 (d, 1H, J = 2.5 Hz), 7.44 (dd, 1H, J = 8.7, 2.5 Hz), 7.31 (d, 1H, J = 8.7 Hz); FAB-MS *m/z*: 437 $[M]^+$, 438 $[M+H]^+$, 439 $[M+2]^+$, 440 $[M+2+H]^+$. Anal. Calcd for $C_{14}H_7Cl_2F_6NO_2S$: C, 38.38; H, 1.61; N, 3.20. Found: C, 38.47; H, 1.75; N, 2.95.

4.4.30. 3,5-Bis(trifluoromethyl)-*N*-2,6-dichlorophenylbenzenesulfonanilide (16e). According to the general procedure (GP-A), **16e** was obtained in 11% yield as white granules after recrystallization from EtOAc/*n*-hexane. Mp 146.0–148.0 °C; IR (KBr) cm^{-1}: 3264 (NH), 1281, 1137 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.75 (br s, 1H), 8.55 (s, 1H), 8.30 (s, 2H), 7.54 (d, 2H, J = 8.5 Hz), 7.38 (t, 1H, J = 8.5 Hz); FAB-MS *m/z*: 437 $[M]^+$, 438 $[M+H]^+$, 439 $[M+2]^+$, 440 $[M+2+H]^+$. Anal. Calcd for $C_{14}H_7Cl_2F_6NO_2S$: C, 38.38; H, 1.61; N, 3.20. Found: C, 38.25; H, 1.77; N, 3.03.

4.4.31. *N*-3,5-Bis(trifluoromethyl)phenyl-4-fluorobenzenesulfonanilide (17a). According to the general procedure (GP-A), **17a** was obtained in 86% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 117.0–119.0 °C; IR (KBr) cm^{-1}: 3267 (NH), 1382, 1130 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.24 (br s, 1H), 7.88 (dd, 2H, J = 8.9, 2.1 Hz), 7.75 (s, 1H), 7.64 (s, 2H), 7.44 (dd, 2H, J = 8.9, 2.1 Hz); FAB-MS *m/z*: 387 $[M]^+$, 388 $[M+H]^+$. Anal. Calcd for $C_{14}H_8F_7NO_2S$: C, 43.42; H, 2.08; N, 3.62. Found: C, 43.12; H, 2.17; N, 3.59.

4.4.32. 3,5-Bis(trifluoromethyl)-*N*-2,3-difluorophenylbenzenesulfonanilide (17b). According to the general procedure (GP-A), **17b** was obtained in 49% yield as white solid after recrystallization from EtOAc/*n*-hexane. Mp 113.0–115.0 °C; IR (KBr) cm^{-1}: 3299 (NH), 1358, 1164 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.87 (br s, 1H), 8.56 (s, 1H), 8.26 (s, 2H), 7.38–7.04 (m, 3H); FAB-MS *m/z*: 405 $[M]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.49; H, 1.74; N, 3.46. Found: C, 41.25; H, 1.90; N, 3.46.

4.4.33. 3,5-Bis(trifluoromethyl)-*N*-2,4-difluorophenylbenzenesulfonanilide (17c). According to the general procedure (GP-A), **17c** was obtained in 84% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 121.0–123.0 °C; IR (KBr) cm^{-1}: 3249 (NH), 1353, 1169 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.61 (br s, 1H), 8.54 (s, 1H), 8.21 (s, 2H), 7.32–7.05 (m, 3H); FAB-MS *m/z*: 405 $[M]^+$, 406 $[M+H]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.49; H, 1.74; N, 3.46. Found: C, 41.45; H, 1.89; N, 3.45.

4.4.34. 3,5-Bis(trifluoromethyl)-*N*-2,5-difluorophenylbenzenesulfonanilide (17d). According to the general procedure (GP-A), **17d** was obtained in 41% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 121.0–123.0 °C; IR (KBr) cm^{-1}: 3254 (NH), 1349, 1171 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.87 (s, 1H), 8.54 (s, 1H), 8.29 (s, 2H), 7.30–7.10 (m, 3H); FAB-MS *m/z*: 405 $[M]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.49; H, 1.74; N, 3.46. Found: C, 41.30; H, 2.04; N, 3.37.

4.4.35. 3,5-Bis(trifluoromethyl)-N-2,6-difluorophenylbenzenesulfonanilide (17e). According to the general procedure (GP-A), **17e** was obtained in 55% yield as white solid after recrystallization from EtOAc/*n*-hexane. Mp 150.0–152.0 °C; IR (KBr) cm^{-1}: 3257 (NH), 1352, 1167 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.63 (br s, 1H), 8.55 (s, 1H), 8.28 (s, 1H), 7.40 (t, 1H, J = 8.6 Hz), 7.14 (t, 2H, J = 8.6 Hz); FAB-MS *m/z*: 405 $[M]^+$, 406 $[M+H]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.49; H, 1.74; N, 3.46. Found: C, 41.30; H, 1.88; N, 3.54.

4.4.36. 3,5-Bis(trifluoromethyl)-N-3,4-difluorophenylbenzenesulfonanilide (17f). According to the general procedure (GP-A), **17f** was obtained in 93% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 97.0–100.0 °C; IR (KBr) cm^{-1}: 3216 (NH), 1281, 1165 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.74 (br s, 1H), 8.51 (s, 1H), 8.26 (s, 2H), 7.41–6.88 (m, 3H); FAB-MS *m/z*: 405 $[M]^+$, 406 $[M+H]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.49; H, 1.74; N, 3.46. Found: C, 41.52; H, 1.95; N, 3.54.

4.4.37. 3,5-Bis(trifluoromethyl)-*N*-3,5-difluorophenylbenzenesulfonanilide (17g). According to the general

procedure (GP-A), **17g** was obtained in 86% yield as white plates after recrystallization from $CHCl_3$/*n*-hexane. Mp 98.0–101.0 °C; IR (KBr) cm^{-1}: 3234 (NH), 1363, 1167 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.10 (br s, 1H), 8.50 (s, 1H), 8.34 (s, 2H), 6.96 (tt, 1H, J = 9.2, 2.2 Hz), 6.80 (dd, 2H, J = 9.2, 2.2 Hz); FAB$^-$MS *m*/*z*: 405 $[M]^+$, 406 $[M+H]^+$. Anal. Calcd for $C_{14}H_7F_8NO_2S$: C, 41.48; H, 1.74; N, 3.46. Found: C, 41.25; H, 1.89; N, 3.40.

4.4.38. 3,5-Dichloro-*N*-3,5-difluorophenylbenzenesulfonanilide (17h). According to the general procedure (GP-A), **17h** was obtained in 80% yield as white plates after recrystallization from CH_2Cl_2/*n*-hexane. Mp 115.0–118.0 °C; IR (KBr) cm^{-1}: 3238 (NH), 1344, 1177 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 11.07 (br s, 1H), 7.99 (td, 1H, J = 2.0, 0.8 Hz), 7.81 (dd, 2H, J = 2.0, 0.8 Hz), 6.96 (tt, 1H, J = 9.2, 2.0 Hz), 6.80 (dd, 2H, J = 9.2, 2.0 Hz); FAB-MS *m*/*z*: 337 $[M]^+$, 338 $[M+H]^+$, 339 $[M+2]^+$, 340 $[M+2+H]^+$. Anal. Calcd for $C_{12}H_7Cl_2F_2NO_2S$: C, 42.62; H, 2.09; N, 4.14. Found: C, 42.75; H, 1.74; N, 3.99.

4.4.39. *N*-3,5-Bis(trifluoromethyl)phenyl-3,5-dichloro-*N*-methylbenzenesulfonanilide (18a)-methylbenzenesulfonanilide (18a). According to the general procedure (GP-C), **18a** was obtained in 33% yield as white granules after recrystallization from EtOAc/*n*-hexane. Mp 105.0–107.0 °C; IR (KBr) cm^{-1}: 1279, 1175 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.85 (s, 1H), 7.63 (t, 1H, J = 2.0 Hz), 7.58 (s, 2H), 8.10 (s, 1H, J = 0.8 Hz), 7.45 (s, 2H), 3.28 (s, 3H); FAB-MS *m*/*z*: 451 $[M]^+$, 452 $[M+H]^+$, 453 $[M+2]^+$, 454 $[M+2+H]^+$. Anal. Calcd for $C_{15}H_9Cl_2F_6NO_2S$: C, 39.84; H, 2.01; N, 3.10. Found: C, 39.71; H, 2.18; N, 2.95.

4.4.40. 3,5-Bis(trifluoromethyl)-*N*-3,5-dichlorophenyl-*N*-methylbenzenesulfonanilide (18b)-methylbenzenesulfonanilide (18b). According to the general procedure (GP-C), **18b** was obtained in 58% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 85.0–86.0 °C; IR (KBr) cm^{-1}: 1282, 1142 (SO_2); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 7.98 (s, 2H), 7.36 (t, 1H, J = 1.8 Hz), 7.00 (d, 2H, J = 1.8 Hz), 3.18 (s, 3H); FAB-MS *m*/*z*: 451 $[M]^+$, 452 $[M+H]^+$, 453 $[M+2]^+$, 454 $[M+2+H]^+$. Anal. Calcd for $C_{15}H_9Cl_2F_6NO_2S$: C, 39.84; H, 2.01; N, 3.10. Found: C, 39.60; H, 2.28; N, 2.99.

4.4.41. 3,5-Dichloro-*N*-3,5-dichloro-*N* -4-hydroxyphenylbenzenesulfonanilide (19a). According to the general procedure (GP-A), **19a** was obtained in 89% yield as brown needles after recrystallization from EtOAc/*n*-hexane. Mp 207.0–210.0 °C; IR (KBr) cm^{-1}: 3419, 3282 (OH), 3093 (NH), 1345, 1159 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 10.42 (s, 1H), 10.20 (s, 1H), 7.99 (s, 1H), 7.67 (s, 2H), 7.03 (s, 2H); FAB-MS *m*/*z*: 385 $[M]^+$, 386 $[M+H]^+$, 387 $[M+2]^+$, 388 $[M+2+H]^+$, 389 $[M+4]^+$. Anal. Calcd for $C_{12}H_7Cl_4NO_3S$: C, 37.24; H, 1.82; N, 3.62. Found: C, 32.28; H, 1.78; N, 3.57.

4.4.42. 3,5-Bis(trifluoromethyl)-*N*-3,5-dichloro-*N*-4-hydroxyphenylbenzenesulfonanilide (19b). According to the general procedure (GP-A), **19b** was obtained in 69% yield as white solid after recrystallization from EtOAc/*n*-hexane. Mp 155.0–156.0 °C; IR (KBr) cm^{-1}: 3339, 3268 (OH), 3087 (NH), 1279, 1155 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.15 (s, 2H), 8.10 (s, 1H), 7.04 (s, 2H), 6.41 (br s, 1H), 5.86 (br s, 1H); FAB-MS *m*/*z*: 453 $[M]^+$, 454 $[M+H]^+$, 455 $[M+2]^+$. Anal. Calcd for $C_{14}H_7Cl_2F_6NO_3S$: C, 37.02; H, 1.55; N, 3.08. Found: C, 37.13; H, 1.83; N, 2.97.

4.4.43. 3,5-Dichlorobenzenesulfonamide (20). 3,5-Dichlorobenzenesulfonylchloride (3.0 mmol) was added to NH_3 aq (8.0 mL). The mixture was stirred at room temperature for 3 h, then extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure. Compound **20** was obtained in 88% yield as white needles after recrystallization from EtOAc/*n*-hexane. Mp 158.0–160.0 °C; ^1H NMR (300 MHz, DMSO-d_6) δ: 7.94 (td, 1H, J = 1.8, 0.3 Hz), 7.80 (dd, 2H, J = 1.8, 0.3 Hz), 7.66 (s, 2H).

4.4.44. 3,5-Dichloro-*N*-(5-trifluoromethyl-2-pyridinyl)benzenesulfonanilide (21a). To mixture of **20** (1.4 mmol) and 2-chloro-5-(trifluoromethyl)pyridine (1.4 mmol) in dry DMF (2.0 mL) were added K_2CO_3 (1.4 mmol) and Cu (catalytic amount). The mixture was refluxed at 175 °C for 5 h, then poured into 10% AcOH. The whole was filtered through a pad of Celite, and the filtrate was extracted with EtOAc (3× 30 mL). The organic layer was collected, washed with H_2O (2× 50 mL) and brine (100 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/*n*-hexane = 1:4) and obtained in 32% yield as yellow needles after recrystallization from EtOAc/*n*-hexane. Mp 179.0–181.0 °C; IR (KBr) cm^{-1}: 3083 (NH), 1332, 1128 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.53 (s, 1H), 7.91 (d, 1H, J = 9.0 Hz), 7.81 (d, 2H, J = 1.8 Hz), 7.56 (s, 1H), 7.40 (d, 1H, J = 9.0 Hz); FAB-MS *m*/*z*: 371 $[M+H]^+$, 372 $[M+2]^+$, 373 $[M+2+H]^+$. Anal. Calcd for $C_{12}H_7Cl_2F_3N_2O_2S$: C, 38.83; H, 1.90; N, 7.55. Found: C, 38.78; H, 1.85; N, 7.47.

4.4.45. *N*-(2,6-Dichloro-4-pyridinyl)-3,5-dichlorobenzenesulfonanilide (21b). According to the general procedure (GP-A), **21b** was obtained in 22% yield as white solid after recrystallization from EtOAc/*n*-hexane. Mp 155.0–156.0 °C; IR (KBr) cm^{-1}: 3077 (NH), 1351, 1174 (SO_2); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.78 (d, 2H, J = 1.8 Hz), 7.64 (t, 1H, J = 1.8 Hz), 7.01 (s, 2H); FAB-MS *m*/*z*: 371 $[M+H]^+$, 372 $[M+2]^+$, 373 $[M+2+H]^+$, 374 $[M+4]^+$, 375 $[M+4+H]^+$. Anal. Calcd for $C_{11}H_6Cl_4N_2O_2S$: C, 35.51; H, 1.63; N, 7.53. Found: C, 35.24; H, 1.81; N, 7.15.

4.4.46. 3,5-Bis(trifluoromethyl)-*N*-(2,6-dichloro-4-pyridinyl)benzenesulfonanilide (21c). According to the general procedure (GP-A), **21c** was obtained in 65% yield as white granules after recrystallization from EtOAc/*n*-hexane. Mp 171.0–172.0 °C; IR (KBr) cm^{-1}: 3095 (NH), 1354, 1140 (SO_2); ^1H NMR (300 MHz, DMSO-d_6) δ: 8.54 (s, 1H), 8.48 (s, 2H), 7.12 (s, 2H); FAB-MS *m*/*z*: 439 $[M+H]^+$, 440 $[M+2]^+$, 441 $[M+2+H]^+$, 442 $[M+4]^+$,

443 $[M+4+H]^+$. Anal. Calcd for $C_{13}H_6Cl_2F_6N_2O_2S$: C, 35.55; H, 1.38; N, 6.38. Found: C, 35.75; H, 1.73; N, 6.26.

4.5. Preparation of bacterial culture

Test strains were methicillin-resistant *S. aureus* OM584 as MRSA and *Enterococcus faecium* FN-1 as VRE. A single colony of each strain was transferred into 3 mL of nutrient broth (Eiken Chemical Co., Ltd, Tokyo, Japan) in a 15-mL test tube, and the tube was capped and placed in incubator overnight at 35 °C. After 12–18 h of incubation, the broth was directly used for the disc diffusion method. On the other hand, for the microdilution susceptibility test, a 20 μL aliquot of the overnight broth culture was transferred to 2 mL of sensitivity test broth (Nissui Pharmaceutical, Tokyo, Japan) in a shaking incubator (140 shakes/min) at 37 °C for about 1.5 h for MRSA and for about 4 h for VRE until the optical density at 620 nm reached 0.1. An optical density of 0.1 was regarded as approximately equivalent to 10^8 CFU/mL (colony-forming unit/mL).

4.6. Disc diffusion method (first screening)

Muller-Hinton agar (Nissui Pharmaceutical, Tokyo, Japan) was prepared according to the supplier's instructions. After autoclave treatment, the Muller-Hinton agar (MH agar) was poured into 90-mm Petri dishes (about 20 mL/dish). After solidification, on the surface of the agar was spread 100 μL of overnight broth culture as described above. Seven 8-mm paper discs were placed on the agar, evenly spaced apart, and a solution of test compound in DMSO (20 mM) was transferred onto each paper disc. After overnight incubation of the plate at 37 °C, the diameters of the inhibitory zones were measured. The size was categorized into five classes: minus (no inhibition zone), plus/minus (0–1 mm), plus 1 (1–15 mm), plus 2 (15–20 mm), and plus 3 (>20 mm).

4.7. The microdilution susceptibility test

The microdilution susceptibility test in sensitivity test broth was carried out for determination of the minimum inhibitory concentration (MIC) according to Standard method of Japan Society of Chemotherapy. Vancomycin was used as a standard antibacterial agent. Solutions of compounds in DMSO were prepared at a concentration of 10 mg/mL. Vancomycin was dissolved in water at a concentration of 10 mg/mL. A 95 μL aliquot of autoclaved sensitivity test broth was poured into the first line wells of 96-well plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan) and 50 μL of autoclaved sensitivity test broth was poured into the remaining wells, then 5 μL of test compound solution was poured into the first line wells, and 50 μL of the mixture was transferred to the adjacent wells successively to generate concentrations of 500, 250, ..., 0.2 μg/mL. Finally, 50 μL of test strain suspension, adjusted to $\approx 10^5$ CFU/mL using the bacterial cultures mentioned above and sensitivity test broth, was inoculated into the corresponding wells. Plates were incubated under sufficient humidity at 37 °C for 20 h. After incubation, MIC was determined by visual inspection as the lowest concentration of the test compound at which bacterial growth was clearly inhibited. Controls with media, DMSO, and water were run in parallel with the test compounds under the same conditions. The experiment was performed at least in duplicate.

4.8. Disc diffusion test against isolated clinical strains

Antimicrobial activities of the test compounds against clinical isolates from patients with urinary tract infection at Okayama University hospital were assessed by the disc diffusion method as described above.

4.9. Acute toxicity test in mice

Male mice, weighing 15–30 g, were acquired from Charles River Co. Ltd. Only water was provided ad libitum during the 12 h before experimentation. The study was conducted according to internationally accepted principles of laboratory animal use. Solutions of each compound dissolved in 0.5% carboxymethyl cellulose (CMC) were administered at doses up to 100 mg kg^{-1} ip to mice (n = 5/group). After drug administration, food, and water was provided ad libitum. The weight gain of animals was observed up to 14 days as an indicator of toxicity.

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References and notes

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