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ARTICLE

Design, synthesis, and antibacterial evaluation of novel derivatives of NPS-2143 for the treatment of methicillin-resistant *S. aureus* (MRSA) infection

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a significant global health challenge due to the emergence of strains exhibiting resistance to nearly all classes of antibiotics. This necessitates the rapid development of novel antimicrobials to circumvent this critical problem. Screening of compounds based on phenotypes is one of the major strategies for finding new antibiotics at present. Hence, we here performed a phenotypic screening against MRSA and identified NPS-2143 exhibiting activity against MRSA with an MIC value of 16 μg ml^{-1}. In order to discover more potent anti-MRSA agents, a series of derivatives of NPS-2143 were designed and synthesized. The most promising compounds **48** and **49** exhibited favorable antimicrobial activity with an MIC value of 2 μg ml^{-1}.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most important multidrug-resistant organisms causing healthcare infections. Studies indicate that the incidence of MRSA in the past few years has extensively increased worldwide. Infection due to MRSA imposes a high and increasing burden on healthcare resources, as well as increasing morbidity and mortality. In a recently published report, it is estimated that by 2050, 10 million lives a year will be at risk due to the rise of drug-resistant infections [1–3]. Indeed, clinical isolates of both community-associated MRSA (CA-MRSA) [2] and healthcare-associated MRSA (HA-MRSA) have been documented that exhibit resistance to an array of different antibiotic classes including the β-lactams [4, 5], macrolides [6], quinolones [7, 8], tetracyclines [9, 10], and lincosamides [9]. Further exacerbating the problem, strains have emerged which exhibit resistance to first-line antibiotics (such as mupirocin [9, 11] for the treatment of MRSA skin infections) and agents of last resort (such as vancomycin [12, 13] and linezolid [10, 14, 15]). Therefore, there is an urgent need for the development of novel therapeutic agents and treatment strategies to circumvent this significant global health issue.

The discovery of new antibacterial agents which relies on new structures and new action modes plays a significant role in achieving the goal of overcoming bacterial resistance [16, 17]. Several strategies have been employed in the process of traditional antimicrobials discovery, i.e., high-throughput screening based on biological targets, structural modification of the known antimicrobial agents, and phenotypic compounds screening. Although many hit compounds [17, 18] were found by target-based high-throughput screening method, most of these hit compounds exhibit low phenotypic antibacterial activities, difficulty in crossing cell membrane or not absorbed because of the intrinsic efflux pump mechanism of bacteria [17]. Some

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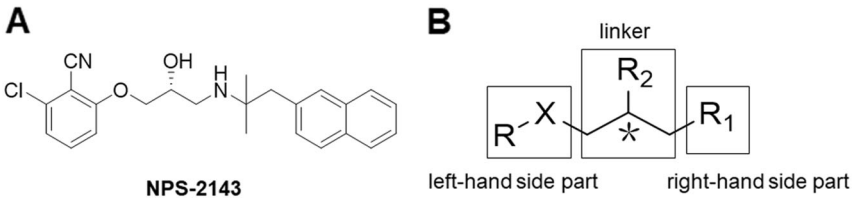


Fig. 1 (**a**) The chemical structure of NPS-2143; (**b**) Scheme of the three parts to modify

leading pharmaceutical companies have carried out a large number of high-throughput screening based on molecular targets, but few hit compounds advanced to the clinical research [19]. In contrast, phenotypic screening is highly productive in the history of antimicrobial agents discovery campaign, and a brand new action mechanism may be disclosed with new agents were found in this way [20]. Notably, most antibiotics used clinically are directly obtained through phenotypic screening [17]. Furthermore, novel antibiotic discovery studies, with high originality and new skeletons, are often derived from phenotypic screening. For example, the listed antimicrobial agents bedaquiline [21], retapamulin [22], and GSK299423 [23] currently in clinical research stage in recent years, were all found by phenotypic screening strategy.

Hence, we here performed a phenotypic screening of around 2600 compounds from MCE library against MRSA and identified NPS-2143 (Fig. 1) as a potential hit, which exhibiting activity against MRSA with minimal inhibitory concentration (MIC) value of 16 μg ml^{-1}. The hit molecule, named NPS-2143, is a calcilytic drug which acts as an antagonist at the calcium-sensing receptor (CaSR). The chemical structure of NPS-2143 [24] consists of three parts, i.e., a linear linker with heteroatom incorporated in and substituted, the two aromatic groups in left-hand and right-hand side. In order to find more potent antibacterial candidates and explore the structure-activity relationships (SAR), a series of NPS-2143 derivatives with modifications to these three parts (Fig. 1b) were designed and synthesized.

Result and discussion

Chemistry

In order to examine the SAR of the right-hand side part of NPS-2143 (Fig. 1b), compounds **3–18** were synthesized following the routes detailed in Fig. 2a. The *(R)*-2-chloro-6-(2-(oxiran-2-yl)ethoxy)benzonitrile (**2a**) was synthesized by a regioselective nucleophilic substitution reaction of 2-chloro-6-hydroxybenzonitrile (**1a**) with commercially available *(R)*-glycidyl nosylate [25–27]. It was previously shown by Sharpless that such nucleophilic displacement proceeded with no racemization [28, 29]. Then, solutions of different amines in ethanol reacted with **2a** providing a range of substituted analogs by changing its right-hand side part (**3–18**) (Table 1).

Analogs **19–30** and **38–40** were designed to explore the SAR of left-hand side part of NPS-2143. As outlined in Fig. 2a, b), the synthesis started from nucleophilic substitution reaction of various phenols **1b-m** [27] or thiophenols **36a-c** [30] with *(R)*-glycidyl nosylate and *(S)*-epichlorhydrin. Treatment of phenols **1c-i** with NaOH, followed by *(R)*-glycidyl nosylate provided epoxides **2c–2i**. By heating substituted phenols **1b**,**1j-m** with excess *(S)*-epichlorhydrin, K_2CO_3 and KI, we obtained the corresponding epoxides **2b**,**2j-m**. Thiophenols **36a-c** were captured with *(S)*-epichlorhydrin to yield epoxides **37a-c**. Then, solutions of 2-methyl-1-(naphthalen-2-yl)propan-2-amine in ethanol reacted with epoxides **2b-m, 37a-c** providing a range of substituted analogs in the left-hand side part **(19–30, 38–40)**. Enantiomers **31** and **32** were obtained in the same ways as NPS-2143 and **30**, replacing *(R)*-glycidyl nosylate and *(S)*-epichlorhydrin with *(S)*-glycidyl nosylate and *(R)*-epichlorhydrin. The chemical structures of analogs **19–30**, **38–40** were recorded in Table 2.

Synthesis of analogs **43**, **44**, **48**, and **49** was outlined in Fig. 2c, d. Treatment of 7-bromo-6-chloro-4-(3 *H*)quinazolinone (**41**) with *(S)*-epichlorhydrin and *(R)*-epichlorhydrin, respectively, in the presence of K_2CO_3 afforded epoxides **42a** and **42b**. 3,5-Bis(trifluoromethyl)aniline (**45**) was reacted with *(R)*-epichlorhydrin or *(S)*-epichlorhydrin to yield substituted propanolamines **46a** and **46b** which afforded epoxides **47a** and **47b** by treating with KF. Then, solutions of commercially available 2-methyl-1-(naphthalen-2-yl)propan-2-amine in ethanol reacted with corresponding epoxides provide target analogs **43**, **44**, **48** and **49**.

As showed in Fig. 2a, d), analog **33** was produced by treatment of **31** with 1,1′-carbonyldiimidazole (CDI) and triethylamine. Methylation of secondary amine of **31** and analog **48** by using iodomethane afforded target compounds **34** and **50**. Methylation of hydroxyl group of **32**, whose secondary amine was protected by using di-*tert*-butyl dicarbonate (Boc_2O), produced analog **35** after removal of the protective group.

Anti-MRSA activity

Phenotypic screening results in the identification of the moderately potent hit compound NPS-2143 against MRSA

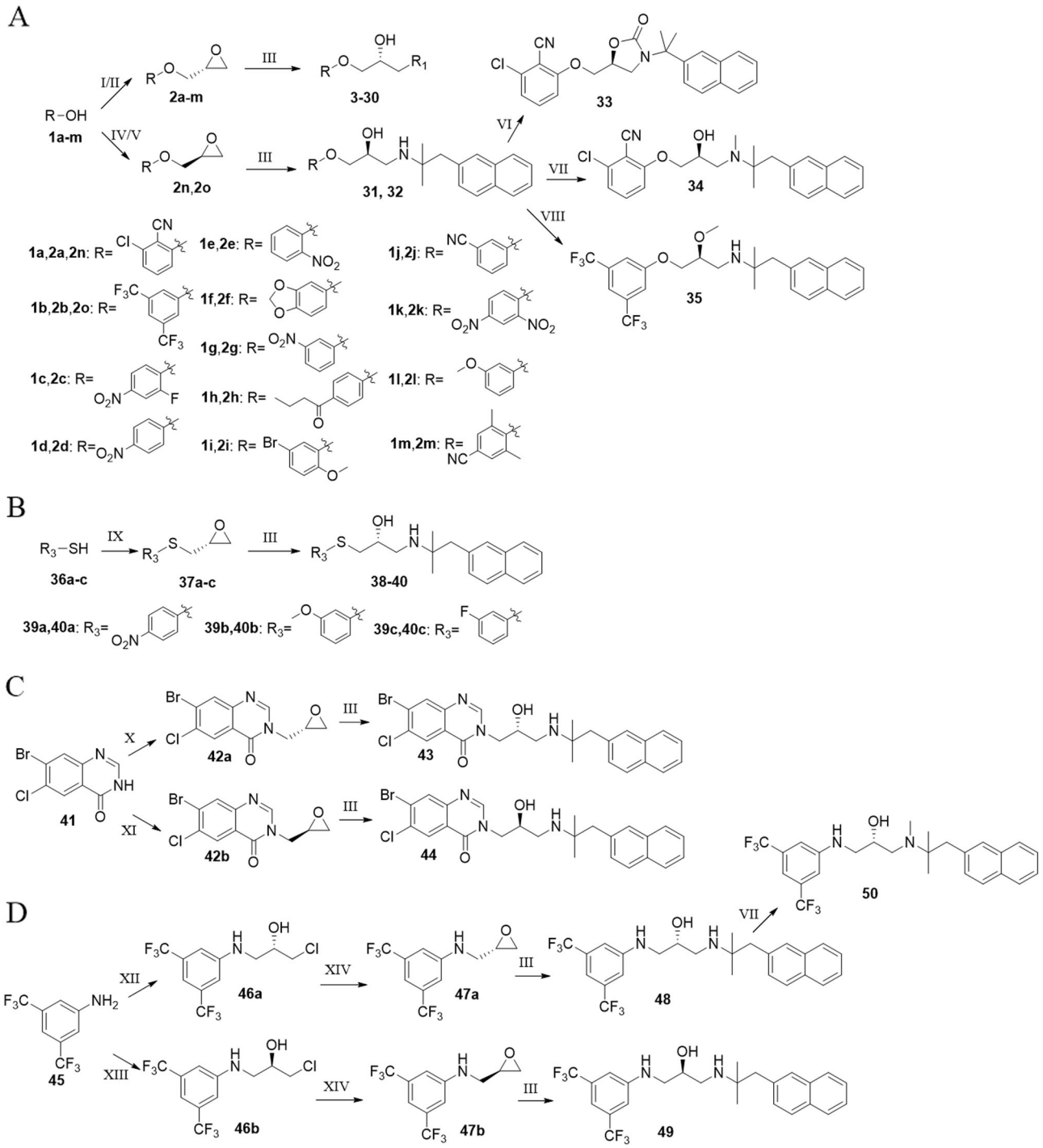
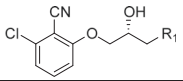


Fig. 2 Synthetic routes of target compounds. Reagents and conditions: (I) *(R)*-glycidyl nosylate and NaOH, DMF, rt, 5 h; (II) *(S)*-epichlorhydrin, K_2CO_3, KI, DMF, 60 °C,4 h; (III) HNR_1R_2, Et_3N, EtOH, reflux, 7 h; (IV) *(S)*-glycidyl nosylate NaOH, DMF, rt, 5 h; (V) *(R)*-epichlorhydrin, K_2CO_3, KI, DMF, 60 °C,4 h; (VI) CDI, Et_3N, DCM, r. t. 30 min; (VII) CH_3I, NaOH, MeOH, r.t. 1 h; (VIII) Boc_2O, Et_3N, DCM, r.t. 40 min. CH_3I, NaH, DMF, r.t. 1 h. HCl, EtOH, r.t. 20 min; (IX) $NaS_2O_3{:}5H_2O$, EtOH, 0–5 °C, 1 h; (X) *(S)*-epichlorhydrin, K_2CO_3, acetone, reflux, 8 h; (XI) *(R)*-epichlorhydrin, K_2CO_3, acetone, reflux, 8 h; (XII) *(R)*-epichlorhydrin, AcOH, 75 °C, 8 h; (XIII) *(S)*-epichlorhydrin, AcOH, 75 °C, 8 h; (XIV) KF, MeCN, reflux, 5 h

(MIC = 16 μg ml^{-1}). To ascertain the SAR of the lead compound more thoroughly, our initial efforts focused on the SAR of the right-hand side moiety as detailed in Table 1. Replacement of the right-hand side aromatic moiety with alkyl groups provided analogs **3** and **4**, which were inactive to MRSA versus NPS-2143 and warranted no

Table 1 Structure and activity against MRSA and MSSA of compounds **3–18** and **NPS-2143**



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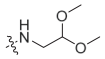
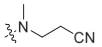
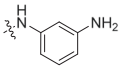
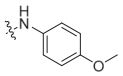
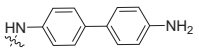
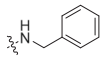
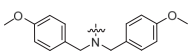
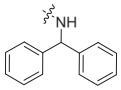
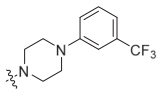
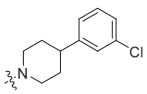
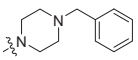
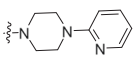
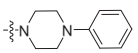
3		> 30	> 30
4		> 30	> 30
5		> 30	> 30
6		> 30	> 30
7		> 30	> 30
8		> 30	> 30
9		> 30	> 30
10		> 30	> 30
11		> 30	> 30
12		> 30	> 30
13		> 30	> 30
14		> 30	> 30
15		> 30	> 30
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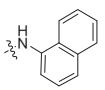
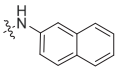
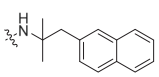
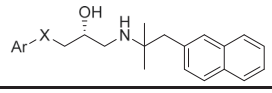
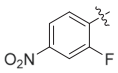
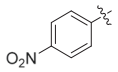
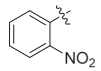
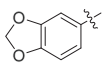
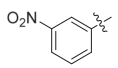
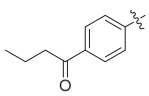
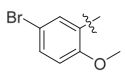
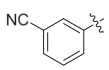
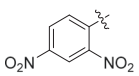
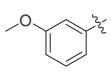
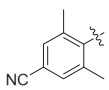
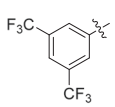
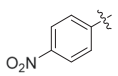
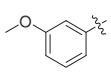
17		> 30	> 30
18		> 30	> 30
NPS-2143		16	16

Table 2 Structure and activity against MRSA and MSSA of compounds **19–30** and **38–40**



Cmpd	X	Ar	MIC (μg/mL)	
			ATCC 25923	ATCC 33591
19	O		16	16
20	O		16	16
21	O		32	32
22	O		64	64
23	O		16	16
24	O		16	16
25	O		32	32
26	O		32	32
27	O		32	32
28	O		32	32
29	O		16	16
30	O		8	8
38	S		32	32
39	S		16	16
40	S		16	16

further investigation. Thereby, we designed and synthesized a series of analogs **5–16** which contained a benzene ring or pyridine ring in hopes of identifying analogs with greater potency. Unfortunately, each of analogs **5–16** did not exhibit any activities against MRSA at all. Analogs **17** and **18** which contained a naphthalene ring without geminal dimethyl group were also inactive. These results strongly suggest that the geminal dimethyl moiety contained within this amino alcohol template plays a critical role in anti-MRSA activity.

The structure-activity studies to explore the role of the left-hand side part began with holding the right-hand side part beta-naphtyl amine group with the geminal dimethyl moiety as in lead compound NPS-2143 invariant, focusing on variations of substitution on the benzene ring as detailed in Table 2. The 5-hydroxy-1,3-benzodioxole (compound **22**, MIC = 64 μg ml^{-1}), 5-bromo-2-methoxyphenyl (compound **25**, MIC = 32 μg ml^{-}), 3-methoxyphenyl (compound **28**, MIC = 32 μg ml^{-1}) analogs exhibited a 2 or 4-fold decrease in activity versus the lead compound NPS-2143, which clearly demonstrates that electron donating groups on the left-hand side part is detrimental to increasing antibacterial activity. A series of analogs **19**, **20**, **21**, **23**, **24**, **26** and **27** which contained electron withdrawing groups only afforded an approximately equipotent anti-MRSA activity versus the lead compound NPS-2143 except the analog **30** (MIC = 8 μg ml^{-1}). Replacement of left-hand side moiety with phenyl-sulfhydryl (compounds **38–40**) did not give an improvement of anti-MRSA activity neither. However, analog **43** (MIC = 4 μg ml^{-1}) exhibited a 4-fold increase in anti-MRSA activity. As above, we obtained analog **48** which gave a gain in activity anti-methicillin-susceptible *Staphylococcus aureus* which is not resistant to methicillin (MSSA, MIC = 2 μg ml^{-1}).

Assessment of the role of the stereochemistry of the C-2 secondary hydroxyl group in influencing potency was performed utilizing analogs **30**, **43**, **49**, and the lead compound NPS-2143. As we can see from the Table 3, the C2 S enantiomers **31**, **44**, and **48** were 2-fold more active than the C2 R enantiomers NPS-2143, **30**, **43**, and **49**. The structure-activity studies focused on the central propanolamine linker were initiated around analogs **31**, **32**, and **48** (Table 3). Either methylation of the hydroxyl group or cyclization between the hydroxyl group and the secondary amine as in analogs **35** and **33** resulted in a significant loss in activity, suggesting that hydrogens on oxygen atom is critical for anti-MRSA activity. Methylation of the secondary amine as in analogs **34** and **50** did not provoke an obvious increase in activity.

After confirming that analogs **48** and **49** possessed strong antimicrobial activity against MRSA ATCC 33591 and MSSA ATCC 25923, we next assessed their activity against two other MSSA strains which are not resistant to methicillin, MRSA ATCC 43300 and *Escherichia coli* (*E. coli*) (Table 4). We found analogs **48** and **49** also have strong activity against other MRSA and MSSA strains. However, they do not have obvious antimicrobial activity against *E. coli*.

Conclusion

We present herein a novel series of NPS-2143 derivatives exhibiting potent activity against MRSA. A rigorous analysis of the SAR of these analogs reveals the geminal dimethyl groups contained within this amino alcohol moiety and unsubstituted hydroxyl group are critical for the compound's anti-MRSA activity. Furthermore, substitution with aniline contained electron withdrawing groups results in a compound that exhibits favorable anti-MRSA activity with an MIC value of 2 μg ml^{-1}. But the most potent compounds **48** and **49** did not possess obvious antimicrobial activity against Gram-negative bacteria *E. coli*. Consequently, it is the first time that naphthylamine compounds were reported to display such low MIC on MRSA strains, and the results present critical information necessary for further analysis and development of these compounds containing an amino alcohol linker and geminal dimethyl moiety as novel antimicrobial agents for treatment of infections caused by MRSA.

Experimental

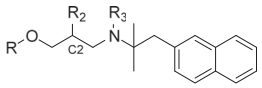
Chemistry

Chemical reagents and solvents used were purchased from commercial sources (mainly Sigma-Aldrich. Acros and Fisher Scientific).^{1}H-NMR and ^{13}C-NMR spectra were recorded in a DMSO-d_6 or $CDCl_3$ solution on a Bruker 400 spectrometer (400 and 100 MHz) using tetramethylsilane as internal standard. Monitoring the reactions and checking the purity of the final products were carried out by thin layer chromatography using visualization with ultraviolet light (UV) at 365 and 254 nm.

General procedure for the synthesis of compounds 3–18

To a solution of 2-chloro-6-hydroxybenzonitrile **1a** (1.315 g, 10.6 mmol) in DMF (8 ml) was added NaOH (625 mg, 14.5 mmol). After stirring for 30 min, a solution of *(R)*-glycidyl nosylate (2.5 g, 9.65 mmol) in DMF (8 ml) was added. The mixture was stirred at room temperature for 5 h (TLC-monitoring). Then the reaction was quenched by adding water (100 ml) and the resulting mixture was extracted with ethyl acetate (3 × 100 ml). The organic phase was washed with

Table 3 Structure and activity against MRSA and MSSA of compounds **NPS-2143**, **30**–**35**, **43**, **44**, and **48–50**



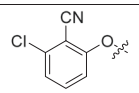
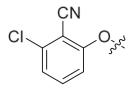
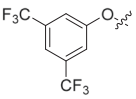
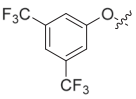
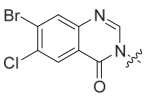
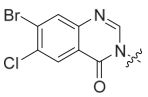
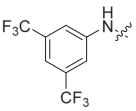
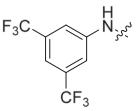
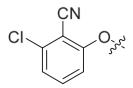
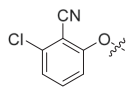
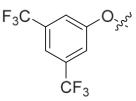
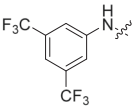
Cmpd	C2 chirality	R	R_2	R_3	MIC (μg/mL)	
					ATCC 25923	ATCC 33591
NPS-2143	R		OH	H	16	16
31	S		OH	H	8	8
30	R		OH	H	8	8
32	S		OH	H	4	4
43	R		OH	H	8	4
44	S		OH	H	4	4
48	S		OH	H	2	2
49	R		OH	H	2	4
33	S		see Figure 2A	see Figure 2A	64	64
34	S		OH	CH_3	8	8
35	S		OCH_3	H	64	64
50	S		OH	CH_3	4	4

Table 4 Minimum inhibitory concentration (MIC) of Linezolid, **48** and **49** against two MRSA, three MSSA and *Escherichia coli (E. coli)*

Compound	MRSA[a] ATCC 33591	MRSA[b] ATCC 43300	MSSA[c] ATCC 25923	MSSA[d] ATCC 29213	MSSA[e] ATCC 6538	*E.coli*[f] ATCC 25922
Linezolid	2	2	2	2	2	>64
48	2	2	2	2	2	>64
49	2	2	4	2	2	>64

[a–e]Colony of each *Staphylococcus aureus* strain is yellow, circular, entire and low convex on blood agar plates

[f]*Escherichia coli* (ATCC 25922)

brine (100 ml), dried over sodium sulfate, filtrated, and evaporated. Thereafter, column chromatography was performed over silica gel (petroleum ether/CH_2Cl_2 = 2:1), 1.3 g (68%) of *(R)*-2-chloro-6-(oxiran-2-yl)methoxybenzonitrile (**2a**) was obtained as a white solid.

A mixture of the appropriate amine (1.2 mmol) and compound **2a** (1.0 mmol) in 20 ml of ethanol was refluxed for 7 h. After cooling, the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compounds **3–18**.

General procedure for the synthesis of compounds 19–32 and 38–40

To a solution of the phenol **1c-i** (1.315 g, 10.6 mmol) in DMF (8 ml) was added NaOH (625 mg, 14.5 mmol). After stirring for 30 min, a solution of *(R)*-glycidyl nosylate (2.5 g, 9.65 mmol) in DMF (8 ml) was added. The mixture was stirred at room temperature for 5 h (TLC-monitoring). Then the reaction was quenched by adding water (100 ml) and the resulting mixture was extracted with ethyl acetate (3 × 100 ml). The organic phase was washed with brine (100 ml), dried over sodium sulfate, filtrated, evaporated, and purified by silica gel column chromatography (EtOAc/petroleum benzin = 1:8) to give the desired compounds **2c-i**.

To a solution of the phenol **1b, 1j-m** (1.315 g, 10.6 mmol) in DMF (8 ml) was added K_2CO_3 (625 mg, 14.5 mmol) and KI. *(S)*-epichlorhydrin (3.2 ml, 40.8 mmol) was added dropwise and reaction stirred at 60 °C for 6 h (TLC-monitoring). Then the reaction mixture was cooled and quenched by adding water (100 ml) and the resulting mixture was extracted with ethyl acetate (3 × 100 ml). The organic phase was washed with brine (100 ml), dried over sodium sulfate, filtrated, evaporated, and purified by silica gel column chromatography (EtOAc/petroleum benzin = 1:8) to furnish the desired compounds **2b** and **2j-m**. Enantiomers **2n** and **2o** were obtained in the same ways as **2a** and **2b**, replacing *(R)*-glycidyl nosylate and *(S)*-epichlorhydrin with *(S)*-glycidyl nosylate and *(R)*-epichlorhydrin.

A solution of the thiophenol **36a-c** (7.46 g, 41.6 mmol) and $Na_2S_2O_3$ (13.15 g, 83.2 mmol) in aqueous EtOH (5 ml, 1:1) was cooled to 0 °C. *(S)*-Epichlorhydrin (3.2 ml, 40.8 mmol) was added dropwise and reaction stirred at 0 °C for 1 h before warming to room temperature for 5 h (TLC-monitoring). The EtOH was removed in vacuo, and the residual aqueous phase was saturated with NaCl and extracted with EtOAc (3 × 10 ml). The combined extract was washed with brine (1 × 10 ml), dried over sodium sulfate, filtrated, evaporated, and purified by silica gel column chromatography (EtOAc/petroleum benzin = 1:8) to give the desired compounds **37a-c**.

A mixture of epoxides **2b-o**, **37a-c** (1.0 mmol), 2-methyl-1-(naphthalen-2-yl)propan-2-amine hydrochloride (1.2 mmol) and trimethylamine (1.2 mmol) in 20 ml of ethanol was refluxed for 7 h (TLC-monitoring). After cooling, the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compounds **19–32** and **38–40**.

General procedure for the synthesis of compounds 43 and 44

To a solution of 7-bromo-6-chloro-4-(3 *H*)quinazolinone **41** (2.0 mmol) in acetone (40 ml) was added K_2CO_3 (2.4 mmol) and *(S)*-epichlorohydrin or *(R)*-epichlorhydrin (2.3 mmol), respectively. After refluxing for 12 h, the resulting mixture was concentrated under vacuum and the product was extracted with AcOEt (3 × 100 ml). The AcOEt extract was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified on silica gel to afford compounds **42a** or **42b**.

A mixture of epoxide **42a** (0.47 mmol) or **42b** (0.47 mmol) and 2-methyl-1-(naphthalen-2-yl)propan-2-amine hydrochloride (0.47 mmol) in 20 ml of ethanol was refluxed for 7 h (TLC-monitoring). After cooling, the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compounds **43** or **44**.

General procedure for the synthesis of compounds 48 and 49

A mixture of 3,5-bis(trifluoromethyl)aniline (1.31 mmol) (**45**) and *(R)*-epichlorhydrin (6.55 mmol) or *(S)*-

epichlorhydrin (6.55 mmol) in acetic acid (10 ml) was heated to 75 °C. After 2 h, the reaction mixture was cooled to room temperature. The resulting mixture was concentrated under vacuum and the product was extracted with AcOEt (3 × 100 ml). The AcOEt extract was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude was purified on the silica gel to afford compound **46a** or **46b**. The solution of compound **46a** (0.83 mmol) or **46b** (0.83 mmol) and KF (2.49 mmol) in acetonitrile (10 ml) was refluxed for 3 h. Then the resulting mixture was filtered and the filtrate was concentrated under vacuum, the product was purified on the silica gel to afford epoxides **47a** or **47b**.

A mixture of epoxide **47a** (0.29 mmol) or **47b** (0.29 mmol), 2-methyl-1-(naphthalen-2-yl)propan-2-amine hydrochloride (0.43 mmol), and triethylamine (0.43 mmol) in 15 ml of ethanol was refluxed for 7 h (TLC-monitoring). After cooling, the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compounds **48** or **49**.

General procedure for the synthesis of compounds 33–35, 50

A mixture of **31** (0.073 mmol), 1,1′-carbonyldiimidazole (CDI) (0.183 mmol) and trimethylamine (0.073 mmol) in 10 ml of dichloromethane was stirred at room temperature for 5 h (TLC-monitoring). Then the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compound **33**.

A mixture of **31** (0.145 mmol) or compound **48** (0.145 mmol), iodomethane (0.290 mmol), and NaOH in 10 ml of methanol was stirred at room temperature for 3 h (TLC-monitoring). Then the solvent was removed in vacuo. The crude product was purified on a silica gel column to give pure compounds **34** and **50**.

To a solution of compound **32** (0.30 mmol) and NaOH (0.90 mmol) in H_2O (4 ml) and 1,4-dioxane (4 ml) was added di-*tert*-butyl dicarbonate (Boc_2O) (0.90 mmol). The mixture was stirred at room temperature for 1 h (TLC-monitoring). Then the resulting mixture was extracted with AcOEt (3 × 10 ml). The AcOEt extract was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude was purified on the silica gel to afford *N*-Boc intermediate. The intermediate was treated with NaH (2.40 mmol) and iodomethane (3.40 mmol) in DMF (3 ml), after stirring for 3 h (TLC-monitoring) at room temperature. Then the resulting mixture was extracted with AcOEt (3 × 10 ml). The AcOEt extract was dried with Na_2SO_4, filtered and evaporated in vacuo. The crude was purified on the silica gel to afford another *N*-protected methoxy intermediate. Then this intermediate was treated with hydrochloric acid in ethanol (10 ml). After stirring for 1 h at room temperature, the resulting mixture was concentrated under vacuum and the product was extracted with AcOEt (3 × 10 ml). The AcOEt extract was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified on the silica gel to afford compound **35**.

Microbiology

In vitro anti-MRSA activity

Bacteria strains MRSA ATCC 33591, MRSA ATCC 43300, MSSA ATCC 25923, MSSA ATCC 6538, MSSA ATCC 25913, and *E. coli* ATCC 25922 were purchased from the American Type Culture Collection. All synthesized compounds were tested for their in vitro antibacterial activity against MRSA, MSSA, and *E. coli* by performing a microdilution (MIC). Methicillin-resistant *Staphylococcus aureus* (MRSA and MSSA) was cultured in Mueller–Hinton broth (MH, Oxoid, Basingstoke, England) at 37 °C under aerobic conditions. MICs of NPS-2143 analogs used against MRSA were determined in triplicate using flat-bottom 96-well microtiter plates (TPP, Trasadingen, Switzerland).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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