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Synthesis and structure–activity relationship of dicationic diaryl ethers as novel potent anti-MRSA and anti-VRE agents

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ABSTRACT

A series of dicationic diaryl ethers have been synthesized and evaluated for in vitro antibacterial activities, including drug resistant bacterial strains. Most of these compounds have shown potent antibacterial activities. Several compounds, such as piperidinyl and thiomorpholinyl compounds **9e** and **9l**, improved the antimicrobial selectivity and kept potent anti-MRSA and anti-VRE activity. The most potent bis-indole diphenyl ether **19** exhibited anti-MRSA MIC value of ⩽0.06 μg/mL and enhanced antimicrobial selectivity.
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Interest in novel antimicrobial agents has been stimulated by the emergence of multi-drug resistant Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and vancomycin-resistant *Enterococcus faecium* (VRE).[1] Severe nosocomial and community-acquired infections caused by these pathogens has become a significant challenge in the clinic.[2]

During the course of our efforts to develop novel antimicrobial agents, we have discovered a new class of dicationic bis-benzimidazole derivatives which displayed potent anti-MRSA and anti-VRE activities.[3] The lead compound **1** (Fig. 1) has shown significant antibacterial activity including MRSA and VRE (MIC ⩽ 0.5 μg/mL). Optimization of the central linker of lead compound **1** resulted in the discovery of 4,4′-bis-[2-(5-*N*-isopropylamidino)benzimidazolyl] diphenyl ether **2**, which displayed more potent anti-MRSA and anti-VRE activity than lead compound **1**.[4] Interestingly, the mono-amidino benzimidazolyl diphenyl ether derivative **3** has been previously reported as an inhibitor of bacterial two-component system (TCS), which showed anti-MRSA and anti-VRE activity (MIC = 16 μg/mL).[5] Another diamidino benzimidazolyl diphenyl ether derivative **4**, a DNA minor groove binder, displayed inhibitory activity of arginine-specific esteroproteases and antifungal activity.[6] A 6-amidino indole analogue **5** of the diamidino benzimidazole compound **4** exhibited antimicrobial activity and human mitogen-activated protein kinase phosphatase-3 (MKP-3) inhibitory activity.[7,8] In order to investigate the structure–activity relationship (SAR) of this series of novel dicationic bis-benzimidazolyl diphenyl ether compounds, we designed and syn-

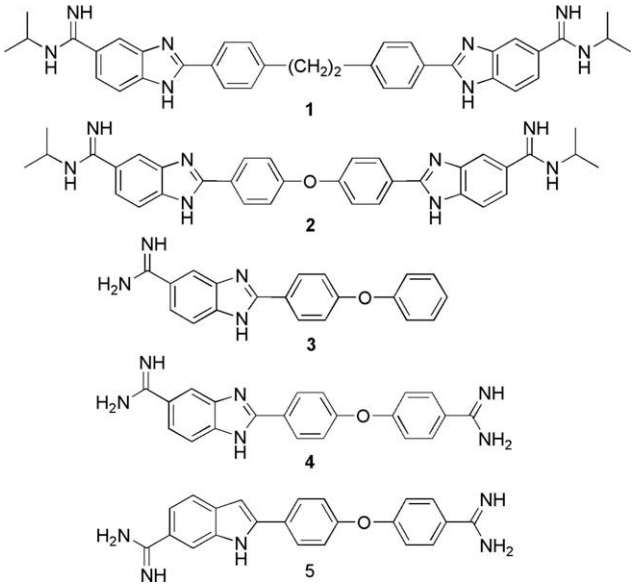

Figure 1. Benzimidazole and indole amidine compounds.

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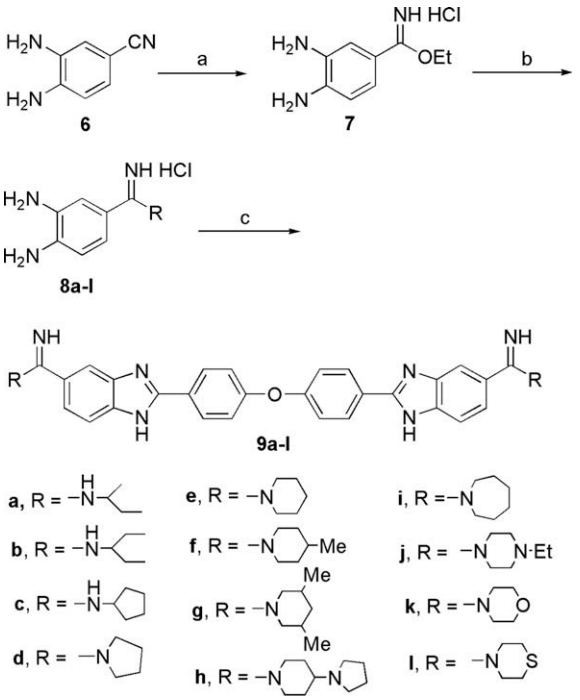
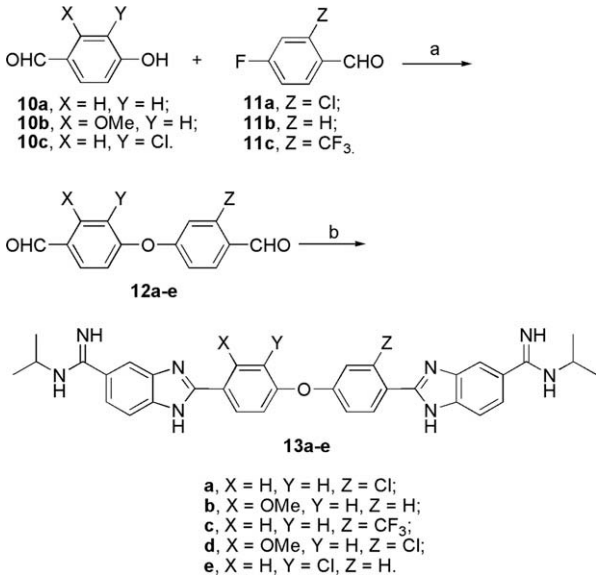
thesized analogues of the new lead compound **2**. In this article, we report the synthesis, in vitro antibacterial activity and SAR of these compounds as novel potent anti-MRSA and anti-VRE agents.

We have reported the synthesis of lead compound **2** by the condensation of 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride with commercial available 4-(4-formylphenoxy)benzaldehyde in presence of benzoquinone as oxidative reagent.[4] The analogs **9a–l** of the lead compound **2** was prepared by following this procedure as shown in Scheme 1. The cyano group of starting material 3,4-diaminobenzonitrile **6** was converted into the imidate ester **7** by using the Pinner method.[9] Then, the imidate ester was used directly to react with suitable commercially available amines to yield the 4-(N-substituted amidino)-1,2-phenylenediamines **8a–l**. Condensation of these derivatives with 4-(4-formylphenoxy)benzaldehyde in presence of benzoquinone as oxidative reagent afforded the corresponding dicationic bis-benzimidazolyl diphenyl ethers **9a–l**. Various derivatives **13a–e** substituted on the phenyl ring of the lead compound **2** were prepared from bis-benzaldehydes **12a–e** by condensation with 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride using the same approach as described above (Scheme 2). Nucleophilic aromatic substitution reactions of 4-hydroxybenzaldehydes **10a–c** with 4-fluorobenzaldehydes **11a–c** generated bis-benzaldehydes 12a–e in reasonable yields.[10] The preparation of pyridinyl bis-benzimidazole amidines **15a–b** was achieved by using the previously described procedure in two steps from 4-hydroxybenzaldehyde **10a**, as shown in Scheme 3. 4,4′-Bis-[2-(6-cyanoindolyl)] diphenyl ether **18** was prepared from 4-methyl-3-nitrobenzonitrile **16** by condensation with 4-(4-formylphenoxy)benzaldehyde and followed by heating with neat triethylphosphite (Scheme 4).[7,8] The dicyano compound **18** was converted to the desirable 6-(*N*-isopropylamidino)indole compound **19** by using the Pinner method as previously reported.[7,8]

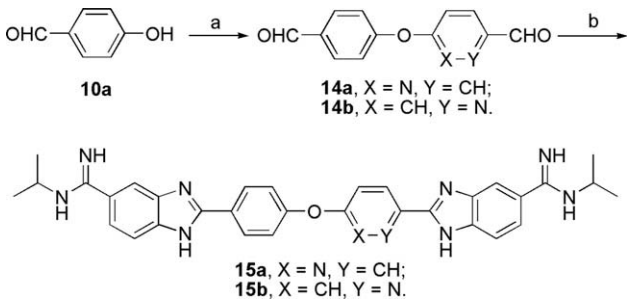
All the dicationic diaryl ether hydrochloride compounds prepared herein were screened for their potential antibacterial activities in vitro against ten selected Gram-positive bacterial strains



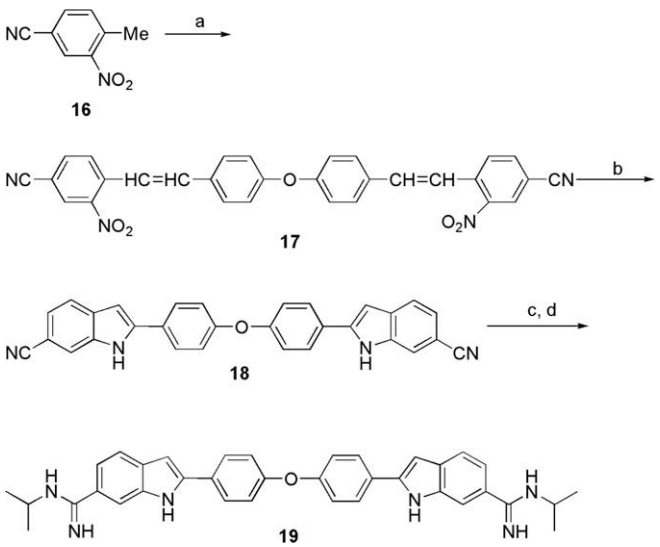
Scheme 1. Reagents and conditions: (a) HCl (gas), EtOH; (b) R–NH_2, EtOH, reflux, 45–90% in two steps; (c) 4-(4-formylphenoxy)benzaldehyde, 1,4-benzoquinone, EtOH, reflux, 36–85%.



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMAC, 150 °C, 60–76%; (b) 4-(*N*-isopropylamidino)-1,2-phenylenediamine, 1,4-benzoquinonE, EtOH. reflux, 45–78%.



Scheme 3. Reagents and conditions: (a) 2-fluoro-5-formylpyridine or 5-fluoro-2-formylpyridine, K_2CO_2, DMAC, 150 °C, 73% or 68%; (b) 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 58% or 67%.



Scheme 4. Reagents and conditions: (a) 4-(4-formylphenoxy)benzaldehyde, piperidine, 100 °c, 50%; (b) $P(OEt)_3$, 160 °C, 39%; (c) HCl (gas), EtOH; (d) I–$PrNH_2$, EtOH, reflux, 70% in two steps.

and two anaerobic bacterial strains, including MRSA, multi-drug resistant *S. aureus* (MDRSA), MRSE and VRE strains.[3] We also screened their antifungal activity against *Candida albicans* in order to evaluate the specificity of their antimicrobial activity. Penicillin G (Pen G), ciprofloxacin (CPLX) and vancomycin (VCM)) were used as reference standards. The minimum inhibitory concentration (MIC) results for the test compounds are shown in Table 1. Of the twenty compounds tested against the Gram-positive aerobic strains, almost all of these compounds showed potent antibacterial activities, including MRSA and VRE strains (MIC value $\leqslant 1$ μg/mL); only one compound **9h** was found to have only moderate antibacterial activity. Nine compounds **9b**, **9d–g**, **9i**, **9l**, **13a** and **19** also showed good activity against two anaerobic bacterial strains (MIC value $\leqslant 4$ μg/mL).

The SAR study of the lead compound **1** has shown that bis-5-*N*-substituted amidine of 1*H*-benzimidazole is very important for achieving potent antibacterial activity.[3] The parent diamidine compound of the lead compound **1** exhibited almost no antibacterial activity. Antibacterial activity of the *sec*-butyl and *c*-pentyl compounds were comparable to that of the lead compound **1**, however, the *c*-pentyl compound gave decreased anti-anaerobic bacterial activity. *N*-butyl and *c*-propyl substituents led to some loss of antibacterial potency. The pyrrolidinyl derivative showed similar anti-*S. aureus* and anti-*S. epidermidis* activities but was less active against the other bacterial strains compared to that of lead compound **1**. Further SAR study of this lead compound **1** by replacement of the central ethylene linker with various two-atom or one-atom groups has shown that the antibacterial activity of the dicationic bis-benzimidazole compounds is related to the relative electronegativity of the central linkers, however, the central linkers of one-atom and two-atoms showed opposite effects on activity. This result may be related to the difference in geometry of the two series since the single-atom linker produces a much more bent molecule than the two-atom linker.[4] Based on the above SAR information, we further designed and prepared the *N*-substituted analogs **9a–l** of lead compound **2** for probing the SAR of this series of novel dicationic bis-benzimidazolyl diphenyl ether compounds. *sec*-Butyl, *sec*-pentyl and *c*-pentyl compounds **9a–c** showed similar effects on antibacterial activity to that

Table 1
In vitro antibacterial activity of benzimidazole amidine compounds[a]

Strain/compound	MICs (μg/mL)								
	2	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**
S. aureus ATCC 29213	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.5	8
S. aureus BAA-39[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	4
S. aureus ATCC 33591[c]	0.12	0.25	0.25	0.5	0.25	0.12	0.25	0.25	4
S. epidermidis ATCC 12228	<0.06	0.12	0.12	0.12	0.12	<0.06	0.12	0.25	1
S. epidermidis ATCC 51625[d]	<0.06	0.12	0.25	0.12	0.12	0.12	0.12	0.5	1
S. pneumoniae ATCC 6301	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	0.12	0.5	>32
E. faecalis ATCC 51575[e]	0.12	0.25	0.5	0.25	0.25	0.25	0.5	1	8
E. faecium ATCC 700221[e]	<0.06	<0.06	<0.06	<0.06	<0.06	0.12	0.25	0.5	2
B. subtils ATCC 23857	<0.06	<0.06	0.12	<0.06	<0.06	<0.06	0.12	0.25	4
B. cereus ATCC 11778	<0.06	<0.06	0.5	<0.06	0.12	0.25	<0.06	1	1
B. fragilis ATCC 23745	2	16	4	32	2	2	4	4	>32
C. perfringens ATCC 10388	0.5	0.5	0.5	0.25	0.5	0.25	1	2	>32
C. albicans ATCC 90028	1–2	2	4	2	4	8	4	8	16

	9i	**9j**	**9k**	**9l**	**13a**	**13b**	**13c**	**13d**	**13e**
S. aureus ATCC 29213	0.25	1	1	0.5	0.25	0.5	0.5	0.25	0.25
S. aureus BAA-39[b]	0.25	1	0.5	0.5	0.5	0.5	0.5	0.5	0.25
S. aureus ATCC 33591[c]	0.25	1	0.5	0.25	0.12	0.25	0.25	0.25	0.25
S. epidermidis ATCC 12228	0.12	0.5	0.25	0.12	⩽0.06	⩽0.06	⩽0.06	⩽0.06	⩽0.06
S. epidermidis ATCC 51625[d]	0.25	0.5	0.5	0.25	⩽0.06	0.12	0.12	⩽0.06	⩽0.06
S. pneumoniae ATCC 6301	0.12	0.5	0.5	0.25	⩽0.06	⩽0.06	⩽0.06	⩽0.06	⩽0.06
E. faecalis ATCC 51575[e]	0.5	1	0.5	0.5	0.12	0.5	0.12	0.25	0.25
E. faecium ATCC 700221[e]	0.5	1	0.5	0.5	⩽0.06	⩽0.06	⩽0.06	⩽0.06	⩽0.06
B. subtils ATCC 23857	0.25	0.5	0.5	0.25	⩽0.06	⩽0.06	⩽0.06	⩽0.06	0.12
B. cereus ATCC 11778	0.5	0.5	0.12	0.5	0.12	0.12	0.12	0.12	0.12
B. fragilis ATCC 23745	4	16	8	4	4	>32	8	>32	16
C. perfringens ATCC 10388	2	16	2	1	0.25	0.25	0.25	0.25	0.5
C. albicans ATCC 90028	8	32	16	16	2	2	4	4	1

	15a	**15b**	**19**	Pen-G	CPLX	VCM
S. aureus ATCC 29213	1	0.5	0.12	1	0.5	0.5
S. aureus BAA-39[b]	1	0.5	⩽0.06	>32	8	1
S. aureus ATCC 33591[c]	0.5	0.5	⩽0.06	>32	⩽0.12	1
S. epidermidis ATCC 12228	0.12	0.12	⩽0.06	32	⩽0.12	1
S. epidermidis ATCC 51625[d]	0.12	0.12	⩽0.06	32	⩽0.12	1
S. pneumoniae ATCC 6301	0.12	⩽0.06	⩽0.06	<0.06	0.5	1
E. faecalis ATCC 51575[e]	0.25	0.12	0.25	4	0.5	>64
E. faecium ATCC 700221[e]	⩽0.06	⩽0.06	⩽0.06	>32	>64	>64
B. subtils ATCC 23857	0.25	⩽0.06	⩽0.06	<0.06	⩽0.12	0.12–0.5
B. cereus ATCC 11778	0.12	0.12	⩽0.06	4–>32	⩽0.12	1–⩽0.12
B. fragilis ATCC 23745	>32	32	0.125	4–8	0.5	4–8
C. perfringens ATCC 10388	2	0.5	0.5	⩽0.06–0.12	0.25	0.12–0.25
C. albicans ATCC 90028	2	4	0.5	>32	—	>32

[a] NCCLS guidelines M11-A6 and M7-A6 followed.
[b] MDRSA.
[c] MRSA.
[d] MRSE.
[e] VRE.

of the lead compound **1**. However, the pyrrolidinyl substituent **9d** displayed only slightly less antibacterial potency compared to lead compound **1**, which is clearly different from the result in the ethylene series. It is also noteworthy that the compound **9d** decreased the antifungal activity by 2–4-folds. The differences in antimicrobial activity are likely due to the difference in shapes of these two series of compounds. Interestingly, the *N*-piperidinyl group in **9e** further enhanced the selectivity of antimicrobial activity and kept the potent anti-MRSA and anti-VRE activities. 4-Methylpiperidnyl and the 3,5-dimethylpiperidinyl derivatives **9f–g** showed similar effects on the activity in comparison to **9d**. However, the 4-pyrrolidinylpiperidinyl group led to some loss of potency. This result may suggest that the more bulky group is not beneficial to the antibacterial activity. *N*-Hexamethyleneiminyl, ethylpiperazinyl, morpholinyl, and thiomorpholinyl substituents **9i–l** were slightly less potent than lead compound **2**. In particular, the thiomorpholinyl compound **9l** exhibited the best selectivity by a factor of 32 for anti-MRSA and anti-VRE activity (MIC $\leqslant$ 0.5 μg/mL) to antifungal activity (MIC = 16 μg/mL).

The effect of the substituents on the central phenyl ring of the lead compound **2** was examined. Both electron-donating groups, such as methoxy or chloro group, and strong electron-withdrawing group—trifluoromethyl group located at various positions on the phenyl ring (**13a–e**) showed no apparent effect on the activity against Gram-positive bacterial activity including MRSA and VRE, however, decreased anti-anaerobic bacterial activity was noted. Replacement of one of the 2-phenyl rings of **2** by 2-pyridin-5-yl or 5-pyridin-2-yl ring yielded the compounds **15a–b**. Both pyridinyl compounds **15a–b** showed a slight loss of activity against Gram-positive bacterial strains and decreased potency against anaerobic bacterial strains compared to **2**. This result is in contrast to that for the pyridinyl compounds of the lead compound **1** which showed only moderate antibacterial activity.[3] The SAR results for these two series of dicationic bis-benzimidazole compounds containing ethylene and oxygen central linkers suggested that the central linker leads to different effects on the antibacterial activity.

Like imidazoles, benzimidazoles exhibit fast prototropic tautomerism,[11] which leads to an equilibrium mixture of symmetrical tautomers **2a** and **2b** (Figure ure2). Since the 6-amidino indole analogue **5** has been previously reported as an antimicrobial agent,[7] we replaced of the bis-benzimidazole rings with bis-indole rings to yield 4,4′-bis-[2-(6-*N*-isopropylamidino)] diphenyl ether **19**, which is an isostere of one tautomer of **2**, in order to investigate

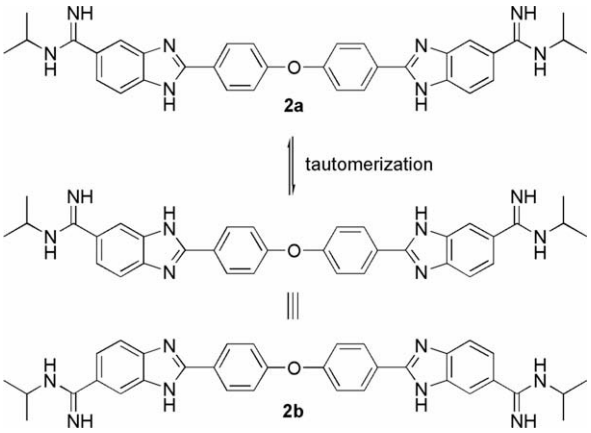


Figure 2. The lead compound **2** tautomers.

the effect of the bis-benzimidazole rings of the lead compound **2** on antibacterial activity. Very interestingly, the bis-indole compound **19** showed the most potent activity (MIC $\leqslant$ 0.5 μg/mL) compared to that of lead compound **2** and the other analogues. In particular, the anti-MDRSA activity of the compound **19**, MIC $\leqslant$ 0.06 μg/mL, was more active than that of the lead compound **2** by 8 times and VCM by 16 times. The compound **19** was also more potent than the lead compound **2** against the anaerobic bacterial strain *Bacillus subtilis* and the fungal strain *Candida albicans*. On the other hand, it is noteworthy that compound **19** exhibited an antimicrobial selectivity factor of 8 for anti-MRSA activity to antifungal activity, which is much better than that of lead compound **2** by a factor of 2. This result suggests that replacement of the bis-benzimidazole rings of the lead compound **2** can lead to improved antibacterial activity and enhanced antimicrobial selectivity.

In conclusion, we have synthesized and evaluated the antibacterial activities of the analogues of lead compound **2** for probing the SAR of this system. Most of the compounds show significant antibacterial activities against Gram-positive bacteria, including drug resistant bacterial strains. Several compounds, such as piperidinyl and thiomorpholinyl compounds **9e**, **9l**, show improved antimicrobial selectivity and at the same time keep potent anti-MRSA and anti-VRE activity. The SAR study of two series of dicationic bis-benzimidazole compounds (**1** and **2**) containing ethylene and oxygen central linker have shown that the central linker causes different effect on the antibacterial activity. Replacement of the benzimidazole ring of the lead compound **2** with an indole ring resulted in improvement of antibacterial activity and enhanced antimicrobial selectivity. This series of dicationic diaryl ethers merits further investigation as novel potent anti-MRSA and anti-VRE agents.

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