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Synthesis and in vitro activity of dicationic bis-benzimidazoles as a new class of anti-MRSA and anti-VRE agents

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ABSTRACT

A new class of novel bis-benzimidazole diamidine compounds have been synthesized and evaluated for in vitro antibacterial activities, including drug-resistant bacterial strains. Anti-MRSA and anti-VRE activities of the most potent compound **1** were more active than Vancomycin. The mechanism of action for this class of compounds appears to be different from existing antibiotics. Bis-benzimidazole diamidine compounds have potential for further investigation as a new class of potent anti-MRSA and anti-VRE agents.

Since methicillin-resistant *Staphylococcus aureus* (MRSA) was firstly identified in 1961,[1] it has been the leading cause of morbidity and mortality in various nosocomial and community-acquired infections.[2] With the increasing use of Vancomycin, as the last line of defense against MRSA in recent decades, vancomycin-resistant *Enterococccus faecium* (VRE) has emerged and can lead to bacteremia and death.[3] Therefore, a tremendous need exists to discover novel anti-MRSA and anti-VRE agents for overcoming these emerging bacterial resistance problems.

Benzimidazole derivatives have received much interest in drug discovery, since they display diverse biological activities, such as antihelminth, antiulcer, antiviral, anticancer, and antihistaminic activity.[4–6] Some benzimidazole-containing compounds, including 2-phenyl-1*H*-benzimidazole monoamidine derivatives, have shown interesting anti-MRSA activity.[7,8] We have previously reported several series of benzimidazole diamidine compounds, which have displayed diverse biological activities against parasites, fungi and Bovine Viral Diarrhea virus (BVDV).[9] Screening our benzimidazole dicationic compounds led to the discovery that compound **1** (Fig. 1), DB325, is a potent anti-MRSA agent. Thus, we designed and synthesized analogues of lead compound **1** to investigate the structure–activity relationship (SAR) of this series of bis-benzimidazole amidine compounds. In this letter, we report the synthesis, in vitro antibacterial activity and initial SAR of these compounds as novel potent anti-MRSA and anti-VRE agents.

We have reported the synthesis of lead compound **1** by the condensation of 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride with 1,2-bis-(4-formylphenyl)ethane in presence of benzoquinone as oxidative reagent.[9a] The analogs **5a–g** of lead compound **1** were prepared by following this procedure as shown in Scheme 1. The cyano group of starting material 3,4-diaminobenzonitrile **2** was converted into the imidate ester **3** by using the Pinner method.[10] Then, the imidate ester was used directly to react with suitable commercially available amines to yield the 4-(*N*-substituted amidino)-1,2-phenylenediamine **4a–g**. Condensation of these derivatives with 1,2-bis-(4-formylphenyl)ethane in the presence of benzoquinone as oxidative reagent afforded the corresponding bis-benzimidazole amidine hydrochloride salts **5a–g**. 1,2-Bis-(4-formylphenyl)ethane was prepared in two steps from 2,3-bis-(4-bromophenyl)propionic acid by the action of copper(I) cyanide in DMF, and reduction with diisobutylaluminum hydride (DIBAL-H), followed by as reported in the preparation of lead compound **1**.[9a]

Thienyl bis-benzimidazole amidines **12a and 12b** were obtained using a similar procedure to that of lead compound **1** in six steps, as shown in Scheme 2. 4-Bromophenylpropionic acids

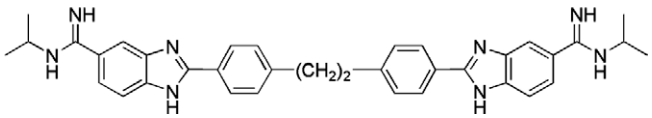

Figure 1. Bis-benzimidazole diamidine lead compound **1** (DB325).

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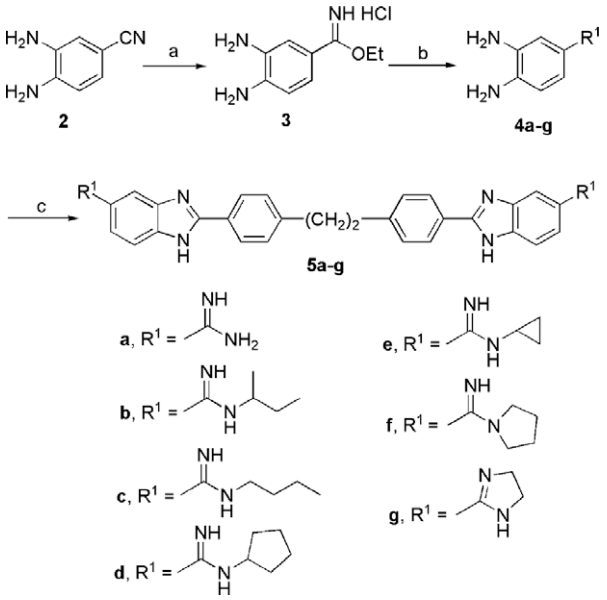
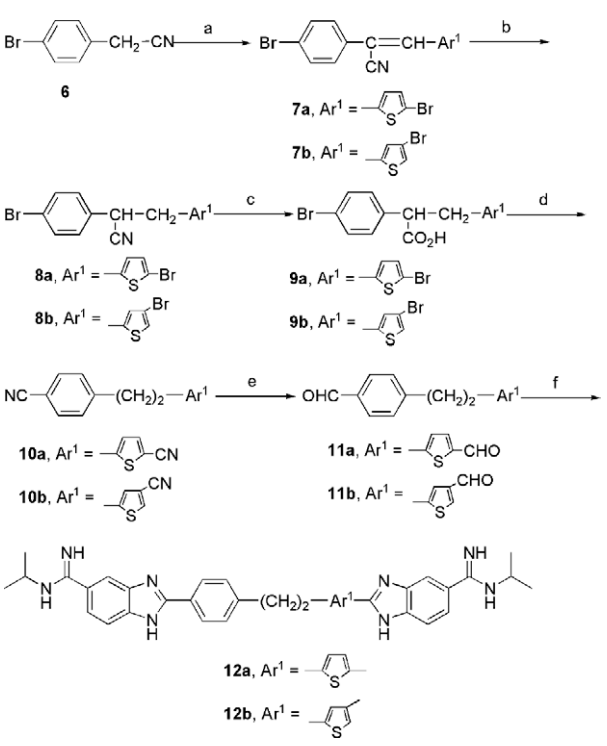


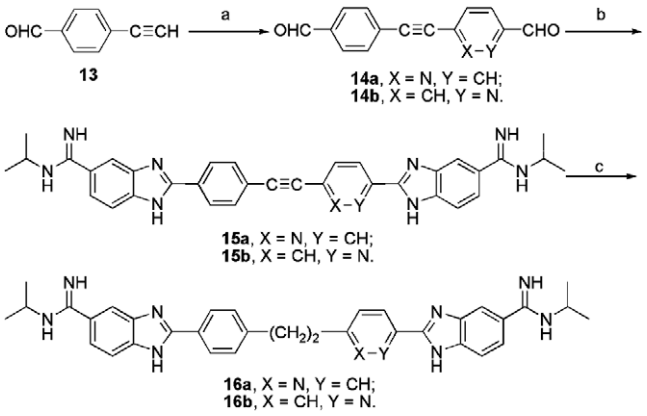
Scheme 1. Reagents and conditions: (a) HCl (gas), EtOH; (b) corresponding amines, EtOH, reflux, 62–90% in two steps; (c) 1,2-bis-(4-formylphenyl)ethane, 1,4-benzoquinone, EtOH, reflux, 53–86%.



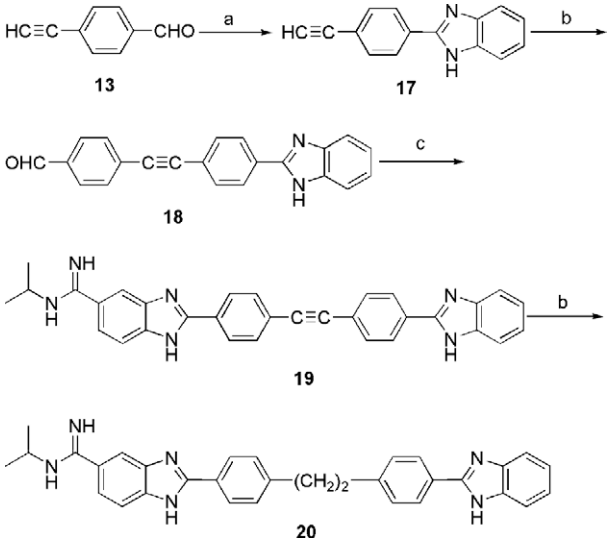
Scheme 2. Reagents and conditions: (a) 5 or 4-bromothiophene-2-aldehyde, 5 N NaOH, MeOH, 63% or 70%; (b) $NaBH_4$, Pyridine, MeOH, 85% or 82%; (c) 20% NaOH, EtOH, reflux, 79% or 85%; (d) CuCN, *N*-methyl-2-pyrrolidone,73% or 68%; (e) DIBAL, CH_2Cl_2, 65% or 62%; (t) 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 58% or 67%.

9a and 9b were prepared from 4-bromophenylacetonitrile **6** by condensation with thiophene-2-aldehydes **7a and 7b** and reduction with sodium borohydride in two steps.

Pyridinyl bis-benzimidazole amidines **16a and 16b**, bis-benzimidazole monoamidine **20** and mono-benzimidazole diamidine **25** were prepared in an alternative approach employing Sonogoshira coupling, shown in Scheme 3–5.[11] 4-Ethynylbenzaldehyde **13** was coupled with bromopyridinyl aldehyde to obtain the dialdehyde intermediates **14a** and **14b** using Sonogoshira conditions.[12] Then, the dialdehyde intermediates were condensed with 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride followed by benzoquinone oxidation to yield the corresponding benzimidazoles **15a** and 1**5b**. The final compounds **16a and 16b** were obtained by hydrogenation of the acetylene group using the Pearlman's catalyst in DMF.[13] The synthesis of bis-benzimidazole monoamidine **20** (Scheme 4) was carried out by a similar procedure for that of **16a and 16b**. 4-Ethynylbenzaldehyde **13** was condensed with 1,2-diaminobenzene to prepare 2-(4-ethynylphenyl)-1*H*-benzimidazole **17**. The target compound **20** was prepared by Sonogoshira coupling, condensation and hydrogenation in three steps similar to the prodedure of **11a and 11b**. The preparation of mono-benzimidazole diamidine compound **25** (Scheme 5) was achieved by using a similar procedure in five steps from ethynylbenzonitrile **21** as starting material.



Scheme 3. Reagents and conditions: (a) 6-bromo-3-pyridinecarboxaldehyde or 5-bromopyridine-2-carboxaldehyde, CuI, $PdCl_2(PPh_3)_2$, $(i\text{-}Pr)_2NEt$, THF, 50 °C, 79% or 56%; (b) 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 59% or 65%; (c) H_2, $Pd(OH)_2$, EtOH, 67% or 60%.

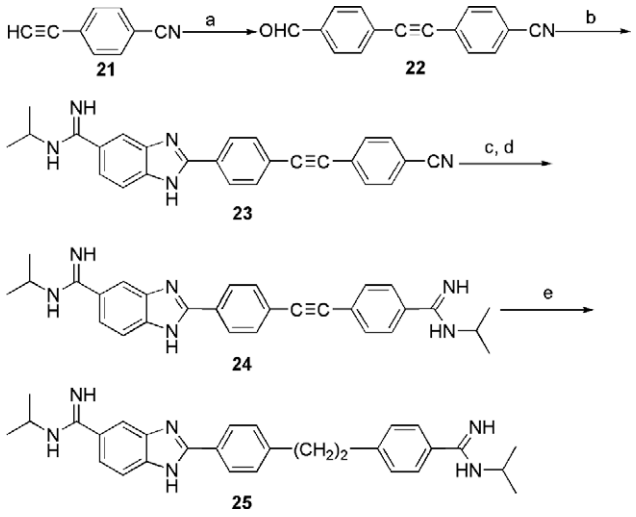


Scheme 4. Reagents and conditions: (a) 1,2-diaminobenzene, 1,4-benzoquinone, EtOH, refulx, 30%; (b) 4-iodobenzaldehyde, CuI, $PdCl_2(PPh_3)_2$, NEt_3, DMF, 50 °C, 91%; (c) 4-(N-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 98%; (d) H_2, $Pd(OH)_2$, EtOH, 68%.

Pyridinyl bis-benzimidazole amidines **16a and 16b**, bis-benzimidazole monoamidine **20** and mono-benzimidazole diamidine **25** were prepared in an alternative approach employing Sonogoshira coupling, shown in Scheme 3–5.[11] 4-Ethynylbenzaldehyde **13** was coupled with bromopyridinyl aldehyde to obtain the dialdehyde intermediates **14a** and **14b** using Sonogoshira conditions.[12] Then, the dialdehyde intermediates were condensed with 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride followed by benzoquinone oxidation to yield the corresponding benzimidazoles **15a** and 1**5b**. The final compounds **16a and 16b** were obtained by hydrogenation of the acetylene group using the Pearlman's catalyst in DMF.[13] The synthesis of bis-benzimidazole monoamidine **20** (Scheme 4) was carried out by a similar procedure for that of **16a and 16b**. 4-Ethynylbenzaldehyde **13** was condensed with 1,2-diaminobenzene to prepare 2-(4-ethynylphenyl)-1*H*-benzimidazole **17**. The target compound **20** was prepared by Sonogoshira coupling, condensation and hydrogenation in three steps similar to the prodedure of **11a and 11b**. The preparation of mono-benzimidazole diamidine compound **25** (Scheme 5) was achieved by using a similar procedure in five steps from ethynylbenzonitrile **21** as starting material.

All the benzimidazole amidine hydrochloride compounds prepared herein were screened for their potential antibacterial activi-

ties in vitro against 10 selected Gram-positive bacterial strains and two anaerobic bacterial strains, including MRSA, multi-drug-resistant *S. aureus* (MDRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and VRE strains according to the NCCLS guidelines. 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 14 compounds tested against the Gram-positive aerobic strains, five compounds **1**, **5b**, **5d**, **12a, and 12b** showed good antibacterial activities, including potent anti-MRSA (MIC value $\leqslant$1 μg/mL) and anti-VRE activity (MIC value $\leqslant$2 μg/mL); six compounds **5c**, **5e**, **5f**, **16a, 16b, and 25** were found to have moderate activity; and three compounds **5a**, **5 g**, and **20** showed poor or no antibacterial activity. Four compounds **1**, **5b**, **12a, and 12b** also showed good activity against two anaerobic bacterial strains (MIC value $\leqslant$4 μg/mL).



Scheme 5. Reagents and conditions: (a) 4-iodobenzaldehyde, CuI, $PdCl_2(PPh_3)_2$, NEt_3, DMF, 50 °C, 78%; (b) 4-(N-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, refulx, 92%; (c) HCl (gas), EtOH; (d) *i*-$PrNH_2$, EtOH, reflux, 46% in two steps; (e) H_2, $Pd(OH)_2$, EtOH, 54%.

The lead compound **1** exhibited potent activity against all selected Gram-positive bacterial strains and anaerobic bacterial strains, including MRSA, MRSE and VRE (MIC value <1 μg/mL). Anti-MRSA activity of the lead compound **1** was more potent than that of VCM by two times and anti-VRE activity was more active by 512 times than that of VCM. It is noteworthy that anti-MRSA or anti-MDRSA activity of the lead compound **1** was equivalent to that of anti-MSSA activity. These results suggested that the mechanism of action of the lead compound **1** could be different from existing antibiotics.

To investigate the effect of *N*-substituent of 5-amidine of 1*H*-benzimidazole on antibacterial activity, we designed and prepared the *N*-substituted analogs **5a–g** of the lead compound **1**. It is noteworthy that the parent diamidine compound **5a** exhibited almost no antibacterial activity. Antibacterial activity of the *sec*-butyl and *c*-pentyl compounds **5b** and **5d** were comparable to that of lead compound, however, compound **5d** decreased anti-anaerobic bacterial activity. *N*-butyl and *c*-propyl substituents **5c** and **5e** led to some loss of antibacterial potency. Replacement of the isopropylamino group of the amidine with a pyrrolidinyl group yielded the compound **5f** which showed similar anti-*S. aureus* and anti-*S. epidermidis* activities and but was less active against the other bacterial strains compared to that of lead compound **1**. However, replacement of 5-*N*-isopropylamidine substituent of benzimidazole by imidazoline led to total loss of antibacterial

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

Strain/compound	MICs (μg/mL)								
	1	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**12a**
S. aureus ATCC 29213	0.25–0.5	>32	1	4	1	8	0.5	>64	1
S. aureus BAA-39 [b]	0.5	>32	0.5	4	1	16	0.5	>64	0.5
S. aureus ATCC 33591[c]	0.25–0.5	>32	0.25	2	0.5	8	0.25	>64	0.5
S. epidermidis ATCC 12228	<0.06	4	<0.06	0.25	<0.06	0.12	0.25	>64	0.12
S. epidermidis ATCC 51625 [d]	0.125	32	0.12	0.5	0.12	0.25	0.25	>64	0.12
S. pneumoniae ATCC 6301	<0.06	>32	0.12	1	0.25	<0.06	>32	>64	⩽0.06
E. faecalis ATCC 51575 [e]	0.25–0.5	>32	2	32	2	32	16	>64	0.5
E. faecium ATCC 700221 [e]	0.12	32	0.5	2	0.25	1	2	>64	⩽0.06
B. subtils ATCC 23857	0.12	32	0.25	1	1	1	2	>64	0.5
B. cereus ATCC 11778	0.12	>32	0.5	4	0.5	0.25	1	>64	0.25
B. fragilis ATCC 23745	0.5–1	>32	2	32	>32	32	2	>64	4
C. perfringens ATCC 10388	0.25–0.5	>32	0.5	0.5	4	1	2	>64	0.25
	12b	**16a**	**16b**	**20**	**25**	DB788	Pen-G	CPLX	VCM
S. aureus ATCC 29213	1	16	4	>32	4	4	1	0.5	0.5
S. aureus BAA-39[b]	1	8	8	>32	8	8	>32	8	1
S. aureus ATCC 33591[c]	0.25	8	4	>32	4	4	>32	⩽0.12	1
S. epidermidis ATCC 12228	⩽0.06	2	0.25	4	0.25	4	32	⩽0.12	1
S. epidermidis ATCC 51625[d]	0.25	4	1	8	1	4	32	⩽0.12	1
S. pneumoniae ATCC 6301	0.12	8	0.12	>32	0.12	8	<0.06	0.5	1
E. faecalis ATCC 51575[e]	1	4	2	>32	2	32	4	0.5	>64
E. faecium ATCC 700221[e]	0.25	16	1	16	1	8	>32	>64	>64
B. subtils ATCC 23857	0.5	32	1	>32	1	4	<0.06	⩽0.12	0.12–0.5
B. cereus ATCC 11778	0.25	8	0.5	32	0.5	4	4->32	⩽0.12	1-⩽0.12
B. fragilis ATCC 23745	1	16	32	>32	32	>32	4–8	0.5	4–8
C. perfringens ATCC 10388	0.25	0.5	8	1	>32	16	⩽0.06–0.12	0.25	0.12–0.25

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

activity. These results clearly indicated that *N*-substituent of the amidine of 1*H*-benzimidazole is very important for antibacterial activity.

Replacement of one of the 2-phenyl rings of bis-benzimidazole lead compound **1** by 2-thien-5-yl, 2-thien-4-yl, 2-pyridin-5-yl or 5-pyridin-2-yl ring yielded bis-benzimidazole amidine compounds **12a, 12b** and **16a, 16b**. Thienyl compounds **12a and 12b** showed a slight loss of activity compared to lead compound **1**. However, pyridinyl compounds **16a and 16b** exhibited only moderate antibacterial activity compared to the lead compound **1**.

Removal one of 5-*N*-isopropylamidino group in the lead compound **1** led to much loss of antibacterial activity for **20**. This result is consistent with the SAR results for the *N*-substituents of 5-amidine of 1*H*-benzimidazole analogues and further demonstrates that *N*-substituent of 5-amidine of 1*H*-benzimidazole is important for achieving potent antibacterial activity. Keeping both isopropylamidino groups and removal of one of benzimidazole rings of lead compound **1** yielded compound **25** which gave reduced anti-MRSA and anti-anaerobic bacterial activities. Replacement of the 5-*N*-isopropylamidino group with 4-methoxy group on the phenyl ring of the compound **25**, the previously reported DB788 exhibited similar activities against MRSA and anaerobic bacteria.[9d] However, DB788 showed decreased activities against other bacterial strains, such as *S. epidermidis* and VRE, compared to the 5-isopropylamidino compound **25**. These results suggest that bis-5-*N*-substitued amidino groups of 1*H*-benzimidazole is necessary for potent antibacterial activity.

In conclusion, we have identified the bis-benzimidazole diamidine lead compound **1** as a potent anti-MRSA and anti-VRE agent and prepared a number of analogues for probing the SAR of this system. Five compounds **1**, **5b**, **5d**, **12a, and 12b** have shown good antibacterial activities against Gram-positive bacteria, including drug-resistant bacterial strains. The SAR study of this series of compounds has shown that bis-5-*N*-substitued amidine of 1*H*-benzimidazole is very important for achieving potent antibacterial activity. The mechanism of action of the lead compound **1** could be different from existing antibiotics. Further structural optimization, study of the mechanism of action and in vivo efficacy of this new class of potent anti-MRSA and anti-VRE agents is underway and will be reported in due course.

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