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Optimization of the central linker of dicationic bis-benzimidazole anti-MRSA and anti-VRE agents

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ABSTRACT

A series of bis-benzimidazole diamidine compounds containing different central linkers has been synthesized and evaluated for in vitro antibacterial activities, including drug-resistant bacterial strains. Seven compounds have shown potent antibacterial activities. The anti-MRSA and anti-VRE activities of compound **1h** were more potent than that of the lead compound **1a** and vancomycin.

The worldwide spread of drug-resistant Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococccus faecium* (VRE), has become a serious global clinical problem for the treatment of various nosocomial and community-acquired infections.[1] In order to overcome these emerging bacterial resistance problems, numerous efforts have focused on discovering novel anti-MRSA and anti-VRE agents in recent decades.[2]

During a study seeking to discover novel anti-MRSA agents, we found a new class of dicationic bis-benzimidazole derivatives which displayed potent anti-MRSA and anti-VRE activities.[3] The lead compound **1a** (Fig. 1) has shown potent activity against Gram-positive bacterial strains and anaerobic bacterial strains, including both MRSA and VRE (MIC $\leqslant$ 0.5 μg/mL). A structure–activity relationship (SAR) study of this series of compounds has shown that bis-5-N-substituted amidine of 1*H*-benzimidazole is very important for achieving potent antibacterial activity. Replacement of one of the 2-phenyl rings of the lead compound **1a** by thienyl or pyridyl ring also resulted in loss of activity compared to the lead compound **1a**. The mechanism of action of this class compounds appears to be different from existing antibiotics. Therefore, we have continued to explore structural modifications of this new class of dicationic bis-benzimidazole compounds. With the goals of improving the anti-MRSA activity and to further investigate the SAR of this system, we designed and synthesized analogues **1b–m** and **2** of lead compound **1a**. Replacement of ethylene central linker with various one atom or two atoms groups in the lead compound **1a** was extensively investigated. In this Letter, we report the synthesis, in vitro antibacterial activity and SAR of this series of bis-benzimidazole compounds containing different central linkers.

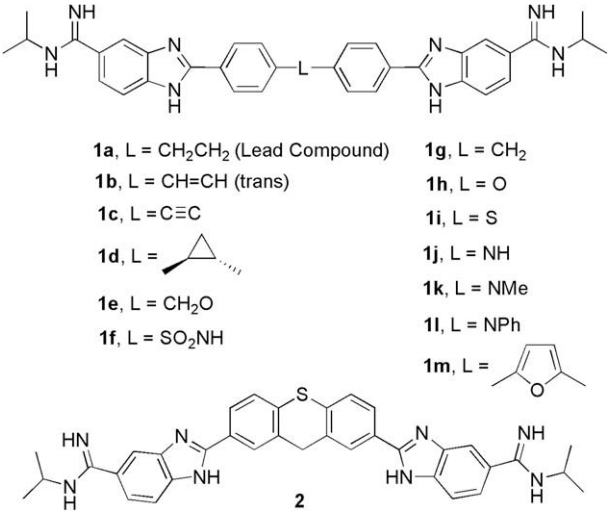

Figure 1. Bis-benzimidazole diamidine compounds.

We have reported the syntheses **1a, 1b, 1d,** and **1m** by the condensation of 4-(*N*-isopropylamidino)-1,2-phenylenediamine

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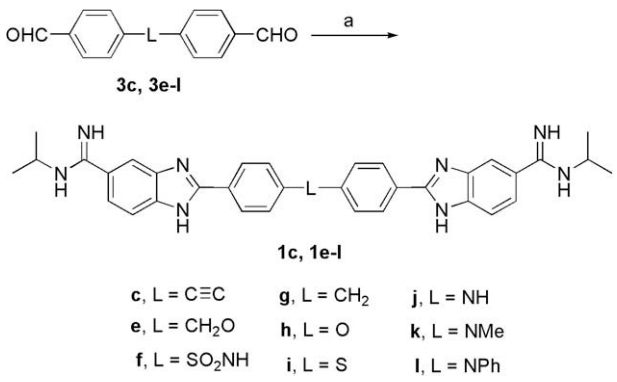
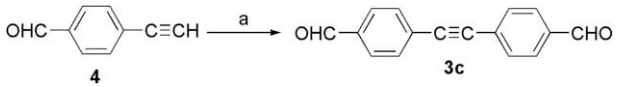
hydrochloride with the corresponding bis-benzaldehydes in presence of benzoquinone as oxidative reagent.[4] The analogs **1c** and **1e–l** were prepared from the bis-benzaldehydes **3c** and **3e–l** by following this procedure as shown in Scheme 1. The bis-benzaldehydes **3h** and **3l** are commercially available. The intermediate **3c** was prepared by Sonogoshira coupling of 4-ethynylbenzaldehyde **4** with 4-bromobenzaldehyde in reasonable yield (Scheme 2).[5] The starting material 4-bromomethylbenzonitrile **5** was converted into the benzaldehyde **6** by reduction with diisobutylaluminum hydride (DIBAL-H) in toluene (Scheme 3).[6] 4-Bromomethylbenzaldehyde **6** was reacted with 4-hydroxybenzaldehyde to yield the bis-benzaldehyde **3e** by using potassium carbonate in the presence of tetrabutylammonium iodide (TBAI) in DMF.[7] The bis-benzaldehydes **3f**, **3g**, and **3i–k** were obtained from the corresponding cyano compounds by DIBAL-H reduction, as shown in Schemes 4–7.[4a] The sulfonamide compound **8** was prepared from 4-cyanobenzenesulfonylchloride **7** by reaction with 4-aminobenzonitrile (Scheme 4). 4,4′-Dibromodiphenylmethane **9** on reaction with cuprous cyanide in DMF gave the 4,4′-dicyanodiphenylmethane **10** (Scheme 5).[8] The dicyano compound **12** was prepared from 4-chlorobenzonitrile **11** using sodium sulfide under reflux condition in DMF (Scheme 6).[9] 4,4′-Dicyanodiphenylamine **14a** was readily prepared by the reaction of 4-aminobenzonitrile **13** with 4-fluorobenzonitrile using potassium *tert*-butoxide as a base (Scheme 7).[10] The dinitrile compound **14a** was converted to *N*-methylamino derivative **14b** by methyl iodide in the presence of sodium hydride in DMF. The thioxanthene dicationic bis-benzimidazole **2** was prepared from 3,6-dicyanothioxanthene **15**[11] in two steps by using a procedure similar to that reported for the preparation of lead compound **1a** (Scheme 8).

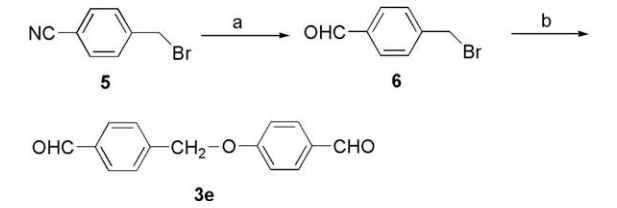
All of the bis-benzimidazole amidine hydrochloride compounds **1b–m** and **2** were screened for their potential antibacterial activities in vitro against 10 selected Gram-positive bacterial strains and two anaerobic bacterial strains, including MRSA, multi-drug resistant *S. aureus* (MDRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and VRE strains compared to the lead compound **1a**. 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 13 compounds tested against the Gram-positive aerobic strains, seven compounds **1b**, **1d**, **1g**, **1h**, and **1j–l** showed potent antibacterial activities comparable to or better than lead compound **1a**, including anti-MRSA (MIC value $\leqslant$ 1 μg/mL) and anti-VRE activity (MIC value $\leqslant$ 0.5 μg/mL); two compounds **1c**, **1e** were found to have good anti-MRSA activity (MIC value $\leqslant$ 4 μg/mL); and



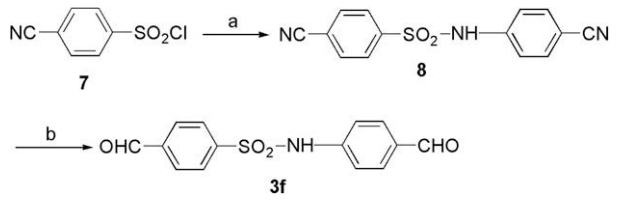
Scheme 1. Reagents and conditions: (a) 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 62–85%.



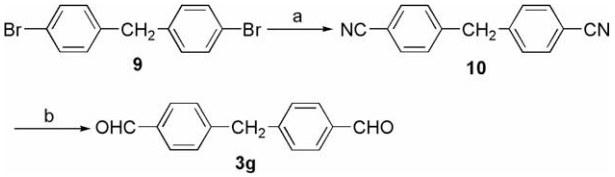
Scheme 2. Reagents and conditions: (a) 4-bromobenzaldehyde, CuI, $PdCl_2(PPh_3)_2$, $(i\text{-Pr})_2NEt$, THF, 50 °C, 69%.



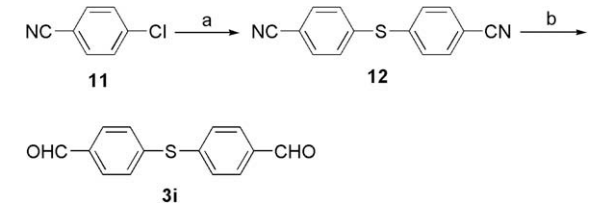
Scheme 3. Reagents and conditions: (a) DIBALH, toluene, 0 °C, 81%; (b) 4-hydroxybenzaldehyde, TBAI, K_2CO_3, DMF, 98%.



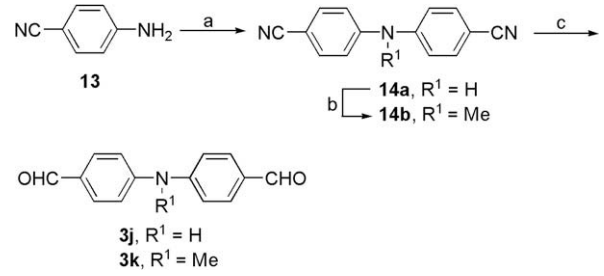
Scheme 4. Reagents and conditions: (a) 4-aminobenzonitrile, Py, CH_2Cl_2, 49%; (b) DIBALH, toluene, 0 °C, 35%.



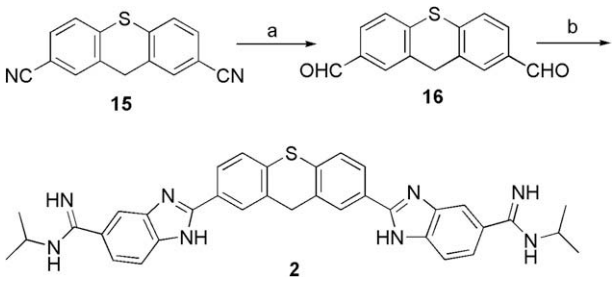
Scheme 5. Reagents and conditions: (a) CuCN, DMF, reflux, 38%; (b) DIBALH, toluene, 0 °C, 61%.



Scheme 6. Reagents and conditions: (a) Na_2S, DMF, 48%; (b) DIBALH, toluene, 0 °C, 55%.



Scheme 7. Reagents and conditions: (a) 4-fluorobenzonitrile, BuOK, DMF, 91%; (b) MeI, NaH, DMF, 87%; (c) DIBALH, toluene, 0 °C, 21% or 28%.



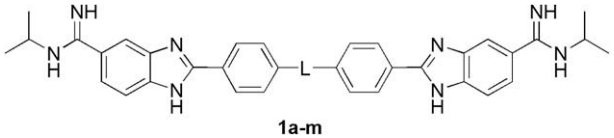
Scheme 8. Reagents and conditions: (a) DIBALH, toluene, 0 °C, 62%; (b) 4-(*N*-isopropylamidino)-1,2-phenylenediamine hydrochloride, 1,4-benzoquinone, EtOH, reflux, 75%.

four compounds **1f**, **1i**, **1m**, and **2** showed moderate or poor antibacterial activity. Four compounds **1b**, **1d**, **1g** and **1h** also showed good activity against two anaerobic bacterial strains (MIC value $\leqslant$ 2 μg/mL).

To investigate the effect of ethylene central linker of the lead compound **1a** on antibacterial activity, we first evaluated the antibacterial activities of analogs in which the ethylene group of **1a** has been replaced by other two-atom linkers (**1b–f**). Antibacterial activity of the vinyl and ethynyl compounds **1b** and **1c** were comparable to that of the lead compound **1a**. However, compound **1c** showed dramatically decreased anti-anaerobic bacterial activity. The antibacterial potency of *c*-propyl analog **1d** was almost equal to the lead compound **1a**. It is noteworthy that these dicationic bis-benzimidazole compounds **1a–d** generally decrease in anti-MRSA and anti-VRE activity as the relative electronegativities of these two-atom linker groups increase: ethylene < *c*-propyl < vinyl < ethynyl. A similar effect was found on replacement of the ethylene group with the more electronegative –CH_2O– group to give compound **1e** which showed lower antibacterial activity compared to that of lead compound **1a**. Replacement of the ethylene group by sulfonamide group led to poor antibacterial activity. The significant difference in activity of the sulfonamide **1f** relative to the other compounds with two-atom linkers may be due to the larger size and unique geometry of the sulfonamide linkage.[12] These results indicated that the central linker is well tolerated by vinyl and *c*-propyl group, however, ethynyl and –CH_2O– groups result in some loss of potency and the sulfonamide group is not tolerated.

Replacement of ethylene group of bis-benzimidazole lead compound **1a** by one-atom groups yielded bis-benzimidazole amidine compounds **1g–l**. The methylene compound **1g** showed a slight loss of activity compared to the lead compound **1a**. Interestingly, when the ethylene group of the lead compound **1a** was replaced by oxygen to yield the ether compound **1h** the antibacterial activity was enhanced compared to the lead compound **1a**. The activi-

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



1a-m

Strain/compound	MICs (μg/mL)								
	1a L = CH_2CH_2	**1b** CH=CH	**1c** C≡C	**1d** *c*-Pr	**1e** CH_2O	**1f** SO_2NH	**1g** CH_2	**1h** O	**1i** S
S. aureus ATCC 29213	0.25–0.5	1	2	1	1	>32	1–2	0.25	>32
S. aureus BAA-39[b]	0.5	1	4	0.5	4	16	1	0.5	32
S. aureus ATCC 33591[c]	0.25–0.5	0.5	2	0.25	0.5	16	0.25–0.5	0.12	16
S. epidermidis ATCC 12228	<0.06	0.12	0.12	≤0.06	≤0.06	2	0.12	<0.06	8
S. epidermidis ATCC 51625[d]	0.12	0.25	0.25	0.12	0.25	4	0.25	<0.06	16
S. pneumoniae ATCC 6301	<0.06	0.25	≤0.06	≤0.06	≤0.06	1	<0.06	<0.06	>32
E. faecalis ATCC 51575[e]	0.25–0.5	0.5	32	0.5	1	2	0.25–0.5	0.12	>32
E. faecium ATCC 700221[e]	0.12	0.25	0.5	0.12	0.12	4	0.12	<0.06	16
B. subtilis ATCC 23857	0.12	0.25	4	0.12	0.12	2	0.25	<0.06	16
B. cereus ATCC 11778	0.12	0.25	0.5	0.12	0.12	2	0.25	<0.06	8
B. fragilis ATCC 23745	0.5–1	0.5–2	>32	0.5	32	2	1-2	2	>32
C. perfringens ATCC 10388	0.25–0.5	0.5–1	4	0.25	16	4	1-2	0.5	>32

	MICs (μg/mL)							
	1j NH	**1k** NMe	**1l** NPh	**1m** 2,5-Furyl	**2**	Pen G	CPLX	VCM
S. aureus ATCC 29213	0.25	1	1	>32	16	1	0.5	0.5
S. aureus BAA-39[b]	0.25	0.5	0.5	32	8	>32	8	1
S. aureus ATCC 33591[c]	0.25	0.5	0.5	2	2	>32	≤0.12	1
S. epidermidis ATCC 12228	0.12	0.12	0.25	4	2	32	≤0.12	1
S. epidermidis ATCC 51625[d]	0.25	0.12	0.25	2	0.5	32	≤0.12	1
S. pneumoniae ATCC 6301	0.12	0.12	0.12	0.12	0.12	<0.06	0.5	1
E. faecalis ATCC 51575[e]	0.25	0.25	0.5	>32	16	4	0.5	>64
E. faecium ATCC 700221[e]	0.12	≤0.06	0.12	2	0.5	>32	>64	>64
B. subtilis ATCC 23857	0.12	0.12	≤0.06	2	1	<0.06	≤0.12	0.12–0.5
B. cereus ATCC 11778	0.12	0.25	0.12	2	1	4–>32	≤0.12	1–≤0.12
B. fragilis ATCC 23745	>32	>32	4	>32	>32	4–8	0.5	4–8
C. perfringens ATCC 10388	2	1	1	1	0.5	≤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.

ties of **1h** against MRSA ATCC 33591, MRSE ATCC 51625, VRE ATCC 51575 and 700221 were twofold more active than that of the lead compound **1a**. However, compound **1i** with sulfur as a linker showed a dramatic loss of antibacterial activity. Antibacterial activity of the compounds with –NH, –NMe and –NPh linkers (**1j–l)** were similar to that of the lead compound **1a**. However, the compounds **1j–l** showed decreased anti-anaerobic bacterial activity. These results were in contrast to that for those compounds 1a–**e** with two-atom linkers: the greater the electronegativity of the central one-atom linker group (the order of relative electronegativities: –O– > –NH– > $–CH_2–$), the greater the antibacterial activity of the compounds **1h**, **1j** and **1g**. Replacement of oxygen with less electronegative sulfur in the compound **1h** resulted in loss of antimicrobial activity. These results suggest that the antibacterial activity of the dicationic bis-benzimidazole compounds is related to the 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. This may suggest different binding modes for the two series. Replacement of ethylene group with 2,5-furyl ring in lead compound **1a** to yield the bis-benzimidazole amidine compound **1m** resulted in significant loss of antibacterial activity. When the diphenyl ethylene of the lead compound **1a** is replaced by a thioxanthenyl ring, the antibacterial potency is reduced compared to the lead compound **1a**. However, it is noteworthy that compound **2** showed better activity than the sulfur compound **1i**. The order of anti-MRSA and anti-VRE activity of these dicationic bis-benzimidazole compounds containing different central linkers was: **lh > 1a**, **1d**, **1j–l > 1b**, **1g > 1e > 1c > 2 > 1f > 1m > 1i**. These results clearly demonstrate that the central linker of the dicationic bis-benzimidazole compounds is very important for achieving potent antibacterial activity.

In conclusion, we have synthesized and evaluated the antibacterial activities of analogues of lead compound **1a** by replacement of the central ethylene linker with various two-atom or one-atom groups for probing the SAR of this system. Seven compounds have shown potent antibacterial activities against Gram-positive bacteria, including drug-resistant bacterial strains. The SAR study of this series compounds has shown that the antibacterial activity of the dicationic bis-benzimidazole compounds can be related to the relative electronegativity of the central linkers, however, the central linkers of one-atom and two-atoms showed opposite effects on activity. The most potent compound **1h** is more active than the lead compound **1a** against MRSA, MRSE and VRE strains. The anti-MRSA or anti-MDRSA activity of the most potent compound **1h** as well as the lead compound **1a** was equivalent to that of anti-MSSA (methicillin-susceptible *S. aureus*) activity, which was clearly different from that of the reference antibiotics Pen G and CPLX. The anti-VRE activity of compound **1h** was more active by 512 times than that of VCM. These results suggest that the mechanism of action of the compound **1h** might be different from the reference antibiotics. The compound **1h** merits further investigation as a new lead of this class of dicationic bis-benzimidazole anti-MRSA and anti-VRE agents.

References and notes

1. (a) Levy, S. B.; Marshell, B. *Nat. Med.* **2004**, *10*, S122; (b) Barrett, J. F. *Expert Opin. Ther. Targets* **2005**, *9*, 253; (c) Bryskier, A. *Expert Rev. Anti Infect. Ther.* **2005**, *3*, 505.
2. (a) Loffler, C. A.; MacDougall, C. *Expert Rev. Anti Infect. Ther.* **2007**, *5*, 961; (b) Mitchell, M. O. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 243.
3. Hu, L.; Kully, M. L.; Boykin, D. W.; Abood, N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1291.
4. (a) Del Poeta, M.; Schell, W. A.; Dykstra, C. C.; Jones, S.; Tidwell, R. R.; Czarny, A.; Bajic, M.; Bajic, M.; Kumar, A.; Boykin, D.; Perfect, J. R. *Antimicrob. Agents Chemother.* **1998**, *42*, 2495; (b) Kang, Z.; Dykstra, C. C.; Boykin, D. W. *Molecules* **2004**, *9*, 158; (c) Hopkins, K. T.; Wilson, W. D.; Bender, B. C.; McCurdy, D. R.; Hall, J. E.; Tidwell, R. R.; Kumar, A.; Bajic, M.; Boykin, D. W. *J. Med. Chem.* **1998**, *41*, 3872.
5. (a) Sonogahsira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467; (b) Khabnadideh, S.; Pez, D.; Musso, A.; Brun, R.; Ruiz Pérez, L. M.; González-Pacanowska, D.; Gilbert, I. H. *Bioorg. Med. Chem.* **2005**, *13*, 2637.
6. Wen, L.; Li, M.; Schlenoff, J. B. *J. Am. Chem. Soc.* **1997**, *119*, 7726.
7. Fernandes, R. A.; Bodas, M. S.; Kumar, P. *Tetrahedron* **2002**, *58*, 1223.
8. Young, J. D.; Stevenson, G. R.; Bauld, N. L. *J. Am. Chem. Soc.* **1972**, *94*, 8790.
9. Evans, T. L.; Kinnard, R. D. *J. Org. Chem.* **1983**, *48*, 2496.
10. Murase, T.; Fujita, M. *J. Org. Chem.* **2005**, *70*, 9269.
11. Chauhan, P. M. S.; Rao, K. V. B.; Lyer, R. N.; Shankhadhar, V.; Guru, P. Y.; Sen, A. B. *Indian J. Chem., Sect. B: Org. Chem. Including Med. Chem.* **1987**, *26B*, 248.
12. Petrov, V.; Petrova, V.; Girichev, G. V.; Oberhammer, H.; Giricheva, N. I.; Ivanov, S. *J. Org. Chem.* **2006**, *71*, 2952.