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Antibacterial activity of substituted dibenzo[*a,g*]quinolizin-7-ium derivatives

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Abstract

Berberine is a substituted dibenzo[*a,g*]quinolizin-7-ium derivative whose modest antibiotic activity is derived from its disruptive impact on the function of the essential bacterial cell division protein FtsZ. The present study reveals that the presence of a biphenyl substituent at either the 2- or 12-position of structurally-related dibenzo[*a,g*]quinolizin-7-ium derivatives significantly enhances antibacterial potency versus *Staphylococcus aureus* and *Enterococcus faecalis*. Studies with purified *S. aureus* FtsZ demonstrate that both 2- and 12-biphenyl dibenzo[*a,g*]quinolizin-7-ium derivatives act as enhancers of FtsZ self-polymerization.

Keywords

Antibacterial; Dibenzo[*a,g*]quinolizinium; FtsZ-targeting; *Staphylococcus aureus*; *Enterococcus faecalis*

Bacterial resistance to current clinical antibacterial drugs has created a critical need for new therapeutic antibacterial agents with novel targets and modes of action. Infections associated with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) represent an increasing nosocomial health concern for both patients and healthcare professionals.[1,2] FtsZ is a key protein involved in bacterial cell division (cytokinesis).[3,4] It is highly conserved among bacterial pathogens and, in several genetic studies, has been shown to be indispensable.[5–8] Cell division in bacteria occurs at the site of formation of a cytokinetic Z-ring polymeric structure, which is comprised of FtsZ subunits.[9] The essential role that FtsZ plays in bacterial cell division makes this protein a promising therapeutic target, and recent advances in the development of small molecules that target FtsZ have been the subject of several recent reviews.[10–13] FtsZ-targeting antibacterial agents can exert their disruptive effects on the Z-ring by either enhancing or inhibiting FtsZ self-polymerization.[14–21]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.123.

Berberine (Fig. 1) is a plant alkaloid that exhibits weak antibacterial activity, with MIC values typically on the order of 100–400 μg/mL versus Gram-positive bacteria and >500 μg/mL versus Gram-negative bacteria.[22–24] Recent studies have suggested that the antibacterial activities of berberine and the structurally-related benzo[*c*]phenanthridine alkaloid sanguinarine are derived from the inhibitory effects of the compounds on FtsZ polymerization and Z-ring formation.[22–24] We have recently demonstrated that the presence of phenyl or biphenyl substituents at the 1- or 12-position of substituted 5-methylbenzo[*c*]phenanthridinium derivatives structurally similar to sanguinarine enhanced relative antibacterial activity versus both *S. aureus* and *E. faecalis* (including MRSA and VRE strains). In this study, we explore the effect of aryl substituents at the 2- and the 12-position on the antistaphylo coccal and antienterococcal activities of a series of dibenzo[*a,g*]quinolizin-7-ium and 8-methyldibenzo[*a,g*]quinolizin-7-ium derivatives that are structurally-related to berberine.

Two key intermediates were used for the preparation of the 1-substituted dibenzo[*a,g*]quinolizin-7-ium salts evaluated for antibacterial activity in this study, Table 1. Commercially available 4-bromo-3-methoxyphenethylamine was treated with 3, 4-dimethoxybenzoylchloride as outlined in Scheme 1 to form *N*- [2-[1-(4-bromo-3-methoxyphenyl)]ethyl]-3,4-dimethoxyphenylacetamide derivative, intermediate **A**. As outlined in Scheme 2, intermediate A was employed for the synthesis of **1–4** in Table 1. For the preparation of intermediate **B**, veratrole was treated with excess *n*-butyllithium and then reacted with bromine. The resulting 1,4-dibromo-2,3-dimethoxy-benzene was then treated with *n*-butyllithium and DMF to provide the intermediate 4-bromo-2,3,-dimethoxybenzaldehyde in Scheme 1. Condensation of this benzaldehyde with nitromethane provided the 2-nitroethylene intermediate, which was reduced with $NaBH_4$ and then treated with Zn powder in acetic acid to yield 2-bromo-2,3-dimethoxyphenethylamine. Treatment of this phenethylamine with 3,4-dimethoxyphenylacetylchloride provided the *N*-[2-[1-(4-bromo-2, 3-dimethoxyphenyl)]ethyl]phenylacetamide derivative as intermediate **B**. This intermediate was used as outlined in Scheme 2 for the preparation of **6–13** in Table 1.

Both intermediate **A** and **B** were subject to Suzuki coupling conditions using various arylboronates (Scheme 2). In the presence of $POCl_3$ in acetonitrile, these derivatives provided the requisite precursors for the formation of various 7-substituted 3,4-dihydro-1-(3,4-dimethoxybenzyl)isoquinolines. Oxidation in the presence of palladium on carbon in tetralin provided the aromatized 1-benzylisoquinoline derivatives. Treatment of these derivatives with $POCl_3$ in dimethylformamide yielded the desired 2-substituted 3,4,10,11-tetramethoxydibenzo[*a,g*]quinolizin-7-ium chlorides. Alternatively, treatment of these 1-benzylisoquinoline derivatives with acetic anhydride in the presence of fuming (20%) H_2SO_4 gave 2-substituted 8-methyl-3,4,10,11-tetramethoxydibenzo[*a,g*]quinolizin-7-ium acetylsulfonates.

The synthesis of 12-aryl substituted dibenzo[*a,g*]quinolizin-7-ium salts was accomplished as outlined in Scheme 3. Treatment of either 2,3-dimethoxyphenethylamine or 3,4-dimethoxyphenethylamine with the acid chloride derivative of 2-bromo-3,4-dimethoxyphenylacetic acid provide the *N*-[2-[1-(2,3-dimethoxyphenyl)]- ethyl]2-bromo-3,4-dimethoxyphenylacetamide or the *N*-[2-[1-(3,4-dimethoxyphenyl)]ethyl]-2-bromo-3,4-dimethoxyphenylacetamide, respectively. These bromo derivatives were reacted under Suzuki coupling conditions with several arylboronates, cyclized in the presence of $POCl_3$ in acetonitrile and oxidized to their respective 1-benzylisoquinoline derivatives. Treatment of these derivatives with $POCl_3$ in dimethylformamide yielded the desired 12-substituted dibenzo [*a,g*]quinolizin-7-ium chlorides, **14** and **16**. Alternatively, treatment of these 1-benzylisoquinoline derivatives with acetic anhydride in the presence of fuming

(20%) H_2SO_4 gave the 12-substituted 8-methyldibenzo[*a,g*]quinolizin-7-ium acetylsulfonates, **15** and **17**.

These dibenzo[*a,g*]quinolizin-7-ium derivatives were evaluated for their antibacterial activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) as well as vancomycin-sensitive *Enterococcus faecalis* (VSE) and vancomycin-resistant *Enterococcus faecalis* (VRE). Their relative antibacterial activities are listed in Table 2. Berberine did not exhibit appreciable antibiotic activity when evaluated against the strains of *S. aureus* or *E. faecalis* used in this study. Compounds **1–4** exhibit significant antibacterial activity against MSSA. The 2-(4-toluyl) derivatives **3** and **4** are more active than the 2-phenyl derivatives **1** and **2** against both *S. aureus* strains and VSE. The presence or absence of an 8-methyl substituent has a modest effect on antibacterial activity among these trimethoxy derivatives, with this effect being variable and typically reflected by a two-fold difference in MIC values.

Antibacterial activities of the 2-aryl 3,4,10,11-tetramethoxydibenzo[*a,g*]quinolizin-7-ium derivatives **5–13** were also consistently associated with an increase in antibacterial activity against both *S. aureus* strains relative to **1** and **2**. The effect of an 8-methyl substituent among these tetramethoxy derivatives on antibacterial activity is modest and, in general, tends toward only a slightly greater antibacterial effect. Only in the case of **9** when evaluated against VRE is a slightly greater antibiotic activity observed relative to its 8-methyl derivative, **10**.

There was a notable difference between the 3,10,11-trimethoxy- and 3,4,10,11-tetramethoxydibenzo[*a,g*]quinolizin-7-ium derivatives with regard to activity against *E. faecalis*. None of the trimethoxy derivatives has an MIC value less than 64 μg/mL against the vancomycin-resistant strain. Interestingly, several of the 2-aryl-3,4,10,11-tetramethoxy derivatives exhibited enhanced activity toward both strains of *E. faecalis*. The 8-isopropyl derivative, **11**, was the most potent dibenzo[*a,g*]quinolizin-7-ium analog evaluated in both strains of *S. aureus* and *E. faecalis*.

Comparison of the antibiotic activities of **9** and **10** with the 1-naphthyl derivatives (**12**,**13**) and the 12-biphenyl derivatives(**14,15**) reveals that these compounds exhibit similar activity. The relative antibiotic activities of **14** and **15** tend to be somewhat less than **9** and **10** against the resistant *S. aureus* and *E. faecalis* strains. A similar trend is observed in comparing the antibacterial activities of 12-biphenyl-2,3,10,11-trimethoxydibenzo[*a,g*]quinolizin-7-ium derivatives **16** and **17** with the 2-biphenyl-3,4,10,11-tetramethoxydibenzo[*a,g*]quinolizin-7-ium derivatives **9** and **10**.

A 90°-angle light scattering assay was used to monitor the impact, if any, of select dibenzo[*a,g*]quinolizin-7-ium compounds on the dynamics of self-polymerization by purified *S. aureus* FtsZ (SaFtsZ). In this assay, FtsZ polymerization is detected in solution by a time-dependent increase in light scattering. As an illustrative example for a dibenzo[*a,g*]quinolizin-7-ium compound with an aryl (biphenyl) substitution at the 2-position, Figure 2A shows the time-dependent light scattering profiles of SaFtsZ in the absence and presence of **11** at concentrations of 10 μg/mL and 20 μg/mL. The time-dependent increase in light scattering observed in the presence of vehicle only is reflective of FtsZ self-polymerization in the absence of test compound. Significantly, the increase in light scattering observed in the presence of **11** is greater than that in the absence of compound, with the extent of this light scattering enhancement being dependent on compound concentration. The lack of light scattering change associated with 20 μg/mL **11** in the absence of FtsZ (Fig. 2A) confirms that the enhanced light scattering induced by **11** in

the presence of FtsZ reflects a corresponding stimulation of FtsZ self-polymerization and not simply compound aggregation or precipitation.

As an illustrative example for the impact on SaFtsZ polymerization of a dibenzo[*a*,*g*]quinolizin-7-ium compound with an aryl (biphenyl) substitution at the 12-position, Figure 2B shows the time-dependent light scattering profiles of SaFtsZ in the absence and presence of **17** at a concentration of 20 μg/mL. Oxacillin is also included (at 20 μg/mL) as a control non-FtsZ-targeting antibiotic. Note that oxacillin exerts a negligible impact on SaFtsZ polymerization, an expected result given that the antibacterial target of oxacillin is the bacterial cell wall rather than FtsZ. A comparison of the light scattering profiles of **11** and **17** at 20 μg/mL (the green profiles in Fig. 2A and B) reveals the following two differential features: (i) **17** stimulates FtsZ polymerization more rapidly than **11**, as reflected by the light scattering profile of **17** reaching a plateau earlier than that of **11**. (ii) After reaching a plateau, the light scattering profile of **17** also undergoes a time-dependent decrease, suggestive of a corresponding time-dependent dissociation from larger to smaller FtsZ polymers or polymeric bundles. These two differential features may contribute, at least in part, to the reduced activities of **17** relative to **11** against both the MSSA and MRSA strains tested.

Stimulation of FtsZ polymerization has been shown to be the antibacterial mechanism of action for agents such as the substituted benzamides (e.g., PC190723).[14,17,18] In addition, FtsZ has been implicated as the antibacterial target of the naturally occurring dibenzoquinolizinium berberine, although the antibacterial potency of berberine is substantively weaker than that of our substituted dibenzoquinolizinium compounds.[23] Viewed as a whole, the light scattering results point to the stimulation of FtsZ polymerization dynamics as underlying the antibacterial activity of the 2- and 12-substituted dibenzo[*a*,*g*]quinolizin-7-ium compounds.

In summary, the presence of an aryl substituent at either the 2- or 12-position of tetramethoxy dibenzoquinolizinium significantly increases FtsZ-targeted antistaphylococcal and antienterococcal activity relative to berberine, with the compounds having notable antibacterial activity against multidrug-resistant strains of both MRSA and VRE. Against VRE, all of the biphenyl-substituted derivatives have MIC values lower than those of the clinical antibiotics used as controls in this study. Studies are in progress to develop suitable formulations for assessment of their relative antibacterial activity in vivo using appropriate animal models of infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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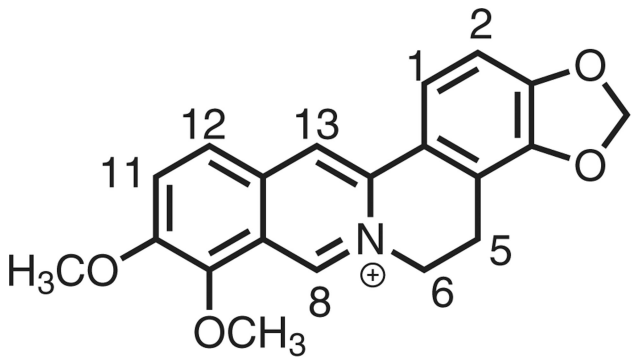
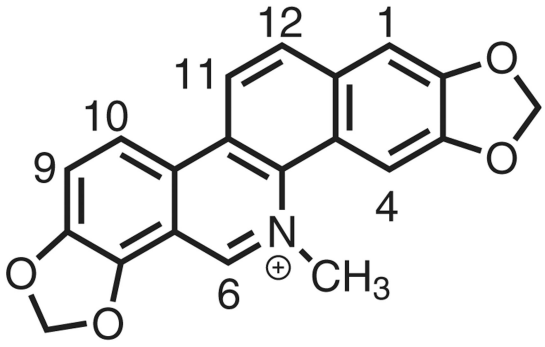


Berberine



Sanguinarine

Figure 1.
The structure and numbering of berberine and sanguinarine.

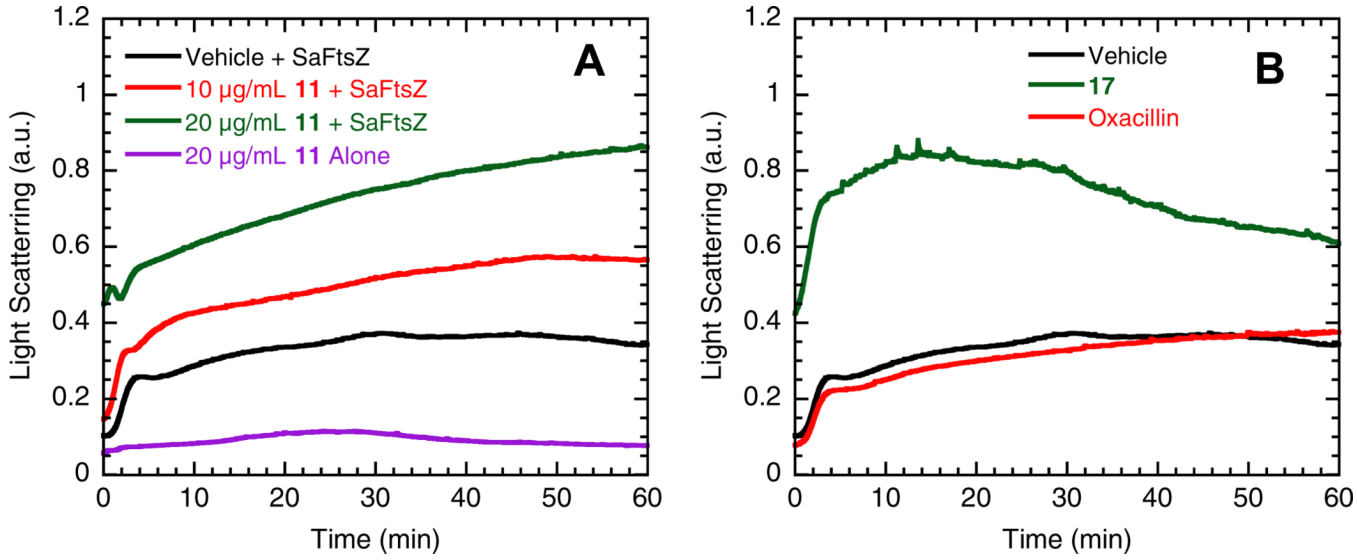
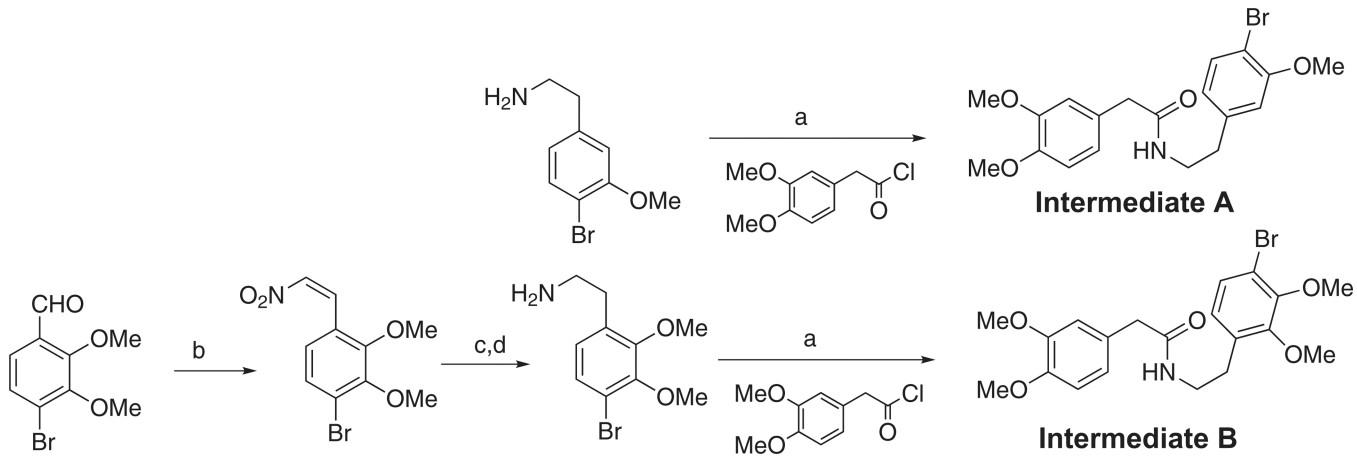


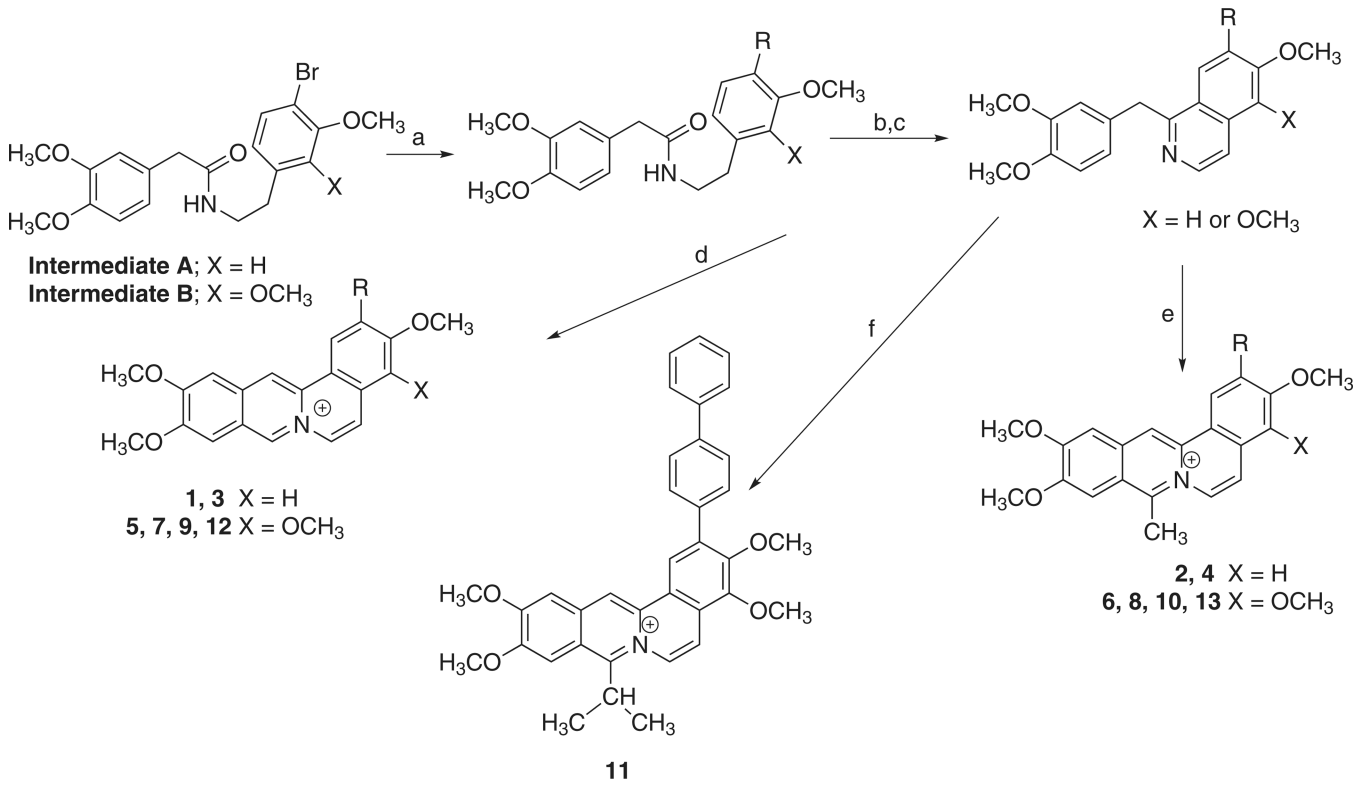
Figure 2.

Impact of select dibenzo[*a*,*g*]quinolizin-7-ium compounds on the self-polymerization of purified *S. aureus* FtsZ (SaFtsZ), as determined by monitoring time-dependent changes in 90°-angle light scattering. (A) Light scattering profiles of SaFtsZ (8.3 μM) in the presence of DMSO vehicle (black) or **11** at a concentration of either 10 (red) or 20 (green) μg/mL. For comparative purposes, the corresponding light scattering profile of 20 μg/mL **11** alone (violet) is also included as a no-protein control. (B) Light scattering profiles of SaFtsZ (8.3 μM) in the presence of DMSO vehicle (black) or 20 μg/mL of either **17** (green) or the comparator antibiotic oxacillin (red). Experiments were conducted at 25 °C in solution containing 50 mM Tris•HCl (pH 7.4), 50 mM KCl, 2 mM magnesium acetate, 1 mM $CaCl_2$, and 1 mM GTP. GTP was combined with vehicle, test compound, or control drug, and the reactions were initiated by addition of the protein. The reactions (150 μL total volume) were continuously monitored in quartz ultramicro cells (pathlength of 10 mm in the excitation direction and 2 mm in the emission direction) using an AVIV ATF 105 spectrofluorimeter, with the excitation and emission wavelengths set at 470 nm (at which the dibenzo[*a*,*g*]quinolizin-7-ium compounds absorb little or no light) and the corresponding excitation and emission bandwidths set at 2 nm. Readings were acquired in 5-second intervals with a corresponding time constant of 1 second.



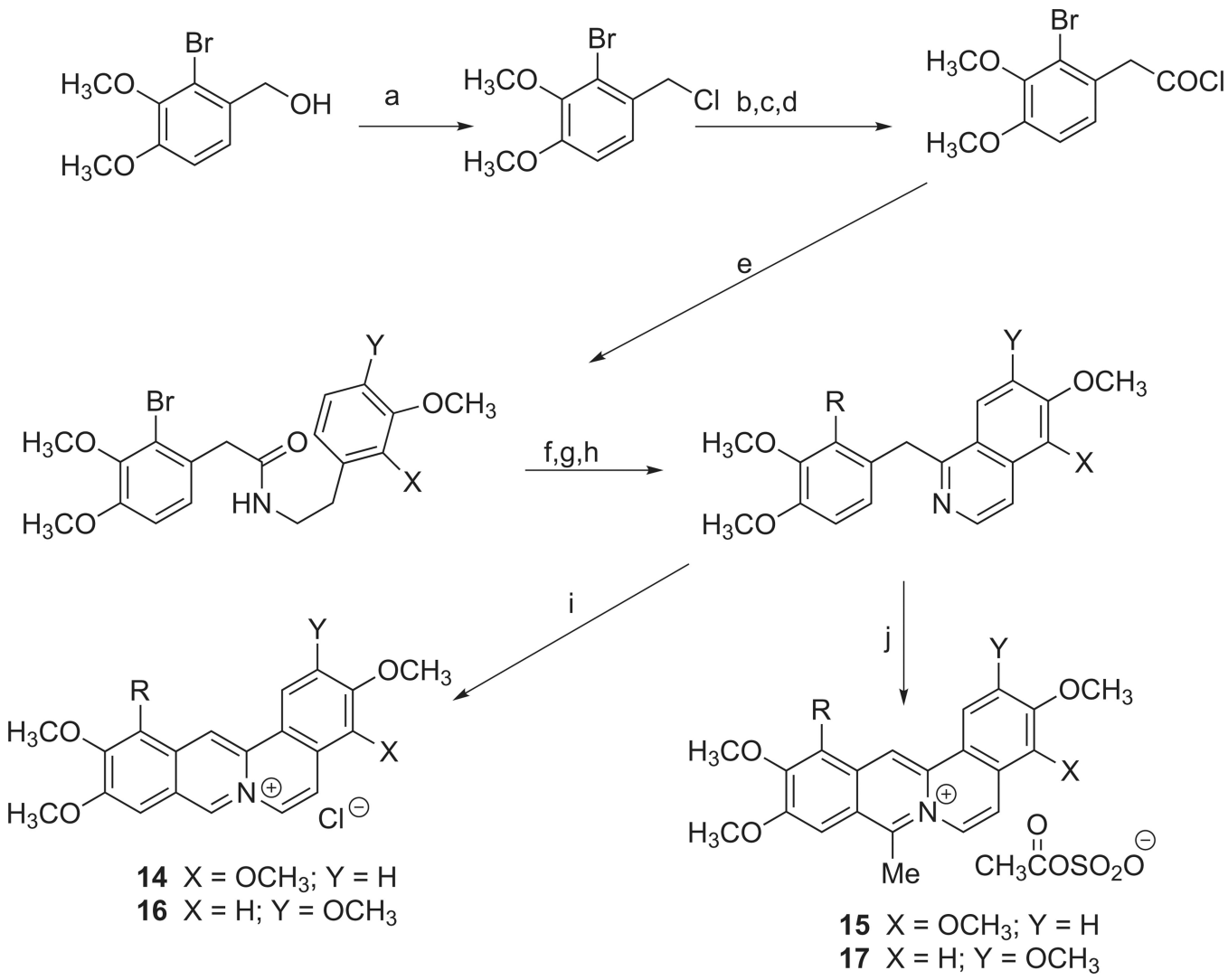
Scheme 1.

Preparation of intermediate A and intermediate B. Reagents and conditions: (a) 2M Na_2CO_3, $CHCl_3$, 0 °C; (b) NH_4OA_c, CH_3NO_2, glacial acetic acid, 120 °C, 72%; (c) $NaBH_4$, 1,4-dioxane/EtOH (2:1), 91%; (d) acetic acid/Zn powder, 57%.



Scheme 2.

Methods used for the preparation of 2-substituted dibenzo[*a*,*g*]quinolizin-7-ium salts. Reagents and conditions: (a) R-B(OH)$_2$, K_2CO_3, Pd (PPh_3)4, dioxane:H_2O (3:1), 100 °C; (b) $POCl_3$, ACN, reflux, 1–2 h; (c) Pd/C, tetralin, 210–220 °C; (d) $POCl_3$, DMF, HCl; (e) acetic anhydride, fuming H_2SO_4; (f) isobutyric anhydride, fuming H_2SO_4.

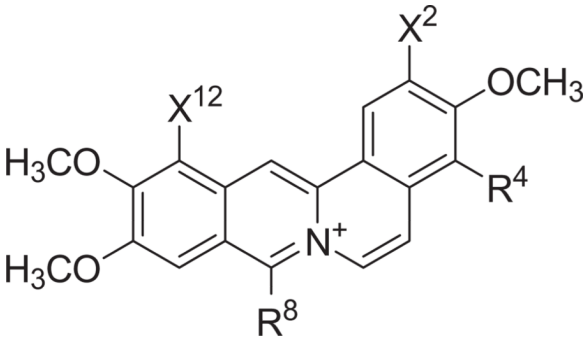


Scheme 3.

Methods used for the preparation of 12-substituted dibenzo[*a*,*g*]quinolizin-7-ium salts. Reagents and conditions: (a) 12N HCl, toluene, 88%; (b) KCN, Me_2SO, 90%; (c) HOAC, H_2O, conc. H_2SO_4, quant.; (d) (COCl)2, DCM, 2 h; (e) 2M Na_2CO_3, $CHCl_3$, 0 °C; (f) R-$B(OH)_2$, K_2CO_3, Pd $(PPh_3)_4$, dioxane:H_2O (3:1), 100 °C; (g) $POCl_3$, ACN, reflux, 1–2 h; (h) Pd/C, tetralin, 210–220 °C; (i) $POCl_3$, DMF, HCl; (j) acetic anhydride, fuming H_2SO_4

Table 1

Dibenzo[*a*,*g*]quinolizin-7-ium salts synthesized with either a 2-aryl or a 12-aryl substituent



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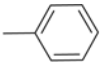
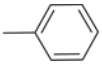
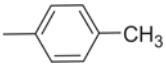
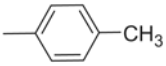
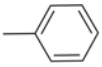
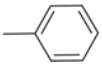
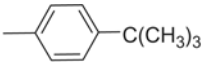
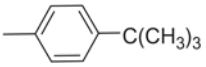
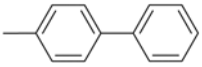
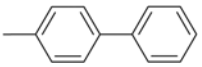
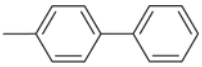
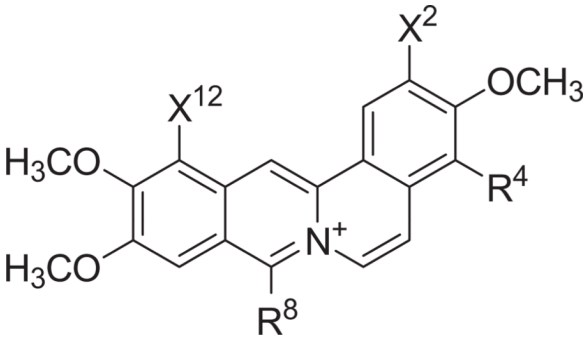
1		H	H	H
2		H	−CH_3	H
3		H	H	H
4		H	−CH_3	H
5		−OCH_3	H	H
6		−OCH_3	−CH_3	H
7		−OCH_3	H	H
8		−OCH_3	−CH_3	H
9		−OCH_3	H	H
10		−OCH_3	−CH_3	H
11		−OCH_3	$CH(CH_3)_2$	H



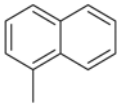
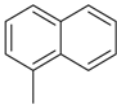
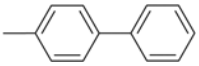
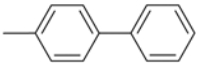
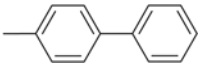
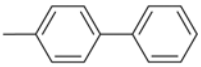
	X^2	R^4	R^8	X^{12}
12		$-OCH_3$	H	H
13		$-OCH_3$	$-CH_3$	H
14	H	$-OCH_3$	H	
15	H	$-OCH_3$	$-CH_3$	
16	$-OCH_3$	H	H	
17	$-OCH_3$	H	$-CH_3$	

Table 2

Antistaphylococcal and antienterococcal activities of ibenzo[*a*,*g*]quinolizin-7-ium salts with either 2- or 12-aryl substituents

	MIC[a] (μg/mL)			
	S. aureus **8325-4 (MSSA)**	***S. aureus*** **ATCC 33591 (MRSA)**	***E. faecalis*** **ATCC 19433 (VSE)**	***E. faecalis*** **ATCC 51575 (VRE)**
1	8	32	32	64
2	8	>64	>64	>64
3	2	8	16	>64
4	4	8	8	>64
5	4	8	16	32
6	2	8	8	16
7	4	4	4	8
8	1	2	4	4
9	2	2	8	4
10	1	2	4	8
11	0.5	0.5	2	2
12	4	2	8	8
13	1	2	4	8
14	2	8	8	16
15	1	4	16	16
16	2	4	8	32
17	1	4	4	32
Berberine	>64	>64	>64	>64
Oxacillin	0.06	>64	8	>64
Vancomycin	1	2	1	>64
Erythromycin	0.13	>64	1	>64
Tetracycline	0.06	64	0.5	>64
Clindamycin	0.03	>64	2	>64

[a]Minimum inhibitory concentration (MIC) assays were conducted in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines for broth microdilution.[25] MIC is defined as the lowest compound concentration at which bacterial growth is ≥90% inhibited.