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Synthesis of riccardin D derivatives as potent antimicrobial agents

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ABSTRACT

We describe the synthesis and biological evaluation of riccardin D derivatives, a novel class of antimicrobial molecules. Structural diversification of these derivatives was achieved by introducing hydroxy, methoxy, and bromine into the aromatic rings of riccardin D. The antimicrobial evaluation of these compounds was performed as in vitro assays against clinically isolated bacteria and fungi. The introduction of bromine atom into the arene *B* of riccardin D led to several strongly active antibacterial compounds with a MIC value ranging from 0.5 to 4 μg/mL for *Staphylococcus aureus*, both methicillin-sensitive and -resistant strains. Antifungal tests found compound **34** was the most potent molecule with a MIC value of 2 μg/mL against *Candida albicans*. This initial biological evaluation suggests that these novel molecules merit further investigation as potential antimicrobial agents.

Macrocyclic bisbibenzyls are a series of phenolic natural products that are almost exclusively found in liverworts.[1–4] These natural products exhibit versatile and often potent biological activities, including cyclooxygenase, calmodulin, and 5-lipoxygenase inhibitory effects and antifungal, antibacterial, antioxidative, antivirus, anti-mitotic, cytotoxic, muscle-relaxing, NOS-inhibiting, and LXR-modulating activities.[1,3,5–13] Bisbibenzyls, therefore, are of great interest to natural product researchers for their potential applications as pharmacological agents.[5,14–18]

Riccardin D, a macrocyclic bisbibenzyl isolated from *Marchantia polymorpha* L., has been the focus of considerable biological investigation and pharmacological testing in our group[19–25] and has been shown to possess potent antifungal activity, with a MIC value of 16 μg/mL against *Candida albicans*.[26–28] Moreover, it was recently reported that riccardin D also exhibits in vitro antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with MIC values in the 4–8 μg/mL range.[7–9]

These encouraging antifungal and antibacterial properties of riccardin D motivated us to synthesize additional derivatives to discover more potent molecules as potential new antimicrobial agents. Recently, Miyachi et al. reported the number and position of hydroxyl groups on the benzene ring could impact the anti-MRSA activity of riccardin C.[7,8] These results inspired our group to prepare riccardin D derivatives bearing more hydroxyl groups on arene *A* and arene *C* to determine the influence of these groups on the biological properties of riccardin D. We also methylated the hydroxyl group on the arene *B* and arene *D* to evaluate the effect of methoxyl group on the antimicrobial activity. In addition, Huigens et al. reported brominated phenazines exhibited potent anti-MRSA activity by eradicating biofilms and killing MRSA persister cells.[29] In consideration of these results, the bromination of riccardin D and the evaluation of these derivatives as antimicrobial agents were also studied.

The synthesis of 13-hydroxyl riccardin D (**25**) was achieved in 10 steps, as shown in Scheme 1. The synthetic route began with the Ullmann coupling of the protected 3-hydroxy-4,5-dimethoxyl-benzaldehyde (**2**) with methyl 4-bromobenzoate (**3**), resulting in the formation of the diphenyl ether **5**. Compound **5** was then reduced with lithium aluminum hydride, followed by treatment with triphenylphosphonium bromide to afford *AC* fragment **8** in three steps. The *BD* fragment **15** was prepared from compounds **13** and **14** by a standard Suzuki reaction, following the approach reported previously.[11] The building blocks **8** and **15** were coupled by a Wittig reaction in the presence of potassium carbonate and 18-crown-6. The resulting stilbene, a mixture of geometrical isomers, was then hydrogenated over Pd/C, followed by acidic hydrolysis to afford compound **16**. The distal hydroxyl group was directly treated with triphenylphosphine HBr to afford compound **19** for cyclization. The intramolecular cyclization was then achieved via Wittig reaction in the presence of sodium methoxide, followed by hydrogenation over Pd/C to yield compound **22**. Derivative **25** was obtained after the methyl ether cleavage of com-

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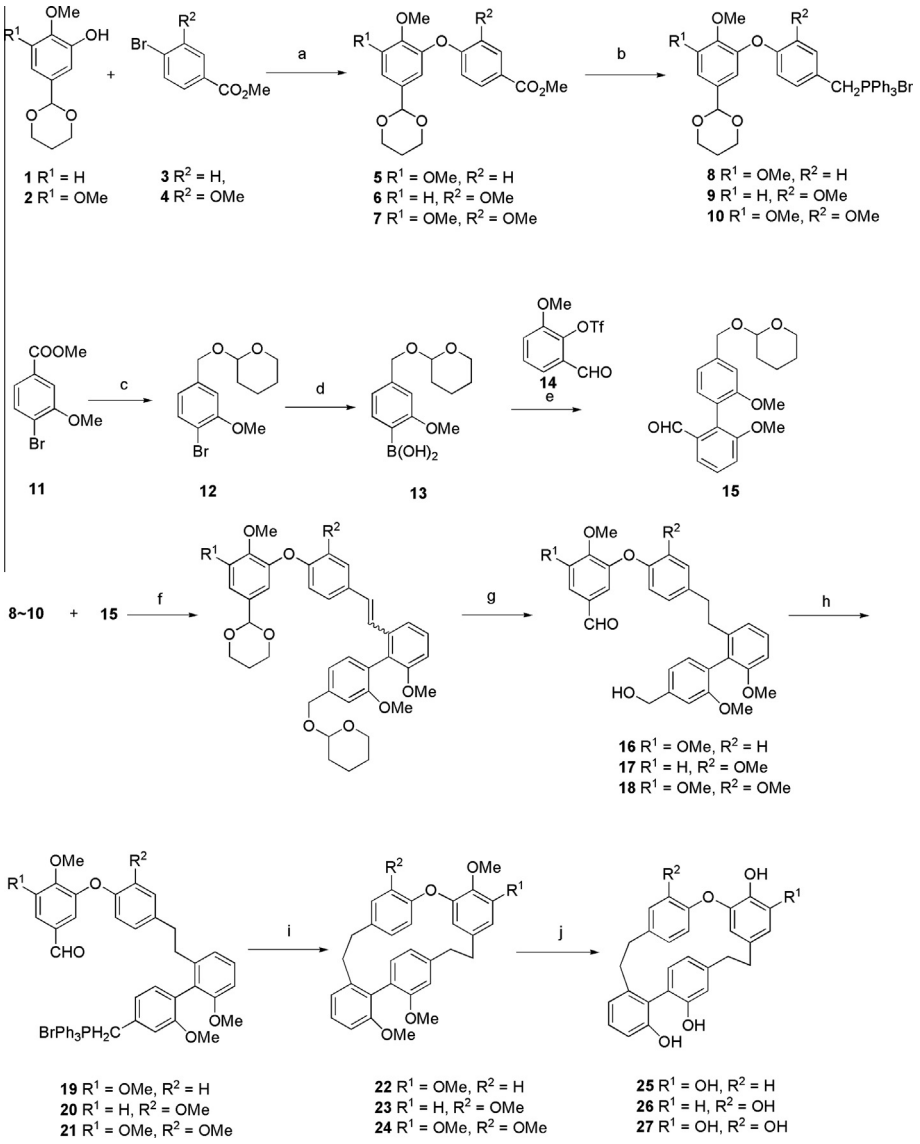


Scheme 1. Synthesis of riccardin D derivatives. Reagents and conditions: (a) CuO, K_2CO_3, Py, reflux, 71–83%; (b) (i) $LiAlH_4$, THF, −40 °C to rt; (ii) PPh_3HBr, MeCN, reflux, 98%; (c) (i) $LiAlH_4$, THF, −40 °C to rt; (ii) 2,3-dihydropyran, *p*-toluenesulfonic acid, DCM, 77%; (d) *n*-BuLi, $B(OMe)_3$, KH_2PO_4, THF, −30 °C to rt, 78%; (e) $Pd(PPh_3)_4$, toluene, EtOH, Na_2CO_3, reflux, 81%; (f) K_2CO_3, 18-crown-6, DCM, reflux, 91%; (g) (i) Pd/C (10%), H_2, Et_3N, EtOAc, rt; (ii) HCl/THF (1:1), rt, 93%; (h) PPh_3HBr, MeCN, reflux, 98%; (i) (i) NaOMe, DCM, rt; (ii) Pd/C (10%), H_2, EtOAc, rt, 81–89%; (j) BBr_3, DCM, −40 °C to rt, 80–86%.

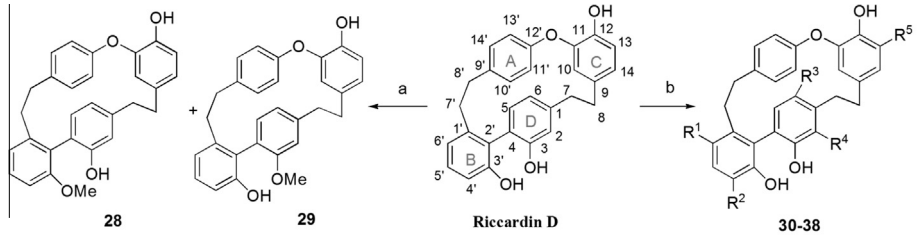
pound **22** by boron tribromide. The derivatives **26** and **27** were synthesized by the method similar to that described for compound **25** (Scheme 1).

The preparation method for the methylated derivatives is shown in Scheme 2. Treatment of riccardin D and iodomethane in the presence of potassium carbonate in acetone afforded compounds **28** and **29**. The methylated position of riccardin D was determined by NOE spectrum as shown in Figure 1. Correlations of H_3-15 with H-4′ and H-2 were determined in compound **28** and compound **29**, respectively (Figs. S5–S6 and S9–S10).

We have previously synthesized two brominated derivatives of riccardin D with *N*-bromosuccinimide (NBS) and evaluated their anticancer activity.[11] In this Letter, a more convenient and efficient method was applied for the bromination. Riccardin D was treated with HBr in the presence of DMSO in ethyl acetate (Scheme 2),[30] and derivatives **30**–**38** were provided by HPLC separation.

The antibacterial activity of the riccardin D derivatives were assessed in vitro against Gram-positive *Staphylococcus aureus* strains SDS15016 and MRSA SDSM1503, both strains were clinical isolates from The Second Hospital of Shandong University China. The minimum inhibitory concentration (MIC, μg/mL) was determined by the liquid microdilution method. Cefotaxime, proctaphlin, ceftazidime and cloxacillin were used as positive antimicrobial agents. The antifungal activity of the synthesized compounds was evaluated against *Candida albicans* SC5314, and fluconazole served as the positive antifungal agent. The susceptibility test was performed according to the guidelines of CLSI.

Antibacterial activities of riccardin D and its derivatives are summarized in Table 1. The lead riccardin D exhibited moderate antibacterial activity and inhibited the growth of *S. aureus* SDS15016 and MRSA SDSM1503 at 4 μg/mL and 8 μg/mL, respectively. Most of the derivatives demonstrated a two- to eight-fold improvement in MIC values. Compound **32**, with a bromine atom at C-4′ of arene *B*, displayed the most potent antibacterial activities against *S. aureus* and MRSA with MIC values of 0.5 μg/mL and 1 μg/mL, respectively, and was eight-fold better than the parent riccardin D. Its activity against *S. aureus* was comparable to that of cloxacillin, and the anti-MRSA activity of compound **32** was much



30 R^1 = H, R^2 = H, R^3 = H, R^4 = Br, R^5 = H
31 R^1 = H, R^2 = Br, R^3 = H, R^4 = Br, R^5 = H
32 R^1 = H, R^2 = Br, R^3 = H, R^4 = H, R^5 = H
33 R^1 = Br, R^2 = H, R^3 = H, R^4 = Br, R^5 = H
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35 R^1 = H, R^2 = H, R^3 = Br, R^4 = H, R^5 = H
36 R^1 = H, R^2 = Br, R^3 = Br, R^4 = H, R^5 = H
37 R^1 = Br, R^2 = H, R^3 = Br, R^4 = H, R^5 = H
38 R^1 = H, R^2 = Br, R^3 = H, R^4 = H, R^5 = Br

Scheme 2. Synthesis of riccardin D derivatives. Reagents and conditions: (a) MeI, K_2CO_3, acetone; (b) HBr, DMSO, EtOAc.

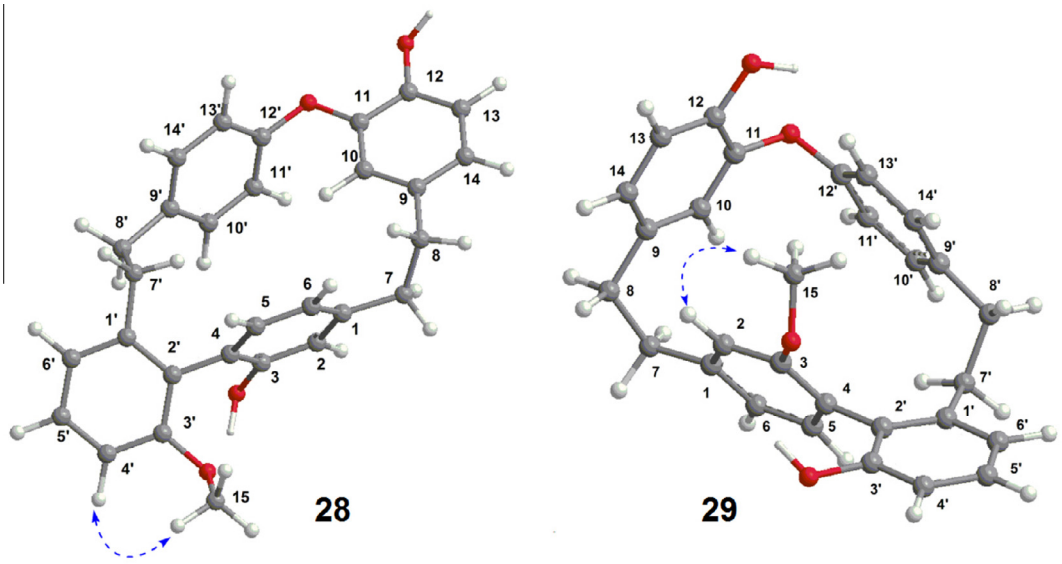

Figure 1. Key NOESY correlations (dashed blue arrows) for compounds **28** and **29**.

Table 1
In vitro antimicrobial activity of riccardin D derivatives[a]

Structures	*S. aureus*	MASA	*C. albicans*	Structures	*S. aureus*	MASA	*C. albicans*
25	2	8	>64	**33**	2	4	8
26	2	4	>64	**34**	1	2	2
27	8	16	>64	**35**	2	4	>64
28	2	4	>64	**36**	2	4	>64
29	2	8	>64	**37**	2	4	8
30	2	8	>64	**38**	8	16	>64
31	4	16	>64	Riccardin D	4	8	16
32	0.5	1	>64	Ceftazidime	4	>128	N/T[b]
Cefotaxime	4	>128	N/T[b]	Cloxacillin	0.5	>128	N/T[b]
Proctaphlin	8	>128	N/T[b]	Fluconazole	N/T[b]	N/T[b]	2

[a] Mean values based on three independent experiments; all data are minimum inhibitory concentrations (MIC), given as μg/mL.
[b] N/T means not tested.

better than the reference drugs cefotaxime, proctaphlin, ceftazidime and cloxacillin. In addition, introduction of bromine at C-6′ of arene *B* led to another excellent antibacterial compound **34**, which showed four-fold enhancement of the MIC. Compounds **30** and **35**, both with a bromine atom on arene D, also exhibited slightly improved antibacterial activity compared with riccardin D. Unexpectedly, di-bromination of riccardin D did not significantly enhance the antibacterial activity and the MIC values of all di-brominated derivatives **31**, **33**, **36**, **37**, and **38** were higher than the values of mono-brominated derivatives **32** and **34**. This finding indicates that bromination improves antibacterial activity, and the mono-brominated derivatives, especially with the bromine atom on arene *B*, exhibit optimum potency against both *S. aureus* and MRSA. Furthermore, the MIC for **25**, **26** (containing four hydroxyl

groups) and **28**, **29** (containing one methoxyl group and two hydroxyl groups) matched or exceeded the MIC attained for riccardin D. The presence of five hydroxyl groups reduced the antibacterial activity of riccardin derivative **27**, which is most likely due to poor lipophilicity.

Riccardin D and its derivatives were demonstrated to inhibit the growth of *Candida albicans*, and their MICs are shown in Table 1. Compound **34** exhibited excellent antifungal activity with a MIC value of 2 μg/mL, eight-fold better than riccardin D. Its activity against *C. albicans* is comparable to that of the positive control drug fluconazole. Compounds **33** and **37** both display moderate antifungal activity with MIC values of 8 μg/mL, two-fold better than riccardin D. The MIC values of other derivatives exceeded the MIC for riccardin D and were more than 64 μg/mL. This suggests the addition of bromine at the 6′- position of arene B is critical for increased the antifungal activity.

In conclusion, we have prepared a series of riccardin D derivatives and evaluated their in vitro antimicrobial activity against *S. aureus* SDS15016, MRSA SDSM1503, and *C. albicans* SC5314. Compound **32** exhibited excellent antibacterial activity against both methicillin-sensitive and -resistant *S. aureus*, with MIC values of 0.5 μg/mL and 1 μg/mL, respectively. Compound **34** also displayed potent antifungal activity with a MIC value of 2 μg/mL. Further explanation of the molecular target of these novel riccardin D derivatives will be presented in a future publication. The characterization of these novel compounds presents an intriguing step in the development of a novel class of therapeutic agents effective for treating bacterial and fungal infections.

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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.2016.06.006.

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