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Synthesis and antibacterial activity of emodin and its derivatives against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Synthesis of the antibacterial emodin was improved using Friedel-Crafts acylation as a key step leading to 37% overall yield. In addition, 21 analogues were synthesized by structural modification of the hydroxyl and methyl groups, as well as the aromatic ring of emodin. Antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and cytotoxicity against noncancerous Vero cells were evaluated. A structure-activity relationship (SAR) study indicated that the hydroxyl groups and the methyl group in the emodin skeleton were crucial for anti-MRSA activity. Furthermore, the presence of an iodine atom or ethylamino group on the aromatic ring enhanced the anti-MRSA activity with higher selectivity indices, while derivatives containing bromine, chlorine atoms or quaternary ammonium salt were as active as emodin. The quaternary ammonium group on the aromatic ring also led to non-cytotoxicity against Vero cells.

Introduction

The widespread use of antibiotics, especially their clinical misuse, has resulted in an increase of multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is becoming a global problem [1]. Only a few antibiotics such as vancomycin, linezolid, daptomycin, and ceftaroline have been used against infections caused by MRSA. Emodin (Fig. 1), which belongs to the anthraquinone family, is an active component of a traditional Chinese medicinal herb (Da Huang, rhubarb) [2]. It has been shown to exhibit various biological activities including anti-inflammatory [3–5], anti-cancer [3], antiviral [4], antiulcerogenic [6], vasorelaxant [7] and T-cell and B-cell immunosuppressive [4] effects. In particular, it showed antibacterial activities against MRSA252 and 36 clinical MRSA strains with MIC values in the range of 2–8 μg/mL [8], as well as MRSA-SK1 with a MIC value of 4 μg/mL [9]. The anti-MRSA mechanism of emodin which involves damaging the cell membrane has been investigated [8]. There have been a number of literature precedents for the anti-MRSA activity of emodin analogues obtained either from natural sources or simple semisyntheses [10–22]. However, there are only a few derivatives, for example aloe emodin, which displayed strong activity [10]. There have been several reports regarding the synthesis of emodin. Usually, a Diels-Alder reaction [23–27] or Friedel-Crafts acylation [28–31] was utilized as a key step. However, there are some drawbacks of the reported syntheses, such as the requirement of expensive, toxic or controlled substances, difficult set-up, harsh conditions or low yields.

As part of an ongoing search for biologically active compounds from fungi, our research group has found that emodin and its derivatives: isorhodoptilometrin and penicillanthranin A, isolated from the marine-derived fungus *Trichoderma aureoviride* PSU-F95 [12] and the sea fan-derived fungus *Penicillium citrinum* PSU-F51 [13], respectively, displayed antibacterial activity against MRSA with respective MIC values of 4, 16 and 16 μg/mL. Interestingly, emodin was weakly cytotoxic to noncancerous Vero cells (African green monkey kidney fibroblasts) with an IC_{50} value of 42.5 μM, and its citrinin-substituted derivative, penicillanthranin A, was non-cytotoxic to Vero cells. Owing to the antibacterial potential of emodin and its derivatives, we pursued the improved synthesis and chemical structure modification of emodin as well as an evaluation of the antibacterial activity of emodin analogues against MRSA, together with cytotoxicity against noncancerous Vero cells. Accordingly, emodin derivatives with stronger activity and higher selectivity indices were discovered, and a structure-activity relationship (SAR) was also established.

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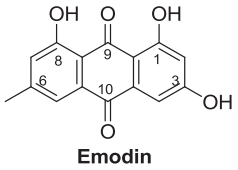


Fig. 1. Structure of emodin.

Results and discussion

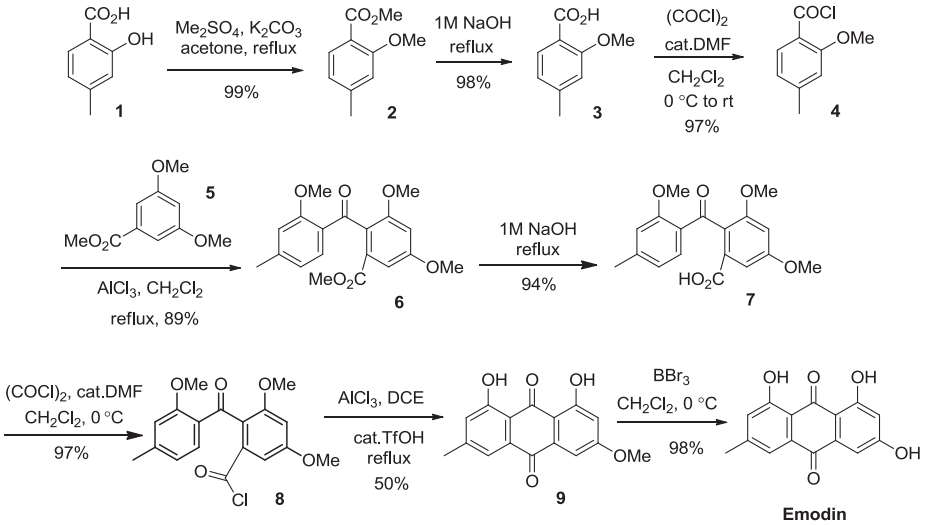
The improved synthesis of emodin commenced with the methylation of 4-methylsalicylic acid (**1**), followed by hydrolysis of the resulting methyl ester **2** to give carboxylic acid **3** (Scheme 1). Acid chloride **4** was obtained by treating the carboxylic acid **3** with oxalyl chloride and cat. DMF. An intermolecular Friedel-Crafts acylation between acid chloride **4** and methyl 3,5-dimethoxybenzoate (**5**) in the presence of aluminum chloride resulted in the formation of benzophenone **6**. In contrast to the previously reported synthesis [30], deprotection of the methyl groups was not observed under these conditions, and it also provided a better yield. Conversion of **6** into the corresponding acid chloride **8** was achieved in two high-yielding steps: hydrolysis of the methyl ester **6** to give the carboxylic acid **7**, followed by reaction with oxalyl chloride and cat. DMF. Intramolecular Friedel-Crafts acylation of **8** was accomplished using aluminum chloride and cat. triflic acid [32] to furnish anthraquinone **9**. This step required a much lower temperature (83 °C) than that (170 °C) used in the reported intramolecular cyclization of carboxylic acid **7** [30]. Finally, demethylation using boron tribromide gave emodin. The synthesis afforded emodin in 8 steps in 37% overall yield, which was higher than the previously reported one from the same starting material (11% overall yield) [30].

In order to evaluate the effect of the hydroxyl groups of emodin on anti-MRSA activity, the first series of analogues were synthesized by chemical modification at these groups (Scheme 2). The methylation of emodin was achieved by treatment with either iodomethane or methyl sulfate in the presence of K_2CO_3 to give monomethylated emodin, 3-methoxyemodin (**9**), and fully methylated emodin, 1,3,8-trimethoxyemodin (**10**), respectively. Treatment of **10** with boron tribromide resulted in demethylation at only the 8-position to give 1,3-dimethoxyemodin (**11**). Since previous study disclosed that the antibacterial activity of methacrylate monomers against *Staphylococcus aureus* increased with an increase in the alkyl chain length by penetration through bacterial cells to disrupt membranes [33], alkylation of the nonchelated hydroxyl group was performed by treating emodin with 2-[2-(2-iodoethoxy)ethoxy]ethanol or 1-bromododecane in the presence of K_2CO_3 to give alkoxy analogues **12** and **13**, respectively.

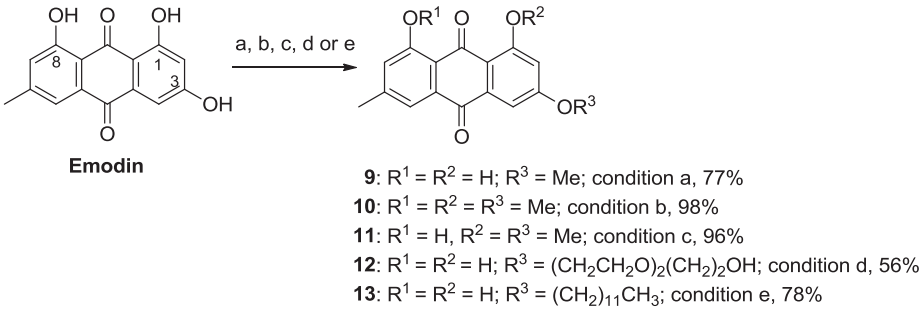
With the aim to identify the importance of the methyl group, which is located at C-6, the second series of emodin analogues were prepared (Scheme 3). The bromination of trismethylated emodin **10** using *N*-bromosuccinimide and dibenzoyl peroxide as an initiator, followed by oxidation with silver nitrate were performed according to literature procedures to give emodin analogues **14** and **15**, respectively [34]. Oxime **16**, carboxylic acid **17** and primary alcohol **18** were synthesized from aldehyde **15** by condensation with hydroxylamine hydrochloride, oxidation with sodium chlorite, and reduction with sodium borohydride, respectively. Derivatives **19**–**21** were obtained after the demethylation of **16**–**18**, respectively, with boron tribromide. However, attempts to demethylate aldehyde **15** were unsuccessful.

Structure modification at the aromatic ring afforded the third series of emodin analogues (Scheme 4). 2,4-Dichloro, 2,4-dibromo and 2,4-diiodo emodin (**22**, **23** and **25**) were synthesized by treatment of emodin with *N*-chlorosuccinimide, *N*-bromosuccinimide and excess iodine, respectively [35]. Monoiodination at the 2-position could be achieved using only 1.5 eq of iodine to give 2-iodoemodin (**24**). Carbon-carbon bond formation at the C-2 position could be achieved *via* a Mannich reaction using dimethylamine and benzaldehyde to provide emodin Mannich base **26** [36]. Amination at the 4-position gave analogues **27** and **28** using the corresponding amines [37]. Quaternary ammonium compounds have been reported as broad spectrum cationic antimicrobials by binding to the cell membrane to cause cytoplasmic leakage, and have low toxicity [38]. Therefore, amino analogue **28** was doubly methylated to afford quaternary ammonium iodide **29**.

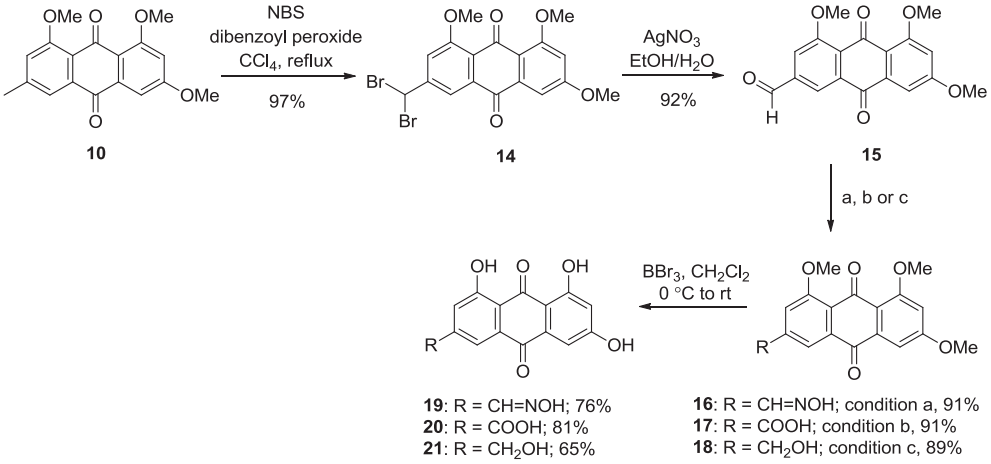
Emodin and its analogues **9**–**29** were evaluated for antibacterial activity against MRSA-SK1 using a colorimetric broth microdilution test [39] and cytotoxic activity against noncancerous Vero cells using the green fluorescent protein (GFP)-based assay [40]. Results of active compounds **22**–**29** are shown in Table 1. Vancomycin and ellipticine were used as positive controls for the anti-MRSA and cytotoxic activities, respectively. The parent compound, emodin, exhibited moderate anti-MRSA activity with a MIC value of 4 μg/mL and weak cytotoxicity against Vero cells with



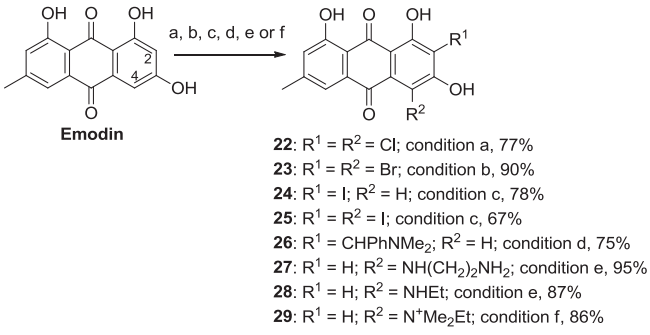
Scheme 1. Synthesis of emodin.



Scheme 2. Structure modification at the hydroxyl groups. Reagents and conditions: (a) MeI, K_2CO_3, acetone, reflux; (b) Me_2SO_4, K_2CO_3, MeCN, reflux; (c) MeI, K_2CO_3, acetone, reflux, then BBr_3, CH_2Cl_2, 0 °C to rt; (d) 2-[2-(2-iodoethoxy)ethoxy]ethanol, K_2CO_3, acetone, reflux; (e) 1-bromododecane, K_2CO_3, DMF, 70 °C.



Scheme 3. Structure modification at the methyl group. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOH, $EtOH/H_2O$, reflux; (b) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O, 0 °C to rt; (c) $NaBH_4$, MeOH, 0 °C to rt.

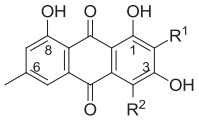


Scheme 4. Structure modification at the aromatic ring. Reagents and conditions: (a) NCS, cat. $ZrCl_4$, 1,4-dioxane, 70 °C; (b) NBS, THF, rt; (c) I_2 (1.5 or 8 eq), $NaHCO_3$, THF/H_2O, 0 °C to rt; (d) Me_2NH, PhCHO, 1,4-dioxane, 75 °C; (e) $NH_2(CH_2)_2NH_2$ or NH_2Et, $PhI(OAc)_2$, rt; (f) NH_2Et, $PhI(OAc)_2$, rt, then MeI, MeCN, 60 °C.

an IC_{50} value of 42.5 μM. All analogues modified at the chelated and non-chelated hydroxyl groups (**9**–**13**) were inactive towards MRSA at 200 μg/mL, and non-cytotoxic against Vero cells at 50 μg/mL. Analogues **12** and **13** with long chain alkoxy groups at the 3-position also lacked anti-MRSA activity and cytotoxicity. The importance of all of the hydroxyl groups of emodin to anti-MRSA activity was emphasized by the previous observation that pachybasin, an anthraquinone containing hydrogen atoms at the 1- and 3-positions instead of the hydroxyl groups in emodin, was inactive against MRSA-SK1 [12]. The absence of the hydroxyl group at the 3-position resulted in the inactivity of chrysophanol against MRSA [16]. Physcion (**9**) containing the methoxyl group at the 3-position also showed no antibacterial effects on four strains of MRSA [10]. Moreover, emodin-8-*O*-glucoside has been reported to exhibit no activity against MRSA-252 [14]. Similarly, all derivatives modified at the 6-methyl group (**14**–**21**) were also inactive and non-cytotoxic, although it has been shown that ω-hydroxyemodin (**21**) could limit *Staphylococcus aureus* quorum sensing-mediated pathogenesis and inflammation [41]. These results were supported by the inactivity of 1,3,6,8-tetrahydroxyanthraquinone and other hydroxyl alkylated analogues against MRSA-252 [14], and strongly suggested that the methyl group played an important role on anti-MRSA activity.

All modified analogues at the aromatic ring (**22**–**29**) were active against MRSA (Table 1). Three derivatives: 4-ethylaminoemodin (**28**), 2,4-diiodoemodin (**25**) and 2-iodoemodin (**24**), showed stronger anti-MRSA activity than emodin with MIC values of 0.5, 1 and 2 μg/mL, respectively, and higher selectivity indices (ratio of cytotoxic IC_{50} (μg/mL) to MIC). The order of selectivity index is as follows: 2,4-diiodoemodin **25** (14.8), 4-ethylaminoemodin **28** (9.3), 2-iodoemodin **24** (7.6). The higher selectivity index indicates the greater selective toxicity towards pathogens over host cells [42]. An iodine atom on the aromatic ring (**24** and **25**) increased the antibacterial activity, whereas chlorine (**22**) and bromine (**23**) substitution maintained the activity with higher cytotoxic activities. It has been reported that 2,4-diiodoemodin (**25**) [15] and naturally chlorinated emodin, 1,3,8-trihydroxy-4-chloro-6-methylanthraquinone [17], inhibits the growth of various strains of Gram-positive bacteria including MRSA in the range of 2–32 μg/mL. 2,4-Dibromoemodin (**23**) could be considered as a bactericidal agent since its MBC was 4-fold greater than its MIC [43]. In contrast, the MBCs of emodin and other active derivatives

Table 1
Antibacterial activity against MRSA and cytotoxic activity against Vero cells of compounds **22**–**29**.



Compound	R^1	R^2	Anti-MRSA (μg/mL)		Cytotoxicity (Vero) (μM)	Selectivity index
			MIC	MBC		
Emodin	H	H	4	>200	42.5	2.9
22	Cl	Cl	4	200	9.7	0.28
23	Br	Br	4	16	18.7	1.9
24	I	H	2	>200	38.6	7.6
25	I	I	1	32	28.4	14.8
26	$CHPhNMe_2$	H	16	>200	23.0	0.58
27	H	$NH(CH_2)_2NH_2$	32	>200	NA	0
28	H	NHEt	0.5	8	14.9	9.3
29	H	N^+Me_2Et	4	64	NA	0
Vancomycin[a]			1	1	–	–
Ellipticine[b]			–	–	3.97	–

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration.
NA refers to "not active" which is indicative of no inhibition at >50 μg/mL of a compound tested.
Selectivity Index is ratio of cytotoxic IC_{50} (μg/mL) to MIC (μg/mL).
[a] Positive control for antibacterial assay.
[b] Positive control for cytotoxicity assay.

(**22** and **24**–**29**) were at least 16-fold greater than their MICs. Accordingly, they could only be used as bacteriostatic agents. In contrast to 2-iodoemodin (**24**), Mannich base **26**, an analogue solely substituted at C-2, was 4-fold less active than emodin. While an ethylamino substituent at C-4 (**28**) significantly increased the anti-MRSA activity of emodin by 8-fold, an ethylenediamino substituent (**27**) reduced the activity to the same extent. Interestingly, quaternary ammonium iodide analogue **29**, derived from 4-ethylaminoemodin (**28**), was as potent as emodin against MRSA without cytotoxicity against Vero cells.

Conclusion

In conclusion, we report the improved synthesis of emodin in 37% overall yield using Friedel-Crafts acylation as a key step. To the best of our knowledge, this is the first SAR study of emodin derivatives. Simple chemical modification of the hydroxyl and methyl groups as well as the aromatic ring of emodin provided 21 analogues. The resulting antibacterial activity and cytotoxicity established a SAR, which revealed that the hydroxyl groups as well as the methyl group were crucial for anti-MRSA activity. Analogues modified at the aromatic ring were all active against MRSA. The presence of an iodine atom or ethylamino group enhanced the anti-MRSA activity of emodin, whereas analogue containing quaternary ammonium group was as active as emodin, and non-cytotoxic against Vero cells. Moreover, the presence of two iodine atoms on the aromatic ring led to the greatest selective toxicity towards MRSA over Vero cells.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2019.151004.

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