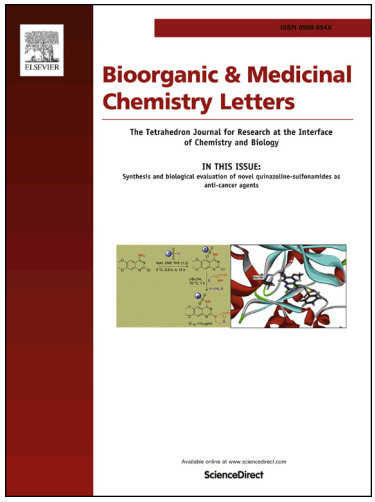



# Syntheses of benzophenone-xanthone hybrid polyketides and their antibacterial activities

Takeshi Kodama, Takuya Ito, Dya Fita Dibwe, So-Yeun Woo, Hiroyuki Morita

PII: S0960-894X(17)30379-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.04.017
Reference: BMCL 24870

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 February 2017
Revised Date: 22 February 2017
Accepted Date: 5 April 2017

Please cite this article as: Kodama, T., Ito, T., Dibwe, D.F., Woo, S-Y., Morita, H., Syntheses of benzophenone-xanthone hybrid polyketides and their antibacterial activities, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.04.017

# Syntheses of benzophenone-xanthone hybrid polyketides and their antibacterial activities

Takeshi Kodama[a], Takuya Ito[a, *], Dya Fita Dibwe[a], So-Yeun Woo[a], Hiroyuki Morita[a, *]

[a] Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan

To whom correspondence should be addressed:

Hiroyuki Morita, Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama, 930-0194, Japan; Tel: +81-76-434-7625, Fax: +81-76-434-5059; E-mail: hmorita@inm.u-toyama.ac.jp.

Takuya Ito, Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama, 930-0194, Japan; Tel: +81-76-434-7627, Fax: +81-76-434-5059; E-mail: itot@inm.u-toyama.ac.jp.

**Abstract**

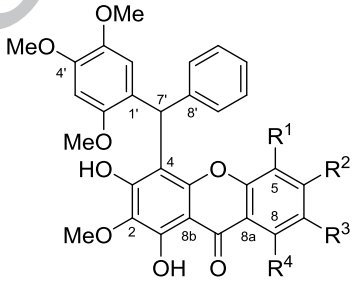
Muchimangins are benzophenone-xanthone hybrid polyketides produced by *Securidaca longepedunculata*. However, their biological activities have not been fully investigated, since they are minor constituents in this plant. To evaluate the possibility of muchimangins as antibacterial agent candidates, five muchimangin analogs were synthesized from 2,4,5-trimethoxydiphenyl methanol and the corresponding xanthones, by utilizing *p*-toluenesulfonic acid monohydrate for the Brønsted acid-catalysis. The antibacterial assays against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and Gram-negative bacteria, *Klebsiella pneumoniae* and *Escherichia coli*, revealed that the muchimangin analogs (±)-1,3,6,8-tetrahydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**1**), (±)-1,3,6-trihydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**2**), and (±)-1,3-dihydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**3**) showed significant activities against *S. aureus*, with MIC values of 10.0, 10.0, and 25.0 μM, respectively. Analogs (±)-**1** and (±)-**2** also exhibited antibacterial activities against *B. subtilis*, with MIC values of 50.0 and 12.5 μM, respectively. Furthermore, (+)-**3** enhanced the antibacterial activity against *S. aureus*, with a MIC value of 10 μM.

Keywords: muchimangin, benzophenone-xanthone hybrid polyketide, Brønsted acid-catalysis, antibacterial activity

Xanthone and benzophenone dimers are a widespread and structurally diverse family of natural products frequently found in plants, fungi, and lichens.[1,2] These natural product dimers possess antimicrobial activity.[2,3] Dicerandrols A-C are examples of the xanthone dimers produced by *Phomopsis longicolla*, with antibacterial activities against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.[4] In contrast, microsphaerin A is an example of a benzophenone dimer derived from *Microsphaeropsis* sp. with antibacterial activity against *S. aureus*.[5] Interestingly, the benzophenone dimer, phomalevone B isolated from *Phoma* sp., reportedly displayed a broad range of antimicrobial activities against *S. aureus*, *B. subtilis*, *Escherichia coli*, and *Candida albicans*.[6] However, the benzophenone monomer lacks these antimicrobial activities.

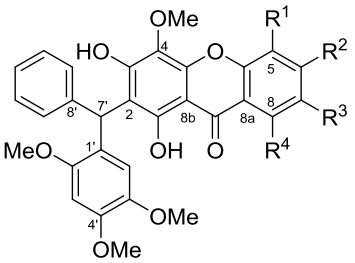
Muchimangins are benzophenone-xanthone hybrid polyketides isolated from the roots of the medicinal plant, *Securidaca longepedunculata* Fresen (Polygalaceae)[7-11] (Fig. 1), used in traditional Congolese medicine. These muchimangins are structurally divided into two types, the C-2 and C-4 diphenylmethyl xanthones. Among them, muchimangin B, a C-2 type diphenylmethyl xanthone, has antiausteric activity against the human pancreatic cancer PANC-1 cell line.[7] However, since muchimangins are minor components in *S. longepedunculata*, their other biological activities have remained unclear. In our ongoing research for the discovery of antibacterial agents, we synthesized new five muchimangin analogs, 1,3,6,8-tetrahydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-

C-4 substituted muchimangins




Muchimangin A: $R^1$=H, $R^2$,$R^3$=-$OCH_2O$-, $R^4$=H
Muchimangin D: $R^1$=H, $R^2$=OH, $R^3$=OMe, $R^4$=H
Muchimangin K: $R^1$=OMe, $R^2$=OH, $R^3$=H, $R^4$=OH

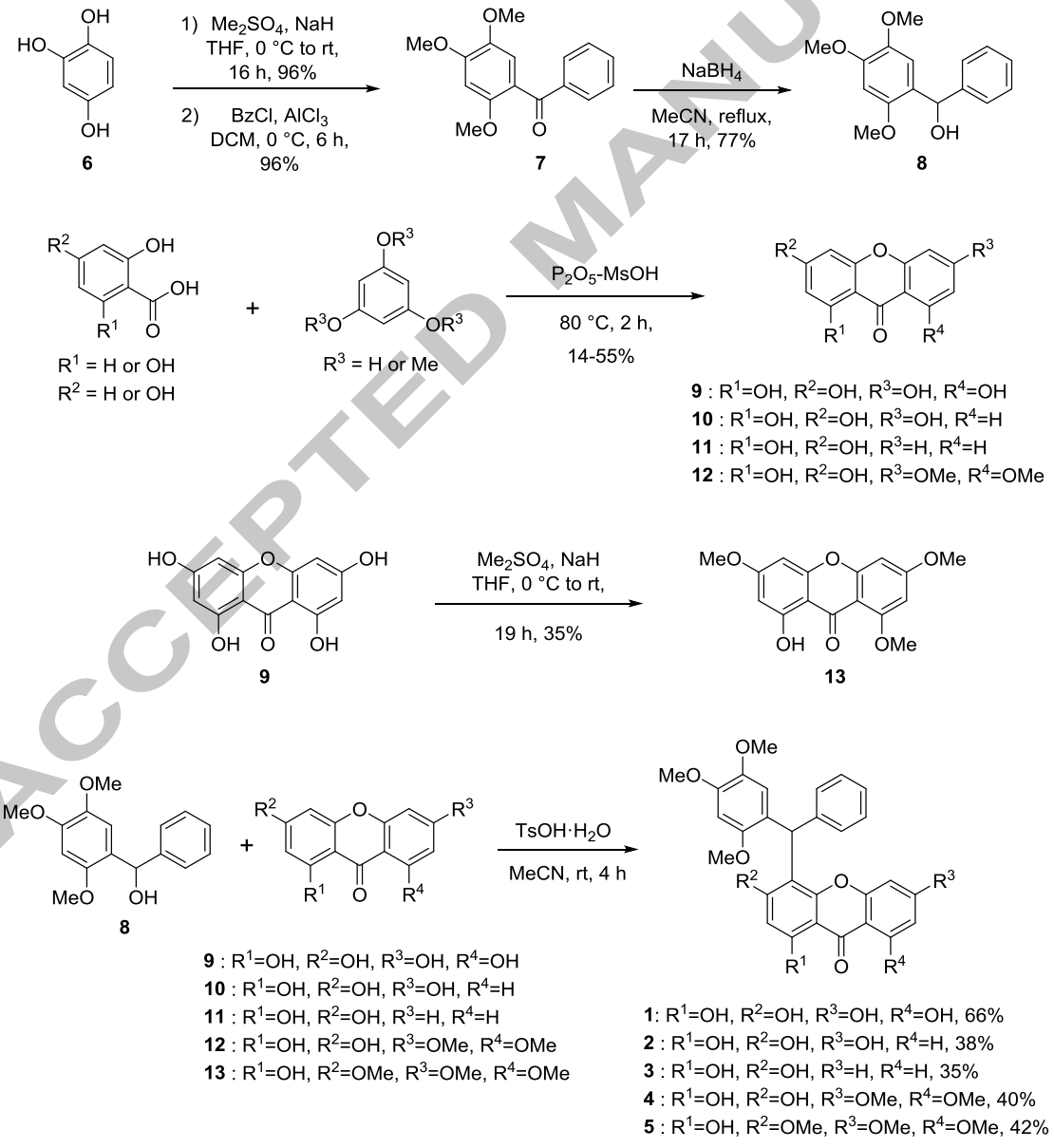
C-2 substituted muchimangins




Muchimangin B: $R^1$=H, $R^2$=OH, $R^3$=OMe, $R^4$=OH
Muchimangin C: $R^1$=OMe, $R^2$=OH, $R^3$=OMe, $R^4$=H
Muchimangin E: $R^1$=OH, $R^2$=OMe, $R^3$=OMe, $R^4$=H

**Fig. 1** Examples of the structures of muchimangins isolated from *Securidaca longepedunculata*.

xanthone (**1**), 1,3,6-trihydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**2**), 1,3-dihydroxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**3**), 1,3-dihydroxy-6,8-dimethoxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)methyl)-xanthone (**4**), and 1-hydroxy-3,6,8-trimethoxy-4-(phenyl-(2',4',5'-trimethoxyphenyl)-xanthone (**5**), and found that (±)-**1** and (±)-**2** display antibacterial activities against *S. aureus* and *B. subtilis*, whereas (±)-**3** shows selective antibacterial activity against *S. aureus*. Herein, we report the syntheses of these analogs and their detailed antibacterial activities, as well as the structure activity relationships of the analogs against *S. aureus* and *B. subtilis*.




**Scheme 1.** Syntheses of muchimangin analogs **1**−**5**.

The synthetic route for the muchimangin analogs is outlined in Scheme 1. Commercially available 1,2,4-trihydroxybenzene (**6**) and dimethyl sulfate were coupled in the present of sodium hydride to give 1,2,4-trimethoxybenzene. Subsequently, the acylated 1,2,4-trimethoxybenzene was treated with benzoyl chloride and aluminium chloride in dichloromethane, to produce 2,4,5-trimethoxybenzophenone (**7**) in 96% yield.[12] Compound **7** was reduced by sodium borohydride in acetonitrile, to furnish part of the muchimangin analog skeleton, 2,4,5-trimethoxydiphenyl methanol (**8**), in 77% yield.[13]

The 1,3,6,8-tetrahydroxy (**9**)-,[14] 1,3,6-trihydroxy (**10**)-,[14] 1,3-dihydroxy (**11**),[15] and 1,3-dihydroxy-6,8-dimethoxy (**12**)[16]-xanthones were synthesized from the corresponding salicylic acid and phenol in phosphorus pentoxide-methanesulfonic acid (Eaton reagent, v/v = 1:10) at 80 °C, according to the published method.[17] Compounds **8** (1.0 equiv.) and **9** (1.0 equiv.) were coupled by Brønsted acid-catalyzed nucleophilic substitution, using *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$, 0.4 equiv.) in acetonitrile,[18] to obtain (±)-**1** as racemic mixture in 66% yield. The structure of (±)-**1** was elucidated on the basis of 1D and 2D NMR, and HR-ESI-MS analyses. The HMBC correlations from 1-OH ($\delta_H$ 12.10, s) to C-1 ($\delta_C$ 161.2), C-2 ($\delta_C$ 100.0), and C-8b ($\delta_C$ 102.4), and from H-7' ($\delta_H$ 6.27, s) to C-3 ($\delta_C$ 162.73), C-4 ($\delta_C$ 108.1), and C-4a ($\delta_C$ 154.7) confirmed the connectivity between C-7' in the diphenylmethyl unit and C-4 in the xanthone unit. The muchimangin analogs (±)-**2**, (±)-**3**, and (±)-**4**, with C-7' of the diphenylmethyl unit linked to C-4 of the xanthone skeleton, were also prepared from the corresponding xanthones **10**, **11**, and **12** and the diphenyl methanol **8**, respectively, using the same procedure as that for the synthesis of **1**. The yields of (±)-**2**, (±)-**3**, and (±)-**4** were 38%, 35%, and 40%, respectively. In addition, the muchimangin analog (±)-**5** was synthesized from 1-hydroxy-3,6,8-trimethylxanthone (**13**) and the diphenyl methanol **8** in 42% yield, using the same procedure as that for (±)-**1**, after **9** was methylated with dimethyl sulfate in tetrahydrofuran to give **13**. Despite the use of $TsOH \cdot H_2O$, which is reported to be an effective Brønsted acid catalyst for the formation of carbon-carbon and carbon-heteroatom bonds,[18-20] none of the C-2 diphenyl methyl substituted muchimangin analogs was produced in these preparations. Presumably, the steric repulsion between the C-1 and C-3

substitutions of the xanthones prevented the nucleophilic substitution of the diphenyl methyl unit to the C-2 of the xanthone scaffold.

The *in vitro* antibacterial activities of the synthesized muchimangin analogs (±)-**1**−**5**, as well as the synthetic intermediates **6**−**13**, were assessed against two Gram-positive bacteria, *S. aureus* NBRC 100910 and *B. subtilis* NBRC 13719, and two Gram-negative bacteria, *Klebsiella pneumoniae* NBRC 14940 and *E. coli* NBRC 102203 (Table 1).[21-24] As in the case of the reported benzophenone monomer, the benzophenone monomer **7** did not display any antibacterial activities against the tested strains. Furthermore, all compounds were inactive against the tested Gram-negative bacteria. In contrast, (±)-**1** showed antibacterial activity against the Gram-positive bacterium, *S. aureus*, with a MIC value of 10.0 μM. However, the antibacterial activity of the corresponding xanthone **9** against *S. aureus* was 5 times weaker than that of (±)-**1**. The muchimangin analogs (±)-**2** and (±)-**3** also displayed antibacterial activities against *S. aureus*, with MIC values of 10.0 μM and 25.0 μM, respectively. Compounds **10** and **11**, the corresponding xanthone skeletons of (±)-**2** and (±)-**3**, respectively, showed weak antibacterial activities against *S. aureus*, in contrast to those of (±)-**2** and (±)-**3**. Furthermore, the muchimangin analogs (±)-**4** and (±)-**5** lacked antibacterial activity against *S. aureus*. In the case of the antibacterial activity against *B. subtilis*, the muchimangin analog (±)-**2** showed the highest activity with a MIC value of 12.5 μM, among the tested compounds. In addition, the muchimangin analog (±)-**1** displayed weak antibacterial activity. However, the muchimangin analogs (±)-**3**, (±)-**4**, and (±)-**5** lacked antibacterial activity against *B. subtilis*. Furthermore, **9**−**13**, corresponding to the xanthone skeletons of (±)-**3**, (±)-**4**, and (±)-**5**, also did not show antibacterial activity against this strain.

Thus, the antibacterial assays revealed that the benzophenone-xanthone hybrid structure enhanced the antibacterial activities. Furthermore, the structure activity relationship analysis of the tested muchimangin analogs suggested that the presence of the hydroxy group at C-6 would be important for the growth inhibitory activities against *S. aureus* and *B. subtilis*. To further clarify the enantiomeric efficacy of the antibacterial activities of **1**, **2**, and **3**, the potent and active muchimangin

**Table 1.** Antibacterial activities of muchimangin analogs **1**−**5** and synthetic intermediates **6**−**13**.

| Compound | MIC (μM) | | Compound | MIC (μM) | |
|---|---|---|---|---|---|
| | *S. aureus* | *B. subtilis* | | *S. aureus* | *B. subtilis* |
| (±)-**1** | 10.0 | 50.0 | **6** | >100 | >100 |
| (+)-**1** | 10.0 | 50.0 | **7** | >100 | >100 |
| (−)-**1** | 12.5 | 100 | **8** | >100 | >100 |
| (±)-**2** | 10.0 | 12.5 | **9** | 50.0 | >100 |
| (+)-**2** | 10.0 | 10.0 | **10** | 100 | >100 |
| (−)-**2** | 10.0 | 12.5 | **11** | 100 | >100 |
| (±)-**3** | 25.0 | >100 | **12** | >100 | >100 |
| (+)-**3** | 10.0 | >100 | **13** | >100 | >100 |
| (−)-**3** | 50.0 | >100 | Ampicillin[a] | 1.25 | 1.25 |
| (±)-**4** | >100 | >100 | Kanamycin[a] | 1.25 | 1.25 |
| (±)-**5** | >100 | >100 | | | |

[a]Positive control.

analogs (±)-**1**, (±)-**2**, and (±)-**3** were separated on a chiral stationary phase column [CHIRALPAK-AD-H, 10 mm × 250 mm (Daicel); detection: UV 340 nm; flow rate: 3.0 mL/min; column temperature: 40 °C], using hexane/ethanol/acetic acid as an eluent to furnish each enantiomer, and their antibacterial activities were evaluated against *S. aureus* and *B. subtilis*. The enantiomers (+)-**1** and (−)-**1** were eluted at retention times of 29.1 min and 20.7 min and showed optical rotations of $[\alpha]_D^{24}$ +25 (*c* 0.02 $CHCl_3$) and −28 (*c* 0.02 $CHCl_3$), respectively. The muchimangin analogs (±)-**2** and (±)-**3** were also separated using the same procedure, to furnish the corresponding enantiomers (+)-**2**, (−)-**2**, (+)-**3**, and (−)-**3**. The antibacterial assays revealed that both enantiomers (+)-**1** and (−)-**1** display antibacterial activities against *S. aureus* at levels comparable to that of (±)-**1**. In the case of the antibacterial assay against *B. subtilis*, (+)-**1** exhibited the same MIC value as that of (±)-**1**, while the activity was significantly decreased with (−)-**1**. In contrast, the enantiomers (+)-**2** and (−)-**2** retained the same efficacy as that of (±)-**2** against both strains. However, the antibacterial activity of (+)-**3** against *S. aureus* was 2.5 and 5 times higher than those of (±)-**3** and (−)-**3**, respectively, although the enantiomers lacked antibacterial activity against *B. subtilis*, as in the case of (±)-**3**. Thus, (+)-**3** possessed the highest activity against *S. aureus*, followed by (±)-**1** and (±)-**2**, suggesting the importance of the stereo-differentiation for the further development of antibacterial muchimangin

analogs.

In conclusion, we demonstrated the antibacterial activities of the muchimangin analogs, as well as the syntheses of the C-4 benzophenone-substituted xanthones, using the corresponding salicylic acids and phloroglucinols. Further evaluations of the structure activity relationships of the muchimangin analogs might contribute to the development of structurally interesting anti-*S. aureus* and anti-*B. subtilis* agents.

**Acknowledgement**

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (T.I. and H.M.).

**A. Supplementary material**

Supplementary data associated with this article can be found, in the online version, at XXX.

**References and Notes**

1. Masters, K. S.; Bräse, S. *Chem. Rev.* **2012**, *112*, 3717.
2. Wu, S. B.; Long, C.; Kennelly, E. J. *Nat. Prod. Rep*. **2014**, *31*, 1158.
3. Wezeman, T.; Bräse, S.; Masters, K. S. *Nat. Prod. Rep*. **2015**, *32*, 6.
4. Chaesung, L.; Kim, J.; Choi, J. N.; Ponnusamy, K.; Jeon, Y.; Kim, S. U.; Kim, J. G.; Lee, C. H. *J. Microbiol. Biotechnol*. **2010**, *20*, 494.
5. Yoganathan, K.; Cao, S.; Crasta, S. C.; Aitipamula, S.; Whitton, S. R.; Ng, S.; Buss, A. D.; Butler, M. S. *Tetrahedron* **2008**, *64*, 10181.
6. Shim, S. H.; Baltrusaitis, J.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2011**, *74*, 395.
7. Dibwe, D. F.; Awale, S.; Kadota, S.; Tezuka, Y. *Tetrahedron Lett*. **2012**, 53, 6186.
8. Dibwe, D. F.; Awale, S.; Kadota, S.; Morita, H.; Tezuka, Y. *Bioorg. Med. Chem*. **2013**, *21*, 7663.

9. Dibwe, D. F.; Awale, S.; Kadota, S.; Morita, H.; Tezuka, Y. *Tetrahedron Lett*. **2014**, *55*, 1916.

10. Dibwe, D. F.; Awale, S.; Kadota, S.; Morita, H.; Tezuka, Y. *J. Nat. Prod*. **2014**, *77*, 1241.

11. Dibwe, D. F.; Awale, S.; Kadota, S.; Morita, H.; Tezuka, Y. *Nat. Prod. Commun.* **2014**, *9*, 655.

12. Kamino, S.; Ichikawa, H.; Wada, S.; Horio, Y.; Usami, Y.; Yamaguchi, T.; Koda, T.; Harada, A.; Shimanuki, K.; Arimoto, M.; Doi, M.; Fujita, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4380.

13. Waterlot, C.; Couturier, D.; Backer, M. D.; Rigo, B. *Can. J. Chem.* **2000**, *78*, 1242.

14. Liu, Y.; Zou, L.; Ma, L.; Chen, W. H.; Wang, B.; Xu, Z. L. *Bioorg. Med. Chem.* **2006**, *14*, 5683.

15. Zhou, T.; Shi, Q.; Chen, C. H.; Huang, L.; Ho, P.; Morris-Natschke, S. L.; Lee, K. H. *Eur. J. Med. Chem.* **2012**, *47*, 86.

16. Brahmachari, G.; Gorai, D.; Mondal, S.; Gangopadhyay, A.; Chatterjee, D. *J. Chem. Res. Synopses* **2003**, 362.

17. Kiang Lim, C.; Tho, L. Y.; Hoe Lim, C.; Mooi Lim, Y.; Adnan Ali Shah, S.; Faizal Weber, J. F. *Lett. Drug. Des. Discov.* **2001**, *8*, 523.

18. Sanz, R.; Martínez, A.; Miguel, D.; Álvarez-Gutiérrez, J. M.; Rodríguez, F. *Adv. Synth. Catal.* **2006**, *348*, 1841.

19. Urgaonkar, S.; La Pierre, H. S.; Meir, I.; Lund, H.; RayChaudhuri, D.; Shaw, J. T. *Org. Lett*. **2005**, *7*, 5609.

20. Iovel, I.; Mertins, K.; Kischel, J.; Zapf, A.; Beller, M. *Angew. Chem. Int. Ed*. **2005**, *44*, 3913.

21. *Antimicrobial Assay:* The microdilution method described by Eloff was used to determine the minimum inhibitory concentration (MIC) values for the synthetic compounds against bacteria, with some modifications. The respective MIC values of the samples were determined using two Gram-positive bacteria, *S. aureus* NBRC 100910 and *B. subtilis* NBRC 13719, and two Gram-negative bacteria, *Klebsiella pneumoniae* NBRC 14940 and *E. coli* NBRC 102203. Briefly, 2 µL of pure compounds (1 mM) in duplicate, dissolved in dimethyl sulfoxide (DMSO, concentration less than 5%), were added to 198 µL of bacterial culture in YP medium (1% polypeptone, 0.2% yeast extract, and 0.1% $MgSO_4 \cdot 7H_2O$) and serially diluted two-fold in 96-

well microtiter plates. The densities of the bacterial cultures were approximately $1.5 \times 10^6$ cfu/mL, obtained by dilution (1:1,000) from the overnight bacterial cultures in YP medium. The plates were incubated overnight at 37 °C, and the MICs were determined as the lowest concentration inhibiting microbial growth. The plates were further incubated at 37 °C overnight. Ampicillin and kanamycin were used as reference reagents for the bacterial strains.

22. Eloff, J. N. *Planta Medica* **1998**, *64*, 711.

23. Nisa, K.; Ito, T.; Subehan; Matsui, T.; Kodama, T.; Morita, H. *Phytochem. Lett.* **2016**, *15*, 42.

24. Awouafack, M. D.; Ito, T.; Tane, P.; Kodama, T.; Tanaka, M.; Asakawa, Y.; Morita, H. *Phytochem. Lett.* **2016**, *15*, 220.