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Caged-Tetraprenylated Xanthones from *Garcinia scortechinii*

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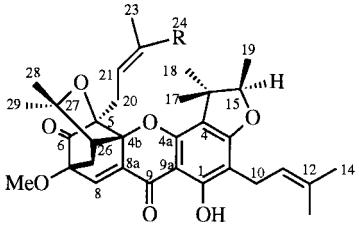
Abstract—Three new caged tetraprenylated xanthones, named scortechinones A–C (**1**–**3**) were isolated from twigs of *Garcinia scortechinii* together with friedelin and stigmasterol. The scortechinone structures were elucidated by analysis of spectroscopic data and comparison of the NMR data with those of gaudichaudione H (**4**). The antibacterial activity against methicillin-resistant *Staphylococcus aureus* was evaluated.

Introduction

Garcinia scortechinii is a small slender tree which is distributed throughout Malaysia and Southern Thailand. In our continuing phytochemical studies of *Garcinia* species in the Ton Nga-Chang Wildlife Sanctury in Songkhla Province, scortechinone A–C (**1**–**3**), new caged tetraprenylated xanthones, were isolated from twigs of *G. scortechinii*. We describe herein the isolation and structural determination of the new compounds by spectroscopic methods. The effect on the inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) is also reported.



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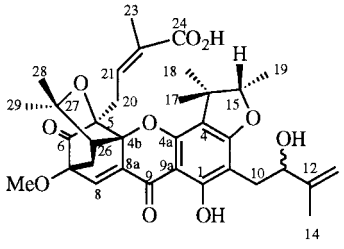
2 R = CO_2H



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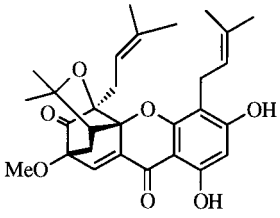

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Results and Discussion

The twigs of *G. scortechinii* were extracted with MeOH and the MeOH extract was then separated into two parts with EtOAc. The EtOAc soluble part, upon repeated chromatography, afforded scortechinone A, B and C (**1**, **2** and **3**)

Keywords: *Garcinia scortechinii*; guttiferae; twigs; caged tetraprenylated xanthones; methicillin-resistant *Staphylococcus aureus*; antibacterial activity.

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Table 1. ^{1}H NMR data of scortechinone A (**1**), B (**2**) and C (**3**)

H	1	2	3
1-OH	13.15 (s, 1H)	13.10 (s, 1H)	13.15 (s, 1H)
8	7.49 (d, *J*=1.4 Hz, 1H)	7.56 (d, *J*=1.2 Hz, 1H)	7.51 (d, *J*=1.4 Hz, 1H)
10	3.22 (md, *J*=7.2 Hz, 2H)	3.17 (mdd, *J*=14.4, 7.2 Hz, 1H) 3.11 (mdd, *J*=14.4, 7.2 Hz, 1H)	2.98 (dd, *J*=14.0, 3.4 Hz, 1H) 2.64 (dd, *J*=14.0, 11.1 Hz, 1H)
11	5.22 (ht, *J*=7.2, 1.4 Hz, 1H)	5.20 (ht, *J*=7.2, 1.5 Hz, 1H)	4.32 (brdd, *J*=11.1, 3.4 Hz, 1H)
13	1.68 (brq, *J*=1.2 Hz, 3H)	1.65 (q, *J*=1.5 Hz, 3H)	5.07 (m, 1H) 4.92 (m, 1H)
14	1.75 (brd, *J*=1.2 Hz, 3H)	1.72 (brs, 3H)	1.87 (m, 3H)
15	4.37 (q, *J*=6.4 Hz, 1H)	4.46 (q, *J*=6.6 Hz, 1H)	4.56 (q, *J*=6.6 Hz, 1H)
17	1.16 (s, 3H)	1.37 (s, 3H)	1.56 (s, 3H)
18	1.58 (s, 3H)	1.37 (s, 3H)	1.37 (s, 3H)
19	1.41 (d, *J*=6.6 Hz, 3H)	1.23 (d, *J*=6.6 Hz, 3H)	1.45 (d, *J*=6.6 Hz, 3H)
20	2.69 (ddh, *J*=14.4,4.5,1.5 Hz, 1H) 2.55 (dd, *J*=14.4, 10.5 Hz, 1H)	3.27 (brdd, *J*=16.0, 9.6 Hz, 1H) 2.83 (ddq, *J*=16.0,4.5,2.0 Hz, 1H)	3.81 (dd, *J*=15.2, 11.8 Hz, 1H) 2.73 (ddq, *J*=15.2,3.4,2.5 Hz, 1H)
21	4.39 (m, 1H)	5.67 (ddq, *J*=9.6, 4.5, 1.5 Hz, 1H)	5.20 (ddq, *J*=11.4,3.4,1.4 Hz, 1H)
23	1.36 (brt, *J*=1.5 Hz, 3H)	1.72 (s, 3H)	1.65 (dd, *J*=2.5, 1.4 Hz, 3H)
24	1.07 (brt, *J*=1.4 Hz, 3H)	–	–
25	2.33 (dd, *J*=12.8, 1.4 Hz, 1H) 1.65 (dd, *J*=12.8, 9.6 Hz, 1H)	2.33 (dd, *J*=13.2, 1.2 Hz, 1H) 1.68 (dd, *J*=13.2, 9.2 Hz, 1H)	2.35 (dd, *J*=13.0, 1.4 Hz, 1H) 1.70 (dd, *J*=13.0, 9.3 Hz, 1H)
26	2.55 (d, *J*=9.6 Hz, 1H)	2.60 (d, *J*=9.2 Hz, 1H)	2.64 (d, *J*=9.3 Hz, 1H)
28	1.71 (s, 3H)	1.72 (s, 3H)	1.71 (s, 3H)
29	1.29 (s, 3H)	1.28 (s, 3H)	1.29 (s, 3H)
OMe	3.62 (s, 3H)	3.52 (s, 3H)	3.65 (s, 3H)

Table 2. ^{13}C NMR data of scortechinone A (**1**), B (**2**) and C (**3**) (* interchangeable)

C	Type of C	1	2	3
1	C	163.26	163.46	163.83
2	C	105.77	105.81	101.66
3	C	166.87	167.08	167.71
4	C	113.03	112.30	112.50
4a	C	153.82	154.07	155.78
5	C	84.19	83.77	84.35
4b	C	89.30	89.38	89.20
6	C	202.26	202.30	203.20
7	C	84.90	84.93	85.15
8	CH	133.96	135.09	134.79
8a	C	132.38	132.38	132.54
9	C	178.23	177.60	178.18
9a	C	101.39	101.27	101.38
10	CH_2	21.42	21.35	28.80
11	CH	121.75	121.69	73.72
12	C	131.98	132.05	146.84
13	CH_3	25.70	25.66	–
	CH_2	–	–	110.31
14	CH_3	17.73	17.72	18.66
15	CH	90.61	91.40	92.43
16	C	43.47	43.50	43.94
17	CH_3	21.07	19.95*	19.28
18	CH_3	24.06	28.09*	28.66
19	CH_3	13.57	15.81	16.77
20	CH_2	28.93	29.91	29.07
21	CH	117.17	136.99	135.65
22	C	135.59	128.68	129.58
23	CH_3	25.47	20.57	21.11
24	C	–	170.67	166.63
	CH_3	16.87	–	–
25	CH_2	30.85	30.54	30.42
26	CH	49.94	49.75	49.74
27	C	83.23	83.71	83.46
28	CH_3	30.78	30.93	30.81
29	CH_3	28.97	28.79	28.88
OMe	CH_3	53.94	53.88	53.82

together with two known compounds, friedelin and stigmasterol.

Scortechinone A (**1**) was found to have the molecular formula $C_{34}H_{42}O_7$ by HRMS. The IR spectrum exhibited absorption bands at 3550 (a hydroxy group), 1748 (an

Table 3. Major correlations for **1**, **2** and **3** in the HMBC experiments

Proton	^{2}J correlation	^{3}J correlation	Notes
H-8	C-7, C-8a	C-4b, C-6, C-9, C-25	
H-10	C-2, C-11	C-1, C-3, C-12	
H-11	C-10, C-12	C-13, C-14	No observed correlations with C-12 in **1** and **2**
Me-13	C-12	C-11, C-14	CH_2 in **3**
Me-14	C-12	C-11, C-13	
H-15	C-16, C-19	C-3, C-4, C-17, C-18	
Me-17	C-16	C-4, C-15, C-18	
Me-18	C-16	C-4, C-15, C-17	
Me-19	C-15	C-16	
H_a-20	C-5, C-21	C-4b, C-6, C-22	
H_b-20	C-5, C-21	C-4b, C-6, C-22	
H-21	C-22	C-23	Also C-24 in **1**
Me-23	C-22	C-21, C-24	
Me-24	C-22	C-21, C-23	**2** and **3** contained no hydrogen at C-24
H_a-25	C-7, C-26	C-4b, C-6, C-8, C-27	
H_b-25	C-7	C-6, C-8, C-27	
H-26	C-4b, C-7, C-27	C-5, C-7, C-28	
Me-28	C-27	C-26, C-29	
Me-29	C-27	C-26, C-28	
1-OH	C-1	C-2, C-3, C-9a	Weak correlation with C-9 (H-bonding)
OMe	–	C-7	

unconjugated carbonyl group) and 1636 cm^{-1} (a chelated *ortho*-hydroxyl carbonyl group). The presence of these carbonyl functionalities was confirmed by the signals at δ_C 202.26 and 178.23 in the ^{13}C NMR spectrum (Table 2). The absorption band at λ_{max} 363 nm was similar to that of caged polyprenylated xanthonoids recently isolated from *Garcinia gaudichaudii*.[1–2] The ^1H NMR (Table 1) and ^1H–^1H COSY spectra together with the data from decoupling experiments revealed the presence of one chelated hydroxyl proton (δ_H 13.15, s, 1-OH), one olefinic proton (δ_H 7.49, d, J=1.4 Hz, H-8, showing W-coupling with H_a-25), one methoxy group (δ_H 3.62, s), one secondary methyl group, 9 tertiary methyl groups and four proton-coupled systems: two units belonging to 3-methylbut-2-enyl groups [δ_H 5.22 (ht, J=7.2, 1.4 Hz, 1H, H-11), 3.22 (md, J=7.2 Hz, 2H, H-10), 1.68 (brq, J=1.2 Hz, 3H, Me-13), 1.75 (brd, J=1.2 Hz, 3H, Me-14); 4.39 (m, 1H, H-21), 2.69 (ddh, J=14.4, 4.5, 1.5 Hz, 1H, H_a-20), 2.55, (dd, J=14.4, 10.5 Hz, 1H, H_b-20); 1.36 (brt, J=1.5 Hz, 3H, Me-23), 1.07 (brt, J=1.4 Hz, 3H, Me-24)], one unit of a 2,2,3-trimethyldihydrofuran ring [δ_H 4.37 (q, J=6.4 Hz, 1H, H-15), 1.58 (s, 3H, Me-18), 1.41 (d, J=6.6 Hz, 3H, Me-19), 1.16 (s, 3H, Me-17)] and one unit of –$C(Me)_2$–$CHCH_2$–C(OMe)–CH═C– [δ_H 7.49 (d, J=1.4 Hz, 1H, H-8), 2.55 (d, J=9.6 Hz, 1H, H-26), 2.33 (dd, J=12.8, 1.4 Hz, 1H, H_a-25), 1.71 (s, 3H, Me-28), 1.65 (dd, J=12.8, 9.6 Hz, 1H, H_b-25), 1.29 (s, 3H, Me-29)]. ^{13}C NMR (Table 2), DEPT and HMQC spectra showed resonances for 16 quaternary carbons, 5 methine carbons, 3 methylene carbons and 10 methyl carbons.

In the HMBC spectrum of **1** (Table 3), the olefinic proton H-8 (δ_H 7.49/δ_C 133.96) showed ^2J correlations with C-7 and C-8a (δ_C 84.90 and 132.38), and ^3J correlations with C-4b, C-6, C-9 and C-25 (δ_C 89.30, 202.26, 178.23 and 30.85). The methylene proton H_a-25 (δ_H 2.33/δ_C 30.85) caused ^2J cross peaks with C-7 and C-26 (δ_C 84.90 and 49.94) and ^3J cross peaks with C-4b, C-6, C-8 and C-27 (δ_C 89.30, 202.26, 133.96 and 83.23). Data from the above HMBC correlations together with the value of ^{13}C chemical shift of C-4b allowed for the construction of a bicyclo[2.2.2]octane ring bearing carbonyl and ether functionalities at C-8a and C-4b, respectively. Further HMBC correlations of the two methyl groups (δ_H 1.71/δ_C 30.78, Me-28; δ_H 1.29/δ_C 28.97, Me-29) to C-26 (δ_C 49.94) and C-27 (δ_C 83.23) suggested that a 2,2-dimethyltetrahydrofuran ring was fused to positions C-5 and C-26 of the bicyclooctane ring to form a tricyclo-4-oxa-[4.3.1.$0^{3,7}$]decan-2-one system. According to the chemical shifts of C-5 and C-26, the oxygen atom of the tetrahydrofuran ring was located at C-5 rather than C-26. The methylene protons H_{ab}-20 (δ_H 2.69 and 2.55/δ_C 28.93) exhibited ^3J correlations with C-4b, C-6 and C-22 (δ_C 89.30, 202.26 and 135.59), indicating the attachment of a 3-methylbut-2-enyl group at C-5. The methoxy group (δ_H 3.62/δ_C 53.94) was placed at C-7 according to the correlation between its protons with C-7 (δ_C 84.90); C-7 had correlations also with H-8, H_a-25, H_b-25 and H-26. The structure of the left-hand part was thus determined as shown, which was identical to that of gaudichaudione H (**4**).[1] The ^3J correlations of the chelated hydroxy proton (δ_H 13.15, 1-OH) with C-2 and C-9a (δ_C 105.77 and 101.39), the methylene protons H-10 (δ_H 3.22/δ_C 21.42) of the other 3-methylbut-2-enyl group with C-1 and C-3 (δ_C 163.26 and 166.87), and two methyl protons [Me-17 (δ_H 1.16/δ_C 21.07; Me-18 (δ_H 1.58/δ_C 24.06)] of the 2,2,3-trimethyldihydrofuran ring with C-4 (δ_C 113.03) suggested that the right-hand part contained a phloroglucinol-type aromatic ring with a chelated hydroxyl group at C-1, a carbonyl group *peri* to the hydroxyl group, the 3-methylbut-2-enyl group at C-2 and the 2,2,3-trimethyldihydrofuran ring at C-3 and C-4. The chemical shift of C-3 (δ_C 166.87) and C-4 (δ_C 113.03) confirmed that the dihydrofuran ring was fused to the aromatic ring by linkage of its *gem*-dimethyl carbon and ring oxygen atom with C-4 and C-3, respectively. The structure of scortechinone A was thus assigned to be **1**.

Support for the relative stereochemistry shown in **1** was provided by NOED and NOESY results. Firstly, Me-13 (δ_H 1.68) and Me-23 (δ_H 1.36) were assigned since they gave selective NOEDs and cross peaks with H-11 and H-21, respectively. Secondly, Me-18 (δ_H 1.58), which gave selective correlations with H-15 (δ_H 4.37), also gave correlations with H_a-20 (δ_H 2.69) and Me-24 (δ_H 1.07); these results indicated that the C-5 methylbutenyl group was located on the same side of the molecule as Me-18 and H-15-the α side in **1**. Dreiding models show that the butenyl group is disposed orthogonally to the plane of the chromanone ring and the group can lie over the ring. This allows the observed interactions and also explains the extraordinary chemical shift of the olefinic H-21 (δ_H 4.39) as the proton can lie in the shielding zone below the C-8, C-8a, C-9 enone system in **1** (this idea was further supported by weak but significant NOE correlations of H-8 with H-21 and Me-23). Finally, Me-28 (δ_H 1.71) showed selective NOED and NOESY correlations with Me-17 (δ_H 1.16) and Me-18 (δ_H 1.58) as well as with H-26 (δ_H 2.55) and Me-29 (δ_H 1.29) whereas Me-29 gave correlations only with H_a-25 (δ_H 2.33), H-26 and Me-28.

Scortechinone B (**2**) with the molecular formula $C_{34}H_{40}O_9$ determined by HRMS showed an additional IR band due to an α,β-unsaturated carboxyl group at 1692 cm^{-1}. The UV absorption band (λ_{max} 366 nm) was similar to that of **1**. Its ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectra revealed that scortechinone B has the same phoroglucinol-type aromatic ring as **1**. The minor difference was found in the prenyl group attached to the tricyclodecane ring. The olefinic proton H-21 (δ_H 5.67, ddq, J=9.6, 4.5, 1.5 Hz) was shifted to lower field, suggesting the replacement of a 3-methylbut-2-enyl substituent with a 3-carboxybut-2-enyl group. This was confirmed by the absence of one methyl group in the ^1H and ^{13}C NMR spectra and the presence of an additional carboxyl carbon (C-24) at δ_C 170.67. The configuration at the C-21 double bond was determined as *Z* by NOESY and NOED experiments which exhibited interactions between H-21 and Me-23. The attachment of the 3-carboxybut-2-enyl substituent at C-5, not C-2, was confirmed by correlations in the HMBC spectra (Table 3). The structure of scortechinone B was therefore determined as **2**. Because of accidental equivalence of the ^1H resonances of Me-17 and Me-18 (δ_H 1.37) it was not possible to use data from the NOESY and NOED experiments to determine the relative stereochemistry of **2** as was done for **1**. Nevertheless, correlations were observed

between Me-17/Me-18 and H_a-20 and Me-28, as well as with H-15.

Scortechinone C (**3**) exhibited similar IR and UV spectra as **2**. From ^1H and ^{13}C NMR data the tricyclo-4-oxa[4.3.1.$0^{3,7}$]decan-2-one moiety of **3** was identical to that of **2**, but the substituent at C-2 on the phloroglucinol-type aromatic ring was different from that of **2**. The characteristic signals of olefinic methylene protons of a terminal double bond (δ_H 5.07 and 4.92, H-13/δ_C 110.31) instead of a methyl signal of a prenyl group and the hydroxymethine proton (δ_H 4.32, brdd, J=11.1, 3.4 Hz, 1H, H-11) established the 2-hydroxy-3-methylbut-3-enyl group. Consequently, scortechinone C was assigned as **3**. The HMBC correlations (Table 3) confirmed the proposed structure. NOED and NOESY correlations observed between Me-17 and Me-28 as well as with H-15, and between Me-18 and H_a-20, suggest that the relative configuration at C-15 in **3** is opposite to that in **1**.

The minimum inhibitory concentration (MIC) of scortechinone A–C (**1**–**3**) against MRSA were found to be 128, 2 and 32 μg/ml, respectively. Scortechinone B and C thus showed promising antibacterial activity while scortechinone A exhibit nonsignificant activity, indicating that the carboxyl group of the isoprenyl side chain might play an important role in the antibacterial activity.

Caged polyprenylated xanthones are exclusively found in several plants which belong to the genus *Garcinia*, e.g. *G. gaudichaudii*,[1–2] *G. forbesii*,[3] *G. hanburyi*[4–5] and *G. morella*.[6–11] However, there has been only one report on the isolation of gaudichaudione H, the first bridgehead methoxylated tetraprenylated xanthone, from *Garcinia gaudichaudii*.[1] We now add three new members to the list of compounds of this type. Caged polyprenylated xanthones are considered to have a mixed shikimate-triacetate and isoprenoid biosynthetic origin.[12] Quillinan et al.[13] proposed that construction of the novel heterocyclic bicyclo[2,2,2]octenone structure involved Claisen rearrangement of a 5,6-diallyl ether followed by an intramolecular Diels–Alder reaction on the intermediate cyclohexane dienone.

Experimental

Plant material

The twigs of *G. scortechinii* were collected at Ton Nga-Chang Wildlife Sanctury in Hat Yai, Songkhla, Thailand in August 1998. The plant material was identified by Mr. Prakart Sawangchote, Department of Biology, where a voucher specimen (No 0006871) has been deposited.

Extraction and isolation

Twigs (860 g) were ground and extracted with MeOH. The MeOH extract was then evaporated under red. pres. to yield a dark-brown residue. The MeOH extract (60 g) was separated into EtOAc soluble and insoluble parts. The EtOAc soluble part (10.5 g) was subjected to silica gel CC and eluted with a gradient of CH_2Cl_2–MeOH, affording 11 frs. Fr 1 (157 mg), upon standing at room temperature, yielded friedelin (10 mg) as white crystals. Fr 3 (345 mg) was purified by silica gel CC using 80% CH_2Cl_2–petrol as eluant followed by reversed-phase CC using Cosmosil 75C_{18}-OPN and eluted with MeOH to give scortechinone A (**1**) (15 mg) as a yellow solid. Fr 4 (340 mg), upon standing at room temperature, afforded stigmasterol (18 mg) as white crystals. Fr 6 was chromatographed over silica gel using CH_2Cl_2–EtOAc with increasing polarity to 5% MeOH–CH_2Cl_2 to give 4 frs. The second fraction was separated by reversed-phase CC using Cosmosil 75C_{18}-OPN and eluted with 40% MeOH–H_2O to give 2 frs. The first fraction was further subjected to preparative TLC with 2% MeOH–CH_2Cl_2 as a developing solvent to obtain scortechinone C (**3**) (9 mg) as a yellow oil. The other fraction was separated by reversed-phase CC with Cosmosil 75C_{18}-OPN followed by preparative TLC using 5% MeOH–CH_2Cl_2 as developing solvent to yield scortechinone B (**2**) (90 mg) as a yellow solid.

Scortechinone A (1). Yellow solid, mp 153–155°C; $[\alpha]_D^{29}$=+18 (c=2.8×10^{-2}, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm: 363 (log ε 3.30); IR $[\nu]_{KBr}$ cm^{-1}: 3550 (O–H), 1748 (C=O), 1636 (C=O); MS *m/z* (rel. int): 562 $[M]^+$ (0.4), 550 (3), 535 (68), 534 (100), 465 (54), 437 (27), 381 (35), 339 (9), 289 (11), 245 (26), 233 (12), 203 (16), 177 (13), 135 (10), 121 (13), 69 (46); HRMS *m/z* 562.29532 for $C_{34}H_{42}O_7$ (calcd 562.29303); ^1H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

Scortechinone B (2). Yellow solid, mp 162–163°C; $[\alpha]_D^{29}$=−105 (c=9.5×10^{-2}, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm: 366 (log ε 3.30); IR $[\nu]_{KBr}$ cm^{-1}: 3600–2500 (O–H), 1745 (C=O), 1692 (C=O), 1644 (C=O); MS *m/z* (rel. int): 592 $[M]^+$ (38), 577 (18), 564 (77), 537 (28), 495 (27), 437 (38), 381 (59), 371 (27), 277 (25), 233 (31), 217 (29), 177 (25), 121 (38), 43 (100); HRMS *m/z* 592.26838 for $C_{34}H_{40}O_9$ (calcd 592.26721); ^1H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

Scortechinone C (3). Yellow oil; $[\alpha]_D^{29}$=−107 (c=1.4×10^{-2}, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm: 365 (log ε 3.91); IR $[\nu]_{KBr}$ cm^{-1}: 3504–2500 (O–H), 1744 (C=O), 1694 (C=O), 1624 (C=O); MS *m/z* (rel. int): 608 $[M]^+$ (2.3), 590 (3.6), 580 (28), 562 (10), 537 (100), 509 (89), 435 (16), 383 (20), 371 (17), 277 (23), 233 (46), 177 (29), 109 (32), 43 (95); HRMS *m/z* 580.26669 for $C_{34}H_{40}O_{10}$–CO (calcd 580.26723); ^1H NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

Antifungal activity testing

MICs were determined by the agar microdilution method.[14] The test substances were dissolved in DMSO (Merck, Germany). Serial two-fold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 128–0.03 μg/ml. MRSA isolated from a clinical specimen, Songklanakarin Hospital, was used as test strain. Inoculum suspensions (10 μl) were spotted on agar-filled wells. The innoculated plates were incubated at 35°C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth.

Vancomycin was used as positive control drug. Growth controls were performed on agar containing DMSO.

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