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p-Terphenyl and Diterpenoid Metabolites from Endophytic *Aspergillus* sp. YXf3

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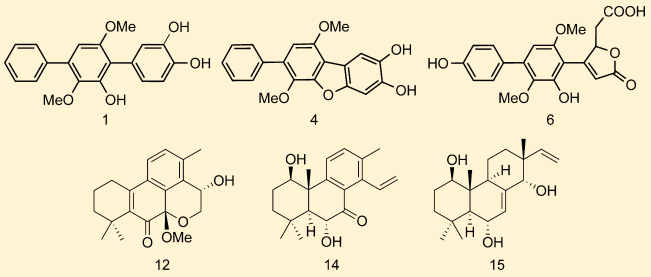
Supporting Information

ABSTRACT: Six new *p*-terphenyl derivatives, named 4″-deoxy-3-hydroxyterphenyllin (**1**), 4″-deoxy-5′-desmethylterphenyllin (**2**), 5′-desmethylterphenyllin (**3**), 4″-deoxycandidusin A (**4**), 4,5-dimethoxycandidusin A (**5**), and terphenolide (**6**), four new diterpenoids with norcleistanthane (aspergiloid A (**12**) and aspergiloid B (**13**)), cleistanthane (aspergiloid C (**14**)), and isopimarane (aspergiloid D (**15**)) type skeletons, and five known *p*-terphenyl compounds (**7**−**11**) were isolated from the fermentation broth of the plant endophytic fungus *Aspergillus* sp. Their structures were elucidated on the basis of detailed spectroscopic analysis and by comparison of their NMR data with those reported in the literature. Compounds **4**, **6**, **7**, and **9** displayed moderate neuraminidase inhibitory activity with IC_{50} values ranging from 4.34 to 9.17 μM.



Fungi belonging to the genus *Aspergillus* (Trichocomaceae) are one of the most prolific resources for such compounds as alkaloids,[1] polyketides,[2] terpenes,[3] cyclopeptides,[4] and *p*-terphenyls.[5,6] Fungal endophytes have attracted considerable attention as a source for finding novel biologically active compounds.[7] Some endophytic *Aspergillus* fungi have been identified from leaves or stems of plants, and chemical investigations have afforded a variety of secondary metabolites with novel structures and interesting bioactivities.[8] In our continued screening for new bioactive secondary metabolites from plant endophytic fungi, 15 compounds (**1**−**15**) were isolated from the fermentation broth extract of *Aspergillus* sp. YXf3, an endophytic fungus isolated from a healthy leaf of *Ginkgo biloba*. Characterization of these compounds by extensive spectroscopic methods revealed six new *p*-terphenyl derivatives, 4″-deoxy-3-hydroxyterphenyllin (**1**), 4″-deoxy-5′-desmethylterphenyllin (**2**), 5′-desmethylterphenyllin (**3**), 4″-deoxycandidusin A (**4**), 4,5-dimethoxycandidusin A (**5**), and terphenolide (**6**), and four new diterpenoids, aspergiloids A−D (**12**−**15**), together with five known *p*-terphenyls identified as 4″-deoxyterphenyllin (**7**),[10] terphenyllin (**8**),[10,11] 3-hydroxyterphenyllin (**9**),[9,12] candidusin C (**10**),[6] and candidusin A (**11**).[13] In this paper, we describe the isolation, structure elucidation, and biological activity of these compounds.

RESULTS AND DISCUSSION

The ethyl acetate extract from a 15 L fermentation broth of *Aspergillus* sp. was fractionated by a combination of column chromatographic methods, resulting in the isolation of 15 metabolites (**1**−**15**).

4″-Deoxy-3-hydroxyterphenyllin (**1**), obtained as colorless columnar crystals, had the molecular formula $C_{20}H_{18}O_5$ (12 unsaturations) according to its HRESIMS data. Analysis of the ^1H, ^{13}C, and HMQC NMR data of **1** revealed the presence of two oxygenated methyl groups, three exchangeable protons, and 18 aromatic or olefinic carbons (nine of which were protonated), which suggested compound **1** has a terphenyl-type structure. Analysis of the splitting patterns for the coupled aromatic proton signals in the ^1H and COSY spectra led to the identification of a monosubstituted, a 1,2,4-trisubstituted, and a pentasubstituted benzene ring. These assignments were further confirmed by relevant HMBC correlations. Key HMBC correlations from H-2 and H-6 to C-4′ and from H-2″ and H-6″ to C-1′ indicated the connection of C-1 with C-4′, and C-1′ with C-1″, respectively. Two oxygenated methyl protons present in compound **1** showed HMBC correlations to C-2′ and C-5′, respectively, indicating that these two carbons bear methoxyl groups, while three hydroxyl protons showed correlations with C-3, C-4, and C-3′, respectively, suggesting that these three carbons carried a hydroxyl group. A NOESY experiment further confirmed the substitution pattern of the central benzene ring in **1**. Strong NOE correlations of H-2″/H-6″ with H-6′ and the OMe-2′ were observed. Moreover, another methoxyl signal at δ_H

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3.65 showed a NOE correlation to H-6′, locating the methoxyl group at C-5′. Thus the structure for compound **1** was assigned as shown in Figure 1, which was confirmed by analysis of HMQC, HMBC, ^{1}H−^{1}H COSY, and NOESY data (Figure 2).

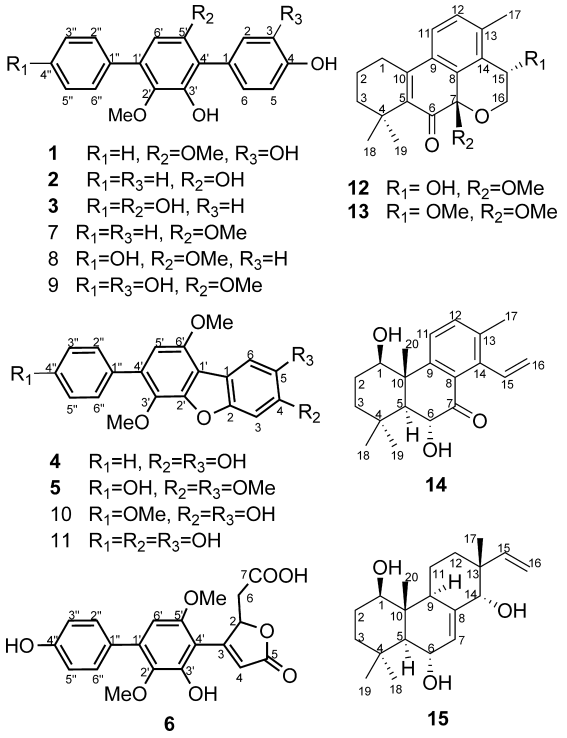

Figure 1. Structures of compounds **1**−**15**.

4″-Deoxy-5′-desmethylterphenyllin (**2**) was isolated as a yellow oil, and it had the molecular formula $C_{19}H_{16}O_4$ by analysis of HRESIMS and NMR data. Its mass was found to be 14 Da lower than that of 4″-deoxyterphenyllin (**7**).[10] The ^{1}H NMR spectrum of **2** was very similar to that of **7**, but with a new phenolic hydroxyl group instead of a methoxyl substituent, thereby suggesting that **2** was a desmethyl analogue of 4″-deoxyterphenyllin (**7**). Further observation of HMBC correlations from OMe-2′ (δ_H 3.26) to C-2′ and NOESY correlations of H-2″/H-6″ with H-6′ and OMe-2′ unambiguously verified the structure of **2**. Overall analysis of 2D NMR data from HMQC, ^{1}H−^{1}H COSY, HMBC, and NOESY experiments led to the full assignment of the structure as shown in Figure 1.

5′-Desmethylterphenyllin (**3**) was obtained as a light green oil. Its molecular formula was determined as $C_{19}H_{16}O_5$ on the basis of HRESIMS analysis and NMR spectra. Initial interpretation of the MS and ^{1}H, ^{13}C, and HMQC NMR data in DMSO-d_6 indicated that **3** was a 5′-desmethyl derivative of terphenyllin (**8**).[10,11] The HMBC spectrum showed a correlation from OMe-2′ (δ_H 3.26) to C-2′, and NOESY correlations of H-2″/H-6″ with H-6′ and OMe-2′ were observed. Thus, the structure of **3** was established as shown in Figure 1.

4″-Deoxycandidusin A (**4**) gave a molecular formula of $C_{20}H_{16}O_5$ (13 unsaturations), as determined by HRESIMS in combination with ^{1}H and ^{13}C NMR data, which were very close to those for candidusin A (**11**).[13] The major differences observed in the NMR spectra for **4** relative to those of **11** revealed their similar structural features except for the presence of a monosubstituted benzene ring and the absence of OH-4″ in **4**. Key HMBC correlations from H-2″/H-6″ to C-4′ and from

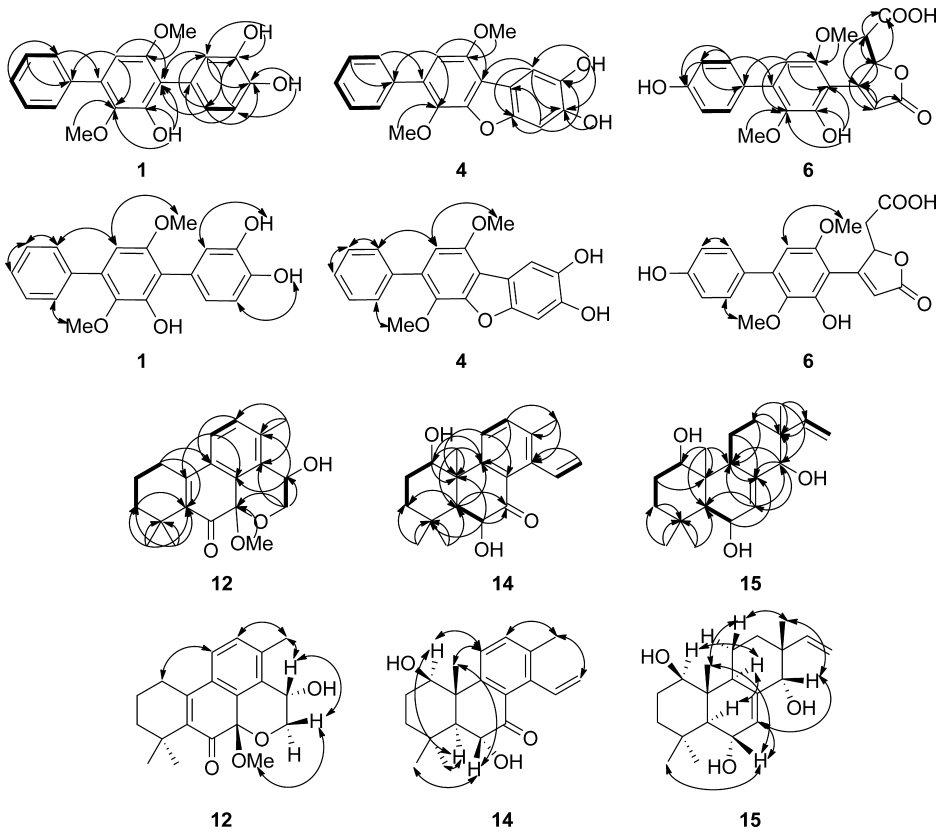

Figure 2. Significant ^{1}H−^{1}H COSY (—), HMBC (→), and NOE correlations (↔) observed for compounds **1**, **4**, **6**, **12**, **14**, and **15**.

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H-5′ to C-1″ linked the monosubstituted ring with the dibenzofuran unit. Strong NOESY correlations of H-2″/H-6″ with H-5′ and the C-3′ methoxyl signal (δ_H 3.79) and of H-5′ with the C-6′ methoxyl signal (δ_H 3.99) supported the assignment of the structure. Complete analysis of NMR data including 1D NMR, HMQC, HMBC, COSY, and NOESY (Figure 2) provided evidence for assignment of the structure of compound **4** as 4″-deoxycandidusin A. Given the close structural relationship of compound **4** with the new terphenyllin analogue **1**, this new candidusin derivative can be viewed as arising from oxidative cyclization of the 3′-hydroxyl group with C-6 in **1**.[14]

4,5-Dimethoxycandidusin A (**5**), a minor metabolite of the fungus, was obtained as a greyish white powder that analyzed for the molecular formula $C_{22}H_{20}O_6$ (13 unsaturations) on the basis of HRESIMS and NMR data. Analysis of these data suggested it was a derivative of candidusin A (**11**),[13] with the presence of only one exchangeable proton and two more methoxyl groups for **5**. HMBC spectroscopic data showed the methoxyl protons at δ_H 4.07, 3.96, 3.92, and 3.84 correlated with C-6′, C-4, C-5, and C-3′, respectively. Significant NOE effects of H-3 with OMe-4, of H-6 with OMe-5, and of H-5′ (δ_H 6.80) with OMe-6′ also confirmed the positions of these methoxyl groups. NOESY correlations of H-5′ with H-2″/H-6″ were also observed. Extensive NMR analysis allowed the assignment of the structure for this compound as shown in Figure 1.

Terphenolide (**6**) was isolated as an off-white solid that analyzed for the molecular formula $C_{20}H_{18}O_8$ (12 unsaturations) by interpretation of HRESIMS and NMR data. The ^{13}C and DEPT NMR spectra displayed resonances for 20 carbons, including 14 aromatic or olefinic carbons, two carbonyl carbons, one oxygenated methine, two oxygenated methyl carbons, and one methylene. Analysis of splitting patterns of protons in the ^1H NMR spectrum and ^{13}C NMR data showed the presence of a 1,4-disubstituted benzene ring and a pentasubstituted benzene ring. The IR data (1738.9 cm^{-1}), together with the UV spectrum [317 nm (3.98)], argued for the presence of a butenolide moiety.[15] The above information indicated that compound **6** could be a terphenyl-like derivative. Key ^3J HMBC correlations (Figure 2) from H-2″/H-6″ to C-1′ and from H-6′ to C-1″ permitted the connectivity of C-1′ to C-1″. HMBC correlations from the methoxyl group at δ_H 3.82 to C-5′ and from the methoxyl group at δ_H 3.28 to C-2′ and NOE effects between OMe-5′ and H-6′ and between OMe-2′ and H-2″/H-6″ demonstrated that the methoxyl groups should be located at C-5′ and C-2′, respectively. The hydroxyl group (δ_H 9.90) was placed on C-3′ on the basis of HMBC correlations from OH-3′ to C-2′, C-3′, and C-4′. The $^1H-^{13}C$ long-range couplings from H-2 to C-3, C-4, C-6, and carboxyl carbon C-7 (δ_C 171.5), from H_2-6 to C-2, C-3, and C-7, and from H-4 to C-3 and carbonyl carbon C-5 (δ_C 173.1) were observed. Finally, the connection between the butenolide moiety and pentasubstituted benzene ring was confirmed by the ^3J HMBC correlation from H-4 to C-4′. Thus, the structure of **6** was elucidated as shown in Figure 1.

The molecular formula of compound **12**, $C_{20}H_{24}O_4$ (9 unsaturations), was determined by HRESIMS in combination with NMR data. The IR spectrum revealed the presence of a hydroxyl group (3415.8 cm^{-1}), a ketone carbonyl (1683.5 cm^{-1}), and a strong absorption for an ether linkage (1026.0 cm^{-1}). Analysis of the ^1H, ^{13}C, DEPT, and HMQC NMR spectra revealed three methyl groups (one aromatic methyl), two *ortho* aromatic protons, one oxygenated methyl group, one oxygenated methylene, an oxygenated methine, and three mutually coupled aliphatic methylene groups. These data for compound **12** were indicative of a nor-cleistanthane structure similar to diterpenoids isolated from Velloziaceae.[16] This suggestion was further supported by 2D NMR analysis. The ^3J HMBC correlations from the aromatic methyl Me-17 to C-12, C-13, and C-14, oxymethine H-15 to C-8 and C-13, oxymethylene H_2-16 to C-14, H-12 to C-9, and H-11 to C-8 defined the 1,2,3,4-tetrasubstituted pattern of the aromatic ring. Interpretation of the HMBC spectrum from H_2-1 to C-5 and C-9, H_2-3 to C-5, Me-18, Me-19 to C-3, C-4, and C-5, and H-11 to C-10 revealed the connections of C-10 to C-5 and C-9 and of C-4 to C-3 and C-5. The COSY correlations between H_2-2 and H_2-1, H_2-3 and between H-15 and H_2-16 revealed the connectivity of C-2 to C-1 and C-3 and of C-15 to C-16. The characteristic ^3J HMBC correlation from the methoxyl protons (δ_H 3.25) to C-7 confirmed their location at C-7. Significant ^3J HMBC correlations from H_2-16 to C-7 supported the connectivity of C-16 to C-7 through an ether bridge, which was also supported by the downfield chemical shift of C-7 (δ_C 92.9). So far, eight degrees of unsaturation had been assigned, while the ketone carbonyl C-6 (δ_C 196.3) was assigned as a conjugated ketone to connect with C-5 and C-7 to complete the last degree of unsaturation. The NOESY correlations of OMe-7 with H-16β (δ_H 4.34) and of H-15 with H-16β suggested the β-orientation of OMe-7 and α-orientation of OH-15. Therefore, the structure of compound **12**, named aspergiloid A, was elucidated as shown in Figure 1. It was possible that the methoxyl group at C-7 resulted from a reaction with methanol during the isolation procedure. It is of interest to note that aspergiloid A was isolated as a racemic metabolite with almost no CD absorption.

Aspergiloid B (**13**) was isolated as a pale yellow powder, with the molecular formula $C_{21}H_{26}O_4$ (9 unsaturations) as derived from HRESIMS data. Its ^1H and ^{13}C NMR spectra were almost identical to those for **12**, except for the presence of one new oxygenated methyl proton at δ_H 3.38 in **13**, suggesting this compound was the 15-methoxyl derivative of **12**. This suggestion was further confirmed by ^3J HMBC correlations of OMe-15 protons with C-15 (δ_C 69.3), and H-15 with the OMe-15 carbon signal. On the basis of the NOESY data, it could be deduced that compound **13** had the same relative configuration as compound **12**. The NOESY correlations of OMe-7 with H-16β (δ_H 4.17) and of H-16α (δ_H 4.43) with OMe-15 protons revealed the β-orientation of OMe-7 and α-orientation of OMe-7. Thus, compound **13** was proposed as shown in Figure 1. However, the methoxyl group at C-7 could also be derived from methanol used in the isolation procedure. Aspergiloid B was also a racemic mixture with almost no CD absorption.

Aspergiloid C (**14**) was isolated as a white powder that gave a molecular formula of $C_{20}H_{26}O_3$ (8 unsaturations) by HRESIMS. The IR spectrum displayed absorption bands at ν_{max} 3426.8 and 1684.9 cm^{-1}, which suggested the presence of hydroxyl and keto carbonyl groups. Analysis of the ^1H NMR spectrum (Table 3) revealed one terminal vinyl group signals at δ_H 7.00 (H-15), 5.47 (H-16a), and 5.13 (H-16b) and a pair of *ortho* aromatic protons at δ_H 8.00 (H-11) and 7.38 (H-12). The ^{13}C NMR spectrum (Table 4) showed a ketone carbonyl signal at δ_C 201.4 (C-7). These data indicated its structure was similar to 8,11,13-cleistanthantrien-7-on-19-oic acid,[17] a cleistanthane diterpenoid. The correlation between H-5 at δ_H 1.70 and H-6 at δ_H 4.54 was observed in the $^1H-^1H$ COSY spectrum. The key

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Table 1. ^{1}H and ^{13}C NMR Data (DMSO-d_6) for Compound 1−3

	1[a]		2		3[b]	
position	δ_C	δ_H (J in Hz)	δ_C[b]	δ_H[a] (J in Hz)	δ_C	δ_H (J in Hz)
1	124.8 C		124.8 C		124.9 C	
2	118.4 CH	6.70, d (1.9)	131.8 CH	7.16, d (8.6)	131.8 CH	7.15, d (8.5)
3	144.3 C		114.1 CH	6.75, d (8.6)	114.1 CH	6.74, d (8.5)
3-OH		8.75, s				
4	144.0 C		155.7 C		155.6 C	
4-OH		8.76, s		9.27, s		9.24, s
5	114.8 CH	6.72, d (8.1)	114.1 CH	6.75, d (8.6)	114.1 CH	6.74, d (8.5)
6	121.9 CH	6.56, dd (8.1, 1.9)	131.8 CH	7.16, d (8.6)	131.8 CH	7.15, d (8.5)
1′	132.3 C		132.2 C		132.1 C	
2′	139.4 C		138.0 C		137.8 C	
2′-OMe	60.3 CH_3	3.30, s	60.2 CH_3	3.26, s	60.0 CH_3	3.26, s
3′	148.2 C		148.1 C		148.0 C	
3′-OH		8.53, s		8.44, s		8.33, s
4′	118.1 C		116.2 C		115.4 C	
5′	153.2 C		151.0 C		150.9 C	
5′-OMe	55.6 CH_3	3.65, s				
5′-OH				8.93, s		8.82, s
6′	103.2 CH	6.43, s	106.8 CH	6.34, s	106.5 CH	6.29, s
1″	138.2 C		138.2 C		128.7 C	
2″	128.6 CH	7.61, d (7.3)	128.2 CH	7.53, d (8.0)	129.4 CH	7.35, d (8.5)
3″	128.3 CH	7.46, t (7.3)	128.3 CH	7.44, t (8.0)	115.1 CH	6.82, d (8.5)
4″	127.2 CH	7.36, t (7.3)	126.9 CH	7.34, t (8.0)	156.5 C	
4″-OH						9.47, s
5″	128.3 CH	7.46, t (7.3)	128.3 CH	7.44, t (8.0)	115.1 CH	6.82, d (8.5)
6″	128.6 CH	7.61, d (7.3)	128.2 CH	7.53, d (8.0)	129.4 CH	7.35, d (8.5)

[a]Recorded at 300 MHz. [b]Recorded at 500 MHz. δ in ppm.

HMBC correlations from methine H-5 to C-3, C-7, and C-9, from oxymethine H-6 to C-4, C-7, and C-10, and from Me-20 to C-1, C-5, C-9, and C-10 were observed. The other oxymethine proton at δ_H 4.04, which showed HMBC correlations to C-9 and C-10, was designated as H-1. The vinyl group at C-14 and the methyl group at C-13 were established by the HMBC correlations from H_2-16 to C-14 and from Me-17 to C-13 and C-14, respectively. The NOE correlations between H-11 and H-1, Me-20 and between H-16b and Me-17 also supported the assignment of **14**. The NOESY spectrum of **14** showed correlations of H-5α with H-1 and Me-19 (δ_H 1.14) and of H-6β with Me-18 (δ_H 1.20) and Me-20, indicating that the α-oriented hydroxyl group at C-6 and OH-1 adopted a β-orientation. The total assignment for **14** was elucidated by a series of 2D NMR experiments, including HMQC, ^{1}H−^{1}H COSY, HMBC, and NOESY spectra, as shown in Figure 2.

Aspergiloid D (**15**) was obtained as a greyish white powder. The molecular formula was estabished as $C_{20}H_{32}O_3$ (5 unsaturations) by HRESIMS and ^{1}H and ^{13}C NMR data. The ^{1}H NMR spectrum displayed characteristic signals at δ_H 5.86 (H-15), 5.20 (H-16a), and 5.14 (H-16b) for a terminal vinyl group, and three oxymethines at δ_H 3.49 (H-1), 4.33 (H-6), and 3.65 (H-14), which were consistent with the results deduced from ^{13}C and DEPT NMR data. Through analysis of the NMR data, compound **15** could be recognized as a new isopimarane derivative closely related to agallochin K,[18] but with different positions of the hydroxyl groups. The key HMBC correlations from H-14 to C-7, C-8, C-9, and C-13, from H-15 to C-14, from H-6 to C-8, and from Me-20 to C-1, C-5, C-9, and C-10 were observed. The ^{1}H−^{1}H COSY correlations between H-1, H_2-3 and H_2-2, between H-9, H_2-12 and H_2-11, and between

Table 2. ^{1}H and ^{13}C NMR Data (500 MHz) for Compound 4−6

	4[a]		5[b]		6[a]	
position	δ_C	δ_H (J in Hz)	δ_C[c]	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	113.4 C		115.1 C			
2	149.4 C		152.5 C		81.4 CH	6.13, dt (9.5, 1.5)
3	98.4 CH	7.08, s	96.1 CH	7.33, s	161.5 C	
4	142.6 C		150.7 C		117.7 CH	6.49, d (1.5)
4-OH		9.43, s				
4-OMe			56.3 CH_3	3.96, s		
5	146.0 C		147.7 C		173.1 C	
5-OH		9.11, s				
5-OMe			56.5 CH_3	3.92, s		
6	107.1 CH	7.39, s	106.0 CH	7.59, s	39.0 CH_2	2.74, dd (16.5, 2.2)
						2.20, dd (16.5, 9.5)
7-COOH					171.5 COOH	12.52, br s
1′	114.7 C		114.9 C		137.0 C	
2′	148.1 C		149.5 C		139.6 C	
2′-OMe					60.6 CH_3	3.28, s
3′	136.0 C		137.3 C		150.7 C	
3′-OH						9.90, s
3′-OMe	60.7 CH_3	3.79, s	56.9 CH_3	3.84, s		
4′	130.3 C		131.1 C		106.1 C	
4′-OH						
5′	105.8 CH	6.76, s	106.6 CH	6.80, s	154.5 C	
5′-OMe					56.3 CH_3	3.82, s
6′	149.4 C		150.8 C		103.5 CH	6.48, s
6′-OMe	55.9 CH_3	3.99, s	56.1 CH_3	4.07, s		
1″	138.1 C		130.3 C		128.0 C	
2″	129.2 CH	7.59, d (7.4)	131.5 CH	7.49, d (8.6)	130.3 CH	7.48, d (8.5)
3″	128.1 CH	7.46, t (7.4)	115.9 CH	6.94, d (8.6)	115.8 CH	6.86, d (8.5)
4″	127.0 CH	7.37, t (7.4)	157.1 C		157.9 C	
4″-OH				8.49, s		9.65, br s
5″	128.1 CH	7.46, t (7.4)	115.9 CH	6.94, d (8.6)	115.8 CH	6.86, d (8.5)
6″	129.2 CH	7.59, d (7.4)	131.5 CH	7.49, d (8.6)	130.3 CH	7.48, d (8.5)

[a]In DMSO-d_6. [b]In Acetone-d_6. [c]Assignment based on HMBC and HMQC data. δ in ppm.

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Table 3. ^{1}H NMR Data (500 MHz, $CDCl_3$) for Compounds 12−15[a]

position	12	13	14	15
1	2.78, dt (18.8, 5.9)	2.77, dt (19.0, 6.0)	4.04, dd (5.3, 9.4)	3.49, dd (11.4, 4.0)
	2.46, m	2.46, dt (19.0, 6.0)		
2	1.80, m	1.81, m	1.81, m	1.65, m
				1.51, m
3	1.64, m	1.63, m	1.56, dt (4.1, 13.5)	1.38, m
	1.51, m	1.50, m	1.42, m	
5			1.70, d (11.1)	1.15, m, overlapped
6			4.54, d (11.1)	4.33, br d (9.2)
7				5.64, br s
9				2.34, m
11	7.32, d (8.0)	7.32, d (8.0)	8.00, d (8.3)	2.27, m
				1.43, m
12	7.20, d (8.0)	7.19, d (8.0)	7.38, d (8.3)	1.97, dt (13.5, 3.7)
				1.32, m
14				3.65 s
15	4.55, br s	4.20, d (3.0)	7.00, dd (11.5, 17.9)	5.86, dd (17.7, 10.8)
16	4.24, d (12.4)	4.17, dd (13.0, 3.0)	5.47, dd (1.5, 11.5)	5.20, d (10.8)
	4.34, dd (12.4, 2.3)	4.43, d (13.0)	5.13, dd (1.5, 17.9)	5.14, d (17.7)
17	2.42, s	2.34, s	2.33, s	0.91, s
18	1.38, s	1.25, s	1.20, s	1.11, s
19	1.26, s	1.37, s	1.14, s	1.15, s, overlapped
20			1.31, s	0.93, s
7-OMe	3.25, s	3.24, s		
15-OMe		3.38, s		

[a] δ in ppm. J in Hz.

Table 4. ^{13}C NMR Data (125 MHz, $CDCl_3$) for Compounds 12−15[a]

position	12	13	14	15
1	27.9 CH_2	27.2 CH_2	75.9 CH	80.5 CH
2	19.2 CH_2	18.4 CH_2	30.0 CH_2	29.6 CH_2
3	41.0 CH_2	40.3 CH_2	39.3 CH_2	41.3 CH_2
4	34.7 C	33.9 C	33.8 C	33.2 C
5	137.2 C	136.4 C	53.8 CH	56.6 CH
6	196.3 C	194.7 C	73.7 CH	68.9 CH
7	92.9 C	91.9 C	201.4 C	131.8 CH
8	132.4 C	132.6 C	127.8 C	138.4 C[b]
9	131.1 C	130.1 C	152.6 C	47.0 CH
10	146.9 C	145.9 C	44.2 C	42.8 C
11	125.5 CH	124.8 CH	124.8 CH	22.9 CH_2
12	132.2 CH	131.2 CH	135.9 CH	27.7 CH_2
13	139.0 C	138.6 C	135.0 C	41.1 C
14	133.7 C	130.4 C	139.7 C	79.3 CH
15	62.7 CH	69.3 CH	136.3 CH	146.0 CH
16	66.3 CH_2	60.4 CH_2	117.5 CH_2	114.2 CH_2
17	18.8 CH_3	18.2 CH_3	20.7 CH_3	22.1 CH_3
18	30.2 CH_3	27.7 CH_3	22.2 CH_3	22.8 CH_3
19	28.4 CH_3	29.5 CH_3	34.2 CH_3	36.2 CH_3
20			18.8 CH_3	8.8 CH_3
7-OMe	51.8 CH_3	50.9 CH_3		
15-OMe		55.5 CH_3		

[a] δ in ppm. [b] Assigned by HMBC corelations.

H-5 and H-6 indirectly supported the location of three hydroxyl groups. The relative configuration of **15** was assigned from the NOESY experiment and analysis of the related coupling constants. The NOESY correlations from Me-18, Me-20 to H-6 and from H-5α, H-1α to H-9α indicated the hydroxyl group at C-1 adopted the β-orientation and OH-6 was α-oriented. The key NOE effects between Me-17, Me-20 and H-11β (δ_H, 1.43) and between Me-17 and H-14 indicated that OH-14 and Me-17 adopted an α- and β-orientation, respecitvely. Therefore, compound 15 was assigned as shown in Figure 1.

All of these isolates were tested for neuraminidase inhibitory activity. Metabolites **4** and **6** showed moderate inhibitory activity with IC_{50} values of 9.05 and 5.79 μM, respectively, as compared to oseltamivir, a clinical drug for the treatment of influenza, which had an IC_{50} value of 0.14 μM. Two known compounds, **7** and **9**, also displayed moderate inhibitory activity with IC_{50} values of 4.34 and 9.17 μM, respectively, whereas other compounds exhibited very weak activity, with IC_{50} values greater than 30 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points (mp) were determined with a Boetius micromelting apparatus and were uncorrected. Optical rotations were recorded on a Rudolph Autopol III automatic polarimeter. The UV spectra were obtained on a Hitachi U-3000 spectrophotometer, and the IR spectra (KBr) were obtained on a Nexus 870 FTIR spectrometer. NMR data were acquired using a Bruker DRX500 NMR spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) or a DPX300 NMR spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR), and TMS (tetramethylsilane) was used as the internal standard (δ in ppm, J in Hz). HRESIMS spectra were measured on an Agilent 6210 TOF LC-MS spectrometer. Silica gel (200−300 mesh) for column chromatography was produced by Qingdao Marine Chemical Factory, Qingdao (China). Sephadex LH-20 was purchased from Pharmacia Biotech (Sweden). Semi-preparative HPLC was performed using a Waters ODS (250 × 4.6 mm) on a Hitachi HPLC system consisting of a L-7110 pump (Hitachi) and a L-7400 UV/vis detector (Hitachi). Neuraminidase inhibitory test kits were purchased from Beyotime Institute of Biotechnology (Haimen, China). All other chemicals used in this study were of analytical grade.

Fungal Material and Cultivation. The strain YXf3 was isolated by one of the authors (Z.K.G.) from a healthy leaf of *Ginkgo biloba* collected in the campus of Nanjing University (Nanjing, P. R. China), in October 2008. The isolate was identified by Dr. Y. C. Song as *Aspergillus* sp. according to its morphological characteristics on potato dextrose agar medium composed of potato (20 g/L), sucrose (20 g/L), agar (20 g/L), and deionized water. The voucher specimen (IFB-YX) is deposited in the Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University. The fungus was subcultured on MEA medium (consisting of 20 g/L malt extract, 20 g/L sucrose, 1 g/L peptone, 20 g/L agar, and deionized water) at 28 °C for 5 days. Agar plugs were used to inoculate 1000 mL Erlenmeyer flasks, each containing 300 mL of ME liquid media. Fermentation was carried out on a rotary shaker (140 rpm) at 26 °C for 13 days in 50 × 1000 mL Erlenmeyer flasks.

Extraction and Isolation. The filtrate (15 L) of the fermented culture broth was extracted four times with EtOAc (15 L × 4) at room temperature, and the organic solvent was evaporated to dryness under reduced pressure to afford a brown crude extract (3.5 g), which was then fractionated by silica gel (35 g, 200−300 mesh) column chromatography (4 × 50 cm) eluted with a gradient of $CHCl_3$−MeOH (v/v 100:0, 100:1, 100:2, 100:4, 100:8, 100:16, 100:32, and 0:100, each 500 mL) to give eight fractions. Fraction 2 (680 mg) was

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subsequently separated by Sephadex LH-20 CC (column chromatography) (1.5 × 30 cm) eluting with MeOH (500 mL) to give six subfractions. Subfraction 2 (105.4 mg) of fraction 2 was further purified by semipreparative reversed-phase HPLC (Waters Spherisorb ODS column; 5 μm, 10.0 × 25.0 cm, 2 mL/min; 80% MeOH in H_2O) to yield **5** (3.1 mg, t_R 18.7 min), **7** (4.2 mg, t_R 9.2 min), and **10** (1.3 mg, t_R 11.9 min), and subfraction 4 (63.3 mg) was also purified by semipreparative RP HPLC to yield **12** (6.3 mg, t_R 12.0 min, 93% MeOH in H_2O), **13** (4.4 mg, t_R 25.0 min, 80% MeOH in H_2O), and **14** (3.3 mg, t_R 21.5 min, 64% MeOH in H_2O). Fraction 3 (197 mg), fraction 4 (780 mg), and fraction 5 (410 mg) were respectively subjected to ODS column (2.5 × 40 cm) chromatography with a gradient of MeOH−H_2O (v/v 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 100:0, each 300 mL) to give seven subfractions, respectively. The fifth subfraction (37.6 mg, MeOH−H_2O, 70:30) of fraction 3 was further fractioned by Sephadex LH-20 CC (1.5 × 30 cm) using MeOH (500 mL) as eluent. Purification of the resulting subfractions by RP HPLC (80% MeOH in H_2O) afforded **2** (4.1 mg, t_R 7.8 min). The third (83.3 mg, MeOH−H_2O, 50:50) and fourth subfractions (45.5 mg, MeOH−H_2O, 60:40) of fraction 4 were purified only by Sephadex LH-20 CC (1.5 × 30 cm) with MeOH (each 500 mL) to yield **8** (35.6 mg) and to yield **1** (10.2 mg), **4** (4.2 mg), and **11** (7.4 mg), respectively. The fifth subfraction (32.4 mg, MeOH−H_2O, 70:30) of fraction 4 was further purified by semipreparative RP HPLC (65% MeOH in H_2O) to yield **15** (2.8 mg; t_R 26.1 min). Subfractions 2 (28.6 mg, MeOH−H_2O, 40:60) and 3 (71.2 mg, MeOH−H_2O, 50:50) of fraction 5 were also purified by Sephadex LH-20 CC (1.5 × 30 cm) with MeOH (each 500 mL) to yield **3** (3.3 mg) and **9** (17.9 mg), respectively. Compound **6** (13 mg) was obtained as an off-white precipitate, which was filtered and washed by $CHCl_3$−MeOH (1:1) from the brown crude extract. All these compounds were stored under 4 °C and not exposed to light.

4″-Deoxy-3-hydroxyterphenyllin (1): colorless column crystals (MeOH); mp 202−204 °C; UV (MeOH) λ_{max} (log ε) 205 (5.08), 277 (4.66) nm; IR (KBr) ν_{max} 3319.6, 1603.5, 1506.5, 1485.5, 1454.5, 1408.4, 1314.9, 1269.7, 1227.3, 1213.9, 1119.3, 1110.6, 1071.0, 1015.2, 939.3, 869.8, 832.8, 771.9, 700.3 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 361.1051 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_5Na$, 361.1046).

4″-Deoxy-5′-desmethylterphenyllin (2): yellow oil; UV (MeOH) λ_{max} (log ε) 205 (4.79), 270 (4.43), 374 (3.59) nm; IR (KBr) ν_{max} 3389.9, 1609.5, 1408.6, 1384.4, 1263.6, 1242.3, 1174.6, 1037.1, 840.0, 701.7 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 331.0948 $[M + Na]^+$ (calcd for $C_{19}H_{16}O_4Na$, 331.0941).

5′-Desmethylterphenyllin (3): light green oil; UV (MeOH) λ_{max} (log ε) 205 (4.61), 266 (4.29) nm; IR(KBr) ν_{max} 3255.5, 1608.9, 1596.5, 1528.9, 1494.1, 1456.2, 1410.0, 1384.3, 1242.6, 1173.6, 1038.1, 1019.3, 998.4, 834.4, 820.7, 790.5 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 347.0898 $[M + Na]^+$ (calcd for $C_{19}H_{16}O_5Na$, 347.0890).

4″-Deoxycandidusin A (4): pale brown powder; UV (MeOH) λ_{max} (log ε) 210 (4.69), 278 (4.38), 333 (4.47) nm; IR(KBr) ν_{max} 3209.8, 1598.5, 1517.1, 1469.8, 1395.3, 1336.4, 1228.3, 1124.7, 1105.2, 1064.0, 995.1, 915.1, 831.5, 814.9, 707.9 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 359.0879 $[M + Na]^+$ (calcd for $C_{20}H_{16}O_5Na$, 359.0890).

4,5-Dimethoxycandidusin A (5): greyish white powder; UV (MeOH) λ_{max} (log ε) 212 (3.77), 279 (3.44), 295 (3.43), 331 (3.50) nm; IR (KBr) ν_{max} 3194.0, 2922.6, 1599.2, 1483.5, 1384.5, 1205.8, 1133.1, 1105.0, 1019.2, 916.0, 833.4 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 403.1140 $[M + Na]^+$ (calcd for $C_{22}H_{20}O_6Na$, 403.1152).

Terphenolide (6): off-white solid; $[\alpha]^{25}_D$ +9.33 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.22), 317 (3.98) nm; IR (KBr) ν_{max} 3320.3, 2924.4, 2853.3, 1738.9, 1723.6, 1671.3, 1608.9, 1597.2, 1466.6, 1408.6, 1275.5, 1176.1, 1123.5, 1070.7, 1026.6, 1000.0, 880.6, 844.4, 824.1, 768.8, 713.8 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 409.0895 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_8Na$, 409.0894).

Aspergiloid A (12): greyish white powder; $[\alpha]^{25}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.76), 242 (3.54), 333 (3.03) nm; IR (KBr) ν_{max} 3415.8, 2930.5, 1683.5, 1457.8, 1383.2, 1026.0 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 351.1571 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4Na$, 351.1567).

Aspergiloid B (13): pale yellow powder; $[\alpha]^{25}_D$ 0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.87), 242 (4.62), 333 (4.00) nm; IR (KBr) ν_{max} 2930.8, 1689.9, 1458.6, 1383.5, 1088.6, 1059.5, 1035.6, 947.7 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 365.1727 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_4Na$, 365.1723).

Aspergiloid C (14): white powder; $[\alpha]^{25}_D$ −89.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.80), 210 (4.77), 219 (4.77), 258 (4.31), 305 (3.76) nm; IR (KBr) ν_{max} 3426.8, 2928.9, 1684.9, 1384.7, 1087.4, 992.1 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 337.1776 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_3Na$, 337.1774).

Aspergiloid D (15): greyish white powder; $[\alpha]^{25}_D$ −2.05 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.31), 245 (4.04) nm; IR (KBr) ν_{max} 3411.4, 2928.5, 1703.4, 1457.2, 1387.8, 1037.0, 1003.6, 912.4 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS *m/z* 343.2239 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

Neuraminidase Inhibitory Assay (ref 19). The neuraminidase inhibitory experiments were assayed on a 96-well plate. The purity of the tested compounds was determined to be over 95% by using the HPLC-DAD method. For the inhibitory test, 90 μL of the assay system containing 70 μL of neuraminidase assay buffer, 10 μL of neuraminidase solution, and 10 μL of different concentrations of the tested compounds **1**−**15** and oseltamivir (positive control) (dissolved in DMSO and diluted with 0.1 M PBS buffer (pH = 7.8)) (10, 5, 2.5, 1.25, or 0.625 μM) was mixed by gently shaking and placed on ice. Then 10 μL of fluorogenic substrate was added to each tested cell, and the plate was incubated for 20 min at 37 °C. The fluorescence absorbance was measured with a TECAN Sunrise microplate reader at 360−440 nm. Each experiment was performed in triplicate. The IC_{50} value was defined as the concentration of neuraminidase inhibitor that inhibited 50% of neuraminidase activity. For the standard kinetic test, the reaction was performed according to the method above with different amounts of neuraminidase solution (0, 1, 2, 3, 7.5, and 10 μL) and not adding test compound. Each experiment was also performed in triplicate.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra of compounds **1**−**6** and **12**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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