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γ- and δ-Lactams from the Leaves of *Clausena lansium*

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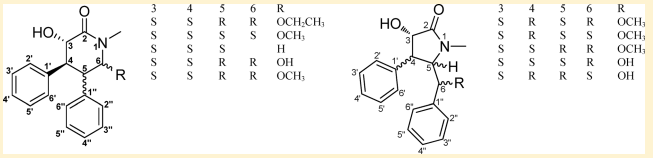
Supporting Information

ABSTRACT: Eight new clausenamides, including three γ-lactams (**1**–**3**), four δ-lactams (**4**–**7**), and an amide (**8**), and seven known lactams, including compounds **9**–**11**, which were purified from natural sources for the first time, were characterized from the leaves of *Clausena lansium*. Their structures were elucidated using spectroscopic methods, and the absolute configurations were determined using electronic circular dichroism and single-crystal X-ray diffraction analyses with Cu Kα radiation. Compound **2** (50 μM) protected 22.24% of cortical neurons against $A\beta_{25-35}$-induced cell death.



Clausena lansium Skeels (Rutaceae), also known as "wampee", has been commonly cultivated in Southern mainland China, Southeast Asia (especially from northern to central Vietnam), and North America for many years.[1] Because this species has commercial and medicinal importance traditionally, extensive phytochemical investigations have led to the characterization of physiologically active compounds, including volatile components, lactams, coumarins, and carbazole alkaloids.[2−8] γ- and δ-Lactams have attracted interest for their biological activities, particularly the neuroprotective effects of the clausenamides.[9−11] In addition, significant anti-acetylcholinesterase (anti-AChE) activity has been reported for the compounds from this plant, implying that they may have potential to treat Alzheimer's disease (AD).[11−14]

AD, the most common cause of dementia among the elderly, is a progressive neurodegenerative disorder characterized by gradual loss of learning, memory, and other cognitive functions.[15] Aβ deposition is considered a crucial event in that it initiates neuronal degeneration in AD.[16] The apoptosis cascades activated by Aβ have been confirmed, although the mechanism of Aβ-induced cell damage is still unclear.[17]

In this work, eight new lactams, including γ-lactams (**1**–**3**), δ-lactams (**4**–**7**), and amide **8**, along with seven known lactams (**9**–**15**) were characterized from the leaves of *C. lansium*. The isolation and structural elucidation of these new compounds and the determination of their absolute configurations through spectroscopic analysis, electronic circular dichroism (ECD), and single-crystal X-ray diffraction experiments are described here. The purified compounds were assayed for the protective effects on cortical neurons.

RESULTS AND DISCUSSION

The methanol extract of the dried leaves of *C. lansium* was partitioned between H_2O and $CHCl_3$. Purification of the $CHCl_3$ fraction by a combination of column chromatographic methods afforded eight new (**1**–**8**) and seven known lactams. The known compounds were identified by comparing their physical and spectroscopic data with reported values.

6-*O*-Methylneoclausenamide (**1**) was obtained as colorless crystals (MeOH) with $[\alpha]_D^{20}$ −85 (*c* 1.3, MeOH), and the enantiomeric excess (ee %) of compound **1** was determined to be >93% by chiral HPLC analysis (Supporting Information). The molecular formula, $C_{19}H_{21}NO_3$, was established by ^{13}C NMR and HRESIMS data (334.1416 $[M + Na]^+$, calcd for 334.1419), suggesting 10 indices of hydrogen deficiency (IHD). The IR spectrum displayed absorptions characteristic of hydroxy (3316 cm^{-1}), amide carbonyl (1692 cm^{-1}), and monosubstituted benzene ring (1598, 1543, 1491, 1447, 756, and 700 cm^{-1}) functionalities, respectively. The benzenoid nature was supported by the absorption maximum λ_{max} at 259 nm in the UV spectrum. The ^1H NMR spectrum revealed the presence of two monosubstituted benzene rings [δ_H 6.72 (2H), 7.10−7.38 (8H)], two oxygenated methines (δ_H 4.10 and 4.58 for H-3 and H-6, respectively), an N-bearing methine (δ_H 3.65, H-5), a methine (δ_H 3.25, H-4), and an *N*-methyl group (δ_H 3.03). The structure was verified by the following HMBC correlations (Figure 1): H-3 with C-2/C-5/C-1′, H-4 with C-3/C-5/C-6/C-1′, H-5 with C-4/C-6/C-1′, H-6 with C-4/C-5/C-

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Chart 1

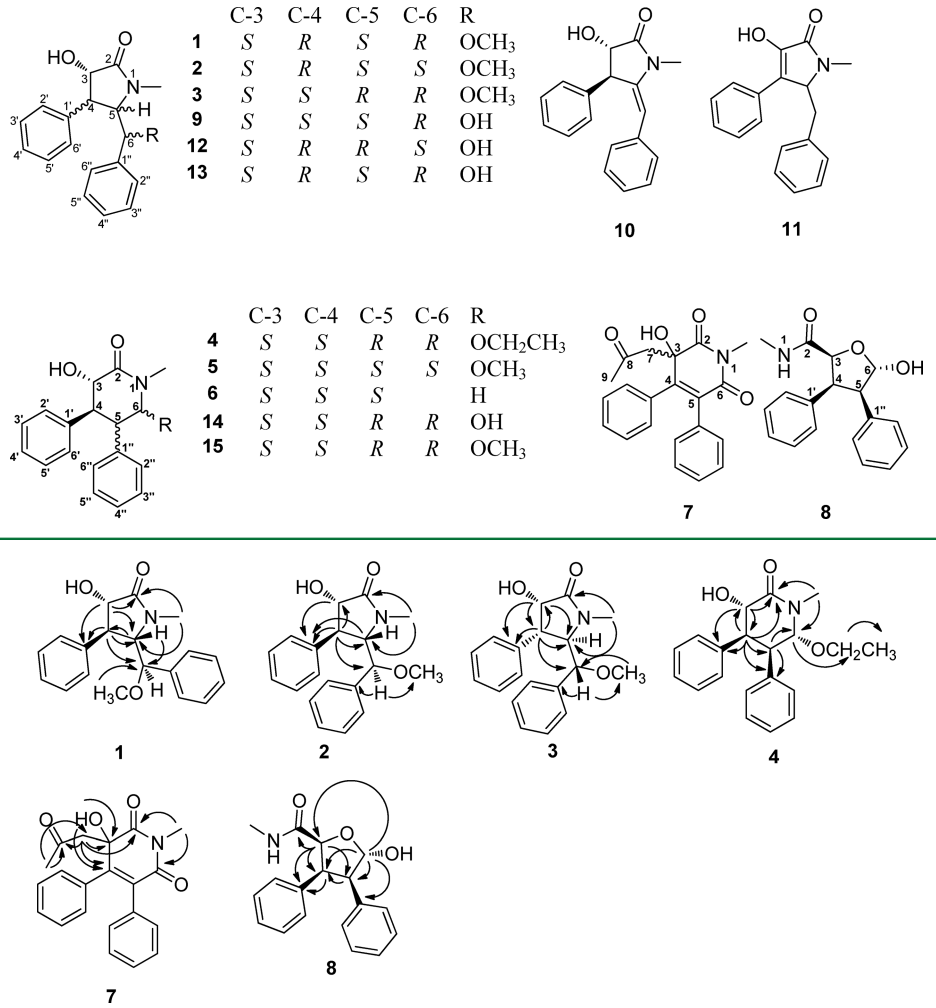

Figure 1. Selected HMBC (→) correlations for compounds **1**, **2**, **3**, **4**, **7**, and **8**.

Table 1. ^{1}H NMR [δ, mult, (J in Hz)] Data for Compounds 1−3 and 9−13 (400 MHz)

position	1[a]	2[a]	3[a]	9[a]	10[a]	11[a]	12[b]	13[a]
3	4.10 dd (8.8, 2.8)	4.16 dd (5.6, 3.2)	3.73 s	4.50 dd (6.4, 6.4)	4.27 m		4.02 dd (10.8, 2.0)	4.15 dd (8.0, 2.4)
4	3.25 dd (2.8, 2.8)	2.86 dd (5.6, 5.6)	3.72 d (5.2)	3.79 dd (6.4, 6.4)	4.33 m		3.72 dd (10.8, 8.4)	3.16 dd (2.4, 2.4)
5	3.65 dd (2.8, 2.8)	3.83 dd (6.8, 5.6)	4.27 dd (5.2, 3.2)	4.15 dd (6.4, 6.4)		4.62 dd (5.2, 4.0)	4.20 dd (8.4, 3.2)	3.75 dd (2.4, 2.4)
6	4.58 d (2.8)	4.22 d (6.8)	4.17 d (3.2)	4.84 dd (6.4, 6.4)	6.04 d (1.6)	3.27 dd (14.6, 4.0)	4.79 dd (3.2, 3.2)	5.22 m
						2.98 dd (14.6, 5.2)		
N-CH_3	3.03 s	3.02 s	3.02 s	2.61 s	3.23 s	3.06	2.97 s	3.11 s
OH-3	3.70 d (8.8)	3.28 d (3.2)	2.70 s	2.60 d (6.4)	3.71 br s	6.55 br s	3.02 br	4.67 s
OH-6				3.73 d (6.4)			1.92 br	4.69 s
OCH_3-6	3.43 s	3.22 s	2.91 s					
2″,6″			6.66 d (6.8)			6.84 m	6.78 m	
2′−6′, 3″−5″			7.17−7.40 m				7.15−7.39 m	
3″−5″						7.15 m		
2′, 6′	6.72 m	6.71 m				7.60 d (7.6)		6.65 m
3′, 5′						7.44 t (7.6)		
4′						7.31 t (7.6)		
3′−5′, 2″−6″	7.10−7.38 m	7.10−7.20 m						7.10−7.37 m
2′−6′, 2″−6″				7.15−7.25 m	6.99−7.16 m			

[a]Recorded in $CDCl_3$. [b]Recorded in methanol-d_4.

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Table 2. ^{13}C NMR Data for Compounds 1−3 and 9−13 (100 MHz)

carbon	1[a]	2[a]	3[a]	9[a]	10[a]	11[a]	12[b]	13[a]
2	174.0	173.6	174.7	175.7	173.6	167.0	177.2	174.6
3	76.2	77.1	69.1	71.7	77.3	142.1	70.7	76.7
4	46.3	49.9	50.1	47.3	50.8	120.4	51.3	45.7
5	71.2	69.2	65.3	66.1	142.1	60.2	67.4	72.4
6	80.5	87.2	83.2	71.6	106.4	36.7	74.1	69.6
1′	141.7	140.4	135.1	134.2	139.0	131.6	136.6	141.9
2′−6′	126.2−128.7	126.6−128.5	127.2−128.6	126.5−130.0	125.9−128.7	126.8−129.2	127.9−129.8	126.2−128.8
1″	136.2	137.0	136.0	140.5	134.8	135.0	141.2	139.4
2″−6″	126.2−128.7	126.6−128.5	127.2−128.6	126.5−130.0	125.9−128.7	126.8−129.2	128.4−128.7	125.6−128.5
N-CH_3	28.7	30.7	31.5	29.5	27.3	28.7	31.5	28.6
OCH_3-6	57.8	56.4	56.4					

[a]Recorded in $CDCl_3$. [b]Recorded in methanol-d_4.

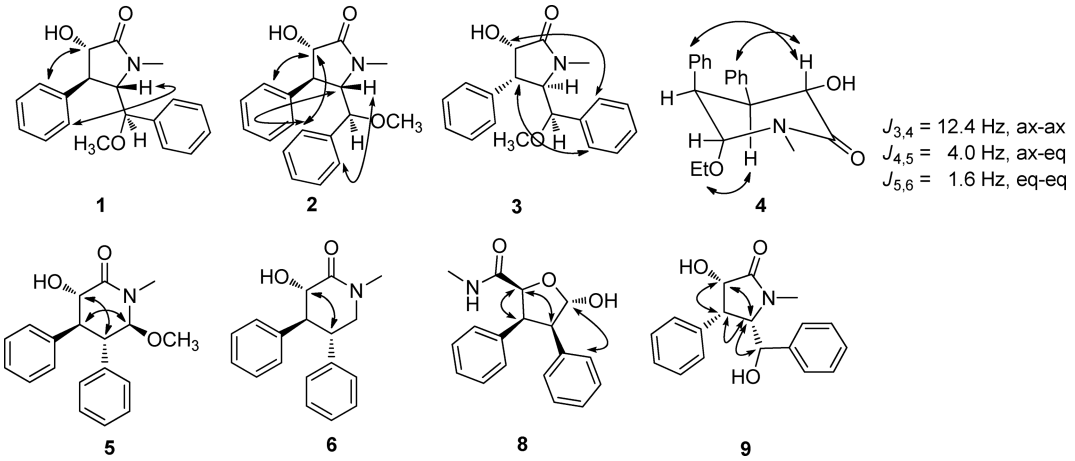

Figure 2. Selected NOESY (↔) correlations for compounds 1−6, 8, and 9.

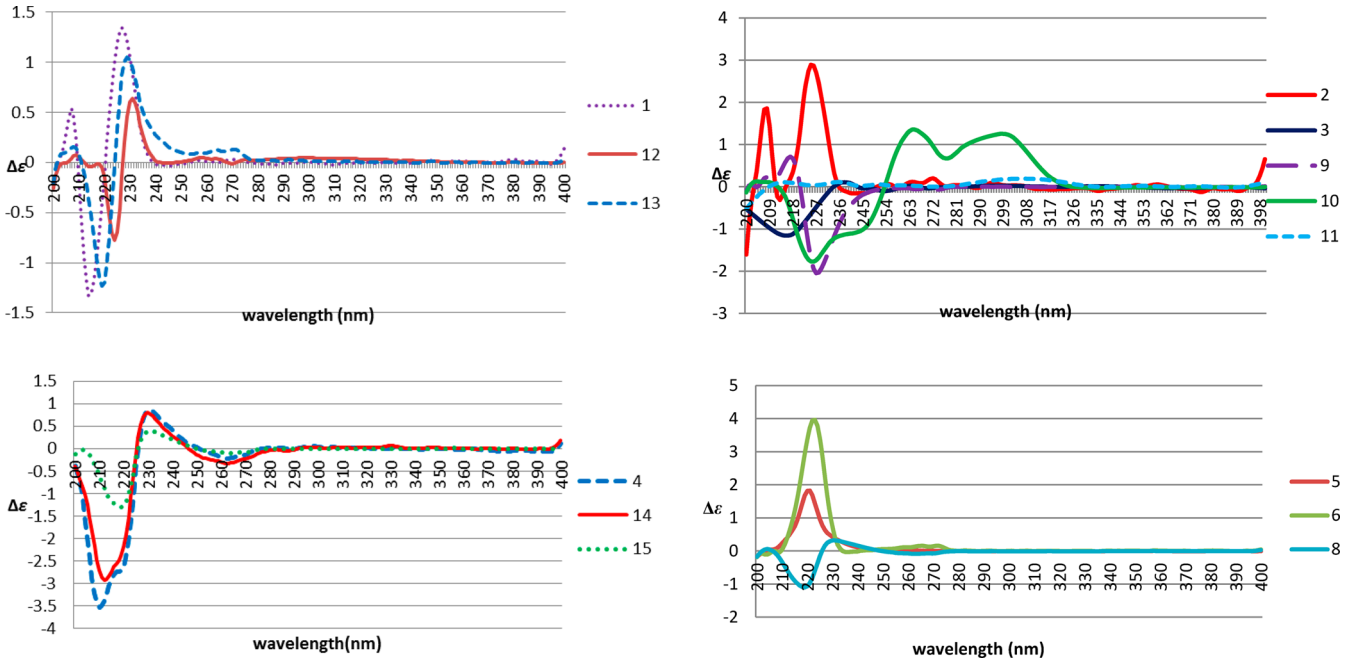

Figure 3. ECD spectra of compounds 1−6 and 8−15.

1″/OCH_3, and N-CH_3 with C-2/C-5. A comparison of the ^{1}H and ^{13}C NMR data of compound **1** and (−)-neoclausenamide (**13**)[2] (Tables 1 and 2) indicated that the two compounds differed only in the presence of a methoxy group (δ_H 3.43, δ_C 57.8) in compound **1** instead of the hydroxy group in (−)-neoclausenamide. In the HMBC experiment (Figure 1), the ^3J-correlation between the OMe (δ_H 3.43) and C-6 indicated the location of the methoxy group at C-6. The relative

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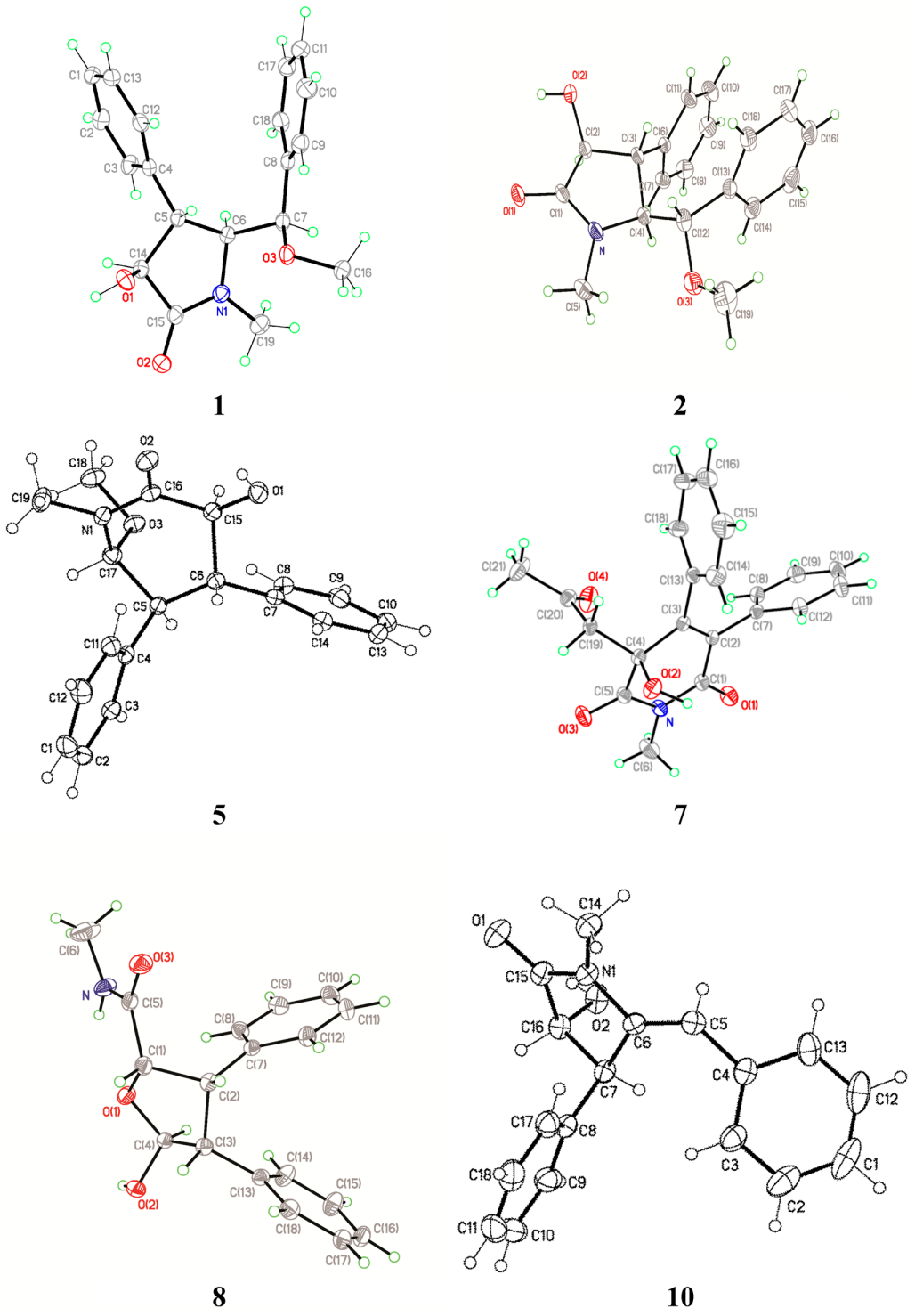

Figure 4. ORTEP drawings of compounds **1**, **2**, **5**, **7**, **8**, and **10**.

configuration of the lactam ring in compound **1** was assigned from the NOESY spectrum (Figure 2), in which the correlations of H-2′ and H-6′ with H-3/H-5 indicated *cis* configurations of H-3, H-5, and the C-4 phenyl group. Meguro and co-workers proposed an extension of the axial-haloketone rule to deduce the configuration of an inherently dissymmetric lactam chromophore between the carbonyl and lone pair electrons of the C-α heteroatom substituent. The ECD sign and red shift of the Cotton effect were shown to experimentally determine the C-α configuration and the sign and the magnitude of the n → π^* Cotton effect, which are sensitive to the nature of the C-3 substituent.[18] Therefore, the C-3 configuration of the lactam derivative with a hydroxy functionality was determined as *S* because it displayed a positive Cotton effect near 230 nm. In addition, the absolute configuration of compound **1** was unambiguously defined by a single-crystal X-ray diffraction analysis with Cu Kα radiation as 3*S*, 4*R*, 5*S*, and 6*R* (Figure 4). Consequently, the structure of 6-*O*-methylneoclausenamide (**1**) was characterized as shown in Figure 1.

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Table 3. ^{1}H NMR [δ, mult, (J in Hz)] Data for Compounds 4−8, 14, and 15 (400 MHz)

position	4[a]	5[a]	6[a]	7[a]	8[b]	14[a]	15[a]
3	4.36 d (12.4)	4.65 dd (11.2, 2.4)	4.55 d (10.0)		5.10 d (4.8)	4.40 d (12.0)	4.36 d (12.4)
4	3.89 dd (12.4, 4.0)	3.04 dd (11.2, 8.8)	3.19 dd (10.0, 9.6)		4.03 dd (7.2, 4.8)	3.89 dd (12.0, 4.0)	3.85 dd (12.4, 4.0)
5	3.35 dd (4.0, 1.6)	3.36 dd (8.8, 2.8)	3.11 dd (9.6, 6.4)		3.86 dd (7.2, 7.2)	3.40 dd (4.0, 1.6)	3.38 dd (4.0, 1.6)
6	4.69 d (1.6)	4.57 d (2.8)	3.79 dd (10.0, 6.4)		6.11 d (7.2)	5.14 dd (6.4, 1.6)	4.61 d (1.6)
			3.25 d (10.0)				
7				3.15 d (17.6)			
				2.83 d (17.6)			
9				2.00 s			
N-CH_3	3.20 s	3.17 s	3.01 s	3.36 s	2.47 s	3.23 s	3.21 s
OH-3	3.45 s	3.79 d (2.4)	5.74 s	3.59 s		3.48 s	3.44 s
OH-6						3.06 d (6.4)	
OCH_3-6		3.34 s					3.53 s
O$\underline{CH_2}CH_3$	3.73 dt (9.2, 7.2)						
	3.61 dt (9.2, 7.2)						
$OCH_2\underline{CH_3}$	1.33 t (7.2)						
2′, 6′	6.90 m	7.15 m	6.87 m			6.91 m	6.91 m
2″, 6″	6.63 d (7.2)	7.01 m	6.79 m			6.61 d (7.2)	6.63 d (8.0)
3′−5′, 3″−5″	7.14−7.23 m	7.17−7.28 m	7.18−7.25 m			7.13−7.22 m	7.15−7.23 m
2′−6′, 2″−6″				7.01−7.17 m	6.97−7.07 m		

[a]Recorded in $CDCl_3$. [b]Recorded in methanol-d_4.

Table 4. ^{13}C NMR Data for Compounds 4−8, 14, and 15 (100 MHz)

carbon	4[a]	5[a]	6[a]	7[a]	8[b]	14[a]	15[a]
2	173.4	174.2	176.5	174.4	172.7	173.3	173.3
3	66.7	70.3	72.6	71.9	82.8	66.8	66.6
4	43.9	53.1	51.1	149.6	55.5	43.7	43.9
5	49.5	53.0	40.4	133.3	59.0	52.7	48.5
6	91.9	95.2	56.0	164.2	103.4	85.0	93.5
7				51.9			
8				205.8			
9				30.4			
1′	138.1	140.3	140.2	133.9	137.5	137.7	138.0
1″	136.1	141.0	140.5	134.9	137.9	135.8	135.9
2′−6′	126.9−128.4	126.9−128.8	127.4−128.5	127.4−130.5	127.4−130.7	127.0−128.4	126.9−128.4
2″−6″	127.4−128.9	127.2−128.5	127.4−128.5	127.4−130.5	127.4−130.7	127.5−128.7	127.5−128.9
N-CH_3	33.4	34.8	29.7	27.1	25.5	33.1	33.7
OCH_3-6		55.7					57.0
O$\underline{CH_2}CH_3$	65.0						
$OCH_2\underline{CH_3}$	15.4						

[a]Recorded in $CDCl_3$. [b]Recorded in methanol-d_4.

6-*O*-Methyl-*epi*-neoclausenamide (**2**) was isolated as colorless crystals (MeOH), $[\alpha]_D^{20}$ −54. The HRESIMS data of compound **2** showed a sodium adduct ion at m/z 334.1417, which was consistent with the molecular formula of $C_{19}H_{21}NO_3$. Its ^{1}H and ^{13}C NMR, IR, and UV data were similar to those of compound **1**, indicating the same Clausenamide skeleton. The methoxy group was also located at C-6, which was supported by the long-range correlation from OMe (δ_H 3.22) to C-6 (δ_C 56.4) in the HMBC spectrum (Figure 1). Thus, the 2D structure of compound **2** was the same as compound **1**, and the relative configuration of the lactam ring was assigned as being the same as that of compound **1** through the analysis of their NOESY spectra (Figure 2). In addition, the absolute configurations at C-4, C-5, and C-6 were determined from the single-crystal X-ray diffraction pattern using the anomalous scattering of Cu Kα radiation (Figure 4). Therefore, the absolute configuration was determined as 3*S*, 4*R*, 5*S*, and 6*S*. Thus, the structure of 6-*O*-methyl-*epi*-neoclausenamide (**2**) was assigned as shown.

The 2D structure of compound **3** was assigned as identical to those of compounds **1** and **2** by comparison of their UV, IR, MS, and NMR data. In the Konno report,[18] the negative Cotton effect observed for (*R*,*R*)-3,4-dihydroxy-2-pyrrolidone constitutes the sum of the contributions from the 3- and 4-substituents. The ECD spectrum of **3** showed a low-amplitude positive Cotton effect near 236 nm. The ECD spectrum of compound **12** showed a high-amplitude positive Cotton effect at 230 nm. Thus, the low-amplitude positive Cotton effect at 238 nm in the ECD spectrum of **3** (Figure 3) suggested 3*S* and 4*S* absolute configurations. By comparing the specific rotation and absolute configuration of compound **3** with the 16 stereoisomers of clausenamide,[19] the (3*S*, 4*S*, 5*R*, 6*S*) and (3*S*, 4*S*, 5*R*, 6*R*) configurations could be considered further. The $J_{5,6}$ value in compound **3** was 3.2 Hz. This value is consistent with the 6*R* stereoisomer of clausenamide ($J_{5,6}$ = 2.7

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Hz), whereas the isomer with 6*S* configurations exhibited a significantly different coupling constant ($J_{5,6}$ = 5.4 Hz). Therefore, the absolute configuration of 6-*O*-methyl-*epi*-*cis*-neoclausenamide (**3**) was established as 3*S*, 4*S*, 5*R*, and 6*R*.

Lansamide-5 (**4**) was isolated as a colorless powder. The molecular formula $C_{20}H_{23}NO_3$ was determined from the ^{13}C NMR data and HRESIMS ion at *m*/*z* 348.1578 $[M + Na]^+$ (calcd for $C_{20}H_{23}NO_3Na$, 348.1576), suggesting 10 IHD. The IR spectrum displayed absorptions characteristic of hydroxy (3386 cm^{-1}) and amide carbonyl (1656 cm^{-1}) functionalities, respectively. The benzenoid nature was supported by the absorption maximum λ_{max} at 248 nm in the UV spectrum. The ^1H NMR spectrum revealed the presence of two monosubstituted benzene rings [δ_H 6.63 (2H), 6.90 (2H), 7.14−7.23 (6H)], two oxygenated methines (δ_H 4.36 and 4.69 for H-3 and H-6, respectively), two methines (δ_H 3.35 and 3.89 for H-5 and H-4, respectively), an ethoxy group (δ_H 1.33 (3H, t, *J* = 7.2 Hz), 3.61 (1H, dt, *J* = 9.2, 7.2 Hz), and 3.73 (1H, dt, *J* = 9.2, 7.2 Hz)), and an *N*-methyl group (δ_H 3.20). The structure was verified by the following HMBC correlations (Figure 1): H-3 with C-2/C-4/C-1′; H-4 with C-2/C3/C-5/C-1′/C-1″; H-5 with C-3/C-4/C-6/C-1′/C-1″; H-6 with C-2/C-4/C-1″/$O\underline{C}H_2CH_3$/*N*-$\underline{C}H_3$; *N*-$C\underline{H}_3$ with C-2/C-6; and $OC\underline{H}_2CH_3$ with C-6. Comparison of the ^1H and ^{13}C NMR data of compound **4** and lansamide 4 (**15**)[5] (Tables 3 and 4) indicated that the two compounds differed only in the presence of an ethoxy group (δ_H 1.33, t, 3H; 3.73, dt, H; 3.61, dt, H; δ_C 65.0, 15.4) in compound **4** instead of the methoxy group found in lansamide 4 (**15**). Moreover, the HMBC correlations of H-6 (δ_H 4.69, d) to the oxymethylene carbon confirmed that the ethoxy group was located at C-6 (Figure 1). The relative configuration of compound **4** was proposed by analyzing the $^1H-^1H$ coupling constants and the NOESY spectrum (Figure 2). H-3 was assigned an axial (or pseudoaxial) orientation by virtue of a large *trans*-diaxial coupling constant (*J* = 12.4 Hz) with H-4. H-5 was assigned an equatorial orientation because of a small coupling constant (*J* = 4.0 Hz) with H-4. H-6 was also assigned an equatorial orientation because of the small coupling constant (*J* = 1.6 Hz) with H-5. In addition, the relative configuration of compound **4** was also determined by the following analysis of the NOESY data (Figure 2). The *β*-orientation of H-3 was established because NOE correlations to H-2′ and -6′ as well as H-2″ and -6″ were observed. The absolute configuration of C-3 in compound **4** was deduced by the ECD spectrum. In this case, the ECD spectrum of compound **4** (Figure 3) showed a positive Cotton effect at 231 nm, which evidenced a 3*S* absolute configuration.[20] Consequently, the absolute configuration of compound **4** was deduced as 3*S*, 4*S*, 5*R*, and 6*R*, and the structure was illustrated as shown.

Lansamide-6 (**5**) was shown to have the same molecular formula, $C_{19}H_{21}NO_3$, as compound **15** based on analyses of the HRESIMS and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra of compound **5** closely resembled those of compound **15**, but with a deshielded H-3 (δ_H 4.65) and shielded H-4, -5, and -6 (δ_H 3.04, 3.36, and 4.57) resonances, indicating that compound **5** was a stereoisomer of compound **15**. The large *J* values between H-3/H-4 (11.2 Hz) and H-4/H-5 (8.8 Hz) and NOE correlations of H-3/H-5 and H-4/H-6 (Figure 2) confirmed that H-3/H-4, H-4/H-5, and H-5/H-6 were *trans*-oriented. To determine the absolute configuration, compound **5** was subjected to a single-crystal X-ray diffraction analysis with Cu K*α* radiation (Figure 4), which unambiguously confirmed the structure. Therefore, the absolute configuration was established as 3*S*, 4*S*, 5*S*, and 6*S* (Figure 4). Thus, compound **5** was characterized as lansamide-6.

Lansamide-7 (**6**) was obtained as a colorless, amorphous powder. Its molecular formula was determined as $C_{18}H_{19}NO_2$ by the HRESIMS ion at *m*/*z* 304.1310 $[M + Na]^+$ (calcd for $C_{18}H_{19}NO_2Na$, 304.1313). The NMR data (Tables 3 and 4) of compound **6** were similar to those of compound **5**, except that the C-6 aminal group in compound **5** was replaced by an aminomethylene (δ_H 3.79 and 3.25) group in compound **6**. The large coupling constants of 10.0 and 9.6 Hz between H-3/H-4 and H-4/H-5 indicated that the three protons occupied axial (or pseudoaxial) orientations. In addition, the NOE correlation (Figure 2) between H-3 and H-5, together with the absence of NOE correlations between H-4 and H-3/H-5, suggested that the orientations of H-3/H-4 and H-4/H-5 were in the *trans* form. A positive Cotton effect at 223 nm in the ECD spectrum (Figure 3) suggested a 3*S* absolute configuration.[20] Thus, the absolute configuration was established as 3*S*, 4*S*, and 5*S*, and the structure of lansamide-7 was characterized as shown.

Lansamide-8 (**7**) was obtained as colorless crystals (MeOH). The molecular formula, $C_{21}H_{19}NO_4$, was established by HRESIMS (*m*/*z* 372.1204, $[M + Na]^+$, calcd 372.1206) and suggested its IHD of 13. The IR spectrum displayed absorptions characteristic of a hydroxy group (3376 cm^{-1}) and a carbonyl group (1746 and 1696 cm^{-1}). The benzenoid nature was supported by the UV absorption at 258 nm. A comparison of the ^1H and ^{13}C NMR data of compounds **7** and **6** (Tables 3 and 4) indicated their related structures, differing in the presence of a CH_3COCH_2- [δ_H 2.00 (3H, s), 3.15 (1H, d), 2.83(1H, d); δ_C 30.4, 205.8, 51.9] and an *α*,*β*-unsaturated carbonyl group [δ_C 149.6, 133.3, 164.2] in **7**. In the HMBC experiment (Figure 1), the correlations from H-7 to C-2/C-3/C-4/C-8, from OH-3 to C-3/C-4, and from *N*-CH_3 to C-2/C-6 revealed a 3-hydroxypyridine-2,6-dione moiety. On the basis of these results and the single-crystal X-ray diffraction analyses using Cu K*α* radiation (Figure 4), the structure of lansamide-8 was identified as shown. The crystals of compound **7** were orthorhombic and belonged to space group *Pbca*. As shown in the ORTEP drawing (Figure 4), the X-ray analysis revealed that compound **7** is a racemic mixture presumably originating from reaction of a pyridine-2,3,6-trione and acetone.

Lansamide-9 (**8**) exhibited a sodium adduct ion at *m*/*z* 320.1261 in the HRESIMS, which is in agreement with the molecular formula $C_{18}H_{19}NO_3$ (calcd for $C_{18}H_{19}NO_3Na$, 320.1263). Its ^{13}C NMR data (Table 4) showed 18 carbon resonances, including 12 aromatic carbons, four aliphatic methines (δ_C 82.8, C-3; 55.5, C-4; 59.0, C-5; 103.4, C-6), and one *N*-methylaminocarbonyl group (δ_H 2.47; δ_C 172.7, 25.5). In addition, four methine signals (δ_H 5.10, H-3; 4.03, H-4; 3.86, H-5; 6.11, H-6) were observed (Table 3). The COSY cross-peaks suggested the sequential connections from C-3 to C-6. This was also supported by the HMBC correlations (Figure 1) of H-3 to C-4/C-5, H-4 to C-5/C-6, H-5 to C-4/C-6, and H-6 to C-3/C-4/C-5. The deshielding of H-3 and H-6 revealed the presence of oxygenated functionalities at C-3 and C-6. Moreover, the HMBC correlation of H-6/C-3 displayed that C-3 and C-6 are connected via an oxygen atom to form a tetrahydrofuran ring. The ^{13}C NMR chemical shift of C-6 (δ_C 103.4) was indicative of the presence of hemiacetal. In the HMBC spectrum, the correlations of H-3/C-2, H-4/C-1′, and H-5/C-1″, as well as their chemical shifts, suggested that an *N*-

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methylaminocarbonyl group, two phenyl groups, and a hydroxy group are attached to C-3, C-4, C-5, and C-6, respectively. In addition, the significant NOE correlation (Figure 2) between H-3 and H-4/H-5 together with the absence of NOE correlations between H-6 and H-5 suggested that the orientations of H-3/H-4/H-5 and H-5/H-6 were in the *cis* and *trans* form, respectively. From the spectroscopic analysis and the single-crystal X-ray diffraction data (Figure 4), the absolute configuration was confirmed by the Flack parameter 0.0(2) and defined as 3*S*, 4*S*, 5*R*, and 6*S*.

The structures of compounds **9** and **10** were confirmed by HRESIMS data and single-crystal X-ray diffraction analysis (Figure 4). These structures had been reported as synthetic products,[19,21] but they were isolated from natural sources for the first time. Compound **11** was identified as anhydroclausenamide, which had been reported as a synthetic product.[4] However, the specific rotation of anhydroclausenamide was $[\alpha]_D^{24}$ −2.3 (*c* 1.3, MeOH), which was expected to be a racemate.

Compounds **12** and **13** were identified as (−)-clausenamide and (−)-neoclausenamide via ^{1}H and ^{13}C NMR (Tables 1 and 2), the positive Cotton effect in the ECD spectrum [at 230 and 229 nm] (Figure 3), single-crystal X-ray diffraction analysis (Figure 4), and its negative specific rotation [−148.5 (*c* 0.8, MeOH) and −71.8 (*c* 1.8), MeOH]. Racemic mixtures of compounds **12** and **13** had previously been isolated from the leaves of *C. lansium*,[2,4] and their enantiomer had been synthesized in Feng's report.[19] Compound **12** was subjected to chiral HPLC, and its ee value was >89%. Therefore, optically active compounds **12** and **13** are naturally occurring enantiomers.

Compounds **14** and **15** were reported as racemates in a previous study,[5] but negative specific rotation [−107.8 (*c* 1.4, MeOH) and −117.1 (*c* 0.7, MeOH)] and a high-amplitude Cotton effect (Figure 3) confirmed that they were pure enantiomers. Their structures were confirmed by the positive Cotton effects in their ECD spectra [at 230 and 231 nm] (Figure 3) and single-crystal X-ray diffraction analyses (Figure 4).

From the above results, some relationship between the ECD spectra and the absolute configurations could be found. In the ECD spectra, *δ*-lactams **4**, **14**, and **15** with 3*S*, 4*S*, and 5*R* absolute configurations exhibited negative and positive Cotton effects near 210 and 230 nm, respectively. Compounds **5** and **6**, possessing 3*S*, 4*S*, and 5*S* absolute configurations, displayed ECD spectra with a positive Cotton effect at 220 nm. For the *γ*-lactam group, compounds **1**, **12**, and **13**, with 3*S* and 4*R* stereochemistry, exhibited similar ECD spectra. However, the absolute configurations at C-5 and C-6 of compound **12** were different from those of compounds **1** and **13**. This implied that the absolute configuration of C-5 and C-6 had little contribution to the ECD spectra. In contrast, compounds **3** and **9** possessed 3*S* and 4*S* absolute configurations and showed different ECD spectra compared to those of compounds **1**, **12**, and **13**. This indicated that the C-4 phenyl group may have a significant influence on the Cotton effect near 230 nm. Furthermore, a comparison of the ECD spectra of compounds **3** and **9** showed that the absolute configuration at C-5 may influence the wavelength of the Cotton effect.

The purified compounds were examined for their neuroprotective activity on A*β*-induced neurotoxicity (Supporting Information). The most active compound (**2**) reduced A*β*-induced neurotoxicity by 22.24% at a concentration of 50 *μ*M (S 101). The neuroprotective activity of compound **2**, but not of its stereoisomers **1** and **3**, suggested the involvement of a stereoselective protein in the neuroprotective mechanism. A similar effect among stereoisomers was suggested for compound **12** in facilitating synaptic transmission[11,12,19,21] and protecting PC12 cells[22] and cortical neurons[23] against A*β*-induced neurotoxicity.

In the present study, most of the purified clausenamides are optically active, while racemic mixtures were obtained in previous studies. In addition, *γ*-lactams are reported from the *Clausena* species collected in China, while *δ*-lactams were from those cultured in India. Comparatively, both types of clausenamides are characterized from the species collected in Vietnam. These results may support the theory of genuine medicinal materials commonly mentioned in Traditional Chinese Medicine. Also, it may provide chemotaxonomic evidence to differentiate these *Clausena* species.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured with a JASCO P-2000 digital polarimeter in a 0.5 dm cell. The UV spectra were obtained with a Hitachi UV-3210 spectrophotometer, and the IR spectra were measured with a Shimadzu FTIR Prestige-21 spectrometer. ECD spectra were recorded on a JASCO J-720 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured using Bruker AMX-400 and AV500 spectrometers with TMS as the internal reference, and chemical shifts are expressed in *δ* (ppm). The ESIMS and HRESIMS were collected on a Bruker Daltonics APEX II 30e spectrometer. Silica gel (70−230 and 230−400 mesh; Merck), Diaion HP-20 resin (Mitsubishi, Chemical, Tokyo, Japan), and silica gel 60 F_{254} (Merck) were used for preparative TLC and TLC, respectively. HPLC was performed on a Shimadzu LC-10AT$_{VP}$ (Japan) system equipped with a Shimadzu SPD-M20A diode array detector at 250 nm, a Purospher STAR RP-8e c (5 *μ*m, 250 × 4.6 mm), Cosmosil 5C$_{18}$ ARII (250 × 4.6 mm i.d. Nacalai Tesque Inc.), and Astec Cellulose DMP (150 × 4.6 mm i.d. 5 *μ*m) columns. The X-ray diffraction experiments were performed on a Bruker D8 Venture with a Photon 100 CMOS detector system equipped with a Cu Incoatec I*μ*S microfocus source (*λ* = 1.541 78 Å).

Plant Material. The leaves of *C. lansium* were collected from the Quyhop District, Nghean Province, Vietnam (N 19°19′ 32.69″, E 105°11′36.57″) during July 2011. The plant material was identified and authenticated by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (Viet-TSWu-20110703) has been deposited in the Herbarium of the Faculty of Biology, Vinh University, Vietnam.

Extraction and Isolation. The air-dried *C. lansium* leaves (25 kg) were extracted with MeOH (6 × 20 L) under reflux for 8 h. The filtrate was evaporated under reduced pressure to yield a dark brown residue (810 g). The extract was suspended in H_2O and partitioned successively with $CHCl_3$ (4 × 3 L). The $CHCl_3$ extract (310 g) was subjected to column chromatography and eluted with *n*-hexane−EtOAc (3:1) to afford six fractions. Fraction 3 was chromatographed on silica gel with *n*-hexane−diisopropyl ether (3:1) to give six subfractions (Subfr. 3.1−3.6). Subfr. 3.5 was chromatographed on silica gel with $CHCl_3$−acetone (59:1) to yield **15** (4.6 mg). Fraction 4 was chromatographed on silica gel using $CHCl_3$−acetone (14:1) to give six subfractions (Subfr. 4.1−4.6). Subfr. 4.2 was chromatographed on silica gel with $CHCl_3$−acetone (19:1) to give **11** (6.1 mg). Subfr. 4.6 was chromatographed on silica gel with *n*-hexane−acetone (2:1) to afford **1** (43.2 mg) and **10** (5.3 mg). Fraction 5 was subjected to column chromatography on silica gel using $CHCl_3$−acetone (19:1) to give five subfractions (Subfr. 5.1−5.5). Subfr. 5.2 was chromatographed on silica gel with *n*-hexane−EtOAc (2:1) to give **7** (2.3 mg), **8** (3.6 mg), and **9** (4.3 mg). Subfr. 5.5 was chromatographed on silica gel with diisopropyl ether−acetone (7:1) to give **14** (57.6 mg). Fraction 6 was subjected to column chromatography on silica gel using $CHCl_3$−MeOH (9:1) to give six subfractions (Subfr. 6.1−6.6). Subfr. 6.2 was

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chromatographed on silica gel with diisopropyl ether−MeOH (14:1) to yield **2** (5.6 mg), **3** (3.1 mg), **4** (4.4 mg), **5** (4.2 mg), **6** (0.9 mg), and **12** (6.2 mg). The H_2O-soluble layer (390 g) was directly subjected to Diaion HP-20 column chromatography using H_2O containing increasing proportions of MeOH to give six fractions. Fraction 6 was subjected to column chromatography on silica gel using $CHCl_3$−MeOH (6:1) to give six subfractions (Subfr. 6.1−6.6). Subfr. 6.6 was chromatographed on silica gel using $CHCl_3$−MeOH (5:1) to give **13** (5.6 mg).

*6-O-Methylneoclausenamide (**1**):* colorless crystals (MeOH); $[\alpha]_D^{24}$ −85 (*c* 1.3, MeOH); mp 152−154 °C; UV (MeOH) λ_{max} (log ε) 206 (3.62), 216 (3.38), 259 (2.66) nm; ECD (MeOH, $c = 2.20 \times 10^{-4}$ M) 207 ($\Delta\varepsilon$ +0.53), 214 ($\Delta\varepsilon$ −1.33), 227 ($\Delta\varepsilon$ +1.35) nm; IR (KBr) ν_{max} 3316, 2931, 1692, 1491, 1447, 1129, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 334 $[M + Na]^+$; HRESIMS *m*/*z* 334.1416 (calcd for $C_{19}H_{21}NO_3Na$, 334.1419).

*6-O-Methyl-epi-neoclausenamide (**2**):* colorless crystals (MeOH); mp 120−122 °C; $[\alpha]_D^{24}$ −54 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.39), 217 (3.12), 257 (2.43) nm; ECD (MeOH, $c = 5.30 \times 10^{-5}$ M) 208 ($\Delta\varepsilon$ +1.85), 213 ($\Delta\varepsilon$ −0.31), 225 ($\Delta\varepsilon$ +2.89), 241 ($\Delta\varepsilon$ −0.15) nm; IR (KBr) ν_{max} 3318, 2926, 2853, 1680, 1457, 1097 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 334 $[M + Na]^+$; HRESIMS *m*/*z* 334.1417 (calcd for $C_{19}H_{21}NO_3Na$, 334.1419).

*6-O-Methyl-epi-cis-neoclausenamide (**3**):* colorless, amorphous powder; $[\alpha]_D^{24}$ −22 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.72), 215 (3.58), 258 (2.21) nm; ECD (MeOH, $c = 1.32 \times 10^{-4}$ M) 215 ($\Delta\varepsilon$ −1.16), 238 ($\Delta\varepsilon$ +0.11), 253 ($\Delta\varepsilon$ −0.10) nm; IR (KBr) ν_{max} 3380, 2928, 1686, 1496, 1450, 1156, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 334 $[M + Na]^+$; HRESIMS *m*/*z* 334.1412 (calcd for $C_{19}H_{21}NO_3Na$, 334.1413).

*Lansamide-5 (**4**):* colorless, amorphous powder; $[\alpha]_D^{24}$ −89 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.84), 248 (2.85) nm; ECD (MeOH, $c = 1.23 \times 10^{-4}$ M) 210 ($\Delta\varepsilon$ −3.52), 219 sh ($\Delta\varepsilon$ −2.71), 231 ($\Delta\varepsilon$ +0.86), 263 ($\Delta\varepsilon$ −0.22) nm; IR (KBr) ν_{max} 3386, 2920, 2866, 1656, 1596, 1493, 1446, 1400, 1056 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 348 $[M + Na]^+$; HRESIMS *m*/*z* 348.1578 (calcd for $C_{20}H_{23}NO_3Na$, 348.1576).

*Lansamide-6 (**5**):* colorless crystals (MeOH); mp 168−170 °C; $[\alpha]_D^{24}$ +20 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.14), 260 (2.32) nm; ECD (MeOH, $c = 6.41 \times 10^{-4}$ M) 221 ($\Delta\varepsilon$ +1.83) nm; IR (KBr) ν_{max} 3442, 2913, 1663, 1490, 1460, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 334 $[M + Na]^+$; HRESIMS *m*/*z* 334.1422 (calcd for $C_{19}H_{21}NO_3Na$, 334.1419).

*Lansamide-7 (**6**):* colorless, amorphous powder; $[\alpha]_D^{24}$ +11 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.27), 217 (3.08), 258 (2.24) nm; ECD (MeOH, $c = 5.45 \times 10^{-4}$ M) 223 ($\Delta\varepsilon$ +3.97), 272 ($\Delta\varepsilon$ +0.16) nm; IR (KBr) ν_{max} 3446, 2919, 2853, 1670, 1500, 1440, 1300, 1023 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 304 $[M + Na]^+$; HRESIMS *m*/*z* 304.1310 (calcd for $C_{18}H_{19}NO_2Na$, 304.1313).

*Lansamide-8 (**7**):* colorless crystals (MeOH); mp 118−120 °C; $[\alpha]_D^{24}$ +1 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.51), 225 (3.37), 258 (3.12), 304 (2.88) nm; ECD (MeOH, $c = 8.43 \times 10^{-4}$ M) 204 ($\Delta\varepsilon$ −0.14), 210 ($\Delta\varepsilon$ +0.06), 229 ($\Delta\varepsilon$ +0.07) nm; IR (KBr) ν_{max} 3376, 2923, 2852, 1746, 1696, 1436, 1253, 1033 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 372 $[M + Na]^+$; HRESIMS *m*/*z* 372.1204 (calcd for $C_{21}H_{19}NO_4Na$, 372.1206).

*Lansamide-9 (**8**):* colorless crystals (MeOH); mp 135−137 °C; $[\alpha]_D^{24}$ −50 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.34), 258 (2.05) nm; ECD (MeOH, $c = 8.32 \times 10^{-4}$ M) 219 ($\Delta\varepsilon$ −1.09), 231 ($\Delta\varepsilon$ +0.32), 263 ($\Delta\varepsilon$ −0.08) nm; IR (KBr) ν_{max} 3373, 2925, 1643, 1494, 1039 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 320 $[M + Na]^+$; HRESIMS *m*/*z* 320.1261 (calcd for $C_{18}H_{19}NO_3Na$, 320.1263).

*(+)-cis-Clausenamide (**9**):* colorless crystals (MeOH); mp 200−202 °C; $[\alpha]_D^{24}$ +29 (*c* 0.03, $CHCl_3$); lit. $[\alpha]_D^{20}$ +6.30 (*c* 0.46, $CHCl_3$);[19] UV (MeOH) λ_{max} (log ε) 204 (3.60), 217 (3.41), 259 (2.07) nm; ECD (MeOH, $c = 6.74 \times 10^{-4}$ M) 217 ($\Delta\varepsilon$ +0.71), 227 ($\Delta\varepsilon$ −2.04) nm; IR (KBr) ν_{max} 3297, 1660, 1476, 1450, 1403, 1022 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 320 $[M + Na]^+$; HRESIMS *m*/*z* 320.1262 (calcd for $C_{18}H_{19}NO_3Na$, 320.1263).

*(+)-(3S,4S)-Δ5,6-Clausenamide (**10**):* colorless crystals (MeOH); mp 124−126 °C; $[\alpha]_D^{24}$ +51 (*c* 2.5, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.02), 275 (3.06) nm; ECD (MeOH, $c = 4.82 \times 10^{-4}$ M) 204 ($\Delta\varepsilon$ +0.13), 226 ($\Delta\varepsilon$ −1.77), 243 sh ($\Delta\varepsilon$ −1.07), 265 ($\Delta\varepsilon$ +1.36), 277 ($\Delta\varepsilon$ +0.67), 299 ($\Delta\varepsilon$ +1.25) nm; IR (KBr) ν_{max} 3371, 1712, 1646, 1476, 1436, 1309, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 302 $[M + Na]^+$; HRESIMS *m*/*z* 302.1158 (calcd for $C_{18}H_{17}NO_2Na$, 302.1157).

*Anhydroclausenamide (**11**):* colorless, amorphous powder; $[\alpha]_D^{24}$ −2 (*c* 1.3, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.43), 251 (2.97), 290 (3.25) nm; ECD (MeOH, $c = 3.21 \times 10^{-4}$ M) 236 ($\Delta\varepsilon$ +0.11), 307 ($\Delta\varepsilon$ +0.19) nm; IR (KBr) ν_{max} 3062, 1666, 1593, 1486, 1385, 1126 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 302 $[M + Na]^+$; HRESIMS *m*/*z* 302.1159 (calcd for $C_{18}H_{17}NO_2Na$, 302.1157).

*(−)-Clausenamide (**12**):* colorless crystals (MeOH); mp 162−164 °C; $[\alpha]_D^{24}$ −149 (*c* 0.9, MeOH); lit. $[\alpha]_D^{20}$ −146 (*c* 0.2, MeOH);[19] UV (MeOH) λ_{max} (log ε) 202 (3.39), 261 (2.13) nm; ECD (MeOH, $c = 7.24 \times 10^{-4}$ M) 224 ($\Delta\varepsilon$ −0.80), 231 ($\Delta\varepsilon$ +0.64) nm; IR (KBr) ν_{max} 3492, 2882, 1682, 1254, 1061 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 320 $[M + Na]^+$; HRESIMS *m*/*z* 320.1265 (calcd for $C_{18}H_{19}NO_3Na$, 320.1263).

*(−)-Neoclausenamide (**13**):* colorless crystals (MeOH); mp 178−180 °C; $[\alpha]_D^{24}$ −72 (*c* 1.8, MeOH); lit. $[\alpha]_D^{20}$ −89 (*c* 0.2, MeOH);[19] UV (MeOH) λ_{max} (log ε) 206 (3.41), 216 (3.22), 258 (1.94) nm; ECD (MeOH, $c = 3.57 \times 10^{-4}$ M) 219 ($\Delta\varepsilon$ −1.23), 229 ($\Delta\varepsilon$ +1.05) nm; IR (KBr) ν_{max} 3349, 2920, 1679, 1493, 1450, 1310, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 320 $[M + Na]^+$; HRESIMS *m*/*z* 320.1257 (calcd for $C_{18}H_{19}NO_3Na$, 320.1257).

*Lansamide-3 (**14**):* colorless crystals (MeOH); mp 190−192 °C; $[\alpha]_D^{24}$ −109 (*c* 1.4, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.34), 258 (1.84) nm; ECD (MeOH, $c = 1.20 \times 10^{-4}$ M) 212 ($\Delta\varepsilon$ −2.92), 230 ($\Delta\varepsilon$ +0.80), 263 ($\Delta\varepsilon$ −0.33) nm; IR (KBr) ν_{max} 3381, 2926, 1643, 1493, 1450, 1047, 699 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 320 $[M + Na]^+$; HRESIMS *m*/*z* 320.1261 (calcd for $C_{18}H_{19}NO_3Na$, 320.1263).

*Lansamide-4 (**15**):* colorless crystals (MeOH); mp 118−120 °C; $[\alpha]_D^{24}$ −117 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.25), 295 (3.00) nm; ECD (MeOH, $c = 6.92 \times 10^{-4}$ M) 219 ($\Delta\varepsilon$ −1.30), 231 ($\Delta\varepsilon$ +0.39), 263 ($\Delta\varepsilon$ −0.10) nm; IR (KBr) ν_{max} 3369, 2925, 1653, 1491, 1447, 1392, 1073, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; ESIMS *m*/*z* 334 $[M + Na]^+$; HRESIMS *m*/*z* 334.1413 (calcd for $C_{19}H_{21}NO_3Na$, 334.1413).

Single-Crystal X-ray Diffraction Analysis and Crystallographic Data for Compounds 1, 2, 5, 7, 8, 9, 10, 12, 13, and 15. The diffraction intensity data for compounds **2**, **5**, **8**, **9**, **10**, **12**, **13**, and **15** were acquired on a Bruker D8 Venture with a Photon 100 CMOS detector system equipped with a Cu INCOATEC IμS microfocus source (λ = 1.541 78 Å). The diffraction intensity data for compound **7** were measured with Mo Kα radiation. The data were collected by Bruker APEX2 software. The data reductions were conducted with Bruker SAINT. Structure solutions and refinements were performed with the SHELXTL program package. The crystal structures of selected compounds **1**, **2**, **5**, **7**, **8**, and **10** were drawn by ORTEP and are shown in Figure 4, and those for compounds **9**, **12**, **13**, and **15** were placed in the Supporting Information.

*Crystallographic data of **1**:* $C_{19}H_{21}NO_3$, formula weight 311.37, crystal size 0.20 × 0.13 × 0.12 mm^3, crystal system monoclinic, space group $P2_1$, a = 8.5617(2) Å, b = 5.50850(10) Å, c = 17.2021(5) Å, $\alpha = \gamma = 90°$, β = 103.0150(10)°, V = 790.45(3) $Å^3$, Z = 2, D_{calcd} = 1.308 mg/m^3, μ(Cu Kα) = 0.710 mm^{-1}, F(000) = 332; total 5748 reflections, 2138 independent reflections [R(int) = 0.0209]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0288, wR_2 = 0.0739; R indices (all data) R_1 = 0.0291, wR_2 = 0.0741, Flack parameter 0.00(10), Hooft parameter 0.01(11).

*Crystallographic data of **2**:* $C_{19}H_{21}NO_3$, formula weight 311.37, crystal size 0.60 × 0.14 × 0.07 mm^3, crystal system monoclinic, space group $P2_1$, a = 8.7190(8) Å, b = 5.6224(6) Å, c = 17.2361(12) Å, $\alpha = \gamma$

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= 90°, β = 96.032(8)°, V = 840.26(13) Å^3, Z = 2, D_{calcd} = 1.231 mg/m^3, μ(Cu Kα) = 0.668 mm^{-1}, F(000) = 332; total 6781 reflections, 2918 independent reflections [R(int) = 0.0303]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0471, wR_2 = 0.1243; R indices (all data) R_1 = 0.0534, wR_2 = 0.1322, Flack parameter −0.1(3).

Crystallographic data of ***5****:* $C_{19}H_{21}NO_3$, formula weight 311.37, crystal size 0.25 × 0.25 × 0.15 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, a = 7.54260(10) Å, b = 12.0156(2) Å, c = 17.4134(3) Å, $\alpha = \beta = \gamma$ = 90°, V = 1578.16(4) Å^3, Z = 4, D_{calcd} = 1.310 mg/m^3, μ(CuKα) = 0.711 mm^{-1}, F(000) = 664; total 6852 reflections, 2627 independent reflections [R(int) = 0.0213]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0270, wR_2 = 0.0692; R indices (all data) R_1 = 0.0274, wR_2 = 0.0697, Flack parameter 0.07(16), Hooft parameter 0.04(6).

Crystallographic data of ***7****:* $C_{21}H_{19}NO_4$, formula weight 349.37, crystal size 0.64 × 0.56 × 0.42 mm^3, crystal system orthorhombic, space group *Pbca*, a = 9.4116(3) Å, b = 15.8331(5) Å, c = 24.7415(10) Å, $\alpha = \beta = \gamma$ = 90°, V = 3686.9(2) Å^3, Z = 8, D_{calcd} = 1.259 mg/m^3, μ(MoKα) = 0.087 mm^{-1}, F(000) = 1472; total 11 035 reflections, 4362 independent reflections [R(int) = 0.0220]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0476, wR_2 = 0.1284; R indices (all data) R_1 = 0.0867, wR_2 = 0.1384.

Crystallographic data of ***8****:* $C_{18}H_{19}NO_3$, formula weight 297.34, crystal size 0.62 × 0.32 × 0.12 mm^3, crystal system monoclinic, space group $P2_1$, a = 9.9132(8) Å, b = 7.0748(6) Å, c = 11.5133(11) Å, $\alpha = \gamma$ = 90°, β = 104.337(9)°, V = 782.32(12) Å^3, Z = 2, D_{calcd} = 1.262 mg/m^3, μ(Cu Kα) = 0.694 mm^{-1}, F(000) = 316; total 5676 reflections, 2741 independent reflections [R(int) = 0.0266]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0334, wR_2 = 0.0834; R indices (all data) R_1 = 0.0371, wR_2 = 0.087, Flack parameter 0.0(2), Hooft parameter −0.01(17).

Crystallographic data of ***9****:* $C_{18}H_{19}NO_3$, formula weight 297.34, crystal size 0.43 × 0.37 × 0.09 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, a = 5.9065(3) Å, b = 7.9113(3) Å, c = 32.9027(14) Å, $\alpha = \beta = \gamma$ = 90°, V = 1537.48(12) Å^3, Z = 4, D_{calcd} = 1.285 mg/m^3, μ(Cu Kα) = 0.707 mm^{-1}, F(000) = 632; total 36 159 reflections, 2739 independent reflections [R(int) = 0.0411]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0436, wR_2 = 0.1316; R indices (all data) R_1 = 0.0444, wR_2 = 0.1323, Flack parameter 0.3(4), Hooft parameter 0.18(6).

Crystallographic data of ***10****:* $C_{18}H_{17}NO_2$, formula weight 279.33, crystal size 0.30 × 0.30 × 0.20 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, a = 6.0722(2) Å, b = 8.1150(3) Å, c = 29.7039(10) Å, $\alpha = \beta = \gamma$ = 90°, V = 1463.69(9) Å^3, Z = 4, D_{calcd} = 1.268 mg/m^3, μ(Cu Kα) = 0.659 mm^{-1}, F(000) = 592; total 5946 reflections, 2415 independent reflections [R(int) = 0.0205]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0289, wR_2 = 0.0817; R indices (all data) R_1 = 0.0304, wR_2 = 0.0924, Flack parameter 0.1(2).

Crystallographic data of ***12****:* $C_{18}H_{21}NO_4$, formula weight 315.36, crystal size 0.39 × 0.36 × 0.13 mm^3, crystal system monoclinic, space group $C2$, a = 14.0792(9) Å, b = 6.7979(3) Å, c = 17.7480(8) Å, $\alpha = \gamma$ = 90°, β = 95.958(3)°, V = 1689.47(15) Å^3, Z = 4, D_{calcd} = 1.240 mg/m^3, μ(Cu Kα) = 0.715 mm^{-1}, F(000) = 672; total 22 268 reflections, 2963 independent reflections [R(int) = 0.0453]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0359, wR_2 = 0.1012; R indices (all data) R_1 = 0.0374, wR_2 = 0.1029, Flack parameter 0.0(2).

Crystallographic data of ***13****:* $C_{18}H_{19}NO_3$, formula weight 297.34, crystal size 0.43 × 0.14 × 0.08 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, a = 14.0792(9) Å, b = 6.7979(3) Å, c = 17.7480(8) Å, $\alpha = \beta = \gamma$ = 90°, V = 1523.95(14) Å^3, Z = 4, D_{calcd} = 1.296 mg/m^3, μ(Cu Kα) = 0.713 mm^{-1}, F(000) = 632; total 15 716 reflections, 2727 independent reflections [R(int) = 0.0442]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0403, wR_2 = 0.1114; R indices (all data) R_1 = 0.0441, wR_2 = 0.1150, Flack parameter 0.1(3).

Crystallographic data of ***15****:* $C_{19}H_{21}NO_3$, formula weight 311.37, crystal size 0.49 × 0.12 × 0.06 mm^3, crystal system orthorhombic, space group $P2_12_12_1$, a = 7.3552(2) Å, b = 13.6032(4) Å, c = 17.2196(5) Å, $\alpha = \beta = \gamma$ = 90°, V = 1722.89(9) Å^3, Z = 4, D_{calcd} = 1.200 mg/m^3, μ(Cu Kα) = 0.652 mm^{-1}, F(000) = 664; total 16 808 reflections, 3074 independent reflections [R(int) = 0.0414]; final R indices [$I > 2\sigma(I)$] R_1 = 0.0384, wR_2 = 0.0885; R indices (all data) R_1 = 0.0510, wR_2 = 0.0943, Flack parameter 0.0(3).

The crystallographic data for the structures of compounds **1**, **2**, **5**, **7**, **8**, **9**, **10**, **12**, **13**, and **15** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposit numbers CCDC 991717−991726, respectively). Copies of these data can be obtained, free of charge, on application to Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Neuroprotective and Neurotoxicity Assay. The neuroprotective activity on Aβ-induced neurotoxicity of purified compounds was determined according to the method reported previously.[23] The neurotoxicity was also measured by the MTT reduction assay as reported.[23]

Statistical Analysis. All experiments were performed at least three times, and the results are expressed as the means ± SEM. The statistical analyses were based on the Student's t test or the Mann−Whitney U test, and all calculations were performed with SigmaPlot (Systat Software, San Jose, CA, USA). A p value < 0.05 was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00148.

Copies of the spectra of compounds **1**−**15**, the X-ray structures of **9**, **12**, **13**, and **15**, and the chiral HPLC profiles of compounds **1** and **12** (PDF)

Crystallographic data for compounds **1**, **2**, **5**, **7**−**10**, **12**, **13**, and **15** (ZIP)

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Notes

The authors declare no competing financial interest.

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