Amides and Benzenoids from *Zanthoxylum ailanthoides* with Inhibitory Activity on Superoxide Generation and Elastase Release by Neutrophils

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Five new compounds, ailanthamide (**1**), *N*-(4-methoxyphenethyl)-*N*-methylbenzamide (**2**), (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde (**4**), and (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (**5**), and 17 known compounds have been isolated from the stem bark of *Zanthoxylum ailanthoides*. The structures were determined through spectroscopic and MS analyses. Compounds **1**, **3**, xanthyletin, decarine, (+)-episesamin, (−)-hinokinin, and evofolin-B exhibited inhibition (IC$_{50}$ ≤ 5.34 μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds **1**, xanthyletin, decarine, and (+)-episesamin also inhibited fMLP/CB-induced elastase release with IC$_{50}$ values ≤ 5.53 μg/mL.

Zanthoxylum ailanthoides Sieb. & Zucc. (Rutaceae) is a medium- to large-sized tree found at low altitude in forests of China, Korea, Japan, the Philippines, and Taiwan.[1] Its leaves are used as a folk medicine to treat the common cold in Taiwan.[2] Benzo[c]phenanthridines, quinolines, coumarins, flavonoids, lignans, and terpenoids have been identified as constituents of this plant.[3–12] Antiplatelet aggregation[11] and anti-HIV[12] activities have been reported for some of these compounds. In our studies on constituents of Formosan plants for *in vitro* inhibitory activity on neutrophil pro-inflammatory responses, *Z. ailanthoides* was found to be an active species. Three new amides, ailanthamide (**1**), *N*-(4-methoxyphenethyl)-*N*-methylbenzamide (**2**), and (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), two new benzenoids, 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde (**4**) and (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (**5**), and 17 known compounds have been isolated and identified from the stem bark of *Z. ailanthoides*. This paper describes the structural elucidation of **1**−**5** and the inhibitory activities of all isolates on superoxide generation and elastase release by neutrophils.

Results and Discussion

Chromatographic purification of the EtOAc-soluble fraction of a MeOH extract of stem bark of *Z. ailanthoides* on a silica gel column (CC) and preparative thin-layer chromatography (TLC) afforded five new (**1**−**5**) and 17 known compounds.

Ailanthamide (**1**) was isolated as a colorless oil. The HRESIMS gave an [M + Na]$^+$ ion at *m/z* 334.1421 (calcd for C$_{19}$H$_{21}$NO$_3$Na, 334.1419), consistent with a molecular formula of C$_{19}$H$_{21}$NO$_3$. The UV absorptions at 225, 294, and 313 nm were similar to those of *N-p*-coumaroyltyramine,[13] and the bathochromic shift in alkaline solution suggested the presence of an *N-p*-coumaroyltyramine nucleus.[13] IR absorptions for OH (3345 cm^{-1}) and carbonyl (1650 cm^{-1}) functions were observed. The ^{1}H NMR data of **1** were similar to those of beecheyamide,[14] except that the 4-hydroxyphenyl group at C-7 of **1** replaced the 3,4-dimethoxyphenyl group at C-7 of beecheyamide.[14] This was supported by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between H-2 and both H-3 and H-7; (b) HMBC correlations were
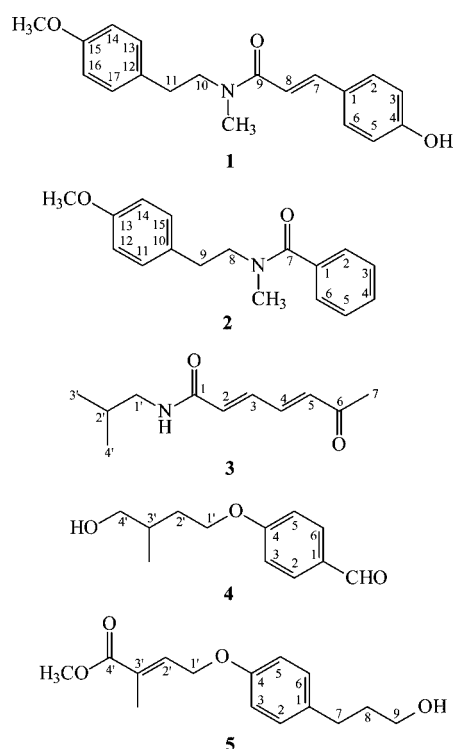
observed between H-2 and C-4 and C-7; (c) HMBC correlations were observed between H-3 and C-1 and C-5. At room temperature, two isomers (**1a** and **1b**), with integrated intensities of 2 (**1a**):3 (**1b**), were detected in the ^{1}H NMR spectrum of **1**, but at a temperature above 70 °C, only one component could be detected in the ^{1}H NMR spectrum of **1**, as in the case of beecheyamide.[14] Consequently, the temperature-dependent ^{1}H NMR spectra can be explained in terms of slow interconversion of the *syn* and *anti* amide isomers due to *syn*−*anti* isomerism.[15] The *syn* and *anti* isomers of **1** were determined by the NOESY experiments (Figure 1). The major component (**1b**) exhibited a NOESY correlation between H-8 (δ 6.38) and H-10 (δ 3.65), while the same experiment for H-8 (δ 6.69) in the minor component (**1a**) correlated with N-Me (δ 3.03). Thus, the amide configurations in the major (**1b**) and minor (**1a**) components are *syn* and *anti* form, respectively. On the basis of the above data, the structure of **1** was elucidated as 3-(4-hydroxyphenyl)-*N*-[2-(4-methoxyphenyl)ethyl]-*N*-methylacryla-

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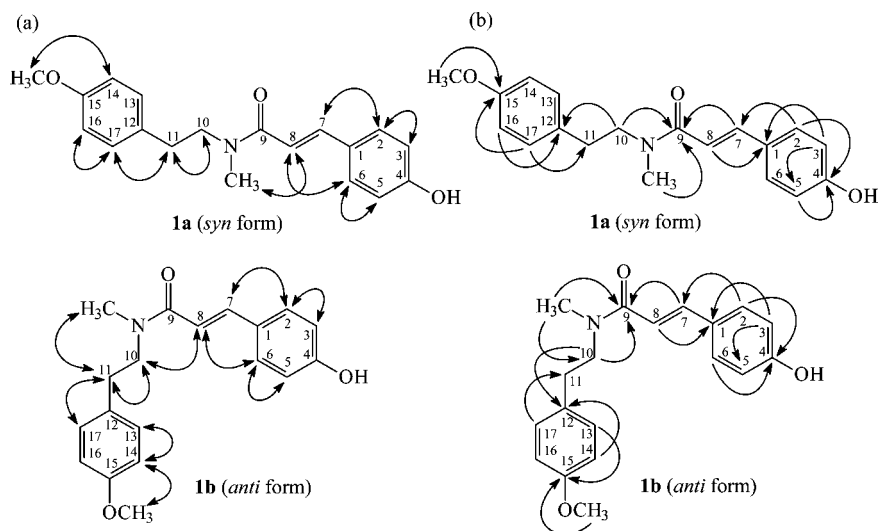§ Taitung District Agricultural Research and Extension Station.

Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

mide. This was confirmed by $^1H-^1H$ COSY and NOESY experiments (Figure 1). Assignments of the carbon resonances were confirmed by DEPT, HSQC, and HMBC techniques.

Compound **2** was isolated as a yellowish, amorphous powder. The ESIMS afforded the sodiated ion $[M + Na]^+$ at m/z 292, implying a molecular formula of $C_{17}H_{19}NO_2$, which was confirmed by the HRESIMS (m/z 292.1314 $[M + Na]^+$, calcd for $C_{17}H_{19}NO_2Na$, 292.1313). UV absorptions at 224, 274, and 283 nm were similar to those of dihydroalatamide[16] and suggested the presence of a benzoylamide moiety. The presence of a carbonyl group was revealed by the band at 1628 cm^{-1} in the IR spectrum of **2** and was confirmed by a signal at δ 172.2 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **2** showed the presence of a benzoyl group, an N-methyl group, a 4-methoxyphenyl group, and four mutually coupled methylene protons very similar to signals described previously for dihydroalatamide,[16] except for the presence of an additional N-methyl group [δ 3.14 (3H, s)] of **2**, replacing an NH group of dihydroalatamide.[16] This was supported by HMBC correlations observed between N-Me (δ 3.14) and both C-7 (δ 172.2) and C-8 (δ 53.1) and by NOESY correlations between N-Me (δ 3.14) and both H-6 (δ 7.33) and H-9 (δ 2.74). The ^1H NMR spectrum of **2** also exhibited the *syn* and *anti* amide isomers due to *syn–anti* isomerism[15] as in the case of **1** and beecheyamide.[14] The *syn* amide component (**2a**) exhibited a NOESY correlation between N-Me (δ 3.14) and H-6 (δ 7.33), while the same experiment for N-Me (δ 2.82) in the *anti* amide component (**2b**) correlated with H-9 (δ 2.95). Assignments of the carbon resonances were confirmed by DEPT, HSQC, and HMBC techniques (Figure 2). On the basis of the above data, the structure of **2** was elucidated as N-(4-methoxyphenethyl)-N-methylbenzamide, which was confirmed by the $^1H-^1H$ COSY and NOESY experiments (Figure 2).

Compound **3** was isolated as a colorless oil with molecular formula $C_{11}H_{17}NO_2$ as determined by positive-ion HRESIMS, showing an $[M + Na]^+$ ion at m/z 218.1159 (calcd for $C_{11}H_{17}NO_2Na$, 218.1157). The presence of NH and carbonyl groups in **3** was revealed by the bands at 3292, 1666, and 1627 cm^{-1}, respectively, in the IR spectrum. The ^1H NMR spectrum of **3** showed resonances for an N-isobutyl group, an NH group, an acetyl group, and four mutually coupled protons of a 6-oxohepta-2,4-dienamide moiety. The locations of the above groups of **3** were determined by the following NOESY and HMBC correlations. NOESY correlations were observed between H-2′ (δ 1.83) and both H-1′ (δ 3.20) and H-3′ (δ 0.95); between NH (δ 5.64) and both H-1′ (δ 3.20) and H-2 (δ 6.22); between H-2 (δ 6.22) and both NH (δ 5.64) and H-4 (δ 7.15); and between H-5 (δ 6.42) and both H-3 (δ 7.30) and H-7 (δ 2.32). HMBC correlations were observed
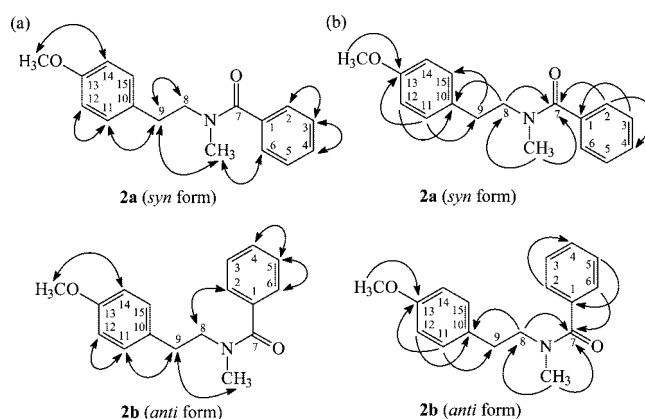

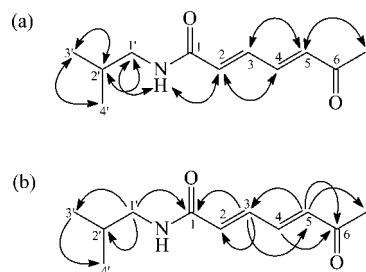**Figure 2.** NOESY (a) and HMBC (b) correlations of **2**.

Figure 3. NOESY (a) and HMBC (b) correlations of **3**.

between H-1′ (δ 3.20) and both C-1 (δ 165.3) and C-3′ (δ 20.1); between H-3 (δ 7.30) and C-1 (δ 165.3), C-2 (δ 131.7), and C-5 (δ 135.1); and between H-5 (δ 6.42) and C-3 (δ 137.9), C-6 (δ 198.3), and C-7 (δ 28.1). In addition, the 2E,4E-configuration of **3** was established by the coupling constants between H-2/H-3 (J = 15.0 Hz) and H-4/H-5 (J = 15.5 Hz) and by the NOESY correlations between H-2/H-4 and H-3/H-5. Thus, the structure of **3** was elucidated as (2E,4E)-N-isobutyl-6-oxohepta-2,4-dienamide. This structure was confirmed by the $^1H-^1H$ COSY, NOESY, DEPT, HSQC, and HMBC techniques (Figure 3).

Compound **4** had the molecular formula $C_{12}H_{16}O_3$, as indicated by the sodiated HRESIMS ion peak at m/z = 231.0996 $[M + Na]^+$ (calcd for $C_{12}H_{16}O_3Na$, 231.0997). IR absorptions for OH (3425 cm^{-1}) and conjugated carbonyl functions (1680 cm^{-1}) were observed. The ^1H NMR spectrum of **4** showed resonances for a 4-hydroxy-3-methylbutoxy group [δ 1.02 (3H, d, J = 6.5 Hz, Me-3′), δ 1.69, 1.98 (each 1H, each m, H-2′), δ 1.94 (1H, m, H-3′), δ
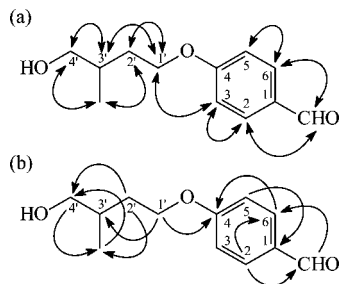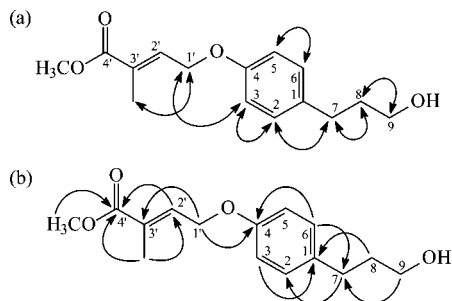
Figure 4. NOESY (a) and HMBC (b) correlations of **4**.

Figure 5. NOESY (a) and HMBC (b) correlations of **5**.

3.57 (2H, d, J = 5.5 Hz, H-4′), and δ 4.14 (2H, m, H-1′)], a formyl group [δ 9.89 (1H, s, CHO-1)], and an AA′BB′ spin system [δ 7.00 (2H, d, J = 9.0 Hz, H-3 and H-5) and δ 7.84 (2H, d, J = 9.0 Hz, H-2 and H-6)]. On the basis of NOESY correlations between H-2 (δ 7.84) and both CHO-1 (δ 9.89) and H-3 (δ 7.00), and between H-3 (δ 7.00) and both H-2 (δ 7.84) and H-1′ (δ 4.14), the 4-hydroxy-3-methylbutoxy group was assigned to C-4. The structure of **4** was thus elucidated as 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde. This was confirmed by ^{1}H$-^1$H COSY and NOESY experiments (Figure 4). The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC techniques.

Compound **5** was isolated as colorless needles having the molecular formula $C_{15}H_{20}O_4$ as deduced from the sodiated ion at m/z 287.1254 [M + Na]$^+$ in the HRESI mass spectrum. The presence of OH and carbonyl groups in **5** was revealed by bands at 3394 and 1715 cm^{-1}, respectively, in the IR spectrum. Comparison of the ^{1}H NMR data of **5** with that of cuspidiol[17] suggested that their structures were closely related except that a 3′-methoxycarbonyl group [δ 3.76 (3H, s)] of **5** replaced the 3′-hydroxymethyl group of cuspidiol.[17] This was supported by HMBC correlations observed between OMe-4′ (δ 3.76) and C-4′ (δ 167.8) and between H-2′ (δ 6.93) and C-4′ (δ 167.8). NOESY correlations of **5** were observed between H-1′ (δ 4.70) and Me-3′ (δ 1.93). Thus, the E-configuration of **5** was established. According to the above data, the structure of **5** was elucidated as (E)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate, which was further supported by the ^{1}H$-^1$H COSY, NOESY, DEPT, HSQC, and HMBC experiments (Figure 5). This is the first isolation of **5** from a natural source, although it has been synthesized by Ishii et al.[18]

The known isolates were readily identified by a comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, [α]$_D$, and MS) with corresponding authentic samples or literature values. The known compounds were identified as xanthyletin,[7] bergapten,[19] aesculetin dimethyl ether,[20] 6,7,8-trimethoxycoumarin,[21] decarine,[22] norchelerythrine,[22] γ-fagarine,[23] (+)-episesamin,[24] (−)-hinokinin,[25] methyl (E)-p-hydroxycinnamate,[26] *trans*-p-coumaryaldehyde,[27] methyl 4-hydroxybenzoate,[28] ailanthoidiol,[29] evofolin-B,[30] syringaldehyde,[31] 4-hydroxybenzaldehyde,[32] and β-amyrin.[33]

Human neutrophils are known to play significant roles in host defense against microorganisms and in pathogenesis of various diseases such as rheumatoid arthritis, chronic obstructive pulmonary

Table 1. Inhibitory Effects of Compounds Isolated from Stem Bark of *Z. ailanthoides* on Superoxide Radical Anion Generation and Elastase Release by Human Neutrophils in Response to fMet-Leu-Phe/Cytochalasin B^a

	IC$_{50}$ (μg/mL)^b or (Inh %)^c	
compound	superoxide anion	elastase
1	3.71 ± 0.06***	4.23 ± 1.01**
2	8.49 ± 0.31***	7.48 ± 0.58***
3	4.20 ± 0.72**	6.20 ± 0.72***
xanthyletin	4.10 ± 0.33***	5.53 ± 0.23***
aesculetin dimethyl ether	8.25 ± 1.52***	(43.1 ± 5.2)***
decarine	1.29 ± 0.19***	1.94 ± 0.30***
γ-fagarine	7.17 ± 0.44***	(19.1 ± 3.9)**
(+)-episesamin	4.30 ± 0.59***	1.38 ± 0.17***
(−)-hinokinin	0.60 ± 0.12***	(24.7 ± 6.2)*
evofolin-B	5.34 ± 0.41***	6.15 ± 1.10***
diphenyleneiodonium	0.54 ± 0.21***	
phenylmethylsulfonyl fluoride		35.24 ± 5.62***

^{a} Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls. Results are presented as average ± SEM (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001 compared with the control. ^{b} Concentration necessary for 50% inhibition (IC$_{50}$). ^{c} Percentage of inhibition (Inh %) at 10 μg/mL.

disease (COPD), ischemia-reperfusion injury, and asthma.[34−38] In response to different stimuli, activated neutrophils secrete a series of cytotoxins, such as the superoxide anion radical ($O_2^{\bullet-}$), a precursor to other reactive oxygen species (ROS), granule proteases, bioactive lipids, and neutrophil elastase, a major contributor to destruction of tissue in chronic inflammatory disease.[35,39−41] Suppression of the extensive or inappropriate activation of neutrophils by drugs has been proposed as a way to ameliorate inflammatory diseases. The effects on neutrophil pro-inflammatory responses of compounds isolated from the stem bark of *Z. ailanthoides* were evaluated by suppressing fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced superoxide anion ($O_2^{\bullet-}$) generation and elastase release by human neutrophils. The inhibitory activity data on neutrophil pro-inflammatory responses are summarized in Table 1. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls for $O_2^{\bullet-}$ generation and elastase release, respectively. From the results of our biological tests, the following conclusions can be drawn: (a) Ailanthamide (**1**), (2E,4E)-N-isobutyl-6-oxohepta-2,4-dienamide (**3**), xanthyletin, decarine, (+)-episesamin, (−)-hinokinin, and evofolin-B exhibited potent inhibitory activities (IC$_{50}$ ≤ 5.34 μg/mL) on human neutrophil $O_2^{\bullet-}$ generation. Among them, (−)-hinokinin was the most effective, with an IC$_{50}$ value of 0.60 ± 0.12 μg/mL against fMLP-induced superoxide anion generation. (b) Compounds **1**, xanthyletin, decarine, and episesamin inhibited fMLP/CB-induced elastase release with IC$_{50}$ values ≤ 5.53 μg/mL. Among them, (+)-episesamin exhibited the most effective inhibition, with an IC$_{50}$ value of 1.38 ± 0.17 μg/mL against fMLP-induced elastase release. (c) Ailanthamide (**1**), with an N-(E)-3-(4-hydroxyphenyl)acryloyl group, exhibited more effective inhibition than its analogue, N-(4-methoxyphenethyl)-N-methylbenzamide (**2**), with an N-benzoyl substituent, against fMLP-induced $O_2^{\bullet-}$ generation and elastase release. (d) Among the coumarin analogues, xanthyletin, with a 2,2-dimethyl-3,4-dehydroxypyran moiety at C-6,7, exhibited more effective inhibition than analogues bergapten, aesculetin dimethyl ether, and 6,7,8-trimethoxycoumarin against fMLP-induced $O_2^{\bullet-}$ generation and elastase release. (e) Decarine, with an 8-hydroxy group, showed strong inhibition against fMLP-induced superoxide production, but its analogue, norchelerythrine, with a 8-methoxy group, was inactive.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and were uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter in

CHCl₃. UV spectra were obtained on a Jasco UV-240 spectrophotometer. IR spectra (KBr or neat) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Unity 400 or a Varian Inova 500 spectrometer operating at 400 and 500 MHz (^{1}H) and 100 and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. EI, ESI, and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. FAB and HRFAB mass spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. Silica gel (70−230, 230−400 mesh) (Merck) was used for CC. Silica gel 60 F-254 (Merck) was used for TLC and preparative TLC.

Plant Material. The stem bark of *Z. ailanthoides* was collected from Taitung District Agricultural Research and Extension Station, Taitung County, Taiwan, in June 2007 and identified by one of the authors (J.F.C.). A voucher specimen (Chen 5645) was deposited in the herbarium of the Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation. The dried stem bark of *Z. ailanthoides* (7.1 kg) was pulverized and extracted three times with MeOH (20 L each) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and the residue (730 g) was partitioned between EtOAc and H₂O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 125 g). The water layer was further extracted with *n*-BuOH, and the *n*-BuOH-soluble part (fraction B, 230 g) and the water-solubles (fraction C, 365 g) were separated. Fraction A (125 g) was chromatographed on silica gel (70−230 mesh, 4.9 kg), eluting with CH₂Cl₂, gradually increasing the polarity with MeOH to give 13 fractions: A1 (8 L, CH₂Cl₂), A2 (5.5 L, CH₂Cl₂/MeOH, 98:1), A3 (5 L, CH₂Cl₂/MeOH, 95:1), A4 (6 L, CH₂Cl₂/MeOH, 90:1), A5 (6 L, CH₂Cl₂/MeOH, 80:1), A6 (4 L, CH₂Cl₂/MeOH, 70:1), A7 (7 L, CH₂Cl₂/MeOH, 50:1), A8 (7 L, CH₂Cl₂/MeOH, 30:1), A9 (7 L, CH₂Cl₂/MeOH, 10:1), A10 (6 L, CH₂Cl₂/MeOH, 5:1), A11 (6 L, CH₂Cl₂/MeOH, 3:1), A12 (5.5 L, CH₂Cl₂/MeOH, 1:1), A13 (5 L, MeOH). Fraction A1 (10.5 g) was chromatographed further on silica gel (70−230 mesh, 410 g) eluting with *n*-hexane/acetone (8:1) to give 14 fractions (each 1.0 L, A1-1−A1-14). Fraction A1-5 (850 mg) was washed with MeOH and filtered to yield β-amyrin (236 mg) after recrystallization (MeOH). Fraction A1-6 (185 mg) was separated by preparative TLC (PTLC) (silica gel, *n*-hexane/EtOAc, 2:1) to afford norchelerythrine (3.7 mg). Fraction A1-8 (230 mg) was separated by PTLC (silica gel, *n*-hexane/EtOAc, 2:1) to yield xanthyletin (5.7 mg), bergapten (6.4 mg), and (+)-episesamin (16.8 mg). Fraction A1-11 (200 mg) was subjected to PTLC (silica gel, *n*-hexane/acetone, 2:1) to obtain (−)-hinokinin (21.5 mg). Fraction A2 (9.8 g) was chromatographed on silica gel (70−230 mesh, 350 g) eluting with CH₂Cl₂/acetone (20:1) to give 12 fractions (each 1.0 L, A2-1−A2-12). Fraction A2-5 (780 mg) was purified by MPLC (33 g silica gel, 230−400 mesh, CH₂Cl₂/MeOH, 20:1, 200 mL fractions) to obtain 11 subfractions: A2-5-1−A2-5-11. Fraction A2-5-4 (82 mg) was purified further by PTLC (silica gel, CH₂Cl₂/acetone, 30:1) to give 6,7,8-trimethoxycoumarin (12 mg). Fraction A2-5-5 (90 mg) was purified further by PTLC (silica gel, *n*-hexane/EtOAc, 2:1) to afford syringaldehyde (3.7 mg). Fraction A2-5-6 (98 mg) was washed with *n*-hexane and filtered to yield aesculetin dimethyl ether (5.3 mg) after recrystallization (CH₂Cl₂/MeOH). Fraction A3 (9.4 g) was chromatographed on silica gel (70−230 mesh, 385 g) eluting with CH₂Cl₂/MeOH (40:1) to afford 12 fractions (each 1.3 L, A3-1−A3-12). Fraction A3-5 (1.1 g) was separated by MPLC (38 g silica gel, 230−400 mesh, *n*-hexane/EtOAc, 2:1, 250 mL fractions) to obtain nine subfractions: A3-5-1−A3-5-9. Fraction A3-5-3 (165 mg) was purified by PTLC (silica gel, CH₂Cl₂/acetone, 20:1) to yield methyl 4-hydroxybenzoate (5.8 mg). Fraction A3-5-5 (155 mg) was fractionated by PTLC (silica gel, CH₂Cl₂/acetone, 20:1) to obtain methyl (E)-p-hydroxycinnamate (3.5 mg). Fraction A4 (9.5 g) was chromatographed on silica gel (70−230 mesh, 390 g) eluting with *n*-hexane/EtOAc (1:1) to afford 12 fractions (each 500 mL, A4-1−A4-12). Fraction A4-3 (840 mg) was purified by MPLC (37 g silica gel, 230−400 mesh, CH₂Cl₂/MeOH, 40:1, 500 mL fractions) to obtain 11 subfractions: A4-3-1−A4-3-11. Fraction A4-3-4 (120 mg) was subjected to PTLC (silica gel, CHCl₃/MeOH, 30:1) to obtain decarine (3.5 mg). Fraction A4-3-5 (110 mg) was purified by PTLC (silica gel, *n*-hexane/EtOAc, 1:1) to yield **2** (2.5 mg). Fraction A4-3-6 (95 mg) was purified further by PTLC (silica gel, *n*-hexane/EtOAc, 1:1) to obtain γ-fagarine (6.3 mg). Fraction A4-3-7 (115 mg) was separated by PTLC (silica gel, CHCl₃/acetone, 10:1) to afford **5** (2.3 mg). Fraction A4-3-8 (98 mg) was purified further

by PTLC (silica gel, CHCl₃/acetone, 10:1) to give **4** (2.3 mg). Fraction A4-4 (160 mg) was subjected to PTLC (silica gel, CHCl₃) to yield *trans-p*-coumarylaldehyde (3.6 mg) and 4-hydroxybenzaldehyde (3.2 mg). Fraction A4-5 (190 mg) was subjected to PTLC (silica gel, *n*-hexane/acetone, 3:2) to yield **3** (2.4 mg). Fraction A5 (4.6 g) was chromatographed on silica gel (70−230 mesh, 205 g) eluting with *n*-hexane/acetone (5:1) to afford 15 fractions (each 600 mL, A5-1−A5-15). Fraction A5-9 (175 mg) was fractionated further by PTLC (silica gel, CHCl₃/MeOH, 15:1) to give evofolin-B (3.2 mg). Fraction A7 (7.6 g) was chromatographed on silica gel (70−230 mesh, 315 g) eluting with CHCl₃/EtOAc (5:1) to afford 13 fractions (each 900 mL, A7-1−A7-13). Fraction A7-6 (195 mg) was separated by PTLC (silica gel, *n*-hexane/acetone, 3:2) to give **1** (2.8 mg). Fraction A7-8 (180 mg) was separated by PTLC (silica gel, *n*-hexane/acetone, 1:1) to give ailanthoidiol (5.7 mg).

Biological Assay. The effects of the isolated compounds on neutrophil pro-inflammatory responses were evaluated by inhibiting the generation of O₂•⁻ and the release of elastase in fMLP-activated human neutrophils in a concentration-dependent manner.

Preparation of Human Neutrophils. Human neutrophils from venous blood of healthy, adult volunteers (20−28 years old) were isolated using a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes.[42] Purified neutrophils containing >98% viable cells, as determined by the trypan blue exclusion method,[43] were resuspended in a Ca²⁺-free HBSS buffer at pH 7.4 and were maintained at 4 °C prior to use.

Measurement of O₂•⁻ Generation. Measurement of O₂•⁻ generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[44,45] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca²⁺, neutrophils (6 × 10⁵/mL) were equilibrated at 37 °C for 2 min and incubated with different concentrations of compounds or DMSO (as control) for 5 min. Cells were activated with 100 nM fMLP for 10 min. When fMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by peptide (fMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ϵ = 21.1/mM/10 mm).

Measurement of Elastase Release. Degranulation of azurophilic granules was determined by measuring elastase release as described previously.[45] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6 × 10⁵/mL) were equilibrated at 37 °C for 2 min and incubated with compounds for 5 min. Cells were stimulated with fMLP (100 nM)/cytochalasin B (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously in order to assay elastase release. The results were expressed as the percent of elastase release in the fMLP/cytochalasin B-activated, drug-free control system.

Statistical Analysis. Results are expressed as the mean ± SEM, and comparisons were made using Student's *t*-test. A probability of 0.05 or less was considered significant. The software SigmaPlot was used for the statistical analysis.

Ailanthamide (1): colorless oil; UV (MeOH) λ_{max} (log ϵ) 225 (4.09), 294 (4.13), 313 (4.15) nm; IR (neat) ν_{max} 3345 (OH), 1650 (C=O) cm⁻¹; ^{1}H NMR (CDCl₃, 400 MHz) of **1a** (*syn* form) δ 2.85 (2H, t, *J* = 7.4 Hz, H-11), 3.03 (3H, s, N-Me), 3.63 (2H, t, *J* = 7.4 Hz, H-10), 3.79 (3H, s, OMe-15), 5.32 (1H, br s, D₂O exchangeable, OH-4), 6.69 (1H, d, *J* = 15.6 Hz, H-8), 6.82 (2H, d, *J* = 8.8 Hz, H-3 and H-5), 6.84 (2H, d, *J* = 8.4 Hz, H-14 and H-16), 7.17 (2H, d, *J* = 8.4 Hz, H-13 and H-17), 7.43 (2H, d, *J* = 8.8 Hz, H-2 and H-6), 7.63 (1H, d, *J* = 15.6 Hz, H-7); **1b** (*anti* form) δ 2.85 (2H, t, *J* = 7.4 Hz, H-11), 3.03 (3H, s, N-Me), 3.65 (2H, t, *J* = 7.4 Hz, H-10), 3.72 (3H, s, OMe-15), 5.32 (1H, br s, D₂O exchangeable, OH-4), 6.38 (1H, d, *J* = 15.6 Hz, H-8), 6.75 (2H, d, *J* = 8.8 Hz, H-3 and H-5), 6.84 (2H, d, *J* = 8.4 Hz, H-14 and H-16), 7.09 (2H, d, *J* = 8.4 Hz, H-13 and H-17), 7.31 (2H, d, *J* = 8.8 Hz, H-2 and H-6), 7.46 (1H, d, *J* = 15.6 Hz, H-7); ^{13}C NMR (CDCl₃, 100 MHz) of **1a** (*syn* form) δ 33.9 (C-11), 36.2 (N-Me), 50.7 (C-10), 55.1 (MeO-15), 113.9 (C-14), 113.9 (C-16), 115.7 (C-3), 115.7 (C-5), 118.2 (C-8), 127.6 (C-1), 129.7 (C-13), 129.7 (C-17), 130.1 (C-2), 130.1 (C-6), 131.2 (C-12), 141.8 (C-7), 157.8 (C-4), 158.0 (C-15), 166.7 (C-9); **1b** (*anti* form) δ 34.3 (C-11), 34.3 (N-Me),

52.0 (C-10), 55.1 (MeO-15), 113.9 (C-14), 113.9 (C-16), 115.5 (C-3), 115.5 (C-5), 118.1 (C-8), 127.3 (C-1), 129.7 (C-13), 129.7 (C-17), 130.0 (C-2), 130.0 (C-6), 130.2 (C-12), 141.6 (C-7), 157.7 (C-4), 158.4 (C-15), 166.3 (C-9); ESIMS m/z 334 [M + Na]$^+$; HRESIMS m/z 334.1421 [M + Na]$^+$ (calcd for $C_{19}H_{21}NO_3Na$, 334.1419).

***N*-(4-Methoxyphenethyl)-*N*-methylbenzamide (2):** yellowish, amorphous powder; UV (MeOH) λ_{max} (log ϵ) 224 (4.29), 274 (3.43), 283 (3.32) nm; IR (neat) ν_{max} 1628 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) of **2a** (*syn* form) δ 2.74 (2H, t, J = 7.6 Hz, H-9), 3.14 (3H, s, N-Me), 3.43 (2H, t, J = 7.6 Hz, H-8), 3.78 (3H, s, OMe-13), 6.87 (2H, d, J = 8.8 Hz, H-12 and H-14), 7.22 (2H, d, J = 8.8 Hz, H-11 and H-15), 7.33 (2H, m, H-2 and H-6), 7.35 (2H, m, H-3 and H-5), 7.36 (1H, m, H-4); **2b** (*anti* form) δ 2.82 (3H, s, N-Me), 2.95 (2H, t, J = 7.2 Hz, H-9), 3.74 (2H, t, J = 7.2 Hz, H-8), 3.80 (3H, s, OMe-13), 6.78 (2H, d, J = 8.4 Hz, H-12 and H-14), 6.86 (2H, d, J = 8.4 Hz, H-11 and H-15), 7.14 (2H, d, J = 7.6 Hz, H-2 and H-6), 7.34 (2H, m, H-3 and H-5), 7.36 (1H, m, H-4); ^{13}C NMR (CDCl$_3$, 100 MHz) of **2a** δ 33.1 (N-Me), 33.9 (C-9), 53.1 (C-8), 55.3 (OMe-13), 113.9 (C-12), 113.9 (C-14), 126.9 (C-2), 126.9 (C-6), 128.3 (C-3), 128.3 (C-5), 129.4 (C-4), 129.8 (C-11), 129.8 (C-15), 131.5 (C-10), 136.9 (C-1), 158.1 (C-13), 172.2 (C-7); **2b** δ 32.5 (C-9), 38.3 (N-Me), 49.5 (C-8), 55.3 (OMe-13), 114.0 (C-12), 114.0 (C-14), 126.4 (C-2), 126.4 (C-6), 128.3 (C-3), 128.3 (C-5), 129.2 (C-4), 129.7 (C-11), 129.7 (C-15), 131.2 (C-10), 136.6 (C-1), 158.0 (C-13), 171.3 (C-7); ESIMS m/z 292 [M + Na]$^+$; HRESIMS m/z 292.1314 [M + Na]$^+$ (calcd for $C_{17}H_{19}NO_2Na$, 292.1313).

(2*E*,4*E*)-*N*-Isobutyl-6-oxohepta-2,4-dienamide (3): colorless oil; UV (MeOH) λ_{max} (log ϵ) 272 (4.30) nm; IR (neat) ν_{max} 3292 (NH), 1666 (C=O), 1627 (C=O); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.95 (6H, d, J = 6.5 Hz, H-3' and H-4'), 1.83 (1H, m, H-2'), 2.32 (3H, s, H-7), 3.20 (2H, t, J = 6.5 Hz, H-1'), 5.64 (1H, br s, D$_2$O exchangeable, NH), 6.22 (1H, d, J = 15.0 Hz, H-2), 6.42 (1H, d, J = 15.5 Hz, H-5), 7.15 (1H, dd, J = 15.5, 11.5 Hz, H-4), 7.30 (1H, d, J = 15.0, 11.5 Hz, H-3); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 20.1 (C-3'), 20.1 (C-4'), 28.1 (C-7), 28.6 (C-2'), 47.1 (C-1'), 131.7 (C-2), 135.1 (C-5), 137.9 (C-3), 139.4 (C-4), 165.3 (C-1), 198.3 (C-6); ESIMS m/z 218 [M + Na]$^+$; HRESIMS m/z 218.1159 [M + Na]$^+$ (calcd for $C_{11}H_{17}NO_2Na$, calcd 218.1157).

4-(4'-Hydroxy-3'-methylbutoxy)benzaldehyde (4): colorless, amorphous powder; $[\alpha]^{25}_D$ −34 (c 0.11, CHCl$_3$). UV (MeOH) λ_{max} (log ϵ) 283 (4.13) nm; IR (neat) ν_{max} 3425 (OH), 1680 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 1.02 (3H, d, J = 6.5 Hz, Me-3'), 1.69 (1H, m, H-2'), 1.94 (1H, m, H-3'), 1.98 (1H, m, H-2'), 3.57 (2H, d, J = 5.5 Hz, H-4'), 4.14 (2H, m, H-1'), 7.00 (2H, d, J = 9.0 Hz, H-3 and H-5), 7.84 (2H, d, J = 9.0 Hz, H-2 and H-6), 9.89 (1H, s, CHO-1); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 16.7 (Me-3'), 32.5 (C-2'), 33.0 (C-3'), 66.5 (C-1'), 67.9 (C-4'), 114.7 (C-3), 114.7 (C-5), 129.9 (C-1), 132.0 (C-2), 132.0 (C-6), 163.9 (C-4), 190.8 (CHO); ESIMS m/z 231 [M + Na]$^+$; HRESIMS m/z 231.0996 [M + Na]$^+$ (calcd for $C_{12}H_{16}O_3Na$, 231.0997).

(*E*)-Methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (5): colorless needles (CHCl$_3$/MeOH); mp 47−49 °C; UV (MeOH) λ_{max} (log ϵ) 223 (4.35), 285 (3.99), 308 (4.02) nm; IR (neat) ν_{max} 3394 (OH), 1715 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 1.87 (2H, tt, J = 7.6, 6.4 Hz, H-8), 1.93 (3H, s, Me-3'), 2.66 (2H, t, J = 7.6 Hz, H-7), 3.67 (2H, t, J = 6.4 Hz, H-9), 3.76 (3H, s, OMe-4'), 4.70 (2H, d, J = 5.6 Hz, H-1'), 6.82 (2H, d, J = 8.8 Hz, H-3 and H-5), 6.93 (1H, br t, J = 5.6 Hz, H-2'), 7.12 (2H, d, J = 8.8 Hz, H-2 and H-6); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 13.0 (Me-3'), 31.2 (C-7), 34.4 (C-8), 51.5 (OMe), 62.3 (C-9), 64.9 (C-1'), 114.6 (C-3), 114.6 (C-5), 129.4 (C-2), 129.4 (C-6), 129.6 (C-3'), 134.5 (C-1), 137.0 (C-2'), 156.6 (C-4), 167.8 (C-4'); ESIMS m/z 287 [M + Na]$^+$; HRESIMS m/z 287.1254 [M + Na]$^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

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