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Article



# Gerardiins A–L and Structurally Related Phenanthrenes from the Halophyte Plant *Juncus gerardii* and Their Cytotoxicity against Triple-Negative Breast Cancer Cells

Dóra Stefkó, Norbert Kúsz, Anita Barta, Zoltán Kele, László Bakacsy, Ágnes Szepesi, Csilla Fazakas, Imola Wilhelm, István A. Krizbai, Judit Hohmann, and Andrea Vasas*

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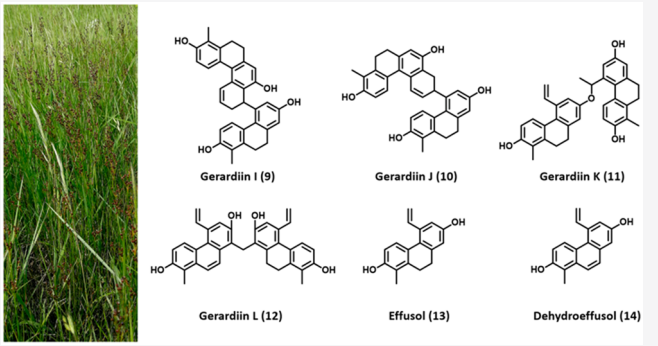
**ABSTRACT:** Species in the Juncaceae accumulate different types of secondary metabolites, among them phenanthrenes and 9,10-dihydrophenanthrenes in substantial amounts. These compounds have chemotaxonomic significance and also possess interesting pharmacological activities. The present study has focused on the isolation, structure determination, and pharmacological investigation of phenanthrenes from *Juncus gerardii*. Twenty-six compounds, including 23 phenanthrenes, have been isolated from a methanol extract of this plant. Twelve compounds, the phenanthrenes gerardiins A–L (**1**–**12**), were obtained as new natural products. Eleven phenanthrenes [effusol (**13**), dehydroeffusol (**14**), effususin A (**15**), compressin A, 7-hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene, juncusol, 2-hydroxy-7-hydroxymethyl-1-methyl-5-vinyl-9,10-dihydrophenanthrene, 2,7-dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene, effususol A, 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene, and jinflexin C], 1-*O*-*p*-coumaroyl-3-*O*-feruloyl-glycerol, and the flavones apigenin and luteolin were isolated for the first time from this plant. The cytotoxicity of the 23 isolated phenanthrenes in both mouse (4T1) and human (MDA-MB-231) triple-negative breast cancer cells and in a nontumor (D3, human cerebral microvascular endothelial) cell line was tested using an MTT viability assay. The results obtained showed that the dimeric compounds gerardiins I (**9**), J (**10**), K (**11**), and L (**12**), derived biogenetically from effusol and dehydroeffusol, were cytotoxic to both tumor and nontumor cell lines, while the monomeric compounds exerted no or very low cytotoxicity. Impedance measurements were consistent with the results of the MTT assays performed.






The plant family Juncaceae is an abundant source of phenanthrene derivatives. These compounds can be found in almost all parts (e.g., roots, medulla, leaves) of the plants belonging to this family.[1] In terms of biosynthesis, phenanthrenes and dihydrophenanthrenes are classified as stilbenoids, with 9,10-dihydrophenanthrenes being formed from bibenzyls by an oxidative coupling reaction. These compounds are thus products of the phenylpropanoid metabolism in combination with polyketide formation. Phenanthrenes are synthesized by stilbene synthase from cinnamic acids through stilbene precursors.[2] The biosynthesis of these compounds can be enhanced by different stress conditions, such as fungal infection or wounds or by increases in the salt concentration of the soil.[3]

Phenanthrenes occurring in Juncaceae species have chemotaxonomic significance, as several of them contain a vinyl group in the molecule. To date, almost 100 monomers and dimers, substituted with methyl, hydroxy, vinyl, methoxy, oxymethylene, methoxyethyl, ethoxyethyl, and formyl groups, have been isolated from different *Juncus* and *Luzula* species.[1] Phenanthrenes possess a wide range of biological activities, including antimicrobial, antiproliferative, anti-inflammatory, anxiolytic, and spasmolytic effects.[4]

In a continuation of our work aiming at the isolation of biologically active compounds from Juncaceae species, the halophyte plant *Juncus gerardii* Loisel. was investigated. Halophytes are specialized plants able to survive and thrive in saline soils. Apart from their physiological adaptation, improved biochemical strategies such as improved antioxidant capacity and transporters determine the tolerance against oxidative stress caused by high salinity conditions.[5] Moreover, these antioxidant systems, including enzymes and bioactive

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compounds, produce a plethora of further interesting biological activities.[6]

The phytochemistry and pharmacology of *J. gerardii* have not been previously investigated. The isolation procedure was carried out by using combined chromatographic methods. The structures of the isolated compounds were elucidated by 1D and 2D NMR spectroscopic and HRMS methods. Twenty-six compounds were obtained, including 23 phenanthrenes, a glycerol derivative, and two flavonoids. Twelve phenanthrenes, named as gerardiins A–L (**1**–**12**), were new natural products. The isolated phenanthrenes were tested for their cytotoxic activity against three different cell lines.

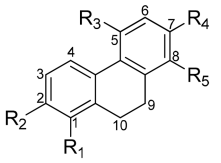
## RESULTS AND DISCUSSION

The dried and ground whole plant material (3.6 kg) was extracted with methanol at room temperature. After evaporation, the extract was dissolved in 50% aqueous methanol, and solvent–solvent partition was performed with *n*-hexane, chloroform, and EtOAc. The chloroform and EtOAc phases were purified by a combination of different techniques, including CC, VLC, MPLC, gel filtration, preparative TLC, and HPLC, to afford 26 compounds. The structure determinations for the new compounds were carried out by spectroscopic data analysis, using 1D ($^1$H, JMOD) and 2D NMR ($^1$H–$^1$H COSY, HSQC, HMBC, NOESY) spectroscopy, HRESIMS measurement, and comparison of the spectroscopic data obtained with reported literature values for related compounds.

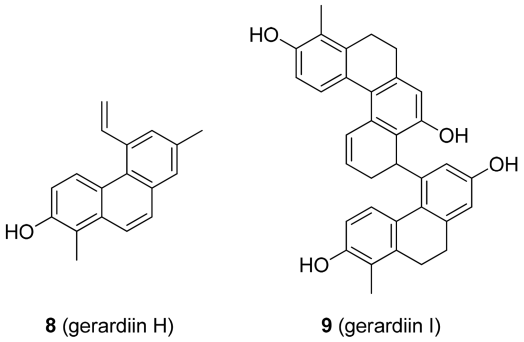
Compound **1** was obtained as an amorphous solid. Its HRESIMS provided the molecular formula $C_{18}H_{18}O_3$ through the presence of a peak at *m*/*z* 251.1068 ([M + H − $CH_3OH$]$^+$, calcd $C_{17}H_{18}O_2$, 251.1072). The $^1$H NMR spectrum (Table 1) displayed signals of two *ortho*- ($\delta_H$ 6.69 and 7.32, each 1H, d, *J* = 8.5 Hz) and two *meta*-coupled ($\delta_H$ 6.64 and 6.84, each 1H, d, *J* = 2.4 Hz) aromatic protons, three methylenes ($\delta_H$ 2.62, 2.75 m, and 4.65 s, each 2H), a vinyl moiety ($\delta_H$ 6.91, 5.21, and 5.65, each 1H, dd), and a methoxy group ($\delta_H$ 3.40, 3H, s). The JMOD spectrum contained 18 carbon resonances attributable to a vinyl- and methoxy-substituted phenanthrene derivative (Table 1). The presence of two adjacent methylene signals ($H_2$-9, $H_2$-10) in the COSY spectrum suggested that compound **1** is a 9,10-dihydrophenanthrene derivative. Further COSY correlations were observed between the signals at $\delta_H$ 6.69 and $\delta_H$ 7.32 (H-3/H-4), as well as $\delta_H$ 6.91 and $\delta_H$ 5.21 and 5.65 (H-12/H-13). The connectivities of the COSY fragments were determined by means of relevant HMBC correlations (Figure 1).

Heteronuclear long-range correlations of C-4a with H-3 and $H_2$-10, C-5a with H-4, H-6, H-8, and $H_2$-9, C-1a with H-4, $H_2$-9, and $H_2$-11, C-9 with H-8, and C-8a with $H_2$-10 were used to establish a 9,10-dihydrophenanthrene skeleton for compound **1**. HMBC cross-peaks of C-2 ($\delta_C$ 156.0) with H-3, H-4, and $H_2$-11, together with C-7 ($\delta_C$ 156.7) with H-6 and H-8, showed the presence of two hydroxy groups at C-2 and C-7. The vinyl moiety was placed at C-5 based on the C-5a/H-12, C-6/H-12, and C-5/$H_2$-13 correlations. In addition, the strong cross-peak of the methoxy group and the hydroxymethylene at $\delta_C$ 66.5 confirmed its location on C-11. The NOE interactions of H-4/H-12, H-8/$H_2$-9, and $H_2$-10/$H_2$-11 corroborated the proposed structure of gerardiin A (**1**), as shown.

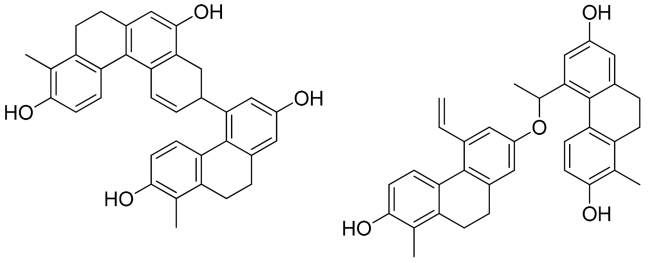
Compound **2** (gerardiin B) was obtained as an amorphous solid. The HRESIMS peak at *m*/*z* 249.1275 established the molecular formula as $C_{19}H_{20}O_2$ ([M + H − $CH_3OH$]$^+$, calcd $C_{18}H_{17}O$, 249.1279). In the $^1$H NMR spectrum, signals of a methyl group ($\delta_H$ 2.18, 3H, s), four *ortho*-coupled aromatic protons ($\delta_H$ 7.53 and 7.25, each 1H, d, *J* = 8.0 Hz, 7.43 and 6.72, each 1H, d, *J* = 8.5 Hz), and a vinyl group ($\delta_H$ 6.85, 5.57, and 5.25, each 1H, dd, *J* = 17.4 and 10.8 Hz, 17.4 and 1.3 Hz, and 10.8 and 1.3 Hz, respectively) were observed (Table 1). The presence of saturated methylene protons at $\delta_H$ 2.70 and 2.82 (each 2H, m) indicated this compound to be a 9,10-dihydrophenanthrene. The HMBC cross-peaks C-7/H-5, C-8a/H-5, C-8/H-6, C-12/H-6, C-8/$H_2$-12, C-7/$H_2$-12, C-7/H-13, C-8a/H-13, and C-8/H-14 demonstrated that the oxymethylene and vinyl groups are situated on the adjacent carbons C-7 and C-8, respectively. The three-bond correlation of the methoxy group with the carbon resonating at $\delta_C$ 74.3



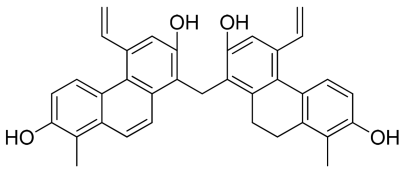

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Trivial name |
|---|---|---|---|---|---|---|
| **1** | $CH_2OCH_3$ | OH | $CH{=}CH_2$ | OH | H | gerardiin A |
| **2** | $CH_3$ | OH | H | $CH_2OCH_3$ | $CH{=}CH_2$ | gerardiin B |
| **3** | $CH_3$ | *O*-glc | $CH{=}CH_2$ | OH | H | gerardiin C |
| **4** | $CH_3$ | OH | $CH{=}CH_2$ | *O*-glc | H | gerardiin D |
| **5** | $CH_3$ | OH | $CH(CH_3)OH$ | OH | H | gerardiin E |
| **6** | $CH_3$ | *O*-glc | $CH(CH_3)OH$ | OH | H | gerardiin F |
| **7** | $CH_3$ | OH | $CH(CH_3)OH$ | *O*-glc | H | gerardiin G |




**8** (gerardiin H) **9** (gerardiin I)




**10** (gerardiin J) **11** (gerardiin K)




**12** (gerardiin L)



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Table 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data of Compounds 1−3

| | 1[a] | | 2[a] | | 3[b] | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type |
| 1 | | 121.8, C | | 122.3, C | | 123.5, C |
| 1a | | 142.2, C | | 137.2, C[c] | | 138.3, C |
| 2 | | 156.0, C | | 156.2, C | | 154.2, C |
| 3 | 6.69, d (8.5) | 112.9, CH | 6.72, d (8.5) | 114.0, CH | 7.00, d (8.6) | 111.7, CH |
| 4 | 7.32, d (8.5) | 131.1, CH | 7.43, d (8.5) | 123.5, CH | 7.18, d (8.6) | 126.5, CH |
| 4a | | 127.4, C | | 127.7, C | | 127.4, C |
| 5 | | 137.3, C | 7.53, d (8.0) | 123.1, CH | | 135.7, C |
| 5a | | 127.2, C | | 136.8, C | | 124.9, C |
| 6 | 6.84, d (2.4) | 113.6, CH | 7.25, d (8.0) | 128.6, CH | 6.80, d (2.2) | 112.6, CH |
| 7 | | 156.7, C | | 134.3, C | | 155.8, C |
| 8 | 6.64, d (2.4) | 115.0, CH | | 138.5, C[c] | 6.66, br s | 114.3, CH |
| 8a | | 141.8, C | | 135.1, C | | 140.3, C |
| 9 | 2.62, m | 31.4, $CH_2$ | 2.82, m | 27.1, $CH_2$ | 2.59, m | 29.7, $CH_2$ |
| 10 | 2.75, m | 26.5, $CH_2$ | 2.70, m | 26.2, $CH_2$ | 2.64, m | 25.1, $CH_2$ |
| 11 | 4.65, s | 66.5, $CH_2$ | 2.18, s | 11.6, $CH_3$ | 2.21, s | 11.9, $CH_3$ |
| $OCH_3$-11 | 3.40, s | 58.1, $CH_3$ | | | | |
| 12 | 6.91, dd (17.4, 10.8) | 140.2, CH | 4.44, s | 74.3, $CH_2$ | 6.84, dd (17.3, 10.8) | 138.4, CH |
| $OCH_3$-12 | | | 3.36, s | 58.1, $CH_3$ | | |
| 13 | 5.65, dd (17.4, 1.3) | 113.9, $CH_2$ | 6.85, dd (17.9, 11.5) | 135.4, CH | 5.64, d (17.3) | 114.1, $CH_2$ |
| | 5.21, dd (10.8, 1.3) | | | | 5.27, d (10.8) | |
| 14 | | | 5.57, dd (11.5, 2.1) | 120.9, $CH_2$ | | |
| | | | 5.25, dd (17.9, 2.1) | | | |
| 1′ | | | | | 4.79, d (7.1) | 101.3, CH |
| 2′ | | | | | 3.28, m | 73.4, CH |
| 3′ | | | | | 3.26, m | 76.7, CH |
| 4′ | | | | | 3.16, m | 69.8, CH |
| 5′ | | | | | 3.31, m | 77.1, CH |
| 6′ | | | | | 3.70, br d (11.4) | 60.8, $CH_2$ |
| | | | | | 3.46, m | |

[a]Measured in MeOD. [b]Measured in DMSO-$d_6$. [c]Interchangeable signals.

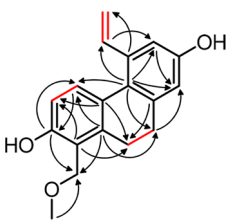


**Figure 1.** $^{1}H-^{1}H$ COSY (red) and diagnostic HMBC (C→H) correlations of **1**.

unambiguously showed that it is attached to C-12. NOE correlations of H-4/H-5, H-6/$H_2$-12, $H_2$-12/H-14b, $H_2$-9/H-13, and $H_2$-10/$H_3$-11 supported the above findings and afforded the structure of gerardiin B (**2**), as shown.

According to the sodiated molecular ion exhibited at $m/z$ 437.1565 $[M + Na]^+$ in the HRESIMS spectrum, compound **3** (gerardiin C) has the molecular formula $C_{23}H_{26}O_7$ (calcd $C_{23}H_{26}O_7Na$, 437.1576). The $^{1}H$ NMR spectrum contained the signals of two *ortho*- ($\delta_H$ 7.18 and 7.00, each 1H, d, $J$ = 8.6 Hz) and two *meta*-coupled ($\delta_H$ 6.80, 1H, d, $J$ = 2.2 Hz and 6.66, 1H, br s) aromatic methines, a vinyl group ($\delta_H$ 6.84, 1H, dd, $J$ = 17.3 and 10.8 Hz, and 5.64 and 5.27, each 1H, d, $J$ = 17.3 and 10.8 Hz, respectively), and a methyl group ($\delta_H$ 2.21, 3H, s), two methylenes ($\delta_H$ 2.64 and 2.59, each 2H, m), and a sugar moiety. The monosaccharide was identified as D-glucose based on its $^{1}H$ and $^{13}C$ chemical shift values. The large coupling constant of the anomeric H-1′ proton ($J$ = 7.1 Hz) indicated that the glucose unit is attached to the phenanthrene skeleton through a $\beta$-glycosidic bond. The 1D and 2D NMR data of compound **3** were similar to those of effusol, and the HMBC cross-peaks C-2/H-4, C-2/$H_3$-11, and C-2/H-1′ revealed that gerardiin C (**3**) is a 2-*O*-glycoside of a known aglycon, as shown.

The same molecular formula, $C_{23}H_{26}O_7$ ($m/z$ 437.1564 $[M + Na]^+$, calcd $C_{23}H_{26}O_7Na$, 437.1576), was assigned to gerardiin D (**4**) as to **3**, suggesting that these compounds are structural isomers. The markedly upfield shifted H-3 ($\delta_H$ 6.72, 1H, d, $J$ = 8.4 Hz vs 7.00, 1H, d, $J$ = 8.6 Hz in **3**) and the deshielded *meta*-coupled protons assigned to ring C ($\delta_{H-6}$ 7.06 and $\delta_{H-8}$ 6.92, each 1H, d, $J$ = 2.1 Hz vs 6.80, 1H, d, $J$ = 2.2 Hz, and 6.66, 1H, br s in **3**) implied that the glucose unit is attached to the C-7 hydroxy group of the aglycone (Table 2). This conclusion was confirmed by HMBC cross-peaks between C-7 ($\delta_C$ 155.4), H-6, and H-8, as well as by the NOE interactions of H-6 and H-8 with H-1′, with the structure proposed for **4** as shown.

Compound **5** (gerardiin E) was obtained as a colorless, amorphous solid. Its molecular formula was determined as $C_{17}H_{18}O_3$ by the HRESIMS data ($m/z$ 253.1226 $[M + H - H_2O]^+$, calcd $C_{17}H_{17}O_2$, 253.1229). The presence of four aromatic methines (two *meta*- and two *ortho*-coupled), two saturated methylenes, and one methyl group in the $^{1}H$ NMR spectrum revealed that **5** is a 1,2,5,7-tetrasubstituted 9,10-dihydrophenanthrene derivative (Table 2). However, the lack of any characteristic resonances of a vinyl moiety, and the


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Table 2. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data of Compounds 4−6 in DMSO-$d_6$

| | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type |
| 1 | | 120.7, C | | 120.3, C | | 123.4, C |
| 1a | | 138.9, C | | 138.9, C | | 138.8, C |
| 2 | | 154.3, C | | 153.7, C | | 153.9, C |
| 3 | 6.72, d (8.4) | 111.6, CH | 6.71, d (8.4) | 111.6, CH | 7.00, d (8.6) | 111.8, CH |
| 4 | 7.10, d (8.4) | 126.9, CH | 7.13, d (8.4) | 125.9, CH | 7.26, d (8.6) | 125.9, CH |
| 4a | | 124.3, C | | 125.0, C | | 127.7, C |
| 5 | | 135.0, C | | 144.2, C | | 144.6, C |
| 5a | | 128.0, C | | 124.8, C | | 124.4, C |
| 6 | 7.06, d (2.1) | 113.6, CH | 6.92, d (2.2) | 112.0, CH | 6.94, d (2.2) | 112.1, CH |
| 7 | | 155.4, C | | 155.4, C | | 155.8, C |
| 8 | 6.92, d (2.1) | 115.1, CH | 6.56, d (2.2) | 113.1, CH | 6.58, d (2.2) | 113.1, CH |
| 8a | | 139.9, CH | | 139.4, C | | 139.8, C |
| 9 | 2.59−2.65, m | 29.9, $CH_2$ | 2.48−2.55, m | 30.5, $CH_2$ | 2.51−2.57, m | 30.4, $CH_2$ |
| 10 | 2.59−2.65, m | 25.0, $CH_2$ | 2.59, m | 25.4, $CH_2$ | 2.61, m | 25.3, $CH_2$ |
| | | | 2.49, m | | 2.51−2.57, m | |
| 11 | 2.13, s | 11.7, $CH_3$ | 2.12, s | 11.7, $CH_3$ | 2.22, s | 12.0, $CH_3$ |
| $OCH_3$-11 | | | | | | |
| 12 | 6.85, dd (17.3, 10.8) | 138.0, CH | 5.05, q (6.2) | 64.2, CH | 5.04, q (5.9) | 64.2, CH |
| $OCH_3$-12 | | | | | | |
| 13 | 5.74, d (17.4) | 114.4, $CH_2$ | 1.39, d (6.2) | 25.6, $CH_3$ | 1.40, d (5.9) | 25.6, $CH_3$ |
| | 5.26, d (10.8) | | | | | |
| 14 | | | | | | |
| 1′ | 4.88, d (7.4) | 100.7, CH | | | 4.81, d (7.1) | 101.2, CH |
| 2′ | 3.23, m | 73.3, CH | | | 3.27, m[a] | 73.4, CH |
| 3′ | 3.26, m | 76.7, CH | | | 3.27, m[a] | 76.7, CH |
| 4′ | 3.14, dd (8.9, 8.3) | 69.9, CH | | | 3.16, m | 69.8, CH |
| 5′ | 3.33, m | 77.2, CH | | | 3.31, m (overlaps with $H_2O$) | 77.0, CH |
| 6′ | 3.70, br d (11.4) | 60.8, $CH_2$ | | | 3.70, br d (11.4) | 60.8, $CH_2$ |
| | 3.45, m | | | | 3.47, dd (11.4, 5.5) | |

[a]Interchangeable signals.

additional oxymethine and methyl signals detected at $\delta_H$ 5.05 (1H, q, $J$ = 6.2 Hz) and $\delta_H$ 1.39 (3H, d, $J$ = 6.2 Hz), suggested the presence of an $\alpha$-hydroxyethyl group in the molecule. HMBC cross-peaks of C-12 ($\delta_C$ 64.2) with H-6 ($\delta_H$ 6.92), C-5a ($\delta_C$ 124.8) with H-4 ($\delta_H$ 7.13), H-6, and H-12 ($\delta_H$ 5.05), and C-5 ($\delta_C$ 144.2) with H-12 and $H_3$-13 ($\delta_H$ 1.39) revealed that the hydroxyethyl substituent is attached to C-5. From a biosynthetic point of view, this side chain is most likely formed from a vinyl group through hydration of the vinylic double bond. NOEs between H-4, H-12, and $H_3$-13, as well as between H-6 and $H_3$-13, were in line with the proposed structure of gerardiin E (**5**), as shown.

The molecular formula $C_{23}H_{28}O_8$ of gerardiin F (**6**) was deduced from the sodium adduct ion $[M + Na]^+$ observed at $m/z$ 455.1671 (calcd $C_{23}H_{28}O_8Na$, 455.1682) in the HRESIMS. Apart from the signals of a D-glucose moiety, the $^1$H NMR data closely resembled those of compound **5**. On examining the chemical shifts, the deshielded nature of H-3 and H-4 ($\delta_H$ 7.00 and 7.26, each 1H, d, $J$ = 8.6 Hz in **6** vs 6.71 and 7.13, each 1H, d, $J$ = 8.4 Hz in **5**) clearly suggested that the sugar unit is attached to the skeleton at C-2 ($\delta_C$ 153.9) (Table 2). The position of the $\beta$-D-glucose was substantiated by HMBC correlations of C-2 ($\delta_C$ 153.9) with H-4, $H_3$-11, and the anomeric H-1′ and by the NOE cross-peak between H-3/H-1′, and the structure of **6** was proposed as shown.

Gerardiin G (**7**) was shown to be a structural isomer of **6** by the sodium adduct HRESIMS ion at $m/z$ 455.1675 $[M + Na]^+$ (calcd $C_{23}H_{28}O_8Na$, 455.1682). As in the case of gerardiin D, the position of the $\beta$-D-glucose at C-7 ($\delta_C$ 155.5) was determined with the aid of diagnostic HMBC and NOE cross-peaks, leading to the structure of **7** as shown.

HRESIMS data provided the molecular formula of $C_{18}H_{16}O$ for gerardiin H (**8**) through the presence of a peak at $m/z$ 249.1274 ($[M + H]^+$, calcd $C_{18}H_{17}O$, 249.1279). The $^1$H NMR data of **8** were similar to those of the known 9,10-dehydrophenanthrene juncunol, except for the replacement of the $H_2$-9 and $H_2$-10 methylene signals by two *ortho*-coupled aromatic protons ($\delta_{H\text{-}10}$ 7.89 and $\delta_{H\text{-}9}$ 7.65, each 1H, d, $J$ = 9.1 Hz) (Table 3). The presence of a double bond between C-9 and C-10 was corroborated by the HMBC cross-peaks of C-1a ($\delta_C$ 134.9), C-5a ($\delta_C$ 128.7), C-8 ($\delta_C$ 129.2) with H-9 and C-1 ($\delta_C$ 118.7), C-4a ($\delta_C$ 126.0), and C-8a ($\delta_C$ 133.3) with H-10, as well as by NOE interactions between H-8 ($\delta_H$ 7.59 s) and H-9, and H-10 and $H_3$-11 ($\delta_H$ 2.56).

Compound **9** (gerardiin I) was isolated as an amorphous solid. The peak of the protonated molecule exhibited at $m/z$ 503.2210 $[M + H]^+$ in the HRESIMS suggested a molecular formula of $C_{34}H_{30}O_4$ (calcd $C_{34}H_{31}O_4$, 503.2222). The 34 carbon signals, including five methylenes detected in the JMOD spectrum, indicated that **9** is a dimer consisting of two 9,10-dihdyrophenanthrene units (Table 4). The $^1$H−$^1$H COSY spectrum defined two $-CH_2-CH_2-$ fragments [$\delta_H$ 2.53 and 2.47, each 2H, m; 2.87, 2.59, 2.42 and 2.40, each 1H, m), two pairs of *ortho*-coupled aromatic protons ($\delta_H$ 7.62 and 6.74, each 1H, d, $J$ = 8.4 Hz; $\delta_H$ 7.04 and 6.72, each 1H, d, $J$ = 8.4 Hz), a pair of *meta*-coupled sp$^2$ methines ($\delta_H$ 6.40 and 6.12,


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Table 3. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data of Compounds 7 and 8

| | 7[a] | | 8[b] | |
|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type |
| 1 | | 120.5, C | | 118.7, C |
| 1a | | 139.4, C | | 134.9, C |
| 2 | | 154.0, C | | 154.1, C |
| 3 | 6.73, d (8.4) | 111.6, CH | 7.10, d (9.1) | 115.8, CH |
| 4 | 7.21, d (8.4) | 126.2, CH | 8.58, d (9.1) | 128.1, CH |
| 4a | | 124.6, C | | 126.0, C |
| 5 | | 144.0, C | | 138.2, C |
| 5a | | 127.6,C | | 128.7, C |
| 6 | 7.13, d (2.3) | 113.6, CH[c] | 7.42, br s | 131.3, CH |
| 7 | | 155.5, C | | 135.7, C |
| 8 | 6.88, d (2.3) | 113.6, CH[c] | 7.59, br s | 129.2, CH |
| 8a | | 139.8, C | | 133.3, C |
| 9 | 2.54−2.64, m | 30.6, $CH_2$ | 7.65, d (9.1) | 128.4, CH |
| 10 | 2.54−2.64, m | 25.3, $CH_2$ | 7.89, d (9.1) | 124.0, CH |
| 11 | 2.13, s | 11.7, $CH_3$ | 2.56, s | 11.4, $CH_3$ |
| $OCH_3$-11 | | | | |
| 12 | 5.07 m, (overlaps with $H_2O$) | 64.3, CH | 7.48, dd (17.2, 11.0) | 143.6, CH |
| $OCH_3$-12 | | | | |
| 13 | 1.39, d (6.0) | 25.5, $CH_3$ | 5.73, dd (17.2, 1.7) | 114.3, $CH_2$ |
| | | | 5.40, dd (16.9, 1.7) | |
| 14 | | | 2.51, s | 21.3, $CH_3$ |
| 1′ | 4.85, d (7.5) | 100.7, CH | | |
| 2′ | 3.24, m | 73.3, CH | | |
| 3′ | 3.27, m | 76.7, CH | | |
| 4′ | 3.18, m | 69.7, CH | | |
| 5′ | 3.30, m | 77.1, CH | | |
| 6′ | 3.68, br d (11.4) | 60.7, $CH_2$ | | |
| | 3.49, m | | | |

[a]Measured in DMSO-$d_6$. [b]Measured in MeOD. [c]Interchangeable signals.

each 1H, d, $J$ = 2.0 Hz), and a further sequence of correlated protons as follows: −CH−$CH_2$−CH═CH− ($\delta_H$ 5.19, 1H, d, $J$ = 9.3 Hz, 2.62 and 2.20, each 1H, m 5.76, 1H, m, and 6.77, 1H, dd, $J$ = 9.7 and 2.6 Hz). The latter structural portion was incorporated into a cyclohexadiene ring and connected the two phenanthrene units between C-6′−C-12, and C-13′−C-13, as confirmed by the key HMBC correlations of C-12 ($\delta_C$ 30.8) with H-6 ($\delta_H$ 6.12) and C-5, C-5′, and C-7′ ($\delta_C$ 142.5, 130.5, and 152.3, respectively) with H-12 ($\delta_H$ 5.19) (Figure 2). Further relevant HMBC correlations from C-5 ($\delta_C$ 142.5) and C-12′ ($\delta_C$ 127.5) to H-13, from C-5′ to H-13′ ($\delta_H$ 5.76), and from C-6′ ($\delta_C$ 123.9) to H-12′ ($\delta_H$ 6.77) and H-8′ ($\delta_H$ 6.56) were also observed.

The substitution pattern of the monomers, excepting the connection sites at C-5, C-5′, and C-6′, was identical with the known effusol, which possesses a C-5 vinyl group. Thus, it is postulated that **9** is biosynthesized from two effusol monomers that are connected through their vinyl substituents. NOESY cross-peaks of H-12 with H-4 and of H-4′ with H-12′ were in agreement with these conclusions. While the planar structure of gerardiin I (**9**) could be elucidated, the configuration of C-12 still remained uncertain. The specific rotation value $[\alpha]_D^{25}$ of

Table 4. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data of Compounds 9 and 10 in DMSO-$d_6$

| | 9 | | 10 | |
|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | $\delta_H$ ($J$ in Hz) | $\delta_C$, type |
| 1 | | 120.1, C | | 120.6, C[a] |
| 1a | | 139.2, C | | 139.3, C |
| 2 | | 153.3, C | | 153.7, C |
| 3 | 6.74, d (8.4) | 111.7, CH | 6.68 (d, overlaps with H-8′) | 111.6, CH |
| 4 | 7.62, d (8.4) | 125.1, CH | 7.03, d (7.8) | 125.1, CH |
| 4a | | 126.2, C | | 125.1, C |
| 5 | | 142.5, C | | 142.8, C |
| 5a | | 125.1, C | | 125.9, C |
| 6 | 6.12, d (2.0) | 114.2, CH | 6.76, d (2.2) | 113.7, CH |
| 7 | | 154.3, C | | 155.2, C |
| 8 | 6.40, d (2.0) | 111.9, CH | 6.57, d (2.2) | 112.7, CH |
| 8a | | 139.9, C | | 140.1, C |
| 9 | 2.47, m | 31.0, $CH_2$ | 2.52−2.58, m | 30.7, $CH_2$ |
| 10 | 2.53, m | 25.7, $CH_2$ | 2.64, m | 25.4, $CH_2$ |
| | | | 2.52, m[a] | |
| 11 | 2.15, s | 11.7, $CH_3$[a] | 2.12, s | 11.7, $CH_3$ |
| 12 | 5.19, d (9.3) | 30.8, CH | 4.13, m | 35.4, CH |
| 13 | 2.62, m | 30.0, $CH_2$ | 3.17, m | 30.7, $CH_2$ |
| | 2.20, m | | 2.59, m[a] | |
| 14 | | | | |
| 1′ | | 120.5, C | | 120.5, C[a] |
| 1a′ | | 138.6, C | | 133.7, C |
| 2′ | | 153.6, C | | 153.8, C |
| 3′ | 6.72, d (8.4) | 111.5, CH | 6.72, d (8.1) | 111.6, CH |
| 4′ | 7.04, d (8.4) | 126.7, CH | 7.05, d (8.1) | 126.6, CH |
| 4a′ | | 124.9, C | | 124.7, C |
| 5′ | | 130.5, C | | 129.5, C |
| 5a′ | | 124.3, C | | 124.5, C |
| 6′ | | 123.9, C | | 119.4, C |
| 7 | | 152.3, C | | 151.9, C |
| 8′ | 6.56, s | 113.4, CH | 6.67 (s, overlaps with H-3) | 113.8, CH |
| 8a′ | | 137.3, C | | 137.1, C |
| 9′ | 2.59, m | 29.7, $CH_2$ | 2.59, m[a] | 29.7, $CH_2$ |
| | 2.42, m | | 2.44, m | |
| 10′ | 2.87, m | 25.3, $CH_2$ | 2.80, m | 25.3, $CH_2$ |
| | 2.40, m | | 2.42, m | |
| 11′ | 2.14, s | 11.9, $CH_3$[a] | 2.13, s | 11.8, $CH_3$ |
| 12′ | 6.77, dd (9.7, 2.6) | 127.5, CH | 6.78, dd (9.8, 2.0) | 127.0, CH |
| 13′ | 5.76, m | 124.2, CH | 5.93, br d (9.8) | 132.3, CH |

[a]Interchangeable signals.

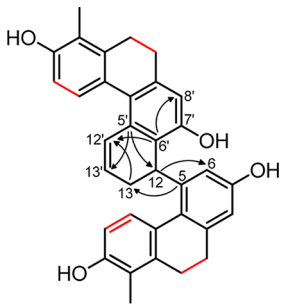


Figure 2. $^1$H−$^1$H COSY (red) and diagnostic HMBC (C→H) correlations of **9**.


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the compound was 0 (*c* 0.1, MeOH). When gerardiin I was injected onto a chiral HPLC column, it eluted with two well-separated peaks with a peak ratio area of 1:1. The peaks also exhibited the same UV spectra, suggesting that **9** is a racemic mixture, with the structure shown.

The protonated molecular ion peak of compound **10** at *m/z* 503.2213 $[M + H]^+$ (calcd $C_{34}H_{31}O_4$, 503.2222) in the HRESIMS data provided the same molecular formula ($C_{34}H_{30}O_4$) as for **9**. Careful analysis of the 1D NMR spectra implied that compound **10** also comprises two effusol units (Table 4). Based on the −CH($CH_2$)−CH═CH− structural portion ($\delta_H$ 4.13, 1H, m, 3.17 and 2.59, each 1H, m, 5.93, 1H, br d, *J* = 9.8 Hz, 6.78, 1H, dd, *J* = 9.8 and 2.0 Hz), as defined by the $^1$H−$^1$H COSY spectrum, it was assumed that the effusol monomers in **10** are connected via C-13′ → C-12 and C-13 → C-6′ linkages. This hypothesis was supported by HMBC cross-peaks of C-12 ($\delta_C$ 35.4) with H-6 ($\delta_H$ 6.76), C-5 ($\delta_C$ 142.8), C-6′ ($\delta_C$ 119.4), and C-7′ ($\delta_C$ 151.9) with H-13 ($\delta_H$ 3.17), C-5 and C-5′ ($\delta_C$ 129.5) with H-13′ ($\delta_H$ 5.93), and C-5′ and C-6′ with H-12′ ($\delta_H$ 6.78). The specific optical rotation of **10** was recorded as zero. When compound **10** was investigated by HPLC using the same chiral stationary phase as in the case of **9**, only one peak was observed. Thus, the structure of gerardiin I (**10**) was assigned as shown.

Compound **11** (gerardiin K) was obtained as an amorphous solid. According to its protonated molecular ion peak seen at *m/z* 505.2375 $[M + H]^+$ (calcd $C_{34}H_{33}O_4$, 505.2379) in the HRESIMS, the molecular formula of $C_{34}H_{32}O_4$ was assigned to this compound. The JMOD spectrum displayed 34 signals, which suggested that compound **11** is also a phenanthrene dimer (Table 5). The subunits were identified based on their 1D NMR data as effusol (**15**). The HMBC cross-peak of C-7 ($\delta_C$ 156.9) with H-12′ ($\delta_H$ 5.80), and a strong NOE from H-6 to H-12′ revealed that the monomers are linked through an ether bond formed between the OH-7 group of one effusol monomer and the vinyl side chain of the other effusol molecule. The specific optical rotation of **11** was recorded as zero. By HPLC investigation on chiral stationary phase, only one peak was observed. Accordingly, the structure determined for gerardiin K (**11**) is as shown.

Gerardiin L (**12**) was obtained as an amorphous solid. Its HRESIMS provided the molecular formula $C_{35}H_{30}O_4$ through the presence of its protonated molecular ion peak at *m/z* 515.2213 $[M + H]^+$ (calcd $C_{35}H_{31}O_4$, 515.2222). The JMOD spectrum of **12** displayed 35 carbon resonances, which suggested that it is a dimer (Table 5). Since two vinyl groups ($\delta_H$ 7.36, 5.74, and 5.33, each 1H, dd, *J* = 17.2, 10.7 Hz, 17.2, 1.3 Hz, and 10.7, 1.3 Hz, respectively, and 6.85, 5.65, and 5.13, each 1H, dd, *J* = 17.4 and 10.8 Hz, 17.4 and 1.2 Hz, 10.8 and 1.2 Hz, respectively) were identified in the $^1$H NMR and $^1$H−$^1$H COSY spectra, the phenanthrene monomers had to be linked together in a different manner as in the cases of gerardiins I−K (**9**−**11**). Moreover, the presence of a −$CH_2$−$CH_2$− ($\delta_H$ 2.47 and 2.42, each 2H, m) subunit and three pairs of *ortho*-coupled aromatic protons ($\delta_H$ 8.36 and 6.96, each 1H, d, *J* = 9.1 Hz, 8.02 and 7.68, each 1H, d, *J* = 9.6 Hz, and 6.98 and 6.50, each 1H, d, *J* = 8.4 Hz) showed that **12** is composed of a phenanthrene and a 9,10-dihydrophenanthrene unit. These monomers could be characterized as effusol (**15**) and dehydroeffusol (**22**) based on their $^1$H and $^{13}$C NMR chemical shifts. However, the signals of H-8 and H-8′ were missing, and a downfield-shifted, isolated methylene fragment appeared instead at $\delta_H$ 4.58 and $\delta_C$ 22.8. In order to clarify the exact structure, a series of 2D NMR experiments was recorded. Considering the HMBC cross-peaks of C-7 and C-7′ ($\delta_C$ 152.8 and 154.1), C-8 and C-8′ ($\delta_C$ 122.9 and 127.5), and C-8a and C-8a′ ($\delta_C$ 133.2 and 141.3) with the aforementioned methylene ($H_2$-14), it was concluded that the phenanthrene monomers are connected through the $CH_2$-14 group attached to the corresponding carbons C-8 and C-8′ (Figure 3). These data, combined with NOE interactions of H-4/H-12, H-6/H-13, H-9/H-14, H-4′/H-12′, H-6′/H-13′, and $H_2$-9′/H-14, allowed the structure of **12** to be depicted as in Figure 3.

**Table 5. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR Data of Compounds 11 and 12 in MeOD**

| | 11 | | 12 | |
|---|---|---|---|---|
| position | $\delta_H$ (*J* in Hz) | $\delta_C$, type | $\delta_H$ (*J* in Hz) | $\delta_C$, type |
| 1 | | 121.9, C | | 118.1, C |
| 1a | | 140.2, C | | 133.1, C[a] |
| 2 | | 155.1, C | | 153.1,C |
| 3 | 6.56, d (8.6) | 112.2, CH | 6.96, d (9.1) | 115.2, CH |
| 4 | 7.02, d (8.6) | 128.4, CH | 8.36, d (9.1) | 127.8, CH |
| 4a | | 126.7, C[a] | | 126.3, C |
| 5 | | 136.5, C | | 137.1, C |
| 5a | | 128.2, C | | 124.9, C |
| 6 | 6.25, d (1.9) | 112.7, CH | 7.22, s | 118.6, CH |
| 7 | | 156.9, C | | 152.8, C |
| 8 | 6.33, br s | 115.6, CH | | 122.9, CH |
| 8a | | 141.0, C | | 133.2, C[a] |
| 9 | 2.36−2.44, m | 31.3, $CH_2$ | 8.02, d (9.6) | 125.3, CH |
| 10 | 2.63, m | 26.5, $CH_2$ | 7.68, d (9.6) | 123.2, CH |
| | 2.48, m | | | |
| 11 | 2.14, s | 11.7, $CH_3$ | 2.46, s | 11.1, $CH_3$ |
| 12 | 6.70, dd (17.4, 10.9) | 139.8, CH | 7.36, dd (17.2, 10.7) | 143.4, CH |
| 13 | 5.00, br d (17.4) | 113.3, $CH_2$ | 5.74, dd (17.2, 1.3) | 113.1, $CH_2$ |
| | 4.92, br d (10.9) | | 5.33, dd (10.7, 1.3) | |
| 14 | | | 4.58, s | 22.8, CH |
| 1′ | | 122.7, C | | 121.3, C |
| 1a′ | | 141.4, C | | 140.0, C |
| 2′ | | 155.5, C | | 154.7, C |
| 3′ | 6.79, d (8.6) | 112.6, CH[a] | 6.50, d (8.4) | 112.1, CH |
| 4′ | 7.04, d (8.6) | 127.3, CH | 6.98, d (8.4) | 128.5, CH |
| 4a′ | | 126.7, C[a] | | 127.4, C[a] |
| 5′ | | 141.8, C | | 134.7, C |
| 5a′ | | 128.6, C | | 128.7, C |
| 6′ | 6.94, d (2.1) | 112.6, CH[a] | 7.00, s | 112.7, CH |
| 7 | | 156.9, C | | 154.1, C |
| 8′ | 6.58, d (2.1) | 114.7, CH | | 127.5, CH[a] |
| 8a′ | | 141.3, C | | 141.3, C |
| 9′ | 2.65, m | 31.8, $CH_2$ | 2.42, m | 27.2, $CH_2$ |
| | 2.52, m | | | |
| 10′ | 2.98, br d (14.6) | 27.2, $CH_2$ | 2.47, m | 26.4, $CH_2$ |
| | 2.30, m | | | |
| 11′ | 2.27, s | 12.1, $CH_3$ | 2.09, s | 11.6, $CH_3$ |
| 12′ | 5.80, q (6.2) | 72.1, CH | 6.85, dd (17.4, 10.8) | 140.4, CH |
| 13′ | 1.86, d (6.2) | 24.1, $CH_3$ | 5.65, dd (17.4, 1.2) | 112.5, $CH_2$ |
| | | | 5.13, dd (10.8, 1.2) | |

[a]Interchangeable signals.



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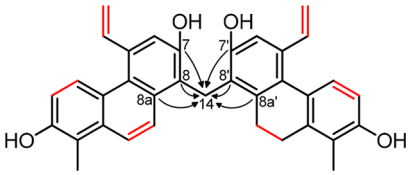



**Figure 3.** $^{1}$H−$^{1}$H COSY (red) and diagnostic HMBC (C → H) correlations of **12**.

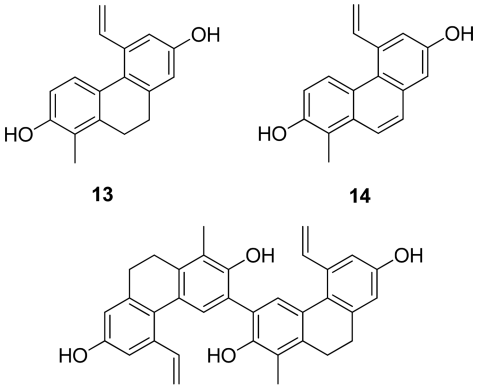
Besides the new compounds, gerardiins A−L (**1**−**12**), 11 known phenanthrenes, the monomers effusol (**13**) and dehydroeffusol (**14**),[7] compressin A,[8] 7-hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene,[9] juncusol,[10] 2-hydroxy-7-hydroxymethylene-1-methyl-5-vinyl-9,10-dihydrophenanthrene,[11] 2,7-dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene,[12] effususol A,[13] 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene,[9] jinflexin C,[14] the diphenanthrene effususin A (**15**), 1-*O*-*p*-coumaroyl-3-*O*-feruloyl-glycerol,[15] and the flavones apigenin and luteolin,[16] were also isolated from *J. gerardii*. Their identifications were made by analysis of their HRESIMS and 1D and 2D NMR spectra and by comparison of their $^{1}$H and $^{13}$C NMR chemical shifts with literature values. In this report, complete $^{1}$H and $^{13}$C NMR assignments are provided for compressin A, 7-hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene, 2-hydroxy-7-hydroxymethylene-1-methyl-5-vinyl-9,10-dihydrophenanthrene, 2,7-dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene, effususol A, 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene, effususin A, and 1-*O*-*p*-coumaroyl-3-*O*-feruloyl-glycerol measured in different solvents than those previously reported; these data are listed in the Experimental Section. All compounds were isolated for the first time from the plant. All of the known compounds, with the exception of 1-*O*-*p*-coumaroyl-3-*O*-feruloyl glycerol, were isolated previously from other *Juncus* species.

Gerardiin A (**1**) and gerardiin B (**2**) are substituted with a methoxymethylene group at C-1 (**1**) or C-7 (**2**). The structure of compound **1** is very similar to that of effusol, with the only difference being the presence of a methoxy group at C-11. Gerardiins C (**3**) and D (**4**) are glycosides of effusol, substituted with a D-glucose unit at C-2 (**3**) or C-7 (**4**), respectively. Gerardiins F (**6**) and G (**7**) are also substituted with a D-glucose moiety. Gerardiin E (**5**) contains a hydroxyethyl group at C-5, instead of a vinyl group. The only difference between gerardiin H (**8**) and juncunol, isolated previously from other *Juncus* species (*J. acutus*, *J. effusus*, *J. roemerianus*, *J. subulatus*),[10,17−19] is the presence of an unsaturated B ring in the former phenanthrene.

Phenanthrenoid dimers represent a rare class of secondary metabolites; to date, less than 20 have been reported from species in the plant family Juncaceae. In gerardiins I (**9**) and J (**10**), the two effusol monomers are connected through their vinyl groups. Gerardiin K (**11**) is composed of two effusol monomers that are joined through an ether bond, while in gerardiin L (**12**) an effusol and a dehydroeffusol unit are attached via a C−C linkage formed between C-8−C-8′. The individual monomers [effusol (**13**) and dehydroeffusol (**14**)] were also isolated from the plant.

In order to gain insight into the biological effects of the isolated phenanthrenes, 4T1 mouse breast cancer cells were treated with the isolated compounds, and changes in the viability and impedance were assessed, which reflects proliferation, degree of adhesion, spreading, and viability of the cells. At a concentration of 20 μM, compounds **1**−**8** (gerardiin A−H) had no cytotoxic effects on 4T1 cells, as assessed by an MTT assay (Figure S73A, Supporting Information) or impedance measurements (Figure S73B, Supporting Information).

In contrast to this, the viability of 4T1 cells was reduced significantly in a concentration-dependent manner in response to compounds **9**−**12** (gerardiins I−L) (Figure S74, Supporting Information). The effect of these phenanthrenes was comparable to that of doxorubicin, which was applied as a positive control to measure cytotoxicity. Since all these compounds are dimers of effusol (**13**) (compounds **9**−**11**) or of effusol and dehydroeffusol (**14**) (compound **12**), the cytotoxic effects of the monomers and dimers in both mouse and human tumor cells and in a nontumor cell line (D3) were compared. Besides the aforementioned phenanthrenes, effususin A (**15**) was also included in this study, since it is also a dimer of effusol (Figure S74, Supporting Information).




The present results show unequivocally that the dimeric compounds **9**−**12** and **15** comprising effusol (**13**) and dehydroeffusol (**14**) monomers are cytotoxic to both tumor and nontumor cell lines, while the monomers (**13**, **14**) alone displayed no or very low cytotoxicity (Figure S74, Supporting Information). Among the diphenanthrenes tested, effususin A (**15**) exerted the lowest cytotoxicity, while gerardiins I−L (**9**−**12**) proved to be the most active. Indeed, moderate toxicity of effususin A (**15**) in A2780 human ovarian cancer cells was reported by Bús et al.[8] Impedance measurements were in line with the results of the MTT assay, indicating a concentration-dependent toxicity of the dimers (Figure S76, Supporting Information).

$IC_{50}$ values of both **9** and **10** were below 10 μM in the two tested tumor cell lines (Table 6). In the case of compound **11**,

**Table 6. $IC_{50}$ Values[a] and Their 95% Confidence Intervals [95% C.I.] for Compounds 9−12**

| | cell line | |
|---|---|---|
| compound | MDA-MB-231 | 4T1 |
| **9** | 8.0 [7.3−8.8] | 7.8 [6.5−9.5] |
| **10** | 6.6 [6.2−7.1] | 5.6 [5.1−6.1] |
| **11** | >10 | 8.1 [7.7−8.6] |
| **12** | 7.3 [6.7−7.9] | >10 |
| doxorubicin | 0.8 [0.7−0.9] | 3.4 [3.1−3.7] |

[a]Half-maximal inhibitory concentration (μM), $n$ = 6−8.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the concentration that caused 50% inhibition of cell viability was lower than 10 $\mu$M only in the mouse (4T1) ($IC_{50}$ 8.1 $\mu$M), but not in the human breast cancer (MDA-MB-231) cell line (10.1 $\mu$M). On the other hand, compound **12** was more cytotoxic ($IC_{50}$ 7.3 $\mu$M) to the human breast cancer cells ($IC_{50}$ was 11.7 $\mu$M on 4T1 cells). D3 endothelial cells were the less sensitive to these diphenanthrenes, all of them having $IC_{50}$ values above 10 $\mu$M (21.6 $\mu$M for **9**, 15.7 $\mu$M for **10**, 10.6 $\mu$M for **11**, and 13.7 $\mu$M for **12**, respectively) (Table S1, Supporting Information).

Considering the already known isolated phenanthrenes, only juncusol (in MTT and impedance assays) and jinflexin C (in impedance measurements) displayed moderate cytotoxicity, while compressin A, 7-hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene, 2-hydroxy-7-hydroxymethylene-1-methyl-5-vinyl-9,10-dihydrophenanthrene, 2,7-dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene, effususol A, and 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene were not cytotoxic in 4T1 cells at the concentration of 20 $\mu$M (Figure S75, Supporting Information). The present results are in agreement with previous findings that demonstrated the antiproliferative activity of juncusol against HeLa cervical cancer cells.[20]

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were determined in MeOH at ambient temperature using a PerkinElmer 341 polarimeter. NMR spectra were recorded in MeOD and DMSO-$d_6$ on a Bruker Avance DRX 500 spectrometer at 500 MHz ($^1$H) and 125 MHz ($^{13}$C). The signals of the deuterated solvents were taken as references. The chemical shift values ($\delta$) were given in ppm, and coupling constants ($J$) are in Hz. Two-dimensional (2D) experiments were performed with standard Bruker software. In the COSY, HSQC, and HMBC experiments, gradient-enhanced versions were used. The HRMS were acquired on a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer equipped with ESI ion source in the positive ionization mode. The resolution was over 1 ppm. The data were acquired and processed with MassLynx software.

Vacuum-liquid chromatography (VLC) was carried out on silica gel (15 $\mu$m, Merck); LiChroprep RP-18 (40−63 $\mu$m, Merck) stationary phase was used for reversed-phase VLC; column chromatography (CC) was performed on polyamide (MP Biomedicals). Medium-pressure liquid chromatography (MPLC) was processed with a Combi Flash Rf$^+$ Lumen instrument (Teledyne Isco) on a reversed-phase RediSep Rf HP Gold (50 g) column. Preparative thin-layer chromatography (prep. TLC) was performed on silica gel 60 $F_{254}$ plates (Merck) and on reversed-phase silica gel 60 RP-18 $F_{254}$ plates (Merck). Sephadex LH-20 (25−100 $\mu$m, Sigma-Aldrich) was used for gel filtration. HPLC was carried out on a Waters HPLC, using normal (Phenomenex Luna silica, 3 $\mu$m 100 A) and reversed-phase [Phenomenex, Kinetex 5 $\mu$m C18 100A and LiChrospher LiChroCART 250-4 RP-18e (5 $\mu$m)] columns. For the investigation of compounds with a chiral carbon atom, a Lux amylose-1 column (250 × 21.2 mm) (Phenomenex, USA) was used with cyclohexane−isopropanol (85:15) as mobile phase. All solvents used for CC were of at least analytical grade (VWR Ltd., Hungary).

**Plant Material.** *Juncus gerardii* (whole plant, 3.6 kg) was collected during the flowering period in June 2017, near Mórahalom, Hungary (GPS coordinates: 46°12.017″ N; 019°58.955″ E). Botanical identification of the plant material was performed by one of the authors (L.B., Department of Plant Biology, University of Szeged, Szeged, Hungary), and a voucher specimen (No. 881) has been deposited at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

**Extraction and Isolation.** The air-dried whole plant of *J. gerardii* (3.6 kg) was percolated with MeOH (60 L) at room temperature. The crude methanolic extract was concentrated under reduced pressure (421 g) and subjected to solvent−solvent partitioning with *n*-hexane (4 × 1 L), chloroform (4 × 1 L), and ethyl acetate (4 × 1 L), respectively.

The concentrated chloroform-soluble fraction (52 g) was separated by polyamide open column chromatography with a gradient system of MeOH−$H_2O$ {2:3 (A), 3:2 (B), 2:1 [10 L (C), 8 L (D), and 8 L (E), respectively], each eluent was collected as a fraction}. The fraction obtained from the polyamide column with MeOH−$H_2O$ (3:2, 12 g) was subjected to VLC on silica gel, with a gradient system of cyclohexane−EtOAc−MeOH [from 98:2:0 to 1:1:1 (1500 mL/eluent) and finally with MeOH; volume of collected fractions was 100 mL], to yield 16 major fractions (B/1−16). The fractions were combined according to their TLC patterns. Fractions B/1−3 (45.5, 21.5, and 38.9 mg, respectively) were purified using Sephadex LH-20 gel chromatography with $CH_2Cl_2$−MeOH (1:1) as eluent, to yield compounds **8** (1.2 mg) and compressin A (5.8 mg) from B/1, 7-hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene (6.8 mg) from B/2, and compound **2** (11.8 mg) from B/3. Fraction B/4 (1.64 g) was separated by reversed-phase (RP) MPLC, by using a gradient system of MeOH−$H_2O$ [from 1:1 to 1:0 (40 mL/min); volume of collected fractions was 20 mL], to yield eight subfractions (B/4/1−8). Subfractions B/4/2 and B/4/4 were pure for compounds **13** (1.3 g) and juncusol (185 mg), respectively. Fraction B/6 (494.0 mg) was also separated by RP-MPLC, using a gradient system of MeOH−$H_2O$ [from 1:1 to 1:0 (40 mL/min); volume of collected fractions was 20 mL)], to afford eight subfractions (B/6/1−8). Fractions B/6/2 (31.8 mg) and B/6/5 (42.6 mg) were purified by prep. TLC on silica gel using cyclohexane−EtOAc−EtOH (20:10:1) as solvent system, to yield compounds **1** (2.1 mg) and **14** (3.9 mg). Subfraction B/6/6 contained 2-hydroxy-7-hydroxymethylene-1-methyl-5-vinyl-9,10-dihydrophenanthrene (109 mg). Fraction B/7 (771.1 mg) was separated by RP-MPLC with a gradient system of MeOH−$H_2O$ [from 1:1 to 1:0 (40 mL/min); volume of collected fractions was 20 mL] to yield 11 subfractions (B/7/1−11). Fraction B/7/2 (179.5 mg) was purified by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as solvent system to afford five subfractions (B/7/2/1−5). Further purification of subfraction B/7/2/4 by normal-phase (NP) HPLC under gradient conditions, using cyclohexane−EtOAc (9:1 to 1:9 in 10 min at a flow rate of 1.5 mL/min) as mobile phase, yielded two fractions ($t_R$ = 7.5 min and $t_R$ = 7.9 min), which were purified by preparative TLC on silica gel using cyclohexane−EtOAc−EtOH (20:10:1) as solvent system, to yield 2,7-dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene (3.1 mg) and 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene (2.0 mg). Fraction B/7/6 (8.6 mg) was purified by prep. TLC on silica gel using cyclohexane−EtOAc−EtOH (20:10:1) as mobile phase to yield compound **11** (6.2 mg).

Fractions B/8 (63 mg) and B/9 (230.2 mg), B/12 (421.3 mg) and B/14 (548.6 mg) were separated by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as solvent system to yield compound **12** (5.5 mg, from B/8), jinflexin C (8.2 mg, from B/9/2), apigenin (18.7 mg, from B/12/2), and luteolin (22.8 mg, from B/14/2). Fraction B/10 (154.5 mg) was purified by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as solvent system to afford six subfractions (B/10/1−6). Fraction B/10/4 (30.1 mg) was purified by preparative TLC on silica gel using $CH_2Cl_2$−MeOH (9:1) as solvent system to yield compound **5** (9.2 mg). Fraction B/11 (474.1 mg) was purified by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as solvent system to afford four subfractions (B/11/1−4). Fractions B/11/2 (112.2 mg) and B/11/3 (54.6 mg) were further purified by preparative TLC on silica gel using $CH_2Cl_2$−MeOH (9:1) as solvent system, to yield 1-*O*-*p*-coumaroyl-3-*O*-feruloyl-glycerol (9.2 mg) and 2,7-dihydroxy-5-hydroxymethyl-1-methyl-9,10-dihydrophenanthrene (11.8 mg).

The fraction obtained from the polyamide column with MeOH−$H_2O$ 2:1 (3 g) was subjected to VLC on silica gel with a gradient system of cyclohexane−EtOAc−MeOH [from 95:5:0 to 1:1:1 (200 mL/eluent), and finally with MeOH; the volumes of the collected fractions were 50 mL], to yield six major fractions (D/1−6). The fractions were combined according to their TLC patterns. Fraction


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D/4 (130.1 mg) was purified by Sephadex LH-20 gel chromatography applying $CH_2Cl_2$−MeOH (1:1) as solvent system to afford four subfractions (D/4/1−4). Subfraction D/4/4 (15.8 mg) was purified by preparative TLC on silica gel using $CH_2Cl_2$−MeOH (9:1) as solvent system to yield compound **9** (5.9 mg). Fraction D/5 (124 mg) was purified by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as solvent system to afford four subfractions (D/5/1−4). Further purification of subfraction D/5/3 by reversed-phase HPLC under gradient conditions, using $CH_3CN$−$H_2O$ (from 55:44 to 7:3 in 11 min as mobile phase, flow rate 1.5 mL/min), resulted in the isolation of compound **15** ($t_R$ = 8.1 min, 4.6 mg). Fraction D/5/4 (20.1 mg) was purified by prep. TLC on silica gel using $CH_2Cl_2$−MeOH (95:5) as solvent system, to yield compound **10** (4.0 mg).

The concentrated ethyl acetate-soluble fraction (37 g) was separated by VLC on silica gel with a gradient system of $CHCl_3$−MeOH [from 98:2 to 6:4 (1500 mL/eluent), and finally with MeOH; volume of collected fractions were 100 mL], to yield 15 major fractions (F/1−15). The fractions were combined according to their TLC patterns. Fraction F/8 (705.3 mg) was purified by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as eluent and then by RP-TLC on RP silica gel using MeOH−$H_2O$ (1:1) as mobile phase. Further purification of subfraction F/8/2/1 by RP-HPLC under gradient conditions, using $CH_3CN$−$H_2O$ (from 21:79 to 26:74 in 10 min as mobile phase, flow rate 1.5 mL/min), resulted in the isolation of compounds **3** ($t_R$ = 8.35 min, 1.8 mg) and **4** ($t_R$ = 9,4 min, 2.0 mg). Fraction F/10 (720.4 mg) was separated by Sephadex LH-20 gel chromatography using $CH_2Cl_2$−MeOH (1:1) as eluent and RP-TLC on RP silica gel using MeOH−$H_2O$ (2:3) as solvent system. Further purification of subfraction F/10/2/1 by RP-HPLC under gradient conditions, using $CH_3CN$−$H_2O$ (from 1:9 to 35:65 in 12 min, flow rate 1.5 mL/min) as mobile phase, afforded compounds **6** ($t_R$ = 11.3 min, 6.8 mg) and **7** ($t_R$ = 12.1 min, 1.8 mg).

*Gerardiin A (**1**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 251.1068 $[M + H - CH_3OH]^+$ (calcd for $C_{17}H_{18}O_2$, 251.1072).

*Gerardiin B (**2**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 249.1275 $[M + H - CH_3OH]^+$ (calcd for $C_{18}H_{17}O$, 249.1279).

*Gerardiin C (**3**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 437.1565 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_7Na$, 437.1576).

*Gerardiin D (**4**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 437.1564 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_7Na$, 437.1576).

*Gerardiin E (**5**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 253.1226 $[M + H - H_2O]^+$ (calcd for $C_{17}H_{17}O_2$, 253.1229).

*Gerardiin F (**6**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 455.1671 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_8Na$, 455.1682).

*Gerardiin G (**7**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 455.1675 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_8Na$, 455.1682).

*Gerardiin H (**8**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 249.1274 $[M + H]^+$ (calcd for $C_{18}H_{17}O$, 249.1279).

*Gerardiin I (**9**):* amorphous solid; $[\alpha]_D^{25}$ 0 (*c* 0.1, MeOH); $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 503.2210 $[M + H]^+$ (calcd for $C_{34}H_{31}O_4$, 503.2222).

*Gerardiin J (**10**):* amorphous solid; $[\alpha]_D^{25}$ 0 (*c* 0.1, MeOH); $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 503.2213 $[M + H]^+$ (calcd for $C_{34}H_{31}O_4$, 503.2222).

*Gerardiin K (**11**):* amorphous solid; $[\alpha]_D^{25}$ 0 (*c* 0.1, MeOH); $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 505.2375 $[M + H]^+$ (calcd for $C_{34}H_{33}O_4$, 505.2379).

*Gerardiin L (**12**):* amorphous solid; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 515.2213 $[M + H]^+$ (calcd for $C_{35}H_{31}O_4$, 515.2222).

*Compressin A:* $^1$H NMR (500 MHz, methanol-$d_4$) $\delta$ 7.53 (1H, d, *J* = 8.7 Hz, H-4), 6.79 (1H, dd, *J* = 18.0, 11.4 Hz, H-12), 6.70 (1H, d, *J* = 8.7 Hz, H-3), 6.63 (1H, s, H-8), 5.42 (1H, d, *J* = 11.4 Hz, H-13a), 5.12 (1H, d, *J* = 18.0 Hz, H-13b), 3.81 (3H, s, $OCH_3$), 2.66 (2H, m, H-10), 2.55 (2H, m, H-9), 2.21 (3H, s, $CH_3$), 2.18 (3H, s, $CH_3$).

*7-Hydroxy-2-methoxy-1-methyl-5-vinyl-9,10-dihydrophenanthrene:* $^1$H NMR (500 MHz, methanol-$d_4$) $\delta$ 7.33 d (1H, d, *J* = 8.6 Hz, H-4), 6.93 (1H, dd, *J* = 17.5, 10.9 Hz, H-12), 6.86 (1H, d, *J* = 2.4 Hz, H-6), 6.78 (1H, d, *J* = 8.6 Hz, H-3), 6.65 (1H, d, *J* = 2.4 Hz, H-8), 5.64 (1H, d *J* = 17.4 Hz, H-13a), 5.20 (1H, d, *J* = 10.9 Hz, H-13b), 3.83 (3H, s, $OCH_3$), 2.68 (2H, m, H-10), 2.62 (2H, m, H-9), 2.20 (3H, s, $CH_3$).

*2-Hydroxy-1-methyl-7-hydroxymethylene-5-vinyl-9,10-dihydrophenanthrene:* $^1$H NMR (500 MHz, methanol-$d_4$) $\delta$ 7.39 (1H, s, H-6), 7.28 (1H, d, *J* = 8.4 Hz, H-4), 7.14 (1H, s, H-8), 6.95 (1H, dd, *J* = 17.4, 10.9 Hz, H-12), 6.67 (1H, d, *J* = 8.4 Hz, H-3), 5.70 (1H, d, *J* = 17.4, H-13a), 5.22 (1H, d, *J* = 10.9 Hz, H-13b), 4.59 (2H, s, H-14), 2.12 (3H, s, $CH_3$); $^{13}$C NMR (125 MHz, methanol-$d_4$) $\delta$ 155.8 (C-2), 140.9 (C-1a), 140.4 (C-12), 140.0 (C-7), 135.9 (C-5), 128.9 (C-4), 126.6 (C-4a), 126.5 (C-8), 126.2 (C-6), 122.1 (C-1), 113.8 (C-13), 112.4 (C-3), 65.1 (C-14), 31.1 (C-9), 26.6 (C-10), 11.8 ($CH_3$-1).

*2,7-Dihydroxy-5-formyl-1-methyl-9,10-dihydrophenanthrene:* $^1$H NMR (500 MHz, methanol-$d_4$) $\delta$ 9.95 (1H, s, H-12), 7.14 (1H, d, *J* = 2.5 Hz, H-6), 6.97 (1H, d, *J* = 2.5 Hz, H-8), 6.77 (1H, d, *J* = 8.3 Hz, H-4), 6.74 (1H, d, *J* = 8.3 Hz, H-3), 2.78 (2H, m, H-10), 2.72 (2H, m, H-9), 2.24 (3H, s, $CH_3$); $^{13}$C NMR (125 MHz, methanol-$d_4$) $\delta$ 194.6 (C-12), 157.3 (C-7), 156.9 (C-2), 142.9 (C-8a), 140.3 (C-1a), 135.1 (C-5), 123.1 (C-1), 129.7 (C-4), 124.4 (C-4a), 120.9 (C-8), 113.1 (C-3), 112.7 (C-7), 30.4 (C-9), 26.4 (C-10), 11.8 ($CH_3$-1).

*Effususol A:* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.87 (1H, d, *J* = 8.3 Hz, H-4), 6.78 (1H, d, *J* = 2.3 Hz, H-6), 6.73 (1H, d, *J* = 8.3 Hz, H-3), 6.60 (1H, d, *J* = 2.0 Hz, H-8), 4.70 (1H, q, *J* = 6.1 Hz, H-12), 2.86 (3H, s, $OCH_3$), 2.76 (1H, m, H-10a), 2.62 (1H, m, H-9a), 2.42 (1H, m, H-9b), 2.37 (1H, m, H-10b), 2.12 (3H, s, $CH_3$), 1.48 (3H, d, *J* = 6.2 Hz, $CH_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 155.7 (C-7), 153.8 (C-2), 140.8 (C-5), 139.6 (C-8a), 139.0 (C-1a), 126.2 (C-5a), 125.7 (C-4), 124.5 (C-4a), 120.51 (C-1), 113.6 (C-8), 111.6 (C-3), 111.3 (C-6), 74.4 (C-12), 55.0 ($OCH_3$-12), 30.4 (C-9), 25.4 (C-10), 23.1 (C-13), 11.75 ($CH_3$-1).

*2,7-Dihydroxy-1-methyl-5-hydroxymethyl-9,10-dihydrophenanthrene:* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.26 (1H, d, *J* = 8.5 Hz, H-4), 6.87 (1H, d, *J* = 2.3 Hz, H-6), 6.70 (1H, d, *J* = 8.5 Hz, H-3), 6.58 (1H, d, *J* = 2.3 Hz, H-8), 4.49 (2H, s, H-12), 2.55 (2 × 2H, brs, H-9, H-10), 2.11 (3H, s, $CH_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 155.1 (C-7), 153.7 (C-2), 139.3* (C-5), 139.1* (C-8a), 138.4 (C-1a), 125.7 (C-4), 125.7 (C-5a), 125.2 (C-4a), 120.3 (C-1), 114.8 (C-6), 113.2 (C-8), 111.6 (C-3), 62.1 (C-12), 30.2 (C-9), 25.3 (C-10), 11.7 ($CH_3$-1) (*interchangeable signals).

*Effususin A (**15**):* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.15 (1H, s, H-4, H-4′), 6.90 (1H, dd, *J* = 17.3, 10.8 Hz, H-12, H-12′), 6.79 (1H, d, *J* = 1.7 Hz, H-6, H-6′), 6.63 (1H, d, *J* = 1.6 Hz, H-8, H-8′), 5.54 (1H, d, *J* = 17.4 Hz, H-13a, H-13a′), 5.06 (1H, d, *J* = 10.8 Hz, H-13b, H-13b′), 2.60 (2 × 2H, m, H-9, H-9′, H-10, H-10′), 2.18 (3H, s, $CH_3$-1, $CH_3$-1′); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 157.3 (C-2, C-2′), 154.7 (C-7, C-7′), 139.8 (C-8a, C-8a′), 138.9 (C-12, C-12′), 135.9 (C-1a, C-1a′), 134.5 (C-5, C-5′), 128.5 (C-4, C-4′), 126.7 (C-5a, C-5a′), 125.7 (C-3, C-3′), 122,3 (C-1, C-1′), 114.3 (C-8, C-8′), 112.6 (C-13, C-13′), 112.1 (C-6, C-6′), 30.2 (C-9, C-9′), 25.5 (C-10, C-10′), 12.7 (C-11, C-11′).

*1-O-p-Coumaroyl-3-O-feruloyl-glycerol:* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.58 (1H, d, *J* = 15.9 Hz), 7.57 (1H, d, *J* = 15.9 Hz), 7.53 (1H, d, *J* = 8.6 Hz), 7.31 (1H, d, *J* = 1.2 Hz), 7.10 (1H, dd, *J* = 8.2, 1.2 Hz), 6.78 (1H, d, *J* = 8.2 Hz), 6.77 (2 × 1H, d, *J* = 8.6 Hz), 6.48 (1H, d, *J* = 15.9 Hz), 6.39 (1H, d, *J* = 15.9 Hz), 4.15 (2H, d, *J* = 5.1 Hz), 4.11 (2H, m), 4.01 (1H, m), 3.80 (3H, s, $OCH_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 2 × 166.6, 160.2, 149.7, 148.0, 145.1, 145.4, 130.4, 125.4, 124.9, 123.3, 115.9, 115.6, 114.1, 113.8, 111.1, 66.4, 65.1, 55.7.

**Cell Culture and Cytotoxicity Testing.** 4T1 (mouse triple-negative breast cancer cells) were cultured in RPMI (Roswell Park


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Memorial Institute) 1640 medium supplemented with 5% fetal bovine serum (FBS) (both from Thermo Fisher Scientific, Waltham, MA, USA). MDA-MB-231 (human triple-negative breast cancer cells, abbreviated as MDA) were cultured in DMEM (Dulbecco's modified Eagle's medium, Thermo Fisher Scientific) + 5% FBS. D3 (hCMEC/D3 human cerebral microvascular endothelial cells) were kept in rat tail collagen-coated dishes in EBM-2 (endothelial basal medium-2, Lonza, Basel, Switzerland) complemented with 2% FBS and an EGM-2MV kit (Lonza).

Viability and impedance measurements were performed in the log growth phase of tumor cells and in the stationary phase of endothelial cells.

For the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) viability assay, cells were plated in 96-well plates (Corning, Corning, NY, USA). The seeding density of the cells was 5000/well (for 4T1 or MDA cells) or 25 000/well (for D3 cells). After 48 h, half of the medium was replaced with serum-free medium, containing the test compounds in a final concentration of 10 or 20 $\mu$M. Control wells received solvent (DMSO) in up to a 0.2% concentration. After 48 h, MTT reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to the cells in a final concentration of 2.5 mg/mL. After incubation at 37 °C for 30 min, acidified isopropanol solution was added to each well. Absorbance was measured at 595 nm with a FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg, Germany). Doxorubicin was used as a positive control, at a concentration of 10 $\mu$M.

For impedance measurements, cells were plated in 96-well E-plates having microelectrodes integrated on the bottom (ACEA Biosciences, San Diego, CA, USA) and allowed to attach onto the electrode surface for 48 h. Afterward, cells were treated with the test compounds as described above. Electrical impedance was recorded every 30 min for 48 h using an xCELLigence real-time cell analysis (RTCA) instrument (ACEA Biosciences). Cell index was automatically calculated by the software of the instrument.

For determining $IC_{50}$ values, nine-step, 2-fold serial dilutions of the test compounds were applied, starting from 100 $\mu$M. Cells were treated for 48 h, and viability was measured with the MTT assay, as described above. Half-maximum inhibitory concentrations ($IC_{50}$) were calculated via nonlinear dose−response curve fitting by the log(inhibitor) vs response (variable slope) model of GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA) by using automatic outlier elimination at $Q = 1.0$.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00631.

1D and 2D NMR spectra for new compounds (**1**−**12**); results of pharmacological investigations (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

**Andrea Vasas** − *Department of Pharmacognosy, Interdisciplinary Excellence Centre, University of Szeged, 6720 Szeged, Hungary;* orcid.org/0000-0002-1818-7702; Phone: +36-62-546451; Email: vasasa@pharm.u-szeged.hu

### Authors

**Dóra Stefkó** − *Department of Pharmacognosy, Interdisciplinary Excellence Centre, University of Szeged, 6720 Szeged, Hungary*

**Norbert Kúsz** − *Department of Pharmacognosy, Interdisciplinary Excellence Centre, University of Szeged, 6720 Szeged, Hungary*

**Anita Barta** − *Department of Pharmacognosy, Interdisciplinary Excellence Centre, University of Szeged, 6720 Szeged, Hungary*

**Zoltán Kele** − *Department of Medical Chemistry, University of Szeged, 6720 Szeged, Hungary*

**László Bakacsy** − *Department of Plant Biology, University of Szeged, 6726 Szeged, Hungary*

**Ágnes Szepesi** − *Department of Plant Biology, University of Szeged, 6726 Szeged, Hungary*

**Csilla Fazakas** − *Institute of Biophysics, Biological Research Centre, 6726 Szeged, Hungary*

**Imola Wilhelm** − *Institute of Biophysics, Biological Research Centre, 6726 Szeged, Hungary; Institute of Life Sciences, Vasile Goldiş Western University of Arad, RO-310414 Arad, Romania*

**István A. Krizbai** − *Institute of Biophysics, Biological Research Centre, 6726 Szeged, Hungary; Institute of Life Sciences, Vasile Goldiş Western University of Arad, RO-310414 Arad, Romania*

**Judit Hohmann** − *Department of Pharmacognosy, Interdisciplinary Excellence Centre and Interdisciplinary Centre of Natural Products, University of Szeged, 6720 Szeged, Hungary;* orcid.org/0000-0002-2887-6392

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c00631


### Notes

The authors declare no competing financial interest.

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