Bioactive Metabolites from the Endophytic Fungus *Stemphylium globuliferum* Isolated from *Mentha pulegium*

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The endophytic fungus *Stemphylium globuliferum* was isolated from stem tissues of the Moroccan medicinal plant *Mentha pulegium*. Extracts of the fungus, which was grown on solid rice medium, exhibited considerable cytotoxicity when tested in vitro against L5178Y cells. Chemical investigation yielded five new secondary metabolites, alterporriol G (**4**) and its atropisomer alterporriol H (**5**), altersolanol K (**11**), altersolanol L (**12**), stemphypyrone (**13**), and the known compounds 6-*O*-methylalaternin (**1**), macrosporin (**2**), altersolanol A (**3**), alterporriol E (**6**), alterporriol D (**7**), alterporriol A (**8**), alterporriol B (**9**), and altersolanol J (**10**). The structures were determined on the basis of one- and two-dimensional NMR spectroscopy and mass spectrometry. Among the alterporriol-type anthranoid dimers, the mixture of alterporriols G and H (**4/5**) exhibited considerable cytotoxicity against L5178Y cells with an EC_{50} value of 2.7 μg/mL, whereas the other congeners showed only modest activity. The compounds were also tested for kinase inhibitory activity in an assay involving 24 different kinases. Compounds **1**, **2**, **3**, and the mixture of **4** and **5** were the most potent inhibitors, displaying EC_{50} values between 0.64 and 1.4 μg/mL toward individual kinases.

An endophyte is a fungal or bacterial microorganism that spends the whole or part of its life cycle colonizing healthy tissues of its host plant, typically causing no apparent symptoms of disease.[1] The relationship between the endophyte and its host plant may range from symbiotic to near-pathogenic.[2] The nature of interaction between host plants and endophytes in natural populations and communities is, however, poorly understood. Endophyte−host plant symbioses represent a broad continuum of interactions, from pathogenic to mutualistic, even within the lifespan of an individual microorganism and its host plant.[2−4] Moreover, endophytic fungi are thought to interact mutualistically with their host plants, for example by increasing resistance to herbivores, and thus have been termed "acquired plant defenses".[5−7] Accordingly, there are numerous examples of endophytes producing secondary metabolites with agricultural or pharmaceutical potential.[1,8] In some cases, endophytic fungi have also been found to produce commercially important natural products that were previously only known from plants. Some renowned examples include paclitaxel,[9] camptothecin,[10] podophyllotoxin,[11] and hypericin,[12] although it is far from clear to what extent endophytes actually contribute to the biosynthesis of these compounds within their host plants.

The purpose of this study was to investigate natural products produced by the endophytic fungus *Stemphylium globuliferum*, isolated from stem tissues of the traditional medicinal plant *Mentha pulegium* (Lamiaceae) growing in Morocco. Teas brewed from the leaves of *M. pulegium* are used traditionally to treat common colds and disorders of the liver and gall-bladder, as a carminative, as a diuretic, and to stimulate digestive action.[13] The essential oil has been reported to have antifungal,[14] larvicidal,[15] acaricidal,[16] and cytotoxic activities.[17] The stimulus that prompted our investigation of the endophyte *S. globuliferum* was the strong cytotoxic activity of its crude EtOAc extract against murine L5178Y cells. *Stemphylium* species have a widespread distribution, with many species occurring as plant pathogens. A number of metabolites of polyketide origin, including altersolanol A[18] (initially described as stemphylin[19]), stemphyltoxins I−IV,[20] stemphyperylenol,[20] and stemphyloxins I and II,[21,22] have been isolated previously from *Stemphylium* spp., and most of them were described as phytotoxins. The present study provides the first comprehensive analysis of natural products produced by *S. globuliferum.*

Results and Discussion

A crude EtOAc extract of *S. globuliferum* grown on solid rice cultures was partitioned between *n*-hexane and 90% MeOH. From the 90% MeOH fraction, the known compounds 6-*O*-methylalaternin (**1**),[23−26] macrosporin (**2**),[24,25] and altersolanol A (**3**)[24,25,27] were obtained. Compounds **1**−**3** were also identified as building blocks for a series of dimeric anthranoids, including two new congeners, obtained as an inseparable mixture of alterporriol G (**4**) and its atropisomer alterporriol H (**5**), together with the known derivatives alterporriol E (**6**),[28−30] alterporriol D (**7**),[28−30] alterporriol

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Table 1. NMR Data of Alterporriol G (**4**), Alterporriol H (**5**), Altersolanol K (**11**), and Altersolanol L (**12**)

	4/5	**4**	**5**	**11**	**12**	
position	δ_C[a,b]	δ_H mult. (*J* Hz)[a]	δ_H mult. (*J* Hz)[a]	δ_H mult. (*J* Hz)[c]	δ_C[c]	δ_H mult. (*J* Hz)[c]
1	112.2	7.55 s	7.53 s	4.08 br s[d]	72.1	3.81[e]
2	163.4				72.2	
3	133.4			3.46 br d (12.2)	69.5	3.54 m
4	131.7	8.00 s[f]	8.00 s[f]	ax 1.48 q (12.0)	29.5	ax 1.42 q (12.1)
				eq 2.07 dt (12.0, 4.9)		eq 2.10 dt (12.6, 4.5)
4a	126.9			2.30 m	41.5	2.65 m
5	103.6	7.45 s	7.48 s		164.3	
6	166.2			6.58 d (2.5)	98.8	6.33 d (2.4)
7	112.5				165.7	
8				6.89 d (2.5)	104.2	6.74 dd (2.4, 1.0)
8a	120.3				151.9	
9	188.9				66.1	4.63 m
9a	135.0			2.65 dd (13.1, 2.1)	45.7	2.19 td (10.9, 2.9)
10	183.1			4.80 d (10.2)	204.4	
10a					108.8	
1′	70.4	4.740 d (7.6)	4.738 d (7.3)			
2′	75.4	3.82[e,f]	3.82[e,f]			
3′	74.8					
4′	70.1	4.34 s	4.33 s			
4a′	143.8					
5′	118.3					
6′ (in **4**)	164.5					
6′ (in **5**)	165.1					
7′	104.9	6.89 s[f]	6.89 s[f]			
8′	166.3					
8a′	111.1					
9′						
9a′	143.8					
10′	185.7					
10a′						
CH_3-2				1.20 s	23.6	1.22 s
CH_3-3	16.4	2.34 s[f]	2.34 s[f]			
OCH_3-6	57.4	3.81 s	3.80 s			
OCH_3-7				3.74 s	55.6	3.81 s
OH-1				4.88 d (5.4)		4.94 d (5.3)
OH-2				4.12 s		3.96 s
OH-3				4.18 br s		4.22 br s
OH-5				n.d.[g]		12.96 s
OH-9						5.42 d (7.8)
OH-10				4.08[d]		
CH_3-3′	22.2	1.363 s	1.357 s			
OCH_3-6′	56.8	3.83 s	3.85 s			

[a] Measured in MeOH-d_4 at 500 (^{1}H), 125 (^{13}C) MHz. [b] Derived from HMBC spectrum (missing signals, no correlations observed). Signals for both atropisomers are overlapping if not indicated otherwise. [c] Measured in DMSO-d_6 at 600 (^{1}H) and 150 (^{13}C) MHz. [d] Overlapped. [e] Overlapped by methoxyl signal. [f] Overlapping signal of both **4** and **5**. [g] n.d., not detected.

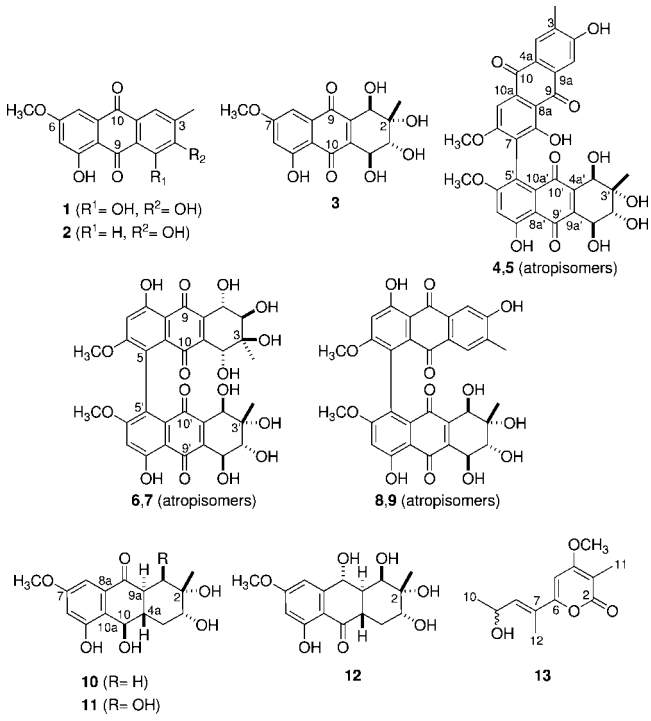
A (**8**),[31,32] and alterporriol B (**9**).[33] Additionally, the known hexahydroanthronol altersolanol J (**10**)[34] two new congeners, altersolanol K (**11**) and altersolanol L (**12**), and a new α-pyrone, stemphypyrone (**13**), were isolated. The known compounds were identified on the basis of their spectroscopic data and $[\alpha]_D$ values as well as by comparison with published data.[35]

Compounds **4** and **5** were obtained as an inseparable mixture forming a red, amorphous powder. Upon LC-MS analysis, the mixture exhibited two partially overlapped peaks (area ratio ca. 4:3), which displayed virtually identical UV spectra and ESI mass spectra. From the ^{1}H NMR spectrum and ESIMS (*m*/*z* 619.0 $[M + H]^+$), they were assumed to be dimeric anthraquinones each comprising a macrosporin (**2**) and an altersolanol A (**3**) subunit. This was confirmed by a pseudomolecular ion peak at *m*/*z* 641.128 $[M + Na]^+$, consistent with the molecular formula $C_{32}H_{26}O_{13}$ (HRESIMS). Even though the ^{1}H NMR spectrum (Table 1) showed a considerable degree of overlapping, it was possible to distinguish most signals for compounds **4** and **5** and to assign them to either atropisomer on the basis of the integrals. The COSY spectrum showed long-range correlations between CH_3-3 and H-4 as well as a cross-peak between H-1′ and H-2′, the latter obscured by the OCH_3 groups. Furthermore, interpretation of the HMBC spectrum showed that both H-4 and H-5 correlated to C-10 (δ 183.1), whereas H-1 correlated to C-9 (δ 188.9). A strong correlation was observed for H-7′ to both C-6′ (δ 165.1 in **5**, 164.5 in **4**) and C-8′ (δ 166.3), thus establishing the link between the two monomers to reside between C-7 and C-5′.

In order to determine the relative configuration of compounds **4/5** in the aliphatic ring, a ROESY experiment was performed. The CH_3-3′ signals showed correlations to H-2′ and H-4′ as well as OH-4′, while H-2′ correlated to OH-1′, and in turn, H-1′ to OH-2′. Furthermore, correlations were observed from H-2′ to OH-4′, indicating the relative configuration of the aliphatic ring to be identical to the one present in altersolanol A (**3**). Thus, compounds **4** and **5** were identified as new natural products, alterporriol G and alterporriol H, and representing a pair of atropisomers as observed previously for other alterporriols.[28–33]

The HRESIMS of compound **11** exhibited a peak at *m*/*z* 325.129 $[M + H]^+$ indicating a molecular formula of $C_{16}H_{20}O_7$. Comparison of the ^{1}H NMR spectrum of **11** (Table 1) with that of altersolanol J (**10**) showed a close structural relationship between both compounds except for the absence of one aliphatic proton and the presence of an additional OH group in compound **11**. This was confirmed by the corresponding signals observed for OH-1 (δ 4.88), thus placing the additional OH group at C-1.

The relative configuration of **11** was deduced from the ROESY spectrum as well as from the coupling constants extracted from the ^{1}H NMR spectrum (Table 1). The large values of $J_{3,4ax}$ (12.2



Hz), $J_{4ax,4a}$ (12.0 Hz), $J_{4a,9a}$ (13.1 Hz), and $J_{4a,10}$ (10.2 Hz) indicated that all of these protons exhibited mutual diaxial relationships. The equatorial position of H-1 was evident from the small coupling constant of 2.1 Hz to H-9a. Furthermore, H-3 was found to correlate to H-4_{eq} and H-4a in the ROESY spectrum, as well as CH_3-2 to OH-1, while on the other hand, H-4_{ax} displayed correlations to both H-9a and H-10. On this basis, **11** was identified as altersolanol K, representing a new natural product.

Compound **12** was obtained as a brown powder. Its HRESIMS exhibited a prominent peak at *m/z* 325.127 $[M + H]^+$ indicating a molecular formula of $C_{16}H_{20}O_7$, identical to that of **11**. The ^{1}H NMR spectrum of compound **12** (Table 1) was similar to that of **10** and **11**, with the most obvious difference consisting in the presence of an additional singlet appearing at δ 12.96, attributed to the chelated phenolic group OH-5. Importantly, in the COSY spectrum, the less shielded aryl proton H-8 exhibited a long-range correlation to a *peri*-proton (H-9) (d, $J = 1.2$ Hz), reminiscent of tetrahydroaltersolanol B.[36] In turn, H-9 coupled to both the OH signal at δ 5.42 (9-OH) and the ring junction proton at δ 2.19 (H-9a). This indicated that the OH group at C-10 present in **10** and **11** was oxidized to a carbonyl group in **12**, while in turn, the keto function at C-9 was reduced to an OH. Confirmation of this assumption was provided by the observed HMBC correlations.

The relative configuration of **12** was deduced from analysis of the coupling constants, similar to those described above for **11**, indicating a series of mutual diaxial relationships for H-3, H-4_{ax}, H-4a, H-9a, and H-9, as well as an equatorial orientation for H-1. Confirmation was obtained from a complete set of mutual NOEs in the ROESY spectrum, which also allowed assignment of the relative stereochemistry of CH_3-2. The compound was thus identified as a new natural product, for which the name altersolanol L is proposed.

The HRESIMS of compound **13** displayed a molecular ion peak at *m/z* 225.112 $[M + H]^+$, indicating the molecular formula $C_{12}H_{16}O_4$ and thus implying five degrees of unsaturation. The ^{1}H NMR spectrum (Table 2) indicated the presence of three methyl groups, one methoxy group, and three methine protons. The structure of **13** was deduced from analysis of the COSY and HMBC spectra. In the former, a spin system including H_3-10, H-9, H-8, and H_3-12 was evident, while the latter allowed for the α-pyrone core structure as well as placing the side chain identified previously

Table 2. NMR Data of Stemphypyrone (**13**) at 500 (^{1}H) and 125 (^{13}C) MHz (MeOD-d_4)

position	δ_C[a]	δ_H mult. (*J* Hz)
2	167.4	
3	102.7	
4	168.7	
5	94.9	6.53 s
6	161.2	
7	127.7	
8	138.7	6.47 dd (8.3, 1.2)
9	65.2	4.69 dq (8.3, 6.4)
10	23.1	1.28 d (6.4)
11	8.5	1.88 s
12	12.5	1.98 d (1.2)
OCH_3-4	57.2	3.98 s

[a] Obtained from the HMBC spectrum.

at C-6 through a full set of all possible ^2J and ^3J correlations for H-5, H_3-11, and H_3-12. The *trans*-geometry of the double bond was indicated by the upfield shift observed for CH_3-12 as well as the distinct allylic coupling between H-8 and H_3-12 ($J = 1.2$ Hz), similar to the known pestalopyrone and hydroxypestalopyrone.[37] Attempts to determine the absolute configuration of **13** by a modified Mosher procedure[38] proved inconclusive.[39] The compound was thus identified as a new natural product, for which the name stemphypyrone is proposed.

Fungi are well-known producers of both anthraquinones such as macrosporin (**2**) and hydrogenated anthranoid congeners such as altersolanol A (**3**). Biogenetically, monomeric anthranoids have been identified as octaketides produced through condensation of acetate (or malonate) units,[25] and the incorporation of altersolanol A (**3**) into macrosporin (**2**) has been demonstrated,[40] indicating that these metabolites are part of a common biogenetic grid. Altersolanol K (**11**) represents the second example of a fungal-derived hexahydroanthronol with an oxidized C-9 and a reduced C-10, as found in altersolanol J (**10**).[34] The opposite pattern, i.e., oxidized C-10 and reduced C-9, was first described for tetrahydroaltersolanol B,[36] and later for ampelanol,[41] with altersolanol L (**12**) representing the third hexahydroanthronol congener with this structural feature. Remarkably, the relative configuration in all these derivatives has been found identical for the corresponding positions, so if present, OH-9 and OH-10 are equatorial, while OH-1 is axial.

Dimeric alterporriols have so far only been described from *Alternaria porri*[29,31,33,42] and *A. solani*;[28,43,44] thus the present communication represents the first report of alterporriols outside this genus. In terms of the underlying monomers, alterporriols are either homodimers made of two altersolanol A units, i.e., alterporriols D (**7**) and E (**6**), or more commonly, heterodimers incorporating one macrosporin and one altersolanol A unit, i.e., alterporriols A (**8**), B (**9**), C,[42,44] G (**4**), and H (**5**). With regard to the coupling positions of the monomers, alterporriols A (**8**), B (**9**), D (**7**), and E (**6**) feature a C-5−C-5′ linkage, while alterporriol C shows a C-1−C-7′ connection. Thus, alterporriols G (**4**) and H (**5**) represent the first dimers with a C-7−C-5′ linkage.

The biosynthesis of alterporriol A and the atropisomer pair D and E has been studied in *A. porri* through feeding studies using single- and double-labeled acetate.[30,32] Although no experimental proof of enzymatic coupling of monomers was obtained, in both cases the correspoding preanthranoids could not be detected in the culture media by HPLC analysis, and it was presumed that alterporriols might be formed by oxidative coupling.[30,32] Thus, it is noteworthy that in this study **2** and **3** were detected together with the corresponding dimers **4**−**9**.

α-Pyrones are a common motif in fungal polyketides, and various congeners with a 4-methoxy-3-methyl substitution as well as a branched unsaturated side chain at C-6 have been described. Examples include infectopyrone from *Alternaria infectoria*,[45] dihydroinfectopyrone and further congeners from *Petriella* sp.,[46]

Table 3. Cytotoxicity Assay for Isolated Compounds

compound tested	L5178Y growth in % (at 10 μg/mL)	EC_{50} (μg/mL)
1[a]	4.9	4.2
2[a]	54.5	
3[a]	1.5	
4/5	0.1	2.27
6[a]	92.1	
7[a]	64.8	
8/9	31.1	
10[a]	20.1[a]	
11	41.4	
12	51.4	
13	n.t.[b]	
emodin	77.0	
kahalalide F (positive control)		6.3

[a] Data from ref 41. [b] n.t., not tested.

nectriapyrone from *Gyrostroma missouriense*,[47] and phomapyrone A[48] as well as phomapyrones D and E[49] from *Leptosphaeria maculans* (asexual stage: *Phoma lingam*). Stemphypyrone (**13**) differs from phomapyrone D by the reduction of the keto function at C-9 to the hydroxyl group present in **13**.

All compounds isolated from the endophytic fungus *S. globuliferum* were tested for cytotoxicity toward L5178Y mouse lymphoma cells (Table 3).[50] For comparison, macrosporin-2-*O*-sulfate, 6-*O*-methylalaternin-2-*O*-sulfate, and ampelanol, which had previously been obtained from the fungal endophyte *Ampelomyces* sp.,[41] as well as the known anthraquinone emodin were included in the bioassay. Among the monomeric anthranoids, the tetrahydroanthraquinone **3** was the most active compound, while the hexahydroanthronol derivatives **10**, **11**, **12**, and ampelanol (data in ref 41) showed only moderate to weak activities, suggesting that the *para*-quinone moiety is important for cytotoxic activity. Furthermore, 6-*O*-methylalaternin (**1**) was the most active derivative among the anthraquinone derivatives tested, while emodin and **2** showed only moderate activity, indicating that the *ortho* dihydroxy substitution pattern of **1** greatly increases cytotoxic activity. Moreover, sulfate substitution as present in macrosporin-2-*O*-sulfate and 6-*O*-methylalaternin-2-*O*-sulfate resulted in significantly reduced activity, probably due to the increased polarity, which could affect cellular uptake.

Among the alterporriol-type dimers, the mixture of alterporriols G and H (**4/5**) was found to possess considerable cytotoxic activity (EC_{50} 3.7 μM), while the other alterporriols isolated from *S. globuliferum* displayed only moderate to weak activity. This could possibly be explained by the different shape adopted by **4** and **5** (as well as the previously reported alterporriol C), since a 3D model of these compounds clearly shows that they adopt an angular, "L"-like shape, with both tricyclic aromatic systems arranged more or less perpendicular to each other, as opposed to the remaining alterporriols, in which the dimers are arranged more or less parallel to each other. Thus it seems rewarding to also test alterporriol C and its atropisomer, for which no such activity had been reported previously, but which, however, were not detected in the culture of the fungus under investigation.

Testing of all compounds at a dose of 1 μg/mL in the biochemical protein kinase activity assays revealed a pattern of activity similar to that found in the MTT assay for L5178Y cells (Tables 3 and 4). Compounds that were most active in the MTT assay including **1**, **3**, and the mixture of **4** and **5** proved also to be active as kinase inhibitors. Compounds that lacked significant activity in the MTT assay were inactive in the protein kinase assays (data not shown). Whereas both **1** and the mixture of **4** and **5** inhibited a broad panel of kinases with similar IC_{50} values, **3** proved to be more specific. Among the 24 different enzymes tested, Aurora-B and CDK4/CycD1 were the most susceptible to this natural product (IC_{50} values 0.76 and 0.64 μg/mL), whereas inhibition of other kinases was only observed at higher concentrations (Table 4). The similar pattern of activity in the cellular assay and in the biochemical protein kinase assays observed for compounds **1**, **3**, and **4/5** suggests that inhibition of protein kinases may be one of the major mechanisms contributing to the cytotoxic activity of these compounds.

For **2** no such parallelism of activity in both assay systems was observed. The compound was only moderately active in the MTT assay (Table 3), but was a strong kinase inhibitor similar to **3**. IC_{50} values of **2** against Aurora-B and FLT3 kinases amounted to 0.65 and 0.85 μg/mL (Table 4). Other kinases were inhibited only at clearly higher concentrations. This discrepancy of pronounced inhibitory activity in the biochemical protein kinase assay and only moderate activity in the cellular assay may perhaps be explained by the low solubility of **2**, which would lead to reduced uptake into the tested cell line.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer-241 MC polarimeter. 1D and 2D NMR spectra were recorded on Bruker ARX 500 or AVANCE DMX 600 NMR spectrometers. ESIMS was conducted on a Finnigan LCQ Deca mass spectrometer, and HRESIMS spectra were obtained on a Micromass Qtof 2 mass spectrometer. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. HPLC analysis was carried out on a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S). Routine detection was at 235, 254, 280, and 340 nm. The separation column (125 × 4 mm, L × i.d.) was prefilled with Eurospher-10 C_{18} (Knauer, Germany), and the following gradient was used (MeOH/0.02% H_3PO_4 in H_2O): 0 min, 10% MeOH; 5 min, 10% MeOH; 35 min, 100% MeOH; 45 min, 100% MeOH.

Fungal Material. Fresh, healthy stems of *Mentha pulegium* L. (Lamiaceae) were collected in January 2006 from the mountain of Beni-Mellal, Morocco. The plant was identified by Prof. A. Boulli, Faculty of Sciences and Techniques, Beni-Mellal, Morocco. Voucher specimens have been deposited in the Laboratoire des Substances Naturelles et Thermolyse Éclair, University Mohammed V Agdal, Rabat, Morocco (accession number M.P.01/2006). Stems were rinsed twice in sterilized distilled water. Surface sterilization was done by immersing the stems in 70% ethanol for 2 min (two times) followed by rinsing two times in sterilized distilled water. Then, the stem was cleaved aseptically into

Table 4. IC_{50} Values of Selected Compounds against 24 Different Protein Kinases[a]

cpd	AKT1	ARK5	Aurora-A	Aurora-B	B-RAF-VE	CDK2/CycA	CDK4/CycD1	COT	EGF-R	EPHB4	ERBB2	FAK
1	n.a.	n.a.	3.0	1.8	n.a.	n.a.	3.6	7.3	4.0	n.a.	n.a.	n.a.
2	n.a.	n.a.	1.9	0.65	6.4	n.a.	n.a.	7.7	2.7	n.a.	7.3	n.a.
3	6.7	n.a.	1.3	0.76	n.a.	n.a.	0.64	n.a.	6.0	n.a.	n.a.	1.5
4/5	n.a.	3.8	2.8	2.4	6.5	n.a.	4.7	4.2	2.7	4.2	4.2	5.5

cpd	IGF1-R	SRC	VEGF-R2	VEGF-R3	FLT3	INS-R	MET	PDGFR-beta	PLK1	CK2-alpha1	SAK	TIE2
1	2.8	1.8	1.2	2.7	2.5	3.9	4.3	n.a.	n.a.	n.a.	4.4	4.5
2	n.a.	n.a.	2.4	n.a.	0.85	3.5	4.3	n.a.	n.a.	n.a.	4.3	1.9
3	3.5	2.4	7.3	9.9	4.8	n.a.	n.a.	n.a.	4.8	n.a.	2.5	8.9
4/5	1.9	1.7	3.4	4.0	2.8	3.7	6.4	6.4	n.a.	n.a.	1.4	3.3

[a] Inhibitory potentials of compounds at various concentrations were determined in biochemical protein kinase activity assays. Listed are IC_{50} values in μg/mL. n.a.: not active, i.e., $IC_{50} > 10$ μg/mL.

small segments (≈ 1 cm in length). The material was placed on a Petri dish (malt agar medium) containing an antibiotic to suppress bacterial growth (medium composition: 15 g/L malt extract, 15 g/L agar, and 0.2 g/L chloramphenicol in distilled water, pH 7.4−7.8) and incubated at room temperature (25 °C). After several days hyphae growing from the plant material were transferred to fresh plates with the same medium, incubated again for 10 days, and periodically checked for culture purity.

Identification of Fungal Cultures. Fungal cultures were identified according to a molecular biological protocol by DNA amplification and sequencing of the ITS region as described previously.[51] The sequence data have been submitted to GenBank, accession number EU859960. The fungal strain was identified as *Stemphylium globuliferum*. A voucher strain (strain designation DA8) is kept at one of the authors' laboratory (P.P.).

Cultivation. Two Erlenmeyer flasks (1 L each) containing 100 g of rice and 100 mL of distilled water were autoclaved. A small part of the medium from a Petri dish containing the purified fungus was transferred under sterile conditions to the rice medium. The fungal strain was grown on solid rice medium at room temperature (22 °C) for 40 days.

Extraction and Fractionation. The culture was extracted with 300 mL of EtOAc (two times). The EtOAc extract was taken to dryness and partitioned between *n*-hexane and 90% MeOH. Evaporation of the 90% MeOH fraction gave 460 mg of extract, which was chromatographed over a Sephadex LH-20 column with 100% MeOH as solvent. Based on detection by TLC (silica gel F_{254}, Merck, Darmstadt, Germany) using EtOAc−MeOH−H_2O (77:13:10) as solvent system, collected fractions were combined and subjected to semipreparative HPLC (Merck, Hitachi L-7100) using a Eurosphere 100-10 C_{18} column (300 × 8 mm, L × i.d.) with the following gradient (MeOH−H_2O): 0 min, 10% MeOH; 5 min, 10% MeOH; 35 min 100% MeOH; 45 min, 100% MeOH. Yields of compounds were as follows: **1** 5.2 mg, **2** 6.9 mg, **3** 4.2 mg, **4/5** 9.4 mg, **6** 5.2 mg, **7** 3.2 mg, **8/9** 6.2 mg, **10** 3.3 mg, **11** 2.0 mg, **12** 1.5 mg, and **13** 2.0 mg.

Alterporriol G (4) and Alterporriol H (5) (obtained as an inseparable 4:3 mixture): red powder; $[\alpha]_D^{22}$ −316 (*c* 0.05, EtOH); UV λ_{max} (PDA) 222, 288, 420 nm; ^{1}H and ^{13}C NMR in MeOH-d_4, see Table 1; ^{1}H NMR (DMSO-d_6, 400 MHz, signals overlapping if not indicated otherwise) δ 12.69, 12.64 (each 1H, br s, OH-8, OH-8′), 7.98 (1H, s, H-4), 7.57 (1H, s, H-1 in **4**), 7.56 (1H, s, H-1 in **5**), 7.42 (1H, s, H-5 in **5**), 7.38 (1H, s, H-5 in **4**), 6.99 (1H, s, H-7′), 5.62 (1H, br s, OH-4′), 5.03 (1H, br s, OH-1′), 4.85 (1H, br s, OH-2′), 4.49 (1H, d, J = 7.0 Hz, H-1′), 4.41 (1H, br s, OH-3′), 4.12 (1H, s, H-4′), 3.85 (3H, s, OCH_3-6 in **5**), 3.81 (3H, s, OCH_3-6 in **4**), 3.79 (3H, s, OCH_3-6′), 3.59 (1H, d, J = 7.0 Hz, H-2′), 2.30 (3H, s, CH_3-3), 1.16 (3H, s, CH_3-3′ in **4**), 1.15 (3H, s, CH_3-3′ in **5**); ESIMS pos *m/z* 619.1 $[M + H]^+$ (100), ESIMS neg *m/z* 617.7 $[M - H]^-$ (100); HRESIMS *m/z* 641.128 $[M + Na]^+$ (calcd for $C_{32}H_{26}O_{13}Na$, 641.1226).

Altersolanol K (11): yellowish white powder; $[\alpha]_D^{22}$ −57 (*c* 0.4, MeOH); UV λ_{max} (PDA) 218, 271, 331 nm; ^{1}H and ^{13}C NMR, see Table 2; ESIMS pos *m/z* 670.8 $[M + Na]^+$ (100), ESIMS neg *m/z* 323.3 $[M - H]^-$ (100); HRESIMS *m/z* 325.129 $[M + H]^+$ (calcd for $C_{16}H_{21}O_7$, 325.1282).

Altersolanol L (12): yellowish-white powder; $[\alpha]_D^{22}$ −73 (*c* 0.3, MeOH); UV λ_{max} (PDA) 217, 282 nm; ^{1}H and ^{13}C NMR, see Table 2; ESIMS pos *m/z* 325.1 $[M + H]^+$ (100), ESIMS neg *m/z* 323.5 $[M - H]^-$ (100); HRESIMS *m/z* 325.127 $[M + H]^+$ (calcd for $C_{16}H_{21}O_7$, 325.1282).

Stemphypyrone (13): red powder; $[\alpha]_D^{22}$ −82 (*c* 0.01, MeOH); UV λ_{max} (PDA) 229, 330 nm; ^{1}H and ^{13}C NMR, see Table 3; ESIMS pos *m/z* 225.1 $[M + H]^+$ (100), ESIMS neg *m/z* 223.3 $[M - H]^-$ (100); HRESIMS *m/z* 225.112 $[M + H]^+$ (calcd for $C_{12}H_{17}O_4$, 225.1121).

Cell Proliferation Assay. Cytotoxicity was tested against L5178Y mouse lymphoma cells using a microculture tetrazolium (MTT) assay and compared to that of untreated controls as described previously.[52] All experiments were carried out in triplicate and repeated three times. As controls, media with 0.1% EGMME−DMSO were included in the experiments. The depsipeptide kahalalide F isolated from *Elysia grandifolia*[52] was used as a positive control.

Biochemical Protein Kinase Activity Assay. Protein kinase inhibitory activity was determined in 96-well plates as described previously.[53]

Chiral Derivatization. The reaction was performed according to a convenient Mosher ester procedure.[54] Compound **13** (1 mg each) dried under vacuum was dissolved in pyridine-d_5 (0.5 mL) and transferred into a NMR tube. Both (*R*)-(−)-α-(trifluoromethyl)phenylacetyl chloride (MTPA) and (*S*)-MTPA chloride were added separately into NMR tubes immediately under a N_2 stream. The reagent was added to the compound at a ratio of 0.14 mM reagent to 0.10 mM compound. The NMR tubes were shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand at room temperature and was monitored by ^{1}H NMR spectroscopy. The reaction was complete after 72 h. The assignment of the signals was confirmed by COSY.

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Supporting Information Available: Comparison of NMR data of **2**, **3**, and **4/5** in MeOH-d_4. This material is available free of charge via the Internet at http://pubs.acs.org.

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