Pochonins A−F, New Antiviral and Antiparasitic Resorcylic Acid Lactones from *Pochonia chlamydosporia* var. *catenulata*

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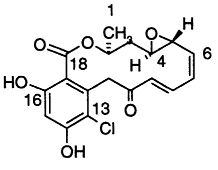
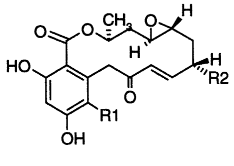
Monorden (**1**) and the novel resorcylic acid lactones pochonins A (**2**), B (**4**), C (**6**), D (**7**), and E (**8**) as well as tetrahydromonorden (**5**) and pseurotin A (**22**) were isolated from cultures of the clavicipitaceous hyphomycete *Pochonia chlamydosporia* var. *catenulata* strain P 0297. Fermentation of P 0297 in bromide-containing culture media led to a shift in secondary metabolite production and yielded monocillins III (**3**) and II (**9**) as major metabolites besides monorden (**1**) as well as the novel compounds pochonin F (**10**) and a monocillin II glycoside (**11**) as minor metabolites. Most of these compounds showed moderate activities in a cellular replication assay against Herpes Simplex Virus 1 (HSV1) and against the parasitic protozoan *Eimeria tenella*. In contrast to the structurally related zearalenone derivatives none of the metabolites of strain P 0297 were found to be active in a fluorescence polarization assay for determination of modulatory activities on the human estrogenic receptor ERβ. β-Zearalenol (**17**), but not zearalenone (**15**) and α-zearalenol (**16**), showed antiherpetic effects. We report the production, isolation, and structure elucidation of compounds **1**−**11** and their biological characterization.

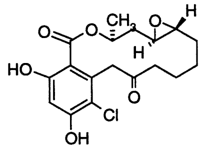
The vast majority of the world population is infected by at least one member of the human herpes virus family. These viruses remain latent in their hosts after primary infection and may be reactivated from a pool of latently infected cells upon diverse internal and external stimuli, thus causing serious symptoms. Since the late 1970s, herpes virus infections have been mostly treated with nucleoside antibiotics such as acyclovir (Zovirax). Developing resistance to these drugs and increasing numbers of immunocompromised patients have created medical need for novel antivirals. This has led to high-throughput based screening (HTS) approaches, which recently resulted in the development of nonnucleosidic antiviral agents that act on the helicase-primase in Herpes Simplex Virus (HSV).[1] With the same HTS system, microbial extracts were screened for potent antiherpetic metabolites. In the following we wish to report the isolation and biological and physicochemical characterization of a new group of biologically active metabolites **1**−**11** from the fungal strain P 0297.



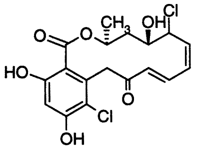
Monorden (**1**)



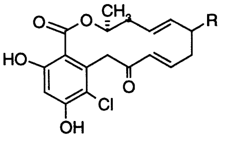
R¹ = Cl, R² = H: Pochonin A (**2**)
R¹ = H, R² = H: Monocillin III (**3**)
R¹ = Cl, R² = OH: Pochonin B (**4**)



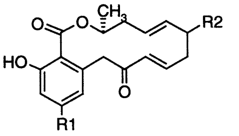
Tetrahydromonorden (**5**)



Pochonin C (**6**)



R = H: Pochonin D (**7**)
R = OH: Pochonin E (**8**)



R¹ = H, R² = H: Monocillin II (**9**)
R¹ = H, R² = OH: Pochonin F (**10**)
R¹ = : R² = H: Monocillin II glycoside (**11**)

Results and Discussion

Shake flask fermentations of P 0297, *Pochonia chlamydosporia* var. *catenulata* (Kamyschko ex Barron & Onions) Zare & W. Gams (see Figure 1), were carried out in the culture media YMG, Q6/2, MGP, and MGP-Br. Aliquots of the culture broth were analyzed during the fermentation course for determination of growth parameters, estimation of secondary metabolite production, and biological activities (Figure 2) in order to harvest the fermentors at the optimal production rate and pH value regarding the instability of the bioactive metabolites in alkaline medium. MGP (and later MGP-Br) were chosen for scale-up in 10 L fermentors due to slightly higher production rates in this medium compared with Q6/2 and YMG. The isolation procedures using reversed-phase (RP) HPLC and, if necessary, subsequent high-performance gel-permeation chromatography (HPGPC) are summarized in Table 1. From stirring fermentors and corresponding shake flask fermentation carried out in the same media, similar production rates of the resorcylic acid lactones (RAL) **1**−**11** were obtained.

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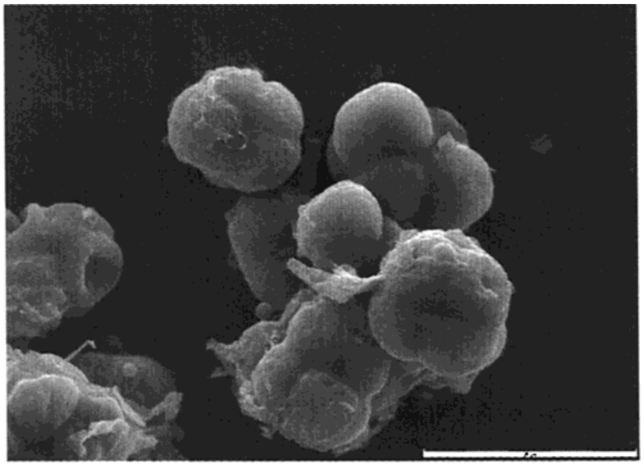

Figure 1. *Dictyochlamydospores* of *Pochonia chlamydosporia* var. *catenulata*. SEM micrograph of strain P 0297 (YMG, 500×).

Table 1. Isolation of **1**−**11** and **22**

		retention time t_R [min]		
	preparation[a]	system A RP-HPLC small scale	system B RP-HPLC large scale	system C HPGPC
1	all media[b]	78−80	60−72	36−39
2	all media[c]	84−86	74−76	−[d]
3	MGP-Br[c]	74−75	77−79	−
4	all media[c]	59−60	42−45	−
5	MGP or MGP-Br[b]	−	80−85	34−35
6	all media[b]	−	58−59	29−32
7	all media[b]	−	125−129	39−40
8	all media[c]	64−66	52−58	−
9	MGP-Br[c]		101−111	−
10	MGP-Br[b]	−	69−74	19−24
11	MGP-Br[b]	−	60−62	23−30
22	Q6/2[c]	53−55	−	−

[a] If not indicated otherwise, all culture media described in the Experimental Section are suited to produce the respective compounds. [b] Purification by RP-HPLC followed by HPGPC. [c] RP-HPLC in either system A or B (see Experimental Section) yielded the pure compounds at the indicated t_R. [d] −: Method not employed for purification.

The main metabolite was produced in yields of 15−20 mg/L and identified by LC-MS and ^{1}H NMR data as monorden (**1**). This well-known RAL, also named as radicicol in the literature, had previously been reported from various ascomycetes,[2−5] including *Pochonia chlamydosporia* (albeit under the synonym "*Verticillium chlamydosporium*").[6]

The bioassay-guided isolation procedure yielded several bioactive co-metabolites of monorden (**1**), which were produced in minor quantities (1−2 mg/L, less than 1 mg/L for **5**) and differ in the substitution pattern of the 14-membered macrocyclic lactone ring.

Pochonin A (**2**) represents a dihydro derivative of monorden (**1**) due to its molecular formula $C_{18}H_{19}ClO_6$ from HREIMS. Indeed, the ^{1}H NMR signals (Table 2) for only one double bond in conjugation to the carbonyl moiety (δ_H 6.06, δ_H 6.92) are detected. Due to corresponding ^{1}H−^{1}H COSY correlation signals, the two additional methylene groups (δ_H 1.20−1.25, 2.20−2.29 and δ_H 2.20−2.29, 2.38) are located between the epoxide and the α,β-unsaturated carbonyl moiety. Pochonin A (**2**) differs only in the halogen substitution from monocillin III (**3**).[7]

Pochonin B (**4**), $C_{18}H_{19}ClO_7$, contains an additional oxygen atom in comparison to pochonin A (**2**). The ^{1}H NMR signals (Table 2) for a secondary alcohol function at δ_H 4.26 (δ_C 68.04; OH: δ_H ca. 5.3) as well as its ^{1}H−^{1}H COSY correlation to the β-H atom of the α,β-unsaturated carbonyl indicate formula **4** for pochonin B. ^{1}H NMR signals for a diastereotopic methylene group at δ_H 1.15, 2.36 (δ_C 39.98) are assigned to position 6 between the secondary alcohol function and the epoxide ring.

Compound **5** exhibits a molecular ion peak at *m/z* 368 for $C_{18}H_{21}ClO_6$ and represents a tetrahydro derivative of monorden (**1**). No signals other than the one for H-15 are detected in the low-field region of the ^{1}H NMR spectrum, but additional signals for a $(CH_2)_4$ moiety appear in the aliphatic shift region. The natural product is identical with the semisynthetic tetrahydromonorden **5** (see below).[2,3]

For pochonin C (**6**) the HRMS of the molecular peak at *m/z* 400 with a characteristic isotopic pattern for two chlorine atoms resulted in the molecular formula $C_{18}H_{18}Cl_2O_6$. The ^{1}H NMR spectrum (Table 2) contains the signals for the Michael system as in monorden (**1**), but lacks those for the *trans* epoxide ring. Instead a signal for a secondary hydroxyl function at δ_H 3.98 (δ_C 70.70; OH; δ_H ca. 5.45)

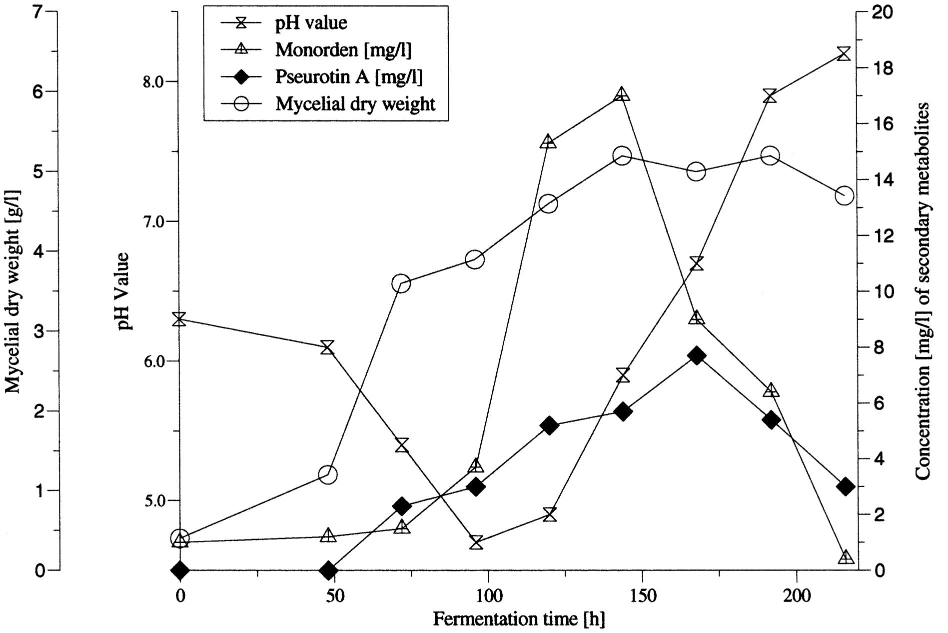

Figure 2. Time course of production of monorden (**1**) and pseurotin A (**22**) in Q6/2 medium (150 mL shake flasks) by strain P 0297.

Table 2. ^{1}H NMR Data [500 MHz] of Pochonins A−C (**2**, **4**, **6**) in [D$_6$]DMSO

	2			**4**			**6**		
proton	δ_H [ppm]		J_{HH} [Hz]	δ_H [ppm]		J_{HH} [Hz]	δ_H [ppm]		J_{HH} [Hz]
Me-1	1.31	d	6.3	1.31	d	6.4	1.37	d	6.1
2	5.12	m		5.13	m		5.28	m	
3	1.77	m		1.77	m		H$_a$: 1.91	m	
							H$_b$: 1.83	m	
4	2.80	m		2.80	m		3.98	m	
							OH: 5.45	d	5.0
5	2.59	dm	8.6	2.60	dm	10.2	5.10	dd	10.5, 5.0
6	α: 1.20−1.25	m		α: 1.15	ddd	12.1, 11.4, 10.2	5.78	dd	10.5, 10.5
	β:2.20−2.29	m		β: 2.36	dm	12.1			
7	H$_a$: 2.20−2.29	m		4.26	m		6.26	dd	10.5, 10.5
	H$_b$: 2.38	m		OH: 5.34[a]	d	3.5			
8	6.92	ddd	15.6, 10.2, 5.0	6.77	dd	15.9, 8.1	7.12	dd	16.1, 11.2
9	6.06	d	15.6	6.10	d	15.9	6.04	d	16.1
10									
11	H$_a$: 4.17	d	17.5	H$_a$: 4.18	d	17.7	H$_a$: 4.04	d	16.0
	H$_b$: 4.08	d	17.5	H$_b$: 4.07	d	17.7	H$_b$:3.61	d	16.0
14	OH: 10.81[a]	s, br		OH: 10.81	s		OH: 10.12[b]	s	
15	6.52	s		6.52	s		6.56	s	
16	OH: 10.73[a]	s		OH: 10.81	s		OH: 10.57[b]	s	

[a] Signals may be interchanged. [b] δ_H dependent on concentration.

Table 3. ^{1}H NMR Data [500 MHz] of Pochonins D−E (**7**, **8**) in [D$_6$]DMSO

	7			**8**		
proton	δ_H [ppm]		J_{HH} [Hz]	δ_H [ppm]		J_{HH} [Hz]
Me-1	1.23	d	6.2	1.23	d	6.1
2	5.00	m		4.98	m	
3	H$_a$: 2.32	dm	14.4	H$_a$: 2.33−2.40	m	
	H$_b$: 2.19	m[a]		H$_b$: 2.10−2.23[b]	m	
4	5.21	m		5.30	ddd	15.6, 8.9, 8.9
5	5.21	m		5.23	dd	15.6, 6.5
6	2.21−2.01	m		4.00	m	
				OH: 5.03	d	4.1
7	2.21−2.01	m		H$_a$: 2.33−2.40	m	
				H$_b$: 2.06−2.17[b]	m	
8	6.61	ddd	15.5, 7.4, 7.4	6.60	ddd	15.8, 7.9, 7.9
9	5.78	d	15.5	5.78	d	15.8
11	H$_a$: 3.86	d	17.1	H$_a$: 3.87	d	17.2
	H$_b$: 3.70	d	17.1	H$_b$: 3.71	d	17.2
12						
13						
14	OH: 10.64[c]	s br		OH: 10.63[c]	s	
15	6.53	s		6.52	s	
16	OH: 10.28[c]	s		OH: 10.23[c]	s	

[a] Signal hidden by signal of 6-CH$_2$ and 7-CH$_2$. [b] Overlapping signals. [c] Signals may be interchanged.

shows ^{1}H−^{1}H COSY correlation signals to the methylene group in position 3 at δ_H 1.91 and 1.83 as well as to a secondary methine proton at δ_H 5.10 (δ_C 60.26). The latter shows ^{1}H−^{1}H COSY correlation signals to the proton δ_H 5.78 in the δ position of the Michael system. Therefore, formal ring opening of the epoxide ring yields a chlorhydrin functionality in **6**. This structure is already part of a patent describing tyrosine kinase inhibitors, but it is described there as a semisynthetic derivative of monorden (**1**).[8]

For two further metabolites the ^{1}H NMR signals (Table 3) corresponding with the epoxide ring are missing. Instead, signals for olefin protons in position H-4 and H-5 at δ_H 5.2−5.3 and the corresponding ^{1}H−^{1}H COSY correlation signals are detected. Pochonin D (**7**), with the molecular formula $C_{18}H_{19}ClO_5$, contains one oxygen less in comparison with pochonin A (**2**). Pochonin E (**8**), with the molecular formula $C_{18}H_{19}ClO_6$, is a formal oxidation product of pochonin D (**7**). In the ^{1}H NMR spectrum there are signals for a secondary alcohol function (δ_H 4.00, 5.03 (OH)) in position 6, in agreement with a ^{1}H−^{1}H COSY correlation signal from δ_H 4.00 to δ_H 5.23 of the isolated double bond.

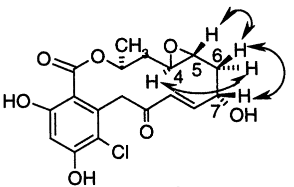

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Figure 3. Selected NOESY correlation signals of pochonin B (**4**) (500 MHz, [D$_6$]DMSO).

The three asymmetric carbon atoms in monorden (**1**) were determined to have *R* configurations from single-crystal X-ray diffraction through an analysis of the anomalous scattering.[9] The sample from strain P 0297 has the same absolute configuration, as evidenced by its optical rotation. The stereochemistry of pochonins A (**2**), B (**4**), C (**6**), D (**7**), and E (**8**) is proposed to be analogous to that of monorden (**1**). The relative configuration of the secondary alcohol function in pochonin B (**4**) was assigned by a NOESY experiment (see Figure 3). The *trans* epoxide ring as described for monorden (**1**) is assumed due to the very weak NOESY correlation signal between the two protons H-4 and H-5. H-4 shows a NOESY correlation signal to H-6α at δ_H 1.15, while H-5 correlates with the diastereotopic H-6β at δ_H 2.36. The methine proton of the secondary alcohol function at C-7 shows strong NOESY correlation signals to H-5 and to H-6β at δ_H 2.36, but only a weak one to H-6α at δ_H 1.15. Due to the lack of the epoxide ring, pochonin C (**6**) as well as E (**8**) are too flexible to enable determination of the relative configuration of their secondary alcohol function by NOESY correlation spectroscopy.

The pochonins obtained by fermentation of P 0297 with standard conditions have a chlorine substitution in the aromatic nucleus in common. Considering previous results on the influence of halogen salts to increase the metabolic diversity in fungi by fermentation,[10,11] the MGP medium was thus supplemented with bromide salts in substitution for the chloride salts in the standard protocol (see Experimental Section). While monorden (**1**) still constituted a main component in MGP-Br medium, some further biologically active compounds, **3**, **9**, **10**, and **11**, were isolated as described in the Experimental Section and Table 1, which could not be detected in bromide-free culture media.

NMR and MS data (see Experimental Section) identified dechloro analogues of monorden and the pochonins, some

Table 4. ^{13}C NMR Data [125 MHz] of Monorden (**1**), Pochonins A−D (**2**, **4**, **6**, **7**), and Tetra- (**5**) and Hexahydromonorden (**12**) in [D$_6$]DMSO

	δ_C [ppm][a]						
carbon	**1**	**2**	**4**	**5**	**12**	**6**	**7**
1	18.52	17.76	17.51	19.97	20.49	18.91	19.01
2	69.82	70.66	70.98	70.30	69.12	69.11	71.48
3	36.46	36.63	36.31	37.36	43.66	37.30	37.89
4	55.08	54.40	54.58	54.95	63.77	70.70	127.74[b]
5	55.08	55.84	53.74	56.72	32.95	60.26	131.47[b]
6	136.13	29.90	39.98	29.15	22.84	136.51	30.57
7	129.78	27.81	68.04	23.18	25.28	129.51	30.57
8	138.02	148.70	149.75	23.18	23.06	139.09	147.49
9	130.67	129.95	127.08	39.77	40.91	132.04	128.69
10	195.92	195.66	195.88	206.65	205.54	196.98	195.25
11	45.11	42.90	43.84	44.90	43.76	44.38	44.44
12	131.93	134.33	134.11	132.37	132.72	133.11	133.59
13	115.43[b]	113.61[b]	113.37[b]	115.04[b]	114.64[b]	115.02[b]	114.41[b]
14	155.19	157.97[b]	158.38[b]	154.93[b]	154.69[b]	155.27[b]	156.74[b]
15	102.85	102.70	102.80	102.51	102.47	102.59	103.00
16	155.19	156.73[b]	156.69[b]	155.24[b]	155.48[b]	155.39[b]	155.71[b]
17	111.98[b]	111.55[b]	111.12[b]	112.28	112.57[b]	112.10[b]	112.90[b]
18	166.61	168.09	167.94	167.56	167.36	166.39	167.90

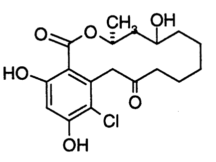
[a] The ^{13}C NMR shifts are deduced from the HSQC and HMBC correlation spectra. [b] Signals may be interchanged.

of which were described as monocillins from *Monocillium nordinii*.[7] Monocillin III (**3**) is the dechloro analogue of pochonin A (**2**) and monocillin II (**9**) that of pochonin D (**7**).

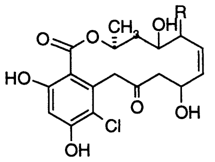
The LCMS data of the dechloro analogue **10** of pochonin E (**8**) show a [M + H]$^+$ peak at *m*/*z* 333, and its ^{1}H NMR data (Table 5) are in good agreement with that of pochonin E (**8**) concerning the 14-membered lactone ring. As in the other monocillins, two aromatic signals for protons in meta position to each other (δ_H 6.07 and 6.20) are detected. The new compound is called pochonin F (**10**).

Compound **11** constitutes a novel glycoside of monocillin II, as the spectroscopic data of the aglycone moiety are almost identical to that of monocillin II (**9**). Only one ^{1}H NMR signal for a phenolic proton is detected at δ_H 10.25, which is located at position 16 due to its HMBC signals to C-15 (δ_C 102.62), C-16 (δ_C 158.18), and C-17 (δ_C 114.00). The remaining NMR signals (Table 5) belong to a aldofuranose moiety linked to position 14, as evidenced by its HMBC signal from the anomeric proton H-1′ (δ_H 5.47) to the quaternary carbon C-14 (δ_C 159.27). Exact determination of the sugar was not performed due to the limited amount of sample available.

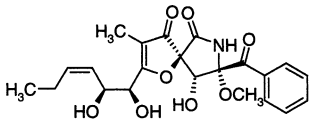
Monorden (**1**) has been shown to be unstable in alkali.[3] Hydrogenation of monorden (**1**) with H$_2$/Pd−C under atmospheric pressure yielded two products: tetrahydromonorden (**5**), with an intact oxirane ring, and hexahydromonorden (**12**), with a secondary alcohol function from oxirane ring opening.[2,3] Microscale epoxide ring opening of monorden (**1**) with HCl in methanol yielded **13** and **14** as main products.



Hexahydromonorden (**12**)



R = Cl: **13**
R = OH: **14**



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In the HSV1 replication assay,[1] monorden (**1**) showed inhibitory activity in the nM range accompanied by cyto-

Table 5. ^{1}H and ^{13}C NMR Data [500 and 125 MHz] of **10** and **11**

	10				**11**			
pos	δ_C [ppm][a]	δ_H [ppm]		J_{HH} [Hz]	δ_C [ppm][a]	δ_H [ppm]		J_{HH} [Hz]
1	19.39	1.25	d	6.1	19.34	1.28	d	6.6
2	70.79	5,11	m		71.29	5.08	m	
3	37.90	H$_a$: 2.39−2.47	m		38.06	H$_a$: 2.39	dm	14.9
		H$_b$: 2.20	ddd	16.1, 7.0, 7.0		H$_b$: 2.21[b]	m	
4	126.87	5.42	ddd	15.6, 9.9, 4.8	131.08[c]	5.35[c]	m	
5	136.47[b]	5.30	dd	15.6, 6.9	128.47[c]	5.33[c]	m	
6	71.01	4.01	m		30.34	H$_a$: 2.23[b]	m	
		OH: 5.01	d	3.9		H$_b$: 2.02	m	
7	40.03	H$_a$: 2.39−2.47	m		30.05	H$_a$: 2.23[b] H$_b$: 2.13	m	
		H$_b$: 2.12	m				m	
8	146.03	6.54	ddd	15.7, 7.6, 7.6	n.d.	6.68	ddd	15.7, 7.4, 7.4
9	131.20	5.88	d	15.7	129.92	5.91	d	15.7
10	197.12				196.43			
11	44.90	H$_a$: 3.94	d	14.9	44.38	H$_a$: 3.94[b]	m	
		H$_b$: 3.45	d	14.9		H$_b$: 3.47[b]	m	
12	136.47[b]				135.53			
13	108.35	6.07	s		108.82	6.31	d	2.0
14	160.33	OH: 9.84	s		159.27			
15	101.56	6.20	s		102.62	6.50	d	2.0
16	159.40	OH: 10.19	s		158.18	OH: 10.25	s	
17	111.16				114.00			
18	168.75				n.d.			
1′					100.09	5.47	d	4.8
2′					71.50	4.04	ddd	9.1, 5.0, 5.0
						OH: 4.70	d	9.1
3′					69.31	3.89[b] OH: 4.91	m	
							d	5.05
4′					86.39	3.93[b]	m	
5′					61.41	3.45[b] OH: 4.80	m	
							t	5.5

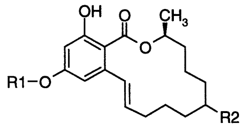
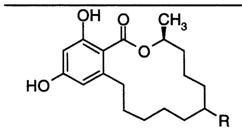
[a] The ^{13}C NMR shifts are deduced from the HSQC and HMBC correlation spectra. [b] Overlapped signals. [c] Signals may be interchanged.

Table 6. Antiviral (HSV1-F) Effects and Antiparasitic (versus *E. tenella*) Activities of **1**−**11** and **22**

	antiviral (HSV 1)[a]		antiparasitic (*E. tenella*)[b]	
	IC$_{50}$ [μM]	SI	10 ppm	1 ppm
1	0.2−0.8	n.d. (T)	2/T	0
2	2	8	2	1
3	0.4	SI 7.5	1	n.d.
4	10	8	T	n.d.
5	1.5	12	2	0
6	6	15	T	n.d.
7	not active	T (2.5 μM)	T	n.d.
8	1.5	6	T	n.d.
9	not active	T (5 μM)	2/(T)	n.d.
10	2	SI 10	2/(T)	n.d.
11	T (10 μM)		T	n.d.
22	50	(SI 6)	2	0

[a] Concentrations tested in cellular replication assay using Vero cells:[1] 0.1 nM to 125 μM; SI: selectivity index = Tox$_{50}$/IC$_{50}$; T: cytotoxic. [b] Concentrations tested against *E. tenella* grown in PCKC cells:[37,38] 200, 10, 1, and 0.1 ppm; 2: full activity, 1: weak activity; 0: no activity, T: cytotoxic. *N. caninum* was not sensitive.

Table 7. Antiviral (HSV1−F) Effects and Agonistic Effects against the the ERβ Receptor of Zearalenone (**15**) and Analogues **16**−**21**

Compound[a]		Antiviral[b] (HSV 1)		Erß[c]
		IC$_{50}$	SI	EC$_{50}$
	**15**: Zearalenone R^1 = H; R^2 = =O	not active		110 nM
	16: α-Zearalenol R^1 = H; R^2 = α−OH	not active		38 nM
	17: β-Zearalenol R^1 = H; R^2 = β−OH	1.5 μM	> 40	220 nM
	18: Methyl-β-zearalenol R^1 = CH$_3$; R^2 = β−OH	not active		inactive
	**19**: Zearalanone R = =O	not active		660 nM
	20: α-Zearalanol R = α−OH	not active		24 nM
	21: β-Zearalanol R = β−OH	15 μM	> 8	72 nM

[a] None of the compounds were active against *E. tenella* and *N. caninum*. [b] Concentrations tested in cellular replication assay using Vero cells:[1] 0.1 nM to 125 μM. [c] Concentrations tested: 10 nM to 10 μM.

static effects. The pochonins (**2**−**5**) with epoxide moieties exhibited bioactivities in the low μM range, while hexahydromonorden (**12**), with a secondary alcohol function, was found devoid of activity (Table 6). Probably, the chlorine substituent is not essential for the antiviral activity, as monocillin III (**3**) showed inhibitory activity in the nM range. Pochonin D (**7**) and monocillin II glycoside (**11**), which contain a double bond instead of the epoxide ring, showed only cytostatic effects. For those pochonins with an allyl alcohol moiety (**8**, **10**) moderate inhibitory activity was determined. Inhibition of HSV was generally accompanied by weak cytostatic effects on the host cell, as observed microscopically (Table 6). This results in rather low tolerability in vitro described by the selectivity indices (SI, i.e., the ratio of Tox$_{50}$/IC$_{50}$ with IC$_{50}$ or Tox$_{50}$ for the concentration at which a drug reduced 50% of the cytopathic effects of viral replication or cell viability).

Tanaka et al. had described antimalarial in vivo effects for monorden (**1**) and discussed the heme-dependent radical generation in *Plasmodium falciparum* as a possible mode of action.[12−14] Therefore we evaluated the antiparasitic potential of the pochonins and the effectiveness of monorden (**1**) against economically important animal parasites. Compounds **1**−**11** were tested against *Eimeria tenella* and *Neospora caninum*, both of which are taxonomically related to *Plasmodium*. While *N. caninum* was not susceptible, the partly hydrogenated pochonins containing an epoxide function showed moderate activities against *E. tenella*. Among these, pochonin A (**2**) and tetrahydromonorden (**5**) exhibited the best selectivity toward the parasite (Table 6). Recently, Isaka et al. reported moderate antimalarial activity of the resorcylic acid derivative hypothemycin and the related aigialomycins from the marine mangrove fungus *Aigialus parvus*.[15]

Among the several other biological effects reported for monorden (**1**) are cytotoxic activities. As a result, the use of monorden (**1**) and semisynthetic derivatives as anticancer agents was therefore evaluated extensively in past years. Compound **1** is known to inhibit tyrosin kinases[16] and to induce the differentiation of HL-60 cells in the 100 nM range, probably addressing this target.[17] Recently, a semisynthetic derivative of monorden was reported to exhibit tyrosin kinase inhibition at 20 nM (as compared to 370 nM for **1**) and was patented as an anticancer agent.[18] Monorden and related compounds were also proven to modulate MAP kinase activity, thus acting as promoters of nerve regeneration in vitro.[19] Moreover, inhibitory effects of monorden and derivatives on cytokine (particularly interleukin 1) release were described.[20] Recently, the biochemical mode of action of the antagonistic effects of monorden against the heat shock protein HSP90 was elucidated.[21] The use of monorden (**1**) as an antiinflammatory agent was claimed as well.[22] These previous results indicated the potential usefulness of monorden and related metabolites as anticancer agents and in a wide range of further pharmaceutical indications. Its antiviral activities and effects on coccidial parasites other than *Plasmodium*, however, had not been noted previously.

Various other resorcylic acid lactones had previously been obtained from fungi; that is, the zearalenones are well-known estrogenic mycotoxins from several *Fusarium* spp.[23] These compounds act on the human estrogen receptor in competition with 17-estradiol.[24] In our own studies, zearalenone (**15**), both stereoisomers of zearalenol (**16**, **17**), and semisynthetic derivatives from hydrogenation, methylation, and acetylation were studied in comparison with the metabolites of P 0297.[25−27] Zearalenone (**15**) and α-zearalenol (**16**) and their derivatives did not exhibit any activity against HSV1, while β-zearalenol (**17**) was the most potent among all compounds examined in this study (Table 7). Hydrogenation of β-zearalenol (**17**) to β-zearalanol (**21**) resulted in decreased activities, while the methylation and acetylation products of **17** were inactive. However, the HSV1 inhibition of these zearalenone type compounds was not accompanied by any cytostatic effects. The structure−activity relationships (SAR) of the antiviral properties of these zearalenols are not parallel to their estrogenic properties (Table 7). However, neither of the zearalenone derivatives were found effective against the parasites.

On the other hand, all pochonins, the derivatives of monorden, and the monocillins were inactive against the ERβ receptor up to 10 μM, suggesting that other determinants are also relevant for the estrogenic activity, e.g., the lactone chain of RAL and not only the aromatic substitution pattern,[23] which is identical at least in the monocillins and zearalenone derivatives.

In conclusion, these results suggest that neither the antiviral nor the antiparasitic modes of action of RAL are related to their effects on the estrogenic receptor. The antiherpetic activity of β-zearalenol derivatives may thus be coincidental. Unfortunately, β-zearalenol (**17**) seems to be an inappropriate antiviral lead structure because of the estrogenic side effects and the rather narrow SAR observed upon preliminary studies. Concerning monorden (**1**) and the pochonins, it was not possible to retain the strong bioactivities of monorden and reduce the cytostatic effects at the same time. The antiparasitic and antiviral target sites of the metabolites of P 0297 may be different from one another as well. Multiple targets possibly are addressed by these fungal metabolites.

During our investigations, the spirocyclic alkaloid pseurotin A (**22**) was found to be a main metabolite in most of the *P. chlamydosporia* isolates examined when propagated in Q6-medium. Compound **22** was isolated and also subjected to all biological investigations performed with the pochonins. Pseurotin A (**22**) had been obtained previously from other fungi such as *Pseudeurotium ovalis*[28] and, repeatedly, from *Aspergillus fumigatus*.[29]

The distribution of the pochonins in submerged cultures of several *Pochonia* species and further conidial fungi was studied with HPLCUV and LCMS. Until recently, these fungal species with verticillium-like anamorphs were all included in *Verticillium* sect. *Prostrata*. The results from these chemotaxonomical evaluations support a recent generic segregation by Zare et al.,[30] as the pochonins were found to occur exclusively in species of the genus *Pochonia*. With few exceptions, the production of pochonins and monorden appears to be a rather constant feature in cultures of *P. chlamydosporia* from around the world. The chemotaxomical investigations as well as results from minisatellite PCR fingerprinting studies on *Pochonia* and other conidial fungi with verticillium-like anamorphs are reported concurrently.[31]

Experimental Section

General Experimental Procedures. Unless stated otherwise, culture media ingredients and solvents for chromatography and spectroscopy were obtained from Merck (Darmstadt, Germany) and all solid chemicals, including zearalenone and zearalenols, from Sigma-Aldrich (Deisenhofen, Germany).

HPLCUV characterization of the natural products and semisynthetic derivatives was performed with system 1[32,33] and system 2, respectively. System 2 was carried out using a HP 1090 (Agilent, Waldbronn, Germany) unit with automated sample injector, diode array detector, and Sedex 55 light scattering detector (ERC, Alteglofsheim, Germany), employing the following instrumental conditions: column, Eurospher-100 C_{18}, 5 μm; 2 × 125 mm (Knauer); eluant A, water + 0.05% TFA; eluant B, ACN + 0.05% TFA; gradient 0 min 10% B; 1 min 10% B; 15 min 100% B; 17 min 100% B; 18 min 0% B; flow 0.4 mL/min; temperature, 40 °C. UV spectra were recorded from 200 to 400 nm.

HPLCMS was performed using the following conditions. System 3: HP 1100 liquid chromatograph (Agilent, Waldbronn, Germany) directly coupled with a Micromass Quattro LCZ mass spectrometer (Micromass, Manchester, UK) in the positive electrospray ionization (ESIpos) mode with the following instrumental conditions: column, Symmetry-C18, 3.5 μm; 2.1 × 50 mm (Waters); eluant A, H_2O + 0.1% HCOOH; eluant B, ACN + 0.1% HCOOH; gradient 0 min 10% B; 4 min 90% B; 6 min 90% B; 6.1 min 10% B, 7.5 min 10% B; flow 0.5 mL/min; temperature, 40 °C. MS parameters: scan speed, 1.0 s/decade against scan; scan range, 100−1200; heated capillary temperature, 120 °C. System 4: TSP liquid chromatograph directly coupled with a MAT 900S mass spectrometer (Finnigan, Bremen, Germany) in the ESIpos mode with the following instrumental conditions: column, Symmetry-C_{18}, 5 μm; 2.1 × 150 mm (Waters); eluant A, 0.01 M HCl, eluant B, ACN; gradient 0 min 10% B; 9 min 90% B; 18 min 90% B; flow 0.6 mL/min; temperature, 50 °C. MS parameters: scan speed, 1.5 s/decade against scan; scan range, 150−1200; resolution, 2000; capillary voltage, 4.750 V; identity of nebulizer gas, N_2 99.999%; nebulizer gas pressure, 5 bar; heated capillary temperature, 220 °C. System 5: HP 1100 liquid chromatograph directly coupled with a Micromass-LCT mass spectrometer in ESIpos. and ESIneg. mode with the following instrumental conditions: column, Symmetry-C_{18}, 3.5 μm; 2.1 × 50 mm (Waters); eluant A, H_2O + 0.1% HCOOH; eluant B, acetonitrile + 0.1% HCOOH; gradient 0 min 0% B; 1 min 0% B 10% B; 5 min 90% B; 6 min 90% B; 6.1 min 100% B; flow 0.5 mL/min; temperature, 40 °C. MS parameters: source temperature, 100 °C; desolvation temperature, 200 °C; capillary voltage, 3.2 kV; gas flow (total), 550l/h; nebulizer gas, N_2; flow, 3l/h; resolution, 4000; scan time, 1 s; scan range, 100−1200.

NMR spectra were recorded at 300 K with a Bruker DRX 500 spectrometer (500 MHz) with the solvent peak as internal reference (CD_3OD: δ_H 3.35, δ_C 49.0; [D_6]DMSO: δ_H 2.50, δ_H 39.8). Direct ($^1J_{CH}$) and long-range $^1H-^{13}C$ connectivity were determined by inverse experiments using gradient pulses. The HMBC experiment was optimized for a coupling constant of 10 Hz ($D_6 = 0.05$ s).

Biological Material. P 0297 was isolated from a sample of plant debris collected in Germany. A voucher specimen of the fungal strain is kept under liquid N_2 in 10% glycerol at the Bayer Pharma Research Center, Wuppertal, Germany. Morphological studies, DNA extraction, and sequencing of the nuclear ribosomal DNA (nrDNA) were carried out according to the literature,[30] while SEM was performed according to the previously published procedure.[34] Morphological studies showed the production of chlamydospores (Figure 1) in abundance besides typical verticillate conidiophores producing phialioconidia, corresponding to species of *Verticillium* sect. *Prostrata*.[35] According to the currently valid taxonomy, P 0297 keyed out with *Pochonia chlamydosporia* var. *catenulata* (Kamyschko ex Barron & Onions) Zare & W. Gams. Sequence data of the ITS/5.8S nrDNA region of P 0297 showed 100% identity to the sequence AJ292398 (deposited as *Verticillium chlamydosporium* var. *catenulatum* in EMBL[36]) of CBS 504.66, the *ex*-type strain of this variety. The correspondence of P 0297 with this taxon was further confirmed by extensive morphological, PCR, and HPLC profiling studies on related strains.[31]

Fermentation, Extraction, and Isolation. Small-scale fermentations of P 0297 were carried out by adding 2 mL of a 10% glycerol culture to 500 mL Erlenmeyer flasks containing 150 mL of Q6/2 (D-glucose 0.2%, glycerol 1%, cotton seed meal 0.5%; tap water 1 L, pH 7.2), YMG medium (yeast extract 0.4%, malt extract 1%, glucose 0.4%, tap water 1 L, pH 6.3), or MGP.[10] In further experiments MGP was also supplemented with 100 mM $CaBr_2$ as previously described.[11] Free glucose in the culture media was estimated by "Diastix Harnzuckerstreifen" (Bayer Diagnostics, Munich, Germany), and the pH was determined. The flasks were sterilized at 1 bar and 121 °C for 30 min. The cultures were propagated in the dark on a rotary shaker at 140 rpm. Daily samples (25 mL) of the broth were withdrawn under sterile conditions and extracted with ethyl acetate (EtOAc) for 30 min in an ultrasonic bath. The organic phases were dried over Na_2SO_4 and evaporated to dryness under reduced pressure, and aliquots thereof were used for biological assays and HPLC analyses.[37] The shake cultures were harvested after 7 or 8 days, freeze-dried, and extracted with MeOH in an ultrasonic bath. The residue was resuspended in 1 L 1:1 H_2O/EtOAc, and the organic layers were dried over Na_2SO_4 and evaporated in vacuo. Fermentations in 10 flasks each yielded 500 mg of crude product from Q6/2, 450 mg from YMG, and 600 mg from MGP medium. These crude extracts were subjected in portions of 100−200 mg to preparative HPLC (system A) as described below.

Fermentations in 10 L scale were carried out using a 10 L Braun (Melsungen, Germany) Biostat E stirring fermentor with aeration (3 L/min) and agitation (100 rpm) at 23 °C. The culture media were sterilized in situ for 30 min at 121 °C with steam. The cultures were inoculated with 300 mL of well-grown YMG shake cultures propagated for 72−96 h, and 3 mL of Sigma Antifoam A was added to the medium to avoid extensive foaming. Daily samples were taken and analyzed as described above. The cultures were harvested after 168 h. After separation from the fluid by filtration, the wet mycelium was extracted with acetone in an ultrasonic bath. The aqueous residue was diluted and extracted with EtOAc, and the organic layer was dried over Na_2SO_4 to yield an oily residue (5 g from 10 L of culture fluid). The culture filtrate was applied onto a column packed with 500 g of Bayer Lewapol CA 9225 resin. The resin was rinsed with H_2O (5 L) and subsequently eluted with acetone (6 L). The organic eluates were extracted with EtOAc to yield 4.5 g of crude product. The crude products were subjected to preparative HPLC (system B, see below) in portions of 1 g each.

HPLC system A was used for small scale: column, LichroSorb RP18, 7 μm; 25 × 250 mm (Merck Darmstadt, Germany); eluant A, H_2O; eluant B, acetonitrile; gradient, 0 min 20% B; 15 min 20% B; 35 min 55% B; 50 min 55% B; 70 min 100% B; flow 9 mL/min. System B was used for large scale: column, Kromasil RP 18, 7 μm; 40 × 250 mm (MZ Analysentechnik, Mainz, Germany); eluant A, H_2O, eluant B, acetonitrile; gradient, 0 min 20% B; 30 min 40% B; 70 min 55% B; 100 min 55% B; 150 min 100% B; flow 9 mL/min. System C was used in some cases for final purification by isocratic high-performance gel permeation chromatography (HPGPC) at RT on LichroGel PS1, 10 μm; 250 × 25 mm (Merck Darmstadt, Germany); isocratic elution with 2-propanol; flow 7 mL/min. The mode of isolation and their t_R in different gradients are summarized in Table 1.

Biological Assays. The assay for evaluation of antiviral activities against HSV1, using strain HSV1-F, including determination of IC_{50} and selectivity indices (SI) of all compounds was recently described in detail.[1] For evaluation of anticoccidial activities, the parasites *Eimeria tenella* (strain Houghton) and *Neospora caninum* (strain NC-1)[38,39] were used for primary screening and propagated at Bayer Animal Health. Primary chicken kidney cells (PCKC, *E. tenella*) or African green monkey cells (Vero, *N. caninum*), respectively, were used for in vitro studies. Cells were grown to confluence in 25 cm^2 flasks in DMEM or RMPI medium supplemented with 10% fetal bovine serum, glutamine, and essential amino acids. Cell cultures were incubated at 37 °C (*E. tenella*) or 41 °C (*N. caninum*) in a humidified atmosphere containing 5% CO_2 and 95% air. For growth of parasites, Vero cell monolayers were infected with tachyzoites and examined with an inverted microscope for the development of lesions or the presence of extracellular tachyzoites. Once lesions were observed, the monolayers were scraped and a small amount of tachyzoite-containing fluid was transferred to infect new flasks of fresh cells. Tachyzoites of *N. caninum* were passaged in this manner every 3 to 4 days as infectious material for the test system. *E. tenella* was maintained in 3-week-old chickens. Recovery, sporulation, and sterilization of oocysts were accomplished from the feces, and excystation and column fractionation served to isolate the sporozoite stages. For determination of the effectiveness of test samples, microtiter monolayer disruption assays (MMDA) were employed. Flat-bottomed 96-well microtiter plates were inoculated with PCKC or Vero cells, and the resulting monolayers were used to determine the effects of substances on merozoite or tachyzoite production as measured by plaque formation. Monolayers were inoculated with parasites (*E. tenella* or *N. caninum*) and inoculated with test compounds 2 h post infection. Untreated and uninfected monolayer wells served as parasite controls, and uninfected agent treated host cells served as toxicity controls. The assay was stopped either 120 h post infection (*E. tenella***)** or when 90−100% of the untreated infected cells had lysed (*N. caninum*)**.** Determination of viability of monolayer cells was done by microscopic control or after fixation with MeOH for 5 min and protein staining (crystal violet solution). The plates were evaluated microscopically or by using an ELISA plate reader (Alpha Diagnostic, San Antonio, TX) to quantify the protein dye incorporation and to determine the concentration of test compounds that inhibited monolayer destruction.

A commercially available fluorescence polarization assay (Pan Vera Inc., Madison, WI) was used for the estrogen receptor-β (ERβ) assays. In this competition type assay, a fluorescent tracer (fluorescein derivative of estrogen) is displaced from an estrogen receptor β/tracer complex by active compounds resulting in fluorescence depolarization. This assay does not discriminate between ERβ agonists and antagonists. The assays were performed as described in the PanVera assay protocol[40] in 96-well microtiter plates. 13-β-Hydroxyestradiol served as control (EC_{50} = 19 nM). The plates were incubated for 2 h in a Thermo Labsystems (Cambridge, UK) iEMS incubator without shaking. After incubation, the fluorescence polarization of the sample was determined using a Tecan (Crailsheim, Germany) Polarion polarization reader. The analysis was performed at $\lambda_{ex} = 485$ nm (excitation wavelength) and $\lambda_{em} = 535$ nm (emission wavelength). Low mP values (low polarization) indicated release of fluorescent tracer by an ERβ ligand. EC_{50} values of compounds were calculated using GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, CA).

Monorden (1): colorless crystals; HPLC, t_R 7.1 min (system 1); $[\alpha]^{20}_D$ +108° (*c* 0.05, $CHCl_3$); ^1H NMR ($[D_6]$DMSO, 500 MHz) δ 10.49 (1H, s, br, 14-O*H* or 16-O*H*), 10.09 (1H, s, 14-O*H* or 16-O*H*), 7.37 (1H, dd, *J* = 15.7, 9.1 Hz, H-8), 6.65 (1H, s, H-15), 6.25 (1H, dd, *J* = 10.2, 10.2 Hz, H-7), 6.10 (1H, d, *J* = 15.7 Hz, H-9), 5.71 (1H, dd, *J* = 10.8, 5.0 Hz, H-6), 5.20 (1H, m, H-2), 3.87 (1H, d, *J* = 16.2 Hz H-11a), 3.65 (1H, d, *J* = 16.2 Hz, H-11b), 3.39 (1H, m, H-5), 3.14 (1H, dm, *J* = 8.8 Hz, H-4), 2.38 (1H, dm, *J* = 14.4 Hz, H-3a), 1.54 (1H, m, H-3b), 1.42 (3H, d, *J* = 6.2 Hz, CH_{3}–1); ^{13}C NMR ($[D_6]$DMSO, 125 MHz), see Table 4; LCMS, t_R 3.6 min; ESIMSpos *m/z* 733 (13), 731 (53), 729 (69) $[2M + H]^+$, 367 (34), 365 (100) $[M + H]^+$, 347 (18) $[M - H_2O + H]^+$ (system 3); LCMS, t_R 5.6 min; ESIMSpos *m/z* 733 (27), 731 (32), 729 (54) $[2M + H]^+$, 408 (25), 406 (100) $[M + CH_3CN + H]^+$, 367 (12), 365 (88) $[M + H]^+$, 350 (38), 348 (45) $[M - O + H]^+$, 293 (14), 291 (31) (system 4); EIMS *m/z* 366 (4), 364 (7) $[M]^+$, 203 (7), 201 (21), 186 (34), 184 (100), 121 (31); HREIMS *m/z* 364.0734 (calcd for $C_{18}H_{17}ClO_6$, 364.0714).

Pochonin A (2): brownish oil; HPLC, t_R 7.3 min (system 1); ^1H NMR ($[D_6]$DMSO, 500 MHz), see Table 2; ^{13}C NMR ($[D_6]$DMSO, 125 MHz), see Table 4; LCMS, t_R 6.4 min; ESIMSpos *m/z* 369 (3), 367 (7) $[M + H]^+$, 351 (2), 349 (3) $[M - H_2O + H]^+$, 292 (25), 291 (100), 157 (65), 99 (35) (system 3); LCMS, t_R 5.7 min; ESIMSpos *m/z* 408 (37) $[M + CH_3CN + H]^+$, 369 (38), 367 (100) $[M + H]^+$ (system 4); HREIMS *m/z* 366.0864 (calcd for $C_{18}H_{19}ClO_6$, 366.0870).

Pochonin B (4): brownish oil; HPLC, t_R 6.3 min (system 1); ^1H NMR ($[D_6]$DMSO, 500 MHz), see Table 2; ^{13}C NMR ($[D_6]$DMSO, 125 MHz), see Table 4; LCMS, t_R 5.3 min; ESIMSpos *m/z* 767 (1), 765 (2) $[2M + H]^+$, 385 (38), 383 (100) $[M + H]^+$ (system 3); EIMS *m/z* 384 (3), 382 (7) $[M]^+$, 186 (12), 184 (39), 130 (26), 81 (58), 69 (100); HREIMS *m/z* 382.0809 (calcd for $C_{18}H_{19}ClO_7$, 382.0819).

Pochonin C (6): brownish oil; HPLC, t_R 6.6 min (system 1); ^1H NMR ($[D_6]$DMSO, 500 MHz), see Table 2; ^{13}C NMR ($[D_6]$DMSO, 125 MHz), see Table 4; LCMS, t_R 3.3 min; ESIMSpos *m/z* 803 (8), 801 (5) $[2M + H]^+$, 403 (19), 401 (30) $[M + H]^+$, 387 (7), 385 (41), 383 (60), $[M - H_2O + H]^+$, 365 (24) $[M - Cl]^+$, 349 (43), 347 (100) (system 5); EIMS *m/z* 402 (7), 400 (11) $[M + H]^+$, 366 (6), 364 (6), 314 (3), 312 (7), 229 (12), 203 89), 201 (26), 186 (33), 184 (100), 157 (16), 155 (16), 121 (25), 96 (32); HREIMS *m/z* 400.0472 (calcd for $C_{18}H_{18}Cl_2O_6$, 400.0480).

Pochonin D (7): brownish oil; HPLC, t_R 8.4 min (system 1); ^1H NMR ($[D_6]$DMSO, 500 MHz), see Table 3; ^{13}C NMR ($[D_6]$DMSO, 125 MHz), see Table 4; LCMS, t_R 7.0 min; ESIMSpos *m/z* 394 (11), 392 (29) $[M + CH_3CN + H]^+$, 353 (30), 351 (100) $[M + H]^+$, 335 (9), 333 (34), $[M - H_2O + H]^+$, 315 (6) $[M - Cl]^+$ (system 4); EIMS *m/z* 352 (9), 350 (27) $[M]^+$, 254 (6), 252 (16), 186 (13), 184 (39), 81 (100); HRESIMS *m/z* 349.0795 $[M - H]^-$ (calcd for $C_{18}H_{18}ClO_5$, 349.0843).

Pochonin E (8): brownish oil; HPLC, t_R 6.6 min (system 1); ^1H NMR ($[D_6]$DMSO, 500 MHz), see Table 3; LCMS t_R 5.6 min; ESIMSpos *m/z* 369 (5), 367 (14) $[M + H]^+$, 233 (100), 157 (72), 99 (50), 97 (31) (system 3). LCMS, t_R 4.8 min; ESIMSpos *m/z* 408 (9) $[M + CH_3CN + H]^+$, 369 (32), 367 (100) $[M + H]^+$, 351 (5), 349 (14) $[M - H_2O + H]^+$ (system 4); EIMS *m/z* 368 (4), 366 (9) $[M]^+$, 252 (20), 224 (8), 203 (3), 201 (9), 186 (16), 184 (42), 175 (12), 157 (4), 155 (9), 117 (40), 97 (34), 91 (12), 81 (8), 69 (17), 59 (100); HREIMS *m/z* 366.0867 (calcd for $C_{18}H_{19}ClO_6$, 366.0870).

Monocillin III (3): colorless solid; HPLC, t_R 6.9 min (system 1); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 11.36 (1H, s, 16-O*H*), 10.23 (1H, s, 14-O*H*), 6.94 (1H, ddd, $J = 16.0, 10.0, 4.8$ Hz, H-8), 6.23 (1H, d, $J = 2.3$ Hz, H-15), 6.20 (1H, d, $J = 2.3$ Hz, H-13), 6.01 (1H, d, $J = 16.0$ Hz, H-9), 5.13 (1H, m, H-2), 4.42 (1H, d, $J = 17.0$ Hz, H-11a), 3.78 (1H, d, $J = 17.0$ Hz, H-11b), 2.85 (1H, m, H-4), 2.59 (1H, dm, $J = 9.3$ Hz, H-5), 2.42 (1H, m, H-7a), 2.30−2.22 (2H, m, H-6β and H-7b), 1.85 (1H, m, H-3a) 1.68 (1H, ddd, $J = 15.9, 4.9, 4.9$ Hz, H-3b), 1.34 (3H, d, $J = 6.4$ Hz, Me-1), 1.22 (1H, m, H-6α); ^{13}C NMR ([D$_6$]DMSO, 125 MHz), shifts from HSQC/HMBC dataset, δ 197.45 (C-10), 170.55 (C-18), 163.94 (C-16), 162.34 (C-14), 149.63 (C-8), 139.49 (C-12), 131.00 (C-9), 112.38 (C-13), 107.09 (C-17), 101.83 (C-15), 71.17 (C-2), 55.98 (C-5), 54.75 (C-4), 46.20 (C-11), 36.27 (C-3), 30.43 (C-6), 28.47 (C-7), 18.14 (C-1); LCMS, t_R 5.2 min; ESIMSpos *m*/*z* 665 (5) [2M + H]$^+$, 374 (16) [M + CH$_3$CN + H]$^+$, 334 (21), 333 (100) [M + H]$^+$, 315 (7) [M − H$_2$O + H]$^+$ (system 4); EIMS *m*/*z* 332 (56) [M]$^+$, 150 (100); HREIMS *m*/*z* 332.1252 (calcd for $C_{18}H_{20}O_6$, 332.1260).

Monocillin II (9): colorless solid; HPLC, t_R 8.1 min (system 1); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 10.29 (1H, s, 16-O*H*), 9.91 (1H, s, br, 14-O*H*), 6.67 (1H, m, H-8), 6.21 (1H, d, $J = 2.2$ Hz, H-15), 6.10 (1H, d, $J = 2.2$ Hz, H-13), 5.88 (1H, d, $J = 15.9$ Hz, H-9), 5.31 (2H, m, H-4 and H-5), 5.11 (1H, m, H-2), 3.92 (1H, d, $J = 15.5$ Hz, H-11a), 3.50 (1H, d, $J = 15.5$ Hz, H-11b), 2.40 (1H, dm, $J = 14.1$ Hz, H-3a), 2.27−2.17 (3H, m, H-3b, H-7a, and H-7b), 2.12 (1H, m, H-6a), 2.06 (1H, m, H-6b), 1.25 (3H, d, $J = 6.3$ Hz, Me-1); ^{13}C NMR ([D$_6$]DMSO, 125 MHz), shifts from HSQC/HMBC dataset, δ 196.51 (C-10), 168.32 (C-18), 159.93 (C-14), 159.21 (C-16), 148.39 (C-8), 136.72 (C-12), 131.65 (C-5), 130.03 (C-9), 128.36 (C-4), 110.70 (C-17), 108.66 (C-13), 101.57 (C-15), 71.08 (C-2), 45.81 (C-11), 37.65 (C-3). 30.41 (C-7), 30.28 (C-6),19.24 (C-1); LC-MS, t_R 6.8 min; ESIMSpos *m*/*z* 633 (6) [2M + H]$^+$, 358 (33) [M + CH$_3$CN + H]$^+$, 318 (20), 317 (100) [M + H]$^+$, 299 (6) [M − H$_2$O + H]$^+$ (system 4); EIMS *m*/*z* 317 (8) [M + H]$^+$, 316 (36) [M]$^+$, 288 (5), 270 (6), 236 (9), 218 (25), 190 (13), 150 (70), 81 (100); HREIMS *m*/*z* 316.1315 (calcd for $C_{18}H_{20}O_5$, calcd 316.1311).

Pochonin F (10): brownish oil; HPLC, t_R 6.3 min (system 1); ^{1}H NMR ([D$_6$]DMSO, 500 MHz), see Table 5; ^{13}C NMR ([D$_6$]-DMSO, 125 MHz), see Table 5; LCMS, t_R 4.4 min; ESIMSpos, *m*/*z* 374 (11) [M + CH$_3$CN + H]$^+$, 333 (100) [M + H]$^+$ (system 4); HRESIMS *m*/*z* 333.2514 [M + H]$^+$ (calcd for $C_{18}H_{21}O_6$, 333.1338); HRESIMS *m*/*z* 331.1167 [M − H]$^-$ (calcd for $C_{18}H_{19}O_6$, 331.1182).

Monocillin II glycoside (11): brownish oil; HPLC: t_R 7.0 min (system 1); ^{1}H NMR ([D$_6$]DMSO, 500 MHz), see Table 5; ^{13}C NMR ([D$_6$]DMSO, 125 MHz), see Table 5; LCMS, t_R 5.4 min; ESIMSpos *m*/*z* 449 (18) [M + H]$^+$, 358 (15) [aglycone + CH$_3$CN + H]$^+$, 317 (100) [aglycone + H]$^+$, 299 (98) [aglycone − H$_2$O + H]$^+$ (system 4).

Hydrogenation of Monorden (1) to Tetra- (5) and Hexahydromonorden (12). To a solution of 20 mg (0.055 mmol) of monorden (**1**) in methanol is added 7 mg of palladium (10% Pd/C). After hydrogenation for 1 h at room temperature under atmospheric pressure, the catalyst is removed by filtration and the residue of the filtrate is purified by preparative reversed-phase HPLC using the following conditions: Nucleosil C$_{18}$, 7 μm; 8 × 125 mm (MZ Analysentechnik); eluant A, water; eluant B, ACN; gradient, 0 min 20% B; 40 min: 100% B, flow 5 mL/min. t_R(**5**) = 8−9 min and t_R(**12**) = 10−11 min.

Tetrahydromonorden (5):[2,3] yield 4.3 mg (0.01 mmol, 18%); HPLC: t_R 7.5 min (system 1); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 10.42 (1H, s, 14-O*H* or 16-O*H*), 9.99 (1H, s, 14-O*H* or 16-O*H*), 6.51 (1H, s, H-15), 5.08 (1H, m, H-2), 4.01 (1H, d, $J = 18.3$ Hz, H-11a), 3.86 (1H, d, $J = 18.3$ Hz, H-11b), 2.71 (1H, dm, $J = 8.3$ Hz, H-4), 2.68 (1H, m, H-5), 2.41 (2H m, H$_2$-9), 2.21 (1H, ddd, $J = 14.2, 10.0, 3.6$ Hz, H-3a), 1.89 (1H, m, H-6β), 1.61 (1H, m, H-8a), 1.53 (1H, m, H-8b), 1.45 (1H, m, H-7a), 1.40 (1H, ddd, $J = 14.2, 8.3, 1.9$ Hz, H-3b), 1.27 (3H, d, $J = 6.2$ Hz, Me-1), 1.07 (1H, m, H-6α); ^{13}C NMR ([D$_6$]DMSO, 125 MHz), see Table 4; LCMS, t_R 5.9 min; ESIMSpos *m*/*z* 410 (5) [M + CH$_3$CN + H]$^+$, 371 (31), 369 (100) [M + H]$^+$, 351 (8) [M − H$_2$O + H]$^+$ (system 4); EIMS *m*/*z* 370 (18), 368 (53) [M]$^+$, 186 (30), 184 (100); HREIMS *m*/*z* 368.1043 (calcd for $C_{18}H_{21}ClO_6$, 368.1027).

Hexahydromonorden (12): yield: 3.5 mg (0.009 mmol, 16%); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 10.37 (1H, s, br, 14-O*H* or 16-O*H*), 9.85 (1H, s, 14-O*H* or 16-O*H*), 6.49 (1H, s, H-15), 5.03 (1H, m, H-2), 4.37 (1H, d, $J = 5.2$ Hz, 4-O*H*), 4.12 (1H, d, $J = 18.5$ Hz, H-11a), 3.85 (1H, d, $J = 18.5$ Hz, H-11b), 3.39 (1H, m, H-4), 2.62 (1H, ddd, $J = 13.0, 9.0, 3.2$ Hz, H-9a), 2.12 (1H, ddd, $J = 13.0, 9.4, 3.4$ Hz, H-9b), 1.78 (1H, ddd, $J = 14.1, 10.2, 3.7$ Hz, H-3a), 1.56 (2H, m, H-8a and H-3b), 1.50−1.36 (3H, m, H-8b, H-6a, and H-5a), 1.32 (1H, m, H-7b), 1.25 (3H, d, $J = 6.2$ Hz, Me-1), 1.21−1.11 (3H, m, H-7b, H-6b, and H-5b); ^{13}C NMR ([D$_6$]DMSO, 125 MHz), see Table 4; LCMS, t_R 5.5 min; ESIMS pos *m*/*z* 743 (9), 741 (12) [2M + H]$^+$, 373 (35), 371 (100) [M + H]$^+$, 355 (9), 353 (30) [M − H$_2$O + H]$^+$, 337 (9), 335 (19) (system 4); HREIMS *m*/*z* 370.1167 (calcd for $C_{18}H_{23}ClO_6$, 370.1183).

Epoxide Ring Opening. To a solution of 5 mg (0.014 mmol) of monorden (**1**) in methanol is added 1 N HCl. After stirring for 1 day at room temperature, the reaction mixture is evaporated and purified by preparative reversed-phase HPLC using the following conditions: Eurospher C$_{18}$, 7 μm; 16 × 125 mm (Knauer); eluant A, water + 0.05% TFA; eluant B, ACN + 0.05% TFA; gradient, 0 min 0% B; 60 min 100% B; flow 7 mL/min. Retention time of the products: t_R(**14**) = 30 min and t_R(**13**) = 34 min.

6,13-Dichloro-5,9,14,16-tetrahydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1*H*-2-benzoxacyclotetradecine-1,11(12H)-dione (13): yield 0.6 mg (0.0014 mmol, 10%); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 10.31 (1H, s, 14-O*H* or 16-O*H*), 9.91 (1H, s, 14-O*H* or 16-O*H*), 6.49 (1H, s, H-15), 5.91 (1H, dm, $J = 10.1$ Hz, H-7), 5.84 (1H, dm, $J = 10.1$ Hz, H-6), 5.12 (1H, m, H-2), 4.62 (1H, dm, $J = 12.3$ Hz, H-8), 4.40 (1H, dm, $J = 6.7$ Hz, H-5), 3.96 (2H, m, H$_2$–11), 3.83 (1H, dd, $J = 8.0, 8.0$ Hz, H-4), 3.12 (1H, dd, $J = 13.4, 12.3$ Hz, H-9a), 2.51 (H-9b, under solvent signal), 2.02 (1H, dm, $J = 14.7$ Hz, H-3a), 1.75 (1H, m, H-3b), 1.27 (3H, d, $J = 6.1$ Hz, Me-1); ^{13}C NMR ([D$_6$]DMSO, 125 MHz), shifts from HSQC/HMBC data, only detected signals δ 131.72 (C-7), 127.04 (C-6), 102.21 (C-15), 72.73 (C-4), 72.29 (C-2), 69.49 (C-8), 56.10 (C-5), 45.86 (C-11), 43.39 (C-9), 38.12 (C-3), 20.74 (C-1); LCMS, t_R 7.1 min; ESIMSpos *m*/*z* 405 (10), 404 (15), 403 (68), 402 (22), 401 (100) [M − H$_2$O + H]$^+$ (system 4); EIMS *m*/*z* 404 (5), 403 (7), 402 (33), 401 (10), 400 (47), 367 (2), 366 85), 365 (9), 364 (7), 231 (18), 229 (54), 203 (18), 201 (58), 186 (34), 184 (100), 159 (30), 157 (97), 139 (45), 121 (90); HRESIMS *m*/*z* 401.0579 [M − H$_2$O + H] (calcd for $C_{18}H_{19}Cl_2O_6$, 401.0559).

13-Chloro-5,6,9,14,16-pentahydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1*H*-2-benzoxacyclotetradecine-1,11(12H)-dione (14): yield 0.6 mg (0.0015 mmol, 11%); ^{1}H NMR ([D$_6$]DMSO, 500 MHz) δ 10.30 (1H, s, 14-O*H* or 16-O*H*), 9.90 (1H, s, 14-O*H* or 16-O*H*), 6.48 (1H, s, H-15), 5.95 (1H, dm, $J = 9.6$ Hz, H-6), 5.24 (1H, dm, $J = 9.6$ Hz, H-7), 5.09 (1H, m, H-2), 4.53 (1H, dm, $J = 12.9$ Hz, H-8), 4.00 (1H, d, $J = 17.6$ Hz, H-11a), 3.90 (1H, d, $J = 17.6$ Hz, H-11b), 3.58 (1H, dd, $J = 8.1, 8.1$ Hz, H-4), 3.35 (5-H, hidden by water signal), 3.02 (1H, dd, $J = 13.9, 12.8$ Hz, H-9a), 2.45 (1H, d, $J = 13.9$ Hz, H-9b, under solvent signal), 1.94 (1H, dm, $J = 15.7$ Hz, H-3a), 1.66 (1H, ddd, $J = 15.7, 7.5, 7.5$ Hz, H-3b), 1.25 (3H, d, $J = 6.1$ Hz, Me-1).

Zearalenone Derivatives (15−21). Compounds **15**−**17** were obtained from Sigma-Aldrich, and **18** was obtained from **17** by methylation with CH$_3$I/K$_2$CO$_3$ in acetone. Compounds **19**−**21** were prepared from the respective educts **15**−**17** by hydrogenation in methanol at RT and 1 bar with Pd/C as catalyst.

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