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Melithiazols A–N: New Antifungal β-Methoxyacrylates from Myxobacteria

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Dedicated to Professor Hans Zähner on the occasion of his 70th birthday

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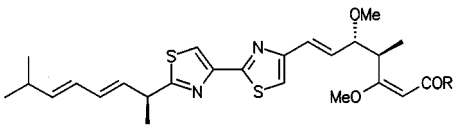
From cultures of *Melittangium lichenicola*, *Archangium gephyra* and *Myxococcus stipitatus*, thirteen new β-methoxyacrylate (MOA) fungicides related to myxothiazols (**1**) have been isolated. Melithiazols A (**2a**), D (**2b**), K (**2c**), and L (**2d**) are characterized by a thiazoline–thiazole system, whereas melithiazols B (**3a**), E (**3b**), F (**3c**), G (**3d**), H (**3e**), I (**3f**), M (**3g**), and N (**3h**) are bis(thiazoles). Melithiazol C (**4**), as the first representative of this class of compounds, contains only one thiazole ring. The structures were established on the basis of spectroscopic data, and confirmed in the case of melithiazol E (**3b**), including its relative configuration, by an X-ray structure analysis. The absolute configuration of melithiazols A (**2a**) and B (**3a**) was determined by degradation and CD spectroscopy. Antifungal and cytotoxic activities, inhibition of NADH oxidation, and lipophilicities of melithiazols **2–4**, myxothiazols **1**, and strobilurin-type compounds are compared.

In the course of our ongoing screening of myxobacteria for biologically active metabolites, we noted that strains of *Melittangium lichenicola*, *Archangium gephyra* and *Myxococcus stipitatus* exhibit a broad spectrum of antifungal activities. Although the crude extracts of these cultures showed complex peak patterns in HPLC/DAD analyses, none of the peaks could be correlated with the antifungal activity. The reason for this, as we subsequently discovered, was not the lack of a chromophore, but rather the production of only minute amounts of highly active metabolites. However, using TLC/bioautography with the indicator organism *Botrytis cinerea*, the compounds responsible for the activity could be easily localized and monitored during the isolation process. They turned out to be a new group of β-methoxyacrylates (MOAs) related to myxothiazols **1** (Scheme 1),[2][3] and were named melithiazols (Scheme 2).[4]

More recently, further close analogues of melithiazols named cystothiazoles have been isolated by a Japanese group from another myxobacterium, *Cystobacter fuscus*.[5] Here, we report the isolation and structure elucidation of the melithiazols A–N (**2**, **3**, **4**) (see Scheme 2). The production, detailed biological properties,[1] and semisyntheses[6] of these compounds from myxothiazol A (**1a**) will be described separately.



Myxothiazol A (**1a**) R = NH_2
Z (**1b**) R = OMe

Scheme 1. Structures of Myxothiazol A (**1a**) and Myxothiazol Z (**1b**)

Isolation of the Melithiazols and Structure Elucidation

Isolation was started from a 300 L fermentation batch of *Melittangium lichenicola*, strain Me l26, grown in the presence of 1% (*v*/*v*) of the adsorber resin XAD-16. The culture was harvested by passage through a process filter to collect the adsorber resin. Adherent cells were largely eliminated by repeated washing with water. Elution of the resin with methanol and acetone yielded a crude extract of 62 g, which was separated by consecutive chromatography on Sephadex LH-20, silica gel, and C-18 reversed-phase silica gel with monitoring of the antifungal activity against *Botrytis cinerea.* In the final step, the activity could be correlated with two peaks detected by UV absorption at 220 nm. From the fraction corresponding to the major peak, 22 mg of melithiazol A (**2a**) was isolated. The fraction corresponding to the smaller peak was further purified by preparative TLC to give 2 mg of melithiazol C (**4**). The yields of **2a** and **4** correspond to a production of 70 and 5 μg/L and correlate well with the initially observed level of antifungal activity.

Similarly, a 300 L fermentation batch of *Archangium gephyra,* strain Ar 7747, yielded 95 g of crude extract. This

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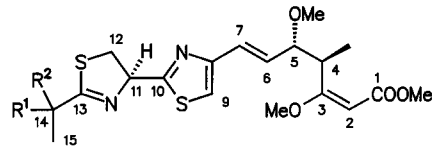
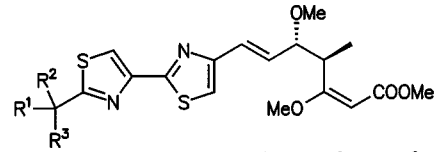
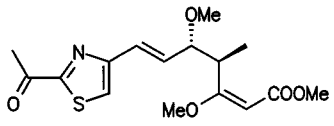


	R¹	R²
Melithiazol A (**2a**)	−CH₂−	
D (**2b**)	Me	H
K (**2c**)	−CH₂O−	
L (**2d**)	CH_2OMe	H



	R¹	R²	R³
Melithiazol B (**3a**)	−CH₂−		Me
E (**3b**)	Me	Me	H
F (**3c**)	Ph	H	H
G (**3d**)	Et	Me	H
H (**3e**)	Me	H	H
I (**3f**)	iProp	H	H
M (**3g**)	Me	−O−	
N (**3h**)	−CH₂O−		Me



Melithiazol C (**4**)

Scheme 2. Structures of Melithiazols A−H (**2**, **3**, **4**)

was fractionated as described above, however, the first two chromatographic steps were performed in reversed order. Productivity in this fermentation was comparable with that in the above case, and 21 mg of melithiazol A (**2a**), 16 mg of melithiazol B (**3a**), 1 mg of melithiazol D (**2b**), 7 mg of melithiazol K (**2c**), 6 mg of melithiazol L (**2d**), 4 mg of melithiazol N (**3h**), and 2 mg of melithiazol M (**3g**) were obtained.

Myxococcus stipitatus, strain Mx s64, was found to be considerably more productive, yielding 5 g of crude extract from a 10 L fermentation. This was separated by chromatography on Sephadex LH-20, silica gel, and C-18 reversed-phase silica gel to give 39 mg of melithiazol E (**3b**), 12 mg of melithiazol F (**3c**), 2 mg of melithiazol G (**3d**), 1 mg of melithiazol H (**3e**), and 0.4 mg of melithiazol I (**3f**).

Inspection of the ^{1}H-NMR spectra of the melithiazols (Table 1 and Experimental Section) reveals that all have in common a methyl β-methoxyacrylate pharmacophore and a linker part of C-4 to C-7 including one thiazole ring, as is known for myxothiazol Z (**1b**).[3] These structural features were easily identified from the ^{1}H-NMR spectra, which showed singlets for the methoxy groups on *C*-1, *C*-3, *C*-5, the vinyl proton 2-H, and 9-H of the thiazole at δ = 3.66, 3.60, 3.32, 4.96 and 7.00, respectively. In addition, signals were observed for the allylic system C-5 to C-7 at δ = 3.80, 6.34, 6.52 and for the adjacent CH_3CH group at δ = 1.20 and 4.16, showing the same coupling pattern as in myxothiazols A (**1a**)[2] and Z (**1b**).[3]

Of all the isolated melithiazols, only E (**3b**) gave crystals of sufficient quality to allow a X-ray crystal structure analysis. The stereoscopic view (Figure 1) confirms the partial structure assigned above and identifies the rest of the molecule as a 2-isopropylthiazole. Thus, like the myxothiazols, melithiazol E (**3b**) contains a bis(thiazole) system, but with the 13-nonadienyl side chain replaced by 13-isopropyl. According to spectroscopic data, **3b** seems to be identical to cystothiazole A, recently described as a metabolite of *Cystobacter fuscus*.[5][7]

From the NMR and mass spectral data given in Table 1 and in the Experimental Section, seven further melithiazols were identified differing from melithiazol E (**3b**) only in their C-13 substituents. Thus, B (**3a**) is the 13-isopropenyl, F (**3c**) the 13-benzyl, G (**3d**) the 13-(2′-butyl), H (**3e**) the 13-ethyl, I (**3f**) the 13-isobutyl, M (**3g**) the 13-acetyl, and N (**3h**) the 13-(1′-methyl)epoxyethyl analogue.

On the basis of HR MS and its ^{1}H-NMR spectrum, melithiazol A (**2a**) differs from B (**3a**) in that it has two more hydrogens. These can be assigned as being attached to C-11 and C-12 of a thiazoline ring giving rise to an isolated ABX spin system with signals at δ = 3.82, 3.66 and 5.93. This structural assignment is supported by a hypsochromic shift of the long-wavelength UV band at 307 nm by > 30 nm and the chemical shifts of C-11 (δ = 78.2) and C-12 (δ = 38.8). Besides melithiazol A (**2a**), having a 2-isopropenyl-substituted thiazoline moiety, three further thiazolines were identified by their spectral data, namely melithiazol D (**2b**) with a 13-isopropyl group, K (**2c**) with a 13-(1′,2′-epoxy-2′-propyl) group, and L (**2d**) with a 13-(1′-methoxy-2′-propyl) group.

Melithiazol C (**4**) differs from all the other melithiazols in that it contains only one thiazole ring, which bears a 2-acetyl substituent (δ_{Me} = 2.65). Furthermore, it is the only melithiazol occurring as a (6*E*)/(6*Z*) mixture (3.5:1), however, the isomers cannot be separated by chromatography.

According to NMR data and the crystal structure analysis, all the melithiazols have the same relative and absolute configuration at *C*-4 and *C*-5 as found for the myxothiazols **1**.[2][3] This was confirmed by correlation with myxothiazol A (**1a**) of the CD spectra of natural and semisynthetic **3a** prepared from **1a**.[6] The configuration at *C*-11 in the thiazolines **2** was determined in the case of melithiazol A (**2a**) by Raney Ni desulfurization and oxidative cleavage of the thiazole ring by singlet oxygen[8] to liberate C-10 as a carboxy group upon hydrolysis. The resulting alanine was derivatized as its *N*-TFA/isopropyl ester and identified by GC on L-Chirasil-Val as (*S*)-alanine. Thus, melithiazol A (**2a**) and presumably also D (**2b**), K (**2c**) and L (**2d**) have (11*S*)-configuration. Interestingly, the opposite configuration was observed for the closely related thiazoline−thiazole system of phleomycin.[9]

Table 1. ^{1}H- and ^{13}C-NMR data of melithiazols A (**2a**), B (**3a**) and C (**4**) in $CDCl_3$ (300 MHz and 75 MHz, respectively)

Atom	**2a**[a]	**3a**	**4**	Atom	**2a**	**3a**
2-H	4.96 s	4.96	5.00	C-1	167.7	167.8
4-H	4.16 dq	4.17	4.23	C-2	91.1	91.1
5-H	3.80 t	3.81	3.79	C-3	176.8	176.8
6-H	6.34 dd	6.41	6.46	C-4	39.9	39.9
7-H	6.52 d	6.57	6.67	C-5	84.4	84.4
9-H	7.00 s	7.09	7.82	C-6	131.4	131.7
11-H	5.93 dd	–	–	C-7	125.6	125.6
12-H_a	3.82 dd	7.88[b]	2.65[b]	C-8	153.8	154.5
12-H_b	3.66 dd	–	–	C-9	114.7	115.6
15-H_3	2.14 s, br	2.27	–	C-12	38.8	115.3
16*E*-H	5.60 s, br	5.35	–	C-10	171.2	162.3
16*Z*-H	5.70 s, br	5.90	–	C-11	78.7	149.5
1-OCH_3	3.66 s	3.66	3.64	C-13	172.8	169.6
3-OCH_3	3.60 s	3.60	3.59	C-14	139.4	137.8
4-CH_3	1.20 d	1.21	1.19	C-15	20.0	20.5
5-OCH_3	3.32 s	3.33	3.30	C-16	124.2	117.4
				1-OCH_3	50.8	50.9
				3-OCH_3	55.6	55.6
				4-CH_3	14.1	14.1
				5-OCH_3	57.0	57.1

[a] $J_{4,5}$ = 7.8 Hz, $J_{4,4\text{-Me}}$ = 6.9 Hz, $J_{5,6}$ = 7.8 Hz, $J_{6,7}$ = 15.7 Hz, $J_{11,12a}$ = 8.8 Hz, $J_{11,12b}$ = 7.5 Hz, $J_{12a,12b}$ = 11.2 Hz. – [b] Singlet.

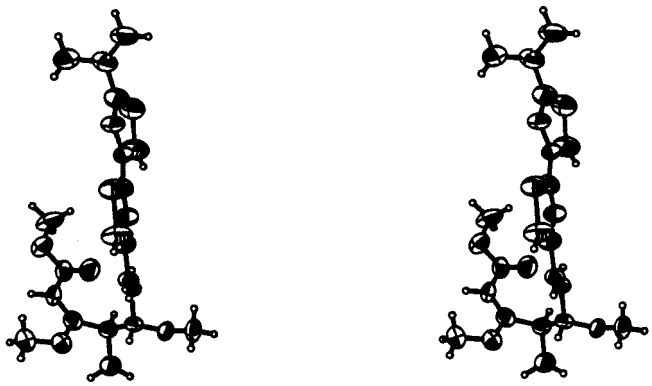


Figure 1. Stereoscopic view of melithiazol E (**3b**) in the crystal

On the basis of feeding experiments with ^{13}C-labelled precursors, myxothiazol A[10] and, analogously, melithiazols are evidently synthesized by a polyketide synthase/peptide synthetase hybrid. As starter units for melithiazols, acyl-CoA esters produced by oxidative degradation of valine (A, B, D, E), phenylalanine (F), isoleucine (G), α-aminobutyric acid (H), and leucine (I) are clearly recognizable from their C-13 substituents. Remarkably, in the biosynthesis of myxothiazol A (**1a**), the starter unit isobutyryl-CoA is derived from leucine.[10] The *N*-acyl cysteine intermediates are presumably cyclized to thiazolines, which are subsequently dehydrogenated to their thiazole counterparts at various stages of the biosynthesis of melithiazols. The origin of melithiazol C (**4**) remains obscure; it may be formed by incorporation of only one cysteine residue, or more likely, from e.g. melithiazol A (**2a**) by opening of the thiazoline ring to give an *N*-thioacyl enamine,[11] followed by hydrolysis.

Since the discovery of myxothiazol A in 1980[2] and the elucidation of its mode of action,[12] the remarkable similarity with the fungal metabolites oudemansin[13] and strobilurin[14][15] has been recognized. These three groups of compounds, including the melithiazols, contain a β-methoxyacrylate pharmacophore (MOA)[16] and exert their antifungal properties by inhibiting mitochondrial respiration at the cytochrome bc_1 complex. However, an important difference is also apparent: in oudemansin and strobilurin the pharmacophore is linked through the α-carbon to the rest of the molecule, whereas in myxothiazol and melithiazol it is linked through the β-carbon. Thus, the latter group of compounds may be collectively referred to as isostrobilurins. Nevertheless, both types of inhibitors adopt the same ″bent″ conformation of the linker region *C*-4/*C*-5, which appears to be essential for binding to the target and for activity.[15,17,18] In the crystalline state, the conformations of melithiazol E (**3b**) and oudemansin A[13][19] are almost identical, with the exception of the torsion angle between

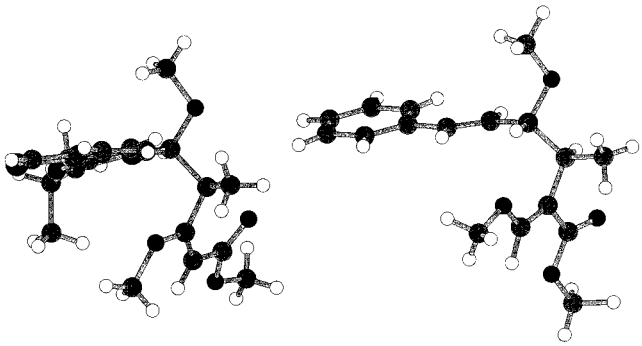


Figure 2. Conformations of the strobilurin (right) and isostrobilurin (left) pharmacophores in the crystal structures of oudemansin A[13] and melithiazol E (**3b**)

Table 2. Biological activities and lipophilicities of melithiazols A−N (**2−4**), myxothiazols A and Z (**1a**,**b**), strobilurin A, oudemansin A, kresoxim methyl, and azoxystrobin

Compound		*Botrytis cinerea* IC_{50} [ng/mL][a]	Inhibition of NADH oxidation IC_{50} [ng/mL][b]	Cytotoxity IC_{50} [ng/mL][c]	Lipophilicity log P_{ow}[d]
melithiazol	A (**2a**)	40	33	50	3.85
melithiazol	B (**3a**)	40	18	30	4.75
melithiazol	C (**4**)	1500	1600	700	2.92
melithiazol	D (**2b**)	150	70	30	3.67
melithiazol	E (**3b**)	80	46	50	4.35
melithiazol	F (**3c**)	300	147	25	4.42
melithiazol	G (**3d**)	300	41	60	4.88
melithiazol	H (**3e**)	150	80	450	3.77
melithiazol	I (**3f**)	300	75	130	4.74
melithiazol	K (**2c**)	300	250	650	2.75
melithiazol	L (**2d**)	80	30	50	3.67
melithiazol	M (**3g**)	40	30	50	3.37
melithiazol	N (**3h**)	150	60	150	3.37
myxothiazol	A (**1a**)	40	11	0.5	5.29
myxothiazol	Z (**1b**)	150	24	2	7.17
strobilurin A		150	83	180	3.90
oudemansin A		800	400	800	3.55
kresoxim-methyl		100	72	400	3.70[e]
azoxystrobin		150	110	400	2.06[f]

[a] The MIC was determined by a serial broth dilution assay; for details see ref.[1] − [b] The inhibition of NADH oxidation was measured with submitochondrial particles isolated from beef heart; for details see ref.[1] − [c] The cytotoxicity was measured by a growth inhibition assay using the mouse fibroblast cell line L929 (ATTCC CCL1); for details see ref.[1] − [d] Estimated by RP-18 TLC according to ref.[23] − [e] In ref.[18]: 3.4 and 3.5. − [f] In ref.[17]: 2.64.

the planar β-methoxyacrylate and 4-H which is 4° in the former (C-2/4-H) and 25° in the latter (C-1/4-H), as can be seen in Figure 2. From the vicinal coupling constants of $J_{4,5}$ and $J_{5,6}$ = 7.8 Hz, the solution conformation of melithiazol is evidently less rigid than that of oudemansin (J = 9.8 and 8.3 Hz). However, the gross conformation of melithiazol shown in Figure 2 is preserved in solution, as can be deduced from NOEs between 4-Me, 5-H and 3-OMe in **2a** and the strong deshielding of 4-H (δ = 4.16) by the ester carbonyl oxygen.

Clearly, the conformations of the strobilurin and isostrobilurin inhibitors bound to the target site in the cytochrome bc_1 complex may be different, as should be seen when details of the recently published crystal structure analyses[20−22] become available.

Biological Activity

The biological properties and lipophilicities of melithiazols A−N (**2−4**), myxothiazols A (**1a**) and Z (**1b**), and strobilurin-type inhibitors are summarized in Table 2. For comparison of their in vitro antifungal activities *Botrytis cinerea* was used, while for assessment of their inhibition of NADH oxidation, beef heart submitochondrial particles were chosen as a model system. With melithiazols A−N, the inhibitory activities observed in the two systems correlate fairly well. The most active compounds, with activities comparable to those of myxothiazols, are melithiazols A, B, L and M, whereas D, E, H and N still show good activities against *B. cinerea* at lower target activities. Melithiazol C and the structurally related oudemansin A are the least active.

Most remarkably, the cytotoxicity of the highly antifungal melithiazols A, B and M is seen to be *lower* than that of myxothiazol A by a factor of 60 to 100, while the moderately active melithiazols H, N and strobilurin A show comparable activities in all three test systems. Thus, the extremely high in vitro and in vivo toxicity observed with myxothiazols A[2][17] and Z[3] is evidently not an inherent property of the β-linked (isostrobilurin) pharmacophore. Moreover, there does not seem to be a correlation between lipophilicity, as estimated by mobility on RP-18 TLC,[23] and toxicity, although the least toxic melithiazols C and K are particularly polar. From a comparative inspection of all the structural features, it would appear that it is not the overall lipophilicity that determines toxicity, but rather that of the side chain. Presumably, the lipid-like side chain of myxothiazols A and Z selectively favours uptake and transport to the target site in the mitochondrial membranes of animal cells. This is in good agreement with the observation of markedly increased toxicity for strobilurins D[24] and E[25] having lipophilic prenyl-derived side chains. Interestingly, strobilurin E and myxothiazols A and Z exhibit similar cytotoxic and target site activities. On the other hand, with melithiazol A and strobilurin A about the same relative antifungal, target and cytotoxic activities are observed (Table 2). Clearly, on the basis of the knowledge acquired in the early 1980s, only strobilurin A was selected as a lead compound for the development of the environmentally safe agricultural fungicides kresoxim-methyl[17] and azoxystrobin.[18] Now, a similar optimization of the isostrobilurin-

type compounds would be feasible, since this pharmacophore shows no inherent toxicity.

Experimental Section

General Methods: Analytical TLC: TLC aluminium sheets coated with Merck silica gel Si 60 F_{245}, 0.2 mm; solvent system: dichloromethane/acetone (95:5); detection: UV absorption at $\lambda = 254$ nm and spraying with vanillin/sulfuric acid [0.5 g vanillin in 100 mL of sulfuric acid/ethanol (8:2) and heating to 120°C]. − Analytical HPLC: Nucleosil RP-18−125−5, 125 × 2 mm (Macherey−Nagel), eluent methanol/water (75:25), 0.3 mL/min; UV detection at 220 nm. − Preparative HPLC: Unless specified otherwise, Lichrosorb Si60 (7 μm, Merck), 4 × 25 cm, peak detection at $\lambda = 220$ nm, flow rate 17 mL/min. − All eluates and chromatographic fractions were concentrated to dryness in vacuo in a rotary evaporator with a bath temperature of 35°C. − NMR spectra: Spectra were recorded on a Bruker AM-300 (^{1}H: 300 MHz, ^{13}C: 75.5 MHz) or a Bruker AM-400 spectrometer (^{1}H: 400 MHz, ^{13}C: 100 MHz), with TMS or residual solvent signals as internal standard. − UV spectra: Shimadzu UV-2102 PC spectrophotometer. − CD spectra: Jasco J600. − IR spectra: Nicolet 20 DXB FT-IR spectrometer. − EI mass spectra: Kratos MS 9/50 or Finnigan MAT 95 spectrometer; resolution $M/\Delta M = 1000$; high-resolution data from peak matching $M/\Delta M = 10000$.

Isolation of Melithiazols A and C from *Melittangium lichenicola*, Strain Me l26: A 300-L fermentation batch containing 1% (*v*/*v*) Amberlite XAD-16 adsorber resin was harvested by filtration with a process filter (0.3 m^2, 210 μm mesh). The collected adsorber resin was washed several times with water and then applied to the top of a chromatographic column. After elution with 10 L of methanol and 5 L of acetone (corresponding to 5 bed volumes) at a flow rate of 1 bed volume/h, the organic solvents were evaporated from the eluate under reduced pressure. The remaining aqueous phase (750 mL) was extracted with ethyl acetate (5 × 600 mL). The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated to dryness. The crude extract of 62 g was dissolved in acetone and separated by chromatography on Sephadex LH-20 (Pharmacia) (column 100 × 10 cm; 12 batches; eluent: acetone; flow rate 4 mL/min; detection at 365 nm). The biologically active fractions (agar diffusion test, *B. cinerea*) were combined, concentrated to dryness (residue: 3.2 g), and separated by chromatography on Merck silica gel 60 (9.5 × 75 cm; particle size 0.063−0.200 mm; eluents: 350 mL dichloromethane, 800 mL dichloromethane/acetone, 80:20, 100 mL methanol). Fractions containing melithiazols were combined according to TLC analyses (dichloromethane/acetone, 9:1) and concentrated to dryness (residue: 710 mg). This material was divided into two portions, which were further separated by medium-pressure RP-18 chromatography [column 47 × 380 mm, HD-SIL Labogel 18−20−60 (Kronlab); eluent: methanol/water, 80:20, for 2 h, gradient to 100% methanol in 30 min, and then 30 min with 100% methanol; flow rate 10 mL/min; detection by UV absorption at 227 nm] to give two fractions of 26 mg, $t_R = 85$ min, and 60 mg, $t_R = 185$ min. The latter fraction was divided into three batches, which were each separated by Si-HPLC [column: 20 × 250 mm, Nucleosil 100 B10-Y55, 7 μm (Macherey−Nagel); eluent: petroleum ether/TBME/methanol, 3:6:0.01; flow rate 10 mL/min; detection by UV absorption at 227 nm] to give melithiazol A (**2a**) (22 mg; $t_R = 10$ min). The first fraction was divided into two batches and separated by TLC (Merck silica gel Si 60 F_{254}, 0.25 mm, eluent: dichloromethane/acetone, 97:3, $R_f = 0.4$) to give 5 mg of a fraction containing melithiazol C (**4**), which was further purified by Si-HPLC [column 20 × 250 mm, Nucleosil 100 B10-Y55, 7 μm (Macherey−Nagel); eluent: petroleum ether/TBME/methanol, 3:6:0.01; flow rate 10 mL/min; detection by UV absorption at 225 nm] to yield melithiazol C (**4**) [2 mg; $t_R = 12$ min, 6*E*/6*Z* mixture (3.5:1)].

Isolation of Melithiazols B, D, K−N and A from *Archangium gephyra*, Strain Ar 7747: Adsorber resin from a 300 L fermentation batch was harvested and the resin was eluted as described above. From the combined eluate of 19.5 L of methanol and 6 L of acetone (corresponding to 8.5 bed volumes), the organic solvents were evaporated under reduced pressure. The remaining aqueous phase of 1.2 L was extracted with ethyl acetate (5 × 700 mL). The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated to dryness. The crude extract (95 g) was dissolved in acetone and separated by column chromatography on Merck silica gel 60 (9.5 × 75 cm; particle size 0.063−0.200 mm; eluents 2 L dichloromethane, 2 L dichloromethane/acetone, 85:15, 1 L methanol, 2 L dichloromethane/acetone, 80:20). The biologically active fractions were combined, concentrated to dryness (residue: 7 g) and separated on Sephadex LH-20 (Pharmacia) (column: 7 × 70 cm; 3 batches; eluent: dichloromethane/methanol, 80:20; flow rate 1.2 mL/min). The biologically active fraction (240 mg) was divided into ten batches, which were further separated by RP-18 HPLC [column: 16 × 250 mm, Nucleosil 100 C_{18} 7 μm (VDS optilab); eluent: methanol/water 80:20; flow rate 10 mL/min; detection by UV absorption at 220 nm] to give 7 mg of melithiazol K (**2c**), 6 mg of melithiazol L (**2d**), 21 mg of melithiazol A (**2a**), 16 mg of melithiazol B (**3a**), and 7 mg of a mixture of other melithiazols. This mixture was further separated by preparative TLC (Merck silica gel Si 60 F_{254}, 0.5 mm, eluent: petroleum ether/diethyl ether, 1:1) to yield 4 mg of melithiazol N (**3h**), 2 mg of melithiazol M (**3g**), and 1 mg of melithiazol D (**2b**).

Isolation of Melithiazols E−I from *Myxococcus stipitatus* Strain Mx s64: Adsorber resin from a 10 L fermentation batch was harvested as described above and the resin was eluted with 4.5 L of methanol and 5 L of acetone (corresponding to 12.5 bed volumes). The organic solvents were evaporated from the eluate under reduced pressure and the remaining aqueous phase (500 mL) was extracted with ethyl acetate (3 × 500 mL). The combined organic layers were washed with brine, dried with sodium sulfate, and concentrated to dryness. The crude extract (5 g) was dissolved in dichloromethane and separated by gel permeation chromatography on Sephadex LH-20 (Pharmacia) (7 × 70 cm; 2 batches; eluent: dichloromethane/methanol, 80:20; flow rate 1.2 mL/min). The biologically active fractions were combined, concentrated to dryness (1.0 g), and separated by column chromatography on Merck silica gel 60 (5 × 30 cm; particle size: 0.063−0200 mm; eluents: 650 mL dichloromethane, 600 mL dichloromethane/acetone, 80:20, 400 mL methanol). The biologically active fraction (150 mg) was further separated by seven RP-18 HPLC runs [column: 16 × 250 mm, Nucleosil 100 C_{18} 7 μm (VDS optilab); eluent: methanol/water, 78:22, flow rate 8 mL/min, detection by UV absorption at 220 nm] to give 39 mg of melithiazol E (**3b**), 12 mg of melithiazol F (**3c**), 2 mg of melithiazol G (**3d**), 1 mg of melithiazol H (**3e**), and 0.4 mg of melithiazol I (**3f**).

Melithiazol A (2a): Colourless oil. − TLC: $R_f = 0.20$; hexane/ethyl acetate (1:1): $R_f = 0.56$. − HPLC: $t_R = 4.4$ min. − ^{1}H NMR ($CDCl_3$, 300 MHz): See Table 1. − ^{13}C NMR ($CDCl_3$, 150 MHz): See Table 1. − Optical rotation (methanol): $[\alpha]_D^{22} = +0.32$ ($c = 1$). − UV (CH_3CN): λ_{max} (lg ε) = 211 (4.33, sh), 227 (4.47), 256 (4.28, sh), 263 (4.11, sh), 309 (3.27, sh), 311 (3.27, sh), 327 (3.07, sh), 335 nm (2.83, sh). − IR (KBr): $\tilde{\nu} = 3093$ (w), 2980 (m, sh),

2931 (s), 2864 (w), 2822 (w, sh), 1711 (vs), 1624 (vs), 1582 (m), 1501 (w), 1449 (m), 1441 (m), 1383 (m), 1264 (m), 1281 (w, sh), 1195 (m), 1150 (vs), 1125 (w), 1091 (m), 973 (w), 925 (w), 828 (w), 804 cm^{-1} (w). − EI-MS: *m/z* (%) = 422 (2) [M^+], 407 (2), 375 (3), 359 (3), 279 (100), 180 (10). − $C_{20}H_{26}N_2O_4S_2$: calcd. 422.1334; found 422.1324. − $C_{13}H_{15}N_2OS_2$: calcd. 279.0626; found 279.0623 (HR-EI-MS).

Determination of the Absolute Configuration at *C*-11 of Melithiazol A (2a): To **2a** (0.7 mg) was added an ethanolic suspension of Raney nickel (freshly prepared from 100 mg of aluminium/nickel alloy). The mixture was kept for 4 h at 60°C in a screw-capped vial and then filtered. To the filtrate was added 200 µL of 1 N HCl and after 10 min. the mixture was concentrated to dryness. The residue was redissolved in 3 mL of methanol and, in the presence of methylene blue, a stream of singlet oxygen was bubbled through the solution for 5 h. After evaporation of the solvent, the product was hydrolyzed under nitrogen by treatment with 6 N HCl at 100°C for 18 h. The mixture was then concentrated to dryness under nitrogen. The residue was treated with 0.5 mL of 1 N HCl in 2-propanol and the resulting solution was heated for 30 min. at 100°C. After removal of the excess reagent with a stream of nitrogen, 0.5 mL of dichloromethane and 0.1 mL of trifluoroacetic acid anhydride were added and the solution was heated for a further 10 min. at 100°C. After concentration to dryness, the residue was redissolved in dichloromethane and analyzed by GC (on a Shimadzu GC-17A) in comparison with the *N*-TFA-isopropyl esters of D,L- and L-alanine on a Permabond L-Chirasil-Val column (Macherey−Nagel, 0.25 mm, 25 m); carrier gas hydrogen at 70 kPa, column temperature 80 to 160°C, 4°C/min.; t_R (D-alanine) = 3.84 min; t_R (L-alanine) = 4.16 min. In a separate experiment, the identity of the reaction product was proved by comparison with an authentic sample by GC/MS (on a Shimadzu GC-17A/QP-5000) using a Restek Crossbond XTI-5 column (0.25 mm, 30 m); carrier gas helium, 70 kPa, column temperature 80 to 160°C, 4°C/min; t_R = 7.75 min; yield 7%.

Conversion of Melithiazol A (2a) into Melithiazol B (3a): Melithiazol A (2 mg) was dissolved in 1 mL of chloroform and treated with NiO_2 (5 mg) according to the procedure described for phleomycin.[8] The reaction product was filtered and purified by RP-18 chromatography to yield 1 mg of melithiazol B.

Melithiazol B (3a): Colourless oil. − TLC: R_f = 0.46. − HPLC: t_R = 7.2 min. − ^{1}H NMR ($CDCl_3$, 600 MHz; only selected signals distinct from those of melithiazol A are listed): δ = 7.89 (s, 12-H), 7.10 (s, 9-H), 6.58 (d, *J* = 15.8 Hz, 7-H), 6.42 (dd, *J* = 15.8, 7.6 Hz, 6-H), 5.92 (br. s, 15*Z*-H), 5.36 (br. s, 15*E*-H), 2.88 (br. s, 14-CH_3). − UV (CH_3CN): λ_{max} (lg ε) = 235 (4.38), 307 nm (3.83). − CD (MeOH): λ_{max} (Δε) = 237 nm (+2.8). − EI MS; *m/z* (%): 420 [M^+] (3), 405 (3), 373 (3), 357 (4), 277 (100). − $C_{20}H_{24}N_2O_4S_2$: calcd. 420.1178; found 420.1187. − $C_{13}H_{13}N_2OS_2$: calcd. 277.0469; found 277.0465 (HR-EI-MS).

Melithiazol C (4): Colourless oil. − TLC: R_f = 0.46. − HPLC: t_R = 2.6 min. − ^{1}H NMR: See Table 1; (6*Z*)-isomer: δ = 4.90 (s, 9-H), 6.67 (d, *J* = 10 Hz, 7-H), 5.55 (dd, *J* = 7.8; 10 Hz, 6-H), 4.90 (s, 2-H), 4.20 (m, 4-H), 5.22 (t, *J* = 8 Hz, 5-H), 3.64 (s, 1-OMe), 3.61 (s, 3-OMe), 3.31 (s, 5-OMe), 2.70 (s, 11-Me), 1.21 (d, *J* = 6.9 Hz, 4-Me). − UV (CH_3OH): λ_{max} (ε_{rel}) = 235 (100), 329 (10) nm. − EI MS; *m/z* (%): 339 [M^+] (1), 324 (1), 307 (5), 292 (3), 276 (5), 196 (100). − $C_{16}H_{21}NO_5S$: calcd. 339.1140; found 339.1156. − $C_9H_{10}NO_2S$: calcd. 196.0432; found 196.0418 (HR-EI-MS).

Melithiazol D (2b): Colourless oil. − TLC: R_f = 0.13; hexane/ethyl acetate (1:1): R_f = 0.45. − HPLC: t_R = 4.0 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol A are listed): δ = 5.81 (dd, *J* = 7.5, 8.8 Hz, 11-H), 3.76 (dd, *J* = 11.2, 7.5 Hz, 12-H_a), 3.63 (dd, *J* = 11.2, 8.8 Hz, 12-H_b), 2.90 (dq, *J* = 7.1 Hz, 14-H), 1.28 (d, *J* = 7.1 Hz, 14-CH_3). − UV (CH_3OH): λ_{max} (ε_{rel}) = 215 (84), 239 (100) nm. − EI MS; *m/z* (%): 424 [M^+] (5), 409 (4), 392 (3), 377 (4), 361 (5), 281 (100). − $C_{20}H_{28}N_2O_4S_2$: calcd. 424.1491; found 424.1497. − $C_{13}H_{17}N_2OS_2$: calcd. 281.0782; found 281.0778 (HR-EI-MS).

Melithiazol E (3b): Colourless needles (methanol), m.p. 109°C. − TLC: R_f = 0.56. − HPLC: t_R = 5.7 min. − ^{1}H NMR ($CDCl_3$, 300 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 7.82 (s, 12-H), 3.38 (dq, *J* = 6.9 Hz, 14-H), 1.44 (d, *J* = 6.9 Hz, 14-CH_3). − ^{13}C NMR ($CDCl_3$, 75.5 MHz; only selected signals distinct from those of melithiazol A are listed): δ = 176.7 (C-13), 162.6 (C-10), 154.4 (C-8), 148.7 (C-11), 115.0 (C-9), 114.8 (C-12), 33.3 (C-14), 23.1 (14-CH_3). − UV (CH_3OH): λ_{max} (lg ε) = 223 (4.41), 242 (4.36), 311 (3.90) nm. − Optical rotation: $[\alpha]_D^{21}$ = + 100.6 (*c* = 5, methanol) (ref.: 109 in $CHCl_3$[5]). − EI MS; *m/z* (%): 422 [M^+] (4), 407 (3), 375 (3), 359 (3), 279 (100). − $C_{20}H_{26}N_2O_4S_2$: calcd. 422.1334; found 422.1358. − $C_{13}H_{15}N_2OS_2$: calcd. 279.0626; found 279.0622 (HR-EI-MS).

Crystallographic Data of 3b: Crystal data: $C_{20}H_{26}N_2O_4S_2$, M_r = 422.55, monoclinic, space group $P2_1$, *Z* = 2, *a* = 10.348(1), *b* = 8.934(1), *c* = 12.787(1) Å, β = 108.80(1)°, *V* = 1119.1(2) Å^3, ρ_{calcd} = 1.254 g/cm^3, λ(Cu-K_α) = 1.54178 Å − Data collection was carried out by the ωscan technique (2° < θ < 57°) on a Siemens P4 diffractometer. Three standard reflections were monitored periodically and were used to apply a correction for crystal decay (< 10%). Of the 1830 measured reflections, 1698 had $I > 2\sigma(I)$. The structure was solved by direct methods and refined on F^2 for all reflections with positive F^2 using SHELXTL (Siemens). Hydrogen atoms were generated with SHELXTL after anisotropic refinement of the non-hydrogen atoms and were included in the full-matrix least-squares refinement restrained to the corresponding nonhydrogen atom and with isotropic temperature factors. The resulting *R* value for 259 variables and 1829 observations was 0.0769, based on *F* and using the weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.1773\ P)^2 + 1.0433\ P]$ where $P = (F_o^2 + 2F_c^2)/3$. The Flack parameter,[26] used as a criterion for determination of the absolute configuration, was −0.03(6).

Melithiazol F (3c): Colourless oil. − TLC: R_f = 0.56. − HPLC: t_R = 6.4 min. − ^{1}H NMR ($CDCl_3$, 300 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 7.84 (s, 12-H), 4.39 (s, 14-CH_2), 7.35 (m, phenyl-H_5). − ^{13}C NMR ($CDCl_3$, 100.6 MHz; only selected signals distinct from those of melithiazol E are listed): δ = 176.8 (s, C-13), 149.2 (s, C-11), 137.4 (phenyl), 129.2, 128.9 and 127.4 (phenyl), 116.3 (C-12), 39.7 (C-14). − UV (CH_3OH): λ_{max} (lg ε) = 223 (4.43), 241 (4.36), 312 (3.90) nm. − EI MS; *m/z* (%): 470 [M^+] (4), 455 (3), 423 (3), 407 (4), 327 (100). − $C_{24}H_{26}N_2O_4S_2$: calcd. 470.1334; found 470.1348. − $C_{17}H_{15}N_2OS_2$: calcd. 327.06258; found 327.06142 (HR-EI-MS).

Melithiazol G (3d): TLC: R_f = 0.57. − HPLC: t_R = 7.2 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 7.84 (s, 12-H), 3.15 (ddq, *J* = 7.0 Hz, 14-H), 1.85 (m, 15-H_a), 1.72 (m, 15-H_b), 1.40 (d, *J* = 7.0 Hz, 14-CH_3), 0.96 (t, *J* = 7 Hz, 16-H_3). − UV (CH_3OH): λ_{max} (lg ε) = 223 (4.28), 241 (4.24), 312 (3.80) nm. − EI MS; *m/z* (%): 436 [M^+] (4), 421 (3), 389 (3), 373 (3), 341 (3), 293 (100). − $C_{21}H_{28}N_2O_4S_2$: calcd. 436.1491; found 436.1510; [(M − $C_7H_{11}O_3$), $C_{14}H_{17}N_2OS_2$]: calcd. 293.0782; found 293.0784 (HR-EI-MS).

Melithiazol H (3e): Colourless oil. − TLC: R_f = 0.32. − HPLC: t_R = 5.3 min. − ^{1}H NMR (CD_3OD, 300 MHz): δ = 1.26 (d, *J* = 7 Hz, 4-Me), 1.46 (t, *J* = 7 Hz, 15-CH_3), 3.12 (q, *J* = 7 Hz, 14-

CH_2), 3.37 (s, 5-OCH_3), 3.65 (s, 1-OCH_3, 3-OCH_3), 3.82 (t, J = 8.5 Hz, 5-H), 4.22 (dq, J = 8.5, 7 Hz, 4-H), 5.08 (s, 2-H), 6.40 (dd, J = 16, 8.5 Hz, 6-H), 6.61 (d, J = 16 Hz, 7-H), 7.40 (s, 9-H), 8.05 (s, 12-H). − UV (CH_3OH): λ_{max} (ε_{rel}) = 225 (100), 244 (96), 312 nm (32). − EI-MS; *m/z* (%): 408 [M^+] (2), 393 (1), 345 (1), 265 (100), 234 (4). − $C_{19}H_{24}N_2O_4S_2$: calcd. 408.1176; found 408.1191 (HR-EI-MS).

Melithiazol I (3f): Colourless oil. − TLC: R_f = 0.57. − HPLC: t_R = 6.9 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 7.83 (s, 12-H), 2.91 (d, J = 7.2 Hz, 14-H), 2.15 (m, 15-H), 1.01 (d, J = 6.6 Hz, 15-CH_3). − UV (CH_3OH): λ_{max} (ε_{rel}) = 223 (100), 241 (93), 312 (36) nm. − DCI MS (isobutane); *m/z*: 437 [M + H]$^+$. − $C_{21}H_{28}N_2O_4S_2$: calcd. 436.1491; found 436.1490 (HR-DCI-MS).

Melithiazol K (2c): Colourless oil. − TLC: R_f = 0.27; hexane/ethyl acetate (1:1): R_f = 0.23. − HPLC: t_R = 2.9 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol A are listed): δ = 5.86 (dd, J = 8.6, 9.2 Hz, 11-H), 3.79 (dd, J = 11.3 Hz, 12-H_a), 3.56 (dd, 12-H_b), 3.13 (d, J = 5.2 Hz, 15-H_a), 2.92 (d, 15-H_b), 1.70 (s, 14-CH_3). − ^{13}C NMR ($CDCl_3$, 100.6 MHz, only selected signals distinct from those of melithiazol A are listed): δ = 177.1 (s, C-13), 78.8 (d, C-11), 56.7 (s, C-14), 54.3 (t, C-15), 38.5 (t, C-12), 19.2 (q, 14-CH_3). − UV (CH_3OH): λ_{max} (lg ε) = 217 (4.07), 239 (4.12), 305 nm (3.16, sh). − DCI MS (isobutane); *m/z*: 439 [M + H]$^+$. − $C_{20}H_{26}N_2O_5S_2$ [M^+]: calcd. 438.1283; found 438.1252 (HR-DCI-MS).

Melithiazol L (2d): Colourless oil. − TLC: R_f = 0.06; hexane/ethyl acetate (1:1): R_f = 0.30. − HPLC: t_R = 3.3 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazole A are listed): δ = 5.83 (dd, J = 9.1 Hz, 11-H), 3.78 (dd, 12-H_a), 3.64 (dd, 15-H_a), 3.63 (dd, 12-H_b), 3.48 (dd, J = 6.4 Hz, 9.4 Hz, 15-H_b), 3.37 (s, 15-OCH_3), 3.06 (m, 14-H), 1.28 (d, J = 7.0 Hz, 14-CH_3). − ^{13}C NMR ($CDCl_3$, 75.5 MHz, only selected signals distinct from those of melithiazol A are listed): δ = 178.3 (s, C-13), 77.2 (d, C-11), 75.7 (t, C-15), 59.0 (q, 15-OCH_3), 39.7 (d, C-14), 38.7 (t, C-12), 16.2 (q, 14-CH_3). − UV (CH_3OH): λ_{max} (lg ε) = 217 (4.07), 240 (4.11), 280 nm (3.53, sh). − DCI MS (isobutane); *m/z*: 455 [M + H]$^+$. − $C_{21}H_{30}N_2O_5S_2$ [M^+]: calcd. 454.1596; found 454.1586 (HR-DCI-MS).

Melithiazole M (3f): Colourless oil. − TLC: R_f = 0.44; petroleum ether/diethyl ether (1:1), R_f = 0.44. − HPLC: t_R = 4.0 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 8.28 (s, 12-H), 7.14 (s, 9-H), 2.77 (s, 14-CH_3). − UV (CH_3OH): λ_{max} (ε_{rel}) = 234 (100), 310 (35) nm. − DCI MS (isobutane); *m/z*: 423 [M + H]$^+$. − $C_{19}H_{22}N_2O_5S_2$ [M^+]: calcd. 422.0970; found 422.0985 (HR-DCI-MS).

Melithiazol N (3g): Colourless oil. − TLC: R_f = 0.44; petroleum ether/diethyl ether (1:1), R_f = 0.38. − HPLC: t_R = 4.0 min. − ^{1}H NMR ($CDCl_3$, 400 MHz, only selected signals distinct from those of melithiazol B are listed): δ = 3.11 (d, J = 5.1 Hz, 15-H_a), 3.08 (d, 15-H_b), 1.89 (s, 14-CH_3). − UV (CH_3OH): λ_{max} (ε_{rel}) = 227 (100), 243 (93), 311 (33) nm. − DCI MS (isobutane); *m/z*: 437 [M + H]$^+$. − $C_{20}H_{24}N_2O_5S_2$ [M^+]: calcd. 436.1127; found 436.1113 (HR-DCI-MS).

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