Synthesis of Melithiazol B and Related Compounds via Oxidative Degradation of Myxothiazol A and Z

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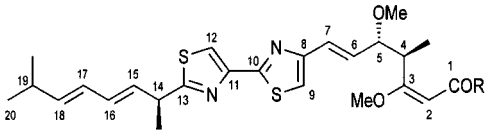
The synthesis of melithiazol B (**4**) has been accomplished in five steps and 19% overall yield starting from myxothiazol A (**1**). Key steps include the conversion of the amide into the methyl ester **4**, oxygenation to hydroperoxides **7** and **9**, and subsequent Hock cleavage to ketones **11** and **12**, followed by a Wittig reaction to give **4** and **13**. The biological activities of intermediates, melithiazol B and derivatives thereof are compared.

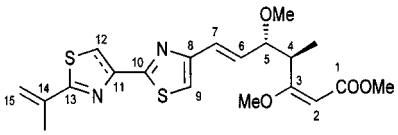
Introduction

Recently we have isolated a new group of β-methoxyacrylate fungicides, named melithiazols A–N, from various species of myxobacteria.[1] Later, further close analogs were described from *Cystobacter fuscus* and named Cystothiazols.[2] The melithiazols, and the myxothiazols A (**1**)[3] and Z (**2**),[4] contain a β-substituted β-methoxyacrylate pharmacophore, and act as inhibitors of the cytochrome bc_1 complex.[5] However, contrary to myxothiazols, all melithiazols occur only as methyl esters and lack the lipophilic heptadienyl side chain. Fortunately, these changes have no major influence on the inhibition of NADH oxidation or the antifungal activity, yet reduce their cytotoxicity for animal cell cultures by a factor of 50 to 100.[1a] As a consequence, mammalian toxicity is also greatly reduced making these compounds interesting for the development of agricultural fungicides such as the structurally related strobilurins.[6]

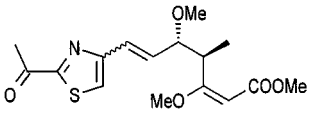
However, due to the minute amounts of melithiazols usually obtained from fermentation it has not been possible to determine their biological properties in greater detail or start a derivatisation program to investigate structure-activity relationships. Compared to a total synthesis of melithiazols,[7] a semi-synthesis from the easily accessible myxothiazols A (**1**) and Z (**2**) seemed to be advantageous as it would lead automatically to enantiopure products. To this end the unsaturated side-chain had to be cleaved next to the second thiazole ring, to give melithiazol B (**4**), or the corresponding thiazole ring had to be reduced or cleaved to give melithiazols A (**3**) and C (**5**). In addition, in the case where myxothiazol A (**1**) was the starting material, the amide also had to be converted into the methyl ester. Here we report on the semi-synthesis of melithiazol B (**4**), and in a following paper that of melithiazol C (**5**).[8]



myxothiazol A (**1**) R = NH_2
myxothiazol Z (**2**) R = OMe

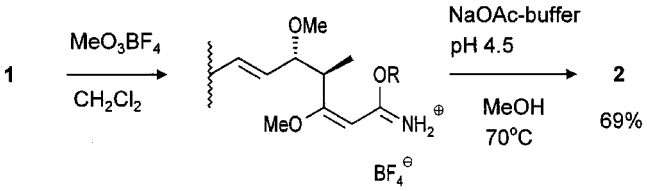


melithiazol A (**3**) 11,12 single bond
melithiazol B (**4**) 11,12 double bond



melithiazol C (**5**)

First the conversion of the amide to an ester group was investigated. Following established methods, **1** was treated with Meerwein's reagent to give the methyl **(6)** and ethyl **(6a)**[9] imidates in good yields (Scheme 1). Cleavage of these



6 R = OMe
6a R = OEt

Scheme 1. Synthesis of myxothiazol Z (**2**) from myxothiazol A (**1**): (a) Me_3OBF_4, CH_2Cl_2; (b) NaOAc-buffer pH 4.5, MeOH, 70°C (69% overall yield)

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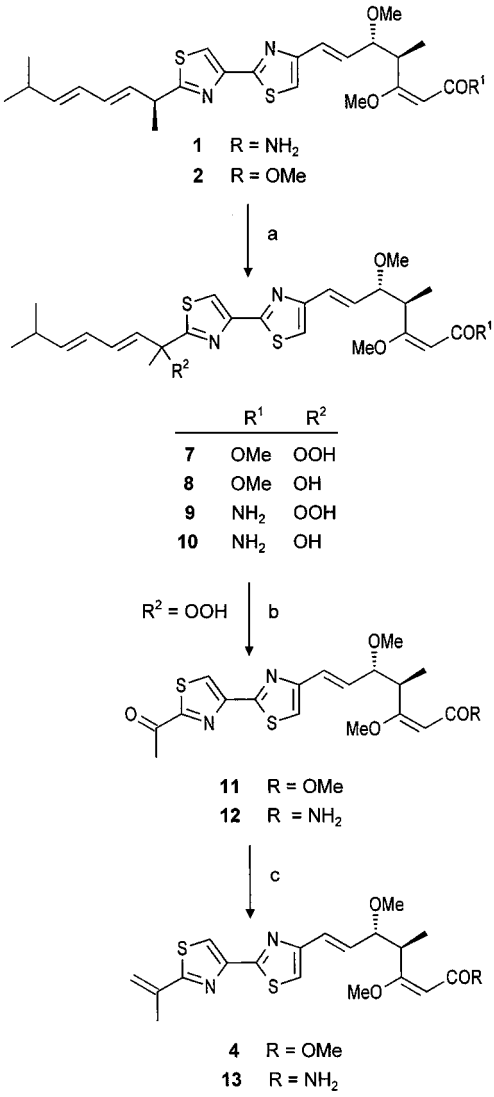
imidates under acidic conditions[10] unexpectedly reformed the amide with concomitant hydrolysis of the 2,3-enol ether.[9] After some experimentation we found that the desired methyl ester was only obtained in good yield within a narrow pH range of 4.5 ± 0.5 in an aqueous methanolic sodium acetate buffer from the methyl imidate **6** (R = CH_3). With lower or higher pH the rate of conversion into **2** drops sharply, and side-reactions become dominant. Apparently this behavior is a consequence of the conjugation of the protonated imidate with the 2,3-enol ether. When methanol was replaced by ethanol or *n*-propyl alcohol the corresponding ethyl and propyl esters were obtained preferentially from methyl imidate **6**.[11] Interestingly, at that time the methyl ester **2** was also identified for the first time as co-metabolite of the amide **1** in a *Myxococcus fulvus* strain and named myxothiazol Z.[12]

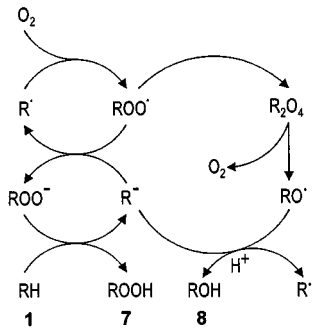
For the degradation of the C-13 side-chain, cleavage of the 15,17-diene system with ozone or osmium tetraoxide was envisaged. However, even at temperatures down to –90 °C no selectivity was observed and complex mixtures resulted from additional partial cleavage of the 2,3- and 6,7-double bonds. Similarly, according to an earlier observation,[13] myxothiazol A (**1**) is rapidly degraded to complex mixtures by atmospheric oxygen in the presence of a base. However, in this case, the ^1H NMR spectra showed clear signals for the C-1 to C-7 side-chain and the adjacent thiazole, which indicated that reactions occurred exclusively in the heptadienyl side-chain. After extensive variation of reaction conditions the best results were obtained with 5 equivalents of potassium *tert*-butoxide in *tert*-butyl alcohol saturated with molecular oxygen at room temperature. Under these conditions oxidation was complete within five minutes to give only two products in a 3:1 ratio according to HPLC analysis (Scheme 2).

They were identified as the 14-hydroperoxide **7** and the corresponding alcohol **8**. From the specific reaction conditions this is the result of a radical chain reaction[14] which is initiated by formation of a C-14 carbanion and its oxidation to a radical (see Scheme 3). The C-14 radical is captured by O_2 to give a 14-hydroperoxy radical which is reduced by another C-14 carbanion to hydroperoxide **7**, thus propagating the chain. In a side-reaction two hydroperoxy radicals recombine to a tetraoxide,[15] which spontaneously decays into an oxygen molecule and two 14-alkoxide radicals. The latter are reduced by a 14-carbanion to alcohol **8**, a reaction which also propagates the chain.[16] Accordingly, when the reaction was performed in presence of $^{18}O_2$ only [14-$^{18}O_2$]hydroperoxide and [14-^{18}OH]alcohol were isolated and identified by MS. Products of chain termination reactions were not detected.

The hydroperoxide **7** was an ideal intermediate for a Hock cleavage[17] of either the C-13/C-14 or C-14/C-15 bond. Fortunately, on treatment of the crude oxidation mixture **7/8** with BF_3–diethyl ether[18] and aqueous workup, only one product, the methyl ketone **11**,[19] was obtained in an overall yield of 46% from ester **2**. This two-step procedure could also be applied to myxothiazol A (**1**) leading to the corresponding ketoamide **12**,[20] which in turn was transformed into **11** by the imino ester procedure described above.



Scheme 2. Synthesis of melithiazol B (**3**) and derivatives: (a) O_2, potassium *tert*-butoxide, *tert*-butyl alcohol; (b) $BF_3{\cdot}Et_2O$, diethyl ether (46% based on **2**); (c) $Ph_3PCH_2Br/NaNH_2$, THF (60%)



Scheme 3. Proposed radical chain mechanism for the oxidation of myxothiazol **1** to 14-hydroperoxide **7** and 14-alcohol **8** (R represents the C-14 myxothiazol residue)

The final step of the synthesis of melithiazol B (**4**) was a Wittig reaction of ketone **11** with "instant-ylide" (a mixture of methyl triphenylphosphonium bromide and sodium

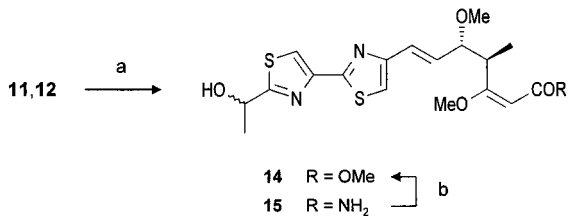
Table 1. Biological activities and lipophilicities of selected myxothiazol and melithiazol derivatives

Compound (pharmacophore)[a]	*Botrytis cinerea* Inhibition zone	*Hansenula anomala* at 2 μg/disc (mm)	Cytotoxicity IC_{50} (ng/mL)[b]	Inhibition of NADH oxidation IC_{50} (ng/mL)[c]	Lipophilicity log P_{OW}[d]
1 (A)	16	15	1	11	5.29
2 (E)	13	16	2	17	7.17
13 (A)	17	12	250	37	3.26
4 (E)	29	22	20	18	4.75
12 (A)	12	<7	400	230	2.25
11 (E)	39	26	55	29	3.37
15 (A)	<7	<7	2500	4400	1.05
14 (E)	19	20	220	37	2.36
10 (A)	<7	<7	30	76	3.47
8 (E)	17	12	40	20	4.25

[a] A = amide type, E = ester type. – [b] The cytotoxity was measured by an MTT assay with the mouse fibroblast cell line L929 for details see ref.[1a] – [c] The inhibition of the NADH oxidation was measured with submitochondrial particles isolated from beef heart, for details see ref.[1a] – [d] Estimated by RP-18 TLC according to ref.[23]

amide)[21] affording **4** in 60% yield. The chromatographic and spectroscopic properties, including the CD spectra, of the semi-synthetic product were in full agreement with those of the natural melithiazol B (**4**).[22] In addition, it follows from the CD experiments that melithiazol B (**4**), and thus the other melithiazols, has the same absolute configuration at C-4 and C-5 as myxothiazol A (**1**).

Further derivatives of melithiazol B were obtained by reduction of the ketones **11** and **12** with $NaBH_4$, leading to the alcohols **14** and **15** (Scheme 4),[19] and by treating **12** with "instant-ylide" to give amide **13**. Alternatively the 14-hydroxy ester **14** could also be obtained from amide **15** by using the imino ester procedure mentioned above.



Scheme 4. (a) $NaBH_4$, MeOH; (b) 1. Me_3OBF_4, CH_2Cl_2, 2. NaOAc-buffer, MeOH, 70°C (50%)

The biological activities and the overall lipophilicities expressed by the octanol/water partition coefficients, log P_{OW}, of selected compounds are summarized in Table 1. These data confirm earlier results[1a] according to which the highest activity at the target is observed with myxothiazols A (**1**) and Z (**2**), and remains high over a broad range of decreasing lipophilicity. Only with compounds having two polar end-groups, such as amides **12** and **15**, does the activity drop sharply.

Contrary to target activity, cytotoxicity is decreased significantly with decreased lipophilicity as can be see by comparison of the esters **2**, **11** and **14**. The antifungal activity of compounds having a lipophilic side-chain is moderate-to-low regardless of target activity and overall lipophilicity, whereas compounds with an ester pharmacophore and short polar side-chain (**4**, **11** and **14**) show high antifungal activity. In connection with their moderate to low toxicity they are interesting compounds for synthetic fungicides.

Experimental Section

General: Analytical TLC: TLC aluminum sheets, silica gel Si 60 F_{254}, 0.2 mm (Merck), detection: UV absorption = 254 nm. – Preparative TLC: Precoated TLC plates, silica gel Si 60 F_{254}, 0.25, 0.5 and 1.0 mm layer thickness (Merck). – Analytical HPLC: column A: Nucleosil RP-18-7-100, 250 × 4 mm (Macherey Nagel), UV detection 254 nm, flow rate 1.5 mL/min; column B: ET 250/4 Nucleosil 100–7 (Macherey-Nagel), UV-detection 254 nm, flow rate 1.0 mL/min. – Preparative HPLC: column C: Nucleosil RP-18-7-100, 250 × 20 mm (Macherey Nagel), UV-detection 254 nm, flow rate 12 mL/min; column D: Nucleosil 100 (Knauer), 7 μ, 250 × 20 mm), UV-detection 254 nm, flow rate 15 mL/min. – IR: FT-IR spectrometer 20 DXB (Nicolet). – Column chromatography: silica gel (SiO_2, 0.063–0.200 mm mesh, Merck). – UV: spectrometer UV-2102 PC (Shimadzu), solvent: MeOH (Uvasol, Merck). – CD: Jasco J1600. – NMR: Spectrometer WM-400 and AM-300 (Bruker), ^1H: 400 and 300 MHz, ^{13}C: 100.6 and 75.5 MHz, $CDCl_3$ as solvent standard δ = 7.25. – MS: EI and DCI: spectrometer MAT 95 (Finnigan), resolution M/ΔM = 1000, high-resolution data from peak matching $M/\Delta M$ = 10000.

Myxothiazol Z (2): Myxothiazol A (**1**) (75% purity, 1.0 g, 1.54 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2, trimethyloxonium tetrafluoroborate (342 mg, 2.32 mmol) was added and the mixture stirred for 2.5 h at room temp. The solvent was evaporated in vacuo and the residue of methyl imidate **6** dissolved in 300 mL of MeOH and 150 mL of 1 M NaOAc buffer (pH = 4.5). After stirring for 4 h at 75°C the MeOH was removed in vacuo, and the aqueous layer extracted four times with diethyl ether. The combined organic layers were dried with $MgSO_4$, and evaporated in vacuo. Purification of the crude product by column chromatography (SiO_2, petroleum ether/diethyl ether 75:25) gave **2** (532 mg, 69%). – R_f = 0.49 (petroleum ether/diethyl ether 60:40). – IR (KBr): $\tilde{\nu}$ = 2963 cm^{-1} (m), 2934 (m), 1712 (s), 1625 (s), 1193 (m), 1147 (s), 1095 (m). – UV (MeOH): λ_{max} (lg ε) = 233 nm (4.83), 313 (4.15). – ^1H NMR (300 MHz): δ = 1.00 (d, J = 6.8 Hz, 3 H, 20-H_3), 1.00 (d, J = 6.8 Hz, 3 H, 19-CH_3), 1.20 (d, J = 6.9 Hz, 3 H, 4-CH_3), 2.33 (qqd, J = 6.7 and 6.8 Hz, 1 H, 19-H), 3.32 (s, 3 H, 5-OCH_3), 3.59 (s, 3 H, 3-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 3.80 (dd, J = 7.7 and 7.7 Hz, 1 H, 5-H), 3.92 (dq, J = 6.9 and 7.5 Hz, 1 H, 14-H), 4.16 (dq, J = 6.9 and 7.7 Hz, 1 H, 4-H), 4.94 (s, 1 H, 2-H), 5.67 (dd, J = 6.7 and 15.1 Hz, 1 H, 18-H), 5.78 (dd, J = 7.5 and 15.0 Hz, 1 H, 15-H), 6.01 (dd, J = 10.3 and 15.1 Hz, 1 H, 17-H), 6.17 (dd, J = 10.3 and 15.0 Hz, 1 H, 16-H), 6.39 (dd, J = 7.7

and 15.7 Hz, 1 H, 6-H), 6.56 (d, J = 15.7 Hz, 1 H, 7-H), 7.10 (s, 1 H, 9-H), 7.83 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz): δ = 14.1 (4-CH_3), 20.9 (14-CH_3), 22.3 (C-20), 22.3 (19-CH_3), 31.1 (C-19), 39.8 (C-4), 41.3 (C-14), 50.8 (1-OCH_3), 55.6 (3-OCH_3), 57.0 (3-OCH_3), 84.4 (C-5), 91.5 (C-2), 115.2 (C-9), 115.6 (C-12), 125.6 (C-7), 126.6 (C-17), 131.6 (C-6), 131.9 (C-16), 132.5 (C-15), 142.4 (C-18), 149.0 (C-11), 154.4 (C-8), 162.6 (C-10), 167.3 (C-1), 176.3 (C-13), 176.8 (C-3). – DCI MS (120 eV, *i*-butane): *m*/*z* = 503 [M + H$^+$], 471. – $C_{26}H_{35}N_2O_4S_2$: calcd. 503.2038; found 503.2001 (DCI MS).

14-Hydroperoxide 7 and 14-Alcohol 8: Compound **2** (49 mg, 98 μmol) was dissolved in 10 mL of anhydrous *tert*-butyl alcohol. Oxygen was bubbled through the solution for 15 min and 0.5 mL of a 1 M solution of potassium *tert*-butoxide in *tert*-butyl alcohol was added with continued bubbling of oxygen through the solution. After stirring for 10 min the reaction was quenched with 1 mL of water and the solvent was removed in vacuo. The residue was partitioned between diethyl ether and water and the aqueous layer was extracted twice with diethyl ether. The combined organic layers were dried with $MgSO_4$ and evaporated in vacuo. Analysis of the crude product by analytical HPLC (column B, eluent: petroleum ether/*tert*-butyl methyl ether 75:25, 2% MeOH) indicated a product mixture which consisted of **7** (75%) and **8** (25%). Separation by preparative HPLC (column D, eluent: petroleum ether/*tert*-butyl methyl ether 75:25, 0.5% MeOH) yielded **7** (22 mg, 42%) and **8** (11 mg, 22%).

7: R_f = 0.55 (petroleum ether/diethyl ether 30:70). – IR (KBr): $\tilde{\nu}$ = 2960 cm^{-1} (s), 2932 (m), 1711 (s), 1624 (s), 1456 (m), 1439 (m), 1383 (m), 1294 (m), 1265 (m), 1194 (m), 1146 (s), 1093 (s), 1040 (m), 991 (m), 970 (m), 926 (m), 825 (m). – UV (MeOH): λ_{max} (lg ε) = 233 nm (4.56), 310 (3.98). – ^{1}H NMR (300 MHz) (selected signals): δ = 0.99 (d, J = 6.8 Hz, 3 H, 20-H_3), 0.99 (d, J = 6.8 Hz, 3 H, 19-CH_3), 1.86 (s, 3 H, 14-CH_3), 2.33 (m, 1 H, 19-H), 5.74 (dd, J = 6.7 and 15.5 Hz, 1 H, 18-H), 5.93 (dd, J = 15.6 Hz, 1 H, 15-H), 6.02 (dd, J = 10.2 and 15.5 Hz, 1 H, 17-H), 6.24 (dd, J = 10.2 and 15.6 Hz, 1 H, 16-H), 7.09 (s, 1 H, 9-H), 7.96 (s, 1 H, 12-H), 9.32 (br. s, 1 H, 14-OOH). – ^{13}C NMR (75.5 MHz) (selected signals): δ = 22.2 (C-20), 22.2 (19-CH_3), 24.5 (14-CH_3), 31.2 (C-19), 85.8 (C-14), 115.3 (C-9), 116.7 (C-12), 126.1 (C-17), 130.2 (C-15), 134.1 (C-16), 145.1 (C-18), 148.7 (C-11), 154.6 (C-8), 174.1 (C-13). – DCI MS (120 eV, *i*-butane): *m*/*z* = 534 [M$^+$], 516, 422. – $C_{26}H_{34}N_2O_6S_2$: calcd. 534.1858; found 534.1858 (DCI MS).

8: R_f = 0.46 (petroleum ether/diethyl ether 30:70). – IR (KBr): $\tilde{\nu}$ = 2960 cm^{-1} (m), 2927 (m), 1712 (s), 1624 (s), 1438 (m), 1383 (m), 1262 (m), 1193 (m), 1146 (s), 1125 (s), 1094 (s), 1040 (s), 1031 (s), 992 (m), 970 (m), 801 (m). – UV (MeOH): λ_{max} (lg ε) = 230 nm (4.55), 314 (3.91). – ^{1}H NMR (300 MHz) (selected signals): δ = 0.99 (d, J = 6.7 Hz, 3 H, 20-H_3), 0.99 (d, J = 6.7 Hz, 3 H, 19-CH_3), 1.79 (s, 3 H, 14-CH_3), 2.32 (m, 1 H, 19-H), 5.72 (dd, J = 6.7 and 15.2 Hz, 1 H, 18-H), 5.96 (dd, J = 15.3 Hz, 1 H, 15-H), 6.01 (dd, J = 10.2 and 15.2 Hz, 1 H, 17-H), 6.32 (dd, J = 10.2 and 15.3 Hz, 1 H, 16-H), 7.08 (s, 1 H, 9-H), 7.90 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz) (selected signals): δ = 22.2 (C-20), 22.2 (19-CH_3), 29.7 (14-CH_3), 31.1 (C-19), 74.9 (C-14), 115.2 (C-9), 116.3 (C-12), 126.1 (C-17), 129.8 (C-15), 134.8 (C-16), 143.9 (C-18), 149.0 (C-11), 154.5 (C-8), 177.8 (C-13). – DCI MS (120 eV, *i*-butane): *m*/*z* = 519 [M + H$^+$], 487, 375, 349, 139. – $C_{26}H_{34}N_2O_5S_2$: calcd. 518.1909; found 518.1897 (DCI MS).

14-Alcohol 10: Compound **1** (20 mg, 41.1 μmol) was oxidized as described for the synthesis of **7** and **8**. The reaction mixture was not worked up but treated with 500 μL MeOH and $NaBH_4$ (5 mg, 129 μmol). After stirring for a further 20 min the solvent was removed in vacuo. The residue was partitioned between diethyl ether and water and the aqueous layer was extracted twice with diethyl ether. The combined organic layer was dried with $MgSO_4$, and evaporated in vacuo. Isolation by preparative HPLC (column C: eluent CH_3CN/water 50:50) gave **9** (10 mg, 48%). – R_f = 0.61 (dichloromethane/methanol 90:10). – IR (KBr): $\tilde{\nu}$ = 3340 (m), 2961 (m), 2932 (m), 1662 (s), 1621 (m), 1594 (s), 1453 (m), 1411 (m), 1327 (m), 1215 (s), 1184 (m), 1125 (m), 1093 (s), 1041 (m), 991 (m), 969 (m). – UV (MeOH): λ_{max} (lg ε) = 231 nm (4.69), 313 (3.99). – ^{1}H NMR (300 MHz) (selected signals): δ = 0.99 (d, J = 6.7 Hz, 3 H, 20-H_3), 0.99 (d, J = 6.7 Hz, 3 H, 19-CH_3), 1.79 (s, 3 H, 14-CH_3), 2.32 (m, 1 H, 19-H), 5.72 (dd, J = 6.7 and 15.2 Hz, 1 H, 18-H), 5.96 (dd, J = 15.3 Hz, 1 H, 15-H), 6.01 (dd, J = 10.2 and 15.2 Hz, 1 H, 17-H), 6.32 (dd, J = 10.2 and 15.3 Hz, 1 H, 16-H), 7.12 (s, 1 H, 9-H), 7.89 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz) (selected signals): δ = 29.6 (14-CH_3), 22.2 (C-20), 22.2 (19-CH_3), 31.2 (C-19), 74.9 (C-14), 115.2 (C-9), 116.4 (C-12), 126.1 (C-17), 129.8 (C-15), 134.9 (C-16), 143.8 (C-18), 149.0 (C-11), 154.4 (C-8), 178.0 (C-13). – DCI MS (120 eV, *i*-butane): *m*/*z* = 504 [M + H$^+$]. – $C_{25}H_{33}N_3O_4S_2$: calcd. 503.1913; found 503.1877 (DCI MS).

Keto Ester 11: Compound **1** (49 mg, 98 μmol) was oxidized as described above for the synthesis of **7** and **8** and the crude product dissolved in 20 mL anhydrous diethyl ether. Under nitrogen 50 μL BF_3–diethyl ether (48%) was added, the mixture was stirred for 20 min at room temp. and then quenched with an aqueous $NaHCO_3$ solution. The organic solvent was removed in vacuo and the aqueous layer extracted three times with ethyl acetate. The combined organic layers were dried with $MgSO_4$ and evaporated in vacuo. Purification of the crude product by preparative TLC (solvent: petroleum ether/diethyl ether 50:50) yielded **11** (23 mg, 46%). – R_f = 0.46 (petroleum ether/diethyl ether 50:50). – IR (KBr): $\tilde{\nu}$ = 2928 cm^{-1} (m), 1710 (s), 1692 (s), 1623 (s), 1480 (m), 1469 (m), 1457 (m), 1438 (m), 1413 (m), 1382 (m), 1360 (m), 1263 (s), 1192 (m), 1147 (s), 1126 (s), 1093 (s), 1055 (s), 1036 (s), 971 (m), 927 (m), 824 (m), 805 (m). – UV (MeOH): λ_{max} (lg ε) = 234 nm (4.37), 310 (3.85).– ^{1}H NMR (300 MHz): δ = 1.21 (d, J = 6.9 Hz, 3 H, 4-CH_3), 2.77 (s, 3 H, 15-H_3), 3.33 (s, 3 H, 5-OCH_3), 3.60 (s, 3 H, 3-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 3.82 (dd, J = 7.5 and 7.5 Hz, 1 H, 5-H), 4.16 (dq, J = 7.0 and 7.5 Hz, 1 H, 4-H), 4.96 (s, 1 H, 2-H), 6.45 (dd, J = 7.5 and 15.7 Hz, 1 H, 6-H), 6.57 (d, J = 15.7 Hz, 1 H, 7-H), 7.14 (s, 1 H, 9-H), 8.27 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz): δ = 26.0 (C-15), 115.8 (C-9), 122.9 (C-12), 125.1 (C-7), 132.3 (C-6), 151.3 (C-11), 154.8 (C-8), 161.3 (C-10), 167.1 (C-13), 191.4 (C-14). – DCI MS (120 eV, *i*-butane): *m*/*z* = 423 [M + H$^+$], 391, 279. – $C_{19}H_{22}N_2O_5S_2$: calcd. 422.0970; found 422.0967 (DCI MS).

Ketoamide 12: The procedure described above for the synthesis of **11** was applied to myxothiazol A (**1**) (48 mg, 98 μmol). Isolation by preparative HPLC (column C: eluent MeOH/water 70:30) gave **12** (14 mg, 35%). – R_f = 0.51(dichloromethane/methanol 90:10). – IR (KBr): $\tilde{\nu}$ = 1685 cm^{-1} (s), 1601 (s), 1472 (m), 1456 (m), 1437 (m), 1416 (m), 1361 (m), 1327 (m), 1271 (m), 1215 (m), 1189 (m), 1090 (s), 964 (m), 825 (m), 668 (m). – UV (MeOH): λ_{max} (lg ε) = 232 nm (4.54), 309 (4.03). – ^{1}H NMR (300 MHz): δ = 1.16 (d, J = 6.9 Hz, 3 H, 4-CH_3), 2.77 (s, 3 H, 15-H_3), 3.33 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.82 (dd, J = 7.3 and 7.5 Hz, 1 H, 5-H), 4.13 (dq, J = 6.9 and 7.3 Hz, 1 H, 4-H), 4.93 (s, 1 H, 2-H), 6.45 (dd, J = 7.5 and 15.8 Hz, 1 H, 6-H), 6.57 (d, J = 15.8 Hz, 1 H, 7-H), 7.17 (s, 1 H, 9-H), 8.28 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2 (4-CH_3), 26.0 (C-15), 39.4 (C-4), 55.1 (3-OCH_3), 56.8 (5-OCH_3), 85.0 (C-5), 94.0 (C-2), 115.9 (C-9), 122.9 (C-12), 125.5 (C-7), 131.9 (C-6), 151.2 (C-11), 154.7 (C-8), 161.3 (C-10),

167.0 (C-13), 169.2 (C-1), 172.0 (C-3), 191.4 (C-14). – DCI MS (120 eV, *i*-butane): *m/z* = 408 [M + H$^+$], 376. – $C_{18}H_{22}N_3O_4S_2$: calcd. 408.1052; found. 408.1016 (DCI MS).

Melithiazol B (4): Anhydrous THF (400 µL) was added to a mixture of methyl triphenylphosphonium bromide/sodium amide (Fluka; 25 mg, 60 µmol). After stirring for 15 min at room temp. a solution of **11** (10 mg, 23.7 µmol) in 400 µL of THF was added. The reaction mixture was stirred for 1.5 h and the solvent was removed in vacuo. The residue was partitioned between diethyl ether and water and the aqueous layer was extracted twice with diethyl ether. The combined organic layer was dried with $MgSO_4$, and evaporated in vacuo. Isolation by preparative HPLC (column C: eluent MeOH/water 75:25) gave **4** (6 mg, 60%). – R_f = 0.58 (petroleum ether/diethyl ether 50:50). – IR (KBr): $\tilde{\nu}$ = 1711 cm^{-1} (m), 1624 (s), 1490 (m), 1454 (m), 1438 (m), 1413 (m), 1382 (m), 1270 (m), 1263 (m), 1193 (m), 1146 (s), 1226 (s), 1093 (s), 1052 (m). – UV (MeOH): λ_{max} (lg ε) = 234 nm (4.38), 311 (3.83). – CD (MeOH): λ_{max} (Δε) = 237 nm (+2.07).[22] – ^{1}H NMR (300 MHz): δ = 1.21 (d, *J* = 6.9 Hz, 3 H, 4-CH$_3$), 2.27 (s, 3 H, 14-CH$_3$), 3.33 (s, 3 H, 5-OCH$_3$), 3.60 (s, 3 H, 3-OCH$_3$), 3.66 (s, 3 H, 1-OCH$_3$), 3.81 (dd, *J* = 7.5 and 7.5 Hz, 1 H, 5-H), 4.17 (dq, *J* = 6.7 and 7.5 Hz, 1 H, 4-H), 4.96 (s, 1 H, 2-H), 5.35 (s, 1 H, 15-H$_A$), 5.90 (s, 1 H, 15-H$_B$), 6.41 (dd, *J* = 7.5 and 15.8 Hz, 1 H, 6-H), 6.57 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.09 (s, 1 H, 9-H), 7.88 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz) (selected signals): δ = 20.5 (14-CH$_3$), 115.3 and 115.6 (C-9 and C-12), 117.2 (C-15), 125.6 (C-7), 131.7 (C-6), 149.7 (C-11), 154.5 (C-8), 162.4 (C-10), 169.8 (C-13), 137.8 (C-14). – DCI MS (120 eV, *i*-butane): *m/z* = 421 [M + H$^+$], 389. – $C_{20}H_{24}N_2O_4S_2$: calcd. 420.1177; found 420.1173 (DCI MS).

Amide Analog of Melithiazol B (13): The method described above for the preparation of **4** was applied to **12** (10 mg, 24.6 µmol). Isolation by preparative HPLC (column C: eluent MeOH/water 70:30) gave **13** (6 mg, 60%). – R_f = 0.59 (dichloromethane/methanol 90:10). – IR (KBr): $\tilde{\nu}$ = 3326 cm^{-1} (m), 2931 (m), 1684 (s), 1653 (s), 1600 (s), 1490 (m), 1457 (m), 1209 (m), 1127 (m), 1215 (m), 1098 (s), 967 (m). – UV (MeOH): λ_{max} (lg ε) = 234 nm (4.64), 310 (4.13). – ^{1}H NMR (300 MHz): δ = 1.16 (d, *J* = 6.9 Hz, 3 H, 4-CH$_3$), 2.26 (s, 3 H, 14-CH$_3$), 3.32 (s, 3 H, 5-OCH$_3$), 3.56 (s, 3 H, 3-OCH$_3$), 3.80 (dd, *J* = 7.5 and 7.5 Hz, 1 H, 5-H), 4.10 (dq, *J* = 6.9 and 7.5 Hz, 1 H, 4-H), 4.93 (s, 1 H, 2-H), 5.35 (s, 1 H, 15-H$_A$), 5.90 (s, 1 H, 15-H$_B$), 6.41 (dd, *J* = 7.5 and 15.8 Hz, 1 H, 6-H), 6.57 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.17 (s, 1 H, 9-H), 7.87 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz): δ = 14.3 (4-CH$_3$), 20.5 (14-CH$_3$), 39.6 (C-4), 55.1 (3-OCH$_3$), 56.8 (5-OCH$_3$), 85.2 (C-5), 94.3 (C-2), 115.4 and 115.6 (C-9 and C-12), 117.4 (C-15), 126.0 (C-7), 131.3 (C-6), 137.8 (C-14), 149.6 (C-11), 154.4 (C-8), 162.5 (C-10), 169.9 (C-13), 169.2 (C-1), 171.7 (C-3). – DCI MS (120 eV, *i*-butane): *m/z* = 406 [M + H$^+$], 374. – $C_{19}H_{23}N_3O_3S_2$: calcd. 405.1181; found 405.1170 (DCI MS).

14-Hydroxy Ester 14: a) A solution of **11** (10 mg, 23.6 µmol) in 1 mL of methanol was treated with sodium borohydride (1 mg, 26.3 µmol). After stirring for 20 min the solvent was removed in vacuo. The residue was partitioned between ethyl acetate and water and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried with $MgSo_4$ and evaporated to give **14**, quantitatively. b) Amide **15** (25 mg, 61.1 µmol) was treated with Meerwein's salt and hydrolyzed as described above. Isolation by preparative HPLC (column C: eluent MeOH/water 60:40) gave **14** (13 mg, 50%). – R_f = 0.47 (petroleum ether/diethyl ether 70:30). – IR (KBr): $\tilde{\nu}$ = 2934 cm^{-1} (m), 1709 (s), 1623 (m), 1455 (m), 1438 (m), 1413 (m), 1383 (m), 1294 (m), 1265 (m), 1194 (m), 1147 (s), 1226 (s), 1093 (s), 1050 (m), 970 (m). – UV (MeOH): λ_{max}(lg ε) = 222 nm (4.55), 243 (4.51), 312 (4.03). – ^{1}H NMR (300 MHz): δ = 1.21 (d, *J* = 6.7 Hz, 3 H, 4-CH$_3$), 1.67 (d, *J* = 6.5 Hz, 3 H, 15-H$_3$), 3.33 (s, 3 H, 5-OCH$_3$), 3.60 (s, 3 H, 3-OCH$_3$), 3.66 (s, 3 H, 1-OCH$_3$), 3.81 (dd, *J* = 7.5 and 7.5 Hz, 1 H, 5-H), 4.17 (dq,, *J* = 6.7 and 7.5 Hz 1 H, 4-H), 4.96 (s, 1 H, 2-H), 5.18 (q, *J* = 6.5 Hz, 1 H, 14-H), 6.41 (dd, *J* = 7.5 and 15.8 Hz, 1 H, 6-H), 6.57 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.09 (s, 1 H, 9-H), 7.92 (s, 1 H, 12-H). – ^{13}C NMR (75.5 MHz): δ = 24.1 (C-14), 68.2 (C-14), 115.2 and 116.1 (C-9 and C-12), 125.5 (C-7), 131.8 (C-6), 149.1 (C-11), 154.5 (C-8), 162.1 (C-10), 176.4 (C-13). – DCI MS (120 eV, *i*-butane): *m/z* = 425 [M + H$^+$], 393. – $C_{19}H_{25}N_2O_5S_2$: calcd. 425.1205; found 425.1187 (DCI MS).

14-Hydroxyamide 15: The procedure described for the preparation of **14** was applied to **13** (5.0 mg, 12.3 µmol) yielding **15** (5.0 mg, 99%). – R_f = 0.47 (dichloromethane/methanol 90:10). – ^{1}H NMR (300 MHz): δ = 1.16 (d, *J* = 6.9 Hz, 3 H, 4-CH$_3$), 1.67 (d, *J* = 6.5 Hz, 3 H, 15-H$_3$), 3.32 (s, 3 H, 5-OCH$_3$), 3.56 (s, 3 H, 3-OCH$_3$), 3.80 (dd, *J* = 7.5 and 7.5 Hz, 1 H, 5-H), 4.10 (dq, *J* = 6.9 and 7.5 Hz, 1 H, 4-H), 4.93 (s, 1 H, 2-H), 5.18 (q, *J* = 6.5 Hz, 1 H, 14-H), 6.41 (dd, *J* = 7.5 and 15.8 Hz, 1 H, 6-H), 6.57 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.09 (s, 1 H, 9-H), 7.92 (s, 1 H, 12-H). – DCI MS (120 eV, *i*-butane): *m/z* = 410 [M + H$^+$], 378. – $C_{18}H_{23}N_3O_4S_2$: calcd. 409.1130; found 409.1101 (DCI MS).

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