Neighbouring-Group Assisted Thiazole-Ring Cleavage by DIBAL-H: An Expeditious Synthesis of Melithiazol C from Myxothiazol A

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The semi-synthesis of melithiazol C (**2b**) has been accomplished in 4 steps and 41% overall yield starting from myxothiazol A (**1a**) produced by fermentation of *Myxococcus fulvus*. Key steps are a novel reductive cleavage of a thiazole ring by DIBAL-H and the conversion of the amide **2a** into the methyl ester **2b** via an imino ester. The biological activities of **2b** and of derivatives of its 10-acetyl group are described.

Introduction

Recently we and others have isolated new β-methoxyacrylate fungicides named melithiazols[1] and cystothiazols from various species of myxobacteria (Figure 1).[2] Compared to the well-known myxothiazol A (**1a**)[3] they lack the lipophilic heptadienyl side-chain which reduces their mammalian toxicity by a factor of 50−100 while the antifungal activity is retained or, in some cases, even significantly improved.[1] This makes the melithiazols interesting leads for the development of agricultural fungicides.

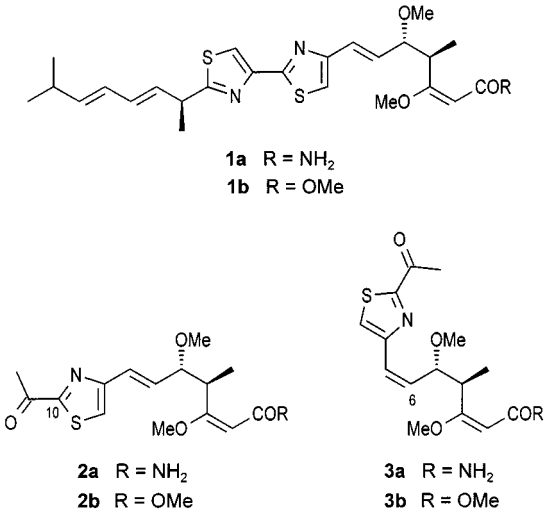

Figure 1. Structures of myxothiazol A (**1a**) and Z (**1b**), melithiazol C (**2b**), (6*Z*)-melithiazol C (**3b**) and their amide analogs **2a** and **3a**

However, due to the extremely low productivity of the fermentation process, sufficient material for the evaluation of their biological properties in-vitro and on plants was not available. This applies in particular to melithiazol C (**2b**), which in addition was obtained only as an inseparable 7:2 mixture of (6*E*)/(6*Z*) isomers **2b**, **3b**.[1a]

In a preceding paper we described the synthesis of melithiazol B from the readily available myxothiazol A (**1a**) by oxidative cleavage of the side-chain.[4] Here we report on a novel reductive transformation of myxothiazol A into melithiazol C (**2b**), and the derivatisation of its 10-acetyl group.

Results and Discussion

Synthesis of Melithiazol C (2b)

In the course of derivatisation of the β-methoxyacrylate pharmacophore of myxothiazol A (**1a**) and Z (**1b**)[5] we observed that the ester function in **1b** was smoothly reduced to the corresponding alcohol by DIBAL-H in THF.[6] Surprisingly, when the solvent was changed to dichloromethane the NMR spectra indicated that, in addition to ester reduction, significant changes in the side-chain had occurred. After hydrolytic workup the 1-hydroxy analogue of melithiazol C was isolated in low yield and identified by spectroscopic methods.

For optimization of this interesting side reaction myxothiazol A (**1a**) was chosen in order to avoid concomitant reduction of the ester group of the pharmacophore by DIBAL-H. After some experimentation we found that a minimum of three mol of DIBAL-H was necessary for complete conversion into the melithiazol C amide **2a**. However, depending on the quality of **1a** and of the DIBAL-H used, up to 7.5 mol of DIBAL-H had to be added to obtain the green-black colour indicating an excess of the reducing agent. Furthermore, temperatures close to −50 °C were found to be optimal.[7] As a second cleavage product the amine **5** could be extracted from the aqueous phase after pH adjustment, and was purified by bulb-to-bulb distillation. Obviously this reductive cleavage of myxothiazol A (**1a**) opens a short route to the desired melithiazol C (**2b**). Thus, amide **2a** was transformed along established lines[4]

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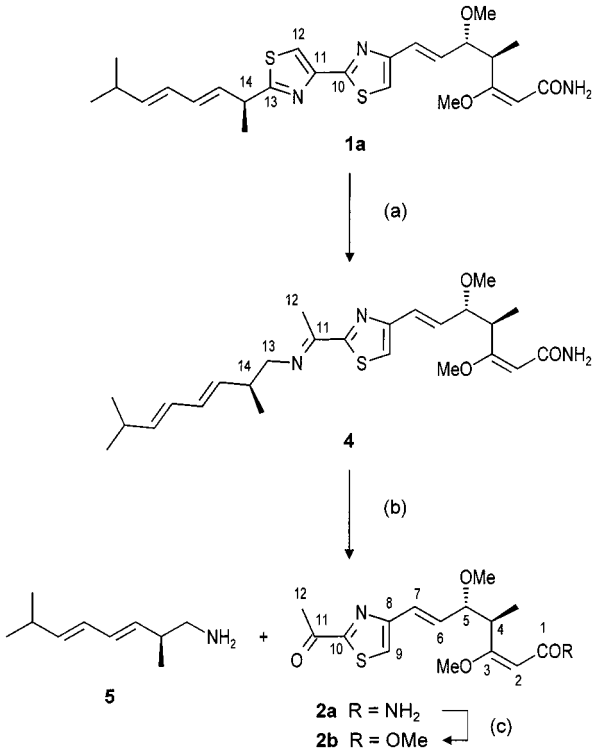


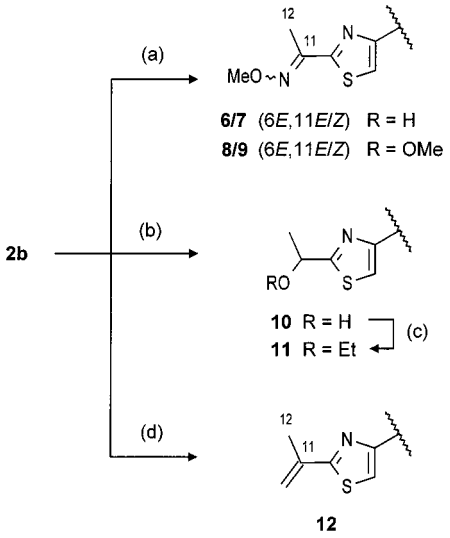
Scheme 1. Synthesis of melithiazol C (**2b**): (a) DIBAL-H, CH_2Cl_2, −50°C; (b) NaOAc-buffer, pH 4.5, MeOH, 75% for 2 steps; (c) 1. MeO_3BF_4, CH_2Cl_2, 2. NaOAc-buffer, MeOH, 70°C, 54% for 2 steps

into the corresponding iminoester, which was then hydrolysed to melithiazol C (**2b**) in 55% yield (Scheme 1).

While this reaction sequence was repeated several times in batches of up to several grams without any problems, in a single case the desired amide was obtained as a 5:1 mixture of (6*E*)/(6*Z*) isomers **2a**, **3a** instead of the pure (6*E*) isomer **2a**. Attempts to induce this isomerisation by light or by various chemical methods failed. However, it may be assumed that the same mechanism was operative here as in the isomerisation of natural melithiazol C during isolation.[1a] Pure (6*Z*)-melithiazol C (**3b**) was obtained after enrichment by reversed phase chromatography at the amide stage and separation of the esters **2b** and **3b** by TLC on silica gel plates impregnated with silver nitrate. After isolation the (6*E*) and (6*Z*) isomers were found to be perfectly stable.

To obtain a first insight into the structure-activity relationships of melithiazol C some derivatives of the 10-acetyl group were prepared. Thus, treatment with hydroxylamine and *O*-methylhydroxylamine hydrochlorides in ethanol/pyridine gave oximes **6/7** and **8/9** as (11*E*)/(11*Z*) mixtures in quantitative yields (Scheme 2). Separation of **8** and **9** was achieved by preparative HPLC on RP-18 and assignment of the configuration by inspection of the ^{13}C NMR chemical shift of the neighbouring C-12 methyl group (11*E*): δ = 11.9, (11*Z*): δ = 18.8.[8]

Reduction of the keto group with sodium borohydride in ethanol furnished alcohol **10** as an inseparable mixture of diastereomers which could be transformed to their ethyl ethers[11] by treatment with Ag_2O/ethyl iodide. Finally,

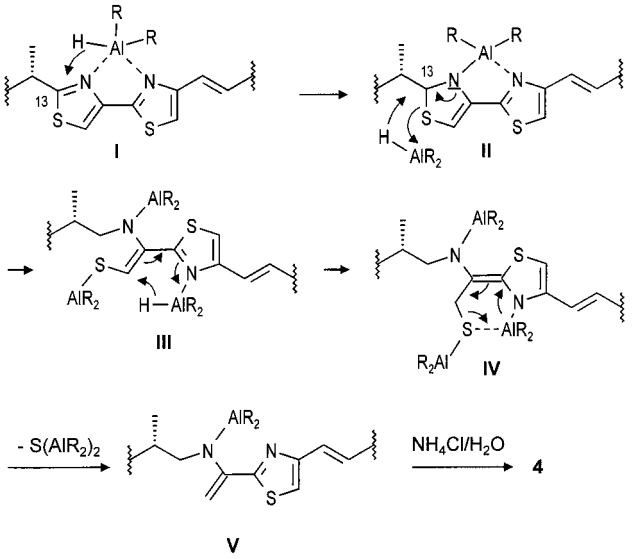


Scheme 2. Derivatisation of melithiazol C (**2b**): (a) $RONH_3Cl$, pyridine, EtOH; (b) $NaBH_4$, EtOH; (c) EtI, Ag_2O, diethyl ether; (d) $Ph_3PCH_2Br/NaNH_2$, THF

olefination of **2b** with Schlosser's "instant ylide"[9] gave the 11-methylene derivative **12** which may be considered as an analogue of melithiazol B.[1a]

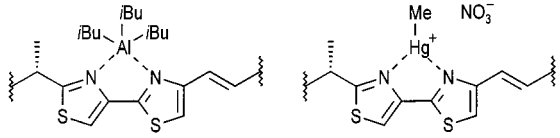
Mechanism of the Thiazole Ring Cleavage

To the best of our knowledge the reductive cleavage of a thiazole ring by DIBAL-H has no precedence. Model studies with 2-methyl-4-phenylthiazole confirmed that an isolated thiazole is resistant towards DIBAL-H at −50 °C.[12] On the other hand the corresponding α-pyridyl-substituted thiazole was degraded by DIBAL-H in dichloromethane at this temperature. This suggests that the reduction of myxothiazol (**1**) is initiated by the formation of a bidentate aluminium complex **I** (Scheme 3). After hydrogen transfer to C-13 the thiazoline ring in **II** is opened, and the sulfur atom binds a second molecule of DIBAL-H which transfers its hydrogen to C-13 to give intermediate **III**.



Scheme 3. Proposed mechanism of the DIBAL-H reduction of myxothiazol A (**1a**)

Now a third molecule of DIBAL-H binds to the remaining thiazole and transfers its hydrogen to C-12. Elimination of $(DIBAL)_2S$ from **IV** gives an enamine **V** which, on aqueous workup, isomerizes to the imine **4**. At this stage ^1H NMR spectra indeed showed signals at $\delta = 2.32$ (12-H_3) and $\delta = 2.63$ (14-H) which could be assigned to **4**. Independently amine **5** and ketone **2a** were condensed in the presence of molecular sieves to the supposed imine which showed the same NMR signals and HPLC retention time as observed above. However, attempts to isolate this imine by chromatography failed due to rapid hydrolysis.



13 **14**

Scheme 4. Complexes formed from myxothiazol *Z* (**1b**) and triisobutylaluminium or methylmercury nitrate

In support of the proposed mechanism, the reaction of myxothiazol *Z* (**1b**) with other Lewis acids was investigated. Thus, with equimolar amounts of triisobutylaluminium in $CDCl_3$ complex **13** (Scheme 4) was formed exhibiting characteristics down-field shifts of 9-H and 12-H by $\Delta\delta = 0.80$ and 0.61.[9] Methylmercury nitrate in methanol formed complex **14** (Scheme 4) which, in addition to shifts of the 9-H and 12-H protons (0.70 and 0.72 ppm), showed the same geminal $^1H,^{199}Hg$ coupling constant of 239.8 Hz as related trigonal planar mercury(II) complexes.[11] Attempts to induce ring cleavage by reduction of **13** and **14** led to recovery of myxothiazol *Z* (**1b**).

Biological Activity

For comparison of the biological properties the antifungal activity against filamentous fungi and yeasts, toxicity for an animal cell line and target activity with submitochondrial particles were determined. The lipophilicity was estimated by the octanol/water partition coefficient log P_{OW} (Table 1). Compared to melithiazol C (**2b**) the (6*Z*)-isomer **3b** was essentially inactive in all test systems. Very low antifungal activity was observed for the polar amide-analogue **2a** of melithiazol C and the 14-hydroxy derivative **10**, whereas the 14-ethoxy derivative **11** showed good antifungal activity and only slightly increased cytotoxicity and target activity.

Transformation of the keto group to the oximes **6/7**, on the other hand, reduces the antifungal activity and increases cytotoxicity. Exceptionally high antifungal activity at low cytotoxicity is observed with the 14-vinyl derivative **12** and the (14*E*) and (14*Z*) methoximes **8** and **9**. Interestingly, cytotoxicity, target activity and lipophilicity of these three derivatives are very similar to the commercial fungicide kresoxim-methyl,[14] whereas their activity against, for example, *Botrytis cinerea* is significantly higher.

Experimental Section

General: Analytical TLC: TLC aluminium sheets, silica gel Si 60 F_{254}, 0.2 mm (Merck), detection: UV absorption at $\lambda = 254$ 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: Nucleosil RP-18-7-100, 250 × 4 mm (Macherey & Nagel), UV detection 254 nm, flow rate 1.5 mL/min. − Preparative HPLC: column: Nucleosil RP-18-7-100, 250 × 20 mm (Macherey & Nagel), UV detection 254 nm, flow rate 12 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). − 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 $\delta = 7.25$. − DCI-MS: spectrometer MAT 95 (Finnigan), resolution $M/\Delta M = 1000$, high-resolution data from peak matching $M/\Delta M = 10000$.

Amide Analog of Melithiazol C (2a): To a solution of myxothiazol A (**1a**) (70% purity, 0.80 g, 1.15 mmol) in 15 mL of anhydrous CH_2Cl_2 was slowly added a 1 M solution of DIBAL-H in hexane (8.8 mL, 8.8 mmol) at −70 °C under nitrogen. After stirring for 1 h the reaction was quenched with aqueous NH_4Cl solution. The mixture was warmed to room temperature and the solvent removed

Table 1. Activity of selected melithiazol derivatives **2**−**12**, myxothiazol A (**1a**) and kresoxim-methyl against filamentous fungi and yeast, mammalian cells and in inhibition of NADH oxidation

	Botrytis cinerea Inhibition zone at 2 µg/disc (mm)	*Hansenula anomala*	Cytotoxity IC_{50} (ng/mL)[a]	Inhibition of NADH oxidation IC_{50} (ng/mL)[b]	Lipophilicity logP_{OW}[c]
1a[d]	16	15	1	11	5.29
2a	10	<7	8000	7000	1.25
2b	18	14	700	730	2.92
3b	<7	<7	25000	>8000	2.92
6/7	15	10	150	820	2.47
8	42	42	500	42	3.97
9	42	42	400	85	3.62
10	10	<7	4000	>8000	1.99
11	30	22	400	350	3.35
12	35	35	2000	250	3.81
Kresoxim-methyl[d]	33	30	400	72	3.70

[a] The cytotoxicity was measured by an MTT assay with the mouse fibroblast cell line L929, for details see ref.[1b] − [b] The inhibition of the NADH oxidation was measured with submitochondrial particles isolated from beef heart, for details see ref.[1b] − [c] Estimated by RP-18 TLC according to ref.[13] − [d] Values taken from ref.[1]

in vacuo. The residue was dissolved in 50 mL of MeOH and 20 mL of 1 M NaOAc buffer (pH = 4.5). After stirring for 2 h the MeOH was removed in vacuo and the aqueous layer was extracted four times with diethyl ether. The combined organic layer was washed with 0.1 M HCl (to separate the amine **5**) and a satd. aqueous solution of $NaHCO_3$, dried with $MgSO_4$, and the solvents evaporated in vacuo. Purification of the crude **2a** by MPLC [column: Eurosil Bioselect 100−20-C18 (15−20 μm), 30 mm × 320 mm, eluent: first 1. MeOH/H_2O 55:45 for 60 min, then 55:45 to 80:20 in 90 min, then 80:20 for 60 min, UV detection 254 nm, flow rate 20 mL/min] yielded **2a** (209 mg, 56%) and starting material **1a** (73 mg, 13%). When this reaction was repeated with reduction at −50°C no starting material was left and the isolated yield of **2b** was 75%. − **2a**: R_f (dichloromethane/methanol 90:10) = 0.50. − R_t (methanol/water 55:45) = 4.7 min. − IR (KBr): $\tilde{\nu}$ = 668 cm^{-1} (m), 935 (m), 953 (m), 1057 (m), 1093 (s), 1125 (m), 1186 (m), 1216 (s), 1274 (m), 1329 (m), 1259 (m), 1274 (m), 1329 (m), 1413 (m), 1441 (m), 1485 (m), 1599 (s), 1685 (s), 2935 (m), 2970 (m), 3191 (m), 3342 (m). − UV (MeOH): λ_{max}(lg ε) = 232 nm (4.59), 325 (3.61). − ^{1}H NMR (300 MHz): δ = 1.15 (d, *J* = 7.0 Hz, 3 H, 4-CH_3), 2.70 (s, 3 H, 12-H_3), 3.32 (s, 3 H, 5-OCH_3), 3.57 (s, 3 H, 3-OCH_3), 3.81 (dd, *J* = 7.0 and 7.6 Hz, 1 H, 5-H), 4.14 (dq, *J* = 7.0 and 7.0 Hz, 1 H, 4-H), 4.93 (s, 1 H, 2-H), 6.45 (dd, *J* = 7.6 and 15.8 Hz, 1 H, 6-H), 6.58 (d, *J* = 15.6 Hz, 1 H, 7-H), 7.40 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ = 14.1 (4-CH_3), 39.5 (C-4), 55.1 (3-OCH_3), 56.9 (3-OCH_3), 84.9 (C-5), 94.1 (C-2), 121.5 (C-9), 125.0 (C-7), 132.9 (C-6), 191.9 (C-11), 155.6 (C-8), 166.5 (C-10), 169.1.3 (C-1), 172.0 (C-3). − DCI-MS (120 eV, *i*-butane): *m*/*z* = 325 [M + H^+], 293. − $C_{15}H_{21}N_2O_4S$: calcd. 325.1222; found 325.1202 (DCI-MS).

For isolation of **5** the acidic aqueous layer obtained above was adjusted to pH 12. After repeated extraction with diethyl ether the combined organic layers were dried with $MgSO_4$ and the solvents evaporated in vacuo yielding 102 mg of crude **5**. Bulb-to-bulb distillation gave **5** (55 mg, 31%) b.p. 60°C/0.015 Torr. − **5**: R_f (dichloromethane/ methanol 90:10) = 0.47. − IR (KBr): $\tilde{\nu}$ = 801 cm^{-1} (m), 990 (m), 1021 (m), 1097 (m), 1093 (s), 1262 (m), 1364 (m), 1458 (m), 1575 (m), 2868 (s), 2926 (s), 2960 (s). − UV (MeOH): λ_{max}(lg ε) = 225 nm (sh), 230, 237 (sh). − ^{1}H NMR (300 MHz): δ = 0.96 (d, *J* = 6.7 Hz, 3 H, 2-CH_3), 0.98 (d, *J* = 6.7 Hz, 3 H, 7-CH_3), 0.98 (d, *J* = 6.7 Hz, 3 H, 8-H_3), 2.13 (m, 1 H, 2-H), 2.28 (qqd, *J* = 6.7, 6.7 and 6.7 Hz, 1 H, 7-H), 2.49 (dd, *J* = 7.7 and 12.6 Hz, 1 H, 1-H_A), 2.58 (dd, *J* = 5.5 and 12.6 Hz, 1 H, 1-H_B), 5.37 (dd, *J* = 8.0 and 14.6 Hz, 1 H, 3-H), 5.55 (dd, *J* = 6.7 and 14.6 Hz, 1 H, 6-H), 5.94 (dd, *J* = 10.3 and 14.6 Hz,1 H, 5-H), 6.01 (dd, *J* = 10.3 and 14.6 Hz, 1 H, 4-H). − ^{13}C NMR (75.5 MHz): δ = 17.2 (2-CH_3), 22.3 (7-CH_3), 31.1 (C-7), 40.6 (C-2), 48.1 (C-1), 127.1 (C-5), 130.8 (C-4), 135.3 (C-3), 140.3 (C-6). − DCI-MS (120 eV, *i*-butane): *m*/*z* = 154 [M + H^+]. − $C_{10}H_{20}N_2$: calcd. 154.1595; found 154.1585 (DCI-MS). − $[\alpha]_D^{22}$ = +48.5 (*c* = 0.65 in MeOH).

Melithiazol C (2b): Compound **2a** (0.80 g, 2.50 mmol) was dissolved in 15 mL of anhydrous CH_2Cl_2 and trimethyloxonium tetrafluoroborate (0.55 g, 3.70 mmol) was added. The mixture was stirred for 2.5 h at room temperature. The solvent was then evaporated in vacuo and the residue was dissolved in 300 mL of MeOH and 180 mL of 1 M NaOAc-buffer (pH = 4.5). After stirring for 3 h at 80 °C the mixture was cooled to room temp. and MeOH was removed in vacuo. The remaining aqueous layer was extracted four times with diethyl ether. The combined organic layers were dried with $MgSO_4$, and the solvents evaporated in vacuo. Purification of the crude product by column chromatography (SiO_2, petroleum ether/diethyl ether 75:25) gave **2b** (0.45 g, 54%). − R_f (petroleum ether/diethyl ether 50:50) = 0.52. − IR (KBr): $\tilde{\nu}$ = 2975 cm^{-1} (m), 2936 (s), 1710 (s), 1689 (s), 1624 (s), 1486 (m), 1383 (m), 1360 (m), 1273 (s), 1194 (m), 1147 (s), 1126 (s), 1094 (s), 1054 (m), 972 (m), 953 (m), 927 (m), 826 (m). − UV (MeOH): λ_{max}(lg ε) = 234 nm (4.47), 325 (3.51). − ^{1}H NMR (300 MHz): δ = 1.20 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 2.70 (s, 3 H, 12-H_3), 3.32 (s, 3 H, 5-OCH_3), 3.59 (s, 3 H, 3-OCH_3), 3.81 (dd, *J* = 7.4 and 7.5 Hz, 1 H, 5-H), 4.16 (dq, *J* = 6.9 and 7.4 Hz, 1 H, 4-H), 4.96 (s, 1 H, 2-H), 6.45 (dd, *J* = 7.5 and 15.8 Hz, 1 H, 6-H), 6.58 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.37 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ = 14.0 (4-CH_3), 39.8 (C-4), 50.8 (1-OCH_3), 55.6 (3-OCH_3), 57.2 (5-OCH_3), 84.2 (C-5), 91.1 (C-2), 121.4 (C-9), 124.7 (C-7), 133.3 (C-6), 191.9 (C-11), 155.6 (C-8), 166.5 (C-10), 167.7 (C-1), 176.6 (C-3). − DCI-MS (120 eV, *i*-butane): *m*/*z* = 340 [M + H^+], 308. − $C_{16}H_{21}NO_5S$: calcd. 339.11405; found 339.1142 (DCI-MS). − $[\alpha]_D^{22}$ = +169.0 (*c* = 0.3 in MeOH).

(6*Z*)-Melithiazol C (3b): Compound **1a** (0.80 g) was reduced with DIBAL-H at −70 °C as described above to give a crude product containing the (6*E*) and (6*Z*)-isomers **2a** and **3a** in a ratio of 5:1 according to the ^{1}H NMR spectrum. Reversed phase chromatography yielded an enriched fraction of 120 mg containing **2a** and **3a** in a ratio of 3:1. This material was converted into a mixture of the esters **2a** and **3b**, which were separated on a small scale by TLC on an $AgNO_3$-impregnated silica gel plate (20 × 20 cm, 0.25 mm, dichloromethane/acetone 95:5). From 7 mg mixture/plate (6*E*)-melithiazol C (**26**) (4.5 mg) and (6*Z*)-melithiazol C (**3b**) (1.5 mg) were obtained.
2b: R_f (dichloromethane/acetone 95/5, $AgNO_3$-impregnated silica gel) = 0.50. − **3b:** R_f (dichloromethane/acetone 95/5) = 0.61. − UV (MeOH): λ_{max}(lg ε) = 237 nm (4.53), 322 (3.58). − ^{1}H NMR (300 MHz): δ = 1.22 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 2.72 (s, 3 H, 12-H_3), 3.32 (s, 3 H, 5-OCH_3), 3.48 (s, 3 H, 3-OCH_3), 3.66 (1-OCH_3), 4.21 (dq, *J* = 6.9 and 9.0 Hz, 1 H, 4-H), 4.91 (s, 1 H, 2-H), 5.03 (dd, *J* = 9.6 and 9.0 Hz, 1 H, 5-H), 5.68 (dd, *J* = 9.9 and 12.0 Hz, 1 H, 6-H), 6.56 (d, *J* = 12.0 Hz, 1 H, 7-H), 7.52 (s, 1 H, 9-H). − DCI-MS (120 eV, NH_3): *m*/*z* = 357 [M + NH_4^+], 340 [M + H^+]. − $C_{16}H_{22}NO_5S$: calcd. 340.1219; found 340.1194 (DCI-MS).

Methoximes 8 and 9: Pyridine (165 μL, 2.10 mmol) and *O*-methylhydroxylamine hydrochloride (127 mg, 1.50 mmol) were added to a solution of **2b** (353 mg, 1.04 mmol) in 34 mL of ethanol. After stirring for 14 h at room temperature, the mixture was concentrated in vacuo, diluted with 0.1 N HCl and extracted with diethyl ether. The organic layer was washed with an aqueous $NaHCO_3$ solution and dried with Na_2SO_4. After evaporation of the solvent in vacuo 380 mg (100%) of product was obtained which, according to analytical HPLC (MeOH/water 75:25), consisted of a mixture of **8** (57%) and **9** (43%). Separation of the isomers was achieved by preparative HPLC (MeOH/water 75:25).
8: R_f (petroleum ether/ diethyl ether 50:50): 0.57. − R_t (methanol/ water 80/20): 3.6 min. − IR (KBr): $\tilde{\nu}$ = 1383 cm^{-1} (m), 1438 (s), 1625 (s), 1712 (s). − UV (MeOH): λ_{max}(lg ε) = 239 nm (4.62), 313 (3.81). − ^{1}H NMR (300 MHz): δ = 1.20 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 2.44 (s, 3 H, 12-H_3), 3.32 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 3.79 (dd, *J* = 7.5 and 7.7 Hz, 1 H, 5-H), 4.08 (s, 3 H, $NOCH_3$), 4.17 (dq, *J* = 6.9 and 7.5 Hz, 1 H, 4-H), 4.95 (s, 1 H, 2-H), 6.40 (dd, *J* = 7.7 and 15.8 Hz, 1 H, 6-H), 6.59 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.29 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ = 14.1 (4-CH_3), 18.8 (C-12), 39.8 (C-4), 50.8 (1-OCH_3), 55.5 (3-OCH_3), 57.1 (5-OCH_3), 62.4 ($NOCH_3$), 84.4 (C-5), 91.2 (C-2), 118.6 (C-9), 125.5 (C-7), 132.1 (C-6), 148.1 (C-11), 152.6 (C-8), 154.8 (C-10), 167.7 (C-1), 176.6 (C-3). − DCI-MS (120 eV, *i*-butane): *m*/*z* = 369 [M + H^+], 337. − $C_{17}H_{24}N_2O_5S$: calcd. 368.1406; found 368.1386 (DCI-MS).

9: R_f (petroleum ether/diethyl ether 50:50) = 0.64. − R_t (methanol/water 80:20) = 3.9 min. − ^{1}H NMR (300 MHz): δ = 1.20 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 2.44 (s, 3 H, 12-H_3), 3.32 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.65 (s, 3 H, 1-OCH_3), 3.79 (dd, *J* = 7.5 and 7.7 Hz, 1 H, 5-H), 4.08 (s, 3 H, $NOCH_3$), 4.15 (dq, *J* = 6.9 and 7.5 Hz, 1 H, 4-H), 4.95 (s, 1 H, 2-H), 6.37 (dd, *J* = 7.7 and 15.7 Hz, 1 H, 6-H), 6.59 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.29 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ =14.1 (4-CH_3), 18.8 (C-12), 39.8 (C-4), 50.8 (1-OCH_3), 55.5 (3-OCH_3), 57.1 (5-OCH_3), 62.4 ($NOCH_3$), 84.4 (C-5), 91.2 (C-2), 118.6 (C-9), 125.5 (C-7), 132.1 (C-6), 148.1 (C-11), 152.6 (C-8), 154.8 (C-10), 167.7 (C-1), 176.6 (C-3).

Oximes 6 and 7: According to the procedure described for **8** and **9**, compound **2b** (15 mg, 44 μmol) and hydroxylammonium hydrochloride (5 mg, 72 μmol) yielded **6/7** (16 mg) as a 2:3 mixture of (*E,Z*)-isomers **6** and **7** (by NMR spectroscopy). − R_f (petroleum ether/diethyl ether 50:50) = 0.31 and 0.39. − ^{1}H NMR (300 MHz): δ = 1.20 (d, *J* = 7.1 Hz, 3 H, 4-CH_3), 2.39 and 2.45 (s, 3 H, 12-H_3), 3.31 and 3.32 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 3.79 and 3.80 (dd *J* = 7.6 and 7.6 Hz, 1 H, 5-H,), 4.17 (m, 1 H, 4-H), 4.96 (s, 1 H, 2-H), 6.41 and 6.39 (dd, *J* = 7.6 and 15.8 Hz, 1 H, 6-H), 6.60 and 6.54 (d, *J* = 15.7 Hz, 1 H, 7-H), 7.01 and 7.30 (s, 1 H, 9-H). − DCI-MS (120 eV, *i*-butane): *m/z* = 355 [M + H^+], 323. − $C_{16}H_{23}N_2O_5S$: calcd. 355.1328; found 355.1326 (DCI-MS).

11-Hydroxy Derivative 10: A solution of **2b** (8.0 mg, 24 μmol) in 500 μL of methanol was treated with sodium borohydride (1.0 mg, 26 μmol). After stirring for 20 min the solvent was removed in vacuo. The residue was partitioned between ethyl acetate and water, and the water layer was extracted twice with ethyl acetate. The combined organic layers were dried with $MgSO_4$, and the solvents evaporated in vacuo. Purification of the crude product by preparative HPLC (MeOH/H_2O 55:45) gave **10** (7 mg, 87%). − R_f (dichloromethane/ methanol 95/5) = 0.26. − IR (KBr): $\tilde{\nu}$ = 3420 cm^{-1} (m, br), 2978 (m), 2937 (m), 1710 (s), 1624 (s), 1455 (m), 1440 (m), 1384 (m), 1307 (m), 1266 (m), 1195 (s), 1148 (s), 1126 (s), 1093 (s), 1051 (m), 969 (m), 926 (m), 826 (m). − UV (MeOH): λ_{max}(lg ε) = 223 nm (4.27), 241 (4.36). − ^{1}H NMR (300 MHz): δ = 1.19 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 1.62 (d, *J* = 6.5 Hz, 3 H, 12-H_3), 3.30 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.65 (s, 3 H, 3-OCH_3), 3.77 (dd, *J* = 7.7 and 7.7 Hz, 1 H, 5-H), 4.13 (dq, *J* = 6.9 and 7.7 Hz, 1 H, 4-H), 4.94 (s, 1 H, 2-H), 5.10 (m, 1 H, 11-H), 6.33 (dd, *J* = 7.7 and 15.8 Hz, 1 H, 6-H), 6.50 (d, *J* = 15.8 Hz, 1 H, 7-H), 7.01 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ = 14.1 (4-CH_3), 24.1 (C-12), 39.9 (C-4), 50.8 (1-OCH_3), 55.6 (3-OCH_3), 57.0 (5-OCH_3), 68.2 (C-11), 84.3 (C-5), 91.1 (C-2), 114.4 (C-9), 125.3 (C-7), 131.7 (C-6), 153.3 (C-8), 175.2 (C-10), 167.7 (C-1), 176.7 (C-3). − DCI-MS (120 eV, *i*-butane): *m/z* = 342 [M + H^+], 310. − $C_{16}H_{23}NO_5S$: calcd. 341.1297; found 341.1291 (DCI-MS).

Ethyl Ether 11: A solution of **11** (5.0 mg, 15 μmol) in 500 μL of diethyl ether was stirred with Ag_2O (30 mg) and 30 μL of ethyl iodide for 23 h. The mixture was filtered through celite and the solvent was removed in vacuo. Purification of the crude product by preparative TLC (petroleum ether/diethyl ether 50:40) gave **11** (3 mg, 54%). − R_f (petroleum ether/diethyl ether 60:40) = 0.46. − IR (KBr): $\tilde{\nu}$ = 2978 (m), 2935 (m), 1712 (s), 1624 (s), 1440 (m), 1382 (m), 1265 (m), 1231 (s), 1193 (m), 1146 (s), 1095 (s), 1080 (m), 969 (m), 926 (m). − UV (MeOH): λ_{max} (lg ε) = 215 nm (4.20), 241 (4.34). − ^{1}H NMR (300 MHz): δ = 1.20 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 1.22 and 1.23 (q, *J* = 7.2 Hz, 3 H, 11-OCH_2CH_3), 1.54 and 1.55 (d, *J* = 6.5 Hz, 3 H, 12-H_3), 3.30 (s, 3 H, 5-OCH_3), 3.57 (m, 2 H, 11-OCH_2CH_3), 3.65 (s, 3 H, 3-OCH_3), 3.78 (dd, *J* = 7.7 and 7.7 Hz, 1 H, 5-H), 4.13 (m, 1 H, 4-H), 4.72 (q, *J* = 6.5 Hz, 1 H, 11-H), 4.94 (s, 1 H, 2-H), 6.29 (dd, *J* = 6.7 and 15.7 Hz, 1 H, 6-H), 6.51 (d, *J* = 15.7 Hz, 1 H, 7-H), 7.02 (s, 1 H, 9-H). − DCI-MS (120 eV, *i*-butane): *m/z* = 370 [M + H^+]. − $C_{18}H_{26}NO_5S$: calcd. 370.16882; found 370.17232 (DCI-MS).

11-Vinyl Derivative 12: 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 temperature a solution of **26** (8.0 mg, 24 μmol) in 400 μL of THF was added. The reaction mixture was stirred for 1.5 h and the solvent 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 the solvents evaporated in vacuo. Preparative HPLC (MeOH/water 65:35) gave **12** (5 mg, 63%). − R_f (dichloromethane/ methanol 95:5) = 0.63. − IR (KBr): $\tilde{\nu}$ = 2980 cm^{-1} (m), 2937 (m), 1711 (s), 1624 (s), 1454 (m), 1440 (m), 1383 (m), 1265 (m), 1194 (m), 1147 (s), 1094 (s), 1054 (m), 970 (m), 927 (m), 825 (m). − UV (MeOH): λ_{max}(lg ε) = 233 nm (4.57), 301 (3.67). − ^{1}H NMR (300 MHz): δ = 1.19 (d, *J* = 6.9 Hz, 3 H, 4-CH_3), 2.22 (s, 3 H, 11-CH_3), 3.31 (s, 3 H, 5-OCH_3), 3.58 (s, 3 H, 3-OCH_3), 3.78 (dd, *J* = 7.8 and 7.8 Hz, 1 H, 5-H), 4.15 (dq, *J* = 6.9 and 7.8 Hz, 1 H, 4-H), 4.95 (s, 1 H, 2-H), 5.29 (s, 1 H, 12-H_A), 5.83 (s, 1 H, 12-H_B), 6.36 (dd, *J* = 7.8 and 15.7 Hz, 1 H, 6-H), 6.52 (d, *J* = 15.7 Hz, 1 H, 7-H), 6.97 (s, 1 H, 9-H). − ^{13}C NMR (75.5 MHz): δ = 14.0 (4-CH_3), 39.8 (C-4), 50.8 (1-OCH_3), 55.6 (3-OCH_3), 57.2 (5-OCH_3), 84.2 (C-5), 91.1 (C-2), 121.4 (C-9), 124.7 (C-7), 133.3 (C-6), 191.9 (C-11), 155.6 (C-8), 166.5 (C-10), 167.7 (C-1), 176.6 (C-3). − DCI-MS (120 eV, *i*-butane): *m/z* = 338 [M + H^+], 306. − $C_{17}H_{23}NO_4S$: calcd. 337.1348; found 337.11348 (DCI-MS).

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