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Melithiazols, New β-Methoxyacrylate Inhibitors of the Respiratory Chain Isolated from Myxobacteria

Production, Isolation, Physico-chemical and Biological Properties†

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New antibiotic compounds, melithiazols, were isolated from the culture broth of strains of the myxobacteria *Melittangium lichenicola*, *Archangium gephyra*, and *Myxococcus stipitatus*. The compounds belong to the group of β-methoxyacrylate (MOA) inhibitors and are related to the myxothiazols. The melithiazols show high antifungal activity, but are less toxic than myxothiazol A and its methyl ester in a growth inhibition assay with mouse cell cultures. The melithiazols inhibit NADH oxidation by submitochondrial particles from beef heart. Melithiazol A blocks the electron transport within the bc_1-segment (complex III) and causes a red shift in the reduced spectrum of cytochrome *b*.

During our screening for antibiotics from myxobacteria, we found antifungal activities in the culture broth of two strains of *Melittangium lichenicola*, Me ℓ26 and Me ℓ46. The activities were due to new compounds, which we named melithiazols[1]. Later, we also found these compounds in a strain of *Archangium gephyra*, Ar 7747, and of *Myxococcus stipitatus*, Mx s64. In this paper we describe the production, isolation, and the physico-chemical and biological properties of the melithiazols. Fig. 1 shows the structures of the melithiazols isolated so far, the elucidation of which will be published elsewhere[2]. The melithiazols are related to myxothiazol[3] and the recently published cystothiazoles[4,5], which were also isolated from myxobacteria. All three groups contain a β-methoxyacrylate (MOA) system as a terminal segment. Thus, this class of substances were termed MOA-inhibitors[6] and are grouped with the other β-methoxyacrylates, oudemansin[7] and strobilurin[8]. The latter are substituted in the α-position, while melithiazol and myxothiazol are β-substituted β-methoxyacrylates. Strobilurin was taken as the lead compound to develop agricultural fungicides such as kresoxim-methyl and azoxystrobin[9].

Microorganisms and Culture Conditions

The producing organisms were isolated at the GBF as follows: *Melittangium lichenicola* strain Me ℓ26 from rabbit dung in 1989 and strain Me ℓ46 from a soil sample in 1995; *Archangium gephyra* strain Ar 7747 and *Myxococcus stipitatus* strain Mx s64 were isolated from soil samples in 1993 and 1995, respectively.

The *Melittangium* strains were normally grown on VY/2 agar and liquid medium[10], the *Archangium* strain on M7 liquid medium[11], and *Myxococcus stipitatus* on MD1[10] liquid medium with 0.6% peptone. Batch cultures of 100 or 500 ml in 250-ml or 1,000-ml Erlenmeyer flasks, respec-

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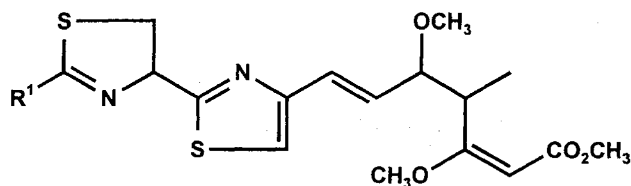
Fig. 1. The chemical structures of the melithiazols.

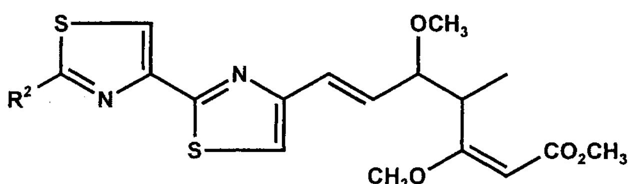


Melithiazol A R^1 = - $C(CH_3)=CH_2$

Melithiazol D R^1 = - $CH(CH_3)_2$

Melithiazol K R^1 = (2-methyloxiranyl: CH_3, O)

Melithiazol L R^1 = - $CH(CH_3)CH_2OCH_3$



Melithiazol B R^2 = - $C(CH_3)=CH_2$

Melithiazol E R^2 = - $CH(CH_3)_2$

Melithiazol F R^2 = - $CH_2C_6H_6$

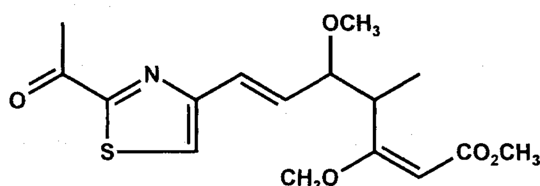
Melithiazol G R^2 = - $CH(CH_3)CH_2CH_3$

Melithiazol H R^2 = - C_2H_5

Melithiazol I R^2 = - $CH_2CH(CH_3)_2$

Melithiazol M R^2 = - $C(O)CH_3$

Melithiazol N R^2 = (2-methyloxiranyl: CH_3, O)



Melithiazol C

tively, were incubated at 30°C on a gyratory shaker at 160 rpm for 3~5 days.

Production

All four strains were used for production on a larger scale. They were grown on SY-medium containing soy flour (defatted) 0.7%, bakers' yeast 0.3%, $CaCl_2 \cdot 2H_2O$ 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, glucose 0.2%, and cyanocobalamin 0.1 mg/liter. For example, a 500-ml culture in a 1000-ml-Erlenmeyer flask grown for three days on a gyratory shaker at 160 rpm was inoculated into 10 liter medium in a 15-liter fermentor with a flat-blade turbine stirrer. After three days the content of the seed fermentor was inoculated into a bioreactor with 300 liter of SY- medium to which 1% (v/v) of an adsorber resin, *e.g.*, Amberlite XAD-16 (Rohm & Haas, Frankfurt) was added. Both fermentors were kept at 30°C and agitated at 150 rpm. In order to reduce foam formation, 0.02% silicon antifoam agent (Tegosipon, Goldschmidt AG, Essen) was added. The aeration rate was 0.1 volume air per volume culture and minute. The pH was adjusted initially to 7.2. At harvest, the adsorber resin, which contained over 90% of the melithiazol, was separated from the culture broth by passing the culture through a process filter of 210 μm mesh size. Me ℓ26 produced 0.5 mg/liter, Me ℓ46 1.5 mg/liter, and Ar 7747 up to 10 mg/liter melithiazol A as the main compound, Mx s64 produced 5 mg/liter melithiazol E. Fig. 2 shows a fermentation of Ar 7747 in a 15-liter draft tube bioreactor (Giovanola Frères SA, Monthey, Switzerland). As the strain grew in lumps, the oxygen consumption was monitored as a growth parameter. The pO_2 dropped to nearly zero within two days. The pH was not regulated, went down in the beginning into the acid range and then slowly rose again. Melithiazol A was produced during the logarithmic and early stationary phase. In the given example, the yield was 2.9 mg/liter after 4 days.

Isolation and Quantitative Determination

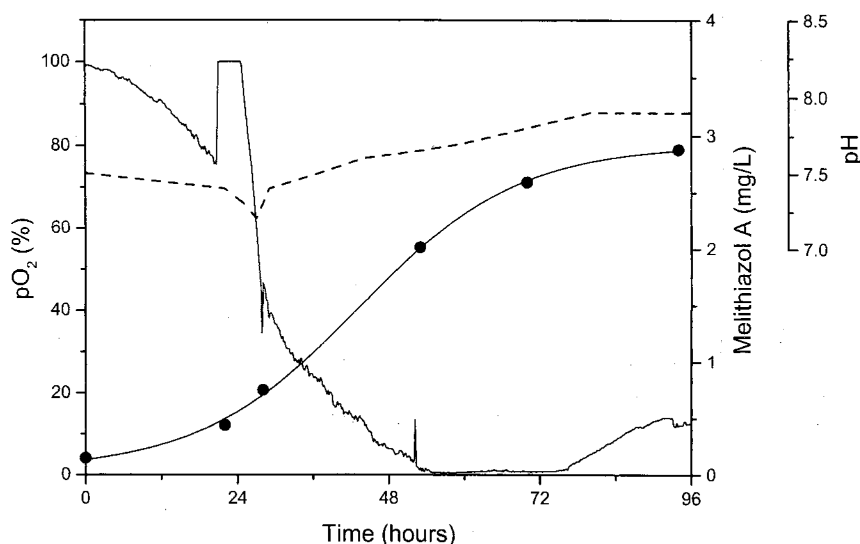
The melithiazols could be eluted from the resin with methanol or acetone. The organic solvent was evaporated, and the remaining aqueous phase was extracted with ethyl acetate. The organic phase was separated and evaporated again. The resulting crude extract was dissolved in acetone or dichloromethane, and fractionated by column chromatography on Sephadex LH20 (solvent: acetone or dichloromethane/methanol 80 : 20) and silica gel 60 (solvent: dichloromethane, dichloromethane/methanol 85 : 15 and 80 : 20, methanol). Active fractions were further purified by medium pressure RP18-chromatography (methanol/water gradient from 80 to 100% methanol), and Si-HPLC (petroleum ether/TBME/methanol 3 : 6 : 0.01) or RP18-HPLC (methanol/water 80 : 20).

Melithiazols A, B and C were isolated from strain Me ℓ26 and Me ℓ46, and melithiazols A, B, D, K, L, M, and N from strain Ar 7747. The melithiazols E, F, G, H, and I were only found in strain Mx s64.

The melithiazols were quantitatively determined by HPLC (column 125×2 mm, nucleosil RP-18-120-5, Macherey-Nagel, Oensingen, Switzerland; solvent: methanol/water

Fig. 2. Fermentation of *Archangium gephyra* Ar 7747 in a 15-liter bioreactor with a flatblade turbine stirrer (10 liter culture volume, 120 rpm, aeration rate 1 liter/minute).

—— pO_2, ----- pH, ● melithiazol A. At 96 hours, the culture was in stationary phase and collapsed a few hours later.



75:25; flow rate: 0.3 ml/minute; detection: 210 nm). The retention times were as follows: melithiazol A 4.4 minutes, B 7.2 minutes, C 2.6 minutes, D 4.0 minutes, E 5.7 minutes, F 6.4 minutes, G 7.2 minutes, H 5.3 minutes, I 6.9 minutes, K 2.9minutes, L 3.3 minutes, M 4.0 minutes, and N 4.0 minutes.

Physico-chemical Properties

The melithiazols are soluble in methanol, acetone, ethyl acetate, and chloroform. The Rf values in TLC on silica gel 60 F_{254} with dichloromethane/acetone (90:10) as the solvent were 0.55 for melithiazol A and 0.69 for melithiazol B. High resolution mass spectroscopy with a Kratos MS 9/50 or a Finnigan MAT 95 gave the following molecular masses (and calculated elemental compositions): melithiazol A 422.1324 ($C_{20}H_{26}N_2O_4S_2$), B 420.1178 ($C_{20}H_{24}N_2O_4S_2$), C 339.1156 ($C_{16}H_{21}NO_5S$), D 424.1497 ($C_{20}H_{28}N_2O_4S_2$), E 422.1358 ($C_{20}H_{26}N_2O_4S_2$)[a], F 470.1348 ($C_{24}H_{26}N_2O_4S_2$), G 436.1510 ($C_{21}H_{28}N_2O_4S_2$), H 408.1191 ($C_{19}H_{24}N_2O_4S_2$), I 436.1490 ($C_{21}H_{28}N_2O_4S_2$), K 438.1252 ($C_{20}H_{26}N_2O_4S_2$), L 454.1586 ($C_{21}H_{30}N_2O_5S_2$), M 422.0985 ($C_{21}H_{30}N_2O_5S_2$), and N 436.1113 ($C_{20}H_{24}N_2O_5S_2$). The UV spectra of melithiazols A and B were recorded with a Shimadzu UV-2102 PC spectrophotometer (Fig. 3), the IR spectrum of melithiazol A with a Nicolet 20 DXB FT-IR spectrometer (Fig. 4).

Biological Activity

The melithiazols had no effect on bacteria, but high antifungal activity (Table 1). We used two yeasts (*Hansenula anomala* and *Metschnikowia pulcherrima*) and two phytopathogenic fungi (*Botrytis cinerea* and *Pythium debaryanum*) as indicator organisms. The inhibition zones of an agar diffusion assay and the minimal inhibitory concentration (MIC) in a serial broth dilution assay were taken as a measure of the antifungal potency. The activities of the melithiazols were compared to myxothiazol A, its methyl ester, and the α-substituted β-methoxyacrylates strobilurin A, oudemansin A, kresoxim-methyl and azoxystrobin. The methyl ester of myxothiazol A was isolated from *Myxococcus fulvus*, strain Mx f333[12)], which also produced myxothiazol A. The ester was named myxothiazol Z. With the exception of the derivatives C and F, the melithiazols showed bigger inhibition zones in the agar diffusion assays than the

[a] A comparison of the spectroscopic data revealed that melithiazol E is identical to cystothiazol A[4)].

Fig. 3. The UV spectrum of melithiazol A (——) and B (----) in methanol.

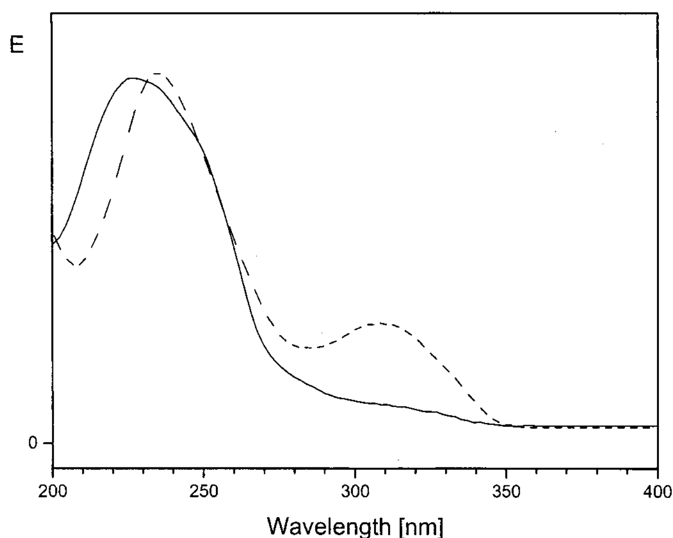
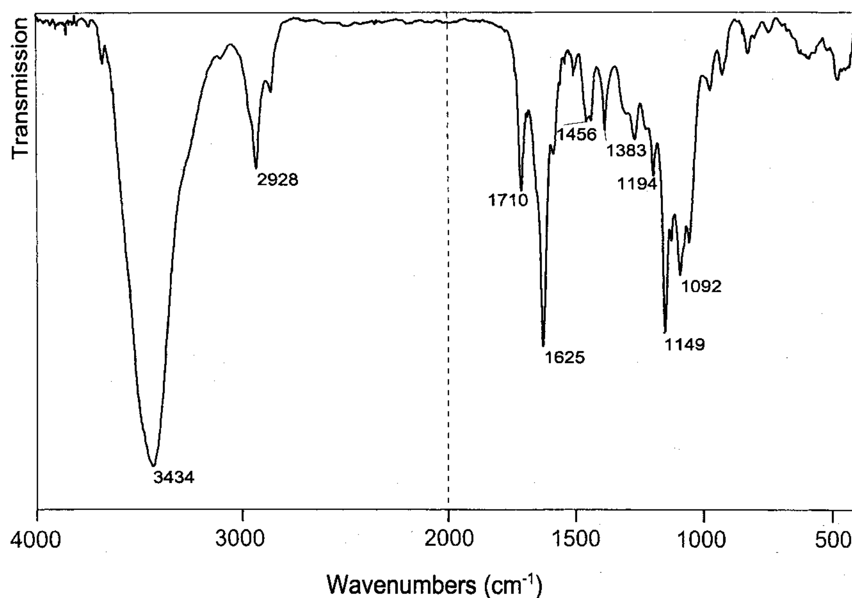


Fig. 4. IR spectrum of melithiazol A in KBr.



myxothiazols A and Z. The MIC values of myxothiazols and melithiazols with the exception of melithiazol C, were similar. The melithiazols showed lower MIC values than the natural α-substituted β-methoxyacrylates, strobilurin A and oudemansin A. The efficacies of the chemical derivatives kresoxim-methyl and azoxystrobin were comparable to those of the melithiazols. Melithiazol C, which has only one thiazole ring, was the least active of the melithiazols.

The toxicity of the compounds was tested by a growth inhibition assay with L929 mouse fibroblast cells. Approximately 6,000 cells were seeded into each well of a 96-well microtiterplate containing sequentially diluted concentrations of the test compounds. The cells were grown in Dulbecco's modified Eagle medium (high glucose; GIBCO)

Table 1. Antifungal activity of the melithiazols in comparison with other MOA-inhibitors.

Compound	Inhibition zone[a,b] (mm)				MIC[c] (μg/ml)			
	Hn a	Mt p	Bo c	Py d	Hn a	Mt p	Bo c	Py d
Melithiazol A	27	31	32	38	0.15	0.04	0.04	0.15
Melithiazol B	22	24	29	27	0.08	0.04	0.04	0.15
Melithiazol C	16	17	18	22	4	3	2.5	> 5
Melithiazol D	25	30	27	27	0.15	0.08	0.15	0.15
Melithiazol E	29	29	33	29	0.08	0.08	0.08	0.15
Melithiazol F	14	16	18	19	0.2	0.2	0.3	0.3
Melithiazol G	21	21	24	22	0.15	0.15	0.3	0.6
Melithiazol H	28	30	31	27	0.15	0.15	0.15	0.15
Melithiazol I	20	24	25	22	0.15	0.15	0.3	0.3
Melithiazol K	28	29	29	24	0.15	0.15	0.3	0.3
Melithiazol L	29	31	33	25	0.15	0.08	0.08	0.15
Melithiazol M	26	30	34	29	0.08	0.04	0.04	0.04
Melithiazol N	30	33	34	28	0.15	0.08	0.15	0.15
Myxothiazol A	15	14	16	13	0.08	0.04	0.04	0.3
Myxothiazol Z	13	13	16	14	0.15	0.15	0.15	0.15
Strobilurin A	21	24	24	28	0.8	0.8	1.5	1.5
Oudemansin A	26	28	26	37	0.8	0.4	0.8	1.5
Kresoxim-methyl	30	35	33	40	0.1	0.05	0.1	0.4
Azoxystrobin	27	34	30	35	0.6	0.6	0.15	0.15

[a] Test organisms were: *Hansenula anomala* (Hn a; DSM 70263), *Metschnikowia pulcherrima* (Mt p; DSM 70321), *Botrytis cinerea* (Bo c; DSM 877), and *Pythium debaryanum* (Py d; DSM 62946). All strains were from Deutsche Sammlung von Mikroorganismen und Zellkulturen, and were grown in a medium containing glycerol 2 %, casein peptone 1 %, yeast extract 1 %, and phosphate 50 mM. The pH value was adjusted to 6.3.

[b] Determined by an agar diffusion test using paper discs of 6 mm diameter with 2 μg of the compound in 20 μl methanol.

[c] Determined by a serial dilution assay.

plus 10% newborn calf serum. The same medium was used for the dilutions. The culture volume was 180 μl/well. The metabolic activity of the grown cells was measured by an MTT assay[13] after 5 days of incubation at 37°C under 10% CO_2. The IC_{50} values derived from the resulting inhibition curves are given in Table 2. The melithiazols were clearly less toxic than myxothiazols A and Z. The melithiazols with the exception of C showed nearly the same antifungal activity as myxothiazol A, but were 50 to 1000 times less toxic. The natural and synthetic α-substituted β-methoxyacrylates had, in general, low toxicity.

Because of the structural relationship to myxothiazol, we assumed that the melithiazols, too, interfere with the respirative energy metabolism. Beef heart submitochondrial particles (SMP) were chosen to study the inhibition of the respiratory chain. The isolation and characterization of SMP as well as the execution of the experiment has been described previously[14]. In all assays, SMP were suspended in 75 mM sodium phosphate buffer at pH 7.4 containing EDTA and $MgCl_2$, each at 1 mmol/liter. The antibiotics were dissolved in methanol, the concentration of which did not exceed 2% in the test.

As expected, the melithiazols inhibited NADH oxidation by SMP, as recorded with a UV2 Unicam UV/VIS spectrometer (Table 2). But when we compared melithiazols and myxothiazols, the inhibition of NADH oxidation did not correlate with cytotoxicity. While the inhibition values of myxothiazol A and Z were clearly higher than the corre-

Table 2. Cytotoxicity of melithiazols and their inhibition of NADH oxidation in SMP in comparison with other MOA-inhibitors.

Compound	Cytotoxicity[a] IC_{50} (ng/ml)	NADH oxidation[b] IC_{50} (ng/ml)
Melithiazol A	50	33
Melithiazol B	30	18
Melithiazol C	500	1600
Melithiazol D	30	70
Melithiazol E	50	46
Melithiazol F	25	147
Melithiazol G	60	41
Melithiazol H	450	79
Melithiazol I	130	75
Melithiazol K	650	250
Melithiazol L	50	31
Melithiazol M	50	31
Melithiazol N	150	59
Myxothiazol A	0.5	11
Myxothiazol Z	1.7	24
Strobilurin A	180	83
Oudemansin A	800	400
Kresoxim-methyl	350	72
Azoxystrobin	400	110

[a] Cytotoxicity was measured by a growth inhibition assay with the mouse cell line L929 (ATTCC CCL1), NADH oxidation by submitochondrial particles (SMP) isolated from beef heart.

[b] The SMP were suspended in air-saturated buffer at a concentration of 90 µg/ml. NADH was added to a final concentration of 0.16 mmol/liter.

sponding cytotoxicity values (22 and 14 times, respectively), the relation of NADH oxidation inhibition to cytotoxicity with melithiazols was generally below 1. The only exceptions were melithiazol C, D, and F. Within the group of strobilurins we observed the same correlation as found with most melithiazols.

The site of inhibition within the electron transport chain was investigated by difference spectroscopy using a DW 2000 UV/VIS SLM double beam spectrophotometer (SLM Instruments, Inc., Il., U.S.A.). Upon reduction with physiological substrates such as NADH, fully oxidized cytochromes in front of the block become reduced, while those behind it remain oxidized. As can be seen in Fig. 5, the difference spectrum of NADH-reduced minus air-oxidized SMP showed the characteristic absorption maxima for the different cytochromes. In the presence of melithiazol A, only cytochrome b of complex III (α band at 563 nm) became reduced, whereas the cytochromes cc_1 (α band at 553 nm) and aa_3 (α band at 608 nm) remained in the oxidized state. This indicated that melithiazol inhibited the electron flow within the cytochrome bc_1 segment of the respiratory chain between cytochrome b and cytochromes cc_1. Apparently it blocked reduction of only one of the two cytochrome b centers, most likely the outer one (b-566, or b_1). The binding of complex III inhibitors to cytochrome b, *e.g.*, myxothiazol, causes a bathochromic shift in the α band of reduced cytochrome b[14]. When added to a suspension of SMP, which had been reduced by dithionite, melithiazol A caused a red shift in the spectrum of cytochrome b with a maximum at 565 nm and a minimum at 560 nm (Fig. 6).

Fig. 5. The effect of melithiazol A on the reduction of cytochromes by NADH.

Beef heart submitochondrial particles (SMP) were suspended in air-saturated buffer at a concentration of 3.2 mg protein/ml. —— Difference spectrum (reduced minus oxidized) of SMP reduced with NADH (final concentration 2 mM) without inhibitor. ----- Difference spectrum of SMP reduced with NADH (final concentration 2 mM) in the presence of 10 μg/ml melithiazol A. ········ Baseline.

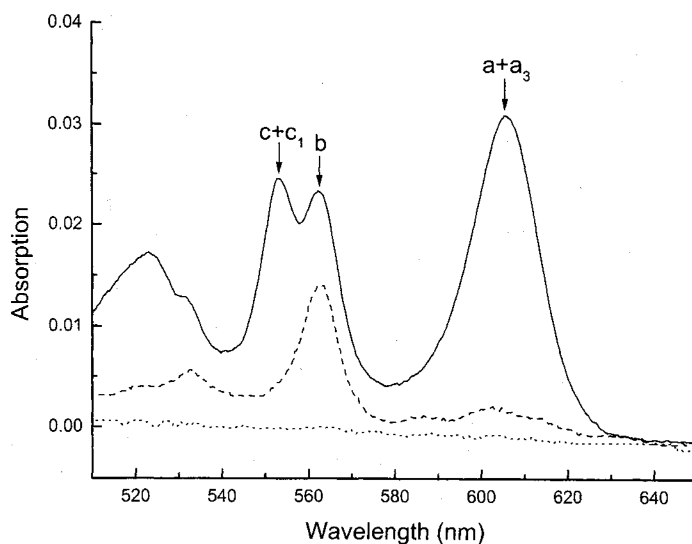
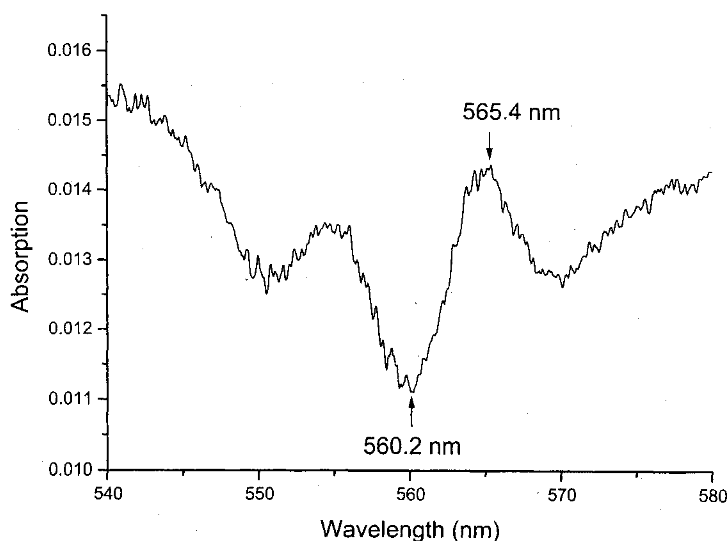


Fig. 6. Spectral shift induced by melithiazol A in dithionite reduced beef heart submitochondrial particles.



A suspension of SMP (15 mg protein/ml) was reduced with dithionite and filled into sample and reference cuvettes with a light path of 1 cm. After adjustment of the baseline, melithiazol A dissolved in methanol was added to the sample cuvette (final concentration 20 μg/ml). The same amount of solvent was added to the reference cuvette. The difference spectrum was recorded with a bandwidth of 1.5 nm.

Discussion

Inhibition of electron transport is a relatively rare mechanism of action among bacterial compounds, but is much favored by myxobacteria. Thus far we isolated and characterized nearly 20 new substances with this effect. The melithiazols are the first antibiotics isolated from a strain of the genus *Melittangium*. After their discovery in *Melittangium lichenicola* they were also found in *Archangium gephyra* and *Myxococcus stipitatus*. The closely related cystothiazoles were discovered in *Cystobacter fuscus*[4]. All those genera belong to the suborder Cystobacterineae of the myxobacteria[15]. The melithiazols and cystothiazoles are related to myxothiazol, which was found in different genera of the Cystobacterineae[16]. All these compounds are β-substituted β-methoxyacrylates. They are highly active antifungal substances, which inhibit the electron flow within the cytochrome bc_1 segment of the respiratory chain.

The melithiazols and cystothiazoles turned out to be less toxic in cell culture assays than myxothiazol A. In contrast to myxothiazol, which is an amide, the melithiazols and most of the cystothiazoles are methyl esters. It may be assumed that this would be the reason for reduced toxicity. We therefore compared the melithiazols with myxothiazol methyl ester (myxothiazol Z), which was isolated from *Myxococcus fulvus* strain Mx f333[12]. As the methyl ester of myxothiazol proved to be highly toxic, too, we assume that the left side of the molecule is important for the lower toxicity in animal cell cultures.

The differences in toxicity of the melithiazols and myxothiazols can not be deduced from the inhibitory activity in NADH oxidation by beef heart SMP. The inhibition values rather corresponded to the antifungal activity. Thus, the lower toxicity of the melithiazols with respect to myxothiazol A can not be simply explained by a difference in the inhibition of fungal and mammalian respiratory chains. It may rather be due to different uptake or detoxification mechanisms.

Our investigations showed that, in contrast to observations with myxothiazol, β-substituted β-methoxyacrylates are not necessarily highly toxic. The relation of antifungal activity and toxicity is an important criterion for the evaluation of an antifungal substance. Melithiazols are in the same range as α-substituted β-methoxyacrylates in that respect.

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References

1) HÖFLE, G.; H. REICHENBACH, B. BÖHLENDORF & F. SASSE: DE4410449 A1, 1995 [Chem. Abstr. 123: 339531, 1995]
2) BÖHLENDORF, B.; M. HERRMANN, H.-J. HECHT, F. SASSE, E. FORCHE & B. KUNZE: Eur. J. Org. Chem. 1999, in print
3) GERTH, K.; H. IRSCHIK, H. REICHENBACH & W. TROWITZSCH: Myxothiazol, an antibiotic from *Myxococcus fulvus* (Myxobacterales). J. Antibiotics 33: 1474~1479, 1980
4) OJIKA, M.; Y. SUZUKI, A. TSUKAMOTO, Y. SAKAGAMI, R. FUDOU, T. YOSHIMURA & S. YAMANAKA: Cystothiazoles A and B, new bithiazole-type antibiotics from the myxobacterium *Cystobacter fuscus*. J. Antibiotics 51: 275~281, 1997
5) SUZUKI, Y.; M. OJIKA, Y. SAKAGAMI, R. FUDOU & S. YAMANAKA: Cystothiazoles C~F, new bithiazole-type antibiotics from the myxobacterium *Cystobacter fuscus*. Tetrahedron 54: 11399~11404, 1998
6) BECKER, W. F.; G. von JAGOW, T. ANKE & W. STEGLICH: Oudemansin, strobilurin B and myxothiazol: new inhibitors of the bc_1 segment of the respiratory chain with an α-β-methoxyacrylate system as common structural element. FEBS Letters 132: 329~333, 1981
7) ANKE, T.; H. J. HECHT, G. SCHRAMM & W. STEGLICH: Antibiotics from basidiomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (SCHRADER ex FR.) HOEHNEL (AGARICALES). J. Antibiotics 32: 1112~1117, 1979
8) ANKE, T.; F. OBERWINKLER, W. STEGLICH & G. SCHRAMM: The strobilurins — new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (PERS. ex FR.) SING. J. Antibiotics 30: 808~810, 1977
9) SAUTER, H.; E. AMMERMANN, R. BENOIT, S. BRAND, R. E. GOLD, W. GRAMMENOS, H. KÖHLE, G. LORENZ, B. MÜLLER, F. RÖHL, U. SCHIRMER, J. B. SPEAKMAN, B. WENDEROTH, & H. WINGERT: Mitochondrial respiration as a target for antifungals: Lessons from research on strobilurins. *In* Antifungal Agents, Discovery and Mode of Action. *Ed.*, G. K. DIXON, L. G. COPPING, D. W. HOLLOMON, pp. 173~191, BIOS Scientific Publishers Ltd., Oxford, 1995
10) REICHENBACH, H. & M. DWORKIN: The myxobacteria. *In* The Prokaryotes. Vol. 4. *Ed.*, A. BALOWS, H. G. TRÜPER, M. DWORKIN, W. HARDER, K.-H. SCHLEIFER, pp. 3416~3487, Springer-Verlag, New York, 2nd ed.1992
11) SASSE, F.; H. STEINMETZ, G. HÖFLE & H. REICHENBACH: Gephyronic acid, a novel inhibitor of eukaryotic protein

synthesis from *Archangium gephyra* (myxobacteria). J. Antibiotics 48: 21~25, 1995
12) FORCHE, E.; H. STEINMETZ, G. HÖFLE & H. REICHENBACH: Unpublished results
13) MOSMANN, T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assay. J. Immunol. Meth. 65: 55~63, 1983
14) THIERBACH, G. & H. REICHENBACH: Myxothiazol, a new inhibitor of the cytochrome bc_1 segment of the respiratory chain. Biochim. Biophys. Acta 638: 282~289, 1981
15) REICHENBACH, H.: Biology of the myxobacteria: Ecology and taxonomy. *In* Myxobacteria II. *Ed.*, DWORKIN, M. & D. KAISER, pp. 13~61, American Society for Microbiology, Washington, DC, 1993
16) REICHENBACH, H. & G. HÖFLE: Myxobacteria as producers of secondary metabolites. *In* Drug Discovery from Nature. *Ed.*, GRABLEY, S. & R. THIERICKE, pp. 149~179, Springer, Verlag, Berlin, 1998