seco-Cyclothialidines: New Concise Synthesis, Inhibitory Activity toward Bacterial and Human DNA Topoisomerases, and Antibacterial Properties[§]

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seco-Cyclothialidines are a promising class of bacterial DNA gyrase B subunit inhibitors. A new *seco*-cyclothialidine derivative containing a dioxazine moiety, BAY 50-7952, was synthesized through a new concise pathway. One key step of the synthesis is the straightforward formation of the 2-aminothiazole derivative of *S*-tritylcysteine. In biological tests, BAY 50-7952 and other known *seco*-cyclothialidines exhibited high and selective activity toward bacterial DNA gyrase and toward Gram-positive bacteria. The dioxazine moiety and other similar groups were found to be important for the ability of the *seco*-cyclothialidines to penetrate bacterial membranes. The opposite enantiomer ((*S*)-form) of BAY 50-7952 was also synthesized, and neither significant target activity nor in vitro antibacterial activity were found, suggesting a highly selective fit of the (*R*)-form. Despite promising in vitro activity, only poor activity was found in the murine infection model.

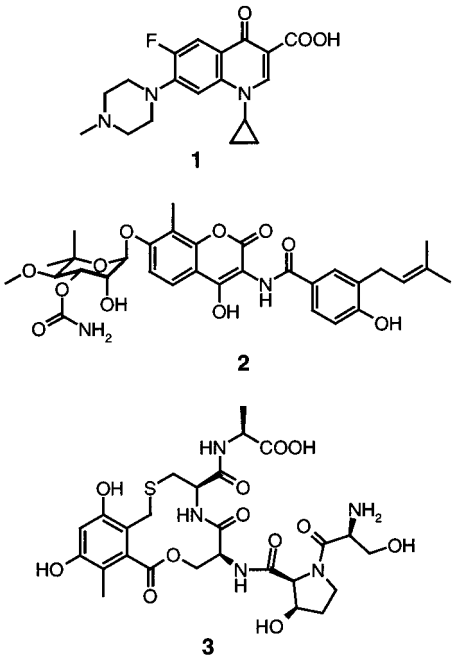
Introduction

Due to the rapidly growing number of resistant bacterial strains, the search for antibacterial agents with new modes of action will always remain an important and challenging task.[1] In the past, the bacterial DNA gyrase has drawn much attention as a selectively addressable target. In particular the fluoroquinolones, e.g. ciprofloxacin (**1**; Chart 1), proved to be highly successful inhibitors of bacterial DNA gyrase and were extensively investigated in the pharmaceutical industry.[2]

Apart from the quinolones, other naturally occurring bacterial DNA gyrase inhibitors, such as the coumarins (e.g. novobiocin (**2**; Chart 1) and coumermycin), have been known for a long time. However, no pharmaceutically useful lead compounds have been derived from them until now.[3,4] In 1994, cyclothialidine (**3**; Chart 1) was described as a new potent DNA gyrase-inhibiting natural product.[5] Its structure features a unique 12-membered lactone ring that is fused to a highly substituted benzene ring and partly integrated into a pentapeptide chain. Shortly after its discovery, the potential of this compound was recognized by Hoffmann-La Roche and has been intensively investigated since that time.[6] Different from the quinolones which bind to the A subunit of the DNA gyrase, the cyclothialidines act as competitive inhibitors of the B subunit of this enzyme.

Despite their high activity on the target, cyclothialidine and its natural analogues barely exert any bacterial activity against intact bacterial cells, probably due to insufficient penetration of the cytoplasmic membrane.

Chart 1



In further investigations, it was shown that the lactone ring is not a mandatory requirement for target activity, leading to the development of the open-chain analogues. In fact, these compounds, called *seco*-cyclothialidines, were able to penetrate bacterial membranes. The *seco*-cyclothialidines were patented by Hoffmann-La Roche,[7a] and the compound Ro-61-6653 was later identified as an interesting lead candidate.[7b] As shown in Table 1, Ro-61-6653 exerts excellent activity against Gram-positive bacteria; however, it is inactive against Gram-negative strains. Prior to our own investigations, no results were reported concerning the in vivo antibacterial potential of the *seco*-cyclothialidines in animal infection models.

[§] Dedicated to Professor K. Barry Sharpless on the occasion of his 60th birthday.

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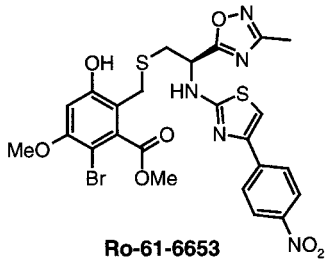
Table 1. In Vitro Antibacterial Activity of Ro-61-6653



Ro-61-6653

	MIC (μg/mL)
S. aureus 25923	0.12
S. aureus QR-54	0.25
S. epidermidis	0.12
S. pyogenes	0.12
E. faecalis	0.25

Our initial goal was the synthesis of known and new *seco*-cyclothialidine derivatives to carefully study their biological profile, especially the activity in animal models. Since the previously described *seco*-cyclothialidine synthesis is relatively long and complicated, we also explored a shorter and more flexible synthetic pathway. As the starting point for our own investigations, we focused on varying the carboxylic acid part of the cysteine core of the *seco*-cyclothialidines. In Ro-61-6653, this part is structurally represented by an oxadiazole moiety. We speculated that this portion of the molecule plays an important role for its penetration properties. On the basis of our speculation, we decided to synthesize new analogues, including the simplest case, the corresponding methyl ester, and a derivative containing a dioxazine ester mimic.[8]

Results and Discussion

Chemistry. *seco*-Cyclothialidines are trifunctionalized cysteines. According to a known synthesis, Ro-61-6653 is obtained in a 10-step synthesis starting with the amino acid cystine (Scheme 1).[7a] The initial carboxy functionalization is followed by modification of the side chain thio group, and the amino group is transformed into the corresponding aminothiazole in the last part of the sequence.

Since the dioxazine moiety is chemically more labile than the oxadiazole residue, we explored a synthetic pathway that introduces this moiety at a later stage of the synthetic sequence. Furthermore, since the aromatic group attached to the thio functionality is highly substituted and requires a multistep synthesis, we preferred the introduction of this group at the final stage of the synthetic pathway.

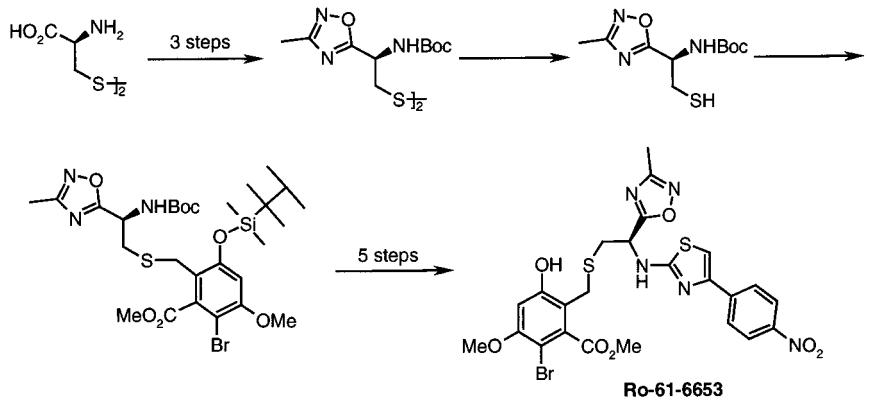
In the course of our work, we developed a new improved synthesis of the *seco*-cyclothialidines which includes only seven steps (Scheme 2). This synthesis was applied in the preparation of the *seco*-cyclothialidine derivatives BAY 50-7952 and Ro-61-6653.

Starting with the amino acid L-cysteine, the *S*-trityl protecting group was attached in the first step (**4**). To build the aminothiazole group, different methods were tried. Originally, we planned to transform the methyl ester derivative of compound **4** into the corresponding thiourea, which would serve as the starting material for a later Hantzsch type reaction to form the corresponding aminothiazole. However, we were unsuccessful with this approach. Alternatively, a more simple and direct approach was successfully applied. Compound **4** reacts with *ω*-thiocyanato-4-nitroacetophenone (**5**) in ethanol at 50 °C with good conversion to the aminothiazole derivative **6**, which could be further used without purification. It is especially noteworthy that this method tolerates the presence of a free carboxylic acid group. Compound **5** was easily prepared by treating *ω*-bromo-4-nitroacetophenone with sodium thiocyanate (Scheme 3). Alternatively, **5** can be generated in situ and directly converted to the aminothiazole derivative in the same pot.[9]

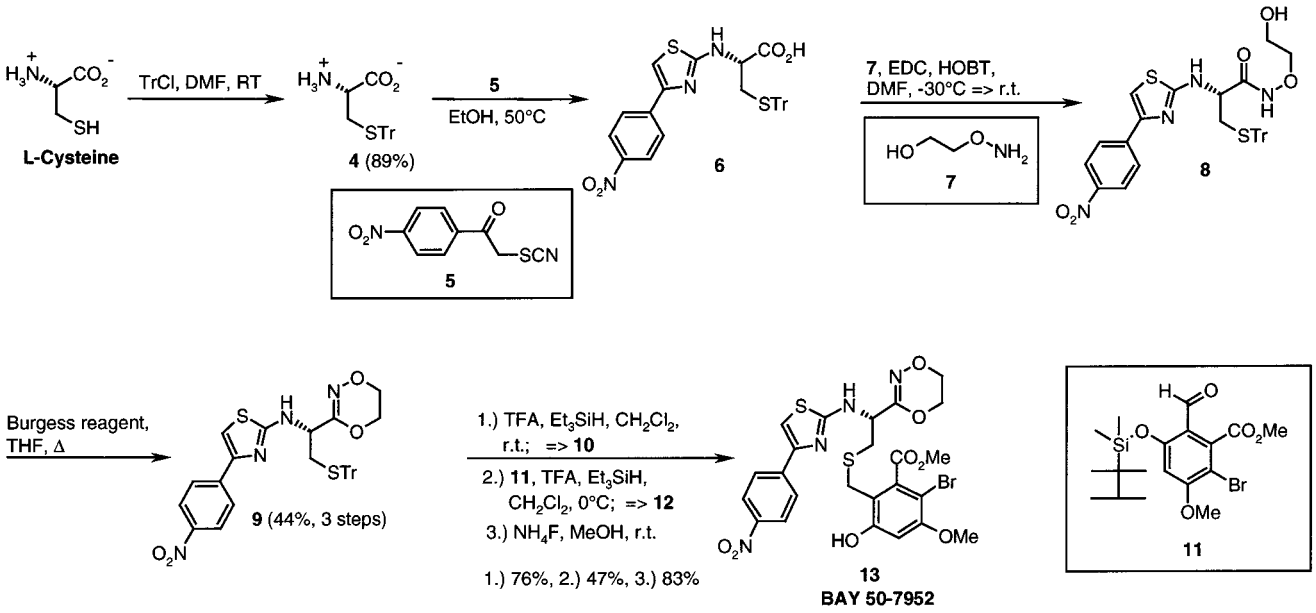
In the next step of the sequence, the dioxazine moiety was formed. When we applied the classical way of introducing this group, reaction of the methyl ester derivative of **6** with hydroxylamine and subsequent basic cyclization with dibromoethane, we observed only decomposition.[10] Therefore, we switched to a different strategy using aminoglycol **7** as building block.[11] Compound **7** was attached to the carboxylic acid residue of **6** under standard peptide coupling conditions (EDC, HOBT). Subsequent cyclization of **8** to **9** was achieved by using the Burgess reagent. This very mild method has been widely used for cyclodehydration reactions, especially for the formation of five-membered rings.[12]

In the last part of the synthesis, the *S*-trityl group was cleaved (**10**) and the highly substituted aromatic portion **11**[13] was attached via reductive thiolation reaction, i.e. reaction of the thiol function with the

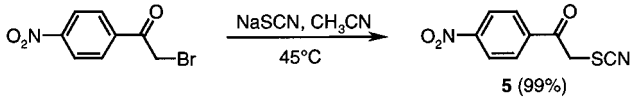
Scheme 1



Scheme 2



Scheme 3



aldehyde group of the aromatic moiety.[7,14] Subsequent silyl deprotection of compound **12** led to the final product **13** (BAY 50-7952). We also prepared the racemate **14** of compound **13**. This provided us with the reference compound needed for chiral HPLC to determine whether racemization had occurred in the course of the synthesis of **13**. In this determination, we found that the product **13** still contained 90% of the (*R*)-enantiomer originally derived from L-cysteine. We also separated the racemate **14** via preparative HPLC to obtain the pure (*S*)-enantiomer of **13**, BAY 51-4137 (**15**), and to compare their antibacterial activities.

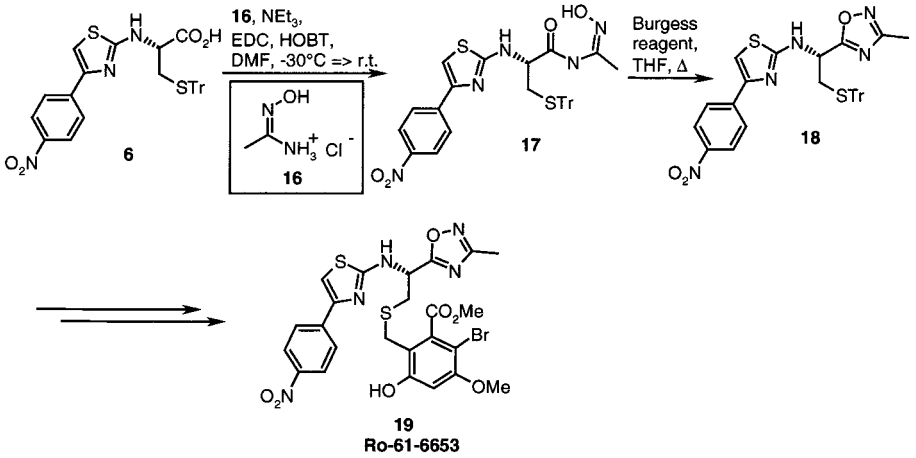
Compound Ro-61-6653 (**19**) was also synthesized via the improved synthesis, as shown in Scheme 4. Compound **6** was treated with *N*-hydroxyacetamidine hydrochloride[15] (**16**) to form **17**. Cyclization of **17** with Burgess reagent led to compound **18** which was further converted to the final product Ro-61-6653 (**19**) in an analogous manner as in the case of **13**.

For the synthesis of the methyl ester derivative **22** (BAY 50-7950), *S*-trityl-L-cysteine (**4**) was converted to the methyl ester **20** and subsequently transformed into the corresponding aminothiazole derivative **21** (Scheme 5). The further course of the synthesis was carried out analogously as described for the preparation of **13** or **19**.

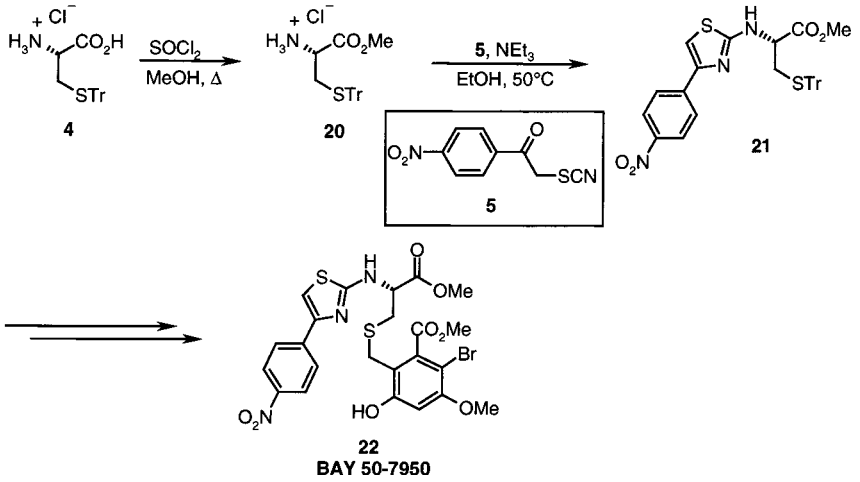
The sequences developed and described in this report allow a rapid and flexible access to new cyclothialidine derivatives. It is especially noteworthy that the synthesis does not require amino and carboxyl protecting groups, in particular, thanks to a very efficient formation of the aminothiazole portion.

Antibacterial Activity. (a) Inhibitory Effects on DNA Gyrase and Other DNA Topoisomerases. The activity of BAY 50-7952 (**13**), the methyl ester derivative BAY 50-7950 (**22**), and Ro-61-6653 toward the DNA gyrase target (*Micrococcus luteus*) (Figure 1) and various other DNA topoisomerases is shown in Table 2. In the case of bacterial DNA gyrase, the IC_{50} values of the (*R*)-enantiomers **13**, **19**, and **22** are all similar and indicate potent inhibition. For comparison, Novobiocin has an IC_{50} of 0.1 μg/mL in the present assay. We were also interested to find out if the target activity is restricted

Scheme 4



Scheme 5



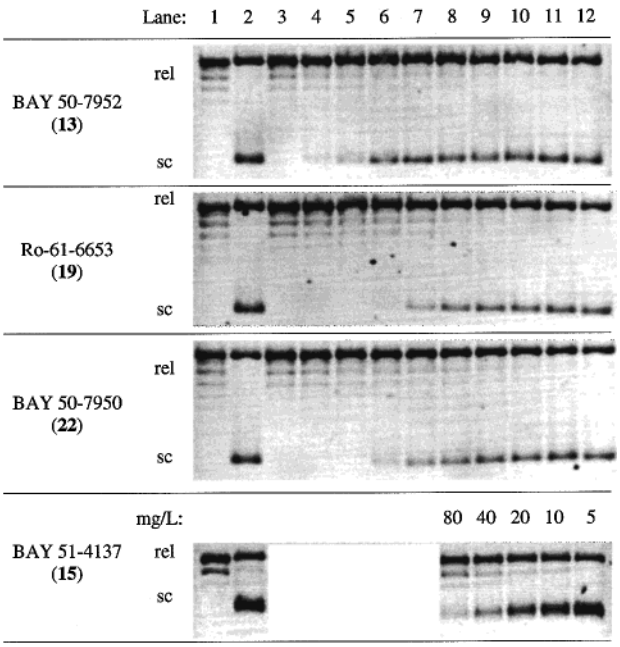


Figure 1. Gel photographs of reactions with *M. luteus* DNA gyrase: rel, relaxed DNA; sc, supercoiled DNA; lane 1, 100 ng relaxed DNA only; lanes 2−12, relaxed DNA and DNA gyrase; lanes 3−12, 1:2 dilutions of test compound starting from 1.25 mg/L in lane 3, except for BAY 51-4137, where test compound concentrations are indicated above the gel picture.

only to the (*R*)-enantiomers, as already known for the cyclothialidines.[6b,c] Therefore, as a representative of the (*S*)-enantiomers, BAY 51-4137 (**15**), the enantiomer of BAY 50-7952, was tested and found to be only weakly active, as expected.

The active compounds inhibit DNA gyrase from Gram-positive *M. luteus* and from Gram-negative *E. coli* equally well. In contrast, the three active compounds inhibit human type II DNA topoisomerase only at very high concentrations and are essentially inactive against eukaryotic type I DNA topoisomerases. In addition, only very weak activity is observed against *E. coli* type I DNA topoisomerases.

(b) Minimal Inhibitory Concentrations for Various Bacteria. As shown in Table 3, the dioxazine compound BAY 50-7952 (**13**) and Ro-61-6653 (**19**) both exhibit excellent activity against Gram-positive strains. No activity was observed against Gram-negative strains.

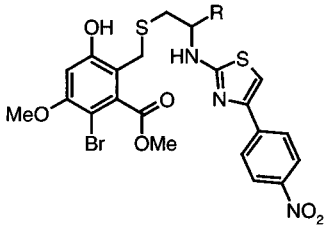
The inferior activity of the methyl ester derivative **22** is likely to be caused by poorer penetration of this compound through the bacterial membrane. As we speculated earlier, the choice of appropriate ester mimics such as the dioxazine moiety in **13** or the oxadiazole moiety in **19** has a strong influence on the transport properties of the *seco*-cyclothialidines. As expected from the target tests, no antibacterial activity was observed for the (*S*)-enantiomer **15**. For compounds **13**, **19**, and **22**, we also carried out MIC determinations in the presence of blood (Table 3). The antibacterial activity of all compounds decreased significantly, indicating strong serum binding.

(c) Therapeutic Efficacy in the Murine Infection Model. Encouraged by the high target activities and MIC values, we also studied the in vivo efficacy of compounds **13**, **19**, and **22**. For this investigation, we used a murine sepsis model with *Staphylococcus aureus* 133 as the infecting organism. Compounds were applied intraperitoneally with 100 mg/kg 30 min after challenge. As shown in Figure 2, administration of the test compounds leads in all cases to prolongation of survival in comparison to an untreated control group. Compound **19** (Ro-61-6653) is more active than compounds **22** and **13**. However, in comparison to marketed antibiotics, all compounds have only minor therapeutic efficacy. As indicated by the in vitro studies in the presence of blood, we speculate that a high level of serum binding may contribute to the low in vivo efficacy. To confirm this hypothesis, we determined the log *P* value of compound **13** (BAY 50-7952). We found a value of 3.1 which clearly demonstrates the lipophilic nature of these compounds.

Summary of the Biological Results and Conclusion

The *seco*-cyclothialidines derivatives described in this report show strong and highly selective target activity toward the bacterial DNA gyrase B subunit and exert high inhibitory activity against Gram-positive bacteria. No substantial activity was observed against bacterial topoisomerase I or human topoisomerases I and II. We also confirmed that only the (*R*)-enantiomers exhibit activity, whereas the corresponding (*S*)-enantiomers are only weakly active in the target and completely inactive in the whole cell assay. Among the three compounds

Table 2. Inhibitory Effects of Selected *seco*-Cyclothialidine Derivatives Against Bacterial DNA Gyrase



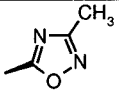
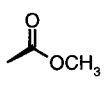
IC_{50} (μg/mL)	 BAY 50-7952 **13**	 BAY 51-4137 **15**	 Ro-61-6653 **19**	 BAY 50-7950 **22**
M. luteus DNA Gyrase	**0.4**	**40**	**0.1**	**0.2**
E. coli DNA Gyrase	**0.5**		**0.1**	**0.3**
human DNA Topo II	**60**		**60**	**15**
human DNA Topo I	**> 240**		**> 240**	**> 240**
Calf thymus Topo I	**> 240**		**> 240**	**> 240**
E. coli DNA Topo I	**120**		**120**	**120**

Table 3. In Vitro Antibacterial Activities of Selected *seco*-Cyclothialidine Derivatives[a]

	MIC (μg/mL)			
strain	**13**	**15**	**19**	**22**
S. epidermidis 193	≤0.12 (8)	>64	≤0.12 (8)	0.5 (>64)
S. hämolyticus 146	≤0.12 (16)	>64	0.25 (16)	1 (>64)
S. saprophyticus 2916	≤0.12 (16)	>64	0.25 (16)	1 (>64)
S. aureus 133	0.25 (16)	>64	0.25 (16)	1 (>64)
S. aureus 48 N	0.25 (16)	>64	0.25 (16)	1 (>64)
S. aureus 9TV	0.25 (16)	>64	0.25 (16)	1 (>64)
S. aureus 44 (25508)	0.25 (16)	>64	0.25 (16)	2 (>64)
S. aureus 25701	≤0.12 (16)	>64	≤0.12 (16)	1 (>64)
S. aureus 25470	0.25 (16)	>64	0.25 (16)	1 (>64)
S. pyogenes Wacker	0.25 (32)	>64	0.25 (32)	1 (>64)
S. pyogenes 4851	0.25 (16)	>64	0.25 (16)	1 (>64)
S. pyogenes 4333	0.25 (32)	64	≤0.12 (32)	1 (>64)
S. pyogenes 7029	0.5 (16)	>64	0.5 (16)	2 (>64)
E. faecium L4001	0.5 (64)	>64	0.5 (32)	2 (>64)
E. faecium 27266	0.5 (64)	>64	0.5 (32)	2 (>64)
E. faecalis 27251	0.5 (64)	>64	0.5 (32)	2 (>64)
E. faecalis 27253	0.5 (64)	>64	0.25 (64)	1 (>64)
P. vulgaris 1017	>64 (>64)	>64	>64 (>64)	>64 (>64)
P. mirabilis 1235	>64 (>64)	>64	>64 (>64)	>64 (>64)
K. pneumoniae 8085	>64 (>64)	>64	>64 (>64)	>64 (>64)
K. pneumoniae 63	>64 (>64)	>64	>64 (>64)	>64 (>64)
E. coli Neumann	>64 (>64)	>64	>64 (>64)	>64 (>64)

[a] MIC values in the presence of 10% horse blood are given in parentheses.

investigated, the nature of the residue at the carboxylic acid moiety of the cysteine core unit does not have a strong influence on the target activity. However, a significant activity difference can be observed in the whole cell bacterial test. The derivatives bearing a dioxazine or oxadiazole moiety are strikingly more active than the corresponding methyl ester derivative, suggesting a pivotal role of this portion for the transport properties of the *seco*-cyclothialidines. Despite the high antibacterial activity of the compounds investigated, only weak activity was found in the murine infection model. Correspondingly, we found high levels of serum binding which is likely to be caused by the lipophilic nature of the compounds.

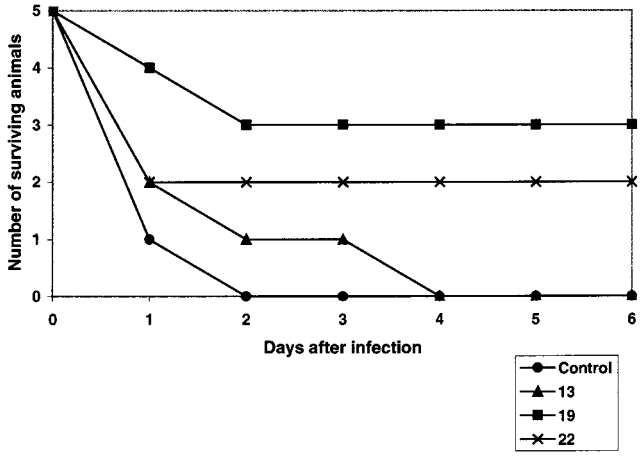


Figure 2. Therapeutic efficacy of selected *seco*-cyclothialidine derivatives. Murine sepsis model with *S. aureus* 133 ip therapy with 100 mg/kg, 30 min post-infection.

Experimental Section

Chemistry. General. Melting points were obtained on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded on Bruker DPX400 spectrometers in $CDCl_3$ or DMSO-d_6 using the solvent signals as internal references. The chemical shifts are reported in δ (ppm) relative to TMS and the coupling constants (J) are given in hertz (Hz). Electrospray-high-resolution mass spectrometry (ESI-HRMS) was performed on an Finnigan-MAT 900 double focusing mass spectrometer. The ESI source was operated in the positive ion mode, spray needle voltage 3.5 kV, nitrogen sheath gas pressure 500 kPa, and the heated metal capillary held at 250 °C.

All reactions were monitored by analytical TLC on aluminum-backed TLC plates coated with silica gel (Merck Si 60 F_{254}), and visualization of spots was accomplished by UV detection at 254 nm. Column chromatography was carried out using Amicon silica gel (30−70 μm). Preparative HPLC racemate separations were performed on a Gilson-Abimed M 305 system (flow: 30 mL/min) with a stationary silica gel phase based on poly(*N*-methacryloyl-L-isoleucine-3-pentylamide) (10 μm) and

ethyl acetate as eluent (UV detection: 280 nm). Analytical HPLC racemate separations were performed on a Jasco system (flow: 1 mL/min) with a Kromasil CHI-TBB stationary phase (5 μm) and *n*-heptane/THF 1:1 (v/v) as eluent (UV detection: 280 nm).

***S*-Trityl-L-cysteine (4).** L-Cysteine hydrochloride (10.0 g, 63.4 mmol) and trityl chloride (27.0 g, 96.9 mmol) were stirred in 40 mL DMF for 2 days at room temperature. A 10% sodium acetate solution (350 mL) was then added, and the precipitate was filtered and washed with distilled water. Afterward, the residue was stirred in acetone at 50 °C for 30 min and filtered after cooling. The residue was washed with little acetone and diethyl ether. After drying in vacuo, 20.5 g (89%) of **4** was obtained as a white powder: mp 195 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.49, (dd, J = 9 Hz, 12 Hz, 1H, CH_2), 2.58 (dd, J = 4.4 Hz, 12 Hz, 1H, CH_2), 2.91 (m, 1H, CH), 7.22−7.36 (m, 15H, arom H).

***ω*-Thiocyano-4-nitroacetophenone (5).** ω-Bromo-4-nitroacetophenone (30.0 g, 123.2 mmol) and sodium thiocyanate (11.0 g, 135.8 mmol) were dissolved in 300 mL acetonitrile and stirred for 3 h at 45 °C. After removal of the solvent, 50 mL distilled water was added, and the mixture was extracted three times with ethyl acetate. The organic layers were dried with Na_2SO_4, and the solvent was removed in vacuo. The product was obtained as an ocre, finely crystalline powder in high purity: yield 27.0 g (99%); mp 118.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.35 (s, 2H, CH_2), 8.25 (d, J = 13 Hz, 2H, arom H), 8.39 (d, J = 13 Hz, 2H, arom H).

***N*-[4-(4-Nitrophenyl)thiazol-2-yl]-*S*-trityl-L-cysteine (6).** Compound **4** (15.0 g, 41.3 mmol) was dissolved in 400 mL ethanol, and compound **5 (**10.1 g, 45.4 mmol) was subsequently added. After stirring for 6 h at 55 °C, the solvent was removed in vacuo. The crude material (yellow powder) was used without further purification. NMR data of a purified sample: ^{1}H NMR (DMSO-d_6) δ 2.59 (m, 1H, CH_2), 2.68 (m, 1H, CH_2), 3.99 (m, 1H, CH), 6.98 (s, 1H, thiazole-H), 7.14−7.34 (m, 15H, trityl-H), 8.0 (d, J = 9 Hz, 2H, arom H), 8.22 (d, J = 9 Hz, 2H, arom H).

***N*^{1}-(2-Hydroxyethoxy)-*N*^{2}-[4-(4-nitrophenyl)-1,3-thiazol-2-yl]-*S*-trityl-L-cysteinamide (8)**. The crude product **6** (10.0 g, ∼17.6 mmol) was dissolved in 50 mL DMF and cooled to −30 °C. HOBT (4.56 g, 30 mmol) and EDC (5.70 g, 30 mmol) were then added. After 15 min of stirring, a solution of aminoglycol (1.93 g, 25 mmol) in 50 mL DMF was added. The reaction mixture was allowed to warm to room temperature and stirred for 14 h. Ethyl acetate (250 mL) was added, and the solution was washed with water (200 mL). The aqueous layer was extracted again with ethyl acetate (150 mL). The combined organic layers were successively extracted with 5% citric acid solution, 10% $NaHCO_3$ solution and twice with brine. The organic layer was dried with Na_2SO_4, and the solvent was removed in vacuo. The crude material (yellow powder) was used without further purification. Analytical data of a purified sample: mp 100 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 2.82 (dd, J = 5.5 Hz, 13.6 Hz, 1H, CH_2), 2.96 (dd, J = 5.5 Hz, 13.6 Hz, 1H, CH_2), 3.63 (m, 2H, CH_2), 3.88 (m, 2H, CH_2), 3.94 (dd, J = 5.5 Hz, 6 Hz, 1H, CH), 4.88 (d, J = 6 Hz, 1H, NH), 6.95 (s, 1H, thiazole-H), 7.21−7.34, 7.45−7.50 (m, 15H, trityl-H), 7.87 (d, J = 9 Hz, 2H, arom H), 8.23 (d, J = 9 Hz, 2H, arom H), 9.11 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 32.8, 55.7, 59.2, 67.6, 78.5, 106.5, 124.1, 126.3, 127.1, 128.2, 129.5, 140.0, 144.1, 146.9, 148.5, 167.0, 170.5; MS (ESI, +) *m*/*z* 627 [M + H]$^+$.

***N*-[(1*R*)-1-(5,6-Dihydro-1,4,2-dioxazin-3-yl)-2-(tritylsulfanyl)ethyl]-*N*-[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amine (9).** The crude product **8** (5.0 g, ∼8 mmol) was dissolved in 200 mL THF, and Burgess reagent [(methoxycarbonylsulfamoyl)triethylammonium *N*-betaine] (2.1 g, 8.8 mmol) was added. After refluxing for 3 h, the solvent was removed in vacuo. The crude product was absorbed onto silica gel and purified via column chromatography using an EtOAc/cyclohexane 2:1 mixture as the eluent. The product was obtained as a dark yellow powder: yield 2.66 g (total yield from three steps: 44%); mp 77 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 2.73 (dd, J = 6 Hz, 12 Hz, 1H, CH_2), 2.83 (dd, J = 6 Hz, 12 Hz, 1H, CH_2), 4.05 (m, 2H, dioxazine-CH_2), 4.31 (m, 1H, CH), 4.32 (m, 2H, dioxazine-CH_2), 5.42 (d, J = 8 Hz, 1H, NH), 6.93 (s, 1H, thiazole-H), 7.18−7.29, 7.39−7.44 (m, 15H, trityl-H), 7.89 (d, J = 9 Hz, 2H, arom H), 8.21 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 34.1, 50.9, 63.7, 67.0, 105.8, 124.0, 126.5, 126.9, 128.0, 129.6, 140.6, 144.4, 146.8, 148.9, 154.9, 167.0; MS (ESI, +) *m*/*z* 243 (100%), 609 (100%, [M + H]$^+$).

(2*R*)-2-(5,6-Dihydro-1,4,2-dioxazin-3-yl)-2-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amino}ethanethiol (10). In an inert atmosphere, compound **9** (3.74 g, 4.93 mmol) was dissolved in 35 mL CH_2Cl_2. Afterward, triethylsilane (2.6 mL, 16.2 mmol) and trifluoroacetic acid (37.4 mL) were added subsequently. After 2 h, the solvent was removed in vacuo, and the crude product was absorbed onto silica gel. The product was purified via column chromatography using an EtOAc/cyclohexane 1:2 ⇒ 2:3 gradient (1% triethylamine was added to each solvent). The product was obtained as a bright yellow powder: yield 1.37 g (76%); mp 189 °C; ^{1}H NMR (DMSO-d_6) δ 3.03 (m, 1H, CH_2), 3.12 (m, 1H, CH_2), 4.12 (m, 2H, dioxazine-CH_2), 4.41 (m, 2H, dioxazine-CH_2), 4.77 (m, 1H, CH), 6.96 (d, J = 8 Hz, 1H, NH), 7.00 (s, 1H, thiazole-H), 7.98 (d, J = 9 Hz, 2H, arom H), 8.22 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR (DMSO-d_6) δ 25.6, 57.9, 63.3, 64.5, 107.0, 123.9, 126.3, 140.6, 146.0, 147.4, 154.7, 167.2; MS (ESI, +) *m*/*z* 367 [M + H]$^+$.

Methyl 2-Bromo-6-{[((2*R*)-2-(5,6-dihydro-1,4,2-dioxazin-3-yl)-2-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amino}ethyl)-sulfanyl]methyl}-5-{[dimethyl(1,1,2-trimethylpropyl)-silyl]oxy}-3-methoxybenzoate (12). In an inert atmosphere, trifluoroacetic acid (1.5 mL) was cooled to 0 °C and 2-bromo-5-dimethylthexylsilyloxy-6-formyl-3-methoxybenzoic acid methyl ester (**11**;[13] 0.385 g, 0.955 mmol) was added. A suspension of compound **10** (0.35 g, 0.955 mmol) and triethylsilane (0.14 g, 1.2 mmol) in 2 mL CH_2Cl_2 was added. The mixture was stirred for 18 h at 0 °C. After removal of the solvent, ethyl acetate was added to the mixture, and it was washed successively with water, saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4 and purified via column chromatography using an EtOAc/cyclohexane 1:2 ⇒ 2:1 gradient. The product **12** was obtained in a yield of 0.35 g (47%) as a dark-yellow powder: mp 63 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 6H, $SiCH_3$), 0.91 (d, J = 6.9 Hz, 6H, TDS−CH_3), 0.97 (s, 6H, TDS−CH_3), 1.70 (m, 1H, TDS−H), 3.07 (d, J = 6 Hz, 2H, CH_2), 3.68 (d, J = 13 Hz, 1H, CH_2), 3.76 (s, 3H, OCH_3), 3.82 (d, J = 13 Hz, 1H, CH_2), 3.96 (s, 3H, OCH_3), 4.08 (m, 2H, dioxazine-CH_2), 4.34 (m, 2H, dioxazine-CH_2), 4.60 (m, 1H, CH), 5.80 (br, 1H, NH), 6.33 (s, 1H, arom H), 6.92 (s, 1H, thiazole-H), 7.94 (d, J = 9 Hz, 2H, arom H), 8.22 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 0.0, 20.5, 22.1, 27.3, 35.8, 36.3, 54.7, 57.5, 58.3, 65.7, 66.8, 103.0, 106.2, 107.9, 121.9, 126.0, 128.4, 139.5, 142.7, 148.8, 150.7, 156.1, 157.0, 157.5, 169.2, 170.0; MS (ESI, +) *m*/*z* 782 [M + H]$^+$.

Methyl 2-Bromo-6-{[((2*R*)-2-(5,6-dihydro-1,4,2-dioxazin-3-yl)-2-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amino}ethyl)-sulfanyl]methyl}-5-hydroxy-3-methoxybenzoate (BAY 50-7952, 13). Compound **12** (0.6 g, 0.767 mmol) was dissolved in 20 mL methanol. Ammonium fluoride (280 mg, 7.67 mmol, 10 equiv) was then added. After addition of 100 mL ethyl acetate, the reaction mixture was washed with 60 mL H_2O. The aqueous layer was extracted once more with ethyl acetate (50 mL). The combined organic layers were washed with 60 mL brine and dried with Na_2SO_4. After removal of the solvent in vacuo, the residue was dissolved in ethyl acetate and precipitated by the addition of hexane. The precipitate was filtered and washed with hexane. Compound **13** was obtained as bright yellow powder: yield 0.41 g (82.8%); mp 93 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 2.99 (dd, J = 14 Hz, 6.3 Hz, 1H, CH_2), 3.08 (dd, J = 14 Hz, 6.3 Hz, 1H, CH_2), 3.74 (s, 3H, OCH_3), 3.76 (d, J = 14 Hz, 1H, CH_2), 3.83 (d, J = 14 Hz, 1H, CH_2), 3.95 (s, 3H, OCH_3), 4.10 (m, 2H, dioxazine-CH_2), 4.39 (m, 2H, dioxazine-CH_2), 4.75 (m (br), 1H, CH), 5.96 (br, 1H, NH), 6.44 (s, 1H, arom H), 6.93 (s, 1H, thiazole-H), 7.90 (d, J = 9 Hz, 2H, arom H), 8.21 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ

29.2, 33.2, 52.8, 55.9, 56.3, 63.8, 65.0, 99.7, 102.4, 106.0, 113.6, 124.0, 126.5, 137.6, 140.4, 146.8, 148.8, 155.5, 155.9, 156.2, 167.4, 167.9; MS (ESI, +) *m*/*z* 640 $[M + H]^+$. Anal. ($C_{24}H_{23}BrN_4O_8S_2$) C, H, N.

Methyl 2-Bromo-6-{[((2*S*)-2-(5,6-dihydro-1,4,2-dioxazin-3-yl)-2-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amino}ethyl)sulfanyl]methyl}-5-hydroxy-3-methoxybenzoate (BAY 51-4137, 15, enantiomer of 13). Starting from DL-cysteine, the corresponding racemate **14** of **13** was synthesized in the same manner and in similar yields as **13**. The racemic mixture was separated via chiral HPLC (conditions given in the general experimental part). Under preparative HPLC conditions, the (*R*)-enantiomer **13** and the (*S*)-enantiomer **15** have retention times of 18.0 and 23.5 min, respectively. Under analytical conditions, the (*R*)-enantiomer **13** and the (*S*)-enantiomer **15** have retention times of 7.67 and 8.42 min, respectively. Compound **15** was obtained in an optical purity of 99.5% according to HPLC analysis.

***N*^{1}-[(1*Z*)-*N*-Hydroxyethanimidoyl]-*N*^{2}-[4-(4-nitrophenyl)-1,3-thiazol-2-yl]-*S*-trityl-L-cysteinamide (17).** The crude product **6** (13.4 g, 23.0 mmol) was dissolved in 120 mL DMF, and the solution was cooled to −30 °C. HOBT (5.95 g, 39.1 mmol) and EDC (7.43 g, 39.1 mmol) were then added. After 15 min, a solution of *N*-hydroxyacetamidine hydrochloride[15] (3.6 g, 32.6 mmol) and triethylamine (3.3 g, 32.6 mmol, 4.6 mL) in 120 mL DMF was slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 14 h. Water (200 mL) was added subsequently, and the mixture was extracted twice with 200 mL ethyl acetate. The combined organic layers were successively washed 5% citric acid, 10% $NaHCO_3$ solution and twice with brine. The organic layer was dried with Na_2SO_4, and the solvent was removed in vacuo. Crude yield: 13.3 g. The crude material was used without further purification. Analytical data of a purified sample: mp 100 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 1.88 (s, 3H, CH_3), 2.71 (dd, J = 5.5 Hz, 13.5 Hz, 1H, CH_2), 2.84 (dd, J = 5.5 Hz, 13.5 Hz, 1H, CH_2), 4.50 (m, 1H, CH), 7.00 (s, 1H, thiazole-H), 7.16−7.42 (m, trityl-H), 7.92 (d, J = 9 Hz, 2H, arom H), 8.19 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 16.8, 33.7, 56.0, 67.3, 106.1, 124.0, 126.5, 127.0, 128.1, 129.5, 140.3, 144.2, 146.9, 148.6, 156.8, 167.0, 168.4.

***N*-[(1*R*)-1-(3-Methyl-1,2,4-oxadiazol-5-yl)-2-(tritylsulfanyl)ethyl]-*N*-[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amine (18).** Compound **17** (4 g, 6.41 mmol) was dissolved in THF (160 mL) and treated with Burgess reagent [(methoxycarbonylsulfamoyl)triethylammonium *N*-betaine] (1.68 g, 7.05 mmol). After refluxing for 3 h, the mixture was cooled to room temperature, and the solvent was removed in vacuo. The crude product was absorbed onto silica gel and purified via column chromatography using EtOAc/cyclohexane 1:3. The product **18** was obtained as a bright yellow powder: yield 0.97 g (25% over three steps starting from **4**); mp 90 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, oxadiazole-CH_3), 2.98 (m, 2H, CH_2), 4.95 (m, 1H, CH), 5.36 (br, 1H, NH), 6.93 (s, 1H, thiazole-H), 7.18−7.41, 7.40−7.44 (m, 15H, trityl-H), 7.82 (d, J = 9 Hz, 2H, arom H), 8.20 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.6, 34.9, 51.1, 67.6, 106.3, 124.0, 126.4, 127.1, 128.0, 129.4, 140.3, 144.0, 146.8, 148.8, 166.1, 167.2, 177.9; MS (ESI, +) *m*/*z* 606 $[M + H]^+$.

The further conversion of **18** to **19** was carried out in an analogous manner and in similar yields as described for the preparation of **13** from **9**.

Methyl 2-Bromo-5-hydroxy-3-methoxy-6-{[((2*R*)-2-(3-methyl-1,2,4-oxadiazol-5-yl)-2-{[4-(4-nitrophenyl)-1,3-thiazol-2-yl]amino}ethyl)sulfanyl]methyl}benzoate (BAY 50-7954, 19). Bright yellow powder: mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H, oxadiazole-CH_3), 3.12 (dd, J = 14.4 Hz, 6.9 Hz, 1H, CH_2), 3.25 (dd, J = 14.4 Hz, 5.2 Hz, 1H, CH_2), 3.74 (d, J = 4.1 Hz, 1H, CH_2), 3.80 (s, 3H, OCH_3), 3.83 (d, J = 4.1 Hz, 1H, CH_2), 3.99 (s, 3H, OCH_3), 5.59 (br, 1H, CH), 6.08 (br, 1H, NH), 6.47 (s, 1H, arom H), 6.97 (s, 1H, thiazole-H), 7.89 (d, J = 9 Hz, 2H, arom H), 8.23 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 11.6, 29.4, 33.9, 52.0, 53.0, 56.4, 102.2, 106.5, 113.4, 124.1, 126.5, 137.6, 140.2, 146.9, 148.8, 155.7, 156.3, 166.7, 167.1, 168.1, 178.0; MS (ESI, +) *m*/*z* 638 $[M + H]^+$. Anal. ($C_{24}H_{22}BrN_5O_7S_2$) C, H, N.

***S*-Trityl-L-cysteine Methyl Ester Hydrochloride (20).** *S*-Trityl-L-cysteine (50 g, 0.138 mol) was added to 1000 mL methanol to form a suspension. After cooling to 0−5 °C, thionyl chloride (75 mL, 1.03 mmol) was added, and the reaction mixture was allowed to warm to room temperature. The mixture was then refluxed for 5 h, cooled, and evaporated in vacuo. The residue was dried for 2 d at 30 °C in high vacuum. The product **20** was obtained as a white, finely crystalline solid: yield 55.52 g (97.2%); mp 78 °C (phase change); ^{1}H NMR (DMSO-d_6) δ 2.60 (m, 2H, CH_2), 3.71 (s, 3H, CO_2CH_3), 3.85 (dd, J = 6 Hz, 6 Hz, 1H, CH), 7.28−7.41 (m, 15H, trityl-H), 8.50 (br, 3H, NH_3^+).

***N*-[4-(4-Nitrophenyl)thiazol-2-yl]-*S*-trityl-L-cysteine Methyl Ester (21).** Compound **20** (2.67 g, 6.46 mmol) was dissolved in 50 mL ethanol, and triethylamine (0.65 g, 6.46 mmol, 0.9 mL) was added. Afterward, compound **5** (1.44 g, 6.46 mmol) was added, and the mixture was stirred at 50 °C for 16 h. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate and subsequently washed with water. The aqueous layer was extracted three times with ethyl acetate, and the combined organic layers were dried with Na_2SO_4. The product **21** was obtained as a golden yellow solid (3.6 g, 96%, purity 90−95%). It was used without further purification: mp 131−133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (m, 1H, CH_2), 2.71 (m, 1H, CH_2), 3.60 (s, 3H, OCH_3), 4.32 (m, 1H, CH), 7.20−7.33 (m, 15H, trityl-H), 7.52 (s, 1H, thiazole-H), 7.99 (d, J = 8.9 Hz, 2H, ArH), 8.26 (d, J = 8.9 Hz, 2H, ArH), 8.36 (d, J = 7.8 Hz, 1H, NH); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 33.5, 52.8, 56.3, 67.0, 105.8, 124.0, 126.4, 126.9, 128.0, 129.5, 140.6, 144.3, 146.8, 148.9, 166.6, 171.3.

The further conversion of **21** to **22** was carried out in an analogous manner and in similar yields as described for the preparation of **13** from **9**.

BAY 50-7950 (22). Bright yellow powder: mp 67 °C (phase change); ^{1}H NMR ($CDCl_3$) δ 3.04 (dd, J = 14 Hz, 6 Hz, 1H, CH_2), 3.26 (dd, J = 14 Hz, 4.7 Hz, 1H, CH_2), 3.72 (d, J = 14 Hz, 1H, CH_2), 3.77 (s, 3H, OCH_3), 3.80 (d, J = 14 Hz, 1H, CH_2), 3.81 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.86 (br, 1H, CH), 5.97 (br, 1H, NH), 6.42 (s, 1H, arom H), 6.94 (s, 1H, thiazole-H), 7.90 (d, J = 9 Hz, 2H, arom H), 8.23 (d, J = 9 Hz, 2H, arom H); ^{13}C NMR ($CDCl_3$) δ 29.7, 32.8, 52.9, 53.0, 56.4, 57.4, 99.8, 102.3, 106.1, 113.2, 124.0, 126.5, 137.6, 140.3, 140.5, 146.8, 148.8, 155.8, 156.3, 167.1, 168.0, 171.5; MS (ESI, +) *m*/*z* 614 $[M + H]^+$. Anal. ($C_{23}H_{22}BrN_3O_8S_2$) C, H, N.

Biology. 1. DNA Topoisomerase Tests. *M. luteus* DNA gyrase and calf thymus DNA topoisomerase I were purchased from Life Technologies (Karlsruhe, Germany), *E. coli* DNA gyrase from Lucent Ltd. (Leicester, U.K.), and human DNA topoisomerases I and II from TopoGEN (Columbus, OH). The *E. coli* DNA topoisomerase I gene was cloned in the pET28a vector (Novagen), and the resulting N-terminally His-tagged enzyme was overproduced in *E. coli* BL21(DE3)pLys and purified on Ni-NTA support (Qiagen). All reactions were performed in buffers as suggested by the respective manufacturers. Supercoiled pBR322 plasmid DNA (Life Technologies) was used as substrate for the enzymatic reaction. For DNA gyrase tests, the plasmid was first relaxed using calf thymus DNA topoisomerase I and then purified using standard procedures.[16]

Test compounds were diluted to the desired concentrations in 2 μL DMSO. Topoisomerases (1 unit) and 100 ng substrate DNA were added in the respective reaction buffers to a final volume of 20 μl. DNA gyrase and *E. coli* DNA topoisomerase I reactions were incubated at 37 °C for 60 min, the eukaryotic enzyme reactions were incubated for 30 min. Reactions were stopped by addition of 3 μL stop solution (0.3% SDS, 0.3% bromophenyl blue, 40% sucrose). DNA topoisomers were separated by electrophoresis on 1% agarose gels at 45 V for 15 h. Gels were then stained in 1 μg/mL ethidium bromide for 10 min followed by 20 min destaining in water. The gels were photographed under UV light and visually inspected and scored for IC_{50} values.

2. MIC Determination (minimal inhibitory concentration). The MICs were determined by the agar dilution method using IsoSensitest agar or Mueller Hinton agar supplemented with 10% horseblood and IsoVitaleX. Overnight cultures were grown in BHI broth (staphylococci, enterobacteria) or BHI broth supplemented with 10% bovine serum (streptococci, enterococci). After dilution (1:100), agar plates containing the test compounds (concentration range: 0.125−64 μg/mL) were inoculated with the test bacteria by a Denley inoculator. Plates were incubated aerobically at 37 °C and read after approximately 20 h. The MIC was considered the first concentration with no visible growth.

3. Systemic Infection with *S. aureus* 133. Bacteria from an overnight culture were grown to the logarithmic growth phase in BHI broth. The culture was precipitated and washed twice with phosphate-buffered saline. Bacteria were suspended in phosphate-buffered saline containing 10% mucin to a cell number of 4×10^6 cells/mL. Female CFW1 mice (20 g) were infected with 0.25 mL of the bacterial suspension. The test compounds were dosed ip at 30 min post-infection. The survival in each treatment group was monitored for 6 days.

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(14) See for example: (a) Kikugawa, Y. A new synthesis of sulfides from thiols and aldehydes or ketones with pyridine-borane in trifluoroacetic acid. *Chem. Lett.* **1981**, 1157−1158. (b) Olah, G. A.; Wang, Q.; Trivedi, N. J.; Surya Prakash, G. K. Boron trifluoride monohydrate catalyzed one-flask preparation of sulfides from carbonyl compounds with thiols and triethylsilane. *Synthesis* **1992**, 465−466.

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