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Short communication

Antimicrobial activity of *N*-hydroxyalkyl 1,2-benzisothiazol-3(2*H*)-ones and their thiono analogues

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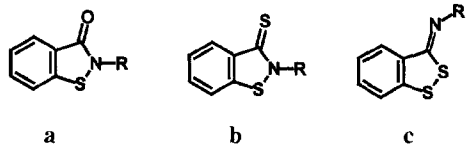
Summary — *N*-Hydroxyalkyl derivatives of 1,2-benzisothiazol-3(2*H*)-one and 1,2-benzisothiazol-3(2*H*)-thione have been prepared and their antifungal and antibacterial activity evaluated. Several compounds were active against selected fungi and Gram-positive microorganisms. Interesting activity was observed against the anaerobic strain *Clostridium perfringens*. Generally the more active compounds belong to the class of 1,2-benzisothiazol-3(2*H*)-ones. The retardation matches R_M of the compounds was also evaluated but the results obtained show that lipophilicity has only a minor effect on the antimicrobial activity.

1,2-benzisothiazol-3(2*H*)-one / 1,2-benzisothiazol-3(2*H*)-thione / antifungal activity / antibacterial activity

Introduction

The 2-substituted 1,2-benzisothiazol-3(2*H*)-ones **a** (fig 1) have been reported to show a variety of biological activities [1], and their antifungal and antibacterial properties have attracted considerable attention [2, 3]. The general interest in these compounds has shifted to their industrial application as biocides [4] even if the parent compound 1,2-benzisothiazol-3(2*H*)-one (BIT) is not recommended for pharmaceutical, cosmetic and toiletry preparations since it is a skin sensitizer [5]. Nevertheless, the lack of data about the antimicrobial properties of BIT derivatives has led to much research on the effect of substitutions at different positions in the molecule. The influence of the *N*-substitution with alkyl chains has been widely studied [6] but only marginal attention has been paid to the hydroxyalkyl derivatives. Such a modification could be interesting as alkyl chains can act as modulators of the lipophilicity of these structures and prompted us to synthesize some new *N*-hydroxyalkyl 1,2-benzisothiazol-3(2*H*)-ones.



a b c

Fig 1. 1,2-Benzisothiazol-3(2*H*)-ones **a**, 1,2-benzisothiazol-3(2*H*)-thiones **b**, and 3-imino-3*H*-1,2-benzodithioles **c**.

The 2-substituted 1,2-benzisothiazol-3(2*H*)-thiones **b** (fig 1) have been claimed to possess useful fungicide activity [7–9]. To assess the influence of the thiocarbonyl group and extend the studies to both classes of compounds **a** and **b**, we prepared the corresponding thiono analogues of the *N*-hydroxyalkyl 1,2-benzisothiazol-3(2*H*)-ones.

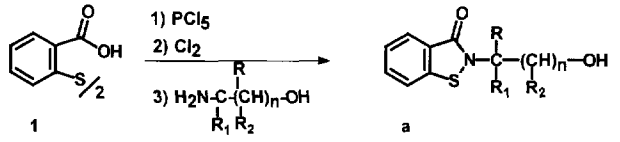
All the synthesized compounds have been tested against representative fungal and bacterial microorganisms. The antifungal activity was determined by using traditional screenings. The antibacterial activity was evaluated by a new, fast and reliable method that monitors the in vitro kinetic growth of cells by using the Bioscreen Analyzer [10]. We also present a growth evaluation of the anaerobic bacteria *Clostridium perfringens*, the most common organism cultured from cases of human foodborne enterocolitis [11]. The lipophilicity of all compounds was also evaluated in order to establish eventual correlation with antimicrobial activity.

Chemistry

Derivatives **a** were synthesized starting from 2,2'-dithiobis(benzoic acid) **1** by treatment with phosphorus pentachloride according to the McClelland procedure [12] (scheme 1). The intermediate 2,2'-dithiobis(benzoyl chloride) was not isolated and immediately treated with dry chlorine and then with the appropriate hydroxyalkylamine to give the desired *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-ones **2a–6a**.

Compounds characterized by structure **b** were prepared by reaction between the 3*H*-1,2-benzodithiole-3-thione **7** and a primary amine [13]. Depending on the nature of the amine, the solvent polarity and the temperature, different ratios of isomers **b** and **c** were obtained [14–16]. Using ethanolamine as coupling reagent a dynamic equilibrium of the two forms **b** and **c** occurs in solution, making the isomers inseparable by usual liquid chromatographic techniques [17–19].

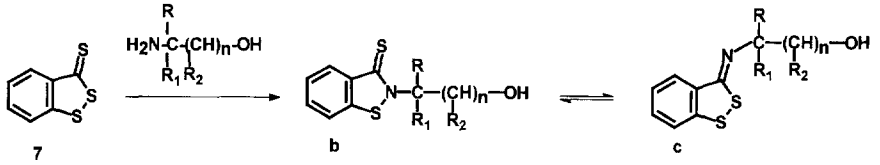
Thus, *N*-hydroxyalkylthio derivatives **8b,c–10b,c** were synthesized by treatment of 3*H*-1,2-benzodithiole-3-thione **7** [13] with the appropriate hydroxyalkylamine to give mixtures of *N*-substituted 1,2-benzisothiazol-3(2*H*)-thiones **8b–10b** and 3-imino-3*H*-1,2-benzodithioles **8c–10c** (scheme 2). This procedure gave unsatisfactory results in the synthesis of compounds **11b,c–12b,c**, which were obtained in better yields by sulfuration of the corresponding oxocarbonyl derivatives **5a** and **6a** with Lawesson's reagent [20] (scheme 3).



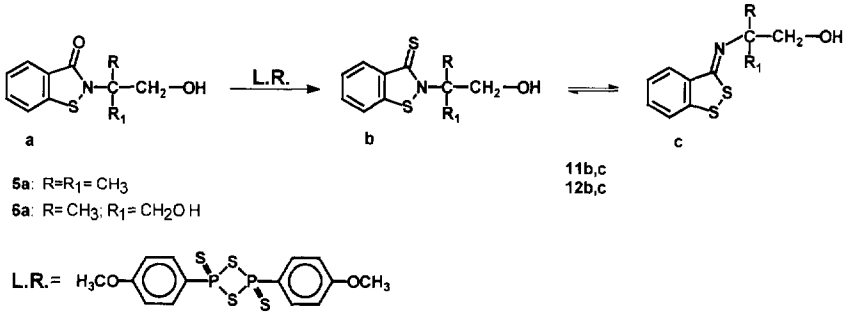
Scheme 1. 2a: R = R_1 = R_2 = H, *n* = 1; **3a**: R = R_1 = R_2 = H, *n* = 2; **4a**: R = R_1 = H, R_2 = CH_3, *n* = 1; **5a**: R = R_1 = CH_3, R_2 = H, *n* = 1; **6a**: R = CH_3, R_1 = CH_2OH, R_2 = H, *n* = 1.

The isomeric forms **8b–12b** and **8c–12c** could not be isolated by usual preparative liquid chromatography; the identity of synthesized compounds was thus reliably confirmed by ^{1}H-NMR of the mixture. As previously reported [15, 18, 19] a good diagnostic feature of isomers **b** and **c** is the chemical shift of the methylene protons adjacent to the nitrogen atom, the protons of isomers **b** being shifted downfield (δ 4.11–4.61) and the protons of isomers **c** being shifted upfield (δ 3.51–3.93). Further evidence was obtained from the ^{13}C-NMR spectra of compounds **8b** and **8c** showed two peaks at δ 185.75 (C=S) and 163.28 (C=N), respectively.

All new compounds gave satisfactory elemental analyses (C, H, N) within 0.35% of the theoretical values and the structures were in accordance with their spectroscopic data, reported in tables I and II.



Scheme 2. 8b,c: R = R_1 = R_2 = H, *n* = 1; **9b,c**: R = R_1 = R_2 = H, *n* = 2; **10b,c**: R = R_1 = H, R_2 = CH_3, *n* = 1.



Scheme 3.

Table I. Physicochemical data of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-ones **2a–6a**.

Compound	*Mp °C (solvent[a])*	*R_f (eluent[a])*	*R_M*	*Analyses (C, H, N)*	*Mass*	*^{1}H-NMR ($CDCl_3$)*
2a	110–113 [27] (A)	0.24 (A/D 9:1)	–0.57	$C_9H_9NO_2S$	195 (M^+, 28), 164 (90), 152 (38), 151 (100), 136 (41)	δ 3.95 (t, NCH_2, 2H), 4.05 (t, $OCH_2$2, 2H), 7.41 (dt, H-5, $J_{4,5}$ = $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.55 (dt, H-7, $J_{6,7}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.62 (dt, H-6, $J_{7,6}$ = $J_{5,6}$ = 8.0 Hz, $J_{4,6}$ = 1.0 Hz, 1H), 8.03 (dt, H-4, $J_{5,4}$ = 8.0 Hz, $J_{6,4}$ = 1.0 Hz, 1H)
3a	71–75 [27] (A)	0.28 (A/D 9:1)	–0.48	$C_{10}H_{11}NO_2S$	209 (M^+, 54), 165 (40), 164 (38), 151 (57), 136 (100)	δ 1.92 (m, $CH_2CH_2CH_2$, 2H), 3.58 (t, NCH_2, 2H), 4.0 (br s, OH, 1H), 4.10 (t, OCH_2, 2H), 7.43 (dt, H-5, $J_{4,5}$ = $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.66 (dt, H-7, $J_{6,7}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.76 (dt, H-6, $J_{7,6}$ = $J_{5,6}$ = 8.0 Hz, $J_{4,6}$ = 1.0 Hz, 1H), 8.16 (dt, H-4, $J_{5,4}$ = 8.0 Hz, $J_{6,4}$ = 1.0 Hz, 1H)
4a	Oil [27]	0.31 (A/D 6:4)	–0.47	$C_{10}H_{11}NO_2S$	209 (M^+, 4), 195 (13), 177 (10), 168 (18), 151 (100), 136 (15), 104 (56)	δ 1.28 (d, CH_3, 3H), 3.47 (br s, OH, 1H), 3.83 (dd, CH_2, ^2J = 14.5 Hz, ^3J = 7 Hz , 1H), 3.84 (dd, CH_2, 1H, ^2J= 14.5 Hz, ^3J = 3.5 Hz), 4.21 (m, CH, 1H), 7.39 (dt, H-5, $J_{4,5}$ = $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.53 (dt, H-7, $J_{6,7}$ = 8.0 Hz, $J_{5,7}$ = 1.0 Hz, 1H), 7.60 (dt, H-6, $J_{7,6}$ = $J_{5,6}$ = 8.0 Hz, $J_{4,6}$ = 1.0 Hz, 1H), 8.00 (dt, H-4, $J_{5,4}$ = 8.0 Hz, $J_{6,4}$ = 1.0 Hz, 1H)
5a	89 (B)	0.64 (E/F 95:5)	–0.14	$C_{11}H_{13}NO_2S$	223 (M^+, 18), 192 (21), 152 (58), 151 (100), 136 (12)	δ 1.61 (s, CH_3, 6H), 3.96 (s, CH_2, 2H), 7.39 (t, H-5, $J_{4,5}$ = $J_{5,6}$ = 7.0 Hz, 1H), 7.51 (d, H-7, $J_{6,7}$ = 8.0 Hz, 1H), 7.61 (t, H-6, $J_{7,6}$ = $J_{5,6}$ = 8.0 Hz, 1H), 7.96 (d, H-4, $J_{5,4}$ = 8.0 Hz, 1H)
6a	136–140 (C)	0.33 (E/F 95:5)	–0.35	$C_{11}H_{13}NO_3S$	239 (M^+, 18), 208 (23), 190 (15), 178 (16), 152 (69), 151 (100), 137 (20)	δ 1.60 (s, CH_3, 3H), 3.88 (d, CH_A-OH, 2H), 4.23 (d, CH_BOH, 2H), 7.42 (t, H-5, $J_{4,5}$ = $J_{5,6}$ = 7.0 Hz, 1H), 7.52 (d, H-7, $J_{6,7}$ = 8.0 Hz, 1H), 7.64 (t, H-6, $J_{7,6}$ = $J_{5,6}$ = 8.0 Hz, 1H), 7.99 (d, H-4, $J_{5,4}$ = 8.0 Hz, 1H)

[a]A: ethyl acetate; B: ethyl ether; C: ethanol; D: hexane; E: methylene chloride; F: methanol.

Table II. Physicochemical data of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-thiones **8b,c–12b,c**.

Compound	*Mp °C (solvent[a])*	*R_f **b**; **c** (eluent[a])*	*R_M **b**; **c***	*Analyses (C, H, N)*	*Mass*	*^{1}H-NMR ($CDCl_3$)*
8b,c	107 (A)	0.31; 0.48 (A/C 4:6)	–0.12; 0.30	$C_9H_9NOS_2$	211 (M^+, 47), 180 (100), 178 (78), 167 (43), 152 (8), 134 (18)	δ 3.51 (t, **8c**: NCH_2, 2H), 4.00 (t, **8c**: OCH_2, 2H), 4.11 (t, **8b**: OCH_2, 2H), 4.51 (t, **8b**: NCH_2, 2H), 7.22–7.70 (m, Ar, 6H), 8.01 (d, **8c**: H-4, 1H), 8.34 (d, **8b**: H-4, 1H)
9b,c	70–71 (B)	0.31; 0.52 (A/C 5:5)	–0.05; 0.00	$C_{10}H_{11}NOS_2$	225 (M^+, 49), 192 (100), 180 (24), 167 (74), 152 (15), 134 (13)	δ 2.08 (m, **9b,c**: $CH_2CH_2CH_2$, 4H), 3.58 (t, **9b,c**: OCH_2, 4H), 3.93 (t, **9c**: NCH_2, 2H), 4.62 (t, **9b**: NCH_2, 2H), 7.27–7.70 (m, Ar, 6H), 7.93 (d, **9c**: H-4, 1H), 8.33 (d, **9b**: H-4, 1H)
10b,c	Oil	0.36; 0.53 (A/C 4:6)	0.00; 0.09	$C_{10}H_{11}NOS_2$	225 (M^+, 38), 192 (43), 180 (100), 167 (61), 152 (18), 134 (20)	δ 1.31 and 1.34 (d, CH_3, 4H), 3.22 (dd, **10c**: NCH, ^2J = 14.2 Hz, ^3J = 7.8 Hz, 1H), 3.42 (dd, **10c**: NCH, ^2J = 14.2 Hz, ^3J = 3.4 Hz, 1H), 4.29 (dd, **10b**: NCH, ^2J = 13.6 Hz, ^3J = 7.8 Hz , 1H), 4.60 (dd, **10b**: NCH, ^2J = 13.6 Hz, ^3J = 2.7 Hz, 1H), 7.26–7.77 (m, Ar, 6H), 8.03 (d, **10c**: H-4, 1H), 8.36 (d, **10b**: H-4, 1H)
11b,c	Oil	0.28; 0.40 (A/C 2:8)	0.64; 0.80	$C_{11}H_{13}NOS_2$	239 (M^+, 7), 206 (100), 152 (23), 135 (12), 109 (8)	δ 1.34 (s, **11b**: CH_3, 6H), 1.42 (s, **11c**: CH_3, 6H), 4.05 (br s, **11b**: CH_2, 2H), 4.12 (br s, **11c**: CH_2, 2H), 7.15–7.76 (m, Ar, 6H), 7.80 (d, **11c**: H-4, 1H), 8.08 (d, **11b**: H-4, 1H)
12b,c	85 (B)	0.28; 0.46 (A/C 5:5)	0.07; 0.17	$C_{11}H_{13}NO_2S_2$	255 (M^+, 10), 241 (13), 224 (7), 209 (10), 178 (38), 168 (100), 152 (29), 136 (40)	δ 1.40 (s, CH_3, 6H), 3.50 (d, **12c**: CH_AOH, 2H), 3.80 (d, **12c**: CH_BOH, 2H), 4.11 (d, **12b**: CH_AOH, 2H), 4.45 (d, **12b**: CH_BOH, 2H), 7.20–7.81 (m, Ar, 6H), 7.73 (d, **12c**: H-4, 1H), 8.13 (d, **12b**: H-4, 1H)

[a]A: ethyl acetate; B: ethyl acetate/petroleum ether; C: hexane.

Results and discussion

Antimicrobial activity

The in vitro antifungal and antibacterial activities of 1,2-benzisothiazol-3(2*H*)-ones **2a–6a** and isomeric mixtures of 1,2-benzisothiazol-3(2*H*)-thiones and 3-imino-3*H*-1,2-benzodithioles **8b,c–12b,c** derivatives are reported in table III. The microbiological results illustrate the good antifungal and antibacterial activity, especially against Gram-positive bacteria. The best results were obtained with compound **6a** bearing two hydroxyl groups on the side chain; the activity against the anaerobic strain *C perfringens* is particularly interesting.

Activity against fungi (Tricophyton mentagrophytes and Candida albicans)

The fungitoxicity of the compounds tested was generally higher against *T mentagrophytes (Tm)* (MIC between 1 and 15 μg/mL) than *C albicans (Ca)* (MIC between 5 and 20 μg/mL). The most active compounds were **6a** (MIC_{Tm} = 1 μg/mL; MIC_{Ca} = 5 μg/mL) and **8b,c** (MIC_{Tm} = 1.5 μg/mL; MIC_{Ca} = 5 μg/mL). Worthwhile activity was also seen with **2a** (MIC_{Tm} = 5 μg/mL; MIC_{Ca} = 10 μg/mL) and **9b,c** (MIC_{Tm} = 3 μg/mL; MIC_{Ca} = 15 μg/mL).

Activity against Gram-positive bacteria (Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis and C perfringens)

Activities against Gram-positive bacteria (*S aureus (Sau), S albus (Sal), B subtilis (BSu)* and *C perfringens (Cp)*) were quite interesting with MIC between 1.5 and 30 μg/mL. In these assays the 1,2-benzisothiazol-3(2*H*)-one derivatives were generally more active than the thiono derivatives. Compound **6a** had the lowest MIC (MIC_{Sau} = 2 μg/mL; MIC_{Sal} = 1.5 μg/mL; MIC_{Bsu} = 3 μg/mL). Interesting activities were also shown by compounds **2a** ($MIC_{Sau;Sal;Bsu}$ = 5 μg/mL) and **3a** (MIC_{Sau} = 3 μg/mL; $MIC_{Sal;Bsu}$ = 5 μg/mL). The most active compound against *C perfringens* was **6a** (MIC = 1.5 μg/mL), followed by **2a** and **8b,c** with MICs of 5 μg/mL.

Table III. In vitro antifungal and antibacterial activity of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-ones **2a–6a** and thiones **8b,c–12b,c** (MIC in μg/mL).

Compound	*Fungi*		*Gram-positive*				*Gram-negative*			
	T mentagrophytes	*C albicans*	*S aureus*	*S albus*	*B subtilis*	*C perfringens*	*E coli*	*S typhi*	*P aeruginosa*	*K pneumoniae*
2a	5	10	5	5	5	5	20	20	30	20
3a	5	10	3	5	5	10	20	25	40	20
4a	10	20	15	10	10	15	40	40	>50	40
5a	5	10	10	10	10	10	50	50	>50	50
6a	1	5	2	1.5	3	1.5	50	50	50	50
8b,c	1.5	5	10	5	10	5	50	50	>50	50
9b,c	3	15	15	10	10	10	>50	>50	>50	>50
10b,c	5	15	20	10	10	20	>50	>50	>50	>50
11b,c	15	20	30	20	15	20	50	50	>50	>50
12b,c	10	20	30	30	20	20	50	>50	>50	>50
Gentamicin	–	–	2.5	1.5	0.5	0.25	2.5	2.5	12.5	2.5
Cefotaxime	–	–	1.5	0.5	0.5	–	1.5	1.5	10	1.5
Clotrimazole	0.5	1.25	–	–	–	–	–	–	–	–

Activity against Gram-negative bacteria (Escherichia coli, Salmonella typhi, Klebsiella pneumoniae and Pseudomonas aeruginosa)

Activity against Gram-negative bacteria was generally poor, though compound **2a** showed some antibacterial activity, particularly against *S typhi*, *E coli* and *K pneumoniae* (MIC = 20 μg/mL). Compounds **2a** and **3a** showed a limited activity against *P aeruginosa*, with MIC in the range of 30 and 40 μg/mL.

Structure–activity relationships

Fuller et al suggested that the benzisothiazolone mode of antibacterial action could be explained by interaction with the thiol groups of glutathione, cysteine or biomacromolecules [21]. The S-S bond formation with biological targets is strictly related to the lability of S-N bond, which in turn can be affected by a combination of different factors [10].

The substitution of sulphur for oxygen in the keto-benzisothiazole system increases the strength of the S-N bond [13, 22]; this is in accordance with the lower antibacterial activity shown by the 1,2-benzisothiazol-3(2*H*)-thiones **b**. Moreover any rigorous structure–activity relationship considerations for these compounds are difficult because structures **b** and **c** are in equilibrium in the biological test conditions.

Lipophilicity parameters are attractive physicochemical properties in QSAR studies and chromatographic parameters are often used as substitutes for partition coefficients. Retardation matches (R_M) are true measures of lipophilicity and closely correlated with log *P* [23–26]; higher R_M values indicate higher lipophilicity.

In figure 2 lipophilicity parameters are reported as $R_M + 1$ in order to give positive values (see *Experimental protocols*). Compounds **2a–6a** are less lipophilic than the mixtures **8b,c–12b,c** where $R_M + 1$ are calculated as average values between the values of isomers **b** and **c**.

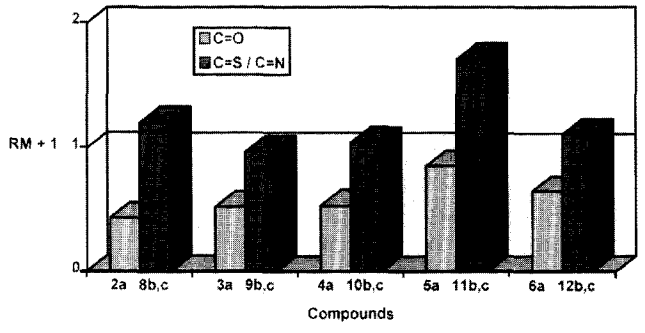


Fig 2. Lipophilicity parameters of 1,2-benzisothiazol-3(2*H*)ones **2a–6a** and their thiono analogues **8b,c–12b,c**.

In the series considered, similarly to several other antibacterial structures [24], no linear relationship was observed between activity and lipophilicity (see fig 2 and table III). The present findings show that factors influencing the lipophilicity of the molecules, such as different *N*-side chains or oxo-thiono substitution, cannot be considered important in determining differences in the antimicrobial activity.

In order to obtain new more active compounds, several parameters, including electronic and steric factors, could be considered while lipophilicity could be of interest in modulating the pharmacokinetics of these classes of compounds.

Experimental protocols

Chemistry

Melting points were measured using a Kofler hot-stage apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR were recorded in $CDCl_3$ at 300 and 75.46 MHz using a Bruker ACE-300 spectrometer. ^{1}H chemical shifts (δ) were reported with Me_4Si (δ = 0.00 ppm) as internal standard. ^{13}C chemical shifts (δ) were reported with $CDCl_3$ (central peak, δ = 77.00 ppm) as internal standard. The following abbreviations are used: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets and m = multiplet. Mass spectra were obtained on a Finnigan MAT 8222 spectrometer using the direct inlet. Electron ionization was performed at 70 eV and 0.5 mA with a source temperature of 250 °C. Elemental analyses were within 0.35% of the theoretical values and were performed on a Carlo Erba 1106 Elemental Analyser. All reactions were monitored by thin-layer chromatography on 0.25 mm Merck silica gel (60 F_{254}) and visualised by UV light (λ = 264 or 365 nm); flash chromatography was performed using silica gel 60 (60–200 μm, ICN Biomedicals Gmbh).

*General preparation of N-hydroxyalkyl 1,2-benzisothiazol-3(2H)-ones **2a–6a***

Dry Cl_2 was bubbled into a suspension of 2,2'-dithiobis(benzoylchloride) [12] (1 mmol) in dry CCl_4 until complete dissolution. Excess Cl_2 was removed by a dry nitrogen stream. The resulting solution of 2-(chlorothio)benzoylchloride was added dropwise to a stirred solution (10 mmol) of the appropriate hydroxyalkylamine in dry CCl_4. The reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo and the residue treated with dilute HCl. The mixture was extracted with ethyl acetate, the collected organic layers were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The residue was purified through flash chromatography.

*General preparation of N-hydroxyalkyl 1,2-benzisothiazol-3(2H)-thiones and 3-imino-3H-1,2-benzodithioles **8b,c–10b,c***

The appropriate hydroxyalkylamine (1.5 mmol) was added to a solution of 3*H*-1,2-benzodithiole-3-thione **7** [13] (1 mmol) in ethanol (for compounds **8b,c**) or xylene (for compounds

9b,c–10b,c). The mixture was refluxed for 3 h and then the solvent was removed under reduced pressure and the residue was purified through flash chromatography (*n*-hexane/ethyl acetate 50:50).

General preparation of N-hydroxyalkyl 1,2-benzisothiazol-3(2H)-thiones and 3-imino-3H-1,2-benzodithioles ***11b,c*** *and* ***12b,c***
A solution of the appropriate 1,2-benzisothiazol-3(2*H*)-one (1 mmol) and Lawesson's reagent (1 mmol) in dry toluene was refluxed under stirring and under nitrogen for 2 h. After a second addition of Lawesson reagent (0.5 mmol), the reaction mixture was refluxed for a further 2 h. The solvent was evaporated under reduced pressure and the residue purified through flash chromatography (**11b,c**: *n*-hexane/ethyl acetate 80:20; **12b,c**: *n*-hexane/ethyl acetate 55:45).

Lipophilicity tests

The relative lipophilicity of the compounds was measured by reverse-phase thin-layer chromatography [23, 24]. Silanized silica-gel plates Merck 60 F_{254} were used as the nonpolar stationary phase. The plates were dried at 105 °C for 1 h before use. The polar mobile phase was a 2:1 v/v mixture of acetone and water. Each compound was dissolved in chloroform (3 mg/mL) and 5 µL of the solution was applied to the plate. Experiments were repeated five times with different arrangements of the compounds on the plate. R_f are expressed as means of the five determinations. R_M calculated from the experimental R_f according to the equation: $R_M = \log [(1/R_f) - 1]$ are reported in tables I and II.

Microbiology

The antimicrobial activity was determined against a series of bacterial strains: *S aureus* (ATCC 6538), *S albus* (ATCC 12228), *B subtilis* (ISM 6513), and *C perfringens* (ATCC 12916) (Gram-positive species); and *E coli* (ISM 6585), *S typhi* (ATCC 19430), *K pneumoniae* (ATCC 4352), and *P aeruginosa* (ATCC 15442) (Gram-negative species). The mycetes tested were *C albicans* (ATCC 2091) and *T mentagrophytes* (ATCC 9129). Minimum inhibitory concentrations (MIC) were established as previously reported [10] by the medium dilution technique for the fungal strains and by using the Bioscreen Analyzer for the bacterial strains.

Some modifications in the Bioscreen method were adopted for *C perfringens*: the bacteria were incubated at 37 °C on Perfringens Agar (OPSP), in anaerobic conditions (NO_2 80%; H_2 10%; CO_2 10%). In the antibacterial tests cultures were grown at 37 °C for 24 h in Bacto Minimal Broth Davis containing 5% Tryptone, and after 1:10 serial dilution, were directly inoculated (600 µL) in Bioscreen cuvettes; 100 µL of sterile liquid paraffin was added to the cuvette surface in order to maintain anaerobiosis.

The test substances were dissolved in acetone/water solution (3:1) and the concentrations examined were in the range 1–50 µg/mL. The results of all measurements are shown as kinetic growth curves and their elaboration provided the antibacterial activities, expressed as MIC and presented in µg/mL. Gentamicin and cefotaxime, for antibacterial activity, and clotrimazole, for antimycotic activity, were employed as reference substances.

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