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Antimicrobial and genotoxic activities of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-thione carbamic esters

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Abstract

The antimicrobial properties of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-thione (**1a**,**b**) and its carbamic esters **2a**,**b**–**6a**,**b** were tested *in vitro* against Gram positive and Gram negative bacteria, yeasts and dermatophytes. All compounds markedly inhibit the growth of Gram positive bacteria exhibiting MIC values ranging from 1.25 to 10 μg/ml. A strong antifungal activity is exerted against dermatophytes with MICs, in general, between 0.7 and 12 μg/ml. Structure–activity relationship studies show that these compounds are, in most cases, more effective than the corresponding benzisothiazolone analogues **7**–**12**. None of the tested compounds shows genotoxic properties by *Bacillus subtilis rec*-assay and *Salmonella*-microsome test.

Keywords: 1,2-Benzisothiazole derivatives; Antimicrobial activity; Genotoxicity

1. Introduction

The antimicrobial activity of 1,2-benzisothiazoles has been extensively studied and various 1,2-benzisothiazol-3(2*H*)-ones are known to be effective against a wide range of bacteria and fungi [1–3]. Furthermore, many microbicidal compositions containing 1,2-benzisothiazolin-3-one have been reported in patent applications as industrial preservatives [4–10].

In recent papers the antimicrobial properties of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-ones [11] and related thiono analogues [12,13] have been reported. In this context a series of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-one and -thione carbamic esters was demonstrated to be active against *Mycobacterium avium*, a microorganism responsible for frequent complications in AIDS patients [13].

In the present study we enlarge the investigation on the antimicrobial properties of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-thione (**1a**,**b**) and its carbamic esters **2a**,**b**–**6a**,**b** and of their oxo analogues **7** and **8**–**12** (Fig. 1) against representative bacteria and fungi in order to better evaluate the extent of their activity spectrum.

Since carbamates have been found to act as electrophilic agents by alkylating or acylating DNA constituents [14–18], we looked also for the in vitro genotoxic properties of these compounds by determining their DNA-damaging activity in the *Bacillus subtilis*

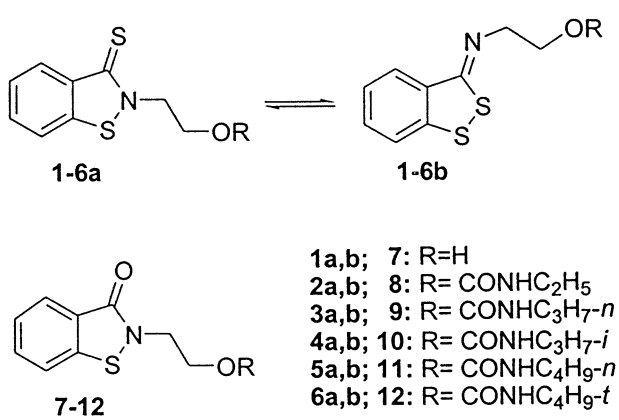

Fig. 1. Structure of tested compounds.

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Table 1
Antimicrobial activity against Gram positive bacteria (MIC, μg/ml)

Microorganism	Compounds							
	1a,b[a]	**2a,b**	**3a,b**	**4a,b**	**5a,b**	**6a,b**	Cefotaxime	Gentamicin
B. subtilis	10	10	10	5	10	10	0.5	0.5
C. perfringens	5	2.5	2.5	1.25	2.5	5	[b]	0.25
S. albus	5	5	5	5	5	10	0.5	1.5
S. aureus	10	5	5	5	5	10	1.5	2.5

[a] Values previously reported [12].
[b] Not tested.

rec-assay and their mutagenicity in the *Salmonella*-microsome test (Ames test).

2. Results and discussion

The in vitro antimicrobial activity of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-thione **1a,b** and its carbamic esters **2a,b–6a,b** against Gram positive bacteria is reported in Table 1. All compounds exhibit a good growth inhibition at concentrations ranging from 1.25 to 10 μg/ml. *Clostridium perfringens* is the most sensitive microorganism (MICs 1.25–5 μg/ml).

A poor activity against Gram negative bacteria is observed: compounds **2a,b–6a,b** exhibit MIC values > 50 μg/ml (data not shown), whereas the parent compound **1a,b** is effective against *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi* at the concentration of 50 μg/ml [12].

Table 2 reports the inhibitory activity of benzisothiazol-3(2*H*)-thione (**1a,b**) and its carbamic esters **2a,b–6a,b** against yeasts and moulds. The effects of the corresponding benzisothiazolone analogues **7** and **8–12** towards the same microorganisms are also reported. Generally compounds show moderate antifungal properties against yeasts, the best susceptibility being shown by *Cryptococcus neoformans*, which is inhibited by **1a,b–6a,b** at concentrations between 6 and 12 μg/ml. Benzisothiazolthiones and many benzisothiazolones possess good antifungal properties against dermatophytes *Epidermophyton floccosum* and *Trichophyton* spp. (MICs 0.7–12 μg/ml). However, the most interesting activity is exhibited against *Microsporum gypseum* with MIC values only 1.2–5 times higher than that of the reference compound.

Although for the studied compounds it is not possible to state a correlation between antimicrobial activity and chemical structure, the following can be stated:

1. the substitution in 2-position does not determine significant differences in the activity of the studied compounds;
2. concerning the antibacterial activity, the comparison of the MICs of compounds **1a,b–6a,b** with those previously reported for the corresponding oxo analogues [11] shows an increased potency of the thione carbamic esters towards *C. perfringens*;
3. regarding the antifungal activity, benzisothiazolones **8–12** seem to be more active upon *Candida* spp. than the corresponding benzisothiazolthiones **2a,b–6a,b**. The differences between the two classes are less evident by observing their activity towards moulds;
4. in all cases the antimicrobial activity of the studied compounds is lower than that of the reference substances.

None of the benzisothiazolthiones shows DNA-damaging activity in the *B. subtilis rec*-assay (Table 3) or mutagenicity in the *Salmonella*-microsome test (Table 4), although the carbamoyl group is cited as 'alerting structure' [22] and 'biofore' [23] for genotoxicity. This confirms the hypothesis that similar chemical structures may not exhibit similar biological activity.

Thus, it is interesting to underline that the lack of genotoxicity detected in benzisothiazolthiones and in the corresponding oxo analogues [11] may be of great importance in the development of new benzisothiazol-carbamates as potential antimicrobial agents.

3. Experimental

3.1. Chemistry

Compounds **1a,b–6a,b** and **7–12** were synthesized as previously reported and analytical data are in agreement with those published [11,13].

3.2. Biological assays

3.2.1. Antimicrobial activity

The *in vitro* antimicrobial activity of compounds was evaluated by the measure of the minimal inhibitory concentration (MIC, μg/ml) required to prevent visible growth of the tested microorganisms. The antibacterial activity was assayed against *Bacillus subtilis* ISM 6513, *Clostridium perfringens* ATCC 12916, *Staphylococcus albus* ATCC 12228 and *Staphylococcus aureus* ATCC

Table 2
Antimicrobial activity against fungi (MIC, μg/ml)

Microorganism	Compounds												
	1a,**b**	**2a**,**b**	**3a**,**b**	**4a**,**b**	**5a**,**b**	**6a**,**b**	**7**	**8**	**9**	**10**	**11**	**12**	Miconazole
Yeasts													
C. albicans[a]	50	50	>100	>100	>100	>100	100	25	25	25	12	25	6
C. guilliermondii	25	50	>100	>100	>100	>100	>100	50	25	50	25	50	0.3
C. parapsilosis	12	50	>100	>100	>100	>100	>100	100	50	100	25	50	0.7
C. tropicalis	50	50	>100	>100	>100	>100	50	25	12	25	12	12	6
C. neoformans	6	12	6	12	6	12	100	25	12	25	6	12	0.3
S. cerevisiae	25	25	25	50	25	100	100	50	25	50	25	25	12
Moulds													
E. floccosum	3	3	1.5	1.5	0.7	3	12	6	3	6	1.5	6	0.01
M. gypseum	50	25	12	25	25	12	>100	100	50	50	50	50	10
T. interdigitalis	3	12	6	25	6	12	50	50	25	25	12	25	1
T. mentagrophytes[a]	12	6	6	12	6	6	100	25	12	25	6	12	1
T. rubrum	3	1.5	1.5	6	3	3	25	12	12	12	6	6	1
T. soudanense	3	6	0.7	12	3	0.7	25	25	3	12	3	3	0.1

[a] The strain used in this study is different to that employed in Ref. [12].

6538 (all Gram positive bacteria) and against *Escherichia coli* ISM 6585, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 15442 and *Salmonella typhi* ATCC 19430 (all Gram negative bacteria). Cefotaxime and gentamicin were used as reference substances. The test was carried out by the Bioscreen Analyzer as previously reported [12].

The antifungal activity was evaluated against yeasts and moulds of dermatological interest by the serial twofold dilution technique [19]. The compounds were dissolved in DMSO and further progressive double dilutions with the test media furnished the required concentrations in the range from 0.15 to 100 μg/ml. Miconazole was employed as standard control. The following yeasts were used: *Candida albicans* ATCC 10231, *C. tropicalis* ATCC 1369, *Saccharomyces cerevisiae* ATCC 9763 and clinical isolates of *Candida guilliermondii*, *C. parapsilosis* and *Cryptococcus neoformans*. Well-growing cultures were obtained in Sabouraud liquid medium (pH 5.7, Oxoid) and then diluted to ensure an inoculum size of 10^3 CFU/ml. Tubes containing serial twofold dilutions of the compounds in Sabouraud liquid medium were inoculated with the test microorganisms and the MICs were recorded by observing the fungal growth after 48 h of incubation at 30°C. Dermatophytes freshly isolated from pathological materials were tested as moulds: *Epidermophyton floccosum*, *Microsporum gypseum*, *Trichophyton interdigitalis*, *T. mentagrophytes*, *T. rubrum* and *T. soudanense*. Dermatophytes were cultivated on Sabouraud dextrose agar (Oxoid). The inocula, prepared by suspending the mycelia in phosphate buffer (pH 7.4), were streaked on Sabouraud dextrose agar plates containing different concentrations of the test substances. MIC values were determined after 14 days of incubation at 25°C.

Table 3
DNA-damaging activity in the *Bacillus subtilis rec*-assay

Compound	Inhibition halo diameter (mm)		DNA-damaging activity[a]
	Rec$^+$	*Rec*$^-$	*Rec*$^-$/*rec*$^+$
1a,**b**	28	28	1
2a,**b**	16	16	1
3a,**b**	14	14	1
4a,**b**	13	13	1
5a,**b**	16	16	1
6a,**b**	15	15	1
Controls			
Ampicillin	35	35	1
Chloramphenicol	20	21	1.05
Methyl methanesulfonate	10	33	**3.30**

[a] Values in bold indicate positive response.

Table 4
Mutagenic activity on *Salmonella typhimurium* strains

Compound	Amount tested[a] (μg/plate)	*His*$^+$ revertants/plate[b]							
		TA 1535		TA 1537		TA 98		TA 100	
		−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
1a,**b**	50	15	11	9	10	41	48	156	164
2a,**b**	200	16	15	11	10	43	47	142	166
3a,**b**	500	15	21	6	8	36	52	155	133
4a,**b**	500	16	14	7	7	36	50	151	150
5a,**b**	500	12	15	14	13	39	37	173	153
6a,**b**	500	15	17	6	10	30	39	178	175
Controls[c]									
Spontaneous		11	11	6	7	32	36	142	124
2AAF	50	12	13	10	8	33	**>3000**	149	**>3000**
EtBr	10	13	15	9	8	34	**>2000**	160	141
MNNG	10	**>5000**	**>5000**	8	9	30	43	**>5000**	**>5000**

[a] The highest subtoxic dose is reported.
[b] Mean values from three experiments without correction for background are given. Values in bold print indicate mutagenic activity.
[c] 2AAF, 2-acetylaminofluorene; EtBr, ethidium bromide; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

3.2.2. Genotoxicity

3.2.2.1. Bacillus subtilis rec-assay. DNA-damaging activity was evaluated by using two strains of *Bacillus subtilis*: PB 1652 (*rec*$^+$) and PB 1791 (*rec*$^-$) [20]. Cultures of the test bacteria were grown overnight at 37°C in Mueller Hinton broth (Difco). Compounds were dissolved in DMSO and 30 μl of each solution (1200 μg) were adsorbed onto sterile paper disks (8 mm diameter) placed on the surface of Nutrient agar (Difco) plates seeded separately with 100 μl of each bacterial culture. After an incubation of 24 h at 37°C, the resulting zones of growth inhibition around the disks were measured in mm. Ampicillin (20 μg/disk) and chloramphenicol (30 μg/disk) were used as negative standard drugs, methyl methanesulfonate (300 μg/disk) as a positive one. DNA-damaging activity is estimated by comparing the diameter of the inhibition zone on the *rec*$^-$ strain with that on the *rec*$^+$ one. Active substances produce a *rec*$^-$/*rec*$^+$ value higher than 1.2. Each assay was repeated three times.

3.2.2.2. Salmonella-microsome test (Ames test). The mutagenic potential was measured by the histidine reversion assay [21] against *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 strains. Compounds were tested by the plate incorporation procedure at different concentrations from 5 μg/plate to the highest subtoxic dose in the absence and presence of rat liver microsomes (as S9 fraction). Each substance was incorporated in the molten top agar together with 10^8 cells of the indicator strain and, in the experiment in which activation and detoxification were determined, 500 μl of S9 mix. The mixture was homogenized and poured into minimal Vogel–Bonner agar plates. When solidified, the plates were incubated at 37°C for 72 h. *His*$^+$ revertant colonies were counted. An increase in spontaneous reversion of a factor of three for TA 1535 and TA 1537, and a factor of two for TA 98 and TA 100 indicates mutagenic activity. 2-Acetylaminofluorene, ethidium bromide and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine were employed in the screening as positive reference drugs.

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References

[1] A. De, in: G.P. Ellis, G.B. West (Eds.), Progress in Medicinal Chemistry, vol. 18, Elsevier/North-Holland Biomedical Press, 1981, pp. 117–133.
[2] M. Davis, in: A.R. Katritzky, A.J. Boulton (Eds.), Advances in Heterocyclic Chemistry, vol. 38, Academic Press, New York, 1985, pp. 114–116.
[3] R. Okachi, H. Niino, K. Kitaura, K. Mineura, Y. Nakamizo, Y. Murayama, T. Ono, A. Nakamizo, J. Med. Chem. 28 (1985) 1772–1779.

[4] H. Kluschanzoff, R. Lehmann, H.T. Leinen (Henkel K.-G.a.A.), Ger. Offen. DE 4 217 881 [Chem. Abstr. 120 (1994) 71556t].
[5] H. Eggensperger, P. Oltmanns, K.H. Scheler, K.H. Diehl (Sterling Winthrop, Inc.), U.S. US 5 276 047 [Chem. Abstr. 120 (1994) 144174d].
[6] J.D. Payne (Zeneca Ltd), PCT Int. Appl. WO 94 16,564 [Chem Abstr. 121 (1994) 295086y].
[7] P.W. Austin (Zeneca Ltd), PCT Int. Appl. WO 94 20 479 [Chem. Abstr. 121 (1994) 300882m].
[8] H. Egawa (Shinto Paint Co Ltd), Jpn. Kokai Tokkyo Koho JP 06 305 917 [94 305 917] [Chem. Abstr. 122 (1995) 99324f].
[9] H. Sugano, T. Nita, H. Yamamoto T. Kamagami, M. Odagiri (Junsei Chemical), Jpn. Kokai Tokkyo Koho JP 07 02 606 [95 02 606] [Chem. Abstr. 122 (1995) 180993y].
[10] S. Hiraiwa, T. Nakano, K. Suzuki, H. Nakao (Toray Industries; Osaka Kasei Kk), Jpn. Kokai Tokkyo Koho JP 06 313 269 [94 313 269] [Chem. Abstr. 122 (1995) 242251n].
[11] M.L. Carmellino, G. Pagani, M. Terreni, M. Pregnolato, P. Borgna, F. Pastoni, F. Zani, Il Farmaco 52 (1997) 359–366.
[12] P. Borgna, M.L. Carmellino, M. Natangelo, G. Pagani, F. Pastoni, M. Pregnolato, M. Terreni, Eur. J. Med. Chem. 31 (1996) 919–925.
[13] G. Pagani, P. Borgna, C. Piersimoni, D. Nista, M. Terreni, M. Pregnolato, Arch. Pharm. Pharm. Med. Chem. 329 (1996) 421–425.
[14] S.J. Rinkus, M.S. Legator, Cancer Res. 39 (1979) 3289–3318.
[15] J. Schlatter, W.K. Lutz, Fd. Chem. Toxic. 28 (1990) 205–211.
[16] A. Ashby, R.W. Tennant, Mutat. Res. 257 (1991) 229–306.
[17] D. Wild, Mutat. Res. 260 (1991) 309–310.
[18] K. Park, A. Liem, B.C. Stewart, J.A. Miller, Carcinogenesis 14 (1993) 441–450.
[19] P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tonover, R.H. Yolken (Eds.), Manual of Clinical Microbiology, 6th ed., American Society of Microbiology, Washington, DC, 1995, section X.
[20] P. Mazza, Appl. Environ. Microbiol. 43 (1982) 177–184.
[21] D.M. Maron, B.N. Ames, Mutat. Res. 113 (1983) 173–215.
[22] J. Ashby, R.W. Tennant, Mutat. Res. 204 (1988) 17–115.
[23] H.S. Rosenkranz, G. Klopman, Mutagenesis 5 (1990) 333–361.