ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 669–679

IL FARMACO

3*H*-[1,2]Dithiolo[3,4-*b*]pyridine-3-thione and its derivatives Synthesis and antimicrobial activity

Massimo Pregnolato [a,*], Marco Terreni [a], Daniela Ubiali [a], Giuseppe Pagani [a], Pietro Borgna [a], Fiorenzo Pastoni [b], Fabrizio Zampollo [b]

[a] *Dipartimento di Chimica Farmaceutica, Università degli Studi, via Taramelli 12, 27100 Pavia, Italy*
[b] *Joint Research Centre, Environment Institute, Food and Drug, Laboratory of Microbiology, via E. Fermi, 21020 Ispra (Va), Italy*

Received 22 February 2000; accepted 31 July 2000

Abstract

A series of 2-substituted isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones, isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones, *N*-substituted 2-sulfanylnicotinamides and the corresponding carbothioamide derivatives were synthesized and evaluated for their antimicrobial activity against several strains of Gram + and Gram − bacteria and fungi. Chemical syntheses were resumed into a comprehensive cyclic route that enables the reversible conversion for each derivative of the series considered. Among the tested compounds the *N*-(aralkyl)-2-sulfanylnicotinamides show the highest fungitoxicity (MIC = 1.25–5 μg/ml). The best activity towards Gram-positive bacteria was in the range of 2.5–5 μg/ml. Activity against Gram-negative bacteria was generally very poor for all compounds.

Keywords: 3*H*-[1,2]Dithiolo[3,4-*b*]pyridine-3-thiones; Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones; Isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones; 2-Sulfanylnicotinamides; 2-Sulfanylpyridine-3-carbothioamides; Antimicrobial activity

1. Introduction

During the last two decades, a large number of 2-substituted 1,2-benzisothiazolo-3(2*H*)-ones (**A**) [1–3] and 1,2-benzisothiazolo-3(2*H*)-thiones (**B**) [4–6] (Fig. 1) have been claimed to have several biological activities. The antifungal and antibacterial properties of these compounds have opened up the possibility of their potential use as products of industrial interest (agrochemicals, veterinary medicines and preservative addictives for inks, paints and photographics) [7–9]. An extensive study on benzene substituents has been done concluding that the position and the nature of the substituent influence the S–N bond reactivity and, as a consequence, the antimicrobial activity [10]. It has also been determined that the *N*-substitutions can influence the lipophilicity of the structure with a minor influence upon the antimicrobial activity [11]. The substitution of sulfur (B) for oxygen (A) in the ketobenzisothiazole system generally increases the strength of the S–N bond; although a strict structure–activity relationship could not be determined since a dynamic equilibrium occurs in the biological test medium with the corresponding 3-imino-3*H*-1,2-benzodithiole [12,13]. Considerable attention has been successfully devoted to the investigation of 1,2-benzisothiazolo-3(2*H*)-one isosters, namely isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**C**), where benzene is replaced by a pyridine ring [14,15]; some patents claimed their fungicidal and antibacterial properties [16], their activity as inhibitors of blood platelet aggregation [17] and as antiacne agents [18]. In our

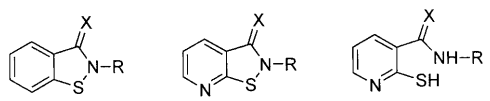
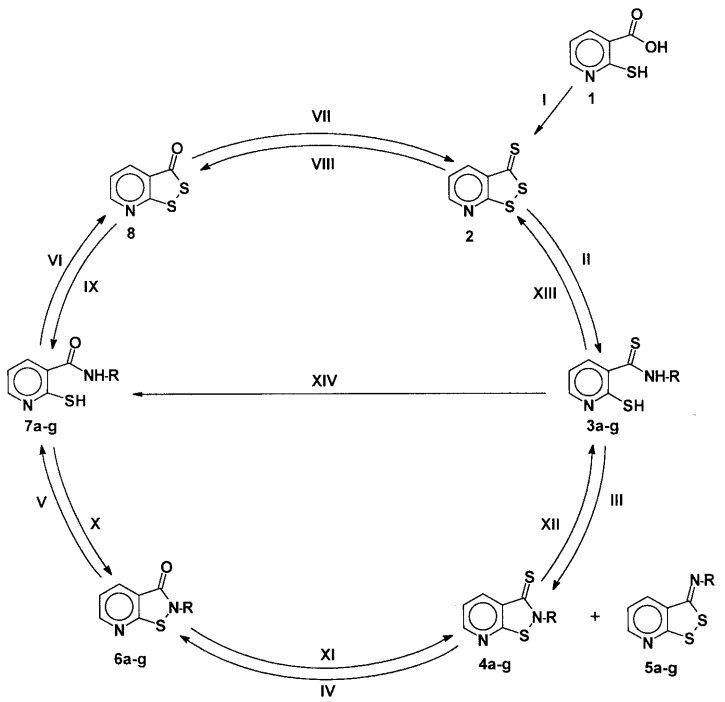


Fig. 1. 1,2-benzisothiazol-3(2*H*)-ones (**A**); 1,2-benzisothiazol-3(2*H*)-thiones (**B**); isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**C**); isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones (**D**); 2-sulfanylnicotinamides (**E**); 2-sulfanylpyridine-3-carbothioamides (**F**).

* Corresponding author. Tel.: + 39-0382-507 583; fax: + 39-0382-507 583.
E-mail address: maxp@pbl.unipv.it (M. Pregnolato).

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00084-7



a: R= *n*-C_3H_7 **e**: R= CH_2Ph
b: R= *n*-C_4H_9 **f**: R= $(CH_2)_2Ph$
c: R= *n*-C_5H_{11} **g**: R= $(CH_2)_3Ph$
d: R= *n*-C_6H_{13}

Scheme 1. **I**: Lawesson's reagent, toluene, 5 h, 75% yield. **II**: R–NH_2, abs. EtOH, 75–83% yields. **III**: I_2/$NaHCO_3$, abs. EtOH, 60–70% yields. **IV**: $Hg(CH_3COO)_2$, $CHCl_3$/CH_3COOH, 35–40% yields. **V**: H_2S, EtOH, 80–85% yields. **VI**: Lawesson's reagent, toluene, 30 min, 90% (ponderal yield). **VII**: Lawesson's reagent, toluene, 3 h, 70–77% yields. **VIII**: $Hg(CH_3COO)_2$, $CHCl_3$/CH_3COOH, 45–59% yields. **IX**: R–NH_2 abs EtOH, 63–90% yields. **X**: I_2/$NaHCO_3$, abs. EtOH, 74–81% yields. **XI**: Lawesson's reagent, toluene, 1.5 h, 26–31% yields. **XII**: H_2S, EtOH, 78–82% yields. **XIII**: P_4S_{10}, xylene, 2 h, 60–66% yields. **XIV**: $Hg(CH_3COO)_2$, $CHCl_3$/CH_3COOH, 32–37% yields.

previous paper [19] we studied the chemistry of isothiazolo[5,4-*b*]pyridine-3(2*H*)-thione (**D**) synthesizing some *N*-substituted derivatives. Structure (**D**), the thio-analogue of isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones (**C**), is expected to have a similar biological activity and in this paper some *N*-alkyl and *N*-aralkyl isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones have been synthesized as potential novel antimicrobial agents.

Following new synthetic protocols, a cyclic route of synthesis has been developed starting from 2-mercaptonicotinic acid (**1**) to give 3*H*-[1,2]dithiolo[3,4-*b*]pyridine-3-thione (**2**). From compound **2** both the thiocarbonyl- (**D** and **F**) and the carbonyl- derivatives (**C** and **E**) can be easily prepared through different pathways according to Scheme 1. All the obtained products **3**–**7** (**a**–**g**) were tested for their antimicrobial activity against several strains of Gram +, Gram − bacteria and fungi, in order to evaluate both the effect of the thiocarbonyl-oxocarbonyl substitution on the S–N isothiazolo[5,4-*b*]pyridine ring opening (to give structures **3** and **7**), and the influence of the lipophilicity apported by different *N*-alkyl or *N*-aralkyl chains.

A lipophilicity study has also been done in order to establish a potential structure–activity relationship for these classes of compounds.

2. Results and discussion

2.1. Chemistry

Chemical syntheses are included into a comprehensive cyclic route that enables the reversible conversion for each derivative of the series considered (Scheme 1). In order to obtain the desired compounds **3**–**7** (**a**–**g**) and **8**, novel synthetic procedures were exploited involving the interconversion between oxo-carbonyl and thio-carbonyl derivatives by using Lawesson's reagent (C=O → C=S) (Reactions I, VII, XI) [20] or mercuric acetate (C=S → C=O) (Reactions IV, VIII, XIV) [14].

3*H*-[1,2]dithiolo[3,4-*b*]pyridine-3-thione (**2**) is usually prepared by cyclization of 2-mercaptonicotinic acid (**1**) with phosphorous pentasulfide in anhydrous pyridine in

a good yield (95%) [14]; in this study, a novel approach was attempted using Lawesson's reagent and sulfur but a lower yield was obtained (Reaction I, 75% yield). By reaction between **2** and the appropriate primary amine, the *N*-substituted 2-sulfanylpyridine-3-carbothioamides **3a**–**g** were obtained in rapid equilibrium with the 2-thioxo-1,2-dihydropyridine-3-carbothioamides forms [19] (Reaction II, 75–83% yields). The preparation of isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **4a**–**g** was performed with iodine by oxidative cyclization of the corresponding 2-sulfanylpyridine-3-carbothioamides **3a**–**g** in ethanol (Reaction III, 60–70% yields). As previously described [19], the reaction afforded a mixture of *N*-substituted isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **4a**–**g** and *N*-substituted-*N*-[3*H*-[1,2]-dithiolo-[3,4-*b*]pyridin-3-ylidene]amines **5a**–**g**, respectively, which can be separated by flash chromatography. Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones **6a**–**g** were obtained by desulfurization of the corresponding thioderivatives **4a**–**g** by mercuric acetate in glacial acetic acid in 35–40% yield (Reaction IV) [14]. The complete conversion of bicyclic **6a**–**g** into the 2-sulfanylnicotinamides **7a**–**g** occurred after treatment with hydrogen sulfide (Reaction V, 80–85% yield). All synthesized compounds were fully characterized and analytical data are reported in Tables 1–5. In order to facilitate the formation of derivatives to be tested, **3**–**7** (**a**–**g**), alternative synthetic routes have been accomplished. Thus, the starting material (**2**) can easily be desulfurized by treatment with mercuric acetate (Reaction VIII, 45–59% yields) to give 3*H*-[1,2]-dithiolo[3,4-*b*]pyridin-3-one (**8**), the starting material of the derivatives **6**–**7** (**a**–**g**). In fact, compounds **7a**–**g** were obtained from **8** by reacting the appropriate amine in absolute ethanol

Table 1
2-Sulfanylpyridine-3-carbothioamides **3a**–**g**

Comp.	R	m.p. (°C)	R_f (eluent [a])	R_M	Analyses (C, H, N)
3a	*n*-C_3H_7	167–169	0.41 (A/B (6:4))	−0.217	$C_9H_{12}N_2S_2$
3b	*n*-C_4H_9	141–143	0.20 (A/B (6:4))	−0.128	$C_{10}H_{14}N_2S_2$
3c	*n*-C_5H_{11}	123–126	0.38 (A/B (5:5))	−0.045	$C_{11}H_{16}N_2S_2$
3d	*n*-C_6H_{13}	122–124	0.37 (A/B (6:4))	+0.084	$C_{12}H_{18}N_2S_2$
3e	Ph–CH_2	162–164	0.24 (A/B (6:4))	−0.089	$C_{13}H_{12}N_2S_2$
3f	Ph–$(CH_2)_2$	144–148	0.27 (A/B (6:4))	−0.085	$C_{14}H_{14}N_2S_2$
3g	Ph–$(CH_2)_3$	133–135	0.28 (A/B (6:4))	+0.002	$C_{15}H_{16}N_2S_2$

[a] A hexane; B ethyl acetate; C chloroform; D acetone. ^{1}H NMR ($CDCl_3$) are in accordance with those reported in Ref. [19].

Table 2
Isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **4a**–**g**

Comp.	R	m.p. (°C)	R_f (eluent [a])	R_M	Analyses (C, H, N)
4a	*n*-C_3H_7	125–127	0.58 (A/B (7:3))	−0.087	$C_9H_{10}N_2S_2$
4b	*n*-C_4H_9	105–107	0.61 (A/B (6:4))	+0.002	$C_{10}H_{12}N_2S_2$
4c	*n*-C_5H_{11}	73–75	0.47 (A/B (8:2))	+0.118	$C_{11}H_{14}N_2S_2$
4d	*n*-C_6H_{13}	55–60	0.23 (A/B (92:8))	+0.220	$C_{12}H_{16}N_2S_2$
4e	Ph-CH_2	162–164	0.26 (A/B (85:15))	+0.339	$C_{13}H_{10}N_2S_2$
4f	Ph-$(CH_2)_2$	111–115	0.70 (A/B (65:35))	+0.357	$C_{14}H_{12}N_2S_2$
4g	Ph-$(CH_2)_3$	88–90	0.38 (A/B (8:2))	+0.410	$C_{15}H_{14}N_2S_2$

[a] A hexane; B ethyl acetate; C chloroform; D acetone. ^{1}H NMR ($CDCl_3$) are in accordance with those reported in Ref. [19].

Table 3
N-[3*H*-[1,2]-Dithiolo[3,4-*b*]pyridin-3-ylidene]amines **5a**–**g**

Comp.	R	m.p. (°C)	R_f (eluent [a])	R_M	Analyses (C, H, N)
5a	*n*-C_3H_7	oil	0.75 (A/B (7:3))	−0.005	$C_9H_{10}N_2S_2$
5b	*n*-C_4H_9	42–46	0.73 (A/B (6:4))	+0.077	$C_{10}H_{12}N_2S_2$
5c	*n*-C_5H_{11}	50–52	0.60 (A/B (8:2))	+0.173	$C_{11}H_{14}N_2S_2$
5d	*n*-C_6H_{13}	60–61	0.36 (A/B (9:1))	+0.311	$C_{12}H_{16}N_2S_2$
5e	Ph–CH_2	61–62	0.35 (A/B (85:15))	+0.382	$C_{13}H_{10}N_2S_2$
5f	Ph–$(CH_2)_2$	111–113	0.82 (A/B (65:35))	+0.426	$C_{14}H_{12}N_2S_2$
5g	Ph–$(CH_2)_3$	oil	0.48 (A/B (8:2))	+0.496	$C_{15}H_{14}N_2S_2$

[a] A hexane; B ethyl acetate; C chloroform; D acetone. ^{1}H NMR ($CDCl_3$) are in accordance with those reported in Ref. [19].

Table 4
Isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones **6a**–**g**

Comp.	R	m.p. (°C)	R_f (eluent [a])	R_M	Analyses (C, H, N)	^{1}H NMR ($CDCl_3$)
6a	*n*-C_3H_7	71–72	0.26 (A/B (7:3))	−0.162	$C_9H_{10}N_2OS$	1.10 (t, 3H, CH_3, $J = 7.4$ Hz), 1.82 (sextet, 2H, CH_2), 3.91 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.38 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.29 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.78 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6b	*n*-C_4H_9	68–69	0.28 (A/B (6:4))		$C_{10}H_{12}N_2OS$	0.99 (t, 3H, CH_3, $J = 7.4$ Hz), 1.44 (sextet, 2H, CH_2), 1.79 (quintet, 2H, CH_2), 3.95 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.33 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.27 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.77 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6c	*n*-C_5H_{11}	oil	0.17 (A/B (8:2))	+0.078	$C_{11}H_{14}N_2OS$	0.90 (t, 3H, CH_3, $J = 7.4$ Hz), 1.30–1.49 (m, 4H, CH_2), 1.79 (quintet, 2H, CH_2), 3.92 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.42 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.52 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.78 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6d	*n*-C_6H_{13}	oil	0.33 (A/B (7:3))	+0.194	$C_{12}H_{16}N_2OS$	0.89 (t, 3H, CH_3, $J = 7.4$ Hz), 1.28–1.47 (m, 6H, CH_2), 1.80 (quintet, 2H, CH_2), 3.92 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.42 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.53 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.78 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6e	Ph–CH_2	oil	0.30 (A/B (7:3))		$C_{13}H_{10}N_2OS$	5.60 (s, 2H, N–CH_2), 7.42 (m, 6H, 5H and phenyl), 8.55 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.78 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6f	Ph–$(CH_2)_2$	oil	0.27 (A/B (65:35))	−0.022	$C_{14}H_{12}N_2OS$	3.05 (t, 2H, CH_2–Ph, $J = 7.4$ Hz), 4.18 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.32 (m, 5H, phenyl), 7.49 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.55 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.80 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)
6g	Ph–$(CH_2)_3$	oil	0.32 (A/B (6:4))	+0.0653	$C_{15}H_{14}N_2OS$	2.18 (quintet, 2H, CH_2–C*H*$_2$–CH_2), 2.75 (t, 2H, CH_2–Ph, $J = 7.4$ Hz), 3.99 (t, 2H, N–CH_2, $J = 7.4$ Hz), 7.65 (dd, 1H, 5H, $J_{6,5} = 4.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.45 (dd, 1H, 4H, $J_{6,4} = 1.5$ Hz, $J_{5,4} = 8.0$ Hz), 8.85 (dd, 1H, 6H, $J_{6,5} = 1.5$ Hz, $J_{6,4} = 4.5$ Hz)

[a] A hexane; B ethyl acetate; C chloroform; D acetone.

Table 5
2-Sulfanylnicotinamides **7a–g**

Comp.	R	m.p. (°C)	R_f (eluent [a])	R_M	Analyses (C, H, N)	^{1}H NMR ($CDCl_3$)
7a	*n*-C_3H_7	145–150	0.23 (A/B (2:8))	−0.231	$C_9H_{12}N_2OS$	1.05 (t, 3H, CH_3, $J = 7.4$ Hz), 1.71 (sextet, 2H, CH_2), 3.48 (q, 2H, N–CH_2), 6.99 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.78 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.90 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.80 (bs, 1H, NH or SH).
7b	*n*-C_4H_9	128–130	0.25 (A/B (2:8))	−0.122	$C_{10}H_{14}N_2OS$	0.97 (t, 3H, CH_3, $J = 7.4$ Hz), 1.48 (sextet, 2H, CH_2), 1.68 (quintet, 2H, CH_2), 3.52 (q, 2H, N–CH_2), 7.00 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.75 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.90 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.70 (bs, 1H, NH or SH)
7c	*n*-C_5H_{11}	127–130	0.35 (A/B (2:8))	0.000	$C_{11}H_{16}N_2OS$	0.98 (t, 3H, CH_3, $J = 7.4$ Hz), 1.45–1.60 (m, 4H, CH_2), 1.70 (quintet, 2H, CH_2), 3.50 (q, 2H, N–CH_2), 6.95 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.75 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.90 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.70 (bs, 1H, NH or SH)
7d	*n*-C_6H_{13}	108–112	0.40 (C/D (8:2))	+0.0959	$C_{12}H_{18}N_2OS$	0.90 (t, 3H, CH_3, $J = 7.4$ Hz), 1.20–1.50 (m, 6H, CH_2), 1.55 (quintet, 2H, CH_2), 3.48 (q, 2H, N–CH_2), 6.91 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.69 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.82 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.78 (bs, 1H, NH or SH)
7e	Ph–CH_2	174–175	0.37 (C/D (8:2))		$C_{13}H_{12}N_2OS$	4.74 (d, 2H, N–CH_2, $J = 7.0$ Hz), 6.96 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.37 (m, 5H, Ph), 7.66 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.88 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 11.14 (bs, 1H, SH or NH)
7f	Ph–$(CH_2)_2$	155–160	0.36 (C/D (8:2))	−0.104	$C_{14}H_{14}N_2OS$	2.98 (t, 2H, CH_2–Ph, $J = 7.4$ Hz), 3.76 (q, 2H, N–CH_2), 6.90 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz),), 7.25 (m, 5H, Ph), 7.66 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.82 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.78 (bs, 1H, SH or NH)
7g	Ph–$(CH_2)_3$	117–120	0.38 (A/B (8:2))	−0.022	$C_{15}H_{16}N_2OS$	2.14 (quintet, 2H, CH_2–CH_2–CH_2), 2.72 (t, 2H, CH_2–Ph, $J = 7.4$ Hz), 3.86 (q, 2H, N–CH_2), 7.25 (m, 5H, Ph), 7.37 (dd, 1H, 5H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 8.29 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.77 (dd, 1H, 6H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.93 (bs, 1H, SH or NH)

[a] A hexane; B ethyl acetate; C chloroform; D acetone.

Table 6
In vitro antimicrobial activity of *N*-substituted 2-sulfanylpyridine-3-carbothioamides (**3a**–**g**), MIC in μg/ml

Comp.	Fungi		Gram-positive bacteria			Gram-negative bacteria			Anaerobic
	T. mentagrophytes	*C. albicans*	*S. aureus*	*S. albus*	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. typhi*	*C. perfringens*
3a	5	20	10	10	10	40	40	40	10
3b	15	25	30	20	15	50	>50	50	20
3c	15	30	20	15	15	50	40	50	15
3d	10	20	10	10	10	50	50	40	15
3e	10	20	10	10	10	50	50	50	10
3f	15	30	20	15	15	>50	50	50	15
3g	10	10	30	20	20	50	>50	50	15
BIT-5-F [a]	2.5	2.5	10	10	2	40	30	40	
BIT-6-CF$_3$ [a]	5	7.5	5	0.5	5	>50	>50	>50	
Gentamicin			2.5	1.5	0.5	2.5	2.5	2.5	0.25
Cefotaxime			1.5	0.5	0.5	1.5	1.5	1.5	NT
Clotrimazole	0.5	1.25							

[a] **BIT-5-F** *N*-butyl-5-fluoro-1,2-benzisothiazol-3(2*H*)-one [10]; **BIT-6-CF$_3$** *N*-butyl-6-trifluoromethyl-1,2-benzisothiazol-3(2*H*)-one [10].

(Reaction IX, 63–90% yield). The cyclization of **7a**–**g** was performed by treatment with iodine and afforded **6a**–**g** in high yields (Reaction X, 74–81% yields). A mixture of compounds **4** and **5** (**a**–**g**) was obtained after refluxing **6a**–**g** with Lawesson's reagent in toluene but in this case the yields were low (Reaction XI, 26–31% yields). The cyclic *N*-substituted isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **4a**–**g** were easily opened by hydrogen sulfide treatment (Reaction XII, 78–82% yields). Finally, the conversion of 2-sulfanylpyridine-3-carbothioamides **3a**–**g** into their analogues **7a**–**g** was performed using mercuric acetate (Reaction XIV, 32–37% yields).

2.2. Antimicrobial activity

The in vitro antifungal and antibacterial activities of 2-sulfanylpyridine-3-carbothioamides **3a**–**g**, isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones **4a**–**g**, *N*-[3*H*-[1,2]-dithiolo[3,4-*b*]pyridin-3-ylidene]amines **5a**–**g**, isothiazolo[5,4-*b*]pyridin-3(2*H*)-ones **6a**–**g** and 2-sulfanylnicotinamides **7a**–**g** derivatives are reported in Tables 6–8. Among the investigated compounds **7f** and **7g**, bearing an arylalkyl group as *N*-substituent, displayed the most interesting activities.

2.2.1. Activity against fungi (Tricophyton mentagrophytes [Tm] and Candida albicans [Ca])

The fungitoxicity of the compounds tested was generally higher against *T. mentagrophytes* (MIC: 1.25–15 μg/ml) than *C. albicans* (MIC: 5–40 μg/ml). The most active compounds were **7f** and **7g** ($MIC_{Tm} = 1.25$ μg/ml; $MIC_{Ca} = 5$ μg/ml) and **7e** ($MIC_{Tm} = 2.5$ μg/ml) (Table 8).

2.2.2. Activity against Gram-positive bacteria (Staphylococcus aureus [Sau], Staphylococcus albus [Sal], Bacillus subtilis [Bsu], Clostridium perfringens [Cp])

Activities against Gram-positive bacteria were interesting with MIC between 2.5 and 5 μg/ml. Compound **7g** had the lowest MIC ($MIC_{Sau;Sal;Bsu} = 2.5$ μg/ml). Interesting activities were also shown by compound **7f** ($MIC_{Bsu} = 2.5$ μg/ml; $MIC_{Sal;Sau} = 5$ μg/ml). The most active compounds against *C. perfringens* were **7f**–**g** (MIC = 2.5 μg/ml) and **7e** (MIC = 5 μg/ml) (Table 8).

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

Activity against Gram-negative bacteria was generally very poor for all compounds (Tables 6–8). Particularly, the MIC values against *Pseudomonas aeruginosa* were >50 μg/ml (data not reported).

2.3. Structure–activity relationships

The benzisothiazolone mechanism for antibacterial action has been reported by Fuller et al. [21] and could be explained by the reaction of the sulfur with the thiol group of the glutathione, cysteine and other biomacromolecules. The lability of the benzisothiazolone S–N bond is supposed to be important for S–S bond formation with biological targets [10] but a direct relationship between this property and the activity has never been demonstrated [12,13].

The presence of the pyridinic nitrogen in isothiazolo[5,4-*b*]pyridine moiety should increase the reactivity of the S–N bond with respect to the analogues 1,2-benzisotiazolones. In order to compare our compounds

with some 1,2-benzisotiazolone derivatives, we included in Tables 6–8 the *N*-butyl-5-fluoro-1,2-benzisothiazol-3(2*H*)-one (**BIT-5-F**) and the *N*-butyl-6-trifluoromethyl-1,2-benzisothiazol-3(2*H*)-one (**BIT-6-CF$_3$**) [10] chosen as reference compounds because they have been tested upon the same strains according to the same

Table 7
In vitro antimicrobial activity of 2-substituted isothiazolo[5,4-*b*]pyridine-3(2*H*)-thiones (**4a**–**g**) and *N*-[3*H*-[1,2]-dithiolo[3,4-*b*]pyridin-3-ylidene]amines (**5a**–**g**), MIC in μg/ml

Comp.	Fungi		Gram-positive bacteria			Gram-negative bacteria			Anaerobic
	T. mentagrophytes	*C. albicans*	*S. aureus*	*S. albus*	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. typhi*	*C. perfringens*
4a	10	25	15	10	10	50	40	50	10
4b	10	30	30	20	15	40	>50	50	15
4c	5	20	10	10	10	40	50	50	10
4d	15	30	20	15	20	50	50	50	15
4e	15	20	25	15	20	40	>50	50	15
4f	15	30	30	20	15	50	50	50	20
4g	10	30	15	10	15	50	50	50	15
5a	10	20	10	10	10	40	40	40	10
5b	15	25	30	20	20	50	>50	50	20
5c	15	30	20	15	15	50	40	50	20
5d	10	25	10	10	15	50	50	50	15
5e	10	20	25	15	15	40	>50	50	20
5f	15	30	30	20	20	50	50	50	20
5g	10	30	15	10	15	50	50	50	15
BIT-5-F [a]	2.5	2.5	10	10	2	40	30	40	
BIT-6-CF$_3$ [a]	5	7.5	5	0.5	5	>50	>50	>50	
Gentamicin			2.5	1.5	0.5	2.5	2.5	2.5	0.25
Cefotaxime			1.5	0.5	0.5	1.5	1.5	1.5	NT
Clotrimazole	0.5	1.25							

[a] **BIT-5-F** *N*-butyl-5-fluoro-1,2-benzisothiazol-3(2*H*)-one [10]; **BIT-6-CF$_3$** *N*-butyl-6-trifluoromethyl-1,2-benzisothiazol-3(2*H*)-one [10].

Table 8
In vitro antimicrobial activity of 2-substituted isothiazolo[5,4-b]pyridin-3(2H)-ones (**6a**–**g**) and 2-sulfanylnicotinamides (**7a**–**g**), MIC in μg/ml

Comp.	Fungi		Gram-positive bacteria			Gram-negative bacteria			Anaerobic
	T. mentagrophytes	*C. albicans*	*S. aureus*	*S. albus*	*B. subtilis*	*K. pneumoniae*	*E. coli*	*S. typhi*	*C. perfringens*
6a	15	30	30	20	15	50	>50	50	20
6b	5	20	10	5	5	40	>50	40	10
6c	10	20	15	10	15	40	40	40	15
6d	15	30	20	15	15	50	50	50	10
6e	15	30	30	20	20	40	>50	50	20
6f	15	30	30	20	20	50	50	50	20
6g	5	20	10	10	10	40	>50	50	10
7a	5	15	50	20	25	>50	>50	>50	20
7b	10	20	50	20	50	50	50	50	20
7c	20	40	25	20	25	>50	>50	>50	20
7d	10	10	20	20	10	>50	>50	>50	20
7e	2.5	10	10	10	2.5	>50	>50	>50	5
7f	1.25	5	5	5	2.5	>50	>50	>50	2.5
7g	1.25	5	2.5	2.5	2.5	>50	>50	>50	2.5
BIT-5-F [a]	2.5	2.5	10	10	2	40	30	40	
BIT-6-CF$_3$[a]	5	7.5	5	0.5	5	>50	>50	>50	
Gentamicin			2.5	1.5	0.5	2.5	2.5	2.5	0.25
Cefotaxime			1.5	0.5	0.5	1.5	1.5	1.5	NT
Clotrimazole	0.5	1.2							

[a] **BIT-5-F** *N*-butyl-5-fluoro-1,2-benzisothiazol-3(2*H*)-one [10]; **BIT-6-CF$_3$** *N*-butyl-6-trifluoromethyl-1,2-benzisothiazol-3(2*H*)-one [10].

experimental protocol. If we compare the *N*-butylisothiazolo[5,4-*b*]pyridin-3(2*H*)-one **6b** with **BIT-5-F** or **BIT-6-CF$_3$** it is possible to state that the influence of the pyridinic ring is not determinant for the activity; in fact, the activities on Gram-positive bacteria and fungi strains are almost comparable (Table 8). A low activity on Gram-negative bacteria is observed for both classes of compounds.

Lipophilicity parameters are attractive physicochemical properties in QSAR studies and often chromatographic parameters are used as substitutes for partition coefficients. Retardation matches (R_M) are true measures of lipophilicity and closely correlated with log *P* [22–25]: higher R_M values indicate higher lipophilicity. The R_M values have been determined for compounds **3**–**7** (see Section 3) and are reported in Tables 1–5. The results indicate that, in the series considered, similar to several other antibacterial structures [23], no linear relationship between activity and lipophilicity is observed. The present findings show that factors influencing the lipophilicity of the molecules such as different *N*-side chains or oxo-thiono substitution do not play a pivotal role in determine the antimicrobial activity.

In conclusion some considerations can be stated. The presence of the pyridinic nitrogen in the isothiazolo[5,4-*b*]pyridine moiety does not increase the activity. The arylakyl moiety confers a better activity if compared with the alkyl *N*-side chains. Among the synthesized compounds, 2-sulfanylnicotinamides **7** are the most active substances. This finding suggests that the bicyclic structure is not determinant for the activity. In all cases the antimicrobial activity of the studied compounds is lower than that of the reference substances.

In order to better clarify the structure–activity relationships of these compounds, a chemometric approach should be considered. In this concern a chemometric study using WHIM descriptors [26] is in progress.

3. Experimental

3.1. Chemistry

Melting points were measured using a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz using a Bruker ACE-300 spectrometer in $CDCl_3$. ^{1}H chemical shifts (δ) were reported with Me_4Si ($\delta = 0.00$ ppm) as internal standard. The following abbreviations are used: br broad, s singlet, d doublet, dd double doublet, t triplet, and m multiplet. Elemental analyses, indicated by the symbols, were performed on a Carlo Erba 1106 elemental analyser and were within $\pm 0.4\%$ of the theoretical values. All reactions were monitored by thin layer chromatography carried out on 0.25 mm Merck silica gel (60 F_{254}) and visualized by UV light ($\lambda = 254$ or 365 nm); flash chromatography was performed using silica gel 60 (60–200 μm, Merck). All other reagents are commercial grade and were used without further purification.

3.2. Synthesis of 3H-[1,2]dithiolo[3,4-b]pyridine-3-thione (2)

Reaction I. A mixture of 2-mercaptonicotinic acid (**1**) (10 g, 0.064 mol), sulfur (4 g, 0.0155 mol) and Lawesson's reagent (62.5 g, 0.155 mol) was refluxed in anhydrous toluene (300 ml) for 5 h and then allowed to stand at room temperature overnight. A solid material was collected by filtration and purified by dry-column flash chromatography (SiO_2; eluent: gradient from *n*-hexane 100 to *n*-hexane/ethyl acetate 90–10). Recrystallization from ligroin afforded 8.77 g (75% yield) of pure **2** as orange crystals.

R_f 0.34 (*n*-hexane/ethyl acetate 9:1); m.p. 175–177°C; ^{1}H NMR ($CDCl_3$): δ 7.41 (dd, 1H, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 5.0$ Hz), 8.42 (dd, 1H, $J_{4,5} = 7.5$ Hz, $J_{4,6} = 1.5$ Hz), 8.85 (dd, 1H, $J_{6,5} = 5.0$ Hz, $J_{6,4} = 1.5$ Hz). *Anal.* $C_6H_3NS_3$ (C, H, N).

Reaction VII. A solution of 3*H*-[1,2]-dithiolo[3,4-*b*]pyridin-3-one (**8**) (0.5 g, 2.95 mmol) and Lawesson's reagent (0.6 g, 1.48 mmol) in anhydrous toluene was refluxed for 3 h. After cooling to room temperature, the resultant precipitate was isolated by filtration under reduced pressure (70–77% yields). Analytical data were consistent with those reported above.

Reaction XIII. A solution of 2-sulfanylpyridine-3-carbothioamide **3a**–**g** (0.023 mmol) and phosphorous pentasulfide (0.029 mmol) in anhydrous xylene (5 ml) was refluxed for 2 h. After cooling to room temperature, the solvent was removed in vacuo and the residual raw material was purified by flash chromatography (*n*-hexane/ethyl acetate 60–40), giving **2** in 60–66% yields. Analytical data were consistent with those reported above.

3.3. Synthesis of 2-sulfanylpyridine-3-carbothioamides 3a–g

Reaction II. A mixture of 3*H*-[1,2]dithiolo[3,4-*b*]pyridine-3-thione (**2**) (16 mmol) and the appropriate amine (19 mmol) was refluxed in 150 ml of absolute ethanol for 30–60 min, the optimum reaction time being determined by TLC (eluent: see Table 1). After cooling to room temperature, the solvent was removed under reduced pressure and the residue was recrystallized from the suitable solvent (75–83% yields). Analytical data are reported in Table 1.

Reaction XII. Hydrogen sulfide was bubbled into a solution of isothiazolo[5,4-*b*]pyridine-3(2*H*)-thione **4a**–**g** (0.17 mmol) in ethanol at pH 7.4 (phosphate buffer) till disappearance of the starting material (TLC, eluent: see Table 2). The reaction mixture was extracted with

ethyl acetate and the collected organic layers were washed with phosphate buffer (pH 7.4) till neutrality. After drying over anhydrous sodium sulfate, the solvent was removed in vacuo affording the title compound (78–82% yields).

3.4. Synthesis of isothiazolo[5,4-b]pyridine-3(2H)-thiones **4a–g** and N-[3H-[1,2]-dithiolo[3,4-b]pyridin-3-ylidene]amines **5a–g**

Reaction III. A solution of iodine in ethanol (6% v/v) was added dropwise to a stirred mixture of the appropriate 2-sulfanylpyridine-3-carbothioamide **3a–g** (6 mmol) and sodium bicarbonate (10 mmol) in absolute ethanol (100 ml), till the persistance of the brown colour. A precipitate was removed by filtration and washed with dichloromethane. The collected organic phases were evaporated under reduced pressure and the resultant residue was purified by flash chromatography (eluent: see Tables 2 and 3) affording 30–35% yields of pure **4a–g** and 30–35% yields of pure **5a–g**. Analytical data are reported in Tables 2 and 3.

Reaction XI. A solution of isothiazolo[5,4-*b*]pyridin-3(2*H*)-one **6a–g** (0.28 mmol) and Lawesson's reagent (0.14 mmol) in anhydrous toluene (5 ml) was refluxed for 30 min. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (eluent: see Table 4) giving the desired compounds with yields around 26–30%.

3.5. Synthesis of isothiazolo[5,4-b]pyridin-3(2H)-ones **6a–g**

Reaction IV. A solution of isothiazolo[5,4-*b*]pyridine-3(2*H*)-thione **4a–g** (0.6 mmol) in chloroform (2 ml) was added to a stirred suspension of mercuric acetate (1.3 mmol) in glacial acetic acid (5 ml) at room temperature. After 30 min (TLC, eluent: see Table 4) the mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: see Table 4) (35–40% yields). Analytical data are reported in Table 4.

Reaction X. This reaction was performed by the same method used for Reaction III except that the appropriate 2-sulfanylnicotinamide **7a–g** was employed as starting material. Compounds **6a–g** were obtained in 74–81% yields.

3.6. Synthesis of 2-sulfanylnicotinamides **7a–g**

Reaction V. Hydrogen sulfide was bubbled into a solution of isothiazolo[5,4-*b*]pyridin-3(2*H*)-one **6a–g** (0.17 mmol) in ethanol at pH 7.4 (phosphate buffer) till disappearance of the starting material (TLC, eluent: see Table 5). The reaction mixture was extracted with ethyl acetate and the collected organic layers were washed with phosphate buffer (pH 7.4) till neutrality. After drying over anhydrous sodium sulfate, the solvent was removed in vacuo affording the title compound (80–85% yields). Analytical data are reported in Table 5.

Reaction IX. This reaction was performed by the same method used for Reaction II except that 3*H*-[1,2]-dithiolo[3,4-*b*]pyridin-3-one (**8**) was employed as starting material. Compounds **7a–g** were obtained with yields between 63 and 90%.

Reaction XIV. This reaction was performed by the same method used for Reaction IV except that the appropriate 2-sulfanylpyridine-3-carbothioamide **3a–g** was employed as starting material. Compounds **7a–g** were obtained with yields around 32–37%.

3.7. Synthesis of 3H-[1,2]-dithiolo[3,4-b]pyridin-3-one (**8**)

Reaction VI. A solution of 2-sulfanylnicotinamide **7a–g** (0.24 mmol) and Lawesson's reagent (0.57 mmol) in anhydrous toluene (5 ml) was refluxed for 30 min. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (*n*-hexane/ethyl acetate 80–20). Yield 90%; R_f 0.63 (*n*-hexane/ethyl acetate 60–40); m.p. 94–96°C; ^{1}H NMR ($CDCl_3$): δ 7.39 (dd, 1H, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 5.0$ Hz), 8.23 (dd, 1H, $J_{4,5} = 7.5$ Hz, $J_{4,6} = 1.5$ Hz), 8.80 (dd, 1H, $J_{6,5} = 5.0$ Hz, $J_{6,4} = 1.5$ Hz). *Anal.* $C_6H_3NOS_2$ (C, H, N).

Reaction VIII. This reaction was performed by the same method used for Reaction IV except that 3*H*-[1,2]dithiolo[3,4-*b*]pyridine-3-thione (**2**) was employed as starting material. Product **8** was obtained in 59% yield.

3.8. Lipophilicity tests

The relative lipophilicity of the compounds was measured by reverse-phase thin-layer chromatography [22,23]. Silanized silica gel plates Merck 60 F_{254} were used as the non-polar 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 were calculated from the experimental R_f according to the equation:

$$R_M = \log[(1/R_f) - 1]$$

The calculated R_M values are presented in Tables 1–5.

3.9. Microbiology

The in vitro antimicrobial activity of the compounds was determined against *S. aureus* (ATCC 6538), *S. albus* (ATCC 12228), *B. subtilis* (ISM 6513) as Gram positive; *E. coli* (ISM 6585), *S. typhi* (ATCC 19430), *K. pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 15442) as Gram negative; *C. albicans* (ATCC 2091) and *T. mentagrophytes* (ATCC 9129) as fungi. The minimum inhibitory concentrations (MICs) were defined as the lowest concentrations of the substances that prevent visible growth. The evaluation of MIC was carried out as previously reported [15] by the Bioscreen Analyzer for bacterial strains and by the medium dilution technique for the fungal strains. Gentamicin and cefotaxime were used as reference drugs for antibacterial activity, while clotrimazole was used for antifungal activity. Test and reference compounds were dissolved in acetone–water solution (3:1) at concentrations of 1–100 μg/ml. It was determined that the solvent exhibited no antimicrobial activity against any of the test organisms and was used as negative control. The results of all measurements are shown as kinetic growth curves and their elaboration provided the reported antibacterial activities, expressed as MICs (μg/ml).

Acknowledgements

Financial support for this research by the FAR 1999, Università di Pavia, is gratefully acknowledged. We thank Dr Luca Daniele for his help in chemical syntheses.

References

[1] L.L. Bambas, 1,2-Benzisothiazol-3(2)-ones. In: A. Weissburger (Ed.), The Chemistry of Heterocyclic Compounds, Wiley-Interscience, New York, 1952, pp. 253–270.
[2] M. Davis, Benzisothiazoles. In: A.R. Katritzky, A.J. Boulton (Eds.), Advances in Heterocyclic Chemistry, Academic Press, New York, 1972, pp. 43–98.
[3] M. Davis, Recent advances in the chemistry of benzisothiazoles and other polycyclic isothiazoles. In: A.R. Katritzky, A.J. Boulton (Eds.), Advances in Heterocyclic Chemistry, Academic Press, New York, 1985, pp. 105–133.
[4] L.L. Bambas, 1,2-Benzisothiazol-3(2)-thiones. In: A. Weissburger (Ed.), The Chemistry of Heterocyclic Compounds, Wiley-Interscience, New York, 1952, pp. 271–273.
[5] A. Baruffini, P. Borgna, F. Gialdi, R. Ponci, Benzoisotiazolin-3-tioni ad attività antifungina, Farmaco Ed. Sci. 23 (1968) 3–15.
[6] P. Borgna, L. Vicarini, M.L. Carmellino, G. Pagani, Nuovi derivati dell'1,2-benzoisotiazolin-3-tione ad attività antimicotica, Farmaco Ed. Sci. 38 (1983) 801–810.
[7] J. Shimizu, Antimicrobial agent-containing aqueous jet-printing inks, Jpn. Kokai Tokkyo Koho JP, 05,171,081. Chem. Abstracts 120 (1994) 56889k.
[8] P.W. Austin, *N*-(Beta-branched alkyl)-1,2-benzisothiazolin-3-ones, their preparation and use as industrial biocides (Zeneca Ltd), PCT Int. Appl. WO 9416564. Chem. Abstracts 121 (1994) 295086y.
[9] S. Hirawa, T. Nakano, K. Suzuki, H. Nakao, Antifungal and waterproof fabric and its production (Toray Industries; Osaka Kasei Kk), Jpn. Kokai Tokkyo Koho JP, 06, 313 269. Chem. Abstracts 122 (1995) 242251n.
[10] M.L. Carmellino, G. Pagani, M. Pregnolato, M. Terreni, F. Pastoni, Antimicrobial activity of fluorinated 1,2-benzisothiazol-3(2*H*)-ones and 2,2′-dithiobis(benzamides), Eur. J. Med. Chem. 29 (1994) 743–751.
[11] M.L. Carmellino, G. Pagani, M. Terreni, M. Pregnolato, P. Borgna, Antimicrobial and genotoxic activities of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-one carbamic esters, Farmaco 52 (1997) 359–366.
[12] P. Borgna, M.L. Carmellino, N. Natangelo, G. Pagani, F. Pastoni, M. Pregnolato, M. Terreni, Antimicrobial activity of *N*-hydroxyalkyl-1,2-benzisothiazol-3(2*H*)-ones and their thionoanalogues, Eur. J. Med. Chem. 31 (1996) 919–925.
[13] M. Pregnolato, P. Borgna, M. Terreni, Synthesis of 3*H*-thieno[3,2-*c*]-1,2-dithiole-3-thione and its reaction with butylamine, J. Heterocyclic Chem. 32 (1995) 847–850.
[14] K.H. Baggaley, L.J.A. Jennings, A.W.R. Tyrrell, Synthesis of 2-substituted isothiazolopyridin-3-ones, J. Heterocyclic Chem. 19 (1982) 1393.
[15] A. Monge, V. Martinez-Merino, E. Fernandez-Alvarez, Synthesis of 2-substituted 3-oxoisothiazolo[5,4-*b*]pyridines, J. Heterocyclic Chem. 22 (1985) 1353.
[16] J.L. Raney, M.C. Seidel (Rohm and Haas Co), Isothiazolopyridinones, U.S. Patent 3,965,107 (Cl. 260–294.8C; C07D513/04), 22 June 1976, Appl. 486,550, 08 Jul 1974. Chem Abstracts 85 (1976) 160072h.
[17] K.H. Baggaley (Beecham Group Ltd.), Isothiazolopyridines, Ger. Offen. 2,718,707 (Cl. C07D513/04), 10 November 1977, Brit. Appl. 76/17,165, 28 April 1976. Chem Abstracts 88 (1978) 50843q.
[18] B. Shroot, J. Maignam (Oreal S.A., Centre International de Recherches Dermatologiques (CIRD)), Acne treatment with isothiazolopyridinones, Ger. Offen. DE 3,342,538 (Cl. A61K31/425), 30 May 1984. Chem. Abstracts 101 (1984)116736c.
[19] P. Borgna, M. Pregnolato, I.A. Gamba, G. Mellerio, On the reaction between 3*H*-1,2-dithiolo[3,4-*b*]pyridine-3-thione and primary alkyl and arylalkylamines, J. Heterocyclic Chem. 30 (1993) 1079–1084.
[20] S. Scheibye, B.S. Pedersen, S.O. Lawesson, Studies on organophosphorus compounds XXI. The dimer of *p*-methoxyphenylthionophosphine sulfide as thiation reagent. A new route to thiocarboxamides, Bull. Soc. Chim. Belg. 87 (1978) 229–238.
[21] S.J. Fuller, S.P. Denyer, W.B. Hugo, D. Pemberton, P.M. Woodcock, A.J. Buckley, The mode of action of 1,2-benzisothiazolin-3-one on *Staphylococcus aureus*, Lett. Appl. Microbiol. 1 (1985) 13–15.
[22] C.B.C. Boyce, B.V. Milborrow, A simple assesment of partition data for correlating structure and biological activity using thin-layer chromatography, Nature 208 (1965) 537–539.
[23] E. Tomlison, Chromatographihic hydrophobic parameters in correlation analysis of structure–activity relationships, J. Chromatogr. 113 (1975) 1–45.
[24] G.L. Biagi, M. Recanatini, A.M. Barbaro, M.C. Guerra, A. Sapone, P.A. Borea, M.C. Pietrogrande, Chromatographic

parameters as lipophilicity indices for QSAR studies. In: H. Kalasz, L.S. Ettre (Eds.), New Approaches in Chromatography '93, Fekete Sas Könyvkiadó, Budapest, 1993, pp. 215–234.
[25] K.P. Dross, R. Mannhold, R.F. Rekker, Drug lipophilicity in QSAR practice II. Aspects of RM-determinations and their interrelations with partition coefficients, Quant. Struct.-Act. Relat. 11 (1992) 36–44.
[26] R. Todeschini, M. Lasagni, E. Marengo, New molecular descriptors for 2D and 3D structures. Theory, J. Chemometrics 8 (1994) 263–272.