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Antimicrobial activity of fluorinated 1,2-benzisothiazol-3(2*H*)-ones and 2,2'-dithiobis(benzamides)

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Summary — Fluoro and trifluoromethyl derivatives of 1,2-benzisothiazol-3(2*H*)-ones and the 2,2'-dithiobis(benzamides) have been prepared and their antifungal and antibacterial activity evaluated. Several compounds were found highly active against fungi and Gram-positive microorganisms and a few derivatives displayed some activity against Gram-negative strains. Structure–activity relationships are proposed.

1,2-benzisothiazol-3(2*H*)-one / 2,2'-dithiobis(benzamide) / antifungal activity / antibacterial activity / fluorosubstitution

Introduction

Many 1,2-benzisothiazol-3(2*H*)-one and 2,2'-dithiobis(benzamide) derivatives have received considerable attention for their antifungal and antibacterial properties [1–5]. The effects of the nitro-, methyl-, methoxy- and chloro-substitution at different aromatic ring positions [6–10] and the role of the *N*-substitution with a linear or branched alkylic chain, have been extensively studied [11].

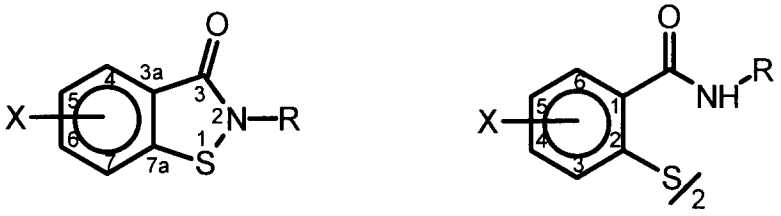
Taking into account that the selective introduction of a fluorine atom or a fluorinated residue in pharmaceuticals modifies the physicochemical and biological behaviour [12–14], we synthesized the unsubstituted or *N*-butyl-substituted fluoro- and trifluoromethyl-1,2-benzisothiazol-3(2*H*)-ones **A** and **B** and the difluoro- and bis(trifluoromethyl)-2,2'-dithiobis(benzamides) **C** and **D** (fig 1). The substances **A–D**, all unknown, were tested against representative strains of Gram-positive, Gram-negative and fungal microorganisms. The synthesis and the microbiological assays are reported in this paper.

Chemistry

The 4-, 5- and 6-fluoro-2-aminobenzoic acids **7f–h** are commercially available, whereas the 3-, 4-, 5- and 6-trifluoromethyl-2-aminobenzoic acids **7a–d** and the 3-fluoro-2-aminobenzoic acid **7e** were synthesized as shown in scheme 1. The 2,2'-dithiobenzoic acids **8a–h**, obtained from the suitable aminobenzoic acids, were selected as starting materials for the synthesis of the substances to be tested, as reported in scheme 2 (pathway A or B).

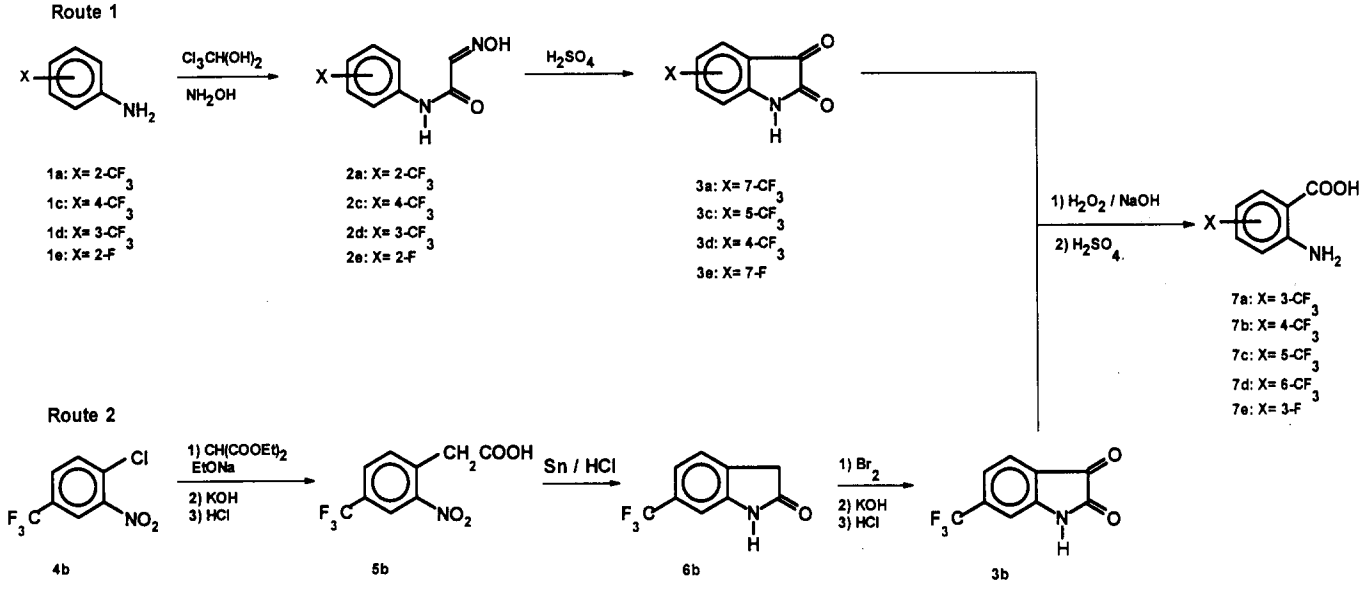
The 2-, 4- or 3-trifluoromethylphenyl-substituted 2-oxyiminoacetanilides **2a,c,d** were prepared according to Magginity and Gaulin [15], while the 2-fluoro analogue **2e** was obtained according to Yen *et al* [16] (scheme 1, route 1). The condensation of the appropriate trifluoromethyl- or fluoro-substituted anilines, **1a,c–e**, with chloral hydrate and hydroxylamine hydrochloride was performed according to the Marvel and Hiers modification [17] of the general Sandmeyer synthesis [18].

Compounds **2a,c–e** were then cyclized to the substituted isatins **3a,c–e** by heating at 60–80°C in concentrated sulphuric acid. The ring closures of *N*-(2-trifluoromethylphenyl)- **2a**, *N*-(4-trifluoromethylphenyl)- **2c** and *N*-(2-fluorophenyl)-2-oxyiminoacetamide **2e** were unambiguous and resulted in the formation of 7-trifluoromethyl-1*H*-indol-2,3-dione **3a** [17], 5-trifluoromethyl-1*H*-indol-2,3-dione **3c** [19] and 7-fluoro-1*H*-indol-2,3-dione **3e** [20], respectively. As reported in the literature, the 3-phenyl-substituted 2-oxyiminoacetanilides usually give a mixture of separable 4- and 6-substituted isatins, but in this case it was possible to obtain by **2d** cyclization only the 4-trifluoromethyl-1*H*-indol-2,3-dione **3d** [21]. Thus the 6-trifluoromethyl-1*H*-indol-2,3-dione isomer **3b**



X	R	X	R	X	R	X	R
A1: 4-F	H	A5: 4-F	n-C_4H_9	C1: 3,3'-F	H	C5: 3,3'-F	n-C_4H_9
A2: 5-F	H	A6: 5-F	n-C_4H_9	C2: 4,4'-F	H	C6: 4,4'-F	n-C_4H_9
A3: 6-F	H	A7: 6-F	n-C_4H_9	C3: 5,5'-F	H	C7: 5,5'-F	n-C_4H_9
A4: 7-F	H	A8: 7-F	n-C_4H_9	C4: 6,6'-F	H	C8: 6,6'-F	n-C_4H_9
B1: 4-CF_3	H	B5: 4-CF_3	n-C_4H_9	D1: 3,3'-CF_3	H	D5: 3,3'-CF_3	n-C_4H_9
B2: 5-CF_3	H	B6: 5-CF_3	n-C_4H_9	D2: 4,4'-CF_3	H	D6: 4,4'-CF_3	n-C_4H_9
B3: 6-CF_3	H	B7: 6-CF_3	n-C_4H_9	[D3: 5,5'-CF_3]*	H	D7: 5,5'-CF_3	n-C_4H_9
B4: 7-CF_3	H	B8 : 7-CF_3	n-C_4H_9	[D4: 6,6'-CF_3]*	H	D8: 6,6'-CF_3	n-C_4H_9

Fig 1. 1,2-Benzisothiazol-3(2*H*)-ones **A**, **B** and 2,2'-dithiobis(benzamides) **C**, **D**. *Compounds **D3** and **D4** were not obtained.

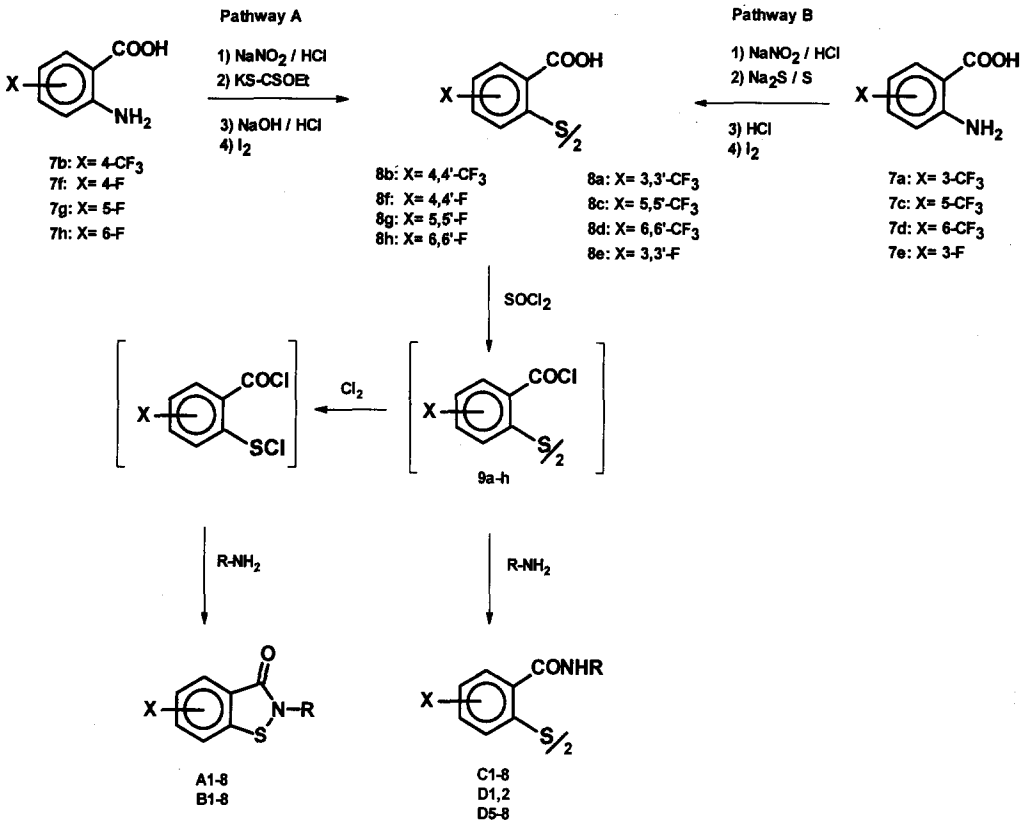


Scheme 1.

had to be prepared according to Simet [22] (scheme 1, route 2), by bromine oxidation of 6-trifluoromethyl-oxindole **6b**, resulting from reduction of **5b**. Compound **5b** was prepared by ethyl malonate arylation with 4-chloro-3-nitrobenzotrifluoride **4b**, followed by hydrolysis and decarboxylation of the resulting malonate.

The key substituted 2-aminobenzoic acids **7a–e** were obtained by alkaline hydrogen peroxide oxidation of the corresponding isatins **3a–e**, according to Baker [23].

Iodine oxidation of the intermediate thiosalicylic acids, prepared according to Katz *et al* [24] (scheme 2, pathway A) or Allen and McKay [25]



Scheme 2.

(scheme 2, pathway B), gave the 2,2'-dithiobis-(benzoic acids) **8a–h**, which were then converted into the corresponding acid dichlorides **9a–h** by treatment with thionyl chloride.

The fluoro-1,2-benzisothiazol-3(2*H*)-ones **A1–4** (R = H), **A5–8** (R = *n*-C_4H_9) and the trifluoromethyl-1,2-benzisothiazol-3(2*H*)-ones **B1–4** (R = H), **B5** and **B7**, **8** (R = *n*-C_4H_9) were prepared by reaction of the appropriate 2,2'-dithiobis(benzoylchloride) **9** with dry chlorine, followed by treatment with ammonia or *n*-butylamine [26]. This procedure failed for the 5-trifluoromethyl-1,2-benzisothiazol-3(2*H*)-one **B6** that was obtained by cyclization of 4,4'-bis(trifluoromethyl)-2,2'-dithiobis(benzamide) **D7** with thionyl chloride [27]. The difluoro-2,2'-dithiobis(benzamides) **C1–4** (R = H), **C5–8** (R = *n*-C_4H_9) and the bis(trifluoromethyl)-2,2'-dithiobis(benzamides) **D1**, **2** (R = H), **D5–8** (R = *n*-C_4H_9) were prepared by treating the corresponding chlorides **9a–h**, with ammonia or with *n*-butylamine in dioxane solution [5]. In some cases a large excess of base had no effect on the yield, in other cases the treatment of the acylchloride with an excess of ammonia or *n*-butylamine resulted in a mixture of the cyclic and acyclic compound. This difficulty was readily overcome either by using only a 2.6 molar ratio of the appropriate base, resulting merely in the 2,2'-dithiobis(benzamides), or by performing chromatographic separation of the mixture. Finally, ammonia treatment of acid dichlorides **9c**, **d**, gave exclusively compounds **B2** and **B1**, respectively, free of 5,5'-bis(trifluoromethyl)-2,2'-dithiobis(benzamide) **D3** and 6,6'-bis(trifluoromethyl)-2,2'-dithiobis(benzamide) **D4**. All attempts to obtain compounds **D3** and **D4** failed, although several modifications of experimental procedures (temperature, pH, reaction time) were performed. 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–III or in the *Experimental protocols*. ^{1}H-NMR spectral data of compounds **8a–h**, not included in table I, were consistent with the assigned structures.

Table I. Physicochemical data of 2,2'-dithiobis(benzoic acids) **8a–h**.

Compd	*X*	*Solvent*[a]	*mp (° C)*	*Yield (%)*
8a	3,3'-CF_3	A	268-270	28
8b	4,4'-CF_3	A	250-255	27
8c	5,5'-CF_3	A	301-308	24
8d	6,6'-CF_3	C	116	31
8e	3,3'-F	A	230	30
8f	4,4'-F	B	310	27
8g	5,5'-F	A	180-185	26
8h	6,6'-F	A	265 dec	21

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

Results and discussion

The *in vitro* antifungal and antimicrobial activities of fluoro (**A**, **C**) and trifluoromethyl (**B**, **D**) derivatives are reported in tables IV and V, respectively.

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

The fluoroderivatives **A1–8** and **C1–8** showed a fungitoxicity higher than the trifluoromethyl derivatives **B1–8**, **D1**, **2** and **D5–8**. In particular, among the fluoro derivatives, **A6** (MIC_{Tm} = 2.5 μg/ml; MIC_{Ca} = 2.5 μg/ml) and **C4** (MIC_{Tm} = 2 μg/ml; MIC_{Ca} = 5 μg/ ml) were the most active substances and relevant activities were also exhibited by **C8** towards both fungi (MIC_{Tm} = 2.5 μg/ml; MI_{Ca} = 5 μg/ml) and by **C7** against *T mentagrophytes* (MIC_{Tm} = 2.5 μg/ml). Concerning the trifluoromethyl derivatives, the lowest MICs were observed towards both *T mentagrophytes* and *C albicans* with **B3**, **B7**, **D2** and **D6** (MIC_{Tm} = 5 μg/ml; MIC_{Ca} = 7.5 μg/ml).

Activity against Gram-positive bacteria (Staphylococcus aureus [Sau], Staphylococcus albus [Sal], Bacillus subtilis [Bsu])

In these assays the fluoroderivatives display relevant activities and therefore very interesting results were also obtained for trifluoromethyl derivatives. The lowest MICs were exhibited by compounds **C2** (MIC_{Sau} = 2.5 μg/ml; MIC_{Sal} = 0.5 μg/ml; MIC_{Bsu} = 2.5 μg/ml), **A2** (MIC_{Sau} = 5 μg/ml; MIC_{Sal} = 1 μg/ml; MIC_{Bsu} = 1.25 μg/ml) and **A3** (MIC_{Sau} = 5 μg/ml; MIC_{Sal} = 1 μg/ml; MIC_{Bsu} = 2.5 μg/ml) and among the trifluoromethyl derivatives by **B7** (MIC_{Sau} = 5 μg/ml; MIC_{Sal} = 0.5 μg/ml; MIC_{Bsu} = 5 μg/ml) and by **D2** (MIC_{Sau} = 10 μg/ml; MIC_{Sal} = 1.5 μg/ml; MIC_{Bsu} = 5 μg/ml).

Activity against Gram-negative bacteria (Salmonella thyphi [Sty], Klebsiella pneumoniae [Kpn], Escherichia coli [Eco])

The activities against Gram-negative bacteria were lower than against Gram-positive. Nevertheless compound **A3** showed good antibacterial activity towards all tested strains and in particular towards *S typhi* (MIC_{Sty} = 2 μg/ml). Some activity was also exhibited by **A2** (MIC_{Kpn} = 10 μg/ml; MIC_{Eco} = 10 μg/ml; MIC_{Sty} = 10 μg/ml) and **C2** (MIC_{Sty} = 7.5 μg/ml). Among the trifluoromethyl derivatives **B3** (MIC_{Eco} = 10 μg/ml) and **D2** (MIC_{Sty} = 10 μg/ml) were the most active compounds.

Structure–activity relationships

The fluoroderivatives (**A**, **C**) are generally more active than the trifluoromethyl analogues (**B**, **D**) towards both fungi and bacteria. As regards the antifungal action, the difluoro-2,2'-dithiobis(benzamides) showed higher activity than the corresponding benzisothiazolones, whereas among the trifluoromethyl derivatives substantial differences were not observed. Moreover the *N*-butyl substitution in structure **A** increased the activity towards fungi but had an opposite effect against bacteria; on the other hand, unsubstituted and substituted structures **B**, **C** and **D** generally gave comparable results.

Concerning the benzene substitution, as observed for methyl, methoxy and chloro derivatives previously studied [8–10], the benzisothiazolones and the dithiobis(benzamides) fluorinated in the 5 or 6 and in the 4,4' or 5,5' positions, respectively, showed the highest activities, whereas when the substituents were *ortho* to the sulphur function, the activity was strongly decreased. In the past, a correlation between the steric hindrance of an *ortho* substituent and decreasing activity was suggested. However, as regards the derivatives reported in this paper, a decrease in activity occurs for both the bulky trifluoromethyl group and, although to a lesser extent, the fluorine atom, whose hindrance is comparable to hydrogen. Thus it is possible to hypothesize that not only size-related phenomenon but also electronic, steric and proximity combined effects play a role in determining the biological activity.

On the other hand, it is well known that a fluoro substitution induces an increased electronic density in the *ortho* and *para* positions, whereas a trifluoromethyl group causes a decrease of electronic density in the same positions [12–14]. Fuller *et al* supposed that the benzisothiazolone mode of antibacterial

Table II. Physicochemical data of fluoro-1,2-benzisothiazol-3(2*H*)-ones **A1–8** and difluoro-2,2'-dithiobis(benzamides) **C1–8**.

Compd	*X*	*R*	*Solvent*[a]	*mp (° C)*	*^1H NMR δ (DMSO-d_6)*
A1	4-F	H	A	215-217	7.0-8.0 (m, 3H, Ar), 11.9 (bs, 1H, NH)
A2	5-F	H	-	210 dec	7.4-8.2 (m, 4H, Ar and NH)
A3	6-F	H	B	210 dec	7.1-8.4 (m, 3H, Ar), 11.6 (bs, 1H, NH)
A4	7-F	H	A	176-178	7.4-7.9 (m, 3H, Ar), 12.3 (bs, 1H, NH)
A5	4-F	*n*-C_4H_9	-	oil	0.9 (t, 3H, CH_3), 1.1-1.9 (m, 4H, CH_2CH_2), 3.8 (t, 2H, J=7 H_z, CH_2-N), 7.1-7.9 (m, 3H, Ar)
A6	5-F	*n*-C_4H_9	-	50-53	0.9 (t, 3H, CH_3), 1.1-1.8 (m, 4H, CH_2CH_2), 3.8 (t, 2H, J=7 Hz, CH_2-N), 7.4-8.3 (m, 3H, Ar)
A7	6-F	*n*-C_4H_9	C	38-40	0.9 (t, 3H, CH_3), 1.1-1.8 (m, 4H, CH_2CH_2), 3.8 (t, 2H, J=7 Hz, CH_2-N), 7.2-8.1 (m, 3H, Ar)
A8	7-F	*n*-C_4H_9	-	oil	0.9 (t, 3H, CH_3), 1.2-1.9 (m, 4H, CH_2CH_2), 3.9 (t,2H, J=7 Hz, CH_2-N), 7.4-7.9 (m, 3H, Ar)
C1	3,3'-F	H	B	188-91	7.2-7.5 (m, 6H, Ar), 7.5 (s, 2H, NH), 7.8 (s, 2H, NH)
C2	4,4'-F	H	D	258 dec	7-8 (m, 6H, Ar), 7.7 (s, 2H, NH), 8.1 (s, 2H, NH)
C3	5,5'-F	H	E	175 dec	7-8.3 (m, 10H, Ar and NH_2)
C4	6,6'-F	H	E	243-7	7-7.6 (m, 6H, Ar), 8 (s, 2H, NH), 8.2 (s, 2H, NH)
C5	3,3'-F	*n*-C_4H_9	F	124-8	0.9 (t, 6H, CH_3), 1.2-1.4 (m, 8H, CH_2CH_2), 2.8 (m, 4H, CH_2-NH), 7.1-7.6 (m, 6H, Ar), 8.3 (bt, 2H, J= 4 Hz, NH)
C6	4,4'-F	*n*-C_4H_9	G	200	0.85 (t, 6H, CH_3), 1.1-1.7 (m, 8H, CH_2CH_2), 3.3 (m, 4H, CH_2-NH), 7-7.8 (m, 6H, Ar), 8.6 (bt, 2H, J= 4 Hz, NH)
C7	5,5'-F	*n*-C_4H_9	-	169-72	0.9 (t, 6H, CH_3), 1.1-1.6 (m, 8H, CH_2CH_2), 3.2 (m, 4H, CH_2-NH), 7.2-7.7 (m, 6H, Ar), 8.6 (bt, 2H, J= 4 Hz, NH)
C8	6,6'-F	*n*-C_4H_9	H	145-7	0.9 (t, 6H, CH_3), 1.1-1.7 (m, 8H, CH_2CH_2), 3.3 (m, 4H, CH_2-NH), 7-7.6 (m, 6H, Ar), 8.7 (bt, 2H, J= 4 Hz, NH)

[a]A: ethyl acetate; B: ethyl acetate/petroleum ether; C: petroleum ether; D: dioxane; E: methanol; F: ethyl acetate/hexane; G: ethanol; H: ethanol/water.

action could be explained by interaction with thiol groups of glutathione, cysteine or biomacromolecules [28]. Therefore, decreased activity of *ortho*-fluorinated benzisothiazolones could result from a concurrence of steric effects and changes of S-N bond reactivity, induced by the electronic effects of substituents. In order to better elucidate the role of the various chemical effects on the mode of antimicrobic action, further chemometric and biochemical studies are in progress.

Table III. Physicochemical data of trifluoromethyl-1,2-benzisothiazol-3(2*H*)-ones **B1–8** and bis(trifluoromethyl)-2,2'-dithiobis(benzamides) **D1**, **2** and **D5–8**.

Compd	*X*	*R*	*Solvent*[a]	*mp (° C)*	*^{1}H NMR δ (DMSO-d_6)*
B1	4-CF_3	H	A	218-219	7.70-7.90 (m, 3H, Ar), 12.8 (bs, 1H, NH)
B2	5-CF_3	H	A	215	7.90-8.40 (m, 3H, Ar), 12.1 (bs, 1H, NH)
B3	6-CF_3	H	B	245 dec	7.70-8.70 (m, 3H, Ar), 12.1 (bs, 1H, NH)
B4	7-CF_3	H	B	170-172	7.40-8.40 (m, 3H, Ar), 11.45 (bs, 1H, NH)
B5	4-CF_3	*n*-C_4H_9	-	oil	0.92 (t, 3H, J=7 Hz, CH_3), 1.35 (m, 2H, CH_2CH_3), 1.72 (m, 2H, CH_2CH_2), 3.90 (t, 2H, J=7 Hz, CH_2-N), 7.80-8.40 (m, 3H, Ar)
B6	5-CF_3	*n*-C_4H_9	-	54-58	0.92 (t, 3H, J=7 Hz, CH_3), 1.30 (m, 2H, CH_2CH_3), 1.70 (m, 2H, CH_2CH_2), 3.90 (t, 2H, J=7 Hz, CH_2-N), 7.80-8.40 (m, 3H, Ar)
B7	6-CF_3	*n*-C_4H_9	-	oil	0.98 (t, 3H, J=7 Hz, CH_3), 1.30 (m, 2H, CH_2CH_3), 1.70 (m, 2H, CH_2CH_2), 3.90 (t, 2H, J=7 Hz, CH_2-N), 7.60-8.80 (m, 3H, Ar)
B8	7-CF_3	*n*-C_4H_9	-	oil	0.97 (t, 3H, J=7 Hz, CH_3), 1 30 (m, 2H, CH_2CH_3), 1.70 (m, 2H, CH_2CH_2), 3.95 (t, 2H, J=7 H_z, CH_2-N), 7.10-8.50 (m, 3H, Ar)
D1	3,3'-CF_3	H	C	195-198	7.30-7.90 (m, 10H, Ar and NH_2)
D2	4,4'-CF_3	H	D	214-218	7.60-8.60 (m, 10H, Ar and NH_2)
D5	3,3'-CF_3	*n*-C_4H_9	D	175-178	0.75 (t, 6H, J= 7 Hz, CH_3), 1.15 (m, 4H, CH_2CH_3), 1.30 (m, 4H, CH_2CH_2), 3.20 (m, 4H, CH_2-NH), 7.60-8.00 (m, 6H, Ar), 8.40 (bt, 2H, J= 6 Hz, NH)
D6	4,4'-CF_3	*n*-C_4H_9	A	232 dec	0.92 (t, 6H, J= 7 Hz, CH_3), 1.25 (m, 4H, CH_2CH_3), 1.55 (m, 4H, CH_2CH_2), 3.20 (m, 4H. CH_2-NH), 7.70-8.00 (m, 6H, Ar), 8.80 (bt, 2H, J= 6 Hz, NH)
D7	5,5'-CF_3	*n*-C_4H_9	A	251 dec	0.95 (t, 6H, J= 7 Hz, CH_3), 1.30 (m, 4H, CH_2CH_3), 1.70 (m, 4H, CH_2CH_2), 3.20 (m, 4H, CH_2-NH), 7.60-8.30 (m, 6H, Ar), 8.90 (bt, 2H, J= 6 Hz, NH)
D8	6,6'-CF_3	*n*-C_4H_9	C	158-162	0.90 (t, 6H, J= 7 Hz, CH_3), 1.35 (m, 4H, CH_2CH_3), 1.48 (m, 4H, CH_2CH_2), 3.20 (m, 4H, CH_2-NH), 7.60-8.00 (m, 6H, Ar), 8.80 (bt, 2H, J= 6 Hz, NH)

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

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 300 and 80 MHz, in DMSO-d_6 as solvent by using a Bruker ACE-300 or a Bruker WP80 SY spectrometer. Proton chemical shifts (δ) were reported with TMS (δ = 0.00 ppm) as internal standard. Splitting patterns are designated as follows: bs = broad singlet; s = singlet; d = doublet; dd = doublet of doublets; bt = broad triplet; t = triplet; m = multiplet. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck silica gel (60 F-254) and visualised by UV light (λ = 264 or 365 nm);

Table IV. The *in vitro* antifungal and antimicrobial activity of compounds **A1–8** and **C1–8** (MIC in μg/ml).

Compound	*Fungi*		*Gram-positive*			*Gram-negative*			
	T mentagrophytes	*C albicans*	*S aureus*	*S albus*	*B subtilis*	*K pneumoniae*	*E coli*	*S typhi*	*P aeruginosa*
A1	7.5	20	25	12.5	5	25	25	25	50
A2	7.5	30	**5**	**1**	**1.25**	10	10	10	50
A3	10	15	**5**	**1**	**2.5**	**5**	20	**2**	40
A4	15	20	40	40	30	> 50	50	50	> 50
A5	**5**	10	30	20	20	25	30	40	> 50
A6	**2.5**	**2.5**	10	10	**2**	40	30	40	> 50
A7	**5**	15	50	25	25	50	50	50	> 50
A8	10	20	40	20	20	> 50	50	> 50	> 50
C1	20	30	40	25	25	50	50	50	> 50
C2	**5**	7.5	**2.5**	**0.5**	**2.5**	20	10	7.5	20
C3	15	30	7.5	7.5	5	20	20	30	50
C4	**2**	**5**	50	40	25	50	50	50	> 50
C5	10	20	20	15	15	50	50	50	50
C6	5	7.5	15	2.5	5	> 50	> 50	> 50	50
C7	**2.5**	20	50	25	**2**	> 50	> 50	> 50	> 50
C8	**2.5**	**5**	50	50	25	50	50	50	> 50
Gentamycin	–	–	2.5	1.5	0.5	2.5	2.5	2.5	12.5
Cefotaxime	–	–	1.5	0.5	0.5	1.5	1.5	1.5	10
Clotrimazole	0.5	1.25	–	–	–	–	–	–	–

Table V. The *in vitro* antifungal and antimicrobial activity of compounds **B1–8** and **D1**, **2** and **D5–8** (MIC in μg/ml).

Compound	*Fungi*		*Gram-positive*			*Gram-negative*			
	T mentagrophytes	*C albicans*	*S aureus*	*S albus*	*B subtilis*	*K pneumoniae*	*E coli*	*S typhi*	*P aeruginosa*
B1	15	30	30	20	20	50	50	50	> 50
B2	**5**	10	15	10	10	50	40	40	> 50
B3	**5**	7.5	10	20	2	30	10	20	> 50
B4	10	20	30	30	20	50	50	50	> 50
B5	25	30	50	40	40	> 50	50	50	> 50
B6	20	40	40	30	40	50	50	40	> 50
B7	**5**	7.5	**5**	**0.5**	**5**	> 50	> 50	> 50	> 50
B8	40	40	40	30	40	50	50	50	> 50
D1	25	30	50	40	30	50	50	50	> 50
D2	**5**	7.5	10	1.5	5	20	50	10	50
D5	25	30	40	25	40	50	50	50	> 50
D6	**5**	7.5	20	20	20	> 50	> 50	> 50	> 50
D7	20	40	40	40	40	> 50	> 50	50	> 50
D8	25	30	50	40	40	> 50	> 50	50	> 50
Gentamycin	–	–	2.5	1.5	0.5	2.5	2.5	2.5	12.5
Cefotaxime	–	–	1.5	0.5	0.5	1.5	1.5	1.5	10
Clotrimazole	0.5	1.25	–	–	–	–	–	–	–

flash column chromatography was performed using silica gel 60 (60–200 μm, Merck). Elemental analyses were performed on a Carlo Erba 1106 Elemental Analyser (C, H, N), data obtained were within ± 0.35% of the theoretical values.

5-Trifluoromethyl-2-aminobenzoic acid ***7c***
To a stirred suspension of 5-trifluoromethyl-1*H*-indol-2,3-dione **3c** (3.8 mmol), in 20 ml of 5% sodium hydroxide (13.2 mmol), 20 ml of 30% hydrogen peroxide was added dropwise. The reaction mixture was stirred at 50°C for 30 min and was then allowed to reach room temperature. The filtered solution was acidified to pH 4 and the solid product collected. Recrystallization from ethanol/water provides a 82% yield of the pure product: mp 187–190°C; ^{1}H-NMR (DMSO-d_6) δ 3.30 (bs, 2H, NH_2), 6.85 (d, 1H, H-3), 7.55 (dd, 1H, H-4), 8.00 (s, 1H, H-5), 9.00 (bs, 1-H, COOH). Anal $C_8H_6F_3NO_2$ (C, H, N).

2-Aminobenzoic acids ***7a, b, d and e***
These compounds were prepared from corresponding substituted 1*H*-indol-2,3-dione **3**, following the same procedure described above for the preparation of compound **7c**. 3-Trifluoromethyl-2-aminobenzoic acid **7a** [29]: mp: 155–156°C (water). 4-Trifluoromethyl-2-aminobenzoic acid **7b** [22]: mp: 174–175°C (water). 6-Trifluoromethyl-2-aminobenzoic acid **7d** [23]: mp: 127–131°C (chloroform/petroleum ether). 3-Fluoro-2-aminobenzoic acid **7e** [30]: mp: 183–184°C (chloroform).

General preparation of 1,2-benzisothiazol-3(2H)-ones ***A1–4*** *and* ***B1–4***
Dry Cl_2 was bubbled into a suspension of the appropriate 2,2'-dithiobis(benzoylchloride) (4.0 mmol) in dry CCl_4 (60 ml) until complete dissolution (45 min). Excess Cl_2 was removed by a dry nitrogen stream. The resulting solution of 2-(chlorothio)benzoylchloride was treated with dry NH_3. The reaction mixture was set aside at room temperature for 2 h, and then the solvent was evaporated under reduced pressure. The resulting solid material was treated with water and then filtered. Analytical purification was obtained by recrystallization from a suitable solvent.

General preparation of N-butyl-1,2-benzisothiazol-3(2H)-ones ***A5–8, B5 and B7, 8***
A solution of the appropriate 2-(chlorothio)benzoylchloride (8.0 mmol) in dry CCl_4 was added dropwise to a stirred solution (28 mmol) of *n*-butylamine in dry CCl_4 (60 ml) at 50°C. The reaction mixture was stirred at this temperature for 1 h and then at room temperature for 4 h. The solvent was evaporated *in vacuo* and the residue was treated with aqueous HCl. The mixture was extracted with ethyl acetate (3 x 100 ml), collected organic layers were dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography on neutral alumina (eluent: ethyl acetate).

5-Trifluoromethyl-1,2-benzisothiazol-3(2H)-one ***B6***
To a stirred suspension of 5,5'-bis(trifluoromethyl)-2,2'-dithiobis(*N*-butylbenzamide) **D7** (1.8 mmol) in 150 ml dry CH_2Cl_2 a solution of $SOCl_2$ (11 ml) in the same solvent (40 ml) was added dropwise for over 1 h. The resulting mixture was stirred at reflux overnight and then the solvent removed *in vacuo*. Chromatography of the residue on silica gel with 33% ethyl acetate/*n*-hexane afforded the desired product.

General preparation of 2,2'-dithiobis(benzamides) ***C2–4*** *and* ***D2***
Dry NH_3 was bubbled into a cooled dioxane solution (ice-bath) of the appropriate 2,2'-dithiobis(benzoylchloride) (4.0 mmol) under stirring. The saturated solution was held at room temperature for 2 h and, after water addition, was acidified with 0.1 N HCl. The crude product was filtered and washed with water. Analytical purification was obtained by recrystallization from a suitable solvent.

General preparation of 2,2'-dithiobis(benzamides) ***C1*** *and* ***D1***
A solution of NH_3 (10.4 mmol) in 8 ml dioxane was added dropwise, with stirring, to a cooled solution (ice-bath) of the 2,2'-dithiobis(benzoylchloride) (4.0 mmol) in the same solvent (40 ml). After 30 min at room temperature, ice and aqueous HCl was added and the mixture was extracted with ethyl acetate (3 x 100 ml); the combined organic layers were dried (anhydrous Na_2SO_4) and then evaporated under reduced pressure. The residue was recrystallized from a suitable solvent.

General preparation for 2,2'-dithiobis(N-butylbenzamides) ***C6–8***
A solution of the appropriate 2,2'-dithiobis(benzoylchloride) (4.0 mmol) in dioxane was added dropwise with stirring to a cooled solution of *n*-butylamine (16 mmol) in the same solvent. After 15 min at 50–60°C, aqueous HCl was added and the resulting product was filtered and washed with a diluted solution of Na_2CO_3 and then with water. The crude solid was recrystallized from a suitable solvent.

General preparation for 2,2'-dithiobis(N-butylbenzamides) ***C5, D5 and D7***
A solution of *n*-butylamine (10.4 mmol) in dioxane (8 ml) was added dropwise to a cooled solution of 2,2'-dithiobis(benzoylchloride) (4.0 mmol) under stirring. After 10 min, ice and diluted HCl were added and the resulting mixture was extracted with ethyl acetate (3 x 100 ml). The collected organic layers were dried (anhydrous Na_2SO_4) and the solvent was removed under reduced pressure. The crude residue was recrystallized from a suitable solvent.

Microbiology

The antimicrobial activity of the prepared compounds was determined against a series of bacterial strains: *S aureus* (ATCC 6538), *S albus* (ATCC 12228) and *B subtilis* (ISM 6513) (Gram-positive species); and *E coli* (ISM 6585), *S typhi* (ATCC 19430), *K pneumoniae* (ATCC 4352) and *Pseudomonas aeruginosa* (ATCC 15442) (Gram-negative species). The mycetic strains tested were *C albicans* (ATCC 2091) and *T mentagrophytes* (ATCC 9129). The evaluation of minimum inhibitory concentrations (MIC) was carried out by the medium dilution technique for the fungal strains [31] and by the Bioscreen Analyzer for the bacterial strains [32].

Bioscreen is a fully automated analyzing system for measuring the *in vitro* kinetic growth of cells. The bacterial growth is monitored by a vertical pathway turbidimetry system in which the light beam passes through the bottom of the cuvette, the bacterial suspension and the cover of the cuvette to a detector. The vertical light path makes it possible to measure the turbidity of liquids every 10 or 20 min during a selected period (from 24 h to several days). The results of all measurements are shown as kinetic growth curves and their elaboration provided the reported antibacterial activities expressed as MIC.

The tested substances were dissolved in acetone/water solution (3:1), this solution exhibited no antimicrobial activity against the test organisms and was used as a negative control. The concentrations examined were in the range 1–100 μg/ml.

In the antibacterial testing procedures, Nutrient Broth (Oxoid) was used as a test medium. Cultures, grown at 37°C (overnight) in Nutrient Broth (Oxoid), were directly inoculated (approximately 10^4 cells) in Bioscreen cuvettes.

C albicans and *T mentagrophytes* were cultivated in Sabouraud Dextrose Broth (Oxoid) (25°C for 48–72 h and for 15 d, respectively) and then inoculated on the test plates containing both Sabouraud Dextrose Agar and the substances to test in the concentrations range of 1–100 μg/ml. The results were detected after incubation at 25°C for 48–72 h *(C albicans)* and for 15 d *(T mentagrophytes)*.

Gentamycin and cefotaxime, for antibacterial activity, and clotrimazole, for antimycotic activity, were employed as reference substances. All the results are presented in μg/ml.

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