Synthesis and Antimicrobial Activity of 2-(Acyl or carboxyalkyl)-3-(H or alkyl or aryl)-5 (or -6 or -8)-monochloro,7-fluoro-substituted-4*H*-1,4-benzothiazines

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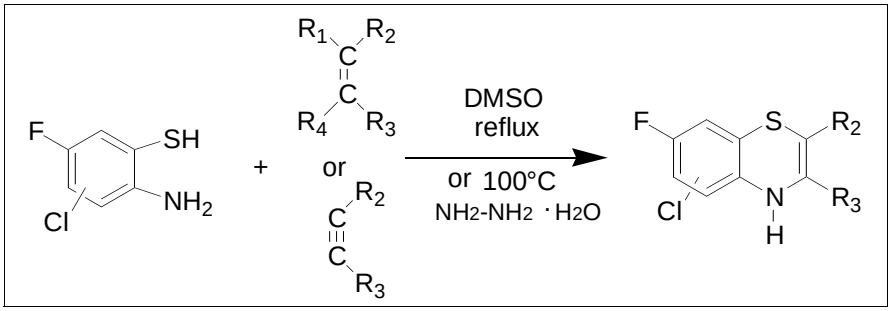
Received November 4, 2005



A series of 2-(acyl or carboxyalkyl)-3-(H or alkyl or aryl)-5 (or -6 or -8)-monochloro,7-fluoro-substituted-4*H*-1,4-benzothiazines **3a-x** were prepared to investigate their potential biological activity. In this work the **3a-w** structures are supported with physical and analytical data and the results of their *in vitro* antimicrobial activity against some strains Gram positive, Gram negative, and Fungi are reported. It was found that compounds **3a**, **3d** displayed interesting antibacterial activity, whereas compound **3f** displayed interesting antifungal activity.

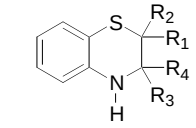
J. Heterocyclic Chem., **43**, 1371 (2006).

Introduction.

The 4*H*-1,4-benzothiazine heterocyclic nucleus, even when part of complex molecules [1a-c], possesses a wide spectrum of pharmacological and biological activities [1a-c,2] similar to phenothiazines. For example, a large number of type **1** and **2** (Figure 1) compounds, endowed with innumerable biological activities [3], including antibacterial and antifungal, have been known for some time. Recently, in addition, some dihydro1,4-benzothiazin-3-one compounds of type **1b** (Figure 1) were described as histamine H_1-receptor antagonists with anti-pruritic activities [4] and inhibitory effects on xanthine oxidase [5].

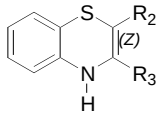
Moreover, some dihalogenated benzothiazines **3** (Figure 1) have been cited for their activities. In particular, 1-(5-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-yl)ethanone (**3d**) and ethyl 5-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate (**3s**) have been mentioned as potential anticancer agents [2]. Furthermore, the corresponding acid of methyl 6-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate (**3k**) [6], has also been tested because of its analogy to the antibacterial quinolones.

Continuing our studies aimed at synthesizing aza and thiaza ethero bi- and tri-cyclic potentially bio-active compounds [1b,c], as an enlargement of our researches on the dehalogenated benzothiazoles type **4** (Figure 1) activity [7a], we recently reported the synthesis and



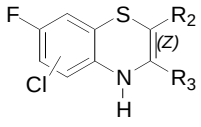
1

a) R_1, R_4 = H; R_2, R_3 = H, alkyl, etc.
b) R_3 + R_4 = O



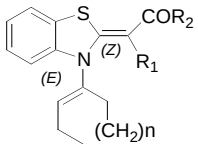
2

Cpd	R_2	R_3
2a[1b]	$COCH_3$	H
2d[1b]	$COCH_3$	CH_3
2g[1b]	$COCH_3$	C_6H_5
2j[1d]	$COOCH_3$	H
2m[1a]	$COOCH_3$	CH_3
2p[1b]	$COOC_2H_5$	H
2s[1b]	$COOC_2H_5$	CH_3
2v[1e]	$COOC_2H_5$	C_6H_5



3

		R_2	R_3
3d[2]	5-Cl	$COCH_3$	CH_3
3k[6]	6-Cl	$COOCH_3$	H
3s[2]	5-Cl	$COOC_2H_5$	CH_3



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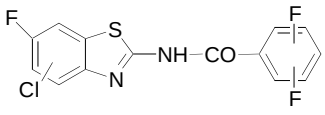


5

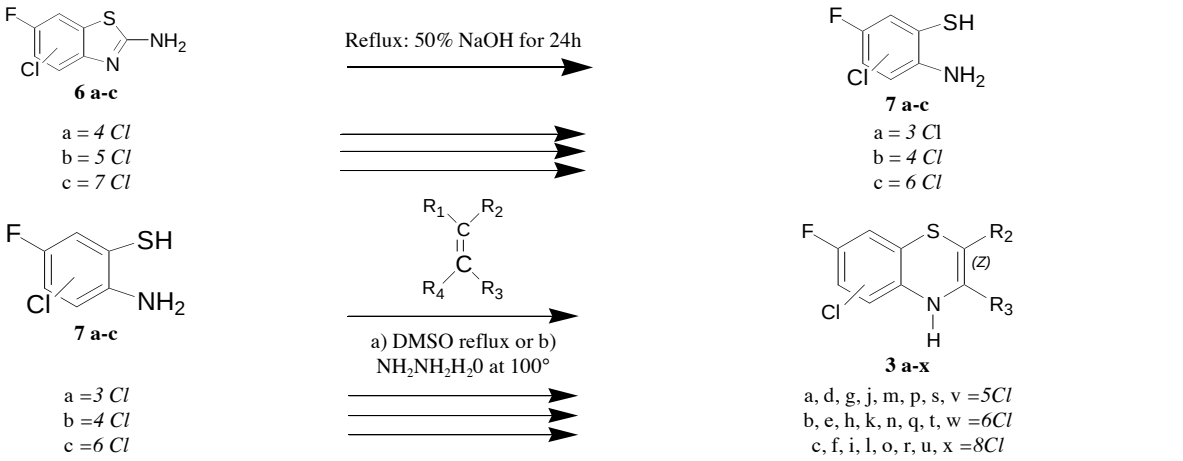
Figure 1

antifungal activities of a series of 6-fluoro-4(5 or 7)-chloro-2-(difluorobenzoyl) aminobenzothiazoles type **5** (Figure 1) [7b].

Taking into account these findings, and as an extension of our syntheses of non-halogenated benzothiazine compounds [1b] type **2** (Figure 1), with the intention of enlarging the number of compounds analogous to the three already known compounds [2,6] of type **3** (Figure 1), we decided to synthesize a large number of dihalogenated benzothiazines variously substituted with appropriate pharmacophoric groups.

In particular, the synthesis of 2-(acyl or carboxyalkyl)-3-(H or alkyl or aryl)-5 (or -6 or -8)-monochloro,7-fluoro-substituted 4*H*-1,4-benzothiazine compounds **3** (Figure 1)

Scheme 1



Cpd			Cpd [method]	Yield %	Yield av.[*]	5	6	8	R_2	R_3	R_1	R_4
7a	I	3-Butyn-2-one	**3a**[a]	47.7		Cl			$COCH_3$	H		–
7b		$CH_3-CO-C\equiv CH$	**3b**[a]	29.2	46,8		Cl		$COCH_3$	H		–
7c			**3c**[a]	63.5				Cl	$COCH_3$	H		–
7a	II	2,4-Pentanedione (enol-form)	**3d**[a]	32.2		Cl			$COCH_3$	CH_3	H	OH
7b		$CH_3-CO-CH=C(OH)-CH_3$	**3e**[a]	48.6	49.8		Cl		$COCH_3$	CH_3	H	OH
7c			**3f**[a]	68.5				Cl	$COCH_3$	CH_3	H	OH
7a	III	4-Phenyl-3-Butyn-2-one	**3g**[b]	2.1		Cl			$COCH_3$	C_6H_5		–
7b		$CH_3-CO-C\equiv C-C_6H_5$	**3h**[b]	5.0	4.2		Cl		$COCH_3$	C_6H_5		–
7c			**3i**[a]	5.5				Cl	$COCH_3$	C_6H_5		–
7a	IV	Methyl-propiolate	**3j**[a]	17.7		Cl			$COOCH_3$	H		–
7b		CH_3-O-CO−C≡C−H	**3k**[a]	71.2	31.8		Cl		$COOCH_3$	H		–
7c			**3l**[b]	6.4				Cl	$COOCH_3$	H		–
7a	V	Methyl-acetoacetate (enol form)	**3m**[a]	26.0		Cl			$COOCH_3$	CH_3	H	OH
7b		CH_3-O-CO−CH=C(OH)−CH_3	**3n**[a]	84.4	46.9		Cl		$COOCH_3$	CH_3	H	OH
7c			**3o**[a]	30.2				Cl	$COOCH_3$	CH_3	H	OH
7a	VI	Ethyl propiolate	**3p**[a]	47.9		Cl			$COOC_2H_5$	H		–
7b		C_2H_5-O−CO−C≡CH	**3q**[a]	42.5	31.8		Cl		$COOC_2H_5$	H		–
7c			**3r**[b]	5.0				Cl	$COOC_2H_5$	H		–
7a	VII	Ethyl acetoacetate (enol form)	**3s**[a]	49.0		Cl			$COOC_2H_5$	CH_3	H	OH
7b		C_2H_5-O−CO−CH=C(OH)−CH_3	**3t**[a]	43.2	47.2		Cl		$COOC_2H_5$	CH_3	H	OH
7c			**3u**[a]	49.4				Cl	$COOC_2H_5$	CH_3	H	OH
7a	VIII	Phenyl-ethyl--propiolate	**3v**[a]	14.8		Cl			$COOC_2H_5$	C_6H_5		–
7b		C_2H_5-O-CO·C≡C−C_6H_5	**3w**[a]	35.9	16.9		Cl		$COOC_2H_5$	C_6H_5		–
7c			**3x**[b]	0				Cl	$COOC_2H_5$	C_6H_5		–

(*) Average yield of three isomers. For example, for **3a-c**; (47.7+29.2+63.5)/3 = 46.8.

is part of our current research program aimed at discovering new aza-sulfurated antimicrobial agents [2,7]. It is also, from an SAR viewpoint, an attempt to evaluate the relationship between the antimicrobial activity of derivatives **3a-w** and their substituent groups.

Results and Discussion.

Chemistry.

As shown in Scheme 1, the desired compounds **3a-x** were prepared following a two-step procedure involving the synthesis of the appropriate 3(4 or 6)-monochloro-5-fluoro-2-amino benzenthioles (**7a-c**) by hydrolytic basic cleavage [8] of 4(5 or 7)-monochloro-6-fluoro-2-aminobenzothiazoles (**6a-c**) [7b]. Next, all the eight series of three possible isomers, namely the twenty four compounds **3a-x**, were prepared by cyclization of compounds **7a-c** with the eight suitable unsaturated (enolic or acetylenic) reagents as shown in Scheme 1, using two different procedures a and b. Briefly, these were as follows, procedure a: refluxing in dimethyl sulfoxide for three hours, procedure b: using hydrazine monohydrate as catalyst at 100°C for ten minute. Further details of these procedures are described below in the section Experimental Chemistry.

Generally, procedure a gave the better yields. Nevertheless, in cases where it failed (**3g,h,l,r,x**), procedure b had the merit of producing the desired compound (except for **3x**), albeit in low yields (max. 5.2% for **3l**).

In Scheme 1, both cyclization procedures, the yields of compounds **3a-x** and the eight average yields of all the three possible isomers of compounds **3** (*i.e.* 8 "three possible isomers": **3a-c**, **3d-f**,…) are also presented. The scheme also reports the structures of compounds **6a-c** [7b] and **7a-c**, those of eight unsaturated (enolic or acetylenic) reagents employed, as well as of the compounds 2-(acyl or carboxyalkyl)-3-(H or alkyl or aryl)-5(6 or 8)-monochloro,7-fluoro-4*H*-1,4-benzothiazines (**3a-x**).

The structures of the new compounds are fully supported by microanalytical and spectral (ir, ^{1}H nmr, and mass) data. Compounds **3d** [2], **3k** [6] and **3s** [2] show spectral data consistent with those reported in the literature. Tables 1-4 show the IUPAC names, the elemental analyses, the main physical properties and spectral data for compounds **7a-c** and **3a-w**.

In connection with the yields, we note that they can alter depending on the position of the chloro substituent in the benzene ring and on the chemical structure of the reagent employed. The highest yields were observed with procedure a for compounds **3n** (84.4%), **3k** (71.2%), **3f** (68.5%), **3c** (63.5%). Considering that each of the eight reagents yielded three isomeric benzothiazines (**3**), Scheme 1 allows two interpretations. In the first of these it is possible to discern the influence of the chloro substituent. Thus, selecting the highest yield of each of eight groups of isomers (**3a-c, d-f,...**),the results indicate that there is no straightforward dependency between Cl-position and the yields. In fact, with each of the eight reagents, the highest yield of **3** compounds often occurred for different positions (5 or 6 or 8) of the chloro substituent (**). The second interpretation of Scheme 1 is based on the consideration that the combination of the yields of various three isomers can remove the effect of the dependency between the yields and Cl-position. This way, considering, for each reagent, the average yields of each of the three isomers, the yields obtained by each reagent can be compared without regard to the chloro position (***).

In short, from a synthetic point of view, the results indicate that, with regard to reactivity, the reagents themselves are more influential than the position of the chloro substituent in the benzene ring. Moreover, among the reagents, the enol forms are more reactive than acetylenic compounds. The latter are, in fact, inactive when a phenyl group is present α to the ethyne group (see: **3x,** yield=0%).

Microbiology.

Benzo[1,4]thiazine compounds are known to exhibit a wide range of pharmacological properties including antidepressant, anxiolytic, and calcium antagonist activity [3]. In our search, devoted, as it was, to exploring the antimicrobial properties of 1,4-benzothiazine compounds, all the synthesized compounds were tested *in vitro* against some Gram-positive and Gram-negative bacteria and the fungus *Candida albicans*. The results are summarized in Table 5 in which only the most significant findings are listed (*i.e.* compounds showing MIC ≤ 250 μg/ml).

As shown in Table 5, as regards Gram-positive bacteria, the compounds **3a** and **3d** displayed an interesting antibacterial activity against strains of *Bacillus subtilis* and *Bacillus cereus* (MIC 15.6 μg/ml) and a moderate activity (MIC 62.5 μg/ml) against two strains of *Staphylococcus aureus* and against the *Enterococcus faecalis*. Moderate activity (MIC 62.5 μg/ml) was displayed respectively by compound **3w** against four strains (*Bacillus subtilis*, *Bacillus cereus* and two strains of *Staphylococcus aureus),* by compound **3r** against three strains (*Enterococcus faecalis* and two strains of *Staphylococcus aureus*), and finally by compound **3p** against the two strains of *Staphylococcus aureus*.

There are no compounds with any significant antibacterial activity against the Gram-negative strains tested.

As regards the fungi, the compound **3f** displayed an interesting antifungal activity (MIC 31.2–15.6 μg/ml) against the four strains of *Candida albicans*, and the compounds **3h**, **3m**, **3n** displayed against the same strains a moderate activity (MIC 62.5 μg/ml). Actually, the above compounds, together with the compounds **3a** and **3d** (MIC 125 μg/ml), exhibit an antifungal activity between those of our standards fluconazole (MIC:1–0.5 μg/ml) and sorbic acid (MIC:500–250 μg/ml).

In conclusion, from an SAR standpoint, we observe that, of all compounds tested (**3a-w** of Table 5), the highest antimicrobial activity occurs in compounds **3a**, **3d** when the molecule 7-fluoro-4*H*-1,4-benzothiazine possesses the acetyl substituent in position 2 and the chloro in position 5 (Scheme 1).

Concerning the activity against the strain *Bacillus subtilis* ATCC 6633, it is interesting to compare the activities of the two more active compounds **3a** and **3d** (type **3**, Figure 1) (MIC 15.6 μg/ml) with the activities of two analogous non-halogenated compounds 1-(4*H*-1,4-benzothiazin-2-yl)ethanone (MIC 62.5 μg/ml [1b]) and 1-(3-methyl-4*H*-1,4-benzothiazin-2-yl)ethanone (MIC >250 μg/ml [1b]) (**2a** and **2d**, Figure 1). The comparison reveals that the 5-chloro position (and not the 6 or the 8 position) causes a remarkable increase in the activities.

EXPERIMENTAL

Chemistry.

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Ir spectra were obtained on a Perkin-Elmer 283 spectrophotometer (nujol mull). ^{1}H nmr spectra were recorded on Varian-Mercury instrument operating at 300 MHz. Chemical shifts are given in δ values (ppm) and the coupling constants are expressed in J values (Hz) downfield from tetramethylsilane as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC-MS low$_1$ resolution spectrometer. All compounds showed appropriate ir, ^{1}H nmr and mass spectra. Elemental analyses were carried out with Eurovector EuroEA 3000 analyzer and the results were within ± 0.4% of the theoretical values. Column chromatography on silica gel 60 (Merck 70-230 mesh) was carried out by using light petroleum ether (bp 40-70°)-ethyl acetate (7:3 v/v) as eluent. Thin layer chromatography was performed using light petroleum ether-ethyl acetate (7:3 v/v) or hexane-ethyl acetate (7:3 v/v) as eluent, in each case specified.

General Procedure for the Preparation of Compounds **7** [8]

A solution of 2-amino-(4 or 5 or 7)-chloro-6-fluorobenzothiazolo (**6a** or **6b** or **6c**) (5 g, 24.7 mmoles) in 82.80 ml of 50% NaOH was refluxed vigorously until the evolution of ammonia ceased (about 24 h). After cooling, the reaction mixture was diluted with distilled water (30 ml) and extracted with ethyl acetate (3x100). The combined organic layers were washed with water, dried (anhydrous sodium sulfate) and evaporated under reduced pressure. The crude product was separated by column-chromatography on silica gel (eluent: *n*-hexane-ethyl acetate 7:3 v/v) to obtain the pure compound **7a** (3.27 g) or **7b** (3.80 g) or **7c** (3.13 g), respectively. Each of the three isomers showed m/z 177 (M^+, base), 142, 132. The yields and the physical and analytical data are reported in Tables 1 and 2.

General Procedure for the Preparation of Compounds **3**.

Method a [2] for **3 a-f, i-k, m-q, s-w**.

To a well-stirred solution of **7** (2.8 mmoles) in dimethyl sulfoxide (10 ml) was added the appropriate enolic or acetylenic reagent (2.8 mmoles) at room temperature. The mixture was refluxed for 3 h. The crude mixture was purified by column chromatography on silica gel using light petroleum ether (bp 40-70°)-ethyl acetate (7:3 v/v) as eluent. The yield, the elemental analyses and the chemical and physical properties are reported in Tables 3 and 4.

Method b [1a] for **3 g, h, l, r,x**.

A well stirred mixture of **7** (0.5 g, 2.8 mmoles) and hydrazine monohydrate (14 mg, 0.28 mmoles) was heated at 100°C for 2-3 minutes before introducing the appropriate enolic or acetylenic reagent (2.8 mmol) and warming the reaction to 100°C for a further 10 minutes. After cooling to room temperature, the crude mixture was purified by column chromatography on silica gel using light petroleum ether (bp 40-70°)-ethyl acetate (7:3 v/v) as eluent. The yield, the elemental analyses and the chemical and physical properties are reported in Tables 3 and 4.

Table 1

Yield, Physical and Analytical Data for the Benzenethiol Compounds **7**

N° [a]	Compound	Yield %	State [b]	Mp (°C)	Molecular Formula (Molecular Weight)	Analysis (%) Calcd/Found			Rf [c]
						C	**H**	**N**	
7a[2]	2-amino-3-chloro-5-fluorobenzenethiol	74.65	Y	100-106	C_6H_5ClFNS 177.628	40.57 40.20	2.84 2.76	7.89 7.82	0.88
7b[8]	2-amino-4-chloro-5-fluorobenzenethiol	86.75	G	121-124		40.57 40.22	2.84 2.85	7.89 7.56	0.30
7c[8]	2-amino-6-chloro-5-fluorobenzenethiol	71.46	G	124-128		40.57 40.90	2.84 2.64	7.89 7.57	0.52

[a] compounds mentioned in references have been characterized more completely;[b] Y= yellow solid, G = green solid; [c] Eluant: *n*-hexane-ethyl acetate 7:3 v/v (TLC).

Table 2

Physical and Analytical Data for the Benzothiazine Compounds **3**

N°	Compound	State [a]	Mp (°C)	Molecular Formula (Molecular Weight)	Analysis (%) Calcd/Found			Rf [b]
					C	**H**	**N**	
3a	1-(5-chloro-7-fluoro-4*H*-1,4-benzothiazin-2-yl)ethanone	O	251-253	$C_{10}H_7ClFNOS$ (243.686)	49.29 48.92	2.90 3.30	5.75 5.37	0.45
3b	1-(6-chloro-7-fluoro-4*H*-1,4-benzothiazin-2-yl)ethanone	O	276-277		49.29 49.55	2.90 3.24	5.75 6.02	0.09
3c	1-(8-chloro-7-fluoro-4*H*-1,4-benzothiazin-2-yl)ethanone	O	236-239		49.29 49.24	2.90 3.29	5.75 5.55	0.03
3d[2]	1-(5-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazin-2-yl) ethanone	O	51-55	$C_{11}H_9ClFNOS$ (257.712)	51.27 50.94	3.52 3.82	5.44 5.83	0.68
3e	1-(6-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazin-2-yl)ethanone	O	262-265		51.27 50.89	3.52 3.58	5.44 5.33	0.50
3f	1-(8-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazin-2-yl)ethanone	O	274-275		51.27 51.50	3.52 3.90	5.44 5.05	0.13
3g	1-(5-chloro-7-fluoro-3-phenyl-4*H*-1,4-benzothiazin-2-yl)ethanone	O	131-135	$C_{16}H_{11}ClFNOS$ (319.782)	60.09 59.88	3.47 3.79	4.38 4.05	0.71
3h	1-(6-chloro-7-fluoro-3-phenyl-4*H*-1,4-benzothiazin-2-yl)ethanone	R	173-177		60.09 60.12	3.47 3.70	4.38 4.28	0.62
3i	1-(8-chloro-7-fluoro-3-phenyl-4*H*-1,4-benzothiazin-2-yl)ethanone	B	172-180		60.09 59.83	3.47 3.12	4.38 4.00	0.60
3j	methyl 5-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	Y	157-162	$C_{10}H_7ClFNO_2S$ (259.685)	46.25 46.60	2.72 3.07	5.39 5.79	0.70
3k[6]	methyl 6-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	B	206-209		46.25 46.46	2.72 3.02	5.39 5.01	0.49
3l	methyl 8-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	B	222-226		46.25 46.53	2.72 3.00	5.39 5.32	0.21
3m	methyl 5-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	B	110-113	$C_{11}H_9ClFNO_2S$ (273,712)	48.27 48.33	3.31 3.43	5.12 5.09	0.74
3n	methyl 6-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	Y	230-235		48.27 47.98	3.31 3.42	5.12 5.07	0.66
3o	methyl 8-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	O	225-230		48.27 48.08	3.31 3.30	5.12 5.06	0.38
3p	ethyl 5-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	Y	107-112	$C_{11}H_9ClFNO_2S$ (273,712)	48.27 47.90	3.31 3.70	5.12 4.97	0.70
3q	ethyl 6-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	O	196-199		48.27 48.51	3.31 3.44	5.12 4.80	0.50
3r	ethyl 8-chloro-7-fluoro-4*H*-1,4-benzothiazine-2-carboxylate	O	178-181		48.27 48.27	3.31 3.63	5.12 4.89	0.37
3s[2]	ethyl 5-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	B	66-70	$C_{12}H_{11}ClFNO_2S$ (287.738)	50.09 49.69	3.85 3.71	4.87 4.99	0.81
3t	ethyl 6-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	B	171-174		50.09 49.97	3.85 3.72	4.87 4.84	0.45
3u	ethyl 8-chloro-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate	B	218-220		50.09 49.78	3.85 3.76	4.87 5.25	0.28
3v	ethyl 5-chloro-7-fluoro-3-phenyl-4*H*-1,4-benzothiazine-2-carboxylate	B'	oil	$C_{17}H_{13}ClFNO_2S$ (349.808)				0.75
3w	ethyl 6-chloro-7-fluoro-3-phenyl-4*H*-1,4-benzothiazine-2-carboxylate	O	60		58.37 58.75	3.75 4.10	4.00 3.88	0.82

[a] O = orange solid; Y = yellow solid; R = red solid; B = brown solid; B' = brown oil; [b] Eluant: petrol ether-ethyl acetate 7:3 v/v (TLC).

Microbiology.

Determination of Antibacterial Activity.

Organisms.

The synthesised compounds **3a-w** were tested for antimicrobial activity against a variety of Gram-positive (*Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 11778, Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 6538p, Enterococcus faecalis ATCC 29212*) and Gram-negative bacteria (*Escherichia coli ATCC 8739, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853*) and fungal strains too (*Candida albicans* ATCC 10231, *Candida albicans* ATCC 14053, *Candida albicans* NRRL y 869 and *Candida albicans* y NRRL 12983).

For bacterial strains the MICs were determined by the microdilution method, the inocula were prepared as follows: colonies derived from fresh mature strains on MHA (Mueller Hinton agar) were suspended in saline solutions (0.85%) and suspensions were adjusted to 10^8 cfu ml^{-1} (0.5 MacFarland) as determined by viable count.

Table 3

Spectral Data for the Benzenethiol Compounds **7**

N° [a]	IR (nujol mull) (cm^{-1}) NH_2	^1H nmr δ (ppm) [b]
7a[2]	3489 and 3392	($CDCl_3$) δ 4.56 (s, broad, 3H, SH + NH_2), 6.83 and 6.86 (dd, 1H, J = 2.5 Hz, H_6), 7.04 and 7.07 (dd, 1H, J = 2.5 Hz, H_4)
7b[8]	3470 and 3380	($CDCl_3$) δ 4.52 (s, broad, 3H, SH + NH_2), 6.72 (d, 1H, J = 6.0 Hz, H_3), 6.95 (d, 1H, J = 9.0 Hz, H_6)
7c[8]	3449 and 3352	(DMSO-d_6) δ 4.30 (s, broad, 3H, SH + NH_2), 6.50 and 6.53 (dd, 1H, J = 5.0 Hz, H_3), 6.95 (t, 1H, J = 8.0 Hz, H_4)

[a] compounds mentioned in references have been characterized more fully; [b]= All NH_2 signals disappear with D_2O.

Table 4

Spectral Data for the Benzothiazine Compounds **3**

N°	IR (nujol mull) (cm^{-1}) NH , CO	^1H nmr δ (ppm) [a]
3a	3247, 1590	($CDCl_3$) 2.19 (s, 3H, $COCH_3$), 6.1 (s, br, 1H, NH), 6.43 and 6.46 (dd, 1H, J = 2.0 Hz, H_8), 6.62 and 6.65 (dd, 1H, J = 2.0 Hz, H_6), 7.02 (d, 1H, J = 7.5 Hz, H_3)
3b	3244, 1554	($CDCl_3$) 2.10 (s, 3H, $COCH_3$), 6.57 (d, 1H, J = 7.0 Hz, H_5), 6.82 (d, 1H, J = 9.0 Hz, H_8), 7.24 (d, 1H, J = 7.0 Hz, H_3), 8.9 (s, br, 1H, NH)
3c	3240, 1568	($CDCl_3$) 1.6 (s, br, 1H, NH), 2.11 (s, 3H, $COCH_3$), 6.80 and 7.64 (dd, 1H, J = 3.4 Hz, H_3), 7.1-7.2 (m, 1H, H_5), 7.50 (t, 1H, J = 8.4 Hz, H_6)
3d	3330, 1590	($CDCl_3$) 1.8 (s, br, 1H, NH), 2.30 (s, 3H, CH_3), 2.38 (s, 3H, $COCH_3$), 6.62 and 6.66 (dd, 1H, J = 2.7 Hz, H_8), 6.78 and 6.82 (dd, 1H, J = 2.7 Hz, H_6)
3e	3238, 1564	(Acetone deut.) 2.86 (s, 3H, CH_3), 2.88 (s, 3H, $COCH_3$), 6.76 and 6.79 (dd, 1H, J = 2.2 Hz, H_5), 6.88 and 6.92 (dd, 1H, J = 2.2 Hz, H_8), 8.1 (s, br, 1H, NH)
3f	3286, 1560	(DMSO-d_6) 1.5 (s, br, 1H, NH), 2.18 (s, 3H, CH_3), 2.23 (s, 3H, $COCH_3$), 6.6-6.4 (m, 1H, H_5), 6.89 (t, 1H, J = 8.0 Hz, H_6)
3g	3406, 1603	($CDCl_3$) 2.10 (s, 3H, $COCH_3$), 6.25 (s, br, 1H, NH), 6.61 and 6.63 (dd, 1H, J = 2.75 Hz, H_8), 6.73 and 6.76 (dd, 1H, J = 2.75 Hz, H_6), 7.4-7.6 (m, 5H, aromatic-H)
3h	3264, 1565	($CDCl_3$) 2.10 (s, 3H, $COCH_3$), 7.19 (d, 1H, J = 7.5 Hz, H_5), 7.43 (t, 3H, J = 7.5 Hz, *o-p* aromatic-H), 7.51 (1H, d, J = 6.75, H_8), 7.58-7.60 (m, 2H, *m* aromatic-H), 11.61 (s, br, 1H, NH)
3i	3260, 1576	($CDCl_3$) 2.62 (s, 3H, $COCH_3$), 6.26 and 6.29 (dd, 1H, J = 5.05 Hz , H_5), 6.70 (t, 1H, J = 7.58 Hz, H_6,), 7.3-7.6 (m, 5H, aromatic-H), 11.48 (s, br, 1H, NH)
3j	3333, 1635	($CDCl_3$) 3.73 (s, 3H, $COOCH_3$), 6.06 (s, br, 1H, NH), 6.42 and 6.44 (dd, 1H, J = 2.7 Hz, H_8), 6.66 and 6.67 (dd, 1H, J = 3.3 Hz, H_6), 7.06 (d, 1H, J = 5.9 Hz, H_3)
3k	3285, 1633	($CDCl_3$) 3.73 (s, 3H, $COOCH_3$), 5.59 (s, br, 1H, NH), 6.29 (d, 1H, J = 5.9 Hz, H_5), 6.55 (d, 1H, J = 8.3 Hz, H_8), 7.06 (d, 1H, J = 5.9 Hz, H_3)
3l	3278, 1629	($CDCl_3$) 3.73 (s, 3H, $COOCH_3$), 5.45 (s, br, 1H, NH), 6.00 and 6.03 (dd, 1H, J = 3.8 Hz, H_5), 6.57 (t, 1H, J = 8.5 Hz, H_6), 6.95 (d, 1H, J = 6.3 Hz, H_3)
3m	3415, 1620	($CDCl_3$) 2.25 (s, 3H, CH_3), 3.70 (s, 3H, $COOCH_3$), 6.0 (s, br, 1H, NH), 6.59 and 6.52 (dd, 1H, J = 2.9 Hz, H_8), 6.77 and 6.70 (dd, 1H, J = 2.9 Hz, H_6)
3n	3324, 1640	($CDCl_3$) 2.30 (s, 3H, CH_3), 3.73 (s, 3H, $COOCH_3$), 5.52 (s, br, 1H, NH), 6.42 (d, 1H, J = 5.8 Hz, H_5), 6.67 (d, 1H, J = 8.3 Hz, H_8)
3o	3328, 1639	($CDCl_3$) 2.25 (s, 3H, CH_3), 3.73 (s, 3H, $COOCH_3$), 5.4 (s, br, 1H, NH), 6.11 and 6.14 (1H, dd, J = 3.8 Hz, H_5), 6.25 (t, 1H, J = 8.3 Hz, H_6)
3p	3331, 1632	(DMSO-d_6) 1.18 (t, 3H, J = 7.0 Hz, CH_2CH_3), 4.07 (q, 2H, J = 7.0 Hz, CH_2CH_3), 6.70 and 6.73 (dd, 1H, J = 3..0 Hz, H_8), 6.95 (d, 1H, J = 7.0 Hz, H_3), 6.98 and 7.00 (dd, 1H, J = 3.0 Hz, H_6), 8.28 (d, 1H, *J* = 7.0 Hz, NH)
3q	3291, 1635	(DMSO-d_6) 1.15 (t, 3H, J = 7.0 Hz, CH_2CH_3), 4.05 (q, 2H, J = 7.0 Hz, CH_2CH_3), 6.57 (d, 1H, J = 7.0 Hz, H_5), 6.88 (d, 1H, J = 10 Hz, H_8), 7.03 (d, 1H, J = 7.0 Hz, H_3) , 8.72 (d, 1H, J = 7.0 Hz, NH)
3r	3297, 1632	($CDCl_3$) 1.15 (t, 3H, J = 7.0 Hz, CH_2CH_3), 4.05 (q, 2H, J = 7.0 Hz, CH_2CH_3), 5.5 (s, br, 1H, NH), 6.00 (d, 1H, J = 9.0 Hz, H_6), 6.55 (d, 1H, J = 7.0 Hz, H_5), 6.93 (d, 1H, J = 7.0 Hz, H_3)
3s	3335, 1635	(DMSO-d_6) 1.17 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.27 (s, 3H, CH_3), 4.05 (q, 2H, J = 7.0 Hz, CH_2CH_3), 6.05 (d, 1H, J = 8.5, H_6), 7.05 (d, 1H, J = 8.5 Hz, H_8), 7.8 (s, br, 1H, NH)
3t	3331, 1622	($CDCl_3$) 1.30 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.28 (s, 3H, CH_3), 4.19 (q, 2H, J = 7.0 Hz, CH_2CH_3), 5.56 (s, br, 1H, NH), 6.42 (d, 1H, J = 6.5 Hz, H_5), 6.68 (d, 1H, J = 9.5 Hz, H_8)
3u	3333, 1627	(DMSO-d_6) 1.18 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.1 (s, 3H, CH_3), 4.03 (q, 2H, J = 6.0 Hz, CH_2CH_3), 6.4-6.5 (m, 1H, H_6), 6.82 (d, 1H, J = 8.0 Hz, H_5), 8.7 (s, br, 1H, NH)
3v	3357, 1622	($CDCl_3$) 1.18 (t, 3H, J = 6.7 Hz, CH_2CH_3), 4.18 (q, 2H, J = 6.7 Hz, CH_2CH_3), 6.98 (d, 1H, J = 9.0 Hz, H_6), 7.30 (s, 1H, H_8), 7.35-7.53 (m, 5H, aromatic-H), 8.00 (d, 1H, J = 8.0 Hz, NH)
3w	3304, 1613	($CDCl_3$) 1.09 (t, 3H, J = 7.0 Hz, CH_2CH_3), 4.08 (q, 2H, J = 7.0 Hz, CH_2CH_3), 6.0 (s, br, 1H, NH), 7.1-7.6 (m, 5H, aromatic-H), 7.12 (d, 1H, J = 7.0 Hz, H_5), 8.00 (d, 1H, J = 7.0 Hz, H_8)

[a]= All NH signals disappear with D_2O.

Susceptibility Testing.

A series of two fold dilutions of each product were prepared in DMSO at required dilutions (500, 250, 125, 62.5, 31.2, 15.6, 7.8 μg/ml) and placed in 96 wells microdilution trays, containing broth media. Each well was inoculated with 20 μl of a final inoculum concentration that approximately contained $5 \cdot 10^4$ cfu/ml [9-11]. A growth control well test was also prepared; this control well contained the broth media with DMSO at the working concentrations (5%). Under these conditions DMSO was inactive against our strains. The trays were incubated at 37° for 24 hours. Each strain was tested at least three times in duplicate before selection of the modal value. The MICs were determined as the lowest concentration of the agent resulting in no growth. To determine the MIC, we compared the growth in every well visually with that of the growth control well [9-11]. Appropriate controls employed the antibiotic norfloxacin; this was tested as international reference standard in accordance with the procedures suggested by CLSI-NCCLS (MS100-S13). The microdilution technique was based on the procedures recommended by CLSI-NCCLS (M7A6). [12]

Determination of Antifungal Activity.

Organisms.

Standard strains of *Candida albicans* ATCC 10231, *Candida albicans* ATCC 14053, *Candida albicans* NRRL y 869 and *Candida albicans* NRRL y 12983 was grown and maintained on sabouraud agar slants. The fungal strains were grown in Sabouraud dextrose agar. Five colonies were selected and the suspensions adjusted to approximately $1 \cdot 10^6$ cfu/ml. They were diluted appropriately in yeast malt broth medium (Clinical and Laboratory Standard Institute, USA -, m27-a2) to give an inoculum of $1 \cdot 10^3$ cfu/ml. [9, 12]

Susceptibility Testing.

The broth microdilution assay was based on that recommended by the Clinical and Laboratory Standard Institute [12]. The stock solutions of the compounds were adjusted in DMSO [9]. Final concentration ranges for each compound were from 500 to 7.8 μg/ml. Fluconazole was dissolved in sterile distilled water and used as positive controls. Solvent and media controls were also included for reference. A series of twofold dilutions of each agent were prepared in yeast malt broth in a 96 wells microdilution tray using fluconazole as reference compound. Each well was inoculated with a final concentration of approximately $1 \cdot 10^3$ to $3 \cdot 10^3$ cfu/ml as confirmed by viable counts. Microdilution trays were incubated for 48 h at 30° and then the growth was monitored visually. The minimum inhibitory concentration was defined as the lowest concentration required to arrest the growth of the fungi at the end of 48 h of incubation at 30°C. Each isolate was tested at least twice in duplicate [12].

Acknowledgements.

This work was supported by a grant of the Ministero dell'Istruzione, dell'Università e della Ricerca (M.I.U.R.), Rome.

REFERENCES AND NOTES

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Table 5

Antifungal and Antibacterial Activity of Compounds **3** against Fungi, Gram-positive and Gram-negative Bacteria (MIC, μg/ml)

	Bacteria								Fungi			
	Gram positive					Gram negative						
N°	*B.subt.* *ATCC* 6633	*B.cer.* *ATCC* 11778	*S.aur.* *ATCC* 29213	*S.aur.* *ATCC* 6538p	*E.faec.* *ATCC* 29212	*E.coli* *ATCC* 8739	*E.coli* *ATCC* 25922	*P.aerug* *ATCC* 27853	*C.alb* ATCC 10231	*C.alb.* *ATCC* 14053	*C.alb.* NRRL Y869	*C.alb.* NRRL Y12983
3a	15.6	15.6	62.5	62.5	62.5	250		250	125	125	62.5	125
3b	125	250	250	62.5	250	----	----	----	----	----	----	----
3c	125	125	125	62.5	125	250	250	250	250	250	250	250
3d	15.6	15.6	62.5	62.5	62.5	250		250	125	125	62.5	125
3e	----	----	----	----	----	----		----	250	250	250	250
3f	----	----	----	----	----	----		----	31.2	15.6	31.2	31.2
3g	125	250	125	125	250	250	250	----	250	250	125	250
3h	125	250	125	125	125	----	----	250	62.5	62.5	125	62.5
3i	125	----	125	125	250	----	250	----	250	250	250	250
3j	125	125	125	62.5	125	250	250	250	250	250	250	250
3k	125	125	125	62.5	125	250	125	----	**---**	**---**	**---**	**---**
3m	250	250	125	125	----	----	250	----	62.5	62.5	62.5	62.5
3n	125	----	125	125	----	----	----	----	62.5	62.5	62.5	62.5
3o	125	250	125	125	250	----	250	----	---	---	---	---
3p	125	125	62.5	62.5	----	----	----	----	----	----	----	----
3q	125	125	125	62.5	125	----	----	----	----	----	----	---
3r	125	125	62.5	62.5	62.5	----	----	----	----	----	----	----
3s	250	250	125	125	----	----	----	----	250	125	125	125
3t	125	125	125	125	250	250	250	250	125	125	125	125
3u	125	125	125	125	125	----	----	----	----	----	----	----
3w	62.5	62.5	62.5	62.5	125	125	125	250	----	----	----	----
a	0.5	0.25	1	0.5	1	2		2				
b									0.5	0.5	1	1
c									250	500	500	250

Reference compounds: a = Norfloxacine, b = Fluconazole, c = Sorbic acid; "----" = MIC > 250 μg/ml.

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** For 8-Cl there are four compounds with the highest yield (*i.e.* **3c** (63.5%), **3f** (68.5%), **3i** (5.5%), **3u** (49.4%)) that are derived from reagents I°, II°, III° and VII° respectively. For 6-Cl there are three compounds with the highest yield (*i.e.* **3k** (71.2%), **3n** (84,4%), **3w** (35,9%)) that are derived from reagents IV°, V°, VIII°. For 5-Cl there is one only compound with the highest yield (*i.e.* **3p** (47,9%) that is derived from reagent VI°.

*** The average yields into each of the three isomers is about 50% for reagent II°, 47-40% in decreasing order for reagents VII°, V° and I°, 31-17% for reagents IV°, VI° and VIII°, and finally 4% for reagent III°.

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