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Original article

Synthesis, antibacterial and antifungal activities of several new benzo- naphtho- and quinolino-1,4-thiazine and 1,5-thiazepine derivatives*

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Summary — The synthesis of a number of thiosemicarbazone, phenylthiosemicarbazone, oxime and oxime *O*-ester derivatives of benzo- naphtho- and quinolino-1,4-thiazines and 1,5-thiazepines is described. All the compounds were tested *in vitro* for their antimicrobial activity. Compounds **4b, 5b, 5d, 5g** and **8f** showed interesting antifungal activity.

Résumé — Synthèse, activité antibactérienne et antifongique de plusieurs nouveaux dérivés benzo-, naphtho- et quinoléino-1,4-thiazines et 1,5-thiazépines. *Plusieurs dérivés, thiosemicarbazones, phénylthiosemicarbazones, oximes et O-acyloximes, ont été synthétisés et leur activité antimicrobienne a été évaluée. Cinq substances **4b, 5b, 5d, 5g** et **8f** se sont montrées actives vis-à-vis de certaines souches fongiques.*

thiosemicarbazones / oximes / *O*-acyloximes / benzo-, naphtho-, quinolino-1,4-thiazines and -1,5-thiazepines / antimicrobial activity

Introduction

The pharmacological importance of substituted 1,4-benzothiazines and 1,5-benzothiazepines and their annelated derivatives has been well established [1–6]. Some derivatives are also of interest as antimicrobials [7, 8].

Recently, the antiviral, antibacterial and/or antifungal activities of some compounds containing either the thiosemicarbazone [9] or the hydroxyimino group [10, 11] have been reported. These findings prompted us to continue our work on 1,4-benzothiazine and 1,5-benzothiazepine systems [12–14] and we now describe the synthesis of a number of their derivatives containing the above functionalities and therefore with potential antibacterial and/or antifungal activity.

Chemistry

As starting material we used benzo- naphtho- and quinolino-1,4-thiazine-3(4H)-thione and -1,5-thiazepine-4(5H)-thione derivatives [12, 14–18], whose thiolactame function is more reactive towards nucleophilic reagents. They were prepared in excellent yields by reacting the corresponding thiazinones or thiazepinones with the Lawesson's reagent, as described recently by us [12, 14, 19].

Compounds **3a–s** were easily and conveniently obtained using the methylthiolactime ethers **2a–m** as intermediates; the thiones were alkylated by an improved procedure [12] and the methyl derivatives were then refluxed for 3–4 h with thiosemicarbazide or 4-phenylthiosemicarbazide in ethanol to afford compounds **3a–s** and **8a, b**.

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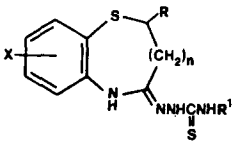
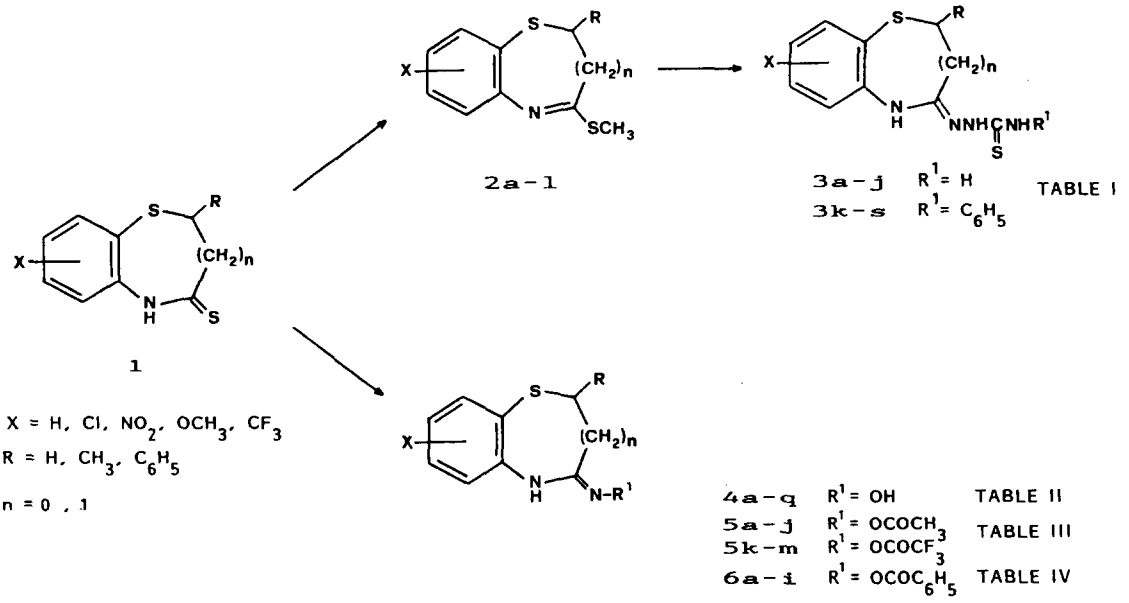
The same thiones were also reacted with hydroxylamine hydrochloride to give the oximes **4a–q** (table II) and **8c, 8d, 8g** (table V) which were then acylated to yield the following series of compounds **5a–j, 5k–m, 6a–i** and **8e, f** whose physico-chemical properties are summarized in tables III, IV and V.

Table I. Physical data of derivatives **3a–s**.

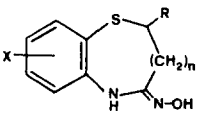


Compd	*X*	*R*	*R'*	*n*	*Yield* %	*Mp* °C	*Colour-Cryst form Recryst solvent*	*Formula*	*MW*
3a	H	H	H	0	69	190–191	white prisms MeOH	$C_9H_{10}N_4S_2$	238
3b	6Cl	H	H	0	79	188–192 dec	white prisms EtOH	$C_9H_9ClN_4S_2$	272
3c	$6NO_2$	H	H	0	70	221–222	yellow prisms MeOH	$C_9H_9N_5O_2S_2$	283
3d	7Cl	H	H	0	81	204–206	yellow plates MeOH	$C_9H_9ClN_4S_2$	272
3e	H	H	H	1	75	183–185	white cubes EtOH	$C_{10}H_{12}N_4S_2$	252
3f	7Cl	H	H	1	30	162–166	white prisms MeOH	$C_{10}H_{11}ClN_4S_2$	286
3g	8Cl	H	H	1	72	195–197	white cubes MeOH	$C_{10}H_{11}ClN_4S_2$	286
3h	H	CH_3	H	1	84	190–191	white cubes MeOH	$C_{11}H_{14}N_4S_2$	266
3i	7Cl	CH_3	H	1	77	180–182	yellow needles MeOH	$C_{11}H_{13}ClN_4S_2$	300
3j	H	C_6H_5	H	1	82	185–187	white cubes EtOH	$C_{16}H_{16}N_4S_2$	328
3k	H	H	C_6H_5	0	79	197–199	white prisms MeOH	$C_{15}H_{14}N_4S_2$	314
3l	6Cl	H	C_6H_5	0	43	264-266	white prisms EtOH	$C_{15}H_{13}ClN_4S_2$	348
3m	$6NO_2$	H	C_6H_5	0	72	186–189	gold yellow prisms EtOH	$C_{15}H_{13}N_5O_2S_2$	359
3n	7Cl	H	C_6H_5	0	75	202–204	white prisms MeOH	$C_{15}H_{13}ClN_4S_2$	348
3o	$7NO_2$	H	C_6H_5	0	54	193–195	dark yellow prisms MeOH	$C_{15}H_{13}N_5O_2S_2$	359
3p	H	C_6H_5	C_6H_5	0	42	183–185	yellow cubes EtOH	$C_{21}H_{18}N_4S_2$	390
3q	H	H	C_6H_5	1	77	173–176	white prisms EtOH	$C_{16}H_{16}N_4S_2$	328
3r	7Cl	H	C_6H_5	1	81	182–185	yellow prisms MeOH	$C_{15}H_{15}ClN_4S_2$	362
3s	H	C_6H_5	C_6H_5	1	99	185–189	white plates MeOH	$C_{22}H_{20}N_4S_2$	404



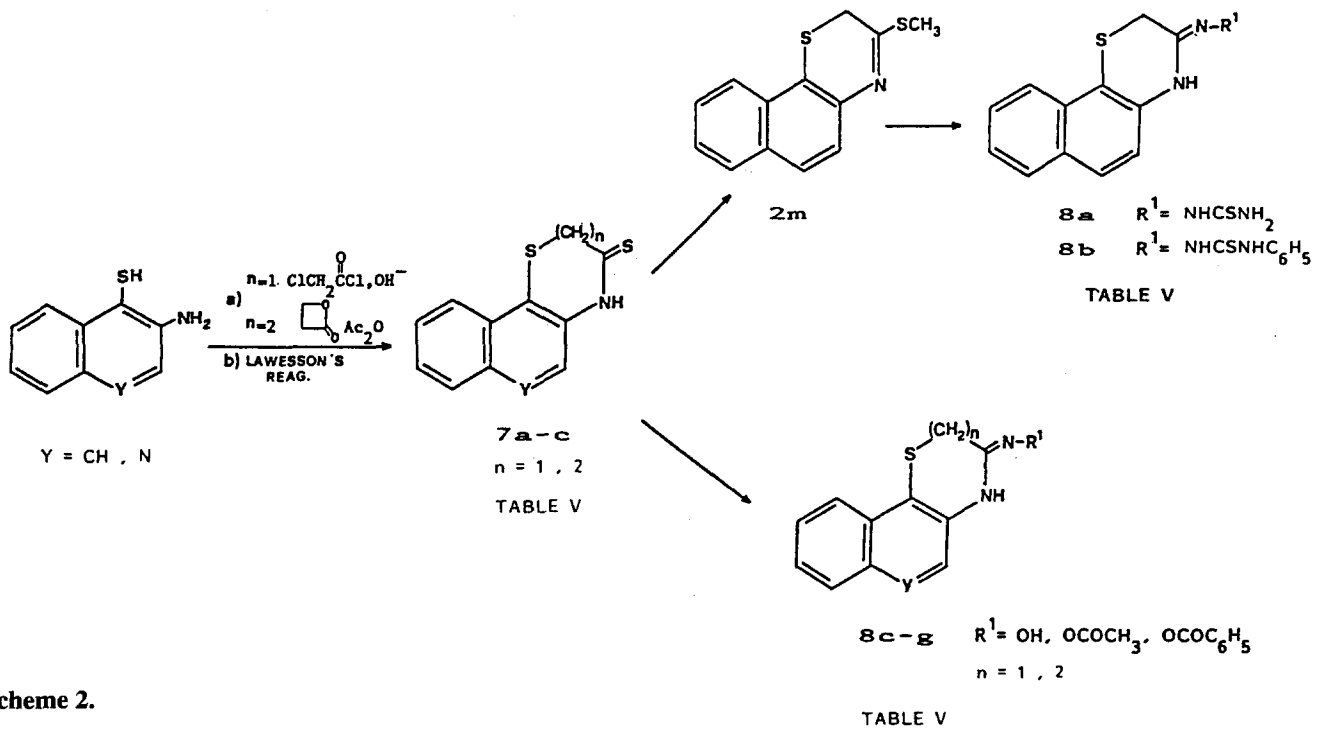
Scheme 1.

Table II. Physical data of derivatives **4a–q**.



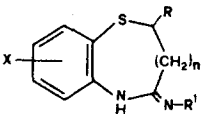
Compd	*X*	*R*	*n*	*Yield* %	*Mp* °C	*Colour-Cryst form* [a]	*Formula*	*MW*
4a	H	H	0	98	159–162	white prisms	$C_8H_8N_2OS$	180
4b	6Cl	H	0	99	243–246	white prisms	$C_8H_7ClN_2OS$	214
4c	$6OCH_3$	H	0	80	195–198	pale yellow prisms	$C_9H_{10}N_2O_2S$	210
4d	$6NO_2$	H	0	77	202–205	gold yellow needles	$C_8H_7N_3O_3S$	225
4e	$6CF_3$	H	0	53	116–119	white cubes	$C_9H_7F_3N_2OS$	248
4f	7Cl	H	0	48	184–187	pale yellow prisms	$C_8H_7ClN_2OS$	214
4g	$7NO_2$	H	0	83	150–153	gold yellow prisms	$C_8H_7N_3O_3S$	225
4h	H	C_6H_5	0	86	181–184	yellow prisms	$C_{14}H_{12}N_2OS$	256
4i	H	H	1	83	195–196	white needles	$C_9H_{10}N_2OS$	194
4j	7Cl	H	1	94	237–239	white prisms	$C_9H_9ClN_2OS$	228
4k	$7CF_3$	H	1	79	228–230	white prisms	$C_{10}H_9F_3N_2OS$	262
4l	8Cl	H	1	53	206–208	white needles	$C_9H_9ClN_2OS$	228
4m	$8OCH_3$	H	1	68	213–215	pale yellow needles	$C_{10}H_{12}N_2O_2S$	224
4n	H	CH_3	1	85	147–149	white prisms	$C_{10}H_{12}N_2OS$	208
4o	7Cl	CH_3	1	87	161–164	white prisms	$C_{10}H_{11}ClN_2OS$	242
4p	8Cl	CH_3	1	40	123–126	yellow prisms	$C_{10}H_{11}ClN_2OS$	242
4q	H	C_6H_5	1	69	184–187	white prisms	$C_{15}H_{14}N_2OS$	270

[a]With the exception of **4p, 4q** (ethyl acetate) and **4j** (dioxane: MeOH 1:1) all the other compounds were recrystallized from EtOH.



Scheme 2.

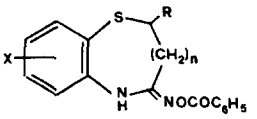
Table III. Physical data of derivatives **5a–m**.



Compd	*X*	*R*	*R1*	*n*	*Yield %*	*Mp °C*	*Colour-Cryst form* [a]	*Formula*	*MW*
5a	H	H	$OCOCH_3$	0	99	171–173	white prisms	$C_{10}H_{10}N_2O_2S$	222
5b	6Cl	H	$OCOCH_3$	0	84	203–205	white needles	$C_{10}H_9ClN_2O_2S$	256
5c	7Cl	H	$OCOCH_3$	0	89	172–174	orange plates	$C_{10}H_9ClN_2O_2S$	256
5d	H	C_6H_5	$OCOCH_3$	0	52	146–149	yellow prisms	$C_{16}H_{14}N_2O_2S$	298
5e	H	H	$OCOCH_3$	1	99	168–171	white prisms	$C_{11}H_{12}N_2O_2S$	236
5f	7Cl	H	$OCOCH_3$	1	85	221–223	white needles	$C_{11}H_{11}ClN_2O_2S$	270
5g	8Cl	H	$OCOCH_3$	1	86	177–179	white needles	$C_{11}H_{11}ClN_2O_2S$	270
5h	H	CH_3	$OCOCH_3$	1	83	184–187	white prisms	$C_{12}H_{14}N_2O_2S$	250
5i	7Cl	CH_3	$OCOCH_3$	1	86	186–188	white needles	$C_{12}H_{13}ClN_2O_2S$	284
5j	H	C_6H_5	$OCOCH_3$	1	87	173–175	white needles	$C_{17}H_{16}N_2O_2S$	312
5k	H	H	$OCOCF_3$	0	60	166–168 dec	pink prisms	$C_{10}H_7F_3N_2O_2S$	276
5l	H	C_6H_5	$OCOCF_3$	0	43	145–148	yellow prisms	$C_{16}H_{11}F_3N_2O_2S$	352
5m	H	C_6H_5	$OCOCF_3$	1	74	207–210	yellow prisms	$C_{17}H_{13}F_3N_2O_2S$	366

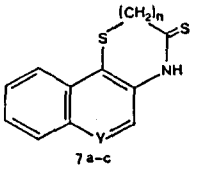
[a]All the compounds were recrystallized from EtOH.

Table IV. Physical data of derivatives **6a–i.**

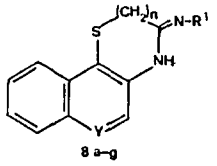


Compd	*X*	*R*	*n*	*Yield* %	*Mp* °C	*Colour-Cryst form Recryst solvent*	*Formula*	*MW*
6a	H	H	0	86	185–187	dark yellow prisms EtOH	$C_{15}H_{12}N_2O_2S$	284
6b	6Cl	H	0	99	199–201	pink prisms EtOH	$C_{15}H_{11}ClN_2O_2S$	318
6c	7Cl	H	0	99	195–197	pink prisms MeOH	$C_{15}H_{11}ClN_2O_2S$	318
6d	H	H	1	92	122–125	light yellow prisms EtAc	$C_{16}H_{14}N_2O_2S$	298
6e	7Cl	H	1	99	127–129	yellow prisms EtOH	$C_{16}H_{13}ClN_2O_2S$	332
6f	8Cl	H	1	99	186–188	dark yellow cubes EtOH	$C_{16}H_{13}ClN_2O_2S$	332
6g	H	CH_3	1	85	130–132	dark yellow prisms EtAc	$C_{17}H_{16}N_2O_2S$	312
6h	7Cl	CH_3	1	88	142–145	yellow cubes EtAc	$C_{17}H_{15}ClN_2O_2S$	346
6i	H	C_6H_5	1	99	184–186	yellow cubes EtAc	$C_{22}H_{18}N_2O_2S$	374

Table V. Physical data of derivatives **7a–c** and **8a–g.**



7 a–c



8 a–g

Compd	*Y*	*n*	*R¹*	*Yield* %	*Mp* °C	*Colour-Cryst form* [a]	*Formula*	*MW*
7a	CH	1	–	90	208–211 dec	yellow needles	$C_{12}H_9NS_2$	231
7b	CH	2	–	84	197–200	yellow needles	$C_{13}H_{11}NS_2$	245
7c	N	1	–	93	203–205	white prisms	$C_{11}H_8N_2S_2$	232
8a	CH	1	$NHCSNH_2$	91	207–210	light yellow needles	$C_{13}H_{12}N_4S_2$	288
8b	CH	1	$NHCSNHC_6H_5$	68	234–237	white prisms	$C_{19}H_{16}N_4S_2$	364
8c	CH	1	OH	79	180–183	pale yellow prisms	$C_{12}H_{10}N_2OS$	230
8d	CH	2	OH	52	213–215	pale yellow needles	$C_{13}H_{12}N_2OS$	244
8e	CH	1	$OCOCH_3$	85	186–188	pink prisms	$C_{14}H_{12}N_2O_2S$	272
8f	CH	1	$OCOC_6H_5$	86	111–113	violet prisms	$C_{19}H_{14}N_2O_2S$	334
8g	N	1	OH	51	215–218	pale yellow prisms	$C_{11}H_9N_3OS$	231

[a]All the compounds were recrystallized from EtOH.

Since acylation could occur either at the nuclear nitrogen or at the oxime oxygen, we performed a substitution reaction (methylation) on oximes **4a, 4h, 4i** and **4q** in order to evaluate the preferred targeted site.

We have indeed shown, by spectroscopic and chemical evidences, that the substitution reaction occurred exclusively and regioselectively at the oxime oxygen atom. In fact, the site of methylation follows from either the permanence, in the PMR spectra (DMSO–d_6 + 10% $CDCl_3$) of the alkylated compounds **9a–d**, of the signal (broad singlet) at δ 8.2–9.5 attributed to the NH group, and the disappearance of the peak relative to the OH of the hydroxyimino group (sharp singlet at δ 9.3–10.1). Moreover, structures **9a–d** were independently confirmed by preparing the isomers **12a–d**, as shown in scheme 3. The most characteristic PMR (DMSO–d_6 + 10% $CDCl_3$) peaks of the **12a–d** were the singlet at δ 3.2–3.3 due to the CH_3 and the singlet at δ 9.3–9.8 relative to the OH group.

The other significant peaks were the singlet at δ 3.3–3.6 due to the CH_2 group of the thiazine-derivatives and the A_2B_2 system at δ 2.3–2.6 and 3.1–3.4 corresponding to the CH_2CH_2 group of the thiazepine-compounds.

Biological investigation and results

All the compounds tested *in vitro* for their antibacterial activity against Gram-positive and Gram-negative bacteria exhibited only weak or no activity at all against the test organisms.

On the contrary, some compounds showed a significant antifungal activity especially against *Candida krusei* CBS 1910 and *Cryptococcus neoformans* IMAT 4711 at a similar concentration to that of Nystatin used as the reference compound (table VI).

These results are not sufficient for an accurate evaluation of the structure-activity relationship, but they show that the oxime derivative **4b**, the acetyloxyimino compounds **5b, 5d, 5g** and the benzoyloxyimino derivative **8f** might be of interest for the development of antimycotic derivatives against some fungal species.

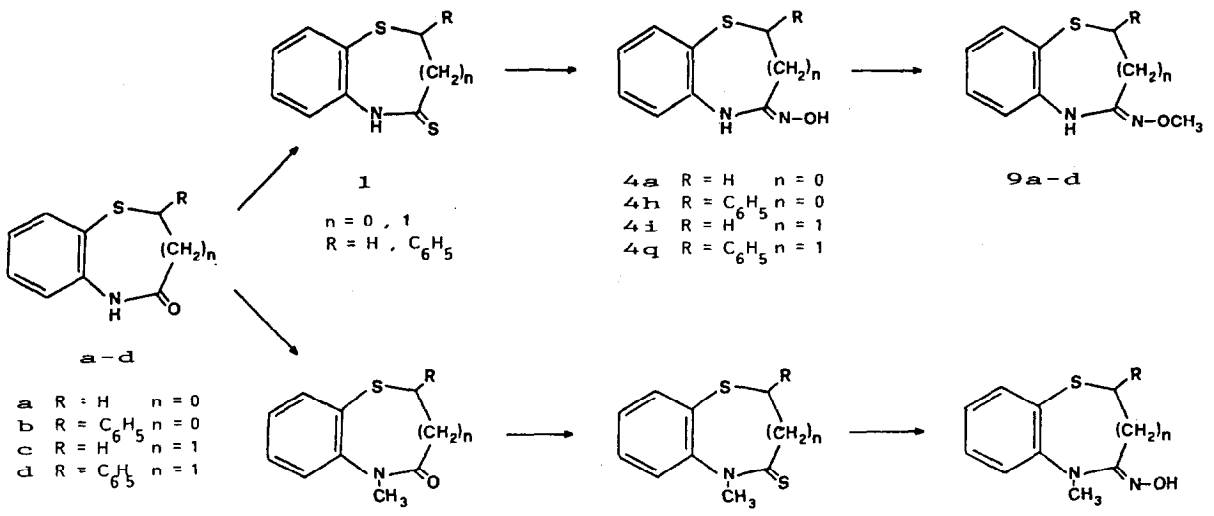
Experimental protocols

Chemical synthesis

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. The PMR spectra were recorded with a Varian EM-390 (90 MHz) instrument in the solvents indicated. The chemical shift values in δ (ppm) are relative to tetramethylsilane as an internal standard. Mass spectra were measured with a LKB 2091 spectrometer at 70 eV. Elemental analyses were carried out with a Carlo Erba Elemental Analyzer mod 1106 and all the new compounds gave satisfactory analytical results (within ±0.4% of the theoretical values). Precoated Kieselgel 60 F254 plates from Merck were used for TLC controls.

Thiones 1,7a–c and 11a–d

Thiones **1** [12, 14–16, 18], **11a** [20] and **11b–d** were prepared by the method described in ref [12].



Scheme 3.

Table VI. *In vitro* antimycotic activity (MIC values μg/ml).

Fungi	Compound														
	4b	***4q***	***5b***	***5d***	***5g***	***6d***	***6f***	***6e***	***6g***	***8b***	***8d***	***8e***	***8f***	*nystatin*	*nalidixic ac*
Candida utilis ISS 4870	>100	>100	10	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	2	>100
Candida albicans CBS 562	>100	>100	25	>100	50	>100	>100	>100	>100	50	>100	>100	>100	1	>100
Candida tropicalis IMAT 5711	>100	>100	25	>100	50	>100	>100	>100	>100	>100	>100	50	>100	1	>100
Candida guilliermondii IMAT 5313	>100	>100	5	>100	5	50	50	>100	>100	>100	>100	50	>100	< 2	>100
Candida krusei CBS 1910	5	50	5	10	5	>100	>100	>100	>100	>100	>100	>100	5	1	25
Cryptococcus laurentii IMAT 4688	50	50	25	50	50	>100	>100	>100	>100	>100	>100	>100	50	2	>100
Cryptococcus neoformans IMAT 4711	10	50	10	10	25	>100	50	50	50	50	50	50	25	1	50
Geotrichum candidum ISS 1214	6	50	25	25	25	50	50	50	50	50	>100	50	5	< 2	>100

ISS (Ist Sup Sanità, Roma); IMAT (Ist di Microbiologia Agraria, PG); CBS (Centraalbureau voor Shimmelcultures, Baarn, NL).

Compounds **7a–c*** were prepared according to the same procedure and their physical data are reported in table V.

2-*Phenyl*-4-*methyl*-2H-1,4-*benzothiazin*-3(4H)-*thione* **11b** (R = C_6H_5, *n* = 0): yield 75%, mp 109–110°C (ethanol), yellow plates, $C_{15}H_{13}NS_2$, MW 271.

5-*Methyl*-2,3-*dihydro*-1,5-*benzothiazepin*-4(5H)-*thione* **11c** (R = H, *n* = 1): yield 75%, mp 74–75°C (ethanol), white needles, $C_{10}H_{11}NS_2$, MW 209.

2-*Phenyl*-5-*methyl*-2,3-*dihydro*-1,5-*benzothiazepin*-4(5H)-*thione* **11d** (R = C_6H_5, *n* = 1): yield 84%, mp 110–112°C (ethanol), white prisms, $C_{16}H_{15}NS_2$, MW 285.

The most characteristic PMR ($CDCl_3$) peak was the singlet at δ 3.7–4 due to the NCH_3.

*Methylthioderivatives **2a–m***

Methylthioderivatives **2a–e** [12], **2f** [18], **2l** [15] and **2g, h–k, m** were prepared by the method described in ref [12]. The crude products were employed without further purification.

4-*Methylthio*-2,3-*dihydro*-1,5-*benzothiazepine* **2g** (X = H, R = H, *n* = 1): yield 95%, mp 50–51°C (petroleum ether 50–70°), yellow prisms, $C_{10}H_{11}NS_2$, MW 209.

4-*Methylthio*-7-*chloro*-2,3-*dihydro*-1,5-*benzothiazepine* **2h** (X = 7-Cl, R = H, *n* = 1): yield 87%, mp 55–57°C (ethanol), yellow prisms, $C_{10}H_{10}ClNS_2$, MW 243,5.

4-*Methylthio*-8-*chloro*-2,3-*dihydro*-1,5-*benzothiazepine* **2i** (X = 8-Cl, R = H, *n* = 1): yield 80%, mp 53–54°C (ethanol), yellow prisms, $C_{10}H_{10}ClNS_2$, MW 243,5.

2-*Methyl*-4-*methylthio*-2,3-*dihydro*-1,5-*benzothiazepine* **2j** (X = H, R = CH_3, *n* = 1): yield 82%, yellow oil, $C_{11}H_{13}NS_2$, MW 223.

2-*Methyl*-4-*Methylthio*-7-*chloro*-2,3-*dihydro*-1,5-*benzothiazepine* **2k** (X = 7-Cl, R = CH_3, *n* = 1): yield 87%, mp 67–70°C (ethanol), yellow prisms, $C_{11}H_{12}ClNS_2$, MW 257,5.

3-*Methylthio*-2H-*naphtho*[1,2-b]-1,4-*thiazine* **2m** (scheme 2): yield 97%, yellow oil, $C_{13}H_{11}NS_2$, MW 245.

The most characteristic PMR ($CDCl_3$) peak of the methylthioderivatives **2a–m** was the singlet at δ 2.4–2.6 due to the SCH_3.

*Thiosemicarbazone derivatives **3a–j, 8a** and 4-phenylthiosemicarbazone derivatives **3k–s, 8b** (tables I and V)*

General procedure

A mixture of the methylthioderivative (3 mmoles), thiosemicarbazide or 4-phenylthiosemicarbazide (3 mmoles) and AcOH (3 drops) in dry ethanol (20 ml) were refluxed for *ca* 3–4 h. The resulting solution was cooled to room temperature. The separated crude product was filtered and purified by crystallization from a suitable solvent.

*Oxime derivatives **4a–q, 8c, 8d, 8g** (tables II and V) and **12a–d***

General procedure

Method A. A mixture of the thione (10 mmoles), hydroxylamine hydrochloride (15 mmoles) and sodium acetate (15 mmoles) in dry ethanol was refluxed for *ca* 40 min.

The mixture was cooled to room temperature, filtered and the solid washed with water. The crude product was purified by crystallization.

Method B. To a solution of the thione (5 mmoles) in dry pyridine (10 ml) hydroxylamine hydrochloride (10 mmoles) was added; the reaction mixture was heated on a steam bath for 1.5 h.

After cooling, the solution was poured into ice-water and the precipitate was filtered, washed and recrystallized.

Method B affords the hydroxyimino derivatives in poorer yields than method A.

*The synthetic procedures for the preparation of the intermediates 2-amino-1-thionaphthol, 3-amino-4-mercaptoquinoline and their thiazino and thiazepino tricyclic derivatives will be referred in a separate, forthcoming paper.

3-*Hydroxyimino*-4-*methyl*-2,4-*dihydro*-1,4-*benzothiazine* **12a** (R = H, *n* = 0): yield 30%, mp 108–110°C (ethanol), pink prisms, $C_9H_{10}N_2OS$, MW 194.

2-*Phenyl*-3-*hydroxyimino*-4-*methyl*-2,4-*dihydro*-1,4-*benzothiazine* **12b** (R = C_6H_5, *n* = 0): yield 30%, mp 194–196°C (ethanol), white prisms, $C_{15}H_{14}N_2OS$, MW 270.

4-*Hydroxyimino*-5-*methyl*-2,3-*dihydro*-5H-1,5-*benzothiazepine* **12c** (R = H, *n* = 1): yield 25%, mp 115–118°C (ethanol), yellow prisms, $C_{10}H_{12}N_2OS$, MW 208.

2-*Phenyl*-4-*hydroxyimino*-5-*methyl*-2,3-*dihydro*-5H-1,5-*benzothiazepine* **12d** (R = C_6H_5, *n* = 1): yield 25%, mp 184–188°C (ethanol), light yellow needles, $C_{16}H_{16}N_2OS$, MW 284.

The corresponding starting thiones **11a–d** (*ca* 50%) were usually recovered.

*Acetyloxyimino esters **5a–j, 8e** (tables III and V)*

General procedure. Freshly distilled acetic anhydride (2.5 ml) was added to a suspension of the oxime (0.5 g) in dry pyridine (3 ml). The reaction mixture was kept at room temperature for *ca* 15 min and the precipitated product was filtered and purified by crystallization.

*Trifluoracetyloxyimino esters **5k–m** (table III)*

General procedure. Trifluoroacetic anhydride (3 ml) was added, dropwise and with cooling, to a suspension of the oxime (0.5 g) in dry pyridine (3 ml).

The reaction mixture was poured into ice-water and the precipitated product was filtered and recrystallized.

*Benzoyloxyimino esters **6a–i, 8f** (tables IV and V)*

General procedure. To a solution of the oxime (30 mmoles) in dry benzene (30 ml), benzoyl chloride (30 mmoles) and dry pyridine (3 drops) were added.

The reaction mixture was refluxed for 2 h. After cooling, the solvent was evaporated *in vacuo* and the residue, taken up with chloroform, was washed with water, dried over sodium sulfate and brought to dryness *in vacuo*. The residue was induced to crystallize by adding small amounts of ethylacetate and the crude product was then recrystallized.

*Methyloxyimino derivatives **9a–d***

General procedure. Dimethylsulfate (10 mmoles) was added dropwise to a vigorously stirred suspension of the oxime (10 mmoles) and 10% sodium hydroxide (10 mmoles) in ethanol (5 ml). After a few minutes the methyl derivative crystallized. The reaction mixture was kept at room temperature for *ca* 15 min, then the crude product was filtered, washed first with a solution of sodium hydroxide (10%) and then with water, purified by chromatography, using chloroform as eluent and finally recrystallized.

3-*Methyloxyimino*-2,4-*dihydro*-1,4-*benzothiazine* **9a** (R = H, *n* = 0): yield 45%, yellow oil, $C_9H_{10}N_2OS$, MW 194.

2-*Phenyl*-3-*methyloxyimino*-2,4-*dihydro*-1,4-*benzothiazine* **9b** (R = C_6H_5, *n* = 0): yield 35%, mp 114–117°C (ethanol), white needles, $C_{15}H_{14}N_2OS$, MW 270.

4-*Methyloxyimino*-2,3-*dihydro*-5H-1,5-*benzothiazepine* **9c** (R = H, *n* = 1): yield 37%, mp 110-112°C (ethanol), white prisms, $C_{10}H_{12}N_2OS$, MW 208.

2-*Phenyl*-4-*methyloxyimino*-2,3-*dihydro*-5H-1,5-*benzothiazepine* **9d** (R = C_6H_5, *n* = 1): yield 40%, mp 105–110°C (ethanol), light yellow prisms, $C_{16}H_{16}N_2OS$, MW 284.

The corresponding starting oximes **4a, 4h, 4i** and **4q** (*ca* 40%) were usually recovered.

The most characteristic PMR (DMSO–d_6 + 10% $CDCl_3$) peaks of **9a–d** were the broad singlet at δ 8.2–9.5 attributed to the NH group and the sharp singlet at δ 3.7–3.8 due to the OCH_3.

N–*Methyl derivatives **10a–d** [18, 20, 21, 22]*

General procedure. To a stirred solution of the benzothiazinone or benzothiazepinone (10 mmoles) (scheme 3), tetrabutylammonium bromide (1 mmole) and methyliodide (10 mmoles) in tetrahydrofuran (10 ml), finely powdered potassium hydroxide (10 mmoles) was added.

The reaction mixture was kept at room temperature under stirring for 3 h and then filtered. The filtrate was evaporated under reduced pressure and the residue was taken up with chloroform. This solution was washed with water, dried over sodium sulfate and brought to dryness *in vacuo*. The crude product was recrystallized from a suitable solvent.

5-*Methyl*-2,3-*dihydro*-1,5-*benzothiazepin*-3(5H)-*one* **10c** (R = H, *n* = 1): yield 80%, mp 89–92°C (ethanol), white prisms, $C_{10}H_{11}NOS$, MW 193.

The most characteristic PMR (DMSO–d_6 + 10% $CDCl_3$) peak was the singlet at δ 3.2 due to the CH_3.

In vitro *antimicrobial assays*

All the products synthesized to be tested for the antibacterial and antimycotic activities showed limited solubility in water; therefore they were suspended in either dimethylsulfoxide (DMSO) or PEG 400 and then diluted with distilled water. A preliminary screening was conducted against a panel of Gram-positive and Gram-negative bacteria as well as strains of fungi. Agar plates with the compounds included as an ingredient were used. Strains were inoculated with a multi-point inoculator. Those compounds with significant activity were successively tested against the following strains:

Gram-positive: *Bacillus subtilis* ICI, *Micrococcus luteus* 9341, *Bacillus subtilis* var *niger*, *Bacillus cereus* B43 1335, *Staphylococcus aureus*.

Gram-negative: *Pseudomonas aeruginosa* 6750, *Salmonella typhimurium*, *Proteus vulgaris*, *Escherichia coli* 982, *Pseudomonas fluorescens* C3.

Fungi: *Candida utilis* ISS 4870, *Candida albicans* CBS 562, *Candida tropicalis* IMAT 5711, *Candida guilliermondii* IMAT 5313, *Candida krusei* CBS 1910, *Cryptococcus laurentii* IMAT 4688, *Cryptococcus neoformans* IMAT 4711, *Geotrichum candidum* ISS 1214.

The strains used come from 3 different collections: IMAT (Istituto di Microbiologia Agraria e Tecnica, University of Perugia, Italy), ISS (Istituto Superiore di Sanità, Rome, Italy), CBS (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands).

The minimum inhibitory concentration (MIC) values (μg/ml) were determined using a multi-point inoculator on nutrient agar prepared with progressively increasing concentrations of each compound. Inocula were prepared from cultures incubated at 37°C for 48 h in nutrient broth (Gram-positive and Gram-negative bacteria) or Sabouraud broth (Fungi).

Precultures were centrifuged, washed twice in sterile water, resuspended in sterile water and colorimetrically calibrated.

MIC values were determined after 48 h incubation at 37°C against positive controls of Cephaloridine for Gram-positive bacteria, Nalidixic Acid for Gram-negative bacteria, Nystatin for fungi.

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