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Synthesis, antimicrobial and cytostatic activities of some derivatives of indolo[2,3-*b*]-1,5-benzothiazepine, a novel heterocyclic ring system*

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Summary — Some derivatives of indolo[2,3-*b*]-1,5-benzothiazepine, a novel heterocyclic ring system, and of its 5,6-dihydroderivative have been obtained by Fischer indolization of corresponding phenylhydrazones. All the tetracyclic compounds were studied for their antimicrobial and cytostatic activities and several of them showed good activity against Gram-positive bacteria and *Cryptococci*.

indolo[2,3-*b*]-1,5-benzothiazepine / antimicrobial activity / cytostatic activity / structure–activity relationship

Introduction

Over the years, the family of natural indole alkaloids has been a source of antineoplastic principles, and structural prototypes possessing the indole nucleus as a part of a polycyclic system have given rise to a number of useful drugs.

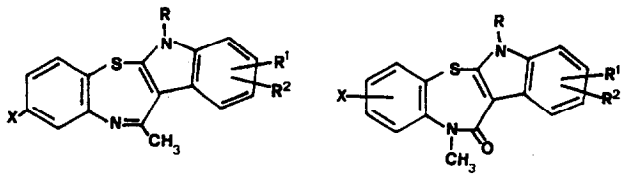
Therefore, as an extension of our previous investigations on 1,5-benzothiazepines [1, 2], it seemed interesting to synthesize derivatives of indolo[2,3-*b*]-1,5-benzothiazepine, a new heterocyclic ring system obtained by fusion of the indole nucleus with the 1,5-benzothiazepine system.

Thus, in this paper we report the synthesis and the preliminary results of *in vitro* antimicrobial and cytostatic activities of some derivatives of the indolo[2,3-*b*]-1,5-benzothiazepine **A** and of its 5,6-dihydroderivative **B**. Moreover, the data obtained allow us to make some comments on the structure–activity relationship.

Chemistry

The indolo[2,3-*b*]-1,5-benzothiazepines **A** and **B** were prepared by Fischer indolization of phenylhydrazone derivatives of the benzothiazepinones **1a, b** and **7d–f** respectively.

The 4-methyl-2,3-dihydro-1,5-benzothiazepin-2-one **1a** [3] and its 7-chloro derivative **1b** were synthesized as previously described [4] and both exist as a tautomeric mixture (5:4) (scheme 1). Such a mixture



A **B**

X = H, Cl

R = H, CH_3

R^1 = H, CH_3, Cl

R^2 = H, CH_3

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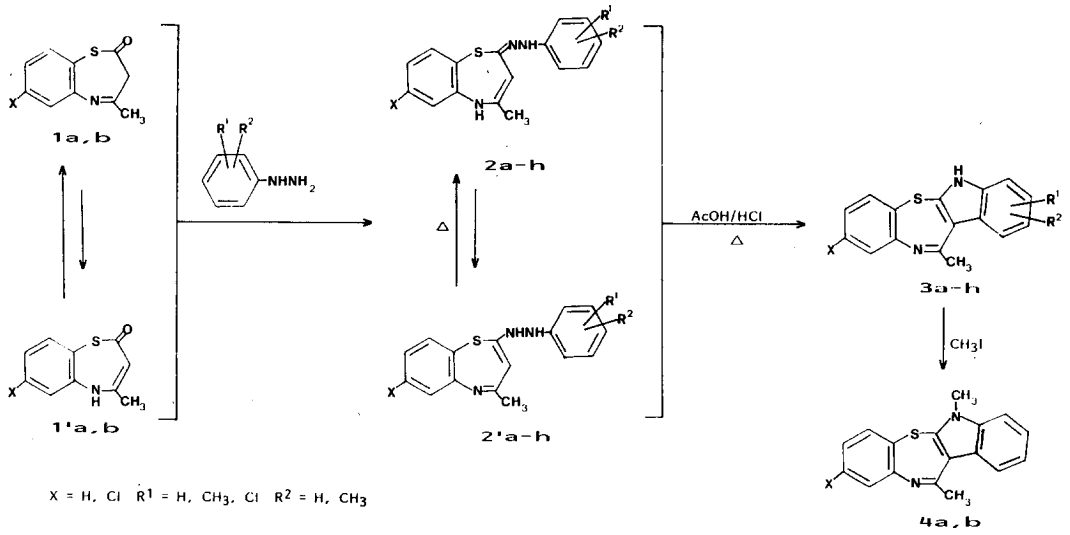
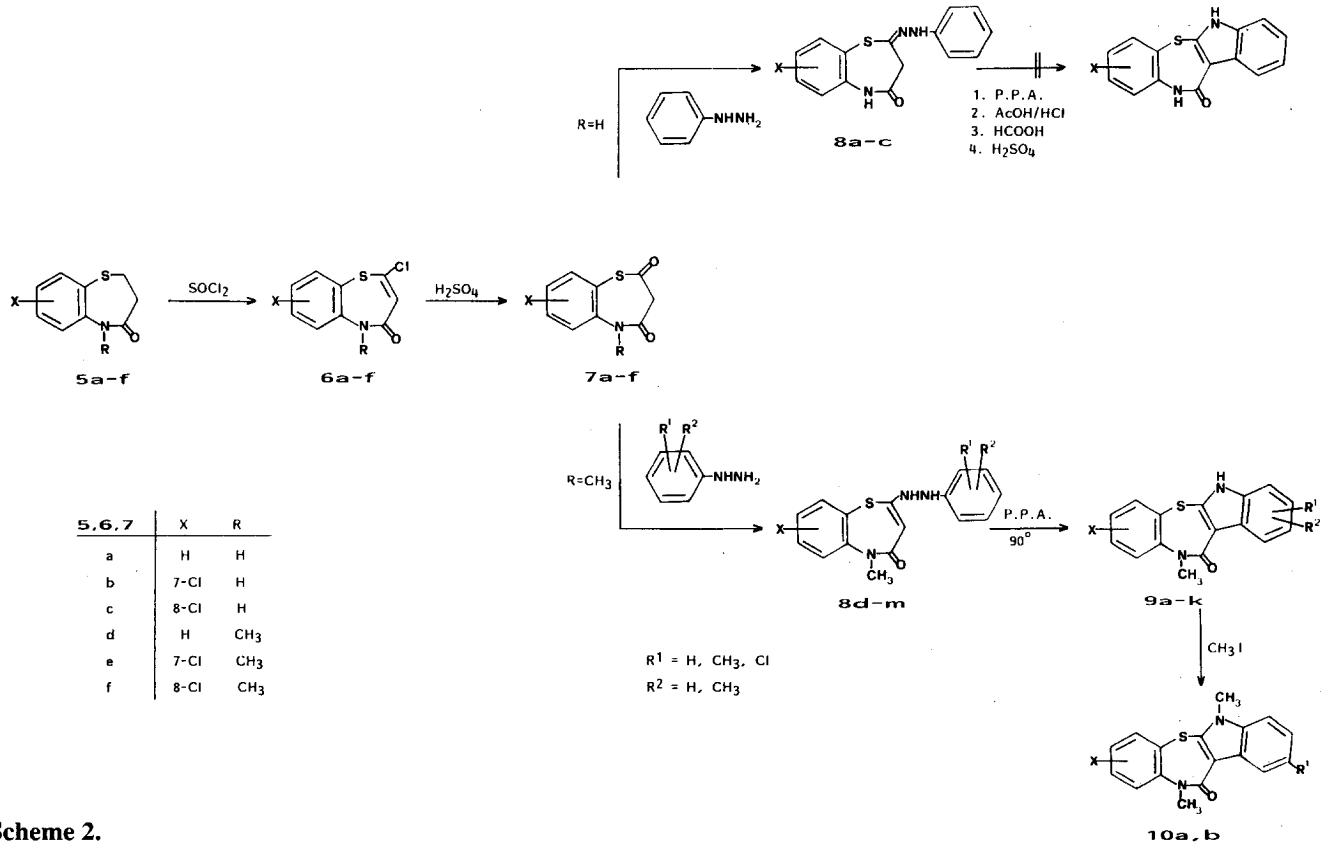


Scheme 1.



5,6,7	X	R
a	H	H
b	7-Cl	H
c	8-Cl	H
d	H	CH_3
e	7-Cl	CH_3
f	8-Cl	CH_3

Scheme 2.

was directly treated with the appropriate phenylhydrazine in anhydrous ethanol to afford the corresponding phenylhydrazones also obtained as a mixture of isomers (**2a–h** and **2'a–h**) as shown by ^{1}H-NMR spectra, in which the signal for the methylene group was absent. By heating, the mixture was resolved giving rise to a unique compound, the more stable 1,5-benzothiazepino derivative **2a–h**, as also confirmed by NOE experiments.

The desired indolobenzothiazepines **3a–h** (compounds **A**) were obtained, although in low yields, by refluxing **2a–h** with acetic acid saturated with hydrochloric acid. Several other attempts performed with various reagents *eg* formic acid or polyphosphoric acid in order to increase the yield, were unsuccessful (scheme 1).

The 1,5-benzothiazepin-2,4(3*H*,5*H*)-diones **7a–f** were the key intermediates for the synthesis of 5,6-dihydro-indolo[2,3-*b*]-1,5-benzothiazepines **B**. Compounds **7a–f** were prepared starting from the 1,5-benzothiazepin-4(5*H*)-ones **5a–c** [5] and their *N*-methyl derivatives **5d–f** [6], through the 2-chloroderivatives **6a–f**, properly modifying the reported reaction conditions [7].

Compounds **7a–f** were then heated in dry ethanol with phenylhydrazine or its benzosubstituted derivatives to give the phenylhydrazones **8a–m** in good yields. The structure of **8a–c** resulted from the presence in ^{1}H-NMR spectra either of a singlet at δ 4.16–4.20 for the methylene group and of a broad signal at *ca* 10.20 δ due to the amidic proton. In compounds **8d–m** such 2 signals were absent and, on the contrary, their ^{1}H-NMR spectra showed 2 singlets at δ 3.36–3.43 and δ 5.30–5.50 attributable to the *N*-methyl group and to the vinylic proton respectively. Accordingly, the IR spectra showed a band at *ca* 1660 cm^{-1} (amidic CO) for **8a–c** and a band at *ca* 1610 cm^{-1} (α,β unsaturated CO) for **8d–m**.

Numerous reaction conditions (*eg* acetic acid saturated with hydrochloric acid, formic acid, polyphosphoric acid or diluted sulphuric acid) were employed to perform the Fischer indole cyclization on compounds **8**. In case of phenylhydrazones **8a–c** all attempts proved futile; on the contrary, the *N*-methylated compounds **8d–m** cyclized easily in hot polyphosphoric acid to give the desired tetracyclic compounds **9a–k** (scheme 2).

It is noteworthy that the cyclization of **8** gave rise, as expected, to 2 indolobenzothiazepine isomers, **9c** and **9e**, while the phenylhydrazone **2c** cyclized only into the tetracycle **3c**, perhaps because of the hindrance exerted by the methyl at the 4-position.

Several indolobenzothiazepines were *N*-methylated by phase transfer catalysis (PTC) method to give **4a, b** and **10a, b**.

Biological results and discussion

All compounds were submitted to *in vitro* antimicrobial essays; all indolo[2,3-*b*]-1,5-benzothiazepines, with the exception of **3c**, **4b** and **10b** which showed too low solubility, were also tested for *in vitro* cytostatic activity.

Indolo[2,3-*b*]-1,5-benzothiazepines **A** displayed interesting antimicrobial activity. In fact compounds **3b, d, f, h** showed good activity against Gram-positive bacteria and some yeasts (MIC 3–12 μg/ml) whereas they were inactive against the tested Gram-negative bacteria (MIC ≥ 200 μg/ml).

From the reported data it is evident that the presence of a substituent on the aromatic ring of the indole nucleus greatly improves the antibacterial and antifungal activities, while methylation at the indolic nitrogen is unfavorable. No clear-cut biological conclusions can be drawn when a chloro substituent is present in the aromatic ring of the benzothiazepine system.

All intermediate products (**1, 2, 5, 6, 7**) and 5,6-dihydroindolo[2,3-*b*]-1,5-benzothiazepines **B** showed very weak if any antimicrobial activity (MIC ≥ 200 μg/ml) with the only exceptions of compounds **2h, 6f** and **8b**.

The most interesting data on the antimicrobial activity are given in table V.

The potential cytostatic activity of indolobenzothiazepines **A** and **B** was evaluated by testing these compounds *in vitro* against the growth of human KB tumor cells (table VI).

Several compounds showed moderate cytostatic activity, among them compound **3h** resulted the most active. However, from the reported data some observations can be drawn about structure–cytostatic effect relationship for both series of compounds **A** and **B**, as follows.

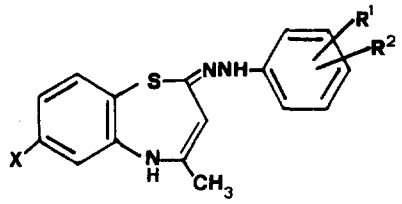
In indolo[2,3-*b*]-1,5-benzothiazepines **A** the presence of a substituent on the indolobenzothiazepine skeleton seems to be essential for the activity. The position of methyl groups on the indole ring plays a critical role. In fact, the introduction of one methyl group at 3 or 2 methyl groups at 1 and 4 positions remarkably raised the activity. On the other hand, 1- or 5-methyl substituted compounds resulted in inactivity.

The introduction of a chlorine atom either at the 3- or 8-position led to compounds which were more active than the unsubstituted **3a**; however, they were found to be poorly effective. The strongest activity was obtained when both the positions 3 and 8 were substituted by the chlorine (**3h**).

In 5,6-dihydro-indolo[2,3-*b*]-1,5-benzothiazepines **B** the structure–activity relationship led to conclusions similar to those reached about the other series **A**.

The presence of 1 or 2 methyl groups on the aromatic indolic ring at 2 or 3 or 4 or 1,4-positions

Table I. Physicochemical properties of derivatives **2a–h**.



Compd.	X	R^1	R^2	m.p. °C	Yield %	Recryst. solvent Colour - cryst.form	Formula
2a	H	H	H	127-128	80	EtOH Yellow prisms	$C_{16}H_{15}N_3S$
2b	H	2-CH_3	H	110-111	60	EtAc Yellow prisms	$C_{17}H_{17}N_3S$
2c	H	3-CH_3	H	---	51	oil	$C_{17}H_{17}N_3S$
2d	H	4-CH_3	H	143-144	34	EtOH Yellow prisms	$C_{17}H_{17}N_3S$
2e	H	2-CH_3	5-CH_3	---	68	oil	$C_{18}H_{19}N_3S$
2f	H	4-Cl	H	164	68	EtAc Yellow prisms	$C_{16}H_{14}ClN_3S$
2g	7-Cl	H	H	68-70	58	EtAc Yellow prisms	$C_{16}H_{14}ClN_3S$
2h	7-Cl	4-Cl	H	144-145	60	EtOH Yellow prisms	$C_{16}H_{13}Cl_2N_3S$

caused a significant enhancement in cytostatic activity, while the introduction of the methyl group at 1-position had a deleterious effect.

No clear conclusions can be drawn when a chloro substituent is present on the aromatic rings. The most active compound was the 8-chloro derivative whose activity was comparable to those of the methyl derivatives **9c–f**. On the contrary, the presence of a chlorine atom at other positions, as well as the contemporaneous presence of another substituent (methyl), led to less active compounds.

Experimental protocols

Chemistry

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with a Bruker AC-200 (200 MHz) instrument in the solvents indicated. The chemical shifts values in δ (ppm) are relative to tetramethylsilane as internal standard. IR spectra were detected in nujol using a Beckman Acculab TM5 spectrophotometer. UV spectra were measured in 95% ethanol solution on a Perkin–Elmer 5515 instrument. Mass spectra were measured with a LKB 2091 spectrometer at 70 eV. Elemental analyses were carried out on Carlo–Erba Analyzer model 1106 and all compounds gave satisfactory analytical results (within ± 0.4% of theoretical values). The purity of compounds was verified by thin-layer chromatography (TLC) which was run on precoated Kieselgel 60 F_{254} plates from Merck with chloroform/methanol (95:5), toluene/ethyl acetate (50:50 and 90:10) as eluents.

*4-Methyl-1,5-benzothiazepin-2(3H)-one **1a**, 4-methyl-1,5-benzothiazepin-2(5H)-one **1'a** and their 7-chloroderivatives **1b**, **1'b**. General procedure*

These compounds were obtained according to a previously reported method [4].

*7-Chloro-4-methyl-1,5-benzothiazepin-2(3H)-one **1b** and 7-chloro-4-methyl-1,5-benzothiazepin-2(5H)-one **1'b***

Yield: 45%; mp: 130–131°C, yellow prisms from ethanol; ^{1}H-NMR (DMSO–d_6): 2.06 (s, 3H, CH_3 of tautomer **1'b**), 2.28

(s, 3H, CH_3 of tautomer **1b**), 4.44 (s, 2H, CH_2 of tautomer **1b**), 5.97 (s, 1H, CH of tautomer **1'b**), 7.06–8.28 (m, 3H, aromatic protons).

4-Methyl-1,5-benzothiazepin-2(5H)-phenylhydrazones ***2a–h***
A mixture of the appropriate benzothiazepinone **1** (10 mmol), phenylhydrazine or the suitable substituted phenylhydrazine hydrochloride (10 mmol), and sodium acetate (10 mmol) in dry ethanol (10 ml) was refluxed for *ca* 1 h. After evaporation of the solvent, the oily residue as taken up with chloroform and this solution 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 ethyl acetate or hexane/ethyl acetate or was purified by flash chromatography using toluene/ethyl acetate 98:2 as eluent. The crude product was then recrystallized from a suitable solvent (table I). The most characteristic ^{1}H-NMR ($CDCl_3$) peaks were: 2.34–2.40 (s, 3H, CH_3), 5.90–6.10 (s, 1H, CH), 6.37–7.56 (m, aromatic protons).

5-Methyl-12H-indolo[2,3-b]-1,5-benzothiazepines ***3a–h****. General procedure*
A solution of the appropriate phenylhydrazone **2** (1 mmol), as obtained above, in acetic acid saturated with hydrochloric acid (*ca* 10 ml) was refluxed for 15 min until there was no starting material (TLC). After cooling, the desired indolobenzothiazepine **3** crystallized out. It was collected by filtration and recrystallized from a suitable solvent (table II).

The most characteristic ^{1}H-NMR (DMSO–d_6) peaks were: 2.80–3.03 (s, 3H, CH_3), 6.90–8.80 (m, aromatic protons), 9.50–12.60 (s, 1H, NH).

5-Methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-ones ***5d–f****. General procedure*
Compound **5d** [6], **5e** and **f** were prepared starting from **5a–c** [5] according to a previously reported method [6].

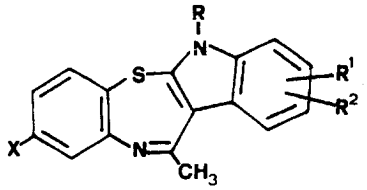
7-Chloro-5-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one ***5e***
Yield: 95%; mp: 117–118°C, white needles from ethanol; ^{1}H-NMR ($CDCl_3$): 2.58 and 3.36 (dt, 4H, CH_2-CH_2), 3.36 (s, 3H, CH_3), 7.06–7.60 (m, 3H, aromatic protons).

8-Chloro-5-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one ***5f***
Yield: 94%; mp: 121°C, white plates from ethanol; ^{1}H-NMR ($CDCl_3$): 2.58 and 3.40 (dt, 4H, CH_2-CH_2), 3.35 (s, 3H, CH_3), 7.10–7.65 (m, 3H, aromatic protons).

2-Chloro-1,5-benzothiazepin-4 (5H)-ones ***6a–f****. General procedure*
Compounds **6a–f** were prepared according to the previously reported method [7].

2,8-Dichloro-1,5-benzothiazepin-4 (5H)-one ***6c***
Yield: 43%; mp: 264–266°C, white prisms from ethyl acetate; ^{1}H-NMR (DMSO–d_6): 6.50 (s, 1H, CH), 7.19–7.60 (m, 3H, aromatic protons), 10.76 (s, 1H, NH).

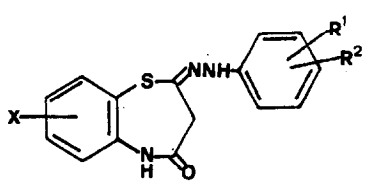
Table II. Physicochemical properties of derivatives **3a–h** and **4a, b**.



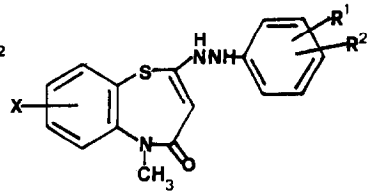
Compd.	X	R	R^1	R^2	m.p.[a] °C	Yield %	Formula (M^+)	UV λ_{max} (Log ε)
3a	H	H	H	H	167-168	30	$C_{16}H_{12}N_2S$ (264)	332.4(4.39), 226.1(4.54)
3b	H	H	1-CH_3	H	144-145	27	$C_{17}H_{14}N_2S$ (278)	334.7(4.31), 224.5(4.49)
3c	H	H	2-CH_3	H	189-190	20	$C_{17}H_{14}N_2S$ (278)	335.3(4.06), 227.1 (4.26)
3d	H	H	3-CH_3	H	188-190	43	$C_{17}H_{14}N_2S$ (278)	334.5(4.41), 227.1(4.54)
3e	H	H	1-CH_3	4-CH_3	180-182	13	$C_{18}H_{16}N_2S$ (292)	326.8(4.15), 256.1(4.36), 223.8(4.88)
3f	H	H	3-Cl	H	182-184	25	$C_{16}H_{11}ClN_2S$ (298.5)	328.9(4.51), 228.8(4.63)
3g	8-Cl	H	H	H	175-177	27	$C_{16}H_{11}ClN_2S$ (298.5)	339.2(4.45), 227.9(4.57)
3h	8-Cl	H	3-Cl	H	156-158	39	$C_{16}H_{10}Cl_2N_2S$ (333)	335.1(4.41), 230.2(4.55)
4a	H	CH_3	H	H	143-144	85	$C_{17}H_{14}N_2S$ (278)	335.5(4.77), 279.3(4.37), 226.5(4.89)
4b	8-Cl	CH_3	H	H	178-180	80	$C_{17}H_{13}ClN_2S$ (312.5)	342.4(4.63), 228.8(4.77)

[a]All compounds were crystallized from ethanol as yellow prisms with the exception of **3b**, **3f** and **4a** (yellow needles) and **4b** (white needles).

Table III. Physicochemical properties of derivatives **8a–m**.



8a-c



8d-m

Compd.	X	R^1	R^2	m.p. °C	Yield %	Recryst.[a] solvent	Formula
8a	H	H	H	157-159	46	EtOH	$C_{15}H_{13}N_3OS$
8b	7-Cl	H	H	177-178	40	EtOH	$C_{15}H_{12}ClN_3OS$
8c	8-Cl	H	H	174-175	61	EtOH	$C_{15}H_{12}ClN_3OS$
8d	H	H	H	186-187	84	EtOH	$C_{16}H_{15}N_3OS$
8e	H	2-CH_3	H	220	70	MeOH	$C_{17}H_{17}N_3OS$
8f	H	3-CH_3	H	189-190	73	EtOH	$C_{17}H_{17}N_3OS$
8g	H	4-CH_3	H	175	43	EtOH	$C_{17}H_{17}N_3OS$
8h	H	2-CH_3	5-CH_3	224-225	70	EtOH	$C_{18}H_{19}N_3OS$
8i	H	4-Cl	H	202-203	70	EtOH	$C_{16}H_{14}ClN_3OS$
8j	7-Cl	H	H	194-195	92	MeOH	$C_{16}H_{14}ClN_3OS$
8k	7-Cl	4-CH_3	H	196-197	60	EtOH	$C_{17}H_{16}ClN_3OS$
8l	8-Cl	H	H	214-215	88	EtAc	$C_{16}H_{14}ClN_3OS$
8m	8-Cl	4-CH_3	H	193-194	77	EtAc	$C_{17}H_{16}ClN_3OS$

[a]Recryst form: white prisms with the exception of **8c** (white needles), **8d** (yellow prisms), **8e** (orange prisms).

5-Methyl-2,7-dichloro-1,5-benzothiazepin-4 (5H)-one ***6e***
Yield: 74%; mp:132–133°C, white needles from ethyl acetate; ^{1}H-NMR ($CDCl_3$): 3.50 (s, 3H, CH_3), 6.40 (s, 1H, CH), 7.15–7.53 (m, 3H, aromatic protons).

5-Methyl-2,8-dichloro-1,5-benzothiazepin-4 (5H)-one ***6f***
Yield: 60%; mp: 94–95°C, white prisms from ethanol; ^{1}H-NMR ($CDCl_3$): 3.40 (s, 3H, CH_3), 6.30 (s, 1H, CH), 7.10–7.50 (m, 3H, aromatic protons).

1,5-Benzothiazepin-2,4(3H, 5H)-diones ***7a–f****. General procedure*
The reported procedure [7] was properly modified giving improved yields. The appropriately substituted 2-chlorobenzothiazepinone **6** (1 mmol) was added portionwise, with stirring and cooling in an ice-bath, to a solution of conc sulphuric acid. The reaction mixture was kept at room temperature under stirring for *ca* 1 h, then was poured into ice-water and extracted with methylene chloride. The organic layer was washed with water and dried over sodium sulphate. The evaporation of the solvent gave the required **7a–f** which were recrystallized from a suitable solvent.

1,5-Benzothiazepin-2,4(3H, 5H)-dione ***7a***
Yield: 87%; mp: 208°C (lit [7] yield: 75%; mp: 208°C), white prisms from isopropyl alcohol.

7-Chloro-1,5-benzothiazepin-2,4(3H, 5H)-dione ***7b***
Yield: 60%; mp: 221–222°C (lit [7] yield: 16%; mp: 218°C), white needles from isopropyl alcohol.

8-Chloro-1,5-benzothiazepin-2,4(3H, 5H)-dione ***7c***
Yield: 66%; mp: 216–217°C (lit [8] yield: 5%; mp: 210°C), white needles from isopropyl alcohol.

5-Methyl-1,5-benzothiazepin-2,4(3H, 5H)-dione ***7d***
Yield: 83%; mp: 136°C (lit [7] yield: 40%; mp: 136°C), white prisms from isopropyl alcohol.

7-Chloro-5-methyl-1,5-benzothiazepin-2,4(3H, 5H)-dione ***7e***
Yield: 85%; mp: 156–157°C, orange cubes from isopropyl alcohol. ^{1}H-NMR (DMSO–d_6): 3.40 (s, 3H, CH_3), 3.35, 4.09 (d, 2H, J = 14 Hz, CH_2), 7.42–7.88 (m, 3H, aromatic protons).

8-Chloro-5-methyl-1,5-benzothiazepin-2,4 (3H, 5H)-dione ***7f***
Yield: 76%; mp: 130–131°C; white prisms from isopropyl alcohol. ^{1}H-NMR (DMSO–d_6): 3.42 (s, 3H, CH_3), 3.38, 4.10 (d, 2H, J = 9 Hz, CH_2), 7.61–7.80 (m, 3H, aromatic protons).

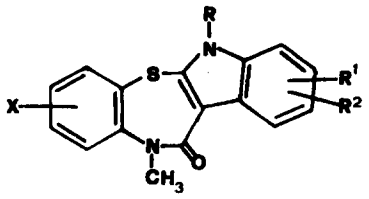
1,5-Benzothiazepin-2 (3H)-phenylhydrazon-4 (5H)-ones ***8a–m***
A mixture of benzothiazepindione **7** (10 mmol), phenylhydrazine or the appropriately substituted phenylhydrazine hydrochloride (10 mmol), and sodium acetate (10 mmol) in dry ethanol (20 ml) was refluxed for 1 h (compounds **8a–d, 8j, 8l**) or *ca* 30 min (compounds **8e–i, 8k, 8m**). After cooling, the precipitate was collected, washed with water and recrystallized from a suitable solvent (table III).

The most characteristic ^{1}H-NMR (DMSO–d_6) peaks were: **8a–c**: 4.16–4.20 (s, 2H, CH_2), 6.63–8.26 (m, aromatic protons), *ca* 10.20 (br s, 1H, NHCO), **8d–m**: 3.36–3.43 (s, 3H, NCH_3), 5.30–5.50 (s, 1H, CH), 6.53–7.90 (m, aromatic protons), 7.43–7.80 (br s, 1H, NH), 8.90–9.06 (br s, 1H, NH).

5,6-Dihydro-6-methyl-12H-indolo [2,3-b]-1,5-benzothiazepin-5-ones ***9a–k***
Phenylhydrazone **8** (1 mmol) was added to an excess of polyphosphoric acid (*ca* 2g) and the mixture was heated in oil bath at 100°C for *ca* 45 min with stirring. The reaction mixture was poured into ice-water and neutralized with 10% ammonium hydroxide. The product was extracted with chloroform and the organic layer was washed with water, dried over sodium sulfate and brought to dryness *in vacuo*. The residue was taken up in ethyl acetate, collected by filtration and recrystallized from a suitable solvent (table IV).

The most characteristic ^{1}H-NMR (DMSO–d_6) peaks were: 3.70–3.96 (s, 3H, N-CH_3), 6.53–8.00 (m, aromatic protons), 10.25–10.55 (s, 1H, NH).

Table IV. Physicochemical properties of derivatives **9a–k** and **10a, b.**



Compd.	X	R	R^1	R^2	m.p. °C	Yield %	Recryst. solvent[a]	Formula(M^+)	UV λ max (Log ε)
9a	H	H	H	H	273-275	53	EtAc	$C_{16}H_{12}N_2OS$(280)	396.1(4.43), 285.2(4.09)
9b	H	H	1-CH_3	H	323-324	41	Dioxane	$C_{17}H_{14}N_2OS$(294)	398.5(4.46), 285.0(4.07)
9c	H	H	2-CH_3	H	234-235	46	EtOH	$C_{17}H_{14}N_2OS$(294)	399.0(4.41), 290.1(4.01)
9d	H	H	3-CH_3	H	260-262	47	Dioxane	$C_{17}H_{14}N_2OS$(294)	398.7(4.45), 290.0(4.09)
9e	H	H	4-CH_3	H	228-230	25	EtOH	$C_{17}H_{14}N_2OS$(294)	407.0(4.21), 289.0(3.78)
9f	H	H	1-CH_3	4-CH_3	289-290	68	Dioxane	$C_{18}H_{16}N_2OS$(308)	408.5(4.10), 288.0(3.64)
9g	H	H	3-Cl	H	210-211	50	Dioxane	$C_{16}H_{11}ClN_2OS$(314.5)	399.0(4.28), 291.7(3.89)
9h	8-Cl	H	H	H	269-271	53	Dioxane	$C_{16}H_{11}ClN_2OS$(314.5)	394.5(4.36), 286.3(3.83)
9i	8-Cl	H	3-CH_3	H	303-304	30	Dioxane	$C_{17}H_{13}ClN_2OS$(328.5)	399.0(4.29), 290.0(3.76)
9j	9-Cl	H	H	H	289-291	50	EtOH	$C_{16}H_{11}ClN_2OS$(314.5)	397.5(4.43), 286.7(4.00)
9k	9-Cl	H	3-CH_3	H	264-265	42	EtOH	$C_{17}H_{13}ClN_2OS$(328.5)	394.9(4.37), 290.7(3.89)
10a	H	CH_3	H	H	185-187	75	EtOH	$C_{17}H_{14}N_2OS$(294)	395.0(4.49), 287.5(4.21)
10b	H	CH_3	3-Cl	H	258-259	72	EtOH	$C_{17}H_{13}ClN_2OS$(328.5)	398.0(4.36), 293.7(4.01)

[a]Recryst form: yellow prisms with the exception of **9e** and **9f** (orange prisms).

*5,12-Dimethylindolo [2,3-b]-1,5-benzothiazepines **4a**, **b** and 6,12-dimethyl-5,6-dihydroindolo[2,3-b]-1,5-benzothiazepin-5-ones **10a**, **b**. General procedure*

To a stirred solution of the suitable indolo[2,3-*b*]-1,5-benzothiazepine **3** or **9** (1 mmol), tetrabutylammonium bromide (0.1 mmol) and methyl iodide (1 mmol) in tetrahydrofuran (*ca* 50 ml), finely powdered potassium hydroxide (1 mmol) was added. The reaction mixture was kept at room temperature under stirring for 1–2 h and then filtered. The filtrate 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 crude product was then recrystallized (tables II and IV).

^{1}H-NMR (DMSO–d_6): **4a**, **b**: 2.90 (s, 3H, CH_3), 3.80 (s, 3H, N-CH_3), 7.18–8.36 (m, aromatic protons). **10a**, **b**: 3.35–3.40 (s, 3H, N_{12}-CH_3), 3.95–3.98 (s, 3H, N_6-CH_3), 6.90–7.93 (m, aromatic protons).

Biological evaluation

In vitro antimicrobial assays

A preliminary screening was conducted against the Gram-positive and Gram-negative bacteria and the strains of yeasts reported in table V. Agar plates with the compounds (200 μg/ml) included were used. Strains were inoculated with a multipoint-inoculator in Petri disks with nutrient agar in duplicate and prepared from cultures incubated at 32°C for 48 h in nutrient broth (Gram-positive and Gram-negative bacteria) or Sabouraud broth (yeasts). The precultures were centrifuged, washed twice in isotonic sodium chloride solution, resuspended in the same medium, and calibrated with a Spectronic 20 Bausch and Lomb colorimeter at λ = 550 nm, OD 0.2. Those compounds with significant activity were successively tested at 2-fold serial dilutions (see table V). The minimum inhibitory concentration (MIC) values were determined after 48 h incubation at 32°C.

NB: 37°C is not the best temperature for the bacterial strains used in this screening; the procedure used for the antimicrobial screening and the temperature of incubation (32°C) allow us to test different bacteria and yeast strains at the same time.

In vitro cytostatic activity evaluation

An established cell line derived from an oral epidermoid human carcinoma (KB) [9] was used for cytostatic effect evaluation. The KB cells were maintained and tested as monolayers

Table V. MIC values (μg/ml).

Microorganisms and fungi strains	Compounds												
	2h	**3b**	**3d**	**3e**	**3f**	**3g**	**3h**	**4b**	**6f**	**8b**	**Cephaloridine**	**Nalidixic acid**	**Nystatin**
Bacillus subtilis ICI B35 ISS	>200	6	6	100	3	25	6	200	50	>200	<1	25	>100
Micrococcus luteus 9341 ISS	>200	12	12	100	6	50	6	100	25	>200	<1	>50	>100
Bacillus subtilis var. *niger* 6455 ATCC	>200	6	6	50	6	50	3	200	50	>200	1	25	>100
Bacillus cereus B43 1335 ISS	>200	6	6	50	3	50	6	200	100	>200	1	>50	>100
Staphylococcus aureus B124 ISS	>200	6	6	100	3	50	3	200	100	>200	1	>50	>100
Pseudomonas aeruginosa 6750 ISS	>200	>200	>200	100	>200	>200	>200	>200	100	>200	>100	>100	>100
Salmonella typhimurium B109 ISS	>200	>200	>200	>200	>200	>200	>200	>200	100	>200	10	5	>100
Proteus vulgaris B66 ISS	>200	>200	>200	>200	>200	>200	>200	>200	100	>200	10	5	>100
Escherichia coli 982 ISS	>200	>200	>200	>200	>200	>200	>200	>200	100	>200	10	5	>100
Citrobacter freundii B198 ISS	>200	>200	>200	>200	>200	>200	>200	>200	100	>200	10	5	>100
Pseudomonas fluorescens C3 ISS	>200	>200	>200	100	>200	100	>200	>200	50	>200	>100	>100	>100
Cryptococcus laurentii 4685 IMAT	100	12	12	100	12	50	25	>200	100	100	>100	>100	2
Candida utilis var. *maior* 4870 IMAT	100	100	100	100	100	100	200	>200	100	100	>100	>100	2
Candida albicans ser. A CBS 562	100	100	200	>200	200	100	200	>200	100	100	>100	>100	1
Candida tropicalis 5711 IMAT	100	200	200	>200	200	100	200	>200	100	100	>100	>100	1
Candida krusei 1910 CBS	100	200	200	>200	200	100	200	>200	50	100	>100	25	1
Cryptococcus neoformans 4711 IMAT	100	25	3	50	6	100	50	200	25	100	>100	50	1

ISS: Istituto Superiore di Sanità (Italy); ATCC: American Type Culture Collection; IMAT: Istituto Microbiologia Agraria e Tecnica (Perugia, Italy); CBS: Central Bureau Voor Shimmelcultures Delft (ND).

Table VI. Effect of several indolobenzothiazepines on the growth of KB cells.

Compound	IC_{50}		Compound	IC_{50}	
	μg/ml	μM		μg/ml	μM
3a	>10	-	**9c**	3.06	10.4
3b	>10	-	**9d**	2.23	7.6
3d	2.40	8.6	**9e**	3.07	10.4
3e	1.95	6.7	**9f**	2.60	8.4
3f	9.11	30.5	**9g**	10.00	31.8
3g	7.26	24.3	**9h**	3.51	11.2
3h	0.79	2.4	**9i**	8.79	26.8
4a	>10	-	**9j**	9.13	29.0
9a	>10	-	**9k**	8.62	26.2
9b	>10	-	**10a**	7.42	25.2
6-Mercaptopurine	0.13	0.86	**Cisplatin**	0.13	0.43

in buffered Eagle's Minimal Essential Medium (MEM) supplemented with 10% newborn calf serum, 1% nonessential amino acids as previously described [10, 11]. The cell population doubling time was *ca* 24 h.

For the *in vitro* assay the cells in exponential growth phase were refed 24 h before testing and seeded at 10^5 cells per Leighton tube. The compounds were added 24 h after seeding in order to allow a cellular adhesion to substrate.

The compounds were dissolved immediately before use in sterile dimethylsulfoxide. Further dilutions were performed with the growth medium to the desired drug concentration. The final solvent concentration in culture medium (0.5% in every tube) was previously tested by us and did not show any cytotoxic effect. At least 5 concentration levels were used for each compound and each concentration value was tested in triplicate. Each compound was assayed on at least 2 separate occasions.

The incubation was carried out at 37°C for 72 h, time interval in which exponential growth occurs. Cell growth was estimated by counting the viable cells (Trypan blue exclusion test). The cytostatic activity was evaluated on the basis of cell growth inhibition in the treated cultures with respect to the controls. The significance of these results was evaluated by use of the Student's-*t* test ($p < 0.01$). The drug concentration (μg/ml medium and micromolar) at which cell proliferation was 50% of that in control cultures (IC_{50}) was determined by linear regression analysis, setting the activity threshold at 10 μg/ml medium since this appears to be a fairly realistic cutoff point for most compounds [12].

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