SYNTHESIS OF SOME NOVEL ANNULATED 1,4-BENZOTHIAZINE AND 1,5-BENZOTHIAZEPINE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AND CYTOSTATIC AGENTS(1)

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ABSTRACT: Several series of polyheterocyclic annulated 1,4-benzothiazine and 1,5-benzothiazepine derivatives were synthesized starting from 1,4-benzothiazin-3(4H)-one and 1,5-benzothiazepin-4(5H)-one, respectively.

All the compounds were tested for their antimicrobial and cytostatic activities. None of them showed any significant antimicrobial activity, whereas some of them were found to be moderate cytostatic agents.

INTRODUCTION

Several derivatives of tetracyclic systems containing the quinazoline nucleus are known to possess antimicrobial (2,3) or antitumoral (4-6) activities. Therefore, as a continuation of our work on 1,4-benzothiazines and 1,5-benzothiazepines with potential antimicrobial and cytostatic activities (7-9), it appeared interesting to synthesize compounds containing both quinazoline and 1,4-benzothiazine or 1,5-benzothiazepine ring systems fused in the same molecule.

Moreover, in order to extend the studies on structure-activity relationships, we have synthesized some derivatives of new polycyclic systems in which the benzene ring of the quinazoline is replaced by that one of pyridine, thiophene, pyrazine or naphthalene.

EXPERIMENTAL

1 - Chemistry

Melting points were taken on a Kofler hot-stage apparatus (uncorrected). ^{1}H-NMR spectra were recorded using a Varian EM-390 (90 MHz) in DMSO-d_6 (added of 10% $CDCl_3$) for compounds 2f, 2h, 5e, 8a, 8c, and 8d and in $CDCl_3$ solution for other products; TMS was used

as an internal standard and chemical shifts are reported in δ values (ppm). Mass spectra were recorded on a LKB 2091 spectrometer at 70 eV, IR spectra on a Beckman Acculab T.M. 5 spectrophotometer in nujol and UV spectra on a Perkin Elmer 551 S in EtOH solution. Elemental analyses were carried out on a Carlo Erba Elemental Analyzer mod. 1106.

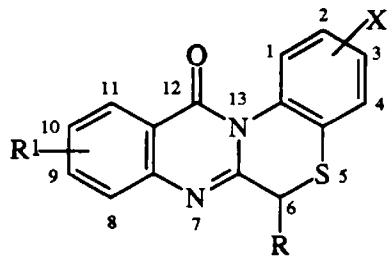
General procedure for synthesis of quinazolino[2,3-*c*][1,4]benzothiazines 2a-h. quinazolino[2,3-*d*][1,5]benzothiazepines 3a-t. pyrido[2',3':4,5]pyrimido[2,1-*c*][1,4] benzothiazines 4a.b. pyrido[2',3':4,5]pyrimido[2,1-*d*][1,5]benzothiazepines 4c-g. thieno [3',2':4,5]pyrimido[2,1-*c*][1,4]benzothiazines 5a.b. thieno[3',2':4,5]pyrimido[2,1-*d*][1,5]benzothiazepines 5c-e. pyrazino[2',3':4,5]pyrimido[2,1-*d*][1,5]benzothiazepines 6a-d. naphtho[2',3':4,5]pyrimido[2,1-*c*][1,4]benzothiazines 7a.b and naphtho[2',3':4,5] pyrimido[2,1-*d*][1,5]benzothiazepines 7c-f (scheme)

Triethylamine (20 mmol) and the appropriate aminoacid (10 mmol) were added slowly, in succession and with stirring, to a cooled suspension of 2H-1,4-benzothiazin-4(3H)-one (10, 11) or 2,3-dihydro-1,5-benzothiazepin-4(5H)-one (12) (10 mmol) in phosphorylchloride (20 mmol). The mixture was then stirred at 60-70°C for 0.5-3 h in the case of compounds 2a-h and 3a-t. at 80°C for 2-3 h in the case of compounds 4a-g. at 30-40°C for 3-4 h in the case of compounds 6a-d. at 120°C for 1h in the case of compounds 7a-f and for 2h in the case of compounds 5c-e and at 130°C for 5-7 h in the case of compounds 5a.b. At last the reaction mixture was brought to dryness *in vacuo* and the residue was treated with ethanol. The precipitate was filtered and recrystallized from a suitable solvent or purified by flash chromatography.
Physicochemical and spectral data of all the compounds were listed in Tables 1-4.

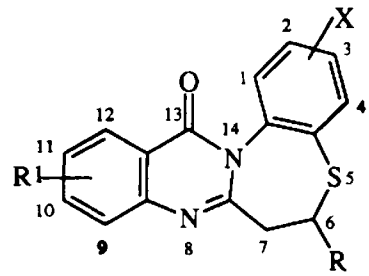
2 - Antimicrobial activity

A preliminary screening was conducted *in vitro* against the following strains:
Gram-positive: *Bacillus subtilis* ICI, *Micrococcus luteus* 9341 ISS, *Bacillus subtilis* var. *niger*, *Bacillus cereus* B43 ISS, *Staphylococcus aureus* ISS.
Gram-negative: *Pseudomonas aeruginosa* 6750, *Salmonella typhimurium* ISS, *Proteus vulgaris* ISS, *Escherichia coli* 982 ISS, *Pseudomonas fluorescens* C3 ISS.
Fungi: *Candida utilis* 4870 ISS, *Candida albicans* 562 CBS, *Candida tropicalis* 5711 IMAT, *Candida guilliermondii* 5313 IMAT, *Candida krusei* 1910 CBS, *Cryptococcus laurentii* 4688 IMAT, *Cryptococcus neoformans* 4711 IMAT.
The strains used originate from four different collections: ICI (Imperial Chemical Industries, England), 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).

Table 1: Physicochemical data of compounds 2a-h and 3a-t



2a-h



3a-t

Compound*	X	R	R^1	Mp(°C)	Yield(%)	Recryst. Solv. Colour-cryst. form	Molecular Formula (Mol.W.)
2a	H	H	H	152-4**	75	ethanol yellow prisms	$C_{15}H_{10}N_2OS$ (266.3)
2b	H	H	8-CH_3	244-6	56	dioxane ivory needles	$C_{16}H_{12}N_2OS$ (280.3)
2c	H	H	9-Cl	176-7***	72	ethanol ivory prisms	$C_{15}H_9ClN_2OS$ (300.8)
2d	H	H	9-NO_2	182-3	60	ethanol yellow needles	$C_{15}H_9N_3O_3S$ (311.3)
2e	3-Cl	H	H	203-5****	70	ethanol yellow prisms	$C_{15}H_9ClN_2OS$ (300.8)
2f	3-Cl	H	8-CH_3	255-6	60	dioxane white prisms	$C_{16}H_{11}ClN_2OS$ (314.8)
2g	3-Cl	H	9-Cl	239-40	66	dioxane/ethanol yellow needles	$C_{15}H_8Cl_2N_2OS$ (335.2)
2h	3-Cl	H	9-NO_2	240	50	ethyl acetate/ethanol yellow needles	$C_{15}H_8ClN_3O_3S$ (345.8)
3a	H	H	H	209-10*****	81	ethanol white needles	$C_{16}H_{12}N_2OS$ (280.3)
3b	H	H	9-CH_3	195	60	ethanol white needles	$C_{17}H_{14}N_2OS$ (294.4)
3c	H	H	10-Cl	204-5	55	ethanol white prisms	$C_{16}H_{11}ClN_2OS$ (314.8)

Table 1 (continued)

Compound*	X	R	R^1	Mp(°C)	Yield(%)	Recryst. Solv. Colour-cryst. form	Molecular Formula (Mol.W.)
3d	H	H	10-NO_2	210-1	70	ethanol yellow prisms	$C_{16}H_{11}N_3O_3S$ (325.3)
3f	2-Cl	H	10-Cl	231-2	63	ethanol yellow prisms	$C_{16}H_{10}Cl_2N_2OS$ (349.2)
3g	2-Cl	H	10-NO_2	226-8	73	methanol/ethanol yellow prisms	$C_{16}H_{10}ClN_3O_3S$ (359.8)
3h	2-Cl	H	11-NO_2	240	68	dioxane yellow prisms	$C_{16}H_{10}ClN_3O_3S$ (359.8)
3i	3-Cl	H	H	195	70	ethanol white needles	$C_{16}H_{11}ClN_2OS$ (314.8)
3j	3-Cl	H	9-CH_3	267-8	60	ethanol white prisms	$C_{17}H_{13}ClN_2OS$ (328.8)
3k	3-Cl	H	10-Cl	240-1	62	dioxane ivory needles	$C_{16}H_{10}Cl_2N_2OS$ (349.2)
3l	3-Cl	H	10-NO_2	263-4	75	ethyl acetate white needles	$C_{16}H_{10}ClN_3O_3S$ (359.8)
3m	3-Cl	H	11-NO_2	232-4	74	dioxane yellow prisms	$C_{16}H_{10}ClN_3O_3S$ (359.8)
3n	H	CH_3	H	141	50	ethanol white prisms	$C_{17}H_{14}N_2OS$ (294.4)
3o	H	CH_3	10-Cl	202-4	65	ethanol white needles	$C_{17}H_{13}ClN_2OS$ (328.8)
3p	H	CH_3	10-NO_2	176-7	50	ethanol light yellow needles	$C_{17}H_{13}N_3O_3S$ (339.4)
3q	3-Cl	CH_3	H	225-6	45	ethanol white prisms	$C_{17}H_{13}ClN_2OS$ (328.8)
3r	3-Cl	CH_3	10-Cl	226-7	62	dioxane yellow needles	$C_{17}H_{12}Cl_2N_2OS$ (363.3)
3s	3-Cl	CH_3	10-NO_2	213-4	50	ethanol yellow needles	$C_{17}H_{12}ClN_3O_3S$ (409.3)

Table 1 (continued)

Compound*	X	R	R^1	Mp(°C)	Yield(%)	Recryst. Solv. Colour-cryst. form	Molecular Formula (Mol.W.)
3t	3-Cl	CH_3	11-NO_2	289-91	54	dioxane yellow needles	$C_{17}H_{12}ClN_3O_3S$ (409.3)

* Analyses agree within 0,4 % of the theoretical values

** Lit. (20), 147°C

*** Lit. (19), 156°C

**** Lit. (20), 172°C

***** Lit. (21), 208°C

Table 2: Spectral data of compounds 2a-h and 3a-t

Compound	UV_{EtOH} (log ε) λ_{max}	IR_{nujol} C=O cm^{-1}	^{1}H-NMR δ (ppm) and multiplicity*
2a	228.0(4.57)	1680	3.88 (s, 2H, CH_2), 7.16-7.80 (m, 6H, arom.), 8.05 (m, 1H, C_1 arom.), 8.33 (br d, J = 7Hz, 1H, C_{11} arom.)
2b	236.9(4.42)	1680	2.64 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 7.16-7.65 (m, 5H, arom.), 8.05 (m, 1H, C_1 arom.), 8.20 (m, 1H, C_{11} arom.)
2c	242.7(4.45)	1685	3.86 (s, 2H, CH_2), 7.16-7.67 (m, 5H, arom.), 8.05 (m, 1H, C_1 arom.), 8.24 (br d, J = 8Hz, 1H, C_{11} arom.)
2d	252.9(4.46)	1685	3.91 (s, 2H, CH_2), 7.20-8.53 (m, 7H, arom.)
2e	230.3(4.68)	1685	3.90(s, 2H, CH_2), 7.26-7.80 (m, 5H, arom.), 8.01 (br d, J = 7Hz, 1H, C_1 arom.), 8.32 (m, 1H, C_{11} arom.)
2f	241.5(4.73)	1680	2.55(s, 3H, CH_3), 4.08 (s, 2H. CH_2), 7.40-7.80 (m, 4H, arom.), 7.99 (br d, J = 8Hz, 1H, C_1 arom.), 8.05 (br d, J = 8Hz, 1H, C_{11} arom.)
2g	244.0(4.53)	1690	3.93 (s, 2H, CH_2), 7.29-7.73 (m, 4H, arom.), 8.00 (br d, J = 8Hz, 1H, C_1 arom.), 8.24 (br d, J = 8Hz, 1H, C_{11} arom.)
2h	252.2(4.59)	1700	4.10 (s, 2H. CH_2), 7.40-8.45 (m, 6H, arom.)
3a	228.1(4.58)	1678	2.46-3.43 (m, 4H, CH_2-CH_2), 7.26-7.83 (m, 7H, arom.), 8.27 (m, 1H, C_{12} arom.)
3b	233.3(4.65)	1680	2.66 (s, 3H, CH_3), 7.23-7.76 (m, 6H, arom.), 8.16 (m, 1H, C_{12} arom.)

Table 2 (continued)

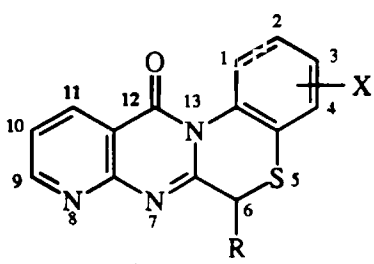
Compound	UV_{EtOH} (log ε) λ_{max}	IR_{nujol} C=O cm^{-1}	^{1}H-NMR δ (ppm) and multiplicity*
3c	241.6(4.84)	1675	2.40-3.54 (m, 4H, CH_2-CH_2), 7.26-7.80 (m, 6H, arom.), 8.22 (br d, J = 7Hz, 1H, C_{12} arom.)
3d	241.6(4.84)	1695	2.43-3.60 (m, 4H, CH_2-CH_2), 7.36-8.56 (m, 7H, arom.)
3e	241.9(4.64)	1690	2.45-3.53 (m, 4H, CH_2-CH_2), 7.23-7.83 (m, 6H, arom.), 8.28 (m, 1H, C_{12} arom.)
3f	256.0(4.58)	1680	2.43-3.45 (m, 4H, CH_2-CH_2), 7.26-7.73 (m, 5H, arom.), 8.22 (br d, J = 7Hz, 1H, C_{12} arom.)
3g	218.4(4.78)	1690	2.53-3.48 (m, 4H, CH_2-CH_2), 7.32-7.74 (m, 3H, arom.), 8.13-8.56 (m, 3H, arom.)
3h	309.3(4.74) 232.0(5.22)	1695	2.41-3.57 (m, 4H, CH_2-CH_2), 7.32-7.86 (m, 4H, arom.), 8.54 (dd, J = 8Hz, J = 2Hz, 1H, C_{10} arom.), 9.14 (d, J = 3Hz, 1H, C_{12} arom.)
3i	230.5(4.56)	1680	2.40-3.46 (m, 4H, CH_2-CH_2), 7.30-7.80 (m, 5H, arom.), 8.26 (dd, J = 7Hz, J = 1Hz, 1H, C_{12} arom.)
3j	234.7(4.70)	1675	2.66 (s, 3H, CH_3), 2.50-3.46 (m, 4H, CH_2-CH_2), 7.30-7.76 (m, 5H, arom.), 8.13 (dd, J = 9Hz, J = 2Hz, 1H, C_{12} arom)
3k	241.9(4.69)	1695	2.53-3.56 (m, 4H, CH_2-CH_2), 7.33-7.80 (m, 5H, arom.), 8.20 (br d, J = 8Hz, 1H, C_{12} arom.)
3l	257.1(4.46)	1695	2.53-3.60 (m, 4H, CH_2-CH_2), 7.47-8.60 (m, 6H, arom.)
3m	327.8(4.20)	1680	2.44-3.60 (m, 4H, CH_2-CH_2), 7.46-7.86 (m, 4H, arom.), 8.53 (dd, J = 9Hz, J = 3Hz, 1H, C_{10} arom.), 9.10 (d, J = 3Hz, 1H, C_{12} arom.)
3n	269.7(4.29)	1680	1.33 (d, 3H, CH_3), 2.30, 3.40, 3.73 (ABX system, 3H, CH-CH_2), 7.33-7.83 (m, 6H, arom.), 8.30 (m, 1H, C_{12} arom.)
3o	241.7(4.59)	1680	1.33 (d, J = 5.4 Hz, 3H, CH_3), 2.26, 3.10, 3.66 (ABX system, 3H, $CHCH_2$), 7.30-7.80 (m, 6H, arom.), 8.21 (br d, J = 6Hz, 1H, C_{12} arom.)
3p	256.0(4.47)	1680	1.33 (d, J = 5.4 Hz, 3H, CH_3), 2.26, 3.10, 3.66 (ABX system, 3H, $CHCH_2$), 7.30-7.80 (m, 6H, arom.), 8.21 (br d, J = 6Hz, 1H, C_{12} arom.)

Table 2 (continued)

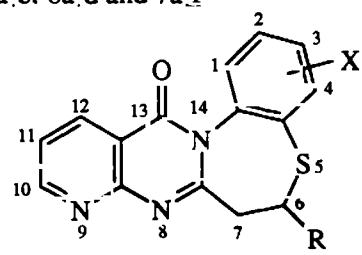
Compound	UV_{EtOH} (log ε) λ_{max}	IR_{nujol} C=O cm^{-1}	^1H-NMR δ (ppm) and multiplicity*
3q	310.1(3.73) 270.1(4.22) 230.2(4.65)	1675	1.36 (d, J = 5.4 Hz, 3H, CH_3), 2.30, 3.40, 3.67 (ABX system, 3H, CH-CH_2), 7.30-7.83 (m, 6H, arom.), 8.28 (m, 1H, C_{12} arom.)
3r	241.2(4.68)	1690	1.37 (d, J = 5.4 Hz, 3H, CH_3), 2.27, 3.14, 3.73 (ABX system, 3H, CH-CH_2), 7.20-7.77 (m, 5H, arom.), 8.21 (br d, J = 6Hz, 1H, C_{12} arom.)
3s	257.5(4.45)	1695	1.40 (d, J = 5.4 Hz, 3H, CH_3), 2.30, 3.19, 3.73 (ABX system, 3H, CH-CH_2), 7.46-7.80 (m, 3H, arom.), 8.13-8.56 (m, 3H, arom.)
3t	329.1(4.23)	1690	1.30 (d, J = 5.4 Hz, 3H, CH_3), 2.25, 2.30, 3.80 (ABX system, 3H, CH-CH_2), 7.67-8.00 (m, 4H, arom.), 8.60 (dd, J = 9Hz, J = 3Hz, 1H, C_{10} arom.), 8.83 (d, J = 3Hz, 1H, C_{12} arom.)

* Abreviations have their usual significance

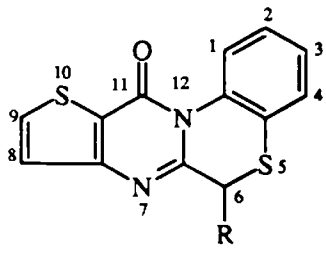
Table 3: Physicochemical data of compounds 4a-g. 5a-e. 6a-d and 7a-f



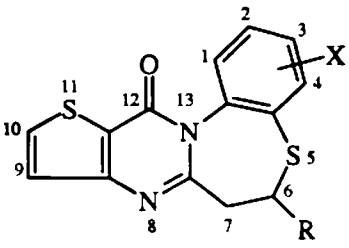
4a,b



4c-g



5a,b



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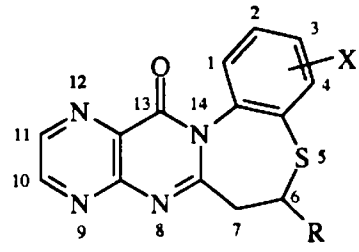

6a-d

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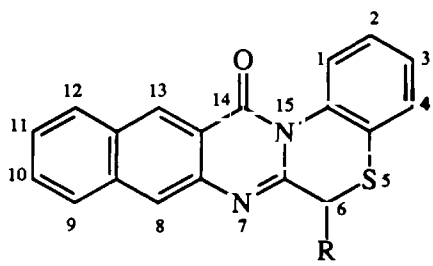
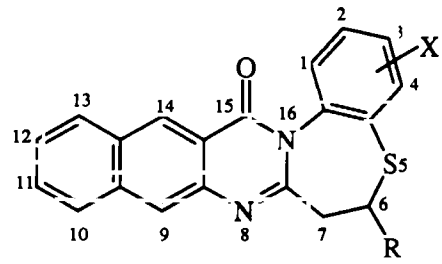


7a,b



7c-f

Compound*	X	R	Mp(°C)	Yield%	Recryst. solvent Colour cryst. form	Molecular Formula (Mol.W.)
4a	H	H	125-7	16	**	$C_{14}H_9N_3OS$ (267.3)
4b	H	CH_3	121-4	10	**	$C_{15}H_{11}N_3OS$ (281.3)
4c	H	H	198-200	57	ethyl acetate yellow prisms	$C_{15}H_{11}N_3OS$ (281.3)
4d	2-Cl	H	214-7	36	ethyl acetate yellow prisms	$C_{15}H_{10}ClN_3OS$ (315.8)
4e	3-Cl	H	232-4	35	ethanol orange prisms	$C_{15}H_{10}ClN_3OS$ (315.8)
4f	H	CH_3	194-5	41	ethanol white prisms	$C_{16}H_{13}N_3OS$ (295.4)
4g	3-Cl	CH_3	212-4	40	ethanol white needles	$C_{16}H_{12}ClN_3OS$ (329.8)
5a	H	H	224-5	63	ethanol white prisms	$C_{13}H_8N_2OS_2$ (272.3)
5b	H	CH_3	164-5	70	ethanol white prisms	$C_{14}H_{10}N_2OS_2$ (286.4)
5c	H	H	224-5	100	ethanol white prisms	$C_{14}H_{10}N_2OS_2$ (286.4)
5d	2-Cl	H	228-30	87	ethanol white prisms	$C_{14}H_9ClN_2OS_2$ (320.8)
5e	H	CH_3	195-7	40	ethanol white prisms	$C_{15}H_{12}N_2OS_2$ (300.4)

Table 3 (continued)

Compound*	X	R	Mp(°C)	Yield%	Recryst. solvent Colour cryst. form	Molecular Formula (Mol.W.)
6a	H	H	251-3	15	ethanol dark red cubes	$C_{14}H_{10}N_4OS$ (282.3)
6b	2-Cl	H	140-2	11	ethanol dark red cubes	$C_{14}H_9ClN_4OS$ (316.8)
6c	3-Cl	H	270	13	ethanol yellow cubes	$C_{14}H_9ClN_4OS$ (316.8)
6d	H	CH_3	170-3	12	ethyl acetate white prisms	$C_{15}H_{12}N_4OS$ (296.3)
7a	H	H	238-40	95	dioxane/ethanol ivory needles	$C_{19}H_{12}N_2OS$ (316.4)
7b	H	CH_3	213-4	60	***	$C_{20}H_{14}N_2OS$ (330.4)
7c	H	H	299-300	80	dioxane white prisms	$C_{20}H_{14}N_2OS$ (330.4)
7d	2-Cl	H	278-80	70	dioxane white cubes	$C_{20}H_{13}ClN_2OS$ (364.8)
7e	3-Cl	H	323-6	70	dioxane white prisms	$C_{20}H_{13}ClN_2OS$ (364.8)
7f	H	CH_3	253-4	75	ethyl acetate white cubes	$C_{21}H_{16}N_2OS$ (344.4)

* Analyses agree within 0,4 % of the theoretical values
** Purified by chromatography using chloroform as eluant and not recrystallized
*** Purified by chromatography using hexane:chloroform 80:20 as eluant and not recrystallized

Table 4: Spectral data of compounds 4a-g, 5a-e, 6a-d and 7a-f

Compound	UV_{EtOH}(log ε) λ max	IR nujol C=O cm^{-1}	^{1}H-NMR δ (ppm) and multiplicity*
4a	273.0(4.06)	1680	3.36(s, 2H, CH_2), 6.60 - 7.00(m, 4H, arom.), 7.45(m, 1H, C_1arom), 8.06(dd, J=6Hz,J= 2Hz, 1H, C_9arom), 8.36(dd, J=5Hz, J=2Hz, 1H, C_{11}arom)

Table 4 (continued)

Compound	UV_{EtOH}(log ε) λ max	IR nujol C=O cm^{-1}	^{1}H-NMR δ (ppm) and multiplicity*
4b	273.4(4.11)	1670	1.71(d, J=7Hz, 3H, CH_3), 4.03(q, J=7Hz, 1H, CH), 7.10 - 7.60(m, 4H, arom.), 8.00(m, 1H, C_1arom), 8.36(dd, J=2Hz, J=7Hz, 1H, C_9arom), 8.95(dd, J=4Hz, J=2Hz, 1H C_{11}arom)
4c	275.0(4.13)	1680	2.50 - 3.50 (m, 4H, CH_2-CH_2), 7.30 - 7.83 (m, 5H, arom.), 8.60 (dd, J= 7Hz, J=2Hz, 1H, C_{10}arom.), 8.96 (dd, J= 3Hz, J = 2Hz, 1H, C_{12} arom
4d	275.6(4.36)	1690	2.50 - 3.43 (m, 4H, CH_2-CH_2), 7.23 - 7.80 (m, 4H, arom), 8.50 (dd, J = 7Hz, J = 2Hz, C_{10} arom.), 8.83 (dd, J = 4Hz, J = 2Hz, 1H, C_{12} arom.)
4e	277.0(4.15)	1690	2.56 - 3.53 (m, 4H, CH_2-CH_2), 7.20 - 7.80 (m, 4H, arom.), 8.60 (dd, J = 7Hz, J = 2Hz, 1H, C_{10} arom.), 9.00 (dd, J = 4Hz, J = 2Hz , 1H, C_{12} arom.)
4f	277.8(4.18)	1680	1.33 (d, J = 6Hz, 3H, CH_3), 2.30, 3.24, 3.76 (ABX system, 3H, CH-CH_2), 7.23-7.83 (m, 5H, arom.), 8.60 (dd, J = 7Hz, J = 3Hz, 1H, C_{10} arom.), 8.97 (dd, J = 5Hz, J = 2Hz, 1H, C_{12} arom.)
4g	276.5(4.08)	1685	1.36 (d, J = 5Hz, 3H, CH_3), 2.30, 3.26, 3.76 (ABX system, 3H, CH-CH_2), 7.30 - 7.76 (m, 4H, arom.) 8.56 (dd, J = 6Hz, J = 3Hz,1H, C_{10} arom.), 8.96 (dd, J = 4Hz, J = 3Hz, 1H, C_{12} arom.)
5a	315.0(3.92) 307.0(3.92) 270.0(4.01)	1660	3.90 (5, 2H, CH_2), 7.16 - 7.80 (m, 5H, arom.), 8.14 (m, 1H, C_1 arom)
5b	318.0(4.00) 307.0(3.99) 269.0(3.98)	1680	1.60 (d, J = 6Hz, 3H,CH_3), 3.98 (q, J=6Hz,1H, CH), 7.16 - 8.83 (m, 5H, arom.), 8.10 (m, 1H, C_1 arom)
5c	273.0(4.42)	1670	2.40-3.56 (m, 4H, CH_2-CH_2), 7.26-7.86 (m, 6H, arom.)

Table 4 (continued)

Compound	UV_{EtOH}(log ε) λ max	IR nujol C=O cm^{-1}	^{1}H-NMR δ (ppm) and multiplicity*
5d	292.0(4.00) 270.0(4.06)	1680	2.36-3.53 (m, 4H, CH_2-CH_2), 7.20-7.90 (m, 5H, arom.)
5e	270.5(3.97)	1680	1.24 (d, J = 5Hz, 3H, CH_3), 2.13, 3.13, 3.70 (ABX system, 3H, CH-CH_2), 7.30 - 7.73 (m, 5H, arom.), 8.16 (d, J = 4Hz, 1H, C_9 arom.)
6b	275.0(4.08)	1715	2.52 - 3.56 (m, 4H, CH_2-CH_2), 7.03 - 7.76 (m, 3H, arom.), 8.88, 8.92 (2d, J = 11Hz, 2H, C_{10}, C_{11} arom.)
6c	272.5(4.19)	1700	2.63 - 3.56 (m, 4H, CH_2-CH_2), 7.23 - 7.93 (m, 3H, arom.), 8.84, 9.97(2d, J = 11Hz, 2H, C_{10}, C_{11} arom.)
6d	272.5(4.16)	1700	1.37 (d, J = 6Hz, 3H, CH_3), 2.33, 3.30, 3.76 (ABX system, 3H, CH-CH_2), 7.33 - 7.80 (m, 4H, arom.), 8.86, 8.90 (2d, J = 10 Hz, 2H, CH_{10}, C_{11} arom.)
7a	331.5(4.85) 271.5(5.02)	1690	3.92 (s, 2H, CH_2), 7.07 - 8.20 (m, 9H, arom.), 8.92 (s, 1H, C_{13} arom.)
7b	270.5(4.88)	1690	1.66 (d, J = 6Hz, 3H, CH_3), 4.00 (q, J = 6Hz,1H, CH), 7.06 - 8.16 (m, 9H, arom.), 8.90 (s, 1H, C_{13} arom.)
7c	278.0(*) 270.0(*)	1690	2.58 - 3.48 (m, 4H, CH_2-CH_2), 7.40 - 8.20 (m, 9H, arom.), 8.95 (s, 1H, C_{14} arom.)
7d	278.0(*) 271.0(*)	1680	2.57 - 3.50 (m, 4H, CH_2-CH_2), 7.37 - 8.33 (m, 8H, arom.), 8.93 (s, 1H, C_{14} arom.)
7e	270.0(5.21)	1690	2.57 - 3.50 (m, 4H, CH_2-CH_2), 7.50 - 8.20 (m, 8H, arom.), 8.92 (s, 1H, C_{14} arom.)
7f	278.0(4.88) 269.0(4.48)	1685	1.36 (d, J = 5Hz, 3H, CH_3), 2.30, 3.19, 3.73 (ABX system, 3H, $CHCH_2$), 7.30 - 8.23 (m, 9H, arom.), 8.90 (s, 1H, C_{14} arom)

* Abreviations have their usual significance

As compounds showed limited solubility in water, they were dissolved in dimethylsulfoxide and the solutions were then diluted with water. Agar plates with the compounds (200 μg/ml) included were used. Strains were inoculated with a multi-point inoculator 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.

3 - *In vitro* cytostatic activity

An established cell line derived from an oral epidermoid human carcinoma (KB) (13) was used for cytostatic effect evaluation. The KB cells were maintained and tested as monolayers in buffered Eagle's Minimal Essential Medium (MEM) supplemented with 10% newborn calf serum, 1% nonessential aminoacids as previously described (14,15). The cell population doubling time was *ca.* 24 h.

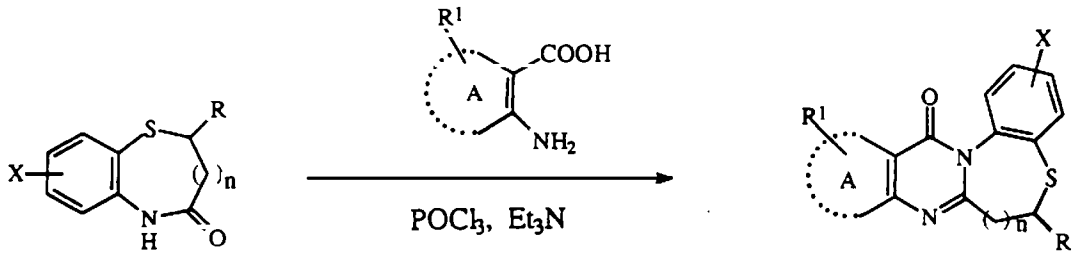
For the *in vitro* assay the cells in exponential growth phase were refed 24 h before testing, and seeded at $1x10^5$ cells per Leighton tube. The compounds were added 24 h after seeding in order to allow a cellular adhesion to substrate.

The compounds were dissolved immediately before use in sterile DMSO. 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 five concentration levels were used for each compound and each concentration value was tested in triplicate. Each compound was assayed on at least two 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 (16).

RESULTS AND DISCUSSION

Several methods for the synthesis of quinazolino[2,3-*c*][1,4]benzothiazine 2a and some of its derivatives are known (17-20). In this paper the tetracyclic compounds 2a-h (Table 1) were prepared modifying and improving a known method (20) to give the desired quinazolino[2,3-*c*][1,4]benzothiazines in increased yields and in a shorter time. Thus, the 1,4-benzothiazinones 1 were treated one-pot with phosphorylchloride, triethylamine and anthranilic acid and the mixture was heated at 60-70°C for 0.5-3h to give directly the tetracyclic compounds 2, without isolating the imidoyl chloride intermediate (Scheme).



1

X = H, Cl
R = H, CH_3
n = 0, 1

2a-h, 3a-t, 4a-d
5a-e, 6a-c, 7a-f

R1 = H, Cl, NO_2
A = benzo, pyrido, thieno, pyrazino, naphtho

This method was extended here to the synthesis of quinazolino[2,3-*d*][1,5]benzothiazepine 3a , a heterocyclic system of which only two derivatives are hitherto known (21,22), and of a number of its derivatives 3b-t (Table 1). The reported method (21) for the synthesis of the quinazolino[2,3-*d*][1,5]benzothiazepines started from the 1,5-benzothiazepin-4(5H)-one and consisted of a multistep procedure which gave moderate overall yield.

The ^{1}H-NMR spectra (Table 2) of benzothiazine derivatives 2a-h showed that the resonance signals for the two aromatic protons on C_1 and C_{11} appeared relatively downfield (δ 7.99-8.05 and 8.05-8.33, respectively). The deshielding effect on C_1 proton, due to the C=O group, depends on the planarity of the tetracyclic structure. In fact, the ^{1}H-NMR spectra of the benzothiazepine derivative 3a-t showed only the aromatic proton C_{12} (δ 8.13-9.14) downfield, as expected, because of the non-planar molecular conformation of the 1,5-benzothiazepine system.

The synthetic procedure described above was used also to prepare some derivatives of pyrido-, pyrazino-, thieno- and naphtho-pyrimidobenzothiazines and pyrimidobenzothiazepines which are new heterocyclic systems. To compare the synthetic procedures, these compounds were prepared according to both experimental conditions. Compounds 4a-g, 5a-e and 7a-f (Table 3) were obtained more easily and always in higher yields (80-100%) when our conditions were used, but no improvement was observed in the preparation of 4a,b and 6a-d: the reaction between benzothiazinones or benzothiazepinones and methyl-3-amino-2-thiophenecarboxylate gave rise directly to the tetracyclic compounds 5a-e, whereas, when the reported experimental conditions (17) were used, the open intermediate (methyl esters) were obtained.

It is notheworthy that, in general, 1,4-benzothiazines proved less reactive than 1,5-benzothiazepines and also that 2-aminonicotinic acid and 2-aminopyrazin-3-carboxylic acid showed less reactivity than the other reagents used (methyl-3-amino-2-thiophenecarboxylate and 3-amino-2-naphthoic acid). In fact, whereas the quinazolino, thieno and naphtho derivatives

were obtained in good yields, the pyridopyrimidobenzothiazines and the pyrazinopyrimidobenzothiazepines were formed in very low yields and the pyrazinopyrimidobenzothiazines could not be obtained in any case. In fact, in both conditions, the starting material or decomposition products were obtained, depending on the temperature conditions (above or below 40 °C).

All synthesized compounds were tested for antimicrobial activity but none of them showed remarkable results (MIC > 200 μg/ml).

The cytostatic activity evaluation of all synthesized compounds was performed *in vitro* against the growth of human KB tumor cells.

Whereas compounds 4a-g, 6a-d and 7a-f proved inactive, some compounds of the other series showed moderate cytostatic activity (Table 5).

Taking into account the obtained results it can be observed that the presence of the benzothiazepine skeleton seems to be favourable for the activity. Furthermore, it is worthy of note that the presence of electron-withdrawing substituents at the 10-position (nitro group or chloro) and at the 2- or 3-position (chloro) (3f, 3g, 3k, 3l) on the quinazolinobenzothiazepine remarkably improves the activity.

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Table 5 : Effect on the growth of KB cells

Compound	IC_{50}	
	μg/ml	μM
2a	5.23	19.7
2c	8.42	28.0
3a	>10	–
3d	6.31	19.4
3f	4.62	13.2
3g	2.30	6.4
3h	7.50	20.9
3i	9.79	31.1
3j	8.27	25.2

Table 5 (continued)

Compound	IC_{50}	
	μg/ml	μM
3k	2.55	7.3
3l	2.36	6.6
3m	7.57	21.1
3n	8.27	28.1
3p	8.96	26.4
3q	6.03	18.4
3s	5.18	13.8
5a	5.35	19.7
5e	9.66	32.2
6-Mercaptopurine*	0.13	0.86
Cisplatin*	0.13	0.43

* Drug used as reference

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