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Thioanalogues of anti-tumor antibiotics. II. Synthesis and preliminary *in vitro* cytotoxicity evaluation of tricyclic [1,4]benzothiazepine derivatives*

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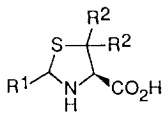
Summary — The synthesis of tricyclic [1,4]benzothiazepine derivatives starting from optically active cyclic amino acids and amino alcohols is described. The absolute configurations of the target compounds were assigned by X-ray and ^{1}H-NMR analyses and by molecular modeling studies. The cytotoxic activity of the tricyclic derivatives was tested *in vitro* by growth inhibition assays using murine L1210 and human lymphoblastic CCRF-CEM leukemias. Compounds **5**, **9**, and **10** exhibited marked cytotoxic activity.

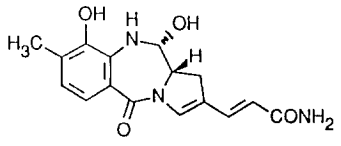
cytotoxicity / leukemia L1210 / 1,4-benzothiazepine / L-thioproline

Introduction

Recently (*R*)(–)-thiazolidine-4-carboxylic acid (L-thioproline, timonacic, norgamem) **1** and some related compounds, such as 2-phenylthiazolidine-4-carboxylic acid **2** and 5,5-dimethyl-2-phenylthiazolidine-4-carboxylic acid **3**, elicited much interest owing to their anti-tumor activities associated with low toxicity [1–9]. L-Thioproline, in particular, induced complete remission in most of the patients affected by epidermoid carcinoma of the head and neck when administered at doses of 40 mg/kg daily for 1 month, but was found to be inactive in several experimental tumor systems in mice, including L1210 and P388 leukemias, Lewis lung cancer and human xenograft in nude mouse. Such apparently contradictory results have been explained by Bassleer *et al* [5–6]. L-Thioproline is thought to act on the cell membrane of tumor cells by causing reverse transformation to normal cells. Moreover, L-thioproline seemed to be completely non-toxic over a wide range of doses (LD_{50} in mice was 300 mg/kg).



1 $R^1 = R^2 = H$
2 $R^1 = Ph$, $R^2 = H$
3 $R^1 = Ph$, $R^2 = CH_3$



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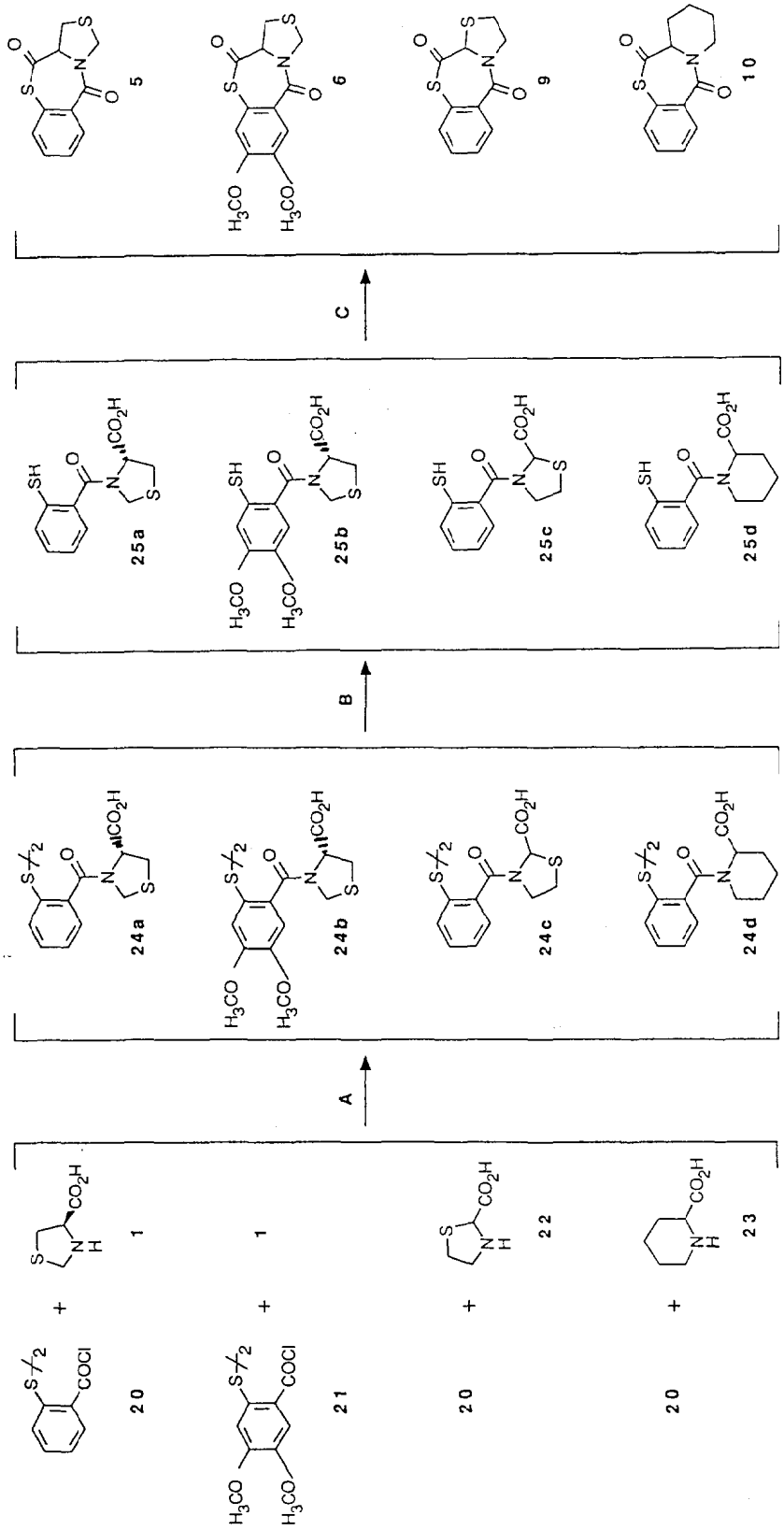
During the last few years, we have been engaged in the synthesis and biological evaluation of pyrrolo[2,1-*c*][1,4]benzothiazepine derivatives [10–14] as sulfur-containing analogues of anthramycin **4** and other pyrrolo[2,1-*c*][1,4]benzodiazepine anti-tumor antibiotics [15]. As an extension of our research, we decided to synthesize novel polycyclic systems annelating on a benzothiazepine skeleton the thiazolidine and its bioisosterically related piperidine ring. This paper describes the preparation of thiazolo[4,3-*c*][1,4]-benzothiazepine derivatives **5–8**, thiazolo[2,3-*c*][1,4]-benzothiazepine **9**, pyrido[2,1-*c*][1,4]benzothiazepine **10** and some 11-substituted 5-oxo-2,3,11,11a-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepine **11–14**. These compounds, along with the previously described pyrrolo[2,1-*c*][1,4]benzothiazepine derivatives **15–19** [13] (fig 1), have been tested for their growth inhibitory activity on L1210 and human lymphoblastic CCRF-CEM leukemia cells.

*Dedicated to Pr R Giuliano on the occasion of his 80th birthday.
**Correspondence and reprints.



Scheme 1. A: Na_2CO_3, THF/H_2O; B: $NaBH_4$, EtOH; C: CDI, THF.

15 R = H
16 R = OCH_3
17 RR = OCH_2O

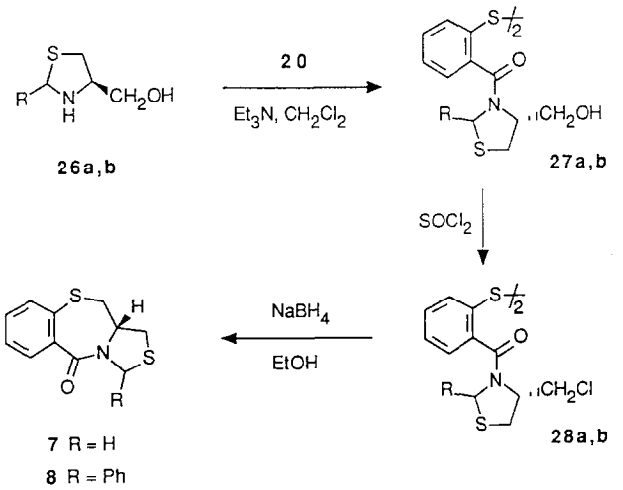
18 R = H
19 R = OCH_3

Fig 1. Compounds **15–19**.

Chemistry

The synthetic route followed in the preparation of compounds **5**, **6**, **9** and **10** is outlined in scheme 1. Schotten–Baumann reaction between dithiosalicylic acid chloride **20** [16] or the corresponding tetramethoxy derivative **21** [17] and the cyclic amino acids **1**, **22** [18] and **23** gave disulfides **24a–d**. Sodium borohydride reduction of the disulfides **24a–d** furnished the corresponding thiols **25a–d** in very good yields. Compounds **24a–d** and **25a–d** are amorphous and were used in the next step without thorough characterization. The final cyclization reaction was carried out with *N,N'*-carbonyldiimidazole (CDI) in dry tetrahydrofuran (THF) leading to optically inactive compounds due to racemization at C-11a. Other condensing reagents (dicyclohexylcarbodiimide/ copper(II) chloride, dicyclohexylcarbodiimide/1-hydroxybenzotriazole) were unable to avoid racemization.

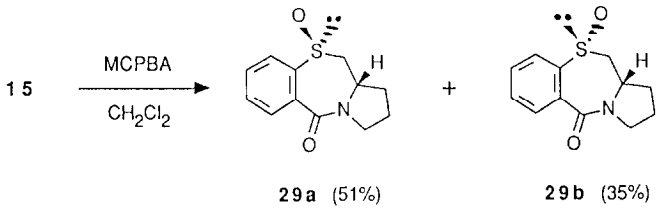
Compounds **7** and **8** were prepared as depicted in scheme 2. Acid chloride **20** was reacted with amino alcohols **26a**, **b** [19] to provide the amorphous diols **27a**, **b**, which were converted into the dichloro derivatives **28a**, **b** by treatment with thionyl chloride.



Scheme 2. a: R = H; **b**: R = Ph.

Reaction of **28a** and **28b** with sodium borohydride in refluxing ethanol directly afforded benzothiazepinones **7** and **8**, which were obtained as single stereoisomers, although configuration at C-3 of **8** was not assigned. 11-Substituted 5-oxo-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepines **11–14** were prepared starting from the previously reported 5-oxo-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]-benzothiazepine **15** (scheme 3). 3-Chloroperbenzoic acid oxidation of **15** gave the sulfoxides **29a** and **29b**, in a 1.5:1 ratio, which were separated by flash chromatography on silica gel. Since crystals of both diastereomers were not suitable for X-ray crystallographic analysis, their structure was elucidated on the basis of ^{1}H-NMR and molecular modeling studies. The first eluted component showed in the 200 MHz ^{1}H-NMR spectrum a doublet of doublets (J = 11.5 and 5.5 Hz) centered at δ 2.99 and an apparent triplet (J = 11.5 Hz) at δ 4.17 for the C-11 protons. Decoupling experiments revealed that the triplet was actually a doublet of doublets with $J_1 = J_2$ = 11.5 Hz. C-11 protons appeared in the spectrum of the second eluted compound as 2 doublets of doublets at δ 3.25 (J = 14.8 and 3.6 Hz) and δ 3.46 (J = 14.8 and 4.9 Hz). Inspection of Dreiding molecular models suggested that the α-proton at C-11 was deshielded in the case of the equatorial sulfoxide, whereas for the axial one both C-11 protons were equally affected by the SO group. In order to verify this hypothesis, we undertook a computer-assisted molecular modeling study.

Input geometry for **29a** and **29b** was generated and initially minimized by using the program MODEL (version KS 2.95) [20]. First a thorough conformational analysis using the grid option in MODEL was carried out in order to evaluate the putative global minimum energy conformations. A geometric optimization was then carried out, as far as convergence was reached, for the low energy conformations with the program MMX (version 89) [21]. Very similar global energies were obtained for both sulfoxides (ΔE = 0.05 kcal/mol), while noticeably different dipole moments were found: 3.65 D for the equatorial sulfoxide and 6.05 D for the axial one. The computer generated structures at minimum energy



Scheme 3.

showed a mutual spatial arrangement for the C-11 protons and the SO group, in agreement with that deduced by ^{1}H-NMR data and Dreiding models.

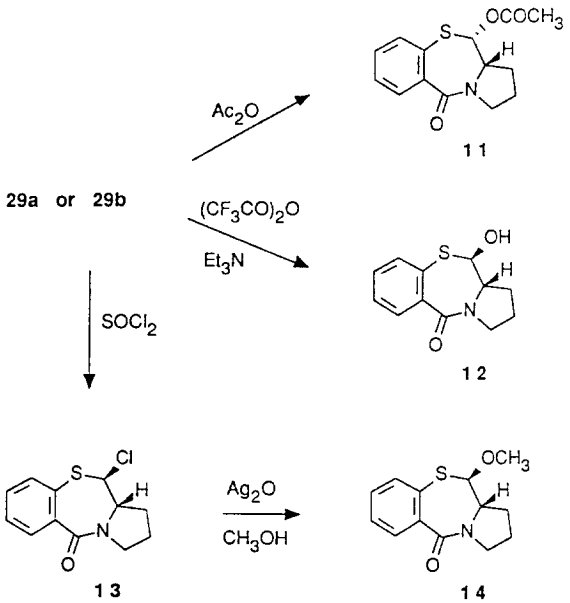
In light of these results, the less and more polar sulfoxides were identified as, respectively (10*S*,11*aS*)-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]-benzothiazepin-5-one 10-oxide **29a** (equatorial) and the (10*R*,11*aS*)-isomer **29b** (axial).

Both the sulfoxides **29a** and **29b** were subjected to Pummerer rearrangement in order to functionalize the 11 position of the pyrrolo[2,1-*c*][1,4]benzothiazepine nucleus. According to previous observations of Glue *et al* [22], the rearrangement reaction did not proceed stereospecifically, giving the same results irrespectively of the stereochemistry of the starting sulfoxide (scheme 4). When **29a** or **29b** was treated with acetic anhydride [23], (11*S*,11*aS*)-11-acetoxy-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepin-5-one **11** was obtained as a single isomer. Similarly, reaction with trifluoroacetic anhydride/triethylamine [23] afforded only the isomer (11*R*,11*aS*)-11-hydroxy-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]-benzothiazepin-5-one **12**. On the other hand, the reaction with thionyl chloride afforded a diastereomeric mixture of 11-chloro derivatives, from which only the isomer (11*S*,11*aS*)-11-chloro-2,3,11,11*a*-tetrahydro-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepin-5-one **13** was isolated by column chomatography (80% yield). Attempts to isolate the other diastereomer by high performance liquid chromatography (HPLC) fractional crystallization were unsuccessful. Treatment of **13** with silver oxide in methanol led to the methoxy derivative **14** with retention of configuration. The absolute stereochemistry of compound **13** was unambiguously assigned by X-ray crystallography (unpublished results) (fig 2). The pyrrolidine ring adopts a half-chair conformation with the C-1 and C-2 atoms out of the plane defined by C-3, N-4 and C-11a atoms of 0.21 Å and –0.31 Å, respectively. The 7-membered ring has a twisted boat-like conformation with the C-11 atom in the *S*-configuration. The structure of compounds **11**, **12** and **14** was inferred through correlation of their ^{1}H-NMR spectra with that of **13**. In fact, the coupling constant values for protons at C-11 and C-11a are 9.5–10.2 Hz or 1–3 Hz, depending upon a relative *trans*- or *cis*-configuration, respectively.

With the data available, despite our attempts to deduce a plausible reaction mechanism taking into account energetic, steric and stereoelectronic factors, we are not able to explain the stereoselective course of the above-described rearrangement reactions.

Results and discussion

The tricyclic compounds described above were subjected to preliminary *in vitro* anti-tumor evaluation against murine L1210 and human lymphoblastic CCRF-CEM leukemia cell growth. Table I shows the growth inhibition caused by each test compound at the dose of 100 μg/ml at the end of 24 h treatment and after 72 h in which L1210 cells were maintained in drug-free medium. Compounds **11**, **14** and **17** were



Scheme 4.

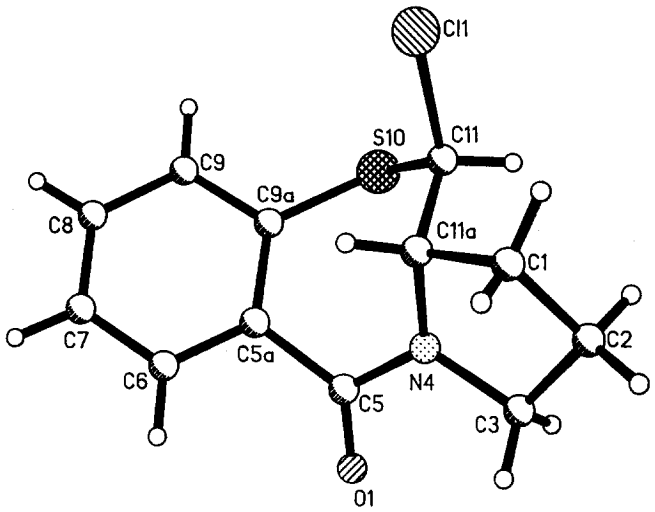


Fig 2. A perspective view of **13** with the atom numbering scheme.

Table I. Growth inhibition (%) of L1210 mouse leukemia cells[a].

Compd	*Concentration (μg/ml)*	*After 24 h exposure*	*72 h after drug wash-out*
5	100	42.6[b]	91.6[b]
	50	26.5[b]	29.3
	25	19.8	24.3
6	100	12.0	19.6[b]
8	100	23.2[b]	33.0[b]
9	100	42.4[b]	93.6[b]
	50	49.3[b]	56.1[b]
	25	24.2[b]	30.1
10	100	49.4[b]	75.8[b]
	50	36.7[b]	31.3[b]
	25	14.4	7.8
11	100	–3.7	–0.5
12	100	8.6[b]	13.9[b]
14	100	10.4[b]	7.1
15	100	37.4[b]	22.8[b]
16	100	19.2[b]	22.9[b]
17	100	4.6	–0.4
18	100	12.7	20.7[b]
19	100	23.5[b]	22.3[b]
1	100	11.2[b]	3.7
22	100	7.2	16.4
23	100	12.9[b]	2.1

[a]The data are the average of 4 replications. Standard deviations never exceeded 10% of the mean values. [b]$P < 0.01$; Student's *t*-test.

Table II. Growth inhibition (%) of CCRF-CEM human leukemia cells[a].

Compd	*Concentration (μg/ml)*	*After 24 h exposure*	*72 h after drug wash-out*
5	100	41.1[b]	70.2[b]
	50	12.3	6.6
	25	3.3	0.2
9	100	53.7[b]	95.1[b]
	50	11.6	15.2
	25	2.0	–1.1
10	100	55.3[b]	97.6[b]
	50	22.3[b]	34.9[b]
	25	0.1	–0.8

[a]The data are the average of 4 replications. Standard deviations never exceeded 10% of the mean values. [b]$P < 0.01$; Student's *t*-test.

devoid of any detectable activity. Compound **12** caused a small, but statistically significant growth inhibition. Growth inhibition of between 20 and 30% at 72 h, was observed for compounds **6**, **8**, **15**, **16**, **18** and **19**. High activity was exhibited by compounds **5**, **9** and **10** (75–94% inhibition at 72 h). These latter substances retained outstanding cell growth inhibitory activity when tested at 50 μg/ml. Compound **9** was even active at 25 μg/ml. Compounds **5**, **9** and **10** were also effective against CCRF-CEM human acute lymphoblastic leukemia cells at a concentration of 100 μg/ml (table II). Compound **10** was also active at a concentration of 50 μg/ml.

High irreversible cytotoxic activity was only exhibited by compounds bearing a thiolactone moiety. The only slight activity shown by dimethoxy derivative **6** was likely due to its poor solubility, which caused partial reprecipitation in the test medium.

The association found between cytotoxic effects and thiolactone structure could mean that compounds **5**, **9** and **10** act as carriers of L-thioproline **1** and related amino acids **22** and **23**, which would, in all cases, be released into the test culture. However, the inactivity of **1**, **22** and **23** against L1210 leukemia is well documented in the literature and was further demonstrated by our own results. Therefore, the growth-inhibitory activity shown by compounds **5**, **9** and **10** has to be attributed to the whole molecule.

Experimental protocols

Chemistry

Melting points were taken on an Electrothermal 8103 apparatus and are uncorrected. Optical rotations were measured on an optical activity polarimeter. IR spectra (nujol mulls) were obtained using a Perkin–Elmer 398 spectrophotometer. Mass spectra were recorded on a Kratos MS 80 spectrometer with an electron beam of 70 eV. ^{1}H-NMR spectra were run on a Varian XL 200 spectrometer with tetramethylsilane as the internal standard. Kieselgel 60 Merck (230–400 mesh) was used for flash chromatography columns. Dowex 50 x 2 200 resin (Aldrich) was used for ion-exchange chromatography. Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values and were performed on a Perkin–Elmer 240C elemental analyzer in our department. Anhydrous sodium sulfate was used to dry organic extracts. All the reactions were carried out under a nitrogen atmosphere.

Chemical and spectroscopic data of the new compounds are reported in tables III and IV.

General preparation of disulfides ***24a–d***

The appropriate amino acid **1**, **22** or **23** (3 mmol) was dissolved in water (30 ml) containing sodium carbonate (1.5 mmol). The mixture was cooled with an ice-water bath. The acid chloride **20** or **21** (1.5 mmol) in dry THF (100 ml) was added dropwise and small portions of sodium carbonate were added from time to time to maintain a weakly alkaline pH. The reaction was stirred at room temperature overnight, then concentrated and made acidic by adding 37% HCl. The gummy precipitate was extracted into ethyl acetate. The organic layer was washed with water, dried and evaporated to give a foam. An analytical sample was obtained by ion–exchange column chromatography with methanol as the eluent. After evaporation of the eluate, the residue was taken up in chloroform and the solution was washed with water, dried and evaporated to furnish pure **24a–d**.

Table III. Chemical data of new compounds.

Compd	*Molecular formula* [a]	*Yield (%)*	*mp (°C)*	*Solv* [b]	$[\alpha]_D^{20}$ [c]
5	$C_{11}H_9NO_2S_2$	85	145–146	A	0
6	$C_{13}H_{13}NO_4S_2$	71	234–236	B	0
7	$C_{11}H_{11}NOS_2$	83	[d]		+583
8	$C_{17}H_{15}NOS_2$	88	219–220	C	–
9	$C_{11}H_9NO_2S_2$	64	177	C	0
10	$C_{13}H_{13}NO_2S$	65	124–125	D	0
11	$C_{14}H_{15}NO_3S$	58	183	E	+528
12	$C_{12}H_{13}NO_2S$	76	226–227	F	+115
13	$C_{12}H_{12}ClNOS$	80	170–172	G	+84
14	$C_{13}H_{15}NO_2S$	83	139–140	H	–120
24a	$C_{22}H_{20}N_2O_6S_4$	91	[d]		–208
24b	$C_{26}H_{28}N_2O_{10}S_4$	86	[d]		–50
24c	$C_{22}H_{20}N_2O_6S_4$	90	[d]		–
24d	$C_{26}H_{28}N_2O_6S_2$	89	[d]		–
25a	$C_{11}H_{11}NO_3S_2$	82	[d]		–198
25b	$C_{13}H_{15}NO_5S_2$	77	[d]		–
25c	$C_{11}H_{11}NO_3S_2$	76	[d]		–
25d	$C_{13}H_{15}NO_3S$	77	[d]		–
27a	$C_{22}H_{24}N_2O_4S_4$	88	[d]		–
27b	$C_{34}H_{32}N_2O_4S_4$	85	[d]		–
28a	$C_{22}H_{22}Cl_2N_2O_2S_4$	83	[d]		–185
28b	$C_{34}H_{30}Cl_2N_2O_2S_4$	75	[d]		–
29a	$C_{12}H_{13}NO_2S$	51	167–168	I	+259
29b	$C_{12}H_{13}NO_2S$	35	172–173	A	+446

[a]Anal C, H, N; [b]crystallization solvent: A: benzene/petroleum ether; B: toluene; C: benzene; D: ethyl ether; E: isopropanol; F: ethyl acetate/ethanol; G: carbon tetrachloride; H: cyclohexane; I: 2-butanone; [c]measured for 1% chloroform solutions; [d]amorphous or glassy solid.

*General preparation of thiols **25a–d***
To a solution of the proper disulfide **24a–d** (5 mmol) in 85% ethanol (40 ml) and NaOH (0.41 g), a solution of sodium borohydride (10 mmol) in ethanol (15 ml) was added dropwise. The resulting solution was stirred for 0.5 h at room temperature and for 0.5 h at 80°C. After concentration *in vacuo*, ice–water (100 ml) was added. The mixture was stirred for 15 min, then filtered and cooled. Acidification with conc HCl gave a gum which was extracted into ethyl acetate. The organic layer was washed with water and dried. Evaporation of the solvent gave a foam which underwent preliminary purification by passage thorough an ion-exchange resin column as described above to obtain foamy products **25a–d** of no distinct melting point.

*General preparation of tricyclic compounds **5, 6, 9 and 10***
To a solution of the appropriate thiol **25a–d** (6 mmol) in dry THF (25 ml) *N,N'*-carbonyldiimidazole (6 mmol) was added in portions. The mixture was stirred for 40 h at room temperature, then refluxed for 7 h. The solvent was evaporated and the residue was taken up in chloroform. The organic solution was washed successively with 0.5 N HCl, sodium bicarbonate saturated solution, water and brine. After drying and evaporation, the pasty residue was chromatographed on silica gel eluting with 8% methanol in dichloromethane to afford the title compounds.

*General preparation of disulfides **27a, b***
To a solution of amino alcohol **26a** or **26b** (9 mmol) and triethylamine (9 mmol) in dry dichloromethane (50 ml), kept at –10°C, a solution of **20** (4.5 mmol) in the same solvent (50 ml) was added dropwise. The mixture was stirred overnight at room temperature, then washed with water and dried. Evaporation of the solvent left a foamy residue, which was chromatographed on silica gel eluting with 8% methanol in dichloromethane to give the pure diols **27a, b.**

*General preparation of the chlorides **28a, b***
Freshly distilled thionyl chloride (48 mmol) was added dropwise to a cooled (0°C) solution of the diol **27a, b** (12 mmol) in chloroform (60 ml). After 0.5 h at 0°C, the temperature was gradually raised to reflux (1 h). Evaporation of the volatiles left a residue which was azeotropically dried with benzene and purified by column chromatography on silica gel eluting with dichloromethane to give **28a, b** as flaky products of no distinct melting point.

*General preparation of the tricyclic compounds **7 and 8***
Sodium borohydride tablets (24 mmol) were added to a solution of **28a, b** (5 mmol) in ethanol (100 ml). The mixture was refluxed for 2 h, then concentrated and diluted with water. The pH was adjusted to 6 by means of 10% acetic acid. Extraction with dichloromethane, followed by the usual work-up, afforded a pasty residue, which was purified by column chromatography on silica gel eluting with 20% light petroleum ether in ethyl acetate to give the title compounds.

Table IV. Spectroscopic data of new tricyclic compounds.

Compd	*IR (cm^{-1})*	*^{1}H-NMR (δ, ppm)* [a]	*MS (m/z): (%)*
5	1695, 1630	3.17 (dd, J = 12 and 6.5 Hz, 1H, 1-H), 3.64 (dd, J = 12 and 1.7 Hz, 1H, 1-H), 4.58 (dd, J = 6.5 and 1.7 Hz, 1H, 11*a*-H), 4.70 and 4.89 (AB q, J = 10.5 Hz, 2H, 3-CH_2), 7.50 (m, 3H, Ar-H), 7.96 (m, 1 H, Ar-H)	251 (M^+, 9)
6	1700, 1640	3.16 (dd, J = 12 and 6.6 Hz, 1H, 1-H), 3.64 (dd, J = 12 and 1.8 Hz, 1H, 1-H), 3.91 and 3.94 (each s, 3H, OCH_3), 4.60 (dd, J = 6.6 and 1.8 Hz, 1H, 11*a*-H), 4.66 and 4.91 (AB q, J = 10.4 Hz, 2H, 3-CH_2), 6.85 (s, 1H, 6-H), 7.43 (s, 1H, 9-H)	311 (M^+, 13)
7	1625	2.76 (dd, J = 11.9 and 1 Hz, 1H) and 3.25 (m, 3H, 1- and 11-CH_2), 3.96 (m, 1H, 11*a*-H), 4.74 (s, 2H, 3-CH_2), 7.3–7.7 (m, 4H, Ar-H)	237 (M^+, 75)
8	1645	2.85 (dd, J = 12 and 2 Hz, 1H) and 3.42 (m, 3H, 1- and 11-CH_2), 4.22 (m, 1H, 11*a*-H), 6.44 (s, 1H, 3-H), 7.2–7.8 (m, 9H, Ar-H)	313 (M^+, 76)
9	1700, 1630	3.16 (m, 2H, 2-CH_2), 4.03 (m, 2H, 3-CH_2), 5.36 (s, 1 H, 11*a*-H), 7.49 (m, 3H, Ar-H), 7.96 (m, 1H, Ar-H)	251 (M^+, no peak)
10	1710, 1645	1.35–2.15 (m, 6H, 1-, 2- and 3-CH_2), 2.74 (ddd, J = 13.7 and 2.7 Hz, 1H, 4-H), 4.49 (d, J = 4.8, 1H, 12*a*-H), 4.73 (dd, J = 13.7 and 2.8 Hz, 1H, 4-H), 7.30–7.50 (m, 3H, Ar-H), 7.83 (m, 1 H, Ar-H)	247 (M^+, no peak)
11	1740, 1630	1.85–2.25 (m, 4H, 1- and 2-CH_2), 2.16 (s, 3H, CH_3), 3.81–3.95 (m, 3H, 3-CH_2 and 11*a*-H), 6.29 (d, J = 1 Hz, 1H, 11 -H), 7.25–7.48 (m, 3H, Ar-H), 7.78 (m, 1 H, Ar-H)	277 (M^+, 16)
12	3210, 1605	1.91–2.11 (m, 4H, 1- and 2-CH_2), 3.37–3.80 (m, 3H, 3-CH_2 and 11*a*-H), 4.23 (d, J = 5.7 Hz, 1H, OH, exchangeable), 5.11 (dd, J = 5.7 and 9.7 Hz, 1 H, 11 -H), 7.30–7.50 (m, 3H, Ar-H), 7.73 (m, 1 H, Ar-H)	235 (M^+, 20)
13	1635	1.95–2.25 (m, 4H, 1- and 2-CH_2), 3.55–3.90 (m, 3H, 3-CH_2 and 11*a*-H), 5.27 (d, J = 10.2 Hz, 1H, 11-H), 7.41–7.65 (m, 3H, Ar-H), 7.79 (m, 1 H, Ar-H)	253 (M^+, 29)
14	1640	2.00 (m, 4H, 1- and 2-CH_2), 3.30–3.83 (m, 3H, 3-CH_2 and 11*a*-H), 3.45 (s, 3H, OCH_3), 4.67 (d, J = 9.6 Hz, 1H, 11-H), 7.33–7.50 (m, 3H, Ar-H), 7.72 (m, 1 H, Ar-H)	249 (M^+, 100)
29a	1645, 1070	1.75–2.40 (m, 4H, 1- and 2-CH_2), 2.99 (dd, J = 11.5 and 5.5 Hz, 1H, β-11-H), 3.44–3.73 (m, 2H, 3-CH_2), 3.92 (m, 1H, 11*a*-H), 4.17 (t, J = 11.5 Hz, 1H, α-11-H), 7.65–7.91 (m, 4H, Ar-H)	235 (M^+, 26)
29b	1645, 1045	1.75–2.28 (m, 4H, 1- and 2-CH_2), 3.25 (dd, J = 14.8 and 3.6 Hz, 1 H, 11-H), 3.46 (dd, J = 14.8 and 4.9 Hz, 1 H, 11-H), 3.52–3.82 (m, 2H, 3-CH_2), 4.00 (m, 1 H, 11*a*-H), 7.61–7.80 (m, 4H, Ar-H)	235 (M^+, 8)

[a]All the spectra were run in $CDCl_3$ except for compounds **29a** and **29b** (CD_3OD).

(10S,11aS)-2,3,11,11a-Tetrahydro-1H,5H-pyrrolo[2,1-c][1,4]-benzothiazepin -5-one 10-oxide ***29a*** *and (10R,11aS)-2,3,11,11a-tetrahydro-1H,5H-pyrrolo[2,1-c][1,4]benzothiazepin-5-one 10-oxide* ***29b***

To a stirred and cooled (0°C) solution of **15** (15.5 mmol) in dry dichloromethane (50 ml) 3-chloroperbenzoic acid (15.5 mmol) in the same solvent (80 ml) was added dropwise over about 1 h. After an additional 2 h at 0°C, the reaction mixture was filtered and the filter cake was rinsed with dichloromethane. The combined solution was washed twice with 5% aqueous potassium carbonate, dried and evaporated to give the crude **29**, which solidified on trituration with light petroleum ether. After flash chromatography (5% methanol in ethyl acetate), 1.85 g (51% yield) of **29a** were obtained. Further elution gave 1.27 g (35% yield) of **29b**.

(11S,11aS)-11-Acetoxy-2,3,11,11a-tetrahydro-1H,5H-pyrrolo-[2, 1-c][1,4]benzothiazepin-5-one ***11***

A mixture of **29a** or **29b** (22 mmol) and acetic anhydride (30 ml) was refluxed for 2.5 h (0.5 h for **29b**), then cooled and poured into crushed ice. A solid was obtained and separated by filtration, then washed thoroughly with water. Recrystallization from isopropanol gave **11** (4.6 g, 75%) as colorless needles.

(11R,11aS)-11-Hydroxy-2,3,11,11a-tetrahydro-1H,5H-pyrrolo-[2, 1-c][1,4]benzothiazepin-5-one ***12***

Trifluoroacetic anhydride (13.6 mmol) in dichloromethane (10 ml) was added dropwise to a solution of **29a** or **29b** (1.6 g, 6.8 mmol) and triethylamine (13.6 mmol) in dichloromethane (25 ml), at 0–5°C. After stirring for 1 h at the same temperature, the solution was diluted with diethyl ether (50 ml) and

quenched with 5% sodium hydroxide (100 ml). The organic layer was separated, washed with water and dried. Removal of the solvent gave a solid which was treated with cold ethyl acetate and filtered. Recrystallization from ethyl acetate–ethanol gave **12** (1.1 g, 68%) as colorless prisms.

(11S,11aS)-11-Chloro-2,3,11,11a-tetrahydro-1H,5H-pyrrolo-[2,1-c][1,4]benzothiazepin-5-one ***13***

The sulfoxide **29a** or **29b** (1.5 g, 6.4 mmol) was added portionwise to a stirred solution of thionyl chloride (5 ml) in dichloromethane (25 ml). After stirring for 2 h, the solution was poured into ice–water (250 ml) containing potassium hydroxide (10 g). The organic layer was separated and the aqueous layer was extracted twice with the same solvent. The combined organic solution was washed with water, dried and evaporated to leave a solid. Flash chromatography on silica gel (ethyl acetate: petroleum ether, 1:1, as the eluent) and subsequent recrystallization gave **13** (1.3 g, 80%) as a pure diastereomer (yellowish prisms).

(11R,11aS)-11-Methoxy-2,3,11,11a-tetrahydro-1H,5H-pyrrolo-[2,1 -c][1,4]benzothiazepin-5-one ***14***

Freshly prepared silver oxide, obtained from a methanolic solution of silver nitrate (2.3 g) and potassium hydroxide (0.5 g), was suspended in dry methanol. To this suspension a solution of **13** (1.77 g, 7 mmol) in methanol (150 ml) was added dropwise. The mixture was stirred at room temperature overnight in the dark, then filtered through Celite. Evaporation of the solvent gave a residue which solidified on cooling. Recrystallization afforded **14** (1.45 g, 83%) as colorless needles.

Biological methods

L1210 mouse lymphocytic leukemia cells were grown as a suspension culture in RPMI 1640 medium (Gibco Europe, Glasgow, Scotland) supplemented with 10% heat-inactivated (56°C, 0.5 h) fetal bovine serum (Gibco) and 2-mercaptoethanol (10 μM). CCRF-CEM human acute lymphoblastic leukemia cells were grown as a suspension culture in the same medium as above without 2-mercaptoethanol. Stock cultures of both cell lines were maintained in exponential growth at a density between 0.1×10^6 and 1×10^6 cells/ml.

Test compounds were dissolved as stock solutions in dimethylsulfoxide at a concentration of 10 mg/ml and afterwards diluted to 100 μg/ml in culture medium immediately before each experiment. Compounds **5**, **9** and **10** were also tested at 50 and 25 μg/ml.

Cells were seeded at a concentration of 1×10^5/ml in Nunc T25 flasks (Gibco). 24 h later, cells were treated with test compounds for 24 h at the concentrations reported above. After this time, cultures were washed by centrifugation with prewarmed phosphate-buffered saline (PBS) and resuspended in fresh drug-free medium. 24 h after treatment with chemical compounds and 72 h after drug washout, cell growth inhibition was evaluated by counting treated and untreated (control) cells in a Coulter Counter (Electronics Ltd, Harpenden Herts, UK). Data are the averages of 4 replicates. Controls and treated samples were diluted in fresh medium 48 h after seeding in order to maintain the cells in logarithmic phase growth.

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