Eur J Med Chem (1995) 30, 107–114

Synthesis of new alkylaminoalkyl thiosemicarbazones of 3-acetylindole and their effect on DNA synthesis and cell proliferation

T Siatra-Papastaikoudi[1], A Tsotinis[1], CP Raptopoulou[2], C Sambani[3], H Thomou[4]

[1]*Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopoli, Zografou, 157 71 Athens;*
[2]*Institute of Material Sciences, NCSR Demokritos;*
[3]*Institute of NT–Radiation Protection, NCSR Demokritos;*
[4]*Institute of Biology, NCSR Demokritos, 15310 Ag Paraskevi, Attica, Greece*

(Received 8 November 1993; final version received and accepted 28 September 1994)

Summary — The preparation of a number of thiosemicarbazones of 3-acetylindole is described. These compounds were evaluated *in vitro* for their effect on proliferation and cell-division delays in cultured human peripheral blood lymphocytes, and their effect on DNA synthesis in T-cell leukemia Molt-4 cells.

3-acetylindole / thiosemicarbazone / proliferation / cell division / cytotoxicity / human peripheral lymphocyte / Molt-4 cell

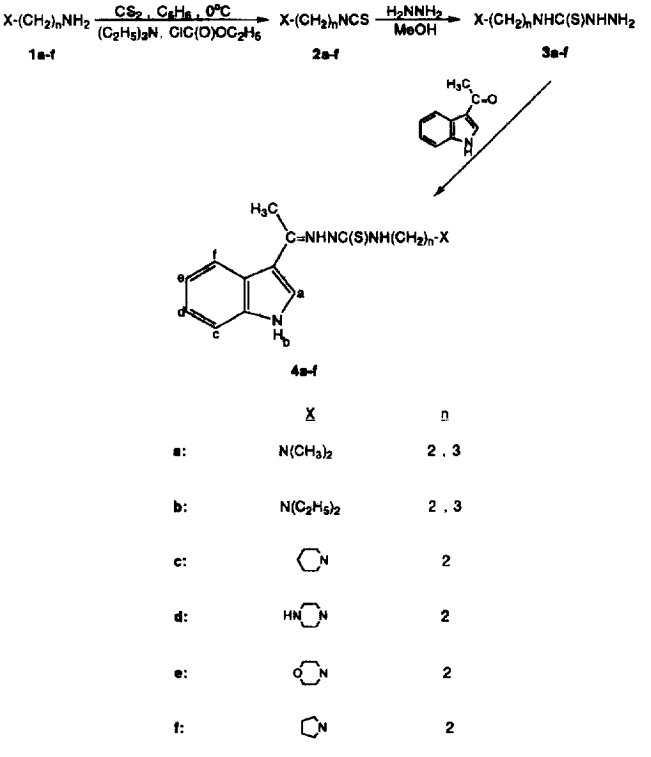
Introduction

Thiosemicarbazones of heterocyclic aldehydes and ketones exhibit various biological responses [1–3] and are particularly active against certain carcinomas [4] and viruses [5], including those belonging to the group of herpes viruses of DNA [6].

Structure–activity relationship studies on a large number of thiosemicarbazones of heterocyclic aldehydes and ketones revealed that the presence of an intact -HN-(C=S)-NH- moiety is essential for their anticancer activity [7]. It has also been demonstrated that their antineoplastic properties are due to the protogenic blocking of the synthesis of DNA in mammal cells by inhibiting the action of ribonucleoside-diphosphate anagogase or transcriptase [8–12]. These important findings encouraged us to explore the synthesis of new derivatives of thiosemicarbazones of 3-acetylindole (scheme 1). All the compounds were tested *in vitro* for their effect on proliferation and cell-division delays in cultured human peripheral blood lymphocytes [13–14], and their cytotoxic effect in human T-cell leukemia Molt-4 cells by measurement of [^{3}H]thymidine incorporation into DNA [15].

Chemistry

The alkylaminoalkylisothiocyanates **2a–f** were prepared by the reaction of the corresponding alkylaminoalkyl amines **1a–f** with carbon disulphide [16]. The products were isolated by fractional distillation under reduced pressure and were characterised by their spectral properties. Treatment of **2a–f** with hydrazine in methanol gave the corresponding alkylaminoalkyl



Scheme 1.

thiosemicarbazides **3a–f** [17], the structures of which were elucidated by IR and ^{1}H-NMR spectroscopy. Condensation of **3a–f** with 3-acetylindole in ethanol afforded the desired products **4a–f**. The ^{1}H-NMR spectrum of **4a** (n = 2) is in full accordance with the structure deduced from the X-ray analysis, which in addition clearly shows that the *E*-isomer was obtained (fig 1). The ^{1}H-NMR spectra of compounds **4a** (n = 3) and **4b–f** were also completely in accord with the proposed structures.

Results and discussion

As depicted in table I, the mitotic indices (MI) decrease as the concentrations of the products added are increased in the lymphocyte cultures. In the case of compounds **4a** (n = 2) and **4b** (n = 3), it was found that at the concentration value of 40 μg/ml, the mitotic indices are lowered by almost 50% compared to the untreated cultures. The relatively low MI indicates that at this concentration fewer cells reach metaphase as compared to the control. It is clear from these results that there is a concentration-dependent increase in the cell damage. As far as cell-division delay is concerned, the proliferation rate index (PRI) values (table II) are indicative of the observed case of cell-cycle delay. This fact constitutes evidence of biological damage provoked by the chemicals used. Moreover, as it can be seen, the cultures treated with the highest concentrations exhibit significantly lower PRIs compared to the untreated control. Measurement of [^{3}H]thymidine incorporation into DNA is one of the most commonly used methods of quantitation of drug cytotoxicity [15]. In order to evaluate the antineoplastic potential of the products, an established tumour cell line, the human T-cell leukemia Molt-4 cells, was used. Specifically, the cytotoxic effect of the products on DNA synthesis in Molt-4 cells was tested. As it can be seen (fig 2), a dramatic decline of DNA synthesis was observed as the concentration of the products was increased.

Table I. MI in cultured human lymphocytes after 48 h incubation with the products **4a** (n = 2), **4b** (n = 3), **4e** and **4f**.

Product	*Concentration (μg/ml)*	*MI (‰)*
Control	–	112
4a (n = 2)	20	92
	30	84
	40	51
4b (n = 3)	20	89
	30	73
	40	58
4e	20	102
	30	89
	40	62
4f	20	106
	30	90
	40	68

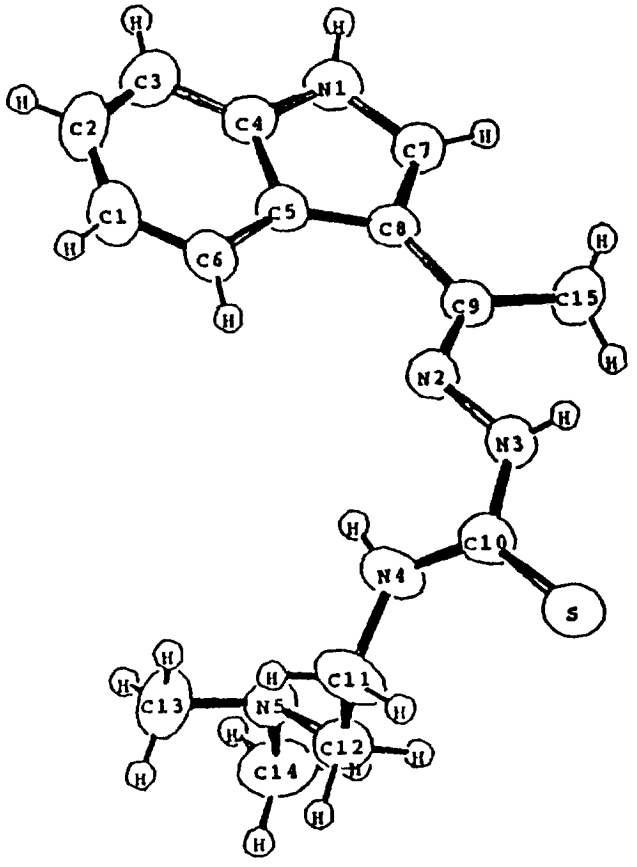


Fig 1. X-ray structure of 2-dimethylaminoethyl thiosemicarbazone of 3-acetylindole. Papaia. Rotations: –5.0 about x, –5.0 about y, and –90.0 about z.

Experimental protocols

Chemistry

Melting points were determined on a Büchi melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 177 spectrophotometer. Fourier transform proton magnetic resonance (^{1}H-NMR) spectra were recorded

Table II. Cell-division delays in cultured human lymphocytes treated with the products **4a** (n = 2), **4b** (n = 3), **4e** and **4f** after 72 h of incubation.

Product	*Concentration* ($\mu g/ml$)	*% of cells at* M_1	M_2	M_{3+}	*PRI*[a]
Control	–	1	20	79	2.78
4a (n = 2)	20	1	32	67	2.66
	30	3	51	46	2.43
	40	4	65	31	2.27
4b (n = 3)	20	1	29	70	2.69
	30	3	41	56	2.53
	40	4	65	31	2.27
4e	20	2	26	72	2.70
	30	4	37	59	2.55
	40	4	56	40	2.36
4f	20	1	24	75	2.74
	30	3	31	66	2.63
	40	5	48	47	2.42

[a]PRIs were calculated as $(1M_1 + 2M_2 + 3M_{3+})/100$, where M_1 is the percentage of cells in the first, M_2 in the second and M_{3+} in the third and higher divisions.

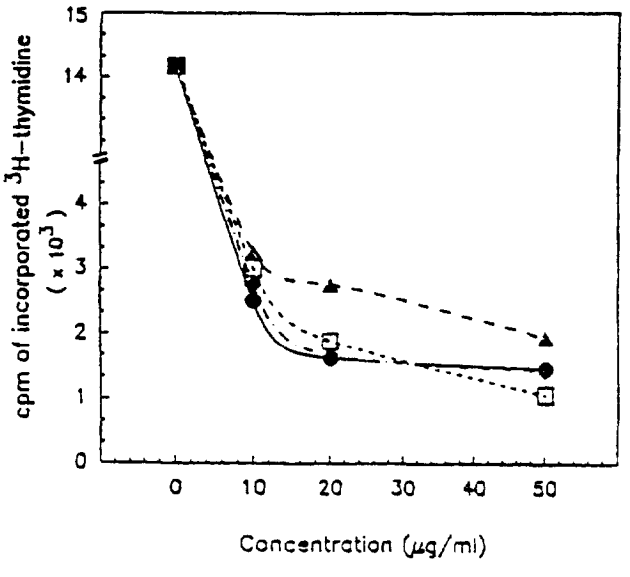


Fig 2. Decline of DNA synthesis in cultures of Molt-4 cells. Cells were treated with increased concentrations of the products **4a** (n = 2) (◆), **4b** (n = 3) (▲), **4e** (□) and **4f** (●). DNA synthesis was measured by incorporation of [^{3}H]thymidine. Each point represents the mean value of three independent experiments.

on a Bruker AC 200 MHz spectrometer in deuterated dimethyl sulphoxide (DMSO-d_6) and are reported in δ units relative to tetramethylsilane (TMS) as a standard. Analyses indicated by the symbols of the elements were within ±0.4% of theoretical values and were carried out by the Microanalytical Section of Service Central (CNRS, Paris).

Alkylaminoalkyl isothiocyanates ***2a–f***

These derivatives were synthesised by the following general method. Carbon disulphide (6.7 ml, 0.1 mol) was added to a stirred solution of the appropriate alkylaminoalkylamine **1a–f** (0.1 mol) in benzene (25 ml) at 0°C. Triethylamine (14 ml, 0.1 mol) was then added and the mixture was stirred for 2 h. The precipitated triethylammonium dithiocarbamate salt was filtered off, washed with anhydrous diethyl ether and then dissolved in chloroform (100 ml). The solution was treated with triethylamine (14 ml, 0.1 mol) and chilled to 0°C. Ethyl chloroformate (9.6 ml, 0.1 mol) was then added dropwise, taking care that the temperature was maintained at about 0°C. The resulting solution was then stirred at room temperature for 1 h and then treated with HCl (3 N). The chloroform layer was extracted with H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by fractional distillation under reduced pressure. The desired compounds **2a–f** were obtained as pale-yellow oils.

2a (n = 2). Yield: 61%. Bp 95°C, 15 mmHg. IR (cm^{-1}): 2180–2100 (N=C=S). ^{1}H-NMR: 2.03 (s, 6H, $N(CH_3)_2$), 2.50 (t, 2H, $CH_2N(CH_3)_2$, J = 7.02 Hz), 3.60 (t, 2H, CH_2NCS, J = 6.95 Hz).

2a *(n = 3).* Yield: 70%. Bp 120°C, 15 mmHg. IR (cm^{-1}): 2130–2100 (N=C=S). ^{1}H-NMR: 1.45 (m, 2H, $CH_2CH_2N(CH_3)_2$), 2.05 (s, 6H, $N(CH_3)_2$), 2.48 (m, 2H, $CH_2N(CH_3)_2$), 3.58 (m, 2H, CH_2NCS).

2b *(n = 2).* Yield: 65%. Bp 125°C, 15 mmHg. IR (cm^{-1}): 2050–2000 (N=C=S). ^{1}H-NMR: 1.10 (br, s, 6H, $N(CH_2CH_3)_2$), 2.40 (t, 2H, $CH_2N(C_2H_5)_2$, J = 7.01 Hz), 3.40 (t, 2H, CH_2NCS, J = 7.02 Hz), 3.60 (br, s, 4H, $N(CH_2CH_3)_2$).

2b *(n = 3).* Yield: 81%. Bp 130°C, 15 mmHg. IR (cm^{-1}): 2200–2180 (N=C=S). ^{1}H-NMR: 1.0 (br, s, 6H, $N(CH_2CH_3)_2$), 1.40 (m, 2H, $CH_2CH_2N(C_2H_5)_2$), 2.45 (m, 2H, $CH_2N(C_2H_5)_2$), 3.50 (m, 2H, CH_2NCS), 3.65 (br, s, 4H, $N(CH_2CH_3)_2$).

2c. Yield: 67%. Bp 119°C, 16 mmHg. IR (cm^{-1}): 2080–2000 (N=C=S). ^{1}H-NMR: 1.50 (m, 6H, $N(CH_2CH_2)_2CH_2$), 2.49 (t, 2H, $CH_2N(CH_2CH_2)_2CH_2$, J = 7.00 Hz), 2.74 (m, 4H, $N(CH_2CH_2)_2CH_2$), 3.60 (t, 2H, CH_2NCS, J = 7.02 Hz).

2d. Yield: 80%. Bp 158°C, 17 mmHg. IR (cm^{-1}): 2080–2000 (N=C=S). ^{1}H-NMR: 2.10 (m, 1H, *NH* piperazine), 2.39 (m, 2H, $CH_2N(CH_2CH_2)_2NH$), 2.62 (m, 4H, $N(CH_2CH_2)_2NH$), 2.88 (m, 4H, $N(CH_2CH_2)_2NH$), 3.58 (m, 2H, CH_2NCS).

2e. Yield: 66%. Bp 131°C, 14 mmHg. IR (cm^{-1}): 2100–2120 (N=C=S). ^{1}H-NMR: 2.50 (m, 2H, $CH_2N(CH_2CH_2)_2O$), 2.85 (m, 4H, $N(CH_2CH_2)_2O$), 3.58 (m, 2H, CH_2NCS), 3.65 (m, 4H, $N(CH_2CH_2)_2O$).

2f. Yield: 88%. Bp 143°C, 16 mmHg. IR (cm^{-1}): 2080–2000 (N=C=S). ^{1}H-NMR: 1.60 (m, 4H, $N(CH_2CH_2)_2$), 2.55 (m, 2H, $CH_2N(CH_2CH_2)_2$), 2.76 (m, 4H, $N(CH_2CH_2)_2$), 3.60 (m, 2H, CH_2NCS).

Alkylaminoalkyl thiosemicarbazides ***3a–f***

These compounds were prepared by the following general method. The appropriate alkylaminoalkyl isothiocyanate **2a–f** (0.1 mol) in methanol (25 ml) was added over a period of 15 min to a stirred solution of hydrazine (0.125 mol) in methanol (100 ml) at 0°C. The mixture was then allowed to reach ambient temperature and stirring was continued for 24 h. The solvent was removed by evaporation *in vacuo* and the crude product was recrystallised from ethyl alcohol. The compounds were obtained as yellowish solids.

3a *(n = 2).* Yield: 88%. Mp 130°C. IR (cm^{-1}): 1230 (C=S). Anal $C_5H_{14}N_4S$ (C, H, N). ^{1}H-NMR: 2.25 (s, 6H, $N(CH_3)_2$), 2.50 (m, 2H, $CH_2N(CH_3)_2$), 2.90 (m, 2H, CH_2NH), 4.10 (br, s, 2H, $NHNH_2$), 4.25 (m, 2H, $NHC(S)NHNH_2$).

3a *(n = 3).* Yield: 90%. Mp 139°C. IR (cm^{-1}): 1225 (C=S). Anal $C_6H_{16}N_4S$ (C, H, N). ^{1}H-NMR: 1.92 (m, 2H, $CH_2CH_2N(CH_3)_2$), 2.25 (s, 6H, $N(CH_3)_2$), 2.55 (m, 2H, $CH_2N(CH_3)_2$), 2.88 (m, 2H, CH_2NH), 4.10 (br, s, 2H, $NHNH_2$), 4.22 (m, 2H, $NHC(S)NHNH_2$).

3b *(n = 2).* Yield: 72%. Mp 115°C. IR (cm^{-1}): 1235 (C=S). Anal $C_7H_{18}N_4S$ (C, H, N). ^{1}H-NMR: 1.09 (br, s, 6H, $N(CH_2CH_3)_2$), 2.45 (m, 2H, $CH_2N(C_2H_5)_2$), 2.84 (m, 2H, CH_2NH), 3.55 (br, s, 4H, $N(CH_2CH_3)_2$), 4.12 (br, s, 2H, $NHNH_2$), 4.20 (m, 2H, $NHC(S)NHNH_2$).

3b *(n = 3).* Yield: 84%. Mp 165°C. IR (cm^{-1}): 1235 (C=S). Anal $C_8H_{20}N_4S$ (C, H, N). ^{1}H-NMR: 1.10 (br, s, 6H, $N(CH_2CH_3)_2$), 1.91 (m, 2H, $CH_2CH_2N(C_2H_5)_2$), 2.55 (m, 2H, $CH_2N(C_2H_5)_2$), 2.75 (m, 2H, CH_2NH), 3.45 (br, s, 4H, $N(CH_2CH_3)_2$), 4.10 (br, s, 2H, $NHNH_2$), 4.20 (m, 2H, $NHC(S)NHNH_2$).

3c. Yield: 85%. Mp 140°C. IR (cm^{-1}): 1230 (C=S). Anal $C_8H_{18}N_4S$ (C, H, N). ^{1}H-NMR: 1.55 (m, 6H, $N(CH_2CH_2)_2CH_2$), 2.42 (m, 2H, CH_2-$N(CH_2CH_2)_2CH_2$), 2.75 (m, 4H, $N(CH_2CH_2)_2CH_2$), 2.88 (m, 2H, CH_2NH), 4.11 (br, s, 2H, $NHNH_2$), 4.18 (m, 2H, $NHC(S)NHNH_2$).

3d. Yield: 80%. Mp 167°C. IR (cm^{-1}): 1230 (C=S). Anal $C_7H_{17}N_5S$ (C, H, N). ^{1}H-NMR: 2.10 (m, 1H, *NH* piperazine), 2.37 (m, 2H, $CH_2N(CH_2CH_2)_2NH$), 2.45 (m, 4H, $N(CH_2CH_2)_2NH$), 2.88 (m, 4H, $N(CH_2CH_2)_2NH$), 2.95 (m, 2H, CH_2NH), 4.09 (br, s, 2H, $NHNH_2$), 4.16 (m, 2H, $NHC(S)NHNH_2$).

3e. Yield: 92%. Mp 170°C. IR (cm^{-1}): 1220 (C=S). Anal $C_7H_{16}N_4SO$ (C, H, N). ^{1}H-NMR: 2.45 (m, 2H, $CH_2N(CH_2CH_2)_2O$), 2.87 (m, 4H, $N(CH_2CH_2)_2O$), 2.90 (m, 2H, CH_2NH), 3.64 (m, 4H, $N(CH_2CH_2)_2O$), 4.10 (br, s, 2H, $NHNH_2$), 4.18 (m, 2H, $NHC(S)NHNH_2$).

3f. Yield: 82%. Mp 120°C. IR (cm^{-1}): 1225 (C=S). Anal $C_7H_{16}N_4S$ (C, H, N). ^{1}H-NMR: 1.60 (m, 4H, $N(CH_2CH_2)_2$), 2.42 (m, 2H, $CH_2N(CH_2CH_2)_2$), 2.70 (m, 4H, $N(CH_2CH_2)_2$), 2.90 (m, 2H, CH_2NH), 4.11 (br, s, 2H, $NHNH_2$), 4.17 (m, 2H, $NHC(S)NHNH_2$).

Alkylaminoalkyl thiosemicarbazones of 3-acetylindole ***4a–f***

The 3-acetylindole derivatives were synthesised by the following general method. The appropriate alkylaminoalkyl thiosemicarbazide **3a–f** (0.03 mol) in ethanol (20 ml) was added to an ethanolic solution (20 ml) of 3-acetylindole (0.03 mol) at ambient temperature. The mixture was then heated under reflux for 4 h. Upon cooling, the product crystallised, filtered off, washed with cold aqueous ethanol and dried. The crude solid was recrystallised from ethyl alcohol. The desired compounds were obtained as pale-yellow solids.

4a *(n = 2).* Yield: 88%. Mp 147°C. IR (cm^{-1}): 3335 (N-H), 1625 (C=N), 1225 (C=S). Anal $C_{15}H_{21}N_5S$ (C, H, N). ^{1}H-NMR: 2.06 (s, 6H, $N(CH_3)_2$), 2.18 (s, 3H, CH_3), 2.52 (m, 2H, $CH_2N(CH_3)_2$), 2.90 (m, 2H, CH_2NH), 4.12 (br, s, 1H, $NHCH_2$), 6.95 (s, 1H, C=NN*H*), 7.09 (ddd, 1H, [H_e], J_{ed} = 7.10 Hz, J_{ef} = 7.90 Hz, J_{ec} = 1.28 Hz), 7.19 (ddd, 1H, [H_d], J_{de} = 7.10 Hz, J_{dc} = 8.09 Hz, J_{df} = 1.18 Hz), 7.30 (d, 1H, [H_a], J_{ab} = 2.52 Hz), 7.42 (ddd, 1H, [H_c], J_{cd} = 8.09 Hz, J_{cf} = 0.90 Hz, J_{ce} = 1.28 Hz), 7.56 (ddd, 1H, [H_f], J_{fe} = 7.90 Hz, J_{fd} = 1.18 Hz, J_{fc} = 0.90 Hz) and 10.30 (d, 1H, [H_b], J_{ba} = 2.52 Hz).

4a *(n = 3).* Yield: 80%. Mp 270°C. IR (cm^{-1}): 3340 (N-H), 1625 (C=N), 1225 (C=S). Anal $C_{16}H_{23}N_5S$ (C, H, N). ^{1}H-NMR: 1.95 (m, 2H, $CH_2CH_2N(CH_3)_2$), 2.01 (s, 6H, $N(CH_3)_2$, 2.18 (s, 3H, CH_3), 2.58 (m, 2H, $CH_2N(CH_3)_2$), 2.88 (m, 2H, CH_2NH), 4.10 (br, s, 1H, $NHCH_2$), 6.92 (s, 1H, C=NN*H*), 7.10–8.15 (m, 5H, aromatic), 10.28 (s, 1H, [H_b]).

4b *(n = 2).* Yield: 85%. Mp 165°C. IR (cm^{-1}): 3330 (N-H), 1635 (C=N), 1230 (C=S). Anal $C_{17}H_{25}N_5S$ (C, H, N). ^{1}H-NMR: 1.10 (br, s, 6H, $N(CH_2CH_3)_2$), 2.20 (s, 3H, CH_3), 2.40 (m, 2H, $CH_2N(C_2H_5)_2$), 2.78 (m, 2H, CH_2NH), 3.58 (br, s, 4H, $N(CH_2CH_3)_2$), 4.10 (br, s, 1H, $NHCH_2$), 6.96 (s, 1H, C=NN*H*), 7.08–8.15 (m, 5H, aromatic), 10.29 (s, 1H, [H_b]).

4b *(n = 3).* Yield: 65%. Mp 180°C. IR (cm^{-1}): 3330 (N-H), 1630 (C=N), 1225 (C=S). Anal $C_{18}H_{27}N_5S$ (C, H, N). ^{1}H-NMR: 1.12 (br, s, 6H, $N(CH_2CH_3)_2$), 1.92 (m, 2H, CH_2CH_2-$N(C_2H_5)_2$), 2.20 (s, 3H, CH_3), 2.50 (m, 2H, $CH_2N(C_2H_5)_2$), 2.80 (m, 2H, CH_2NH), 3.55 (br, s, 4H, $N(CH_2CH_3)_2$), 4.12 (br, s, 1H, $NHCH_2$), 7.00 (s, 1H, C=NNH), 7.40–8.15 (m, 5H, aromatic), 10.30 (s, 1H, [H_b]).

4c. Yield: 88%. Mp 172°C. IR (cm^{-1}): 3335 (N-H), 1630 (C=N), 1225 (C=S). Anal $C_{18}H_{25}N_5S$ (C, H, N). ^{1}H-NMR: 1.52 (m, 6H, $N(CH_2CH_2)_2CH_2$), 2.25 (s, 3H, CH_3), 2.43 (m, 2H, $CH_2N(CH_2CH_2)_2CH_2$), 2.76 (m, 4H, $N(CH_2CH_2)_2CH_2$), 2.88 (m, 2H, CH_2NH), 4.10 (br, s, 1H, $NHCH_2$), 6.99 (s, 1H, C=NNH), 7.08–8.15 (m, 5H, aromatic), 10.25 (s, 1H, [H_b]).

4d. Yield: 72%. Mp 160°C. IR (cm^{-1}): 3330 (N-H), 1630 (C=N), 1230 (C=S). Anal $C_{17}H_{24}N_6S$ (C, H, N). ^{1}H-NMR: 2.09 (m, 1H, NH piperazine), 2.21 (s, 3H, CH_3), 2.35 (m, 2H, CH_2-$N(CH_2CH_2)NH$), 2.45 (m, 4H, $N(CH_2CH_2)NH$), 2.90 (m, 4H, $N(CH_2CH_2)NH$), 2.95 (m, 2H, CH_2NH), 4.08 (br, s, 1H, $NHCH_2$), 6.95 (s, 1H, C=NNH), 7.08–8.16 (m, 5H, aromatic), 10.26 (s, 1H, [H_b]).

4e. Yield: 75%. Mp 167°C. IR (cm^{-1}): 3335 (N-H), 1630 (C=N), 1225 (C=S). Anal $C_{17}H_{23}N_5SO$ (C, H, N). ^{1}H-NMR: 2.20 (s, 3H, CH_3), 2.45 (m, 2H, $CH_2N(CH_2CH_2)_2O$), 2.80 (m, 4H, $N(CH_2CH_2)_2O$), 2.90 (m, 2H, CH_2NH), 3.65 (m, 4H, $N(CH_2CH_2)_2O$), 4.10 (br, s, 1H, $NHCH_2$), 6.95 (s, 1H, C=NNH), 7.06–8.15 (m, 5H, aromatic), 10.30 (s, 1H, [H_b]).

4f. Yield: 65%. Mp 170°C. IR (cm^{-1}): 3330 (N-H), 1625 (C=N), 1230 (C=S). Anal $C_{17}H_{23}N_5S$ (C, H, N). ^{1}H-NMR: 1.60 (m, 4H, $N(CH_2CH_2)_2$), 2.20 (s, 3H, CH_3), 2.45 (m, 2H, CH_2-$N(CH_2CH_2)_2$), 2.71 (m, 4H, $N(CH_2CH_2)_2$), 2.90 (m, 2H, CH_2-NH), 4.10 (br, s, 1H, $NHCH_2$), 6.96 (s, 1H, C=NNH), 7.10–8.17 (m, 5H, aromatic), 10.32 (s, 1H, [H_b]).

Biology

Lymphocyte cultures were prepared by adding 0.3 ml heparinised whole blood from normal subjects to 5 ml McCoy's 5A medium (Seromed) supplemented with 10% FCS (fetal calf serum), glutamine, antibiotics and phytohemagglutinin (PHA-L, Seromed). The products tested (**4a** ($n = 2$), **4b** ($n = 3$), **4e** and **4f**) were those that could be dissolved. Thus, these compounds were dissolved in ethanol, appropriate dilutions were made in McCoy's 5A culture medium without calf serum and added at the beginning of the cultures. The final concentration of ethanol used to dissolve the products (2 µl/ml of culture medium) was added to the control cultures.

In order for the MI to be evaluated, a period of incubation of 48 h at 37°C was required. Three hours prior to harvesting, colcemid was added at 0.2 µg/ml. The cells were then fixed in methanol/acetic acid (3:1) after being pre-treated with hypotonic solution (0.075 M, KCl). Next, they were dropped on wet slides, air-dried and finally stained with Giemsa. MIs were calculated as the amount of cells in mitosis after 1000 cells per tested sample had been analysed (table I) [18].

As far as cell-division analysis is concerned, the cultures were incubated for 72 h at 37°C. The BrdU-Giemsa technique, a method for the differential staining of sister chromatids, was employed [19]. 5-Bromodeoxyuridine (BrdU, 20 µM) was added 24 h after initiation of culture. The cultures were maintained in the dark throughout to minimise photolysis of BrdU.

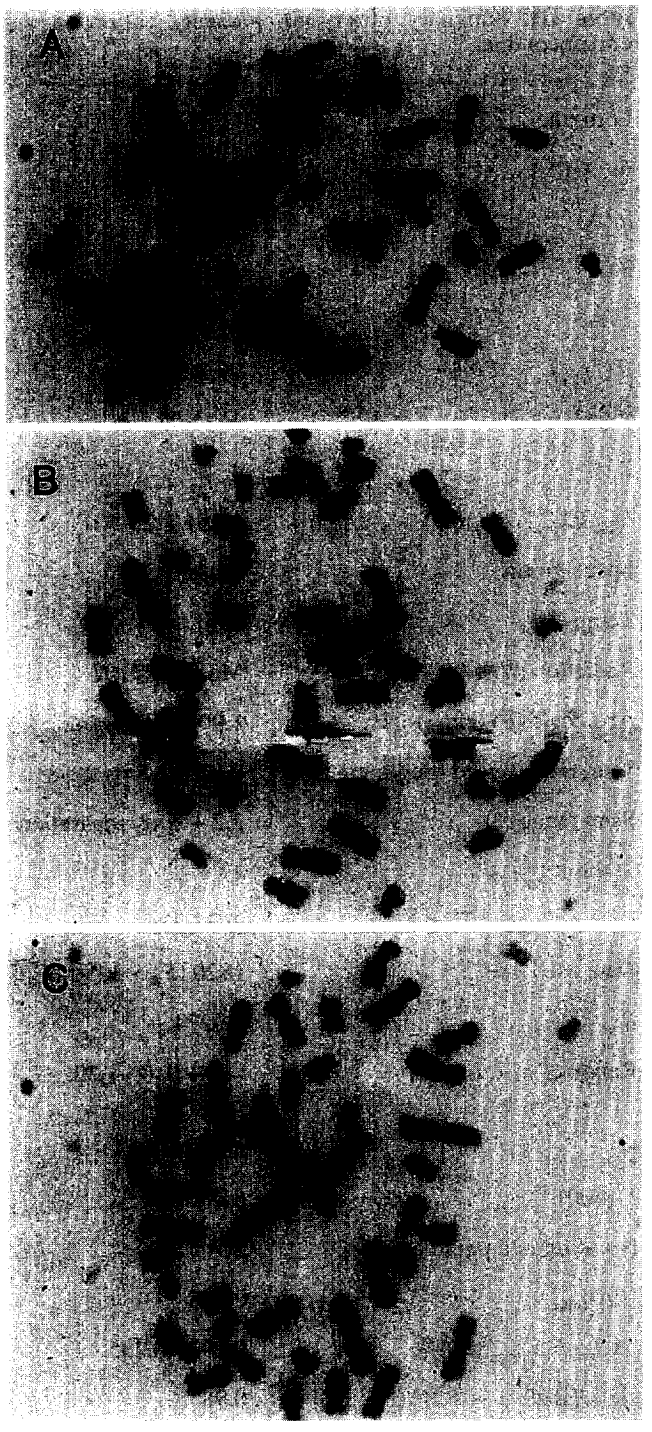


Fig 3. Microphotographs of metaphases stained by the BrdU-Giemsa method. The cell shown in A has undergone replication for one cell cycle (M_1), whereas the cells in B and C have completed two (M_2) and three (M_{3+}) cell cycles respectively, in the presence of BrdU.

Table III. Summary of crystal, intensity collection and refinement data.

Formula	$C_{15}H_{21}N_5S$
fw	303.42
a (Å)	7.921 (1)
b (Å)	18.318 (1)
c (Å)	11.252 (1)
β (°)	99.322 (3)
V (Å^3)	1611.02
Z	4
D_{calc}/D_{meas} (mg m^{-3})	1.251/1.23
Space group	$P2_1/c$
Temp (K)	296
Radiation (λ)	Mo K_α 0.7107
Abs coeff (μ) (cm^{-1})	1.90
Scan mode/speed (deg/min)	$\theta - 2\theta/1.5$
Scan range (°)	2.2 + $\alpha_1\alpha_2$ separation
2θ limit (°)	47
Data collected/unique	2633/2362
Data used	1820 [$F_0 > 3.0\ \sigma\ (F_0)$]
R_{int}	0.0274
Range of *h*, *k*, *l*	$-8 \rightarrow 0$, $0 \rightarrow 20$, $-12 \rightarrow 12$
w	unit weights
F (000)	648
No of refined parameters	274
$[\Delta/\sigma]_{max}$	0.028
$[\Delta_\rho]_{max}/[\Delta_\rho]_{min}$ (e/Å^3)	0.293/–0.235
sig = $[\Sigma w(\|F_0\| - \|F_c\|)^2/(N - P)]^{1/2}$	0.98
$R = \Sigma\|\|F_0\| - \|F_c\|\|/\Sigma\|F_0\|$	0.0500
$R_w = (\Sigma w(\|F_0\| - \|F_c\|)^2/\Sigma w\|F_0\|^2)^{1/2}$	0.0476
R/R_w (all data)	0.0732/0.0640

Then, 3 h before harvesting, colcemid was added and after hypotonic treatment of the harvested cells, fixation and slide preparation, the slides were stained on Hoechst 33258, UV irradiated and finally stained with Giemsa [20]. For cell-cycle analysis, 100 metaphases per culture were microscopically examined. Each metaphase was classified as being in the first (M_1), second (M_2), or third and further (M_{3+}) mitosis (fig 3). The proliferation rate index (PRI) was determined as an indirect measure of cell-division kinetics (table II) and calculated according to the formula proposed by Schneider and coworkers [21].

Molt-4 human T-cell leukemia cells (Flow) were grown in RPMI 1640 culture medium (Seromed) supplemented with 10% FCS, glutamine and antibiotics. Approximately 1.5 x 10^6 Molt-4 cells were transferred to each flask containing 5 ml medium and cultures were incubated at 37°C for 48 h. The products were then added in the appropriate concentrations at the beginning of the cultures. Four hours prior to harvesting, [^{3}H]thymidine (1 µCi/ml; spec act 5 Ci/mM) was added to the cultures. At the end of the labelling period, a fraction (1 ml) of each culture was removed, the cells were washed three times with Hanks' balanced salt solution to remove unincorporated isotope and then taken up in a solution of sodium dodecyl sulphate (1.5%) in 0.01 M Tris buffer pH 7.4 with 0.001 M EDTA; sodium hydroxide was added at a final concentration of 0.3 M, the mixture was precipitated with trichloroacetic acid at a final concentration of 5% and collected on 0.45 µM HA Millipore filters. These were washed three times with 5% trichloroacetic acid, dried and counted in a scintillation counter [18]. The decline of DNA synthesis in Molt-4 cells treated with increased concentrations of the products used is depicted in figure 3.

X-ray crystal structure determination

Slow crystallisation from ethyl alcohol yielded pale-yellow prismatic crystals of compound **4a** (n = 2). A crystal with approximate dimensions 0.28 x 0.22 x 0.30 mm was mounted in air. Diffraction measurements were made on a $P2_1$ Nicolet diffractometer upgraded by Crystal Logic using Zr-filtered Mo radiation. Unit cell dimensions were determined and refined using the angular settings of 25 automatically centred reflections in the range $11 < 2\theta < 24$ and they appear in table III. Intensity data were recorded using a θ–2θ scan to $2\theta_{max} = 47°$ with scan speed 1.5 deg/min and scan range 2.2 plus $\alpha_1\alpha_2$ separation. Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay. Lorentz and polarisation corrections were applied using Crystal Logic software.

Symmetry equivalent data were averaged with R = 0.0274 to give 2362 independent reflections from a total of 2633 collected. The structure was solved by direct methods using ShelXS-86 [22] and refined by full-matrix least-squares techniques with ShelX-76 [23] using only 1820 reflections with $F > 3\sigma$ (F) and refining 274 parameters. All hydrogen atoms were located by difference maps and their positions were refined isotropically. All non-hydrogen atoms were refined anisotropically.

The final values for R, R_w and GOF for observed data are presented in table III, and are 0.0732, 0.0640 and 1.14, respectively for all data. The maximum and minimum residual peaks in the final difference map were 0.293 and –0.235 e/Å^3. The largest shift/esd in the final cycle was 0.028. Positional and U_{eq} thermal parameters are given in table IV and selected bond distances and angles in table V.

Table IV. Positional and equivalent thermal parameters (x 10^4) of the non-H atoms.

Atom	*X*	*Y*	*Z*	U_{eq}
C(1)	2135 (5)	11573 (3)	–3948 (4)	606
C(2)	1855 (6)	11915 (3)	–5063 (5)	635
C(3)	2962 (6)	11828 (3)	–5868 (4)	555
C(4)	4404 (5)	11397 (2)	–5514 (3)	415
C(5)	4726 (4)	11052 (2)	–4386 (3)	369
C(6)	3534 (5)	11143 (2)	–3597 (3)	482
N(1)	5752 (4)	11256 (2)	–6104 (3)	490
C(7)	6911 (5)	10840 (2)	–5379 (3)	430
C(8)	6363 (4)	10694 (2)	–4305 (3)	364
C(9)	7356 (4)	10293 (2)	–3318 (3)	384
N(2)	6691 (4)	10181 (2)	–2363 (2)	439
N(3)	7797 (4)	9844 (2)	–1414 (3)	485
C(10)	7156 (5)	9443 (2)	–592 (3)	467
S	8427 (2)	9178 (1)	681 (1)	650
N(4)	5506 (5)	9270 (2)	–880 (3)	575
C(11)	4549 (7)	8821 (3)	–145 (4)	663
C(12)	4071 (6)	8087 (3)	–711 (4)	579
N(5)	2933 (4)	8131 (2)	–1861 (3)	454
C(13)	1281 (7)	8426 (4)	–1750 (6)	849
C(14)	2680 (10)	7391 (3)	–2379 (5)	843
C(15)	9093 (6)	10030 (3)	–3485 (4)	575

Esd's in parentheses. $U_{eq} = 1/3\ (U_{11} + U_{22} + U_{33})$.

Table V. Bond distances (Å) and angles (°).

C(1)-C(2)	1.388 (6)	C(9)-N(2)	1.288 (4)
C(1)-C(6)	1.365 (6)	C(9)-C(15)	1.498 (5)
C(2)-C(3)	1.367 (6)	N(2)-N(3)	1.409 (4)
C(3)-C(4)	1.393 (5)	N(3)-C(10)	1.344 (4)
C(4)-C(5)	1.404 (5)	C(10)-S	1.683 (4)
C(4)-N(1)	1.370 (5)	C(10)-N(4)	1.333 (5)
C(5)-C(6)	1.407 (5)	N(4)-C(11)	1.462 (5)
C(5)-C(8)	1.443 (5)	C(11)-C(12)	1.510 (7)
N(1)-C(7)	1.359 (5)	C(12)-N(5)	1.454 (5)
C(7)-C(8)	1.375 (5)	N(5)-C(13)	1.439 (6)
C(8)-C(9)	1.452 (5)	N(5)-C(14)	1.477 (6)
C(2)-C(1)-C(6)	121.7 (4)	C(8)-C(9)-N(2)	118.3 (3)
C(1)-C(2)-C(3)	121.4 (4)	C(8)-C(9)-C(15)	117.5 (3)
C(2)-C(3)-C(4)	117.4 (4)	N(2)-C(9)-C(15)	124.1 (3)
C(3)-C(4)-C(5)	122.3 (4)	C(9)-N(2)-N(3)	114.5 (3)
C(3)-C(4)-N(1)	129.5 (4)	N(2)-N(3)-C(10)	120.2 (3)
C(5)-C(4)-N(1)	108.2 (3)	N(3)-C(10)-S	120.2 (3)
C(4)-C(5)-C(6)	118.4 (3)	N(3)-C(10)-N(4)	115.3 (3)
C(4)-C(5)-C(8)	106.9 (3)	S-C(10)-N(4)	124.5 (3)
C(6)-C(5)-C(8)	134.6 (3)	C(10)-N(4)-C(11)	125.0 (4)
C(1)-C(6)-C(5)	118.8 (4)	N(4)-C(11)-C(12)	112.3 (4)
C(4)-N(1)-C(7)	108.6 (3)	C(11)-C(12)-N(5)	113.7 (4)
N(1)-C(7)-C(8)	111.0 (3)	C(12)-N(5)-C(13)	112.6 (4)
C(5)-C(8)-C(7)	105.4 (3)	C(12)-N(5)-C(14)	108.9 (4)
C(5)-C(8)-C(9)	130.4 (3)	C(13)-N(5)-C(14)	108.2 (5)
C(7)-C(8)-C(9)	124.1 (3)		

Acknowledgment

The authors wish to thank Boutari and Sons SA for their financial support.

References

1 Malatesta P, Accineli GP, Quaglia G (1959) *Ann Chim (Rome)* 49, 397–403
2 Wagner WH, Winkelmann E (1972) *Arzneim Forsch* 22, 1713–1716
3 Klayman DL, Lin AJ, McCall JW *et al* (1991) *J Med Chem* 34, 1422–1425
4 Domagk G, Behnisch R, Mietzsch F, Schmidt H (1946) *Naturwissenschaften* 33, 315
5 Drain DJ, Goodacre CL, Seymour DE (1949) *J Pharm Pharmacol* 1, 784–787
6 Brockman RW, Thompson JR, Schabel FM Jr MJ, Skipper HI (1956) *Cancer Res* 16, 788–795
7 Logan S, Fox M, Morgan H, Makohon Pfau G (1975) *J Gen Virol* 28, 271–276
8 Thomson R, Minton S, Officer J, Hitchings G (1953) *J Immunol* 70, 229–234
9 Sartorelli A (1967) *Bioch Biophys Res Comm* 27, 26–32
10 Sartorelli A (1967) *Pharmacologist* 9, 192
11 Brockman RW, Sidwell RW, Arnett G, Shaddix S (1970) *Proc Soc Exp Biol Med* 133, 609–614
12 Winkelmann E, Rolly H (1972) *Arzneim Forsch* 22, 1704–1711
13 Gonzalez Cid M, Matos E (1984) *Mutat Res* 138, 175–179
14 Petrou C, Mourelatos D, Dozi-Vassiliades J, Catsoulacos P (1990) *Mutat Res* 243, 109–113
15 Silvestrini R, Sanfilippo O, Daidone MG (1983) *In: Human Tumour Drug Sensitivity Testing* in vitro (Dendy PP, Hill BT, eds), Academic Press, New York, USA, 281
16 Caujolle R, Amarouch H, Payard M *et al* (1989) *Eur J Med Chem* 24, 287–292
17 Kiritsy JA, Yung DK (1978) *J Med Chem* 21, 1301–1307
18 Papadaki-Valiraki A, Papaioannou G, Siatra-Papastaikoudi T, Sambani C, Thomou H (1989) *Eur J Med Chem* 24, 455–457
19 Perry P, Wolff S (1974) *Nature (Lond)* 251, 156–158
20 Wolff S, Perry P (1974) *Chromosoma* 48, 341–353
21 Schneider EL, Nakanishi Y, Lewis J, Sternberg H (1981) *Cancer Res* 41, 4973–4975
22 Sheldrick GM (1986) ShelXS-86: *Structure Solving Program*. University of Gottingen, Germany
23 Sheldrick GM (1976) ShelX-76: *Program for Crystal Structure Determination*. University of Cambridge, UK