Anti-Cancer Drugs 1994, **5**, pp. 207–212

The cytotoxicity of 3-imino-1-oxoisoindolines in murine and human tissue culture cells

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Certain types of hypolipidemic agents have been observed to also function as cytotoxic agents. Previously reported hypolipidemic agents, 3-imino-1-oxoisoindolines, were evaluated for their anti-neoplastic activity. Selected agents were effective at inhibiting L1210, $Tmolt_3$, HeLa-S^3, KB nasopharynx, lung, osteosarcoma and glioma growth. 2-Propyl-3-imino-1-oxoisoindoline, (4), a representative compound of the class of agents, inhibited DNA and RNA syntheses of L1210 cells. The major site of inhibition was the purine pathway at IMP dehydrogenase. Other enzyme sites which were affected by (4) marginally were t-RNA and r-RNA polymerases, dihydrofolate reductase, aspartate transcarboxylase, and nucleoside kinases. d(NTP) pools of L1210 cells were reduced after 60 min. incubation with (4).

Key words: 3-Imino-1-oxoindolines, cytotoxicity.

Introduction

A series of hypolipidemic agents, 1,2,4-triazolidine-3,5-diones,[1] 2,3-dihydrophthalazine-1,4-diones,[2] indazolones,[3] indan-1,3-diones[4] and diphenimides,[5] have cytotoxic activity. These derivatives inhibited DNA synthesis by reducing purine *de novo* synthesis at the PRPP amidotransferase and IMP dehydrogenase enzyme sites. Compounds with a similar chemical structure, e.g. 3-imino-1-oxoisoindolines,[6,7] have been shown to have analogous hypolipidemic activity. Other hypolipidemic agents, e.g. Compactin, inhibit DNA synthesis. Thus, this study was undertaken to investigate whether 3-imino-1-oxoisoindolines inhibit tumor cell growth.

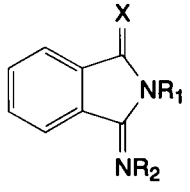
Methods and materials

Source of compounds

The substituted 3-imino-1-oxoisoindolines were synthesized as previously outlined.[6] The chemical and physical characteristics were identical to those reported.[6]

Cytotoxic activity

Compounds **1–23** were tested for cytotoxic activity by preparing 1 mM solutions of each drug in 0.05% Tween 80/H_2O by homogenization. The solutions were sterilized by passing them through an Acrodisc (45 μM). The following cell lines were maintained by the literature techniques:[8] murine L1210 lym-



Compound	X	R_1	R_2
1	O	—H	—H
2	O	—CH_3	—H
3	O	—CH_2H_5	—H
4	O	—C_3H_7	—H
5	O	—C_4H_9	—H
6	O	—C_5H_{11}	—H
7	O	—C_2H_5	—C_2H5
8	O	—C_3H_7	—C_3H_7
9	O	—C_4H_9	—C_4H_9
10	O	—C_5H_{11}	—C_5H_{11}
11	O	—$(CH_2)_2COCH_3$	—$(CH_2)_2COCH_3$
12	O	—H	—C_3H_7 · HCl
13	O	—H	—C_4H_9 · HCl
14	O	—H	—C_5H_{11} · HCl
15	—NC_3H_7	—H	—NC_3H_7
16	—NC_4H_9	—H	—NC_4H_9
17	—NC_5H_{11}	—H	—NC_5H_{11}
18	O	—COOEt	—H
19	O	—CH_2COOEt	—H
20	O	—$(CH_2)_2$COOEt	—H
21	O	—$(CH_2)_3$COOEt	—H
22	O	—$(CH_2)_4$COOEt	—H
23	O	—$CH_2CH_2C(O)OA?$	—H

Figure 1. Structures of 3-imino-1-oxolisoindolines.

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Table 1. The cytotoxicity of 3-imino-1-oxoisoindolines against murine and human tissue cultured cells (ED_{50} = μg/ml) (N = 6)

Compound	Murine L1210	Human						
		$Tmolt_3$	SW480 adeno-carcinoma (colon)	HeLa-S^3	KB naso-pharyngeal	broncho-genic lung	osteo-sarcoma	brain glioma
1	1.72	1.73	2.43	2.45	4.65	1.59	2.20	3.55
2	1.97	2.26	6.10	2.45	4.66	1.59	5.93	3.54
3	1.44	1.20	0.95	1.52	3.57	1.59	5.58	6.93
4	0.84	1.86	2.30	1.40	4.82	3.35	3.47	6.16
5	2.40	0.78	0.95	1.92	4.65	3.81	2.99	3.23
6	1.40	1.07	2.99	1.40	3.18	2.98	4.52	4.65
7	0.96	2.66	0.95	1.22	6.37	2.59	3.84	2.60
8	1.41	1.66	1.80	1.17	3.73	1.73	7.48	5.82
9	2.99	2.06	1.84	1.57	3.41	4.20	5.27	5.21
10	2.32	2.53	3.79	1.11	0.93	0.39	6.29	2.68
11	3.98	3.33	2.76	2.86	5.70	6.49	0.96	1.24
12	0.84	1.86	2.95	1.63	3.57	2.42	1.63	2.16
13	2.61	2.06	2.31	2.27	5.90	1.59	1.76	4.63
14	0.67	0.80	2.00	1.45	0.74	0.85	0.83	1.23
15	1.19	1.33	4.35	2.62	4.19	3.49	1.70	5.30
16	0.95	1.46	1.90	1.63	0.93	0.61	0.67	0.80
17	0.60	1.13	1.37	5.31	0.72	7.46	2.16	1.85
18	1.84	1.60	3.06	2.39	4.19	1.20	2.33	2.89
19	2.40	1.59	2.42	2.45	4.19	4.21	8.55	2.69
20	1.96	1.13	2.66	1.75	5.75	6.36	3.21	4.93
21	1.76	0.99	2.18	1.57	5.33	4.20	6.79	2.68
22	2.15	2.26	2.22	1.92	6.91	3.04	5.48	1.24
23	2.32	1.86	2.42	1.51	7.92	7.94	8.32	2.96
5-FU	1.41	2.14	3.09	2.47	1.25	5.69	—	1.28
Ara-C	2.76	2.67	3.42	2.13	2.84	4.60	—	1.88
Hyroxyurea	2.67	3.18	4.74	1.96	5.29	7.37	7.57	2.57

Values < 4 μg/ml are considered significant.

phoid leukemia, rat UMR 106 osteosarcoma, human $Tmolt_3$ acute lymphoblastic T cell leukemia, colorectal adenocarcinoma SW480, HCT-8 ileum, lung bronchogenic lines MB-9812 and A349, osteosarcoma TE418, KB epidermoid nasopharynx, A431 epidermoid carcinoma, and HeLa-S^3 suspended and solid cervical carcinoma. The protocol of Geran *et al.*[8] was used to assess cytotoxicity; standards were determined for each cell line. The compounds' cytotoxicities were expressed as ED_{50} values, i.e. the concentration in μg/ml which inhibits 50% of cell growth determined by the Trypan blue exclusion techniqe. Solid tumor cytotoxicity was determined by the method of Leibovitz *et al.*,[9] using 0.2% crystal violet/20% MeOH staining and evaluation at 580 nm.

Incorporation studies

Incorporation of labeled precursors into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein into 10^6 L1210 cells was determined by the method of Liao *et al.*[10] The concentration response of compound **4** for inhibition of DNA, RNA and protein syntheses was determined after 60 min at 25, 50 and 100 μM. [1-^{14}C]Glycine (53.0 mCi/mol) incorporation into purines was determined by the method of Cadman *et al.*[11] [^{14}C]Formate (53.0 mCi/mol) incorporation into pyrimidines was determined by the method of Christopherson *et al.*[12]

Enzyme assays

Inhibition of various enzyme activities was carried out by first preparing the appropriate L1210 cell homogenate or subcellular fraction, then adding the test drug during the enzyme assay. For the concentration response studies, inhibition of enzyme activity was determined at 25, 50 and 100 μM after incubation for 60 min. DNA polymerase α activity was determined in a cytoplasmic extract isolated by

Table 2. The effects of 3-imino-1-oxolisoindolines on L-1210 DNA, RNA and protein syntheses after 24 h incubation at the ED_{50} value ($N = 6$)

Compound	Percent of control (mean ± SD)		
	DNA synthesis	RNA synthesis	protein synthesis
Control	100 ± 6[a]	100 ± 7[b]	10 ± 8[c]
1	88 ± 5	67 ± 6*	110 ± 7
2	72 ± 6*	69 ± 5*	89 ± 6
3	72 ± 7*	69 ± 6*	111 ± 7
4	63 ± 5*	32 ± 4*	97 ± 8
5	88 ± 4*	52 ± 5*	85 ± 6
6	70 ± 5*	58 ± 4*	94 ± 7
7	62 ± 6*	47 ± 5*	112 ± 6
8	70 ± 7*	77 ± 5*	95 ± 6
9	73 ± 6*	82 ± 7	101 ± 7
10	60 ± 6*	97 ± 7	120 ± 5
11	95 ± 8	129 ± 5	97 ± 6
12	81 ± 7	73 ± 6*	30 ± 3*
13	108 ± 5	67 ± 5*	82 ± 6
14	69 ± 6*	41 ± 4*	13 ± 3*
15	72 ± 6*	61 ± 5*	92 ± 6
16	52 ± 3	42 ± 4*	114 ± 5
17	56 ± 5	40 ± 5*	113 ± 6
18	88 ± 6	73 ± 6*	72 ± 5*
19	80 ± 7	80 ± 7	102 ± 6
20	82 ± 6	72 ± 6	87 ± 7
21	81 ± 8	80 ± 5	109 ± 5
22	115 ± 6	82 ± 6	110 ± 6
23	81 ± 5	91 ± 5	114 ± 7

[a] 46314 d.p.m./24 h; [b] 60840 d.p.m./24 h; [c] 10452 d.p.m./24 h.
*$p \leq 0.001$ by Student's t-test.

the method of Eichler *et al.*[13] Nuclear DNA polymerase β was determined by isolating nuclei by the method of Mamaril *et al.*[14] The polymerase assay for both α and β was that of Sawada *et al.*,[15] with [^{3}H]TTP. Messenger-, ribosomal- and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate[16,17] and the individual RNA polymerase activities were determined using [^{3}H]UTP. Ribonucleoside reductase activity was measured with [^{14}C]CDP with and without dithioerythritol.[18] The deoxyribonucleotides labeled with [^{14}C]dCDP were separated from [^{14}C]rCDP from the ribonucleotides by thin layer chromatography (TLC) on PEI plates. Thymidine, TMP and TDP kinase activities were measured with [^{3}H]thymidine (58.3 mCi/mol) in the medium of Maley and Ochoa.[19] PRPP amidotransferase activity was determined by the method of Spassova *et al.*[20] and IMP dehydrogenase activity was determined with [^{14}C]IMP (Amersham, Arlington Heights, IL) where XMP was separated on PEI plates (Fisher Scientific Raleigh, NC) by TLC.[21] Carbamyl phosphate synthetase activity was determined by the method of Kalman *et al.*[22] and citrulline was determined colorimetrically.[23] Aspartate transcarbamylase activity was determined by the method of Kalman *et al.*[22] and carbamyl aspartate was determined colorimetrically.[24] OMP decarboxylase activity was determined by the method of Appel.[25] Thymidylate synthetase activity was analyzed by the method of Kampf *et al.*[26] The ^3H_2O measured was proportional to the amount of TMP formed from [^{3}H]dUMP. Dihydrofolate reductase activity was determined by the spectrophotometric method of Ho *et al.*[27] Protein was determined for all of the enzymatic assays.[28]

DNA assays

Deoxyribonucleoside triphosphates were extracted by the method of Bagnara and Finch.[29] Deoxyribonucleoside triphosphates were determined by the method of Hunting and Henderson,[30] with calf thymus DNA, *Escherichia coli* DNA polymerase I, nonlimiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 µCi of [^{3}H-methyl]dTTP or [5-^{3}H]dCTP.

The effects of compound **4** on DNA strand scission were determined by the methods of Suzuki *et al.*,[31] Pera *et al.*[32] and Woynarowski *et al.*[33] L1210 lymphoid leukemia cells were incubated with 10 µCi thymidine methyl-^{3}H, 84.0 Ci/mmol and drug at 100 µM for 24 h at 37°C. After harvesting the $Tmolt_3$ cells (10^7), the cells were centrifuged at 600 *g* for 10 min in PBS, washed and suspended in 1 ml of PBS. Lysis buffer (0.5 ml; 0.5 M NaOH, 0.02 M EDTA, 0.01% Triton X-100 and 2.5% sucrose) was layered onto a 5–20% alkaline–sucrose gradient (5 ml; 0.3 M NaOH, 0.7 KCl and 0.01 M EDTA) followed by 0.2 ml cell preparation. After incubating for 2.5 h at room temperature, the gradient was centrifuged at 12000 r.p.m. at 20°C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the top of the gradient, neutralized with 0.2 ml of 0.3N HCl, and radioactivity measured. Thermal calf thymus DNA denaturation studies, UV absorption studies and DNA viscosity studies were conducted after incubating compound **4** at 100 µM in PBS buffer pH 7.2 at 37°C for 24 h.[34]

Results

Compounds **4**, **7**, **14**, **16** and **17** all afforded L1210 lymphoid leukemia cytotoxicity with ED_{50} values of less than 1 µg/ml. All of the 23 compounds were

active against L1210 growth with ED_{50} values less than 4 μg/ml. $Tmolt_3$ lymphoid leukemia growth was reduced by **5**, **14** and **21** with ED_{50} values less than 1 μg/ml. Against this cell line, all compounds tested were active.

Adenocarcinoma colon SW480 growth was inhibited by **3**, **5** and **7** with ED_{50} values less than 1 μg/ml. Compounds **2** and **15** were inactive. Hela-S^3 cell growth was inhibited by all of the compounds except **17**, with ED_{50} values between 1.11 and 2.86 μg/ml. KB nasopharyngeal growth was inhibited significantly by **10**, **14**, **16** and **17**. Lung bronchogenic growth was inhibited by **10**, **14** and **16**; compounds **9**, **11**, **17**, **19**, **21** and **23** were inactive. Osteosarcoma growth was inhibited by **11**, **14** and **16** with ED_{50} values less than 1 μg/ml. Glioma growth was inhibited by **16** (ED_{50} value less than 1 μg/ml); such growth was inhibited by **11**, **14**, **17** and **22** with ED_{50} values less than 2 μg/ml.

L1210 DNA and RNA syntheses were inhibited significantly by all compounds with low L1210 ED_{50} values. Compounds **4**, **7**, **14**, **16** and **17** caused at least 35% inhibition of DNA synthesis, and the same compounds reduced RNA syntheses greater than 50%. There did not appear to be any significant correlations between protein synthesis inhibition and the compounds' ED_{50} values in the L_{1210} screen. Compound **4** caused a concentration dependent reduction of both L1210 DNA and RNA syntheses. Purine synthesis was markedly reduced by 65% at 100 μM. Whereas PRPP amidotransferase activity was marginally inhibited (27%), IMP dehydrogenase activity seemed to be the site maximally effected by **4** with 63% inhibition at 100 μM. Carbamyl phosphate synthetase was marginally inhibited 20% at 50 and 100 μM but aspartate transcarbamylase activity was inhibited 42% at 100 μM. DNA polymerase α activity was inhibited 22%; m-RNA polymerase activity was not affected by **4**. r-RNA and t-RNA polymerase activities were inhibited 35–39%. Ribonucleoside reductase activity was inhibited 36% by **4** at 100 μM. This correlated positively with the reductions in d(ATP), d(GTP), d(CTP), and d(TTP) pool levels between 14–36%. Other enzymes which when inhibited by **4** contribute to reduced d(NTP) pool levels are nucleoside/nucleotide kinases. Activity of these kinase enzymes was inhibited 40–52% by **4** at 100 μM.

Experiments with cDNA showed that compound **4** caused no changes in DNA viscosity, thermal denaturation, T_m values or absorption of DNA at 260 nm. Incubation of compound **4** at 100 μM with L1210 cells for 24 h showed that DNA fragmentation did occur at that concentration (Figure 2).

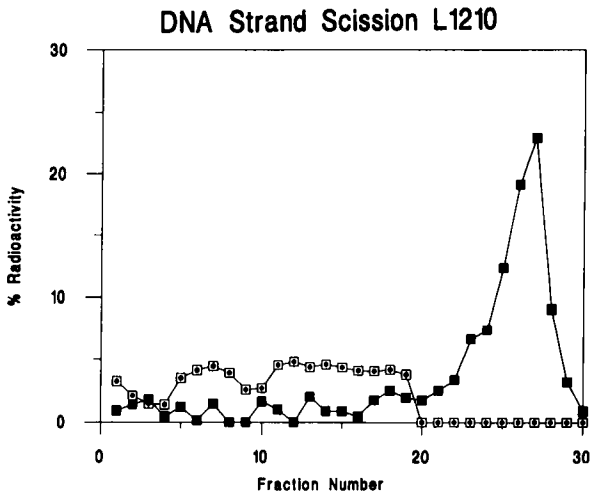

Figure 2. DNA strand scission L1210: ⊡, **4**; ▩, control.

Discussion

3-Imino-1-oxoisoindolines proved to be potent cytotoxic agents against single suspended cells, e.g. L1210, $Tmolt_3$ and HeLa-S^3 cells. Selected agents were active against solid tumors, e.g. colon adenocarcinoma, KB, lung, osteosarcoma and glioma. Selected derivatives were potent across the whole battery of tumor lines, e.g. compounds **13** and **17**. The derivatives are similar to the purine nucleus structure in both size and shape. This may explain why regulation enzymes in the purine pathway are inhibited, i.e. by feedback regulation. Inhibition of the purine pathway after 60 min incubations would account for reduction in both RNA and DNA syntheses in tumor cells. Nevertheless, other metabolic sites were inhibited by the componds. Inhibition of the *de novo* pyrimidine pathway may be due to agent-induced reductions in aspartate transcarbamylase activity. The inhibition of dihydrofolate reductase activity would add to reduced purine and pyrimidine syntheses since that enzyme is responsible for one carbon transfer in both pathways. Inhibition of ribonucleotide reductase, reducing conversion of ribonucleoside to deoxyribonucleotides, would be reflected in lower d(NTP) pools after treatment. The agents' reduction of nucleoside and nucleotide kinase activities would contribute to decrements in d(NTP) pool levels observed after 60 min exposure to the drug.

The 3-imino-1-oxoisoindolines demonstrated a mode of action similar to other cyclic imides, e.g. 2,3-dihydrophthalazine-1,4-diones, indazolones and diphenimides. The purine synthetic path-

Table 3. The effects of compound **4** on L1210 lymphoid leukemia cell metabolism after 60 min incubation ($N = 6$)

Assay	Percent of control (mean ± SD)			
	control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 6[a]	74 ± 5*	67 ± 6*	59 ± 5*
RNA synthesis	100 ± 7[b]	76 ± 6*	60 ± 5*	54 ± 6*
Protein synthesis	100 ± 6[c]	135 ± 7*	120 ± 6	111 ± 5
DNA polymerase α	100 ± 6[d]	99 ± 5	79 ± 5	78 ± 6
m-RNA polymerase	100 ± 5[e]	102 ± 7	104 ± 8	95 ± 6
r-RNA polymerase	100 ± 6[f]	101 ± 7	66 ± 6*	65 ± 4*
t-RNA polymerase	100 ± 5[g]	71 ± 6*	64 ± 5*	61 ± 5*
Ribonucleoside reductase	100 ± 6[h]	76 ± 7*	66 ± 6*	64 ± 5*
Dihydrofolate reductase	100 ± 7[i]	84 ± 6	68 ± 5*	64 ± 5*
Purine synthesis	100 ± 8[j]	47 ± 5*	46 ± 5*	35 ± 4*
PRPP amidotransferase	100 ± 7[k]	79 ± 6*	79 ± 7*	73 ± 5*
IMP dehydrogenase	100 ± 6[l]	65 ± 5*	58 ± 5*	37 ± 3*
Pyrimidine synthesis	100 ± 7[m]	134 ± 5*	113 ± 6	53 ± 4*
Carbamyl phosphate synthetase	100 ± 6[n]	81 ± 6	74 ± 5*	74 ± 6*
Aspartate transcarbamylase	100 ± 7[o]	84 ± 6	72 ± 4*	58 ± 5*
Thymidylate synthetase	100 ± 7[p]	104 ± 7	98 ± 6	87 ± 6
Thymidine kinase	100 ± 8[q]	73 ± 6*	64 ± 5*	54 ± 5*
Thymidine monophosphate kinase (TMP)	100 ± 5[r]	73 ± 5*	63 ± 4*	48 ± 3*
Thymidine diphosphate kinase (TDP)	100 ± 6[s]	93 ± 8	70 ± 6*	60 ± 4*
dATP	100 ± 6[t]	—	—	64 ± 5*
dGTP	100 ± 7[u]	—	—	78 ± 6*
dCTP	100 ± 6[v]	—	—	86 ± 5
dTTP	100 ± 5[w]	—	—	71 ± 6*

*$p \leq 0.0001$

Control values are for 10^6 cells/h: [a]7719 d.p.m.; [b]1014 d.p.m.; [c]17492 d.p.m.; [d]5318 d.p.m.; [e]1343 d.p.m.; [f]325 d.p.m.; [g]400 d.p.m.; [h]48780 d.p.m.; [i]0.133 ΔOD units; [j]28614 d.p.m.; [k]19375 d.p.m.; [l]0.0878 ΔOD units; [m]19758 d.p.m.; [n]0.273 μmol citrulline; [o]57387 d.p.m.; [p]44743 d.p.m.; [q]4362 d.p.m.; [r]646 d.p.m.; [s]275 d.p.m.; [t]32.39 d.p.m.; [u]23.79 pmol; [v]86.24 pmol; [w]22.04 pmol.

way is one of the key targets of this class of chemical compounds. The cyclic imide nucleus may function much like the purine nucleus resulting in reduction of the *de novo* synthesis of purines by affecting feedback inhibition. Alternatively, the compounds may be incorporated into DNA- or RNA-reducing template activities, thus causing DNA fragmentation.

References

1. Hall IH, Wong OT, Simlot R, *et al.* Antineoplastic activities and cytotoxicity of 1-acyl and 1,2-diacyl-1,2,4-triazolidine-3,5-diones in murine and human tissue culture cells. *Anticancer Res* 1992; **12**: 1355–62.
2. Hall IH, Hall ES, Wong OT. The antineoplastic activity of 2,3-dihydrophthalazine-1,4-dione and *N*-butyl-2,3-dihydrophthalazine-1,4-dione in human and murine tumor cells. *Anti-Cancer Drugs* 1992; **3**: 55–62.
3. Hall IH, Wong OT, Chi LK, *et al.* The cytotoxicity of substituted indan-1,3-diones in murine and human tissue culture cells. *Anti-Cancer Drugs* 1994; submitted.
4. Hall IH, Wong OT, Hall ES, *et al.* The cytotoxicity of *N*-substituted indazolones in murine and human tumor cells. *Anti-Cancer Drugs*, **4**, 389–93.
5. Hall IH, Wong OT, Chi LK. The cytotoxicity of *N*-substituted diphenimides and 6,7-dihydro-5H-dibenbz (*c,e*)azepine. *Anti-Cancer Drugs* 1993; **4**: 665–70.
6. Murthy ARK, Wong OT, Hall IH. Synthesis and hypolipidemic activity of 3-imino-1-oxoisoindolines. *Pharm Res* 1987; **4**: 21–7.
7. Wong OT, Williams WL Jr, Oswald BS, *et al.* The effect of cyclic imides on lipoprotein receptor binding-degradation of rat and human cells, and effects on regulatory enzymes of lipid metabolism. *Res Commun Chem Pathol Pharmacol* 1992; **76**: 3–32.
8. Geran RJ, Greenburg NH, MacDonald MM, *et al.* Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep* 1972; **3**: 9–24.
9. Leibovitz AL, Stinson JC, McComb WB III, *et al.* Classification of human colorectal adenocarcinoma cell lines. *Cancer Res* 1976; **36**: 4562–9.
10. Liao LL, Kupchan SM, Horwitz SB. Mode of action of the antitumor compound bruceatin, an inhibitor of protein synthesis. *Mol Pharmacol* 1976; **12**: 167–76.
11. Cadman E, Heimer R, Benz C. The influence of methotrexate pretreatment on *5*-flaxorouracil metabolism in L1210 cells. *J Biol Chem* 1981; **256**: 1695–704.
12. Christopherson RI, Yu ML, Jones ME. An overall radioassay for the first three reactions of *de novo* pyrimidine synthesis. *Anal Biochem* 1981; **11**: 240–7.
13. Eichler DC, Fisher PA, Korn D. Effect of calcium on the recovery distribution of DNA polymerase from cultured human cells. *J Biol Chem* 1977; **252**: 4011–4.
14. Mamaril FP, Dobrjasky A, Green S. A rapid method for isolated of nuclei of Ehrlich ascites tumor cells. *Cancer*

Res 1970; **30**: 352–6.
15. Sawada H, Tatsumi K, Sadada M, *et al.* Effects of neocarzinostatin on DNA synthesis in L_{1210} cells. *Cancer Res* 1974; **34**: 3341–6.
16. Anderson KM, Mendelson IS, Guzik G. Solubilized DNA-dependent nuclear RNA polymerases from the mammary glands of late-pregnant rats. *Biochem Biophys Acta* 1975; **383**: 56–66.
17. Hall IH, Carlson GL, Abernathy GS, *et al.* Cycloalkanones IV. Antifertility agents. *J Med Chem* 1974; **17**: 1253–7.
18. Moore EC, Hurlbert RB. Regulation of mammalian deoxy-ribonucleotide biosynthesis by nucleotide or activators and inhibitors. *J Biol Chem* 19866; **241**: 4802–9.
19. Maley F, Ochoa S. Enzymatic phosphorylation of deoxycytidylic acid. *J Biol Chem* 1958; **233**: 1538–43.
20. Spassova MK, Russev GC, Goovinsky EV. Some pyrazoles as inhibitors of purine biosynthesis *de novo*. *Biochem Pharmacol* 1976; **25**: 923–4.
21. Becker JH, Lohr GW. Inosine-5^1-phosphate dehydrogenase activity in normal and leukemic blood cells. *Klin Wochenschr* 1979; **57**: 1109–15.
22. Kalman SM, Duffield PH, Brzozwki TJ. Purification and properties of a bacterial carbamyl phosphate synthetase. *J Biol Chem* 1966; **241**: 1871–7.
23. Archibald RM. Determination of citrulline and allontoin and demonstration of citrulline in blood plasma. *J Biol Chem* 1944; **156**: 121–42.
24. Koritz SB, Gohen PP. Colorimetric determination of carbamyl amino acid and related compounds. *J Biol Chem* 1954; **209**: 145–50.
25. Appel SH. Purification and kinetic properties of brain orotidine 5′-phosphate decarboxylase. *J Biol Chem* 1968; **243**: 3924–9.
26. Kampf A, Barfknecht RL, Schaffer PJ, *et al.* Synthetic inhibitors of *Escherichia coli* calf thymus and Ehrlich ascites tumor thymidylate synthetase. *J Med Chem* 1976; **19**: 903–8.
27. Ho YK, Hakala T, Zakrzewski SF. 5-(1-Adamantyl) pyrimidines as inhibitors of folate metabolism. *Cancer Res* 1971; **32**: 1023–8.
28. Lowry OH, Rosebrough J, Farr AL, *et al.* Protein measurement with folin phenol reagent. *J Biol Chem* 1951; **193**: 265–75.
29. Bagnara AS, Finch LR. Quantitative extraction and estimation of intracellular nucleostide-triphosphate in *Escherichia coli*. *Anal Biochem* 1971; **45**: 24–34.
30. Hunting D, Henderson JF. Determination of deoxyribonucleoside triphosphates using DNA polymerase: a critical evaluation. *Can J Biochem* 1982; **59**: 723–7.
31. Suzuki H, Nishimura T, Muto SK, *et al.* Mechanism of action of macromomycin: DNA strand scission, inhibition of DNA synthesis mitosis. *J Antibacteriol* 1978; **32**: 875–83.
32. Pera JF Sr, Rawlings CJ, Shackleton J, *et al.* Quantitative aspects of the formation and loss of DNA. *Biochim Biophys Acta* 1981; **655**: 152–66.
33. Woynarowski JW, Beerman TA, Konopa J. Introduction of deoxyribonucleic acid damage in HeLa S^3 cells by cytotoxic and antitumor sesquiterpine lactones. *Biochem Pharmacol* 1981; **30**: 3005–7.
34. Zhao Y, Hall IH, Oswald CB, *et al.* Anti-malarial agents III. Mechanism of action of artesunate against *Plasmodium berghi* infection. *Chem Phar Bull* 1987; **35**: 2052–61.

(Received 28 December 1993; accepted 24 January 1994)