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The cytotoxic activity of cyclic imido alkyl ethers, thioethers, sulfoxides, sulfones and related derivatives

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Cyclic imides such as N-substituted alkyl ethers, thioethers, sulfoxides, sulfones and related derivatives were potent agents against human single cell tumors and selected solid tumor growths, *eg* adenocarcinoma of the colon and glioma. These agents in the L_{1210} lymphoid leukemia tumor model preferentially inhibited DNA synthesis. The regulatory enzyme sites in the purine pathway were targets of the agents. Other sites of inhibition were DNA polymerase α and thymidylate synthetase activities. d(NTP) pool levels were also reduced by the agents over 60 min.

Key words: Cyclic imides, cytotoxic, DNA synthesis, purine synthesis inhibition.

Introduction

Cyclic imides and related derivatives have demonstrated potent hypolipidemic activity in rodents. A number of chemical classes, e.g. amine carboxyboranes, heterocyclic amine boranes, sesquiterpene lactones and triazolidinediones, have demonstrated crossover between hypolipidemic and antineoplastic pharmacological activities. Compactin, an HMG–CoA reductase agent that is a hypocholesterolemic agent, also inhibits DNA synthesis in L_{929} cells.[1] 2,3-Dihydrophthalazine-1,4-diones,[2] indazolones,[3] diphenimides and reduced diphenimides,[4] have all demonstrated such crossover activity. At this time, a series of alkyl ethers, thioethers, sulfoxides and sulfones of a variety of cyclic imides have been examined for their cytotoxic activity and their mode of action in blocking cellular metabolism in L_{1210} leukemia cells.

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Materials and methods

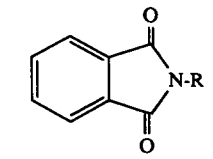
Source of compounds

Compounds **1–5**[5] and compounds **6** and **7**[6] were synthesized according to published methods. The structures of these compounds are shown in Figure 1.

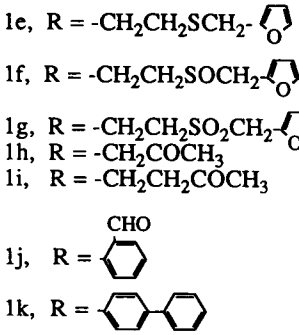
Pharmacological methods

Compounds **1a–7** (Table 1) were tested for cytotoxic activity by homogenizing drugs in a 1 mM solution in 0.05% Tween 80/H_2O. These solutions were sterilized by passing them through an acrodisc (45 μM). The following cell lines were maintained by literature techniques:[7–10] murine L_{1210} lymphoid leukemia,[11] human $Tmolt_3$ acute lymphoblastic T cell leukemia, colorectal adenocarcinoma SW480, lung bronchogenic MB-9212, osteosarcoma TE418, KB epidermoid nasopharynx, HeLa-S[3] suspended cervical carcinoma and glioma EH 118 MG. The protocol of Geran *et al.*[11] was used to assess the cytotoxicity of the compounds and standards in each cell line. Values for cytotoxicity were expressed as ED_{50} = μg/ml, i.e. the concentration of the compound inhibiting 50% of cell growth. ED_{50} values were determined by the trypan blue exclusion technique. A value of less than 4 μg/ml was required for significant activity of growth inhibition. Solid tumor cytotoxicity was determined according to Liebovitz *et al.*,[12] using 0.2% crystal violet/20% MeOH and read at 580 nm (Molecular Devices).

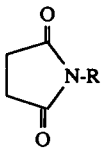
Incorporation of labelled precursors into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein for 10^6 L_{1210} cells was obtained.[13] The concentration response at 25, 50 and 100 μM required for inhibition of DNA, RNA and protein syntheses was determined



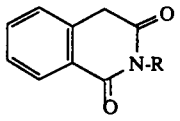
1a, R = $-CH_2CH_2OCH_3$
1b, R = $-CH_2CH_2SCH_3$
1c, R = $-CH_2CH_2SOCH_3$
1d, R = $-CH_2CH_2SO_2CH_3$



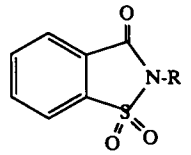
1e, R = $-CH_2CH_2SCH_2-$
1f, R = $-CH_2CH_2SOCH_2-$
1g, R = $-CH_2CH_2SO_2CH_2-$
1h, R = $-CH_2COCH_3$
1i, R = $-CH_2CH_2COCH_3$
1j, R =
1k, R =



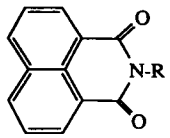
4a, R = $-CH_2CH_2OCH_3$
4b, R = $-CH_2CH_2SCH_3$
4c, R = $-CH_2CH_2SOCH_3$
4d, R = $-CH_2CH_2SO_2CH_3$



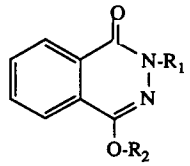
6a, R = $-CH_3$
6b, R = $-CH_2CH_2CH_2CH_3$
6c, R = $-CH_2CH_2COOH$
6e, R =
6f, R =
6g, R =



2a, R = $-CH_2CH_2OCH_3$
2b, R = $-CH_2CH_2SCH_3$
2c, R = $-CH_2CH_2SOCH_3$
2d, R = $-CH_2CH_2SO_2CH_3$



3a, R = $-CH_2CH_2OCH_3$
3b, R = $-CH_2CH_2SCH_3$
3c, R = $-CH_2CH_2SOCH_3$
3d, R = $-CH_2CH_2SO_2CH_3$



5a, R_1 = $-CH_2CH_2OCH_3$, R_2 = H
5b, R_1 = $-CH_2CH_2SCH_3$, R_2 = H
5c, R_1 = $-CH_2CH_2SOCH_3$, R_2 = H
5d, R_1 = $-CH_2CH_2SO_2CH_3$, R_2 = H
5e, R_1 = $-CH_2CH_2COOH$, R_2 = H
5f, $R_1 = R_2 = CH_2CH_2OCH_3$

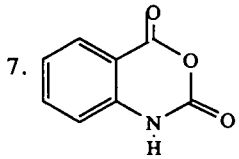


Figure 1. Figure structures of cytotoxic agents.

after 60 min incubations. The incorporation of [^{14}C]glycine (53.0 mCi/mmol) into purines was obtained by the method of Cadman *et al.*[14] Incorporation of [^{14}C]formate (53.0 mCi/mmol) into pyrimidines was determined by the method of Christopherson *et al.*[15]

Enzyme assays

Inhibition of various enzyme activities was performed by first preparing the appropriate L_{1210} cell homogenates or subcellular fractions, then adding the drug to be tested during the enzyme assay. For

Table 1. Cytotoxicity of compounds against murine and human cell lines (ED_{50} = μg/ml)

Compound no.	Murine	Human						
	L_{1210}	$Tmolt_3$	SW 48 colon adenocarcinoma	$HeLaS^3$	KB nasopharynx	bronchogenic lung	osteosarcoma	brain glioma
1a	0.55	1.88	4.19	1.98	6.85	9.41	2.63	2.36
1b	1.78	2.68	1.65	1.77	7.17	6.89	7.79	6.36
1c	1.25	3.79	2.16	2.26	6.83	7.31	7.98	8.04
1d	1.29	3.63	2.72	1.65	6.77	8.18	8.19	3.88
1e	0.46	5.27	1.60	2.25	5.52	8.39	6.09	5.02
1f	0.90	3.36	2.61	3.66	5.13	7.74	7.54	5.88
1g	0.82	3.57	0.52	2.95	6.77	6.21	4.01	6.26
1h	3.15	2.92	6.22	1.61	8.05	5.04	7.84	—
1i	2.14	1.29	1.42	2.48	1.82	2.51	1.67	3.05
1j	1.42	1.55	6.60	2.29	4.49	7.61	2.10	5.25
1k	4.79	2.56	1.84	2.63	8.01	4.44	6.23	5.24
2a	1.34	2.57	1.58	1.75	6.79	8.27	7.84	6.17
2b	0.78	2.62	1.02	2.44	2.49	2.82	6.68	1.37
2c	0.32	2.52	1.92	1.95	8.08	6.90	5.57	5.57
2d	1.45	1.52	2.04	2.09	8.04	7.65	6.79	6.57
3a	1.27	2.72	2.95	2.00	6.74	5.74	4.65	3.27
3b	3.18	3.53	1.63	1.15	3.58	7.16	6.92	7.67
3c	1.43	0.87	4.11	3.76	5.46	6.26	2.79	8.25
3d	1.21	2.95	1.32	5.58	4.56	7.36	7.44	8.10
4a	2.80	2.01	7.60	2.30	5.57	7.17	7.66	3.48
4b	1.76	1.64	1.50	2.73	7.26	5.40	8.78	3.33
4c	2.02	3.76	1.25	1.77	7.23	5.06	4.06	4.67
4d	0.64	4.71	5.23	1.65	5.42	8.18	7.05	4.65
5a	1.74	1.20	1.33	2.75	3.91	6.73	6.06	6.61
5b	1.54	3.56	0.75	2.64	3.18	6.07	5.62	8.76
5c	1.18	1.92	2.17	4.27	7.23	6.77	6.38	2.68
5d	1.55	3.48	1.69	2.97	3.52	6.46	5.29	2.17
5e	1.51	1.09	3.38	2.13	4.49	6.51	4.08	8.61
5f	2.32	2.66	8.01	1.51	7.91	7.49	2.65	3.52
6a	1.30	3.05	2.93	2.30	3.82	7.89	3.99	6.40
6b	1.44	3.43	1.84	1.91	7.98	7.79	4.06	3.45
6c	2.97	6.18	0.43	2.61	2.50	7.88	6.63	2.09
6d	3.20	4.13	1.27	2.70	2.50	7.92	4.51	3.54
6e	1.05	2.62	3.07	1.36	6.19	5.75	4.08	3.95
6f	2.43	2.24	0.75	2.43	3.21	5.04	3.45	2.57
6g	2.74	1.83	0.66	1.19	3.03	2.74	2.16	3.54
7	2.01	4.51	4.12	1.60	7.31	7.57	2.78	1.94
5–FU	1.41	2.14	3.09	2.47	1.25	5.69	—	128
Ara C	2.76	2.67	3.42	2.13	2.84	4.60	—	1.88
Hydroxyurea	2.67	3.18	4.74	1.96	5.29	7.37	7.57	2.57

the concentration–response studies, inhibition of enzyme activity was determined at 25, 50 and 100 μM of compound **1f**, **2b** and **5a** after 60 min incubations. DNA polymerase α activity was determined in cytoplasmic extracts isolated by the method of Eichler *et al.*[16] Nuclear DNA polymerase (β) was determined by isolating nuclei.[17] The polymerase assay for both α and β was as described by Sawada *et al.*[18] with [^{3}H]TTP. Messenger-, ribosomal- and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate; individual RNA polymerase activities were determined using [^{3}H]UTP.[19,20] Ribonucleoside reductase activity was measured using [^{14}C]-CDP with and without dithioerythritol.[21] The deoxyribonucleotides [^{14}C]dCDP were separated from the ribonucleotides by TLC on PEI plates. Thymidine, TMP and TDP kinase activities were determined using [^{3}H]thymidine (58.3 mCi/mmol) in the medium of Maley and Ochoa.[22] Carbamyl phosphate synthetase activity was determined with the method of Kalman *et al.*;[23] citrulline was determined colorimetrically.[24] Asparate transcarbamylase activity was measured by the method of Kalman *et al.*;[23] carbamyl asparate was determined colorimetrically.[25] OMP decarboxylase activity was determined

Table 2. Effects of compound **1f** on enzyme activities of L_{1210} leukemia cells after 60 min

$n = 5$	Control	Percent of control		
		25 μM	50 μM	100 μM
DNA synthesis	100 ± 6[a]	79 ± 5*	77 ± 6*	69 ± 5*
RNA synthesis	100 ± 7[b]	140 ± 6*	97 ± 6	95 ± 6
Protein synthesis	100 ± 5[c]	123 ± 6	84 ± 5	83 ± 6
DNA polymerase α	100 ± 5[d]	56 ± 5*	55 ± 4*	49 ± 5*
mRNA polymerase	100 ± 6[e]	115 ± 6	150 ± 7*	227 ± 9*
rRNA polymerase	100 ± 7[f]	95 ± 6	90 ± 6	84 ± 6
tRNA polymerase	100 ± 6[g]	61 ± 5*	61 ± 4*	33 ± 3*
Purine synthesis	100 ± 5[h]	47 ± 6	41 ± 5	39 ± 6
PRPP amido transferase	100 ± 7[i]	51 ± 7*	29 ± 7*	19 ± 6*
IMP dehydrogenase	100 ± 6[j]	57 ± 5*	41 ± 5*	34 ± 5*
Carbamyl phosphate synthetase	100 ± 5[k]	106 ± 6	80 ± 5*	74 ± 5*
Aspartate transcarbamylase	100 ± 7[l]	104 ± 5	88 ± 6	87 ± 5
OMP decarboxylase	100 ± 5[m]	119 ± 6	116 ± 7	89 ± 5
Thymidylate synthetase	100 ± 7[n]	119 ± 5	110 ± 6	44 ± 6
Thymidine kinase	100 ± 6[o]	61 ± 5*	54 ± 5*	43 ± 5*
Thymidine monophosphate kinase	100 ± 5[p]	81 ± 6	84 ± 5	85 ± 6
Thymidine diphosphate kinase	100 ± 7[q]	82 ± 5	97 ± 6	130 ± 5*
Ribonucleoside reductase	100 ± 6[r]	99 ± 7	69 ± 6*	60 ± 5*
Dihydrofolate reductase	100 ± 5[s]	123 ± 5	101 ± 6	54 ± 5*
d(ATP)	100 ± 6[t]	—	—	77 ± 5*
d(GTP)	100 ± 5[u]	—	—	6 ± 2*
d(CTP)	100 ± 5[v]	—	—	69 ± 5*
d(TTP)	100 ± 6[w]	—	—	72 ± 5*

Control values for 10^6 cells/h: [a]7719 dpm; [b]1014 dpm; [c]17492 dpm; [d]5318 dpm; [e]1343 dpm; [f]325 dpm; [g]400 dpm; [h]28614 dpm; [i]19375 dpm; [j]0.0878 Δ OD units; [k]0.273 μmol citrulline; [l]57387 dpm; [m]19758 dpm; [n]44743 dpm; [o]4362 dpm; [p]646 dpm; [q]275 dpm; [r]48780 dpm; [s]0.133 Δ OD units; [t]32.39 dpm; [u]23.79 pmol; [v]86.24 pmol; [w]2.204 pmol.

Table 3. Effects of **2b** on enzyme activities of L_{1210} leukemia cells after 60 min

$n = 5$	Control	Percent of control		
		25 μM	50 μM	100 μ
DNA synthesis	100 ± 6[a]	81 ± 5	73 ± 5*	64 ± 4*
RNA synthesis	100 ± 7[b]	123 ± 6	133 ± 5*	150 ± 6*
Protein synthesis	100 ± 5[c]	88 ± 5	84 ± 6	81 ± 6
DNA polymerase α	100 ± 5[d]	98 ± 6	62 ± 5*	54 ± 5*
mRNA polymerase	100 ± 6[e]	178 ± 9*	138 ± 6*	90 ± 6
rRNA polymerase	100 ± 7[f]	81 ± 7	79 ± 5*	62 ± 6*
tRNA polymerase	100 ± 6[g]	87 ± 7	76 ± 5*	60 ± 5*
Purine synthesis	100 ± 5[h]	95 ± 7	45 ± 6	15 ± 4*
PRPP amido transferase	100 ± 7[i]	104 ± 6	17 ± 2*	12 ± 3*
IMP dehydrogenase	100 ± 6[j]	72 ± 3*	61 ± 5*	39 ± 4*
Carbamyl phosphate synthetase	100 ± 5[k]	93 ± 6	77 ± 4*	75 ± 4*
Aspartate transcarbamylase	100 ± 7[l]	82 ± 6	79 ± 5*	87 ± 5
OMP decarboxylase	100 ± 5[m]	97 ± 6	91 ± 7	81 ± 6
Thymidylate synthetase	100 ± 7[n]	71 ± 6*	52 ± 5*	30 ± 3*
Thymidine kinase	100 ± 6[o]	79 ± 5*	69 ± 6*	44 ± 5*
Thymidine monophosphate kinase	100 ± 5[p]	79 ± 7	61 ± 5*	31 ± 4*
Thymidine diphosphate kinase	100 ± 7[q]	81 ± 6	65 ± 5*	39 ± 5*
Ribonucleoside reductase	100 ± 6[r]	79 ± 7	72 ± 5*	58 ± 4*
Dihydrofolate reductase	100 ± 5[s]	137 ± 8*	97 ± 6	96 ± 5
d(ATP)	100 ± 6[t]	—	—	82 ± 5
d(GTP)	100 ± 5[u]	—	—	8 ± 2*
d(CTP)	100 ± 5[v]	—	—	87 ± 5
d(TTP)	100 ± 6[w]	—	—	83 ± 5

Control values for 10^6 cells/h: [a]7719 dpm; [b]1014 dpm; [c]17492 dpm; [d]5318 dpm; [e]1343 dpm; [f]325 dpm; [g]400 dpm; [h]28614 dpm; [i]19375 dpm; [j]0.0878 Δ OD units; [k]0.273 μmol citrulline; [l]57387 dpm; [m]19758 dpm; [n]44743 dpm; [o]4362 dpm; [p]646 dpm; [q]275 dpm; [r]48780 dpm; [s]0.133 Δ OD units; [t]32.39 dpm; [u]23.79 pmol; [v]86.24 pmol; [w]2.204 pmol.

Table 4. Effects of **5a** on enzyme activities of L_{1210} leukemia cells after 60 min

$n = 5$	Control	Percent of control		
		25 μM	50 μM	100 μM
DNA synthesis	100 ± 6[a]	75 ± 6	64 ± 4	60 ± 5
RNA synthesis	100 ± 7[b]	103 ± 5	83 ± 4	73 ± 6*
Protein synthesis	100 ± 5[c]	48 ± 5*	42 ± 6*	37 ± 5*
DNA polymerase α	100 ± 5[d]	85 ± 6	77 ± 6	75 ± 4*
mRNA polymerase	100 ± 6[e]	144 ± 9*	87 ± 6	78 ± 6*
rRNA polymerase	100 ± 7[f]	89 ± 7	112 ± 6	133 ± 7*
tRNA polymerase	100 ± 6[g]	94 ± 6	105 ± 6	262 ± 7*
Purine synthesis	100 ± 5[h]	139 ± 8*	76 ± 5*	53 ± 5*
PRPP amido transferase	100 ± 7[i]	22 ± 5*	20 ± 3*	14 ± 3*
IMP dehydrogenase	100 ± 6[j]	73 ± 6*	54 ± 5*	51 ± 5*
Carbamyl phosphate synthetase	100 ± 5[k]	78 ± 6*	72 ± 5*	71 ± 6*
Aspartate transcarbamylase	100 ± 7[l]	81 ± 7	80 ± 6	79 ± 6*
OMP decarboxylase	100 ± 5[m]	120 ± 6	129 ± 6	80 ± 6
Thymidylate synthetase	100 ± 7[n]	238 ± 11*	151 ± 7*	17 ± 4*
Thymidine kinase	100 ± 6[o]	70 ± 6*	68 ± 7*	52 ± 6*
Thymidine monophosphate kinase	100 ± 5[p]	55 ± 5*	48 ± 6*	47 ± 5*
Thymidine diphosphate kinase	100 ± 7[q]	69 ± 6*	71 ± 6*	49 ± 4*
Ribonucleoside reductase	100 ± 6[r]	75 ± 6*	62 ± 6*	68 ± 5*
Dihydrofolate reductase	100 ± 5[s]	113 ± 6	97 ± 6	90 ± 5
d(ATP)	100 ± 6[t]	—	—	83 ± 6
d(GTP)	100 ± 5[u]	—	—	24 ± 4*
d(CTP)	100 ± 5[v]	—	—	83 ± 5
d(TTP)	100 ± 6[w]	—	—	35 ± 5*

Control values for 10^6 cells/h: [a]7719 dpm; [b]1014 dpm; [c]17492 dpm; [d]5318 dpm; [e]1343 dpm; [f]325 dpm; [g]400 dpm; [h]28614 dpm; [i]19375 dpm; [j]0.0878 Δ OD units; [k]0.273 μmol citrulline; [l]57387 dpm; [m]19758 dpm; [n]44743 dpm; [o]4362 dpm; [p]646 dpm; [q]275 dpm; [r]48780 dpm; [s]0.133 Δ OD units; [t]32.39 dpm; [u]23.79 pmol; [v]86.24 pmol; [w]2.204 pmol.

using orotidine-5-monophosphate [carboxyl-^{14}C][34.9 μCi/mmol] by Appel's method.[26] Thymidylate synthetase activity was analyzed by Kampf *et al.*'s method.[27] 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.*[28] PRPP amidotransferase activity was determined by Spassova *et al.*'s method;[29] IMP dehydrogenase activity was analyzed with [8-^{14}C]-IMP (54 mCi/mmol) (Amersham, Arlington Heights, IL) after separating XMP on PEI plates (Fisher Scientific) by TLC.[30] Protein content was determined for the enzymatic assays by the Lowry technique.[31]

After deoxyribonucleoside triphosphates were extracted,[32] levels were determined by the method of Hunting and Henderson[33] with calf thymus DNA, *Escherischia coli* DNA polymerase I, non-limiting amounts of the three deoxyribonculeoside triphosphates not being assayed, and either 0.4 μCi of [^{3}H-methyl]-dTTP or [5-^{3}H]dCTP.

The effects of compounds **1f**, **2b** and **5a** on DNA strand scission was determined by the methods of Suzuki *et al.*,[34] Pera *et al.*[35] and Woynarowski *et al.*[36] L_{1210} lymphoid leukemia cells were incubated with 10 μCi thymidine methyl-^{3}H, 84.0 Ci/mmol for 24 h at 37°C. L_{1210} cells (10^7) were harvested and then centrifuged at 600 ***g*** for 10 min in PBS. They were later 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); this was followed by 0.2 ml of the cell preparation. After the gradient was incubated for 2.5 hr at room temperature, it was centrifuged at 12000 rpm at 20°C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the bottom of the gradient, neutralized with 0.2 ml of 0.3 N HCl, and measured for radioactivity. Thermal calf thymus DNA denaturation studies and DNA viscosity studies were conducted after incubation of compounds **1f**, **2b** and **5a** at 100 μM at 37°C for 24 h.[37]

Statistics

The mean and standard deviation are designated by $x \pm$ SD. The probable level of significance (p) between test and control samples was determined by the Student's t-test with the raw data.

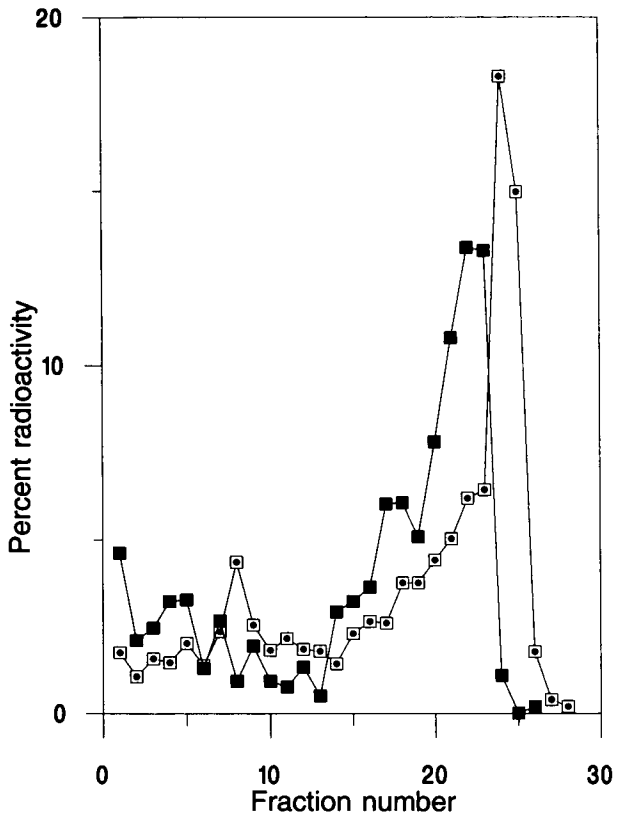


Figure 2. DNA strand scission-1. ⊡, control; ▩, **1f**.

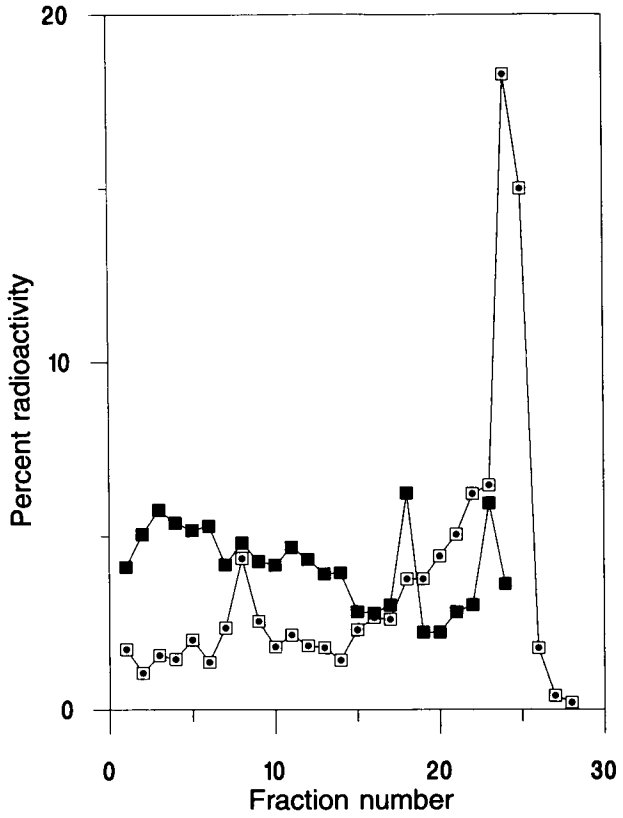


Figure 3. DNA strand scission-4. ⊡, control; ■, **2b**.

Results

Cytotoxicity was demonstrated by a large number of the compounds against the growth of murine and human cultured cells. All of the compounds were active against L_{1210} lymphoid leukemia growth with ED_{50}'s less than 4 μg/m, except **1k**. Compounds **1e**, **1f**, **1g**, **3b**, **3c** and **4d** were particularly effective with ED_{50} values less than 1.0 μg/ml. Growth of human $Tmolt_3$ leukemia was effectively blocked by all of the compounds except **1e**, **4d**, **6a**, **6c**, **6d** and **7**. HeLaS[3] uterine carcinoma growth was effectively inhibited by all compounds with the exception of **3d** and **5c**. Cells cultured from human solid tumors were also selectively reduced by the compounds. Growth of adenocarcinoma colon tumors (SW48) was significantly reduced by **1g**, **5b**, **6c**, **6f** and **6g** with ED_{50} values of less than 1 μg/ml. Compounds **1h**, **1j**, **3e**, **4a**, **4d**, **5f** and **7** were inactive in this screen. KB nasopharyngeal growth was inhibited by **1i**, **2b**, **3b**, **5a**, **5b**, **5d**, **6c**, **6d**, **6f**, and **6g**; all other compounds were inactive. Lung brochogenic growth was reduced by **1i**, **2b** and **6g**; all other compounds were inactive. Brain glioma growth was reduced by **1a**, **1d**, **1i**, **2b**, **3a**, **4a**, **4b**, **5c**, **5d**, **5e**, **5f**, **6b**, **6c**, **6d**, **6e**, **6f**, **6g** and **7**. Osteosarcoma growth was inhibited by **1a**, **1i**, **1j**, **3c**, **5f**, **6b** and **6g** (Table 1).

Compounds **1f**, **2b** and **5a** were selected for a mode of action study in L_{1210} lymphoid leukemia cells, since they were representative of each chemical class. In these cells, all compounds significantly inhibited DNA synthesis over 60 min (Tables 2, 3 and 4). Only compound **5a** marginally inhibited L_{1210} RNA synthesis and significantly reduced protein synthesis. L_{1210} DNA polymerase α activity was inhibited after 60 min by 1f and reduced by **5a**. rRNA and tRNA polymerase activities were inhibited by **1f** and **2b**, but **5a** stimulated both polymerase activities. All three compounds markedly suppressed L_{1210} *de novo* purine synthesis over 60 min. Both regulatory enzyme sites, PRPP amidotransferase and IMP dehydrogenase, were inhibited by the three agents. Regulatory enzymes in the pyrimidine *de novo* synthetic pathway were only marginally inhibited by the agents. Thymidylate synthetase activity was inhibited significantly by all of the compounds. Nucleoside kinase activities were inhibited by **2b** and **5a**. Compound **1f** inhibited only thymidine kinase activity. Ribonucleoside reductase activity was reduced after 60 min incubations with all three compounds. Dihydrofolate re-

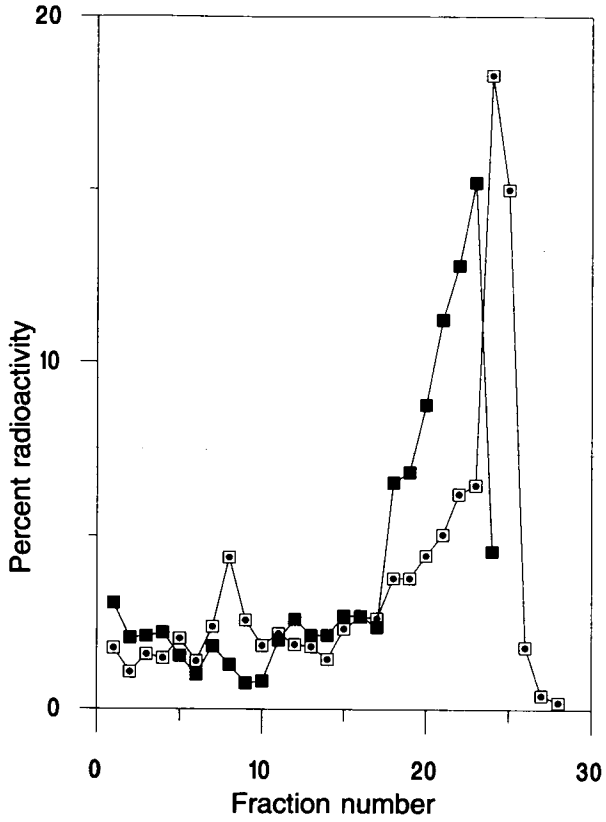


Figure 4. DNA strand scission. ⊡, control; ▩, **5a**.

ductase activity was inhibited only by **1f**. d(NTP) pools were reduced by drug treatment but d(GTP) pools were more significantly reduced. d(TTP) pool levels were markedly reduced by **5a**. Interaction of the agents with cDNA over a 24 h period showed that DNA viscosity did not change. Thermal DNA denaturation (as measured by Im values) was 74°C for the control, 57°C for compound **1f**, 56°C for compound **2b** and 61°C for compound **5a**. Compound **5a** showed no changes in DNA absorption at 260 nm but **1f** and **2b** demonstrated a hyperchromic shift with the peak absorption of DNA to a lower UV wavelength. When the compounds at 100 μM were incubated with L_{1210} cells for 24 h, DNA strand scission occurred with **2b** (Figure 3). Compounds **1f** and **5a** appeared to shift the double strand DNA molecule slightly in the gradient, but did not cause significant fragmentation of the strands (Figures 2, 4).

Discussion

The phthalimide, saccharin, 1,8-naphthalimide, succinimides, homophthalimide, and 2,3-dihydrophthazine-1,4-dione *N*-substituted derivatives demonstrated potent cytotoxicity against single cell suspended cells, e.g., L_{1210}, $Tmolt_3$ and HeLa-S^3. Solid tumor growth was more selectively inhibited by the derivatives. Adenocarcinoma colon carcinoma growth was inhibited by most of the compounds. The 2,3-dihydrophthalazine-1,4-diones and homophthalimide *N*-substituted derivatives showed more activity against KB nasopharynx growth, and only selected compounds from any chemical group demonstrated activity against lung bronchogenic or osteosarcoma growths. Approximately half of the compounds demonstrated activity against glioma growth but no clear pattern emerged regarding functional groups needed for cytotoxicity. Examination of the mode of action of these derivatives in L_{1210} lymphoid leukemia cells demonstrated that DNA synthesis was a major target. The *de novo* synthesis of purines was markedly reduced because both of its regulatory enzymes in the pathways, *ie*, PRPP amido transferase and IMP dehydrogenase, were inhibited markedly by the agents. If the cyclic imide ring is present in the structure of these compounds, it usually inhibits *de novo* purine synthesis.[2–4] An additional target for the agents is the DNA polymerase a enzyme. Since the template and d(NTP) pools are added exogenous in this assay, the agents are inhibiting the polymerase enzyme activity directly. Thus, the DNA template did not appear to be a target of the agents. Only the saccharin derivative **2b** showed any ability to fragment L_{1210} DNA. This is of a magnitude to account for the DNA synthesis reduction afforded by the compounds. Other sites of minor inhibition by the agents are the regulatory enzymes in the pyrimidine pathway. Although these inhibitory effects are probably additive they are not a major site of the agents. Ribonucleoside reductase, thymidylate synthetase and thymidine kinase appear to be a major sites of inhibition for the agents. Inhibition of these enzymes would reduce d(NTP) levels which was observed after 60 min. Reduction of the regulatory enzymes in the purine pathway would help reduce d(ATP) and d(GTP) pool levels. Lowering of the pools would reduce the incorporation of deoxyribonucleosides into DNA and ultimately cause cell death.

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