Anti-Cancer Drugs 1996, **7**, pp. 613–620

Experimental report

Antineoplastic activities of 2,3,4-chloro-substituted β-alkylaminopropiophenone derivatives in CF_1 mice and in murine and human tumor cells

Yunsheng Huang and Iris H Hall

Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA. Tel: (+1) 919 966-1121; Fax: (+1) 919 966-6919.

A series of β-alkylaminopropiophenone derivatives were demonstrated to be potent antineoplastic agents. Several compounds showed activity against Ehrlich ascites carcinoma growth in CF_1 mice by demonstrating over 70% inhibition. Most of these agents proved to be potent cytotoxic agents in inhibiting the growth of a number of murine and human cancer cell lines grown in tissue culture. Their ED_{50} values were comparable to those of the selected standard anticancer drugs, such as 6-MP, ara-C, hydroxyurea, 5-FU, 6-aza-UMP, etoposide, antimycin A, actinomycin D and cycloheximide. In the mode of action studies in $Tmolt_3$ cells, β-(3″,5″-dimethyl)piperidinopropiophenone was observed to reduce DNA and RNA synthesis significantly at 25 μM within 60 min incubation. The site of action of this agent appears to involve the reduction of the activities of $Tmolt_3$ DNA polymerase α, dihydrofolate reductase, PRPP-amido transferase and ribonucleoside reductase.

Key words: Cytotoxicity, β-alkylaminopropiophenones, purine synthesis inhibitors.

Introduction

Previously, a series of β-alkylaminopropiophenone derivatives have been reported to be potent hypolipidemic agents by lowering both serum cholesterol and triglyceride levels.[1–4] These agents also demonstrated potent anti-inflammatory activity.[5] More recently, a number of these agents were observed to be potent antineoplastic agents.[6] Similar agents have been reported to inhibit the growth of P388 leukemia, L1210 leukemia, EMT6 mammary carcinoma, etc.[7–17] In this study, a series of β-alkylaminopropiophenone derivatives with substituted amino moities or with chloro-substituted phenyl rings were selected to test their *in vivo* inhibition of Ehrlich ascites carcinoma growth and *in vitro* cytotoxicity against a number of murine and human tumor cultured cell lines.

Correspondence to IH Hall

Materials and methods

Source of compounds

All tested compounds were synthesized and characterized previously.[1–4] All radioisotopes were purchased from New England Nuclear (Boston, MA) unless otherwise indicated. Radioactivity was determined in Fisher Scintiverse scintillation fluid with correction for quenching. Substrates and cofactors for enzymes assays were obtained from Sigma (St Louis, MO).

Pharmacological methods

In vivo studies. CF_1 mice (25–30 g) were inoculated with 2×10^6 cells i.p. in isotonic saline (pH 7.0) on day 0. Drugs were suspended and homogenized in 0.05% Tween 80/H_2O and administered to the mice i.p. at 8 mg/kg/day from day 1 to day 9. On day 10, animals were sacrificed by cervical dislocation. A transverse incision was made across the abdomen, the ascitic fluid drained and the volume measured. Samples of ascitic fluid were collected, centrifuged and astrocrits calculated. Percents of inhibition values were calculated for all compounds.[6] The antineoplastic agents 5-FU and 6-MP were used as standards in this screen.

Cytotoxicity screens. All compounds were tested for cytotoxicity by preparing a 1 mM solution in 0.05% Tween 80/H_2O. The following cell lines were selected and maintained by the literature methods:

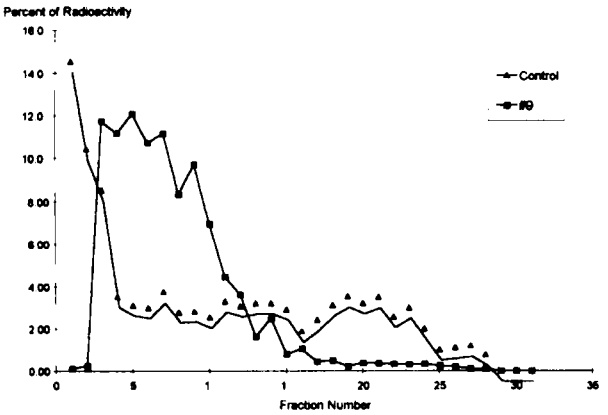


Figure 1 Effect of analog **9** on DNA strand scisson in $Tmolt_3$ cells.

mouse L1210 lymphoid leukemia,[18] human $Tmolt_3$ leukemia,[19] mouse P388 leukemia,[20] human HeLa-S^3 carcinoma,[21] human HuT-78 lymphoma,[22] human HeLa carcinoma,[23] human HCT-8 adenocarcinoma,[24] human colon SW-480 carcinoma,[25] human lung A-549 carcinoma,[26] human lung MB-9812 carcinoma,[27] human KB nasopharynx carcinoma,[28] human skin A-431 carcinoma,[26] rat bone UMR-106 sarcoma,[29] human Hs-683 glioma,[30] mouse L-929 cells,[31] human G-361 melanoma[32] and mouse WEHI-164 fibrosarcoma.[33] The protocol used to assess cytotoxicity was that of Geran *et al.*[18] Standard anti-cancer agents were used in each cell line. Values were expressed as $ED_{50} = \mu g/ml$, i.e. the concentration which inhibits 50% of the cell growth, which was determined by the Trypan blue exclusion technique. A value less than 4 μg/ml was required for significant activity of growth inhibition. The ED_{50} values for solid tumor cells were determined by the method of Liebovitz *et al.*[25] using crystal violet determined at 580 nm in a 96-well plate with a microplate reader.

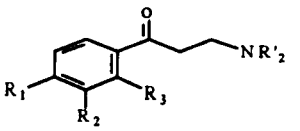
Incorporation studies. Incorporation of labeled precursors into [^{3}H]DNA [^{3}H]RNA, and [^{3}H]protein for $Tmolt_3$ cells was determined by the method of Liao *et al.*[34] The concentration (25, 50 and 100 μM) responsible for inhibition of DNA, RNA and protein synthesis was determined after 60 min incubation. [^{14}C]Glycine (53.0 mCi/mmol) incorporation into purines was determined by the method of Cadman *et al.*[35] [^{14}C]Formate (53.0 mCi/mmol) incorporation into pyrimidines was determined by the method of Christopherson *et al.*[36] Deoxyribonucleoside triphosphates were extracted by the method of Bagnara and Finch,[37] and deoxyribonucleoside triphosphate pool levels were determined by the method of Hunting and Henderson[38] with calf thymus DNA, *Escherichia coli* DNA polymerase I, non-limiting amounts of the three deoxyribonucleoside triphosphates and either 0.4 μCi [^{3}H-methyl]dTTP or [5-^{3}H]dCTP.

Enzyme assays. Inhibition of various enzyme activities was carried out first by preparing the appropriate $Tmolt_3$ cell homogenate or subcellular fraction, then adding the drug to be tested during the enzyme assay. For the concentration response studies, the inhibition of enzyme activity was determined at 25, 50 and 100 μM of drugs for 60 min incubation. DNA polymerase α activity was determined by the method of Sedwick *et al.*[39] and Eichler *et al.*[40] mRNA, rRNA and tRNA polymerase activities were assayed by the methods of Hall *et al.*[41] and Anderson *et al.*[42] PRPP-amidotransferase activity was determined spectrophotometrically by the method of Spassova *et al.*[43,44] IMP dehydrogenase activity was measured by the method of Becker and Lohr.[45] Dihydrofolate reductase activity was assayed by the method of Ho *et al.*[46] Ribonucleotide reductase activity was determined by the method of Moore and Hulbert.[47] Carbamyl phosphate synthetase activity was ascertained by the method of Kalman *et al.*[48] Aspartate transcarbamylase activity was determined by the method of Koritz and Cohen.[49] Thymidylate synthetase activity was measured with [5-^{3}H]UMP (14 Ci/mmol) by the method of Kampf *et al.*[50] Thymidine kinase activities were determined using [methyl-^{3}H]thymidine (84 Ci/mmol) by the method of Maley and Ochoa[51]

DNA studies

The effects of the compounds on DNA strand scission were determined by the methods of Suzuki *et al.*,[52] Pera *et al.*[53] and Woynarowski *et al.*[54] $Tmolt_3$ lymphoid leukemia cells were incubated with 100 μM drug in PBS with 10 μCi [^{3}H-methyl]thymidine for 24 h. The cells were harvested and washed 2 times in isotonic 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 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 0.3 N HCl and radioactivity measured. Thermal calf thymus DNA

Table 1. Antineoplastic activity against Ehrlich ascites carcinoma growth of β-alkylaminopropiophenone analogs in CF_1 mice at 8 mg/kg/day, i.p.



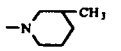
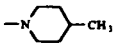
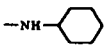
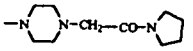
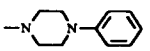
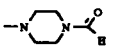
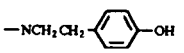
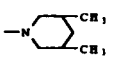
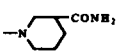
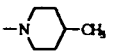
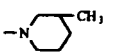
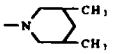
Analog ($n = 6$)	R_1	R_2	R_3	$—NR'_2$	Inhibition of Ehrlich ascites carcinoma growth (%)
1	H	H	H		26
2	H	H	H		0
3	H	H	H		82
4	H	H	H		63
5	H	H	H		0
6	H	H	H		74
7	H	H	H		67
8	H	H	H		55
9	H	H	H		70
10	H	H	H		38
11	H	H	Cl		24
12	Cl	Cl	H		46
13	Cl	Cl	H		16
14	Cl	Cl	H		33
15	Cl	Cl	H		43
16	H	Cl	H		0
17	H	Cl	H		20
18	Cl	H	H		21
19	Cl	H	H		0
20	Cl	H	Cl		14
21	Cl	H	H		0
22	Cl	H	H		0
5-Fluorouracil (27.5 mg/kg/day)					90
6-Mercaptopurine (0.5 mg/kg/day)					100

Table 2. *In vitro* cytotoxicity screen of β-alkylaminopropiopenone analogs against different murine or human tumor tissue cultured cell lines (ED_{50} μg/ml)

Analog	L1210 (mouse)	$Tmolt_3$ (human)	P-388 (mouse)	HeLa-S^3 (human)	HuT-78 (human)	HeLa (human)	HCT-8 (human)	SW-480 (human)	A-549 (human)
1	3.05	3.85	2.84	3.64	3.15	3.67	3.71	2.04	2.67
2	3.52	3.92	3.14	2.95	2.48	5.57	7.30	4.99	5.81
3	4.35	5.96	1.73	2.68	3.94	9.54	11.24	8.86	6.56
4	2.97	2.31	2.70	3.57	2.14	3.72	3.58	2.32	4.22
5	1.78	1.38	3.78	3.43	3.49	1.17	1.01	0.28	4.02
6	2.95	2.15	1.73	4.46	2.92	1.32	0.66	0.32	4.97
7	4.28	4.09	2.05	3.85	5.07	8.75	7.89	0.50	9.03
8	3.52	4.07	1.73	3.06	2.69	7.28	2.59	5.38	6.91
9	2.82	1.99	0.65	2.89	2.11	3.56	2.57	1.47	3.48
10	2.10	2.23	1.51	3.91	3.72	0.53	0.60	0.33	2.30
11	2.71	1.04	3.56	4.80	4.17	1.29	1.17	0.24	1.06
12	2.86	1.85	4.00	3.43	3.15	9.60	8.00	7.18	8.52
13	3.03	2.84	4.86	3.85	3.49	8.80	6.76	5.33	6.27
14	2.43	1.96	3.46	3.23	3.72	5.47	2.95	2.53	5.47
15	2.88	3.00	2.48	3.09	2.82	4.40	5.05	4.39	7.23
16	3.67	3.42	2.81	2.40	3.49	8.38	10.76	5.92	9.51
17	2.16	1.31	2.27	2.67	1.13	4.31	5.32	2.74	6.40
18	2.52	1.96	3.03	4.80	1.80	2.77	2.86	0.98	5.85
19	1.81	1.85	2.92	4.67	1.92	3.55	3.39	0.69	5.43
20	2.33	1.73	2.27	3.50	1.69	2.74	4.54	3.38	5.28
21	2.27	1.27	1.62	4.33	2.48	1.74	7.49	4.07	5.87
22	1.21	1.60	3.24	3.36	5.75	3.73	8.06	2.32	6.40
6-MP	2.43	1.62	2.16	2.12	5.47	5.61	1.15	3.61	4.71
Ara-C	2.43	2.67	2.38	2.13	2.86	4.74	2.54	3.42	6.28
Hydroxyurea	2.67	4.47	1.30	1.96	3.87	8.12	1.77	7.33	8.89
5-FU	1.41	2.14	1.41	2.47	5.81	4.11	1.12	3.09	3.58
6-Aza UMP	1.20	1.54	1.41	2.48	4.46	4.69	0.75	5.73	2.63
Etoposide	1.83	—	3.03	7.87	3.20	3.05	3.78	3.34	4.74
Antimycin A	1.79	—	1.08	5.83	4.13	4.29	4.64	6.44	6.49
Actinomycin D	1.98	—	1.41	5.88	4.88	2.46	3.71	3.18	0.90
Cycloheximide	1.44	—	1.84	3.57	6.31	0.86	0.81	3.62	1.34

denaturation studies, UV absorption studies and DNA viscosity studies were conducted after incubating compound **9** at 100 μM in PBS buffer pH 7.2 at 37°C for 24 h.[55]

Results

In the *in vivo* Ehrlich ascites carcinoma screen selected derivatives at 8 mg/kg/day i.p. demonstrated anti-neoplastic action (Table 1). Compound **3** afforded 82% inhibition of tumor growth, while compound **6** resulted in 74% and compound **9** resulted in 70% inhibition of tumor growth. All of the remaining compounds were significantly less active. Substitution of the chloride atom on any of the positions of the aromatic ring did not improve the antineoplastic activity at this dose.

Cytotoxicity in murine and human tumor cell lines was demonstrated by a number of the compounds. Whereas an ED_{50} value of 4 μg/ml or less is considered active in these screens, noted for the discussion here are the compounds with ED_{50} values less than 2 μg/ml. In the murine L1210 lymphoid leukemia screen compounds **5**, **19** and **22** and in the P-388 lymphocytic leukemia screen compounds **3**, **6**, **8** and **9** demonstrated excellent activity. In the human $Tmolt_3$ leukemia screen compounds **5**, **9**, **11**, **12**, **14**, **17–22** and cutaneous lymphoma HuT-8 screen compounds **17**, **18**, **19** and **20** afforded significant activity. Compounds **2**, **3**, **9**, **16** and **17** inhibited the growth of HeLa-S^3 suspended uterine carcinoma while compounds **5**, **6**, **10**, **11** and **21** reduced solid HeLa growth. Compounds **5**, **6**, **10** and **11** reduced the growth of adenocarcinoma growth of ileum HCT-8 growth, and compounds **5–7**, **9**, **11**, **18** and **19** reduced the growth of adenocarcinoma SW-480 colon growth. Lung A549 growth was inhbited only by compound **11** with an ED_{50} value of 1.01 μg/ml.

Table 2 (continued). *In vitro* cytotoxicity screen of β-alkylaminopropiophenone analogs against different murine or human tumor tissue cultured cell lines (ED_{50} = μg/ml)

Analog	MB-9812 (human)	KB (human)	A-431 (human)	UMR-106 (rat)	Hs-683 (human)	L-929 (mouse)	G-361 (human)	WEHI-164 (mouse)
1	1.33	5.63	3.92	2.64	7.32	2.05	11.12	6.89
2	1.68	6.61	8.42	3.37	7.76	9.07	14.60	7.57
3	7.65	7.14	9.29	6.74	5.79	8.15	14.84	7.67
4	1.74	5.84	2.20	2.78	4.09	4.72	12.73	7.43
5	0.52	2.63	1.42	2.06	1.18	2.48	1.86	7.58
6	0.62	2.21	1.31	1.66	1.78	4.03	3.06	1.52
7	1.27	3.82	10.74	2.54	6.56	5.23	3.16	6.18
8	4.81	0.42	4.10	0.34	0.96	1.60	0.62	0.46
9	0.71	0.89	0.79	0.68	0.94	1.17	2.85	0.56
10	1.46	1.51	0.79	2.28	1.48	3.44	3.02	0.38
11	0.06	0.68	0.95	1.30	0.89	0.74	0.98	0.96
12	6.53	7.09	9.67	6.39	5.97	7.28	10.08	4.75
13	3.22	6.83	7.12	5.12	9.12	4.95	13.25	4.71
14	1.42	7.70	3.43	4.48	7.74	8.59	8.58	4.95
15	2.39	7.83	5.25	4.79	6.58	7.25	7.16	3.89
16	4.47	7.25	9.18	4.64	6.61	5.31	9.98	8.26
17	1.14	5.60	8.76	2.92	4.12	6.22	1.82	8.47
18	1.39	7.11	3.94	1.91	6.96	6.26	3.82	5.62
19	0.18	7.52	4.71	2.11	6.97	5.65	8.78	6.51
20	0.47	5.46	4.12	1.65	6.09	5.94	0.23	6.38
21	0.51	5.22	2.49	1.92	6.76	6.76	1.52	7.15
22	0.97	8.40	5.84	1.96	5.06	6.62	2.60	6.74
6-MP	4.29	11.04	3.42	9.13	4.46	5.41	12.07	6.36
Ara-C	6.16	2.84	0.92	0.86	1.88	3.87	6.54	3.90
Hydroxyurea	7.18	5.27	3.21	2.87	2.27	6.68	9.15	5.22
5-FU	5.64	1.25	0.61	3.52	1.28	5.41	2.45	5.28
6-Aza UMP	2.39	3.57	1.09	4.02	1.93	4.27	4.78	2.80
Etoposide	3.50	3.32	0.71	3.57	2.44	5.13	4.65	3.49
Antimycin A	2.96	5.40	2.28	2.05	3.90	3.90	3.88	3.37
Actinomycin D	1.28	0.93	0.30	0.33	1.15	3.11	2.48	1.63
Cycloheximide	1.18	0.57	0.61	0.60	2.04	1.70	10.10	3.39

Whereas lung bronchogenic tumor growth was reduced significantly by compounds **1**, **2**, **4–7**, **9–11**, **14** and **17–22**. Compounds **8–11** inhbited KB nasopharynx growth and compounds **5**, **6** and **9–11** inhibited skin A431 epidermoid growth. Human G-361 melanoma growth was reduced by compounds **5**, **8**, **11**, **17**, **20** and **21**, and human Hu-683 glioma growth was suppressed by compounds **5**, **6**, **8–10** and **11**. Rat UMR-106 osteosrarcoma growth was reduced by compounds **6**, **8**, **9**, **11**, **18** and **20–22**. Mouse L929 fibrosarcoma growth was reduced by compounds **8** and **11** and WEHI-164 fibrosarcoma growth was inhibited by compounds **8–11**. See Table 2.

A mode of action study was undertaken with compound **9** as being representative of the chemical class in human $Tmolt_3$ leukemia cells (Table 3). DNA and RNA syntheses were inhibited by compound **9** greater than 50% at all concentration within 60 min. Protein synthesis was not significantly reduced by the agent. DNA polymerase α activity was reduced 48% but only mRNA polymerase activity was marginally reduced by 21%. Ribonucleoside reductase activity was reduced 57% at 100 μM after 60 min. *De novo* purine syntheis was reduced 24% after 60 min; the regulatory enzyme PRPP-amidotransferase was inhibited 80% by compound **9** at 25 μM and 92% at 100 μM. An additional important site of the drug action is dihydrofolate reuctase activity, which was suppressed 98% at 100 μM. Neither *de novo* pyrimidine synthesis nor the activities of its regulatory enzymes were inhibited significantly by the agent. Thymidylate synthetase activity was inhibited 36% at 100 μM. The thymidine kinase activities were actually stimulated by the agent. d[GTP], d[CTP] and d[TTP] pool levels were reduced after 60 min incubation with the drug at 100 μM.

Table 3. Effects of analog **9** on $Tmolt_3$ cell metabolism after incubation for 60 min [percent of control ($X \pm SD$)]

Assay ($N=6$)	Control	25 μM	50 μM	100 μM
DNA synthesis	100 ± 6^a	$42 \pm 4^*$	$41 \pm 3^*$	$37 \pm 4^*$
RNA synthesis	100 ± 5^b	$41 \pm 3^*$	$39 \pm 3^*$	$24 \pm 2^*$
Protein synthesis	100 ± 6^c	$180 \pm 6^*$	$137 \pm 5^*$	85 ± 6
DNA polymerase α	100 ± 5^d	$75 \pm 5^*$	$68 \pm 6^*$	$52 \pm 4^*$
mRNA polymerase	100 ± 4^e	119 ± 5	110 ± 6	$79 \pm 5^*$
rRNA polymerase	100 ± 6^f	90 ± 5	88 ± 5	83 ± 4
tRNA polymerase	100 ± 6^g	110 ± 6	110 ± 5	86 ± 5
Ribonucleotide reductase	100 ± 6^h	$69 \pm 4^*$	$62 \pm 5^*$	$43 \pm 4^*$
De novo purine synthesis	100 ± 6^i	92 ± 7	91 ± 5	$76 \pm 5^*$
PRPP-amido transferase	100 ± 6^j	$20 \pm 2^*$	$18 \pm 3^*$	$8 \pm 2^*$
IMP dehydrogenase	100 ± 7^k	109 ± 6	97 ± 7	92 ± 5
De novo pyrimidine synthesis	100 ± 6^l	96 ± 6	$78 \pm 5^*$	$76 \pm 5^*$
Carbamyl phosphate synthetase	100 ± 6^m	$78 \pm 6^*$	$78 \pm 5^*$	$70 \pm 5^*$
Aspartate transcarbamylase	100 ± 7^n	99 ± 7	95 ± 5	93 ± 6
Thymidylate synthetase	100 ± 6^o	99 ± 7	98 ± 6	$64 \pm 5^*$
Thymidine Kinase	100 ± 5^p	$184 \pm 6^*$	$278 \pm 6^*$	$250 \pm 6^*$
TDP kinase	100 ± 4^q	$155 \pm 6^*$	$141 \pm 6^*$	117 ± 6
TTP kinase	100 ± 4^r	$138 \pm 6^*$	$139 \pm 5^*$	$164 \pm 7^*$
Dihydrofolate reductase	100 ± 6^s	$26 \pm 4^*$	$21 \pm 3^*$	$2 \pm 1^*$
d[ATP]	100 ± 4^t	—	—	97 ± 4
d[GTP]	100 ± 6^n	—	—	$73 \pm 5^*$
d[CTP]	100 ± 6^v	—	—	$45 \pm 4^*$
d[TTP]	100 ± 6^w	—	—	$78 \pm 5^*$

*$p \leq 0.001$; control values based on 10^6 $Tmolt_3$ cells.
[a]12349 d.p.m., [b]2569 d.p.m., [c]17 492 d.p.m., [d]9019 d.p.m., [e]1343 d.p.m., [f]325 d.p.m., [g]400 d.p.m., [h]48 780 d.p.m., [i]24 500 d.p.m., [j]0.087 OD units, [k]1487 d.p.m., [l]19 758 d.p.m., [m]0.850 μmol citrulline, [n]0.807 mol *N*-carbamyl aspartate, [o]14260 d.p.m., [p]1317 d.p.m., [q]1179 d.p.m., [r]1891 d.p.m., [s]0.144 OD units, [t]17.07 pmol, [u]13.58 pmol, [v]33.60 pmol, [w]31.40 pmol.

ctDNA studies showed that the agent did not interact with the bases of the DNA molecule as determined by UV absorption from 220 to 340 nm. However, the thermal denaturation studies showed that the control T_m value was 90°C whereas treated ctDNA resulted in at T_m value of 70°C. The DNA viscosity after treatment with compound **9** at 100 μM for 24 h did not significantly change. $Tmolt_3$ cells incubated for 24 h at 100 μM of drug showed minor DNA fragmentation.

Discussion

Chloride substitution in the aromatic ring of the β-alkylaminopropiophenones did not significantly increase the *in vivo* anti-neoplastic activity at 8 mg/kg/day i.p. Previous studies have shown that nitro substitutions in the aromatic ring also led to a reduction in pharmacological activity when comparing the the identical heterocyclic ring structure in the NR′2 position.[6] This suggests that electron withdrawing groups substituted on the aromatic ring cause a reduction in anti-neoplastic activity *in vivo*. The NR′2 substitution of a piperidino ring resulted in 85% indihibtion of Ehrlich ascites tumor growth.[6] Variation of the heterocyclic ring did not improve the inhibition of Ehrlich ascites carcinoma tumor growth at 8 mg/kg/day and the same observation can be made for the aromatic substituted chloride derivatives.

Cytotoxicity of the compounds in human and rodent tissue culture screens showed that selected derivatives retained activity against the growth of certain histological types of tumors. Among the 22 compounds there were derivatives which afforded ED_{50} values which were favorable when compared to standard clinical useful antineoplastic drugs. Compounds **5**, **9** and **11** demonstrated good activity in most of the screens, demonstrating better broad spectrum cytotoxicity. The functional groups substituted on the β-alkylaminopropiophenone probably dictated the individual ED_{50} values in each of the tumor screens.

β-(3″,5″-Dimethyl)piperidinopiophenone as a representative member of the chemical class inhibited $Tmolt_3$ leukemia DNA and RNA syntheses in 60 min from 25 to 100 μM. *De novo* purine synthesis was inhibited at the regulatory site of PRPP-amido transferase but not at the secondary regu-

latory site, IMP dehydrogenase. Dihydrofolate reductase activity was markedly reduced at all concentrations employed. Inhibition of this enzyme would block one-carbon transfer for purine synthesis, thus reducing both DNA and RNA syntheses. The suppression of ribonucleoside reductase activity would reduce the deoxyribonucleotides for incorporation into DNA. These d[NTP] pools are affected quicker than ribonucleotide pools because they represent only 10% of the total triphosphate pools in mamalian cells and this enzyme regulates their synthesis as they cross the nuclear membrane. Thus it is not unexpected to observe after 60 min that d[GTP], d[CTP] and d[ATP] pools are reduced as well as DNA sysnthsis. Conversely, the inhibition of DNA polymerase α activity by the agent after 60 min would tend to cause an increase in d[NTP] pools because the deoxyribonucleosides were not being incorporated into the new strand of DNA and would accumulate in the cell. The increase in the nucleoside kinase activities afforded by the agent would increase the nucleotide levels. Thus, the overall effects on the nucleotide pools are the result of a number of effects afforded by the agent.

The DNA molecule itself probably is not a major target of the drug even though the T_m value changed, suggesting the possibility of intercalation. Although the DNA viscosity was lower in the presence of the drug, only a small amount of DNA fragmentation occurred. These studies would have to be performed in more detailed to answer this question but the effects appear to be non-specific interaction of the drug with DNA or latent effects from metabolic effects.

References

1. Huang Y, Hall IH. Hypolipidemic effects of β-alkylaminophenone analogs in rodents. *Eur J Med Chem* 1996; **31**: 281.
2. Huang Y, Hall IH. Synthesis and pharmacological studies of α-methyl-β-alkylaminopropiophenone analogs as hypolipidemic agents in rodents. *Arch Pharm* 1996; in press.
3. Huang Y, Hall IH. Hypolipidemicactivity of β-alkylamino-(2'-, 3'-, and 4'-mononitro-, or mono-and di-halogen substituted)propiophenone analogs in rodents. *Arch Pharm [Weinheim]* 1996; in press.
4. Huang Y, Hall IH. Synthesis and hypolipidemic evaluation of β-alkylaminopropiophenone and β-alkylaminopropio-2'-naphthone derivatives in rodents. *der Pharmazie* 1996; in press.
5. Huang Y, Hall IH. *In vivo* and *in vitro* anti-inflammatory activity of α-, β-, and γ-alkylaminoketones in CF_1 mice and in macrophages. *Res Commun Pharm Toxicol* 1966; **1**: 17.
6. Huang Y, Hall IH. Antineoplastic activities of α-, β-, and γ-alkylaminopropiophenone derivatives in mice and in murine and human tissue culture cells. *Anticancer Res* 1996; **16**: 597.
7. Dimmock JR, Raghavan SK, Logan BM, Bigam GE. Antileukemic evaluation of some Mannich bases derived from 2-arylielene-1,3-diketones. *Eur J Med Chem* 1983; **18**: 248.
8. Dimmock JR, Taylor WG. Evaluation of nuclear-substituted styryl ketones and related compounds for antitumor and cytotoxic properties. *J Pharm Sci* 1975; **64**: 241.
9. Dimmock JR, Raghavan SK, Bigam GE. Evaluation of Mannich bases of 2-arylidene-1,3-diketones versus murine P-388 leukemia. *Eur J Med Chem* 1988; **23**: 111.
10. Dimmock JR, Jonnalagadda SS, Leek DM, Warrington RC, Fang WD. Evaluation of Mannich bases of styryl ketone and related hydrazones for activity against P-388 leukemia. *Neoplasma* 1988; **35**: 715.
11. Dimmock JR, Smith LM. Syntheses and evalation of ketals, hemithioketals, and dithioketals of conjugated styryl ketones principally for antineoplastic activity. *J Pharm Sci* 1980; **69**: 575.
12. Dimmock JR, Phillips OA, Wonko SL, *et al.* Evaluation of some Mannich bases of conjugated styryl ketones and related compounds versus the WiDr colon cancer *in vitro*. *Eur J Med Chem* 1989; **24**: 217.
13. Dimmock JR, Erciyas E, Bigam GE, Kirkpatrick DL, Duke MM. Cytotoxicity of Mannich bases of α-arylidene-β-ketoesters and related compounds against EMT6 mammary carcinoma cells. *Drug Design Del* 1990; **7**: 51.
14. Dimmock JR, Erciyas E, Bigam GE, Kirkpatrick DL, Duke MM. Intramolecular cyclization and cytotoxicities of some Mannich bases of styryl ketones. *Eur J Med Chem* 1989; **24**: 379.
15. Eshba NH, Salama HM. 5-(2-Oxo-3-indolinylidene) thiazolidine-2,4-dione-1,3-di-Mannich base derivatives: synthesis and evaluation for antileukemic activity. *Pharmazie* 1985; **40**: 320.
16. Batra JK, Jurd L, Hamel E. Morpholino derivatives of benzyl-benzodioxole, a study of structural requirements for drug interactions at the colchincine/podophyllotoxin binding site of tubulin. *Biochem Pharmacol* 1986; **35**: 4013.
17. Werner W, Jungstand W, Gutsche W, Wohlrabe KL. Struktur-wirkung-beziehungen bei Mannich-basen mit und ohne stickstoff-lostgruppen und einigen von β-aminoketonen abgeleiteten reduktionsprodukten auf grund eines cancerostatica-3-stufentests mit transplantationstumoren. *Pharmazie* 1977; **32**: 341.
18. Geran RJ, Greenberg NH, Macdonald MM, Schumacher AM, Abbott BJ. Protocol for screening chemical agents and natural products against animal tumor and other biological systems. *Cancer Chemother Rep* 1973; **3**: 7.
19. Minowada J, Ohnuma T, Moore GE. Rosette-forming human lymphoid cell lines: I. Establishment and evidence for origin of thymus derived lymphocytes. *J Natl Cancer Inst* 1972; **49**: 891.
20. Dawe CJ, Potter M. Morphologic and biologic progression of a lymphoid neoplasm of the mouse *in vivo* and *in vitro*. *Am J Pathol* 1957; **33**: 603.
21. Puck TT, Marcus P, Cieciura S. Clonal growth of mammalian cells *in vitro*. *J Exp Med* 1956; **103**: 273.

22. Gootenberg JE, Ruscetti FW, Mier JW, Gazdar A, Gallo RC. Human actaneous T cell lymphoma and leukemia cell lines produce and respond to T cell growth factor. *J Exp Med* 1981; **154**: 1403.
23. Scherer WF, Syverton JT, Gey GO. Studies on the propagation *in vitro* of poliomyelities viruses: IV. Viral multiplication in a stable strain of human malignant epithelial cells (Strain Hela) derived from epidermoid carcinoma of the cervix. *J Exp Med* 1953; **97**: 695.
24. Tompkins WAF, Watrach AM, Schmale JD, Schultz RM, Harris JA. Cultural and antigenic properties of newly-established cell strains derived from adenocarcinoma of the human colon and rectum. *J Natl Cancer Inst* 1974; **52**: 1101.
25. Leibovitz AL, Stinson JC, McComb III WB, McCoy CE, Mazur KC, Mabry ND. Classification of human colorectal ademocarcinoma cell lines. *Cancer Res* 1976; **36**: 4562.
26. Giard DJ, Aronson SA, Todaro GJ, *et al. In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* 1973; **51**: 1417.
27. Aronson SA, Todaro GJ, Freeman AE. Human sarcoma cells in culture. *Exp Cell Res* 1970; **61**: 1.
28. Eagle H. Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. *Proc Soc Exp Biol Med* 1955; **89**: 362.
29. Martin TJ, Ingleton PM, Underwood JCE, Michelangeli VP, Hunt NH. Parathyroid hormone-responsive adenylate cyclase in induced transplantable osteogenic rat sarcoma. *Nature* 1976; **260**: 436.
30. Lutton JK, Frederich Jr, RC, Perkins JP. Isolation of adenylate cyclase-enriched membranes from mammalian. *J Biol Chem* 1979; **254**: 11181.
31. Earle WR. Production of malignancy *in vitro* IV. The mouse fibroblast cultures and changes seen in the living cells. *J Natl Cancer Inst* 1943; **4**: 165.
32. Peebles PT, Trisch T, Papageorge AG. Isolation of four unusual pediatric solid tumor cell lines. *Pediatr Res* 1978; **12**: 485.
33. Rollinghoff M, Warner NL. Specificity of *in vivo* tumor rejection assessed by mixing immune spleen cells with target and unrelated tumor cells. *Proc Soc Exp Biol Med* 1973; **144**: 813.
34. Liao L, Kupchan SM, Horwitz SB. Mode of action of the antitumor compound bruceantin, an inhibitor of protein synthesis. *Mol Pharmacol* 1976; **12**: 167.
35. Cadman E, Heimer R, Benz C. The influence of methotrexate pretreatment on S-flaxorouracil metabolism in L1210 cells. *J Biol Chem* 1981; **256**: 1695.
36. Christopherson RI, Yu ML, Jones M. An overall radioassay for the first three reactions of *de novo* pyrimidine synthesis. *Anal Biochem* 1981; **111**: 240.
37. Bagnara AS, Finch LR. Quantitative extraction and estimation of intracellular nucleoside triphosphates in *E. coli*. *Anal Biochem* 1972; **45**: 24.
38. Hunting D, Henderson J. Determination of deoxyribonucleoside triphosphates using DNA polymermase α: a critical evaluation. *Can J Biochem* 1981; **59**: 723.
39. Sedwick WD, Wang TS, Korn D. Purification and properties of nuclear and cytoplasmic deoxyribonucleic acid polymerases from human KB cells. *J Biol Chem* 1972; **247**: 5026.
40. Eichler DC, Fisher PA, Korn D. Effect of calcium on the recovery distribution of DNA polymerase α from cultured human cells. *J Biol Chem* 1977; **252**: 4011.
41. Hall IH, Carlson GL, Abernathy GS, Piantadosi C. Cycloalkanones IV. Antifertility agents. *J Med Chem* 1974; **17**: 1253.
42. Anderson KM, Mendelson IS, Guizik G. Solubilized DNA-dependent nuclear RNA polymerases from the mammary glands of late-pregnant rats. *Biochim Biophys Acta* 1975; **383**: 56.
43. Spassova MK, Russev GC, Goovinsky EV. Some pyrazoles as inhibitors of purine biosynthesis *de novo*. *Biochem Pharmacol* 1976; **25**: 923.
44. Wyngaarden JB, Ashton DM. The regulation of activity of phosphoribosylpyrophosphate amidotransferse by purine ribonucleotides: a potential feedback control of purine biosynthesis. *J Biol Chem* 1959; **234**: 1492.
45. Becker JH, Lohr GW. Inosine 5′-phosphate dehydrogenase activity in normal and leukemic blood cells. *Klin Wochenschr* 1979; **57**: 1109.
46. Ho YK, Hakala T, Zakrzewski S. 5-(1-Adamantyl)pyrimidines as inhibitors of folate metabolism. *Cancer Res* 1972; **32**: 1023.
47. Moore EC, Hulbert RB. Regulation of mammalian deoxyribonucleotide biosynthesis by nucleotides as activators and inhibitors. *J Biol Chem* 1966; **241**: 4802.
48. Kalman SM, Duffield PH, Brzozwki TJ. Purification and properties of a bacterial carbamyl phosphate synthetase. *J Biol Chem* 1966; **241**: 1871.
49. Koritz SB, Cohen PP. Colorimetric determination of carbamyl amino acid and related compounds. *J Biol Chem* 1968; **243**: 3924.
50. Kampf A, Bariknecht R, Schaffer P, Osaki S, Mertes MP. Synthetic inhibitors of *E. coli* calf thymus and Ehrlich ascites tumor thymidylate synthetase. *J Med Chem* 1976; **19**: 903.
51. Maley F, Ochoa S. Enzymatic phosphorylation of deoxycytidylic acid. *J Biol Chem* 1958; **233**: 1538.
52. Suzuki H, Nishimura T, Muto SK, Tanaka N. Mechanism of action of macromomycin: DNA strand scission, inhibition of DNA synthesis, mitosis. *J Antibacteriol* 1978; **32**: 875.
53. Pera JF Sr, Rawlings CJ, Shackleton J, Robert JJ. Quantitation aspects of the formation and loss of DNA. *Biochim Biophys Acta* 1981; **655**: 152.
54. Woynarowski JW, Beerman TA, Konopa J. Introduction of deoxyribonucleic acid damage in HeLa S^3 cells by cytoxic and antitumor sesquiterpine lactones. *Biochem Pharmacol* 1981; **30**: 3005.
55. Zhao Y, Hall IH, Oswald CB, Yokoi T, Lee KH. Antimalarial agents III. Mechanism of action of artesunate aganist *Plasmodium berghi* infeaction. *Chem Pharm Bull* 1987; **35**: 2052.

(Received 12 April 1996; received in revised form 2 May 1996; accepted 19 May 1996)