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In vivo and in vitro anticancer activity of the structurally novel and highly potent antibiotic CI-940 and its hydroxy analog (PD 114,721)*

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Summary. CI-940, PD 114,721, and PD 118,607 are structurally novel antibiotics, which were isolated from fermentation beers of a previously unknown actinomycete. They are highly lipophilic acids characterized by unsaturated lactone and branched, polyunsaturated aliphatic side-chain moieties. All three agents demonstrated significant cytotoxic activity in vitro against a number of human and mouse tumor lines which encompassed a wide range of tissue types. CI-940 retained full activity in vitro against lines of P388 leukemia that are resistant to Adriamycin, amsacrine, and mitoxantrone. Activity was confirmed for both CI-940 and PD 114,721 against a number of murine experimental tumor systems in vivo, which included the P388 and L1210 leukemias and also B16 melanoma, Ridgway osteogenic and M5076 sarcomas, and mammary adenocarcinoma 16/C. PD 118,607 was also highly active against B16 melanoma. All three agents demonstrated anticancer activity at very low dosages compared with current clinically useful anticancer agents. No significant activity was seen against the MX-1 human mammary xenograft or pancreas 02 tumor models. The primary target for host toxicity of CI-940 and PD 114,721 appeared to be gastrointestinal in nature. Neither CI-940 nor PD 114,721 caused delayed lethality when given either IP or IV. In schedule studies, the toxicities of both CI-940 and PD 114,721 were moderately dependent on the regimen used, with total maximum tolerated dosages for intermittent (q4dx2), daily (qdx5), and divided daily (q4hx3, qdx5) dosing schedules of 1, 0.25, and 0.12 mg/kg, respectively.

CI-940 is being developed for clinical trial on the basis of its potent activity against seven different tumor models, its novel structure, and its apparently novel mechanism of action.

Introduction

CI-940 (NSC 364372, PD 114,720, CL 1957A), PD 114,721 (NSC 364373, CL 1957B), and PD 118,607 were discovered in fermentation beers of a novel actinomycete which was isolated from a soil sample collected in Pennsylvania [17]. All three compounds were components of a complex that showed activity against L1210 leukemia in vitro and P388 leukemia in vivo.

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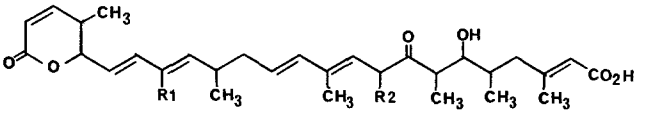
The three compounds were all characterized as highly lipophilic acids containing unsaturated lactone and branched polyunsaturated aliphatic side-chain moieties. The previously reported structures for CI-940 and PD 114,721 [14] are shown with that for PD 118,607 in Fig. 1.

Similar compounds were reported by Hamamoto et al. and shown to have antifungal activity [7, 8]. An antitumor antibiotic called Kazusamycin, which appears to resemble PD 114,721, was recently described by Umezawa and associates [11, 18].

The novel structures of CI-940 and PD 114,721 and their activity in initial tests against leukemias and solid tumors prompted further studies to assess their potential anticancer activity.

Materials and methods

In vitro cytotoxicity assays. The drug-resistant cell lines of P388 leukemia were established in vitro from lines previously passaged in vivo. These lines were obtained from the following sources: Adriamycin-resistant P388 leukemia, Mr Donald Dykes, Southern Research Institute, Birmingham, Ala; amsacrine-resistant P388 leukemia, Dr David Kessel, Wayne State University, Detroit, Mich; mitoxantrone-resistant P388 leukemia, Dr J. Clement, A. D. Little, Inc., Cambridge, Mass. The L1210 in vitro cytotoxicity assays were performed as previously reported [12]; the P388 leukemia in vitro cytotoxicity assays followed the same procedure, except that cells were dispensed at 5×10^4 cells/ml and grown in Fischer's medium supplemented with 10% horse serum, 10 μM 2-mercaptoethanol, and 50 μg/ml gentamicin sulfate.



	R1	R2
CI-940	CH_2CH_3	CH_3
PD 114721	CH_2CH_3	CH_2OH
PD 118607	CH_3	CH_3

Fig. 1. Structures of CI-940, PD 114,721, and PD 118,607

Cytotoxic levels of CI-940, PD 114,721, and PD 118, 607 were established for a panel of in vitro solid tumor lines consisting of three human tumor cell lines (MCF-7 mammary, A549 lung, HCT-8 colon) and three murine tumor cell lines (Lewis lung carcinoma, mammary adenocarcinoma 16/C, pancreas adenocarcinoma 02). The in vitro population-doubling times of these lines were 50.2, 74.3, 26.3, 22.0, 21.2, and 29.9 h, respectively. The growth media employed were RPMI 1640 (HCT-8 colon, mammary adenocarcinoma 16/C), Dulbecco MEM (MCF-7 mammary, pancreas adenocarcinoma 02), and enriched MEM (A549 lung, Lewis lung carcinoma). Each contained 10% fetal bovine serum and 50 μg/ml gentamicin sulfate. Drug testing was performed according to the microculture method developed by Finlay et al. [3] and consisted of adding the test agents to 2-day-old cultures established in microtiter plates. Drug exposure was for 4 days (or 5 days in the case of MCF-7 mammary and A549 lung). Cells were stained with methylene blue (or crystal violet for MCF7 mammary and A549 lung), washed, and solubilized with 1% Sarkosyl (Sigma). Absorbance was determined with a Dynatech MR600 plate reader. The relationship between optical density and cell number was linear.

Tumor passage. All mice were purchased from Charles River Breeding Laboratories Inc., Portage, Mich, except for the AKR mice, which were obtained from Jackson Laboratories, Bar Harbor, Me. The inbred host of tumor origin was used for all tumor passages. L1210 and P388 leukemias were passed IP into male DBA/2 mice at weekly intervals with 10^4 and 10^5 cell inocula, respectively. The solid tumors were passed SC as tumor fragments or tumor brei prepared from 1 g SC masses.

Chemotherapy. All the testing at Warner-Lambert/Parke-Davis Research Laboratories reported herein was performed as previously described [1, 12] and as described below, while that done by the NCI followed standard NCI protocols [6].

F_1 hybrid mice derived from the host of origin of each tumor were used in all the chemotherapy testing except for the experiments using the Ridgway osteogenic sarcoma (ROS), for which AKR mice were used.

The animals were inoculated with a counted number of cells or a trocar needle was used to implant a tumor fragment on day 0 of the experiment. All mice were then randomized and distributed into either control or treatment groups. All mice in life-span studies were weighed on the first and last days of treatment or on each treatment day, for the daily and intermittent dosing regimens, respectively. The mice in the other studies were weighed on each treatment day, 4 days after the last treatment, and weekly thereafter until the maximum treatment-related weight loss was determined. All dosing was based on the mean group weights.

Each compound was made up as a stock solution in absolute ethanol and diluted in various diluents: distilled water, 0.9% aqueous NaCl, 0.5% Tween 80 plus 0.9% aqueous NaCl, or phosphate-buffered saline (PBS). Because of solubility problems with CI-940 and PD 114,721, various diluents were used in the earlier tests. It was found that the solubility of both agents was heavily dependent upon the pH of the diluent used. Therefore, PBS (0.01 *M* potassium phosphate buffer, pH 7.2 in 0.8% saline) with a maximum ethanol concentration of 2% became the diluent of choice and was utilized in most of these studies. All drug solutions were administered within 30 min of preparation.

The quantitative end points used in these tests to assess antitumor activity have been previously described [1, 12, 13]. The results of the tests using the LC12 squamous cell lung and ROS tumors were evaluated for % T/C on days 25 and 35, respectively. The dosages reported are either the optimal dosages used (life-span studies) or the maximum tolerated dosages ($\leq LD_{10}$).

Results

In vitro anticancer activity

CI-940 and PD 118,607 showed comparable potency in vitro against L1210 leukemia and all cell lines of the solid tumor panel. For both compounds the IC_{50} values ranged from 0.2 to 3.4 n*M* (Table 1). PD 114,721 was generally 2- to 4-fold less potent than either CI-940 or PD 118,607. All three agents had markedly lower IC_{50} values against these tumor cell lines than the clinically useful anticancer agents of Adriamycin, cisplatin, and methotrexate (Table 1).

CI-940 was examined for its activity against pleiotropically resistant lines of P388 leukemia (Table 2). It retained full activity against lines resistant to Adriamycin, amsacrine, or mitoxantrone. Marked resistance to each of the appropriate standard agents was confirmed in the same

Table 1. Comparison of in vitro cytotoxicity of CI-940, PD 114,721, and PD 118,607 and three clinically useful anticancer agents

	IC_{50} (n*M*)[a]					
Tumor cell line	CI-940	PD 114,721	PD 118,607	Adriamycin	Cisplatin	Methotrexate
L1210 Leukemia	0.22	0.33	0.17	41	1100	21
Lewis lung carcinoma	0.37	1.6	0.58	250	640	66
Pancreatic carcinoma 02	2.8	8.0	3.4	71	1500	89
Mammary adenocarcinoma 16/C	0.61	–	0.97	38	220	7.3
HCT-8 Human colon	0.37	1.7	0.51	290	1300	14
A549 Human lung	0.45	1.2	0.54	90	22000	3600
MCF-7 Human mammary carcinoma	0.19	0.66	0.27	91	3900	180

[a] IC_{50}, concentration of drug that will reduce tumor cell growth to 50% of the control value

Table 2. In vitro activity of CI-940 against three pleiotropically drug-resistant lines of P388 leukemia

Drug	IC_{50} (n*M*)[a]			
	P388	P388/ADR	P388/AMSA	P388/MITOX
Adriamycin	62.1	9320	140	1480
Amsacrine	23.7	907	1030	70
Mitoxantrone	6.30	373	24.7	2000
CI-940	0.463	0.704	0.352	0.463

[a] IC_{50}, concentration of drug that will reduce tumor cell growth to 50% of the control value

test. Thus, none of these pleiotropically resistant lines of P388 leukemia were cross-resistant to CI-940.

Spectrum of in vivo anticancer activity

CI-940 and PD 114,721 were tested IP against two leukemias (P388, L1210) and against IP-implanted B16 melanoma, mammary adenocarcinoma 16/C, and M5076 sarcoma (Tables 3 and 4). CI-940 and PD 114,721 were most active against the M5076 sarcoma and the B16 melanoma tumor models producing percentage T/C ratios of 152–217 and some cures against the M5076 sarcoma and percentage T/C ratios of 151–201 against B16 melanoma. Both compounds retained activity against IP-implanted B16 melanoma when therapy was given SC (Tables 5 and 6). PD 118,607 injected IP at 0.025 mg/kg/inj on days 1–9 was also highly active (% T/C = 223) against IP-implanted B16 melanoma.

No activity was shown for either compound against P388, M5076 sarcoma, colon adenocarcinoma 38, pancreas 02, or the MX-1 human mammary tumor xenograft when the tumor inoculum and the drug treatment were given by separate routes (Tables 5 and 6). CI-940 and PD 114,721, given intraperitoneally, had equivocal activity against SC mammary adenocarcinoma 16/C. CI-940 but not PD 114,721 had slight activity against advanced stage LC12 squamous cell lung carcinoma. CI-940 had strong activity against ROS with a significant percentage of the treated mice tumor-free on day 35 (when the control tumor sizes ranged between 3 and 10 g). CI-940 (given either IP or IV) retained activity against ROS at an advanced stage (100–400 mg at first treatment). In every test where CI-940 and PD 114,721 were directly compared in sensitive models, CI-940 was slightly more active than PD 114,721.

Table 3. Evaluation of CI-940 injected IP against IP-implanted leukemias and solid tumors[a]

Tumor	Schedule	Optimal dose (mg/kg/dose)	Total dose (mg/kg)	% T/C[b]	Activity rating[c]
P388	d3-7	0.05	0.25	150	++
	d3-6	0.05	0.2	138	+
	d3,7	0.5	1.0	153	++
	d3,6	0.5	1.0	135	+
	q4hx3,d3-7	0.008	0.12	132	+
	q4hx3,d3-6	0.005	0.06	126	+
	d1-5	0.05	0.25	148	++
	d1-5	0.05	0.25	146	++
	d1-9	0.00625	0.056	146	++
	d1-9	0.0125	0.1125	135	+
L1210	d1-5	0.05	0.25	128	+
	d1-9	0.1	0.9	127	+
B16 Melanoma	d1-9	0.0125	0.1125	198	+++
	d1-9	0.0125	0.1125	156	++
	d1-9	0.0125	0.1125	159	++
	d1,5,9	0.075	0.225	201	+++
Mammary adenocarcinoma 16/C	d1,5	0.38	0.76	130	+
	d1,5,9	0.625	1.875	163	++
	d1,5,9	0.5	1.5	161	++
M5076	d1,5,9,13	0.2	0.8	195	+++
	d1,5,9,13	0.4	1.6	195 (1/10)[d]	+++
	d1,5,9,13	0.8	3.2	217 (9/10)	++++
	d1,5,9,13	0.4	1.6	152	++

[a] Some of the data were produced under the auspices of the screening program of the Drug Evaluation Branch, Division of Cancer Treatment, NCI

[b] %T/C = (median life-span of the treated group divided by the median life-span of the control group) × 100. Tumor-free survivors (cures) were excluded from these calculations

[c] Activity rating %T/C

++++	>220
+++	181–220
++	141–180
+	125–140
–	>85 <125

[d] Numbers in parentheses represent the number of mice surviving for 60 days over the total number of mice in that group

Table 4. Evaluation of PD 114,721 injected IP against IP-implanted leukemias and solid tumors [a]

Tumor	Schedule	Optimal dose (mg/kg/dose)	Total dose (mg/kg)	% T/C [b]	Activity rating [c]
P388	d3-7	0.05	0.25	161	++
	d3-6	0.05	0.2	149	++
	d3,7	0.5	1.0	132	+
	d3,6	1.0	2.0	162	++
	q4hx3,d3-7	0.008	0.12	132	+
	q4hx3,d3-6	0.01	0.12	152	++
	d1-5	0.05	0.25	161	++
	d1-5	0.1	0.5	154	++
	d1-9	0.025	0.225	161	++
	d1-9	0.0125	0.1125	155	++
	d3,7,11	3.0	9.0	163	++
L1210	d1-5	0.05	0.25	142	++
	d1-9	0.025	0.225	131	+
B16 Melanoma	d1-9	0.00625	0.05625	184	+++
	d1-9	0.025	0.225	165	++
	d1-9	0.0125	0.1125	178 (1/10) [d]	++
	d1,5,9	0.094	0.282	151	++
Mammary adenocarcinoma 16/C	d1,5	0.19	0.38	141	++
	d1,5,9	0.312	0.936	161	++
M5076	d1,5,9,13	0.8	3.2	217 (7/10)	++++
	d1,5,9,13	0.4	1.6	152 (2/10)	++
	d1,5,9,13	0.8	3.2	208 (2/10)	+++

[a] Some of the data were produced under the auspices of the screening program of the Drug Evaluation Branch, Division of Cancer Treatment, NCI

[b] %T/C = (median life-span of the treated group divided by the median life-span of the control group) × 100. Tumor-free survivors (cures) were excluded from these calculations

[c] Activity rating %T/C

++++	>220
+++	181–220
++	141–180
+	125–140
–	>85 <125

[d] Numbers in parentheses represent the number of mice surviving for 60 days over the total number of mice in that group

Schedule dependence

The schedule dependence of CI-940 and PD 114,721 was examined in mice bearing P388 leukemia (Tables 3 and 4). Both CI-940 and PD 114,721 were approximately 10-fold more toxic when administered on a frequent (q4hx3; qdx5) dose schedule than when given on an intermittent (q4dx2) schedule. Thus the maximum tolerated dosages of CI-940 on the q4hx3; qdx5 and q4dx2 schedules were 0.12 and 1.0 mg/kg, respectively. The activities of CI-940 and PD 114,721 against P388 leukemia were not significantly dependent on the treatment schedule (Tables 3 and 4).

Toxicity

Mice that received lethal doses of CI-940 and PD 114,721 died with severe diarrhea within 2–7 days of treatment, suggesting that the dose-limiting toxicity for both agents was gastrointestinal in nature. The toxicity of both agents was significantly greater upon oral administration than after IP, SC, or IV administration (Tables 4–6). Both CI-940 and PD 114,721 were assessed for their long-term toxicities. No significant delayed lethality (deaths more than 20 days after treatment) was observed for either compound given at its maximum tolerated dosage levels. Two deaths (out of 89 total mice) occurred, on days 117 and 142, when PD 114,721 was injected IV. These deaths may have been drug-related, but were probably related to the urinary tract complications that sometimes occur in AKR mice held for long periods.

Discussion

The anticancer activity of CI-940 is of interest for several reasons: (a) Its spectrum of anticancer activity in preclinical test systems is quite broad. CI-940 had significant activity in 7 of the 10 tumors against which it was tested. One of the tumor models (pancreas 02) in which CI-940 showed no activity is also insensitive to all of more than 40 other experimental and clinically active agents tested [2]. (b) CI-940 is a structurally novel antibiotic with unusual potency as an antitumor agent. The maximum tolerated dosages on a q4dx3 schedule are typically about 0.5 mg/kg/inj. (c) The mechanism of action of CI-940 as an anticancer agent, while still unknown, appears to be novel (T. J. Boritzki, personal communication). (d) CI-940 may not be subject to the phenomenon of pleiotropic drug resistance, as evidenced by our in vitro studies.

The structure-activity relationships for CI-940 have not been determined. Each of the three related compounds de-

Table 5. Effect of CI-940 against solid tumors at sites distant from that of drug injection [a]

Tumor	Schedule	Site of tumor	Drug route	Optimal or maximum tolerated dose [b] (mg/kg/dose)	Total dose (mg/kg)	% T/C	T-C [c] (days)	Gross log_{10} cell kill [d]	Activity rating [e]
ROS	d3, 7	SC	IP	0.25	0.5	38			A
	d1, 5, 9	SC	IV	0.5	1.5	0			AA
	d2, 6, 10	SC	IP	0.5	1.5	0			AA
	d11, 15, 19	SC	IV	1.0	3.0		6.0	1.0	+
	d11, 15, 19	SC	IP	1.0	3.0		5.3	0.8	+
	d11, 15, 19	SC	SC	0.5	1.5		2.3	0.4	−
	d11, 15, 19	SC	PO	0.25	0.75		2.7	0.4	−
B16 Melanoma	d1−9	IP	SC	0.2	1.8	135			+
Mammary	d1, 5	SC	IP	0.95	1.9		0.6	0.2	−
Adenocarcinoma	d1, 5, 9	SC	IP	1.6	4.8		4.0	1.3	++
16/C	d1, 5, 9	SC	IP	0.5	1.5		−0.9	0	−
M5076	d1, 5, 9	SC	IP	1.0	3.0		2.3	0.4	−
	d1, 5, 9, 13	IP	SC	0.8	3.2	119			−
	d1, 5, 9, 13	SC	IP	0.8	3.2	101			−
	q4hx3, d1−2	SC	IP	0.047	0.282		1.7	0.3	−
Colon 38	d2, 9	SC	IP	1.6	3.2	98			−
MX-1	d1, 5, 9	Subrenal	IP	0.6	1.8	102			−
	d1, 5, 9	Subrenal	IP	4.0	16.0	71			−
Pan 02	d1, 5, 9	SC	IP	1.0	3.0		1.7	0.1	−
LC 12	d7,11, 15, 19	SC	IV	2.0	8.0	32			A

[a] Some of the data were produced under the auspices of the screening program of the Drug Evaluation Branch, Division of Cancer Treatment, NCI

[b] Maximum tolerated dose is $\leq LD_{10}$

[c] T-C (tumor growth delay) in days = the median time required for the treated tumors to reach a predetermined size (750 mg) minus the median time required for the control tumors to reach the same size. Tumor-free survivors were excluded from these calculations

[d] Gross log_{10} cell kill = (T-C) [3.32 (tumor volume-doubling time)]

[e] *Activity ratings based on gross log_{10} cell kill*

Activity rating	Gross log_{10} kill
++++	> 2.8
+++	2.0−2.8
++	1.3−1.9
+	0.7−1.2
−	< 0.7

Activity ratings based on % T/C for life-span assays (see Table 4)

Activity ratings based on % T/C for tumor ratio assays (ROS, LC12)

Activity rating	%T/C
AA	0−10
A	11−42
−	> 42

scribed in this study had roughly comparable anticancer activity where tested. Thus hydroxylation of the methyl group at C(9) (PD 114,721) or substitution of methyl for ethyl at C(17) did not appreciably alter activity or potency. Although the differences between CI-940 and PD 114, 721 in individual test were usually insignificant, CI-940 appeared to have the best overall activity against our tumor panel as a whole. Thus, in every test in which CI-940 and PD 114,721 were directly compared in a sensitive tumor model, CI-940 had slightly better activity.

Recently, we and others have described the anticancer activity and biochemistry of another fermentation-derived anticancer antibiotic, CI-920 [9, 12, 15, 16]. The structures of CI-920 and CI-940 are somewhat similar, both compounds being characterized by unsaturated lactone and polyene side-chain moieties. Considerable evidence has accumulated, however, that indicates that these compounds are fundamentally different in their anticancer activities. CI-920 activity has an absolute requirement for a phosphate ester moiety [12] that CI-940 lacks. Both compounds are potent inhibitors of DNA synthesis, but their time courses of inhibition of macromolecular synthesis differ [4, 10; T. J. Boritzki, personal communication). Preliminary data from our laboratory indicate that CI-940 retains full activity in vitro and in vivo against a line of L1210 leukemia made specifically resistant to CI-920. This cell line is resistant to CI-920 by virtue of a defect in the transport of reduced folates into the cell [5]. These data

Table 6. Effect of PD 114, 721 against leukemias and solid tumors at sites distant from that of drug injection[a]

Tumor	Schedule	Site of tumor	Drug route	Optimal or maximum tolerated dose[b] (mg/kg/dose)	Total dose (mg/kg)	% T/C	T-C[c] (days)	Gross $\log_{10}$ cell kill[d]	Activity rating[e]
P388	d3, 7	IP	PO	0.62	1.24	107		−1.5	–
	d3, 7, 11	IP	SC	2.0	6.0	112		−1.9	–
	d3, 7, 11	IP	IV	3.0	9.0	111		−1.9	–
ROS	d3, 7	SC	IP	0.5	1.0	105			–
	d1, 5, 9	SC	IV	0.5	1.5	15			A
	d2, 6, 10	SC	IP	0.375	1.125	17			A
B16 Melanoma	d1–9	IP	SC	0.05	0.45	130			+
Mammary	d1, 5	SC	IP	0.95	1.9		1.3	0.4	–
Adenocarcinoma	d1, 5, 9	SC	IP	1.6	4.8		3.7	1.2	+
16/C	d1, 5, 9	SC	IP	0.5	1.5		−0.7	0	–
M5076	d1, 5, 9, 13	SC	IP	0.8	3.2	75			–
MX-1	d1, 5, 9	Subrenal	IP	0.6	1.8	94			–
	d1, 5, 9	Subrenal	IP	4.0	12.0	68			–
	d1, 5, 9	Subrenal	IP	32.0	96.0	121			–
Pan 02	d1, 5, 9	SC	IP	1.0	3.0		0.4	0	–
LC 12	d7,11, 15, 19	SC	IV	4.0	16.0	59			–

[a] Some of the data was produced under the auspices of the screening program of the Drug Evaluation Branch, Division of Cancer Treatment, NCI

[b] Maximum tolerated dose is $\leq LD_{10}$

[c] T-C (tumor growth delay) in days = the median time required for the treated tumors to reach a predetermined size (750 mg) minus the median time required for the control tumors to reach the same size. Tumor-free survivors were excluded from these calculations

[d] Gross $\log_{10}$ cell kill = (T-C) [3.32 (tumor volume-doubling time)]

[e] See Tables 4 and 5 for definitions of activity ratings

suggests that CI-920 and CI-940 enter tumor cells by different pathways. The targets for host toxicity for CI-920 and CI-940 also differ. The time course of CI-920-induced lethality suggests that marrow suppression is dose-limiting [12], while the rapid onset of CI-940-induced lethality with severe diarrhea indicates that damage to the gastrointestinal epithelium may be dose-limiting. In support of this hypothesis, our data indicate that CI-940 is considerably more toxic when given orally than by other routes of administration. Other studies have suggested that the cell cycle effects of these agents also differ. Treatment of 1210 cells with CI-920 at low concentrations causes a delay in the progression of cells through the G2 phase of the cell cycle, while incubation of cytotoxic concentrations leads to arrest of cells in S phase [10]. Early work with CI-940 (data not shown) indicates that incubation of Chinese hamster ovary cells with cytotoxic levels of CI-940 causes cell cycle arrest at the G1-S interface, while treatment with lower concentrations has no effect on the cell cycle distributions of asynchronous populations. Thus, CI-940 and CI-920, although somewhat similar structurally, differ in spectrum of anticancer activity, host toxicity, transport, resistance, and cell cycle effect.

CI-940 has been selected for development to clinical trial on the basis of its spectrum of anticancer activity, potency, novel structure, and apparently novel mechanism of action. Details of its properties with respect to resistance and cross-resistance, cell cycle effects, route- and schedule-dependence, and efficacy in combination with other anticancer agents are under study and will be the topics of forthcoming publications.

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