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Preclinical Antitumor Activity of 6-Hydroxymethylacylfulvene, a Semisynthetic Derivative of the Mushroom Toxin Illudin S

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ABSTRACT

6-Hydroxymethylacylfulvene (HMAF; MGI 114) is a novel semisynthetic antitumor agent derived from the sesquiterpene mushroom toxin illudin S. *In vitro* cytotoxicity determinations produced IC_{50} concentrations (concentrations required for 50% inhibition of growth) ranging from 160 nM in sensitive MCF-7 human mammary carcinoma cells to 17 μM in relatively insensitive murine B16 melanoma cells. *In vivo* antitumor activity was consistent with *in vitro* sensitivity. HMAF was very effective in human tumor xenograft models, including MX-1 breast carcinoma, MV522 lung adenocarcinoma, and HT-29 colon carcinoma, but not murine B16 melanoma or P388 leukemia. Excellent responses were observed in animals bearing MX-1 tumors administered i.v. or i.p. doses of 3–7.5 mg/kg daily for 5 days, with complete regression recorded in 29 of 30 animals administered i.v. HMAF. Extensive tumor shrinkage was also observed with MV522, and significant tumor growth inhibition was obtained with HT-29 when animals received 5 daily i.p. doses ranging from 3.75 to 7.5 mg/kg. Complete regressions were also observed in individual animals with MV522 and HT-29. The excellent activity of HMAF in several human solid tumor xenografts, including the more refractory MV522 and HT-29 models, warrants the further investigation of this novel agent in clinical trials.

INTRODUCTION

Illudins are sesquiterpene natural products isolated from mushrooms of the genus *Omphalotus* (*O. illudens*) or the closely related *Lampteromyces* (*L. japonicus*). The function or purpose of illudin production by these organisms is unknown. Illudin S was tested previously by the National Cancer Institute as a potential antitumor agent against a variety of rodent solid tumors and leukemias. No increases in life span or tumor regression were observed in solid tumors, including lung carcinoma, sarcoma, and melanoma, whereas activity against leukemias was limited by increased mortality in illudin S-treated animals (1). Despite this dose-limiting toxicity, illudin S and various derivatives have generally demonstrated several properties that make them unique as potential antitumor agents. In sensitive tumor cell types, illudin S has been shown to be a potent inhibitor of DNA synthesis that causes cell cycle arrest in S phase (1). Illudin S and the semisynthetic analogue acylfulvene are also actively taken up by sensitive tumor cell types and damage DNA in a unique manner that appears to require functional DNA helicase activity for DNA repair processes to occur (2–4). In addition, illudin S, acylfulvene, and dehydroilludin M have been shown to retain antitumor activity against a broad range of drug-resistant tumor cell lines *in vitro* (1, 4, 5).

On the basis of the unique properties of the illudins, an effort has been made to synthesize chemical derivatives of illudin S with a better therapeutic index. MGI 114, HMAF[2] (Fig. 1), is a semisynthetic analogue of illudin S (6) with favorable *in vitro* and *in vivo* antitumor activity (7) that is currently being evaluated in a human Phase I clinical trial. The present studies were conducted to evaluate the activity of HMAF in several tumor xenograft models and compare this activity to conventional cytotoxic anticancer drugs.

MATERIALS AND METHODS

Cytotoxic Drugs. Illudin S obtained from still cultures of *O. illudens* was used to synthesize HMAF (6). Cyclophosphamide, etoposide, Taxol, and cisplatin were obtained from Bristol-Myers Squibb (Princeton, NJ). Navelbine was obtained from Burroughs Wellcome (Research Triangle Park, NC).

Tumors. Murine B16 melanoma and P388 leukemia and human MX-1 breast carcinoma cell lines were obtained from the National Cancer Institute-Division of Cancer Treatment Tumor Repository (Frederick, MD). Human MCF-7M breast carcinoma cells were obtained from Dr. C. K. Osborne (University of Texas Health Science Center, San Antonio, TX). HT-29 human colon carcinoma cells were obtained from the American Type Culture Collection (Rockville, MD), and MV522 human lung adenocarcinoma was derived as described previously (8, 9).

Cell Culture. Murine B16 melanoma cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 25 μg/ml gentamycin, 0.75% sodium bicarbonate, 10 mM HEPES buffer (pH 7.4), and 0.06 mg/ml anti-pleuropneumonialike organism. Murine P388 leukemia and HT-29 colon adenocarcinoma cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. MCF-7M human breast adenocarcinoma cells were maintained in improved minimal essential medium supplemented with 5% non-heat-inactivated fetal bovine serum and 1 nM insulin.

***In Vitro* Growth-inhibitory Activity.** Exponentially growing cells (1–2×10^3 cells, unless otherwise specified) in 0.1 ml medium were seeded on day 0 in 96-well microtiter plates. On day 1, 0.1-ml aliquots of medium containing graded concentrations of HMAF were added in duplicate to the cell plates. After incubation at 37°C in a humidified incubator for 3 days (P388 and B16) or 6 days (HT-29 and MCF-7M), the plates were centrifuged briefly (1–2 min at 1000 rpm), and 100 μl of the growth medium were removed. Cell cultures were incubated with 50 μl of 3-(4,5-dimethylthiazyl-2-yl)-2,5-diphenyltetrazolium bromide MTT, 1 mg/ml in Dulbecco's PBS) for 4 h at 37°C. The resulting purple formazan precipitate was solubilized with 200 μl of 0.4 N HCl in isopropyl alcohol. Absorbance was quantitated using a Bio-Rad Model 3550 microplate reader at a test wavelength of 570 nm and a reference wavelength of 630 nm. Absorbance values were transferred to a 486 personal computer, and the IC_{50} values were determined using a computer program (EZ-ED50) that fit all the data to the following four-parameter logistic equation:

$$Y = \frac{A_m - A_o}{1 + \left(\frac{X}{IC_{50}}\right)^n} + A_o$$

where A_m is the absorbance of control cells, A_o is the absorbance of cells in the presence of the highest HMAF concentration, Y is the observed absorbance, X

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[2] The abbreviations used are: HMAF, 6-hydroxymethylacylfulvene; MTD, maximally tolerated dose; daily ×1, regimen of one dose; daily ×5, regimen of five consecutive daily doses; TGI, tumor growth inhibition; CHO, Chinese hamster ovary.

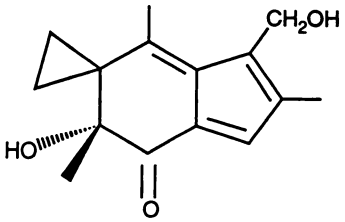

Fig. 1. Structure of HMAF.

is the HMAF concentration, IC_{50} is the concentration of HMAF that inhibits cell growth by 50% of control cells (based on the absorbance), and n determines the slope of the curve.

***In Vivo* Evaluation in B16 and P388 Murine Tumor Models.** HMAF was administered i.p. to non-tumor-bearing B6D2F1 mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) at four graded doses (five mice/dose) to estimate the MTD to be administered for determination of *in vivo* antitumor activity (data not shown). In the B16 melanoma model, female B6D2F1 mice received i.p. inocula of B16 murine melanoma brei prepared from B16 tumors growing s.c. in mice (day 0). In the P388 model, B6D2F1 mice received i.p. inocula of 1×10^6 P388 cells (day 0) obtained by removal of ascites fluid from tumor-bearing mice, centrifugation of the cells, and resuspension of cells in normal saline. On day 1, B16- or P388-inoculated mice were treated i.p. with HMAF, cyclophosphamide, or vehicle. HMAF was administered on 5 consecutive days by i.p. or i.v. injections at doses of 7.5 or 5 mg/kg. Cyclophosphamide was administered as a single i.p. injection in saline at 300 mg/kg. Control animals received five daily i.p. injections of 10% cremophor, 20% ethanol, and 70% isotonic saline. Mean survival times of all groups were calculated and results expressed as mean survival of treated mice/mean survival of control mice (T/C) $\times$ 100%.

***In Vivo* Evaluation in Human Tumor Xenograft Models.** In the MX-1 mammary carcinoma, HT-29 colon adenocarcinoma, and MV522 lung adenocarcinoma models, female nude mice (Harlan Sprague Dawley, Inc.) were implanted s.c. by trocar with fragments harvested from s.c. growing tumors in nude mouse hosts. When tumors were approximately 5 $\times$ 5 mm (usually about 10 days after implantation), the animals were pair-matched into treatment and control groups (day 1). Each group contained 10 tumor-bearing mice that were ear-tagged and followed individually throughout the experiment. Administration of drugs or vehicle began on day 1 and consisted of a single i.p. dose (daily $\times$1) or i.p. or i.v. doses on 5 consecutive days (daily $\times$5). The cremophor/ethanol/saline vehicle, used in the B16 and P388 experiments, was used in the initial MX-1 experiment. A vehicle of 1% ethanol in 5% dextrose in water, the vehicle employed in the current human clinical trial, was used in a subsequent study with MX-1, employing i.p. and i.v. administration of HMAF. HMAF was administered by i.p. injection in normal saline in studies with MV522 and HT-29 xenografts. Other cytotoxic drugs were administered i.p. in 0.9% NaCl (cyclophosphamide, cisplatin, etoposide, and Taxol) or water (navelbine). Following tumor implantation, mice were weighed twice weekly and tumor measurements were made using calipers twice weekly beginning on day 1. Tumor measurements were converted to tumor weight (mg) using an established formula:

$$\text{Weight (mg)} = \frac{\text{Width (mm)}^2 \times \text{Length (mm)}}{2}$$

Experiments were terminated when tumors in control animals reached a size of 1–2 g. At termination, all mice were weighed, sacrificed, and their tumors excised. Tumors were then weighed, and mean tumor weights per group were calculated. TGI was calculated for each group as

$$100\% - \frac{\text{Mean treated tumor weight}}{\text{Mean control tumor weight}} \times 100\%$$

Tumor shrinkage is calculated for individual animals as the initial tumor weight (day 1) minus the final tumor weight. The difference divided by the initial tumor weight is the percentage of shrinkage.

RESULTS

Growth-inhibitory Activity against Tumor Cells *in Vitro*

IC_{50} values for HMAF in two murine and two human tumor cell lines are shown in Table 1. HMAF was much more potent in inhibiting growth of the human tumor cell lines than the murine lines. IC_{50} values were 2–10-fold lower for HT29 and MCF-7 compared to values for P388 murine leukemia and nearly two orders of magnitude lower than values for B16.

Mouse Tumor Models

Following dose range-finding studies to determine a maximally tolerated HMAF dose for a daily $\times$5 schedule, antitumor efficacy was evaluated in the mouse P388 and B16 models at daily i.p. doses of 5 or 7.5 mg/kg. Consistent with low-potency growth inhibition *in vitro*, HMAF was weakly active against P388 (T/C = 140) and was inactive against B16 (T/C = 108). The positive control agent, cyclophosphamide (300 mg, daily $\times$1), was very active against both tumor types, with 10 of 10 long-term survivors with P388 and T/C = 212 with no long-term survivors with B16.

Human Tumor Xenografts

MX-1 Breast Carcinoma. HMAF activity against human MX-1 breast carcinoma was tested because of the good degree of predictability for activity in human neoplastic disease for this solid tumor xenograft model (10–12). Results of two experiments using the MX-1 xenograft model are summarized in Table 2. The initial experiment utilized doses tested against murine B16 melanoma and P388 and the cremophor/ethanol/saline vehicle. The second test against MX-1 utilized slightly lower doses, both i.p. and i.v. administration, and the ethanol/5% dextrose in water vehicle. HMAF demonstrated dose-related shrinkage of MX-1 in nude mouse hosts. Antitumor activity was better than with the positive control agent cyclophosphamide, with HMAF producing complete tumor regressions in most of the animals tested. With i.v. administration of HMAF, 100% tumor shrinkage was observed at 3 mg/kg, the lowest dose tested. Curative activity in all animals using a daily $\times$5 schedule of i.v. administration over the dose range of 3–7 mg/kg indicates that HMAF has a therapeutic margin against solid tumors superior to that of previously reported illudin analogues.

MV522 Lung Adenocarcinoma. To confirm and extend previously reported activity of HMAF against the relatively difficult-to-treat MV522 human lung adenocarcinoma xenograft, HMAF and other conventional antitumor agents were tested for activity against this tumor line in nude mice. In the first MV522 experiment, HMAF was administered at estimated maximally tolerated single doses of 15 mg/kg and 7.5 mg/kg on a daily $\times$5 schedule. Body weight loss of approximately 18% was observed following a single dose of 15 mg/kg and 25% following 7.5 mg/kg (daily $\times$5), with one animal death in the 5-dose regimen. The high dose for the second MV522 experiment was therefore reduced to 7 mg/kg (daily $\times$5), with no drug-related deaths and mean body weight loss of 13% at day 9. All other agents tested in this model were administered at their estimated MTD (daily $\times$5) and, with the exception of etoposide, also at their estimated ½MTD.

Table 1 *Comparative growth Inhibition by HMAF against murine and human tumor cell lines in vitro*

Cell type	Cell line	IC_{50} (μM)
Murine melanoma	B16	17 ± 1.9
Murine leukemia	P388	1.0 ± 0.3
Colon carcinoma	HT-29	0.26 ± 0.16
Mammary carcinoma	MCF-7	0.16 ± 0.01

Table 2 *Activity of HMAF against MX-1 human breast carcinoma in nude mice*

Drug	Dose (mg/kg)	Regimen and route	% tumor growth inhibition[a]	Mean % tumor shrinkage	Mice with partial shrinkage	Mice with complete shrinkage
Experiment 1						
HMAF	5	Daily ×5, i.p.	[b]	88	8 of 10	2 of 10
	7.5	Daily ×5, i.p.	[b]	91	6 of 10	4 of 10
Cyclophosphamide	300	Daily ×1, i.p.	[b]	88	10 of 10	0 of 10
Experiment 2						
HMAF	3	Daily ×5, i.p.	99.8	88.5	7 of 10	2 of 10
	5	Daily ×5, i.p.	[b]	100	0 of 10	10 of 10
	7	Daily ×5, i.p.	[b]	100	0 of 10	10 of 10
HMAF	3	Daily ×5, i.v.	[b]	100	0 of 10	10 of 10
	5	Daily ×5, i.v.	[b]	100	0 of 10	10 of 10
	7	Daily ×5, i.v.	[b]	100	0 of 10	9 of 10[c]
Cyclophosphamide	125	Daily ×5, i.p.	100	95.6	7 of 10	2 of 10

[a] Values exclude mice with partial or complete shrinkage of tumor.
[b] All treated mice exhibited partial or complete tumor shrinkage.
[c] One toxic death in group.

High-dose cisplatin, Taxol, and etoposide produced body weight loss of 3–16% by day 8 or 9 after administration, with no toxic deaths. High-dose navelbine (4 mg/kg, daily ×5) produced 90% mortality, without tumor shrinkage, and the lower dose produced complete body weight gain suppression compared to controls. Antitumor activity of HMAF and comparative agents is summarized in Table 3.

HMAF administered at 7.5 mg/kg on a daily ×5 schedule produced dose-related TGI and tumor shrinkage in half the animals. Lesser TGI was observed following a single dose. Tumor shrinkage was most apparent approximately 15 days postdose (Fig. 2). Terminal tumor weight data expressed in Table 4 reflect regrowth of tumors that had been reduced in mass at earlier time points. Taxol was also highly effective in producing strong TGI and tumor shrinkage of MV522. As in HMAF-treated animals, tumor regrowth after day 15 was also observed with Taxol (Fig. 2). Cyclophosphamide and cisplatin exhibited modest TGI, and navelbine and etoposide were ineffective in this xenograft model.

HT-29 Colon Carcinoma. HT-29 is another tumor cell line that grows as a xenograft, and against this line, conventional cytotoxic drugs exhibit limited growth-inhibitory activity. On the basis of body weight loss and one animal death following single i.p. doses of 15 mg/kg or daily ×5 i.p. doses of 7.5 mg/kg in the MV522 xenograft experiments, the high doses for single-dose and daily ×5 schedules for HMAF were selected at 12 and 6 mg/kg, respectively. At these doses, group mean body weight loss at day 8 after initiation of treatment was <12%. Antitumor activity of HMAF and comparative agents is summarized in Table 4.

On a daily ×5 schedule, 6 mg/kg HMAF produced TGI comparable to a daily ×5 schedule of cyclophosphamide. However, in contrast to cyclophosphamide, one animal administered a single 10 mg/kg dose of HMAF exhibited an 81% tumor shrinkage, and one animal administered 6 mg/kg HMAF daily for 5 days exhibited complete tumor regression. This is considered to be a highly significant response, because tumor shrinkage is not typically observed in the HT-29 xenograft model with other cytotoxic drugs.

DISCUSSION

HMAF (MGI 114) is a semisynthetic analogue of the mushroom toxin illudin S that exhibits excellent activity, including complete regressions, in several human tumor xenograft models. On the basis of preclinical efficacy in these models and the unique antitumor properties of illudin analogues (1–7), preclinical safety studies were conducted with this analogue and the compound is currently in Phase I clinical studies in cancer patients. The activity demonstrated in the studies reported here is particularly significant, because of the dem-

Table 3 *Activity of HMAF against MV522 human lung adenocarcinoma in nude mice*

Drug	Dose (mg/kg)	Regimen and route	% tumor growth inhibition[a]	Mean % tumor shrinkage	Mice with partial shrinkage	Mice with complete shrinkage
Experiment 1						
HMAF	10	Daily ×1, i.p.	32	0	0 of 10	0 of 10
	15	Daily ×1, i.p.	70	0	0 of 10	0 of 10
	3.75	Daily ×5, i.p.	54	0	0 of 10	0 of 10
	7.5	Daily ×5, i.p.	93	51	5 of 10	0 of 10
Cyclophosphamide	300	Daily ×1, i.p.	71	0	0 of 10	0 of 10
	125[b]	Daily ×5, i.p.	80	0	0 of 10	0 of 10
Etoposide	12.5	Daily ×5, i.p.	0	0	0 of 10	0 of 10
Experiment 2						
HMAF	5	Daily ×5, i.p.	74	0	0 of 10	0 of 10
	6	Daily ×5, i.p.	81	0	0 of 10	0 of 10
	7	Daily ×5, i.p.	91	43	1 of 10	1 of 10
Cisplatin	1.5	Daily ×5, i.p.	44	0	0 of 10	0 of 10
	3	Daily ×5, i.p.	64	0	0 of 10	0 of 10
Taxol	10	Daily ×5, i.p.	53	0	0 of 10	0 of 10
	20	Daily ×5, i.p.	98	66	4 of 10	3 of 10
Navelbine	2	Daily ×5, i.p.	15	0	0 of 10	0 of 10
	4[c]	Daily ×5, i.p.	57	0	0 of 10	0 of 10

[a] Values exclude mice with partial or complete shrinkage of tumor.
[b] One toxic death in group.
[c] 9 of 10 toxic deaths in group.

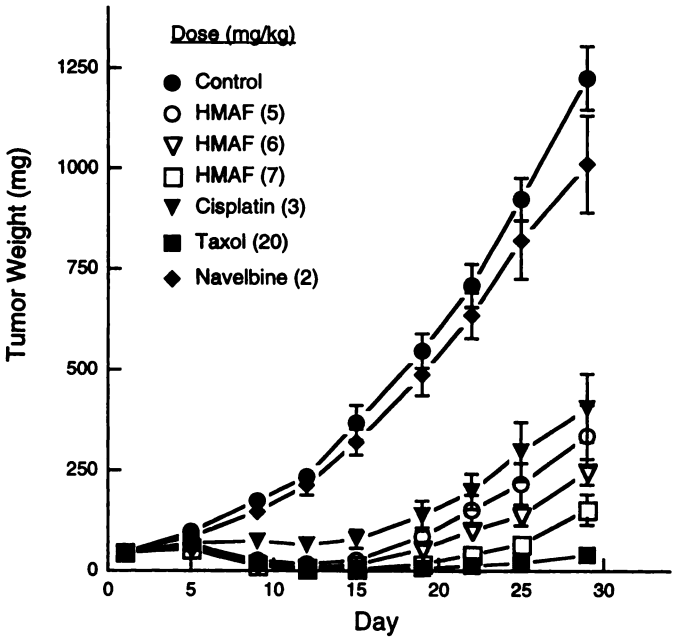

Fig. 2. MV522 human lung adenocarcinoma fragments were implanted s.c. by trocar in nude mice. Treatment was initiated when the primary tumor reached a size of approximately 5 × 5 mm (day 0). HMAF was administered by five daily i.p. injections in sterile saline at doses of 0, 5, 6, or 7 mg/kg. Cisplatin (3 mg/kg), Taxol (20 mg/kg), and navelbine (2 mg/kg) were also administered as five daily i.p. injections. Tumors were measured using calipers twice weekly, and volumes were calculated using the formula weight (mg) = [width (mm)^2 × length (mm)]/2. Mean tumor size for each treatment group is plotted (n = 10); *bars*, SD.

onstrated correlation of activity against MX-1 breast carcinoma for existing approved cytotoxic drugs (10–12) and the fact that lung and colon tumors currently represent some of the most refractory tumors to conventional cytotoxic drug therapy.

Despite one toxic death at the highest dose tested, the complete regression of 29 of 30 MX-1 breast carcinoma xenografts at i.v. doses of 3, 5, and 7 mg/kg on a daily ×5 schedule of HMAF indicates that there is an acceptable therapeutic index for this illudin analogue compared to the natural product illudin S, the solid tumor efficacy of which is limited by toxicity (1). Greater efficacy with i.v. relative to i.p. administration is also an important finding, because the i.v. route will be used in initial clinical trials with this compound. A daily ×5 schedule was also found to provide superior antitumor activity to single doses in both the HT-29 and MV522 xenograft models.

The activity demonstrated against MV522 lung adenocarcinoma is also highly significant, because this tumor cell line has previously been demonstrated to be resistant to a broad range of conventional cytotoxic drugs when grown as a xenograft in nude mice (9). Furthermore, HMAF is the most active illudin analogue tested to date against this xenograft. At MTDs, illudin S and deoxyilludin M do not inhibit primary tumor growth or significantly extend life span of MV522-bearing nude mice, whereas dehydroilludin M and acylfulvene extend life span and inhibit primary tumor growth but do not produce any tumor shrinkage (4, 5). As shown in Fig. 2, shrinkage of MV522 tumors of greater than 90% is maximal at day 15 following daily ×5 i.p. administration at 7 mg/kg. MV522 tumor regressions have also been demonstrated for HMAF administered on a three times per week schedule for 3 consecutive weeks (7).

Despite the excellent activity of HMAF against the human tumor xenografts tested, HMAF showed only modest *in vivo* activity against murine P388 leukemia (*T/C* = 140) and no activity against murine B16 melanoma (*T/C* = 108). This *in vivo* activity profile is consistent with lower cytotoxic potency against these cell lines *in vitro* relative to human tumor cell lines (Table 1). A similar apparent human tumor selectivity has been reported previously for bis-intercalating naphthalimides (13–15). As with the bis-naphthalimides, the mechanism of the apparent human tumor selectivity of HMAF is unknown. However, it has been demonstrated previously that some human tumors that are less sensitive to illudin S toxicity *in vitro* do not possess the energy-dependent uptake mechanism present in sensitive tumor cell types (2). Decreased uptake could therefore explain the relative lack of sensitivity of murine tumors to HMAF, because HMAF is also accumulated in sensitive tumor cell types by a saturable uptake mechanism (unpublished data). Alternatively, murine tumors may be deficient in bioactivation capacity for HMAF or illudins, because the illudins may require intracellular metabolism to reactive intermediates that covalently bind to cellular DNA and are ultimately responsible for the cytotoxicity of this class of compounds (3, 16).

Although the mechanisms of action of HMAF or the illudins are not fully elucidated, existing data for this class of compounds suggests that the mechanism is unique. In addition to the evidence for an uptake mechanism and rapid inhibition of DNA synthesis in sensitive tumor cell types, illudins and their derivatives have several other unique properties. Of particular significance for the therapeutic potential of these agents, illudin S, acylfulvene, and dehydroilludin M have been shown to retain cytotoxic activity against a broad range of multidrug-resistant tumor cell phenotypes *in vitro* (1, 4, 5). Studies in DNA repair-deficient CHO cell lines also indicate a novel interaction of illudins and their derivatives with cellular DNA. Illudin S, acylfulvene, and conventional anticancer drugs, such as cisplatin, *N,N'*-bis (2-chloroethyl)-*N*-nitrosourea, and doxorubicin, are more active against CHO cell lines deficient in the DNA repair enzymes ERCC1, ERCC4, ERCC5, and ERCC6 than parental cell lines (3, 4). However, in contrast to conventional antitumor drugs, illudin S and acylfulvene are also active against CHO cells lacking ERCC2 and ERCC3 helicase activities, suggesting that functional helicase activity may be an important element for repair of the DNA damage produced by this class of compounds. Recent studies have shown that HMAF, in particular, potently inhibits DNA synthesis, blocks cell cycle progression in S phase, and induces apoptotic death of CEM cells in

Table 4 *Activity of HMAF against HT-29 human colon carcinoma in nude mice*

Drug	Dose (mg/kg)	Regimen and route	% tumor growth inhibition[a]	% tumor shrinkage	Mice with partial shrinkage	Mice with complete shrinkage
HMAF	10	Daily ×1, i.p.	29	81	1 of 10	0 of 10
	12	Daily ×1, i.p.	35	0	0 of 10	0 of 10
	4	Daily ×5, i.p.	41	0	0 of 10	0 of 10
	6	Daily ×5, i.p.	68	0	0 of 10	1 of 10
Cyclophosphamide	300	Daily ×1, i.p.	32	0	0 of 10	0 of 10
	125	Daily ×5, i.p.	67	0	0 of 10	0 of 10
Etoposide	12.5	Daily ×5, i.p.	0	0	0 of 10	0 of 10

[a] Values exclude mice with partial or complete shrinkage of tumor.

culture.[3] Additional studies will be required to fully characterize the nature of the uptake, activation, DNA interactions, and ultimate mechanism of cytotoxic action of HMAF.

The present studies clearly demonstrate dramatic antitumor activity of HMAF in several human tumor xenograft models. The degree of activity demonstrated against the MX-1 human breast carcinoma xenograft and activity against refractory xenograft models such as MV522 and HT-29 clearly warrant clinical investigation of this novel agent for possible use against human solid tumors.

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