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ORIGINAL ARTICLE

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A new analogue of 10-deazaaminopterin with markedly enhanced curative effects against human tumor xenografts in mice

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Abstract *Purpose*: These studies sought to evaluate the biochemical and cellular pharmacokinetic properties, cytotoxicity and antitumor efficacy of a new analogue of 10-deaza-aminopterin (PDX) against human tumors. *Methods*: Studies were conducted with a group of human tumor cell lines in culture examining PDX and other folate analogues as permeants for mediated membrane transport, as inhibitors of dihdrofolate reductase and as substrates for folylpolyglutamate synthetase. These same analogues were examined for their cytotoxicity following a 3-h pulse exposure, in experiments providing a value for IC_{50}. Other studies with these analogues were conducted in nude mice bearing subcutaneously implanted human tumors. Treatment of the mice was initiated 4 days after implantation of the tumor using a schedule of administration of one dose per day for 5 days. The tumors were measured 6 days after cessation of therapy and compared to controls for assessment of response. *Results*: In the CCRF-CEM cell system, PDX was 2- to 3-fold less effective as an inhibitor of dihydrofolate reductase than aminopterin (AMT), methotrexate (MTX) or edatrexate (EDX) but much more effective as a permeant for one-carbon, reduced folate transport inward (PDX > AMT $\simeq$ EDX > MTX) and substrate for folylpolyglutamate synthetase (PDX > AMT > EDX > MTX). As predicted by these results, PDX was 15- to 40-fold more cytotoxic than MTX and 3- to 4-fold more cytotoxic than the highly potent EDX following a 3-h pulse exposure in culture of CCRF-CEM cells and cells from a panel of three human breast and two human nonsmall-cell (NSC) lung cancers. The same relative differences were shown for the therapeutic efficacy of these three analogues at equitoxic doses in studies with the human MX-1 and LX-1 tumors and the human A549 NSC lung tumor xenografted in nude mice. On a schedule of qd × 5 given 3–4 days posttransplant, MTX was minimally active (modest tumor growth delay) against all three tumors. EDX was highly active (25–35% complete regressions and 5–10% cures) against the MX-1 and LX-1 tumors but very modestly active (no regressions) against the A549 tumor. In contrast, PDX was even more active (75–85% complete regressions and 25–30% cures) than EDX against the MX-1 and LX-1 tumors and highly active (30% complete regressions and 20% cures) against the A549 tumor. *Conclusions*: These studies showed significantly enhanced antitumor properties of PDX compared with MTX and EDX. Based upon these results, clinical trials of PDX in patients with metastatic breast and NSC lung cancer appear to be warranted.

Key words 10-Propargyl-10-deazaaminopterin · Cytotoxicity · Efficacy · Human tumors

Abbreviations *ALL* acute lymphocytic leukemia · *AMT* aminopterin · *CA* carcinoma · *DHFR* dihydrofolate reductase · *EDX* edatrexate · *FPGS* folypolyglutamate synthetase · IC_{50} 50% inhibition value · *Isotonic saline* 0.14 *M* NaCl plus 0.01 *M* potassium phosphate · *MTD* maximum tolerated dose · *MTX* methotrexate · *NSCLC* nonsmall cell lung cancer · *PDX* 10-propargyl-10-deazaaminopterin

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Introduction

The 10-deazaaminopterins are a class of folate analogues targeted to dihydrofolate reductase (DHFR) that were developed as antitumor agents during the

mid-1980s [1–4]. The design of these analogues emerged from structure-activity studies examining the effect of various modifications of the 4-aminofolate structure on its interaction with specific cytotoxic determinants [5–7]. It was found that alkyl substitution of 10-deazaaminopterin at the C10 position markedly improves the efficacy of this class of compound compared with the unsubstituted analogue with effects against murine tumor models and human tumors xenografts in nude mice considerably superior to that of methotrexate (MTX) [1–4]. One of these analogues, edatrexate (EDX, 10-ethyl-10-deazaaminopterin) has subsequently been shown in phase II clinical trials to yield a major response rate significantly better than MTX in patients treated on a weekly schedule presenting with metastatic nonsmall-cell (NSC) lung cancer [8] breast cancer [9] and one form (MFH) of soft tissue sarcoma [10].

The increased cytotoxicity and antitumor activity of EDX compared with MTX in the model systems utilized in the above studies is attributable to findings that the former is more effectively internalized and polyglutamylated in tumor cells [1, 4]. This is related to the observation [1, 11] that EDX is a more efficient permeant for one-carbon, reduced folate transport and substrate for folylpolyglutamate synthetase (FPGS), both of which are determinants of cytotoxicity in these cells [12–14]. Moreover, the greater therapeutic selectivity seen with EDX in these model tumor systems compared with MTX appears to be explained by findings showing that the former is less effectively internalized and polyglutamylated in normal proliferative tissue where drug-limiting toxicity is seen [1, 4].

Despite the provocative nature of these findings as they pertain to EDX and the encouraging clinical results, the potential for the clinical utility of this agent is as yet not fully realized. In the meantime, further studies on the effect of modification at the C10 position and elsewhere on 10-deazaaminopterin have continued. This has resulted more recently in the synthesis of a new analogue of 10-deazaaminopterin (PDX) bearing a propargyl substituent at C10 [15]. The results of our studies showing that PDX has more interesting pharmacologic properties and markedly enhanced cytotoxicity and antitumor efficacy compared with EDX against human tumors are reported here.

Materials and methods

Biochemical and cellular pharmacokinetics

Measurements of influx [^{3}H]MTX and the nonradioactive analogues by CCRF-CEM cells were carried out by procedures described in detail previously [1, 5, 12]. The analysis of the influx velocity data to derive the appropriate kinetic constants has been given in previous reports [1, 5, 12]. The determination of DHFR activity has been described as well as the method carried out at pH 6.9 for determining the appropriate inhibition constants [1, 5, 12] for the various folate analogues. The methodology used for preparing cell-free extracts and for measurements of FPGS activity and deriving the related kinetic constants has also been described in detail [11].

Methods of cell culture

CCRF-CEM cells were obtained for the various biochemical and cellular pharmacokinetic experiments by growth in RPMI medium. The methods used and the manner of harvesting cells have been described previously [1, 14]. For experiments measuring the cytotoxicity of various folate analogues, the cells were grown in RPMI medium using 96-well plates. Cytotoxicity was determined following exposure of the cells as monolayers (10^3 cells/well) to different concentrations of each analogue in a 3-h pulse. Following this, the medium was removed, the cells washed with isotonic saline (pH 7) and, RPMI medium without drug added prior to further incubation of the plates for 72–96 h. Cell numbers at this time were determined by the XTT assay [16]. IC_{50} (half-maximal inhibition) values were calculated from the data obtained in an automatic plate reader. The other cell lines used in these studies were obtained from various colleagues at MSKCC or the American Type Culture Collection. Maintenance and cytotoxicity determinations with these cells were carried out in DMEM-HG (MDA-468 cells) or α-MEM-F12 (SK-BRIII, ZR-75-1, SK-L16 cells) medium.

In vivo antitumor experiments

The human tumors utilized for these studies were obtained from either the National Cancer Institute, Developmental Therapeutics Program (MX-1, mammary carcinoma, and LX-1, lung carcinoma) or the American Type Culture Collection (A549 squamous cell carcinoma of the lung). These tumors were maintained by transplantation in Swiss nu/nu mice. The transplant for maintenance and during the conduct of the experiments was made by trocar in the form of a segment of tumor (2–3 mm in diameter) inserted subcutaneously into the flank of the mouse. Following randomization of the tumor-bearing mice, treatment with each analogue in isotonic saline (pH 7) was initiated 3–4 days after implant when the tumor mass was on average 62.5 ± 12.5 mg. The schedule of administration was qd × 5 at the MTD (maximum tolerated dose) for each analogue studied. The MTD was obtained in preliminary experiments determining the amount of each analogue that could be administered on this schedule with weight loss no greater than 10%. The average tumor diameter in control and treated groups was measured with a caliper 14 days after implantation (6 days posttreatment). The data are expressed as milligrams of tumor mass as calculated from the average diameter (mass = milligrams = $4/3\pi r^3$). The animals with no palpable tumor were scored as a complete regression and those animals with no evidence of tumor 28 days after cessation of treatment were deemed cured. Other methodological details relevant to the studies described have been given in previous reports [2, 3]. Statistical analysis was carried out by the method of Zar [17].

These studies were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

Other materials and methods

Protein determinations were carried out as previously described [11]. The purity of the various analogues used in these studies were shown to be greater than 98% as determined by HPLC analysis [14]. 3′,5′,9[^{3}H]MTX (specific activity 15–20 ci/mmol) was purchased from Moravek Biochemicals (City of Industry, Calif.) and was used for experiments after HPLC purification [14] to >98%.

Results and discussion

Biochemical and cellular pharmacokinetic studies

In a number of different experiments, PDX was compared with three other 4-amino folate analogues (AMT,

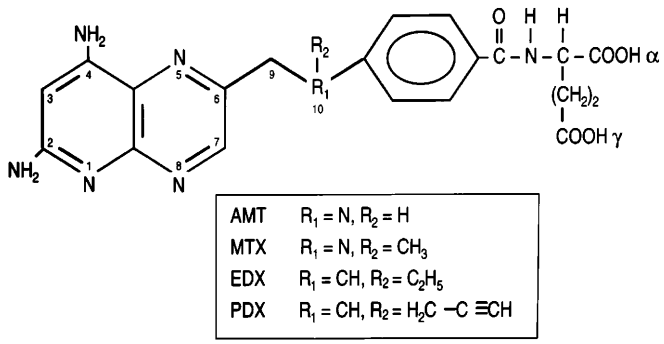

Fig. 1 Structural differences among the various folate analogues utilized in these studies

MTX and EDX) as an inhibitor of DHFR, a permeant for one-carbon, reduced folate transport inward and as a substrate for FPGS. The structure of these analogues are shown in Fig. 1 and the data obtained with them is summarized in Table 1. From these data it can be seen that all four analogues were potent inhibitors of DHFR in the low picomolar range. However, the value for K_i obtained with PDX was two- to threefold higher than with the other three analogues, suggesting that its interaction with this enzyme at least in the unpolyglutamylated form, is slightly less effective than AMT, MTX or EDX.

In other studies, it was shown (Table 1) that PDX was the most efficient permeant for one-carbon, reduced folate transport in CCRF-CEM cells when compared with the three other analogues examined. While maximum capacity for influx (V_{max}) was similar in each case, saturability for influx ($1/K_m$) was in the descending order, PDX > AMT $\simeq$ EDX > MTX. Calculated values for the first-order rate constant (V_{max}/K_m) were 4-fold (AMT and EDX) and 12-fold (PDX) greater than for MTX.

As a substrate for FPGS, PDX was also the most effective among the analogues examined (Table 1) in the assay utilized. PDX exhibited saturability ($1/K_m$) similar to that of AMT and EDX which was fourfold greater than that of MTX. However, unlike MTX and EDX, which exhibited lower values for maximum capacity (V_{max}) than AMT, the maximum capacity obtained with PDX was somewhat higher than with AMT. Thus, first-order rate constants (V_{max}/K_m) derived for FPGS activity were in the descending order of PDX > AMT > EDX > MTX and calculated values were 1.5-, 3- and 10-fold greater, respectively, than AMT, EDX and MTX. HPLC assays carried out (data not shown) on the polyglutamate content in the cytosol of cells exposed to these analogues for 3 h in culture were consistent with these enzymatic findings. Longer-chain polyglutamates (two to four additional glutamates residues) were generated by this catabolism for all of the analogues except AMT in the order PDX > EDX > MTX. As we have reported previously [14], AMT was converted primarily to a polyglutamate with one additional glutamyl residue. Overall, the data obtained from the various studies described above suggest that substantial differences in the order of PDX > AMT $\simeq$ EDX > MTX will be realized in terms of the cytotoxicity of these agents against CCRF-CEM cells and probably other human tumor cells.

Cytotoxicity studies

Other experiments carried out with PDX and two other folate analogues were used to determine their cytotoxicity in culture utilizing a 3-h pulse exposure to these agents. This method of comparison was chosen because it was more relevant to bolus dosing or short-term infusion in patients. Also, we have shown in prior studies [14] that short-term exposure appears to be more appropriate than long-term exposure when comparisons are made between analogues that vary substantially in their ability to be polyglutamylated.

From the data summarized in Table 2, it can be seen that the relative cytotoxicity of these agents against CCRF-CEM cells in culture was consistent with the results of biochemical and cellular pharmacokinetic experiments described above. EDX and PDX were significantly more cytotoxic than MTX ($P = < 0.001$) while PDX was more cytotoxic than EDX ($P = < 0.005$). Comparisons of these three analogues with regard to cytotoxicity to five other human tumor cell lines gave similar results, although the incremental differences seen between each analogue against each cell line were not the same. Among the three mammary carcinomas and two

Table 1 Biochemical and cellular pharmacokinetic properties of various folate analogues in CCRF-CEM cells. The values shown are means ± SE from three to five separate experiments. Experimental details are provided in the text

Compound	DHFR inhibition[a] K_i (pM)	Influx K_m (μM)	Influx V_{max} (pmol/min/mg protein)	V_{max}/K_m	FPGS activity K_m (μM)	FPGS activity V_{max} (pmol/h/mg protein)	V_{max}/K_m
AMT	4.9 ± 1	1.2 ± 0.2	3.6 ± 1	3.0	5.8 ± 1	117 ± 18	20.2
MTX	5.4 ± 2	4.8 ± 1	4.1 ± 1.2	0.9	32.2 ± 5	70 ± 10	2.2
EDX	5.8 ± 1	1.1 ± 0.1	3.9 ± 0.9	3.5	6.3 ± 1	65 ± 9	10.3
PDX	13.4 ± 3	0.3 ± 0.1	3.8 ± 1.3	12.6	5.9 ± 1	137 ± 26	23.2

[a] Reaction carried out at pH 6.9

Table 2 Cytotoxicity of various folate analogues against human tumors in cell culture. The cells were exposed to different concentrations of each agent in a 3-h pulse. The values are means ± SE from three to five experiments. Other experimental details are given in the text

	Tissue type	IC_{50}		
		MTX (μM)	EDX (μM)	PDX (μM)
CCRF-CEM	Acutelymphocytic leukemia	1.2 ± 0.2	0.14 ± 0.03	0.04 ± 0.01
MDA-468	Breast carcinoma	4.5 ± 0.6	0.39 ± 0.05	0.11 ± 0.02
SK-BR III	Breast carcinoma	4.2 ± 0.5	0.99 ± 0.2	0.28 ± 0.04
ZR-75-1	Breast carcinoma	3.5 ± 0.5	0.86 ± 0.1	0.26 ± 0.05
SK-LC8	NSCLC[a]	10.3 ± 2	1.24 ± 0.2	0.42 ± 0.1
SK-LC16	NSCLC[b]	2.1 ± 0.3	0.26 ± 0.05	0.11 ± 0.02

[a] Squamous cell carcinoma
[b] Adenocarcinoma

NSC lung tumors, the latter were the more sensitive (adenocarcinoma) and the more resistant (squamous cell carcinoma) to this group of agents. In a very consistent fashion, EDX was substantially more cytotoxic than MTX ($P = < 0.001$) to the extent of 4- to 11-fold depending upon the cell line. Moreover, PDX was 3- to 4-fold more cytotoxic than EDX ($P = < 0.005$) against the same cell lines.

Studies of in vivo antitumor activity

PDX was evaluated against three different human solid tumors xenografted in nude mice. For comparison, this analogue was examined in parallel with both MTX and EDX at equitoxic doses which represented the MTD for each agent on the schedule of administration used. The results obtained in the initial experiments (data not shown) showed that tolerance of these mice to these agents varied somewhat in the order PDX > EDX > MTX. Against the MX-1 tumor, only a small delay in growth was obtained (Table 3) with MTX. In contrast to this analogue, EDX was substantially more effective resulting on average in frank regression of the tumor. Moreover, 35% of the EDX-treated animals showed complete regression of their tumor and some cures were obtained. In contrast, PDX was even more effective. All of the animals in this treated group had marked regressions of their tumor and a high percentage (75–80%) of these animals exhibited complete regression of their tumor (EDX versus PDX, $P = < 0.005$) and a greater number of cures (30–35%) were observed (EDX versus PDX, $P = < 0.01$).

The superiority of PDX versus EDX or MTX was even more striking in studies (Tables 4 and 5) with the LX-1 and A549 tumors. In the case of the LX-1 tumor, MTX was again without appreciable activity, yielding

Table 3 Antitumor properties of various folate analogues against human MX-1 mammary carcinoma in nude mice. The animals were treated on a schedule of qd × 5 beginning 3–4 days after subcutaneous implantation of tumor. The tumor volume was determined 6 days after cessation of therapy. Additional details are given in the text. The values shown are means ± SE from two to five experiments

R_x group	R_x MTD (mg/kg)	Tumor mass (mg)	Complete regressions[a] (no./total)	Cures[b] (no./total)
Control[c]	–	430 ± 35	0/25	0/25
MTX	1.5	303 ± 28	0/10	0/10
EDX	2.0	9 ± 3	9/25	3/25
PDX	3.0	< 1	19/25	8/25

[a] No palpable mass 6 days after cessation of therapy
[b] No evidence of tumor anywhere in the animal 28 days after cessation of therapy
[c] Treated with solvent (isotonic saline) only

Table 4 Antitumor properties of various folate analogues against human LX-1 lung cancer in nude mice. See text and legend of Table 3 for additional experimental details. Values shown are means ± SE from two to four experiments

R_x group	R_x MTD (mg/kg)	Tumor mass (mg)	Complete regressions[a] (no./total)	Cures[b] (no./total)
Control[c]	–	567 ± 68	0/20	0/20
MTX	1.5	409 ± 52	0/10	0/20
EDX	2.0	58 ± 14	6/20	1/20
PDX	3.0	< 1	17/20	5/20

[a] No palpable tumor mass 6 days after cessation of therapy
[b] No evidence of tumor anywhere in the animal 28 days after cessation of therapy
[c] Treated with solvent (isotonic saline) only

Table 5 Antitumor properties of various folate analogues against the human A549 squamous cell lung carcinoma in nude mice. Values shown are the means from two experiments. See text and legend of Table 3 for additional details

R_x group	R_x MTD (mg/kg)	Tumor mass (mg)	Complete regressions[a] (no./total)	Cures[b] (no./total)
Control[c]	–	470 ± 48	0/10	0/10
MTX	1.5	395 ± 49	0/10	0/10
EDX	2.0	148 ± 31	0/10	0/10
PDX	3.0	54 ± 16	3/10	2/10

[a] No palpable tumor mass 6 days after cessation of therapy
[b] No evidence of tumor anywhere in the animal 28 days after cessation of therapy
[c] Treated with solvent (isotonic saline) only

only a small delay in tumor growth. Treatment with EDX resulted in complete inhibition of tumor growth along with some complete regressions (30%) and only an occasional cure. By comparison, all of the animals treated with PDX showed frank regression of their tumor with a large number of complete regressions (85%; EDX versus PDX, $P = < 0.005$). Moreover, 25% of the animals in this treated group were cured (EDX versus PDX, $P = < 0.01$). Against the A549 tumor, MTX was essentially without activity. Tumors in animals treated with EDX showed a growth delay ($P = < 0.05$) but no complete regressions or cures were observed. In contrast, the growth of tumors in animals treated with PDX was completely inhibited (EDX versus PDX, $P = < 0.01$) and many animals showed complete regression (30%) and were cured (20%).

From an analysis of the data presented above, it would appear that PDX is a significantly superior cytotoxic and antitumor agent to either MTX or EDX in the model systems used in these studies. Moreover, the biochemical and cellular pharmacokinetic data also derived during these studies provides a reasonable pharmacologic basis for this superiority. Although a direct comparison of the pharmacologic and biological data was made only in the case of CCRF-CEM cells, the overall consistency of the results obtained with each analogue among the different cell types would appear to provide justification for such a conclusion. The biochemical and cellular pharmacokinetic parameters examined in these studies are generally accepted as major determinants of cytotoxicity and therapeutic response to this class of agents at least in these model systems [6, 7, 11–14, 18]. Moreover, among the 4-aminofolate analogues, PDX appears to be the most effective permeant for one-carbon, reduced folate transport and substrate for FPGS so far examined.

As such, the data presented above also clearly suggest that PDX represents a further advance in the development of agents targeted to DHFR which may have significantly improved therapeutic potential in patients with neoplastic disease. The data offer additional proof-of-principle for the premise that optimizing through structural design drug interactions with pharmacologic determinants relevant to cytotoxicity and therapeutic response will ultimately lead to improved efficacy compared to therapy with standard agents, in this case, MTX. This would appear to represent ample reason for an evaluation of PDX in the appropriate clinical trials, particularly in breast and NSC lung cancers. These studies are now underway at the Memorial Sloan-Kettering Cancer Center.

The improvement in antitumor activity of PDX compared to EDX shown in the current studies with these human tumor xenograft systems was unanticipated in light of our other previous studies [15, 19]. Further increase in bulk in the form of a fully saturated alkyl substituent at C10 beyond two carbons as in the case of *n*-propyl-10-deazaaminopterin has been found to diminish antitumor efficacy at least in one murine tumor model [15]. Similarly, the data pertaining to the therapeutic activity of PDX in this same model system [15] were not particularly encouraging. Moreover, a propargyl substituent at N10 in another series of 4-aminofolates has been shown to result in very extensive loss of DHFR inhibition by this analogue [19]. Thus, enhanced cytotoxicity and antitumor efficacy against human tumors that was observed in the case of PDX may relate to the different effect of propargyl substitution at C10 rather than N10 and also to the unsaturated nature of this substituent.

References

1. Sirotnak FM, DeGraw JI, Moccio DM, Samuels LL, Goutas LJ (1984) New folate analogs of the 10-deaza-aminopterin series. Basis for structural design and biochemical and pharmacologic properties. Cancer Chemother Pharmacol 12: 18–25
2. Sirotnak FM, DeGraw JI, Schmid FA, Goutas LL, Moccio DM (1984) New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methotrexate in ascitic and solid murine tumor models. Cancer Chemother Pharmacol 12: 26–30
3. Schmid FA, Sirotnak FM, Otter GM, DeGraw JI (1985) New folate analogs of the 10-deaza-aminopterin series. Markedly increased antitumor activity of the 10-ethyl analog compared to the parent compound and methotrexate against some human tumors xenografts in nude mice. Cancer Treat Rep 69: 551–553
4. Sirotnak FM, Schmid FA, Samuels LL, DeGraw JI (1987) 10-ethyl-10-deazaaminopterin: Structural design and biochemical, pharmacologic and antitumor properties. NCI Monogr. 5: 127–131
5. Sirotnak FM, Chello PL, Brockman RW (1979) Potential for exploitation of transport systems in anticancer drug design. Methods Cancer Res. 16, 381–447
6. Sirotnak FM (1983) Concepts in new folate analog design. In: Cheng Y-C, Goz B, Minkoff M (eds.) Development of target-oriented anticancer drugs. Vol. 28 Progress in cancer research and therapy. Raven Pres, New York, pp 77–96
7. Sirotnak FM, DeGraw JI (1984) Selective antitumor action of folate analogs. In: Sirotnak FM, Burchall JJ, Ensminger WD, Montgomery JA (eds) Folate antagonists and therapeutic agents, vol 1. Academic Press, New York, pp 173–207
8. Shum KY, Kris MG, Gralla RJ, Patanovich LM, Marks LD, Hellan RT (1988) Phase II study of 10-ethyl-10-deazaaminopterin in patients with stage IIIb and IV nonsmall cell lung cancer. J Clin Oncol 6: 446–450
9. Vandenberg TA, Pritchard KI, Eisenhauer EA, Trudeau ME, Norris BD, Lopez P, Verma SS, Buchman RA, Muddal A (1992) Phase II study of weekly edatrexate as first-line chemotherapy for metastatic breast cancer: A National Cancer Institute of Canada clinical trials group study. J Clin Oncol 11, 1241–1244
10. Casper ES, Christman KL, Schwartz GK, Johnson B, Brennan MF, Bertino JR (1993) Edatrexate in patients with soft tissue sarcoma. Cancer 72: 766–770
11. Rumberger BG, Barrucco JR, Sirotnak FM (1990) Differing specificities for 4-amino folate analogs of folylpolyglutamate synthetase from tumors and proliferative intestinal epithelium of the mouse with significance for selective antitumor action. Cancer Res. 50: 4639–4643
12. Sirotnak, FM (1980) Correlates of folate analog transport, pharmacokinetics and selective antitumor action. Pharmacol. Ther 8: 71–103
13. Goldman ID, Matherly LH (1986) Cellular pharmacology of methotrexate. In: Goldman ID, (ed) Membrane transport of antineoplastic agents. International encyclopedia of

pharmacology therapy, sect 118. Pergamon Press, New York, pp 283–302
14. Samuels LL, Moccio DM, Sirotnak FM (1985) Similar differential for polyglutamylation and cytotoxicity among various folate analogs and tumor cells in vivo. Cancer Res 45: 1488–1495
15. DeGraw JI, Colwell WT, Piper JR, Sirotnak FM (1992) Synthesis and antitumor activity of 10-propargyl-10-deazaaminopterin. J Med Chem 36: 2228–231
16. Chou T-C, Otter GM, Sirotnak FM (1996) Schedule-dependent synergism of taxol and taxotere with edatrexate against human breast cancer cells in vivo. Cancer Chemother Pharmacol. 37: 222–228
17. Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs, pp 145–146
18. Chabner BA (1982) Methotrexate. In: Chabner BA (ed) Pharmacologic principles of cancer treatment. WB Saunders, Philadelphia, pp. 229–258
19. Piper JR, McCaleb GS, Montgomery JA, Kisliuk RL, Goumont Y, Sirotnak FM (1992) 10-propargylaaminopterin and alkyl homologues of methotrexate as inhibitors of folate metabolism. J Med Chem 25: 877–880