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SYNTHESIS AND BIOLOGICAL EVALUATION OF A NEW SERIES OF DIHYDROFOLATE REDUCTASE INHIBITORS BASED ON THE 4-(2,6-DIAMINO-5-PYRIMIDINYL)ALKYL-L-GLUTAMIC ACID STRUCTURE‡

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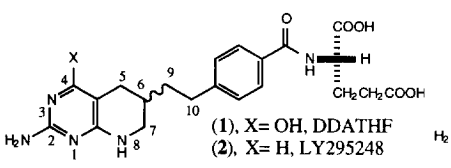
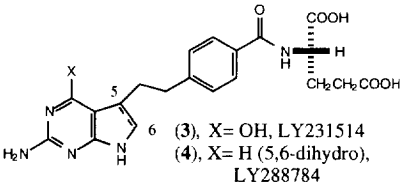
Abstract: A novel series of dihydrofolate reductase inhibitors was uncovered during an expansion of the SAR of 5,10-dideazatetrahydrofolic acid, and their biological activity was evaluated. These new analogs do not possess an oxygen at the 4 position and contain a monocyclic pyrimidine ring.

5,10-Dideazatetrahydrofolic acid[1] (DDATHF, **1**) is a potent inhibitor of glycinamide ribonucleotide formyltransferase (GARFT). The 6R diastereomer of DDATHF (Lometrexol) is currently in Phase I clinical trials. LY231514[2] (**3**), a pyrrolo[2,3-*d*]pyrimidine-based antifolate which inhibits primarily at thymidylate synthase (TS) is currently undergoing Phase II clinical trials. As part of the continuing SAR study of these novel classes of deazafolates, we have examined the role of the (3H)4-oxo group on the pyrimidine ring of both DDATHF and LY231514. The replacement of the 4-oxo group of DDATHF and LY231514 with a hydrogen atom has resulted in compounds such as LY295248[3] (**2**) and LY288784[3] (**4**) with complete loss of inhibitory activity against GARFT and TS. Cell culture end products reversal and enzyme inhibition studies have



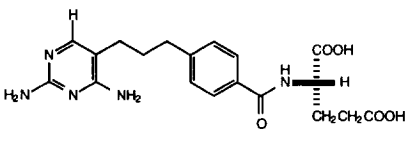
(**1**), X= OH, DDATHF
(**2**), X= H, LY295248



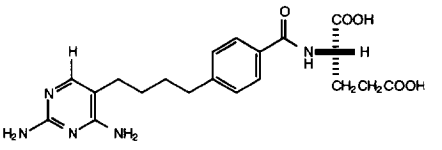
(**3**), X= OH, LY231514
(**4**), X= H (5,6-dihydro), LY288784

‡ Dedicated to the memory of Dr. Gerald B. Grindey.

demonstrated that these 4-deoxy analogs do not inhibit GARFT and/or TS, but instead are potent inhibitors of human dihydrofolate reductase (hDHFR). This finding has prompted us to examine the corresponding less rigid, monocyclic pyrimidine based analogs with the methylene moiety in both the reduced pyridine and pyrrole rings being removed. Herein we describe the synthesis and biological evaluations of these two monocyclic pyrimidine compounds LY316373[4] (**5**) and LY298207[4] (**6**).



(**5**) LY316373



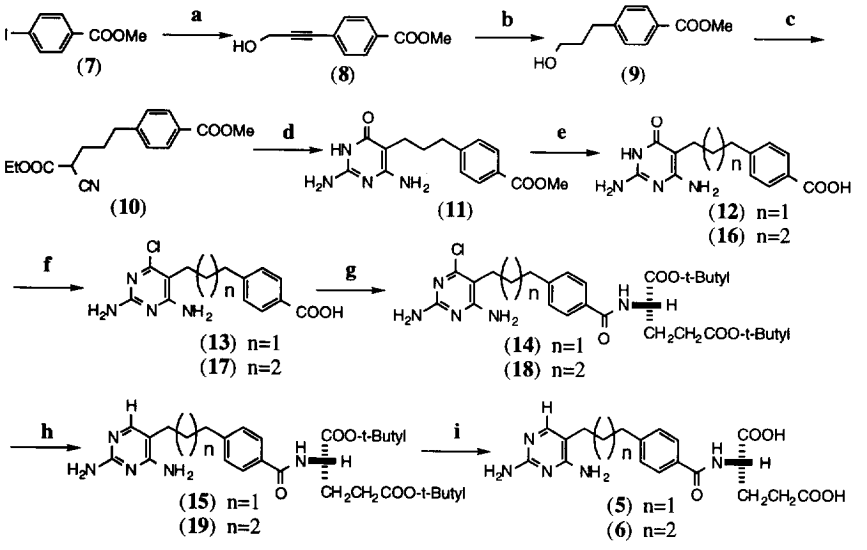
(**6**) LY298207

The synthesis (Scheme 1) of compound (**5**) begins by reacting 4-iodobenzoic acid methyl ester (**7**) with propargyl alcohol using a palladium (0) catalyzed Heck reaction to give (**8**) in 95% yield. The alkyne was reduced catalytically (5% Pd/C, 95% yield) to produce (**9**), and the resulting alcohol was then reacted with ethyl cyanoacetate under Mitsunobu conditions to generate compound (**10**) in 40% yield. This intermediate was then reacted with guanidine to give the cyclized 2,6-diamino-4-oxo-5-substituted-pyrimidine (**11**) in 68% yield. After the cleavage of the methyl ester in 1.0 N NaOH, the corresponding benzoic acid derivative (**12**) was then refluxed in phosphorous oxychloride[5] to give (**13**) as a crude solid. This crude solid was then coupled with L-glutamic acid di-t-butyl ester using 2-chloro-4,6-dimethoxy-1,3,5-triazine in DMF to afford (**14**) in 2 steps. The chlorine was then reduced via hydrogenolysis using palladium black to give (**15**) in 80% yield. Finally, after treatment with trifluoroacetic acid, N-{[4-(2,6-diamino-5-pyrimidinyl)propyl]}-L-glutamic acid (**5**) was isolated in 80% yield. To synthesize compound (**6**), 4-[2,6-diamino-4-oxo-5-pyrimidinyl)butyl benzoic acid (**16**) was treated in a similar fashion, (chlorination, coupling with L-glutamic acid dialkylester, reduction and final saponification), to give N-{[4-(2,6-diamino-5-pyrimidinyl)butyl]}-L-glutamic acid (**6**) in comparable yields.

Both compounds (**5**) and (**6**) were found to be extremely cytotoxic against CCRF-CEM leukemia cells in vitro with IC_{50}'s = 1 ng/mL. (Methotrexate has an IC_{50} = 5 ng/mL.) Cell culture end products reversal studies indicated that the primary target of inhibition for these compounds is DHFR since both thymidine (5 μM) and hypoxanthine (100 μM) are required to reverse the cytotoxic activity of these compounds (data not included). This

observation is confirmed by the extremely potent enzyme inhibition of both compounds (**5**, K_i = 120 pM) and (**6**, K_i = 5 pM) against recombinant human DHFR. The measured K_i's of (**5**) and (**6**) against DHFR and other folate requiring enzymes are shown in Table 1. Neither compound possesses potent inhibitory activity against TS or GARFT. Both

Scheme 1



(a) $PdCl_2$, propargyl alcohol, diethylamine (b) 5% Pd/C, H_2, MeOH (c) DEAD, ethylcyanoacetate, THF (d) guanidine, t-butanol (e) 1 N NaOH (f) $POCl_3$, dimethylaniline (g) L-glutamic acid dialkylester, 2-chloro-4,6-dimethoxy-1,3,5-triazine, DMF (h) palladium black, H_2, EtOH (i) TFA

compounds were also evaluated against the enzyme folylpolyglutamate synthetase (FPGS, Table 2), and it was discovered that compound (**5**), for example, is an excellent substrate for FPGS (K_m = 5.1 μM). Its first order rate constant (V_{max}/K_m) of 149 is one of the best antifolates ever reported. This value is significantly better than the well-known DHFR inhibitor methotrexate, and compares favorably with the DHFR inhibitor 10-EDAM[6] (K_m = 13.3 μM, V_{max}/K_m = 19). In summary, both (**5**) and (**6**) were found to be very potent and selective DHFR inhibitors. Their excellent polyglutamation profile suggests that these compounds may be retained in the cells more efficiently than other DHFR inhibitors and thus be more efficacious as antitumor agents. *In vivo* animal studies are currently in progress to further evaluate these new DHFR inhibitors.

Table 1. Measured K_i's Against Various Folate Requiring Enzymes

Compound	DHFR[a]	TS[b]	GARFT[c]
5	120 pM	>5 μM	54.3 μM
6	5 pM[d]	>5 μM	62.0 μM

(a) recombinant human DHFR, *Biochemistry* **1989**, *27*, 3664 (b) recombinant human TS, *J. Biol. Chem.* **1989**, *264*, 9145 (c) human monofunctional GARFT, supplied by Agouron Pharmaceuticals (d) methotrexate also has a K_i = 5 pM

Table 2. Substrate Activity of Various Compounds For FPGS[a]

Compound	K_m (μM)	V_{max}[b]	V_{max}/K_m
5	5.1	758	149
6	20	685	34
Methotrexate	116	498	4.3
10-EDAM	13.3	258	19
DDATHF	16.4	977	60
LY231514	1.9	725	381

(a) isolated from hog liver (b) nmol/hr·mg

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