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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-DESAMINO AND 4-DEOXY ANALOGS OF 5,10-DIDEAZATETRAHYDROFOLIC ACID (DDATHF)

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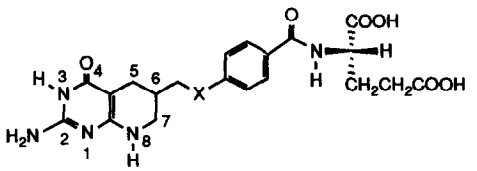
Abstract: Either the 2-amino or the 4-oxo group of the potent GAR formyltransferase inhibitor 5,10-dideazatetrahydrofolic acid (DDATHF) was replaced with a hydrogen atom and their biological activity evaluated.

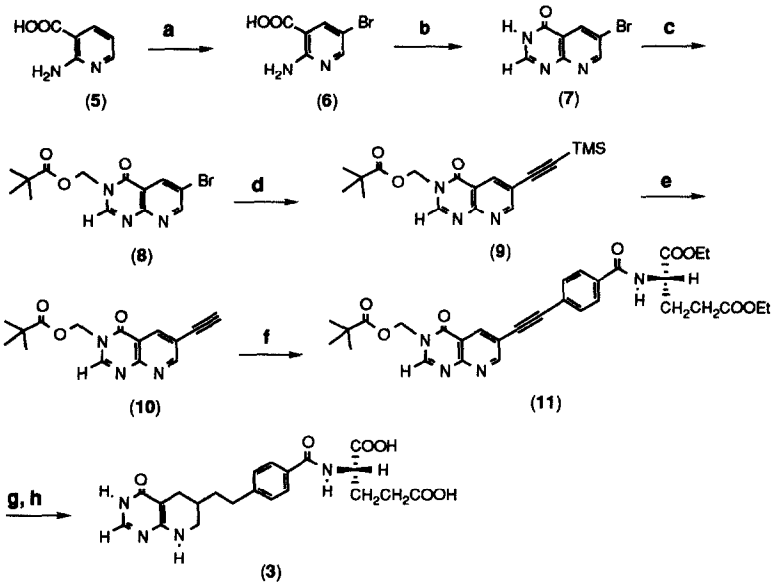
5,10-Dideazatetrahydrofolic acid (DDATHF, **1**) is the prototype of a new class of folate antimetabolites that inhibit the folate requiring enzyme glycinamide ribonucleotide formyltransferase (GARFT, EC 2.1.2.1) in the purine de novo biosynthesis.[1] DDATHF and its analogs form a tight inhibitory ternary complex with the enzyme GARFT and its natural substrate β-glycinamide ribonucleotide. The structures of the apo and complexed *Escherichia coli* GARFT have recently been determined.[2,3] Analysis of the ternary complex indicated that hydrogen bonding interactions existed between the folate ligand such as 5-deaza-5,6,7,8-tetrahydrofolic acid (5dTHF, an analog of DDATHF) and the active site residues. The parts of the inhibitor that participated in the hydrogen bonding interactions included the hydrogen bond acceptors such as the N1 and the 4-oxo groups and donors such as the 2-NH_2, 8-NH and possibly the 3-NH groups of the 5-deaza-5,6,7,8-tetrahydropteridine ring system.[2] In order to evaluate and assess the relative importance of each of these hydrogen bonding interactions, we have prepared analogs which do not possess the 2-amino group (2-desamino-DDATHF, **3**) or the 4-oxo group (4-deoxy-DDATHF, **4**) of DDATHF and examined their inhibitory activity against human monofunctional GARFT[4].



1, X=CH2, DDATHF
2, X=NH, 5dTHF

The synthesis of 2-desamino-DDATHF began with 2-aminonicotinic acid (**5**, Scheme 1). Treatment of (**5**) with bromine in acetic acid gave the corresponding 2-amino-5-bromonicotinic acid (**6**) in 80% yield. Reaction of (**6**) with neat formamide at 180°C then yielded the cyclized product 4-oxo-7-bromopyrido[2,3-d]pyrimidine (**7**, 80%). The nitrogen at position 3 was first protected with trimethylacetyloxymethyl chloride / NaH (75%), and then the trimethylsilyl ethynyl side chain was introduced into the 6 position through a standard palladium catalyzed Heck reaction[1h] between compound (**8**) and trimethylsilylacetylene (55%). The trimethysilyl group in (**9**) was subsequently removed with fluoride ion

SCHEME 1

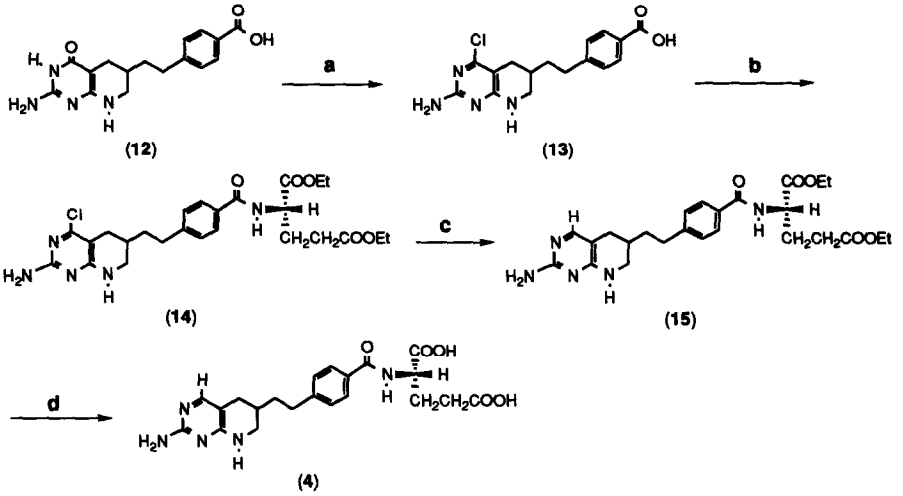


(a) Br_2, HOAc; (b) $HCONH_2$, 180° C; (c) NaH, $(CH_3)_3COOCH_2Cl$; (d) trimethylsilylacetylene, $PdCl_2$, PPh_3, CuI, Et_3N; (e) KF, HOAc; (f) diethyl-p-iodobenzoyl-l-glutamate, $PdCl_2$, PPh_3, CuI, Et_3N; (g) H_2, 5% Pd/C; (h) 0.4 N NaOH.

(KF/HOAc, 93%) and the resulting ethynyl compound (**10**) was coupled with diethyl p-iodobenzoyl-l-glutamate again using palladium (0) as the catalyst and gave compound (**11**) in 73% yield. Catalytic hydrogenation (5% Pd/C) followed by saponification in 0.4N NaOH then led to the fully reduced 2-desamino-DDATHF (**3**) as a white solid.

The corresponding 4-deoxy analog (**4**) was prepared from the pteoric acid derivative (**12**)[5] of DDATHF (Scheme 2). Treatment of (**12**) with phosphorus oxychloride in the presence of tetraethylammonium chloride and dimethylaniline gave the imminium chloride (**13**). Without purification, compound (**13**) was coupled directly with diethyl-l-glutamate by using 2-chloro-4,6-dimethoxy-1,3,5-triazine as the activating agent to give compound (**14**) (33%, 2 steps). Hydrogenolysis using palladium black replaced the chlorine atom at the 4 position with hydrogen (73%) and gave compound (**15**). Final saponification (1.0 N NaOH) then gave the desired 4-deoxy-DDATHF (**4**).

SCHEME 2



(a) $POCl_3$, Et_4NCl, $C_6H_5N(CH_3)_2$, CH_3CN; (b) l-glutamic acid diethyl ester hydrochloride, 2-chloro-4,6-dimethoxy-1,3,5-triazine, 4-methylmorpholine; (c) Pd black, H_2; (d) 1.0 N NaOH.

Both compounds (**3**) and (**4**) were evaluated against a recombinant human monofunctional GARFT (hGARFT) and the results are shown in Table 1. Enzyme inhibition studies have indicated that neither compounds (**3**) or (**4**) possess potent inhibitory activity toward hGARFT. The Ki values of these compounds (DDATHF and **3**) on the human monofunctional GARFT correlated

Table 1. GARFT Inhibition and Cellular Cytotoxicity of DDATHF Analogs

compd	K_i (μM) hGARFT[1] (L1210 GARFT)[2]	IC_{50} (μg/ml) CCRF-CEM[3]
DDATHF	0.13 (0.12)	0.007
3[4]	82.1 (27.0)	>20
4[4]	186	0.004

1. obtained from Dr. Cheryl Janson of Agouron Pharmaceuticals Inc., San Diego, CA
2. trifunctional GARFT isolated from murine L1210 cells, see Reference 1e
3. 72 hours growth inhibition assay, CCRF-CEM is a human T-cells derived lymphoblastic leukemic cells.
4. both compounds (**3**) and (**4**) were prepared and tested as mixture of diastereomers at C-6.

well with the earlier reported Ki values on the trifunctional GARFT isolated from the murine L1210 cells. The removal of either the 2-amino or 4-oxo group on the pyrimidine ring portion of DDATHF has rendered a major loss of activity (630-fold and 1,400-fold, respectively) of these compounds against the target enzyme. This result has confirmed the X-ray observations that hydrogen bonding interactions involved with each of these groups are crucial to the overall binding of DDATHF toward mammalian GARFT. In contrast to the completely non-cytotoxic nature of 2-desamino-DDATHF (**3**), the 4-deoxy analog (**4**) turned out to be highly cytotoxic against the CEM cells (IC_{50} =0.004 μg/ml). Testing against other folate requiring enzymes has revealed that compound (**4**) is a potent inhibitor against the human dihydrofolate reductase (IC_{50} = 6.5×10^{-8} M)[6]. Cell culture reversal studies showed that the cytotoxicity of (**4**) can only be reversed with the simultaneous addition of thymidine and hypoxanthine, which also suggests it is mainly targeted at DHFR. Compound (**4**) thus represents the first potent folate antagonist that inhibits DHFR without the "prerequisite" 2,4-dihydroamino configuration on the pyrimidine ring.[7] More detailed enzymatic and pharmacological studies on this novel DHFR inhibitor is currently underway and the results will be presented elsewhere.

References and Notes:

(1) (a) Taylor, E.C.; Harrington, P.J.; Fletcher, S.R.; Beardsley, G.P.; Moran, R.G. *J. Med. Chem.* **1985**, *28*, 914. (b) Beardsley, G.P.; Taylor, E.C.; Grindey, G.B.; Moran, R.G. In *Chemistry and Biology of Pteridines*; Cooper, B.A.; Whitehead, V.M. Eds.; Walter de Gruyter: Berlin, 1986; p.953. (c) Beardsley, G.P.; Moroson, B.A.; Taylor, E.C.; Moran, R.G. *J. Biol. Chem.* **1989**, *264*, 328. (d) Baldwin, S.W.; Tse, A.; Grindey, G.B.; Taylor, E.C.; Rosowsky, A.; Shih, C.; Moran, R.G. *Proc. Amer. Assoc. Cancer Res.* **1990**, *31*, 341. (e) Baldwin, S.W.; Tse, A.; Gossett, L.S.; Taylor, E.C.; Rosowsky, A.; Shih, C.; Moran, R.G. *Biochemistry*, **1991**, 1997. (f) Beardsley, G.P.; Taylor, E.C.; Shih, C.; Poore, G.A.; Grindey, G.B.; Moran, R.G. *Proc. Amer. Assoc. Cancer Res.* **1986**, *27*, 259. (g) Shih, C.; Grindey, G.B.; Houghton, P.J.; Houghton, J.A. *Proc. Amer. Assoc. Cancer Res.* **1988**, *29*, 283. (h) Taylor, E.C.; Wong, G.S.W. *J. Org. Chem.* **1989**, *54*, 3618.

(2) Almassy, R.J.; Janson, C.A.; Kan, C.; Hostomska, Z. *Proc. Natl. Acad. Sci. USA* **1992**, 89, 6114-6118.

(3) Chen, P.; Schulze-Gahmen, U.; Stura, E. A.; Inglese, J.; Johnson, D. L.; Marolewski, A.; Benkovic, S.J.; Wilson, I.A. *J. Mol. Biol.* **1992**, 227, 283-292.

(4) Kan, C.; Gehring, M. R.; Nodes, B. R.; Janson, C. A.; Almassy, R. J.; Hostomska, Z. *J. Protein Chem.* **1992**, 11, 5, 467-473.

(5) Obtained either by using synthetic route reported in Reference 1h or by treating DDATHF with 6N HCl (reflux, 6h).

(6) In comparison, DHFR inhibitor Methotrexate has an IC50 of 2.5×10^{-8} M against the recombinant hDHFR. We thank Dr. James H. Freisheim of Medical College of Ohio for testing compound (**4**).

(7) For a recent detailed review on structure modifications and requirements on folates and antifolates, see Rosowsky, A. in *Progress in Medicinal Chemistry*, Ellis, G. P.; West, G. B. Eds.; Elsevier Science Publishing, B. V. **1989**, 26, p. 1-252.