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BILIMSEL TARAMALAR / SCIENTIFIC REVIEWS

The Chemistry and Pharmacological Importance of 5,10-dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF)

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Summary : Over the past 30 years, big efforts have been devoted to the development of novel folate antimetabolites. In fact, there are more than 18 folate -dependent enzymes. All of the potent antifolates reported thus far have been inhibitors of dihydrofolate reductase. In 1985, Taylor and his co-workers reported the synthesis of 5,10-dideaza- 5,6,7,8-tetrahydrofolate, DDATHF, as a new class of folate antimetabolites. DDATHF is a new potent antitumor agent that specially inhibits purine biosynthesis, primarily through inhibition of glycinamide ribonucleotide transformylase, the first of the tetrahydrofolate-requiring enzymes in the de novo synthesis pathway. It is a close analog of tetrahydrofolic acid, differs only by replacement of the 5-and 10-position nitrogen atoms by carbon. It retains the structural features of tetrahydrofolates which are necessary for membrane transport. It is incapable of participating in any of the one-carbon transfers and cofactor interconversions characteristic of folate metabolism. DDATHF can exist in two diastereomeric forms, differing in configuration at a carbon 6.(6R)-DDATHF has the configuration analogous to that of natural tetrahydrofolate. Both diastereomers of DDATHF are potent inhibitor of cell growth in culture. The 6R-diastereomer is currently undergoing clinical trials.

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5,10-dideaza-5,6,7,8-tetrahidrofolik asit (DDATHF)'in Kimyası ve Eczacılıktaki Önemi

Özet : *Son 30 yıl içinde, yeni folat antimetabolitlerinin geliştirilmesi için büyük çabalar sarfedilmiştir. Gerçekte, 18 in üzerinde folat-bağımlı enzim bulunmaktadır. Bugüne kadar bulunmuş güçlü antifolatların hepsi dihidrofolat reduktaz enziminin inhibitörleridir. 1985'de, Taylor ve grubu, yeni bir sınıf folat antimetaboliti olan DDATHF'in sentezini gerçekleştirdi. DDATHF, başta glisinamid ribonucleotid transformilaz olmak üzere tetrahidrofolat gerektiren enzimleri inhibe ederek, özellikle purin biosentezini durduran yeni bir antitumor reaktifdir. Kimyasal açıdan tetrahidrofolik aside benzediğinden, hücre zarı geçirgenliğine sahiptir. DDATHF'de, 5- ve 10-yerlerinde azot atomlarının karbonla yerdeğiştirmesi DDATHF'in kimyasal kararlılığını arttırır ve folat metabolizmasına bir kofaktör olarak katılmasını önler. DDATHF, 6-yerinde konfigürasyonun değiştiği iki diastereomerik şekilde bulunur. (6R)-DDATHF, doğal tetrahidrofolat ile konfigürasyon benzerliğini gösterir. DDATHF'in her iki diastereomeri kültür içinde hücre büyümesini önleyen güçlü inhibitörlerdir. (6R)-diastereomeri ile klinik araştırmalar devam etmektedir.*

Anahtar sözcükler : *Aminopterin, Metotraksat, Tetrahidrofolat, Antikanser ajan, de novo Purin biyosentezi, Glisinamidoribonükleotid transformilaz inhibitörü*

Introduction

The history of antimetabolite cancer chemotherapy began when aminopterin, and methotrexate (MTX), both inhibitors of folate metabolism, were found to induce remission of acute lymphoblastic leukemia[1]. The clinical record with MTX has compiled as an antineoplastic and immunosuppressive drug testifies to the value of folate antimetabolites as antiproliferative agents[2]. All of the potent antifolates reported thus far have been inhibitors of dihydrofolate reductase (DHFR), and none has supplanted MTX in clinical usefulness.

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Reduced folate cofators are necessary for a wide spectrum of 1-carbon transfer reactions in intermediary metabolism, including critical steps in the de novo pathways for thymidylate and purine biosynthesis, the regeneration of methionine from homocystein, the interconversion of serine and glycine, and the catabolism of certain amino acids.

Recently, Taylor et al. reported the synthesis and preliminary evaluation of a new class of tetrahydrofolate analogs, which were designed as inhibitors of folate metabolism other than DHFR[3-7]. The lead member of this series is 5,10- dideaza - 5,6,7,8-tetrahydrofolic acid (DDATHF), whose structure is shown in Figure 1. The nitrogen atoms at 5 and 10 positions of DDATHF are essential participants in all of the 1-carbon transfers and cofactor interconversions of reduced folate metabolism, and DDATHF is structurally precluded from serving as a substrate in any of these reactions. It posseses extraordinary and selective antitumor activity. Its therapeutic index and its broad spectrum of activity against a variety of murine solid tumors and human colon xenografts in mice are unrivaled among known antitumor agents[3-11].

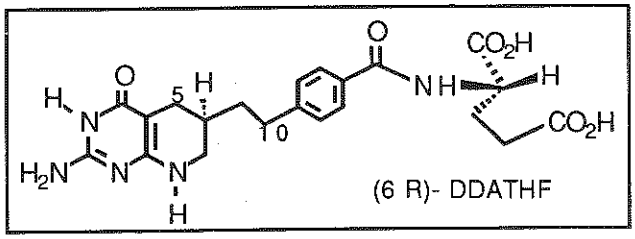


Figure 1

The current level of interest in the synthesizing and biological evaluation of DDATHF and analogs is indicated by the increasing number of publications in this area. In this review, primary emphasis will be on synthesis of DDATHF and analogs and also recent reports on intracellular metabolism of DDATHF isomers will be given.

Some Synthetic Pathways to 5,10-dideaza-5,6,7,8-Tetrahydrofolic Acid

The first synthesis of DDATHF was accomplished by Taylor et al[8]. in 14 steps from thiocyanoacetamide and ß-ethoxymethacrolein. 3-cyano-5-methyl-2 (1H) pyridinethione was initially formed[12]. A Wittig condensation of its derivative with t-butyl-p-formyl benzoate gave 2. Guanidine cyclization of 2 to give pyrido [2,3-d] pyrimidine 3, followed by hydrolysis and acetylation. Coupling with diethyl-L-glutamate (DEG.HCl) afforded 4. Catalytic reduction of 4 followed by hydrolysis of both the acetyl and ester functionalities to give DDATHF, 1 (Scheme 1).

Reaction of 5 with d-10-camphorsulfonic acid gave a mixture (ddL and dLL) of salts, 6, seperated by fractional crystallization.

Related to different synthetic pathways for DDATHF, Piper et al.[13,14] has developed an alternative synthesis of 5,10-Dideazatetrahydrofolic acid and its methyl analogue 7 (Figure 2). Both of these compounds, 1 and 7 were transported into cells more efficiently than methotrexate (Figure 2).

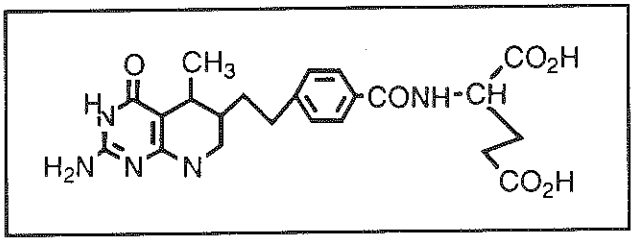
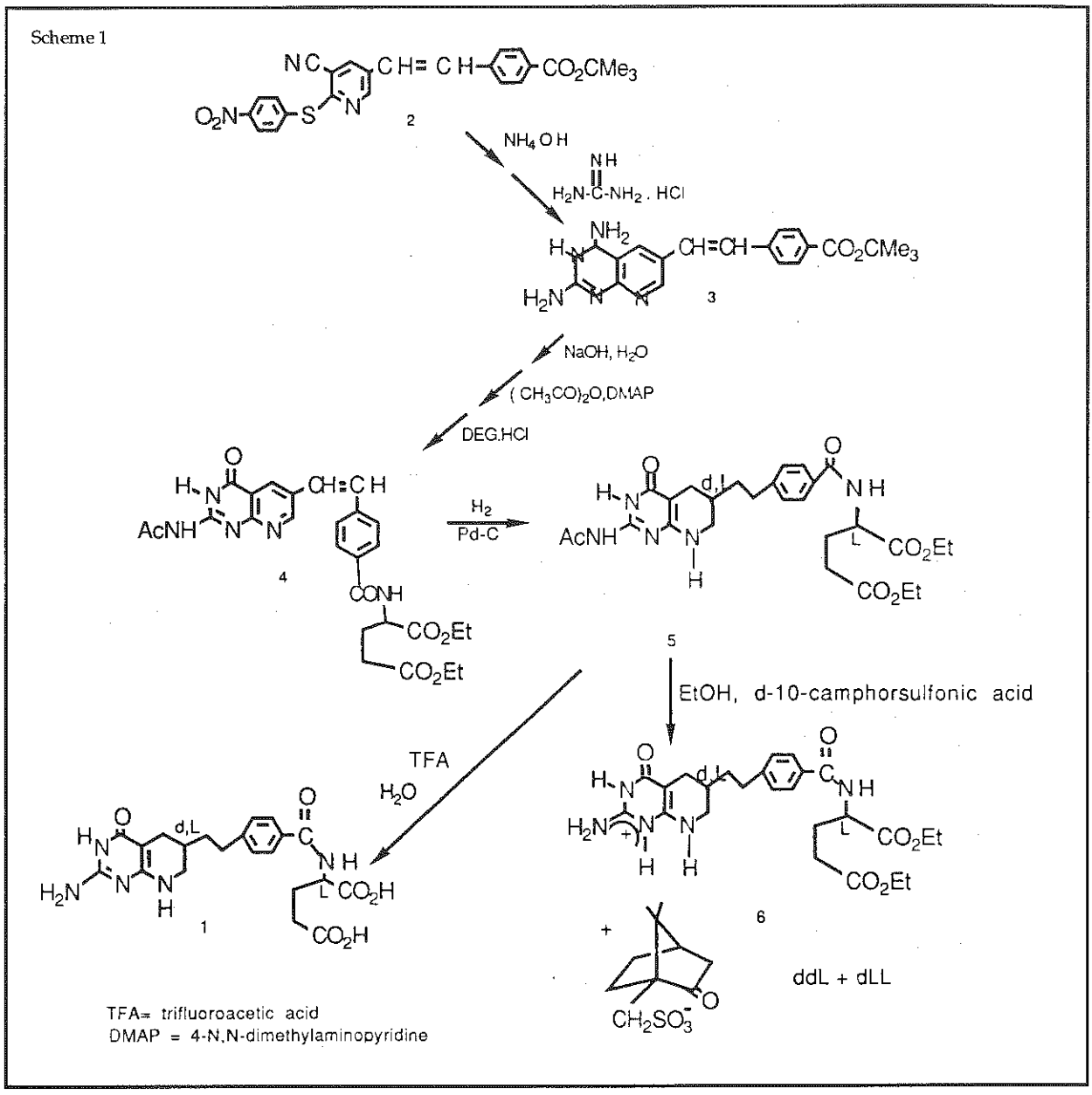


Figure 2

Taylor and co-workers[15] have prepared dideazapterote 11 by an inverse electron demand Diels-Alder reaction[16] between pyrrolidine enamine 8 and 6-azapterin 9, followed by the desulfurization of the resulting compound 10 by Raney nickel. Compound 11 has been used as a key intermediate for the preparation of DDATHF[5-8,14] (Scheme 2).

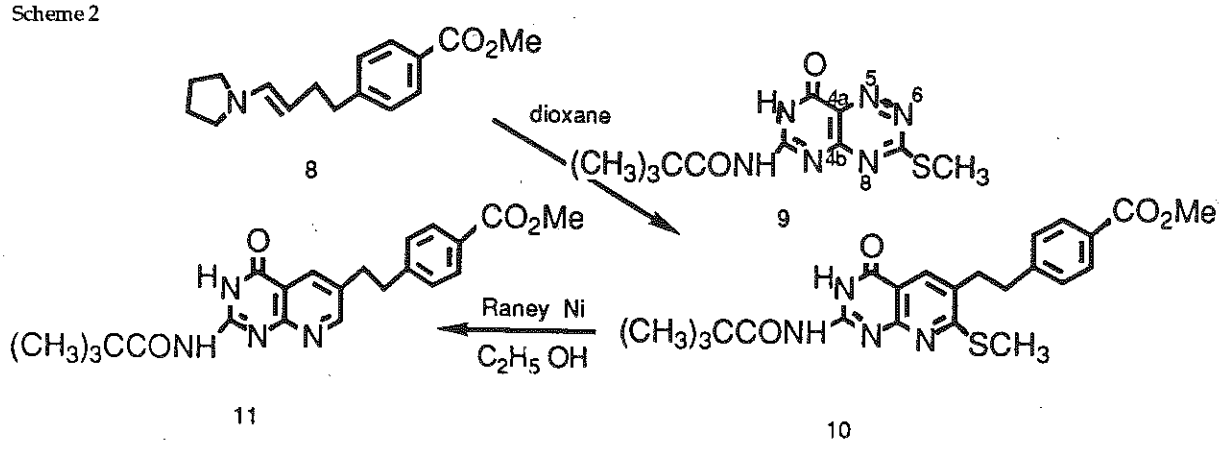
Taylor and Wong have reported[17] efficient Palladium-effected synthesis of DDATHF. A key feature of the synthesis was a double exploitation of a Pd catalyzed carbon-carbon[18-20] bond coupling reaction. Coupling of tert-butyl-4-ethynyl benzoate with methyl 2-chloro, 5-iodo-3-pyridine carboxylate with palladium chloride and cuprous iodide lead to 12. Cyclization with guanidine gave 13 and tert-butyl group was removed to give 14. Pivaloylation of 14 followed by peptide coupling which was achieved in N-methylpyrrolidone as solvent with phenyl-N-phenylphosphoramidochloridate as the coupling agent. DDATHF was prepared by



reduction of 15 with Pd/C, followed by hydrolysis of the 2-pivaloyl and glutamate ester groups (Scheme 3).

Barnett et al.[21] have reported the asymmetric synthesis of DDATHF 1 and related 5,10-dideaza-5,6,7,8-tetrahydropteroic acids. They have utilized enzymatic enantiodifferentiation of prochiral 1,3-diol 17 in the process[22] (Scheme 4). Reaction of 17 with MeOAc in the presence of porcine pancreatic lipase PPL, gave the monoacetate (R)-(+)-18, 85 % ee. Mesylation of (R)-(+)- 18, treatment with NaN_3 and hydrolysis produced azidoalcohol (R)-(+)-21. Azidoalcohol (R)-(+)21 was converted to 22 by tosylation and reaction with sodium diethylmalonate. Reduction of 22 gave 23, reaction with the Meerwein reagent and exposure of the resulting lactim ether to guanidine[23] produced 24. Reaction of 24 with CuCN afforded nitrile 25. Hydrolysis of 25 gave (S)-(+)-5,10-dideaza-5,6,7,8- tetrahydropteroic acid 26. Chlorodimethoxytriazine was efficient and selective reagent for coupling of dideazatetrahydropteroic acid 26 with diethyl L-glutamate[24].

Scheme 2



De Graw et al.[25-27] has reported the synthesis and activity of 10-methyl and 10-ethyl analogs of 5,10-dideazaaminopterin 27 a,b (Figure 3). They have evaluated these compounds as inhibitors of ACIAR formly transferase, another key enzyme in purin biosynthesis, besides GAR formyl transferase[28].

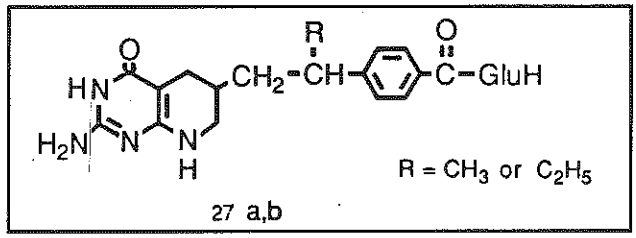
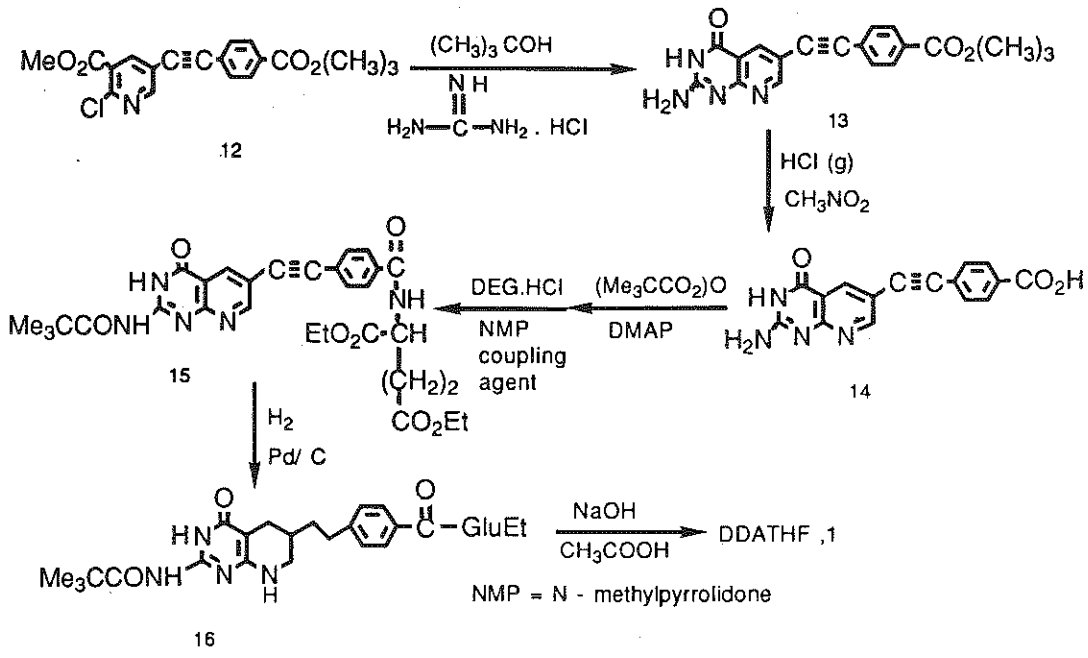


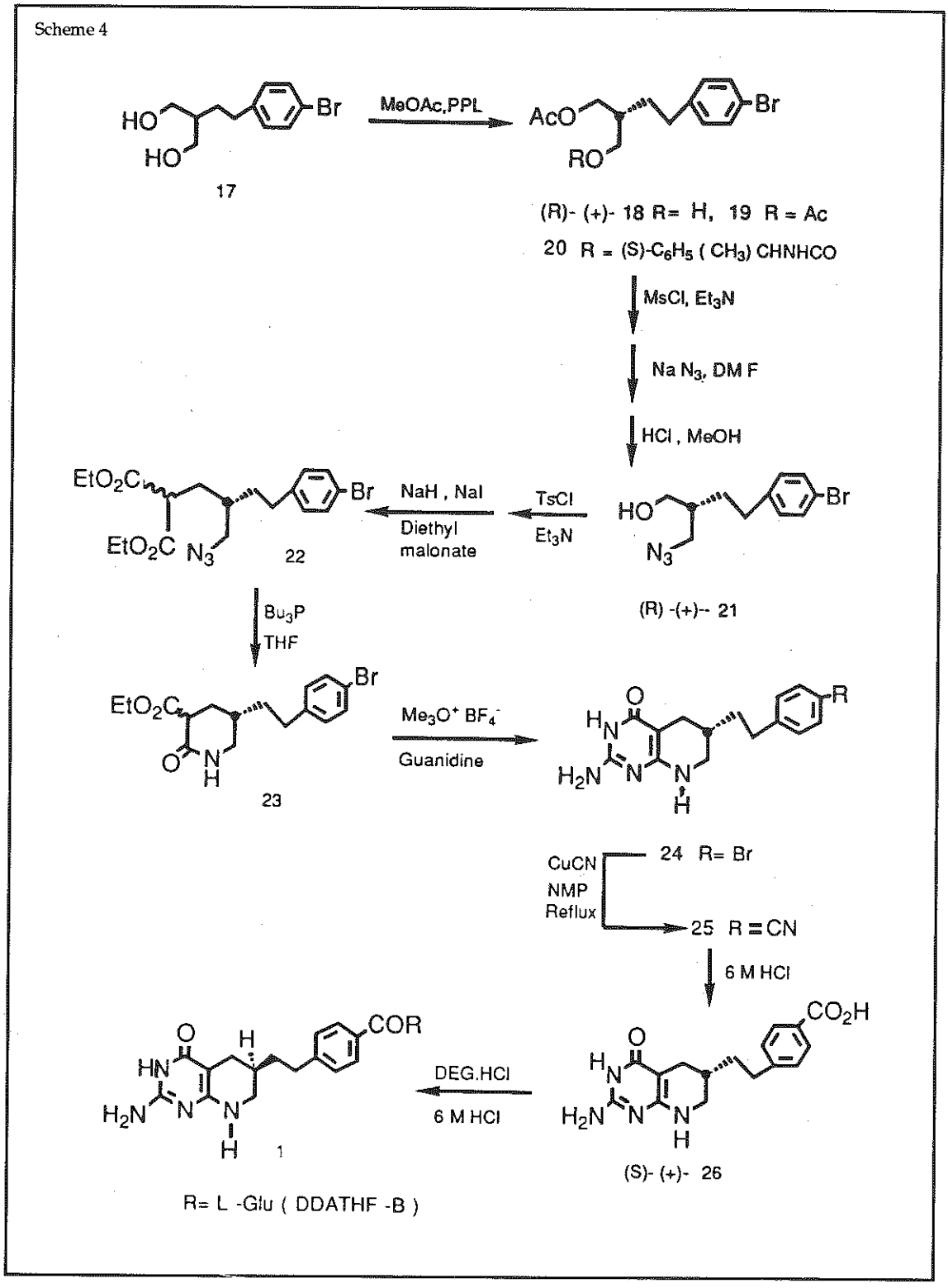
Figure 3

Taylor and Harrington[29] have synthesized 5,10-dideaza-5,6,7,8-tetrahydrofolic acid 1 and its homolog 5,10-dideaza-5,6,7,8-tetrahydrohomofolic acid (HDDATHF) by a new convergent strategy (Scheme 5). A key feature of the synthesis is the

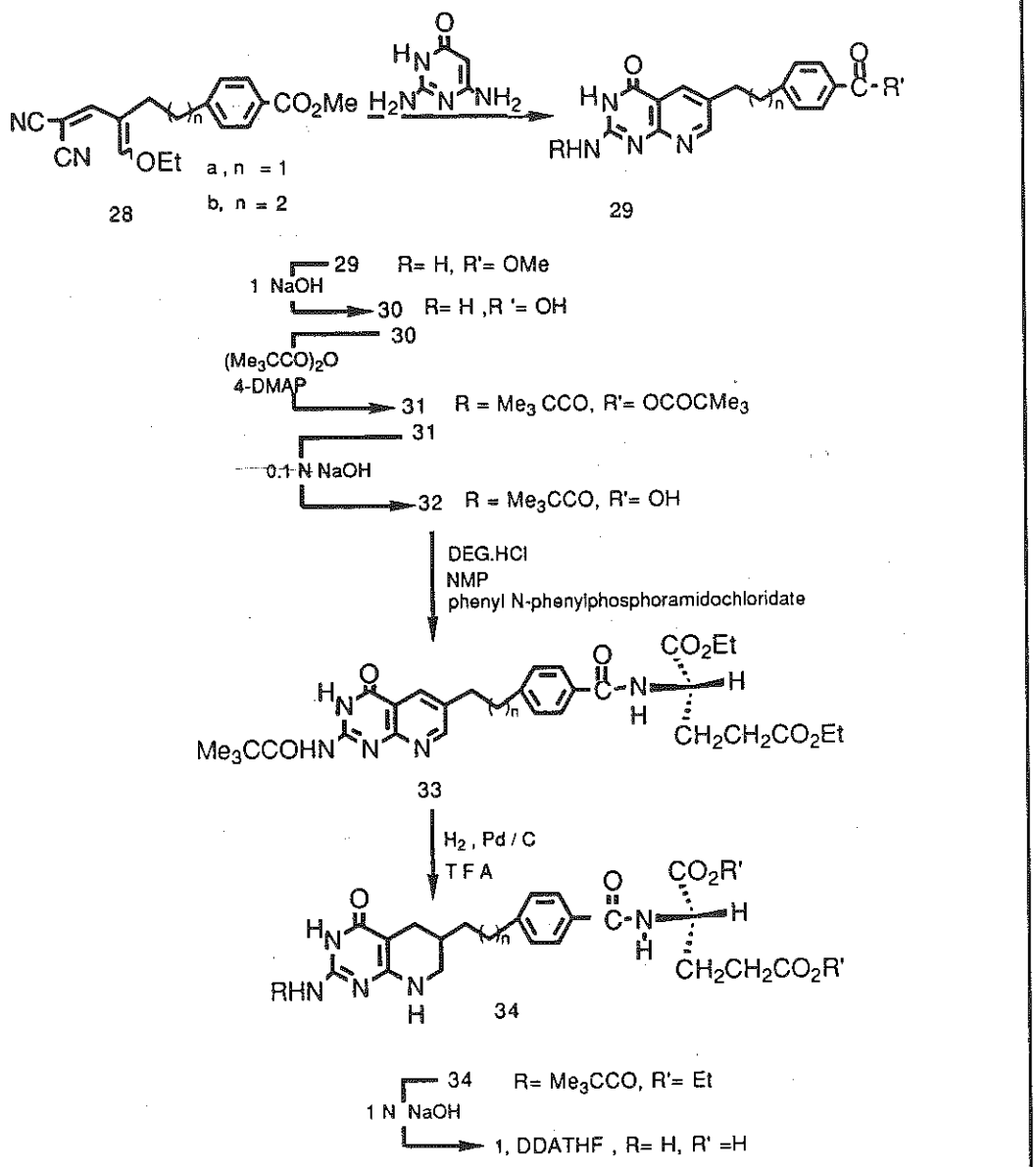
Scheme 3



Scheme 4


MeOAc,PPL
17
(R)- (+)- 18 R= H, 19 R = Ac
20 R = (S)-C6H5 (CH3) CHNHCO
MsCl, Et3N
Na N3, DM F
HCl , MeOH
(R) -(+)-- 21
TsCl
Et3N
NaH , NaI
Diethyl malonate
22
Bu3P
THF
23
Me3O+ BF4-
Guanidine
CuCN
NMP
Reflux
24 R= Br
25 R = CN
6 M HCl
(S)- (+)- 26
DEG.HCl
6 M HCl
1
R= L -Glu (DDATHF -B)

Scheme 5



activation of carbonyl groups by aldol condensation with malononitrile. Condensation of 28 as a malondialdehyde equivalent[30] with 2,4-diamino-6 (1H)-pyrimidone might be excepted to take place more readily than with tle elusive malondialdehyde itself. The Michael condensation of 28 with 2,6-diamino-4 (1H) - pyrimidone led to 29. This step must be facilitated by the greater electrophicity of - C = C $(CN)_2$ group as compared with carbonyl group.

Previous studies with DDAHF indicated that parent or a polyglutamyl derivative was a potent inhibitor of de novo purine nucleotide biosynthesis[6,9]. Since the cytotoxicity of DDATHF in cultured cells appears to dependent on the depletion of intracellular purine nucleotide levels, it was of interest to study the effects of DDATHF on the growth and differentation of HL-60 promyelocytic leukemia cells. Galivan et al. have described that different

concentrations of lipid-soluble DHFR inhibitors and MTX could cause synergistic growth inhibition and cell kill of hepatoma cells in vitro, when coupled with PDDF[31]. Their results have suggested the DHFR inhibitors act by increasing the capacity of PDDF to inhibit TS[32]. Sokoloski et al.[33] has demonstrated that DDATHF was a potent inducer of maturation of HL-60 leukemia cells. The induction of differentiation by DDATHF was associated with the inhibition of de novo purine nucleotide biosynthesis, presumably at the reaction catalyzed by GARFT[6,10,17]. These findings efforts the importance of purine nucleotides to both the growth and differentiation of HL-60 leukemia cells[6-15].

DDAHF has no inhibitor activity against either. DHFR or TS in vitro. But it was found to be an excellent substrate[9] for FPGS and to deplete cellular ATP and GTP at concentrations in the range of 10-30 nM. as inhibitor to leukemic cell growth[34, 35]. The 6S- and 6R-diastereomers of DDATHF are moderately inhibitors of 5'-phosphoribosylglycinamide formyl transferase[36,37]. The two diastereomers were also efficient substrates for mouse liver FPGS [9,38]. Pizzorno et al.[3] have reported that (6R)-DDATHF was rapidly converted polyglutamates in the cultured human leukemia cell lines. Polyglutamylation of (6R)-DDATHF represented a mechanism for trapping the drug inside the cells producing a more potent inhibitor of the target enzyme.

Conclusion

DDATHF showed as chemotherapeutic agent against experimental rodent solid tumors[6,11] and also, during its first use in patients with cancer[40]. Moran et al[41] has compared the activity of a series of DDATHF analogs as inhibitors of GARFT purified to electrophoretic homogeneity from mouse L1210 cells. They have indicated that a reduced pyridopyrimidine ring, N-8 and 2-amino group of DDATHF played an important role in the binding of tetrahydrofolate analogs to GARFT[38] and the glutamic acid in the side chain of DDATHF did not play a role in this ligand-enzyme interaction. Polyglutamates of DDATHF are much more active inhibitors of GARFT than is the parent molecule. Activity of DDATHF analogs has retained in case of replacing phenyl ring by a cyclohexyl ring or by methylene groups.

The abbreviations used ase: DHFR, dihydrofolate reductase; PDDF, N^{10}-propargyl-5,8-dideazafolate N{4-[N-(2-amino-4-hydroxy-6-quinazolinyl) methyl] prop-2-ynl amino} benzoyl-L-glutamic acid; AICAR, aminoimidazolecarboxamideribotide; GARFT, glycinamidoribonucleotide transformylase; TS, thymidylate synthetase; FPGS, folypolyglutamate synthetase; ATP, adenosine triphosphate; GTP, glucose-3-phosphate.

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References

1. Farber, S., Diamond, L. K., Mercer, L. D., Sylvester, R. F., Wolff, J. A., "Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonist, 4-Aminopteroyl-Glutamic Acid (Aminopterin)", *N. Engl. J. Med.*, 238, 787-793, 1948.

2. Palmer, D.C., Skotnicki, J. S., Taylor, E.C., Ellis, G. P., *Progress in Medicinal Chemistry*, Ellis G. P., West, G. B., (eds), Elsevier, Amsterdam, 25, 85-231, 1988.

3. Taylor, E. C., Harrington, R. J., Fletcher, S. R., Beardsley, G. P., Moran R.G., "Synthesis of the Antileukemic Agents 5,10-Dideazaaminopterin and 5,10-Dideaza-5,6,7,8-tetrahydroaminopterin", *J. Med. Chem.*, 28, 914-921, 1985.

4. Moran, R. G., Taylor, E.C., Beardsley, G. P., "5,10-Dideaza-5,6,7,8-Tetrahydrofolic Acid (DDATHF), A Potent Antifolate Inhibitory to de novo Purine Synthesis", *Proc. Amer. Assoc. Cancer Res.*, 26, 231, 1985.

5. Beardsley, G. P., Taylor, E. C., Shih, C., Poore, G. A., Grindey, G. B., Moran, R. G., "A New Class of Antifolates, 5,10-Dideazatetrahydrofolic Acid (DDATHF), an Inhibitor of GAR Transformylase with Broad in vivo Activity", *Proc. Amer. Assoc. Cancer Res.*, 27, 259, 1986.

6. Beardsley, G. P., Taylor, E. C., Grindey, G. B., Moran, R. G., "Deaza Derivatives of Tetrahydrofolic Acid. A New Class of Folate Antimetabolites", Cooper, B. A., Whitehead V. M. (eds), *Chemistry*

and Biology of Pteridines, New York, Walter de Grayter&Co., 953-957, 1986.

7. Moran, R. G., Taylor, E. C., Beardsley, G. P., "The Two Diastereomers of 5,10-Dideazatetrahydrofolate are Equiactive", *Proc. Am. Assoc. Cancer Res.*, 28, 274, 1987.

8. Wong, G. S. K., Fletcher, S. R., Harrington, P. J., Beardsley, G. P., Shih, C. J. Taylor, E. C., "Synthesis of 5,10-Dideaza-5,6,7,8-Tetrahydrofolic Acid (DDATHF) and Analogs", Cooper, B. A., Whitehead V. M. (eds), *Chemistry and Biology of Pteridines*, Berlin, Walter de Gruyter & Co., 61-64,1986.

9. Pizzorno, G., Moroson, B. A., Cashmore, A. R., Taylor, E. C., Beardsley, G. P., "Biochemical Determinants of Sensivity and Resistance to 5,10-Dideaza-5,6,7,8- tetrahydrofolic Acid, DDATHF, in CCRF-CEM Cells", *Proc. Am. Assoc. Cancer Res.*, 29, 281, 1988.

10. Beardsley, G. P., Moroson, B. A., Taylor, E. C., Moran, R. G., "A New Folate Antimetabolite, 5,10-Dideaza-5,6,7,8-tetrahydrofolate is a Potent Inhibitor of de novo Purine Synthesis", *J. Biol. Chem.*, 264, 328-333, 1989.

11. Taylor, E. C., Hamby, J. M., Shih, C., Grindey, G. B., Rinzel, S. M., Beardsley, G. P., Moran R.G., "Synthesis and Antitumor Activity of 5-Deaza-5,6,7,8-tetrahydrofolic Acid (5-DATHF) and N^{10} - Substituted Analogs", *J. Med. Chem.*, 32, 1517-1522, 1989.

12. Taylor, E. C., Palmer, D.C., George, T. J., Fletcher, S. R., Tseng, C. P., Harrington, P. J., "Synthesis and Biological Activity of L-5-Deazaaminopterin: Synthetic Strategies to 5-Deazapteridines", *J. Org. Chem.*, 48, 4852-4860, 1983.

13. Piper, J. R., McCaleb, G. S., Montgomery, J. A., Kisliuk, R. L., Gaumont, Y., Thorndike, J., Sirotnak, F. M., "Synthesis and Antifolate Activity of 5-Methyl-5,10-Dideaza Analogs of Aminopterin and Folic Acid and an Alternative Synthesis of 5,10-Dideazatetrahydrofolic Acid, a Potent Inhibitor of Glycinamide Ribonucleotide Formyltransferase", *J. Med. Chem.*, 31, 2164-2169, 1988.

14. Struck, R. F., Shealy, Y. F., Montgomery, J. A., "Potential Folic Acid Antagonists. 5. Synthesis and Biologic Evaluation of N^{10} - Deazafolic Acid and Their 9,10-Dehydro Derivatives", *J. Med. Chem.*, 14, 693-698, 1971.

15. Taylor, E. C., Harrington, P. M., Warner, J. C., "Diels-Alder Reactions of 6-Azapterins. An Alternative Strategy for the Synthesis of 5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF)", *Heterocycles*, 27, 1925-1928, 1988.

16. Taylor, E. C., McDaniel, K. F., Warner, J. C., "Diels-Alder Reactions of Bicyclic 1,2,4-Triazines. The Conversion of Pyrimido [4,5-e]-1,24-triazines to Pyrido [2,3-d] pyrimidines", *Tet. Lett.*, 28, 1977-1980, 1987.

17. Taylor, E. C., Wong, G. S. K., "Convergent and Efficient Palladium-effected Synthesis of 5,10-Dideaza-,5,6,7,8-tetrahydrofolic Acid", *J. Org. Chem.*, 54, 3618-3624, 1989.

18. For representative example of palladium-catalyzed C-C coupling reactions of halo-substituted pyridines and condensed pyridines: Sakamoto, T., Shiraiwa, M., Kondo, Y., Yamanaka, H., "A Facile Synthesis of Ethynyl-Substituted Six-Membered N-Heteroaromatic Compounds", *Synthesis*, 312-314, 1983.

19. Sakamoto, T., Kondo, Y., Watanabe, R., Yamanaka, H., "Condensed Heteroaromatic Ring Systems. VII. Synthesis of Thienopyridines, Thienopyrimidines and Furopyridines from o-Substituted N-Heteroarylacetylenes", *Chem. Pharm. Bull.*, 34, 2719-2724, 1986.

20. Taylor, E. C., Yoon, C. M., "A Convenient Synthesis of 6-Formyl-5-Deazapterin", *Syn. Commun.*, 18, 1187-1191, 1988.

21. Barnett, C. J., Wilson, T. M. "Asymmetric Synthesis and Absolute Configuration of 5,10-Dideaza-5,6,7,8-tetrahydropteroic Acid and 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF)", *Tet. Lett.*, 30, 6291-6294, 1989.

22. Ramos Tombo, G. M., Schär, H. P., Fernandez I Busquets X., Ghisalba, O., "Synthesis of Both Enantiomeric Forms of 2-Substituted 1,3-Propanediol Monoacetates Starting from a Common Prochiral Precursor, Using Enyzmatic Transformations in Aqueous and in Organic Media", *Tet. Lett.*, 27, 5707-5710, 1986.

23. Pyatin, B. M., Glushkov, R. G., "Investigation of Lactams. XII. Lactim Ethers of 3-Ethyl-3-Carboethoxy and 3-Carboethoxy-2-piperidones in the Synthesis of Piperido [2,3-d] pyrimidines", *Khim. Farm. Zh.*, 2, 17-20, 1968.

24. Kaminski, Z. J., "2-Chloro-4,6-disubstituted- 1,3,5-triazines. A Novel Group of Condensing Reagents", *Tet. Lett.*, 26, 2901-2904, 1985.

25. De Graw J. I., Christie P. H., Kisliuk, R. L., Gaumont, Y., Sirotnak, F. M., "Synthesis and Antifolate Properties of 10-Alkyl-5,10-Dideaza Analogs of Methotrexate and Tetrahydrofolic Acid", *J. Med. Chem.*, 33, 673-677, 1990.

26. Rieser, J., Friedrich, K., "Reaktionen von Sulfoniumdicyanmethyliden", *Liebigs. Ann. Chem.*, 648-655, 1976.

27. Josey, A. D., Dickinson, C. L, Dewhirst, K. C., McKusick, B. C., "Trifluoromethyl malononitrile. The Reaction of 1,1-Dichloro-2,2-dicyanoethylene with Argentous Fluoride", *J. Org. Chem.*, 32, 1941-1944, 1967.

28. De Graw, J. I., Tagawa, H., Christie, P. H., Lawson, J. A., Brown, E. G., Kisliuk, R. L., Gaumont, Y., "Synthesis of 5,10-Didezaaminopterin", *J. Heterocycl. Chem.*, 23, 1-4, 1986.

29. Taylor, E. C., Harrington, P. M., "A Convergent Synthesis of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid and 5,10-Dideaza-5,6,7,8-tetrahydrohomofolic Acid. An Effective Principle for Carbonyl Group Activation", *J. Org. Chem.*, 55, 3222-3227, 1990.

30. The Condensation of 6-Aminopyrimidines with 1,3-Dicarbonyl Compounds to Give [2,3-d] pyrimidines is well known: Bernetti, R., Mancini, F., Price C.C., "Pyrido [2,3-d] pyrimidines from Malonaldehydes", *J. Org. Chem.*, 27, 2863-2865, 1962.

31. Galivan, J., Nimec, A., Rhee, M., "Synergistic Growth Inhibition of Hepatoma Cells Exposed in vitro to N^{10}-propargyl-5,8-dideazafolate with Methotrexate or The Lipophilic Antifolates Trimetrexate or Metoprine", *Cancer Res.*, 47, 5256-5260, 1987.

32. Galivan, J., Nimec, Z., Rhee, M., Boschelli, D., Oronsky, A. L., Kerwar, S. S. "Antifolate Drug Interactions: Enhancement of Growth Inhibition Due to The Antipurine 5,10-Dideazatetrahydrofolic Acid by The Lipopophilic Dihydrofolate Reductase Inhibitors Metoprine and Trimetrexate1", *Cancer Res.*, 48, 2421-2425, 1988.

33. Sokoloshi, J. A., Beardsly, G. P., Sartorelli, A. C., "Induction of HL-60 Leukemia Cell Differentiation by the Novel Antifolate 5,10-Dideazatetrahydrofolic Acid", *Cancer Res.*, 49, 4824-4828, 1989.

34. Beardsley, G. P., Rosowsky, A., McCaffrey, R. P., Abelson, H. T., "Methotrexate Di-n-Butyl Ester Effects on Thymidine Metabolism", *Biochem. Pharmacol.*, 28, 3069-3974, 1979.

35. Olempska-Beer, Z., Freese, E. B., "Optimal Extraction Conditions for High-Performance Liquid Chromatographic Determination of Nucleotides in Yeast", *Anal. Biochem.*, 140, 236-245, 1984.

36. Rode, W., Scanlon, K. J., Hynes, J., Bertino, J. R. "Purification of Mammalian Tumor (L1210) Thymidylate Synthetase by Affinity Chromotography on Stale Biospesific Adsorbent", *J. Biol. Chem.*, 254, 11538-11543, 1979.

37. Daubne, S. C., Benkovic, S. J., "Characterization of Mamalian Phosphoribosylglycineamide Formyltransferase from Transformed Cells[1]", *Cancer Res.*, 45, 4990-4997, 1985.

38. Moran, G. M., Baldwin, S. W., Taylor, E. C., Shih, C., "The 6S-and 6R-Diastereomers of 5,10-Dideaza-5,6,7,8-tetrahydrofolate are Equiative Inhibitors of de novo Purine Synthesis", *J. Biol. Chem.*, 264, 21047-21049, 1989.

39. Pizzorno, G., Sokoloski, J. A., Cashmore, A. R., Moroson, B. A., Cross, A. D., Beardsley, G.P., "Intracellular Metabolism of 5,10-Dideazatetrahydrofolic Acid in Human Leukemia Cell Lines", *Mol. Pharmacol.*, 39, 85-89, 1990.

40. Muggia, F., Martin. T., Ray, M., Leichman, C. G., Grunberg, S., Gill, I., Moran, R. G., Dyke, R., Grindey, G., "Phase I Study of Weekly 5,10-Dideazatetrahydrofolate (LY 264618, DDATHF-B)", *Proc. Am. Assoc. Clin. Pharm.*, 9, 74, 1990.

41. Baldwin, S. W., Tse, A. Gossett, L. S., Taylor, E. C., Rosowsky, A., Shih, C., Moran, R. G., "Structural Features of 5,10-Dideaza-5,6,7,8-tetrahydrofolate that Determine Inhibiton of Mamalian Glycinamide Ribonucleotide Formly Transferase", *Biochemistry*, 30, 1997-2006, 1991.