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NOVEL OPEN-RING ANALOGUES OF N^5,N^{10}-METHYLENETETRAHYDROFOLIC ACID WITH SELECTIVE ACTIVITY AGAINST BRAIN TUMOR

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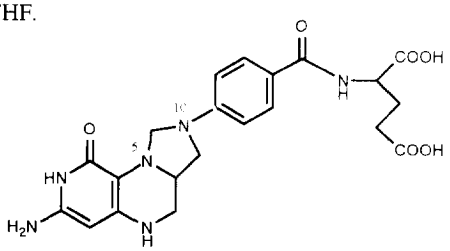
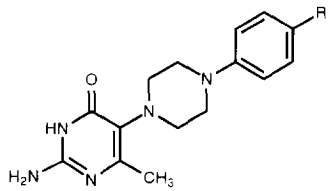
Abstract. A series of 5-(4-substituted-1-piperazinyl)pyrimidines were synthesized as open-ring analogues of N^5,N^{10}-methylene-tetrahydrofolic acid. Compounds **6**, **7**, **8**, **9** and **11** showed selective cytotoxicity on SNB75 human CNS cancer cell line with growth inhibition (GI_{50}) ranged from 1.39×10^{-7} M to less than 10^{-8} M.

Recent studies on folate antimetabolites have focused on inhibiting enzymes along purine and pyrimidine *de novo* biosyntheses.[1-5] Compounds, such as 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF) known as lometrexol currently in clinical trial, are basically designed as antimetabolites of N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolic acid (MTHF), a cofactor for thymidylate synthetase and a precursor of the cofactor for glycinamide ribonucleotide transformylase (GAR-Tfase).[6] Interest in this new target in designing antitumor agents prompted us to prepare a series of MTHF analogues. Since the problem of drug resistance frequently emerged as unfavorable outcome of antifolates, it is important to take into consideration on how to prevent this problem. The acquired drug resistance for antifolates, mostly revealed with studies on methotrexate (MTX), is due to either an alteration of the target enzymes[7] or to an impaired process of cellular uptake.[8,9] We previously reported a series of 2, 4-diaminopyrimidines as open ring analogues of MTX.[10] The pyrimidinyl moiety on these molecules in replacing the pteridine ring of MTX was thought to eliminate conformational rigidity and, in consequence, to enhance the accessibility of the compounds into the active site of the target enzyme. In contrast to the carrier-mediated cellular uptake of MTX, these analogues are transported into the cell by passive diffusion. Some of the compounds with high lipophilicity are active against WI-L2/MTX^r resistant strain of cancer cells.[11] With the same consideration of maintaining molecular flexibility and lipophilicity for cellular uptake, we report here the preparation and *in vitro* antitumor activities on a series of 2-amino-6-methyl-5-(4-substituted-1-piperazinyl)pyrimidin-4(3H)-ones as MTHF antimetabolites. The significance of the structural features on these compounds is basically a piperazine substituted at the C-5 of 2-aminopyrimidin-4(3H)-ones as a bridge for the pyrimidine and the phenyl ring so as to maintain a similar yet more flexible conformation to that of MTHF.

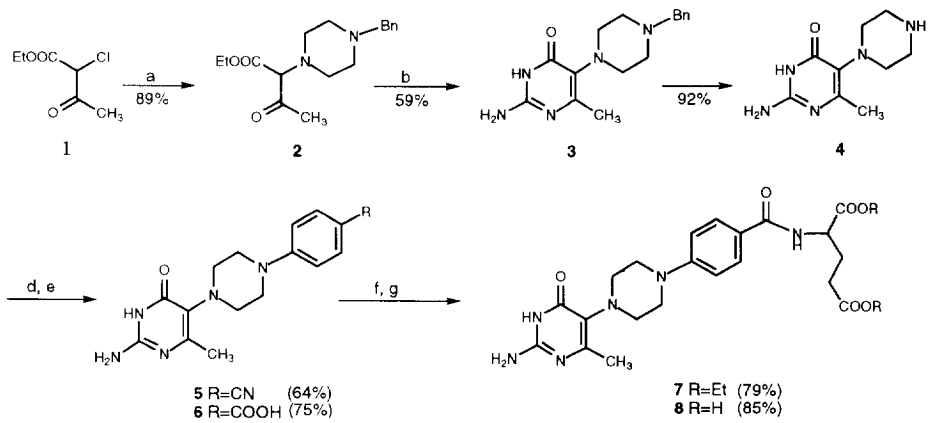


MTHF



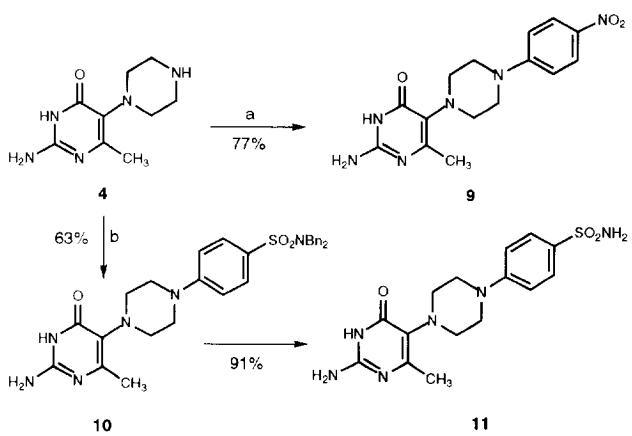
Target compounds 5-11

Preparation of compounds is outlined in Schemes **1** and **2**.[12] Condensation of N-benzylpiperazine with ethyl 3-oxo-2-chlorobutyrate (**1**) gave intermediate **2**, which was allowed to condense with guanidine to afford intermediate **3**. Debenzylation of **3** by hydrogenolysis under hydrogen in the presence of palladium-on-charcoal gave compound **4**. Coupling of **4** with 4-fluorobenzonitrile followed by hydrochloric acid treatment gave compound **6**. Coupling of **6** and L-diethyl glutamate with 1-ethyl-3- (3-dimethylaminopropyl)carbodiimide (EDCI) gave compound **7**, which upon saponification gave the final product **8**.



(a) N-benzylpiperazine/K_2CO_3/AcCN/50°C/24h (b) Na/guanidine HCl/$MeOCH_2CH_2OH$/Δ/13h (c) Pd/C/H_2/AcOH/15psi/34h (d) 4-fluorobenzonitrile/K_2CO_3/DMF/Δ/7h (e) HCl/Δ/12h (f) diethyl glutamate HCl/EDCI/HOBt/NMM/DMF/50°C/28h (g) i. $NaOH_{(aq)}$ ii. HCl.

Scheme 1



(a) 4-fluoronitrobenzene/K_2CO_3/EtOH/Δ/11h (b) N,N-dibenzyl-4-fluorobenzene sulfonamide/K_2CO_3/DMSO/Δ/9h (c) H_2SO_4/rt/7.5h

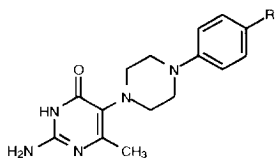
Scheme 2

Analogues without glutamic acid side chain were also synthesized. Thus, compound **9** was prepared by coupling intermediate **4** with 4-fluoronitrobenzene. Condensation of intermediate **4** with N,N-dibenzyl 4-fluorobenzenesulfonamide followed by debenzylation[13] gave compound **11** (Scheme **2**).

Cytotoxic activity was conducted on 60 cancer cell lines in the disease-oriented antitumor screening program by the National Cancer Institute of the U. S. A..[14-15] Interestingly, brain tumor specificity was noticed and only SNB 75 cancer cell line was subjected to be inhibited by most of these compounds (Table **1**). The GI_{50}'s, the concentration corresponding to 50% of growth inhibition, for compounds **7**, **8** and **9** on SNB 75 cancer cell line are less than 0.01 μM. Whether the selectivity came from a preferential transport of these compounds due to their lipophilicity and consequent higher intracellular bioavailability in the sensitive cell line[16] needs further investigation. Besides inhibiting brain tumor, compound **10** showed wide spectrum of marginal cytotoxic activity on cell lines among non-small cell lung cancer, colon, ovarian, renal, prostate and breast cancers.

In summary, based on the rationale of increasing conformational flexibility and improving cellular uptake of MTHF antimetabolites, a series of 2-amino-6-methyl-5-(4-substituted-1-piperazinyl)pyrimidin-4(3H)-ones were synthesized. These analogues exhibited selective cytotoxicity on brain tumors.

Table **1**. Cytotoxic activity of compounds **5** -**11** on human cancer cell lines.



compound	R	Cytotoxic activity (GI_{50}, μM)[a]						
		lung	colon	CNS	ovarian	renal	prostate	breast
5	-CN	--[b]	--	0.04[c]	93	--	--	--
6	-COOH	--	--	--	--	1.39	--	--
7	-CONH-CH(COOEt)-$(CH_2)_2$COOEt	--	--	< 0.01[c]	--	--	--	--
8	-CONH-CH(COOH)-$(CH_2)_2$COOH	--	--	< 0.01[c]	--	--	--	--
9	-NO2	--	--	< 0.01[c]	--	--	--	--
10	$SO_2N(CH_2Ph)_2$	4.8-79	55	3.68[d]	0.7-16	3.1-27	1.9-3.5	2.1-37
11	SO_2NH_2	--	--	0.10[c]	--	--	--	--

[a]The value represents the range of GI_{50}'s for the cell lines of each respective cancer unless specified. [b]-- denotes $GI_{50} > 10^{-4}$ M. [c]CNS SNB-75 cell line. [d]CNS U251 cell line.

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