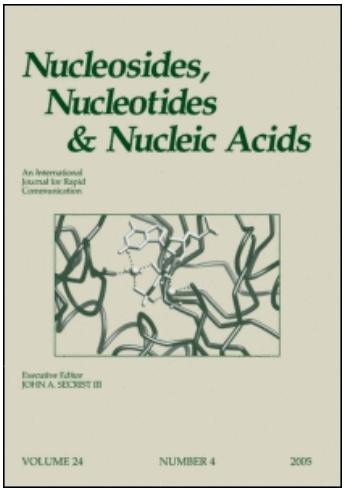

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Thiated Pyrimidine Deoxynucleoside Analogues, Potential Chemotherapeutic Agents, and Substrates/Inhibitors in Various Enzyme Systems

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THIATED PYRIMIDINE DEOXYNUCLEOSIDE ANALOGUES, POTENTIAL CHEMOTHERAPEUTIC AGENTS, AND SUBSTRATES/INHIBITORS IN VARIOUS ENZYME SYSTEMS.

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ABSTRACT:The synthesis of thiated nucleoside and nucleotide analogues, and determination of their structures, conformations, potential chemotherapeutic activities, and substrate/inhibitor properties in various enzyme systems, with emphasis on enzymes related to chemotherapeutic activities, are reported.

Thiopyrimidine nucleosides and nucleotides are of considerable biological importance e.g. they are components of the tRNA of various organisms, play a significant role in translation and its control, and 4-thioUrd inhibits the growth of EAC and L1210 cells. This prompted us to synthesize and investigate other new thiated pyrimidine nucleoside and nucleotide analogues as potential antitumor, antiviral and antiparasitic agents.

In the series of thionated inhibitors of thymidylate synthase (TS), potential antitumor agents, regioselective syntheses based mainly on Lewis acid-catalysed nucleoside condensations or direct transformations of pyrimidine, were elaborated for 2- and 4-thio-, and 2,4-dithio derivatives of dUrd, f^{5}dUrd, and other 5-substituted pyrimidine nucleosides and nucleotides. The 5-fluoro-, 5-bromo-, and 5-trifluoromethyl- 4-thio congeners were obtained with use of a "one-pot procedure" involving regioselective thiation employing the Lawesson reagent in different solvents[1]. 2-Thio derivatives of dUrd, f^{5}dUrd and their α-anomers were synthesized *via* $TiCl_4$-catalysed condensation of silylated bases with O-acylated 1-chloro-dRib[2]. 2,4-Dithio derivatives of dUrd and f^{5}dUrd were obtained *via* direct thiation of O-acylated 2-thio congeners[3]. Similarly, regioselective syntheses were elaborated for s^{2}dCyd and f^{5}s^{2}dCyd

TABLE 1. Solution conformations of thiated 2'- and 2',3'-dideoxyuridines.

Compound	Conformer Population in D_2O								
	S	g^+	t	g^-	Ng^+	Nt	Sg^+	St	Sg^-
f^5s^4dUrd	0.70	0.74	0.19	0.07	0.25	0.06	0.49	0.13	0.07
f^5s^2dUrd	0.59	0.56	0.32	0.12	0.28	0.13	0.29	0.19	0.12
$f^5s^2s^4$dUrd	0.51	0.55	0.35	0.10	0.32	0.17	0.23	0.18	0.10
s^2f_3ddUrd	1.00	0.75[a]							
$m^5s^2f_3$ddUrd	1.00	0.81[a]							
$f^5s^4f_3$ddUrd	~1.00	0.77	0.17	0.07	-0.06	-0.01	0.82	0.18	0.07
$m^5s^4f_3$ddUrd	~1.00	0.49[a]							
$cl^5s^4f_3$ddUrd	~1.00	0.78[a]							

[a] for equivalent H-5', H-5" only the g^+ population can be estimated.

derivatives and 2',3'-dideoxy-2-thiopyrimidine nucleosides. The nucleosides were selectively converted to the corresponding 5'-monophosphates with the aid of the wheat shoot phosphotransferase system or by a modified Yoshikawa procedure.

Solution conformations of thiated nucleosides were deduced from high resolution (500 MHz) ^{1}H NMR spectra. S and g^- conformations of 2-thio- and 2,4-dithio-2'-deoxynucleosides differ only slightly while 4-thio nucleosides exhibit somewhat larger differences. 3'-Fluoro derivatives of thiated pyrimidine 2',3'-dideoxynucleosides exhibit only the S-type conformation.

Biological results. Thymidylate synthase (EC 2.1.1.45) is a target enzyme in anticancer, antiviral, antifungal and antiprotozoan chemotherapy. Whereas β-s^2dUMP (but not the α -anomer) was a good substrate of the enzyme, and both β -f^5s^2dUMP and β -f^5s^4dUMP proved to be potent competitive, slow-binding inhibitors, *vs* dUMP, not much weaker than β-f^5dUMP, the β-s^2s^4 analogues of dUMP and f^5dUMP were weak competitive inhibitors, with the latter showing slow-binding behaviour (TABLE 2). Similarly, β-$f^5s^2s^4$dUrd was a much weaker inhibitor of tumour cell growth than its f^5, f^5s^2 or f^5s^4 congeners, while with β-s^2dUrd or β-s^2s^4dUrd no influence on cell growth could be observed (TABLE 3). Comparative studies with thymidylate synthases isolated from various sources showed that substitution of O-4 (but not O-2) by S-4 in f^5dUMP may alter the specificity for enzyme forms differing in sensitivity to slow-binding inhibition by f^5dUMP. A similar, albeit less pronounced, effect was observed

TABLE 2. Parameters for interaction of thymidylate synthases from different sources with dUMP, f^5dUMPand its analogues [2-4]

Enzyme source	K_m (μM) for compounds with substrate activity				K_i (μM) for compounds with classical inhibition			K_i* (μM) for compounds behaving as slow-binding inhibitors							
	dUMP	s^2-dUMP[a]	oh^5-dUMP	oh^5s^4-dUMP	s^4cl^5-dUMP	s^4br^5-dUMP	s^2s^4-dUMP[b]	f^5dUMP[3]	f^5s^2-dUMP[c]	f^5s^4-dUMP[c]	$f^5s^2s^4$-dUMP[b]	f^5s^2-dCMP[d]	hm^5-dUMP	hm^5s^2-dUMP	hm^5s^4-dUMP[5]
Ehrlich ascites carcinoma	1.3	20[e]	0.60		12	9.3		0.006	0.079[f]	0.06					
Mouse leukemia L1210	2.6	20[e]	3.2	6.6			32	0.002	0.041	0.10	68	34	85	58	85
Mouse leukemia L1210 R	2.5		6.8	5.0			55	0.012	0.30	0.014	141	16			
Rat colon tumour K-12	3.2							0.12		0.40					
Regenerating rat liver	3.4							0.010	0.064	0.85					
Colon tumour HCT-8	2.8							0.13		0.14					
Leukemia CCRF-CEM	2.3							0.004		0.18					
Hymenolepis diminuta	5.4							0.11	0.63	0.91					

[a]Ref. [2]; [b]Ref. [3]; [c]Ref. [4]; s^4dUMP was a substrate with the *L. casei* enzyme (K_m = 70 μM; for dUMP K_m = 5 μM)[5]; [d]f^5dCMP was slow-binding inhibitor of the *L. casei* enzyme (K_i^* = 27 μM)[6]; [e]Apparent Km under conditions where apparent Km for dUMP was 10 μM; [f]α-anomer showed K_i^* = 27 μM.

TABLE 3. Inhibition by 2'-deoxynucleosides of cell growth and [^{14}C]Leu or [^{3}H]Thd incorporation

Growth assay	IC_{50} (μM) for						
	s^2dUrd	s^2s^4-dUrd	f^5dUrd	f^5s^2-dUrd	f^5s^4-dUrd	$f^5s^2s^4$-dUrd	f^5s^2-dCyd
Cell count	>10	>100	0.0020	0.26	0.047	30	6.0
[^{14}C]Leu incorporation	>10	-	0.0024	0.30	0.045	44	6.7
[^{3}H]Thd incorporation	>10	-	0.0020	0.28	0.038	10	-

with f^5s^2dCMP, a moderately potent competitive, slow-binding, inhibitor of the enzyme (TABLE 2), its nucleoside being a relatively good inhibitor of cell growth (TABLE 3). Interestingly, both cl^5s^4dUMP and br^5s^4dUMP were merely classical competitive inhibitors, without symptoms of slow-binding, pointing to each of the two being recognized by thymidylate synthase as an analogue of the enzyme reaction product rather than of the substrate (TABLE 3). oh^5s^2dUMP, hm^5s^2dUMP and hm^5s^4dUMP, as well as the parent oh^5 and hm^5 congeners, were competitive (*vs* dUMP) inhibitors of the enzyme. Surprisingly, while hm^5-substituted analogues showed moderate slow-binding inhibition, reflected by time- and $N^{5,10}$-methylenetetrahydrofolate-dependent inactivation, neither oh^5-substituted analogue did. On the contrary, spectrophotometric monitoring at 340 nm of an incubation mixture with 0.04 mM oh^5dUMP, 0.25 mM $N^{5,10}$-methylenetetrahydrofolate and thymidylate synthase, demonstrated a slow, time-dependent increase in extinction, pointing to dihydrofolate production and suggesting substrate activity of the analogue, characterized by a maximal velocity at least an order of magnitude lower than that observed with dUMP.

Substrate/inhibitor properties of s^2dCyd and f^5s^2dCyd with respect to human leukemic spleen deoxycytidine kinase have been examined. Both are substrates, and as expected, good competitive inhibitors of phosphorylation of dCyd and dAdo.

Biological properties of thiated pyrimidine 2',3'-dideoxy-3'-fluoronucleosides were investigated. 2-Thio-3'-fluoronucleosides were moderate substrates for thymidine phosphorylase and were quite inactive *vs* uridine phosphorylase, while 4-thio congeners were inactive *vs* both enzymes. The dideoxynucleosides were evaluated against a syncytia inducing HIV-1 strain (cat#3) isolated in our laboratory. One of them (s^2f_3ddThd) showed promising

anti-HIV activity in CEM cells (EC_{50}<0.1 μM) comparable to the activity of the known antiretroviral agent AZT.

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