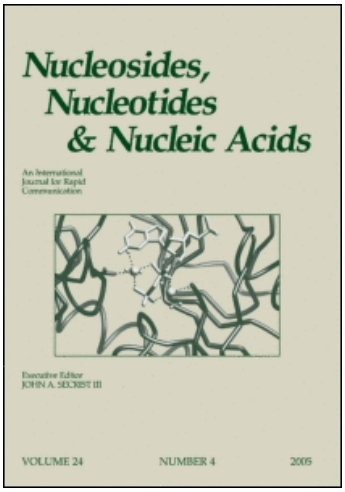

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5-PROPYNYLPYRIMIDINE NUCLEOSIDE DERIVATIVES: RATIONALLY DESIGNED MECHANISM-BASED INACTIVATORS OF THYMIDYLATE SYNTHASE

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ABSTRACT

A novel series of 5-propynyl-dUMP derivatives, with a variety of leaving groups on the side-chain, was designed as potential mechanism-based inhibitors of thymidylate synthase (TS), and synthesized from 5-iodo-2′-deoxyuridine by Pd(0)-catalyzed coupling, followed by direct phosphorylation with $POCl_3$. All members of the series inhibited TS competitively with K_i-values of 0.015–18 μM. Analogs with fluorine or imidazole-based leaving groups caused rapid, irreversible inactivation of TS.

INTRODUCTION

Thymidylate synthase (TS) is a target of important anticancer agents, such as the widely used 5-fluorouracil (FU) and its derivatives, and raltitrexed, a representative of clinically effective antifolate inhibitors of this enzyme. Intrinsic and acquired drug resistance is a major limitation of the chemotherapy of cancer using 5-fluoropyrimidines, reducing the response rates to 20–40%. The mechanisms of resistance to FU are often related to factors preventing prolonged inhibition of TS activity, such as the reversibility of inactive ternary complex formation, or inadequate levels of polyglutamylated folate cofactors necessary for stable complex formation. This prompted the search for novel inhibitors that do not require folate cofactors for inhibitory activity, and are able to inactivate the enzyme irreversibly to achieve prolonged inhibition of TS activity.

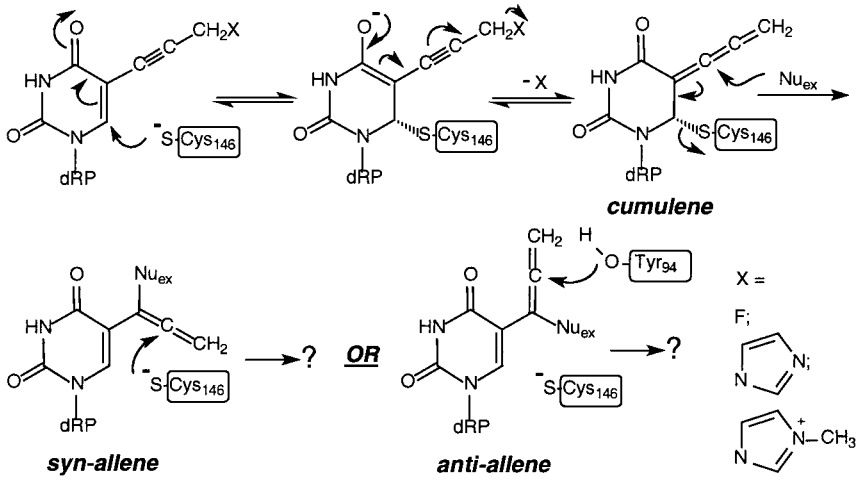

Figure 1. Hypothetical covalent interactions of 5-propynyl-dUMP derivatives with TS. Nu_{ex}: external nucleophile; X: leaving group.

RESULTS AND DISCUSSION

The rationale for the design of the target compounds was based on the hypothesis that analogs of the substrate, dUMP, with a propynyl side-chain carrying a suitable leaving group, would undergo enzyme-mediated, conjugate elimination, generating a covalently-linked, reactive cumulene derivative (Fig. 1), which may form additional covalent bonds with adjacent amino acid side-chains at the active site of the enzyme (1,2). Such covalent interactions were predicted to be irreversible and their formation not to require the presence of the cofator, 5,10-methylenetetrahydrofolate (CH_2H_4 folate) (2).

Table 1. K_i-values of 5-substituted dUMP Analogs

Abbreviation	5-Substituent	K_i (μM)[a]
EdUMP	C≡CH	0.10[b]
PdUMP	C≡CCH$_3$	1.0
NH$_2$PdUMP	C≡CCH$_2$NH$_2$	18
ImdPdUMP (Imd)	C≡CCH$_2$-N(imidazole)	0.54
Imd$^+$PdUMP (Imd$^+$)	C≡CCH$_2$-N(imidazolium)-CH$_3$	0.50
FPdUMP	C≡CCH$_2$F	0.015

[a]A value of 6.8 ± 2.7 μM was obtained for the K_m of the substrate, dUMP; K_i-values were calculated using K_m values determined in the same experiment.
[b]In excellent agreement with published values (3).

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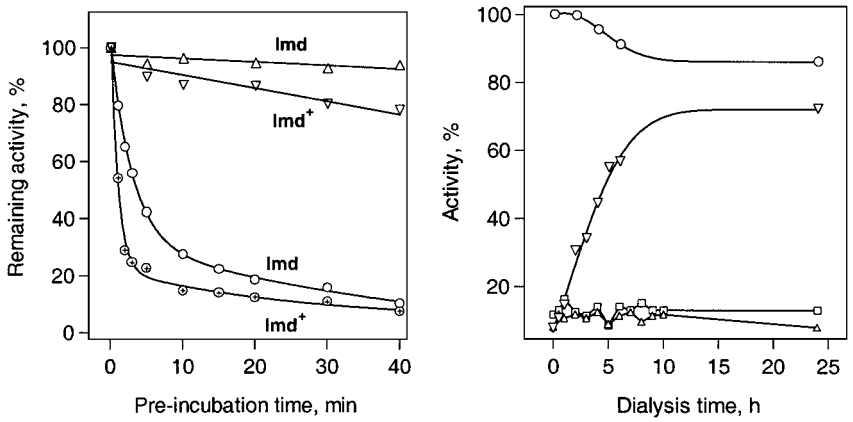

Figure 2. *Left:* Time-dependent inactivation of TS by Imd and Imd$^+$ in the presence (*triangles*) or absence (*circles*) of 20 mM dUMP. *Right:* Inactivation of TS by Imd (□), or Imd$^+$ (Δ), is not reversible by dialysis, in contrast to that by EdUMP (▽); control, (O).

Consistent with this hypothesis was the finding that 5-fluoropropynyl-dUMP (FPdUMP), which has a leaving group X = F, irreversibly inactivated TS in the absence of the cofactor (2). The potential of the fluoropropynyl side-chain to undergo reactions with S_N2 displacement of F, rather than via conjugate elimination, prompted the synthesis of as series of analogs with varying, or no leaving group abilities. All of these analogs inhibited TS competitively with K_i-values ranging from 0.015 to 18 μM (Table 1). Among these, only FPdUMP (2) and the two imidazole derivatives, Imd and Imd$^+$, which have leaving groups, showed irreversible inactivation upon incubation of TS in the absence of the cofactor, in contrast to the cofactor-requiring EdUMP, which was readily reversible (Fig. 2). The results demonstrate that the inactivation mechanism involves conjugate elimination, rather than S_N2 displacement (which is not possible for Imd or Imd$^+$), providing experimental evidence for the validity of the proposed hypothesis.

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