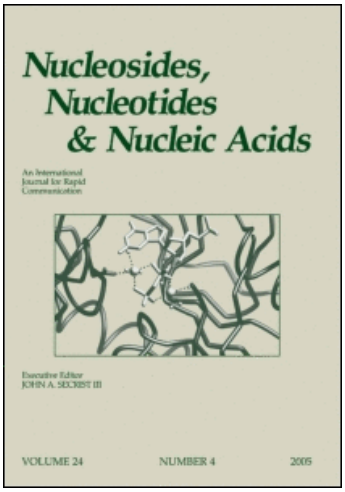

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5-HALO-6-ALKOXY-5,6-DIHYDRO-PYRIMIDINE NUCLEOSIDES: ANTIVIRAL NUCLEOSIDES OR NUCLEOSIDE PRODRUGS?

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ABSTRACT

5-Halo-6-alkoxy-5,6-dihydro derivatives of azidothymidine (AZT) and ethyldeoxyuridine (EDU) were developed and evaluated using *in vitro* and *in vivo* models. Although most of these 5,6-dihydro derivatives served as prodrugs in improving site-specific delivery and pharmacokinetic parameters, some unique characteristics were exhibited.

Prodrugs are drugs modified chemically to mask their pharmacological properties. They are designed to regenerate the parent drug under specified physiological conditions. The prodrug may be designed to increase solubility, increase oral bioavailability, improve stability, increase site-specific delivery, decrease toxicity and possibly to serve as a chemical depot to provide sustained release of the drug. In the cases of 3'-azidothymidine (AZT) and 5-ethyl-2'-deoxyuridine (EDU), we have utilized the prodrug approach to improve their delivery to the brain, in order to develop localized, therapeutically-effective concentrations without increasing toxicity. The brain delivery objective was based on the sheltering reservoir effect of the CNS, which protects HIV-infected macrophages and herpes viruses in the brain.[1,2] Since AZT penetrates the blood-brain barrier (BBB) mainly by diffusion[3], and since hydrophilic nucleosides such as EDU (octanol-phosphate buffer partition coefficient <0.1) enter brain only slowly, our prodrug approach was to improve brain entry by increasing lipophilicity, so that diffusion across the BBB would be facilitated. Disubstitution of the 5,6-double bond of AZT and EDU was used to prepare the corresponding 5-halo-6-alkoxy-5,6-dihydro prodrug derivatives.

RESULTS AND DISCUSSION

Synthesis of the 5-halo-6-alkoxy-5,6-dihydro pyrimidine nucleosides by reaction of the parent nucleoside with the appropriate halogen in the desired alcohol as solvent yields

primarily the two diastereomers that are formed via *trans* addition (5R,6R and 5S,6S), although appreciable yields of *cis* (5S,6R)-5-chloro-6-alkoxy diastereomers were also obtained.[4,5] The resulting diastereomeric products of several anti viral nucleosides had increased lipophilicity (Table 1), with P values for I > Br > Cl, for *trans* > *cis*, and for *trans* (-)(5S,6S) > *trans* (+) (5R,6R).

These 5-halo-6-alkoxy derivatives were stable in aqueous solution, but were subject to degradation to the parent nucleoside under pseudo physiological conditions *in vitro*. Upon challenge with glutathione (2 molar equivalents), whole blood and murine liver homogenate (soluble fraction), the 5-iodo-and 5-bromo derivatives underwent facile conversion (Table 2), whereas the corresponding *cis* (+)-(5S,6R)- and *trans* (+)-(5R,6R)-6-methoxy-5-chloro AZT derivatives were refractory to elimination.

AZT and these 5,6-dihydro derivatives of AZT were resistant to glycosidic cleavage by E. coli thymidine phosphorylase, whereas EDU and d4T were susceptible to phosphorylases, their 5,6-disubstituted derivatives were stable (data not shown).[4] Clearly, 5,6-disubstitution in this manner produces prodrugs that are resistant to deglycosylation by phosphorylases, a major cause of nucleoside inactivation *in vivo*.

In vitro antiviral activities of the prodrug derivatives of both antiviral agents reflected the ease with which the parent (active) nucleosides were generated under incubation conditions. The anti-HIV activities of selected AZT-derivatives (Table 3) show clearly that the 5-bromo compounds with short chain alkoxy substituents at C^6 are equally active to AZT, whereas the corresponding 5-chloro compounds, which do not eliminate at C^5-C^6 to regenerate AZT, are inactive. Similarly, the 5-bromo-6-methoxy derivatives of EDU are equally active to EDU against HSV-1 and HSV-2, whereas the 5-chloro analogous are inactive (data not shown).[5] The activities of these EDU derivatives against HCMV do not provide a clear picture of the prodrug effect, but the potency of *cis*-(+) (5S,6R)-CMEDU, which is stable *in vivo*, suggests that this derivative may have antiviral activity without conversion to EDU.

The rapid *in vivo* generation of AZT from the 5-bromo/iodo-6-ethoxy AZT derivatives demonstrates that they also provide rapid release of AZT under physiological conditions. Furthermore, the presence of AZT in blood 150 min after the iv injection of the most lipophilic compound [*trans*-(-)-(5S,6S)-5-iodo-6-methoxy-5,6-dihydro-3'-azidothymidine; P = 19] demonstrates sustained release, most probably due to a depot effect (data not shown).[6]

Pharmacokinetic data for EDU and its 5,6-disubstituted derivatives show that with the exception of *trans*-(+)-(5R,6R)-5-chloro-6-methoxy-5,6-dihydro-5-ethyl-2'-deoxyuridine, these compounds have surprisingly similar blood clearance half-lives (0.59-0.69 hr), and with the exception of the *cis* 5-chloro compound, all provide similar areas

TABLE 1. Partition coefficients (P)[a] of selected pyrimidine nucleosides and their 5-halo-6-methoxy-5,6-dihydro derivatives.

	AZT	FLT	EDU	d4T
Parent nucleoside	1.3	0.5	0.08	0.1
Trans-(+)-(5R,6R)-ClOMe	7.6	5.2	2.5	2.6
Cis-(+)-(5S,6R)-ClOMe	3.3	1.5	0.38	1.0
Trans-(+)-(5R,6R)-BrOMe	13.2	4.7	1.9	3.4
Trans-(-)-(5S,6S)-BrOMe	16.7	3.4	2.5	1.5
Trans-(+)-(5R,6R)-IOMe	10.6	2.8	-	2.6
Trans-(-)-(5S,6S)-IOMe	18.8	4.0	-	1.3

[a]$P=C_{1\text{-octanol}}/C_{\text{phosphate buffer}}$

TABLE 2.Conversion of 5-halo-6-methoxy-5,6-dihydro-3'-azidothymidine to AZT *in vitro.*

	% Conversion		
Compound	Glutathione[a]	Mouse blood[b]	Soluble fraction of mouse liver[c]
Trans-(+)-(5R,6R)-AZTIOMe[d]	100	100	80
Trans-(-)-(5S,6S)-AZTIOMe	100	100	95
Trans-(+)-(5R,6R)-AZTBrOMe[d]	98	59	26
Trans-(-)-(5R,6R)-AZTBrOMe	90	88	43
Cis-(+)-(5S,6R)-AZTClOMe	0	0	0
Trans-(+)-(5R,6R)-AZTClOMe[d]	0	0	0

[a]30 min incubation at 37°C,glutathione:substrate= 2:1; [b]10 min incubation at 37°C; [c]30 min incubation at 37°C; [d]No measurable conversion in phosphate buffer (pH=7, 0.06M) at 37°C for 24 hr.

TABLE 3. *In vitro* activity of *trans*-(+)-(5R,6R)-5-bromo/chloro-6-alkoxy-5,6-dihydro-3'-azidothymidines against HIV-1 in CEM cells.[a]

C^5 halogen	C^6Alkoxy	EC_{50} (M)[b]	TI[c]
Br	OCH_3	3.27×10^{-9}	5260
Br	OCH_2CH_3	6.75×10^{-9}	2741
Br	$OCH(CH_3)_2$	5.72×10^{-6}	35
Br	$O(CH_2)7CH_3$	8.56×10^{-7}	14
Br	$O(CH_2)15CH_3$	inactive	-
Cl	OCH_3	5.79×10^{-6}	155
Cl	OCH_2CH_3	inactive	-
Cl	$OCH(CH3)_2$	inactive	-
Cl	$O(CH2)_7CH_3$	inactive	-
Cl	$O(CH_2)15CH_3$	inactive	-

[a]*In vitro* tests were performed by the Antiviral Evaluation Branch, National Cancer Institute, USA; [b]Concentration for 50% survival in HIV-1 infected cells (AZT=3 $\times10^{-9}$ M); [c]$TI=IC_{50}/EC_{50}$ (cytotoxicity/antiviral activity) ($AZT\geq10^4$).

TABLE 4. Pharmacokinetic parameters for EDU and its 5,6-dihydro derivatives.

Compound injected	AUC[a](μmol.h.ml^{-1})			Cl[b](lit.hr^{-1}),	$t_{1/2}$[c] (hr),
	Prodrug	EDU	EU		
EDU	-	0.33	0.44,	5.1	0.69
(5R,6R)-BMEDU	0.11	0.34	0.96	6.3	0.67
(5S,6S)-BMEDU	0.06	0.40	1.05	5.9	0.59
(5R,6R)-CMEDU	0.85	0.28	0.63	0.7	2.21
(5S,6R)-CMEDU	0.30	0.10	-	2.3	0.67

All parameters calculated using Lagran program; [a]Area under the curve of blood concentration *vs* time (0 → last sample); [b]Total body clearance; [c]Terminal half-life in blood.

TABLE 5. Thymidine influx inhibition constants (k_i)[a] for AZT and its 5-halo-6-methoxy-5,6-dihydro analogs in murine erythrocytes (mean ± SD; n=3)

Compound	k_i (mM)
AZT	1.33 ± 0.04
Cis-(5S,6R)-AZTClOMe	>> 1.5
Trans-(+)-(5R,6R)-AZTClOMe	0.36 ± 0.01
Trans-(+)-(SR,6R)-AZTBrOMe	0.45 ± 0.07
Trans-(-)-(5S,6S)-AZTBrOMe	0.50 ± 0.04
Trans-(+)-(5R,6R)-AZTIOMe	0.35 ± 0.03
Trans-(-)-(SS,6S)-AZTIOMe	0.20 ± 0.00

[a]NBMPR sensitive equilibrative facilitative transporter.

under drug concentration *vs* time curves (AUC's) for EDU (0.28-0.40 μmol.h.ml^{-1}). Importantly, these data demonstrate not only that the 5-chloro compounds are prodrugs for EDU *in vivo*, but that as prodrugs, they do not provide any obvious pharmacokinetic advantage over the nucleoside (EDU) itself (Table 4).[5]

Surprisingly, these 5-halo-6-alkoxy-5,6-dihydro-3'-azidothymidines demonstrated an interaction with the NBMPR-sensitive facilitative equilibrative nucleoside transporter in murine erythrocytes, whereas AZT did not inhibit thymidine influx in this system. The conformation of the 5,6-disubstituted AZT derivatives, as influenced by the configurations of C^5 and C^6, also strongly influenced interaction with the transporter, since *cis* (5S,6R)-5-chloro-6-methoxy-5,6-dihydro-5-ethyl-2'-deoxyuridine also had no effect on thymidine influx (Table 5).[6]

Based on these *in vitro* and *in vivo* studies, it is possible to conclude that the 5,6-disubstituted derivatives of AZT and EDU are prodrugs of their respective nucleosides when the C^5 halogen is Br or I and the C^6 alkoxy substituent is a short chain, whereas the

stability of the C^6-chloro analogous makes them poor quality prodrugs at best. However, in terms of anti viral (HCMV) activity, *cis* (+)-(5S,6R)-5-chloro-6-methoxy-5-ethyl-2'-deoxyuridine may be an antiviral agent in its own right. Furthermore, the *trans* (+)-5-halo-6-methoxy-3'-azidothymidine derivatives are weak inhibitors of facilitated equalibrative nucleoside transport, whereas AZT itself does not interact with this system.

EXPERIMENTAL

The synthesis of 5-halo-6-alkoxy-5,6-dihydro derivatives of AZT and EDU, and selected ^{14}C-labelled derivatives have been reported previously.[4,5,7] ^{14}C-AZT was purchased from Amersham International. Procedures for partition coefficients (P), phosphorolysis studies, nucleoside transport and pharmacokinetic studies in Spraque-Dawley rats and/or Balb-C mice are described in the references. [4-7] Antiviral tests against HIV, HSV-1, HSV-2 and HCMV were performed by the Antiviral Testing Branch, NCI, Bethesda, U.S.A., according to their published procedures as described elsewhere.[8]

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