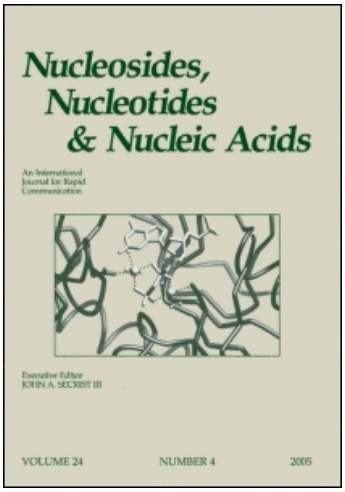


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# HEPT DERIVATIVES: 6-BENZYL-1-ETHOXYMETHYL-5-ISOPROPYLURACIL (MKC-442)

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**Abstract** - The 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) derivatives have been found to be potent and specific inhibitors of human immunodeficiency virus type 1 (HIV-1) replication *in vitro*. Among the compounds, MKC-442 (6-benzyl-1-ethoxymethyl-5-isopropyluracil) has recently been chosen as a candidate for clinical efficacy and safety studies in patients with the acquired immune deficiency syndrome (AIDS).

In the fight against human immunodeficiency virus type 1 (HIV-1), the causative agent of the acquired immune deficiency syndrome (AIDS), several classes of compounds have been identified as highly specific inhibitors of HIV-1.[1] Among the compounds, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) has been described in 1989 as a novel lead for specific anti-HIV-1 agents.[2,3] After the discovery of HEPT, we have attempted at increasing its potency by introducing various chemical modifications and obtained several HEPT derivatives that inhibit HIV-1 replication in the nanomolar concentration range.[4,5] We have also investigated their toxicologies and pharmacologies in animals to select the best candidate(s) for clinical trials. From these studies, we have chosen MKC-442 (6-benzyl-1-ethoxymethyl-5-isopropyluracil or I-EBU) as a candidate for clinical efficacy and safety studies in AIDS patients.[6]

**Chemistry**

It is well-known that glycosidation of 6-substituted uracils almost invariably results in the predominant formation of $N^3$-glycosylated products due to steric hindrance by the substituents. Therefore, the synthesis of 6-substituted uridines can be carried out best by using uridine as the starting material. We reported that regiospecific C-6 lithiation of the uracil moiety took place when 2',3'-*O*-isopropylideneuridine was deprotonated with lithium diisopropylamide (LDA).[7] Since various types of electrophiles react with the C-6 lithiated species, this method disclosed a simple entry to a wide range of 6-substituted uridines, most of which are difficult to synthesize by any other methods.

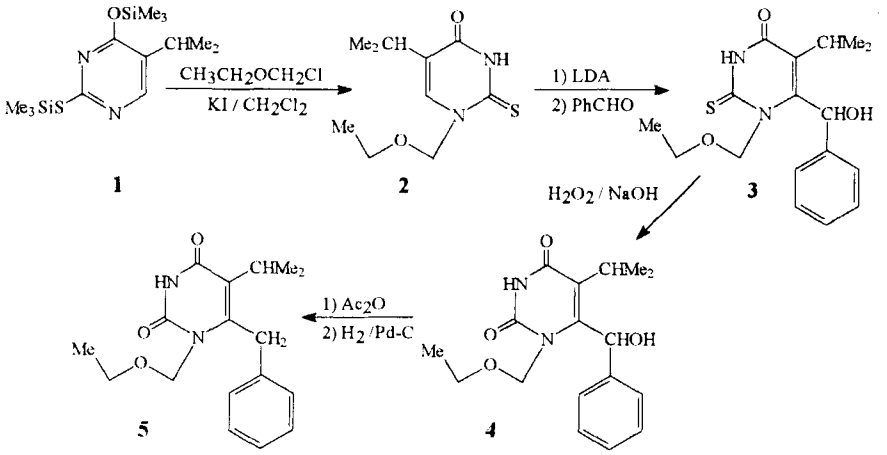
HEPT was discovered during the preparation of acyclic 6-substituted uridines based on the LDA lithiation chemistry.[2] Although a number of acyclonucleosides had been known, no such 6-substituted pyrimidine derivatives had appeared in literature when we embarked upon the evaluation of HEPT for its antiviral activities. Extensive synthetic efforts to improve the anti-HIV-1 activity of HEPT finally led to MKC-442.



The synthesis of MKC-442 is outlined in Scheme 1. For the reasons outlined above, the 6-substituent has to be introduced after the acyclic portion has been introduced. In this particular case of MKC-442, the silylated 5-isopropyl-2-thiouracil **1** was used as the starting material, because the $N^1$-ethoxymethyl derivative **2** gave a much higher yield of the 6-substituted product **3** than the corresponding 5-isopropyluracil derivative did upon the LDA lithiation and subsequent reaction with benzaldehyde. Oxidative hydrolysis of **3** readily afforded **4**, which was then converted to MKC-442 by conventional hydrogenolysis.

**Anti-HIV-1 activity**

MKC-442 completely protected MT-4 cells against HIV-1-induced cell destruction at a concentration of 160 nM (data not shown). The 50% effective concentration ($EC_{50}$) of MKC-442 for the $III_B$ strain was 15 nM (Table 1). In contrast, MKC-442 did not reduce the growth and viability of mock-infected MT-4 cells at concentrations up to 20 μM (data not shown). Its 50% cytotoxic concentration ($CC_{50}$) was 102 μM (Table 1). MKC-442 was also effective against HIV-1 replication in MOLT-4 cells, peripheral blood lymphocytes (PBL), and monocyte-macrophages (M/M).




Scheme 1. Synthesis of MKC-442



However, as previously noted for HEPT, MKC-442 did not inhibit HIV-2 replication in MT-4 cells (Table 1). MKC-442 was equally active against 3'-azido-3'-deoxythymidine (AZT)-resistant mutants (A012D and A018C)[8,9] and AZT-susceptible strains (Table 1).

**Inhibition of HIV-1 RT**

Although the HEPT derivatives are structurally related to nucleosides, previous studies on their mechanism of action have revealed that they functionally behave as HIV-1-specific nonnucleoside RT inhibitors.[4,5] MKC-442 also markedly inhibited HIV-1 RT activity (Table 2). When poly(C)/oligo(dG) was used as the template/primer, the 50% inhibitory concentration ($IC_{50}$) of MKC-442 was 0.012 μM. However, MKC-442 was totally inactive against HIV-1 RNase H, avian myeloblastosis virus (AMV) RT, Moloney murine leukemia virus (MLV) RT, and calf thymus DNA polymerase α (Table 2). In contrast, AZTTP was inhibitory to these RTs and DNA polymerase α. The kinetic analysis revealed that the inhibition of HIV-1 RT by MKC-442 was noncompetitive, linear-mixed, and uncompetitive with respect to the substrates (dTTP and dGTP) and templates [poly(A) and poly(C)], respectively.[10]

Table 1. Antiviral activity of MKC-442 and AZT in cell cultures

| Virus | Strain | Cells | MKC-442 | | AZT | |
|---|---|---|---|---|---|---|
| | | | $EC_{50}$[a] (μM) | $CC_{50}$[b] (μM) | $EC_{50}$ (μM) | $CC_{50}$ (μM) |
| HIV-1 | $III_B$ | MT-4 | 0.015 | 102 | 0.0033 | 10 |
| | | MOLT-4 | 0.0031 | 181 | 0.00080 | 19 |
| | | PBL | 0.0038 | 95 | 0.0021 | 12 |
| | $III_{RF}$ | MT-4 | 0.012 | - | 0.0059 | - |
| | HE | MT-4 | 0.019 | - | 0.0066 | - |
| | JR-FL | M/M | 0.011 | > 100 | 0.0038 | > 100 |
| | A012D | MT-4 | 0.0047 | - | 0.19 | - |
| | A018C | MT-4 | 0.0076 | - | 0.22 | - |
| HIV-2 | ROD | MT-4 | > 102 | - | 0.0025 | - |
| | EHO | MT-4 | > 102 | - | 0.0039 | - |

[a] Except for A012D and A018C strains, the 50% effective concentration ($EC_{50}$) was based on the inhibition of HIV-induced cytopathic effect in MT-4 and MOLT-4 cells or the reduction of p24 antigen in culture supernatants of PBL and M/M. For A012D and A018C, the $EC_{50}$ was based on the inhibition of HIV-1 antigen expression in MT-4 cells.

[b] The 50% cytotoxic concentration ($CC_{50}$) was based on the reduction of viability of mock-infected cells.

Data are taken from reference 6.



**Combination with other compounds**

The combined inhibitory effect of MKC-442 and AZT, 2',3'-dideoxycytidine (DDC), or 2',3'-dideoxyinosine (DDI) on HIV-1 replication was found to be synergistic in MT-4 cells.[6] Furthermore, unlike other non nucleoside RT inhibitors, MKC-442 acted also synergistically with AZTTP at the level of the HIV-1 RT.[10] In agreement with these observations,

Table 2. Effect of MKC-442 and AZT on various RTs and DNA polymerase α

| Enzyme | Template/primer | $IC_{50}$[a] (μM) | |
|---|---|---|---|
| | | MKC-442 | AZTTP[c] |
| HIV-1 RT | poly(A)/oligo(dT) | 0.21 | 0.007 |
| | poly(C)/oligo(dG) | 0.012 | > 1000 |
| HIV-1 RNaseH | - | > 1000 | > 1000 |
| AMV RT | poly(A)/oligo(dT) | > 1000 | 0.12 |
| MLV RT | poly(A)/oligo(dT) | > 1000 | 0.20 |
| DNA pol α[b] | activated DNA | > 1000 | 540 |

[a] 50% Inhibitiory concentration, or concentration required to inhibit enzyme activity by 50%.

[b] Calf thymus DNA polymerase α.

[c] AZT 5'-triphosphate.

Data are taken from reference 10.



we found that the simultaneous treatment of HIV-1-infected MT-4 cells with MKC-442 and AZT could prevent the breakthrough of virus for more than 60 days (Fig. 1).

**Drug resistance**

Recent studies on HIV-1 mutants resistant to nonnucleoside RT inhibitors have demonstrated that single amino acid changes in the RT are responsible for resistance.[12-15] We have also obtained drug-resistant HIV-1 mutants upon several passages in cell culture in the presence of MKC-442. The mutants were cross-resistant to nevirapine[16] and L-696,229,[17] but not to AZT (Table 3). Sequence analysis of RT revealed that the HIV-1 mutant completely resistant to MKC-442 ($HE_{442-2}$) had two amino acid substitutions ($Lys^{103} \rightarrow$ Arg and $Tyr^{181} \rightarrow$ Cys), whereas the mutants partially resistant to MKC-442 ($III_{B-R}$ and $HE_{442-1}$) had only the $Tyr^{181} \rightarrow$ Cys substitution.[18]

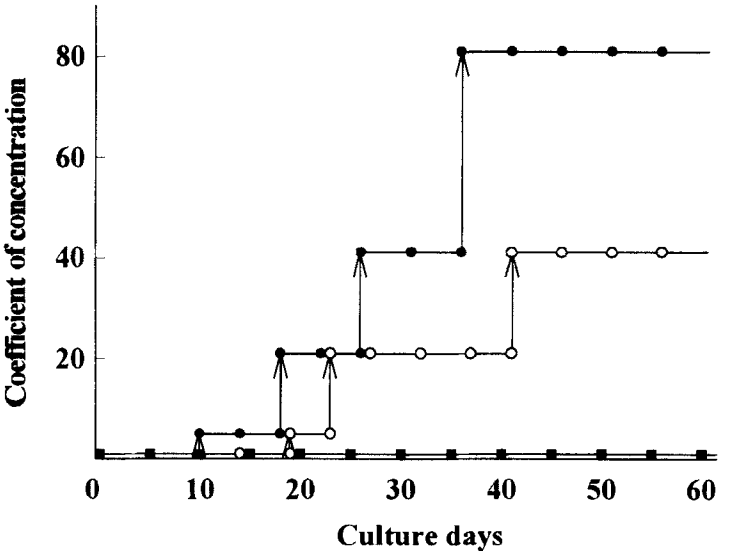



Fig. 1. Selection of drug-resistant HIV-1 in the presence of compounds. MT-4 cells were infected with $III_B$ strain and cultured in the presence of either MKC-442 alone (●), AZT alone (○), or combination of MKC-442 and AZT (■). The starting concentrations of MKC-442 alone and AZT alone were 56 and 16 nM, respectively. For the combination, 28 and 8 nM were used as the initial concentrations of MKC-442 and AZT, respectively. After a 4- or 5-day incubation at 37°C, the cells were passed by 1:5 dilution into fresh culture medium containing the same concentration of the compound. When the number of viable cells was less than $1 \times 10^5$ per ml due to the HIV-1-induced cytopathic effect, subsequent passage was not performed. In this case, the culture supernatant was collected and examined for its infectivity. Then MT-4 cells were infected with the virus at a MOI of 0.1 and cultured in 1 ml of medium containing MKC-442 at a concentration which was 2- to 5-fold higher than that in the previous passage. The concentration of compounds in each passage was expressed as a ratio to the starting concentration (coefficient of concentration). Data are taken from reference 11.

Table 3. Antiviral activity of MKC-442, nevirapine, L-696,229, and AZT against MKC-442-resistant strains

| Strain | $EC_{50}$[a] (μM) | | | |
|---|---|---|---|---|
| | MKC-442 | Nevirapine | L-696,229 | AZT |
| IIIB | 0.015 | 0.077 | 0.022 | 0.0033 |
| $III_{B-R}$ | 53 | > 224 | > 188 | 0.0023 |
| HE | 0.016 | 0.073 | 0.031 | 0.0060 |
| $HE_{442-1}$ | 88 | > 191 | > 179 | 0.0026 |
| $HE_{442-2}$ | > 119 | > 191 | > 179 | 0.0047 |

[a] The 50% effective concentration ($EC_{50}$) was based on the inhibition of HIV-1-induced cytopathic effect in MT-4 cells.
Data are taken from references 6 and 18.

Table 4. Antiviral activity of MKC-442, nevirapine, L-696,229, and AZT against A018A, A018C, and A018CR

| Compound | $EC_{50}$[a] (μM) | | |
|---|---|---|---|
| | A018A | A018C | A018CR |
| AZT | 0.0015 | 0.31 | 0.070 |
| DDI | 11 | 11 | 6.8 |
| MKC-442 | 0.0059 | 0.0088 | 1.5 |
| Nevirapine | 0.030 | 0.043 | 0.074 |
| L-696,229 | 0.027 | 0.036 | 0.28 |

[a] The 50% effective concentration ($EC_{50}$) was based on the inhibition of HIV-1 antigen expression in MT-4 cells.
Data are taken from reference 11.

The HIV-1 mutant co-resistant to MKC-442 and AZT could be obtained in cell culture when the selection experiment was started with the AZT-resistant strain A018C. Table 4 shows the inhibitory effects of various HIV-1 RT inhibitors on the replication of A018A (AZT-susceptible), A018C (AZT-resistant), and A018CR (co-resistant to MKC-442 and AZT) in MT-4 cells. A018CR was found to be highly resistant to MKC-442. Interestingly, A018CR retained almost normal sensitivity to nevirapine; it was also more susceptible to AZT than was A018C, although it was less AZT-susceptible than A018A. In addition to the amino acid substitutions that have been reported to confer AZT-resistance,[9] A018CR showed the substitutions $Leu^{74} \rightarrow Ile$, $Lys^{103} \rightarrow Glu$, and $Val^{108} \rightarrow Ile$.[11] It remains to be elucidated whether the combination chemotherapy will achieve prolonged suppression of drug-resistant mutants.



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