J. Microbiol. Biotechnol. (1998), **8**(3), 229 – 236

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

Effect of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) Derivatives on Bacterial Growth

GANG, JIN-GU, HONG-CHUL YUN, JONG-CHAN SON[1], AND SE-YOUNG HWANG*

Graduate school of Biotechnology, Korea University, 5-1 Anam-Dong, Sungbuk-Gu, Seoul 136-701, Korea
[1]Korea Research Institute of Chemical Technology, Yusong-Gu, Taejon 305-343, Korea

Received: January 22, 1998

Abstract 6-Substituted derivatives of 1-[(2-hydroxyethoxy) methyl]-6-(phenylthio)thymine (HEPT) were synthesized by introducing alkyl groups with the aid of chlorotrimethylsilane, and then purified ranging 40 to 81% of yield. Because of their peculiar structures, we presumed that HEPT derivatives would contain extra biological activities other than their already known anti-human immunodeficiency viral (HIV-1) activities. In this study, we investigated the possible effects of the HEPT derivatives on bacterial growth and found their selective antibiotic activities against gram-positive strains. We could not observe the corresponding activity from a disc-zone test, but confirmed the activity by liquid cultivation. Since the growth rate of cells was easily recovered, the antibiotic function was suggested to be bacteriostatic. We also suggested that the intracellular fate of HEPT derivatives would be fast. A HEPT derivative f-3 was shown to synergize unidirectionally toward chloramphenicol (Chr). With 0.1 mM f-3, the Chr-directed growth-inhibitory curve appeared 4 hours earlier than found without the additive. Interestingly, from the data of SDS-polyacrylamide gel electrophoresis (PAGE), we found that a membrane-bound protein having a molecular weight of 70-kDa was overexpressed by f-3 in *S. aureus*.

Key words: 1-[2-(Hydroxyethoxy)methyl]-6-(phenylthio) thymine (HEPT), HEPT derivatives, synthesis, AIDS, antibacterial activity

HEPT, a 6-substituted acyclouridine derivative, was first developed [16] for the purpose of medical treatment of acquired immunodeficiency syndrome (AIDS) [2]. This compound, known as a potent and selective inhibitor for human immunodeficiency virus type 1 (HIV-1), is totally inactive against HIV-2, and yet exhibits no activity toward any other retroviruses [4]. HEPT or its derivatives are therefore strongly suggested as promising agents for the treatment of AIDS in the future. Literally, the currently-used chemotherapeutic agents [e.g., 3'-azido-3'-deoxythimine (AZT) [15], 2',3'-dideoxynucleosides (ddNs) [14], 2',3'-dideoxy-cytidine (DDC) [18], 2',3'-dideoxy-inosine (DDI) [24]] are generally nonspecific and toxic to the host. On the contrary, because of HEPT's unusual specificity [3], the world's attention is greatly focused on its immediate application for patients with AIDS. Its numerous derivatives have been synthesized and studied.

Basically, HEPT derivatives are nucleoside analogs. Nevertheless, their skeletal structures seem unusual because the cores consisting of an acyclouridine and aryl ring are asymmetrical via the sulfur atom, etc. This unnatural nucleus suggests that HEPT derivatives may contain extra biological properties other than anti-HIV activities.

Unexpected changes in the population of human microflora can be happened by successive doses of certain xenobiotics. In fact, the differential distribution in microbial inhabitants may possibly change the type of secondary infections, the causative agents for opportunistic pathogenesis [9, 20]. Upon an immunosuppressed state such as AIDS, this change would be even more significant. It should be noteworthy that, in patients with AIDS, life-threatening factors are rather closely related with the infectious diseases than AIDS itself. For instance, bacteremic patients with immunodefects are ultimately fatal [19, 23]. In this regard, it is important to study the cytopathic effect of drugs against microorganisms prior to their clinical application. With this purpose in mind, we first synthesized various HEPT derivatives in a novel way and examined their effects on bacterial growth.

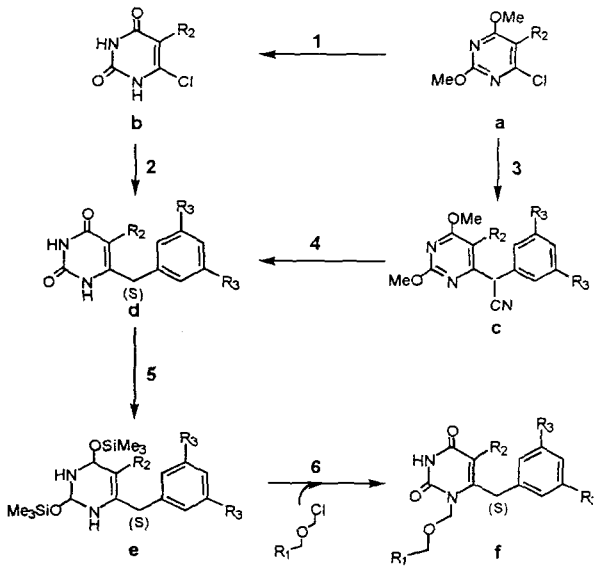
In this paper, we first introduce a rational way of preparing the HEPT derivatives, avoiding extreme conditions. Then, we describe their characteristics as antibiotics and synergism with Chr. In addition, the putative role of the HEPT derivatives *in vivo* is discussed.

**Corresponding author*
Phone: 82-415-60-1412; Fax: 82-415-865-3339;
E-mail: shwang@tiger.korea.ac.kr

MATERIALS AND METHODS

Synthesis of HEPT Derivatives

Briefly, as presented in Scheme 1, methylated acyclouridines (R_2 substituents; a and b) were linked with aryl compounds (R_3 substituents; 2 and 3) via either -S or -CH_2. The resulting aryl acyclouridines were subsequently masked with hexamethyldisilazane in the presence of chlorotrimethylsilane (e), followed by condensation with alkylethers (R_1 substituents; 6) at the N-1 position. The yields of the resulting products (f), harvested as white powders, were in the range of 30 to 80%. Melting points were determined with the MFB 595-030G capillary system (1.2 mm; Quallankamp Co.). ^{1}H NMR spectral data were obtained with a Varian GEMINI-200 NMR spectrometer at 200 MHz, and chemical shifts were recorded in parts per million (ppm). ^{13}C NMR spectra were recorded on a Bruker AMX-300 NMR spectrometer. FAB-mass spectra were observed with the GCMS-QP 1000 at 70 cV, and IR spectra were taken on a Shimadzu IR-435 spectrometer. TLC was performed on silica gel (precoated silica gel plate 60 F_{254}, Merk). Column chromatography was carried out using silica gel resin (230-400 mesh). **(f-1) 1-benzyloxymethyl-5-isopropyl-6-(3,5-dimethylphenylthio)-2,4-pyrimidinedione:** mp, 157~158°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.16 (6H, d, J=7.0 Hz), 2.24 (6H, s), 3.47 (1H, m), 4.64 (2H, s), 5.58 (2H, s), 6.76 (2H, s), 6.84 (1H, s), 7.25~7.36 (5H, m), 8.65~8.71 (1H, brs). IR (KBr) 3430, 3049, 1711, 1657, 1585, 1436, 1425, 1079. **(f-2) 1-ethoxymethyl-5-isopropyl-6-(3,5-dimethylphenylthio)-2,4-pyrimidinedione:** mp, 151~153°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.13 (3H, t, J =7.0 Hz), 1.16 (6H, d, J=7.0 Hz), 2.27 (6H, s), 3.47 (1H, m), 3.58 (2H, q, J=7.0 Hz) 5.51 (2H, s), 6.80 (2H, s), 6.86 (1H, s), 9.15~9.20 (1H ,brs). IR (KBr) 3460, 3054, 1713, 1651, 1563, 1452, 1097. **(f-3) 1-acetoxyethoxymethyl-5-isopropyl-6-(3,5-dimethylphenylthio)-2,4-pyrimidinedione:** mp, 98~99°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18 (6H, d, J=7.0 Hz), 2.04 (3H, s), 2.27 (6H, s), 3.48 (1H, m), 3.76 (2H, m), 4.12 (2H, m), 5.55 (2H, s), 6.79 (2H, s), 6.87 (1H, s), 8.75~8.80 (1H ,brs). IR (KBr) 3484, 3052, 1747, 1666, 1580, 1458, 1237, 1088. **(f-4) 1-acetoxyethoxymethyl-5-ethyl-6-(3,5-dimethylbenzyl)-2,4-pyrimidinedione:** mp, 119~193°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.06 (3H, t, J=7.4 Hz), 2.05 (3H, s), 2.27 (6H, s), 2.51 (2H, q, J= 7.4 Hz), 3.76~3.80 (2H, m), 4.04 (2H, s), 4.15~4.20 (2H, m), 5.14 (2H, s), 6.67 (2H, s), 6.88 (1H, s), 8.82~8.90 (1H, brs). IR (KBr) 3477, 3036, 1742, 1712, 1664, 1480, 1238, 1075. **(f-5) 1-benzyloxymethyl-5-isopropyl-6-(3,5-dimethylbenzyl)-2,4-pyrimidinedione:** mp, 129~131°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.27 (6H, s), 2.83 (1H, m), 4.08 (2H, s), 4.66 (2H, s), 5.20 (2H, s), 6.64 (2H, s), 6.88 (1H, s) 7.32 (5H, m), 8.70 (1H, brs). IR (KBr) 3440, 3185, 3054, 1705, 1636, 1458, 1360, 1073. **(f-6) 1-ethoxymethyl-5-isopropyl-6-(3,5-dimethylbenzyl)-2,4-pyrimidinedione:** mp, 134~136°C. ^{1}H NMR (200 MHz, $CDCl_3$) 1.18 (3H, t, J=7.0 Hz), 1.28 (6H, d, J=7.0 Hz), 2.27 (6H, s), 2.83 (1H, m), 3.61 (2H, q, J=7.0 Hz), 4.08 (2H, s), 5.10 (2H, s), 6.69 (2H, s), 6.88 (1H, s), 8.77 (1H, s). IR (KBr) 3428, 3064, 1711, 1687, 1647, 1457, 1358, 1104.



Scheme 1. Synthetic procedure for HEPT derivatives.

This procedure involves the trimethylsilination prior to alkylation by R_1-O-Cl [20]. Experimental conditions were briefly as follows; 1, conc. HCl in reflux. 2, arylthiol-KOH-ethanol in reflux. 3, arylacetonitrile-NaH-dimethylformamide at 0°C. 4, conc. HCl in reflux. 5, chlorotrimethylsilane-hexamethyldisilazane at 130~140°C. 6, tetrachlorotin in 1 *M* dichloromethane-acetonitrile at room temperature. R_1-C_6H_5, CH_3, $OCOCH_3$. R_2-CH_2CH_3, isopropyl. R_3-H, CH_3. (*Cf.*) HEPT; R_1-CH_2OH, R_2-CH_3, R_3-H.

Microorganisms and Culture Condition

Bacterial strains used were as follows: *Bacillus megaterium, Corynebacterium glutamicum* ATCC 13059, *Enterobacter aerogens* KCTC 2190, *Enterococcus fuecium* ATCC 2022, *Escherichia coli* No. 20 [7], *Pseudomonas aeruginosa* KCTC 1750, and *Staphylococcus aureus* KCTC 1916. Strains were inoculated and cultivated overnight (reciprocal shaking; 150 rpm, 37°C) in 5 ml nutrient medium (NM; 0.5% polypeptone, 0.1% beef extract, 0.3% yeast extract, 0.5% NaCl, pH 7.0) using culture tubes (1.5×20 cm). The grown cells were transfered into 100 ml NM using 250-ml Erlenmyer flask and cultivated by rotary shaker (90 rpm, 37°C) until cell turbidities of $A_{660} \simeq 1.0$ were observed.

Intact Cells and Uptake of Aryl Acyclouridine

Cells were harvested by centrifugation (9,000×g, 10 min) and washed three times by resuspending in 50 mM phosphate buffer (PB, pH 7.0). Typically, the

washed cells in PB (pH 7.0) were adjusted to give a final volume of 1 ml with O.D. of 1.0~3.0 at 660 nm. The resulting cell suspensions were directly used as intact cell systems (9 mls) [10]. The uptake rate of a given HEPT derivative was assessed as follows: 1 ml of 1 mM compound (f-3, dissolved in conc. EtOH) was added to the reaction system (screw capped-vial) on ice and 1 ml was taken as a control. The vial was then placed on a water bath (37°C), and time course portioning was undertaken at 1-min intervals. Extracellular amounts of the compound were then determined after removing cells by centrifugation at 0°C (9,000×g, 10 min). The uptake rate was determined by measuring its decreasing absorbance with time at 253 nm (ε_{253}=15,465 $M^{-1}cm^{-1}$), and the rate was expressed as nmoles per minute.

Isolation of Membrane-bound Proteins

Cells incubated with or without an additive (f-3, 0.1 mM) were thoroughly washed as described above. Washed cells were then suspended in PB containing 0.1 mg lysozyme per ml, 3 mM [ethylenediaminedinitrilo]tetraacetate (EDTA), 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM $MgCl_2$, pH 7.0, and incubated for 30 min at 4°C. Following the incubation, the cells were disrupted using an ultrasonic processor (amplitude 20%, pulse 5 min: VCX 400, Sonic & Materials, Inc.). After discarding the cell debris by centrifugation (9,000×g, 10 min), the supernatant was subjected to ultracentrifugation (190,000×g, 1 h). Sedimented membrane pellet [25] was then solubilized with 1% SDS and directly applied to electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Electrophoresis was performed according to the method of Laemmli [8]. Heat-treated samples with 1% SDS were loaded on a stacking gel (4% in polyacrylamide) and electrophoresis was carried out at a currency of 15 mA for 3.5 h on a separating gel (10% in polyacrylamide: 180×160×1 mm). Protein bands were visualized with 0.1% Coomassie Brilliant Blue R_{250}, and were destained with 10% methanol in 10% acetic acid.

Protein Determination

Protein concentration was assayed by the method of Lowry *et al.* [11] using bovine serum albumin as a standard.

RESULTS

Synthesis of HEPT Derivatives

In literatures, various analogs lacking a hydroxyl function in the acyclic structure of HEPT were reported to be synthesized from the corresponding pyrimidine bases by employing the lithiating method [21, 22]. The lithiation, however, is inconvenient because it entails the use of unwanted extreme-condition (e.g., under THF at -70°C for 1 h). For this reason, we developed an easy way of performing the trimethylsilination of aryl 2,4-pyrimidinedione and subsequent alkylation at the N-1 position. This strategy was found to be useful in preparing any aryl acyclouridine analogs (see Scheme 1). Furthermore, since the procedure was practically simple, we inferred that the related compounds could be prepared at the laboratory level. In addition, by using either arylthiol or arylacetonitrile instead of diaryl disulfide as a substituent at the C-6 position (halide; a, b in Scheme 1), the linkage between aryl and acyclouridine groups could easily be changed from sulfur atom to methine. Referred data in Table 1 may indicate that the structure of the linker significantly affects the cytotoxicity of HEPT derivatives [4, 22].

Cellular Uptake of HEPT Derivatives

Using fresh cells of *E. coli* and *S. aureus* the cellular uptake rate of a HEPT derivative, 1-acetoxyethoxymethyl-5-isopropyl-6-(3,5-dimethylphenylthio)-2,4-pyrimidinedione (f-3), was determined (Fig. 1). From the slopes in the figure, the individual uptake rate was computed. The data are presented in Table 2. From the data, it was apparent that the HEPT derivative f-3 was taken up by *E. coli* over 2 times faster than by *S. aureus*. Energetic factors such as glucose, H^+, or ATPase inhibitors did not change the cellular uptake of f-3. This observation

Table 1. Summary of *in vitro* anti-HIV-1 properties of the synthetic HEPT derivatives.

Compd	R_1	R_2	R_3	Linker	Yield (%)	m.p. (°C)	CC_{50} (μM)	EC_{50} (μM)	S.I.
f-1	$-C_6H_5$	$-CH(CH_3)_2$	$-CH_3$	S	54	157~158	155±27	0.016±0.0021	9,688
f-2	$-CH_3$	$-CH(CH_3)_2$	$-CH_3$	S	80	151~153	79.7±4.7	0.004±0.001	19,925
f-3	$-OCOCH_3$	$-CH(CH_3)_2$	$-CH_3$	S	40	98~99	128	0.0027	47,407
f-4	$-CH_3$	$-CH_2CH_3$	$-CH_3$	CH_2	81	161~163	207	0.0016	130,000
f-5	$-OCOCH_3$	$-CH_2CH_3$	$-CH_3$	CH_2	55	119~123	280	0.013	21,538
f-6	$-C_6H_5$	$-CH(CH_3)_2$	$-CH_3$	CH_2	57	129~131	148	0.0054	2,740

In vitro efficacy of the compounds were referred from references 4 and 22. EC_{50}: effective concentration of compound required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1. CC_{50}: cytotoxic concentration of compound required to reduce 50% of the viability of mock-infected MT-4 cells. S.I.: selective index indicating the ratio of CC_{50} over EC_{50}.

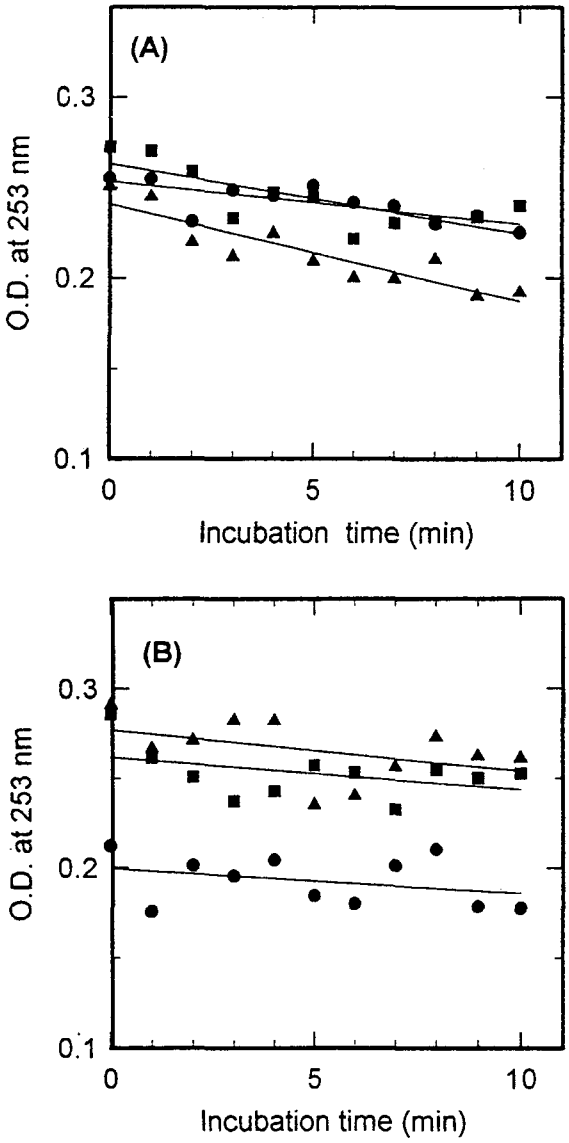


Fig. 1. Time course determination of bacterial uptakes of a HEPT derivative.

Initial concentration of a HEPT derivative; f-3 was 0.1 mM. The extracellular amounts of the compound in the intact cell system was measured after discarding the cells with centrifugation at 9,000 ×g for 10 min. For other conditions, see Materials and Methods. A. Uptake into *E. coli* cells. B. Uptake into *S. aureus* cells. Cell turbidities in both figures were A_{660}=1.0 (●), 2.0 (■), and 3.0 (▲), respectively.

Table 2. Bacterial uptake rate of HEPT derivative.

Cell density (O.D. at 660 nm)	Uptake rate (nmole/min)	
	E. coli	*S. aureus*
1.0	0.180	0.095
2.0	0.231	0.115
3.0	0.404	0.147

Individual uptake rate was evaluated by using the slopes presented in Fig. 2.

strongly suggested that f-3 was taken up via facilitated diffusion (data not shown). The cytopathic effect of such lipophilic agents are generally concentration-dependent. Hence, we anticipated that the effect of HEPT derivatives on these strains would be strictly related to their rates of intracellular uptake.

Bacteriostatic Effect of HEPT Derivatives

We could not observe any antibiotic activities of HEPT derivatives from an agar diffusion test for growth

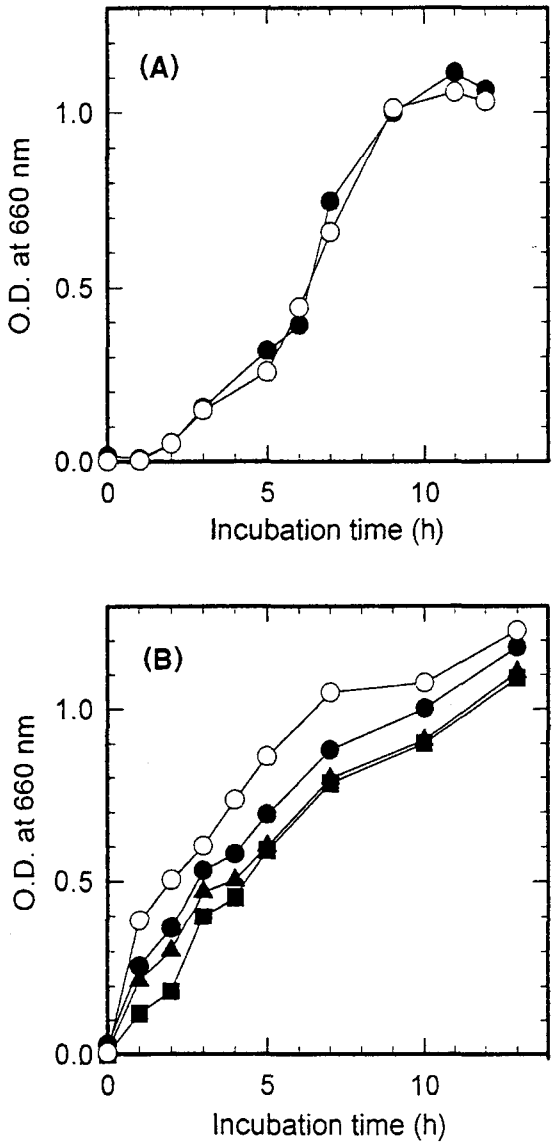


Fig. 2. Effect of HEPT derivative on the bacterial growth.

0.1 mM f-3 was added at time 0. Portions of culture broth at each time were taken and kept frozen. The resulting culture broths were then thawed, and was recorded the absorbance at 660 nm. A. Effect of f-3 on the growth of *E. coli*. Symbols used: (○), control; (●), with 0.1 mM f-3. B. Effect of f-3 on the growth of *S. aureus*. Symbols: (○), control; with f-3 at 0.05 mM (●), 0.1 mM (▲), and 0.2 mM (■).

inhibition, but we did find the activities by monitoring the cell growth quantitatively. In the course of vegetative growth of the above strains in liquid NM, the compound f-3 (0.1 mM) exerted a considerably time-delayed growth curve only in the case of *S. aureus* (Fig. 2). This result was against expectation as noted above. The lag time to achieve the same turbidity was proportionally extended with increasing concentration of the compound. Notably, the shape of the inhibitory curve was not sigmoid but rectangularly hyperbolic, indicating that the mode of inhibition of staphylococcal growth should be bacteriostatic.

Structural Requirement and Antibiotic Significance of HEPT Derivatives

Various HEPT derivatives including those that differed in linkers (S or CH_2) were examined for their putative differences in antibiotic activities against *S. aureus*. Data in Fig. 3 showed that, surprisingly, all of the compounds' activities appeared similarly not only in their growth-inhibitory shapes but also even in the lag time. This result indicated that the HEPT skeleton *per se* might be involved in bacteriostasis. This in turn, indicated that the cellular target may not be differential to the compounds. In other words, the mechanism of growth inhibition by HEPT derivatives was thought to be unusual. We then postulated that the compounds would be rather gram-specific. Accordingly, the effect of HEPT derivative f-3 was tested against some gram-positive and gram-negative bacteria. As predicted, the positive activity was found only in gram-positive strains (data not shown). To understand its precise mechanism, we calculated percent levels of growth inhibition of individuals relative to their respective controls at each time. The resulting values were then plotted as a function of incubation time. As shown in Fig. 4, the inhibitory peaks were found within a few hours of cell cultivation, suggesting that the compound might either be rapidly metabolized or destroyed. Unfortunately, we could not obtain clear data

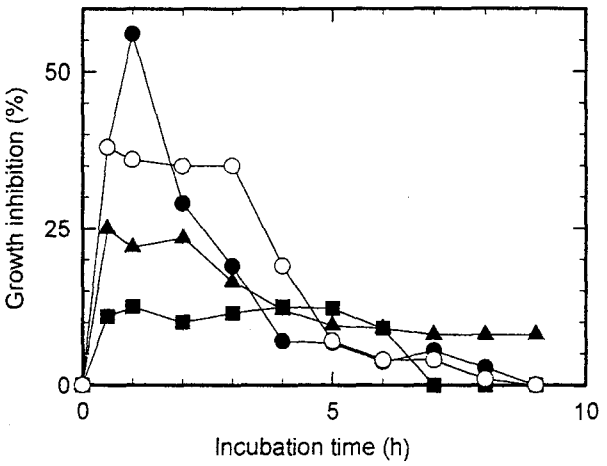


Fig. 4. Antibiotic property of HEPT derivative against gram-positive bacteria.

0.1 mM f-3 was supplemented to each of the cell suspensions (in NM) and incubation was performed at 37°C. Using the growth curves obtained in the presence or absence of the compound, the relative ratio of growth inhibition at the given time was evaluated. The resulting values were then plotted as a function of incubation time. Note that the peaks were likely to appear shortly after the beginning of the incubation of *C. glutamicum* (●), *S. aureus* (○), *B. megaterium* (▲), and *E. fuecium* (■).

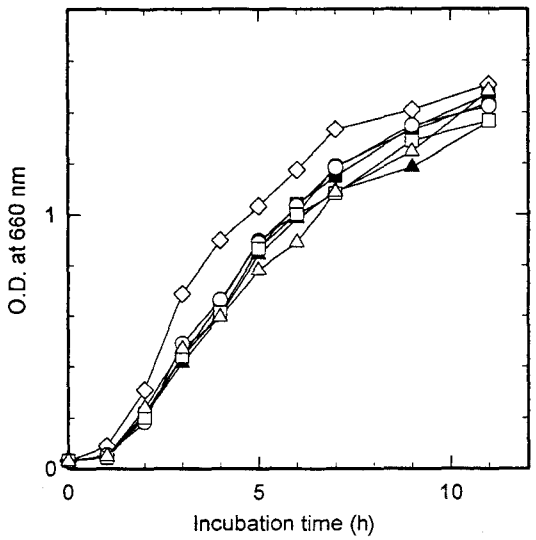


Fig. 3. Significance of aryl acyclouridine constituents (e.g., alkyl substituents or linkers) for bacteriostatic activity.

Logarithmically grown cells of *S. aureus* were used. Cell suspension containing NM was incubated at 37°C in absence (◇) or presence of 0.1 mM f-1 (●), f-2 (△), f-3 (□), f-4 (○), f-5 (■), or f-6 (▲).

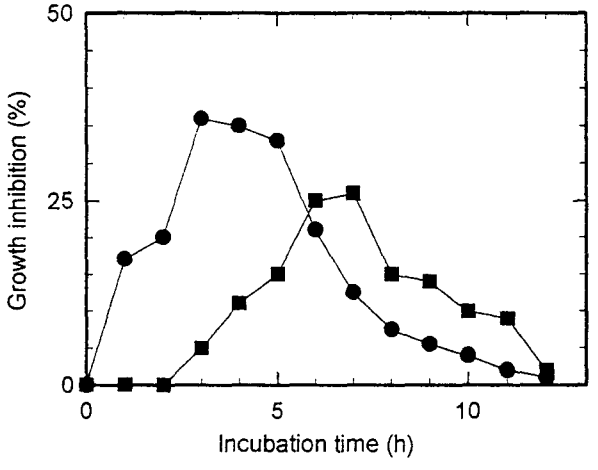


Fig. 5. Synergy of HEPT derivative with Chr.

Cells of *S. aureus* (1×10^4 cells/ml) were incubated at 37°C (in NM) with or without additives (additives; 1 μg/ml Chr or 1 μg/ml Chr plus 0.1 mM f-3). The curves, indicated as the percent ratio of growth inhibition relative to the control, were obtained in the same way as described in Fig. 4. Symbols: (■), Chr only; (●), Chr plus f-3. Note that the synergistic peak (●) was apparently shifted toward early period of incubation.

with *E. fuecium* because of its limited-growing property under the culture conditions employed.

Synergistic Effect of Chloramphenicol (Chr) with HEPT Derivatives

Various antibiotics were examined for their presummed synergy with f-3. Among those tested, the antistaphylococcal activity of Chr was distinctively enhanced (ca. 10%) with the aid of 0.1 mM f-3. Interestingly, the synergistic peak was achieved at about 3 hours, a similar period as that found in Fig. 4. The time shift of this peak was about 4 hours from that found without f-3 (Fig. 5). This observation strongly suggests that the synergy induced by f-3 may be unidirectional.

Effect of HEPT Derivatives on the Cellular Protein's Phenotype

An experiment to determine the cytopathic effect of f-3 was carried out for vegetative cells of *E. coli* and *S. aureus*. Aiming to access the nature of the bacteriostatic function of this compound, we attempted SDS-PAGE to analyse the effect of f-3 on the cellular protein's phenotype. Among the above two strains, we found that f-3 affected only staphylococcal physiology in terms of protein expression. It was noteworthy that there were no apparent changes in the protein phenotypes of cell-free extract except cell membranes. As can be seen in Fig. 6, a membrane-bound protein (70-kDa) was overexpressed from the growing cells of *S. aureus*. This event did not occur in nongrowing cells (intact cells) of *S. aureus*, strongly suggesting that the cell's growing state is essential for the compound-directed antibiosis.

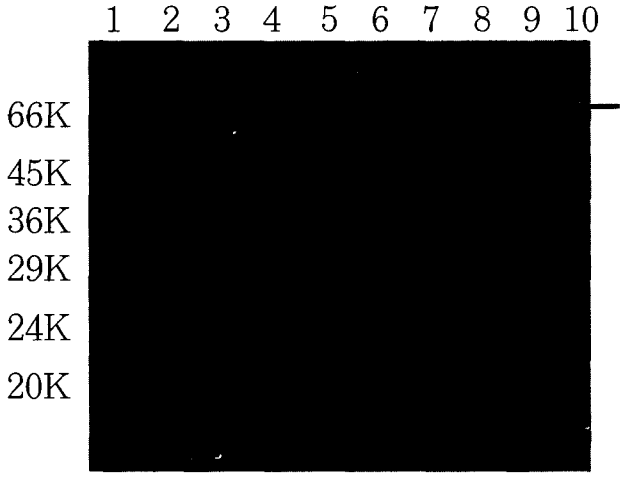


Fig. 6. Determination of cytopathic effect of HEPT derivative with SDS-PAGE.

Nongrowing or growing cells of *S. aureus* were treated with 0.1 mM f-3 and incubated for 2 h. After incubation, membrane-bound proteins were isolated (see Materials and Methods for conditions), and SDS-PAGE was carried out. Lanes from left to right: molecular weight markers (lane 1), nongrowing cells with ethanol for 2 h at 37°C (lane 2), with f-3 for 0 h at 0°C (lane 3), with ethanol for 0 h at 0°C (lane 4), and with f-3 for 2 h 37°C (lane 5); growing cells in the same way (lane 6 through 9) and the marker proteins (lane 10). Arrow points to a protein band overexpressed by the presence of f-3.

DISCUSSION

In this paper, we first described some of the antibacterial properties of HEPT derivatives other than their anti-HIV activities. It was interesting that their antibiotic activities appeared to be gram-specific. That is, the HEPT derivatives exhibited anti-gram-positive bacterial activities, as opposed to that found with AZT. The growth of *S. aureus* was not inhibited with AZT at a concentration of 0.1 mg/ml, whereas in *E. coli*, the inhibitory growth-curve was sigmoid (5 hours of time-lag; data not shown). These antibacterial activities are considered to be indispensable factors concerning the secondary diseases occasioned by bacteria.

Diverging nucleosides are generally known to interfere with mitochondrial-DNA replication [13]. The disturbing effect of AZT on mitochondrial bioenergetic function [17] is thought to be different from the above cytotoxicity. The relationship between these drugs (nucleoside analogs) and cellular targets is still controversial [5]. Meanwhile, although the nature of the 70-kDa protein (Fig. 6) was not clarified here, a putative role of serving HEPT derivatives in disturbing the cell membrane's energetics would be presumable. Among the membrane-resident proteins in bacteria, ~70-kDa proteins are often found as being part of cation motive proton pumps [1, 6, 20, 26]. To elucidate the relationship between this protein and HEPT derivatives, studies are now being pursued in our laboratory.

In contrast with AZT, HEPT or its derivatives are not currently applied to patients with AIDS. Nonetheless, HEPT or its analogs are possibly of great potential as an alternative to AZT etc., for medication of the disease, as their cytopathic effects are known to be highly selective [4]. In addition, their synergistic properties with antibiotics (e.g., Chr) seem to be a matter of no little interest. Chr is usually bacteriostatic in action, but may become bacteriocidal at high concentrations. This drug is recognized to inhibit primarily peptide bond formation [12]. Clinically, the use of Chr is strictly restricted because of its incompatibility with many drugs. Therefore, the data in Fig. 5 indicating a HEPT derivative-directed synergy are remarkable. Also, it is noteworthy that HEPT derivatives and AZT are active selectively against gram-positive and negative bacteria, respectively.

Concerning their antibacterial properties, it is readily anticipated that the types of secondary infections, often lethal to the immunologically suppressed patients, can be

influenced by the parental drug of choice. For this reason, we would like to emphasize here the significance of HEPT derivatives as antibiotics. By their long-term administrations in such patients, the bodily distribution of bacterial species would be changed along with differential typing of lethal pathogenesis.

Acknowledgments

This work was supported in part by the grant 971-0711-096-1 from The Korea Science & Engineering Foundation, Korea.

REFERENCES

1. Apell, H. J. and M. Solioz. 1990. Electrogenic transport by the *Enterococcus hirae* ATPase. *Biochim. Biophys. Acta* **1017:** 221–228.
2. Baba, M., E. De Clercq, H. Tanaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, K.Umezu, H. Nakashima, S. Mori, S. Shigeta, R. T. Walker, and T. Miyasaka. 1991. Potent and selective inhibition of human immunodeficiency virus type 1 (HIV-1) by 5-ethyl-6-phenylthiouracil derivatives through their interaction with the HIV-1 reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **88:** 2356–2360.
3. Baba, M., S. Shigeta, H. Tanaka, T. Miyasaka, M. Ubasawa, K. Umezu, R. T. Walker, R. Pauwels, and E. De Clercq. 1992. Highly potent and selective inhibition of HIV-1 replication by 6-phenylthiouracil derivatives. *Antiviral Res.* **17:** 245–264.
4. Baba, M., H. Tanaka, E. De Clercq, R. Pauwels, J. Balzarini, D. Schols, H. Nakashima, C. F. Perno, R. T. Walker, and T. Miyasaka. 1989. Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. *Biochem. Biophys. Res. Commun.* **165:** 1375–1381.
5. Chen, C. H., M. Vazquez-Padua, and Y.-C. Cheng. 1991. Effect of anti-human immunodeficiency virus nucleoside analogs on mitochondrial DNA and its implication for delayed toxicity. *Mol. Pharmacol.* **39:** 625–628.
6. Epstein, W., V. Whitelaw, and J. Hesse. 1978. A K^+ transport ATPase in *Escherichia coli*. *J. Biol. Chem.* **253:** 6666–6668.
7. Hwang, S.-Y., M.-R. Ki, S.-Y. Cho, W.-J. Lim, and I.-D. Yoo. 1996. Competitive spectrophotometry for microbial dipeptide transport systems. *J. Microbiol. Biotechnol.* **6:** 92–97.
8. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
9. Lerner, C. W. and M. L. Tapper. 1984. Opportunistic infection complicating acquired immune deficiency syndrome. *Medicine* **63:** 155–158.
10. Lim, W.-J., K.-M. Choi, and S.-Y. Hwang. 1993. Optimization of an intact cell system of *Rhodocyclus gelatinosus* KUP-74 for δ-aminolevulinic acid production. *J. Microbiol. Biotechnol.* **3:** 244–251.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
12. Mcevoy, G. K. 1991. *AHFS Drug Information* [A]. pp. 191–194. American Society of Hospital Pharmacists, Inc. U.S.A.
13. Mhiri, C., M. Baudrimount, G. Bonne, C. Geny, F. Degoul, C. Marsac, E. Roullet, and R. Gherardi. 1991. Zidovudine myopathy: a distinctive disorder associated with mitochondrial dysfunction. *Annu. Neurol.* **29:** 606–614.
14. Mitsuya, H. and S. Broder. 1985. Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA* **83:** 1911–1915.
15. Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder. 1985. 3'-Azido-3'-deoxythymine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. USA* **82:** 7096–7100.
16. Miyasaka, T., H. Tanaka, M. Baba, H. Hayakawa, H. Walker, J. Balzarini, and E. De Clercq. 1989. A novel lead for specific anti-HIV-1 agents: 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thimine. *J. Med. Chem.* **32:** 2507–2509.
17. Modica-Napolitano, J. S. 1993. AZT causes tissue-specific inhibition of mitochondrial bioenergetic function. *Biochem. Biophys. Res. Commun.* **194:** 170–177.
18. Munch-Petersen, B. L. Cloos, G. Trysted, and S. Eriksson. 1991. Diverging substrate specificity of pure human thymidine kinases-1 and 2 against antiviral dideoxynucleosides. *J. Biol. Chem.* **266:** 9032–9038.
19. Pitchenik, A. E. and H. A. Rubinson. 1985. The radiographic appearance of tuberculosis in patients with the aquired immunodeficiency syndrome (AIDS) and pre-AIDS. *An. Rev. Resp. Dis.* **131:** 393. 1986.
20. Solioz, M., S. Mathews, and P. Furst. 1987. Cloning of the K^+-ATPase of *Streptococcus faecalis*: Structural and evolutionary implications of its homology to the KdpB-protein of *Escherichia coli*. *J. Biol. Chem.* **262:** 7358–7362.
21. Tanaka, H., M. Baba, H. Hayakawa, T. Sakamaki, T. Miyasaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, S. Shigeta, R. T. Walker, J. Balzrin, and E. De Clercq. 1991. A new class of HIV-1-specific 6-substituted acyclouridine derivatives: Synthesis and anti-HIV-1 activity of 5- or 6-substituted acyclouridine analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thimine (HEPT). *J. Med. Chem.* **34:** 349–357.
22. Tanaka, H., H. Takashima, M. Ubasawa, K. Sekiya, I. Nitta, M. Baba, S. Shigeta, R. T. Walker, E. De Clercq, and T. Miyasaka. 1992. Synthesis and antiviral activity of deoxy analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thimine (HEPT) as potent and selective anti-HIV agents. *J. Med. Chem.* **35:** 4713–4719.

23. Wofsy, C. B. 1986. Antimicrobial therapy of infections in patients with aquired immunodeficiency syndrome, pp. 388–390. *In* P. K. Peterson and J. Verhoef (eds.), *The Antimicrobial Agents Annual 1*. Elsevier Science Publishers B. V. Amsterdam.
24. Yarchoan, R., P. Brouwers, J. M. Pluda, K. M. Wyvill, R. V. Thomas, N. Hartman, H. Mitsuya, D. G. John, and S. Broder. 1990. Long-term toxicity/activity profile of 2',3'-dideoxyinosine in AIDS or AIDS-related complex. *Lancet* **336:** 526–529.
25. Yun, S.-K. and S.-Y. Hwang. 1997. Characteristics of ATPases present in everted membrane vesicles of *Helicobacter pylori*. *J. Microbiol. Biotechnol.* **7:** 167–173.
26. Yun, S.-K. and S.-Y. Hwang. 1997. Expression of latent P-type ATPases and their presumptive roles in cell membranes of *Helicobacter pylori*. *J. Microbiol. Biotechnol.* **7:** 378–385.