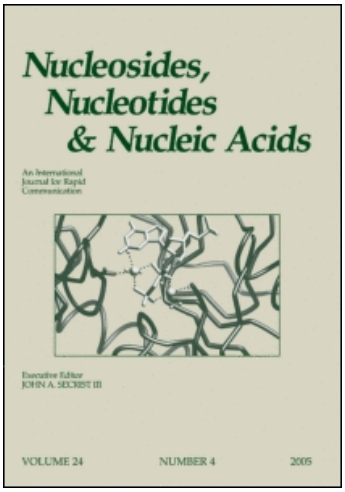

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SYNTHESIS AND IN VITRO ANTIBACTERIAL ACTIVITY OF NOVEL 5'-O-ANALOG DERIVATIVES OF ZIDOVUDINE AS POTENTIAL PRODRUGS

Guillermo N. Moroni[a]; Patricia M. Bogdanov[a]; Margarita C. Briñón[a]
[a] Departamento de Farmacia, Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina

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SYNTHESIS AND IN VITRO ANTIBACTERIAL ACTIVITY OF NOVEL 5′-O-ANALOG DERIVATIVES OF ZIDOVUDINE AS POTENTIAL PRODRUGS

Guillermo N. Moroni, Patricia M. Bogdanov, and Margarita C. Briñón*

Departamento de Farmacia, Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, 5000-Córdoba, Argentina

ABSTRACT

An efficient, short synthesis of four potential prodrugs of 3′-azido-3′-deoxythymidine (AZT) and their antibacterial activity are reported. The 5′-OH group of AZT was functionalized with oxalyl chloride obtaining an acyl chloride derivative (AZT-Ox), which by further transformation with leucine, isoleucine and valine amino acids led to the corresponding AZT analogs, namely AZT-Leu, AZT-iLeu and AZT-Val. A carboxyl acid derivative (AZT-Ac) was also obtained by hydrolysis of AZT-Ox. These compounds, which exhibit anti HIV activity, have killed collection and clinical strains of some opportunistic infectious agents in AIDS-related complex. Thus, the clinical strains, *K. oxytoca*, *S. typhi* and *K. pneumoniae*, and collection strain *K. pneumoniae* ATCC 10031 showed sensitivity to antibiotics. The activity order for the studied compounds against the most sensitive strain (*K. pneumoniae* ATCC 10031) was AZT-Leu > AZT-iLeu > AZT-Val > AZT-Ac > AZT. On the other hand, the activity order for the second most sensitive strain (*K. oxytoca*) was AZT-Leu > AZT-Val = AZT-Ac > AZT-iLeu > AZT. The most effective antibacterial drug AZT-Leu, M.I.C. = 0.125 μg mL^{-1}) was 16 times more active than AZT (AZT, M.I.C. = 2 μg mL^{-1}) against *K.*

*Corresponding author. Fax: 54-351-4334127; E-mail: macribri@dqo.fcq.unc.edu.ar.

pneumoniae ATCC 10031. Thus, this compound may therefore have better clinical potential than AZT for the treatment of AIDS-related complex.

3′-azido-3′-deoxythymidine (AZT, zidovudine, 1), the first drug to show clinical efficacy in the treatment of acquired immunodeficiency syndrome (AIDS) or AIDS-related complex is a powerful inhibitor of the replication of human immunodeficiency virus (HIV-1)[1–3]. AZT, which is intracellularly converted into its 5′-O-triphosphate analog, competes with the natural nucleoside thymidine-5′-triphosphate for binding to the retroviral transcriptase reverse enzyme, and upon incorporation into viral DNA results in premature termination of DNA synthesis[4,5]. Although AZT therapy reduces mortality and morbidity in some AIDS patients[6–8]. with clinical improvement leading to a decrease of opportunistic infections[2], bone marrow toxicity resulting in anemia and leucopoenia detracts from its clinical utility[7,9].

The failure of existing nucleosidic drugs to totally stop progression of AIDS is due partly to their failure to maintain adequate triphosphate levels at the site of replication[10], and because of their relatively short half-lives[11]. Despite these undesirable effects, this nucleoside transcriptase reverse inhibitor (NRTI) continues to play an important role in the therapy of AIDS extending the life expectancy of individuals with AIDS, particularly in combination with other NRTI, non-nucleoside transcriptase reverse and protease inhibitors[12–14].

In HIV-infected individuals, the primary target of therapy is the HIV-1, but most of the clinical signs are related to the effect of HIV on the immune system, which leads to progressive immunodeficiency. Consequently, the other potential therapeutic applications are aimed at the immune system and treatment of opportunistic infections[2,15–18]. The use of AZT in AIDS-patients showed an effective activity against etiologic agents of opportunistic infections, with in vitro bactericidal action against members of Enterobacteriaceae family, including *Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri, Enterobacter aerogenes or Escherichia coli* strains, but no activity against *Pseudomonas aeruginosa* and Gram-positive bacteria strains[17–23].

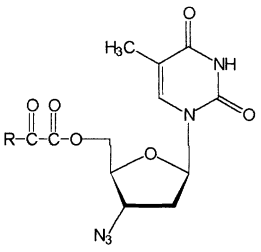
Regarding efficient anti-HIV-1 agents, we have recently developed more lipophilic AZT derivatives with antiviral and bactericidal activity[24,25]. In attempts to overcome the problems of rapid elimination of AZT and to increase its therapeutic efficacy, numerous AZT prodrugs have been reported in the literature[26–31]. The mechanism of the antiviral action of these AZT prodrugs is based on the enzymatical hydrolysis of the labile 5′-O-bonds between the drug (AZT) and its spacer group[32]. Focusing our attention on opportunistic infections in AIDS-related complex patients, we here report an efficient, short synthesis and antibacterial activity of four novel potential prodrugs of zidovudine, which have also proved anti HIV-1 activity[33].

RESULTS AND DISCUSSION

Chemistry

In the current study, our synthesis strategy was focused on the association of 5′-OH of the zidovudine with essential amino acids. The first step was the reaction of 3′-azido-3′-deoxylthymidine (AZT, **1**) with oxalyl chloride, obtaining 3′-azido-3′-deoxy-5′-O-oxalylthymidine chloride (AZT-Ox, **2**) in quantitative yields. Although reactions with acyl chlorides could take place either with the amine or acid groups[34], the reaction conditions of AZT-Ox with the amino acids, L-leucine, L-isoleucine and L-valine, yielded the corresponding, 3′-azido-3′-deoxy-5′-O-oxalyl-*N*-leucinethymidine (AZT-Leu, **3**), 3′-azido-3′-deoxy-5′-O-oxalyl-*N*-isoleucine-thymidine (AZT-iLeu, **4**) and 3′-azido-3′-deoxy-5′-O-oxalyl-*N*-valinethymidine (AZT-Val, **5**) amides (Fig. 1). The hydrolysis of the halogen group (C1) of **2** with a solution of $NaHCO_3$ 0.05 M, yielded 3′-azido-3′-deoxy-5′-O-oxalylthymidine acid (AZT-Ac, **6**) (Fig. 1).

The structures of nucleoside derivatives **3–6** were characterized by spectroscopic data. The ^1H NMR, ^{13}C NMR, DEPT 135 and COSY homo and heteronuclear spectra of **3–6** were performed in DMSO-d_6, using TMS as internal standard. The proton signals of CH_3-base, H-1′, H-2′, H-3′ and H-6 correlated well with those of AZT[24], while H-4′ ($\delta \cong 4.14$ ppm) and H-5′ ($\delta \cong 4.40$ ppm) showed certain chemical shift differences with the parent compound of about 0.35 ppm and 0.80 ppm, respectively. Proton signals were successfully assigned using COSY homo (H-H) and heteronuclear (C-H) spectra. The assignment of all exchangeable protons was confirmed by the addition of D_2O, which showed that the NH signals of the base ($\delta \cong 11.3$ ppm) and amino acid moieties ($\delta \cong 9$ ppm) disappear, as well as the fact that no 5′-hydroxyl groups were present in compounds **3–6**. CH attached to NH of the amino acid in **3–5** evidenced several chemical shift differences with the corresponding free amino acid of about 1.10 ppm for the NH proton with a difference of about 5 ppm.



Comp.	R
AZT-Ox, 2	Cl
AZT-Leu, 3	NH-CH(COOH)CH_2-CH$(CH_3)_2$
AZT-iLeu, 4	NH-CH(COOH)CH$(CH_3)CH_2$-CH_3
AZT-Val, 5	NH-CH(COOH)CH$(CH_3)_2$
AZT-Ac, 6	OH

Figure 1. Chemical structures of zidovudine analogs (**2–6**).

The most significant features in the ^{13}C NMR spectra were the signals at $\delta \cong 157$–159 and $\delta \cong 159$–160 corresponding to the CO carbons of the oxalyl moiety as well as the carboxylic group of the amino acids at $\delta \cong 173$.

In the IR spectra of **3–6** characteristic vibrational regions of the structural features were found. The intense absorption at $2105\,cm^{-1}$ and $1703\,cm^{-1}$ indicated the presence of azido and carbonyl groups, respectively, while those at $3400\,cm^{-1}$ (NH) and $3261\,cm^{-1}$ (OH) corresponded to amino acid attached moieties.

Antibacterial Activity

Considering the great amount of opportunistic infections associated with AIDS, the inhibition effect of novel zidovudine derivatives (**3–6**) on twenty two bacterial species was studied. Results demonstrated that these compounds are able to kill some culture collection and clinical strains of specimens isolated from infected patients, thereby exhibiting a bactericidal property (Table 1).

According to data from Table 1, AZT-Leu, AZT-iLeu, AZT-Val and AZT-Ac showed in vitro antibacterial activity against different *Klebsiella* strains. Thus, *Klebsiella pneumoniae* ATCC 10031 exhibited a Minimum Inhibitory Concentration (M.I.C.) of 0.125–16 $\mu g\,mL^{-1}$, the clinical strains ranged between 2–16 $\mu g\,mL^{-1}$ and *Klebsiella oxytoca* a M.I.C. of 0.5–4 $\mu g\,mL^{-1}$. AZT-Leu has shown a higher inhibitory effect than other drugs. *Klebsiella oxytoca* was fairly more sensitive to AZT-Leu than to AZT-Val, AZT-Ac, and AZT-iLeu. In addition, only AZT-Leu and AZT-iLeu have evidenced activity against *Salmonella typhi* with M.I.C. of 2 and 4 $\mu g\,mL^{-1}$ respectively, while *Enterobacter cloacae* and *Enterobacter aerogenes* were inhibited only by AZT-Val (M.I.C. = 2–4 $\mu g\,mL^{-1}$ and 64 $\mu g\,mL^{-1}$, respectively) and AZT-Ac (M.I.C. = 16 and 64 $\mu g\,mL^{-1}$, respectively). In all cases, the MIC's were the same as Minimum Bactericidal Concentration (M.B.C.). The studied compounds displayed no activity against all tested strains of Gram-positive bacteria and *Pseudomonas* species (M.I.C. $> 128\,\mu g\,mL^{-1}$) in agreement with previous reports by other authors on zidovudine[19].

It is well known that the advantageous therapy for opportunistic infections, used by HIV-infected patients leads to a complex drug administration schedule with numerous agent interactions[35]. Additionally, it has been shown that a combined treatment of an antiviral and an antibiotic presented advantages as regard single drug treatment in AIDS and the AIDS-related complex[36]. Due to the above reasons, the results obtained for these novel drugs suggest a potential applicability in the treatment of AIDS.

Preliminary studies[37] about the antibiotic effect of these novel compounds (**3–6**) indicate that the mechanism could be carried out through free radical production, especially by the generation of superoxide anion, and a

Table 1. Antibacterial Activity of the Novel Compounds **3–6** Measured by M.I.C. ($\mu g\,mL^{-1}$)

Strain	AZT-Leu	AZT-iLeu	AZT-Val	AZT-Ac
	$\mu g\,mL^{-1}$			
Klebsiella pneumoniae ATCC 10031*,[a]	0.125	2	4	16
Escherichia coli ATCC 25922*,[b]	> 128	> 128	> 128	> 128
Escherichia coli ATCC 35218*,[b]	> 128	> 128	> 128	> 128
Pseudomonas aeruginosa ATCC 27853*,[b]	> 128	> 128	> 128	> 128
Staphylococcus aureus ATCC 29213*,[b]	> 128	> 128	> 128	> 128
Staphylococcus aureus ATCC 25923*,[b]	> 128	> 128	> 128	> 128
Staphylococcus epidermidis ATCC 12228*,[b,]	> 128	> 128	> 128	> 128
Enterococcus faecalis ATCC 29212*,[b,]	> 128	> 128	> 128	> 128
Staphylococcus aureus meticillin R**,[b]	> 128	> 128	> 128	> 128
*Staphylococcus haemolyticus***,[b]	> 128	> 128	> 128	> 128
*Enterobacter cloacae***,[b]	> 128	> 128	2–4	16
*Enterobacter aerogenes***,[b]	> 128	> 128	64	64
*Klebsiella pneumoniae***,[c]	4	4	4	16
*Klebsiella pneumoniae***,[c]	8	8	8	16
*Klebsiella pneumoniae***,[c]	2	16	8	8
*Klebsiella oxytoca***,[d]	0.5	4	2	2
*Salmonella typhi***,[d]	2	4	–	–
*Shigella dysenteriae***,[b]	> 128	> 128	> 128	> 128
*Shigella flexneri***,[b]	> 128	> 128	> 128	> 128
*Enterococcus faecalis***,[b]	> 128	> 128	> 128	> 128
*Enterococcus faecalis***,[b]	> 128	> 128	> 128	> 128
*Enterococcus faecalis***,[b]	> 128	> 128	> 128	> 128

*American Type Culture Collection strains; ** clinical strains.
MICs determined for AZT under the same experimental conditions that of **3–6**: a) M.I.C. = 2 $\mu g\,mL^{-1}$; b) M.I.C. = > 128 $\mu g\,mL^{-1}$; c) MICs = 1–32 $\mu g\,mL^{-1}$; d) MIC = 16 $\mu g\,mL^{-1}$.

subsequent oxidative injury at cellular level. However, further assays need to be performed to propose a general mechanism of antibacterial action for these novel compounds.

CONCLUSION

We have synthesized and characterized four novel potential prodrugs of AZT, AZT-Leu, AZT-iLeu, AZT-Val and AZT-Ac with antibiotic activity. Previous studies on peripheral blood mononuclear cells (PBMC) using AZT as standard compound have demonstrated that these compounds present anti HIV activity. In the mentioned study, AZT-Leu showed to be the most active compound ($IC_{50} = 0.01\,\mu g\,mL^{-1}$), while the other including AZT exhibited the following anti HIV decreasing order activity, AZT-Val ($IC_{50} = 0.015$

$\mu g\,mL^{-1}$), AZT ($IC_{50} = 0.02\,\mu g\,mL^{-1}$), AZT-Ac ($IC_{50} = 0.025\,\mu g\,mL^{-1}$ and AZT-iLeu ($IC_{50} = 0.035\,\mu g\,mL^{-1}$)[33].

Among twenty two bacteria strains studied, the collection strain *K.pneumoniae* ATCC 10031 and the clinical strains *K. pneumoniae*, *K. oxytoca* and *S. typhi* proved to be the most sensitive strains to antibiotic killing effect. The decrease activity order for the studied compounds was AZT-Leu > AZT-iLeu > AZT-Val > AZT-Ac > AZT against the most sensitive strain (*K. pneumoniae* ATCC 10031) and AZT-Leu > AZT-Val > AZT-Ac > AZT-iLeu > AZT against the following one (*K. oxytoca*). AZT-Leu, the most effective antibacterial agent (M.I.C. $= 0.125\,\mu g\,mL^{-1}$), was 16 times more active against *K. pneumoniae* ATCC 1003 as compared to AZT (M.I.C. $= 2\,\mu g\,mL^{-1}$), assayed under the same experimental conditions.

These compounds were more effective against Gram negative bacteria than Gram positive strains, demonstrating their potential biologic activity against *Enterobacteriaceae* family. These results are in agreement with data reported for AZT[15]. It is relevant to point out that the major pathogen for community-acquired pneumonia (CAP) is *K. pneumoniae*[38]. Taking into account the rising prevalence of CAP in patients with HIV-1 infection, as well as the increasing resistance to antibiotics against Gram-negative bacilli, these new agents could be successful pharmacological agents in the treatment of AIDS due to not only their antiviral but also their antibacterial activities.

EXPERIMENTAL SECTION

Apparatus. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer at 200.13 MHz, using DMSO-d_6 (99%, SIGMA) as solvent and tetramethylsilane (TMS) as internal standard. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. IR spectra were obtained from potassium bromide discs on a Nicolet 5 SXC FT-IR.

Materials. All chemicals and reagents were of analytical grade. THF (Sintorgan) was dried with potassium hydroxide. Oxalylchloride was obtained from Merk C. Leucine, isoleucine and valine 99% were obtained from SIGMA Co and used as their sodium salts. Analytical thin layer chromatography (TLC) was performed on the corresponding precoated silicagel 60 F254 plates (Merk). The nucleoside 3′-azido-3′-deoxythymidine (AZT), generously supplied by Filaxis (Buenos Aires, Argentina), was used without purification. The studied compounds AZT-Leu (**3**), AZT-iLeu (**4**), AZT-Val (**5**) and AZT-Ac(**6**) (Fig. 1) were synthesized as follows.

3′-Azido-3′-deoxy-5′-O-oxalylthymidine chloride (AZT-Ox, 2). Zidovudine (0.400 g, 1.5 mmol) was added rapidly to oxalyl chloride (1 mL, 11.66 mmol). The reaction mixture was stirred in an ice bath for 30 min

and then for 2 h at room temperature. TLC (ethyl acetate – acetone, 10:4 – v/v) showed the absence of zidovudine. The solution was concentrated to dryness under reduced pressure, at a temperature not exceeding 40°C, in order to remove hydrogen chloride and excess oxalyl chloride. AZT-Ox (**2**) was obtained in quantitative yield (0.495–0.500 g).

General procedure for the amino acid esters synthesis. To a suspension of sodium amino acid salt (3 mmol) in dry THF (15 mL) was added drop wise a solution of **2** (1.5 mmol) in dry THF (5 mL) while stirring at room temperature for 2 hours, when the TLC assay (ethyl acetate – acetone – glacial acetic acid, 10:6:0.1) revealed the absence of **2**. The remaining amino acid salt was removed by filtration and the solution was then concentrated to dryness under reduced pressure, at a temperature not exceeding 40°C, to obtain the desired product (**3–5**) with yields of 85–95%. Products were precipitated from acetyl acetate (minimum volume) by addition of cool hexane. The solid residue was washed with a cool solution of Hcl (0.5 M) and dried under reduced pressure.

3′-Azido-3′-deoxy-5′-O-oxalyl-N-leucinethymidine (AZT-Leu, 3). m.p.: 121–122°C. ^{1}H NMR (DMSO-d_6) δ 0.86 [d, $J = 6.65$, 6 H, $(CHCH_3)_2$], 1.57 [dd, $J = 4.30$, $J = 6.26$, 2 H, $CH_2CH(CH_3)_2$], 1.75 [s, 3 H, CH_3-base], 1.73 [m, $J = 6.65$, 1 H, $(CHCH_3)_2$], 2.29 [m, $J = 6.65$, 1 H, H-2′$_b$], 2.36 [m, $J = 7.04$, 1 H, H-2′$_a$], 4.16 [m, $J = 3.52$, 1 H, H-4′], 4.32 [dd, $J = 8.22$, 1 H, $NHCH(COOH)CH_2$], 4.33[dd, $J = 10.56$, 2 H, H-5′], 4.55 [m, $J = 3.52$, 1 H, H-3′], 6.19 [t, $J = 6.65$, 1 H, H-1′], 7.58 [s, 1 H, H-6], 8.75 [s, 1 H, COOH], 9.15 [d, $J = 8.22$, 1 H, NH-*aa*], 11.32 [s, 1 H, NH-*base*]; ^{13}C NMR (DMSO-d_6), 11.82 [CH_3-base], 20.98 [$CH(CH_3)CH_3$], 22.83 [$CH(CH_3)CH_3$], 24.36 [$CH(CH_3)CH_3$], 35.50 [CH_2-2′], 39.03 [$NHCH(COOH)CH_2$], 50.75 [$NHCH(COOH)CH_2$], 61.00 [CH-3′], 65.80 [CH_2-5′], 80.46 [CH-4′], 83.61 [CH-1′], 110.21 [C-5], 135.85 [CH-6], 150.49 [CO-2], 156.82 [AZT-OC-(-O)C(O)-], 160.02 [AZT-OC(O)C(O)-], 163.66 [CO-4], 172.78 [NHCH-COOH]. λ_{max}(water)/nm 211.2 and 266.0; ν_{max}(KBr/cm^{-1}) 3309.5 (NH-base); 3189.4 (OH acid); 2105.8 (N_3); 1701.2 (CO); 1527.2 (NH-amino acid).

3′-Azido-3′-deoxy-5′-O-oxalyl-N-isoleucinethymidine (AZT-iLeu, 4). m.p.: 85–86°C. ^{1}H NMR (DMSO-d_6) δ 0.85 [t, $J = 6.21$, 3 H, $CH(CH_3)$-CH_2CH_3], 0.87 [d, $J = 8.04$, 3 H, $CH(CH_3)CH_2CH_3$], 1.55 [m, $J = 6.21$, 2 H, $CH(CH_3)CH_2CH_3$)], 1.58 [m, $J = 4.02$, 1 H, $CH(CH_3)CH_2CH_3$], 1.75 [s, 3 H, CH_3-*base*], 2.26 [m, $J = 6.57$, 1 H, H-2′$_b$], 2.33 [m, $J = 7.30$, 1 H, H-2′$_a$], 4.15 [m, $J = 3.65$, 1 H, H-4′], 4.30 [dd, $J = 8.04$, 1 H, NHCH-(COOH)CH], 4.42 [dd, $J = 11.14$, 2 H, H-5′], 4.56 [m, $J = 3.28$, 1 H, H-3′], 6.19 [t, $J = 6.58$, 1 H, H-1′], 7.58 [s, 1 H, H-6], 8.78 [s, 1 H, COOH], 9.18 [d, $J = 8.41$, 1 H, NH-*aa*], 11.33 [s, 1 H, NH-*base*]; ^{13}C NMR (DMSO-d_6),

11.86 [CH_3-*base*], 20.97 [$CH(CH_3)CH_2CH_3$], 22.85 [$CH(CH_3)CH_2CH_3$], 24.36 [$CH(CH_3)CH_2CH_3$], 35.52 [CH_2-2′], 39.03 [$CH(CH_3)CH_2CH_3$], 50.72 [NH*C*H(COOH)CH], 61.05 [*C*H-3′], 65.82 [CH_2-5′], 80.48 [*C*H-4′], 83.58 [*C*H-1′], 110.23 [*C*-5], 135.86 [*C*H-6], 150.50 [*C*O-2], 156.81 [AZT-OC(O)*C*(O)-], 159.99 [AZT-O*C*(O)C(O)-], 163.65 [*C*O-4], 172.82 [CH(NH)*C*OOH]. λ_{max}(water)/nm 210.8 and 266.4; ν_{max}(KBr/cm^{-1}) 3276.9 (NH-base); 3078.4 (OH acid); 2105.8 (N_3); 1698.4 (CO); 1520.2 (NH-amino acid).

3′-Azido-3′-deoxy-5′-O-oxalyl-N-valinethymidine (AZT-Val, 5). m.p.: 76–77°C. ^{1}H NMR (DMSO-d_6) δ 0.89 [d, $J = 5.48$, 6 H, $(CHCH_3)_2$], 1.76 [s, 3 H, CH_3-base], 2.16 [m, $J = 6.65$, 1 H, $(CHCH_3)_2$], 2.26 [m, $J = 6.65$, 1 H, H-2′$_b$], 2.32 [m, $J = 7.43$, 1 H, H-2′$_a$], 4.12 [dd, $J = 7.83$, 1 H, NHC*H*(COOH)CH], 4.15 [m, $J = 3.52$, 1 H, H-4′], 4.43 [dd, $J = 10.56$, 2 H, H-5′], 4.55 [m, $J = 3.52$, 1 H, H-3′], 6.18 [t, $J = 6.65$, 1 H, H-1′], 7.58 [s, 1 H, H-6], 7.67 [s, 1 H, COO*H*], 8.70 [d, $J = 7.83$, 1 H, N*H-aa*], 11.33 [s, 1 H, N*H-base*]; ^{13}C NMR (DMSO-d_6), 11.80 [CH_3-base], 18.06 [$CH(CH_3)CH_3$], 19.07 [$CH(CH_3)CH_3$], 29.73 [$CH(CH_3)CH_3$], 35.42 [CH_2-2′], 58.11 [NH*C*H(COOH)], 60.83 [*C*H-3′], 65.56 [CH_2-5′], 80.38 [*C*H-4′], 83.50 [*C*H-1′], 110.09 [*C*-5], 135.79 [*C*H-6], 150.37 [*C*O-2], 157.02 [AZT-OC(O)*C*(O)-], 160.31 [AZT-O*C*(O)C(O)-], 163.54 [*C*O-4], 171.66 [CH(NH)*C*OOH]. λ_{max}(water)/nm 211.4 and 265.8; $\nu_{max.}$(KBr/cm^{-1}) 3399.2 (NH); 3261.7 (OH acid); 2105.8 (N_3); 1703.5 (CO); 1530.4 (NH-amino acid).

3′-Azido-3′-deoxy-5′-O-oxalylthymidine acid (AZT-Ac, 6). -3′-azido-3′-deoxy-5′-O-oxalylthymidine chloride (537 mg, 1.5 mmol) was added to a stirred solution of $NaHCO_3$ (10 mL, 0.05 M) at room temperature until TLC showed the absence of **2**. Then the solution was lyophilized and the residue was washed with cool HCl (0.5 M) and dried under reduced pressure (458 mg, 95% yield). m.p.: 97–98°C. ^{1}H NMR (DMSO-d_6) δ 1.78 [s, 3 H, CH_3], 2.29 [m, $J = 6.84$, 1 H, H-2′$_b$], 2.37 [m, $J = 7.81$, 1 H, H-2′$_a$], 4.11 [m, $J = 3.90$, 1 H, H-4′], 4.42 [dd, $J = 9.77$, 2 H, H-5′], 4.52 [m, $J = 3.41$, 1 H, H-3′], 6.17 [t, $J = 6.84$, 1 H, H-1′], 7.51 [s, 1 H, H-6], 11.32 [s, 1 H, N*H*]; ^{13}C NMR (DMSO-d_6), 11.91 [CH_3], 35.68 [CH_2-2′], 60.48 [*C*H-3′], 65.28 [CH_2-5′], 80.37 [*C*H-4′], 83.47 [*C*H-1′], 110.13 [*C*-5], 135.70 [*C*H-6], 150.39 [*C*O-2], 158.37 [AZT-O*C*(O)C(O)-], 158.56 [AZT-OC(O)-*C*(O)OH], 163.60 [*C*O-4]. λ_{max}(water)/nm 208.8 and 266.0; ν_{max}(KBr/cm^{-1}) 3475.5 (NH); 3180.2 (OH); 2105.8 (N_3); 1708.6 (CO).

Antibacterial Activity

Antibiotics. The Minimum Inhibitory Concentration (M.I.C., μg mL^{-1}) was determined in a Müeller Hinton broth (pH = 7.4) using the

standard tube dilution method according to the procedures of the NCCLS[39]. The Minimum Bactericidal Concentration (M.B.C., $\mu g\,mL^{-1}$) was assayed in a Müeller Hinton agar (pH 7.4) using subcultures which exhibited the 99.9% death of bacterial population. The drugs were used as freshly made solution stock in DMSO/Sorensen buffer pH 7.4 at a concentration of $5120\,\mu g\,mL^{-1}$, and for M.I.C. assays in dilution test, within a 0.125 to $128\,\mu g\,mL^{-1}$ range.

Bacterial strains. Strains were obtained from the American Type Culture Collection: *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213; *S. aureus* ATCC 25923; *Staphylococcus epidermidis* ATCC 12228; *Enterococcus faecalis* ATCC 29212, and clinical strains of *Enterobacter cloacae* (n = 1), *Enterobacter aerogenes* (n = 1), *Klebsiella pneumoniae* (n = 3), *Klebsiella oxytoca* (n = 1), *Salmonella typhi* (n = 1), *Shingella dysenteriae* (n = 1), *Shigella flexneri* (n = 1), *Enterococcus faecalis* (n = 3), (n = 1), *Staphylococcus aureus* meticillin R (n = 1), *Staphylococcus haemolyticus* (n = 1). Strains isolated from clinical specimens were typified according to a taxonomic *Bergey' Manual of Systematic Bacteriology*. Stock cultures were maintained on Trypticase soy agar slants at 4°C.

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