The Effect of Dihydronicotinate N-Substitution on the Brain-Targeting Efficacy of a Zidovudine Chemical Delivery System[1]

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Enhanced brain delivery of zidovudine (AZT) has been demonstrated using a redox-based chemical delivery system (CDS). Optimization of the prototype AZT-CDS (5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]-3′-azido-3′-deoxythymidine) was investigated by manipulation of the *N*-methyl group present on the dihydronicotinate portion of the molecule and examining the release of AZT *in vivo* in a rat model. Of the five compounds examined, all produced higher brain levels and lower blood levels of AZT than did AZT itself. In comparing the novel AZT-CDS analogues to the *N*-methyl benchmark, the *N*-propyl system proved to be the most efficient of the compounds tested.

KEY WORDS: azidothymidine (zidovudine); chemical delivery system; brain; blood–brain barrier; dihydronicotinates; organ targeting.

INTRODUCTION

Brain-enhanced delivery of zidovudine (AZT; azidothymidine) and other antiretroviral drugs would be beneficial in the management of acquired immune deficiency syndrome (AIDS) encephalopathy. AIDS encephalopathy is caused by brain infection of the AIDS pathogen, HIV-1, and is associated with a constellation of debilitating symptoms that affect a large percentage of individuals stricken with the disease (1–3). Treatment of the central components of AIDS is difficult due to the inaccessibility of the infection site. The brain is protected from many blood borne substances by the blood–brain barrier (BBB), a system derived from the cerebral microvasculature (4,5). This barrier effectively impedes the entry of many polar molecules, such as antiviral nucleosides, into brain parenchyma. As a result, AZT administration to test animals is associated with low brain extracellular fluid (ECF) concentrations relative to plasma or cerebrospinal fluid levels (6).

Several chemical methods have been proposed to enhance brain delivery of AZT including ester prodrugs, AZT phosphotriesters, and AZT chemical delivery systems (CDS). Since the BBB is lipoidal in character, the preparation of lipophilic prodrugs may improve transit of the drug into the CNS. Esters of AZT and various fatty acids of the type prepared by Kawaguchi *et al.* may be suited for this purpose (7). A variation of this theme was proposed by Namane *et al.* and involves lipophilic phosphotriesters which, rather than delivering AZT, would deliver the 5′-phosphate of AZT (8). This is a potential advantage since AZT must be phosphorylated (ultimately to the 5′-triphosphate) before it can exert biological activity. These simple prodrugs of either AZT or AZT 5′-phosphate have several important kinetic limitations (9,10). Since the lipophilicity of the compounds is high, the derivatives may, in fact, be extracted into the CNS to a greater extent than is the parent drug. Unfortunately, the extraction of the lipophilic prodrug into other tissues is often increased, meaning that there is no selectivity in this process and that the tissue burden of the drug may generally be increased. This can have untoward toxicological ramification in the case of AZT since peripheral side effects such as myleosupression are dose-limiting.

The CDS approach which has been applied by several groups to various antiviral nucleosides, including AZT, may represent an advantage (11,12). The method involves covalent attachment of a molecular targetor to the drug of interest. In the case of AZT, the only targetor thus far examined has been 1-methyl-1,4-dihydronicotinate. The targetor is designed to undergo conversion subsequent to BBB transit, resulting in the formation of an AZT-methylnicotinate salt. While the AZT–targetor conjugate [AZT-CDS (7) 5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyl]-3′-azido-3′-deoxythymidine] is lipophilic and membrane permeable, the oxidation product, i.e., the corresponding nicotinate salt (AZT-Q^+; 1), is relatively hydrophilic and membrane impermeable. As a result of this dramatic change in physicochemical properties, that portion of the dose which is present in the CNS at the time of oxidation is trapped there. On the other hand, the polar AZT-methylnicotinate species (AZT-Q^+) is readily eliminated from the systemic circulation. The result of this manipulation is that CNS levels of the AZT salt are higher and more sustained than blood levels. The conjugate that is "locked in" the CNS can subsequently degrade through hydrolysis to generate the AZT where it can exert its virustatic activity.

Experimental studies have vindicated these assertions. Little *et al.* found that systemic administration of the AZT-CDS (7) to rats and dogs produced three times as much AZT in brain than did AZT treatment and less AZT in blood than did AZT dosing (13). Other studies found significantly improved brain levels and improved brain/blood ratios in rabbits and mice (14–17). Aggarwal *et al.* found that not only was the AZT-CDS more effective *in vitro* in inhibiting HIV replication than AZT, but it was also less toxic to the host cells (H9 lymphocytes) than AZT (18,19). While several investigators have examined the AZT-CDS and similar nucleosides, no attempt has been made to chemically optimize the

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delivery system. As a first step toward this goal, we have examined the effect of manipulating the *N*-methyl substituent of the dihydronicotinate targetor on release and delivery of AZT in the CNS.

MATERIALS AND METHODS

Chemistry

Microcombustion analysis of compounds synthesized was performed by Atlantic Microlabs, Atlanta, GA. Uncorrected melting points (m.p.) were determined with either an Electrothermal or a Thomas-Hoover melting point apparatus. Ultraviolet spectra (UV) were obtained on either a Hewlett-Packard 8451A diode array or a Shimadzu UV-160 rapid scan spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Varian XL 200 (200 MHz, FT mode). Samples were dissolved in an appropriate solvent and chemical shifts (δ) reported relative to tetramethylsilane. Thin-layer chromatography was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with indicated silica gel (60 mesh). All chemicals were reagent grade and were obtained from either Aldrich Chemical Co. or Sigma Chemical Corp. Azidothymidine was purchased from ACIC, Inc. (Bradford, Ontario, Canada). The AZT 5′-nicotinate (5′-[(pyridin-3-yl)carbonyl]-3′-azido-3′-deoxythymidine), the AZT-Q$^+$ (1; 1-methyl-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azidol]-β-D-ribofuranos-5-ylcarbonyoxy}pyridinium iodide), and the AZT-CDS (7; 5′-[(1-methyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3′-azido-3′,5′-dideoxythymidine) were prepared based on literature procedures (13).

General Procedure for the Preparation of the Nicotinate Salts (AZT-Q$^+$)

To a solution of 2.25 g (6 mmol) of AZT 5′-nicotinate in 100 mL of nitromethane was added 6.6 mmol of the appropriate alkyl halide. The solution was allowed to stir at 50°C for 24 hr. The solvent was removed *in vacuo* and the residue recystallized from ethanol/ethyl ether.

1-Ethyl-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azido]-β-D-ribofuranos-5-ylcarbonyoxy}pyridinium Iodide (2). Yield, 95%; purity (HPLC), 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.35 (1H, s, NH); 9.65 (1H, s, 2-C pyridinium); 9.36 (1H, d, $J_{5,6}$ = 5.07, 6-C pyridinium); 9.05–9.02 (1H, m, H-4 pyridinium); 8.35–8.30 (1H, m, H-5 pyridinium); 7.52 (1H, s, =CH); 6.15 (1H, t, 1′-C); 4.80–4.40 (1H, m, 3′-C); 4.75 (1H, m, $^2J_{A,B}$ = 12 Hz 5′-C H_A); 4.60 (1H, dd, $^2J_{A,B}$ = 12 Hz 5′-C H_B); 4.76 (2H, q, CH_2-CH_3); 4.13 (1H, m, 4′-C); 2.60 (1H, dd, $^2J_{A,B}$ = 14 Hz, 2′-C H_A); 2.40 (1H, m, $^2J_{A,B}$ = 14, Hz, 2′-C H_B); 1.71 (3H, s, =C-CH_3); 1.56 (3H, t, -CH_2-CH_3). *Calcd. for $C_{18}H_{21}O_5N_6I$.* Theory: C, 40.92%; H, 4.01%; N, 15.91%; I, 24.02. Found: C, 40.73%; H, 4.25%; N, 16.21%; I, 23.89%.

1-Propyl-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azido]-β-D-ribofuranos-5-ylcarbonyoxy}pyridinium iodide (3). Yield, 97%; purity (HPLC), 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.35 (1H, s, NH); 9.64 (1H, s, 2-C pyridinium); 9.34–9.32 (1H, m, 6-C pyridinium); 9.06–9.03 (1H, m, H-4 pyridinium); 8.35–8.30 (1H, m, H-5 pyridinium); 7.52 (1H, s, =CH); 6.15 (1H, t, 1′-C); 4.73–4.60 (3H, m, 3′-C, 5′-C_2); 4.65 (2H, m, CH_2 -CH_2-CH_3); 4.13 (1H, m, 4′-C); 2.60 (1H, m, $^2J_{A,B}$ = 13.8 Hz, 2′-C H_A); 2.40 (1H, m, $^2J_{A,B}$ = 13.8 Hz, 2′-C H_B); 1.96 (2H, m, -CH_2-CH_2-CH_3); 1.71 (3H, s, =C-CH_3); 0.90 (3H, m, -CH_2-CH_2-CH_3). *Calcd. for $C_{19}H_{23}O_5N_6I$.* Theory: C, 42.08%; H, 4.27%; N, 15.50%; I, 23.40. Found: C, 41.79%; H, 4.35%; N, 15.31%; I, 23.55%.

1-(2-Propyl)-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azido]-β-D-ribofuranos-5-yl-carbonyoxy}pyridinium iodide (4). Yield, 93%; purity (HPLC), 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.36 (1H, s, NH); 9.61 (1H, s, 2-C pyridinium); 9.45–9.43 (1H, m, 6-C pyridinium); 9.04–9.01 (1H, m, 4-C pyridinium); 8.35–8.30 (1H, m, H-5 pyridinium); 7.50 (1H, s, =CH); 6.16 (1H, t, 1′-C); 5.2 (1H, m, N-CH); 4.73–4.60 (3H, m, 3′-C, 5′-C_2); 4.13 (1H, m, 4′-C); 2.60–2.40 (2H, m, 2′-C_2); 1.71 (3H, s, =C-CH_3); 1.65 [6H, m, =$(CH_3)2$]. *Calcd. for $C_{19}H_{23}O_5N_6I$.* Theory: C, 42.08%; H, 4.27%; N, 15.50%; I, 23.40. Found: C, 41.93%; H, 4.28%; N, 15.47%; I, 23.29%.

1-Benzyl-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azido]-β-D-ribofuranos-5-ylcarbonyoxy}pyridinium bromide (5). Yield, 63%; purity (HPLC), 97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.36 (1H, s, NH); 9.83 (1H, s, 2-C pyridinium); 9.44–9.42 (1H, m, 6-C pyridinium); 9.08–9.02 (1H, m, H-4 pyridinium); 8.36–8.32 (1H, m, H-5 pyridinium); 7.70–7.40 (6H, m, =CH, C_6-C_5); 6.15 (1H, t, 1′-C); 6.05 (2H, s, N-CH_2); 4.76–4.59 (3H, m, 3′-C, 5′-C_2); 4.14 (1H, m, 4′-C); 2.60–2.40 (2H, m, 2′-C_2); 1.67 (3H, s, =C-CH_3). *Calcd. for $C_{23}H_{23}O_5N_6Br \cdot 0.5\ H_2O$.* Theory: C, 50.01%; H, 4.38%; N, 15.21%; Br, 14.47%. Found: C, 50.13%; H, 4.26%; N, 15.17%; Br, 14.71%.

1-(Methylcyclopropyl)-3-{1′-[(thymin-1-yl)-3′,5′-dideoxy-3′-azido]-β-D-ribofuranos-5-ylcarbonyoxy} pyridinium iodide (6). Yield, 88%; purity (HPLC), 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.35 (1H, s, NH); 9.68 (1H, s, 2-C pyridinium); 9.43–9.41 (1H, m, 6-C pyridinium); 9.08–9.05 (1H, m, H-4 pyridinium); 8.37–8.32 (1H, m, H-5 pyridinium); 7.53 (1H, s, =CH); 6.15 (1H, t, 1′-C); 4.75–4.59 (5H, m, 3′-C, 5′-C_2, N-CH_2); 4.12 (1H, m, 4′-C); 2.62–2.40 (2H, m, 2′-C_2); 1.70 (3H, s, =C-CH_3); 1.50 [1H, m, CH(cyclopropyl)]; 0.65 [4H, m, =$(CH_2)_2$(cyclopropyl)]. *Calcd. for $C_{20}H_{23}O_5N_6Br$.* Theory: C, 47.35%; H, 4.57%; N, 16.56%; Br, 15.75%. Found: C, 47.09%; H, 4.65%; N, 16.42%; N, 16.42%; Br, 15.63%.

General Procedure for the Preparation of the AZT-CDS Analogues

To a solution of the appropriate AZT-Q$^+$ (2 mmol) in a deaerated biphasic system on ethyl acetate and water at 0°C was added 23 mmol of $NaHCO_3$ and 18 mmol of $Na_2S_2O_4$ over a period of 1 min. After 30 min of stirring, an additional quantity of $NaHCO_3$ (6 mmol) was added and the system stirred for 2 hr at 0°C and then for 1 hr at room temperature. The organic layer was separated and the aqueous layer extracted with 5 portions of ethyl acetate. The combined organic layers were then washed with water and brine and dried over Na_2SO_4. The solvent was reduced by 80% under reduced pressure, at which time the produce precipitated. The suspension was the cooled to 0°C, filtered, and washed with ethyl acetate and ether.

5'-[(1-Ethyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3'-azido-3',5'-dideoxythymidine (8). Yield, 72%; purity (HPLC), 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.35 (1H, s, NH); 7.40 (1H, s, =CH); 7.14 (1H, d, J=2.4 Hz, 2-C dihydropyridine); 6.10, (1H, t, J=6.5 Hz 1'-C); 5.94 (1H, dd, $J_{6,5}$=8.06 Hz, $J_{6,2}$=1.6 Hz, 6-C dihydropyridine); 4.78–4.73 (1H, m, 5-C dihydropyridine); 4.52–4.47 (1H, m, 3'-C); 4.32 (1H, dd, $^2J_{A,B}$=12 Hz, $^3J_{A,4'}$=4.0 Hz, 5'-C H_A); 4.20 (1H, dd, $^2J_{A,B}$=12, Hz, $^3J_{B,4'}$=4.6 Hz, 5'-C H_B); 4.0 (1H, m, 4'-C); 3.2 (2H, q, J=7.0 Hz, CH_2-CH_3); 3.0 (2H, s, 4-CH_2 dihydropyridine); 2.44–2.32 (2H, m, 2'-C_2); 1.80 (3H, s, =C-CH_3); 1.06 (3H, t, J=7.0 Hz, CH_2=CH_3). *Calcd. for* $C_{18}H_{22}O_5N_6$. Theory: C, 53.72%; H, 5.51%; N, 20.89%. Found: C, 53.44%; H, 5.64%; N, 20.64%.

5'-[(1-Propyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3'-azido-3',5'-dideoxythymidine (9). Yield, 80%; purity (HPLC), 92%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.39 (1H, s, =CH); 7.12 (1H, d, $J_{6,2}$=1.6 Hz, 2-C dihydropyridine); 6.10, (1H, t, J=6.5 Hz 1'-C); 5.9 (1H, dd, $J_{6,5}$=8.08 Hz, $J_{6,2}$=1.6 Hz, 6-C dihydropyridine); 4.76–4.71 (1H, m, 5-C dihydropyridine); 4.52–4.46 (1H, m, 3'-C); 4.32 (1H, dd, $^2J_{A,B}$=12.0 Hz, $^3J_{A,4'}$=3.4 Hz, 5'-C H_A); 4.21 (1H, dd, $^2J_{A,B}$=12, Hz, $^3J_{B,4'}$=4.6 Hz, 5'-C H_B); 4.04–3.98 (1H, m, 4'-C); 3.10 (2H, t, J=7.0 Hz, N-CH_2); 3.0 (2H, s, 4-CH_2 dihydropyridine); 2.43–2.31 (2H, m, 2'-C_2); 1.79 (3H, s, =C-CH_3); 1.45 (2H, m, J=7.0 Hz, CH_2-CH_2-CH_3); 0.83 (3H, t, J=7.0 Hz, CH_2=CH_3). *Calcd. for* $C_{19}H_{24}O_5N_6$. Theory: C, 54.80%; H, 5.81%; N, 20.18%. Found: C, 54.62%; H, 5.74%; N, 20.34%.

5'-[(1-Isopropyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3'-azido-3',5'-dideoxythymidine (10). Yield, 64%; purity (HPLC), 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.39 (1H, s, =CH); 7.15 (1H, d, $J_{6,2}$=1.6 Hz, 2-C dihydropyridine); 6.10, (1H, t, J=6.5 Hz 1'-C); 6.0 (1H, dd, $J_{6,5}$=8.2 Hz, $J_{6,2}$=1.6 Hz, 6-C dihydropyridine); 4.79–4.74 (1H, m, 5-C dihydropyridine); 4.53–4.47 (1H, m, 3'-C); 4.34 (1H, dd, $^2J_{A,B}$=12.0 Hz, $^3J_{A,4'}$=4.0 Hz, 5'-C H_A); 4.18 (1H, dd, $^2J_{A,B}$=12, Hz, $^3J_{B,4'}$=4.6 Hz, 5'-C H_B); 4.0 (1H, m, 4'-C); 3.5 (1H, m, J=6.60 Hz, N-CH); 3.0 (2H, s, 4-CH_2 dihydropyridine); 2.43–2.32 (2H, m, 2'-C_2); 1.80 (3H, s, =C-CH_3); 1.13 [6H, d, J=6.6 Hz, =$(CH_3)]_2$. *Calcd. for* $C_{19}H_{24}O_5N_6$. Theory: C, 54.80%; H, 5.81%; N, 20.18%. Found: C, 54.84%; H, 5.90%; N, 20.45%.

5'-[(1-Benzyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3'-azido-3',5'-dideoxythymidine (11). Yield, 74%; purity (HPLC), 95%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.37 (1H, s, NH); 7.35 (1H, s, =CH); 7.4–7.2 (6H, m, 2-C dihydropyridine, $-C_6$-C_5); 6.10, (1H, t, J=6.6 Hz 1'-C); 5.97 (1H, dd, $J_{6,5}$=8.0 Hz, $J_{6,2}$=1.1 Hz, 6-C dihydropyridine); 4.79–4.74 (1H, m, 5-C dihydropyridine); 4.50–4.46 (1H, m, 3'-C); 4.42 (2H, s, N-CH_2); 4.34 (1H, dd, $^2J_{A,B}$=12.2 Hz, $^3J_{A,4'}$=3.7 Hz, 5'-C H_A); 4.18 (1H, dd, $^2J_{A,B}$=12.2 Hz, $^3J_{B-,4'}$=4.6 Hz, 5'-C H_B); 4.06–3.98 (1H, m, 4'-C); 3.0 (2H, s, 4-CH_2 dihydropyridine); 2.37–2.32 (2H, m, 2'-C_2); 1.75 (3H, s, =C-CH_3). *Calcd. for* $C_{23}H_{24}O_5N_6 \cdot 0.5\ H_2O$. Theory: C, 58.33%; H, 5.32%; N, 17.75%. Found: C, 58.62%; H, 5.44%; N, 17.66%.

5'-[(1-Methylcyclopropyl-1,4-dihydropyridin-3-yl)carbonyloxy]-3'-azido-3',5'-dideoxythymidine (12). Yield, 64%; purity (HPLC), 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.35 (1H, s, NH); 7.38 (1H, s, =CH); 7.17 (1H, d, $J_{6,2}$=0.9 Hz, 2-C dihydropyridine); 6.10, (1H, t, J=6.5 Hz 1'-C); 5.98 (1H, dd, $J_{6,5}$=8.0 Hz, $J_{6,2}$=0.9 Hz, 6-C dihydropyridine); 4.76–4.71 (1H, m, 5-C dihydropyridine); 4.5 (1H, dd, J=12.6 Hz, J=5.6 Hz, 3'-C); 4.33 (1H, dd, $^2J_{A,B}$ =12.2 Hz, $^3J_{A,4'}$=3.9 Hz, 5'-C H_A); 4.19 (1H, dd, $^2J_{A,B}$= 12.2 Hz, $^3J_{B,4'}$=4.4 Hz, 5'-C H_B); 4.02–3.98 (1H, m, 4'-C); 3.0 (4H, m, N-CH_2, 4-CH_2 dihydropyridine); 2.40–2.34 (2H, m, 2'-C_2); 1.80 (3H, s, =C-CH_3); 0.94 [1H, m, $CH_{(cyclopropyl)}$]; 0.48–0.42 [2H, m, $CH_ACH_{A'(cyclopropyl)}$]; 0.22–0.20 [2H, m, $CH_BCH_{B(cyclopropyl)}$]. *Calcd. for* $C_{20}H_{24}O_5N_6$. Theory: C, 56.05%; H, 5.65%; N, 19.62%. Found: C, 55.78%; H, 5.76%; N, 19.41%.

Analytical Methodology

HPLC systems were developed to separate, detect, and quantify the chemical delivery systems, their components and AZT. In all determinations the following served as the system configuration: a Spectra-Physics SP 8810 ternary solvent pump, a SP 8430 Ultraviolet detector, a SP 4270 integrator, and a Perkin-Elmer ISS-100 autosampler.

For determination of log k' values (20), a Spherisorb ODS-2 reversed-phase column (5-μm particle size, 25 cm × 4.6-mm i.d., Alltech/Applied Science) was used. Samples of dihydronicotinates (7–12) were prepared in acetonitrile and a small volume (5 μL) was injected on the analytical column. After each chromatographic run the eluting power of the mobile phase (acetonitrile:water) was increased by increasing the proportion of acetonitrile (from 20:80 to 90:10 acetonitrile:water). For each run the retention time of the compound in question was recorded. The flow rate was maintained at 1.0 mL/min and all determinations were made at ambient temperature. The log k' was determined from the following expression:

$$\log k' = \log\left[\frac{(t_r - t_o)}{t_o}\right]$$

where t_r refers to the retention time of the compound in question at a particular mobile phase composition and t_o is the retention time of an unretained peak (formaldehyde). The collected log k' data are then plotted against the water content in the mobile phase and the value of 100% water is estimated by extrapolation. The results are given as the mean of three experiments ± the standard deviation.

For determination of AZT, AZT-Q^+, and AZT-CDS in biological samples, a Spherisorb C8 column (5-μm particle size, 25 cm × 4.6-mm i.d., Alltech/Applied Sciences) fitted with a guard column was used. The mobile phase contained 45:55 acetonitrile:0.05 M $NH_4C_2H_3O_2$ (pH adjusted to 6.0 with acetic acid), the flow rate was 1.0 mL/min, and all determinations were made at ambient temperature. All compounds were detected at 266 nm. The retention time for AZT in this system was 3.0 min, while that for AZT-Q^+ was 7.0 (1), 6.5 (2), 8.2 (3), 8.0 (4), 9.6 (5), and 7.2 (6) min. For the corresponding dihydronicotinates, the retention times were 7.7 (8), 10.2 (9), 9.6 (10), 12.1 (11), and 10.8 (12) min.

In Vitro Stability in Brain Homogenate

An adult, male Sprague Dawley rat was anesthetized with pentobarbital (65 mg/kg), the thoracic cavity opened,

the vena cava cut, and 20 mL of phosphate-buffered saline perfused through the left ventricle. The brain was removed and homogenized in a sufficient volume of phosphate-buffered saline to give a 2% homogenate (w/v). The homogenate (2.75 mL) was then placed in a cuvette fitted with a Teflon septum and the cuvette was placed in the thermostated cell holder of a Hewlett-Packard 8451A diode array spectrophotometer and equilibrated at 37°C. At $t = 0$, 27.5 μL of a 5×10^{-3} M solution of the appropriate dihydronicotinate was introduced into the cuvette via a Hamilton syringe. The disappearance of the band III absorbance (360 nm) was monitored. The pseudo-first-order rate constants were calculated by plotting the change in the log (absorbance$_{360}$) with time using the HP 85 dedicated microprocessor.

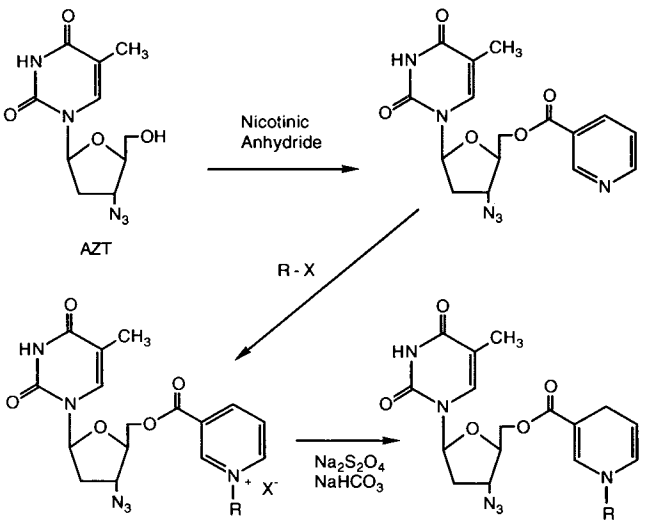
In Vivo Tissue Distribution

Adult, male Sprague Dawley rats, weighing 175 to 225 g, were administered a single dose of an AZT-CDS analogue (**7–12**) via the tail vein. Each analogue or AZT was given equimolar to 50 mg/kg of the AZT-CDS (**7**) [which represents a dose of 34.4 mg/kg (0.13 mmol/kg) based on the parent drug, AZT]. The doses of the analogues (**7–12**) were 52.4, 53.6, 53.6, 59.9, and 55.3 mg/kg, respectively. All compounds were dissolved in dimethyl sulfoxide (DMSO) and injected at a volume of 0.5 mL/kg. Animals were killed by rapid decapitation at 0.25, 1.0, and 4.0 hr after injection of each compound and five rats were included at each time point. Immediately upon sacrifice, trunk blood was collected into heparinized tubes (0.15 mL per tube), capped, and inverted thrice to thoroughly mix the blood and heparin and prevent clotting. The entire brain and approximately 1 g of liver was removed next, weighed, and frozen on dry ice. The tissues or blood were then stored at −5°C prior to homogenization.

One milliliter of deionized water was added to either 1.0 mL of whole blood, whole brain, or 1.0 g of liver. Each tissue was thoroughly homogenized with a ground-glass pestle (Kontes Duall 23). To each homogenate was then added 4.0 mL of acetonitrile and the system was vortexed. Concentrated brine (1.0 mL) was then added and the system was allowed to settle at −5°C for 1 hr. The organic phase which separated under these conditions were removed and transferred to autosampler vials and submitted for HPLC analysis as described above.

RESULTS AND DISCUSSION

The design of compounds used in this study was based on manipulation of two parameters, namely, lipophilicity and stability to oxidation. Based on model studies (21), it was suggested that extending the *N*-alkyl chain would increase the lipophilicity of the CDS without strongly affecting the chemical stability, while placement of a *N*-benzyl group in the CDS would act to increase the stability of the CDS to oxidation. In the preparation of the derivatives selected, AZT was treated with nicotinic anhydride to give the AZT 5′-nicotinate, which served as common intermediate for all compounds prepared (Fig. 1). The nicotinate was then alkylated with ethyl iodide, propyl iodide, isopropyl iodide, benzyl bromide, or methylcyclopropyl bromide to give the corresponding quaternary salts. The ethyl (**2**), propyl (**3**), isopropyl (**4**), benzyl (**5**), and methylcyclopropyl (**6**) salts were then reduced using aqueous basic sodium dithionite to yield the appropriate 1-substituted 1,4-dihydronicotinates (**8–12**, respectively). The preparation of longer *N*-alkyl homologues is described in a separate study (22).



1: R = CH_3, X = I
2: R = CH_2CH_3, X = I
3: R = $CH_2CH_2CH_3$, X = I
4: R = $CH(CH_3)_2$, X = I
5: R = $CH_2C_6H_5$, X = Br
6: R = $CH_2C_3H_5$, X = Br

7: R = CH_3
8: R = CH_2CH_3
9: R = $CH_2CH_2CH_3$
10: R = $CH(CH_3)_2$
11: R = $CH_2C_6H_5$
12: R = $CH_2C_3H_5$

Fig. 1. Synthesis of various AZT-CDS derivatives.

The lipophilicity of the AZT-CDS derivatives is important since they should be sufficiently lipoidal to readily pass the BBB. Relative lipophilicity information was accessed by calculating the logs of the HPLC capacity factors (log k') of the compound of interest as suggested by Yamana *et al.* (20). The results, which are presented in Table I, indicate the expected trend of increased lipophilicity with increased chain length or substituent size. The most lipoidal derivative prepared was the benzyl (**11**) AZT-CDS, which was 8 and 100 times more lipophilic than the benchmark compound (**7**) and AZT, respectively. The calculated log k' values and their magnitudes relative to the methyl AZT-CDS (**7**) suggest rapid BBB penetration.

The stability of the AZT-CDS analogues was examined in rat brain homogenate to ensure that the compound would convert from the dihydronicotinate transport form to the

Table I. HPLC-Derived log k' Values for a Series of AZT-CDS Analogues[a]

Derivative	Extrapolated logk'	Correlation coefficient
AZT	0.55 ± 0.02	0.99
Methyl (**7**)	1.64 ± 0.22	0.99
Ethyl (**8**)	2.15 ± 0.53	0.99
Propyl (**9**)	2.34 ± 0.19	0.98
Isopropyl (**10**)	2.39 ± 0.15	0.98
Benzyl (**11**)	2.54 ± 0.07	0.98

[a] log k' values are extrapolated to a 100% aqueous mobile phase.

Table II. Half-Lives ($t_{1/2}$), Pseudo-First-Order Rate Constants (k), and Correlation Coefficients (r) for the Degradation of Several AZT-CDS Analogues in 2% Brain Homogenate at 37°C

Derivative	$t_{1/2}$ (min)	$k \pm$ SE (min^{-1})	r
Ethyl (**8**)	79.5	$(8.70 \pm 0.09) \times 10^{-3}$	0.99
Propyl (**9**)	64.8	$(1.07 \pm 0.04) \times 10^{-2}$	0.99
Isopropyl (**10**)	73.3	$(9.45 \pm 0.14) \times 10^{-3}$	0.99
Benzyl (**11**)	98.8	$(7.01 \pm 0.15) \times 10^{-3}$	0.99
Methyl-cyclopropyl (**12**)	91.2	$(7.60 \pm 0.17) \times 10^{-3}$	0.99

brain-sequestered nicotinate salt form. The data (Table II) indicate that the degradation of the *N*-alkyl derivatives (**8–10**) occurred at similar rates, while the methylcyclopropyl (**12**) and the benzyl systems (**11**) were somewhat more stable. The reason for this can be explained only partially by the chemical stabilities of the compounds, in that while the benzyl derivative (**11**) is more stable to chemical oxidation than the *N*-alkyl analogues, the methylcyclopropyl compound (**11**) is not (21). This may indicate that enzyme affinities are involved. It has been suggested that a family of NAD-NADH transhydrogenases is responsible for the enzymatic oxidation of dihydronicotinates (16). If this is the case, bulky groups in the N-position, a location where a methyl group is expected, may decrease enzyme-substrate binding.

Administration of AZT itself to rats gave high initial concentrations in blood and liver. These levels were rapidly cleared, with 1-hr blood and liver concentrations being only 10 and 3%, respectively, of the 15-min values. No AZT was detected at 4 hr in any tissue. Small amounts of AZT were detected in homogenates of whole brain at the 15-min time point but not thereafter. The amounts recorded in brain were less than 0.5 μg/g tissue and represent, in addition to AZT in brain tissue, AZT present in the CSF as well as in the CNS circulatory system. The rapid systemic elimination of the AZT as well as its poor penetration of the CNS is consistent both with its polar structure and with the reported clinical and pharmacokinetic profiles of the drug (23,24).

Administration of the AZT-CDS compounds (**7–12**) produced a dramatically different profile. No intact dihydronicotinate was observed in any tissue after systemic administration of the AZT-CDS or its analogues. This finding is consistent with the metabolic lability of the dihydronicotinates, with their large volumes of distribution and with previously published work (13). Levels of the various AZT-nicotinate salts (**1–6**; AZT-Q^+) are given in Table III. Levels of the methylcyclopropyl salt (**6**) were indeterminate in all tissues. Blood levels were highest for the methyl (**1**) and isopropyl (**4**) salts at 15 min, with some quantities of the ethyl (**2**) pyridinium cation detected. The levels dropped by 1 hr, although significant amounts of (**4**) were still detectable. At 4 hr, none of the quaternary salts were present. In liver, high concentrations of the isopropyl (**4**) salt were observed, with smaller quantities of the other pyridinium species after administration of the corresponding AZT-CDS compounds. At 1 hr, the level of **4** had fallen considerably and was only 9% of the 15-min value. None of the pyridinium salts were detected in significant amounts past 2 hr. In brain, relatively high concentrations of the quaternary salts were produced subsequent to CDS administration, with the most dramatic result coming from the isopropyl derivative. Levels of **4** in brain were recorded at 49 μg/g at 15 min, 12.13 μg/g at 1 hr, and 1.59 μg/g at 4 hr. These levels not only are significantly higher than those produced by other analogues, but also are higher than those produced by **10** in either blood or liver. The next-best system in this respect was the propyl (**9**) analogues which generated levels of the quaternary salt (**3**) in brain greater than 10 μg/g at 15 min and greater than 6 μg/g at 1 hr. The prototype AZT-CDS (**7**) system ranked only third in terms of AZT-Q^+ delivery.

AZT produced from the various AZT-Q^+ salts formed

Table III. Concentration of Various AZT-Q^+ Salts (μg/g or μg/mL) in Brain, Blood, and Liver at 0.25, 1.0, and 4.0 hr After an i.v. Dose of the Corresponding AZT-CDS Derivatives (0.13 mmol/kg)

		AZT-Q^+ level (μg/g or μg/mL ± SE)		
Derivative	Time (hr)	Brain	Blood	Liver
Methyl (**7** → **1**)	0.25	3.95 ± 0.94	6.81 ± 1.54	0.36 ± 0.11
	1.0	2.26 ± 0.85	0.18 ± 0.11	0.00
	4.0	0.00	0.00	0.00
Ethyl (**8** → **2**)	0.25	2.07 ± 0.63	0.59 ± 0.06	0.83 ± 0.07
	1.0	0.00	0.00	0.00
	4.0	0.00	0.00	0.00
Propyl (**9** → **3**)	0.25	10.43 ± 4.65	0.00	1.05 ± 0.64
	1.0	6.34 ± 2.04	0.00	0.00
	4.0	0.00	0.00	0.00
Isopropyl (**10** → **4**)	0.25	49.00 ± 9.09	4.65 ± 0.51	26.94 ± 2.38
	1.0	12.13 ± 0.11	1.98 ± 0.35	2.45 ± 0.24[a]
	4.0	1.59 ± 0.50	0.00	0.00
Benzyl (**11** → **5**)	0.25	1.68 ± 0.32	0.30 ± 0.00	1.46 ± 0.18
	1.0	0.10 ± 0.10[b]	0.00	0.00
	4.0	0.00	0.00	0.00

[a] Three of five detectable.
[b] Two of five detectable.

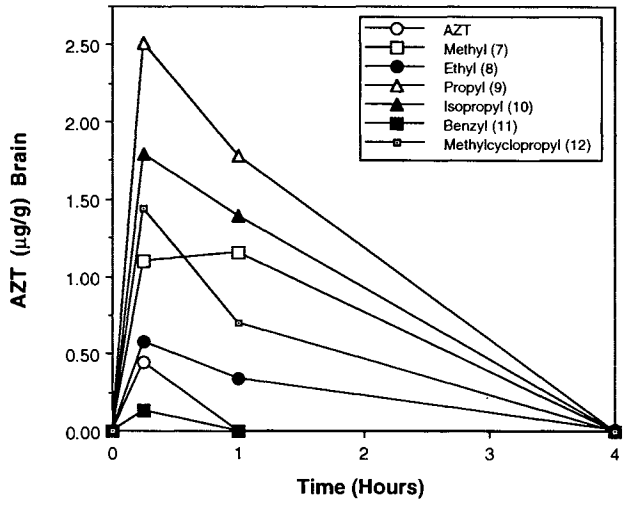


Fig. 2. Brain concentration of AZT after i.v. administration of AZT or various AZT-CDS derivatives at doses of 0.13 mmol/kg.

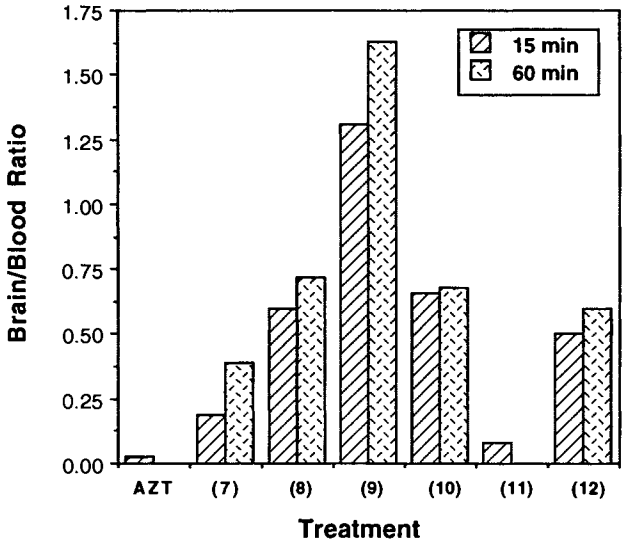


Fig. 3. Brain/blood ratios of AZT after treatment with AZT or various AZT-CDS derivatives.

subsequent to AZT-CDS administration was measured in all tissues (Fig. 2 and Table IV). The highest blood levels of AZT were observed at 15 min subsequent to administration of the methyl AZT-CDS (7) (19). Levels associated with release from the isopropyl, benzyl, and methylcyclopropyl (4–6) salts were similar (1–2 μg/mL), a trend that was also observed at the 1-hr time point. Some AZT derived from the methyl derivative (7) was present even at 4 hr. In liver, concentrations of AZT greater than 12 μg/g were demonstrated subsequent to dosing with the isopropyl (10) and methylcyclopropyl (12) analogues. AZT produced from other derivatives (7–9, 11) was present at levels between 2 and 6 μg/g. By 1 hr the level of AZT derived from all of the AZT-Q^+ salts had fallen to less than 2 μg/g. Levels at 4 hr were at or below the limits of detection. In brain, AZT was readily released from the quaternary salt in all cases except for the benzyl (6) salt. The highest AZT concentrations were produced by the propyl (3) analogues (2.5 μg/g), followed by the isopropyl (4) (1.93 μg/g), methylcyclopropyl (6) (1.44 μg/g), methyl (1) (1.10 μg/g), and finally, ethyl (2) (0.59 μg/g) derivatives. Brain concentrations of AZT were fairly sustained through 2 hr as shown in Table IV but fell to undetectable values by 4 hr.

In comparing brain and blood levels of AZT subsequent

Table IV. Concentration of AZT (μg/g or μg/mL) in Brain, Blood, and Liver at 0.25, 1.0, and 4.0 hr After an i.v. Dose of Either AZT or AZT-CDS Derivatives (0.13 mmol/kg)

Derivative	Time (hr)	AZT level (μg/g or μg/mL ± SE)		
		Brain	Blood	Liver
AZT	0.25	0.45 ± 0.07	17.69 ± 0.97	10.96 ± 0.83
	1.0	0.00	2.12 ± 0.16	0.40 ± 0.24
	4.0	0.00	0.00	0.00
Methyl (7)	0.25	1.10 ± 0.15	5.88 ± 0.55	5.56 ± 0.43
	1.0	1.16 ± 0.31	2.93 ± 0.44	1.31 ± 0.26
	4.0	0.00	1.70 ± 0.64	0.00
Ethyl (8)	0.25	0.58 ± 0.14	0.98 ± 0.12	2.46 ± 0.30
	1.0	0.34 ± 0.15	0.47 ± 0.06	0.90 ± 0.20
	4.0	0.01 ± 0.01	0.00	0.00
Propyl (9)	0.25	2.51 ± 0.28	1.92 ± 0.38	4.47 ± 0.59
	1.0	1.78 ± 0.33	1.09 ± 0.14	1.51 ± 0.24
	4.0	0.00	0.07 ± 0.01	0.11 ± 0.11
Isopropyl (10)	0.25	1.79 ± 0.15	2.72 ± 0.14	>12
	1.0	1.39 ± 0.25	2.03 ± 0.88	1.12 ± 0.28
	4.0	0.02 ± 0.01	0.21 ± 0.04	0.00
Benzyl (11)	0.25	0.13 ± 0.05	1.58 ± 0.07	1.63 ± 0.18
	1.0	0.00	0.52 ± 0.17	0.28 ± 0.10
	4.0	0.00	0.00	0.00
Methylcyclopropyl (12)	0.25	1.44 ± 0.28	2.90 ± 0.37	>12
	1.0	0.70 ± 0.21	1.16 ± 0.18	0.83 ± 0.12
	4.0	0.00	0.00	0.00

to either AZT dosing or AZT-CDS analogue administration, it is clear that for all of the analogues, an advantage is seen (Fig. 3). Blood levels of AZT are significantly lower and brain levels significantly higher after AZT-CDS (7–12) treatment than after an equimolar dose of AZT. The data indicate that the propyl (9) derivative is the best in this respect, with a brain/blood ratio of 1.3 at 15 min. The ethyl (8) and isopropyl (10) analogues give a ratio of approximately 0.6 at 15 min, compared to 0.025 for AZT itself. Importantly, as long as the quaternary salt is present in CNS, the ratio continues to increase. At 1 hr, the AZT brain/blood ratio was 1.6 for the propyl (9) system and small increases were seen for most of the other systems, especially for the methyl derivative. In examining brain/liver ratios, similar statements can be made except that the concentration of AZT derived from isopropyl (10) and methylcyclopropyl (12) AZT-CDS compounds is high in liver, yielding a low ratio brain/organ ratio. Of the systems examined, the propyl (8) appears to provide the greatest advantage over the prototype methyl AZT-CDS (7). The superiority seems to be associated with fine adjustments on the lipophilicity of the AZT-CDS as well as its corresponding pyridinum salt. Attempts to increase the stability of the AZT-CDS by introducing a benzyl substituent were not fruitful.

These data suggest that the *N*-propyl AZT-CDS may offer some improvement over the prototype system. One point which should be examined for these delivery systems is the toxicity of the new targetors. The 1-methylnicotinate salt is a natural product (trigonelline), and various systematic safety evaluations have shown that, when attached to a number of drugs, it is without toxic ramifications. Similar assurance will be required for the novel targetors.

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