Synthesis and Biological Evaluation of Prodrugs of Zidovudine

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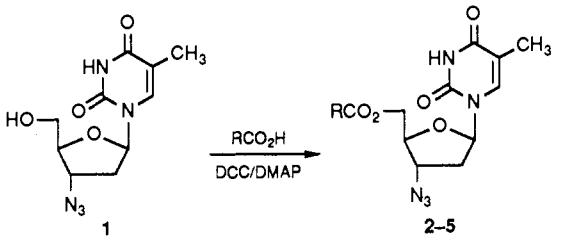
A series of prodrugs of zidovudine (AZT) has been synthesized in an effort to enhance the uptake of the prodrugs by the HIV-1 infected cells and to increase the plasma half-life of AZT. The 5'-OH function of AZT was esterified with various acids in the presence of DCC and 4-(dimethylamino)pyridine (DMAP). The prodrug moieties included (a) morpholine and *N*-phenylpiperazine-1-acetic acid, (b) 1,4-dihydro-1-methyl-3-nicotinic acid, (c) retinoic acid, and (d) certain amino acids. The anti-HIV-1 activity of the esters was determined in peripheral blood lymphocytes. The IC_{50} for AZT in this system was 0.12 μM whereas for prodrugs it ranged from 0.05 to 0.2 μM. The prodrugs were generally less cytotoxic than AZT except the retinoic acid ester. In vitro hydrolysis of the various esters in human plasma indicated that these agents were relatively stable toward plasma esterases with $t_{1/2}$ ranging from 10 to 240 min. Drug uptake studies in H9 cells with radiolabeled analogues demonstrated that the retinoic acid ester achieved approximately 4-fold higher intracellular concentration than [^{3}H]AZT. However, 1,4-dihydro-1-methyl-3-[(pyridylcarbonyl)oxy] ester (**5**) was the most active agent of this series and had a higher therapeutic index than AZT.

Intensive efforts are underway worldwide to develop chemotherapeutic agents effective against the human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS). Zidovudine (3'-azido-2',3'-dideoxythymidine, AZT, azidothymidine, Retrovir) is the only FDA-approved drug available at the present time for the treatment of patients suffering from AIDS and AIDS-related complex (ARC). Treatment with AZT has resulted in improving immunologic function and other clinical abnormalities. This therapy has led to a decrease in the mortality rate and frequency of opportunistic infections in AIDS patients.[1] However, a significant dose-related toxicity associated with the administration of AZT resulting in anemia and leucopenia remains a limiting factor for its effective utilization in the treatment strategies.[2,3] Pharmacokinetic studies of AZT in phase I trials indicate that the plasma half-life of AZT is approximately 1 h,[4] thus necessitating frequent administration of AZT to maintain therapeutic drug levels. Although AZT has been shown to penetrate into the cerebrospinal fluid (CSF), the levels of AZT achieved were approximately 35 and 70% of the plasma concentrations after the administration of 2.5 and 5 mg/kg doses, respectively.[4] These data suggest the necessity of administering higher doses of AZT to achieve adequate antiviral concentrations in the CSF; the increased dose levels however resulted in bone marrow toxicity.[2,3] Furthermore, AZT does not penetrate into the brain tissue from the cerebrospinal fluid[5] and therefore may not suppress viral replication in the brain. In attempts to overcome the problem of rapid elimination and decreased permeability of AZT through the blood–brain barrier and to increase its therapeutic efficacy, we have synthesized a variety of 5'-esters of AZT and have investigated their in vitro stability in human plasma. Enzymatic hydrolysis of these esters was studied upon incubation with rat hepatic microsomes and selected agents were tested for anti-HIV activity. Various strategies have been employed in designing the prodrug moiety of the 5'-esters. Synthesis of alkyl or aryl esters of an acid or an alcohol is a general approach for developing prodrugs. These derivatives can increase stability and lipophilicity of parent compounds, thus improving their biotransport through plasma membranes or lipid barriers. However, the bioavailability of the parent compound may differ intracellularly, depending upon the rate of hydrolysis of the prodrug in T-lymphocytes or macrophages, the primary target cells for HIV replication. Thus design of an ester which is relatively stable to plasma esterases, and yet is hydrolyzed by the cellular enzymes of the target cells, may seem to provide an approach for developing prodrugs of various anti-HIV agents.

Scheme I



Accordingly, the following esters of 5'-OH function of AZT were synthesized with various prodrug moieties: (a) N-substituted piperazine and morpholine esters to utilize their high lipophilicity as demonstrated in a recent report on the uptake of 1-alkyl-4-phenylpiperazines with brain/blood ratio of >20,[6] (b) 1,4-dihydro-1-methyl-3-[(pyridylcarbonyl)oxy] ester to employ a redox chemical drug delivery system of dihydropyridine and pyridinium salt as described by Bodor,[7] (c) retinoic acid ester to simultaneously exploit the biological effects of the prodrug moiety since retinoic acid by itself has been reported to inhibit HIV replication,[8] and (d) amino acid esters to explore the amino acid active transport system in attempts to deliver AZT to target cells at higher concentrations than can be achieved by AZT alone. The use of amino acid transport system L, the classical sodium-independent leucine-preferring transport system, was recently utilized

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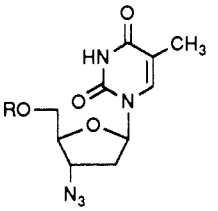
Table I. Physicochemical Properties of Various Prodrugs of Zidovudine



no.	R	% yield	crystn solv	mp, °C	formula[a]
2	4-phenylpiperazine-1-acetyl	70		foam	$C_{22}H_{27}N_7O_5$
3	N-morpholinoacetyl	50	hexane–Et_2O	37–40	$C_{16}H_{22}N_6O_6$
4	3-nicotinyl	69	ETOAc	137–139	$C_{16}H_{16}N_6O_5$
5	1-methyl-1,4-dihydro-3-[(pyridylcarbonyl)oxy]	51	EtOH	132–135	$C_{17}H_{20}N_6O_5$
6	retinoyl	54		70–72	$C_{30}H_{39}N_5O_5 \cdot 2H_2O$
7	*N*-α-*t*-Boc-L-phenylalaninyl	86		foam	$C_{24}H_{30}N_6O_7$
8	*N*-α-*t*-Boc-L-tyrosinyl	32		85–95[b]	
9	*N*-α-*t*-Boc-L-isoleucinyl	91		foam[b]	
10	*N*-α,*N*-ε-bis-*t*-Boc-L-lysinyl	46		foam[b]	
11	*O*-*t*-butyl-α-*t*-Boc-L-serinyl	37		foam	$C_{22}H_{34}N_6O_8$
12	phenylalanyl[c]	93		foam	$C_{21}H_{23}F_3N_6O_7 \cdot 2H_2O$
13	tyrosinyl[c]	87	MeOH–Et_2O	124–128	$C_{21}H_{23}F_3N_6O_8 \cdot H_2O$
14	isoleucinyl[c]	93	MeOH–Et_2O	52–54	$C_{17}H_{25}F_3N_6O_7$
15	lysinyl[c]	92	MeOH–Et_2O	foam	$C_{20}H_{27}F_6N_7O_9$
16	*N*-α-*t*-Boc-γ-glutamyl-α-*tert*-butyl ester	83		foam[b]	
17	glutamyl	35	MeOH–Et_2O	126–129	$C_{15}H_{20}N_6O_8$

[a] Analyzed for C, H, N; analytical results were within ±0.4% of the theoretical values. [b] Used directly in the next step. [c] Obtained as trifluoroacetate salt.

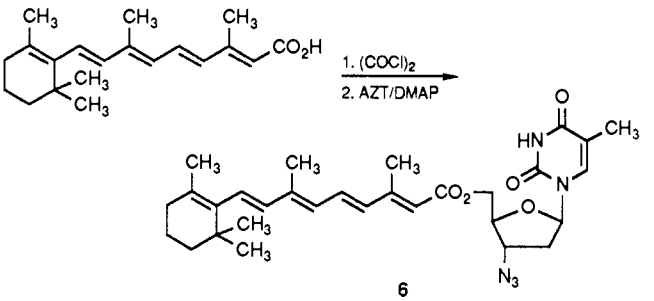
by Haines et al.[9] in the design and synthesis of analogues of L-phenylalanine nitrogen mustard. Since bone marrow progenitor cells may lack amino acid transport system,[10] the amino acid prodrugs of AZT may be expected to be less toxic to bone marrow.

Chemistry

During recent years, carbodiimides, and especially dicyclohexylcarbodiimide (DCC), have been widely used as condensing agents in ester synthesis.[11,12] The esterification reaction in the case of AZT was found to be dependent upon the requirement for 4-(dimethylamino)pyridine (DMAP) as a catalyst. Use of triethylamine for catalysis of condensation as a base was found to be unsuccessful. The yield of the ester was usually decreased because of the simultaneous formation of an *N*-acylurea derivative as byproduct. The esterification reactions in the presence of catalytic amounts of DMAP remained incomplete even after long periods of reaction time. However, addition of 1.5 molar equiv of DMAP in the presence of DCC produced the corresponding esters (**2**–**4**) (Scheme I) in approximately 50–70% yields.

The synthesis of compound **5**, the 1,4-dihydro-1-methyl-3-[(pyridylcarbonyl)oxy] ester, has been recently published by Torrence et al.[13] However, this compound has also been reported earlier from our laboratory[14] and subsquently preliminary biological results have been published.[15] Palomino et al. have also recently reported

Scheme II



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the dihydropyridine derivative of 3′-deoxy-2′,3′-didehydrothymidine (d4t).[16] The synthesis of **5** involved a three-step conversion of AZT according to the published procedure,[7,13] which included the formation of its nicotinyl ester (**4**) followed by quaternization with iodomethane in acetone and subsequent reduction with sodium dithionite and aqueous sodium bicarbonate. In our hands, the reduction was best carried out in a biphasic system by using ethyl acetate in the aqueous mixture. The analytical data for compound **5**, obtained under these conditions, did not require one-half molecule of water as reported earlier.[13]

The synthesis of retinoyl ester of AZT (**6**) when carried out under the identical conditions as described for compounds **2**–**4** resulted in approximately 12% yield. However, a greater than 50% yield of the ester **6** was obtained when retinoic acid was first reacted with oxalyl chloride in benzene and then followed by its reaction with AZT in the presence of an equivalent amount of DMAP (Scheme II).

The amino acid (phenylalanine, tyrosine, isoleucine, and lysine) esters containing free amino function were synthesized by coupling the *t*-BOC-amino acids with AZT in the presence of DCC and DMAP (Scheme III). Deblocking of these esters was conveniently achieved in a 1:1 mixture of methylene chloride and TFA, thus providing

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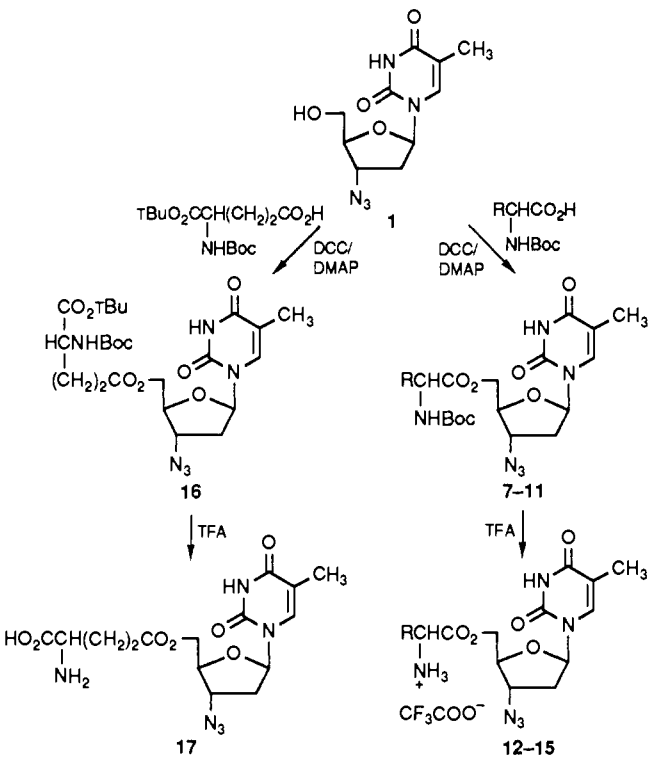
Table II. Partition Coefficients and Biological Properties of Various Prodrugs of Zidovudine

no.	R	PC[a]	in vitro hydrolysis $t_{1/2}$,[b] min: human plasma	in vitro hydrolysis $t_{1/2}$,[b] min: rat hepatic microsomes	IC_{50},[c] μM: HIV-1	IC_{50},[c] μM: H9
1	H	1.2			0.12	150
2	4-phenylpiperazine-1-acetyl	9.2	120	47	0.18	250
3	*N*-morpholinoacetyl	2.2	12	28		320
4	3-nicotinyl	1.6	10	30		
5	1-methyl-1,4-dihydro-3-[(pyridylcarbonyl)oxy]	7.2	>240	110	0.05	336
6	retinoyl	17.9	120	60	0.20	25
12	Phe	3.3	20	30	0.11	350
13	Tyr	0.94	60	5	0.11	300
14	Ile	10.3	>240	19	0.10	250
15	Lys	0.01	30	14		300
17	Glu	0.12	70	30		320

[a] Partition coefficient in *n*-octanol and phosphate buffer pH 7.4.[17] [b] $t_{1/2}$ is the time required for 50% hydrolysis of the AZT ester at 37 °C upon incubation in human plasma and in rat hepatic microsomes. [c] IC_{50} is the concentration required to produce 50% inhibition of replication of HIV-1 and of proliferation of H9 lymphocytes under the conditions described in the Experimental Section.

Scheme III



the corresponding esters as their trifluoroacetate salts. Attempts to deprotect the *tert*-butoxy-*t*-BOC-serine ester of AZT under similar conditions resulted in the hydrolysis of the ester bond. Use of other groups such as benzyloxy for the protection of CH_2OH of serine was also found to be unsuccessful in obtaining the desired compound since the ester bond was hydrolyzed during deprotection. Glutamic acid ester at the γ-COOH function was obtained by the interaction of *N*-α-*t*-BOC-L-glutamic-α-*tert*-butyl ester with AZT in the presence of DCC and DMAP (Scheme III). The desired prodrug **17** was obtained by removal of both the protective groups, *tert*-butyl and BOC, from the COOH and NH_2 functions, respectively, in a 1:1 mixture of TFA and methylene chloride. The physicochemical properties of various prodrugs of AZT are described in Table I.

Results and Discussion

The primary objective of this project was to design and synthesize prodrugs of AZT with improved permeability properties for achieving higher concentrations of the prodrug in the central nervous system (CNS) and in the HIV target cells, the T-lymphocytes, and macrophages. Therefore, the partition coefficients of the newly synthesized esters were first determined by equilibrating their solutions in *n*-octanol with phosphate buffer (0.2 M, pH 7.4) at room temperature according to the procedure published earlier.[17] The data in Table II indicate that all the esters (**2–6**) except certain amino acid analogues were more lipophilic than AZT. Among the five amino acid substituted esters of AZT, the phenylalanine (**12**) and isoleucine (**14**) analogues were more lipophilic, as expected, than the tyrosine (**13**), lysine (**15**), and glutamic acid (**17**) esters. The partition coefficient of isoleucine ester **14** was 10.3, highest in this series.

The usefulness of the prodrugs of AZT should depend not only on the stability of the prodrug for its transport across the cell membrane and into the CNS but also upon its reversion to the parent compound intracellularly, especially in the virally infected cells. The half-lives ($t_{1/2}$) of hydrolysis of the esters were therefore determined in phosphate buffer (pH 7.4) and in human plasma. The various prodrugs of AZT were found to be stable in phosphate buffer for up to 4 h at 37 °C except the Tyr (**13**) and Lys (**15**) analogues, which were found to be unstable and resulted in hydrolysis of approximately 75% of the esters under these conditions. The data in Table II indicate that the various esters of AZT were susceptible to the action of plasma esterases with $t_{1/2}$ in the range of 10 to 120 min except the compounds **5** and **14**, which did not undergo enzymatic hydrolysis for up to 4 h. These results suggest that the esters **5** and **14** may have increased plasma $t_{1/2}$ under the in vivo conditions.

Since esters have been shown to undergo extensive first-pass metabolism, the stability of the prodrugs of AZT was also assessed upon incubation with hepatic microsomes isolated from rat. The $t_{1/2}$ of the prodrugs in this system varied from 5 to 110 min, indicating that the microsomal enzymatic hydrolysis was relatively rapid and complete. Compound **5** had the largest $t_{1/2}$ of 110 min, suggesting that this agent may possess better pharmacokinetic properties than other members of this series.

The extent of cell membrane permeability for diffusion of AZT and its prodrugs was measured by comparing the uptake of radiolabeled drugs in H9 cells. These experiments were conducted at the level of 5 μM AZT or its prodrug, a concentration that can be achieved clinically by the administration of AZT. Previous studies have reported that AZT crosses the cell membranes by nonfaci-

(17) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.

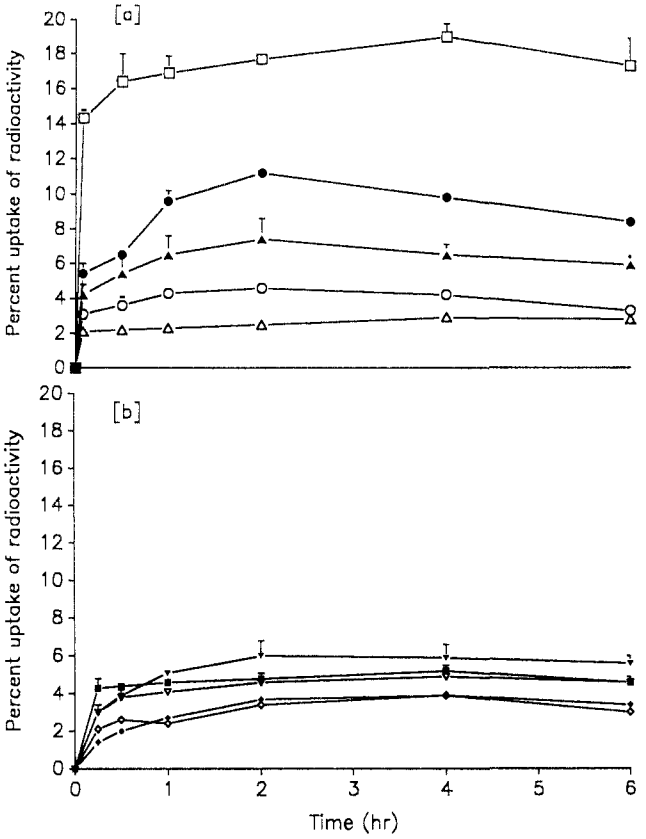


Figure 1. Uptake of [^{3}H]AZT and its analogues (5 μM) in H9 cells (5 × 10^5 at 37 °C at various time intervals: a, AZT (○), **2** (▲), **3** (△), **5** (●), and **6** (□); b, **12** (▽), **13** (▼), **14** (■), **15** (◆), **17** (◇).

litated diffusion and that its uptake is insensitive to the inhibitors of nucleoside transport,[18] indicating that the partition coefficient of AZT analogues might have a significant role in their diffusion. Most prodrugs that had higher partition coefficients than AZT except compound **3** diffused into the cells at a higher level than AZT, suggesting an important influence of partition coefficient on the diffusion of AZT analogues into the cells (Figure 1a). The data in Figure 1, parts a and b, indicate that the uptake of retinoyl derivative (**6**) in H9 cells was highest in this series (approximately 4-fold more than AZT), followed by compound **5**, which was taken up by the cells approximately 2 times more than AZT. Among amino acid derivatives, compound **14**, which had the highest partition coefficient, diffused into the cells to a greater extent than other analogues at short time intervals (Figure 1b). The two amino acid analogues **15** and **17** had significantly lower partition coefficients than AZT and were less permeable to the cells.

The antiviral activity of AZT and its prodrugs against HIV-1 was determined in vitro in peripheral blood lymphocytes (PBL) obtained from HIV-seronegative donors. The replication of HIV-1 was followed by determining the concentration of virus-specific p24 antigen in the supernatant fractions of the culture. The inhibition of production of the p24 antigen was therefore used as an indicator to determine the effectiveness of the antiviral drugs. The data are presented as the nontoxic concentration of the drugs required to produce 50% inhibition of production of p24 antigen (Table II). The IC_{50} value for AZT was 0.12 μM under these conditions and most of the prodrugs tested were found to be similar in activity except compound **5**, which was approximately 2.5 times more potent than AZT in inhibiting the viral replication. These results suggest that the increased inhibition of HIV-1 replication by compound **5** may be due to higher intracellular levels achieved by this prodrug in comparison to that of AZT (Figure 1a). However, retinoyl derivative **6**, which had the highest cellular uptake, was approximately similar in antiviral activity with an IC_{50} value of 0.2 μM. This lack of increase in antiviral activity by **6** may be related to incomplete intracellular hydrolysis of the prodrug under the in vitro conditions. Although retinoic acid has been reported to inhibit HIV replication,[8] the partially released retinoic acid from the prodrug did not produce a synergistic or an additive effect.

The cytotoxicity of AZT and the prodrugs was determined against H9 cells by trypan blue dye exclusion method for viability. Most prodrugs except compound **6** were less cytotoxic than AZT (Table II), suggesting that the slow release of AZT from the prodrugs may be related to the reduction in toxicity. However, a 6-fold increase in toxicity observed with **6** may be due to the partial release of the prodrug moiety, the retinoic acid, which had been shown to be relatively more toxic to the cells than AZT.[8] Compound **5**, which was 2.5-fold more active in inhibiting the virus replication, was in addition approximately 2 times less toxic than AZT. Compound **14**, among the amino acid analogues, was not susceptible to plasma esterases and was also less toxic than AZT under in vitro conditions. These results suggest that compounds **5** and **14** may have better clinical potential than AZT for the treatment of AIDS.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Silica gel plates (Merck F254) were used for thin-layer chromatography. The compounds were detected by visual examination under short- and long-wavelength UV light. IR spectra were recorded on Perkin-Elmer FTIR 1600 series spectrophotometer. ^{1}H NMR spectra were recorded at 80 MHz on a varian FT-80A spectrophotometer using tetramethylsilane as the internal reference. UV spectra were recorded on a Beckman DB-G spectrophotometer.

Radiolabeled compounds were synthesized from [*methyl*-^{3}H]AZT. Specific activity and radiochemical purity of the crude compounds were as follows: compound **2** (0.83 mCi/mmol, 87%), compound **3** (0.64 mCi/mmol, 88%), compound **4** (1.5 mCi/mmol, 92%), compound **5** (0.46 mCi/mmol, 88%), compound **6** (5.1 mCi/mmol, 98%), compound **12** (0.85 mCi/mmol, 93%), compound **13** (0.12 mCi/mmol, 95.5%), compound **15** (0.48 mCi/mmol, 92%), compound **17** (0.14 mCi/mmol, 93%). The radiopurity was determined by employing a Beckman (Model 340) high-performance liquid chromatographic system equipped with an on-line Flow-One Beta radioactive flow detector (Radiomatic Instruments, Model CR, 2.5 mL cell type). All compounds were detected at 267 nm. A reverse-phase Econosphere C18 column (150 × 4.6 mm, 5 μm) was used with various mobile phases as follows. Esters **4** and **5** were detected with methanol/water (1:1) as the mobile phase with retention times of 4.0 and 11.2 min, respectively, with a flow rate of 1.5 mL/min. Esters **2** and **6** were detected with methanol/25 mM ammonium phosphate buffer, pH 2.2 (9:1), at peak retention times of 5.2 and 4.8 min with a flow rate of 0.5 and 2 mL/min, respectively. The same mobile phase was used with various amino acid esters, i.e. phenylalanine (**12**), tyrosine (**13**), isoleucine (**14**), lysine (**15**), and glutamic acid (**17**) analogues with peak retention times of 4.4, 5.3, 3.4, 2.6, and 5.4 min with a flow rate of 1.0, 1.0, 2.0, 2.5 and 1.0 mL/min, respectively. Ester **3** was detected with acetonitrile/25 mM ammonium phosphate buffer pH 2.2 (15:85) as the mobile phase at a peak retention time of 3.2 min with a flow rate of 2.0 mL/min. For detection of radioactivity, a simultaneous flow of scintillation cocktail (Flo-Scint II) was used at a flow rate of 3 times the flow rate of the HPLC system. The elemental analyses were performed by Baron-Consulting Co., Orange, CT, and are within ±0.4% of

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the calculated value when specified by symbols.

AZT was synthesized by a five-step procedure as published by Glinski et al.[19] [*methyl*-^{3}H]AZT was synthesized according to the procedure reported from our laboratory.[20] Morpholine-1-acetic acid was synthesized from morpholine and chloroacetic acid in the presence of aqueous sodium hydroxide by the reported method.[21] 4-Phenylpiperazine-1-acetic acid was obtained by the hydrolysis of its ethyl ester in 20% HCl as reported.[22]

General Procedure for the Esterification of AZT. DCC (1.2 equiv) was added to a stirred solution consisting of AZT (1 equiv), DMAP (1.5 equiv) and the appropriate acid (1.1 equiv) in ethyl acetate (50 mL/g of AZT) under anhydrous conditions. The progress of the reaction was monitored by TLC. After the reaction was complete (~36–72 h), the separated DCU was filtered off. The filtrate was evaporated to dryness under reduced pressure with rotary evaporator and the residue consisting of one major product was purified by column chromatography over silica gel using a mixture of ethyl acetate/hexane (8.5:1.5) as eluent. ^{1}H NMR and IR spectra of all new compounds were consistent with the assigned structures. Compound **5** was synthesized by a variation of the published procedure,[13] as described below.

3′-Azido-3′-deoxy-5′-*O*-[(1,4-dihydro-1-methyl-3-pyridyl)carbonyl]thymidine (5). Compound 4[13] (1.35 g, 3.6 mmol) was converted to its methiodide by refluxing for 6 h with iodomethane (3 g, 21 mmol) in acetone (50 mL). The solvent was removed and the residue was directly used in the next step. To a suspension of crude methiodide in deaerated water (50 mL) under nitrogen were added sodium dithionite (2.7 g, 15.5 mmol), sodium bicarbonate (1.3 g, 15.5 mmol), and ethyl acetate (50 mL). The mixture was stirred in an ice bath for 1 h followed by 2 h at ambient temperature. The ethyl acetate layer was separated and the aqueous layer was further extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate solution was washed with water (2 × 15 mL), dried over anhydrous sodium sulfate, and evaporated to dryness to produce ester **5** as a yellow solid, which was crystallized two times from absolute ethanol to yield 0.65 g (50.6%): mp 132–135 °C (dec); IR (KBr) 3420 (NH), 2080 (N_3), 1710 (C=O ester), 1680, 1650 (CONH) cm^{-1}; UV_{max} (methanol) 217 nm (ϵ 13521), 265 (ϵ 11673), 359 (ϵ 8035); ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3 H, 5-Me), 2.19–2.50 (m, 2 H, H-2′), 2.94 (s, 3 H, NMe), 3.12–3.31 (b, 2 H, C_4 pyridine H) 3.81–4.40 (m, 4 H, H-3′, H-4′, and H-5′), 4.75 (d, 1 H, pyridine C_5H), 5.62 (m, 1 H, pyridine C_6 H), 6.12 (m, 1 H, H-1′), 6.81 (s, 1 H, H-6), 7.31 (b, 1 H, pyridine C_2 H), 11.12 (b, 1 H, NH, exchangeable with D_2O). Anal. ($C_{17}H_{20}N_6O_5$) C, H, N.

3′-Azido-3′-deoxy-5′-*O*-retinoylthymidine (6). A reaction mixture consisting of retinoic acid (0.53 g, 1.8 mmol), oxalyl chloride (0.34 g, 2.7 mmol), and anhydrous benzene (25 mL) was stirred at ambient temperature for about 1 h. The reddish yellow solution thus obtained was evaporated to dryness under reduced pressure. The residual oil was dissolved in benzene (25 mL) and the solution was added dropwise to an ice-cold, stirred solution consisting of the AZT (0.47 g, 1.8 mmol), DMAP (0.32 g, 2.6 mmol), and benzene (25 mL) under anhydrous conditions. The solution was stirred in an ice bath for 1 h and then refluxed in an oil bath for about 3 h. The mixture was cooled and diluted with benzene (100 mL). The organic solution was washed with saturated aqueous sodium carbonate (2 × 15 mL) and then with water (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate and was evaporated to dryness. The residue consisting of one major product was purified by chromatography over silica gel using hexane/ethyl acetate (2:3) as eluent to yield 0.55 g (54%) of **6** as a yellow foam: mp 70–72 °C; IR (KBr) 3327 (NH), 2105 (N_3), 1728 (C=O ester), 1681, 1640 (CONH) cm^{-1}; UV_{max} (methanol) 222 nm (ϵ 19650), 267 (ϵ 23165), 364 (ϵ 51610); ^{1}H NMR ($CDCl_3$) δ 0.88–2.50 (m, 26 H, 6 × Me, cyclohexane H and H-2′), 4.12–450 (m, 4 H, H-3′, H-4′, and H-5′), 5.56–6.40 (m, 6 H, olefinic H and H-1′), 7.10–7.40 (m, 1 H, olefinic H), 7.50 (s, 1 H, H-6), 8.56 (s, 1 H, NH, exchangeable with D_2O). Anal. ($C_{30}H_{39}N_5O_5 \cdot 2H_2O$) C, H, N.

General Procedure for Deblocking of BOC-Amino Acid Esters of AZT. The BOC-Amino acid esters of AZT (7–11 and 16) were initially synthesized by using the various BOC-amino acids in the general procedure described under the section General Procedure for the Esterification of AZT.

The BOC-amino acid ester of AZT (500 mg) was added slowly to a mixture of TFA and methylene chloride (1:1, 10 mL). The clear solution was stirred under anhydrous conditions for 30 min. The solvent was then removed under vacuum and the residue was purified by column chromatography using ethyl acetate/methanol (4:1) as eluent. The yields and physicochemical data for various amino acid esters are shown in Table I.

Uptake Studies of the Esters in H9 Cells. H9 cells (a lymphoblastoid cell line) were grown in RPMI 1640 medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), and 15% fetal bovine serum (FBS) at 37 °C in 5% CO_2/95% humidified air in an incubator. A suspension of 5 × 10^5 cells (prewarmed at 37 °C in Eppendorf tubes) was incubated with various esters (5 μM) at 37 °C for various time intervals. The reactions were stopped by centrifugation at 13000*g* for 1 min. The cell-free supernatants were discarded by aspiration and the cell pellets were washed with ice-cold PBS to remove free radioactive drugs. The cell pellets were then resuspended in PBS and counted for radioactivity diffused into the cells in a Tri-carb Packard liquid scintillation analyzer Model 1500 (Packard Instruments Co., Dowers Grove, IL) and the percentage of radioactivity diffused into the cells was calculated. (All experiments were repeated at least three times).

Hydrolysis of the Esters in Human Plasma, Rat Hepatic Microsomes, and Phosphate Buffer. To 990 μL of human plasma, a solution of rat hepatic microsomes (15.5 mg protein/mL),[23] or phosphate buffer (0.2 M, pH 7.4) was added 10 μL of a solution of one of the esters (10 mg/mL in dimethyl sulfoxide) and the mixture was incubated at 37 °C in a water bath. At various time intervals (0–4 h), 100 μL of the samples were withdrawn and added immediately to ice-cold methanol (400 μL). The samples were centrifuged and the supernatants were filtered through nylon 66 filters (0.45 μm) and analyzed by HPLC using appropriate solvents as described in the beginning of the Experimental Section.

In Vitro Inhibition of HIV Replication in Peripheral Blood Lymphocytes. Inhibition of HIV-1 replication was determined with peripheral blood lymphocytes (PBL) from seronegative donors. PBL activated with phytohemagglutinin (2 μg/mL) for 72 h were washed with medium and 10 × 10^6 cells were incubated for 4 h at 37 °C in 5 mL of medium containing the appropriate concentrations of the drugs. The cells were then infected with 10^4 $TCID_{50}$ of HIV (isolate CD 451) by the addition of 0.1 mL of virus preparation and gentle rocking of the cultures at 15-min intervals for 90 min at room temperature. The fluid volumes were increased to 10 mL and the cultures were maintained at 37 °C in a humidified 5% CO_2/95% air atmosphere. On day 3 postinfection, half of the cell-free fluids in each cultures were removed and replaced with fresh medium control and the experimental cultures were evaluated 7 days postinfection by quantifying the concentration of HIV-specific p24 antigen in an undiluted and 5-fold serial dilutions of the culture supernatants with commercial antigen-capture assay kits (Du Pont, Billerica, MA). The concentration of HIV specific p24 antigen in the 7-day supernatant fluid of a drug-free control culture was on an average approximately 372835 pg/mL.

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Registry No. 1, 30516-87-1; **2**, 125762-96-1; **3**, 125762-97-2; 4, 116333-43-8; **5**, 116333-41-6; **6**, 125780-97-4; **7**, 125780-75-8; **8**, 125780-76-9; **9**, 125780-98-5; **10**, 125780-77-0; 11, 125780-78-1;

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12·CF_3CO_2H, 125780-80-5; 13·CF_3CO_2H, 125780-82-7; 14·CF_3CO_2H, 125780-96-3; 15, 125780-84-9; 16, 125780-85-0; 17, 125780-86-1; 4-phenyl-1-piperazineacetic acid, 119378-70-0; 4-morpholineacetic acid, 3235-69-6; nicotinic acid, 59-67-6; retinoic acid, 302-79-4; *N*-Boc-L-phenylalanine, 13734-34-4; *N*-Boc-L-tyrosine, 3978-80-1; *N*-Boc-L-isoleucine, 13139-16-7; *N,N'*-di-Boc-L-lysine, 2483-46-7; *N*-Boc-L-glutamic acid, 1-*tert*-butyl ester, 24277-39-2; *N*-Boc-*O*-*tert*-butyl-L-serine, 13734-38-8.

Dihydropyrimidine Calcium Channel Blockers: 2-Heterosubstituted 4-Aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Potent Mimics of Dihydropyridines

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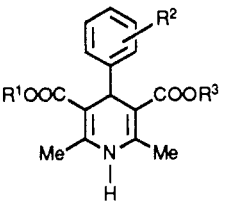
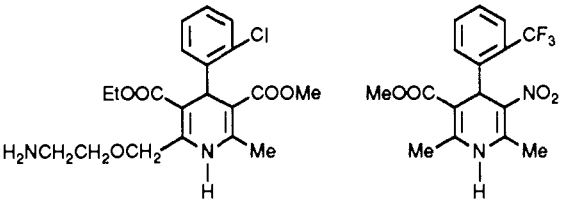
2-Heterosubstituted-4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic acid esters **8**, which lack the potential C_s symmetry of dihydropyridine calcium channel blockers, were prepared and evaluated for biological activity. Biological assays using potassium-depolarized rabbit aorta and radioligand binding techniques showed that some of these compounds are potent mimics of dihydropyridine calcium channel blockers. The combination of a branched ester (e.g. isopropyl, *sec*-butyl) and an alkylthio group (e.g. SMe) was found to be optimal for biological activity. When compared directly with similarly substituted 2-heteroalkyldihydropyridines **9**, dihydropyrimidines **8** were found to be 30-fold less active. The solid-state structure of dihydropyrimidine analogue **8g** shows that these compounds can adopt a molecular conformation which is similar to the reported conformation of dihydropyridine calcium channel blockers.

Dihydropyridines are the largest and most studied class of organic calcium channel blockers.[1] In addition to their proven clinical utility in cardiovascular medicine, dihydropyridines are employed extensively as biological tools for the study of voltage-activated calcium channel structure and function.[2] The structures of some of these compounds are shown in formulae **1–5**. Biological activity appears to depend on an axial aromatic ring, the plane of which bisects the dihydropyridine ring in a boat conformation.[3] When the substituents at C2/C6 and the esters at C3/C5 are equivalent, the molecule possesses C_s symmetry and is nonchiral. Unsymmetrical modification of these substituents generates a chiral center at C4. In certain cases (e.g. **6** and **7**), individual isomers possess opposite actions on the calcium channel; one showing blocking activity and the other demonstrating activating activity.[4] Additional modification of both the ester (e.g. **1–4**) and alkyl substituents (e.g. **5**) can affect duration of action in vivo and is claimed to alter tissue selectivity.[5,6]

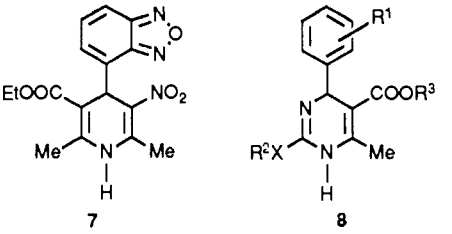
The study of inherently unsymmetrical molecules facilitates obtaining information about structural requirements important to biological activity and the investigation of the effects of absolute stereochemistry. It also affords an opportunity to expand existing structure–activity relationships and, potentially, to discover additional structural modifications consistent with improved biological activity. We have recently demonstrated that the potential C_s symmetry of dihydropyridine calcium channel blockers



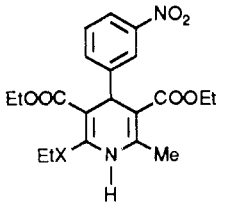
1, nifedipine: R^1 = Me, R^2 = 2 - NO_2, R^3 = Me
2, nitrendipine: R^1 = Me, R^2 = 3 - NO_2, R^3 = Et
3, nimodipine: R^1 = CH_2CH_2OMe, R^2 = 3 - NO_2, R^3 = iPr
4, nicardipine: R^1 = $CH_2CH_2N(Me)Bn$, R^3 = 3 - NO_2, R^3 = Et



5, amlodipine **6**, Bay K - 8644



7 **8**



9a: X = S
b: X = O

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