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Bisheteroarylpiperazine Reverse Transcriptase Inhibitor in Combination with 3′-Azido-3′-Deoxythymidine or 2′,3′-Dideoxycytidine Synergistically Inhibits Human Immunodeficiency Virus Type 1 Replication In Vitro

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Bisheteroarylpiperazine compounds are nonnucleoside reverse transcriptase inhibitors of human immunodeficiency virus type 1 (HIV-1). To provide a rationale for combination therapy with a second-generation bisheteroarylpiperazine, we investigated the effect of U-90152 in combination with 3′-azido-3′-deoxythymidine (AZT) or 2′,3′-dideoxycytidine (ddC). HIV-1-infected cells were cultured in the presence of test compounds, and drug effects on p24 core antigen production were measured by an enzyme-linked immunosorbent assay. In a CD4$^+$ T-cell line (H9) infected with HIV-1$_{IIIB}$, the 50% effective concentrations for U-90152, AZT, and ddC were 6.0, 80.4, and 31.8 nM, respectively. In human peripheral blood mononuclear cells infected with the molecularly cloned clinical isolate HIV-1$_{JRCSF}$, the 50% effective concentrations for U-90152, AZT, and ddC were 5.3, 5.9, and 25.0 nM, respectively. Over a range of drug concentrations (U-90152 and AZT at 0.3, 1, 3, 10, and 30 nM; ddC at 3, 10, 30, and 100 nM), U-90152 in combination with AZT or ddC synergistically inhibited the replication of a laboratory-adapted strain and a clinical isolate of HIV-1.

The bisheteroarylpiperazine (BHAP) class of compounds has been shown to inhibit the reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) and block HIV-1 replication in culture cells (1, 3, 7, 8, 28, 33). The BHAPs belong to a structurally diverse group of compounds known as the nonnucleoside RT inhibitors. These compounds inhibit the HIV-1 RT by binding to a common region of the enzyme away from the substrate binding site (8, 12, 33). In contrast, nucleoside analog inhibitors, such as 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine, and 2′,3′-dideoxycytidine (ddC), mimic the normal deoxynucleotide triphosphate substrate for RT and thus act as chain terminators during proviral synthesis (31). Other nonnucleoside RT inhibitors include dipyridodiazepinones (nevirapine) (18, 23), pyridinones (L-697,661) (12), thiobenzimidazolone compounds (TIBO Ro82150) (25), and the more recently reported TSAO [2′,5′-bis-*O*-(*tert*-butyl-dimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine] and HEPT {1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine} compounds (2, 32).

Drug-resistant HIV-1 can emerge as a result of treatment of HIV-1-infected individuals with a nucleoside RT inhibitor, such as AZT (21), and also when a laboratory strain of HIV-1 is passaged in cell culture in the presence of increasing amounts of AZT (20). Similar in vitro findings have been reported for the nonnucleoside RT inhibitors (12, 23). Therefore, much research has been directed toward therapy with combinations of various anti-HIV agents (13). Several investigators have reported synergistic inhibition of HIV-1 replication in cell cultures by combining AZT with various nucleoside (7, 11) and nonnucleoside RT inhibitors (12, 27). When combined with AZT, a first-generation BHAP, U-87201, was reported to be synergistic against AZT-resistant isolates, but showed only additive effect against AZT-susceptible isolates. The chemistry and biology of a second-generation highly potent BHAP compound, U-90152 {1-(5-methanesulfonamido-1*H*-indol-2-yl-carbonyl)-4-[3-(1-methyl-ethylamino)pyridinyl] piperazine, methanesulfonate}, have been recently described (9, 29). Currently, U-90152 is undergoing clinical evaluation both as single therapy and in combination with AZT and other nucleoside inhibitors. To provide a rationale for combination therapy with this second-generation BHAP, we

TABLE 1. Comparison of antiviral activities of U-90152 in cell culture with those of AZT and ddC[a]

HIV-1 isolate	Cell type	U-90152		AZT		ddC	
		EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
IIIB	H9	6.0 ± 2.0 (4–8)	45.0 ± 16.0 (26–65)	80.4 ± 60.0 (38–123)	1,575.2 ± 1,440 (437–2,713)	31.8 ± 14.0 (13–47)	206.2 ± 148.0 (81–435)
JRCSF	PBMC	5.3 ± 4.0 (1–8)	39.3 ± 16.0 (18–47)	5.9 ± 4.0 (3–10)	19.0 ± 5.0 (16–25)	25.0 ± 9.0 (17–34)	152.7 ± 83.0 (102–248)

[a] Data represent mean EC_{50} and EC_{90} values in nanomoles ± standard errors of the means of five separate experiments (each using three replicate wells) for HIV-1$_{IIIB}$/H9 determinations and three experiments for HIV-1$_{JRCSF}$/PBMC determinations. Values in parentheses are the ranges of values.

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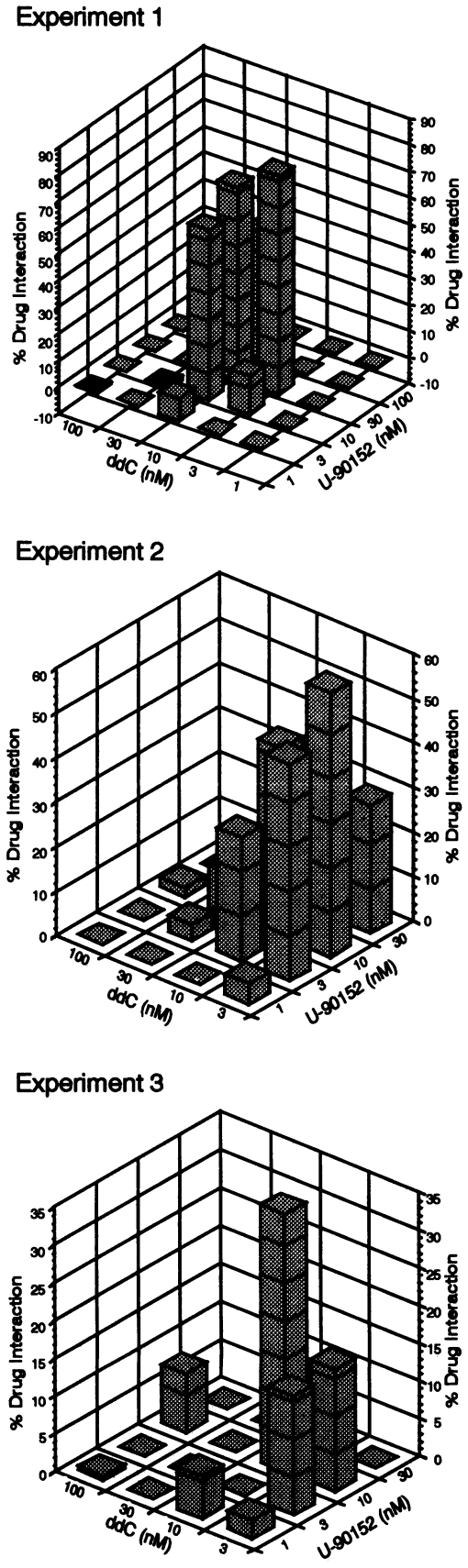

investigated the effect of U-90152 in several combination ratios with AZT or ddC in H9 cells acutely infected with HIV-1_{IIIB} ($HTLV_{IIIB}$) and in human peripheral blood mononuclear cells (PBMC) infected with a molecularly cloned clinical isolate, HIV-1_{JRCSF}.

MATERIALS AND METHODS

Compounds. Stocks of AZT and ddC (Sigma), as well as U-90152 (Upjohn Laboratories, Kalamazoo, Mich.), were prepared as 10 mM stocks in dimethyl sulfoxide and maintained at −70°C.

Virus and cells. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. HIV-1_{JRCSF} came from Irvin Chen. H9 cells and $HTLV_{IIIB}$/H9 came from Robert Gallo; MT-4 cells came from Douglas Richman. HIV-1_{JRCSF} was selected because it retained properties similar to those of primary patient isolates, such as replication only in primary human lymphocytes or mononuclear phagocytes (19). Stock viruses were harvested as culture supernatant: HIV-1_{JRCSF} was from infected primary human PBMC culture, and HIV-1_{IIIB} was from infected H9 cells. The 50% cell culture infective dose per milliliter was determined in MT-4 cells by the detection of cytopathic effect and confirmed by p24 assay on day 5 after virus infection.

Antiviral assay. H9 human T-lymphocytic cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum. Human PBMC were obtained from fresh plasmapheresis preparations taken from HIV-1-seronegative donors and prepared by density gradient centrifugation with Ficoll-Hypaque (Organon Teknika, Durham, N.C.). Nonadherent cells were collected and incubated for 3 days in culture medium containing 4 μg of phytohemagglutinin per ml. The activated lymphoblasts were cultured in antiviral assay medium containing natural human interleukin-2 (64 U/ml; Pharmacia). H9 cells and PBMC assays were set up as described previously (4). Under our experimental conditions and without change of culture media, uninfected PBMC reached a peak cell number (1.85×10^6 cells per ml) on day 5 after seeding and showed 93.4% survival on culture day 7. The percent inhibition of HIV replication by the compound was determined by comparing HIV p24 antigen levels in the supernatant of infected cells treated with inhibitor with those in supernatant from the control cultures without compound.

Combination studies. The inhibition of HIV replication by combinations of U-90152 and AZT or ddC was evaluated in experiments involving either single or multiple molar ratios of the drugs. For an experiment involving multiple ratios, four to five concentrations of each drug (U-90152 and AZT at 0.3, 1.0, 3.0, 10.0, and 30.0 nM; ddC at 3.0, 10.0, 30.0, and 100 nM), or drug combinations, were assayed in a checkerboard manner. At the most relevant drug interaction ratios, three to four datum points for each molar ratio were available for analysis. With a computer software program (5), combination indices (CI) were calculated by the multiple drug effect equation of Chou and Talalay (6), assuming a mutually nonexclusive drug interaction condition. The CI values at various fractional inhibitions (50, 90, and 95%) were used to determine whether

FIG. 1. Drug interaction analysis of ddC and U-90152 in acutely infected H9 cells (experiments 1 and 2) and PBMC (experiment 3). The amount of synergy is represented by the height of bars in the graphs.

TABLE 2. Volumes of synergy and CI values for two-drug combination regimens of U-90152 with AZT or ddC against HIV-1 replication in H9 cells and in PBMC

Expert no.	Cell type and virus	Combination (ratio)	CI[a] at % inhibition			Vol[b] of synergy at 95% confidence level
			50	90	95	
1	H9; HIV-1_{IIIB}	U-90152 plus ddC				229.8
		1:1	0.68	0.57	0.57	
		1:3	0.52	0.62	0.67	
		1:10	0.34	0.89	1.32	
2	H9; HIV-1_{IIIB}	U-90152 plus ddC				234.6
		1:1	0.33	0.38	0.45	
		1:3	0.24	0.45	0.63	
		1:10	0.30	0.40	0.46	
3	PBMC; HIV-1_{JRCSF}	U-90152 plus ddC				82.0
		1:1	0.66	0.46	0.45	
		1:3	0.57	0.38	0.37	
		1:10	0.14	0.65	1.26	
4	PBMC; HIV-1_{JRCSF}	U-90152 plus AZT				372.0
		1:1	0.42	0.38	0.39	
		1:3	0.20	0.47	0.68	
		3:1	0.21	0.53	0.78	
5	PBMC; HIV-1_{JRCSF}	U-90152 plus AZT				292.9
		1:1	0.29	0.51	0.64	
		1:3	0.56	0.73	0.82	
		3:1	0.19	0.49	0.72	

[a] CI values of <1, 1, and >1 indicate synergism, additive effects, and antagonism, respectively.
[b] Volume of synergy was computed from a surface area plot of percent drug interaction versus drug concentrations. Values of between 50 and 100 $\mu M^2\%$ indicate moderate synergy whereas values greater than 100 $\mu M^2\%$ indicate strong synergy.

the combinations were synergistic (CI at 50% < 1), additive (CI at 50% = 1), or antagonistic (CI at 50% > 1). Drug interactions were also analyzed by the method of Prichard and Shipman (26) employing the MacSynergy computer program (C. Shipman, University of Michigan). The amount of synergy observed with combinations of the two compounds is represented by the height of bars in the graph when the percentage of drug interaction is plotted versus drug concentrations. Additive levels were subtracted out by the program and are represented by the floor of the graph. The extent of synergistic interaction can be quantitated by the computation of the volume of synergy from a surface area plot of the same data.

Assay of cytotoxicity. The cytotoxicities of the compounds in uninfected H9 cells and in PBMC cultures were evaluated by counting viable cells by the trypan blue exclusion method and by measuring the formation of formazan, a tetrazolium dye, in a 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay (24). Cytotoxicity assays were performed by incubating uninfected cells in the presence of the highest concentrations of the drugs tested, individually and in combination for a period of 5 and 7 days under conditions similar to those for antiviral assays.

RESULTS

Effects of U-90152, AZT, and ddC alone on HIV replication in PBMC and H9 cells. We first determined the antiviral effect of U-90152 in comparison with AZT and ddC in acutely infected PBMC and in H9 cells. In H9 cells acutely infected with a laboratory-adapted HIV-1_{IIIB}, U-90152 showed a dose-dependent inhibition of HIV-1 with mean 50% effective concentration (EC_{50}) and EC_{90} values of 6.0 and 45.0 nM, respectively (Table 1). In human PBMC cultures acutely infected with the clinical isolate HIV-1_{JRCSF}, U-90152 showed similar activities with EC_{50} and EC_{90} values of 5.3 and 39.3 nM, respectively. Thus, U-90152 was active in a T-cell line infected with a laboratory-adapted HIV-1 and also in primary target cells (PBMC) infected with a clinical isolate. As expected, AZT was less active in H9 cells, which is thought to reflect a poorer phosphorylation into its active triphosphate by these cells. The activity of ddC was marginally less in H9 cells, whereas U-90152 was equally active in H9 and in human PBMC. We also determined the toxicity of these compounds to the cell type used in this study. U-90152, AZT, and ddC showed 50% cytotoxicity at concentrations greater than 100 μM in H9 and PBMC cultures (data not shown). Thus, the selectivity index of U-90152 is about 20,000 in human PBMC, which is similar to that of AZT and about five times better than that of ddC. From these data, optimal concentration ranges of U-90152, AZT, and ddC that would best demonstrate drug interactions in the subsequent combined therapy experiments under similar assay conditions were selected.

Effects of U-90152 and ddC combinations in H9 cells. We studied the effects of combinations with these two drugs on HIV-1 replication and also on potential cytotoxicity of these agents to H9 cells. Combinations of U-90152 and ddC in the dose range tested were consistently more effective than either agent alone. Analysis by the MacSynergy program indicated strong synergistic interactions at the 95% confidence level (Fig. 1 and Table 2).

The dose effects for each single agent and the combination were used to make a median effect plot. From this, we computed CI at combination ratios of U-90152 to ddC of 1:1, 1:3, and 1:10 because this reflected U-90152 as a more active compound than ddC and reflected that both compounds were evaluated with a similar activity range. Synergistic interactions at these molar ratios were observed with CI of <1 at 50, 90, and 95% effective doses (Table 2, experiments 1 and 2). At the 1:10 combination ratio, one of the experiments showed a CI value of >1 at the 95% inhibition level. However, the MacSynergy program indicated that there was no antagonism at the 95% confidence level in any of the test ratios (Fig. 1). We observed no antiproliferative activity and toxicity to cultured cells with either of these single agents or their combinations.

Thus, the enhanced antiviral activity observed was not related to effects resulting from the combination of the agents on cell proliferation. The concentrations of each agent (U-90152 in test ranges from 0.3 to 30 nM and ddC at 3 to 100 nM) used in the combination experiments were about 1,000-fold less than their toxicity concentration (data not shown).

Combinations of U-90152 and ddC in PBMC. To extend these findings to a clinical isolate, we evaluated combination therapy against the replication of HIV-1_{JRCSF} in human PBMC. At dose ranges of each compound tested similar to those above, the combination of U-90152 and ddC in PBMC was also more active than either agent alone. This effect was synergistic at the 95% confidence level over a range of concentrations of the two inhibitors as analyzed by MacSynergy and demonstrated CI values of <1 at the ratios shown (Table 2, experiment 3). We also noted a CI value of >1 at the 1:10 ratio in this PBMC assay (Table 2). However, the analysis of all test combination ratios by MacSynergy indicated no statistically significant antagonism (Fig. 1, experiment 3). As with the H9 cells, these compounds and their combinations also did not show antiproliferative effect or cytotoxicity to PBMC culture.

Combinations of U-90152 and AZT in PBMC. AZT was about 10 times less active than U-90152 in our H9 cell assay. In fixed-ratio experiments with a ratio of U-90152 to AZT of 1:10, we noted synergy (CI < 1) at 50, 90, and 95% effective doses (data not shown). For a more detailed analysis of the combination effect of U-90152 with AZT, we used as target cells human PBMC infected with the clinical isolate HIV-1_{JRCSF}. Since U-90152 and AZT were equally active in our PBMC antiviral assays, we assayed each drug at 0.3, 1.0, 3.0, 10.0, and 30 nM in a checkerboard manner to allow for combinations of comparable activity from each agent. These data were analyzed by the MacSynergy program, which indicated strong synergistic interactions at the 95% confidence level (Fig. 2 and Table 2). Analysis of CI also indicated synergistic interactions (CI < 1) at the molar ratios of U-90152 to AZT of 1:1, 1:3, and 3:1 (Table 2, experiments 4 and 5).

DISCUSSION

We showed that U-90152 in combination with AZT or ddC over a range of concentrations of each inhibitor synergistically inhibited HIV-1 replication in cell cultures. We analyzed the effects of drug interactions by the CI method of Chou and Talalay (6) and the MacSynergy program of Prichard and Shipman (26). The CI method has been widely used in combination studies involving various inhibitors of HIV-1 replication, including nucleoside and nonnucleoside RT inhibitors (7, 11, 12, 15, 16, 32). However, the existing CI program did not provide computation for the extent of synergy and for a statistical analysis of the observed synergy. Therefore, we have also analyzed our data by the Prichard and Shipman approach. Both methods of synergy analyses indicated that the combination of U-90152 with AZT or ddC consistently showed synergy against drug-susceptible virus. When U-90152 and ddC were combined at a 1:10 ratio in the HIV-1_{IIIB}/H9 assay and in the HIV-1_{JRCSF}/PBMC assay, we noted CI values of >1 in two of three experiments at only the 95% inhibition level, which may indicate an antagonistic effect (Table 2). We are not clear whether this effect (CI of about 1.3) is due to experimental variations or alternately to the computation of CI. However, we believe that this indication of antagonism was probably not significant because the MacSynergy program indicated no significant (95% confidence level) antagonism in these experiments at all levels of viral inhibition.

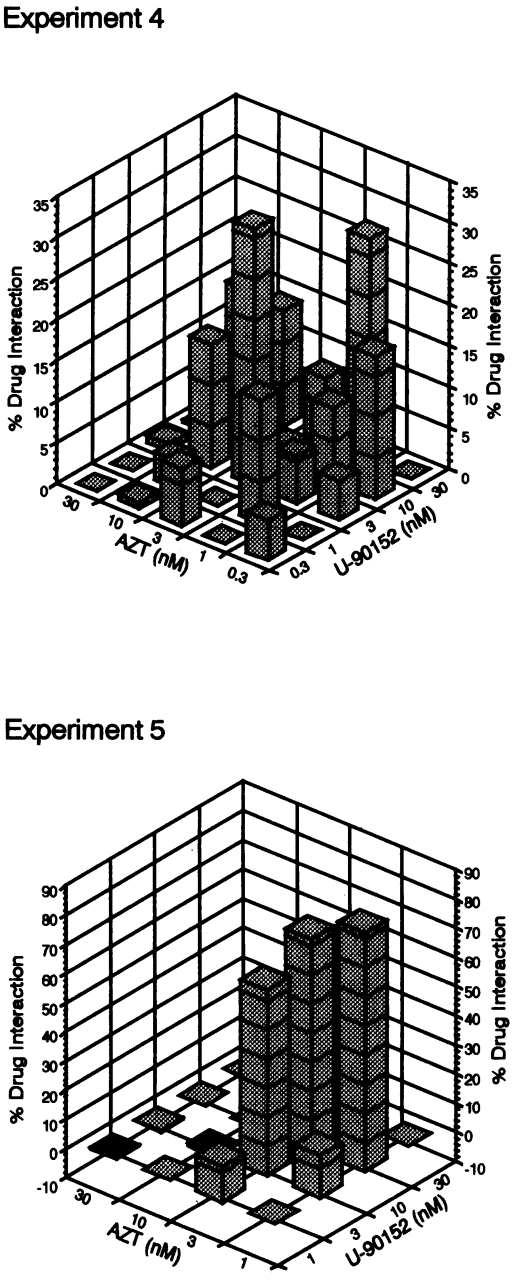

FIG. 2. Drug interaction analysis of AZT and U-90152 in acutely infected PBMC.

To the best of our knowledge, this is the first description of a synergistic effect of the combination of a BHAP compound with AZT or ddC against drug-susceptible HIV strains. Recently, Campbell et al. reported that BHAP U-87201 when combined with AZT synergistically inhibited the replication of an AZT-resistant isolate. However, they observed only an additive effect with the combination when tested against AZT-susceptible isolates (3). It is not clear whether this discrepancy is due to some properties of the clinical isolates or to the use of a different BHAP in that study. However, our finding of synergy in the antiviral assay was consistent with the reported synergistic effects of combinations of BHAP (U-85961) and AZT triphosphate or ddTTP on inhibition of

purified HIV-1 RT (28). Other nonnucleoside RT inhibitors such as nevirapine (27) and pyridinone L-697,639 (13) have also been reported to possess synergistic antiviral effects when combined in vitro with various nucleoside inhibitors including AZT. The synergistic effect that we demonstrated may also account for the finding that the combination of BHAP and AZT resulted in a more complete inhibition of HIV-1 spread in HIV-1_{IIIB}-infected MT4 cells (9).

Recent clinical evaluation indicated that combination therapy with AZT and ddC was more effective than therapy with single agents (22). For this drug combination, clinical results appeared to correlate with in vitro data obtained in cell culture experiments on synergy (11). The BHAP is likely to be an important additional anti-HIV agent because it may have antiviral activity different from that of other nonnucleoside RT inhibitors. This is indicated by a recent report that HIV-1 resistance to the BHAPs appeared to result from RT mutations that are different from RT mutations associated with resistance to other nonnucleoside inhibitors (10). Therefore, our findings that BHAP U-90152 acts synergistically with nucleoside RT inhibitors in vitro provide a strong argument for a combination therapy for patients with HIV-1 infection with the BHAPs and nucleoside analog RT inhibitors.

It should be pointed out that when BHAP U-90152 is administered orally to normal or HIV patients at 200 to 400 mg/kg of body weight, three times a day, the observed peak plasma concentrations were 30 to 50 μM and trough concentrations were 1 to 5 μM (data not shown). With intravenous doses of 5 mg/kg or oral doses of 10 mg of AZT per kg, plasma concentrations were maintained at above 1 μM in HIV-infected patients (17). Thus, the concentrations of the drugs we employed to show in vitro synergy are much lower (1 to 30 nM for U-90152 and AZT) than the plasma concentration that can be achieved in the patients. This may imply that similar antiviral effects may be achievable in the clinical situation. However, at this time, we do not have data on the distribution of BHAP compounds to various tissues and target cells and whether intracellular levels of BHAP are directly dependent on plasma levels. Until the clinical results are known, caution should be used in extrapolating in vitro findings to in vivo situations because of the involvement of complex host and viral factors, such as viral burden and the existence of viral reservoirs.

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REFERENCES

1. **Althaus, I. W., J. J. Chou, A. J. Gonzales, M. R. Deibel, K.-C. Chou, F. J. Kezdy, D. L. Romero, P. A. Aristoff, W. G. Tarpley, and F. Reusser.** 1993. Steady-state kinetic studies with the nonnucleoside HIV-1 reverse transcriptase inhibitor U-87201E. J. Biol. Chem. **268**:6119–6124.
2. **Balzarini, J., M.-J. Perez-Perez, A. San-Felix, D. Schols, C.-F. Perno, A.-M. Vandamme, M.-J. Camarasa, and E. D. De Clercq.** 1992. 2′,5′-Bis-O-(*tert*-butyl-dimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine (TSAO) nucleoside analogues: highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. Proc. Natl. Acad. Sci. USA **89**:4392–4396.
3. **Campbell, T. B., R. K. Young, J. J. Eron, R. T. D'Aquila, W. G. Tarpley, and D. R. Kuritzkes.** 1993. Evaluation of the bisheteroarylpiperazine reverse transcriptase inhibitor U-87201E in combination with zidovudine or didanosine against HIV-1 infection *in vitro*. 1993. J. Infect. Dis. **168**:318–326.
4. **Chong, K. T., M. J. Ruwart, R. R. Hinshaw, K. F. Wilkinson, B. D. Rush, M. F. Yancey, J. W. Strohbach, and S. Thaisrivongs.** 1993. Peptidomimetic HIV protease inhibitors: phosphate prodrugs with improved biological activities. J. Med. Chem. **36**:2575–2577.
5. **Chou, J., and T.-C. Chou.** 1987. Dose effect analysis with microcomputers, p. 26. Biosoft, Cambridge.
6. **Chou, T.-C., and P. Talalay.** 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. **22**:27–55.
7. **Dornsife, R. E., M. H. St. Clair, A. T. Huang, T. J. Panella, G. W. Koszalka, C. L. Burns, and D. R. Averett.** 1991. Anti-human immunodeficiency virus synergism by zidovudine (3′-azidothymidine) and didanosine (dideoxyinosine) contrasts with their additive inhibition of normal human marrow progenitor cells. Antimicrob. Agents Chemother. **35**:322–328.
8. **Dueweke, T. J., F. J. Kezdy, G. A. Waszak, M. R. Deibel, and W. G. Tarpley.** 1992. The binding of a novel bisheteroarylpiperazine mediates inhibition of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. **267**:27–30.
9. **Dueweke, T. J., S. M. Poppe, D. L. Romero, S. M. Swaney, A. G. So, K. M. Downey, I. W. Althaus, F. Reusser, M. Busso, L. Resnick, D. L. Mayers, J. Lane, P. A. Aristoff, R. C. Thomas, and W. G. Tarpley.** 1993. U-90152, a potent inhibitor of human immunodeficiency virus type 1 replication. Antimicrob. Agents Chemother. **37**:1127–1131.
10. **Dueweke, T. J., T. Pushkarskaya, S. M. Poppe, S. M. Swaney, J. Q. Zhao, I. S. Y. Chen, M. Stevenson, and W. G. Tarpley.** 1993. A mutation in reverse transcriptase of bis(heteroaryl)piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other nonnucleoside inhibitors. Proc. Natl. Acad. Sci. USA **90**:4713–4717.
11. **Eron, J. J., Jr., V. A. Johnson, D. P. Merrill, T.-C. Chou, and M. S. Hirsch.** 1992. Synergistic inhibition of replication of human immunodeficiency virus type 1, including that of a zidovudine-resistant isolate, by zidovudine and 2′,3′-dideoxycytidine in vitro. Antimicrob. Agents Chemother. **36**:1559–1562.
12. **Goldman, M. E., J. H. Nunberg, J. A. O'Brien, J. Quintero, W. A. Schleif, K. F. Freund, S. L. Gaul, W. S. Saari, J. S. Wai, J. M. Hoffman, P. S. Anderson, D. J. Hupe, E. A. Emini, and A. M. Stern.** 1991. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. Proc. Natl. Acad. Sci. USA **88**:6863–6867.
13. **Hall, M. J., and I. B. Duncan.** 1988. Antiviral drug and interferon combinations, p. 29–84. *In* H. J. Fields (ed)., Antiviral agents: the development and assessment of antiviral chemotherapy, vol. II. CRC Press, Inc., Boca Raton, Fla.
14. **Hayashi, S., R. L. Fine, T.-C. Chou, M. J. Currens, S. Broder, and H. Mitsuya.** 1990. In vitro inhibition of the infectivity and replication of human immunodeficiency virus type 1 by combination of antiretroviral 2′,3′-dideoxynucleosides and virus-binding inhibitors. Antimicrob. Agents Chemother. **34**:82–88.
15. **Johnson, V. A., M. A. Barlow, D. P. Merrill, T.-C. Chou, and M. S. Hirsch.** 1990. Three-drug synergistic inhibition of HIV-1 replication by zidovudine, recombinant soluble CD4, and recombinant interferon-alpha a. J. Infect. Dis. **161**:1059–1067.
16. **Johnson, V. A., D. P. Merrill, J. A. Videler, T. C. Chon, R. E. Byington, J. J. Eron, R. T. D'Aquila, and M. S. Hirsch.** 1991. Two-drug combinations of zidovudine, didanosine, and recombinant interferon-alpha a inhibit replication of zidovudine-resistant human immunodeficiency virus type 1 synergistically *in vitro*. J. Infect. Dis. **164**:646–655.
17. **Klecker, R. W., Jr., J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers.** 1987. Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′-deoxythymidine: a novel pyridine analog with potential application for the treatment of patients with AIDS and related diseases. Clin. Pharmacol. Ther. **41**:407–412.
18. **Koup, R. A., V. J. Merluzzi, K. D. Hargrave, J. Adams, K. Grozinger, R. J. Eckner, and J. L. Sullivan.** 1990. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by the dipyridodiazepinone BI-RG-587. J. Infect. Dis. **163**:966–970.
19. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V.**

Vinters, and I. S. Y. Chen. 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236:**819–822.

20. **Larder, B. A., K. E. Coates, and S. D. Kemp.** 1991. Zidovudine-resistant human immunodeficiency virus selected by passage in cell culture. J. Virol. **65:**5232–5236.
21. **Larder, B. A., G. Darby, and D. D. Richman.** 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science **243:**1731–1734.
22. **Meng, T. C., M. A. Fischl, A. M. Boota, S. A. Spector, D. Bennett, Y. Bassiakos, S. H. Lai, B. Wright, and D. D. Richman.** 1992. Combination therapy with zidovudine and dideoxycytidine in patients with advanced human immunodeficiency virus infection. Ann. Intern. Med. **116:**13–20.
23. **Merluzzi, V. J., K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C.-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosenthal, R. Faanes, R. J. Eckner, R. A. Koup, and J. L. Sullivan.** 1990. Inhibition of HIV-1 replication by a non-nucleoside reverse transcriptase inhibitor. Science **250:**1411–1413.
24. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65:**55–63.
25. **Pauwels, R., K. Andries, J. Desmyter, D. Schols, M. J. Kukla, H. J. Breslin, A. Raeymaeckers, J. VanGelder, R. Woestenborghs, J. Heykants, K. Schellelens, M. A. C. Janssen, E. DeClerq, and P. A. J. Janssen.** 1990. Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. Nature (London) **343:**470–474.
26. **Prichard, M. N., and C. Shipman, Jr.** 1990. Review: a three dimensional model to analyze drug-drug interactions. Antiviral Res. **14:**181–206.
27. **Richman, D. D., A. S. Rosenthal, M. Skoog, R. J. Eckner, T.-C. Chou, J. P. Sabo, and V. J. Merluzzi.** 1991. BI-RG-587 is active against zidovudine-resistant human immunodeficiency virus type 1 and synergistic with zidovudine. Antimicrob. Agents Chemother. **35:**305–308.
28. **Romero, D. L., M. Busso, C.-K. Tan, F. Reusser, J. R. Palmer, S. M. Poppe, P. A. Aristoff, K. M. Downey, A. G. So, L. Resnick, and W. G. Tarpley.** 1991. Non-nucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc. Natl. Acad. Sci. USA **88:**8806–8810.
29. **Romero, D. L., R. A. Morge, M. J. Genin, C. Biles, M. Busso, L. Resnick, I. W. Althaus, F. Reusser, R. C. Thomas, and W. G. Tarpley.** 1993. Bisheteroarylpiperazine (BHAP) reverse transcriptase inhibitors: structure activity relationships of novel substituted indole analogues and the identification of 1′[(5-methanesulfonamido-1H-indol-2-yl)carbonyl]-4-[3-(1-methylethylamino) pyridinyl] piperazine monomethanesulfonate (U-90152), a second generation clinical candidate. J. Med. Chem. **36:**1505–1508.
30. **Smith, M. S., J. A. Kessler, C. D. Rankin, J. S. Pagano, J. Kurtzberg, and S. G. Carter.** 1992. Evaluation of synergy between carbovir and 3′-azido-2′,3′-deoxythymidine for inhibition of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. **37:**144–147.
31. **St. Clair, M. H., C. A. Richards, T. Spector, K. J. Weinhold, W. H. Miller, A. J. Langlois, and P. A. Furman.** 1987. 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob. Agents Chemother. **31:**1972–1977.
32. **Tanada, H., M. Baba, H. Hayakawa, T. Sakamaki, T. Miyasaka, M. Ubasawa, H. Takashima, K. Sekiya, I. Nitta, S. Shigeta, R. T. Walder, J. Balzarini, and E. D. 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 analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). J. Med. Chem. **34:**349–357.
33. **Vasudevachari, M. B., C. Battista, H. C. Lane, M. C. Psallidopoulos, B. Zhao, J. Cook, J. R. Palmer, D. L. Romero, W. G. Tarpley, and N. P. Salzman.** 1992. Prevention of the spread of HIV-1 infection with non-nucleoside reverse transcriptase inhibitors. Virology **190:**269–277.