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Biological and biochemical anti-HIV activity of the benzothiadiazine class of nonnucleoside reverse transcriptase inhibitors

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Abstract

A series of benzothiadiazine derivatives were screened against the human immunodeficiency virus (HIV) and certain structure-activity relationships were defined for anti-HIV activity in this chemical class. The selected representative NSC 287474 was a highly potent inhibitor of HIV-induced cell killing and HIV replication in a variety of human cell lines, as well as in fresh human peripheral blood lymphocytes and macrophages. The compound was active against a panel of biologically diverse laboratory and clinical strains of HIV-1, including the AZT-resistant strain G910–6. However, the agent was inactive against HIV-2, and also against both nevirapine- and pyridinone-resistant strains (N119 and A17) of HIV-1, which are cross-resistant to several structurally diverse nonnucleoside reverse transcriptase inhibitors. The compound selectively inhibited HIV-1 reverse transcriptase, but not HIV-2 reverse transcriptase. Combination of NSC 287474 with AZT synergistically inhibited HIV-1-induced cell killing in vitro. The compound did

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not inhibit the replication of the Rauscher murine leukemia retrovirus or the simian immunodeficiency virus. The benzothiadiazine class of compounds represents a new active anti-HIV-1 chemotype within the diverse group of nonnucleoside reverse transcriptase inhibitors.

Key words: Benzothiadiazine; Nonnucleoside reverse transcriptase inhibitor; Mechanism of action; Combination therapy; Structure-activity relationship

1. Introduction

The only agents found to be clinically useful in the treatment of AIDS patients to date have been reverse transcriptase (RT) inhibitors (Connolly and Hammer, 1992). These inhibitors include the nucleoside analogs, such as 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI) and 2′,3′-dideoxycytidine (ddC) (Furman et al., 1986; Ahluwalia et al., 1987; Yarchoan et al., 1986, 1989), as well as the new, general pharmacologic class of structurally diverse nonnucleoside RT inhibitors (Pauwels et al., 1990; Merluzzi et al., 1990; Goldman et al., 1991; Romero et al., 1991; Klunder et al., 1992; McMahon et al., 1993; Buckheit et al., 1993a; Maas et al., 1993). The nonnucleoside reverse transcriptase inhibitors represent very different chemotypes and are potent and selective inhibitors of HIV-1 replication and cell killing. As a class, the compounds have no activity against isolates of HIV-2 and most other retroviruses. Moreover, viral mutants which are cross-resistant to these nonnucleoside RT inhibitors have been easily obtained in tissue culture (Nunberg et al., 1991; Richman et al., 1991a; Wu et al., 1991; Mellors et al., 1992; De Vreese et al., 1992; Balzarini et al., 1993) as well as in patients (Saag et al., 1993). Sequence analysis of the nonnucleoside RT inhibitor-resistant viruses has identified specific amino acid changes, corresponding to a common site on the RT enzyme at which the different inhibitors interact (Nunberg et al., 1991; Cohen et al., 1991; Condra et al., 1992; Grob et al., 1992). Recently, subgroups of the pharmacologic class of nonnucleoside RT inhibitors have been identified, suggesting that this class of inhibitor may be more heterogeneous than first appreciated (Buckheit et al., 1993b).

The National Cancer Institute (NCI) operates a high capacity screening program in which synthetic and natural products submitted from various sources throughout the world are tested for in vitro anti-HIV activity (Boyd et al., 1988; Weislow et al., 1989). The subject compounds, the benzothiadiazines, were submitted to the NCI program by chemists from Parke-Davis Pharmaceutical Research Division, Warner Lambert Company (Ann Arbor, Michigan). Fourteen of thirty-two structurally-related analogs which exhibited activity in the anti-HIV screen are presented herein. A detailed characterization of the range of antiviral properties of one of the most active compounds (NSC 287474), alone and in combination with AZT, is presented.

2. Materials and methods

Chemistry. The chemical structures of the compounds screened for antiviral activity are shown in Fig. 1 and Table 1. Compounds 1–12 were synthesized by the method of Stoss and Satzinger (1976). Compound 13, the dimer, was synthesized by a standard oxidative coupling reaction.

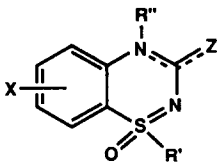

Fig. 1. Base structure of benzothiadiazine compounds listed in Table 1.

Cells and viruses. The CEM-SS cell line (Nara and Fischinger, 1988) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). Other tissue culture cell lines utilized included AA5, MT2, 174xCEM, and U937, all of which were obtained from the NIAID AIDS Research and Reference Reagent Program and were cultured in the same medium. Fresh human cells were obtained from the American Red Cross (Baltimore, MD, USA). Peripheral blood lymphocytes and macrophages were isolated following Ficoll-Hypaque centrifugation as described (Gartner and Popovic, 1990). The HIV-1 isolates included the common laboratory HIV-1 strains III_B, LAV, MN, and RF, as well as a panel of laboratory-derived and clinical HIV-1 isolates cultured from peripheral blood lymphocytes of patients at Duke University Medical Center (Durham, NC) and the University of Alabama at Birmingham (Birmingham, AL). The biological and biochemical properties of the panel of laboratory-derived isolates have been previously described (Cloyd and Moore, 1989; Buckheit and Swanstrom, 1991). The HIV-2 isolate ROD (Nr. I-532) was obtained from Dr. Luc Montagnier. The HIV-2 isolate MS, the AZT-resistant isolate G910–6, the nevirapine-resistant strain N119 and the SIV isolate $Delta_{B670}$ were obtained from the AIDS Research and Reference Reagent Program, AIDS Program,

Table 1
Structure and anti-HIV activity of selected compounds

Compound	Substituent				Antiviral activity (μM)				RT inhibition ID_{50}
	R'	R''	X	Z	CEM-SS		MT2		
					EC_{50}	IC_{50}	EC_{50}	IC_{50}	
1	=O	H	H	O	NA	1260	ND	ND	ND
2	OH	CH_3	5-Cl	S	NA	> 951	ND	ND	ND
3	Bu	H	H	O	NA	> 280	ND	ND	ND
4	Ph	H	H	O	155	> 970	215	> 365	11.3
5	Ph	CH_3	H	O	NA	> 160	ND	ND	ND
6	Ph	H	H	S	4.04	> 130	9.5	175	1.2
7	Ph	H	7-Cl	S	0.16	> 120	0.04	91	> 0.4
8	Ph	H	6-Cl	S	23	> 120	ND	ND	ND
9	3-Cl-Ph	H	4-Cl	S	0.71	> 7.9	0.04	32.8	4.4
10	2-Cl-Ph	H	5-Cl	S	0.63	> 15	0.03	65.7	0.03
11	Ph	H	H	SCH_3	32	> 90	6.2	87.6	> 0.3
12	Ph	H	H	NH_2	119	290	ND	ND	ND
13 [a]	Ph	H	H	S	NA	> 6.9	ND	ND	ND

[a] Compound 13 is the disulfide dimer of compound 6.

NIAID, NIH (Kanki et al., 1989; Larder et al., 1989; Richman et al., 1991a; Murphey-Corb et al., 1986). The pyridinone-resistant isolate A17 was obtained from Dr. E. Emini at Merck, Sharp and Dohme Laboratories (West Point, PA) (Nunberg et al., 1991). Cells and viruses utilized in the performance of Rauscher MuLV assays have been previously described (Shannon et al, 1974).

Materials. All experimental antiviral agents were synthesized at Parke-Davis Pharmaceutical Research Division, Warner Lambert Company. Crystalline stock materials were stored at −70°C and solubilized in 100% dimethylsulfoxide (DMSO). The reference anti-HIV compounds used in these studies were 3′-azido-3′-deoxythymidine (AZT, NSC 602670) and 2′,3′-dideoxycytidine (ddC, NSC 606170). Biscarboxyethyl-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF) was purchased from Molecular Probes (Eugene, OR) and dissolved immediately before use in DMSO (1 mg/ml); a working solution of 2 μg/ml was prepared in Dulbecco's phosphate-buffered saline (PBS) (GIBCO, Grand Island, New York). 4′,6-Diamidino-2-phenylindole (DAPI) was purchased from Sigma Chemical (St. Louis, MO). Stock solutions of DAPI were prepared at 100 μg/ml in distilled water by sonication, passed through a 0.45-μm filter and stored at −20°C. Working solutions of DAPI were prepared at 10 mg/ml in PBS containing 0.5% nonidet P-40 (NP-40) (Sigma). Poly (rA):$p(dT)_{12-18}$, poly(rC):$p(dG)_{12-18}$, poly(rA), oligo(dT), dATP, dGTP, dCTP, and dTTP were purchased from Pharmacia LKB Biotechnology (Piscataway, NJ). [Methyl,1′2′-^{3}H]dTTP (100 Ci/mmol) and [8,5-^{3}H]dGTP (31.9 Ci/mmol) in Tricine buffer were obtained from New England Nuclear Research Products (Wilmington, DE). The sodium salt of lauryl sulfate (SDS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical (St. Louis, MO). A solution of 16S and 23S *E. coli* ribosomal RNA was obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN). The 15 base primer, complementary to a sequence in the 16S *E. coli*, was purchased from Genosys Biotechnologies (The Woodlands, TX). The sequence of the 15 base primer was 5′-TAACCTTGCGGCCGT-3′. Affi-Gel Heparin Gel was purchased from Bio-Rad (Richmond, CA). DE81 and GF/A chromatography paper were from Whatman International (Maidstone, England). Tissue culture medium and additives, were obtained from GIBCO. Fetal bovine serum was obtained from HyClone (Logan, UT). Human serum was obtained from volunteer donors and was stored at −70°C prior to use. Rat serum was obtained from Fisher rats housed at Southern Research Institute. ELISA plates were obtained from Coulter Cytometry (Hialeah, FL). 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt, XTT, was obtained from the National Cancer Institute.

Anti-HIV assays. Evaluation of the antiviral activity of test compounds was performed as previously described (Buckheit et al, 1993a). Antiviral and toxicity data is reported as the concentration of drug required to inhibit 50% of virus induced cell killing or virus production (EC_{50}) and the concentration of drug required to reduce cell viability by 50% (IC_{50}). The activity and toxicity of an active compound were further analyzed in a multiparameter microtiter anti-HIV assay by simultaneous quantitation of both virus and cellular growth as described (Gulakowski et al., 1991).

Confirmatory assays of virus growth in supernatant samples. Samples of virus-containing supernatants were removed from each well of the microtiter plate prior to

staining with XTT. These samples were analyzed for their content of virus by RT activity assay, p24 ELISA, and CEM-SS infectivity assay as previously described (Buckheit et al., 1993a).

Effect of compounds on chronically-infected cells. Chronically-infected cell lines ($H9\text{-}III_B$) were obtained from the outgrowth of HIV-infected, virus-producing cells following acute infection of H9 cells. These cells were cultured in the presence of serial, one-log dilutions of antiviral compounds. Cell-free supernatant samples were obtained on a daily basis and analyzed for virus content by RT assay, p24 ELISA and CEM-SS infectivity assay as described above.

Reverse transcriptase enzyme inhibition assays. Evaluation of the activity of compounds using homopolymer and heteropolymer templates was performed as previously described (Buckheit et al., 1993a). Coomassie Blue staining of SDS-PAGE showed that the purified recombinant RT ($HIV\text{-}1_{BH10}$) contained approximately 60% full length p66 and 40% processed p51 polypeptides (White et al., 1993). RT enzyme from virus pools was purified by a modification of the method described (White et al., 1991). The cell-free viral pellet lysed in Triton X-100 was partially purified by chromatography on a Heparin-Affi-gel column equilibrated with buffer containing 25 mM potassium phosphate buffer (pH 7.4), 10% glycerol, 1 mM PMSF, and 10 mM β-mercaptoethanol. The reverse transcriptase was eluted with a gradient of 0.1 to 0.8 N KCl. Fractions containing HIV-1 reverse transcriptase activity were pooled and used for the inhibitor studies. The assays utilizing various template : primer systems to evaluate the inhibition of HIV-1 and HIV-2 RT have been previously described (White et al., 1991; Parker et al., 1991; Buckheit et al., 1993a). All assays contained 2% DMSO.

Cross-resistance evaluation. Compounds were screened for activity against isolates resistant to several nonnucleoside RT inhibitors by the microtiter anti-HIV assay described above.

Combination antiviral analysis. Analysis of drug combination assays was performed as previously described (Buckheit et al., 1993c,d) utilizing the anti-HIV assay methodology described above, with statistical evaluations performed according to the method of Prichard and Shipman (1990).

3. Results and discussion

3.1. Structure activity relationships of the benzothiadiazine class

One of the earliest and most potent inhibitors of HIV-1 replication discovered in this study was compound 6 (NSC 287474), a benzothiadiazine-1-oxide ($EC_{50} = 4.04\ \mu M$). Table 1 shows the anti-HIV screening results obtained for compound 6 as well as several analogs from which a relationship between chemical structure and antiviral activity was discerned.

Regarding substitutions at the R′ position, the sulfone (compound 1) was not active, therefore it was reasoned that this position probably requires an aliphatic or aromatic group. Substituting a hydroxyl or butyl group at R′ resulted in compounds 2 and 3, respectively, which were not active. Compound 4 showed moderate anti-HIV activity

and carried a phenyl ring in the R′ position demonstrating that an aromatic group was required at R′ for activity.

It should be noted that compound 4 was less active than compound 6, so it could be concluded that the carbonyl at the Z position rendered the structure less potent, which is in agreement with structure activity relationships (SAR) described for the TIBO class of nonnucleoside RT inhibitors (Kukla et al., 1991). A thiomethyl (compound 11) or amino (compound 12) substitution at the Z site also significantly reduced the activity relative to compound 6. It appears that the Z position is not flexible with regard to substitution of the thio group.

In order to determine whether substitution on the N (position R″) improved activity, compound 5 was synthesized and tested. The methyl group in the R″ position significantly decreased the antiviral potency of the compound. This loss of activity with the methyl substitution implies that simple alkyl substitution is not beneficial to inhibitory activity.

It remained possible that substitutions on the aromatic rings could improve the potency of the compound. In particular, since it has been shown for the TIBO class of inhibitor that chloro-substituted analogs were more potent (Kukla et al., 1992), compounds 7–10 were tested. Compound 7 shows that a chloro group on the 7 position improved the antiviral activity by greater than one order of magnitude ($EC_{50} = 0.16$ μM) while not affecting the cytotoxicity profile. However, a chloro substitution on the 6 position (compound 8) decreased the activity dramatically ($EC_{50} = 23$ μM). A 4-chlorophenyl or a 3-chlorophenyl group attached to the sulfur (compounds 9 and 10, respectively) did not improve the antiviral activity but did increase the cytotoxicity, indicating that chloro substitutions on this ring were not desirable.

Given the propensity of aromatic sulfides to form dimers under oxidative conditions, the disulfide dimer of benzothiadiazine 6, compound 13, was synthesized by standard methodology and tested to evaluate the exact nature of the active species. The results with compound 13 showed that the dimer had no detectable antiviral activity thus indicating that the monomer was the active species.

3.2. Biological activity of benzothiadiazines

Assays were performed to determine the effect of the benzothiadiazine class of compounds on the ability of HIV-1 to initiate a productive, cytopathic infection in CEM-SS and MT2 cells. All of the active compounds showed the same relative activity against the III_B strain of HIV-1 in both of these cell lines (Table 1). The selected representative (NSC 287474) of the class reproducibly protected CEM-SS cells from HIV-1 induced cytopathicity with 50% cellular protection (EC_{50}) achieved with approximately 3 μM (Fig. 2C). Microscopic examination of the cells in wells protected by effective doses of the compound confirmed the complete absence of HIV-induced giant-cell formation. NSC 287474 was nontoxic to CEM-SS and MT2 cells at concentrations of at least 130 μM (Fig. 2C). Measures of in vitro therapeutic index for NSC 287474 performed with the BCECF (Fig. 2A) and DAPI (Fig. 2B) assays were fully consistent with the XTT assay results. In cell-free supernatants from infected cells, profound decreases in supernatant RT activity, p24 core protein, and infectious virus

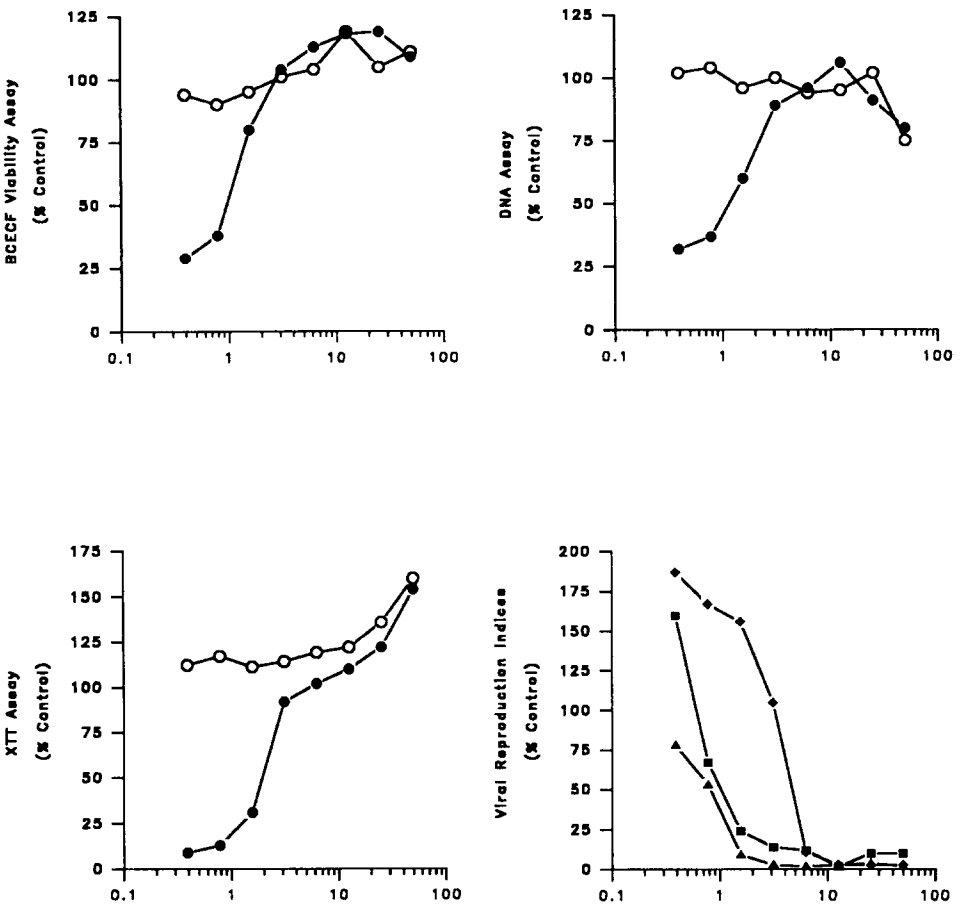

Fig. 2. Antiviral and cytotoxic effects of NSC 287474 in CEM-SS cells as measured by a multiparameter microtiter assay. Panels A, B and C show the effects of a range of concentrations on uninfected CEM-SS cells (○) and HIV-1-infected CEM-SS cells (●). (A) Effects of NSC 287474 on cell viability measured by BCECF assay. (B) Effects of NSC 287474 on relative DNA contents of cells measured by DAPI assay. (C) Effects of NSC 287474 on total cell number and cell viability measured by XTT assay. (D) Panel D shows the effects of NSC 287474 upon indices of virus replication in CEM-SS cells, including supernatant RT activity (▲), supernatant core protein (◆), and infectious virus (■). In panels A, B, and C the data are reported as the percent of uninfected, non-drug treated control values. In panel D, the data are reported as the percent of infected, non-drug treated control values.

were found after incubation with cytoprotective doses of NSC 287474 (Fig. 2D). The EC_{50} values for the effects of the drug on these indices of virus replication were similar to those obtained in the anti-HIV assays.

3.3. Range of activity of NSC 287474

NSC 287474 and AZT were tested against 12 isolates of HIV-1 and two isolates of HIV-2 (Table 2) in CEM-SS cells. These HIV-1 isolates included common laboratory strains as well as a group of clinical isolates obtained from patients. NSC 287474 was active against all of the HIV-1 isolates. However, no activity was detected against HIV-2. The EC_{50} values obtained for NSC 287474 for these various HIV-1 strains fell into a narrow range; concentrations of NSC 287474 from 0.95–5.11 μM were required to inhibit virus-induced cell killing by 50%. AZT was equally active against both HIV-1 and HIV-2 isolates (EC_{50} values of 0.002–0.008 μM for HIV-2 and 0.002–0.037 μM for HIV-1) (Table 2).

Further biological experiments were performed to determine the range of action of NSC 287474. The compound was active against HIV-1 in all established cell lines tested including human T cells, B cells, and macrophages (Table 3). Antiviral activity in fresh human peripheral blood lymphocytes and macrophages was obtained at drug concentrations similar to those which were active in the continuous cell lines (Table 3). Assays in fresh human cells also confirmed the ability of the compound to inhibit the replication of clinical strains of HIV-1, which have only been passaged in fresh human cells. Further, NSC 287474 was fully active when solubilized in fresh human, dog or mouse serum (data not shown).

Limited pretreatment experiments showed that the compound had to be present continuously to be maximally active in cell based assays (data not shown). Further, in assays in which the compound was removed from treated cells by extensive washing prior to HIV infection, no protection from HIV-1 induced cytopathic effects was detected. Experiments involving variations in the MOI or the time of drug addition

Table 2
Range of activity of NSC 287474 against HIV isolates in cell culture based assays

Virus/strain	EC_{50} (μM) [a]	
	NSC 287474	AZT
HIV-1 III_B	3.28	0.012
HIV-1 RF	1.39	0.037
HIV-1 LAV	0.95	0.002
HIV-1 SK-1	0.99	0.004
HIV-1 214	1.20	0.002
HIV-1 205	1.75	0.002
HIV-1 G	1.13	0.004
HIV-1 TP-1	3.58	0.006
HIV-1 KELL	5.07	0.003
HIV-1 PM16	5.11	0.006
HIV-1 McK	1.68	0.002
HIV-1 C	4.16	0.008
HIV-2 ROD	> 73	0.002
HIV-2 MS	> 73	0.008
SIV MAC251	> 73	0.015

[a] EC_{50} values determined by XTT-based cytopathicity assay in CEM-SS cells.

Table 3
Range of activity of NSC 287474 in different human cell lines

Cell line	Phenotype [a]	Assay [b]	EC_{50} (μM) [c]
CEM-SS	T	XTT	1.39
MT2	T (HTLV-I +)	XTT	0.44
AA5	B (EBV +)	XTT	2.88
U937	M	RT	5.04
174 × CEM	B × T	XTT	3.28
PBL	lymphocyte	RT	0.40
macrophage	macrophage	p24	0.07
H9-III_B	T (HIV-1 +)	RT	> 73

[a] T = T cell; B = B cell; M = macrophage; HTLV-I = human T cell leukemia virus type-I; EBV = Epstein-Barr virus.
[b] Drug-induced inhibition of virus replication was quantitated by XTT assay in cytopathically infected cells and by RT activity assay or p24 ELISA in noncytopathically infected cells.
[c] $HIV\text{-}1_{RF}$ utilized in all antiviral testing involving cultured cell lines; fresh PBL and macrophage cells infected with clinical HIV-1 isolates.

suggest that the compound acts similarly to other RT inhibitors such as AZT and TIBO (data not shown). Like AZT and TIBO, NSC 287474 exhibited activity in CEM-SS cells when challenged at various MOIs or when added at different periods of time post-infection. In addition, NSC 287474 had no effect on virus reproduction in chronically-infected cells in which the virus was already integrated (Table 3).

HIV-1 isolates resistant to AZT or other RT inhibitors were tested for susceptibility to NSC 287474 in order to define further the range of action and to obtain preliminary information regarding the mechanism of action of the compound. NSC 287474 was fully active against the AZT-resistant strain G910–6, exhibiting an EC_{50} of 4.20 μM (Table 4). The pyridinone-resistant virus strain, A17, and the nevirapine-resistant strain, N119, however, were able to replicate in the presence of high concentrations (73 μM) of NSC 287474 (Table 3). Control data demonstrating the inhibition of replication of drug

Table 4
Antiviral activity of NSC 287474 against drug-resistant HIV-1 strains in cell culture based assays

Virus isolate (mutation)	Resistance phenotype	EC_{50} [a] (μM)	IC_{50} [a] (μM)
III_B	sensitive	1.39	> 73
III_B	sensitive	0.44 [b]	> 73
G910-6	AZT	4.20 [b]	> 73
A17 (Y181C, K103N)	pyridinone	> 73	> 73
N119 (Y181C)	nevirapine	> 73	> 73
THZ1-5 (V108I)	thiazolobenzimidazole	> 73	> 73
DPS1-5 (Y181C)	diphenylsulfone	> 73	> 73
OC1-5 (L100I)	oxathiin carboxanilide	19.1	> 73
CALO1-3 (undefined)	calanolide A	6.02	> 73
TIBO1-6 (A98G, V108I)	TIBO (R82150)	> 73	> 73
HEPT1-6 (P236L)	HEPT	5.04	> 73

[a] Efficacy and toxicity was evaluated by XTT assay in CEM-SS cells with all isolates except for those noted.
[b] Efficacy and toxicity was evaluated by XTT assay in MT2 cells.

sensitive HIV-1 (III_B) is presented in Table 3 for comparison in both CEM-SS and MT2 cells. The activity of the benzothiadiazines against the AZT-resistant strain G910–6 and the lack of activity against the pyridinone-resistant strain A17 was also observed with all of the active compounds reported in Table 1 (data not shown).

3.4. Biochemical activity of NSC 287474

The cytoprotective activity of NSC 287474 against the drug resistant virus strains suggested that the compound might be a nonnucleoside reverse transcriptase inhibitor. Therefore the ability of the compound to inhibit recombinant HIV-1 RT was examined. When ribosomal RNA was used in the assay to mimic negative strand synthesis, NSC 287474 inhibited the activity of the enzyme by 50% (ID_{50}) at 0.36 μM. If either of the synthetic homopolymer RNA templates poly(rA):$p(dT)_{12-18}$ or poly(rC):$p(dG)_{12-18}$ were used as the template the ID_{50} was much higher than that seen with the ribosomal RNA template. With poly (rA):$p(dT)_{12-18}$ the ID_{50} value was found to be greater than 365 μM. Tested with the poly (rC):$p(dG)_{12-18}$ template primer system the 50% inhibitory concentration was 10.2 μM. As noted above NSC 287474 was inactive against HIV-2 in cell based assays (see Table 1). Consistent with this finding, NSC 287474 did not inhibit the recombinant RT of HIV-2. The ID_{50} value with the ribosomal RNA template was greater than 365 μM. Further investigation of the ability of the benzothiadiazines to inhibit reverse transcription was performed with the active compounds listed in Table 1. Each of these compounds was able to inhibit recombinant HIV-1 reverse transcriptase (Table 1), as well as partially purified RT from AZT-sensitive and AZT-resistant viruses (data not shown).

3.5. Combination antiviral activity with AZT

NSC 287474 was tested for anti-HIV activity in combination with AZT using the in vitro XTT anti-HIV assay. Five concentrations of NSC 287474 were tested in all combinations with eight concentrations of AZT. Data analyses were performed using the three-dimensional model of Prichard and Shipman (1990). Effects of the drug combination were calculated based on the activity of the two compounds when tested alone. The results of these assays demonstrated that the combined antiviral activity was much greater than that predicted based on additivity, indicating significant synergy. The three-dimensional plot of the data demonstrated a surface extending above the plane of additivity (Fig. 3). The peak above the plane represented a maximal antiviral activity (protection from HIV-induced cell killing) from a combination of NSC 287474 and AZT which was 20–50% greater than would have been expected if the antiviral effects were merely additive. Synergistic interaction of nonnucleoside RT inhibitors and AZT have been previously reported (Richman et al., 1991b; Buckheit et al., 1993a, 1993c, 1993d).

3.6. Activity against virus isolates resistant to other nonnucleoside RT inhibitors

Drug resistant virus isolates were generated to the nonnucleoside reverse transcriptase inhibitors thiazolobenzimidazole (Buckheit et al., 1993a), diphenylsulfone (McMahon et al., 1993), calanolide A (Kashman et al., 1992), and oxathiin carboxanilide (Bader et al., 1989) and to the HIV-1-specific nucleoside inhibitor HEPT. The mutations responsible

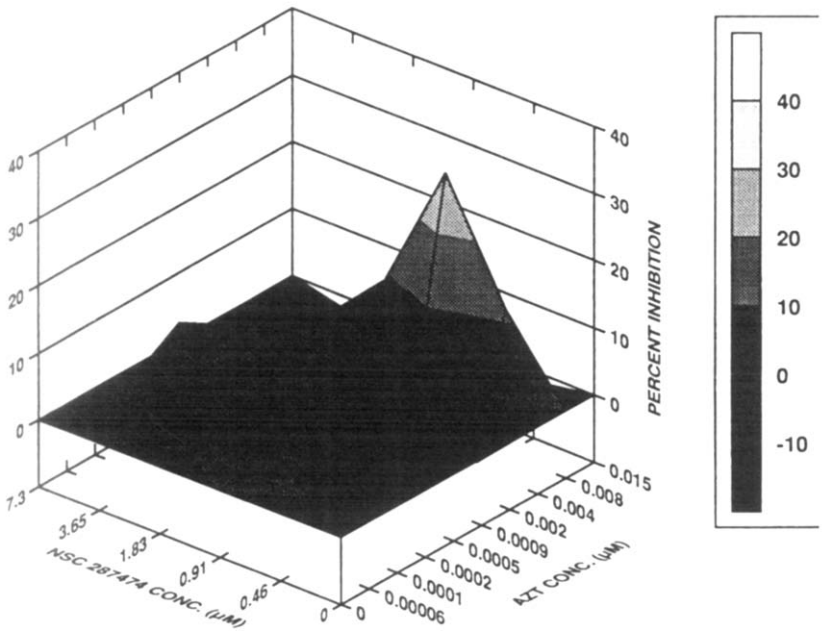

Fig. 3. Combination antiviral activity of NSC 287474 with AZT. The three dimensional synergy plot demonstrating the synergistic activity of the two compounds.

for resistance to each of these inhibitors are presented in Table 4. All of the drug-resistant isolates, with the exception of the calanolide A-resistant isolate and the HEPT-resistant isolate, were cross resistant to NSC 287474 (Table 4). The calanolide A-resistant isolate and the HEPT-resistant isolate remained sensitive to the anti-HIV effects of NSC 287474. This data indicates that NSC 287474 falls within subgroup III (Buckheit et al., 1993b) of the nonnucleoside RT inhibitors with nevirapine, the diphenylsulfones and pyridinone.

Based on cross-resistance data, combination therapy with NSC 287474 may be most effective with calanolide A or with the nucleoside HEPT since isolates resistant to one of the individual compounds may remain sensitive (or exhibit enhanced sensitivity) to the second inhibitor. It has been shown that virus isolates may become resistant to a combination of two HIV-1-specific compounds through generation of the Y181C mutation (Balzarini et al., 1993). Thus, the possible generation of resistance to the simultaneous presence of two or more compounds should also be considered. The results presented in this report support that combination therapy of NSC 287474 with AZT (which was found to synergistically inhibit HIV-1 in cell culture) and a second nonnucleoside RT inhibitor should be considered.

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