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Anti-HIV-1 activity of thiadiazole derivatives: structure–activity relationship, reverse transcriptase inhibition, and lipophilicity

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Abstract

The structure–activity relationship of the non-nucleoside HIV-1-specific reverse transcriptase (RT) inhibitors 4-phenyl-1,2,5-thiadiazol-3-yl *N,N*-dialkylcarbamate (TDA) derivatives was investigated with respect to their anti-HIV-1 activity, RT inhibition, and lipophilicity. 4-Phenyl-1,2,5-thiadiazol-3-yl *N,N*-dimethylcarbamate inhibited HIV-1-induced cytopathic effect (CPE) by 50% at a concentration of 28.8 μM in MT-4 cells. The activity increased more than 100-fold when the hydrogens at the 2-position and the 6-position in phenyl moiety were substituted by chlorines. However, the derivative with a chlorine at the 4-position of phenyl moiety did not show any inhibition of HIV-1 replication at its non-toxic concentrations. All of the 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-methyl-*N*-alkylcarbamates proved inhibitory to HIV-1 replication in the nanomolar concentration range. The TDA derivatives that showed anti-HIV-1 activity also inhibited RT activity in an enzymatic assay. However, the TDA derivatives did not show any specific inhibition of a non-nucleoside RT inhibitor (NNRTI)-resistant mutant and its RT activity. When the TDA derivatives were examined for their inhibitory effect on HIV-1 replication in the presence of 50% human serum, the activity significantly decreased depending on their lipophilicity.

Keywords: NNRTI; HIV-1-specific reverse transcriptase inhibitor; Lipophilicity; Structure–activity relationship; TDA derivatives

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1. Introduction

Several structurally diverse non-nucleoside compounds have been reported as non-nucleoside human immunodeficiency virus type 1 (HIV-1)-specific reverse transcriptase (RT) inhibitors (NNRTIs) (De Clercq, 1993, 1995). They include 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) derivatives (Baba et al., 1991), tetrahydroimidazo-[4,5,1-jk][1,4]benzodiazepin-2(1*H*)-one and -thione (TIBO) derivatives (Pauwels et al., 1990), nevirapine (Merluzzi et al., 1990), pyridinone derivatives (Goldman et al., 1991), and α-anilinophenylacetamide (α-APA) derivatives (Pauwels et al., 1993). All of the compounds are highly inhibitory to HIV-1 RT but not to RTs of other retroviruses, including HIV-2, or cellular DNA polymerases (Goldman et al., 1991; Pauwels et al., 1990). Since cross-resistance to HIV-1 mutants has been observed among these non-nucleoside HIV-1-specific RT inhibitors (Nunberg et al., 1991; Richman et al., 1991), they appear to interact with a similar site in the RT enzyme (Cohen et al., 1991; Grob et al., 1992; Nunberg et al., 1991).

In our large-scale screening for effective anti-HIV-1 agents, we have recently found that 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*,*N*-dialkyl carbamate (TDA) derivatives are potent and selective inhibitors of HIV-1 replication in vitro (Ijichi et al., 1995; Hanasaki et al., 1995). Studies on their mechanism of action have revealed that the TDA derivatives also belong to the family of non-nucleoside HIV-1-specific RT inhibitors. In this study, we have examined the structure–activity relationship of TDA derivatives with respect to their anti-HIV-1 activity, RT inhibition, and lipophilicity and found that 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-methyl-*N*-propylcarbamate is the most promising congener for further development as an anti-HIV-1 agent.

2. Materials and methods

2.1. Compounds

The detailed synthesis of 4-phenyl-1,2,5-thiadiazol-3-yl *N*,*N*-dialkylcarbamate (TDA) derivatives has been reported elsewhere (Hanasaki et al., 1995). The TDA derivatives and nevirapine were synthesized by the Tokyo Research Laboratory of Tosoh Co., Ltd. (Tokyo, Japan) and Yamanouchi Pharmaceutical Co., Ltd. (Tsukuba, Japan), respectively. 3′-Azido-3′-deoxythymidine (AZT) was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO). These compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C until use. AZT 5′-triphosphate (AZT-TP) was prepared in the Research Laboratory of Yamasa Co., Ltd. (Chiba, Japan).

2.2. Cells and virus

MT-4 cells (Harada et al., 1985) were used in the anti-HIV-1 assay. The cells were grown and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin G (100 units/ml), and gentamicin (20 mg/ml). HIV-1 (HIV-1-III_B and HIV-1-$III_{B\text{-}R}$) was used in the anti-HIV-1 assay. HIV-1-III_B (HTLV-III_B) was obtained from R.C. Gallo (National Cancer Institute, Bethesda, MD). HIV-1-$III_{B\text{-}R}$ is a non-nucleoside HIV-1-specific RT inhibitor-resistant mutant isolated by serial passage of HIV-III_B in cell culture in presence of escalating concentrations of the HEPT derivative 6-benzyl-1-ethoxymethyl-5-isopropyluracil (MKC-422). This mutant has a single amino acid change $Tyr^{181} \rightarrow$ Cys in its RT (Baba et al., 1994). These viruses were propagated in MT-4 cells and stored at −80°C until use.

2.3. Antiviral assays

Determination of the antiviral activity of test compounds against HIV-1 replication was based on the inhibition of the virus-induced CPE in MT-4 cells, as previously described (Baba et al., 1991). Briefly, MT-4 cells were suspended at 1×10^5 cells/ml and infected with HIV-1 at a multiplicity of infection (m.o.i.) of 0.02. Immediately after infection, 100 μl of the cell suspension was

brought into each well of a flat-bottomed microtiter culture plate containing various concentrations of the test compounds. After a 4-day or 5-day incubation at 37°C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method (Pauwels et al., 1988). The anti-HIV-1 activity of compounds was expressed as the 50% effective concentration (EC_{50}). Cytotoxicity of the compounds was determined in parallel with their antiviral activity. It was based on the viability of mock-infected MT-4 cells and expressed as the 50% cytotoxic concentration (CC_{50}). In some experiments, the anti-HIV-1 assays were carried out in culture medium containing 50% human serum (HS) instead of 10% FCS, as previously described (Baba et al., 1993). HS was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan).

2.4. Reverse transcriptase assay

The inhibitory effect of TDA derivatives on RT activity was evaluated with recombinant HIV-1 wild-type RT (wt-RT) and mutant RT (mu-RT). The latter has a single amino acid change of $Tyr^{181} \rightarrow Cys$. The wt-RT was purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan) and the mu-RT was obtained from Aros Biotech (Uppsala, Sweden). These recombinant RTs were composed of 66-kDa and 51-kDa proteins. The assays were based on the following modifications of the previously described methods (Baba et al., 1991). The assays were performed at 37°C for 15 min in a reaction mixture (50 μl) containing 50 mM Tris-HCl (pH 8.4), 2 mM dithiothreitol, 100 mM KCl, 10 mM $MgCl_2$, 0.01% Triton X-100, 2 mM EGTA, 0.01 OD_{260} of poly(C)/oligo$(dG)_{12-18}$, 1 μCi of [1′,2′-^{3}H]dGTP (33 Ci/mmol), test compound, and 0.02 unit of HIV-1 wt-RT or 2 ng of mu-RT. The reaction was stopped with 200 μl of 5% cold trichloroacetic acid, and the precipitated materials were analyzed for radioactivity.

2.5. Lipophilicity determination

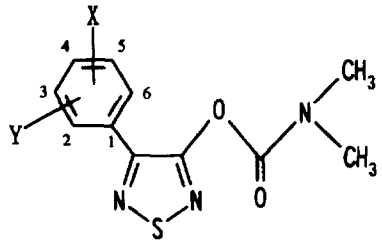
The lipophilicity of compounds was determined by the log *P* value that was calculated using Tsar Quantitative Structure–Activity Relationships (QSAR) system software (Oxford Molecular Ltd., UK). Atomic contribution factors to molecular lipophilicity are classified by the substituent properties of elements into various and numerous atomic types in the Tsar database. The total molecular lipophilicity was estimated as the sum of individual fragment contribution values in the compound.

3. Results

When we evaluated the TDA derivatives for their inhibitory effect on the replication of HIV-III_B in MT-4 cells, the lead compound 4-phenyl-1,2,5-thiadiazol-3-yl *N*,*N*-dimethylcarbamate (RD3-2105) inhibited HIV-1-induced CPE by 50% at a concentration of 28.8 μM (Table 1). Substitution of the hydrogen at the 2-position of the phenyl moiety by chlorine (RD3-2356) markedly increased the anti-HIV-1 activity of RD3-2105, whereas substitution of the hydrogen at the 4-position by chlorine (RD3-2107) completely annihilated its anti-HIV-1 activity. Further substitution of the hydrogen of the 2-chlorophenyl moiety at the 3-, 5-, or 6-position by chlorine retained or even enhanced the activity of TDA derivatives. Among the TDA derivatives, 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*,*N*-dimethylcarbamate (RD3-2220) showed the highest anti-HIV-1 activity. The 50% effective concentration (EC_{50}) of RD3-2220 was 0.20 μM. However, all of the *N*-dimethylcarbamate derivatives failed to show any inhibition of a non-nucleoside HIV-1-specific RT inhibitor-resistant mutant (HTLV-$III_{B\text{-}R}$) (Table 1).

In the next set of experiments, we synthesized various 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*,*N*-dialkylcarbamates and examined their inhibitory effect on HIV-1 replication. All compounds having the *N*-methyl-*N*-alkylcarbamate structure strongly inhibited the HIV-1-induced CPE at concentrations below 1 μM (Table 2). Furthermore, potent inhibition of HIV-1 repli-

Table 1
Structure–activity relationship of the TDA derivatives against HIV-1 replication (part 1)



Compound	X	Y	EC_{50} [a] (μM)		CC_{50} [b] (μM)
			HIV-1-III_B [c]	HIV-1-$III_{B\ R}$ [c]	
RD3-2105	H	H	29 ± 8	>210	210 ± 39
RD3-2356	2-Cl	H	0.44 ± 0.2	>162	162 ± 9
RD3-2107	4-Cl	H	>353	>353	353 ± 19
RD3-2236	2-Cl	3-Cl	0.94 ± 0.1	>127	127 ± 59
RD3-2219	2-Cl	4-Cl	8.3 ± 0.5	>77	77 ± 20
RD3-2233	2-Cl	5-Cl	0.37 ± 0.09	>111	111 ± 21
RD3-2220	2-Cl	6-Cl	0.20 ± 0.05	>190	190 ± 61
RD3-2218	3-Cl	4-Cl	>315	>315	315 ± 18

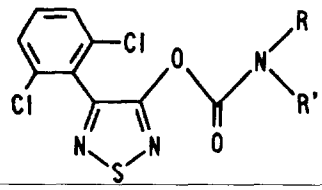
[a] 50% Effective concentration, required to inhibit HIV-1-induced CPE by 50% in MT-4 cells.
[b] 50% Cytotoxic concentration, required to reduce cell viability by 50% in mock-infected MT-4 cells.
[c] HIV-1-III_B and HIV-1-$III_{B\text{-}R}$ represent a wild-type and a non-nucleoside HIV-1-specific RT-resistant mutant, respectively.
All data represent mean ± S.D. for at least three independent experiments.

cation was also identified with piperidin-1-yl carbamate (RD3-2222), *N*,*N*-diethylcarbamate (RD3-2101), and *N*-butyl-*N*-ethylcarbamate (RD4-2023). The most potent congener of the TDA derivatives was 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-methyl-*N*-propylcarbamate (RD4-2024), which inhibited HIV-1 replication by 50% in MT-4 cells at a concentration of 0.013 μM. In contrast, the *N*-monoalkylcarbamate derivative (RD3-2380) did not show any inhibition. RD4-2025, RD4-2024, RD3-2102, and RD3-2101 were found to be active against the HIV-$III_{B\text{-}R}$ strain (Table 2). However, their activities were 40–360-fold lower for the mutant than for the wild type (HIV-III_B).

The TDA derivatives proved highly inhibitory to HIV-1 wt-RT activity when poly(C)/oligo$(dG)_{12-18}$ was used as the template/primer. In this assay system, 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*,*N*-dialkylcarbamates inhibited wt-RT activity by 50% at a concentration of 0.16–9.5 μM (Table 3). Among the test compounds, 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-ethyl-*N*-methylcarbamate (RD4-2025) was found to be the most potent inhibitor of HIV-1 RT. RD3-2380 did not suppress the wt-RT activity in the enzymatic assay system or HIV-1 replication in MT-4 cells (Table 2). Although the *N*,*N*-dialkylcarbamates derivatives strongly inhibited the wt-RT activity, little, if any, inhibition was observed for the mu-RT (Table 3). These results correlate well with those obtained in cell culture assays (Table 2).

The anti-HIV-1 assays were carried out in the presence of 50% HS to evaluate the TDA derivatives under more physiological conditions. When the compounds were examined for their inhibitory effects on the replication of HIV-1 in the presence of 50% HS, their activities showed a 10–200-fold decrease in comparison with those examined in the presence of 10% FCS (Table 4). The degree of reduction clearly depended on their lipophilicity, so that greater reduction was observed with the more lipophilic compounds. In contrast, the lipophilicity of nevirapine and AZT was much lower than that of the TDA derivatives, so that the anti-HIV-1 activity of these compounds was not affected in the presence of 50% HS.

Table 2
Structure–activity relationship of the TDA derivatives against HIV-1 replication (part 2)



Compound	R	R′	EC_{50} [a] (μM)		CC_{50} [b] (μM)
			HIV-1-III_B [c]	HIV-1-III_{B-R} [c]	
RD3-2220	CH_3	CH_3	0.20 ± 0.05	>190	190 ± 61
RD4-2025	CH_3	C_2H_5	0.037 ± 0.010	5.6 ± 3.0	29 ± 6
RD4-2024	CH_3	nC_3H_7	0.013 ± 0.002	2.6 ± 2.0	28 ± 1
RD3-2102	CH_3	nC_4H_9	0.020 ± 0.010	7.3 ± 2.0	23 ± 3
RD4-2031	CH_3	nC_6H_{13}	0.12 ± 0.02	>19	19 ± 8
RD3-2380	H	nC_4H_9	>139	>139	139 ± 13
RD3-2222	-C_5H_{10}-(cyclopentyl)		0.96 ± 0.50	>221	221 ± 33
RD3-2101	C_2H_5	C_2H_5	0.25 ± 0.08	9.4 ± 4.0	314 ± 106
RD4-2023	C_2H_5	nC_4H_9	0.33 ± 0.04	>44	44 ± 15
Nevirapine			0.21 ± 0.08	>235	235 ± 107
AZT			0.004 ± 0.002	0.001 ± 0.001	3.2 ± 0.2

Footnotes as in Table 1.

4. Discussion

We have recently reported that the TDA derivatives are potent and selective inhibitors of HIV-1 replication in vitro (Ijichi et al., 1995; Hanasaki et al., 1995). Among the 200 TDA derivatives examined, more than 100 compounds could inhibit HIV-1 replication. This prompted us to analyze the structure–activity relationship of TDA derivatives. One of the most important findings is that the anti-HIV-1 activity of TDA derivatives was markedly enhanced by the substitution of hydrogen by chlorine at the 2-position of the phenyl moiety (Table 1). Furthermore, when the chlorine was substituted by another halogen such as bromine or fluorine, a similar anti-HIV-1 activity was observed (data not shown). On the other hand, the substitution of hydrogen by chlorine at the 4-position of the phenyl moiety totally annihilated or greatly weakened the anti-HIV-1 activity (Table 1), suggesting that the 4-position, as well as the 2-position, plays a crucial role in the activity of the TDA molecules. Another important finding is that the length of *N*,*N*-alkyl chains in the carbamate moiety affected the HIV-1 activity of TDA derivatives. Interestingly, the *N*-monoalkylated carbamate derivative (RD3-2380) was inactive against HIV-1 replication or RT activity (Tables 2 and 3). These results indicate that the *N*-alkyl chains are also an important determinant in the anti-HIV-1 activity of TDA molecules.

It has been demonstrated that nevirapine and other NNRTIs bind to a hydrophobic pocket close to the catalytic site of HIV-1 RT (Kohlstaedt et al., 1992; Tantillo et al., 1994; Ren et al., 1995; Kroeger Smith et al., 1995). Since several other HIV-1-specific RT inhibitors including the TDA derivatives showed cross-resistance to HIV-1 mutants, these inhibitors may bind to the same hydrophobic pocket (Baba et al., 1994; Boyer et al., 1993; Ijichi et al., 1995). The RT

Table 3
Structure–activity relationship of the TDA derivatives against HIV-1 RT

Compound	R [a]	R' [a]	IC_{50} [b] (μM)	
			wt-RT	mu-RT [c]
RD3-2220	CH_3	CH_3	5.1 ± 0.05	>200
RD4-2025	CH_3	C_2H_5	0.16 ± 0.03	66.4 ± 17
RD4-2024	CH_3	nC_3H_7	0.53 ± 0.01	111 ± 15
RD3-2102	CH_3	nC_4H_9	4.5 ± 0.57	155 ± 26
RD4-2031	CH_3	nC_6H_{13}	9.5 ± 0.57	>200
RD3-2380	H	nC_4H_9	>200	>200
Nevirapine			0.56 ± 0.09	>200
AZT-TP [d]			0.01 ± 0.001	0.02 ± 0.003

[a] See Table 2.
[b] 50% Inhibitory concentration.
[c] mu-RT has $Tyr^{181} \rightarrow Cys$ mutation.
[d] AZT-TP (AZT 5'-triphosphate) was evaluated using poly(A)/oligo$(dT)_{12-18}$ as a template/primer.
All data represent means $\pm$ S.D. for at least three independent experiments.

sequence analysis of mutants resistant to TDA derivatives revealed that the mutants have a point mutation at position 181 [$Try^{181} \rightarrow Cys$ (data not shown)]. This result also suggests that TDA derivatives bind to the same hydrophobic pocket as nevirapine and the other NNRTIs.

Table 4
Influence of human serum on anti-HIV-1 activity

Compound	$EC_{50/HS}$ [a] (μM)	Ratio [b]	Lipophilicity [c] (log P)
RD3-2220	2.6 ± 0.54	13	3.754
RD4-2025	1.0 ± 0.01	27	4.097
RD4-2024	0.54 ± 0.01	42	4.565
RD3-2102	3.9 ± 0.22	195	4.962
RD4-2031	23 ± 1.05	192	5.754
Nevirapine	0.15 ± 0.11	0.7	1.897
AZT	0.002 ± 0.001	0.5	0.225

[a] EC_{50} value in the presence of 50% human serum (HS).
[b] Ratio of $EC_{50/HS}$ to $EC_{50/FCS}$ ($EC_{50/FCS}$ values are taken from Table 2, where they correspond to the EC_{50} values for HIV-1-III_B).
[c] Predicted log P value calculated by the atomic log P values (Viswanadhan et al., 1989).

We determined the lipophilicity of compounds using Tsar QSAR system software and the theoretical values were reflected in the ratio of serum protein binding (data not shown). The lipophilicity of RT inhibitors may be related to the intensity of hydrophobic binding to the HIV-1 RT. However, the inhibitory effect of the TDA derivatives on HIV-1 RT activity did not correlate with their lipophilicity (Tables 3 and 4). Since the TDA derivatives have higher lipophilicity than other HIV-1-specific RT inhibitors, they could be expected to penetrate more easily through the blood–brain barrier. Considering the fact that the central nervous system is damaged by HIV-1 at an early stage of the virus infection (Janssen et al., 1989), the TDA derivatives might be preferable to other compounds from this point of view. On the other hand, the anti-HIV-1 activity of the TDA derivatives with higher lipophilicity was affected to a greater extent in the presence of 50% HS (Table 4). The TDA derivatives were bound to serum protein by more than 90% under these conditions, and this protein binding capacity was clearly correlated with their lipophilicity (data not shown). Evidently, further studies on pharmacokinetics and toxicity in vivo are required to select the best candidate for clinical trials with the TDA derivatives.

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