Communications to the Editor

Thiadiazole Derivatives: Highly Potent and Specific HIV-1 Reverse Transcriptase Inhibitors

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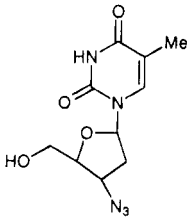
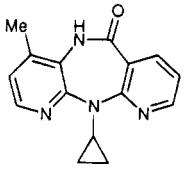
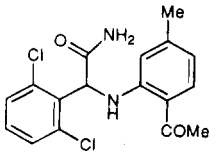
Reverse transcriptase (RT) is an essential enzyme for the replication of HIV and thus is regarded as one of the most important targets for anti-HIV agents. So far, only the nucleoside RT inhibitors 3′-azido-3′-deoxythymidine (AZT, Zidovudine), 2′,3′-dideoxyinosine (ddI, Didanosine), 2′,3′-dideoxycytidine (ddC, Zalcitabine), and 2′,3′-didehydro-3′-deoxythymidine (d4T, Stavudine) have been approved for the treatment of HIV-infected patients. However, their long-term use leads to toxic side effects such as bone marrow suppression.[1] In the meantime, non-nucleoside inhibitors, such as 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT),[2] dipyridodiazepinone (Nevirapine),[3] and α-anilinophenylacetamide (R-89439, Loviride),[4] have been found and developed as specific and potent inhibitors of HIV-1 RT. Several of them have proceeded to clinical evaluation.[5]



Zidovudine



Nevirapine



Loviride

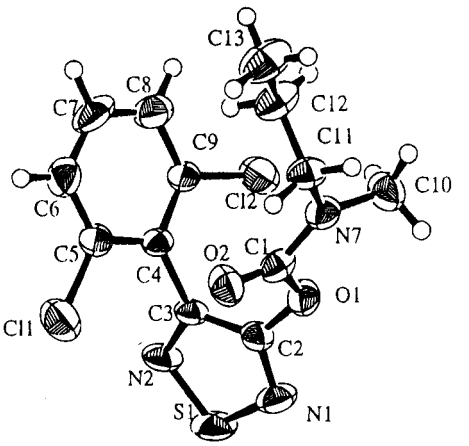


Figure 1. ORTEP drawing of compound **19**.

From a random screening program of compounds having herbicidal activities,[6] we identified a series of 1,2,5-thiadiazoles (TDA) as inhibitors of HIV-1. In this communication, we describe the efficient synthesis and the anti-HIV-1 activity as well as the structure–activity relationship of novel non-nucleoside inhibitors having the thiadiazole skeleton.

Chemistry. The compounds used in this investigation were synthesized as shown in Scheme 1. The 3-hydroxyl-4-substituted-1,2,5-thiadiazole nucleus was constructed in good yield by condensation of α-amino acid amides **1**–**5** with sulfur monochloride in dimethylformamide.[7] Compound **10** could also be synthesized by the hydrolysis of 3-chloro-4-(2,6-dichlorophenyl)-1,2,5-thiadiazole (**12**) which was prepared from α-amino-α-(2,6-dichlorophenyl)acetonitrile (**11**) with sulfur monochloride. The introduction of a carbamoyl moiety at the 3-hydroxy position of the thiadiazole skeleton was achieved using the following two methods: method A, treatment of hydroxythiadiazoles **6**–**10** with carbamoyl chlorides, and method B, reaction of hydroxythiadiazole with triphosgene followed by treatment with secondary amines giving the carbamates **13**–**21**. Under these reaction conditions, the carbamoyl groups were site-selectively introduced at the 3-hydroxy group (not at the N-2 position), which was confirmed by X-ray crystallography of 4-(2,6-dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-methyl-*N*-propylcarbamate (**19**), as depicted in Figure 1.[8]

Antiviral Activity and Discussion. MT-4 cells and peripheral blood lymphocytes (PBLs) as cells, HTLV-III_B strain of HIV-1 and LAV-2_{EHO} strain of HIV-2 as viruses were used for the assay. Antiviral activity was based on the inhibition of virus-induced cytophathic effect (CPE) in MT-4 cells or the quantitative detection of HIV-1 p24 antigen in PBLs.[9,10] The number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[11] Table 1 summarizes the anti-HIV-1 activity and cytotoxicity of the compounds synthesized in this study. 4-Phenyl-1,2,5-thiadiazol-3-yl *N*,*N*-dimethylcarbamate (**15**) exhibited antiviral activity (50% effective concentration (EC_{50}) = 23 μM). Cytotoxicity was observed at the concentration that was 8-fold higher than the EC_{50}.

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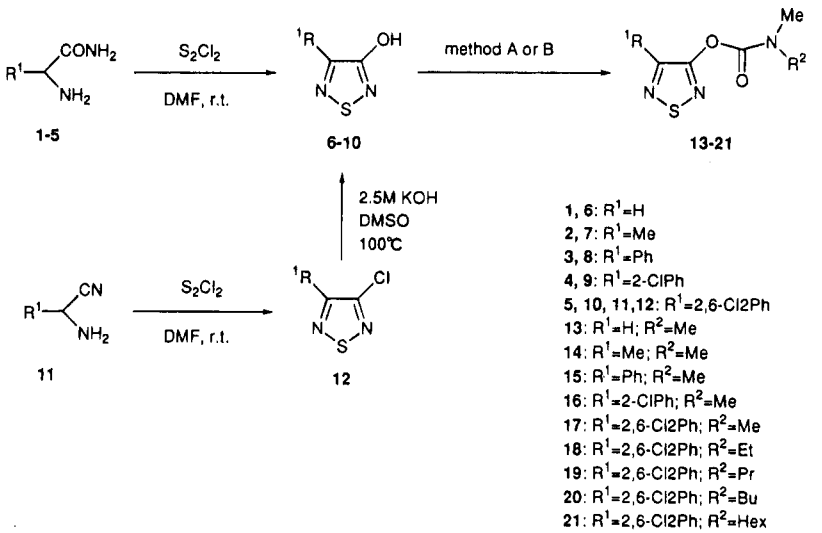
Scheme 1[a]



[a] Method A: ClC(=O)N(Me)R^2, K_2CO_3, CH_3CN, reflux. Method B: (1) triphosgene, pyridine, CH_2Cl_2, rt, (2) HN(Me)R^2, CH_2Cl_2, rt.

Table 1. Inhibition of HIV-1 and HIV-2 Replication in MT-4 Cells and Peripheral Blood Lymphocytes (PBLs) by TDA Derivatives

compd	virus	cells	EC_{50}[a], μM	CC_{50}[b], μM	SI[c]
10	HIV-1 ($HTLV\text{-}III_B$)	MT-4	>212	221	<1
13	HIV-1 ($HTLV\text{-}III_B$)	MT-4	>560	560	<1
14	HIV-1 ($HTLV\text{-}III_B$)	MT-4	>535	535	<1
15	HIV-1 ($HTLV\text{-}III_B$)	MT-4	23	183	8
16	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.32	161	503
17	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.24	145	604
18	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.039	136	3487
19	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.013	131	10 077
		PBL	0.004	150	37 500
	HIV-2 ($LAV\text{-}2_{EHO}$)	MT-4	>131	131	<1
20	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.039	29	744
21	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.10	16	160
AZT	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.004	3.2	800
		PBL	0.003	83	27 667
Nevirapine	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.073	290	3973
Loviride	HIV-1 ($HTLV\text{-}III_B$)	MT-4	0.006	137	22 833
		PBL	0.11	48	436

[a] 50% effective concentration based on the inhibition of HIV-1-induced CPE in MT-4 cells or the reduction of p24 antigen in culture supernant of PBLs. [b] 50% cytotoxic concentration based on the reduction of viability of mock-infected cells. [c] Selectivity index: ratio of CC_{50} to EC_{50}. All data represent mean values of at least two separate experiments.

The introduction of the chlorine atoms at the 2- or 2,6-positions of the phenyl group led to an increase in activity (2-ClPh (**16**), EC_{50} = 0.32 μM; 2,6-diClPh (**17**), EC_{50} = 0.24 μM). However, the replacement of the 4-phenyl group in the TDA skeleton by hydrogen (**13**) or a methyl group (**14**) resulted in complete loss of activity. 3-Hydroxy-4-(2,6-dichlorophenyl)-1,2,5-thiadiazole (**10**) was also inactive. These results indicated that the presence of both the aromatic ring at the C-4 position and the carbamate moiety at the C-3 position of the thiadiazole ring was essential. Furthermore, the effect of the alkyl group (R^2) in the carbamate moiety was also examined. 4-(2,6-Dichlorophenyl)-1,2,5-thiadiazol-3-yl *N*-methyl-*N*-propylcarbamate (**19**) exhibited the most potent activity; in the MT-4 cells, its EC_{50} and CC_{50} values were 0.013 and 131 μM, respectively (SI = 10 077). Under the same assay conditions, Nevirapine, Loviride, and AZT displayed EC_{50}s of 0.073, 0.006, and 0.004 μM, respectively.

When **19** was evaluated for its inhibition of HIV-1 ($HTLV\text{-}III_B$) in PBLs, it proved to be extremely potent inhibitor (EC_{50} = 0.004 μM). However, it had no effect on the replication of HIV-2 ($LAV\text{-}2_{EHO}$) at concentrations up to 131 μM. The effect of compounds **18**–**20** on recombinant HIV-1 RT (HIV-1 rRT) was examined with poly(rC)-oligo(dG) and poly(rA)-oligo(dT) as the template-primers (Table 2). We found that these compounds inhibited HIV-1 rRT and that the inhibition was dependent on the template-primer used.

Table 2. Inhibitory Effect of Compound **19** on HIV-1 RT Activity[a]

compd	IC_{50}[b], μM poly(rA)-oligo(dT)[c]	IC_{50}[b], μM poly(rC)-oligo(dG)[c]
18	3.4	0.30
19	5.8	0.80
20	12	1.7
AZT-TP	0.006	>50
Loviride	16	0.50

[a] The assay procedure has been described previously.[12] [b] 50% inhibition concentration. [c] Template-primer used for assay. All data represent mean values of at least two separate experiments.

In conclusion, we have shown that the TDA derivatives were highly potent and specific inhibitors of HIV-1. Considering that the TDA derivatives are easy to synthesize, they have potential as candidate drugs for the chemotherapy of HIV-1 infections. Our recent studies on their mechanism of action have revealed that the TDA derivatives belong to the family of non-nucleoside HIV-1 RT inhibitors. The investigations on further structure–activity relationships and emergence of drug resistance are now in progress.

Acknowledgment. The strain of HIV-2 ($LAV\text{-}2_{EHO}$) was provided by L. Montanier (Pasteur Institute, Paris, France), while the HIV-1 ($HTLV\text{-}III_B$) strain was originally obtained from R. C. Gallo (National Cancer Institute, Bethesda, MD).

Supplementary Material Available: Experimental Procedures, physical and spectral data for compounds **10** and **12**–**21**, and X-ray crystallographic data for compound **19** (26 pages). Ordering information is given on any current masthead page.

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(8) Physical data for compound **19** is as follows: mp 55–58 °C (cyclohexane); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.73 (t, J = 7.4 Hz, 3H), 1.42 (sextet, J = 7.4 Hz, 2H), [2.87 (s), 2.91 (s), 3H], [3.15 (t, J = 7.4 Hz), 3.20 (t, J = 7.4 Hz), 2H], 7.22–7.43 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.2, 20.7, 21.5, 35.1, 35.5, 51.6, 51.7, 128.6, 130.2, 131.6, 131.7, 136.2, 148.9, 149.0, 151.8, 156.6, 156.8; IR (KBr, cm^{-1}) υ_{max} 1740, 1430, 1380, 1300, 1235, 1150, 785; MS (EI) *m/z* 349 (0.3), 347 (0.6), 345 (M^+, 0.9), 171 (4.3), 100 (100); MS (CI) *m/z* 350 (2.7), 348 (9.7), 346 (MH^+, 15), 100 (100). Anal. ($C_{13}H_{13}N_3O_2SCl_2$) C, H, N.

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