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Acridone derivatives are selective inhibitors of HIV-1 replication in chronically infected cells

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Abstract

In our extensive screening of anti-HIV-1 agents in chronically infected cell lines, we have found acridone derivatives to be selective inhibitors of HIV-1 replication. Among the acridone derivatives, 1-hydroxy-10-methyl-9,10-dihydroacrid-9-one (RD6-5071) suppressed tumor necrosis factor (TNF)-α-induced HIV-1 expression in the latently infected cell line OM-10.1, U1, and ACH-2. Its 50% effective concentration for HIV-1 p24 antigen production was 2.0 μg/ml in OM-10.1 cells, while its 50% cytotoxic concentration was 18 μg/ml. The compound also inhibited phorbol 12-myristate 13-acetate (PMA)-induced HIV-1 expression in these cell lines. Furthermore, RD6-5071 was inhibitory to HIV-1 replication in acutely infected U937 and peripheral blood mononuclear cells. The compound was found to suppress TNF-α-induced HIV-1 long terminal repeat-driven gene expression. An inhibition assay for protein kinase C (PKC) revealed that RD6-5071 could reduce the enzyme activity. Furthermore, the compound was a moderate inhibitor of PMA-induced nuclear factor κB (NF-κB) activation, as determined by a gel mobility shift analysis. These results suggest that the acridone derivatives suppress HIV-1 replication at the transcriptional level primarily through a mechanism of PKC inhibition.

Keywords: HIV-1; Acridone; PKC; Transcription; Chronic infection

1. Introduction

Several steps of the HIV-1 replication cycle have been identified as possible targets for inhibition of HIV-1 (De Clercq, 1995). Among the

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targets, the transcriptional step of viral genome seems attractive, because the intervention in this step may be able to control HIV-1 replication not only in acutely infected cells but also in chronically infected cells. Therefore, various attempts have been made to find a lead compound that is inhibitory to viral mRNA synthesis without affecting host cellular functions (Baba, 1997a). Several structurally unrelated compounds have been identified as selective inhibitors of HIV-1 transcription. These include the antioxidant *N*-acetyl-L-cysteine (NAC) (Roederer et al., 1990, 1991), sodium salicylate (Kopp and Ghosh, 1994), some protein kinase C (PKC) inhibitors, and the tumor necrosis factor (TNF)-α production inhibitor thalidomide (Moreira et al., 1993). Benzothiophene derivatives and certain flavonoids were found to selectively inhibit HIV-1 replication by preventing HIV-1 mRNA accumulation in chronically infected cell cultures (Butera et al., 1995; Critchfield et al., 1996). Although their mechanism of action has not fully been elucidated yet, these compounds have proved inhibitory to the activity of human casein kinase II (Critchfield et al., 1997). More recently, some fluoroquinoline derivatives were shown to be potent and selective inhibitors of HIV-1 transcription (Baba et al., 1997b). These fluoroquinolines are assumed to target a cellular factor associated with the viral transactivator Tat (Baba et al., 1998).

Acridone derivatives are known to possess antiviral activities. 10-Carboxymethyl-9-acridanone (CMA) protected at least 50% of mice against infections with Semliki forest virus, coxsackie virus type B1, Columbia SK virus, Western equine encephalitis virus, herpes simplex virus, and pseudorabies virus due to the induction of interferon-like substances (Kramer et al., 1976). Yamamoto et al. (1989) reported that the acridone derivative citrusine-I was a potent inhibitor of herpes virus replication. The compound suppressed the synthesis of herpes simplex virus type 2 and human cytomegalovirus DNA, but it did not inhibit viral DNA polymerases in cell-free assay systems. They postulated that the target of the compound was a virus-encoded ribonucleotide reductase. In addition, Turpin et al. (1998) reported that a bistriazoloacridone analog selectively inhibited HIV-1 transcription. In this study, we have examined a variety of acridone derivatives for their inhibitory effects on HIV-1 replication in cell cultures and found that one of the derivatives, 1-hydroxy-10-methyl-9,10-dihydroacrid-9-one (RD6-5071), is a selective inhibitor of HIV-1 replication in chronically infected cells. Furthermore, the compound seems inhibitory to HIV-1 replication at the transcriptional level primarily through a mechanism of PKC inhibition.

2. Materials and methods

2.1. Chemicals

The acridone derivatives used in this study are listed in Table 1. The chemical synthesis of these derivatives will be described elsewhere. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (St. Louis, MO). TNF-α was purchased from Genzyme (Cambridge, MA). The PKC inhibitor HA-100 (Hagiwara et al., 1987) was obtained from Alexis (San Diego, CA). The test compounds were dissolved in dimethyl sulfoxide (DMSO) at appropriate concentrations and stored at −20°C until use. For all experiments, they were dissolved in culture medium where the final concentration of DMSO did not exceed 1% (V/V).

2.2. Cells and virus

The promyelocytic cell line OM-10.1 (Butera et al., 1991), the promonocytic cell line U1 (Folks et al., 1988), and the T-cell line ACH-2 cells (Clouse et al., 1989) were used for the antiviral assays in chronic infection. These cells are latently infected with HIV-1. They produce little or no HIV-1 under basal culture conditions but do produce significant levels of virions and antigens after stimulation with TNF-α and PMA (Butera et al., 1994). MOLT-4/III_B cells, a chronically infected clone derived from the T-cell line MOLT-4 (Kikukawa et al., 1986), were also used for the assays. MT-4 (Miyoshi et al., 1982), MOLT-4, CEM, U937 and peripheral blood mononuclear cells (PBMCs) were used for the antiviral assays

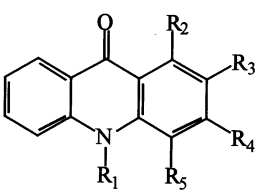
in acute infection. MT-4, MOLT-4, CEM and U937 cells were grown and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin G, and 100 μg/ml streptomycin. PBMCs were isolated from HIV-1-negative donors by Ficoll-Hypaque centrifugation. PBMCs were stimulated with 2 μg/ml phytohemagglutinin for 3 days, and cultured with RPMI 1640 medium containing 20% FCS, antibiotics and 50 U/ml interleukin 2. HIV-1 (III_B strain) was used for the assays in acute infection. The virus was propagated in MT-4 cells, and titers of viral stocks were determined in MT-4 cells. The stocks were stored at −80°C until use.

2.3. Antiviral assays

The activities of the test compounds against chronic HIV-1 infection were based on the inhibition of p24 antigen production in OM-10.1, U1, ACH-2 and MOLT-4/III_B cells. OM-10.1 and U1 cells (1×10^5 cells/ml) were incubated in the absence or presence of the compounds for 30 min, stimulated with 20 U/ml TNF-α or 20 nM PMA, and further incubated for 2 days. ACH-2 cells (2×10^5 cells/ml) were also incubated in the absence or presence of the compounds for 30 min, stimulated with 200 U/ml TNF-α or 100 nM PMA, and further incubated for 4 days. MOLT-4/III_B cells (1×10^5 cells/ml) were incubated for 4 days without stimulation. The culture supernatants were collected and examined for their p24 antigen levels by a p24 antigen-capture ELISA kit (Cellular Products, Buffalo, NY).

The activities of the compounds against acute HIV-1 infection were based on the inhibition of virus-induced cytopathicity in MT-4, MOLT-4 and CEM cells. These cells were suspended in culture medium (1×10^5 cells/ml) and infected with HIV-1 at a multiplicity of infection (MOI) of 0.02. Immediately after viral infection, the cell suspension (100 μl) was added into each well of a flat-bottomed microtiter tray containing various

Table 1
Inhibitory effects of acridinone derivatives on HIV-1 replication in TNF-α-stimulated OM-10.1 cells



Compound	R_1	R_2	R_3	R_4	R_5	EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)
6-5070	H	OCH_3	H	H	H	>17	17
6-5071	CH_3	OH	H	H	H	2.0	18
6-5072	H	OCH_3	OCH_3	H	H	>32	32
6-5073	CH_3	OCH_3	H	OCH_3	H	3.2	8.0
6-5075	CH_3	OCH_3	H	H	H	8.8	13
7-5039	CH_3	H	H	H	H	4.9	16
7-5040	CH_3	OH	H	OCH_3	H	>0.8	0.8
7-5043	CH_3	OCH_3	OCH_3	H	H	>28	28
7-5044	CH_3	OH	OH	H	H	>7.8	7.8
7-5053	CH_3	OH	H	H	OCH_3	>16	16
7-5054	CH_3	OH	H	H	OH	>0.36	0.36

[a] Fifty percent effective concentration, based on the reduction of p24 antigen in culture supernatants.
[b] Fifty percent cytotoxic concentration, based on the reduction of viable cell number. All data represent mean values for two separate experiments.

concentrations of the test compounds. After a 5-day incubation at 37°C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels et al., 1988). The activities of the compounds against acute infection were also examined in U937 cells and PBMCs. These cells were infected with HIV-1 at a MOI of 0.2. After viral adsorption for 2 h, the cells were extensively washed to remove unadsorbed virus particles and incubated at 37°C in the presence of various concentrations of the test compounds. After a 7-day incubation, the amount of p24 antigen in culture supernatants was measured by the p24 antigen-capture ELISA kit. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activities. It was based on the viability of mock-infected cells by the MTT method.

2.4. Flow cytometry

Expression of cell surface CD4 molecules was determined by direct immunofluorescence and laser flow cytometry, as previously described (Fujiwara et al., 1996). Briefly, OM-10.1 cells were treated with RD6-5071 at a concentration of 10 μg/ml and stimulated with 20 U/ml TNF-α. MOLT-4/III_B cells were treated with the compound but without stimulation. The cells were incubated at 37°C. On days 0, 4, 7, and 10, the cells were stained with a FITC-labeled anti-CD4 monoclonal antibody (Becton Dickinson, Mountain View, CA) and analyzed for their CD4 expression by FACScan™ (Becton Dickinson, Mountain View, CA).

2.5. Transfection assays

HeLa cells (2×10^6 cells) were transfected with 5 μg of pUC-BENN-CAT, a plasmid expressing chloramphenicol acetyltransferase (CAT) under the control of the HIV-1 long terminal repeat (LTR) (Gendelman et al., 1986), by a liposome-mediated transfection method, as previously described (Okamoto et al., 1998). After transfection, the cells were incubated at 37°C in the absence or presence of the compound for 24 h, stimulated with 10 ng/ml TNF-α or 20 nM PMA, and further incubated. In the assay for Tat-induced transactivation, the cells were cotransfected with 5 μg of pUC-BENN-CAT and 0.5 μg of a plasmid expressing HIV-1 Tat under the control of the simian virus 40 promotor (pSV2*tat*72). After a 12-h incubation, total cell extracts (200 μg) were incubated with ^{14}C-labeled chloramphenicol and acetyl coenzyme A, and their acetylated forms were determined by thin-layer chromatography.

2.6. PKC assay

Effects of the compounds on PKC activity were determined by the incorporation of ^{32}P from [γ-^{32}P]ATP into H1 histone, according to the established method (Kikkawa et al., 1982). Briefly, the test compounds were added to the reaction mixture (0.25 ml), containing 20 mM of Tris–HCl (pH 7.0), 10 mM magnesium acetate, 20 μM calcium chloride, 50 μg of H1 histone, 10 μM of [γ-^{32}P]ATP, 6 μg phosphatidylserine, 6 μg diacylglycerol, and 0.5 μg of PKC, and incubated for 10 min at 37°C. All reactions were carried out in siliconized tubes. The reactions were stopped by adding 25% trichloroacetic acid, and the acid-precipitable materials were collected on a membrane filter. The radioactivity of the test samples was determined with a liquid scintillation spectrometer.

2.7. Gel-mobility shift assay

U1 cells (5×10^6 cells) were incubated in the absence or presence of the test compounds for 2 h, stimulated with 20 nM PMA and further incubated. At 30 min after stimulation, the cells were collected, and their nuclear protein extracts were prepared as described by Schreiber et al., (1989). Equivalent amounts (10 μg) of nuclear protein were examined for their NF-κB activities with a gel-shift assay kit (Promega, Madison, WI), according to the manufacturer's protocol.

Table 2
Inhibitory effects of RD6-5071 and HA-100 on HIV-1 replication in chronically infected cells

Compound	Cells	Inducer	EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)
RD6-5071	OM-10.1	TNF-α	2.0 ± 0.1	18 ± 0.0
	OM-10.1	PMA	1.9 ± 0.0	34 ± 24
	U1	TNF-α	1.1 ± 0.7	67 ± 9
	U1	PMA	1.7 ± 0.0	>100
	ACH-2	TNF-α	6.9 ± 1.2	57 ± 34
	ACH-2	PMA	5.9 ± 4.6	>100
	MOLT-4/III_B	None	>2.1	2.1 ± 0.0
HA-100	OM-10.1	TNF-α	1.3 ± 0.8	12 ± 1
	U1	TNF-α	0.54 ± 0.06	11 ± 3
	ACH-2	TNF-α	0.68 ± 0.50	8.6 ± 2.0
	ACH-2	PMA	1.4 ± 0.1	11 ± 2
	MOLT-4/III_B	None	>14	14 ± 5

[a] Fifty percent effective concentration, based on the reduction of p24 antigen in culture supernatants.
[b] Fifty percent cytotoxic concentration, based on the reduction of viable cell number. All data represent mean values ± SD for more than three separate experiments.

3. Results

When we evaluated 11 acridone derivatives for their inhibitory effects on HIV-1 production in TNF-α-stimulated OM-10.1 cells, four compounds displayed anti-HIV-1 activities at non-toxic concentrations to the host cells (Table 1). Among them, RD6-5071 was found to be the most potent inhibitor of HIV-1. Its 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were 2.0 and 18 μg/ml, respectively. Thus, the selectivity index (CC_{50}/EC_{50}) was 9.0. Under the same assay conditions, the PKC inhibitor HA-100 also suppressed HIV-1 production with an EC_{50} of 1.3 μg/ml (Table 2). However, other PKC inhibitors, including staurosporine, chelerythrine and calphostin C, did not show selective anti-HIV-1 activities due to their remarkable cytotoxicity to OM-10.1 cells (data not shown). RD6-5071 was also inhibitory to HIV-1 production in PMA-stimulated OM-10.1 cells (Table 2). Furthermore, the compound could inhibit viral production in U1 and ACH-2 cells without affecting their proliferation, as determined by trypan exclusion as well as the MTT method (data not shown). However, RD6-5071 did not show any inhibition of HIV-1 production in MOLT-4/III_B cells (Table 2), which are constitutively producing a large amount of HIV-1 antigens and virions without any stimulation. A similar result was obtained with HA-100 in MOLT-4/III_B cells.

In the next experiment, we examined whether RD6-5071 was inhibitory to HIV-1 replication in acutely infected cells. RD6-5071 also proved to be a selective inhibitor of HIV-1 in U937 cells and PBMCs. Its EC_{50}s were 7.0 and 1.4 μg/ml in U937 cells and PBMCs, respectively (Table 3). The CC_{50}s of RD6-5071 were 35 μg/ml for U937 cells

Table 3
Inhibitory effects of RD6-5071 and HA-100 on HIV-1 (III_B strain) replication in acutely infected cells

Compound	Cells	EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)
RD6-5071	MT-4	>15	15 ± 6
	MOLT-4	>15	15 ± 5
	CEM	>5.6	5.6 ± 2.0
	U937	7.0 ± 3.0	35 ± 24
	PBMCs	1.4 ± 0.5	12 ± 1
HA-100	MT-4	>15	15 ± 6
	MOLT-4	>8.4	8.4 ± 0.6
	U937	>11	11 ± 3

[a] Fifty percent effective concentration, based on the inhibition of HIV-1-induced cytopathicity for MT-4, MOLT-4, and CEM cells and the reduction of p24 antigen in culture supernatants for U937 cells and PBMCs.
[b] Fifty percent cytotoxic concentration, based on the reduction of viable cell number. All data represent mean values ± SD for more than three separate experiments.

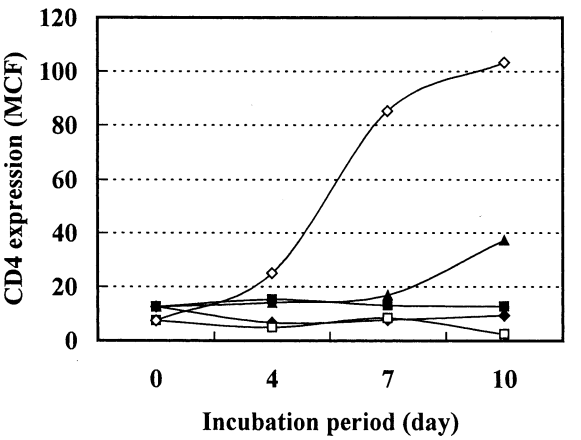


Fig. 1. Effect of RD6-5071 on surface CD4 expression in TNF-α-stimulated OM-10.1 cells and MOLT-4/III_B cells. OM-10.1 cells were either untreated (filled square and filled diamond) or treated (filled triangle) with 10 μg/ml RD6-5071 and either unstimulated (filled square) or stimulated (filled diamond and filled triangle) with 20 U/ml TNF-α. MOLT-4/III_B cells were either untreated (open square) or treated (open diamond) with the compound without any stimulation. On days 0, 4, 7, and 10, the cells were stained with a FITC-labeled anti-CD4 monoclonal antibody and analyzed for their CD4 expression by FACScan™. The amounts of CD4 molecules are expressed as the mean channel fluorescence (MCF). All data represent mean values for two separate experiments.

and 12 μg/ml for PBMCs. However, it was not effective against HIV-1 replication in the T-cell lines MT-4, MOLT-4 or CEM cells. Unlike RD6-5071, HA-100 did not show any selective inhibition of HIV-1 replication in U937 cells (Table 3).

Since the down-regulation of surface CD4 molecules is associated with the TNF-α-induced HIV-1 expression in OM-10.1 cells (Feorino et al., 1993), we examined whether the treatment with RD6-5071 could affect the CD4 down-regulation. In the absence of RD6-5071, the stimulation of OM-10.1 cells with TNF-α slightly reduced the number of CD4-positive cells, as determined by their mean channel fluorescence (Fig. 1). When TNF-α-stimulated OM-10.1 cells were cultured in the presence of RD6-5071 (10 μg/ml), the expression of CD4 molecules increased with increasing culture periods (Fig. 1). Interestingly, marked increase of the CD4 expression was observed in MOLT-4/III_B cells, when cultured in the presence of RD6-5071.

To determine whether RD6-5071 exerted its antiviral activity through the inhibition of HIV-1 gene expression, we examined whether the compound could inhibit the HIV-1 LTR-driven CAT gene expression induced by TNF-α, PMA, or HIV-1 Tat in HeLa cells. Although a considerable amount of the acetylated forms was identified in the absence of TNF-α stimulation, the expression was clearly enhanced when the cells were stimulated with TNF-α (Fig. 2A) or Tat (Fig. 2B). RD6-5071 significantly suppressed the TNF-α-induced enhancement in a dose dependent fashion (Fig. 2A). A similar result was obtained in the cells stimulated with PMA (data not shown). However, RD6-5071 was found to be a much weaker inhibitor of the Tat-induced gene expression. Only marginal inhibition of the CAT gene expression was observed in the Tat-stimulated HeLa cells (Fig. 2B). The mechanism of inhibition by RD6-5071 was further confirmed by a dot-blot hybridization analysis, where the compound reduced the accumulation of HIV-1 mRNA at a concentration of 10 μg/ml (data not shown). Since PKC participates in the signal transduction pathways following the stimulation of cells with TNF-α or PMA, we examined whether RD6-5071 was inhibitory to PKC activity in a cell-free assay system. RD6-5071 displayed PKC inhibition similar to that by HA-100 (Table 4). In addition, a gel-mobility shift assay revealed that RD6-5071 had some inhibitory effect on NF-κB activation in PMA- or TNF-α-stimulated U1 cells, and this effect was slightly more potent than that of the PKC inhibitor HA-100 (Fig. 3 and data not shown).

4. Discussion

HIV-1 infection is characterized by a rapid turnover and a high level of viral replication even at a latent period in patients (Ho et al., 1995). On the other hand, recent studies have demonstrated that replication-competent virus can be recovered from resting CD4-positive T-cells even in the patients with prolonged suppression of plasma viremia for more than 100 weeks by the current three-drug combination chemotherapy (Finzi et

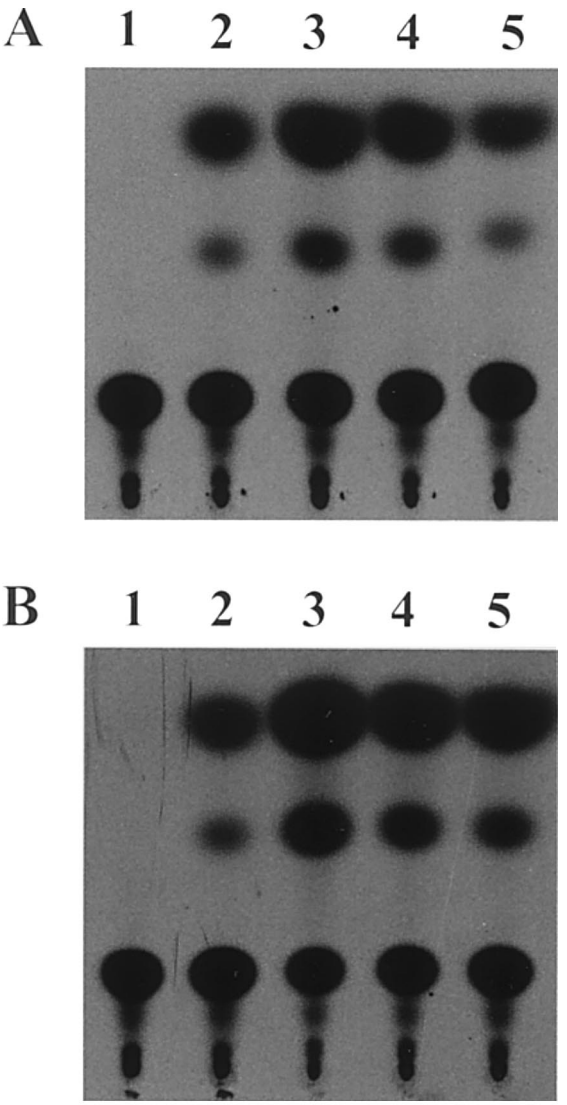


Fig. 2. Inhibitory effects of RD6-5071 on (A) TNF-α-induced or (B) Tat-induced HIV-1 LTR-driven gene expression in HeLa cells. The cells were untreated (lane 1) or transfected with 5 μg of pUC-BENN-CAT (lanes 2–5). After transfection, the cells were incubated at 37°C in the absence (lanes 2 and 3) or presence of the compound at 1 μg/ml (lane 4) or 10 μg/ml (lane 5). After a 24-h incubation, the cells were unstimulated (lanes 1–2) or stimulated with 10 ng/ml TNF-α (lanes 3–5). and further incubated. For the assay for Tat-induced transactivation, the cells were untreated (lane 1) or cotransfected with 5 μg of pUC-BENN-CAT and 0.5 μg of pSV2*tat*72 (lanes 2-5). After a 2-day incubation, total cell extracts (200 μg) were incubated with ^{14}C-labeled chloramphenicol and acetyl coenzyme A, and their acetylated forms were determined by thin-layer chromatography.

al., 1997; Wong et al., 1997). Thus, the mechanisms of latency and reactivation of HIV-1 in infected cells have not completely been understood yet. TNF-α is thought to play a considerable role in the pathogenesis of HIV-1 infection in patients, because stimulation of the infected cells with TNF-α immediately leads to the enhanced HIV-1 expression by activating the cellular transcriptinal factor NF-κB (Duh et al., 1989). TNF-α signaling is in part mediated through the activation of PKC in monocytic cells (Schütze et al., 1990), and the PKC activator PMA is also able to induce HIV-1 expression in chronically infected cells (Castagna et al., 1982). Therefore, PKC could be a possible target for inhibition of HIV-1 replication.

In the present study, we have shown that the acridone derivative RD6-5071 inhibits HIV-1 replication in TNF-α- or PMA-stimulated chronically infected cells (Table 2). We have also demonstrated that RD6-5071 is inhibitory to PKC activity in a cell-free assay system (Table 4). Transcriptional activation of HIV-1 from the chronically infected cell lines OM-10.1 and U1 is suggested to be mediated by PKC (Butera et al., 1991; Kinter et al., 1990). Our results support the role of PKC in the reactivation of HIV-1 in latently infected cells. In fact, indrocarbazole derivatives as PKC inhibitors could prevent the reactivation of HIV-1 in U1 cells. These compounds strongly inhibited the release of HIV-1 antigens and virions into the culture supernatants of chronically infected cells stimulated with PMA or TNF-α (Pätzold et al., 1993; Qatsha et al., 1993). The biological activities of HA-100, which is another class of PKC inhibitors, seem quite similar to those of RD6-5071. In addition, RD6-5071 restored the surface expression of CD4 molecules in chronically infected cells (Fig. 1). Since PKC phosphorylates the CD4 molecules and induces their internalization and subsequent degradation (Acres et al., 1986), the effect of RD6-5071 on the surface CD4 molecules may also be mediated by the inhibition of PKC.

It is generally accepted that Tat play a more important role in the activation and maintenance

Table 4
Inhibition of PKC activity by RD6-5071 and HA-100[a]

Compound	Concentration (μg/ml)	Inhibition (%)
RD6-5071	100	78 ± 12
	20	63 ± 19
	4	52 ± 28
	0.8	18 ± 16
	0.16	12 ± 11
HA-100	100	64 ± 8
	20	39 ± 14
	4	41 ± 31
	0.8	14 ± 5
	0.16	24 ± 15

[a] All data represent mean values ± SD for more than three separate experiments.

of high level of HIV-1 replication than PKC and its downstream factor NF-κB in acutely infected cells, in particular, T-cell lines. Thus, PKC inhibitors, including HA-100, are not potent and selective inhibitors of HIV-1 replication in acutely infected cells. Our observations in this study also support this hypothesis. In fact, RD6-5071 could not inhibit acute HIV-1 infection in MT-4, MOLT-4, and CEM cells (Table 3). Although the compound displayed HIV-1 inhibition in U937 cells and PBMCs, its selectivity indices were less than 10. In accordance with the observations in antiviral assays, RD6-5071 was found to preferentially inhibit the HIV-1 LTR-driven gene expression induced by PKC activators rather than that

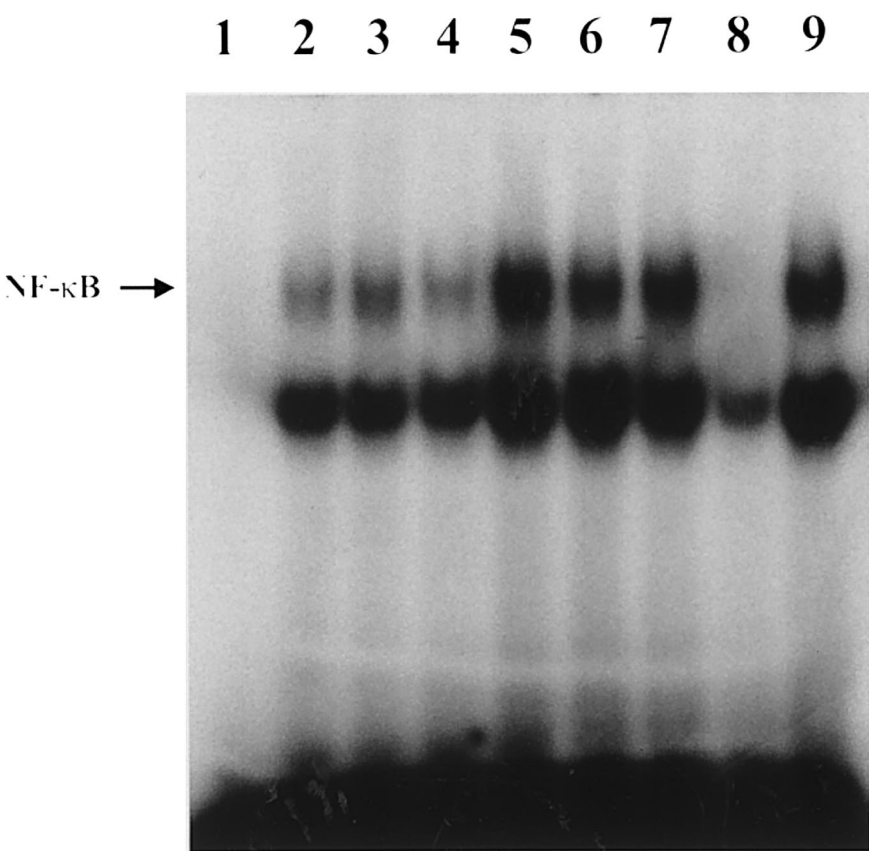


Fig. 3. Effects of RD6-5071 and HA-100 on NF-κB activation in U1 cells. The cells were incubated for 150 min in the absence or presence of the compounds at a concentration of 10 μg/ml and treated with 20 nM PMA for 30 min. The cells were collected, and their nuclear protein extracts were subjected to a gel-mobility shift assay, as described in Materials and methods. Lanes: (1) no nuclear protein extracts; (2) no compound + no PMA; (3) RD6-5071 + no PMA; (4) HA-100 + no PMA; (5) PMA; (6) RD6-5071 + PMA; (7) HA-100 + PMA; A 100-fold molar excess of (8) unlabeled specific DNA probe or (9) random DNA probe was added for competition.

induced by Tat (Fig. 2). However, a gel-mobility shift assay revealed that RD6-5071 and HA-100 had modest inhibition of NF-κB activation in PMA- or TNF-α-stimulated U1 cells (Fig. 3 and data not shown), indicating that PKC inhibition is not the only mechanism of action by these compounds, but another target molecule may also exist.

It has recently been reported that HIV-1 Tat activates viral transcription through binding to CDK9/human cyclin T1, a regulatory subunit of the positive transcription elongation factor b (P-TEFb) (Wei et al., 1998; Zhou et al., 1998). P-TEFb is a cellular cofactor required for the Tat-mediated transcriptional elongation. Mancebo et al. (1997) found that several kinase inhibitors, including HA-100, blocked the transcriptional elongation by Tat in cell-free and cell culture systems. These kinase inhibitors are assumed to interact with P-TEFb. Thus, it would be of particular interest whether P-TEFb could be a target molecule of the acridone derivatives. In conclusion, the acridone derivatives provide unique properties, as described here, and should be further pursued for their chemotherapeutic potential for HIV-1 infection.

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