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Synthesis of new phorbol derivatives having ethereal side chain and evaluation of their anti-HIV activity

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Abstract—Several new phorbol derivatives having ethereal substituents at the 12-position were synthesized and subjected to biological evaluation to find new candidates of an anti-HIV agent. Among them, 12-*O*-(methoxymethyl)phorbol 13-decanoate showed potent inhibitory activity against infection of HIV-1 in MT-4 cells (EC_{50}: 1.3 ng/mL) and relatively low cytotoxicity (CC_{50}: 8.3 μg/mL). This compound was also found to have sufficient stability in mouse plasma compared with the corresponding 12-acetate derivative, which was an equipotent HIV-1 inhibitor, but with an activity that decreased considerably after plasma treatment.

1. Introduction

According to the worldwide spread of acquired immunodeficiency syndrome (AIDS), development of an anti-HIV drug has been made widely for extermination of the causative agent. Many compounds originating from plants have been investigated to find a new potent inhibitor of the replication of HIV-1 or its essential enzymes.[1] Recently, phorbol esters isolated from the seed of *Croton tiglium* were reported to show anti-HIV-1 activity in vitro.[2] Phorbol esters have been reported to exhibit a wide variety of biological activities, including inflammation, cell proliferation, platelet activation, and many other biological responses.[3] Especially, tumor-promoting activity as well as the mechanisms of its causal PKC activation has been studied extensively.[4] Also, much research has revealed that these biological effects can be influenced by the nature (lipophilicity and hydrophilicity) and the structure (carbon-chain length, etc.) of the substituents on the C12- and/or C13-hydroxyl group(s) of the parent phorbol (Fig. 1).[5] Indeed, for example, it was disclosed that tuning of the C-12 ester moiety is an essential determinant of PKC agonist/antagonist activity.[6] With regard to anti-HIV-1 activity, TPA (12-*O*-tetradecanoylphorbol 13-acetate)[7] and prostratin (12-deoxyphorbol 13-acetate)[8] have been reported to have the HIV-1 inhibitory effect, the former of which acts with direct PKC activation and the latter without such a tumor-promoting action. Recently, a comprehensive study of the structure–activity relationship of phorbol esters has been performed on inhibition of an HIV-1-induced cytopathic effect. Among the many phorbol esters examined, 12-*O*-acetylphorbol 13-decanoate (**3**) was found to be the most effective inhibitor of HIV-1, obtained from culture supernatant of MOLT-4 cells, which were persistently infected with LAV-1.[9] Additionally, it did not influence

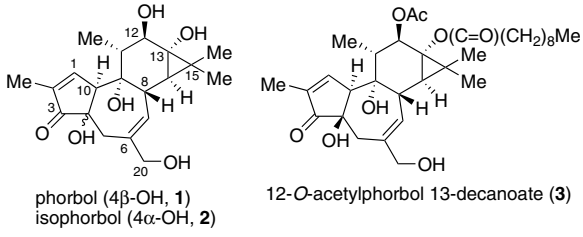

Figure 1. Parent phorbol and its known derivatives.

Keywords: Phorbol; Anti-HIV agents; Ethereal side chain; Safety index.
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the cell growth and viability of MT-4 cells at a concentration exhibiting anti-HIV-1 activity.[2,9]

In this study, we evaluated the anti-HIV-1 activity of compound **3** and its stability in a living body, and newly found that the activity was considerably decreased by pre-incubation with mouse plasma. Furthermore to search for much more stable and potent HIV-1 inhibitors under physiological conditions, we investigated the synthesis and the bioactivity of new 12-*O*-alkylated and α-oxyalkylated phorbol derivatives, as well as their in vivo stability and safety.

2. Results and discussion

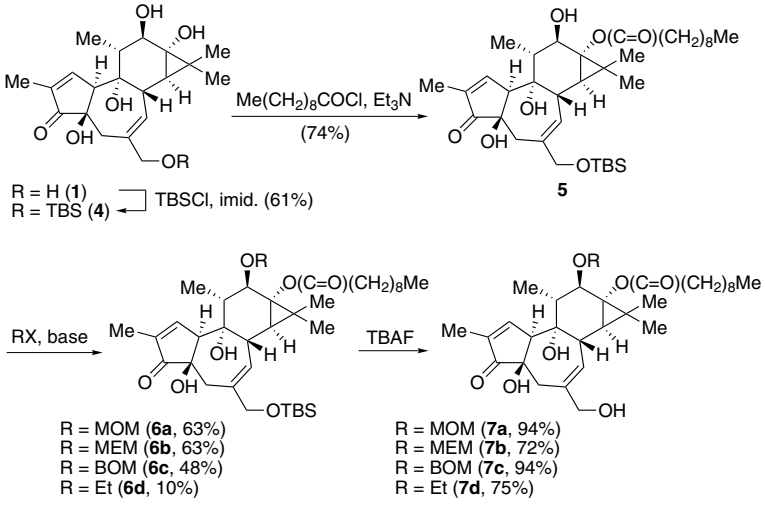
2.1. Chemistry

We designed new phorbol derivatives possessing a decanoyloxy group on the 13-position and an ethereal linkage on the 12-hydroxyl group, in light of the previous finding about the suitable chain length of the acyl group at the 13-position[9] and the expected in vivo stability. Parent phorbol (**1**), which is available by the reported procedure[10] or from commercial sources, has five hydroxyl groups of different reactivity. The method for stepwise introduction of substituents onto each hydroxyl group has been well established for the preparation of various phorbol 12- and 13-diesters, the representative of which employ a trityl ether as a protection of the most reactive primary hydroxyl group at the 20-position.[11] This protecting group is removable by treatment with perchloric acid; however, because one of our synthetic goals is to introduce α-oxyalkyl groups to 12-oxygen, which are expected to be susceptible to hydrolysis under acidic conditions, we chose the TBS group for protection of the 20-hydroxyl group. Thus, the synthesis was performed according to Scheme 1.

The protection of the primary alcohol of parent phorbol (**1**) as a corresponding TBS ether (**4**) was carried out under the standard condition using TBS chloride and imidazole in DMF in 61% yield. Introduction of the decanoyl group to tertiary 13-OH was achieved by treatment with decanoyl chloride and triethylamine in CH_2Cl_2 with a high selectivity, probably due to the orientation of the 13-OH to less hindered space compared to the other hydroxyl groups, including the secondary 12-OH group. On the other hand, when using pyridine as a base, competitive 12-acylation was observed to afford the mixture of the product **5** and the 12-decanoyl isomer (ca. 2:1) concomitant with a small amount of 12,13-diacylated product. The structure assignment could be simply carried out based on the change of the chemical shifts of the 12-proton in their ^1H NMR spectra. The 13-decanoyl-derivative **5** was subsequently subjected to etherification at the 12-position; namely, methoxymethyl, (methoxy)ethoxymethyl, and benzyloxymethyl derivatives (**6a**–**c**, respectively) were synthesized by reaction with the corresponding chlorides in the presence of diisopropylethylamine, and additionally, 12-ethyl ether (**6d**) using ethyl triflate and 2,6-di-*tert*-butyl-4-methylpyridine. The compounds **6a**–**d** were allowed to react with TBAF in THF at room temperature to afford the deprotected products **7a**–**d** in high yields. Thus, our target phorbol derivatives having 12-ethereal side chains were satisfactorily synthesized in four steps from parent phorbol (**1**). In addition, 20-*O*-methyl-12-*O*-methoxymethylphorbol 13-decanoate (**8**) was also prepared from **7a** for the purpose of comparison of the bioactivity.

2.2. Biology

12-*O*-Acetylphorbol 13-decanoate (**3**), reported to be the most effective anti-HIV-1 phorbol derivative without PKC activation,[9] was examined for inhibitory activity against HIV-1 infection in MT-4 cells with and without pre-incubation with mouse plasma. The results are summarized in Table 1. As previously reported, the compound **3** potently inhibited the infection of HIV-1 in MT-4 cells (EC_{50}: 1.7 ng/mL), and its cytotoxicity in uninfected MT-4 cells was weak (CC_{50}: 3.3 μg/mL). On the other hand, the inhibitory activity of **3** against



Scheme 1. Synthesis of 12-*O*-alkylphorbol 13-decanoate derivatives.

Table 1. Comparison of anti-HIV activity of 12-*O*-acetylphorbol 13-decanoate (**3**) in MT-4 cells with and without mouse plasma pretreatment

Entry	Plasma	Anti-HIV activity (EC_{50}, ng/mL)	Cytotoxicity (CC_{50}, μg/mL)	Safety index (CC_{50}/EC_{50})
1	–	1.7	3.3	1941
2	+	70.7	>10,000	>10,000

HIV-1 was obviously decreased after incubation with mouse plasma (EC_{50}: 70.7 ng/mL), suggesting a lack of in vivo efficacy as an anti-HIV agent.

We presumed that the undesirable aspect might be attributed to the inherent instability of **3** and consequential inactivation by a certain kind of enzyme (maybe pseudocholine esterase) under physiological circumstances. Assuming that the instability originates from susceptibility of the ester bond to hydrolytic degradation, new phorbol derivatives synthesized in this study, having 12-ethereal side chain rather than ester function, would be expected to show a proper stability and good in vivo efficacy. Thus, the modified compounds (**7a**–**d**, and **8**), as well as the basal compounds phorbol (**1**) and isophorbol (**2**), were evaluated for inhibition of infection of HIV-1 in MT-4 cells. Cytotoxicity in uninfected MT-4 cells was also determined, and the selectivity indexes were calculated based on the ratio of CC_{50} to EC_{50} values. These results are listed in Table 2.

The change of the acetyl group at the 12-position in **3** to the methoxymethyl group (**7a**) brought about the almost equipotent activity against HIV-1 to that of **3** (entries 3 and 4), and it was found that the activity was not reduced by pretreatment with plasma (Table 3). Furthermore, its CC_{50} value was ca. 2.5-fold as compared with that of **3**. However, extension of the 12-side chain to (methoxy)ethoxymethyl group (**7b**) led to slight decrease in inhibitory activity (entry 5). Introduction of the benzyloxymethyl group (**7c**) largely enhanced the activity (EC_{50}: 0.27 ng/mL), although this conversion resulted in an increase of cytotoxicity compared with **7a** (entry 6). On the other hand, simple 12-ethyl ether (**7d**) exhibited considerably decreased inhibitory activity (entry 7, ca. two-order lower than **7a**). These results clearly suggest that the α-oxyalkyl side chain (acetal structure) at the 12-position plays an important role in exhibiting the effective anti-HIV-1 activity. In addition, the compound **8** with methyl group on the 20-OH group showed lower activity than **7a** (entry 8). Parent compounds (**1** and **2**) exhibited no inhibitory activity against HIV-1 (entries 1 and 2), which is consistent with the previously reported findings.[9] Thus, among the compounds having the ethereal substituent at the 12-position examined in this study, compound **7a** was found to be the most effective phorbol derivative as a new anti-HIV agent in terms of potency, cytotoxicity, and stability against degradation in plasma.

Table 3. Influence of plasma pretreatment on anti-HIV activity of compounds **3** and **7a**

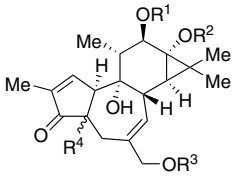
Compound	Plasma	Anti-HIV activity[a] (%)
3	–	100
	+	60.2
7a	–	100
	+	100

[a] These values indicate percentages of the activity based on the plasma (–) values as 100% for each compound at the dose of 100 ng/mL.

3. Conclusion

In this study, we synthesized and evaluated a new series of 12-*O*-alkylphorbol 13-decanoate, aiming at the discovery of new effective anti-HIV agents with in vivo efficacy. As a result of in vitro screening of the bioactivity, 12-*O*-(methoxymethyl)phorbol 13-decanoate (**7a**) was newly found to exhibit high potency and low cytotoxicity, resulting in a good safety index. Moreover, this compound was demonstrated to keep its anti-HIV-1 activity even after pre-incubation with mouse plasma, implying that it has sufficient stability under physiological conditions in contrast to the corresponding 12-acetate (**3**). Further exploration to find more efficient phorbol derivatives with an in-depth in vivo assay is

Table 2. Anti-HIV activity and safety index of new phorbol derivatives



Entry		R^1	R^2	R^3	R^4	Anti-HIV activity (EC_{50}, ng/mL)	Cytotoxicity (CC_{50}, μg/mL)	Safety index (CC_{50}/EC_{50})
1	**1**	H	H	H	β-OH	>10,000	>10,000	—
2	**2**	H	H	H	α-OH	>10,000	>10,000	—
3	**3**	Ac	Decanoyl	H	β-OH	1.7	3.3	1941
4	**7a**	MOM	Decanoyl	H	β-OH	1.3	8.3	6385
5	**7b**	MEM	Decanoyl	H	β-OH	3.72	3.3	887
6	**7c**	BOM	Decanoyl	H	β-OH	0.27	4.4	16,296
7	**7d**	Et	Decanoyl	H	β-OH	195.7	3.3	17
8	**8**	MOM	Decanoyl	Me	β-OH	46.9	4.0	85

ongoing in the search for new clinically useful anti-HIV agents.

4. Experimental

4.1. Chemistry

All nonaqueous reactions were carried out under an Ar atmosphere. Reagents were purchased from commercial sources and used as received. Anhydrous solvents were obtained from commercial sources or prepared by distillation over CaH_2 or P_2O_5. ^1H and ^{13}C NMR spectra were obtained on a Varian Gemini 300 (300 MHz for ^1H and 75.46 MHz for ^{13}C) instrument or a Varian UNITY plus 500 (500 MHz for ^1H and 125 MHz for ^{13}C) instrument, using tetramethylsilane or chloroform as an internal reference. Mass spectra were measured on a JEOL D-200 or a JEOL AX 505 mass spectrometer, and the ionization method was electron impact (EI, 70 eV). IR spectra were recorded on a Perkin–Elmer 1600 spectrometer. The optical rotations were determined on a JASCO DIP-1000 instrument. Melting points were taken with a Yanagimoto micro-melting point apparatus and are uncorrected. Column chromatography was carried out by employing Cica Silica Gel 60N (spherical, neutral, 40–50 or 63–210 μm). Phorbol (**1**) and isophorbol (**2**) were obtained by reported procedure.[10] 12-*O*-Acetylphorbol 13-decanoate (**3**) was identified with the reported one.[12]

4.1.1. 20-*O*-(*tert*-Butyldimethylsilyl)phorbol (4). To a solution of phorbol (**1**, 364 mg, 1 mmol), *N*,*N*-dimethylaminopyridine (12 mg, 0.1 mmol), and imidazole (201 mg, 3 mmol) in DMF (2 mL) at 0 °C was added *tert*-butyldimethylsilyl chloride (166 mg, 1.1 mmol). The solution was stirred for 1 h at room temperature and the mixture was directly subjected to column chromatography on silica gel (AcOEt). The collected fractions were concentrated and the residue containing DMF was dissolved in CH_2Cl_2–hexane (1:1) and then cooled. The precipitates formed were collected by filtration and dried to afford **1** (292 mg, 61%) as a colorless solid. Mp 270–273 °C (decomp.); ^1H NMR (acetone-d_6): δ 0.07 (3H, s), 0.08 (3H, s), 0.71 (1H, d, J = 5.2 Hz), 0.89 (9H, s), 1.06 (3H, d, J = 6.3 Hz), 1.15 (3H, s), 1.26 (3H, s), 1.69 (3H, dd, J = 3.0, 1.3 Hz), 1.95–2.00 (1H, m), 2.46 (2H, s), 3.09–3.14 (2H, m), 3.24 (1H, br s), 3.51 (1H, br), 4.05 (2H, s), 4.09 (1H, br), 4.10 (1H, d, J = 9.8 Hz), 4.65 (1H, s), 5.61 (1H, d, J = 5.6 Hz), 7.58 (1H, s); ^{13}C NMR (acetone-d_6): δ −5.1, 10.2, 15.5, 17.8, 18.8, 24.1, 26.2, 26.5, 37.2, 38.3, 40.1, 46.1, 58.2, 63.0, 68.6, 74.5, 78.8, 81.4, 130.3, 133.1, 140.9, 159.8, 208.5; IR (KBr) cm^{-1}: 3315, 1675. Anal. Calcd for $C_{26}H_{42}O_6Si$: C, 65.24; H, 8.84. Found: C, 64.95; H, 8.69; MS *m*/*z* 478 (M^+−*t*-Bu); HRMS Calcd for $C_{22}H_{33}O_6Si$: 421.2046 (M^+−*t*-Bu). Found: 421.2027; $[\alpha]_D^{25}$ +83.39 (*c* 0.85, MeOH).

4.1.2. 20-*O*-(*tert*-Butyldimethylsilyl)phorbol 13-decanoate (5). To a solution of **4** (48 mg, 0.1 mmol) and triethylamine (42 μL, 0.3 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added decanoyl chloride (41 μL, 0.2 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 10% HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$. The solvent was evaporated off to leave a residue, which was chromatographed on silica gel (CH_2Cl_2–AcOEt = 4:1) to afford **5** (47 mg, 74%) as a colorless oil. ^1H NMR ($CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.87 (3H, t, J = 6.8 Hz), 0.88 (9H, s), 0.99 (1H, d, J = 5.6 Hz), 1.03 (3H, d, J = 6.8 Hz), 1.19 (3H, s), 1.23 (3H, s), 1.22–1.36 (12H, m), 1.58–1.65 (2H, m), 1.76 (3H, dd, J = 3.0, 1.3 Hz), 1.97–2.04 (1H, m), 2.33–2.37 (3H, m), 2.46 (1H, d, J = 19 Hz), 2.71 (1H, s), 3.13 (2H, br), 3.96 (1H, d, J = 9.8 Hz), 4.00 (2H, s), 5.59 (1H, d, J = 5.8 Hz), 7.56 (1H, d, J = 1.3 Hz); ^{13}C NMR ($CDCl_3$): δ −5.3, 10.1, 14.1, 15.1, 16.9, 18.4, 22.6, 23.7, 24.8, 25.8, 25.9, 26.6, 29.1, 29.20, 29.22, 29.4, 31.8, 34.3, 35.5, 38.3, 39.0, 44.9, 56.8, 67.9, 73.5, 77.5, 78.2, 127.6, 132.9, 140.5, 160.4, 176.9, 209.0; IR (neat) cm^{-1}: 3396, 1706; MS *m*/*z* 575 (M^+−*t*-Bu); HRMS Calcd for $C_{32}H_{51}O_7Si$: 575.3404 (M^+−*t*-Bu). Found: 575.3432; $[\alpha]_D^{25}$ +65.95 (*c* 0.71, $CHCl_3$).

4.1.3. General procedure for the synthesis of 20-*O*-(*tert*-butyldimethylsilyl)-12-*O*-(α-oxyalkyl)phorbol 13-decanoate (6a–c). To a solution of **5** and *N*,*N*-diisopropylethylamine (2 equiv) in CH_2Cl_2 was added chloromethyl methyl ether, chloromethyl 2-methoxyethyl ether, or benzyloxymethyl chloromethyl ether (1.2 equiv) at 0 °C, and the mixture was stirred at room temperature for 20 h. The mixture was diluted with CH_2Cl_2 and washed with 10% HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent left a residue, which was chromatographed on silica gel (CH_2Cl_2–AcOEt = 9:1) to afford **6a** (63%), **6b** (63%), or **6c** (48%) as a colorless oil, respectively. Compound **6a**: ^1H NMR ($CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.87 (9H, s), 0.88 (3H, t, J = 6.8 Hz), 0.97 (1H, d, J = 5.6 Hz), 1.05 (3H, d, J = 6.4 Hz), 1.18 (3H, s), 1.24 (3H, s), 1.22–1.33 (12H, m), 1.60–1.66 (2H, m), 1.77 (3H, dd, J = 3.0, 1.3 Hz), 2.02–2.07 (1H, m), 2.18 (1H, s), 2.32 (2H, td, J = 7.7, 2.6 Hz), 2.39 (1H, d, J = 19 Hz), 2.46 (1H, d, J = 19 Hz), 3.12 (1H, t, J = 5.6 Hz), 3.23 (1H, br s), 3.36 (3H, s), 3.94 (1H, d, J = 9.4 Hz), 4.00 (2H, s), 4.54 (1H, d, J = 6.8 Hz), 4.88 (1H, d, J = 6.8 Hz), 5.61 (1H, d, J = 6.3 Hz), 5.65 (1H, s), 7.61 (1H, s); ^{13}C NMR ($CDCl_3$): δ −5.30, −5.28, 10.1, 14.1, 15.2, 16.8, 18.4, 22.6, 23.8, 24.6, 25.6, 25.9, 29.1, 29.23, 29.24, 29.4, 31.8, 34.5, 36.1, 38.4, 39.1, 44.2, 55.7, 56.1, 65.6, 68.2, 73.7, 77.8, 79.8, 95.0, 128.5, 132.4, 139.9, 161.2, 176.2, 209.2; IR (neat) cm^{-1}: 3404, 1709; MS *m*/*z* 676 (M^+); HRMS Calcd for $C_{38}H_{64}O_8Si$: 676.4370 (M^+). Found: 676.4402; $[\alpha]_D^{25}$ +78.75 (*c* 0.53, $CHCl_3$). Compound **6b**: ^1H NMR ($CDCl_3$): δ 0.02 (6H, s), 0.81–0.90 (12H, m), 0.94 (1H, d, J = 5.6 Hz), 0.98 (3H, d, J = 6.3 Hz), 1.13 (3H, s), 1.15–1.30 (15H, m), 1.57–1.68 (2H, m), 1.72 (3H, d, J = 1.4 Hz), 1.98–2.05 (1H, m), 2.29–2.35 (2H, m), 2.37 (1H, d, J = 19 Hz), 2.40 (1H, d, J = 19 Hz), 2.72 (1H, br), 3.08 (1H, br), 3.17 (1H, br), 3.35 (3H, s), 3.46–3.55 (2H, m), 3.61–3.75 (2H, m), 3.94 (1H, d, J = 9.6 Hz), 3.97 (2H, s), 4.64 (1H, d, J = 6.6 Hz), 4.94 (1H, d, J = 6.6 Hz), 5.57 (1H, d, J = 4.1 Hz), 5.60 (1H, s), 7.55 (1H, s); ^{13}C NMR ($CDCl_3$): δ −5.0, 10.3, 14.3, 15.4,

17.1, 18.6, 22.9, 24.0, 24.8, 25.8, 26.2, 29.3, 29.5, 29.6, 32.0, 34.7, 36.3, 38.5, 39.3, 44.3, 56.3, 59.1, 65.6, 67.2, 68.3, 71.8, 73.8, 77.9, 80.1, 93.8, 128.4, 132.5, 140.0, 160.9, 176.1, 209.0; IR (neat) cm^{-1}: 3411, 1711; MS *m/z* 720 (M^+); HRMS Calcd for $C_{40}H_{68}O_9Si$: 720.4633 (M^+). Found: 720.4622; $[\alpha]_D^{25}$ +79.98 (*c* 0.90, $CHCl_3$). Compound **6c**: ^1H NMR ($CDCl_3$): δ 0.04 (3H, s), 0.05 (3H, s), 0.82–0.92 (12H, m), 0.98 (1H, d, J = 5.2 Hz), 1.05 (3H, d, J = 6.3 Hz), 1.20 (3H, s), 1.20–1.32 (15H, m), 1.50–1.58 (2H, m), 1.75 (3H, d, J = 1.6 Hz), 2.02–2.15 (1H, m), 2.21 (2H, t, J = 7.7 Hz), 2.41 (1H, d, J = 19 Hz), 2.45 (1H, d, J = 19 Hz), 2.63 (1H, br), 3.12 (1H, br), 3.22 (1H, br s), 4.00 (2H, s), 4.05 (1H, d, J = 9.6 Hz), 4.58 (1H, d, J = 11 Hz), 4.65 (1H, d, J = 11 Hz), 4.72 (1H, d, J = 6.9 Hz), 5.01 (1H, d, J = 6.9 Hz), 5.61 (1H, d, J = 5.5 Hz), 5.65 (1H, s), 7.25–7.38 (5H, m), 7.59 (1H, s); ^{13}C NMR ($CDCl_3$): δ −4.9, 10.4, 14.4, 15.5, 17.2, 18.6, 22.9, 24.1, 24.8, 25.8, 26.2, 29.3, 29.4, 29.5, 29.6, 32.1, 34.7, 36.4, 38.6, 39.4, 44.4, 56.3, 65.6, 68.3, 69.8, 73.9, 77.9, 80.3, 93.2, 127.7, 127.8, 128.4, 128.5, 132.5, 138.0, 140.0, 161.0, 176.2, 209.0; MS *m/z* 751 (M^+−H); HRMS Calcd for $C_{44}H_{67}O_8Si$: 751.4605 (M^+−H). Found: 751.4603.

4.1.4. 12-*O*-Ethyl-20-*O*-(*tert*-butyldimethylsilyl)phorbol 13-decanoate (6d). To a solution of **5** (275 mg, 0.43 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (356 mg, 1.74 mmol) in CH_2Cl_2 (4 mL) was added ethyl trifluoromethanesulfonate (0.13 mL, 1.04 mmol) at 0 °C. The reaction was continued for 65 h at room temperature and quenched with H_2O. After diluting with CH_2Cl_2, the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, successively, and dried over $MgSO_4$. The solvent was evaporated to leave a residue, which was chromatographed on silica gel (CH_2Cl_2–AcOEt = 4:1) to afford **6d** (28 mg, 10%) as a colorless oil. ^1H NMR ($CDCl_3$): δ 0.05 (6H, s), 0.81–0.96 (13H, m), 1.01 (3H, d, J = 6.6 Hz), 1.11–1.18 (3H, m), 1.13 (3H, s), 1.22 (3H, s), 1.20–1.39 (12H, m), 1.58–1.70 (2H, m), 1.78 (3H, s), 1.98–2.03 (1H, m), 2.22–2.38 (3H, m), 2.43 (1H, d, J = 19 Hz), 2.61 (1H, br), 3.17 (1H, br), 3.27–3.38 (1H, m), 3.39–3.49 (2H, m), 3.99 (3H, br s), 4.28 (1H, s), 5.61 (1H, d, J = 3.3 Hz), 7.47 (1H, s); ^{13}C NMR ($CDCl_3$): δ −4.9, 10.5, 14.4, 16.0, 16.1. 17.5, 18.7, 23.0, 23.6, 25.3, 26.2, 27.5, 29.4, 29.5, 29.7, 30.4, 32.1, 34.4, 35.3, 38.5, 39.0, 46.5, 49.7, 59.4, 68.2, 69.7, 73.7, 78.6, 83.7, 128.8, 133.6, 137.5, 160.6, 177.7, 209.0; IR (neat) cm^{-1}: 3419, 1711; MS *m/z* 659 (M^+−H); HRMS Calcd for $C_{38}H_{63}O_7Si$: 659.4343 (M^+−H). Found: 659.4383; $[\alpha]_D^{25}$ +81.97 (*c* 0.120, $CHCl_3$).

4.1.5. General procedure for the synthesis of 12-*O*-alkyl or 12-*O*-(α-oxyalkyl)phorbol 13-decanoate (7a–d). To a solution of **6** in THF was added tetra-*n*-butylammonium fluoride (1 M solution in THF; 2 equiv) at 0 °C, and the solution was stirred for 1.5 h at room temperature. Saturated aqueous NH_4Cl was added to the reaction mixture, and the aqueous solution was extracted with $CHCl_3$ and dried over $MgSO_4$. Removal of the solvent left a residue, which was chromatographed on silica gel (CH_2Cl_2–AcOEt = 4:1) to afford **7a** (94%), **7b** (72%), **7c** (94%), and **7d** (75%) as a colorless oil, respectively. Compound **7a**: ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.3 Hz), 1.00 (1H, d, J = 5.6 Hz), 1.06 (3H, d, J = 6.8 Hz), 1.19 (3H, s), 1.22–1.35 (15H, m), 1.59–1.62 (2H, m), 1.78 (3H, d, J = 1.7 Hz), 2.02–2.07 (1H, m), 2.24 (1H, s), 2.29–2.35 (2H, td, J = 7.7, 3.4 Hz), 2.46 (1H, d, J = 19 Hz), 2.53 (1H, d, J = 19 Hz), 3.18 (1H, t, J = 5.6 Hz), 3.23 (1H, s), 3.36 (3H, s), 3.94 (1H, d, J = 9.8 Hz), 3.98 (1H, d, J = 13 Hz), 4.04 (1H, d, J = 13 Hz), 4.54 (1H, d, J = 6.8 Hz), 4.88 (1H, d, J = 6.8 Hz), 5.67 (1H, d, J = 4.7 Hz), 5.76 (1H, s), 7.61 (1H, s); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.4, 15.4, 17.1, 22.9, 24.1, 24.8, 25.8, 29.1, 29.3, 29.5, 29.6, 32.1, 34.8, 36.3, 38.7, 39.2, 44.3, 55.9, 56.2, 65.7, 68.1, 73.8, 78.2, 79.9, 95.1, 129.3, 132.7, 140.6, 160.9, 176.3, 209.1; IR (neat) cm^{-1}: 3409, 1709; MS *m/z* 501 (M^+−OCH_2OCH_3); HRMS Calcd for $C_{30}H_{45}O_6$: 501.3216 (M^+−OCH_2OCH_3). Found: 501.3200; $[\alpha]_D^{25}$ +89.27 (*c* 0.11, $CHCl_3$). Compound **7b**: ^1H NMR ($CDCl_3$): δ 0.86 (3H, t, J = 7.1 Hz), 0.98 (1H, d, J = 5.6 Hz), 1.00 (3H, d, J = 6.8 Hz), 1.16 (3H, s), 1.21 (3H, s), 1.21–1.35 (12H, m), 1.55–1.63 (2H, m), 1.72 (3H, d, J = 1.4 Hz), 2.00–2.10 (1H, m), 2.31 (2H, td, J = 7.4, 2.5 Hz), 2.44 (1H, d, J = 19 Hz), 2.51 (1H, d, J = 19 Hz), 3.05–3.19 (3H, m), 3.36 (3H, s), 3.52 (2H, t, J = 4.9 Hz), 3.60–3.75 (2H, m), 3.90–4.02 (3H, m), 4.65 (1H, d, J = 6.9 Hz), 4.94 (1H, d, J = 6.9 Hz), 5.63 (1H, d, J = 5.2 Hz), 5.75 (1H, s), 7.55 (1H,s); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.4, 15.5, 17.1, 22.9, 24.1, 24.8, 25.8, 29.4, 29.5, 29.6, 32.1, 34.8, 36.3, 38.7, 39.2, 44.3, 56.3, 59.2, 65.7, 67.3, 68.2, 71.9, 73.8, 78.2, 80.1, 94.0, 129.3, 132.7, 140.5, 161.0, 176.3, 209.1; IR (neat) cm^{-1}: 3406, 1709; MS *m/z* 606 (M^+); HRMS Calcd for $C_{34}H_{54}O_9$: 606.3768 (M^+). Found: 606.3779; $[\alpha]_D^{25}$ +85.88 (*c* 1.00, $CHCl_3$). Compound **7c**: ^1H NMR ($CDCl_3$): 0.87 (3H, t, J = 6.9 Hz), 1.01 (1H, d, J = 5.2 Hz), 1.06 (3H, d, J = 6.1 Hz), 1.11–1.36 (18H, m), 1.45–1.60 (2H, m), 1.73 (3H, s), 2.02–2.15 (1H, m), 2.20 (2H, t, J = 7.4 Hz), 2.46 (1H, d, J = 19 Hz), 2.54 (1H, d, J = 19 Hz), 3.20 (3H, br), 3.90–4.04 (3H, m), 4.56 (1H, d, J = 12 Hz), 4.64 (1H, d, J = 12 Hz), 4.71 (1H, d, J = 6.9 Hz), 4.99 (1H, d, J = 6.9 Hz), 5.66 (1H, d, J = 5.5 Hz), 5.81 (1H, s), 7.22–7.37 (5H, m), 7.58 (1H, s); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.4, 15.5, 17.2, 22.9, 24.1, 24.8, 25.9, 29.3, 29.4, 29.5, 29.6, 32.1, 34.7, 36.3, 38.7, 39.2, 44.4, 56.2, 65.6, 68.2, 69.8, 73.8, 78.3, 80.3, 93.2, 127.7, 127.8, 128.4, 129.4, 132.7, 137.9, 140.6, 161.0, 176.4, 209.2; IR (neat) cm^{-1}: 3399, 1708; MS *m/z* 638 (M^+); $[\alpha]_D^{25}$ +70.75 (*c* 3.45, $CHCl_3$). Compound **7d**; ^1H NMR ($CDCl_3$): 0.82–0.95 (4H, m), 1.03 (3H, d, J = 6.6 Hz), 1.13–1.17 (6H, m), 1.20–1.39 (15H, m), 1.59–1.70 (2H, m), 1.78 (3H, d, J = 1.6 Hz), 1.95–2.07 (2H, m), 2.30–2.41 (3H, m), 2.50 (1H, d, J = 19 Hz), 2.97 (1H, br s), 3.17 (1H, br), 3.25–3.37 (1H, m), 3.40–3.49 (2H, m), 3.97–4.03 (3H, m), 4.30 (1H, s), 5.63 (1H, br s), 7.48 (1H, s); ^{13}C NMR ($CDCl_3$): δ 10.5, 14.4, 15.9, 16.1, 17.6, 23.0, 23.6, 25.3, 25.9, 27.5, 29.4, 29.5, 29.7, 32.1, 34.4, 35.2, 38.6, 39.1, 46.5, 49.7, 59.4, 68.4, 69.6, 73.7, 78.7, 83.7, 130.1, 133.9, 138.1, 160.6, 177.7, 209.2; IR (neat) cm^{-1}: 3449, 1708; MS *m/z* 546 (M^+); HRMS Calcd for $C_{32}H_{50}O_7$: 546.3557 (M^+). Found: 546.3548; $[\alpha]_D^{25}$ +109.81 (*c* 0.745, $CHCl_3$).

4.1.6. 12-*O*-Methoxymethyl-20-*O*-methylphorbol 13-decanoate (8). According to the procedure for the synthesis of **6d**, the compound **7a** (49 mg, 0.087 mmol)

was treated with 2,6-di-*tert*-butyl-4-methylpyridine and methyl trifluoromethanesulfonate to afford **8** (44 mg, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, t, J = 7.1 Hz), 0.99 (1H, d, J = 5.2 Hz), 1.03 (3H, d, J = 6.3 Hz), 1.17 (3H, s), 1.18–1.35 (15H, m), 1.55–1.68 (2H, m), 1.75 (3H, t, J = 1.4 Hz), 2.00–2.10 (1H, m), 2.32 (2H, t, J = 7.4 Hz), 2.44 (1H, d, J = 19 Hz), 2.48 (1H, d, J = 19 Hz), 2.57 (1H, br), 3.14–3.24 (2H, m), 3.27 (3H, s), 3.36 (3H, s), 3.73 (1H, d, J = 12 Hz), 3.81 (1H, d, J = 12 Hz), 3.93 (1H, d, J = 9.6 Hz), 4.54 (1H, d, J = 6.7 Hz), 4.88 (1H, d, J = 6.7 Hz), 5.63 (1H, s), 5.66 (1H, s), 7.58 (1H, s); ^{13}C NMR ($CDCl_3$): δ 10.4, 14.4, 15.4, 17.1, 22.9, 24.1, 24.8, 25.8, 29.4, 29.5, 29.6, 32.1, 34.8, 36.3, 39.1, 39.5, 44.4, 55.9, 56.4, 58.2, 65.7, 73.8, 78.1, 80.0, 95.2, 131.3, 132.7, 137.6, 161.0, 176.2, 209.0; IR (neat) cm^{-1}: 3411, 1710; MS *m*/*z* 576 (M^+); HRMS Calcd for $C_{33}H_{52}O_8$: 576.3662 (M^+). Found: 576.3613; $[\alpha]_D^{25}$ +103.53 (*c* 2.00, $CHCl_3$).

4.2. Biology

4.2.1. Preparation of the test compound solutions. The test compounds were dissolved in DMSO and then diluted with physiological saline or medium solution. In the stability test, the test compounds were diluted with plasma collected from male BALB/cA mice and the mixed solutions were incubated on the water bath at 37 °C for 30 min. The plasma was prepared with 3.8% sodium citrate added blood, collected from the inferior vena cava.

4.2.2. Cells and virus. T-cell lines MT-4 and Molt-4 cells, and the HIV-1 IIIB infected Molt-4 cells, were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Cansera international Inc., Canada) and antibiotics (100 μg/mL penicillin/100 μg/mL streptomycin). HIV-1 IIIB viral stocks were prepared by propagation in co-culturing of HIV-1 IIIB infected Molt-4 and uninfected Molt-4 cells.

4.2.3. MTT assay. To determine susceptibility to HIV-1 and cytotoxicity, MT-4 cells were infected with HIV-1 IIIB strains at multiplicity of infection (MOI) of 0.01, and infected or uninfected MT-4 cells (mock infection) were cultured in the presence or absence of serial concentrations of the test compounds for 5 days. Cell viability was quantified with 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT, Dojindo, Japan) assay. The absorbances were read at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The concentration achieving 50% protection in HIV-1 infected cells was determined as the 50% effective concentration (EC_{50}) for drug susceptibility, and 50% cytotoxic concentration (CC_{50}) values were calculated at uninfected cells for drug cytotoxicity.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmc.2005.04.056.

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