Chinese Journal of Natural Medicines

Synthesis, and anti-HIV activities of phorbol derivatives

Xiaolei HUANG, Chengrun TANG, Xusheng HUANG, Yun YANG, Qirun LI, Mengdi MA, Lei ZHAO, Liumeng YANG, Yadong CUI, Zhenqing ZHANG, Yongtang ZHENG, Jian ZHANG

Citation: Xiaolei HUANG, Chengrun TANG, Xusheng HUANG, Yun YANG, Qirun LI, Mengdi MA, Lei ZHAO, Liumeng YANG, Yadong CUI, Zhenqing ZHANG, Yongtang ZHENG, Jian ZHANG, Synthesis, and anti-HIV activities of phorbol derivatives, Chinese Journal of Natural Medicines, 2024, 22(2), 1-16. doi: 10.1016/S1875-5364(23)60460-1.

View online: https://doi.org/10.1016/S1875-5364(23)60460-1

Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2024, **22**(2): 146-160
doi: 10.1016/S1875-5364(24)60587-X
Chinese Journal of Natural Medicines

•Original article•

Synthesis and anti-HIV activities of phorbol derivatives

HUANG Xiaolei[1Δ], TANG Chengrun[2Δ], HUANG Xusheng[2, 3Δ], YANG Yun[1], LI Qirun[1], MA Mengdi[2], ZHAO Lei[1], YANG Liumeng[2], CUI Yadong[1], ZHANG Zhenqing[1*], ZHENG Yongtang[2, 3*], ZHANG Jian[1*]

[1] *College of Pharmaceutical Science, Soochow University, Suzhou 215123, China;*
[2] *Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences/Key Laboratory of Bioactive Peptides of Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China;*
[3] *Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China*

Available online 20 Feb., 2024

[ABSTRACT] In this study, 37 derivatives of phorbol esters were synthesized and their anti-HIV-1 activities evaluated, building upon our previous synthesis of 51 phorbol derivatives. 12-Para-electron-acceptor-*trans*-cinnamoyl-13-decanoyl phorbol derivatives stood out, demonstrating remarkable anti-HIV-1 activities and inhibitory effects on syncytia formation. These derivatives exhibited a higher safety index compared with the positive control drug. Among them, 12-(*trans*-4-fluorocinnamoyl)-13-decanoyl phorbol, designated as compound **3c**, exhibited the most potent anti-HIV-1 activity (EC_{50} 2.9 nmol·L^{-1}, CC_{50}/EC_{50} 11 117.24) and significantly inhibited the formation of syncytium (EC_{50} 7.0 nmol·L^{-1}, CC_{50}/EC_{50} 4891.43). Moreover, compound **3c** is hypothesized to act both as an HIV-1 entry inhibitor and as an HIV-1 reverse transcriptase inhibitor. Isothermal titration calorimetry and molecular docking studies indicated that compound **3c** may also function as a natural activator of protein kinase C (PKC). Therefore, compound **3c** emerges as a potential candidate for developing new anti-HIV drugs.

[KEY WORDS] Phorbol esters; Anti-HIV-1 activity; Syncytia formation; 12-(*Trans*-4-fluorocinnamoyl)-13-decanoyl phorbol; Safety index; HIV-1 entry inhibitor; HIV-1 reverse transcriptase inhibitor; PKC activator

[CLC Number] R284, R965 **[Document code]** A **[Article ID]** 2095-6975(2024)02-0146-15

Introduction

The relentless spread of acquired immunodeficiency syndrome (AIDS) globally has catalyzed the extensive development of anti-HIV medications, targeting the eradication of causative agents [1]. In this quest, a multitude of plant-derived compounds are being scrutinized for their potential to inhibit HIV-1 replication and its crucial enzymes. Central to this exploration are phorbol esters, tetracyclic diterpenoids predominantly found in the *Euphorbiaceae* and *Thymelaeaceae* families [2-6]. These esters are known for their diverse biological activities, including inhibition of HIV-1 protease, activation of protein kinase C (PKC), platelet aggregation, cell differentiation, anti-tuberculosis properties, cytotoxic activities, and the activation of mesenchymal stem cells [3-5, 7]. Interestingly, some phorbol esters, such as 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), are known for their tumor-promoting and pro-inflammatory properties and are used to induce inflammation in pharmacological and biochemical research. In contrast, compounds like 12-deoxyphorbol-13-acetate (prostratin) and phorbol 12-acetate-13-decanoate have shown non-tumor-promoting activities, along with pronounced anti-HIV effects [4, 6-8]. Moreover, phorbol 12-acetate-13-decanoate and its 12-benzoyl derivative have demonstrated notable anti-HIV activities [9-11]. The compound 4-deoxy-4*β*-phorbol-12-tiglate-13-phenylacetate, effective in reactivating 70%−75% of latent HIV at concentrations ranging from 9.1−0.091 μmol·L^{-1}, has been identified as a promising lead for the development of anti-HIV drugs targeting latency reactivation [5].

Extracting phorbol esters from plants is fraught with challenges such as labor-intensive processes, difficult separations, and low yields. Consequently, the focus has shifted to

[Received on] 19-Aug.-2023
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 81202882 and 82060670); Suzhou Science and Technology Planning Project in Jiangsu Province of China (No. SNG2021022); the Priority Academic Program Development of the Jiangsu Higher Education Institutes, China (PAPD); and the Project of Innovative Research Team of Yunnan Province (No. 202005AE160005).
[*Corresponding author] E-mails: z_zhang@suda.edu.cn (ZHANG Zhenqing); zhengyt@mail.kiz.ac.cn (ZHENG Yongtang); jianzhang@suda.edu.cn (ZHANG Jian)
[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

the structural modification of phorbol, a field that has reached maturity, using methods such as selective hydroxyl group protection, esterification, and deprotection. Notably, the hydroxyl groups in phorbol exhibit a reactivity order of C20-OH > C13-OH > C12-OH > C4-OH > C9-OH [6], guiding the structural modifications primarily towards the C20, C13 and C12 hydroxyl groups. Previous research primarily explored the esterification of phorbol with long-chain saturated fatty acids [6].

The concept of privileged substructures and pharmacophore merging is pivotal in drug design and the structural modification of natural products [9, 12, 13]. In this study, 35 phorbol esters derivatives, including phorbol monoesters, diesters and triesters, 4*α*-phorbol esters, and 4*α*-4-deoxyphorbol esters were designed and synthesized by coupling with cinnamic acid derivatives, oleanolic acid, glycyrrhetinic acid, unsaturated and saturated fatty acids, and terminal brominated saturated fatty acids (Figs. 1 and 2). This study also encompassed the evaluation of the anti-HIV-1 activities of these derivatives, along with 51 previously synthesized phorbol ester derivatives [14, 15].

Results and Discussion

Synthesis of phorbol esters

In this research, we synthesized an array of 35 phorbol ester derivatives, including variants of 4*α*-phorbol ester and 4*α*-4-deoxyphorbol ester. The initial step in the synthesis involved protecting the C20-OH group of phorbol with trityl chloride, followed by esterification using cinnamic acid, its derivatives, or other carboxylic acids in different proportions. The subsequent removal of the trityl group with 3% $HClO_4$/MeOH resulted in the production of 12,13-phorbol diester derivatives (**1a**−**1k**, **3a**−**3g** and **4a**−**4b**) and a 13-phorbol ester (**6c**). In a parallel synthesis route, phorbol was acylated with acetic anhydride and subsequently esterified with carboxylic acids. The C-20-*O*-acetyl or C-13,20-*O*-diacetyl groups were then removed using either 3% $HClO_4$/MeOH or 0.05 mol·L^{-1} KOH/MeOH, yielding 12,13-phorbol diester derivatives (**2a**−**2h**) and a 13-phorbol ester (**5a**). The synthesis of the 4*α*-phorbol ester and 4*α*-4-deoxyphorbol ester (**10**, **11**) mirrored the approach used for compounds **1a**−**1k**. The synthetic pathways for these phorbol derivatives (**1a**−**1k**, **2a**−**2h**, **3a**−**3g**, **4a**−**4b**, **5a**, **6c**, **10** and **11**) are detailed in Schemes 1−5.

For the synthesis of the target compounds **4c**, **6a**−**6b**, and **9a**−**9b** containing dual pharmacophores, we employed methods outliuned in Schemes 6−7. The process began with the esterification of phorbol-13,20-diacetate with 6-bromohexanoic acid, followed by coupling with uracil to yield intermediate **A** and the target compound **9a**. To create intermediate **B_1**−**B_3**, 20-*O*-trityl-phorbol was first esterified with acetic

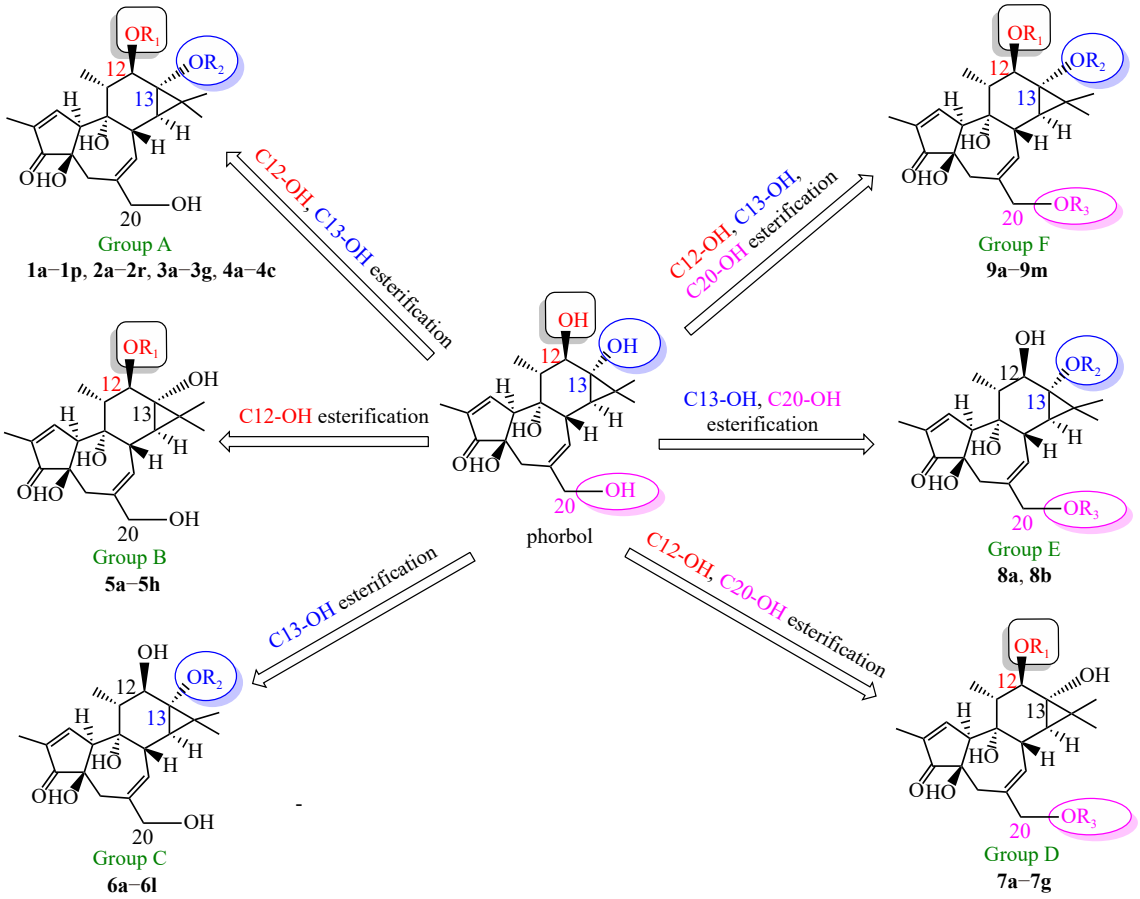
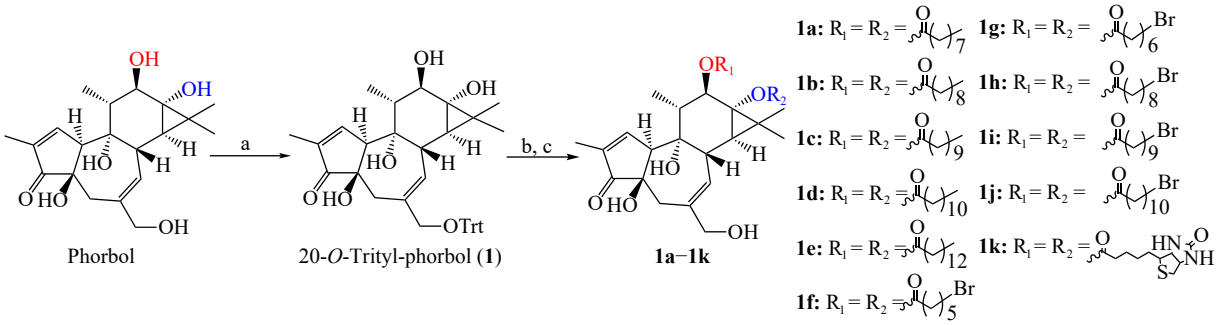
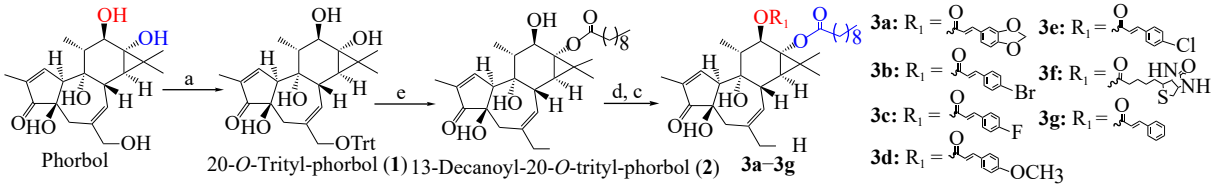


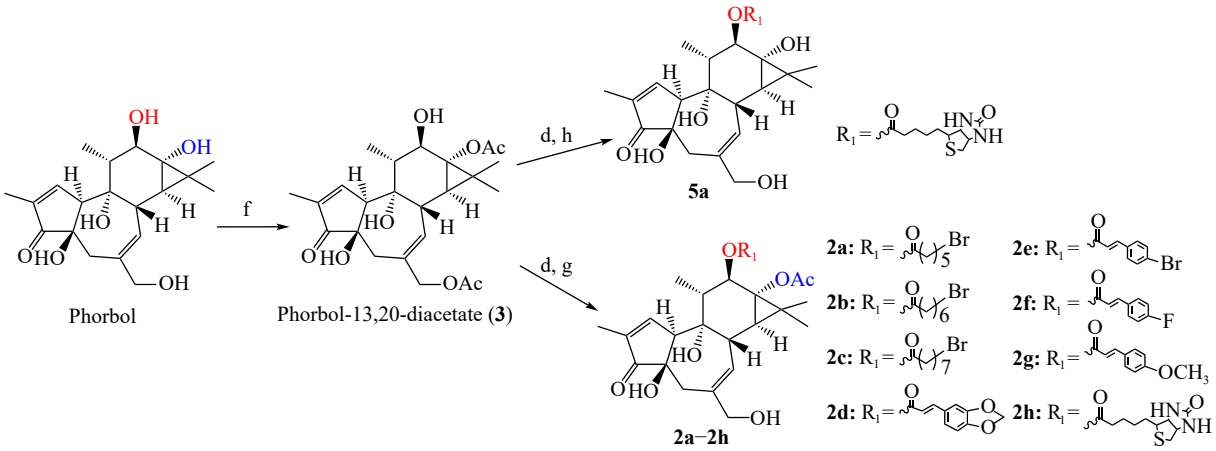
Fig. 1 The classifications of phorbol derivitives.



Scheme 1 Synthesis of compounds 1a–1k reagents and conditions: (a) Ph_3CCl, pyridine, 3 d; (b) 3 eq RCOOH, DMAP, EDCI, DCM, overnight; (c) 3% $HClO_4$/MeOH, 60 min.



Scheme 2 Synthesis of compounds 3a–3g reagents and conditions: (a) Ph_3CCl, pyridine, 3 d; (c) 3% $HClO_4$/MeOH, 60 min. (d) 1.5 eq RCOOH, DMAP, EDCI, DCM, overnight; (e) 1.5 eq decanoic acid, DMAP, EDCI, DCM overnight.

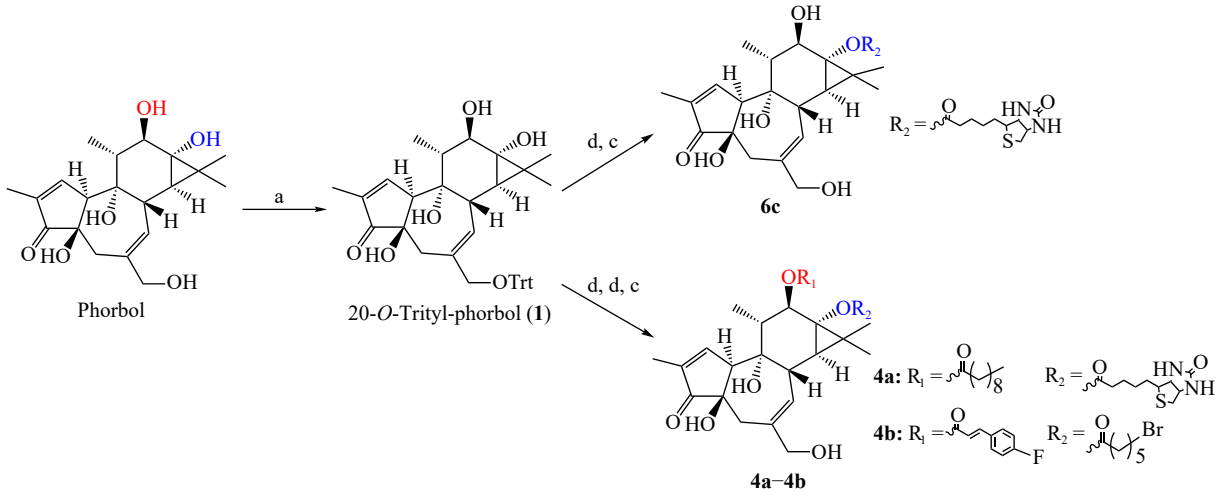


Scheme 3 Synthesis of compounds 2a–2h, 5a reagents and conditions: (d) 1.5 eq RCOOH, DMAP, EDCI, DCM, overnight; (f) acetic anhydride, Et_3N, DCM, overnight; (g) 3% $HClO_4$/MeOH, overnight; (h) 0.05 mol·L^{-1} KOH/MeOH, 30 min.

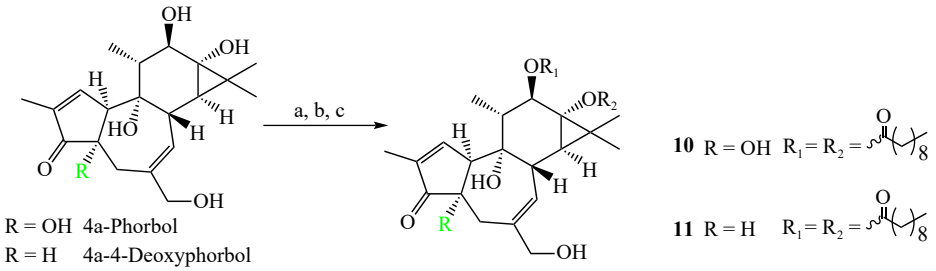
anhydride and either 6-bromohexanoic acid or 9-bromononanoic acid. After removing the C-20-*O*-acetyl group using 3% $HClO_4$/MeOH, the intermediates were coupled with uracil or piperazine. Furthermore, oleanolic acid and glycyrrhetic acid were reacted with 1,4-dibromobutane to produce intermediate **C**, which was subsequently coupled with intermediate **A** or $\mathbf{B_1}$–$\mathbf{B_3}$ to synthesize the target compounds **9b**, **4c**, and **6a**–**6b**.

Anti-HIV-1 activities of phorbol esters

The anti-HIV-1 activities of 88 phorbol ester derivatives, including 51 phorbol esters derivatives synthesized by our team, were evaluated [14, 15]. As shown in Table 1, 12,13-didecanoyl-phorbol (**1b**, EC_{50} 4.6 nmol·L^{-1}) exhibited a much higher anti-HIV-1 activity than 12,13-didecanoyl-4α-phorbol (**10**, EC_{50} 122 510 nmol·L^{-1}) and 12,13-didecanoyl-4α-4-deoxyphorbol (**11**, EC_{50} 3763.3 nmol·L^{-1}), highlighting the critical influence of the 4β-hydroxyl group. As shown in Tables 1–4, esterification with the same acid leads to varying levels of anti-HIV-1 activity, depending on the number of ester groups in the phorbol molecule. Phorbol-12,13,20-triesters generally show significantly lower activity than monoesters and diesters, such as phorbol-12,13,20-tricinnamate (**9c**, EC_{50} > 200 000 nmol·L^{-1}), phorbol-12,20-dicinnamate (**7e**, EC_{50} 27 010.0 nmol·L^{-1}), phorbol-12-cinnamate (**5d**, EC_{50} 19 785.0 nmol·L^{-1}), phorbol-13-cinnamate (**6f**, EC_{50} 6655.0 nmol·L^{-1}), phorbol-12,13-dicinnamate (**1l**, EC_{50} 123.6 nmol·L^{-1}), phorbol-12,13,20-triacetate (**9f**, EC_{50} 8955.0 nmol·L^{-1}), and phorbol-12,13-diacetate (**2l**, EC_{50} 323.5 nmol·L^{-1}). In a striking finding presented in Table 4, phorbol esters with C13-OH and C20-OH of phorbol esterified by the same acid but with C12-



Scheme 4 Synthesis of compounds 4a–4b, 6c reagents and conditions: (a) Ph_3CCl, pyridine, 3 d; (c) 3% $HClO_4$/MeOH, 60 min. (d) 1.5 eq RCOOH, DMAP, EDCI, DCM, overnight.

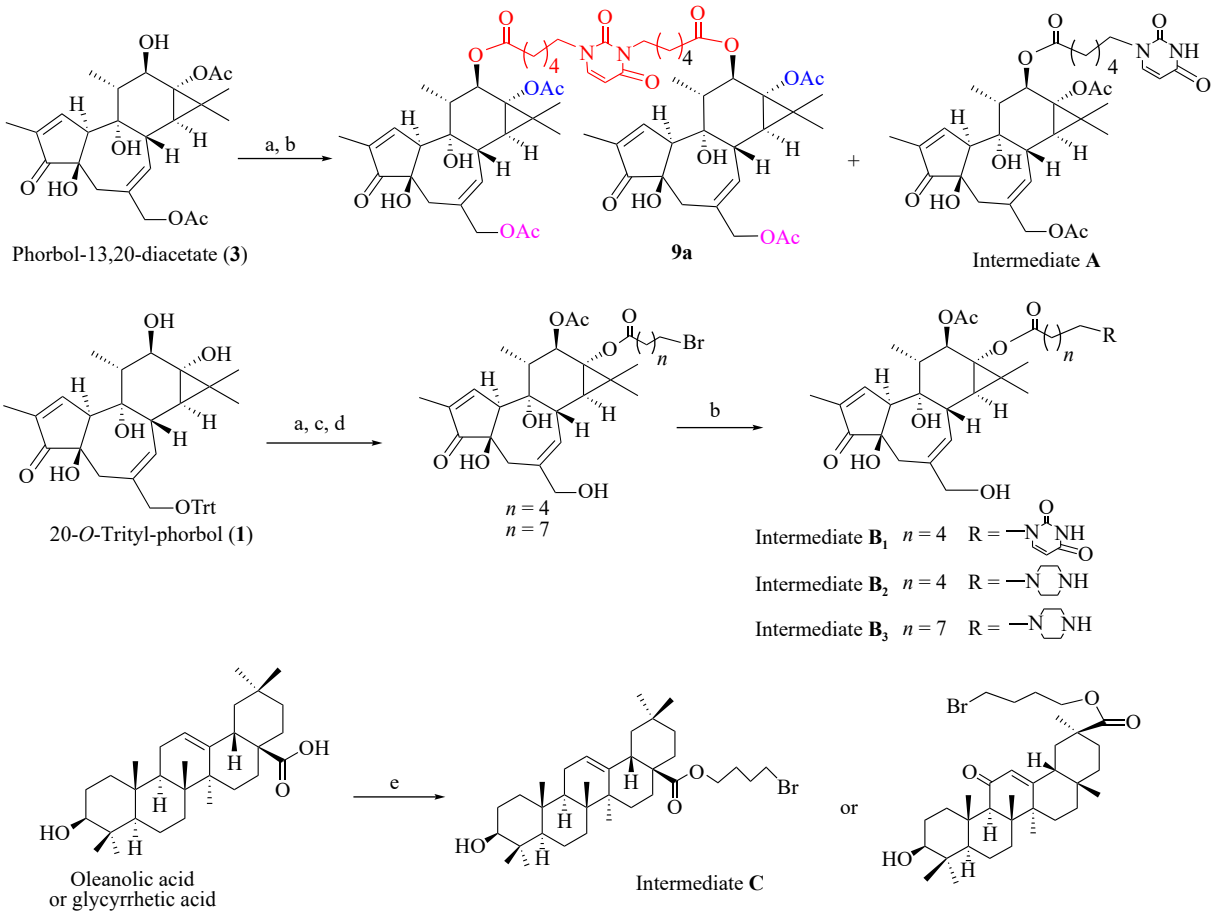


Scheme 5 Synthesis of compounds 10 and 11 reagents and conditions: (a) Ph_3CCl, pyridine, 3 d; (b) 3 eq RCOOH, DMAP, EDCI, DCM, overnight; (c) 3% $HClO_4$/MeOH, 60 min.

OH modified by a different acid exhibited highly effective anti-HIV activities, such as 12-eicosapentaenoyl phorbol-13, 20-diacetate (**9j**, EC_{50} 70.5 nmol·L^{-1}) and 12-*trans*-cinnamoyl phorbol-13,20-dibutyryl esters (**9d**, EC_{50} 54.9 nmol·L^{-1}). In contrast, compounds coupled with oleanolic acid (**9b**) and phorbol itself (**9a**) showed minimal activity (EC_{50} > 200 000 nmol·L^{-1}). Furthermore, the 12-*trans*-cinnamoyl-13-dibutyryl-20-(3,4-methylenedioxycinnamoyl)-phorbol esters (**9l**), synthesized with different acids, also demonstrated a significant anti-HIV-1 activity (EC_{50} 671.4 nmol·L^{-1}).

As shown in Tables 1, 2 and 4, phorbol esters coupled with oleanolic acid (**9b**) and phorbol itself (**9a**) showed no anti-HIV-1 activity. Conversely, those coupled with glycyrrhetic acids (**4c**, **6a**, and **6b**) exhibited weak anti-HIV activity (EC_{50}: **6a** > **6b** > **4c**). Compounds **6a** and **6b** were phorbol-13-monoester. Compound **4c** was 12-acetyl-phorbol-13-ester. It is speculated that the anti-HIV-1 activity is influenced by the number of methylene groups in the linker arm and the ester group at the C12 position, especially when phorbol is combined with large pharmacophores like glycyrrhetic acid.

As shown in Table 2, phorbol was esterified with the same acid to produce phorbol-12-monoesters or phorbol-13-monoesters. Phorbol-13-monoesters consistently demonstrated stronger anti-HIV-1 activities than their 12-monoester counterparts. For example, 13-eicosanyol phorbol (**6k**) had an EC_{50} of 20.2 nmol·L^{-1}, notably lower than 12-eicosanyol phorbol (**5c**, EC_{50} 39 955.0 nmol·L^{-1}), and 13-eicosapentaenoyl phorbol (**6i**) exhibited a notably lower EC_{50} than 12-eicosapentaenoyl phorbol (**5g**) (136.2 nmol·L^{-1} *vs* 5325.0 nmol·L^{-1}). This trend was consistent, with the exception of biotinylated derivatives (**5a**, **6c**, EC_{50} > 200 000 nmol·L^{-1}). As shown in Tables 1 and 3, the anti-HIV-1 activity of phorbol-13,20-diesters or phorbol-12,20-diester was much lower than that of phorbol-12,13-diester. For instance, 13,20-dieicosapentaenoyl phorbol (**8b**, EC_{50} of 12 740.0 nmol·L^{-1}) and 12-eicosapentaenoyl phorbol-20-acetate (**7d**, EC_{50} 5080.0 nmol·L^{-1}) were markedly less potent than 12-eicosapentaenoyl phorbol-13-acetate (**2m**, EC_{50} 4.1 nmol·L^{-1}). Phorbol was esterified by the same saturated fatty acid, yielding phorbol-12,13-diester. As shown in Table 1, the anti-HIV-1 activity of phorbol-12,13-diester did not increase with the increase in methylene number. 12,13-Didecanoyl phorbol exhibited the strongest anti-HIV-1 activity (**1b**, EC_{50} 4.6 nmol·L^{-1}) with a high safety index (CC_{50}/EC_{50} 23 665.22); 12,13-di (3-phenylpropanoyl)-phorbol showed the stronger anti-HIV-1 activity (**1m**, EC_{50} 26.4 nmol·L^{-1}) than phorbol-12,13-diester obtained by combining phorbol with unsaturated acid; 12,13-di (6-bromohexanoyl)-phorbol exhibited high anti-HIV-1 activity (**1f**, EC_{50} 5.7 nmol·L^{-1}). As shown in Table 1, the anti-HIV-1 activity of phorbol-12,13-diester obtained by combining phorbol with terminal brominated satur-

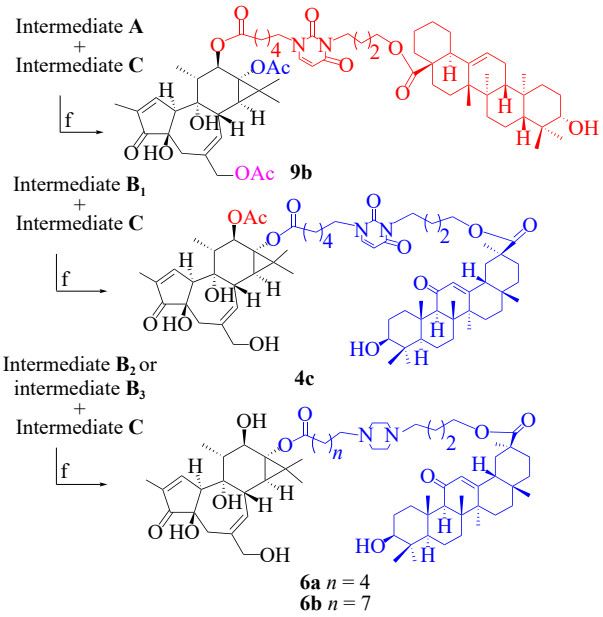


Scheme 6 Synthesis of compound 9a reagents and conditions: (a) 6-bromohexanoic acid or 9-bromononanoic acid, DMAP, EDCI, DCM overnight; (b) uracil or piperazine, K_2CO_3, DMF, 60 °C or 50 °C, 12 h; (c) acetic anhydride, Et_3N, DCM, overnight; (d) 3% $HClO_4$/MeOH, 60 min; (e) 1,4-dibromobutane, K_2CO_3, DMF, 12 h.

ated fatty acids decreased with the increase in methylene number. For example, 12,13-di (9-bromononanoyl)-phorbol (**1h**) had a lower EC_{50} value of 17 413.2 nmol·L^{-1} than 12,13-di (10-bromodecanoyl)-phorbol (**1i**, EC_{50} 19 873.3 nmol·L^{-1}). As shown in Table 1, most 13-acetyl-phorbol-12-esters exhibited significant anti-HIV-1 activities, such as 12-docosahexaenoyl-phorbol-13-acetate (**2n**, EC_{50} 8.3 nmol·L^{-1}). However, 12-(6-bromohexanoyl)-13-acetyl-phorbol did not show anti-HIV-1 activity (**2a**, EC_{50} > 200 000 nmol·L^{-1}). As shown in Table 1, the anti-HIV-1 activities of compounds **2b** and **2c** increased with the increase in methylene number; the anti-HIV-1 activities of 13-acetyl-phorbol-12-*trans*-cinnamoyl derivatives decreased with the introduction of *para* electron-acceptor groups in the cinnamoyl moiety. For instance, 12-*trans*-cinnamoyl phorbol-13-acetate (**2i**) exhibited a dramatically lower EC_{50} value than 12-feruloyl-13-acetyl-phorbol (**2j**) (14.0 nmol·L^{-1} *vs* 77 920.0 nmol·L^{-1}). As shown in Table 1, 13-butyryl phorbol-12-unsaturated acid derivatives showed high anti-HIV-1 activity, such as 12-*trans*-cinnamoyl-13-butyryl phorbol (**2o**, EC_{50} 7.9 nmol·L^{-1}). As shown in Table 1, a series of 13-decanoyl-phorbol-12-*trans*-cinnamoyl derivatives all exhibited remarkable anti-HIV-1 activities with a high safety index. Moreover, the anti-HIV activity could be increased by incorporating the *para* electron-acceptor groups into the cinnamoyl group (**3a**–**3e**), so the anti-HIV-1 activities of these derivatives were stronger than that of positive control drug (AZT, EC_{50} 5.4 nmol·L^{-1}). Among these derivatives, 12-(*trans*-4-fluorocinnamoyl)-13-decanoyl phorbol emerged as the most potent compound (**3c**, EC_{50} 2.9 nmol·L^{-1}) with a high safety index (CC_{50}/EC_{50} 11 117.24), but its anti-HIV-1 activity decreased after 13-decanoyl was replaced by 6-bromohexanoyl (**3f**, EC_{50} 301.9 nmol·L^{-1}), as shown in Table 1.

Inhibition of syncytia formation induced by HIV-1$_{IIIB}$

The data in Table 5 illustrated that compounds **1b**, **2m**, **2n**, **2q**, and **3a**–**3d**, recognized for their remarkable anti-HIV-1 activities, also demonstrated significant inhibition of syncytia formation induced by HIV-1$_{IIIB}$. In particular, compounds **1b** and **3a**–**3d** outperformed the positive control drug 3TC in terms of inhibition strength and safety (**1b**, EC_{50} 5.2 nmol·L^{-1}, CC_{50}/EC_{50} 20 550.00; **3a**, EC_{50} 26.2 nmol·L^{-1}, CC_{50}/EC_{50} 1438.36; **3b**, EC_{50} 9.6 nmol·L^{-1}, CC_{50}/EC_{50} 3368.23; **3c**, EC_{50} 7.0 nmol·L^{-1}, CC_{50}/EC_{50} 4891.43; **3d**, EC_{50} 33.7 nmol·L^{-1}, CC_{50}/EC_{50} 1564.24; 3TC, EC_{50} 175.8



Scheme 7 Synthesis of compounds 6a, 6b, 9b, and 4c reagents and conditions: (f) K_2CO_3, KI, MeCN or DMF, 75 °C, 36 h.

nmol·L^{-1}, CC_{50}/EC_{50} > 1137.66).

A comprehensive analysis of anti-HIV-1 activities and syncytia formation inhibition highlighted the superior performance of 13-decanoyl-phorbol-12-*trans*-cinnamoyl derivatives, especially those with *para* electron-acceptor groups in the cinnamoyl structure. The introduction of *para* electron-withdrawing groups, such as fluorine (F), chlorine (Cl), or bromine (Br), into the 12-*trans*-cinnamoyl phorbol derivatives (compounds **3c**, **3e**, and **3b**) significantly enhanced their anti-HIV-1 activities compared with derivatives with electron-donating groups like -OCH_3 or -OCH_2O- (compounds **3d** and **3a**). There is a clear trend indicating that the stronger the electron absorption capacity of the substituents (ranked as -F > -Cl > -Br), the greater the anti-HIV-1 activity.

Potential of compound 3c as an inhibitor of HIV-1$_{IIIB}$ replication at the entry step

We investigated the mechanism by which compound **3c** inhibits HIV-1 replication, particularly examining its impact on HIV-1 reverse transcription and virus entry processes. To understand the role of compound **3c** in HIV-1 replication, we analyzed its effect on the intermediates during reverse transcription, specifically single-stranded DNA (ssDNA) and 2-long terminal repeat (2LTR) circles. The results demonstrated that compound **3c** significantly reduced the expression of these intermediate products, indicating its inhibitory role in HIV-1 replication either during the HIV-1 reverse transcription stage or in preceding steps (Fig. 3).

To further determine whether compound **3c** functions as an inhibitor at the virus entry step, we treated C8166 cells with this compound at two critical points: during the infection by HIV-1$_{IIIB}$ and after the virus had entered the cells. The results revealed that the treatment with compound **3c** during the HIV-1$_{IIIB}$ infection phase effectively suppressed virus replication. However, its inhibitory effect was notably reduced after the virus had entered the cells. These observations suggest that compound **3c** likely functions as an HIV-1 entry inhibitor, in a manner similar to T-20, a known inhibitor of this stage (Fig. 4).

Effects of compound 3c on PKC

We then explored the role of compound **3c** as a potential natural activator of PKC, inspired by its structural similarity to diacylglycerol (C3-C=O, C4-*β*-OH, and C20-OH). This similarity suggests that phorbol esters, such as compound **3c**, might activate PKC. Furthermore, the specific substitutions at C-12 and C-13 in the phorbol esters are believed to influence their interaction with PKC's hydrophobic region, affecting both the depth and orientation of their insertion into the enzyme [16]. A molecular docking study was conducted to analyze the interaction between compound **3c** and PKC. As depicted in Fig. 5, compound **3c** exhibited high binding energy (−8.32 kcal·moL^{-1}) at the active site of PKC-δ. The interactions between compound **3c** and PKC-δ primarily involved the formation of hydrogen bonds with GLN-25 and hydrophobic interactions with other amino acid residues, such as PHE-27, ALA-23 and, ASN-24. The binding affinity of compound **3c** for PKC-C1 protein was further quantified using isothermal titration calorimetry (ITC). The results revealed that compound **3c** exhibits a high affinity for PKC, with a dissociation constant (K_d) of 10.0 ± 1.3 nmol·L^{-1}. This finding indicates that compound **3c** may act as a natural activator of PKC.

Stability of compound 3c in plasma in vitro

The stability of compound **3c** in plasma *in vitro* was investigated. Specifically, the concentration of compound **3c** in rat plasma over time was monitored using high-performance liquid chromatography (HPLC). As shown in Fig. 6, the findings indicated that compound **3c** maintained its stability in rat plasma *in vitro* throughout the duration of the experiment. Notably, there was no significant degradation of the compound, nor was there any metabolization back to its parent compound, phorbol.

Experimental

General experimental procedures

Reagents and solvents were of commercial quality and used without further purification. ^{1}H and ^{13}C NMR spectra were obtained from Varian UNITY INOVA-600 (Agilent, USA) with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra of synthesized compounds were recorded on Agilent 6540 UHD Q-TOF (Agilent, USA) with an electrospray ionization (ESI) interface. Thin-layer chromatography (TLC) analysis was performed on Huanghai silica gel GF-254 plates (Yantai Huanghai, 02025892, Y.K.X. Material Technology Co., Ltd., China). Compounds were purified by silica gel column (Yantai Huanghai, AKX-H0090, Y.K.X. Material Technology Co., Ltd., China). The structures of all the phorbol esters (**1a**–**9b**), 4*α*-phorbol ester (**10**)

Table 1 Anti-HIV-1 activity and safety index of 12,13-phorbol diesters.

No.	R_1	R_2	R_3	Anti-HIV activity (EC_{50}, nmol·L^{-1})	Cytotoxicity (CC_{50}, μmol·L^{-1})	Safety index (CC_{50}/EC_{50})
1a	Pelargonyl	Pelargonyl	H	35 270 ± 18215.1	22.7291 ± 2.3457	/
1b	Decanoyl	Decanoyl	H	4.6 ± 1.1	106.865 ± 13.9371	23 665.22
1c	Undecanoyl	Undecanoyl	H	3180 ± 381.8	> 200	> 62.89
1d	Dodecanoyl	Dodecanoyl	H	16 510 ± 1866.8	> 200	> 19.60
1e	Myristoyl	Myristoyl	H	18670 ± 2775.5	> 200	> 12.12
1f	6-Bromohexanoyl	6-Bromohexanoyl	H	5.7 ± 4.1	22.205 ± 1.1384	3895.61
1g	7-Bromoheptanoyl	7-Bromoheptanoyl	H	180 ± 8.1	79.71 ± 7.3822	4428.33
1h	9-Bromononanoyl	9-Bromononanoyl	H	17130 ± 400.5	74.0345 ± 10.9126	> 11.63
1i	10-Bromodecanoyl	10-Bromodecanoyl	H	19873.3 ± 5354.7	26.1641 ± 2.7342	/
1j	11-Bromoundecanoyl	11-Bromoundecanoyl	H	80 850 ± 17 740	> 200	> 2.96
1k	Biotin	Biotin	H	> 200 000	> 200	/
1l	*trans*-Cinnamoyl	*trans*-Cinnamoyl	H	123.6 ± 73.5	26.145 ± 10.7268	211.53
1m	3-Phenylpropanoyl	3-Phenylpropanoyl	H	26.4 ± 10.5	26.34 ± 4.7659	997.73
1n	*trans*-2-Butenoyl	*trans*-2-Butenoyl	H	622.4 ± 29.1	77.565 ± 15.9877	124.62
1o	Eicosapentaenoyl	Eicosapentaenoyl	H	1280.6 ± 409.3	> 200	> 156.18
1p	Eicosanoyl	Eicosanoyl	H	87 425 ± 1732.4	> 200	> 2.29
2a	6-Bromohexanoyl	Ac	H	> 200 000	> 200	/
2b	7-Bromoheptanoyl	Ac	H	47.4 ± 37.3	73.6 ± 2.6446	1554.47
2c	8-Bromooctanoyl	Ac	H	33.5 ± 8.1	80.565 ± 1.0253	2441.21
2d	3,4-Methylene dioxycinnamoyl	Ac	H	22.5 ± 15.3	104.845 ± 9.7934	4659.78
2e	4-Bromocinnamoyl	Ac	H	18.8 ± 0.8	41.15 ± 9.1358	2188.83
2f	4-Fluorocinnamoyl	Ac	H	26.4 ± 1.1	107.125 ± 8.2237	4057.76
2g	4-Methoxycinnamoyl	Ac	H	47 ± 20.1	108.575 ± 9.5106	2310.11
2h	Biotin	Ac	H	> 200 000	> 200	> 11.26
2i	*trans*-Cinnamoyl	Ac	H	14 ± 9.5	75.055 ± 20.4425	5361.07
2j	Feruloyl	Ac	H	77 920 ± 18 144.4	97.47 ± 9.9985	1.25
2k	4-Chlorocinnamoyl	Ac	H	24.6 ± 8.3	23.17 ± 4.2992	945.71
2l	Ac	Ac	H	323.6 ± 116.6	> 200	> 618.24
2m	Eicosapentaenoyl	Ac	H	4.1 ± 0.8	26.405 ± 11.2076	6440.24
2n	Docosahexaenoyl	Ac	H	8.3 ± 4.0	44.12 ± 2.5173	5315.66
2o	*trans*-Cinnamoyl	Butyryl	H	7.9 ± 2.4	32.335 ± 9.8924	4093.04
2p	Feruloyl	Butyryl	H	95.7 ± 22.5	77.84 ± 0.9192	813.38
2q	Eicosapentaenoyl	Butyryl	H	7.4 ± 2.8	23.265 ± 3.8537	3165.31
2r	Docosahexaenoyl	Butyryl	H	28.3 ± 11.2	26.7 ± 2.6304	946.99
3a	3,4-Methylene dioxycinnamoyl	Decanoyl	H	4.8 ± 2.0	37.685 ± 1.5627	7851.04
3b	4-Bromocinnamoyl	Decanoyl	H	3.8 ± 0.5	32.335 ± 8.0539	8841.43
3c	4-Fluorocinnamoyl	Decanoyl	H	2.9 ± 0.8	34.24 ± 1.7819	11 117.24
3d	4-Methoxycinnamoyl	Decanoyl	H	4.5 ± 0.0013	52.715 ± 7.3893	11 714.44
3e	4-Chlorocinnamoyl	Decanoyl	H	3.4 ± 0.1	33.485 ± 8.2519	9848.53
3f	*trans*-Cinnamoyl	Decanoyl	H	301.9 ± 61.5	128.8774 ± 57.872	562.49
3g	Biotin	Decanoyl	H	17260 ± 354.2	17.1095 ± 1.0309	/
4a	Decanoyl	Biotin	H	18545 ± 120.2	16.3950 ± 0.5673	/
4b	4-Fluorocinnamoyl	6-Bromohexanoyl	H	153.62 ± 41.3	48.1037 ± 28.6126	444.86
4c	Ac	A	H	75 870 ± 33 474.4	> 200	> 2.01
10	Decanoyl	Decanoyl	H	122510 ± 6912.7	> 200	1.63
11	Decanoyl	Decanoyl	H	6230 ± 3488.4	59.5516 ± 2.5928	/
AZT				5.4 ± 3.0	> 200	> 26 666.60

Compounds **1l**–**1p** and **2i**–**2r** were phorbol esters previously synthesized by our team [14, 15]; compound **10** was 4*α*-phorbol derivative; compound **11** was 4*α*-4-deoxyphorbol derivative; A: 3-(4-butyl glycyrrhetinic acid)-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione; AZT: zidovudine.

Table 2 Anti-HIV-1 activity and safety index of 12-phorbol esters and 13-phorbol esters.

No.	R_1	R_2	R_3	Anti-HIV activity (EC_{50}, nmol·L^{-1})	Cytotoxicity (CC_{50}, μmol·L^{-1})	Safety index (CC_{50}/EC_{50})
5a	Biotin	H	H	> 200 000	> 200	/
5b	Docosahexaenoyl	H	H	5060 ± 1060.7	46.3 ± 0.5657	9.15
5c	Eicosanoyl	H	H	39 955 ± 18 151.4	> 200	> 5.01
5d	*trans*-Cinnamoyl	H	H	19 785 ± 120.2	114.055 ± 17.8403	5.76
5e	3-Phenylpropanoyl	H	H	51 575 ± 6936.7	> 200	> 3.88
5f	*trans*-2-Butenoyl	H	H	> 200 000	> 200	/
5g	Eicosapentaenoyl	H	H	5325 ± 473.8	44.795 ± 17.5433	8.41
5h	Myristoyl	H	H	1625 ± 544.5	23.885 ± 1.7324	14.70
6a	H	B	H	98 200 ± 14 241.1	16.3522 ± 0.4254	/
6b	H	C	H	96 005 ± 4278	18.9147 ± 0.1387	/
6c	H	Biotin	H	> 200 000	> 200	/
6d	H	Docosahexaenoyl	H	95.1 ± 27.4	46.865 ± 0.4596	492.80
6e	H	3-Methoxy-4-acet oxycinnamoyl	H	9540 ± 2404.2	92.23 ± 8.556	9.67
6f	H	*trans*-Cinnamoyl	H	6655 ± 1958.7	> 200	> 30.65
6g	H	3-Phenylpropanoyl	H	11 140 ± 7028.6	> 200	> 17.95
6h	H	*trans*-2-Butenoyl	H	21 990 ± 3139.6	> 200	> 9.09
6i	H	Eicosapentaenoyl	H	136.2 ± 52.3	72.985 ± 18.2363	535.87
6j	H	Angeloyl	H	44 515 ± 20 810.2	> 200	> 4.49
6k	H	Eicosanoyl	H	41.7 ± 8.7	90.195 ± 14.1351	4478.41
6l	H	Decanoyl	H	563.5 ± 196.3	117.56 ± 7.9196	208.62
AZT				5.4 ± 3.0	> 200	> 26 666.60

Compounds **5b**−**5h** and **6d**−**6l** were phorbol esters previously synthesized by our team [14, 15]; B: 1-(4-butyl glycyrrhetinic acid)-4-(6-oxohexyl)-piperazine; C: 1-(4-butyl glycyrrhetinic acid)-4-(9-oxononyl)- piperazine; AZT: zidovudine.

Table 3 Anti-HIV-1 activity and safety index of 12,20-phorbol diesters and 13,20-phorbol diesters.

No.	R_1	R_2	R_3	Anti-HIV activity (EC_{50}, nmol·L^{-1})	Cytotoxicity (CC_{50}, μmol·L^{-1})	Safety index (CC_{50}/EC_{50})
7a	Myristoyl	H	Ac	4415 ± 459.6	25.26 ± 6.5195	> 5.72
7b	*trans*-Cinnamoyl	H	Ac	29 420 ± 1117.2	108.39 ± 22.3304	3.68
7c	3-Phenylpropanoyl	H	Ac	46 345 ± 22 478.9	118.8 ± 1.5415	2.56
7d	Eicosapentaenoyl	H	Ac	5080 ± 84.9	33.91 ± 9.2207	6.68
7e	*trans*-Cinnamoyl	H	*trans*-Cinnamoyl	27 010 ± 16 136.2	> 200	> 7.41
7f	Eicosanoyl	H	Ac	> 200 000	> 200	/
7g	Docosahexaenoyl	H	Ac	15 660 ± 2220.3	55.9 ± 12.5865	3.57
8a	H	*trans*-2-Butenoyl	*trans*-2-Butenoyl	64 790 ± 17 903.9	144.25 ± 13.1098	2.23
8b	H	Eicosapentaenoyl	Eicosapentaenoyl	12 740 ± 9772.2	86.42 ± 16.7584	6.78
AZT				5.4 ± 3.0	> 200	> 26 666.60

Compounds **7a**−**7g** and **8a**−**8b** were phorbol esters previously synthesized by our team [14, 15]; AZT: zidovudine.

and 4α-4-deoxyphorbol ester (**11**) were characterized through 1D (^{1}H and ^{13}C) NMR and HR-ESI-MS data analysis. Following literature methods [17], 20-*O*-trityl-phorbol (intermediate compound **1**, 70%) was synthesized from phorbol (isolated and identified by our laboratory, HPLC purity: 96.61%). From this compound, 13-decanoyl-20-*O*-trityl-phorbol (inter-

Table 4 Anti-HIV-1 activity and safety index of phorbol-12,13,20-triesters.

No.	R_1	R_2	R_3	Anti-HIV activity (EC_{50}, nmol·L^{-1})	Cytotoxicity (CC_{50}, μmol·L^{-1})	Safety index (CC_{50}/EC_{50})
9a	D	Ac	Ac	> 200 000	> 200	/
9b	E	Ac	Ac	> 200 000	> 200	/
9c	*trans*-Cinnamoyl	*trans*-Cinnamoyl	*trans*-Cinnamoyl	> 200 000	> 200	/
9d	*trans*-Cinnamoyl	Butyryl	Butyryl	55 ± 11.7	> 200	> 3639.67
9e	3-Methoxy-4-acet oxycinnamoyl	Butyryl	Butyryl	293.2 ±186.3	> 200	> 682.24
9f	Ac	Ac	Ac	8955 ± 770.7	> 200	> 22.33
9g	Eicosapentaenoyl	Eicosapentaenoyl	Eicosapentaenoyl	41 740 ± 24 621.5	129.78 ± 23.278	3.12
9h	3-Phenylpropanoyl	Ac	Ac	208.7 ± 53.5	125.08 ± 12.7986	599.47
9i	*trans*-2-Butenoyl	Ac	Ac	2180 ± 989.9	> 200	> 91.74
9j	Eicosapentaenoyl	Ac	Ac	70.5 ± 45.6	54.22 ± 7.2832	1015.99
9k	*trans*-Cinnamoyl	Butyryl	*trans*-Cinnamoyl	469.6 ± 11.2	> 200	> 425.94
9l	*trans*-Cinnamoyl	Butyryl	3,4-Methylene dioxycinnamoyl	671.4 ± 205.6	> 200	> 297.89
9m	3,4-Methylene dioxycinnamoyl	Ac	Ac	210.1 ± 44.9	> 200	> 952.15
AZT				5.4 ± 3.0	> 200	> 26 666.60

Compounds **9c**−**9m** were phorbol esters previously synthesized by our team [14, 15]; D: 3-[6-(12-*O*-hexanoyl phorbol-13,20-diethyl ester)]-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione; E: 3-(4-butyl oleanolic acid)-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione; AZT: zidovudine.

Table 5 Inhibition of syncytia formation induced by HIV-1_{IIIB} and safety index of phorbol derivatives.

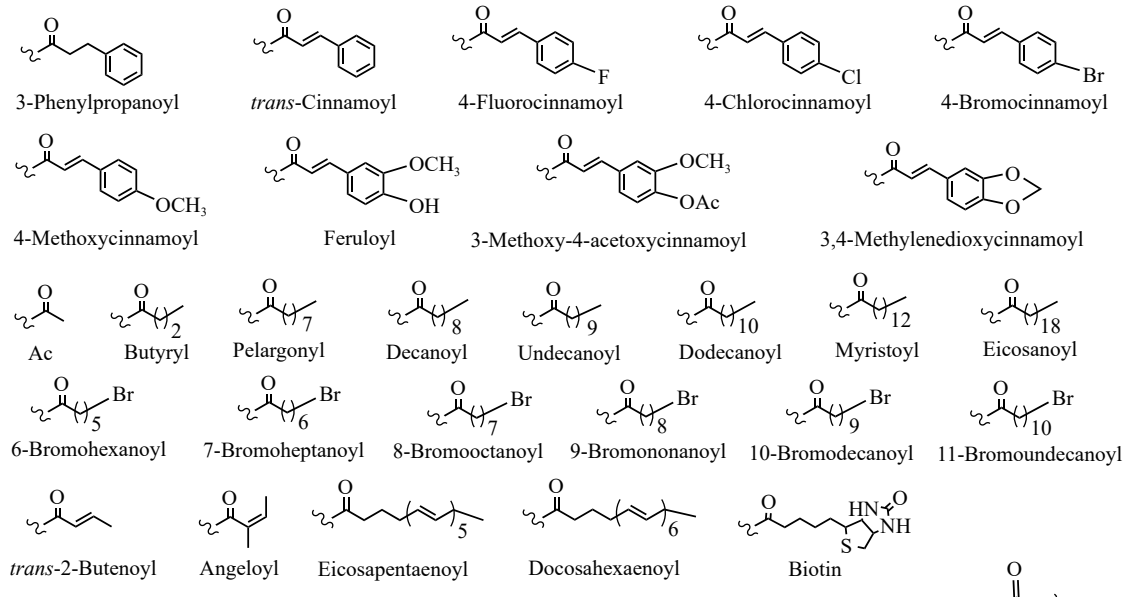
No.	R1	R2	R3	Anti-HIV activity (EC_{50}, nmol·L^{-1})	Cytotoxicity (CC_{50}, μmol·L^{-1})	Safety index (CC_{50}/EC_{50})
1b	Decanoyl	Decanoyl	H	5.2 ± 1.4	106.865 ± 13.9371	20 550.00
2m	Eicosapentaenoyl	Ac	H	60.6 ± 9.6	26.405 ± 11.2076	435.73
2n	Docosahexaenoyl	Ac	H	943.1 ± 63.4	44.120 ± 2.5173	46.78
2q	Eicosapentaenoyl	Butyryl	H	11 66.7 ± 76.1	23.265 ± 3.8537	19.94
3a	3,4-Methyl enedi oxycinnamoyl	Decanoyl	H	26.2 ± 5.1	37.685 ± 1.5627	1438.36
3b	4-Bromocinnamoyl	Decanoyl	H	9.6 ± 5.2	32.335 ± 8.0539	3368.23
3c	4-Fluorocinnamoyl	Decanoyl	H	7.0 ± 2.1	34.240 ± 1.7819	4891.43
3d	4-Methoxycinnamoyl	Decanoyl	H	33.7 ± 2.3	52.715 ± 7.3893	1564.24
3TC				175.8 ± 9.2	> 200	> 1137.66

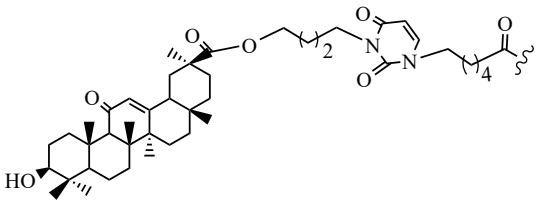
Compounds **2m**, **2n**, and **2q** were phorbol esters derivatives previously synthesized by our team [14, 15]; 3TC: lamivudine.

mediate compound **2**) was obtained with an 80% yield. Additionally, phorbol-13,20-diacetate (intermediate compound **3**) was synthesized from phorbol, achieving a 78% yield (Schemes 1−3). The detailed procedures for the synthesis of intermediate compounds **1**−**3** are available in the Supporting Information.

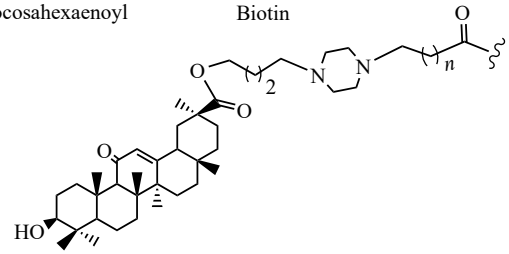
Synthesis of target compounds ***1a***−***1k***, ***2a***−***2h***, ***3a***−***3g***, ***4a***−***4b***, ***5a***, ***6c***, ***10***, *and* ***11***

A solution of 20-*O*-trityl-phorbol (**1**, 0.5 mmol) in 12 mL dichloromethane (DCM) was treated with various acids (nonanoic acid, decanoic acid, hendecoic acid, dodecanoic acid, myristic acid, 6-bromohexanoic acid, 7-bromoheptanoic acid, 9-bromononanoic acid, 10-bromodecanoic acid, 11-bromohendecoic acid, and biotin) in 3.0 equialents (eq). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (1.5 eq) and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (1.0 eq) were added. Chemicals and reagents were purchased from Adamas, HPLC purity 97%−99%, Titan Technology Co., Ltd. (Shanghai, China). After overnight stirring, the reaction was quenched with 20 mL of saturated $NaHCO_3$, and then the mixture was extracted three times with DCM. The combined organic layers were concentrated under reduced pressure and further purified by silica gel column chromatography (petroleum ether: ethyl acetate, 5:1) to yield phorbol-12,13,20-triesters. These triesters were then treated with 10 mL of 3% $HClO_4$/MeOH. After stirring for 1 h, the mixture was neutral-

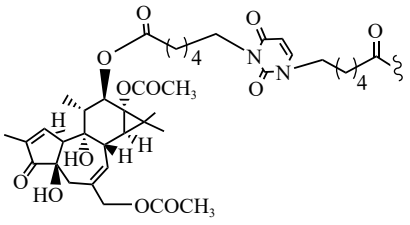




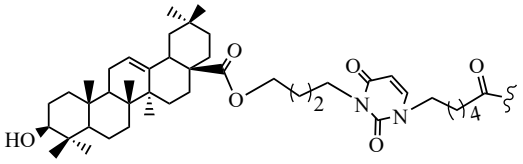
A: 3-(4-Butyl glycyrrhetinic acid)-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione



B: n = 4 1-(4-Butyl glycyrrhetinic acid)-4-(6-oxohexyl)-piperazine
C: n = 7 1-(4-Butyl glycyrrhetinic acid)-4-(9-oxononyl)-piperazine



D: 3-(6-(12-*O*-Hexanoyl phorbol-13,20-diethyl ester))-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione



E: 3-(4-Butyl oleanolic acid)-1-(6-oxohexyl) pyrimidine-2,4(1*H*,3*H*)-dione

Fig. 2 The acyl groups of phorbol esters.

ized with 1 mol·L^{-1} NaOH and then extracted with DCM. The organic layers were concentrated under reduced pressure. The final products were purified by silica gel column chromatography (petroleum ether: ethyl acetate, 3:1 to 1:1) to afford compounds **1a** (80%), **1b** (75%), **1c** (83%), **1d** (75%), **1e** (81%), **1f** (75%), **1g** (65%), **1h** (88%), **1i** (90%), **1j** (86%) as colorless oil, and **1k** (60%) as white amorphous powder, respectively (Scheme 1).

The procedures for the synthesis of target compounds **2a**–**2h** and **3a**–**3g** were similar to those for **1a**–**1k**. Phorbol-12,13,20-triesters were synthesized by esterification of phorbol-13,20-diacetate (**3**, 0.5 mmol) with various acids (6-bromohexanoic acid, 7-bromoheptanoic acid, 8-bromooctanoic acid, 3,4-methylenedioxycinnamic acid, 4-bromocinnamic acid, 4-fluorocinnamic acid, 4-methoxycinnamic acid, and biotin) in 1.5 eq. The triesters were treated with 3% $HClO_4$/MeOH for 12 h. Subsequently, the products were isolated and purified by silica gel chromatography and eluted with (petroleum ether ∶ ethyl acetate, 2 ∶ 1) to afford **2a** (80%), **2b** (83%), **2c** (85%), **2d** (77%), **2e** (82%), **2f** (76%), **2g** (88%), and **2h** (38%) as white amorphous powders (Scheme 3). Phorbol-12,13,20-triesters were synthesized by esterification of phorbol-13,20-diacetate (**3**, 0.5 mmol) and biotin (1.5 eq). Later, these triesters were treated with 0.05 mol·L^{-1} KOH/MeOH for 30 min to obtain compound **5a** (60%) as a white amorphous powder (Scheme 3).

20-*O*-Trityl-13-decanoyl-phorbol (**2**, 0.5 mmol) was reacted separately with 3,4-methylenedioxycinnamic acid, 4-bromocinnamic acid, 4-fluorocinnamic acid, 4-methoxycinnamic acid, 4-chlorcinnamic acid, cinnamic acid, and biotin (1.5 eq) to yield compounds **3a**–**3g**. Reaction mixtures were eluted with petroleum ether and ethyl acetate (5 ∶ 1) by silica gel chromatography to yield **3a** (70%), **3b** (86%), **3c** (90%), **3d** (83%), **3e** (88%), **3f** (86%), and **3g** (48%) as white

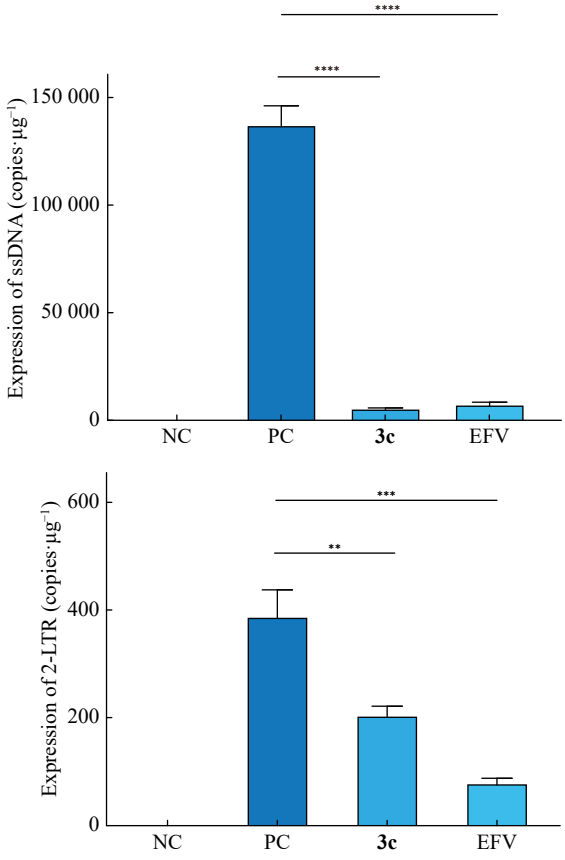


Fig. 3 Effects of compound 3c on the intermediate products of HIV-1 reverse transcription. ssDNA: single-stranded DNA; 2LTR: 2 long terminal repeat; EFV: efavirenz, HIV-1 reverse transcriptase inhibitor, used as a approved drug control; NC: negative control, only C8166 cells, used as a negative control; PC: positive control, only virus infected C8166 cells, without drug treatment, serve as a positive control. Data are presented as means ± SD of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ *vs* positive control group.

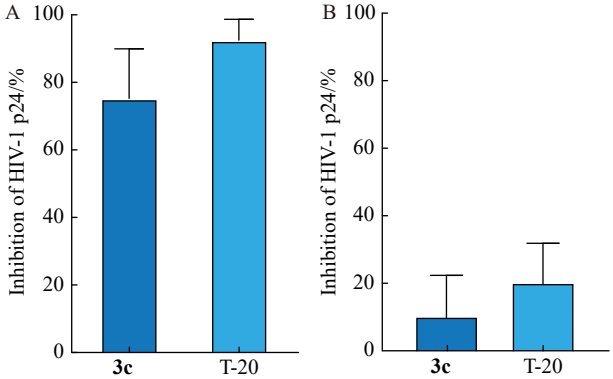


Fig. 4 Effects of compound 3c after the virus had entered the cells. (A) When HIV-1_{IIIB} entered C8166 cells, the cells were treated with compound 3c; (B) After the HIV-1_{IIIB} entered C8166 cells, the cells were treated with compound 3c. T-20: enfuvirtide, HIV-1 entry inhibitor. Data are presented as means ± SD of three independent experiments.

amorphous powders (Scheme 2).

As shown in Scheme 4, compound **6c** (65%, white amorphous powder) was synthesized by esterification of 20-*O*-trityl-phorbol (**1**, 0.5 mmol) and biotin (1.5 eq); compound **4a** (35%, a white amorphous powder) was synthesized by esterification of 20-*O*-trityl-phorbol (**1**, 0.5 mmol), biotin (1.5 eq), and decanoic acid (1.5 eq); compound **4b** (55%, a white amorphous powder) was synthesized similarly by using 20-*O*-trityl-phorbol (**1**, 0.5 mmol) and 6-bromohexanoic acid (1.5 eq), 4-fluorocinnamic acid (1.5 eq).

As shown in Scheme 5, 20-*O*-trityl-4*α*-phorbol and 20-*O*-trityl-4*α*-4-deoxyphorbol were synthesized following a procedure similar to that for 20-*O*-trityl-phorbol. Compounds **10** (75%, colorless oil) and **11** (80%, colorless oil) were separately synthesized by esterification of 20-*O*-trityl-4*α*-phorbol and 20-*O*-trityl-4*α*-4-deoxyphorbol with decanoic acid (3.0 eq), following a similar method to the synthesis of compound **1b**.

*Synthesis of target compounds **4c**, **6a**, **6b**, **9a**, and **9b***

As shown in Scheme 6, 1.2 mmol 6-bromohexanoic acid, 1.5 mmol EDCI and 1.0 mmol DMAP were added into the DCM (12 mL) solution of phorbol-13,20-diacetate (**3**, 0.42 mmol) and stirred overnight; and then the crude product (0.42 mmol), uracil (3 eq), and K_2CO_3 (1 eq) were dissolved in *N*,*N*-dimethylformamide (DMF) and stirred at 60 °C for 12 h to produce intermediate **A** and the compound **9a** (32%) as white amorphous powders. 1.2 mmol 6-bromohexanoic acid or 9-bromononanoic acid, 1.5 mmol EDCI, and 1.0 mmol DMAP were added into the DCM (12 mL) solution of 20-trityl-phorbol (**1**, 0.42 mmol), then stirred overnight and further esterified with acetic anhydride and hydrolyzed C20 protection group to produce crude product. This crude product (0.42 mmol), uracil (3 eq) or piperazine (4 eq), and K_2CO_3 (1 eq) were dissolved in DMF and stirred at 60 or 50 °C for 12 h to produce intermediate $\mathbf{B_1}$−$\mathbf{B_3}$. Oleanolic acid or glycyrrhetic acid (5 mmol), 1,4-dibromobutane (6 eq) and K_2CO_3 (1 eq) were dissolved in DMF and stirred at 60 °C for 12 h to yield intermediate **C**. As shown in Scheme 7, intermediate **A** or $\mathbf{B_1}$−$\mathbf{B_3}$ (0.5 mmol), intermediate **C** (3 equiv), K_2CO_3 (1 eq), and KI (0.2 eq) were dissolved in methyl cyanide or DMF and stirred at 75 °C for 36 h to yield the compounds **4c** (11%), **6a** (35%), **6b** (34%) and **9b** (21%) as white amorphous powders, respectively.

Data of target compounds

Compound **1a**, colorless oil; HR-MS (ESI): *m/z*, Calcd. for $C_{38}H_{60}NaO_8$ $[M + Na]^+$: 667.4186, Found 667.4196. ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.55 (1 H, s, H-1), 5.70 (1H, s, -OH), 5.66 (1 H, s, H-7), 5.37 (1 H, d, *J* = 10.3 Hz, H-12), 4.01 (1 H, d, *J* = 12.8 Hz, H-20), 3.94 (1 H, d, *J* = 12.8 Hz, H-20), 3.24 (1 H, s, H-8), 3.23 (1 H, s, H-10), 2.59 (1 H, d, *J* = 18.9 Hz, H-5*β*), 2.47 (1 H, d, *J* = 19.0 Hz, H-5*α*), 2.33−2.25 (4 H, m, -$COCH_2$- × 2), 2.15−2.12 (1 H, m, H-11), 1.74 (3 H, s, H-19), 1.61−1.57 (4 H, m, -$COCH_2CH_2$- × 2), 1.26−1.23 (23 H, m, -CH_2- × 10, -CH_3), 1.18 (3 H, s, H-17), 1.04 (1 H, d, *J* = 4.7 Hz, H-14), 0.85 (9 H, brs, -CH_3 × 3). ^{13}C NMR (150

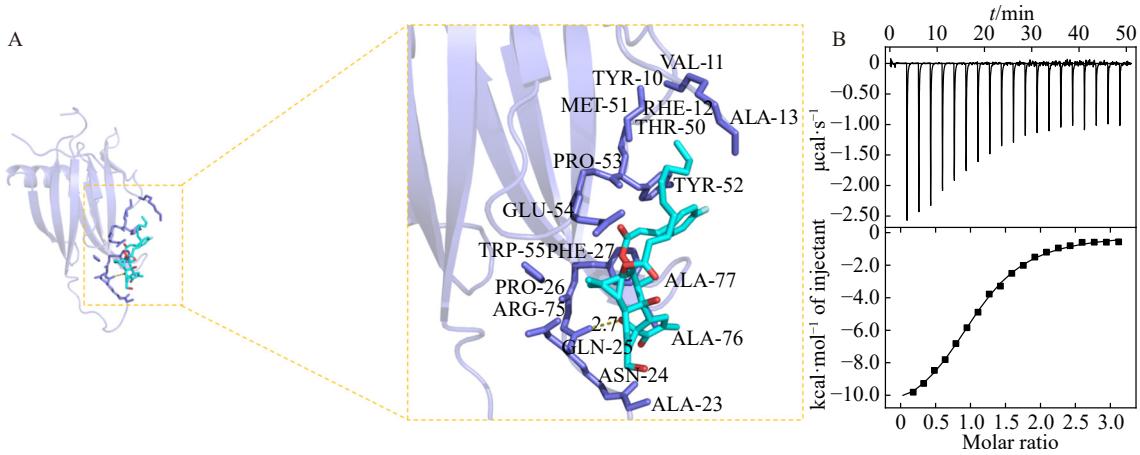


Fig. 5 Effects of compound 3c on PKC. (A) Molecular docking; (B) PKC-δ protein affinity constant (K_d) of compound 3c.

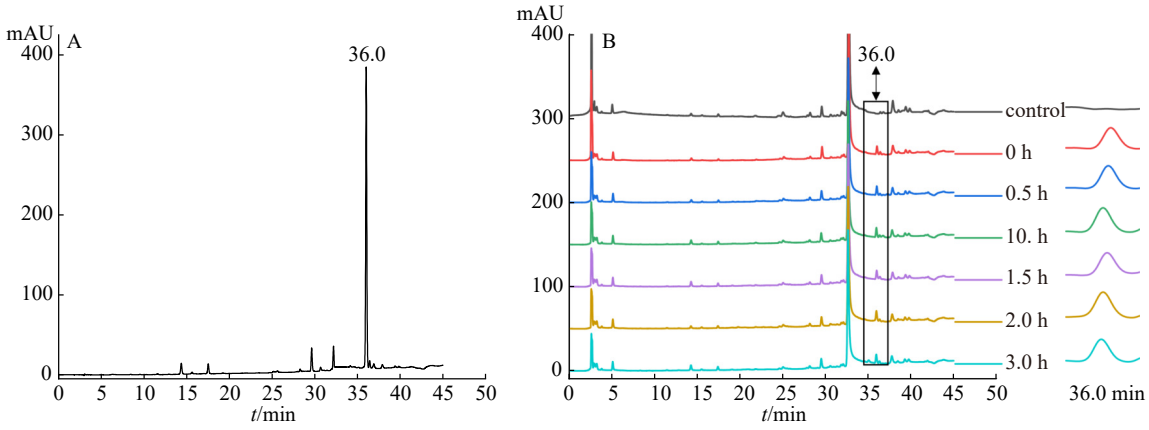


Fig. 6 Stability of compound 3c in plasma *in vitro*. (A) HPLC of compound 3c; (B) HPLC of compound 3c in rat plasma at different reaction time points.

MHz, $CDCl_3$) δ: 209.40, 176.48, 173.70, 160.98, 140.77, 132.89, 129.29, 78.48, 76.68, 73.76, 68.08, 65.51, 56.12, 42.98, 39.05, 38.50, 36.42, 34.67, 34.50, 31.91, 31.88, 29.34, 29.28, 29.26, 29.18 (2 × C), 29.09, 25.81, 25.32, 24.62, 23.94, 22.72 (2 × C), 16.94, 14.52, 14.17, 14.16, 10.17.

Compound **2a**, a white amorphous powder; HR-MS (ESI): *m/z*, Calcd. for $C_{28}H_{39}BrNaO_8$ $[M + Na]^+$: 605.1726, found 605.1713. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.57 (1 H, s, H-1), 5.66 (1 H, d, J = 4.8 Hz, H-7), 5.65 (1 H, s, -OH), 5.35 (1 H, d, J = 10.3 Hz, H-12), 4.02 (1 H, d, J = 12.9 Hz, H-20), 3.96 (1 H, d, J = 12.9 Hz, H-20), 3.40 (2 H, t, J = 6.8 Hz, $BrCH_2$-), 3.24 (2 H, m, H-8, H-10), 2.56 (1 H, d, J = 19.0 Hz, H-5β), 2.48 (1 H, d, J = 19.0 Hz, H-5α), 2.39−2.31 (2 H, m, -$COCH_2$-), 2.16−2.13 (1 H, m, H-11), 2.06 (3 H, s, -$COCH_3$), 1.99−1.95 (2 H, m, $BrCH_2CH_2$-), 1.74 (3 H, d, J = 1.5 Hz, H-19), 1.68−1.63 (2 H, m, -$COCH_2CH_2$-), 1.50−1.45 (2 H, m, -CH_2), 1.23 (3 H, s, H-16), 1.21 (3 H, s, H-17), 1.06 (1 H, d, J = 5.2 Hz, H-14), 0.87 (3 H, d, J = 6.5 Hz, H-18). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 209.27, 176.22, 171.07, 160.86, 140.76, 133.02, 129.24, 78.41, 77.11, 73.79, 68.09, 65.68, 56.19, 43.07, 39.11, 38.61, 36.50, 34.20, 33.61, 32.49, 27.67, 26.00, 24.02, 23.79, 21.16, 16.87, 14.56, 10.24.

Compound **3c**, a white amorphous powder; HR-MS (ESI): *m/z*, Calcd. for $C_{39}H_{51}FNaO_8$ $[M + Na]^+$: 689.3466, found 689.3474. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.62 (1 H, d, J = 16.2 Hz, -CH=CH-), 7.60 (1 H, s, H-1), 7.51 (2 H, dd, J = 8.6, 5.3 Hz, -Ph) 7.08 (2 H, t, J = 8.5 Hz, -Ph), 6.35 (1 H, d, J = 16.0 Hz, -CH=CH-), 5.76 (1 H, s, -OH), 5.71 (1 H, d, J = 4.9 Hz, H-7), 5.51 (1 H, d, J = 10.3 Hz, H-12), 4.05 (1 H, d, J = 12.8 Hz, H-20), 3.99 (1 H, d, J = 12.8 Hz, H-20), 3.29 (1 H, t, J = 5.4 Hz, H-8), 3.27 (1 H, s, H-10), 2.59 (1 H, d, J = 19.0 Hz, H-5β), 2.50 (1 H, d, J = 19.0 Hz, H-5α), 2.42−2.29 (2 H, m, $COCH_2$-), 2.26−2.21 (1 H, m, H-11), 1.77−1.76 (3 H, m, H-19), 1.65−1.61 (2 H, m, -CH_2-), 1.31−1.26 (15 H, m, -CH_2- × 6, H-16), 1.22 (3 H, s, H-17), 1.08 (1 H, d, J = 5.2 Hz, H-14), 0.92 (3 H, d, J = 6.5 Hz, H-18), 0.87 (3 H, t, J = 6.9 Hz, -CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 209.22, 176.67, 166.76, 164.08, 160.96, 143.93, 140.65, 133.02, 130.69, 130.12, 129.38, 117.82, 116.22, 78.42, 77.16, 73.83, 68.17, 65.52, 56.28, 43.35, 39.22, 38.72, 36.66, 34.53, 32.00, 29.54, 29.42, 29.39, 29.23, 26.08, 24.69, 24.00, 22.81, 17.07, 14.63, 14.26, 10.27.

Compound **5a**, a white amorphous powder; HR-MS (ESI): *m/z*, Calcd. for $C_{30}H_{43}N_2O_8S$ $[M + H]^+$: 591.2740, found 591.2827. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.62 (1 H, s, H-1), 5.63 (1 H, d, J = 4.8 Hz, H-7), 5.32 (1 H, d, J = 10.1

Hz, H-12), 4.56−4.45 (1 H, m, -N-CH-), 4.32 (1 H, dd, *J* = 7.6, 4.5 Hz, -N-CH-), 3.97 (2 H, s, H-20), 3.22 (1 H, dt, *J* = 9.6, 5.0 Hz, -S-CH-), 3.16 (1 H, t, *J* = 5.4 Hz, H-8), 3.14 (1 H, s, H-10), 2.94 (1 H, dd, *J* = 12.8, 5.0 Hz, -S-CH_2-), 2.73 (1 H, d, *J* = 12.8 Hz, -S-CH_2-), 2.56 (1 H, d, *J* = 19.2 Hz, H-5*β*), 2.47 (1 H, d, *J* = 18.8 Hz, H-5*α*), 2.41 (2 H, t, *J* = 7.3 Hz, $COCH_2$-), 2.20−2.10 (1 H, m, H-11), 1.77 (3 H, s, H-19), 1.76−1.68 (2 H, m, -CH_2-), 1.63 (2 H, dt, *J* = 13.5, 7.3 Hz, -CH_2-), 1.50 (2 H, dt, *J* = 17.5, 8.6 Hz, -CH_2-), 1.22 (3 H, s, H-16), 1.14 (3 H, s, H-17), 0.97 (3 H, d, *J* = 6.4 Hz, H-18), 0.86 (1 H, d, *J* = 5.8 Hz, H-14). ^{13}C NMR (150 MHz, CD_3OD) *δ*: 210.56, 176.81, 166.12, 160.85, 142.26, 134.53, 130.64, 86.68, 79.62, 74.73, 68.26, 63.43, 62.15, 61.62, 58.68, 57.05, 44.71, 41.03, 40.03, 38.56, 37.69, 35.24, 29.81, 29.51, 27.86, 26.09, 23.58, 17.86, 15.63, 10.23.

Compound **6a**, a white amorphous powder; HR-MS (ESI): *m/z*, Calcd. for $C_{67}H_{105}N_2O_{11}$ $[M + H]^+$: 1113.7718, found 1113.7668. ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.52 (1 H, s, H-1), 5.60−5.58 (2 H, m, H-7, -CH=CH-), 5.27 (1 H, d, *J* = 3.3 Hz, -OH), 4.09−4.06 (2 H, m, -$COOCH_2$-), 3.99−3.92 (3 H, m, H-12, H-20), 3.19−3.17 (2 H, m, H-8, -OCH-), 3.10 (1 H, brs, H-10), 1.72 (3 H, s, H-19), 1.33 (3 H, s, H-16), 1.26−1.09 (18 H, -CH_3 × 5, H-17), 1.00−0.96 (6 H, -CH_3, H-18), 0.76 (3 H, s, -CH_3). ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 209.03, 200.17, 176.79, 176.43, 169.32, 160.44, 140.97, 132.92, 128.79, 128.42, 78.68, 78.35, 77.23, 77.15, 73.45, 67.56, 61.78, 57.89, 56.70, 56.50, 54.90, 52.79 (2 × C), 52.61 (2 × C), 48.39, 45.38, 44.92, 43.96, 43.19, 41.05, 39.08, 38.90, 38.47, 37.71, 37.05, 35.61, 34.27, 32.73, 31.81, 31.08, 29.08, 28.89, 28.85, 28.55, 28.38, 28.08, 27.34, 27.22, 26.75, 26.45, 26.37, 26.31, 26.26, 24.62 (2 × C), 23.77, 23.37, 23.22, 18.65, 17.46, 16.93, 16.34, 15.59, 15.05, 14.16, 10.14.

Compound **9a**, a white amorphous powder; HR-MS (ESI): *m/z*, Calcd. for $C_{64}H_{84}N_2O_{20}$ $[M + Na]^+$: 1223.5515, found 1223.5513. ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.59 (2 H, s, H-1 × 2), 7.07 (1 H, d, *J* = 7.8 Hz, -CH=CH-), 5.70−5.68 (3 H, m, H-7 × 2, -CH=CH-), 5.52 (2 H, -OH × 2), 5.38 (2 H, d, *J* = 10.3 Hz, H-12 × 2), 4.48−4.42 (4 H, m, H-20 × 2), 3.88−3.85 (2 H, m, -NCH_2-), 3.71−3.69 (2 H, m, -NCH_2-), 3.26−3.23 (4 H, m, H-8 × 2, H-10 × 2), 2.55 (2 H, dd, *J* = 19.4, 6.2 Hz, H-5), 2.40 (2 H, dd, *J* = 19.1, 5.6 Hz, H-5), 2.34−2.32 (4 H, m, $COCH_2$- × 2), 2.13−2.10 (2 H, m, H-11 × 2), 2.09 (3 H, s, -$COCH_3$), 2.08 (3 H, s, -$COCH_3$), 2.04 (6 H, s, -$COCH_3$ × 2), 1.77−1.76 (6 H, m, H-19 × 2), 1.70−1.65 (6 H, m, -CH_2- × 3), 1.62−1.58 (2 H, m, -CH_2-), 1.42−1.32 (4 H, m, -CH_2- × 2), 1.22 (3 H, s, H-16), 1.21 (3 H, s, H-16), 1.20 (3 H, s, H-17), 1.20 (3 H, s, H-17), 1.07−1.06 (2 H, m, H-14 × 2), 0.90 (3 H, d, *J* = 6.1 Hz, H-18), 0.86 (3 H, d, *J* = 6.1 Hz, H-18). ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 208.76, 208.72, 173.72, 173.63, 173.31, 173.14, 170.76, 170.74, 163.12, 160.61 (2 × C), 151.25, 142.00, 135.67, 135.64, 133.00, 132.94, 132.52, 132.44, 101.58, 78.08, 78.04, 76.87, 76.72, 73.52, 73.48, 69.35, 69.29, 65.49, 65.48, 56.02 (2 × C), 49.58, 42.94, 42.88, 41.08, 39.21, 39.16, 38.79, 38.69, 36.06, 36.01, 34.32, 34.15, 29.66, 28.68, 27.12, 26.30, 25.87, 25.67, 24.70, 24.49, 23.81, 23.77, 21.06 (2 × C), 20.94 (2 × C), 16.81, 16.75, 14.53, 14.41, 10.12, 10.10.

Compound **10**, colorless oil; HR-MS (ESI): *m/z*, Calcd. for $C_{40}H_{64}NaO_8$ $[M + Na]^+$: 695.4515, found 695.4521. ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.06 (1 H, s, H-1), 5.47 (1 H, d, *J* = 10.3 Hz, H-12), 5.40 (1 H, s, -OH), 5.21 (1 H, s, H-7), 3.92 (2 H, brs, H-20), 3.75 (1 H, d, *J* = 14.1 Hz, H-5*β*), 3.29−3.28 (1 H, m, H-10), 2.37−2.34 (2 H, m, -$COCH_2$-), 2.33−2.25 (3 H, m, H-5*α*, -$COCH_2$-), 2.17 (1 H, brs, -CH_2-), 1.97 (1 H, brs, -CH_2-), 1.89 (1 H, s, H-8), 1.75 (3 H, s, H-19), 1.70−1.64 (3 H, m, H-11, -CH_2-), 1.62−1.55 (2 H, m, -CH_2-), 1.37−1.25 (22 H, m, -CH_2- × 11), 1.23 (3 H, s, H-16), 1.18 (3 H, s, H-17), 1.06 (3 H, d, *J* = 6.4 Hz, H-18), 0.88 (6 H, m, -CH_3 × 2). ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 210.43, 176.28, 173.42, 155.83, 140.62, 131.88, 126.07, 77.93, 76.71, 75.62, 68.62, 65.14, 55.84, 43.06, 40.94, 36.24, 34.68, 34.51, 34.23, 31.99, 31.96, 29.61, 29.48, 29.43, 29.39, 29.37, 29.33, 29.18, 29.14, 25.42, 25.35, 24.61, 24.21, 22.78 (2 × C), 16.61, 14.21 (2 × C), 12.00, 10.69.

Compound **11**, colorless oil; HR-MS (ESI): *m/z*, Calcd. for $C_{40}H_{64}NaO_7$ $[M + Na]^+$: 679.4550, found 679.4577. ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.00 (1 H, s, H-1), 5.41 (1 H, d, *J* = 10.3 Hz, H-12), 5.17 (1 H, s, -OH), 5.07 (1 H, brs, H-7), 3.95 (1 H, d, *J* = 12.5 Hz, H-20), 3.84 (1 H, d, *J* = 12.5 Hz, H-20), 3.45 (1 H, d, *J* = 6.2 Hz, H-10), 3.39 (1 H, d, *J* = 15.5 Hz, H-5*β*), 2.73 (1 H, t, *J* = 7.0 Hz, H-4), 2.41 (1 H, dd, *J* = 15.5, 4.8 Hz, H-5*α*), 2.35−2.18 (5 H, m, -$COCH_2$- × 2, H-8), 1.91 (1 H, brs, H-11), 1.73 (3 H, s, H-19), 1.65−1.50 (6 H, m, -CH_2- × 3), 1.37−1.16 (22 H, m, -CH_2- × 11), 1.15 (3 H, s, H-16), 1.12 (3 H, s, H-17), 1.03 (3 H, d, *J* = 6.3 Hz, H-18), 0.82 (6 H, m, -CH_3 × 2), 0.73 (1 H, d, *J* = 4.9 Hz, H-14). ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 213.09, 176.05, 173.33, 156.11, 143.25, 136.92, 126.15, 78.00, 75.31, 69.12, 64.89, 49.52, 47.29, 43.08, 40.62, 36.99, 34.51, 34.35, 31.81, 31.78, 29.43, 29.29, 29.25, 29.21, 29.15, 29.00, 28.94, 25.19 (2 × C), 25.15, 25.05, 24.42, 24.08, 22.60 (2 × C), 16.33, 14.04 (2 × C), 11.82, 10.37.

Cytotoxicity Assay

C8166 cell suspension (100 μL, 4 × 10^5/mL) was mixed with different drug solutions, establishing triplicate wells for each treatment. Control wells without any drug treatment were also set up, with AZT (Lot: D1115A, MeilunBio, Dalian, China) serving as the positive control drug. The cultures were incubated at 37 °C in a 5% CO_2 environment for 3 d. Post-incubation, cytotoxicity was detected using the MTT assay. The absorbances at 570 and 690 nm were recorded using an ELx800 microplate reader (Bio-Tek Instruments, Winooski, VT, USA). The 50% cytotoxic concentration (CC_{50}) was subsequently calculated [4].

Anti-HIV-1 activity assay

C8166 cells (8 × 10^5/mL) were infected with HIV-1_{IIIB} using a multiplicity of infection (M.O.I) of 0.01. After 4-h infection, cells were washed twice with PBS and then resuspended in fresh medium (4 × 10^5/mL). This suspension was added to 96-well plates with gradient diluted compounds. 3TC (Lot: M20220A, MeilunBio, Dalian, China) was the positive control drug. The plates were incubated at 37 °C in a humidi-

fied incubator with 5% CO_2 for 72 h. Post-incubation, cell culture supernatants were harvested for the subsequent enzyme linked immunosorbent assay (ELISA) [4].

ELISA for HIV-1 p24 antigen

HIV-1 p24 antigen in cell-free culture medium was measured using an antigen capture ELISA assay. Briefly, anti-p24 McAb P6F4 (homemade by our laboratory) was added to 96 well microtiter plates coated with Fc-specific anti-mouse IgG (Merck KGaA, Darmstadt, Germany). Triton X-100 (Merck KGaA, Darmstadt, Germany)-treated cell culture supernatant was then added to the wells. The plates underwent sequential incubations: first with diluted rabbit anti-p24 serum, followed by HRP-labeled goat anti-rabbit IgG (Merck KGaA, Darmstadt, Germany). Finally, the OPD substrate solution was added. The optical density of the plates was read at 490/630 nm using an ELISA reader after terminating the colorimetric reaction by H_2SO_4. The percent inhibition of p24 antigen expression was calculated, and the concentration resulting in a 50% reduction in p24 antigen expression (EC_{50}) was calculated [18, 19].

Inhibition of syncytia formation assay

The cytopathic effect (CPE) of HIV-1 on C8166 cells was measured by the Johnson & Byington method. The Tissue Culture Infectious Dose 50 (TCID 50) was determined according to the Reed & Muench method. A suspension of C8166 cells (4×10^5/mL) was mixed with HIV-1_{IIIB} (2000 TCID 50/well) and treated with different concentrations of the compounds (100 μL each). The positive control drugs used were 3TC and T-20 (Lot: M20220A, Roche Laboratories Inc., Basel, Switzerland). Cultures were incubated at 37 °C in a 5% CO_2 environment for 72 h. The number of syncytia was counted under an inverted microscope, and the concentration causing a 50% reduction in syncytia formation (EC_{50}) was calculated [19, 20].

Detection of HIV-1 replication intermediate products

C8166 cells were treated with either compound **3c** or EFV (Lot: A1212AS, MeilunBio, Dalian, China), followed by infection with HIV-1_{IIIB} for 24 h, Subsequent to the infection, the cells were harvested for total DNA extraction. HIV-1 reverse transcriptional intermediate products (ssDNA and 2LTR) were detected by quantitative PCR on an ABI PRISM 7500 Fast Real-time PCR system (Applied Biosystems, Waltham, MA, USA) using primers and probes of ssDNA (Forward primer: 5′-GCCTCAATAAAGCTTGCCTTGA-3′, reverse primer: 5′-TGACTAAAAGGGTCTGAGGGATCT-3′, probe: 5′-FAM-AGAGTCACACAACAGACGGGCACA-CACTA-TAMRA-3′) and 2LTR (Forward primer: 5′-GCCT-GGGAGCTCTCTGGCTAA-3′, reverse primer: 5′-AGGTA-GCCTTGTGTGTGGTAGATCC-3′, probe: 5′-FAM-TAGT-GTGTGCCCGTCTGTTGTGTGAC-TAMRA-3′). The PCR protocol involved 40 cycles, consisting of pre-denaturation at 95 °C for 2 min, denaturation at 95 °C for 30 s, and and annealing-extension at 60 °C for 30 s [20].

Statistical analysis

The experimental data were visualized using GraphPad Prism 9.0.0 (GraphPad Software, Boston, MA, USA). The EC_{50} and CC_{50} were calculated by the Reed & Muench method. The therapeutic index of the anti-HIV-1 activity was calculated using the formula $TI = CC_{50}/EC_{50}$.

Molecular docking analyses of compound 3c and PKC-δ

Protein Data Bank (PDB, Research Collaboratory for Structural Bioinformatics, https://www.pdbus.org/) was used to obtain the crystal structure of PKC-δ. The 3D structure of compound **3c** was optimized using ChemBio3D Ultra 16.0.0.82 (PerkinElmer, Waltham, USA). This optimization included hydrogenation, charge calculation, and distribution, performed with The Autodock Tool 1.5.6 (ADT, Scripps Research, La Jolla, USA). The molecular docking and estimation of the binding affinities of ligands with proteins were performed using the AutoDock Vina 1.1.2 (The Center for Computational Structural Biology, La Jolla, USA). The final protein-ligand complexes, including cartoon structures and 2D interactions, were visualized using PyMol 2.5.2.0 (DeLano Scientific LLC, San Carlos, USA) [21].

ITC of human-PKC-δ protein binding constants of compound 3c

The PKC-δ protein, expressed and purified *as per* established protocols [22], was assessed using an iTC-200 microcalorimeter (MicroCal, Inc, USA) at 25 °C. For the ITC measurements, the protein solution was prepared in 20 mmol·L^{-1} Tris-Cl (pH 7.5) with 50 mmol·L^{-1} NaCl (ITC buffer), and the concentration of DMSO was adjusted to 0.5%. Compound **3c**, initially prepared in DMSO, was diluted in the ITC buffer to achieve the desired concentrations. The experiment utilized 50 mmol·L^{-1} protein and 1.0 mmol·L^{-1} compound **3c**. The protein was placed in the cell chamber, and compound **3c**, loaded in the syringe, was injected in 20 successive intervals of 150 s each. The obtained data were fitted using the single-site binding model within the origin software package (MicroCal, Inc, USA).

Stability of compound 3c in rat plasma in vitro

Blood samples were obtained from the femoral artery of male rats, anticoagulated with heparin sodium, and centrifuged at 4 °C, 3000 × *g* for 10 min), and the upper plasma was obtained. The stock solution of P63 was prepared at 1 mg·mL^{-1} in methanol. To assess stability, we mixed 198 μL of blank plasma with 2 μL of 1 mg·mL^{-1} compound **3c** methanol solution, and the mixture was then incubated for at 37 °C 0, 0.5, 1.0, 1.5, 2.0, 3.0 h, respectively. 400 μL of cold-ice methanol was added to quench the reactions. Then the samples were vortex-mixed and centrifuged (4 °C, 10 000 × *g*, 10 min). The supernatant was taken, filtered and transferred to vials for HPLC analysis. The blank plasma control group was treated similarly except that 2 μL of methanol instead of compound **3c** was used. HPLC was performed using an Agilent 1260 HPLC system (Agilent Technologies, USA) and a JADE-PAK ODS-AQ column (250 mm × 4.6 mm I.D., 5 μm) (Echway, China) at room temperature. The mobile phase was a mixture of methanol (A) and water (B) with gradient elution (0 min: 30% A, 30 min: 90% A, 45 min: 100% A). The

flow rate was 1 mL·min^{-1}, the detection wavelength was set at 234 nm, and the injection volume was 10 μL. Each sample underwent triplicate analyses [23].

Conclusions

In conclusion, a series of phorbol esters were innovatively designed and synthesized, leveraging the concepts of privileged substructure and pharmacophore merging. These compounds were evaluated for their anti-HIV-1 activities. The findings revealed that the anti-HIV-1 efficacy of phorbol esters varied depending on the nature and position of the esterification groups. Notably, 13-decanoyl-phorbol-12-*trans*-cinnamoyl derivatives and 12,13-didecanoyl-phorbol demonstrated superior anti-HIV-1 activities compared to the control compound, AZT. Particularly significant was the enhanced anti-HIV-1 activity observed in 12-*trans*-cinnamoyl phorbol derivatives substituted with *para* electron withdrawing group (F, Cl, or Br). These derivatives also exhibitedremarkable inhibition activities on syncytia formation with a high safety index. Thus, this study provides evidence that 13-decanoyl-phorbol-12-*trans*-cinnamoyl derivatives with the *para* electron withdrawing groups in the cinnamoyl moiety and 12,13-didecanoyl-phorbol, especially the most active compound **3c**, are promising candidates for further preclinical trials. Compound **3c** not only inhibits HIV-1 replication and itst entry into cells but also shows potential as a natural activator of PKC.

Supporting Information

Supporting information of this paper can be requested by sending E-mails to the corresponding authors.

References

[1] Song YH, Zhang HG, Wang YM, *et al*. Importin KPNA2 confers HIV-1 pre-integration complex nuclear import by interacting with the capsid protein [J]. *Antivir Res*, 2022, **200**: 105289.

[2] Korinek M, Wagh VD, Lo IW, *et al*. Antiallergic phorbol ester from the seeds of *Aquilaria malaccensis* [J]. *Int J Mol Sci*, 2016, **17**(3): 398.

[3] Zhang DD, Zhou B, Yu JH, *et al*. Cytotoxic tigliane-type diterpenoids from *Croton tiglium* [J]. *Tetrahedron*, 2015, **71**(52): 9638-9644.

[4] Chen H, Zhang R, Luo RH, *et al*. Anti-HIV activities and mechanism of 12-*O*-tricosanoylphorbol-20-acetate, a novel phorbol ester from *Ostodes katharinae* [J]. *Molecules*, 2017, **22**(9): 1498.

[5] Tostes JB, Carvalho A, Silva A, *et al*. Phorbol esters from the latex of *Euphorbia umbellata*: bioguided isolation of highly potent HIV-1 latency interupters in virus reservoir cells [J]. *J Nat Prod*, 2021, **84**(5): 1666-1670.

[6] Wang HB, Wang XY, Liu LP, *et al*. Tigliane diterpenoids from the *Euphorbiaceae* and *Thymelaeaceae* Families [J]. *Chem Rev*, 2015, **115**(9): 2975-3011.

[7] Lee HK, Kim HS, Pyo M, *et al*. Phorbol ester activates human mesenchymal stem cells to inhibit B cells and ameliorate lupus symptoms in MRL Fas^{lpr} mice [J]. *Theranostics*, 2020, **10**(22): 10186-10199.

[8] Gulakowski RJ, McMahon JB, Buckheit RW, *et al*. Antireplicative and anticytopathic activities of prostratin, a non-tumor-promoting phorbol ester, against human immunodeficiency virus (HIV) [J]. *Antivir Res*, 1997, **33**(2): 87-97.

[9] Matsuya Y, Yu Z, Yamamoto N, *et al*. Synthesis of new phorbol derivatives having ethereal side chain and evaluation of their anti-HIV activity [J]. *Bioorg Med Chem*, 2005, **13**(14): 4383-4388.

[10] Mekkawy SE, Meselhy MR, Nakamura N, *et al*. Anti-HIV-1 phorbol esters from the seeds of *Croton tiglium* [J]. *Phytochemistry*, 2000, **53**(4): 457-464.

[11] Asada Y, Sukemori A, Watanabe T, *et al*. Isolation, structure determination, and anti-HIV evaluation of tigliane-type diterpenes and biflavonoid from *Stellera chamaejasme* [J]. *J Nat Prod*, 2013, **76**(5): 852-857.

[12] Evans BE, Rittle KE, Bock MG, *et al*. Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists [J]. *J Med Chem*, 1988, **31**(12): 2235-2246.

[13] Ueda R, Suzuki T, Mino K, *et al*. Identification of cell-active lysine specific demethylase 1-selective inhibitors [J]. *J Am Chem Soc*, 2009, **131**(48): 17536-17537.

[14] Li QR. *Preparation and Biological Activity of Phorbol Derivatives* [D]. Soochow University, 2018: 22-65.

[15] Li QR, Zhang XQ, Cui XJ, *et al*. Optimization of preparation process of phorbol and synthesis, characterization and cytotoxicity of its derivatives [J]. *Nat Prod Res Dev*, 2019, **31**(6): 1091-1100.

[16] Silinsky EM, Searl TJ. Phorbol esters and neurotransmitter release: more than just protein kinase C [J]. *Brit J Pharmacol*, 2003, **138**(7): 1191-1201.

[17] Boudreault PL, Mattler JK, Wender PA. Studies on the regio- and diastereo-selective epoxidation of daphnanes and tiglianes [J]. *Tetrahedron Lett*, 2015, **56**(23): 3423-3427.

[18] Liu YY, Liu YP, Wang XP, *et al*. Bioactive daphnane diterpenes from *Wikstroemia chuii* with their potential anti-inflammatory effects and anti-HIV activities [J]. *Bioorg Chem*, 2020, **105**: 104388.

[19] Wang RR, Gao YD, Ma CH, *et al*. Mangiferin, an anti-HIV agent targeting protease effective against resistant strains [J]. *Molecules*, 2011, **16**(5): 4264-4277.

[20] Tian CJ, Li SF, Huang N, *et al*. Daphnane diterpenoids from *Trigonostemon lii* and inhibition activities against HIV-1 [J]. *Nat Prod Bioprospect*, 2020, **10**(1): 37-44.

[21] Kumar KS, Rao AL, Rao MVB, *et al*. Synthesis and hypoglycemic and anti-inflammatory activity screening of novel substituted 5-[morpholino(phenyl)methyl]-thiazolidine-2,4-diones and their molecular docking studies [J]. *Turk J Pharm Sci*, 2019, **16**(4): 380-391.

[22] Fisher HF, Singh N. Calorimetric methods for interpreting protein-ligand interactions [J]. *Methods Enzymol*, 1995, **259**: 194-221.

[23] Chayrov1 RL, Stylos EK, Chatziathanasiadou MV, *et al*. Tailoring acyclovir prodrugs with enhanced antiviral activity: rational design, synthesis, human plasma stability and in vitro evaluation [J]. *Amino Acids*, 2018, **50**: 1131-1143.

Cite this article as: HUANG Xiaolei, TANG Chengrun, HUANG Xusheng, YANG Yun, LI Qirun, MA Mengdi, ZHAO Lei, YANG Liumeng, CUI Yadong, ZHANG Zhenqing, ZHENG Yongtang, ZHANG Jian. Synthesis and anti-HIV activities of phorbol derivatives [J]. *Chin J Nat Med*, 2024, **22**(2): 146-160.