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Synthesis and HIV-1 Inhibitory Activities of Dicaffeoyl and Digalloyl Esters of Quinic Acid Derivatives

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Abstract: Twenty analogues of the anti-HIV-1 integrase (IN) inhibitors dicaffeoylquinic acids (DCQAs) were prepared. Their IC_{50} values for 3'-end processing and strand transfer against recombinant HIV-1IN were determined *in vitro*, and their cell toxicities and EC_{50} against HIV-1 were measured in cells (*ex vivo*). Acetylated or benzylated and/or with cyclohexylidene group compounds exhibited no inhibition of integration in biochemical assays or viral replication in HIV-infected cells, with the exception of **16** and **36**. Removal of these groups, however, correlated with potent inhibition. Compounds **19**, **31**, and **38**, all digalloyls, exhibited the most robust inhibitory performance in biochemical assays as well as in cell culture and less toxicity than other molecules in the current study.

Keywords: Integrase inhibitor, HIV-1, dicaffeoyl, digalloyl, second generation inhibitor, esters, quinic acid, cyclitol, anti-HIV agents, catechol.

INTRODUCTION

Human immunodeficiency virus (HIV-1) is the etiologic agent of acquired immune deficiency syndrome (AIDS), a serious global public health problem. HIV integrase (IN) is one of three enzymes required by HIV to infect a host cell. The other two, reverse transcriptase and protease, have traditionally been exploited as antiviral targets [1]. IN inhibition is relatively novel, and only two U.S. FDA-approved inhibitors, raltegravir (RGV) and elvitegravir (EVG), exist. IN catalyzes the insertion of the HIV cDNA into the host cell DNA, which is essential for the production of progeny viruses. Therapeutic agents that can inhibit this process are effective anti-HIV agents [1-3].

The dicaffeoylquinic acids (DCQAs, for example, compound **1**) and dicaffeoyltartaric acids (DCTAs, for example, compound **2**) Fig. (**1**) are potent and selective inhibitors of IN. They also inhibit HIV-1 replication at nontoxic concentrations [4]. Other derivatives of caffeic acid (**3**) and gallic acid (**4**) condensates with different classes of substrates, specifically carbohydrates and cyclohexanepolyols, also show inhibition of IN [1-9]. It has been proposed that the catechol moiety plays a major role in the inhibition by these compounds by chelation with divalent metals Mg (II) and Mn (II) in the catalytic site of IN [9-11].

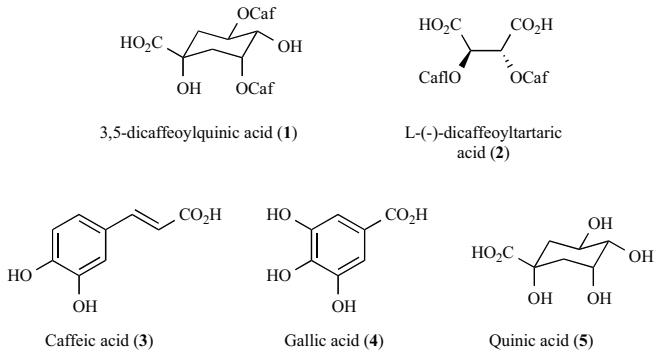
The quinic acid (**5**) Fig. (**1**) is a carboxylated cyclohexanepolyol that is widespread in the plant kingdom, where it is free or in the form of various esters with caffeic acid (**3**) and gallic acid (**4**) Fig. (**1**). It is widely used as an optically-active synthetic precursor in multistep chemical synthesis [12]. Barco and co-workers [12] published a review that reports the different modifications on all carbon atoms from quinic acid. Among all applications, quinic acid is widely used to obtain carbasugar derivatives [13-17].

In this context, we describe the synthesis of dicaffeoyl and digalloyl esters of quinic acid derivatives and their anti-HIV and anti-IN activities.

RESULTS

Synthesis

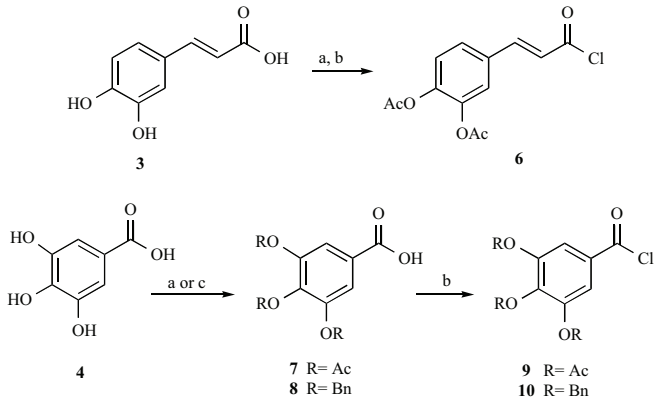
The acyl chlorides **6** and **9** were prepared from caffeic acid (**3**) and gallic acid (**4**), respectively, by acetylation of phenolic hydroxyl groups followed by reaction with oxalyl chloride [18]. The spectroscopic data of **6** and **9** are reported in the literature [18, 19]. The acid chloride **10** was prepared by perbenzylation of gallic acid followed by basic hydrolysis of the ester group and reaction with oxalyl chloride (Scheme **1**) [20, 21].



3,5-dicaffeoylquinic acid (**1**) L-(-)-dicaffeoyltartaric acid (**2**)

Caffeic acid (**3**) Gallic acid (**4**) Quinic acid (**5**)

Fig. (1). Structure of the 3,5-dicaffeoylquinic, L-(-)-dicaffeoyltartaric, caffeic, gallic and quinic acids.



Scheme 1. a: Ac_2O, py, 0°C-r.t.; **b:** $(COCl)_2$, toluene, 0°C-r.t.; **c:** i) NaH, DMF, r.t. for 30 min. then BnBr, 100°C; ii) NaOH, acetone, r.t.; iii) HCl.

The isomers **11** and **12** were obtained from quinic acid (**5**) in four steps in 10% and 57% yield, respectively, as previously reported [22]. These compounds were condensed with **6** or **9** in the presence of pyridine followed by hydrolysis of the acetyl and cyclohexylidene groups using hydrochloric acid in THF to furnish the diesters **17-20** (Scheme **2**).

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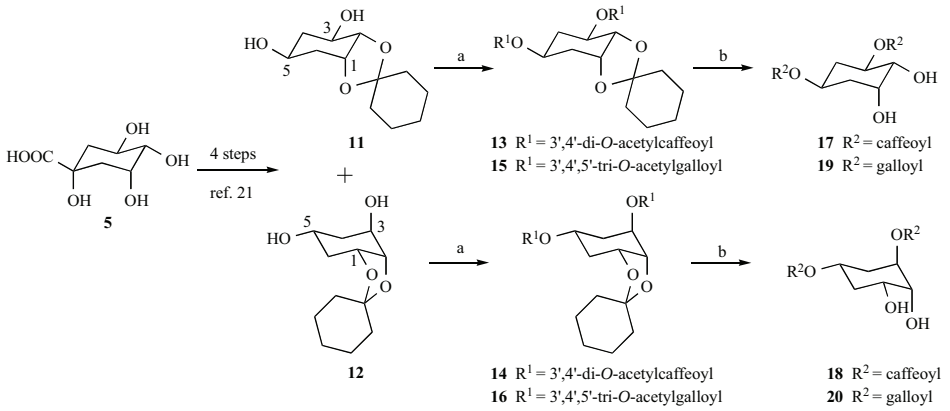


Scheme 2. a: **6** or **9**, py, toluene, 0°C-r.t.; **b:** HCl (1 mol.L^{-1}), THF, r.t.

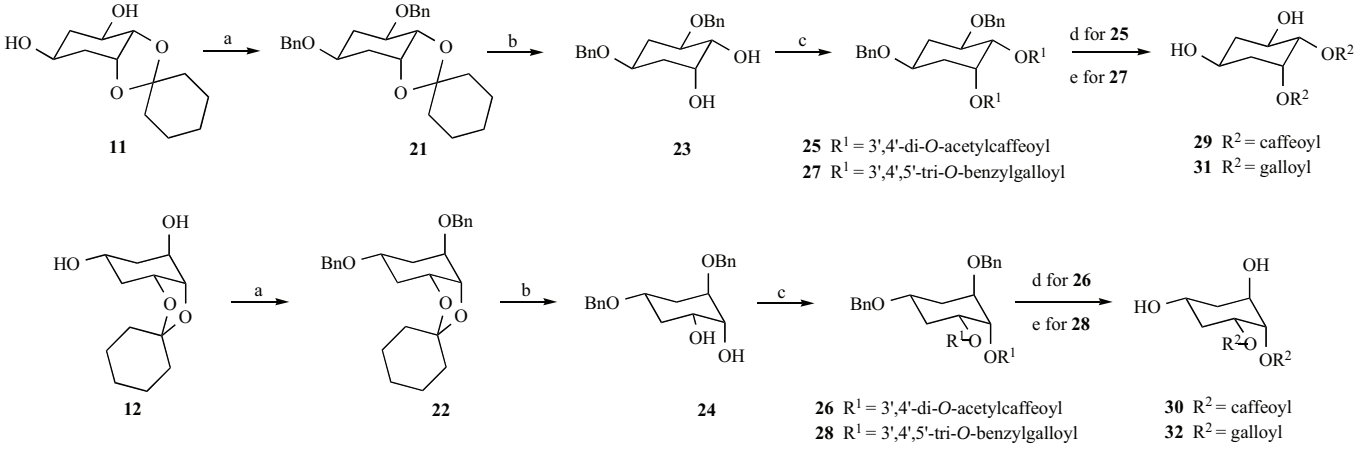
The compounds **21** and **22** were obtained by benzylation of diols **11** and **12**, respectively, by treatment with benzyl bromide using phase transfer conditions, followed by the hydrolysis of the cyclohexylidene group with trifluoroacetic acid (TFA) to give **23** and **24** (Scheme **3**). These diols were subsequently condensed with **6** or **10** in the presence of pyridine leading to **25-28**. Then the compounds **25** and **26** were treated with palladium (0) in methanol leading to compounds **29** and **30**, [23] respectively, while **27** and **28** were hydrogenated in ethyl acetate and presence of palladium 10% leading to compounds **31** and **32**, respectively (Scheme **3**).

The quinic acid (**5**) was transformed into the γ–lactone **33** by reaction with cyclohexanone in the presence of Amberlite IR120 (Scheme **4**) [24]. The γ-lactone was reduced using lithium aluminium hydride (LAH) in THF to give the triol **34** in 90% yield. The primary and secondary hydroxyl groups of **34** were condensed with **6** or **9** in the presence of pyridine to give compounds **35** and **36**, which were treated with hydrochloric acid in THF leading to diesters **37** and **38** (Scheme **4**).

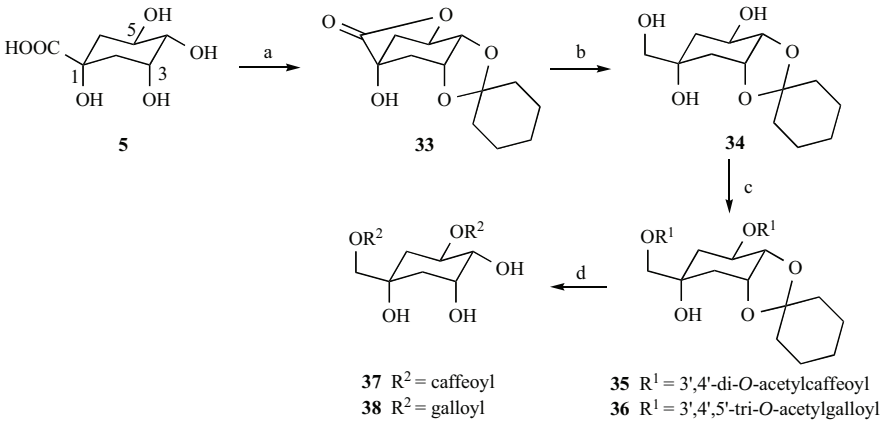
Acetylated Caffeoyl, Galloyl Moieties and Cyclohexylidene Groups Abolish Inhibitory Efficacy

To evaluate the hypothesis that caffeoyl or galloyl side chains linked to quinic acid-derived cyclohexanepolyols could inhibit integration, compounds **13-16** and **35-36** were incubated in the presence of purified, recombinant IN and radiolabeled oligonucleotide substrates. Integrase activities can be measured using several substrates, including the disintegration substrate, which quantifies the reversal of integration, the 3'-end processing (3'-EP) substrate, which quantifies the removal of two oligonucleotides from the 3' end of the oligonucleotide substrate, and the strand transfer (ST) substrate, which quantifies the covalent joining of the processed oligonucleotide into another oligonucleotide. The latter two of these reactions, 3'-EP and ST, quantify what is, in effect, the integration reaction. Following incubation, the reaction products were resolved on a urea-PAGE gel, which was subsequently exposed to phosphorimager analysis. The inhibition of percent product conversion was used to calculate the 50% inhibitory concentration (IC_{50}) Fig. (**2**). Epimers **13** and **14** exhibited no inhibition of integration in biochemical assays or viral replication in HIV-infected cells. When the compounds were deacetylated and the cyclohexylidene group hydrolyzed to yield **17** and **18**, both inhibited *in vitro* integration at low to submicromolar concentrations (Table **1**) and displayed mild selectivity for the 3'-EP and ST reactions. Compound **17** exhibited modest inhibition of integration in HIV-infected cells.

Compounds **15** and **16** possess acetylated digalloyl and cyclohexylidene side chains linked to cyclitol cores. The acetylated **15** did not exhibit any inhibitory activity during biochemical or cellular integration assays. Compound **16**, despite its acetylated hydroxyl groups, inhibited integration *in vitro* at low micromolar concentrations with clear selectivity towards the ST reaction. The removal of the cyclohexylidene and acetyl groups from **15** and **16** to produce the compounds **19** and **20** again correlated with a dramatic increase in anti-IN activity. Compound **20** exhibited modest inhibition of integration *in vitro* and slight ST selectivity, but did not prevent integration in infected cells. Compound **19**, potently inhibited all reactions of integrase *in vitro* at submicromolar concentrations. Compound **19** was also active in cellular assays and inhibited integration at low micromolar concentrations with low cytotoxicity.



Scheme 3. a: BnBr, *n*-Bu_4NBr, CH_2Cl_2, NaOH 50% (m/v), r.t.; **b:** CF_3COOH, H_2O, CH_2Cl_2, reflux; **c:** **6** or **10**, py, toluene, 0°C-50°C; **d:** Pd/C (10%), MeOH, reflux for **25** and **26**; **e:** H_2, Pd/C (10%), EtOAc, r.t. for **27** and **28**.



Scheme 4. a: i) cyclohexanone, toluene, DMF, reflux for 50 h; ii) Amberlite IR 120, reflux; **b:** $LiAlH_4$, THF, r.t.; **c: 6** or **9**, py, toluene, r.t.; **d:** HCl (1 $mol.L^{-1}$), THF, r.t.

Table 1. Biochemical and Antiviral Activities in µM of Active Compounds 16 – 38[a]

Compound	Disinteg, IC_{50} (SD)[b]	3'-EP IC_{50} (SD)[b]	ST IC_{50} (SD)[b]	Anti-HIV_{LAI} Activity EC_{50} (SD)[c]	Cell Toxicity CT_5 (SD)[d]	Cell Toxicity CT_{50} (SD)[d]	TI[e]
RGV[f]	35.2 (9.2)	2.50 (0.64)	0.11 (0.01)	0.004 (0.002)	41.2 (0.8)	291 (15)	72.750
EVG[g]	>100	2.82 (0.13)	0.24 (0.06)	0.001 (0.0001)	8.68(1.62)	24.7(2.63)	24.700
3,5-DCQA[h]	5.53 (0.24)	1.71 (0.06)	1.68 (0.75)	0.41(0.13)	>80.6	>80.6	>196
16	>100	20.6 (1.16)	4.5 (0.55)	>10	6.6 (2.7)	31.3 (3.3)	na[i]
17	7.29 (0.47)	0.71 (0.16)	0.67 (0.9)	149 (9.8)	142 (47.5)	352 (117)	2.47
18	24 (0.7)	2.69 (0.18)	1.58 (0.34)	>264	161 (9.5)	261 (20)	na
19	0.79 (0.05)	0.33 (0.01)	0.24 (0.05)	2.73 (0.37)	69.3 (4.5)	182 (3.6)	66
20	71 (11.4)	34.3 (1.47)	15.1 (1.84)	>34.5	39 (11.3)	136 (9.3)	na
29	52.2 (7.9)	3.78 (3.3)	3.67 (0.48)	>264	111 (42.5)	5415 (6202)	na
30	62.3 (7.9)	5.39 (0.49)	1.83 (0.25)	>66.2	34.6 (3.7)	268 (7.0)	na
31	1.21 (0.8)	0.71 (0.16)	0.46 (0.01)	3.19 (0.5)	82.6 (7.6)	196 (6.6)	61.4
32	11.4 (0.6)	3.33 (0.19)	3.89 (0.41)	>69	33.6 (2.5)	205 (8.0)	na
36	>100	>100	30.5 (1.34)	>61	51.3 (0.65)	75.2 (1.6)	na
37	23.3 (3.2)	6.82 (0.63)	3.99 (0.84)	56.1 (18.3)	56.2 (7.2)	487 (137)	8.66
38	11.4 (0.65)	2.79 (0.17)	3.2 (0.14)	12.2 (1.1)	>207.2	>207.2	>17
29	52.2 (7.9)	3.78 (3.3)	3.67 (0.48)	>264	111 (42.5)	5415 (6202)	na
30	62.3 (7.9)	5.39 (0.49)	1.83 (0.25)	>66.2	34.6 (3.7)	268 (7.0)	na

[a]Values represent the mean of triplicate assays; parenthetical values are 1 standard deviation (SD). [b]IC_{50}, the concentration of compound required to inhibit 50% of enzymatic activity. [c]EC_{50}, the concentration of compound required to inhibit 50% of HIV-induced cytopathic effect. [d]CT_5, the concentration of compound required to inhibit cellular replication by 5% relative to an untreated control, i.e., cells are 95% viable and the concentration of compound is nontoxic. [e]TI, therapeutic index, ratio of the inhibitor concentration that induces 50% toxicity-related cell death (CT_{50}) to the effective dose of a compound (EC_{50}). [f]RGV and EVG exhibit increased potency *in vivo* due integrase higher-order multimer formation. [g]3,5-DCQA: 3,5-dicaffeoylquinic acid. [h]na, not applicable, TI unable to be calculated for this compound. Disinteg.=disintegration reaction, 3'-EP=3'-EP reaction, ST=ST reaction.

Compounds **35** and **36** contain acetylated 3,5-dicaffeoyl or digalloyl, respectively, linkages to the cyclohexylidene modified core, which possesses a hydroxymethylene group (pseudosugar) instead of carboxylic group. Though **35** does not exhibit biochemical or antiviral efficacy, **36** selectively inhibits the ST reaction and is slightly active in cellular assays. This ST-specificity is mimicked by compound **16**, suggesting that digalloyl groups, even when acetylated, are sufficient to inhibit IN.

Benzylated Caffeoyl, Galloyl Moieties Reduce Anti-IN and Antiviral Efficacy

To investigate the hypothesis that acetylated dicaffeoyl or benzylated galloyl residues in the presence of a benzylated cyclitol core would inhibit integration activity, compounds **25-28** were employed in biochemical and cellular assays. Similar to previous experiments involving compounds **13** and **14**, neither compound **25** nor **26**, which possessed acetylated caffeoyl groups, exhibited any inhibition of integration. Likewise, the introduction of benzylated digalloyl residues, compounds **27** and **28** did not produce inhibitory activity. Removal of the benzyl and acetyl groups, however, correlated with potent inhibition.

Compounds **29** and **30** both exhibited low micromolar inhibition of the 3'-EP and ST reactions. Neither compound inhibited viral replication in HIV-infected cells. Compounds **31** and **32** also inhibited integration in biochemical assays at low micromolar concentrations. Indeed, **31** inhibited both 3'-EP and ST reactions at submicromolar concentrations and exhibited low micromolar potency in anti-HIV assays, yet exhibited a CT_5 25-fold higher than the compound's EC_{50}.

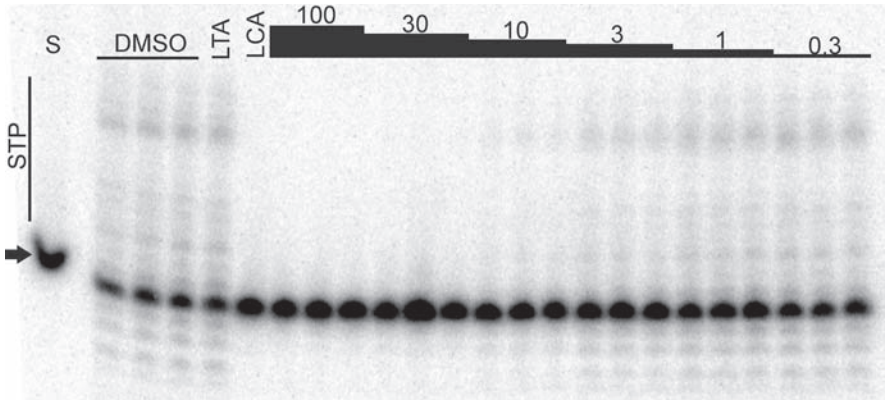


Fig. (2). Compound **17** inhibits HIV-IN mediated strand transfer catalysis. 100 nM HIV IN was incubated for one hour at 37˚C in presence of 0.1 pmol ^{32}P-labelled oligonucleotide end-processed substrate homologous to the viral long-terminal repeat region, reaction buffer, and decreasing μM concentrations of the inhibitor, compound **17**. Reactions were stopped with the addition of 18 mM EDTA and formamide. Products were resolved via denaturing urea-PAGE. Phosphorimager analysis was used to quantify percent-product conversion, and the IC_{50} was obtained with CalcuSyn software (Biosoft, Cambridge, UK). STP: Strand transfer products. Arrow: Strand transfer substrate. S: Reaction performed in the absence of IN. DMSO: Reactions performed in the presence of inhibitor solvent, 100% dimethylsulfoxide. LTA: Denotes addition of 25 μM L-tartaric acid, an inactive analogue of L-chicoric acid [25]. LCA: Denotes addition of 25 μM L-chicoric acid [25]. 100-0.3: Denotes μM concentration of compound **17** in which reactions were incubated.

3,5 Digalloyl, But Not Dicaffeoyl, Linkages Inhibit Integration

Of those compounds that inhibited integration *in vitro*, **17-20**, **29-32**, and **36-38**, only five, **17**, **19**, **31**, **37**, and **38** prevented HIV-induced cytopathic effect in cells. Both **17** and **37** have caffeoyl or caffeoyloxymethyl moieties linked to the 3 and 5 carbon atoms of the core molecule. The 3,5-linkage proved critical to caffeoyl-based compounds' inhibition of HIV replication, as no other arrangement of caffeoyl pharmacophores around the cyclitol core was effective in cellular assays. Compounds involving the arrangement of galloyls were more pronounced in their antiviral performance and exhibited less cytotoxicity. Compounds **19** and **38** are comprised of galloyl or galloyloximethyl groups linked to the 3 and 5 cyclitol carbon atoms and **31** contains 1,2-linkages of the same. These compounds inhibit integration, *in vitro* and *ex vivo*, at submicromolar and low micromolar concentrations, respectively. Compounds that consisted of galloyls linked to other carbon atoms, for example **32**, did not possess inhibitory activity in cellular assays.

Raltegravir-Resistant IN Remains Susceptible to Compounds 17, 19, and 31

To compare the novel cyclitols with current IN inhibitors, both RGV and EVG were investigated for their inhibition of IN catalysis and viral replication. RGV was approved by the FDA in 2007, and EVG is currently enrolled in extended Phase III clinical studies. Both inhibitors are selective for the ST reaction and exhibit low-nanomolar efficacy *in vivo*. Accordingly, neither RGV nor EVG was capable of inhibiting the disintegration reaction at attractive concentrations (Table **1**). In addition, RGV and EVG exhibited micromolar potency for the inhibition of the 3'-EP reaction. Only in the ST reaction, as well as *in vivo*, was nanomolar potency observed for either drug.

Due to their persistent efficacy in biochemical and cellular assays, compounds **17**, **19**, and **31** were examined for their inhibition of integration performed by IN that exhibited mutations commonly identified as RGV-resistant (Table **2**). These IN mutations included Q148H, N155H, and the double mutant G140S+Q148H. Similar to previous biochemical assays, each IN mutant was examined for its inhibitor susceptibility in the disintegration, 3'-EP, and ST reactions. Q148H, G140S+Q148H, and N155H IN mutants were not resistant to the inhibitory effects of **17**. Q148H was slightly resistant to **19** in the 3'-EPST assay, and N155H was slightly resistant to inhibition by **19** in ST assay. No IN mutant exhibited >10-fold resistance to inhibition by **31**. These data indicate that cyclitol-based compounds are effective at prohibiting integration by highly drug-resistant IN variants.

DISCUSSION AND CONCLUSION

Initial studies employing quinic acid as the inhibitory molecule for integration concluded that the compound was ineffective at prohibiting integrase activity [1, 25]. Similar results have been obtained for chlorogenic acid [10, 25]. While ineffective alone, both of these compounds, when used as scaffolds for hydroxylated-phenyl moiety attachment, potently inhibit integration in biochemical assays [3, 25, 26]. Indeed, DCQAs and DCQA-like compounds comprised the bulk of early structure-activity-relationship (SAR) studies aimed at discovering potential anti-IN leads [10].

Multiple SAR studies have determined that the inhibitory activities of DCQAs depend upon a quinic acid core possessing at least one free carboxyl and a bisphenol [25]. Complete acetylation, methylation, or benzylation of the caffeoyl hydroxyls abolishes anti-IN as well as antiviral activity [1, 26]. Partial acetylation or methylation of the hydroxyls restores minimal inhibitory behavior in biochemical contexts, though such compounds remain inactive in cellular assays [3]. Stereoisomers of DCQAs perform similarly; [26] the same is not true for more recent inhibitory molecules [27]. Examination of the ester linkages revealed that substitution with diamides does not reduce potency, [3, 26] though increasing the length of the catechol's poly-carbon chain linker correlates with a precipitous decline of inhibitory efficacy [3]. Finally, cyclohexanediol cores, a category that includes all compounds in the current study, improve the inhibitory performance of biscatechols at the expense of antiviral potency [28].

Similar to previous reports, acetylation or benzylation of the biscatechol groups abolished inhibitory activity for all compounds analyzed (Table **I**), with the exception of **16** and **36**. Also similar to previous findings, [4, 26] the removal of these groups restored potency. The compounds **17-20** and **31** exerted anti-IN activity. This inhibitory activity persisted in the absence of a carboxylated quinic acid, previously identified as essential for inhibition [25, 26]. Indeed, a singular result of this study is the demonstration that IN catalytic inhibition does not require the presence of hydroxylated quinic acid. The carboxyl group of the quinic acid and the diacid moeties of such compounds as LCA that exhibit low micromolar potency have been predicted, through modeling studies, [29] to

Table 2. Biochemical Activities in μM[a] of Compounds 17, 19, and 31 Against Drug-Resistant IN

IN Mutant	Disinteg. IC_{50} (SD)	3'-EP IC_{50} (SD)	ST IC_{50} (SD)	Inhibitory Compound
Reference	7.29 (0.47)	0.71 (0.16)	0.67 (0.9)	**17**
Q148H	0.64 (0.14)	0.91 (0.08)	5.64 (1.56)†	**17**
G140SQ148H	0.63 (0.17)	6.28 (1.29)†	1.75 (0.5)†	**17**
N155H	1.24 (0.24)	1.25 (0.31)	0.32 (0.04)	**17**
Reference	0.7 (0.05)	0.33 (0.01)	0.24 (0.05)	**19**
Q148H	0.16 (0.02)	2.31 (0.5)	0.84 (0.17)†	**19**
G140SQ148H	0.26 (0.02)	1.13 (0.11)†	1.37 (0.61)†	**19**
N155H	0.26 (0.02)	0.25 (0.04)	5.75 (3.36)	**19**
Reference	1.21 (0.8)	0.71 (0.16)	0.46 (0.01)	**31**
Q148H	0.73 (0.04)	0.99 (0.14)	70.1 (13.3)†	**31**
G140SQ148H	0.94 (0.07)	2.72 (0.17)†	3.04 (0.96)†	**31**
N155H	1.03 (0.15)	1.29 (0.29)	1.24 (0.56)	**31**

[a]Values represent the mean of triplicate assays; parenthetical values are 1 standard deviation (SD). †Assays performed in the presence of 350 nM IN due to diminished catalytic activity of IN mutants. 350 nM reference IN micromolar 3'-EP IC_{50}(SD) for compounds **17**, **19**, and **31** are, respectively, 5.22 (0.54), 0.88 (0.14), and 2.95 (0.64). 350 nM reference IN ST IC_{50}(SD) for compounds **17**, **19**, and **31** are, respectively, 1.76 (0.12), 4.06 (0.78), and 16.02 (7.99). Disinteg.=disintegration reaction, 3'-EP=3'-EP reaction, ST=ST reaction.

interfere with IN's requisite divalent metal positioning. All of the free-catechol compounds that exhibited potency (**17-20**, **31**, **37**, and **38**), while absent carboxyls, do possess consecutive hydroxyl groups in the 1,2 (**17-20**), 3,5 (**31**) or 1,3,4 (**37, 38**) positions. It is possible that these hydroxyl groups perturb active site architecture, either by hydrogen bonding to the active site residues or to the divalent metals themselves, [11, 30] of an order sufficient to disrupt catalysis.

Compounds **19**, **31**, and **38** exhibited the most robust inhibitory performance in biochemical assays as well as in cell culture. Indeed, these compounds were the only molecules evaluated that prevented viral replication at concentrations substantially lower than those at which they exerted minimal toxicity. While TIs of 66 are unsuitable for actual therapeutic application, these values do render these molecules attractive as scaffolds for further modification. Galloyl-based biscatechols generally present with greater inhibition of integration as well as greater cellular toxicity [3, 6, 10, 27]. Compounds **19**, **31**, and **38** exhibited less toxicity than other molecules in the current study, perhaps due to the lack of the carboxylated cyclohexanepolyol core.

Galloyl linkages have also been described as reducing IN selectivity, [1] a feature consistent with the results obtained in this study. Compounds **19**, **31**, and **38** prevent IN biochemical activities at submicromolar concentrations; however, they exert little or no selectivity for any of the three reactions IN is capable of performing. This selectivity could perhaps be restored with the addition of a third galloyl group, as trigallic acids linked to a glucose scaffold strongly and selectively inhibit HIV-IN [27]. Additionally, galloyl groups linked to pyrimidine cores, which resemble the molecular geometry of **19** and **31**, also inhibit IN [28]. Combined, these data suggest that galloyl-modified molecular scaffolds present an attractive base for future SAR studies.

The anti-IN activity of compounds **16** and **36** was surprising, as the compounds not only possess acetylated digalloyl groups, but a cyclohexane group bound to the 3,4 or 1,2 hydroxyls, respectively. Modification of the cyclitol core does not typically result in favorable performance in inhibitory studies, [3, 5, 25] though highly-negatively charged linkages, such as trifluorocarbons, can enhance anti-IN activity [28]. The acetylation of the galloyls would render the hydrogen-bonding potential, and thus binding affinity, of the compound within the IN active site relatively inert. Therefore, another mechanism must exist.

While many inhibitory assays are performed in the presence of Mg^{2+}, the biochemical assays completed herein were performed in the presence of Mn^{2+} as the requisite metal cofactor. Initial descriptions of the anti-IN potency of DCQA compound were based upon biochemical assays performed in the presence of Mn^{2+} [1, 25]. Hence, Mn^{2+} was utilized herein as an internal control. Reports describe increased potency of anti-IN compounds, such as DCQAs or RGV, in the presence of Mg^{2+} [30-32]. However, IN catalysis is exquisitely sensitive to reaction conditions in the presence of Mg^{2+} and consequently exhibits lower specific activity [32, 33]. Thus, diminished enzymatic activity reduces the concentration of anti-IN compounds necessary for prescribed inhibition, which can account for the enhanced "potency" of drugs in the presence of Mg^{2+}. IN is far more catalytically robust in Mn^{2+} and thus requires more inhibitor present to elicit inhibition [33]. Those compounds that exhibited the greatest potency *in vitro* were also the most potent *in vivo*, which provided a useful comparison to the generated biochemical data. These data indicate that the selection of metal ion by IN does not alter the relative inhibitory profile of the experimental compounds described herein.

The data contained herein continue to refine our understanding of the essential DCQA and digalloylquinic acid (DGQA) moieties involved in inhibition of HIV IN. The previous finding that a free carboxylic acid moiety is necessary for DCQA and DGQA-based inhibition of HIV-IN is no longer supported. Furthermore, these data confirm the robust activity of DGQAs and present three compounds, **19**, **31**, and **38** that retain both potent inhibitory profiles and attractive TIs. Finally, the DCQA **17** and DGQAs **19** and **31** effectively inhibited IN mutants resistant to two IN inhibitors, RGV and EVG. Combined, these data present attractive scaffolds for future SAR evaluation and chemical modification.

EXPERIMENTAL SECTION

Chemical Syntheses. General

The solvents were pretreated, when necessary, according to the appropriate standard procedures before being used. The compounds were purified by column chromatography on silica gel (70-230 mesh ASTM) with visualization under UV light and H_2SO_4 charring. All reaction mixtures were stirred magnetically. The hydrogenolysis reactions were performed on an A16CA 4L hydrogenation apparatus. The melting points were recorded on a MQAPF-

Microquímica. NMR spectra were obtained with a BRUKER AVANCER DRX/300 spectrometer using tetramethylsilane as the internal standard. IR spectra were recorded on a BOMEM-FTIR MB-120 spectrometer. Mass spectra were recorded on a KRATOS MS-80 spectrometer. Optical rotations were measured on a Bellingham Stanley ADP410 polarimeter.

General Procedure to Prepare 13-16

To a solution of **11** or **12** (1.14 g, 5 mmol) in pyridine (10 mL) was added at 0°C a solution of **6** or **9** (20 mmol) in toluene (40 mL). The reaction mixture was stirred for 24 h at room temperature. Then the mixture was acidified with HCl (4 mol.L^{-1}) until pH 3 and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 / MeOH) leading to compounds **13-16**.

(1*R*,2*S*,3*R*,5*S*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-(3',4'-di-*O*-acetyl)-caffeoyl-1,2,3,5-cyclohexanetetrol (**13**): 57% (2.1 g; 2.9 mmol); White solid; mp 87-89°C; $[\alpha]_D$: -40.0 (*c* 0.2; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.31 (s, 12 H), 1.20-2.60 (m, 14 H), 4.16 (t, *J* 5.9 Hz, 1H), 4.49 (s, 1H), 5.17 (m, 1H), 5.31 (m, 1H), 6.28 (t, *J* 16.0 Hz, 2H), 7.20-7.40 (m, 6H), 7.61 (d, *J* 16.0 Hz, 1H), 7.65 (d, *J* 16.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.5, 20.6, 23.7-32.8, 34.7, 37.5, 66.8, 72.0, 72.5, 75.8, 110.0, 119.1, 122.6-143.2, 143.5, 165.5, 167.9, 168.0; HRMS calcd for $C_{38}H_{40}O_{14}Na$ [M + Na^+] 743.2316, found 743.2337.

(1*R*,2*S*,3*R*,5*R*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-(3',4'-di-*O*-acetyl)-caffeoyl- 1,2,3,5-cyclohexanete-trol (**14**): 56% (2.0 g; 2.8 mmol); White solid; mp 79-81°C; $[\alpha]_D$: +30 (*c* 0.2; CH_2Cl_2); (300 MHz, $CDCl_3$): δ (ppm) 2.31 (s, 12H), 1.39-2.20 (m, 14H), 4.12 (t, *J* 5.0 Hz, 1H), 4.42 (m, 1H), 5.23 (s, 1H), 5.52 (s, 1H), 6.38 (d, *J* 15.8 Hz, 1H), 6.41 (d, *J* 15.8 Hz, 1H), 7.20-7.45 (m, 6H), 7.64 (d, *J* 15.8 Hz, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ (ppm) 20.8, 20.9, 23.9-32.1, 35.3, 38.3, 67.5, 70.6, 72.6, 75.3, 110.1, 119.2, 119.6, 122.9-143.7, 143.8, 165.8, 166.0, 168.2, 168.3; ESI MS: m/z 759.1 ($M+K^+$).

(1*R*,2*S*,3*R*,5*S*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-(3',4',5'-tri-*O*-acetyl)-galloyl-1,2,3,5-cyclohexanete-trol (**15**): 50% (2.0 g; 2.5 mmol); White solid; mp 89-91°C; $[\alpha]_D$: -40.0 (*c* 0.2; CH_2Cl_2); ^{1}H NMR: (300 MHz, $CDCl_3$): δ (ppm) 2.29 (s, 18H), 1.50-2.66 (m, 14H), 4.19 (t, *J* 5.3 Hz, 1H), 4.49 (s, 1H), 5.29 (s, 1H), 5.42 (s, 1H), 7.75 (s, 2H), 7.81 (s, 2H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ (ppm) 20.3, 20.7, 23.8-33.1, 34.9, 37.7, 68.1, 72.7, 73.3, 75.9, 110.4, 122.3-143.6, 163.4, 166.5, 167.8; HRMS calcd for $C_{38}H_{40}O_{18}Na$ [M + Na^+] 807.2112, found 807.2126.

(1*R*,2*S*,3*R*,5*R*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-(3',4',5'-tri-*O*-acetyl)-galloyl-1,2,3,5-cyclohexanete-trol (**16**): 45% (1.8 g; 2.3 mmol); White solid; mp 76-78°C; $[\alpha]_D$: -10.0 (*c* 0.2; CH_2Cl_2); ^{1}H NMR: (300 MHz, $CDCl_3$): δ (ppm) 2.29 (s, 18H), 1.50-2.23 (m, 14H), 4.17 (t, *J* 5.5 Hz, 1H), 4.42 (m, 1H), 5.37 (m, 1H), 5.54 (m, 1H), 7.77-7.81 (m, 4H); ^{13}C NMR: (75 MHz, $CDCl_3$): δ (ppm) 20.3, 20.8, 23.9-35.2, 38.3, 38.4, 68.6, 71.8, 72.6, 75.4, 110.2, 122.5-143.7, 163.6, 166.5, 167.7, 167.8.

General Procedure to Prepare 17-20

To a solution of **13**, **14**, **15** or **16** (2.5 mmol) in THF (25 mL) was added an aqueous solution of 1 mol.L^{-1} HCl (90 mL). The reaction mixture was stirred for 10 days at room temperature, then was saturated with solid NaCl and the aqueous phase extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) leading to compounds **17-20**.

(1*R*,2*S*,3*R*,5*S*)-3,5-Di-*O*-caffeoyl-1,2,3,5-cyclohexanetetrol (**17**): 70% (0.8 g; 1.8 mmol); White solid; mp 117-119°C; $[\alpha]_D$: -10.0 (*c* 0.2; CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.64 (m, 4H), 4.63 (dd, *J* 9.0 and 2.8 Hz, 1H), 5.04 (m, 1H), 6.05 (m, 1H), 6.15 (m, 1H), 6.25 (d, *J* 15.9 Hz, 1H), 6.27 (d, *J* 15.9 Hz, 1H), 6.82-7.16 (m, 6H), 7.54 (d, *J* 15.9 Hz, 1H), 7.57 (d, *J* 15.9 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 35.0, 36.4, 68.1, 69.2, 72.1, 73.7, 115.3-148.8, 166.8, 167.3; HRMS calcd for $C_{24}H_{24}O_{10}Na$ [M + Na^+] 495.1267, found 495.1268.

(1*R*,2*S*,3*R*,5*R*)-3,5-Di-*O*-caffeoyl-1,2,3,5-cyclohexanetetrol (**18**): 55% (0.7 g; 1.4 mmol); White solid; mp 148-150°C; $[\alpha]_D$: -10.0 (*c* 0.2; CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.90-2.04 (m, 4H), 3.82 (m, 1H), 3.94 (m, 1H), 5.06 (m, 1H), 5.20 (m, 1H), 6.22 (d, *J* 15.7 Hz, 1H), 6.26 (d, *J* 15.9 Hz, 1H), 6.73-7.04 (m, 6H), 7.52 (d, *J* 15.9 Hz, 1H), 7.54 (d, *J* 15.9 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 31.6, 34.8, 68.4, 69.4, 70.3, 73.1, 115.0-149.8, 168.2, 168.8; HRMS calcd for $C_{24}H_{24}O_{10}Na$ [M + Na^+] 495.1267, found 495.1288.

(1*R*,2*S*,3*R*,5*S*)-3,5-Di-*O*-galloyl-1,2,3,5-cyclohexanetetrol (**19**): 45% (0.5 g; 1.1 mmol); Colorless oil; $[\alpha]_D$: +266.7 (*c* 0.24; CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.20-2.48 (m, 4H), 3.77 (dd, *J* 9.1 and 2.7 Hz, 1H), 4.18 (m, 1H), 5.27-5.36 (m, 2H), 7.03 (s, 2H), 7.09 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 35.8, 36.8, 68.3, 69.8, 72.4, 74.1, 109.9-146.1, 166.2, 166.7.

(1*R*,2*S*,3*R*,5*R*)-3,5-Di-*O*-galloyl-1,2,3,5-cyclohexanetetrol (**20**): 50% (0.6 g; 1.3 mmol); White solid; mp 138-140°C; $[\alpha]_D$: -72.7 (*c* 0.11; CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.26-1.89 (m, 4H), 3.89-3.97 (m, 2H), 5.22-5.30 (m, 2H), 7.01-7.06 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 35.2, 38.0, 64.5, 68.6, 71.7, 74.0, 110.2-146.7, 167.3, 168.0; HRMS calcd for $C_{20}H_{20}O_{12}Na$ [M + Na^+] 475.0852, found 475.0862.

Preparation of 21 and 22

To a solution of **11** or **12** (2.28 g, 10 mmol) in dichloromethane (40 mL) was added tetrabutylammonium bromide (0.65 g, 2 mmol), sodium hydroxide 50 % v/v (20 mL) and benzyl bromide (4.79 mL, 40 mmol) at room temperature. The reaction mixture was stirred for 96 hours at room temperature and then was extracted with dichloromethane (2 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc / hexane) to give the corresponding dibenzyl ethers.

(1*R*,2*S*,3*R*,5*S*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**21**): 90% (3.7 g; 9.0 mmol); Colorless oil; $[\alpha]_D$: +50.0 (*c* 0.16; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 0.80-1.70 (m, 2H), 2.30-2.50 (m, 2H), 3.50 (m, 1H), 3.72 (m, 1H), 4.02 (t, *J* 6.6 Hz, 1H), 4.38 (m, 1H), 4.66 (s, 2H), 4.68 (d, *J* 12.3 Hz, 1H), 4.76 (d, *J* 12.3 Hz, 1H), 7.29-7.33 (m, 10 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 23.9-34.9, 35.4, 38.1, 70.9, 71.5, 72.0, 73.7, 79.8, 109.3, 127.7-138.8; HRMS calcd for $C_{26}H_{32}O_4Na$ [M + Na^+] 431.2198, found 431.2199.

(1*R*,2*S*,3*R*,5*R*)-1,2-*O*-Cyclohexylidene-3,5-di-*O*-benzyl-1,2,3,5-cyclohexane-tetrol (**22**): 53% (2.2 g; 5.3 mmol); Colorless oil; $[\alpha]_D$: +29.2 (*c*, 0.48; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.19-2.01 (m, 14H), 3.73 (m, 1H), 3.97 (m, 1H), 4.05 (m, 1H), 4.28 (m, 1H), 4.40-4.67 (m, 4H), 7.30 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 23.9-33.3, 35.5, 38.2, 70.1, 71.1, 73.1, 75.0, 77.6, 109.3, 127.5-138.8; HRMS calcd for $C_{26}H_{32}O_4Na$ [M + Na^+] 431.2198, found 431.2201.

Preparation of 23 and 24

To a solution of **21** or **22** (2.05 g, 5 mmol) in dichloromethane (20 mL) was added TFA (2.5 mL) and water (2.0 mL). The reaction

mixture was stirred for 96 hours under reflux. After this time the mixture was cooling, water was added (60 mL) and extracted with dichloromethane (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane) giving **23** and **24**, respectively.

(1*R*,2*S*,3*R*,5*S*)-3,5-Di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**23**): 72% (1.2 g; 3.6 mmol); Colorless oil; $[\alpha]_D$: +140 (*c* 0.1; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.21-1.42 (m, 2H), 2.33-2.41 (m, 1H), 2.54-2.60 (m, 1H), 2.62 (s, 1H), 2.91 (s, 1H), 3.52 (dd, *J* 9.2 and 3.1 Hz, 1H), 3.59-3.67 (m, 1H), 3.79-3.86 (m, 1H), 4.14 (m, 1H), 4.44 (d, *J* 11.2 Hz, 1H), 4.55 (s, 2H), 4.70 (d, *J* 11.2 Hz, 1H), 7.25-7.30 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 35.2, 36.1, 68.5, 71.0, 71.3, 71.9, 75.3, 76.4, 127.8-138.7; Anal. calcd. for $C_{20}H_{24}O_4.H_2O$: C, 69.38; H, 7.57. Found: C, 69.40; H, 7.58.

(1*R*,2*S*,3*R*,5*R*)-3,5-Di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**24**): 79% (1.3 g; 4.0 mmol); Colorless oil; $[\alpha]_D$: +77.8 (*c* 0.18; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.48-1.57 (m, 1H), 1.69-1.75 (m, 1H), 2.15-2.29 (m, 2H), 3.60 (dd, *J* 9.2 and 3.1 Hz, 1H), 3.81 (dt, *J* 9.5 and 4.0 Hz, 1H), 3.88 (m, 1H), 4.04 (m, 1H), 4.47 (s, 2H), 4.62 (d, *J* 11.9 Hz, 1H), 4.68 (d, *J* 11.9 Hz, 1H), 7.26-7.35 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 32.8, 34.0, 70.3, 70.8, 72.1, 74.4, 74.7, 75.2, 127.6-137.7; Anal. calcd. for $C_{20}H_{24}O_4.H_2O$: C, 69.38; H, 7.57. Found: C, 69.40; H, 7.01.

General Procedure to Prepare 25-28

To a solution of **23** or **24** (0.82 g, 2.5 mmol) in pyridine (7 mL) was added a solution of **6** or **10** (10 mmol) in toluene (30 mL) at 0°C. The reaction mixture was stirred for 48 h at 50°C. The mixture was acidified with HCl (4 mol.L^{-1}) until pH 3 and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) leading to compounds **25-28**.

(1*R*,2*S*,3*R*,5*S*)-1,2-Di-*O*-(3',4'-di-*O*-acetyl)-caffeoyl-3,5-di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**25**): 71% (1.5 g; 1.8 mmol); White solid; mp 72-74°C; $[\alpha]_D$: -206.0 (*c* 0.32; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.10-1.85 (m, 2H), 2.28-2.31 (m, 12H), 2.57 (m, 2H), 3.76-3.94 (m, 2H), 4.56-4.71 (m, 4H), 5.13 (dd, *J* 9.4 and 2.8 Hz, 1H), 5.64 (m, 1H), 6.34 (d, *J* 16.0 Hz, 1H), 6.37 (d, *J* 16.0 Hz, 1H), 7.25-7.38 (m, 16H), 7.54 (d, *J* 16.0 Hz, 1H), 7.56 (d, *J* 16.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.8, 34.5, 36.2, 69.4, 70.8, 71.4, 72.1, 74.1, 75.2, 119.0-143.8, 165.4, 165.8, 168.1, 168.2; HRMS calcd for $C_{46}H_{44}O_{14}Na$ [M + Na^+] 843.2629, found 843.2611.

(1*R*,2*S*,3*R*,5*R*)-1,2-Di-*O*-(3',4'-di-*O*-acetyl)-caffeoyl-3,5-di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**26**): White solid; 75% (1.5 g; 1.9 mmol); mp 65-67°C; $[\alpha]_D$: +82.4 (*c* 0.17; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.29 (m, 16H), 3.88-3.95 (m, 2H), 4.50-4.71 (m, 4H), 5.30-5.42 (td, *J* 11.0 and 2.8 Hz, 1H), 5.50 (t, *J* 2.8 Hz, 1H), 6.32 (d, *J* 16.0 Hz, 1H), 6.42 (d, *J* 16.0 Hz, 1H), 7.34 (m, 16H), 7.61 (t, *J* 16.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.8, 32.3, 32.6, 68.9, 69.3, 70.9, 71.8, 71.9, 74.2, 119.0-143.9, 165.6, 165.8, 168.1, 168.2; HRMS calcd for $C_{46}H_{44}O_{14}Na$ [M + Na^+] 843.2629, found 843.2640.

(1*R*,2*S*,3*R*,5*S*)-1,2-Di-*O*-(3',4',5'-tri-*O*-benzyl)-galloyl-3,5-di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**27**): 70% (2.1 g; 1.8 mmol); Colorless oil; $[\alpha]_D$: -200.0 (*c* 0.16; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.60-1.90 (m, 2H), 2.33-2.60 (m, 2H), 3.70 (m, 1H), 3.74 (m, 1H), 4.48-5.04 (m, 16H), 5.31 (dd, *J* 9.0 and 2.6 Hz, 1H), 5.77 (m, 1H), 7.22-7.35 (m, 44H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 34.4, 35.9, 69.8-75.3, 109.3-152,8, 164.9, 165.3; Anal. calcd. for $C_{76}H_{68}O_{12}$: C, 77.80; H, 5.58. Found: C, 77.44; H, 5.91.

(1*R*,2*S*,3*R*,5*R*)-1,2-Di-*O*-(3',4',5'-tri-*O*-benzyl)-galloyl-3,5-di-*O*-benzyl-1,2,3,5-cyclohexanetetrol (**28**): 65% (1.9 g; 1.6 mmol); Colorless oil; $[\alpha]_D$: -30.8 (*c* 0.13; CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.61 (m, 2H), 2.26-2.52 (m, 2H), 3.95-3.99 (m, 1H), 4.56-5.13 (m, 16H), 5.46 (td, *J* 10.9 and 3.1 Hz, 1H), 5.63 (t, *J* 3.1 Hz, 1H), 7.21-7.31 (m, 44H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 29.9, 32.7, 69.7-75.2, 109.1-152.8, 165.3; ESI MS: m/z 1196.5 (M+Na^+).

Preparation of 29 and 30

To a solution of **25** or **27** (0.13 g, 0.15 mmol) in methanol (10 mL) was added Pd/C 10% (0.15 g). The reaction mixture was refluxed for 96 hours. After completion of the reaction, the resulting mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 / MeOH) leading to compounds **29** and **30**, respectively.

(1*R*,2*S*,3*R*,5*S*)-1,2-Di-*O*-caffeoyl-1,2,3,5-cyclohexanetetrol (**29**): 60% (0.09 mmol; 0.043 g); White solid; mp 144-146°C; $[\alpha]_D$: 181.8 (*c* 0.1; CD_3OD); ^1H NMR (300 MHz, C_5D_5N): δ (ppm) 0.88-1.69 (m, 4H), 4.06 (m, 2H), 4.59 (m, 1H), 5.58 (m, 1H), 6.72 (t, *J* 15.9 Hz, 2H), 6.76-7.00 (m, 6H), 7.47 (t, *J* 15.9 Hz, 2H); ^{13}C (75 MHz, C_5D_5N): δ (ppm) 38.3, 42.5, 65.4, 66.3, 69.5, 70.6, 114.9-147.4, 168.1, 168.5; HRMS calcd for $C_{24}H_{24}O_{10}Na$ [M + Na^+] 495.1267, found 495.1280.

(1*R*,2*S*,3*R*,5*R*)-1,2-Di-*O*-caffeoyl-1,2,3,5-cyclohexanetetrol (**30**): 60% (0.09 mmol; 0.043 g); White solid; mp 133-135°C; $[\alpha]_D$: -19.6 (*c* 0.1; CD_3OD); ^1H NMR (300 MHz, C_5D_5N): δ (ppm) 1.17-2.01 (m, 4H), 2.64 (s, 2H), 4.03 (m, 2H), 5.13-5.28 (m, 2H), 6.10 (d, *J* 15.9 Hz, 1H), 6.22 (d, *J* 16.0 Hz, 1H), 6.61-6.96 (m, 6H), 7.37 (d, *J* 15.9 Hz, 1H), 7.48 (d, *J* 16.0 Hz, 1H); ^{13}C NMR (75 MHz, C_5D_5N): δ (ppm) 36.4, 38.0, 65.2, 66.7, 69.6, 72.4, 114.9-147.7 168.3, 168.4; HRMS calcd for $C_{24}H_{24}O_{10}Na$ [M + Na^+] 495.1267, found 495.1270.

Preparation of 31 and 32

A solution of **27** or **28** in EtOAc (20 mL) was hydrogenated under pressure of 4 psi in presence of Pd/C 10% (0.30 g). The suspension was stirred for 6 h at room temperature, the catalyst was removed by filtration, washed with methanol (10 mL) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 / MeOH) leading to compounds **31** and **32**, respectively.

(1*R*,2*S*,3*R*,5*S*)-1,2-Di-*O*-galloyl-1,2,3,5-cyclohexanetetrol (**31**): 93% (0.6 g; 1.4 mmol); White solid; mp 179-181°C; $[\alpha]_D$: -270.6 (*c* 0.23; CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ (ppm) 2.12-2.14 (m, 2H), 2.52-2.81 (m, 2H), 4.55 (m, 1H), 4.70 (m, 1H), 5.77 (dd, *J* 8.5 and 2.4 Hz, 1H), 6.29 (m, 1H), 7.90 (s, 4H); ^{13}C NMR (75 MHz, C_5D_5N): δ (ppm) 30.6, 38.8, 65.6, 67.8, 70.2, 77.9, 110.7-150.7, 166.9, 167.8; HRMS calcd for $C_{20}H_{19}O_{12}$ [M - H^+] 451.0877, found 451.0894.

(1*R*,2*S*,3*R*,5*R*)-1,2-Di-*O*-galloyl-1,2,3,5-cyclohexanetetrol (**32**): 95% (0.6 g; 1.4 mmol); White solid; mp 174-176°C; $[\alpha]_D$: +136.4 (*c* 0.15; CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ (ppm) 2.28-2.70 (m, 4H), 4.68 (m, 1H), 4.86 (m, 1H), 6.15 (m, 1H), 6.24 (m, 1H), 7.87 (s, 2H), 7.94 (s, 2H); ^{13}C NMR (75 MHz, C_5D_5N): δ (ppm) 37.7, 39.2, 64.8, 68.2, 70.1 72.9, 110.7-148.2, 167.0, 167.2; HRMS calcd for $C_{20}H_{19}O_{12}$ [M - H^+] 451.0877, found 451.0899.

Preparation of 33

To a solution of **5** (10.91 g, 56.8 mmol) in toluene (40 mL) and DMF (40 mL) was added cyclohexanone (36.0 mL, 237.0 mmol). The reaction mixture was coupled to the dean-stark and refluxed for 50 hours. Then was added amberlite IR 120 (14.0 g) and refluxed for another 18 hours. The solution was filtered and neutralized with solution of sodium bicarbonate (5% m/v). The mixture was extracted with ethyl ether (3 x 200 mL). The combined organic ex-

tracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc / hexane) leading to compound **33** in 50% yield (7.2 g, 28.4 mmol).

(1*R*,3*R*,4*R*,5*R*)-3,4-*O*-Cyclohexylidene-1,3,4,5-cyclohexane tetrol-1,5-carbolactone (**33**): White solid; mp 141-143°C; $[\alpha]_D$: -35.2 (*c* 1.18; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.21-1.90 (m, 10H), 2.18 (dd, *J* 14.7 and 3.1 Hz, 1H), 2.28-2.41 (m, 2H), 2.65 (d, *J* 11.9 Hz, 1H), 3.27 (s, 1H), 4.28-4.32 (m, 1H), 4.48 (td, *J* 6.2 and 2.8 Hz, 1H), 4.74 (dd, *J* 6.2 and 2.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 23.6-34.5, 37.0, 38.4, 71.2, 71.7, 71.8, 76.0, 110.7, 179.2; Anal. calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Anal. calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found: C, 61.20; H, 7.08.

Preparation of 34

To a solution of **33** (2.3 g, 10 mmol) in dry THF (50 mL) was added $LiAlH_4$ (0.8 g, 20.0 mmol). The reaction mixture was stirred for 5 h at room temperature. After the completion of the reaction the excess of $LiAlH_4$ was destroyed with EtOAc. The mixture was filtered and the filtrate extracted with water (100 mL) and EtOAc (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel column to afford **34** in 90% yield (2.3 g; 9.0 mmol).

(1*R*,2*R*,3*R*,5*R*)-1,2-*O*-Cyclohexylidene-5-*C*-(hydroxymethyl)-1,2,3,5-cyclohexanetetrol (**34**): White solid; mp 91-93°C; $[\alpha]_D$= -68.3 (*c* 1.3; $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.43-2.26 (m, 14H), 3.43 (d, *J* 19.7 Hz, 1H), 3.45 (d, *J* 19.7 Hz, 1H), 3.95 (t, *J* 5.9 Hz, 1H), 4.02-4.09 (m, 1H), 4.45 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ (ppm) 23.8-34.8, 38.2, 38.4, 69.3, 70.2, 72.8, 74.0, 76.8, 110.0; HRMS calcd for $C_{13}H_{22}O_5Na$ [M + Na^+] 281.1365, found 281.1367.

Preparation of 35 and 36

To a solution of **34** (0.5 g, 2.0 mmol) in pyridine (5 mL) was added a solution of **6** or **9** (6 mmol) in toluene (30 mL) at 0°C. The reaction mixture was stirred for 24 h at room temperature. Then the mixture was acidified with HCl (4 mol.L^{-1}) until pH 3 and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) leading to **35** and **36**, respectively.

(1*R*,2*R*,3*R*,5*R*)-1,2-*O*-cyclohexylidene-3-*O*-(3',4'-di-*O*-acetyl)-caffeoyl-5-*C*-((3',4'-di-*O*-acetyl)-caffeoyloxymethyl)-1,2,3,5-cyclo hexanetetrol (**35**): 70% (1.1 g; 1.4 mmol); White solid; mp 69-71°C; $[\alpha]_D$= +26.0 (*c* 0.31; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.60-1.80 (m, 12H), 2.31 (m, 12H), 4.09-4.20 (m, 3H), 4.54 (m, 1H), 5.45 (m, 1H), 6.41 (t, *J* 16.3 Hz, 2H), 7.20-7.41 (m, 6H), 7.64 (d, *J* 16.3 Hz, 1H), 7.65 (d, *J* 16.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.8-34.9, 36.2, 37.9, 70.9, 71.4, 71.7, 73.9, 76.4, 110.6, 118.8-143.8, 165.8, 166.6, 168.2, 168.3; HRMS calcd for $C_{39}H_{42}O_{15}Na$ [M + Na^+] 773.2421, found 773.2448.

(1*R*,2*R*,3*R*,5*R*)-1,2-*O*-cyclohexylidene-3-*O*-(3',4',5'-tri-*O*-ace tyl)-galloyl-5-*C*-((3',4',5'-tri-*O*-acetyl)-galloyloxymethyl)-1,2,3,5-cyclohexanetetrol (**36**): 50% (0.8 g; 1.0 mmol); White solid; mp 73-75°C; $[\alpha]_D$= +22.2 (*c* 0.18; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.23-2.10 (m, 14H), 2.27-2.29 (m, 18H), 3.50 (m, 1H), 4.19-4.31 (m, 3H), 4.51 (s, 1H), 5.54 (m, 1H), 7.80 (s, 2H), 7.81 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.4-34.9, 36.3, 37.9, 64.6, 71.6, 72.8, 73.9, 76.4, 110.7, 122.6-143.7, 163.6, 164.4, 166.6, 166.9.

Preparation of 37 and 38

To a solution of **35** or **36** (1.0 mmol) in THF (10 mL) was added an aqueous solution of HCl (1 mol.L^{-1}) (35 mL). The reaction mixture was stirred for 10 days at room temperature and after completion of the reaction, was saturated with solid NaCl. The aqueous phase was extracted with EtOAc (3 x 100 mL) and the combined organic extracts dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) leading to compounds **37** and **38**.

(1*R*,2*R*,3*R*,5*R*)-3-*O*-caffeoyl-5-*C*-(caffeoyloxymethyl)-1,2,3,5-cyclohexanetetrol (37): 58% (0.3 g; 0.6 mmol); White solid; mp 120-122°C; $[\alpha]_D$= -38.5 (*c* 0.10; MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 1.29 (m, 4H), 3.70 (m, 1H), 4.06 (s, 2H), 4.20 (m, 1H), 5.43 (m, 1H), 6.31 (t, *J* 15.6 Hz, 2H), 6.85-7.18 (m, 6H), 7.54 (d, *J* 15.6 Hz, 1H), 7.57 (d, *J* 15.6 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 37.4, 39.1, 71.1, 72.0, 73.9, 74.3, 115.2-149.0, 167.6; HRMS calcd for $C_{25}H_{26}O_{11}Na$ [M + Na^+] 525.1373, found 525.1375.

(1*R*,2*R*,3*R*,5*R*)-3-*O*-galloyl-5-*C*-(galloyloxymethyl)-1,2,3,5-cyclohexanetetrol (38): White solid; 54% (0.3 g; 0.5 mmol); mp 151-153°C; $[\alpha]_D$= -19.0 (*c* 0.21; MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 2.20-2.60 (m, 4H), 4.67 (m, 1H), 4.85 (m, 1H), 6.13 (s, 2H), 7.86 (s, 2H), 7.92 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 38.2, 40.1, 71.7, 72.2, 74.1, 74.9, 110.7-148.2, 167.6; HRMS calcd for $C_{21}H_{21}O_{13}$ [M - H^+] 481.0982, found 481.1003.

BIOLOGICAL ASSAYS

Oligonucleotides

All oligonucleotides employed throughout this study were synthesized by Integrated DNA Technologies and gel-purified and desalted prior to use. The disintegration substrate, a self-annealing oligonucleotide that is homologous to the HIV long terminal repeat regions (LTR), is dBY-1: 5'- TGCTAGTTCTAGCAGGCCC TTGGGCCGGCGCTTGCGCC-3' [34]. The 3'-end processing/ strand transfer substrate (V1/V2) consists of an annealed pair, V1: 5'- ATGTGGAAAATCTCTAGCAGT-3', and V2: 5'- ACTGCTA GAGATTTTCCACAT-3' [31]. The strand transfer substrate consists of U5V1P: 5'- ATGTGGAAAATCTCTAGCA-3' annealed to V2 in order to generate U5V1P/V2, a pre-processed form of V1V2 homologous to the end-processed LTR (2) [31]. This substrate is used to separate the effect of compounds upon 3'-end processing from strand transfer catalysis. Oligonucleotides used for site-directed mutagenesis include: G140S (+): 5'-G ATCAAGCAG-GAATTTAGCATTCCCTACAATC-3; G140S(-): 5'-GATTGTA GGGAATGCTAAATTCCTGCTTGATC-3'; Q148H(+):5'-CAAT CCCCAAAGTCATGGA GTAATAGAATC-3'; Q148H(-):5'-GAT TCTATTACTCCATGACTTTGGGGATTG-3'; N155H(+): 5'-GTAATAGAATCTATGCATAAAGAATTAAAG-3'; N155H(-): 5'-CTTTAATTCTTTATGCATAGATTCTATTAC-3'. Underlined sequence denotes mutated codon.

Generation of Recombinant IN Genes

Mutated IN genes were produced by performing the Quick-Change XL site-directed mutagenesis protocol (Stratagene, La Jolla, CA 92037) upon the stable, infectious molecular clone of HIV, HIV_{NL4-3} (GenBank Accession #M19921). Mutation success was confirmed by fluorophore-labeled dideoxynucleotide DNA sequencing (GeneWiz, La Jolla, CA 92037) and restriction digest. By utilizing engineered silent restriction sites, the mutated IN gene was subsequently cloned into and replaced the reference IN gene in a recombinant protein expression vector [35] pT7.7, which encodes an amino-terminal six-histidine tag.

Generation of Recombinant IN

Recombinant IN protein was expressed from BL21 DE3 pLysS *Escherichia coli* (Stratagene, La Jolla, CA 92037) and purified

following bacterial lysis [36]. Briefly, transformed bacterial cultures were induced to express IN protein by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Following harvest by low-speed centrifugation, cells were lysed by freeze-thaw, ultrasonication, and digestion by the endogenous lysozyme encoded by the pLysS plasmid. The resultant mixture was clarified by centrifugation, and the precipitate was washed with 100 mM NaCl. The suspension was again centrifuged, and the precipitate was slowly stirred in a buffer containing 1 mM NaCl, and 5 mM CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate). Each of the washing steps occurred at 5˚C under reducing conditions (5 mM β-mercaptoethanol) in a buffer containing 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.5. Recombinant IN containing an amino-terminal 6-histidine tag was purified via Ni^{2+}-column affinity chromatography as previously described (10) [37]. IN purity was confirmed via sodium-dodecyl-sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) followed by Coomassie Blue staining; IN resolved at a MW of ~ 32 kDa. Highly-purified fractions containing IN were dialyzed into storage buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 5 mM CHAPS, 10% glycerol, and 5 mM dithiothreitol) and stored at -80˚C.

IC_{50} Determination

Inhibitory susceptibility of recombinant IN to all compounds was determined as previously described [31, 35]. Briefly, 100 nM IN was incubated for 1 h at 37˚C in the presence of 0.1 pmol ^{32}-P labeled dBY-1, the disintegration substrate, V1/V2, the 3'-EP substrate, or U5VIP/V2, the ST substrate, and decreasing concentrations of compounds **13-38** in reaction buffer (20 mM HEPES pH 7.5, 10 mM dithiothreitol, 0.05% Nonidet P-40, 7.5% DMSO, and 10 mM $MnCl_2$). Following incubation, reactions were stopped with the addition of loading buffer (98% deionized formamide, 10 mM EDTA pH 8.0, 0.05% bromophenol blue, 0.05% xylene cyanol) and resolved via 16% urea-PAGE. Substrate to product percent conversion was calculated by phosphorimager analysis using a Molecular Dynamics Storm (GE Healthcare, Piscataway, NJ). The IC_{50} was determined using Calcusyn software.

Viruses and Cell Cultures

H9 cells ($CD4^+$ human T-lymphoblastoid cell line) and MT-2 cells ($CD4^+$ cell line transformed by human T-lymphotrophic virus type 1) were both obtained from the AIDS Research and Reference Reagent Program. All cell cultures were incubated at 37˚C in RPMI 1640 containing 2 mM L-glutamine and 25 mM HEPES supplemented with 11.5% fetal bovine serum (Atlas, Fort Collins, CO). HIV_{LAI} was obtained from H9 cell lysates clarified of cells by low-speed centrifugation followed by filtration through 0.45 μm filters.

Fifty Percent Effective Concentration and Cell Toxicity Analysis (EC_{50} and CT_{50}/CT_5)

Susceptibility of HIV_{LAI} to compounds **13-38** was determined in MT-2 cells as previously described [38]. Triplicate samples of a compound were diluted serially 128-fold in 96-well culture plates. HIV_{LAI} was pre-incubated with each compound (**13-38**) for 1 h prior to the addition of MT-2 cells at a multiplicity of infection (MOI)>1. Upon observation of HIV-induced cytopathic effect, cells were transferred to poly-L-lysine coated plates and stained with Finter's neutral red dye. Plates were then incubated at 37˚C for 1 h. Following incubation, cells were washed 3 times with PBS and lysed in acidified methanol. Viability was subsequently quantified on a microcolorimeter by A_{540}. The percentage of viable cells was calculated based upon eight uninfected cell controls (100% viable) and eight infected-cell controls with HIV_{LAI} in the absence of an inhibitor (virus control, 0% viable). The fifty percent effective concentration was determined using Calcusyn for Windows. Sensitivity of MT-2 cells to the cytostatic and cytotoxic effects of compounds **13-38** was determined in a similar fashion as described, previously [38]. The concentration of compound at which 95% of the cells are viable, CT_5, is a nontoxic concentration of the compound.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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