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Synthesis and Anti-HIV Activity of Cosalane Analogues with Substituted Benzoic Acid Rings Attached to the Pharmacophore through Methylene and Amide Linkers

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The cosalane pharmacophore has been extended by the attachment of two additional substituted benzoic acid rings through amide and methylene linkers. The resultant compounds display significant antiviral activity when tested in vitro for inhibition of the cytopathic effects of HIV-1p24 in CEM-SS cells and HIV-1la in MT-4 cells. The compound containing the methylene linker also shows moderate activity versus HIV-2p24 in MT-4 cells. Because cosalane and related compounds containing extended pharmacophores inhibit the binding of gp120 to CD4, the presently described new compounds are assumed to act by a similar mechanism. A hypothetical model is proposed for the binding of the methylene-linked compound to CD4.

Introduction

Since the design and synthesis of cosalane (1),1,2 a number of studies have been reported by us and others involving the development of new HIV inhibitors based on the cosalane framework.3,4,5 These communications have provided information about the biological effects of (1) changing the length of the alkenyl linker chain between the cholestane and the disaccharide "pharmacophore" of the molecule, (2) altering the site of attachment of the linker chain to the steroid, (3) varying the chemical nature of the linker, and (4) alkylation of the phenolic hydroxyl groups to prepare new inhibitors containing additional benzoic acid rings.

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From the latter study, it was demonstrated that extending the length of the cosalane (1) pharmacophore by functionalization with additional benzoic acid rings provided new compounds with enhanced anti-HIV potency, which could possibly be attributed to stronger cosalane/CD4 interactions. For example, it was found that the cosalane analogue 2 was more potent against HIV-1p24 in CEM-SS cells and HIV-1la in MT-4 cells than
Rationale and Design

The disacylhydrazine portion of cosalane is believed to be the pharmacophore of this compound. This is supported by the observation that the dichlorofuransulfosulfonate fragment of cosalane has low but reproducible activity against HIV-1 in CEM cells. In addition, low molecular weight components of the mixture of polymeric polymers known as aurintricarboxylic acid (ATA), a schematic representation shown in structure [3] that are replaced by normal alkyl chains and anti-HIV activity is retained, although potency is compromised. Cosalane binds to the HIV surface protein gp120 and its lymphocyte receptor CD4. This finding suggests that cosalane may interact with one or both of these target molecules through aminic interactions of the carbonyl groups of the ligand with similar components of the virus. Because the D102 domain of CD4, the portion required for HIV-1 binding, has been crystallized and the structure is available for examination, a hypothetical model of cosalane interacting with this protein has been proposed. The core of this model is that the two carboxyl groups of cosalane could easily span the distance between the surface-exposed, adjacent arginines (Arg62 and Arg59) of CD4. These residues are believed to be important in HIV-1 binding to CD4. Upon examination of the amino acids near this site, it was determined that either amino side chains are in close enough proximity to be reachable by additional amino groups that could be appended to cosalane’s pharmacophore. One such target amino acid would be Lys72. As revealed by the construction of the models of the proposed compounds 4 and 5 bound to the CD4 molecule, both 4 and 5 appear to be capable of interacting with all three of these basic residues on the protein.

A hypothetical model of the binding interaction is detailed in Figure 1. This model was created using the

References:


(2) Chabot, F.; Schell, D.; Furthermore, there is a strong correlation between the CD42 region of CD4 and the binding of these compounds to the reverse transcriptase (RT) of HIV-1. This indicates that these compounds may be useful as inhibitors of HIV-1 replication.
Sculpt molecular modeling program (Interactive Simulations, Inc.) by docking the coalasal analogue 5 onto the CD4 surface. Because free rotation exists about the methylene groups connecting the aromatic rings, it was possible to position this compound so that the carboxylates of the ligand contacted Arg68, Arg69, Lys72, and Arg56 of the protein by simple bend rotation. The protein structure was then “frozen,” and the energy of the ligand was minimized.

Our decision to construct molecules 4 and 5 was influenced by three factors. First, it had been demonstrated that increasing the molecular weight of the polyamionic anti-HIV polymer aminosubcarboxylic acid (3) by increasing the number of salicylic acid units resulted in increased anti-HIV potency in vitro.16 Because coalasal incorporates the disalicylamidemethane structural unit of ATa as a part of its pharmacophore, addition of salicylic acid rings to the coalasal pharmacophore might be expected to increase antiviral potency. Second, molecules 4 and 5 were originally thought to be easily accessible from coalasal by dehalogenation followed by chloroform substitution methods. Unfortunately, this did not prove to be the case, and other synthetic methods eventually had to be pursued. Third, the antiviral potency of low molecular weight: AIA (3) oligomers increases as the number of salicylic acid rings increases.17

Chemistry

The desired analogues 4 and 5 were both expected to be synthesized by convergent routes. This would require the preparation of the aromatic pharmacophore region, as well as a functionalized cholesterol component containing a precursor of the linker chain, and their eventual union late in the synthesis. The decision to execute the convergent step by McMurry olefination, which tolerates a wide range of functional groups, led us to prepare the pharmacophore regions in the form of substituted benzophenones.18

Several problems had to be overcome to achieve the synthesis of the required benzophenone derivative 14 (Scheme 2). The diamine derived from reduction of the two nitro groups of 12 did not undergo acylation with acid chlorides or anhydrides but instead formed resins and intractable mixtures. Additionally, it was not possible to oxidize diphenylmethane 10, accessible via standard chemistry outlined in Scheme 1, to 14 because of the insolvency of 10. However, the temazacilid derived from 10 proved to be valuable itself as a biological probe because it afforded a substance with which to determine the efficacy of a small, amide-containing compound as an inhibitor of HIV-1 infection. Our previous reports with small polyanionic molecules have not included amide derivatives.19,20

The benzophenone 14 was finally prepared according to Scheme 2. The diamino benzhydrol compound 13 was synthesized in one step by simultaneous reduction of the keto and nitro functionalities in 12.13 This route overcomes the inhibitory effect that the ketone functionality apparently imposes on acylating the meta amino groups of the compound resulting when only the two nitro groups of 12 are reduced to amino groups. Alcohol 13 proved to be very unstable and was normally acylated as soon as


Synthesis and Anti-HIV Activity of Covalene Analogues

Scheme 1*

Reagents and conditions: (a) 90% HNO₃, H₂O, 0 to 21 °C, 12 h. (b) NaNO₂, H₂O, 60 °C, 4 h. (c) Pyridine, 60 °C, 12 h. (d) K₂CO₃, aq. NaOH, 95 °C, 12 h.

Benzophenone 24 was prepared by the methods outlined in Schemes 3 and 4. This synthesis makes use of chlorine as a protecting group to control the regiochemistry of aromatic substitution. Thus, 3-bromoacryloyl chloride (15) was formulated to give aldehyde 16. This formulation proved to be temperature-dependent. Under the conditions shown (90 °C, TFA, hexamethyldisilazane), the bond to the bromine atom was stable. At higher temperatures (120 °C), the bromine was removed and a second formulation occurred. By comparison, the corresponding aromatic iodide was very unstable, and iodine sublimed up the condenser during the formulation reaction. Reduction of aldehyde 16 gave the benzyl alcohol 17, which was used to alkylate 6-chlorosalicyl acid (18) to form a diacid that was esterified to yield 19. The yield of the alactetion product was dependent on several variables. Chief among these was the solvent; solvent chosen proceeded in methanol/sulfuric acid, but only benzyl O-acylation occurs in acetic acid/sulfuric acid. Additionally, a slight excess of the benzyl alcohol was needed. Finally, the concentration of the reagents in the solvent were found to be very important. To obtain an analytically pure sample, the easiest method of purification was found to be Fisher esterification and isolation of the diester 19. Reductive removal of the bromide with zinc dust under basic conditions gave 20, which could be used in ensuing reactions but was normally re-esterified to afford 21. This was done so that 21 could be monitored by TLC analysis in the ensuing reactions. The selective

Scheme 2*

Scheme 3*

Scheme 4*
removal of bromine from 19 left the chlorine atom to function as a protecting group and control the regiochemistry during the subsequent Friedel–Crafts reactions.

The electron-rich nature of 20 and 21 suggested that palladium carbonylation methods might prove difficult, so 24 was prepared by Friedel–Crafts alkylation followed by degradation chemistry. Treatment of 21 with 2 equiv of chloroacetetyldehyde dimethyl acetal gave the diphenylethane 22 in fair yield. Unlike the literature precedent, 22 was without doubt undergoing some ether hydrolysis on workup. Attempts to find conditions that circumvented aqueous workup were not successful. Rearrestification of the crude material with diazomethane or by Fisher esterification led to a greater isolated yield. Regardless, there was also some loss of material by base-catalyzed reactions on the silica gel used to separate the compounds.

Etherification of 22 was then accomplished with dimethyl sulfate in DMP solution containing potassium carbonate as the base. It was necessary not to methylate until after 22 was isolated in pure form because it was not otherwise possible to separate the desired ether product 23 from unreacted starting material. Chloride 23 was obtained as an oil that was heat-sensitive and eliminated HCl slowly upon standing.

Intermediate 23 was converted to 24 through three reactions which were performed in series because the intermediates were difficult to isolate or unstable. The transformation of 23 to 24 was done as quickly as possible to minimize rearrangement of the intermediary 1,1-disubstituted olefin. Thus, 23 underwent base-catalyzed elimination of HCl and ester hydrolysis using KOH in methanol to afford the corresponding 1,1-disubstituted olefin along with a small amount of the 1,2-rearranged product (detected by H NMR). Subsequent concomitantly and re-esterification of the crude mixture provided 24, which was purified free of aldehydes (obtained from the 1,2-rearranged product) by column chromatography. Ketones 14 and 24 were appended to aldehyde 26 (Scheme 5). Esters 27 and 28 were readily hydrolyzed to give the free acids 4 and 5. These compounds were converted to their more water-soluble tetrazolium salts for biological evaluation.

**Biological Results and Discussion**

We have demonstrated that extended cosaline-type compounds are accessible by routes that do not utilize cosaline itself as an intermediate or a starting material. Despite the length of these syntheses, two different
Table 1. Anti-HIV Activities of Coleen Analogue

<table>
<thead>
<tr>
<th>Compd</th>
<th>EC50 (μM)</th>
<th>CEM-SS MT-4</th>
<th>HIV-1Δ gag</th>
<th>HIV-1Δ pol</th>
<th>HIV-1Δ env</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1Δ</td>
<td>&lt;200</td>
<td>&lt;125</td>
<td>1</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>1.4</td>
<td>55</td>
<td>76</td>
<td>95</td>
</tr>
<tr>
<td>22</td>
<td>26.6</td>
<td>8.4</td>
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<td>66.4</td>
<td>79.4</td>
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<tr>
<td>3</td>
<td>29.9</td>
<td>12.5</td>
<td>19.9</td>
<td>49.9</td>
<td>41.0</td>
</tr>
<tr>
<td>11</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>31.6</td>
<td>125</td>
</tr>
<tr>
<td>27</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>94.6</td>
<td>78.2</td>
</tr>
</tbody>
</table>

The concentration required to reduce the cytopathic effect of the virus by 50%. The concentration required for a 50% reduction in cell viability in uninfected cells. Determined in CEM-SS cells. Determined in MT-4 cells. *Tested as the tetrahydrox salt. *No activity observed.

routes have been employed to prepare two classes of these compounds. Both with the carboxylic acid to introduce the cytotoxic effect of HIV-1 is virus (Table 1). However, the amide compound 4 was not as potent as coleena and had only moderate activity against HIV-1Δ gag in CEM-SS cells (EC50 29.4 μM and HIV-1Δ gag in MT-4 cells (EC50 8.4 μM). It was inactive as an inhibitor of the cytotoxic effect of HIV-2Δ env in MT-4 cells. Compound 11, which is the pharmacophore portion of 4, proved to be inactive. In general, all of the amide compounds had greater cytotoxicity than is normally seen with similar compounds. As with coleena, the carboxylic acids appear to play a critical role in the antiviral activity, as the tetrahydroxin 27 was inactive. The methylated compound 5 appeared to be more potent than 4 against HIV-1Δ gag in CEM-SS cells (EC50 2.9 μM) and HIV-2Δ env in MT-4 cells (EC50 19.9 μM), but it was slightly less potent than 4 against HIV-1Δ gag in MT-4 cells (EC50 12.5 μM). Compound 5 was slightly more potent than 1 when tested against HIV-1Δ gag in CEM-SS cells, but it was less potent than an inhibitor of HIV-1Δ gag in MT-4 cells. In addition, compound 5 was less potent against both strains of HIV-1 than the compound 2 having a benzyl ether linkage. As shown in the hypothetical model of the binding of 1 to CD4 displayed in Figure 1, the positively charged guanidinium ions of the side chains of Arg86 and Arg91 may interact with two of the carboxylates of the ligand 3 by ionic bonding. The oxygen of a tertiary carboxylate residue of the ligand is also capable of ionic bonding to the positively charged ammonium ion of Lys72, and it may also interact with the carboxylic acid residue of Asp60. A similar model has been proposed for the binding of the coleena analogue 2 to CD4. Thus, although the model in Figure 1 is speculative, it has led to the design of several coleena analogues that display significant anti-HIV activity in in vitro systems. However, this model has limited predictive power due to the nature of several factors. It is known that covalent and structurally related compounds bind to gp120 and CD4, and the gp120/C4 complex is not though to be the primary determinant of the inhibition of the interaction of gp120 with CD4. 1,2,3,10 A more complete understanding of the mechanisms of action and potencies of the present compounds may therefore require a model for their binding to gp120 in addition to the CD4 binding model. It might be possible to propose such a model for gp120 binding in the future as a result of the recent publication of the X-ray crystal structure of gp120 in complex with the CD4 receptor and a neutralizing antibody. 11 In addition, recent mechanisms of action studies on compound 2 have indicated that it inhibits both viral infectivity and viral binding of the subsequent fusion of the viral envelope with the cell membrane, but it is a more potent inhibitor of fusion than attachment. The mechanism of action of 3 and 5 are acting by similar mechanisms, which would further complete an interpretation of the observed inhibition of virus replication based on the model of viral fusion. Thus, the CD4/coleena binding model has provided information for the initial design of more effective coleena analogues but requires further refinement to provide a more direct correlation of in vitro antiviral results to the interaction of coleena with the complex gp120/CD4 receptor array suggested by mechanistic studies for this class of compounds.

Polynions are known to exert their anti-HIV activity by a shielding of gp120/FF3 through an interaction of the negative charges of the polynions, for example, the sulfite groups of disodium sulfite, with the positively charged amino acid residues in the V3 loop. 12 The early inhibitory effect of polyanionic compounds may be the result of a disruption of the ionic interactions between the charged regions of viral surface glycoproteins, including gp120, and the membrane phospholipids and the receptor molecules at the cell surface (i.e., CD4 and chemokine receptors including CCR5 and CXCR4). Enveloped viruses that bud from cells carry part of the cell membrane and therefore may interact more effectively with the negatively charged cell surface if the outer part of the envelope protein contains regions of high positive charge. Such a V3 loop-FV interaction may provide that function, and the binding of polynions to the loop not only neutralizes positive charges but may also add an additional negative region to the loop thereby electrostatically preventing the interaction between the virion and the cell. Prior studies have demonstrated that coleena inhibits the binding of a specific anti-gp120 monoclonal antibody, directed to the V3 loop of HIV-1 gp120, to persistently HIV-1Δ gag-infected HUT-78 cells, thus establishing the possible involvement of the V3 loop of gp120 as a target in the anti-HIV activity of coleena. 11

The synthesis of 24 offers new methodology for the preparation of large quantities of libraries of compounds related to VTA in a controlled fashion. Recent interest in the synthesis of functionalized diphenylethlenamine molecules from their ortho-phenylalanines is in combinatorial chemistry as platforms to support functional groups employed in the search for new pharmacophores. 13

Experimental Section

General. Melting points are uncorrected. Nuclear magnetic resonance spectrums for proton (1H NMR) were recorded on a 300 MHz spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane.

The zinc salts were filtered off, and the solution was concentrated to a small volume. The precipitate obtained in a flask and centrifuged

4.2-Benzyl-3-methoxy-4-methylthiothymine (20). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

3.5-Benzyl-5-methoxycarbonylnaphthalene (14). Diamine 13 (10.38 g, 1 mmol) and 4-methyl-3-methoxycarbonyl-

2.4-Benzyl-4-methoxy-5-methoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.1-Benzamino-2-methoxy-5-methoxycarbonylnaphthalene (10). A solution of 10 g (89.5 mmol) of 5-methoxycarbonylnaphthalene in 50 ml of ethanol was added to a solution of 80.6 g (354 mmol) of zinc dust in 50 ml of ethanol. The mixture was allowed to stand for 2 h and then filtered. The filtrate was evaporated to dryness, and a solid was obtained.

1.2-Benzamino-2-methoxy-3-methoxycarbonylnaphthalene (11). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.1-Benzamino-2-methoxy-3-methoxycarbonylnaphthalene (8). A solution of 7.1 g (25 mmol) in ethyl acetate (100 ml) was stirred at room temperature with carbon dioxide, and the mixture was

1.3-Benzamino-2-methoxy-4-methoxycarbonylnaphthalene (9). A solution of 7.1 g (25 mmol) in ethyl acetate (100 ml) was stirred at room temperature with carbon dioxide, and the mixture was

1.4-Benzamino-2-methoxy-4-methoxycarbonylnaphthalene (12). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.1-Benzamino-2-methoxy-6-methoxycarbonylnaphthalene (15). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.3-Benzamino-2-methoxy-6-methoxycarbonylnaphthalene (16). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

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1.5-Benzyl-2-methyl-4,5-dimethoxycarbonylpyrimidine (17). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.6-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (18). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.7-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.8-Benzyl-2-methyl-4,5-dimethoxycarbonylpyrimidine (17). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.9-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (18). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.10-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.11-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.12-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.13-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (16). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.14-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.15-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.16-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.17-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.18-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.19-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.20-Benzyl-2-methyl-5,6-dimethoxycarbonylpyrimidine (19). A mixture of 17.5 g (0.29 mmol) of 1,4-diethoxycarbonylpiperazine in 50 ml of ethanol was stirred at room temperature for 1 h. After removal of the

1.21-Benzyl-2-methyl-4,5-dimethoxycarbonylnaphthalene (13). A mixture of 18.5 g (89.5 mmol) of 4-methylthiothymine and 250 ml of ethanol was stirred at room temperature for 1 h. After removal of the
zinc acetate (3.0 g). The zinc was removed by filtration through a gelatin membrane coated with concentrated HCl to pH 1, and then washed with water. The solid was air-dried to give the product: mp 263–265°C; IR (KBr): 3441, 1629, 1235 cm⁻¹; 1H NMR (acetone-6H): δ 1.31 (3H, t, J = 7.8 Hz), 7.26 (d, J = 1.8 Hz, 7H), 7.18 (d, J = 1.9 Hz, 7H), 7.11 (d, J = 1.7 Hz, 7H), 7.01 (d, J = 1.3 Hz, 7H), 6.44 (q, J = 7.7 Hz, 1H), 3.44 (a 2H). Anal. Calcd for C₄₂H₂₈O₂Cl₂: C, 85.73; H, 5.73. Found: C, 85.38; H, 5.62.

1.3-Bis[3,3,5,6-tetrahydroxy-2-methoxy-6-ethylphenyl]imidazol-4(3H)-ylidene] (22). A mixture of 21.34 (1.1 g, 5 mol%) chlorine containing phenol xylyl acetate (111.4 g, 1 mol%), and neral acetate (15% mol) was stirred at 0°C while concentrated sulfuric acid (50 ml) was added dropwise. The mixture slowly became red. After stirring overnight while warming to room temperature, the mixture was poured into 200 g of water and the grayish solid produced was filtered and air-dried. Flash chromatography on SiO₂ (50 g, 250–400 mesh) eluting with hexane/ethyl acetate diethyl ether system was used to isolate the 22 product. A mixture of 22 (21.34 g, 1.1 mol), chloroform, and dichloromethane (1 g, 5 ml) was stirred in DMP (5 ml) and the mixture was poured into an ice-water bath. After 1 h, the mixture was stirred with 10% H₂SO₄ solution (10 ml) at room temperature. After 1 h, the mixture was diluted with water (200 ml) and the organic layer was separated. The water was extracted with dichloromethane (3 x 100 ml). The organic layer was concentrated and dried over MgSO₄. The crude material was recrystallized from CH₂Cl₂/CH₃OH (1:1). Anal. Calcd for C₃₂H₂₆Cl₂O₃(C): C, 75.85; H, 4.52. Found: C, 75.86; H, 4.23.

1.3-Bis[3,3,5,6-tetrahydroxy-4-metoxo-6-ethylphenyl]imidazol-4(3H)-ylidene] (23). A solution of 22 (21.34 g, 1.1 mol), K₂CO₃, and dichloromethane (2 ml, 5 ml) was stirred in DMF (5 ml) and the mixture was poured into an ice-water bath. After 1 h, the mixture was stirred with 10% H₂SO₄ solution (10 ml) at room temperature. After 1 h, the mixture was diluted with water (200 ml) and the organic layer was separated. The water was extracted with dichloromethane (3 x 100 ml). The organic layer was concentrated and dried over MgSO₄. The crude material was recrystallized from CH₂Cl₂/CH₃OH (1:1). Anal. Calcd for C₃₂H₂₆Cl₂O₃(C): C, 75.85; H, 4.52. Found: C, 75.86; H, 4.23.

1.3-Bis[3,3,5,6-tetrahydroxy-2-methoxy-6-ethylphenyl]imidazol-4(3H)-ylidene] (24). A solution of 22 (21.34 g, 1.1 mol), K₂CO₃, and dichloromethane (2 ml, 5 ml) was stirred in DMF (5 ml) and the mixture was poured into an ice-water bath. After 1 h, the mixture was stirred with 10% H₂SO₄ solution (10 ml) at room temperature. After 1 h, the mixture was diluted with water (200 ml) and the organic layer was separated. The water was extracted with dichloromethane (3 x 100 ml). The organic layer was concentrated and dried over MgSO₄. The crude material was recrystallized from CH₂Cl₂/CH₃OH (1:1). Anal. Calcd for C₃₂H₂₆Cl₂O₃(C): C, 75.85; H, 4.52. Found: C, 75.86; H, 4.23.

1.3-Bis[3,3,5,6-tetrahydroxy-2-methoxy-6-ethylphenyl]imidazol-4(3H)-ylidene] (25). A solution of 22 (21.34 g, 1.1 mol), K₂CO₃, and dichloromethane (2 ml, 5 ml) was stirred in DMF (5 ml) and the mixture was poured into an ice-water bath. After 1 h, the mixture was stirred with 10% H₂SO₄ solution (10 ml) at room temperature. After 1 h, the mixture was diluted with water (200 ml) and the organic layer was separated. The water was extracted with dichloromethane (3 x 100 ml). The organic layer was concentrated and dried over MgSO₄. The crude material was recrystallized from CH₂Cl₂/CH₃OH (1:1). Anal. Calcd for C₃₂H₂₆Cl₂O₃(C): C, 75.85; H, 4.52. Found: C, 75.86; H, 4.23.
CuH13N6O12Na2SiO4·H2O; C: 58.63; H: 6.89; N: 2.08. Found: C, 57.70; H, 7.06; N, 1.72.

2.0 M soln in THF (0.58 g, 1 mmol) and Zn (0.2 g, 4 mmol) was heated in THF (10 mL) under argon at reflux for 1 h. A solution of iron(II) acetate (24.0 g, 0.32 mmol) and aldehyde (20 g, 0.5 mmol) in THF (20 mL) was added under argon, and the mixture was heated at reflux for 1 h. The reaction was stopped by pouring into 1 N HCl (100 mL) and extracting with ethyl acetate (100 mL). The organic layer was dried over MgSO4, evaporated in vacuo to afford the product.

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In Vitro Anti-HIV Assays. Evaluation of the antiviral activity of compounds against HIV-1p24, HIV-1ms, and HIV-1sc infection in CEM-SS and MT-4 cells were as previously described. 

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