# Articles

## Synthesis of Naphthalenesulfonic Acid Small Molecules as Selective Inhibitors of the DNA Polymerase and Ribonuclease H Activities of HIV-1 Reverse Transcriptase

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Over 25 selected naphthalenesulfonic acid derivatives were evaluated for their inhibitory effect on two different functional domains of the HIV-1 reverse transcriptase (RT), namely the ribonuclease H and DNA polymerase activities. Most of the analogues were found to be either specific toward the DNA polymerase activity or showed nonselective inhibition of both catalytic functions. The most active compounds are either symmetrical derivatives or nonsymmetrical derivatives containing a lipophilic appendage consisting of a palmitoyl or cholesteryl moiety. The six most active compounds in the preliminary screen, derivatives **6**, **16**, **17**, **23**, **26**, and **27**, were subjected to experiments to determine their 50% inhibitory concentration ($IC_{50}$) values in the assays that measure RNA-dependent DNA polymerase (RDDP), DNA-dependent DNA polymerase (DDDP), and ribonuclease H (RNase H) functions of HIV-1 RT. The most potent derivative was a nonsymmetric cholesterol-linked 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid analogue, compound **23**, which demonstrated an $IC_{50}$ value of 0.06 $\mu$M for inhibiting RDDP activity. Inhibition of DDDP and RNase H activity for this compound was demonstrated at concentrations that were over 100-fold of that for inhibiting RDDP activity. However, the potency of this active compound does not correlate in the whole virus assay, probably due to a lack of cellular entry. The cholesterol derivative, **23**, also possesses HIV-1 protease inhibitory activity and belongs to a unique class of multifunctional HIV-1 inhibitors.

Reverse transcriptase (RT), an enzyme crucial to the replicative cycle of HIV-1, has long been regarded as one of the most viable targets for potential anti-AIDS chemotherapy.[1] The HIV-1 RT has been characterized as a heterodimer comprising three well-defined catalytic properties, namely RNA-dependent DNA polymerase (RDDP), DNA-dependent DNA polymerase (DDDP), and ribonuclease H (RNase H) activities. Clinically approved nucleoside drugs such as AZT, DDI, and DDC and several structurally diverse non-nucleoside compounds have been reported to possess potent RT inhibitory activity.[2,3] Most of the nucleoside and non-nucleoside RT inhibitors have been shown to inhibit the polymerase activity of RT and not its RNase H activity. It has been demonstrated that monophosphorylated AZT can inhibit RNase H activity of RT, albeit at higher concentrations than those employed to inhibit its polymerase function.[4] In this vein, we were interested to conduct a systematic search for RT inhibitors capable of specifically interfering with the catalytic activity associated with more than one of the RT functions.

Past work in our laboratories to develop novel anti-AIDS agents has revealed the anti-HIV activity of numerous naphthalenesulfonic acid derivatives in a variety of assays. These have included the inhibition of HIV-1 cytopathogenicity using both a laboratory strain and a clinical isolate, inhibition of HIV-1 induced syncytia formation, and both HIV-1 and HIV-2 RT inhibition.[5–8] In the present study, we report the synthesis and evaluation of the inhibitory effects of over 25 naphthalenesulfonic acid derivatives on the different catalytic functions of HIV-1 RT.

### Chemistry

The synthetic details for compounds **1–4**, **6–10**, **13–16** and **18–22** are reported elsewhere.[5,7–10] 4-Amino-1-naphthalenesulfonic acid (**11**) was palmitoylated to yield derivative **12**. The reaction of 1-hydroxy-3,6-naphthalenedisulfonic acid with 1,5-naphthalenedisulfonyl chloride under aqueous $NaHCO_3$ conditions produced derivative **17** (Scheme 1). Cholesteryl chloroformate was reacted with 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid to produce the *N*-carbamoyl derivative **23** (Scheme 2). A key starting material, 4-amino-3-hydroxy-1-naphthalenesulfonic acid, was reacted with palmitoyl chloride under different reaction conditions to yield derivatives **24–27** (Scheme 3). Derivative **24** was synthesized at an elevated reaction temperature of 150–160 °C using triethylamine as base in the presence of DMF. Oxazole formation during

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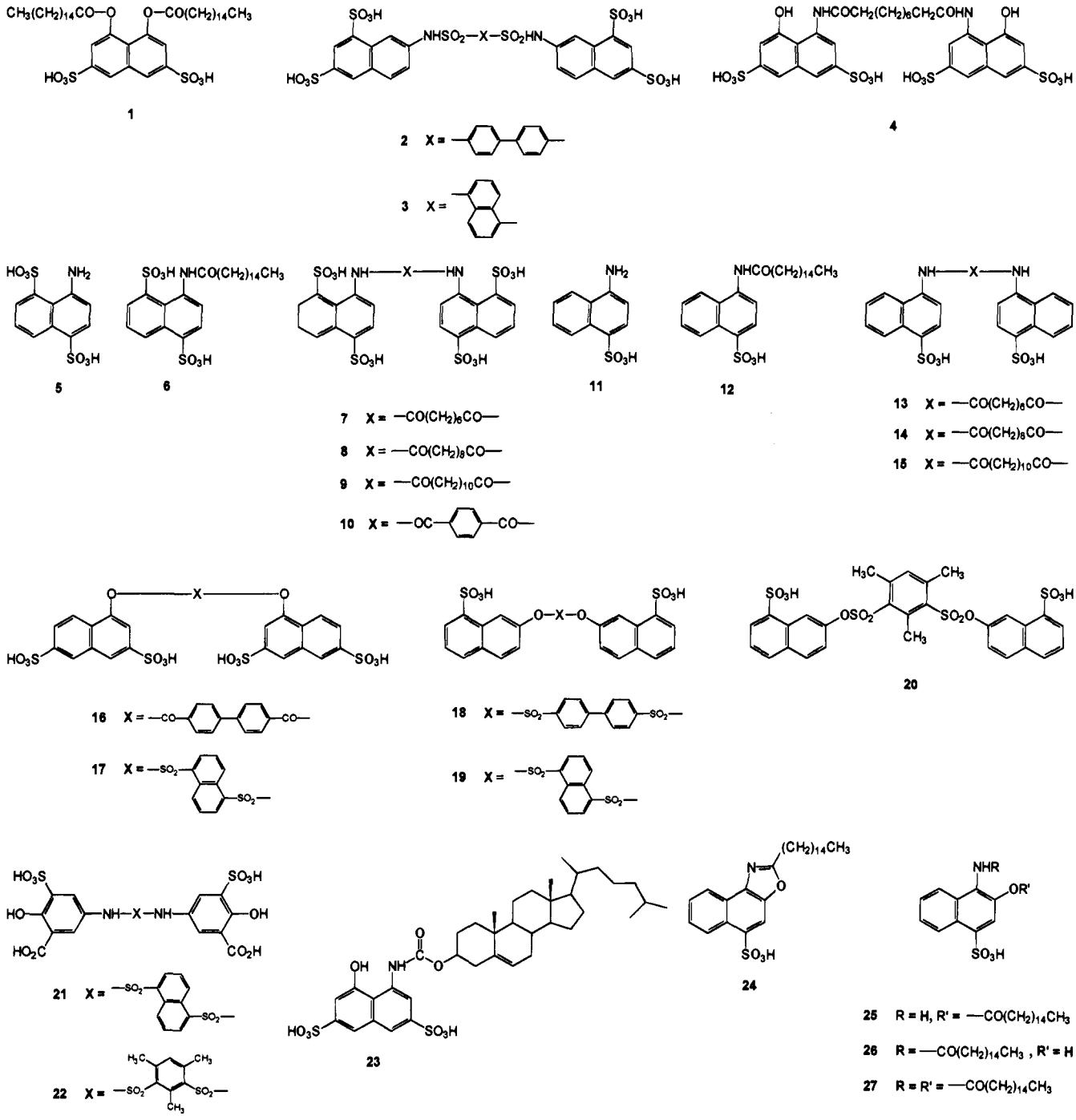
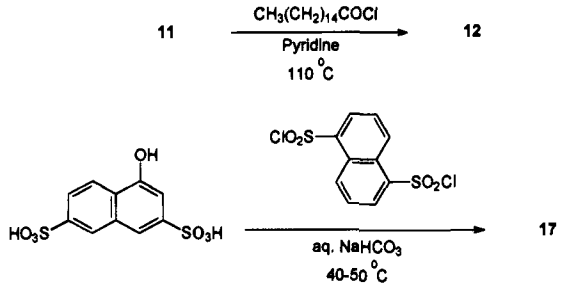
**Chart 1**

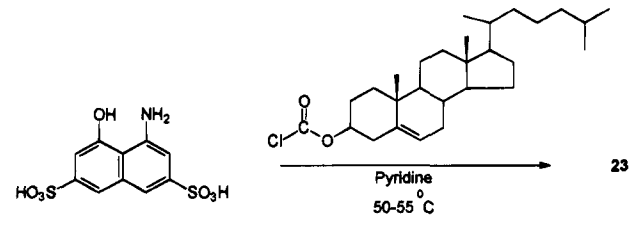



**Scheme 1**

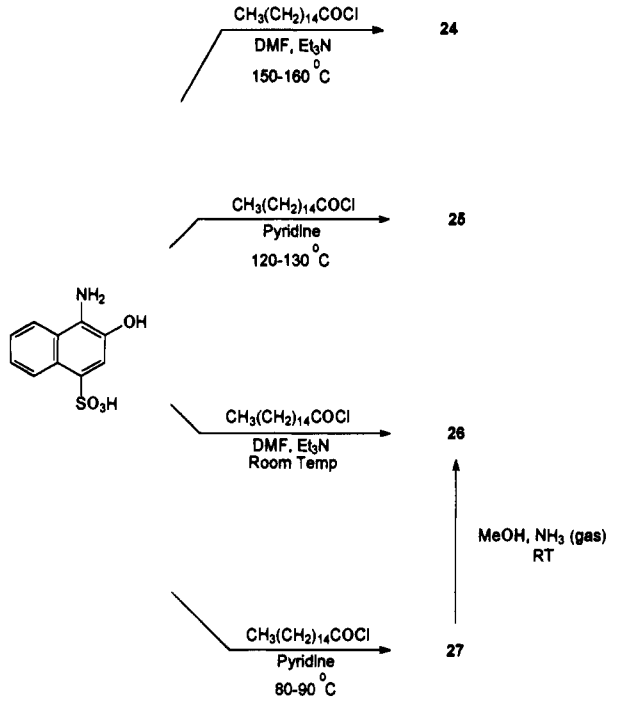



acylation of naphthalenesulfonic acid compounds possessing vicinal amino and phenolic functionalities has been documented[11] and is confirmed by the lack of amino and phenolic proton NMR signals. Analogue **25** was synthesized using pyridine at 120–130 °C. Reaction of 4-amino-3-hydroxy-1-naphthalenesulfonic acid with palmitoyl chloride using triethylamine at room temperature afforded **26**, while use of pyridine with the same reactants at 80–90 °C afforded derivative **27**. Exclusive *O*-palmitoylation as in **25** is corroborated by the presence of the amino proton NMR signal. The unreactivity of the amino functionality in **25** may be

**Scheme 2**



**Scheme 3**




explained by the deactivating effect of the *p*-sulfonic acid group. Selective *N*-palmitoylation as in **26** is verified by the presence of the NMR resonance for the phenolic proton. Similarly, both *N*- and *O*-palmitoylation in **27** was confirmed by $^1$H NMR spectroscopy.[5] The dipalmitoylated product **27** when dissolved in methanol and subjected to bubbling with ammonia at ambient temperature caused *O*-deacylation to yield the *N*-acylated derivative **26**. In agreement with our previous results, several target compounds were isolated as sodium salts containing varied amounts of water of crystallization.[7,8]

## Results and Discussion

In this study, we have screened several representative naphthalenesulfonic acid derivatives from an inventory of over 100 synthesized naphthalenesulfonic acid derivatives differing in length and type of spacer and containing pendant functional groups. As a preliminary screen, the inhibition of DNA polymerase and RNase H activity of HIV-1 RT has been measured (Table 1). In general, among the active compounds, derivatives were more inhibitory to the DNA polymerase function over the RNase H activity of RT. The concentrations of the test compounds used were 50 $\mu$M for DNA polymerase and 200 $\mu$M for RNase H inhibition. Due to the reduced sensitivity of measuring RNase H activity, a higher concentration of test compounds was used for this assay. Of the more than 25 compounds evaluated in this study, derivatives **1**, **4**, **17**, **18**, and **23** completely inhibited the DNA polymerase activity at 50 $\mu$M (0% of initial activity), while derivatives **2**, **6**, **16**, **24**, **26**, and **27** also displayed inhibitory activity by reducing the activity to <1% of the initial activity. In the assay measuring reduction of RNase H activity, derivatives **16** and **23** completely inhibited the enzyme activity (0% of initial activity), while derivatives **6** and **26** inhibited the enzyme activity to ≤3% of initial activity at 200 $\mu$M. In the DNA polymerase assay, the most active naphthalensulfonic acid compounds fall into two broad structural classes. Compounds **2**, **4**, and **16**–**18** belong to the symmetrical class of derivatives consisting of either a flexible polymethylene spacer or an aromatic spacer, while compounds **1**, **6**, **23**, **24**, **26**, and **27** are nonsymmetrical derivatives having lipophilic palmitoyl or cholesteryl moieties. We have previously reported that compound 1 possesses potent inhibitory activities towards recombinant HIV-1 and HIV-2 RT, which was superior to the known RT inhibitor, Suramin.[7]

On the basis of the above data, derivatives **6**, **16**, **17**, **23**, **26**, and **27** were chosen for determinations of 50% inhibitory concentration ($IC_{50}$) values for the inhibition of RNA-dependent DNA polymerase activity (RDDP), DNA-dependent DNA polymerase activity (DDDP), and ribonuclease H (RNase H) activity of RT (Table 2). The dose response curves for inhibiting these three parameters of RT activity for compounds **16**, **23**, and **27** are displayed in Figure 1. The most intriguing structure–activity correlation emerged from compound **6**, a *N*-palmitoylated derivative of the parent sulfonic acid **5**. While parent compound **5** was completely inactive in both the DNA polymerase and RNase H assays (Table 1), compound **6** exhibited $IC_{50}$ values of 1.6, 6.7, and 27.5 $\mu$M for RDDP, DDDP, and RNase H inhibition (Table 2). The comparison of derivatives **6** and **12** also reveals that the naphthalenedisulfonic acid nucleus is necessary for enhancing RT inhibitory activity, an observation also valid for potentiating anti-HIV-1 activity at nontoxic concentrations (Table 3). In comparing derivatives **16** and **17**, both derived from the same naphthalenesulfonic

**Table 1.** Inhibition of DNA Polymerase and RNase H Activity of Reverse Transcriptase by Naphthalenesulfonic Acid Derivatives

| | residual HIV-1 RT activity[d] (% of initial activity) | | | residual HIV-1 RT activity[d] (% of initial activity) | |
|---|---|---|---|---|---|
| compd | DNA polymerase (at 50 $\mu$M) | RNase H (at 200 $\mu$M) | compd | DNA polymerase (at 50 $\mu$M) | RNase H (at 200 $\mu$M) |
| **1**[b] | 0 | 27 | **15**[b] | 100 | 32 |
| **2**[c] | 0.9 | 28 | **16**[c] | 0.2 | 0 |
| **3**[c] | 16 | 51 | **17**[c] | 0 | 43 |
| **4**[c] | 0 | 30 | **18**[b] | 0 | 48 |
| **5**[a] | 100 | 100 | **19**[b] | 25 | 17 |
| **6**[b] | 0.16 | 2.8 | **20**[b] | 13 | 23 |
| **7**[b] | 100 | 84 | **21**[c] | 46 | 75 |
| **8**[b] | 54 | 57 | **22**[c] | 2 | 32 |
| **9**[c] | 13 | 16 | **23**[a] | 0 | 0 |
| **10**[b] | 90 | 92 | **24** | 0.2 | 68 |
| **11** | 100 | 88 | **25** | 6.5 | 7.5 |
| **12** | 100 | 98 | **26**[a] | 0.02 | 3 |
| **13**[b] | 90 | 59 | **27**[a] | 0.03 | 5.3 |
| **14**[b] | 94 | 70 | | | |

[a] Monosodium salt. [b] Disodium slat. [c] Tetrasodium salt. [d] All data are the mean result of at least two separate experiments.

**Table 2.** Inhibition of RNA-Dependent DNA-Polymerase (RDDP), DNA-Dependent DNA-Polymerase (DDDP), and Ribonuclease H (RNase H) Activities by Naphthalenesulfonic Acid Derivatives

| | $IC_{50}$ ($\mu$M)[d] | | |
|---|---|---|---|
| compd | RDDP | DDDP | RNase H |
| **6**[b] | 1.6 | 6.7 | 27.5 |
| **16**[c] | 3.4 | 6.9 | 14.4 |
| **17**[c] | 4.3 | 91.3 | 215 |
| **23**[a] | 0.06 | 7.9 | 21.8 |
| **26**[a] | 3.2 | 13.1 | 47.6 |
| **27**[a] | 0.72 | 6.6 | 16.8 |

[a] Monosodium salt. [b] Disodium salt. [c] Tetrasodium salt. [d] 50% Inhibitory concentration. All data are the mean result of at least two separate experiments.

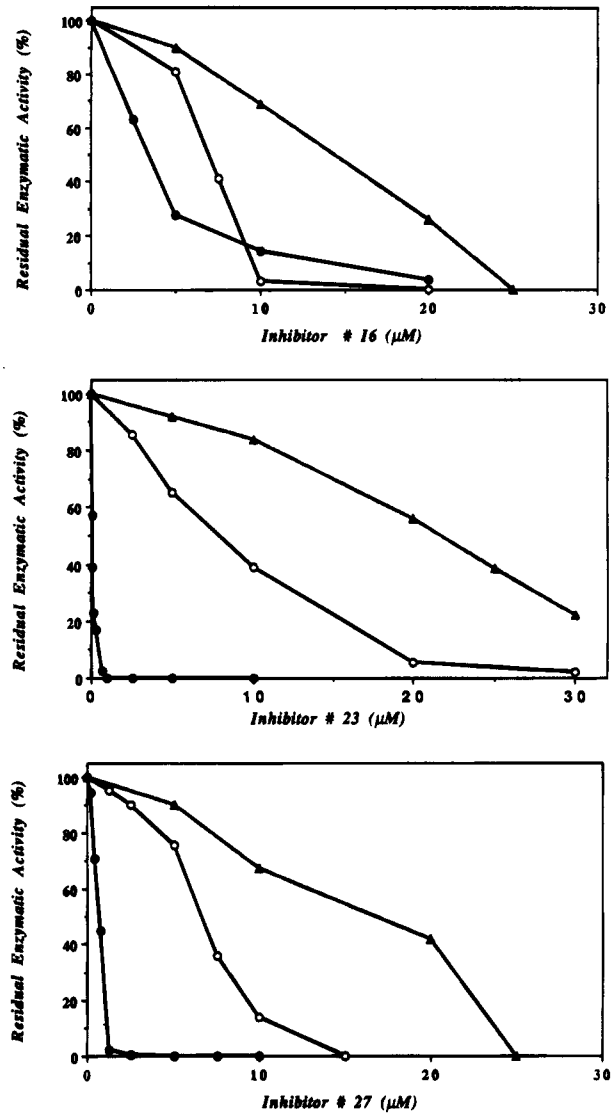



**Figure 1.** Dose-response curves for compounds **16**, **23**, and **27** measuring inhibition of RNA-directed DNA polymerase (●), DNA-directed DNA polymerase (○), and ribonuclease H (▲) activities of HIV-1 RT.

acid moiety, it is clear that a longer aromatic spacer is beneficial for potency. The biphenyl spacer derivative **16** has $IC_{50}$ values of 3.4, 6.9, and 14.4 $\mu$M for RDDP, DDDP, and RNase H inhibition, respectively. The corresponding values for the naphthalene spacer analog **17** are 4.3, 91.3, and 215 $\mu$M (Table 2).

The important role of the palmitoyl functionality in imparting RT inhibitory activity was further substantiated by the evaluation of derivatives **24**–**27**, all of which are derivatives of 4-amino-3-hydroxy-1-naphthalenesulfonic acid. These analogs differ in the position and number of palmitoyl groups on the naphthalene ring. The evaluation of derivatives **26** and **27**, derived from the same naphthalenesulfonic acid moiety, demonstrated that the analog with two palmitoyl functionalities, derivative **27**, was more active than derivative **26** containing one palmitoyl functionality. Compound **27** exhibited $IC_{50}$ values of 0.72, 6.6, and 16.8 $\mu$M for RDDP, DDDP, and RNase H inhibition, respectively (Table 2). Considering the beneficial effect of the palmitoyl functionality in potentiating RT inhibitory activity, we decided to investigate whether other lipophilic appendages would fulfil this apparent hydrophobic requirement

**Table 3.** Inhibition of Sulfonic Acid Derivatives on the Replication of a Laboratory Strain of HIV-1 (HTLV-$III_B$) in MT-4 Cells[d]

| compd | $EC_{50}$ ($\mu$M)[e] | $CC_{50}$ ($\mu$M)[f] |
|---|---|---|
| **6**[b] | 100 | 225 |
| **12** | >47 | 47 |
| **15**[b] | 260 | >500 |
| **16**[c] | 81.5 | >500 |
| **17**[c] | 39 | 321.5 |
| **20**[b] | 42 | 228 |
| **23**[a] | >500 | >500 |
| **24** | >42 | 42 |
| **25** | >38 | 38 |
| **26**[a] | >21 | 21 |
| **27**[a] | >41 | 41 |

[a] Monosodium salt. [b] Disodium salt. [c] Tetrasodium salt. [d] All data are the mean result of at least two separate experiments. [e] 50% effective antivirial concentration. [f] 50% cytotoxic concentration.

for the inhibition of RT activity. Hence, we evaluated derivative **23** consisting of a bulky cholesteryl group linked to 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid. Compound **23** emerged as the most potent derivative, displaying $IC_{50}$ values of 0.06, 7.9, and 21.8 $\mu$M for inhibition of RDDP, DDDP, and RNase H activity respectively (Table 2).

In analyzing the structural parameters required for RT inhibition in the 4-amino-3-hydroxy-1-naphthalenesulfonic acid series, the pronounced activities (Table 2) of analogs **26** ($IC_{50}$ = 3.2 $\mu$M for RDDP inhibition) and **27** ($IC_{50}$ = 0.72 $\mu$M for RDDP inhibition) suggests the need for at least one free hydrogen on the nitrogen atom to produce significant inhibition. However, if an extra sulfonic acid group is present on the naphthalene nucleus, as in **6**, there is no stringent requirement for the 3-phenolic functionality. Nevertheless, the palmitoyl function is important because parent compound **5** is inactive in both of the assays (Table 1). Similarly, the cholesteryl moiety has an influential role since the parent 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid moiety in **23** is inactive in inhibiting both HIV-1 induced cytopathogenesis and RT (Data not shown). Further RT studies on the fragments (potential hydrolysis products) of the most active derivatives revealed inactivity (data not shown), suggesting that potency was due to the intact molecule. In this regard, it is interesting that among the fragments, inhibitory activity was revealed only for the 4-hydroxy-2,7-naphthalenedisulfonic acid against the polymerase function (9.7% of initial activity at 50 $\mu$M concentration and no activity against the RNase H function at 200 $\mu$M concentration) of the enzyme. The fact that fragment 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid is inactive in both the polymerase and RNase H RT assays also suggests that the amino functionality is detrimental to the RT activity of the fragment naphthalene molecule.

In considering the anti-HIV-1 activity of these compounds as measured in the whole virus assay, active symmetrical derivatives **16** ($EC_{50}$ = 81.5 $\mu$M, $CC_{50}$ = >500 $\mu$M) and **17** ($EC_{50}$ = 39 $\mu$M, $CC_{50}$ = 321.5 $\mu$M) were moderately active at nontoxic concentrations (Table 3). On the contrary, the nonsymmetrical steroid analog **23** and the palmitoylated analogs **24**–**27** were all active at toxic concentrations. In this regard, cosalane, a steroid aurintricarboxylic monomer analog, demonstrates *in vitro* anti-HIV-1 activity at nontoxic concentrations.[12] The mechanism of action of these

naphthalenesulfonic acid analogues in the whole virus assay is probably not due to RT inhibition, since it is unlikely that they enter cells. Instead, like other analogues in this series, their activity is probably due to the inhibition of virus adsorption involving specific interaction with the envelope glycoprotein gp120.[13] Nevertheless, the discovery of nonsymmetrical small molecule agents capable of inhibiting both the DNA polymerase and RNase H functions of RT is not common. For example, TIBO derivative R81250 inhibits the DNA polymerase function but not the RNase H activity of the enzyme. Nevirapine, the pyridinones, and a quinoline derivative act on the polymerase function of the enzyme.[14] However, a polymeric derivative, the ammonium salt of poly(vinylsulfonic acid), inhibits both the polymerase and RNase functions of HIV-1 RT.[15] Sulfated polysaccharides such as dextran sulfate have been shown to be more potent inhibitors of the RNase H function than the polymerase function of RT.[16]

The marked inhibitory potential of naphthalenesulfonic acid moieties tethered to lipophilic substituents is an interesting and unique observation. These studies substantiate our previous report on the governing role of the palmitoyl functionality in inhibiting the activity of RT[7]. The function of the palmitoyl or the steroid moiety is not apparent at the present time. It is possible that these lipophilic groups interact with the hydrophobic core of the enzyme and the naphthalenesulfonic acid units chelate with the metal ions that are essential for the catalytic activity of the RT enzyme.[17,18] Due to the charged nature of these compounds, structural modifications will be required in order to achieve cell penetration. Such a modification will be necessary to realize the RT inhibitory potential of these derivatives in the whole virus assay. This is a worthwhile endeavor because the steroid derivative **23** also has activity against HIV-1 protease[10] and joins a class of unique multifunctional inhibitors of HIV-1[19] that deserve further chemical development.

## Experimental Section

**Synthetic Procedures.** Melting points were determined on a Mel-Temp II apparatus and are uncorrected. NMR spectra were recorded on Varian XL-300 (300 MHz) instrument in DMSO-$d_6$. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. IR spectra were recorded on MIDAC FT IR system using KBr pellets. Analytical thin-layer chromatography was performed with Baker-flex silica gel IB2-F sheets. Elemental analyses were carried out at Midwest Microlab (Indianapolis, IN). Pyridine was distilled from potassium hydroxide and stored over fresh potassium hydroxide. Gel permeation chromatography was performed using Spectra/Gel 05 (Fisher; Itasca, IL; filtration range 300–2500 MW) and using deionized water under $N_2$ pressure as eluent.

**4-(Palmitoylamino)-1-naphthalenesulfonic Acid (12).** 4-Amino-1-naphthalenesulfonic acid (1.00 g, 4.48 mmol) was heated to 110 °C for 30 min, palmitoyl chloride (1 mL, 2.5 mmol) was added, and the reaction mixture was further heated for 15 min under argon atmosphere. To this mixture was added dry pyridine (3 mL), and the reaction mixture was stirred for 24 h at 110 °C. An additional amount of palmitoyl chloride (1.5 mL, 3.75 mmol) was added to the reaction mixture and stirred for another 65 h at 110 °C. The reaction mixture was evaporated to dryness, and chloroform (30 mL) was added. The solution was filtered, and the filtrate was evaporated to give a dark-colored dense liquid which on addition of anhydrous ether (35 mL) produced a light pink solid. The solid was filtered and was twice recrystallized from hot methanol to yield the pure product (0.086 g, 4.15%): mp 266–267 °C; IR 3235 (NH), 2920, 2849, 1647 (C=O), 1547 (NH), 1182 (S=O), 1063, 856, 769, 694 $cm^{-1}$; NMR $\delta$ 9.89 (s, 1H, exchangeable with $D_2O$), 8.87 (m, 1H), 8.04 (m, 1H), 7.91 (d, $J$ = 7.9 Hz, 1H), 7.58 (d, $J$ = 8.0 Hz, 1H), 7.52 (m, 2H), 7.12 (s, 2H, exchangeable with $D_2O$), 2.47 (m, 2H), 1.65 (m, 2H), 1.24 (s, 24H), 0.85 (t, $J$ = 6.6 Hz, 3H); (−)-FABMS *m/e* 460, calcd for $[C_{26}H_{39}NO_4S - H]^-$ 460. Anal. ($C_{26}H_{39}NO_4S \cdot H_2O$) C, H, N.

**4,4′-[1,5-Naphthalenediylbis(sulfonyloxy)]bis(2,7-naphthalenedisulfonic Acid) (17).** A mixture of 1-hydroxy-3,6-naphthalenedisulfonic acid disodium salt (0.35 g, 1 mmol), 1,5-naphthalenedisulfonyl chloride (0.25 g, 0.75 mmol), and $NaHCO_3$ (0.17 g, 2 mmol) in deionized water (20 mL) was stirred at 40–50 °C for 40 h. The reaction mixture was evaporated to dryness, and the resulting solid was dissolved in methanol and filtered. The filtrate was concentrated and triturated with ether to produce a white solid (0.42 g). A portion of this solid (0.25 g) was dissolved in water (2 mL) and introduced onto a gel permeation column. The column was eluted with water, and appropriate fractions were pooled to yield the pure product isolated as the tetrasodium salt (0.12 g, 50%): mp >360 °C; IR 3445, 1635, 1371 (S=O, sulfonyloxy), 1190 (S=O, sulfonyloxy), 1120 (S=O), 1037, 926, 804 $cm^{-1}$; NMR $\delta$ 9.32 (d, $J$ = 9.3 Hz, 2 H), 8.59 (d, $J$ = 8.6 Hz, 2 H), 8.29 (s, 2 H), 8.22 (s, 2 H), 8.17 (d, $J$ = 8.2 Hz, 2 H), 7.96 (d, $J$ = 7.9 Hz, 2 H), 7.84 (d, $J$ = 7.8 Hz, 2 H), 7.42 (s, 2 H). Anal. ($C_{30}H_{16}O_{18}S_6Na_4 \cdot 4H_2O$) C, H.

**4-[[(3-Cholesteryloxy)carbonyl]amino]-5-hydroxy-2,7-naphthalenedisulfonic Acid (23).** 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid monosodium salt (2.05 g, 6 mmol) and cholesteryl chloroformate (2.92 g, 6.5 mmol) were reacted together at 50–55 °C for 15 min, after which time anhydrous pyridine (15 mL) was added. An initial effervescence was observed, and the reaction was stirred for 21 h at 50–55 °C. The reaction mixture was evaporated to dryness, and the residue was washed thoroughly with $CHCl_3$ (3 × 10 mL) and then triturated with methanol (25 mL). The methanolic solution was coevaporated with *n*-heptane (4 × 5 mL). The dried mass was dissolved in methanol (20 mL), and the insolubles were discarded. $NH_3$ gas was passed through the methanolic solution for *ca.* 20 s followed by coevaporation with *n*-heptane (3 × 5 mL) to yield a dark gray solid (0.957g, 22%). An analytically pure sample was obtained by recrystallizing the solid four times from methanol/acetone/water (8:1:1): mp 227–229 °C dec; IR 3477 (NH/OH), 3123 (NH/OH), 1614 (C=O), 1372, 1187 (S=O), 1047, 684 $cm^{-1}$; NMR $\delta$ 10.57 (s, 1H, exchangeable with $D_2O$), 8.47 (s, 1H), 7.65 (s, 1H), 7.52 (s, 1H), 7.15 (s, 1H), 5.41 (s, 1H), 4.54 (bs, 2H), 1.29 (m, 43H). Anal. ($C_{38}H_{52}O_9S_2NNa \cdot 3H_2O$) C, H, N, $H_2O$ verified by Karl–Fischer analysis.

**2′-Pentadecylnaphth[3,4-*d*]oxazole-1-sulfonic Acid (24).** A mixture of 4-amino-3-hydroxy-1-naphthalenesulfonic acid (0.24 g, 1 mmol), palmitoyl chloride (0.73 g, 3 mmol), triethylamine (0.40g, 4 mmol), and DMF (10 mL) was heated at 150–160 °C for 20 h under a nitrogen atmosphere. After cooling, the reaction mixture was triturated with ether (100 mL), and the separated solid was filtered and washed with ether (4 × 20 mL). The resulting solid was stirred in methanol (40 mL) for 1 h. The resulting mixture was filtered, and the solid so obtained was washed with methanol (3 × 20 mL) to yield a sky blue solid which was recrystallized from water/DMSO (3:1) to afford the pure product (0.40 g, 87%): mp 276–277 °C dec; IR 2924, 2858, 1593 (C=N), 1444, 1371, 1203 (S=O), 1103, 1037, 864 $cm^{-1}$; NMR $\delta$ 8.98 (d, $J$ = 8.6 Hz, 1 H), 8.34 (d, $J$ = 7.8 Hz, 1 H), 8.21 (s, 1 H), 7.66 (t, $J$ = 7.3 Hz, 1 H), 7.58 (t, $J$ = 7.1 Hz, 1 H), 3.04 (t, $J$ = 7.4 Hz, 2 H), 1.86 (m, 2 H), 1.23 (br s, 24 H), 0.84 (t, $J$ = 6.4 Hz, 3 H); (−)-FABMS *m/e* (relative intensity) 458 ($[M - H]^-$, 100). Anal. ($C_{26}H_{37}NO_4S$) C, H, N.

**4-Amino-3-(palmitoyloxy)-1-naphthalenesulfonic Acid (25).** 4-Amino-3-hydroxy-1-naphthalenesulfonic acid (0.24 g, 1 mmol) and palmitoyl chloride (0.73 g, 3 mmol) were heated at 120–130 °C for 10 min, after which time dry pyridine (5 mL) was added. After the mixture was refluxed for 25 h under a nitrogen atmosphere, pyridine was evaporated and the resulting solid was washed with ether (6 × 20 mL) and dissolved in methanol. After having stood overnight, the

solution was filtered and evaporated to yield a white solid (0.40 g, 84%) which was recrystallized from ethanol to yield the product: mp 272 °C; IR 3466 (NH), 3186, 2922, 2847, 1593 (C=O), 1444, 1371, 1203 (S=O), 1103, 864 $cm^{-1}$; NMR $\delta$ 8.98 (d, $J$ = 8.3 Hz, 1 H), 8.34 (d, $J$ = 7.4 Hz, 1 H), 8.21 (s, 1 H), 7.67 (t, $J$ = 8.2 Hz, 1 H), 7.57 (t, $J$ = 8.6 Hz, 1 H), 7.12 (s, 2 H, exchangeable with $D_2O$), 3.05 (t, $J$ = 7.4 Hz, 2 H), 1.67 (m, 2 H), 1.24 (br s, 24 H), 0.86 (t, $J$ = 7.0 Hz, 3 H); (−)-FABMS *m/e* (relative intensity) 458 ([M − H − $(H_2O)$]$^-$, 100). Anal. ($C_{26}H_{39}NSO_5$) C, H.

**4-(Palmitoylamino)-3-hydroxy-1-naphthalenesulfonic Acid (26).** 4-Amino-3-hydroxy-1-naphthalenesulfonic acid (0.24 g, 1 mmol) was added to dry DMF (5 mL) at 0 °C followed by palmitoyl chloride (0.73 g, 3 mmol) and triethylamine (0.40 g, 4 mmol). The reaction mixture was allowed to attain room temperature and stirred for 16 h at room temperature. After this time, the mixture was triturated with ether (80 mL). The separated solid was filtered and washed with ether (4 × 25 mL). The obtained solid was stirred with methanol (60 mL) for 2 h, filtered, and washed with warm methanol (3 × 10 mL). The filtrate was concentrated and triturated with ether. The separated solid was filtered and stirred for 4 h with 2% $NaHCO_3$ solution (40 mL). The insoluble residue was filtered and washed with cold water (5 × 20 mL). The product was recrystallized from aqueous methanol (5:1) to produce white needles isolated as the pure monosodium salt (0.21 g, 42%): mp 130 °C dec; IR 3342 (NH/OH), 2927, 2849, 1652 (C=O), 1520 (NH), 1275, 1181 (S=O), 1058 $cm^{-1}$; NMR $\delta$ 9.46 (s, 1 H, exchangeable with $D_2O$), 9.41 (s, 1 H, exchangable with $D_2O$), 8.71 (d, $J$ = 7.9 Hz, 1 H), 7.70 (s, 1 H), 7.66 (d, $J$ = 8.2 Hz, 1 H), 7.38 (t, $J$ = 7.6 Hz, 1 H), 7.27 (t, $J$ = 7.2 Hz, 1 H), 2.41 (t, $J$ = 7.0 Hz, 2 H), 1.61 (m, 2 H), 1.20 (br s, 24 H), 0.81 (t, $J$ = 6.8 Hz, 3 H); (−)-FABMS *m/e* (relative intensity) 476 ([M − Na − H]$^-$, 100). Anal. ($C_{26}H_{38}NO_5SNa$) C, H.

**4-(Palmitoylamino)-3-(palmitoyloxy)-1-naphthalenesulfonic Acid (27).** 4-Amino-3-hydroxy-1-naphthalenesulfonic acid (0.24 g, 1 mmol) was dissolved in dry pyridine (8 mL), and palmitoyl chloride (1.10 g, 4 mmol) was added. The reaction mixture was then stirred at 80−90 °C for 4 h. Pyridine was evaporated, and ether (80 mL) was added. The separated solid was filtered, washed with ether (5 × 20 mL), and stirred for 2 h with 2% $NaHCO_3$ solution (30 mL). The separated solid was filtered, washed with water and dissolved in minimum amount of cold methanol (*ca.* 10 mL), and the solution was filtered. The filtrate was warmed, and to it was added dropwise water until the cloud point was reached. After this, the solution was allowed to stand overnight. The separated white solid was filtered and dried to yield the pure product as the monosodium salt (0.23 g, 31%): mp 256 °C dec; IR 3311 (NH), 2955, 2859, 1738 (C=O, amide), 1663 (C=O, ester), 1593 (NH), 1448, 1371, 1203 (S=O), 1103, 864 $cm^{-1}$; NMR $\delta$ 9.75 (s, 1 H, exchangeable with $D_2O$), 8.88 (d, $J$ = 9.8 Hz, 1 H), 7.93 (d, $J$ = 9.7 Hz, 1 H), 7.77 (s, 1 H), 7.58 (m, 2 H), 2.59 (t, $J$ = 7.5 Hz, 2 H), 2.45 (t, $J$ = 7.5 Hz, 2 H), 1.68 (m, 4 H), 1.29 (br s, 48 H), 0.90 (t, $J$ = 6.9 Hz, 6 H); (+)-FABMS *m/e* (relative intensity) 739 ([M+H]$^+$, 60), 380 (100). Anal. ($C_{42}H_{68}NOS_6Na \cdot 0.5H_2O$) C, H, N.

**Antiviral Assays. Cytopathogenesis Assay.** Activity of the compounds against the replication of HIV-1 was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells, as previously described.[20] Briefly, MT-4 cells were infected with HTLV-$III_B$ (a laboratory strain of HIV-1) at a multiplicity of infection of 0.02 and incubated in the presence of various concentrations of test compounds. After a 4-day incubation, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) method, as previously described.[20]

**Reverse Transcriptase Assays. Enzymes.** The reverse transcriptase used in this study was recombinant enzyme expressed in *Escherichia coli*. HIV-1 RT was derived from BH-10 proviral clone of HIV-1.[21] The enzyme was purified to homogeneity according to the protocol described previously,[22] yielding polypeptide with apparent molecular weight of approximately 66 kDa for HIV-1. Prolonged storage of enzyme was performed in 50% glycerol (v/v), 2 mM dithiothreitol, 25 mM Tris-Cl, pH 8.0 at −80 °C.

**Enzymatic Assays.** Enzymatic assays for HIV-1 RT was carried out as described previously.[23,24] In all inhibition experiments the enzymes were preincubated for 5 min at 30 °C in the absence or in the presence of inhibitor at various concentrations. The enzymatic reactions were initiated by adding the appropriate substrate followed by an incubation for 30 min at 37 °C. Enzymatic residual activity was calculated relative to the initial linear reaction rates observed when no drug was added. The inhibitor concentration leading to 50% inhibition ($IC_{50}$ values) of the enzymatic activities was calculated from the inhibition curves as a function of the inhibitor concentrations. Enzymatic activities were defined as follows: One unit of DNA polymerase activity is the amount of enzyme that catalyzes the incorporation of 1 pmol of dNTP into DNA product after 30 min at 37 °C under the standard assay conditions. One unit of the RNaseH activity is the amount of enzyme that catalyzes the hydrolysis of 1 pmol of AMP after 30 min at 37 °C under the outlined assay conditions.

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