5.06 (s, 2 H, OCH_2Ph), 5.21 (d, J = 5.7 Hz, 1 H, H-4'), 5.80 (bs, 1 H, H-1'), 5.94 (bs, 1 H, H-2'), 6.28 (d, J = 2.0 Hz, 1 H, H-3), 6.57 (dd, J = 6.0 Hz, J' = 2.0 Hz, 1 H, H-5), 7.25–7.45 (m, 10 H, Ph), 8.04 (d, J = 6.0 Hz, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 26.1, 27.5, 66.8 (+, C-6'), 69.8, 72.8, 83.0 (–, C-1'), 83.4 (–, C-5'), 83.9 (–, C-4'), 95.2 (–, C-5), 106.8 (–, C-3), 112.2, 126.4 (–, C-2'), 127.5, 127.7, 128.3, 128.4, 128.7, 135.8, 138.0, 147.8 (+, C-3'), 147.8 (–, C-6), 164.8 (+, C-2), 167.0 (+, C-4). Anal. ($C_{28}H_{29}NO_5$) C, H, N.

(1'*R*,4'*R*,5'*S*)-1-[3-(Hydroxymethyl)-4,5-dihydroxy-2-cyclopenten-1-yl]-4-hydroxy-2-pyridone (6, 3-Deaza-CPE-U). A solution of protected nucleoside 15 (0.245 g, 0.534 mmol) in dry CH_2Cl_2 was stirred at −78 °C under a nitrogen atmosphere. This solution was treated with 3.2 mL of a 1 M solution of BCl_3 in CH_2Cl_2 and stirred at −78 °C for 3.5 h. Methanol (5 mL) was added and the solution was allowed to reach room temperature. The solution was concentrated in vacuo and coevaporated three more times with MeOH. The residual compound was dissolved in water (6 mL) and lyophilized. Reversed-phase (C-18) column chromatography with 5% aqueous methanol as eluant afforded 0.041 g (32%) of the desired target compound **6** as a white amorphous powder; $[\alpha]^{25}_D$ = −55.6° (c 1.34, H_2O); UV (H_2O, pH 1) λ_{max} 280 nm (log ϵ 3.65), UV (H_2O, pH 11) λ_{max} 268 (sh, log ϵ 3.80), 255 nm (log ϵ 3.91); ^1H NMR (CD_3OD) δ 4.01 (t, J = 5.5 Hz, 1 H, H-5'), 4.55 (bs, 2 H, H-6'$_{a,b}$), 5.70 (d, J = 5.7 Hz, 1 H, H-4'), 5.70 (bd, J = 1.8 Hz, 1 H, H-2'), 5.77 (m, 1 H, H-1'), 5.83 (d, J = 2.6 Hz, 1 H, H-3), 6.07 (dd, J = 7.5 Hz, J' = 2.6 Hz, 1 H, H-5), 7.33 (d, J = 7.6 Hz, 1 H, H-6); ^{13}C NMR (Me_2SO-d_6) δ 58.6 (+, C-6'), 64.3 (–, C-1'), 72.5 (–, C-5'), 77.4 (–, C-4'), 98.2 (–, C-5), 100.2 (–, C-3), 124.0 (–, C-2'), 135.4 (–, C-6), 151.1 (+, C-3'), 163.3 (+, C-2), 166.2 (+, C-4); FAB mass spectrum, m/z (relative intensity) 332 (MH + glycerol, 3), 240 (MH^+, 100), 112 (b + 2 H, 98). Anal. ($C_{11}H_{13}NO_5$) C, H, N.

Biological Tests. In vitro cytotoxicity evaluations were performed by Dr. Richard L. Cysyk and Ms. Nancy M. Malinowski of the Laboratory of Biological Chemistry, DTP, DCT, NCI. Antiviral activity evaluations were conducted under NIH purchase order 263-MD-610174 at Southern Research Institute, Birmingham, AL, under the direction of Dr. William Shannon and Ms. Gussie Arnett.

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Potential Anti-AIDS Agents. Synthesis and Antiviral Activity of Naphthalenesulfonic Acid Derivatives against HIV-1 and HIV-2

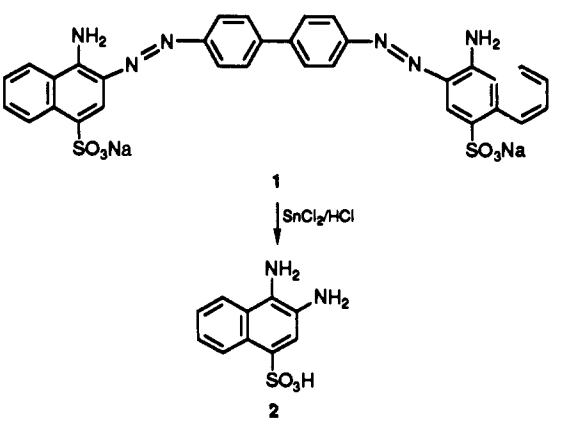
Prem Mohan,*,† Rajendra Singh,†,‡ and Masanori Baba§

Department of Medicinal Chemistry and Pharmacognosy (M/C 781), College of Pharmacy, University of Illinois at Chicago, Box 6998, Chicago, Illinois 60680, and Department of Bacteriology, Fukushima Medical College, Hikarigaoka 1, Fukushima 960-12, Japan. *Received March 22, 1990*

Certain naphthalenesulfonic acid analogues have been synthesized and evaluated for their inhibitory effects on HIV-1- and HIV-2-induced cytopathogenicity, HIV-1 giant cell formation, and HIV-1 reverse transcriptase (RT) activity. A bis(naphthalenedisulfonic acid) derivative having a biphenyl spacer emerged as the most potent and selective inhibitor of virus-induced cytopathogenicity in MT-4 cells. The ED_{50} values for this compound were 7.6 and 36 μM for HIV-1 and HIV-2, respectively. No toxicity to the host cells was detected at 98 μM. This compound also inhibited giant cell formation and was superseded in potency by a bis(naphthalenedisulfonic acid) derivative having a flexible decamethylene spacer. In the cell-free RT assay, a long-chain amide derivative exhibited the most inhibition of RT. All the compounds that achieved complete inhibition of virus-induced cytopathogenicity at concentrations not toxic to host cells were derivatives of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid. These analogues represent new leads for the development of anti-HIV agents.

Since the discovery of a retrovirus as the causative agent for AIDS,[1,2] only 3'-azido-2',3'-dideoxythymidine (AZT) has gained wide-scale FDA approval for treatment of patients with AIDS and ARC.[3] However, AZT use has been associated with toxicity,[4] resumption of viral replication,[5] the occurrence of cell to cell virus transmission,[6] and the emergence of AZT-resistant HIV-1 strains during prolonged therapy.[7] It has been demonstrated that common mutations in the HIV-1 reverse transcriptase gene can confer resistance to AZT.[8] Many nucleoside analogues with potential anti-AIDS activities have been reported[9-16] and the mechanism of action of most dideoxynucleosides is established.[17] It is pertinent to note that, so far, this approach has proved to be incapable of completely halting

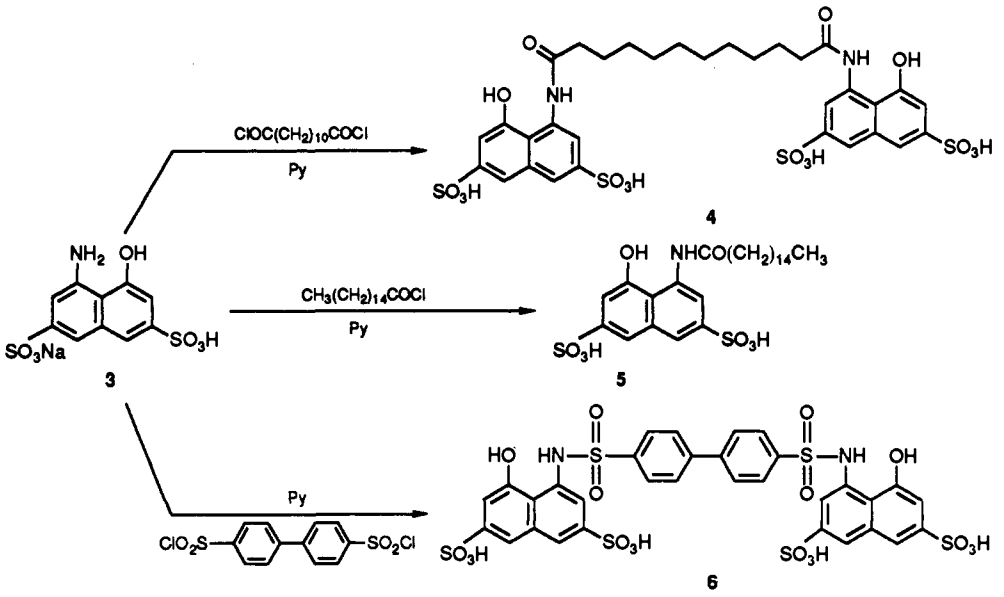
Scheme I



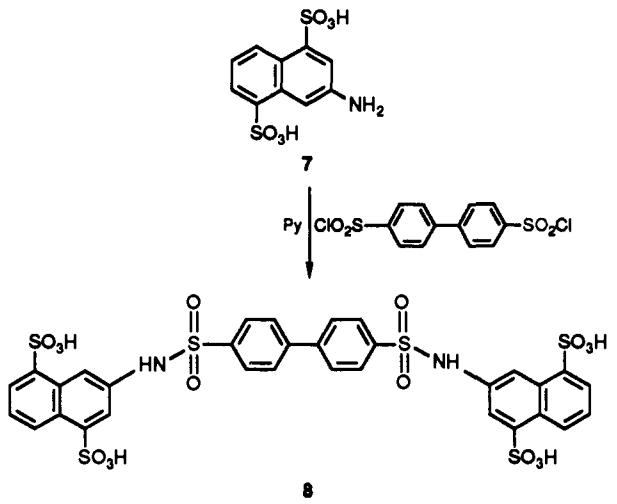
* Scholar of the American Foundation for AIDS Research. To whom reprint requests should be addressed.
† University of Illinois at Chicago.
‡ On leave from the Department of Applied Chemistry, Institute of Technology, Banares Hindu University, Varanasi, India.
§ Fukushima Medical College.

0022-2623/91/1834-0212$02.50/0

Scheme II



Scheme III



and/or eradicating the virus in toto. Novel anti-HIV mechanisms of action have been demonstrated by several *non-nucleoside* agents.[18] These include various natural products and/or their analogues[19-33] and other diverse

Table I. Inhibitory Effects of Naphthalenesulfonic Acids on HIV-1- and HIV-2-Induced Cytopathogenicity in MT-4 Cells[d]

		% inhibn		
no.	concn μg/mL (μM)	HIV-1	HIV-2	cell viability
2	100 (420)	0	0	97
	20 (84)	0	0	1
	4 (16.8)		3	0
	0.8 (3.4)	0	1	13
4[a]	100 (107)	100	100	0
	20 (21.4)	14	4	9
	4 (4.3)	2	3	5
	0.8 (0.9)	0	3	4
5[b]	100 (167)	100	100	0
	20 (33.4)	7	12	1
	4 (6.7)	0	3	0
	0.8 (1.3)	5	6	0
6[c]	100 (98)	100	100	0
	20 (19.6)	100	20	0
	4 (3.9)	15	7	0
	0.8 (0.8)	5	6	0
8[c]	100 (101)	100	94	0
	20 (20.2)	8	9	0
	4 (4)	1	1	0
	0.8 (0.8)	0	0	1

[a] Disodium salt. [b] Monosodium salt. [c] Tetrasodium salt. [d] All data represent mean values for two separate experiments.

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Table II. ED_{50} of Naphthalenesulfonic Acids for Virus-Induced Cytopathogenicity and CD_{50} for Cell Viability[a]

no.	ED_{50}, μg/mL (μM) HIV-1	ED_{50}, μg/mL (μM) HIV-2	CD_{50}, μg/mL (μM)
2	>45 (>189)	>45 (>189)	45 (189)
4[b]	39 (42)	43 (46)	>100 (107)
5[c]	42 (70)	40 (67)	>100 (167)
6[d]	7.8 (7.6)	37 (36)	>100 (98)
8[d]	42 (42)	43 (43)	>100 (101)

[a] ED_{50} = 50% antiviral dose, based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells. CD_{50} = 50% cytotoxic dose, based on the reduction of viability of mock-infected MT-4 cells. All data represent mean values for two separate experiments. [b] Disodium salt. [c] Monosodium salt. [d] Tetrasodium salt.

chemical entities.[34-44] As part of an ongoing search for new anti-AIDS leads, a previous study of the in vitro anti-HIV-1 activity of certain naphthalenedisulfonic acid derivatives[45,46] provided the impetus to further design and biologically evaluate other analogues of this class of compounds for potential anti-AIDS activity. In addition, since Congo Red and related anti-HIV dyes are known to be metabolized to carcinogenic aromatic amines in vivo,[47] we

Table III. Inhibitory Effect of Naphthalenesulfonic Acids on Giant Cell Formation[a]

no.	concn, μM	% inhibn	ID_{50}, μg/mL (μM)
2	2100	T	>100 (>420)
	420	0	
	84	1	
	16.8	2	
	3.4	7	
4[b]	535	100	24 (26)
	107	99	
	21.4	44	
	4.3	15	
	0.9	2	
5[c]	835	T	46 (77)
	167	95	
	33.4	4	
	6.7	9	
	1.3	0	
6[d]	490	100	32 (31)
	98	100	
	19.6	30	
	3.9	0	
	0.8	4	
8[d]	505	100	45 (45)
	101	100	
	20.2	0	
	4	0	
	0.8	0	

[a] ID_{50} = 50% inhibitory dose. T = toxic. All data indicate representative results. [b] Disodium salt. [c] Monosodium salt. [d] Tetrasodium salt.

Table IV. Inhibitory Effect of Naphthalenesulfonic Acids and Suramin on the Activity of Cell-Free HIV-1 Reverse Transcriptase[a]

no.	concn, μM	% inhibn	ID_{50}, μg/mL (μM)
2	2100	T	>500 (>2100)
	420	0	
	84	1	
	16.8	2	
4[b]	535	100	160 (171)
	107	99	
	21.4	44	
	4.3	15	
5[c]	835	T	50 (83.5)
	167	95	
	33.4	4	
	6.7	9	
6[d]	490	100	190 (186)
	98	100	
	19.6	30	
	3.9	0	
8[d]	505	100	370 (374)
	101	100	
	20.2	0	
	4	0	
suramin	350	92	42 (29.4)
	70	83	
	14	22	
	2.8	18	

[a] ID_{50} = 50% inhibitory dose. All data represent mean values for two separate experiments. [b] Disodium salt. [c] Monosodium salt. [d] Tetrasodium salt.

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wanted to explore the potential of using other spacers for the naphthalene units. Indeed, in this report we show that this approach has been fruitful.

Chemistry

Congo Red (1) was reduced with stannous chloride and hydrochloric acid to afford 3,4-diaminonaphthalenesulfonic acid (2) (Scheme I). This reagent was more suitable, in terms of purification, over the dithionite reduction[48] method. Starting material 3 was reacted with dodecanedioyl dichloride in pyridine to give the bis(naphthalenedisulfonic acid) compound 4. Similarly, reaction of 3 with palmitoyl chloride and pyridine produced amide derivative 5. Reaction of 3 with 4,4'-biphenyldisulfonyl chloride afforded derivative 6 (Scheme II). The isolated reaction products 4–6 showed acylation or sulfonylation of the amino group as observed by the presence of the phenolic signal in the NMR. This is similar to the acylation reaction of aminohydroxynaphthalenesulfonic acids with nitrobenzoyl chloride.[49] Treatment of 7 with the disulfonyl chloride in pyridine afforded 8 (Scheme III). The target compounds were only amenable to negative ion FAB mass spectral analysis, as documented for many naphthalenesulfonic acid compounds.[50] In agreement with the observation that naphthalenesulfonic acids tend to bear an undefined number of waters of crystallization,[51] derivatives 4–6 and 8 were isolated as hydrates containing one or three molecules of water.

Biological Results and Discussion

Inhibition of HIV-1 and HIV-2 cytopathogenicity was studied in MT-4 cells. These results illustrate that compounds 4–6 effect 100% inhibition of both HIV-1 and HIV-2 at doses that are nontoxic to the host cells (Table I). Compound 6 has ED_{50} (50% antiviral dose) values of 7.6 and 36 μM agaisnt HIV-1 and HIV-2, respectively, demonstrating greater potency in the HIV-1 cytopathogenicity assay. The biphenyl spacer compound 6 has a CD_{50} value of >98 μM and also demonstrates the best in vitro therapeutic index (ratio of CD_{50} to ED_{50}, obtained from Table II). In the giant cell formation inhibition assay, the flexible spacer compound 4 is the most potent derivative, demonstrating 99% inhibition at 107 μM and showing no toxicity in this assay at a dose of 535 μM. In this assay, derivative 4 exhibits an ID_{50} value of 26 μM. The biphenyl spacer compound 6 also has significant activity in this assay, showing an ID_{50} value of 31 μM (Table III). In the cell-free RT assay, a long chain amide derivative 5, exhibited potent inhibition of RT activity (ID_{50} = 83.5 μM) (Table IV). Compounds 6 inhibits HIV-1-induced cytopathogenicity at a dose of 19.6 μm (Table I). However, at this dose this compound exhibits little or no activity against RT (Table IV). These results indicate that the mechanism of inhibition of cytopathogenicity in this compound may not be due to RT inhibition.

Polar compounds have provided novel potential anti-HIV leads and fostered research in the development of new anti-AIDS agents. Castanospermine,[20] a polar natural product, probably provided the impetus to develop *N*-butylnojirimycin,[21] a glucosidase inhibitor that has entered clinical trials. Many sulfated polysaccharides have shown anti-HIV activity below the anticoagulant threshold with a favorable therapeutic index.[37,52-55] Dextran sulfate and heparin[56] exhibit their antiviral activity by inhibiting viral adsorption to the target cell.

It is worthy to note that the sulfated polysaccharides do not need to enter cells to exhibit antiviral activity. In a clinical study it has been shown that oral dextran sulfate is poorly absorbed.[57] The activity of these agents seems to be related to the sulfate content and molecular weight of the molecule.[58] Indeed, certain workers have used the strategy of sulfation to enhance or induce anti-HIV activity.[59] Another polar compound, aurintricarboxylic acid (ATA),[60] a triphenylmethane dye, has been shown to have anti-HIV activity and specific interaction with the CD4 receptor.[61] A new structure for ATA has been assigned.[62]

Few sulfonic acid compounds have displayed anti-HIV activity. The first such compound was the hexasulfonic acid antitrypanosomial drug suramin,[63] also the first agent to be used in patients with AIDS and found to be clinically ineffective.[64] Other sulfonic acid dyes have also demonstrated anti-HIV activity.[43,65] However, the dye properties of these molecules and their known ability to be metabolized to mutagenic and/or carcinogenic phenyl or biphenyl amine fragments will preclude their use as antiviral agents.[47,66] Among the sulfonic acid containing natural products, a lignin[67] and a group of sulfolipids[68] have shown anti-HIV activity. Synthetic analogues of suramin have also demonstrated in vitro activity better than that of suramin.[69]

Replacement of the spacer unit with a biphenyldisulfonyl or a decamethylene unit does retain anti-HIV activity in these compounds. Substituting the biphenyldisulfonyl spacer (as in 6) for the decamethylene spacer

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(as in 4) increases potency against both HIV-1 and HIV-2. The activity of the decamethylene spacer derivative 4, previously studied in H9 and MOLT-3 cells,[46] is now confirmed in MT-4 cells. The general inactivity of compound 2 and the activity of Congo Red[43] suggests the need for the bis(naphthalenesulfonic acid) moiety for anti-HIV activity. The detailed mechanism of action of these agents remains to be determined.

However, the mode of action against RT may be due to the chelating potential of these compounds. The anti-HIV dyes, Congo Red and Evans Blue, are documented to form metal complexes. Congo Red is known to complex with rare earth salts[70] and Evans blue is used for the estimation of magnesium.[71] Examination of the structures of the described bis(naphthalenesulfonic acid) derivatives reveals that they also have the potential to form metal complexes. It is known that HIV-1 RT is a zinc metalloenzyme requiring magnesium for optimum activity.[72] Complexation may be expected to interfere with the activity of RT. The antiviral activity of chelating agents is well-known.[73] It remains to be determined whether the activity of these compounds is due to the complexes they form with these crucial metal ions or whether they form in vivo complexes with other metals and it is these metal complexes that are responsible for the anti-HIV activity.

It may be envisioned that the activity of these compounds is due to their in vivo hydrolysis products. Many naphthalenesulfonic acids have demonstrated antiviral activity in a variety of screens other than for HIV.[74] For example, the activity of compounds 4–6 may be due to the common naphthalenedisulfonic acid moiety 3. Although this unit 3 has shown activity in H9 and MOLT-3 cells,[45] activity in MT-4 cells was only evident at toxic doses (>279 μM). The varied sensitivity of anti-HIV agents to different screens is well-documented.[75] Similarly, 1,10-decanedicarboxylic acid, the potential in vivo hydrolysis product of compound 4, did not show any anti-HIV activity in MT-4 cells (data not shown). These studies indicate that the activity of these compounds is not due to any in vivo hydrolysis products.

Conclusions

Our studies have demonstrated that the naphthalenedisulfonic acids have potential for future development in the area of potential anti-AIDS therapy. These observations suggest that the bis(naphthalenedisulfonic acid) unit may be a requirement for increased activity of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid. Studies to prepare prodrugs and analogues of these agents targeted for optimizing anti-HIV activity are in progress.

Experimental Section

Synthetic Procedures. All reactions were performed under a nitrogen atmosphere. Melting points were determined on a Meltemp apparatus and are uncorrected. NMR spectra were recorded with a Varian XL-300 (300 MHz) instrument in DMSO-d_6 except where noted. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. IR spectra were recorded with an IBM FT IR-32 system 9000, using KBr pellets, in the Department of Chemistry. Analytical thin-layer chromatography was performed with Baker-flex silica gel IB2-F or cellulose sheets. Elemental analyses were performed at Midwest Microlab, Indianapolis, IN. Negative ion FAB mass spectra were obtained on a Finnigan MAT-90 mass spectrometer using a matrix of either glycerol or a mixture of dithiothreitol and dithioerythritol. All reaction mixtures were evaporated on a rotary evaporator. Pyridine was distilled from potassium hydroxide or barium oxide and stored over fresh potassium hydroxide or molecular sieves.

3,4-Diaminonaphthalenesulfonic Acid (2). A solution of stannous chloride (20 g) in concentrated HCl (75 mL) was added dropwise to a water–ethanol (2:1) solution of Congo Red (1, Aldrich, 10 g) and the mixture was stirred on a heated water bath until decolorization. The solution was further heated at reflux on a water bath for 2–3 h. After cooling of the reaction mixture in ice, the separated, crystalline, yellow solid was filtered and vigorously shaken with methanol (20 mL). The insoluble solid was filtered to yield the product (2.15 g, 32%). An analytical sample was prepared by recrystallizing three times from water to yield a brown powder: mp 278–280 °C dec; NMR δ 8.76 (m, 1 H), 8.12 (m, 1 H), 7.85 (s, 1 H), 7.49 (m, 2 H). Anal. ($C_{10}H_{10}N_2O_3S$) C, H, N.

4,4′-[1,10-Decanediylbis(carbonylamino)]bis(5-hydroxy-2,7-naphthalenedisulfonic acid) (4). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid monosodium salt (3, 1 g, 2.9 mmol) and dodecanedioyl dichloride (1 g, 3.7 mmol) were heated to a temperature of 120–130 °C, after which time dry pyridine (10 mL) was added to the reaction mixture and the mixture was heated under reflux for 5 h. The pyridine was evaporated and the resulting product was washed with chloroform (5 × 10 mL). The obtained solid was dissolved in methanol (15 mL) and filtered. The filtrate was triturated with ether (50 mL) to yield the product isolated as the disodium salt (0.72 g, 53%). An analytical sample was prepared by recrystallizing three times from glacial acetic acid–water (1:10) to afford a light yellow powder: mp 345–347 °C dec; IR 3437, 3109, 2928, 2855, 1672, 1626, 1549, 1433, 1390, 1194, 1043 cm^{-1}; NMR δ 11.38 (s, 2 H, exchangeable with D_2O), 11.08 (s, 2 H, exchangeable with D_2O), 8.73 (s, 2 H), 7.67 (s, 2 H), 7.51 (s, 2 H), 7.16 (s, 2 H), 2.36 (t, 4 H, J = 7.2 Hz), 1.62 (m, 4 H), 1.26 (br s, 12 H); (–)-FABMS m/e (relative intensity) 875 ([M – 2H]$^-$, 2), 853 ([M – Na]$^-$, 6), 530 (100). Anal. ($C_{32}H_{34}N_2O_{16}S_4Na_2 \cdot 3H_2O$) C, H.

4-(Palmitoylamino)-5-hydroxy-2,7-naphthalenedisulfonic Acid (5). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid monosodium salt (3, 1 g, 2.9 mmol) and palmitoyl chloride (5 mL, 0.026 mol) was heated to 120–125 °C. Dry pyridine (20 mL) was added and the mixture heated under reflux for 4 h. The pyridine was evaporated and the solid mass thus obtained was washed with chloroform (4 × 20 mL). The residue was dissolved in methanol (15 mL) and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product, isolated as the monosodium salt (0.90 g, 51%). An analytical sample was prepared by recrystallizing three times from glacial acetic acid: mp 320–322 °C dec; IR 3455, 2924, 2853, 1688, 1678, 1641, 1626, 1500, 1468, 1441, 1192, 1043 cm^{-1}; NMR δ 11.38 (s, 1 H, exchangeable with D_2O), 11.08 (s, 1 H, exchangeable with D_2O), 8.76 (s, 1 H), 7.70 (s, 1 H), 7.54 (s, 1 H), 7.19 (s, 1 H), 2.39 (t, 2 H, J = 7.2 Hz), 1.66 (m, 2 H), 1.23 (br s, 24 H), 0.85 (t, 3 H, J = 6.6 Hz); (–)-FABMS m/e (relative intensity) 579 ([M – H]$^-$, 6), 557 ([M – Na]$^-$, 21), 556 (100). Anal. ($C_{26}H_{38}NO_8S_2Na \cdot H_2O$) C, H, N.

4,4′-[4,4′-Biphenyldiylbis(sulfonylamino)]bis(5-hydroxy-2,7-naphthalenedisulfonic acid) (6). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid monosodium salt (3, 1 g, 2.9 mmol) and 4,4′-biphenyldisulfonyl chloride (1 g, 2.8 mmol) were heated to a temperature of 120–125 °C after which time dry pyridine (10 mL) was added and the mixture was heated under reflux for 5 h. The pyridine was evaporated and the solid mass obtained was washed with chloroform (5 × 10 mL). The residue was dissolved in methanol (15 mL) and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered, dissolved in 0.1 N NaOH (10 mL), heated on a water bath for 1 h, and evaporated to dryness. The solid was washed with absolute ethanol (5 × 10 mL) to give a light pink powder, isolated as the tetrasodium salt (0.79 g, 53%). An analytical sample was prepared by recrystallizing four times from glacial acetic acid–water mixture

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(1:8): mp 296-298 °C dec; IR 3453, 3237, 3073, 1599, 1529, 1487, 1375, 1327, 1167, 1093 cm^{-1}; NMR δ 11.35 (s, 2 H, exchangeable with D_2O), 11.08 (s, 2 H, exchangeable with D_2O), 7.79 (m, 10 H), 7.67 (s, 2 H), 7.50 (s, 2 H), 7.11 (s, 2 H); (-)-FABMS *m/e* (relative intensity) 982 ($[M - Na]^-$, 9), 962 (38), 961 (30), 960 (42), 556 (100). Anal. ($C_{32}H_{20}N_2O_{18}S_6Na_4 \cdot H_2O$) C, H, N.

3,3'-[4,4'-Biphenyldiylbis(sulfonylamino)]bis(1,5-naphthalenedisulfonic acid) (8). 3-Amino-1,5-naphthalenedisulfonic acid (7, 1 g, 3.3 mmol) and 4,4'-biphenyldisulfonyl chloride (1 g, 2.8 mmol) were heated to 120 °C, after which time dry pyridine (10 mL) was added and the mixture was heated under reflux for 5 h. Pyridine was evaporated and the remaining solid was washed with chloroform (5 × 10 mL), and the residue was dissolved in methanol (ca. 10-15 mL) and filtered. The filtrate was triturated with ether (50 mL) to produce a solid which was filtered. The solid was dissolved in 0.1 N NaOH (10 mL), warmed on a water bath for 1 h, and evaporated to dryness to yield a solid. The product was washed with absolute ethanol (5 × 10 mL) to produce a yellow powder isolated as the tetrasodium salt (0.91 g, 56%). An analytical sample was prepared by recrystallization from methanol-chloroform: mp 338-340 °C dec; IR 3445, 1624, 1597, 1333, 1309, 1196, 1163, 1093, 1041 cm^{-1}; NMR δ 10.56 (s, 2 H, exchangeable with D_2O), 8.83 (s, 2 H), 8.73 (d, 2 H, J = 8.7 Hz), 8.00 (d. 4 H, J = 7.8 Hz), 7.92 (d, 4 H, J = 7.8 Hz), 7.90 (s, 2 H), 7.83 (d, 2 H, J = 8.4 Hz), 7.31 (t, 2 H, J = 7.8 Hz); (-)-FABMS *m/e* (relative intensity) 949 ($[M - Na - H]^-$, 43), 905 (100). Anal. ($C_{32}H_{20}N_2O_{16}S_6Na_4 \cdot H_2O$) C, H, N.

Antiviral Assay Procedures. Activity of the compounds against the replication of HIV-1 (HTLV-III_B strain) and HIV-2 (ROD strain) was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells. MT-4 cells were infected with HIV 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.[76] The giant cell formation assay was carried out according to established protocol.[77] MOLT-4 cells were cultured with an equal number of HUT-78/HTLV-III_B for 24 h, and the number of giant cells was determined microscopically. The HIV RT assay was carried out as follows. HIV-1 RT was obtained from disrupted virions which had been partially purified and concentrated. The assay was performed at 37 °C for 30 min with 50 μL of a reaction mixture containing 50 mM Tris-HCl (pH 8.4), 2 mM dithiothreitol, 100 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, 1 μCi of [*methyl*-^{3}H]dTTP (30 Ci/mmol), 0.01 unit of poly(rA)·oligo(dT), test compound, and enzyme. The reaction was terminated with 200 μL of trichloroacetic acid (5%, v/v). The precipitated materials were collected on glass-fiber filters and analyzed for their radioactivity in a liquid-scintillation counter. Variation in the experiments was 10% at maximum.

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Potential Antitumor Agents. 61. Structure-Activity Relationships for in Vivo Colon 38 Activity among Disubstituted 9-Oxo-9*H*-xanthene-4-acetic Acids

Gordon W. Rewcastle, Graham J. Atwell, Li Zhuang, Bruce C. Baguley, and William A. Denny*

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Aukland, New Zealand.
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Analogues of 9-oxo-9*H*-xanthene-4-acetic acid (XAA) bearing small, lipophilic 5-substituents are among the most dose-potent compounds yet reported with the capability of causing hemorrhagic necrosis of implanted colon 38 tumors in mice. To further extend structure-activity relationships among this class of compound, a series of XAA derivatives bearing two small lipophilic groups at various positions have been prepared and evaluated. The 5,6-disubstituted compounds in particular show consistently high levels of both dose potency and activity, suggesting this is the optimal configuration among substituted 9-oxo-9*H*-xanthene-4-acetic acids. The 5,6-dimethyl and 5-methyl-6-methoxy are the most effective analogues, showing in vivo colon 38 activity comparable to that of FAA at 10-15-fold lower doses and superior activity to FAA at the respective optimal doses, and the former has been selected for detailed evaluation.

The drug flavone-8-acetic acid (1; FAA, NSC 347512) has come under intense scrutiny as a potential antitumor agent, since it shows remarkable activity against advanced experimental colon tumors in mice,[1-3] high activity as a biological response modifier,[4,5] good ability to induce cytokines,[6] and has dramatic effects on shutting down blood flow in solid tumors.[7,8] However, the drug has low dose potency and has not shown activity in man, despite extensive clinical trials.[9]

We have previously reported[10] on the synthesis and evaluation of a related class of compounds, the 9-oxo-9*H*-xanthene-4-acetic acids, which have a similar biological profile. The parent compound (2; XAA) is as active as FAA against colon 38 tumors in mice, while being somewhat more dose-potent.[10] Studies[10,11] of structure-activity relationships among monosubstituted analogues of XAA

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