Coumarin-Based Inhibitors of HIV Integrase[1]

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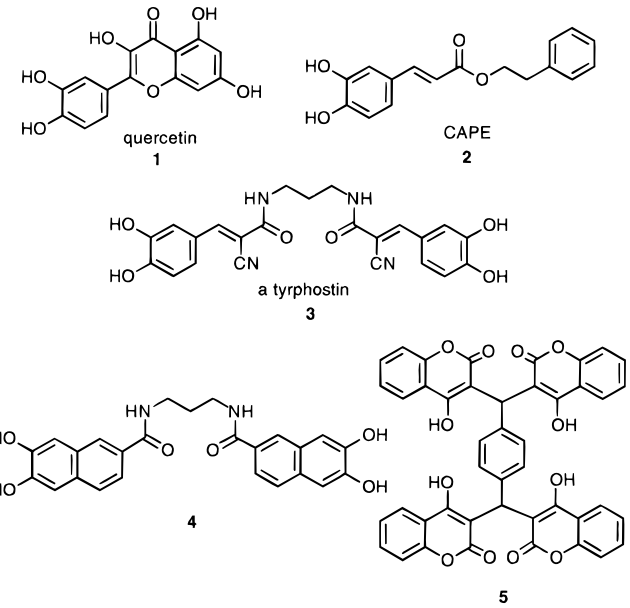
The structures of a large number of HIV-1 integrase inhibitors have in common two aryl units separated by a central linker. Frequently at least one of these aryl moieties must contain 1,2-dihydroxy substituents in order to exhibit high inhibitory potency. The ability of *o*-dihydroxy-containing species to undergo *in situ* oxidation to reactive quinones presents a potential limitation to the utility of such compounds. The recent report of tetrameric 4-hydroxycoumarin-derived inhibitor **5** provided a lead example of an inhibitor which does not contain the catechol moiety. Compound **5** represents a large, highly complex yet symmetrical molecule. It was the purpose of the present study to determine the critical components of **5** and if possible to simplify its structure while maintaining potency. In the present study, dissection of tetrameric **5** ($IC_{50} = 1.5\ \mu M$) into its constituent parts showed that the minimum active pharmacophore consisted of a coumarin dimer containing an aryl substituent on the central linker methylene. However, in the simplest case in which the central linker aryl unit consisted of a phenyl ring (compound **8**, $IC_{50} = 43\ \mu M$), a significant reduction in potency resulted by removing two of the original four coumarin units. Replacement of this central phenyl ring by more extended aromatic systems having higher lipophilicity improved potency, as did the addition of 7-hydroxy substituents to the coumarin rings. Combining these latter two modifications resulted in compounds such as 3,3′-(2-naphthalenomethylene)bis[4,7-dihydroxycoumarin] (**34**, $IC_{50} = 4.2\ \mu M$) which exhibited nearly the full potency of the parent tetramer **5** yet were structurally much simpler.

Development of resistance is a major problem associated with traditional therapeutic approaches toward AIDS. One promising means of overcoming this is to direct agents at multiple points which are required for HIV replication.[2] The thought here is that the simultaneous mutation of more than one critical enzyme, which would be necessary for this type of multidrug resistance, either would be quite rare or would result in decreased virulence. Among HIV enzymes, three have been identified as major targets for therapeutic development. The first two, reverse transcriptase and protease, have inhibitors either on the market or in clinical trial. The third enzyme, integrase, is equally appealing as a site for antagonist action; however, this enzyme has not yet been as extensively investigated as the first two. Recent reports have appeared on HIV integrase inhibitory potency of a variety of compounds. In many cases, multiple aromatic rings and aryl ortho-hydroxylation are required for good inhibitory potency. Examples of these compounds include flavones, such as quercetin (**1**),[3] caffeic acid phenethyl ester (CAPE, **2**),[3] and analogues[4] as well as certain "tyrphostins" (**3**),[5] arctigenin-based compounds,[6] and bis-catechols such as *β*-conidendrol.[7] These inhibitors may be described in general as consisting of two aryl units, at least one of which contains the 1,2-dihydroxy pattern, separated by an appropriate linker segment. Based on this model, a series of bis-arylamides have recently been prepared, with the dimeric 6,7-dihydroxynaphtho-2-yl analogue **4** exhibiting the best potency of the series.[8]



The practical utility of catechol-containing inhibitors is significantly diminished by cytotoxicity, presumably attributable in part to the *in situ* oxidation of the catechol moiety to reactive quinone species. This is exemplified by the finding that CAPE-like compounds can cross-link cellular protein at micromolar concentrations, most likely via Michael-type addition of protein nucleophiles to such quinone intermediates.[9] In an effort to derive new inhibitors of the general type **1**−**4**,

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Table 1. Examination of Constituent Components of Tetramer **5**[a]

Compound	IC50 Values (μM)	
	3'-Processing	Integration
5 (Lead Tetramer)	1.5 ± 0.5	0.8 ± 0.3
6	> 100	> 100
7	> 100	> 100
8	43.4 ± 23.7	38.8 ± 25.9
9	80.6 ± 23.0	69.2 ± 34.6

[a] IC_{50} values were obtained against HIV-1 integrase as outlined in the Experimental Section. Results are expressed as the average of three independent experiments.

which do not contain catechol functionality, note was taken of the high HIV integrase inhibitory potency of certain coumarin-based analogues such as coumermycin A1[10] and, most notably, the tetrameric compound **5**.[11] Inhibitor **5** bears several planes of symmetry which allow it to be viewed in multiple orientations. More importantly, though, is the absence of catechol functionality. Based on these considerations, a study was undertaken to delineate structural features of **5** necessary for high inhibitory potency and to explore modifications which might simplify the structure without losing potency.

Syntheses

Synthesis of final products was accomplished by one of two general methods. Geminal dicoumarins were prepared by reaction of an appropriate aldehyde linker with either 4-hydroxycoumarin or 4,7-dihydroxycoumarin, while nongeminally substituted analogues were synthesized by heating coumarin monomers neat with an α,α′-dibromoxylene.

Results and Discussion

One purpose of this study was to examine features of tetracoumarin analogue **5** which contribute to its high integrase inhibitory potency. A general arrangement of two aromatic units separated by a central linker has emerged as a common motif for a large number of HIV integrase inhibitors. As a prelude, it was necessary to first determine the optimum arrangement of structural components of **5**. An initial series of compounds were therefore assembled (**6**−**9**) to address this question (Table 1). These four coumarin-containing analogues

Table 2. Rotation of the Site of Coumarin Attachment about a Central Phenyl Linker[a]

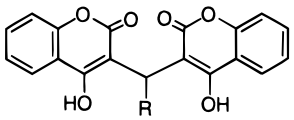
Compound	IC50 Values (μM)	
	3'-Processing	Integration
8	43.4 ± 23.7	38.8 ± 25.9
10	46.0 45.0	20.5 33.2
11	34.0 40.0	11.1 27.5
9	80.6 ± 23.0	69.2 ± 34.6
12	3.0 2.9	1.2 2.0

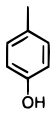
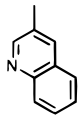
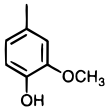
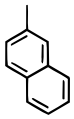
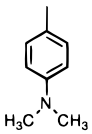
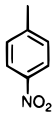
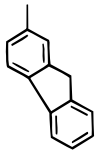
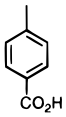
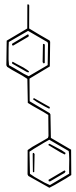
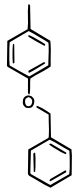
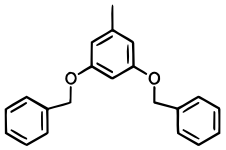
[a] IC_{50} values were obtained against HIV-1 integrase as outlined in the Experimental Section. Where standard errors are given, results are expressed as the average of three independent experiments. In other cases, results of two independent experiments are explicitly listed.

represent a dissection of the parent tetramer **5** into its possible dimeric components (**7**−**9**) as well as the monomeric building block, 4-hydroxycoumarin (**6**). None of the analogues retained the full potency of the parent tetramer **5** (IC_{50} = 1.5 μM), with the monomeric **6** and partial "horizontal" dimer **7** being inactive within the test range. Inclusion of the phenyl ring in the horizontal dimer (compound **8**, IC_{50} = 43 μM) exhibited approximately 2-fold better inhibitory potency than the corresponding "vertical" dimer **9** (IC_{50} = 81 μM).

The above results suggested that both the nature of the phenyl portion of the central "linker" and the position of attachment of the coumarin units affected affinity. In order to more closely examine the effect of attachment position, additional analogues were therefore prepared, **10** and **11**, which placed the coumarins in 1,2- and 1,3-orientations relative to the phenyl spacer. The resulting set of isomeric dimers (**8**−**11**) sequentially rotated the coumarins about the phenyl spacer from the

Table 3. Examination of the Effect of Central Linkers on Inhibitory Potency[a]

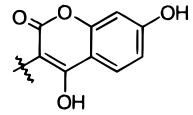


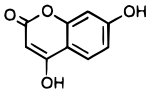
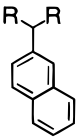
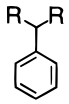
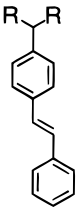
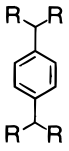
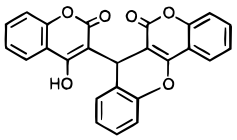
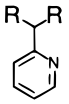
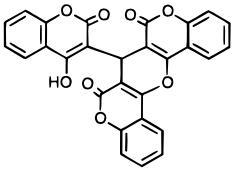
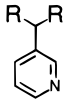
No.	R	IC50 Values (μM) 3'-Processing	IC50 Values (μM) Integration	No.	R	IC50 Values (μM) 3'-Processing	IC50 Values (μM) Integration
13		141 128	75 74	**20**		40 29	23 14
14		198 177	76 108	**21**		19 20	16 9.0
15		100 88	50 88	**22**		14 16	13 28
16		58 50	12 24	**23**		10 11	9.5 7.8
17		66 48	54 49	**24**		5.5 9.2	3.7 9.5
18		100 110	148 149	**25**		19 8.5	14 24
19		75 34	133 112	**26**		8.0 11	12 3.7
				27		11 3.6	7.0 5.0

[a] IC_{50} values were obtained against HIV-1 integrase as outlined in the Experimental Section. Results of two independent experiments are explicitly expressed.

1,1- to the 1,4-positions (Table 2). While inhibition of integration is slightly increased in going sequentially from the 1,1- to 1,2- to 1,3-orientations (compounds **8**−**11**, respectively), there is no significant corresponding effect on 3′-processing until the 1,4-orientation is achieved, at which point potency decreases by a factor of 2 (compound **9**). Because rotation about the phenyl spacer has the double effect of both increasing intercoumarin spacing and torquing relative coumarin orientations, the modest effect on inhibitory potency indicates surprising latitude in both of these parameters. Finally, since the 1,3-orientation was more potent

Table 4. Examination of the Effect of 7-Hydroxylation on Inhibitory Potency[a]

R = 

Compound	IC_{50} Values (μM)		Compound	IC_{50} Values (μM)	
	3'-Processing	Integration		3'-Processing	Integration
 **28**	300	325	 **34**	4.2 ± 0.74	3.55 ± 1.2
 **29**	46.3 ± 24	44.9 ± 20			
 **30**	17.2 ± 11.2	22.2 ± 9.6	 **35**	7.0 ± 0.07	1.8 ± 0.5
 **31**	0.37 ± 0.10	0.33 ± 0.30	 **36**	121 127	122 163
 **32**	94 84	49 70		35.7 ± 2.5	22.5 ± 2.2
 **33**	62 116	25 97	**37**		

[a] IC_{50} values were obtained against HIV-1 integrase as outlined in the Experimental Section. Where standard errors are given, results are expressed as the average of three independent experiments. In other cases, results of two independent experiments are explicitly listed.

than the 1,4-orientation in this series of dimers, it was also interesting to note that the corresponding 1,3-arranged tetramer (**12**, IC_{50} = 2.9 μM) did not exhibit greater inhibitory potency than the 1,4-substituted parent **5** (Table 2).

Having examined positional isomerism, it was next of interest to study effects of variation in the chemical structure of the central linker. Since the "horizontal" dimer is more potent than the "vertical" dimer (compounds **8** and **9**, respectively, Table 1), the former pattern was utilized, and a series of aryl-containing geminal coumarins (**13**−**27**) was prepared (Table 3). Using as a reference compound **8**, which has an unsubstituted benzyl central linker, addition of hydroxyl (compounds **13** and **14**) or amino (compound **15**) functionality to the 4-position reduced inhibitory activity approximately 3−4-fold, while either nitro or carboxyl substituents at this position had little effect (compounds **16** and **17**, respectively). Replacement of the phenyl ring with a heteroaryl ring decreased inhibitory potency, with 2-thiopheno (**18**) being less potent than the 2-furano analogue **19**.

The number of aryl rings on the central linker was increased next. Utilizing a 3-quinolyl ring (compound **20**, IC_{50} = 35 μM) resulted in a slight increase in potency relative to **8**, while removal of the nitrogen, yielding the 2-naphthyl ring system (compound **21**, IC_{50} = 20 μM), gave a further enhancement. In the next series, the length of the aryl system was extended first as the 4′-biphenyl **22**, then as the closely related fluorenyl **23**, and finally as the stilbene analogue **24**. Each modification sequentially enhanced inhibitory potency, with analogue **24** (IC_{50} = 7.3 μM) being approximately 6-fold more potent than starting phenyl compound **8**. Replacement of the styryl component of **24** with a benzyloxy group (compound **25**) caused a 2.5-fold loss of potency. In this case, both **24** and **25** have phenyl rings separated by 2-unit spacers; however, the vinyl bridge of **24** is significantly more rigid than the methyleneoxy bridge of **25**. Addition of a second benzyloxy group (compound **26**) restored most of the lost potency, with the compound now appearing reminiscent of a simplified version of the tetrameric structure of parent **5**. Finally, ferrocene analogue **27** displayed potency equivalent to

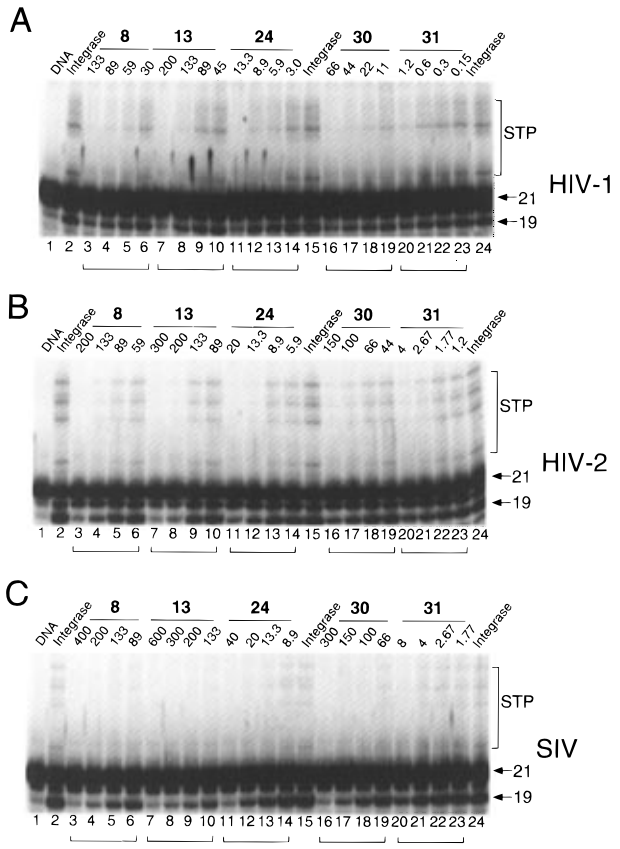


Figure 1. Inhibition of the related retroviral integrases from HIV-1, HIV-2, and SIV by five representative coumarin derivatives: phosphorimager pictures of the concentration-dependent inhibition of HIV-1 (A), HIV-2 (B), and SIV (C) integrases. The DNA strand transfer products (STP) are indicated by brackets, while the 3′-processing product (19-mer) and the DNA substrate (21-mer) are shown by arrows. Coumarin concentrations (μM) are indicated above each lane: lane 1, DNA alone; lanes 2, 15, and 24, plus integrase without inhibitor; lanes 3−6, 7−10, 16−19, and 20−23, plus integrase in the presence of coumarin inhibitors **8**, **13**, **24**, **30**, and **31**, respectively.

stilbene analogue **24**, which was the best compound of the series to this point.

Having examined modifications of the central linker, the next group of analogues (Table 4) was designed to assess the effect of altering the coumarin ring itself by addition of a 7-hydroxyl moiety. Precedence for hydroxylation at the 7-position was established by the recent finding that a 7-hydroxycoumarin-containing analogue showed good integrase inhibitory potency.[11] In the present study, monomeric 4,7-dihydroxycoumarin **28** showed extremely weak potency (IC_{50} = 300 μM); however, conversion to the simple dimer **29** significantly enhanced potency (IC_{50} = 46 μM). Addition of a phenyl ring (compound **30**, IC_{50} = 17 μM) more than doubled the potency, while elaboration as the full tetramer **31** resulted in a 4-fold enhancement of inhibitory potency (IC_{50} = 0.37) relative to the parent compound **5**. For comparative purposes five representative compounds (**8**, **13**, **24**, **30**, and **31**) were tested for inhibition of related retroviral integrases from HIV-2 and Simian immunodeficiency virus (SIV). Gels for these assays as well as for an HIV-1 assay are shown in Figure 1. All five compounds inhibited both 3′-processing and strand transfer activities against HIV-1, HIV-2, and SIV integrases, with no remarkable selectivity noted.

The above results firmly established the value of 7-hydroxylation, and it was of further interest to examine the relative effect of this modification when combined with alterations in the central linker. Replacement of the phenyl ring of **30** with 2- and 3-pyridyl rings (**32** and **33**, IC_{50} = 81 and 89 μM, respectively) decreased inhibitory potency from 4- to 5-fold. Extension of the phenyl ring as the 2-naphthyl and 4-stilbene analogues (**34**, IC_{50} = 4.2 μM; **35**, IC_{50} = 7 μM, respectively) increased inhibitory potency, similar to the effect observed in compounds **21** and **24**, which lacked the 7-hydroxyl substituents.

As a final study, compounds **36** and **37** were prepared as conformationally constrained analogues in which the aryl ring of the central linker is locked via an ether bridge to one of the coumarin units. The cumulative effect of this ether bridge is to lock both center linker and the coumarin ring into fixed conformations. This type of restraint proved deleterious to inhibitory potency (Table 4).

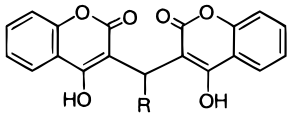
In summarizing the findings of Tables 1−4, it can be concluded that adding 7-hydroxy groups to the coumarin rings increased inhibitory potency across a wide range of analogues. Additionally, modification of the central linker by extending its aryl component also enhanced affinity. This latter effect may be dependent on both the shape and the hydrophobicity of the central linker unit. In order to ascertain the possible role hydrophobicity may have in differential potency, the logarithm of the partition coefficient in an *n*-octanol/water system (log *P*) was approximated using the PROLOGP program, which calculates a theoretical log *P* based on chemical structure. In several systems quantitative considerations of lipophilicity have proven to be useful parameters in the bioactivity. As seen in Table 5, a general trend was observed in which increased hydrophobicity of the central linker substituent was associated with increased potency. Compounds fell within two groups: Those having $IC_{50} > 30$ μM exhibited $\Delta\log P < 2$, while those with $IC_{50} \leq 20$ had $\Delta\log P > 2.5$. The lack of strict correlation between hydrophobicity and potency may indicate that additional parameters must be considered. These may include steric factors and specific bonding interactions which could occur between the enzyme and the central linker moiety. Additionally, the possibility of multiple binding orientations within a given binding site, as well as multiple binding sites, may all contribute to the lack of a strict correlation.

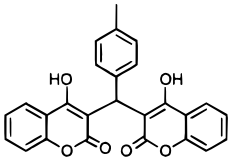
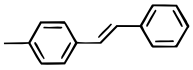
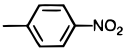
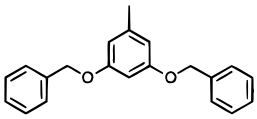
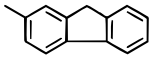
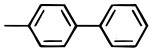
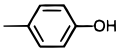
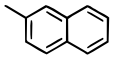
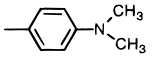
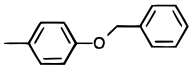
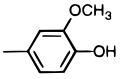
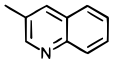
It was hoped that this study would clarify some of the structural requirements for the high inhibitory potency of parent tetramer **5** and, in so doing, allow the generation of smaller, simpler coumarin-based structures which retained high inhibitory potency. Several of the analogues reported in this study have reached this latter objective. Of note are compounds **34** and **35**, which have effectively replaced the "bottom" half of parent tetramer **5** with simple aryl rings without significantly sacrificing potency.

Experimental Section

Preparation of Oligonucleotide Substrates. The HPLC-purified oligonucleotides AE117 (5′-ACTGCTAGAGATTTTC-CACAC-3′) and AE118 (5′-GTGTGGAAAATCTCTAGCAGT-3′) were purchased from Midland Certified Reagent Co. (Midland, TX). Purified recombinant wild-type HIV-1 was prepared as described.[12] The extent of 3′-processing and

Table 5. Comparison of Inhibitory Potency Determined against HIV-1 versus Hydrophobicity of the Central Linker[a]



No.	R	3'-Processing IC50 Values (μM)	△ log P[a]	No.	R	3'-Processing IC50 Values (μM)	△ log P[a]
5		2	2.84	**8**		43	1.60
24		7	4.14	**16**		54	1.37
26		10	5.15	**19**		54	0.45
23		10	3.4	**18**		105	0.86
22		15	3.33	**13**		130	1.10
21		20	2.60	**15**		190	1.88
25		20	3.06	**14**		190	1.05
20		35	1.60	**7**	H	>300	0

[a] Δlog *P* values represent the hydrophobic contribution of the R group, taking the value of R = H as zero.

strand transfer was determined using 5′-end-labeled substrate, with 5′-labeled AE118 having been prepared using T4 polynucleotide kinase (Gibco BRL) and [γ-^{32}P]ATP (DuPont NEN). The kinase was heat inactivated prior to adding AE117 to the same final concentration. The resulting mixture was heated to 95 °C, allowed to cool slowly to room temperature, and run on a G-25 Sephadex quick spin column (Boehringer Mannheim, Indianapolis, IN) to separate double-stranded oligonucleotide from unincorporated label.

HIV-1 Integrase Assay. Integrase was preincubated with inhibitor at 30 °C for 30 min at a final concentration of 200 nM in reaction buffer [50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM MOPS, pH 7.2]. Following preincubation, 5′-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional 1 h at 30 °C. Reactions were quenched by addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue), and an aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M Tris-borate, pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed in a Molecular Dynamics phosphorimager cassette, and analyzed using a Molecular Dynamics phosphorimager (Sunnyvale, CA). Percent inhibition was calculated using the equation: $100 \times [1 - (D - C)/(N - C)]$, where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processed product) or strand transfer product for DNA alone, DNA plus integrase, and integrase plus drug, respectively. Determination of IC_{50} values was achieved by plotting drug concentration versus percent inhibition and measuring the concentration at which 50% inhibition occurred.

HIV-1 and SIV Integrase Assays. A plasmid encoding the HIV-2 integrase was generously provided by Dr. R. H. A. Plasterk (Netherlands Cancer Institute). This integrase was purified essentially as described for the HIV-1 integrase.[12] Purified recombinant wild-type SIV integrase was a generous gift of R. Craigie (NIDDK). Assays were run as indicated above for HIV-1, except that a final enzyme concentration of 600 nM was employed for SIV assays.

Molecular Modeling. log *P* values were calculated using the program PROLOGP, contained in the PALLAS package.[13] PALLAS is a package of powerful tools for the prediction of certain physicochemical parameters of organic compounds based solely on structural information. PROLOGP is an optional component of the PALLAS system, which predicts the logarithm of the partition coefficient (log *P*) of organic compounds in an *n*-octanol/water system based on chemical structure.

Synthesis. Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA, and are within 0.4% of theoretical values unless otherwise indicated. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. ^{1}H NMR data were obtained on a Bruker AC250 (250 MHz) spectrometer and are reported in ppm relative to TMS and referenced to the solvent in which they were run. Anhydrous solvents were obtained commercially and used without further drying.

Method A: Synthesis of Coumarin Dimers and Tetramers by Condensation of Aldehydes with 4-Hydroxycoumarin or 4,7-Dihydroxycoumarin. 4-Hydroxycoumarin or 4,7-dihydroxycoumarin[14] (2 or 4, for tetramers, mmol) was dissolved in 6 mL of hot ethanol, 1 mmol of the corresponding aldehyde was added, and the mixture was refluxed for 24 h. After cooling to room temperature, the solid was filtered off and crystallized to give the product.

3,3′-Benzylidenebis[4-hydroxycoumarin] (8): prepared as previously described.[15]

3,3′,3″,3‴-Isophthalalidenetetrakis[4-hydroxycoumarin] (12): yield 77%; mp 230−234 °C; IR (KBr) 3448 (br), 1655, 1618, 1561, 1342, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.68 (d, J = 7.8 Hz, 4H), 7.54 (m, 4H), 7.25 (m, 4H), 7.22 (m, 4H), 6.95 (m, 4H), 6.29 (s, 2H); FABMS m/z 747.6 (MH$^+$). Anal. ($C_{26}H_{18}O_8 \cdot H_2O$) C, H.

3,3′-(4-Hydroxybenzylidene)bis[4-hydroxycoumarin] (13): prepared as previously described.[15]

3,3′-(4-Hydroxy-3-methoxybenzylidene)bis[4,7-dihydroxycoumarin] (14): yield 77%; mp 217−219 °C; IR (KBr) 3495 (br), 3070 (br), 1671, 1617, 1561, 1271, 768 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.90 (dd, J = 7.8, 1.1 Hz, 2H), 7.59 (dd, J = 7.8, 1.3 Hz, 2H), 7.38 (m, 4H), 6.67 (m, 3H), 6.24 (s, 1H), 3.57 (s, 3H); FABMS m/z 459 (MH$^+$). Anal. ($C_{26}H_{18}O_8$) C, H.

3,3′-[4-(Dimethylamino)benzylidene]bis[4-hydroxycoumarin] (15): prepared as previously described.[16]

3,3′-(4-Nitrobenzylidene)bis[4-hydroxycoumarin] (16): prepared as previously described.[15]

3,3′-(4-Carboxybenzylidene)bis[4-hydroxycoumarin] (17): yield 72%; mp 280−282 °C; IR (KBr) 3398 (br), 1684, 1654, 1618, 1560, 1340, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.82 (dd, J = 7.8, 1.4 Hz, 2H), 7.78 (d, J = 8.3 Hz, 2H), 6.76 (m, 2H), 7.30 (m, 6H), 6.34 (s, 1H); FABMS m/z 456 (MH$^+$). Anal. ($C_{26}H_{16}O_8$) C, H.

3,3′-(2-Thiophenomethylene)bis[4-hydroxycoumarin] (18): prepared as previously described.[17]

3,3′-(2-Furanomethylene)bis[4-hydroxycoumarin] (19): prepared as previously described.[17]

3,3′-(4-Quinolinomethylene)bis[4-hydroxycoumarin] (20): yield 78%; mp 279−281 °C; IR (KBr) 3448 (br), 3069 (br), 1686, 1609, 1559, 1389, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.11 (d, J = 1.6 Hz, 1H), 8.87 (s, 1H), 8.31 (d, J = 8.2 Hz, 1H), 8.20 (d, J = 5.0 Hz, 1H), 8.03 (m, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.79 (dd, J = 7.8, 1.5 Hz, 2H), 7.55 (m, 2H), 7.24 (m, 4H), 6.53 (s, 1H); FABMS m/z 464 (MH$^+$). Anal. ($C_{28}H_{17}O_6 \cdot {}^1/_2H_2O$) C, H, N.

3,3′-(2-Naphthalenomethylene)bis[4-hydroxycoumarin] (21): yield 97%; mp 292−294 °C; IR (KBr) 3058 (br), 1663, 1615, 1561, 1347, 765 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.90 (d, J = 7.6 Hz, 2H), 7.77 (m, 3H), 7.62 (d, J = 7.6 Hz, 2H), 7.58 (m, 1H), 7.40 (m, 3H), 7.36 (m, 2H), 7.32 (m, 2H), 6.49 (s, 1H); FABMS m/z 463 (MH$^+$). Anal. ($C_{29}H_{18}O_6$) C, H.

3,3′-(4-Phenylbenzylidene)bis[4-hydroxycoumarin] (22): yield 88%; mp 227−229 °C; IR (KBr) 3448 (br), 3028 (br), 1671, 1606, 1567, 1347, 761 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.89 (dd, J = 7.9, 1.2 Hz, 2H), 7.60 (m, 3H), 7.49 (m, 4H), 7.39 (d, J = 8 Hz, 2H), 7.33 (m, 4H), 7.21 (d, J = 8.1 Hz, 2H), 6.36 (s, 1H); FABMS m/z 489 (MH$^+$). Anal. ($C_{32}H_{20}O_6 \cdot {}^1/_4H_2O$) C, H.

3,3′-(2-Fluorenomethylene)bis[4-hydroxycoumarin] (23): yield 97%; mp 279−280 °C; IR (KBr) 3448 (br), 3071 (br), 1656, 1618, 1567, 1351, 760 cm^{-1}; ^{1}H NMR (DMSO) δ 7.90 (dd, J = 7.9, 1.2 Hz, 2H), 7.80 (d, J = 7.2 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 7.60 (m, 2H), 7.52 (d, J = 7.2 Hz, 1H), 7.38 (m, 2H), 7.35 (m, 2H), 7.32 (m, 2H), 7.24 (m, 1H), 7.20 (m, 1H), 6.42 (s, 1H), 3.81 (s, 2H); FABMS m/z 501 (MH$^+$). Anal. ($C_{32}H_{20}O_6 \cdot {}^1/_4H_2O$) C, H.

3,3′-(4-Stilbenomethylene)bis[4-hydroxycoumarin] (24): yield 90%; mp 238−240 °C; IR (KBr) 3448 (br), 3026 (br), 1656, 1618, 1568, 1348, 766 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.88 (dd, J = 7.8, 1.2 Hz, 2H), 7.16 (m, 4H), 7.44 (d, J = 8.3 Hz, 2H), 7.32 (m, 6H), 7.16 (m, 5H), 6.33 (s, 1H); FABMS m/z 515 (MH$^+$). Anal. ($C_{33}H_{22}O_6$) C, H.

3,3′-[4-(Benzyloxy)benzylidene]bis[4-hydroxycoumarin] (25): yield 85%; mp 180−182 °C; IR (KBr) 3448 (br), 3070 (br), 1671, 1619, 1607, 1569, 1351, 763 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.89 (d, J = 7.8Hz, 2H), 7.59 (m, 2H), 7.37 (m, 9H), 7.05 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 6.27 (s, 1H), 5.01 (s, 2H); FABMS m/z 519 (MH$^+$). Anal. ($C_{32}H_{22}O_7$) C, H.

3,3′-[3,5-Bis(benzyloxy)benzylidene]bis[4-hydroxycoumarin] (26): yield 85%; mp 207−209 °C; IR (KBr) 3028 (br), 1663, 1618, 1561, 1349, 764 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.85 (dd, J = 7.8, 1.2 Hz, 2H), 7.54 (m, 2H), 7.27 (m, 14H), 6.46 (s, 1H), 6.32 (s, 2H), 6.20 (s, 1H), 4.93 (s, 4H); FABMS m/z 625.6 (MH$^+$). Anal. ($C_{39}H_{28}O_8$) C, H.

3,3′-(Ferrocenomethylene)bis[4-hydroxycoumarin] (27): yield 35%; mp >360 °C; IR (KBr) 3448 (br), 2365, 2344, 1655, 1608, 1560, 1398, 759 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.74 (s, 2H), 7.42 (s, 2H), 7.15 (s, 4H), 6.21 (s, 1H), 4.00 (m, 9H); FABMS m/z 520.4 (MH$^+$).

3,3′-Benzylidenebis[4,7-dihydroxycoumarin] (30): yield 72%; mp 287−289 °C; IR (KBr) 3409 (br), 3087 (br), 1655, 1619, 1572, 1354, 793 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.71 (d, J = 8.7 Hz, 2H), 7.18 (m, 3H), 7.11 (m, 2H), 6.76 (dd, J = 8.7, 2.2 Hz, 2H), 6.67 (d, J = 2.2 Hz, 2H), 6.26 (s, 1H); FABMS m/z 445 (MH$^+$). Anal. ($C_{25}H_{16}O_8 \cdot {}^1/_2H_2O$) C; H: calcd, 3.78; found, 4.19.

3,3′,3″,3‴-(1,4-Dimethylenophenyl)tetrakis[4,7-dihydroxycoumarin] (31): yield 72%; mp >380 °C; IR (KBr) 3180 (br), 1652, 1619, 1569, 1256, 780 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.69 (d, J = 8.7 Hz, 4H), 6.95 (s, 4H), 6.74 (dd, J = 8.8, 2.1 Hz, 4H), 6.65 (d, J = 2.0 Hz, 4H), 6.20 (s, 2H); FABMS m/z 811.1 (MH$^+$). Anal. ($C_{26}H_{18}O_8 \cdot 3{}^1/_2H_2O$) C, H.

3,3′-(2-Pyridinomethylene)bis[4,7-dihydroxycoumarin] (32): yield 71%; mp >320 °C; IR (KBr) 3448 (br), 3179 (br), 1686, 1611, 1560, 1404, 760 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.21 (s, 2H), 8.66 (d, J = 5.6 Hz, 1H), 8.52 (s, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.88 (dd, J = 8.1, 5.7 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 6.67 (dd, J = 8.6, 2.2 Hz, 2H), 6.60 (d, J = 2.1 Hz, 2H), 6.30 (s, 1H); FABMS m/z 446 (MH$^+$). Anal. ($C_{24}H_{15}NO_8 \cdot {}^1/_2H_2O$) C, H, N.

3,3′-(3-Pyridinomethylene)bis[4,7-dihydroxycoumarin] (33): yield 54%; mp 306−308 °C; IR (KBr) 3448 (br), 2364, 2344, 1654, 1617, 1561, 1410, 777 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.26 (s, 2H), 8.59 (d, J = 5.5 Hz, 1H), 8.41 (m, 1H), 7.80 (m, 2H), 7.60 (d, J = 8.6 Hz, 2H), 6.68 (dd, J = 8.6, 2.1 Hz, 2H), 6.62 (d, J = 2.1 Hz, 2H), 6.39 (s, 1H); FABMS m/z 446 (MH$^+$). Anal. ($C_{24}H_{15}NO_8 \cdot 1{}^3/_4H_2O$) C, H, N.

3,3′-(2-Naphthalenomethylene)bis[4,7-dihydroxycoumarin] (34): yield 51%; mp 284−285 °C; IR (KBr) 3356 (br), 1654, 1610, 1560, 1364, 786 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.73 (m, 5H), 7.58 (s, 1H), 7.42 (d, J = 3.3 Hz, 1H), 7.40 (d, J = 3.3 Hz, 1H), 7.28 (d, J = 8.6 Hz, 1H), 6.76 (dd, J = 8.6, 2.1 Hz, 2H), 6.69 (d, J = 2.1 Hz, 2H), 6.40 (s, 1H); FABMS m/z (MH$^+$) 495. Anal. ($C_{29}H_{18}O_8$) C, H.

3,3′-(4-Stilbenomethylene)bis[4,7-dihydroxycoumarin] (35): yield 46%; mp 215−217 °C; IR (KBr) 3083 (br), 1655, 1619, 1561, 1355, 813 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.75 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 7.4 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 7.35 (m, 2H), 7.17 (m, 5H), 6.79 (dd, J = 8.7, 2.2 Hz, 2H), 6.71 (d, J = 2.1 Hz, 2H), 6.29 (s, 1H); FABMS m/z 546 (MH$^+$). Anal. ($C_{33}H_{22}O_8 \cdot {}^1/_4H_2O$) C, H.

Method B: Synthesis of Coumarin Dimers by Alkylation of 4-Hydroxycoumarin with α,α′-Dibromoxylene. 4-Hydroxycoumarin (324 mg, 2 mmol) and α,α′-dibromoxylene (1 mmol) were heated in the molten state at a temperature of 130−180 °C for 2 h under argon, and the solid was formed. Then the solid was dissolved in hot ethanol and gave crystals after cooling.

3,3′-*p*-Xylenebis[4,7-dihydroxycoumarin] (9): yield 73%; mp 320−322 °C; IR (KBr) 3219 (br), 1668, 1631, 1570, 1171, 759 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.95 (dd, J = 8.4, 1.6 Hz, 2H),

7.60 (m, 2H), 7.37 (m, 4H), 7.11 (s, 4H), 3.81 (s, 4H); FABMS *m/z* 427 (MH^+). Anal. ($C_{26}H_{18}O_6 \cdot {}^1/_4H_2O$) C, H.

3,3′-*o*-Xylenebis[4,7-dihydroxycoumarin] (23): yield 27%; mp 326−328 °C; IR (KBr) 3230 (br), 1678, 1635, 1570, 1395, 751 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99 (d, J = 7.8 Hz, 2H), 7.64 (m, 2H), 7.38 (m, 4H), 7.01 (m, 2H), 6.94 (m, 2H), 3.99 (s, 4H); FABMS *m/z* 427 (MH^+). Anal. ($C_{26}H_{18}O_6 \cdot {}^1/_4H_2O$) C, H.

3,3′-*m*-Xylenebis[4,7-dihydroxycoumarin] (11): yield 20%; mp 316−317 °C; IR (KBr) 3240 (br), 1670, 1635, 1570, 1396, 754 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.60 (br, 2H), 7.92 (dd, J = 8.6, 1.6 Hz, 2H), 7.61 (m, 2H), 7.35 (m, 4H), 7.14 (m, 2H), 7.02 (m, 2H), 3.83 (s, 4H); FABMS *m/z* 427 (MH^+). Anal. ($C_{26}H_{18}O_6 \cdot {}^1/_4H_2O$) C, H.

Method C: Synthesis of 7,7′-Dicoumarol (7). 4,7-Dihydroxycoumarin (356 mg, 2 mmol) was dissolved in 25 mL of hot water to which an excess of 36% formalin (0.1 mL) was added. The precipitate was produced immediately and continued refluxing for 0.5 h. After cooling, the solid was filtrated and crystallized from ethanol to give the title compound in 81% yield: mp 326−328 °C; IR (KBr) 3327, 3105 (br), 1627, 1597, 1572, 1355, 1312, 1246, 1158, 795 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.51 (br, 2H), 7.74 (d, J = 8.6 Hz, 2H), 6.80 (dd, J = 8.7, 2.2 Hz, 2H), 6.69 (d, J = 2.2 Hz, 2H), 3.68 (s, 2H); FABMS *m/z* 369 (MH^+). Anal. ($C_{26}H_{18}O_6$) C, H.

3-[6-Oxo(1)benzopyrano[4,3-*b*]-(1)10-hydroxybenzopyran-7-yl]-4-hydroxycoumarin (36): prepared as previously described.[15]

Coumarin trimer 37: prepared as previously described.[15]

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