Design, Synthesis, and Testing of Potential Antisickling Agents. 10. (2,2-Dimethylchroman-6-yl)alkanoic Acids

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Five (2,2-dimethylchroman-6-yl)alkanoic acids were synthesized and tested for antigelling activities. It was envisioned that these agents might bind via hydrophobic bonding to nonpolar sites of the "donor–acceptor" regions of hemoglobin S. Several (2,2-dimethylchroman-6-yl)alkanoic acids containing 1–4 carbon atoms on the side-chain residue were designed to interact at the acceptor site, were synthesized, and were found to be moderately potent antigelling agents. The weak activity observed for two of the acids at low concentrations is rationalized in terms of weak binding affinities or multiple binding to active and nonactive sites. The effect of these compounds on shifting the allosteric equilibrium was small or negligible. The low toxicity of one of the (2,2-dimethylchroman-6-yl)alkanoic acids demonstrates the potential use of yet another class of compounds that can be modified in the development of antisickling agents.

The molecular basis for the pathophysiology of sickle cell disease is recognized to arise from a mutation of one amino acid [Glu to Val] at position 6 on the β-chain of normal hemoglobin (HbA) that results in the production of sickle hemoglobin (HbS). Increased understanding of the gelling and sickling phenomena have permitted varied approaches to the discovery of drugs that could be effective in treating sickle cell disease. To date, only treatment for symptoms of the disease is available and no clinically acceptable therapy has been approved for general use in treating sickle cell disease itself.

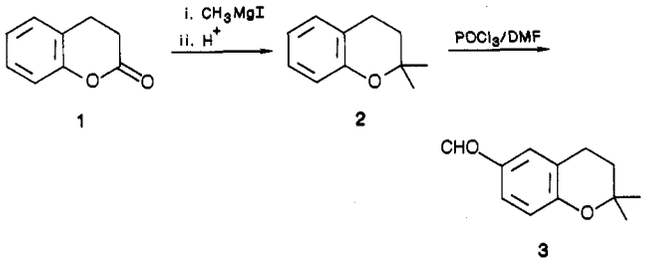
The problem of adopting a therapy for sickle cell anemia is compounded by the extreme diversity and variability in the clinical and hematological expressions of the disease.[1,2] It is however generally accepted that the underlying cause in the manifestation of sickle cell disease is related to the formation of intracellular HbS polymer under anoxic conditions. Therefore, agents have been sought to inhibit the polymerization process.

Three pharmacological approaches[3–5] used to prevent gelation or sickling include designing new agents or selecting known compounds or drugs that (a) bind stereospecifically to HbS and alter its structure to prevent interaction with other HbS molecules, (b) shift the allosteric equilibrium toward the more soluble oxy (R) state, or (c) reduce the mean corpuscular hemoglobin concentration (MCHC) of the red cell, decreasing the HbS concentration and increasing the delay time in polymerization.

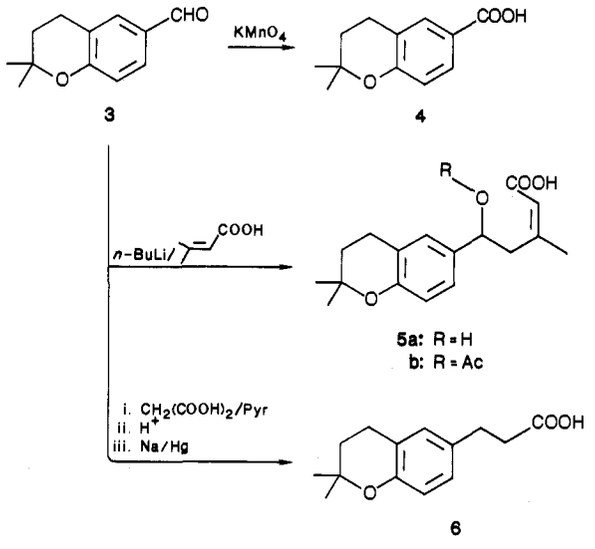
Comprehensive hematological studies based on these pharmacological approaches have led to the discovery of a number of compounds, some of which are capable of covalent or noncovalent interactions in inhibiting HbS gelation. Recent reviews and articles have summarized these findings.[3,4,6,7] In this paper, we report the synthesis and testing of a class of moderately active molecules which demonstrate significant activity that must arise from stereochemical distortions of hemoglobin structure.

Noncovalent Stereospecific Inhibitors. We have found noncovalently acting molecules to be generally less potent antigelling agents under Hofrichter–Ross–Eaton[8] solubility assay conditions than compounds that covalently bind to HbS.[9–11] However, the majority of potent covalent inhibitors of gelation are not therapeutically useful because of their high affinity for other serum and cellular constituents, toxicity, undesired side effects, or inability to transport into red cells.[11–15] Therefore noncovalently acting molecules that might exhibit better in vivo biological properties have been sought by several groups.[9,10,16–19]

Scheme I



Scheme II



Many of the noncovalently acting chemotherapeutic agents have been designed or proposed to disrupt the

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(1) Steinberg, M. H. In *Approaches to the Therapy of Sickle Cell Anemia*; Beuzard, Y., Charache, S., Galacteros, F., Eds.; INSERM: Paris, 1986; pp 457–470.
(2) Pagneir, J.; Labie, D. In *Approaches to the Therapy of Sickle Cell Anemia*; Beuzard, Y., Charache, S., Galacteros, F., Eds.; INSERM: Paris, 1986; pp 471–475.
(3) Edelstein, S. J.; *Annu. Rep. Med. Chem.* **1985**, *20*, 247–255.
(4) Abraham, D. J.; Perutz, M.F. In *Approaches to the Therapy of Sickle Cell Anemia*; Beuzard, Y., Charache, S., Galacteros, F., Eds.; INSERM: Paris, 1986; pp 141–149.
(5) Dean, J.; Schechter, A. N. *N. Engl. J. Med.* **1978**, *299*. 752–763; 804–811; 863–870.
(6) Beuzard, Y.; Garel, M. C.; Mahieu, J.-P.; Domenget, C.; Caburi-Martin, J.; Galacteros, F.; Sebille, B. In *Approaches to the Therapy of Sickle Cell Anemia*; Beuzard, Y., Charache, S., Galacteros, F., Eds.; INSERM: Paris, 1986; pp 173–184.

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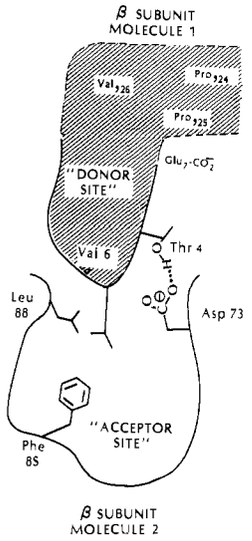
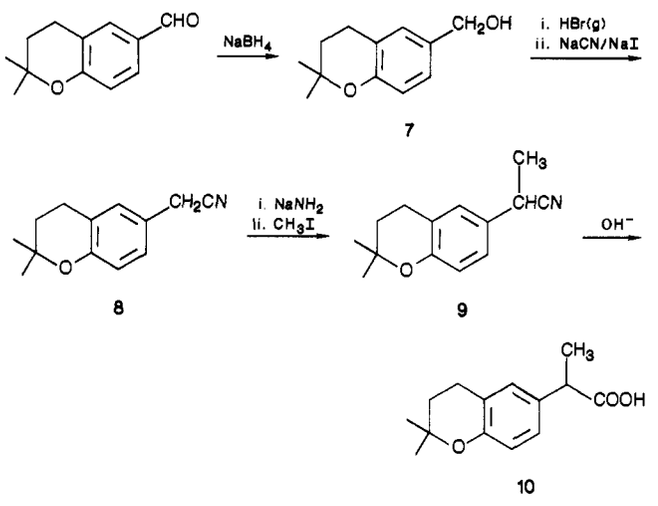


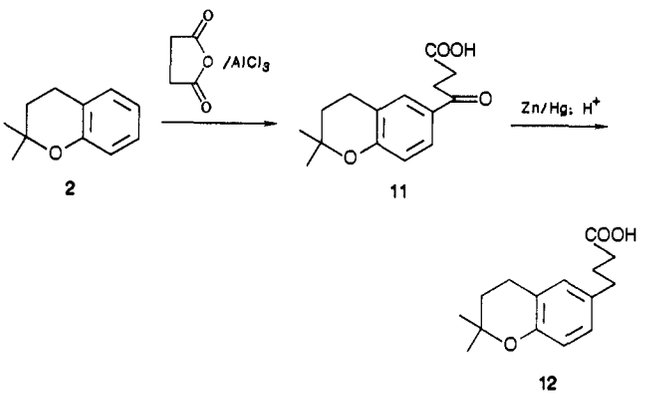
Figure 1. Representation of the donor site–acceptor site interaction between HbS molecules in the deoxy state.

Scheme III



contacts between the "donor" site (mutation area on β_2-strand of HbS tetramer) and the "acceptor" site (a cluster of amino acid residues on the β_1-chain of another HbS tetramer).[10,18,20] The profile of the "donor–acceptor" region includes β_2Thr$_4$, β_2Val$_6$, β_1Phe$_{85}$, β_1Asp$_{73}$, and β_1Leu$_{88}$ (Figure 1). Stereospecific inhibitors could theoretically bind covalently or noncovalently within the contact area between the "donor" arm and "acceptor" cavity. Although other fiber-stabilizing contacts are recognized in deoxy sickle hemoglobin (dHbS),[21,22] the inability of human deoxyhemoglobin A (dHbA) to gel at normal cellular concentrations, even under drastic conditions, suggests that interactions at the donor–acceptor site near the mutation area are crucial to the stabilization of the HbS fiber.

Scheme IV



It was envisioned that the hydrophobic aromatic ends of the proposed molecules might interact noncovalently at the donor–acceptor sites and inhibit gelation directly in a fashion similar to that proposed by Ross et al.[18] for phenylalanine inhibition. It was further expected that contact of such agents at these sites would not interfere with the allosteric equilibrium and delivery of oxygen to tissues.

Chemistry. The key reagent **3** in the synthesis of coompounds **4**, **5b**, **6**, and **10** (Schemes II and III) was obtained from Vilsmeier–Haack formylation of 2,2-dimethylchroman (**2**) using $POCl_3$–DMF complex as a formylation agent. Aldehyde **3** obtained by this route (Scheme I) is structurally identical with that obtained by Knight and Pattenden[23] from acid cyclization of 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde. 2,2-Dimethylchroman (**2**) was efficiently prepared through Grignard addition to dihydrocoumarin and acid cyclization of the resulting carbinol.

2,2-(Dimethylchroman-6-yl)alkanoic Acids. The syntheses of compounds **4**, **5b**, **6**, and **10** were readily achieved. The oxidation of **3** with aqueous potassium permanganate afforded **4**. The condensation of **3** with lithium dianion of 3-methyl-2-butenoic acid gave hydroxy acid **5a**. The most important property of the dianion lies in its ability to introduce the prenyl unit stereospecifically,[24] leading exclusively to the *Z* isomer.

The major problem in the synthesis of **5b** (Scheme II) was the preparation of the δ-hydroxy acid **5a** in pure form. Compound **5a** is converted readily to a mixture of lactone and diene in the presence of acid at room temperature. Consequently, the workup procedure for the condensation

(7) Keidan, A. J.; White, R. D.; Joy, M.; Huehns, E. R.; Franklin, I. M.; Stuart, J. In *Approaches to the Therapy of Sickle Cell Anemia*; Beuzard, Y., Charache, S., Galacteros, F., Eds.; INSERM: Paris, pp 129–140.
(8) Hofrichter, J.; Ross, P. D.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3035–3039.
(9) Abraham, D. J.; Kennedy, P. E.; Mehanna, A. S.; Patwa, D.; Williams, F. L. *J. Med. Chem.* **1984**, *27*, 967–978.
(10) Abraham, D. J.; Gazze, D.; Kennedy, P. E.; Mokotoff, M. *J. Med. Chem.* **1984**, *27*, 1549–1559.
(11) Kennedy, P. E.; Williams, F. L.; Abraham, D. J. *J. Med. Chem.* **1984**, *27*, 103–105.
(12) Harkness, D. R.; Roth, S. *Prog. Hematol.* **1975**, *9*, 157–184.
(13) Balcerzak, S. P.; Grever, M. R.; Sing, D. E.; Bishop, J. N.; Segal, M. L. *J. Lab. Clin. Med.* **1982**, *100*, 345–355.
(14) Roth, R. F., Jr.; Nagel, R. L.; Bookchin, R. M.; Grayzel, A. I. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 612–618.
(15) Zak, S. J.; Geller, G. R.; Finkel, B.; Tukey, D. P.; McCormack, M. K.; Krivit, W. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4153–4156.
(16) Poillon, W. N. *Biochemistry* **1982**, *21*, 1400.
(17) Noguchi, C. T.; Schechter, A. N. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 637–642.
(18) Ross, P. D.; Subramanian, S. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 1217–1223.
(19) Gorecki, M.; Acquaye, C. T. A.; Wilcheck, M.; Votano, J. R.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 181–185.
(20) Abraham, D. J.; Mokotoff, M.; Sheh, L.; Simmons, J. E. *J. Med. Chem.* **1983**, *26*, 549–554.
(21) Wishner, B. C.; Ward, K. B.; Lattman, E. E.; Love, W. E. *J. Mol. Biol.* **1975**, *98*, 179–194.
(22) Dykes, G. W.; Crepeau, R. H.; Edelstein, S. J. *J. Mol. Biol.* **1979**, *130*, 451–472.
(23) Knight, D. W.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1*, **1979**, 70–76.
(24) Cainelli, G.; Cardillo, G.; Orena, M. *J. Chem. Soc., Perkin Trans 1* **1979**, 1597–1599.

Table I. Antigelling Activity of (2,2-Dimethylchroman-6-yl)alkanoic Acids

compd[a]	$[HbS_{drug}]/[HbS_{control}]$[b] 5 mM	10 mM	20 mM	40 mM
12[c]	1.052 (0.004)	1.086 (0.007)	1.144 (0.004)	1.299[d]
6	1.045 (0.004)	1.077 (0.010)	1.164 (0.021)	1.297 (0.018)
4	1.044 (0.005)	1.043 (0.009)	1.108 (0.012)	1.226 (0.054)
10	1.011 (0.011)	1.028 (0.009)	1.095 (0.016)	1.210 (0.002)
5b	1.010 (0.018)	1.030 (0.007)	1.085 (0.001)	1.188 (0.027)
Phe	1.036	1.048	1.093	1.178
GFZ	1.040	1.073	1.117	1.234
CFA	1.018	1.064	1.155	1.302

[a] All compounds except Phe, GFZ, and CFA were prepared as outlined in the Experimental Section. Phe and CFA were reported in ref 9 and GFZ was reported in ref 31. [b] The ratios are calculated as soluble HbS drug (g/dL)/soluble HbS control (g/dL). The values listed in the table represent the average of duplicate runs. The values in the parentheses represent the spread between the duplicate assays. [c] Compound 12 has been evaluated previously by a solubility assay[35] with somewhat lower activity reported. We estimate from the plot shown in ref 35, p 638, that the solubility ratios at 5, 10, 20, and 40 mM are 1.019, 1.043, 1.081, and 1.166, respectively. Our duplicate assays were run on different hemoglobin preparations on different dates and agree well. [d] Only one run for this concentration.

reaction was carried out at neutral pH, and the crude hydroxy acid was acetylated in pyridine/acetic anhydride (1:1) to give **5b** in 36% yield after purification by chromatography on a silica gel column. Doebner condensation of carboxaldehyde **3** with malonic acid gave *E*-3-(2,2-dimethylchroman-6-yl)-2-propenoic acid via an addition–decarboxylation mechanism.[25] This was quantitatively reduced to **6** by using 5 g/100 mL Na/Hg.

Compound **8** is a convenient intermediate in the synthesis of **10**. It was prepared as shown in Scheme III. A number of procedures[26-28] have appeared in the literature for preparing compound **12**. We have employed the procedure outlined in Scheme IV for preparing **12** because of the large quantity of 2,2-dimethylchroman available in our laboratory.

Biological Results

Antigelling Activity. Compounds **4**, **5b**, **6**, **10**, and **12** were tested for antigelling activity by using the solubility assay described by Hofrichter et al.[8] This assay involves the incubation of drugs at various concentrations with HbS in the presence of dithionite as a deoxygenating substance. Activity is reported as a ratio of HbS solubility in the presence of drug to HbS solubility in the absence of drug, i.e., $[HbS_{drug}]/[HbS_{control}]$. Solubility ratios of 1.06–1.17 have been estimated[29] as necessary for decreasing the clinical severity of sickle cell anemia. When drugs are compared at a given concentration, the higher the solubility ratio, the higher the activity.

For comparison, Table I shows the results for the five compounds, phenylalanine (a reference antigelling compound), and the antilipidemic drugs clofibric acid (CFA)[30,31] and gemfibrozyl (GFZ).[31]

At 20 mM, all the compounds attain the ratio suggested for potentially observable therapeutic benefits. The three most active chromanyl acids at 5 mM are **4**, **6**, and **12**, and their activities are comparable with those of noncovalently acting antigelling drugs CFA and GFZ. Although the antigelling activities of **6** and **12** at 10 mM are within the dosage range needed for observation of positive clinical effects, the concentrations (in the red blood cell) needed to achieve this activity are not therapeutically practical.

Oxygen Equilibrium Studies. The shifts in the oxygen equilibrium curves of HbA solutions in the presence of compounds **4**, **5b**, **6**, **10**, and **12** are shown in Table II. The compounds had small or no significant effects in shifting the allosteric equilibrium. The lowering of Hb affinity for oxygen would indicate that these compounds may bind to pockets in the Hb central water cavity similar to the binding sites found for bezafibrate[32] and clofibric acid.[31] It is not clear by structural comparison why only **4** (at high concentrations), and not the closely related molecules **6** and **10**, exhibits a small but reproducible right shift in the allosteric mechanism. Compound **5b**, which is structurally much different from the other molecules, appears to produce a small increase in oxygen affinity (left shift) at high concentrations. None of the molecules are significant allosteric regulators.

Conclusions

The antigelling results indicate that increased hydrophobicity in the acid chain does not give rise to a large increase in antigelling activity in this class of compounds. The activity observed for **5b** and **10** at high concentrations (40 mM) probably reflects either the low binding constants of these agents or their binding to multiple sites with the secondary and *not* primary sites possessing antigelling properties. In general, these acids do not have adverse effects on the oxygen equilibrium as was hoped for in the original design of molecules that might interact at the mutation site interface.

The antisickling property and mechanism of action of **12** has attracted the interest of several investigators.[33-36] The reported low toxic effect of **12** is encouraging for the continued pursuit of modifications within this class of compounds.

Experimental Section

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Acculab TM 10 spectrometer. ^1H NMR spectra were recorded either on a Varian EM 360L 60-MHz instrument or on a Bruker WM-300 spectrometer available at the Ohio State University Campus Chemical Instrument Center. The spectra are reported in parts per millin (δ) downfield with tetramethylsilane as an internal standard. Mass spectra were recorded on either a Perkin-Elmer Hitachi RMU-6E or a Varian 620 Data System spectrometer. Microanalyses were performed at the Chemistry Department, University of Ibadan, Nigeria. Reactions were monitored by TLC using precoated Merck silica gel (60 F_{254}), and spots were visualized under UV light. Products were purified by column chromatography on Merck silica gel 60 (70–230 mesh

(25) Corey, E. J.; Fraenkel, G. *J. Am. Chem. Soc.* **1953**, *75*, 1168–1172.
(26) Okpako, D. T.; Oriowo, M. A.; Okogun, J. I.; Ekong, D. E. U.; Enyenihi, V. U. *Planta Med.* **1983**, *47*, 112–116.
(27) Fatope, M. O.; Okogun, J. I. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1601–1603.
(28) Nakanishi, K. *Bioorg. Chem.* **1977**, *3*, 166–169.
(29) Sunshine, H. R.; Hofrichter, J.; Eaton, W. A. *Nature (London)* **1978**, *275*, 238–239.
(30) Abraham, D. J.; Mehanna, A. S.; Williams, F. L. *J. Med. Chem.* **1982**, *25*, 1015–1017.
(31) Abraham, D. J.; Perutz, M. F.; Phillips, S. E. V. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 324–328.
(32) Perutz, M. F.; Fermi, G.; Abraham, D. J.; Poyart, C.; Bursaux, E. *J. Am. Chem. Soc.* **1986**, *108*, 1064–1078.
(33) Ekong, D. E. U.; Okogun, J. I.; Enyenihi, V. U.; Balogh-Nair, V.; Nakanishi, K.; Natta, C. *Nature (London)* **1975**, *258*, 743–746.
(34) Honig, G. R.; Vida, L. N.; Ferenc, C. *Nature (London)* **1978**, *272*, 833–834.
(35) Poillon, W. N.; Bertles, J. F. *Biochem. Biophys. Res. Commun.* **1977**, *75*, 636–642.
(36) Natta, C. L.; Balogh-Nair, V.; Nakanishi, K. In *Biochemical and Clinical Aspects of Hemoglobin Abnormality*; Caughey, W. S., Ed.; Academic: New York, 1978; pp 143–149.

Table II. Oxygen Equilibrium Effects of (2,2-Dimethylchroman-6-yl)alkanoic Acids

	shift in P_{50} values (mmHg with drug − mmHg control)					
	10 mM		20 mM		control	
compd	av (σ_{n-1})	no. of runs	av (σ_{n-1})	no. of runs	P_{50} (σ_{n-1})	no. of runs
12	0.0 (1.5)	2	1.0 (0.7)	2	18.5 (0.8)	4
6	0.0 (0.7)	2	−0.3 (1.1)	2	17.7 (0.3)	3
4	0.8 (2.0)	4	2.3[a] (1.9)	4	18.0 (1.2)	9
10	−1.0 (0.0)	2	0.3 (1.4)	3	18.2 (0.8)	9
5b[b]	−0.5 (2.1)	2	−1.5 (0.5)	3	18.2 (0.7)	9

[a] Three of the runs averaged a shift of 3.2 mmHg with $\sigma_{n-1} = 0.8$. The other run produced the results that raised the standard deviation. [b] There were solubility problems with this compound. In some assays of **5b**, **4**, and **10**, 5 μL of *N*-methylpiperazine was added to help solubilize the compounds.

ASTM) using petroleum ether (40–60 °C)/diethyl ether mixture as the gradient elution solvent. All evaporations were performed in vacuo by using a Büchi rotavapor. The organic solvents were purified by standard techniques.

Antigelling Tests. All compounds were dissolved in 0.15 M phosphate buffer, pH 7.4, with 2 equiv of $NaHCO_3$ to make the sodium salt at a concentration of 0.18 M (5–10 μL of *N*-methylpiperazine was added to enhance solubility of **4**, **5b**, **6**, and **10**). Aliquots of this solution (10, 20, 40, and 80 μL) were mixed with buffer to equal 90 μL. The 90-μL solutions were added to 250 μL of HbS (0.15 M phosphate buffer), usually around 34 g/100 mL, and then 20 μL of dithionite (1.06 M) was added before the EPR tubes were sealed. Final concentrations of Hb were around 24 g/100 mL, and the drug concentrations were 5, 10, 20, and 40 mM in four separate tubes. A set of six tubes was spun on each run, which included the four drug concentrations, one dHbS control (90 μL of buffer, no drug), and a 40 mM phenylalanine control. The above dilution procedure produces indentical initial concentrations of HbS for all six tubes.

Oxygen Equilibrium. Oxygen equilibrium curves were determined on a Minco HEM-O-SCAN oxygen dissociation analyzer (Travenol Laboratories).

HbA solution was prepared as follows. Whole blood (20 mL) from a nonsmoking donor was drawn into a heparinized Vacutainer. The blood was immediately packed in ice (to prevent MetHb formation) and then centrifuged (10 min at 2500 rpm) to separate the plasma and buffy coat from the packed erythrocytes. The plasma and buffy coat were removed by aspiration and the cells washed three times with 0.9 g/100 mL NaCl (40 mg of EDTA/L) and then once with 1.0 g/100 mL NaCl (40 mg of EDTA/L). The cells were lysed by the addition of 1–2 volumes of deionized water containing 40 mg of EDTA/L. This was allowed to stand for 30 min with occasional mixing before being centrifuged for 1 h at 17 000 rpm at 4 °C. The supernatant was decanted into a 50-mL tube. NaCl (60 mg/mL of Hb supernatant) was added, mixed, and centrifuged as described above to remove any remaining cell stroma. The supernatant was further purified by gel filtration with Sephadex G-25 (Sigma) that was equilibrated with 0.05 M HEPES, pH 7.4, containing 0.1 M Cl ion. Concentration of the Hb solution was accomplished by first using an Amicon stirred cell (Amicon Corp.) and then a Schleicher and Schuell collodion bag apparatus (Schleicher and Schuell Inc.). The Hb solution was concentrated to approximately 6 mM for the experiment. Less than 5 g/100 mL methemoglobin was noted even after several days storage at 4 °C.

All compounds were dissolved in the HEPES buffer described above to give 0.04 M solutions. Dilutions were prepared to give two or three stock solutions. Just prior to the running of the O_2 equilibrium curve, the Hb (6 mM) and the drug were mixed in a 1:1 ratio (10 μL of Hb + 10 μL of drug) to give approximately 3 mM Hb with drug concentrations of 10 and 20 mM. The control was prepared by the addition of 50 μL of Hb to 50 μL of the HEPES bufer.

2,2-Dimethylchroman (2). To a suspension of 0.6 mol of CH_3MgI in 300 mL of dry ether under N_2(g) was added dropwise a solution of dihydrocoumarin (29.6 g, 0.2 mol) in 500 mL of dry ether. The mixture was stirred overnight at room temperature and decomposed in ice chips containing 150 mL of 2 M H_2SO_4. After 30 min, the ether layer was separated and the aqueous layer was extracted with ether. The combined extracts were washed with H_2O, 2 M $NaHCO_3$, and 2 M $Na_2S_2O_3$, dried (Na_2SO_4), and evaporated to give 24 g of 4-(*o*-hydroxyphenyl)-2-methyl-2-butanol as a white solid: yield 67%; mp 115–116 °C. A mixture of 18 g (0.1 mol) of 4-(*o*-hydroxyphenyl)-2-methyl-2-butanol and 200 mL of a concentrated H_2SO_4/H_2O mixture (1:4) was refluxed at 140 °C for 12 h, before dilution with 400 mL of water and extraction with hexane. The hexane solution was washed with H_2O and 2 M NaOH, dried ($MgSO_4$), and evaporated to give 15 g of 2,2-dimethylchroman (**2**) as a yellow oil: yield 93%; bp 52–54 °C (3 mm); NMR ($CDCl_3$) δ 6.9–7.3 (4 H, m, ArH's), 2.8 (2 H, t, CH_2Ar), 1.8 (2 H, t, CH_2CO), 1.3 (6 H, s, $C(CH_3)_2$); IR (neat) 1110 (COC), 760 cm^{-1}.

2,2-Dimethylchroman-6-carboxaldehyde (3). Dimethylformamide (DMF, 14.25 g, 0.195 mol, 15 mL) and phosphorus oxychloride ($POCl_3$, 33.40 g, 0.22 mol, 20 mL) were mixed at 0 °C. After 15 min, 12 g of 2,2-dimethylchroman (**2**) (0.074 mol) was added. The mixture was stirred magnetically at 60 °C for 10 h, then decomposed in 400 mL of H_2O containing ice chips, and left overnight. The solution was extracted with ether, and the ether extract was washed with water, dried ($MgSO_4$), and evaporated to give 10 g of crude aldehyde **3**: yield 71%; bp 110–114 °C (2 mm); NMR ($CDCl_3$) δ 9.6 (1 H, s, CHO), 7.6 (2 H, m, ArH's), 6.9 (1 H, d, J = 8 Hz, ArH), 2.8 (2 H, t, CH_2Ar), 1.8 (2 H, t, CH_2CO), 1.3 (6 H, s, $C(CH_3)_2$); IR (neat) 2900 (aldehydic, CH), 1690 (ArCHO); MS, m/e 190 (M^+). Anal. ($C_{12}H_{14}O_2$) C, H.

2,2-Dimethylchroman-6-carboxylic Acid (4). A solution of $KMnO_4$ (2.2 g in 50 mL of water) was added to a mechanically stirred solution of 2 g (0.01 mol) of **3** in 40 mL of water at 100 °C over a period of 45 min. After 1.5 h, the reaction mixture was made alkaline with aqueous KOH and filtered. The solid was washed with 30 mL of hot water twice. The combined filtrate was acidified with dilute HCl and cooled, and the resulting precipitate was filtered and recrystallized from a water/methanol mixture to give 600 mg of a white solid: yield 29%; mp 177–180 °C; NMR ($CDCl_3$, 300 MHz) δ 10.66 (1 H, br, COOH), 7.85–7.82 (2 H, m, ArH), 6.79 (1 H, d, J = 9 Hz, ArH), 2.80 (2 H, t, CH_2Ar), 1.82 (2 H, t, CH_2CO), 1.34 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1640 (ArCOOH), 850, 760 cm^{-1}; MS, m/e 206 (M^+), 191, 171, 151, 105. Anal. ($C_{12}H_{14}O_3$) C, H.

5-(2,2-Dimethylchroman-6-yl)-5-acetoxy-3-methyl-2-pentenoic Acid (5b). To a solution of diethylamine (0.015 mol, 1.5 mL) in 10 mL of hexane at 0 °C under N_2(g) was slowly added 10 mL of 1.55 M *n*-BuLi (0.015 mol) in *n*-hexane solution. The mixture was stirred at 0 °C for 1 h and at 40 °C for 6 h. 3-Methyl-2-butenoic acid (0.75 g, 0.0075 mol) in 5 mL of hexane was then added at 0 °C. The mixture was stirred for 30 min at this temperature and at room temperature overnight. The suspension was cooled to −60 °C before a solution of 1.43 g (0.0075 mol) of **3** in 5 mL of hexane/THF (5:1) was added in 20 min, and then the mixture was stirred overnight. The mixture was diluted with 100 mL of water saturated with NH_4Cl, neutralized, and extracted with ether. The ether extract was washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo. The oily residue (one major product, TLC, ethyl acetate/benzene, 3:1) was acetylated[37] overnight at room temperature in a mixture of pyridine/acetic anhydride (1:1, 10 mL) and chromatographed on silica gel after workup to give 0.9 g of **5b**: yield 36%; eluant, diethyl ether/hexane (2:3); mp 134–136 °C; NMR ($CDCl_3$), 300 MHz) δ 8.5 (1 H, exchanged D_2O, COOH), 7.14–7.26 (2 H, m, ArH's), 6.77 (1 H, d, J = 8 Hz, ArH), 5.90 (1 H, complex, =CH), 5.33–5.29 (1 H, dd,

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ArCHOAc) 2.77 (2 H, t, CH_2Ar), 2.43 (2 H, dd, CH_2C=), 2.18 (3 H, s, OAc), 2.03 (3 H, s, CH_3C=), 1.80 (2 H, t, CH_2CO), 1.33 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1730 (OAc), 1710 (CO_2H), 1640 (C=C), 1140, 1160 cm^{-1}; MS, *m/e* (relative intensity) 332 (M^+, 0.6), 273 (14), 272 (18), 227 (10), 191 (35), 190 (89), 171 (29), 161 (18), 135 (31), 82 (100), 69 (21). Anal. ($C_{19}H_{24}O_5$) C, H.

3-(2,2-Dimethylchroman-6-yl)propanoic Acid (6). Aldehyde **3** (4.5 g, 0.024 mol), 5 g (0.048 mol) of malonic acid, and 10 mL of pyridine were mixed at 80 °C to ensure dissolution. Piperidine, 0.8 mL, was then added, and the mixture was maintained at 80 °C for 1 h and refluxed at 115 °C for 3 h. On cooling, the mixture was poured into 80 mL of water containing ice chips and acidified with 6 M HCl. The precipitate was filtered and dissolved in 60 mL of water containing 2 g of NaOH pellets. This was diluted with H_2O to 75 mL, acidified, and filtered to give 3.8 g (0.016 mol) of *E*-3-(2,2-dimethylchroman-6-yl)-2-propenoic acid: yield 68.2%; mp 208–210 °C (methyl ester, mp 53–54 °C); NMR ($CDCl_3$) δ 9.3 (1 H, exchanged D_2O, COOH), 7.6 (1 H, d, J = 16 Hz, ArCH=), 7.3 (2 H, m, ArH's), 6.7 (1 H, d, J = 8 Hz, ArH), 6.2 (1 H, d, J = 16 Hz, =CHCOOH), 2.8 (2 H, t, CH_2Ar), 1.8 (2 H, t, CH_2CO), 1.3 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1690 cm^{-1} (α,β-unsaturated carbonyl); MS, *m/e* 232 (M^+).

The propenoic acid was reduced to **6** with Na/Hg (5 g/100 mL) and recrystallized from petroleum: mp 88–89 °C; NMR ($CDCl_3$, 300 MHz) δ 6.89–6.92 (2 H, m, ArH's), 6.69 (1 H, d, J = 9 Hz), 2.73 (2 H, t, $ArCH_2$), 2.64 (2 H, t, $ArCH_2$), 2.62 (2 H, t, CH_2COOH), 1.77 (2 H, t, CH_2CO), 1.3 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1710, 1110, 940, 890, 815 cm^{-1}; MS, *m/e* 234 (M^+), 219, 200, 179, 175, 159, 133. Anal. ($C_{14}H_{18}O_3$) C, H.

(2,2-Dimethylchroman-6-yl)methanol (7). Aldehyde **3**, 10 g (0.053 mol) was dissolved in 120 mL of isopropyl alcohol, and 4 g (0.15 mol) of crystalline $NaBH_4$ was added in three portions with vigorous stirring. After 4 h, the solvent was boiled off in vacuo and the residue was diluted with 160 mL of cold water. This was extracted with ether. The ether extract was washed with H_2O, dried (Na_2SO_4), and evaporated. The alcohol was separated from unreacted aldehyde by chromatography on silica gel. Diethyl ether/petroleum (4:6) eluted 8 g of **7** (0.042 mol, 78.6% yield): NMR ($CDCl_3$) δ 7.04 (2 H, m, ArH's), 6.74 (1 H, d, J = 8 Hz, ArH), 4.52 (2 H, s, CH_2O), 2.8 (2 H, t, CH_2Ar), 1.82 (2 H, t, CH_2CO), 1.78 (1 H, s, OH), 1.3 (6 H, s, $C(CH_3)_2$); IR (neat) 3600 cm^{-1} (OH).

(2,2-Dimethylchroman-6-yl)acetonitrile (8). Alcohol **7**, 22.5 g (0.12 mol), was dissolved in 200 mL of a mixture of dry benzene/petroleum ether (30–40 °C) (3:1) at −15 °C. This was saturated with dry HBr(g) generated by dropping liquid bromine on tetraline. The reaction was monitored by TLC. The solution was dried over $CaCl_2$ for 0.5 h and evaporated in vacuo at 20 °C to give 25 g of brown solid. This was dissolved in 450 mL of dry acetone containing 30 g of NaCN and 2.5 g of NaI. The mixture was magnetically stirred at room temperature for 3 days. The solid was filtered off and washed with acetone (120 mL), and the filtrate was evaporated. The oily residue was dissolved in 400 mL of benzene, washed with hot water, dried ($MgSO_4$), and evaporated to give 18 g of **8** (0.090 mol, 74.6% yield): bp 120–125 °C (2 mm); mp 39–40 °C; NMR ($CDCl_3$) δ 7.01 (2 H, m, ArH), 6.78 (1 H, d, J = 9 Hz), 3.6 (2 H, s, CH_2CN), 2.8 (2 H, t, CH_2Ar), 1.80 (2 H, t, CH_2CO), 1.3 (6 H, s, $C(CH_3)_2$); IR (neat) 2200 cm^{-1} (C≡N); MS, *m/e* 201 (M^+), 186, 172, 161, 159, 146, 137, 131, 117.

(±)-2-(2,2-Dimethylchroman-6-yl)-2-methylacetic Acid (10). A solution of 4.5 g (0.022 mol) of **8** in dry toluene was added dropwise to a suspension of 80–90% sodamide (1.1 g, ~0.023 mol) in 50 mL of toluene, at 0 °C, under a N_2(g) atmosphere. After the mixture was stirred for 1 h, 14.1 g (0.1 mol) of CH_3I was added and the mixture was heated at 80 °C for 4 h before being diluted with 50 mL of H_2O. The organic layer was separated, and the aqueous layer was extracted with toluene. The combined extracts were dried over Na_2SO_4 and evaporated to give 3.8 g of brown oil. Alkaline hydrolysis of the oil gave a mixture of acids, which were separated on a silica gel column. Diethyl ether/petroleum ether (1:9) eluted 2.4 g (0.010 mol) of **10**: yield 47%; mp 128–129 °C; NMR ($CDCl_3$, 300 MHz) δ 8.3 (1 H, br, exchanged D_2O, COOH), 7.00–7.04 (2 H, m, ArH's), 6.81 1 H, d, J = 8 Hz, ArH), 3.62 (1 H, q, ArCHCO), 2.74 (2 H, t, CH_2Ar), 1.77 (2 H, t, CH_2CO), 1.46 (3 H, d, J = 8 Hz, CH_3CH), 1.31 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1710, 1110 cm^{-1}; MS, *m/e* 234 (M^+), 220, 190, 189, 179, 173, 165, 159. Anal. ($C_{14}H_{18}O_3$) C, H.

Diethyl ether/petroleum ether (1:5) eluted (2,2-dimethylchroman-6-yl)acetic acid; mp 99–100 °C.

4-(2,2-Dimethylchroman-6-yl)butyric Acid (12). To a suspension of 6.2 g (0.062 mol) of succinic anhydride in 125 mL of a symmetrical tetrachloroethane/nitrobenzene mixture (4:1) was added 18 g (0.134 mol) of anhydrous $AlCl_3$. The mixture was mechanically stirred at 0 °C. After 1 h, 10 g (0.062 mol) of **2** was added over 30 min, and the mixture was stirred for another 6 h and allowed to stand at 0 °C for 3 days. The complex was decomposed in crushed ice containing 100 mL of 5 M HCl. The solvents were removed by steam distillation. The solid obtained was filtered off and dissolved in 200 mL of 5 g/100 mL $NaHCO_3$. After clearing with charcoal, the solution was acidified with 2 M HCl. The precipitate was crystallized from benzene/petroleum ether to give 9 g of keto acid **11** (0.034 mol): yield 55%; mp 129–130 °C (methyl ester, mp 75–76 °C). The methyl ester, 8 g (0.03 mol), was reduced by Clemmensen (Zn/Hg, 28 g; CH_3OH, 50 mL; concentrated HCl, 100 mL) and hydrolyzed (CH_3OH, 75 mL; KOH, 10 g; H_2O, 25 mL) at 100 °C in 4 h to give 5.6 g of **12** (0.023 mol): mp 68–69 °C (petroleum); NMR ($CDCl_3$, 300 MHz) δ 6.89 (2 H, m, ArH's), 6.72 (1 H, d, J = 8 Hz), 2.74 (2 H, t), 2.56 (2 H, t), 2.35 (2 H, t), 1.94 (2 H, t), 1.78 (2 H, t), 1.32 (6 H, s, $C(CH_3)_2$); IR (Nujol) 1710, 1110, 820, 710 cm^{-1}; MS, *m/e* 248 (M^+).

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Registry No. 1, 119-84-6; 2, 1198-96-5; 3, 61370-75-0; 4, 2039-47-6; 5a, 109978-80-5; 5b, 109978-85-0; 6, 109978-81-6; 7, 61370-82-9; 8, 61370-76-1; 9, 109978-82-7; 10, 109978-83-8; 11, 109978-84-9; 11 (methyl ester), 109996-87-4; 12, 58821-97-9; 4-(*o*-hydroxyphenyl)-2-methyl-2-butanol, 4167-73-1; (*E*)-3-(2,2-dimethylchroman-6-yl)-2-propenoic acid, 12772-83-7; methyl (*E*)-3-(2,2-dimethylchroman-6-yl)-2-propanoate, 109978-86-1; (2,2-dimethylchroman-6-yl)acetic acid, 109978-87-2; 3-methyl-2-butenoic acid, 541-47-9; malonic acid, 141-82-2; succinic anhydride, 108-30-5.