(A2-3), 122172-84-3; **25** (A2-4), 122173-90-4; **25** (A2-5), 122173-91-5; **26** (A2-1), 122173-92-6; **26** (A2-2), 122173-93-7; **26** (A2-3), 122172-85-4; **26** (A2-4), 122173-94-8; **26** (A2-5), 122173-95-9; **27** (A2-1), 122173-96-0; **27** (A2-2), 122173-97-1; **27** (A2-3), 122188-75-4; **27** (A2-4), 122173-98-2; **27** (A2-5), 122173-99-3; **28** (A2-1), 122174-00-9; **28** (A2-2), 122174-01-0; **28** (A2-3), 122172-86-5; **28** (A2-4), 122174-02-1; **28** (A2-5), 122174-03-2; **29** (A2-1), 122174-04-3; **29** (A2-2), 122174-05-4; **29** (A2-3), 122188-76-5; **29** (A2-4), 122174-06-5; **29** (A2-5), 122174-07-6; **30** (A2-1), 120562-31-4; **30** (A2-2), 120538-93-4; **30** (A2-3), 122188-77-6; **30** (A2-4), 120538-95-6; **30** (A2-5), 120538-96-7; **31** (A2-1), 120562-32-5; **31** (A2-2), 120538-97-8; **31** (A2-3), 120538-98-9; **31** (A2-4), 120562-52-9; **31** (A2-5), 120562-53-0; **32** (A2-2), 122174-16-7; **32** (A2-3), 122172-88-7; **32** (A2-4), 122174-17-8; **32** (A2-5), 122174-18-9; **33** (A2-2), 120562-55-2; **33** (A2-3), 120562-56-3; **33** (A2-4), 122189-06-4; **33** (A2-5), 120562-58-5; **34**, 122172-90-1; **35**, 122188-78-7; **36**, 122188-79-8; **37**, 122172-91-2; **38**, 122188-80-1; **39**, 122172-92-3; **40**, 122172-93-4; **41**, 122188-81-2; **42**, 122188-82-3; **43**, 122188-83-4; **44**, 122188-84-5; **45**, 122188-85-6; **46**, 122188-86-7; **47**, 122188-87-8; **48**, 122188-88-9; **49**, 117251-07-7; **50**, 122172-94-5; **51**, 122172-95-6; **52**, 122172-96-7; **53**, 122172-97-8; **54**, 122172-98-9; **55**, 122188-89-0; **56**, 122188-90-3; **57**, 122172-99-0; **58**, 122173-00-6; **59**, 122173-01-7; **60**, 122173-02-8; **61**, 122173-03-9; **62**, 117226-72-9; **63**, 122173-04-0; **64**, 122173-05-1; **65**, 122173-06-2; **66**, 122173-07-3; **67**, 122173-08-4; **68**, 122173-09-5; **69**, 122188-91-4; **70**, 122173-10-8; **71**, 122173-11-9; **72**, 122173-12-0; CTA (A2-1), 91032-34-7; CTA (A2-2), 91032-26-7; CTA (A2-3), 91032-36-9; CTA (A2-4), 91032-37-0; CTA (A2-5), 91032-38-1; TB, 93616-27-4; TC, 91032-39-2; TD, 89139-42-4; TD (N^{15}-Cbz derivative), 104581-72-8; $HNMe_2$, 124-40-3; $H_2NCH_2CH_2NH_2$, 107-15-3; $H_2N(CH_2)_3NH_2$, 109-76-2; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2NCH_2CH_2NHMe$, 109-81-9; $H_2N(CH_2)_3NHMe$, 6291-84-5; $H_2NCH_2CH_2NHEt$, 110-72-5; $H_2N(CH_2)_3NHEt$, 10563-23-2; $H_2NCH_2CH_2NMe_2$, 108-00-9; $H_2N(CH_2)_3NMe_2$, 109-55-7; $H_2N(CH_2)_4NMe_2$, 3529-10-0; $H_2N(CH_2)_5NMe_2$, 3209-46-9; $H_2N(CH_2)_7NMe_2$, 22078-09-7; $H_2N(CH_2)_3NEt_2$, 104-78-9; $H_2N(CH_2)_3NBu_2$, 102-83-0; $MeNHCH_2CH_2NHMe$, 110-70-3; $MeNH(CH_2)_3NHMe$, 111-33-1; $MeNHCH_2CH_2NMe_2$, 142-25-6; H-Gly-OEt·HCl, 623-33-6; H-DL-Glu(OCH_2Ph)-OCH_2Ph·TsOH), 120538-51-4; thiomorpholine, 123-90-0; *N*-methylpiperazine, 109-01-3; *N*-benzyl-4-piperidinamine, 50541-93-0; 3-amino-1-azabicyclo[2.2.2]octane, 6238-14-8; 2-(aminomethyl)pyridine, 3731-51-9; *N*-ethyl-2-(aminomethyl)pyrrolidine, 26116-12-1; *N*-(2-aminoethyl)pyrrolidine, 7154-73-6; *N*-(2-aminoethyl)morpholine, 2038-03-1; *N*-(3-aminopropyl)morpholine, 123-00-2; morpholine, 110-91-8; N^{ϵ}-aminocaproyl-β-D-galactopyranosylamine, 122211-76-1.

Design, Synthesis, and Testing of Potential Antisickling Agents. 7. Ethacrynic Acid Analogues

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In search of a drug to treat sickle cell anemia, several analogues of the diuretic ethacrynic acid (ECA) have been synthesized and found equivalent in antigelling potency to ECA, but they have moderate or little diuretic activity. Structure–activity studies revealed that most of the highly active derivatives contain an acryloyl moiety. The latter functionality reacts covalently with protein sulfhydryl groups via a Michael addition reaction. Other derivatives, which lack the acryloyl moiety, showed notably lower antigelling activity. Since the antigelling assay is run under anaerobic conditions, activity implies a stereochemical inhibition of polymerization of deoxyhemoglobin S. The solubility ratios obtained from [HbS drug]/[HbS control] of several compounds (Table I) are near those expected for a drug with clinical potential (1.06–1.20 at tolerable doses in vivo).

Our discovery of the potent antigelling and antisickling activity of ethacrynic acid (ECA)[1] (compound 1, Table I) suggested the exploitation of this lead by extensive structure–activity studies since the strong diuretic properties exhibited by ECA preclude its use as an oral therapeutic agent for the treatment of sickle cell anemia. Therefore, we set out to find ECA analogues which might retain strong antigelling activity but would lack the diuretic properties.

We now report the synthesis and antigelling activity of 32 ECA analogues. Some of the new compounds are equivalent in antigelling potency to ECA but have moderate or little diuretic activity. Structural modification of ECA included both the phenoxyacetic acid and the acryloyl moieties. Table I lists 9 derivatives of ECA with the same acryloyl group but different phenoxy acid substituents, while Table II lists 12 ECA derivatives varied in either or both moieties. Table III lists five analogues with the same phenoxyacetic acid moiety, but the vinyl moieties have been saturated, and Table IV lists six miscellaneous structurally related cyclic analogues of ECA.

Chemistry

The compounds listed in Table I–IV can be grouped into three categories with regard to their source: (1) compounds previously prepared at Merck Sharp and Dohme Co.; these include compounds **20** and **21**, (Table II), compounds **22–26** (Table III), and compounds **27**, **28**, and **31** (Table IV); (2) compounds synthesized as previously reported; these include compound 1 (ECA)[2] (Table I), compounds **10**,[3] **12**,[2] **15**,[4] **16**,[5] **17**,[5] and **19**,[5] (Table II), and compounds **29**,[6] **30**,[7] and **32**[8] (Table IV); (3) newly synthesized com-

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§ Merck Sharp and Dohme Research Laboratories.

(1) Kennedy, P. E.; Williams, F. S.; Abraham, D. J. *J. Med. Chem.* **1984**, *27*, 103.
(2) Schultz, E. M.; Cragoe, E. J., Jr.; Bicking, J. B.; Bolhofer, W. A.; Sprague, J. M. *J. Med. Pharm. Chem.* **1962**, *5*, 66.
(3) Cragoe, E. J., Jr. U.S. Patent 4,390,537, June 28, 1983.
(4) Bicking, J. B.; Holtz, W. J.; Watson, L. S.; Cragoe, E. J., Jr. *J. Med. Chem.* **1976**, *19*, 530.
(5) Schultz, E. M.; Bicking, J. B.; Deana, A. A.; Gould, N. P.; Strobaugh, T. P.; Watson, L. S.; Cragoe, E. J., Jr. *J. Med. Chem.* **1976**, *19*, 783.
(6) de Solms, S. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr. *J. Med. Chem.* **1978**, *21*, 437.
(7) Cragoe, E. J., Jr.; Schultz, E. M.; Schneeberg, J. D.; Stokker, G. I.; Woltersdorf, O. W., Jr.; Fanelli, G. M.; Watson, L. S. *J. Med. Chem.* **1975**, *18*, 225.

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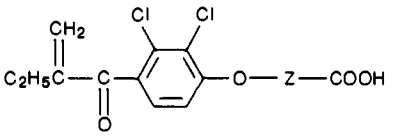
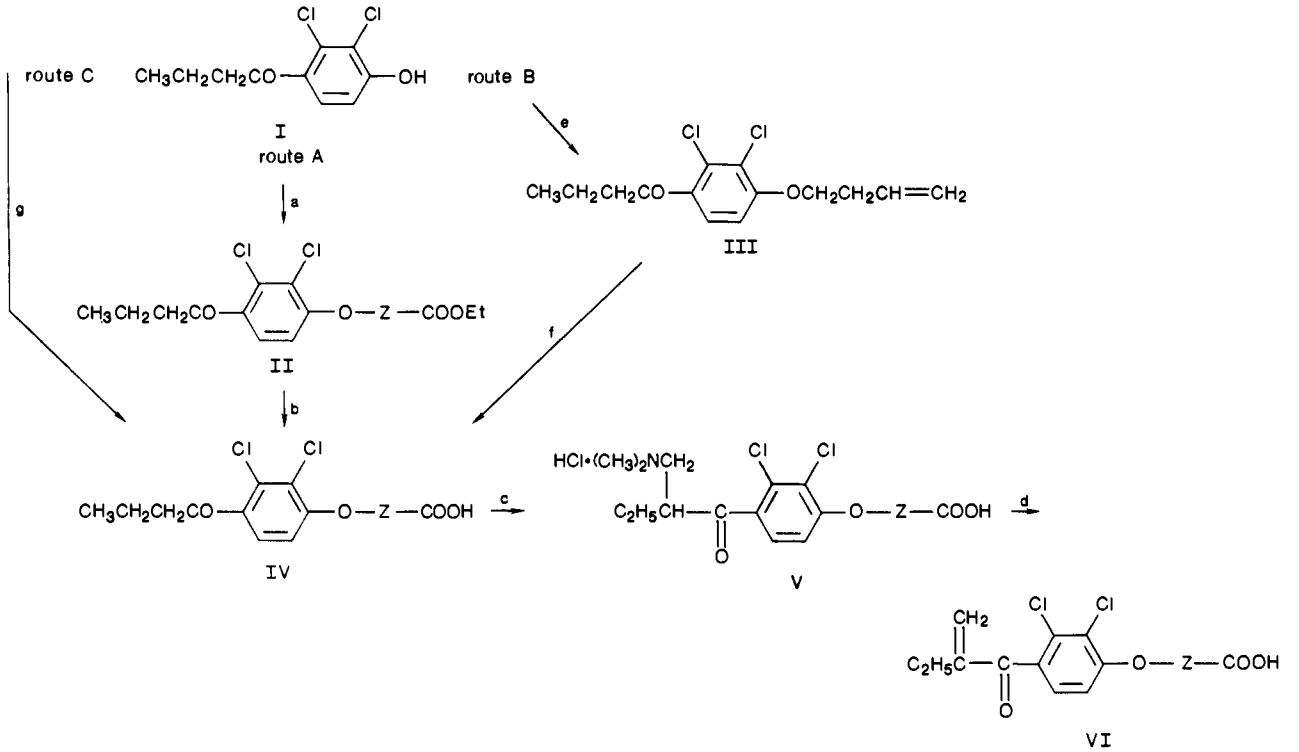
Table I. ECA Analogues with Modified Oxyalkanoic Acid Moiety



compd	Z	solubility ratio 3 mM	5 mM	7 mM	9 mM	no. of runs	activity rank at 7 mM	diuretic activity[a]
1[b]	CH_2	1.070	1.125	1.151	1.184	2	7	6
2	$(CH_2)_2$	1.112	1.145	1.188	1.302	2	4	
3	$(CH_2)_3$	1.084	1.122	1.168	1.196	3	6	±
4	$C(CH_3)_2$	1.050	1.094	1.183	1.237	3	5	0
5	$(CH_2)_4$	1.081	1.129	1.200	1.308	2	3	
6	$c\text{-}C_4H_6$	1.080	1.127	1.147	1.202	3	8	
7	$(CH_2)_5$	1.063	1.086	1.125	1.164	2	9	
8	$CH_2CONHCH_2$	1.109	1.145	1.222	1.327	2	2	3
9	$(CH_2)_3CONHCH_2$	1.107	1.224	1.287	1.309	1	1	

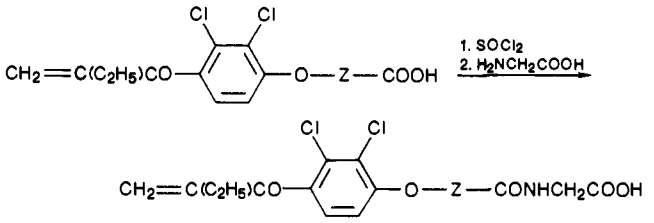
[a] Refer to the text for the significance of the numbers, and see ref 12 for details on diuretic activity and its relationship to natriuretic activity. [b] Compound 1 is ECA.

Scheme I[a]



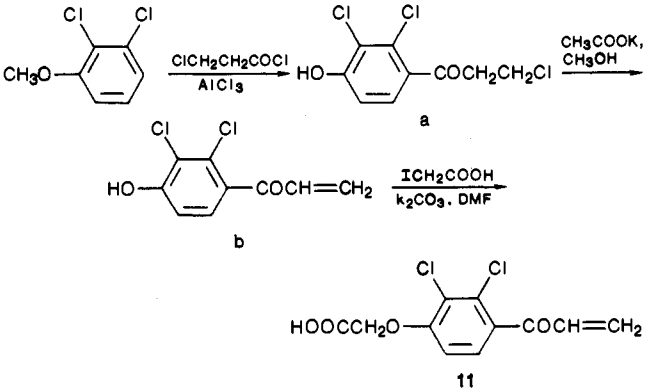
[a] (a) Br–ZCOOEt, K_2CO_3, DMF. (b) 1. NaOH, H_2O. 2. HCl. (c) $(CH_3)_2NH{\cdot}HCl$, $(CHO)_x$, CH_3COOH. (d) DMF, K_2CO_3, 60 °C. (e) $CH_2{=}CHCH_2CH_2Br$, K_2CO_3, DMF. (f) $KMnO_4$. (g) CH_3COCH_3, $CHCl_3$, KOH.

Scheme II[a]



[a] Z = CH_2 and $(CH_2)_3$ for compounds 8 and 9, respectively.

Scheme III



pounds; these include compounds **2–9** (Table I) and compounds **11**, **13**, **14**, and **18** (Table II).

The synthetic pathways for the novel compounds are outlined in Schemes I–IV (for more details on synthesis, see refs 9 and 10).

(8) Hoffman, W. F.; Woltersdorf, O. W.; Novello, F. C., Jr.; Cragoe, E. J., Jr. *J. Med. Chem.* 1981, *24*, 865.

Table II. ECA Analogues Varied in either the Acryloyl, the Oxyalkanoic acid, or Both Moieties

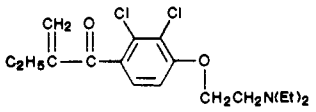
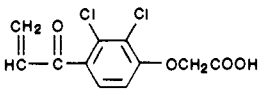
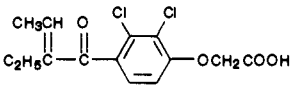
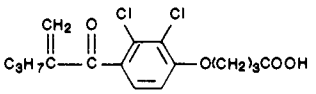
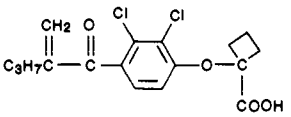
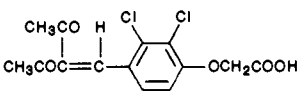
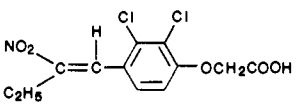
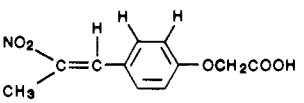
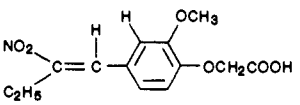
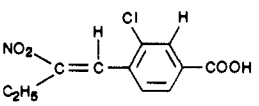
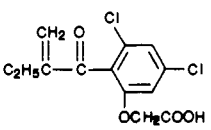
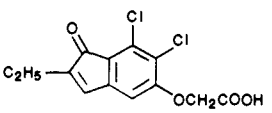
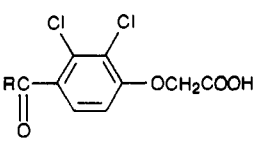
compd	structure	solubility ratio 3 mM	5 mM	7 mM	9 mM	no. of runs	diuretic activity
10		1.073	1.077	1.089	1.113	2	
11		1.033	1.060	1.067	1.095	2	±
12		1.017	1.033	1.062	1.078	1	5
13		1.082	1.115	1.155	1.149	2	
14		1.057	1.085	1.099	1.143	3	
15		1.019	1.010	0.994	1.060	1	6
16		1.051	1.086	1.096	1.131	3	
17		1.038	1.076	1.075	1.099	3	1
18		1.085	1.128	1.159	1.207	2	
19		1.052	1.100	1.106	1.150	1	
20		1.048	1.080	1.103	1.129	2	
21		1.074	1.090	1.103	1.121	2	5

Table III. ECA Analogues with Saturated Acryloyl Moiety



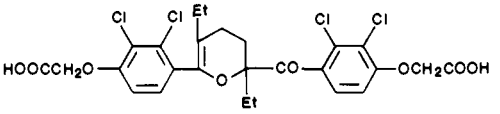
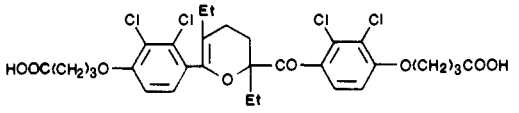
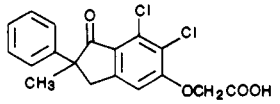
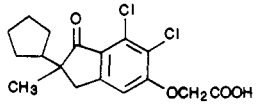
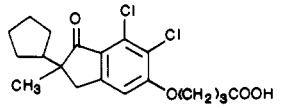
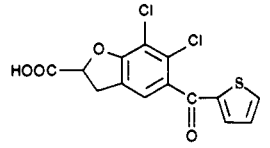
compd	R	solubility ratio 5 mM	10 mM	no. of runs	diuretic activity
22	$CH(CH_3)(C_2H_5)$	1.061	1.088	1	2
23	$CH[CH_2N(CH_3)_2 \cdot HCl](C_2H_5)$	1.018	1.168	1	4
24	$CHBr[CH(CH_3)_2]$	0.966	0.983	1	3
25	$CH(SCH_3)(C_2H_5)$	1.016	1.073	1	
26	$CH(SO_2CH_3)(C_2H_5)$	1.029	1.052	1	

Route A, Scheme I, is followed to prepare the intermediate compounds with formula IV when Z is $(CH_2)_3$, $(CH_2)_4$, 1,1-c-C_4H_6, or $(CH_2)_5$. By reacting, 2,3-dichloro-4-butyrylphenol (I) with the appropriate bromo ester, an ester of formula II is obtained. The reaction is conducted in *N,N*-dimethylformamide (DMF) in the presence of potassium carbonate or sodium carbonate at 55–60 °C for a period of 10–20 h. The esters of formula II are directly converted to the corresponding carboxylic acids of formula IV by saponification with aqueous sodium hydroxide followed by acidification.

Route B, Scheme I, is used to prepare the intermediate compound of formula IV when Z is $(CH_2)_2$. Reaction of phenol I with 4-bromo-1-butene in *N,N*-dimethylformamide and potassium carbonate or sodium carbonate gives

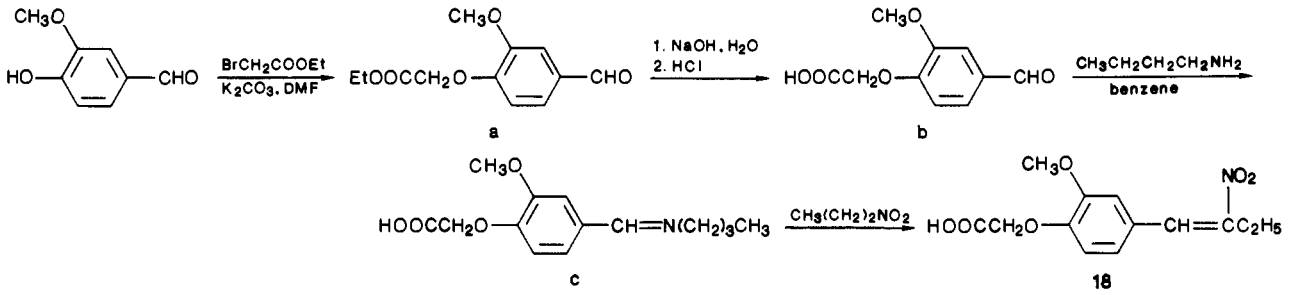
(9) Abraham, D. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr. U.S. Patent 4,669,926, Oct 13, 1987.
(10) Abraham, D. J.; Woltersdorf, O. W., Jr.; Cragoe, E. J., Jr. U.S. Patent 4,751,244, June 14, 1988.

Table IV. Miscellaneous ECA Analogues

compd	structure	solubility ratio 5 mM	solubility ratio 10 mM	no. of runs	diuretic activity[a]
27		0.993	0.996	1	0
28		1.059	1.039 (9 mM)	1	
29		0.989	1.000	1	3 (po)
30		1.010	1.015	2	2 (po)
31		0.961	1.006	1	
32		1.076	1.080	1	0 (po)

[a] po means that the compounds were administered by oral route rather than by iv.

Scheme IV



structure III. III is converted by oxidation to structure IV [Z = $(CH_2)_2$] by stirring a two-phase mixture of the phenol III in methylene chloride with an aqueous solution of potassium permanganate at 0–10 °C for 1.5 h. A detergent, e.g., benzyltriethylammonium chloride, is used to promote the two-phase reaction. The product is isolated by extracting the methylene chloride layer with sodium hydroxide followed by acidification of the aqueous extract with hydrochloric acid.

Route C, Scheme I, is used to prepare the intermediate of formula IV, where Z = $C(CH_3)_2$. The reaction of the phenol I with acetone and chloroform in the presence of sodium hydroxide produces a compound of formula IV, with Z = $C(CH_3)_2$. The reaction is conducted by adding chloroform dropwise to a mixture of phenol I and sodium hydroxide in acetone followed by refluxing the mixture for a period of 3 h. The product is isolated by evaporating the acetone, treating the residue with water, acidifying the mixture, extracting with ether, drying the ether, and removing the solvent. The product is conveniently purified by chromatography.

A compound of formula IV is reacted under Mannich conditions with dimethylamine hydrochloride, paraformaldehyde, and a catalytic amount of acetic acid to form a compound of formula V. The reaction is generally conducted without a solvent by heating on a steam bath for periods of 5–12 h. It is generally not convenient to isolate the compounds of formula V, but rather to convert them directly to the desired products VI by heating with DMF. The reaction is generally complete when heating at 75–110 °C is carried out for 5–12 h.

Compounds **8** and **9** (Table I) are the glycine conjugates of compounds **1** and **3**, respectively, and are prepared by conversion of the corresponding acid to the acid chloride using thionyl chloride followed by reaction with glycine (Scheme II).

Compound **11** (Table II) is synthesized according to Scheme III. The first step involved a Friedel-Crafts reaction with 3-chloroproprionyl chloride and 2,3-dichloroanisole in the presence of aluminum chloride in methylene chloride to produce the phenol a. The reaction of phenol a with potassium acetate in methanol produces the acrylophenone b. Reaction of compound b with iodoacetic acid in acetone in the presence of potassium carbonate at reflux temperature produces, after acidification, compound **11**.

Compounds **13** and **14** (Table II) are prepared via the reactions described for compounds **3** and **6**, respectively

Table V

score	change in Na^+ excretion, μequiv/min[a]
±	active only at higher doses
0	0–100
1	100–499
2	500–699
3	700–899
4	900–1099
5	1100–1299
6	over 1300

[a] Other criteria, such as chlorouresis or diuresis, generally ran parallel to this.

(Scheme I, route A), except that equimolar amounts of 2,3-dichloro-4-valerylphenol are substituted for 2,3-dichloro-4-butyrylphenol.

Compound 18 (Table II) is prepared according to Scheme IV. The conversion of vanillin to the corresponding phenoxyacetic acid derivative b is accomplished via route A, Scheme I. Aldehyde b is heated with butylamine to form Schiff base c. Addition of nitropropane to compound c with heating gives compound 18 (Table II).

Biological Activity

Antigelling Properties. The antigelling testing of all compounds was performed by using the assay developed by Hofrichter et al.[11] This assay involves deoxygenation of concentrated HbS with sodium dithionite in the presence of different concentrations of drug. Samples are then sealed in quartz EPR tubes under anaerobic conditions and spun at about 150000*g* for 2.5 h at 35 °C in an ultracentrifuge. This procedure pellets the polymerized HbS to the bottom of the tubes, and the supernatant (soluble HbS) is measured as the cyanmethemoglobin derivative. See Experimental Section for details.

Phenylalanine was used as an internal standard for each compound run plus the control with no compound. The experimental error in the HbS solubility of 173 runs is 17.03 g % ± 0.46. Therefore, single runs should not vary by more than that. Each compound, even though some are only evaluated once, was measured at various concentrations to give a dose–response curve. The dose–response curve approach was found suitable as a means of screening to ensure that active compounds would not be missed.

Diuretic Effect.[12] Compounds that have been previously tested for a diuretic effect are listed in Tables I–IV.[12] The usual biological evaluation of each compound consisted of intravenous administration of 5 mg/kg to dogs in which urinary Na^+, K^+, Cl^-, and urine volume were measured in comparison to a control phase. The 5 mg/kg dose was given over a period of 5 min, and 15-min collections of urine were taken over a period of 2 h.

To simplify the data, the natriuretic results (change in Na^+ excretion) were scored according to the criteria shown in Table V.

Structure–Activity Results. Since the solubility assay is performed under anaerobic conditions, it monitors activity that must arise from a stereochemical distortion in Hb tertiary or quaternary structure, which in turn destabilizes polymer formation. The ratio values in the activity tables signify the solubility of HbS in the presence of the compound to the solubility of HbS without compound (i.e., control). Therefore, the higher the ratio, the higher the activity of the compound at that concentration. Because a measurement is made for each of four concentrations (3, 5, 7, and 9 mM, Tables I and II), it may be somewhat difficult to compare small differences in activities from compound to compound. Therefore we have ranked the compounds in Table I in order of their activity ratios as measured at 7 mM.

Sunshine et al. have published the solubility ratios and kinetic parameters (under these assay conditions) for correlation of clinical severity with inhibition of sickle cell hemoglobin gelation.[13] Compounds with ratios above 1.06 at low concentrations in vivo (1–3 mM) meet the minimum ratio estimated by Sunshine et al. for the observation of an improved clinical course. Such compounds would be of interest for other antigelling or antisickling assays as well as be candidates for further molecular modification. As can be seen in Tables I–IV, several of our most active compounds (at 3 mM) fit this criteria. The saturation effects of the more active molecules can be observed in the tables by looking at the solubility ratios vs the concentration of the compound.

Of the 32 compounds studied, analogues 1–9 (Table I) demonstrated the highest antigelling activity, with **9** > **8** > **5** > **2** > **4** > **3** > **1** > **6** > **7** at 7 mM. Compounds 1–7 (Table I) vary in structure only by addition of various aliphatic substituents to the acetic acid moiety of ECA. Compounds **8** and **9** are the glycine conjugates of ECA and compound **3**, respectively. The solubility results for compounds 1–9 at 7 mM (approximately 2 drug/1 HbS; see experimental where HbS concentration is around 3.7 mM) indicate that these derivatives are very strong antigelling agents and have similar potency. The rank order of activity for compounds 1–9 (Table I) at all concentrations is about the same with **9** the most active and **7** the least. At 7–9 mM all nine compounds exhibit activity that should theoretically reduce clinical symptoms of the disease.[13]

The presence of a 2-methylenebutyryl moiety comprises a common structural feature of compounds 1–9. Any modification made at the acryloyl linkage leads to lower antigelling activity. Compound **11**, which lacks the ethyl group on the acryloyl moiety, showed only moderate activity, while compound **12** with a methyl group at the terminal vinyl carbon shows greatly reduced antigelling activity.

In addition to the highly active analogues listed in Table I, Table II also contains several compounds with moderate activity comparable to that of compound **7** in Table I. These derivatives include the 2-methylenepentanoyl analogues (compounds **13** and **14**), the 2-nitroalkenyl compounds (compounds **16–19**), and the ECA cyclic analogue (compound **21**).

Compound **10** (Table II) (a basic analogue of ECA) also demonstrated moderate activity; however, solubility at concentrations used in the assay posed a serious problem.

Compound **20** (Table II), which is an isomer of ECA that has the acryloyl group adjacent to the oxyacetic acid moiety and chlorino groups in the 2- and 6-positions, showed only mild activity.

Compounds with saturated acyl side chain (analogues **22–26**, Table III) were studied as well as compounds **27** and **28** (dimers of compounds **1** and **3**, respectively) and the cyclic derivatives **29–32** (Table IV). None of the above (compounds **22–32**, Tables III and IV) demonstrated interesting antigelling activity.

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Discussion and Conclusions

The nine very active compounds in Table I illustrate the basic nucleus for high activity. The 2-methylenebutyryl moiety is unaltered, and the oxyalkanoic acid moiety can be modified without major alteration in activity. If the vinyl group is saturated and the oxyalkanoic acid kept constant, activity is greatly reduced or totally lost (Table III). Substitution at the terminal vinyl carbon atom reduces the antigelling activity (compound 12).

The acryloyl moiety in these molecules has been shown to react covalently with hemoglobin surface sulfhydryl groups.[14] Therefore, it was expected that compound 11 with the unsubstituted acryloyl group would have equal or higher activity since it should be as chemically reactive to β Cys 93 as is ECA. Surprisingly, we found that compound 11 has only moderate activity. One explanation for the unexpected low antigelling activity of compound 11 would be that there is a loss of steric interaction between the ethyl moiety found in ECA and a key salt bridge (F-helix; β His 146 and β Asp 94).[14] Breaking this salt bridge permits the F-helix to move and perturb the acceptor pocket (β Phe 85 and β Leu 88) for the donor β Val 6 of HbS that is involved in polymerization under anaerobic conditions.[15] An alternate explanation is that the unsubstituted acryloyl group would react with a variety of nucleophiles other than sulfhydryl, thus diluting this specific reaction.

Compounds 13 and 14, with the propyl for ethyl substituents on the vinyl group, were synthesized to see if the longer alkyl chain would cause greater steric interaction with the F-helix. Both compounds exhibited activity similar to those in Table I, but not as great as expected.

Compounds 15–19 (Table II) were of interest since the reactive vinyl moiety has been modified in such a manner that they can still undergo a Michael addition with β Cys 93. Compound 18 was one of the most active compounds. Attempts to determine the nature of its reactivity and binding with hemoglobin are under way.

A recent report indicates that the ECA cyclic analogue (compound 21) has interesting membrane activity with sickle cells,[16] but we found it to have only moderate activity as an antigelling compound.

Two of the very active compounds listed in Table I [compounds 1 (ECA) and 3] possess antigelling and antisickling[17,18] properties that make this series attractive for more detailed evaluation. Many of these agents increase the oxygen affinity of HbS,[19] which gives them a dual mechanism of action: one by stereochemical inhibition of polymerization and one by shifting the allosteric equilibrium toward the more soluble oxygenated (*R*) form of HbS. The latter mechanism of action has been attributed to the efficacy of antisickling agents such as cyanate,[20,21] BW_{12C},[22] and aromatic aldehydes.[23,24] However, no clinical proof exists that such agents will be therapeutically effective. Recent studies of some of these agents (Tables I–IV) demonstrate that they alter erythrocyte membrane chemistry, reduce red cell volume, and increase erythrocyte rigidity.[25] If this class of agents can be modified to circumvent these adverse membrane effects, as well as exhibit low or no toxicity, a first-generation drug to treat sickle cell anemia can be envisioned.

Experimental Section

Synthesis of Compounds 3 and 5–7 (Table I) and Compounds 13 and 14 (Table II). 4-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]butyric Acid (Compounds 3, Table I). Step A: 4-(2,3-Dichloro-4-butyrylphenoxy)butyric Acid [Structure IV, Scheme I, Z = $(CH_2)_3$]. A mixture of 2,3-dichloro-4-butyrylphenol (69.9 g, 0.30 mol), potassium carbonate (75 g, 0.54 mol) and ethyl 4-bromobutyrate (87.8 g, 0.45 mol) in *N,N*-dimethylformamide (900 mL of sieve-dried material) was stirred and heated at 55–60 °C for 16.5 h. The mixture was then treated with 10 N sodium hydroxide solution (120 mL) and water (600 mL) and stirred at 100 °C for 2 h. The solution was cooled and poured into ice water (4 L) containing concentrated hydrochloric acid (250 mL). The solid that separated was removed by filtration, washed with water, and dried. The product (88 g) was recrystallized from butyl chloride (500 mL) to give 78.6 g (82%) of 4-(2,3-dichloro-4-butyrylphenoxy)butyric acid, mp 111–113 °C. Anal. ($C_{14}H_{16}Cl_2O_4$) C, H, Cl.

By conducting the same reaction described for compound **3**, step A, the following compounds were obtained:

5-(2,3-Dichloro-4-butyrylphenoxy)valeric acid [structure IV, Scheme I, Z = $(CH_2)_4$]: 98% yield, mp 102–103 °C. Anal. ($C_{15}H_{18}Cl_2O_4$) C, H.

1-(2,3-Dichloro-4-butyrylphenoxy)cyclobutane-1-carboxylic Acid (Structure IV, Scheme I, Z = *c*-C_4H_6). After being heated for 16.5 h, the reaction mixture was poured into ice water, and the resulting mixture was extracted with ether. The ether extract was washed with a 1% sodium hydroxide solution and then with water and finally dried over $MgSO_4$. The ether was evaporated in vacuo and the residue treated with acetic acid (600 mL) and 5% hydrochloric acid (150 mL). The mixture was stirred and refluxed overnight. After the solvents were evaporated in vacuo, the residue was chromatographed over a column containing silica using methylene chloride/tetrahydrofuran/acetic acid, 50/1/1, as the eluent. The pertinent fractions were combined, treated with toluene, and evaporated in vacuo to obtain 1-(2,3-dichloro-4-butyrylphenoxy)cyclobutane-1-carboxylic acid (in 27% yield) as a liquid whose identity was confirmed by NMR and purity by thin-layer chromatography.

6-(2,3-Dichloro-4-butyrylphenoxy)hexanoic acid [structure IV, Scheme I, Z = $(CH_2)_5$]: 96% yield, mp 94–95 °C. Anal. ($C_{16}H_{20}Cl_2O_4$) C, H.

4-(2,3-Dichloro-4-valerylphenoxy)butyric acid (precursor of compound 13, Table II): 38% yield, mp 92–94 °C. Anal. ($C_{15}H_{18}Cl_2O_4$) C, H.

1-(2,3-Dichloro-4-valerylphenoxy)cyclobutane-1-carboxylic acid (precursor of compound 14, Table II): 32% yield, as confirmed by NMR.

Step B: 4-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]butyric Acid (Compound 3, Table I). A mixture of 4-(2,3-dichloro-4-butyrylphenoxy)butyric acid (71.8 g, 0.225 mol), paraformaldehyde (16.5 g, 0.549 equiv), dimethylamine hydrochloride (82.0 g, 1.0 mol), and acetic acid (5.5 mL) was stirred on a steam bath for 7 h. *N,N*-Dimethylformamide (172 mL) was added, and stirring and heating were continued for another hour.

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The solution was next poured into cold 1 N hydrochloric acid (1.5 L) with stirring. The product that separated slowly solidified. This material was pulverized, filtered, washed with water, and dried to give 70.8 g (95%) of product. This material was recrystallized from butyl chloride to give 4-[2,3-dichloro-4-(2-methylenebutyryl)phenoxy]butyric acid melting at 87–89 °C. Anal. ($C_{15}H_{16}Cl_2O_4$) C, H, Cl.

By conducting the same reaction described for compound 3, step B, the following compounds were obtained:

5-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]valeric acid (compound 5, Table I): 54% yield, mp 107–109 °C. Anal. ($C_{16}H_{18}Cl_2O_4$) C, H.

1-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]cyclobutane-1-carboxylic acid (compound 6, Table I): 43% yield, mp 120 °C. Anal. ($C_{16}H_{16}Cl_2O_4$) C, H.

6-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]hexanoic acid [compound 7, Table I]: 98.6% yield, mp 106–107 °C. Anal. ($C_{17}H_{20}Cl_2O_4$) C, H.

4-[2,3-Dichloro-4-(2-methylenevaleryl)phenoxy]butyric acid (compound 13, Table II): 93% yield, mp 85–87 °C. Anal. ($C_{16}H_{18}Cl_2O_4$) C, H.

1-[2,3-Dichloro-4-(2-methylenevaleryl)phenoxy]cyclobutane-1-carboxylic acid (compound 14, Table II): 47% yield, mp 105–106 °C. Anal. ($C_{17}H_{18}Cl_2O_4$) C, H.

Preparation of 3-[2,3-Dichloro-4-(2-methylenebutyryl)-phenoxy]propionic Acid (Compound 2, Table I). Step A: 2,3-Dichloro-4-(3-butenyloxy)butyrophenone (Structure III, Route B, Scheme I). A mixture of 2,3-dichloro-4-butyrophenol (21 g, 0.090 mol) and potassium carbonate (37 g, 0.27 mol) in *N,N*-dimethylformamide (100 mL) was stirred and heated to 60 °C. 4-Bromo-1-butene (26.7 g, 0.2 mol) was added and the mixture stirred and heated at 60–65 °C for 5 h. The reaction mixture was poured into ice water and the mixture extracted with ether. The ether layer was washed with water and then with brine and finally dried over $MgSO_4$. The ether was removed by evaporation at reduced pressure to give 25 g (quantitative yield) of 2,3-dichloro-4-(3-butenyloxy)butyrophenone as confirmed by elementary analysis. Anal. ($C_{14}H_{16}Cl_2O_2$) C, H.

Step B: 3-(2,3-Dichloro-4-butyrylphenoxy)propionic Acid [Structure IV, Scheme I, Z = $(CH_2)_2$]. A solution of 2,3-dichloro-4-(3-butenyloxy)butyrophenone (31.5 g, 0.11 mol) and benzyltriethylammonium chlorde (2.3 g) in methylene chloride (350 mL) was added dropwise to a solution of potassium permanganate (45.8 g) in water (900 mL) at 5 °C. The mixture was then stired at 20 °C for 1.5 h after which tests showed that the oxidation was complete. Sodium bisulfite and dilute hydrochloric acid were added until the $KMnO_4$ color disappeared. The mixture was filtered to remove a small amount of solid, and the methylene chloride layer from the filtrate was separated. This layer was washed with water and then extracted with a dilute sodium hydroxide solution. The aqueous layer was poured into ice water containing dilute hydrochloric acid. The solid that separted was removed by filtration, washed with water, dried, and recrystallized (butyl chloride) to give 9.0 g (14%) of 3-(2,3-dichloro-4-butyrylphenoxy)propionic acid, mp 138–139 °C. Anal. ($C_{13}H_{14}Cl_2O_4$) C, H.

Step C: 3-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]propionic Acid (Compound 2, Table I). By conducting the same reaction described for the preparation of compound 3, step B, there was obtained compound 2 in 83% yield, mp 136–138 °C. Anal. ($C_{14}H_{14}Cl_2O_4$) C, H.

2-[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]-2-methylpropionic Acid (Compound 4, Table I). Step A: 2-(2,3-Dichloro-4-butyrylphenoxy)-2-methylpropionic Acid [Structure IV, Scheme I, Z = $C(CH_3)_2$]. A mixture of 2,3-dichloro-4-butyrylphenol (15 g, 0.064 mol), acetone (140 mL), and sodium hydroxide (14.5 g, 0.36 mol) was stirred and heated to reflux. Chloroform (12 g, 0.1 mol) was added to the mixture dropwise over a period of 15 min. Refluxing was continued for 3 h, and the acetone was evaporated in vacuo. The residue was treated with ice water containing hydrochloric acid. The product was extracted with ether. The ether extract was washed with water and then with brine and finally dried over $MgSO_4$. The ether was evaporated in vacuo and the residue chromatographed on a column containing silica gel (320 g) and eluted with methylene chloride/tetrahydrofuran/acetic acid, 50/1/1. The appropriate fractions were combined and evaporated in vacuo to give 10 g (49%) of 2-(2,3-dichloro-4-butyrylphenoxy)-2-methylpropionic acid, which was identified by NMR and the purity confirmed by thin-layer chromatography.

Step B. By application of the same reaction described to prepare compound 3, step **B**, there was obtained compound 4 in 61.4% yield after recrystallization from cyclobutane, mp 88–89 °C. Anal. ($C_{15}H_{16}Cl_2O_4$) C, H.

***N*-{[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]-acetyl}glycine (Compound 8, Table I)**. To a solution of [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid (18.19 g, 0.06 mol) in dry benzene (45 mL) was added thionyl chloride (19.04 g, 0.12 mol) and the mixture stirred and refluxed for 75 min. The volatile materials were removed by evaporation at reduced pressure, and the residue, which consisted of [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetyl chloride, was dissolved in dry tetrahydrofuran (50 mL).

To a stirring suspension of finely ground glycine (6.01 g, 0.08 mol) in tetrahydrofuran (50 mL) was added, dropwise, the solution of [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetyl chloride over a period of 15 min. The mixture was stirred at ambient temperature for 2.25 h and then at reflux for 1 h. The mixture was cooled, filtered, and washed with tetrahydrofuran. The solid was suspended in water (60 mL), filtered, washed with water, and dried to give 7.15 g (33%) of *N*-{[2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetyl}glycine. Upon recrystallization from isopropyl alcohol, the product melted at 201.5–202.5 °C. Anal. ($C_{15}H_{15}Cl_2NO_5$) C, H, N.

***N*-{[2,3-Dichloro-4-(2-methylenebutyryl)phenoxy]-butyryl}glycine (Compound 9, Table I)**. When the reaction is carried out essentially as described for compound 8, except that the [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid is replaced by an equimolar quantity of 4-[2,3-dichloro-4-(2-methylenebutyryl)phenoxy]butyric acid, there is obtained *N*-{4-[2,3-dichloro-4-(2-methylenebutyryl)phenoxy]butyryl}glycine in 84% yield, mp 76–78 °C dec. Anal. ($C_{17}H_{19}Cl_2NO_5$) C, H, N.

(2,3-Dichloro-4-acrolylphenoxy)acetic Acid (Compound 11, Table II). Step A: 2,3-Dichloro-4-(3-chloropropionyl)-phenol (Compound a, Scheme III). A stirred mixture of 2,3-dichloroanisole (71 g, 0.6 mol) and 3-chloropropionyl chloride (76.5 g, 0.6 mol) in dry methylene chloride (300 mL) was cooled to 5 °C and aluminum chloride (80 g, 0.6 mol) added portionwise over a period of 30 min. After stirring an additional 2 h, the methylene chloride was removed by distillation. More methylene chloride (150 mL) was added and removed by distillation. The residue was treated with more methylene chloride (200 mL) and aluminum chloride (80 g, 0.6 mol) and the mixture stirred and refluxed for 2.5 h. The reaction mixture was cooled and poured into a mixture of ice and concentrated hydrochloric acid (70 mL). The mixture was extracted with ether and the organic layer washed with water, dried over $MgSO_4$, and filtered, and the solvents were removed by evaporation in vacuo. The residue was treated with benzene to give 28 g of a solid which was removed by filtration. Treatment of the filtrate with cyclohexane gave another 6.5 g of product. The combined products (22.7%) were recrystallized from benzene to give 2,3-dichloro-4-(3-chloropropionyl)phenol, mp 109–111 °C. Anal. ($C_9H_7Cl_3O_2$) C, H.

Step B: 2,3-Dichloro-4-acryloylphenol (Compound b, Scheme III). A solution of 2,3-dichloro-4-(3-chloropropionyl)-phenol (8.4 g, 0.033 mol) in methanol (30 mL) and potassium acetate (3.5 g, 0.033 mol) in methanol (30 mL) were combined and refluxed for 20 min. The mixture was treated with chloroform (200 mL), washed with water, dried over $MgSO_4$, and filtered and the solvent removed from the filtrate by evaporation in vacuo. The residue was recrystallized from carbon tetrachloride to give 6.0 g (83.8%) of 2,3-dichloro-4-acryloylphenol, mp 135–137 °C. Anal. ($C_9H_6Cl_2O_2$) C, H, Cl.

Step C: (2,3-Dichloro-4-acryloylphenoxy)acetic Acid (Compound 11, Table II). A mixture of 2,3-dichloro-4-acryloylphenol (4.85 g, 0.0224 mol), iodoacetic acid (4.5 g, 0.024 mol), potassium carbonate (3.33 g, 0.024 mol), and acetone (200 mL) was stirred and refluxed for 20 h. The reaction mixture was cooled and the solid removed by filtration, dissolved in water (100 mL), and acidified to pH 1 with concentrated hydrochloric acid. The material that separated was extracted with chloroform, the organic layer removed, washed with water, dried over $MgSO_4$, and filtered,

and the solvent removed by evaporation in vacuo. The residue was recrystallized from carbon tetrachloride to give 2.2 g (35.7%) of (2,3-dichloro-4-acryloylphenoxy)acetic acid, mp 111–113 °C. Anal. ($C_{11}H_8Cl_2O_4$) C, H, Cl.

(*E*)-[2-Methoxy-4-(2-nitro-1-butenyl)phenoxy]acetic Acid (Compound 18, Table II). Step A: (2-Methoxy-4-formylphenoxy)acetic Acid (Compound b, Scheme IV). A mixture of vanillin (16.3 g, 0.107 mol), ethyl bromoacetate (21.6 g, 0.128 mol), potassium carbonate (17.8 g, 0.128 mol), and *N*,*N*-dimethylformamide (75 mL) was stirred and heated at 50–55 °C for 35 min. Then a 10% aqueous sodium hydroxide solution (100 mL) and water (300 mL) were added, and the mixture was stirred on a steam bath for 1 h. The solution was cooled and acidified with hydrochloric acid. The solid that separated was removed by filtration, washed with water, and dried to give 20.4 g (90.7%) of the desired product, mp 177–178 °C. Anal. ($C_{10}H_{10}O_5$) C, H.

Step B: (*E*)-[2-Methoxy-4-(2-nitro-1-butenyl)phenoxy]acetic Acid (Compound 18, Table II). A mixture of (2-methoxy-4-formylphenoxy)acetic acid (10.5 g, 0.05 mol) and butylamine (14.6 g, 0.2 mol) in benzene was refluxed in a flask equipped with a Dean–Stark water separator. The mixture was refluxed for 4 h and the benzene removed by distillation at reduced pressure. The residue consisted of [2-methoxy-4-[(butylimino)-methylene]phenoxy]acetic acid, which was treated with nitropropane (17.8 g, 0.2 mol) and acetic acid (50 mL) and heated to boiling. After standing for 30 min, the mixture was poured into crushed ice and acidified with hydrochloric acid. The solid that separated was removed by filtration, washed with water, dried, and recrystallized first from a mixture of water and isopropyl alcohol (3/1) and then from a mixture of benzene and hexane (4/3) to give the desired product in 93% yield, mp 116–117 °C. Anal. ($C_{13}H_{15}NO_6$) C, H, N.

Preparation of Pure HbS Solution. Homozygous sickle cell blood obtained from nonsmoker donors was centrifuged for 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 were washed 3 times with 0.9 g % sodium chloride solution and then once with 1.0 g % sodium chloride solution. The cells were lysed by the addition of 1–2 volumes of pH 8.6 Tris buffer (50 mM, containing 40 mg of EDTA/L). This was allowed to stand at 4 °C for 30 min with occasional mixing before being centrifuged for 2 h at 10 000 rpm at 4 °C. The supernatant was decanted into a flask, and sodium chloride (60 mg/mL of hemoglobin supernatant) was added, mixed, and centrifuged as described above to remove any remaining cell stroma. The supernatant was further purified by either gel filtration with Sephadex G-25 or dialysis against pH 8.6 Tris buffer (50 mM, containing 40 mg of EDTA/L). The sodium chloride free hemoglobin solution was chromatographed on DEAE-Sephacel ion-exchange resin (Sigma) preequilibrated with pH 8.6 Tris buffer. After elution of the A_2 hemoglobin fraction by Tris buffer (pH 8.6, 50 mM, containing EDTA), the HbS fraction was then eluted with pH 8.4 Tris buffer. The pure HbS fraction (identified by electrophoresis) was concentrated in an Amicon stirred cell (Amicon, Model 402) and further concentrated by using a Schleicher and Schuell collodion bag apparatus (Schleicher and Schuell Inc.) with phosphate buffer (150 mM, pH 7.4) as the exchange buffer. The hemoglobin concentration was then determined by using the cyanmethemoglobin derivative.[26,27] The hemoglobin concentration at this point was usually found to be around 35 g% (approximately 5.5 mM).

Antigelling Assay. All compounds were analyzed as outlined under Biological Activity. Compounds tested at 3, 5, 7, and 9 mM (Tables I and II) were dissolved in 0.15 M, phosphate buffer, pH 7.4, with equivalent amounts of sodium bicarbonate to make the sodium salt at a concentration of 0.036 M. Appropriate aliquots of this solution (30, 50, 70, and 90 μL) were mixed with buffer to equal 90 μL. The 90-μL solutions were added to 250 μL of HbS (35 g % in phosphate buffer, pH 7.4, prepared as described above), and the mixture was incubated overnight at 4 °C to allow hemoglobin–drug interaction before deoxygenation (see ref 1 for explanation). After overnight incubation, 20 μL of sodium dithionite (1.06 M) was added before the EPR tubes were sealed and spun at 150000*g* for 2.5 h at 35 °C in an ultracentrifuge. The above dilution procedure produces final compound concentrations of 3, 5, 7, and 9 mM, respectively, and a HbS concentration of around 24 g % (3.7 mM) as well as a final dithionite concentration of 58.88 mM. A set of six tubes was spun on each run, which included the four compound concentrations, one HbS control (90 μL of buffer, no compound), and a 40 mM phenylalanine control. After the tubes were spun, the HbS concentration in the supernatant was determined by using the cyanmet method and the pH was measured for each tube. The pH was found to be at 7.00 ± 0.05 for all tubes including the controls. This pH change from 7.4 to 7.0 could be attributed to the acidic nature of the dithionite in aqueous medium.

For compounds that were tested only at 5 and 10 mM (Tables III and IV), the same procedure was followed with the exception that the stock compound solution was prepared at 0.18 M concentration, and 10- or 20-μL aliquots were used to produce final compound concentrations of 5 and 10 mM, respectively.

It should be indicated that compounds **10**, **16**, **21** (Table II), **24** (Table III), **27**, **29**, and **30** (Table IV) did not dissolve completely at pH 7.4 and were applied to the assay mixture as fine suspension. The solubility ratios (Tables I–IV) were calculated as [solubility of HbS (g %) in the presence of the test compound]/[solubility of HbS (g %) in control (no compound)].

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Registry No. 1, 58-54-8; **2**, 113239-16-0; **3**, 59708-09-7; 4, 20287-91-6; **5**, 113239-17-1; **6**, 113239-19-3; **7**, 113239-18-2; **8**, 29922-97-2; **9**, 113262-76-3; **10**, 58247-26-0; 11, 23228-14-0; **12**, 1431-30-7; **13**, 113262-79-6; 14, 113239-20-6; **15**, 17796-20-2; **16**, 16861-27-1; **17**, 24634-68-2; **18**, 122358-38-7; **19**, 113239-43-3; **20**, 6467-47-6; **21**, 27366-23-0; **22**, 5378-94-9; **23**, 1592-83-2; **23** (free base), 1160-10-7; **24**, 5310-76-9; **25**, 122358-39-8; **26**, 122358-40-1; **27**, 25355-92-4; **28**, 122358-41-2; **29**, 56049-88-8; **30**, 54197-05-6; **31**, 54778-08-4; **32**, 67287-20-1; **I**, 2350-46-1; **III**, 113239-47-7; **IV** (Z = $(CH_2)_2$), 113239-48-8; **IV** (Z = $(CH_2)_3$), 113239-49-9; **IV** (Z = $(CH_2)_4$), 113239-51-3; **IV** (Z = $(CH_2)_5$), 113239-52-4; **IV** (Z = CMe_2), 113239-56-8; $Br(CH_2)_3CO_2Et$, 2969-81-5; $Br(CH_2)_4CO_2Et$, 14660-52-7; $Br(CH_2)_5CO_2Et$, 25542-62-5; $CH_2{=}CH(CH_2)_2Br$, 5162-44-7; $NH_2CH_2CO_2H$, 56-40-6; 2,3-$(Cl)_2C_6H_3OMe$, 1984-59-4; $Cl(CH_2)_2COCl$, 625-36-5; ICH_2CO_2H, 64-69-7; $BrCH_2CO_2Et$, 105-36-2; 2,3-dichloro-4-valerylphenol, 55507-79-4; 1-bromo-1-carbethoxycyclobutane, 35120-18-4; 1-(2,3-dichloro-4-butyrylphenoxy)cyclobutane-1-carboxylic acid, 113239-53-5; 4-(2,3-dichloro-4-valerylphenoxy)butyric acid, 113239-50-2; 1-(2,3-dichloro-4-valerylphenoxy)cyclobutane-1-carboxylic acid, 113239-55-7; [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetyl chloride, 113239-57-9; [2,3-dichloro-4-(2-methylenebutyryl)phenoxy]butyryl chloride, 122358-42-3; 2,3-dichloro-4-(3-chloropropionyl)phenol, 23228-15-1; 2,3-dichloro-4-acryloylphenol, 27223-12-7; vanillin, 121-33-5; (2-methoxy-4-formylphenoxy)acetic acid, 1660-19-1; [2-methoxy-4-[(butylimino)methylene]phenoxy]acetic acid, 113239-62-6.

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