Allosteric Modifiers of Hemoglobin. 1. Design, Synthesis, Testing, and Structure-Allosteric Activity Relationship of Novel Hemoglobin Oxygen Affinity Decreasing Agents

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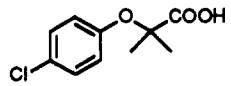
Three isomeric series of 2-(aryloxy)-2-methylpropionic acids were prepared and studied for their ability to decrease the oxygen affinity of human hemoglobin A. The isomeric aryloxy groups included 4-[[(aryloyl)amino]methyl]phenoxy, 4-(arylacetamido)phenoxy, and 4-[[(arylamino)carbonyl]methyl]phenoxy. A total of 20 compounds were synthesized and tested. Structure-activity relationships are presented. Several of the new compounds were found to be strong allosteric effectors of hemoglobin. The two most active compounds are 2-[4-[[(3,5-dichloroanilino)carbonyl]-methyl]phenoxy]-2-methylpropionic acid and the corresponding 3,5-dimethyl derivative. The latter two compounds have been compared to other known potent allosteric effectors in the same assay and show greater activity. Both compounds also exhibit a right shift in the oxygen equilibrium curve when incubated with whole blood. The new compounds may be of interest in clinical or biological areas that require or would benefit from a reversal of depleted oxygen supply (i.e., ischemia, stroke, tumor radiotherapy, blood storage, blood substitutes, etc.). They are also structurally related to several marketed antilipidemic agents.

Introduction

During our search for a drug to treat sickle cell anemia, we discovered that while the antilipidemic drug clofibrate (CFA, structure 1) possessed antisickling activity,[1] it also



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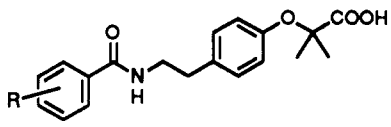
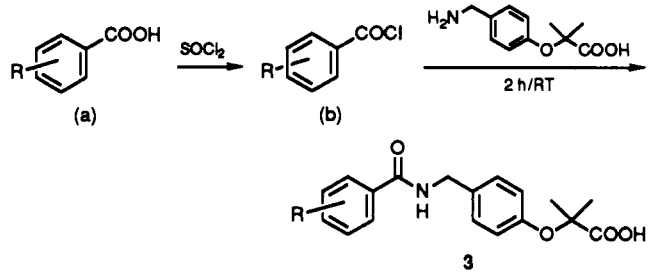
shifted the hemoglobin allosteric equilibrium toward the low oxygen affinity (T) state.[2,3] Later bezafibrate, another antilipidemic agent, was evaluated as a potential antisickling agent (suggested by Perutz) but was found to be progelling.[3] However, Perutz and Poyart[4] discovered that bezafibrate (BZF formula **2**, R = Cl) was a much stronger allosteric effector than CFA. After these initial reports, both CFA and BZF were evaluated for their ability to enhance radiosensitization of tumors by delivering oxygen in vivo.[5] The X-ray binding studies of BZF and CFA-deoxyhemoglobin complexes revealed that the drugs bind to specific sites in the hemoglobin central water cavity via multiple polar interactions.[6,7] Structure-allosteric activity studies with CFA, BZF, and other antigelling compounds[3] designated all binding in the central cavity in terms of four CFA sites (CFA 1, 2, 3 and 4).[3,7] These structural studies suggested the synthesis of a number of BZF analogues. One of these derivatives (a dehalogenated BZF—structure **2**, R = H) was found to be a more potent allosteric effector



2a: R = Cl (BZF)
b: R = H

than BZF.[8] It was proposed that the reason for this increase in activity was due to a relief in steric strain between the *p*-chloro atom in the terminal ring of BZF and the aromatic carbons at the 3- and 4- positions of the phenyl ring in Phe 36α1. Recently, several urea derivatives have been reported[9-11] to effectively decrease the oxygen affinity of Hb in solutions and red cells. These workers demonstrated that shortening of the four-atom bridge between the aromatic rings in BZF to a three-atom urea linkage produced very strong allosteric inhibitors. These observations prompted us to systematically investigate different three-atom arrangements between the aromatic rings, viz, 2-[4-[(aryloylamino)methyl]phenoxy]-2-methylpropionic acids, 2-[4-(arylacetamido)phenoxy]-2-methylpropionic acids, and 2-[4-[[(arylamino)carbonyl]methyl]phenoxy]-2-methylpropionic acids (structure formulae **3**, **4**, and **5**, Schemes I, II, and III, respectively). The position of the alkyl and halogen moieties on the terminal aromatic ring were also varied in each series. The three series have common structural features with interchanging positions of the CH_2, CO, and NH groups as shown in formulae **3-5**. We also synthesized and tested three of the previously reported most active urea compounds[10] (**6-8**, Table I) in an attempt to compare their activity with our most active compounds under the same conditions.

Scheme I



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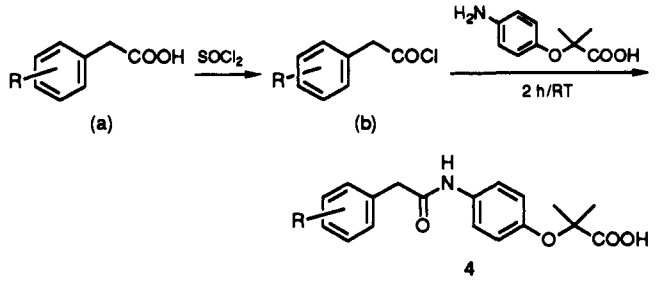
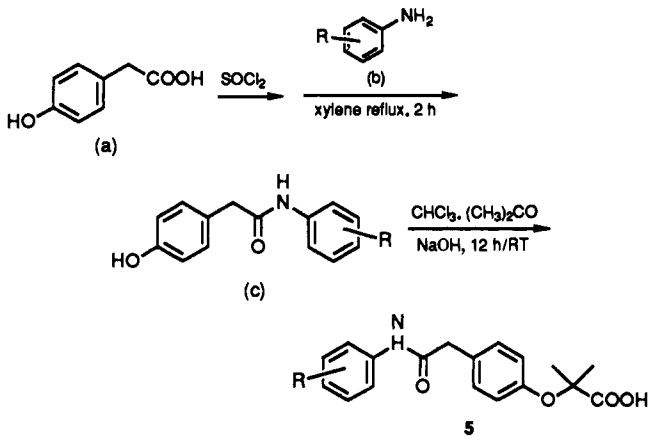
Scheme II



Scheme III



Chemistry

Syntheses of the new compounds listed in Table I are outlined in Schemes I–III. Schemes IV and V demonstrate the synthetic pathways to prepare the key intermediates employed in Schemes I–III.

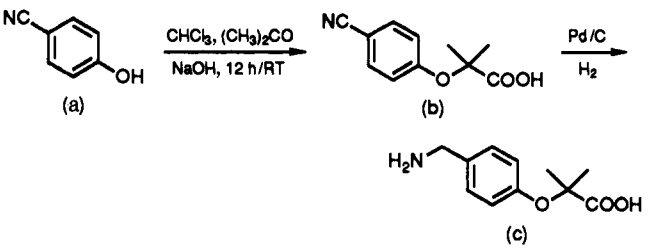
The compounds 2-[4-[(aryloylamino)methyl]phenoxy]-2-methylpropionic acids (scheme I, formula **3**) were prepared as outlined in Scheme I. Conversion of substituted benzoic acids (Scheme I, formula a) into the corresponding aryloyl chloride of formula b followed by reaction with 2-[4-(aminomethyl)phenoxy]-2-methylpropionic acid (compound c, Scheme IV) in aqueous NaOH (2 N) produced compounds of formula **3** (Scheme I).

Likewise, the synthesis of 2-[4-(arylacetamido)phenoxy]-2-methylpropionic acids (Scheme II, formula **4**) was accomplished by converting substituted phenylacetic acid (Scheme II, formula a) to the corresponding acid chloride (formula b, Scheme II) followed by the reaction with 2-(4-aminophenoxy)-2-methylpropionic acid (compound b, Scheme V) to give the desired compounds of formula **4** (Scheme II).

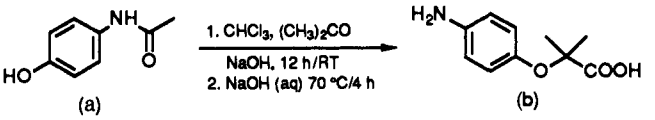
The synthesis of 2-[4-[[(arylamino)carbonyl]methyl]-phenoxy]-2-methylpropionic acids (Scheme III, structure formula **5**) was accomplished by reacting *p*-hydroxyphenylacetic acid (compound a, Scheme III) with thionyl chloride followed by condensation of resulting polymeric ester with the properly substituted aniline in refluxing xylene to give compounds of formula c in Scheme III. The reaction of the latter with chloroform–acetone in presence of sodium hydroxide provided the corresponding 2-[4-[[(arylamino)carbonyl]methyl]phenoxy]-2-methylpropionic acid derivatives (Scheme III, formula **5**). Alternatively, compounds of formula c (scheme III) were prepared by one-pot reaction of *p*-hydroxyphenylacetic acid with substituted anilines in presence of 0.25 equiv of PCl_5 in refluxing mesitylene.

The intermediate 2-[4-(aminomethyl)phenoxy]-2-methylpropionic acid (compound c, Scheme IV) was prepared as outlined in Scheme IV. Reaction of 4-cyanophenol with chloroform and acetone in presence of sodium hydroxide afforded 2-(4-cyanophenoxy)-2-methylpropionic acid (compound b, Scheme IV). Hydrogenation of b with 10% Pd on C in ethanol containing a catalytic amount of concentrated HCl provided 2-[4-(aminomethyl)phenoxy]-2-methylpropionic acid (compound c, Scheme IV) in quantitative yield.

Scheme IV



Scheme V



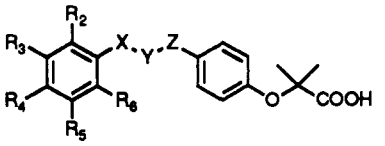
The intermediate 2-(4-aminophenoxy)-2-methylpropionic acid (compound b, Scheme V) was prepared by the reaction of 4-acetaminophenol (compound a, Scheme V) with chloroform and acetone in presence of NaOH followed by hydrolysis.

Biological Evaluation

The allosteric effector activity of the compounds was measured from the shift in the hemoglobin oxygen dissociation curve with an Aminco Hem-O-Scan oxygen dissociation analyzer (Travenol Laboratories). Table I summarizes the P_{50}, mean P_{50}, and Hill coefficient values (n_{50}) at half-saturation for the new derivatives, two BZF analogues, and three urea derivatives previously reported.[9-11] Hemoglobin affinity toward oxygen is expressed by the term P_{50}, which denotes the partial pressure of oxygen at which 50% of hemoglobin is oxygenated. A compound with high activity produces a large right shift in P_{50} relative to that of the control. Therefore it was found useful to express the activity or potency of each compound by the ratio P_{50}(analogue)/P_{50}(control). Since the shift in the P_{50} is dependent on the drug/hemoglobin ratio, we performed all comparisons at a ratio of 4/1 [analogue]/[Hb], i.e. 10 mM analogue/2.7 mM Hb. The high Hb molarity (2.7 mM) used in the oxygen equilibrium studies was intended to approximate the red cell Hb concentration (5 mM).

Table II presents the results of a dose–response study in whole blood with two of our most active new compounds (**5h** and **5i**). Since urea derivatives **6–8** are reported to lose activity in the presence of physiological concentrations of serum albumin, even when palmitin or tripalmitinglyceride was added to inhibit **6–8** from binding to serum albumin,[11] we also included compound **7** for evaluation. Our studies indicate that **5h** and **5i** at a ratio of 0.4 compound to 1.0 Hb retain allosteric activity while **7** under the same conditions is not active. All three compounds are effective at higher dose ratios. It appears that **5h** and **5i** are more effective at lower concentrations than urea derivative **7** in bypassing plasma binding proteins such as serum albumin. Complete hemolysis was observed when 10 mM **5h** and **7** were incubated with whole blood overnight at 4 °C and to a smaller extent with **5i**.

Table I. Ability of Synthesized Compounds To Right Shift the Oxygen Dissociation Curve of Hemoglobin[a]



no.	R_2	R_3	R_4	R_5	R_6	X	Y	Z	$P_{50}c$[b]	$P_{50}d$[c]	$P_{50}d/P_{50}c$	average ± SD	n_{50}[d]
2a (BZF)	H	H	Cl	H	H	CO	$NHCH_2$	CH_2	18	33	1.83		
									17	30	1.76		
									17	29	1.70		
									18.5	31	1.67	1.74 ± 0.06	2.4
2b	H	H	H	H	H	CO	$NHCH_2$	CH_2	18	32	2.05		
3a	H	H	H	H	H	CO	NH	CH_2	18	35	1.94		
									19	35.5	1.86	1.90 ± 0.04	2.6
3b	Cl	H	H	H	H	CO	NH	CH_2	19	28	1.47		2.6
3c	H	Cl	H	H	H	CO	NH	CH_2	18	37	2.05		
									19	37	1.94	1.99 ± 0.05	2.3
3d	H	H	Cl	H	H	CO	NH	CH_2	19	48	2.52		
									18	49	2.72		
									19	48	2.52	2.58 ± 0.09	1.7
3e	H	Cl	Cl	H	H	CO	NH	CH_2	19	38.5	2.02		
									18	40	2.22	2.12 ± 0.10	2.2
3f	H	Cl	H	Cl	H	CO	NH	CH_2	19	48.5	2.55		
									19	47	2.47		
									19	46	2.42	2.48 ± 0.05	2.0
3g	H	Cl	Cl	Cl	H	CO	NH	CH_2	19	40	2.10		
									19	42	2.21		
									11.5	24	2.08	2.13 ± 0.08	2.7
4a	H	H	H	H	H	CH_2	CO	NH	19.5	35	1.79		2.6
4b	H	H	Cl	H	H	CH_2	CO	NH	19	44	2.31		
									19	43	2.26		
									19	41	2.15		
									21	51	2.48		
									19	47	2.23	2.28 ± 0.11	2.3
5a	H	H	H	H	H	NH	CO	CH_2	20.5	63	3.07		
									19	54	2.84	2.95 ± 0.12	2.4
5b	H	H	CH_3	H	H	NH	CO	CH_2	15	40	2.66		
									15	41	2.70	2.68 ± 0.02	2.4
5c	H	H	Cl	H	H	NH	CO	CH_2	15.5	43	2.77		
									19	54	2.84		
									18.5	51	2.75		
									18.5	52	2.81	2.79 ± 0.04	2.2
5d	H	H	F	H	H	NH	CO	CH_2	20	52	2.60		
									17.5	44	2.51	2.55 ± 0.05	2.5
5e	H	H	CF_3	H	H	NH	CO	CH_2	20	48	2.40		
									17.5	40.5	2.31		
									17.5	39	2.23		
									18	44	2.44	2.35 ± 0.08	2.5
5f	H	H	*i*Pr	H	H	NH	CO	CH_2	19.5	57	2.92		
									19	56.5	2.97		
									19	57.8	3.04	2.98 ± 0.05	2.2
5g	H	Cl	Cl	H	H	NH	CO	CH_2	18.2	65	3.57		
									18	65	3.61	3.59 ± 0.02	2.2
5h	H	Cl	H	Cl	H	NH	CO	CH_2	10.3	45	4.39		
									11	47	4.27		
									11	46	4.18		
									19.2	84	4.37		
									19.2	85	4.42	4.32 ± 0.08	2.0
5i	H	CH_3	H	CH_3	H	NH	CO	CH_2	19	75.5	3.97		
									19.2	76.5	3.98		
									19.2	78	4.06	4.01 ± 0.04	2.2
5j	Cl	H	H	H	Cl	NH	CO	CH_2	18	34	1.89		
									18	35	1.94	1.91 ± 0.03	2.1
5k	H	Cl	Cl	Cl	H	NH	CO	CH_2	12	37	3.08		
									19	60	3.15		
									19	63	3.30	3.17 ± 0.09	2.1
6	H	Cl	Cl	H	H	NH	CO	NH	18	32	1.77		
7	H	Cl	H	Cl	H	NH	CO	NH	11.8	44	3.72		
									18	65	3.61		
									18	63	3.50	3.61 ± 0.09	2.1
8	H	Cl	Cl	Cl	H	NH	CO	NH	18	48	2.66		
									14	35	2.50		
									14	36	2.57	2.57 ± 0.07	1.6

[a] All studies were carried out at 2.7 mM hemoglobin concentration in the presence of 10 mM drug concentration. All solutions were prepared in 50 mM, pH 7.4 HEPES buffer. For more details, see the Experimental Section. [b] $P_{50}c$ is the oxygen pressure in mmHg at which the control hemoglobin solution (no drug) is 50% saturated with oxygen. [c] $P_{50}d$ is the oxygen pressure in mmHg at which the hemoglobin solution (in presence of 10 mM drug) is 50% saturated with oxygen. [d] The Hill coefficient at half-saturation is calculated from the Hill equation by the linear-regression analysis of data points between 40 and 60% oxygen saturation.

Table II. Oxygen-Equilibrium Whole-Blood Studies[a]

concn, mM	ratio comp/Hb	$P_{50}d/P_{50}c$ compound 7	compound 5h	compound 5i
0.5	0.4	0.96	1.09	1.07
1.0	0.8	1.15	1.52	1.53
2.0	1.6	1.70	2.00	1.93
4.0	3.2	1.84	2.72	2.61
10.0	8.0	2.25	2.93	3.00

[a] The concentration of Hb in whole blood after addition of the test compounds was approximately 1.25 mM. The ratio of compound to Hb was 0.4, 0.8, 1.6, 3.2, and 8, respectively.

Structure–Activity Relationships

The new isomers (formulae **3–5**, Table I) differ only in arrangement of atoms in the central chain separating the two aromatic rings. The central chain sequence in class 3 is $CONHCH_2$, class 4 CH_2CONH, and class 5 $NHCOCH_2$. These new analogues have a wide range of allosteric effector activity (Table I). All of the new compounds except **3b** induce a greater right shift in the Hb oxygen dissociation curve than bezafibrate. In general, class 5 compounds seem to be more active than the corresponding isomeric compounds of classes 3 or 4. Compounds **3a** and **3d** have been previously reported in a patent, indicating their potential use as antilipidemic agents.[12]

In classes 3 and 4, the unsubstituted derivatives (compounds 3a and 4a, R = H) are less active than the corresponding monosubstituted 4-chloro derivatives (compounds 3d and 4b, respectively). This order is reversed with BZF derivatives (compounds **2a** and **2b**) and with class 5, where **5a** and **5c** are about equal in potency. It appears that monosubstitution at position 4 is important for enhancing activity (compare the activity of compounds **3b**, **3c**, and **3d**, where 4-Cl > 3-Cl > 2-Cl). Furthermore, the nature of the substituent was found to also affect allosteric effector activity. Replacement of 4-Cl with a CH_3 group exhibited about the same activity (see compounds **5b** and **5c**), while the 4-F (**5d**) and 4-CF_3 (**5e**) were found to be less active. It is interesting to note that an increase in steric volume with an isopropyl group was tolerated in the 4-position (compound 5f). The order of allosteric effector activity for monosubstitution at position 4 in class 5 is *i*-Pr = H > Cl > Me > F > CF_3. In general, ring disubstitution demonstrated higher activity than the corresponding mono derivatives. The 3,5-disubstituted derivatives were more active than the corresponding 3,4-compounds (compare **5h** with **5g** and **3f** with **3e**). Further substitution by a third Cl atom resulted in less active compounds relative to the dichloro compounds (see **3g** and **5k**). The 3,5-dichloro (**5h**) and its dimethyl analogue (**5i**) are the most potent hemoglobin oxygen dissociation curve right-shifting agents reported to date.

In general, the urea derivatives (**6–8**) were found to be less active than the corresponding derivatives of class 5 and exhibited comparable or better activity than those of class 3.

The majority of the new compounds show good cooperativity (n_{50} = 2.0–2.7) except for **3d** (1.7). We have confirmed the low n_{50} reported for the cooperativity at half-saturation for compound 8 and the values published for compounds **6** and **7**.[11]

The differences in the detailed binding interactions between the three classes, bezafibrate, and the urea derivatives with hemoglobin will be presented in the accompanying paper based on X-ray crystallographic and molecular modeling studies.[13]

Summary and Conclusions

Three new classes of allosteric effectors of hemoglobin have been synthesized and tested in buffered solutions and in whole blood (compounds **5h** and **5i**). All new compounds were shown to be strong allosteric effectors of Hb and two of them (**5h** and **5i**) are the most potent reported to date. Both **5h** and **5i** appear to be active at low concentrations in whole blood. We confirmed the lack of allosteric activity for one of the urea derivatives (**7**) in the presence of physiological concentrations of serum albumin.[11] However, **7** also shows allosteric effector activity at higher concentrations.

Our findings indicate that the position of the atoms in the three-chain bridge is important and for maximum activity a halogenated ring–$NHCOCH_2$–aromatic acid arrangement is preferred.

The new molecules represent potential agents for use in ischemia, radiotherapy of tumors,[5] stroke, stabilization of blood products or blood substitutes, and antilipidemic therapy.[12] The most active compounds in class 5 are undergoing evaluation for these potential uses.

Experimental Section

Melting points are measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on FT90 spectrophotometer using TMS as internal standard. All final compounds gave satisfactory elemental analysis for C, H, N, and Cl.

Synthesis. Scheme I Compounds. Aryloyl Chlorides (Formula b, Scheme I). These starting materials were prepared by refluxing the corresponding carboxylic acid (formula a) in excess thionyl chloride for 1 h followed by evaporation of thionyl chloride under vacuo. The aryloyl chlorides were used directly in the next step without furtherpurification.

2-[4-[(Aryloylamino)methyl]phenoxy]-2-methylpropionic Acids (Formula 3, Scheme I). 2-[4-[(Benzoylamino)-methyl]phenoxy]-2-methylpropionic Acid (Compound 3a, Table I). General Procedure. A solution of benzoyl chloride (0.14 g, 1 mmol, compound b, Scheme I, R = H) in THF (3 mL) was added over a 15-min period to a stirred solution of 2-[4-(aminomethyl)phenoxy]-2-methylpropionic acid (0.24 g, 1 mmol, compound c, Scheme IV) and NaOH (0.08 g, 2 mmol) in 10 mL of water. After the addition of the benzoyl chloride was completed, the reaction mixture was stirred for 1 h at room temperature. THF was evaporated in vacuo. Acidification of the residue provided the desired compound as an oil which was extracted with ether. The organic layer was washed with water, brine, and dried over anhydrous $MgSO_4$. Subsequent addition of petroleum ether precipitated compound **3a** (Table I), as a white solid (0.15 g, 48%), mp 176–9 °C. NMR: (DMSO-d_6) δ 1.45 (6 H, s, 2 CH_3), 4.4 (2 H, d, CH_2), 6.8–7.2 (4 H, dd, J = 9 Hz, aromatic *H*, para disubstitution), 7.4–8 (5 H, m, aromatic *H*), 9 (1 H, br t, N*H*). Anal. ($C_{18}H_{19}NO_4$): C, H, N. Following the above procedure described to synthesize compound **3a**, there were obtained the following.

2-[4-[[(2-Chlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3b, Table I) was obtained in 58% yield, mp 135–7 °C. Anal. ($C_{18}H_{18}ClNO_4$): C, H, N.

2-[4-[[(3-Chlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3c, Table I) was obtained in 53% yield, mp 145–6 °C. Anal. ($C_{18}H_{18}ClNO_4$): C, H, N.

2-[4-[[(4-Chlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3d, Table I), obtained in 63% yield, crystallized from aqueous ethanol, mp 186–9 °C. Anal. ($C_{18}H_{18}ClNO_4$): C, H, N.

2-[4-[[(3,4-Dichlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3e, Table I) was obtained in 57% yield, mp 154–6 °C. Anal. ($C_{18}H_{17}Cl_2NO_4$): C, H, Cl, N.

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2-[4-[[(3,5-Dichlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3f, Table I) was obtained in 43% yield, mp 110–3 °C. Anal. ($C_{18}H_{17}Cl_2NO_4$): C, H, Cl.

2-[4-[[(3,4,5-Trichlorobenzoyl)amino]methyl]phenoxy]-2-methylpropionic acid (compound 3g, Table I) was obtained in 59% yield and crystallized from ether/petroleum ether, mp 151–2 °C. Anal. ($C_{18}H_{16}Cl_3NO_4$): C, H, Cl.

Scheme II Compounds. Substituted phenylacetyl chlorides of formula b were prepared from the corresponding carboxylic acids of formula a, as described above for Scheme I compounds. They were also directly used without further purification.

2-[4-(Arylacetamido)phenoxy]-2-methylpropionic Acids (Scheme II, Formula 4). 2-[4-(Phenylacetamido)phenoxy]-2-methylpropionic Acid (Compound 4a, Table I). General Procedure. A solution of 2-(4-aminophenoxy)-2-methylpropionic acid (1 g, 5 mmol) was prepared in water (10 mL) containing NaOH (0.41 g, 10 mmol). To this solution was gradually added phenylacetyl chloride (0.79 g, 5 mmol) in 5 mL of THF, over a period of 15 min. After complete addition of the phenylacetyl chloride, the pH of the reaction mixture was alkaline (if not, a few drops of 2 N NaOH were added). The reaction mixture was stirred for an additional hour at room temperature and the THF was evaporated in vacuo. Water (5 mL) was added and the mixture was acidified with concentrated hydrochloric acid. The product was extracted with ether (2 × 20 mL), washed with water (3 × 20 mL), and dried over anhydrous $MgSO_4$. Addition of petroleum ether precipitated the desired compound as a pale brown solid, **4a** (0.9 g, 56%), mp 173–5 °C. Anal. ($C_{18}H_{19}NO_4$): C, H, N.

Following the same procedure described above for the preparation of compound **4a**, there was obtained the following.

2-[4-[(*p*-Chlorophenyl)acetamido]phenoxy]-2-methylpropionic acid (compound 4b, Table I) was obtained in 57% yield, mp 168–71 °C. Anal. ($C_{18}H_{18}NO_4Cl$): C, H, Cl, N.

Scheme III Compounds. 4-[[(Arylamino)carbonyl]methyl]phenols (Scheme III, Formula c). 4-[[(3,5-Dichloroanilino)carbonyl]methyl]phenol (Precursor of Compound 5h, Table I). General Procedure. *p*-Hydroxyphenylacetic acid (2.6 g, 17 mmol) was heated to reflux in 10 mL of thionyl chloride for 30 min. The reaction mixture was then cooled; excess of $SOCl_2$ was removed under vacuum. To the residue, presumably polymeric aryl ester, 3,5-dichloroaniline (5.67 g, 35 mmol), and xylene (50 mL) were added. The reaction mixture was refluxed for 3 h then cooled. The resulting solid was collected by filtration, washed with dilute HCl, water, sodium bicarbonate, and brine, and extracted with aqueous NaOH (1 N, 2 × 15 mL). The combined alkaline layer was washed with ether and acidified to give the precursor of **5h** (4.4 g, 86% yield), recrystallized from methanol–water, mp 176 °C. NMR (DMSO-d_6): δ 6.6–7.6 (7 H, m, aromatic *H*).

Following the same procedure described above to prepare the precursor of compound **5h**, there were obtained the following.

4-[(Anilinocarbonyl)methyl]phenol (precursor of compound 5a, Table I) was obtained in 90% yield, mp 138 °C.

4-[(*p*-Toluidylcarbonyl)methyl]phenol (precursor of compound 5b, Table I) was obtained in 94% yield, mp 144 °C.

4-[[(4-Chloroanilino)carbonyl]methyl]phenol (precursor of compound 5c, Table I) was obtained in 84% yield, mp 163 °C.

4-[[(4-Fluoroanilino)carbonyl]methyl]phenol (precursor of compound 5d, Table I) was obtained in 89% yield, mp 132–6 °C.

4-[[[4-(Trifluoromethyl)anilino]carbonyl]methyl]phenol (precursor of compound 5e, Table I) was obtained in 84% yield, mp 140 °C.

4-[[(4-Isopropylanilino)carbonyl]methyl]phenol (precursor of compound 5f, Table I) was obtained in 85% yield as thick liquid.

4-[[(3,4-Dichloroanilino)carbonyl]methyl]phenol (precursor of compound 5g, Table I) was obtained in 84% yield, mp 158 °C.

4-[[(3,5-Dimethylanilino)carbonyl]methyl]phenol (precursor of compound 5i, Table I) was obtained in 89% yield, mp 164–6 °C.

4-[[(2,6-Dichloroanilino)carbonyl]methyl]phenol (precursor of compound 5j, Table I) in 85% yield, mp 200–2 °C.

4-[[(3,4,5-Trichloroanilino)carbonyl]methyl]phenol (precursor of compound 5k, Table I) was obtained in 85% yield, mp 217–219 °C.

2-[4-[[(Arylamino)carbonyl]methyl]phenoxy]-2-methylpropionic Acids (Scheme III, Formula 5). 2-[4-[[(3,5-Dichloroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic Acid (Compound 5h, Table I). General Procedure. Chloroform (1.25 mL) was added dropwise during 15 min to a stirred and ice-cooled mixture of 4-[[(3,5-dichloroanilino)carbonyl]methyl]phenol (1.5 g, 5 mmol, formula c, Scheme III) and powdered NaOH (2.75 g) in acetone (25 mL). The reaction mixture was allowed to warm to room temperature and stirring continued for 12 h. Excess acetone was removed under vacuum, and the residue was dissolved in water, filtered, and acidified to give an oil which was extracted with ether (3 × 20 mL). The combined organic layer was washed with water and brine, dried, and concentrated to provide a semisolid. Upon crystallization from acetone–hexane (2:1), a white solid, **5h** (0.8 g, 42%), was obtained, mp 138 °C. NMR (DMSO-d_6): δ 1.46 (6 H, s, 2 CH_3), 3.76 (2 H, s, CH_2), 6.92–7.3 (4 H, dd, J = 6 Hz, aromatic para disubstituted *H*), 7.72 (3 H, m, aromatic *H*). Anal. ($C_{18}H_{17}Cl_2NO_4$): C, H, Cl, N.

With the above procedure there were obtained the following.

2-[4-[(Anilinocarbonyl)methyl]phenoxy]-2-methylpropionic acid (compound 5a, Table I) was obtained in 76% yield, mp 198 °C, and recrystallized from acetone–petroleum ether (2:1). Anal. ($C_{18}H_{19}NO_4$): C, H, N.

2-[4-[(4-Toluidylcarbonyl)methyl]phenoxy]-2-methylpropionic acid (compound 5b, Table I) was obtained in 65% yield, mp 164 °C. Anal. ($C_{19}H_{21}NO_4$): C, H, N.

2-[4-[[(4-Chloroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5c, Table I) was obtained in 50% yield, mp 196 °C. Anal. ($C_{18}H_{18}ClNO_4$): C, H, Cl, N.

2-[4-[[(4-Fluoroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5d, Table I) was obtained in 45% yield, mp 198 °C. Anal. ($C_{18}H_{18}FNO_4$): C, H, N.

2-[4-[[[4-(Trifluoromethyl)anilino]carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5e, Table I) was obtained in 44% yield, mp 197 °C. Anal. ($C_{19}H_{18}F_3NO_4$): C, H, N.

2-[4-[[(4-Isopropylanilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5f, Table I) was obtained in 62% yield, mp 141 °C. Anal. ($C_{21}H_{25}NO_4$): C, H, N.

2-[4-[[(3,4-Dichloroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic Acid (compound 5g, Table I) was obtained in 40% yield, mp 174 °C. Anal. ($C_{18}H_{17}Cl_2NO_4$): C, H, Cl, N.

2-[4-[[(3,5-Dimethylanilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5i, Table I) was obtained in 68% yield, mp 85 °C. Anal. ($C_{20}H_{23}NO_4$): C, H, N.

2-[4-[[(2,6-Dichloroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid (compound 5j, Table I) was obtained in 40% yield, mp 198 °C. Anal. ($C_{18}H_{17}Cl_2NO_4$): C, H, Cl, N.

2-[4-[[(3,4,5-Trichloroanilino)carbonyl]methyl]phenoxy]-2-methylpropionic Acid (compound 5k, Table I) was obtained in 43% yield, mp 160 °C. Anal. ($C_{18}H_{16}Cl_3NO_4$): C, H, Cl, N.

Scheme IV Compounds. 2-(4-Cyanophenoxy)-2-methylpropionic Acid (Compound b, Scheme IV). Pulverized sodium hydroxide (7 g, 120 mmol) was gradually added to a stirred solution of 4-cyanophenol (1.19 g, 10 mmol) in dry acetone. To this mixture was added chloroform (1.19 g, 10 mmol) dropwise over a period of 20 min. The reaction mixture was stirred and heated to reflux for 4 h. Acetone was evaporated in vacuo and the solid residue dissolved in H_2O (50 mL), charcoalated, and filtered. Acidification with concentrated hydrochloric acid afforded the desired product as a pale brown precipitate (1.6 g, 78%). Crystallization from ethanol gave a compound of mp 118–20 °C. NMR (DMSO-d_6): δ 1.5 (6 H, s, 2 CH_3), 7.7–6.9 (4 H, dd, J = 6 Hz, aromatic *H*).

2-[4-(Aminomethyl)phenoxy]-2-methylpropionic Acid Hydrochloride (Compound c, Scheme IV). In a 250-mL Parr hydrogenation bottle was placed 75 mL of absolute ethanol and 2-(4-cyanophenoxy)-2-methylpropionic acid (2 g, 9 mmol, compound b, Scheme IV). The solution was acidified with concen-

trated hydrochloric acid (3 mL), then 10% palladium on activated charcoal (0.2 g, 10% wt) was added to the mixture. The reaction mixture was placed on a Parr hydrogenator apparatus at 45 psi of hydrogen pressure and shaken for a period of 2 h. The mixture was filtered to remove the catalyst, and the filtrate concentrated under vacuum. Addition of ether precipitated the desired product (compound c, Scheme IV) as white, shiny crystals (2.1 g, 87%), mp 234–36 °C. NMR (DMSO-d_6 + TMS): δ 1.51 (6 H, s, CH_3), 3.9 (2 H, s, CH_2), 7.4–6.7 (4 H, dd, J = 9 Hz, ArH).

Scheme V Compound. 2-(4-Aminophenoxy)-2-methylpropionic Acid (Compound b, Scheme V). Eight grams (200 mmol) of pulverized sodium hydroxide was added to a solution of *p*-acetaminophenol (5.28 g, 35 mmol, compound a, Scheme V) in acetone (50 mL). The reaction mixture was stirred at room temperature for $^1/_2$ h. Subsequently, 4 mL of chloroform (50 mmol) was added dropwise over the course of 30 min. The reaction mixture was stirred overnight at room temperature and acetone removed under vacuum. The residue was dissolved in water (10 mL), followed by acidification with 37% hydrochloric acid to produce a pale yellow precipitate of 2-(4-acetaminophenoxy)-2-methylpropionic acid (5 g, 60% yield), which upon crystallization from methanol had mp 69–71 °C. NMR (CD_3OD): δ 1.4 (6 H, s, 2 CH_3), 2.05 (3 H, s, CH_3), 7.1 (4 H, m, ArH).

2-(4-Acetaminophenol)-2-methylpropionic acid (1.18 g, 5 mmol) was boiled in 10% KOH (60 ml) for $^1/_2$ h. The cooled solution was acidified with acetic acid to give 0.6 g (62%) of compound b (Scheme V) as a yellowish white powder, mp 214–6 °C dec. NMR (DMSO-d_6 + TMS): δ 1.35 (6 H, s, CH_3), 6.6 (4 H, m, ArH).

Oxygen Equilibrium Studies. Preparation of Pure Hemoglobin A. A solution of HbA was prepared according to the following procedure: 20 mL of whole blood from a nonsmoking donor (blood bank, MCV, Richmond, VA) 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. After centrifugation was completed, the plasma and buffy coat were removed by aspiration and the cells washed three times with 0.9% NaCl (40 mg of EDTA/L) and then once with 1.0% 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 2 h at 10000 rpm at 4 °C. The supernatant was decanted into 50-mL tubes and NaCl (60 mg/mL of Hb supernatant) was added, mixed, and centrifuged at 10000 rpms at 4 °C for 2 h to remove the 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 Tris buffer, pH 8.6. After elution of the A2 hemoglobin fraction by Tris buffer (pH 8.6, 50 mM, containing 40 mg EDTA/L), the HbA fraction was then eluted with pH 8.4 Tris buffer. The pure HbA fraction (identified by electrophoresis) was concentrated by using a Schleicher and Schuell collodion bag apparatus (Schleicher and Schuell, Inc.) with HEPES buffer (50 mM, pH 7.4) as the exchange buffer. The hemoglobin concentration was then determined by using the cyanmet method. The hemoglobin concentration at this point was usually found to be around 35 g% (approximately 5.4 mM). Less than 5% methemoglobin was noted even after several days at 4 °C. Oxygen dissociation curves were recorded on an AMINCO Hem-O-Scan oxygen dissociation analyzer (Travenol Laboratories).

All compounds were dissolved in 50 mM HEPES buffer, pH 7.4, to give 20 mM of drug solution. Just prior to running oxygen dissociation curves the Hb (5.4 mM, in HEPES, pH 7.4) and the test compounds were mixed in a 1:1 ratio (50 μL of Hb + 50 μL of compound solution) to give final concentration of 2.7 mM of Hb and test compound concentration of 10 mM with a molar ratio of test compound to hemoglobin of approximately 4:1. A control experiment was prepared by the addition of 50 μL of Hb to 50 μL of HEPES buffer.

Whole-Blood Studies. The effect of compounds **5h**, **5i**, and **7** (Table II) on oxygen affinity of whole blood was determined by mixing 50 μL of freshly drawn heparinized whole blood and 50 μL of test compound (isosmotic and isotonic 50 mM HEPES buffer, pH 7.4, containing 140 mM NaCl, 10 mM glucose). The mixture was equilibrated for 1 h at 37 °C before analysis.

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Registry No. **3a**, 41859-98-7; **3b**, 131179-81-2; **3c**, 131179-82-3; **3d**, 41859-99-8; **3e**, 131179-83-4; **3f**, 131179-84-5; **3g**, 131179-85-6; **4a**, 131179-86-7; **4b**, 131179-87-8; **5a**, 131179-88-9; **5b**, 131179-89-0; **5c**, 58609-31-7; **5d**, 131179-90-3; **5e**, 131179-91-4; **5f**, 131179-92-5; **5g**, 131179-93-6; **5h**, 131179-94-7; **5i**, 131179-95-8; **5j**, 131179-96-9; **5k**, 131179-97-0; $C_6H_5CO_2H$, 65-85-0; 2-$ClC_6H_4CO_2H$, 118-91-2; 3-$ClC_6H_4CO_2H$, 535-80-8; 4-$ClC_6H_4CO_2H$, 74-11-3; 3,4-$Cl_2C_6H_3CO_2H$, 51-44-5; 3,5-$Cl_2C_6H_3CO_2H$, 51-36-5; 3,4,5-$Cl_3C_6H_2CO_2H$, 51-39-8; $C_6H_5CH_2CO_2H$, 103-82-2; 4-$ClC_6H_4CH_2CO_2H$, 1878-66-6; $C_6H_5NH_2$, 62-53-3; 4-$H_3CC_6N_4NH_2$, 106-49-0; 4-$ClC_6H_4NH_2$, 106-47-8; 4-$FC_6H_4NH_2$, 371-40-4; 4-$CF_3C_6H_4NH_2$, 455-14-1; 4-*i*-$PrC_6H_4NH_2$, 99-88-7; 3,4-$Cl_2C_6H_3NH_2$, 95-76-1; 3,5-$Cl_2C_6H_3NH_2$, 626-43-7; 3,5-$(CH_3)_2C_6H_3NH_2$, 108-69-0; 2,6-$Cl_2C_6H_3NH_2$, 608-31-1; 3,4,5-$Cl_3C_6H_2NH_2$, 634-91-3; 4-$HOC_6H_4CH_2CONHC_6H_5$, 131179-71-0; 4-$HOC_6H_4CH_2CONH$-4-$C_6H_4CH_3$, 58609-19-1; 4-$HOC_6H_4CH_2CONH$-4-C_6H_4CL, 58609-17-9; 4-$HOC_6H_4CH_2CONH$-4-C_6H_4F, 131179-72-1; 4-$HOC_6H_4CH_2CONH$-4-$C_6H_4CF_3$, 131179-73-2; 4-$HOC_6H_4CH_2CONH$-4-C_6H_4Pr-*i*, 131179-74-3; 4-$HOC_6H_4CH_2CONH$-3,4-$C_6H_3Cl_2$, 131179-75-4; 4-$HOC_6H_4CH_2CONH$-3,5-$C_6H_3Cl_2$, 131179-76-5; 4-$HOC_6H_4CH_2CONH$-2,6-$C_6H_3Cl_2$, 131179-78-7; 4-$HOC_6H_4CH_2CONH$-3,5-$C_6H_3(CH_3)_2$, 131179-77-6; 4-$HOC_6H_4CH_2CONH$-3,4,5-$C_6H_2Cl_3$, 131179-79-8; 4-$HOC_6H_4CH_2CO_2H$, 156-38-7; 4-NCC_6H_4OH, 767-00-0; 4-$NCC_6H_4OC(CH_3)_2CO_2H$, 79925-16-9; 4-$H_3CCONHC_6H_4OC(CH_3)_2CO_2H$, 17413-84-2; 4-$H_2NCH_2C_6H_4OC(CH_3)_2CO_2H$, 131179-80-1; 4-$H_2NC_6H_4OC(CH_3)_2CO_2H$, 117011-70-8; 4-$H_3CCONHC_6H_4OH$, 103-90-2; hemoglobin A, 9034-51-9.