Nine milliliters of acetic anhydride, 18 mL of 30% hydrogen peroxide, and ca. 20 mg selenium dioxide were added, and the mixture was heated under reflux for 4 h. Upon cooling, the product precipitated and was collected, washed with water, and dried. This gave 1.3 g (51%) of white solid, which showed the following: mg >300 °C; IR (KBr) 3420 (vb), 3220, 1780 (sh), 1760, 1720, and 1690 cm^{-1}; ^1H NMR (300 MHz, Me_2SO-d_6) δ 12.35 (br s, CO_2H), 11.50 and 11.09 (s, NH), 3.76 (m), 3.11 (br s), and 2.90 (br s). Anal. ($C_{14}H_{12}N_2O_8 \cdot 0.66H_2O$) C, H, N.

2,6-Bis(hydroxymethyl) Derivative 9b. With use of the procdure described for the synthesis of **9a**, 2.0 g (6.3 mmol) **7a** gave 1.2 g (46%) of a white solid, which showed the following: mp 294–296 °C dec; IR (KBr) 3520, 3420, 2960, 1770, and 1700 cm^{-1}; ^1H NMR (300 MHz, MeSO-d_6) δ 12.44 (br s, CO_2H), 6.42 and 6.32 (br s, OH), 4.78 and 4.74 (s, CH_2O), 3.78 and 3.21 and 2.94 and 2.85 (br s). Anal. ($C_{16}H_{16}N_2O_{10} \cdot 1.0H_2O$) C, H, N.

Cyclobutane Diimide 11a. This compound was synthesized according to the method of Boule.[32]

Diimides 11b-d. General Procedure. A 10-g portion of the corresponding commercially available dianhydride was mixed with 50 mL of concentrated aqueous ammonia until a clear solution was obtained. After evaporation of the solvent and oven drying at 100 °C, the solid was placed in sublimation apparatus and heated to about 200 °C in vacuo. After several hours the temperature was gradually raised to about 300 °C, and the product sublimed. The crude products were recrystallized from water or water/DMF mixtures. All of the compounds have been reported in the literature, but no useful physical properties were recorded. Compound **11b** showed the following: mp >300 °C; IR (KBr) 1767, 1690, cm^{-1}; ^1H NMR (60 MHz, Me_2SO-d_6) δ 11.40 (br s, NH), 7.93 (s, arom). Anal. ($C_{10}H_4N_2O_4$) C, H, N. Compound **11c** showed the following: mp 287–291 °C; MS, m/z 208 (M^+, 10), 165 (33), 137 (36), 66 (100); ^1H NMR (60 MHz, TFA-d) δ 12.07 (br s, NH), 4.27–3.67 (m), 3.00–2.63 (m). Anal. ($C_9H_8N_2O_4$) C, H, N. Compound **11d** showed the following: mp >300 °C; MS, m/z 246 (M^+, 6), 176 (15), 131 (13), 78 (100); ^1H NMR (300 MHz, Me_2SO-d_6) δ 11.14 (br s, NH), 6.11 (m, vinyl), 3.27 (m), 3.04 (br s). Anal. ($C_{14}H_{14}N_2O_6 \cdot 0.25H_2O$) C, H, N.

Bis(hydroxymethyl) Derivative 12a. This compound was synthsized by use of the same method as in the synthesis of **7a**. This gave a 50% yield (based on crude **11d**) of white needles, which showed the following: mp >300 °C; ^1H NMR (60 MHz, TFA-d) δ 6.25 (m, olefin), 5.17 (s, CH_2), 3.92 (m), 3.43 (br s). Anal. ($C_{14}H_{14}N_2O_6 \cdot 0.25H_2O$) C, H, N.

Mannich Base Derivative 12b. With use of the same procedure (method A) as in the synthesis of **7b**, compound **12b** was produced directly in 96% yield and showed the following: mp 295 °C dec; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.10 (m, olefin), 4.16 (s, CH_2), 3.3 (m), 3.18 (br s), 2.34 (br s), 2.21 (br s), 2.08 (s). Anal. ($C_{24}H_{34}N_6O_4 \cdot 0.5H_2O$) C, H, N. For screening this compound was formulated as an hydrochloride salt, by the addition of dilute hydrochloric acid to pH 7.0, followed by freeze-drying.

Acknowledgment. This investigation was supported by the National Cancer Institute, National Institutes of Health (Contract NO1-CM-27517). The technical assistance of Laureen S. McGowan and Nancy S. Poineal was greatly appreciated.

Registry No. **1b**, 10403-51-7; **7a**, 96488-31-2; **7b**, 96488-32-3; **7c**, 102520-22-9; **7d**, 96488-34-5; **7e**, 96509-07-8; **7f**, 102572-81-6; **7g**, 96488-35-6; **7h**, 102520-23-0; **7i**, 102520-24-1; **7j**, 102520-25-2; **7k**, 31844-12-9; **7l**, 102520-26-3; **7m**, 102520-27-4; **8a**, 102520-28-5; **8b**, 102520-29-6; **8c**, 102520-30-9; **8d**, 102520-31-0; **9a**, 68644-36-0; **9b**, 102520-32-1; **10a**, 102572-82-7; **10b**, 102572-83-8; **10c**, 102572-84-9; **10d**, 102520-33-2; **10f**, 102535-13-7; **11b**, 2550-73-4; **11b** (dianhydride), 89-32-7; **11c**, 22031-22-7; **11c** (dianhydride), 6053-68-5; **11d**, 6787-63-9; **11d** (dianhydride), 1719-83-1; **12a**, 102520-34-3; **12b**, 102520-35-4; **12b**·HCl, 102520-36-5; maleimide, 541-59-3; benzene, 71-43-2; *N*-methylpiperazine, 109-01-3; thiamorpholine, 123-90-0; morpholine, 110-91-8; diethylamine, 109-89-7; glyoxylic acid, 298-12-4; anisole, 100-66-3; *p*-xylene, 106-42-3; mesitylene, 108-67-8.

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Comparative Antiaggregatory Activity in Human Platelets of a Benzopyranone *aci*-Reductone, Clofibric Acid, and a 2,3-Dihydrobenzofuran Analogue[1]

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A synthetic method for the preparation of *aci*-reductone 6-chloro-3,4-dihydroxy-2*H*-1-benzopyran-2-one (**3**) from 5-chlorosalicylate is presented. In human platelets, the benzopyranone derivative **3**, clofibric acid (**1**), and the 2,3-dihydrobenzofuran analogue **4** inhibited aggregation and serotonin secretory responses to adenosine diphosphate (ADP) with a rank order of potency $3 \geq 4 > 1$. Only analogues **3** and **4** consistently blocked the aggregatory responses (>50%) to arachidonic acid (AA) and U46619, a thromboxane A_2 agonist. Further, the rank order of inhibitory potency against U46619-induced serotonin secretion was $4 > 3 > 1$. Benzopyranone **3** is of interest since it was the most potent inhibitor of thrombin-induced [^{3}H]AA release ($3 \gg 4 = 1$) and more potent than **1** or **4** for the blockade of the ADP- or AA-mediated pathway of platelet aggregation.

Comparative antiaggregatory activities of clofibric acid (**1**) and 2-hydroxytetronic acid *aci*-reductone **2** previously have been reported.[2] Such studies are important since blood platelets are involved in the genesis of atherosclerosis.[3] Both **1**[4] and **2**[2] inhibit collagen-induced human platelet aggregation and secretion of [^{14}C]serotonin in a concentration-dependent manner at equivalent molar concentrations. Preliminary results indicated that **1** and **2** may be inhibiting platelet function by a similar mechanism—possibly involving arachidonic acid (AA) release.[2,4] However, it also might be anticipated that redox analogues such as **2** could function as antioxidants in membranes or interfere with free-radical processes involved in the biosynthetic elaboration of cyclic prostaglandin

(1) We gratefully acknowledge support of this work through U.S. Public Health Service Grant HL-12740 from the National Heart, Lung and Blood Institute. A preliminary account of this work was presented to the Division of Medicinal Chemistry, 191st National Meeting of the American Chemical Society, New York. Kim, S. K.; Tehim, A. K.; Witiak, D. T.; Romstedt, K.; Feller, D. R.; Kamanna, V. S.; Newman, H. A. I., *Abstracts of Papers*, 191st National Meeting of the American Chemical Society, New York; American Chemical Society: Washington, DC, 1986; MEDI 5.

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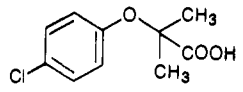
endoperoxides (PGG_2 and PGH_2) and subsequently thromboxane A_2 from AA.[5,6]



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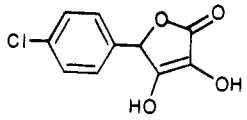

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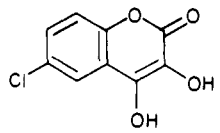

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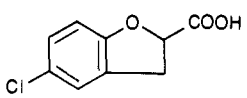
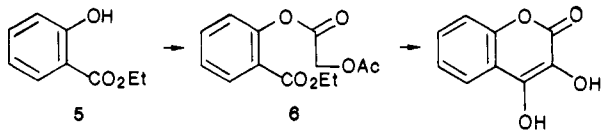


4

To provide leads for in-depth exploration of such mechanism-based structure–activity relationships, we investigated the synthesis and antiaggregatory properties of Brønsted acid benzopyranone *aci*-reductone **3**. Comparison is made with **1** and its cyclic 2,3-dihydrobenzofuran analogue **4**[7] on the prostaglandin-dependent pathway of platelet activation.

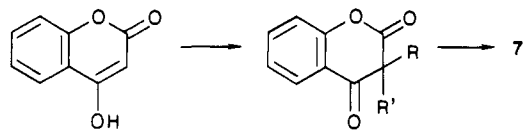
Synthetic Aspects

Benzopyranone *aci*-reductone (**7**) previously had been sythesized by Ghosh[8] from salicylate **5**. Reaction with acetoxyacetyl chloride afforded **6** (93%), which upon reaction with Na in refluxing benzene afforded **7** (no yield reported). In our laboratory attempted conversion of **6** to **7** was unsuccessful.



5 6 7

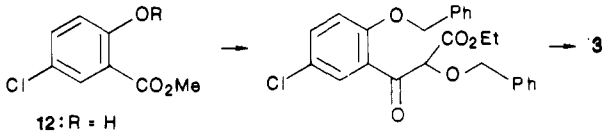
More recently, Schank and Blattner[9] prepared **7** in 44% overall yield from commercially available 4-hydroxycoumarin (**8**). This interesting four-step conversion involved reaction with TsN_3 in DMF/Et_3N affording azo compound **9**, which upon treatment with *t*-BuOCl in HCO_2H produced **10**. Reduction (NaI, $Na_2S_2O_5$) afforded **11** and acid-catalyzed hydrolysis yielded **7**. However, our requirements for large amounts of 6- and 7-substituted analogues to be assessed in vivo for antilipidemic properties necessitated development of shorter sequences from readily available starting materials.



8

9: R, R' = $=N_2$
10: R = Cl, R' = O_2CH
11: R = H, R' = O_2CH

One applicable synthetic method devised afforded target **3** in 39% overall yield, starting from 5-chlorosalicylate **12**. The procedure is anticipated to be general and fulfill our synthetic requirements for structure–activity studies. The benzyl-protected derivative **13** was treated with the enolate anion of ethyl phenylmethoxyacetate in THF at −78 °C, affording **14** in 56% yield. Two processes were employed for the conversion of **14** to **3**. Catalytic hydrogenation (H_2, 10% Pd/C, EtOAc) and lactonization in acidic MeOH afforded **3** in 69% yield. Alternatively, deprotection under transfer hydrogenation[10] conditions (10% Pd/C, cyclohexene, EtOH, reflux) followed by acid-catalyzed lactonization produced **3** in 75.5% yield.



12: R = H
13: R = CH_2Ph

14

Table I. Comparative Potencies of Clofibric Acid (**1**), Benzopyranone *aci*-Reductone **3**, and 2,3-Dihydrobenzofuran **4** as Inhibitors of Human Platelet Aggregation and Serotonin Secretion by Adenosine Diphosphate (ADP), Arachidonic Acid (AA), and Thromboxane A_2 Agonist U46619[a]

	IC_{50},[b] μM		
inducer	1	3	4
aggregation			
ADP	980 ± 245	363 ± 159	533 ± 71
AA	>3071	739 ± 86	851 ± 85
U46619 + ASA	>4000	1506 ± 160	1485 ± 277
serotonin secretion			
ADP	847 ± 92	466 ± 90	419 ± 85
AA	>3428	814 ± 90	1007 ± 142
U46619 + ASA	3501 ± 514	2378 ± 394	1438 ± 239

[a] Inhibitors were incubated 1 min before the stimulation of platelets by ADP (1–5 μM), AA (0.2–1 mM), or U46619 (0.3 μM). Aspirin (ASA, 1 mM) was added to U46619 samples. ADP data is for inhibition of secondary aggregation only. [b] Data are expressed as the mean IC_{50} ± SEM (n = three to nine donors). Maximal drug concentrations used were 4000 μM.

Biological Results

Experiments were conducted to examine the comparative potency of clofibric acid (**1**), benzopyranone *aci*-reductone **3**, and the cyclic clofibric acid related analogue 5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid (**4**) as inhibitors of human platelet aggregation induced by adenosine diphosphate (ADP), arachidonic acid (AA), and a stable epoxymethano-PGH_2 analogue U46619, a thromboxane A_2 agonist[11] (Figure 1). Each inducer is known to activate at a different site in the prostaglandin-dependent pathway of platelet function.[12] All compounds provided a concentration-dependent inhibition of AA-induced aggregation and of the secondary wave of ADP-induced aggregation. Both the *aci*-reductone **3** and the cyclic analogue **4** were approximately 4-fold more potent inhibitors of AA-induced aggregation than was clofibric acid (**1**). Unlike clofibric acid (**1**), **3** and **4** were concentration-dependent inhibitors of U46619-induced aggregation.

Comparative potencies of these compounds to the induction of aggregation and secretory responses in human platelets are summarized in Table I. For AA-induced aggregation and serotonin secretion, the rank order of inhibitory potency was **3** > **4** > **1**. Likewise, **3** and **4** were about 2-fold more potent than **1** as inhibitors of ADP-induced platelet activation and U46619-evoked serotonin secretion.

To test whether analogues **1**, **3**, and **4** inhibited AA release or synthesis of AA metabolites, concentration-de-

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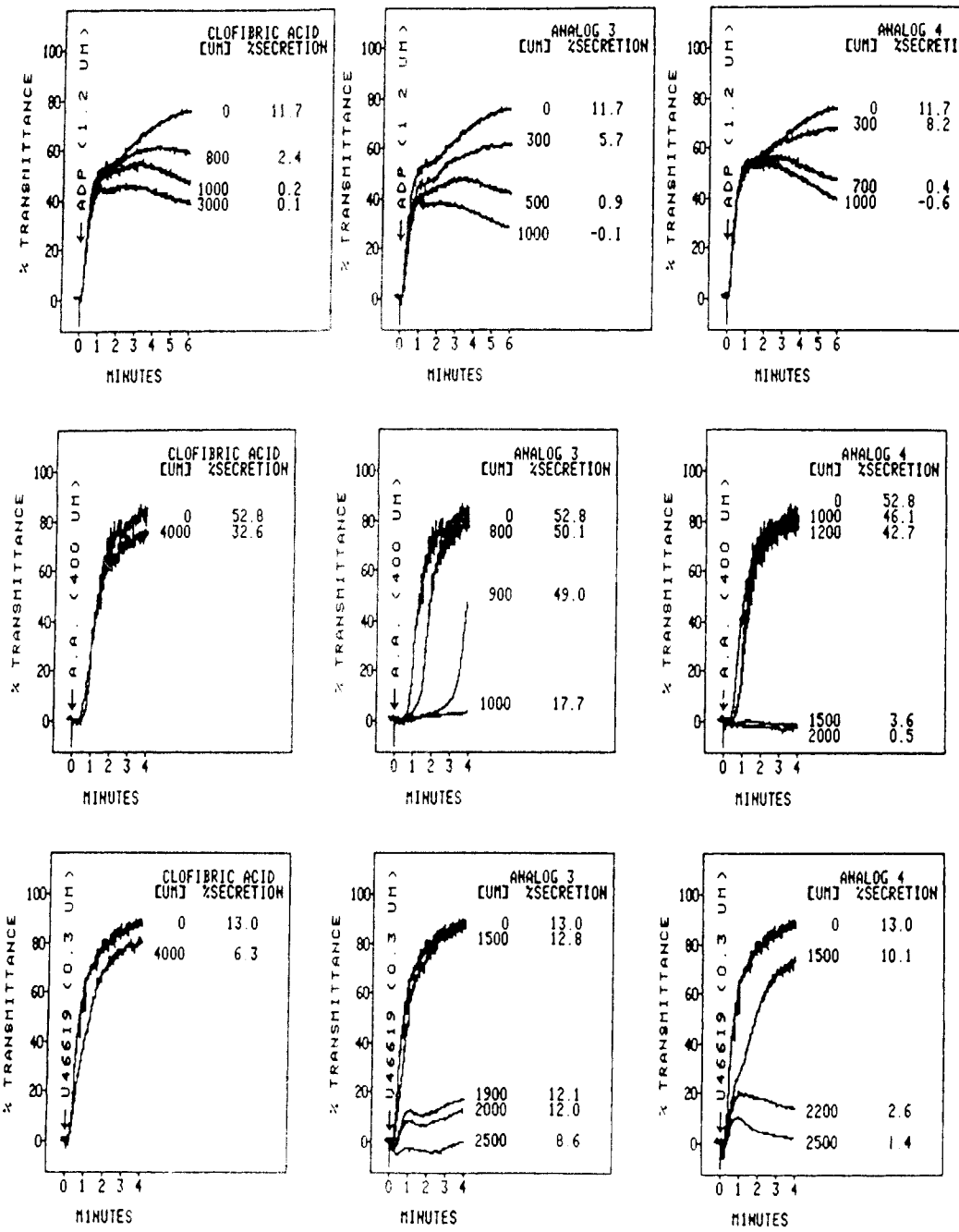

Figure 1. Comparative effects of clofibric acid (1), benzopyranone *aci*-reductone 3, and 2,3-dihydrobenzofuran 4 as inhibitors of human platelet aggregation and serotonin secretion induced by adenosine diphosphate (ADP), arachidonic acid (AA), and thromboxane A_2 agonist U46619. Upper three panels, ADP-induced responses; middle three panels, AA-induced responses; lower three panels, U46619-induced responses. This figure illustrates representative responses of analogues tested in a single platelet preparation for each inducer. This provides for a direct comparison of concentration-dependent effects. Experiments were repeated with each analogue (n = 3–9), and results are summarized in Table I.

pendent inhibitor effects were examined against thrombin-induced [^{3}H]AA release and AA-induced malondialdehyde (MDA) formation in human platelets, respectively. The data given in Figure 2 show that all compounds blocked the release of [^{3}H]AA from platelets with a rank order of inhibitory potency of 3 ≫ 1 = 4 (IC_{50} values = 432, 3311, and 3868 μM, respectively). Formation of MDA by AA was blocked by 1 and 4 which possess IC_{50} values (mean ± SE, N = 3–5) of >2955 and 908 ± 137 μM, respectively. *aci*-Reductone 3 interfered with this assay and was not tested against MDA formation.

Conclusions

Previously we observed clofibric acid (1) to inhibit collagen- and ADP-induced human platelet activation with little activity against AA-induced aggregation.[4] The effect of 1 was attributed to both a blockade of AA release from phospholipids and of subsequent AA metabolism in human platelet preparations. Current studies confirm these earlier observations and also demonstrate that both cyclic analogue 4 and *aci*-reductone 3 are more potent antiaggregatory agents than 1.

Using ADP, AA and U46619 as inducers of the prostaglandin- or AA-dependent pathway of platelet activation, we have shown that 4 not only blocked the release and subsequent metabolism of AA but also antagonized the proaggregatory actions of the thromboxane A_2 agonist U46619. Similarly, 3 blocked AA release from platelet membrane phospholipids and inhibited U46619-induced responses in human platelets. Like 4, 3 may act as an

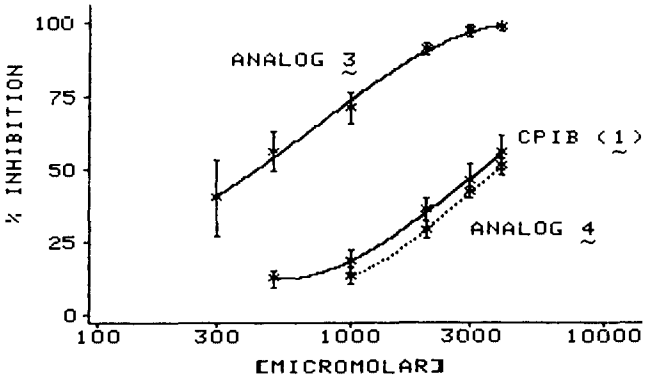

Figure 2. Percent inhibition of thrombin-induced arachidonic acid (AA) release by clofibric acid (1), benzopyranone *aci*-reductone 3, and 2,3-dihydrobenzofuran 4. Inhibitors were added 1 min prior to the addition of thrombin (0.5 U/mL). Reactions were terminated at 4 min and [^{3}H]AA was sampled from platelet supernatants and analyzed as described in the Experimental Section. Data are averages ± SEM from three to five donors.

inhibitor of AA metabolism since it is a more potent inhibitor of AA-mediated responses than of U46619. Although 4 may be construed as a cyclic analogue of 1, 3 has a redox functionality and, if examined in detail, is anticipated to reveal differences in mechanism of biological activity from that of 1 and 4. To our knowledge, 3 and 4 are the first "analogues" of clofibric acid (1) that have been shown to block the proaggregatory effect of U46619. In addition, both compounds (3 and 4) were more potent inhibitors of ADP- and AA-mediated aggregation and secretory responses than of U46619-induced platelet activation.

The *aci*-reductone system would appear to offer promise for mechanism-based structure–activity studies, since 3 was the most potent inhibitor of thrombin-induced AA release and ADP- or AA-induced aggregation. Such structure–activity studies are under investigation because clofibrate therapy in hyperlipidemic patients reversed hyperaggregatory responses to inducers of the prostaglandin-dependent pathway[13] and produces therapeutic plasma 1 concentrations of 370–840 μM.[14] Analogues 3 and 4 are more potent antiaggregatory agents than 1 and are active in the same concentration range achieved for 1 in hyperlipoproteinemic patients.[14] The greater potency observed for blockade of thrombin-induced AA release by 3 (3 $\gg$ 4 = 1) is of interest. Possibly, unlike the phenoxyacetic acid analogues, inhibition by *aci*-reductone 3 may be mediated by a redox-related mechanism in the membrane. Like the phenoxyacetic acids, *aci*-reductone 3 also blocks events of the prostaglandin-dependent pathway subsequent to AA release. Exploration of the mechanism of action of *aci*-reductones is important since such studies may provide us with a potentially effective drug for the treatment of thromboembolic disorders.

Experimental Section

Chemistry. Melting points were determined in open capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Infrared spectra were recorded with a Beckman Model 4230 spectrophotometer. Nuclear magnetic resonance spectra were recorded with a Hx-90 E spectrophotometer. Me_4Si ($CDCl_3$, Me_2SO-d_6) was used as internal standards unless otherwise specified. Chemical shifts are reported on th δ scale with peak multiplicities: d, doublet; m, multiplet; q, quartet; s, singlet; and t, triplet. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Methyl 5-Chloro-2-(phenylmethoxy)benzoate (13). To a solution of methyl 5-chlorosalicylate (12)[15] (5.58 g, 0.03 mol) in 20 mL of dry acetone were added anhydrous K_2CO_3 (4.55 g, 0.033 mol) and benzyl bromide (5.64 g, 0.033 mol). The resulting solution was refluxed for 5 h and the solvent evaporated under reduced pressure. The residue was partitioned between ether and water. The ether layer was washed with 10% aqueous NaOH solution and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting residue was recrystallized from absolute ethanol, affording 7.6 g (91.7%) of 13 as white crystals: mp 54.5–55 °C; IR (KBr) 1740 (ester) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 3.90 (s, 3 H, CO_2CH_3), 5.16 (s, 2 H, OCH_2Ph), 6.93 (d, 1 H, Ar H, J = 8.9 Hz), 7.2–7.6 (m, 6 H, Ar H), 7.79 (d, 1 H, Ar H, J = 2.9 Hz). Anal. ($C_{15}H_{13}ClO_3$) C, H, Cl.

Ethyl 5-Chloro-β-oxo-α,2-bis(phenylmethoxy)benzenepropanoate (14). In a flame-dried flask were placed dry THF (4 mL) and diisopropylamine (1.21 g, 0.012 mol). *n*-Butyllithium (1.55 M in hexane, 7.7 mL, 0.012 mol) was added dropwise at −10 °C under argon. After the mixture was stirred for 0.5 h at 0 °C, a solution of ethyl (phenylmethoxy)acetate[16] (2.33 g, 0.012 mol) in dry THF (4 mL) was added dropwise at −78 °C. After 1 h 13 (2.21 g, 0.008 mol) in dry THF (4 mL) was added. Stirring was continued for an additional 2 h at −78 °C, and the reaction mixture was quenched with 2 mL of EtOH at −20 °C. The quenched mixture was poured into 50 mL of 10% aqueous HCl solution and extracted with ether. The ether layer was washed with water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure, affording a light yellow oil. Purification by flash chromatography (silica gel) using toluene–$CHCl_3$ (5:1) as eluent afforded 1.96 g (56%) of 14 as white crystals (EtOH): mp 64–65 °C; IR (KBr) 1735 (ester) and 1685 (conjugated carbonyl) cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.16 (t, 3 H, OCH_2CH_3), 4.13 (q, 2 H, OCH_2CH_3), 4.55 (d, 1 H, OCH_2Ph, J_{AB} = 12.6 Hz), 4.60 (d, 1 H, OCH_2Ph, J_{AB} = 12.6 Hz), 5.07 (s, 2 H, OCH_2Ph), 5.28 (s, 1 H, $CHCO_2Et$), 6.85 (d, 1 H, Ar H, J = 8.9 Hz), 7.0–7.5 (m, 11 H, Ar H), 7.64 (d, 1 H, Ar H, J = 2.5 Hz). Anal. ($C_{25}H_{23}ClO_5$) C, H, Cl.

6-Chloro-3,4-dihydroxy-2*H*-1-benzopyran-2-one (3). Transfer Hydrogenation.[10] To a solution of 14 (0.88 g, 0.002 mol) in absolute EtOH (14 mL) were added 10% Pd/C (0.18 g, 20% by weight of 14) and cyclohexene (7 mL). The resulting solution was refluxed for 1 h under argon. The mixture was filtered and the filtrate evaporated under reduced pressure. To a solution of the residue in 10 mL of MeOH was added 2 mL of concentrated HCl. The resulting solution was refluxed for 1 h, and the solvent was removed under reduced pressure. The residue was partitioned between EtOAc (20 mL) and 10% aqueous $NaHCO_3$ solution (30 mL). After acidification using a mixture of ice and concentrated HCl, the aqueous layer was extracted with EtOAc and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was recrystallized from EtOAc/petroleum ether, yielding 0.32 g (75.5%) of 3 as a white solid: mp 244–247 °C dec; IR (KBr) 3360 (OH), 3280 (OH), 1710 (C=O), 1668 (C=O), 1635 (C=C) cm^{-1}; NMR (Me_2SO-d_6, 90 MHz) δ 7.2–7.9 (m, 3 H, Ar H), 9.1–9.9 (br s, 1 H, OH), 10.8–12.0 (br s, 1 H, OH). Anal. ($C_9H_5ClO_4$) C, H, Cl.

Catalytic Hydrogenation. A solution of 14 (0.88 g, 0.002 mol) in 20 mL of EtOAc was hydrogenated over 0.18 g of 10% Pd/C at room temperature and 35 psi for 2 h. After filtration, the solvent was removed under reduced pressure, affording a light yellow oil. The oil was treated as previously described, affording 0.293 g (69%) of 3 as a white solid.

Biology. ADP and AA were obtained from Sigma Chemical Co. (St. Louis, MO). [^{14}C]Serotonin (57 mCi/mmol) and [^{3}H]AA (210 Ci/mmol) were supplied by Amersham (Arlington Heights, IL). U46619 (15(*S*)-hydroxy-11α,9α-epoxymethanoprosta-5-

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(Z),13(E)-dienoic acid) was purchased from Upjohn Diagnostics (Kalamazoo, MI).

Platelet Antiaggregatory Activities. Blood was collected from normal human volunteers who reported to be free of medication for at least 10 days prior to blood collection. Platelet-rich plasma was prepared as described previously[4] and used for all studies with the exception of thrombin-induced AA release. For the latter experiments, washed platelets were prepared and suspended in a modified Tyrode's solution, pH 7.4 as previously described.[17] The platelet count was adjusted to 3×10^8/mL for aggregation, secretion, and biochemical studies.

Platelet aggregation studies were performed according to the turbidometric method of Born[18] in a Payton Model 600 dual-channel aggregometer interfaced to an Apple microcomputer for acquisition, quantitation, presentation, and management of platelet aggregation data.[19] All inducers were used at the minimum concentrations required to stimulate maximal aggregation. Inhibitors were added 1 min prior to induction of platelet activation, and inhibitory concentration-50 (IC_{50}) values for each inhibitor were determined from changes in the amplitude of light transmittance after 6 min for ADP and 4 min for AA and U46619. Secretion of the contents of platelet-dense granules was measured by monitoring the release of radioactivity from platelets prelabeled with [^{14}C]serotonin.[17] Malondialdehyde formation induced by AA was determined as previously described.[17]

To study the release of AA from platelet phospholipids, washed platelets were incubated with [^{3}H]AA (3.6 μCi/10^9 platelets) for 2 h at room temperature prior to the final centrifugation and resuspension of the washing procedure. Released [^{3}H]AA was quantified[17] from supernatants of stimulated platelet samples in a manner identical with the determination of serotonin secretion.

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Registry No. **3**, 103620-90-2; **12**, 4068-78-4; **13**, 103620-87-7; **14**, 103620-88-8; **14** (diol), 103620-89-9; ethyl (phenylmethoxy)-acetate, 32122-09-1.

Nonquaternary Cholinesterase Reactivators. 3. 3(5)-Substituted 1,2,4-Oxadiazol-5(3)-aldoximes and 1,2,4-Oxadiazole-5(3)-thiocarbohydroximates as Reactivators of Organophosphonate-Inhibited Eel and Human Acetylcholinesterase in Vitro

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As an extension of an earlier investigation (*J. Med. Chem.* **1984**, *27*, 1431), we prepared a series of 3-substituted 5-[(hydroxyimino)methyl]-1,2,4-oxadiazoles and the corresponding 5-thiocarbohydroximic acid 2-(*N*,*N*-dialkylamino)ethyl *S*-esters. The compounds were evaluated in vitro as reactivators of phosphonylated electric eel and human erythrocyte (RBC) acetylcholinesterases (AChE). The compounds were characterized with respect to (hydroxyimino)methyl acid dissociation constant, nucleophilicity, octanol/buffer partition coefficient, reversible AChE inhibition, and kinetics of reactivating ethyl methylphosphonylated AChE. One compound was also tested for effectiveness in preventing AChE phosphonylation. All of the tested compounds significantly reactivate ethyl methylphosphonylated AChE: the 3-*n*-octyl- and 3-(1-naphthyl)-substituted aldoximes are as reactive (within a factor of 5–10) toward the inhibited enzymes as the benchmark pyridinium reactivators, 2-PAM and HI-6. All of the substituted thiocarbohydroximic acid *S*-esters are powerful reversible inhibitors of AChE's: the 3-*n*-octyl- and 3-(1-naphthyl)-substituted thiocarbohydroximates inhibit eel AChE to 50% initial activity at concentrations <5 μM. When added to an eel AChE solution at concentrations between 5 and 50 μM, the 3-phenyl-substituted thiocarbohydroximate effectively antagonizes AChE inhibition by ethyl *p*-nitrophenyl methylphosphonate (EPMP), suggesting the potential utility of this compound for preventing anti-AChE-agent poisoning.

Various organophosphorus esters irreversibly inhibit acetylcholinesterase (AChE) by phosphonylating a serine hydroxyl at the enzyme active site.[1-7] Appropriately designed nucleophiles can reversibly bind to phosphonylated AChE and displace the phosphorus moiety, thereby restoring enzymatic activity. The therapeutic use of such cholinesterase "reactivators" to treat poisoning by organophosphorus anticholinesterase agents is well-known.[8-11] Our own research[12-15] has focused on compounds that combine the structural elements required for activity vs. phosphonylated AChE but that are devoid of any quaternary functionality. These nonquaternary reactivators should afford significant advantages over conventional

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