71780-69-3; (±)-19·$C_4H_4O_4$, 71780-70-6; (+)-19, 83434-65-5; (+)-19·$C_4H_4O_4$, 89495-77-2; (−)-19, 83434-64-4; (−)-19·$C_4H_4O_4$, 89495-78-3; (±)-20, 71780-77-3; (±)-20·$C_4H_4O_4$, 71780-78-4; (±)-21, 71780-75-1; (±)-21·$C_4H_4O_4$, 71780-76-2; (±)-22, 71780-80-8; (±)-22·$C_4H_4O_4$, 71780-81-9; (±)-23, 97949-05-8; (±)-23·$C_4H_4O_4$, 97995-34-1; (±)-24, 97949-06-9; (±)-24·HCl, 97995-35-2; (±)-25, 97889-82-2; (±)-25·$C_4H_4O_4$, 97889-83-3; (±)-26, 97948-98-6; (±)-26·$C_4H_4O_4$, 97948-99-7; $CH_3SNa$, 5188-07-8; 4-$CH_3SC_6H_4CH_2Cl$, 874-87-3; $(CH_3)_2NCSCl$, 16420-13-6; $C_6H_5COCl$, 98-88-4; *N*-methyl-3,4-lutidinium iodide, 6283-41-6; (±)-1,2-dihydro-2-[4-(methylthio)benzyl]-1,3,4-trimethylpyridine, 97907-56-7.

# Inhibitors of Blood Platelet Aggregation. Effects of Some 1,2-Benzisothiazol-3-ones on Platelet Responsiveness to Adenosine Diphosphate and Collagen

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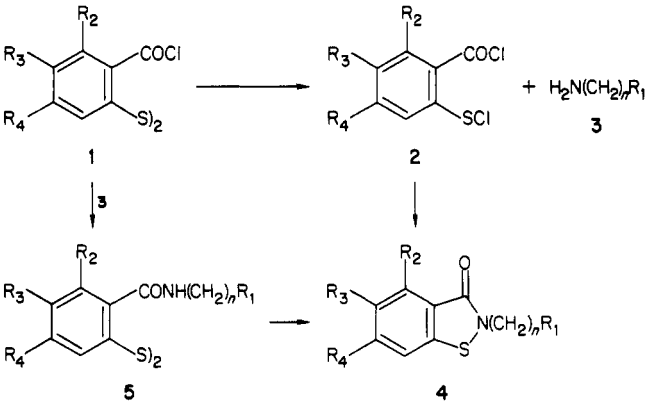
A series of substituted 1,2-benzisothiazol-3-ones was synthesized, and the compounds were tested for ability to inhibit platelet aggregation induced by adenosine diphosphate and collagen in rats and guinea pigs ex vivo. Alkyl substituents at the 2-position bearing a basic group were necessary for ex vivo activity. Several of the compounds were potent inhibitors of adenosine diphosphate induced first-phase aggregation, but adverse toxicological findings terminated their further development. Preliminary studies suggested that inhibition of aggregation was not attributable to inhibition of prostanoid synthesis or to raised levels of cyclic 3′,5′-adenosine monophosphate.

Activation of blood platelets is the first step in hemostasis, the aggregation of platelets stemming blood flow initially and then serving as a nidus for fibrin deposition and permanent closure of the wound[1]. This same sequence of events within vessels probably causes thrombosis, and, hence, one rational approach in the search for antithrombotic drugs is to look for inhibitors of platelet aggregation. Certainly much effort has been spent in this direction since the introduction of a simple photometric method of measuring platelet aggregation[2]. When applied to human citrated platelet-rich plasma (PRP), the technique shows adenosine diphosphate (ADP) to induce aggregation in two phases. The second phase is readily inhibited by nonsteroidal antiinflammatory drugs such as aspirin when tested in vitro[3] or ex vivo[4]. The first phase of aggregation, however, has proved more resistant to therapeutic manipulation. Although there has been some success in the use of aspirin as an antithrombotic agent,[5,6] it could be argued that an inhibitor of first-phase or primary aggregation may have greater potential in the prevention and treatment of platelet-initiated thrombotic events. This proposition is supported by the increasingly wide application[7] of epoprostenol (prostacyclin), a potent inhibitor of primary aggregation induced by a range of aggregating agents in vitro.[8]

Here we report studies on a series of 1,2-benzisothiazol-3-ones, directed toward the selection of potential clinical candidates, the goal being a drug that inhibits first-phase aggregation ex vivo in man and hence a potential antithrombotic agent. When this study was well advanced,[9] a patent application disclosed similar work by another group.[10]

**Chemistry.** The 1,2-benzisothiazol-3-ones (**4**; Table I) were prepared by a number of methods, most well reported in the literature[11,12] (Scheme I). Reaction of diazotized anthranilic acids with potassium ethyl xanthate followed by hydrolysis and oxidation gave 2,2′-dithiosalicylic acids,[13] which were converted into **1** with thionyl chloride. Some amines of type **3** were commercially available; otherwise

**Scheme I**




they were synthesized by lithium aluminium hydride reduction of the corresponding nitriles.[14]

The yields in the reactions between **2** and **3**[15] were only moderate but satisfactory for this study. No obvious side

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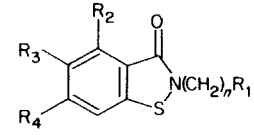
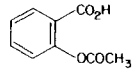
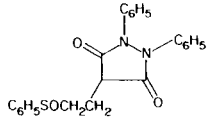
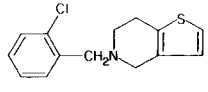
(1) French, J. E. *Pathol. Microbiol.* **1967**, *30*, 653.
(2) Born, G. V. R. *Nature* **1962**, *194*, 927.
(3) Zucker, M.; Peterson, J. *Proc. Soc. Exp. Biol. Med.* **1968**, *127*, 547.
(4) Weiss, H. J.; Aledort, L. M. *Lancet* **1967**, *2*, 495.
(5) Fields, W. S.; Lemak, N. A.; Frankowski, R. F.; Hardy, R. J. *Stroke* **1977**, *8*, 301.
(6) The Canadian Cooperative Stroke Study Group *New. Engl. J. Med.* **1978**, *229*, 53.
(7) *Drugs Fut.* **1982**, *7*, 770.
(8) Moncada, S.; Vane, J. R. *Pharmacol. Rev.* **1979**, *30*, 293.
(9) Baggaley, K. H. Ger. Off. 2652201 (Beecham Group); *Chem. Abstr.* **1977**, *87*, 117847*u*.
(10) Boshagen, H.; Haerlein, U.; Meng, K.; Seuter, F. Ger. Off. 2602643 (Bayer A.-G.); *Chem. Abstr.* **1978**, *88*, 190809*h*.
(11) Bambas, L. L. In "The Chemistry of Heterocyclic Compounds"; Weissburger, A., Ed.; Wiley-Interscience: New York, **1952**; Vol. 4, pp 225–227.
(12) Davis, M. *Adv. Heterocycl. Chem.* **1972**, *14*, 43.
(13) Katz, L.; Karger, L. S.; Schroeder, W.; Cohen, M. S. *J. Org. Chem.* **1953**, *18*, 1380.
(14) (a) Nystrom, R. F.; Brown, W. G. *J. Am. Chem. Soc.* **1948**, *70*, 3738. (b) Micovic, V. M.; Mihailovic, M. L. J. *J. Org. Chem.* **1953**, *18*, 1190.
(15) McClelland, E. W.; Gait, A. J. *J. Chem. Soc.* **1926**, 921.

0022-2623/85/1828-1661$01.50/0 

Table I. 1,2-Benzisothiazol-3-ones, Chemical Data and Inhibition of Platelet Aggregation ex Vivo




| compd | $R_1$ | $n$ | $R_2$ | $R_3$ | $R_4$ | method[a] | yield,[b] % | mp or bp, °C (mmHg) [lit.[c] values] | cryst[d] solvent | formula[e] | platelet responsiveness ex vivo[a] dose, mmol/kg | rat collagen | rat ADP | guinea pig collagen | guinea pig ADP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | H | 0 | H | H | H | A | 73 | 158 [158][f] | A | | 0.3 | 1.0 | | | |
| 7 | $C_6H_5$ | 0 | H | H | H | A | 56 | 147-148 [145][g] | B | | 0.15 | 1.1 | 0.9 | | |
| 8 | 2-pyridyl | 0 | H | H | H | A | 14 | 198-199 [198][g] | B | | 0.3 | 0.9 | | | |
| 9 | H | 5 | H | H | H | A | 54 | 161 (1.5) [152-156 (0.2)][g] | | | 0.3 | 1.2 | | | |
| 10 | OH | 2 | H | H | H | A | 70 | 103-105 [104-106][g] | A/B | | 0.3 | 1.3 | | | |
| 11 | $C_6H_5(CH_3)N$ | 2 | H | H | H | E | 63 | 127-130 | B | | 0.15 | 1.1[h] | 1.0 | | |
| 12 | 1-pyrrolidinyl | 2 | H | H | H | A | 24 | 87-88 [87-88][i] | B | | 0.15 | 2.3[j] | 0.9 | 13.9[j] | 1.9 |
| | | | | | | | | | | | 0.08 | | | 6.6[j] | 1.5[j] |
| 13 | 1-pyrrolidinyl | 3 | H | H | H | A | 11 | oil | C | $C_{14}H_{18}N_2OS$ | 0.08 | | | 6.2[j] | 2.0[j] |
| 14 | 1-pyrrolidinyl | 4 | H | H | H | A | 21 | oil | C | $C_{15}H_{20}N_2OS$ | 0.08 | | | 1.9 | 1.4 |
| 15 | 1-pyrrolidinyl | 2 | H | $CH_3$ | $CH_3$ | B | 27 | 105-107 | B/D | $C_{15}H_{20}N_2OS$ | 0.15 | 2.7[h] | 1.4[j] | >10[h] | 1.9[h] |
| 16 | 1-pyrrolidinyl | 2 | H | $CH_3O$ | $CH_3O$ | A | 45 | 93-96 | E/D | $C_{15}H_{20}N_2O_3S$ | 0.15 | 1.8 | 1.1 | | |
| 17 | 1-pyrrolidinyl | 2 | H | Cl | Cl | A | 38 | 152-154 | B | $C_{13}H_{14}Cl_2N_2OS$ | 0.15 | 1.8 | 1.4 | | |
| 18 | 1-pyrrolidinyl | 2 | $CH_3$ | H | $CH_3$ | C | 27 | gum | C | $C_{15}H_{20}N_2OS$ | 0.08 | | | >23[j] | >32[j] |
| | | | | | | | | | | | 0.04 | | | >11[j] | 1.8[j] |
| 19 | 1-pyrrolidinyl | 2 | $CH_3O$ | H | $CH_3O$ | D | 21 | 105-106 | F | $C_{15}H_{20}N_2O_3S$ | 0.08 | | | >31[j] | 4.6[j] |
| 20 | 1-piperidinyl | 2 | H | H | H | A | 30 | 95-97 | B | $C_{14}H_{18}N_2OS$[k] | 0.08 | 4.0[j] | 1.7[j] | 5.0[j] | 1.4[j] |
| 21 | 1-piperidinyl | 3 | H | H | H | A | 62 | 72-73 | F | $C_{15}H_{20}N_2OS$ | 0.08 | 2.4[j] | 1.0 | >26[j] | 3.7[j] |
| 22 | 1-piperidinyl | 4 | H | H | H | A | 24 | 73-74 | F | $C_{16}H_{22}N_2OS$ | 0.08 | | | 3.4[j] | 1.1 |
| 23 | 1-piperidinyl | 5 | H | H | H | A | 25 | oil | C | $C_{17}H_{24}N_2OS$ | 0.08 | | | 1.1 | 1.3 |
| 24 | 1-piperidinyl | 2 | H | Cl | Cl | A | 41 | 150-151 | G | $C_{14}H_{16}Cl_2N_2OS$ | 0.15 | 1.5 | 1.2 | | |
| 25 | 1-piperidinyl | 2 | H | $CH_3O$ | $CH_3O$ | A | 59 | 100-102 | F | $C_{16}H_{22}N_2O_3S$ | 0.15 | 2.2[j] | 1.1 | 7.3[j] | 1.9[j] |
| 26 | 1-piperidinyl | 2 | $CH_3O$ | H | $CH_3O$ | D | 22 | 72-75 | F | $C_{16}H_{22}N_2O_3S$ | 0.08 | | | >10[j] | >27[j] |
| | | | | | | | | | | | 0.04 | | | >32[j] | 1.8[j] |
| 27 | 1-piperidinyl | 2 | $CH_3$ | H | $CH_3$ | C | 32 | oil | C | $C_{16}H_{22}N_2OS$[l] | 0.08 | | | >21[j] | >24[j] |
| | | | | | | | | | | | 0.04 | | | >21[j] | 2.1[j] |
| 28 | 1-piperidinyl | 3 | H | $CH_3O$ | $CH_3O$ | A | 24 | 108-110 | F | $C_{17}H_{24}N_2O_3S$ | 0.08 | | | 2.5[j] | 1.1 |
| 29 | 1-piperidinyl | 3 | $CH_3$ | H | $CH_3$ | C | 31 | 91-93 | F | $C_{17}H_{24}N_2OS$ | 0.08 | | | 6.7[j] | 1.4[j] |
| 30 | 1-piperidinyl | 4 | H | $CH_3O$ | $CH_3O$ | A | 25 | 75-77 | E/D | $C_{18}H_{26}N_2O_3S$[m] | 0.08 | | | 1.6 | 1.1 |
| 31 | 3-azabicyclo[3.2.2]non-3-yl | 2 | H | H | H | E | 60 | 91-93 | B | $C_{17}H_{22}N_2OS$ | 0.15 | 2.2[j] | 1.6 | 1.7 | 1.1 |
| 32 | 3-azabicyclo[3.2.2]non-3-yl | 3 | H | H | H | A | 30 | 80-82 | H | $C_{18}H_{24}N_2OS$ | 0.08 | | | 1.8[j] | 1.2 |
| 33 | 3-azabicyclo[3.2.2]non-3-yl | 4 | H | H | H | A | 44 | 86-87 | I | $C_{19}H_{26}N_2OS$ | 0.08 | | | 4.1[j] | 1.6[j] |
| 34 | 3-azabicyclo[3.2.2]non-3-yl | 5 | H | H | H | A | 51 | oil | C | $C_{20}H_{28}N_2OS$ | 0.08 | | | 2.8 | 1.2 |
| 35 | 3-azabicyclo[3.2.2]non-3-yl | 2 | H | Cl | Cl | E | 61 | 160-162 | B | $C_{17}H_{20}Cl_2N_2OS$ | 0.3 | 1.0 | | | |
| 36 | 3-azabicyclo[3.2.2]non-3-yl | 2 | H | $CH_3O$ | $CH_3O$ | A | 50 | 139-141 | F | $C_{19}H_{26}N_2O_3S$ | 0.3 | >5.8[j] | 1.9[j] | 1.9[j] | 1.0 |
| | | | | | | | | | | | 0.15 | 2.9[j] | 1.4[j] | 1.6[j] | 1.3 |
| | | | | | | | | | | | 0.08 | | | 1.7[j] | 1.1 |
| 37 | 3-azabicyclo[3.2.2]non-3-yl | 2 | H | $CH_3$ | $CH_3$ | B | 4 | 126-128 | B | $C_{19}H_{26}N_2OS$ | 0.15 | 1.0 | 1.3 | 0.7[h] | 1.0[h] |
| 38 | 3-azabicyclo[3.2.2]non-3-yl | 3 | H | $CH_3O$ | $CH_3O$ | A | 22 | 126-128 | A/B | $C_{20}H_{28}N_2O_3S$ | 0.08 | | | 1.6[j] | 0.9 |
| 39 | 3-azabicyclo[3.2.2]non-3-yl | 4 | H | $CH_3O$ | $CH_3O$ | A | 60 | 132-134 | D | $C_{21}H_{30}N_2O_3S$ | 0.08 | 2.4[j] | 1.4[j] | 5.4[j] | 2.6[j] |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 3-azabicyclo[3.2.2]non-3-yl | 5 | H | $CH_3O$ | $CH_3O$ | A | 35 | 139–140 | D | $C_{22}H_{32}N_2O_3S$ | 0.08 | | | 4.1[j] | 1.7[j] |
| 41 | 3-azabicyclo[3.2.2]non-3-yl | 6 | H | $CH_3O$ | $CH_3O$ | E | 47 | oil | C | $C_{23}H_{34}N_2O_3S$[n] | 0.08 | | | 2.0[j] | 1.1[j] |
| 42 | 3-azabicyclo[3.2.2]non-3-yl | 4 | $CH_3$ | H | $CH_3$ | C | 9 | 83–84 | H | $C_{21}H_{30}N_2OS$ | 0.04 | | | 1.1[h] | 0.8[h] |
| 43 | 2-pyridyl | 2 | H | H | H | A | 62 | 110–111 | C | $C_{14}H_{12}N_2OS$ | 0.3 | 0.7 | | | |
| 44 | 2-pyridyl | 2 | H | Cl | Cl | B | 35 | 168–170 | B | $C_{14}H_{10}Cl_2N_2OS$ | 0.15 | 1.0 | 1.0 | | |
| 45 | 2-pyridyl | 2 | H | $CH_3O$ | $CH_3O$ | A | 70 | 155–157 | G | $C_{16}H_{16}N_2O_3S$ | 0.15 | 2.8[j] | 1.8[j] | 1.5 | 1.1 |
| 46 | 2-pyridyl | 2 | H | $CH_3$ | $CH_3$ | B | 10 | 178–179 | B | $C_{16}H_{16}N_2OS$ | 0.15 | | | 0.8[h] | 0.9[h] |
| 47 | 2-pyridyl | 2 | $CH_3$ | H | $CH_3$ | C | 37 | 70–71 | H | $C_{16}H_{16}N_2OS$ | 0.04 | | | 1.6 | 1.0 |
| 48 | 2-pyridyl | 3 | H | $CH_3O$ | $CH_3O$ | A | 46 | 113–115 | F/G | $C_{17}H_{18}N_2O_3S$ | 0.15 | | | >27[j] | 2.4[j] |
| 49 | 2-pyridyl | 4 | H | $CH_3O$ | $CH_3O$ | A | 73 | 113–114 | D | $C_{13}H_{18}N_2OS$ | 0.15 | | | 2.7[h] | 2.4[h] |
| 50 | $(C_2H_5)_2N$ | 2 | H | H | H | A | 56 | gum | C | | 0.15 | 2.6[h] | 1.3 | | |
| 51 | $(C_2H_5)_2N$ | 2 | H | Cl | Cl | A | 18 | 81–84 | D | $C_{13}H_{16}Cl_2N_2OS$[o] | 0.3 | 6.5[j] | 1.2 | | |
| | | | | | | | | | | | 0.15 | 2.1 | 0.8 | | |
| 52 | $(C_2H_5)_2N$ | 2 | H | $CH_3O$ | $CH_3O$ | A | 33 | 83–85 | D | $C_{15}H_{22}N_2O_3S$ | 0.15 | 1.5 | 1.1 | >47[j] | 2.2[j] |
| 53 | $(C_2H_5)_2N$ | 2 | $CH_3$ | H | $CH_3$ | C | 36 | oil[p] | C | | 0.04 | | | >17[j] | 2.2 |
| 54 | $(C_2H_5)_2N$ | heminapsylate salt of 53 | | | | | | 224–226[p] | A/B | $C_{20}H_{26}N_2O_4S_2H_2O$[q] | | | | >36[j] | 2.8 |
| 55 | *N*-morpholinyl | 2 | H | H | H | A | 62 | 66–68 | C | $C_{13}H_{16}N_2O_2S$ | 0.15 | 1.0 | 1.0 | | |
| 56 | *N*-morpholinyl | 3 | H | H | H | A | 78 | 93–95 [82][i] | H | $C_{14}H_{18}N_2O_2S$[r] | 0.08 | | | 2.5[j] | 1.3 |
| 57 | *N*-morpholinyl | 4 | H | H | H | A | 34 | 55–57 | I | $C_{15}H_{20}N_2O_2S$ | 0.08 | | | 2.4[j] | 1.1 |
| 58 | aspirin |  | | | | | | | | | 0.15 | 1.5[s] | 1.1 | 1.8[s] | 1.0 |
| | | | | | | | | | | | 0.3 | 1.8[j] | 1.0 | 2.5[s] | 1.0 |
| 59 | sulfinpyrazone |  | | | | | | | | | 0.02 | | | 2.9[s] | 1.1 |
| | | | | | | | | | | | 0.04 | 1.3 | 1.0 | 2.0[j] | 1.1 |
| | | | | | | | | | | | 0.08 | 1.8[j] | 1.1 | 6.2[j] | 1.6[j] |
| | | | | | | | | | | | 0.15 | 1.1 | 1.2 | 3.2[t] | 1.2 |
| 60 | ticlopidine |  | | | | | | | | | 0.33 | 1.9[j] | 1.1 | 2.0[j] | 1.1 |
| | | | | | | | | | | | 1.0 | 3.7[s] | 1.1 | 2.0[j] | 1.6[j] |

HCl

[a] See the Experimental Section. [b] Refers to recrystallized product. [c] Literature location of known compounds cited. [d] Key: A, $H_2O$; B, EtOH; C, product purified by column chromatography; D, 60–80 °C petroleum ether; E, $CH_2Cl_2$; F, IPE; G, IPA; H, product purified by column chromatography and oil crystallized on standing; I, $Et_2O$. [e] Analytical data for new compounds were within ±0.4% of the theoretical values unless otherwise stated. [f] Reference 15. [g] Fischer, R.; Hurni, H. *Arzneim.-Forsch.* **1964**, *14*, 1301. [h] Result obtained on pooled samples, see the Experimental Section. [i] Reference 20. [j] $p < 0.05$; Student's t-test. [k] C: calcd, 64.08; found, 64.56. [l] C: calcd, 66.21; found, 65.78. N: calcd, 9.63; found, 9.06. No molecular ion observed on electron-impact MS determination but $MH^+$ ion at *m/e* 291 in isobutane chemical ionization MS: calcd for $C_{16}H_{22}N_2OS + 1$, 291. [m] C: calcd, 61.68; found, 60.04. Exact mass at *m/e* 350.1691; calcd for $C_{18}H_{26}N_2O_3S$, 350.1664. [n] C: calcd, 66.02; found, 66.90. [o] H: calcd, 5.05; found, 5.46. [p] Reference 10 reported no physical data for these compounds. [q] S: calcd, 14.55; found, 15.20. [r] C: calcd, 60.40; found, 60.89. [s] $p < 0.01$; Student's t-test. [t] $p < 0.001$; Student's t-test.

**Table II.** Effects of Standard Compounds and Representative 1,2-Benisothiazol-3-ones on Human Platelet Aggregation in Vitro

| compd | $IC_{50}$,[a] μM vs. collagen | $IC_{50}$,[a] μM vs. ADP |
|---|---|---|
| aspirin (**58**) | 68 ± 6 (3)[b] | >1000 (3) |
| sulfinpyrazone (**59**) | 870 ± 90 (2) | >1000 (3) |
| ticlopidine (**60**) | 1057 ± 70 (3) | 2000 (2) |
| **6** | 34 ± 4 (6) | >200 (2) |
| **12** | 30 ± 3 (2) | 83 ± 3 (2) |
| **21** | 17 ± 1 (2) | 57 ± 11 (4) |
| **36** | 63 (1) | 110 ± 35 (3) |
| **39** | 16 (2) | 99 ± 13 (4) |
| **43** | 12 ± 2 (2) | 20 ± 5 (2) |

[a] Concentration required to inhibit aggregation by 50%. Values for ADP refer to primary aggregation. [b] Figures are mean ± SEM ($n$) or range when $n$ = 2.

products were noted, and attempts to improve yields by carrying out the reaction in the presence of triethylamine were unsuccessful and gave colored products.

In method A, the sulfenyl chlorides, **2**, were generally prepared by reaction of **1** with chlorine[15] and were not isolated prior to reaction with amine **3**. To prepare 5,6-dimethoxy-substituted compounds, however, sulfuryl chloride[16] was used instead of chlorine to produce 6-(chlorosulfenyl)-3,4-dimethoxybenzoyl chloride (**2**) ($R_2$ = H, $R_3$ = $R_4$ = $CH_3O^-$).

The 2,2′-dithiobis(benzamides) **5**, prepared from the acid chlorides **1**, were not isolated. They were cyclized either by aqueous alkali (method B),[17] by thionyl chloride (method C),[18] or via the corresponding Bunte salts (method D).[19]

The only route that did not form the benzisothiazolone in the final step involved the displacement of a tosyl group in **4** ($R_1$ = OTs) by a secondary amine (method E).[20] The tosylate was prepared by treatment of **4** ($R_1$ = OH) with tosyl chloride in pyridine.

**Biology.** Although an inhibitor of ADP-induced primary aggregation was the aim of this program, activity of compounds against both ADP- and collagen-induced aggregation was usually assessed as it indicated the selectivity of action of the compound and also afforded the possibility of finding novel activity directed against responsiveness to collagen. Potency in human citrated PRP was always measured first, for a total lack of inhibitory activity here at high concentrations (up to 200 μM) would discourage further investigation. However, this initial screen did not necessarily dictate the fate of new analogues because it soon became clear that potency in vitro was not related to activity in rats and guinea pigs ex vivo.[21] For example, compound **43** was one of the most potent of the series in human PRP in vitro (Table II) but showed no activity in rats ex vivo (Table I). Activity ex vivo was assumed to give a better reflection of antithrombotic potential than potency in vitro, because the former was taken as an indication that the compound had reached sufficient concentration in the blood stream for a sufficient period of time to affect circulating platelet responsiveness. At the start of this program, compounds were administered orally (0.3 mmol/kg) to either rats or guinea pigs or both species. The rat was later selected for the initial screen because it gave more stable PRP preparations and presented a more rigorous test (see Table I). Compounds failing, at 0.15 mmol/kg, to inhibit ADP-induced aggregation or displaying no remarkable activity against responsiveness to collagen were not usually studied further. Compounds with the required activity were examined in more detail in the guinea pig, where larger effects could be obtained. In the context of studies of particular structure–activity relationships, some analogues were tested only in the guinea pig. Responsiveness to collagen was always more sensitive to inhibition than responsiveness to ADP. Thus, compounds with no anticollagen activity were occasionally not examined for anti-ADP properties.

**Structure–Activity Relationships.** Variation of structure included modification of the groups on the 2-position of the benzisothiazol-3-one nucleus, extension of the length of the alkylene chain at position 2, and substitution in the aromatic ring (Table I). With respect to inhibitory activity in human PRP, there were no discernible structure–activity relationships. Most compounds caused 50% inhibition of collagen-induced aggregation at concentrations of 10–60 μM, some 2–6-fold higher concentrations being required to cause 50% inhibition of primary aggregation in response to ADP. Representative results are given in Table II.

In contrast, clear structure–activity relationships emerged from studies ex vivo. The necessity for a side chain bearing a basic tertiary amine for activity ex vivo in the rat was apparent from the lack of activity of the 2-phenyl (**7**), 2-*n*-pentyl (**9**), and hydroxyethyl (**10**) compounds, the *N*-methylaniline derivative (**11**), and 1,2-benzisothiazol-3-one (**6**).

With no aromatic substituents and $n$ = 2, the pyrrolidinyl (**12**) and piperidinyl (**20**) moieties gave appreciably better activity than 2-pyridyl (**43**), 3-azabicyclo[3.2.2]-non-3-yl (**31**), and morpholino (**55**) groups. Extension of the alkylene chain gave maximal anti-ADP activity at $n$ = 3 in the pyrrolidinyl and piperidinyl series (**13** and **21**), activity decreasing at $n$ = 4 (**14** and **22**), whereas with the 3-azabicyclo[3.2.2.]non-3-yl moiety the $n$ = 4 derivative **33** was the most active. Extending the chain in the morpholino series did not appreciably affect activity.

A number of derivatives with aromatic substituents were prepared but only 4,6- and 5,6-disubstitution produced noteworthy effects. In the latter case, results in the rat in the $n$ = 2 series showed that 5,6-dichloro substitution always rendered compounds less active (**17**, **24**, **35**), whereas 5,6-dimethyl substitution reduced activity in the azabicyclononyl derivative (**37**) but did not appreciably affect potency of the pyrrolidinyl compound (**15**). Similarly, in the rat, 5,6-dimethoxy analogues could be less active than the corresponding unsubstituted compound as in the pyrrolidinyl and piperidinyl series (**16**, **25**), or slightly more active, as in the aza bicyclo series (**36**) and 2-pyridyl series (**45**). Both **36** and **45** were considered to be of sufficient interest for a study in the guinea pig, in which extensions of the alkylene chain were also examined. Both anticollagen and anti-ADP activities were maximal at $n$ = 4 for the bicyclic moiety (**39**), potency falling progressively in compounds with $n$ = 5 (**40**) and $n$ = 6 (**41**). In the 2-pyridyl series both activities peaked at $n$ = 3 (**48**). With $n$ = 2, appropriate 4,6-disubstitution clearly improved activity in the pyrrolidinyl series (**18**) and piperidinyl series (**26**) and (**27**) and produced a very potent diethylamino compound (**53** and the salt **54**) as judged by

(16) Morrison, D. C. *J. Am. Chem. Soc.* **1955**, *77*, 181.
(17) Reissert, A.; Manns, E. *Chem. Ber.* **1928**, *61*, 1308.
(18) Vitali, T.; Amoretti, L.; Plazzi, V. *Farmaco, Ed. Sci.* **1968**, *23*, 1075.
(19) Tyrrell, A. W. R. *Tetrahedron Lett.* **1985**, *26*, 1753.
(20) Belgian Patent 617 384 (Knoll, A.-G.); *Chem. Abstr.* **1963**, *58*, 12579*d*.
(21) Analogous observations have been made by other groups, cf.: Theyes, M.; Lehmann, H. D.; Gries, J.; Konig, H.; Kretzschmar, R.; Kunze, J.; Lebkücher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800.

anti-ADP activity in the guinea pig ex vivo. On the other hand, 4,6-dimethyl substitution did not produce remarkable activity in the 2-pyridyl derivative **47**.

## Discussion

Interest was stimulated in this series of benzisothiazolones because of the marked capacity of some members to inhibit ADP-induced primary aggregation ex vivo. Such activity unequivocally distinguished them from the clinically used antiplatelet agents aspirin (**58**) and sulfinpyrazone (**59**) (Table I), though not necessarily from the newer agent ticlopidine (**60**). The latter has been reported to inhibit ADP-induced primary aggregation ex vivo in both rats[22] and man.[23] However, results in this laboratory have not confirmed either claim. As shown in Table I, the inhibitory activity of ticlopidine in rats was confined to collagen-induced aggregation, which contrasts with the findings of Ashida and Abiko.[22] Although the reason for this discrepancy may lie in the use of different rat strains, it may also be connected with the practice of recalcifying PRP upon addition of ADP.[22] Certainly, ticlopidine inhibited primary aggregation in the guinea pig but only in a weak manner and at a high dose (Table I). The weak effects of the three standard agents aspirin, sulfinpyrazone, and ticlopidine in rats and guinea pigs were consistent with our findings in human volunteers[24,25] though the ticlopidine results again did not accord with others in the literature.[23] Thus, ticlopidine did not actually inhibit primary aggregation but rather increased disaggregation.[25] It is important to distinguish between inhibition of aggregate formation and enhancement of aggregate dispersal for they presumably represent different modes of action. Whether or not the two can be distinguished in conventional aggregometry may depend upon methodological detail. For example, in this laboratory, platelet aggregation is measured in unusually small volumes (0.1 mL) of PRP where stirring is extremely efficient. With larger volumes and less efficient stirring, enhanced disaggregation may impinge upon net rate of aggregation (which is dependent upon stirring) and so appear as inhibition of aggregation. Far from being discouraged by our different results on ticlopidine, we inferred that the main action of this drug may be to enhance disaggregation whereas the benzisothiazolones appeared truly to inhibit aggregate formation. Taken together, the animal and human data encouraged the view that certain members of the benzisothiazolone series were both more potent than and probably different from currently available antiplatelet drugs.

Accordingly, selected compounds, notably **21**, **36** and **39**, were progressed to more detailed pharmacological evaluation. However, these studies were terminated by early, adverse toxicological findings;[26] for example, compound **36** caused dose-related, acute, superficial erosive, and hemorrhagic gastritis in rats and dogs. Hypopigmentary changes were also noted in both species, further work in the rat showing these changes to be related histochemically to decreased or absent tyrosinase activity. Such reports became available before the mechanism of action on platelets could be identified. However, it is perhaps worth recording that none of three benzisothiazolones (**21**, **36**, **39**) tested at 200 $\mu$M had any effect on cyclooxygenase activity in microsomal preparations from bovine seminal vesicles.[27] Furthermore, studies on another compound (**12**), tested at 50 $\mu$M showed no effect on human platelet levels of cyclic 3',5'-adenosine monophosphate (cyclic AMP). Nor was there any potentiation of the effect of prostaglandin $E_1$ (0.2 $\mu$M) on human platelet cyclic AMP levels. The capacity of benzisothiazolones to inhibit platelet aggregation in the absence of an effect on cyclic AMP distinguishes them from prostaglandin $E_1$ and epoprostenol.[8] Hence, further studies on benzisothiazolones may be of interest in elucidating novel mechanisms by which platelet aggregation may be inhibited.

## Experimental Section

**Chemical Methods.** Melting points were determined with a Reichert thermopan apparatus. Melting points and boiling points are uncorrected. IR and NMR spectra, which were in agreement with the structures cited, were recorded on Perkin-Elmer 257 and Perkin-Elmer Hitachi R24A (60-MHz) instruments. Mass spectra were recorded on VG 70-77, VG ZAB, and AEI MS9 instruments. GC was performed on a Pye Model 104 instrument with a 152.5-cm glass column (0.4 cm i.d.) packed with 5% XE 60 coated on 80–100 mesh gas chromatography Q support.

2,2'-Dithiobis(benzoic acids) were prepared by the method of Katz[13] and converted to the diacid chlorides[28,29,10] with $SOCl_2$ without purification.

Intermediate $\omega$-(substituted amino)alkane nitriles were prepared from alicyclic amines by the use of the following homologation reagents: glyconitriles[30] for $n = 2$ derivatives;[31] acrylonitrile for $n = 3$;[32] $\omega$-haloalkane nitriles[33] for $n = 4$–6. Hydrolysis and decarboxylation[34] of ethyl 2-cyano-4-(2'-pyridyl)butyrate[35] produced a 4-(2'-pyridyl)butyronitrile. 3-(2'-Pyridyl)propionitrile was synthesized from 2-vinylpyridine.[35] The nitriles and corresponding amines obtained by $LiAlH_4$ reduction[14b] were >95% pure by GC analysis, showed the requisite spectral properties, and were used directly in the next stage. The physical constants of new compounds are as follows [$m$, mp/bp, °C, yield, %]. For $\omega$-3-azabicyclo[3.2.2]non-3-yl-$(CH_2)_m$CN: 1, 56–57.5, 58; 2, 122–126 (0.02 mm), 98; 3, 132–134 (0.6 mm), 86; 4, 160–165 (4 mm), 58. For the corresponding amines, $\omega$-3-azabicyclo[3.2.2]non-3-yl-$(CH_2)_nNH_2$: 2, 97–101 (1 mm), 72; 3, 96–105 (0.6 mm), 86; 4, 131–132 (1.3 mm), 85; 5, 130 (0.5 mm), 90.

**6-(Chlorosulfenyl)-3,4-dimethoxybenzoyl Chloride.** To 4,4',5,5'-tetramethoxy-2,2'-dithiobis(benzoic acid) (250 g, 0.59 mol) and $C_6H_6$ (500 mL) in a 3-L flask fitted with a stirrer, thermometer, and reflux condenser was added $SOCl_2$ (1.25 L, 17.1 mol) with stirring. Copious gas evolution occurred immediately with some frothing. The mixture was heated gently until a clear solution was obtained at the boiling point, and heating under reflux continued for a further 2 h. The mixture was cooled slightly, and the solvent and excess $SOCl_2$ were evaporated off under vacuum. Benzene (ca. 200 mL) was added to the mixture and reevaporated from the residue. The dark brown solid residue was suspended in $C_6H_6$ (500 mL), $SO_2Cl_2$ (250 mL, 3.1 mol) added, and the mixture heated under reflux for 1 h; then, the solvent


(22) Ashida, S.; Abiko, Y. *Thromb. Haemostasis* **1978**, *40*, 542.
(23) Bruno, J. J. *Thromb. Res.* **1983**, Suppl IV; 59.
(24) Nunn, B.; James, F. J. *Br. J. Clin. Pharmacol.* **1980**, *9*, 239.
(25) Nunn, B.; Lindsay, R. *Thromb. Res.* **1980**, *18*, 807.
(26) Gracon, S. I.; Martin, R. A.; Barsoum, N.; Mitchell, L.; Sturgess, J. M.; de la Inglesia, F. A. *Fed. Proc.* **1982**, *41*, 2526 (Abstr).
(27) Hamberg, M.; Svensson, J.; Samuelsson, B. *Lancet* **1974**, *1*, 223.
(28) Gialdi, F.; Ponci, R.; Barruffini, A. *Farmaco, Ed. Sci.* **1961**, *16*, 509.
(29) Hoerlein, U.; Boeshagen, H.; Seuter, F. Ger. Off. 2656227 (Bayer A.-G.); *Chem. Abstr.* **1978**, *89*, 109123*f*.
(30) Gaudry, R. "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 436.
(31) Stewart, T. D.; Li, C.H. *J. Am. Chem. Soc.* **1938**, *60*, 2782.
(32) Corse, J.; Bryant, J. J.; Shante, H. A. *J. Am. Chem. Soc.* **1946**, *68*, 1911.
(33) Whitmore, F. C.; Moscher, H. S.; Adams, R. R.; Taylor, R. B.; Chapin, E. C.; Weisel, C.; Yanko, W. *J. Am. Chem. Soc.* **1944**, *66*, 725.
(34) Mills, J. U. S. Patent 2903459 (Eli Lilly Co.); *Chem. Abstr.* **1960**, *54*, 5702*a*.
(35) Boekelheide, V.; Linn,, W. J.; O'Grady, P.; Lamberg, L. *J. Am. Chem. Soc.* **1953**, *75*, 3243.

and excess reagents were removed in vacuo. The solid residue was dissolved in boiling $C_6H_6$ (1 L), treated with charcoal, and filtered hot through Celite. After washing the bed with hot $C_6H_6$ the combined filtrate and washings were reheated to give a clear solution. *n*-Hexane (ca. 500–750 mL) was added until crystallisation commenced. This mixture was allowed to stand at 5 °C overnight, and then the yellow-brown product was filtered off, washed with $C_6H_6$–*n*-hexane (1:4) and then *n*-hexane, and dried in a fan-oven at 50 °C: yield 200 g (64%); mp 149–150 °C; chlorine content 26.0% ($C_9H_8Cl_2OS$ requires 26.6%).

**Method A. 2-(3-Piperidinopropyl)-1,2-benzisothiazol-3-one (21).** Dry $Cl_2$ was passed into a suspension of 2,2′-dithiobis(benzoyl chloride) (13.72 g, 0.04 mol) in dry $CCl_4$ (150 mL) until the solid had dissolved (ca. 45 min). Excess $Cl_2$ was removed by passing dry nitrogen through the reaction mixture for 1 h. The resulting solution of *o*-(chlorothio) benzoyl chloride (0.08 mol) was filtered and added dropwise with stirring to a suspension of *N*-(3-aminopropyl)piperidine (31.73 g, 0.223 mol) in $CCl_4$ (125 mL) at 0–5 °C over 35 min. After the addition was complete, stirring was continued at room temperature for a further 3 h. The reaction mixture was transferred to a separatory funnel with $CH_2Cl_2$ (150 mL) and the suspension washed successively with 10% aqueous NaOH (1 × 100 mL), $H_2O$ (2 × 100 mL), and brine (1 × 100 mL). The organic layer was dried ($MgSO_4$) and evaporated and the residue chromatographed on alumina (Camag neutral, Brockman activity 1, 150 g, deactivated with 1% $H_2O$) with $CH_2Cl_2$–MeOH (98:2) as eluant. Crystallization of the product from IPE gave pure **21** as colorless needles: 13.7 g (62%); mp 72–73 °C.

**Method B. 5,6-Dichloro-2-(2-pyridylethyl)-1,2-benzisothiazol-3-one (44).** A mixture of 4,4′,5,5′-tetrachloro-2,2′-dithiobis(benzoic acid) (10.8 g) and $SOCl_2$ (54 mL) was boiled under reflux for 16 h, cooled, and filtered. Excess $SOCl_2$ was removed in vacuo, and the residue was dissolved in dry $C_6H_6$ (100 mL). Half of this solution (50 mL), estimated to contain ca 0.08 mol of diacid chloride, was mixed with pyridine (10 mL), and after 45 min, 2-(2-aminoethyl)pyridine (5.1 g, 0.42 mol) in $C_6H_6$ (40 mL) was added dropwise with stirring over 15 min. The suspension was stirred for a further 4 h and then left overnight. The mixture was extracted with 2 M HCl (2 × 100 mL), and the combined acid layers were made alkaline by addition of 10% NaOH solution. The precipitate was collected, washed with water, and recrystallized from EtOH, yielding **44** (1.89 g (35%)) as brown needles. A second recrystallization gave an analytically pure product as grey needles (1.33 g), mp 168–170 °C.

**Method C. 4,6-Dimethyl-2-(2-piperidinoethyl)-1,2-benzisothiazol-3-one (27).** To a stirred solution of 4,4′,6,6′-tetramethyl-2,2′-dithiobis(benzoyl chloride) (4.0 g, 0.01 mol) in dry THF (100 mL) was added a solution of *N*-(2-aminoethyl)piperidine (2.56 g, 0.02 mol) in THF (50 mL) dropwise over 10 min at room temperature. The mixture was stirred at room temperature for a further 2 h, and then the solvent removed under vacuum. The residue was dissolved in $CH_2Cl_2$ (50 mL) and the resultant mixture stirred at 30–40 °C. To this solution was added $SOCl_2$ (10 mL, 0.13 mol) in $CH_2Cl_2$ (50 mL) dropwise over 1 h and the resulting mixture stirred at 30–40 °C overnight. Solvent and excess $SOCl_2$ were removed under vacuum, and the residue was dissolved in $H_2O$ (150 mL). The solution was filtered and extracted with $Et_2O$ (2 × 50 mL) and the aqueous phase basified with 10% aqueous NaOH solution. The mixture was extracted with $Et_2O$ (3 × 50 mL), and the combined extracts were washed with water (2 × 50 mL) and brine (1 × 50 mL) and dried ($MgSO_4$). Evaporation of the solvent gave an oil that was purified by column chromatography on neutral alumina (80 g) as in method A. Elution with $CH_2Cl_2$–MeOH (99:1) gave **27** (1.85 g (32%)) as a yellow oil.

**Method D. 4,6-Dimethoxy-2-(2-piperidinoethyl)-1,2-benzisothiazol-3-one (26).** To 4,4′,6,6′-tetramethoxy-2,2′-dithiobis(benzoic acid) (3.0 g, 0.07 mol) was added a solution of $SOCl_2$ (1.8 g, 0.15 mol) in dry benzene (25 mL), and the mixture was boiled under reflux for 2.5 h. The solution was cooled and filtered, and the filtrate was evaporated to dryness under reduced pressure; last traces of thionyl chloride were removed by coevaporation with benzene. The residual diacid chloride was dissolved in THF (50 mL), and a solution of *N*-(2-aminoethyl)piperidine (2.40 g, 0.019 mol) in THF (25 mL) was added dropwise over 10–15 min at room temperature. The mixture was stirred for a further 3 h, and the solvent was removed under vacuum. The residue was suspended in $CH_2Cl_2$ (150 mL), and the mixture was shaken with 40% w/v aqueous sodium hydrogen sulfite solution (50 mL) for 5 min. The crude Bunte salt (2.25 g) so obtained ($\nu_{max}$ 1010 $cm^{-1}$; for structure, see ref 19) was collected and suspended in water (30 mL), and 10% aqueous NaOH solution was added. Ether (75 mL) was added and the mixture was stirred for 15-20 min. The ether layer was separated, washed with brine (150 mL), dried, and evaporated. The residue was chromatographed on alumina (150 g) with $CH_2Cl_2$, followed by $CH_2Cl_2$–MeOH (98:2), as eluant. Fractions containing the pure major product were pooled and evaporated, and the residue was recrystallized from IPE–light petroleum ether (bp 60–80 °C), yielding **26** (1.0 g, 22%) as colorless needles. A second recrystallization gave analytically pure material, mp 72–75 °C.

**Method E. 2-[(3-Azabicyclo[3.2.2]non-3-yl)ethyl]-5,6-dichloro-1,2-benzisothiazol-3-one (35).** 5,6-Dichloro-2-(2-hydroxyethyl)-1,2-benzisothiazol-3-one (3.0 g, 0.11 mol), prepared in the same way as compound **10** and having a melting point of 204–205 °C [Anal. ($C_9H_7Cl_2NO_2S$) C, H, N, Cl, S] was dissolved in pyridine (25 mL). Toluene-*p*-sulfonyl chloride (4.35 g, 0.23 mol) was added, and after 24 h the mixture was added to ice and water (800 mL) with stirring. The precipitate was collected, washed with water, and dried in vacuo, yielding the crude *tosylate* (4.18 g (88%)) as a pale yellow solid. A sample recrystallized from ethanol for analysis had a melting point of 204–206 °C [Anal. ($C_{16}H_{13}Cl_2NO_4S_2$) C, H, N, Cl, S]. The crude tosylate (3.0 g, 0.072 mol) and 3-azabicyclo[3.2.2]nonane (1.80 g, 0.143 mol) were boiled in toluene (60 mL) for 2 h under reflux. The cooled solution was extracted with 2 M HCl (2 × 100 mL), and the combined acid layers were made alkaline with 10% NaOH. The mixture was extracted with $CH_2Cl_2$, and the organic layer was dried ($MgSO_4$) and evaporated under vacuum. Recrystallization of the residue from EtOH gave pure **35** as colorless needles: 1.61 g (60.5%); mp 160–162 °C.

**Biological Methods. Determination of the Inhibition of Platelet Aggregation in Vitro.** Human blood was citrated and centrifuged to prepare platelet-rich plasma (PRP) and the platelet count adjusted to 350 000/μL with autologous platelet-poor plasma as previously described.[36] Platelet aggregation was measured photometrically[2] in PRP stirred at 1100 rpm in aggregometers (Albert Browne Ltd.) coupled to Vitatron pen recorders. Aliquots (90 μL) of PRP were warmed to 37 °C with 10 μL of 154 mM NaCl (control) or compound for exactly 3 min before addition of aggregating agent. Water-insoluble compounds were dissolved in dimethylformamide (DMF) and the resultant solutions added to PRP in a volume of 0.5 μL. DMF was included in the control samples of PRP where appropriate.

Responses to collagen[36] were quantified by measuring the maximum increase in light transmission. For the study of inhibitors, a concentration of collagen producing a just-maximal response (typically 0.7–1.4 μg/mL) was selected for each PRP sample, and the concentration of each compound producing 50% inhibition ($IC_{50}$) determined.[36] ADP-induced aggregation is biphasic, and only the first phase was of interest here. For the study of inhibitors, a concentration of ADP producing a clearly discernible first-phase response (typically 1 μM ADP) was selected for each preparation. Such first-phase aggregation always occurred within 1 min and was quantified by measuring the maximum increase in light transmission that occurred within the first minute of adding ADP. The $IC_{50}$ for each compound was determined as before.[36]

**Determination of the Inhibition of Platelet Aggregation ex Vivo.** Groups ($n$ = 5–10) of male rats (≃200 g) or guinea pigs (≃300 g) were fasted overnight and then orally dosed (5 mL/kg) with 1% (w/v) methylcellulose alone (control) or containing the compound under test. Exactly 2 h later, each animal was placed in a chamber of $CO_2$ until respiration ceased. The abdomen was rapidly opened, and 4.5 mL of blood was drawn from the inferior vena cava into a syringe containing 0.5 mL of trisodium citrate (102 mM for rats, 129 mM for guinea pigs). Each blood sample was centrifuged at 450*g* for 5–7 min at 20 °C to prepare PRP and the platelet count determined on a Thrombocounter C (Coulter Electronics Ltd., Harpenden, Herts, U.K.). Platelet count was

(36) Nunn, B. *Thromb. Haemostasis* **1981**, *45*, 1.

adjusted to 800 000/μL (rat) or 500 000/μL (guinea pig) with autologous platelet-poor plasma. Aggregation in response to collagen was measured as already described.[36] For each PRP sample, responses to collagen were obtained such that one response was less than and one response was greater than half-maximal (50 mm). The difference in the two collagen concentrations to achieve this was always 1.4–2-fold. Responses (mm) were plotted against log concentration of collagen, and the concentration required to induce a response of 50 mm ($EC_{50}$) was read by interpolation for each PRP sample. Typical control $EC_{50}$ values were 5 and 2.5 μg/mL in rat and guinea pig PRP, respectively. ADP-induced aggregation in guinea pig citrated PRP is biphasic. As only the first-phase or primary aggregation was of interest here, primary aggregation was quantified by measuring the maximum increase in light transmission that occurred within the first minute of adding ADP. It was found that the maximum increase at or within 1 min was about 50 mm. Hence, for each PRP preparation, responses to ADP were obtained such that one response was less than and one response was greater than half-maximal (25 mm). The difference in the two ADP concentrations to achieve this was always 2-fold. Responses (mm) were plotted against the log concentration of ADP, and the concentration required to induce a response of 25 mm ($EC_{25}$) was read by interpolation for each PRP sample. ADP-induced aggregation in rat PRP is monophasic and so could safely be quantified as the maximum increase in light transmission. However, for the sake of uniformity, $EC_{25}$ (rather than $EC_{50}$) values for ADP were obtained in rat PRP as described for the guinea pig. Typical control $EC_{25}$ values were 0.5 and 0.3 μM for rat and guinea pig PRP, respectively.

**Calculation of Effect of Compounds ex Vivo.** The effect of each compound on responsiveness to each aggregating agent was expressed as a dose ratio calculated by dividing the mean $EC_{50}$(collagen) or $EC_{25}$(ADP) in the PRP samples from the treated animals by the corresponding value in the PRP samples from the control animals tested essentially at the same time. Thus, inactive compounds had dose ratios of 1.0, and the greater the activity of a compound, the greater the dose ratio. The maximum concentrations of aggregating agents tested were 50 μg/mL (collagen) an 8 μM (ADP). If aggregation could not be induced in a given sample of PRP, it was assigned an $EC_{50}$ of 50 μg/mL or an $EC_{25}$ of 8 μM for the purposes of statistical comparison of groups by Student's t-test. On some occasions, a preliminary assessment of the activity of a compound was made by pooling equal volumes of PRP from each treated animal to give one PRP sample for the group ($n = 5$). The $EC_{50}$ or $EC_{25}$ was determined as described for the individual samples and compared with the corresponding values obtained in the PRP sample pooled from the control animals.

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**Registry No.** **1** ($R_2$, $R_3$, $R_4$ = H), 19602-82-5; **1** ($R_2$ = H; $R_3$, $R_4$ = Cl), 82735-37-3; **1** ($R_2$, $R_4$ = Cl; $R_3$ = H), 97655-90-8; **1** ($R_2$, $R_4$ = $CH_3O$; $R_3$ = H), 97655-93-1; **1** ($R_2$ = H; $R_3$, $R_4$ = $CH_3$), 82735-39-5; **2** ($R_2$, $R_3$, $R_4$ = H), 3950-02-5; **2** ($R_2$ = H; $R_3$, $R_4$ = $CH_3O$), 97655-98-6; **2** ($R_2$ = H; $R_3$, $R_4$ = Cl), 97655-99-7; **3** ($n$ = 3; $R_1$ = 1-piperidinyl), 3529-08-6; **3** ($n$ = 2; $R_1$ = 2-pyridyl), 2706-56-1; **3** ($n$ = 2; $R_1$ = 1-piperidinyl), 27578-60-5; **3** ($n$ = 0; $R_1$ = 2-pyridyl), 504-29-0; **3** ($n$ = 4; $R_1$ = $CH_3$), 110-58-7; **3** ($n$ = 2; $R_1$ = OH), 141-43-5; **3** ($n$ = 2; $R_1$ = 1-pyrrolidinyl), 7154-73-6; **3** ($n$ = 3; $R_1$ = 1-pyrrolidinyl), 23159-07-1; **3** ($n$ = 4; $R_1$ = 1-pyrrolidinyl), 24715-90-0; **3** ($n$ = 4; $R_1$ = 1-piperidinyl), 74247-30-6; **3** ($n$ = 5; $R_1$ = 1-piperidinyl), 70403-69-9; **3** ($n$ = 3; $R_1$ = 3-azabicyclo[3.2.2]non-2-yl), 3437-28-3; **3** ($n$ = 4; $R_1$ = 3-azabicyclo-[3.2.2]non-3-yl), 97655-96-4; **3** ($n$ = 5; $R_1$ = 3-azabicyclo[3.2.2]-non-3-yl), 97655-97-5; **3** ($n$ = 3; $R_1$ = 2-pyridyl), 15583-16-1; **3** ($n$ = 4; $R_1$ = 2-pyridyl), 34974-00-0; **3** ($n$ = 2; $R_1$ = 4-morpholinyl), 2038-03-1; **3** ($n$ = 3; $R_1$ = 4-morpholinyl), 123-00-2; **3** ($n$ = 4; $R_1$ = 4-morpholinyl), 6321-07-9; **3** ($n$ = 2; $R_1$ = 3-azabicyclo[3.2.2]-non-3-yl), 1199-72-0; **4** ($n$ = 2; $R_1$ = OH; $R_2$ = H; $R_3$, $R_4$ = Cl), 97655-95-3; **4** ($n$ = 2; $R_1$ = tosyloxy; $R_2$ = H; $R_3$, $R_4$ = Cl), 82735-38-4; **4** ($n$ = 2; $R_1$ = tosyloxy; $R_2$ = H; $R_3$, $R_4$ = $CH_3O$), 97656-04-7; **5** ($n$ = 2; $R_1$ = 1-piperidinyl; $R_2$, $R_4$ = $CH_3$; $CH_3$; $R_3$ = H), 97655-91-9; **5** ($n$ = 2; $R_1$ = 1-piperidinyl; $R_2$, $R_4$ = $CH_3O$; $R_3$ = H), 97655-94-2; **5** ($n$ = 2; $R_1$ = 1-pyrrolidinyl; $R_2$, $R_4$ = $CH_3$; $R_3$ = H), 97656-00-3; **5** ($n$ = 3; $R_1$ = 1-piperidinyl; $R_2$, $R_4$ = $CH_3$; $R_3$ = H), 97656-01-4; **5** ($n$ = 4; $R_1$ = 3-azabicyclo[3.2.2]non-3-yl; $R_2$, $R_4$ = $CH_3$; $R_3$ = H), 97656-02-5; **5** ($n$ = 2; $R_1$ = 2-pyridyl; $R_2$, $R_4$ = $CH_3$; $R_3$ = H), 97673-93-3; **5** ($n$ = 2; $R_1$ = $(C_2H_5)_2N$; $R_2$, $R_4$ = $CH_3$; $R_3$ = H), 64324-77-2; **5** ($n$ = 2; $R_1$ = 2-pyridyl; $R_2$, $R_4$ = $CH_3O$; $R_3$ = H), 97656-03-6; **6**, 2634-33-5; **7**, 2527-03-9; **8**, 4322-83-2; **9**, 4299-08-5; **10**, 4299-09-6; **10** (tosylate), 49549-96-4; **11**, 67388-03-8; **12**, 69577-10-2; **13**, 97655-57-7; **14**, 97655-58-8; **15**, 64016-19-9; **16**, 97655-59-9; **17**, 64016-06-4; **18**, 97655-60-2; **19**, 97655-61-3; **20**, 69577-09-9; **21**, 70316-73-3; **22**, 97655-62-4; **23**, 97655-63-5; **24**, 97655-64-6; **25**, 97655-65-7; **26**, 97655-66-8; **27**, 97655-67-9; **28**, 97655-68-0; **29**, 97655-69-1; **30**, 97655-70-4; **31**, 97655-71-5; **32**, 97655-72-6; **33**, 97655-73-7; **34**, 97655-74-8; **35**, 64016-04-2; **36**, 71998-53-3; **37**, 97655-75-9; **38**, 97655-76-0; **39**, 71998-54-4; **40**, 97655-77-1; **41**, 97655-78-2; **42**, 97655-79-3; **43**, 97655-80-6; **44**, 64016-02-0; **45**, 64015-95-8; **46**, 97655-81-7; **47**, 97655-82-8; **48**, 97655-83-9; **49**, 97655-84-0; **50**, 21309-67-1; **51**, 67388-06-1; **52**, 64324-47-6; **53**, 97655-85-1; **54**, 97655-86-2; **55**, 97655-87-3; **56**, 4367-50-4; **57**, 97655-88-4; $C_6H_5NHCH_3$, 100-61-8; $C_6H_5NH_2$, 62-53-3; $NH_3$, 7664-41-7; $(C_2H_5)_2NH$, 109-89-7; 4,4′,5,5′-tetrachloro-2,2′-dithiobis(benzoic acid), 97655-89-5; 4,4′,6,6′-tetramethoxy-2,2′-dithiobis(benzoic acid), 97655-92-0; 4,4′,5,5′-tetramethoxy-2,2′-dithiobis(benzoic acid), 97656-05-8; 3-azabicyclo[3.2.2]nonane, 283-24-9.