[^{3}H]noradrenaline and [^{14}C]serotonin in slices of the mouse brain was determined as described previously.[7] The brain tissue used (40 mg) was taken from the middle part of the mouse brain and includes hypothalamus, thalamus, and midbrain. The slices (about 1-mm thick) were preincubated for 5 min at 37 °C in 2.0 mL of Krebs–Heuseleit's buffer containing 1×10^{-4} M pargyline (this concentration does not inhibit the accumulation of [^{3}H]NA or [^{14}C]5-HT at the experimental conditions used), 5.6 mM glucose, 1.1 mM ascorbic acid, and 1.3×10^{-4} M Na_2EDTA in an atmosphere of 6.5% CO_2 in O_2. After addition of the labeled substrates (1×10^{-7} M [^{3}H]NA and 1×10^{-7} M [^{14}C]5-HT), the incubation was continued for 5 min. The slices were rapidly removed from the incubation bottles, blotted on filter paper, and transferred to counting vials, in which they were dissolved in 1.0 mL of Soluene-350 (Packard) containing 5% distilled water. Ten milliliters of scintillation liquid (Econofluor, NEN) was added and ^{3}H and ^{14}C were determined by the double-labeling technique in a Packard TriCarb scintillation spectrometer. The active accumulation of the amines was determined from the difference between those in the absence and presence of 5×10^{-4} M cocaine. The inhibition of the accumulation was calculated as a percentage of the control accumulation. Four or five of the inhibitors were determined in quadruplicate. The IC_{50} values were determined from semilogarithmic plots.

The in vivo inhibition of the amine accumulation was determined with the same technique as described above. The compounds were injected intraperitoneally, and the animals were sacrificed 60 min later. Brain slices were prepared and incubated with the labeled amines. Three or four different doses were examined with four mice in each dose. ED_{50} values were estimated from semilogarithmic plots.

Acknowledgment. We thank Hans Thorin for MS work, Eivor Eklund and Margareta Johansson for running UV spectra and developing HPLC systems, and Dr. Brian Pring for linguistic advice.

Synthesis and Platelet Aggregation Inhibitory Activity of 4,5-Bis(aryl)-2-substituted-thiazoles

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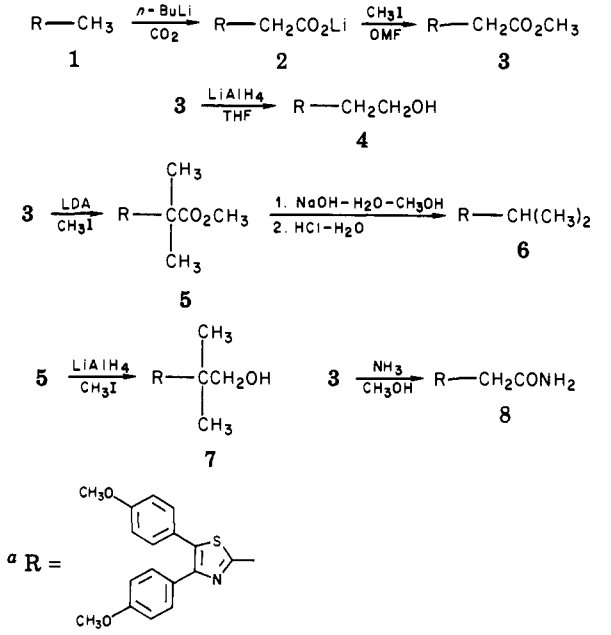
In our continuing effort to discover compounds which inhibit collagen-induced platelet aggregation, we have screened compounds in a mouse pulmonary thromboembolism screen. Methyl 4,5-bis(4-methoxyphenyl)-2-thiazoleacetate (3) was very active in the above screen. However, 3 was active for less than 5 min when given orally to guinea pigs. As a result, our synthetic goal was to prepare 2-substituted thiazoles with a much longer duration of activity. This paper describes the preparation of a number 4,5-bis(aryl)-2-substituted-thiazoles and their in vitro and ex vivo activity against collagen-induced platelet aggregation. It was determined that 4,5-bis(4-methoxyphenyl)-2-(trifluoromethyl)thiazole (16) is the most promising compound.

Many drugs are known that inhibit platelet function. However, in the past all such drugs were first used for other indications, for example, inflammation and gout (aspirin,[1] dipyridamole,[2] flurbiprofen,[3] and Motrin[4]). While these compounds do inhibit platelet aggregation, their antiplatelet potency is low. Understandably, attempts to demonstrate antithrombotic application of these agents in clinical trials have not met with uniform success.

For successful medical use, potential antithrombotic drugs must inhibit the interactions of platelets with one or more in vivo stimuli which promote platelet thrombi formation. Various mechanisms for thrombi formation have been postulated,[5] one of which is the exposure of collagen or collagen-like substances upon injury to the endothelium. Thus, one reasonable approach to antithrombotic therapy is the use of a potent, selective inhibitor of collagen-induced platelet aggregation.

With the availability of a high-volume mouse pulmonary thromboembolism screen,[6] which detects inhibitors of collagen-induced platelet aggregation, we discovered a novel class of compounds, the thiazoles described in this report. Compound 3 was the first of this series to show activity. However, when tested in the guinea pig using a modified ex vivo assay,[7] compound 3 exhibited a duration of antiplatelet activity of less than 5 min. Upon further investigation, it was shown that under acidic conditions 3 hydrolyzes and readily decarboxylates to give compound 1. Our synthetic objective, therefore, was to prepare 2-substituted thiazoles which would have greater stability and longer duration of activity in vivo. Structural mod-

Scheme I[a]

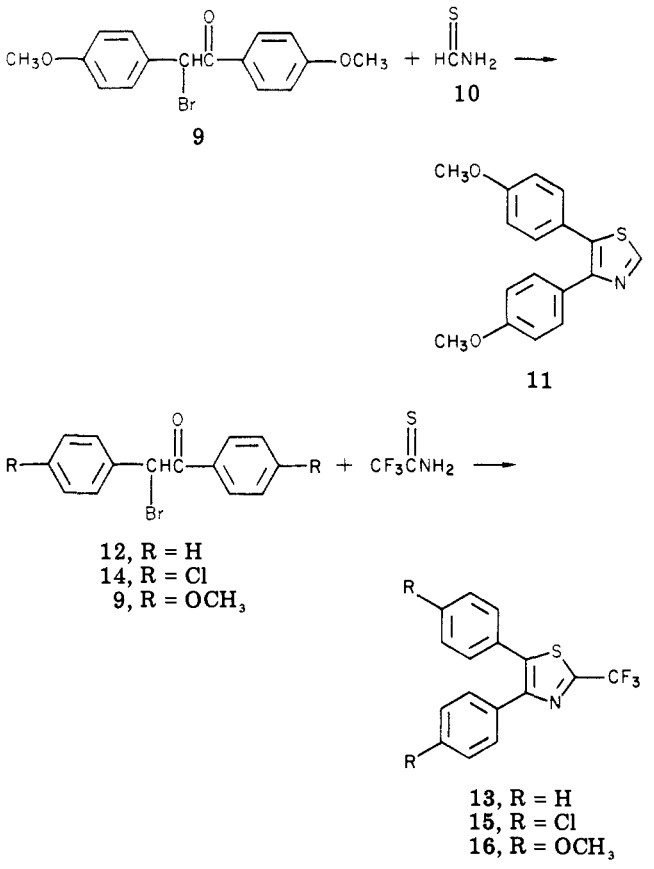


* Deceased June 29, 1981. Address correspondence to The Upjohn Co., Diabetes and Atherosclerosis Research, Kalamazoo, MI 49001.

(1) H. J. Weiss, L. M. Aledort, and S. Kochwa, *J. Clin. Invest.*, **47**, 2169 (1968).
(2) M. P. Cucuianu, E. E. Nishizawa, and J. F. Mustard, *J. Lab. Clin. Med.*, **77**, 958 (1971).

0022-2623/81/1824-1507$01.25/0

Scheme II



ifications of the original active compound (compound 3) led to many active analogues, one of which (compound 16) is of particular interest. Detailed biological studies of compound 16 will be reported elsewhere.[8]

The preparation of compound 3 is shown in Scheme I. Compound 1[9] was treated with *n*-butyllithium, followed by CO_2, to afford the lithium salt (2). Treatment of 2 with methyliodide in DMF afforded 3. Reduction of 3 with lithium tetrahydridoaluminate afforded the alcohol 4. Compound 5 was prepared by treatment of 3 with lithium diisopropyl amide and methyl iodide. Treatment of 5 with aqueous sodium hydroxide, followed by aqueous HCl, afforded the decarboxylated compound 6. Reduction of 5 afforded the alcohol 7. Treatment of 3 with ammonia in methanol afforded the amide 8.

Scheme II shows the preparation of additional thiazoles. Compound 9[10] was converted into 11 by treatment of 9 with thioformamide (10).[11] Compounds 13, 15, and 16 were respectively prepared by the reaction of trifluoromethylthioacetamide[12] with compounds 12[13] 14, and 9.[10]

Table I. Concentrations of Compounds Required to Inhibit Collagen-Induced Platelet Aggregation (in Vitro) by 50% (IC_{50}) in Human and Guinea Pig PRP

compd	IC_{50} human	IC_{50} guinea pig
1	0.32 ng/mL	NT[a]
2	32 ng/mL	NT
3	0.1 ng/mL	NT
4	0.32 ng/mL	3.2 ng/mL
5	3.2 ng/mL	10 ng/mL
6	3.2 ng/mL	10 ng/mL
7	3.2 ng/mL	10 ng/mL
8	3.2 ng/mL	NT
11	32 ng/mL	NT
13	0.32 μg/mL	NT
15	32 ng/mL	NT
16	1.0 ng/mL	10 ng/mL
aspirin	10 μg/mL	
flurbiprofen	0.32 μg/mL	0.32 μg/mL

[a] NT = not tested.

Table II. Percent Inhibition of Platelet Aggregation following Oral Administration of Thiazoles to Guinea Pigs[a] ($n = 5$)

	% inhibn				
no. dose:	10 mg/kg	5 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
4	100[b]	79[b]	9	NT[c]	NT[c]
5	100[b]	100[b]	27	NT	NT
6	100[b]	85[b]	6	NT	NT
8	100[b]	96[b]	39[b]	NT	NT
16	100[b]	100[b]	83[b]	68.5[b]	6

[a] Animals were bled 1 h after dosing. [b] $p < 0.0005$. [c] NT = not tested.

Table III. Duration of Platelet Inhibitory Activity of Compound 16 When Administered Orally to Guinea Pigs at 5 mg/kg ($n = 5$)

time, h, after po dose	% inhibn	p
0	0	
2	97.0	<0.0005
4	68.5	<0.0005
8	93.2	<0.0005
12	48.7	0.001
16	16.2	0.205
24	10.3	0.416

Biological Results

The thiazoles were tested for inhibition of in vitro collagen-induced platelet aggregation, using human platelet-rich plasma (PRP; see Experimental Section for the method). The results are tabulated in Table I. Evaluation of these data in Table I shows that all of the thiazoles are very active in inhibiting collagen-induced human platelet aggregation. Compound 3, the most active, was excluded from additional studies because of its instability and very short duration of in vivo activity.

The remaining thiazoles were evaluated in the guinea pig ex vivo modified assay (see Experimental Section for method). The results are tabulated in Table II. Evaluation of the data in Table II shows that compound 16 is the most active of the thiazoles tested. As a result, compound 16 was tested for its duration of activity (see Table III) in the guinea pig. Examination of the data in Table III shows

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(4) E. E. Nishizawa and D. J. Wynalda, *Thromb. Res.*, 21, 347 (1981).
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(8) E. E. Nishizawa et al., manuscripts in preparation.
(9) U.S. Patent 3560514.
(10) J. Cymerman-Craig, K. V. Martin, and P. C. Wailes, *Aust. J. Chem.*, 8, 385 (1955).
(11) L. R. Cerecdo and J. G. Tolpin, *J. Am. Chem. Soc.*, 59, 1660 (1937).
(12) W. L. Reilly and H. C. Brown, *J. Am. Chem. Soc.*, 78, 6032 (1956).
(13) Aldrich Chemical Co., Milwaukee, WI.

that compound 16 maintained good activity for 12 h after dosing.

Compound 16 was inactive in a wide variety of other biological screens. Suprisingly, although compound 16 had antiinflammatory activity, as reflected by inhibition of carrageenan-induced edema in rats,[14] its potency was only about that of aspirin. In summary, we have discovered that a number of thiazoles are potent inhibitors of collagen-induced platelet aggregation. Compound 16 [4,5-bis(4-methoxyphenyl)-2-(trifluoromethyl)thiazole] appears to be the best of the thiazoles tested.

Experimental Section

All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The structures of all compounds were supported by IR, NMR, and mass spectra. IR spectra were obtained on a Perkin-Elmer Model 421 recording spectrometer in Nujol mulls, the NMR spectra were recorded on a Varian A-60D spectrometer, and the mass spectra were determined on an Atlas CH-4 spectrometer. All elemental analyses were within ±0.4%.

Lithium 4,5-Bis(4-methoxyphenyl)-2-thiazoleacetate (2). A solution of 1.6 M *n*-butyllithium in hexane (26.6 mL) was added by syringe over a 10-min period to a solution of 4,5-bis(4-methoxyphenyl)-2-methylthiazole (1,[9] 11.0 g, 0.0354 mol) in THF (275 mL), which was cooled by a bath of dry ice in 2-propanol. After stirring for 10 min, the reaction mixture was slowly poured with stirring into a slush of powdered CO_2 (500 g) in THF (150 mL). The mixture was then allowed to reach ambient temperature and then concentrated to dryness and dried in a vacuum oven. This material was dissolved in boiling acetone (500 mL), filtered through a fine sintered glass funnel to remove a haze, and cooled to afford 10.2 g (80%) of 2: mp >250 °C. Anal. ($C_{19}H_{16}NO_4SLi$) C, H, N, S, Li.

Methyl 4,5-Bis(4-methoxyphenyl)-2-thiazoleacetate (3). A solution of 2 (8.82 g, 0.024 mol), CH_3I (17.4 g, 0.122 mol), and DMF (100 mL) was stirred at ambient temperature for 16 h. The mixture was poured into H_2O (1.5 L) and extracted with CH_2Cl_2 (500 mL). The organic layer was washed with H_2O (3 × 1.5 L), saturated $NaHCO_3$ (1 L), and H_2O (1.5 L), then dried (Na_2SO_4), and concentrated with reduced pressure. The oily residue was dissolved in acetone (50 ml) and chromatographed on a column packed with Florisil eluting with 5% acetone in hexane. The product was recrystallized from Et_2O–pentane to afford 3.32 g (37%) of 3: mp 79–81 °C. Anal. ($C_{20}H_{19}NO_4S$) C, H, N, S.

4,5-Bis(4-methoxyphenyl)-2-thiazoleethanol (4). A solution of 3 (2.50 g, 6.80 mmol) in THF (10 mL) was added dropwise to a slurry of $LiAlH_4$ (0.55 g, 14 mmol) in THF (20 mL) at 0–5 °C. After the addition was complete, the mixture was stirred for 1.25 h at ambient temperature. The reaction was then treated dropwise with H_2O (2 mL) and then concentrated on a rotary evaporator. The residue was slurried with H_2O (20 mL), taken to pH 4 with 6 N H_2SO_4, and extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with H_2O (50 mL), dried (Na_2SO_4), and concentrated by rotary evaporation. The crude mixture was chomatographed (HPLC) with 50% ethyl acetate in benzene through silica gel and then recrystallized from Et_2O–pentane to afford 0.82 g (35%) of 4: mp 101–103 °C. Anal. ($C_{19}H_{19}NO_3S$) C, H, N, S.

Methyl 4,5-Bis(4-methoxyphenyl)-2,2-dimethyl-2-thiazoleacetate (5). A solution of 1.6 M *n*-butyllithium in hexane (10.6 mL, 17.0 mmol) was added dropwise over 30 min to a solution of diisopropylamine (1.48 g, 17.0 mmol) in THF (15 mL), which was cooled at 0–5 °C. Stirring was continued for an additional 15 min after the addition was completed. The temperature of the mixture was cooled to −78 °C with a dry ice–acetone bath. Compound 3 (5.00 g, 13.6 mmol) dissolved in THF (15 mL) was added by syringe over a 1-h period while the temperature of the reaction mixture was maintained below −60 °C. After the mixture was stirred for an additional 45 min, methyl iodide (3.83 g, 27.0 mmol) was added over a 5-min period. The mixture was stirred at −60 °C for an additional 15 min after the addition was completed and then allowed to warm to ambient temperature. The reaction mixture was poured into H_2O (500 mL) and extracted with CH_2Cl_2 (2 × 250 mL). The combined extracts were washed with H_2O (2 × 500 mL), 1 N HCl (2 × 250 mL), and H_2O (500 mL) and dried (Na_2SO_4). The solvent was removed by rotary evaporation to afford 4.74 g of a solid. This material was dissolved in absolute ethanol (100 mL), treated with Darco, and filtered, and the ethanol was removed by rotary evaporation. The residue was chromatographed on silica gel eluting with 10% ethyl acetate in benzene. The fractions which contained the product were combined, and the solvent was evaporated to afford 2.69 g (50%) of 5 as an oil. Anal. ($C_{22}H_{23}NO_4S$) C, H, N, S.

4,5-Bis(4-methoxyphenyl)-2-isopropylthiazole (6). A solution of 5 (0.70 g, 1.8 mmol), 6 N NaOH (1.5 mL), and CH_3OH (15 mL) was stirred at ambient temperature for 16 h. The CH_3OH was removed in vacuo, and the residue was slurried in H_2O (30 mL) and adjusted to pH 2 with concentrated HCl. Decarboxylation occurred while the mixture was stirred at ambient temperature for 1.75 h. The mixture was extracted with CH_2Cl_2 (50 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo to afford an oil, which solidified on standing. This was recrystallized from pentane to afford 0.19 g (32%) of 6: mp 62–64 °C. Anal. ($C_{20}H_{21}NO_2S$) C, H, N, S.

4,5-Bis(4-methoxyphenyl)-β,β-dimethyl-2-thiazoleethanol (7). A solution of 5 (0.81 g, 2.0 mmol) in THF (5 mL) was added dropwise to a slurry of $LiAlH_4$ (0.16 g, 4.2 mmol) in THF (10 mL) maintained at 0–5 °C. The mixture was then stirred for 1 h at ambient temperature and then cooled to 0–5 °C during the dropwise addition of H_2O (0.5 mL). The solvent was removed in vacuo, and the residue was slurried with H_2O (30 mL), acidified to pH 3 with 6 N H_2SO_4, and then extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with H_2O (50 mL) and dried (Na_2SO_4), and the solvent was removed to afford 0.41 g of crude product. This was recrystallized from Et_2O–pentane to afford 0.19 g (26%) of 7: mp 102–104 °C. Anal. ($C_{21}H_{23}NO_3S$) C, H, N, S.

4,5-Bis(4-methoxyphenyl)-2-thiazoleacetamide (8). A three-neck flask containing a solution of 3 (0.50 g, 1.3 mmol) and MeOH (50 mL) and fitted with a gas frit and a dry ice condenser was cooled to 0–5 °C. Ammonia was bubbled into the solution for 15 min. After 2 h of maintaining the ice–water bath and condenser, the entire appartus was wrapped in towels and aluminum foil and allowed to stand for 18 h. The resulting solution was concentrated in vacuo. The residue was dissolved in MeOH (25 mL), treated with Darco, and concentrated in vacuo. This material was chromatographed through silica gel with 5% 2-propanol in CH_2Cl_2. Recrystallization from Et_2O–pentane afforded 0.06 g (13%) of 8: mp 124–127 °C. Anal. ($C_{19}H_{18}N_2O_3S$) C, H, N, S.

2-Bromo-1,2-bis(4-methoxyphenyl)ethanone (9). This compound was prepared according to the method of Cymerman-Craig and co-workers.[10]

Thioformamide (10). Following the work of Cerecedo and Tolpin,[11] formamide (20.0 g, 0.444 mol) was covered with Et_2O (200 mL) and freshly powdered P_2S_5 (12.0 g, 0.054 mol) was added in several portions with ice–water bath cooling. The flask was refrigerated at 5–10 °C for 72 h and then allowed to warm to ambient temperature. After being on a shaker apparatus at ambient temperature for 16 h, the ethereal solution of thioformamide was used "as is" in the preparation of 11.

4,5-Bis(4-methoxyphenyl)thiazole (11). An ethereal solution of thioformamide (70 mL, 0.153 mol) was concentrated in vacuo at less than 25 °C. The residue was slurried with acetonitrile (10 mL) and cooled in an ice–water bath. Compound 9 (4.27 g, 12.8 mmol) was dissolved in acetonitrile (50 mL) and added via syringe to the above slurry. After stirring for 15 min at 0–5 °C, the reaction was refrigerated at 4 °C for 16 h. The solution was then stirred at ambient temperature for 70 h. The solvent was removed in vacuo and the residue was partitioned with Et_2O (100 mL) and saturated $NaHCO_3$ (100 mL). The Et_2O layer was washed with saturated $NaHCO_3$ (50 mL), H_2O (50 mL), and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chomatographed through silica gel with 5% ethyl acetate in benzene as the eluant. The product was recrystallized from hexane to afford 2.25 g (59%) of 11: mp 98.5–101.5 °C. Anal. (C_{17}-

(14) Method of C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).

$H_{15}NO_2S$) C, H, N, S.

4,5-Diphenyl-2-(trifluoromethyl)thiazole (13). 2-Bromo-1,2-diphenylethanone[13] (12; 10.0 g, 36.0 mmol), trifluoromethylthioacetamide[12] (5.57 g, 43.0 mmol), and acetonitrile (185 mL) were combined and heated at reflux for 20 h. The solution was concentrated in vacuo, and the residue was partitioned with Et_2O (100 mL) and saturated $NaHCO_3$ (100 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL), H_2O (50 mL), and brine (50 mL), and the dried (Na_2SO_4) solution was concentrated in vacuo. The residue was triturated with hexane (100 mL), which was decanted, and the solvent was removed in vacuo to afford a solid residue. This was chromatographed on silica gel with 10% ethyl acetate in Skellysolve B.[15] The fractions which contained the desired product were combined, and the solvent was removed in vacuo to afford a solid. The solid was recrystallized from pentane to afford 3.07 g (28%) of 13: mp 94–97 °C. Anal. ($C_{16}H_{10}F_3NS$) C, H, F, N, S.

2-Bromo-2,3-bis(4-chlorophenyl)ethanone (14). Bromine (2 mL) was added dropwise via syringe to a refluxing solution of 1,2-bis(4-chlorophenyl)ethanone[16] (10 g, 38 mmol), $CHCl_3$ (50 mL), and Et_2O (25 mL). The mixture was heated at reflux for 0.5 h after the addition was complete. After cooling, the mixture was washed with saturated $NaHCO_3$ (2 × 100 mL), dried (Na_2SO_4), and concentrated in vacuo to afford an oil, which crystallized from hexane to afford 10.2 g (78%) of 14: mp 83–84 °C. Anal. ($C_{14}H_9BrCl_2O$) C, H, Br, Cl.

4,5-Bis(4-chlorophenyl)-2-(trifluoromethyl)thiazole (15). This compound was prepared in the same manner as was compound 13, except that compound 14 was substituted for compound 12: mp 101–103 °C. Anal. ($C_{16}H_8Cl_2F_3NS$) C, H, Cl, F, N, S.

4,5-Bis(4-methoxyphenyl)-2-(trifluoromethyl)thiazole (16). Method 1. Compound 16 was prepared in the same manner as was compound 13, except that compound 9 was substituted for compound 12: mp 51–54 °C. Anal. ($C_{19}H_{14}F_3NO_2S$) C, H, F, N, S.

Method 2. Trifluoromethylthioacetamide was prepared in situ[17] by heating a mixture of trifluoromethylacetamide (5.0 g, 44 mmol), freshly pulverized P_2S_5 (1.95 g, 8.80 mmol), and benzene (10 mL) at reflux for 96 h. Compound 9 (7.00 g, 20.8 mmol) was added portionwise over a 15-min period to the refluxing mixture. After refluxing for an additional 1 h, a solution composed of H_2O (1 mL) and concentrated HCl (0.2 mL) was added while heating continued. After an additional 1 h at reflux, the mixture was cooled and concentrated in vacuo. The residue was made strongly basic with 50% NaOH and then heated at 75 °C for 1 h. After cooling, the mixture was extracted with CH_2Cl_2 (2 × 250 mL). The combined extracts were washed with H_2O (200 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was chromatographed through silica gel using 10% ethyl acetate in Skellysolve B[15] as the eluant. The fractions containing the product were combined and the solvents were removed in vacuo. The residue was recrystallized from pentane to afford 2.57 g (34%) of 16: mp 51–54 °C. Anal. ($C_{18}H_{14}F_3NO_2S$) C, H, F, N, S.

In Vitro Assay. Human blood was obtained from the antecubital vein and was treated with 3.8% sodium citrate (1 part citrate to 9 parts of blood). Platelet-rich plasma (PRP) was prepared by centrifuging the citrated blood at 200*g* for 10 min at 10 °C and then separated. The remaining blood was centrifuged at 2000*g* for 10 min at 10 °C to obtain the platelet-poor plasma (PPP). The platelet count was adjusted with autologous PPP to 3×10^5 platelets/mm^3. A collagen concentration[18] required to give slightly less than maximal aggregation was used to test compounds for inhibition of aggregation. The concentrations of compounds in Table I represents those which gave approximately 50% inhibition. Aggregation was measured in a Payton Aggregometer coupled with an Omniscribe recorder. The internal standard was flurbiprofen.

Modified ex Vivo Platelet Assay. Ex vivo platelet inhibitory activity was determined following oral administration of drugs to five male albino guinea pigs (Hartly strain, Kuipers Ranch, Gary, IN) that were fasted overnight. The drug was initially dissolved in Emulfor–ethanol (1:1) to a concentration of 50 mg/mL and subsequently diluted with Sterile Vehicle no. 122 (The Upjohn Co., Kalamazoo, MI: 0.25% methylcellulose in water) to the required concentration such that all animals received 2 mL/kg of body weight; thus, for a dose of 5 mg/kg, 2 mL of a 2.5 mg/mL solution was administered. A control group of guinea pigs was dosed with vehicle containing Emulfor–ethanol (1:1). At specified times after dosing, the animals were anesthetized with an intraperitoneal injection (2 mL/kg) of 5% sodium cyclopal in saline. Blood was collected from the abdominal aorta into syringes containing 0.10 volume of 2.2% sodium citrate (1 part citrate to 9 parts blood). The blood samples were centrifuged at 2000*g* for 30 min to obtained platelet-poor plasma (PPP). The plasma samples were refrigerated at 4 °C for assay on the following day. Pooled platelet-rich plasma (PRP) was prepared from untreated guinea pigs by centrifugation of citrated blood at 200*g* for 10 min. For aggregation studies, aliquots of PRP (0.45 mL) from untreated guinea pigs and 0.45 mL of PPP from treated or placebo guinea pigs were combined and warmed to 37 °C for 1 min. The collagen concentrated required to give slightly submaximal aggregation was determined using PPP from placebo-treated animals and compared with those observed with PPP from drug-treated animals by using the standard one way analysis of variance statistical assay.

(15) Skellysolve B is a petroleum fraction, bp 60–70 °C, sold by the Skelly Oil Co.

(16) S. S. Jenkins and E. M. Richardson, *J. Am. Chem. Soc.*, **55**, 1618 (1933).

(17) R. R. Kurkjy and E. V. Brown, *J. Am. Chem. Soc.*, **74**, 5779 (1972).

(18) Collagen suspension was prepared by homogenizing bovine tendon collagen (type I, acid insoluble) in acid (pH 2.5) to 2 mg/mL. Dilution of this stock solution was prepared using Tyrodes buffer, pH 3.5.