were sacrificed 6 h after single oral administration of test compounds. The stomach was removed and macroscopically observed. The dose (UD_{50}) producing ulcers in 50% of the rats was calculated according to the regression line of each compound.

Acute Lethal Toxicity. LD_{50} was determined from the 7-day mortality after a single dose in Std:ddY male mice.

Acknowledgment. We thank Dr. M. Shimizu, the director of research and development, headquaters, for his encouragement. Thanks are also due to the members of the Analytical Center of these laboratories for the elemental analyses and spectral measurements.

Registry No. **2a**, 100-00-5; **2b**, 99-54-7; **2c**, 611-06-3; **2d**, 6627-53-8; **3**, 609-08-5; **5a**, 19910-33-9; **5b**, 83528-10-3; **5c**, 53455-85-9; **5d**, 83528-11-4; **6a**, 50415-69-5; **6b**, 83528-08-9; **6c**, 24646-28-4; **6d**, 83528-09-0; **7a**, 39718-97-3; **7a**·HCl, 83528-15-8; **7b**, 83528-13-6; **7c**, 67333-29-3; **7d**, 83528-14-7; **8**, 21762-10-7; **9**, 83528-12-5; **10**, 76980-62-6; **11**, 52913-11-8; **12**, 39552-81-3; **12**·HCl, 83528-16-9; **14a**, 76302-28-8; **14a** methyl ester, 83528-17-0; **14b**, 76302-29-9; **14b** methyl ester, 83528-18-1; **14c**, 83528-37-4; **14c** methyl ester, 83528-19-2; **14d**, 83528-38-5; **14d** methyl ester, 83528-20-5; **14e**, 76302-47-1; **14e** methyl ester, 83528-21-6; **14f**, 83528-39-6; **14f** methyl ester, 83528-22-7; **14g**, 83528-40-9; **14g** methyl ester, 83528-23-8; **14h**, 83528-41-0; **14h** methyl ester, 83528-24-9; **14i**, 83528-42-1; 14i methyl ester, 83528-25-0; **14j**, 83528-43-2; 14j methyl ester, 83528-26-1; **14k**, 83528-44-3; **14k** methyl ester, 83528-27-2; **14l**, 83542-58-9; **14l** methyl ester, 83542-57-8; **15a**, 83528-45-4; **15a** methyl ester, 83528-28-3; **15b**, 83528-46-5; **15b** methyl ester, 83528-29-4; **16a**·HCl, 83528-47-6; **16a** methyl ester, 83528-30-7; **16b**·HCl, 83528-48-7; **16b** methyl ester, 83528-31-8; **17a**, 83528-49-8; **17a** methyl ester, 83528-32-9; **17b**·HCl, 83528-50-1; **17b** methyl ester, 83528-33-0; **18a**, 83528-51-2; **18a** methyl ester, 83528-34-1; **18b**, 83528-52-3; **18b** methyl ester, 83528-35-2; **18c**, 83528-53-4; **18c** methyl ester, 83528-36-3; 4-pyridylpyridinium chloride hydrochloride, 5421-92-1.

(11) Y. Yokoyama, *H.* Nakamura and M. Shimizu, *Folia Pharmacol. Jpn*, **75**, 106p (1979).

Replacement of Aromatic or Heteroaromatic Groups in Nonsteroidal Antiinflammatory Agents with the Ferrocene Group

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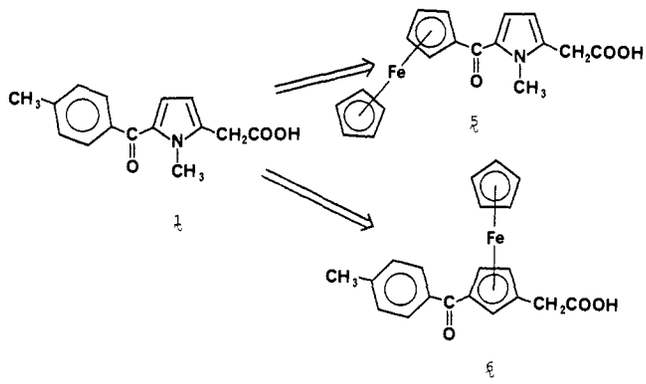
Ferrocene analogues of the antiinflammatory agents tolmetin (**1**), fenbufen (**2**), flurbiprofen (**3**), and fenclofenac (**4**) were synthesized and tested for biological activity. The derivatives exhibited little or no antiarthritic or platelet antiaggregatory activity, indicating that the ferrocene moiety is a poor bioisostere for aromatic or heteroaromatic groups in nonsteroidal antiinflammatory agents.

For many years, medicinal chemists have applied the principle of bioisosterism in drug design to improve bioactivity, reduce toxicity, and develop antagonists of know drugs.[1] In drug molecules containing aromatic rings, the introduction of heterocyclic moieties (e.g., thiophene for phenyl) is a common strategy. When considering potential analogues of tolmetin (**1**),[2] a nonsteroidal antiinflammatory agent for the treatment of arthritis, we sought an isoelectronic group to substitute for the pyrrole nucleus. A cyclopentadienide (Cp) group was an interesting possibility; however, it would be unstable by itself. Thus, a complexed formed was desired. A reasonable coordination unit, such as $Fe(CO)_3$, would afford toxicity problems, so FeCp was considered. Thus, we became intrigued with ferrocene as a bioisosteric[1c] replacement for the pyrrole unit of 1.



Only a few ferrocene analogues of known drugs have been synthesized,[3-6] and their biological activity is generally unexciting. Nevertheless, since no systematic investigation of a therapeutic class of drugs was ever conducted, we

(1) (a) Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563. (b) Korolkovas, A.; Burckhalter, J. H. "Essentials of Medicinal Chemistry"; Wiley: New York, 1976; pp 54–59. Ariens, E. J. In "Drug Design"; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. 1, pp 241–253. (c) The concept of bioisosterism is used here in its broadest sense, as discussed by Thornber (ref 1a).

(2) Carson, J. R.; McKinstry, D. N.; Wong, S. *J. Med. Chem.* **1971**, *14*, 646. Tolmetin sodium dihydrate is sold by McNeil Pharmaceutical under the registered trade name Tolectin. For other compounds in this series, see Carson, J. R.; Wong, S. *J. Med. Chem.* **1973**, *16*, 172.

(3) Kalish, R.; Steppe, T. V.; Walser, A. *J. Med. Chem.* **1975**, *18*, 222. Loev, B.; Flores, M. *J. Org. Chem.* **1966**, *26*, 3595. Popp, F. D.; Moynahan, E. B. *J. Med. Chem.* **1970**, *13*, 1020. Popp, F. D.; Moynahan, E. B. *J. Heterocycl. Chem.* **1970**, *7*, 351.

(4) Edwards, E. I.; Epton, R.; Mars, G. *J. Organometal. Chem.* **1975**, *85*, C23. *Ibid.* **1976**, *107*, 351. *Ibid.* **1976**, *122*, C49.

(5) Hanzlik, R. P.; Soine, P.; Sione, W. H. *J. Med. Chem.* **1979**, *22*, 424.

(6) Epton, R.; Mars, G.; Rogers, G. K. *J. Organometal. Chem.* **1976**, *110*, C42.

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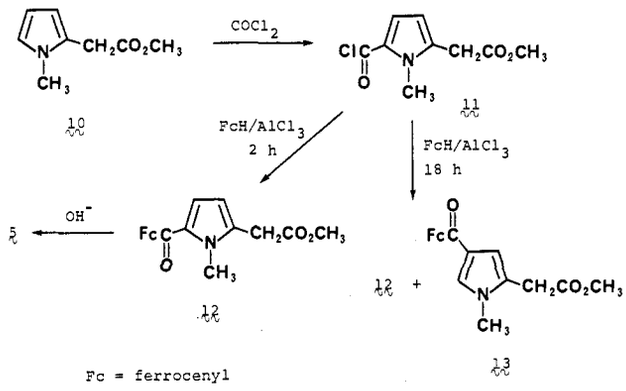
Table I. Biological Testing Results

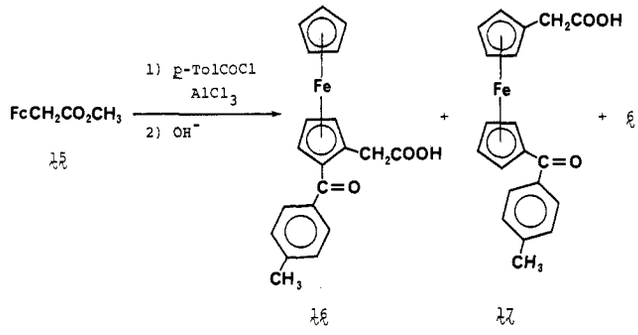
compd	adjuvant arthritis test[a]	platelet aggregation[b] 20 μM	platelet aggregation[b] 100 μM	serotonin release[c] 20 μM	serotonin release[c] 100 μM
5[d]	inact (50)[e]	20	27	32	60
6 (+16, 17)[d]	inact (100)	2	6	0	23
7	inact (25)[f]	0	8	29	64
8	inact (100)[f]	5	6	0	16
9	inact (100)	20	8	10	15
12	inact (100)	0	6	3	0
14 (+5)[d]	inact (100)	2	3	10	12
18	inact (100)	4	15	0	34
19	inact (50)[f]	7	28	3	0
1 (tolmetin)	34 (50)[f,g]	74	85	90	88
2 (fenbufen)	71 (50)[h]				
3 (flurbiprofen)[i]	55 (50)	88	89	86	90

[a] Activity is given as percent inhibition, at the dose (mg/kg, po) in parentheses. Inactivity was determined at the dose level in parentheses. Compounds were administered as suspensions in aqueous gum tragacanth unless otherwise specified. [b] Percent inhibition of collagen-induced platelet aggregation at the compound concentration shown. Significant activity is 30% or greater. [c] Percent inhibition of serotonin release from platelets at the compound concentration. Significant activity is 30% or greater. [d] Tested as the calcium salt. [e] Solution in water. [f] Solution in water with 1 mol equiv of NaOH. [g] I_{50} = 56 mg/kg. [h] Result taken from R. G. Child et al., *Arzeim.-Forsch.*, 30, 695 (1980). [i] Biphenylacetic acid is also active: 55% inhibition at 50 mg/kg (see Child et al., of footnote *e*).

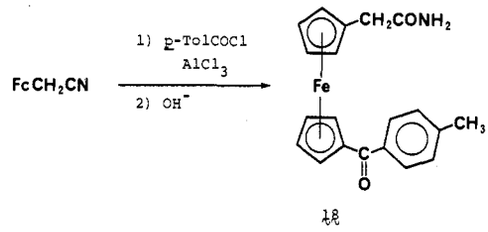
Scheme I



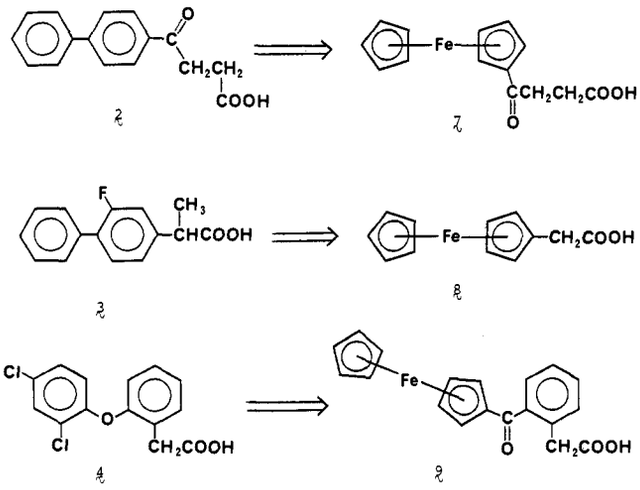
Scheme II



Scheme III



decided to prepare and study several ferrocene bioisosteres of nonsteroidal antiinflammatory agents. In this paper we disclose our work on ferrocene compounds related to four carboxylic acid antiarthritic drugs: tolmetin (**1**),[2,7b] fenbufen (**2**),[7a] flurbiprofen (**3**),[7b] and fenclofenac (**4**)[7] (see molecular transformations: **1** → **5**, **1** → **6**, **2** → **7**, **3** → **8**, and **4** → **9**).[8]



Chemistry. Reaction of pyrroleacetic acid ester **10**[9] with phosgene furnished acid chloride **11**,[10] which was reacted with ferrocene using aluminum chloride as a catalyst to give **12** (Scheme I).[11] When this acylation reaction was allowed to proceed for 18 h, instead of 2 h, a 60:40 mixture of **12** and **13** was obtained.[12] Ester **12** was hydrolyzed with

(7) (a) Lombardino, J. G. *Annu. Rep. Med. Chem.* **1981**, *16*, 189. (b) Wong, S. *Ibid.* **1975**, *10*, 172.

(8) (a) Analogue **8** lacks the α-methyl group of **3**, but such a change probably would not abolish biological activity, since arylacetic and -propionic acid structures both form a basis for potent antiarthritic agents. (b) The ether bridge of **4** was replaced by a carbon bridge (C=O group) in **9** because the latter was more synthetically feasible. The carbonyl group is a reasonable isosteric substitution for ether oxygen.[1a]

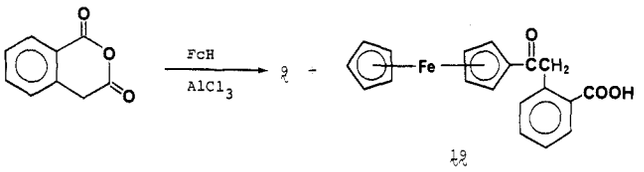
(9) Maryanoff, B. E. *J. Org. Chem.* **1979**, *44*, 4410.

(10) Carson, J. R. U.S. Patent 3 950 355, 1976.

(11) Rausch, M.; Vogel, M.; Rosenberg, H. *J. Org. Chem.* **1957**, *22*, 903.

(12) The 2-h reaction did produce a small amount of **13** (**12**/**13** = ca. 95:5). Treatment of **12** with $AlCl_3$ (in CH_2Cl_2) for 23 h afforded only a minor amount (5–10%) of rearrangement to **13**, whereas the 18-h reaction gave ca. 40% of **13**. Thus, $AlCl_3$ alone is inefficient for effecting the rearrangement of **12** to **13**; the HCl generated in the Friedel–Crafts condensation, possibly in the form $HAlCl_4$, is probably the catalyst.[13]

Scheme IV



NaOH to **5**, which was purified as a calcium salt. Similarly, the mixture of **12** and **13** was converted to an ca. 1:1 mixture of **5** and its 4-position isomer, **14** (purified as a mixture of calcium salts).

Compound **6** was obtained as part of a three-component mixture of isomers (**6**/**16**/**17** = 3:7:10) via toluoylation of acetic acid ester **15**,[14] followed by alkaline hydrolysis (Scheme II; purified as calcium salts). Unfortunately, **6** was a minor constituent of the isomeric mixture, although **16** and **17** seem to be interesting derivatives in themselves. A sample of carboxamide, highly enriched in isomer **18**, was prepared via toluoylation of ferrocenylacetonitrile, followed by hydrolysis (Scheme III).[14]

Keto acid **7** was prepared by reacting ferrocene with succinic anhydride and $AlCl_3$.[15] Acetic acid **8** was obtained commercially.[16]

Compound **9** was produced, along with **19**, by the reaction of ferrocene with homophthalic anhydride,[17] and the two isomers were separated (Scheme IV).

Biological Testing. Ferrocene derivatives **5–9**, **12**, **14**, **18**, and **19** were tested for antiarthritic activity in a rat adjuvant arthritis (AA) model and for possible prostaglandin (PG) synthetase inhibitory activity in a blood platelet aggregation assay (see Table I). The AA test, a standard means for evaluating antiarthritic agents,[18] entails the assessment of a compound's ability to inhibit inflammatory sequelae (measured by edema) consequent to the induction of an inflammatory focus by the injection of 0.75 mg of *mycobacterium butyricum* into the left hind paw of female Wistar/Lewis rats. Testing of these compounds was performed in animals with established disease (phase III). No activity was observed for the compounds in the AA test. Since the lack of activity may have been associated with administration of the compounds orally to intact animals, an in vitro assay that serves to characterize PG synthetase inhibition, on which (nonsteroidal) antiinflammatory activity is based, was also employed. The compounds were screened for inhibition of collagen-induced platelet aggregation, but no significant activity was noted. The effect of the compounds on the platelet serotonin-release reaction, a process which is secondary to aggregation, was also recorded. All of the compounds were inactive except for **7**, which showed weak, dose-dependent inhibition.

Conclusion

It is apparent from our investigation that replacement of aromatic or heteroaromatic groups of nonsteroidal antiinflammatory agents with the ferrocene moiety leads to loss of pharmacological and biochemical activity. We have tested two options for incorporation of ferrocene, both of which proved unsuitable: (1) use of one of the ferrocene cyclopentadienyl rings or (2) use of the entire ferrocene moiety in place of an original "aromatic" unit.[19] Our work considered together with earlier ferrocene substitution studies,[3] indicates that bioisosterism of the ferrocene unit is generally poor.[20]

Experimental Section

General Procedures. Proton NMR spectra were determined on a Varian EM-360 (60 MHz) or Perkin-Elmer R32 (90 MHz) spectrometer with Me_4Si as an internal reference (s = singlet; d = doublet; t = triplet; m = multiplet, br = broad). GLC analysis was conducted on a Perkin-Elmer 3920B instrument with a flame-ionization detector, equipped with a Hewlett-Packard 3352B data system and 18652 A/D converter, with a 3% OV-225 on Chromosorb W HP (100–120 mesh) column (6 ft × $^1/_8$ in.). TLC analysis was performed on Whatman MK1F silica gel (80 Å) plates (1 × 3 in.). Melting points are corrected; melting ranges may be preceded by softening ranges in parentheses. Mass spectra (electron impact) were determined on a Hitashi Perkin-Elmer RMU-6E instrument at 70 eV. Reactions with ferrocene derivatives were conducted under a nitrogen atmosphere with protection from light. The acylferrocene compounds were protected from light, as much as possible, during handling.

Known Compounds. 4-Ferrocenyl-4-oxobutanoic acid (**7**) was prepared according to the published procedure[15] to give orange-brown crystals, mp 165–168 °C (from 75% aqueous ethanol). 2-Ferrocenoylphenylacetic acid (**9**) and α-ferrocenoyl-*o*-toluic acid (**19**) were prepared as described[17] and separated by fractional crystallization from CH_2Cl_2/petroleum ether (**9** separated first). Acid **9** was recrystallized twice from CH_2Cl_2/ethyl ether/petroleum ether (2:1:2) to give dark red crystals: mp 167–169 °C; homogeneous by TLC (ethyl acetate), R_f 0.40. Acid **17** was chromatographed on a dry column of silica gel with ethyl acetate to give an orange-brown solid: mp 158–161 °C; homogeneous by TLC (ethyl acetate), R_f 0.55. Ferrocenylacetic acid (**8**) was obtained commercially[16] as a brown solid, mp 121–124 °C dec. Amide **18** was already prepared by us.[14a]

Methyl 5-Ferrocenoyl-1-methyl-1*H*-pyrrole-2-acetate (12). Methyl *N*-methylpyrrole-2-acetate[9] (**10**; 30.5 g, 200 mmol) in 80 mL of dry ethyl ether was added slowly to a solution of 22.0 g of $COCl_2$ in 40 mL of dry ether with occasional cooling in an ice bath.[10] The reaction was stirred for 2 h and then evaporated to dryness. The brown oil was dissolved in warm CH_2Cl_2/petroleum ether, and some brown oil separated initially on cooling to 20 °C. The yellow solution was decanted and cooled slowly to −20 °C to give 14.8 g of light beige solid, **11**. Acid chloride **11** (10.4 g, 54 mmol) was combined with ferrocene (10.0 g, 54 mmol) in 60 mL of dry CH_2Cl_2. This solution was added slowly to a stirred mixture of anhydrous $AlCl_3$ (6.8 g, 51 mmol) in 50 mL of CH_2Cl_2. After 2 h at 23 °C, the dark blue mixture was poured onto cracked ice, and the layers were separated. The organic phase was rinsed with water, dried ($MgSO_4$), and concentrated to a dark gum, which was chromatographed on a dry column of silica gel with $CHCl_3$ to give 7.2 g of red syrup, which was homogeneous by TLC. The material was dissolved in hexane/ethyl acetate (1:1) and filtered warm, and the filtrate was cooled to −20 °C to give 3.0 g of

(13) Carson, J. R.; Davis, N. M. *J. Org. Chem.* **1981**, *46*, 839.
(14) (a) Maryanoff, B. E. *J. Org. Chem.* **1981**, *46*, 70. (b) We wish to correct a minor error in the *J. Org. Chem.* paper: on p 72, the EM-360 NMR instrument should be attributed to Varian (not Perkin-Elmer).
(15) Rinehart, K. L.; Curby, R. J., Jr.; Sokol, P. E. *J. Am. Chem. Soc.* **1957**, *79*, 3420.
(16) Parish Chemical Co., Provo, UT.
(17) Gautheron, B.; Tirouflet, J. Boichard, J. *Bull. Soc. Chim. Fr.* **1967**, 658.
(18) Swingle, K. F. In "Antiinflammatory Agents: Chemistry and Pharmacology"; Scherrer, R. A.; Whitehouse, M. W., Eds.; Academic Press: New York 1974; Chapter 2, pp 33–122. Wong S. In "Tolmetin, A New Non-Steroidal Anti-Inflammatory Agent"; Ward, J. R., Ed.; Excepta Medica: Amsterdam, **1976**; pp 1–22. Also, see Ward, J. R.; Cloud, R. S. *J. Pharmacol. Expl. Ther.* **1966**, *152*, 116.

(19) The geometry of ferrocene is that of a sandwich complex (local D_{5d} symmetry), wherein two parallel, planar Cp rings surround an Fe(II) center. Although substitution of ferrocene for a biphenyl group (as in **2** → **7** and **3** → **8**) entails some steric incongruity, this substitution is still regarded within the frame of bioisosterism, defined in its broadest sense.[1c]
(20) Biological activity has been reported for ferrocene analogues of β-lactam antibiotics[4] and β-ferrocenylalanine.[5] A ferrocene isostere of aspirin was prepared, but no biological properties were mentioned.[6]

vermillion powder: mp 93–96 °C; IR (KBr) ν_{max} 1735 (CO), 1602 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.68 and 3.73 (2 s, 5, CH_2COO and OCH_3, respectively), 3.85 (s, 3, NCH_3), 4.19 (s, 5, Cp), 4.47 (t, 2, J = 2 Hz, β-H on CpCO), 4.91 (t, 2, J = 2 Hz, α-H on CpCO), 6.08 (d, 1, J = 4.5 Hz, H_3), 7.15 (d, 1, J = 4.5 Hz, H_4); UV (CH_3OH) λ_{max} 474 nm (ϵ 1040), 314.5 (14930), 273 (7300), 235 (10190); MS, m/z 365 ($M^{+\cdot}$), 306, 180, 129, 121, 94, 86, 58, 57. Anal. ($C_{19}H_{19}FeNO_3$) C, H.

Calcium 5-Ferrocenoyl-1-methyl-1*H*-pyrrole-2-acetate (5-Ca). Ester **12** (1.9 g, 5.0 mmol) was dissolved in 10 mL of 95% ethanol and treated with 2.5 mL of 2 N NaOH (5.0 mmol). The solution was heated at reflux for 2 h. Most of the solvent was evaporated, and 10 mL of hot deionized water was added. Addition of a solution of $CaCl_2$ (280 mg, 2.5 mmol) in a minimum of deionized water produced an orange-red precipitate. The solid was rinsed with cold water, rinsed with dry ether, and dried in vacuo for 3 h at 60 °C (1.4 g). Recrystallization from Me_2SO–water gave 0.65 g of orange powder, which was saturated with moisture in a humidity chamber: mp 110–140 °C, darkened and fused to a dense ball; 185 °C, liquid dispersed; UV (CH_3OH) λ_{max} 472 nm (ϵ 1010), 325 (14900), 271 (7130), 237.5 (9020); IR (KBr) ν_{max} 3405 (OH), 1597 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6/$CDCl_3$, 2:1) δ 3.37 (s, 3, NCH_3), 3.86 (br s, 2, CH_2COO), 4.23 (s, 5, Cp), 4.53 (s, 2, β-CpCO), 4.68 (br s, 2, α-CpCO), 6.05 (br s, 1, H_3), 7.05 (br s, 1, H_4); ^1H NMR (Me_2SO-d_6/CD_3OD, 1:1) δ 3.57 (s, 2, CH_2COO), 3.88 (s, 3, NCH_3), 4.23 (s, 5, Cp), 4.57 (m, 2, β-CpCO), 4.90 (m, 2, α-CpCO), 6.08 (d, 1, H_3), 7.10 (d, 1, H_4). Anal. ($C_{18}H_{16}FeNO_3$·0.5Ca·3.3H_2O) C, H, H_2O.

Calcium 4-Ferrocenoyl-1-methyl-1*H*-pyrrole-2-acetate (14-Ca). This compound was obtained in a mixture with the 5-isomer, **5**. Acid chloride **11** (vide supra, 10.4 g, 48 mmol) was reacted with ferrocene (10.0 g, 54 mmol) and $AlCl_3$ (6.8 g, 51 mmol) as described above, but the reaction time was 18 h. Workup and dry-column chromatography (300 g of silica gel; petroleum ether/$CHCl_3$, 1:1) gave 11.0 g of red viscous oil, 6.3 g of which was dissolved in 8 mL of 95% ethanol and hydrolyzed with 8.25 mL of 2.0 N NaOH (reflux for 2 h). The calcium salt was prepared, as above, to give 3.75 g of vacuum-dried, red-orange solid, which was recrystallized from Me_2SO/water. The orange solid (1.06 g) showed an ca. 1:1 mixture of two compounds by TLC (ethyl acetate/95% ethanol): R_f 0.31, orange spot, corresponded to **5**; R_f 0.16, red spot, for **14**. The Ca salt was saturated with moisture in a humdiity chamber: mp (160–185 °C) 185–210 °C dec; UV (CH_3OH) λ_{max} 469 nm (ϵ 1080), 322 (11890), 272 (8770), 232 (11230, sh); IR (KBr) ν_{max} 3420 (OH), 1580 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6/CD_3OD, 1:1) δ 3.44 and 3.49 (2 s, 2, CH_2COO for **14** and **5**, respectively), 3.67 and 3.85 (2 s, 3, NCH_3 for **14** and **5**, respectively), 4.18 and 4.20 (2 s, 5, Cp for **14** and **5**, respectively), 4.26 (s, H_2O), 4.52 (m, 2, β-CpCO), 4.88 (m, 2, α-CpCO), 6.02 (d, 0.6 H_3 in **5**), 6.47 (d, 0.4, H_2 in **14**), 7.03 (d, 0.55, H_4 in **5**), 7.48 (d, 0.3, H_5 in **14**). Anal. ($C_{18}H_{16}FeNO_3$·0.5Ca·1.0H_2O) C, H, H_2O.

Calcium 1′-, 2-, and 3-Toluoylferrocenylacetates. These compounds were prepared as a mixture of isomers in a 10:7:3 ratio, respectively:[12a] UV (CH_3OH) λ_{max} 484 nm (ϵ 1700), 358 (3500), 281 (20925, sh), 256 (30290); IR (KBr) ν_{max} 3428 (OH), 1634 and 1607 (CO) cm^{-1}; ^1H NMR (Me_2SO-d_6/$CDCl_3$, 1:3) δ 2.40 (s, 3, $ArCH_3$), 2.9–3.5 (m, 3.2, H_2O and CH_2COO), 3.9–4.9 (m, 8, Cp), 7.21 (m, 2), 7.74 (m, 2).

Adjuvant Arthritis Assay (Phase III). Adjuvant arthritis was induced in female Wistar/Lewis rats weighing 150–170 g by intraplantar injection into the left hind paw of 0.1 mL (0.75 mg) of heat-killed *M. butyricum* (Difco) suspended in light mineral oil. Eighteen days after induction, edema in the noninjected, contralateral paw was assessed by mercury displacement in a plethysmograph. The animals were then arranged homogeneously by body weight and paw volume into control and treatment groups of five animals each. Compounds were administered orally by gavage, as a suspension in aqueous gum tragacanth (0.5% w/v solution) or solution in water (see Table I), once daily (food and water supplied ad libitum) for 4 days, and paw volumes were determined by plethysmography on the 5th day. Control animals were administered vehicle by gavage once daily. Percent inhibition of paw volume was calculated by comparison to control values, and statistical evaluation was by analysis of variance with significance determined by Dunnett's significant difference ($p \leq 0.05$).

Preparation of Plasma Samples. Blood was drawn from human volunteers who had not ingested any drugs for a period of at least 10 days prior to the study. Blood was taken from the antecubital vein directly into plastic syringes each containing 1 mL of 3.8% w/v trisodium citrate solution (9 vol of blood plus 1 vol of citrate solution) and centrifuged for 5 min at 450 *g* in a Sorvall GLC-1 centrifuge (room temperature). A 2.0-mL aliquot of the upper (platelet-rich plasma or PRP) layer was collected, and the platelet count was determined in duplicate in a thrombocounter (Coulter). The remaining lower layers of plasma and blood cells were recentrifuged at 6000 *g* for 10 min in a Sorvall RC2-B refrigerated centrifuge (4 °C). The entire upper plasma layer was collected and used as a platelet-poor plasma (PPP). During manipulations of plasma at room temperature, all containers were tightly capped in order to minimize drift in plasma pH due to loss of CO_2. All determinations of platelet aggregation were made on PRP adjusted to 300000 platelets/μL by addition of PPP.

Platelet Aggregation Assay. Aggregation was studied with aggregometers (Chrono-Log, Models 330 and 340) with 0.45 mL of PRP at 37 °C and maintained stirring at 1200 rpm. All test drugs were added as 10-μL aliquots (solution in PEG 300) to 0.45 mL of PRP in siliconized glass cuvettes, stirred briefly, and incubated for 8 min at 37 °C prior to a 2-min equilibration period in the aggregometer. Following this equilibration period, aggregation was initiated by the addition of 50 μL of the aggregating agent collagen.[21] Aggregation measurements were initiated at 1 h and completed within 2.5 h following the time the blood was drawn. The extent of platelet aggregation was determined by measuring the maximum increase in light transmission following the addition of stimulating agent and is expressed as percent transmission. Percent inhibition of aggregation was calculated from the extent of aggregation in the presence of a test drug compared to control.

Release of Serotonin from Human Platelets In Vitro. The platelet count was adjusted to 300000/μL. Seventy microliters of [^{14}C]5-HT was added to 7.0 mL of adjusted platelet-rich plasma at room temperature and mixed. The final concentration of radiolabeled 5-HT was 0.5 μM at a specific activity of 50 μCi/μmol. The sample was incubated at room temperature for 30 min with gentle mixing every 10 min. The 5-HT containing PRP (0.45 mL) was placed in siliconized cuvettes, each of which contained a "mini" magnetic stirring bar. The cuvettes were covered with parafilm at all times. The desired concentration of test compound was added to the cuvettes in 10 μL of vehicle (PEG 300) and incubated at 37 °C for 10 min. The cuvettes were then placed in an aggregometer for simultaneous aggregation and release studies. To the stirring cuvettes was added 50 μL of stimulating agent so that the final concentration was 1.6 μL/mL in collagen.[21] After 5 min, the aggregation process was arrested by adding 50 μL of 100 mM EDTA. The samples were transferred to Eppendorf Micro test tubes and centrifuged at 8000*g* for 60 s. To determine the amount of 5-HT released, we counted a 100-μL aliquot of each supernatant fraction in 9 mL of Biofluor scintillation fluid and 1 mL of H_2O. The extent of 5-HT release was calculated from the amount of radioactivity in the supernatant fraction following centrifugation of the aggregated PRP. Percent inhibition of release was calculated from the amount of radioactivity in the supernatant fraction from PRP that was treated with the test compound compared to an identically prepared PRP sample that had been treated with only the vehicle.

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Registry No. **5**-Ca, 83546-55-8; **6**-Ca, 83546-56-9; **7**, 83546-57-0; **8**, 83546-58-1; **9**, 83546-59-2; **11**, 83542-63-6; **12**, 83546-60-5; **14**-Ca, 83546-61-6; **16**-Ca, 83546-62-7; **17**-Ca, 83546-63-8; **18**, 75458-57-0; **19**, 83546-64-9; $COCl_2$, 75-44-5; $AlCl_3$, 7446-70-0; methyl *N*-methylpyrrole-2-acetate, 51856-79-2; ferrocene, 102-54-5.

(21) Collagen solutions were prepared by an established procedure; see Holmer, H.; Ostvold, A. C.; Day, H. J. *Biochem. Pharmacol.* **1973**, *22*, 2599.