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3-Phenyl-5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carbonitrile, a Potent Inhibitor of Prostaglandin Synthetase and of Platelet Aggregation

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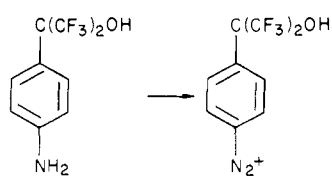
A number of indoles containing the 2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl side chain have been prepared by standard methods. Alternate, novel syntheses of indole-2-carboxamides and indole-2-carbonitriles have been developed. The title compound, **7e**, was found to be a potent inhibitor of bovine prostaglandin synthetase in vitro and to lower serum prostaglandin levels after oral or intraperitoneal administration to rats. Consistent with prostaglandin synthetase inhibition, **7e** prevented arachidonic acid induced diarrhea in mice and also collagen, ADP, or epinephrine induced platelet aggregation in human platelet-rich plasma. In contrast to many prostaglandin synthetase and platelet-aggregation inhibitors, **7e** had neither ulcerogenicity nor systemic antiinflammatory activity in rats.

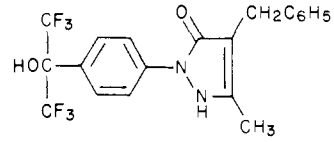
In connection with another project, we had a need to prepare the indole-2-carbonitrile **7e**. A number of 3-phenylindole-2-carbonitriles have been prepared[1-7] in the past as intermediates in the 1,4-benzodiazepine area by functional-group manipulations from the corresponding esters. These esters, in turn, have been prepared by a combination of the Japp–Klingemann reaction[8] and the Fischer indole synthesis.[9] We therefore utilized a similar sequence for the synthesis of **7e**.

Diazotization of **1** gave **2**, which was allowed to react



1 2

with **3a** as shown in Scheme I. Selective elimination of the acetyl group from the resulting **5a** and cyclization of **6a** to the indole **7a** proceeded as expected without isolation of the intermediates. The pyrazolone **8** was isolated as a byproduct of this sequence. Hydrolysis of the ester group of **7a** required at least 2 equiv of sodium hydroxide: the hydroxyl group of the $C(CF_3)_2OH$ side chain is sufficiently



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acidic to neutralize 1 equiv of base. The acid was converted into the acid chloride **7c** on heating with phosphorus pentachloride in ether: other reagents, such as thionyl chloride, would be expected[10] to replace the hydroxyl of the side chain by chlorine. Treatment of the total reaction mixture containing **7c** with ammonia gave the amide **7d**. The ester **7a** was recovered from a number of attempts to prepare the amide from it directly with ammonia under a variety of conditions—probably due to the ionization of the $C(CF_3)_2OH$ side chain and the inability of ammonia to attack the resulting negatively charged molecule. The amide **7d** was dehydrated under a variety of conditions, preferably with polyphosphate ester[11] in chloroform, to give the nitrile **7e** in a total yield from 1 of 38%.

When **7e** was found to be a potent inhibitor of prostaglandin synthetase, alternate, more direct, synthetic routes were considered. A thorough search of the literature failed to disclose any previous synthesis of indole-2-

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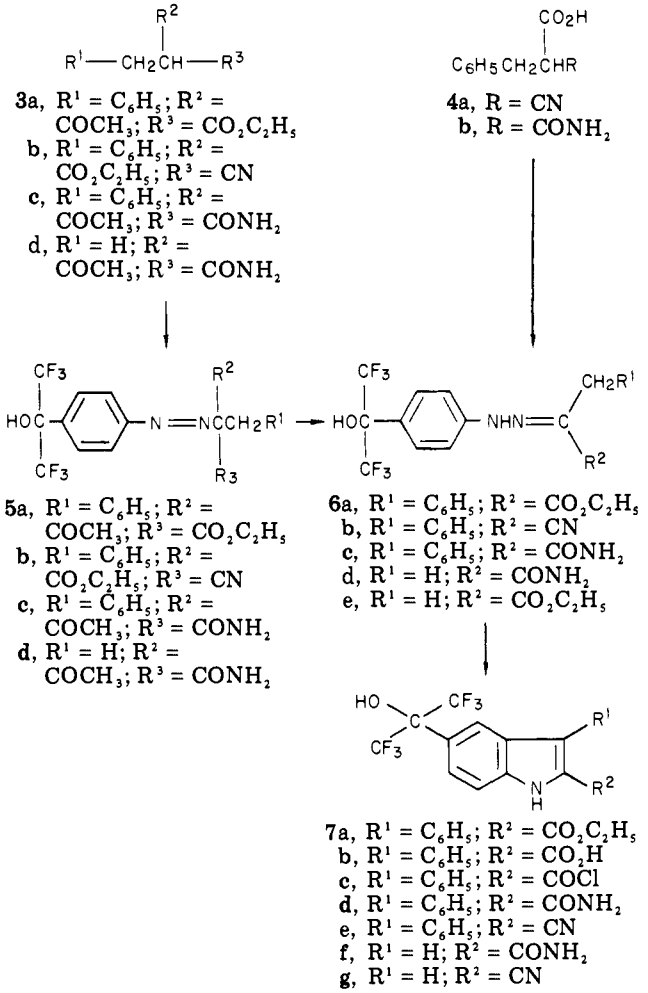
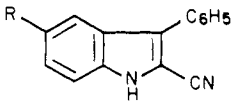
Scheme I



carbonitriles via the Fischer indole synthesis. Nevertheless, we investigated such a route for the preparation of **7e** from both the ester **3b** and the acid **4a**. After the completion of this work, a similar direct synthesis of 3-phenylindole-2-carbonitriles from **4a** was reported.[12]

When **2** was allowed to react with **3b**[13] under essentially neutral conditions, the azo compound **5b** was readily isolated. Treatment of **5b** with acid under mild conditions caused selective loss of the carbethoxy group to give a lower melting isomer of **6b**, also prepared from **2** and **4a**.[14] Treatment of **5b** with acid under somewhat harsher conditions gave a higher melting isomer of **6b**, which also was isolated from the reaction of **2** and **4a** and which, in addition, could be prepared from the lower melting isomer by judicious acid treatment. The reaction of **5b**, either isomer of **6b**, or mixtures thereof under harsher acidic conditions then gave **7e**. This shorter sequence of reactions gave an overall yield of **7e** of 40% from **1** and **3b**. The direct synthesis of **7e** from **1** and **4a** was not as efficient.

The direct synthesis of indole-2-carbonitriles using **3b** was then carried out on 4-aminobenzoic acid to give **9a**,



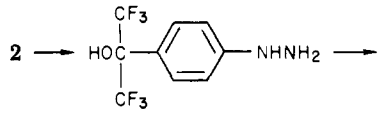
9a, R = CO_2H
b, R = CO_2CH_3

which was best purified via its methyl ester **9b**.

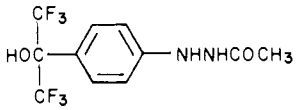
Because the amide **7d** also showed significant prostaglandin synthesis inhibitory activity, more direct syntheses of **7d** were also investigated. The only indole-2-carboxamide previously prepared by a Fischer indole synthesis is the parent compound prepared[15] from the phenylhydrazone of pyruvamide.

The coupling of **3c**[16] and **2** under essentially neutral conditions gave the azo compound **5c**. Acid treatment of **5c** under mild conditions caused the selective cleavage of the acetyl group to give **6c**, which was also prepared (in poor yield) from **4b**[17] and **2**. More vigorous acid treatment of **5c** or **6c** gave a 63% yield (overall from **1** and **3c**) of **7d**. In conjunction with the dehydration step, this procedure thus provides a synthesis of **7e** in 52% yield in essentially two steps.

We also wanted to prepare **7g**, the analogue of **7e** lacking the phenyl ring at position 3. The most straightforward approach to **7g** failed when the hydrazine **10** reacted with



10



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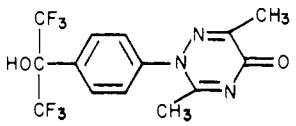
pyruvonitrile not to give the desired hydrazone but rather the hydrazide **11**. We then used an approach analagous to that for **7e** via **5c** and **6c**.

The coupling of **2** with **3d**,[18] followed by mild acid treatment of **5d** to give **6d**, proceeded normally. The hydrazone **6d** was isolated as a lower melting, pale yellow, ether-insoluble isomer, which rapidly at its melting point or slowly on heating in ether was converted into a colorless, more soluble, higher melting isomer. The cyclization of **6d** proved to be considerably more difficult than that of **6c**, since **6d** lacks the phenyl ring to stabilize the enehydrazine tautomer as the first intermediate[9] in the reaction. The amide **6d** on treatment with hydrogen chloride in hot ethanol gave as the only isolated products two isomers of the ester **6e**, the higher melting form of which was also prepared from **2** and ethyl α-methylacetoacetate. The reaction of **6d** with boron trifluoride etherate in acetic acid gave the triazinone **12** as the major product and a



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lesser yield of **7f**. Heating **6d** with zinc chloride gave acceptable yields of **7f**. Dehydration with polyphosphate ester then gave the nitrile **7g**.

Biological Results. As a result of random screening, we found that **7e** was orally active in preventing arachidonic acid induced diarrhea in mice. Similar activity is found with aspirin and indomethacin. Therefore, as a sequel to this result the compounds prepared in this report were tested in comparison with aspirin and indomethacin for their in vitro inhibitory activity on prostaglandin synthetase. Most of the compounds were inactive at 10^{-4} M, except the carboxamide **7d** which had an IC_{50} of 7 μM and the nitrile **7e** which was found to be twice as potent as indomethacin and much more potent than aspirin (Table I). Therefore, detailed investigation of the biological activity of this series was confined to the nitrile **7e**.

One hour after intraperitoneal or oral administration of **7e** to rats a decreased formation of serum $PGF_{2\alpha}$ was found

(Table I). Some variation, due to both the radioimmunoassay procedure for prostaglandins and to the drug response, was found during repeated experiments. However, similar in vivo potencies were found for **7e** and aspirin. In common with aspirin, the effective dose of **7e** after intraperitoneal injection was similar to the oral dose, suggesting that rates of absorption and bioavailability were similar by either route of administration.

Incubation of **7e** with human platelet-rich plasma for 5 min resulted in complete inhibition of platelet aggregation induced by human collagen and of second-wave aggregation induced by adenosine diphosphate (ADP) or epinephrine. Against collagen-induced aggregation, **7e** was more potent than aspirin and slightly less potent than indomethacin (Table I). Against ADP- or epinephrine-induced aggregation, **7e** was less potent.

In contrast to aspirin and indomethacin, **7e** did not cause gastric ulcers even after acute oral administration of 250 mg/kg. It also appears to have low toxicity, both acute (LD_{50} >1000 mg/kg po in mice) and short term (LD_{50} >1000 (mg/kg)/day for 14 days in rats). In contrast to many other prostaglandin synthetase inhibitors or platelet-aggregation inhibitors, **7e** does not have systemic antiinflammatory or analgesic activity. It also appeared to be devoid of central or peripheral pharmacological activity in a variety of primary screening tests.

Experimental Section

Melting points were taken in open capillary tubes using a Thomas-Hoover melting point apparatus and are corrected. Analytical samples had compatible IR, UV, NMR, and mass spectra. Organic solutions were dried by passage over Na_2SO_4.

3-Phenyl-5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carboxylic Acid Ethyl Ester (7a). To a solution of 95.0 g (0.367 mol) of 4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]benzenamine (1) in 300 mL of H_2O and 155 mL of concentrated HCl cooled to and kept at 0 °C was added a solution of 28.5 g (0.40 mol) of $NaNO_2$ in 50 mL of H_2O. The reaction mixture was stirred until it became homogeneous, and this solution of **2** was then added over 45 min to a solution of 81.0 g (0.367 mol) of α-acetylbenzenepropanoic acid ethyl ester **3a** and 155 mL of 50% KOH solution in 800 mL of 50% aqueous ethanol cooled to and kept at −10 °C. The cooling bath was removed, and the reaction was stirred for 20 min and extracted with portions of CH_2Cl_2 until the extract was colorless. The combined extracts were passed over a column of 500 g of silica gel, which was then washed with 1:1 ether–CH_2Cl_2. The combined eluates were concentrated to give 190 g of crude **5a** and/or **6a** as a reddish oil. This was mixed with 250 mL of HOAc and 250 mL of concentrated HCl and heated under reflux for 30 min. The solution was kept in the refrigerator overnight. The resulting precipitate was collected by filtration and washed with water and with $CHCl_3$ to give 81.0 g (51%) of **7a** as yellow crystals, mp 185–193 °C. Recrystallization from ether–CH_2Cl_2 gave colorless crystals, mp 194–195.5 °C. Anal. ($C_{20}H_{15}F_6NO_3$) C, H, F, N.

1,2-Dihydro-5-methyl-4-(phenylmethyl)-2-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-3*H*-pyrazol-3-one (8). The aqueous mother liquor of **7a** was extracted with ether. These ether extracts and the organic mother liquor of **7a** were washed with $NaHCO_3$ solution, dried, and evaporated. The residual oil was triturated with ether, and the resulting precipitate was recrystallized from ethyl acetate to give a 1.3% yield of **8** as colorless crystals, mp 229–231.5 °C. Anal. ($C_{20}H_{16}F_6N_2O_2$) C, H, F, N.

3-Phenyl-5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carboxylic Acid (7b). A solution of 26.65 g (0.062 mol) of **7a** and 6.35 g (0.158 mol) of NaOH in 330 mL of ethanol was heated under reflux for 75 min. Most of the ethanol was evaporated, and the residue was diluted with H_2O and washed with ether. The aqueous solution was then acidified with HCl and extracted with ether which was dried and evaporated. The residual oil was taken up in benzene and evaporated several times to remove occluded ether. The resulting colorless

Table I. Biological Activity of 7e[a]

compd	inhibn of PS:[e] IC_{50}, μM[b]	inhibn of format. of serum $PGF_{2\alpha}$: ED_{50}, mg/kg[c] ip	inhibn of format. of serum $PGF_{2\alpha}$: ED_{50}, mg/kg[c] po	inhibn of platelet aggregation: collagen-induced: IC_{50}, μM[d]	inhibn of platelet aggregation: ADP-induced IC_{50}, μM[d]	inhibn of platelet aggregation: Ep-induced:[e] IC_{50}, μM[d]	inhibn of ArA-induced diarrhea:[e] ED_{50}, mg/kg[c] po	gastric ulcer incidence: ED_{50}, mg/kg[c] po
7e	0.5 (16)	15 (150)	28 (105)	4.2 (3.5–4.9)	7.3 (6.2–8.6)	34 (30–40)	5.0 (6)	>250 (25)
	n = 2	*n* = 5	*n* = 7	*n* = 5	*n* = 6	*n* = 6	*n* = 2	*n* = 2
indomethacin	1.0 (16)	<1.0 (5)	<1.0 (20)	2.8 (2.0–3.5)	0.1 (0.075–0.13)	3.2 (2.0–4.5)	0.8 (6)	3 (25)
	n = 2	*n* = 2	*n* = 2	*n* = 4	*n* = 5	*n* = 5	*n* = 2	*n* = 2
aspirin	400 (16)	10 (5)	6.7 (50)	31 (25–40)	25 (20–30)	18 (17–20)	1.3 (6)	49 (25)
	n = 2	*n* = 2	*n* = 2	*n* = 6	*n* = 5	*n* = 5	*n* = 2	*n* = 2

[a] *n* = number of separate experiments. [b] Numbers in parentheses are the number of individual determinations. [c] Numbers in parentheses are the number of animals. [d] Mean (range of values). [e] Abbreviations used are: PS, prostaglandin synthetase; Ep, epinephrine; ArA, arachidonic acid.

solid was recrystallized from benzene to give in several crops 24.5 g (98%) of **7b**, mp 175–179 °C. The analytical sample was obtained by concentration of a moist ether solution to give colorless crystals of the hemihydrate of **7b**, mp 185.5–190 °C. Anal. ($C_{18}H_{11}F_6NO_3 \cdot 0.5H_2O$) C, H, F, N.

3-Phenyl-5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carboxamide (7d). A. From 7b. A mixture of 24.5 g (0.061 mol) of **7b**, 13.0 g of PCl_5, and 250 mL of ether was heated under reflux for 1 h. The resulting clear yellow solution containing **7c** was added over 15 min to a solution of 100 mL of NH_3 in 350 mL of ether cooled in a dry ice–acetone bath. The cooling bath was removed and with efficient stirring the slurry was gradually warmed to remove the excess NH_3. The resulting colorless suspension was filtered through a filter aid and concentrated to an oil, which soon crystallized. Recrystallization from ether–benzene gave 22.2 g (91%) of **7d**, mp 228–230 °C. The analytical sample was obtained from ether–hexane and had mp 228.5–231 °C. Anal. ($C_{18}H_{12}F_6N_2O_2$) C, H, F, N.

B. From 5c with HCl in Ethanol. Hydrogen chloride was bubbled into an ethanol solution of crude **5c** prepared from 8.19 g (0.0316 mol) of **1**, and the partially saturated solution was heated on the steam bath for 3.5 h with gentle stirring. The heterogeneous (NH_4Cl) reaction was concentrated under vacuum, mixed with H_2O and ether, and made basic with $NaHCO_3$. The ether layer was dried and concentrated with the addition of benzene to give 8.07 g (63% overall from **1**) of **7d** as colorless crystals, mp 227–230 °C.

C. From 6c. Similar treatment of **6c** for 2 h gave after workup a 74% yield of **7d** as tan crystals, mp 227–228 °C.

3-Phenyl-5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carbonitrile (7e). A. From 7d with Polyphosphate Ester. A mixture of 60.7 g (0.15 mol) of **7d** and 400 g of polyphosphate ester[11] in 800 mL of $CHCl_3$ was heated under reflux for 3.5 h. The solvent was removed under vacuum, and the residue was diluted with water, made slightly basic with Na_2CO_3, and extracted with ether. The extracts were dried and concentrated, and the crystalline residue was recrystallized from ether–CH_2Cl_2 to give 48.2 g (83%) of **7e** as colorless crystals, mp 252–254 °C.

The analytical sample of **7e** was obtained from a neat reaction of **7d** and P_2O_5[19] and, after recrystallization from ether–CCl_4, had mp 251–253 °C. Anal. ($C_{18}H_{10}F_6N_2O$) C, H, F, N.

B. From 5b. A similar polyphosphate ester treatment of crude **5b** gave a 43% yield of **7e**.

C. From 6b Isomer A via 6b Isomer B. A solution of 1.85 g (4.60 mmol) of **6b** isomer A in 30 mL of HOAc and 10 mL of concentrated HCl was stirred and gradually heated with an oil bath. After 30 min when the temperature was 35 °C, crystals of **6b** isomer B had formed in the mixture; these gradually dissolved on further heating. After the reaction had been heated to 70–85 °C for 50 min, it was concentrated under vacuum, mixed with $NaHCO_3$ solution, and extracted with ether. The ether was dried and evaporated to 1.50 g of solid residue, which upon recrystallization from ether–CH_2Cl_2 gave 0.93 g (53%) of **7e** as colorless crystals, mp 249–251 °C.

D. From 4a via 6b. A sample of the total crude mixture of **6b** isomers was heated with polyphosphate ester to 190 °C to give a 17% yield (overall from **1**) of **7e**.

α-Cyano-α-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenylazo]benzenepropanoic Acid Ethyl Ester (5b). A solution of 0.033 mol of **2** was treated with 10.0 g of NaOAc and allowed to react with an ethanolic solution of 6.70 g (0.033 mol) of α-cyanobenzenepropanoic acid ethyl ester (**3b**)[13] to give 18.4 g of a red oil. This crude **5b** could be used as such but on occasion was dissolved in benzene and passed over 200 g of silica gel. Elution with increasing amounts of CH_2Cl_2 in benzene gave 15.3 g (98%) of purified **5b** as a yellow oil. After unsuccessful attempts to crystallize this material from various solvents, including ether, excess solvents were removed under vacuum and the residual oil was found by analysis to contain 1 mol of ether, also seen in the NMR spectrum. Anal. ($C_{21}H_{17}F_6N_3O_3 \cdot C_4H_{10}O$) F; C: calcd, 54.84; found, 55.27; H: calcd, 4.97; found, 4.43; N: calcd, 7.68; found, 8.14.

α-[1-[4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]hydrazin-2-ylidene]benzenepropanenitrile (6b Isomer A). A. From 1 and 4a. The reaction of 0.053 mol of **2** with 9.24 g (0.053 mol) of α-cyanobenzenepropanoic acid (**4a**)[14] gave a mixture of the isomers of **6b**, which was absorbed onto silica gel. Elution with 30–80% CH_2Cl_2 in benzene gave fractions rich in **6b** isomer A, while CH_2Cl_2 eluted fractions rich in **6b** isomer B (see below). Recrystallization of the crude **6b** isomer A from CH_2Cl_2–hexane and then from hexane gave 3.71 g (17.5%) of **6b** isomer A as pale yellow crystals, mp 90–92 °C. Anal. ($C_{18}H_{13}F_6N_3O$) C, H, F, N.

B. From 5b. **6b** isomer A was also isolated after treatment of **5b** with HOAc and concentrated HCl for 30 min at room temperature.

6b Isomer B. A. From 1 and 4a. The fractions of crude **6b** isomer B obtained above were recrystallized from ether–hexane to give 0.76 g (4%) of **6b** isomer B as colorless crystals, mp 164.5–167 °C.

B. From 6b Isomer A. A solution of 1.25 g (3.1 mmol) of **6b** isomer A in 15 mL of HOAc and 5 mL of concentrated HCl was gradually warmed over 15 min to 35 °C with an oil bath. The resulting precipitate was recrystallized from CH_2Cl_2–hexane to give 0.48 g (40%) of **6b** isomer B as colorless crystals, mp 164.5–166.5 °C. Further recrystallization from benzene gave the analytical sample, mp 165–167 °C. Anal. ($C_{18}H_{13}F_6N_3O$) C, H, F, N.

2-Cyano-3-phenylindole-5-carboxylic Acid Methyl Ester (9b). A suspension of 13.7 g (0.10 mol) of 4-aminobenzoic acid in 90 mL of 4 N HCl was maintained below 0 °C and stirred while 7.55 g (0.106 mol) of $NaNO_2$ was added, followed by 30.0 g of NaOAc and a solution of 20.3 g (0.10 mol) of **3b** in 50 mL of ethanol. The reaction was allowed to warm to room temperature, diluted with H_2O, and extracted with CH_2Cl_2. The extract was dried and concentrated to leave 41 g of an orange oil, which was dissolved in 300 mL of HOAc and 100 mL of concentrated HCl. This solution was gradually heated with an oil bath and at about 65 °C gas evolution commenced. The reaction was kept at 70 °C; after 1.5 h gas evolution had stopped, after 2.5 h the reaction was heterogeneous, and after 3.5 h it was cooled and filtered. The filtrate was concentrated under vacuum and the residue was mixed with H_2O and CH_2Cl_2. The resulting solid (of impure **9a**) was mixed with the original solid (total weight 21.2 g) and suspended in 200 mL of methanol. The methanol was then saturated with HCl and heated under reflux for 6 h. The benzene-insoluble solid was recrystallized repeatedly from methanol with charcoal, filtered over silica gel in ethyl acetate, and recrystallized from methanol again to give 2.20 g (8%) of **9b** as colorless crystals, mp 250–253 °C. Anal. ($C_{17}H_{12}N_2O_2$) C, H, N.

2-Cyano-3-phenylindole-5-carboxylic Acid (9a). A suspension of 1.50 g (5.4 mmol) of **9b** in 50 mL of 6 N HCl was heated under reflux, enough ethanol (~100 ml) was added to effect solution, and heating was continued for 11 days. The reaction was concentrated to a small volume under vacuum, diluted with water, and made basic with NaOH. Filtration gave 840 mg of recovered **9b**. The basic solution was acidified, and the amorphous precipitate was collected by filtration and crystallized from methanol with charcoal to give 111 mg (18%) of **9a** as colorless crystals, mp 302–304 °C. Anal. ($C_{16}H_{10}N_2O_2$) C, H, N.

α-Acetyl-α-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenylazo]benzenepropanamide (5c). The reaction of 0.04 mol of **2** with 7.65 g (0.04 mol) of α-acetylbenzenepropanamide (**3c**)[16] gave 19.28 g of crude **5c** as an orange oil, which gradually crystallized. This material was recrystallized only with difficulty and was generally used as is for subsequent reactions. An analytical sample was prepared by repeated solution in CH_2Cl_2, dilution with CCl_4, and slow evaporation of the CH_2Cl_2 to give **5c** as yellow crystals, mp 128–133 °C. Anal. ($C_{20}H_{17}F_6N_3O$) C, H, F, N.

α-[1-[4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]hydrazin-1-ylidene]benzenepropanamide (6c). A. From 1 and 4b. A solution of 3.86 g (0.02 mol) of α-(aminocarbonyl)benzenepropanoic acid (**4b**)[17] in 30 mL of H_2O containing sufficient NaOAc to effect solution was allowed to react with 0.02 mol of **2** to give after recrystallization from ether–benzene 701 mg (8%) of the analytical sample of **6c** as cream crystals, mp 211–213 °C. Anal. ($C_{18}H_{15}F_6N_3O_2$) C, H, F, N.

B. From 5c. A 75% yield of **6c** was obtained by HCl in ethanol treatment of crude **5c**.

4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenylhydrazine (10). A solution of 0.10 mol of **2** was added rapidly to a 0 °C solution of 31.5 g (0.25 mol) of Na_2SO_3 in 200 mL of H_2O. The reaction was gradually heated to and kept at 78 °C for 1 h. It was then acidified with HCl and kept at 78 °C overnight. The solution was filtered through a filter aid, cooled, and made basic with Na_2CO_3. The resulting precipitate was collected by filtration and recrystallized from ether–hexane to give 9.74 g (35%) of **10** as colorless crystals, mp 131.5–133 °C. The analytical sample was crystallized from ether–benzene and had an identical melting point. Anal. ($C_9H_8F_6N_2O$) C, H, F, N.

Acetic Acid 2-[4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenylhydrazide] (11). To a solution of 5.48 g (0.02 mol) of **10** in 25 mL of ether was added two drops of HOAc and 2.38 g (0.035 mol) of 2-oxopropanitrile. After the reaction had stood for 2 h it was washed with H_2O, dried, and concentrated with the addition of hexane. The resulting precipitate was recrystallized from ether–CH_2Cl_2 to give 2.58 g (41%) of **11** as tan crystals, mp 177–179 °C. Further recrystallization gave the analytical sample as cream crystals, mp 177.5–179 °C. Anal. ($C_{11}H_{10}F_6N_2O_2$) C, H, F, N.

2-Methyl-3-oxo-2-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenylazo]butanamide (5d). The reaction of **2** and 2-methyl-3-oxobutanamide (**3d**) gave a 91% yield of **5d** as yellow crystals, mp 148.5–151.5 °C. Recrystallization from ether–CH_2Cl_2 gave the analytical sample, mp 149–151 °C. Anal. ($C_{14}H_{13}F_6N_3O_3$) C, H, F, N.

2-[1-[4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]hydrazin-2-ylidene]propanamide (6d). Hydrogen chloride was passed into a solution of 26.8 g (0.070 mol) of **5d** in 300 mL of ethanol until the original gold color had changed to pale yellow and the solution had become just barely warm. The ethanol was removed at room temperature under vacuum, and the resulting solid was slurried with ether and filtered to give 17.0 g of **6d** as very pale yellow, ether-insoluble crystals, mp 212–213.5 °C, after turning colorless at ~170 °C. When this solid was heated enough with ether to effect solution, it was converted into a colorless, rather more readily ether soluble, solid which after recrystallization from ether–CH_2Cl_2 had mp 213.5–215 °C. Anal. ($C_{12}H_{11}F_6N_3O_2$) C, H, F, N.

Concentration of the original ether mother liquor with the addition of CH_2Cl_2 gave additional material of comparable melting point for a total yield of 22.42 g (94%).

2-[1-[4-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]hydrazin-2-ylidene]propanoic Acid Ethyl Ester (6e Isomer A). A. From 6d. A solution of 250 mg (0.73 mmol) of **6d** in ethanol containing some HCl was heated under reflux for 10 h and then concentrated under vacuum. The residue was mixed with ether and filtered to remove just a little unreacted **6d**. The filtrate was concentrated and the resulting yellow oil was scratched with benzene. The resulting solid was recrystallized twice from ether–benzene to give 45 mg (17%) of **6e** isomer A as pale yellow crystals, mp 167–168.5 °C.

B. From 1. The reaction of **2** with 2-methyl-3-oxobutanoic acid ethyl ester gave a 30% yield of **6e** isomer A as pale yellow crystals from ether–CH_2Cl_2, mp 168–170 °C. The analytical sample had mp 169–171 °C. Anal. ($C_{14}H_{14}F_6N_2O_3$) C, H, F, N.

6e Isomer B. The mother liquors of a sample of **6e** isomer A prepared from **6d** were concentrated and passed over a silica gel column in CH_2Cl_2 solution. The first eluted material was isolated and analyzed as a pale yellow oil but which subsequently crystallized and which, after recrystallization from hexane, had mp 67–69 °C. Anal. ($C_{14}H_{14}F_6N_2O_3$) C, H, F, N.

3,6-Dimethyl-2-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-2*H*-1,2,4-triazin-5-one (12). A mixture of 10.00 g (0.029 mol) of **6d**, 50 mL of HOAc, and 10.0 mL of BF_3 etherate was heated on the steam bath for 40 h and then concentrated under vacuum. The residue was shaken with 400 mL of ether and filtered through a filter aid. The filtrate was washed with aqueous $NaHCO_3$, dried, and evaporated. Trituration with a little ether gave some solid, which after recrystallization from methanol–ethyl acetate gave 3.00 g (28%) of **12** as colorless crystals, mp 264–266 °C. The analytical sample had mp 265–266 °C. Anal. ($C_{14}H_{11}F_6N_3O_2$) C, H, F, N.

5-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carboxamide (7f). A mixture of 10.00 g (0.029 mol) of **6d** and 50 g of $ZnCl_2$ was stirred and heated with an oil bath at 145 °C for 6 h. The reaction was allowed to cool somewhat, mixed with 1 N HCl, and extracted with ether. The extracts were washed with 0.5 N HCl and with water, dried, and concentrated. The residue was recrystallized from ether–CH_2Cl_2, passed over some silica gel in ether, and recrystallized again to give 3.76 g (40%) of **7f** as cream crystals, mp 264–266.5 °C. The analytical sample had mp 263–266 °C. Anal. ($C_{12}H_8F_6N_2O_2$) C, H, F, N.

Similar results were obtained at 165 °C (3-h reaction) and at 185 °C (1-h reaction).

The ether triturate mother liquor from the preparation of **12** was concentrated with the addition of CH_2Cl_2 to give a 12% yield of **7f**, mp 262–263 °C, contaminated with a green impurity that was difficult to remove.

5-[2,2,2-Trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]indole-2-carbonitrile (7g). Treatment of **7f** with polyphosphate ester (under the conditions used to convert **7d** to **7e**) gave a 70% yield of **7g** as colorless crystals from CH_2Cl_2, mp 183–185 °C. The analytical sample had mp 181–184 °C. Anal. ($C_{12}H_6F_6N_2O$) C, H, N; F: calcd, 36.99; found, 36.51.

Prostaglandin Synthetase Inhibition. Prostaglandin synthetase was prepared as described,[20] and enzymatic analysis was performed according to the literature procedure.[21] For all rate determinations, 30 μM arachidonic acid served as substrate, and results were plotted as log [inhibitor] vs. percent of control velocity. The data were analyzed by the method of least squares, and the values for the slope and intercept were used to calculate the 50% inhibitory concentrations (IC_{50}) of the compounds. The compounds were dissolved in 95% ethanol and added to the enzyme mixture in a volume of 50 μL or less: control studies showed no effect by up to 100 μL of ethanol. The IC_{50} value for indomethacin was determined after a 10-min preincubation. Preincubation of the other compounds with the enzyme mixture up to 10 min prior to substrate addition demonstrated no time-dependent inhibitory characteristics.

Prostaglandin Formation Inhibition. Male rats, five per group, weighing approximately 200 g, were given various doses of the test compounds either intraperitoneally or orally by intubation. One hour later the animals were sacrificed, blood was collected, and serum was prepared. The serum samples were extracted with ethyl acetate. Aliquots of the extract were evaporated under nitrogen and assayed for prostaglandin-like activity by radioimmunoassay employing antibodies raised to $PGF_{2\alpha}$ in rabbits. The percent inhibition value was plotted against log dose, and a value for 50% inhibition was obtained by inspection (Table I).

Platelet-Aggregation Inhibition. Venous blood was collected from human volunteers in siliconized 20-mL Vacutainer tubes fitted with 20-gauge needles using 3.8% sodium citrate solution as the anticoagulant (9 parts of blood to 1 part of the sodium citrate solution). Platelet-rich plasma (PRP) was separated from the red blood cells by centrifugation at 180*g* for 15 min at room temperature. Platelet-poor plasma (PPP) was prepared by centrifuging PRP at 1000*g* for 2 min. Established techniques[22] were used to study platelet aggregation in vitro employing a Payton dual channel aggregation module. One milliliter of PRP was added to a siliconized cuvette containing a siliconized stirring bar and placed in a densitometer maintained at 37 °C and stirred at 1000 rpm. Various concentrations of test compounds were added in 50 μL of physiological saline and incubated with PRP for 5 min. Aggregation was initiated by the addition of sufficient concentrations of ADP, epinephrine hydrochloride, or human mammary-gland collagen (kindly donated by Dr. Harvey Weiss, Roosevelt Hospital, N.Y.) to give about 60% of the maximum aggregation response. The light transmission through PPP was used to determine maximum response. The percent inhibition of aggregation caused by the drug was calculated from the strip chart recordings at the point of maximum collagen response. The percent inhibition value thus obtained was plotted against log concentrations, and a value for 50% inhibition (IC_{50}) was extrapolated from the graph.

Arachidonic Acid Induced Diarrhea Inhibition. Male mice weighing 18–20 g were administered the test compound orally 1 h prior to the intraperitoneal administration of 4 mg/kg of arachidonic acid. An 0.08-mL aliquot of a stock solution containing 25 mg of arachidonic acid per mL of benzene was diluted with

0.08 mL of 95% ethanol, ground together with 50 mg of dry gum acacia with a mortar and pestle, and brought to a volume of 5 mL with distilled water. A dose of 4 mg/kg of arachidonic acid produced a diarrhea graded 3 to 4+ intensity in all mice. The diarrhea was graded on paper towels as follows: 0 = solid pellet or no bowel movement; 1 = slightly soft pellet with little or no wet ring formation; 2 = moderately soft pellet with definite wet ring formation; 3 = soft pellet with large ring formation; 4 = amorphous pellet with very large wet ring formation. The ED_{50} was the dose which reduced the expected diarrhea score of six pretreated mice by 50% compared to the total diarrhea score of six control mice 30 min after arachidonic acid administration.

Gastric Ulcer Induction. This test is a modification of that described.[23,24] Male rats were deprived of food for 18 h prior to testing, while tap water was permitted ad libitum. The test compounds were administered orally 4 h prior to autopsy, at which time the stomachs were removed. The stomachs were divided along the lesser curvature, everted, rinsed in saline, and examined for the presence of focal petechiae. Ulcers were rated on an all or none basis and, in addition, each stomach was graded for the severity of ulcers formed using the following ratings: 0 = none; 1 = trace; 2 = mild; 3 = moderate; 4 = severe. The results of the ulcer scores were subjected to statistical analysis by the student's *t* test.

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5-Fluoro-2'-deoxyuridine 5'-(*p*-Azidophenyl phosphate), a Potential Photoaffinity Label of Thymidylate Synthetase

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5-Fluoro-2'-deoxyuridine 5'-(*p*-azidophenyl phosphate) (1), a potential photoaffinity labeling reagent for thymidylate synthetase from a methotrexate-resistant strain of *Lactobacillus casei*, has been synthesized and characterized. UV_{254} irradiation of mixtures of thymidylate synthetase with 1, containing ^{14}C-labeled phenyl and ^{3}H-labeled pyrimidine rings, in the presence of excess 5,10-methylenetetrahydrofolate, the cofactor for the reaction, produced two complexes, separable from the native enzyme by polyacrylamide gel electrophoresis, in which only the ^{3}H-containing moiety was bound to the protein. When mixtures of enzyme and 1 were irradiated in the absence of cofactor, complexes separable from the native enzyme were not observed. However, the ^{14}C-containing component of 1 was now bound to the protein in the absence of the ^{3}H-containing portion. The results are discussed in terms of the topography of the enzyme active site.

Thymidylate synthetase, which is essential for the replication of both mammalian and bacterial cells, has been a tempting target for investigation during the past 2 decades because control of its function may have potential utility in cancer chemotherapy. The system is also of interest because of the unique mechanistic role played by 5,10-methylenetetrahydrofolate, which acts both as methylene group donor and reductant in the enzymatic synthesis of thymidylate from 2'-deoxyuridylate. Recently, elegant proteolytic degradation studies of the complex formed between the enzyme and 5-fluoro-2'-deoxyuridylate have culminated in the isolation of active-site peptides bound to the pyrimidine moiety of this substrate analogue.[1,2] However, few investigations have been directed toward the phosphate-binding portion of the receptor site since the initial observation that a phosphate group is essential for substrate or inhibitor activity,[3,4] although a recent report has appeared indicating that arginine is important for enzyme activity and the authors suggest it may form an ionic bond to the phosphate dianion.[5] In

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