Synthesis and Antiinflammatory Activities of 3-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-ones

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A series of 3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-ones was synthesized and evaluated as candidate antiinflammatory/analgesic agents as well as dual inhibitors of prostaglandin and leukotriene synthesis. Some compounds that showed dual inhibitory activity were found to possess equipotent antiinflammatory activities to indomethacin, with reduced ulcerogenic effects. One of the compounds, *N*-methoxy-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one, was found to have a wider safety margin than indomethacin or piroxicam, and was selected for detailed evaluation as a candidate drug for clinical application.

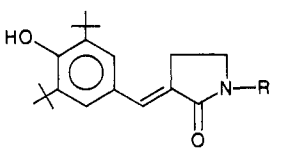
Nonsteroidal antiinflammatory drugs (NSAIDs) are used in the treatment of a number of arthritic conditions, including rheumatoid arthritis and osteoarthritis. The primary mode of action of most NSAIDs is thought to be related to their inhibitory action on prostaglandin biosynthesis.[1] In recent years, another pathway of arachidonic acid metabolism, the 5-lipoxygenase pathway, has been elucidated, and this pathway may also be involved in the inflammatory response.[2] In addition, a possible role of oxygen-derived radicals in the pathology of inflammation has been proposed.[3]

The 2,6-di-*tert*-butylphenol derivatives (Figure 1) R-830,[4] YM-13162,[5] and KME-4[6] appear to represent a new class of NSAIDs with antioxidant properties. As has been shown in the reports on R-830 and KME-4, these compounds are potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase. We have been interested in this class of compounds and have carried out structural modifications in attempts to obtain compounds showing equipotent inhibition of cyclooxygenase and 5-lipoxygenase and offering adequate systemic bioavailability. Amon the compounds tested, 3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one (**1**, Scheme II) showed interesting properties. This paper describes the synthesis and pharmacological properties of analogues of the above pyrrolidin-2-one in which hydrogen at the N atom has been replaced by an alkyl or methoxy group.

Chemistry

The compounds listed in Table I were synthesized by the routes shown in Schemes I and II. γ-Butyrolactone (**8**) was treated with bromine and a catalytic amount of phosphorus tribromide and then refluxed in thionyl chloride to give 2,4-dibromobutyryl chloride (**9**). Reaction of **9** with primary amines, followed by cyclization with base, afforded N-substituted 3-bromopyrrolidin-2-one **10**. The Wittig salts from **10** were treated with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde and a base, such as triethylamine, to give pyrrolidin-2-one derivatives **1–5**. Pyrrolidin-2-one derivatives **6** and **7** were prepared by N-alkylation of compound **1**.

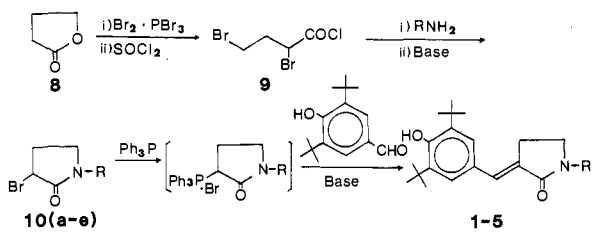
Table I. Antiinflammatory and Analgesic Activities of Pyrrolidin-2-one Derivatives



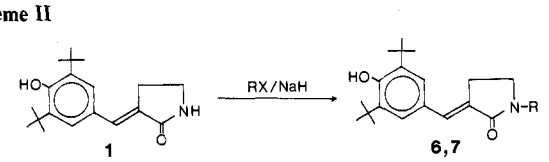
compd	R	hyperthermia:[a] min ED, mg/kg	edema:[b] ED_{30}, mg/kg	pain:[c] ED_{50}, mg/kg
1	H	0.3	1.9, 3.7	2.2
2	OCH_3	0.3	4.8	2.1
3	CH_3	1	10	3.8
4	CH_2CH_3	3	10	12
5	$COCH_3$	3	NA[d]	NA
6	$CH_2CH_2N(CH_3)_2$	10	NT[e]	NT
7	CH_2CH_2OH	10	NT	NT
indomethacin		0.3	1.4, 4.0	0.9
piroxicam		0.3	0.6, 0.9, 2.6	1.0

[a] Minimum effective dose that decreases the surface temperature of rat paw with adjuvant-induced inflammation by more than 2 °C. [b] The values in the case of carrageenin edema were calculated as the mean of five rats/dose with three dose levels. Five experiments were performed with indomethacin or piroxicam as a reference drug. [c] The values were calculated as the mean of 5–14 rats/dose with three dose levels. [d] Not active at the dose level of 10 mg/kg. [e] Not tested.

Scheme I



Scheme II



Biological Results and Discussion

Antiinflammatory activities of the compounds were initially screened by using adjuvant-induced local hyperthermia in rats as the test system.[7] Some compounds were also evaluated in the rat carrageenin foot edema antiinflammatory assay[8] and the rat flection pain test.[9] The

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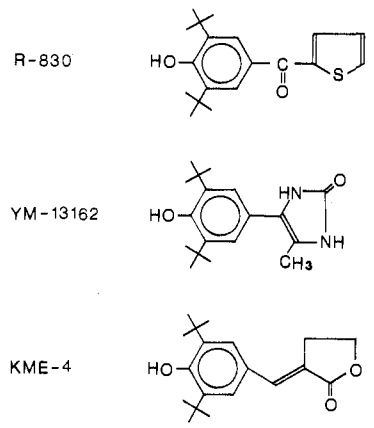

Figure 1. Structural formulas of R-830, YM-13162, and KME-4.

Table II. Inhibition of Adjuvant-Induced Arthritis

compound	dose, mg/kg	edema density[a] exp 1	edema density[a] exp 2	inhibn, %
control		1.00 ± 0.12	1.01 ± 0.14	
1	1	0.64 ± 0.08		36
	3	0.34 ± 0.08		66
2	1	0.30 ± 0.10		70
	3	0.24 ± 0.06		76
3	1		0.50 ± 0.11	50
	3		0.42 ± 0.09	58
4	1		0.71 ± 0.15	30
	3		0.57 ± 0.16	44
5	1		0.47 ± 0.13	53
	3		0.26 ± 0.05	74
indomethacin	1	0.35 ± 0.06		65
	3	0.16 ± 0.03		84
piroxicam	1		0.24 ± 0.08	76
	3		0.15 ± 0.04	85

[a] Each value represents the mean with SE of five rats/dose.

Table III. Inhibitory Effects on Generation of PGE_2 by Synovial Cells and LTB_4 by Neutrophils

compound	PGE_2: IC_{50}, M	LTB_4: IC_{50}, M
1	5×10^{-8}	2.5×10^{-7}
2	3×10^{-8}	2.5×10^{-7}
3	5×10^{-8}	1.5×10^{-7}
4	3×10^{-8}	1.5×10^{-7}
5	2×10^{-7}	3.5×10^{-7}
indomethacin	1×10^{-8}	not active
piroxicam	1×10^{-7}	not active
NDGA	not tested	3.0×10^{-7}

results are shown in Table I. Substituents at the N position of the pyrrolidin-2-one had a negative effect on the antiinflammatory and analgesic activities. Only the methoxy derivative of the pyrrolidin-2-one **2** exhibited the same activity as the unsubstituted compound 1. Antiarthritic activities of the compounds were evaluated in the 21-day adjuvant arthritis assay. As shown in Table II,

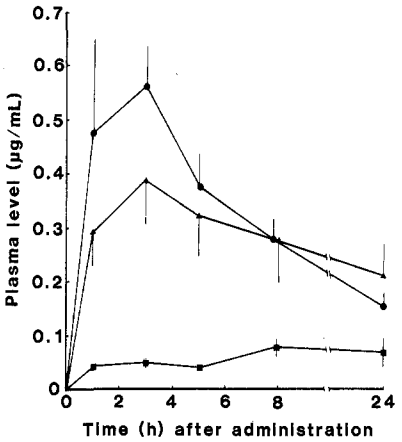

Figure 2. Plasma levels of unchanged compound after oral administration of 10 mg/kg of compound 1 (●), 2 (▲), or 5 (■) in rats. Each value represents the mean with S.E. (n = 3-4).

compounds **2** and **5** each showed a more potent antiarthritic effect than the unsubstituted compound 1.

Table III shows the inhibitory activities of the compounds on the generation of PGE_2 from rat synovial cells[10] and LTB_4 from human neutrophils. Substituents at the N position of the pyrrolidin-2-one showed little effect on the inhibitory activities against cyclooxygenase and 5-lipoxygenase, while the acetyl derivative **5** showed a reduced inhibitory activity against cyclooxygenase. Since compound **5** showed lower potency on acute inflammation but similar potency on adjuvant arthritis to compounds 1 and **2**, plasma levels of these compounds were compared in rats after oral administration. As shown in Figure 2, the plasma levels of compound **5** were lower than those of compound 1 and **2**. The lower potency of compound **5** on acute inflammation might be partly due to slower absorption of the compound from the gastrointestinal tract as compared with the other compounds.

The inhibition of cyclooxygenase by NSAIDs causes characteristic gastrointestinal side effects such as gastric hemorrhage, lesions, and ulcers. Therefore, compound 1 and its methoxy derivative **2** were tested for gastric ulcerogenicity. The results in Table IV show that compounds 1 and **2** are both less ulcerogenic than indomethacin and piroxicam.

Ultimately, compound **2** was chosen for detailed evaluation because of its potent antiinflammatory activity as demonstrated in the carrageenin edema assay and the adjuvant arthritis assay and its low ulcerogenicity.

Experimental Section

General Procedures. Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. NMR spectra were obtained on a JEOL FX-90Q (90 MHz) spectrometer with tetramethylsilane as an internal standard. Where analyses are reported only as elemental symbols, the results

Table IV. Gastric Ulceration in Rats[a]

compound	dose, mg/kg 3	10	30	100
1	1/6 (0.15 ± 0.13)	1/6 (0.33 ± 0.12)	4/6 (1.78 ± 0.27)	3/6 (2.53 ± 1.42)
2	0/6 (0.00)	1/11 (0.26 ± 0.22)	2/12 (0.32 ± 0.27)	2/12 (0.53 ± 0.20)
indomethacin	1/6 (0.30 ± 0.22)	4/6 (1.75 ± 0.51)	6/6 (7.53 ± 1.60)	not tested
piroxicam	1/6 (0.60 ± 0.33)	5/12 (1.01 ± 0.32)	7/12 (4.57 ± 2.18)	not tested

[a] Number of rats with lesions that were more than 0.5 mm in length per total number of rats. The number in parentheses is the mean ulcer index (mm) with SE (see the text).

were within ±0.4% of the calculated values.

2,4-Dibromobutyryl Chloride (9). A mixture of 500 g of γ-butyrolactone (8) and 10 mL of PBr_3 was heated at 100 °C, and 250 mL of Br_2 was added slowly below the surface of the reaction mixture, while the reaction temperature was kept at 110–115 °C. Next, 0.5 mL of dimethylformamide was added at 50 °C, and then 500 mL of $SOCl_2$ was added dropwise at 90 °C (bath temperature). Stirring was continued for a further 3 h. The mixture was distilled twice; the first time, all the distillate was collected, and the second, the fraction boiling at 60–75 °C (1 mmHg) was collected to yield 1.0 kg of the title compound: NMR (δ, $CDCl_3$) 2.4–2.8 (2 H, m), 3.57 (2 H, t, J = 6 Hz), 4.84 (1 H, dd, J = 8, 6 Hz).

3-Bromopyrrolidin-2-one (10a). A solution of 700 g of 2,4-dibromobutyryl chloride in 500 mL of chloroform was added dropwise to a mixture of 400 mL of 28% NH_3 aqueous solution, 500 mL of water, and 2.5 L of chloroform cooled in ice bath, while the reaction temperature was kept below 12 °C. The reaction mixture was heated to 30 °C, and the organic phase was washed with saturated NaCl solution, dried over $MgSO_4$, and evaporated. The solid thus obtained was washed with diethyl ether–hexane and dried to give 360 g of 2,4-dibromobutyramide. A solution of 31 g of Na in 700 mL of ethanol was added dropwise to 800 mL of ethanol containing 360 g of 2,4-dibromobutyramide at room temperature. The mixture was concentrated to give the residue. The residue was dissolved in chloroform, and the solution was washed with water and saturated NaCl solution, dried over $MgSO_4$, and concentrated. The residue was subjected to silica gel column chromatography (acetone–benzene) to give 67 g of the title compound: mp 78 °C; NMR (δ, $CDCl_3$) 2.4–3.0 (2 H, m), 3.3–3.8 (2 H, m), 4.38 (1 H, dd, J = 7, 4 Hz), 7.4 (1 H, br s).

***N*-Methoxy-3-bromopyrrolidin-2-one (10b).** A solution of 1454 g of 2,4-dibromobutyl chloride (9) in 1 L of chloroform was added to a chilled mixture of 520 g of CH_3ONH_2·HCl, 1 L of water, and 5 L of chloroform in an ice bath. With vigorous stirring, 500 g of NaOH in 1 L of water was added slowly to the reaction vessel at below 10 °C, and the mixture was stirred for 1 h. The organic phase was washed with 0.5 N HCl, saturated $NaHCO_3$ solution and saturated NaCl solution, dried over $MgSO_4$, and concentrated in vacuo to give 1400 g of the residue, which was used without further purification. A solution of the above residue in 5 L of benzene was treated with 200 g of NaH (55% in mineral oil) in small portions in an ice bath. The reaction mixture was poured into ice water, and the organic phase was washed with saturated NaCl solution, dried over $MgSO_4$, and concentrated in vacuo to give the residue, which was subjected to silica gel column chromatography (acetone–benzene) to afford 500 g of the title compound: NMR (δ, $CDCl_3$) 2.35 (1 H, ddd, J = 14, 7, 4 Hz), 2.73 (1 H, dt, J = 14, 7 Hz), 3.4–3.9 (2 H, m), 3.81 (3 H, s), 4.38 (1 H, dd, J = 7, 4 Hz).

***N*-Methyl-3-bromopyrrolidin-2-one (10c).** According to the same method as used for **10b**, 77.9 g of **10c** was prepared from 338 g of **9** and 200 mL of 40% methylamine aqueous solution: NMR (δ, $CDCl_3$) 2.2–2.9 (2 H, m), 2.92 (3 H, s), 3.1–3.8 (2 H, m), 4.44 (1 H, dd, J = 7, 4 Hz).

***N*-Ethyl-3-bromopyrrolidin-2-one (10d).** According to the same method as used for **10b**, 140 g of **10d** was prepared from 400 g of **9** and 200 mL of 70% ethylamine aqueous solution: NMR (δ, $CDCl_3$) 1.16 (3 H, t, J = 7 Hz), 2.2–2.9 (2 H, m), 3.2–3.8 (4 H, m), 4.44 (1 H, dd, J = 7, 4 Hz).

***N*-Acetyl-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-pyrrolidin-2-one (5).** A mixture of 16.4 g of 3-bromopyrrolidin-2-one (**10a**) and 30 mL of acetic anhydride was refluxed for 1 h and then concentrated in vacuo to give the residue, which was refluxed with 28.8 g of triphenylphosphine in 50 mL of tetrahydrofuran for 5 h. The reaction mixture was concentrated in vacuo and the residue was stirred with 18.7 g of 3,5-di-tert-butyl-4-hydroxybenzaldehyde, 28 mL of triethylamine, and 200 mL of ethanol at 60 °C for 2 h. The mixture was concentrated, and the residue was dissolved in chloroform. The resultant solution was washed with saturated NaCl solution, dried over $MgSO_4$, and evaporated to give the residue, which was subjected to silica gel column chromatography (ethyl acetate–hexane) to give 22 g of the title compound: mp 228 °C; NMR (δ, $CDCl_3$) 1.47 (18 H, s), 2.62 (3 H, s), 3.03 (2 H, dt, J = 3, 7 Hz), 3.88 (2 H, t, J = 7 Hz), 5.54 (1 H, s), 7.35 (2 H, s), 7.50 (1 H, t, J = 3 Hz). Anal. ($C_{21}H_{29}NO_3$) C, H, N.

3-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one (1). Twenty grams of **5** was stirred with a catalytic amount of potassium carbonate in 200 mL of methanol and 200 mL of ethanol for 1 h at room temperature. After concentration of the reaction mixture, the residue was dissolved in chloroform, and the resulting solution was washed with saturated NaCl solution, dried over $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography (acetone–benzene) and recrystallized from ethyl acetate–hexane to afford 12.7 g of the title compound: mp 210 °C dec; NMR (δ, $CDCl_3$) 1.46 (18 H, s), 3.13 (2 H, dt, J = 3, 6 Hz), 3.52 (2 H, t, J = 6 Hz), 5.45 (1 H, s), 6.98 (1 H, s), 7.32 (1 H, t, J = 3 Hz), 7.37 (2 H, s). Anal. ($C_{19}N_{27}NO_2$) C, H, N.

***N*-Methoxy-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-pyrrolidin-2-one (2).** A mixture of 160 g of *N*-methoxy-3-bromopyrrolidin-2-one (**10b**), 230 g of triphenylphosphine, and 1.5 L of tetrahydrofuran was stirred at 60 °C for 30 h. After cooling of the mixture to 15 °C, the solid was filtered off, washed with tetrahydrofuran, and dried to afford 164 g of the Wittig salt (*N*-methoxy-2-oxopyrrolid-3-yl)triphenylphosphonium bromide. The filtrate obtained above was stirred with 80 g of **10b** and 115 g of triphenylphosphine to give a further 150 g of the Wittig salt according to the same method. A mixture of 300 g of the Wittig salt, 123 g of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 165 mL of triethylamine, and 1.5 L of ethanol was stirred at 60 °C for 2 h and then evaporated. The residue was dissolved in chloroform, and this solution was washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated. The residue was recrystallized from ethanol to give 138 g of the title compound: mp 169 °C; NMR (δ, $CDCl_3$) 1.46 (18 H, s), 3.05 (2 H, dt, J = 3, 7 Hz), 3.66 (2 H, t, J = 7 Hz), 3.88 (3 H, s), 5.44 (1 H, s), 7.30 (2 H, s), 7.35 (1 H, t, J = 3 Hz). Anal. ($C_{20}H_{29}NO_3$) C, H, N.

***N*-Methyl-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-pyrrolidin-2-one (3).** On the basis of the method described for **2**, 25 g of **3** was prepared from 79.9 g of **10c**. The product was purified by silica gel column chromatography (acetone–benzene) and recrystallization from ethyl acetate–hexane: mp 185 °C; NMR (δ, $CDCl_3$) 1.47 (18 H, s), 3.50 (2 H, t, J = 6 Hz), 3.10 (3 H, s), 5.43 (1 H, s), 7.30 (1 H, t, J = 3 Hz), 7.36 (2 H, s). Anal. ($C_{20}H_{29}NO_2$) C, H, N.

***N*-Ethyl-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-pyrrolidin-2-one (4).** By the same method as used for **3**, 23 g of **4** was prepared from 38.4 g of **10d**: mp 186.5 °C; NMR (δ, $CDCl_3$) 1.45 (18 H, s), 2.9–3.1 (2 H, m), 3.3–3.6 (4 H, m), 5.40 (1 H, s), 7.26 (1 H, t, J = 3 Hz), 7.32 (2 H, s). Anal. ($C_{21}H_{31}NO_2$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one (6).** A solution of 500 mg of 3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one (1) in 5 mL of dimethylformamide was treated with 200 mg of NaH (55% in mineral oil) in an ice bath, and then 200 mg of 2-(dimethylamino)ethyl chloride was added and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic phase was washed, dried, and concentrated to give the residue, which was purified by silica gel column chromatography (acetone–benzene) and by recrystallization from ethyl acetate–hexane to give 120 mg of the title compound: mp 150 °C; NMR (δ, $CDCl_3$) 1.46 (18 H, s), 2.26 (6 H, s), 2.50 (2 H, t, J = 7 Hz), 2.9–3.1 (2 H, m), 3.54 (4 H, t, J = 7 Hz), 5.37 (1 H, s), 7.26 (1 H, t, J = 3 Hz), 7.31 (2 H, s). Anal. ($C_{23}H_{36}N_2O_2$) C, H, N.

***N*-(2-Hydroxyethyl)-3-(3,5-di-*tert*-butyl-4-hyroxybenzylidene)pyrrolidin-2-one (7).** *N*-[2-[(2-Tetrahydropyranyl)oxy]ethyl]-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-pyrrolidin-2-one (190 mg; prepared from 150 mg of 1 by the same method as used for **6**) was hydrolyzed with a catalytic amount of concentrated HCl in methanol to yield 110 mg of the title compound: mp 191 °C; NMR (δ, $CDCl_3$) 1.45 (18 H, s), 2.9–3.2 (2 H, s), 3.38 (1 H, t, J = 6 Hz), 3.5–3.7 (4 H, m), 3.84 (2 H, q, J = 6 Hz), 5.41 (1 H, s), 7.2–7.4 (3 H, m). Anal. ($C_{21}H_{31}NO_3$) C, H, N.

Adjuvant-Induced Local Hyperthermia. Male rats of the Fisher strain (F_{344}, 6 week old, Charles River) were given 0.05 mL of a 1% suspension of adjuvant (*Mycobacterium butyricum*) in liquid paraffin by injection into the subplantar tissue of the right hind paw. Test compounds were administered orally as a sus-

pension in 5% gum arabic 3 days after adjuvant injection. The surface temperature of the paw was measured by a contact-type thermometer (Thermomex TH-10, Natsume Seisakusho, Tokyo, Japan) as reported previously.[7]

Carrageenin-Induced Paw Edema. Test compounds as a suspension in 5% gum arabic were orally administered to groups of five male Sprague–Dawley rats (Shizuoka Laboratory Animal Center). Paw edema was induced by subplantar injection of 0.05 mL of 1% λ-carrageenin in 0.9% saline into the right hind paw 1 h after the drug administration. At 3 h after carrageenin injection, the increase in hind paw volume was determined by a water-displacement method.[7,8]

Flection Pain Test. Among the rats used in the local hyperthermia test described above, the rats exhibiting squeaking responses to gentle flection of the affected joints were selected 15–20 days after adjuvant injection.[9] Test compounds as a suspension in 5% gum arabic were orally administered to groups of 5–14 rats, and a series of five flection stimuli were applied at 1, 3, 5, 7, 9, and 11 h after administration. The squeaking responses were counted on each occasion.

Adjuvant-Induced Arthritis. Male Fisher strain rats (F_{344}, 6 week old, Charles River) were given 0.05 mL of a 1% suspension of adjuvant (*M. butyricum*) in liquid paraffin by injection into the subplantar tissue of the right hind paw. Test compounds in 5% gum arabic were orally administered daily from the day of adjuvant injection to day 21. At 24 h after the last administration, the increase in volume of the adjuvant-injected paw was measured by a water-displacement method.

PGE_2 and LTB_4 Generation. Rat synovial cells (2×10^5 cells/well) were cultured for 15 h with a factor derived from rat polymorphonuclear leucocytes which stimulated PGE_2 generation as well as collagenase generation.[10] Test compounds in dimethyl sulfoxide and the factor were simultaneously added; the final concentration of dimethyl sulfoxide was 0.1%. PGE_2 in the culture medium was determined by using a radioimmunoassay kit (NEN). Human neutrophils (2×10^5 cells) were incubated with compounds at 37 °C for 10 min, and A23187 at a final concentration of 1 μg/mL was added to the cells.[11] After 10 min, the cells were pelleted by centrifugation, and LTB_4 in the supernatant was quantitated by using a radioimmunoassay kit (Amersham).

Gastric Ulceration. Male Fisher strain rats (7 week old) were fasted for 24 h. Test compounds as a suspension in 5% gum arabic were administered orally to groups of 6–12 rats. After 6 h, the rats were killed, the stomachs were removed, and gastric lesions on the mucosa were determined by using a stereoscopic microscope. "Ulcer" was defined as at least one lesion that was 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index.

Plasma Levels of Compounds. Test compounds were orally administered as a suspension in 5% gum arabic to Sprague–Dawley rats weighing 300–350 g. Blood samples were obtained by cardiac puncture, and heparinized plasma was stored at −20 °C until analysis. A mixture of 0.2 mL of plasma, 1 mL of water, and 0.2 mL of methanol containing compound **4** as an internal standard was extracted twice with 4 mL of diethyl ether. The ether phase was evaporated to dryness, the residue was dissolved in 200 μL of acetonitrile, and then 20–50 μL of the solution was injected into the HPLC instrument. HPLC analysis was performed on a Zorbax ODS column (8-μm particles, 4.6 mm × 25 cm) with 30% acetonitrile in water as an elution solvent. The flow rate was 1 mL/min, and the absorbance of the effluent was monitored at 310 nm.

Registry No. **1**, 103772-77-6; **2**, 107746-52-1; **3**, 103199-53-7; **4**, 107746-51-0; **5**, 106221-56-1; **6**, 107746-53-2; **7**, 107746-56-5; **8**, 96-48-0; **9**, 82820-87-9; **10a**, 40557-20-8; **10b**, 110027-11-7; **10c**, 33693-57-1; **10d**, 110027-12-8; H_3CONH_2·HCl, 593-56-6; $(H_3C)_2N(CH_2)_2Cl$, 107-99-3; 2,4-dibromobutyramide, 59882-37-0; 3,5-di-*tert*-4-hydroxybenzaldehyde, 1620-98-0; (*N*-methoxy-2-oxopyrrolid-3-yl)triphenylphosphonium bromide, 107746-28-1; (*N*-ethyl-2-oxopyrrolid-3-yl)triphenylphosphonium bromide, 110027-13-9; ((2-tetrahydropyranyl)oxy)ethyl chloride, 5631-96-9; *N*-(2-((2-tetrahydropyranyl)oxy)ethyl)-ethyl)-3-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)pyrrolidin-2-one, 110027-14-0.

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2,4-Diamino-5-benzylpyrimidines as Antibacterial Agents. 8. The 3,4,5-Triethyl Isostere of Trimethoprim. A Study of Specificity[1,2]

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3,4,5-Triethylacetophenone was synthesized in 60% yield by a Friedel–Crafts reaction from 4-ethylacetophenone and converted to 2,4-diamino-5-(3,4,5-triethylbenzyl)pyrimidine (**2**), a trimethoprim (**1**) isostere, by standard techniques. This compound is more lipophilic than **1** by three log units (log *P*, octanol/water). Compound **2** was approximately equipotent with **1** in inhibiting *Escherichia coli* dihydrofolate reductase (DHFR), 2-fold more potent against *P. berghei* and *N. gonorrhoeae* DHFR, and 10 and 25 times better an inhibitor of rat and chicken liver DHFR, respectively. Although the 3,4-dimethoxy analogue **19** was 10-fold less inhibitory to *E. coli* DHFR than **1**, it was 3–4 times *more* potent on the vertebrate isozymes, whereas the diethyl congener **10** followed **19** in its *E. coli* DHFR binding but was less active on rat and chicken DHFR. Therefore, a significant portion of the selectivity of **1** for bacterial, as opposed to vertebrate, DHFR, involves the methoxy functions. An analysis of the X-ray data on **1** and **2** complexed with chicken DHFR, coupled with kinetic data, led to the conclusion that the differences in binding energies of the methoxy and ethyl compounds probably involve desolvation factors, as well as direct energies of interaction with protein atoms. Thus, one cannot invoke lipophilicity or shape alone in explaining the relationship in properties of **1** and **2**.

The 3,4,5-trimethoxy groups of trimethoprim (TMP, **1**) were found at an early date to be very important to its antibacterial activity, as well as to its selectivity for bacterial dihydrofolate reductase (DHFR) over the vertebrate enzymes.[3,4] In this paper we compare the biological

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