Antagonism of amphetamine-induced locomotion was determined by dosing groups of five male rats (210–250 g) with either a graded dose of test compound or vehicle 3.75 h prior to the administration of amphetamine (3.7 mg/kg sc) and then assessing their degree of locomotion after a further 15 min.

Induction of catalepsy was determined by dosing groups of five male rats (200–250 g) with either a graded dose of test compound or vehicle, and then, at appropriate time intervals, placing each paw in turn on a 4.1 cm high rubber cork. A score of 1 was given for each paw which the rat retained on the bung for 15 s. Catalepsy was determined at 1, 2, and 4 h after dosing, and the 4 h figure is quoted.

ED_{50} values and 95% confidence limits for antagonism of apomorphine-induced climbing, amphetamine-induced locomotion, and induction of catalepsy were calculated by the method of Litchfield and Wilcoxon.[26]

Affinities for dopamine D_1 and D_2 receptors were determined in rat striatal tissue by measuring the displacement of *cis*-[^{3}H]-flupenthixol in the presence of spiperone and [^{3}H]spiperone, respectively. Increases in homovanillic acid (HVA) concentrations were determined with groups of five male rats (250–350 g) injected with either a graded dose of test compound or vehicle. After 1 h, the rats were killed swiftly by decapitation, and the corpus striatum and nucleus accumbens were removed and stored in individual sample tubes at −70 °C within 2 min. HVA content was assessed by the method of Westerink and Korf.[27] ED_{200} values (doses to elevate HVA to 200% of control value) and 95% confidence limits were calculated from regression and analysis of variance.

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Registry No. **5a**, 106659-14-7; **5b**, 106659-09-0; **5c**, 106757-54-4; **5d**, 106757-51-1; **5e**, 115114-02-8; **5e**·HCl, 115181-62-9; **5f**, 115114-03-9; **5g**, 115114-04-0; **5g**·HCl, 115114-10-8; **5h**, 115114-05-1; **5h**·HCl, 115114-11-9; **5i**, 115031-78-2; **6e**, 459-22-3; **6f**, 326-62-5; **6g**, 140-29-4; **6h**, 1823-91-2; **6i**, 86-29-3; **8e**, 115031-79-3; **8f**, 115031-80-6; **8g**, 106658-99-5; **8h**, 20946-90-1; **8i**, 28168-63-0; *trans*-**9e**, 115031-84-0; *cis*-**9e**, 115031-87-3; *trans*-**9f**, 115031-82-8; *cis*-**9f**, 115031-88-4; **9g**, 85358-19-6; *trans*-**9h**, 115031-83-9; *cis*-**9h**, 115031-86-2; **9i**, 115031-85-1; **10e**, 115114-06-2; **10f**, 115114-07-3; **10g**, 115114-08-4; **10h**, 115114-09-5; **10i**, 115031-81-7; **11h**, 115115-08-7; $CH_3CH{=}CHCOCH_3$, 625-33-2; 1-bromo-2,2-diethoxyethane, 2032-35-1; but-3-en-2-one, 78-94-4; 4-(acetylamino)-5-chloro-2-methoxybenzoyl chloride, 4516-32-9.

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Etodolac, a Novel Antiinflammatory Agent. The Syntheses and Biological Evaluation of Its Metabolites

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The syntheses of five metabolites of the antiinflammatory drug etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic acid) are described, viz. 6-hydroxyetodolac, *N*-methyletodolac, 4-ureidoetodolac, 8-(1′-hydroxy)etodolac, and 4-oxoetodolac. These syntheses were used to confirm the identities of the metabolites. The metabolites themselves, as well as the previously reported metabolite 7-hydroxyetodolac, were tested in a rat adjuvant edema model and in vitro for their capacity to block prostaglandin production in chondrocyte cells. All either were inactive or possessed only marginal activity. The isolation of *N*-methyletodolac and 4-oxoetodolac from human and rat urine, respectively, is also described.

Etodolac,[1] 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic acid (**1**), is a clinically effective analgesic and antiinflammatory agent, which has been shown to possess an exceptional safety profile with respect to the gastrointestinal tract and renal function and to have the potential to retard the progression of skeletal changes in rheumatoid arthritis.[2] The metabolic disposition of etodolac has been studied in various species, including humans,[3-5] and several metabolites have been isolated. 6-Hydroxyetodolac (**2**), and 7-hydroxyetodolac (**3**), as their methyl esters, were identified in human urine by comparison with samples prepared via a low-yield microbial transformation, wherein the positions of the hydroxyl groups had been assigned on the basis of NMR spectral data.[4] 4-Ureidoetodolac (**4**) and 8-(1′-hydroxy)etodolac (**5**) were identified in human urine, and their structures were assigned on the basis of NMR and mass spectral data of derivatives,[4,6] while 4-oxoetodolac (**6**) was identified in rat urine (see below). *N*-Methyletodolac (**7**) was recently reported, without details, to be present in human urine,[7] and this finding is confirmed by the present study; its structure was tentatively assigned on the basis of NMR and mass spectral data.[7]

In order to confirm the identities of these metabolites by comparison with authentic samples, the syntheses of **2** and **4–7** have been carried out; a synthesis of metabolite **3** was recently reported.[8] We describe also the biological evaluation in vitro and in vivo of metabolites **2–7**, as well

(1) ULTRADOL, LODINE; registered trade marks, Ayerst Laboratories, New York, NY.
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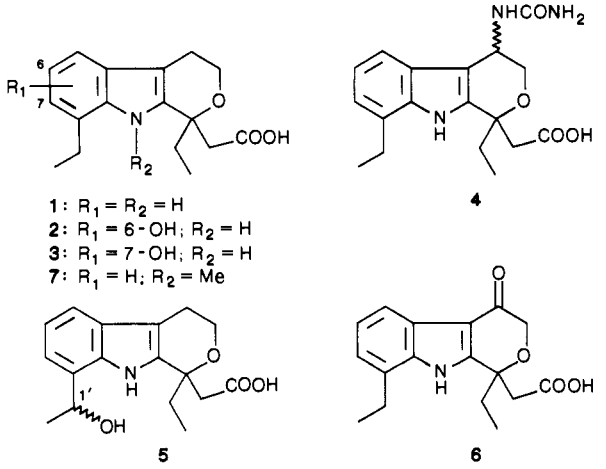


as the isolation of *N*-methyletodolac (**7**) and 4-oxoetodolac (**6**) from human and rat urine, respectively.

Results and Discussion

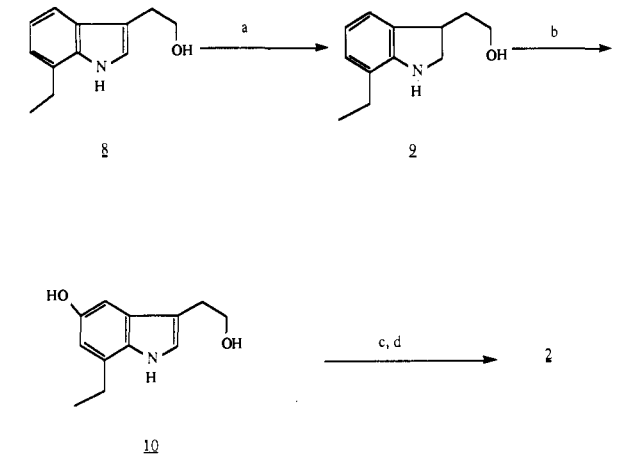
The strategy for introduction of the C-6 hydroxyl group in **2** is based on the recognition that Fremy's salt oxidizes dihydroindoles to their corresponding 5-hydroxylated indoles.[9] Stadler et al.[10] have successfully utilized this oxidation to synthesize 12-hydroxylysergic acid amides from 2,3-dihydrolysergic acid amides, albeit in a low yield.

Scheme I depicts the four-step pathway and begins with 7-ethyltryptophol (**8**), a readily available intermediate in the synthesis of etodolac.[11] Reduction to the corresponding indoline **9** was accomplished by treatment with sodium borohydride pellets in trifluoroacetic acid.[12] Reaction of **9** with potassium nitrosodisulfonate in pH 7 buffer gave an unidentified highly polar product, which, upon standing overnight at room temperature, converted to 7-ethyl-5-hydroxytryptophol (**10**). Flash chromatography provided **10** in a 50% yield along with the dehydrogenated starting material **8**. The remainder of the synthesis was analogous to that of etodolac; condensation of the tryptophol **10** with methyl 3-methoxy-2-pentenoate in methylene chloride in the presence of 2.5 equiv of boron trifluoride etherate afforded the pyrano[3,4-*b*]indole ester, which, upon alkaline hydrolysis, supplied 6-hydroxyetodolac (**2**). It was identical (TLC, melting point, IR, ^{1}H NMR) with that previously obtained by microbial transformation and from human urine.[4]

The synthetic pathway to the 4-ureido- and 4-oxoetodolac metabolites is shown in Scheme II and is analogous to one recently described.[13] The formation of the 4-substituted pyrano[3,4-*b*]indole system was achieved by the condensation of the β-formamidotryptophol **16** with methyl 3-methoxy-2-pentenoate to give 4-formamidoetodolac methyl ester (**17**). The required intermediate, β-formamidotryptophol **16**, was prepared from the 7-ethylindole-3-glyoxylate **11** by conversion to oxime **12** and successive reductions to amino ester **13** and β-aminotryptophol **14**. Attempts to generate 4-aminoetodolac methyl ester (**19**) directly from the β-aminotryptophol **14** were unsuccessful. However, the β-formamidotryptophol **16**, obtained from the *O*,*N*-diformyltryptophol **15** by selective hydrolysis, reacted with methyl 3-methoxy-2-pentenoate in the presence of boron trifluoride etherate to give a 62% yield of the 4-formamidopyrano[3,4-*b*]indole **17** as a 1:1 mixture of diastereomers. After ester hydrolysis to the acids **18**, they were separated into the pure diastereomers by reverse-phase chromatography. Amide hydrolysis of the formamido esters **17** afforded amino esters **19**, which, on reaction with potassium cyanate, afforded the 4-ureido esters **20**, which, in turn, were separated into the individual diastereomers by reverse-phase chromatography. Hydrolysis of esters **20** gave 4-ureidoetodolac (**4**), which was submitted for biological testing as a diastereomeric mixture of benzylamine salts.

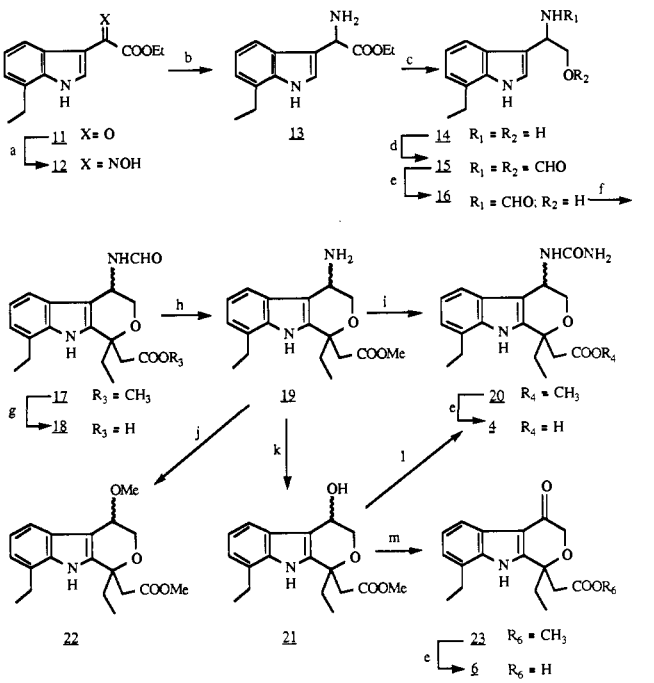
The chemical behavior of 4-aminoetodolac methyl ester (**19**) was investigated and resulted in the discovery of an

Scheme I[a]



[a] (a) $NaBH_4$–CF_3COOH; (b) $(KSO_3)_2NO$; (c) $C_2H_5C(OCH_3)$=$CHCOOCH_3$–BF_3–Et_2O; (d) aqueous KOH.

Scheme II[a]



[a] (a) NH_2OH–NaOAc; (b) H_2–Pd/C–HCl; (c) $LiAlH_4$; (d) $HCOOCOCH_3$; (e) K_2CO_3–MeOH/H_2O; (f) $C_2H_5(OCH_3)$=$CHCOOCH_3$–BF_3·Et_2O; (g) K_2CO_3–H_2O; (h) HCl–MeOH; (i) KCNO–HCl; (j) HCHO–H_2O–MeOH; (k) HCHO–H_2O; (l) $(H_2N)_2$CO–HCl; (m) MnO_2–Et_2O.

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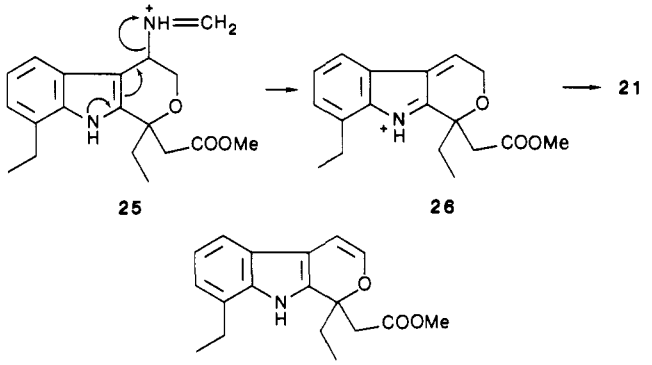
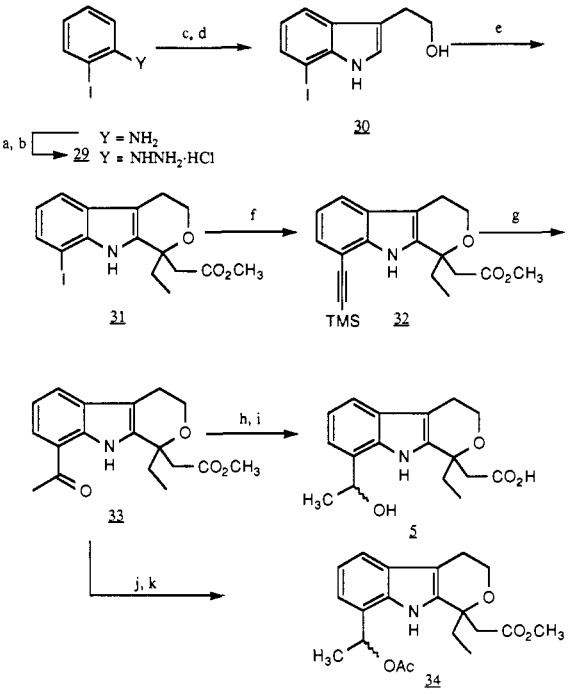
unusual route to 4-ureido- and 4-oxoetodolac. When the 4-amino derivative **19**, as a 1:1 diastereomeric mixture, was treated with paraformaldehyde in aqueous tetrahydrofuran at reflux for 1 h, an 80.5% yield of 4-hydroxyetodolac ester **21** was obtained as a 19:1 mixture of diastereomers. While the precise mechanism of the formation of **21** from **19** is unknown, the simplest route would involve the formation of the indolenine **26**, which could directly generate 4-hydroxyetodolac ester; the required presence of paraformaldehyde for the reaction to proceed suggests that it facilitates the elimination of the 4-substituent by the formation of a species such as **25**. All attempts to obtain the 1,9-dihydropyrano[3,4-*b*]indole system **27**, by elimination of water from **21**, were unsuccessful. When the 4-amino-



noetodolac ester **19** was treated with aqueous formaldehyde in methanol, an 80% yield of 4-methoxyetodolac methyl ester (**22**) was obtained as an 8.5:1 diastereomeric mixture.

Oxidation of 4-hydroxyetodolac methyl ester (**21**) with manganese dioxide followed by hydrolysis afforded 4-oxoetodolac (**6**); its methyl ester **23** was identical, by MS and HPLC analysis, with material isolated from rat urine and treated with diazomethane. When **21** was treated with urea in acidified aqueous tetrahydrofuran, complete conversion to a 1:1 diastereomeric mixture of 4-ureidoetodolac methyl esters (**20**) was observed. This facile conversion of 4-hydroxyetodolac ester to the 4-ureido derivative suggests that the presence of 4-ureidoetodolac in urine might derive from 4-hydroxyetodolac, and Ferdinandi et al. have indeed demonstrated that 4-ureidoetodolac is formed when 4-hydroxyetodolac is incubated in urine at pH 3–6.[6] There are several instances where ureido derivatives have been reported to be metabolites;[14-22] these may have been derived from urea present in both urine and serum.

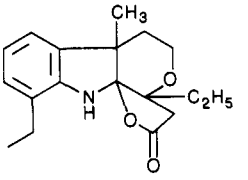
Scheme III[a]



[a] (a) $NaNO_2$, HCl; (b) $SnCl_2$; (c) 2,3-dihydrofuran; (d) $ZnCl_2$, ethylene glycol, 150 °C; (e) $C_2H_5C(OCH_3)=CHCO_2CH_3$, BF_3·OEt_2, CH_2Cl_2; (f) TMSCCH, CuI, $Pd(P(Ph)_3)_4$, $HNEt_2$; (g) HgO, H_2SO_4, THF, H_2O; (h) BH_3·THF; (i) NaOH, CH_3OH; (j) BH_3·THF; (k) Ac_2O, NEt_3, DMAP, CH_2Cl_2.

The diastereomeric pair of 4-ureidoetodolac methyl esters (**20**) were shown to be identical with materials isolated from urinary extracts from rat, dog, and humans, which had been methylated with diazomethane.[6] The isolated esters **20** were found by HPLC analysis to be a 1:1 mixture of diastereomers,[6] showing that there was no diastereoselectivity in the formation of these products in vivo, or when prepared chemically.

The *N*-methyl metabolite **7** was prepared in a facile manner by treatment of etodolac with excess sodium hydride and iodomethane in tetrahydrofuran. A substantial quantity of the C-alkylation product **28** was also obtained.



28

The methyl ester of **7** was identical, by GC–MS analysis, with material obtained from human urine extracts, followed by treatment with (trimethylsilyl)diazomethane. While N-methylation is not a common metabolic route, a number of examples exist; e.g., adamantylamine,[23] 1,4-dihydroxyphthalazine,[24] and a 2-amino-1,3,4-triazole derivative[25] are biotransformed to *N*-methyl derivatives. However, no examples could be found in which an indolic nitrogen was methylated metabolically. A recent reinvestigation[26] of the reported methylation of the indole ring nitrogen of tryptamine by a mammalian *N*-methyl-

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Table I. Biological Evaluation of Etodolac Metabolites

compd	adjuvant edema assay: % inhibn[a]	chondrocyte assay:[b] $IC_{50} \times 10^{-8}$ M
1 (etodolac)	65 ± 12[c]	2.5 (54)
2	10	650 (1)
3	8[d]	5000 (1)
4	37[e,f]	>10000 (3)
5	14	200 (1)
6	21[g]	18.5 (3)
7	11	2500 (1)

[a] Compounds were tested at 25 mg/kg unless noted otherwise. [b] The number of test runs is shown in parentheses. [c] This is a historical value, based on 54 determinations, for etodolac in this assay; $p < 0.01$ relative to control. [d] Tested at 12 mg/kg. [e] Administered as the benzylamine salt at 100 mg/kg, equivalent to 69.9 mg/kg of the free acid. [f] $p < 0.05$ relative to control and to etodolac. [g] $p < 0.01$ relative to etodolac.

transferase revealed that methylation had occurred on the primary amino group.[27]

8-(1′-Hydroxy)etodolac (**5**) was synthesized from the 8-iodopyranoindole **31** via acetylene coupling, hydration of the acetylene, and reduction of the resulting ketone (Scheme III). Diazotization and reduction of 2-iodoaniline gave (2-iodophenyl)hydrazine hydrochloride (**29**), which was reacted with dihydrofuran to afford an intermediate hydrazone, which, in turn, was cyclized to 7-iodotryptophol (**30**). Reaction of tryptophol **30** with methyl 3-methoxy-2-pentenoate gave 8-iodopyranoindole **31**. Palladium-mediated coupling[28] of 8-iodopyranoindole **31** with (trimethylsilyl)acetylene provided the (trimethylsilyl)-acetylene **32**. Mercury-catalyzed hydration[29] of the acetylene with concomitant proto-desilation afforded 8-acetylpyranoindole **33**. Borane reduction of the ketone followed by saponification of the methyl ester gave the desired product **5** as a 3:2 mixture of diastereomers. Attempts to separate the diastereomers as acids or esters were not successful, and **5** was, therefore, tested as a mixture of diastereomers.

The acetoxy ester derivative **34** was prepared by reduction of 8-acetylpyranoindole **33** with borane, followed by acetylation with acetic anhydride, to give a mixture of diastereomers, which were separated by HPLC. The derivatized metabolite isolated from human urine was identical with **34**, isomer B, by HPLC and NMR comparisons.

Metabolites **2–7** were evaluated, in comparison with etodolac, for their capacity to inhibit prostaglandin E_2 production in cultured chondrocyte cells, in vitro, and to prevent adjuvant-induced hindpaw edema in rats. The results are collected in Table I. Only 4-oxoetodolac (**6**) retained significant in vitro activity. With an IC_{50} of 18.5×10^{-8} M in the chondrocyte assay, it was ~7 times less potent than etodolac; it had only slight activity in vivo. The other metabolites showed only marginal activity in vitro. In the adjuvant edema assay, only 4-ureidoetodolac (**4**) exhibited significant ($p < 0.05$) antiinflammatory activity. However, **4** was prepared from a diastereomeric mixture of the corresponding methyl esters **20**, because of the poor recoveries of pure isomers in the preparative reverse-phase HPLC separation of the esters **20**. Thus, **4** was tested as a 2.4:1 mixture of diastereomers, as a benzylamine salt, at 100 mg/kg per day. Under this regimen, the individual diastereomers were administered at doses of 20.5 and 49.1 mg/kg per day, and even if the observed inhibition (37%) was due solely to one of the diastereomers, it would be substantially less potent than etodolac itself (65% inhibition at 25 mg/kg per day). The in vitro result with **4** ($IC_{50} > 10^{-4}$ M) suggests further that the weak activity observed in vivo is probably not ascribable to **4** itself, but more likely to an in vivo transformation product. Attempts to isolate such a product have not been successful.

In summary, the metabolites that have been isolated and identified after the administration of etodolac to various species[30] are all substantially less active than the parent drug as antiinflammatory agents, and the most potent of them, the 4-ureido derivative, is possibly an artifact.

Experimental Section

Melting points were determined on open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 225 spectrophotometer. Except where indicated, ^{1}H NMR spectra were determined in the cited solvent on either a Varian XL-200 (200 MHz) or a Bruker WM-400 (400 MHz) instrument, as indicated, with tetramethylsilane as internal standard. Chemical shifts are given in ppm, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained on an Finnegan 8230 spectrometer and ultraviolet spectra on a Zeiss DMR-21 spectrophotometer. C, H, N were measured on a Perkin-Elmer 240 Analyzer. Silica gel 60 F-254 (Merck) was used for thin-layer chromatography (TLC). Analytical HPLC was performed on a Knauer instrument with a SOTAPHASE C18E column and a Spectroflow 773 detector at $\lambda = 276$ nm (for **21** and **22**) or $\lambda = 214$ nm (for **20**).

2,3-Dihydro-7-ethyl-1*H*-indole-3-ethanol (9). A mixture consisting of 7-ethyl-1*H*-indole-3-ethanol (8 28.0 g, 0.148 mol) and CF_3COOH (250 mL) was stirred at room temperature. $NaBH_4$ pellets (5.4 g, 0.145 mol) were added over a 4-h period. After the addition was complete, the reaction mixture was stirred for 1 h. The reaction mixture was poured onto ice and made alkaline with 50% NaOH (pH 10). The aqueous layer was extracted with Et_2O (3 × 200 mL). The Et_2O layers were combined and extracted with 5% HCl solution (3 × 200 mL). The combined acidic solutions were then made alkaline with 50% NaOH and extracted with Et_2O (3 × 200 mL). The combined Et_2O layers were washed with H_2O (2 × 200 mL) and once with brine (200 mL), dried ($MgSO_4$), filtered, and concentrated to afford 17.3 g of oil. Flash chromatography with 3:2 EtOAc–hexane and then 4:1 EtOAc–hexane afforded 13.5 g (48%) of **9**: mp 73–75 °C (Et_2O–petroleum ether); ^{1}H NMR (200 MHZ, $CDCl_3$) δ 6.98 (d, $J = 7.3$ Hz, 1 H, Ar), 6.93 (d, $J = 5.3$ Hz, 1 H, Ar), 6.74 (t, $J = 7.5$ Hz, 1 H, Ar), 3.7 (m, 3 H, OCH_2OH), 3.68 (m, 1 H, NH_2CH_2), 3.56 (m, 1 H), 3.33(m, 1 H, $NHCH_2$), 2.49 (q, $J = 7.6$ Hz, 2 H, CH_2CH_3), 2.11 (m, 1 H, CH_2CH), 1.80 (m, 1 H, CH_2CH_2), 1.21 (t, $J = 7.6$ Hz, CH_2CH_3); the HCl salt (mp 118 °C, MeOH–Me_2CO–Et_2O) was used for microanalysis. Anal. ($C_{12}H_{17}NO$·HCl) C, H, N.

7-Ethyl-5-hydroxy-1*H*-indole-3-ethanol (10). A solution of potassium nitrosodisulfonate (17.0 g, 0.063 mol) in pH 7 buffer (760 mL) was added over a 20-min period to a stirring solution of **9** (5.0 g, 0.026 mol) in 350 mL of Me_2CO. Ten minutes after the addition was completed, the reaction mixture was extracted with EtOAc (4 × 300 mL). The combined EtOAc layers were washed with distilled H_2O (2 × 200 mL) and once with brine (200 mL), dried ($MgSO_4$), and concentrated to afford 5.6 g of crude. The crude was loaded onto a silica gel flash chromatography column. The next day it was purified by flash chromatography with 7:3 EtOAc–hexane to afford 2.5 g (50%) of **10**, mp 117–118 °C (2-propanol–Et_2O–petroleum ether), and 2.4 g (48%) of starting 7-ethyl-1*H*-indole-3-ethanol (**8**). Attempts at scaling up gave lower yields. **10**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1 H), 6.97 (d, $J = 2.3$ Hz, 2 H, arom), 6.60 (d, $J = 2.1$ Hz, 1 H, arom), 6.40

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(d, J = 2.1 Hz, 1 H, arom), 4.55 (t, J = 5.3 Hz, 1 H, CH_2OH), 3.57 (m, 2 H, 3.32 (s, 1 H, Ar OH, 2.7 (m, 4 H), 1.20 (t, J = 7.5 Hz, 3 H). Anal. ($C_{12}H_{15}NO_2$) C, H, N.

1,8-Diethyl-1,3,4,9-tetrahydro-6-hydroxypyrano[3,4-*b*]-indole-1-acetic Acid (2) and Its Methyl Ester. A mixture consisting of **10** (15.5 g, 0.076 mol), 20% THF–CH_2Cl_2 (1200 mL), methyl 3-methoxy-2-pentenoate (17.5 g, 0.0121 mol), and $BF_3{\cdot}Et_2O$ (23.0 mL, 0.187 mol) was stirred at room temperature for 28 h. The reaction mixture was diluted with 100 mL of CH_2Cl_2 and washed with 5% $NaHCO_3$ (3 × 500 mL) and once with H_2O, dried ($MgSO_4$), and concentrated to give 30.3 g of crude material. Flash chromatography with 3:1 EtOAc–hexane as an eluent afforded 16.0 g (66%) of **6-hydroxyetodolac methyl ester**, mp 153–154 °C (EtOAc–petroleum ether), identical with material isolated from human urine and treated with diazomethane:[4] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (brs, 1 H), 6.74 (d, J = 23 Hz, 1 H, arom), 6.59 (d, J = 2.4 Hz, 1 H, arom), 4.50 (s, 1 H, Ar OH), 4.00 (m, 2 H), 3.70 (s, 3 H), 3.0–2.70 (m, 6 H), 2.20 (m, 2 H), 1.33 (t, J = 7.6 Hz, 3 H), 0.82 (t, J = 7.6 Hz, 3 H). Anal. ($C_{18}H_{23}NO_4$) C, H, N. Hydrolysis by treatment with KOH in MeOH–H_2O (10:1, 1000 mL) under reflux for 5 h was followed by acidification to pH 2 with 6 N HCl and extraction with $CHCl_3$ (4 × 300 mL). The combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford 15 g of foam. Crystallization from CH_3CN–toluene gave 13 g (85%) of **2**: mp 172–173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1 H, NH), 6.51 (d, J = 2.1 Hz, 1 H, Ar), 6.39 (d, J = 1.96 Hz, 1 H, Ar), 3.89 (m, 2 H), 2.89 (q, 2 H), 2.87 (d, J = 13.5 Hz, 1 H, CH_2COOH), 2.75 (d, J = 15.8 Hz, 1 H, CH_2COOH), 2.71–2.57 (m, 2 H), 1.99 (q, J = 7.2 Hz, 2 H), 1.20 (t, J = 7.6 Hz, 3 H), 0.60 (t, J = 8.5 Hz, 3 H); IR (KBr 3590 (OH), 1720 cm^{-1} (CO); UV (MeOH, nm) 276 (9940), 295 (6400); MS, m/e 303 (M^+), 285 ($M^+ - H_2O$), 274 ($M^+ - C_2H_5$), 259 ($M^+ - CO_2$), 244 ($M^+ - OH - CH_2CO$), 230 ($M^+ - CO_2 - C_2H_5$). Anal. ($C_{17}H_{21}NO_4$) C, H, N.

7-Ethyl-α-oxo-1*H*-indole-3-acetic Acid Ethyl Ester (11). To a solution of 7-ethylindole (51 g, 0.35 mol) in dry Et_2O (350 mL) at 0 °C was added dropwise a solution of oxalyl chloride (89.9 g, 0.7 mol) in Et_2O (150 mL). The mixture was stirred for 2 h at 0 °C, then the Et_2O was removed in vacuo, and the residue was triturated with Et_2O and then crystallized from hot EtOH to give the product (60 g, 70%), mp 152–154 °C. Anal. ($C_{14}H_{15}NO_3$) C, H, N.

7-Ethyl-α-(hydroxyimino)-1*H*-indole-3-acetic Acid Ethyl Ester (12). Solutions of NaOAc (43.7 g, 0.53 mol) in H_2O (100 mL) and hydroxylamine hydrochloride (37 g, 0.53 mol) in H_2O (100 mL) were added to ethyl 7-ethylindole-3-glyoxylate (**11**, 17.4 g, 0.07 mol) in EtOH (250 mL), and the mixture was heated at reflux for 12 h. The EtOH was removed by distillation and the resultant precipitate was filtered and crystallized from hot EtOH to give the product (13.8 g, 75%), mp 167–169 °C, as a mixture of isomers. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

7-Ethyl-β-(formylamino)-1*H*-indole-3-ethanol Formate (15). To oxime **12** (7.3 g, 0.028 mol) in EtOH (200 mL) were added concentrated HCl (3 mL) and 10% Pd/C (1.0 g). The mixture was hydrogenated at 40 psi for 4 h at 22 °C. The catalyst and the solvent were removed, and the residue was dissolved in H_2O. After washing with CH_2Cl_2, the aqueous phase was basified with 20% NaOH. The precipitated product, **α-amino-7-ethyl-1*H*-indole-3-acetic acid ethyl ester (13**, 6.0 g, 87%), was collected by filtration, washed with H_2O, and dried. A sample crystallized from hot EtOH had mp 120–122 °C. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

The amino ester **13** (4.9 g, 0.02 mol) in THF (50 mL) was added to $LiAlH_4$ (2.3 g, 0.06 mol) in THF (25 mL), and the mixture was heated at reflux for 2 h. The usual workup procedure gave **β-amino-7-ethyl-1*H*-indole-3-ethanol** (**14**, 3.5 g, 85.7%). A sample crystallized from EtOAc–hexane had mp 84–86 °C.

Amino alcohol **14** (48 g, 0.23 mol) and formic-acetic anhydride (prepared from acetic anhydride (320 mL) and 88% formic acid (135 mL)) were combined and kept at 22 °C for 16 h and then poured onto ice and extracted with EtOAc. The extracts were washed with saturated $NaHCO_3$ solution, dried, and evaporated to give the title product **15** (58.6 g, 98%). A sample crystallized from EtOAc–hexane had the following: mp 159–161 °C; IR (KBr) 3310 (NH), 1735 (O–COH), 1650 cm^{-1} (N–COH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.28 (t, J = 7, 3 H), 2.9 (q, J = 7, 2 H), 4.5 (m, 2 H), 5.6 (m, 1 H), 6.9 (d, 2 H), 7.45 (m, 2 H), 8.3 (m, 3 H), 10.95 (m, 1 H). Anal. ($C_{14}H_{16}N_2O_3$) H, N; C: calcd, 64.6; found, 64.14.

7-Ethyl-β-(formylamino)-1*H*-indole-3-ethanol (16). *O,N*-Diformyl derivative **15** (50 g, 0.19 mol), MeOH (800 mL), and 5% aqueous K_2CO_3 (800 mL) were heated at 55 °C for 2 h. The mixture was cooled to 22 °C and the precipitate was collected by filtration, washed with H_2O, and dried in vacuo to afford the title product (39.7 g, 90%). A sample crystallized from EtOH had the following: mp 195–197 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.28 (t, J = 7, 3 H), 2.88 (q, J = 7, 2 H), 3.5 (br s, 1 H), 3.78 (d, J = 6, 2 H), 5.3 (t, J = 6, 1 H), 6.8–7.8 (m, 4 H), 8.3 (s, 1 H), 11.06 (br s, 1 H). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

1,8-Diethyl-4-(formylamino)-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole-1-acetic Acid (18). To a mixture of indoleethanol **16** (5 g, 0.021 mol) and the enol methyl ether of methyl propionylacetate (50 mL) in CH_2Cl_2 was added boron trifluoride etherate (1 mL) under nitrogen. After stirring at 22 °C for 24 h, the mixture was concentrated in vacuo and chromatographed on silica gel. Elution with 2:1 hexane–EtOAc (2 L) afforded **17**, the methyl ester of **18** (4.5 g, 62.3%), a solid, mp 154–156 °C, as a 1:1 mixture of diastereomers.

The ester **17** (3.5 g, 0.01 mol), MeOH (80 mL), and K_2CO_3 (1.7 g, 0.012 mol) in H_2O (10 mL) were heated at reflux under nitrogen for 8 h, then concentrated in vacuo, diluted with H_2O, and extracted with Et_2O. Acidification of the aqueous phase with 1 N HCl and extraction with EtOAc gave a diastereomeric mixture of the title acid (2.2 g 66.6%). The pure diastereomers were separated by reverse-phase chromatography (C_{18} silica gel, 30% MeCN and 70% 1 mM KH_2PO_4, pH 3.0) to give 500 mg of the more polar isomer A and 430 mg of the less polar isomer B. Isomer A: IR (KBr) 3420 , 3320, 1705, 1600 cm^{-1}; MS, m/e 330. Anal. ($C_{18}H_{22}N_2O_4$) C, H, N. Isomer B: IR (KBr) 3370, 3320, 1725, 1620 cm^{-1}; MS, m/e 330. Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

4-Amino-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic Acid Methyl Ester (19). Formylamino esters **17** (5.5 g, 0.01 mol) in 1 N methanolic HCl (4.2 mL) and MeOH (18 mL) were stirred at 25 °C for 72 h, then concentrated in vacuo, diluted with H_2O, and extracted with EtOAc. The aqueous phase was basified with concentrated $NaHCO_3$ solution and extracted with Et_2O to afford the product (1.58 g, 50%), mp 230 °C dec, after crystallization from Et_2O: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.9 (m, 3 H), 1.38 (t, J = 7, 3 H), 2.12 (m, 2 H), 3.0 (m, 4 H), 3.8 (d, 2 H), 4.0 (s, 3 H), 6.9–7.8 (m, 3 H), 9.5 (m, 1 H). Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

1,8-Diethyl-4-hydroxy-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic Acid Methyl Ester (21). To a solution of amine **19** (1.5 g, 0.0047 mol) in THF (5 mL) was added H_2O (5 mL) and paraformaldehyde (0.5 g). The mixture was heated at reflux for 1 h and then concentrated in vacuo and the residue extracted with Et_2O. The Et_2O extracts were washed with saturated $NaHCO_3$ solution and brine, dried, and concentrated in vacuo. Elution from a silica gel column with a 7:3 hexane–EtOAc mixture gave the product (1.2 g, 80.5%), mp 117–118 °C (Et_2O–hexane). Analytical HPLC showed that the product consisted of two diastereomers in a ratio of 19:1. IR (KBr) 3400, 3280, 1720 cm^{-1}; MS, m/e 317 (M^+), 299 ($M^+ - H_2O$). Anal. ($C_{18}H_{23}NO_4$) C, H, N.

1,8-Diethyl-4-methoxy-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic Acid Methyl Ester (22). To a solution of amine **19** (1.0 g, 0.003 mol) in MeOH (25 mL) was added 37% aqueous formaldehyde (7.5 mL) and the mixture was heated at reflux for 30 min and concentrated in vacuo. The residue was dissolved in EtOAc and the solution was washed with 10% aqueous $NaHCO_3$ and brine. After drying and concentration, the residue was chromatographed on silica gel. Elution with petroleum ether–EtOAc (7:3) gave the product (795 mg, 80%), mp 114–115 °C (EtOAc–hexane), with a diastereomeric ratio of 8.5:1, determined by analytical HPLC: IR (KBr) 3400, 1700 cm^{-1}; MS, m/e 331 (M^+), 299 ($M^+ - CH_3OH$). Anal. ($C_{19}H_{25}NO_4$) C, H, N.

4-[(Aminocarbonyl)amino]-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid Methyl Ester (20). To a solution of amines **19** (1.10 g, 3.48 mmol) in EtOH (20 mL), stirred at room temperature under nitrogen, was added aqueous 1 N HCl (6.96 mL) followed by potassium cyanate (0.565 g, 6.96 mmol) dissolved in a little H_2O. After 2 h the EtOH was removed in

vacuo and the residue was extracted with EtOAc. Drying ($MgSO_4$) and flash chromatography (1% MeOH in EtOAc eluent) afforded the product (0.714 g, 57%) as a mixture of diastereomers, which were separated by reverse-phase chromatography (C_{18} silica gel; 30% MeCN in H_2O). The less polar isomer A (190 mg) had the following: mp 179.5–180.5 °C (CH_2Cl_2–petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (t, 3 H), 1.38 (t, 3 H), 2.15 (m, 2 H), 2.9 (m, 4 H), 3.62 (s, 3 H), 4.02 (m, 2 H), 4.3 (m, 2 H), 4.8 (m, 1 H), 5.0 (m, 1 H), 6.9–7.7 (m, 3 H), 9.05 (br s, 1 H); IR (KBr) 3400 (NH), 1720 (COOMe), 1650 cm^{-1} ($NHCONH_2$); MS, *m/e* 359 (M^+), 286 ($M^+ - CH_2COOCH_3$), 270 ($M - NH_2$). Anal. ($C_{19}H_{25}N_3O_4$) C, H; N: calcd, 11.69; found, 12.16.

The more polar isomer B (100 mg), mp 166–168 °C, had the following spectral characteristics: ^1H NMR (400 MHz, $CDCl_3$) δ 0.9 (t, 3 H), 1.36 (t, 3 H), 2.0 (m, 2 H), 2.9 (m, 4 H), 3.7 (s, 3 H), 4.0 (m, 2 H), 4.2 (br m, 2 H), 5.1 (m, 2 H), 6.9–7.6 (m, 3 H), 9.15 (br s, 1 H); IR (KBr) 3410 (NH), 1735 (COOMe), 1655 cm^{-1} ($NHCONH_2$); MS, *m/e* 359 (M^+), 286 ($M^+ - CH_2COOCH_3$), 270 ($M - NH_2$). Anal. ($C_{19}H_{25}N_3O_4$) C, H; N: calcd, 11.69; found, 11.17.

To a solution of 4-hydroxyetodolac methyl ester (**21**, 100 mg, 0.3 mmol) in THF (3 mL) were added urea (20 mg, 0.35 mmol) in H_2O (0.5 mL) and aqueous 1 N HCl (0.2 mL). The mixture was heated at 55 °C for 1 h. TLC analysis showed the absence of starting material and the appearance of a spot having the same R_f value as authentic 4-ureidoetodolac methyl ester, and HPLC analysis indicated a 1:1 mixture of diastereomers. MS, *m/e* 359 (M^+), 286 ($M^+ - CH_2COOCH_3$), 270 ($M - NH_2$).

4-[(Aminocarbonyl)amino]-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid (4), Benzylamine Salt. A solution of a diastereomeric mixture of esters **20** (2.34 g, 6.51 mmol) and K_2CO_3 (1.07 g, 7.8 mmol) in MeOH (62 mL) and H_2O (7.8 mL) was heated at reflux for 18 h under nitrogen. The mixture was concentrated in vacuo, diluted with H_2O, and washed with Et_2O (2 × 20 mL). The aqueous phase was acidified to pH 2 with 1 N HCl and extracted with EtOAc. The extracts were dried ($MgSO_4$) and evaporated in vacuo to give a foam (2.4 g). A portion (1.7 g, 5.18 mmol) was stirred with Et_2O (17.9 mL) and just enough EtOAc to cause dissolution. To the stirred solution was added benzylamine (555 mg, 5.18 mmol); the precipitated benzylamine salt was collected by filtration and washed with Et_2O to give, after drying, 1.9 g of product (87%), shown to be a 2.4:1 mixture of diastereomers by integration of the triplet NMR signals at δ 0.70 and 0.81 generated by the 1-ethyl group: ^1H NMR (400 MHz, DMSO-d_6 δ 0.70 (t, J = 7.2), 0.81 (t, J = 7.2), 1.23 (t, 3 H, J = 7.4), 1.9–2.16 (m), 2.49–2.56 (m), 3.6–3.72 (m), 3.89–4.0 (m), 4.78 (m), 4.84–5.0 (m), 5.4 (m), 5.52 (m), 5.84 (m), 6.03 (m), 6.86 (m), 7.24–7.42 (m), 11.0 (m), 11.26 (m). Anal. ($C_{25}H_{32}N_4O_4$) C, H, N.

1,8-Diethyl-1,3,4,9-tetrahydro-4-oxopyrano[3,4-*b*]indole-1-acetic acid Methyl Ester (23). Hydroxy ester **21** (2.5 g, 8.0 mmol) was added in one portion to a suspension of manganese dioxide (15 g) in 150 mL of H_2O. The solution was allowed to stir at room temperature for 16 h, after which time an extra 2 g of manganese dioxide was added and the mixture allowed to stir an additional 2 h to complete the reaction. The manganese dioxide was removed by filtration through Celite, and the pad was washed with 500 mL of CH_2Cl_2. The solvent was concentrated in vacuo to provide 2.2 g of crude solid, which was crystallized from hot EtOAc–hexane to yield 1.45 g of colorless crystals, mp 192–193 °C. Concentration of the mother liquor provided a second crop (210 mg) of the title keto ester for a yield of 64%: ^1H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, J = 7, 1 H), 7.14 (t, J = 7, 1 H), 7.07 (d, J = 7, 1 H), 4.27 (d, J = 17, 1 H), 4.21 (d, J = 17, 1 H), 3.52 (s, 3 H), 3.31 (s, 2 H), 2.90 (m, 2 H), 2.23 (m, 1 H), 1.88 (m, 1 H), 1.25 (t, J = 7.5, 3 H), 0.81 (t, J = 7, 3 H); IR (KBr) 1735 (C=O), 1620 cm^{-1} (C=O); MS, *m/e* 315 (27.5, M^+), 286 (79.9, $M - C_2H_5$), 242 (100, $M - CH_2COOH_3$). Anal. ($C_{18}H_{21}NO_4$) C, H, N.

1,8-Diethyl-1,3,4,9-tetrahydro-4-oxopyrano[3,4-*b*]indole-1-acetic Acid (6). The ester **23** (1.5 g, 4.8 mmol) was suspended in 30 mL of MeOH and a solution of K_2CO_3 (3.5 g) in 30 mL of H_2O was added. The mixture was heated to reflux, upon which the solution became homogeneous. After refluxing for 2 h, the solution was cooled to room temperature and the MeOH was removed in vacuo. The aqueous solution was made acidic with 6 N HCl, and the resulting cloudy solution was extracted with Et_2O (2 × 50 mL). The Et_2O layer was washed with 25 mL of saturated NaCl solution and then dried over $MgSO_4$. The $MgSO_4$ was removed and the ether was evaporated. Hexane was gradually added until a slight turbidity was observed. The solution was left at 0 °C overnight and the resultant solid was collected by filtration and dried to provide analytically pure title acid (1.31 g, 93%): mp 200–202 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 11.43 (br s, 1 H), 10.84 (br s, 1 H), 7.77 (d, J = 7, 1 H), 7.14 (t, J = 7, 1 H), 7.06 (d, J = 7, 1 H), 4.28 (d, J = 17, 1 H), 4.21 (d, J = 17, 1 H), 3.19 (d, J = 15, 1 H), 2.89 (q, J = 7.5, 2 H), 2.81 (d, J = 15, 1 H), 2.23 (m, 1 H), 2.01 (m, 1 H), 1.24 (t, J = 7.5, 3 H), 0.81 (t, J = 7.4, 3 H); IR (KBr) 3520 (OH), 1715 (C=O), 1620 cm^{-1} (C=O); MS, *m/e* 301 (59.8, M^+), 272 (100, $M^+ - C_2H_5$), 242 (97.9, $M - CH_2COOH$). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

1,8-Diethyl-1,3,4,9-tetrahydro-9-methylpyrano[3,4-*b*]-indole-1-acetic Acid (7) and 3a,10-Diethyl-5,6,6a,11-tetrahydro-6a-methylfuro[3′,2′:2,3]pyrano[3,4-*b*]indol-2(3*H*)one (28). A solution of etodolac (15 g, 0.052 mol) in THF (150 mL) was added to a stirring suspension of NaH (6.6 g of a 60% oil dispersion, 0.16 mol) in THF (400 mL). This was heated at 50 °C for 2 h. After the mixture was cooled to 0 °C, iodomethane (45.6 g, 0.3 mol) was added dropwise. After the addition was complete, stirring was continued at 50 °C for 2 h. The excess hydride was destroyed by dropwise addition of EtOH, and the reaction concentrated, diluted with H_2O (100 mL), and extracted with $CHCl_3$ (3 × 200 mL). The combined extracts were washed with H_2O (100 mL) and dried ($MgSO_4$), and the solvent was removed to afford 16 g of a thick syrup. Flash chromatography on silica gel eluting with 10:1 toluene–Me_2CO mixture gave lactone **28** (3.2 g, 20%) as a solid, mp 183–185 °C (toluene–petroleum ether), and 2.5 g (16%) of the *N*-methyl product **7**, mp 116–119 °C (Et_2O/petroleum ether). **7**: ^1H NMR (400 MHz, DMSO-d_6) 7.24 (dd, J_1 = 7 Hz, J_2 = 1.5 Hz, 1 H, Ar), 6.90 (m, 2 H, Ar), 3.90 (m, 2 H, CH_2O), 3.88 (s, 3 H, NCH_3), 3.06 (q, J = 7.5 Hz, 2 H), 2.99 (d, J = 13.7 Hz, 1 H, CHHCO), 2.79 (d, J = 13.7 Hz, 1 H, CHHCO), 2.64 (m, 2 H, CH_2CH_3), 2.10 (m, 2 H, CH_2CH_3), 1.24 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 0.65 (t, J = 7.4 Hz, 3 H, CH_2CH_3); IR (KBr) 3440, 1705 cm^{-1}. Anal. ($C_{18}H_{23}NO_3$) C, H, N. Compound **28** had the following spectral characteristics: ^1H NMR (400 MHz, DMSO-d_6) δ 6.88 (dd, J_1 = 13 Hz, J_2 = 7.5 Hz, 1 H, Ar), 6.64 (m, 2 H, Ar), 3.74 (m, 1 H, CHHO), 3.60 (m, 1 H, CHHO), 2.83 (d, J = 7.5 Hz, 1 H, CHHCO), 2.60 (d, J = 7.5 Hz, 1 H, CHHCO), 2.47 (m, CH_2), 1.35 (s, 3 H, CH_3), 1.13 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 0.85 (t, J = 7.4 Hz, 3 H, CH_2CH_3); IR (KBr) 3340, 1780 cm^{-1}. Anal. ($C_{18}H_{23}NO_3$) C, H, N.

(2-Iodophenyl)hydrazine Hydrochloride (29). A mixture of concentrated HCl (115.2 mL) and distilled H_2O (51.2 mL) was cooled in an ice bath while 2-iodoaniline (100 g, 457 mmol) was added slowly with vigorous stirring, resulting in a thick tan suspension. The mixture was cooled to −5 °C and a solution of sodium nitrite (35 g, 492 mmol) in H_2O (51.2 mL) was added dropwise. The resulting yellow mixture was maintained at 0 °C while a solution of tin(II) chloride dihydrate (232 g, 900 mmol) in 6 N HCl (312 mL) was added dropwise over 2.5 h, to form a pale-yellow slurry. After stirring at room temperature for 24 h, the mixture was treated with 50% NaOH, added dropwise with vigorous stirring, until the aqueous layer was basic. The free hydrazine was extracted with Et_2O (3 × 1 L). The Et_2O layers were combined, washed with H_2O (1 L) and saturated NaCl solution (1 L), concentrated to 500 mL, and dried ($MgSO_4$). With cooling and vigorous stirring, HCl gas was introduced into the mixture to yield an off-white precipitate. This was collected by filtration, washed with plenty of Et_2O, and dried under vacuum at room temperature to give the product (112 g, 91%) as an off-white solid: IR (Nujol) 3240, 1450–1550 cm^{-1}; ^1H NMR (60 MHz, D_2O) δ 6.8–8.1 (4 H, m).

7-Iodotryptophol (30). The (2-iodophenyl)hydrazine hydrochloride **29** (50 g, 185 mmol) and 2,3-dihydrofuran (14 mL, 13 g, 185 mmol) were dissolved in THF (300 mL) and H_2O, and the mixture was stirred overnight. The reaction mixture was then diluted with Et_2O (300 mL), and the layers were separated. The aqueous layer was washed with Et_2O (200 mL), and the combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated to an oil. The oil was dissolved in ethylene glycol (200 mL), treated with zinc chloride (48.3 g, 354 mmol), and heated to 170 °C for

1 h with vigorous stirring under nitrogen. The reaction mixture was allowed to cool and was poured into a vigorously stirred mixture of Et_2O (1 L) and 1 N HCl (1 L). The layers were separated, and the organic layer was dried ($MgSO_4$), concentrated, and chromatographed (silica gel, 15 cm i.d. X 15 cm ht, 40% EtOAc in hexane) to give the product (25.3 g, 48%) as an oil: IR (KBr) 1428, 1488k, 2800–3600, 3390 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.00 (2 H, t, J = 6.3 Hz, 3.91 (2 H, t, J = 6.3 Hz, 6.90 (1 H, t, J = 7.7 Hz), 7.16 (1 H, d), 7.56 (1 H, d), 7.59 (1 H, d), 8.13 (1 H, br s).

1-Ethyl-1,3,4,9-tetrahydro-8-iodopyrano[3,4-*b*]indole-1-acetic Acid Methyl Ester (31). 7-Iodotryptophol (**30**, 20.0 g, 69.8 mmol) and methyl 3-methoxypent-2-enoate (12.04 g, 83.6 mmol) were dissolved in CH_2Cl_2 (300 mL), and the mixture was cooled to 0 °C and treated with boron trifluoride etherate (520 mg, 450 μL, 3.66 mmol). The mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was then poured into saturated $NaHCO_3$ solution (500 mL). The layers were separated, and the aqueous layer was washed with CH_2Cl_2 (2 × 200 mL) and Et_2O (100 mL). The combined organic layers were dried ($MgSO_4$), concentrated, and chromatographed (silica gel, 15 cm i.d. X 15 cm ht, 5% EtOAc in hexane) to give the pyranoindole (24.3 g, 87%) as a low-melting solid: mp 74–75 °C; IR (KBr) 1710, 2810–3000, 3120–3610, 3310 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, t, J = 7.4 Hz), 2.10 (2 H, m), 2.77 (2 H, m), 2.93 (1 H, d, J = 16.4 Hz), 3.00 (1 H, d, J = 16.4 Hz), 3.75 (3 H, s), 3.98 (2 H, m), 6.86 (1 H, t, J = 7.7 Hz), 7.46 (1 H, d, J = 7.8 Hz), 7.51 (1 H, d, J = 7.8 Hz), 9.16 (1 H, br s); MS (EI), m/z 399 (M^+), 370 ($M^+ - CH_2CH_3$), 233 ($M^+ - CH_2CH_3 - I$). Anal. ($C_{16}H_{18}INO_3$) C, H, N.

1-Ethyl-1,3,4,9-tetrahydro-8-[(trimethylsilyl)ethynyl]-pyrano[3,4-*b*]indole-1-acetic Acid Methyl Ester (32). The 8-iodopyranoindole ester **31** (10 g, 25.1 mmol), bis(triphenylphosphine)palladium chloride (351.8 mg, 0.5 mmol), and copper(I) iodide (40 mg) were dissolved in benzene (100 mL) and diethylamine (40 mL) and bubbled with nitrogen for 20 min. (Trimethylsilyl)acetylene (7.08 mL, 4.92 g, 50.1 mmol) was added and the reaction mixture stirred at room temperature overnight. The mixture was then concentrated and chromatographed (silica gel, 3.9 cm i.d. X 155 cm ht, 10% EtOAc in hexane) to give the silylacetylene (9.24 g, 99%) as an oil: IR (KBr) 1727, 2150, 2810–3080, 3405 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.2 (9 H, s), 0.72 (3 H, t, J = 7.2 Hz), 2.0 (2 H, m), 2.65 (2 H, m), 2.85 (2 H, 2 d) 3.62 (3 H, s), 3.85 (2 H, m), 6.9 (1 H, t), 7.18 (1 H, d), 7.38 (1 H, d), 9.3 (1 H, br s).

1-Ethyl-8-(1-oxoethyl)-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-acetic Acid Methyl Ester (33). The [(trimethylsilyl)acetylene]pyranoindole **32** (8.08 g, 21.9 mmol) was dissolved in THF (100 mL) and treated with mercuric oxide (1.2 g) and aqueous H_2SO_4 (1.1 mL in H_2O (5 mL)) and heated at reflux for 4 h. The reaction mixture was then cooled and partitioned between Et_2O (400 mL) and saturated $NaHCO_3$ solution (200 mL). The Et_2O layer was washed with brine (200 mL), concentrated, and chromatographed (silica gel, 7.5 cm i.d. X 15 cm ht, 15–25% EtOAc in hexane) to give the product (5.31 g, 77%) as a solid. It was triturated with hexane–EtOAc to give pure material (3.6 gg, 52%): mp 108 °C; IR (KBr) 1667, 1718, 2820–3000, 3120–3620, 3400 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (3 H, t, J = 7.318 Hz), 2.1 (2 H, m), 2.70 (3 H, s), 2.85 (2 H, m), 2.94 (1 H, d, J = 15.5 Hz), 2.98 (1 H, d, J = 15.5 Hz), 3.75 (3 H, s), 3.97 (1 H, m), 4.07 1 H, m), 7.15 (1 H, t, J = 9.8 Hz), 7.77 (2 H, 2 d), 10.73 (1 H, br s); MS (EI), m/z 315 (M^+), 286 ($M^+ - CH_2CH_3$), 242 ($M^+ - CH_2CO_2CH_3$). Anal. ($C_{18}H_{21}NO_4$) C, H, N.

1-Ethyl-8-(1-hydroxyethyl)-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid (5). The acetyl ester **33** (3.0 g, 9.52 mmol) was dissolved in THF (60 mL) under nitrogen and treated dropwise over 45 min with borane (31.7 mL of 1 M solution in THF, 31.7 mmol). After 90 min the reaction was cautiously quenched with saturated $NaHCO_3$ solution (10 mL). The mixture was then filtered through Celite, concentrated, and purified by flash chromatography (3.9 cm X 15 cm, 20% EtOAc in hexane) to give the alcohol (2.97 g, 98%) as an oil. The oil was dissolved in MeOH (10 mL), treated with NaOH (1.83 g, 45.7 mmol), and heated to reflux under nitrogen for 1 h. The reaction mixture was allowed to cool, concentrated, and then partitioned between Et_2O (100 mL) and 2 M HCl (30 mL). The Et_2O layer was washed with brine (50 mL), dried ($MgSO_4$), concentrated, and then triturated with hexane to give the product (2.14 g, 77%), a 3:2 mixture of diastereomers, as a white solid: mp 134–135 °C; IR (KBr) 1714, 2400–3200, 3318, 3420, 3480 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (2 X 2 H, 2 t), 1.57 (2 X 3 H, 1 d), 2.06 (2 X 2 H, m), 2.82–2.98 (2 X 2 H, m), 4.60 (2 X 2 H, m), 5.09 (1 H, q), 5.18 (1 H, q), 6.9–7.7 (2 X 2 H, 4 d), 7.41 (2 X 1 H, 2 t), 9.3 (1 H, br s), 9.35 (1 H, br s). Anal. ($C_{17}H_{21}NO_4$) C, H, N.

8-(1-Acetoxyethyl)-1-ethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic Acid Methyl Ester (34). The acetyl ester **33** (100 mg, 0.317 mmol) was dissolved in THF (2.2 mL) and treated with 1 N borane–THF (1 mL) and stirred at room temperature under N_2. After 2 h, the reaction was quenched with saturated $NaHCO_3$ solution (10 mL). The Et_2O was dried ($MgSO_4$) and concentrated to an oil. The oil was dissolved in CH_2Cl_2 (7 mL) and treated sequentially with triethylamine (48.2 mg, 0.476 mmol, 1.5 equiv), acetic anhydride (38.9 mg, 0.381 mmol, 1.2 equiv), and (dimethylamino)pyridine (DMAP) (one drop). After 30 min the mixture was filtered through a silica gel plug and washed through with CH_2Cl_2–MeOH (19:1). The eluent was concentrated and chromatographed by prep HPLC (Rainin Dynamax, 2.2 cm X 30 cm, 8 μm silica, 20% ethyl acetate in hexane) to give compound A (elutes first, 20 mg) and compound B (elutes second, 45 mg).

Isomer A: ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, t, J = 7.4 Hz), 1.69 (3 H, d, J = 6.67 Hz), 2.09 (2 H, m), 2.14 (3 H, s), 2.79 (2 H, m), 2.92 (1 H, d, J = 16.6 Hz), 3.04 (1 H, d, J = 16.6 Hz), 3.70 (3 H, s), 4.01 (2 H, m), 6.24 (1 H, q, J = 6.67 Hz), 7.06 (1 H, t), 7.10 (1 H, d), 7.45 (1 H, d), 9.63 (1 H, br s); MS (EI), m/z 359 (M^+), 226 ($M^+ - CH_2CO_2CH_3$), 270 ($M^+ - O_2CCH_3 - C_2H_5$), 226 ($M^+ - O_2CCH_3 - C_2H_5 - CH_2CO_2CH_3$).

Isomer B: ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (3 H, t, J = 7.4 Hz), 1.68 (3 H, d, J = 6.67), 2.10 (2 H, m), 2.12 (3 H, s), 2.78 (2 H, m), 2.92 (1 H, d, J = 16.3 Hz), 3.72 (3 H, s), 4.01 (2 H, m), 6.26 (1 H, q, J = 6.67 Hz), 7.07 (1 H, t), 7.12 (1 H, d), 7.45 (1 H, d), 9.39 (1 H, br s); MS (EI), m/z 359 (M^+), 330 ($M^+ - C_2H_5$), 286 ($M^+ - CH_2CO_2CH_3$), 270 ($M^+ - O_2CCH_3 - C_2H_5$), 226 ($M^+ - O_2CCH_3 - C_2H_5 - CH_2CO_2CH_3$).

Isolation of Metabolites. 4-Oxoetodolac Identification in Rat. Male CD-1 Sprague–Dawley rats were given seven repeated doses of 15 mg of etodolac/kg at 24-h intervals. The last dose contained 10 μCi [^{14}C]etodolac.[31] About 9% of the radioactive dose was recovered in the urine. The combined urine from seven daily collections was freeze-dried; the residue was extracted with MeOH containing a trace of antioxidant (2,6-di-*tert*-butyl-4-methylphenol; Aldrich Chemical Co.) and filtered through Celite, and the solvent was evaporated. The recovery of radioactivity was about 16%. The urine extract, methylated with diazomethane generated from Diazald (Aldrich Chemical Co.), was chromatographed by TLC (hexane–EtOAc 60:40, v/v) and a major radioactive band having an R_f of about 0.19 was isolated. Previously, the mass spectrum of a component, isolated from rat urine in the same manner, was consistent for 4-oxoetodolac methyl ester.[6] In this study, HPLC analysis of the TLC-fractionated radioactivity showed a radioactive peak at t_R 9.0 min, the same as that for authentic 4-oxoetodolac methyl ester. A second radioactive peak at t_R 6.0 min was the same as that for authentic 8-(1′-hydroxy)etodolac methyl ester, a metabolite isolated from urine of human subjects given [^{14}C]etodolac.[4]

***N*-Methyletodolac Identification in Humans.** Urine (1–2 mL) from two human subjects given 200 mg of [^{14}C]etodolac[31] and collected during the 0–12-h interval after dosing was hydrolyzed with Glusulase (Dupont Pharmaceuticals; 40 000 units, a 9:1 mixture of β-glucuronidase and arylsulfatase enzymes) by incubation of the specimens at 37 °C in 0.2 M NaOAc buffer, pH 4.6, for 20 h. The hydrolysates were extracted on 20 g of neutral Amberlite XAD-2 resin,[32] eluted with MeOH (40 mL), and methylated with excess (trimethylsilyl)diazomethane[32] (Petrarch Systems Inc.). The recovery of radioactivity from the urine was about 90%.

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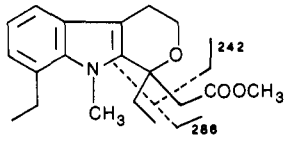
The methylated urine extracts were chromatographed by HPLC on a reverse-phase Microsorb C-18 column (Rainin Instruments; 25 cm X 4.6 mm) under isocratic conditions: 75% MeCN in H_2O mobile phase pumped through the column by a Rabbit HPX pressure module (Rainin Instruments) at a flow rate of 1.5 mL/min. The eluent was monitored at 226 nm by a Gilson Holochrome UV detector. A peak having the same t_R as authentic *N*-methyletodolac methyl ester was collected. The amount of radioactivity associated with this peak was 1–3% of the total urinary radioactivity.

GC–MS analysis on the isolated peak was carried out on a Varian Model 3000 GC instrument interfaced with a Finnigan 8230 mass spectrometer. The following GC conditions were used. The samples were injected onto a RPB 5 capillary column (15 m X 0.25 mm) with the injection temperature at 280 °C and the column temperature initially at 100 °C and then increased after injection at a rate of 25 °C/min to 280 °C. The He carrier gas flow rate was 1.0 mL/min. The interface and spectrometer settings were as follows: heat funnel (interface) temperature, 245 °C; line of sight temperature, 285 °C; MS source temperature, 200 °C; scan rate, 1 s/decade; interscan time, 0.3 s; CO_2 travel time at 100 °C, 18 s; analysis pressure at 100 °C, 9 X 10^{-7} Torr; ionization voltage, 70 eV. The spectrometer was set to monitor ion current for *m/e* 315, the molecular ion of *N*-methyletodolac methyl ester. The t_R of the *m/e* 315 ion current and the mass spectra of the GC peaks were identical for the authentic *N*-methyletodolac ester and the metabolite isolated by HPLC from human urine. The mass spectra showed the characteristic substituted pyranoindole fragmentation pattern: molecular ion (M^+, *m/e* 315), methylene methylacetate cleavage (*m/e* 242), ethyl cleavage (*m/e* 286).



Pharmacology. Adjuvant Edema Assay. Groups of 10 male Sprague–Dawley rats, each weighing 180–200 g, were injected intradermally in the left hindpaw with 0.1 mL of Freund's complete adjuvant (FCA; 0.5 mg of killed and dried *Mycobacterium butyricum* suspended in 0.1 mL of mineral oil). Test compounds or vehicle control (0.5% Tween 80 in distilled water) were administered by gastric lavage immediately before the FCA injection (day 0) and 24 and 48 h after the FCA (days 1 and 2). The volume of the injected hindpaw was measured both before the FCA injection and 24 h after the last drug administration (day 3) by means of a plethysmometer (Buxco Electronics, Sharon, CT). The mean hindpaw volume was calculated for each group, and the mean edema volume represents the difference between the volumes on days 0 and 3. The percent inhibition was calculated as follows:

100 × (mean control edema − mean drug-treated edema)/mean control edema

Statistical comparisons were performed for **1**, **4**, and **6** by using the unpaired *t* test with significance achieved at the $p < 0.05$ level.

In Vitro IC_{50} Determinations. The method for determining IC_{50} values for inhibition of prostaglandin production in stimulated chondrocyte cultures has been described in detail elsewhere.[34] IC_{50} values were estimated from a curve of log dose versus percent inhibition.

Registry No. **1**, 87226-38-8; **2**, 101901-06-8; **3**, 101901-07-9; *cis*-**4**, 114719-97-0; *trans*-**4**, 114719-98-1; *cis*-**4**·$PhCH_2NH_2$, 114719-99-2; *trans*-**4**·$PhCH_2NH_2$, 114720-00-2; **5** (diastereomer 1), 114720-01-3; **5** (diastereomer 2), 114720-02-4; **5** (methyl ester, diastereomer 1), 114720-03-5; **5** (methyl ester, diastereomer 2), 114720-04-6; **6**, 111478-86-5; **7**, 114720-05-7; **8**, 41340-36-7; **9**, 114737-75-6; **9**·HCl, 114737-76-7; **10**, 114720-06-8; **11**, 111478-90-1; (*Z*)-**12**, 114720-07-9; (*E*)-**12**, 114720-08-0; **13**, 111478-93-4; **14**, 111478-94-5; **15**, 111478-95-6; **16**, 111478-96-7; *cis*-**17**, 111478-97-8; *trans*-**17**, 111479-03-9; *cis*-**18**, 111478-98-9; *trans*-**18**, 112059-19-5; *cis*-**19**, 114720-09-1; *trans*-**18**, 114720-10-4; *cis*-**20**, 111478-99-0; *trans*-**20**, 111479-00-6; *cis*-**21**, 114720-11-5; *trans*-**21**, 114720-12-6; *cis*-**22**, 114720-13-7; *trans*-**22**, 114720-14-8; **23**, 111478-84-3; **28**, 114737-77-8; **29**, 60481-34-7; **30**, 114720-15-9; **31**, 114720-16-0; **32**, 114720-17-1; **33**, 114720-18-2; **34** (diastereomer 1), 114720-19-3; **34** (diastereomer 2), 114720-20-6; $C_2H_5C(OCH_3)=CHCO_2CH_3$, 104065-67-0; *o*-$IC_6H_4NH_2$, 615-43-0; 6-hydroxyetodolac methyl ester, 114720-21-7; 7-ethylindole, 22867-74-9; 2,3-dihydrofuran, 1191-99-7.

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3-[1-(2-Benzoxazolyl)hydrazino]propanenitrile Derivatives: Inhibitors of Immune Complex Induced Inflammation[1]

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3-[1-(2-Benzoxazolyl)hydrazino]propanenitrile derivatives were evaluated in the dermal and pleural reverse passive Arthus reactions in the rat. In the pleural test these compounds were effective in reducing exudate volume and accumulation of white blood cells. This pattern of activity was similar to that of hydrocortisone and different from that of indomethacin. The structural requirements for inhibiting the Arthus reactions were studied by systematic chemical modification of **1**. These structure–activity relationship studies revealed that nitrogen 1′ of the hydrazino group is essential for activity and must be electron rich, whereas chemical modifications of other sites of **1** had only a modest effect on activity.

Immune complexes have been implicated in the pathogenesis of rheumatoid arthritis (RA) and other inflammatory diseases.[2-4] The reverse passive Arthus reaction (RPAR) represents a well-characterized experimental model of acute immune complex induced inflammation and tissue injury. The close similarities between the pathogenesis of the Arthus reaction and RA makes it an attractive model to search for new drugs to treat RA and related diseases. Therefore, the Arthus model was chosen to screen for novel antiarthritic agents. This reaction is inhibited by hydrocortisone and other corticosteroids but

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