(S isomer), 83945-75-9; **4a** (R isomer), 93600-86-3; **4b**, 17711-16-9; **5**, 623-71-2; **6**, 1985-88-2; **7**, 76752-38-0; **11**, 10211-88-8; **12**, 63163-38-2; **13**, 79435-62-4; **14a**, 93527-43-6; **14b**, 93527-44-7; **15a**, 83945-76-0; **15b**, 93404-42-3; **16**, 4333-56-6; **17**, 3002-94-6; **18**, 2566-44-1; **19**, 36982-56-6; **20a**, 82937-67-5; **20b**, 82937-68-6; HMG CoA reductase, 9028-35-7; 3,3-dimethylpropyl chloride, 107-84-6; 3,3-dimethylbutyl chloride, 2855-08-5; *tert*-butyllithium, 594-19-4; ethylene oxide, 75-21-8; methyl bromide, 74-83-9.

Analogues of Aminoglutethimide: Selective Inhibition of Aromatase

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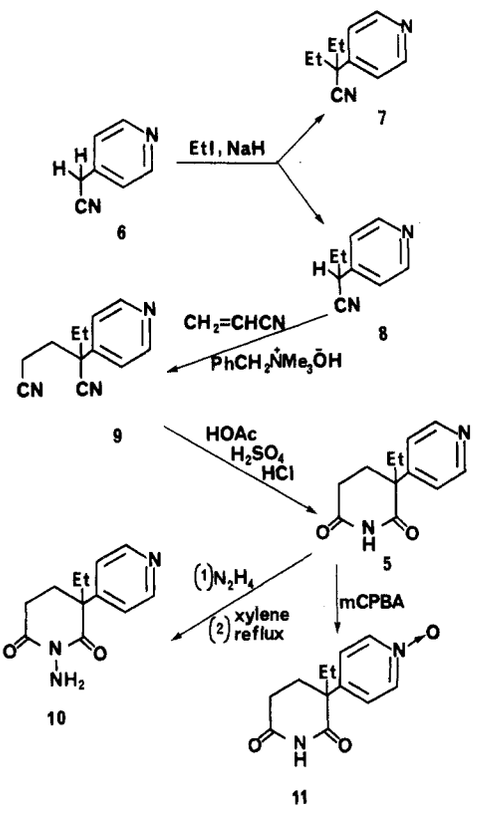
In exploring further the structural features that influence the relative efficacy of analogues of aminoglutethimide [1, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] as inhibitors of the cholesterol side-chain cleavage enzyme system desmolase and the estrogen forming system aromatase, analogues have been synthesized in which the aminophenyl substituent is replaced by pyridyl or substituted pyridyl. The 4-pyridyl analogue **5** [3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione] is a strong competitive inhibitor of aromatase (K_i = 1.1 μM; value for **1**, 0.60 μM), which exhibits a type II difference spectrum (K_s = 0.28 μM; value for **1**, 0.13 μM) but is noninhibitory toward desmolase. The 2- and 3-pyridyl analogues (**3** and **4**) inhibit neither enzyme system. 1-Amino-3-ethyl-3-phenylpiperidine-2,6-dione (**2**) is a strong and selective inhibitor of desmolase but the 4-pyridyl analogue **10** [1-amino-3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione] is a weak inhibitor of desmolase and aromatase. Analogues of **5** having a less basic aromatic substituent, namely, the *N*-oxide **11** and the 2,3,5,6-tetrafluoro derivative **13**, were also prepared. The latter is a weak inhibitor of aromatase and the former inhibits neither enzyme system.

Aminoglutethimide [**1**, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] is becoming increasingly used for the treatment of hormone-dependent metastatic breast carcinoma.[2,3] The drug inhibits the growth of such tumors by interfering with estrogen biosynthesis, the strongest action being against the enzyme complex, aromatase, which converts the androgens androstene-3,17-dione and testosterone into estrone and estradiol.[4] Its other major action is against the initial step in estrogen biosynthesis, namely, the conversion of cholesterol into pregnenolone by the enzyme complex desmolase.[5] Inhibition of desmolase depletes corticosteroid production, and consequently, patients receiving aminoglutethimide require hydrocortisone as replacement therapy to prevent the reflex rise in adrenocorticotropic hormone (ACTH) which might counteract the initial blockade of desmolase. Hence, it might be advantageous therapeutically to use a drug that inhibits aromatase but not desmolase. The steroid analogue 4-hydroxy-4-androstene-3,17-dione is a strong inhibitor of aromatase, and there is indirect evidence, based on its effects on testosterone levels when it is administered in vivo, that it is not inhibitory toward desmolase.[6] In contrast to aminoglutethimide, the binding of 4-hydroxy-4-androstene-3,17-dione to aromatase is irreversible.[7,8] Furthermore, steroidal inhibitors exhibit type I difference spectra in binding to cytochrome P_{450} type enzymes whereas aminoglutethimide gives a type II difference spectrum.[9] Because of these mechanistic differences between the inhibitory actions of the two types of agent, a comparative evaluation as inhibitors of aromatase is desirable and the present study concerns the development of an analogue of aminoglutethimide that selectively inhibits aromatase.

Our previous investigations into the structural features favorable for inhibition of aromatase or desmolase[10] were concerned with the effect of relocating the amino function and of introducing an additional amino group into the molecule. Although 1-amino-3-ethyl-3-phenylpiperidine-2,6-dione (**2**) was identified as a strong inhibitor of desmolase without activity against aromatase, no leads to a pure inhibitor of aromatase emerged. However, these and

Scheme I



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(1) Present address: Department of Biochemistry, University of Oxford, OX1 3QU, England.
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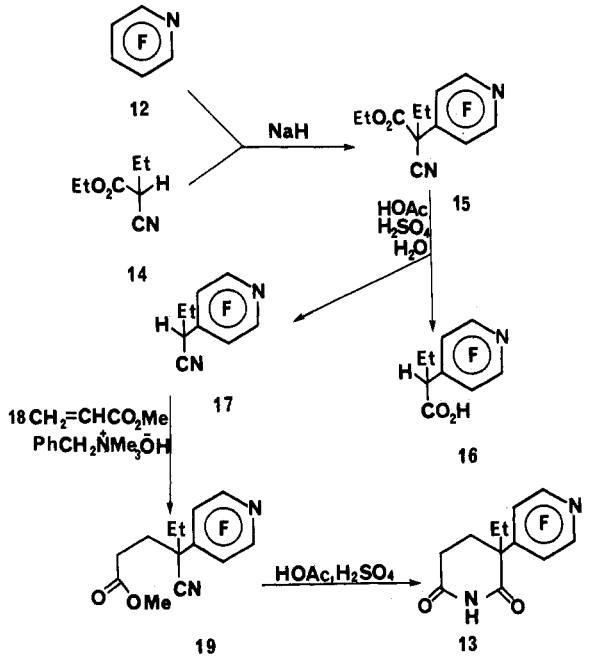
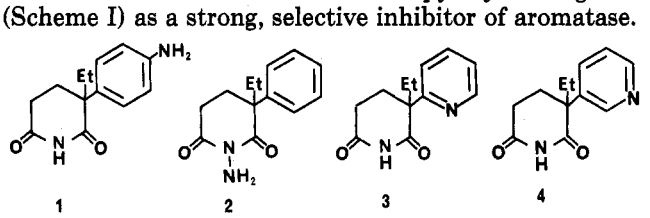
Scheme II[a]



[a] The encircled F denotes that a fluorine atom is attached to all available carbon atoms on the aromatic ring.

other[9] studies confirmed that only basic molecules showed enzyme inhibitory activity. Hence, it was decided to evaluate analogues that would be stronger bases than aminoglutethimide, and since pyridine (pK_a = 5.23) is a stronger base than is aniline (pK_a = 4.58), the isomeric pyridine analogues **3–5** were selected. The 2-pyridyl[11] (**3**) and 3-pyridyl[12] (**4**) analogues have long been known, but the 4-pyridyl derivative has not been described hitherto. The present report is concerned with the enzyme-inhibitory actions of these compounds and of certain analogues and with the identification of the 4-pyridyl analogue **5** (Scheme I) as a strong, selective inhibitor of aromatase.



Results and Discussion

Synthesis of Analogues. Apart from the addition of concentrated HCl in the final cyclization step, which was otherwise incomplete, the route to the synthesis of 3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione (**5**) (Scheme I) was essentially that described for the 2-pyridyl[11] (**3**) and 3-pyridyl[12] (**4**) analogues; **4** had not been obtained crystalline hitherto, nor had elemental analytical data been reported. A diethylated byproduct, 2-ethyl-2-(4-pyridyl)butanenitrile (**7**), was formed during the ethylation of 4-pyridylacetonitrile (**6**), which was not easy to separate from the desired product 2-(4-pyridyl)butanenitrile (**8**) nor from the 4-cyano-4-(4-pyridyl)hexanenitrile (**9**) subsequently formed from **8** by Michael addition of acrylonitrile. Hence, in the preparation of **5**, removal of **7** was deferred to the final cyclization step. The problem[13] of unwanted dialkylation products during attempted monoalkylation of pyridylacetonitriles can be circumvented for the 4-pyridyl derivatives (but not for the 2- or 3-analogues) by using an alternative route to **8**, namely, reaction between *N*-(triphenylmethyl)pyridinium tetrafluoroborate and the lithium derivative of butanenitrile.[14]

Two analogues were prepared from **5** (Scheme I). The *N*-amino analogue **10** was prepared by the route[10] previously developed for the corresponding analogues of glutethimide and aminoglutethimide, namely, ring opening with hydrazine to give the amide hydrazide followed by thermal recyclization. The *N*-oxide **11** was formed from **5** by oxidation with *m*-chloroperbenzoic acid.

Pentafluoropyridine (**12**) is very susceptible to nucleophilic substitution reactions, attack at position 4 being normally preferred.[15] Hence, **12** was a suitable starting

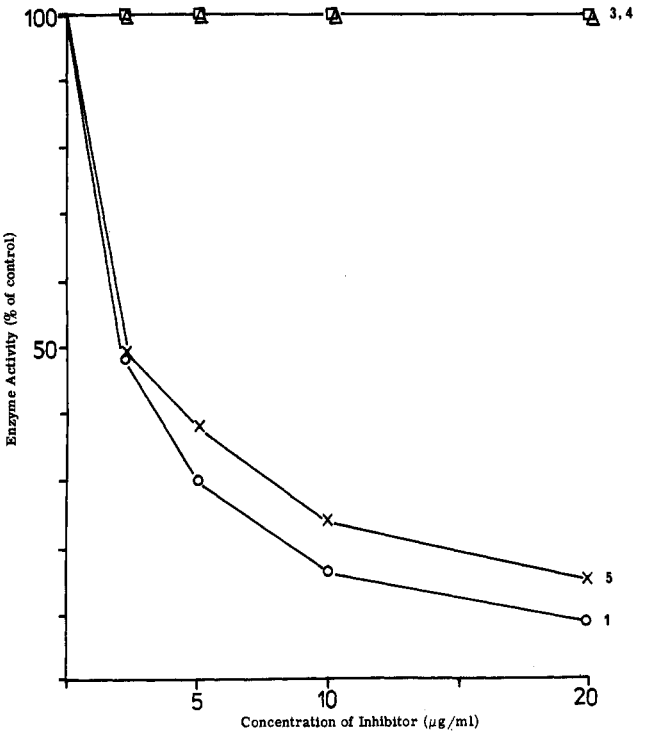


Figure 1. Inhibitory effect of aminoglutethimide (**1**) and the 2-, and 3-, and 4-pyridyl analogues **3–5** toward aromatase from human placenta.

Table I. Apparent K_i and K_a Values for Inhibition of Aromatase by Aminoglutethimide (**1**) and Its 4-Pyridyl Analogue **5**

no.	compd	apparent K_i, μM	apparent K_a, μM
1	aminoglutethimide	0.60	0.13[a]
5	4-pyridyl analogue	1.1	0.28

[a] The published value[17] for **1** (absence of testosterone) is 0.3 μM.

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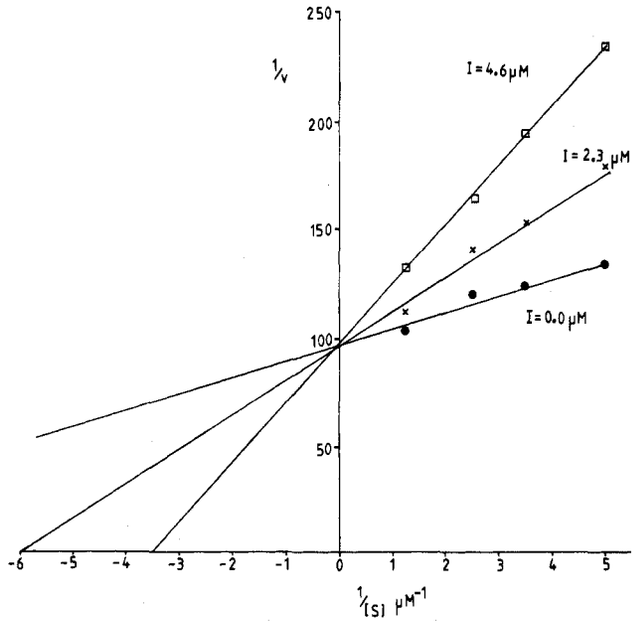

Figure 2. Lineweaver–Burk plot for the 4-pyridyl analogue **5**. Velocity (v) is expressed as nanomoles of testosterone aromatized per minute per milligram of protein. Each point represents duplicate measurements (variation < 10%).

material for the synthesis (Scheme II) of the tetrafluoro analogue (**13**) of **5**. In the base-assisted reaction of ethyl 2-cyanobutanoate[16] (**14**) and **12**, multiple substitution was avoided by a reversed addition technique: the carbanion from **14** was first generated and the reaction mixture containing it was added to **12**. Initially, acidic hydrolysis of the product **15** gave the acid **16**, but with care the nitrile **17** could be made the major component in the mixture of **16** and **17**. No solvent was used during the Michael addition of methyl acrylate (**18**) to the anion formed from **17**, since, in hydroxylic solvents, products arising by further displacements of fluoride ion from the pyridine ring were formed. The cyano ester **19** cyclized under acidic conditions to give **13**.

Enzyme-Inhibitory Activity of Analogues. Figure 1 expresses the inhibitory potency toward aromatase of the pyridyl analogues **3–5** compared with that of **1**. None of these analogues inhibited desmolase at the highest concentration tested (50 μg/mL). The known analogues **3** and **4** did not inhibit aromatase either, but **5** proved to be a strong inhibitor although the K_i values (Table I) indicated it to be a less potent inhibitor than **1**. The Lineweaver–Burk plot (Figure 2) demonstrates that the inhibition exhibited by **5** was competitive. A similar type of inhibition was obtained for **1**.

The type II binding spectrum, exhibited[17] by **1** in its interaction with the cytochrome P_{450} of placental microsomes, was also shown by **5**. Values for the apparent spectral dissociation constant K_s produced by **1** and **5** in the absence of testosterone are compared in Table I and they displayed similar maximal changes in absorption (absorbance units/milligram of protein, 6.25×10^{-4} for **1** and 7×10^{-4} for **5**), suggesting that **5** inhibits aromatase by binding to the cytochrome P_{450} in a manner similar to **1**.

Three analogues of **5** were examined for enzyme-inhibitory activity, namely, the *N*-oxide **11**, which is a major metabolite of **5** in both rat and human,[18] the tetrafluoro derivative **13**, which is less basic than **5** and should have a lower propensity for metabolic N-oxidation, and the *N*-amino analogue **10**. The analogue **10** was prepared because of the high desmolase inhibitory activity associated[10] with the *N*-amino derivative **2**. All three analogues were either poorly or noninhibitory toward the two enzyme systems (Table II).

Table II. Extent of Inhibition of Desmolase and Aromatase at Maximum Concentrations Tested by Weakly Inhibitory or Noninhibitory Analogues Compared with Aminoglutethimide and Its 4-Pyridyl Analogue

no.	compd	% inhibn of desmolase (inhibitor concn, 50 μg/mL)	% inhibn of aromatase (inhibitor concn, 20 μg/mL)
1	aminoglutethimide	85	90
5	4-pyridyl analogue	none	85
10	*N*-amino-4-pyridyl analogue	38	27
11	*N*-oxide of 4-pyridyl analogue	none	none
13	tetrafluoro-4-pyridyl analogue	none	25

Compound **5** is the only analogue of **1** so far examined that is a strong inhibitor of aromatase while being noninhibitory toward desmolase. The study reported earlier[10] coupled with that now described illustrate the marked effect of relatively minor changes in structure in the aminoglutethimide series on the potency and type of enzyme-inhibitory properties. The latter finding is intriguing since both desmolase and aromatase are considered to be cytochrome P_{450} type enzymes. Further studies of the activity against hormone-dependent mammary tumors in animals, metabolism, and CNS effects of **5** and related compounds are in progress.

Experimental Section

NMR spectra for (^{1}H, 60 MHz, internal Me_4Si; ^{19}F, 56.4 MHz, internal CCl_3F, δ) were obtained with a Perkin-Elmer R12B spectrometer. Electron-impact mass spectra ($M^{+\cdot}$ ions) were determined with a VG 7070H spectrometer and VG 2235 data system, using the direct insertion method and an ionizing voltage of 70 eV. IR spectra were obtained with a Perkin-Elmer 257 spectrophotometer. For thin-layer and column chromatography, Merck Kieselgel 60 was used with the specified Art. numbers: 7730 for TLC (UV detection with a Hanovia Chromatolite), 7734 for conventional column chromatography, and 9385 for flash chromatography.[19] Melting points were determined with a Kofler hot-stage or an electrothermal apparatus and are uncorrected.

3-Ethyl-3-(4-pyridyl)-piperidine-2,6-dione (5). To a stirred solution of 4-pyridylacetonitrile[20] (8 g, 0.068 mol) in dry *N,N*-dimethylformamide (130 mL), cooled in an ice–water bath, was added NaH (3.4 g, 50/50 dispersion in oil). After 2.5 h the cooled mixture was treated with EtI (5.7 mL, 11 g, 0.071 mol) and then allowed to warm to room temperature. After a further 18 h the mixture was concentrated under reduced pressure, and the residue was diluted with H_2O (150 mL) and extracted with Et_2O (3 × 300 mL). Flash chromatography (column 15 × 4.5 cm) using $CHCl_3$ afforded a mixture (9.85 g) of 2-(4-pyridyl)butanenitrile (**8**) and 2-ethyl-2-(4-pyridyl)butanenitrile (**7**). To a cooled, stirred solution

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of this mixture in *t*-BuOH (18 mL) containing Triton B (0.32 mL) was added a solution of acrylonitrile (4.46 mL, 5.53 g, 0.104 mol) in *t*-BuOH (5.5 mL). After 2 h the solution was concentrated, diluted with H_2O (150 mL), and extracted with $CHCl_3$ (2 × 50 mL). Flash chromatography using $CHCl_3$ then afforded a mixture (8 g) of **7** and 4-cyano-4-(4-pyridyl)hexanenitrile (**9**), which was heated under reflux with glacial HOAc (15 mL) and concentrated H_2SO_4 (3 mL) for 0.5 h and then, after the addition of 5 M HCl (15 mL), for a further 3 h, cooled, diluted with H_2O, adjusted to pH 7–7.5 with $NaHCO_3$, and extracted with CH_2Cl_2. Column (30 × 4.5 cm) chromatography using $CHCl_3$ then afforded **5** (2.95 g; 20% based on 4-pyridylacetonitrile), which gave colorless crystals from toluene: mp 138–139 °C; mass spectrum, *m/z* 218 (M^+·); IR ν_{max}^{KCl} 3180 (NH), 1784, 1720 (imide C=O), 1605 cm^{-1} (aromatic C=C); ^1H NMR ($CDCl_3$) δ 0.87 (t, 3 H, CH_3CH_2), 1.80–2.82 (m, 6 H, CH_3CH_2, H-4,4,5,5), 7.15 (d, 2 H, aromatic H-3,5, *J* = 4.7 Hz), 8.55 (d, 2 H, aromatic H-2,6, *J* = 4.7 Hz), 9.13 (br s, 1 H, NH). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

2-(4-Pyridyl)butanenitrile (8). The desired intermediate **8** can be separated conveniently from the byproduct (**7**, see above) only on a small scale (0.26 g of mixture) by flash chromatography using $CHCl_3$–MeOH (97:3). The components were not separated by fractional distillation. Eluted first was **7** [^1H NMR ($CDCl_3$) δ 0.87 (t, 6 H, 2 CH_3CH_2, *J* = 7 Hz), 1.97 (q, 4 H, 2 CH_3CH_2), 7.33 (dd, 2 H, pyridine H-3,5, $J_{3,2}$ = 6, $J_{3,6}$ = 1.5 Hz), 8.67 (dd, 2 H, pyridine H-2,6)]. A mixture was eluted next and finally pure **8** (oil, 0.056 g): ^1H NMR ($CDCl_3$) δ 0.84 (t, 3 H, CH_3CH_2, *J* = 7 Hz), 1.74 (quintet, 2 H, CH_3CH_2), 3.60 (t, 1 H, CH_2CH, *J* = 7 Hz), 7.10 (dd, 2 H, pyridine H-3,5, $J_{3,2}$ = 6, $J_{3,6}$ = 1.5 Hz), 8.48 (dd, 2 H, pyridine H-2,6). The product was further characterized as the monopicrate (0.082 g, mp 127 °C) formed by treatment of its ethanolic solution with an ethanolic solution of picric acid (0.126 g). Anal. ($C_{15}H_{13}N_5O_7$) C, H, N. (Note: this compound was previously characterized as its crystalline methotetraphenylborate[14]).

3-Ethyl-3-(3-pyridyl)piperidine-2,6-dione (4). By essentially the foregoing procedure but omitting the concentrated HCl from the final ring closure step, **4** (0.545 g, 11%) was prepared from 3-pyridylacetonitrile[21,22] (2.71 g) and obtained as white crystals from toluene: mp 119–121 °C [lit. bp 164–174 °C (0.1 mmHg)]; IR ν_{max}^{KCl} 3180 (NH), 2950 (aromatic OH), 1725, 1700 cm^{-1} (C=O): picrate (from toluene) mp 166–167.5 °C dec. Anal. **4** ($C_{12}H_{14}N_2O_2$) C, H, N; 4-picrate ($C_{18}H_{17}N_5O_9$) C, H, N.

1-Amino-3-ethyl-3-(4-pyridyl)piperidine-2,6-dione (10). A solution of **5** (0.1 g, 0.46 mmol) in hydrazine hydrate (2 mL) was kept for 3 h at room temperature and then concentrated under reduced pressure and the residue was stored under vacuum over $CaCl_2$. The product [4-(4-pyridyl)-4-carboxamidohexanoic acid hydrazide] (cf. ref 10) was heated under reflux for 3 h with xylene. The solvent was removed and the residue was partitioned between $CHCl_3$ and 1 M HCl. The aqueous layer was neutralized with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 to afford chromatographically pure **10** (0.057 g, 53%), mass spectrum, *m/z* 233 (M^+·). The product did not form a crystalline picrate.

3-Ethyl-3-(4-pyridyl)-piperidine-2,6-dione *N*-Oxide (11). To a solution of **5** (0.0436 g, 0.2 mmol) in benzene (1 mL) was added *m*-chloroperbenzoic acid (0.04 g, 0.3 mmol). After 16 h at room temperature preparative TLC ($CHCl_3$–MeOH, 9:1) separated the *N*-oxide from unreacted *m*-chloroperbenzoic acid and its decomposition product. From ethanol **11** (0.02 g, 43%) afforded large colorless prisms: mp 195–196 °C; mass spectrum, *m/z* 234 (M^+·). Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

Ethyl 2-Cyano-2-(2,3,5,6-tetrafluoropyridyl)butanoate (15). To a stirred suspension of NaH (pellets, 8.0 g, 0.33 mol) in dry *N,N*-dimethylformamide (140 mL) under dry N_2 was added dropwise ethyl 2-cyanobutanoate[16] (**14**; 39.5 g, 0.28 mol) during 30 min and the resulting yellow suspension was stirred at 100 °C for 2.5 h. The clear red solution was cooled to room temperature, diluted with dry *N,N*-dimethylformamide (350 mL), and then added dropwise during 3 h to a rapidly stirred solution of pentafluoropyridine (**12**; 47 g, 0.313 mol) in dry *N,N*-dimethylformamide (500 mL) with cooling to maintain a reaction temperature of 0–2 °C. After a further 1 h of stirring at 0 °C and then at room temperature for 12 h, the solution was poured into ice–water (2 L). The organic layer was extracted into Et_2O (4 × 800 mL). The combined extracts were washed with H_2O (3 × 800 mL), dried ($MgSO_4$), and concentrated to a red oil (74.8 g). Distillation yielded a pale green liquid (46.6 g), bp 112–117 °C (0.25 mmHg), which was shown by GLC to be 93% **15** (i.e., 53% based on **14**). Redistillation [bp 113–114 °C (0.25 mmHg)] afforded analytically pure **15**: IR ν_{max}^{liquid} 2260 (C=N), 1765 cm^{-1} (C=O ester); ^1H NMR ($CDCl_3$) δ 1.18 (t, 3 H, CH_3CH_2C), 1.35 (t, 3 H, CH_3CH_2O), 2.50 (complex q, 2 H, CH_3CH_2C), 4.40 (q, 2 H, CH_3CH_2O); ^{19}F NMR 89.4 (sym m, 2 F, F-2,6), 139 ppm (sym m, 2 F, F-3,5). Anal. ($C_{12}H_{10}F_4N_2O_2$) C, H, F, N.

2-(2,3,5,6-Tetrafluoropyridyl)butanoic Acid (16). A stirred mixture of **15** (0.91 g, 3.14 mmol), concentrated H_2SO_4 (6.44 g), and H_2O (4.15 g) was heated under reflux at 175 °C for 19 h. The cooled solution was poured into ice–water (20 mL), the mixture was extracted with Et_2O (3 × 50 mL), and the combined extracts were washed with aqueous 10% $NaHCO_3$ (3 × 50 mL), H_2O (2 × 50 mL), dried ($MgSO_4$), and concentrated to an orange oil (0.05 g). Reacidification of the aqueous phase (4 M HCl) and further extraction with Et_2O (3 × 50 mL) afforded a red oil (0.6 g) which solidified on storage (mp 85–91 °C). Vacuum sublimation afforded pure **16** (0.4 g, 54%) as a white solid (mp 92–96 °C): IR ν_{max}^{Nujol} 3220–3440 (OH), 1718 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.96 (t, 3 H, CH_3CH_2, *J* = 8 Hz), 3440 (OH), 1718 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.96 (t, 3 H, CH_3CH_2, *J* = 8 Hz), 2.15 (m, 2 H, CH_3CH_2), 4.08 (dd, 1 H, $EtCH$, $J_{2,3}$ = 5 and 9 Hz), 11.54 (s, 1 H, COOH); ^{19}F NMR 90.9 (sym m, 2 F, F-2,6), 143.2 ppm (sym m, 2 F, F-3,5). Anal. ($C_9H_7F_4NO_2$) C, H, F, N.

2-(2,3,5,6-Tetrafluoropyridyl)butanenitrile (17). A stirred solution of **15** (10 g, 0.0345 mol), glacial HOAc (70 g), H_2O (70 g), and concentrated H_2SO_4 (5 g) was heated under reflux during 71 h (oil bath 140 °C). The cooled solution was poured into ice–water (200 mL) and extracted with $CHCl_3$ (3 × 200 mL). The combined extracts were washed with H_2O (200 mL), aqueous 10% $NaHCO_3$ (3 × 200 mL), dried ($CaCl_2$), and concentrated to a pale yellow oil (6.33 g). Distillation afforded **17** (4.5 g, 60%) as a colorless liquid; bp 65–70 °C (0.15 mmHg); IR ν_{max}^{liquid} 2260 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 1.18 (t, 3 H, CH_3CH_2, $J_{3,4}$ = 7 Hz), 2.13 (m, 2 H, CH_3CH_2), 4.22 (t, 1 H, $EtCH$, $J_{2,3}$ = 8 Hz); ^{19}F NMR 89.6 (sym m, 2 F, F-2,6), 143.2 ppm (sym m, 2 F, F-3,5). Anal. ($C_9H_6F_4N_2$) C, H, F, N. The acid **16** (1.42 g, 17%) was isolated from the aqueous 10% $NaHCO_3$ extract.

Methyl 4-Cyano-4-(2,3,5,6-tetrafluoropyridyl)hexanoate (19). To solid benzyltrimethylammonium hydroxide (Triton B; obtained by concentrating with moisture exclusion a 40% solution in 0.5 mL of MeOH) was added **17** (2.46 g, 0.0113 mol). To the stirred solution at room temperature, methyl acrylate (**18**; 1.48 g, 1.55 mL, 0.0172 mol) was added dropwise from a syringe through a septum cap. When the initial exothermic reaction had subsided, the solution was stirred at 95 °C for 4 h and then cooled to room temperature and extracted with $CHCl_3$ (100 mL), the extract was washed with H_2O (3 × 10 mL), dried ($CaCl_2$), and concentrated under reduced pressure, and the residue was distilled to afford **19** (3.20 g, 76%) as a colorless liquid: bp 175–177 °C (0.25 mmHg); IR ν_{max}^{liquid} 2245 (C=N), 1735 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.13 (t, 3 H, CH_3CH_2, $J_{6,5}$ = 7 Hz), 2.04–2.64 (complex q, 2 H, CH_3CH_2), 2.04–2.64 (A_2B_2, 4 H, H-2,2,3,3), 3.64 (s, 3 H, COOMe); ^{19}F NMR 89.3 (2 F, F-2,6) 139.2 ppm (2 F, F-3,5). Anal. ($C_{13}H_{12}F_4N_2O_2$) C, H, F, N.

3-Ethyl-3-(2,3,5,6-tetrafluoropyridyl)piperidine-2,6-dione (13). A stirred solution of **19** (1 g, 3.29 mmol), glacial HOAc (3.30 g), and concentrated H_2SO_4 (1.46 g) was heated with moisture exclusion at 100 °C for 2 h. The cooled solution was poured onto ice (25 mL), and after adjustment to pH 7 with 4M NaOH, the white gluey solid was extracted with $CHCl_3$ (3 × 70 mL), and the combined extracts were washed successively with H_2O (50 mL), aqueous 10% $NaHCO_3$ (50 mL), and H_2O (50 mL), dried ($CaCl_2$), and concentrated. The residue was crystallized twice from $CHCl_3$–light petroleum (bp 60–80 °C) to give **13** (0.8 g, 84%) as short white needles: mp 116–117 °C; IR ν_{max}^{Nujol} 3220 (NH), 3100 (NH), 1733, 1685 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.15 (t, 3 H, CH_3CH_2, *J* = 7 Hz), 2.13–3.25 (complex m, 6 H, CH_2CH_3, CH_2CH_2), 9.0 (br s, 1 H, NH); ^{19}F NMR 90.4 (sym m, 2 F, F-2,6),

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138.9 ppm (sym m, 2 F, F-3,5). Anal. ($C_{12}H_{14}F_4N_2O_2$) C, H, F, N.

Enzyme Preparation and Assay Procedures. The reagents and conditions for the assays for inhibitory activity against desmolase and aromatase were exactly those described in ref 10. Binding spectra were determined as follows. Difference spectra were obtained at room temperature with a Pye Unicam SP8-150 spectrophotometer in the range 360–520 nm. The human placental microsomes were diluted with 0.1 M potassium phosphate buffer (pH 7.4) to give a final protein concentration of 2 mg/mL. The ligands were dissolved in dimethyl sulfoxide and equal volumes of solvent were added to the reference and sample cuvettes. Dimethyl sulfoxide had no effect on the binding spectra with addition up to 10% of the final suspension volume. Spectra were recorded at 1 nm/s with a slit width of 2 nm and a full-scale absorbance of 0.02. The K_s values were determined from a Scatchard plot (absorbance difference vs. absorbance difference/concentration: slope K_s) and are the average of three determinations (±10%).

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Registry No. 4, 92788-14-2; 4·picrate, 92788-15-3; 5, 92788-10-8; 6, 13121-99-8; 7, 92788-11-9; 8, 72434-23-2; 8·picrate, 92788-13-1; 9, 92788-12-0; 10, 92788-16-4; 11, 92788-18-6; 12, 700-16-3; 13, 92788-23-3; 14, 1619-58-5; 15, 92788-19-7; 16, 92788-20-0; 17, 92788-21-1; 18, 96-33-3; 19, 92788-22-2; EtI, 75-03-6; CH_2=CHCN, 107-13-1; 3-pyridineacetonitrile, 6443-85-2; desmolase, 9044-50-2; aromatase, 9039-48-9; 4-(4-pyridyl)-4-carboxamidohexanoic acid hydrazide, 92788-17-5.

Amino Acid Derived Latent Isocyanates: Irreversible Inactivation of Porcine Pancreatic Elastase and Human Leukocyte Elastase

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Several amino acid derived azolides (I) have been synthesized and investigated for their inhibitory activity toward human leukocyte elastase and porcine pancreatic elastase. The inhibitory activity was found to be dependent on the nature of the precursor amino acid ester. Thus, compounds derived from L-valine methyl ester **3**, L-norvaline methyl ester **5**, DL-norleucine methyl ester **9**, and L-methionine methyl ester **10** were found to inhibit irreversibly both enzymes. Compound **10** was found to be a specific and selective inhibitor of human leukocyte elastase. In contrast to these, inhibitors derived from glycine methyl ester **1**, D-valine methyl ester **4**, and D-norvaline methyl ester **6** were found to be inactive. The results of the present study show that latent isocyanates derived from appropriate amino acids can serve as selective inhibitors of serine proteases and are of potential pharmacological value.

Pulmonary emphysema is a disease characterized by alterations in physiological lung function related to the loss of elastic recoil.[1,2] The proteinase–proteinase inhibitor imbalance hypothesis has been proposed as a model for the development of emphysema. It suggests that either an increase in destructive proteolytic activity due to human leukocyte elastase (HLE) and cathepsin G or a decrease in protective serum proteinase inhibitor (α-1-PI) can lead to emphysema.[3,4] The progress of the disease involves the destruction of the lung connective tissues. A similar model may be involved in other related connective tissue diseases, such as arthritis.[5] Thus, the development of biospecific inhibitors of HLE and cathepsin G devoid of cytotoxic effects remains of extreme pharmacological importance.

In designing inhibitors of HLE cognizance must be taken of the fact that potential pharmacological agents must possess the ability to inhibit selectivity HLE and none of the other closely related proteinases. Although HLE, cathepsin G, porcine pancreatic elastase (PPE), and α-chymotrypsin have many features in common, such as a similar catalytic apparatus, an extended binding site, and a preference for hydrophobic substrates (or inhibitors), they differ from each other in their substrate specificities.[6,7] These differences in substrate preference arise from variations in the size of the binding cleft at the catalytic sites.

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