the human leukocyte elastase and cathepsin G used in this study. Special thanks is extended to Mr. Masaru Yamamoto (Georgia Institute of Technology) for his preparation of MeO-Suc-Ala-Ala-Pro-Val-pNA used in the HLE assays, and to Mr. Jeffrey Mathys (University of Akron) who helped in the initial preparations of several anthraquinone analogues as part of the Undergraduate Research Program at Georgia Institute of Technology. This project was financially supported through a Graduate Research Fellowship graciously donated by Union Camp.

Registry No. **2**, 518-82-1; **3**, 481-72-1; **4a**, 14787-38-3; **4b**, 481-39-0; **5**, 28495-22-9; **6a**, 139565-29-0; **7a**, 139565-30-3; **7b**, 139565-31-4; **8a**, 5018-84-8; **8b**, 28785-82-2; **9**, 521-61-9; **10**, 139565-32-5; **11**, 6030-60-0; **12**, 84993-87-3; **13**, 481-73-2; **14**, 478-45-5; **15**, 6414-42-2; **16**, 60699-01-6; **17**, 121210-61-5; **18**, 481-74-3; **19**, 18713-45-6; **20**, 65615-59-0; **21**, 25395-11-3; **22**, 139565-33-6; **23**, 19228-39-8; **24**, 19228-40-1; **25**, 6407-55-2; **26**, 5539-66-2; **27a**, 51996-00-0; **27b**, 139565-34-7; **27c**, 139582-75-5; **27d**, 139565-35-8; **27e**, 139565-36-9; **27f**, 139565-37-0; **27g**, 139565-38-1; **28a**, 34425-60-0; **28b**, 106491-48-9; **28c**, 139565-39-2; **28d**, 76643-51-1; **28e**, 139565-40-5; **28f**, 139565-41-6; **28g**, 139565-42-7; **29**, 139565-43-8; **30**, 139565-44-9; **31**, 139565-45-0; **32**, 139565-46-1; **33**, 85313-87-7; **34**, 85313-88-8; **35**, 85313-89-9; **36**, 139565-47-2; **37**, 54454-84-1; **38**, 6268-09-3; **39**, 20460-44-0; **40**, 84-54-8; **41**, 84-51-5; **42**, 69595-67-1; **43**, 139565-48-3; **44**, 139565-49-4; **45**, 139565-50-7; **46**, 139565-51-8; **47**, 139565-52-9; CatG, 56645-49-9; HLE, 109968-22-1; serine proteinase, 37259-58-8; butyraldehyde, 123-72-8; 1-hydroxyanthraquinone, 129-43-1; propanal, 123-38-6; isobutyraldehyde, 78-84-2; benzaldehyde, 100-52-7; pentanal, 110-62-3; hexanal, 66-25-1; 1-bromopropane, 106-94-5; allyl bromide, 106-95-6; butyl chloroformate, 592-34-7.

Supplementary Material Available: Full spectral characterization for the compounds reported in Table IV, including 300 MHz ^{1}H NMR, EIMS, FTIR, and elemental analyses (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Human Purine Nucleoside Phosphorylase. Synthesis of Pyrrolo[3,2-*d*]pyrimidines, a New Class of Purine Nucleoside Phosphorylase Inhibitors as Potentially T-Cell Selective Immunosuppressive Agents. Description of 2,6-Diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one[1]

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Purine nucleoside phosphorylase (PNP) is a purine-metabolizing enzyme in the purine cascade and has been a target for drug design for sometime. A series of potent human PNP inhibitors, pyrrolo[3,2-*d*]pyrimidines (9-deazaguanines), has been synthesized and evaluated in the enzyme assay and in the cell line assay using MOLT-4 (T-cell) and MGL-8 (B-cell) lymphoblasts for selectivity. One of the compounds, 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11c; CI-972), was found to be moderately potent, competitive, and reversible inhibitor of PNP with K_i = 0.83 μM. It was also found to be selectively cytotoxic to MOLT-4 lymphoblasts (IC_{50} = 3.0 μM) but not to MGL-8 lymphoblasts and was evaluated further. Compound 11c (CI-972) is under development in the clinic.

Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) is an essential enzyme of the purine salvage pathway and has been a target for drug design for sometime. Other enzyme inhibitors of the purine cascade, such as the adenosine deaminase inhibitor 2′-deoxycoformycin (pentostatin; DCF)[2] and the xanthine oxidase inhibitor allopurinol, have proven to be useful drugs, but no PNP inhibitor has yet been developed.

PNP is a purine-metabolizing enzyme which catalyzes the reversible phosphorylosis of inosine, deoxyinosine, guanosine, and deoxyguanosine to the corresponding purines, hypoxanthine and guanine. PNP deficiency in children causes profound impairment in T-cell function with minimal or no effect on B-cell function.[3] Thus, it is theorized that a potent PNP inhibitor could be a potentially useful immunosuppressive agent in the treatment of T-cell-dependent diseases, such as rheumatoid arthritis and psoriasis, and in T-cell leukemia and lymphomas.[4] PNP inhibitors should also be efficacious in the treatment of metabolic disorders such as hyperuricemia because of their ability to block the degradation of nucleosides which are precursors of uric acid. Numerous other potential indications have been postulated.[4]

Previously, we described the synthesis and biological activity of the PNP inhibitor 2,8-diamino-9-(2-thienylmethyl)guanine (1; PD 119229; CI-950).[5,6] Although 1 (CI-950) is an extremely potent PNP inhibitor (K_i = 0.067 μM),[7] physical properties of this compound limited its

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(7) The K_i was determined at a 50 mM inorganic phosphate concentration. At this phosphate concentration, the putatively most potent PNP inhibitor, acyclovir diphosphate, has a K_i of 0.51 μM.

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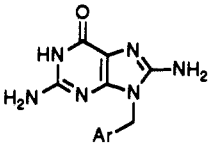
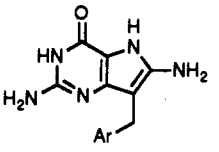
Table I. Comparison of Physical Properties and Solubility of 8-Aminoguanines and 8-Amino-9-deazaguanines



15–17

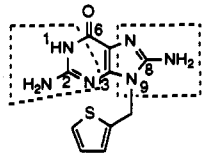


11a–c

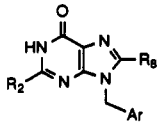
no.	Ar[a]	mp, °C	at pH 7.4 log *P*	at pH 7.4 solubility, mg/mL	no.	Ar[a]	mp, °C	at pH 7.4 log *P*	at pH 7.4 solubility, mg/mL
15	C_6H_5	>300 (B)	0.58	<0.1	11a	C_6H_5	>250 (HCl)	0.67	0.60
16	2-Th	223–6 (HCl)	0.19	0.034	11b	2-Th	220–5 (HCl)	1.01	0.30
17	3-Th	275–8 (HCl)	0.48	<0.1	11c	3-Th	205–15 (HCl)	1.08	0.80

[a] Th = thiophene.

development. Compound 1 (CI-950) is a highly crystalline compound with low log *P* value and very low absolute solubility in water (0.04 mg/mL). It was found to have limited bioavailability in animal models, which could be correlated with its low log *P* value (0.19 in octanol/water in pH 7.4 phosphate buffer) at physiological pH, a measure of lipophilicity. Since 1 contained two amidino groups which made the compound highly crystalline and weakly lipophilic (i.e., low log *P* value), it was reasoned that eliminating one of the nitrogens (N_3, N_7, or N_9) from the ring would make the compound more lipophilic, less crystalline, and therefore more bioavailable.



1 (PD 119229, CI-950)



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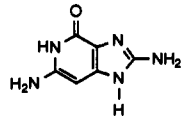

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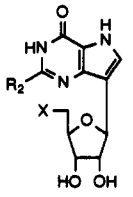
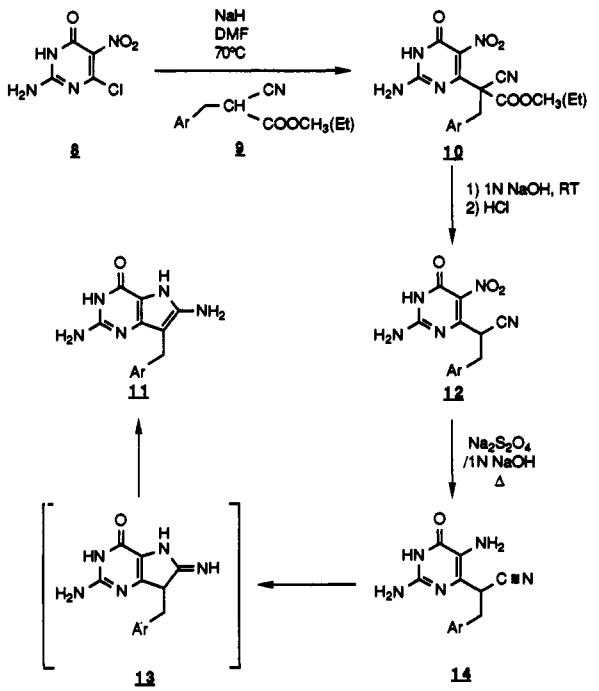


4, R_2 = H; X = OH; Ki = 2.0 μM
5, R_2 = NH_2; X = OH; Ki = 2.9 μM
6, R_2 = H; X = Cl; Ki = 0.20 μM
7, R_2 = H; X = I; Ki = 0.176 μM

Structure–activity relationship (SAR) studies of the CI-950 series, i.e., 8-amino-9-substituted-guanines (2),[8,9] indicated that the N_1-nitrogen and C_2-NH_2 are necessary for activity and potency. The N_3-nitrogen is also necessary since 8-amino-3-deazaguanine[10] (3) is less potent than 8-aminoguanine. The N_9-nitrogen seems to be unnecessary for activity and potency since 9-deazainosine[11] (4), 9-deazaguanosine[11] (5), and 5′-deoxy-5′-chloro/5′-deoxy-5′-iodo-9-deazainosines[11] (6/7) are known potent PNP inhibitors. Thus, as a part of our efforts to develop a PNP inhibitor for autoimmune diseases, we synthesized a series of 9-substituted-9-deazaguanines (pyrrolo[3,2-*d*]pyrimidines) (11; Scheme I) as potential PNP inhibitors. In this article, we report the potent PNP inhibition and T-cell selective cytotoxicity of some of the compounds of this series, and specifically 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (CI-972),

Scheme I



9–14 a. Ar = C_6H_5
b. Ar = 2-Th
c. Ar = 3-Th
d. Ar = 2-$CH_3OC_6H_4$
e. Ar = 2-Fu

23

Table II. PNP Inhibitory Activity of 9-Deazaguanines (11)

no.	Ar[a]	PNP IC_{50} (μM)
11a	C_6H_5	1.6
11b	2-Th	1.0
11c	3-Th	0.9
11d	2-CH_3O-C_6H_4	31.4
11e	2-Fu	5.4
8-aminoguanosine		1.40[b]

[a] Th = thiophene; Fu = furan. [b] Reference 5.

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Table III. Percent Inhibition of PNP as a Function of Time of Dialysis

compound	concn, μM	duration of dialysis (h) 0	1	3	6	24
11c (Cl-972)	5.0	72.2[a]	48.0	34.8	5.4	0
8-aminoguanosine	5.0	72.6	53.0	24.5	7.5	0
1 (PD 119229-2; CI-950)	0.4	75.5	38.0	26.0	7.0	0

[a] Percent inhibition of PNP.

which is presently undergoing clinical trials.

The synthesis of 4*H*-pyrrolo[3,2-*d*]pyrimidines (11) was accomplished following Scheme I. Reaction of 8[12] with the sodium derivative of 9[13,14] in DMF at 70 °C gave **10**, which on hydrolysis and decarboxylation gave **12**. Dithionate reduction of **12** gave the intermediate amino compound **14**,[15] which spontaneously cyclized to **13** and then aromatized to give the 2,6-diamino-3,5-dihydro-7-(aryl- or heteroarylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidines (11).

These compounds have lower melting points than the corresponding 8-amino-9-substituted guanines, i.e., CI-950 series. They are also much more lipophilic, i.e., have higher log *P* values, and are much more water soluble as expected (Table I) than the corresponding purine compounds of the CI-950 series. These compounds were also found to be moderately potent inhibitors of PNP (Table II). The compound **11c** (CI-972) is most lipophilic (log *P* value 1.08 in octanol/water at pH 7.4) and the most water soluble (3.27 mg/mL; HCl salt; pH of the solution 3.3) in the series. Compound **11c** is also quite soluble at physiological pH 7.4 (0.05 M phosphate buffer; 0.80 mg/mL) and was evaluated further. It was found to be a competitive inhibitor of PNP with K_i of 0.83 μM (Figure 1), and is also a reversible inhibitor. The enzyme activity was largely restored after 6 h of dialysis, and could be completely restored after 24 h (Table III). In the presence of 10 μM 2′-deoxyguanosine, **11c** was also found to be selectively inhibitory to human MOLT-4 (T-cell) lymphoblasts (IC_{50} = 3.0 μM) but nontoxic to MGL-8 (B-cell) lymphoblasts (IC_{50} > 50 μM).[16] It was also found to have apparent bioavailability of 69% in dogs at 15 mg/kg in an iv vs po dosing regimen.[17] Compound **11c** is presently being evaluated in the clinic (phase I) for indications including T-cell selective immunosuppression.

Experimental Section

Pharmacology. Human erythrocyte PNP activity was assayed by measuring the conversion of [8-^{14}C]inosine to [8-^{14}C]hypoxanthine according to a chromatographic method previously described.[5,6] For assay of toxicity in the human MOLT-4 T-lymphoblast tissue culture system, compounds were tested for their ability to potentiate the cytotoxicity of 2′-deoxyguanosine in the presence or absence of 10 μM 2′-deoxyguanosine, as quantitated by measurement of [^{3}H]thymidine incorporation.[6]

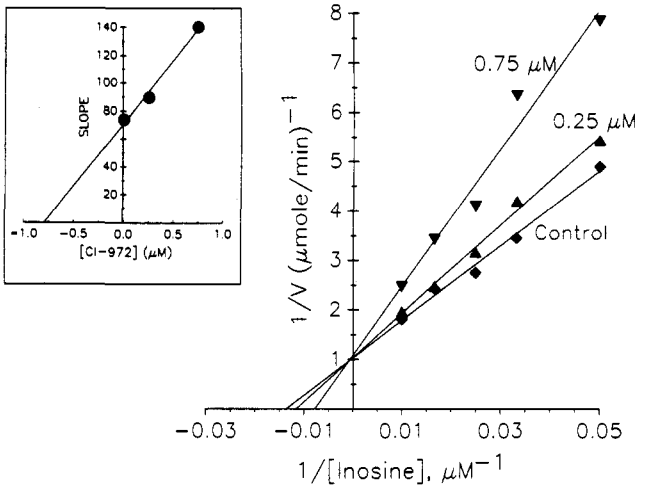

Figure 1. Kinetics of the inhibition of human erythrocyte PNP by 11c (CI-972). The PNP enzyme assay consisted of a 2-min preincubation of 11c (CI-972) with a 1:5000 dilution of enzyme, followed by the addition of [^{14}C]inosine and an additional 10-min incubation. Inosine concentrations of 20–100 μM were used in the kinetics assay. Conversion of inosine to hypoxanthine was used as the endpoint. Hypoxanthine and inosine were separated using thin-layer chromatography, and spots containing these materials were cut out and counted in a liquid scintillation counter. Data are graphed in a Lineweaver–Burk plot (double reciprocal of inosine concentration versus velocity) and the K_i calculated from a replot of these data (inset).

Chemistry. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on either a Varian EM-390 (90 MHz), a IBM WP100SY (100 MHz), a Varian XL-200 (200 MHz), or a Varian XL-300 (300 MHz) spectrometer. Infrared spectra (IR) were obtained on a Nicolet MX-1 FTIR. Mass spectra were obtained on either a Finnigan 4500 spectrometer or a V.G. Analytical 7070E/HF instrument. Spectral data agreed with the proposed structure unless otherwise noted. Elemental analyses were within ±0.4% of theoretical values for the specified elements unless otherwise noted and were performed by the Analytical Chemistry Section of Parke-Davis Pharmaceutical Research Division. Thin-layer chromatography was carried out on 0.25-mm silica gel F254 (E. Merck) glass plates. Merck silica gel 60 (230–400 mesh) was used for chromatography.

2-Amino-α-cyano-1,6-dihydro-5-nitro-6-oxo-α-(phenylmethyl)-4-pyrimidineacetic Acid, Ethyl Ester (10a). A solution of ethyl 2-cyano-3-phenylpropionate[14] (6.0 g) in DMF (25 mL) was added dropwise to a suspension of sodium hydride (1.2 g, 60% suspension in oil, washed with hexane) in DMF (25 mL) under an atmosphere of dry N_2. The reaction mixture was stirred for 15 min at room temperature and then a solution of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone[12] (1.9 g, freshly crystallized from methanol) in DMF (50 mL) was added to the anion. The reaction mixture was heated at 65 °C for 24 h and then was acidified to pH 3 with 1 N HCl.

Most of the DMF was removed by evaporation on a rotary evaporator under high vacuum. The residual oil was partitioned between ethyl acetate (500 mL) and water. The organic layer was dried (Na_2SO_4) and evaporated to a gummy solid. The residue was triturated with ether and collected by filtration. It was dried under vacuum to give **10a** as a partial hydrate (2.25 g, 62%), mp slowly turns brown >170 °C melts at 230–235 °C dec. Anal. ($C_{16}H_{15}N_5O_5 \cdot 0.3H_2O$) C, H, N.

2-Amino-1,6-dihydro-5-nitro-6-oxo-α-(phenylmethyl)-4-pyrimidineacetonitrile (12a). A solution of **10a** (2.0 g) in 1 N NaOH (100 mL) was stirred at room temperature for 1 h and was acidified with 4 N HCl (30 mL). The precipitate was collected by filtration and dried under vacuum to give **12a** (600 mg, 36%), mp 175 °C dec. Anal. ($C_{13}H_{11}N_5O_3$) C, H, N.

2,6-Diamino-3,5-dihydro-7-(phenylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11a). To a solution of **12a** (Ar = C_6H_5) (10.0 g) in 1 N NaOH (600 mL) was added sodium dithionite (35 g). The reaction mixture was heated at 90 °C for 35 min and was

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(17) Black, A.; Woolf, T.; Chang, T. Unpublished data.

acidified to pH 4 with 4 N HCl while still hot. The reaction mixture was cooled in an ice bath and the precipitate was collected by filtration. It was dried over P_2O_5 under vacuum (4.0 g). The crude (undecarboxylated) product was dissolved in 300 mL of concentrated HCl and quickly filtered through a glass frit before it crystallized out. The resulting suspension was boiled for 5 min and cooled. The product **11a** was collected by filtration (1.98 g, 23%), mp >250 °C dec. Anal. ($C_{13}H_{13}N_5O$·HCl·1.1H_2O) C, H, N, Cl.

2-Amino-α-cyano-1,6-dihydro-5-nitro-6-oxo-α-(2-thienylmethyl)-4-pyrimidineacetic Acid, Methyl Ester (10b). Sodium hydride (4.5 g, 60% suspension in oil washed with hexane) was suspended in dry DMF (50 mL) under an atmosphere of dry N_2 and a solution of methyl 2-cyano-3-(2-thienyl)propionate[13] (22.0 g) in dry DMF (50 mL) was added dropwise when a dark blue solution was formed. A solution of freshly recrystallized 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (8.57 g) in DMF (75 mL) was added in one portion. The reaction mixture was heated at 60 °C overnight and cooled and then was acidified to pH 5 with 1 N HCl. It was poured into 1.0 L of ethyl acetate and was extracted with water (4 × 300 mL). The organic layer was evaporated to near dryness and the residue was suspended in ether and collected by filtration. The crude product was washed with hexane until the washings were no longer green. The solid was dried under vacuum to give **10b** (5.0 g; 34%), mp 235–237 °C. Anal. ($C_{13}H_{11}N_5O_5S$·0.1H_2O) C, H, N, S.

2-Amino-1,6-dihydro-5-nitro-6-oxo-α-(2-thienylmethyl)-4-pyrimidineacetonitrile (12b). A solution of **10b** (5.0 g) in 1 N NaOH (200 mL) was stirred at room temperature for 90 min. The reaction mixture was acidified to pH 1 with 4 N HCl and stirred for 5 min. The reaction mixture was neutralized (pH 7) with 1 N NaOH and the product **12b** (4.1 g, 98%) was collected by filtration, mp 192–194 °C dec. Anal. ($C_{11}H_9N_5O_3S$·0.25H_2O) C, H, N; S: calcd, 11.01; found, 9.50.

2,6-Diamino-3,5-dihydro-7-(2-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11b). To a solution of **12b** (4.0 g) in 1 N NaOH (250 mL) was added sodium dithionite (17 g), and the reaction mixture was heated at 90 °C for 20 min. The reaction mixture was acidified to pH 2 with 4 N HCl while still hot and filtered and then was cooled and neutralized with 1 N NaOH. The crude product was added to a stirred solution of concentrated HCl (150 mL) and the hydrochloride salt was collected by filtration. The salt was dissolved in aqueous NaOH and reprecipitated with 1 N HCl. Recrystallization from 2 N HCl gave the analytical hydrochloride salt of **11b** as the monohydrate (0.82, 19%), mp 220–225 °C dec. Anal. ($C_{11}H_{11}N_5OS$·HCl·H_2O) C, H, N, S, Cl.

2-Amino-α-cyano-1,6-dihydro-5-nitro-6-oxo-α-(3-thienylmethyl)-4-pyrimidineacetic Acid, Methyl Ester (10c). Sodium hydride (5.7 g; 60% suspension in oil; washed with hexane) was suspended in dry DMF (50 mL) under an atmosphere of dry N_2 and a solution of methyl 2-cyano-3-(3-thienyl)propionate[13] (28.0 g) in DMF (50 mL) was added dropwise. 2-Amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (9.1 g, freshly recrystallized) in DMF (50 mL) was added. The reaction mixture was heated at 70 °C overnight, cooled, and then acidified to pH 4 with 1 N HCl with ice bath cooling. The reaction mixture was diluted to 1.0 L with cold water, and the resulting precipitate was collected by filtration. It was rinsed with hexane/ethyl acetate and dried to give **10c** (8.8 g, 52%), mp turns brown slowly >200 °C, melts 228–230 °C. This was used in the next step without further purification.

2-Amino-1,6-dihydro-5-nitro-6-oxo-α-(3-thienylmethyl)-4-pyrimidineacetonitrile (12c). A solution of **10c** (5.0 g) in 1 N NaOH (200 mL) was stirred for 2 h at room temperature and then acidified to pH 1 by the dropwise addition of concentrated HCl. The resulting suspension was warmed (45 °C) for 2 min and then cooled. The pH was adjusted to pH 3 with NH_4OH. The solid was collected by filtration, washed with water, and dried under vacuum to give **12c** (3.03 g; 71%), mp 170–173 °C. Anal. ($C_{11}H_9N_5O_3S$·0.2NH_4OH) C, H, N, S.

2,6-Diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11c). To a solution of **12c** (5.0 g) in 1 N NaOH (300 mL) was added sodium dithionite (20 g). The reaction mixture was heated for 30 min at 90 °C and then was acidified (pH 1) with concentrated HCl while still hot. The reaction mixture was cooled and neutralized with ammonium hydroxide. The resulting precipitate was collected by filtration, washed with cold water, and dried under vacuum. The crude product was added to 100 mL of concentrated HCl in small portions with stirring and the hydrochloride salt was collected by filtration. The product was recrystallized from 1 N HCl to give **11c** as the monohydrochloride salt (2.35 g; 44%), mp >185 °C dec. Anal. ($C_{11}H_{11}N_5OS$·HCl·1.2H_2O) C, H, N, S, Cl.

2-Amino-α-cyano-α-(2-furanylmethyl)-1,6-dihydro-5-nitro-6-oxo-4-pyrimidineacetic Acid, Methyl Ester (10e). Sodium hydride (8.4 g, 60% suspension in oil, washed with hexane) was suspended in dry DMF (100 mL) under an atmosphere of dry N_2 and a solution of methyl 2-cyano-3-(2-furanyl)propionate[13] (37.6 g) in DMF (100 mL) was added dropwise. When the addition was complete, a clear red solution was formed. 2-Amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (recrystallized from methanol) (13.34 g) was added as a solid. The reaction mixture was heated at 100 °C for 1 h and at 65 °C overnight. The reaction mixture was cooled in an ice bath, acidified with 10% aqueous HCl, and diluted with enough water to precipitate out the product **10e**. The product was collected by filtration and rinsed with water and ether (18.5 g; 95%), mp 237–241 °C dec. Anal. ($C_{13}H_{11}N_5O_4$) C, H, N.

2-Amino-α-(2-furanylmethyl)-1,6-dihydro-5-nitro-6-oxo-4-pyrimidineacetonitrile (12e). A solution of **10e** (5.0 g) in 1 N NaOH (200 mL) was stirred at room temperature for 2 h. The reaction mixture was acidified (pH 1) by the dropwise addition of concentrated HCl and was stirred at room temperature for 2 min. The precipitate was collected by filtration, washed with water, and dried to give **12e** (3.02 g, 73%), mp 177–178 °C dec. Anal. ($C_{11}H_9N_5O_4$·0.1H_2O) C, H, N.

2,6-Diamino-3,5-dihydro-7-(2-furanylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11e). To a solution of **12e** (4.0 g) in 1 N NaOH (250 mL) was added sodium dithionite (16 g) and the reaction mixture was heated at 90 °C for 30 min. The reaction mixture was cooled in an ice bath and neutralized with 4 N HCl. The resulting precipitate was collected by filtration and purified by a series of acid/base reprecipitations. First the product was reprecipitated from 1 N NaOH by acidifying (pH 2) with a saturated solution of oxalic acid. The NaOH/oxalic acid reprecipitation was repeated. Then it was reprecipitated from 1 N NaOH by adjusting pH to 11 with 4 N HCl. The product **11e** (1.1 g, 26%) was dried under vacuum. An analytical sample of the hydrochloride salt was prepared by dissolving 400 mg of the crude product above in 1 N NaOH (250 mL). The resulting solution was filtered and adjusted to pH 2–3 by the addition of 1 N HCl. The solution was concentrated and the resulting precipitate collected by filtration to give the hydrochloride salt (200 mg), mp 228–231 °C dec. Anal. ($C_{11}H_{11}N_5O_2$·HCl·0.5H_2O) C, H, N.

Methyl (2-Methoxybenzylidene)cyanoacetate. A mixture of 2-anisaldehyde (672.1 g, 4.9 moles) and methyl cyanoacetate (489.2 g, 4.9 mol) in dioxane (700 mL) was cooled to 13 °C and a solution of piperidine (12 mL) in dioxane (60 mL) was added dropwise over 30-min period with stirring and ice-bath cooling. No exothermic reaction was observed. After the addition, the mixture was stirred in ice bath for 2 h and then at room temperature for 18 h. The mixture was triturated with hexane (2 L), and the precipitate was separated by filtration, washed successively with hexane (2 L), ether (1 L), and hexane (1 L), and dried at room temperature to give 875.6 g of a solid, mp 104–5 °C. The solid was suspended in methanol (~3 L) (1 h) and cooled, and the precipitate was collected, washed with cold methanol and hexane, and dried at 56 °C under high vacuum for 18 h to give 781.6 g (73.4%) of a solid, mp 105–6 °C. Recrystallization from methanol gave analytically pure product, mp 105–6 °C. Anal. ($C_{12}H_{11}NO_3$) C, H, N.

Methyl 2-Cyano-3-(2-methoxyphenyl)propionate (9d). To a stirred suspension of methyl (2-methoxybenzylidene)cyanoacetate (761.6 g, 3.5 mol) in methanol (5.6 L) and water (1.4 L) was added 20 drops of 1 N sodium hydroxide, and then sodium borohydride (42.4 g, 1.12 mol) was added portion wise over 75-min period. (The internal temperature was 30–41 °C.) After the addition, the mixture was stirred at room temperature for 17 h and acidified to pH ~6 with acetic acid (~70 mL). After removal of the methanol <40 °C on a rotary evaporator under water aspirator pressure, the residue was extracted twice with toluene (2.2 and 1 L). The toluene extract was washed with saturated

sodium chloride solution (~0.8 L), dried over sodium sulfate, and evaporated, finally at 80 °C under high vacuum to give 536.5 g of oily residue. Distillation gave 483.3 g (62.3%) of pure product as a liquid (solidifies on standing at room temperature), bp 160 °C (0.75 mm). Anal. ($C_{12}H_{13}NO_3$) C, H, N.

2-Amino-α-cyano-1,6-dihydro-α-[(2-methoxyphenyl)-methyl]-5-nitro-6-oxo-4-pyrimidineacetic Acid, Methyl Ester (10d). To a stirred solution of compound **9d** (285 g, 1.3 mol) in dimethyl sulfoxide (650 mL) at 0–30 °C (ice–salt bath cooling) was added potassium *tert*-butoxide (146 g, 1.3 mol) portionwise over 15 min. The reaction mixture was stirred at room temperature for 40 min (internal temperature ~30 °C) and then was cooled to 6 °C, and a solution of 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone monohydrate (108.4, 0.527 mol) in dimethyl sulfoxide (500 mL) was added over a 15-min period (internal temperature 6–12 °C reprecipitated with ice–water bath cooling). After the addition the mixture was stirred at room temperature for 80 min and then was heated in a water bath at 65 °C (internal temperature) for 19 h. After cooling to 12 °C, the mixture was poured into ice water (~8.0 L), acidified to pH ~5.0 with acetic acid (~100 mL), and stirred at room temperature for 40 min. The precipitate was separated by filtration and washed with cold water. Waxy solid was suspended in ether (~2.0 L) and the mixture stirred at room temperature for 30 min, and the solid was collected, washed with cold water (2 × 2 L), ether (1 L), and dried at 78 °C under vacuum for 18 h to give 154.8 g (78.6%) of a light yellow solid, mp 244 °C dec. The solid was suspended in methanol (1 gal) refluxed with stirring on a stream bath for 2 h and cooled, and the precipitate was separated, washed with cold methanol (500 mL) and ether (~800 mL) and dried at 78 °C under vacuum for 4 h to give 116.2 g (59%) of a solid, mp 249 °C dec. Recrystallization (3.3 g) from DMF (40 mL) and ether (~950 mL) gave analytically pure product, mp 247 °C dec. Anal. ($C_{16}H_{15}N_5O_6$) C, N; H: calcd, 4.05; found 4.62.

2-Amino-α-[(2-methoxyphenyl)methyl]-1,6-dihydro-5-nitro-6-oxo-4-pyrimidineacetonitrile (12d). A solution of **10d** (112 g, 0.3 mol) in 1 N sodium hydroxide (1.5 L) was stirred at room temperature under nitrogen atmosphere for 3.75 h. The solution was carefully acidified to pH ~1 with 6 N hydrochloric acid (~425 mL) by dropwise addition over a 15-min period and then stirred for 45 min, 6 N hydrochloric acid (100 mL) was added, and the mixture was stirred for 20 min (internal temperature 25–32 °C). After cooling, the precipitate was collected by filtration, washed with cold water, and dried (house vacuum) overnight to give 74.1 g (78.3%) of a solid, mp ~185 °C dec. This was used in the next step without any further characterization.

2,6-Diamino-3,5-dihydro-7-[(2-methoxyphenyl)methyl]-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11d). To a solution of **12d** (74.1 g, 0.235 mol) in 1 N sodium hydroxide (3.0 L) was added sodium dithionite (263.3 g, 1.51 mol). The reaction mixture was heated on a steam bath with stirring under nitrogen for 50 min, then one additional portion of sodium dithionite (25 g, 0.14 mol) was added, and the mixture heated for 20 min. The hot mixture was filtered (folded paper). After cooling to 30 °C, the solution was acidified to pH ~1 with 6 N hydrochloric acid (~200 mL) (internal temperature was kept at ~30 °C by ice–water cooling) and then stirred at room temperature for 30 min. The mixture was cooled to ~20 °C and the precipitate was separated by filtration, washed with cold water and then with ether (~1.2 L), and dried at 78 °C under vacuum for 17 h to give 56.75 g of **14d**, mp 235–7 °C.

The solid **14d** (56.75 g) was added to concentrated hydrochloric acid (1.2 L), and the mixture was stirred at room temperature for 2 h, then heated to 55 °C, and stirred at 47–55 °C for 45 min. After cooling to 20 °C, the precipitate was separated by filtration, washed with ether (2 × 500 mL), cold water (2 × 250 mL), and then with ether (~500 mL), and dried at 78 °C for 16 h to give 28.7 g of a light-orange colored solid, mp 215–235 °C. A solution of the solid in warm methanol (~700 mL) was filtered, concentrated under reduced pressure to a 250-mL volume, diluted with ether (~1.6 L), and cooled. The precipitate was separated, washed with ether, and dried at 78 °C for 19 h to give 26.95 g (35%) of analytically pure product (**11d**), mp 215–240 °C. Anal. ($C_{14}H_{15}N_5O_2$·HCl·0.33H_2O) C, H, Cl, H_2O; N: calcd 21.37; found, 20.45.

Inhibitors of Acyl-CoA:Cholesterol Acyltransferase. 1. Identification and Structure–Activity Relationships of a Novel Series of Fatty Acid Anilide Hypocholesterolemic Agents

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A series of fatty acid anilides was prepared, and compounds were tested for their ability to inhibit the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) in vitro and to lower plasma total cholesterol and elevate high-density lipoprotein cholesterol in cholesterol-fed rats in vivo. The compounds reported were found to fall into two subclasses with different anilide SAR. For nonbranched acyl analogues, inhibitory potency was found to be optimal with bulky 2,6-dialkyl substitution. For α-substituted acyl analogues, there was little dependence of in vitro potency on anilide substitution and 2,4,6-trimethoxy was uniquely preferred. Most of the potent inhibitors (IC_{50} < 50 nM) were found to produce significant reductions in plasma total cholesterol in cholesterol-fed rats. Additionally, in vivo activity could be improved significantly by the introduction of α,α-disubstitution into the fatty acid portion of the molecule. A narrow group of α,α-disubstituted trimethoxyanilides, exemplified by 2,2-dimethyl-*N*-(2,4,6-trimethoxyphenyl)dodecanamide (**39**), was found to not only lower plasma total cholesterol (–60%) in cholesterol-fed rats but also elevate levels of high-density lipoprotein cholesterol (+94%) in this model at the screening dose of 0.05% in the diet (ca. 50 mg/kg).

Acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is the intracellular enzyme responsible for catalyzing the formation of cholesteryl esters, utilizing cholesterol and fatty acyl-CoA as substrates. Although ACAT activity has been demonstrated in most mammalian tissues, the enzyme itself is poorly characterized.[1] It has been hypothesized that this enzyme may play an important

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