***N*-[*N*-[*N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid (15, *n* = 2).** Side-chain precursor **14** (*n* = 2) from hydrogenolysis of **10** (5.68 g, 5.50 mmol) was dissolved in Me_2NAc (25 mL). Solid **1** (4.58 mmol) was added, and the mixture was stirred at 25 °C for 6 days.[20] The yellow-orange solution was combined with cold H_2O (105 mL) to which 1 N NaOH (20 mL) had been added. The resulting solution (pH 4.7) was treated with 1 N HCl to pH 3.7, and a yellow solid precipitated. After refrigeration overnight, the precipitate was collected, but it changed to a viscous gum on the funnel. Dilution of the filtrate with EtOH (1 L) caused separation of yellow solid. The stiff gum and the EtOH-precipitated solid (1.07 g) were combined while being dissolved in H_2O (80 mL) to which NaOH solution (1 N) was added in small increments to keep the pH near 6. The solution that formed was treated dropwise with 1 N HCl, and a viscous gum-like precipitate began forming at pH 4.0 and continued until the pH was 3.2. After the mixture had been chilled in an ice-H_2O bath, the supernatant was removed by decantation; then the gum was dissolved in a 1:1 solution of AcOH-H_2O (25 mL). Dropwise addition to continuously stirred EtOH (60 mL) followed, and **15** (*n* = 2) separated as an easily managed yellow solid. The collected solid was washed successively with 5:1 (v/v) EtOH-H_2O, EtOH, and then Et_2O and dried at successive temperatures of 25, 58, and 77 °C until it reached a constant weight of 2.26 g (67% yield). Additional data are included in Table II.

***N*-[*N*-[*N*-[*N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid (15, *n* = 3).** A mixture of side-chain precursor **14** (*n* = 3; 4.32 mmol) and **1** (3.60 mmol) in Me_2NAc (20 mL) was stirred at 25 °C for 6 days,[20] and then the solution that formed was combined with cold H_2O (100 mL). Dropwise addition of 1 N NaOH to raise the pH from 2.2 followed, and the precipitate began forming when the pH was 3.0. When the pH had been brought to 3.7, only a small amount of gummy precipitate had formed. Addition of 1 N NaOH was continued until the pH was 6.8 in order to redissolve the precipitate. The solution was then carefully treated with 1 N HCl to lower the pH to 3.8-4.0, where it remained clear. This solution was added dropwise to stirred EtOH (600 mL) to give a yellow solid, which was collected after the mixture had been left overnight in a refrigerator. The collected precipitate was washed with EtOH, followed by Et_2O, and dried in vacuo to give 3.50 g of material whose field-desorption mass spectrum showed peaks of *m/e* 841 (M^+) and 842 [$(M + 1)^+$]. Assay by HPLC showed high purity (98%) with respect to UV-absorbing materials, but elemental analysis results (Found: C, 41.18, 40.99; H, 4.99, 4.93; N, 13.69, 13.62) indicated contamination by inorganic material in addition to the expected solvation by EtOH. This material was dissolved in AcOH-H_2O (1:1, 20 mL), and the solution was added dropwise to stirred EtOH (500 mL). The remainder of the isolation procedure was the same as described above for **15** (*n* = 2). The yield of **15** (*n* = 3) was 2.66 g. More data are given in Table II. The yield was 78% based on the solvate indicated in Table II.

(20) The light-sensitive products were protected during the reaction period by wrapping the flasks with Al foil. Isolated products were stored in a freezer.

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Registry No. 1, 52853-40-4; 4, 2768-50-5; 5, 79974-14-4; 6, 79974-10-0; 7, 30924-93-7; 8, 83816-89-1; 9, 83816-90-4; 10, 83816-91-5; 11, 83816-92-6; 12, 83816-93-7; 13, 83816-94-8; 14 (*n* = 2), 83816-95-9; 14 (*n* = 3), 83816-96-0; 15 (*n* = 0), 59-05-2; 15 (*n* = 1), 41600-13-9; 15 (*n* = 2), 41600-14-0; 15 (*n* = 3), 73610-81-8.

Aromatic Amidines: Comparison of Their Ability to Block Respiratory Syncytial Virus Induced Cell Fusion and to Inhibit Plasmin, Urokinase, Thrombin, and Trypsin

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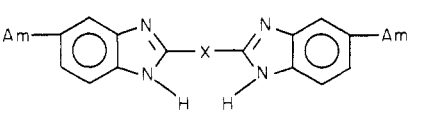
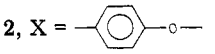
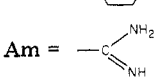
Two series of amidine derivatives consisting of a total of 24 compounds were examined for a correlation between their blocking effect on respiratory syncytial virus induced cell fusion and their inhibitory activity against selected trypsin-like protease. Although no correlation was evident between the two activities, several potentially important discoveries were made. A highly selective inhibitor of plasmin over thrombin (compound **10**) was obtained, and a potent new blocker of virus-induced cell fusion (compound **22**) was identified.

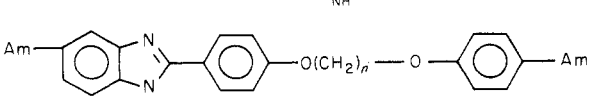
Respiratory syncytial (RS) virus is a common respiratory pathogen and the leading cause of respiratory tract infection in infancy and early childhood.[1] Although the virus commonly produces bronchiolitis and bronchopneumonia and may be life threatening in certain populations, there is no effective vaccine or chemotherapeutic agent yet available. Recently, we reported that certain aromatic amidino derivatives effectively blocked cell fusion induced by RS virus and significantly reduced the yield of RS virus.[2,3] Aromatic amidino compounds are generally recognized as potent reversible inhibitors of arginine- and lysine-specific esteroproteases. Specifically, we observed that our leading compound against virus-induced cell fusion,[2,3] bis(5-amidino-2-benzimidazolyl)methane (BABIM, compound 1), was also an outstanding inhibitor of three esteroproteases (trypsin, urokinase, and plasmin).[4,5] The



1, X = CH_2

2, X = 

Am = 



3, *n* = 2
4, *n* = 3
5, *n* = 4
6, *n* = 5

suggestion of a relationship between the antifusion potency of amidino compounds and their effectiveness against

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Table I. Comparison of the Blocking Effect on Cell Fusion Induced by Respiratory Syncytial Virus with Inhibition Constants for Plasmin, Urokinase, Thrombin, and Trypsin

no.	K_i,[b] μM				min concn, μM, for complete blockage of cell fusion[b]
	plasmin	urokinase	thrombin	trypsin	
1	2.65[c] ± 0.47	2.33[c] ± 0.36	4.15[d] ± 0.65	0.017[d] ± 0.006	0.5[e]
2	~200	nonlinear kinetics	3.94[d] ± 0.52	4.67[d] ± 0.61	NB[f]
3	3.9 ± 1.1	12.0 ± 2.8	2.73[d] ± 0.29	5.98[d] ± 0.71	10[f]
4	8.19 ± 1.96	17.8 ± 4.7	2.09[d] ± 0.44	2.96[d] ± 0.13	10[f]
5	8.18 ± 1.74	4.47 ± 0.82	3.08[d] ± 0.39	4.01[d] ± 0.10	NB[g]
6	3.83 ± 0.54	8.0 ± 1.8	2.24[d] ± 0.26	1.44[d] ± 0.10	NB[g]
7	112[c] ± 13	131[c] ± 35	7.68[h] ± 1.0	29.1[h] ± 3.1	10[e]
8	>1000[c]	196[c] ± 77	35.2[h] ± 8.9	85.3[h] ± 9.1	1[e]
9	>400	280 ± 69	42.8 ± 5.8	137 ± 48	10[f]
10	43 ± 5	10.6 ± 1.6	>1000	25.5 ± 1.9	NB[f]
11	>1000	>100	1890 ± 759	>1000	NB[f]
12	>1000	>100	1510 ± 565	>1000	NB[f]
13	>200	267 ± 49	176 ± 36	>100	NB[f]
14	61.5 ± 8.7	40.3 ± 6.3	55 ± 6	15.3 ± 1.0	NB[f]
15	60[c] ± 18	26.5[c] ± 6.2	24.8[h] ± 7.7	3.18[h] ± 0.95	10[f]
16	>700[c]	19.0[c] ± 2.7	47.8 ± 15.4	5.31 ± 0.46	10[f]
17	65.6[c]	36[c] ± 8	22.4 ± 10.5	8.29 ± 0.83	10[f]
18	164 ± 13	24.4 irregular	15.4 ± 4.3	16.8 ± 1.5	NB[f]
19	>100	>100	6.39 ± 0.92	7.77 ± 0.10	10[f]
20	844 ± 38	191 ± 28	20.1 ± 8.4	15.4 ± 4.0	NB[f]
21	~100[c]	40[c] ± 7	4.8 ± 0.4	5.31 ± 0.32	10[f]
22	111 ± 14	25.6 ± 3.0	13.1 ± 1.5	4.59 ± 0.43	1[f]
23	>400	>200	164 ± 25	3.44 ± 0.38	NB[f]
24	2.93 ± 0.88	9.96 ± 0.23	11.1 ± 0.8	3.2 ± 0.7	10[f]

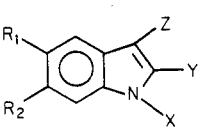
[a] Values are means ± SD; $n = 3$. [b] NB = no blockage of cell fusion at the highest concentration where there was no cytotoxicity. [c] From ref 5. [d] From ref 4. [e] No cytotoxicity observed at concentrations up to 100 μM. [f] Cytotoxic at 100 μM. [g] Cytotoxic at 10 μM. [h] From ref 14.

urokinase and plasmin is particularly noteworthy, since human cells, especially transformed cells, have been shown to form urokinase-like plasminogen activator and plasmin-like enzymes.[6,7] Since we determined antifusion activity of admidines by observing the ability of the compounds to block virus-induced fusion of human transformed cells (HEp-2), the intriguing speculation arose that amidines act to retard the fusion process via inhibition of urokinase and/or plasmin.

In light of these findings, the present study was initiated to determine if the correlation between inhibition of plasmin and/or urokinase with fusion-blocking activity would hold true in a sizable series of compounds. For comparison, the inhibitor values of the compounds against two other esteroproteases, trypsin and thrombin, were included. The two series of compounds examined in this study, amidinobenzimidazole derivatives and amidinoindole derivatives, were chosen because of the high potency against virus-induced cell fusion exhibited by the lead compounds and because of the wide range of antiprotease activity obtained.

Results and Discussion

The inhibition constants (K_i) for the compounds against trypsin, thrombin, plasmin, and urokinase are listed in Table I, along with the minimum concentration of the compounds required to obtain complete blockage of virus-induced cell fusion. Examination of the data reveals that no apparent correlation exists between the inhibitory effect of the amidine derivatives against any of the four protease enzymes and their supressive effect on RS virus-induced cell fusion. For instance, compound 8, though



no.	R_1	R_2	X	Y	Z
7	Am	H	H	H	H
8	Am	H	CH_3	H	H
9	Am	H	CH_2CH_3	H	H
10	H	Am	H	H	CHO
11	H	Am	H	H	$COCH_3$
12	H	Am	H	H	$COCF_3$
13	H	Am	H	H	PhCO
14	H	Am	H	COEt	H

one of the most potent compounds against virus-induced cell fusion, was a weak across-the-board inhibitor of the proteases tested. Although the original objective of this study, the demonstration of a link between antifusion and antiprotease activity, was not realized, several important discoveries can be found in Table I. For example, a new potent inhibitor of plasmin ($K_i = 2.93 \times 10^{-6}$ M) was seen in compound **24**. Also, compound **22** proved to be a highly effective blocker of cell fusion induced by RS virus. This observation was surprising, since earlier structure–activity results pointed to amidine substitution of the heterocyclic ring as the key to maximal antifusion potency. Finally,

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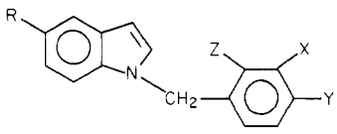
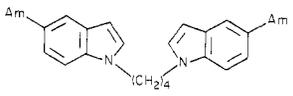


no.	R	X	Y	Z
15	Am	H	Am	H
16	Am	H	Am	Cl
17	Am	Am	H	H
18	Am	NH_2	H	H
19	Am	NO_2	H	H
20	Am	H	H	H
21	Am	H	NO_2	N
22	H	H	Am	H
23	NO_2	H	Am	H
24				

a unique property can be attributed to compound **10**, which demonstrated a relatively high affinity for plasmin when compared to thrombin. While most amidine derivatives demonstrate a higher affinity for thrombin than plasmin, as can be seen in Table I, only compound **10** shows significant selectivity toward plasmin when compared to thrombin. Such an inhibitory characteristic could have both laboratory and clinical significance by allowing selective blockage of the fibrinolytic system without interfering with the key step in the clotting mechanism.

Assimilation of the data seen in Table I leads to the inference that the antifusion effect of amidines is not related to their antiproteolytic efficacy. This conclusion is strengthened by our own observation that several nonamidine inhibitors of proteases were found to be devoid of antifusion activity. For instance, soybean trypsin inhibitor (1 mg/mL), pancreatic trypsin inhibitor (100 g/mL), ϵ-aminocaproic acid (3.3 mg/mL), and nitrophenyl quanidinobenzoate (10^{-4} mol) failed to block RS virus induced cell fusion. Admittedly, the results presented here refute the initial premise that plasmin and/or urokinase could be the sole enzyme(s) involved in cell fusion and specific inhibitors of these enzymes would, therefore, necessarily be the best fusion blockers. However, the possibility that proteolytic activity is necessary for cell fusion and that amidines block cell fusion via antiproteolytic mediation cannot be ruled out from these studies. For instance, if a novel protease or multiple enzymes, i.e., clotting cascade, were needed for expression of cell fusion, then it would be highly unlikely that a relationship between inhibition of any known protease and the blockage of virus-induced cell fusion would be observed. This can be attributed to the high selectivity of amidine derivatives, as well as the non-amidine inhibitors, toward specific proteases. The selectivity of amidines is evidenced by the most potent inhibitor of acrosin, which had a K_i of 5×10^{-8} M against acrosin[9] while exhibiting a K_i of 5×10^{-5} M or greater against five other serine proteases. The remarkable structural specificity of amidine derivatives with regard to their effectiveness against RS virus induced cell fusion still makes a strong argument for protease inhibition as the most likely explanation for their antifusion activity. Ongoing studies directed toward the characterization and isolation of putative proteases or amidine binding sites from virus-infected cell cultures should resolve this intriguing question.

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Experimental Section

Amidase Assays. A number of the dissociation constants (K_i) reported in Table I were obtained from prior communications and have been appropriately referenced in the table. While the details of the amidase assays used in the present work have been previously described,[5,8,10] the the summary of the procedure is given.

The K_i values of the inhibitors with thrombin and trypsin were obtained from amidase assays employing *N*-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride[11] (BANA, Bachem, Inc.) as substrate. In the case of urokinase, the substrate used was L-pyroglutamylglycyl-L-arginine-*p*-nitroanilide hydrochloride (S-2444, Kabi) and for plasmin the amidase assays were carried out with *N*-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA, Bachem, Inc.) as the substrate. All tests were carried out at 37 °C. The K_i values for trypsin, thrombin, and urokinase were determined at pH 8.1, while plasmin values were obtained at pH 7.6. We determined the K_i values for the inhibitors graphically according to Dixon[12] by plotting the reciprocals of the initial reaction velocities at two different substrate concentrations against different inhibitor concentrations.

Thromin (bovine, topical) was purchased from Parke, Davis & Co. Trypsin (bovine, twice crystallized, salt free) was a product of Schwarz-Mann containing 57% active trypsin as determined by active-site titration. Lyophilized huma plasmin was purchased from Kabi, and lyophilized high-molecular-weight urokinase (HMW-UK) was kindly supplied as Winkinase by Winthrop Laboratories.

Blocking Effect on Virus-Induced Cell Fusion. HEp-2 cells were propagated in Eagle minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). For the inhibitor experiments HEp-2 cells were used between passages 370 and 376.

The A_2 strain of RS virus, obtained from R. M. Chanock, NIAID, Bethesda, MD, was grown in HEp-2 cells. After virus was adsorbed to suspensions of HEp-2 cells, the infected cells were seeded in 250-mL plastic culture flasks containing MEM plus 10% FBS. When viral cytopathic effects were maximal (72–96 h), the flasks were stored at −70 °C. Typical lysates contained 10^7 to 10^8 50% tissue infective doses per milliliter.

Using the technique of Chen,[13] we determined all virus stocks and cell lines to be free of mycoplasma contamination.

For cell-fusion assays, HEp-2 cells were seeded in well trays to contain approximately 10^6 cells per well at 72 h. RS virus, added at 0.1 input multiplicity of infection in 0.2 mL, was adsorbed for 2 h at 36 °C. After this period, the wells were rinsed twice with 1 mL of MEM and received 0.5 mL of either inhibitor-containing (100, 10, 1.0, 0.5, and 0.1, M) or control medium consisting of MEM with 2% FBS and 1% Me_2SO. Toxicity and cytopathology were scored at 72 h. The details of the above-mentioned procedure have been previously described.[2,3]

Organic Synthesis. The synthesis and physical data on compounds **1–6**;[4] **7**, **8**, **15**, **16**;[14] and **17** and **21**[5] have been described. Analytical data, melting points, and yields for novel amidines are given in Table II. All melting points were determined on a Thomas-Hoover capillary melting point apparatus. ^1H NMR spectra were recorded as ca. 10% solutions in Me_2SO-d_6 with tetramethylsilane as internal standard on a Hitachi Perkin-Elmer R-24A high-resolution NMR spectrometer. IR spectra were recorded on a Perkin-Elmer Model 710A grating infrared spectrophotometer. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN. Analyses for target compounds were accepted if the determined values were within 0.4% of the theoretical values. In the instances where compounds were analyzed for water of crystallization, the presence of water was also determined by NMR spectra. The amidine products, with the

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Table II. Analytical Data, Melting Points, and Yields of Novel Amidines

no.	mp, °C	yield,[a] %	formula	anal.[b]
9	244 dec	10	$C_{11}H_{13}N_3 \cdot 2HCl \cdot 0.25H_2O$	C, H, N
10	290 dec	40	$C_{10}H_9N_3O \cdot HCl$	C, H, N
11	295 dec	32	$C_{11}H_{11}N_3O \cdot 2HCl \cdot 0.2H_2O$	C, H, N
12	275-282	8	$C_{11}H_8F_3N_3O \cdot HCl$	C, H, N
13	180-182	10	$C_{16}H_{13}N_3O \cdot HCl \cdot 2.75H_2O$	C, H, N
14	252-254	24	$C_{12}H_{13}N_3O_2 \cdot HCl \cdot 1.25H_2O$	C, H, N
18	234-236	78[c]	$C_{16}H_{16}N_4 \cdot HCl \cdot H_2O$	C, H, N
19	99-101	24	$C_{16}H_{14}N_4O_2 \cdot HCl \cdot 1.75H_2O$	C, H, N
20	265-267	25	$C_{16}H_{15}N_3 \cdot HCl \cdot H_2O$	C, H, N
22	240-241	64	$C_{16}H_{15}N_3 \cdot HCl \cdot 1.25H_2O$	C, H, N
23	272-274	41	$C_{16}H_{14}N_4O_2 \cdot HCl \cdot H_2O$	C, H, N
24	255 dec	31	$C_{22}H_{24}N_6 \cdot 4HCl \cdot H_2O$	C, H, N

[a] All yields were calculated from the corresponding cyano derivatives unless so noted. [b] All compounds were recrystallized from H_2O, 10% HCl, or EtOH. Some of the compounds were subjected to multiple recrystallizations with either one or all of the aforementioned solvents. [c] Yield was calculated from the reduction of compound 14.

exception of compound 18, were synthesized from the corresponding nitrile derivatives by a modified version of the Pinner amidine synthesis.[4,15,16] Compound 18 was prepared by reduction of compound 19 with 5% palladium on charcoal in ethanol.[9] Novel intermediates were characterized by ^1H NMR and IR spectra, and purity was determined by TLC. Experimental details for the modified Pinner synthesis, as well as representive synthesis of intermediates, is given as follows.

A. Method of Conversion of Nitriles to Amidines. The nitrile was dissolved in either EtOH alone or EtOH with an appropriate solvent (benzene or chloroform). The resulting solution was cooled to 0 °C, and this temperature was maintained during saturation with dry HCl. Following addition of the HCl, the mixture was allowed to return to room temperature and stirred until aliquots indicated the disappearance of the nitrile band in the IR spectra (2200 cm^{-1}). We precipitated the imino ether hydrochloride from the alcohol solution by either cooling (0–5 °C) or by adding Et_2O. Without further purification, the imino ether was dissolved in ammonia-saturated ethanol, and the solution was heated (50–60 °C) in a capped hydrogenation bottle for 4 h. The crude amidine was normally isolated by Et_2O precipitation, followed by filtration. The crude product was purified by crystallization from EtOH/H_2O or 10% HCl, followed by washing with cold HCl (2 N) and acetone. The product was characterized by the broad and very strong aromatic amidine peak in the IR spectra (KBr, 3050 cm^{-1}) and the ^1H NMR spectra showing the amidine protons at δ 9.0–10.0.

B. 5-Cyano-1-ethylindole (Precursor to 9).[12] To a cooled solution (5 °C) of 5-cyanoindole (3 g, 21.1 mmol) dissolved in 75 mL of acetone was added powdered KOH (5.9 g, 105.5 mmol). The cooled stirring mixture was then treated dropwise with 6.9 g (42.2 mmol) of ethyl iodide. After the mixture was stirred at room temperature for 1 h, dry benzene (30 mL) was added and a white solid was removed by filtration. The filtrate was dried over anhydrous Na_2SO_4, and the solvent was removed by distillation. The residue (oily brown solid) was recrystallized from a mixture of Et_2O and petroeum ether (30–60 °C) to give 1.25 g (33%) of light green crystals: mp 68–70 °C; IR (KBr) 2210 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.32 (3 H, t, CH_3), 4.15 (2 H, q, CH_2), 6.50 (1 H, d, ArC*H*=CH), 7.1–7.9 (4 H, m, aromatic and CH=CHN).

C. 1-Benzyl-5-cyanoindoles (Precursors to Compounds 19, 20, 22, and 23). The nitrile intermediates for compounds 19, 20, 22, and 23 were prepared in like fashion by combining the appropriate indole derivative with the corresponding bromotoluene derivative.[18] As a representive synthesis, the preparation of 5-cyano-1-(3-nitrobenzyl)indole (precursor for compound 19) is detailed as follows.

A solution of 5-cyanoindole (2.0 g, 14 mmol) in anhydrous ether (10 mL) was added dropwise to a stirred solution of sodium (0.35 g, 15.2 mmol) in liquid ammonia (30 mL). To this solution was added dropwise *m*-nitrobenzyl bromide (3.65 g, 17 mmol) in 15 mL of anhydrous ether. The ammonia was allowed to evaporate, and the residue was dissolved in a mixture of 40 mL of MeOH and 40 mL of $CHCl_3$. The $CHCl_3$ solution was washed with 40 mL of H_2O, dried over anhydrous Na_2SO_4, and evaporated, yielding a brown solid. The crude material was decolorized and recrystallized from EtOH to give 2.12 g (55%) of a buff-colored material: mp 157–159 °C; IR 2200 (C≡N), 1510 (asymmetric $ArNO_2$), 1350 (symmetric $ArNO_2$) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.60 (2 H, s, CH_2), 6.75 (1 H, d, ArC*H*=CH), 7.35–8.10 (8 H, m, aromatic and CH=C*H*N).

The following intermediates were made in the same manner.

1-(4-Cyanobenzyl)indole (precursor to compound 22): yield 40%; brown oil; IR (film) 2200 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.45 (2 H, s, CH_2), 6.45 (1 H, d, ArC*H*=CH), 6.9–7.7 (9 H, m, aromatic and CH=C*H*N).

1-Benzyl-5-cyanoindole (precursor to compound 20): yield 40%; mp 101–103 °C; IR (KBr) 2210 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.35 (2 H, s, CH_2), 6.50 (1 H, d, ArC*H*=CH), 7.0–8.0 (9 H, m, aromatic and CH=C*H*NH).

1-(4-Cyanobenzyl)-5-nitroindole (precursor to compound 23): yield 54%; mp 162–164; IR (KBr) 2205 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.60 (2 H, s, CH_2), 6.70 (1 H, d, ArC*H*=CH), 7.2–8.6 (8 H, m, aromatic and CH=C*H*NH_2).

D. 1,4-Bis(5-cyano-1-indolyl)butane (Precursor to Compound 24). To a cooled solution (20 °C) of 2 g of 5-cyanoindole (14 mmol) in 50 mL of acetone was added powdered KOH (3.9 g, 70 mmol). To this cooled stirring mixture was added dropwise 2.17 g (70 mmol) of 1,4-diiodobutane After addition was complete (10 min), the reaction mixture was allowed to stir at ambient temperature for 2 h. A white solid was collected from the rection and washed thoroughly with acetone, followed by cold H_2O. The solid was dried overnight in a vacuum oven at 100 °C to give 1.5 g (63%) of white solid: mp 204–205 °C; IR (KBr) 2210 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.7 (4 H, m, $CH_2CH_2CH_2$), 4.2 (4 H, m, $CH_2CH_2CH_2CH_2$), 6.5 (2 H, ArC*H*=CH), 7.3–8.0 (8 H, m, aromatic and CH=C*H*N).

E. 6-Cyano-3-indolecarboxaldehyde (Precursor to Compound 10). To a stirring and cooled (10 °C) solution of dry DMF (3.4 mL) was added dropwise $POCl_3$ (1 mL, 11 mmol), followed by the dropwise addition of 6 mL of 6-cyanoindole (1.0 g, 7 mmol) dissolved in 20 mL of DMF. After stirring at ambient temperature for 1 h, the mixture was poured onto crushed ice and then treated dropwise with 4.75 N NaOH (8 mL), followed by an additional 4 mL of 4.75 N NaOH added all at once. The solution was heated to boiling and then filtered hot. Upon cooling, the filtrate yielded 0.69 g (58%) of buff solid: mp 241–243 °C; IR (KBr) 2200 (C=N), 1620 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.5–8.5 (4 H, m, aromatic and C=C*H*N), 10.1 (1 H, s, HC=O), 11.3 (1 H, Br, NH).

F. 6-Cyano-3-indolyl Ketones (Precursors to Compounds 11–13). The nitrile precursors to compounds 11–13 were obtained by reacting 6-cyanoindole, prepared according to the method of Ikan and Rapaport,[19] with the appropriate acid anhydride or chloride. The conditions were the same as those described by DeGraw et al.[20] for the synthesis of the corresponding 5-cyano-3-indolyl ketones. The following synthesis of 3-acetyl-6-cyanoindole is given as an example of the acid anhydride reaction, and the acid chloride reaction is demonstrated with the synthesis of 6-cyano-3-benzoylindole.

3-Acetyl-6-cyanoindole (Precursor to Compound 11). A solution of $SnCl_4$ (1.4 mL, 12.2 mmol) in 4 mL of benzene was added dropwise to a cold (0.5 °C) stirring solution of 6-cyanoindole (1.0 g, 7 mmol) and acetyl chloride (0.95 g, 12 mmol) in 16 mL of benzene. The cold mixture was allowed to stir for 1 h and then poured onto 50 mL of ice–water. The precipitate was filtered,

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and the filtrate was washed with cold water and hot acetone to give 0.37 g (28%) of tan solid: mp 275–276 °C; IR (KBr) 2200 (C≡N), 1610 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.5 (3 H, s, CH_3), 7.25–8.5 (4 H, m, aromatic and C=C*H*N), 12.4 (1 H, br, N*H*).

6-Cyano-3-(trifluoroacetyl)indole (precursor to compound 12) was synthesized in the same manner: yield 39% mp 230–235 °C; IR (KBr) 3300 (NH), 2225 (C≡N), 1620 (C=O), 1200 and 1250 (CF_3) cm^{-1}.

6-Cyano-3-benzoylindole (Precursor to Compound 13). To a cold, stirring solution of 6-cyanoindole (1.0 g, 7 mmol) and benzoyl chloride (1.7 g, 14 mmol) was added dropwise stannic chloride (3.6 g, 14 mmol) in 10 mL of benzene. After stirring for 1 h, the mixture was poured onto 125 mL of ice–water and stirred for an additional 30 min. A solid was collected, washed with H_2O, dried, and recrystallized in ethanol to yield 0.4 g (23%) of buff solid: mp 263–265 °C; IR (KBr) 3100 (NH), 2210 (C≡N), 1600 (C=O) cm^{-1}; ^1H NMR δ 7.5–8.5 (9 H, m, aromatic and C=C*H*N), 12.5 (1 H, br, NH).

The precursor to compound **14** is a known compound and was synthesized according to a published method.[21]

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Syntheses of Iron Bis(pyridoxal isonicotinoylhydrazone)s and the in Vivo Iron-Removal Properties of Some Pyridoxal Derivatives

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Pyridoxal isonicotinoylhydrazone (PINH; **1**) and its isomeric *O*-acetates (*E* and *Z*) were synthesized and complexed with ferrous ions to afford the hitherto unisolated chelates iron(II) bis(pyridoxal isonicotinoylhydrazone)s (**11**) and iron(II) bis(*O*-acetylpyridoxal isonicotinoylhydrazone)s (**12**). The analytical and spectroscopic data of the new coordination compounds are presented. In addition, a series of imino derivatives of pyridoxal of structures **2–3** and **5–10** have been prepared and tested in vivo as chelators of storage iron, and the cumulative net excretion of radioiron in urine and in feces was estimated. This study reestablishes that PINH is a potent iron chelator in vivo comparable in efficiency with parenteral desferrioxamine (DF) and indicates that it requires further attention.

Synthetic chemicals containing the pyridoxal moiety exhibit a large variety of biological activities of great importance. One of the most interesting aspects of pyridoxal derivatives is their effect on blood diseases and, in particular, on *thalassemia* and other chronic *anemias* resulting in abnormal accumulation of iron in tissues, which causes severe damage to vital organs.

The only practical method of iron mobilization in such diseases is the use of iron-chelating drugs, of which the commercially available agent desferrioxamine (DF)[1] is distinguished by its lack of significant toxicity. However, because of its limited gastrointestinal absorption and its high cost, current interest is focused on the development of new iron-chelating agents that might be better suited for large-scale clinical use.

We report here the synthesis of some pyridoxylidenimino derivatives, comprising pyridoxal acylhydrazones (**1–4**), a semicarbazone (**5**), alkoxylcarbonylhydrazones (**6–8**), and thiazolylhydrazones (**9** and **10**), for in vivo iron-chelation studies and the isolation of crystalline iron(II) complexes **11** and **12** generated from **1** and **4**, respectively. The binding constants to iron in **11** and **12** were derived from their spectrophotometric studies.

These studies aimed at shedding more light on structure–activity relationships in the new type of iron chelators related to **1**. It was undertaken with a view to develop a new synthetic approach to chelation therapy.

The ability of pyridoxal isonicotinoylhydrazone (**1**, PINH)[2-5] to mobilize iron from both parenchymal and

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