Diarylamidine Derivatives with One or Both of the Aryl Moieties Consisting of an Indole or Indole-like Ring. Inhibitors of Arginine-Specific Esteroproteases

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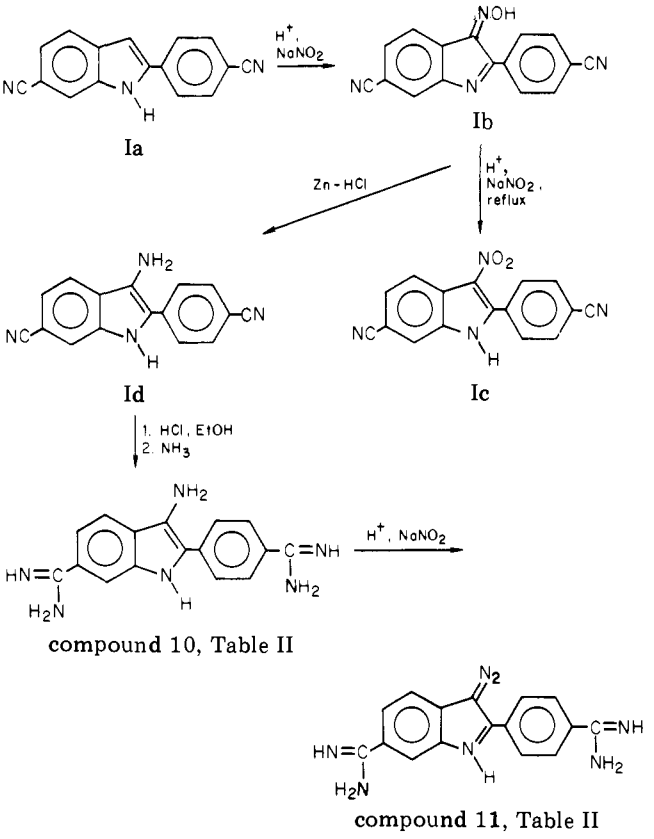
A series of 62 diarylamidine derivatives was evaluated for their antiproteolytic activity. In all but two of the compounds one or both of the amidino-substituted aryl moieties was either an indole or an indole-like ring. The latter included indene, benzimidazole, benzofuran, benzo[β]thiophene, and several other related nitrogen-containing heterocycles. Several of the compounds exhibited considerable inhibitory potency against thrombin, trypsin, and pancreatic kallikrein. An outstanding inhibitor of trypsin was found in bis(5-amidino-2-benzimidazolyl)methane (compound **42**) with a K_i value of 1.7×10^{-8} M (pH. 8.1, 37 °C). Another derivative, 1,2-di(4-amidino-2-benzofuranyl)ethane (compound **21**), proved to be a highly effective inhibitor of the overall blood clotting process. From a general structure–activity standpoint these compounds demonstrate that minor structural variations of low-molecular-weight inhibitors can result in significant changes in specificity and potency with regard to antiproteolytic activity.

In a continuation of the established interest[1] of our laboratories in aromatic amidines as reversible inhibitors of arginine- and lysine-specific esteroproteases, a new series of diarylamidine derivatives is presented. Earlier we investigated the antiproteolytic activity of a number of compounds distinguished by two benzamidine moieties linked by either dioxyalkane,[1i,j] monooxyalkane[1j] or dioxyaryl[1k] bridges. More recently we reported on a series of tris(benzamidine) derivatives with the benzamidine groups linked by an α,α′,α″-trioxymesitylene moiety.[1l] From these series of compounds we obtained highly potent inhibitors of trypsin, thrombin, and pancreatic kallikrein as well as effective inhibitors of the overall coagulation process. The investigation of the current diarylamidine derivatives, where one or both of the aryl moieties consist of an indole or indole-like ring, was initiated to further our efforts to produce more potent, less toxic, and better soluble protease inhibitors. In addition, we sought to obtain highly specific derivatives which would contribute information about the steric and binding characteristics in and around the specificity pockets of the various enzymes. As will be shown below, several of these new compounds exhibited considerable blocking activity against thrombin, trypsin, and pancreatic kallikrein. However, the single most significant finding with these compounds was the striking effect that minor structural changes had on the extent of their antiprotease activity.

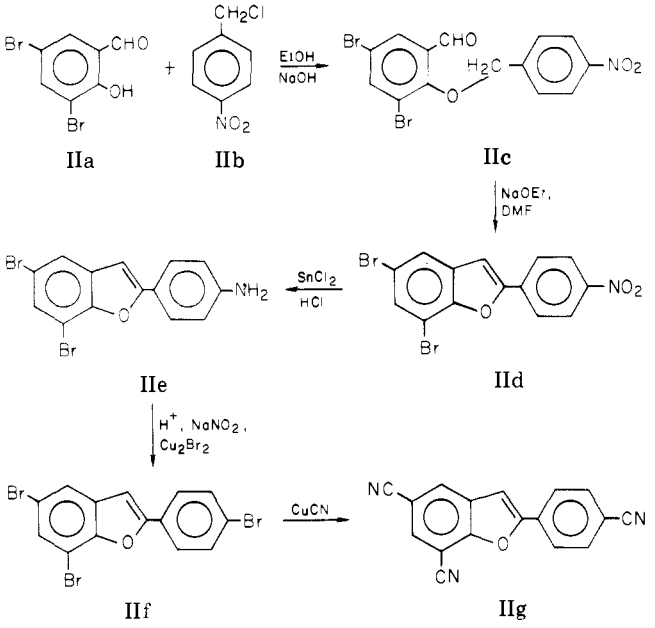
Chemistry. The synthesis and determination of the physical properties of compounds **1–5**, **7**, **8**, **12–22**, **24–36**, **44**, and **54–62** were carried out at the Institut für Pharmazie und Lebensmittelchemie, Friedrich-Alexander-Universität, Erlangen, Germany. This work has been well described by Dann and co-workers.[2-6] Compounds **9–11**, **23**, and **53** have also been reported.[7-9] However, since the synthetic procedures appear in publications not readily accessible to the readers of this journal, the syntheses of these compounds are included in the Experimental Section and outlined in Schemes I–III. Compounds **37–43** and **45–52** are novel and were produced by one of us (H.L.) at Hoechst AG, Frankfurt, Germany. Likewise, compound **6** is a new derivative and was prepared by one of the authors (G.V.). For the preparation of these novel amidines (Schemes IV–VII), one or more of the following reactions comprise the synthetic pathways: (1) the synthesis of 2-substituted benzimidazoles by the condensation of an *o*-phenylenediamine with an imidate in the presence of an equivalent of acid,[10,11] (2) the synthesis of ethers by the standard Williamson reaction, and (3) the Pinner synthesis of an amidine from a nitrile via an imidate intermediate.[12,13] In the case of Schemes IV and V, where each scheme represents the synthesis of several derivatives, the Experimental Section is limited to a typical preparation. However, the yields, melting points, and analytical data of each of these compounds appear in Table I. The procedure outlined in Scheme VIII for the preparation of 7-amidino-2-(4-amidinophenyl)indole follows a method described by Dann et al. for the synthesis of the corresponding 6-amidinoindole derivative.[2]

Scheme I

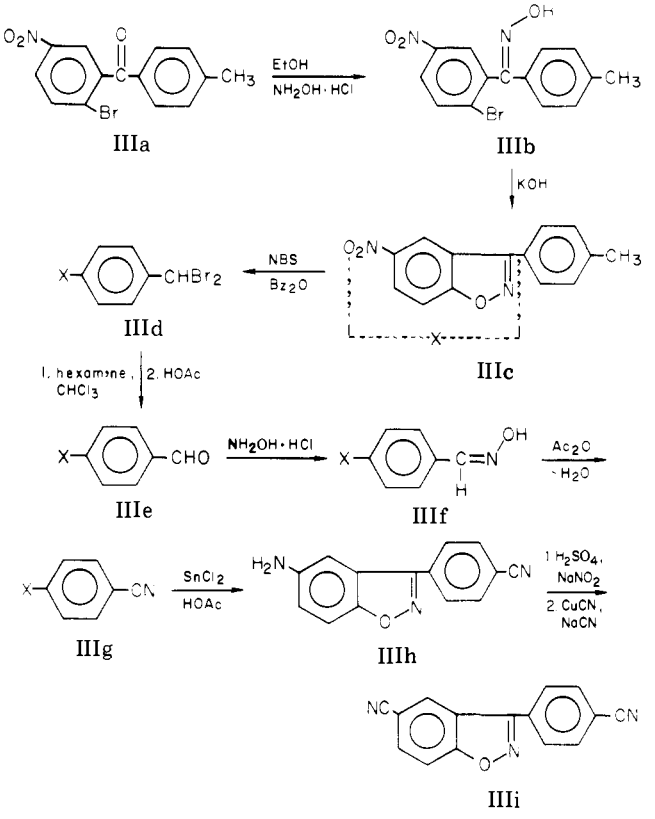


0022-2623/78/1821-0613$01.00/0

Scheme II



Scheme III

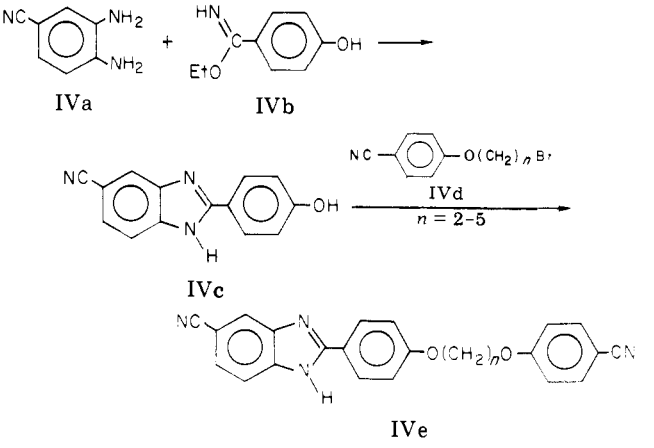


Results and Discussion

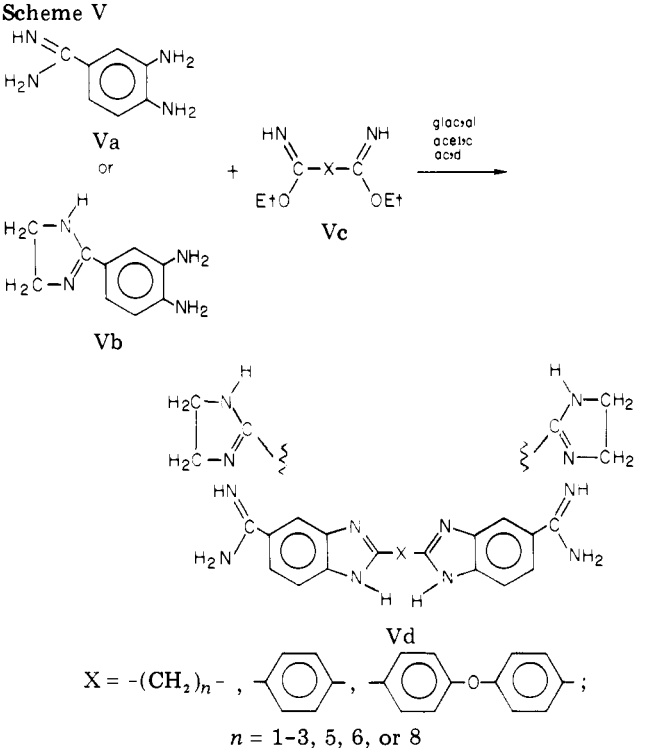
Inhibition of Bovine Thrombin, Porcine Pancreatic Kallikrein, and Bovine Trypsin. The dissociation constants of the inhibitors with the enzymes were determined from rate assays employing synthetic amide substrates. In most cases the reactions followed Michaelis–Menten kinetics and inhibition was strictly competitive and reversible. In the few instances where the kinetics were either slightly irregular or nonlinear, this is so noted.

The compounds are listed in Table II with their structural formulas, their respective K_i values for the three enzymes, and the references for their syntheses. The compounds in Table I are divided into classes according

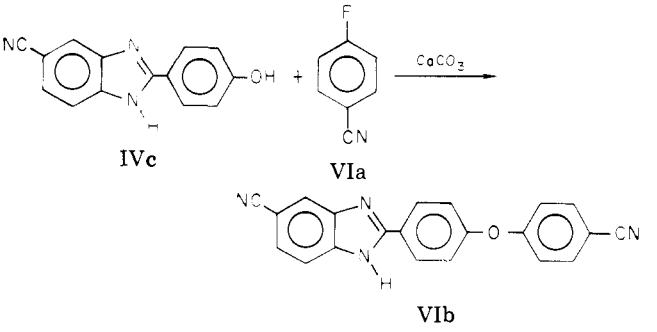
Scheme IV



Scheme V



Scheme VI



to the type of amidino-substituted ring. However, the following discussion will deal with the derivatives in groups based on various consistent structural modifications and the effect of these structural modifications on antiproteolytic activity.

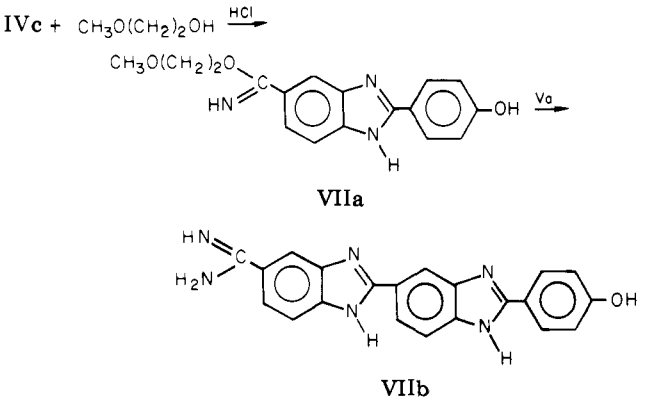
A. Effect of Isosteric Replacement(s). Compounds 7, 19, 25, 35, 36, and 57–60. In an attempt to determine the effect of isosteric replacement on the antiproteolytic activity of 6-amidino-2-(4-amidinophenyl)indole (com-

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

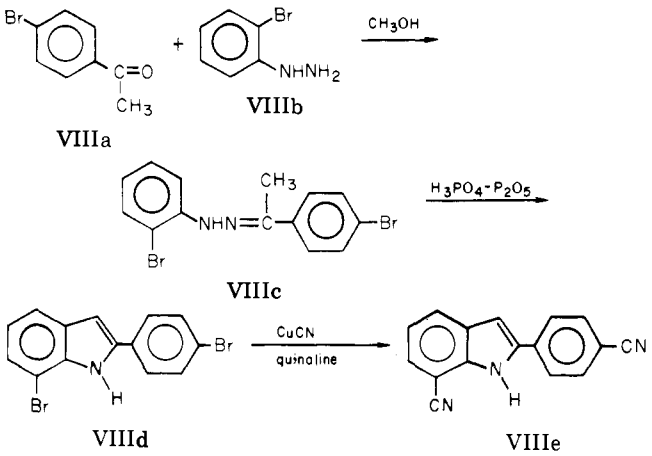
Compd no.	Mp, °C	Yield, %	Formula	Analyses
6	240 dec	15[a]	$C_{16}H_{15}N_5 \cdot 2HCl \cdot 2H_2O$	C, H, N
9	260 dec	52[b]	$C_{16}H_{14}N_6O_2 \cdot 2HCl \cdot 2H_2O$	C, H, N
10	>300	45[b]	$C_{16}H_{16}N_6 \cdot 3HCl$	C, H, N
11	>300	48[b]	$C_{16}H_{14}N_7 \cdot 2HCl \cdot 2.5H_2O$	C, H
23	>300	6[c]	$C_{17}H_{16}N_6O \cdot 3HCl \cdot 3.5H_2O$	C, H, N
37	250–251 dec	47[e]	$C_{21}H_{18}N_6O \cdot 3HCl \cdot 3H_2O$	C, H, N
38	217 dec	8[e]	$C_{23}H_{22}N_6O_2 \cdot 3HCl \cdot 2.5H_2O$	C, H, N
39	195 dec	44[e]	$C_{24}H_{24}N_6O_2 \cdot 3HCl \cdot 2H_2O$	C, H, N
40	281–285 dec	30[e]	$C_{25}H_{26}N_6O_2 \cdot 2HCl \cdot 4H_2O$	C, H, N
41	190 dec	21[e]	$C_{26}H_{28}N_6O_2 \cdot 2HNO_3 \cdot 4H_2O$	C, H, N
42	281–284 dec	25[f]	$C_{17}H_{16}N_8 \cdot 4HCl \cdot H_2O$	C, H, N
43	>300	28[f]	$C_{18}H_{18}N_8 \cdot 4HCl \cdot 2H_2O$	C, H, N
45	200 dec	19[f]	$C_{19}H_{20}N_8 \cdot H_2CO_3 \cdot 0.5H_2O$	C, H, N
46	100	34[f]	$C_{21}H_{24}N_8 \cdot 2HCl \cdot C_2H_5OH \cdot 3H_2O$	C, H, N
47	236–237 dec	13[f]	$C_{22}H_{26}N_8 \cdot 4HNO_3 \cdot 2H_2O$	C, H, N
48	275 dec	25[f]	$C_{24}H_{30}N_8 \cdot 2HCl$	C, H, N
49	>300	34[f]	$C_{22}H_{18}N_8 \cdot 4HCl \cdot 2.5H_2O$	C, H, N
50	>300	11[f]	$C_{28}H_{22}N_8O \cdot 4HCl \cdot 2.5H_2O$	C, H, N
51	285 dec	25[e]	$C_{21}H_{16}N_6O \cdot 3HCl \cdot 3H_2O$	C, H, N
52	>300	15[f]	$C_{22}H_{22}N_8 \cdot 4HCl \cdot 1.5H_2O$	C, H, N
53	245–250 dec	16[d]	$C_{15}H_{13}N_5 \cdot 2HCl \cdot 4H_2O$	C, H

[a] Yield was calculated from 4-bromoacetophenone. [b] Yields were calculated from 6-cyano-2-(4-cyanophenyl)indole. [c] Yield was calculated from 3,5-dibromosalicylaldehyde. [d] Yield was calculated from 2-bromo-4'-methyl-5-nitrobenzophenone. [e] Yields were calculated from 5-cyano-2-(4-hydroxyphenyl)benzimidazole. [f] Yields were calculated from 3,4-diaminobenzamidine hydrochloride.

Scheme VII



Scheme VIII



pound 7), the cited compounds were compared. The substitution of the ring nitrogen of compound 7 by carbon (indene derivative, compound 35) or sulfur (benzo[β]-thiophene derivative, compound 25) resulted in a slight increase in antitrypsin potency while all other isosteric modifications caused a decrease in antitrypsin activity. An approximate fourfold decrease in the antitrypsin potency compared to compound 7 was observed when carbon 3 or carbon 9 was replaced by nitrogen (compounds 36 and 59, respectively). The indene derivative (compound 35) was not only the strongest trypsin inhibitor but also proved to be the most potent antithrombin derivative of the isosteric series, having a K_i value for thrombin of 4.73×10^{-6} M. A tenfold decrease in antithrombin effect was observed in going from the indene compound to the benzofuran isostere (compound 19). Compound 25 proved to be the most outstanding kallikrein inhibitor of the isosteric series, having a K_i value of 7.3×10^{-7} M. In addition to compound 25, the indole derivative (compound 7) also showed a strong antikallikrein effect, while the rest of the isosteric series exhibited considerably less affinity for kallikrein. The indolizene derivative (compound 60) was found to have an antikallikrein effect almost 700 times less that of compound 25.

B. Effect of the Positioning of the Amidino Group(s). Compounds 4–8, 14, 19, 24, and 25. The effect of changing the position(s) of one or both of the amidino groups of 5-amidino-2-(4-amidinophenyl)indole (compound 8) on antiproteolytic activity was demonstrated by compounds 4–7. For instance, there was a substantial decrease in affinity toward all three enzymes when the amidino group on the indole ring was moved to the 7 position (compound 6). This loss in inhibitory potency was greatest with kallikrein where there was a decrease by a factor of almost 20. The influence of amidino positioning on the antikallikrein potency was also seen in the positional isomers of benzofuran (compounds 14 and 19) and benzo[β]thiophene (compounds 24 and 25). When the amidino group on the heterocyclic ring of 5-amidino-2-(4-amidinophenyl)benzo[β]thiophene (compound 24) was moved to the 6 position (compound 25) a tenfold decrease in kallikrein inhibition was observed.

C. Effect of the Variation of the Alkyl Chain Length Connecting Two Amidino-Substituted Rings. Compounds 38–43 and 45–48. The correlation between the antiproteolytic activity and length of the alkyl chain connecting two benzamidine moieties, previously observed in a series of α,ω-di(amidinophenoxy)alkanes,[1h,i] prompted us to study a similar series of compounds where one or both of the amidino substituted rings was a benzimidazole. The first series of these compounds consisted of four homologues of 1-[4-(5-amidino-2-benzimidazolyl)phen-

Table II. Amidino-Substituted Heterocyclic Compounds. Inhibition Constants with Thrombin, Pancreatic Kallikrein, and Trypsin

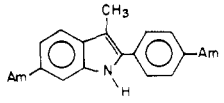
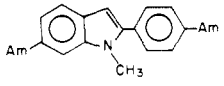
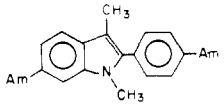
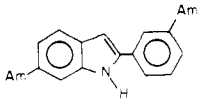
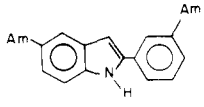
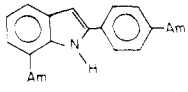
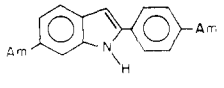
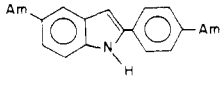
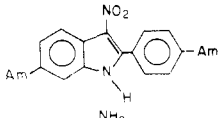
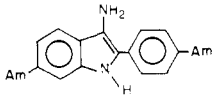
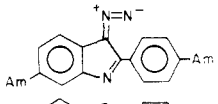
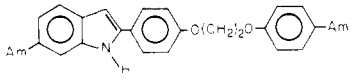
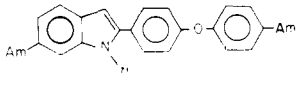
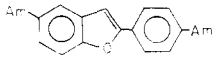
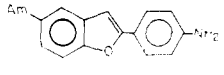
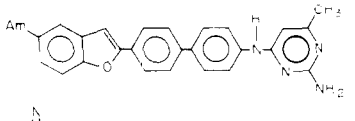
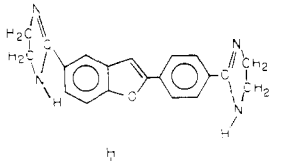
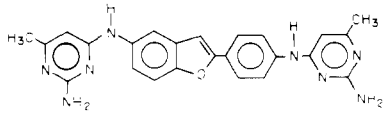
Compd no.	Structure[a]	K_i, μM[b]			Ref synth
		Trypsin	Thrombin	Kallikrein	
	I. Indoles				
1		19.4 ± 2.3	16.1 ± 2.8	25.7 ± 2.1	2
2		4.16 ± 0.08	3.02 ± 0.03	18.9 ± 2.6	2
3		13.1 ± 0.9	11.8 ± 0.5	23.0 ± 7.3	2
4		3.25 ± 0.58	2.45 ± 0.05	12.26 ± 2.3	2
5		4.73 ± 0.31	6.88 ± 0.28	5.07 ± 0.71	2
6		27.2 ± 3.4	22.7 ± 3.8	48.3 ± 7.8	This work
7		5.31 ± 0.09	8.37 ± 0.26	2.70 ± 1.92	3
8		6.17 ± 0.47	6.45 ± 0.12	11.0 ± 7.6	2
9		24.4 ± 3.1	108 ± 16.0	248 ± 31.0	This work
10		2.13 ± 0.25	3.12 ± 0.92	38.0 ± 15.3	This work
11		11.6 ± 1.1	10.5 ± 1.9	17.6 ± 2.4	This work
12		4.19 ± 0.17	1.40 ± 0.94	Nonlinear kinetics	3
13		3.79 ± 0.2	5.22 ± 0.94	Nonlinear kinetics	3
	II. Benzofurans				
14		7.27 ± 1.08	11.3 ± 2.2	9.08 ± 2.33	2
15		6.67 ± 1.03	28.1 ± 1.8	71.7 ± 1.5	4
16		6.57 ± 1.01	4.88 ± 0.64	22.5 ± 1.9	4
17		>>1000	>1000	104 ± 38	4
18		>1000	~700	~300	4

Table II (*Continued*)

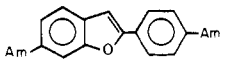
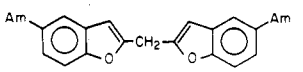
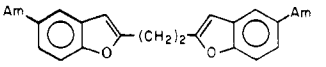
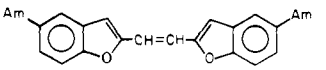
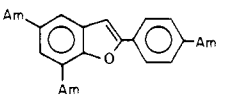
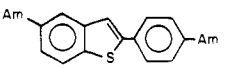
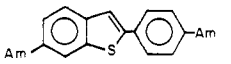
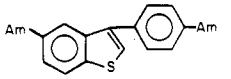
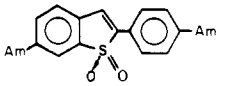
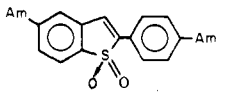
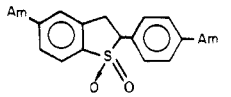
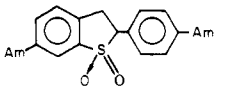
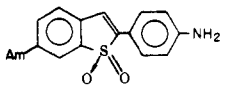
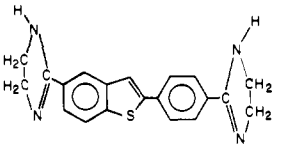
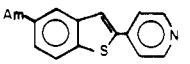
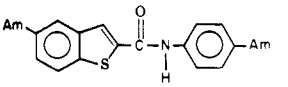
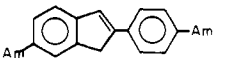
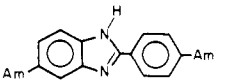
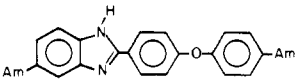
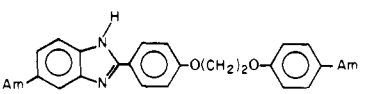
Compd no.	Structure[a]	K_i, μM[b] Trypsin	Thrombin	Kallikrein	Ref synth
19		9.30 ± 0.1	53.5 ± 3.5	56.2 ± 6.7	2
20		3.32 ± 0.14	10.0 ± 1.2	10.6 ± 1.3	5
21		2.24 ± 0.27	4.17 ± 0.87	9.53 ± 1.45	5
22		2.59 ± 0.23	4.13 ± 0.59	2.37 ± 0.32	5
23		67.2 ± 10.7	53.8 ± 22.4	12.3 ± 1.7	This work
	III. Benzo[β]thiophenes				
24		4.17 ± 0.39	2.02 ± 0.59	8.68 ± 1.43	6
25		3.94 ± 0.44	7.68 ± 0.6	0.73 ± 0.06	6
26		32.1 ± 4.0	10.4 ± 0.70	52.9 ± 11.5	4
27		6.52 ± 1.01	19.6 ± 2.5	35.6 ± 2.6	6
28		33.8 ± 8.0	44.7 ± 3.8	29.1 ± 1.6	6
29		2.49 ± 0.33	158 ± 11	300	6
30		3.23 ± 0.33	133 ± 12	61.3 ± 6.4	6
31		>100	~1000	>100	6
32		~1000	~1000	103 ± 17	4
33		31.3 ± 2.1	300	~1000	4
34		5.80 ± 0.37	14.2 ± 2.7	7.76 ± 0.14	4
	IV. Indene				
35		3.39 ± 0.28	4.73 ± 0.21	7.36 ± 0.14	2
	V. Benzimidazoles				
36		17.1 ± 2.8	24.4 ± 6.1	64.5 ± 11.6	3
37		9.80 ± 1.76	6.67 ± 1.50	4.11 ± 0.95	This work
38		5.98 ± 0.71	2.73 ± 0.29	11.3 ± 2.8	This work

Table II (*Continued*)

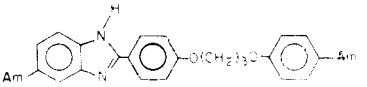
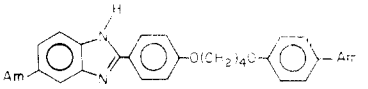
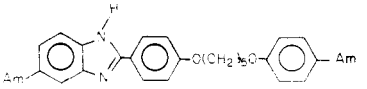
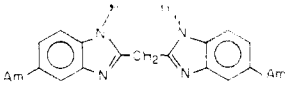
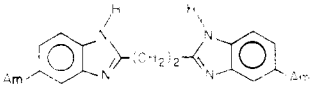
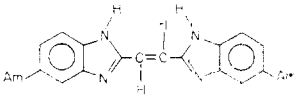
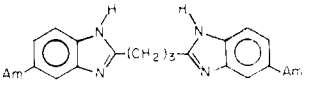
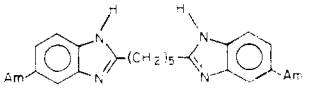
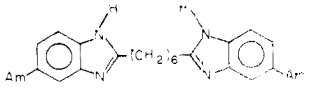
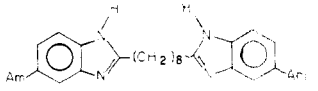
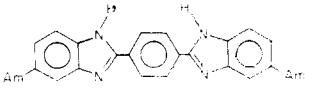
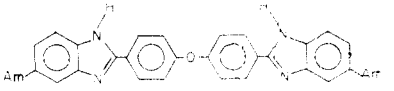
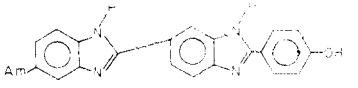
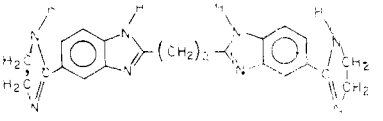
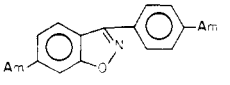
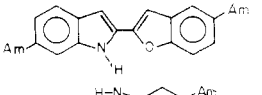
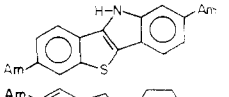
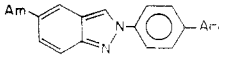
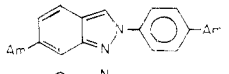
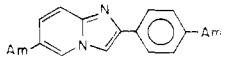
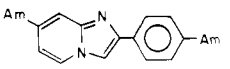
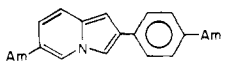
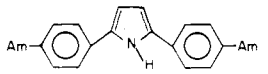
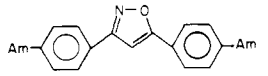
Compd no.	Structure[a]	K_i, μM[b] Trypsin	Thrombin	Kallikrein	Ref synth
39		2.96 ± 0.13	2.09 ± 0.44	3.98 ± 0.74	This work
40		4.01 ± 0.31	3.08 ± 0.39	6.05 ± 0.83	This work
41		1.44 ± 0.10	2.24 ± 0.26	5.16 (irr)	This work
42		0.017 ± 0.006	4.15 ± 0.65	17.3 ± 1.4	This work
43		4.68 ± 0.33	11.6 ± 1.1	36.5 ± 6.6	This work
44		33.2 ± 7.3	>100	~100	5
45		27.8 ± 2.7	79.0 ± 8.5	141 ± 19	This work
46		9.46 ± 1.5	9.87 ± 2.0	31.4 ± 4.9	This work
47		5.88 ± 0.32	4.28 ± 0.51	13.5 ± 0.81	This work
48		4.93 ± 0.76	3.22 ± 0.65	18.1 ± 2.0	This work
49		17.0 ± 2.5	7.83 ± 0.88	15.3 ± 1.6	This work
50		4.67 ± 0.61	3.94 ± 0.52	Nonlinear kinetics	This work
51		42.0 ± 11.8	200 (irr)	206 ± 10	This work
52		617 ± 11.8	>1000	>1000	This work
		VI. Miscellaneous			
53		50.9 ± 1.6	56.7 ± 8.3	~100	This work
54		2.75 ± 0.44	1.39 ± 0.30	4.67 ± 0.61	5
55		9.32 ± 1.67	4.50 ± 0.33	40 (irr)	5
56		14.6 ± 2.7	24.6 ± 2.9	39.7 ± 5.2	4
57		7.11 ± 8.0	10.1 ± 1.2	46.1 ± 5.1	4
58		18.0 ± 1.3	40.7 ± 7.6	61.2 ± 7.6	4

Table II (*Continued*)

Compd no.	Structure[a]	K_i, μM[b] Trypsin	Thrombin	Kallikrein	Ref synth
59		16.2 ± 0.30	23.9 ± 1.5	39.3 ± 6.0	4
60		10.8 ± 1.3	27.7 ± 2.5	~500	4
61		15.6 ± 1.3	17.7 ± 1.7	4.65 ± 0.45	3
62		11.1 ± 0.90	66.2 ± 17.3	8.91 ± 0.60	3

[a] Am, amidino group. [b] K_i, dissociation constants of the inhibitor-enzyme complex. Values are means ± SD; $n = 3$.

oxy]-2-(4-amidinophenoxy)ethane (compounds **38–41**). The derivatives of this series displayed no significant differences in inhibitory potency against thrombin. However, a substantial increase in antitrypsin effect was observed in going from the ethane derivative (compound **38**) to the pentane derivative (compound **41**). For kallikrein, there was an increased inhibitory effect in advancing from the ethane derivative to the propane homologue. However, there was no significant difference in antikallikrein potency between the 3-, 4-, and 5-carbon chain derivatives.

A second series of compounds with a central chain of variable length consisted of bis(5-amidino-2-benzimidazolyl)alkanes (compounds **42**, **43**, and **45–48**). The lengthening of the carbon chain from the methane derivative through the octane derivative affected the inhibitory activity against the three enzymes in much the same manner. The initial lengthening of the connecting chain from 1 to 3 carbons led to a progressive decrease in inhibitory strength for all three enzymes. However, a further increase in the number of carbons resulted in a resurgence of antitrypsin and antithrombin activity through the 8-carbon chain compound. With respect to kallikrein, this secondary rise in inhibitory potency peaked earlier, i.e., with the 6-carbon chain homologue. One of the compounds, bis(5-amidino-2-benzimidazolyl)methane (compound **42**), proved to be an outstanding inhibitor of trypsin with a K_i value of 1.7×10^{-8} M. As can be seen it is also endowed with a significant degree of specificity, being much less effective against thrombin and kallikrein than against trypsin.

D. Effect of Substituents at the 1 and/or 3 Positions of 6-Amidino-2-(4-amidinophenyl)indole. Compounds 1–3, 7, and 9–11. The cited compounds were tested to determine how antiprotease activity would be influenced by the addition of a small group or a single atom at the 1 and/or 3 positions of the parent indole (compound **7**). Examination of the inhibitory data for these compounds reveals that all substitutions resulted in a decrease in antikallikrein activity. Against thrombin and trypsin, however, the introduction of a methyl group in the 1 position (compound **2**) or an amino group in the 3 position (compound **10**) resulted in an increase in potency. In general, the substitutions of the parent indole affected the antikallikrein potency to a greater extent than antithrombin or antitrypsin activity. Also, the least potent inhibitor against all three enzymes was the 3-nitro derivative (compound **9**).

E. Effect of Miscellaneous Structural Modifications. Compounds 14, 15, 23, 25, and 27–31. In order to ascertain if both amidino groups were necessary for maximal inhibitory activity, the amidino groups on the phenyl rings of 5-amidino-2-(4-amidinophenyl)benzofuran (compound **14**) and 6-amidino-2-(4-amidinophenyl)-1,1-dioxobenzo[β]thiophene (compound **30**) were replaced by amino groups. For the benzofuran derivative, the substitution (compound **15**) resulted in no significant loss in antitrypsin activity but did cause a decrease in antithrombin and antikallikrein potency. The corresponding modification in the benzo[β]thiophene derivative, as demonstrated by compound **31**, led to a lowering of potency against all three enzymes. As the loss of one of the amidino groups of the parent benzofuran compound (compound **14**) had a negative effect on inhibitory potency, we examined a triamidino derivative (compound **23**) to determine if adding a third cationic moiety would enhance activity. From the data it can be seen that this was not the case and that, on the contrary, there was a reduction in inhibitory potency against all three enzymes.

In an attempt to determine the importance of molecular planarity on antiprotease activity, 5- and 6-amidino-2-(4-amidinophenyl)benzo[β]thiophene (compounds **24** and **25**) and their corresponding 1,1-dioxy- and 1,1-dioxy-2,3-dihydro derivatives (compounds **27–30**) were tested. The 1,1-dioxy substitutions (compounds **27** and **28**) represented a loss in planarity from the parent compounds. For both the 5- and 6-amidino derivatives this loss of planarity resulted in a decrease in inhibitory potency against all three enzymes. Another alteration of the positional isomers, accomplished by the reduction of the double bond between carbons 2 and 3 (compounds **29** and **30**), reduced the planar state of the molecules to an even greater extent. The reduced derivatives displayed the expected decrease in antithrombin and antikallikrein activity but exhibited an unexpected enhanced antitrypsin potency.

Partial Thromboplastin Time Test (PTT). Since one of our major objectives is the development of new anticoagulants for potential use in man, we also determined the effect of this new series of inhibitors on the partial thromboplastin time test (PTT) of human plasma. The test is sensitive not only to the inhibition of thrombin, but also the blockage of any of the earlier steps of the clotting cascade. For this reason prolongation of the PTT is considered a reliable indicator of the overall anticoagulant activity. On the other hand, since besides thrombin at least one other arginine-specific clotting enzyme, i.e., factor Xa,[14] is susceptible to inhibition by amidine derivatives and since antifactor Xa activity does not necessarily have to parallel antithrombin activity, the K_i values for bovine thrombin alone are not helpful in predicting the usefulness of a compound as an anticoagulant.[1e,h-j] This point is reinforced for the current compounds by a comparison of the inhibitory data for thrombin (Table II) with the PTT

Table III. Inhibitory Effect of Amidine Derivatives on the Partial Thromboplastin Time

Compd no.	Partial thromboplastin time,[a] s (control 59.0 ± 5.4 s), at inhibitor concn (M)		
	10^{-4}	10^{-5}	5×10^{-6}
1	139.3 ± 9.2	77.0 ± 2.4	66.4 ± 1.2
2		119.0 ± 7.5	97.4 ± 3.5
3	192.2 ± 13.9	74.7 ± 3.4	67.9 ± 1.1
4	431.7 ± 9.4	107.5 ± 5.1	78.6 ± 4.0
5	316.3 ± 18.3	75.7 ± 1.3	N.I.[b]
6	73.9 ± 1.1	N.I.	
7	425.7 ± 8.7	141.5 ± 2.0	112.9 ± 2.3
8	222.2 ± 3.3	81.6 ± 1.5	77.8 ± 0.5
9	67.8 ± 2.6	N.I.	
10	91.5 ± 1.0	N.I.	
11	250.8 ± 3.3	92.9 ± 0.7	67.1 ± 1.1
12	497.8 ± 7.1	81.1 ± 0.1	74.5 ± 0.5
13	298.3 ± 18.7	72.6 ± 1.3	69.2 ± 0.8
14	181.9 ± 7.1	92.3 ± 2.4	72.4 ± 1.5
15	76.8 ± 3.7	N.I.	
16	108.0 ± 8.7	N.I.	
17	N.I.		
18	N.I.		
19	256.5 ± 8.2	126.4 ± 4.4	86.5 ± 3.6
20	299.8 ± 10.9	91.1 ± 2.8	69.6 ± 0.6
21	1430.7 ± 104.0	263.8 ± 9.6	175.2 ± 7.4
22	418.2 ± 3.5	85.2 ± 0.5	70.5 ± 1.0
23	83.8 ± 3.4	N.I.	
24	188.2 ± 11.3	78.9 ± 2.4	N.I.
25	166.6 ± 1.9	88.1 ± 0.7	79.3 ± 1.8
26	95.9 ± 4.6	N.I.	
27	121.6 ± 0.7	71.2 ± 0.8	65.9 ± 1.1
28	127.8 ± 7.0	N.I.	
29	N.I.		
30	N.I.		
31	N.I.		
32	N.I.		
33	N.I.		
34	80.4 ± 5.4	74.2 ± 2.3	68.5 ± 2.5
35	318.7 ± 2.6	122.2 ± 3.2	84.1 ± 0.3
36	123.3 ± 3.0	70.3 ± 1.2	66.4 ± 0.8
37	181.7 ± 6.2	84.5 ± 4.6	74.7 ± 1.0
38	336.7 ± 21.2	88.6 ± 2.7	N.I.
39	330.5 ± 13.2	91.3 ± 2.6	72.8 ± 1.9
40	244.0 ± 6.1	79.0 ± 1.9	67.9 ± 0.6
41		69.4 ± 1.8	N.I.
42	424.7 ± 19.3	107.8 ± 4.2	84.0 ± 3.3
43	519.4 ± 33.4	143.6 ± 7.1	122.8 ± 8.4
44	71.7 ± 2.3	N.I.	
45	72.7 ± 2.3	N.I.	
46	149 ± 3.4	70.2 ± 1.5	N.I.
47	309.2 ± 21.4	87.1 ± 2.0	71.5 ± 1.1
48	494.1 ± 20.8	127.2 ± 14.2	91.1 ± 1.9
49		N.I.	
50		67.6 ± 1.8	N.I.
51	117.5 ± 3.3	69.5 ± 1.6	N.I.
52		N.I.	
53	71.8 ± 1.7	N.I.	
54	410 ± 21.0	111.9 ± 12.4	82.3 ± 6.4
55	99.0 ± 1.0	N.I.	
56	85.3 ± 0.8	72.5 ± 2.7	N.I.
57	132.6 ± 1.5	69.6 ± 1.7	N.I.
58	88.2 ± 1.6	68.8 ± 2.9	N.I.
59	85.9 ± 3.4	N.I.	
60	N.I.		
61		66.8 ± 0.5	N.I.
62	83.3 ± 0.8	N.I.	

[a] Values are the means ± SD; $n = 194$ for the control and $n = 3$ for the inhibitor assays. [b] N.I., no inhibition.

results (Table III). It is evident that compound **21** was the most active inhibitor by far in the PTT though its K_i value was considerably higher than for many amidines of lesser anticoagulant effect. Conversely, the two most effective thrombin inhibitors (compounds **12** and **54**) proved to be only average in their ability to prolong the PTT. It was noteworthy that maximal prolongation of the PTT was dependent on very rigid structural requirements. For example, when the ethyl bridge of compound **21** was shortened by one carbon (compound **20**) or substituted by an ethylene link (compound **22**), a decrease in the prolongation of the PTT was observed. Such structural specificity was also noted in a similar, but more extensive series of diamidinobenzimidazoles (compounds **42–48**). As with the dibenzofurans the most effective dibenzimidazole was the derivative with an ethyl bridge (compound **43**). Any modification of this bridge resulted in a marked decrease in inhibition of the PTT.

Conclusions

The inhibitory data obtained from the amidase assays lend further support to our previous conclusions that significant differences exist between the topography of the active sites of trypsin, thrombin, and kallikrein. This difference is evidenced, for example, by the remarkable specificity for trypsin demonstrated by compound **42**. The K_i value of 1.7×10^{-8} M, observed for this compound, makes it the most effective low-molecular-weight reversible inhibitor of trypsin reported to date.

A comparison of the PTT data with the K_i values for thrombin revealed that there is no direct correlation between anticoagulant and antithrombin activity. This finding suggests that, in plasma, inhibition of at least one other clotting enzyme besides thrombin was occurring. Future studies on the inhibition of factor Xa by selected amidines should determine if, in fact, it is the inhibition of this enzyme which is of primary importance for the effect of the inhibitors on the PTT.

Experimental Section

Amidase Assays. In all but two instances the dissociation constants (K_i) of inhibitors with the three enzymes were obtained from amidase assays using α-*N*-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride[15] (BANA, Bachem, Inc.) as the substrate. In the case of compound **25**, having a K_i of 7.3×10^{-7} M, the kallikrein inhibition was determined using the more sensitive substrate, *N*-benzoyl-L-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide hydrochloride (Fox Chemical Co.). Similarly, for the assay of the most potent trypsin inhibitor (compound **42**, K_i of 1.7×10^{-8} M) *N*-benzoyl-L-phenylalanyl-L-valyl-L-arginine-*p*-nitroanilide hydrochloride[16] (A.B. Bofors, Nobel Division) was substituted for BANA. The K_i values for the inhibitors were determined graphically according to Dixon.[17] All tests were carried out at 37 °C and at a pH of 8.1. The details of the assay have been previously described.[11j]

Thrombin (bovine, topical) was purchased from Parke, Davis & Co. Porcine pancreatic Kallikrein was a gift from Farbenfabriken Bayer AG, Wüppertal-Elberfeld, Federal Republic of Germany. The preparation possessed 635 Frey units/mg. Trypsin (bovine, twice crystallized, salt-free) was a product of Schwarz-Mann containing 57% active trypsin as determined by active-site titration.

Partial Thromboplastin Time Test (PTT). The test with human plasma was modified from a method of Nye et al.[18] Citrated plasma (0.1 mL) was incubated for 30 s at 37 °C together with 0.1 mL of partial thromboplastin solution (Thrombofax, Ortho Diagnostics) and 0.1 mL of 0.154 M NaCl solution or 0.154 M NaCl–inhibitor solution. At the end of the incubation period 0.1 mL of 0.02 M $CaCl_2$ solution was added, and the time until formation of a firm clot represented the partial thromboplastin time.

Organic Synthesis. Elemental analyses for all amidine products were carried out by either the Analytical Chemistry Division of Hoechst AG or Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated on the final products, results within ±0.4% of the theoretical values were considered acceptable. The melting or decomposition points of the products were determined on a Thomas-Hoover capillary melting point apparatus. The yields, melting points, and analytical data of the previously

unreported amidines are indicated in Table I.

A. Method of Conversion of Nitriles to Amidines. All mono- and diamidine derivatives were prepared from the corresponding nitriles by modifications of the Pinner reaction.[12,13] A typical amidine synthesis is as follows. The cyano derivative was dissolved in either alcohol alone or an appropriate solvent with alcohol added. The solution was then cooled and saturated with dry HCl at a maximum temperature of 20 °C. After returning to room temperature the solution was allowed to stir until the disappearance of the nitrile band in the IR spectrum (~2250 cm^{-1}). The imino ether hydrochloride intermediate was then precipitated from the solution by either cooling or by the addition of Et_2O. The imino ether was collected and refluxed in an ammonia-saturated EtOH solution. The amidine was isolated by either filtering the EtOH solution or by removal of the solvent. The crude product was normally purified by recrystallization from either acetic acid or HCl (~2 N) followed by washing with 2 N HCl and acetone.

B. 6-Amidino-2-(4-amidinophenyl)-3-nitroindole Dihydrochloride. Compound 9, Table II (See Scheme I).[7] A solution of 5.0 g (20.6 mmol) of 6-cyano-2-(4-cyanophenyl)indole[2] (Ia) dissolved in 300 mL of warm glacial acetic acid was treated dropwise with 1.4 g (20.6 mmol) of $NaNO_2$ in 5 mL of H_2O. The resulting precipitate was filtered and dried at 80 °C to give 5.0 g (89%) of 6-cyano-2-(4-cyanophenyl)-3-hydroxyiminoindoline (Ib). The yellow powder (mp 276–279 °C) was used in the following reaction without further purification.

To a suspension of 5.0 g (18.3 mmol) of Ib in 300 mL of glacial acetic acid was added 1.4 g (20.6 mmol) of $NaNO_2$. The mixture was heated to reflux for 15 min, allowed to cool, and then filtered. The solid was washed with H_2O and then dried. The crude product was recrystallized from a mixture of glacial acetic acid and DMF (7:5) to give 4.1 g (79%) of 6-cyano-2-(4-cyanophenyl)-3-nitroindole (Ic) as a yellow powder (mp 300 °C).

The dinitrile Ic was converted, without further purification, to the corresponding diamidine (compound **9**) by the aforementioned procedure.

C. 3-Amino-6-amidino-2-(4-amidinophenyl)indole Trihydrochloride. Compound 10, Table II (See Scheme I).[7] A suspension of 350 mg (1.2 mmol) of Ib in 10 mL of EtOH was heated to reflux and treated with 1.2 g (18 mmol) of Zn, followed by the dropwise addition of 1.3 mL of concentrated HCl. After the addition of the acid the mixture was allowed to reflux for an additional 10 min. The solution was filtered while still warm and the filtrate made basic with NaOH. The crystalline product was collected and dried in vacuo (P_2O_5). The crude product, 250 mg (80%), was purified by elution on a silica-gel column with a mixture of benzene and acetone (9:1): mp 250–255 °C.

The above material, 3-amino-6-cyano-2-(4-cyanophenyl)indole (Id), was carried to the diamidine (compound **10**) by the standard method.

D. 6-Amidino-2-(4-amidinophenyl)-3-diazoindoline Dihydrochloride. Compound 11, Table II (See Scheme I).[7] A solution of compound **10** (200 mg, 0.5 mmol) in 20 mL of H_2O was cooled to 5 °C and treated with 35 mg (0.5 mmol) of $NaNO_2$ in 1 mL of H_2O. The product was precipitated from the solution by the addition of acetone: yield 170 mg (88%).

E. 2-(4-Amidinophenyl)-5,7-diamidinobenzofuran Trihydrochloride. Compound 23, Table II (See Scheme II).[8] A solution of 42.0 g (150 mmol) of 3,5-dibromosalicylaldehyde (IIa) dissolved in 300 mL of EtOH was treated with 6.0 g (150 mmol) of NaOH in 120 mL of H_2O. The mixture was brought to boiling and 25.5 g (149 mmol) of 4-nitrobenzyl chloride (IIb) in 100 mL of EtOH was added. The stirred mixture was refluxed for 3 h and then allowed to stand at room temperature overnight. The resulting precipitate (IIc) was collected and dried: yield 42.8 g (69%); mp 184–186 °C. This material was used in the following reaction without further purification. However, a small portion was recrystallized once from benzene to give pure 4,6-dibromo-2-formylphenyl-4-nitrobenzyl ether (IIc): mp 185–186 °C. Anal. ($C_{14}H_9Br_2NO_4$) C, N.

A solution of 41.0 g (98.8 mmol) of IIc dissolved in warm DMF was treated with 0.70 g (30.4 mmol) of sodium in 30 mL of MeOH. The mixture was refluxed for 35 min and then cooled. The resulting precipitate was collected giving 26.8 g (69%) of 5,7-dibromo-2-(4-nitrophenyl)benzofuran (IId). This material was used without further purification in the following reaction. A small portion of the product was recrystallized from acetone to give the purified product: mp 219–220 °C. Anal. ($C_{14}H_7Br_2NO_3$) C, H.

To 11.0 g (27.5 mmol) of IId in 270 mL of glacial acetic acid was added 20 g (88.7 mmol) of $SnCl_2 \cdot 2H_2O$ in 30 mL of concentrated HCl. After stirring and refluxing for 2 h the reaction mixture was allowed to stand at room temperature for 12 h and then filtered. The solid was washed with glacial acetic acid and then suspended in 250 mL of H_2O. The suspension was treated with 30 g (125 mmol) of $NaNO_2 \cdot 2H_2O$ in 25 mL of H_2O and 25 mL of NH_4OH. After heating for 1 h at 40–50 °C the mixture was filtered and washed with H_2O until the washings were neutral. The solid was dried and recrystallized from toluene to give 8.3 g (82%) of 5,7-dibromo-2-(4-aminophenyl)benzofuran (IIe). The material was used in the following reaction without further purification. However, a small amount of the pure product was obtained by recrystallization from benzene: mp 175–177 °C. Anal. ($C_{14}H_9Br_2NO$) C, H.

A stirring solution of 3.1 g (45 mmol) of $NaNO_2$ in 25 mL of concentrated H_2SO_4 was cooled and treated slowly (temperature never allowed to exceed 30 °C) with a suspension of 13.8 g (37.6 mmol) of IIe in 125 mL of glacial acetic acid. Following the neutralization of excess $NaNO_2$ with urea, the diazonium solution was treated with a freshly prepared solution of Cu_2Br_2, prepared from 25.0 g (100 mmol) of $CuSO_4 \cdot 5H_2O$. The mixture was warmed on a water bath for 15 min, filtered, and washed with H_2O until the washings were neutral. The product was purified by column chromatography over neutral aluminum oxide. Elution with cyclohexane–benzene (1:1) followed by two recrystallizations from cyclohexane gave 11.3 g (70%) of 5,7-dibromo-2-(4-bromophenyl)benzofuran (IIf): mp 150–152 °C. Anal. ($C_{14}H_7Br_3O$) C, H; C: calcd, 39.02; found, 39.60.

A mixture of 5.0 g (11.6 mmol) of IIf and 15.0 g (167.4 mmol) of CuCN in 35 mL of quinoline was heated under N_2 at 260–270 °C for 45 min. The reaction mixture (cooled to ambient temperature) was poured into a mixture of 500 mL of 10% KOH and 150 mL of ligroine, stirred for 2 h, and filtered. The solid product was purified by column chromatography over aluminum oxide. Elution with benzene–acetone (2:1) was followed by recrystallization from chloroform and then DMF. After purification, 1.6 g (51%) of 5,7-dicyano-2-(4-cyanophenyl)benzofuran (IIg) was isolated: mp >300 °C. Anal. ($C_{17}H_7N_3O$) N.

The triamidine product (compound **23**) was prepared by the standard procedure.

F. 5-Amidino-3-(4-amidinophenyl)-1,2-benzisoxazole Dihydrochloride. Compound 53, Table II (See Scheme III).[9] A solution of 32 g (100 mmol) of 2-bromo-4′-methyl-5-nitrobenzophenone in 400 mL of warm EtOH was treated with 33.6 g (480 mmol) of $NH_2OH \cdot HCl$ in 40 mL of H_2O. After refluxing for 24 h the mixture was poured into 1 L of H_2O, cooled, and filtered. The solid was recrystallized from EtOH to give 32.0 g (95%) of 2-bromo-4′-methyl-5-nitrobenzophenone oxime (IIIb): mp 202 °C. Anal. ($C_{14}H_{11}BrN_2O_3$) Br.

A solution of 6.7 g (20 mmol) of IIIb in 70 mL of EtOH was treated dropwise with 10% KOH until the pH was between 7 and 8. The neutral solution was refluxed for 30 min, poured into 70 mL of H_2O, and filtered. The solid was recrystallized from 30 mL of EtOH to give 3.5 g (68%) of 3-(4-methylphenyl)-5-nitro-1,2-benzisoxazole (IIIc): mp 146 °C. Anal. ($C_{14}H_{10}N_2O_3$) N.

A mixture of 7.0 g (27.6 mmol) of IIIc, 6.6 g (72.6 mmol) of NBS, and 0.4 g of Bz_2O was suspended in 0.5 L of CCl_4 and refluxed for 1 h. The solution was filtered while still hot and the filtrate removed in vacuo. The residue (10.5 g, 96%), 3-[4-(dibromomethyl)phenyl]-5-nitro-1,2-benzisoxazole (IIId), mp 168–172 °C, was used in the following reaction without further purification. The crude product (1.0 g) was recrystallized from EtOH to give colorless crystals: mp 177 °C. Anal. ($C_{14}H_8Br_2N_2O_3$) Br.

A solution of 16.6 g (40.4 mmol) of IIId in 125 mL of $CHCl_3$ was treated with 17.5 g (124 mmol) of hexamethylenetetramine in 125 mL of $CHCl_3$. The mixture was refluxed for 20 min, cooled, and filtered. The crude material was recrystallized twice from a mixture of glycol monomethyl ether and H_2O (4:1) to give a white solid: mp 189–190 °C. A solution of 3.0 g of the solid in 60% acetic acid was refluxed for 4 h, cooled, and filtered. The solid was purified by column chromatography over aluminum oxide

eluted with benzene–ligroine to give 1.9 g (67%) of 3-(4-formylphenyl)-5-nitro-1,2-benzisoxazole (IIIe): mp 186–187 °C. Anal. ($C_{14}H_8N_2O_4$) N.

A mixture of 7.5 g (28.5 mmol) of IIIe, 75 mL of pyridine, and 6.5 g (105 mmol) of NH_2OH·HCl in 225 mL of EtOH was refluxed for 1 h. The hot solution was poured into 1 L of cold H_2O and filtered. The solid was recrystallized from 40 mL of glacial acetic acid to give pure 5-nitro-3-[4-(hydroxyiminomethylene)-phenyl]-1,2-benzisoxazole (IIIf): mp 234–235 °C; 95%. Anal. ($C_{14}H_9N_3O_4$) N.

A solution of 7.5 g (26.5 mmol) of IIIf in 250 mL of Ac_2O was refluxed for 5 h and then poured into 1 L of H_2O. The resulting precipitate was filtered, washed with H_2O, and dried to give 6.7 g (93%) of the crude product (mp 220–224 °C). The solid was purified by recrystallization from a mixture of benzene and cyclohexane to give pure 3-(4-cyanophenyl)-5-nitro-1,2-benzisoxazole (IIIg): mp 228 °C. Anal. ($C_{14}H_7N_3O_3$·H_2O) N.

A solution of 4.5 g of $SnCl_2$·$2H_2O$ in 20 mL of concentrated HCl was added to a heated and stirred solution of 2.0 g of IIIg in 40 mL of glacial acetic acid. The mixture was refluxed for 30 min, cooled, and filtered. The solid was stirred and heated for 30 min in 20 mL of 2 N NaOH and the basic solution filtered. The solid was washed with H_2O, dried, and recrystallized from a mixture of glycol monomethyl ether and H_2O (8:2). The purified 5-amino-3-(4-cyanophenyl)-1,2-benzisoxazole (IIIh) melted at 184 °C: 1.4 g (80%); mol wt calcd 235.3, found 237.6.

A cooled (0 °C) suspension of 2.35 g (10 mmol) of IIIh in 4 mL of concentrated H_2SO_4 and 25 mL of H_2O was treated dropwise with 0.75 g (10.9 mmol) of $NaNO_2$ in 2 mL of H_2O. The excess $NaNO_2$ was destroyed with urea and the solution thus treated was added to a second cooled solution consisting of 3.8 g (42.5 mmol) of CuCN and 5.75 g (11.5 mmol) of NaCN in 30 mL of H_2O. The mixture was heated on a H_2O bath for 10 min and filtered. The solid was suspended in 40 mL of concentrated HCl and 10 mL of H_2O and heated. The acid solution was cooled, made basic with concentrated NH_4OH, and filtered to give 1.9 g (77%) of 5-cyano-3-(4-cyanophenyl)-1,2-benzisoxazole. The crude product was purified by column chromatography over aluminum oxide eluted with benzene, followed by recrystallization from a mixture of benzene and cyclohexane (2:3): mp 208–209 °C. Anal. ($C_{15}H_7N_3O$) mol wt calcd 245.3, found 248.9.

The amidine derivative (compound **60**) was prepared in the standard manner.

G. α-[4-(5-Amidino-2-benzimidazolyl)phenoxy]-ω-(4-amidinophenoxy)alkanes. Compounds 38–41, Table II (See Scheme IV). A mixture of 200 mmol of 3,4-diaminobenzonitrile (IVa) and 250 mmol of 4-hydroxybenzimino ether hydrochloride (IVb) in 150 mL of glacial acetic acid was heated at reflux for 2 h. The mixture was allowed to cool, and the resulting precipitate was filtered. The solid material was washed with glacial acetic acid, followed by warm sodium acetate solution (2 L) until the acid was neutralized. A solid material was collected on cooling, washed with H_2O, and then dried on a steam bath. The yield of 2-(4-hydroxyphenyl)-5-cyanobenzimidazole (IVc) was 75%.

To a solution of 150 mmol of IVc dissolved in 200 mL of dimethylformamide was added 150 mmol of the appropriate α-bromo-ω-(4-cyanophenoxy)alkane (IVd) and anhydrous calcium carbonate. Following 5 h of reflux the reaction mixture was allowed to cool, and a solid material was collected. The solid was recrystallized with DMF to give the pure α-[4-(5-cyano-2-benzimidazolyl)phenoxy]-ω-(4-cyanophenoxy)alkane (IVe).

The diamidine products (compounds 38–41) were prepared in the standard manner.

H. Bis(5-amidino-2-benzimidazolyl)alkanes, 1,4-Bis(5-amidino-2-benzimidazolyl)benzene, Bis[4-(5-amidino-2-benzimidazolyl)phenyl] Ether, and 1,2-Bis[5-(2-imidazolinyl)-2-benzimidazolyl]ethane. Compounds 42, 43, 45–50, and 59, Table II (See Scheme V). A solution of 200 mmol of 3,4-diaminobenzamidine hydrochloride (Va) or 2-(3,4-diaminophenyl)imidazoline (Vb) and 100 mmol of the appropriate bis(imino ether) dihydrochloride (Vc) in acetic acid was heated at reflux for 2 h. After cooling, the acetic acid was removed by rotary evaporation. The resulting residue was dissolved in 200 mL of H_2O, made slightly acidic with 5 mL of HCl, warmed to 60 °C, and decolorized with charcoal. The charcoal was removed by filtration and the filtrate made basic with NaOH. After sitting overnight, a precipitate formed and was filtered. The solid material was washed with H_2O, decolorized, and then placed in HCl-saturated EtOH. After the HCl solution had been allowed to stand for 1 day at room temperature the tetrahydrochloride of the bis(5-amidino-2-benzimidazolyl) compounds precipitated out of solution. The product was collected and purified by washing with 20% HCl and acetone.

I. 5-Amidino-2-[4-(4-amidinophenoxy)phenyl]benzimidazole. Compound 37, Table II (See Scheme VI). A mixture of 40 mmol of IVc, 50 mmol of *p*-fluorobenzonitrile (VIa), and 130 mmol of anhydrous $CaCO_3$ in DMF was heated at reflux for 2 h. The DMF was removed under vacuum and 150 mL of dilute NaOH solution was added to the residue. The basic solution was filtered and the solid was recrystallized from butanol giving 5-amidino-2-[4-(4-cyanophenoxy)phenyl]benzimidazole (VIb) in an 87% yield.

Finally, 70 mmol of VIb was reacted in the same manner as described previously to give the corresponding diamidine (compound **37**).

J. 5-Amidino-2-[2-(4-hydroxyphenyl)-5-benzimidazolyl]benzimidazole. Compound 51 (See Scheme VII). A solution of 60 mmol of IVc in 150 mL of methyl glycol was saturated with dry HCl. On standing at room temperature the mixture became pastelike and after 2 days was stirred with Et_2O and filtered. The solid was washed with anhydrous Et_2O and dried under KOH. A quantitative yield of crude 2-(4-hydroxyphenyl)-5-[(2-methoxyethoxy)(imino)methyl]benzimidazole hydrochloride (VIIa) was recovered and used in the following reaction without further purification.

A mixture of 50 mmol of the crude imino ether VIIa and 50 mmol of 3,4-diaminobenzamidine hydrochloride (Va) in 250 mL of MeOH was refluxed for 3 h. The MeOH was removed and the residue was warmed in 500 mL of H_2O. The aqueous solution was made basic with sodium carbonate, filtered, and washed with H_2O. The solid was dissolved in 2 N acetic acid and warmed on a steam bath. Following the decolorization of the solution with charcoal, concentrated HCl was added, and the solution was allowed to stand overnight in the refrigerator. The solid was filtered and washed with HCl and acetone.

K. 7-Amidino-2-(4-amidinophenyl)indole Dihydrochloride. Compound 6, Table II (See Scheme VIII). A solution of 5.3 g (25 mmol) of 4-bromoacetophenone (VIIIa) and 5.3 g (28 mmol) of 2-bromophenylhydrazine (VIIIb) in MeOH was refluxed for 45 min. After cooling the hydrazone VIIIc precipitated from the MeOH and was filtered. The crude material was recrystallized from cyclohexanol to give 8.9 g (96%) of VIIIc.

The reaction of 4 g (10.9 mmol) of VIIIc with H_3PO_4–P_2O_5 was carried out in the exact manner as described by Dann.[2] A side product, 2-(4-bromophenyl)indole, was removed from the crude product by column chromatography on aluminum oxide. The product, 7-bromo-2-(4-bromophenyl)indole (VIIId), was further purified by recrystallization from a mixture of cyclohexane and benzene: 1.6 g (42%); mp 138–139 °C. Anal. ($C_{14}H_9Br_2N$) Br.

A mixture of 2 g (5.7 mmol) of VIIId and 4 g (44.6 mmol) of CuCN in 12 mL of quinoline was reacted in the same manner as described by Dann.[2] The crude product, 7-cyano-2-(4-cyanophenyl)indole (VIIIe), was purified by column chromatography over aluminum oxide eluted with pyridine, followed by recrystallization from 2-propanol: 0.7 g (50%); mp 252–254 °C. Anal. ($C_{16}H_9N_3$) N.

The diamidine (compound **6**) was prepared according to the standard procedure.

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Synthesis and Antiinflammatory Activity of Trisubstituted Pyrimidines and Triazines

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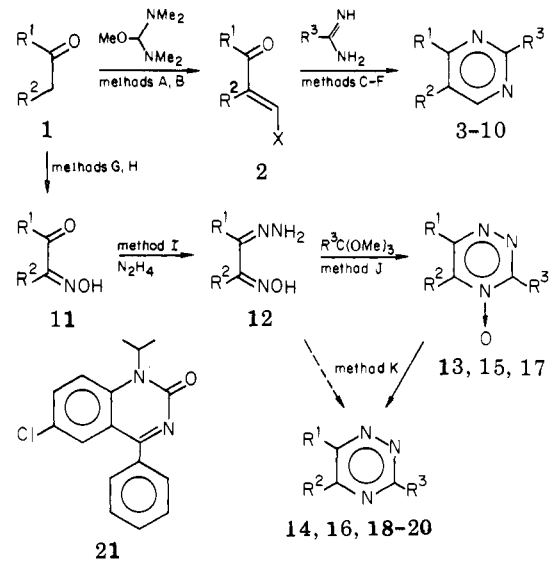
A series of mono-, bi-, and tricyclic pyrimidines and *as*-triazines was prepared and their antiinflammatory activity measured against carrageenan-induced edema in the rat. The more active analogues (ED_{50}), including 4-pyridylpyrimidines **4a** (38), **4b** (47), and **4g** (49) and 2-hydroxypyrimidine **8r** (43), were then tested against adjuvant-induced edema in the rat. None was active in the adjuvant arthritis model.

The search for new classes of nonsteroidal antiinflammatory agents which minimize gastrointestinal toxicity has led to a number of clinical candidates,[1-3] including the nonacidic heterocycle, proquazone (**21**).[3a,25] Despite these agents the need for more effective antirheumatic drugs continues to grow. To this end, a series of mono-, bi-, and tricyclic pyrimidines and *as*-triazines has been prepared and tested for antiinflammatory activity. A description of the synthesis of these compounds as well as a description of the structure–activity relationships is presented in this paper.

Chemistry. Chemically, our aim was to construct both the pyrimidine and triazine ring systems from common ketone precursors. Of the methods available,[4] those outlined in Scheme I were selected due to their general applicability to a variety of commercially available ketone systems as well as the simple nature of the reactions. Pyrimidine construction was accomplished by aminoformylation of an active methylene ketone **1** with Bredereck's reagent,[5] followed by cyclization of the resulting protected β-ketoaldehyde **2** [X = $N(CH_3)_2$] with an amidine equivalent. In some cases the ketones were formylated under standard conditions[6] with the resulting β-ketoaldehyde **2** (X = OH) converted into the corresponding enol ether **2** (X = OCH_2CH_3)[7] prior to cyclization. The cyclization with an amidine equivalent was, in general, most successful when performed in the presence of an equivalent of sodium ethoxide rather than under neutral or acidic conditions. Derivatization of the 2-amino- and

Scheme I



2-chloropyrimidines provided additional analogues (Table I).

The regiospecific preparation of *as*-triazines from the same α-methylene ketones **1** was accomplished by α-nitrosation, reaction of the resulting α-keto oxime **11** with hydrazine to form an α-hydrazino oxime **12**, and subsequent exposure under nonequilibrating conditions to an

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