Nonsteroidal Cardiotonics. 2. The Inotropic Activity of Linear, Tricyclic 5-6-5 Fused Heterocycles[1]

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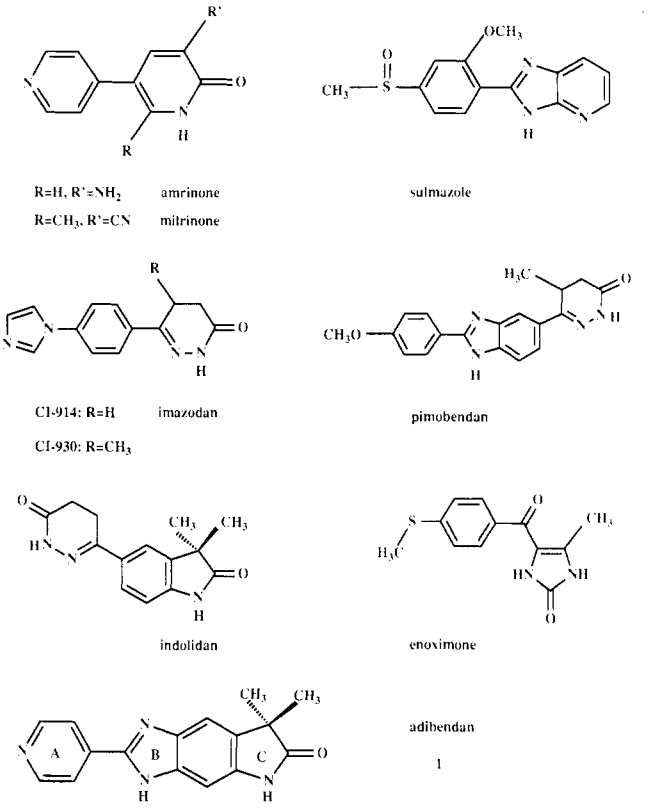
We previously reported the structure–activity relationships (SAR) of adibendan (1), a potent and long-acting cardiotonic. This paper describes the synthesis of a novel series of linear, tricyclic fused heterocycles of the 5-6-5 type. The compounds were evaluated for positive inotropic activity in anesthetized rats, cats, and dogs. Changes in left ventricular dP/dt were measured as an index of cardiac contractility. The increase in contractility was not mediated via stimulation of β-adrenergic receptors. The data revealed the intrinsic positive inotropic activity of the parent compound of this series, 5,7-dihydro-7,7-dimethylpyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (2). The structural features that impart optimal inotropic activity are presented and compared with those of the 4,5-dihydro-3(2*H*)-pyridazinone series. The most potent compounds were evaluated orally in conscious dogs with implanted Konigsberg pressure transducers to measure ventricular pressures, and their effect on left ventricular dP/dt was compared with that of **1**, pimobendan, and indolidan. After administration of 1 mg/kg, **1**, **3**, **7**, **19**, **22**, **24**, **31**, **54**, pimobendan, and indolidan were equipotent, but only with 1, **31**, pimobendan, and indolidan, durations of action exceeded 6 h.

The positive inotropic agents used today for the chronic treatment of congestive heart failure have severe limitations. The cardiac glycosides (digoxin, digitoxin), discovered in the 18th century, are still in use in spite of their low therapeutic index and their propensity to cause life-threatening arrhythmias.[2,3] The newer sympathomimetic agents (dobutamine, dopamine) are orally inactive and may lead to tachyphylaxis due to β-receptor down-regulation.[4-8] The 2-year survival rate of New York Heart Association class III and IV patients is only 30% or less.[9]

Because of this need for safer and orally effective drugs, a series of nonglycosidic, nonsympathomimetic, cardiotonic agents has been developed. Prototypical examples include amrinone,[10] milrinone,[11] sulmazole,[12] imazodan,[13] CI-930,[13] indolidan,[14] pimobendan,[15] and enoximone[16] (Chart I). We have found marked cardiotonic activity with some linear, tricyclic fused compounds including adibendan (1, BM 14.478, 5,7-dihydro-7,7-dimethyl-2-(4-pyridinyl)pyrrolo-[2,3-*f*]benzimidazol-6(1*H*)-one, Chart I).[1] While many of the new cardiotonics appear to derive their inotropic effect from specific inhibition of a phosphodiesterase isozyme, resulting in an increase of intracellular cyclic AMP,[17] sulmazole,[18] pimobendan,[15] and **1**[19] are thought to act, at least in part, by increasing the myofibrillar Ca^{2+} sensitivity.

Molecular modeling studies among pyridazinone cardiotonics have led to the recognition of a pharmacophoric pattern. According to Bristol et al.,[13] five basic structural features are necessary for positive inotropic action. On the basis of our structure–activity relationship (SAR) studies reported earlier,[1] we reasoned that **1** has at least four of the essential features required by the five-point model: (1) the presence of a strong dipole (carbonyl) at one end of the molecule, (2) an adjacent acidic proton, (3) a small lipophilic space (methyl groups), and (4) a basic hydrogen-acceptor site opposite the dipole (pyridine). In addition, Bristol's model suggests "a generally flat topography" for the molecules. Recently, two similar models have been proposed by Leclerc et al.[20a] and Erhardt et al.[20b] which differ from Bristol's model in this respect. The Erhardt model prefers an angle of ca. 15–20° between the perpendicular of the plane of an amide system and the π-electron cloud of an adjacent aromatic ring in order to optimize interaction with the ring. In a Dreiding model of **1**, the oxindol system is puckered because of the sp^3 carbon atom, and the angle required by the Erhardt model can easily be assumed. However, in the single-crystal X-ray structure of indolidan[21] the corresponding angle is only 0.7°, and the carbonyl oxygen atom sits almost in the

Chart I



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(1) Nonsteroidal Cardiotonics. 1: Mertens, A.; Müller-Beckmann, B.; Kampe, W.; Hölck, J.-P.; von der Saal, W. *J. Med. Chem.* **1987**, *30*, 1279. Part of this work was presented at the 11th International Congress of Heterocyclic Chemistry, Heidelberg, West Germany, Aug 16, 1987.
(2) Muller, J. E.; Turi, Z. G.; Stone, P. H.; Rude, R. E.; Raabe, D. S.; Jaffe, A. S.; Gold, H. K.; Gustafson, N.; Poole, W. K.; Passamani, E.; Smith, T. W.; Braunwald, E. *N. Engl. J. Med.* **1986**, *314*, 265.
(3) Yusuf, S.; Wittes, J.; Bailey, K.; Furberg, C. *Circulation* **1986**, *73*, 14.
(4) Su, Y.-F.; Harden, T. K.; Perkins, J. P. *J. Biol. Chem.* **1980**, *255*, 7410.
(5) Mukherjee, C.; Caron, M. G.; Lefkowitz, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1945.
(6) Bristow, M. R.; Ginsburg, R.; Minobe, W.; Cubicciotti, R. S.; Sageman, W. S.; Lurie, K.; Billingham, M. E.; Harrison, Stinson, E. B. *N. Engl. J. Med.* **1982**, *307*, 205.

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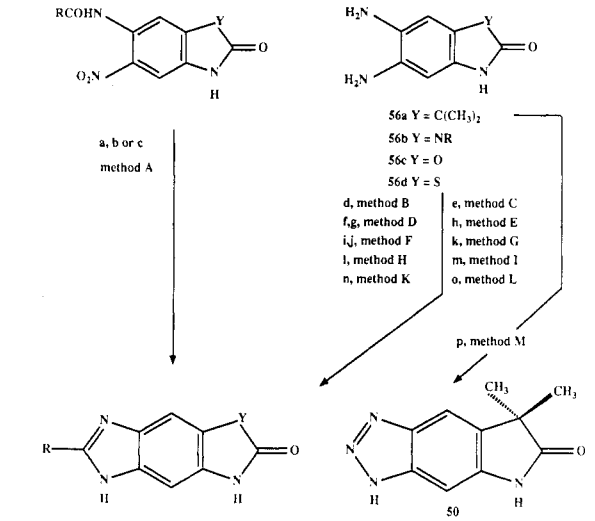
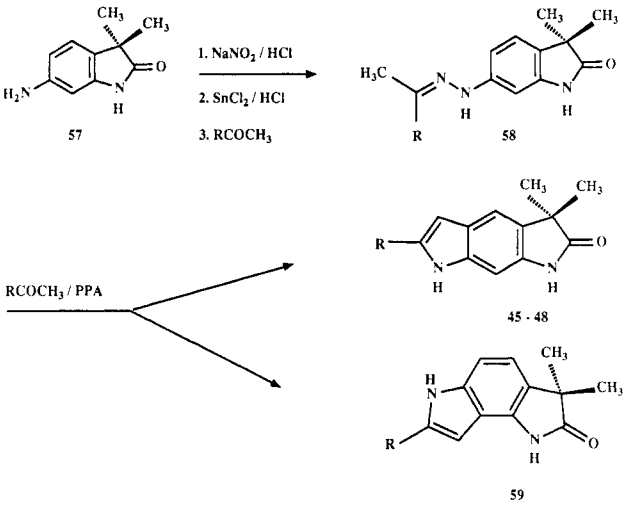
plane (deviation 0.05 Å) of the phenyl ring. We, therefore, initially assumed that 1 and its congeners are almost flat. However, in 10 unsymmetrically 3,3-dialkylated, N-unsubstituted oxindoles included in the Cambridge Crystallographic Data Base,[22] the deviation from coplanarity is much larger (up to 17.7°, and 0.65 Å,[22f] respectively). This suggests a certain degree of conformational flexibility of the lactam system of 1. We subsequently performed MNDO calculations[23] on the conformational flexibility of 1,3-dihydro-3,3-dimethylindol-2(1*H*)-one as a model for 1. The starting set was created with the atomic coordinates of indolidan,[21] replacing the pyridazinone ring by a hydrogen atom, constraining the dihedral angle (carbonyl C)–(N)–(phenyl C)–(phenyl C) to 0° and relaxing all other geometric parameters. The dihedral angle was then rotated from 10° to 25° at 5° intervals, and again all other parameters were relaxed. This procedure created angles between the amide system and the phenyl ring of 8.6°, 11.5°, 16.1°, and 20.4°. In the last two conformations,

Scheme I[a]



[a] (a) H_2/Pd; (b) acetic acid; (c) 2 N HCl; (d) RCHO/EtOH; (e) RCOOH/PPA; (f) RCOCl/NEt_3; (g) concentrated HCl; (h) $RCOOCH_3$; (i) RCOOH/DCC/*N*-hydroxybenzotriazole; (j) EtOH/concentrated HCl; (k) RCOOH; (l) $RCH(OH)SO_3^-Na^+$; (m) $COCl_2$; (n) $CSCl_2$; (o) BrCN; (p) $NaNO_2/H_2SO_4$.

Scheme II

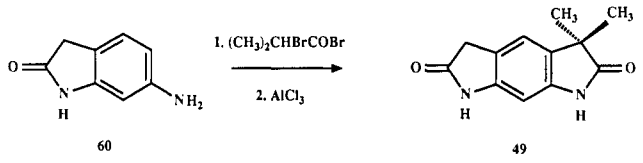


which are in the range predicted by the Erhardt model, the distances between the carbonyl oxygen atom and the plane of the phenyl ring were 0.46 and 0.58 Å, respectively. The corresponding costs in energy were 1.6 and 2.4 kcal/mol. Such energies are easily obtainable at physiological temperature during interaction with a receptor. Therefore, despite the fact that in crystals 3,3-dimethyloxindoles are almost flat, they are flexible enough to assume the angles predicted by the Erhardt model.

In our first paper about the SAR of congeners of 1 we focused primarily on the effects of substituents on the pyridine ring.[1] These pharmacophoric patterns and the apparent structural homology of 1 and several other cardiotonics (Chart I) now led us to question whether it is possible to replace the pyridinyl ring of 1 (ring A) by other groups, including the methoxyphenyl group of pimobendan or by the 2-methoxy-4-(methylthio)phenyl group of sulmazole. In addition, the SAR of the tricyclic system was examined by three types of structural changes. Firstly, we altered region B by replacing the imidazol moiety with a pyrrol, an oxazol, and the triazol system. Secondly, we changed the pyrrolinone ring C to an oxazolone, a thiazolone, and an imidazolone ring. Finally, the free NH

(7) Colucci, W. S.; Alexander, R. W.; Williams, G. H.; Rude, R. E.; Holman, B. L.; Konstam, M. A.; Wynne, J.; Mudge, G. H., Jr.; Braunwald, E. *N. Engl. J. Med.* **1981**, *305*, 185.
(8) Harden, T. K. *Pharmacol. Rev.* **1983**, *35*, 5.
(9) Franciosa, J. A.; Wilen, M.; Zieschej, S. *Am. J. Cardiol.* **1983**, *51*, 831.
(10) Farah, A. E.; Alousi, A. A. *Life Sci.* **1978**, *22*, 1139.
(11) Alousi, A. A.; Canter, J. M.; Montenaro, M. J.; Fort, D. J.; Ferrari, R. A. *Cardiovasc. Pharmacol.* **1983**, *5*, 792.
(12) Diederen, W.; Kadatz, R. *Arzneim.-Forsch.* **1981**, *31*, 141.
(13) (a) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099. (b) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 1963. (c) Sircar, I.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405.
(14) (a) Hayes, J. S.; Pollock, G. D.; Wilson, H.; Bowling, N.; Robertson, D. W. *J. Cardiovasc. Pharmacol.* **1987**, *9*, 425. (b) Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Wyss, V.; Pollock, G. D.; Wilson, H.; Kauffman, R. F.; Hayes, J. S. *J. Med. Chem.* **1986**, *29*, 1832.
(15) van Meel, J. C. A. *Arzneim.-Forsch.* **1985**, *35*, 284.
(16) Schnettler, R. A.; Dage, R. C.; Grisar, M. J. *J. Med. Chem.* **1982**, *25*, 1477.
(17) (a) Sircar, I.; Weishaar, R. E.; Kobylarz, D.; Moos, W. H.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 1955. (b) Endoh, M.; Yanagisawa, T.; Morita, T.; Taira, N. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 267.
(18) Solaro, R. J.; Ruegg, J. C. *Circ. Res.* **1982**, *51*, 290.
(19) Freund, P.; Müller-Beckmann, B.; Strein, K.; Kling, L.; Rüegg, J. C. *Eur. J. Pharmacol.* **1987**, *136*, 243.
(20) (a) Rakhit, S.; Marciniak, G.; Leclerc, G.; Schwartz, J. *Eur. J. Med. Chem.-Chim. Ther.* **1986**, *21*, 511. (b) Erhardt, P. W.; Hagedorn, A. A., III; Sabio, M. *Mol. Pharmacol.* **1988**, *33*, 1.
(21) Robertson, D. W.; Jones, N. D.; Krushinski, J. H.; Don Pollock, G.; Swartzendruber, J. K.; Hayes, J. S. *J. Med. Chem.* **1987**, *30*, 623.
(22) (a) Itai, A.; Iitaka, Y.; Kubo, A. *Acta Crystallogr., Sect. B* **1978**, *B34*, 3775. (b) Kosuge, T.; Tsuji, K.; Hirai, K.; Yamaguchi, K.; Okamoto, T.; Iitaka, Y. *Tetrahedron Lett.* **1981**, *22*, 3417. (c) Wenkert, E.; Moeller, P. D. R.; Piettre, S. R.; McPhail, A. T. *J. Org. Chem.* **1987**, *52*, 3404. (d) Schwartz, R. E.; Hirsch, C. F.; Springer, J. P.; Pettibone, D. J.; Zink, D. L. *J. Org. Chem.* **1987**, *52*, 3704. (e) Silverton, J. V.; Akiyama, T. *J. Chem. Soc., Perkin Trans 1* **1982**, 1263. (f) James, M. N. G.; Williams, G. J. B. *Can. J. Chem.* **1972**, *50*, 2407. (g) Przybylska, M. *Acta Crystallogr.* **1962**, *15*, 301. (h) Polonsky, J.; Merrien, M.-A.; Prangé, T.; Pascard, C. *J. Chem. Soc., Chem. Commun.* **1980**, 601. (i) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135. (j) Dideberg, O.; Lamotte-Brasseur, Jj.; Dupont, L.; Campsteyn, H.; Vermeire, M.; Angenot, L. *Acta Crystallogr., Sect. B* **1977**, *33*, 1796.
(23) (a) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899. (b) Bischof, P.; Friedrich, G. *J. Comput. Chem.* **1982**, *3*, 486.

Scheme III



groups of adibendan **1** were selectively blocked by methylation. The Leclerc model[20a] requires the amide system to be capable of existing in a tautomeric form, and therefore one would expect considerable loss in activity by this methylation. A loss in activity has already been observed in imazodan after alkylation of the pyridazinone nitrogen[13c] and in indolidan after alkylation of the pyridazinone or the oxindol nitrogen.[14b]

Our efforts resulted in the discovery of several potent inotropes. We now detail the synthesis and SAR of these linear, tricyclic fused congeners of **1**.

Chemistry

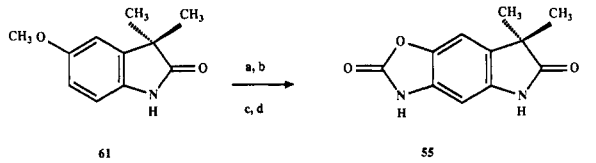
Modification of Region A. The pyrrolo[2,3-*f*]benzimidazoles **1–30** were prepared by one of the methods A–F (Scheme I) according to previously reported procedures[1] for analogues of **1** (Table I). Compounds with alkyl side chains (**2, 9**) were most conveniently prepared by heating the diamino compound **56a** with the corresponding carboxylic acids (method G). In some cases, method B gave only low yields. By replacing the aldehyde by its bisulfite adduct and reacting it with the diamine **56a** (method H), yields were improved considerably.

Congeners bearing a hydroxy, mercapto, or an amino group as substituent R (**31–33, 44**) were prepared by reaction of the requisite diamino compound (**56a,b**) with phosgene (method I), thiophosgene (method K), or cyanogen bromide (method L), respectively.

Modification of Region C. Using similar chemistry, we prepared benzo[1,2-*d*:4,5-*d'*]diimidazoles (**38–43**), imidazo[4,5-*f*]benzoxazoles (**34–36**), and an imidazo[4,5-*f*]-benzothiazole (**37**). The best synthetic methods started from 1,3-dihydro-5,6-diamino-2*H*-benzimidazol-2-ones[24] (**56b**), 2,3-dihydro-5,6-diaminobenzoxazolone[25] (**56c**), or 2,3-dihydro-5,6-diaminobenzothiazol-2-one[25] (**56d**), respectively. Reactions with either carboxylic acids in polyphosphoric acid (PPA)(Scheme I, method C) or with aldehydes in acetic acid (method B) were successful.

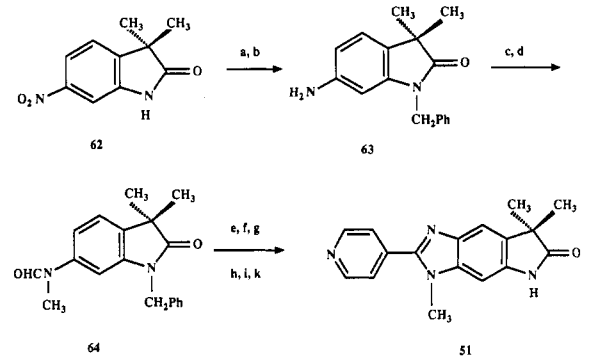
Modification of Region B. Diazotization of the diamine **56a** yielded the pyrrolo[2,3-*f*]benzotriazole **50** (method M). Benzo[1,2-*b*:5,4-*b'*]dipyrroles (**45–48**) were prepared from 1,3-dihydro-6-amino-2*H*-indol-2-ones[1] (Scheme II). Diazotization of **57** and reduction yielded the hydrazines[26] as unstable compounds, which were not isolated, but reacted with ketones to the corresponding hydrazones **58**. The Fischer indole synthesis[26] under acid catalysis, usually PPA, yielded the desired products **45–48**. Only minor amounts of angular isomers (**59**) were formed, as determined by the ^{1}H NMR spectra of the crude products. Compound **49** was prepared by acylation of 1,3-dihydro-6-amino-2*H*-indolin-2-one, **60**,[27] with 2-bromo-2-methylpropanoyl bromide and subsequent ring closure by a Friedel–Crafts reaction[28] (Scheme III).

Scheme IV[a]



[a] (a) HNO_3/H_2SO_4; (b) HBr; (c) H_2/Pd; (d) *N,N'*-carbonyldiimidazole.

Scheme V[a]



[a] (a) $PhCH_2Cl$; (b) H_2/Pd; (c) HCOOH; (d) CH_3I; (e) Na/NH_3; (f) HCOOH; (g) HNO_3/H_2SO_4; (h) HCl/EtOH; (i) H_2/Pd; (k) isonicotinoyl chloride/Et_3N.

The synthesis of a prototype example of pyrrolo[2,3-*f*]benzoxazoles (**55**, Table II) is shown in Scheme IV. Nitration of 1,3-dihydro-3,3-dimethyl-5-methoxy-2*H*-indol-2-one (**61**)[29] followed by ether cleavage of the methoxy group and reduction of the nitro group resulted in 6-amino-1,3-dihydro-3,3-dimethyl-5-hydroxy-2*H*-indol-2-one, which was converted to the pyrrolo[2,3-*f*]benzoxazole **55** with *N,N'*-carbonyldiimidazole.

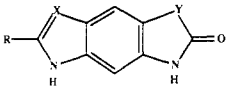
Selective methylation of the free NH groups of 1 (Scheme V) is exemplified with the synthesis of the 3-methyl isomer **51**. Benzylation of 1,3-dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one **62** to protect the NH group was followed by reduction of the nitro group to give **63**, which was subsequently formylated and methylated to yield **64**. The protecting group was removed with sodium in liquid ammonia. The further synthesis followed the method used for the parent compound **1**.[1] By similar schemes we obtained the other *N*-methyl isomers **52** and **53** and one *N,N'*-dimethyl compound, **54**.

The linear structure of the new tricycles was confirmed by ^{1}H NMR spectroscopy: the coupling constant between the two aromatic protons of the central phenyl ring of the tricyclic heterocycle is always smaller than 1 Hz.

Pharmacology. Compounds **1–55** were first tested in rat and cat models to determine their cardiac inotropic, vasodilatory, and chronotropic potency. Anesthetized rats and open-chest cats, pretreated with 0.3 mg/kg of the β-adrenergic antagonist desacetylmetipranolol iv, were prepared for recording left ventricular dP/dt, blood pressure, and heart rate. Dose–response curves were performed for all compounds by iv injection of increasing doses. The hemodynamic parameters were determined 10 min after application and represent "steady-state effects" and not the maximum change in hemodynamics which occurred about 1–3 min after the bolus injection. The effective dose required to increase dP/dt_{50} by 1500

(24) Schindlbauer, H.; Kwiecinski, W. *Monatsh. Chem.* **1976**, *107*, 1307.
(25) Clark, R. L.; Pessolano, A. A. *J. Am. Chem. Soc.* **1958**, *80*, 1662.
(26) Enders, E. In Houben, J.; Weyl, T.; Müller, E. *Methoden der Organischen Chemie* G. Thieme: Stuttgart, 1967; Vol. X/2, 169, p 169.
(27) Ruggli, P.; Grand, R. *Helv. Chim. Acta* **1937**, *20*, 373.
(28) Julian, P. L.; Pikl, J.; Boggess, D. *J. Am. Chem. Soc.* **1934**, *56*, 1797.
(29) Wahl, G. *Monatsh. Chem.* **1917**, *38*, 525.

Table I. Structure and Properties of Pyrrolo[2,3-*f*]benzimidazoles (X = N, Y = CR′R″), Imidazo[4,5-*f*]benzoxazoles (X = N, Y = O), Imidazo[4,5-*f*]benzothiazoles (X = N, Y = S), Benzo[1,2-*d*:4,5-*d*′]diimidazoles (X = N, Y = NR′), and Benzo[1,2-*b*:5,4-*b*′]dipyrroles (X = CR′, Y = CR′R″)



compd no.	R	X	Y	prepn methods[a]	% yield[b]	mp, °C	recrystn solvent[c]	formula
2	H	N	$C(CH_3)_2$	exp part	90	278–280	d	$C_{11}H_{11}N_3O$
3	CH_3	N	$C(CH_3)_2$	A	67	>300	e	$C_{12}H_{13}N_3O{\cdot}H_2O$
4	CF_3	N	$C(CH_3)_2$	A	95	175–177	e	$C_{12}H_{10}F_3N_3O{\cdot}H_2O$
5	$CH_3CH_2CH_2$	N	$C(CH_3)_2$	A	61	284–285	e	$C_{14}H_{17}N_3O{\cdot}0.25H_2O$
6	$CH_3CH{=}CH$	N	$C(CH_3)_2$	C	14	252–254	e	$C_{14}H_{15}N_3O{\cdot}0.5H_2O$
7	$(CH_3)_2CH$	N	$C(CH_3)_2$	A	40	282–284	e	$C_{14}H_{17}N_3O{\cdot}0.5H_2O$
8	$H_2C{=}C(CH_3)$	N	$C(CH_3)_2$	C	14	270–273	f	$C_{14}H_{15}N_3O{\cdot}0.5H_2O$
9	cyclohexyl	N	$C(CH_3)_2$	analog. **2**	53	315–318	d	$C_{17}H_{21}N_3O$
10	phenyl	N	$C(CH_3)_2$	A	81	195–220	g	$C_{17}H_{15}N_3O$
11	2-methoxyphenyl	N	$C(CH_3)_2$	D	23	300-303	h	$C_{18}H_{17}N_3O_2$
12	4-methoxyphenyl	N	$C(CH_3)_2$	D	46	341-343	i/k	$C_{18}H_{17}N_3O_2{\cdot}0.5H_2O$
13	4-hydroxyphenyl	N	$C(CH_3)_2$	B	44	240	h	$C_{17}H_{15}N_3O_2$
14	4-(dimethylamino)phenyl	N	$C(CH_3)_2$	B	23	262–270	l	$C_{19}H_{20}N_4O{\cdot}HCl$
15	4-(1-imidazolyl)phenyl	N	$C(CH_3)_2$	B	28	300	m	$C_{20}H_{17}N_5O{\cdot}1.25H_2O$
16	2-methoxy-4-(methylsulfonyl)phenyl	N	$C(CH_3)_2$	exp part	75	235–237	n	$C_{19}H_{19}N_3O_4S$
17	2-methoxy-4-(methylsulfenyl)phenyl	N	$C(CH_3)_2$	exp part	41	217–220	f	$C_{19}H_{19}N_3O_3S$
18	2-pyrrolyl	N	$C(CH_3)_2$	B	20	>300	l	$C_{15}H_{14}N_4O{\cdot}HCl$
19	2-furanyl	N	$C(CH_3)_2$	A	33	311–316	e	$C_{15}H_{13}N_3O_2$
20	2-thienyl	N	$C(CH_3)_2$	B	30	332–336	h	$C_{15}H_{13}N_3OS$
21	4-imidazolyl	N	$C(CH_3)_2$	D	5	270	m	$C_{14}H_{13}N_5O{\cdot}H_2O$
22	1,2,5-thiadiazol-3-yl	N	$C(CH_3)_2$	C	66	>300	m	$C_{13}H_{11}N_5OS{\cdot}0.5CH_3OH$
23	1,2,4-triazol-3-yl	N	$C(CH_3)_2$	B	28	>350	m	$C_{13}H_{12}N_2O$
24	4-pyridazinyl	N	$C(CH_3)_2$	F	12	>350	e	$C_{15}H_{13}N_5O{\cdot}0.5H_2O$
25	5-pyrimidinyl	N	$C(CH_3)_2$	F	25	310–312	k	$C_{15}H_{13}N_5O$
26	2-pyrazinyl	N	$C(CH_3)_2$	D	16	>300	e	$C_{15}H_{13}N_5O$
27	6-hydroxy-3-pyridazinyl	N	$C(CH_3)_2$	F	32	>350	n	$C_{15}H_{13}N_5O_2{\cdot}0.7H_2O$
28	2-indolyl	N	$C(CH_3)_2$	A	20	>350	e	$C_{19}H_{16}N_4O$
29	3-quinolinyl	N	$C(CH_3)_2$	C	78	>300	m	$C_{20}H_{16}N_4O$
30	4-quinolinyl	N	$C(CH_3)_2$	C	67	210–213	n	$C_{20}H_{16}N_4O$
31	HO	N	$C(CH_3)_2$	exp part	62	>300	m	$C_{11}H_{11}N_3O_2$
32	HS	N	$C(CH_3)_2$	exp part	19	>300	m	$C_{11}H_{11}N_3OS$
33	H_2N	N	$C(CH_3)_2$	exp part	22	300–305	h/n	$C_{11}H_{12}N_4O{\cdot}HBr$
34	CH_3	N	O	analog. **36**	21	355	f	$C_9H_7N_3O_2$
35	$CH_3CH_2CH_2$	N	O	analog. **36**	34	177–180	e	$C_{11}H_{11}N_3O_2{\cdot}0.5H_2O$
36	4-pyridinyl	N	O	exp part	51	>360	o	$C_{13}H_8N_4O_2$
37	4-pyridinyl	N	S	analog. **36**	36	>300	o	$C_{13}H_8N_4OS$
38	4-pyridinyl	N	NH	exp part	22	>300	g/m	$C_{13}H_9N_5O$
39	4-pyridinyl	N	NCH_3	exp part	35	>320	f	$C_{14}H_{11}N_5O$
40	4-pyradizinyl	N	NCH_3	exp part	14	>300	m	$C_{13}H_{10}N_6O$
41	4-pyridinyl	N	NCH_2CH_3	analog. **39**	28	237–239	e	$C_{15}H_{13}N_5O{\cdot}H_2O$
42	4-pyridazinyl	N	NCH_2CH_3	analog. **40**	14	>300	p	$C_{14}H_{12}N_6O$
43	4-pyridinyl	N	$NCH_2CH_2CH_3$	analog. **39**	21	235–237	e	$C_{16}H_{15}N_5O{\cdot}1.5H_2O$
44	HO	N	NCH_3	exp part	19	>300	p	$C_9H_8N_4O_2$
45	CH_3	CH	$C(CH_3)_2$	analog. **47**	13	255	m	$C_{13}H_{14}N_2O$
46	4-(1-imidazolyl)phenyl	CH	$C(CH_3)_2$	analog. **47**	23	>340	m	$C_{21}H_{18}N_4O$
47	4-pyridinyl	CH	$C(CH_3)_2$	exp part	66	>300	g/m	$C_{17}H_{15}N_3O$
48	4-pyridazinyl	CH	$C(CH_3)_2$	analog. **47**	33	305	m	$C_{16}H_{14}N_4O{\cdot}H_2O$
49	HO	CH	$C(CH_3)_2$	exp part	11	280-282	l	$C_{12}H_{12}N_2O_2{\cdot}0.5(CH_3)_2CHOH{\cdot}0.75H_2O$

[a] Capital letters in this column correspond to the methods of preparation in ref 1; exp part means that the synthesis is described in the experimental part of this paper. [b] Yields are not optimized and correspond to the final step. [c] The symbols are as follows: d, ether; e, ethyl acetate; f, flash chromatography; g, dichloromethane; h, acetone; i, water; k, dioxane; l, 2-propanol; m, methanol; n, ethanol; o, dimethylformamide; p, pyridine.

mmHg/s at a LVP of 50 mm ($ED_{1.5}$) was calculated by linear regression analysis. These pharmacological data are summarized in Table III.

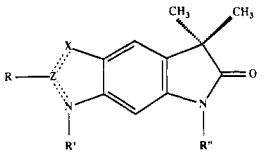
The inotropic effects of **1–55** were not blocked by prior treatment with desacetylmetipranolol, which indicates that these agents do not act via direct stimulation of β-adrenergic receptors or indirectly by release of catecholamines.

Because oral activity is of paramount importance, representatives of compounds which were positive in both front-line screens were tested intravenously and orally in conscious dogs. The dogs were instrumented chronically with a Konigsberg manometer. dP/dt_{60} was obtained as before, but at a LVP of 60 mmHg. Compounds were administered orally to gavage in a suspension of 1% methylcellulose. The results are summerized in Table IV.

Structure–Activity Relationships

The data summarized in Table III provided us with information about structural requirements necessary for optimal inotropic activity. If one considers $ED_{1.5}$ values in rats of compounds **1–9**, then the pyridine ring always shows a greater positive inotropic effect than the simple alkyl derivatives, for which a 10-fold higher dose was required for comparable activity. In conscious dogs, the duration of the inotropic effect of the methyl compound **3** and the propyl compound **7** was shorter when compared

Table II. Structure and Properties of a Pyrrolo[2,3-*f*]benzotriazole (**50**), N-Methylated Pyrrolo[2,3-*f*]benzimidazoles (**51–54**), and a Pyrrolo[2,3-*f*]benzoxazole (**55**)



no.	R–Z	X	bond Z–X	R′	R″	% yield[a]	mp, °C	recrystn solvent	formula[b]
50	N	N	double	H	H	66	288–291	ethanol	$C_{10}H_{10}N_4O \cdot 0.3H_2O$
51	4-pyridinyl-C	N	double	CH_3	H	58	342–344	ethanol	$C_{17}H_{16}N_4O$
52	4-pyridinyl-C	N	double	H	CH_3	36	252–253	ethyl acetate	$C_{17}H_{16}N_4O$
53	4-pyridinyl-C	N	double	CH_3	CH_3	48	223–224	ethanol	$C_{18}H_{18}N_4O$
54	O=C	NCH_3	single	H	H	29	364–367	methanol	$C_{12}H_{13}N_3O_2$
55	O=C	O	single	H	H	51	297–299	2-butanone	$C_{11}H_{10}N_2O_3$

[a] Yields are not optimized and correspond to the final step. [b] The analyses were within 0.4% of the calculated value.

to **1** (Table IV). Because of the reasonable cardiotonic activity of **2**, which may be regarded as the parent compound of this class of heterocycles, we next focused on similar molecules with small substituents. Among compounds **31**, **32**, and **33**, with a hydroxy, mercapto, or amino group, respectively, only **31** exhibits useful activity in rats and cats. A possible explanation for this rank order of potency among **31–33** would be the different tautomeric forms of **31** vs **32** and **33**. Whereas benzimidazolones have a cyclic urea form, the C–S bond of benzimidazolethiones has about 20% single bond character, and 2-aminobenzimidazoles exist as such and not as the imino tautomers.[30] Therefore, **31** possesses a carbonyl oxygen atom three atoms removed from the phenyl ring, which may function as hydrogen-bond acceptor. It has been hypothesized lately that this moiety is essential for the cardiotonic activity of phenylpyridazinones.[14b,31] The oral activity of **31** in dogs was exceptionally high, the positive inotropic effect lasted for more than 6 h (Table IV).

These results clearly show that the pyridinyl group of **1** may be replaced by very small substituents without dramatic loss of activity. There may even be no substituent at all in this position, as in compound **50**, and still a cardiotonic activity may be observed. Compounds **2** and **50** reveal the intrinsic positive inotropic activity of the linear, tricyclic 5-6-5 fused heterocycles.

The deaza analogue of **1**, compound **10**, was examined for inotropic activity to determine whether the beneficial effect of the pyridine ring A was simply due to the presence of an aromatic nucleus regardless of whether a hydrogen-bond-acceptor site was present. **10** was more than 1 order of magnitude less potent than adibendan when administered iv to rats or cats and completely without activity when administered orally to conscious dogs, demonstrating the crucial nature of the pyridine ring nitrogen in maximizing inotropic activity.

Most of the substituted phenyl compounds **11–17** exhibited no useful activity. It is especially worth noting that the *p*-methoxyphenyl compound **12** and its desmethyl analogue **13** are devoid of activity. These two compounds resemble pimobendan and its desmethyl metabolite UD-CG 212 (region A in Chart I). Similarly, **16** and **17**, which may be regarded as analogues of sulmazole, are inactive. The decrease of cardiotonic activity seen upon substition of the phenyl ring is consistent with earlier observations in the pyridinyl series of **1**.[1] In contrast, the "imazodan analogue" **15** exhibits a weak cardiotonic activity. It has been shown lately, however, that in compounds bearing the relationship heterocycle–phenyl–imidazole, the imidazole along with the heterocycle serves as a key substituent necessary for optimal inotropic activity.[13c,34] and the weak cardiotonic activity of **15** may be a consequence of this relationship.

The fact that the size of substituents R (formula I) should not be a larger than a six-membered ring could be further substantiated with the bicyclic substituents for R: Compounds **28–30** lack activity.

The well-established bioisosterism of imidazole and pyridine[31] led us to question whether it is possible to replace the pyridine ring of **1** by five-membered heterocyclic rings. Compounds **18–23** show a superior cardiotonic activity in both front-line tests. The thiadiazole compound **22** is 1 log better than **1** when tested iv in dogs (Table III). Consequently, compounds **19**, **21**, and **22** were further tested orally in conscious dogs (Table IV). The duration of activity was, however, shorter than that of **1**, which may reflect lower bioavailability or a faster metabolism.

Optimal results were obtained with six-membered heterocycles bearing two nitrogen atoms: Compounds **24–27** have similar activities compared to that of **1** (Table III), and the 4-pyridazinyl compound **24** furthermore showed similar oral activity (Table IV).

In a second series of compounds (**34–44**), we replaced the $(CH_3)_2C$ group in the pyrrole ring C of **1** with oxygen, sulfur, or alkylamino groups. Among the imidazo[2,3-*f*]-benzoxazoles **34–36**, only the pyridinyl compound **36** showed a small activity, when administered iv to cats and dogs. Because of the beneficial effect of the pyridine ring in region A it was kept constant in the further series. The low cardiotonic activities of **36** and **37** are especially worth mentioning, because these congeners lack the alkyl substituents in the 3-position of the "lactam" ring, which are of paramount importance for the activity of **1**[1] and indo-

(30) Smith, D. M. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Taylor, E. C., Preston, P. N., Eds.; J. Wiley and Sons: New York, 1981; Vol. 40, p 331.
(31) Robertson, D. W.; Krushinski, J. H.; Pollock, G. D.; Hayes, J. S. *J. Med. Chem.* **1988**, *31*, 461.
(32) Boyer, J. H. In *Heterocyclic Compounds*, Elderfield, R. C., Ed.; J. Wiley and Sons: New York 1961; Vol. 7, p 384.
(33) Robertson, D. W.; Beedle, E. E.; Krushinski, J. H.; Pollock, G. D.; Wilson, H.; Wyss, V. L.; Hayes, J. S. *J. Med. Chem.* **1985**, *28*, 717.
(34) (a) Davey, D.; Erhardt, P. W.; Lumma, W. C., Jr.; Wiggins, J.; Sullivan, M.; Pang, D.; Cantor, E. *J. Med. Chem.* **1987**, *30*, 1337. (b) Hagedorn, A. A., III; Erhardt, P. W.; Lumma, W. C., Jr.; Wohl, R. A.; Cantor, E.; Chou, Y.-L.; Ingebretsen, W. R.; Lampe, J. W.; Pang, D.; Pease, C. A.; Wiggins, J. *J. Med. Chem.* **1987**, *30*, 1342.

Table III. Pharmacological Data of Compounds 1–55 after Intravenous Administration

no.	rat		cat		dog	
	$ED_{1.5}$[a]	max[b] (dose)[c]	$ED_{1.5}$[a]	max[b] (dose)[c]	$ED_{1.5}$[d]	max[b] (dose)[c]
1	0.01	3.7 (0.1)	0.01	2.0 (0.3)	0.20	2.0 (1.0)
2	0.19	3.2 (3.0)	0.13	2.7 (1.0)	NT[e]	
3	0.48	3.5 (10.0)	0.17	2.8 (3.0)	0.46	2.2 (1.0)
4	0.13	2.3 (3.0)	0.08	2.0 (0.3)	NT	
5	0.17	2.5 (1.0)	0.12	2.0 (0.3)	NT	
6	>3	*f*	NT		NT	
7	0.22	2.3 (1.0)	0.09	2.7 (1.0)	0.46	1.9 (1.0)
8	>3	*f*	NT		NT	
9	>3	*f*	NT		NT	
10	0.37	4.2 (10.0)	0.15	3. (3.0)	NT	
11	>3	*f*	>3	*f*	NT	
12	>3	*f*	NT		NT	
13	>3	*f*	NT		NT	
14	>3	*f*	NT		NT	
15	1.0	2.8 (10.0)	0.25	3.7 (10.0)	NT	
16	2.0	1.8 (3.0)	NT		NT	
17	>3	*f*	NT		NT	
18	0.45	2.6 (10.0)	0.07	2.3 (3.0)	0.32	1.9 (1.0)
19	0.07	2.9 (3.0)	0.06	3.2 (10.0)	0.06	2.7 (0.3)
20	0.12	2.1 (0.3)	0.07	2.3 (0.3)	NT	
21	0.12	3.2 (0.3)	NT		0.07	2.7 (1.0)
22	0.007	3.5 (1.0)	0.004	2.6 (0.1)	0.008	2.3 (0.03)
23	0.10	3.1 (3.0)	0.02	3.7 (0.3)	NT	
24	0.04	2.4 (0.3)	0.01	2.4 (0.03)	0.035	2.6 (0.1)
25	0.045	3.1 (3.0)	0.02	3.1 (0.3)	NT	
26	0.10	2.6 (10.0)	0.04	2.7 (0.3)	NT	
27	0.07	3.3 (3.0)	NT		NT	
28	>3	*f*	NT		NT	
29	>3	*f*	NT		NT	
30	2.0	1.8 (3.0)	NT		NT	
31	0.13	3.0 (1.0)	0.08	2.9 (3.0)	0.12	2.0 (0.3)
32	0.55	3.5 (3.0)	0.06	3.6 (1.0)	NT	
33	>3	*f*	NT		NT	
34	>3	*f*	NT		NT	
35	>3	*f*	NT		NT	
36	0.52	4.1 (10.0)	0.06	3.5 (1.0)	0.12	2.6 (0.3)
37	0.20	1.8 (0.3)	NT		NT	
38	>3	*f*	NT		NT	
39	0.33	2.6 (10.0)	0.01	4.7 (1.0)	0.12	2.9 (1.0)
40	0.19	2.7 (1.0)	NT		NT	
41	0.05	2.3 (3.0)	0.007	3.1 (0.1)	NT	
42	0.04	3.2 (3.0)	0.02	2.9 (0.3)	NT	
43	1.0	1.6 (3.0)	NT		NT	
44	>3	*f*	NT		NT	
45	>3	*f*	NT		NT	
46	>3	*f*	NT		NT	
47	0.16	3.4 (3.0)	0.11	3.2 (10.0)	NT	
48	NT		0.01	2.5 (1.0)	NT	
49	0.11	2.0 (0.3)	NT		NT	
50	0.18	2.6 (1.0)	NT		NT	
51	>3	*f*	NT		NT	
52	>3	*f*	NT		NT	
53	>3	*f*	NT		NT	
54	0.03	2.4 (0.1)	0.02	2.1 (0.1)	NT	
55	>3	*f*	NT		NT	
pimobendan	1.3	2.0 (3.0)	0.6	2.9 (10.0)	1.1	1.3 (1.0)

[a] $ED_{1.5}$ is the effective dose (mg/kg) required to produce an increase in dP/dt_5 by 1500 mmHg/s. The values in the table were converted to mHg/s. Each value is the mean of four experiments. [b] Max is the maximum increase in dP/dt (mmHg/s) from control. The values in the table were converted to mHg/s. [c] Dose (mg/kg) at which the maximum increase in dP/dt was achieved. [d] Effective dose required to ptroduce an increase in dP/dt_{60} by 1500 mmHg. [e] NT = not tested. [f] Values not obtained.

lidan.[14b] Better results than for compound **36** and **37** were indeed obtained when small alkyl groups occupied this position, as in compounds **39–44**. The *N*-ethyl group (**41**, **42**) is superior to both the *N*-methyl group (**39**, **40**) and the *N*-propyl group (**43**). In the indolidan series[14b] as well as in the adibendan series,[1] homologation to alkyl groups larger than methyl results in a decreased inotropic potency. Compounds **39–44**, however, resemble enoximone not only in that they contain imidazolone groups as key elements but also in that both methyl or ethyl side chains improve

Table IV. Oral Activities of Tricyclic Cardiotonics in Conscious Dogs

no.	dose[a] (n)[b]	max[c]	(time[d])	duration[e]
1	1 (4)	2.4	45	>6
3	1 (4)	2.1	30	3
7	1 (4)	2.2	30	3
10	1 (4)	0		0
19	1 (4)	2.1	40	2
21	1 (3)	1.3	120	2
22	1 (4)	3.0	10	3
24	1 (7)	2.3	150	5
31	1 (8)	2.2	45	>6
31	0.5 (8)	1.6	60	6
36	1 (4)	0		0
39	1 (4)	0		0
40	1 (4)	0		0
41	1 (4)	0		0
54	1 (4)	2.3	30	>5
pimobendan	1 (4)	2.1	30	6
indolidan	0.3 (4)	2.8	45	>6

[a] Dose in mg/kg. [b] *n* is the number of dogs. [c] max is the maximum increase in dP/dt_{60} recorded in mmHg/s and converted into mHg/s in the table. [d] Time in min after which the maximum increase in contractility was observed. [e] Amount of time during which a significant amount of increase in contracility (>500 mmHg/s) was observed.

potency, and activity then decreases sharply when the alkyl is further lengthened by one carbon atom. In the Erhardt model[20b] this abrupt decrease in potency is related to unfavorable interactions of the side chain with steric boundaries at the receptor surface. Unfortunately, compounds **39–41** were orally inactive (Table IV). A similar observation has been made in indolidan, where *two* methyl groups at the oxindole nucleus are necessary for oral activity.[14b]

In a third series of compounds (**45–49**), we replaced one of the imidazole nitrogens of **1** (ring B) by a CH fragment. The rank order of potency in this series shared some common features with the parent (adibendan) series. Compounds bearing a pyridine (**47**), a pyridazine (**48**), or a carbonyl group (**49**) in region A are more potent than the corresponding methyl or substituted phenyl analogues (**45**, **46**).

With the fourth series of compounds (**51–55**) we were able to show that free NH groups in the tricyclic heterocycle are necessary for inotropic activity. Compounds with methyl groups at one or two nitrogen atoms (**51–53**) have no activity, whereas the similar compound **54** is active. Compound **54** exists most probably as the cyclic urea derivative (vide supra), and therefore one free NH group is present in region B, in contrast to **51** and **53**. To further test the hypothesis that a hydrogen atom is necessary in this region, we prepared and tested the pyrrolo[2,3-*f*]-benzoxazole **55**. To our surprise, this compound has no activity at all.

Conclusions

We have prepared several new linear, tricyclic 5-6-5 fused heterocycles and demonstrated that certain members from this series of compounds possess useful inotropic activity. In our studies we found in general the following. (1) The tricyclic fused system of adibendan has some intrinsic cardiotonic activity. (2) Substituents up to the size of unsubstituted six-membered rings, but not larger substituents, directly bonded to the tricyclic system are well tolerated in region A (Chart I). These aromatic rings must have a hydrogen-bond-acceptor site for the compounds to be active. The exact location of this site seems to be of minor importance. (3) In region C sterically undemanding, lipophilic substituents are advantageous at the 3-position.

Two methyl groups are necessary for oral activity, but not if the compounds are administered iv. Methylation of the nitrogen atom decreases the activity. (4) In region B the imidazole may be replaced by a pyrrol ring for iv activity, but oral activity is lost. At least one free NH group is necessary in this region.

Experimental Section

Chemistry. Prior to analysis, all compounds were dried at 80 °C at 20 mmHg overnight. Some compounds still contained solvent after this procedure. The amount of solvent retained was determined by ^{1}H NMR spectroscopy (organic solvents), differential thermogravimetric analysis, and by Karl Fischer titration (water).

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Identity of all compounds was confirmed by ^{1}H NMR (300 MHz, Varian XL-300, solvent: Me_2SO-d_6, TMS = 0 ppm), mass spectra (Finnigan MAT 312, data system SS300), and microanalytical data, which were provided by the Analytical Department of the Boehringer Mannheim Research Laboratories. All reactions were followed by TLC on Merck F 254 silica gel plates. Baker silica gel (30–60 μm) was used for column chromatography.

The following compounds were prepared according to literature procedures: 1,3-dihydro-3,3-dimethyl-6-nitro-2H-indol-2-one,[1] 5,6-diamino-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one,[1] 5,6-diamino-3*H*-benzoxazol-2-one,[25] 2-methoxy-4-(methylthio)benzaldehyde,[35] 5,6-diamino-3*H*-benzothiazol-2-one,[25] 6-amino-1,3-dihydro-2*H*-indol-2-one,[27] 5,6-diamino-2,3-dihydrobenzimidazol-2-one,[24] 2,4,5-triaminonitrobenzene,[36] 5-methoxy-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one,[29] 1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one,[28] and 5,6-diamino-1,3-dihydro-1-methyl-2*H*-benzimidazol-2-one.[24]

For **methods A–F**, see ref 1.

Method G: 5,7-Dihydro-7,7-dimethylpyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (2). 5,6-Diamino-1,3-dihydro-3,3-dimethyl-(2H)-indol-2-one (288 mg, 1.51 mmol) and formic acid (100 mg, 1.95 mmol) were heated at reflux for 2 h. After trituration with ether (1 mL), the precipitate was filtered and washed with ether (2 × 0.5 mL) to yield 277 mg (90%) of **2**: mp 278–280 °C; ^{1}H NMR δ 1.39 (s, 6 H, 2 CH_3), 6.95 (s, 1 H, aromatic proton), 7.45 (s, 1 H, aromatic proton), 8.05 (s, 1 H, imidazole CH), 10.21 (s, 1 H, lactam NH); MS, *m/e* 201 (M^+). Anal. ($C_{11}H_{11}N_3O$) C, H, N.

Method H: 5,7-Dihydro-7,7-dimethyl-2-[2-methoxy-4-(methylsulfonyl)phenyl]pyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (16). (a) 2-Methoxy-4-(methylthio)benzaldehyde (30.0 g, 165 mmol) and $NaHSO_3$ (150 g, 1.4 mol) in water (300 mL) were stirred at 70–80 °C for 3 h. After cooling to room temperature, the precipitate was collected and washed with ether to yield 46.2 g (98%) of the bisulfite adduct: mp 114–117 °C.

(b) The unpurified bisulfite adduct of 2-methoxy-4-(methylthio)benzaldehyde (46.0 g, 161 mmol) was added in portions to 5,6-diamino-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (30.0 g, 159 mmol) in 500 mL of ethanol. After 3 h at room temperature, 500 mL of water were added, and the precipitate was filtered, washed with ethanol and water, and recrystallized from ethyl acetate to yield 45.5 g (81%) of 5,7-dihydro-7,7-dimethyl-2-[4-(methylthio)-2-methoxyphenyl]pyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one: mp 293–295 °C; MS *m/e* 353 (M^+).

(c) Two hundred milligrams (0.56 mmol) of this compound and 0.4 mL of H_2O_2 in 4 mL of acetic acid were stirred at room temperature for 48 h. Ten milliliters water was added, the solvent was evaporated in vacuo, and the residue was recrystallized from ethanol to yield 160 mg (75%) of pure product: mp 235–237 °C; ^{1}H NMR δ 1.32 (s, 6 H, 2 CH_3), 3.29 (s, 3 H, SCH_3), 4.14 (s, 3 H, OCH_3), 7.09 (s, 1 H, tyricycl aromatic proton), 7.55 (s, 1 H, tricycl aromatic proton), 7.64 (dd, 1 H, phenyl 5-H), 7.66 (d, 1 H, phenyl 3-H), 8.51 (d, 1 H, phenyl 6-H); MS *m/e* 385 (M^+). Anal. ($C_{19}H_{19}N_3O_4S$) C, H, N, S.

(35) Martani, A.; Roussel, O. *Ann. Chim. (Rome)* **1967**, *57*, 121.
(36) Boyer, J. H.; Buriks, R. S. *Organic Syntheses*; J. Wiley and Sons: New York, 1973; Collect. Vol. V, p 1067.

5,7-Dihydro-7,7-dimethyl-2-[2-methoxy-4-(methylsulfenyl)phenyl]pyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (17) was prepared according to the procedure described for compound 16 with half the amount of H_2O_2 in the oxidation step c: yield 41% after column chromatography (dichloromethane/methanol saturated with ammonia = 15:1); mp 217–220 °C; ^{1}H NMR δ 1.32 (s, 6 H, 2 CH_3), 2.82 (s, 3 H, SCH_3), 4.10 (s, 3 H, OCH_3), 7.07 (s, 1 H, tricycl aromatic proton), 7.54 (s, 1 H, tricycl aromatic proton), 7.63 (dd, 1 H, phenyl 5-H), 7.60 (d, 1 H, phenyl 3-H), 8.49 (d, 1 H, phenyl 6-H); MS, *m/e* 369 (M^+). Anal. ($C_{19}H_{19}N_3O_3S$) C, H, N, S.

Method I: 5,7-Dihydro-7,7-dimethyl-2-hydroxypyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (31). A stream of phosgene was passed through a solution of 5,6-diamino-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (4.00 g, 21.0 mmol) in 60 mL of 2 N HCl for 1 h at room temperature. Nitrogen was passed through the solution for 15 min, and the precipitate was collected and recrystallized from methanol to yield 2.80 g (62%) of pure product: mp >300 °C; ^{1}H NMR δ 1.23 (s, 6 H, 2 CH_3), 6.45 (s, 1 H, aromatic proton), 6.81 (s, 1 H, aromatic proton), 9.92 (s, 1 H, NH), 10.25 (s, 1 H, NH), 10.27 (s, 1 H, NH); MS, *m/e* 217 (M^+). Anal. ($C_{11}H_{11}N_3O_2$) C, H, N.

Method K: 5,7-Dihydro-7,7-dimethyl-2-mercaptopyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (32). 5,6-Diamino-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (12.0 g, 63.0 mmol) was treated with 25 mL of thiophosgene for 2 h at room temperature. The mixture was purified by column chromatography (dichloromethane/methanol = 7:3). After evaporation of the solvent, the residue was recrystallized from methanol to yield 2.75 g (19%) of pure product: mp >300 °C; ^{1}H NMR δ 1.26 (s, 6 H, 2 CH_3), 6.64 (s, 1 H, aromatic proton), 7.06 (s, 1 H, aromatic proton), 10.20 (s, 1 H, OCNH), 12.26 (br, NH or SH), 12.38 (br, 1 H, NH or SH); MS, *m/e* 233 (M^+). Anal. ($C_{11}H_{11}N_3OS$), C, H, N, S.

Method L: 2-Amino-5,7-dihydro-7,7-dimethylpyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (33). A suspension of 5,6-diamino-1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one (3.00 g, 16.0 mmol) and cyanogen bromide (1.82 g, 17.0 mmol) in 100 mL of ethanol was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, and the residue was dissolved in ethanol and treated with charcoal. After filtration, acetone was added and the precipitate was filtered and recrystallized from acetone/ethanol 95:5 to yield the pure compound as hydrobromide: mp 300–305 °C; ^{1}H NMR δ 1.28 (s, 6 H, 2 CH_3), 6.92 (s, 1 H), 7.29 (s, 1 H, 2 phenyl H), 8.21 (br, 2 H, NH_2), 10.32 (s, 1 H, OCNH); MS, *m/e* 216 (M^+). Anal. ($C_{11}H_{12}N_4O$·HBr) C, H, N, halogen.

3,5-Dihydro-6-(4-pyridinyl)-2*H*-imidazo[4,5-*f*]benzoxazol-2-one (36). 5,6-Diamino-2,3-dihydrobenzoxazol-2-one (2.80 g, 17.2 mmol), triethylamine (7.2 mL, 51.6 mmol), and 4-pyridinecarbonyl chloride (HCl salt, 4.60 g, 25.8 mmol) in dichloromethane were stirred for 15 min at room temperature. The solvent was removed in vacuo, the residue was triturated with water, and the precipitate was collected and refluxed in ethanol (200 mL) and concentrated hydrochloric acid (25 mL) for 48 h. The solvent was removed in vacuo, the residue triturated with ammonia/water, and the precipitate recrystallized from dimethylformamide to yield 2.21 g (51%) of the pure compound: mp >360 °C; ^{1}H NMR δ 7.1–7.4 (br, 1 H, aromatic proton), 7.5–7.7 (br, 1 H, aromatic proton), 8.05 and 8.74 (m, 4 H, AA'XX' of pyridinyl H). After addition of acetic acid-d_4: 7.27 (s, 1 H, aromatic proton), 7.54 (s, 1 H, aromatic proton), 8.11 and 8.6–8.9 (br, 4 H, pyridinyl H); MS *m/e* 324, 396 (M^+) after silylation. Anal. ($C_{13}H_8N_4O$) C, H, N.

3,5-Dihydro-6-(4-pyridinyl)benzo[1,2-*d*:4,5-*d*']diimidazol-2(1*H*)-one (38). 2,4,5-Triaminonitrobenzene (10.0 g, 59.0 mmol), 4-pyridinecarbonyl chloride hydrochloride (11.6 g, 65.0 mmol), and 20 mL of triethylamine in 500 mL of dichloromethane were stirred for 5 h at room temperature. The solvent was evaporated in vacuo. The residue was refluxed for 36 h in a mixture of 500 mL of ethanol and 80 mL of concentrated HCl. The solvent was evaporated in vacuo, and the residue was treated with 2 N ammonia. The precipitate was filtered. This provided 12.0 g (80%) of 5-amino-6-nitro-2-(4-pyridinyl)-benzimidazole. A 5.0-g sample of this material was, without further purification, dissolved in 300 mL of methanol and hydrogenated at ambient temperature and pressure with 0.4 g of 10% Pd/C until the theoretical amount of hydrogen had been consumed. Filtration and evaporation of

the solvent provided 3.0 g of 5,6-diamino-2-(4-pyridinyl)benzimidazole, which dissolved in 120 mL of 2 N HCl and a stream of phosgene passed through the solution. The precipitate was filtered, washed with 2 N ammonia, and recrystallized from methanol/dichloromethane (4:1 v/v) to yield 720 mg (22%) of pure product: mp >300 °C; ^{1}H NMR δ 7.00 (s, 2 H, phenyl H), 7.95 (m, 2 H), 8.65 (m, 2 H, AA'XX' of pyridinyl H), 10.35 (s, 2 H, NH); MS m/e 251 (M^+). Anal. ($C_{13}H_9N_5O$) C, H, N.

3,5-Dihydro-1-methyl-6-(4-pyridazinyl)benzo[1,2-*d*:4,5-*d*']diimidazol-2(1*H*)-one (40). 2,3-Dihydro-5,6-dinitro-1-methylbenzimidazol-2-one (2.20 g, 9.20 mmol) in 730 mL of methanol was hydrogenated in the presence of 0.44 g of PtO_2 at 60 °C and 100 bar (1400 psi) pressure for 3 h. The solution was filtered and the solvent was removed in vacuo. 4-Pyridazinecarboxylic acid (1.20 g, 10.0 mmol) and polyphosphoric acid (20 g) were added to the residue and heated to 150 °C for 1 h. The mixture was poured into ice/water and neutralized with concentrated ammonia. The precipitate was collected and recrystallized from methanol/dichloromethane to yield 0.34 g (14%) of pure product: mp >300 °C; ^{1}H NMR δ 3.35 (s, 3 H, NCH_3), 7.18 (s, 1 H), 7.27 (s, 1 H, 2 phenyl H), 8.18 (m, 1 H), 9.35 (m, 1 H), 9.85 (m, 1 H, 3 pyridazinyl H), 10.77 (s, 1 H, NH); MS m/e 266 (M^+). Anal. ($C_{13}H_{10}N_6O$) C, H, N.

3,5-Dihydro-6-hydroxy-1-methylbenzo[1,2-*d*:4,5-*d*']diimidazol-2(1*H*)-one (44) was prepared from 5,6-diamino-2,3-dihydro-1-methylbenzimidazol-2-one and phosgene according to the procedure described for compound **31**. After recrystallization from pyridine, 19% of the pure compound was obtained: mp >300 °C; ^{1}H NMR δ 3.29 (s, 6 H, 2 CH_3), 6.58 (s, 1 H), 6.66 (s, 1 H, 2 phenyl H), 10.25 (s, 1 H), 10.38 (s, 1 H), 10.45 (s, 1 H, 3 NH); MS, m/e 204 (M^+). Anal. ($C_9H_8N_4O_2$) C, H, N.

3,7-Dihydro-3,3-dimethyl-6-(4-pyridinyl)benzo[1,2-*b*:5,4-*b*']dipyrrol-2(1*H*)-one (47). $NaNO_2$ (2.80 g, 40.6 mmol) dissolved in 25 mL of H_2O was added dropwise to 6-amino-1,3-dihydro-3,3-dimethyl-2*H*-indolin-2-one (6.80 g, 38.0 mmol, dissolved in 125 mL of 50% H_2SO_4) at 5 °C. The mixture was stirred for 15 min at 5 °C, and 0.5 g of urea was added and stirred for an additional 15 min. $SnCl_2$ (dihydrate, 26.4 g, 117 mmol) dissolved in 20 mL of concentrated HCl was added dropwise, while the temperature was maintained at 5 °C for 2 h. 4-Acetylpyridine (6.90 g, 57.0 mmol) was added, and the mixture was stirred for 2 h at room temperature. The precipitate was filtered, suspended in water, and neutralized with 2 N ammonia. The precipitate was collected and provided 8.5 g (76%) of 4-acetylpyridine (1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-indol-6-yl)hydrazone, mp 272–274 °C. This hydrazone in 500 mL of polyphosphoric acid was stirred for 6 h at 110–120 °C. The mixture was poured on ice and neutralized with concentrated ammonia, and the precipitate was recrystallized from methanol/dichloromethane to yield 5.18 g (66%) of pure product: mp >300 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 6.88 (s, 1 H), 7.43 (s, 1 H, 2 phenyl H), 7.09 (m, 1 H, indole CH), 7.73 (m, 2 H), 8.55 (m, 2 H, AA'XX' of pyridinyl H), 10.21 (s, 1 H, OCNH), 11.55 (br, 1 H, indole NH); MS m/e 277 (M^+). Anal. ($C_{17}H_{15}N_3O$) C, H, N.

5,7-Dihydro-3,3-dimethylbenzo[1,2-*b*:5,4-*b*']dipyrrole-2,6-(1*H*,3*H*)-dione (49). 2-Bromo-2-methylpropanoyl bromide (6.80 g, 29 mmol) was added dropwise to a cooled (5 °C) solution of 6-amino-1,3-dihydro-2*H*-indol-2-one hydrochloride (5.00 g, 27.0 mmol) and triethylamine (6.45 g, 63.0 mmol) in 80 mL of dichloromethane. The solution was stirred for an additional 3 h at room temperature, the solvent was evaporated in vacuo, and the residue was washed with water to yield 2-bromo-*N*-(2-oxoindolin-6-yl)-2-methylpropanamide (4.20 g, 52%), mp. 211–212 °C. A 3.2-g sample of this compound (10.8 mmol) was mixed with $AlCl_3$ (7.1 g) and stirred for 5 h at 160 °C. Water and 2 N HCl were added with cooling, and the precipitate was filtered and purified by column chromatography (dichloromethane/methanol 96:4). Evaporation of the solvent and recrystallization from 2-propanol yielded 270 mg (11%) of the pure compound, which contained 0.5 mol of 2-propanol and 0.75 mol of water per 1 mol of compound: mp 280–282 °C; ^{1}H NMR δ 1.21 (s, 6 H, 2 CH_3), 3.37 (s, 2 H, CH_2), 10.12 (s, 1 H), 10.19 (s, 1 H, 2 NH); MS m/e 216 (M^+). Anal. ($C_{12}H_{12}N_2O_2 \cdot 0.5$ 2-propanol $\cdot 0.75H_2O$) C, H, N.

7,7-Dimethyl-5,7-dihydropyrrolo[2,3-*f*]benzotriazol-6-(1*H*)-one (50). $NaNO_2$ (240 mg, 3.50 mmol) was added portionwise to a suspension of 1,3-dihydro-5,6-diamino-3,3-dimethyl-2*H*-indol-2-one (570 mg, 3.00 mmol) in 6 N hydrochloric acid at 0–5 °C. The red solution was heated to 50–60 °C for 5 min and cooled to room temperature. Filtration of the red crystalline precipitate yielded 380 mg (66%) of the pure compound: mp 288–291 °C; ^{1}H NMR δ 1.39 (s, 6 H, 2 CH_3), 7.05 (s, 1 H, aromatic proton), 7.85 (s, 1 H, aromatic proton), 10.55 (s, 1 H, lactam NH); MS m/e 202 (M^+). Anal. ($C_{10}H_{10}N_4O$) C, H, N.

5,7-Dihydro-2-(4-pyridinyl)-3,7,7-trimethylpyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (51). (a) K_2CO_3 (36.5 g, 264 mmol), followed by benzyl chloride (30 mL, 264 mmol), was added portionwise at room temperature to 1,3-dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one (49.5 g, 240 mmol) in DMF (400 mL). The mixture was stirred for 2 h at 70 °C, water (2 L) was added, and the mixture was extracted with dichloromethane. The organic layer was separated and extracted with water. The organic layer was dried ($MgSO_4$) and evaporated in vacuo, and the residue was triturated with ether to yield 64.4 g (91%) 1-benzyl-1,3-dihydro-3,3-dimethyl-6-nitro(2*H*-indol-2-one: mp 132 °C; ^{1}H NMR δ 1.41 (s, 6 H, 2 CH_3), 5.03 (s, 2 H, CH_2), 7.2–7.4 (m, 5 H, phenyl H), 7.66 (d, 1 H, indole 4-H), 7.69 (d, 1 H, indole 7-H), 7.95 (dd, 1 H, indole 5-H); MS m/e 296 (M^+). Anal. ($C_{17}H_{16}N_2O_3$) C, H, N.

(b) 1-Benzyl-1,3-dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one (64.4 g, 217 mmol) was hydrogenated at ambient temperature and pressure in methanol (600 mL) in the presence of 5 g of 10% Pd/C for 2 h. The solvent was removed after filtration, and the residue (57.8 g of a brown oil) was dissolved in formic acid (200 mL). The formic acid was removed in vacuo, and the residue was heated in toluene (200 mL) in a Dean–Stark trap for 4 h. The solvent was removed in vacuo to yield *N*-(1-benzyl-1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide (68.7 g as a brown oil), 67.0 g (228 mmol) of which in DMSO (200 mL) was added dropwise to sodium hydride (11.9 g, 273 mmol, of a 60% suspension in mineral oil) in DMSO (200 mL), followed after 30 min by a dropwise addition of iodomethane (17.0 mL, 273 mmol). The mixture was poured on ice and extracted with dichloromethane, the solvent was evaporated in vacuo, and the residue was filtered through silica gel (1000 mL, eluent dichloromethane) to yield 56.9 g (69%) of *N*-methyl-*N*-(1-benzyl-1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide: mp 92–93 °C; ^{1}H NMR δ 1.35 (s, 6 H, 2 CH_3), 3.25 (s, 3 H, NCH_3), 4.80 (s, 2 H, CH_2), 6.48 (d, J = 2 Hz, 1 H, oxindole proton, 7-H), 6.65 (dd, 1 H, oxindole proton, 5-H), 7.25 (d, J = 8 Hz, 1 H, oxindole proton, 4-H), 7.30 (s, 5 H, benzyl protons), 8.35 (s, 1 H, CHO); MS m/e 308 (M^+). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

(c) The substance obtained this way (44.4 g, 144 mmol) was added portionwise to sodium (9.90 g, 432 mmol) in liquid ammonia (400 mL). After an additional 30 min, 2 N hydrochloric acid (270 mL) was added, while the ammonia evaporated. Water (300 mL) was added, and the precipitate was collected and washed with toluene and ether to yield 22.1 g (81%) of 1,3-dihydro-3,3-dimethyl-6-(methylamino)-2*H*-indol-2-one: mp 212–213 °C; ^{1}H NMR δ 1.20 (s, 2.60 (d, J = 5 Hz, 3 H, NCH_3), 5.50 (q, J = 5 Hz, 1 H, amine proton), 6.05 (m, 2 H, aromatic protons 5-H, 7-H), 6.90 (d, J = 8 Hz, 1 H, aromatic proton, 4-H), 9.95 (br, 1 H, amide proton); MS m/e 190 (M^+). Anal. ($C_{11}H_{14}N_2O$) C, H, N.

(d) This compound (22.1 g, 116 mmol) was dissolved in 100 mL of formic acid, the solvent was removed in vacuo, and the residue was heated in 100 mL of toluene for 4 h while water was removed by a Dean–Stark trap. The precipitate was collected to yield 19.9 g (79%) of *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide: mp 222–223 °C; ^{1}H NMR δ 1.25 (s, 6 H, 2 CH_3), 3.30 (s, 3 H, NCH_3), 6.75 (d, J. = 2 Hz, 1 H, aromatic proton (7-H), 6.80 (dd, 1 H, aromatic proton 5-H), 7.30 (d, J = 8 Hz, 1 H, aromatic proton 4-H), 8.45 (s, 1 H, CHO), 10.40 (br, 1 H, amide proton); MS m/e 218 (M^+). Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

(e) Nitric acid (100%; 3.8 mL, 90 mmol) was added dropwise at 0 °C to this compound (17.9 g, 82.0 mmol) in 170 mL of acetic anhydride. The solution was stirred for an additional 20 min at room temperature and poured onto ice. The precipitate was collected to yield 9.7 g (45%) of *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-5-nitro-2-oxo-2*H*-6-indolyl)-formamide: mp 195–198 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 3.10 and 3.30 (2:1, 2 s, 3 H, NCH_3), 6.90 and 6.95 (1:2, 2 s, 1 H, aromatic proton 7-H), 8.00 and 8.20 (2:1, 2 s, 1 H, aromatic proton 4-H), 8.15 (s, 1 H, CHO); some

signals are split 2:1 due to hindered rotation around the amide bond; MS m/e 263 (M^+). Anal. ($C_{12}H_{13}N_3O_4$) C, H, N.

(f) This compound (9.70 g, 37.0 mmol) was heated in 100 mL of ethanol and 10 mL of concentrated hydrochloric acid for 90 min. The compound dissolved and a new product precipitated, which was collected to yield 7.80 g (90%) of 1,3-dihydro-3,3-dimethyl-6-(methylamino)-5-nitro-2*H*-indol-2-one: mp 312–313 °C; ^{1}H NMR δ 1.27 (s, 6 H, 2 CH_3), 2.95 (d, 3 H, NCH_3, $J(HNCH_3)$ = 6.5 Hz), 6.30 (s, 1 H, aromatic proton 7-H), 7.95 (s, 1 H, aromatic proton 4-H), 8.55 (q, 1 H, amine proton), 10.75 (br, 1 H, amide proton); MS m/e 235 (M^+). Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

(g) This product (3.90 g, 16.6 mmol) was hydrogenated at ambient temperature and pressure in 100 mL of methanol in the presence of 0.4 g of 10% Pd/C for 4 h. After filtration and evaporation of the filtrate, the residue (5-amino-1,3-dihydro-3,3-dimethyl-6-methylamino-2*H*-indol-2-one (3.40 g, 16.6 mmol)), 4-pyridinecarbonyl chloride (HCl salt, 4.5 g, 25.0 mmol), and 6.9 mL of triethylamine in 60 mL of dichloromethane were stirred at room temperature for 20 min and extracted with water, the organic layer was separated, dried over $MgSO_4$, and filtered, and the sovlent was evaporated in vacuo. The residue (4.50 g) was heated in 150 mL of ethanol and 30 mL of concentrated hydrochloric acid for 24 h. The solvent was evaporated in vacuo, the residue was triturated with ammonia/water, and the precipitate was filtered and purified by column chromatography (1300 mL of silica gel; dichloromethane/methanol saturated with ammonia = 95:5). Appropriate fractions were combined, the solvent was evaporated, and the residue was recrystallized from ethanol to yield 2.81 g (58%) of the pure compound: mp 342–344 °C; ^{1}H NMR δ 1.26 (s, 6 H, 2 CH_3), 3.93 (s, 3 H, NCH_3), 6.95 (s, 1 H, aromatic proton), 7.55 (s, 1 H, aromatic proton), 7.75 and 8.70 (m, 4H, AA'XX' of pyridinyl protons), 10.32 (s, 1 H, amide proton); MS m/e 292 (M^+). Anal. ($C_{17}H_{16}N_4O$) C, H, N.

5,7-Dihydro-2-(4-pyridinyl)-5,7,7-trimethylpyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one (52). (a) Nitric acid (65%; 18 mL, 260 mmol) was added dropwise to 1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one (41.3 g, 236 mmol) in acetic acid (400 mL) at room temperature. After 24 h, the precipitate was filtered, washed with acetic acid, and recrystallized from ethanol to yield 31.8 g (60%) 1,3-dihydro-5-nitro-1,3,3-trimethyl-2*H*-indol-2-one: mp 202–203 °C; ^{1}H NMR δ 1.45 (s, 6 H, 2 CH_3), 3.25 (s, 3 H, NCH_3), 6.5 (d, J = 8 Hz, 1 H, aromatic proton 7-H), 8.15 (dd, 1 H, aromatic proton 4-H), 8.40 (d, J = 2 Hz, 1 H, aromatic proton 6-H); MS m/e 220 (M^+). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

(b) This compound (31.8 g, 144 mmol) was hydrogenated as described for compound **51**, step (b). The resulting amine was treated with acetic anhydride (20.3 mL, 216 mmol) in ethanol (400 mL) at room temperature for 30 min. The solvent was evaporated and the residual red oil purified by column chromatography (1200 mL of silica gel, eluent ethyl acetate) to yield 20.1 g (60%) of N-(1,3-dihydro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)-acetamide: mp 156–158 °C.

(c) Nitric acid (65%; 6.6 mL, 95 mmol) was added dropwise to this substance (20.0 g, 86.1 mmol) in acetic acid (200 mL) at 0–5 °C. After stirring for an additional 15 min at room temperature, the mixture was poured onto ice and filtered to yield 21.7 g (91%) of N-(1,3-dihydro-6-nitro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)acetamide as yellow crystals: mp 211–213 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 2.25 (s, 3 H, CH_3CO), 3.20 (s, 3 H, NCH_3), 7.55 (s, 1 H, aromatic proton), 8.60 (s, 1 H, aromatic proton), 10.30 (br, 1 H, NH); MS m/e 277 (M^+). Anal. ($C_{13}H_{15}N_3O_4$) C, H, N.

(d) This compound (21.7 g, 78.3 mmol) in ethanol (100 mL) and concentrated NaOH (10 mL) was refluxed for 1 h, the solvent was removed, and the residue was triturated with water and filtered to yield 17.8 g (97%) of 5-amino-1,3-dihydro-6-nitro-1,3,3-trimethyl-2*H*-indol-2-one as red crystals: mp 288–290 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 3.10 (s, 3 H, NCH_3), 6.95 (s, 1 H, aromatic proton), 7.45 (s, 1 H, aromatic proton), 7.50 (br, 2 H, NH_2).

(e) 4-Pyridinecarbonyl chloride (HCl salt, 5.40 g, 30.0 mmol) was added portionwise to this compound (5.90 g, 25.0 mmol) in pyridine (50 mL), stirred for an additional 2 h at room temperature, and then poured onto ice and filtered to yield 7.71 g (91%) of *N*-(1,3-dihydro-6-nitro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)-4-pyridinecarboxamide: mp 272–275 °C.

(f) This compound (7.7 g, 22.6 mmol), trimethylamine (3 mL), and 10% Pd/C (0.7 g) in methanol (200 mL) were hydrogenated at ambient temperature and pressure and then treated with boiling hydrochloric acid in ethanol as described for compound **51**, step (g), to yield 2.80 g (36%) of the pure compound after recrystallization from ethyl acetate: mp 252–253 °C; ^{1}H NMR δ 1.31 (s, 6 H, 2 CH_3), 3.20 (s, 3 H, NCH_3), 7.05 (s, 1 H, aromatic proton), 7.55 (s, 1 H, aromatic proton), 8.00 and 8.67 (m, 4 H, AA'XX' system of pyridinyl protons); MS m/e 292 (M^+). Anal. ($C_{17}H_{16}N_4O$) C, H, N.

5,7-Dihydro-2-(4-pyridinyl)-3,5,7,7-tetramethylpyrrolo-[2,3-*f*]benzimidazol-6(1*H*)-one (53). (a) 1,3-Dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one (49.5 g, 240 mmol) was methylated as described for compound **51**, step (b), to yield 45.2 (85%) of 1,3-dihydro-6-nitro-1,3,3-trimethyl-2*H*-indol-2-one: mp 172–173 °C; ^{1}H NMR δ 1.35 (s, 6 H, 2 CH_3), 3.25 (s, 3 H, NCH_3), 7.25 (d, J = 8 Hz, 1 H, aromatic proton 4-H), 7.45 (d, J = 2 Hz, 1 H, aromatic proton 7-H), 7.90 (dd, 1 H, aromatic proton 5-H); MS m/e 220 (M^+). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

(b) This compound (45.2 g, 205 mmol) was reduced as described for compound **51**, step (g), to yield 38.6 g (99%) 6-amino-1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one: mp 176–178 °C; ^{1}H NMR δ 1.18 (s, 6 H, 2 CH_3), 3.04 (s, 3 H, NCH_3), 4.98–5.08 (br, 2 H, NH_2), 6.22 (m, 2 H, oxindole 5-H and 7-H), 6.80 (d, 1 H, oxindole 4-H); MS m/e 190 (M^+). Anal. ($C_{11}H_{14}N_2O$) C, H, N.

(c) This compound (38.6 g, 203 mmol) was formylated as described for compound **51**, step (d), to yield 44.0 g (99%) *N*-(1,3-dihydro-2-oxo-1,3,3-trimethyl-2*H*-6-indolyl)formamide: mp 163–165 °C.

(d) This compound (43.0 g, 197 mmol) was methylated as described for compound **51**, step (b), to yield 37.1 g (81%) *N*-methyl-*N*-(1,3-dihydro-2-oxo-1,3,3-trimethyl-2*H*-6-indolyl)formamide: mp 90–92 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 3.15 (s, 3 H, NCH_3), 3.25 (s, 3 H, NCH_3), 6.60 (d, J = 2 Hz, 1 H, aromatic proton 7-H), 6.75 (dd, 1 H, aromatic proton 5-H), 7.15 (d, J = 8 Hz, 1 H, aromatic proton 4-H), 8.43 (s, 1 H, CHO); MS m/e 232 (M^+). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

(e) Nitric acid (96%; 7.4 mL, 176 mmol) in 80% sulfuric acid (100 mL) was added dropwise to this compound (37.1 g, 160 mmol) in 80% sulfuric acid (500 mL) at 0–5 °C. The solution was stirred for an additional 30 min at 5 °C and then poured onto ice, and the precipitate was filtered to yield *N*-methyl-*N*-(1,3-dihydro-2-oxo-5-nitro-1,3,3-trimethyl(2*H*-6-indolyl)formamide (30.1 g, 68%): mp 173–176 °C; ^{1}H NMR (all signals are split 2:1 due to hindered rotation of the methylformylamino fragment) δ 1.32, 1.33 (1:2, s, 6 H, 2 CH_3), 3.12, 3.40 (2:1, s, 3 H, formylamino CH_3), 2.17, 2.18 (1:2, s, 3 H, pyrrolyl NCH_3), 7.20, 7.32 (1:2, s, 1 H, aromatic proton 7-H), 8.06, 8.17 (2:1, s, 1 H, aromatic proton 4-H), 8.14, 8.18 (1:2, s, 1 H, CHO).

(f) This compound (30.0 g, 108 mmol) was treated with hydrochloric acid in ethanol as described for compound **51**, step (f), to yield 24.0 g (89%) of 1,3-dihydro-3,3-dimethyl-6-(methylamino)-5-nitro-2*H*-indol-2-one: mp 255–256 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), 3.10 (d, J = 5 Hz, 3 H, CH_3NH), 3.20 (s, 3 H, CH_3NCO), 6.05 (s, 1 H, aromatic proton), 7.95 (s, 1 H, aromatic proton), 8.60 (q, br, 1 H, NH).

(g) This compound (6.20 g, 25.0 mmol) was reduced and treated with isonicotinoyl chloride as described for compound **51**, step (g), and the product recrystallized from ethanol to yield the pure title compound (3.60 g, 48%): mp 223–224 °C; ^{1}H NMR δ 1.32 (s, 6 H, 2 CH_3), 3.21 (s, 3 H, $OCNCH_3$), 3.95 (s, 3 H, imidazole CH_3), 7.12 (s, 1H, aromatic proton), 7.62 (s, 1 H, aromatic proton), 7.80 and 8.70 (m, 4 H, AA'XX' of pyridinyl protons); MS m/e 306 (M^+). Anal. ($C_{18}H_{18}N_4O$) C, H, N.

5,7-Dihydro-1,7,7-trimethylpyrrolo[2,3-*f*]benzimidazole-2,6(1*H*,3*H*)-dione (54). (a) 1,3-Dihydro-3,3-dimethyl-5-nitro-2*H*-indol-2-one (82.5 g, 400 mmol) was reacted with benzyl chloride (60 mL, 520 mmol) in DMF (670 mL) in the presence of K_2CO_3 (71.9 g, 520 mmol), as described for compound **51**, step (a), to yield 1-benzyl-1,3-dihydro-3,3-dimethyl-5-nitro-2*H*-indol-2-one (118 g, 99%) as an amber oil: ^{1}H NMR δ 1.42 (s, 6 H, 2 CH_3), 4.99 (s, 2 H, CH_2), 7.13 (d, 1 H, oxindole 7-H), 7.2–7.4 (m, 5 H, phenyl H), 8.15 (dd, 1 H, oxindole 6-H), 8.32 (d, 1 H, oxindole 4-H).

(b) This compound was hydrogenated, formylated, and methylated, as described for compound **51**, step (b), to yield 72.0

g (58%) of *N*-methyl-*N*-(1-benzyl-1,3-dihydro-2-oxo-3,3-dimethyl-2*H*-5-indolyl)formamide: mp 130–132 °C; ^{1}H NMR δ 1.45 (s, 6 H, 2 CH_3), 3.25 (s, 3 H, NCH_3), 4.95 (s, 2 H, CH_2), 6.60 (dd, 1 H, aromatic proton 6-H), 6.80 (dd, 1 H, aromatic proton 4-H), 6.90 (d, 1 H, aromatic proton 7-H), 7.25 (s, 5 H, benzyl protons), 8.35 (s, 1 H, CHO); MS *m/e* 308 (M^+). Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

(c) This compound (36.0 g, 117 mmol) was deformylated and debenzylated as described for compound **51**, step (c), to yield 15.2 g (68%) of 1,3-dihydro-3,3-dimethyl-5-(methylamino)-2*H*-indol-2-one: mp 159–163 °C.

(d) This compound (30.4 g, 160 mmol) was formylated as described for compound **51**, step (d), to yield 29.2 g (84%) of *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-5-indolyl)formamide: mp 170–172 °C.

(e) This compound (14.5 g, 66.4 mmol) was nitrated as described for compound 51, step (e), to yield 18.6 g (83%) *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-6-nitro-2-oxo-2*H*-5-indolyl)formamide: mp 212–216 °C; ^{1}H NMR δ 1.30 (s, 6 H, 2 CH_3), the following signals are split 2:3 due to hindered rotation of the formylamino fragment: 3.05 and 3.40 (2:3, s, 3 H, formylamino NCH_3), 7.40 and 7.49 (2:3, s, 1 H, aromatic proton), 7.55 and 7.65 (2:3, s, 1 H, aromatic proton), 8.05 and 8.15 (3:2, s, 1 H, CHO), 10.80 (br, 1 H, pyrrolyl NH); MS *m/e* 263 (M^+).

(f) This compound (9.50 g, 36.0 mmol) was treated with NaOH in ethanol, as described for compound **52**, step (d), to yield 7.11 g (83%) 1,3-dihydro-3,3-dimethyl-5-(methylamino)-6-nitro-2*H*-indol-2-one: mp 283–285 °C; ^{1}H NMR δ 1.31 (s, 6 H, 2 CH_3), 3.01 (s, 3 H, NCH_3), 7.08 (s, 1 H, oxindole 4-H or 7-H), 7.39 (s, 1 H, oxindole 4-H or 7-H), 8.25–8.35 (br, 1 H, NH), 10.23 (br, 1 H, lactame NH); MS *m/e* 235 (M^+). Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

(g) This compound (7.10 g, 30.0 mmol) was reduced as described for compound **51**, step (b), to yield 6.1 g (99%) 6-amino-1,3-dihydro-3,3-dimethyl-5-(methylamino)-2*H*-indol-2-one: mp 205–208 °C; MS *m/e* 205 (M^+).

(h) This compound (6.10 g, 30.0 mmol) and 1,1′-carbonyldiimidazole (9.8 g, 60.0 mmol) in 250 mL of dioxane were refluxed for 2 h. The solvent was evaporated in vacuo, and the residue was heated in 20% hydrochloric acid for an additional 2 h. The solution was neutralized with concentrated ammonia, and the precipitate was filtered and recrystallized from methanol to yield 2.0 g (29%) of pure compound: mp 364–367 °C; ^{1}H NMR δ 1.26 (s, 6 H, 2 CH_3), 3.26 (s, 3 H, NCH_3), 6.52 (s, 1 H, aromatic proton), 7.06 (s, 1 H, aromatic proton), 10.02 (s, 1 H, NH), 10.60 (s, 1 H, NH); MS *m/e* 231 (M^+). Anal. ($C_{12}H_{13}N_3O_2$) C, H, N.

5,7-Dihydro-7,7-dimethylpyrrolo[2,3-*f*]benzoxazole-2,6-dione (55). (a) 1,3-Dihydro-3,3-dimethyl-5-methoxy-2*H*-indol-2-one was nitrated as described for compound **51**, step (e), and the product treated with boiling 47% hydrobromic acid for 30 min to yield 1,3-dihydro-3,3-dimethyl-5-hydroxy-6-nitro-(2H)-indol-2-one: mp 225–228 °C.

(b) This compound (2.10 g, 9.45 mmol) was hydrogenated at ambient temperature and pressure in a mixture of 50 mL of dioxane and 50 mL of methanol in the presence of 0.4 g of 10% Pd/C at room temperature until 700 mL of hydrogen had been absorbed. The solution was filtered, and the solvent was evaporated in vacuo. The residue (1.80 g) and 1,1′-carbonyldiimidazole (2.50 g, 15.4 mmol) were heated in 50 mL of dioxane for 3 h. The solvent was evaporated in vacuo, and the residue was purified by column chromatography (800 mL of silica gel; dichloromethane/methanol = 15:1). The solvent of the appropriate fractions was evaporated in vacuo, the residue was triturated with dichloromethane, and the precipitate was filtered and recrystallized from 2-butanone to yield 1.05 g (51%) of the pure compound: mp 297–299 °C; ^{1}H NMR δ 1.25 (s, 6 H, 2 CH_3), 6.57 (s, 1 H, aromatic proton), 7.29 (s, 1 H, aromatic proton), 10.17 (s, 1 H, amide NH), 11.38 (s, 1 H, oxazolinone NH); MS *m/e* 218 (M^+). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

Pharmacology: see ref 1.

Registry No. **2**, 109029-65-4; **3**, 109029-66-5; **4**, 109029-71-2; **5**, 109029-68-7; **6**, 120791-10-8; **7**, 109029-67-6; **8**, 120791-11-9; **9**, 109029-70-1; **10**, 104580-81-6; **11**, 104563-89-5; **12**, 104563-78-2; **13**, 104563-84-0; **14**, 120791-12-0; **15**, 120791-13-1; **16**, 120791-14-2; **17**, 120791-15-3; **18**, 120791-16-4; **19**, 104896-32-4; **20**, 104896-35-7; **21**, 120791-17-5; **22**, 120791-18-6; **23**, 120791-19-7; **24**, 104896-40-4; **25**, 120791-20-0; **26**, 104896-37-9; **27**, 104896-45-9; **28**, 116584-59-9; **29**, 116584-57-7; **30**, 116584-58-8; **31**, 109029-72-3; **32**, 109029-75-6; **33**, 120791-21-1; **34**, 120791-22-2; **35**, 120791-23-3; **36**, 107562-20-9; **37**, 120791-24-4; **38**, 107562-19-6; **39**, 107562-18-5; **40**, 120791-25-5; **41**, 120791-26-6; **42**, 120791-27-7; **43**, 120791-28-8; **44**, 120791-29-9; **45**, 107789-02-6; **46**, 107789-00-4; **47**, 107788-95-4; **48**, 107788-99-8; **49**, 120791-30-2; **50**, 115854-48-3; **51**, 120791-31-3; **52**, 116623-01-9; **53**, 120791-32-4; **54**, 120791-33-5; **55**, 120791-34-6; **56a**, 100568-79-4; **56c**, 98279-26-6; **56d**, 120791-35-7; $CH_3CH{=}CHCO_2H$, 3724-65-0; $CH_2{=}C(CH_3)CO_2H$, 79-41-4; *o*-$MeOC_6H_4COCl$, 21615-34-9; *p*-$MeOC_6H_4COCl$, 100-07-2; *p*-HOC_6H_4CHO, 123-08-0; *p*-$Me_2NC_6H_4CHO$, 100-10-7; 2-methoxy-4-(methylthiol)benzaldehyde, 15345-40-1; 2-methoxy-4-(methylthiol)benzaldehyde sodium salt (bisulfite adduct), 120791-72-2; 5,7-dihydro-7,7-dimethyl-2-[4-(methylthio)-2-methoxyphenyl]pyrrolo[2,3-*f*]benzimidazol-6(1*H*)-one, 120791-73-3; 4-pyridinecarbonyl chloride hydrochloride, 39178-35-3; 2,4,5-triaminonitrobenzene, 6635-35-4; 5-amino-6-nitro-2-(4-pyridinyl)benzimidazole, 107562-22-1; 5,6-diamino-2-(4-pyridinyl)benzimidazole, 107562-23-2; 2,3-dihydro-5,6-dinitro-1-methylbenzimidazol-2-one, 120791-42-6; 2,3-dihydro-5,6-dinitro-1-ethyl-2-benzimidazol-2-one, 120791-43-7; 2,3-dihydro-5,6-dinitro-1-propylbenzimidazol-2-one, 120791-44-8; 5,6-diamino-2,3-dihydro-1-methylbenzimidazol-2-one, 115854-53-0; 6-amino-1,3-dihydro-3,3-dimethyl-2*H*-indolin-2-one, 100510-65-4; 4-acetylpyridine, 1122-54-9; 4-acetylpyridine (1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-indol-6-yl)hydrazone, 120791-45-9; 2-bromo-2-methylpropanoyl bromide, 20769-85-1; 6-amino-1,3-dihydro-2*H*-indol-2-one hydrochloride, 101389-22-4; 2-bromo-*N*-(2-oxoindolin-6-yl)-2-methylpropanamide, 120791-46-0; 1,3-dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one, 100510-64-3; 1-benzyl-1,3-dihydro-3,3-dimethyl-6-nitro-2*H*-indol-2-one, 120791-47-1; *N*-(1-benzyl-1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide, 120791-48-2; *N*-methyl-*N*-(1-benzyl-1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide, 120791-49-3; 1,3-dihydro-3,3-dimethyl-6-(methylamino)-2*H*-indol-2-one, 120791-50-6; *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-6-indolyl)formamide, 120791-51-7; *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-5-nitro-2-oxo-2*H*-6-indolyl)formamide, 120791-52-8; 1,3-dihydro-3,3-dimethyl-6-(methylamino)-5-nitro-2*H*-indol-2-one, 120791-53-9; 5-amino-1,3-dihydro-3,3-dimethyl-6-(methylamino)-2*H*-indol-2-one, 120791-54-0; 1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one, 20200-86-6; 1,3-dihydro-5-nitro-1,3,3-trimethyl-2*H*-indol-2-one, 120791-55-1; *N*-(1,3-dihydro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)acetamide, 120791-56-2; *N*-(1,3-dihydro-6-nitro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)acetamide, 120791-57-3; 5-amino-1,3-dihydro-6-nitro-1,3,3-trimethyl-2*H*-indol-2-one, 120791-58-4; *N*-(1,3-dihydro-6-nitro-1,3,3-trimethyl-2-oxo-2*H*-5-indolyl)-4-pyridinecarboxamide, 116623-12-2; 1,3-dihydro-6-nitro-1,3,3-trimethyl-2*H*-indol-2-one, 120791-59-5; 6-amino-1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-one, 120791-60-8; *N*-(1,3-dihydro-2-oxo-1,3,3-trimethyl-2*H*-6-indolyl)formamide, 120791-61-9; *N*-methyl-*N*-(1,3-dihydro-2-one-1,3,3-trimethyl-2*H*-6-indolyl)formamide, 120791-62-0; *N*-methyl-*N*-(1,3-dihydro-2-oxo-5-nitro-1,3,3-trimethyl-2*H*-6-indolyl)formamide, 120791-63-1; 1,3-dihydro-3,3-dimethyl-5-nitro-2*H*-indol-2-one, 100511-00-0; 1-benzyl-1,3-dihydro-3,3-dimethyl-5-nitro-2*H*-indol-2-one, 120791-64-2; *N*-methyl-*N*-(1-benzyl-1,3-dihydro-2-oxo-3,3-dimethyl-2*H*-5-indolyl)formamide, 120791-65-3; 1,3-dihydro-3,3-dimethyl-5-(methylamino)-2*H*-indol-2-one, 120791-66-4; *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-2-oxo-2*H*-5-indolyl)formamide, 120791-67-5; *N*-methyl-*N*-(1,3-dihydro-3,3-dimethyl-6-nitro-2-oxo-2*H*-5-indolyl)formamide, 120791-68-6; 1,3-dihydro-3,3-dimethyl-5-(methylamino)-6-nitro-2*H*-indol-2-one, 120791-69-7; 6-amino-1,3-dihydro-3,3-dimethyl-5-(methylamino)-2*H*-indol-2-one, 120791-70-0; 1,3-dihydro-3,3-dimethyl-5-methoxy-2*H*-indol-2-one, 87234-57-9; 1,3-dihydro-3,3-dimethyl-5-hydroxy-6-nitro-2*H*-indol-2-one, 120791-71-1; 3,3-dimethyl-5-((methylcarbonyl)-amino)-6-nitroindol-2-one, 100510-96-1; 3,3-dimethyl-6-nitro-5-[(1-oxo-2-trifluoroethyl)amino]indol-2-one, 120791-36-8; 3,3-dimethyl-6-nitro-5-[(1-oxobutyl)amino]indol-2-one, 120791-37-9; 3,3-dimethyl-5-[(2-methyl-1-oxopropyl)amino]-6-nitroindol-2-one, 120791-38-0; cyclohexylcarboxylic acid, 98-89-5; 3,3-dimethyl-6-nitro-5-[(phenylcarbonyl)amino]indol-2-one, 120791-39-1; 4-(1-imidazoyl)benzaldehyde, 10040-98-9; 2-pyrrolecarboxaldehyde, 1003-29-8; 3,3-dimethyl-5-[(furan-2-ylcarbonyl)amino]-6-nitroindol-2-one, 120791-40-4; 2-thiophenecarboxaldehyde, 98-03-3; 1*H*-imidazole-4-carbonyl chloride, 56460-32-3; 1,2,5-thiadiazol-

3-carboxylic acid, 13368-86-0; 1,2,4-triazole-3-carboxaldehyde, 31708-25-5; 4-pyridazinecarboxylic acid, 50681-25-9; 5-pyrimidinecarboxylic acid, 4595-61-3; 4-pyrazinecarbonyl chloride, 19847-10-0; 6-hydroxy-3-pyrimidazinecarboxylic acid, 37972-69-3; 3,3-dimethyl-5-[(indol-2-ylcarbonyl)amino]-6-nitroindol-2-one, 120791-41-5; 3-quinolinecarboxylic acid, 6480-68-8; 4-quinolinecarboxylic acid, 486-74-8; 4-imidazol-1-ylbenzaldehyde, 10040-98-9; 4-acetylpyridazine, 50901-46-7.

Effects of N-Substitution on the Activation Mechanisms of 4-Hydroxycyclophosphamide Analogues

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The activation mechanisms of the N-substituted 4-hydroxycyclophosphamide analogues 4-hydroxyifosfamide (**2b**), 4-hydroxytrofosfamide (**2c**), and 3-methyl-4-hydroxycyclophosphamide (**2d**) were compared with that of the unsubstituted parent compound **2a**. The reaction kinetics of *cis*-**2b**, -**2c**, and -**2d** are qualitatively similar to those of **2a** in that they undergo ring opening to the respective aldophosphamide intermediates **3**, which can reclose to the *cis*- or *trans*-4-hydroxy isomers or undergo base-catalyzed β-elimination to generate the corresponding phosphoramide mustard products **4**. In contrast to the general acid catalysis observed for ring opening of **2a** and **2d**, the *N*-(chloroethyl)-substituted analogues **2b** and **2c** undergo specific base-catalyzed ring opening. This mechanistic difference was also illustrated by the rapid reaction of **2a** and **2d** with sodium 2-mercaptoethanesulfonate (Mesna) under acidic conditions to give the 4-(alkylthio)-substituted cyclophosphamide derivatives **5a** and **5d**. Compounds **2b** and **2c** did not react with Mesna to generate **5b** and **5c** under these conditions. Both the fraction of aldehyde/hydrate present at equilibrium and the cytotoxicity against L1210 cells in vitro decreased in the order **2c** > **2b** > **2a** > **2d**. The plasma-catalyzed acceleration of phosphoramide mustard generation previously reported for **2a** was also observed for these analogues.

Cyclophosphamide (**1a**; see Chart I), a widely used antitumor and immunosuppressive agent, is a prodrug that requires initial activation to 4-hydroxycyclophosphamide (**2a**) by the mixed-function oxidase system. The overall activation process and mechanistic details have been extensively studied and reviewed.[1-4] In aqueous solution, *cis*-**2a** undergoes acid-catalyzed ring opening to aldophosphamide (**3a**); this intermediate can recyclize to *cis*- or *trans*-**2a** or undergo base-catalyzed elimination to generate acrolein and the cytotoxic metabolite phosphoramide mustard (**4a**).[5-8] Generation of **4a** from the pseudoequilibrium mixture of **2a** and **3a** is also catalyzed by serum albumin[9,10] and possibly by 3′–5′ exonucleases.[4,11-13]

Numerous structural modifications have been carried out in an attempt to improve the drug's therapeutic index.[2-3,14] Cyclophosphamide analogues of particular clinical interest include mafosfamide (**5a**), ifosfamide (**1b**), and trofosfamide (**1c**). The mechanisms involved in the activation of **5a** were recently reported.[15] Compounds **1b** and **1c** are substituted with a 2-chloroethyl group at the 3-position, and the metabolic activation of these analogues is qualitatively similar to that of cyclophosphamide.[8,16-22]

Table I. Rate Constants for the Ring Opening of 4-Hydroxycyclophosphamide Analogues

compd	[phosphate], mM	pH	k_1, min^{-1}
cis-**2b**	100[a]	6.5	0.083
	100[a]	7.0	0.11
	100[a]	7.4	0.21
	100[b]	7.4	0.23
	150[b]	7.4	0.20
	200[b]	7.4	0.24
cis-**2c**	100[a]	6.5	0.023
	100[a]	7.0	0.049
	100[a]	7.4	0.10
	100[b]	7.4	0.11
	150[b]	7.4	0.11
	200[b]	7.4	0.12
cis-**2d**	100[a]	6.5	0.08[c]
	100[a]	7.0	0.05[c]
	100[a]	7.4	0.03[c]

[a] Ionic strength = 0.28. [b] Ionic strength = 0.6. [c] Upper limit estimated from plasma data.

(1) Friedman, O. M.; Myles, A.; Colvin, M. *Adv. Cancer Chemother.* **1979**, *1*, 143.
(2) Stec, W. J. *Organophosphorus Chem.* **1982**, *13*, 145.
(3) Sladek, N. E. In *Metabolism and Action of Anticancer Drugs*; Powis, G., Prough, R. A., Eds.; Taylor and Francis: London, 1987; p 48.
(4) Hohorst, H.-J.; Bielicki, L.; Voelcker, G. *Adv. Enzyme Regul.* **1986**, *25*, 99.
(5) Borch, R. F.; Hoye, T. R.; Swanson, T. A. *J. Med. Chem.* **1984**, *27*, 490.
(6) Zon, G.; Ludeman, S. M.; Brandt, J. A.; Boyd, V. L.; Okkau, G.; Egan, W.; Shao, K.-L. *J. Med. Chem.* **1984**, *27*, 466.
(7) Borch, R. F.; Millard, J. A. *J. Med. Chem.* **1987**, *30*, 427.
(8) Low, J. E.; Borch, R. F.; Sladek, N. E. *Cancer Res.* **1983**, *43*, 5815.
(9) Sladek, N. E.; Powers, J. F.; Grage, G. M. *Drug. Metab. Dispos.* **1984**, *12*, 553.
(10) Kwon, C.-H.; Maddison, K.; LoCastro, L.; Borch, R. F. *Cancer Res.* **1987**, *47*, 1505.
(11) Voelcker, G.; Bielicki, L.; Horhorst, H.-J. *J. Cancer Res. Clin. Oncol.* **1981**, *99A*, 58.
(12) Bielicki, L.; Voelcker, G.; Horhorst, H.-J. *J. Cancer Res. Clin. Oncol.* **1983**, *105*, 27.
(13) Bielicki, L.; Voelcker, G.; Horhorst, H.-J. *J. Cancer Res. Clin. Oncol.* **1984**, *187*, 195.
(14) Ludeman, S. M.; Boyd, V. L.; Regan, J. B.; Gallo, K. A.; Zon, G.; Ishii, K. *J. Med. Chem.* **1986**, *29*, 716.
(15) Kwon, C.-H.; Borch, R. F.; Engel, J.; Niemeyer, U. *J. Med. Chem.* **1987**, *30*, 395.
(16) Allen, L. M.; Creaven, P. J. *Cancer Chemother. Rep.* **1972**, *56*, 603.
(17) Takamizawa, A.; Matsumoto, S.; Iwata, T.; Tochino, Y.; Katagiri, K.; Yamaguchi, K.; Shiratori, O. *J. Med. Chem.* **1984**, *17*, 1974.
(18) Horhorst, H.-J.; Peter, G.; Struck, R. F. *Cancer Res.* **1976**, *36*, 2278.
(19) Montgomery, J. A.; Struck, R. F. *Cancer Treat. Rep.* **1976**, *60*, 381.
(20) Colvin, M. *Seminars Oncol.* **1982**, *9*, Suppl. 1.
(21) Struck, R. F.; Dykes, D. J.; Corbett, T. H.; Suling, W. J.; Trader, M. W. *Br. J. Cancer* **1983**, *47*, 15.
(22) Brade, W. P.; Herdrich, K.; Varini, M. *Cancer Treat. Rev.* **1985**, *12*, 1.

0022-2623/89/1832-1491$01.50/0